

# IMARIS 9.2

## Reference Manual

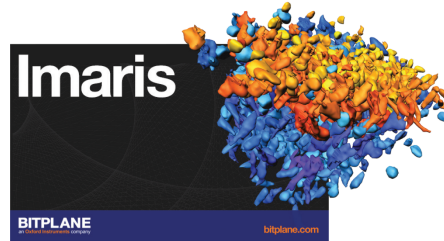


**BITPLANE**  
an Oxford Instruments company

## Section 1 Preface - Getting Started

### Getting Started in Imaris

Today, optical microscopes can record several channels simultaneously to produce multi-channel images. Imaris is an application designed to visualize such complex microscopic data. Imaris uses a special file format to describe images with parameters and can incorporate image files for all major microscopes and image acquisition systems. The images can be viewed in several different ways and processed to provide the optimum amount of information from 2D or 3D still images, time series, and animations.



Once a data set has been loaded into Imaris, individual parameters such as channel colors, geometrical settings or voxel sizes can be adjusted. Imaris has a variety of tools available, such as cropping, threshold cutting and filters for processing the images to bring out the required details.

### An Overview of the Viewing Mode Options

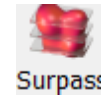
Imaris provides different viewing options for visualization and production of high quality images for presentations, data visualization and storage. This section provides a brief overview of these modes found in the top menu bar, and how they may be used. Each mode is explained in detail in later sections of this manual.



Arena

#### Arena

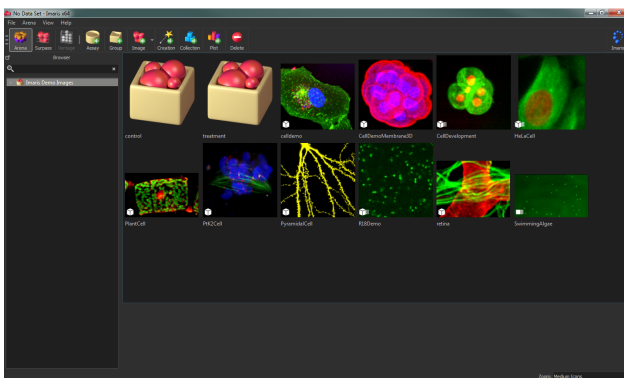
The [Arena](#) is an easy-to-use image management system to manage your image data files. Arena has the ability to batch process through a group of images and produce a statistical data-visualization reports, providing an ideal solution for all your image processing and analysis requirements.



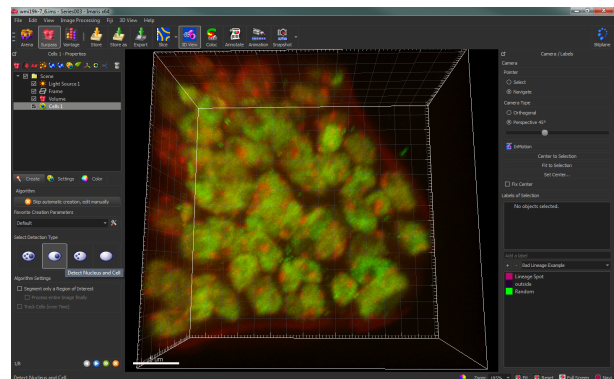
Surpass

#### Surpass View

The [Surpass](#) view offers powerful tools for data preparation, presentation and manipulation of different types of image data. This is presented in a large viewing area (default 3D view) with numerous tools and creation wizards for processing your image data effectively.



Refer to the [Arena](#) section for further information.

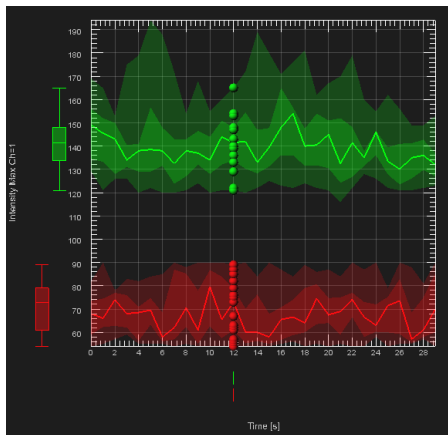


Refer to the [Surpass](#) section for further information.



### Vantage View

[Vantage](#) view is a statistical data-visualization module. This provides a range of tools to create high-dimensional color plots and permits analysis of multi-object images e.g. after processing in Surpass. Its interactive visualization methods help you to enhance the understanding of complex statistical data by creating plots using actual segmented objects.

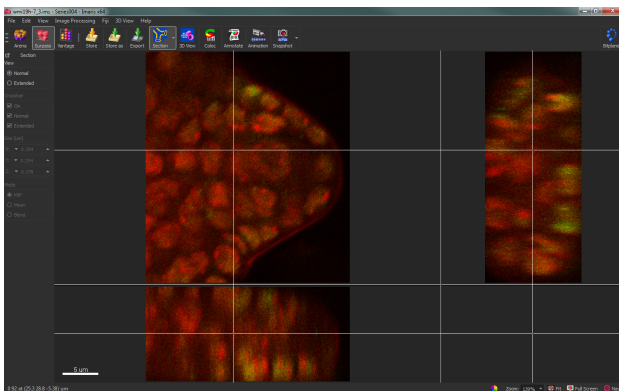


Refer to the [Vantage](#) section for further information.



### Section View

The [Section](#) view option enables simultaneous viewing along three coordinate axes (cross-sectional view).

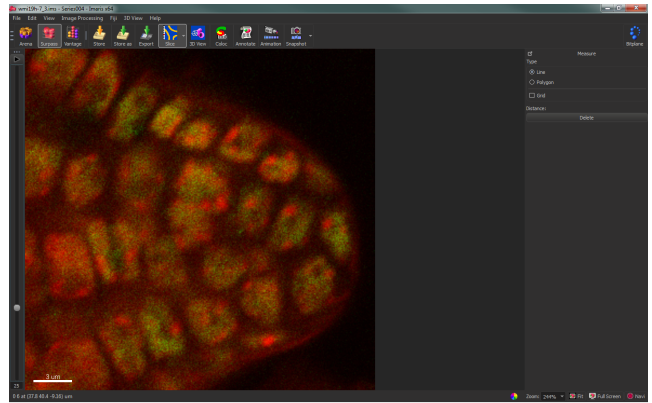


Refer to the [Section](#) section for further information.



### Slice View

[Slice](#) view allows you to view each slice of the image.

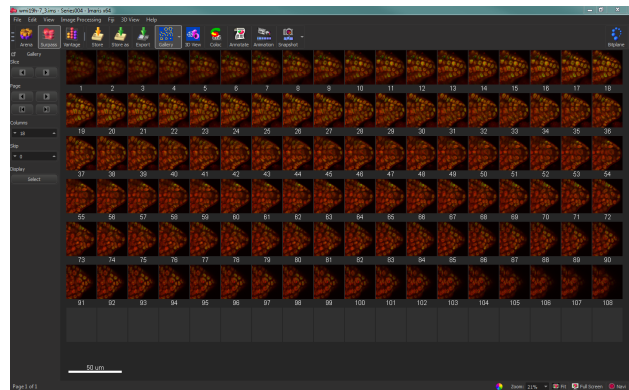


Refer to the [Slice](#) section for further information.



### Gallery View

Use the [Gallery](#) view to see all the image slices or frames in a Gallery overview view.

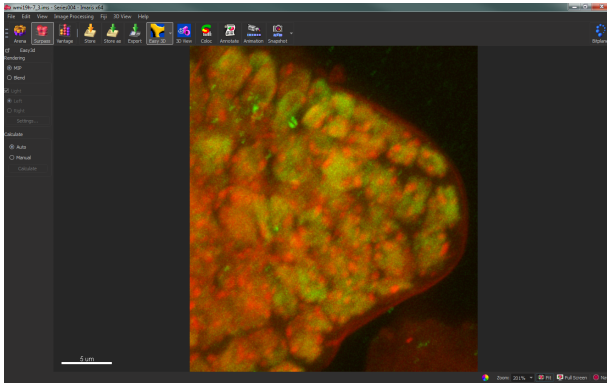


Refer to the [Gallery](#) section for further information.



## Easy 3D View

[Easy 3D](#) provides a quick and simple 3D view of your image - useful as a preview before further processing.

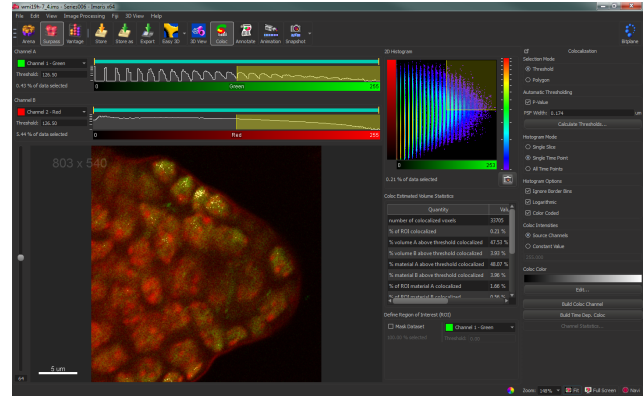


Refer to the [Easy 3D](#) section for further information.



## Colocalization View

This mode is for [Colocalization](#) computations.

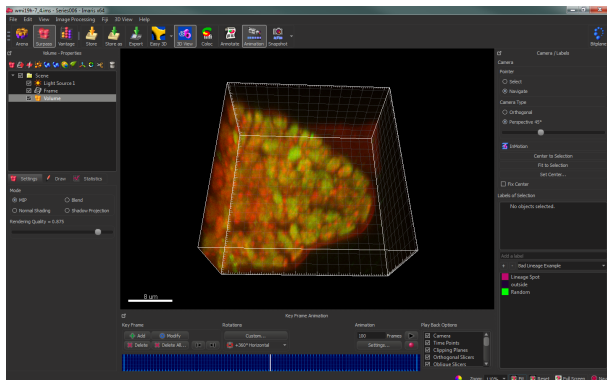


Refer to the [Colocalization](#) section for further information.



## Animation

An [Animation](#) can be created from the Slice and 3D modes, or with the key frame animator in Surpass.



Refer to the [Animation](#) section for further information.



## Snapshot

Use snapshot to copy the current image view. You can adjust the settings e.g. file format, image size etc. making working with multiple images, quick, easy and consistent.

Refer to the [Snapshot](#) section for further information.

## Easy Navigation

Navigate within the Imaris modules using frequently used toolbars, menus, and interactive controls that remain the same, and can all be operated with the mouse buttons.

## View-specific menus

Configure and define the appearance of image data and access processing methods with options specific to the selected view. Toolbars and options update in context with selected options.

## Help

Hover the mouse over an object for a tool-tip. Right-click any any object to go directly to the relevant section of this Imaris reference manual.





## 1.1 Installation and License Information

Imaris software is delivered on a standard CD or downloaded from [www.bitplane.com](http://www.bitplane.com). The CD includes a folder containing the necessary manuals, or the manuals can be downloaded.

Imaris runs on:

- MS Windows 10 x64
- MS Windows 8 x64
- MS Windows 7 x64
- Mac OS X 10.9 to 10.13
- Intel based MAC

**Please note: Windows 8 and 10 do not come with the .net 3.5 and prior framework required for the matlab runtime which Imaris XTensions use. In order to enable this 'Windows Feature' you will need an administrator account. You can set this under Control Panel -> Programs and Features, left hand side panel contains 'Turn Windows features on or off'.**

To find the latest information about minimum and recommended hardware requirements please visit our web site - the Support section.

### Installation

To install the software, please proceed as follows:

1. Insert your Imaris CD-Rom in the computer.
2. Follow the instructions on the screen.
3. The installation is completed automatically.

### Licensing

To run Imaris, the appropriate licenses for the required modules, such as Imaris base, ImarisTime, ImarisColoc or Imaris MeasurementPro. Without licenses, Imaris can only be run in a restricted mode. In case of any license problems, please refer to the support information on our website [www.bitplane.com](http://www.bitplane.com) for detailed instructions.

### Starting Imaris

Imaris can be started by one of the following methods:

- Double-click on the Imaris icon (we recommend copying the icon to the desktop).
- Drag the icon of an image or a file to the Imaris program icon.

The software opens with the main screen.

### Supported File Formats

Imaris (as of version 5.5) can read the following file formats, i.e. it can read the image and the parameters.

- Andor: Multi-TIFF series (\*.tif, \*.tiff)
- Andor: MM (\*.kinetic)

- Andor: iQimage (\*.kinetic)
- Applied Precision, Inc: DeltaVision (\*.r3d, \*.dv)
- Biorad: MRC-600, MRC-1024 (series)(\*.pic)
- BioVision: IPLab Mac (\*.ipm)
- Bitplane: Imaris 5.5 (\*.ims)
- Bitplane: Imaris 3.0 (\*.ims)
- Bitplane: Imaris 2.7 Classic/Old (\*.ims)
- Bitplane: Imaris Scene File (\*.imx)
- BMP (adjustable file series) (\*.mrc, \*.st, \*.rec)
- Carl Zeiss: LSM 510 (\*.lsm)
- Carl Zeiss: LSM 410, LSM 310 (\*.tif, \*.tiff)
- Carl Zeiss: Axiovision (\*.zvi)
- Compix:Simple PCI (\*.cdx)
- Image Cytometry Standard: ICS - used by Nikon, Huygens, and others (\*.ics, \*.ids)
- Gatan Digital Micrograph (serie):(\*.dm3)
- Leica: TCS-NT (\*.tif, \*.tiff)
- Leica: LCS (\*.lei, \*.raw, \*.tif, \*.tiff)
- Leica: series (\*.inf, \*.info, \*.tif, \*.tiff)
- Leica: Image File Format (\*.lif)
- MetaSystem: Meta Viewer (\*.imv)
- Molecular Devices: Metamorph STK (series) (\*.stk)
- Molecular Devices: Metamorph ND (\*.nd)
- MRC: primarily electron density volumes as in cryo-EM (\*.mrc, \*.st, \*.rec)
- Nikon: ND2 (\*.nd2)
- Olympus: FluoViewTIFF (\*.tif, \*.tiff)
- Olympus: 1000 OIF (\*.oif)
- Olympus: 1000 OIB (\*.oib)
- Olympus: Cell^R 1.1/standard (\*.tif, \*.tiff)
- Olympus:Virtual Slide Image (\*.vsi)
- Opellab: liff (series) (\*.liff)
- Openlab: raw (\*.raw)
- Open Microscopy Environment XML (\*.ome)
- Open Microscopy Environment TIF (\*.tif, \*.tiff)
- Perkin Elmer: UltraView (\*.tim, \*.zpo)
- Prairie Technologies: Prairie (\*.xml, \*.cfg, \*.tif, \*.tiff)
- Scanalytics: IPLab (\*.ipl)
- Slide Book (\*.sld)
- TIFF (adjustable file series) (\*.tif, \*.tiff)
- TILL Photonics: TILLvision (\*.rbinf)

Imaris can also read general TIFF series (or BMP series) in the format aaaNNN.tif (where a is a character and N is a number).

## 1.2 About this manual

Information in this manual is subject to change without notice and does not represent a commitment on the part of Bitplane AG. Bitplane AG is not liable for errors contained in this manual or for incidental or consequential damages in connection with the use of this software.

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or contact our support teams:

[eusupport@bitplane.com](mailto:eusupport@bitplane.com)

[ussupport@bitplane.com](mailto:ussupport@bitplane.com)

[asiasupport@bitplane.com](mailto:asiasupport@bitplane.com)

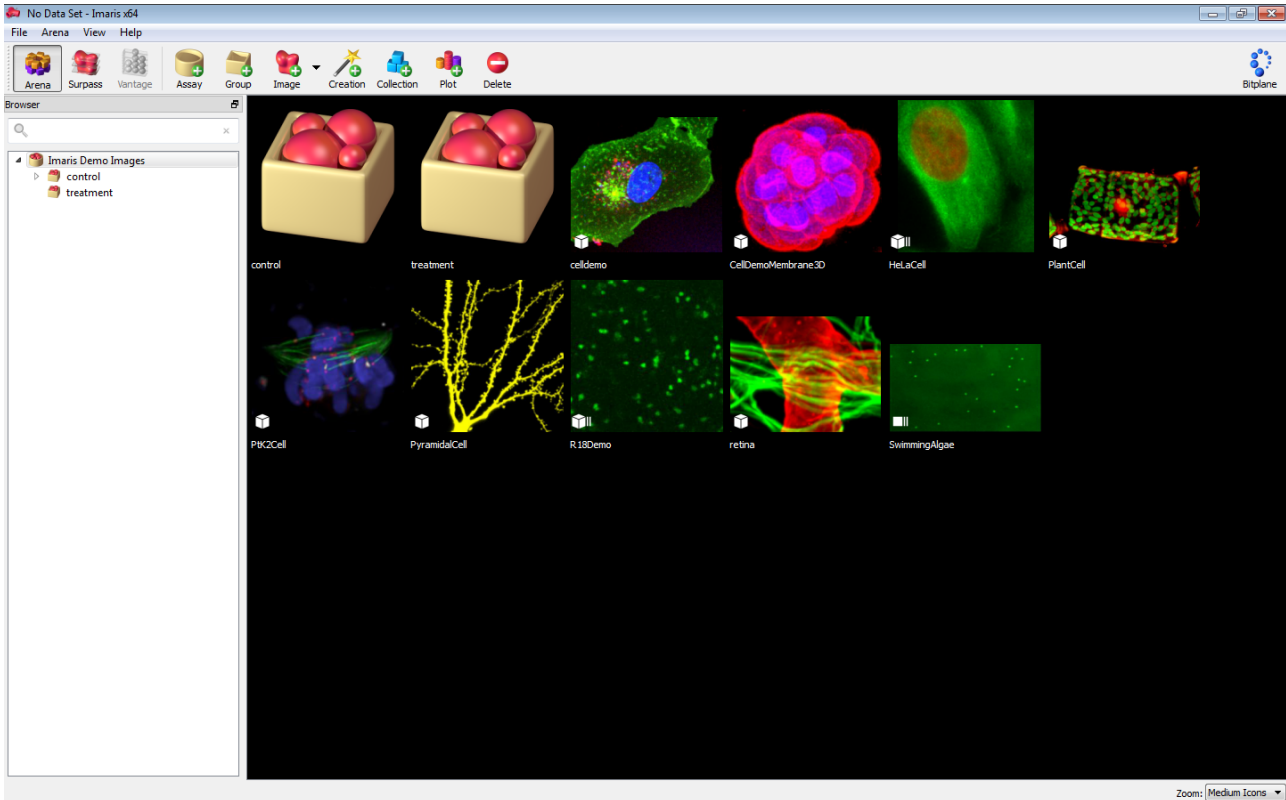
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## Section 2 Arena View

The **Arena View** provides an easy-to-use image management interface. Image analysis often requires the application of the same creation parameters to any number of images. Arena lets you speed up this process by enabling "batch processing" to be performed on multiple images.

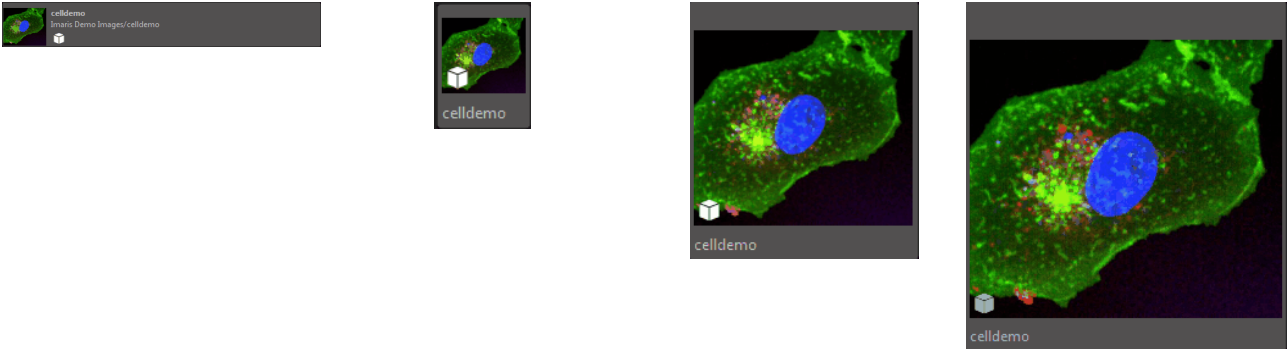


The Arena is your central working area in Imaris and an interface for visualizing, processing, analyzing and interpreting all your images. This is where you add images and creation parameters, create and execute batch runs, and configure all your statistical data-visualization settings.

The Arena view consists of a number of elements.

The search field is at the top left of the Arena view. Below the search field is the [Arena Tree](#). The Arena tree allows easy access to all Arena items. On the left hand side of this view you will find the Arena tree which allows navigation of the folder structure. The pane on the right hand side of the Arena view display contents of the current tree location or any search results. When an item is selected further information about this object will display showing the selected object's Properties, tags and surpass objects where appropriate. The rightmost three quarters of the Arena view display contents of the current tree location or any search results. The lower section of the Arena view displays the [Tags tab](#) or the [Properties](#) area with additional information about the currently selected image. At the bottom right hand side of the Arena view is Zoom.

In Arena view **Zoom** can be used to change how the images are displayed. You can select from: Details that provides a summary of the image files, or Small, Medium or Large Icons, these are shown in order from left to right below. Zoom can help you navigate and find the required image, if you have many, or similar stored images.



Above: From left to right - Details, Small Icon, Medium Icon, Large Icon

### Arena Items

Each Arena item has a number of different properties associated with it. Properties information affects how items are visualized or processed. Some properties are fixed and others are user-defined.

Every Arena item is represented and displayed as an icon with its name written underneath. The following table presents each item along with its associated icon:

Arena item	Icons	
Assay	Empty	Filled
Group	Empty	Filled
Image		
Creation parameters		
Collection	Batch Collection	Manually created Collection
Vantage Plot		

Depending on the selected item, a right-click displays different options. The type of item determines what options and tools are available.

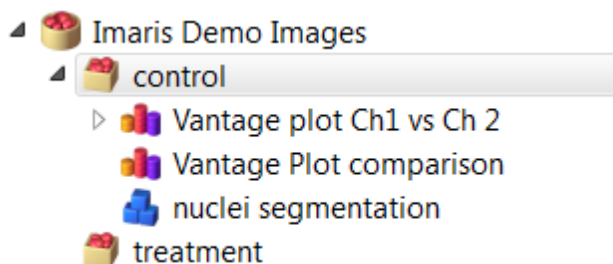
### Using Imaris Without Arena

Imaris has been developed so that the Arena provides the most effective means of accessing and managing your images, and image data. It is however also possible to configure Imaris (version 8.3.0 onwards) so that Arena View is not used. During installation, an option is provided to not use Arena. Using Arena is recommended- if you want to install Imaris without Arena, please contact Bitplane support for further information.

## 2.1 Arena Tree

The Arena Tree is used to organize all the items within the Arena in a hierarchical structure. The Arena tree provides direct access to all the components of your experimental data. To assist you in finding and organizing the Arena items, a specific item's icon is displayed adjacent to the tree hierarchy.

A triangle at the far left of the tree item indicates that this item has multiple entries. Click on the arrow next to the tree item to expand it and display the item's contents, or to contract it.



Clicking on the tree element displays the contents along with all applicable items within. Selecting any of these specific items will restrict your current view to only that item content.

You can manipulate the Arena tree by adding and/or deleting Assay or Groups and items within them.

You can also change the structure of the tree by moving items using the drag and drop method.

### How to Copy/Move Arena Items

To copy or to move an item to a new location, select the item within the Arena view and then simply drag and drop the item into another tree location. The item's icon changes to show a plus sign indicating that you are in drag mode; a minus sign will appear when a drop is not allowed.

Please note that when you drag and drop an item to a new tree location within the Arena, a copy of that item is created in the new location, the item is not moved from the original location.

If the item dragged is a whole Group, Imaris will automatically recognize its contents and add all its files to the new chosen location. Any hierarchy within the items that you drag and drop will be retained.

You can drag as many items or groups as required, and you can add them to any level of the Tree structure. The insertion is indicated and highlighted within the tree. The amount of indentation indicates the level at which an item is located within the tree hierarchy.

To move items you can use drag and drop in combination with the Control key.


Batch Collection item contains the created objects type (based on the selected creation parameter item) and all corresponding statistical values.

If you modify the copied image created from the drag and drop process, the original image is not modified (Imaris 8.3. onwards).

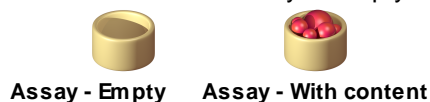
## 2.2 Assay

The top level Arena item is called **Assay**. It should be used to handle a large data set of similar data. It may contain a large amount of experimental image data and any associated information.

To start working with Imaris, you must first create an Assay.

1. To create an Assay, select the Add Assay icon  in the Main toolbar.
2. Enter a descriptive name and a new item, Assay, is created.

As soon as any changes are made the Arena tree is automatically updated. Within the tree a new item, Assay, is created and the corresponding icon indicates that the Assay is empty.



The Assay can hold all Arena items: [Groups](#), [Images](#), [Creation Parameter](#), [Collections](#), [Vantage plots](#). If an Assay item is selected within the tree, the items belonging to the Assay are displayed in the Arena View.

The next, recommended step is to create a [Group](#) item within the Assay structure.

Within one Assay you can create as many Group items as you require.

As soon as any [Group](#) item is created, the Arena view is also automatically updated. The Assay icon is updated, indicating the added Group.

### Right-click options

Right-clicking on an Assay item provides a specific context sensitive menu, giving you access to various actions.

#### Rename

Each item has a name. This name may be changed to assist the organization of data. Right-click once on an item, select the Rename option and change the name in the editable field.


#### Delete

Choose the item(s) to be removed and then select either the Delete option or Delete from the Main toolbar. Verify and confirm your choice and that the selected items are deleted.

## 2.3 Group

The **Group** item is a child of Assay. A Group item helps you keep everything organized by allowing you to gather related items together.

### How to create a Group item

1. To create a Group, select the add Group icon  in the Main toolbar.
2. Enter a descriptive name and a new item, Group, is created.
3. Group is automatically inserted as a child of the currently active Assay, both within the tree-view on the left and the Arena view.

As soon as any changes are made, the Arena view is also automatically updated. The Assay icon is updated, indicating the added Group. Within the tree and the Arena view the Group icon indicates that the Group is empty.





A Group can contain 2D, 3D and 4D images or any other Arena item.

A typical Group consists of many images, creation parameters, Batch results, and/or Vantage plot items. Within the Group, each Arena item is labeled with a special icon. The icon graphically indicates the type of item.

The next step is to add Image items within the Group.

As soon as any item is added within the Group, the Arena view is also automatically updated. The Group icon is updated, indicating that content has been added.

## Right-click options

Right-clicking on a Group item provides a specific context sensitive menu offering you various actions.

### Rename

Each item has a name. This name may be changed to assist organization of data. Right-click once on an item and select the rename option and in an editable field change the name.

### Delete


Select the item(s) to be removed and select the Delete option or Delete from the Main toolbar. Verify and confirm your selection and the selected items are deleted.

Please note: Creating and using a Group is helpful if you want to experiment with a potential [creation parameters](#) sets.

## 2.4 Image

A Group can contain 2D, 3D and 4D images. All images within Arena view are displayed as thumbnails.

### How to add Image items

To add images within the Group select Image in the main toolbar then select the Add Image icon .

This opens a new window from which you can browse and select the required files. In the Open window, select the files you want to add to the Group. Click on the desired file name or select multiple files using standard multiple file selection commands.

### How to add an Image Folder

To add image folders select Image in the main toolbar then select the Add Image Folder icon .

This opens a new window from which you can browse and select the required folder. In the Open window, select the folder you want to add. This will add image files within the selected folder that are in the supported file formats. To add images within subfolders click Recursively add subfolder. This will add images within subfolders, keeping the file structure of the selected folder.

Once you have selected your image(s) and Imaris has finished adding the files, the image's thumbnail appears within the Arena view. Within the tree, the Group icon will change indicating that the Group is populated. Place all relevant images for analysis in a single group.

Imaris collects relevant information from supported file formats when they are added within the Arena and sets the relevant metadata within the [Properties Tab](#) automatically.

You can place the same image file in as many groups as you like. To add an image file to another Group, either repeat the same procedure or alternatively select the image thumbnail and drag and drop it into the required Group.

A small icon in the bottom left corner of the image thumbnails indicates the image type and/or any created objects. Each image may be viewed in a number of different ways. The way that you open the image depends on the type of images and how you want to view and work with it.

The default view is to open the image with the created objects and batch results.

### Right-click options

Image view may be adjusted to the most useful option by right-clicking. Depending on the selected image, the right click opens a list that allows you to choose between different Surpass view visualization options. The following options are available:

#### Open with objects and Batch results

Once the batch run is completed, to validate the Batch segmentation results you can open the image with all created objects and batch results.

#### Open with objects from file only

This option opens image data and all previously created Surpass objects from the selected file.

#### Open with batch results

After the Batch run is completed, the batch segmented images can be compared with the original raw data to make sure that no structure not seen in the original data has been introduced by batch processing.

#### Open image only

This option opens image data only.

#### Open image resampled

The Resampling Open dialog box can be used as a preview before loading the data set and allows you to change the resolution of the data (subsampling) and to select only a part of the data set (cropping) by specifying the parameters in the Resampling Open dialog box. Both options reduce the size of the data set, decreasing the time needed to read the file and speeding up any operations on the data. This is particularly helpful/beneficial when reading large data sets over a network.

### 2.4.1 Image Choice within the Group

If you require your images to be segmented and analyzed in a certain way in the batch run, make sure they are selected in one [Group](#).

Added images within one Group should be scientifically relevant and similar in any other quality attributes, as may be scientifically relevant to the batch results. Due to the essence of [batch processing](#), a well grouped image is essential to ensure reliability between different batch results and ensures the comparability of the different experimental groups.

You should make sure that you are working with a group of images acquired under largely uniform conditions. Also always make sure you have selected all images within the Group that you want to appear in the [Batch Collection](#). Once the Batch job is completed you can modify the image selection within the Group. However, please note that any modifications that you make to the image selection do not affect the previously created Batch Collection item.

When the image files have been successfully added within the Group items, identify the most-representative image of the whole group. The image will be used to define the [Creation Parameters](#) and the entire batch settings.

To inspect the image, double-click on the thumbnail to open it in the [Surpass view](#).

## 2.4.2 Open

Data sets can be loaded from various file formats.

### Image File Series

If the data set consists of a series of images each is stored as an individual file. To open a series of images select the folder and click on Open to open it.

This brings up the Image selection window, which allows you to explore your file system.

The window contains a preview of all the images as well as the following info: Name, Description, Recording date, Size, Time Points, Channels, Total size and Voxel size.

The image series is ordered according to the number, which appears after the file name suffix.

To open the image select a file name from the list and click **Open**.

### Image Preview

Check the box Thumbnail Preview to activate an image preview.

### Search Image name

Enter the image name for instant image selection.

### Resampling Open...

The Resampling Open dialog box can be used as a preview before loading the data set and allows you to change the resolution of the data (subsampling) and to select only a part of the data set (cropping) by specifying the parameters in the Resampling Open dialog box. Both options reduce the size of the data set, decreasing the time needed to read the file and speeding up any operations on the data. This is particularly helpful/beneficial when reading large data sets over a network.

1. In the menu select File – Open.
2. Select a file name from the list and click **Open** or double-click on the requested file entry.
3. The file is loaded.

### Imaris Background Load

If you need to open a large time series data set, Imaris **Background Load** feature loads the data in sequential



time points according to the sort order and demonstrates an advantage in speed performance over the other methods.

## iQ files in Imaris

To open iQ files in Imaris:

1. Locate and select the **DiskInfo5.kinetic** file.
2. Click on the '**Settings**' button to open a new window with a list of images and image information.
3. Check the box Thumbnail Preview to activate an image preview.
4. Select the required image and click on the **OK** button.
5. Then click again on the **Open** button. This will instantly create 3D volume rendered image of the selected data set.

## File Formats

	Format Name	Extension	Remarks
	Imarisbase		
Bitplane	Imaris 5.5	*.ims	
Bitplane	Imaris 3.0	*.ims	
Bitplane	Imaris 2.7	*.ims	
Bitplane	Imaris Scene File	*.imx	
Andor	iQ Image	*.kinetic	
Andor	Multi-TIFF	*.tif,*.tiff	
	TIFF-Series	*.tif,*.tiff	
	BMP-Series	*.bmp	
	Imarisreaderdeltavision		
Applied Precision	DeltaVision	*.r3d,*.dv	
	Imarisreaderbiorad		
Bio-Rad	MRC	*.pic	
	Imaris reader iplab [BioVision purchased IPLab in 2006]		
BioVision	IPLab IPM	*.ipm	Mac
BioVision	IPLab IPL	*.ipl	Windows
	Imarisreadergatan		
Gatan	DigitalMicrograph	*.dm3	
	Imarisreaderhamamatsu		
Hamamatsu Compix	SimplePCI	*.cxd	
	Imarisreaderiii		
Intelligent Imaging Innovations	SlideBook	*.sld	
	Imarisreaderimod		
IMOD	MRC	*.mrc,*.st,*.rec	
	Imarisreaderleica		
Leica	Image File Format LIF	*.lif	
Leica	TCS-NT	*.tif,*.tiff	

Leica	Series	*.tif, *.tiff, *.inf, *.info
Leica	Vista LCS	*.tif, *.tiff, *.lej, *.raw
	Imarisreadermicromanager	
Micro-Manager	Image5D	*.tif, *.tiff, *.txt
	Imarisreaderuniversalimaging [Molecular Devices acquired Universal Imaging in 2002]	
Molecular Devices	MetaMorph STK	*.stk
Molecular Devices	MetaMorph ND	*.nd
	Imarisreadernikon	
Nikon	Image Cytometry Standard ICS	*.ics, *.ids
Nikon	ND2	*.nd2
	Imarisreaderolympus	
Olympus	cellR	*.tif, *.tiff
Olympus	FluoView TIFF	*.tif, *.tiff
Olympus	FluoView OIB	*.oib
Olympus	FluoView OIF	*.oif
Olympus	VSI	*.vsi
	Imarisreaderome	
Open Microscopy Environment	XML	*.ome
Open Microscopy Environment	TIFF	*.tif, *.tiff
	Imarisreaderperkinelmerinc	
PerkinElmer	Improvision Openlab LIFF	*.liff
PerkinElmer	Improvision Openlab RAW	*.raw
PerkinElmer	UltraView	*.tim, *.zpo
	Imarisreaderprairie	
Prairie Technologies	Prairie View	*.xml, *.cfg, *.tif, *.tiff
	Imarisreadertill	
TILL Photonics	TILLvisION	*.rbinf
	Imarisreaderzeiss	
Zeiss	AxioVision	*.zvi
Zeiss	LSM 510	*.lsm
Zeiss	LSM 410, LSM 310	*.tif, *.tiff
Zeiss	CZI	*.czi
TIFF (adjustable file series)		*.tif, *.tiff

**Tip:** If you want to reopen one of the last six images, you can use the **Recent File List** at the bottom of the **File** menu to select the desired image.

### 2.4.3 Remote File Server Authentication

If the Arena image data is retrieved from a remote file server that requires authentication, Imaris uses previously given access credentials to connect to external data sources. Refer to the [Arena](#) Section.

## 2.5 Batch Processing

Image analysis often requires the application of the same creation parameters to any number of grouped images. Batch protocols are the most powerful and efficient way of segmenting and analyzing images. The



ability of Imaris to batch process a group of images to produce a statistical data-visualization report is the essence of Imaris [Arena view](#).

Batch processing enables you to apply the same creation parameters to all images within the selected Group, sparing you the repetitive work.

This section lists the components of the batch processing framework in Imaris and provides an overview of the steps you have to follow to create a [creation parameters](#) item, a batch run and analyze the results of the batch.

Before you start analyzing your images there are some aspects to be confirmed and a few steps to be completed before pressing "go" on any batch run.

The batch process has the following stages:

1. To apply the same creation parameters all images within the Group, first you need to set them up.
2. Storing these creation parameters generates a new Arena item, the [Creation Parameters](#) item 
3. Then double check your [image selection](#) within the Group before selecting the Batch processing option.
4. Right-click on Creation parameter items and select the Run Batch job option.  
The creation parameters are automatically applied to all [images within the Group](#) and simultaneously, a new Arena item, the Batch [Collection](#) item  is created.
5. Once the Batch job is completed you can analyze the results within [Vantage view](#).

## 2.6 Creation Parameters

To be able to apply the creation parameters to all images within the Group you need to set them up.


Identify the most representative image of the whole group.

Double-click on the image thumbnail to open it in the Surpass view.

Complete an appropriate Creation Wizard ([Cells](#), [Filament](#), [Spots](#) and [Surfaces](#)) and create the required objects.

Under the Creation Tab ([Cells](#), [Filament](#), [Spots](#) and [Surfaces](#)) all processing instructions and the parameter's value are listed. The **Store Parameters for the Batch** button allows you to save the complete set of the creation parameters and all relevant settings.

In the Store Creation Parameter window, enter a descriptive name for your creation parameters and select the **Arena** option as the required store location.

When you select the **Arena** option, a new item is automatically created within the Arena view. The Arena view is updated, a new Creation Parameters item  is displayed.

### How to apply the same creation parameters in multiple Groups

As image analysis often requires the application of the same creation parameters to any number of grouped images, the selected creation parameters item can be copied between the Group(s).

In addition to copying the selected creation parameters item, you can also import it into the newly chosen Group. To do so, select the Group and click on the [Creation button](#). The creation parameters file (\*.icpx) is

imported.

Please note that only creation parameters files (\*.icpx) generated by the Export command can be imported.

## How to copy Creation Parameters item


To copy the creation parameters from one Group to a new one, select the creation parameters item that you want to copy and then drag and drop it into the newly chosen Group.

## Right-click options

Right-clicking on a Creation Parameter item provides a specific context sensitive menu offering you various actions.

## Run Batch Job

Before selecting the Run Batch job option double check your [image selection](#) within the Group.

As soon as you press the Run Batch Job option Imaris generates a new Arena item, the Batch [Collection](#) item . To create a Batch Collection item, Imaris applies the selected creation parameters set, (that you created for the chosen image) to all images within the Group. A Batch Collection item contains the image's data and batch segmented objects along with the related statistical calculation.

## Run Batch including Sub-Groups

This option recursively applies the creation parameters to all the images of the chosen Assay/ Group and all its subgroups.

As soon as you press the Run Batch Job option Imaris generates a new Arena item, the Batch [Collection](#) item within the selected Assay/ Group and all its subgroups.

To create a Batch Collection item, Imaris applies the selected creation parameters set, (that you created for the chosen image) to all images within the Group. A Batch Collection item contains the image's data and batch segmented objects along with the related statistical calculation.

## Export

Select the creation parameters item you would like to export and click the Export button. If necessary, rename and save the file in the chosen location. The Imaris creation parameters file will have an .icpx suffix.

## Rename

Each item has a name. This name may be changed to assist organization of data. Right-click once on an item and select the rename option and in an editable field change the name.

## Delete

Select the item(s) to be removed and select the Delete option or Delete from the Main toolbar. Verify and confirm your selection and the selected items are deleted.



## 2.7 Creation

In addition to creating a new set of creation parameters you can also import a creation parameters file. By clicking the Creation button, the creation parameters files (\*.icpx) generated by the Export command can be imported into Imaris Arena or Surpass view.

Under the Edit/Preference/[Creation Parameters](#) the Export and Import buttons provide an easy way to exchange creation parameters between different users or Arena groups.

## 2.8 Batch Collection


As soon as you press the Run Batch Job option Imaris generates a new Arena item, the Batch Collection item



To create a Batch Collection item, Imaris applies the selected creation parameters, to all images within the Group. Within the Group, each image is segmented and analyzed, the selected objects are created and the corresponding statistical data is calculated. All this information is collected and stored within the Batch Collection item.


The Batch Job execution process and status are indicated by a set of overlaid icons placed in the bottom corner of the Batch Collection item and the image thumbnail.

The following status icons are used:


1. Completed - the image is segmented and analyzed 

2. Processing. The proportion of the completed Batch Job is indicated by an icon displaying

corresponding circle segments 

3. Failed - the image(s) have not been segmented and analyzed 

After the creation parameters have been successfully applied to an image, the icon in the bottom corner of the

image thumbnail shows that the batch job is completed .

The Batch Results Collection item appears in the Arena tree directly below the corresponding Group item.

### Batch Collection Name

The Batch Collection is given a default name. This is the creation parameter file name with the Group name prefix.

Once the Batch job is completed you can modify the image selection within the Group. However, please note that any modifications that you make to the image selection do not affect the created Batch Collection.

Batch Collection item lets you retrieve segmented images quickly without affecting your original files. It also allows you to copy the selected batch processed results into your Vantage plot item and then analyze them further.


### Right-click options

Right-clicking on a Batch Collection item provides a specific context sensitive menu offering you various

actions.

## Create Vantage Plot

Vantage plots are the most informative and flexible way to visualize and generate a Batch Collection report.

[Vantage Plot](#) item  provides a statistical data-visualization of the currently selected Batch Collection item.

## Export Statistics

For some further statistical analysis you can export the data either as a Comma Separated Values file, or directly as an Excel file. Imaris offers several data exporting options.

Click on the selected export button and select the file type.

All generated statistical data are exported to Excel, which starts automatically, and sorted in different sheets.

## Rename

Each item has a name. This name may be changed to assist organization of data. Right-click once on an item and select the rename option and in an editable field change the name.

## Delete


Select the item(s) to be removed and select the **Delete** option or Delete from the Main toolbar. Verify and confirm your selection and the selected items are deleted.

### 2.8.1 Batch Results Validation

After the batch run is completed, the batch segmented images can be compared with the original raw data to make sure that no structure not seen in the original data has been introduced by batch processing.

Once the batch run is completed, to validate the Batch segmentation results you can open the image with the default view **Open with objects and Batch results** or with a right-click on the image, then select the **Open with batch results** option.

This option opens the image in the [Surpass view](#).

Within the Surpass tree you will notice that the regular object icon has been changed. A new icon , superimposed on the object type icon, indicates that the required objects were batch created and therefore you will not be able to manually modify them. Please note that these objects do not have an Edit tab. Under the Creation tab, the **Duplicate and Rebuild** button provides two functions:

1. To copy the Batch created objects to a new Surpass Tree object.
2. To re-enter the Creation Wizard and use previous parameter settings and processing instructions as initial values.

If you are not pleased with the Batch segmentation results and you find it necessary to manually modify them, please remember that all the **changes that you make will not be reflected in the Batch Collection item**.

To modify the batched processed image you can open it and start a new creation wizard. As you proceed with the new creation wizard, in each step, all creation parameters are initially set to match the batch creation parameters' values. Adjust them and modify all parameters as necessary to obtain new segmentation results.

These newly segmented objects must be saved using either the [Store](#) or [Store as](#) option. As Imaris does not allow the modification of batch created objects, these newly segmented objects have the same characteristics

as [Manually created](#) ones.

Once the manually created changes have been saved, within the Arena, the image thumbnail is updated. In its corner, a small overlaying object icon appears and indicates that the created objects has been assigned to an image.

To combine and compare these modified objects with the automatic Batch Results Collections, follow the procedure described in the [Manual Collection](#) Section.

## 2.9 Batch Queue

The Batch Queue window displays the batch status. The batch queue table lists the current job(s) as well as a list of all completed batch jobs.


For each job a short description is displayed: name, the current state of the job, the time at which the job has been submitted and finished as well as the progress percentage.


A keyboard shortcut to open the Batch Queue window is Ctrl+Shift+B.

## 2.10 Manual Collection

Sometimes you are confronted with a situation in which a few images that are not automatically well segmented by the Batch creation parameters. In this case a manual object creation should be used for the image segmentation.

The Manual Collection item allows you to combine for manually created objects with the automatic [Batch Collections](#) within one [Vantage Plot](#) item. You can use a Manual Collection item to specify a data set that contains manually created objects as an alternative input to the generate Vantage Plot comparison items.

To create a Manual Collection item select the Collection icon  on the Main toolbar and a new Arena item is created. Within the Arena tree, the Manual Collection item is created.

A small icon in the bottom left corner of the item indicates that contains manually created Surpass objects  .

Within the Surpass view, the manually created objects are not automatically stored and therefore your image changes will not be automatically updated within the Arena. Therefore, please store the image after finishing with a manual object creation. All manually created objects should be stored either by using the [Store](#) or [Stored as](#) option. Once stored, they will appear in the Arena view. This newly created image item has an overlaid icon indicating the image type and segmented surpass objects. Under the [Surpass Objects](#) tab the newly created object is added.

### How to integrate the manually created Surpass objects into the Collection item

In the Arena, under the Surpass Objects option lists all created and stored object types. To assist you in finding and organizing the created objects, each one is labeled with an object icon.

To integrate manually created objects within the Manual Collection item, select the object icon from the [Objects tab](#), then drag and drop it onto the chosen Manual Collection item.


As soon as you have drag and dropped the manually created objects within the Manual Collection item, a new overlaid icon appears and showing the object type.

### Right-click options

Right-clicking on a Batch Collection item provides a specific context sensitive menu offering you various actions.

## Create Vantage Plot

Vantage plots are the most informative and flexible way to visualize and generate a Collection report. [Vantage](#)

[Plot](#) item  provides a statistical data-visualization of the currently selected Collection data.

## Rename

Each item has a name. This name may be changed to assist organization of data. Right-click once on an item, and then select the rename option and in an editable field change the name.

## Delete



Select the item(s) to be removed and select the Delete option or Delete from the Main toolbar. Verify and confirm your selection and the selected items are deleted.

## 2.11 Vantage plot

Vantage plots are the most informative and flexible way to visualize and generate a batch report. It provides a statistical data-visualization of the currently selected collection(s) of Batch Collection data. It facilitates the analysis of the collection results and illustrates patterns and trends that are not immediately apparent by just looking at the raw or segmented data.

Within the [Vantage view](#) there are many different plot types and a selection of various plot dimensions are available so you can easily configure the appearance of the results..

There are two methods to create a Vantage plot item.

1. To visualize the Batch created objects, right-click on the [Collection](#) / [Manual Collection](#) item  and choose the Create Vantage plot option.
2. To create a Vantage Plot item, select the add Vantage icon  in the Main toolbar. Enter a descriptive name and a new item, Vantage Plot, is created. Vantage Plot is automatically inserted as a child of the currently active Assay/ Group, both within the tree-view on the left and within the Arena view.

A Vantage Plot item provides the benefits of [comparing Collection results of multiple groups](#).

Right-click on the [Collection](#) item or double-click on the Vantage plot items to open the [Vantage view](#).

## Right-click options

Right-clicking on a Vantage plot item provides a specific context sensitive menu offering you various actions.

## Open in Vantage

Select this option to visualize the selected Batch Collection created objects within the Vantage view.

## Rename

Each item has a name. This name may be changed to assist organization of data. Right-click once on an item,

and then select the rename option and in an editable field change the name.

## Delete

Select the item(s) to be removed and select the Delete option or Delete from the Main toolbar. Verify and confirm your selection and the selected items are deleted.


### 2.11.1 Comparison of multiple Groups' Collections



A Vantage Plot item generates a separate plot component on a per group basis and allows you to visualize and analyze Batch Collection results in a way that is meaningful and not overwhelming.

It helps you find relationships among several statistical variables, determine their relative importance and therefore draw a comparison between Batch Collection results more clearly.

#### How to compare Batch Collection results

To see if two or more image Groups have scientifically relevant differences between them and assess the degree of variation from one group to the next, create a Vantage plot item.

To create a Vantage plot item, select the add Vantage icon  in the Main toolbar. Enter a descriptive name and a new Vantage Plot item is created.

Then simply drag and drop the chosen Collection items  from the selected Groups into the Vantage Plot item .

To create the Vantage plot either right click on the Vantage Plot item and select the Open in Vantage option or simply double-click on the Vantage Plot item.

Within the Vantage view, the comparison Vantage plot shows an arrangement of Batch Collections results combined in one plot.

The Batch Collection results are overlaid, which helps you to determine how 'different' these image groups are. Comparisons can be drawn between any selected statistical variables that you are interested in.

Within one Vantage Plot item you can drag and drop as many Batch Collection items as you wish to compare.

### 2.11.2 Arena Vantage view

In Vantage view, in the Plot Input area, a Vantage tree displays all source objects. In the Vantage Properties Area you can choose the plot type and which variables are to be assigned to (X, Y, Z, Colour or scale) plot dimensions. For more information on Vantage view and instructions on how to select a plot type and assign plot dimensions, please refer to the [Vantage view](#) Section.

## Store

Selecting the Store option stores the Vantage plot data and created Vantage plot item within the selected Arena Group. Within the Arena tree, a subgroup Vantage appears (within the tree) and is named accordingly.

Selecting the Store option leads a dialog to appear prompting you for a descriptive name for the item.

In the Name field enter a descriptive name for your Vantage Plot item and click the **OK** button. This is the name that will appear within the Arena view. Within the Arena tree, at the bottom of the selected group the newly created Vantage plot item will appear.

## Store as

Selecting the Store as option allows you to modify the already created Vantage plot item and creates a new Vantage plot item within the selected Arena Group.

## 2.12 Properties Tab

The Properties tab contains all image-related metadata.

## 2.13 Tags Tab

Additional experiment or image data plays an essential part in the interpretation, querying and analysis of images. There are many different types of data that may be associated with an image such as sample preparation, experimental conditions or imaging parameters.

To store these types of additional information, the tagging field is available.

Under the Tabs tab, the **Add Tag** button allows further information to be entered about the selected item(s).

Text and description entered in the tag fields are [searchable](#) and may be used to store information about the used creation parameters, the type of the Vantage plots or any other additional information.

The tag has no effect on item properties and it may be used anywhere within the Arena view to provide additional information.

You can add Tagged Names and/or Description to one or more items.

Select the item(s) from the Arena view and dialog choose the Add tag option.

### Add Tag

The Tagged dialog is displayed, which enables you to enter a Name and Value for the tag.

### Delete Tag

To delete this property you must select the item, go to the Tagged Values tab and manually delete the item.

## 2.14 Objects Tab

The Objects lists all manually created and stored object types.


To assist you in finding and organizing the created objects, each one is labeled with an object icon.

To integrate surpass objects within the Manual Collection item, select the object icon then drag and drop it onto the chosen [Manual Collection](#) item.

The Manual Collection item allows you to combine for manually created Surpass objects with the automatic Batch Collections within one Vantage plot item.

## 2.15 Tag Search

The search field allows efficient interactive exploration of items to find a specific item and/or rapid scanning of item tags or labels. It enables you to quickly locate any items within Arena.

In the search field enter your search criteria, an item's [tag](#), [label](#), or description, and click on the **Find** icon  to display the search results. The search field provides you with a text and a description based search.

The Arena window displays the results, a set of items that match the entered search query. There are two ways the search results can be displayed. You can choose to view them within all Arena items, or within the selected

Group. To adjust the search results display, select the appropriate button.

**Arena button**

Selecting this option displays all results that meet the entered search term within the whole Arena view.

**Selected Group (Group name is displayed)**

Selecting this option displays results that meet the entered search term within the selected Group.



## Section 3 Surpass View

The Surpass view provides extended functions for visualization, including:

- A large viewing area with numerous tools for data preparation, presentation and manipulation.
- A selection of different types of data display as well as any combination of them.
- The possibility of loading additional External objects for comparison.
- Surpass Grouping functions.

### How to Open the Surpass View?

- Click on the icon in the Main toolbar.
- Select View - 3D View.
- Press the key combination Ctrl + 5.

### Save and Load Surpass Configuration

The actual Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called Scene and can be stored in a Scene file with the extension \*.imx. The Scene can be loaded again to the same data set or to another data set. For details please refer to Section Surpass View - Overview - [Scene File Concept](#).

See also:

3D View - [Ortho Slice](#)

3D View - [Cells](#)

3D View - [Clipping Plane](#)

3D View - [Filament](#)

3D View - [Reference Frame](#)

3D View - [Frame](#)

3D View - [Surpass Group](#)

3D View - [Measurement Point](#)

3D View - [Oblique Slicer](#)

3D View - [Ortho Slicer](#)

3D View - [Light Source](#)

3D View - [Volume](#)

3D View - [Surfaces](#)

3D View - [Spots](#)

### 3.1 Surpass Toolbar

The Surpass main toolbar provides quick access to frequently used items:

[Arena](#)

[Vantage](#)

[Store](#)

[Store as](#)

[Export](#)

[Slice](#)

[3D View](#)

[Coloc](#)

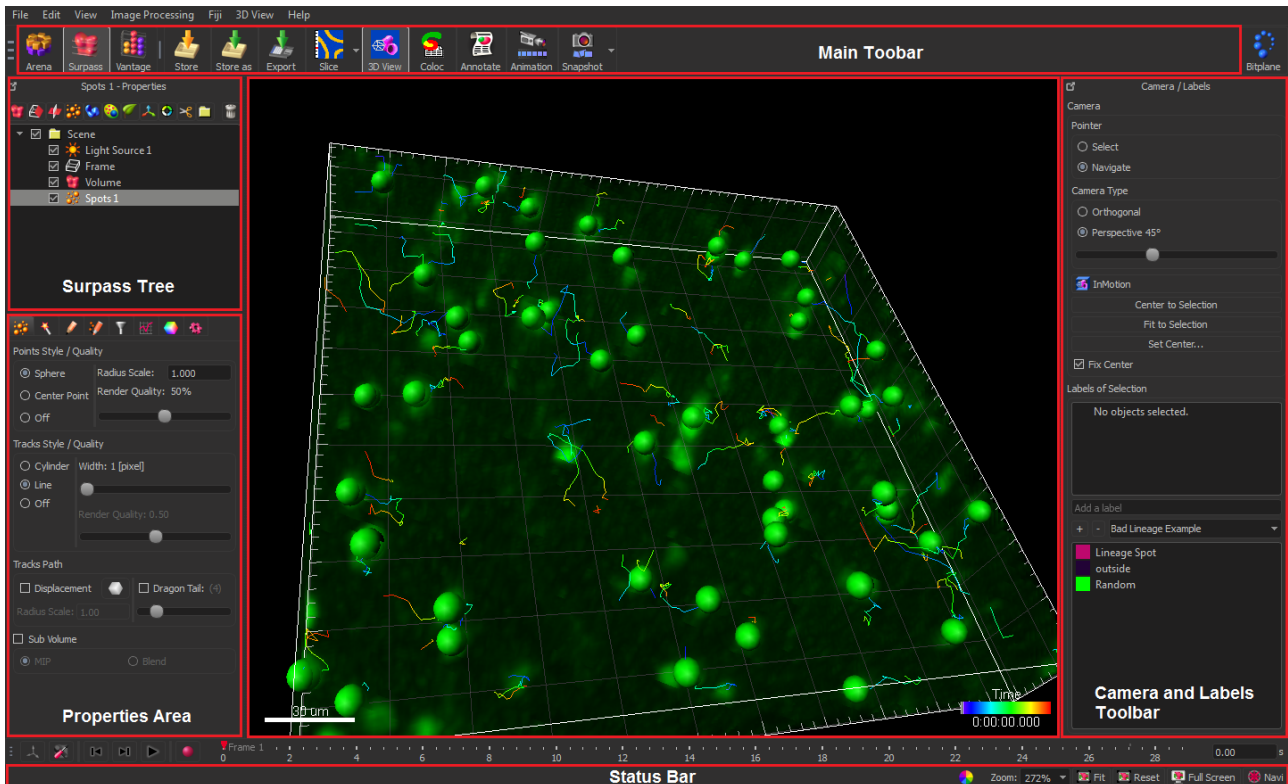
[Annotate](#)



## Animation Snapshot

### 3.2 Overview

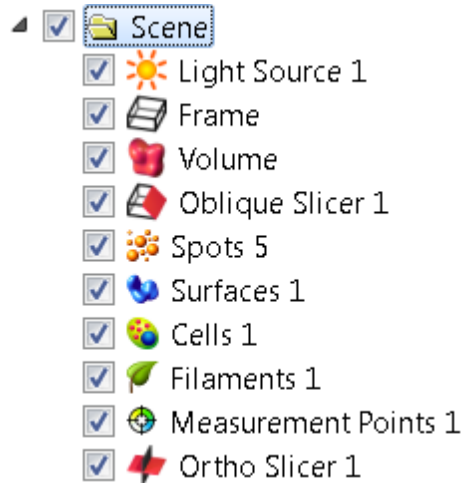
The Surpass view main screen consists of six different areas:



- At the top is the [Main Toolbar](#).
- On the left hand side you find the [Surpass Tree](#) and the [Properties Area](#). In the Properties Area you find all available parameters for the selected Surpass Tree Item. The parameters are grouped on different Parameter Tabs. The content differs depending on the selected Surpass Tree Item.
- The [Viewing Area](#) displays all objects that are added to the Surpass Tree and are checked visible.
- The **Camera and Labels Toolbars** area contains the [Camera Toolbar](#) which features a number of tools for data preparation, presentation and manipulation, most of which are displayed as buttons. The [Labels Toolbar](#) provides tools for labelling your data.
- On the bottom you find the [Status Bar](#). Align to option is available when multi-image mode is applicable.

#### 3.2.1 Surpass Tree

The Tree displays a tree list of all Surpass Tree Items that were added to the viewing area.



## Structure

The tree list is automatically generated and updated when a Surpass Tree Item is added or deleted. The first added object generates a **Surpass Group - Scene**. All following new objects are stored in this **Surpass Group**. A name is generated automatically for each Surpass Tree Item. To change the name, double-click on the item and enter a new name. Move objects or Surpass Groups from one to another by dragging and dropping them with the left mouse button.

## How to Add a new Surpass Tree Item?

All available Surpass Tree Items are available in the menu Surpass. To add new Surpass tree item click on the icon.



You find a list of all Items in the Section Surpass View - Overview - [Properties Area](#).

## Display

Each Surpass Tree Item includes a check-box. Check the box to make the object visible in the viewing area. Uncheck the box to make the object invisible in the viewing area. The currently active object in the viewing area is highlighted in the Surpass Tree.

## Surpass Groups

You can group objects into so-called component groups. Functions applied to the component group apply to all of its members. This facilitates the application of colors or the deletion of objects.

**Please note: If Group folder is checked invisible, all Items in the folder are invisible.**

## Multiple Selection

You may select more than one listed Item at a time for an operation. The selection functions in Surpass correspond to the Windows functions:

- Consecutive: Press and hold the Shift-key down and select the first, then the last entry to be selected from the list. All entries in between the two are also selected.
- Selective: Press and hold the Ctrl-key down and select any required entries from the list.

All selected entries are highlighted and commands or operations apply to all of them.

## Objects Toolbar

In the Objects toolbar you find a selection of Surpass Tree Items. To customize the Objects toolbar please refer to Section Menu Edit - Preferences... - [3D View](#) (Object Creation Buttons).

### Button **Delete...**

To delete Surpass Tree Item, highlight the Item in the Surpass Tree and click the button **Delete...** . The Delete selection window with a confirmation question is displayed.

## Naming Conventions

Objects are automatically named by Surpass as follows:

<a href="#">Cells</a>	Cells n
<a href="#">Clipping Plane</a>	Clipping Plane n
<a href="#">Contour Surface</a>	Surface
<a href="#">External Object</a>	External Object n
<a href="#">Filament</a>	Filament n
<a href="#">Reference Frame</a>	Reference Frame n
<a href="#">Frame</a>	Frame
<a href="#">Surpass Group</a>	Surpass Group n
<a href="#">Surfaces</a>	Surface n
<a href="#">Light Source</a>	Light Source n
<a href="#">Measurement Point</a>	Measurement Points n
<a href="#">Oblique Slicer</a>	Oblique Slicer
<a href="#">Ortho Slicer</a>	Ortho Slicer n
<a href="#">Spots</a>	Spots n
<a href="#">Volume</a>	Volume (only one volume can be created)

## Surpass Tree Item Properties

Each Surpass Tree Item has its own set of adjustable parameters. They are displayed in the properties area.

## Surpass Tree Item Tabs

Each Surpass Tree Item has its own set of adjustable parameters. They are grouped in different Tabs.

## Save and Load Surpass Tree Configuration

The actual Imaris configuration (including Surpass Tree and all existing items) in the Surpass view is called Scene and can be stored in a scene file with the extension \*.imx. The Scene can be loaded again to the same

data set or to another data set. For details please refer to Section Surpass View - Overview - [Scene File Concept](#).

See also:

Menu Edit - Preferences... - [3D View](#) (Object Creation Buttons)

Menu Surpass - [Delete Selected Objects...](#)

Surpass View - Overview - [Properties Area](#)

Surpass View - [Surpass Group](#)

### 3.2.2 Scene File Concept

The actual Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called Scene and can be stored in a scene file with the extension \*.imx. The Scene can be loaded again to the same data set or to another data set.

Surpass View - [Surpass Group](#)

### 3.2.3 Properties Area

The Properties Area displays all available parameters for the selected Surpass Tree Item.

#### Surpass Tree Item - Properties

The name of the heading is a combination of the selected Surpass Tree Item, followed by "- Properties". If you select another Surpass Tree Item the heading changes accordingly.

#### Tab X

The parameters are grouped on different Parameter Tabs. The content differs depending on the selected Surpass Tree Item.

List of available Tabs:

Surpass Tree Item	Parameter Tab
Cells	Tab Rebuild see 3D View - Cells <a href="#">Creation Wizard</a> <a href="#">Tab Settings</a> <a href="#">Tab Rebuild</a> <a href="#">Tab Edit</a> <a href="#">Tab Filter</a> <a href="#">Tab Statistics</a> <a href="#">Tab Color</a>
Clipping Plane	<a href="#">Tab Settings</a>
External Object	<a href="#">Tab Settings</a> <a href="#">Tab Color</a>
Filament	Tab Rebuild see 3D View - Filament <a href="#">Creation Wizard</a> <a href="#">Tab Settings</a> <a href="#">Tab Rebuild</a> <a href="#">Tab Draw</a> <a href="#">Tab Edit</a> <a href="#">Tab Filter</a>

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	<a href="#">Tab Statistics</a> <a href="#">Tab Color</a>
Frame	<a href="#">Tab Settings</a> <a href="#">Tab Color</a>
Surpass Group	<a href="#">Tab Settings</a> <a href="#">Tab Color</a> <a href="#">Tab Statistics</a>
Light Source	<a href="#">Tab Settings</a> <a href="#">Tab Color</a>
Measurement Point	<a href="#">Tab Settings</a> <a href="#">Tab Edit</a> <a href="#">Tab Intensity</a> <a href="#">Tab Statistics</a> <a href="#">Tab Color</a>
Ortho Slicer	<a href="#">Tab Settings</a>
Oblique Slicer	<a href="#">Tab Settings</a>
Spots	Tab Rebuild see 3D View - Spots <a href="#">Creation Wizard</a> <a href="#">Tab Settings</a> <a href="#">Tab Rebuild</a> <a href="#">Tab Edit</a> <a href="#">Tab Filter</a> <a href="#">Tab Statistics</a> <a href="#">Tab Color</a>
Surfaces	Tab Rebuild see 3D View - Surfaces - <a href="#">Creation Wizard</a> <a href="#">Tab Settings</a> <a href="#">Tab Rebuild</a> <a href="#">Tab Draw</a> <a href="#">Tab Edit</a> <a href="#">Tab Filter</a> <a href="#">Tab Statistics</a> <a href="#">Tab Color</a>
Volume	<a href="#">Tab Settings</a> <a href="#">Tab Draw</a> <a href="#">Tab Statistics</a>

### 3.2.4 Creation Wizards

A Creation Wizard is a user interface element where you are guided through a sequence of dialogs. In a Creation Wizard you are prompted to perform a task in a specific sequence.

Examples for Creation Wizards in Imaris are the [Cells Creation Wizard](#), the [Filament Creation Wizard](#), the [Spots Creation Wizard](#), and the [Surfaces Creation Wizard](#). Once you insert a new Item: Filament, Spots, Cell or Surfaces, you are automatically on the Tab Rebuild in the Properties Area.

See also:

3D View - Cells - [Creation Wizard](#)

3D View - Filament - [Creation Wizard](#)

3D View - Spots - [Creation Wizard](#)

3D View - Surfaces - [Creation Wizard](#)

## 3.2.5 Viewing Area

### Pan

To move the image within the Surpass view (pan the object) choose the mouse pointer mode Navigate. Click and hold the right mouse button while dragging the mouse. Release right mouse button to place the image.

#### On a PC or with a three-button mouse or on a Mac:

Right-click & drag                      Pan image

#### On a Mac with a one-button mouse:

ctrl + click & drag                      Pan image

### Rotate

Rotating an image allows to change the viewing angle on a three-dimensional object.

Choose the mouse pointer mode Navigate. Click with the left mouse button in the image and hold the button down while moving the mouse (hold left + drag). The image on screen is rotated towards the direction the mouse is dragged. Be sure to hold the left mouse button down during the whole rotation. Stop moving the mouse and release the left mouse button to stop the rotation.

#### On a PC or with a three-button mouse or on a Mac:

Left-click & drag                      Rotate image

#### On a Mac with a one-button mouse:

Click & drag                      Rotate image

#### How to Keep the Image Continuously Rotated

Choose the mouse pointer mode Navigate. Click with the left mouse button in the image and hold the button down while you move the mouse (hold left + drag). The image on screen is rotated towards the direction the mouse is dragged. Release the left mouse button while still dragging the mouse. The result is a continued rotation (speed of the rotation according to prior mouse motion). To stop the continued rotation re-click in the image area.

### Zoom

In the Surpass view you zoom the image either by using the mouse or by selecting one of the buttons in the Status Bar at the bottom of the screen.





## Using the Mouse

Choose the mouse pointer mode **Navigate**. To zoom in on the image hold the middle mouse button and drag it towards you. To zoom out from the image hold the middle mouse button and drag it away from you.

### On a PC or with a three-button mouse or on a Mac:

Middle-click & drag	Move up: zoom out
	Move down: zoom in

### On a Mac with a one-button mouse:

Shift + ctrl + click & drag	Move up: zoom out
	Move down: zoom in

## Using the Buttons in the Status Bar

### Zoom %

Imaris lets you enlarge or reduce the viewing size of your data set as is required with the Zoom function. You can zoom from 10% to 1000%. To go to a predefined zoom level, click the percentage of enlargement or reduction you want. To specify a custom level, just type a zoom value. At 100% zoom factor one pixel on screen represents one voxel.

### Button **Fit**

Click on this button to pan the position to best fit in the window and adjust the zoom factor.

### Button **Reset**

Click on this button to reset the image to the original position, center the image in the middle and set the optimal zoom factor.

### Button **Full Screen**

Click on this button to maximize the viewing area to full size of the monitor. To return to the standard window re-click on the button **Full Screen** in the lower right corner.

Viewing options for Zoom and rotate can be adjusted in Preferences for 3D View - please refer to [3D\\_View Preferences](#).

See also:

[View](#)

Toolbars - [Status Bar](#)

Surpass View - Overview - [Camera Toolbar](#) (Pointer Navigate)

## 3.2.6 Camera Toolbar

The toolbar to the right hand side provides the controls for the Camera and the Labels functions. Labels functions are outlined in the [Labels](#) section.

### Camera Pointer

#### Select

The cursor becomes an arrow. You use the pointer mode Select whenever you want to mark something in the image, e.g. to set some Measurement Points on the object surface.

#### Navigate

The cursor becomes two rotating arrows. You use the pointer mode Navigate to move, rotate or zoom the image in the viewing area.

**Tip: You can easily switch between the two pointer modes using the ESC-key. The effect is directly visible on screen by the altered mouse pointer display.**

### Camera Type

#### Orthogonal

Orthogonal display using parallel lines.

#### Perspective X

Perspective projection is a type of drawing, or rendering, that graphically approximates on a planar (two-dimensional) surface. If you select Perspective the slider (see below) is active.

#### Slider X

If you select as camera type Perspective X (see above) the slider is active. Drag the slider to adjust the vertical aperture angle of the camera.

**InMotion** is described in the section [InMotion](#)

#### Button Center to Selection

Click on the button **Center to Selection** to move the selection in the center of the viewing area.

#### Button Set Center...

Use this button to select a new center of rotation. Click on the button **Set Center...** and then onto the Scene to define the new center on which the camera zooms in.

**Please note: Rotation centers can be set on Surfaces, Contour Surface, Ortho Slicer and External objects Additional drawing and stereo view options may be enabled- please refer to [3D View](#)**

#### Fix Center

Click the Fix Center checkbox to set the center of the 3D cursor position so it will rotate about this point even if the image object is moved within the image viewing window.

**Note: You can press the shortcut key F6 when the 3D cursor is visible to perform this function (the Fix Center option in the Camera panel gets checked). This shortcut key will have no effect when the 3D cursor is not visible.**

---

**Draw Style (Enable Draw Style Settings enabled)**

Select the draw style of the object from the drop-down list.

**Full Featured**

Shows all objects as they are.

**Wireframe**

Show the objects as a simple wireframe only.

Please note that this will only show the points and edges of the object, without hidden triangle surfaces.

Draws objects; Surfaces, Spots, Filaments, Cells and Measurement Points as red colored wireframe models.

**Hidden Lines**

Draws objects as wireframe models and hides all background lines.

**Please note: Set Volume and OrthoSlicer objects invisible before selecting Hidden Lines.**

**No Texture**

Draws objects without textures.

**Bounding Box**

Shows only the boxes surrounding the objects.

**Wireframe Overlay**

Lays a red wireframe model over objects; Surfaces, Spots, Filaments, Cells and Measurement Points.

**Points**

Draws objects as a point model.

**Stereo (Enable Stereo Camera Settings enabled)****Off**

No stereo display in the viewing area.

**Red/Cyan Anaglyph**

This display mode requires colored glasses.

**Quad Buffer**

This display mode requires shutter glasses.

**Interleaved Rows**

This display mode requires a screen with a lenticular plastic sheet, that overlays the image. The sheet is molded to have the form of dozens of tiny lenses or prisms per inch.

**Interleaved Columns**

This display mode requires a screen with a lenticular plastic sheet, that overlays the image. The sheet is molded to have the form of dozens of tiny lenses or prisms per inch.

**Offset**

Display of the offset (0...5).

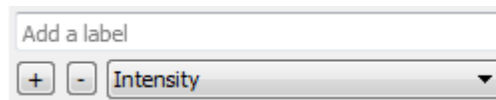
## Slider

Adjust the offset to get an optimized 3D effect. Use a small offset if you are far away from the screen, use a big offset, if you are close to the screen. Click on the slider handle and move it to the desired position.

### 3.2.7 Labels Toolbar

The labels toolbar can be used to label various objects or tracks manually, or according to the filter options available for the data type. Labels provide a useful way to tag and identify a subset of objects (or tracks) for further analysis. The label or label group name (including partial string) may also be used in the Imaris search (refer to [Tag Search](#)) to quickly locate any data sets which use that label.

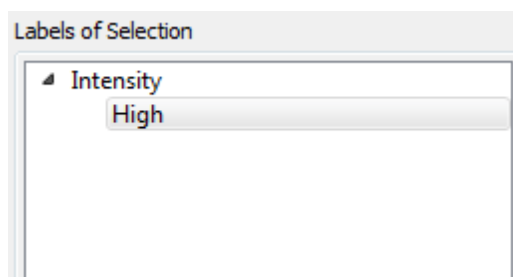
To create a new label, you should first create a new label group. To do this, click the **+** button and enter the name of the new label group (Note this field is highlighted red if unsupported characters are used, or the field is empty). The new label group will now be available from the drop-down menu. If you need to delete a label group, select the label group to be deleted and click the **-** button.



To add a label to the label group, select the label group in the drop-down menu, click the **Add a label** field, or right-click the **label selection box** (lower box) and enter the names for each label that is to be part of the selected label group. The color of the new label is assigned at random. This can be changed using the **Edit Label** option as outlined below.

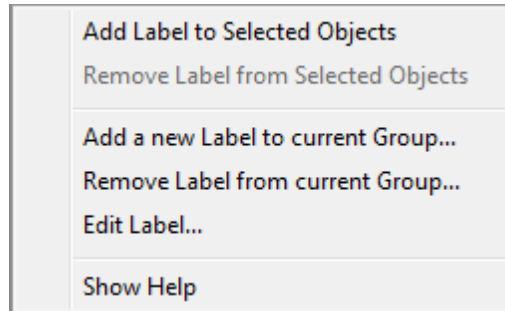
Labels will be assigned to the currently selected object(s) or track(s), for example, manually selected objects or data selected using filter options.

If selected objects (or tracks) have labels associated with them, the Labels of Selection box will update from "No labels found", to display the Label Group name and labels. In the following example, the selected object (or track) has the label group name "Intensity" and a label name of "High". Note that a \* symbol is used to indicate that selected objects (or tracks) have a partial match with the displayed label type.



Double-clicking an object (or track) assigns the selected label to it, double-clicking the object(s) or track(s) again will remove the label.

Right-clicking a label in the lower box brings up the following options:

**Add Label to Selected Objects**

Available when objects (or tracks) are selected. This enables the currently selected object(s) to be added to the selected label type.

**Remove Label from Selected Objects**

Available when objects (or tracks) are selected. This enables the currently selected object(s) to be removed from the selected label type.

**Add a new Label to current Group...**

This option enables new label types to be added to the selected label group.

**Remove Label from current Group...**

This option enables a label type to be removed from the selected label group.

**Edit Label...**

The name of the label can be changed in the name field and the color can also be modified using the color palette options.

**Show Help**

Opens the Imaris Reference Manual at the labels section.

**3.2.8 Mouse & Keyboard PC****General Mouse & Keyboard Functions in the Surpass View**

Ctrl + 5	Activates the 3D View
Esc	Switch mouse mode Navigate/Select

**In the Surpass Tree**

Arrow up	Move Surpass Tree selection up
Arrow down	Move Surpass Tree selection down

**Mouse in Select Mode**

Arrow left	Go to the next time point
Arrow right	Go to the previous time point
Alt	Temporary activate Navigate mode (until key is released)
Left-click	Select object
Ctrl + left-click	Add object to selection/remove object from selection

**Mouse in Navigate Mode**

S	Set center (on Surfaces, Contour Surface, Ortho Slicer and External objects)
Arrow left	Go to the next time point
Arrow right	Go to the previous time point
Left-click & drag	Rotate image (scene)
Right-click & drag	Pan image
Middle-click & drag	Move up: zoom out
	Move down: zoom in
Shift + right-click & drag	Move up: zoom out
	Move down: zoom in

See also:

Addendum - [Mouse & Keyboard PC](#)

### 3.2.9 Mouse & Keyboard Mac

#### General Mouse & Keyboard Functions in the Surpass View

Command + 5	Activates the 3D View
Esc	Switch mouse mode Navigate/Select

#### In the Surpass Tree

Arrow up	Move Surpass Tree selection up
Arrow down	Move Surpass Tree selection down

#### Mouse in Select Mode

Arrow left	Go to the next time point
Arrow right	Go to the previous time point
Click	Select object
Command-click	Add object to selection/remove object from selection

#### Mouse in Navigate Mode

S	Set center (on Surfaces, Contour Surface, Ortho Slices and External objects)
Arrow left	Go to the next time point
Arrow right	Go to the previous time point

#### With a one-button mouse:

Shift + ctrl + click & drag	Move up: zoom out
	Move down: zoom in
ctrl + click & drag	Pan image
Click & drag	Rotate image

#### With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .



- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

**Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.**

Middle-click & drag	Move up: zoom out
	Move down: zoom in
Right-click & drag	Pan image
Click & drag	Rotate image

See also:

Addendum - [Mouse & Keyboard Mac](#)

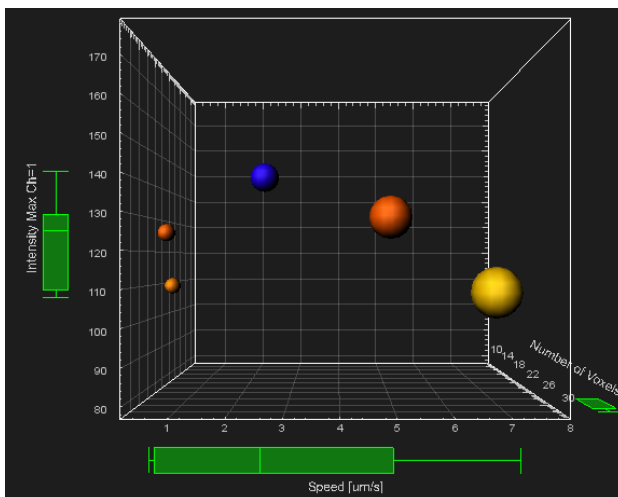
## Section 4 Vantage View

Vantage **Objects View** and [1D\\_View](#) are statistical data-visualization modules that provide a way to "see" abstract numerical values. The interactive visualization methods help you to enhance the understanding of complex statistical data by uncovering hidden relationships between objects and identifying patterns and trends.

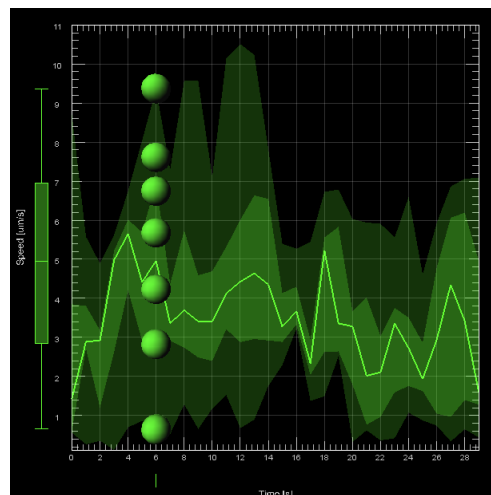
To effectively visualize and analyze the relevant statistical data, Objects View allows you to create clear, concise and colorful high-dimensional plots. The characteristics of Objects View plots are closely linked to the selected statistical variables, and are updated instantly.

In addition to visualizing the statistical values, the created plots are interactive, so you can simply select the object of interest either in Objects View or in the Surpass View to further analyze.

Objects View allows you to compare two or more series of results by creating plots for the multiple sets of data. It lets you visualize the influence of multiple variables assigned to one plot dimension, giving you the possibility to gather more information about the relationship between several independent statistical variables. Imaris Vantage allow you to detect meaningful underlying dimensions and to explain observed phenomena between the investigated objects.

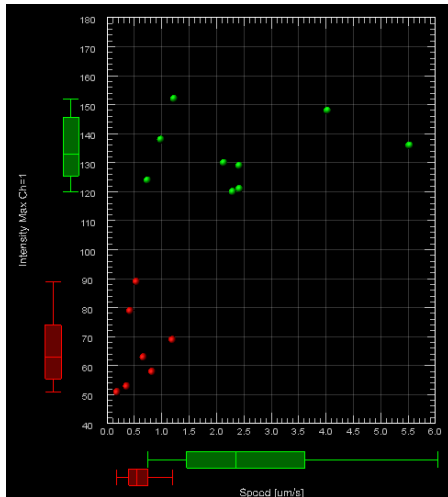


Scatter plot (object multiple dimensions)

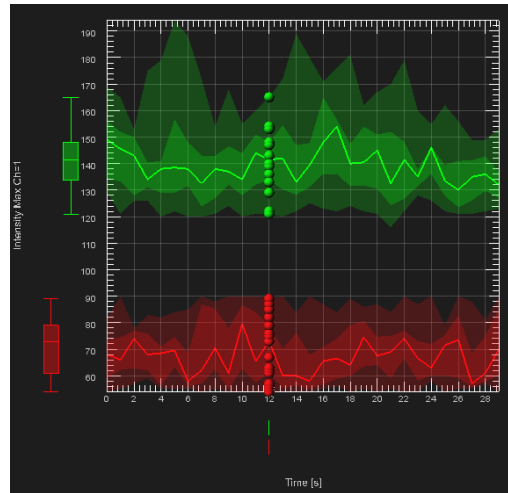


Time plot (one object)

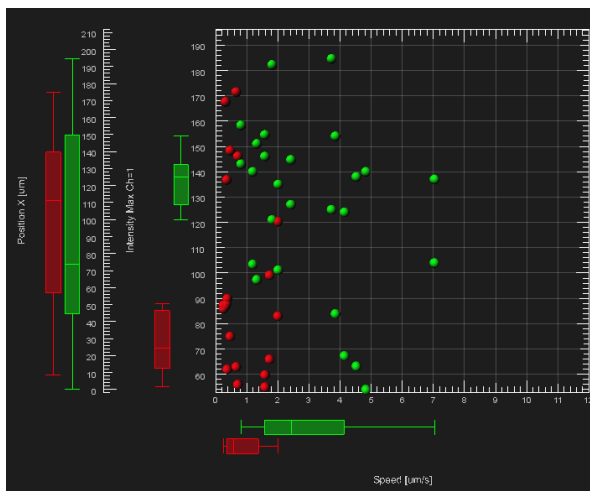




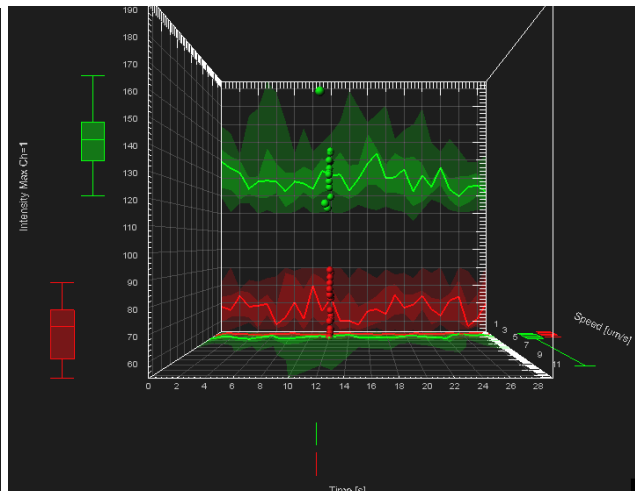
Scatter plot (2 objects 2 dimensions)



Time plot (2 object)



Scatter plot (two objects 2 X variables)



3DTime plot (2 objects)

## 4.1 Vantage Main Toolbar

The Vantage View main toolbar provides quick access to frequently used items.

[Arena](#)

[Surpass](#)


[Store](#)

[Store as](#)

Select between [1D view](#) and Vantage "Objects View".

[Snapshot](#)

### 4.1.1 Store Vantage view


To save an existing data set click **Store** in the menu or Store icon  on the [Main toolbar](#).

Selecting this option stores the Vantage plot data within the selected Arena Group. The Vantage plot item is created if none existed before.

Please note: Store functionality is implemented to be performed at maximum speed. This functionality overwrites the existing file without warning and only writes changed or new data without deleting obsolete ones. As a result, the resulting Save file could actually be larger than the original.

## 4.1.2 Store as Vantage View

To store the changes made to the Vantage plot in a different Vantage plot item whilst maintaining the original

plot click on the Store as option or Store as icon .

This option enables you to modify the Vantage plot and its name and creates a new Vantage plot item within the Arena view.

Enter the name for the file to be saved or confirm the suggestion.  
Select the requested file format and click **OK**.

## 4.2 Vantage Menu

Please refer to the following sections:

[Store](#)

[Store as](#)

[View](#)

[Vantage Plot](#)

[Preferences...](#)

[Help](#)

### 4.2.1 Vantage Plot

#### Vantage Plot

To insert a new Vantage Objects View plot, click the **Objects View** icon.

#### Delete Selected Objects...

Select this option and in the Vantage Tree all highlighted items will be deleted.

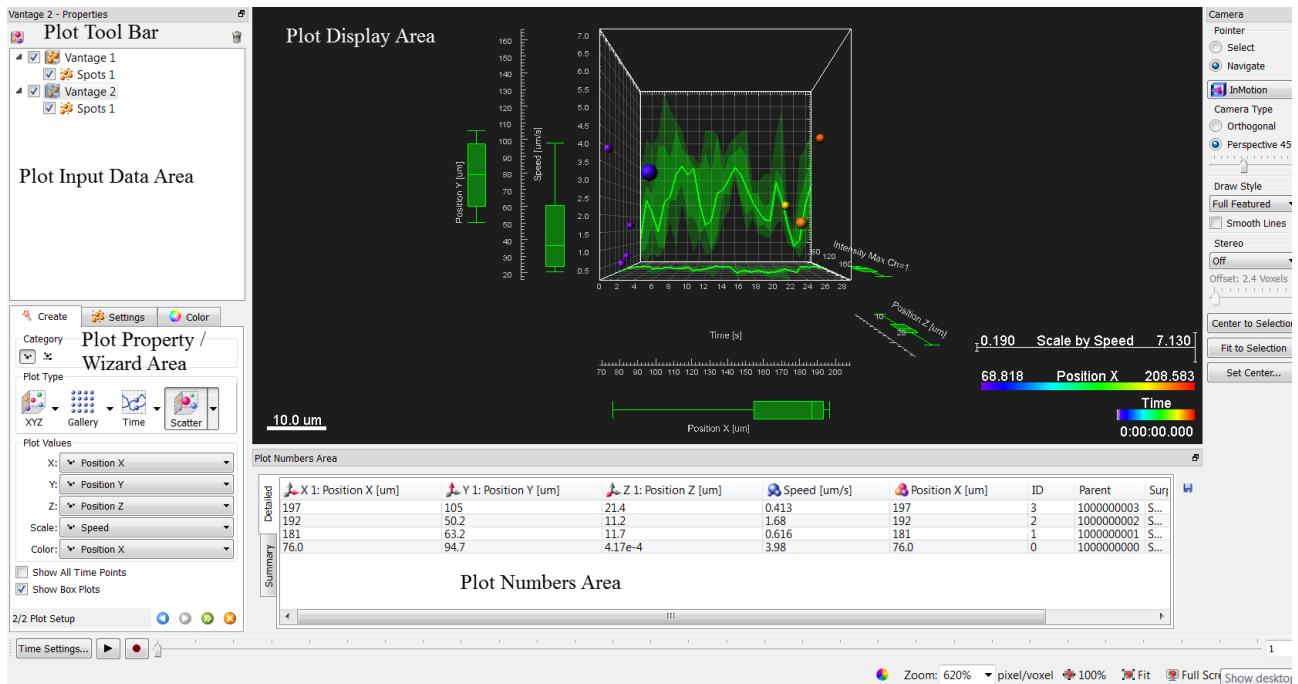
#### Plot Numbers Area

Check the box [Plot Numbers Area](#) to display the tables that contains all statistical values used for the plot creation.

## 4.3 Vantage User Interface

This Section describes the Vantage user interface for **Objects View**, and its various components.

Vantage Objects View consists of five working areas: [Plot Tool Bar](#), [Plot Input Data Area](#), [Plot Property Area](#), [Plot Display Area](#) and [Plot Numbers Area](#).



Follow these steps to start Imaris Vantage:

1. Create object(s) in the Surpass tree.
2. In the Toolbars, click on the Imaris Vantage icon.
3. Imaris Vantage is instantly started.
4. Imaris automatically creates a Vantage plot at the beginning of a Vantage session.

### 4.3.1 Plot Tool Bar

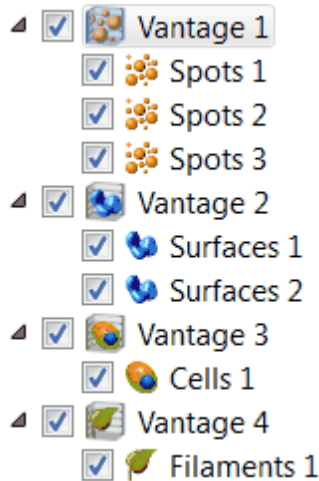
Use the Plot Tool Bar to add or remove plots from Vantage Object View. The Plot Tool Bar contains two icons:



- To create a new plot click on the Imaris Vantage icon and follow the Plot Creation Wizard.
- Click an Imaris Vantage icon to instantly generate a new entry in the Plot Input Data Area.
- To remove plots from Imaris Vantage click on the delete icon.

### 4.3.2 Plot Input Data Area

The Plot Input Data Area in Objects View displays a tree-like list of all created Vantage plots. Each Vantage plot is organized in a tree-structured hierarchy. At the top of the hierarchy is the Vantage plot and all source objects are listed below.



If in the first step of [Plot\\_Creation\\_Wizard](#) the selection of a source object changes, the tree is interactively updated.

Vantage plot symbol reflects the type of the selected source object type. Each element of a Vantage Plot tree has a check-box. Selecting or de-selecting the box next to the plot/object name controls its visibility in the Plot Main Display Area.

The currently active plot/object is selected and highlighted. A set of its adjustable parameters is displayed in Plot Property Area. The selection of the adjustable properties differs depending on the selected tree item; Vantage plot or source object.

For a source object, only tabs that control how objects are individually displayed are available. These are: Settings and Color.

For a Vantage Plot the following tabs are available: [Settings](#), [Creation](#), [View](#), [Edit](#), [Frame](#) and [Color](#).

Changing properties for a Vantage Plot, changes also the corresponding properties for all objects in the tree.

To add a new Imaris Vantage plot click on the Imaris Vantage icon within the [Plot\\_Tool\\_Bar](#). Then in the first step of the creation wizard, select an object category and source data. A new Vantage plot is updated and a name and symbol are automatically generated.

Please note: Imaris automatically creates a Vantage plot at the beginning of a Vantage session.

Please note: In the Imaris Vantage, only one object Category can be chosen.

### 4.3.3 Plot Property Area

In the Properties Area you can create and modify Imaris Vantage plots.

In the Properties Area you can find the Imaris Vantage Plot Creation Wizard and all available parameters for the selected Imaris Vantage object.

The parameters are grouped under different Parameter Tabs:

[Setting](#), [Rebuild](#), [Edit](#), [Frame](#) and [Color](#) tab.

### 4.3.4 Plot Display Area

The Plot Display Area shows a variety of Imaris Vantage interactive plots that can be used to provide the best view of the data.

Plots are highly interactive, allowing you to interact with the data in a variety of ways. As in the Surpass View, in the Plot Display Area you can select the object or group of objects by setting the Pointer mode to the Select option and clicking on the object.

Selecting the object in the Plot Display Area highlights the corresponding row in the [Plot\\_Numbers\\_Area](#) table. For multiple selections press Ctrl+click (Mac:Command-click).

Zoom and Panning features are available in all created Imaris Vantage plots.

In addition, the Plot Main Display Area can accurately arrange multiple plots allowing different types of visualizations simultaneously.

### 4.3.5 Plot Numbers Area

The data displayed in the [Plot Display Area](#) are interactively linked with the numerical data shown in the Plot Numbers Area. The numbers in the tables are based on calculated statistical values in the Surpass View.

Please Note: If the required statistic variable is not listed as an option, you will need to enable it. To do so go under the Edit menu/Preferences click on the [Statistics](#) option.

There you will find all available statistics arranged by category. Only if the box next to a statistical variable is checked, the variable is enabled, it will be calculated. The statistical variable values will appear both under the Statistics tab in the Surpass view and within the Vantage.

In the Plot Numbers' Area two table types are presented: **Detailed** and **Summary**

#### Detailed

This table contains all statistical variables values used for the plot creation.

The first row contains displays the plot dimension icons, variable name and unit, followed by the relevant object information such as ID, Parent number, Labels and Surpass Object name.

In the corresponding columns are the values of the statistical variables.

A click on any individual row of the table automatically highlights the corresponding object in the Imaris Vantage plot and in the Surpass view. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

#### Summary

This table is available only if show the Show [Box Plots](#) option is selected.

The Summary table contains values determined on the basis of all values within a set of the selected variable.

The table displays a five-number statistical summary of variables assigned to the plot dimensions.

The first column contains the most important percentiles:

1. Min - the smallest value within a set of values of the selected variable
2. the lower quartile or first quartile-Q1
3. the median - the middle value of the sorted set of values of the selected variable
4. the upper quartile or third quartile-Q3
5. Max - the largest value within a set of values of the selected variable

In the first row the plot dimension icons, variable name unit and Surpass Object name are listed.

The numerical data displayed in the Summary table are represented graphically within the Plot Display Area using a box plot.

In the case that multiple variables assigned to the same plot dimension (see [Edit tab](#)) the table columns sorting is bases on the following order: X dimensions (X1 primary, X2 secondary, X3 tertiary), all Y (Y1, Y2, Y3), Z dimension (Z1, Z2, Z3), Scale and Color dimension.

To sort the columns click on the arrow in the column heading. Click again to reverse the sorting.

## Exporting Data

Export Button- Export All Statistics to File.

By selecting the Export icon , all of the data that appears in the selected table will be exported.

A click on the Export icon opens the Export Statistics window.

To export data select the file name and the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm).

If you select MS Excel as file type, the program starts automatically.

## 4.4 Creation Wizard

A Plot Creation Wizard is a component of the **Plot Property Area** that guides you through a sequence of plot creating dialogs. It leads you step by step through the process of selecting object categories, source data, plot type and the various dimension options.

To create a plot, you must first create a Surpass object. The object statistical data will be used for the plot creation.

Please Note: If the required statistic variable is not listed as an option, you will need to enable it. To do so go under the Edit menu/Preferences click on the [Statistics](#) option.

There you will find all available statistics arranged by category. Only if the box next to a statistical variable is checked, the variable is enabled, it will be calculated. The statistical variable values will appear both under the Statistics tab in the Surpass view and within the Vantage.

The statistical values of the object Spots, Surfaces, Cells, and Filaments could be used to create the Vantage plots.

To start Imaris Vantage creation wizard follow these steps:

1. Create and select the object in the Surpass tree
2. In the Toolbars, click on the Imaris Vantage icon.
3. Imaris Vantage is instantly started.

The wizard shows a list of all available input components for Imaris Vantage plots.

Select one of the input components from the list (Spots , Surfaces , Cells , or Filaments ).

In the next step of the Creation Wizard, specify the plot type that you want to use.

Then assign the selected statistical variables to the plot dimensions.





Click on the Finish button and the Vantage plot is displayed in the viewing area.

To insert a new Imaris Vantage object, click the Imaris Vantage icon to instantly generate a new Plot Creation Wizard.

Please note that only the currently selected component is shown in the plot Main Display Area.

### 4.4.1 Source Data

All statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Vantage there are the following categories: Spots , Surfaces , Cells  and Filaments . The category buttons allow you to choose the object type for the Vantage plot creation. The category icon is active as long as surpass component is created.

Clicking on the Category button selects the corresponding category. According to the selection, all available source objects data are listed.

Based on your selection, the Vantage tree in the Plot Input Data area displays a list of all source data you added.

To remove an object data from the plot creation wizard, deselect the checkbox next to the object name or use the delete button.

**Please note: If multiple objects are selected in the first step of the creation wizard, the [Gallery](#) plot type is not active in the next step of Creation wizard.**

**To insert a new Imaris Vantage plot, click the Imaris Vantage icon.**

### Load Plots Settings

This option lets you use identical creation parameters for multiple plots.

The option Load Plots Settings is only available if the set of the plot's creation parameters has already been saved using the Remember Parameters button under the Rebuild Tab.

When the wizard is started, all stored creation parameters, corresponding to the chosen object wizard, are automatically loaded. The stored parameters contain all the settings, options, and parameters required for plot creation.

After selecting the Load Plots Settings option, the Load Parameters drop-down list appears, Expand the list by clicking on it. The list shows all the creation parameters sets that have been saved previously, according to the chosen object. Simply load your chosen parameters set, by clicking on it, and run the wizard as you have preset, without again defining all its possible options.

Another option is to customize each creation step to meet your specific needs by modifying some parts of the wizard independently - e.g. the same plot type, with a different plot value selection.

### 4.4.2 Plot Setup

A Category identifies, gathers and summarizes the set of statistical values' calculations of the particular Object Type. Statistical values associated with one particular object type are grouped together in one category. Each Category has its own button, which graphically indicates the Category type.

In Imaris there are following Filter Categories: Surfaces , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filaments , Filament Tracks , Spines , Spine Tracks , Points , Point Tracks , Cells , Cell Tracks , Nucleus , Nucleus Tracks , Vesicles  and Vesicles Tracks .

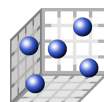
These buttons allow you to choose the Category and determine the list of displayed statistical variables. By clicking one of these buttons the corresponding category is selected and the list of statistical variables will be displayed accordingly.

Please note: In Imaris Vantage, only **one** Object Type Category can be chosen.

### Plot Type

Select the plot type you want to create from the plot type's icons.

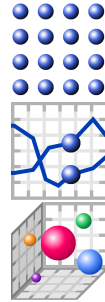
[XYZ](#)



[Gallery](#)

[Time Plots](#)

[Scatter Plots](#)



To view all options of the selected plot type click on the plot type icon and view the available plot subtypes. To select a plot type, click the plot type, and then click on the plot subtype that you want.

By XYZ, Time and Scatter Plots each plot dimension represents one selected statistical variable. By default, these plot types could show up to five statistical variables.

The choices of the available statistical variables for the selected object appear in a drop-down list.

To assign it to the selected Plot Value, select one statistical variable from the list.

Imaris Vantage plots allow you to select the same component for multiple plots.

## Plot Values

Display the statistical variables' name currently being assigned to the chosen plot dimension.

[XYZ](#), [Time](#) and [Scatter Plots](#) show five options for the plot dimensions: **X**, **Y**, **Z**, **Scale** and **Color**.

[Gallery plots](#), a selected statistical value is used to sort the objects. The values specified in the column or row fields, define the number of panels to be displayed. In addition, Scale and/or Color could be chosen as a plot dimensions.

The statistical variables for the Vantage can be chosen from the drop-down list. Click on the list and it offers a set of all available statistical variables for the selected object. To select a particular statistical variable, click on its name in the list and then the selected statistical variables will be assigned to the chosen plot dimension.

The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

Please note: When creating a plot in the creation wizard, only one variable can be assigned to the chosen plot dimension. However, when a plot creation wizard is completed, under the [Edit tab](#), multiple variables could be assigned to X, Y and Z plot dimensions.

## X, Y and Z

Selecting the statistical variable for the X, Y and Z Plot Values define the three-dimensional space of the Vantage plots. The position of the objects within the plot depends on the values of the variables assigned to the X, Y and Z.

The Z-dimension initially points towards the viewer, outward from the screen.

For each Plot type, you can optionally provide an additional statistical variable to individually specify the objects size, color or a combination of these features.

## Scale

If you want to vary the object size across your plots, you need to specify the statistical variable for the Scale dimension. The value of the variable selected for the Scale dimension determines the relative size of the object within the plot.

Initially all the object dimensions are normalized and then the values of the Scale statistical variable is assigned to the object. The lowest value of the selected statistical variable correspond to 0.25 of the normalized object





size, while the highest value of the selected variable is mapped with the 1.25 of the normalized object size. The new values are mapped, providing a new dimension for each object, without altering object shape parameters.

**Please Note: Assigning the statistical variable for the Scale plot dimension is only appropriate when the size of the created object doesn't play a biological role.**

## Color

If you want to vary the object's color across your plots, you need to specify the statistical variable for the Color dimension. The values of the selected variable will determine the color of the object within the plot.

This scalar information of the selected variable is converted into colors using the colormap. The first color of the selected color map is set to the lowest value of the selected statistical variable, while the highest value of the selected variable is mapped with the last color.

## Show All Time Points

Select this box and the created objects are shown for all time points.

## Show Box Plots

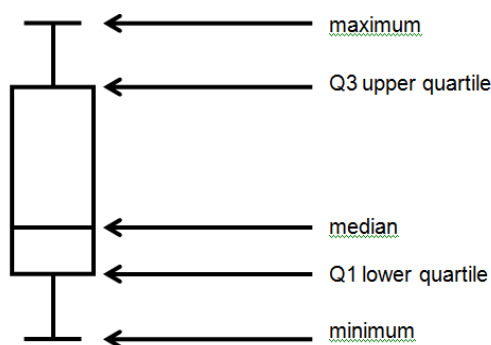
Select this box and in the viewing area, the [box plots](#) are displayed for the selected plot dimensions.

In addition, in the Summary table in the [Plot Numbers' Area](#), all statistical summary numbers are show for all plot dimensions.

Under the [Edit tab](#), you can modify the display of the summary statistical values for individual plot dimension.

### 4.4.2.1 Box Plot

A box plot summarizes the data of the selected statistical variable to only five numbers. It is a convenient way to illustrate graphically large group of statistical data through their summaries: the minimum, lower quartile (Q1), median, upper quartile (Q3), and maximum.



The central box represents the values from the lower Q1 to upper quartiles Q3 quartile (25 to 75 percentile respectively).

The line within the box is indicates the median value. A straight lines extending from the ends of the box represent the maximum and minimum values.

In the viewing area box plots can be drawn in all 3 plot dimensions for multiple variables.

If Vantage plot is composed of multiple objects, in the viewing area a separate box for each data set is created.

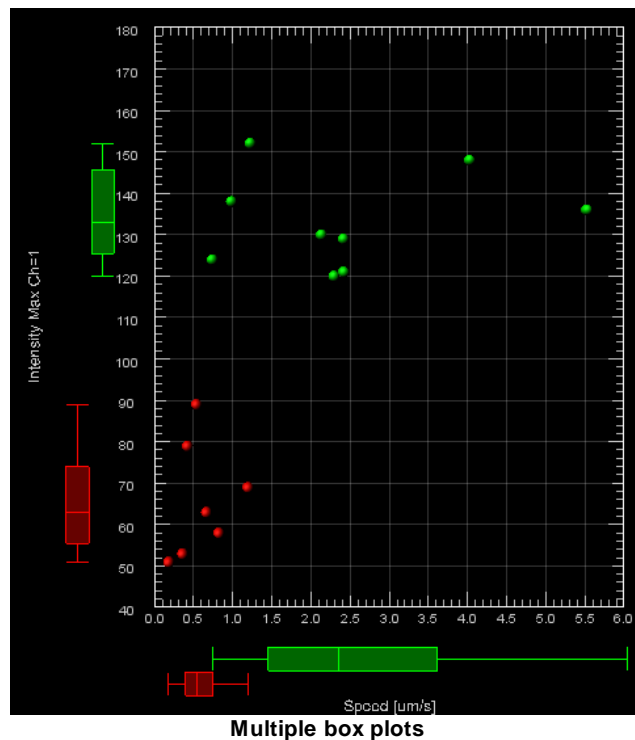
Box Plot is a useful tool offering several benefits to help you improve understanding of the data:

1. A quick visual summary that easily handles extremely large amount of data and shows range, spread and median value of the selected variable values.

2. The distance of the whiskers provides the distribution of the values
3. The space between the different parts of the box indicates the degree of distribution and skewness in the data
4. The position of the median inside the box indicates whether there are more values towards the upper or lower quartile

One of the most important benefits of plot boxes is the possibility to compare more than one data set. Multiple box plots are drawn side by side providing an efficient method to examine value distributions and highlight differences in variability between several data sets.

The Color of the box plot refers to the color of the Surpass object used as a source data.



## Time and box plots

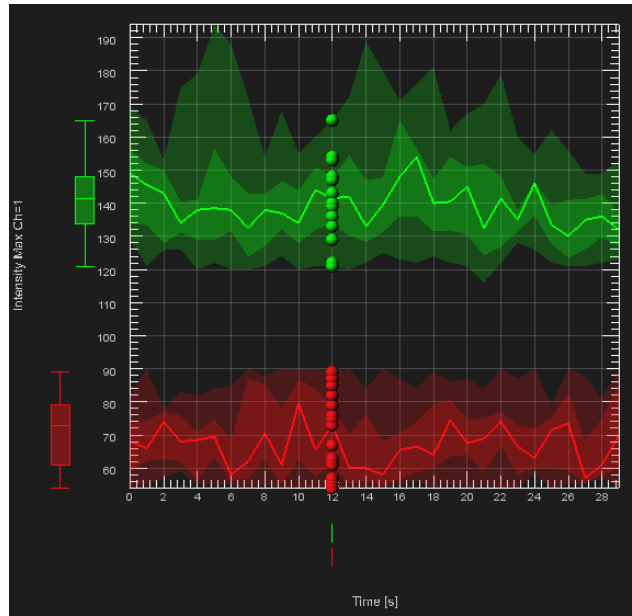
In the case that the Time plot type is selected, the box plots represent a moving statistical summary. The box plot position and shape changes at each time point.

In addition, in the viewing area, 2D summary projection XY, and/or XZ plots show the values of the statistical five-number summary for all time points. The two dimensional plane has the same characteristics as the corresponding box plots and the color of the projections plots correspond to the plot box color.

The summary values of the selected variable are plotted as colored regions and line.

On the projection plot, a tick line indicates where the median value lies. The range between the Q1 and Q3 values is less intensive shade, while the lightly colored area labels the minimum respective the maximum range.

The summary projection plots are especially useful for multiple data sets. You can easily compare how two or more variables change across time based on the line position and size of the regions.



Time box plots

#### 4.4.2.2 XYZ Plot Type

A XYZ plot is used to show the spatial relationship between the selected statistical variables. In XYZ plot type the X, Y and Z Plot Values are predetermined variables, fixed and assigned to the object X, Y and Z position. To uncover other structural connections, an additional statistical variable could be assigned as a Plot value. To add another dimension to the plot, a fourth or fifth variable can be correlated to the color or size of the plotted object, or a combination of these features.

##### Scale

If you want to vary the object size across your plots, you need to specify the statistical variable for the Scale dimension. The value of the variable selected for the Scale dimension determines the relative size of the object within the plot.

##### Color

If you want to vary the object's color across your plots, you need to specify the statistical variable for the Color dimension. The values of the selected variable will determine the color of the object within the plot. This scalar information of the selected variable is converted into colors using the colormap.

##### Plot Dimension

To select the statistical variable for the chosen plot dimensions click on the Plot Values list. This list offers a set of all available statistical variables for the selected object. To select a particular statistical variable, click on its name in the list and the selected variables will then be assigned to the chosen plot dimension.

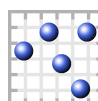
The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

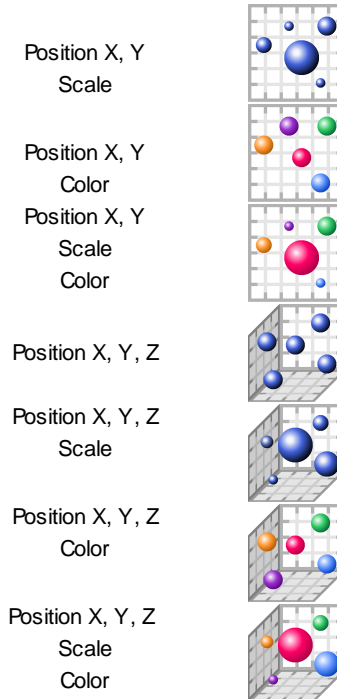
The following XYZ plot types are available:

##### Assigned Plot Dimensions

Position X, Y

##### Icons





**Please Note:** The statistic variable must be enabled under the Edit menu/ Preferences/[Statistics](#) options, otherwise it is not listed in the Plot Dimensions.

#### 4.4.2.3 Gallery Plot Type

Gallery plot creates a systematic panel of row and columns. Each individual pane in a Gallery plot displays an object.

In each panel, an object is placed according to the sorted values of the selected statistical variable.

#### Plot Dimension


##### Sort by

To select the statistical variable for the Gallery dimensions go under the Plot Values and then click on the Sort by list. The list offers a set of all available statistical variables for the selected object. To select a statistical variable, that can be used to order objects in the Gallery plot, click on its name.

The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

The selected variable value is used to arrange the objects into an ascending/descending order.

The position of the object in the Gallery panel, corresponds directly to the ordered value of the selected variable.

If sorting is done in ascending order , the object with the largest value of the selected variable is positioned in the bottom left corner of the Gallery panel.

The object with the lowest value is positioned in the top right corner of the Gallery panel.

If sorting is done in descending order , the position of the objects within the Gallery plot is reversed.

##### Columns and Rows

The Gallery plot type assigns vertical and horizontal dimensions to the predetermined variables, which are then fixed and assigned to the number of Gallery columns and rows. The number of rows and columns defines the Gallery panel size.

The values entered in the column or row fields specify the number of panels to be displayed.

**Please note: If the number of objects is higher than the total number of Gallery panes, a subset of the original objects is displayed and some of the objects are automatically skipped.**

To add another dimension to the plot, a variable can be correlated to the color or size of the plotted object, or a combination of these features.

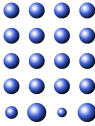



## Scale

If you want to vary the object size across your plots, you need to specify the statistical variable for the Scale dimension. The value of the variable selected for the Scale dimension determines the relative size of the object within the plot.

## Color

If you want to vary the object's color across your plots, you need to specify the statistical variable for the Color dimension. The values of the selected variable will determine the color of the object within the plot. This scalar information of the selected variable is converted into colors using the colormap.

The following Gallery plot types are available:

Assigned Plot Dimensions	Icon
X	
X Scale	
X Color	
X Scale Color	

**Please Note: The statistic variable must be enabled under the Edit menu/ Preferences/[Statistics](#) options, otherwise it will not be listed in the Plot Dimensions.**

### 4.4.2.4 Time Plots Type

A Time Plot is used to show the time dependency between the selected statistical variables. In Time plot, the X Plot dimension is a predetermined variable, which is fixed and assigned to the time. The successive time intervals are uniformly spaced. Y and Z Plot dimensions could be assigned to any available statistical variable. The position of the object within the plot depends on the values of the variables selected for the Y and Z dimensions.

To add another dimension to the plot, a variable can be correlated to the color or size of the plotted object, or a combination of these features.

## Scale

If you want to vary the object size across your plots, you need to specify the statistical variable for the Scale dimension. The value of the variable selected for the Scale dimension determines the relative size of the object within the plot.

## Color

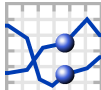
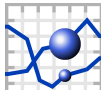
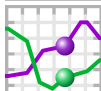
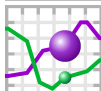
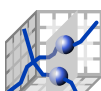
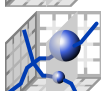
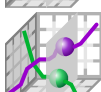
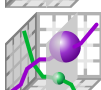
If you want to vary the object's color across your plots, you need to specify the statistical variable for the Color dimension. The values of the selected variable will determine the color of the object within the plot. This scalar information of the selected variable is converted into colors using the colormap.

## Plot Dimension

To select the statistical variable for the chosen plot dimensions click on the Plot Values list. This list offers a set of all available statistical variables for the selected object. To select a particular statistical variable, click on its name in the list and the selected variables will then be assigned to the chosen plot dimension.

The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

The following Time plot types are available:

Assigned Plot Dimensions	Icon
Time Y	
Time Y Scale	
Time Y Color	
Time Y Scale Color	
Time Y, Z	
Time Y, Z Scale	
Time Y, Z Color	
Time Y, Z Scale Color	

**Tip: The statistic variable must be enabled under the Edit menu/ Preferences/Statistics options, otherwise it is not listed in the Plot Dimensions.**

To interact with the plot and to go through all the time points click on the play/pause button. Each successive plot shows the data for the next time point.

### 4.4.2.5 Scatter Plots Type

Scatter plots can show five statistical variables: X, Y, Z, Color, Scale.

Within the Scatter Plot, an object's position depends on the values assigned to the X, Y and Z dimensions.

To add another dimension to the plot, a fourth or fifth variable can be correlated to the color or size of the plotted object, or a combination of these features.

**Scale**

If you want to vary the object size across your plots, you need to specify the statistical variable for the Scale dimension. The value of the variable selected for the Scale dimension determines the relative size of the object within the plot.

**Color**









If you want to vary the object's color across your plots, you need to specify the statistical variable for the Color dimension. The values of the selected variable will determine the color of the object within the plot. This scalar information of the selected variable is converted into colors using the colormap.

**Plot Dimension**

To select the statistical variable for the chosen plot dimensions click on the Plot Values list. The list offers a set of all available statistical variables for the selected object. To select a particular statistical variable, click on its name in the list and the selected variables will then be assigned to the chosen plot dimension.

The exclamation mark sign indicates that the listed statistical parameter does not contain any value.

The following Scatter plot types are available:

Assigned Plot Dimensions	Icons
X, Y	
X, Y Scale	
X, Y Color	
X, Y Scale Color	
X, Y, Z	
X, Y, Z Scale	
X, Y, Z Color	
X, Y, Z Scale Color	

**Tip:** The statistic variable must be enabled under the Edit menu/ Preferences/[Statistics](#) options, otherwise it is not listed in the Plot Dimensions.

## 4.5 Settings Tab

Depending on the object selection the content under the Settings tab differs.

Please click the appropriate link given below:

[Cells](#)

[Filaments](#)

[Spots](#)

[Surfaces](#)

## 4.6 Rebuild Tab

The rebuild option is useful to store your own customized plots' parameters and re-use them. It also enables you to share the plot creation parameters with other people, allowing them to use an identical set of parameters to perform their own analysis and have them perform their own analysis by using identical set of parameters.

Imaris stores the list of plot creation parameters. Select from the list, which step of the Plot Creation Wizard you want to re-enter.

[Source Data](#)

[Plot Setup](#)

### Rebuild Button

Clicking on this button re-starts the wizard.

### Creation Parameters

The Creation Wizard saves parameters so that the Imaris Vantage plot creation can be repeated, and the process of re-creating complex yet standardized plots may be avoided.

The Creation Parameters are listed in the table.

Refer to [Store Creation parameters](#)

### Preference

When you save the Creation Parameter file, it is automatically added to the list that is displayed under the [Edit/Preference/Vantage Plots](#)

## 4.7 Store Creation Parameters

### Store Parameters Button

The Store Parameters button allows you to save the complete set of the creation parameters and the relevant settings shown in the list. Storing the parameters allows them to be easily reused, modified, or exchanged between users. Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the appropriate plot creation wizard.

When you save the Creation Parameter file, it is automatically added to the list which is displayed under the Edit/Preference/[Creation Parameters](#).

Clicking on the Remember Parameters Button opens the Store Creation Parameter window.

### Store Creation Parameter Window

Enter a name for the Creation Parameter set. This name then identifies the Creation Parameter item, in the





Creation Parameter list, used in the Edit/Preference/[Creation Parameters](#) and would be used as default name in the subsequent creation wizard algorithms.

Save all of the attributes of your favorite graph style under the Rebuild tab. Add greater speed and efficiency to your analysis by quickly recalling an existing graph type, and then apply this style to your current data set

## 4.8 View Tab

Under the View Tab you can determine the relative size of the objects, their orientation and their position within the Plots.

### -/ + Button

Selecting this button changes the objects size within the View Area by simultaneously changing the Scale values for all X,Y and Z plot dimensions.

Scaling is measured in percent, starting from 100% as the initial object size.

### Plus Button

The size of the object is **increased** by 10% every time the **Plus** button is pressed.

Also, every time the **Plus** button is clicked, under the Edit tab, the number in the **Scaling** field for the X, Y and Z plot dimensions is **reduced** by 10%.

### Minus Button

The size of the object is **reduced** by 10% every time the **Minus** button is pressed.

Also, every time the **Minus** button is clicked, under the Edit tab, the number in the **Scaling** field for the X, Y and Z plot dimensions is **increased** by 10%.

### Scale Objects to Same Size

Select this button to map all objects with equal size distribution.

All objects within the plot are scaled to the largest object

### Orientation Horizontal /Vertical slider

To rotate the objects horizontally/vertically and change the object orientation use the sliders.

### Align Objects

Select this box to align objects relative to each other.

The objects are aligned along a horizontal or vertical line which represents their original positions.

### Reset Orientation button

Select this button to set an original orientation of the objects.

## 4.9 Edit Tab

















The Edit Tab offers the flexibility to customize every detail of your plot appearance by interactively modifying the various plot elements. It gives you independent control of plot dimensions.

Under the Edit Tab, you can adjust the following characteristics of a plot: modify the number and type of the

assigned statistical variable to the plot dimension, change the position and scale of the objects, define the objects' binning, alter limits and appearance of the plot dimension scale, and add the annotations' labels and text.

## Categories

A Category identifies, gathers and summarizes the set of statistical values' calculations for that particular Object Type. Statistical values associated with one particular object type are grouped together in one category. Each Category has its own button, which graphically indicates the Category type.

In Imaris there are the following Filter Categories: Surfaces , Surfaces Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filaments , Filaments Tracks , Spines , Spine Tracks , Points , Points Tracks , Cells , Cells Tracks , Nucleus , Nucleus Tracks , Vesicles  and Vesicles Tracks .

These buttons allow you to choose the Category and determine the list of displayed statistical variables. By clicking one of these buttons the corresponding category is selected and the list of statistical variables will be displayed accordingly.

**Please note: In Imaris Vantage, only one Object Type Category can be chosen.**

Depending on the selected Plot type the content under the Edit tab differs.

[XYZ](#)

[Gallery](#)

[Time Plot](#)

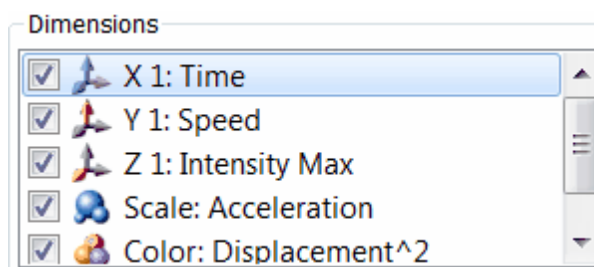
[Scatter Plot](#)

### 4.9.1 XYZ, Time and Scatter Plots

#### Dimensions

The Edit tab gives you independent control of plot dimensions.

Within the Dimensions window, a list displays the checkbox, icon and assigned variable name for all plot dimensions.



In the list dimensions appear in the following order: X1, X2, X3, Y1, Y2, Y3, Z1, Z2, Z3, Scale and Color. Selecting or de-selecting the box next to the plot dimension controls its visibility in the viewing area.





The following table gives an overview of the plot dimensions icons:

Plot Dimension

Icon

X



Y	
Z	
Scale	
Color	

- To adjust the appearance of the plot you can change the various aspects of the plot dimension.
- To modify variables assigned to plot dimensions you first have to select the plot dimension you want to change. Then from the variable list, select a new statistical variable that you want to be mapped.
- To modify the numbers of plot dimensions within the plot, click on the **Add** and/or **Delete** button. By using multiple variables assigned to the plot dimension, more than one criterion can be specified for the data analyses.



Add Button



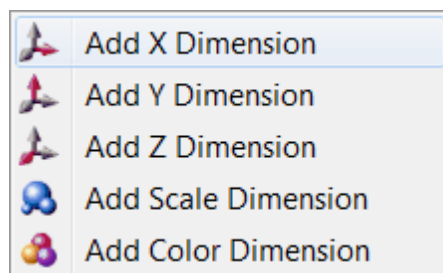
Delete Button

- The statistical variable can be chosen from the drop-down list. The list offers a set of all enabled statistical variables for the selected object. To select a particular statistical variable, click on its name in the list and the selected statistical variable is assigned to the chosen plot dimension.
- The Plot Numerical Table interactively changes with every modification of the plot dimensions, the columns that match the changed dimensions are immediately updated and the new set of values are displayed.

### Add Button

To add a plot dimension, click on the Add button.

From the drop-down list, choose a plot dimension.



Then from the variable list, select a new statistical variable that you want to be assigned to this newly created dimension.

The new dimension appears in the Dimensions list.

When the additional plot dimension is added, the plot is re-drawn and a different set of objects is displayed.

Please note: Vantage plot can have only one Scale and Color plot dimension. If a statistical variable is already assigned to Scale or Color plot dimension, the Scale or Color option under the Add button are disabled.

In order to allow a comparison of trends between several variables placed in one plot dimension all axes variables defining the same plot dimension are mutually parallel.

Please note: From a practical concern, it is most likely very difficult to interpret a graph with more than 3 X, Y, and Z axis and therefore Imaris restricts the number of variables assigned to one plot dimension to three.

## Delete Button

To remove an unwanted plot dimension, select it and click the Delete button. The selected plot dimension is deleted from the plot.

## Plot

The statistical variable can be chosen from the drop-down list. The list offers a set of all enabled statistical variables for the selected object type. To select a particular statistical variable, click on its name in the list and then this selected statistical variable is assigned to the chosen plot dimension.

To remove the assigned plot dimension from the viewing area, deselect the checkbox next to the plot dimension name.

The Plot Number Area interactively changes with every modification of the plot dimensions, the columns that match the changed dimensions are immediately updated and the new sets of values are displayed.

## Scale

A scale function specifies how the scale of each plot dimension should be determined from the value of the selected statistical variable.

As the values of the selected variable may be of different magnitudes, during the plot creation, the offset and scaling values are automatically calculated based on each of the selected variables values.

After automatic scaling, the ranges appear to be the same for all of the objects currently being visualized.

The Scale value provides you with options to adapt how the plot dimensions are scaled.

To change the scaling of the selected plot dimension and enter a number in the Scale field.

The entered Scale value multiplies the initially calculated values of the selected plot dimension by the value entered in the Scale field.

## Offset

This parameter is used to reposition the objects from their original position within the plots.

Offsetting objects is useful when you have more than one object in the same position and you want to evenly distribute the objects in a particular direction. For example, if you have many objects that you want to shift horizontally in a straight line, you can specify the value, in increments, in the Offset field for the selected X dimension.

The offset values change the object's originally assigned value. This new value is calculated by adding the entered offset value to the value of the selected statistical variable.

The offset value can be positive or negative.

For the X plot dimension, positive offset numbers move the objects to the right, and negative numbers move them to the left. For the Y plot dimension, positive offset numbers move the objects up, and negative numbers move them down.

The offset value is given in the same units as the selected statistical variable assigned to the selected plot dimension.



Clicking this arrow sets the Scale and Offset back to the original values.

## Limit Min Limit Max



During plot creation, the range is automatically adjusted to the actual lowest and highest value of the selected variable.

**Min:** This is the smallest value of the selected variable to be displayed.

**Max:** This is the largest value of the selected variable to be displayed.

It is possible to change the Max limit and/or Min limit of the selected plot dimension by entering the values in the Limit Min /Max fields.

Enter a value to the Limit Min /Max to specify the new minimum/maximum values for the selected plot dimension.



Clicking this arrow sets the Limit Min /Max back to the original values.

### Bin Size

Binning allows you to sort your data into different groups or bins. The Binning size value divides a plot into a specific number of groups and specifies the new locations of the objects by distributing them into the different bins according to the value of the selected variable.

### Gamma

The Gamma function allows you to individually manipulate the appearance of the range of the selected variable. The default value of the Gamma is 1, indicating that the range between the lowest and highest value of the selected variable is extrapolated in a linear mode to the full range of the data set.

If the entered value is different than 1, the linear mode is transferred to a nonlinear mode.

Entering the Gamma values higher than 1, will result in a plot in which the range of the object with the lower values will appear to increase relative to the range of the object with higher values.

Alternatively, entering the Gamma values smaller than 1, will result in a plot in which the range of the object with higher values will appear to increase relative to the range of the object with lower values.

The change is directly visible in the viewing area.

### Clamp Limits

If this box is selected, the object outside the plots limits (determined by the frame and range settings) will be repositioned within the limits.

The object with the higher value will be assigned the max value of the selected variable, while the object with the lower value will be assigned the minimum value.

### Show Box Plots

Select this box and in the viewing area, the [box plots](#) are displayed for the selected plot dimension.

### Auto Adjust

Click on this button and the parameters under the Edit tab are returned to the original values.

### Load Colormap

Click on the button Colormap to display a large number of predefined colormaps. If the default colormap (Spectrum) is not appropriate, you can change it by clicking on the drop-down list. The list gives a preview of

what different colormaps look like.

## Show All Time Points

Select this box and the created objects are shown for all time points.

## 4.9.2 Gallery

### Sort by:

To modify the statistical variable for the Gallery dimensions go to Plot Values and then click on the Sort by list.



The icons indicates the sort order in the Gallery, ascending or descending order

### Cell Columns /Row

To readjust the numbers of panels to be displayed in the Gallery enter the values in the column or row fields.

### Skip

The entered value defines the number of displayed objects by defining the skip interval. To display every second object set the value to 1, or to display every third object set the value to 2.

### Offset

Adjusting the Offset value is useful when the number of objects is higher than the total number of Gallery panes.

The offset value specifies the first object to be placed in the Gallery. To display first object, set the offset value to 0. To start with the 10<sup>th</sup> ordered object, set the value to 10.

The Skip and Offset value regulate and limit the Gallery display to a certain number of the displayed objects. In combination, these two values define the subset of the originally ordered objects.

Offset	Skip	Displayed Objects in the Gallery
0	0	all objects
10	0	10 <sup>th</sup> 11 <sup>th</sup> 12 <sup>th</sup> 13 <sup>th</sup> ...
20	15	20 <sup>th</sup> 35 <sup>th</sup> 50 <sup>th</sup> 65 <sup>th</sup> ...



### Cell With/Height

The Gallery panel size is equal to the settings in the width/height numerical fields.

### Plot Dimensions

The Edit tab gives you independent control of plot dimensions.  
Select a plot dimension from the list of icons:



Plot Dimension	Icon
Scale	
Color	

Selecting or de-selecting the box next to the plot dimension name controls its visibility in the viewing area.

- To adjust the appearance of the plot you can change the various aspects of the plot dimension.
- To modify the existing condition, you have to first select the plot dimension you want to change.
- To assign a different statistical variable to the current dimension, select a new statistical variable that you want to be mapped.
- To incorporate a new plot dimension into the plot, select the preferred dimension and assign a new statistical variable.
- The statistical variable can be chosen from the drop-down list. The list offers a set of all available statistical variables for the selected object. To select a particular statistical variable, click on its name in the list and then this selected statistical variable is assigned to the chosen plot dimension.
- To remove the assigned plot dimension from the viewing area, deselect the checkbox next to the plot dimension name.
- The Plot Numerical Table interactively changes with every modification of the plot dimensions, the columns that match the changed dimensions are immediately updated and the new sets of values are displayed.

## Offset

This parameter is used to reposition the objects from their original position within the plots.

Offsetting objects is useful when you have more than one object in the same position and you want to evenly distribute the objects in a particular direction. For example, if you have many objects that you want to shift horizontally in a straight line, you can specify the value, in increments, in the Offset field for the selected X dimension.

The offset values change the object's originally assigned value. This new value is calculated by adding the entered offset value to the value of the selected statistical variable.

The offset value can be positive or negative.

For the X plot dimension, positive offset numbers move the objects to the right, and negative numbers move them to the left. For the Y plot dimension, positive offset numbers move the objects down, and negative numbers move them up.

The offset value is given in the same units as the selected statistical variable assigned to the selected plot dimension.

## Scale

A scale function specifies how the scale of each plot dimension should be determined from the value of the selected statistical variable.

As the values of the selected variables may be of different magnitudes, during the plot creation, the offset and scaling values are automatically calculated based on each of the selected variable values.

After the automatic scaling, the ranges appear to be the same for all of the objects currently being visualized.

The Scale value provides you with options to adapt how the plot dimensions are scaled.

To scale the selected plot dimension enter a number in the Scale field.

The entered Scale value multiplies the initially calculated values of the selected plot dimension by the value entered in the Scale field.



Clicking this arrow sets the Scale and Offset back to the original values.

## Limit Min Limit Max

During the plot creation, the range is automatically adjusted to the actual lowest and highest value of the selected variable.

It is possible to change the max limit and/or min limit of the selected plot dimension by entering the values in the Limit Min /Max fields.

Enter a value to the Limit Min /Max to specify the new minimum/maximum values for the selected plot dimension.



Clicking this arrow sets the Limit Min /Max back to the original values.

## Bin Size

Binning allows you to sort your data into different groups or bins. The Binning size value divides a plot into a specific number of groups and specifies the new locations of the objects by distributing them into the different bins according to the value of the selected variable.

## Gamma

The Gamma function allows you to individually manipulate the appearance of the range of the selected variable. The default Gamma value is 1, indicating that the range between the lowest and highest value of the selected variable is extrapolated in a linear mode to the full range of the data set.

If the entered value is different than 1, the linear mode is transferred to a nonlinear mode.

Entering Gamma values higher than 1, will result in a plot in which the range of the object with the lower values will appear to increase relative to the range of the object with higher values.

Alternatively, entering Gamma values smaller than 1, will result in a plot in which the range of the object with higher values will appear to increase relative to the range of the object with lower values.

The change is directly visible in the viewing area.

## Clam Limits

If this box is selected the object outside the plots limits (determined by the frame and range settings) will be repositioned within the limits.

The object with the higher value will be assigned the max value of the selected variable, while the object with the lower value will be assigned the minimum value.

## Auto Adjust

Click on this button and the parameters under the Edit tab are returned to the original values.

## Show All Time Points

Select this box and the created objects are shown for all time points.





## 4.10 Frame Tab

Common aspects of all frames, such as the thickness and color of frame, grid tickmarks and labels setting can be controlled using this tab.

### Box and Time Settings

#### Box

Check the parameter to display a box around the Frame.

#### Line Width

Adjust the box line width with the respective slider. Moving the slider to the left reduces the line width, moving it to the right increase line width.

#### Shading

Check this parameter to display shading. Use the slider to adjust the shade contrast.

#### Shadow

The entire Scene can produce shadows on the three planes of the Frame object. Check this box to display the shadows. Use the Shading slider (see above) to adjust the contrast. The rendering is done in real-time, and it requires a high performance graphic board.

#### Time Colorbar

Only available if you work with a time data set. Check this box and the Time Colorbar appears in the lower left corner of the viewing area.

### Grid and Tick marks Settings

#### Grid

Check the box Grid to display a grid. Use the slider to adjust the grid width.

The Scale is shown/by a grid behind the object. The frame moves to stay behind the object as it is rotated. The distance represented by each square in the grid, the scale bar, is in the bottom right corner of the screen.

#### Tickmarks

Check the box Tickmarks to display tickmarks on the edges of the frame.

#### Spacing

Specify the grid and tickmark spacing for the X, Y and Z plot dimensions.

The frequency of tick marks can be controlled by selecting the appropriate interval values from the drop-down list.

### Labels Settings

#### Axis Labels

Check the box Axis Labels to display the x, y, and z axes to clearly show the orientation of the object. If this box is checked, the parameter Label Color and Label Font is available (see below).

#### Label Color

Only available if the parameter Axis Labels (see above) is checked. Click on the Color Editor window to change the color of the label on the coordinate axes.

## Label Font

Only available if the parameter Axis Labels (see above) is checked. Click on the Font Editor window to change the font to label the coordinate axes.

## Fix Decoration to Data Set

Check Fix Decoration to Data Set to fix the Frame to the same side when navigating the object.

## 4.11 Color Tab

**Please note: The color coding option is not available under the Vantage Color Tab.**

### RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color, or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

### Light reflection

Quality of the object material is further characterized by reflection properties:

- Diffusion- diffuses reflection of a matte, uneven material.
- Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. The default setting is for no specular intensity.
- Emission- light emitted from the material defines the self-illumination. The default setting is for no emission.

### Transparency

Transparency defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### Palette

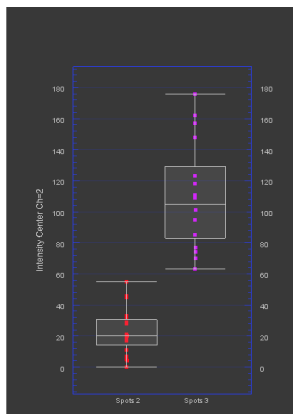
Palette- this option provides a system of preset colors organized into color schemes.

In the Palette, colors are labeled and grouped together. The scrolling window contains 14 color combination groups (Custom, Artdeco, Autumn, Glass, Metal, Neon, Rococo, Santafe, Sheen, Silky, Spring, Summer, Tropical, Winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color palette choices are stored and can be used with other Imaris objects.

## 4.12 1D View

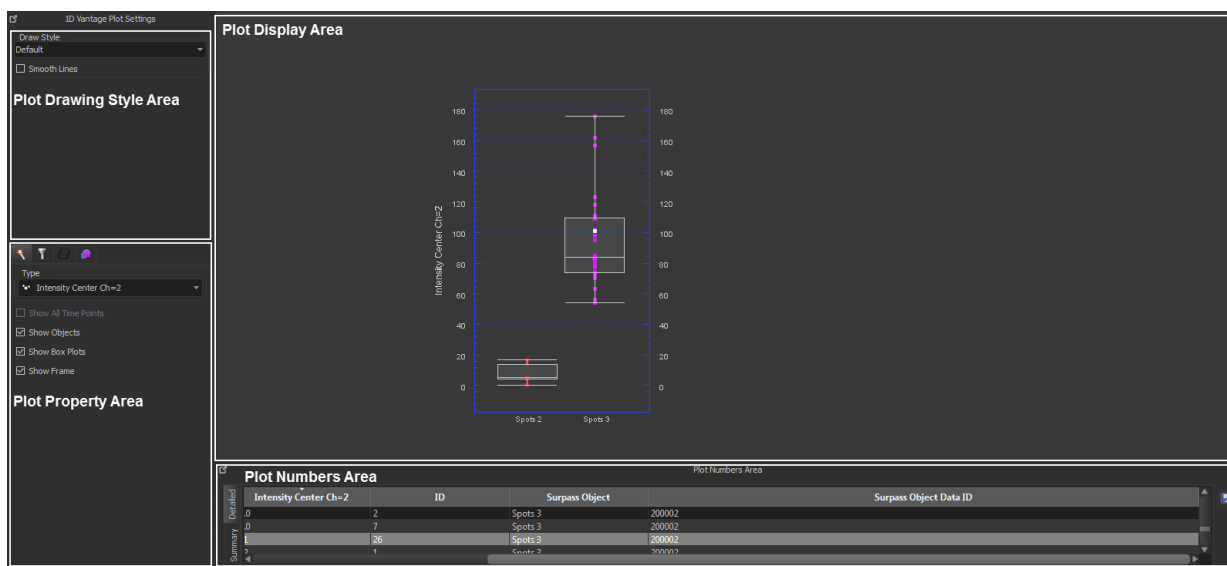
Vantage **1D View** provides an effective way to view patterns and trends in statistical data in the form of one dimensional scatter plots.

1D view lets you analyze data, and compare and derive relationships from one or more data sets. 1D View provides a simpler means to analyze and view data sets (for example spots) than Vantage Objects View, however many of the functions found in Objects View are the same and are described previously in [Vantage View](#). In addition the [Statistics Tests](#) tab provides powerful statistical tests for direct analysis of your data sets within Imaris 1D View.



### 4.12.1 1D User Interface

As with the Vantage Objects View, the 1D User Interface has the following working areas: Plot Display Area, Plot Drawing Style Area, Plot Property Area and Plot Numbers Area.



#### 4.12.1.1 1D Plot Property Area

The settings for the data can be modified. These options are grouped together under the following tabs: Settings Tab, Filter Tab, Frame Tab and Source Tab.

##### Settings Tab

The **Settings Tab** enables you to select the required statistical options to analyze data by for the 1D Plot. The options available will depend on the input components selected e.g. spots, surfaces, cells or filaments.

Use the Setting tab to select display options: Show all Time Points, Show Objects, Show Box Plots and Show Frame.

##### Filter Tab

The **Filter Tab** allows you to select and apply a number of filters. The filters available are specific to the input components selected e.g. spots, surfaces, cells or filaments. Spots for example, includes the filters: by Area,

Diameter, Intensity, Position, Time and Volume. Filters can be added and deleted using the Add and Delete options.

You can use the automatic filter setting, or manually adjust the values as required. Use the slider bar, when this is available, to adjust the parameters for the selected filter. The name of the filter displayed will update if you change the filter settings.

## Frame Tab

The **Frame Tab** provides you with a range of options to change the appearance and settings used for the displayed plot. This includes the Frame Settings, Grid and Tickmarks and Labels.

## Source Tab

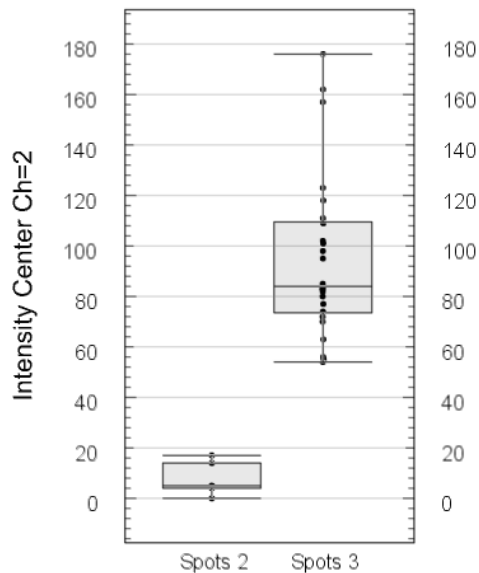
The **Source Tab** allows you select which data sets you want to display. Just tick, or untick, the required data sets and the plot will automatically update.

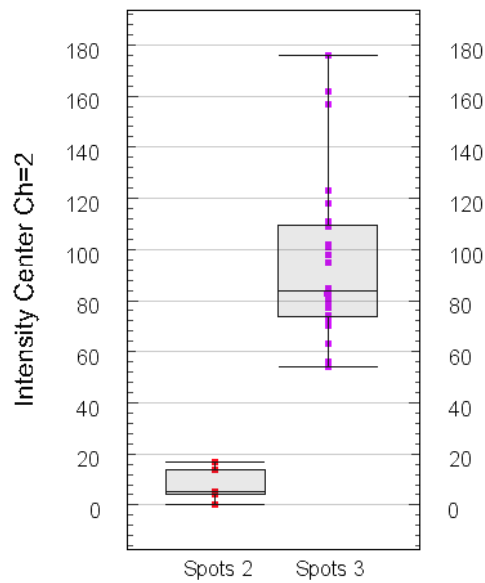
### 4.12.1.2 1D Plot Drawing Style Area

Change the drawing style of the plot from the options available: Default, Print and Color Print.

**Default** style displays the plot according to the settings in Preferences Display, for example, for display on the computer monitor.

**Print** (below left) displays the plot in gray-scale with a white background for printing on paper, and **Color Print** (below right) displays the plot in color with a white background for printing on paper.

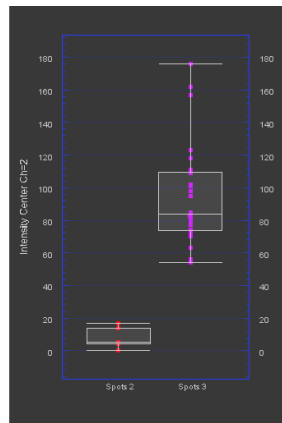




**Tip:** Smooth lines can be used to smooth the appearance of text and lines in the plot.

#### 4.12.1.3 1D Plot Display Area

The Plot Display Area shows the plots for your selected data sets. The plot area is interactive and you can select specific data points, and the data will be updated in the [Plot Numbers Area](#) table. Note that unlike Object View, you can only select one data point in a series at a time.



#### 4.12.1.4 1D Plot Numbers Area

The 1D Plot Numbers Area features the Detailed and Summary tabs that have been described previously for the Objects View in the section, [Plot Numbers Area](#).

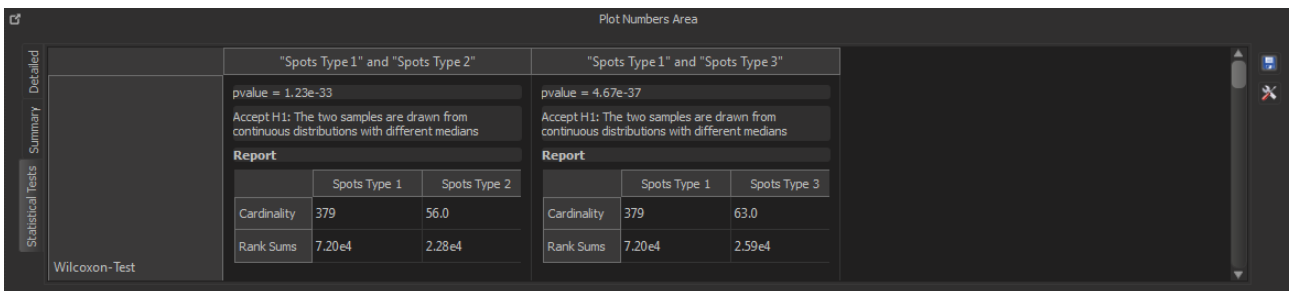
In addition the **Statistical Tests** tab provides automatic statistical data analysis for the surpass objects, meaning that this information can be viewed directly within Imaris. The Statistical Tests functions are outlined below.

#### Statistical Tests

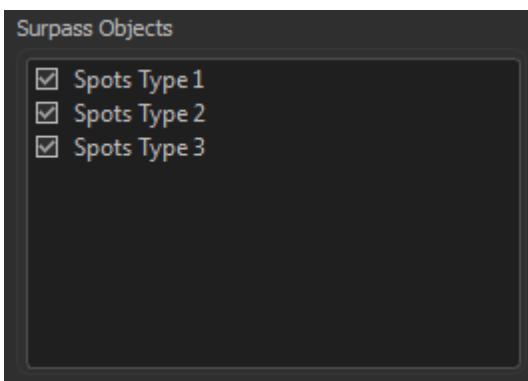
The Statistical Tests tab and functions are available through ID View only- they are not available under Objects View. The following tests are performed:


Test	Summary
<b>Wilcoxon-test</b>	The Wilcoxon-test is also referred to as Mann-Whitney test. The Wilcoxon-test enables you to determine whether two sets of surpass data sets with ordinal data, but without normalised distribution i.e. non-parametric, are statistically different from each other.
<b>t-test</b>	The t-test enables you to test whether the mean of two data sets (for small populations) are statistically different from each other. With this test, there is an option to assume that the variation between the surpass data sets are the same, in this case a <i>Welch's t-test</i> is performed. If this is not the case, a <i>Student's t-test</i> will be performed.
<b>f-test</b>	The f-test enables you to test (the null hypothesis) that the variance of two data sets are equal. This is done by comparing the ratio of the variance between the two surpass data sets.
<b>Kolmogorov-Smirnov test</b>	The KS-test lets you determine if two data sets differ significantly. The KS-test is distribution free.

The statistics are compared for the first of the surpass objects against the others in the list, and the results are displayed in columns as shown in the example below. If the test does not meet the criteria to run, the reason will be displayed.



As the statistics are compared for the first of the surpass objects against the others in the list, to change the order of the data sets drag the items within the list to the required order. In the following example, "Spots Type 1" is compared against "Spots Type 2" and "Spots Type 3". "Spots Type 3" could be compared against "Spots Type 1" and "Spots Type 2" by dragging it to the top of the list.



The settings for Statistical Tests can be viewed and edited e.g. confidence level or test type, using the Settings  button. This is a shortcut to [Statistical Tests](#) preferences.

Note that data for each tab can be exported using the  button when that tab is highlighted.

## Section 5 File

The options available from the file menu will depend on the current view selected:

### Arena Options

The following options can be selected from the **File** menu under Arena:

#### [Batch Convert](#)

Scene Viewer

[Store](#) [Arena](#) [and](#) Ctrl + Shift +

[Restore Arena](#) E

Ctrl + Shift +

I

[Batch Queue](#) Ctrl + Shift +

B

[Preferences...](#) Ctrl + P

List of recently opened

Surpass files

[Exit](#) Ctrl + Q

**In other modes e.g. Surpass** additional options are available and these will change according to what applies to the current image.

[Open...](#) Ctrl + O

Open in a new Ctrl + Shift +

Window O

Revert to File Ctrl + R Use Revert to File to revert to the previously saved version of an image file.

[Store](#) Ctrl + S Use the Store and Store as options to save the current image file as required.

Store as

[Export...](#) Ctrl + E Use Export to save the image file to another location.

Import Scene The actual Imaris configuration (including Surpass Tree and all existing Items) in the Surpass view is called Scene and can be stored in a Scene file with the extension \*.imx. The Scene can be loaded again to the same data set or to another data set. For details please refer to Section Surpass View - Overview - [Scene File Concept](#).

Export Scene

Add Image... Ctrl + Shift + I Add an image into an Imaris Scene for multi-image data sets. Please refer to [Multi-image](#)

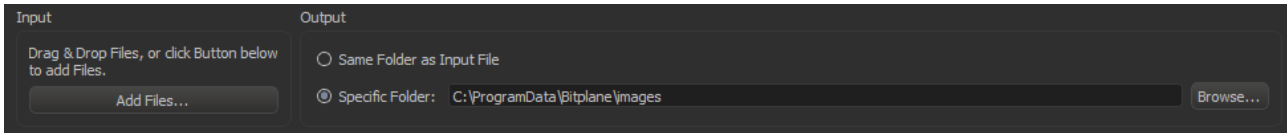
See also:

Addendum - [Mouse & Keyboard Mac](#)

### 5.1 Batch Convert ...

With Batch Convert... (Imaris File Converter) you can convert various image file formats to the Imaris file format \*.ims. Select the menu entry Batch Convert... and the Imaris File Converter window displays. From the Windows Start menu select "ImarisFileConverter".





## Input

### Drag & Drop Files, or click the Button below to add Files.

#### Button **Add Files ...**

The window **Select Files for Conversion** displays. Choose the respective file and click on the **Add Files...** button.

## Output

### Same Folder as Input File

You find the converted image(s) in the same folder as the input files.

### Specific Folder

Here you can select another folder for the converted image(s). Either type in the respective path or use the button **Browse**.

#### Button **Browse**

Click on this button to browse for the specific folder.

### File Format

The format is Bitplane: Imaris 5.5 (\*.ims)

**Note: Bitplane: Imaris 3.0 (\*.ims) and Bitplane: Imaris 2.7 (Classic)(\*.ims) options have been removed as of Imaris 8.4.**

Input	Output
C:\Users\Downloads\Wave FX (XYZ).tif	C:\ProgramData\Bitplane\images\Wave FX (XYZ).ims

## Input

Here you can see the selected input file path(s).

## Output

Here you can see the selected output file path(s).

### Settings

Provides additional settings for the selected file e.g. to specify voxel size


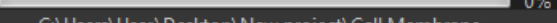

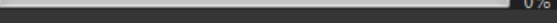






## Remove Selected and Remove All buttons

Click on these buttons to clear either a selected row or all rows in the table respectively.









## Button Start All

Click on this button to start the conversion.

When the conversion starts, files are moved to the bottom table. This displays the progress of the conversion for both read progress and write progress. It is possible to add new files and configure settings while a conversion is running.

Input	Output
 C:\Users\User\Desktop\New project\Cell Membrane Control A.tif  0%	 C:\Results Data\Cell Membrane Control A.ims  0%
 C:\Users\User\Desktop\New project\Cell Membrane Control B.tif	 C:\Results Data\Cell Membrane Control B.ims
 C:\Users\User\Desktop\New project\Cell Membrane Treatment A.tif	 C:\Results Data\Cell Membrane Treatment A.ims
 C:\Users\User\Desktop\New project\Cell Membrane Treatment B.tif	 C:\Results Data\Cell Membrane Treatment B.ims

This then updates on completion of the process:

Input	Output
 C:\Users\User\Desktop\New project\Cell Membrane Control A.tif	 C:\Results Data\Cell Membrane Control A.ims
 C:\Users\User\Desktop\New project\Cell Membrane Control B.tif	 C:\Results Data\Cell Membrane Control B.ims
 C:\Users\User\Desktop\New project\Cell Membrane Treatment A.tif	 C:\Results Data\Cell Membrane Treatment A.ims
 C:\Users\User\Desktop\New project\Cell Membrane Treatment B.tif	 C:\Results Data\Cell Membrane Treatment B.ims

See also:

Imaris Menu - Open - [Reader Configuration](#)

Imaris Menu - Open - [Settings](#)

Imaris Menu - Export ... - [Advanced Save Options](#)

## 5.1.1 Preferences

### Button Preferences

Click on this button to open the Preferences window.

### Window: Preferences

#### Data Cache

Imaris utilizes advanced data caching mechanism that allows you to process images that are significantly larger than the physical memory (RAM) installed in the computer system. This mechanism writes image data blocks to the disk and reads them back into the physical memory when they are needed.

**Cache File Paths** displays the cache file location.

**Please note: Setting the Cache File location to an unused disc, improves the performance when working on larger images. This benefits greatly from a fast SSD with a minimum size of 10 times the biggest data set, which will be used.**

**Memory Limit (MB)**

The value of Data cache limit controls the amount of data blocks Imaris will keep in memory at any time.

**File Paths:**

Display of the cache file paths.

**Button Add**

You can use the buttons to add file paths in the list.

**Button Remove**

You can use the buttons to remove file paths in the list.

**Output Formats**

If you want to extend the list of output formats you have to check the following parameter and enter a valid license in the next step. Find your license number in Imaris as follows: Click on the menu Help, select the menu entry Licenses and copy the license number (in the License Path at the bottom of the window, next to last enter field).

**Include Image Filenames**

Select this option and the output file names are assigned by using a default-naming rule.

**Suffix Alphabetic**

The output file inherits the input file name followed by the successive alphabetic letter.

**Suffix Numeric**

The output file inherits the input file name followed by the number of the output files being created (consecutive integer numbers).

**All Imaris Output File Formats**

Check this box to extend the output file formats.

**License Location**

Type in your license number or use the button **Browse**.

**Button Browse**

Click on this button and select the license path.

**Button OK**

Click on this button and all available Imaris output formats are available.

**Standard formats:**

Bitplane: Imaris 5.5 (\*.ims)

Bitplane: Imaris 3.0 (\*.ims)

Bitplane: Imaris 2.7 (Classic)(\*.ims)

**Additional formats:**

Tiff (series)(\*.tif \*.tiff)

RGBA-Tiff (series)(\*.tif \*.tiff)

ICS file (\*.ics \*.ids)

Olympus: cell^R 1.1/standard (\*.tif \*.tiff)

Open Microscopy Environment XML (\*.ome)  
Open Microscopy Environment Tiff (\*.tif \*.tiff)  
BMP (series)(\*.bmp)

See also:

Imaris Menu - [Export ...](#)

## 5.2 Store Arena and Restore Arena

**Store Arena** and **Restore Arena** allow you to manage your image data files and Arena tree structure.

### Store Arena

Using **Store Arena** you can copy image files to the data storage location that has been set by the user under [Arena Preferences](#). This enables your Arena data files to be stored on a range of local and remote locations. This includes HDD or SSD drives, and removable data storage devices such as USB memory sticks. When using network server locations you will require authentication to access the network server- this is described in [Arena Preferences](#).

Default Data Storage location:

- PC: C:\ProgramData\Bitplane\DataStorage
- Mac: \Library\Bitplane\DataStorage

Arena Files are stored in .ims format. Files in other formats will be converted into .ims format as part of the upload process.

**Note that Store and Restore functions are not available if they have been disabled under Imaris Administrator. This is to prevent overwriting files that may be used by more than one user, for example, image files saved on a remote, network server location that may be accessed and modified independently by different users.**

There are three options:

#### **Arena View only**

Will store only image files currently within the Imaris data store.

#### **Arena View + Local files**

Will store image files within the Imaris data store and any other image files that are present on a local (physical) drive.

#### **Arena View + Local files and Remote files**

Will store image files within the Imaris data store and any other image files on both local and remote locations.

### Restore Arena

With **Restore Arena** you can restore image files in Arena View to the time of the last save. For example, if you have deleted, or modified a file by accident.

**Note that when the Restore Arena function is used, all image data in Arena will be overwritten as the image data will be returned to that of the previous save.**



## 5.3 Open

Data sets can be loaded from various file formats.

### Image File Series

If the data set consists of a series of images each is stored as an individual file. To open a series of images select the folder and click on Open to open it.

This brings up the Image selection window, which allows you to explore your file system.

The window contains a preview of all the images as well as the following info: Name, Description, Recording date, Size, Time Points, Channels, Total size and Voxel size.

The image series is ordered according to the number, which appears after the file name suffix.

To open the image select a file name from the list and click **Open**

### Image Preview

Check the box Thumbnail Preview to activate an image preview.

### Search Image Name

Enter the image name for instant image selection.

### Resampling Open...

The Resampling Open dialog box can be used as a preview before loading the data set and allows you to change the resolution of the data (subsampling) and to select only a part of the data set (cropping) by specifying the parameters in the Resampling Open dialog box. Both options reduce the size of the data set, decreasing the time needed to read the file and speeding up any operations on the data. This is particularly helpful/beneficial when reading large data sets over a network.

1. In the menu select File – Open.
2. Select a file name from the list and click **Open**, or double-click on the requested file entry.
3. The file is loaded.

### Demo Images

A range of demo images is provided so that you may practice with the features and functions of Imaris.

The default location of these files is as follows:

**Windows:** C:\ProgramData\Bitplane\images

**MAC:** /Library/Application Support/Bitplane/images/

### Imaris Background Load

If you need to open a large time series data set, Imaris **Background Load** feature loads the data in sequential time points according to the sort order and demonstrate advantage in speed performance over the best existing methods.

### iQ files in Imaris

To open iQ files in Imaris:

1. Locate and select the **DiskInfo5.kinetic** file.
2. Clicking on the **'Settings'** button opens a new window with a list of images and image information.
3. Check the box Thumbnail Preview to activate an image preview.
4. Select the required image and click on the **OK** button.
5. Then click again on the **Open** button. This will instantly create 3D volume rendered image of the selected data set.

## File Formats

	Format Name	Extension	Remarks
	Imarisbase		
Bitplane	Imaris 5.5	*.ims	
Bitplane	Imaris 3.0	*.ims	
Bitplane	Imaris 2.7	*.ims	
Bitplane	Imaris Scene File	*.imx	
Andor	iQ Image	*.kinetic	
Andor	Multi-TIFF	*.tif, *.tiff	
	TIFF-Series	*.tif, *.tiff	
	BMP-Series	*.bmp	
	Imarisreaderdeltavision		
Applied Precision	DeltaVision	*.r3d, *.dv	
	Imarisreaderbiorad		
Bio-Rad	MRC	*.pic	
	Imaris reader iplab [BioVision purchased IPLab in 2006]		
BioVision	IPLab IPM	*.ipm	Mac
BioVision	IPLab IPL	*.ipl	Windows
	Imarisreadergatan		
Gatan	DigitalMicrograph	*.dm3	
	Imarisreaderhamamatsu		
GE	GE InCell 6000	*.tiff, *.tiff	
Hamamatsu Compix	SimplePCI	*.cxd	
	Imarisreaderiii		
Intelligent Imaging Innovations	SlideBook	*.sld	
	Imarisreaderimod		
IMOD	MRC	*.mrc, *.st, *.rec	
	Imarisreaderleica		
Leica	Image File Format LIF	*.lif	
Leica	TCS-NT	*.tif, *.tiff	
Leica	Series	*.tif, *.tiff, *.inf, *.info	
Leica	Vista LCS	*.tif, *.tiff, *.lei, *.raw	
	Imarisreadermicromanager		
Micro-Manager	Image5D	*.tif, *.tiff, *.txt	
	Imarisreaderuniversalimaging [Molecular Devices acquired Universal Imaging in 2002]		

Molecular Devices	MetaMorph STK	*.stk
Molecular Devices	MetaMorph ND	*.nd
	Imarisreadernikon	
Nikon	Image Cytometry Standard ICS	*.ics,* .ids
Nikon	ND2	*.nd2
	Imarisreaderolympus	
Olympus	cellR	*.tif, *.tiff
Olympus	FluoView TIFF	*.tif, *.tiff
Olympus	FluoView OIB	*.oib
Olympus	FluoView OIF	*.oif
Olympus	VSI	*.vsi
	Imarisreaderome	
Open Microscopy Environment	XML	*.ome
Open Microscopy Environment	TIFF	*.tif, *.tiff
	Imarisreaderperkinelmerinc	
PerkinElmer	Improvision Openlab LIFF	*.liff
PerkinElmer	Improvision Openlab RAW	*.raw
PerkinElmer	UltraView	*.tim, *.zpo
	Imarisreaderprairie	
Prairie Technologies	Prairie View	*.xml,* .cfg,* .tif,* .tiff
	Imarisreadertill	
TILL Photonics	TILLvision	*.rbinf
	Imarisreaderzeiss	
Zeiss	AxioVision	*.zvi
Zeiss	LSM 510	*.lsm
Zeiss	LSM 410, LSM 310	*.tif, *.tiff
Zeiss	CZI	*.czi
TIFF (adjustable file series)		*.tif, *.tiff

If you want to reopen one of the last six images, you can use the Recent File List at the bottom of the File menu to select the desired image.

### 5.3.1 Reader Configuration

#### Read only one Time Point

Loads a single time point of a time series.

#### Resampling Open...

The Resampling Open dialog box can be used as a preview before loading a data set and allows you to change the resolution of the data (subsampling) and to select only a part of the data set (cropping) by specifying parameters in the Resampling Open dialog box. Both options reduce the size of the data set, decreasing the time needed to read the file and speeding up any operations on the data. This can be particularly important when reading large data sets over a network.

The revision applies to all views in Imaris and in Surpass.

- Select Imaris Menu - Open or click on the button **Open** in the Main toolbar.
- Click on a file to highlight it and click on the button **Resampling File Open ...**

## Image Preview

The view on the right side displays as image preview a single time point of the data set. Right-click in the view and move the mouse pointer to the right to increase brightness. Move the pointer upwards to increase contrast.

## Original Size

Display of the original file size.

## Subsampling Factor

### X,Y,Z, Ch, T

You have the option to specify the Subsampling Factor, i.e., the fraction of data points to be retained. The subsampling factor can be specified for the x-, y-, z-directions, the channels (Ch), and the time points (T).

## Crop Limits (Min/Max)

### X, Y, Z, Ch, T, From, To

The Crop Limits (Min/Max) for the x-, y-, z-directions, the channels (Ch), and the time points (T) can also be specified.

## Resampled Size

Display of the resampled file size.

- Click on **OK** and the data set is cropped and resampled while loading.

See also:

Menu Edit - Image Properties... - [Geometry](#)

Menu Edit - [Crop Time...](#)

Menu Edit - [Resample Time...](#)

Menu Edit - [Crop 3D...](#)

Menu Edit - [Resample 3D...](#)

## 5.3.2 Settings

In the Settings you specify options for reading certain file formats.

## Leica LCS Settings

A Leica LCS data set consists of a number of image stacks (or experiments). A dialog box can be opened to select a specific image stack.

- Select File - Open or click **Open** in the Main toolbar.
- Select Files of Type: Leica LCS.
- Click on the \*.lei file to highlight it and click Settings.

A new window appears with a list of images and image information (Name, Description, Recording Date, Data Type, Size, Time Points, Channels, Size (MB), voxel size).

By checking the box **Thumbnail Preview** possibility of activating an image preview is available. The individual images that belong to that stack will display on the left side.



Select the required image stack on the left side of the dialog box.

- Click **OK** to open the image.

### Leica LIF Settings

A Leica LIF data set consists of a number of image stacks (or experiments). A dialog box can be opened to select a specific image stack.

1. Select File - Open or click **Open** in the Main toolbar.
2. Select Files of Type: Leica LIF.
3. Click on the \*.lif file to highlight it and click Settings. A new window appears with a list of images and image information (Name, Description, Recording Date, Data Type, Size, Time Points, Channels, Size (MB), voxel size).
4. By checking the box **Thumbnail Preview** the possibility of activating an image preview is available. The individual images that belong to that stack will display on the left side.
5. Select the required image stack on the left side of the dialog box.
6. Click **OK** to open the image.

### Series Reading Sequence

If the data set consists of a series of images, individual images can be sorted according to various dimensions (i.e. slices, channels, time points, dimension sequence). The reader can handle Tiff series with single and multiple running numbers.

1. Select the Imaris Menu - the Open option
2. Select Files of Type: Tiff (adjustable file series) and not Tiff (series) from the drop-down list.
3. Select the source folder containing the series.
4. The Settings button only becomes enabled when a TIFF file is selected.
5. In the Series Reading Sequence window you can define the options to combine multiple files into one multi-dimensional image.

### Reference File

Display the full path of the selected file.

Depending on the structure of the file system and the file name extension there are two options you can use:

1. File Names with Delimiter
2. File Names without Delimiter

### File names with Delimiter

You can use this option when your files are numbered in sequential order and contain fields of information.

The dialog allows you to specify the order of the dimension within the image series and defines how they should be arranged in the final sequence. These are Slices: Z, channels: C, time points: T and to split into multiple datasets/images: F (Split).

You may apply as many or as few dimensions as you wish, depending on your image file requirements.

Assign the appropriate dimension by clicking on the arrow and selecting a dimension from the drop-down menu.

The selected dimensions are applied to the series of images.

## Summary

Displays the total number of files selected within the file cluster, as defined by your selection criteria, to be used to create multi-dimensional image.

The summary number is updated dynamically.

## File names without Delimiter

You can use this option when the files are not numbered and contain no separator or delimiter.

The dialog allows you to specify the order of the dimension within the image series and defines how they should be arranged in the final sequence. If the file is being loaded, the number of dimensions must be specified by the user so that the algorithm will know when to finish reading the first dimension and continue with the second.

You may apply as many or as few dimensions as you wish, depending on your image file requirements.

Assign the appropriate dimension by clicking on the arrow and select a dimension from the drop-down menu.

## Summary

Displays the total number of files selected within the file cluster, as defined by your selection criteria, to be used to create multi-dimensional image.

The summary number is updated dynamically.

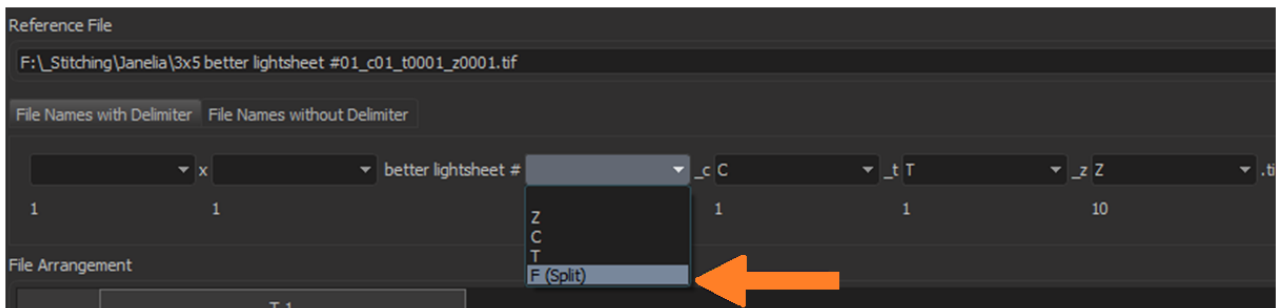
## File Arrangement

In the File Arrangement window, the selected sequence and dimension order is shown in tabular form. This display can be used to validate the sequence parameters.

Validate this information before you apply and open the TIFF series.

## F(Split)

The split option F (Split) lets you split a collection of images files into multiple DataSets / Images.



This Settings Dialog is present both in Imaris and in ImarisFileConverter, this option is available in the two applications:

- In ImarisFileConverter, it allows to convert file series into multiple output IMS Files (with the benefit of configuring the layout and applying the X, Y, Z size)
- In Imaris, it allows, to read a single file (or a subset of files) from file series, or load multiple images from subsets of the series for batch run.

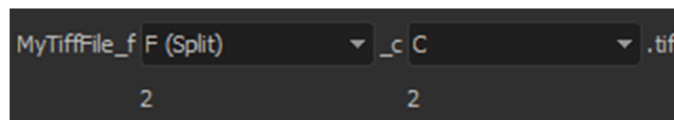
The procedure for how to split a number of files into two ims images, with two channels each is as follows:

In this example there are 4 TIFF files:

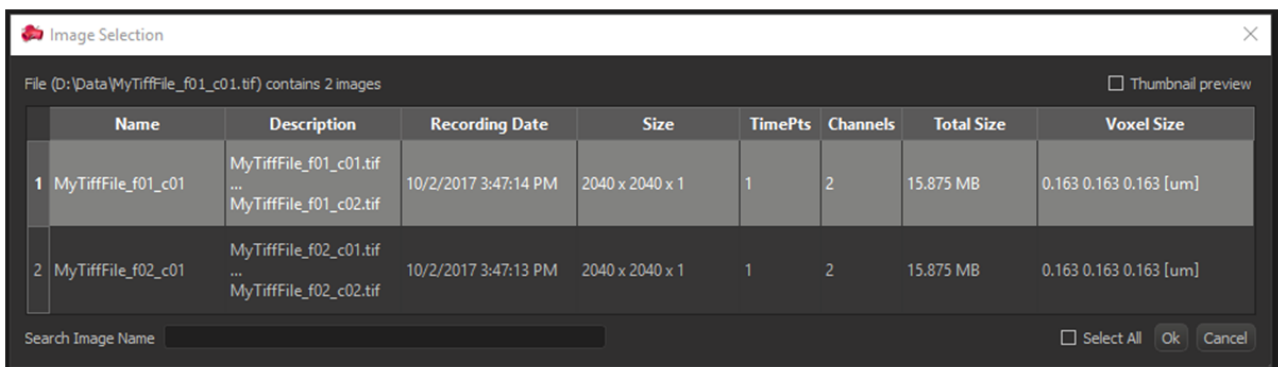
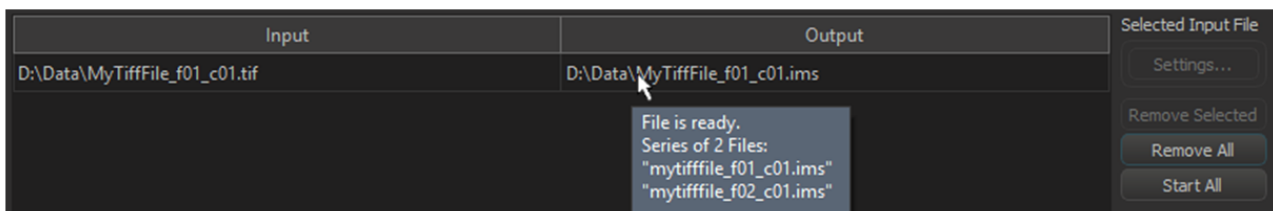
- MyTiffFile\_f01\_c01.tif

- MyTiffFile\_f01\_c02.tif
- MyTiffFile\_f02\_c01.tif
- MyTiffFile\_f02\_c02.tif

1. Add one of one of the TIFF files to ImarisFileConverter.
2. Click Settings the settings button.
3. Select "F (Split)" for the File Split delimiter. In this case it is "\_f".
4. Select "C" for the channel delimiter. In this case it is "\_c".



5. Click OK.
6. By letting the mouse pointer hover over the output, you can see that two IMS Files will be generated (see below).



- The Name and Description categories show which file corresponds to which DataSet.
- The behavior is then the same when loading a multi-image file.

When adding image files as part of a file series, Imaris and ImarisFileConverter will try to automatically detect the layout of image files in the file series. If the detected layout is not as desired, it is possible from to change it from the Series Settings option. It would be cumbersome if you were working with a number of similar image data sets with an incorrect structure to do this each time a new image was opened.

The File Series Delimiters configuration lets you configure the delimiters that are used for the automatic detection. This lets you adjust the process so it matches the image files. (The File Series Delimiters configuration is also accessible in Imaris from Preferences/Loading and in the ImarisFileConverter application under Preferences).

On the left-hand side, you can select the dimension that you want to configure from the drop-down list. The corresponding delimiters will appear in the central area.

For the example above, with "Dimension T" the following images would be correctly detected as time series, because the \_T delimiter is in the Dimension T delimiters' list:

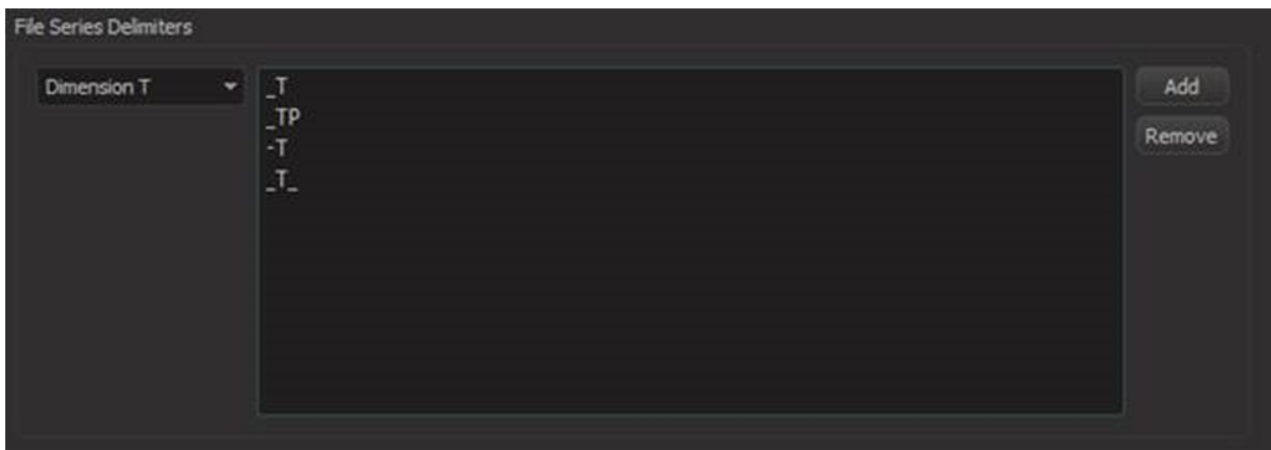
- MyFile\_t1.tif

- MyFile\_t2.tif
- MyFile\_t3.tif
- MyFile\_t4.tif

But the following images would not:

- MyFile\_t\_1.tif
- MyFile\_t\_2.tif
- MyFile\_t\_3.tif
- MyFile\_t\_4.tif

To have these files detected automatically as a time series, the `_T_` delimiter must be added:



Dimension F (Split) is by default not detected automatically (it does not contain any default delimiter). Therefore to have File Series Split detected automatically, some delimiters must be added (e.g. `_F`, `_F_`, `_FLD`).

## Notes

- The delimiters are case insensitive.

## 5.4 Open iQ File ...

1. Click on the **Open iQ files** to open the **Image Selection** window with a list of images and image information.
2. Check the box **Thumbnail Preview** to activate an image preview.
3. Select the required image and click on the **OK** button.
4. This will instantly create a 3D volume rendered image of the selected data set.

### Search File Name

Search for all files that meet a desired criteria

### Resampling Open...

The **Resampling Open** dialog box can be used as a preview before loading a data set and allows you to change the resolution of the data (subsampling) and to select only a part of the data set (cropping) by specifying parameters in the Resampling Open dialog box. Both options reduce the size of the data set, decreasing the time needed to read the file and speeding up any operations on the data.

### Image Preview

The view on the right side displays as image preview a single time point of the data set. Right-click in the view and move the mouse pointer to the right to increase brightness. Move the pointer upwards to increase contrast.

**Original Size**

Display of the original file size.

**Subsampling Factor****X, Y, Z, Ch, T**

You have the option to specify the Subsampling Factor, i.e., the fraction of data points to be retained. The subsampling factor can be specified for the x-, y-, z-directions, the channels (Ch), and the time points (T).

**Crop Limits (Min/Max)****X, Y, Z, Ch, T, From, To**

The Crop Limits (Min/Max) for the x-, y-, z-directions, the channels (Ch), and the time points (T) can also be specified.

**Resampled Size**

Display of the resampled file size.

- Click on **OK** and the data set is cropped and resampled while loading.

## 5.5 Store


To save an existing data set click **Store** in the File menu or Store icon  on the [Main toolbar](#).

Selecting this option stores the image data and (any) created objects within the selected Arena Group. The icon overlaying the image is updated to reflect the newly created surpass objects. The newly created object is added under Properties in the Surpass tab.

**Please note: Store functionality is implemented to be performed at maximum speed. This functionality overwrites the existing file without warning and only writes changed or new data without deleting obsolete ones. As a result, the resulting Save file could actually be larger than the original.**

## 5.6 Store as

To store the changes made to the image in a different file whilst maintaining the original file:

1. Click on the Store as option in the File menu or Store as icon . This option enables you to modify the image name and creates a new image item within the Arena. The selected group now contains one original image file which remains unchanged and the new image file with the additionally segmented object. This newly created image item has an overlaid icon indicating the image type and segmented surpass objects. Under the [Objects tab](#) the newly created object is added.
2. Enter the name for the file to be saved or confirm the suggestion.
3. Select the required file format and click **OK**.

## 5.7 Export

To save a newly created Imaris file that contains both image and scene data on your local storage space click



on the Export option in the File menu or Export icon .

Saving in Imaris format is recommended whenever the data set is cropped or the parameters changed. Saving a data set in Imaris file format provides the advantage of a faster loading process and the possibility of using thumbnails. In addition, parameters are saved with the images.

The file extension, suggested as the default, is \*.ims. The single created Imaris file contains both the image and the scene data.

You can save the file when you are done or if you want you can stop in the middle and resume work later. Simply, choose **Export** from the File menu and give the file a name you will remember. If you then close the file and reopen it at a later time, you can resume work from where you left off.

1. In the menu bar select File - **Export...** The Save as window displays.
2. Select the directory and enter the name for the file to be saved or confirm the suggestion.
3. Select the requested file format and click **OK**.

When the work is done, it is recommended to save the data set by using 'Store as'. Selecting the 'Store as' option could take longer time but will give you the smallest file size possible.

## Available File Formats in Imaris

Bitplane: Imaris 5.5 (\*.ims)  
Bitplane: Imaris 3.0 (\*.ims)  
Bitplane: Imaris 2.7 (Classic)(\*.ims)  
Tiff (series)(\*.tif \*.tiff)  
RGBA-Tiff (series)(\*.tif \*.tiff)  
ICS file (\*.ics \*.ids)  
Olympus: cell^R 1.1/standard (\*.tif \*.tiff)  
Open Microscopy Environment XML (\*.ome)  
Open Microscopy Environment Tiff (\*.tif \*.tiff)  
BMP (series)(\*.bmp)  
Move file (slice ion) (\*.avi, \*.mov, \*.tif, \*.tiff)

### 5.7.1 Advanced Save Options

Button **Format Settings...**

A click on the button **Format Settings...** opens the Imaris Save Options window. See also [Open Microscopy Environment: TIFF](#) and [Export as Movie File \(Slice Animation\)](#).

## Window: Save Options

**Bitplane: Imaris 5.5 (\*.ims)**

### Enable Compression

Check the box to use compression.

### Export Scene

Check the box to save the Imaris scene.

**Bitplane: Imaris 3.0 (\*.ims)**



## Time Series

### Export Single File

The time series are saved in a single file.

### Export Multiple Files

For each time point a new file is generated.

## Compression

### LZW Compression

Check the box to use an LZW compression.

## 5.7.2 Open Microscopy Environment TIFF Advanced Options

Set the advanced save options when exporting as a **Open Microscopy Environment TIFF** format by clicking the **Format Settings** button. It is possible to set the options for this format from the below:

### Dimension order

Select from the following options:

- XY ZCT (default)
- XT TZC
- XT CZT
- XY ZTC
- XY TCZ
- XY CTZ

### Number of dimensions on one file

Set the number of dimensions between 2 and 5.

### Tiff options

Enable/disable **LZW Compression** (lossless data compression, default: Off) and set image to be composed of either **Tiles** or **Strips** (default).

## 5.7.3 Movie File (Slice Animation) Advanced Settings

Set the save options when exporting as a **Movie file (Slice Animation)** by clicking the **Format Settings** button. It is possible to set the options outlined below:

### Format

Select from the following file formats:

- H264 Movie (default - common high definition format, H.264 compression)
- Raw Movie
- Tiff (series).tiff

### Movie Settings

Adjust the Compression factor between 0 (no compression: best quality/largest file size) and 100 (highest compression: lowest quality/smallest file size).

### Frame rate

Set the frame rate for the movie file in frames per second.

## 5.8 Add Image... (multi-image)

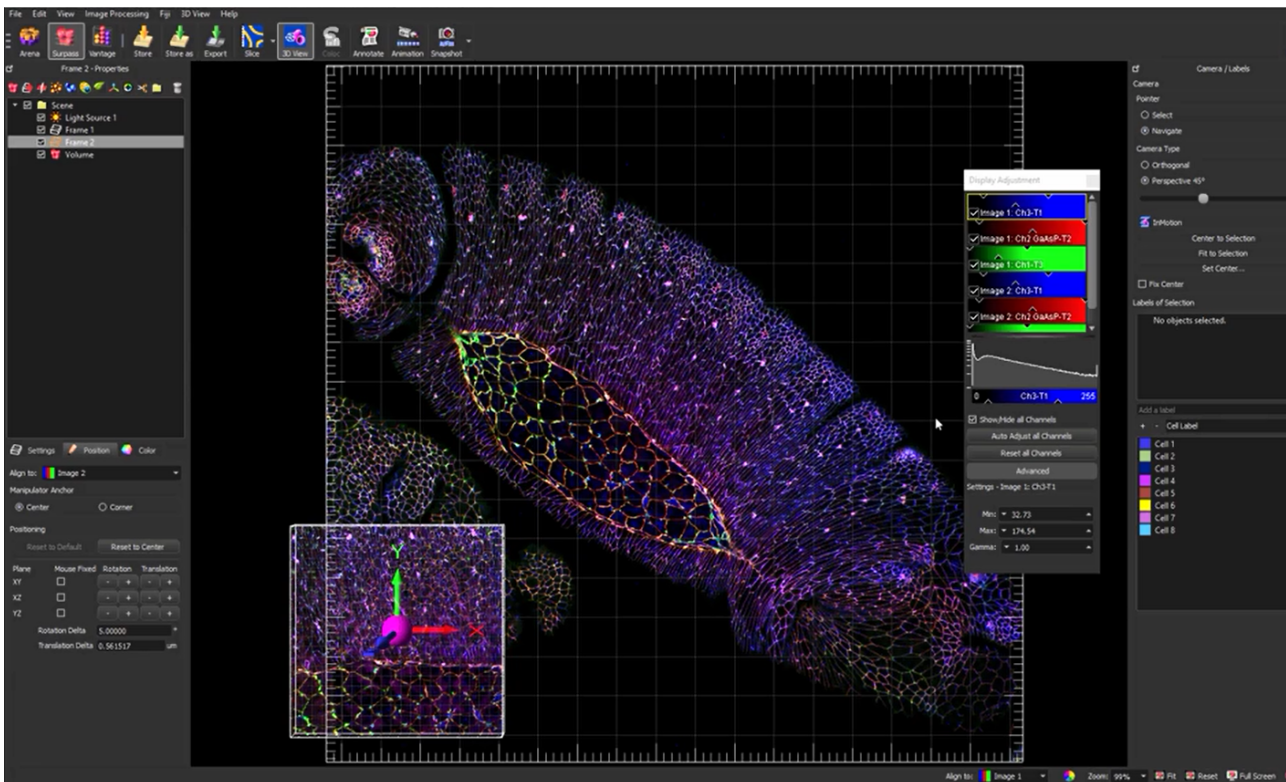
New "multi-image" functionality for working with multiple images in a single Imaris scene was introduced in Imaris 9.1. This feature set may be useful for a range of applications, for example: combining different imaging modalities on the same sample such as electron microscopy and confocal microscopy, or for images of the same modality captured at different magnification levels. It is also possible to perform alignment of 3D objects that were imaged separately.

The features available are described in the following sub-sections along with an overview of how to use these functions on your images.

The first step is how to add additional images:

### How to add your images

1. Open the first image in Surpass view.
2. Load additional images using the **Add images...** from the File menu.
3. Each additional image can then be seen to have been loaded in the viewing window.
  - a. When the position tab is selected an XYZ tripod shows the orientation of the selected image.
  - b. The **Align to:** box displays an indicator color to help identify the appropriate image.
  - c. Use the mouse Pointer **Navigate** mode to navigate through the image area, use the **Select** mode to select the required image.



- The Settings section describes how the images are displayed and labelled.
- If there is more than one image loaded, Imaris will let the user chose which image to perform the required



action on.

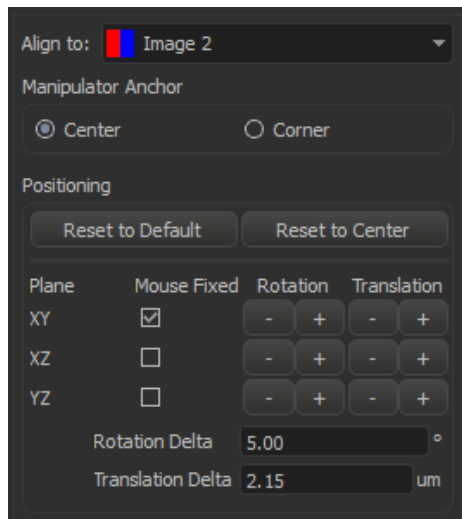
- Image position adjustment tools are described in section Position.

**Note:** To remove images, use the **Remove images...** from the **Edit** menu, see [Remove Images](#).

### Settings Tab Menu Options

Refer to the section [tab settings](#) for further information on frame component settings.

### Position Tab Menu Options



#### Align to:

Select the image you want to align the selected image to. The color indicator displayed is representative of the appropriate image.

#### Manipulator Anchor

Select the adjustment/rotation point to be either at the **Center** or the **Corner** of the image.

#### Positioning

Use the positioning options to adjust the positioning of image in **XY**, **XZ** and **YZ** planes. The **+** and **-** buttons can be used to adjust in increments. The increment size is set in the **Rotation Delta** and **Translation Delta** fields. The positioning can be fixed about the planes by selecting the **Mouse Fixed** checkbox for the appropriate plane. This is useful for locking the movement in the plane/axis as the image is moved.

After making adjustments you can return positioning to the default position or reset to Center using the the **Reset to Default** or **Reset to Center** buttons respectively.

### Color Tab Menu Options

Adjust the color settings.

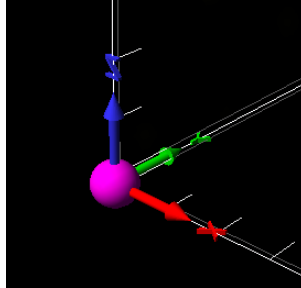
### How to Align Images

Alignment can be split into two steps:

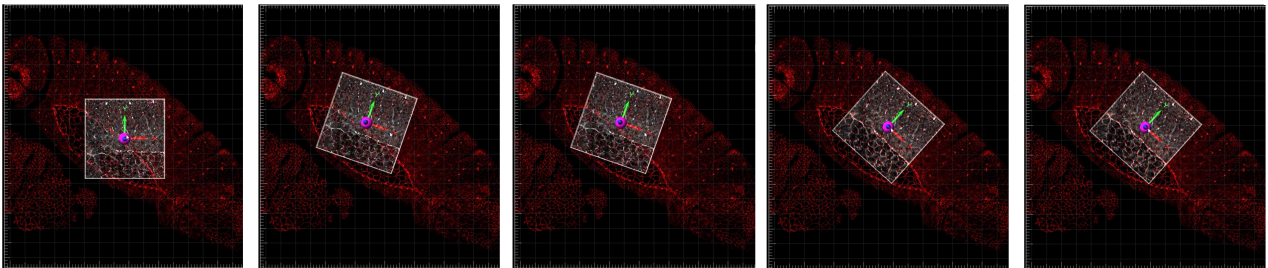
#### Performing the Initial Alignment (Approximate)

1. Add the images as described in [Add Image... \(multi-image\)](#)

2. Images can be repositioned in the **Select** Pointer mode by clicking and holding the left mouse button on the pink central section and moving to the required location.
3. To change the orientation about the axes, click on the required X, Y or Z axis arms of the tripod and it will rotate about the selected axis. The plane can be locked in position if required using the **Mouse Fixed** check box for the required plane (axis).



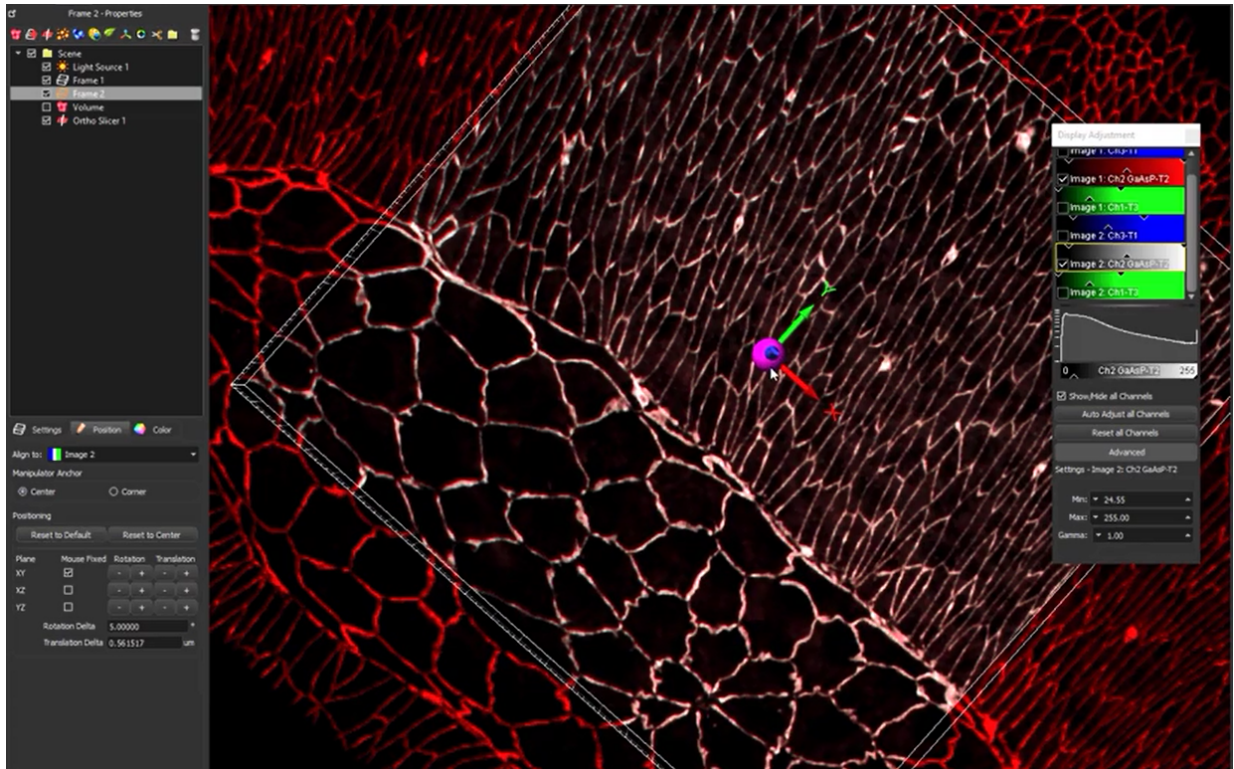
4. When aligning images it is useful to turn off unnecessary channels so you may focus on useful image information to help the alignment. It may also be useful to change the color of the channel you are using for alignment to one that gives good contrast, making alignment easier.



Above: Image is aligned against the underlying image using the tripod to adjust the relative rotation and translation about the XY, XZ and YZ planes. Note that channels for both images have been disabled to help visualize the alignment of the structures during the alignment process.

## Performing Precise Alignment

1. Once the images are aligned approximately you may find it useful to switch to a 2D or Slicer view, zooming in for precise alignment:
2. The plane can be locked in position using the **Mouse fixed** check box for the required plane (axis).
3. The **Rotation** and **Translation** - and + buttons can be used to adjust the position in small increments with a specific plane until the desired alignment is achieved. The size of these increments can be set in the fields below. Note that for rotation you can change between having the adjustment in the **Center**, or the **Corner** of the image.
4. This process can be repeated for the other planes and checked until you are happy with the alignment.



Above: Image has been zoomed in and image plane locked in the XY plane. The rotation and Translation + and - buttons are then used to perform the precise alignment.

- Image position adjustment tools are described in section Position.
- To adjust how the images are displayed and labelled refer to the section Settings.

**Note:** To remove images, use the Remove images... from the Edit menu, see [Remove Images](#).

### Performing Image Analysis

- Once the image has been aligned you can perform analysis on the image scene as required. The source image can be selected (from the Align to drop-down box) at step 1 of the creation wizard.

## 5.9 Preferences...

The adjustable parameters in the Preferences are application specific and IMAVIS stores these preferences for an individual user.

[System](#)

[Display](#)

[Loading](#)

[Calculation](#)

[Time](#)

[3D View](#)

[Creation Parameters](#)

[Vantage Plots](#)

[Statistics](#)

[Statistics Test](#)

[Snapshot](#)

[Custom Tools](#)

[Licenses](#)

[Update](#)  
[Usage Data](#)  
[3D Cursor](#)  
[Annotate](#)  
[Arena](#)  
[Advanced](#)

[Imaris Administrator](#) can be accessed directly from Preferences so that licenses, data storage locations and other Imaris settings can be configured by a user with administrative privileges.

## 5.9.1 System

Displays the basic system parameters of your computer.

### Graphics

**OpenGL Renderer**  
**OpenGL Version**  
**Pixel Shader**  
**OpenGL Extensions**

Information about the OpenGL Renderer, OpenGL Version, Pixel Shader, and OpenGL Extensions.

### Operating System

**OS**  
**Version (Build)**  
**Service Pack**

Information about the operating system, the installed Version and the service pack.

### Memory Status

**Physical Memory installed**  
**Physical Memory available**

Information about the available amount of internal memory.

### Network

#### Network Interfaces

Information about the available network interfaces

#### Proxy Settings

Http Proxy Server Name

Enter a proxy server's name. If this field is empty, no proxy server is used.

#### Http Proxy Server Port

Enter the port to the proxy server. If this field is empty, no proxy server is used.

#### Test Connection Button

Click on this button to test your proxy settings. If the fields are empty, the connection is tested without using a proxy server.

Button **OK**



To apply the changes click on **OK**.

#### Button **Cancel**

If you do not want to save the changes click on **Cancel**.

## 5.9.2 Display

Select the viewing properties and the basic colors for the backgrounds and selection in the gallery.

### Display

#### Interpolate

If checked, the images are automatically interpolated for a smoother display.

#### Texture Cache Limit (MB)

Before displaying any image data, Imaris converts the data into a configuration (called textures) that is optimized by the graphics hardware. The value of the Texture Cache Limit determines how many textures can be stored in RAM. The Texture Cache Limit is automatically determined from the available memory of the graphics board when Imaris is used for the first time. You can also manually set the value to the memory of your graphics card.

### Colors

#### Background Color

The background color appears when there is no image to be displayed or when the displayed image does not fill the whole View Area.

**Please note: The background color setting has not effect on the MIP mode. The default color for MIP(max) is black and MIP(min) is white.**

Click on the color selection button to open the color selection window to change the respective color.

#### Background Color 2

Background marking the original position if an image is moved.

Click on the color selection button to open the color selection window to change the respective color.

#### Checkered Background for Blending

When using blending mode, a checkered background is displayed in Full 3D blend and in Surpass view.

#### Tile Size

Allows definition of the tile size for a checkered background.

#### Linear Color Progress for Blending

The background displays a color gradient in blend progress projections and in Surpass.

#### Selection Color

Color of selection frame and drawing lines in contour surfaces.

Click on the color selection button to open the color selection window to change the respective color.

## Measurement Color

Color of measurements points and lines visible in the image (in the Slice and the 3D view).

Click on the color selection button to open the color selection window to change the respective color.

## User Interface Theme

This option allows you to customize the user interface theme.

You can either choose the Native theme (from OS) or select the Dark theme. Click **OK**. The display updates immediately.

## Coordinate Axis/Scale TimeColor Bar

### Show Coordinates Axis

### Show Scale Bar

### Show Scale Bar Label

### Scale Bar Font Size

### Scale Bar Lower Left Corner x

### Scale Bar Lower Left Corner y

### Show Time Colorbar

### Show Date

### Show Time

These options change the display of the scale bar, labels and time information in the Slice, Selection, Gallery and 3D View.

## Adjusting the Scale Bar

The scale bar position and size can be set in preferences by adjusting the values in the **Scale Bar Font Size**, **Scale Bar Lower Left Corner x**: and **Scale Bar Lower Left Corner y**:

You can also alter the length, width, location and font size of the interactive scale bar when the Pointer is in Select mode. All changes are instantly displayed in the viewing area.

Extend or shorten the scale bar by clicking and dragging one end of it.

Move the scale bar to any location in the viewing by dragging it

Zooming in or out of the image the scale bars are automatically updated.

To choose the style of timestamp to be displayed select either **Show Time** or **Show relative Time**.

**Show Time** option display the acquisition time.

## Show relative Time

For Show relative time the first time point is designated as time zero and all other times are displayed relative to that.

The timestamp presentation format can be chosen and adjusted.

h (hours), min (minutes), s(seconds), f (milliseconds)

Select options to display coordinate axis, date, scale bar, scale bar label or time on screen in the Viewing Area.

**Please note:** To display the Coordinates Axis in the Surpass view the option [Axis Labels](#) in the [Frame-Tab Setting](#) must be also selected.



## Numbers Representation

You can adjust how numbers are represented for the available options:

- Unit of Length
- Unit of Time
- Significant Digits

### Off Screen Rendering (for saving Snapshots and Movies)

If deactivated Imaris will take the images similar like a screenshot directly from the display. Uncheck this option only if you have problems with the Snapshot or Movie export e.g. if the image is distorted. You will not be able to create an output larger than your physical screen size if this option is disabled.

### Show System Monitors

Check this box to display the system monitors in the Status Bar at the bottom of the screen. The first window displays the "reads per sec", the second the "writes per sec", the third the "write requests in queue" and the last the "percentage read cache hits". These are useful information especially if you work with huge data sets.

#### Button **OK**

To apply the changes click on **OK**.

#### Button **Cancel**

If you do not want to save the changes click on **Cancel**.

## 5.9.3 Loading

Allows you to select the color assignment method used when loading data sets and to define the default colors. Images in Imaris format will display in the colors defined in the image file.

### Take Colors from:

#### Default Colors

Use the default color selection to display the loaded data set. The parameter set Default Colors (see below) is available.

#### File Colors (color table or base color if available, otherwise default colors)

Use the original color definition of the loaded data set (usually stored in a lookup table). The parameter set Default Colors (see below) is not available.

#### Emission Wavelength (from file if available, otherwise default colors)

Use the color according to the emitted wavelength from the file (corresponds to the appearance under the microscope). The parameter set Default Colors (see below) is not available.

**Please note that not all file formats support lookup tables and emission wavelength.**

### Default Colors

The parameter set Default Colors is available, if you select Default Colors in the parameter set Take Colors from (see above).

#### First Channel

#### Second Channel

## Third Channel

### Other Channels

Display of the defined color.

## iQ ImageDisk File

1. Either type in the location of the **DiskInfo5.kinetic** file or use the browse button to find it.
2. Use the Browse button to find and select the DiskInfo5.kinetic file.
3. Open iQ files
4. Clicking on the '**Open iQ files**' opens the Image Selection window with a list of images and image information.
5. Check the box Thumbnail Preview to activate an image preview.
6. Select the required image and click on the **OK** button. This will instantly create a 3D volume rendered image of the selected data set.

### Browse Button

Use the Browse button to find and select the DiskInfo5.kinetic file.

### Select... Button

Click on this button to open the color selection window to change the respective color.

### OK Button

To apply the changes click on **OK**.

### Button Cancel

If you do not want to save the changes click on **Cancel**.

## 5.9.4 Calculation

### Calculation

#### Number of Parallel Jobs

Specify the number of threads (processor cores) used in calculations. See maximum number of available threads in the System box.

### History

#### Maximum Number of Commands in History

Defines the maximum number of operations that can be undone/redone.

**Please note: Each level of image processing requires an additional copy of the full image in memory. If your machine runs out of memory, set Maximum Number of Commands in History to 1.**

### Data Cache

Imaris uses a data caching mechanism that allows you to process images that are significantly larger than the physical memory (RAM) installed in the computer system. This mechanism writes image data blocks to the disk and reads them back into the physical memory when they are needed.

### Memory Limit (MB)





The value of data cache limit controls the amount of data blocks Imaris will keep in memory at any time. Enter a value based on the following table.

PC & MAC	
64 bit	Physical memory installed x 0,5

#### Cache File Paths:

Imaris utilize advanced caching when working with large images. You can add a Cache File Path by defining a directory where the temporary files should be stored.

**Cache File Paths** display of the paths to the temporary files (Scratch-disks).

**Please note: Setting the Cache File location to a spare or separate hard drive may improve the performance when working on larger images.**

Button **Add**

Button **Remove**

You can use the buttons to add or remove file paths in the list.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

### 5.9.5 Time

These are the default parameter settings for the Time Bar.

#### Play Back

Specify the play back mode for the Time Bar.

##### Play One Time

All time points of the data set are shown one time. The play back stops when the last time point is reached.

##### Repeat Forever

Once the play back has reached the last time point, it starts at the first time point again (continuous loop).

##### Swing Back and Forth Forever

When the last time point is reached, the time sequence is shown in reverse until the first time point is reached.

##### Frame Rate: ... Frames per Second

You can further specify the frame rate, i.e. the number of frames per second.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

See also:

Toolbars - [Time Bar](#)

## 5.9.6 3D View

### Object Creation Buttons

Check the icons to be displayed on the Objects toolbar in the Surpass View.

Button **Move Up**

Button **Move Down**

Highlight an icon and click Move Up or Move Down to define the order of the icons in the Objects toolbar.

### Key Frame Animation

Specify the play back mode for the Key Frame Animation.

#### Play One Time

All time points of the data set are shown one time. The play back stops when the last time point is reached.

#### Repeat Forever

Once the play back has reached the last time point, it starts at the first time point again (never ending).

#### Frame Rate ... Frames per Second

You can further specify the frame rate, i.e. the number of frames per second.

### Manual tracking auto-connect (Spots and Surfaces)

Delay before auto advancing (s)

You can specify the value between 0.05 and 5 seconds.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

### Camera

#### Enable antialiasing (Smooth Lines)

Check the box to display smooth lines.

#### Enable Draw Style Settings

Check the box to enable the draw options in the Camera Toolbar.

#### Enable Stereo Camera Settings

Check the box to enable the stereo options in the Camera Toolbar.

Refer to the [Camera Toolbar](#) section for further information.

#### Zoom Point Under Cursor

This option enables zoom to be centered on the point under the Cursor automatically. This may be useful when you want to zoom to a specific location, for example when using Filament



Autopath and Filament autodepth tracing. This may be disabled for a standard zoom style in which the zoom will zoom in on the center of the viewing area. Default (On).

### **Auto Adjust Center Of Rotation**

This option enables the rotation point to be centered on the center of the visible part of the image/scene automatically. When not enabled the image will automatically rotate around the center of the viewing area window. Default (On).

### **Zoom Through Data Set**

This option provides an auto focus (based on the distance from the camera to the center of the image for zoom and pan speed and center of rotation) so you may zoom smoothly through an image to the other side. This may be useful when the point that you want to zoom to is close to the rear of the image. Default (On).

## **Torch View**

Adjust the options for Torch View. You can select between the three following options:

- Draw entire dataset (default option)
- Draw dataset only around the torch region (full depth)
- Draw dataset only around the torch region (local depth)

### **5.9.7 Creation Parameters**

When you save the Creation Parameter file, it is automatically added to the list that is displayed under the Edit/Preference/[Creation Parameters](#).

The preference window shows a list of all the parameters sets that have been created. To assist you in finding and organizing the creation parameters, each file is labeled with an object icon, which indicates the type of creation parameters set.

Click on the name of the creation parameter's set, the extended window provides an option to view the entire list of operations, processing instructions and the parameter's value of the selected set.

#### **Remove Button**

This function will remove the selected set of creation parameters from the list.

The Export and Import functions provide an easy way to exchange the creation parameters between different users or groups.

#### **Export Button**

The creation parameters files can be created and exported.

This can be achieved by selecting the creation parameters set from the list and pressing the Export button. Specify the directory, where you want to save the creation parameter's file, select a name and save it.

#### **Import Button**

The creation parameters files, that can be imported, are generated by the Export command.

Specify the directory of the files you want to import and select the file from the chosen location.

## 5.9.8 Vantage Plots

When you save the Vantage Plot file, it is automatically added to the list that is displayed under the Edit/Preference/Vantage Plots.

The preference window shows a list of all the creation parameters' sets. To assist you in finding and organizing the creation parameters, each file is labeled with an object icon, which indicates the type of creation parameters set.

Click on the name of the Vantage Plot, the extended window provides an option to view the entire list of the plot type, dimensions and the selected statistical variables.

### Remove Button

This function will remove the selected Vantage Plot parameters from the list.

The Export and Import functions provide an easy way to exchange the Vantage Plot parameters between different users or groups.

### Export Button

The Vantage Plot parameters files can be created and exported.

This can be achieved by selecting the Vantage Plot parameters set from the list and pressing the Export button.

Specify the directory, where you want to save the Vantage Plot parameter's file, select a name and save it.

### Import Button

The Vantage Plot parameters files, that can be imported, are generated by the Export command.

Specify the directory of the files you want to import and select the file from the chosen location.

## 5.9.9 Statistics

Imaris features many statistical functions that can be utilised to analyse surpass objects. Statistics are accessed through the Statistics tab in the surpass view, the options available are specific to each of the surpass item types e.g. cells or spots. This section provides an introduction to some of the statistical notations and concepts used in the following statistics topics.

- The time indices where the track objects are present are denoted with **t** ranging from **1** to **n**.
- Descriptions for statistic functions are grouped together for vectors "X, Y, Z" or dimensions "A, B, C". For example, for **Cells - Cell Displacement X, Y, Z** there are three individual functions for each component i.e. **Cells - Cell Displacement X, Cells - Cell Displacement Y** and **Cells - Cell Displacement Z**
- Three-dimensional vectors are denoted with an arrow:

$$\vec{r} = [r_x, r_y, r_z]$$

- Length of a vector:

$$|\vec{r}| = \sqrt{r_x^2 + r_y^2 + r_z^2}$$

- Position of an object at time index:

$$\vec{p}(t)$$

- Time of an object at time index:

$$T(t)$$

### Show Statistic Values

Statistics values can be selected (for display, export to MS Excel, or sorting). Check the values to be displayed when you open the tab Statistics in the Surpass View.

#### Show All

Check this box and all statistical values are selected. Uncheck the box and all statistical values are unselected.

For details of the available options please refer to the respective Section:



Menu Edit - Preferences ... [Cell](#)  
- Statistics



Menu Edit - Preferences ... [Filament](#)  
- Statistics



Menu Edit - Preferences ... [Measurement Points](#)  
- Statistics



Menu Edit - Preferences ... [Spots](#)  
- Statistics



Menu Edit - Preferences ... [Surfaces](#)  
- Statistics



Menu Edit - Preferences ... [Volume](#)  
- Statistics

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

See also:

Surpass View - Overview - [Properties Area](#) (Tab Statistics)

Coloc - [Volume Statistics](#)

### 5.9.9.1 Cells

ImarisCell is an Imaris module that performs automated 2D and 3D time-resolved cell image analysis. It extracts a full spectrum of relevant information, present in the data set, which provides a wide range of options to analyze 3D and 4D image data. ImarisCell detects some features that are not easily detected by a human observer, which can lead to the correlation of potential important and otherwise missed variations in the cell structure and function. Spots statistics can be located in the statistics tab.

### Show Statistic Values - Cell

#### Cells - Cell Acceleration

Acceleration is the change in the Cell velocity over time.

Acceleration X, Y, Z are the components of the acceleration vector (at time-point t):

$$\vec{a}(t) = \left( \frac{\vec{p}(t+1) - \vec{p}(t)}{T(t+1) - T(t)} - \frac{\vec{p}(t) - \vec{p}(t-1)}{T(t) - T(t-1)} \right) / \left( \frac{T(t+1) - T(t-1)}{2} \right), \text{ for } 1 < t < n$$

$$\vec{a}(1) = \vec{a}(n) = \vec{0}$$

Acceleration at time-point t:

$$a(t) = |\vec{a}(t)|, \text{ with } \vec{a}(t) \text{ as defined in Acceleration X, Y, Z}$$

Acceleration at the first and last time-point is 0.

### Cells - Cell Acceleration X, Y, Z

Acceleration is the change in the Cell velocity over time along the selected-axis.

### Cells - Cell Area

The area is the sum of the outer voxel faces.

### Cells - BoundingBoxAA Length X, Y, Z

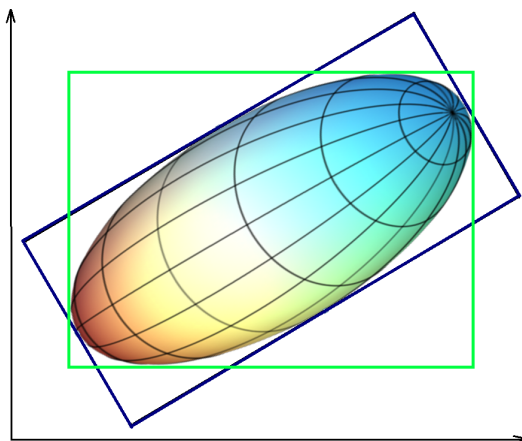
A bounding box is a rectangular cuboid in 3D, or a rectangle in 2D, containing an object.

If the bounding box is aligned with the axes of the coordinate system, it is known as an axis-aligned bounding box (AA). BoundingBoxAA identifies an object by considering the minimal rectangular box which fully encloses the object, whose faces are aligned parallel to the axes of a coordinate system.

BoundingBoxAA Length X = max(X)-min(X)

BoundingBoxAA Length Y = max(Y)-min(Y)

BoundingBoxAA Length Z = max(Z)-min(Z)



axis-aligned bounding box (AA) shown in green  
object-oriented bounding box (OO) shown in blue

Please note the difference between the ellipsoid axis length and bounding box length.

### Cells - BoundingBoxOO Length A, B, C

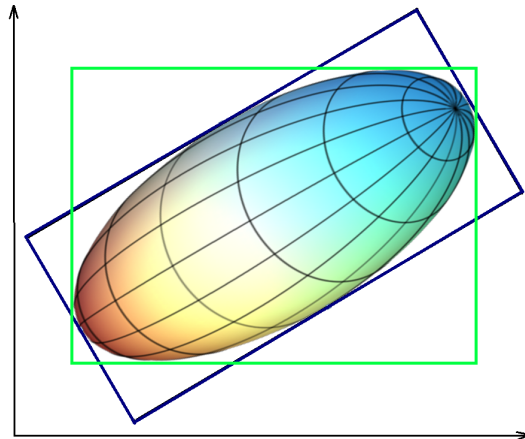
The local axes of the box can be arbitrarily oriented with respect to the coordinate system.

BoundingBoxOO (object oriented) identifies an object by considering the minimal rectangular box, which fully encloses the object. The box dimensions are calculated with no constraints to the orientation.

BoundingBoxOO Length A = Length of the shortest principal axis

BoundingBoxOO Length B = Length of the second longest principal axis

BoundingBoxOO Length C = Length of the longest principal axis



axis-aligned bounding box (AA) shown in green

object-oriented bounding box (OO) shown in blue

Please note the difference between the ellipsoid axis length and bounding box length.

### Cells - Cell Center of Homogeneous Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The algorithm assumes that every voxel has the same intensity (voxel intensities are homogenous).

Assuming a homogeneous intensity, the center of homogeneous mass is the X, Y, Z coordinate of the object's centroid (geometric center) .

It is the location where all of the mass of the system could be considered to be located. At the center of mass, the entire mass of the body could be replaced with a point mass equal to the body's mass. It is equivalent to the concept of centre of gravity for a body, where the intensity value is a substitute for mass.

The center of mass of a solid body does not have to lie within the body. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{i \in \text{object}} r_i$$

**R** : center of homogeneous mass (x, y, z)

$r_i$  : center of a voxel ( $x_i, y_i, z_i$ )

**n** : number of  $i \in \text{Cell}$

### Cells - Cell Center of Image Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The Center of Homogeneous Mass calculations do not consider a variation of the voxels intensity of the inside

the object. In the case of an inhomogeneous distribution of the voxel intensity inside the objects, intensity-based measure may be needed.

The center of mass is calculated using a contribution of each channel and each voxel of the object, based on each voxel intensity value.

Its positions depend on respective intensities of each voxel in the volume.

The Center of Image Mass is the object centroid calculated by taking into account the voxel intensities of each channel separately.

The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{ie\ object} m_i \times r_i$$

**R** : center of image mass (x, y, z)

**m<sub>i</sub>** : voxel intensity

**r<sub>i</sub>** : center of a voxel (x<sub>i</sub>, y<sub>i</sub>, z<sub>i</sub>)

**M** : sum of voxel intensities

### **Cells - Cell Cytoplasm Area**

Measure the area of the cytoplasm within the Cell. It is calculated as sum of Cell area and Nucleus area.

### **Cells - Cell Cytoplasm Vesicle Diameter Max**

The maximum diameter of all Vesicles within the Cell cytoplasm. The value is separately calculated for each vesicle type.

### **Cells - Cell Cytoplasm Vesicle Diameter Mean**

The mean diameter of all Vesicles within the Cell cytoplasm. The value is separately calculated for each vesicle type.

### **Cells - Cell Cytoplasm Vesicle Diameter Min**

The minimum diameter of all Vesicles within the Cell cytoplasm. The value is separately calculated for each vesicle type.

### **Cells - Cell Cytoplasm Intensity Max**

### **Cells - Cell Cytoplasm Intensity Mean**

### **Cells - Cell Cytoplasm Intensity Min**

### **Cells - Cell Cytoplasm Intensity StdDev**

### **Cells - Cell Cytoplasm Intensity Sum**

The voxel intensity statistics describe the voxels enclosed within the Cytoplasm (Center, Max, Mean, Min, StdDev and Sum).

### **Cells - Cell Cytoplasm Number of Vesicles**

The total number of Vesicles objects within the cytoplasm. The value is separately calculated for each vesicle type.

### **Cells - Cell Cytoplasm Number of Voxels**

The total number of voxels within the cytoplasm.

### **Cells - Cell Cytoplasm Volume**





Measure the volume of the cytoplasm within the Cell. It is calculated by subtracting the nucleus volume from the total cell volume.

#### Cells - Cell Cytoplasm Volume of Vesicles

Volume is quantification of how much space the Vesicles object occupies within the cytoplasm. The value is separately calculated for each vesicle type.

#### Cells- Cell Vesicle Diameter Min

The minimum diameter of all Vesicles within the Cell objects.

#### Cells - Cell Vesicle Diameter Mean

The mean value of diameter of all Vesicles within the Cell objects.

#### Cells- Cell Vesicle Diameter Max

The maximum value of diameter of all Vesicles within the Cell objects.

#### Cells - Cell Displacement X, Y, Z

The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

$$\vec{d}(t) = \vec{p}(t) - \vec{p}(1)$$

#### Cells - Cell Displacement ^2

The square of the distance of the object from the starting point of the track (also known as mean square displacement: MSD) when plotted against the relative time since the beginning of the track

$$d^2(t) = |\vec{d}(t)|^2, \text{ with } \vec{d}(t) \text{ as defined in Displacement X Y Z}$$

#### Cells- Cell Distance from Vesicle to Cell Membrane Mean

The mean distance, calculated by the distance map method, between the Vesicle's centers and the Cell surface border.

#### Cells- Cell Distance from Vesicle to Cell Membrane Std.Dev.

This value describes the Standard Deviation of the distance between the Vesicle's centers and the Cell surface border.

#### Cells - Cell Distance from Vesicle to Closest Nucleus Mean

The mean distance, calculated by the distance map method, between the Vesicle's centers and the Nucleus surface border within the Cell object.

#### Cells- Cell Distance from Vesicle to Closest Nucleus Std.Dev.

This value describes the Standard Deviation of the distance between the Vesicle's centers and the Nucleus surface border within the Cell object.

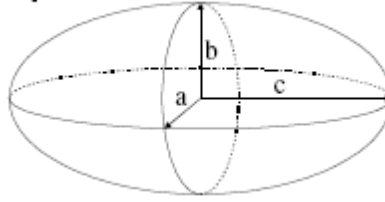
#### Cells- Cell Distance to Image Border X, Y

#### Cells- Cell Distance to Image Border X, Y, Z

The value specifies the inward distances from the image border to be removed.

### Ellipsoid Axis

### Ellipsoid



#### Cells - Cell Ellipsoid Axis A X, Y, Z

These three parameters define the Vector of the Ellipsoid Axis a.

#### Cells - Cell Ellipsoid Axis B X, Y, Z

These three parameters define the Vector of the Ellipsoid Axis b.

#### Cells - Cell Ellipsoid Axis C X, Y, Z

These three parameters define the Vector of the Ellipsoid Axis c.

#### Cells - Cell Ellipsoid Axis Length A, B, C

##### Cells - Cell Ellipsoid (oblate)

##### Cells - Cell Ellipsoid (prolate)

If:

$a \leq b \leq c$ , then when:

- $a = 0$  it is an **Ellipse**
- $a = b = c$  it is a **Sphere** (three equal sides)
- $a \neq b \neq c$  it is a **scalene Ellipsoid** (three unequal sides)

If two of these sides are equal, the Ellipsoid is a **Spheroid**:

- $a = b < c$  it is a prolate Spheroid (cigar-shaped)
- $a < b = c$  it is an oblate Spheroid (disk-shaped)

$$e_{prolate} = \frac{2a^2}{a^2 + b^2} \cdot \left( 1 - \frac{a^2 + b^2}{2c^2} \right)$$

$e_{prolate}$  = prolate Ellipsoid

$$e_{oblate} = \frac{2b^2}{b^2 + c^2} \cdot \left( 1 - \frac{2a^2}{b^2 + c^2} \right)$$

$e_{oblate}$  = oblate Ellipsoid

**Cells - Cell Intensity Center****Cells - Cell Intensity Max****Cells - Cell Intensity Mean****Cells - Cell Intensity Median**

The Cells - intensity median is estimated from the intensity histogram.

**Cells - Cell Intensity Min****Cells - Cell Intensity StdDev****Cells - Cell Intensity Sum**

The voxel intensity statistics describe the voxels enclosed within the Cell (Center, Max, Mean, Median, Min, StdDev and Sum).

**Cells - Cell Intensity Min of Vesicles**

This value describes the minimum voxel intensity of the Vesicles within the Cell object.

**Cells - Cell Intensity Max of Vesicles**

This value describes the maximum voxel intensity of the Vesicles within the Cell object.

**Cells - Cell Intensity mean of Vesicles**

This value describes the mean voxel intensity of the Vesicles within the Cell object.

**Cells - Cell Nucleus to Cytoplasm Volume Ratio**

The value is defined as the ratio between Nucleus volume and Cell cytoplasm volume. The Cell cytoplasm volume is calculated by subtracting the volume of the Nuclei from the whole cell object volume.

**Cells - Cell Number of Nucleus**

The number of the Nucleus objects within the selected Cell object.

**Cells - Cell Number of Vesicles**

The number of the Vesicle objects within the selected Cell object.

**Cells- Cell Number of Tracks**

This value describes the total number of the Cell's object tracks.

**Cells - Cell Number of Voxels**

Number of voxels within the selected Cell.

**Cells - Cell Position X, Y, Z**

Defines the value of X, Y, Z Cell position (x y z).It is equal to Center of Homogeneous Mass of the object

**Cells - Cell Relative Vesicle Position Min****Cells - Cell Relative Vesicle Position Mean****Cells - Cell Relative Vesicle Position Max**

This parameter provides Min Max and Mean values of all Vesicle Relative Positions within the cell object. This value describes the Vesicle position within the cell relative to the Nucleus and Cell object's borders.

Vesicle Relative positions is the ratio between Vesicle distance from the Nucleus border and the sum of Vesicle's distances from the Nucleus and Cell border.

If the Vesicle is located within the Nucleus the value is set to 0. If the value is 1 then the Vesicle is located at the border of Cell object.

**Cells - Cell Speed**

The Cell speed is the instantaneous speed of the object (um/s).

The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

**Cells - Cell Sphericity**

Sphericity is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity,  $\Psi$ , of a particle is the ratio of the Cell surface area of a sphere (with the same volume as the given particle) to the Cell surface area of the particle:

$$\Psi = \frac{\pi^{1/3} (6V_p)^{2/3}}{A_p}$$

**V<sub>p</sub>** = volume of the particle

**A<sub>p</sub>** = surface area of the particle

**Cells- Cell Time**

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

**Cells- Cell Time Index**

The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

**Cells - Cell Total Number of Voxels**

Total number of voxels within the data set for the cell.

**Cells- Cell Track AR1Mean**

TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

**Note for all track statistics, splits or gaps are not taken into account- it is taken that the selected track is continuous in nature.**

**Cells- Cell Track AR1 X, Y, Z**

TrackAR1X is the AR1 coefficient of the model for the x-positions.

For more information, please visit: [http://en.wikipedia.org/wiki/Autoregressive\\_model](http://en.wikipedia.org/wiki/Autoregressive_model)

$$AR1X = \frac{R_x^1}{R_x^0}$$



**AR1X** = TrackAR1X

$$R_x^n = \sum_{t=t_F-1}^{t_L-n} (D_x(t, t-1) - \bar{D}_x) \cdot (D_x(t+n, t+(n-1)) - \bar{D}_x)$$

$t_L$  = last time index of track

$t_F$  = first time index of track

$$D_X(t_F, t_L) = P_X(t_L) - P_X(t_F)$$

$P_X(t)$  = x-position of object at time index t

$$\bar{D}_X = \frac{1}{(t_L - t_F)} \sum_{t=t_F+1}^{t_L} D_x(t, t-1)$$

$t_L$  = last time index of track

$t_F$  = first time index of track

#### Cells-Cell Track Area Mean

The mean of the triangle Cell object surfaces along the track.

#### Cells - Cell Track Center of Homogeneous Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The algorithm assumes that every voxel has the same intensity (voxel intensities are homogenous).

Assuming a homogeneous intensity, the center of homogeneous mass is the X, Y, Z coordinate of the object's centroid (geometric center) .

It is the location where all of the mass of the system could be considered to be located. At the center of mass, the entire mass of the body could be replaced with a point mass equal to the body's mass. It is equivalent to the concept of centre of gravity for a body, where the intensity value is a substitute for mass.

The center of mass of a solid body does not have to lie within the body. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{i \in \text{object}} r_i$$

**R** : center of homogeneous mass (x, y, z)

$r_i$  : center of a voxel ( $x_i, y_i, z_i$ )

**n** : number of  $i \in \text{Surfaces}$

#### Cells - Cell Track Center of Image Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The Center of Homogeneous Mass calculations do not consider a variation of the voxels intensity of the inside the object. In the case of an inhomogeneous distribution of the voxel intensity inside the objects, intensity-based measure may be needed.

The center of mass is calculated using a contribution of each channel and each voxel of the object, based on each voxel intensity value.

Its positions depend on respective intensities of each voxel in the volume.

The Center of Image Mass is the object centroid calculated by taking into account the voxel intensities of each channel separately.

The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{ie\ object} m_i \times r_i$$

**R** : center of image mass (x, y, z)

**m<sub>i</sub>** : voxel intensity

**r<sub>i</sub>** : center of a voxel (x<sub>i</sub>, y<sub>i</sub>, z<sub>i</sub>)

**M** : sum of voxel intensities

### Cells - Cell Track Cytoplasm Intensity Max

This value describes the maximum voxel intensity of the Cytoplasm along the tracks.

### Cells - Cell Track Cytoplasm Intensity Mean

This value describes the mean voxel intensity of the Cytoplasm along the tracks.

### Cells - Cell Track Cytoplasm Intensity Min

This is the minimum value of all minimum (voxel) intensities of the cells along the tracks

### Cells - Cell Track Cytoplasm Intensity Sum

This value describes the sum of voxel intensity of the Cytoplasm along the tracks.

### Cells - Cell Track Cytoplasm Number of Vesicles Mean

The mean number of Vesicle objects along the track within the Cytoplasm.

### Cells - Cell Track Displacement Length

The Track Displacement Length is the distance between the first and last Cell's position.

$$tdl = |\vec{tdl}|, \text{ with } \vec{tdl} \text{ defined in Track Displacement X, Y, Z}$$

### Cells - Cell Track Displacement X, Y, Z

The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

$$\vec{tdl} = \vec{p}(n) - \vec{p}(1)$$

### Cells - Cell Displacement Delta XYZ

The difference in position of the current object and the previous object of the same track.



**Cells - Cell Displacement Delta Length (a scalar value)**

The length of the position difference of the current object and the previous object of the same track.

**Cells - Cell Track Duration**

The Track Duration is the duration between the first and last time point within the track.

$$td = T(n) - T(1)$$

**Cells - Cell Track Ellipsoid Axis A X, Y, Z Mean**

These three parameters define the Vector of the track Ellipsoid Axis a.

**Cells - Cell Track Ellipsoid Axis B X, Y, Z Mean**

These three parameters define the Vector of the track Ellipsoid Axis b.

**Cells - Cell Track Ellipsoid Axis C X, Y, Z Mean**

These three parameters define the Vector of the track Ellipsoid Axis c.

**Cells - Cell Track Ellipsoid Axis Length A, B, C Mean**

These three parameters define the length of the Ellipsoid Axis a, b, and c.

**Cells - Cell Track Ellipsoid Oblate A Mean**

This parameter defines the mean Ellipsoid Oblate for the track (see above for the detailed oblate definition).

**Cells - Cell Track Ellipsoid Prolate A Mean**

This parameter defines the mean Ellipsoid Prolate for the track (see above for the detailed prolate definition).

**Cells - Cell Track Intensity Center Mean**

This value describes the mean voxel intensity in the center of the Cell along the tracks.

**Cells - Cell Track Intensity Max**

This is the maximum value of all maximum (voxel) intensities of the Cell along the tracks

**Cells - Cell Track Intensity Mean**

This is the weighted mean of the objects means, equal to the simple mean of all the intensities of all the objects in a track. Larger objects have a larger impact on the track mean.

**Cells - Cell Track Intensity Median**

The median intensity for a track is calculated as the (approximated) median of the (approximated) medians of its objects, instead of the true median of all the intensities in the track, for performance reasons.

**Cells - Cell Track Intensity Min**

This is the minimum value of all minimum (voxel) intensities of the Cell along the tracks.

**Cells - Cell Track Intensity StdDev**

This value describes the Standard Deviation voxel intensity of the Cell along the tracks.

**Cells - Cell Track Intensity Sum**

This value is the sum of all sum (voxel) intensities of the Cell along the tracks.

**Cells - Cell Track Length**

The Track Length is the total length of displacements within the track.

$$tl = \sum_{t=2}^n |\vec{p}(t) - \vec{p}(t - 1)|$$

**Note for all track statistics, splits or gaps are not taken into account- it is taken that the selected track is continuous in nature.**

### **Cells- Cell Track Number of Nuclei Mean**

The mean number of Nuclei objects along the track.

### **Cells-Cell Track Number of Vesicle Mean**

The mean number of Vesicle objects along the track.

### **Cells - VesicleTrack Number of Branches**

The number of branches along the track.

### **Cells - Vesicle Track Number of Fusions**

The number of fusions along the track.

### **Cells - Cell Tracks Position Mean X, Y, Z**

Defines the mean value of X, Y, Z track positions.

### **Cells - Cell Tracks Position X, Y, Z Start**

Defines the value of the X, Y, Z track start positions.

### **Cells - Cell Track Speed Max**

The maximum value of the object's speed on the track.

### **Cells - Cell Track Speed Mean**

The Average value of the object's speed on the track. If the Track has no Fuse (merge) or Fragments (splits), then the average speed is given by the Track length divided by the time between first and last object in the Track.

### **Cells - Cell Track Speed Min**

The minimum value of the object's speed on the track.

### **Cells - Cell Track Speed Standard Deviation**

The Standard Deviation value of the object's speed on the track.

### **Cells - Cell Track Speed Variation**

The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value. The coefficient of variation is a dimensionless number.

$$v = \frac{\sigma}{\mu}$$

$\sigma$ - Standard Deviation

$\mu$ -Mean value

### **Cells-Cell Track Sphericity Mean**



Describes the mean Cell object Sphericity along the tracks.

### Cells - Cell Track Straightness

$$S = \frac{D}{L}$$

**S** = TrackStraightness

**D** = TrackDisplacement

**L** = TrackLength

### Cells - Cell Track Volume Mean

The mean volume of all Cell objects along the tracks.

**Note for all track statistics splits or gaps are not taken into account- it is taken that the selected track is continuous in nature.**

### Cells - Cell Velocity X, Y, Z

The formulas for calculating the velocity at time points (t) and (1) are calculated as follows:

$$\vec{v}(t) = \frac{\vec{p}(t+1) - \vec{p}(t-1)}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$\vec{v}(1) = \frac{\vec{p}(2) - \vec{p}(1)}{T(2) - T(1)} \text{ and } \vec{v}(n) = \frac{\vec{p}(n) - \vec{p}(n-1)}{T(n) - T(n-1)}$$

### Cells - Cell Volume

Volume is quantification of how much space a Cell object occupies.

### Cells - Cell Volume of Vesicles

Volume is quantification of how much space the Vesicles object occupies within the Cell.

### Cells- Nucleus Acceleration

Acceleration is the change in the Nucleus velocity over time.

Acceleration X, Y, Z are the components of the acceleration vector (at time-point t):

$$\vec{a}(t) = \left( \frac{\vec{p}(t+1) - \vec{p}(t)}{T(t+1) - T(t)} - \frac{\vec{p}(t) - \vec{p}(t-1)}{T(t) - T(t-1)} \right) / \left( \frac{T(t+1) - T(t-1)}{2} \right), \text{ for } 1 < t < n$$

$$\vec{a}(1) = \vec{a}(n) = \vec{0}$$

Acceleration at time-point t:

$$a(t) = |\vec{a}(t)|, \text{ with } \vec{a}(t) \text{ as defined in Acceleration X, Y, Z}$$

Acceleration at the first and last time-point is 0.

### Cells - Nucleus Acceleration X, Y, Z

Acceleration is the change in the Nucleus velocity over time along the selected-axis.

## Cells - Nucleus Area

The area is the sum of the outer Nucleus voxel faces.

## Cells - Nucleus BoundingBoxAA Length X, Y, Z

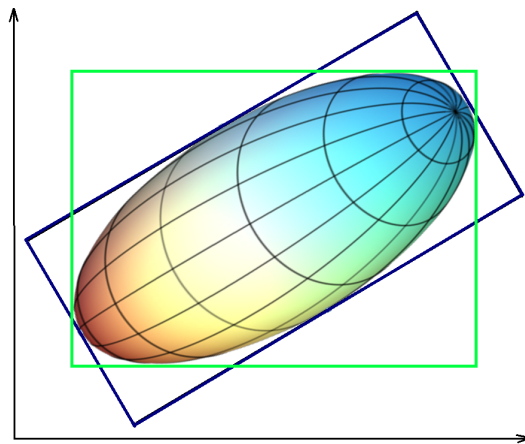
A bounding box is a rectangular cuboid in 3D, or a rectangle in 2D, containing an object.

If the bounding box is aligned with the axes of the coordinate system, it is known as an axis-aligned bounding box (AA). BoundingBoxAA identifies an object by considering the minimal rectangular box which fully encloses the object, whose faces are aligned parallel to the axes of a coordinate system.

BoundingBoxAA Length X =  $\max(X) - \min(X)$

BoundingBoxAA Length Y =  $\max(Y) - \min(Y)$

BoundingBoxAA Length Z =  $\max(Z) - \min(Z)$



axis-aligned bounding box (AA) shown in green  
object-oriented bounding box (OO) shown in blue

**Please note the difference between the ellipsoid axis length and bounding box length.**

## Cells - Nucleus BoundingBoxOO Length A, B, C

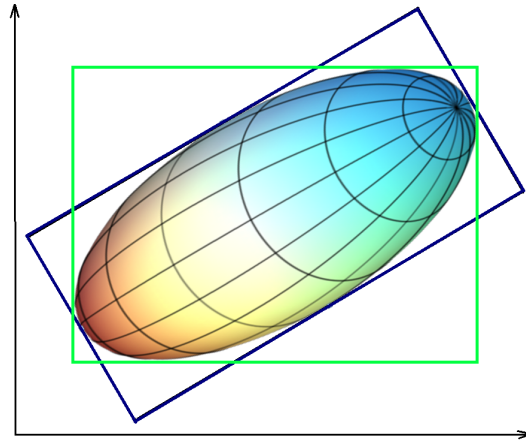
The local axes of the box can be arbitrarily oriented with respect to the coordinate system.

BoundingBoxOO (object oriented) identifies an object by considering the minimal rectangular box, which fully encloses the object. The box dimensions are calculated with no constraints to the orientation.

BoundingBoxOO Length A = Length of the shortest principal axis

BoundingBoxOO Length B = Length of the second longest principal axis

BoundingBoxOO Length C = Length of the longest principal axis



axis-aligned bounding box (AA) shown in green  
 object-oriented bounding box (OO) shown in blue

**Please note the difference between the ellipsoid axis length and bounding box length.**

### Cells - Nucleus Center of Homogeneous Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The algorithm assumes that every voxel has the same intensity (voxel intensities are homogenous).

Assuming a homogeneous intensity, the center of homogeneous mass is the X, Y, Z coordinate of the object's centroid (geometric center) .

It is the location where all of the mass of the system could be considered to be located. At the center of mass, the entire mass of the body could be replaced with a point mass equal to the body's mass. It is equivalent to the concept of centre of gravity for a body, where the intensity value is a substitute for mass.

The center of mass of a solid body does not have to lie within the body. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{i \in \text{object}} r_i$$

**R** : center of homogeneous mass (x, y, z)

**r<sub>i</sub>** : center of a voxel (x<sub>i</sub> y<sub>i</sub> z<sub>i</sub>)

**n** : number of  $i \in \text{Nucleus}$

### Cells - Nucleus Center of Image Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The Center of Homogeneous Mass calculations do not consider a variation of the voxels intensity of the inside the object. In the case of an inhomogeneous distribution of the voxel intensity inside the objects, intensity-based measure may be needed.

The center of mass is calculated using a contribution of each channel and each voxel of the object, based on each voxel intensity value.

Its positions depend on respective intensities of each voxel in the volume.

The Center of Image Mass is the object centroid calculated by taking into account the voxel intensities of each channel separately.

The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{ie \text{ object}} m_i \times r_i$$

**R** : center of image mass (x y z)

**m<sub>i</sub>** : voxel intensity

**r<sub>i</sub>** : center of a voxel (x<sub>i</sub> y<sub>i</sub> z<sub>i</sub>)

**M** : sum of voxel intensities

### Cells - Nucleus Displacement X, Y, Z

The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

$$\vec{d}(t) = \vec{p}(t) - \vec{p}(1)$$

### Cells - Nucleus Displacement<sup>2</sup>

$$d^2(t) = |\vec{d}(t)|^2, \text{ with } \vec{d}(t) \text{ as defined in Displacement X Y Z}$$

### Cells - Nucleus Displacement Delta XYZ

The difference in position of the current object and the previous object of the same track.

### Cells - Nucleus Displacement Delta Length (a scalar value)

The length of the position difference of the current object and the previous object of the same track.

### Cells - Nucleus from Center of Mass to Cell Membrane

The minimum distance between the Nucleus centers of mass and the Cell surface border.

### Cells - Nucleus from Nucleus Membrane to Cell Membrane

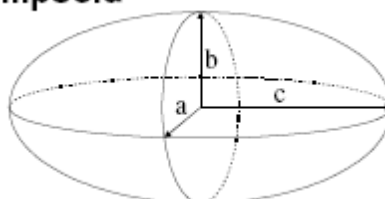
The minimum distance between the Nucleus membrane surface and the Cell surface border.

### Cells - Nucleus from Position to Cell Membrane

The minimum distance between the Nucleus centers of homogeneous mass and the Cell surface border.

## Ellipsoid Axis

Ellipsoid



**Cells - Nucleus Ellipsoid Axis A X, Y, Z**

These three parameters define the Vector of the Ellipsoid Axis a.

**Cells - Nucleus Ellipsoid Axis B X, Y, Z**

These three parameters define the Vector of the Ellipsoid Axis b.

**Cells - Nucleus Ellipsoid Axis C X, Y, Z**

These three parameters define the Vector of the Ellipsoid Axis c.

**Cells - Nucleus Ellipsoid Axis Length A, B, C****Cells - Nucleus Ellipsoid (oblate)****Cells - Nucleus Ellipsoid (prolate)**

If we assume  $a \leq b \leq c$ , then when:

- $a = 0$  it is an **Ellipse**
- $a = b = c$  it is a **Sphere** (three equal sides)
- $a \neq b \neq c$  it is a **scalene Ellipsoid** (three unequal sides)

If two of these sides are equal, the Ellipsoid is a **Spheroid**:

- $a = b < c$  it is a prolate Spheroid (cigar-shaped)
- $a < b = c$  it is an oblate Spheroid (disk-shaped)

$$e_{prolate} = \frac{2a^2}{a^2 + b^2} \cdot \left( 1 - \frac{a^2 + b^2}{2c^2} \right)$$

$e_{prolate}$  = prolate Ellipsoid

$$e_{oblate} = \frac{2b^2}{b^2 + c^2} \cdot \left( 1 - \frac{2a^2}{b^2 + c^2} \right)$$

$e_{oblate}$  = oblate Ellipsoid

**Cells - Nucleus Intensity Center****Cells - Nucleus Intensity Max****Cells - Nucleus Intensity Mean****Cells - Nucleus Intensity Median**

The Cells - Nucleus intensity median is estimated from the intensity histogram.

**Cells - Nucleus Intensity Min**

## Cells - Nucleus Intensity StdDev

### Cells - Nucleus Intensity Sum

The voxel intensity statistics describe the voxels enclosed within the Nucleus (Center, Max, Mean, Median, Min, StdDev and Sum).

## Cells- Nucleus Number of Tracks

This value describes the total number of the Nucleus object tracks.

## Cells- Nucleus Number of Vesicles

The number of the Vesicle objects within the selected Nucleus object.

## Cells-Nucleus Number of Voxels

Number of voxels within the selected Nucleus object.

## Cells- Nucleus Position X, Y, Z

Defines the value of X, Y, Z Nucleus position (x y z ). It is equal to Center of Homogeneous Mass of the object.

## Cells - Nucleus Relative To Cell Acceleration

Acceleration is the change in the Nucleus velocity over time taking into account the relative Nucleus position.

## Cells- Nucleus Relative To Cell Acceleration X, Y, Z

Acceleration is the change in the Nucleus velocity over time along the selected-axis, taking into account the relative Nucleus position.

## Cells - Nucleus Relative To Cell Displacement X, Y, Z

The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position. The calculation is based on the relative Nucleus position.

## Cells - Nucleus Relative To Cell Displacement<sup>2</sup>

The Nucleus squared displacement is calculated taking into account the relative Nucleus position.

$$D^{-2}(\Delta t) = \sum_{t-t_f+\Delta t}^{t_l} D_x(t, t-\Delta t)^2 + D_y(t, t-\Delta t)^2 + D_z(t, t-\Delta t)^2$$

$$D_x(t_f, t_l) = P_x(t_l) - P_x(t_f)$$

$t_l$  = last time index of track

$t_f$  = first time index of track

$P_x(t)$  = x-position of object at time index t

## Cells - Nucleus Relative To Cell Position X, Y, Z

The relative Nucleus position is calculated by subtracting the absolute position of the selected Nucleus from the absolute center position of the parent cell object.

## Cells - Nucleus Relative To Cell Speed

The speed is the instantaneous speed of the object (um/s).The instantaneous speed is the scalar equivalent of

the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

### Cells - Nucleus Relative To Cell Track Displacement Length

The Nucleus Relative To Cell Track Displacement Length is the distance between first and last Nucleus's relative position.

$$D_x(t_n, t_{n-1}) = \sqrt{D_x(t_L, t_F)^2 + D_y(t_L, t_F)^2 + D_z(t_L, t_F)^2}$$

$$D_x(t_F, t_L) = P_x(t_L) - P_x(t_F)$$

**D** = Track Displacement

**t<sub>L</sub>** = last time index of track

**t<sub>F</sub>** = first time index of track

**P<sub>x</sub>(t)** = x-position of object at time index t

### Cells - Nucleus Relative To Cell Track Displacement X, Y, Z

The object's Track Displacement is the distance between the first and last object's position along the selected-axis taking into account the relative Nucleus position.

### Cells - Nucleus Relative To Cell Velocity X, Y, Z

The instant velocity vector  $v$  of an object that has positions  $x(t)$  at time  $t$  and  $x(t + \Delta t)$  at time  $t + \Delta t$ , can be computed as the derivative of the relative Nucleus position along the selected-axis:

$$v = \lim_{\Delta t \rightarrow 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

### Cells - Nucleus Speed

The Nucleus speed is the instantaneous speed of the object (um/s). The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

**Cells - Nucleus Sphericity**

Sphericity is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity,  $\Psi$ , of a particle is the ratio of the Nucleus surface area of a sphere (with the same volume as the given particle) to the Cell surface area of the particle:

$$\Psi = \frac{\pi^{1/3} (6V_p)^{2/3}}{A_p}$$

**V<sub>p</sub>** = volume of the particle

**A<sub>p</sub>** = surface area of the particle

**Cells - Nucleus Time**

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

**Cells - Nucleus Time Index**

The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

**Cells - Nucleus Total Number of Voxels**

Total number of voxels of the Nucleus voxels within the data set.

**Cells - Nucleus Track AR1Mean**

TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

**Cells - Nucleus Track AR1 X, Y, Z**

TrackAR1X is the AR1 coefficient of the model for the x-positions.

For more information, please visit: [http://en.wikipedia.org/wiki/Autoregressive\\_model](http://en.wikipedia.org/wiki/Autoregressive_model)

$$AR1X = \frac{R_x^1}{R_x^0}$$

AR1X = TrackAR1X

$$R_x^n = \sum_{t=t_f-1}^{t_L-n} (D_x(t, t-1) - \bar{D}_x) \cdot (D_x(t+n, t+(n-1)) - \bar{D}_x)$$

**t<sub>L</sub>** = last time index of track

**t<sub>f</sub>** = first time index of track

$$D_x(t_2, t_1) = P_x(t_2) - P_x(t_1)$$



$P_x(t)$  = x-position of object at time index t

$$\bar{D}_X = \frac{1}{(t_L - t_F)} \sum_{t=t_F+1}^{t_L} D_x(t, t-1)$$

$t_L$  = last time index of track

$t_F$  = first time index of track

### Cells - Nucleus Track Area Mean

The mean of the triangle Nucleus object surfaces along the track.

### Cells - Nucleus Track Center of Homogeneous Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The algorithm assumes that every voxel has the same intensity (voxel intensities are homogenous).

Assuming a homogeneous intensity, the center of homogeneous mass is the X, Y, Z coordinate of the object's centroid (geometric center) .

It is the location where all of the mass of the system could be considered to be located. At the center of mass, the entire mass of the body could be replaced with a point mass equal to the body's mass. It is equivalent to the concept of centre of gravity for a body, where the intensity value is a substitute for mass.

The center of mass of a solid body does not have to lie within the body. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{i \in \text{object}} r_i$$

$R$  : center of homogeneous mass (x y z)

$r_i$  : center of a voxel ( $x_i, y_i, z_i$ )

$n$  : number of  $i \in \text{Nucleus}$

### Cells - Nucleus Track Center of Image Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The Center of Homogeneous Mass calculations do not consider a variation of the voxels intensity of the inside the object. In the case of an inhomogeneous distribution of the voxel intensity inside the objects, intensity-based measure may be needed.

The center of mass is calculated using a contribution of each channel and each voxel of the object, based on each voxel intensity value.

Its positions depend on respective intensities of each voxel in the volume.

The Center of Image Mass is the object centroid calculated by taking into account the voxel intensities of each channel separately.

The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{ie \text{ object}} m_i \times r_i$$

**R** : center of image mass (x, y, z)

**m<sub>i</sub>** : voxel intensity

**r<sub>i</sub>** : center of a voxel (x<sub>i</sub> y<sub>i</sub> z<sub>i</sub>)

**M** : sum of voxel intensities

### Cells - Nucleus Track Displacement Length

The Track Displacement Length is the distance between first and last Nucleus position.

$$tdl = |\vec{tdl}|, \text{ with } \vec{tdl} \text{ defined in Track Displacement X, Y, Z}$$

### Cells - Nucleus Track Displacement X, Y, Z

The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

### Cells - Nucleus Track Duration

The Track Duration is the duration between the first and last time point within the track.

$$td = T(n) - T(1)$$

### Cells - Nucleus Track Ellipsoid Axis A X, Y, Z Mean

These three parameters define the Vector of the track Ellipsoid Axis a.

### Cells - Nucleus Track Ellipsoid Axis B X, Y, Z Mean

These three parameters define the Vector of the track Ellipsoid Axis b.

### Cells - Nucleus Track Ellipsoid Axis C X, Y, Z Mean

These three parameters define the Vector of the track Ellipsoid Axis c.

### Cells - Nucleus Track Ellipsoid Axis Length A, B, C Mean

These three parameters define the length of the Ellipsoid Axis a, b, and c.

### Cells - Nucleus Track Ellipsoid Oblate A Mean

This parameter defines the mean Ellipsoid Oblate for the track (see above for the detailed oblate definition).

### Cells - Nucleus Track Ellipsoid Prolate A Mean

This parameter defines the mean Ellipsoid Prolate for the track (see above for the detailed prolate definition).

### Cells- Nucleus Track Intensity Center Mean

This value describes the mean voxel intensity in the center of the Nucleus along the tracks.

### Cells- Nucleus Track Intensity Max

This value describes the maximum voxel intensity of the Nucleus along the tracks.

**Cells - Nucleus Track Intensity Mean**

This value describes the mean voxel intensity of the Nucleus along the tracks.

**Cells - Nucleus Track Intensity Median**

This value describes the Average of all Intensity Medians of all objects at all time points in the track.

**Cells - Nucleus Track Intensity Min**

This value describes the minimum voxel intensity of the Nucleus along the tracks.

**Cells - Nucleus Track Intensity StdDev**

This value describes the Standard Deviation voxel intensity of the Nucleus along the tracks.

**Cells - Nucleus Track Intensity Sum**

This value describes the sum of voxel intensity of the Nucleus along the tracks.

**Cells - Nucleus Track Length**

The Track Length is the total length of displacements within the track.

$$tl = \sum_{t=2}^n |\vec{p}(t) - \vec{p}(t-1)|$$

**Cells - Nucleus Track Number of Branches**

The number of track branches.

**Cells - Nucleus Track Number of Fusions**

The number of track fusions.

**Cells- Track Number of Nucleus**

The number of the all Nucleus objects along the tracks.

**Cells - Nucleus Tracks Position Mean X, Y, Z**

Defines the mean value of X, Y, Z Nucleus track positions.

**Cells - Nucleus Tracks Position X, Y, Z Start**

Defines the value of the X, Y, Z track start positions.

**Cells - Nucleus Track Speed Max**

The maximum value of the object's speed on the track.

**Cells - Nucleus Track Speed Mean**

The Average value of the object's speed on the track. If the Track has no fuses(merges) or fragments(splits), then the average speed is given by the Track length divided by the time between first and last object in the Track.

**Cells - Nucleus Track Speed Min**

The minimum value of the object's speed on the track.

**Cells - Nucleus Track Speed Standard Deviation**

The Standard Deviation value of the object's speed on the track.

**Cells - Nucleus Track Speed Variation**

The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value. The coefficient of variation is a dimensionless number.

$$v = \frac{\sigma}{\mu}$$

$\sigma$ - Standard Deviation

$\mu$ -Mean value

### Cells- Nucleus Track Sphericity Mean

Describes the mean Nucleus object Sphericity along the tracks.

### Cells - Nucleus Track Straightness

$$S = \frac{D}{L}$$

**S** = TrackStraightness

**D** = TrackDisplacement

**L** = TrackLength

### Cells- Nucleus Track Volume Mean

The mean volume of all Nucleus objects along the tracks.

### Cells - Nucleus Velocity X, Y, Z

The formulas for calculating the velocity at time points (t) and (1) are calculated as follows:

$$\vec{v}(t) = \frac{\vec{p}(t+1) - \vec{p}(t-1)}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$\vec{v}(1) = \frac{\vec{p}(2) - \vec{p}(1)}{T(2) - T(1)} \text{ and } \vec{v}(n) = \frac{\vec{p}(n) - \vec{p}(n-1)}{T(n) - T(n-1)}$$

### Cells - Nucleus Volume

Volume is quantification of how much space a Nucleus objects occupies.

### Cells- Number of Cell per Time Point

This value describes the number of Cell object in the selected time point.

### Cells- Number of Nuclei per Time Point

This value describes the number of Nuclei object in the selected time point.

### Cells- Number of Vesicles per Time Point

This value describes the number of Vesicles object in the selected time point.

### Cells- Total Number of Cell

This value describes the total number of Cell objects.

### Cells - Total Number of Nucleus

This value describes the total number of Nucleus objects.

**Cells - Total Number of Vesicles**

This value describes the total number of Vesicles objects.

**Cells- Cell Track Number of Cells**

The number of the all Cell objects along the tracks.

**Cells- Track Number of Nuclei**

The number of the all Nucleus objects along the tracks.

**Cells- Track Number of Vesicles**

The number of the all Vesicles objects along the tracks.

**Cells - Vesicle Acceleration**

Acceleration is the change in the Vesicle velocity over time.

$$\vec{a}(t) = \left( \frac{\vec{p}(t+1) - \vec{p}(t)}{T(t+1) - T(t)} - \frac{\vec{p}(t) - \vec{p}(t-1)}{T(t) - T(t-1)} \right) / \left( \frac{T(t+1) - T(t-1)}{2} \right), \text{ for } 1 < t < n$$

$$\vec{a}(1) = \vec{a}(n) = \vec{0}$$

Acceleration at time-point t:

$$a(t) = |\vec{a}(t)|, \text{ with } \vec{a}(t) \text{ as defined in Acceleration X, Y, Z}$$

Acceleration at the first and last time-point is 0.

**Cells - Vesicle Acceleration X, Y, Z**

Acceleration is the change in the Vesicle velocity over time along the selected-axis.

**Cells- Vesicle Area**

The area is the sum of the outer Vesicles voxel faces.

**Cells- Vesicle Diameter X, Y, Z**

This value describes the Vesicle diameter along the selected-axis.

**Cells - Vesicles Displacement X, Y, Z**

The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

$$\vec{d}(t) = \vec{p}(t) - \vec{p}(1)$$

**Cells - Vesicles Displacement^2**

$$d^2(t) = |\vec{d}(t)|^2, \text{ with } \vec{d}(t) \text{ as defined in Displacement X Y Z}$$

**Cells - Vesicles Displacement Delta XYZ**

The difference in position of the current object and the previous object of the same track.

### **Cells - Vesicles Displacement Delta Length (a scalar value)**

The length of the position difference of the current object and the previous object of the same track.

### **Cells- Vesicle Distance to Cell Membrane**

The distance, calculated by the distance map method, between the selected Vesicle centers and the Cell surface border.

### **Cells- Vesicle Distance to Closest Nucleus**

The distance, calculated by the distance map method, between the selected Vesicle center and the closest Nucleus surface border. For Vesicles located inside a Nucleus, this distance is a negative number.

### **Cells- Vesicle Distance to Closest Nucleus Center**

The distance between the selected Vesicle center and the center of the closest Nucleus. For Vesicles located inside a Nucleus, this distance is a negative number.

### **Cells- Vesicle Intensity Center**

### **Cells- Vesicle Intensity Max**

### **Cells- Vesicle Intensity Mean**

### **Cells- Vesicle Intensity Median**

The Cells- vesicle intensity median is estimated from the intensity histogram.

### **Cells- Vesicle Intensity Min**

### **Cells- Vesicle Intensity StdDev**

### **Cells- Vesicle Intensity Sum**

The voxel intensity statistics describe the voxels enclosed within the Vesicle (Center, Max, Mean, Median, Min, StdDev and Sum).

### **Cells - Vesicle Location in Cytoplasm**

The statistical number either 0 or 1 describing the vesicle parent object. If the value is set to 1 vesicle is located within the cytoplasm, while if the value is 0 the vesicle is located within the nucleus (the value of **Vesicle location in Nucleus** is set to 1).

### **Cells - Vesicle Location in Nucleus**

The statistical number either 0 or 1 describing the vesicle parent object. If the value is set to 1 vesicle is located within the nucleus, while if the value is 0 the vesicle is located outside the nucleus (within the cytoplasm).

### **Cells - Vesicle Number of Tracks**

This value describes the total number of the Vesicle tracks.

### **Cells - Vesicle Number of Voxels**

Number of voxels within the selected Vesicle object.

### **Cells- Vesicle Position X, Y, Z**

Defines the value of X, Y and Z Vesicle position (x y z). It is equal to Center of Homogeneous Mass of the object.

### **Cells- Vesicle Relative Position**

This value describes the Vesicle position within the cell relative to the Nucleus and Cell object's borders.

The value is the ratio between Vesicle distance from the Nucleus border and the sum of Vesicle's distances from the Nucleus and Cell border.

**Cell - Vesicle Relative To Cell Acceleration**

Acceleration is the change in the Vesicles velocity over time taking into account the relative vesicle position.

**Cell - Vesicle Relative To Cell Acceleration X, Y, Z**

Acceleration is the change in the Vesicles velocity over time along the selected-axis, taking into account the relative vesicle position.

**Cells - Vesicle Number of Cell per Time Point**

This value describes the number of Vesicle object in the selected time point.

**Cell - Vesicle Relative To Cell Displacement X, Y, Z**

The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position. The calculation takes into account the relative Vesicle position.

**Cell - Vesicle Relative To Cell Displacement<sup>2</sup>**

The vesicles squared displacement is calculated taking into account the relative vesicle position.

$$D^2(\Delta t) = \sum_{t=t_r+\Delta t}^{t_l} D_x(t, t - \Delta t)^2 + D_y(t, t - \Delta t)^2 + D_z(t, t - \Delta t)^2$$

$$D_x(t_2, t_1) = P_x(t_2) - P_x(t_1)$$

$t_L$  = last time index of track

$t_F$  = first time index of track

$P_x(t)$  = x-position of object at time index t

**Cell - Vesicle Relative To Cell Position X, Y and Z**

The relative Vesicles position is calculated by subtracting the absolute position of the selected Vesicles from the absolute center position of the parent cell object.

**Cell - Vesicle Relative To Cell Speed**

The speed is the instantaneous speed of the object (um/s). The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

**Cells - Vesicle Relative To Cell Track Displacement Length**

The Vesicle Relative To Cell Track Displacement Length is the distance between first and last Vesicle's relative position.

$$D_x(t_n, t_{n-1}) = \sqrt{D_x(t_L, t_F)^2 + D_y(t_L, t_F)^2 + D_z(t_L, t_F)^2}$$

$$D_x(t_1, t_2) = P_n(t_n) - P_n(t_{n-1})$$

**D** = TrackDisplacement

**t<sub>L</sub>** = last time index of track

**t<sub>F</sub>** = first time index of track

**P<sub>X</sub>(t)** = x-position of object at time index t

**Cell - Vesicle Relative To Cell Track Displacement X, Y, Z**

The object's Track Displacement is the distance between the first and last object's position along the selected-axis taking into account the relative Vesicle position.

**Cell - Vesicle Relative To Cell Velocity X, Y, Z**

The instant velocity vector v of an object that has positions x(t) at time t and x(t + Dt) at time t + Δt, can be computed as the derivative of the relative vesicle position along the selected-axis:

$$v = \lim_{\Delta t \rightarrow 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

**Cells - Vesicle Speed**

The Vesicle speed is the instantaneous speed of the object (um/s).The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$



**Cells- Vesicle Time**

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

**Cells- Vesicle Time Index**

The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

**Cells- Vesicle Track AR1Mean**

TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

**Cells- Vesicle Track AR1 X, Y, Z**

TrackAR1X is the AR1 coefficient of the model for the x-positions.

For more information, please visit: [http://en.wikipedia.org/wiki/Autoregressive\\_model](http://en.wikipedia.org/wiki/Autoregressive_model)

$$\vec{ar}1 = \frac{\sum_{t=2}^{n-1} (\vec{d}(t-1,t) - \vec{d}_{avg}) * (\vec{d}(t,t+1) - \vec{d}_{avg})}{\sum_{t=2}^n (\vec{d}(t-1,t) - \vec{d}_{avg})^2}$$

with the displacement between two time points:

$$\vec{d}(t_1, t_2) = \vec{p}(t_2) - \vec{p}(t_1)$$

and the average displacement of the entire track:

$$\vec{d}_{avg} = \sum_{t=2}^n (\vec{p}(t) - \vec{p}(t-1)) / n$$

**Cells- Vesicles Track Area Mean**

The mean area of all Vesicle objects along the tracks.

**Cells- Vesicles Track Diameter Mean**

The mean diameter of all Vesicle objects along the tracks.

**Cells- VesicleTrack Displacement Length**

The Track Displacement Length is the distance between first and last Vesicle's position.

$$tdl = |\vec{tdl}|, \text{ with } \vec{tdl} \text{ defined in Track Displacement X, Y, Z}$$

**Cells- Vesicle Track Displacement X, Y, Z**

The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

$$\vec{tdl} = \vec{p}(n) - \vec{p}(1)$$

**Cells - Vesicle Track Duration**

The Track Duration is the duration between the first and last time point within the track.

$$td = T(n) - T(1)$$

## **Cells- Vesicle Track Intensity Center Mean**

This value describes the mean voxel intensity in the center of the Vesicle along the tracks.

## **Cells- Vesicle Track Intensity Max**

This value describes the maximum voxel intensity of the Vesicle along the tracks.

## **Cells - Vesicle Track Intensity Mean**

This value describes the mean voxel intensity of the Vesicle along the tracks.

## **Cells - Vesicle Track Intensity Median**

This value describes the Average of all Intensity Medians of all objects at all time points in the track.

## **Cells - Vesicle Track Intensity Min**

This value describes the minimum voxel intensity of the Vesicle along the tracks.

## **Cells - Vesicle Track Intensity StdDev**

This value describes the Standard Deviation voxel intensity of the Vesicle along the tracks.

## **Cells - Vesicle Track Intensity Sum**

This value describes the sum of voxel intensity of the Vesicle along the tracks.

## **Cells - Vesicle Track Length**

The Track Length is the total length of displacements within the track.

$$tl = \sum_{t=2}^n |\vec{p}(t) - \vec{p}(t - 1)|$$

## **Cells - VesicleTrack Number of Branches**

The number of track branches.

## **Cells - Vesicle Track Number of Fusions**

The number of track fusions.

## **Cells- Vesicle Tracks Position Mean X, Y, Z**

Defines the mean value of X, Y, Z Vesicles track positions.

## **Cells- Vesicle Tracks Position X, Y, Z Start**

Defines the value of the X, Y, Z track start positions.

## **Cells- Track Number of Vesicle**

The number of the all Vesicles objects along the tracks.

## **Cells - Vesicle Track Speed Max**

The maximum value of the object's speed on the track.

## **Cells - Vesicle Track Speed Mean**

The Average value of the object's speed on the track. If the Track has no Fuses (merges) or fragments (splits), then the average speed is given by the Track length divided by the time between first and last object in the Track.

**Cells - Vesicle Track Speed Min**

The minimum value of the object's speed on the track.

**Cells - Vesicle Track Speed Standard Deviation**

The Standard Deviation value of the object's speed on the track.

**Cells - Vesicle Track Speed Variation**

The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value. The coefficient of variation is a dimensionless number.

$$v = \frac{\sigma}{\mu}$$

$\sigma$ - Standard Deviation

$\mu$ -Mean value

**Cells - Vesicle Track Straightness**

$$S = \frac{D}{L}$$

**S** = TrackStraightness

**D** = TrackDisplacement

**L** = TrackLength

**Cells- Vesicles Track Volume Mean**

The mean volume of all Vesicle objects along the tracks.

**Cells - Vesicle Velocity X, Y, Z**

The instant velocity vector  $v$  of an object that has positions  $x(t)$  at time  $t$  and  $x(t + \Delta t)$  at time  $t + \Delta t$ , can be computed as the derivative of the position:

$$v = \lim_{\Delta t \rightarrow 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

**Cells- Vesicle Volume**

Volume is quantification of how much space a Vesicle objects occupy.

**Velocity Angle**

The velocity angle is the angle between the velocity vector and the corresponding axis.

**Velocity Angle X:** The angle between the velocity vector and the x-Axis (1,0,0).

**Velocity Angle Y:** The angle between the velocity vector and the y-Axis (0,1,0).

**Velocity Angle Z:** The angle between the velocity vector and the z-Axis (0,0,1).

**Lineage Statistics**

There are a number of useful statistics that are specific to the Lineage tracking algorithm:

## Generations

This shows the generation that the selected object belongs to. This is displayed in the format: 0.00 for the initial object, e.g. mother cell and 1.00 for the following 1st generation e.g. daughter cells. Tracked objects without divisions and untracked objects have a generation value of 0.

## Track Number of Generations

This statistic shows the number of generations present in the selected track.

## Time since first Division

This shows the time elapsed since the first division of the selected object(s).

## Time since previous Division

This shows the time between each generation. e.g. the lifespan of an object

## Normalized Time since previous Division

Displays the normalized division time. This value is only calculated for objects that have a previous division. Only defined for objects that have a previous division and a following division and no fuses (merges).

## Displacement Since Previous Division

This describes the displacement that has occurred since the previous division. The Displacement<sup>2</sup> (plotted over Time Since Previous Division obtains the “Displacement Squared Plot”) and its individual components X, Y and Z (which are analogous to the Position statistics). The values are available only for tracked objects after a track split. If there are multiple objects at the last time point, the most distant one (geometrically) is used for the calculation.

## Time since track start

Displays time since the start of the track. This is only available only for objects that belong to a track. It is different to time statistics for objects belonging to tracks that do not start from the first time point. It enables the user to plot the displacement<sup>2</sup> over track time start, as in the example at “Displacement Squared Plot” at [http://bradleymonk.com/Brownian\\_Motion](http://bradleymonk.com/Brownian_Motion).

## Cell Cycle Duration

Time since the previous division for objects that divide again. This is only defined for objects that divide and have a previous division.

## Cell Cycle Displacement

This option introduces multiple values. There is the Cell Cycle Displacement Length, which is equal to the square root of the Displacement Since Previous Division<sup>2</sup> for the last object of a track segment (a track segment is any piece of track that has no bifurcations, a track without bifurcations is constituted by a single segment), and there are the individual components X, Y and Z. As with Track Cycle Duration, the values are only added to the last object of a segment.

### 5.9.9.2 Filament

#### Statistic Values - Filament

A simplified representation of the dendritic tree is shown below with (•) representing a dendritic vertex, and (x) a spine vertex. Dendrite and Spine edges are represented with (—) or (---) respectively. Dendrite segments are noted with D and spine segments with S.

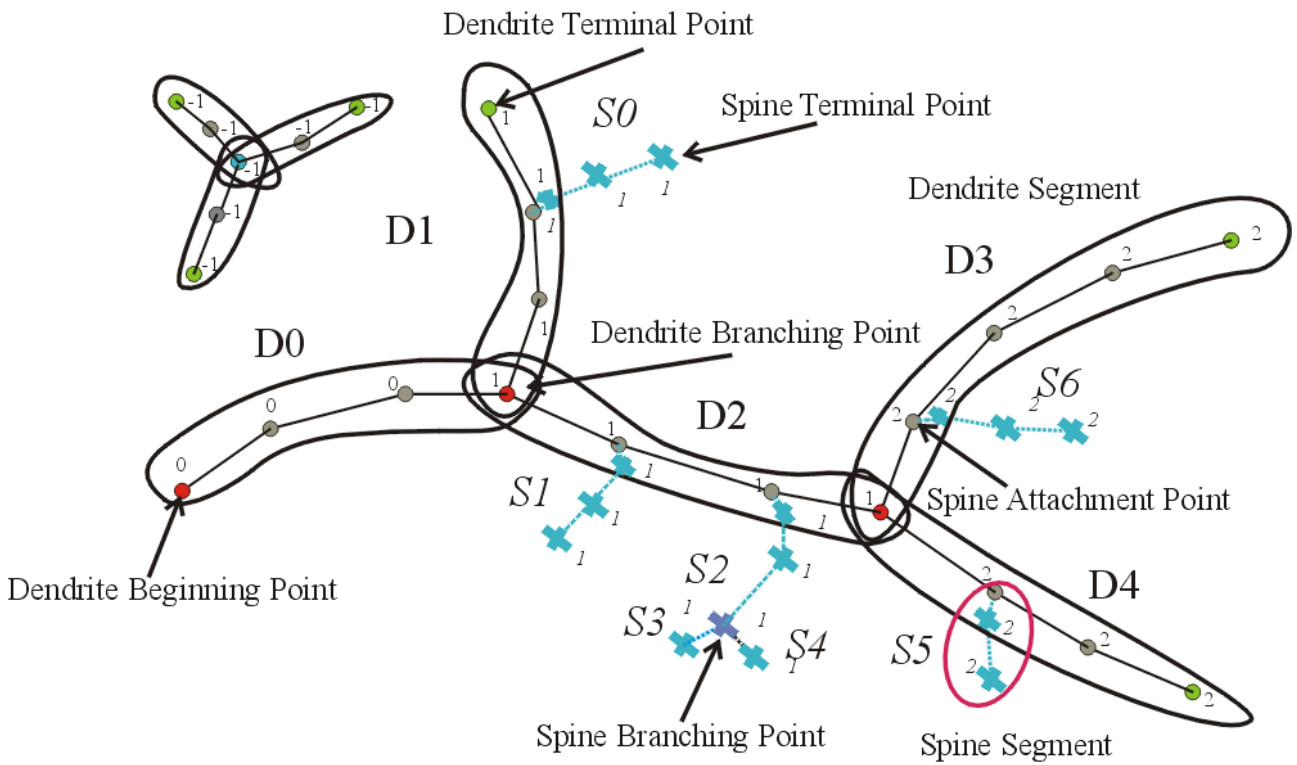


Figure 1

**Filaments - Dendrite Area**

This may be defined as the sum of the areas of all the dendrites segment edges.  
 The area of an edge is defined as a surface area of a frustum (truncated cone).

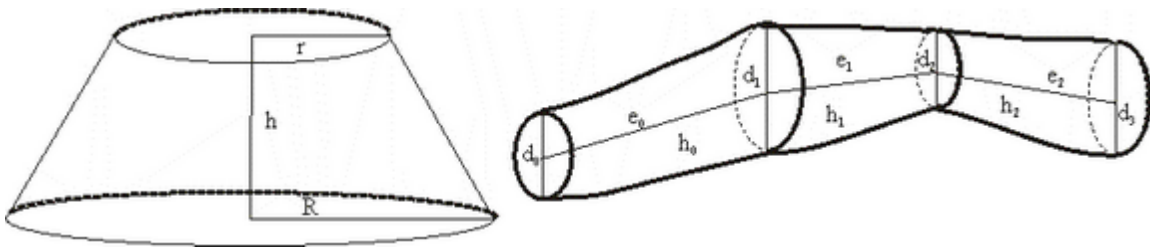


Figure 2

$$A = \sum A(i)_2, i = 0, \dots, n - 1, n$$

$n$  = number of edges of a segment

**Filaments - Dendrite Branch Depth**

Branching Depth is defined as the number of branch points, or bifurcations, in the shortest path from the beginning point to a given point in the dendritic graph.

**Filaments - Dendrite Branch Level**

The Dendrite Branch Level is a numerical structure that starts unfolding from the filament beginning toward the terminal points, assigning Branch Level to dendrite segments at each branching point.

The Branch Level number is assessed according to the diameter calculations of the individual dendrite segments. The initial Branch Level at the beginning point is 1. At each branching point, the dendrite segment with smaller mean diameter sequentially increases Branch Level, while the dendrite segment with a greater diameter maintains the same Branch Level.

In the case of two dendrite segments with the same diameter, the segment with a smaller Branching Angle (refer to Figure 4) keeps the Branch Level, while the dendrite segment with a greater Branching Angle sequentially raises its Branch Level.

In the Figure 3 diameter  $d_1$  is greater than  $d_2$  ( $d_1 > d_2$ ) and  $d_3$  and  $d_4$  are equal ( $d_3 = d_4$ ). The Dendrite Branching Angle  $\alpha_1$  is smaller than  $\alpha_2$  ( $\alpha_1 < \alpha_2$ ).

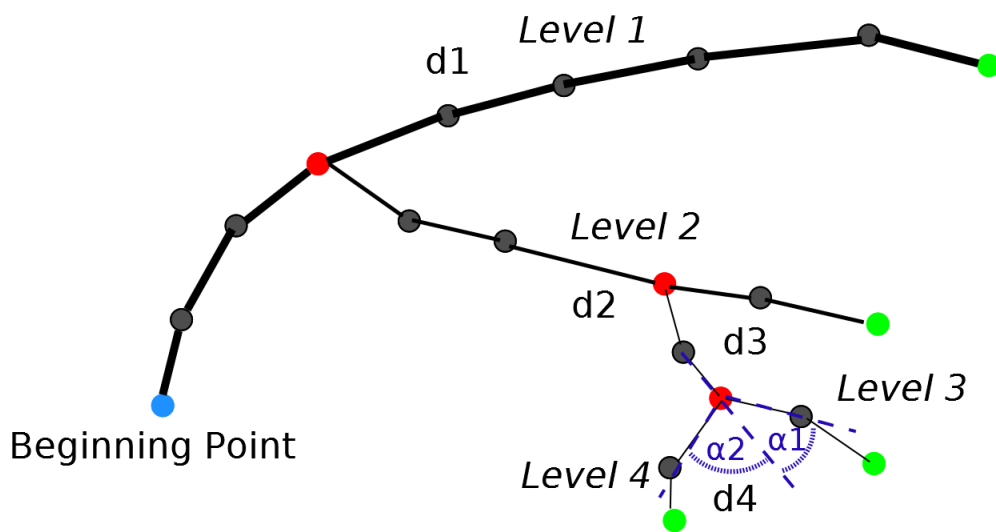


Figure 3

- represents a dendritic vertex

**Filaments - Dendrite Branching Angle**

Defined as the angle between the extending lines from the branch point and its peripheral neighbor vertex (Figure 4 angles marked with dashed blue arcs,  $\alpha_1$  for dendrite segment D1 and  $\alpha_2$  for dendrite segment D2).

Defined only if the beginning point is determined.

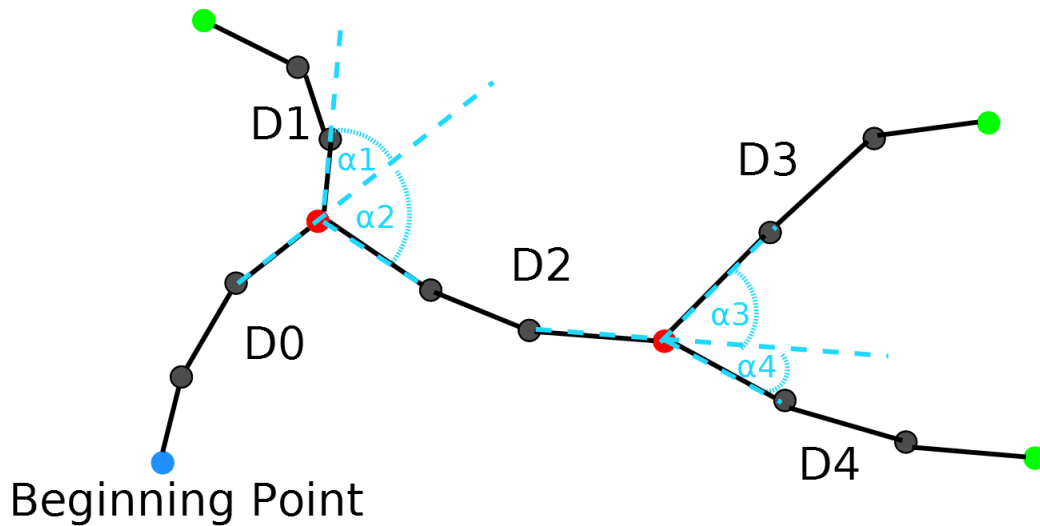


Figure 4

Please note • represents a dendritic vertex

#### Filaments - Dendrite Branching Angle B

The angle between the extending lines connecting the branch point with the neighboring branch points and the terminal points, respectively (Figure 5 angles marked with dashed green arcs,  $\beta$ ). Defined only if the beginning point is determined.

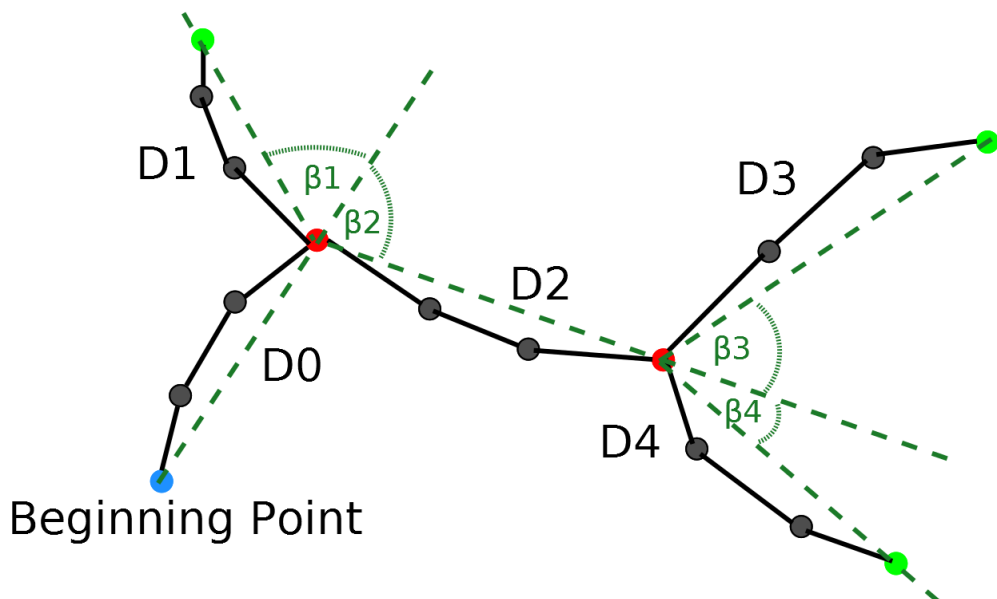


Figure 5

• represents a dendritic vertex

#### Filaments -Dendrite Length

Defined as the sum of all edges between two branch points or between a branch point and a terminal point, respectively. (Figure 2 Dendrite Length  $e_0+e_1+e_2$ ).

#### Filaments -Dendrite Mean Diameter

The mean value of all dendrites diameters measured from the center to the dendrite surfaces using the lower threshold (automatic creation) value.

## Filaments -Dendrite No of Spines

The number of spines branching off a dendrite segment (in Figure1 D0 0, D11, D2 2, D3 1, D4 1)

## Filaments - Dendrite Orientation Angle

The angle formed between extending line connecting distal vertices of the dendrite segment and X-axis of image within the XY plane (Figure 6 angles marked with a dashed orange arcs,  $\gamma$ ).

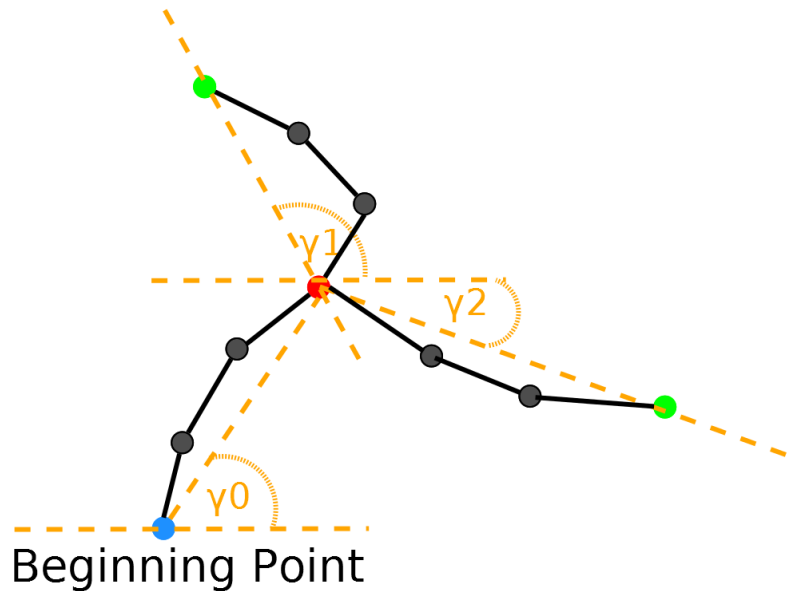


Figure 6

- represents a dendritic vertex

## Filaments – Dendrite Position X, Y, Z

**Dendrite** X,Y and Z positions within the viewing area. The value is equal to Center of Homogeneous Mass of the object.

## Filaments - Dendrite Resistance

Measure of a dendrite ability to conduct an electric current. The dendrite resistance is proportional to its length and inversely proportional to its cross-sectional area taking into account diameter variations. It has units of  $1/\text{mm}$  and must be multiplied by specific material resistance in order to obtain a value of a resistor.

## Filaments - Dendrite Spine Density

Defined as the number of spines per unit length of dendrite.

## Filaments - Dendrite Straightness

Defined as the ratio between dendrite length and radial distance between two branch points ( $h$ ) (Figure 7 value is always smaller than 1. If the Dendrite Straightness is 1 that means that the dendrite is completely straight.



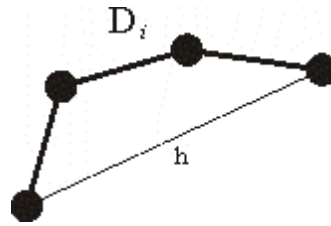


Figure 7

- represents a dendritic vertex

### Filaments - Dendrite Time

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

### Filaments - Dendrite Time Index

The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

### Filaments - Dendrite Volume

Defined as the sum of all (frustum) volumes composing a dendrite (Figure 2).

$$V(i) = \left( \frac{\pi}{3} \right) * h(i) * \left( r(i)^2 + r(i) * r(i+1) + r(i+1)^2 \right)$$

$$V = \sum V(i) \quad i = 0, \dots, n-1, n$$

$n$  = number of edges of a segment

### Filaments - Filament Acceleration

Acceleration is the change in the filament graph velocity over time.

Object position at time-point  $i$ :

$$P(i) = \begin{pmatrix} Px(i) \\ Py(i) \\ Pz(i) \end{pmatrix}$$

Acceleration vector  $x, y, z$  at time-point  $i$

$$A(i) = \frac{\frac{P(i) - P(i-1)}{t(i) - t(i-1)} + \frac{P(i+1) - P(i)}{t(i+1) - t(i)}}{2 * (t(i+1) - t(i-1))}$$

Acceleration at time-point  $i$ :

$$a(i) = |A(i)|$$

Acceleration at the first and last time-point is 0.

## Filaments - Filament Acceleration X, Y, Z

Acceleration is the change in the filament graph velocity over time along the selected-axis.

## Filaments - Filament Dendrite Area (sum)

Defined as the sum of the areas of all dendrites segments within the graph.

## Filaments - BoundingBoxAA Length X, Y, Z

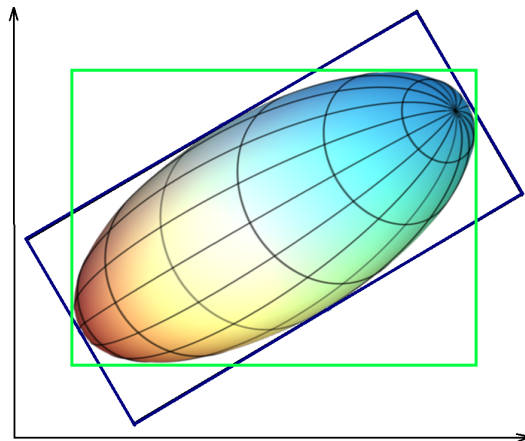
A bounding box is a rectangular cuboid in 3D, or a rectangle in 2D, containing an object.

If the bounding box is aligned with the axes of the coordinate system, it is known as an axis-aligned bounding box (AA). *BoundingBoxAA* identifies an object by considering the minimal rectangular box which fully encloses the object, whose faces are aligned parallel to the axes of a coordinate system.

BoundingBoxAA Length X =  $\max(X) - \min(X)$

BoundingBoxAA Length Y =  $\max(Y) - \min(Y)$

BoundingBoxAA Length Z =  $\max(Z) - \min(Z)$



axis-aligned bounding box (AA) shown in green  
object-oriented bounding box (OO) shown in blue

**Please note the difference between the ellipsoid axis length and bounding box length.**

## Filaments - BoundingBoxOO Length A, B, C

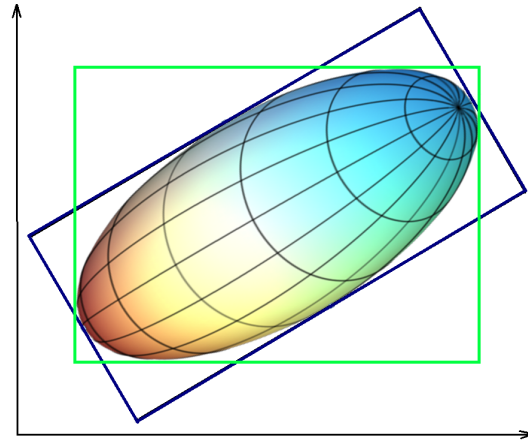
The local axes of the box can be arbitrarily oriented with respect to the coordinate system.

BoundingBoxOO (object oriented) identifies an object by considering the minimal rectangular box, which fully encloses the object. The box dimensions are calculated with no constraints to the orientation.

BoundingBoxOO Length A = Length of the shortest principal axis

BoundingBoxOO Length B = Length of the second longest principal axis

BoundingBoxOO Length C = Length of the longest principal axis



axis-aligned bounding box (AA) shown in green  
object-oriented bounding box (OO) shown in blue

**Please note the difference between the ellipsoid axis length and bounding box length.**

#### **Filaments - Filament Dendrite Area (sum)**

The sum of all dendrites areas of within the entire filament graph.

#### **Filaments - Filament Dendrite Length (sum)**

The sum of all dendrites length of within the entire filament graph.

#### **Filaments - Filament Dendrite Volume (sum)**

The sum of the volumes of all dendrites within the entire filament graph.

#### **Filaments - Filament Displacement X, Y, Z**

The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

#### **Filaments - Filament Displacement <sup>2</sup>**

The square of the distance of the object from the starting point of the track (also known as mean square displacement: MSD) when plotted against the relative time since the beginning of the track

$$D^2(t - t_F) = Dx(t, t_F)^2 + Dy(t, t_F)^2 + Dz(t, t_F)^2$$

$$Dx(t_2, t_1) = Px(t_2) - Px(t_1)$$

**t<sub>F</sub>** = first time index of track

**P<sub>x</sub>(t)** = x-position of object at time index t

#### **Filaments - Filament Full Branch Depth**

Branch Depth is defined as the number of branch points, or bifurcations, in the shortest path from the beginning point to a given point in the filament graph. Full Branch Depth is the highest value of Branching Depth for the graph (in Figure 1 value is 2). If no beginning point is defined, the value is set to "-1" (Figure1).

#### **Filaments - Filament Full Branch Level**

Full Branch Level is the highest value of Branching Level for the entire filament graph (in Figure 3 value is 4).

#### **Filaments - Filament Length (Sum)**

Defined as the sum of the lengths of all edges (both dendrites and spines) within the entire graph.

**Filaments - Filament No. Dendrite Branch Points**

The number of dendrites branching points in the entire filament graph (in Figure1 value is 3).

**Filaments - Filament No. Dendrites Branches**

The statistics are defined for a filament and a distance value. This is calculated by following the filament from the beginning point along all dendrites for the defined distance, and then counting how many different branches have been reached. Imaris calculates this as follows:

$$\text{Distances } 0 \text{ to } n * \text{DistanceIncrement}$$

where *DistanceIncrement* has a default value of 1 μm and *n* is dependent on the maximum dendrite terminal point distance to the beginning point of the filament. Note that the *DistanceIncrement* is equal to the "Sholl Spheres Resolution" and can be adjusted in the Statistics Preferences. The different distance values are visible as Factors "Distance" of the statistics value.

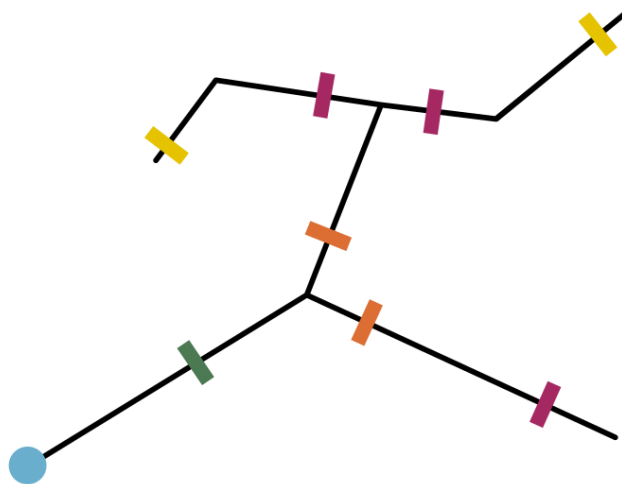


Figure 8 Filament with 7 dendrites, beginning point at the bottom left and indicators marking the dendrite branches for different distances

Value	Distance
0	0
1	1
2	2
3	3
2	4
0	5

**Filaments - Filament No. Dendrite Segments**

The number of dendrite segments in the entire filament graph (Figure 1 value is 8).

**Filaments - Filament No. Dendrite Terminal Points**

The number of dendrite terminal points (excluding the beginning point) in the entire filament graph (Figure 1 value is 6).

**Filaments - Filament No. Edges**

The number of connections between vertices in the entire filament graph (Figure 1 value is 34).

### Filaments - Filament No. Sholl Intersections

Sholl analysis creates a series of concentric circles (spheres in 3D) around the beginning point of the filament. Filament No. Sholl Intersections is defined as the number of dendrite intersections (branches) on concentric spheres, defining dendrite spatial distribution as a function of distance from the beginning point. All spheres have a center at the beginning point (Figure 9). The 1.0  $\mu\text{m}$  step resolution for the spheres can be changed under Preferences -> Advanced -> SurfaceAppFilament

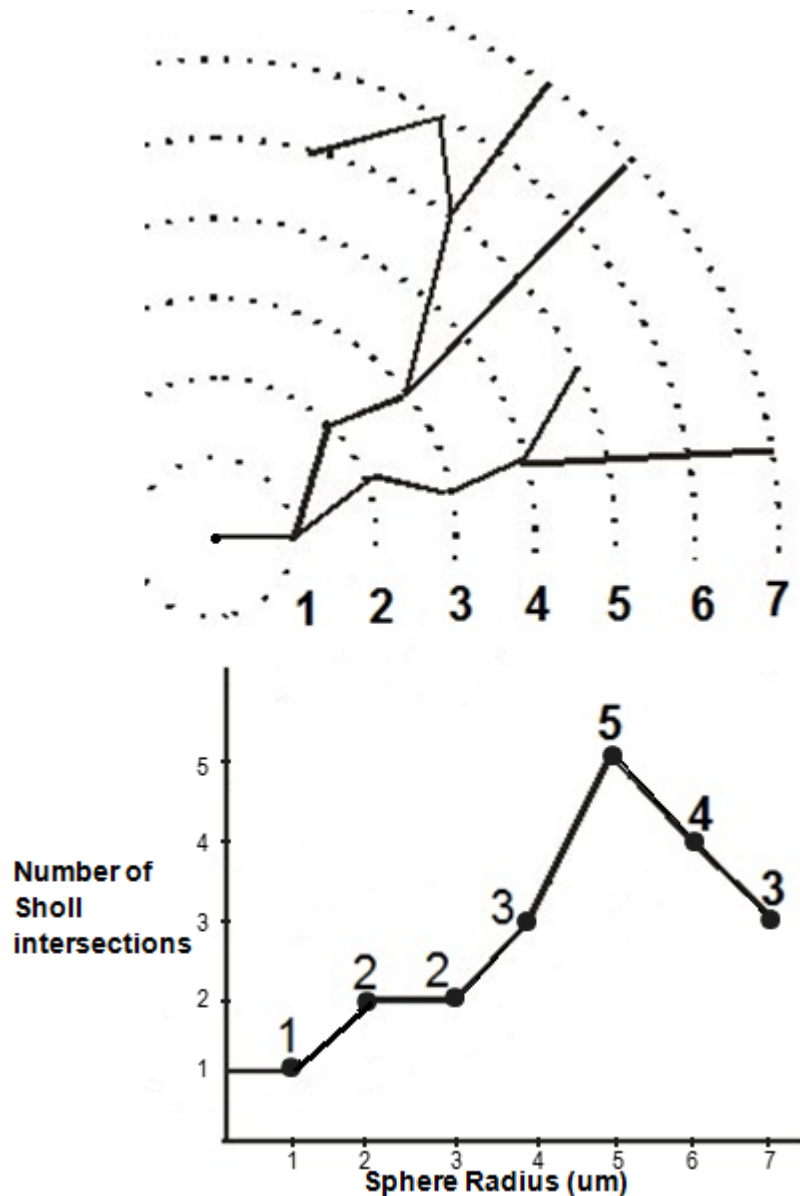


Figure 9

### Filaments - Filament No. Spine Branching Points

The number of spine branch points in the entire filament graph (in Figure 1 value is 1). A spine branch point will only exist in a single spine that has more than one terminal point.

## **Filaments - Filament No. Spine Segments**

The spine segment count in entire filament graph (in Figure 1 value is 7).

## **Filaments - Filament No. Spine Terminal Points**

The number of spine terminal points in the entire filament graph (in Figure 1 value is 6).

## **Filaments - Filament No. Unconnected Parts**

The number of unconnected filament graph within the image.

## **Filaments - Filament No. Vertices**

The number of vertices in the filament graph (Figure 1 value is 36).

## **Filaments – Filament Number of Filament pre Time Points**

Totals number of filament graph within the image at the specific time point.

## **Filaments – Filament Number of Tracks**

Totals number of tracks for the selected filament graph.

## **Filaments – Filament Position X, Y, Z**

Filament graph X, Y, Z position within the viewing area. The value is equal to Center of Homogeneous Mass of the object.

## **Filaments – Filament Speed**

The filament speed is the instantaneous speed of the object (um/s).The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

## **Filaments - Filament Spine Area (sum)**

Defined as the sum of the areas of all spines segments within the filament graph.

## **Filaments -Filament Spine Length (sum)**

The sum of all spines length of within the entire filament graph.

## **Filaments -Filament Spine Volume (sum)**

The sum of the volumes of all spines within the entire filament graph.

## **Filaments -Filament Time**

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

**Filaments -Filament Time Index**

The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

**Filaments -Filament Total Number of Filaments**

The value indicates the total number of filament graph within the data set.

**Filaments -Filament Track AR1Mean**

TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Note for all track statistics splits or gaps are not taken into account- it is taken that the selected tack is continuous.

**Filaments -Filament Track AR1 X, Y, Z**

TrackAR1X is the AR1 coefficient of the model for the x-positions.

$$AR1X = \frac{R_x^1}{R_x^0}$$

AR1X = TrackAR1X

$$R_x^n = \sum_{t=t_F-1}^{t_L-n} (D_x(t, t-1) - \bar{D}_x) \cdot (D_x(t+n, t+(n-1)) - \bar{D}_x)$$

$t_L$  = last time index of track

$t_F$  = first time index of track

$$D_x(t_2, t_1) = P_x(t_2) - P_x(t_1)$$

$P_x(t)$  = x-position of object at time index t

$$\bar{D}_x = \frac{1}{(t_L - t_F)} \sum_{t=t_F+1}^{t_L} D_x(t, t-1)$$

$P_x(t)$  = x-position of object at time index t

$t_L$  = last time index of track

$t_F$  = first time index of track

For more information, please visit: [http://en.wikipedia.org/wiki/Autoregressive\\_model](http://en.wikipedia.org/wiki/Autoregressive_model)

**Filaments - Filament Track Displacement Length**

The Track Displacement Length is the distance between first and last filament graph position.

$$D_x(t_n, t_{n-1}) = \sqrt{D_x(t_L, t_F)^2 + D_y(t_L, t_F)^2 + D_z(t_L, t_F)^2}$$

$$D_x(t_F, t_L) = P_x(t_L) - P_x(t_F)$$

**D** = TrackDisplacement

**t<sub>L</sub>** = last time index of track

**t<sub>F</sub>** = first time index of track

**P<sub>x</sub>(t)** = x-position of object at time index t

**Filaments - Filament Track Displacement X, Y, Z**

The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

**Filaments - Filament Displacement Delta XYZ**

The difference in position of the current object and the previous object of the same track.

**Filaments - Filament Displacement Delta Length (a scalar value)**

The length of the position difference of the current object and the previous object of the same track.

**Filaments - Filament Track Duration**

The Track Duration is the duration between the first and last time point within the track.

$$\text{Duration} = T(t_L) - T(t_F)$$

Duration = TrackDuration

**T(t)** = time in seconds at time point t

**t<sub>L</sub>** = last time index of track

**t<sub>F</sub>** = first time index of track

**Filaments - Filament Track Length**

The Track Length is the total length of displacements along the track.

$$L = \sum_{t=t_F+1}^{t_L} \sqrt{D_x(t, t-1)^2 + D_y(t, t-1)^2 + D_z(t, t-1)^2}$$

**L** = TrackLength

**t<sub>L</sub>** = last time index of track

**t<sub>F</sub>** = first time index of track

$$D_x(t_f, t_l) = P_x(t_l) - P_x(t_f)$$



**Filaments -Filament Track No. Dendrites Branches**

The number of parallel outstretched dendritic branches counted from the beginning point toward the terminal points at constant length increments along the selected track.

**Filaments - Filament Track Number of Filaments**

The total number of filament graphs along the track.

**Filaments - Filament Track Number of Fusions**

A fusion occurs when a filament object is connected to several filament objects of a past time point. The value indicates the number of the related objects.

**Filaments - Filament Track Position X, Y, Z Mean**

Defines the mean value of X, Y, Z Track positions.

**Filaments - Filament Tracks Position X, Y, Z Start**

Defines the value of the X, Y, Z track start positions.

**Filaments - Filament Track Speed Max**

The maximum value of the object's speed on the track.

**Filaments -Filament Track Speed Mean**

The Average value of the object's speed on the track. If the Track has no fuses (merges) or fragments (splits), then the average speed is given by the Track length divided by the time between first and last object in the Track.

**Filaments -Filament Track Speed Min**

The minimum value of the object's speed on the track.

**Filaments - Filament Track Speed Standard Deviation**

The Standard Deviation value of the object's speed on the track.

**Filaments - Filament Track Speed Variation**

The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value. The coefficient of variation is a dimensionless number.

$$v = \frac{\sigma}{\mu}$$

$\sigma$ - Standard Deviation

$\mu$ -Mean value

**Filaments - Filament Track Straightness**

$$S = \frac{D}{L}$$

**S** = TrackStraightness

**D** = TrackDisplacement

**L** = TrackLength

## Filaments - Filament Velocity X, Y, Z

Velocity is the rate of change of object displacement with time. Speed and velocity are related in the same way as distance and displacement. Speed is a scalar and velocity is a vector.

The formula for calculating the velocity  $v(i)$  at time point  $i$  is calculated as follows:

For objects at time point  $i$  at the beginning of a track the velocity  $v(i)$  is computed from the position  $x(i)$  and time  $t(i)$  of the object at time point  $i$  and  $i + 1$ :

$$v(i) = \frac{x(i+1) - x(i)}{t(i+1) - t(i)}$$

For objects at time point  $i$  in the middle of a track the velocity  $v(i)$  is computed from the position  $x(i)$  and time  $t(i)$  of the object at time point  $i - 1$  and  $i + 1$ :

$$v(i) = \frac{x(i+1) - x(i-1)}{t(i+1) - t(i-1)}$$

For objects at time point  $i$  at the end of a track the velocity  $v(i)$  is computed from the position  $x(i)$  and time  $t(i)$  of the object at time point  $i - 1$  and  $i$ :

$$v(i) = \frac{x(i) - x(i-1)}{t(i) - t(i-1)}$$

## Filaments - Filament Volume (sum)

Volume is the quantity of three-dimensional space occupied by an object. The sum of all segment's volumes within the entire filament graph.

## Filaments - Pt Acceleration

Acceleration is the change in the object velocity over time.

Object position at time-point  $i$ :

$$P(i) = \begin{pmatrix} Px(i) \\ Py(i) \\ Pz(i) \end{pmatrix}$$

Acceleration vector x, y, z at time-point  $i$

$$A(i) = \frac{\frac{P(i) - P(i-1)}{t(i) - t(i-1)} + \frac{P(i+1) - P(i)}{t(i+1) - t(i)}}{2 * (t(i+1) - t(i-1))}$$

Acceleration at time-point  $i$ :

$$a(i) = |A(i)|$$

Acceleration at the first and last time-point is 0.

### Filaments - Pt Acceleration X, Y, Z

Acceleration is the change in the filament points velocity over time along the selected-axis.

### Filaments - Pt Branching Angle

The angle between the lines connecting the branch point with either of its two neighboring peripheral vertices (in Figure 10 a angles are marked with  $\delta$ ). This value is defined only for filament graphs with a valid beginning point. If the beginning point has not been defined then Pt. Branching Angle is the smallest of the angles between the branching segments (in Figure 10 b angles marked with  $\delta_2$ ). The value is not calculated for branch points with more than three segments.

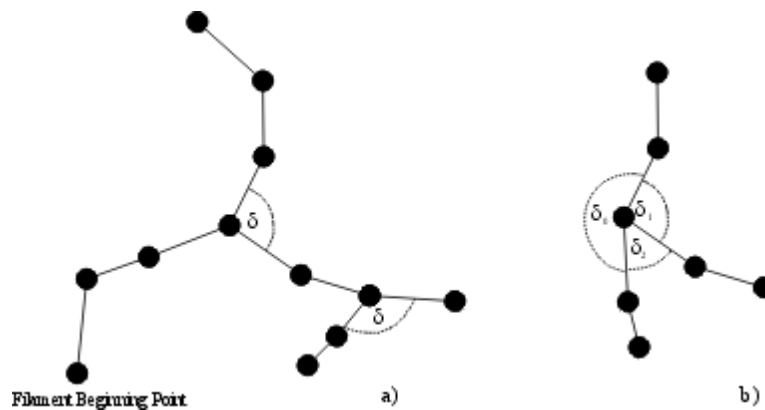


Figure 10

### Filaments - Pt Spine Total Length

This is defined as the sum of the Length of all Spines connected to the Spine Attachment Point.

### Filaments - Pt Spine Total Area

This is defined as the sum of the Area of all Spines connected to the Spine Attachment Point.

### Filaments - Pt Spine Total Volume

This is defined as the sum of the Volume of all Spines connected to the Spine Attachment Point.

### Filaments - Pt Diameter

Defined as the value of the vertices's diameter (Figure 2).

### Filaments - Pt Displacement X, Y, Z

The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

### Filaments - Pt Displacement ^2

$$\bar{D}^{-2}(\Delta t) = \sum_{t-t_r+\Delta t}^{t_1} D_x(t, t-\Delta t)^2 + D_y(t, t-\Delta t)^2 + D_z(t, t-\Delta t)^2$$

$$D_X(t_f, t_l) = P_X(t_l) - P_X(t_f)$$

$t_L$  = last time index of track

$t_F$  = first time index of track

$P_X(t)$  = x-position of object at time index t

**Filaments - Pt Displacement Delta XYZ**

The difference in position of the current object and the previous object of the same track.

**Filaments - Pt Displacement Delta Length (a scalar value)**

The length of the position difference of the current object and the previous object of the same track.

**Filaments - Pt Distance**

Distance of the specific vertex from the beginning point along the dendritic graph structure (Figure 8).

**Filaments - Pt Number of Points per Time Point**

Number of all beginning, branching, attachment and terminal points in the selected time point.

**Filaments - Pt Position X, Y, Z**

The X, Y, Z coordinates of all tracked beginning, branching, attachment and terminal points.

These values are only available if the tracking option is selected.

**Filaments - Pt Speed**

The filaments - Pt speed is the instantaneous speed of the object (um/s). The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

**Filaments -Pt Time**

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

**Filaments -Pt Time Index**

The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

**Filaments -Pt Total Number of Points**

Number of all beginning, branching, attachment and terminal points.



**Filaments - Pt Track AR1Mean**

TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

**Filaments - Pt Track AR1 X, Y, Z**

TrackAR1X is the AR1 coefficient of the model for the x positions.

$$AR1X = \frac{R_x^1}{R_x^0}$$

**AR1X** = TrackAR1X

$$R_x^n = \sum_{t=t_F-1}^{t_L-n} (D_x(t, t-1) - \bar{D}_x) \cdot (D_x(t+n, t+(n-1)) - \bar{D}_x)$$

**t<sub>L</sub>** = last time index of track

**t<sub>F</sub>** = first time index of track

$$D_x(t_2, t_1) = P_x(t_2) - P_x(t_1)$$

**P<sub>X</sub>(t)** = x-position of object at time index t

$$\bar{D}_x = \frac{1}{(t_L - t_F)} \sum_{t=t_F+1}^{t_L} D_x(t, t-1)$$

**t<sub>L</sub>** = last time index of track

**t<sub>F</sub>** = first time index of track

For more information, please visit: [http://en.wikipedia.org/wiki/Autoregressive\\_model](http://en.wikipedia.org/wiki/Autoregressive_model)

**Filaments - Pt Track Displacement Length**

The Track Displacement Length is the distance between first and last point position.

$$D_x(t_n, t_{n-1}) = \sqrt{D_x(t_L, t_F)^2 + D_y(t_L, t_F)^2 + D_z(t_L, t_F)^2}$$

$$D_x(t_F, t_L) = P_x(t_L) - P_x(t_F)$$

**D** = TrackDisplacement

**t<sub>L</sub>** = last time index of track

**t<sub>F</sub>** = first time index of track

**P<sub>X</sub>(t)** = x-position of object at time index t

**Filaments - Pt Track Displacement X, Y, Z**

The object's Track Displacement is the distance between the first and last object's position along the selected-axis.

### Filaments - Pt Track Duration

The Track Duration is the duration between the first and last time point within the track.

$$\text{Duration} = T(t_L) - T(t_F)$$

**Duration** = TrackDuration

**T(t)** = time in seconds at time point t

**t<sub>L</sub>** = last time index of track

**t<sub>F</sub>** = first time index of track

### Filaments - Pt Track Length

The Track Length is the total length of displacements along the track.

$$L = \sum_{t=t_F+1}^{t_L} \sqrt{D_x(t,t-1)^2 + D_y(t,t-1)^2 + D_z(t,t-1)^2}$$

**L** = TrackLength

**t<sub>L</sub>** = last time index of track

**t<sub>F</sub>** = first time index of track

$$D_x(t_F, t_L) = P_x(t_L) - P_x(t_F)$$

**Note for all track statistics splits or gaps are not taken into account- it is taken that the selected track is continuous.**

### Filaments - Pt Track No. Dendrites Branches

The number of parallel outstretched dendritic branches counted from the beginning point toward the terminal points at constant length increments along the selected track.

### Filaments - Pt Track Number of Fusions

A fusion occurs when a filament graph beginning, branching, attachment and terminal points are connected to several graph key points of a past time point. The value indicates the number of the related objects along the selected track.

### Filaments - Pt Track Number of Points

The total number of points (beginning, branching, attachment and terminal) along the track.

### Filaments - Pt Track Position X, Y, Z Mean

Defines the mean value of X, Y, Z Track positions.

### Filaments - Pt Tracks Position X, Y, Z Start

Defines the value of the X, Y, Z track start positions

### Filaments - Pt Track Speed Max



The maximum value of the object's speed on the track.

#### Filaments - Pt Track Speed Mean

The Average value of the object's speed on the track. If the Track has no fuses (merges) or fragments (splits), then the average speed is given by the Track length divided by the time between first and last object in the Track.

#### Filaments - Pt Track Speed Min

The minimum value of the object's speed on the track.

#### Filaments - Pt Track Speed Standard Deviation

The Standard Deviation value of the object's speed on the track.

#### Filaments - Pt Track Speed Variation

The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value. The coefficient of variation is a dimensionless number.

$$v = \frac{\sigma}{\mu}$$

$\sigma$ - Standard Deviation

$\mu$ - Mean value

#### Filaments - Pt Track Spine Area Max

The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 10).

The value is maximal spine area along the track.

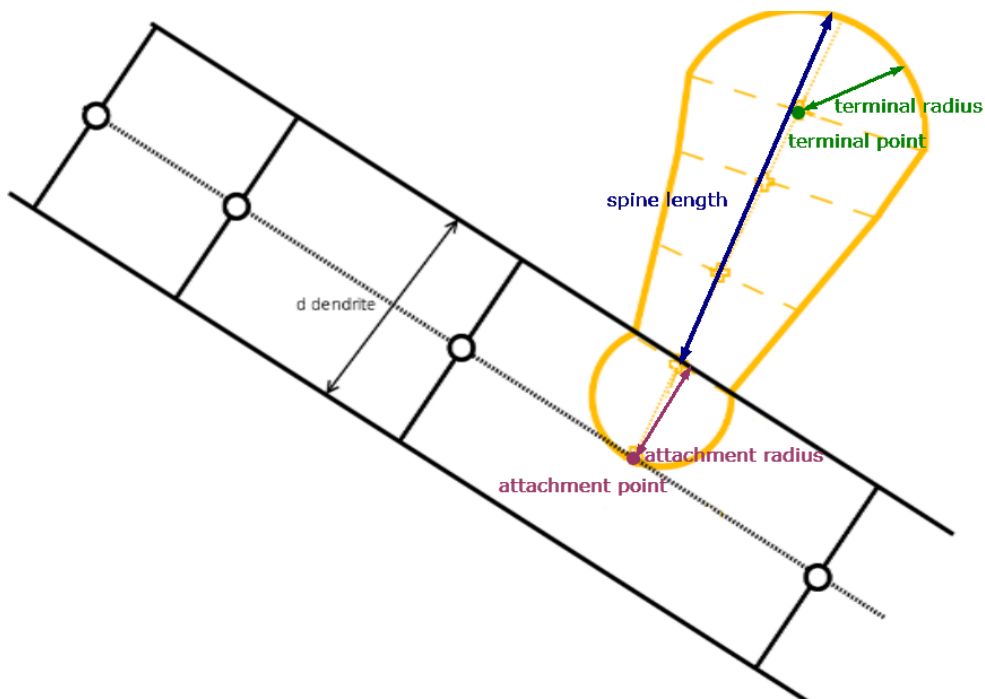


Figure 11

#### Filaments -Pt Track Spine Area Mean

The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 11).

The value is mean spine area along the track.

### **Filaments - Pt Track Spine Area Min**

The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 11).

The value is minimal spine area along the track.

### **Filaments - Pt Track Spine Length Max**

The value is the maximal spine length (see definition of the Spine Length statistics value) of all spines that are part of the track.

### **Filaments - Pt Track Spine Length Min**

The value is the minimal spine length (see definition of the Spine Length statistics value) of all spines that are part of the track.

### **Filaments - Pt Track Spine Length Mean**

The value is the mean spine length (see definition of the Spine Length statistics value) of all spines that are part of the track.

### **Filaments - Pt Track Spine Volume Max**

The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 11).

The value is maximal spine volume along the track.

### **Filaments - Pt Track Spine Volume Min**

The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 11).

The value is minimal spine volume along the track.

### **Filaments - Pt Track Spine Volume Mean**

The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 11).

The value is mean spine volume along the track.

### **Filaments - Pt Track Spine Straightness**

Defined as the ratio between spine length and radial distance between two branch points (h) (defined in the same way as Dendrite Straightness, refer to Figure 7).

### **Filaments - Pt Velocity X, Y, Z**

Velocity is the rate of change of object displacement with time. Speed and velocity are related in the same way as distance and displacement. Speed is a scalar and velocity is a vector. The instant velocity vector  $v$  of an object that has positions  $x(t)$  at time  $t$  and  $x(t + \Delta t)$  at time  $t + \Delta t$ , can be computed as the derivative of the relative object position along the selected-axis:

$$v = \lim_{\Delta t \rightarrow 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

### **Filaments - Spine Area**





The Spine Area is calculated by subtracting the surface area of the hemisphere of spine attachment point from total surface area of the spine frustum and surface of spine ending hemisphere (Figure 11).

#### Filaments - Spine Attachment Pt Acceleration

Acceleration is the change in the object velocity over time.

Object position at time-point  $i$ :

$$P(i) = \begin{pmatrix} Px(i) \\ Py(i) \\ Pz(i) \end{pmatrix}$$

Acceleration vector x, y, z at time-point  $i$

$$A(i) = \frac{\frac{P(i) - P(i-1)}{t(i) - t(i-1)} + \frac{P(i+1) - P(i)}{t(i+1) - t(i)}}{2 * (t(i+1) - t(i-1))}$$

Acceleration at time-point  $i$ :

$$a(i) = |A(i)|$$

Acceleration at the first and last time-point is 0.

#### Filaments - Spine Attachment Pt Acceleration X, Y, Z

Acceleration is the change in the filament points velocity over time along the selected-axis.

#### Filaments - Spine Attachment Pt Branching Angle

The angle between the lines connecting the branch point with either of its two neighboring peripheral vertices (in Figure 10 a angles are marked with  $\delta$ ). This value is defined only for filament graphs with a valid beginning point. If the beginning point has not been defined then Pt. Branching Angle is the smallest of the angles between the branching segments (in Figure 10 b angles marked with  $\delta_2$ ). The value is not calculated for branch points with more than three segments.

#### Filaments - Spine Attachments Pt Diameter

Diameter of a vertex at the spine attachment point to the dendrite.

#### Filaments - Spine Attachments Pt Displacement X, Y, Z

The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

#### Filaments - Spine Attachments Pt Displacement ^2

$$D^{-2}(\Delta t) = \sum_{t=t_r+\Delta t}^{t_l} D_x(t, t-\Delta t)^2 + D_y(t, t-\Delta t)^2 + D_z(t, t-\Delta t)^2$$

$$D_X(t_f, t_l) = P_X(t_l) - P_X(t_f)$$

$t_L$  = last time index of track

$t_F$  = first time index of track

$P_X(t)$  = x-position of object at time index t

**Filaments - Spine Attachments Pt Distance**

Distance of the specific vertex from the beginning point along the dendritic graph structure (Figure 8).

**Filaments - Spine Attachments Pt Position X, Y, Z**

The X, Y, Z coordinates of all tracked beginning, branching, attachment and terminal points. These values are only available if the tracking option is selected.

**Filaments - Spine Attachments Pt Speed**

The object speed is instantaneous speed of the graph beginning, branching, attachment and terminal points (um/s). The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

**Filaments - Spine Attachments Pt Time**

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

**Filaments - Spine Attachments Pt Time Index**

The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

**Filaments - Spine Attachments Velocity X, Y, Z**

Velocity is the rate of change of object displacement with time. Speed and velocity are related in the same way as distance and displacement. Speed is a scalar and velocity is a vector. The instant velocity vector  $v$  of an object that has positions  $x(t)$  at time  $t$  and  $x(t + \Delta t)$  at time  $t + \Delta t$ , can be computed as the derivative of the relative object position along the selected-axis:

$$v = \lim_{\Delta t \rightarrow 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

**Filaments - Spine Branch Depth**



Spine Branching Depth is defined as the number of branch points, or bifurcations, in the shortest path from the spine attachment point to a given point in the dendritic graph.

#### Filaments - Spine Branch Level

A Spine inherits the Dendrite Branch Level from the dendrite segment that it is attached to.

#### Filaments - Spine Branching

The number of spine branching points in a spine segment. The value is typically 0 or 1 (Figure 12 for  $S_0$  0,  $S_1$  1,  $S_2$  0 and  $S_3$  0).

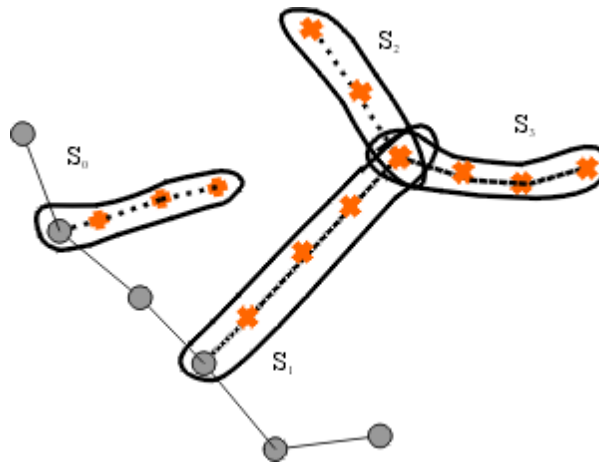


Figure 12

#### Please note:

- The spine neck length is set by default to be 50% of the total spine length.
- Neck length is measured from the base of the spine head toward the dendrite.
- The spine head is 25% of the total spine length.
- The remaining part of the spine, attached to the dendrite tree, is set to be 25% of the total spine length.

#### Filaments - Spine Length

The Spine length is calculated as the sum length of all spine center-line segments including the Spine head radius (the tip of the spine) minus the attachment radius (spine part inside dendrite, see Figure 11).

#### Filaments - Spine Neck Length

The Spine Neck length is calculated by subtracting the terminal radius of the spine terminal point from the sum of spine lengths (see Figure 11).

#### Filaments - Spine Max Diameter

The maximal value of spine diameters including attachment point diameter.

#### Filaments - Spine Min Diameter

The minimal value of spine diameters including attachment point diameter.

#### Filaments - Spine Mean Diameter

The mean value of spine diameters including attachment point diameter.

#### Filaments - Spine Neck Max Diameter

## **Filaments - Spine Neck Mean Diameter**

### **Filaments - Spine Neck Min Diameter**

Maximum, Mean and Minimum value of the spine neck diameter (excluding the spine attachment and terminal points diameters).

### **Filaments - Spine Neck Volume**

The Spine Neck volume is calculated by subtracting the volume of the spine-ending hemispheres (attachment and terminal point) from the spine volume.

### **Filaments - Spine Orientation Angle**

The angle formed between the extending line connecting distal spine vertices in the spine segment and X-axis of the image within the XY plane (defined in the same way as Dendrite Orientation Angle, refer to Figure 6).

### **Filaments - Spine Part Length Ground**

The spine ground length is set by default to be 25% of the total spine vertices (points).

### **Filaments - Spine Part Length Head**

The spine head length is set by default to be 25% of the total spine length.

### **Filaments - Spine Part Length Neck**

The spine neck length is set by default to be 50% of the total spine length.

### **Filaments - Spine Part Max Diameter Ground**

The maximal value of the spine ground diameter.

### **Filaments - Spine Part Max Diameter Head**

The maximal value of the spine head diameter.

### **Filaments - Spine Part Max Diameter Neck**

The maximal value of the spine neck diameter.

### **Filaments - Spine Part Mean Diameter Ground**

The average value of the spine ground diameter.

### **Filaments - Spine Part Mean Diameter Head**

The average value of the spine head diameter.

### **Filaments - Spine Part Mean Diameter Neck**

The average value of the spine neck diameter.

### **Filaments - Spine Part Min Diameter Ground**

The minimal value of the spine ground diameter.

### **Filaments - Spine Part Min Diameter Head**

The minimal value of the spine head diameter.

### **Filaments - Spine Part Min Diameter Neck**

The minimal value of the spine neck diameter.

### **Filaments - Spine Part Volume Ground**

Measure the volume of the spine ground.

**Filaments - Spine Part Volume Head**

Measure the volume of the spine head.

**Filaments - Spine Part Volume Neck**

Measure the volume of the spine neck.

**Filaments - Spine Position X, Y, Z**

Defines the value of X, Y, Z Cell position (x y z). The value is equal to Center of Homogeneous Mass of the object

**Filaments - Spine Resistance**

Measure of a spine's ability to conduct an electric current. The spine's resistance is proportional to its length and inversely proportional to its cross-sectional area taking into account the diameter variations. It has units of 1/um and must be multiplied by specific material resistance in order to obtain a value of resistor (defined in the same way as Dendrite Resistance).

**Filaments - Spine Straightness**

Defined as the ratio between spine length and radial distance between two branch points (h) (defined in the same way as Dendrite Straightness, refer to Figure 7).

**Filaments - Spine Terminal Point Acceleration**

Acceleration is the change in the object velocity over time.

Object position at time-point  $i$ :

$$P(i) = \begin{pmatrix} Px(i) \\ Py(i) \\ Pz(i) \end{pmatrix}$$

Acceleration vector x, y, z at time-point  $i$

$$A(i) = \frac{\frac{P(i) - P(i-1)}{t(i) - t(i-1)} + \frac{P(i+1) - P(i)}{t(i+1) - t(i)}}{2 * (t(i+1) - t(i-1))}$$

Acceleration at time-point  $i$ :

$$a(i) = |A(i)|$$

Acceleration at the first and last time-point is 0.

**Filaments - Spine Terminal Point Acceleration X, Y, Z**

Acceleration is the change in the filament points velocity over time along the selected-axis.

**Filaments - Spine Terminal Point Diameter**

Diameter of a vertex at the spine terminal point.

## Filaments - Spine Terminal Point Displacement X, Y, Z

The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position.

## Filaments - Spine Terminal Point Displacement ^2

$$\bar{D}^2(\Delta t) = \sum_{t=t_r+\Delta t}^{t_l} D_x(t, t-\Delta t)^2 + D_y(t, t-\Delta t)^2 + D_z(t, t-\Delta t)^2$$

$$D_x(t_F, t_L) = P_x(t_L) - P_x(t_F)$$

$t_L$  = last time index of track

$t_F$  = first time index of track

$P_x(t)$  = x-position of object at time index t

## Filaments - Spine Terminal Point Distance

Distance of the specific vertex from the beginning point along the dendritic graph structure (Figure 8).

## Filaments - Spine Terminal Pt Intensity Center

Defined as the intensity of the spine terminal center voxel.

## Filaments - Spine Terminal Point Position X, Y, Z

The X, Y, Z coordinates of all tracked beginning, branching, attachment and terminal points.

These values are only available if the tracking option is selected.

## Filaments - Spine Terminal Point Speed

The object speed is instantaneous speed of the graph beginning, branching, attachment and terminal points (um/s). The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

## Filaments - Spine Terminal Point Time

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

## Filaments - Spine Terminal Point Time Index



The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

### Filaments - Spine Terminal Point Velocity X, Y, Z

Velocity is the rate of change of object displacement with time. Speed and velocity are related in the same way as distance and displacement. Speed is a scalar and velocity is a vector. The instant velocity vector  $v$  of an object that has positions  $x(t)$  at time  $t$  and  $x(t + \Delta t)$  at time  $t + \Delta t$ , can be computed as the derivative of the relative object position along the selected-axis:

$$v = \lim_{\Delta t \rightarrow 0} \frac{x(t + \Delta t) - x(t)}{\Delta t} = \frac{dx}{dt}$$

### Filaments - Spine Terminal Pt. Volume

Spine Terminal Pt. Volume is calculated as a volume of the sphere with the diameter of a spine terminal point vertex (Figure 11).

### Filaments - Spine Time

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

### Filaments - Spine Time Index

The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

### Filaments - Spine Volume

The Spine volume is calculated by subtracting the hemisphere volume of the spine attachment point from the total volume of the spine frustum and volume of the spine ending hemisphere (Figure 11).

### Velocity Angle

The velocity angle is the angle between the velocity vector and the corresponding axis.

**Velocity Angle X:** The angle between the velocity vector and the x-Axis (1,0,0).

**Velocity Angle Y:** The angle between the velocity vector and the y-Axis (0,1,0).

**Velocity Angle Z:** The angle between the velocity vector and the z-Axis (0,0,1).

## 5.9.9.3 Measurement Points

Measurement Point statistics are automatically computed for each Measurement Point.

### Show Statistic Values - Measurement Points

#### Measurement Points - Angle

This is the angle between two adjacent measurement points that share a common point. This statistic is only calculated in Polygon (ABCD...) Line Mode.

#### Measurement Points - Distance

This is the distance between the measurement points.

#### Measurement Points - Distance Sum

The Distance Sum is the sum of all measured distances.

## Measurement Points - Number of Points

This is defined as the number of measurement points.

## Measurement Points - Point Intensity Center

The Point Intensity Center is the value of the point intensity center (average) in the different channels.

## Measurement Points - Point Position X

Point position in x-axis.

## Measurement Points - Point Position Y

Point position in y-axis.

## Measurement Points - Point Position Z

Point position in z-axis.

### 5.9.9.4 Spots

Spots statistics can be located in the statistics tab. Spots store a diameter and a specific time point. The intensity of the spot is defined by their statistics, intensity therefore changes when the image changes, which means that it is not an inherent part of the Spot itself (and not given a different treatment than the other statistics). Position X Y Z, diameter X Y Z and the time point are the values needed for the full description of a Spot. These values can be edited in the Edit tab options.

#### Show Statistic Values - Spots

### Spots- Acceleration

Acceleration is the change in the Spot(s) velocity over time.

Acceleration X, Y, Z are the components of the acceleration vector (at time-point t):

$$\vec{a}(t) = \left( \frac{\vec{p}(t+1) - \vec{p}(t)}{T(t+1) - T(t)} - \frac{\vec{p}(t) - \vec{p}(t-1)}{T(t) - T(t-1)} \right) / \left( \frac{T(t+1) - T(t-1)}{2} \right), \text{ for } 1 < t < n$$

$$\vec{a}(1) = \vec{a}(n) = \vec{0}$$

Acceleration at time-point t:

$$a(t) = |\vec{a}(t)|, \text{ with } \vec{a}(t) \text{ as defined in Acceleration X, Y, Z}$$

The acceleration at the first and last time-point is 0.

### Spots- Acceleration X, Y, Z

The acceleration is the change in the velocity of a spot over time along the selected-axis.

### Spots - Area

The sum of the area of the sphere / ellipsoid surface (surface of the spot).

### Spots - Diameter X, Y, Z

The diameter specifies the size of spherical object (i.e. the spot) along the selected-axis.





**Spots - Displacement X, Y, Z**

The Displacement is a measure of the distance that a spot moves from the first time point position, to its current position. This is calculated for each axis by subtracting the first time point position from a selected time point position:

$$\vec{d}(t) = \vec{p}(t) - \vec{p}(1)$$

**Spots - Displacement ^2**

The square of the distance of the spot from the starting point of the track (also known as mean square displacement: MSD) when plotted against the relative time since the beginning of the track:

$$d^2(t) = |\vec{d}(t)|^2, \text{ with } \vec{d}(t) \text{ as defined in Displacement X Y Z}$$

**Spots - Intensity Center**

The Intensity Center is the intensity of the voxel at the center of the Spot.

**Spots - Intensity Max****Spots - Intensity Mean****Spots - Intensity Median**

The Spots - intensity median is estimated from the intensity histogram.

**Spots - Intensity Min****Spots - Intensity StdDev****Spots - Intensity Sum**

The spots intensity statistics provide a description for the voxels enclosed within a Spot (Max, Mean, Median, Min, StdDev and Sum).

**Spots - Number of Spots per Time Point**

This is the number of spots at each time point.

**Spots - Number of Tracks**

Number of tracks.

**Spots - Number of Voxels**

Number of voxels within a Spot. A voxel is considered in a spot object if the centre of the voxel is located within the spot. When spots have a small diameter compared to the voxel size, this statistic value can be 0

**Tip: When spots with voxel count 0 are reported it can help to resample the image in the z direction so that the voxel z size is smaller than the spot diameter**

**Spots - Position X, Y, Z**

The position specifies the spot position (X, Y, Z). The position of the center of the spot:  $\vec{p} = [p_x, p_y, p_z]$

**Spots - Speed**

The spots speed is the instantaneous speed of the spot  $s(t)$  ( $\mu\text{m/s}$ ). The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

**Spots - Time**

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

**Spots - Time Index**

The value is only available for the time series data sets. The number indicates the number for the current time step in the series

**Spots - Total Number of Spots**

The total number of spot objects.

**Spots - Track AR1Mean**

TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y and TrackAR1Z.

**Note for all track statistics, any splits or gaps are not taken into account- it is taken that the selected track is continuous in nature.**

**Spots - Track AR1 X, Y, Z**

For more information, please visit: [http://en.wikipedia.org/wiki/Autoregressive\\_model](http://en.wikipedia.org/wiki/Autoregressive_model)

$$\vec{ar}1 = \frac{\sum_{t=2}^{n-1} (\vec{d}(t-1,t) - \vec{d}_{avg}) * (\vec{d}(t,t+1) - \vec{d}_{avg})}{\sum_{t=2}^n (\vec{d}(t-1,t) - \vec{d}_{avg})^2}$$

with the displacement between two time points:

$$\vec{d}(t_1, t_2) = \vec{p}(t_2) - \vec{p}(t_1)$$

and the average displacement of the entire track:

$$\vec{d}_{avg} = \sum_{t=2}^n (\vec{p}(t) - \vec{p}(t-1)) / n$$

**Spots - Track Area Mean**

The mean of the triangle Spots surfaces along the track.

**Spots - Track Diameter Mean**

The mean diameter of all spots along the track.

**Track Displacement X, Y, Z**



$$\vec{tdl} = \vec{p}(n) - \vec{p}(1)$$

**Spots - Track Displacement Length**

The Track Displacement Length (TDL) is the distance between first and last spot's position.

$$tdl = |\vec{tdl}|, \text{ with } \vec{tdl} \text{ defined in Track Displacement X, Y, Z}$$

**Spots - Track Displacement X, Y, Z**

The Track Displacement is the distance between the first and last spot's position along the selected-axis.

**Spots - Displacement Delta XYZ**

The difference in position of the current object and the previous object of the same track.

**Spots - Displacement Delta Length (a scalar value)**

The length of the position difference of the current object and the previous object of the same track.

**Spots - Track Duration**

The Track Duration is the duration between the first and last time point within the track.

$$td = T(n) - T(1)$$

**Spots - Track Intensity Center Mean**

This is the mean center intensity of all spots along the track.

**Spots - Track Intensity Max**

This is the maximum value of all maximum (voxel) intensities of the spot along the tracks

**Spots - Track Intensity Mean**

This is the weighted mean of the spot mean values, equal to the simple mean of all the intensities of all the spots in a track. Larger spots have a larger impact on the track mean.

**Spots - Track Intensity Median**

The median intensity for a track is calculated as the (approximated) median of the (approximated) medians of its objects, instead of the true median of all the intensities in the track, for performance reasons.

**Spots - Track Intensity Min**

This is the minimum value of all minimum (voxel) intensities of the spots along the tracks

**Spots - Track Intensity StdDev**

The standard deviation track intensity of all spots along the track.

**Spots - Track Intensity Sum**

The sum of the track intensity of all spots along the track.

**Spots - Track Length**

The Track Length is the total length of displacements within the track.

$$tl = \sum_{t=2}^n |\vec{p}(t) - \vec{p}(t-1)|$$

**Note for all track statistics, splits or gaps are not taken into account- it is taken that the selected track is continuous in nature.**

## Spots - Track Number of Branches

The number of track branches.

## Spots - Track Number of Fusions

The number of track fusions.

## Spots - Track Number of Spots

The number of track Spots.

## Spots - Tracks Position Mean X, Y, Z

Define the mean value of X, Y, Z track coordinates positions.

## Spots - Tracks Position X, Y, Z Start

Define the value of the X, Y, Z track start positions.

## Spots - Track Speed Max

The maximum value of the object's speed on the track.

## Spots - Track Speed Mean

The Average value of the spot's speed on the track. If the Track has no fuses (merges) or fragments (splits), then the average speed is given by the Track length divided by the time between first and last object in the Track.

## Spots - Track Speed Min

The minimum value of the spot's speed on the track.

## Spots - Track Speed Standard Deviation

The Standard Deviation value of the spot's speed on the track.

## Spots -Track Speed Variation

The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value. The coefficient of variation is a dimensionless number.

$$v = \frac{\sigma}{\mu}$$

$\sigma$ - Standard Deviation

$\mu$ - Mean value

## Spots - Track Straightness

$$S = \frac{D}{L}$$

**S** = TrackStraightness

**D** = TrackDisplacement

**L** = TrackLength

## Spots - Track Volume Mean

The mean volume of all Spots along the tracks.



**Spots - Track Velocity X, Y, Z**

The formulas for calculating the velocity at time points (t) and (1) are calculated as follows:

$$\vec{v}(t) = \frac{\vec{p}(t+1) - \vec{p}(t-1)}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$\vec{v}(1) = \frac{\vec{p}(2) - \vec{p}(1)}{T(2) - T(1)} \text{ and } \vec{v}(n) = \frac{\vec{p}(n) - \vec{p}(n-1)}{T(n) - T(n-1)}$$

**Spots - Volume**

Volume is a quantification of how much space an Spots object occupies.

**Velocity Angle**

The velocity angle is the angle between the velocity vector and the corresponding axis.

**Velocity Angle X:** The angle between the velocity vector and the x-Axis (1,0,0).

**Velocity Angle Y:** The angle between the velocity vector and the y-Axis (0,1,0).

**Velocity Angle Z:** The angle between the velocity vector and the z-Axis (0,0,1).

**Lineage Statistics**

There are a number of useful statistics that are specific to the Lineage tracking algorithm:

**Generations**

This shows the generation that the selected object belongs to. This is displayed in the format: 0.00 for the initial object, e.g. mother cell and 1.00 for the following 1st generation e.g. daughter cells. Tracked objects without divisions and untracked objects have a generation value of 0.

**Track Number of Generations**

This statistic shows the number of generations present in the selected track.

**Time since first Division**

This shows the time elapsed since the first division of the selected object(s).

**Time since previous Division**

This shows the time between each generation. e.g. the lifespan of an object

**Normalized Time since previous Division**

Displays the normalized division time. This value is only calculated for objects that have a previous division. Only defined for objects that have a previous division and a following division and no fuses (merges).

**Displacement Since Previous Division**

This describes the displacement that has occurred since the previous division. The Displacement<sup>2</sup> (plotted over Time Since Previous Division obtains the "Displacement Squared Plot") and its individual components X, Y and Z (which are analogous to the Position statistics). The values are available only for tracked objects after a track split. If there are multiple objects at the last time point, the most distant one (geometrically) is used for the calculation.

**Time since track start**

Displays time since the start of the track. This is only available only for objects that belong to a track. It is

different to time statistics for objects belonging to tracks that do not start from the first time point. It enables the user to plot the displacement<sup>2</sup> over track time start, as in the example at “Displacement Squared Plot” at [http://bradleymonk.com/Brownian\\_Motion](http://bradleymonk.com/Brownian_Motion).

## Cell Cycle Duration

Time since the previous division for objects that divide again. This is only defined for objects that divide and have a previous division.

## Cell Cycle Displacement

This option introduces multiple values. There is the Cell Cycle Displacement Length, which is equal to the square root of the Displacement Since Previous Division<sup>2</sup> for the last object of a track segment (a track segment is any piece of track that has no bifurcations, a track without bifurcations is constituted by a single segment), and there are the individual components X, Y and Z. As with Track Cycle Duration, the values are only added to the last object of a segment.

### 5.9.9.5 Surfaces

Surfaces statistics are automatically computed for each Surface. These values can be viewed in the statistics tab.

#### Show Statistic Values - Surfaces

#### Surfaces - Acceleration

Acceleration is the change in the Surface velocity over time.

Acceleration X, Y and Z are the components of the acceleration vector (at time-point t):

$$\vec{a}(t) = \left( \frac{\vec{p}(t+1) - \vec{p}(t)}{T(t+1) - T(t)} - \frac{\vec{p}(t) - \vec{p}(t-1)}{T(t) - T(t-1)} \right) / \left( \frac{T(t+1) - T(t-1)}{2} \right), \text{ for } 1 < t < n$$

$$\vec{a}(1) = \vec{a}(n) = \vec{0}$$

Acceleration at time-point t:

$$a(t) = |\vec{a}(t)|, \text{ with } \vec{a}(t) \text{ as defined in}$$

Acceleration X, Y, Z

The acceleration at the first and last time-point is 0.

#### Surfaces - Acceleration X, Y, Z

Acceleration is the change in the Surfaces velocity over time along the selected-axis.

#### Surfaces - Area

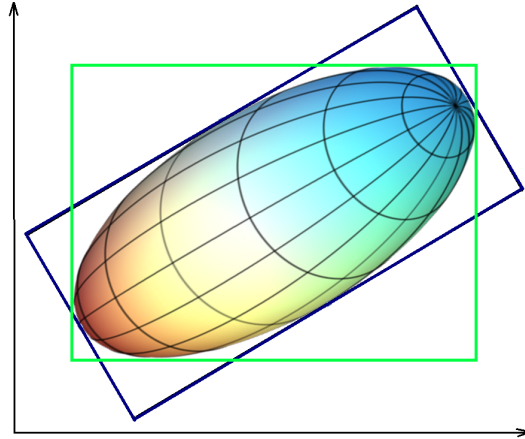
The sum of the triangle surfaces.

#### Surfaces - BoundingBoxAA Length X, Y, Z

A bounding box is a rectangular cuboid in 3D, or a rectangle in 2D, containing an object.

If the bounding box is aligned with the axes of the coordinate system, it is known as an axis-aligned bounding box (AA). BoundingBoxAA identifies an object by considering the minimal rectangular box which fully encloses the object, whose faces are aligned parallel to the axes of a coordinate system.

BoundingBoxAA Length X =  $\max(X) - \min(X)$   
 BoundingBoxAA Length Y =  $\max(Y) - \min(Y)$   
 BoundingBoxAA Length Z =  $\max(Z) - \min(Z)$



axis-aligned bounding box (AA) shown in green  
 object-oriented bounding box (OO) shown in blue

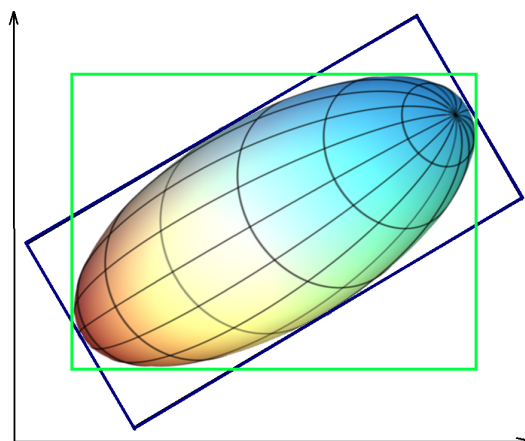
Please note the difference between the ellipsoid axis length and bounding box length.

### Surfaces - BoundingBoxOO Length A, B, C

The local axes of the box can be arbitrarily oriented with respect to the coordinate system.

BoundingBoxOO (object oriented) identifies an object by considering the minimal rectangular box, which fully encloses the object. The box dimensions are calculated with no constraints to the orientation.

BoundingBoxOO Length A = Length of the shortest principal axis  
 BoundingBoxOO Length B = Length of the second longest principal axis  
 BoundingBoxOO Length C = Length of the longest principal axis



axis-aligned bounding box (AA) shown in green  
 object-oriented bounding box (OO) shown in blue

**Please note the difference between the ellipsoid axis length and bounding box length**

## Surfaces - Center of Homogeneous Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The algorithm assumes that every voxel has the same intensity ( i.e. voxel intensities are homogenous).

Assuming a homogeneous intensity, the center of the homogeneous mass is the X, Y, Z coordinate of the object's centroid (geometric center) .

It is the location where all of the mass of the system could be considered to be located. At the center of mass, the entire mass of the body could be replaced with a point mass equal to the body's mass. It is equivalent to the concept of centre of gravity for a body, where the intensity value is a substitute for mass.

The center of mass of a solid body does not have to lie within the body. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{i \in \text{object}} r_i$$

**R** : center of homogeneous mass (X, Y, Z)

**r<sub>i</sub>** : center of a voxel (x<sub>i</sub> y<sub>i</sub> z<sub>i</sub>)

**n** : number of  $i \in \text{Surfaces}$

## Surfaces - Center of Image Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The Center of Homogeneous Mass calculations do not consider a variation of the voxels intensity of the inside the object. In the case of an inhomogeneous distribution of the voxel intensity inside the objects, intensity-based measure may be needed.

The center of mass is calculated using a contribution of each channel and each voxel of the object, based on each voxel intensity value.

Its positions depend on respective intensities of each voxel in the volume.

The Center of Image Mass is the object centroid calculated by taking into account the voxel intensities of each channel separately.

The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{i \in \text{object}} m_i \times r_i$$

**R** : center of image mass (x y z)

**m<sub>i</sub>** : voxel intensity

**r<sub>i</sub>** : center of a voxel (x<sub>i</sub> y<sub>i</sub> z<sub>i</sub>)

**M** : sum of voxel intensities

## Surfaces - Displacement X, Y, Z

The Displacement is a measure the distance that an object moves from the first time point position to its current position. Along each axis, this is calculated by subtracting the first time point position from a selected time point position:



$$\vec{d}(t) = \vec{p}(t) - \vec{p}(1)$$

### Surfaces - Displacement ^2

The square of the distance of the object from the starting point of the track (also known as mean square displacement: MSD) when plotted against the relative time since the beginning of the track:

$$d^2(t) = |\vec{d}(t)|^2, \text{ with } \vec{d}(t) \text{ as defined in Displacement X Y Z}$$

### Surfaces - Distance to Image Border X, Y

### Surfaces - Distance to Image Border X, Y, Z

The value specifies the inward distances from the image border to be removed.

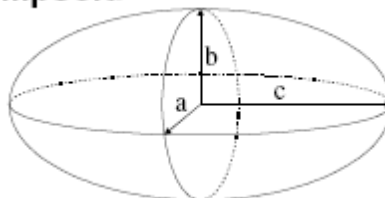
## Ellipsoid Axis

In mathematics, an Ellipsoid is a type of quadric that is a higher dimensional analogue of an Ellipse. The equation of a standard Ellipsoid in an x-y-z Cartesian coordinate system is:

$$\frac{x^2}{a^2} + \frac{y^2}{b^2} + \frac{z^2}{c^2} = 1$$

where a, b and c (the lengths of the three semi-axes) are fixed positive real numbers determining the shape of the Ellipsoid.

### Ellipsoid



## Fitting ellipsoid into the surface

To calculate the dimension of a three principal axes of the best-fitted ellipsoid into the surface, the moments of inertia along the axes of ellipsoid is computed and optimized to match the moment of inertia of the surface. The ellipsoid has homogeneous mass distribution within the surface object. For more information:

[http://en.wikipedia.org/wiki/Moment\\_of\\_inertia#Principal\\_moments\\_of\\_inertia](http://en.wikipedia.org/wiki/Moment_of_inertia#Principal_moments_of_inertia)

**Please note: Only if the shape of the surface matches to an ellipsoid, the lengths of the three ellipsoid axes are exactly equal to the surface axis.**

### Surfaces - Ellipsoid Axis A X, Y, Z

These three parameters define the Vector of the Ellipsoid Axis a.

### Surfaces - Ellipsoid Axis B X, Y, Z

These three parameters define the Vector of the Ellipsoid Axis b.

### Surfaces - Ellipsoid Axis C X, Y, Z

These three parameters define the Vector of the Ellipsoid Axis c.

**Surfaces - Ellipsoid Axis Length A, B, C**  
**Surfaces - Ellipsoid (oblate)**  
**Surfaces - Ellipsoid (prolate)**

If we assume  $a \leq b \leq c$ , then when:

- $a = 0$  it is an **Ellipse**
- $a = b = c$  it is a **Sphere** (three equal sides)
- $a \neq b \neq c$  it is a **scalene Ellipsoid** (three unequal sides)

If two of these sides are equal, the Ellipsoid is a **Spheroid**:

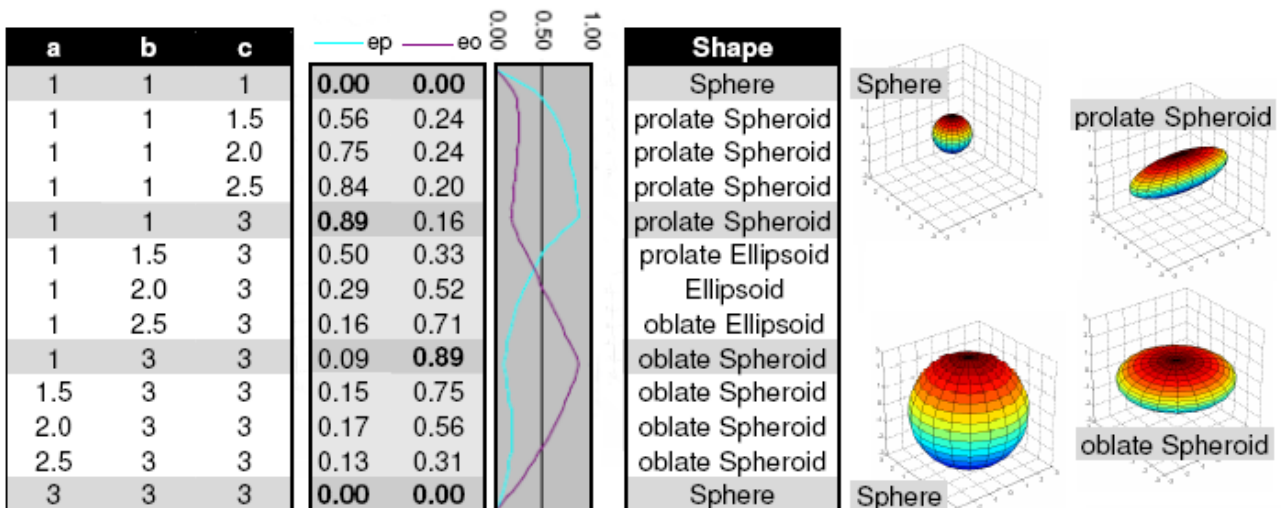
- $a = b < c$  it is a prolate Spheroid (cigar-shaped)
- $a < b = c$  it is an oblate Spheroid (disk-shaped)

$$e_{prolate} = \frac{2a^2}{a^2 + b^2} \cdot \left( 1 - \frac{a^2 + b^2}{2c^2} \right)$$

$e_{prolate}$  = prolate Ellipsoid

$$e_{oblate} = \frac{2b^2}{b^2 + c^2} \cdot \left( 1 - \frac{2a^2}{b^2 + c^2} \right)$$

$e_{oblate}$  = oblate Ellipsoid



**Surfaces - Intensity Center**  
**Surfaces - Intensity Max**  
**Surfaces - Intensity Mean**  
**Surfaces - Intensity Median**

The Surfaces - intensity median is estimated from the intensity histogram.



**Surfaces - Intensity Min****Surfaces - Intensity StdDev****Surfaces - Intensity Sum**

The voxel intensity statistics describe the voxels enclosed within the Surfaces (Center, Max, Mean, Median, Min, StdDev and Sum).

**Surface- Number of Disconnected Components**

The number of disconnected surfaces in the selected Surface object.

**Surface- Number of Disconnected Components per Time Point**

The number of created disconnected surfaces in the selected time point.

**Surfaces - Number of Surfaces per Time Point**

Number of Surfaces per actual Time Point.

**Surfaces - Number of Tracks**

Number of tracks.

**Surfaces - Number of Triangles**

Number of the Surface object triangles.

**Surfaces - Number of Vertices**

Number of the Surface object vertices.

**Surfaces - Number of Voxels**

Number of voxels within the selected Surface object.

**Surfaces - Position X, Y, Z**

The X, Y and Z coordinates of the surface object. The value is equal to the value of the Center of Homogeneous Mass of the object.

**Surfaces - Speed**

The surfaces speed is the instantaneous speed of the object (um/s).The instantaneous speed is the scalar equivalent of the object velocity.

$$s(t) = \frac{|\vec{p}(t+1) - \vec{p}(t)| + |\vec{p}(t) - \vec{p}(t-1)|}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$s(1) = \frac{|\vec{p}(2) - \vec{p}(1)|}{T(2) - T(1)}$$

$$s(n) = \frac{|\vec{p}(n) - \vec{p}(n-1)|}{T(n) - T(n-1)}$$

**Surface - Sphericity**

Sphericity is a measure of how spherical an object is. Defined by Wadell in 1932, the sphericity,  $\Psi$ , of a particle is the ratio of the surface area of a sphere (with the same volume as the given particle) to the surface area of the particle:

$$\Psi = \frac{\pi^{1/3} (6V_p)^{2/3}}{A_p}$$

**V<sub>p</sub>** = volume of the particle

**A<sub>p</sub>** = surface area of the particle

**Surface - Time**

The value is only available for the time series data sets. The number indicates the time associated with each time step for the selected object.

**Surfaces - Time Index**

The value is only available for the time series data sets. The number indicates the number for the current time step in the series.

**Surface- Total Number of Disconnected Components**

Total number of created disconnected surfaces.

**Surfaces - Total Number of Surfaces**

Total number of Surfaces.

**Surfaces - Total Number of Triangles**

Total number of Triangles.

**Surfaces - Total Number of Voxels**

Total number of voxels of the Surface objects within the data set.

**Surfaces - Track AR1Mean**

TrackAR1Mean is the mean of TrackAR1X, TrackAR1Y, TrackAR1Z.

Note for all track statistics splits or gaps are not taken into account- it is taken that the selected tack is continuous.

**Surfaces - Track AR1 X, Y, Z**

TrackAR1X is the AR1 coefficient of the model for the x-positions.

$$AR1X = \frac{R_x^1}{R_x^0}$$

**AR1X** = TrackAR1X

$$R_x^n = \sum_{t=t_f-1}^{t_L-n} (D_x(t, t-1) - \bar{D}_x) \cdot (D_x(t+n, t+(n-1)) - \bar{D}_x)$$

**t<sub>L</sub>** = last time index of track

**t<sub>F</sub>** = first time index of track

$$D_x(t_f, t_l) = P_x(t_l) - P_x(t_f)$$

$P_X(t)$  = x-position of object at time index t

$$\bar{D}_X = \frac{1}{(t_L - t_F)} \sum_{t=t_F+1}^{t_L} D_x(t, t-1)$$

$t_L$  = last time index of track

$t_F$  = first time index of track

### Surfaces - Track Area Mean

The mean of the triangle surfaces along the track.

### Surfaces - Track Center of Homogeneous Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The algorithm assumes that every voxel has the same intensity (voxel intensities are homogenous).

Assuming a homogeneous intensity, the center of homogeneous mass is the X, Y, Z coordinate of the object's centroid (geometric center) .

It is the location where all of the mass of the system could be considered to be located. At the center of mass, the entire mass of the body could be replaced with a point mass equal to the body's mass. It is equivalent to the concept of centre of gravity for a body, where the intensity value is a substitute for mass.

The center of mass of a solid body does not have to lie within the body. Depending on the shape of the object (if the object is concave), the centroid may not be inside the object.

$$R = \frac{1}{n} \sum_{i \in \text{object}} r_i$$

$R$  : center of homogeneous mass (x, y, z)

$r_i$  : center of a voxel ( $x_i, y_i, z_i$ )

$n$  : number of  $i \in \text{Surfaces}$

### Surfaces - Track Center of Image Mass X, Y, Z

The voxel intensity values are taken to be the 'mass' of each voxel.

The Center of Homogeneous Mass calculations do not consider a variation of the voxels intensity of the inside the object. In the case of an inhomogeneous distribution of the voxel intensity inside the objects, intensity-based measure may be needed.

The center of mass is calculated using a contribution of each channel and each voxel of the object, based on each voxel intensity value.

Its positions depend on respective intensities of each voxel in the volume.

The Center of Image Mass is the object centroid calculated by taking into account the voxel intensities of each channel separately.

The X, Y and Z are coordinates of the intensity-weighted centroid of the object.

$$R = \frac{1}{M} \sum_{i \in \text{object}} m_i \times r_i$$

**R** : center of image mass (x, y, z)

**m<sub>i</sub>** : voxel intensity

**r<sub>i</sub>** : center of a voxel (x<sub>i</sub>, y<sub>i</sub>, z<sub>i</sub>)

**M** : sum of voxel intensities

## Surfaces - Track Displacement X, Y and Z

The object's Track Displacement is the distance between the first and last object's position along the selected-axis:

$$t\vec{dl} = \vec{p}(n) - \vec{p}(1)$$

## Surfaces - Track Displacement Length

$$tdl = |t\vec{dl}|, \text{ with } t\vec{dl} \text{ defined in Track Displacement X, Y, Z}$$

## Surfaces - Displacement Delta XYZ

The difference in position of the current object and the previous object of the same track.

## Surfaces - Displacement Delta Length (a scalar value)

The length of the position difference of the current object and the previous object of the same track.

## Surfaces - Track Duration

The Track Duration is the duration between the first and last time point within the Track.

$$td = T(n) - T(1)$$

## Surfaces - Track Ellipsoid Axis A X, Y, Z Mean

These three parameters define the Vector of the track Ellipsoid Axis a.

## Surfaces - Track Ellipsoid Axis B X, Y, Z Mean

These three parameters define the Vector of the track Ellipsoid Axis b.

## Surfaces - Track Ellipsoid Axis C X, Y, Z Mean

These three parameters define the Vector of the track Ellipsoid Axis c.

## Surfaces - Track Ellipsoid Axis Length A, B, C Mean

These three parameters define the length of the Ellipsoid Axis a, b, and c.

## Surfaces - Track Ellipsoid Oblate A Mean

This parameter defines the mean Ellipsoid Oblate for the track (see above for the detailed oblate definition).

## Surfaces - Track Ellipsoid Prolate A Mean

This parameter defines the mean Ellipsoid Prolate for the track (see above for the detailed prolate definition).

## Surfaces - Track Intensity Center Mean

This value describes the mean voxel intensity in the center of the Surfaces.

## Surfaces - Track Intensity Max

This is the maximum value of all maximum (voxel) intensities of the surface along the tracks

## Surfaces - Track Intensity Mean

This is the weighted mean of the objects means, equal to the simple mean of all the intensities of all the objects in a track. Larger objects have a larger impact on the track mean.

#### Surfaces - Track Intensity Median

The median intensity for a track is calculated as the (approximated) median of the (approximated) medians of its objects, instead of the true median of all the intensities in the track, for performance reasons.

#### Surfaces - Track Intensity Min

This is the minimum value of all minimum (voxel) intensities of the surface along the tracks

#### Surfaces - Track Intensity StdDev

This value describes the Standard Deviation voxel intensity of the Surfaces along the tracks.

#### Surfaces - Track Intensity Sum

This value describes the sum of voxel intensity of the Surfaces along the tracks.

#### Surfaces - Track Length

The Track Length is the total length of displacements within the Track.

$$tl = \sum_{t=2}^n |\vec{p}(t) - \vec{p}(t-1)|$$

**Note for all track statistics, splits or gaps are not taken into account- it is taken that the selected track is continuous in nature.**

#### Surfaces - Track Number of Branches

A branch occurs when a Surface object is connected to several other Surface objects of a future time point.

#### Surfaces - Track Number of Fusions

A fusion occurs when a Surface object is connected to several Surface objects of a past time point.

#### Surfaces - Track Number of Surfaces

The total number of Surfaces along the track.

#### Surfaces - Track Number of Triangles

Number of Triangles along the track.

#### Surfaces - Track Number of Voxels

Number of Voxels along the track.

#### Surfaces - Track Position X, Y, Z Mean

Defines the mean value of X, Y, Z track coordinates positions.

#### Surfaces - Tracks Position X, Y, Z Start

Defines the value of the X, Y, Z track start positions.

#### Surfaces - Track Speed Max, Mean, Min, StdDev

Maximum, mean, minimum and standard deviation of the value of the object's speed on the track.

#### Surfaces - Track Speed Variation

The Track Speed Variation is defined as the ratio of the Track Standard Deviation to the Track Speed Mean value. The coefficient of variation is a dimensionless number.

$$v = \frac{\sigma}{\mu}$$

$\sigma$ - Standard Deviation

$\mu$ - Mean value

## Surfaces - Track Sphericity Mean

Describes the mean Surfaces Sphericity along the tracks.

## Surfaces - Track Straightness

$$S = \frac{D}{L}$$

**S** = TrackStraightness

**D** = TrackDisplacement

**L** = TrackLength

## Surfaces - Velocity X, Y, Z

The formula for calculating the velocity  $\vec{v}$  at time point  $t$  is as follows:

$$\vec{v}(t) = \frac{\vec{p}(t+1) - \vec{p}(t-1)}{T(t+1) - T(t-1)}, \text{ for } 1 < t < n$$

$$\vec{v}(1) = \frac{\vec{p}(2) - \vec{p}(1)}{T(2) - T(1)} \text{ and } \vec{v}(n) = \frac{\vec{p}(n) - \vec{p}(n-1)}{T(n) - T(n-1)}$$

## Surfaces - Track Volume Mean

The mean volume of all Surfaces along the tracks.

## Surfaces - Volume

Volume is a quantification of how much space a Surface object occupies.

## Velocity Angle

The velocity angle is the angle between the velocity vector and the corresponding axis.

**Velocity Angle X:** The angle between the velocity vector and the x-Axis (1,0,0).

**Velocity Angle Y:** The angle between the velocity vector and the y-Axis (0,1,0).

**Velocity Angle Z:** The angle between the velocity vector and the z-Axis (0,0,1).

## Lineage Statistics

There are a number of useful statistics that are specific to the Lineage tracking algorithm:

### Generations

This shows the generation that the selected object belongs to. This is displayed in the format: 0.00 for the initial object, e.g. mother cell and 1.00 for the following 1st generation e.g. daughter cells. Tracked objects without divisions and untracked objects have a generation value of 0.

### Track Number of Generations

This statistic shows the number of generations present in the selected track.

### Time since first Division

This shows the time elapsed since the first division of the selected object(s).



**Time since previous Division**

This shows the time between each generation. e.g. the lifespan of an object

**Normalized Time since previous Division**

Displays the normalized division time. This value is only calculated for objects that have a previous division. Only defined for objects that have a previous division and a following division and no fuses (merges).

**Displacement Since Previous Division**

This describes the displacement that has occurred since the previous division. The Displacement<sup>2</sup> (plotted over Time Since Previous Division obtains the “Displacement Squared Plot”) and its individual components X, Y and Z (which are analogous to the Position statistics). The values are available only for tracked objects after a track split. If there are multiple objects at the last time point, the most distant one (geometrically) is used for the calculation.

**Time since track start**

Displays time since the start of the track. This is only available only for objects that belong to a track. It is different to time statistics for objects belonging to tracks that do not start from the first time point. It enables the user to plot the displacement<sup>2</sup> over track time start, as in the example at “Displacement Squared Plot” at [http://bradleymonk.com/Brownian\\_Motion](http://bradleymonk.com/Brownian_Motion).

**Cell Cycle Duration**

Time since the previous division for objects that divide again. This is only defined for objects that divide and have a previous division.

**Cell Cycle Displacement**

This option introduces multiple values. There is the Cell Cycle Displacement Length, which is equal to the square root of the Displacement Since Previous Division<sup>2</sup> for the last object of a track segment (a track segment is any piece of track that has no bifurcations, a track without bifurcations is constituted by a single segment), and there are the individual components X, Y and Z. As with Track Cycle Duration, the values are only added to the last object of a segment.

**5.9.9.6 Volume**

Volume statistics are automatically computed for each Volume object. These can be viewed from the statistics tab.

**Show Statistic Values - Volume****Volume - Data Intensity Mean****Volume - Data Intensity StdDev****Volume - Data Intensity Sum**

The volume intensity statistics describe the voxels of the whole data set (Mean, StdDev and Sum).

**Volume - Data Volume**

The Data Volume is a quantification of how much space an object occupies.

**Volume - Number of Voxels**

Number of the Volume voxels.

**5.9.10 Statistics Tests**

The settings for the Statistics Tests calculated and displayed in the Statistics Tests tab of 1D view can be

viewed or edited as required.

## Common Preferences

Set the Confidence level for all tests. The default is 0.05.

## Wilcoxon Test

Select the type of test performed. Select between Two tails test (default), Left tail test or Right tail test.

## F test

Select the type of test performed. Select between Two tails test (default), Left tail test or Right tail test.

## T Test

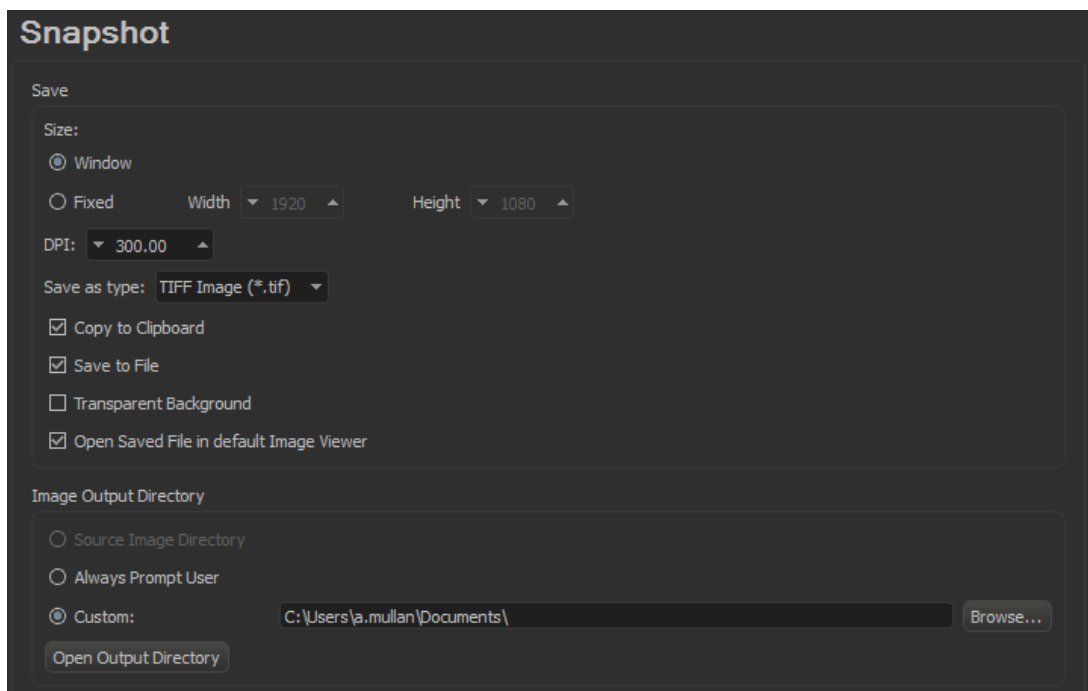
Select the type of test performed. Select between Two tails test (default), Left tail test or Right tail test. Select whether the assumption will be made that the original populations have the same variances. The default is unselected- in this case a *Student's t-test* is performed. If this option is selected, a *Welch's t-test* will be performed.

## Kolmogorov-Smirnov Test

Select the type of test performed. Select between Two tails test (default), Left tail test or Right tail test.

## 5.9.11 Snapshot

Configure the preferences for Snapshot. Note that the default file format is tiff.



## Save

### Size

Adjust the size of the snapshot image. Select the image size either as that of the **Window** size or a **Fixed** size. Set the Fixed size to a required resolution using the Width and Height fields.

**DPI**

Adjust the print resolution of the snapshot image. The default value is 300dpi. This can be increased for higher resolution, or decreased if required.

**Save as type**

Select the file format: TIFF (default), PNG and JPEG).

**Copy to Clipboard**

Set the snapshot image to save to clipboard e.g. so the image may be pasted into a document. Note this mode of copying will not support transparent backgrounds.

**Save to File**

Set Save to file to save the snapshot image directly to file- this will save the image directly to the set image output directory so that multiple snapshot image may be taken quickly and easily.

**Transparent Background**

Save images so that the background of the image is transparent.

**Open Saved File in Default Image Viewer**

Selecting this option will result in the snapshot image opening automatically in your default image browser.

**Image Output Directory**

Set your preferred save location options for Snapshot images.

**Source Image Directory**

Not available when Imaris has been configured for Arena (Arena is the default, recommended option)- this option enables snapshot images to be saved to the source image directory location.

**Always Prompt User**

Select this option to always prompt the save location each time you take a snapshot image.

**Custom**

Imaris will use a logical location by default to save snapshot files to, but you can change this to a more suitable location if required. The current location is shown and the Browse button can be used to set a new location

**Open Output Directory Button**

Open the set output directory to new the folder contents.

**Load and Save Window State**

Custom settings can be saved as a window state- these may be saved and loaded from the View menu options:

**Load Window State** and **Save Window State**.

### 5.9.12 Custom Tools

**ImageJ / Fiji Application**

The **Browse button** allows you to browse to a desired location within the file system.

Here you can specify the Fiji folder in which the Fiji.exe file resides. Either type in the respective path or use the button **Browse**.

Please note: Fiji can be downloaded from the Bitplane Customer Portal.

<http://portal.bitplane.com/>

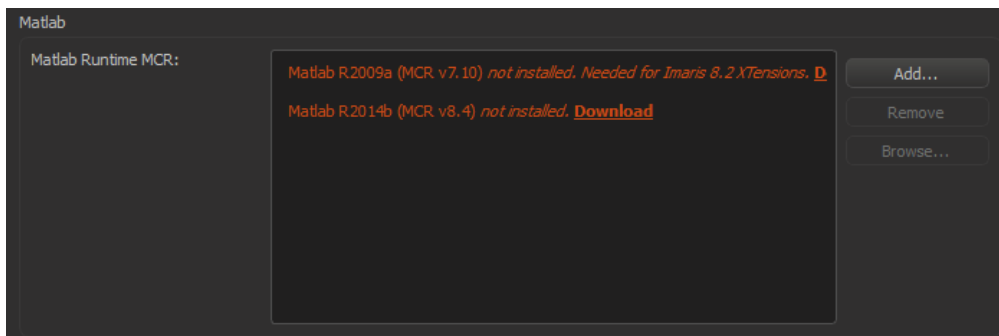
## Default Paths:

Windows: C:\Program Files\Fiji.app\ImageJ-win64.exe

OS X:/Applications/Fiji.app/Contents/MacOS/ImageJ-macosx

## Matlab

MATLAB Compiler Runtime enables the execution of Matlab files on systems without a fully installed Matlab version. Imaris will automatically detect if the required Matlab versions are present, if not, a download link is shown- this is shown in the following image. The Download link accesses the Bitplane Customer Portal <http://portal.bitplane.com/> so that the appropriate MATLAB Compiler Runtime version can be installed.



The **Add** button allows you to add additional compilers to those shown as default e.g. if running different XTensions.

The **Browse...** button allows you to specify the Matlab application if it is not in the default location.

The **Remove** button enable you remove an additional compiler- you can not remove the default versions.

## Python Application

The **Browse** button allows you to specify the Python application.

Either type in the respective path or use the button **Browse**.

## Default Paths:

Windows:C:/Program Files/ /Python27/python.exe

Mac users:/usr/bin/python

## XTension Folders Directories

The **Browse** button allows you to specify the XTensions directories.

Either type in the respective path or use the **Browse** button.

Default Paths (examples shown below for Imaris 9.0)

## Windows:

### Matlab XTensions

\Bitplane\Imaris x64 IMARIS 9.0.0 \XT\matlab\

### Compiled Matlab XTensions

Bitplane\Imaris x64 9.0.0\XT\rtmatlab

### Python XTensions

\Bitplane\Imaris x64 IMARIS 9.0.0 \XT\python

OS X

**Matlab XTensions**

/Applications/Imaris IMARIS 9.0.app/Contents/SharedSupport/XT/matlab

**Compiled Matlab XTensions**

/Applications/Imaris IMARIS 9.0.app/Contents/SharedSupport/XT/rtmatlab

**Python XTensions**

/Applications/Imaris IMARIS 9.0.app/Contents/SharedSupport/XT/python

Please note: Fiji and MATLAB Compiler Runtime can be downloaded from the Bitplane Customer Portal. MATLAB Compiler Runtime enables the execution of Matlab files on systems without a fully installed Matlab version.

<http://portal.bitplane.com/>

**Add Button**

To add a XTensions directory, click on the Add button.

**Remove Button**

Click on the Remove button to remove the selected XTensions directory from the list.

**OK Button**

To apply the changes click on **OK**.

**Cancel Button**

If you do not want to save the changes click on **Cancel**.

### 5.9.13 Licenses

#### Overview

**Node-locked License**

Node-locked licenses grant permission to run the licensed software on one computer (node) only. Node-locked licenses are the easiest way to license the software. You can use the Imaris Administrator to directly download a node-locked license from the Bitplane server. If your computer is not connected to the Internet it is also possible to download this license from another computer and then copy it to the target machine. Please refer to the offline license method for further instructions.

**Floating License**

Floating licenses grant permission to run the licensed software on several computers. One floating license can activate only one instance of the program at any given time. Floating licenses require the installation of a separate license server that runs on a Windows computer. The server installation requires more technical know how.

The Imaris Administrator application easily let you enable a floating license.

**Demo License**

A Demo License is a time-limited (usually one month) license. It is not an official licensing scheme and therefore can't be purchased. It's main purpose is for internal Testing and to grant customers with licensing issues an easy and quick workaround so that their productivity can continue until the problem is solved. Simply open the Imaris Administrator application and open the Demo License you received from your local support representative.

**Evaluation License**

Evaluation Licenses are temporary licenses allowing customers to explore all fascinating possibilities Imaris offers before actually purchasing it. The license is only limited in time (10 days), but not in features. Additionally an Evaluation License is issued per user.

To get an Evaluation License you first have to register at:

<http://www.bitplane.com/go/download/imaris-evaluation>

An automatic mail response should provide you with your personal RegistrationID and its corresponding Passcode. These credentials can then be entered during the application's start up.

## **Imaris Open Developer License**

To get an Imaris Open Developer License you first have to register at:

<http://open.bitplane.com/>

If you can install multiple license locations, (Floating license and a Node-Locked license), Imaris will search all the locations until it finds the license for the requested features.

This Section contains information related to licensing management, options and procedures. It includes the following sections:

[Preferences Dialog](#)

[Installation of Licenses](#)

[Imaris Administrator](#)

### **5.9.13.1 Preferences Dialog**

Imaris Administrator enables the licenses of various Bitplane applications (and Imaris data and system settings) to be managed by the Administrator. It provides a simple interface to add or delete any licenses. When a license is added, the license file gets copied to a system folder where it is secure from (accidental) manipulation by standard users. Although it is possible for administrators to modify license files directly in this system folder we strongly advise you not to do so. It is recommended to go through the Imaris Administrator application instead.

## **Imaris Administrator**

This starts the Imaris Administrator. Unless you want to use an Evaluation license you need to use the Imaris Administrator to set up your licenses.

**Please note that you'll need administrative privileges in order to start the Imaris Administrator.**

Any change of licenses will require Imaris to be restarted.

Except the Evaluation License which is bound to a single user, all other licenses are bound to the whole system. Therefore any license modifications need [administrative privileges](#).

If you have access to an administrative system account, Imaris Administrator will start normally.


## **Administrate**


This button starts Imaris Administrator. This is a tool shipped with Imaris in order to add or modify licenses. Unless you want to use an Evaluation license you need to use Imaris Administrator to set up your licenses. Please note that you'll need administrative privileges in order to start Imaris Administrator.

Any change of licenses will require a new start of Imaris.



## Evaluation License

After you entered your RegistrationID and Passcode you can click on the Light Bulb button  to activate the Evaluation License.

This action requires an internet connection because the licenses are downloaded directly from the Bitplane server. Upon success, the light bulb will turn on .

You can also disable your Evaluation License by clicking again on Light Bulb button and the light bulb will turn off. This indicates that you aren't using any Evaluation Licenses.

Any change of licenses will require a new start of Imaris.

### RegistrationID


Please enter your RegistrationID you received by mail previously.

### Passcode

Please enter your Passcode you received by mail previously.

## Imaris Open Developer License

After you entered your OrderID click on the Imaris Open button  to activate the Developer License.

This action requires an internet connection because the licenses are downloaded directly from the Bitplane server. Upon success, the button will turn on .

Any change of licenses will require a new start of Imaris.

### RegistrationID

Please enter your RegistrationID you received by mail first.

## Available Licenses

The table displays all existing Imaris licenses on the system and additional information for each feature:

### Version

The latest Imaris version this license can be used with.

### Type

Which type of licensing scheme is used. An overview is given in the Licenses section.

### Status

Displays the status of a license.

### Expiration

Shows the number of days until this license expires.

### License colors

In order to gain a quick overview, the validity of a license is also indicated by different colors:

- Green: License is valid.
- Yellow: License is valid, but all licenses are currently in use.
- Red: License has expired.
- No color: License is not available (e.g. this additional license feature hasn't been purchased).

You can select with which license(s) your Imaris should start per default. Any change will require a new start of

Imaris.

## Check/Uncheck All

Check this box to enable or disable all available Imaris licenses on the system.

Please note that the Feature “ImarisBase” can't be disabled because this would render Imaris unusable.

## 5.9.13.2 Installation of Licenses

### First-time Installation

If you have installed Imaris for the very first time, you have to set up a license before you can use Imaris.

A dialog window appears during the start up of the application.

The same dialog window appears if you have accidentally deleted the license .

You now have two options how to proceed:

- If you have a Node-locked or a Floating License you should use [Imaris Administrator](#) application for the further license setup. This is done by simply clicking on the “Start Imaris Administrator” button. Please note that Imaris Administrator application needs administrative privileges.
- If you want to evaluate Imaris first, please register at:  
<http://www.bitplane.com/go/download/imaris-evaluation>

The automatic mail response contains your Registration ID and Passcode.

To enable an Evaluation License enter these values in the corresponding fields and click on the “Use Evaluation License” button.

Please note that a working internet connection is needed to start up Imaris in Evaluation mode.

### Installation of Additional Licenses

If you already have set up a license but you want to add other licenses as well, you can do this in the License Preferences dialog.

Once more, you'll have the option either to start Imaris Administrator application or to use an Evaluation License in addition if you would like to try out a new feature before buying the license.

## 5.9.14 Update

Here you can adjust the default update settings.

### How often should you search for updates?

Daily

Weekly

Never

Select either daily, weekly, or never.

Button **OK**

To apply the changes click on **OK**.





**Button Cancel**

If you do not want to save the changes click on **Cancel**.

### 5.9.15 Usage Data

Here you can enable the capturing of data usage.

**Button OK**

To apply the changes click on **OK**.

**Button Cancel**

If you do not want to save the changes click on **Cancel**.

### 5.9.16 3D Cursor

Here you can adjust the default 3D Cursor settings.

#### 3D Cursor

**Box****Sphere****Cross****Torus**

Here you can select the shape of the 3D Cursor.

**Size****Color**

Here you can select the size and color of the 3D Cursor.

#### Animation

**Frequency**

Adjust the animation frequency.

**Amplitude X****Amplitude Y**

Adjust the amplitude in x-, and y-direction.

**Shear****Rotate**

Select shear or rotate as animation mode.

**Continuous****Discrete**

Select continuous or discrete as animation sequence.

**Button OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

## 5.9.17 Annotate

Here you can adjust the default Annotation settings.

### Format

**Title Format**

**Text Format**

**Legend Format**

Choose title format, text format or legend format and adjust the following parameter set.

### Title, Text or Legend

**Font**

**Font Size**

**Color**

**Format**

**Bold**

**Italic**

**Underlined**

The font, font size, color, and format can be selected (from any installed system font).

**Color Selection** Button

Click on this button to open the color selection window to change the respective color.

### Style

**Arrow Color**

**Arrow Width**

**Arrow Style**

**Text Box Color**

**Text Box Transparency**

**Text Box Style**

You can adjust the arrow color, width and style (Sphere, Arrow, Magnifier), text box color, transparency and style (Unicolor, Black Text, Black Title).

**Color Selection** Button

Click on this button to open the color selection window to change the respective color.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

### 5.9.18 Arena

Change the default storage location for image data sets from the Arena menu of Preferences. This can be either a local drive or remote location e.g. a network server enabling access to files across a network.

If Imaris detects existing data at the new location it will use this data and not overwrite it. If it doesn't detect existing data it will copy the data from the previous storage location.

To see how to modify the Arena Storage location refer to [Modify Arena Storage Location](#).

#### Notes:

- Imaris file server authentication is a secure form of authentication because a hashed version of the password and user name are sent in a cryptographic exchange with the server.
- Imaris 8.3 onwards supports the SMB application layer network protocol which is the default protocol on Microsoft Windows OS and supported by Apple OSX.

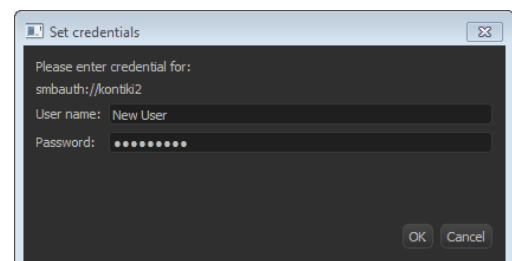
### Using Remote Data Storage Locations

When using a Remote Share location to store data sets you must enter authentication credentials to enable access and sharing of files.

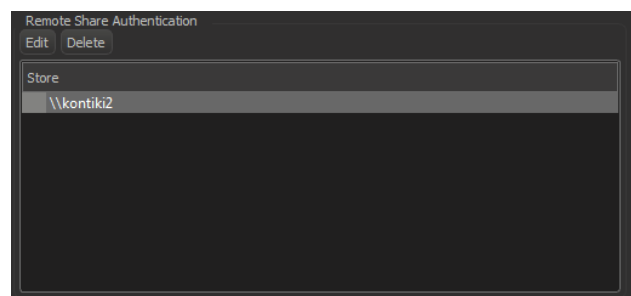
Enter your credentials: a user name and password, when required.

When an incorrect user name or password is used, please retype your credentials and try again.

Then click **OK** to continue.



Remote Share locations that have been set-up are listed under **Store**. Use the **Edit** and **Delete** buttons to manage the Remote Share locations for images.

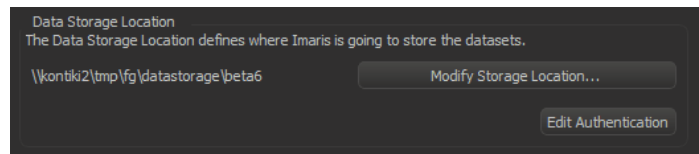


#### 5.9.18.1 Modify Arena Storage Location

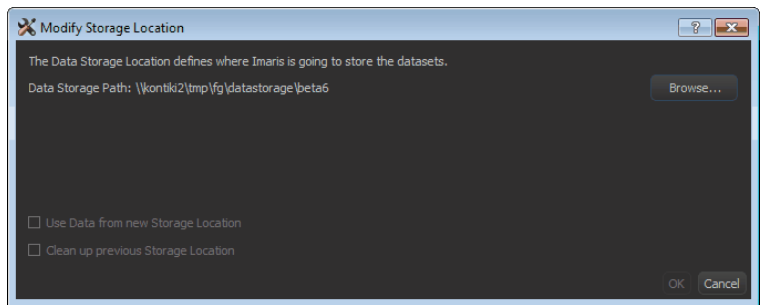
##### Changing the Data Storage Location

To change the storage location proceed as follows:

1. Click **Modify Storage Location**.



2. Click **Browse...**



3. Select the location where Imaris will store the data sets.

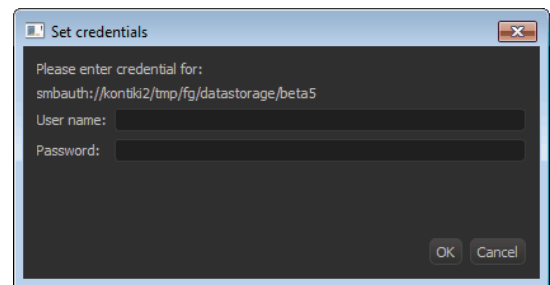
4. Click Select Folder.

The following options may be shown:

**Use data from new Storage Location:** For when you set the data store to a location which previously was used as a data store location. In this instance Imaris will construct the Arena view from the new location, data in the previous location will not be changed or integrated into the new Arena view.

**Clean up previous Storage Location:** For when you migrate the data to a new location so data is going to be copied to this new store. Once all the data has been copied to the new storage location the previous location will be emptied if this option is checked.

5. Enter the authentication credentials (This step is not required if using local file locations on your PC).



6. The data storage path is now updated.

## 5.9.19 Advanced

Displays a window similar to the parameters window. It shows internal settings and parameters in groups and parameter lists.

**Care must be taken when making changes as changes to parameters may affect performance of Imaris. It is therefore recommended to use the default values for most situations.**

### Reset Preferences Button

Click on this button reset preference values to their default settings.

**OK Button**

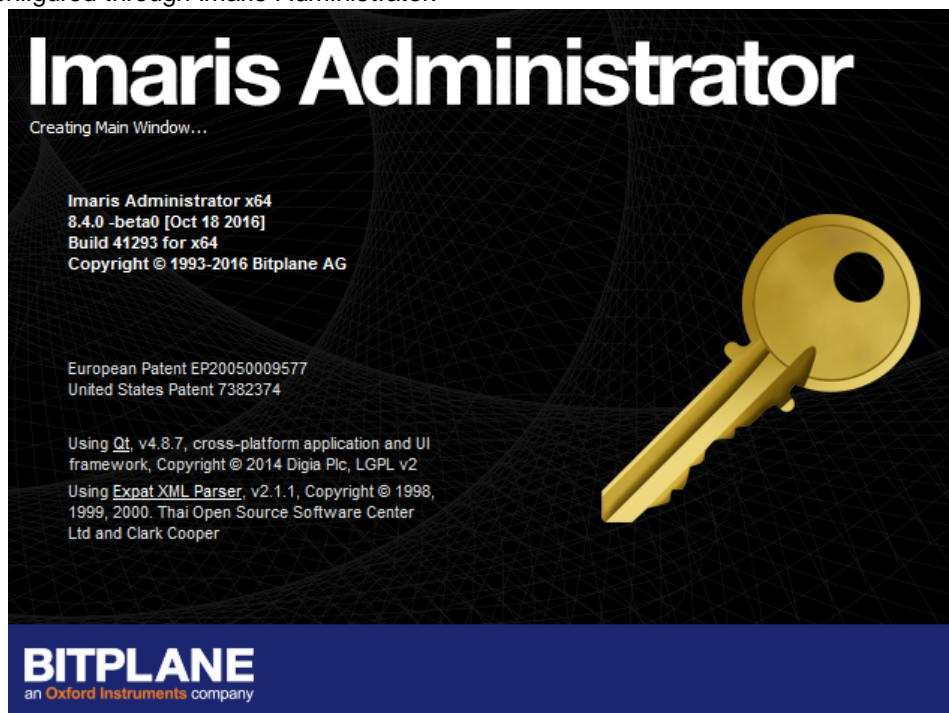
To apply the changes click on **OK**.

**Cancel Button**

If you do not want to save the changes click on **Cancel**.

**5.9.20 Imaris Administrator**

This Section contains information related to licensing management, and the data storage and system options that can be configured through Imaris Administrator.



This Section includes the following sections:

License Management:

[Administrative Privileges](#)

[License Information](#)

[Add License](#)

[Data Management](#)

[System Preferences](#)

[Advanced Preferences](#)

You will need to restart Imaris for any changes to take effect.

**5.9.20.1 Administrative Privileges**

There are some cases where the proper privileges can not be provided automatically and requires you to start the application manually.

**Windows 7:**

If the User Account Control (UAC) is disabled and you are logged in with a standard user no elevation prompt is

shown because the UAC setting omits these prompts. Therefore you can't get the right administrative permissions and you will have to start Imaris Administrator manually. This is done by opening the executable by a right click while holding down the "Shift" key. Then you can select "Run as different User" which prompts you for another account.

## Mac OS X:

No cases known where problems occur.

## Default Imaris Administrator Paths

### Windows 7, 8 and 10

64-bit: C:\Program Files\Bitplane\Imaris 9.0\ImarisAdministrator.exe

### Mac OS X

/Applications/Imaris 9.0.app/Contents/MacOS/ImarisAdministrator

## 5.9.20.2 License Information

### Types of license

A description of the license types available is given in the [Licenses](#) Section.

### License Location

If you did not have set up a license yet (e.g. first-time usage) this window is empty. In that case, please use the **Add License Location** button to add a new license location.

#### Add License Location Button

Use this button to add new licenses. A description is given in the [Add License](#) Section.

#### Delete License Location

If you click this button, the selected license location entry in the table underneath will be deleted. Before using this button, please make sure you have selected the right table entry. In the case of a node-locked license, you are asked if you want to keep the license. It is recommended that you keep all license files.

#### License Location Table

This table lists all available license locations. Normally it would be just enough to have one license location defined. But there are also situations in which it is useful to use multiple locations.

For example you might have a floating license server with just some basic features enabled. Maybe later you purchased some additional features in a node-locked licensing scheme. You can now just define both locations and the licensed features are merged on your system automatically.

The table shows also some additional Information:

- Type: This is either a License Server (floating license scheme) or a License File (all other schemes)
- Number of valid / total Licenses: These numbers show you how many different features your license contains and it lets you easily verify that all licenses are still valid.

If you click on another table entry, the License table underneath gets updated and shows you detailed information about all available features of the current selected license location.

### Licenses Table



In this table you have a detailed overview of all licensed features of a license location. You can switch among different license locations by selecting the corresponding entry in the License Location table.

Each feature provides some information :

**Version**

The latest Imaris version this license can be used with.

**Type**

Which type of licensing scheme is used. An overview is given in the Licenses section.

**Status**

Displays the status of a license.

**Expiration**

Shows the number of days until this license expires.

**License colors**

In order to gain a quick overview, the validity of a license is also indicated by different colors:

- Green: License is valid.
- Yellow: License is valid, but all licenses are currently in use.
- Red: License has expired.
- No color: License is not available (e.g. this additional license feature hasn't been purchased).

**Finish Button**

This button is only enabled if you have added at least one license location. It is an indicator when all necessary steps to set up a license are done. If all features in the above License table are highlighted in green (or only some of them) you successfully managed to add a license. If you click the button, it just closes Imaris Administrator and you can proceed using Imaris.

### 5.9.20.3 Add License

First, you have to select your licensing scheme:

#### Download Node-locked License

If you decide to add a Node-locked License, the software will verify that your computer is connected to the internet. If the connection could be established successfully, the Online-Licensing Wizard is shown; if not, you have the possibility to download the license from another computer which is described in the Offline-Licensing Wizard.

#### Online-Licensing Wizard

This wizard has three steps:

1. Select the Network Interface

You need to select the network interface which is bound to the license. If your Host-ID is already known to our server, the available product for installation will be shown in the table below.

2. Check your Order-ID for newly ordered Products:

If you have ordered new products, you have to enter the Order-ID in this step. Clicking on the "Check Order-ID" button will add the products to the table below. Once you checked an Order-ID, it is bound to the currently selected network interface.

If your Host-ID is already known to the system, the available products will be shown in the tables below and you could skip that step.

3. Select the Licenses for Installation

In this step you just have to mark all products you want to install by checking them in the left table.

The right table is for your information only and shows all currently installed products.

When you click on the "OK" button, the license file is downloaded and the corresponding entry is added to the License Location table.

## **Offline-Licensing Wizard**

If you are not connected to the Internet, this could be due to either of the following:

1. your computer is behind a web proxy server which does not permit a direct connection or
2. the PC is unplugged from the internet.

Therefore you have the following choices:

### A) Set up a Proxy Server

Enter your proxy server's name and its port and click on the **Test Connection** button. If the connection could be established you are automatically redirected to the Online-Licensing Wizard described above.

If not, either the credentials you entered are not correct or the connection is broken at another place in the network. In that case please contact your network administrator or Bitplane Support teams [eusupport@bitplane.com](mailto:eusupport@bitplane.com) or [ussupport@bitplane.com](mailto:ussupport@bitplane.com)

If you want to delete a previously set up proxy server, just delete both fields and click on the button again. The proxy server settings can also be altered in Imaris' system preferences dialog.

### B) Download the Node-locked License from another Computer

You can download the license from another computer connected to the internet.

On that machine log on to [http://www.bitplane.com/activation/acti\\_offline\\_ident.html](http://www.bitplane.com/activation/acti_offline_ident.html)

and enter the Host-ID you wish to bind to the license together with your Order-ID into the web form. The download of the license should immediately start and afterwards you could transfer the file to the offline computer (e.g. by using a USB stick).

The instructions can be saved in a file by clicking the **Save Instructions...** button.

When you have downloaded the license you can just open it with the **Open License...** button or enter its path directly into the field on the left of the button.

When you click **OK** the license file is copied to the proper location.

## **Define Floating License Server**

In order to add a Floating License Server, just enter the server's name and its port to the corresponding fields and click on the **OK** button. The default port is 2700.

## **Install License from Existing File**

If you received a Demo License from your local support representative or you need to add a license file directly you can use this wizard. If you click on the **Open License...** button you can browse to the license you want to add. You can also specify the license file to load by entering its path directly into the field left from the button. If you click on the **OK** button, the license is copied to the right location and it's added to your License Location table.

## **Show floating license selection dialog upon start of Imaris**

This option enables you to enforce active individual selection of all Imaris add-on modules licenses and secure the optimal use of the licenses available on the server. It prevents unintentionally overuse of the purchased add-



on modules licenses and helps you with your short- and long-term plans for Imaris licenses spending and allows you to better control your software cost.

Select the Show floating license selection dialog upon start of Imaris and you are configuring the [License Selection](#) table to starts automatically. So every time Imaris is started Imaris, the License Selection table is displayed for each user.

#### 5.9.20.4 License Selection

It is important to understand that the License Selection option is not designed to minimize your Imaris usage. It lets you manage and optimize use of all Imaris add-on modules licenses that are available within your license purchased pool.

When you launch Imaris, the License Selection table will be shown. With the License Selection table the user has the flexibility to mix and match add-on modules and to suit changing scientific requirements in a flexible form. The License Selection table allows selection of all add-on Imaris modules to be simultaneous used by any number of users up to the maximum number of licenses agreed upon at the time of purchase.

The table displays only the Imaris add-on module license that does not match the overall number of purchased Imaris basic licenses.

This table displays: Imaris product, feature, version, license type and status and expiration date.

From the modules lists, you can select the required license.

Choose from the table the modules that are needed and available and select only the add-on modules that will be needed for the current Imaris session. Activate your license by clicking the appropriate check-box.

After activating the license, for the selected module the total license number available in the pool is reduced.

Once all available licenses are checked out (the activated license number has been exceeded the maximal number of license have been purchased), the corresponding Imaris product will not be shown within the table.

The product will not be available for your future selection until one of the current users is finished and the license is checked back into the licensing pool.

#### Do not show this dialog

If you select this option, you won't have a license table opened on your system, and the opt-in licenses file will not be available for your future selection.

#### 5.9.20.5 Data Management

The administrator can set the default location that the data set and batch data files are saved to using **Set Data Storage Path**. This can be either a local, or a remote network server location. This is described in the [Arena](#) Section. The user can change the default location if they require.

#### 5.9.20.6 System Preferences

System Preferences allows an administrator to set the key system parameters for Imaris users. Default values are detected for the computer by the software and automatically set in the Number of Threads, Data Cache Memory Limit and Texture Cache Limits fields. These values can be set manually if required by free typing or using the up and down arrows in each field.

System Preferences are set by default so they are editable by the user. This can be disabled by deselecting the **Is editable by User** check-box.

Select **Reset to Factory Defaults** to change any modified values back to the original default values. Click the **Finish** button when you are finished making changes. You will need to restart Imaris for any changes to take effect.

Care must be taken when making changes as changes to parameters may affect performance of Imaris. It is therefore recommended to use the default values for most situations. Refer also to the [Calculation](#) Section.

## Calculation

The number of processing threads can be adjusted from the default value depending on the system resource requirements of the computer running Imaris.

## Data Cache

Adjust the size of the **Data Cache Memory Limit** and set and select the **Data Cache File Path** to be used.

## Display

Set the maximum memory limit allocated to the texture cache used for display. Adjusting the Texture Cache Limit can be used to improve image quality or performance or according to the size of the images and the computer resources available.

## Settings

Set the frequency for maintenance and software updates. The default setting is Weekly.

## Usage Data

Enable or disable the capturing of usage data. Refer also to the [Usage Data](#) Section.

### 5.9.20.7 Advanced Preferences

In Advanced Preferences it is possible to edit parameters in Imaris and set whether they may be editable by the user under the Advanced option of preferences. Care must be taken when making changes as changes to parameters may affect performance of Imaris. It is therefore recommended to use the default values for most situations. To return values to the original default values select **Reset to Factory Defaults**. Click the **Finish** button when you are finished making changes. You will need to restart Imaris for any changes to take effect.

## 5.10 Exit

Terminates Imaris and returns to the desktop.

## Section 6 Edit




<a href="#">Undo</a>	Ctrl + Z
<a href="#">Snapshot</a>	Ctrl + C
<a href="#">Image Properties...</a>	Ctrl + I
<a href="#">Show Display Adjustment</a>	Ctrl + D
<a href="#">Annotate</a>	Ctrl + Shift + P
<a href="#">Add Time Points...</a>	
<a href="#">Delete Time Points...</a>	
<a href="#">Add Channels...</a>	Ctrl + Shift + A
<a href="#">Delete Channels...</a>	Ctrl + Shift + B
<a href="#">Add Slices...</a>	
<a href="#">Delete Slices...</a>	
<a href="#">Remove images</a>	
<a href="#">Crop Time...</a>	
<a href="#">Resample Time...</a>	
<a href="#">Crop 3D...</a>	Ctrl + W
<a href="#">Resample 3D...</a>	
<a href="#">Change Data Type...</a>	
<a href="#">File Type Associations...</a>	

See also:

Addendum - [Mouse & Keyboard Mac](#)

### 6.1 Undo

The Undo command reverses the last action, when necessary. It supports multiple undo actions, starting with the most recent one and working backwards in sequence.

All actions in the Draw , Edit  and Edit Tracks  tabs as well as the image processing functions can be undone by selecting the Undo command or by pressing **Ctrl+Z**.

The number of any previously applied command that can be Undone/Redone is defined in the [Edit menu-Preferences-Calculations-Maximum Number of Commands in History](#) field. By default, the number of steps is 4.

### 6.2 Redo

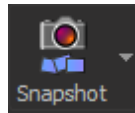
The Redo command repeats the last action, if appropriate. The command supports multiple redo actions, starting with the most recent one and working backwards in sequence.

All action in the Edit, Edit tracks, and Draw tabs as well as image processing functions can be redone by selecting the Redo command or by pressing **Ctrl+Y**.

The number of any previously applied command that can be Undo/Redo is defined in the [Edit menu-Preferences-Calculations-Maximum Number of Commands in History](#) field.

## 6.3 Snapshot

The snapshot button in the main toolbar immediately produces a snapshot of the current view. This may be either the image is Surpass, or a 2D, 3D or 4D Vantage plot as it currently appears in the viewing area. It creates a new file containing your Vantage plot as well any scale information, measurements data, and box plots that are displayed.



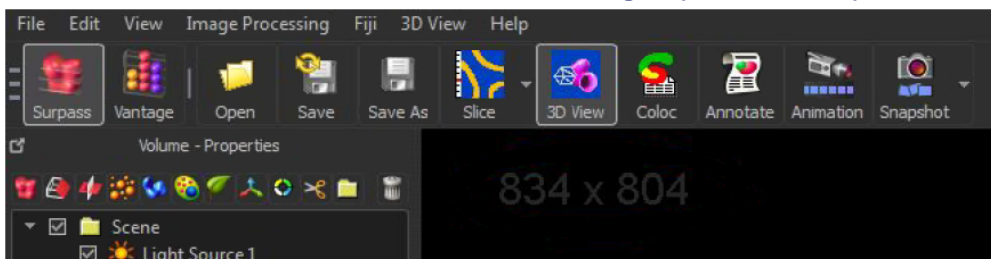
By default the snapshot is saved to a file in the source image directory and after creating the snapshot it is opened in the default image viewer to show the user what the snapshot looks like. The snapshot is by default also copied to the operating system clipboard for use in other applications.

To create the snapshot, simply click the Snapshot button.

To view and adjust the settings of the snapshot, click Preferences from the Snapshot button drop-down box. Refer to [Snapshot Preferences](#) for further information.

### Notes:

- To help the user see the size of the screen render area, Imaris displays this size in the top left hand corner of the render-area whenever the size is changed (shown below).



- The view-menu has “Save Window State” and “Load Window State” options which function on a per viewer basis, to save and load the size and visibility of all windows.
- Snapshots always have the same aspect ratio as the render-area of the current view. (In previous versions of Imaris it was possible to create snapshots with aspect ratios differing from those of the current view).

## 6.4 Image Properties...

[Geometry](#)

[Data Set](#)

[Channels](#)

[\(Channel 1...n\)](#)

[Thumbnail](#)

[Parameters](#)

When opening a data set, the following parameters should be checked or modified as required:

- Name and Description (in Data Set).
- Voxel Sizes (in Geometry).
- Channel Colors (in Channel 1...n).

### 6.4.1 Geometry

The geometrical settings of the actual data set are displayed.

#### Type

##### Data Type

Display of the image type.

#### Size

##### Size X, Y, Z, T

Display of the image size.

##### Image size:

Shows the uncompressed image size.

#### Coordinates (um)

##### Voxel Size, X, Y, Z

The voxel sizes directly influence the views because they control the height of the image relative to its width. Check the parameters and adjust the **Voxel Size** and/or other settings if necessary.

##### Min, X, Y, Z

The minimum value of the coordinate axes.

##### Max, X, Y, Z

The maximum value of the coordinate axes.

#### Time Point

##### First Box

Select the time point.

##### Date

Enter the collection data.

##### Time

Enter the collection time.

##### Button **All Equidistant...**

If the data set is a time series, enter the date/time for each time point or click **All Equidistant** to open the Set Equidistant Time Points dialog box.

- Enter the **Start Date** and **Start Time** and the **Time Interval**.
- Click **OK** when finished. Imaris will calculate the time for each time point in the series.

The data set must be saved to retain the changes. Click **OK** when finished or select another heading for further adjustments.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

## 6.4.2 Data Set

### Name

Data field to enter a data set name.

### Description

Data field to enter a data set description.

### Numerical Aperture (N.A.)

Reads out the numerical aperture (as defined in the menu Edit - Image Properties... - Parameters).

### Log

Display of processing steps.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

## 6.4.3 Channels

There are no parameters available on this menu.

## 6.4.4 Channel 1...n

In the Index of the Image Properties box click the **Channel** entry (Channel 1, Channel 2 etc.) to select the required channel.

### Name

Data field to type in the channel name.

### Description

Data field to type in a channel description.



**Emission Wavelength**

Reads out the emission wavelength.

**Excitation Wavelength**

Reads out the excitation wavelength.

**Pinhole Radius**

Reads out the pinhole radius.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

**6.4.4.1 Tab Base Color****Red, Green, Blue**

Reads out the assigned color. To change the color either adjust the values or move the square in the color circle.

To apply the changes click on **OK**.

**6.4.4.2 Tab Mapped Color****Selected Color**

Button **Edit...**

Click on a square in the grid and click on the button **Edit...** . Select a color and click on **OK**. The selected color is displayed in the square.

Button **Copy**

Click on a square in the grid and then on the button **Copy** to copy the color.

Button **Paste**

Click on a square in the grid and then on the button **Paste** to paste the color.

**Interpolation****Colorspace****RGB**

The RGB color model is an additive model in which red, green and blue are combined in various ways to reproduce other colors.

**HSV**

The HSV (Hue, Saturation, Value) model, defines a color space in terms of three constituent components.

- Hue, the color type.
- Saturation, the vibrancy of the color.
- Value, the brightness of the color.

### Button **Interpolate**

Select two squares in the grid and click on the button **Interpolate**. The colors between the two selected squares are interpolated.

### Color Table File

#### Button **Import ...**

Imaris comes with a set of pre-defined color tables. Click on **Import...** to open the Import Color Table File window. Select a color table file and click on **OK**.

#### Button **Export...**

Click on the button **Export...** to open the Export Color Table File window. Select a destination and click on **Save**.

A large number of predefined colormaps are available. If the default colormap (Spectrum) is not appropriate for your application, you can change it by clicking on the drop-down list. The list gives a preview of what different colormaps look like.

To apply the changes click on **OK**.

### 6.4.4.3 Tab Coloc Statistics

The tab Coloc Statistics is available when a data set contains a Coloc channel and the Coloc channel is selected in the channel selection of the Image Properties.

All statistics about the resulting colocalized volume are displayed. Definitions of the displayed values and further information are given in Section Coloc - Volume Statistics.

#### Button **Export...**

Click on the button **Export...** to open the Export Color Table File window. Select a destination and click on **Save**.

See also:

Coloc - [Volume Statistics](#)

### 6.4.5 Thumbnail

#### Type

##### **None**

Select None if no thumbnail should be displayed.

##### **Middle Slice**

Thumbnail is the middle slice.





**MIP**

Thumbnail in the display mode Maximum Intensity Projection. A Maximum Intensity Projection is a computer visualization method for 3D data that projects in the visualization plane the voxels with maximum intensity that fall in the way of parallel rays traced from the viewpoint to the plane of projection.

**Blend**

Thumbnail in blend projection. Blends all values along the viewing direction and includes their transparency.

**Preview**

Displays a preview of the thumbnail image.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

If you do not want to save the changes click on **Cancel**.

## 6.4.6 Parameters

A set of informational parameters is appended to the image file.

Button **Add Surpass Group...**

Opens a dialog to add a new group.

Button **Delete Surpass Group...**

Deletes a Surpass group.

Button **Add Parameter...**

Opens a dialog to add a new parameter to the group.

Button **Delete Parameter...**

Deletes a parameter.

Button **OK**

To apply the changes click on **OK**.

Button **Cancel**

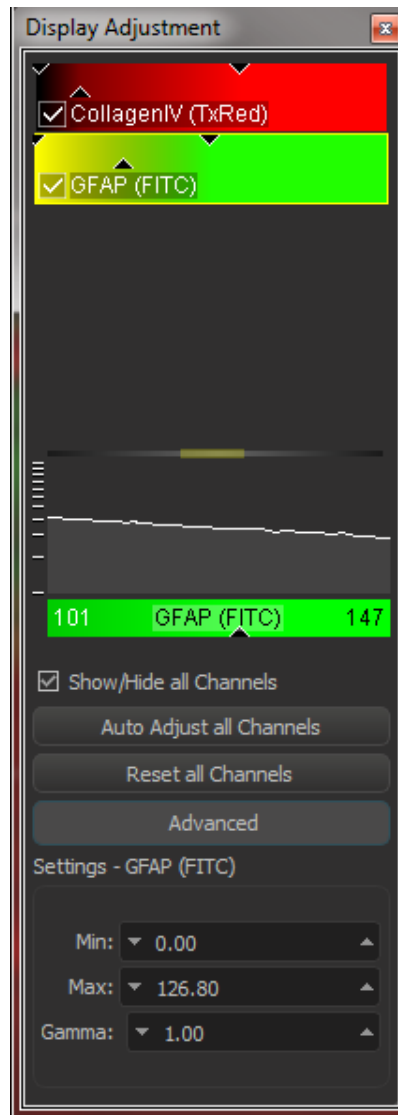
If you do not want to save the changes click on **Cancel**.

## 6.5 Show Display Adjustments

The Display Adjustment options let you choose the channel visibility as well as improve the image display by concentrating on a limited color contrast range of voxels. Usually the color contrast values of the voxels stretch over a wide range (e.g. 0 - 255).

The Display Adjustment function lets you set an upper limit for maximum color and a lower limit for minimum

color (i.e. black). The range between these two limits - represented by triangle symbols, is then extrapolated in a linear mode to the full data set range and the new voxel values are calculated.



## Display Adjustment Dialog (one for each channel)

Switch the individual channels on or off.

- Check or uncheck the required channel check-box to switch the channel visibility.

Change the channel parameters such as name, color and description.

- Click on the channel name to open the Image Properties. For a detailed description please refer to Section Menu Edit - Image Properties - [Channels 1...n](#).

### Show/Hide all Channels

Check or uncheck to show or hide all channels as required.

### Button Auto Adjust all Channels

The auto function works as follows: the minimum value is set to the first mode (maximum) of the histogram. The maximum value is set to a value that is around the 99.8 percentile of the histogram which effectively disregards high intensity outliers.

**Button Reset all Channels**

Click on the button to set the image back to the original values.

**Button Advanced**

Click on the button to open the Advanced settings (see below).

**Advanced Settings****Min****Max**

- Enter direct values in the Min (lower limit for minimum color) and Max (upper limit for maximum color) fields.
- Alternatively drag in the display adjustment dialog the upper or lower handle of the adjustment line to adjust the Min and Max limits.

The effect of the change can be seen on the channels (channels appear brighter or darker).

**Gamma**

The default value of the gamma correction is 1 (the range between lower and upper limit is extrapolated in a linear mode to the full data set range). Enter a value below 1 and the linear mode is transferred to a nonlinear mode, the lower intensities appear brighter. The effect of the change is directly visible in the viewing area.

- Enter the value in the respective field.
- Alternatively click onto the middle triangle in the display adjustment dialog and drag it to the left to increase brightness/to the right to decrease brightness.

**List of Shortcuts in the Display Adjustment Dialog**

Please refer to Section Menu Edit - Show Display Adjustments - [Mouse & Keyboard PC](#).

Please refer to Section Menu Edit - Show Display Adjustments - [Mouse & Keyboard Mac](#).

**Histogram**

A histogram is a statistical data analysis, representing linear voxel within an image of the selected channel.

**Change Channel Color**

Click on the channel name to switch directly to the channel properties (Menu Edit - Image Properties - [Channels 1...n](#)).

See also:

Menu Edit - Image Properties... - [Channel 1...n](#)

Menu Edit - Show Display Adjustments - [Mouse & Keyboard PC](#)

Menu Edit - Show Display Adjustments - [Mouse & Keyboard Mac](#)

**6.5.1 Mouse & Keyboard PC****Mouse & Keyboard Functions in the Display Adjustments Window**

Ctrl + D	Shows the Display Adjustment window
Left-click	Select channel
Left-click on middle triangle and drag	Adjust Gamma Correction
Ctrl + left-click	Add channel to selection, or remove channel from selection

Left-click & drag	Move left: make image channel brighter Move right: make image channel darker Move up: increase image channel contrast Move down: decrease image channel contrast
Right-click & drag	Move left: make image channel transparent Move right: make image channel opaque
Ctrl + right-click	Automatic range for Min and Max

See also:

Addendum - [Mouse & Keyboard PC](#)

## 6.5.2 Mouse & Keyboard Mac

### Mouse & Keyboard Functions in the Display Adjustments Window

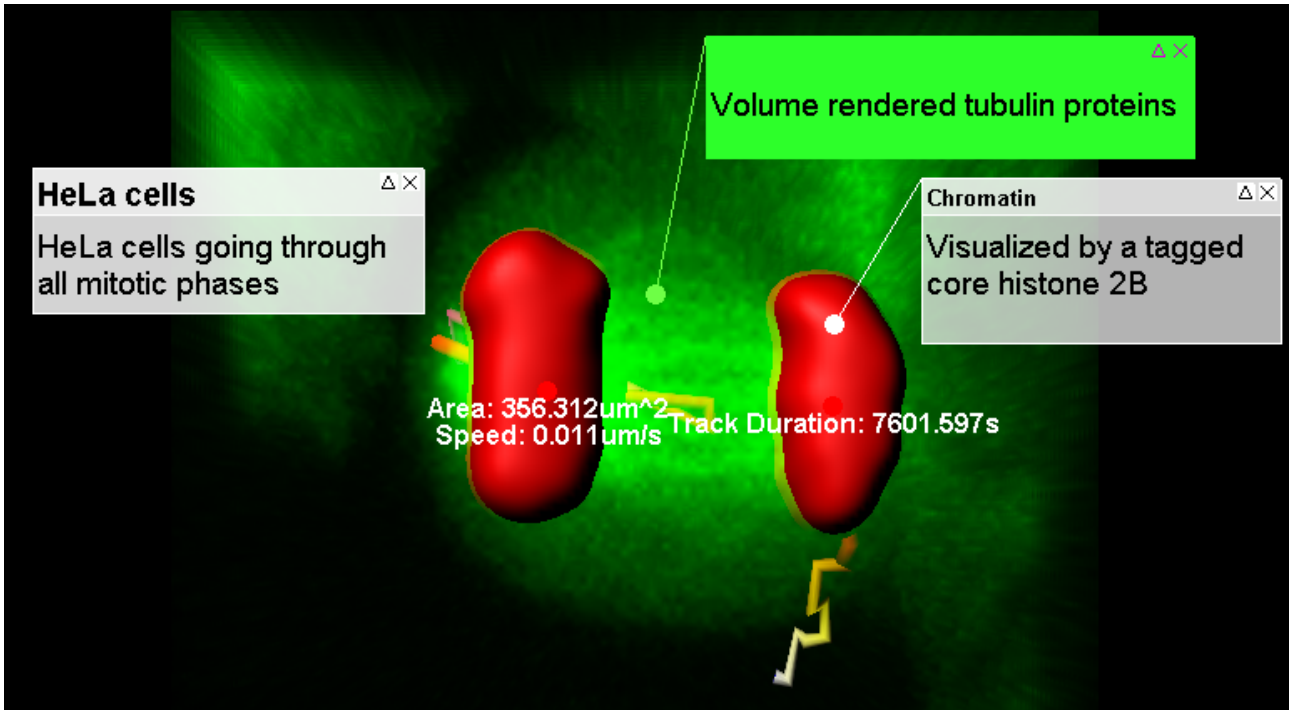
Command + D	Shows the Display Adjustment window
Click	Select channel
Left-click on middle triangle and drag	Adjust Gamma Correction
Command + click	Add channel to selection, or remove channel from selection
Click & drag	Move left: make image channel brighter Move right: make image channel darker Move up: increase image channel contrast Move down: decrease image channel contrast
ctrl + click & drag	Move left: make image channel transparent Move right: make image channel opaque
Command + ctrl + click	Automatic range for Min and Max

See also:

Addendum - [Mouse & Keyboard Mac](#)

## 6.6 Annotate

Annotate allows you to create figures of all Imaris views ready with the selected statistical values, titles, captions, labels, and other highlights for presentations or publications.




Figures are composed of annotations or symbols, a 3D attachment site, and text.

Annotations and symbols are 2D elements which assures optimal reading.

The attachment point and the line are dynamic elements they are updated as you change the view or the camera angle.

Figures can be exported to html.

### 6.6.1 Insert Figures and Annotations

To activate this module click on the Icon **Annotate** , press Ctrl + Shift + P, or in the menu bar select Imaris Menu/Edit – Annotate. To close, press the button **Annotate**.

The functions provides a range of possibilities to create figures with the statistical values, titles, captions and labels.

The **Figures window** appears on the right side of the screen.

The Figures window is open in all Imaris views and applies to all views.

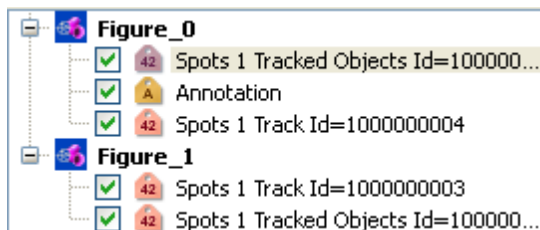
For better handling it can also be undocked by dragging it off its integrated position or clicking on the docking icon (top right, next to x).



Labeling has two main parts: **Figure** and an **Annotation**.


### Figure Tree

The **Figure Tree** is composed of all the existing Figures. In the Figure Tree all existing figures and annotations are displayed.




Within the selected Figure all the annotations' names are displayed.  
For a Figure, Imaris remembers the view that you were in and the camera position.

## Annotations

The labels that are displayed in an image are called Annotations. In Imaris two types of the annotations exist: Text annotation and [Statistical annotation](#) .

The Statistical annotation icon indicates the type of the selected statistical value and for that reason your chose of the selected statistical value type will affect the appearance of the statistical annotation icon.

The following options are available:

Selecting a **statistical variable** that **changes over time** inserts the Statistical Annotation with the **Time icon**  in the selected Figure.

Selecting a **statistical variable** that is **not time dependent** inserts the Statistical Annotation icon  in the selected Figure.

Selecting or de-selecting the box next to the annotation name in the Figure Tree controls the visibility of the annotation in the viewing area.

This Section also provides information on the following topics:

[Annotation Box](#)

[Settings](#)

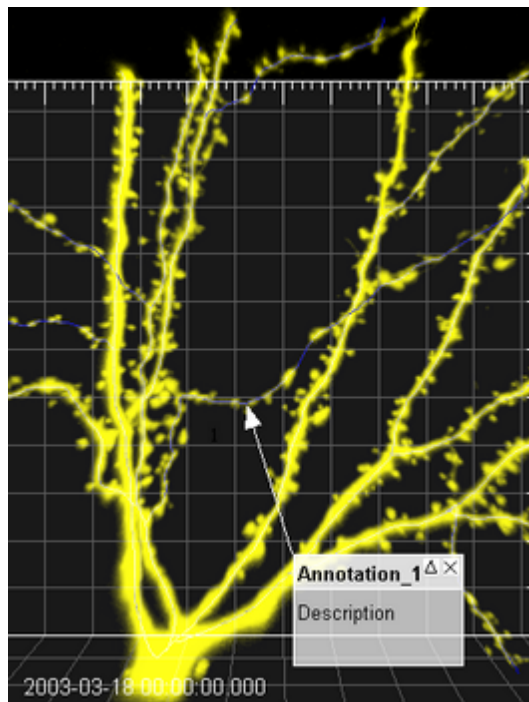
[Tab Text](#)

[Tab Style](#)

[Statistical Annotation](#)

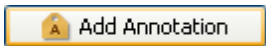
[Tab Export](#)

### 6.6.2 Annotation Box



## Text Annotation

To create a Text annotation using the **Setting** options select the **Add Annotation** button




To create a Text annotation box, click in the image to set the 3D anchor point.

Move the mouse to elongate the line. With the second click you fix the top left corner of the text box.

Move the mouse to adjust the text box size. With the third click you fix the text box size.

## Statistical Annotations

By default, **statistical annotations** are presented in the viewing area without an annotation box. To create the annotation box for the **statistical annotations** select the annotation in the Figure Tree, and then activate the **Text box** option under the [Style tab](#) .

The position of the [statistical annotations](#) can be adjusted by moving the annotation box and the anchor area.

**The statistical annotation is assigned to the selected objects in the image and the anchor point cannot be placed on another object.**

Once created the annotation box and anchor point can be moved to the preferred location.

## Move Annotation Point, Box, Number

To move the **3D anchor point** set the mouse over the anchor point (until it changes to a cross) then click & drag to a new location.

To move the **annotation box** set the mouse on the upper region of the box (until it changes to a cross) then click & drag to a new location.

To move the **annotation number** set the mouse over it (until it changes to a cross) then click & drag to a new location.

## Resize the Annotation Box

To resize the annotation box set the mouse to an edge of the box (until the mouse changes to a resize icon) and then click & drag the edge.

## Annotation Visibility

The description area of the annotation can be closed (hidden) or expanded (shown) by clicking the triangle on the top right of any annotation box.

The annotation can be hidden by clicking the "X" on the top right corner of the box.

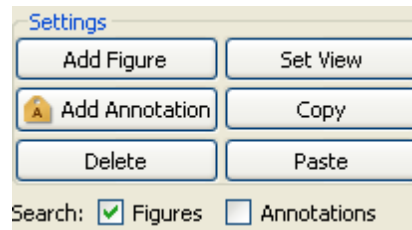
Selecting or de-selecting the box next to the annotation name in the Figure tree controls the visibility of the annotation in the viewing area.

## Save Figures and Annotations in Scene File

The figure legends are saved in the scene file of a data set.



### 6.6.3 Settings



The first buttons in the left row are to **Add Figure**, **Add Annotation** and **Delete** selected figures/annotations. Click on the button **Set View** you can save the actual image view for the selected figure. Click on the button **Copy** to copy the selected figure/annotation. Click on **Paste** to paste the copied figure/annotation.

#### Create a Figure

Click on **Add Figure**. In the first row of the tab Text the standard text header Figure\_0 is displayed. To name a figure type the name in the text box.

In the second row you can add a figure description. A figure label appears in the Annotation Tree.

To jump to the view and camera position associated with a figure double-click the figure name in the Figure Tree. To change the view associated with a particular figure, highlight the name of the figure in the list, move to the Imaris view of interest, and press the button **Set View** from the Settings of Annotation


#### How to Create Text Annotations

Annotations can be added to any figure you want to create.

First you have to select the Figure name in the Figure Tree.

The Figure name is highlighted.

Click on Add Annotation. In the first row of the tab Text the standard text header Annotation\_0 is displayed.


To edit the annotation title select the [Text\\_tab](#) . In the Annotation title description window write new description.

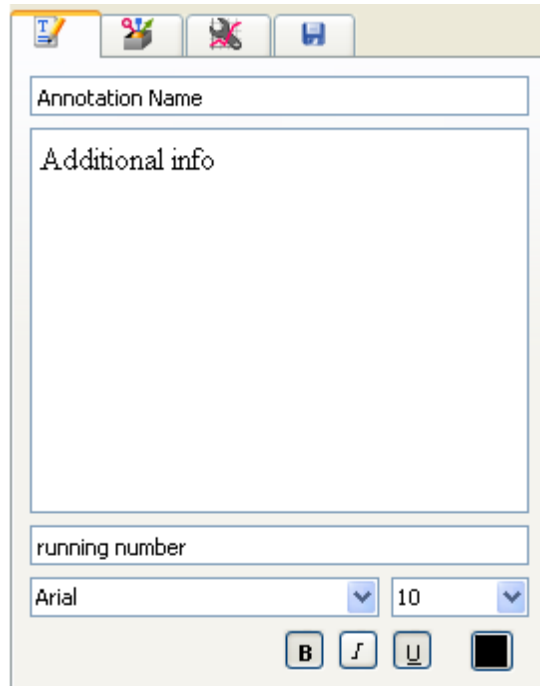
In the second row you can add an annotation text.

#### Search for Figures and Annotations

You can search for the initials in the title field (first row on the tab Text) of a Figure or Annotation.

### 6.6.4 TabText

The Text tab  allows you to modify the object label, insert an additional description, and change the font type, size and color.



## Title

The top line controls the annotation title field.

The title for statistical annotations is by default the object name, category and ID number.

## Additional description

Displays the current text of the annotation controls the description field (text). Edit this field to change the annotation text.

## Description running number

The third box by default contains the annotation running number (legend). To remove the number from the image, delete it from the text field

## Remove an annotation

To remove an annotation from the viewing area, select the entry in the list and then remove the check mark next to it.

## Font

To change the annotation color or font style place the cursor in the running number field. After that, you can select a different color/font in the color/font editor. Click the color/font you wish to use and the annotation text will be changed. Select this option to adjust the appearance of the text.

The parameter specifies the name of the font to use for the text. This font must be supported on your system, in order to be displayed and printed properly. The default font is Arial. This value specifies the font size. The default size is 10.

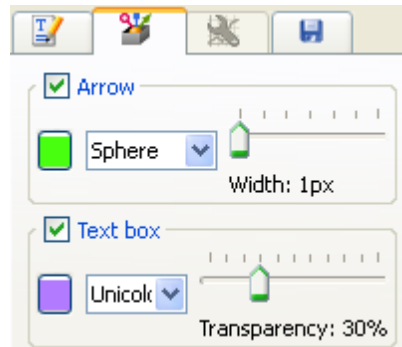
**All changes made in the Text Tab are applied for all time points.**



To display the default settings click on the menu Edit - Preferences... and select [Annotation](#).

### 6.6.5 Tab Style

The **Style Tab**  lists the properties you can modify on an annotation box and annotation anchor point.



#### Arrow

Select this option to adjust the appearance, color, style and width of the arrowhead.

In the drop-down list you can select the shape of the pointer. The pointer can have three possibilities; a sphere, an arrow, or a magnifier

Width of the arrowhead can be modified by specifying this property in points.

Selecting the check-box turns on or off the Arrow.

With a click on the color selection you can change the color of the Arrow.

The slider controls the size of the line and the pointer.

#### Text Box

Select this option to adjust the appearance, the background color and transparency of the annotation box.


With a click on the color selection you can change the color of the Text box.

The pointer can be colored in three ways; uni-color, black text area, or a black title area.

Transparency controls the Text box background. This property defines the degree to which the object's background color is transparent. A value of 100 makes the background completely transparent.

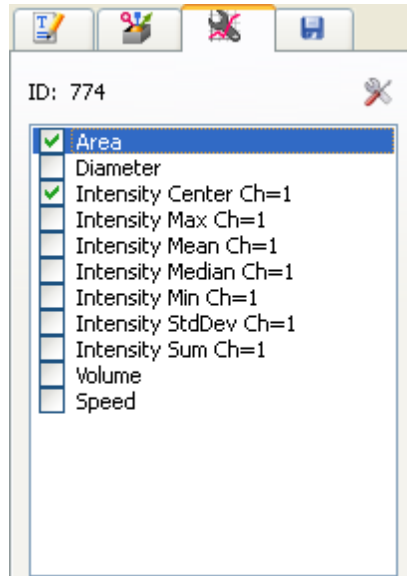
### 6.6.6 Statistical Annotations

The Statistical annotation icon indicates the type of the selected statistical value.

Selecting a **statistical variable** that **changes over time** inserts the Statistical Annotation with the **Time icon**  in the selected Figure.

Selecting a **statistical variable** that is **not time dependent** inserts the Statistical Annotation icon  in the selected Figure.

The statistical annotations name is automatically generated and contains as a default the object name, category and ID number.



The ID number of the selected object appears on the top of the statistical tab.

For all object types that have time dependent statistical parameters, an individual statistical annotation is created for every single time point.



Statistical annotations presents always statistical parameter value for the current time point, both in the viewing area and in the Figure Tree.

Selecting or deselecting the box next to the annotation name in the Figure tree controls the visibility of the annotation in the viewing area for all time points.

Clicking on the statistical annotation name, the annotation is highlighted and the list of statistical values appears in the Annotation window.

This list can be customized, by clicking on the **Preferences** icon .

The option allows you to choose a set of statistical values to be displayed in the viewing area, which differs from the ones selected from the Statistical table.

By default, **statistical annotations** are presented in the viewing area **without a text box**. To create the [annotation box](#) for the **statistical annotations** select the annotation in the Figure Tree, and then activate the **Text box** option under the [Style tab](#) . The text box appears in the viewing area and creates the editable annotation. Select the [Text tab](#)  and in the description windows write additional details.

**Preference** 

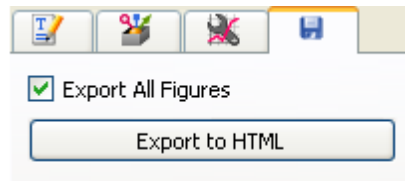
The list of statistical values used in the annotation table, the list can be customized.

Clicking on the icon preferences opens a new window with the list of all possible statistical values available for the selected object type.

Customization of the list of statistical values is possible by selecting the checkbox next to the statistical value name. If the box is checked then the statistical value is visible in the annotation table and if is unchecked then the value is hidden.

**This section does not attempt to describe all features of the Statistical values' calculation in Imaris. For further information please read the description in the Section [Statistics](#).**

### 6.6.7 Tab Export



- Check the check-box if you want to export all figures.
- Highlight the figure in the Figure Tree that you want to Export.
- With a click on the button **Export to HTML** you open the Export Figure as HTML window.
- Choose the desired file path, enter a file name to save the document.
- A web page is created that contains the image name and location, each channel name, the number of time points, the image size, the number of slices, the voxel sizes, a snapshot of the view that is associated with the figure, and the figure name.

## 6.7 Add Time Points...

Additional time points can be added to the data set.

- Select the required image and click **Open**.

The time points to be added must contain the same number of channels and be of the same format as the current data set. A warning message is displayed if the formatting does not match.

### Append with Small Time Gap

By default, the check box **Append with Small Time Gap** is activated. This triggers the same behaviour as in Imaris versions before Imaris 9.0: The original time information of newly added time points is altered by a translation on the time line to the end of existing time data. Relative time differences in the added data set stay the same. The offset between last existing time point and first time point of the added data is set to 1 second.

If the check box is not activated, new time points are added without modifications. This could result in big time gaps on the time line.

If there is an overlap on the time line between existing time points and newly added ones, the same translation is applied as described above.

## 6.8 Delete Time Points...

Any time points that are not needed can be deleted.

### Parameters

#### Delete From, To

Specify the time points to be deleted and click **OK**. The selected time points are automatically deleted from the data set.

## 6.9 Add Channels...

Adding channels is used to extend or complete the data set information if a microscope can only export one channel as a file at one time. All channels of a selected data set are added. The size of the data sets must be identical.

## Window: Add Channels

- Select the required file and click **Open**.

All channels from this data set are added automatically. If the size of the data set does not match a warning message is displayed.

## 6.10 Delete Channels...

Deleting channels is used to erase unwanted information in a data set.

### Select Channels to Delete

#### Channel 1...n

Select the channel to be deleted in the Delete Channels box and click **OK**. The effect is immediately visible on the image.

See also:

Imaris Menu - Open - [Reader Configuration](#)

## 6.11 Remove Images

Use Remove images to remove images from the current set of open images.

To remove images from a multi-image scene:

1. Check the checkbox beside the required images
2. Click OK to remove the image(s) or Cancel to cancel.

For more about multi-image functionality refer to [Add Image... \(multi-image\)](#)

## 6.12 Add Slices...

Adding slices may become necessary, if through manipulation, or during file export or formatting the data set consists of less than the originally acquired number of slices.

## Window: Add Slices

- Select the requested file and click **Open**.

All slices from this data set are added automatically. The x-, and y-values of the two data sets must be identical and the same number of channels is required.

## 6.13 Delete Slice...

### Parameters

#### Slice number [1...n]

Specify the slice to be deleted and click **OK**. The slice is automatically deleted from the data set.

## 6.14 Crop Time...

The total number of time points can be reduced at the beginning or end of the series of images.

### Parameters

#### From, To

Enter the time points to be included in the data set and click **OK**. All other time points are deleted from the data set.

See also:

Imaris Menu - Open - [Reader Configuration](#)

## 6.15 Resample Time...

The total number of time points can be reduced to display the images at a faster speed.

### Parameters

#### New Number of Time Points

Specify the number of time points needed and click **OK**.

The process of resampling the time points takes a while. When the process is finished, the time bar displays the new number of time points.

See also:

Imaris Menu - Open - [Reader Configuration](#)

## 6.16 Crop 3D...

Cropping the data set allows you to crop the images down to the region of interest. Cropping reduces the size of the data set and makes it easier and faster to handle the viewing and storing of the images.

### Preview

Displays a sectional view of the actual image (current time point). A rectangle, representing the region of interest (ROI), is overlaid on all three views.

### Select Crop Dimensions

#### X, Y, Z, From, To, Size

To modify the size and the position of the region of interest you can either enter the ranges of x, y, and z coordinates into the corresponding fields or adjust the region of interest box using the handles directly in the viewer window.

- To move the ROI, click inside the rectangle, hold the mouse button down & drag the entire ROI around.
- To shape the ROI, click on a handle, hold the mouse button it down and reshape the ROI. Side handles affect one direction, corner handles two directions.

The modifications apply to all slices and all time points of the image.  
The displayed values correspond to the changeable voxel value.

Click **OK** when finished. The data set is cut down to the marked ROI. The rest is erased.

See also:

Imaris Menu - Open - [Reader Configuration](#)

## 6.17 Resample 3D...

Resampling reduces the voxel density in a data set to increase the processing speed. Reducing the data size also reduce the resolution. Resampling reduces the number of voxels in a grid but keeps the original relationship between the voxels.

### New Size

#### X, Y, Z

The fields display the current x-, y-, and z-values. The requested values can be directly entered in the fields.

### Aspect Ratio

#### Fixed Ratio X/Y

#### Fixed Ratio X/Y/Z

The Aspect Ratio of the data set's dimensions can be kept by checking the respective Fixed Ratio options. Clicking the **OK** button resamples the data set to the entered values.

Use a Gaussian filter as low-pass before sampling down an image.

See also:

Imaris Menu - Open - [Reader Configuration](#)

Menu Image Processing - Smoothing - [Gaussian Filter](#)

## 6.18 Change Data Type...

### Type

#### From

Displays the current data set type.

#### To

Drop-down list to select the requested data set type from the supported types:

- unsigned 8 bit for the range 0...255.
- unsigned 16 bit (0...65535).
- 32 bit float.

### Range Adjustment

Check field to determine how the data values are translated during the change.

#### None

Data values are imported in the new type.





**Source Range to Target Range**

Maximum data values are scaled to the new range (e.g., 0...255 to 0...65535).

**Data Range to Target Range**

Actual data range values are interpolated to the new range (e.g., 0...150 to 0...65535).

## 6.19 File Type Associations...

The file association dialog for the thumbnail creator (window Imaris Icon and Thumbnail Settings) is displayed.

### File Extensions

Thumbnails will be created and MetaData will be extracted from all checked file types.

**Please note: The feature of the thumbnail extractor is not available for the Macintosh users.**

**.ims**

Imaris Series, Imaris Classic

**.imx**

Imaris Scene

**.ics .ids**

Nikon ICS

**.ism**

Zeiss LSM510

**.zvi**

Zeiss Axiovision

**.lei**

Leica LCS

**.lif**

Leica LIF

**.pic**

Biorad MRC 1024/600

**.rbinf**

TILLvisION

**.stk**

MetaMorph STK

**.r3d .dv**

Delta Vision

**.oib .oif**

Olympus OIB/OIF

**.ipl**

Scanalytics IPLab

**.ome**

Open Microscopy Environment XML

**.inf .info**

Leica Series

**.tif .tiff**

Zeiss LSM 410, Zeiss LSM 310, Leica TCS-NT, Leica Series, Leica LCS, Olympus FluoView, Olympus cellR, PerkinElmer, TIFF series

**More**

Separated by spaces: e.g. ".ims .ics .lsm .zvi".

## 2D/3D Indicator

The generated thumbnails are created with or without a 2D/3D indicator.

**Show**

Check this box to show the indicator at the left upper corner of the thumbnail. There are four different icons to indicate the image type:

- 2D Image (colored square).
- 2D Time Image (pink square and bars).
- 3D Image (green cube).
- 3D Time Image (blue cube and bars).

## Section 7 View

Please refer to Section:

Slice	Ctrl + 1	<a href="#">Slice</a>
Section	Ctrl + 2	<a href="#">Section</a>
Gallery	Ctrl + 3	<a href="#">Gallery</a>
Easy 3D	Ctrl + 4	<a href="#">Easy 3D</a>
3D View	Ctrl + 5	<a href="#">3D View</a>
Coloc	Ctrl + 7	<a href="#">Coloc</a>
Animation	Ctrl + 6	<a href="#">Animation</a>

<a href="#">Fit</a>	Ctrl + B
<a href="#">Reset</a>	Ctrl + A
<a href="#">Full Screen</a>	F11
<a href="#">Navi</a>	Ctrl + N

Load Window State

Save Window State

[Origin Bottom Left](#)

[Origin Top Left](#)

[Origin Bottom Right](#)

[Origin Top Right](#)

[Status Bar](#)

Toolbars - [Status Bar](#)

[Refresh](#) Ctrl + F

[Event log](#)

See also:

Addendum - [Mouse & Keyboard Mac](#)

### 7.1 Slice

#### How to Open the Slice?

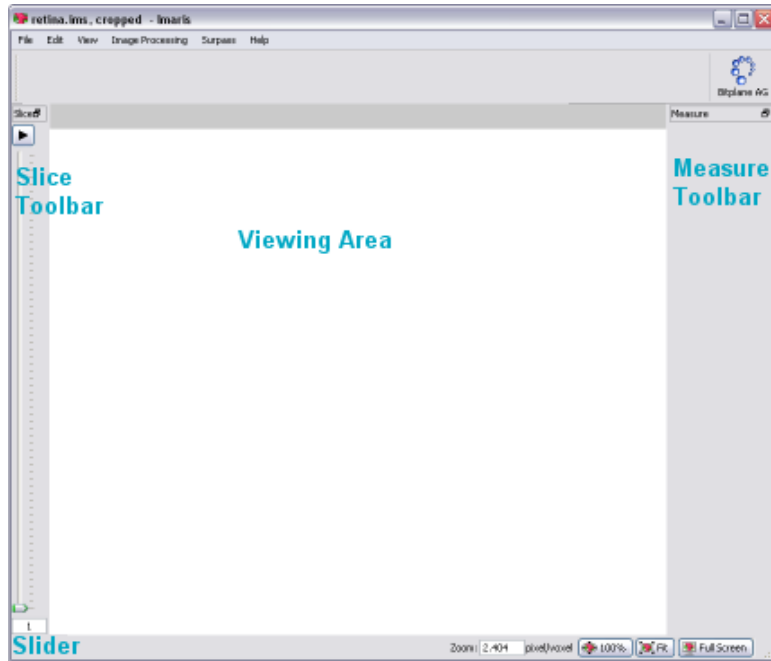
1. Click on the icon in the Main toolbar. If the Slice icon is not displayed click on the arrow in the group: Section, Gallery, Easy 3D, and select Slice.
2. Select View - Slice.
3. Press the key combination Ctrl + 1.

See also:

Slice - [Mouse & Keyboard Mac](#)

#### 7.1.1 Overview

The Slice mode is the view in which an image is displayed when loading a data set into Imaris. The Slice representation shows a 2D view from the top in the z-axis. It shows all images in the data set along the z-axis, so you can inspect any slice along the z-axis for a general overview of the data set.



When moving the cursor over the image, the current voxel intensity and the 3D position in the data set (in brackets) is displayed in the lower left corner of the view.

## Additional Information in the Status Bar

The Slice also displays additional information about any given point in the data set. Click a location in the image display. On the left hand side of the Status Bar the information is displayed as follows:

### Ch 1 ... n at (X, Y, Z)

The display shows the voxel intensity values for the channel(s) and the relative 3D position in the data set (in brackets).

See also:

Toolbars - [Status Bar](#)

## 7.1.2 Slice Toolbar

### Slider

To inspect any slice in the data set along the z-axis you use the Slider. Use the slider as follows:

- Drag the slider handle in the left control bar up and down.
- Select the slider handle with a left mouse click and then use the Arrow-keys up/down on the keyboard.
- Select the slider handle with a left mouse click and then press the Home-key or the End-key to display the first and last slice respectively.
- Select the slider handle with a left mouse click and then press the PageDown-key or the PageUp-key to scroll through the first and last slice respectively in steps.
- Enter the desired slice number in the number field at the bottom of the slider.

Button >

Click on the **Play/Pause** button to see a continuous display through all the slices in the stack. To pause re-click on the button.

**Slice Number Field**

The respective slice number is displayed on the bottom of the slider in the number field.

**7.1.3 Measure Toolbar****Type****Line**

Set the two Measurement Points with two consecutive clicks. The result is displayed in the status field Distance.

**Polygon**

All Measurement Points are consecutively connected by lines and the displayed distance is the sum of the distances between the points.

**Grid**

Check this box to display a grid in the image.

**Distance**

The result of the measurement is displayed in the status field distance.

**Button Delete**

Click on **Delete** to clear the status field and start a new measurement.

**7.1.4 Mouse & Keyboard PC****Mouse & Keyboard Functions in the Slice**

Ctrl + 1	Activates the Slice
Left-click	Set a measurement point
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:

Addendum - [Mouse & Keyboard PC](#)

**7.1.5 Mouse & Keyboard Mac****Mouse & Keyboard Functions in the Slice**

Command + 1	Activates the Slice
Click	Set a measurement point

**With a one-button mouse:**

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

### With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

**Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.**

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:

Addendum - [Mouse & Keyboard Mac](#)

## 7.2 Section

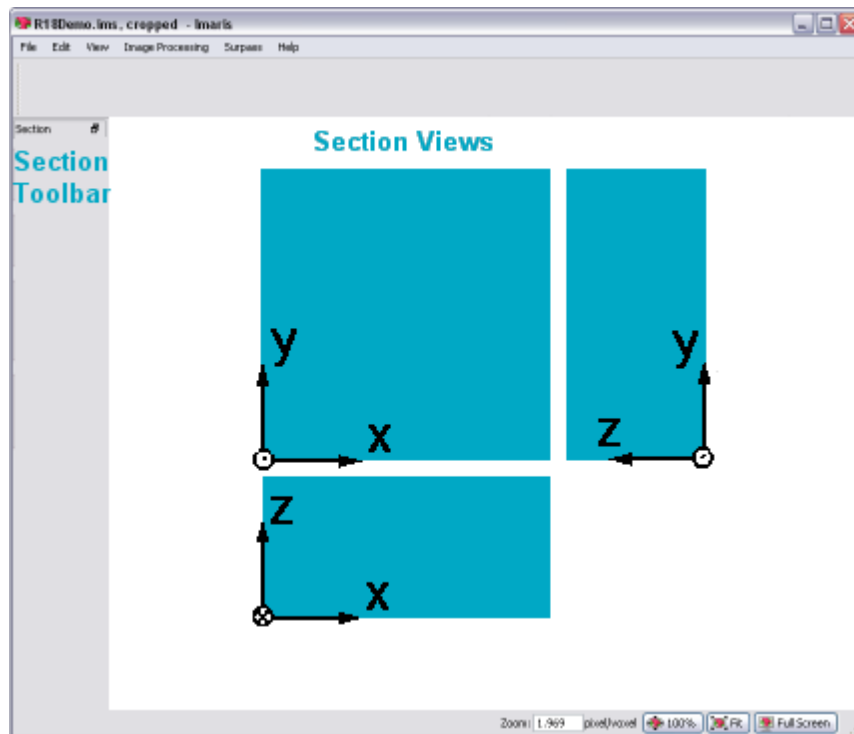
### How to Open the Section?

1. Click on the icon in the Main toolbar. If the icon Section is not displayed click on the arrow in the group: Slice, Gallery, Easy 3D, and select Section.
2. Select View - Section.
3. Press the key combination Ctrl + 2.

### 7.2.1 Overview

The Section lets you inspect the environment of any given point within the data set by showing the cut through the point in the x-, y-, and z-axes or a defined portion of the data set (several slices together).

The illustration below shows the coordinates in the three different display areas of the section.



To select a point, click in any of the three views, hold the button down & drag the crosshairs to the requested point. The crosshairs immediately snap to the cursor when clicking anywhere in the image. The other views, connected by one coordinate, automatically follow the movements.

### Additional Information in the Status Bar

The Section also displays additional information about any given point in the data set. Click a location in the image display. On the left hand side of the Status Bar the information is displayed as follows:

#### Ch 1 ... n at (X, Y, Z)

The display shows the voxel intensity values for the channel(s) and the relative 3D position in the data set (in brackets).

See also:

Toolbars - [Status Bar](#)

## 7.2.2 Section Toolbar

### View

#### Normal

Selects a single cut through the data set.

#### Extended

Additional colored lines are displayed in the viewing area. If Extended is checked the parameter sets Crosshair, Size and Mode are available.

### Crosshair

The parameter set Crosshair is only available, if Extended is checked in the parameter set View (see above).

## Normal

You may eliminate the Crosshair display on the image by unchecking the box. It is then only visible at the edges.

## Extended

Select a portion of the data set in the x-, y-, and z-direction to be displayed. In the main screen, separate the two crosshairs. The planes between the crosshairs are rendered in the views showing the other axis. This allows you to see only certain interior structures without both the top and the bottom.

- The image shows the white crosshair line together with colored focus range lines.
- The focus range can be set in any of the three directions as follows: Move the cursor on a colored line. The cursor changes its form to a straight arrow with a bar.
- Drag the line in the respective direction. The range is extended or reduced. Its depth is reflected in the x-, y-, and z-fields in the Size display. The Size values are indicated in the data set's dimensions, e.g., in  $\mu\text{m}$ . It can also be adjusted directly by entering values in the Size fields (see below) or by means of their up and down arrows.

Uncheck the Crosshair – Normal and/or Crosshair – Extended boxes to eliminate the crosshair and/or the focus range lines display on the image.

## Size fields

The parameter set Size is only available, if Extended is checked in the parameter set View (see above). Display of the actual x-, y-, and z-position of the extended Crosshair. The positions can also be adjusted directly by entering values in the fields or by means of their up and down arrows.

## Mode

The parameter set Mode is only available, if Extended is checked in the parameter set View (see above). Here you may choose the best display mode for the slice portion.

## MIP

Calculated maximum over all slices in the range.

## Mean

Calculated mean value over all slices in the range.

## Blend

Calculated upon the transparency of the slices.

## 7.2.3 Mouse & Keyboard PC

### Mouse & Keyboard Functions in the Section

Ctrl + 2	Activates the Section
Left-click	Set the section center
Middle-click & drag	Move up: zoom out Move down: zoom in





Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:

Addendum - [Mouse & Keyboard PC](#)

## 7.2.4 Mouse & Keyboard Mac

### Mouse & Keyboard Functions in the Section

Command + 2	Activates the Section
Click	Set the section center

#### With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

#### With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

**Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.**

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:

Addendum - [Mouse & Keyboard Mac](#)

## 7.3 Gallery

### How to Open the Gallery ?

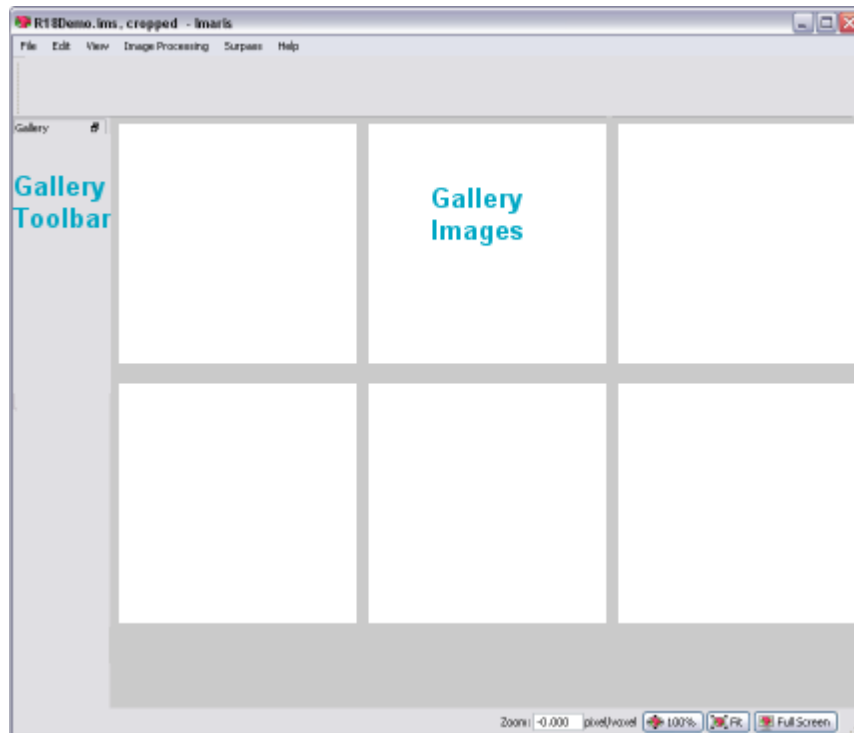
- Click on the icon in the Main toolbar. If the Gallery icon is not displayed click on the arrow in the group: Section, Slice, Easy 3D, and select Gallery.
- Select View - Gallery.
- Press the key combination Ctrl + 3.

See also:

Gallery - [Mouse & Keyboard Mac](#)

## 7.3.1 Overview

The Gallery displays all available slice images of the data set. In the Gallery you can adapt the display to your needs to gain more information from the slice series. When the Gallery is selected, all slices are displayed. Click on images to select or deselect them. Selected images are marked with a colored frame. Holding the Shift-key lets you select a continuous series of images. Hold the Ctrl-key to select various images spread over the gallery.



### Additional Information in the Status Bar

The status bar indicates which of how many available pages is currently being displayed, and how many slices have been selected. Changes in the Gallery only affect the current display and do not crop the data set.

See also:

Toolbars - [Status Bar](#)

## 7.3.2 Gallery Toolbar

In the Gallery the following display and selection controls are available:

### Slice

By clicking on the arrows, you move up and down the images in the gallery one by one. This is especially useful whenever the display on the screen does not include all the slices.

### Page

If there are more images than displayed on screen, move up and down the pages by clicking on the arrows or go directly to the first or last page by using the respective buttons.

## Columns

Specify the number of columns to be currently displayed on the screen by clicking on the up/down arrows or by entering the requested number.

## Skip

Select every second image from the data set by selecting 1 image to skip, or every third image by selecting 2 images to skip (or any other skip interval analogously, by clicking on the up/down arrows or entering the appropriate number directly).

## Display

Button **Select...**

Highlight image(s) and click on the button **Select...** . Only the selected image(s) is(are) displayed in the gallery. Re-click on the button **Select...** and all images are displayed in the Gallery.

## Slice Number Display

The slice number is displayed beneath each slice.

### 7.3.3 Mouse & Keyboard PC

#### Mouse & Keyboard Functions in the Gallery

Ctrl + 3	Activates the Gallery
Left-click	Select slice
Ctrl + left-click	Add single slice to selection
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:

Addendum - [Mouse & Keyboard PC](#)

### 7.3.4 Mouse & Keyboard Mac

#### Mouse & Keyboard Functions in the Gallery

Command + 3	Activates the Gallery
Click	Select slice
Shift + click	Add slices to selection
Command-click	Add single slice to selection

**With a one-button mouse:**

Shift + Ctrl + click & drag	Move up: zoom out
-----------------------------	-------------------

Ctrl + click & drag	Move down: zoom in Pan image
---------------------	---------------------------------

### With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

**Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.**

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:

Addendum - [Mouse & Keyboard Mac](#)

## 7.4 Easy 3D

### How to Open the Easy 3D?

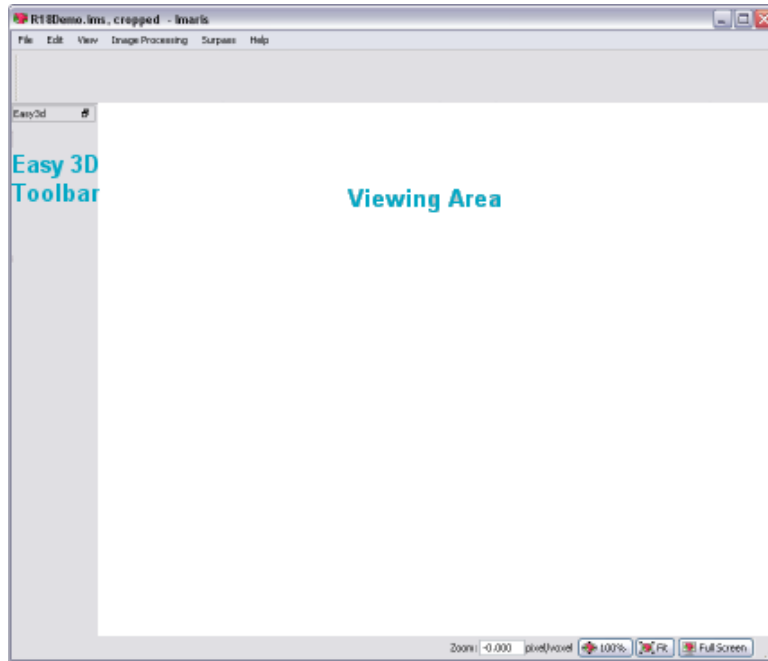
- Click on the icon in the Main toolbar. If the icon Easy 3D is not displayed click on the arrow in the group: Section, Gallery, Slice, and select Easy 3D.
- Select View - Easy 3D.
- Press the key combination Ctrl + 4.

See also:

Easy 3D - [Mouse & Keyboard Mac](#)

#### 7.4.1 Overview

The Easy 3D provides a quick fixed view with a few parameters for rendering and light setting. This lets you explore the data set in the 3D mode before doing any further processing.



## 7.4.2 Easy 3D Toolbar

### Rendering

#### MIP

(Maximum Intensity Projection) - Shows the maximum intensity of all layers along the viewing direction.

#### Blend

Shows the color obtained by blending all values along the viewing direction and including their transparency. If Blend is selected, the parameter set Light is available (see below).

### Light

If Blend is selected (see above), the parameter set Light is available.

#### Light

Check the box to switch the light on and adjust the following parameters. There are two predefined light setting parameters in Easy 3D Blend to choose from.

#### Left

Light from the left, shadow to the right.

#### Right

Light from the right, shadow to the left.

#### Button Settings...

In Blend mode, with light enabled, click the button **Settings...** The Easy 3D Settings dialog box displays (see below).

### Calculate

## Auto

The image is calculated and displayed automatically.

## Manual

The image calculation starts after you click on the button **Calculate**. If you select Manual as calculation mode, the button **Calculate** is available.

## Button **Calculate**

Click on this button to start the calculation for the Easy 3D image view. The button is only available, if you select Manual as calculation mode.

## Window: Easy 3D Settings

### Light

#### Directional Intensity

Controls the brightness of structures that are illuminated from the light source.

#### Ambient Intensity

Controls the brightness of structures that are in the shadow. The default (0) means that structures completely in the shadow are black. The higher the value the brighter the picture and the lower the shadowing contrasts.

### Channel Opacity Combination

Allows you to control the opacity of the image.

#### Opaque

#### Translucent

#### Custom

Select Opaque, Transparent, or Custom opacity. It affects only the behavior of interleaving structures of two or more channels.

## 7.4.3 Mouse & Keyboard PC

### Mouse & Keyboard Functions in the Easy 3D View

Ctrl + 4	Activates the Easy 3D
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

See also:

Addendum - [Mouse & Keyboard PC](#)

## 7.4.4 Mouse & Keyboard Mac

### Mouse & Keyboard Functions in the Easy 3D View

Command + 4                      Activates the Easy 3D

#### With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out
	Move down: zoom in
Ctrl + click & drag	Pan image

#### With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

**Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.**

Middle-click & drag	Move up: zoom out
	Move down: zoom in
Right-click & drag	Pan image

See also:

Addendum - [Mouse & Keyboard Mac](#)

## 7.5 Coloc

In fluorescent microscopy, colocalization describes the presence of two fluorochromes at the same physical location. ImarisColoc provides extended functionality for the analysis and visualization of colocalization in multi-channel data sets. It provides an automated selection of colocalization based on a method introduced by Costes & Lockett at the NIH, NCI/SAIC.

Coloc processes 2D, 3D, and 4D data sets. It operates on two channels simultaneously and measures the degree of overlap of the two channels.

With Coloc you can determine and display locations within the data set with common information from all channels, e.g., to illustrate where all types of proteins can be found simultaneously or to detect low-density tissues in all channels.

The desired range for signal analysis can be limited by defining intensity thresholds or sets of intensity values for each of the involved channels. All voxels falling inside everyone of these channel-specific intensity limits represent a colocalization. They are emphasized in the view and statistics are calculated and displayed. When the selection is changed, the numeric and visual feedback happens in real-time.

The result of the colocalization as a whole can be saved as a separate channel, which can then be processed and viewed as any originally acquired channel in Imaris. Multiple colocalization images are easily combined into a single view in Imaris Surpass.

Coloc is an optional module within the Imaris program and is not available as a standalone program.

## How to Display the Coloc Main Screen?

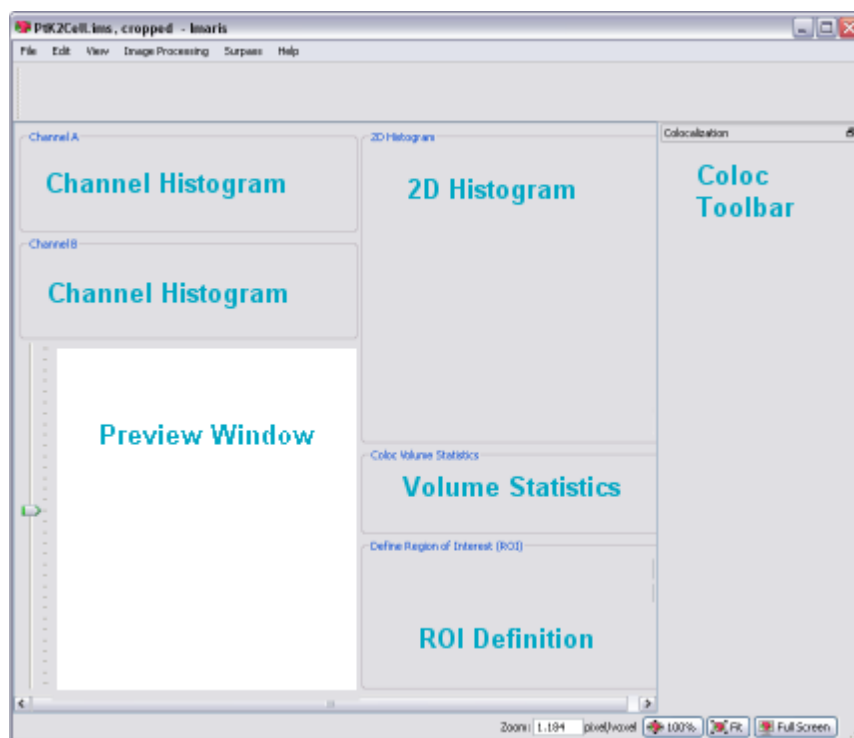
- Click on the Coloc icon in the Main toolbar,
- Select View - Coloc,
- Press the key combination Ctrl + 7.

See also:

Coloc - [Mouse & Keyboard Mac](#)

### 7.5.1 Overview

The Coloc main screen consists of different areas:



- [Channel Histogram](#) (channel A, channel B). This area allows the selection of the two channels for the colocalization analysis.
- [Preview Window](#). This area visualizes the data set together with a preview of the colocalized region.
- [2D Histogram](#). This area displays a two-dimensional intensity histogram of the selected channels. It reflects the distribution of pairs of voxel intensities occurring in the two selected channels. The range of intensity pairs considered as colocalized can be defined on the histogram.
- [Volume Statistics](#). This area displays statistics about the resulting colocalized volume. The display adapts instantly to all modifications in the selection.
- [ROI Definition](#). This feature allows you to define a region of interest (ROI) for the entire analysis.
- [Coloc Toolbar](#). The Coloc toolbar contains several sections with all possible selection and definition tools and settings for the histograms, the thresholding, and the display as well as the buttons for the color definition and the build of the Coloc channel.



## 7.5.2 Channel Histogram

This area allows the selection of the two channels for the colocalization analysis. The intensity histograms are displayed for the currently selected channels.

### Select Channel A and Channel B

The two channels for colocalization detection must be selected first.

- Select channel A and channel B from the drop-down lists. The 1D and 2D intensity histogram displays are updated.

### Adjust Threshold

- The threshold for each channel can then be defined by clicking in the histogram, dragging the colored line directly, or by entering the value in the threshold field.

### Adjust Histogram Settings

If necessary, you can adjust in the Coloc toolbar the histogram mode and histogram options.

See also:

Coloc - [Coloc Toolbar](#)

Coloc - [Mouse & Keyboard PC](#)

Coloc - [Mouse & Keyboard Mac](#)

## 7.5.3 Preview Window

This area visualizes the data set together with a preview of the colocalized region. It displays a Slice representation of the data set, i.e. a 2D view from the top in the z-axis direction. On the left hand side, a sliding bar with a handle allows inspecting and displaying the single slices in the data set along the z-axis. The Preview window updates in real-time the display of the colocalized region based on the actual settings of the colocalization parameters. The original channels can be switched on or off in the Display Adjustments window.

### Time Settings

There are no time-dependent parameters for the colocalization analysis. The Preview window always displays the currently selected time point of the data set. The values in the Statistics window always refer to the volume of the current time point. However, when the **Build Coloc Channel** button is pressed, the Coloc channel and statistical values for all time points are calculated.

See also:

Menu Edit - [Show Display Adjustment](#)

Coloc - [Mouse & Keyboard PC](#)

Coloc - [Mouse & Keyboard Mac](#)

## 7.5.4 2D Histogram

This area displays a two-dimensional intensity histogram of the selected channels. It reflects the distribution of pairs of voxel intensities occurring in the two selected channels. The range of intensity pairs considered as colocalized can be defined on the histogram.

### Adjust Channel Thresholds

Effects of the modification are instantly visible in the channel histogram A and B, and in the Preview window.

## **Selection Mode Threshold**

The channel thresholds are visible in form of a rectangle.

- Point with the cursor on the 2D histogram.
- Click on the histogram to determine the thresholds. The edge of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot.
- Click on the edge and drag it to any another position in the histogram.

## **Selection Mode Polygon**

The channel thresholds are visible as an intensity area in form of a free configurable polygon.

You draw the polygon on the 2D histogram. This method is especially useful for eliminating channel bleed through regions along the border of the lower intensity bins. After checking the Polygon mode, the overlaid threshold rectangle in the 2D histogram is converted into a polygon with four corner points. You can now start to modify this polygon or to draw a completely new one. The initial point of the polygon is visible as white filled.

## **Set Point**

Press and hold the Shift-key. The cursor turns into a cross with a little sign. Click on the 2D histogram with the left mouse button to place a point (vertex). Any additional point connects with lines to the initial point and the last point inserted.

## **Insert Point in a Line**

Press and hold the Shift-key. The cursor turns into a cross with a little " + " sign. Point on the line between the two vertices and click on the line with the left mouse button to add a point.

## **Automatic Point Setting**

Press and hold the Ctrl-key. The cursor turns into a cross with a little " + " sign and a stack symbol. Drag the cursor around. New vertices are added automatically at each change of cursor position.

## **Move Point**

Point on one of the vertices, click & drag the vertex around to the desired location. The polygon changes its shape.

## **Move Line**

Point on a line between two vertices, click & drag the line around to the desired location. The polygon changes its shape.

## **Move Polygon**

Point somewhere beside of the polygon edge, click & drag the polygon around to the desired location.

## **Delete Point**

Shift + click on a point to delete the point.

See also:

Coloc - [Coloc Toolbar](#) (selection Mode Threshold or Polygon)

### 7.5.5 Volume Statistics

The values are updated in real-time and refer to the entire volume data of the current time point, regardless of the settings for the histogram mode. The values are calculated by the following formulas.

**Please note: The calculated Coloc Volume Statistics are not automatically saved in the data set. To save or export the statistics you have to build a coloc channel (Coloc - Coloc Toolbar - [Build Coloc Channel](#)). For publishing Coloc statistical values please use only the statistics reported in the Channel Properties.**

Due to a real-time response, some of the statistical values shown in the main Coloc window are generated from a sub-sampled data set. The accuracy of these values depends on the variability introduced by the sub-sampling procedure.

Depending on the data set, the difference between the values reported in the Channel Properties (a high accuracy values, produced from the entire data set) and these values (calculated from sub-sampled data set) may differ significantly.

#### Definitions

##### Regions:

Data set	All data set voxels
Coloc	Colocalized voxels
Object A	Voxels with channel A intensity above threshold A
Object B	Voxels with channel B intensity above threshold B
ROI	Region of interest

##### Indicators:

N	Number of voxels
SA <sub>i</sub>	Channel A intensity of voxel
S <sub>B</sub> <sub>i</sub>	Channel B intensity of voxel
SA <sub>avg</sub> , Coloc	Average channel A intensity in the Coloc region

Volume A	Number of voxel channel A x voxel volume
Volume B	Number of voxel channel B x voxel volume
Material A	Channel A voxel signal intensity
Material B	Channel B voxel signal intensity

##### Number of colocalized voxels

Total count of colocalized voxels  $N_{coloc}$ .

##### Please note:

- $N_{coloc}$  is the number of colocalized voxels for the defined region of interest (ROI).
- Colocalization statistics are all calculated using the ROI.

##### % of data set colocalized

Percentage of total data set voxels colocalized.

$$\frac{N_{coloc}}{N_{dataset}} \cdot 100\%$$

### **% of volume A above threshold colocalized**

Percentage of channel A voxels above threshold A colocalized.

$$\frac{N_{coloc}}{N_{objectA}} \cdot 100\%$$

### **% of volume B above threshold colocalized**

Percentage of channel B voxels above threshold B colocalized.

$$\frac{N_{coloc}}{N_{objectB}} \cdot 100\%$$

### **% of material A above threshold colocalized**

Percentage of channel A material above threshold A that is colocalized.

$$\frac{\sum_{i \in coloc} SA_i}{\sum_{i \in objectA} SA_i} \cdot 100\%$$

### **% of material B above threshold colocalized**

Percentage of channel B material above threshold B that is colocalized.

$$\frac{\sum_{i \in coloc} SB_i}{\sum_{i \in objectB} SB_i} \cdot 100\%$$

### **% of ROI colocalized**

Percentage colocalization of channel A and channel B volume inside the region of interest.

$$\frac{N_{coloc}}{N_{mask}} \cdot 100\%$$

### **% of ROI material A colocalized**

Percentage colocalization of ROI channel A material.

$$\frac{\sum_{i \in coloc} SA_i}{\sum_{i \in mask} SA_i} \cdot 100\%$$

**% of ROI material B colocalized**

Percentage colocalization of ROI channel B material.

$$\frac{\sum_{i \in coloc} SB_i}{\sum_{i \in mask} SB_i} \cdot 100\%$$

**Pearson`s coefficient in colocalized volume**

Pearson correlation of channel A and channel B inside the colocalized region.

**Please note: The correlation value ranges between 1 and -1. A value of 1 represents perfect correlation, 0 no correlation, and -1 perfect inverse correlation.**

$$R_{AB,coloc} = \frac{\sum_{i \in coloc} (SA_i - SA_{avg,coloc})(SB_i - SB_{avg,coloc})}{\sqrt{\sum_{i \in coloc} (SA_i - SA_{avg,coloc})^2 \sum_{i \in coloc} (SB_i - SB_{avg,coloc})^2}}$$

**Pearson`s coefficient in data set volume**

Pearson correlation of channel A and channel B inside the entire data set volume.

$$R_{AB,dataset} = \frac{\sum_{i \in dataset} (SA_i - SA_{avg,dataset})(SB_i - SB_{avg,dataset})}{\sqrt{\sum_{i \in dataset} (SA_i - SA_{avg,dataset})^2 \sum_{i \in coloc} (SB_i - SB_{avg,dataset})^2}}$$

**Pearson`s coefficient in ROI volume**

Pearson correlation of channel A and channel B inside the region of interest.

$$R_{AB,mask} = \frac{\sum_{i \in mask} (SA_i - SA_{avg,mask})(SB_i - SB_{avg,mask})}{\sqrt{\sum_{i \in mask} (SA_i - SA_{avg,mask})^2 \sum_{i \in mask} (SB_i - SB_{avg,mask})^2}}$$

**Original Mander's coefficient A**

Original Mander's coefficient A = Sum(SA<sub>i</sub>SB<sub>coloc</sub>) / Sum(SA<sub>i</sub>)  
 SB<sub>coloc</sub>=1 if SB<sub>i</sub>>0, otherwise SB<sub>coloc</sub>=0

**Original Mander's coefficient B**

Original Mander's coefficient B = Sum(SB<sub>i</sub>SA<sub>coloc</sub>) / Sum(SB<sub>i</sub>)  
 SA<sub>coloc</sub>=1 if SA<sub>i</sub>>0, otherwise SA<sub>coloc</sub>=0

**Mander's coefficient A (thresholded)**

Thresholded Mander's coefficient A = Sum(SA<sub>i</sub>SB<sub>coloc</sub>) / Sum(SA<sub>i</sub>)  
 SB<sub>coloc</sub>=1 if SB<sub>i</sub>>threshold<sub>B</sub>, otherwise SB<sub>coloc</sub>=0

**Mander's coefficient B (thresholded)**

Thresholded Mander's coefficient B = Sum(SB<sub>i</sub>SA<sub>coloc</sub>) / Sum(SB<sub>i</sub>)  
 SA<sub>coloc</sub>=1 if SA<sub>i</sub>>threshold<sub>A</sub>, otherwise SA<sub>coloc</sub>=0

See also:

Menu Edit - Image Properties... - Channel 1...n - [Tab Coloc Statistics](#) (to export the statistics)  
 Coloc - Coloc Toolbar - [Build Coloc Channel](#) (to build the coloc channel)

**7.5.6 ROI Definition**

**Define Region of Interest (ROI)**

This feature allows you to define a region of interest (ROI) for the entire analysis. A third channel can be selected as a masking area for the entire analysis. A masking channel is e.g. a third microscope channel acquired with settings that allows defining a region of interest by simple intensity thresholding. All voxels outside of the region of interest defined by the mask channel are ignored for the colocalization analysis. The volume excluded by the mask appears hatched in the Preview window. The mask channel is used in conjunction with the Automatic Threshold function. For this function, the selection of an appropriate region of interest is essential.

**Mask data set**

Check this box to activate the function.

**X% of Data selected**

Displays the percentage of selected data.

**Channel Selection**

Select the channel to be used from the drop-down list.

**Channel Histogram**

Point with the cursor into the histogram and click to set the threshold. A bolded line marks the threshold in the



histogram and the corresponding value is indicated in the threshold field. The masked volume of the data set appears hatched in the Preview window. Drag the cursor in the histogram to modify the threshold. Effects of the modification are instantly visible in the threshold field as well as in the Preview window.

### Threshold Value

Editable threshold value field.

## Mask Channel and Automatic Threshold

When the mask is set as required, click the button **Automatic Thresholding** to calculate the thresholds. Build the colocalization channel by clicking the button **Build Coloc Channel**.

See also:

Coloc - Coloc Toolbar - [Automatic Thresholding](#)

Coloc - Coloc Toolbar - [Build Coloc Channel](#)

Coloc - [Mouse & Keyboard PC](#)

Coloc - [Mouse & Keyboard Mac](#)

### 7.5.7 Coloc Toolbar

The Coloc toolbar contains several sections with all possible selection and definition tools and settings for the histograms, the thresholding, and the display as well as the buttons for the color definition and the build of the Coloc channel.

## Selection Mode

You can define, for each of the channels separately, an intensity value as a threshold. Only voxels with an intensity value above the specified threshold will be considered for the colocalization analysis. The two thresholds are visible and can be set in four different ways:

1. As a hatched region with a bold line on the channel histograms.
2. As a value in the Threshold field of each channel (channel histogram).
3. As a hatched region with bold lines on the 2D histogram. The region always contains the top right part of the histogram. The left and lower bold lines define the intensity thresholds for the two channels.
4. As an active contour in the Preview window.

### Threshold

To adjust the threshold value using the mouse (see above 1. and 3.), select this mode.

### Polygon

To draw a polygon line in the 2D histogram (see above 4.) select this selection mode.

## Automatic Thresholding

Please refer to Section Coloc - Coloc Toolbar - [Automatic Thresholding](#).

## Histogram Mode

The histogram mode options define the voxels selection for the colocalization calculation within the data set.

### Single Slice

The 2D histogram is calculated and displayed for the current slice only.

## Single Time Point

The 2D histogram is calculated and shown for the entire 3D stack of the current time point. This mode allows for faster browsing through the data set along the z-axis.

## All Time Points

The 2D histogram is calculated and shown for an entire time data set, i.e. for all time points within the 4D data set.

## Histogram Options

The histogram mode options are used to define the display of the 1D and 2D histograms.

### Ignore Border Bins

A border bin is always the first and last value in a histogram. Often a lot of voxels fall into the bin with the lowest intensity. In a frequency plot this causes a massive and unnecessary downscale of all other intensity bins which are of more interest. The border bins can therefore be ignored when scaling the display of the histogram. Ignore is selected as default. With most data sets, checking the Ignore Border Bin and Logarithmic options allows a more representative histogram display.

### Logarithmic

The frequency plot is shown in a logarithmic scaling instead of a linear scaling.

### Color Coded

Applies a false color look-up table to the histogram to improve the visibility of intensity differences. The high frequencies (bins in the 2D histogram into which a lot of voxels accumulate) are shown in yellow-to-white, bins with little voxels are shown in blue-to-black.

## Coloc Intensities

The Coloc intensities option defines the brightness of the calculated colocalization displayed in the Preview window.

### Source Channels

Uses the intensities of the two channels for displaying the intensity of a colocalized voxel as the square root of the product of the intensities of the original channels.

### Constant Value

Displays the intensity of a colocalized voxel defined by the specified value. No distinction is made whether high-intensity or low-intensity voxels colocalize as long as they are within the selection.

## Coloc Color

Coloc color allows you to specify personal color settings for the colocalization display in the Preview window.

### Button **Edit**

Clicking on the button **Edit** opens the Color window. You can either select one of the predefined colors to apply on the colocalization areas or define, add, and apply your own color definitions.

### Button **Build Coloc Channel**

Please refer to Section Coloc - Coloc Toolbar - [Build Coloc Channel](#).





**Button Build Time Dep. Coloc**

Please refer to Section Coloc - Coloc Toolbar - [Build Coloc Channel](#).

**Button Channel Statistics**

Please refer to Section Menu Edit - Image Properties... - Channel 1...n - [Tab Coloc Statistics](#).

See also:

Coloc - [Channel Histogram](#)

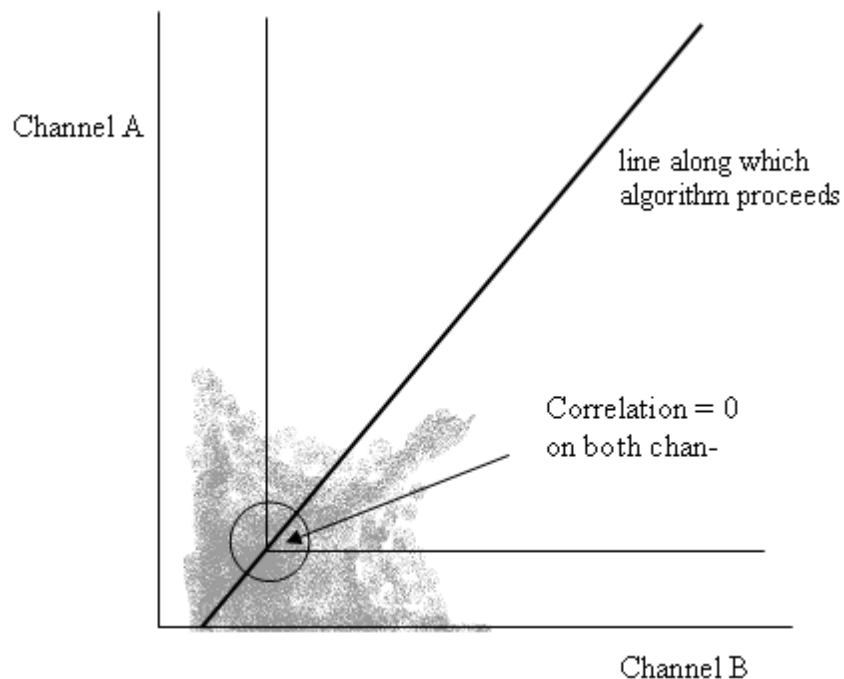
Coloc - [Preview Window](#)

Coloc - [2D Histogram](#)

**7.5.7.1 Automatic Thresholding****Button Calculate Thresholds**

This button performs an automatic threshold run on both channels based on an algorithm developed by Costes and Lockett at the National Institute of Health, NCI/SAIC, which is based on the exclusion of intensity pairs that exhibit no correlation (Pearson's correlation below zero).

The automatic threshold search is done using the 2D histogram. Starting with the highest intensity value, the algorithm reduces the threshold value step by step along a line (see figure below) and computes the correlation coefficient of the image using only voxels with intensities below the threshold. The algorithm continues reducing the thresholds until the correlation reaches 0, thus defining the automatic threshold.

**P-Value****PSF Width**

As the size of the Point Spread Function please enter the length along the Z Axis. This can be a calculated theoretical length.

You can find links to calculators on the web e.g. on the Bitplane homepage.

The default in Imaris is not actually related to the PSF but only to the diagonal of the voxel size.

For more information about the P-Value and PSF Width please refer to the following publication:

Automatic and Quantitative Measurement of Protein-Protein Colocalization in Live Cells

Sylvain V. Costes,\*y Dirk Daelemans,z Edward H. Cho,\* Zachary Dobbin,\* George Pavlakis,z and Stephen Lockett\*

\* Image Analysis Laboratory, National Cancer Institute, Frederick, Maryland;  
y National Cancer Institute/Science Applications International Corporation, Frederick, Maryland; and  
z Human Retrovirus Section, National Cancer Institute, Frederick, Maryland

Biophysical Journal Volume 86 June 2004 3993-4003

## 7.5.7.2 Build Coloc Channel

Button **Build Coloc Channel**

Clicking this button calculates the colocalization channel according to the selected settings and adds it to the data set. The channel can then be saved and is part of the data set.

Button **Build Time Dep. Coloc**

Clicking this button calculates the colocalization channel according to the selected settings (calculating an automatic threshold individually for each time point) and adds it to the data set. The channel can then be saved and is part of the data set.

### Save Coloc Channel

Before the channel is added permanently to the data set it must be saved. In the menu bar select File – Export or click the **Export** button in the toolbar. The Export box is displayed. Select the directory and enter the name for the file to be saved or confirm the suggestion. Select the requested file format and click **OK**. The data set is saved.

Button **Channel Statistics** (for the new Coloc channel)

After building the Coloc channel, its statistics become available in the Image Properties. The button **Channel Statistics** displays directly the Image Properties window with the statistics for the calculated channel. The name of the channel, the description of the source channels and the selection method are indicated and displayed in the Image Properties on the Tab Coloc Statistics.

### Export Statistics

The statistics can be exported.

Button **Export**

In the Image Properties window click the **Export** button. The Export Coloc Statistics window is displayed. Select the directory and enter the name for the file to be saved. The statistics are saved as .csv Excel file and can be opened directly with Microsoft Excel.

See also:

Coloc - [Volume Statistics](#)

## 7.5.8 Basic Operation

The basic use of Coloc in the daily work flow comprises the following steps:

- Select the channels.
- Adjust selection mode and histogram mode.
- Set thresholds.
- Check the statistics.
- Build and save the Coloc channel.

### Select Channel A and Channel B

In the 1D histograms select channel A and channel B from the drop-down lists. The 1D and 2D intensity histogram displays are updated.

### Adjust Selection Mode and Histogram Mode

Before you set the thresholds, please check the appropriate parameters in the Coloc toolbar on the right hand side of the screen:

- Check as Selection Mode Threshold.
- Decide, which Histogram Mode you need. Choose Single Slice for calculating the histogram for the current slice only. Or choose Single Time Point to calculate the histogram for the entire 3D time point. Or choose All Time Points for the entire 4D image.

### Set Thresholds

Colocalization occurs where the voxels are above threshold in channel A and channel B. You can define, for each of the channels separately, an intensity value as a threshold. Only voxels with an intensity value above the specified threshold will be considered for the colocalization analysis. The two thresholds are visible and can be set in four different ways:

1. As a hatched region with a bold line on the channel histograms.
2. As a value in the Threshold field of each channel (channel histogram).
3. As a hatched region with bold lines on the 2D histogram. The region always contains the top right part of the histogram. The left and lower bold lines define the intensity thresholds for the two channels.
4. As an active contour in the Preview window.

#### A. On the 1D Channel Histogram

- Point with the cursor on the histogram. The cursor turns into a cross.
- Click on the histogram to determine a threshold. The bold left line of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot.
- Click on the line and drag it to another position in the histogram. Effects of the modification are instantly visible in the other indications as well as in the Preview window.
- Perform for both channels until the settings are as desired.

#### B. By Entering a Value

- Click in the Threshold field of one of the channels and enter a value, then press Enter. The bold left line of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked

spot. Effects of the modification are instantly visible in the other indications as well as in the Preview display.

- Perform for both channels until the settings are as desired.

### C. On the 2D Histogram

- Point with the cursor on the 2D histogram. The cursor turns into a cross.
- Click on the histogram to determine the thresholds. The edge of the overlaid hatched area (indicating the selected voxel intensity range for colocalization) jumps on the marked spot.
- Click on the edge and drag it to any another position in the histogram.

### D. On the Preview Window

- Click with the cursor on the image in the Preview window. An active contour is displayed, outlining image elements brighter than the location clicked.
- Drag the line on the image to define the threshold of channel A. The selection works best when starting with brighter spots and dragging towards darker areas.
- Press and hold the Shift-key, click with the cursor on the image, then drag the line to determine the threshold of channel B.

### Check the Statistics

The statistical values are updated in real-time and refer to the entire volume data of the current time point, regardless of the settings for the histogram mode. For details please refer to Section Coloc - [Volume Statistics](#).

### Build Coloc Channel

#### Button **Build Coloc Channel**

Clicking this button calculates the colocalization channel according to the selected settings and adds it to the data set.

### Save Coloc Channel

Before the channel is added permanently to the data set it must be saved. In the menu bar select File - Export or click the **Export** button in the toolbar. The Export box is displayed. Select the directory and enter the name for the file to be saved or confirm the suggestion. Select the requested file format and click **OK**. The data set is saved.

## 7.5.8.1 Advanced Examples

The colocalization channel can be visualized like any other channel in Imaris. In the following examples, a Coloc channel was built on the retina data set.

### Visualize the Coloc Channel in Easy 3D

#### Example 1: Visualize and Adjust the Coloc Channel in Imaris Easy 3D

- After building the Coloc channel, click on the **Easy 3D** button in the Imaris menu bar.
- Check the desired Rendering mode parameters and adjust the Settings if necessary, then click the **Calculate** button. The 3D image of channel is calculated and displayed.

As the colocalized region always lays inside the initial channels, making the initial channels invisible or more transparent allows a better view of the colocalized region.

- In the Imaris menu bar select Edit – Show Display Adjustment.
- In the Display Adjustments window uncheck one of the two original channels, then click the button **Calculate** again. The 3D image of the original and the Coloc channel is calculated and displayed.
- In the Imaris menu bar select Edit – Show Display Adjustment.
- In the Display Adjustment window select the Coloc channel and adjust its settings.
- In the Display Adjustment window select the remaining original channel and adjust its settings. In this example the channel is set as partly opaque.
- After each modification, click the **Calculate** button again to calculate the result.

### Visualize the Coloc Channel in Surpass

The colocalization channel can be visualized like any other channel in Surpass.

#### Example 2: Create Surfaces of the Coloc Channel and Add the Two Initial Channels as Volume Rendering

- After building the Coloc channel, click on the **Surpass** button in the Imaris menu bar.
- In the Objects toolbar click the **Add New Surfaces** button.
- In the Surfaces Properties window check and select the Coloc channel.
- Select the desired parameters. In this example, we set the threshold just below the lowest intensity values in the histogram.
- In the menu Surpass select Volume.
- In the Imaris menu bar select Edit – Show Display Adjustment and uncheck the Coloc channel. The Surpass Volume object displays only the two initial channels. Then select the two initial channels and adjust their settings, e.g. decrease the blend opacity.

## 7.5.9 Mouse & Keyboard PC

### Coloc Mouse & Keyboard Functions

Ctrl + 7                      Activates the Coloc view

### Selection Mode Threshold

When using one of the methods to modify the threshold values, the other indications as well as the Preview display are updated in real-time.

### Histogram Channel A and Histogram Channel B

Left-click on histogram      Set new threshold  
 Left-click on line & drag    Move threshold

### 2D Histogram

Left-click                      Set channel A and channel B thresholds  
 Left-click on line & drag    Move channel A and channel B thresholds

### Preview Window

Left-click & drag	Set threshold channel A
Shift + left-click & drag	Set threshold channel B
Middle-click & drag	Move up: zoom out Move down: zoom in
Shift + right-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

## Selection Mode Polygon

### 2D Histogram

Shift + left-click	Add new point (any additional point connects to the last inserted point)
Shift + left-click on connecting line	Insert point in a line
Shift + left-click on point	Delete point
Ctrl + left-click	Add multiple new points
Left-click & drag (on point or line)	Move point or line
Left-click & drag (outside polygon)	Move polygon
Delete	Delete single point

### 7.5.10 Mouse & Keyboard Mac

#### Coloc Mouse & Keyboard Functions

Command + 7	Activates the Coloc view
-------------	--------------------------

#### Selection Mode Threshold

When using one of the methods to modify the threshold values, the other indications as well as the Preview display are updated in real-time.

#### Histogram Channel A and Histogram Channel B

Click on histogram	Set new threshold
Click on line & drag	Move threshold

### 2D Histogram

Click	Set channel A and channel B thresholds
Click on line & drag	Move channel A and channel B thresholds

#### Preview Window

Click & drag	Set threshold channel A
Shift + click & drag	Set threshold channel B

**With a one-button mouse:**

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image

**With a three-button mouse:**

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

**Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.**

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

**Selection Mode Polygon****2D Histogram**

Shift + click	Add new point (any additional point connects to the last inserted point)
Shift + left-click on connecting line	Insert point in a line
Shift + left-click on point	Delete point
Command + click	Add multiple new points
Click & drag (on point or line)	Move point or line
Click & drag (outside polygon)	Move polygon
Delete	Delete single point

**7.6 Animation**

The Key Frame Animation function defines several subsequent views (key frames) of your image, which will be used to create an animation. Imaris generates smooth transitions between the selected views by interpolation.

**How to Open the Key Frame Animation Window?**

1. Click on the icon in the Main toolbar.
2. Select View - Animation.
3. Press the key combination Ctrl + 6.

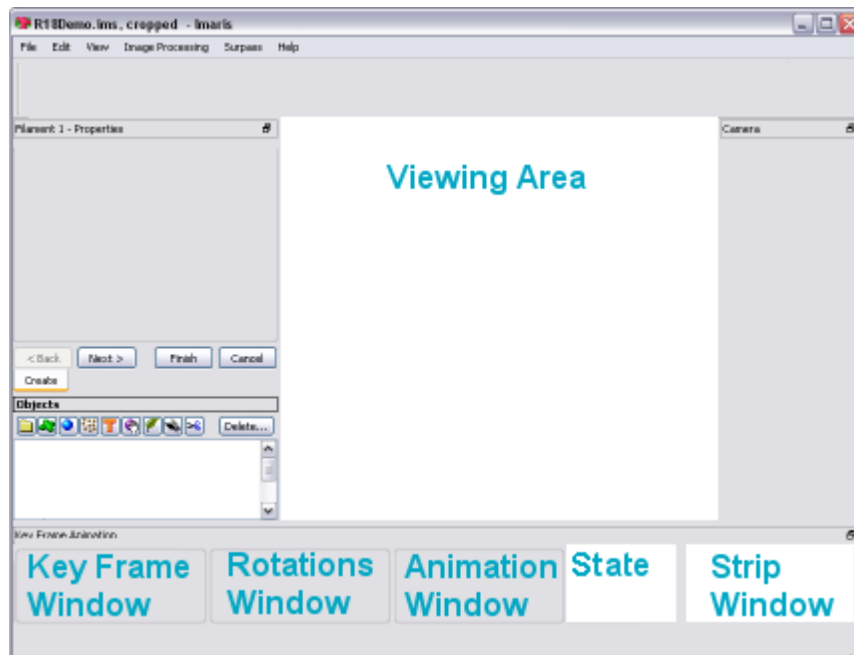
See also:

Menu Edit - Preferences... - [3D View](#)

Animation - [Mouse & Keyboard Mac](#)

## 7.6.1 Overview

The Key Frame Animation consists of different areas:



At the bottom of the screen the following windows are displayed: the [Key Frame Window](#), the [Rotations Window](#), the [Animation Window](#), the [State](#) selection, and the [Strip Window](#).

## 7.6.2 Key Frame Window

In the Key Frame window you define and manage the key frames of your movie.

### Button **Add**

Use this button to insert user-defined key frames in the strip sequence.

### Button **Modify**

Use this button to modify user-defined key frames in the strip sequence.

### Button **Delete**

Use this button to delete user-defined key frames in the strip sequence.

### Button **Delete All**

Click on this button to delete all user-defined key frames in the Strip window.

**Large spaces between the user-defined key frames (thick blue lines) will cause that section of the movie to play more slowly. Placing the lines close together will cause that section of the movie to play more quickly. Use this button to set a constant speed throughout the entire movie.**

### Button **II<**

### Button **II>**

With the arrow buttons you step to the previous (arrow to the left) or next (arrow to the right) user-defined key frame.



## Buttons Overview

<b>Add</b>	<b>Add</b> new user-defined key frame
<b>Mod.</b>	<b>Modify</b> active, user-defined key frame
<b>Del</b>	<b>Delete</b> active, user-defined key frame
<b>Del. All</b>	<b>Delete</b> all key frames
<b>  &lt;</b>	Go to <b>Previous</b> user-defined key frame
<b>  &gt;</b>	Go to <b>Next</b> user-defined key frame

For the complex use of these buttons please refer to Section Animation - [Strip Window](#).

See also:

Animation - [Create Animation](#)

### 7.6.3 Rotations Window

Use the templates to quickly define standard camera motions for the key frame animation system.

#### Templates

Select one of the predefined templates.

You can customize your animation rotation by choosing one of the following options: +360 horizontal, + 180 horizontal, - 360 horizontal, - 180 horizontal, +360 vertical, + 180 vertical, - 360 vertical, - 180 vertical

The key frames are inserted automatically. Click on the button **Play** to see the animation.

#### Button **Custom**

Click on this button to display the Custom Rotation window to freely define the rotation (see below).

## Space

### Screen

The rotation axis is associated to the x-, y-, and z-axis of the screen.

### World

The rotation axis is associated to the x-, y-, and z-axis of the data set.

## Axis

**X**

**Y**

**Z**

Turn along the x-, y-, or z-axis.

## Direction

**+**

Turn to the left.

**-**

Turn to the right.

## Angle

Select or type in the desired angle.

### Button **OK**

Click on this button to accept the changes.

### Button **Cancel**

Click on this button to cancel the changes.

## 7.6.4 Animation Window

### Frames

The number in the field **Frames** indicates the number of thin blue lines within the Strip Panel. This number defines the duration of animations.

### Button **Settings ...**

To open and adjust the Key Frame settings click on this button. For details please refer to Section Menu Edit - Preferences... - [3D View](#).

### Button **>**

Click on this button (arrow to the right - **Play/Pause**) to start the movie preview. To pause the movie preview re-click on this button.

### Button **O**

To record the movie click on this button (red dot - **Record**). Selecting this button the Export Movie window is displayed (see below).

## Window: Export Movie Window

### File name

Enter a file name.

### Export type

Select the desired file type (TIFF series, or AVI).

## Movie Settings

### Compression Factor

Select a compression factor between 0 (high quality) and 100 (low quality), the default setting is 5. Use the slider to set the compression factor between 0 and 100. A lower factor results in a lower compression and therefore a better quality, but also in a larger movie file.

### Frame Rate

Define how many frames are displayed per second.

### Play Movie with default Media Player when finished

Check the box to play the movie with the default media player when finished.

Menu Edit - Preferences... - [Surpass](#) (default Key Frame settings)

## 7.6.5 Play Back State

In the State selection you can adjust the play back settings.

### Camera

- Check: Play back the previously recorded Camera positions for each key frame.
- Uncheck: Do not play back the recorded Camera positions. The Camera stays in the same position. You can change the Camera position manually while playing the animation.

### Time Points

- Check: Play back the previously recorded Time Points for each key frame.
- Uncheck: Do not play back the recorded Time Points. The Time Points do not automatically change during the animation. You can change the Time Points manually while playing the animation.

### Colors

- Check: Play back the previously recorded Colors for each key frame.
- Uncheck: Do not play back the recorded Colors. The Colors do not automatically change during the animation. You can change the Colors manually while playing the animation.

### Display Adjustments

- Check: Play back the previously recorded Display Adjustments for each key frame.
- Un-check: Do not play back the recorded Display Adjustments. The Display Adjustments do not automatically change during the animation. You can change the Display Adjustments manually while playing the animation.

### Clipping Plane (Position)

- Check: Play back the previously recorded Clipping Plane positions for each key frame.
- Uncheck: Do not play back the recorded Clipping Plane positions. The Clipping Plane positions do not automatically change during the animation. You can change the Clipping Plane positions manually while playing the animation.

### Orthogonal Slicer (Position)

- Check: Play back the previously recorded Orthogonal Slicer positions for each key frame.
- Uncheck: Do not play back the recorded Orthogonal Slicer positions. The Orthogonal Slicer positions do not automatically change during the animation. You can change the Orthogonal Slicer positions manually while playing the animation.

### Oblique Slicer (Position)

- Check: Play back the previously recorded Oblique Slicer positions for each key frame.
- Uncheck: Do not play back the recorded Oblique Slicer positions. The Oblique Slicer positions do not automatically change during the animation. You can change the Oblique Slicer positions manually while playing the animation.

### Objects Show/Hide (Object Visibility)

- Check: Play back the previously recorded objects for each key frame.
- Uncheck: The visibility of the objects in the viewing area can be changed manually during the animation. Check or un-check Surpass Tree Items during the animation and the effect is directly visible in the viewing area.

See also:

Animation - [Create Animation](#)

## 7.6.6 Strip Window

The Strip window provides the working area. To display the views of the different key frames left-click on a line to display the corresponding image in the viewing area.

### Lines in the Strip Window

#### Thick Blue Line

A thick blue line indicates a user-defined key frame.

#### White Line

A white line represents the active key frame.

#### Thin Blue Line

A thin blue line stands for an interpolated frame.

### Actions in the Strip Window

#### Add user-defined Key Frame

- Click on a thin blue line (interpolated key frame) in the Strip window. Rotate your image to the desired position. Click on the button **Add** to insert a user-defined key frame in the strip sequence.
- Click on a thick blue line (user-defined key frame) in the Strip window. Rotate your image to the desired position. Click on the button **Add**. Each time you click on the button Add you insert a new user-defined key frame right to the last active key frame.

**Please remember: If you select a thin blue line, you define a new user-defined key frame exactly on this location. If you select an already user-defined key frame the new key frame is halfway between the selected and the next user-defined key frame on the right hand side.**

#### Delete user-defined Key Frame

Click on a key frame and then on the button **Delete**.

#### Move user-defined Key Frame

To move a key frame in the Strip window drag the key frame line while holding down the mouse button. The first and last key frames can not be moved.

#### Modify user-defined Key Frame

Select the key frame in the Strip window. Turn the image to a new position and click on the button **Modify**. The new position for the selected key frame is saved.

#### Copy user-defined Key Frame

To copy a key frame in the Strip window select the initial key frame with a click. The scene in the viewing area changes accordingly. Right-click on the key frame in the Strip window at the desired copy position. The scene in the viewing area does not change. Click on the button **Add** to insert the user-defined key frame.

**Please remember: Click on a key frame to change the camera position in the viewing area accordingly. Right-click (if you use a three button mouse) on a key frame and the view does not change.**

### Strip Window Overview



<b>Click</b> on key frame	Select key frame and change view accordingly
<b>Right-click</b> on key frame	Select key frame, view does not change
Button <b>Add</b>	<b>Add</b> new user-defined key frame
Button <b>Mod.</b>	<b>Modify</b> selected, user-defined key frame
Button <b>Del</b>	<b>Delete</b> selected, user-defined key frame

Menu Edit - Preferences... - [3D View](#) (default interpolated frame settings)

### 7.6.7 Create Animation

The Key Frame Animation feature in Surpass allows you to create an animation from views (key frames). It interpolates frames between the user-defined views to create a smooth movie.

#### 1. Adjust Pre-Settings

- Create an object (Surfaces, Volume, etc) from which to create the animation.
- In the Main toolbar click on the icon **Animation**. The Key Frame Animation window displays on the bottom of the screen and is divided in the Key Frame window, Rotations window, Animation window, State window, and Strip window.
- In the Animation window you find the data field Frames. Enter the number of frames to be included in the movie.
- Click the **Settings** button and enter the number of frames per second.

#### 2. Add Key Frames

- Turn the image view to the first position.
- Capture the first view with a click on the button **Add** in the Key Frame window (records position). The key frame appears in the Strip window.
- Move the image to the next viewing position.
- Click on the button **Add** in the Key Frame window to set the next key frame position (records new position). The new key frame appears in the Strip window.
- Repeat as desired, until all user-defined key frames are added.

#### 3. Select Play Back Settings

- In the State selection you can adjust the play back settings. Please refer to Section Animation - [Play Back State](#) for details.

#### 4. Play the Animation

- In the Animation window click on the button **Play** to start the animation.

#### Example: Handle Camera

##### Use 360° Template

Use the templates to quickly define standard camera motions for the key frame animation system.

- In the Rotations window select +360° Horizontal. Five key frames are inserted automatically. The first and the last key frames are identical.

- In the State window check Camera.
- In the Animation window click on the button **Play** to see the animation. The camera rotates 360° horizontally.

## Capture Individual Camera Positions

- With the pointer in Navigate mode, position the image to create the view for the first key frame.
- In the Key Frame window click the button **Add**. With the first click on **Add** you insert the first and last key frames, they are identical. The key frames appears in the Strip window.
- Position the image to the desired view for the next key frame and click the **Add** button to place the next key frame.
- Repeat as desired, until all user-defined key frames are added.
- In the State window check Camera.
- To preview the movie, click in the Animation window on the button **Play**. The camera position changes automatically.

## Move the Camera Position Manually During the Play Back

- Create an animation.
- In the State window uncheck Camera.
- Play the animation and change the Camera position during the play back manually.

## Example: Handle the Orthogonal Slicer

### Individual Orthogonal Slicer Positions

- Add the Surpass Tree Item Orthogonal Slicer and check the box in the Surpass Tree.
- Adjust the Orthogonal Slicer for the first key frame and click on the button **Add**.
- Move the Orthogonal Slicer before you add the next key frame and so on.
- In the State window check Orthogonal Slicer.
- Play the animation. The Orthogonal Slicer position changes automatically.

### Change Orthogonal Slicer Position Manually During the Play Back

- Create an animation.
- In the State window uncheck Orthogonal Slicer.
- Play the animation and change the Orthogonal Slicer position during the play back manually.

See also:

Animation - [Overview](#)

Animation - [Key Frame Window](#)

Animation - [Rotations Window](#)

Animation - [Animation Window](#)

Animation - [Play Back State](#)

Animation - [Strip Window](#)

Animation - [Save Animation](#)

## 7.6.8 Save Animation

When you are satisfied with the movie, there are two possibilities to save the animation; either as an Imaris Scene File or as a movie-file.

### In Imaris as Scene File

Whenever you save the Scene File in Imaris, the actual animation is saved automatically as part of the Scene

File.

### Save Different Animations in Different Scene Files

Saving different animations of the same data set requires saving of different Scene Files. After generating a new movie by the Key Frame Animation, rename each time the Scene File, e.g. add "Movie" at the end of the Scene File name.

### Record the Animation as Movie

To save the animation as movie click in the Animation window on the button **Record** (red dot). The Export Movie dialog window is displayed on screen. As File name type in the corresponding Scene File name, select the file type and choose the Movie Settings. Click on **Save**.

The **Export type** option allows you to select the movie output file format and save the settings as a preset for future use.

Imaris lets you **save** and **create movie** files on **any platform**. The movie file type specifies the video format and predetermines the codes required to view saved animation.

To playback the movie you must use the appropriate Media player for the selected movie file type.

### Available Movie File Formats

- MPEG-1 Movie (\*.mpg)
- MPEG-4 Movie (\*.mp4)
- AVI Movie (\*.avi)
- WMV Movie (\*.wmv)
- Flash Movie (\*.flv)
- Raw Movie (\*.avi)
- Tiff (series) (\*.tif, \*.tiff)

**Please note:** The number and type of media player codes available depends on your operating system version, video card, and other software installed on your system. If you have experienced problems with viewing the created animation files we recommend installing the free and platform independent, VLC player.

### Multi-threaded Movie Writers

Multi-threading offers a significant (up to 30%) increase in speed during movie creation and are therefore recommended.

The following movie writer is multi-threaded:

- MPEG-4 Movie (\*.mp4)

See also:

Surpass View - Overview - [Scene File Concept](#)

Animation - [Animation Window](#) (Button Record)

## 7.6.9 Mouse & Keyboard PC

### Animation Mouse & Keyboard Functions

Ctrl + 6                      Activates the Key Frame Animation

### In the Strip Window

Left-click on key frame	Select key frame and change view accordingly
Click thick line & drag	Move key frame to new position
Right-click on key frame	Select key frame, view does not change

## 7.6.10 Mouse & Keyboard Mac

### Animation Mouse & Keyboard Functions

Command + 6                      Activates the Key Frame Animation

#### In the Strip Window

##### With a one-button mouse:

Click on key frame	Select key frame and change view accordingly
Click thick line & drag	Move key frame to new position
Ctrl + click on key frame	Select key frame, view does not change

##### With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

Left-click on key frame	Select key frame and change view accordingly
Click thick line & drag	Move key frame to new position
Right-click on key frame	Select key frame, view does not change

## 7.7 Fit

Fits the entire scene into the view.

In the Status Bar you find the respective button **Fit**.

See also:

Toolbars - [Status Bar](#)

## 7.8 Reset

Click on this button to reset the image to the original position, center the image in the middle and set the optimal zoom factor.

## 7.9 Full Screen

Displays the viewing area to full size of the monitor. To return to the standard window, click on the button **Full Screen** in the lower left corner.





In the Status Bar you find the respective button **Full Screen**.

See also:

Toolbars - [Status Bar](#)

## 7.10 Navi

The Navigation window displays an overview of the data set in the upper right corner of the viewing area. If you move the data set, the overview updates in real-time. You can activate the Navigation window individually for each Imaris view.

In the Status Bar you find the respective button **Navi**.

### Change Position

- In the pointer mode Select you can click & drag the Navigation window to any desired corner.
- Double-click onto the Navigation window in order to switch off the automatic "snap-to-corner-mode". You can drag it to any desired location. Double-click the Navigation window again to switch the automatic "snap-to-corner-mode" back on again.
- Right-click into the Navigation window in order to pan to the clicked position (moves the small yellow window).

In the Section the position of the Navigation window is fixed (lower right corner) and can not be moved.

## 7.11 Origin

The location of the coordinate system origin can be set to any corner of all the views. It can be set to: Bottom Left (default), Top Left, Bottom Right, or Top Right. A selection is stored and used each time you start Imaris.

## 7.12 Status Bar

At the bottom of the screen you find the [Status Bar](#).

## 7.13 Event log

It is Web based event log reporting tool.

Event Log is a window that displays detailed information about Imaris Bisque significant events on your computer. This can be helpful when troubleshooting problems and errors.

## 7.14 Refresh

Refresh the screen.

## Section 8 Image Processing

The Image Processing menu provides a range of functions to change the image data. Frequently used functions are Baseline Subtraction, and Threshold Cutoff from the Image Thresholding submenu or the Gaussian Filter from the Image Smoothing submenu.

### Button **Apply**

If a time series is loaded, clicking **Apply** applies the image processing function to the current time point displayed on the screen.

### Button **OK**

Clicking **OK** applies the image processing function to all time points.

[Flip ...](#)

[Rotate...](#)

[Free Rotate](#)

[Channel Shift...](#)

[Swap Time and Z](#)

[Swap Time and Channels](#)

### [Smoothing](#)

[Gaussian Filter...](#)

[Median Filter...](#)

### [Thresholding](#)

[Baseline Subtraction...](#)

[Threshold Cutoff...](#)

[Background Subtraction...](#)

[Connective Baseline...](#)

### [Contrast Range](#)

[Linear Stretch...](#)

[Gamma Correction...](#)

[Invert...](#)

[Normalize Layers...](#)

[Histogram Equalization](#)

[AutoQuant...](#)

[XTensions](#)

## 8.1 Flip...

The channels can be flipped together or separately.

On the left hand side of the window Flip Channel(s) the actual channel(s) are displayed. Select the channels to be flipped by marking the check-box on the left.

### All Channel(s)

#### Direction X, Y, Z

Check the requested Direction box.



Button **Apply**

Button **OK**

Button **Cancel**

Click the **Apply** button. The effect of the change can be seen in the image. If necessary, change the direction. When the results are satisfactory, click the **OK** button. The data set is modified. Click **Cancel** to return to the original image.

## 8.2 Rotate...

Rotate direction applies to all channels together. Single channel application is not possible.

### All Channel(s)

**Axis X, Y, Z**

Enter the requested axis.

**Orientation**

**Counter Clockwise, Clockwise**

Select the respective orientation.

Button **Apply**

Button **OK**

Button **Cancel**

Click the **Apply** button. The effect result can be seen in the image. If necessary, change the direction. When the results are satisfactory, click the **OK** button. The data set is modified. Click **Cancel** to return to the original image.

## 8.3 Free Rotate

The Free Rotation function rotates an image by any degree and offers additional image resizing options. The camera location determines the rotation angles and directions. The center of the image is the center point of the rotation. The operation is performed for every time point and channel.

Please note selecting just one channel for free rotation will create two data sets.

### X, Y, Z

These fields display the current x-, y-, and z axis vector values.

To alter the image position you can write the values directly into the fields or you can as well adjust the image position in the Surpass View.

- The x, y and z axis values are the components of a vector from the origin, to the point with the specified x,y, and z value.
- The rotation will rotate the image around this axis by a specified amount (angle).
- The amount of rotation is specified as an angle. The angle can have any value. 0 means no rotation. Rotations will repeat themselves after 360 degrees. For example, a rotation of 45 degrees is the same as one of 405 degrees, the same as one of -315 degrees and the same as one of 765 degrees etc.

### Angle

This field displays the current value of the angle of rotation.

## Coordinate resize factor

The image and voxel values define the size of the new data set after free rotation.

These values are used to build a map of all the co-ordinates that will be used to display the newly rotated image.

Free rotation can be an expensive memory operation. Adjusting the image dimensions can reduce the size of the data set, decreasing the time needed to read the file and speeding up calculation. This can be particularly important when reading large data sets.

## Voxel

Changing the x, y, and z values creates a new image with different voxel spacing.

These values determine the resampling points. Resampling reduces or increases the voxel density in a data set.

Reducing the resampling points deteriorates the image but speed up any operations on the data.

### X, Y, Z

In the Voxel X, Y and Z fields, enter the factor by which to multiply the current resampling interval.

For decimation (fewer samples), enter a factor greater than 1 to increase the sampling interval by this factor.

For interpolation (more samples), enter a factor less than 1 to decrease the sampling interval by this factor.

## Image

If the border of the new image needs to be adjusted, change the x, y, and z values and create a new image with the different image size. This option helps you to crop (minimize the size) the data set without losing significant data.

### X, Y, Z

The parameters X, Y and Z allow you to specify the size of a rotated image by defining the range of an original image.

In the Image X, Y and Z fields, enter the crop factor of the lower and upper image range.

The values of X, Y and Z should be in the range between 0 and 1.

## Padding Intensity

When you create a new image with free rotation some of the new voxel surroundings are included with the data set. The padding voxels define an area between the previous outside boundary of the original image and newly rotated image content.

This padding intensity field lets you edit the newly created pad voxel intensity.

## Linear interpolation

After rotation and data size adjustment, a new image with different voxel spacing is created and the new voxel coordinates need to be calculated.

The difference between the voxel spacing on the new and old image means that an interpolation scheme is required as the new image's voxels are unlikely to coincide with those in the old image.

The Linear interpolation box allows you to select the interpolation method.

If the box is selected for new voxel coordinates then the tri-linear interpolation method is used (all surrounding

values are used for calculations).

If the box is not selected the voxel spacing will be calculated based on the nearest neighbor interpolation. Depending on the data set and parameter values the interpolation algorithm could involve some rather time intensive internal calculations. When speed is favored, please consider selecting the less-time costly interpolation method (nearest-neighbor).

## 8.4 Channel Shift...

The Channel Shift function allows you to move channels relative to one another.

On the left hand side of the window Channel Shift the actual channel(s) are displayed. Select the channels that should be moved relative to the remaining channels.

### Pixel Shift for All Selected Channel(s)

#### X, Y, Z

The translation is specified in terms of integer numbers of voxels to move along each of the x-, y-, and z-directions.

## 8.5 Swap Time and Z

Exchange the t-axis and the z-axis and re-format the image in such a way that the time dimension is mapped onto the z-axis and the z-dimension is mapped onto the t-axis. The section now shows x-t-, and y-t-views.

### Start Function

- Select in the menu Image Processing - Swap Time and Z.

The z-, and t-axes are exchanged. This allows you to play the images over z and t.

### Play the Series

- Click on Start (arrow to the right) in the Time Bar to play the series of images along the z-axis.

The Time Bar is visible in all display modes (Slice, Section, Gallery 3D Preview, and Surpass). It allows you to view a particular Time Point or to automatically play 4D images. The views change when selecting a new Time Point.

Example:

Your data set has four Time Points, each Time Point has one Slice in z-dimension. Then you swap time and z. The result is a new data set with one Time Point and four Slices in z-dimension.

See also:

Toolbars - [Time Bar](#)

## 8.6 Swap Time and Channels

Exchange the t-axis and the channels and re-format the image.

### Start Function

- Select in the menu Image Processing - Swap Time and Channels.

The t-axes and the channels are exchanged.

Example:

Your data set has four Time Points, each Time Point has one channel. Then you swap time and channel. The result is a new data set with one Time Point and four channels.

See also:

Menu Edit - Image Properties - [Channel 1 ... n](#)

Menu Edit - [Show Display Adjustment](#)

## 8.7 Extract Channel

The Extract Channels compare the color content of an image to reference color information. This function extracts and displays color data from the image referent point and use it as a representative color, for a new color channel creation. The new channel is generated based on the difference in the reference color point and the whole image color values.

The difference relates how closely the color information in the region matches the information represented by the color reference point, assigning the higher intensity for smaller difference. The result of the Extract Channel is a separate channel, which can be processed and viewed as any originally acquired channel in Imaris and used for easier segmentation of similarly colored multi-channel data sets.

1. Click on the **Slice** icon in the Main toolbar.
2. Change pointer mode from Navigate to **Select**.
3. Select the reference color and click on it.
4. Click on the **Get value from the last measurement point** button displays associated RGB values in the numerical fields.
5. Specify Color Difference by selecting the color space (either RGB or Perceptual).
6. Select the Apply button. The effect of the change can be seen on the screen.
7. When the results are satisfactory, click the **OK** button.
8. The new channel is created and it's a part of the data set.
9. To restore the initial image click Cancel.

The new extracted channel can be visualized like any other channel in Imaris by selecting the Edit menu and choosing the option [Display Adjustment Window](#). In the Display Adjustment Window uncheck original channels, select the newly created channel and adjust its setting.

**Please note:**

**Channel selection in the Extract channel window switches to match Display Adjustment channel selection when you click Get value from the last measurement point button**

### Extracted channel values

1. The number of channels switched on in Extract Channel window influence extracted channel values.
2. Display Adjustment Window color parameters (min/max/gamma) influence the extracted channel values.



3. However, the number of channels switched on in Display Adjustment Window does not influence the extracted channel values

### Channels

The channels can be selected by clicking on the check-box.

### Color of Interest

The Color of Interest option allows the reference color point to be present either as separate value for red green and blue or as the intensity value for a selected channel.

The reference color point is shown within the color wheel.

### RGB

The RGB triple value is displayed for the color reference point.

### Intensity

The Intensity of the selected channel is displayed for the color reference point.

Get value from the last measurement point Button

Clicking this button calculates the RGB or intensity values according to the selected color reference point.

### Color difference

This option defines the color space for a newly created channel.

### RGB

The RGB color space is an additive color model in which values for Red, Green and Blue color information are combined together to reproduce any given color.

### Perceptual

Implements the color extraction using Lab color space. The Lab color model provides a perceptually uniform color space. A perceptually uniform color space indicates that a change in a color value produces a change of about the same visual importance. Lab color is designed to approximate human vision.

For more specific information about Lab color space: [http://en.wikipedia.org/wiki/Lab\\_color\\_space](http://en.wikipedia.org/wiki/Lab_color_space)

## 8.8 Smoothing

Whether or not images need to be smoothed depends on their quality. Inspect the image to see if any smoothing is required. For image smoothing several different filters are available:

- [Gaussian Filter](#)
- [Median Filter](#)

### 8.8.1 Gaussian Filter

#### User Interface

The Gaussian filter for a data set can have only one value. This value can be applied separately to any one channel or to all channels. Structures smaller than the filter width will be removed.

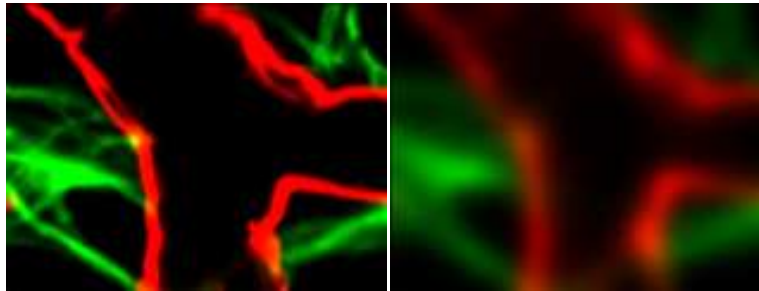
- Select the menu Image Processing - Image Smoothing - Gaussian Filter. The Gaussian Filter box is displayed.

- Select the channels to be filtered by marking the check-box on the left of the channel.
- Enter the requested filter width in the parameter field and click **Apply**. The effect of the change can be seen on the screen.
- If necessary, readjust the value.
- When the results are satisfactory, click the **OK** button.

The data set is modified. To restore the initial image click **Cancel**.

### Function

The Gaussian filter smooths the image.



It has some nice properties:

- Total image intensity is preserved.
- Noise is suppressed evenly across the image.
- No artefacts are introduced.

See also:

Surpass View - Filament - [Data Input and Filtering](#)

### 8.8.2 Median Filter

#### User Interface

The Median filter for a data set can have only one value. This value can be applied separately to any one channel or to all channels. Structures that are smaller than the filter width will be removed.

- Select the menu Image Processing - Image Smoothing - Median Filter. The Median Filter box is displayed.
- Select the channels to be filtered by marking the check-box.
- Check the requested Filter Size and click the **Apply** button. The effect of the change can be seen in the image.
- If necessary, readjust the value.
- When the results are satisfactory, click the **OK** button.

The data set is modified. Click **Cancel** to return to the original image.

### Function

The Median Filter replaces the intensity of each voxel with the statistical median of the intensities of the neighboring voxels. There are several choices for the neighboring voxels to be included in the median calculation. The median filter is useful when the image data are corrupted by 'salt and pepper' noise.



## 8.9 Thresholding

Whether or not it is necessary to apply a thresholding function to an image depends on its quality. Inspect the image to see if any action is required.

For image thresholding a number of functions are available. The following paragraphs describe the user interface to the following filters:

- [Threshold Cutoff](#)
- [Baseline Subtraction](#)
- [Background Subtraction](#)
- [Connective Baseline](#)

### 8.9.1 Threshold Cutoff

Threshold Cutoff compares the intensity of every voxel in the image to the threshold value. If the intensity is greater than the threshold, it remains unchanged. If a voxel has an intensity value less than the threshold value it is assigned a new intensity value. This value is 0 by default. The Threshold Cutoff values for the channels may differ.

1. Select the menu Image Processing - Image Thresholding - Threshold Cutoff. The Threshold Cutoff box is displayed, showing a histogram of the voxels in the data set.
2. Select the channels to be cut by marking the check-box to the left of the channel bar.
3. Highlight a channel.
4. A colored bar in the histogram shows which channel has been selected.
5. Enter the requested values for that channel in the Threshold field or drag the bar in the histogram.
6. Repeat for each channel (if applicable).
7. Click **Apply**. The effect of the changes can be seen in the image.
8. If necessary, readjust the value.
9. When the results are satisfactory, click the **OK** button.

The data set is modified. Click **Cancel** to return to the original image.

### 8.9.2 Baseline Subtraction

Baseline Subtraction subtracts the baseline value from the intensity of every voxel in the image. If the result is positive, this is the new intensity value, if it is negative, the voxels intensity is set to zero. The Baseline Subtraction values for the channels may differ.

1. Select the menu Image Processing - Image Thresholding - Baseline Subtraction. The Baseline Subtraction box is displayed, showing a histogram of the voxels in the data set.
2. Select the channels to be cut by marking the check-box to the left of the channel bar.
3. Highlight a channel.
4. A colored bar in the histogram shows which channel has been selected.
5. Enter the requested value for that channel in the Baseline field or drag the bar in the histogram.
6. Repeat for each channel (if applicable).
7. Click **Apply**. The effect of the changes can be seen in the image.
8. If necessary, readjust the value.

9. When the results are satisfactory, click the **OK** button.

The data set is modified. Click **Cancel** to return to the original image.

### 8.9.3 Background Subtraction

Background Subtraction applies a Gaussian filter to define the background at each voxel and then performs a Baseline Subtraction of this variable background. The filter width is the only parameter of the background subtraction method.

1. Select the menu Image Processing - Thresholding - Background Subtraction. The Background Subtraction box is displayed.
2. Select the channels by marking the check-box on the left of the channel.
3. Enter the requested filter width in the parameter field and click **Apply**. The effect of the change can be seen on the screen.
4. If necessary, readjust the value.
5. When the results are satisfactory, click the **OK** button.

### 8.9.4 Connective Baseline

The Connective Baseline works with two threshold values, a lower and an upper threshold. It applies these thresholds to divide the image into regions and then processes each region with a Baseline Subtraction using either the lower or the upper threshold. The three types of regions the method creates are the following:

- Connected region of voxels where the intensity of every voxel within the region is below the lower threshold (Type A).
- Connected region of voxels where the intensity of every voxel within the region is greater than the lower threshold and no voxel within the region has an intensity greater than the upper threshold (Type B).
- Connected region of voxels where the intensity of every voxel within the region is greater than the lower threshold and at least one voxel within the region has an intensity greater than the upper threshold (Type C).

The intensities of voxels from regions of type A are set to zero, as are the intensities of voxels from regions of type B. Only voxels from regions of type C retain a nonzero intensity, namely the original intensity less the lower threshold value.

See also:

Surpass View - Filament - [Data Input](#)

## 8.10 Contrast Change

For image Contrast Change a number of functions are available:

[Linear Stretch...](#)

[Gamma Correction...](#)

[Invert...](#)

[Normalize Layer\(s\)](#)

[Histogram Equalization](#)

### 8.10.1 Linear Stretch...

Linear stretching may be necessary whenever 12 bit data is read in 16 bit mode. Then the contrast can be extended to the new limits. Picture data is changed when stretching. Linear stretching results in a picture displaying dense data in a wider contrast range.

1. Select the menu Image Processing - Contrast Change - Linear Stretch. The Linear Stretch box is displayed.
2. Select the channels to be stretched by marking the check-box.
3. Enter the requested values in the parameter fields New Maximum and New Minimum and click the Apply button. The effect of the change can be seen in the image.
4. If necessary, readjust the value.
5. When the results are satisfactory, click the **OK** button.

The data set is modified. Click Cancel to return to the original image.

### 8.10.2 Gamma Correction

The Gamma correction function lets you improve the image display by intensifying the gray value of a specific range of voxels. Usually the color contrast values of the voxels stretch linear over a wide range (e.g. 0 - 255). The Gamma correction function lets you set a new gamma value according to which the gamma curve for the voxels is calculated automatically. Thus the brightness of the voxels can be intensified or weakened in certain areas.

1. Select the menu Image Processing - Contrast Change - Gamma Correction. The Gamma Correction box is displayed with the default Gamma Value set to 1, therefore showing a straight line as a linear voxel representation.
2. Select the requested channels by clicking marking the check-box.
3. Enter the requested value in the Gamma value field or click directly in the gray Gamma Curve area to set the new gamma value point. The curve and the field value are set accordingly and the effect of the change can be seen on the channels (channels appear differently).
4. Click the **Apply** button
5. If necessary, readjust the value.
6. When the results are satisfactory, click the **OK** button.

The data set is modified. Click **Cancel** to return to the original image

### 8.10.3 Invert...

The channels can be inverted together or separately.

1. Select the menu Image Processing - Contrast Change - Invert. The Invert box is displayed.
2. Select the channels to be inverted by marking the check-box on the left. The effect of the change can be seen in the image.
3. When the results are satisfactory, click the **OK** button.

The data set is modified.

Click **Cancel** to return to the original data set.

### 8.10.4 Normalize Layer(s)

This normalization adjusts brightness and contrast of individual z-slices to a uniform level. It is most useful for correcting illumination variations of scans from physical sections, and in some cases it can also reduce

attenuation effects from z-stacks.

This image processing function first computes the mean and standard deviation values of the intensity for an entire image z-stack. Then it computes mean and Std.dev. for all individual z-slices. Finally, it adjusts the intensities of each z-slice with a linear transformation, to match the overall mean and Std.dev. value.

This procedure is applied for all z-slices of the data set, for all time points and for each channel independently. Voxels with zero intensity are excluded from this calculation - they are interpreted as background.

1. Select the menu Image Processing - Contrast Change - Normalize.
2. Select the channels to be inverted by marking the check-box on the left and click on the button **Apply**.
3. When the results are satisfactory, click the **OK** button.

The data set is modified.

Click **Cancel** to return to the original data set.

## 8.10.5 Histogram Equalization

Histogram equalization is a method of image processing by contrast adjustment using the image's histogram. The method is useful in images with close contrast values; in particular, this method can lead to better views of electron microscopy images.

Through this adjustment, the dynamic range and contrast of an image is altered. The resulting image features the highest possible contrast, uniformly distributed over the whole contrast range.

This method increases the global contrast by adjusting the distribution of the intensities on the histogram by spreading out the most frequent values. In histogram equalization, each pixel is assigned a new intensity value based on its previous intensity level so that the output image contains a uniform distribution of intensities. This allows for areas of lower local contrast to gain a higher contrast without affecting the global contrast.

### Current minimum/maximum

These values define the limits of data set intensities. Intensities below the Current minimum will be set to the new minimum and intensities above the Current maximum will be set to the new maximum.

### New minimum/maximum

These values define the intensity range of the output data set. By default, they are set to the minimum/maximum achievable intensities of the data set.

To perform histogram equalization on the data set perform the following steps:

1. Select the menu Image Processing - Contrast Change - Histogram Equalization.
2. The Equalize Channel Histogram(s) window is displayed
3. Select the channels to be processed by marking the check-box
4. Enter the requested values in the parameter fields Current Minimum/Maximum, New Maximum/Minimum or accept the automatically optimized Min. and Max. limits.
5. Click the **Apply** button.
6. If necessary, readjust the value.
7. When the results are satisfactory, click the **OK** button.

Click **Cancel** to return to the original data set.

## 8.11 Image Alignment

Two options are available:

### Set Reference Frames



This option analyses the image and automatically corrects the drift. You can select if only **Translational Drift** or **Translational and Rotational Drift** should be corrected. The result of this option is the creation of a Reference Frame surpass object. The image and other objects are not affected.

### Align Image

This option analyses the image and automatically corrects the translational and rotational drift. As a result, the image will be changed. In the Objects options section, you can choose if none, all or the selected surpass objects should be adjusted along with the image.

The **Result dataset size** section lets you control the resulting image size.

**Crop Largest common region** creates a dataset that contains the overlapping region between the original and the corrected dataset.

**New size equal to current size** creates a dataset that has the same size as the original dataset and contains the overlapping region.

**Include entire result** creates a dataset that fully contains the original and corrected dataset.

The **Preview** section illustrates the resulting dataset in pink, compared to the original and corrected image in black.

Drift Correction is also possible from [Tracked Objects](#) and from the [Reference Frame](#).

## 8.12 AutoQuant...

The AutoQuant deconvolution software is integrated into Imaris. It requires a separate license. The pathway to AutoQuant must be set in the menu Edit - Preferences... - Advanced - Files - AutoQuantExeFileName.

When opening AutoQuant, the software will prompt for a destination for the file that is transferred to AutoQuant and will automatically create an image in the ids/ics format and open it in AutoQuant.

- Select the menu Image Processing - AutoQuant ...

An error message appears if the program is not installed or the pathway is not set.

## Section 9 XTensions

This Section gives an overview of all Imaris XTensions.

Imaris XTensions is a multi-functional two-way interface from Imaris to classic programming languages, Matlab and other imaging software programs.

### Please note:

- **Windows 8 and 10 do not come with the .net 3.5 and prior framework required for the Matlab runtime which Imaris XTensions use.**
- **In order to enable this 'Windows Feature' you will need an administrator account. You can set this under Control Panel -> Programs and Features, left hand side panel contains 'Turn Windows features on or off'.**

In order to use the supplied Matlab runtime extensions Matlab runtime also needs to be installed. This can be found on the Bitplane Customer Portal.

[Project to 2D](#)

[Attenuation Correction](#)

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Camera Functions:

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[Spots Split](#)

[Spots Split Into Surface Objects](#)

[Super Resolution Localization Data To Image](#)

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Filaments Functions:

[Filaments Angles Statistics](#)

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[Histogram Equalize Layers](#)  
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Tracks Functions:

[Connect Tracks](#)  
[Plot Angles of selected Tracks](#)  
[Plot Distance Between Tracks](#)  
[Plot Length of selected Track](#)  
[Split Tracks](#)  
[Translate Tracks](#)

[Vesicle Outside Cell](#)

## 9.1 Project to 2D

This XTension compresses the data set into two selected dimensions. The user first selects the projection plane (XY, XZ or YZ) and then selects the projection mode (MIP or Mean). In the projection plane window, select the projection plane XY, XZ or YZ.

### Projection mode MIP or Mean

MIP- max intensities over the projection line

Mean- over the projection line divided by the number of voxels

## 9.2 Attenuation Correction

When working with 3D images you may find that optical sections from deeper within the sample may appear to have lower intensity when compared to the top layers. This XTension corrects this effect, by amplifying the intensity of pixels from slices at the bottom of the z stack.

To apply the AttenuationCorrection XTension to an image:

1. Select the relevant channel.
2. Find a position in the first and last slice of the image stack that should have the same intensities.
3. Determine the intensities at these positions
4. Select the AttenuationCorrection XTension
5. In the Attenuation Correction window, enter the channel number and corresponding intensity of the top and bottom optical slices.
6. Press **OK** and the XTension increases the intensities of the bottom layers while leaving the intensities of the top layers unchanged

Each channel is handled separately with the relevant correction.

It is based on the following calculation:

=log (value2)-log(value1)/ pixel size (z)

$$I_{\text{layer new}} = I_{\text{layer old}} * e^{- (\text{slice number}-1)}$$

## 9.3 Crop Borders

This XTension computes the bounding box of voxels with intensity higher than the user specified value. Starting from the borders, it searches the image for the largest possible border containing an intensity value above the entered threshold. All voxels with intensity values below the threshold are cut away.

This XTension minimizes the size of the data set without losing meaningful data by removing peripheral pixels along any of the specified three axes.

The cropping direction X,Y, or Z should be specified and the threshold voxel intensity should be defined.

## 9.4 Camera Functions

This Section outlines the Camera XTensions:

[Camera Manager](#)

[Set the Angle of the Camera](#)

### 9.4.1 Camera Manager

This XTension saves, loads or clears camera settings in .mat files. With this Xtension you can save the camera position and you can load the same camera positions for other data sets as well.

Available settings are: position, orientation, perspective, orthographic, focus and height. This XTension lets you store and load an arbitrary number of viewpoints. This XTension can be also used for different images with a similar size.

To change the default location (Matlab), select the Browse button. Select the directory and enter the name for the file to be saved.

To save a setting:

1. Adjust the data set to the position you need
2. Start Camera Manager
3. Browse to a location where you want to save the file
4. Save the file

To reload a saved setting:

1. Execute the Camera Manager
2. Browse to the location where you saved the file
3. Confirm the dialogue for the overwrite file
4. Select the Load button to apply the same

If you open in the Camera.mat file (Matlab directly) 10 values can be found.

- 1-3: Camera Position
- 4-6: Camera Orientation
- 7: Angle
- 8: Perspective or Orthogonal
- 9: Focus
- 10: Height (width of the perspective)



## 9.4.2 Set the Angle of the Camera

The angle of the camera is defined by using the Azimuth, Elevation, Angle coordinates. The camera is first rotated around axis Y/the Y axis by Angle, then around axis X/the X axis by Elevation, then around the object axis by Azimuth.

Coordinates can be ambiguous: A different Azimuth, Elevation, and Angle can determine the same camera orientation.

Moving the sliders or entering a numeric value in the selected fields changes the camera position.

## 9.5 Channel Arithmetics

This XTension supports the common arithmetical operators on the channels. Matlab expressions are executed by entering a mathematical expression in the Matlab syntax field. The expression is executed in Matlab itself. As a result, the intensity values are converted into double floating-point precision, calculated and converted back to the original data type and shown in Imaris as an additional channel.

Channels "ch1, ch2, ch3, etc." and numbers can be used.

**Note: avoid using NaNs (Not a Number values) in mathematical expressions.**

The following examples are shown in the following table for your reference:

Example	Expression
New channel is the sum of two existing channel intensities	ch1 + ch2
New channel is the first channel with an intensity of 20 added	ch1 + 20
New channel is the second channel intensity, divided by two (note that you must use "./" and not just "/")	ch2 ./ 2
New channel is the first channel multiplied by 3.5 plus the second channel divided by 2 (note that you must use ".*" and not just "*")	ch1 .* 3.5 + ch2 ./ 2
New channel is 255 minus the first channel intensity, i.e. the inverse of the first channel in case of an 8bit image	255 - ch1
New channel is the square root of the multiplication of the first and second channel intensity	sqrt(ch1 .* ch2)
Masking is also possible. The new channel has intensity 1 for those voxels that have channel1 higher than 200	ch1 > 200
Visualization is easier when the intensity is higher than one. In this case the new channel has intensity 150 for those voxels that have channel1 higher than 200	(ch1 > 200) .* 150
New channel has intensity 150 where the first channel has intensity higher than 50 and the second channel has intensity higher than 30	(ch1 > 50 & ch2 > 30) .* 150
The new channel has intensity 150 where the first and second channel has an intensity of 0	(ch1 == 0 & ch2 == 0) .* 150
New channel has intensity 150 where the first or second channel has an intensity higher than 200	(ch1 > 200   ch2 > 200) .* 150

## 9.6 Add Similarity Statistics Value

The Add Similarity Statistic Value is an XTension that allows quantification of the degree of the similarity of a set of objects with the pre-selected reference object(s).

This function creates a new statistical value, which can be used under the Filter, Statistical and Color tab.

To create a Similarity Statistic Value:

1. Select the reference objects according to your requirements
2. Enter an appropriate name for the new statistical value.
3. In the Similarity-Selection Box select one statistical value or in combination them from the list.
4. To select a statistical value, click on the name of the variable in the list. For multiple selection use Ctrl + click. The newly created statistical value is based upon the aggregate weighted value of the statistical characteristics of the selected object(s).
5. The Matlab window confirms the creation of the new statistical value.

The benefits of the Similarity Statistic Values function are the precise identification of the objects with similar complex statistical properties or on the other hand, it offers the benefit of determining the objects which are the least similar to the reference object.

## 9.7 Distance Transformation

### For Surface objects

This XTension computes and creates a new channel. The created channel intensity indicates the shortest distance to a surface object border. The border of the surface objects defines the origin of the distance transformation. The intensity value of the voxels at the border of the surface is always set to 0.

There are two options for the distance transformation calculation:

- Inside Surfaces object
- Outside Surfaces object

Each voxel is mapped with the intensity representing the measured minimum distance to the nearest surface border. The intensity value correlates directly to the distance to the border. The higher the intensity value corresponds to the larger the distance.

**Please note: In order to calculate the distance correctly, the data should be transformed into a 32-bit float image. To change the data set type, select the Change Data Type option under the Edit menu.**

To modify the channel visibility contrast and range open the Display Adjustment window under the Edit tab.

### For Spots objects

This XTension computes and creates a new channel. The created channel intensity values are based on the distance to a spot object. Each voxel is mapped with an intensity representing the minimum distance measured to the nearest spot's centre. A higher intensity value corresponds to a larger distance to an object. The center of the spot object defines the origin of the distance transformation. Voxels in the centre of the spot object are always set to have an intensity value of 0.

**Please note: In order to calculate the distance correctly, the data should be transformed into 32-bit float images. To change the data set type, select the Change Data Type option under the Edit menu.**

To modify the channel visibility contrast and range open the Display Adjustment window under the Edit tab.

## 9.8 Surfaces Functions

This Section covers the XTensions relevant to surfaces:

Distance Transformation- refer to [Distance Transformation](#)  
[Spots And Surfaces Distance](#)  
[Surfaces Split](#)  
[Surface Merge](#)

### 9.8.1 Spots And Surfaces Distance

This XTension computes the distance between all Surface and Spots objects that are in the same Surpass group.

This XTension first computes the centroid of each object component. The centroid of the Spots objects is equal to the mean of the coordinates of all the single spots. The centroid of a surface object is calculated based on the mass of the object's center.

Then, the XTension calculates and displays the distance between the centroids.  
The results of this XTension are presented in the Matlab window as a table.

### 9.8.2 Surfaces Split

This XTension splits one Surfaces object in multiple Surfaces of disconnected components.  
As a result, in the Surpass tree a new Surfaces objects are created. The number in brackets indicates the number of Surfaces objects.

### 9.8.3 Surface Merge

This XTension merges all the Surfaces objects that are in the same folder into a single Surfaces object.  
As a result, in the Surpass tree a new Surfaces object is created. The number in brackets indicates the number of merged Surfaces objects.

## 9.9 Spots Functions

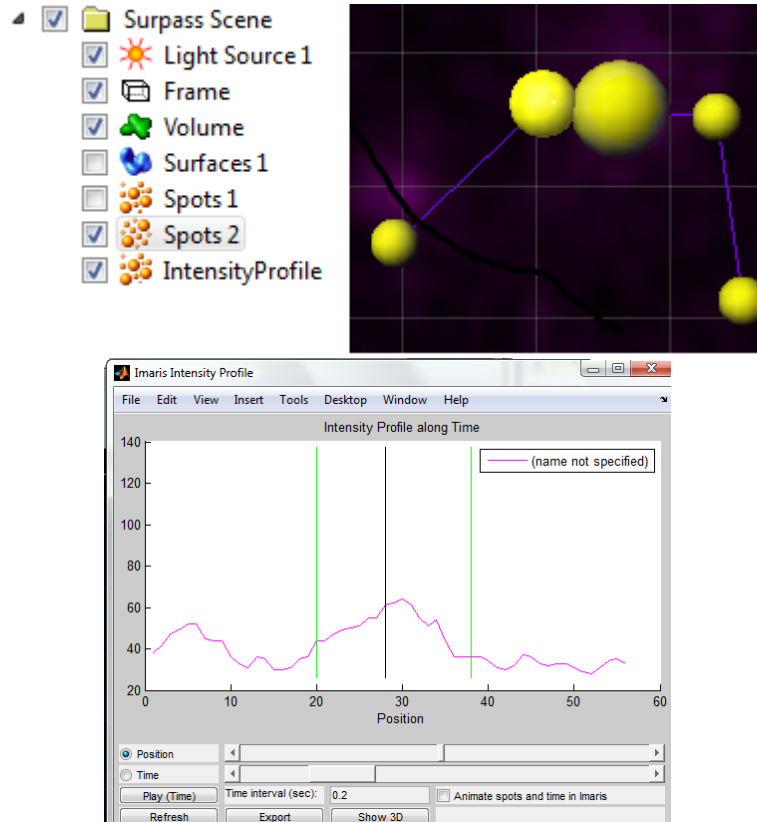
This Section covers the XTensions relevant to spots.

Distance Transformation- refer to [Distance Transformation](#)  
[Intensity Profile](#)  
[Spots And Surfaces Distance](#)  
[Spots to Spots Closest Distance](#)  
[Find Spots Close To Filaments](#)  
[Colocalize Spots](#)  
[Create Channel from Spots](#)  
[Split Spots](#)  
[Split Spots Into Surface Objects](#)  
[Super Resolution Localization Data To Image](#)  
[Super Resolution Localization Data To Spots](#)

### 9.9.1 Intensity Profile

This XTension creates and plots the intensity profile of the created spots. The Matlab window displays the intensity profile as a function of the position or time. The x-axis represents the position /time and the y-axis represents the intensity range.

The black vertical line indicates the current position/time point. The green lines indicate the intermediate positions. To change the position/time use the slider.



The channel colors are displayed in the plot. The intensities of the channels are shown using different colors respective to the channels. Several options allow the user to have control over the parameters of the calculation and how the results are displayed.

The length/time selection is located below the plot.

In the animation line you can find the following options:

- Play/Stop button
- Time interval field
- Animate spots and time in Imaris

Click on the **Play** button to play the time series. To stop the continuous display through all the time points click on the **Stop** Button.

In the **Time intervals** field the current value is displayed. By entering a new value, you can adjust the time interval duration.

Selecting or de-selecting the box next to the **Animate spots and time in Imaris** controls the animation.

## Refresh

Clicking on the Refresh button imports any changes that were made to the data in Imaris and displays the most recent version of the data.

## Export

For some further statistical analysis you can export the data. Click on the button Export to open the Export window and then select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Text file \*.txt).

## Show 3D

3D plots are used to represent data on three axes and to show the relationship between the three variables: time, position and intensities.

**Please note: The best way to analyze the Intensity Profile information is by using a limited amount of data.**

### 9.9.2 Spots Close to Surface

This XTension finds the subset of Spots that are closer to the surface than the user specified threshold (in  $\mu\text{m}$ ). The coordinates of the Spot and Surface objects are used for the distance calculation. The distance is measured for the centre of the spot, not the surface of the spot. As a result, in the Surpass tree two new Spots objects are created; a subset of spots, whose location lies within the specified distance to the surface and the other subset of spots, whose location exceed the specified threshold value. In the viewing area, the subsets of the spots are represented by different colors.

The user can specify which Surfaces and Spots objects should be used. If multiple Spots or Surfaces are selected, each pair of Spots/ Surfaces are analyzed. The number of the Spots objects created is 2 times the 'number of selected Spots items' multiplied by 'number of the selected Surfaces'.

### 9.9.3 Spots to Spots Closest Distance

This XTension calculates and displays the distance to the closest neighbour of the Spots objects.

1. Choose between the 3 possibilities of how the results can be displayed.

The results can be displayed as:

- a. New statistical values under the Statistical tab (distance max, distance mean, distance min)
- b. New spot radius, where the new the spot size corresponds to the calculated minimum distance
- c. New channel, where the channel intensity values depend on the minimum distance.

A possibility of multiple selections is available by pressing the **Select All** button.

2. Select the distance calculation mode.

The distance can be calculated either from the centre of the spot object or from the border.

### 9.9.4 Find Spots Close To Filaments

To run this XTension the user has to create a Spots and a Filaments component.

This XTension splits one Spots object into two Spots objects. One Spots object will include all the spots that are close to the filamentous structure (colored red). The other Spots object will include the spots that are far from the reference filaments (colored blue).

To account for significantly different filament diameters a ratio instead of an absolute distance between spots and filaments is used.

The ratio is given by  $r=a/b$

- a- Distance between the spot location and the centre of the filament
- b- Filament radius

Multiple Spots/Filaments objects:

This XTension produces result for pair of the selected Spots Filaments objects.

The selected spots objects are determined by the fact that you run the XTension from its 'Tool' tab.

If multiple Filaments objects are created, by default, the selected Filament object used in calculation is the one which appears first in the top down order of the Surpass tree

To change the order of Filaments objects in the Surpass tree please use the drag and drop functionality.

## 9.9.5 Colocalize Spots

This XTension creates new Spots objects including spots that exist within a user-defined distance of each other (using the currently set Imaris unit).

If the distance between two spots is less or equal to the user defined value then the two spots are considered to “colocalize” and will be placed within a new Spots object.

In the Surpass tree a new Spots object group is created with an automatically generated name. The name indicates the threshold value and the selected spot's object.

## 9.9.6 Create Channel from Spots

This XTension creates and computes a new channel. The created channel intensity are calculated based on the radius of the selected Spots object. The user can choose how the new channel is created by selecting how the intensity is calculated along the spots radius.

If the channel is created by interpolating along the spot's radius, the maximum intensity will be assigned to the voxel at the centre of the Spots object. If not, the uniform intensity is mapped at the location of the spot.

To modify the channel visibility contrast and range, open the Display Adjustment window under the Edit tab.

## 9.9.7 Split Spots

This XTension splits one Spots object in multiple Spots objects.

Two Spots objects are created if the minimal distance between their spot elements is greater than the user-defined threshold.

All spots elements are assigned to the same Spots object if the shortest distance between two neighbouring spots is less or equal to the user-defined threshold.

Each new Spots object is individually colored.

A spot element can belong to only one Spots object.

In the Surpass tree a new Spots object is created (the number in brackets indicates the threshold value). The names of the newly created Spots objects are automatically generated indicating the number of spots in the Spots object (and the Spots object ID number).

## 9.9.8 Split Spots Into Surface Objects

To run this XTension the user has to create a Spots and a Surface component.

For each Surface object located in the same Surpass group as the Spots object, this XTension creates a new subset of Spots that contains only the spots that lie inside the Surface.

## 9.9.9 Super Resolution Localization Data To Image

The Super Resolution Localization Data To Image XTension reads localization data files produced by super-resolution microscopy. This XTension is very similar to the [Super Resolution Localization Data To Spots](#) XTension, but creates an *Imaris image* as the output.

### Super Resolution Microscopy

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Super-resolution techniques allow you to capture images with a higher resolution than is possible with conventional confocal microscopy techniques.

A super-resolution data set from PALM and STORM methods (also known as PALMIRA, dSTORM, sptPALM, GSDIM, SMLM, 'blink' microscopy) is composed of:

- a) a sequence of a large number of images where individual sub-diffraction particles need to be detected (typically 10.000-100.000 images)
- b) a particle list (typically 1.000.000 - 10.000.000 particles) that is generated after the particle detection analysis.

The particle information that is the result of this technique is composed of the intensity, x-position, y-position, z-position and additional metadata.

For more information please refer to:

[http://en.wikipedia.org/wiki/Super\\_resolution\\_microscopy](http://en.wikipedia.org/wiki/Super_resolution_microscopy)

This XTension allows to load super resolution localization data into Imaris as an image. The supported format of the localization data is the QuickPalm particles tables tif file, as well as CSV ([https://en.wikipedia.org/wiki/Comma-separated\\_values](https://en.wikipedia.org/wiki/Comma-separated_values)) and TXT files.

You can find further QuickPALM information at these locations:

<http://code.google.com/p/quickpalm/>

<http://www.nature.com/nmeth/journal/v7/n5/full/nmeth0510-339.html>

### To perform this XTension:

1. Press **Choose file** in the top left of the Dialog and choose the super-resolution file you want to process. The file name and the first few lines of the file are displayed in the filename and preview area of the dialog. Depending on the format, the column settings in the bottom left area of the dialog will be enabled. For quickpalm files, these settings are not available and step 2 can be skipped.
2. For csv and txt files, the bottom left area of the dialog allows you to specify which columns should be used to generate the spots. The dropdown boxes are filled with the text of the first line of the file.

#### a. Position

Use the dropdown boxes to choose which columns should be used for Position X and Position Y values. Position Z is optional and contains "None" as last element in the dropdown. By choosing "None" for Position Z, all spots will have Position Z set to 0.

#### b. Unit

With the "Unit" dropdown, choose which unit the spot positions should have. The available options are "m", "mm", "µm", and "nm", default is "µm".

#### c. Channel

With the "Channel" dropdown you can specify a column for the channel. The XTension will generate an separate channel in the image for each distinct "Channel" value. It is possible to set "Channel" to None, then the created image will only contain one channel.

3. Press **OK**. A second dialog will appear. Enter the voxel x y z sizes for the each dimension in the input dialog. Default values are 10 µm.
4. Press **OK** to start reading the whole file. Depending on the size of the file, this can take a while. A progress is shown and at the end, the image that Imaris displays is changed. A scene is created if Imaris does not yet contain a scene.

The entered voxel size is used to create a 2D/3D image covering a volume equal to the volume occupied by the

particles. Each voxel has an intensity value corresponding to the number of particles found within. Voxel intensities are initialized to zero and then incremented by one every time a particle is found inside them. The result is voxels with high intensities where many particles were found and lower intensities where fewer particles were found.

**Please note: Smaller voxel size create a higher resolved image, but requires more time and memory for processing. If the value of the voxel size is set to be too large, the benefits of the super resolution technique are lost. Finding the optimal voxel size is a difficult task that might need several tries.**

### 9.9.10 Super Resolution Localization Data To Spots

The Super Resolution Localization Data To Spots XTension reads localization data files produced by super-resolution microscopy.

The XTension is very similar to the [Super Resolution Localization Data To Image](#) XTension, but produces **Spots Components in Imaris** as the output.

#### Super Resolution Microscopy

Super-resolution techniques allow you to capture images with a higher resolution than is possible with conventional confocal microscopy techniques.

A super-resolution data set from PALM and STORM methods (also known as PALMIRA, dSTORM, sptPALM, GSDIM, SMLM, 'blink' microscopy) is composed of:

- a) a sequence of a large number of images where individual sub-diffraction particles need to be detected (typically 10.000-100.000 images)
- b) a particle list (typically 1.000.000 - 10.000.000 particles) that is generated after the particle detection analysis.

The particle information that is the result of this technique is composed of the intensity, x-position, y-position, z-position and additional metadata.

For more information please refer to:

[http://en.wikipedia.org/wiki/Super\\_resolution\\_microscopy](http://en.wikipedia.org/wiki/Super_resolution_microscopy)

This XTension allows to load super resolution localization data into Imaris as an image. The supported format of the localization data is the QuickPalm particles tables tif file, as well as CSV ([https://en.wikipedia.org/wiki/Comma-separated\\_values](https://en.wikipedia.org/wiki/Comma-separated_values)) and TXT files.

You can find further QuickPALM information at these locations:

<http://code.google.com/p/quickpalm/>

<http://www.nature.com/nmeth/journal/v7/n5/full/nmeth0510-339.html>

To perform this XTension:

1. Press **Choose file** in the top left of the Dialog and choose the super-resolution file you want to process. The file name and the first few lines of the file are displayed in the filename and preview area of the dialog. Depending on the format, the column settings in the bottom left area of the dialog will be enabled. For quickpalm files, these settings are not available and step 2 can be skipped.
2. For csv and txt files, the bottom left area of the dialog allows you to specify which columns should be used





to generate the spots. The dropdown boxes are filled with the text of the first line of the file.

#### a. Position

Use the dropdown boxes to choose which columns should be used for Position X and Position Y values. Position Z is optional and contains "None" as last element in the dropdown. By choosing "None" for Position Z, all spots will have Position Z set to 0.

#### b. Load Spot Sizes From File

Check the "Load Spot Sizes From File" checkbox if the spot sizes should be read from the file. If checked, the dropdown box below will be activated to choose a column. If "Load Spot Sizes From File" is checked, the "PSF & Camera Gain" area is disabled. For csv and txt files, if "Load Spot Sizes From File" is not checked, the intensities are set to 1, for quickpalm files, the intensities are read from the file.

#### c. Unit

With the "Unit" dropdown, choose which unit the spot positions should have. The available options are "m", "mm", "μm", and "nm", default is "μm".

#### d. Channel

With the "Channel" dropdown you can specify a column for the channel. The XTension will generate a separate Spots component for each distinct "Channel" value. It is possible to set "Channel" to None, then only one Spots component will be generated.

### 3. PSF & Camera Gain

Quickpalm files contain intensities for every spot. With PSF and Camera Gain and the intensities the spot diameters are calculated according to the formula below. Adjust PSF and Camera Gain accordingly:

$$R = \frac{S}{\sqrt{\frac{I}{G}}}$$

**R** - The diameter of the created spots

**S** - The full-width-half-maximum of the microscope point-spread-function

**I** - Intensity

**G** - The gain factor of the camera to convert particle intensities into photons

4. Press **OK** to start reading the whole file. Depending on the size of the file, this can take a while. A progress is shown and at the end, a group with one or more Spots components is created in Imaris.

## 9.10 Filaments Functions

This Section covers the XTensions relevant to Filaments:

[Filaments Angles Statistics](#)

[Filaments Branch Hierarchy](#)

[Filaments Convex Hull](#)

[Create Channel from Filaments](#)

[Filaments Points Track](#)

[Classify Filaments Spines](#)

[Split Filaments Into Branches](#)

### 9.10.1 Filaments Angles Statistics

This XTension measures and displays the angles statistics of the filament, the angle between segments and branches. The calculated data is plotted. Two plots are created: Angles between the segments and Angles between the branches.

The x-axis represents the measurement of the angle and the y-axis represents the number of segments or number of branches.

**Please note: All the angles calculations are based on dendrite vertex positions.**

## 9.10.2 Filaments Branch Hierarchy

This XTension calculates and displays dendrite branch levels. All dendrites with the same branch level are assigned to a new Filaments object. Each new Filaments object is individually colored. Each dendrite branch can belong only to one Filaments object.

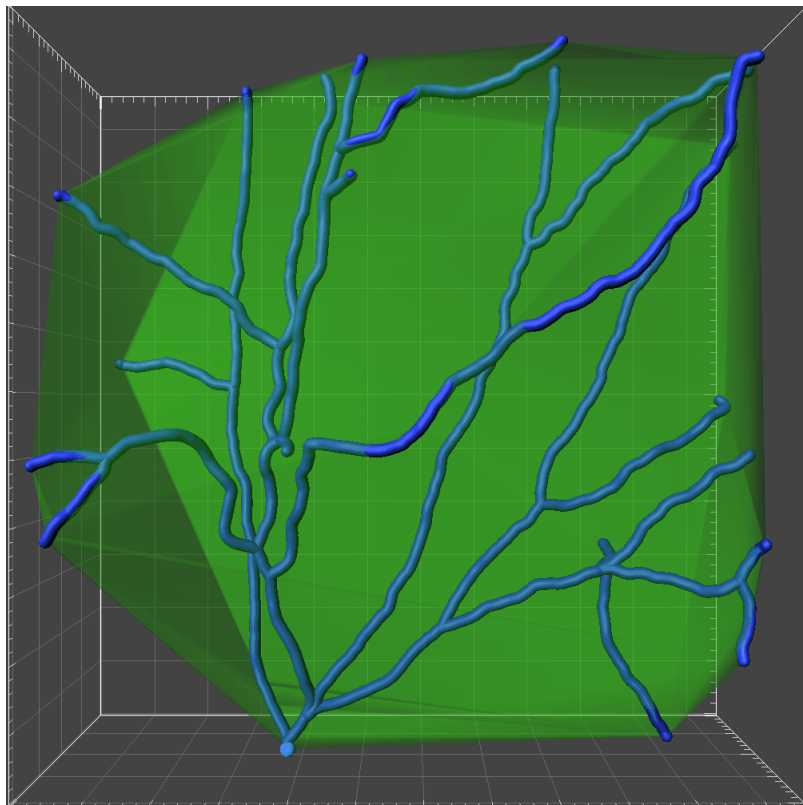
In the Surpass tree multiple Filaments objects are created (the number in brackets indicates the branch level).

## 9.10.3 Filaments Convex Hull

This XTension calculates the convex hull for the filament object.

The convex hull is the minimal convex polyhedron that contains all the points in the selected filament object. A convex hull is defined by the minimal convex set of points that envelops a terminal points of the dendritic arbor. Based on the convex hull calculation, a new Surface is created that completely overlaps the filament object. The region of the filament object is obtained from the statistical values of the newly created surface.

Convex hull measurements are more robust than tortuosity (branch length divided by linearity) to artifacts of optical microscopy, and allow a better measurement of dendrite spread - 'spanned area' preserving the spatial component of the neuron.



## 9.10.4 Create Channel from Filaments

This XTension creates and computes a new channel. The channel intensity values depend on the diameter of the selected filament object. The user can choose how the new channel is created by selecting how the intensity is calculated along the filament diameter.

If the channel is created by interpolating along the dendrite/spine diameter, the maximum intensity will be

assigned to the voxel at the centre of the filament. If not, the uniform intensity is mapped at the location of the filament.

**Tip: To modify the channel visibility contrast and range, open the Display Adjustment window under the Edit tab.**

### 9.10.5 Filaments Points Track

This XTension tracks Filament significant points (beginning, dendrite branching and terminal points, spine attachments and spine branching and terminal points) and plots the track statistics.

It provides several tracking algorithms to choose from. The motion models are used to predict the future positions of Filament significant points (beginning, dendrite branching and terminal points, spine attachments and spine branching and terminal points). Based on these predicted positions, the best matches to candidate Filament significant points can be determined. All of the algorithms except for Connected Components use a Linear Assignment algorithm and Total Cost function to find the best track connections. The first two algorithms compute cost based on change in position (distance), while the Autoregressive Expert mode combines the changes in position and the intensity to compute the total cost.

#### **Brownian Motion**

This algorithm models the motion of each of the Filament significant points as a Brownian motion. This model is appropriate if the Filament significant points perform similar motions to a Brownian motion (the random movement with very frequent changes in direction and no discernible pattern).

The only prediction applied is that the Filament significant points do not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

#### **Autoregressive Motion**

This algorithm models the motion of each of the Filament significant points as an autoregressive AR1 process. This model is appropriate if the Filament significant points perform any kind of continuous motion. The AR1 model looks back to one time-point and predicts that the Filament significant points will move the same distance and in the same direction again. The user-specified maximum distance value is the distance in which the Filament significant points are allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

#### **Autoregressive Motion Expert**

This algorithm models the motion of each of the Filament significant points as an autoregressive AR1 process (see above). It is suitable for the tracing of multiple objects with adjacent or crossing paths, especially if the neighbouring objects have a stable (but different) intensity. In Autoregressive Motion Expert the algorithm called Total Cost determines the most appropriate connections for tracks by seeking the lowest Total Cost. In this case, the Total Cost algorithm combines Intensity Cost and Distance Cost functions (see Intensity Weight below).

#### **Connected Components**

For this algorithm, Filament significant points in adjacent time-points are considered connected if the Filament significant points' spheres occupy some of the same space (the spheres would overlap if two time-points were merged into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. All objects with an overlap will be assigned the same track ID.

This is the only algorithm that automatically handles lineage (Filament significant points that split or merge). It will only work with images that have excellent time resolution because the Filament significant points can't jump a distance larger than their own size, Max Distance.

#### **Max Distance**

The value of the Max Distance specifies the maximum distance between a predicted object location and its actual location for two consecutive time points. This parameter does not allow connections between a Filament graph and a candidate match when the distance between the predicted future position of the Filament graph and the candidate position exceeds the maximum distance.

## Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions and create Track fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the object's connection is automatically established is based on the value defined in the Max Gap Size file.

The Max Gap Size defines the maximum number of the consecutive time points, which are allowed to be missing in order for the track to be continuous.

## Intensity Weight

Based on the information obtained from the measured Filament significant point's intensity and position, the Intensity Weight function is used to balance the relative influence of the Intensity and Distance costs. The Total Cost is determined by combining the Intensity term and Distance term, and minimization of the Total Cost is used to determine the best track from all feasible ones.

$$\text{TotalCost} = (\text{all connections}) \text{DistanceCost} + \text{IntensityWeight} * \text{IntensityCost}$$

## Track Filters

The Track filtering is incorporated into the process. Enter the filtering type to be used in order to classify the tracks and specify the measurement of the criteria.

As a result of this XTension, a Matlab plot can be created. The plot displays the number of Filament significant points as a time plot. Each Filament significant point is represented using a special symbol.

In Imaris, a new Spot's object is created in the Surpass tree.

## 9.10.6 Classify Filaments Spines

This XTension determines the differences in spine morphology. It classifies spines into the following default categories: stubby, mushroom, long/thin, and filopodial based on their length, head and neck morphology.

### Classification Rule

All detected spines are classified according to the current classification rules. All classification rules are written as Matlab syntaxes and are fully editable. These are logic statements returning a value of 1 if true and 0 if false. These rules are applied in order of precedence (i.e. top to bottom). All spines that comply with the first rule (the first classification rule is true-total spine length is less than 1µm) are set as stubby. For all remaining spines, the second rule is tested and the process of classification continues.

Any spine that was categorized by the highest rule in the list, is excluded from further classification. Therefore, a spine can be part of one class only.

### Setting the New Rule

You can classify spines according to the default classification rules, or you may create new classification rules by defining output classes based on the new criteria.

To adjust a classification of the spines morphology into more relevant classes, it is possible to change a set of classification criteria. In the data set, any detected spine can be selected and measured and then the different 3D features obtained from the spine, can be used for classification. The suitable morphological feature could be used for setting the new rule.

The list of variables used for setting rules are displayed.

To create or adjust the classification rules combine the spine morphology categories (**Spine, Head, Neck,**



**Ground**) with the following variables:

Volume, length, mean/ minimum/ maximum/ width.

### **Insert variable button**

Select the Insert variable button to insert the selected combination of spine morphology and statistical variables.

**Any new rule must be based on Matlab syntaxes.** The default classification rules show you a pattern of the syntax needed to complete these logic statements.

- To adjust the rule that controls classification, click on the rule line and enter a new set of classification criteria.
- To change the name of the class, click on the name line and enter a new name.
- To remove the selected class, click on the appropriate **Remove class** button.

In this XTension, the total spine length is divided in 3 parts: ground, neck and head. The spine neck length is set by default to be 50% of the total spine length. Neck length is measured from the base of the spine head toward the dendrite. The spine head is 25% of the total spine length. The remaining part of the spine, attached to the dendrite tree, is set to be 25% of the total spine length. To change the default length ratio enter the new values in the head/ ground length fields.

### **Create one Filament**

Under the Create One Filament option you can find different outputs.

#### **Create one Filament objet for each class**

As a result, in the Surpass tree a new Surpass group is created which is composed of multiple Filament objects. All spines (and dendrites of the source) categorised in the same class are assigned to the same Filaments object. Each Filaments object is individually colored.

#### **Create one Filament with custom statistics**

As a result, in the Surpass tree a new Filaments object is created. Under the Statistical tab a new variable - Spine class is added. The spine class is indicated by a numerical value (this value corresponds to the rule number).

Please note: In both output options every branched spine is replaced with an unbranched one, starting from the attachment point wherever a branching point is encountered, a new spine is created.

#### **Plot class statistics**

A new window plots the selected spine statistics. For each spine class the following statics can be plotted: count, density, average volume, average length, average mean/ min/max with for total spine, head, neck and ground.

Variables can be selected interactively.

Density is defined as the total number of selected spines divided by the total length of the dendrite

### **Add new class button**

Select this button and fields for the new classification rule and name are added.

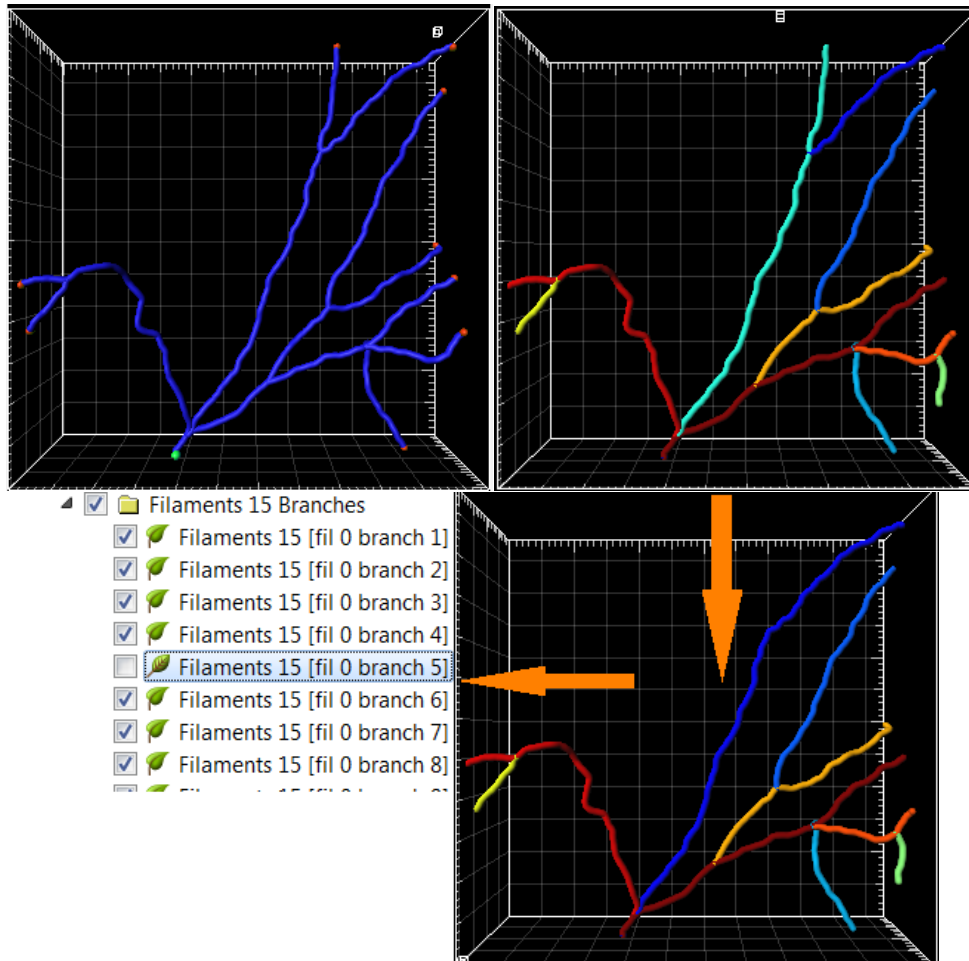
### **Classify spines**

Select this button and the Classify spine XTension is executed.

## 9.10.7 Split Filaments Into Branches

This XTension splits one Filaments object into different Filaments objects. Starting from a common beginning point and going towards a terminal point, whenever a branching point is encountered a new Filament object is created. As a result, the newly created Filaments do not have branches and for each branch point, there is only one beginning and one terminal point. The number of Filaments created is equal to the number of terminals and branch points of the original Filaments object.

In the Surpass tree a new filament object is created.



## 9.11 Histogram Equalize Layers

This XTension adjusts the contrast layer by layer by using the image's histogram. Histograms are created for each layer separately and for the whole image. The XTension enhances the contrast by transforming the intensity values in each of the layer's histograms, so that the resulting histogram approximately matches the whole image histogram.

For more information, please refer to:

<http://www.mathworks.ch/help/toolbox/images/ref/histeq.html>

The resulting image features the highest possible contrast, uniformly distributed over all the layers. This method increases the contrast by adjusting the distribution of the intensities on the histogram by spreading out the most frequent values. This allows for areas of lower local contrast to gain a higher contrast without affecting the global contrast.

Define the value of the lower limits of intensities. Voxels with an intensity below the entered value are excluded from this calculation - they are interpreted as background.

## 9.12 Normalize Time Points

This XTension adjusts the brightness and contrast of the individual time points in a 4D data set to a uniform level.

It first computes the mean and standard deviation values of the intensity for all of the time points. Then, it adjusts the intensities of each time point to match the overall mean and Standard Deviation value.

It adjusts the brightness of all the time points in the data set and each channel independently.

Voxels with intensity below the entered value are excluded from this calculation - they are interpreted as background.

## 9.13 Open Object Manager

This XTension saves, loads or clears single Imaris objects in .mat files.

It can be applied to Spots, Surfaces, Tracks, and Filaments objects and Groups. Groups are saved with all their content. Only first-level objects (mScene direct children) can be saved.

To change the default location (Matlab), select the Browse button. Select the directory and enter the name for the file to be saved.

## 9.14 RGB to Gray

This XTension creates and computes a new gray channel. The intensity values of the gray channel are calculated as a total weighted sum of the selected channels' intensities.

The user defines the coefficients of the sum for each channel.

## 9.15 Smooth Time

This XTension smoothes the intensity of each voxel along the time dimension. The amount of smoothing is defined by the Gaussian filter width.

The user specifies the channel and the filter width value.

## 9.16 Time Projection

This XTension projects all channels of a time data set as a single time point. For each voxel the intensity is computed using a MIP or a mean projection of all time points. The projection mode is defined by the user.

## 9.17 Time Subtract Average

This XTension calculates for each voxel a new intensity value, by subtracting the time-averaged intensity value from the initial intensity. It eliminates all motionless objects since the difference in intensities for motionless objects are negative values.

There are two options:

1. The user can set all negative values to zero
2. To avoid these negative values and loss of data, any intensity which is zero or negative can be equal to absolute values

To speed up the processing of **2D time series** you can use Image processing - **Swap Time and Z** before and after starting the XTension.

1. After exchanging the Time and Z dimensions start the XTension.
2. Define the number of slices that should be processed.
3. Select to set Negative values either to zero or to absolute values
4. Run the XTension
5. Finally, under the Image processing menu, select again the Swap Time and Z option

## 9.18 Tracks Functions

This Section covers the XTensions relevant to tracking functions:

[Connect Tracks](#)

[Plot Angles of selected Tracks](#)

[Plot Distance Between Tracks](#)

[Plot Length of selected Track](#)

[Split Tracks](#)

[Translate Tracks](#)

### 9.18.1 Connect Tracks

For each initial object within the track, this XTension assesses the position at the previous time point. Then at the previous time, it measures the distance between the calculated position and the positions of all the other objects. It creates a track connection if the distance between the anticipated object position and any other object at the previous time point is less than the user specified maximal distance value.

The new connections can be seen under the Edit track tab.

**Please note: Two tracks, which track an object through the same time point, cannot be connected.**

### 9.18.2 Plot Angles of selected Tracks

This XTension creates four plots that display the angles statistics of the selected track. The first plot shows the spatial orientation of the selected track and the following plots are illustrating the XY, XZ and YZ planes.

### 9.18.3 Plot Distance Between Tracks

This XTension creates four plots that display the distance between the selected track objects and all other tracks objects that are in the same group. The first plot shows the absolute spatial distance of the selected track and all other tracks. The following are illustrating planes X, Y and Z distance. The following are illustrating the distance of the selected track and all other tracks in X, Y and Z planes.

### 9.18.4 Plot Length of selected Track

This XTension calculates the difference in the object position within the selected track between two consecutive time points. If the distance between particle (e.g. a vesicle or a cell) positions in two consecutive time points is smaller than the entered threshold value, then it is considered as a pause.

As a result, this XTension creates four plots that display the length of the track steps. The first plot shows the spatial step size of the object within the selected track. The other three plots illustrate the XY, XZ and YZ planes.

### 9.18.5 Split Tracks

This XTension splits tracked objects into individual Spots objects.

- All objects within one track are assigned to the same Spots object.
- Each Spots is individually colored.





- Each track can belong to only one Spots object.
- As a result, in the Surpass tree a new objects group is created. The subgroup names are automatically generated indicating the object type and track number.

### 9.18.6 Translate Tracks

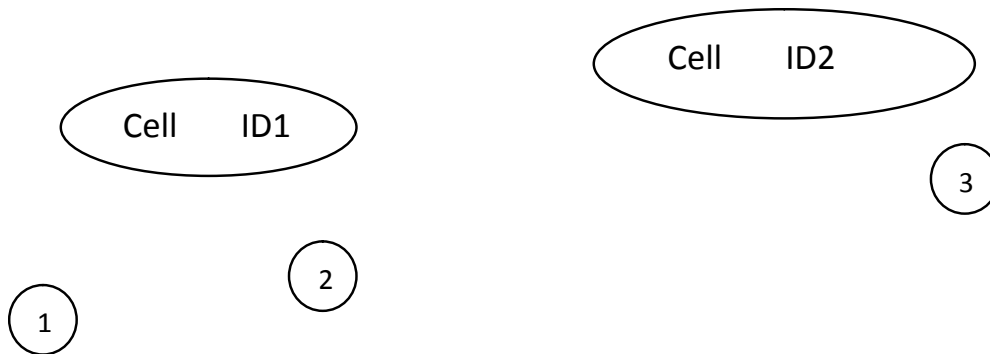
This XTension makes a copy of all tracks that are in the same group of the selected track. The copies are then translated and as a result they all have the same starting point.

In the Surpass tree a new object is created.

### 9.19 Vesicle Outside Cell

The XTensions VesicleOutsideCell function calculates for each Spot object, the closest Cell, and the distance to it.

XTensions VesicleOutsideCell illustration



The figure illustrates two Cells (ID 1, ID 2) and three Spots (1, 2, 3).

The results of the XTensions VesicleOutsideCell function are two statistic values per Spot object: Nearest Cell ID and Nearest Cell Distance.

Spot 1

Nearest Cell ID: 1

Nearest Cell Distance: 10  $\mu\text{m}$

Spot 2

Nearest Cell ID: 1

Nearest Cell Distance: 7  $\mu\text{m}$

Spot 3

Nearest Cell ID: 2

Nearest Cell Distance: 3  $\mu\text{m}$

## Section 10 Fiji

Imaris Fiji compatible plugins are aimed at performing additional image processing and offering a solution for specific problems with image analysis.

The Imaris Fiji menu allows you to use your current Fiji routines and still continue with your work within Imaris. The Fiji plugins are integrated into Imaris and can be executed from within the application, enhancing and extending the functionality of Imaris.

In the Fiji menu you can find a list of plugins organized into a menu structure. Each plugin collection is displayed as a sub menu on this menu. The plugins and macros are stored and run from the Fiji folder.

[Image From Fiji](#)

[Image To Fiji](#)

[File](#)

[Image](#)

[Process](#)

[Analyze](#)

[Plugins](#)

[Options...](#)

[Help..](#)

**Please note: This is not an Fiji tutorial. These Sections describe only the usage of Fiji as an integrated part of Imaris.**

### 10.1 Fiji-Imaris

In this Section you will learn how to integrate Fiji plugins in Imaris by means of the Fiji menu and how to establish connections between the Fiji and Imaris.

If you already have Fiji installed, skip down directly to the to the **Options...** section. If you don't have Fiji installed, you must install it first. The latest version can be downloaded from the following locations:

Fiji: <http://pacific.mpi-cbg.de/wiki/index.php/Downloads>

Depending upon your operating system select the appropriate link.

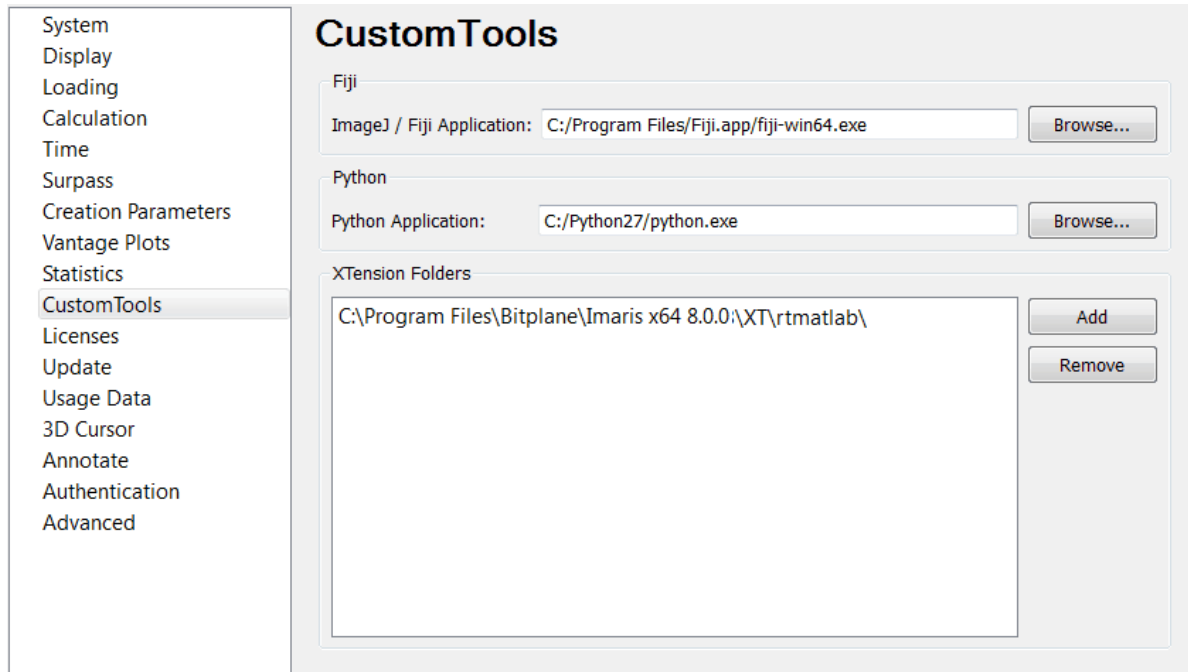
To complete download follow the specific download instructions.

#### Options...

The first step of integrating the Fiji plugins within Imaris is to specify the folder with the Fiji.exe file. To do that open the Fiji menu and choose the last item in the list **Options...**

The Custom Tools window opens. In the field Fiji application either type in the respective path or use the button Browse.

Specify the Fiji folder and select the .exe file. Click on the **OK** button.



### For Mac users:

/Applications/Fiji.app/Contents/MacOS/fiji-macosx

In the Fiji menu all options are now active.

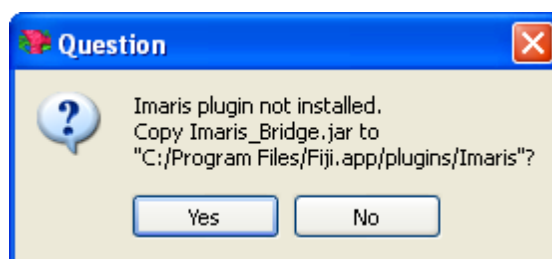
## 10.2 Imaparis Bridge.jar File

The downloaded plugins do not include the file needed to run your Fiji plugins in Imaparis.

In order to run your Fiji plugins in Imaparis you need to install the file, **Imaris\_Bridge.jar file**; which provides the link between the two applications.

The installation starts automatically as soon as you select a plugin from the list.

The new window appears asking you to install Imaparis\_Bridge.jar file



You can confirm installation by selecting **Yes**.

The Imaparis\_Bridge.jar file is placed in a new subfolder, Imaparis, within the Fiji application.

**Security Alert window: Depending upon how your computer security is set, you may see a Security Warning. Select the option Unblock.**



After installing the Imaris bridge file all plugins are ready to be used and no additional steps are required.

## 10.3 Configuration File

This section will show you how to expand the pre-existing collection of plugins and embed initially unlisted plugins into the Imaris Fiji menu.

You can customize the Fiji menu by the integration of new plugins.

### Download Plugin

To incorporate the new Fiji plugin into Imaris, you are not required to modify, or access the Imaris code. Simply select a plugin from the Fiji list, download it and place it in the plugin menu or one of the submenus.

### Configuration File

To properly run the plugin in Imaris it is important to create a **configuration file**.

The configuration file is needed to fully read/execute/and write permissions to the Fiji directory and subdirectories. The configuration file handles all the details of incorporating, executing and presenting the results of this new plugin within Imaris.

The configuration file has two main elements. The first is executed with Imaris. It has several lines that connect the plugin to Imaris. By adding a special header to the file you define the Menu or the Submenu and the name of the plugin in Imaris.

The second part is written as any plugin and executed by Fiji. This part of the file runs the Imaris-Bridge file, which provides a connection between the Fiji and Imaris, loads data set information, performs some actions, sends images back to Imaris, closes Fiji and terminates the connection.

Once a file is created, copy the file to the Imaris Fiji plugins folder in the current Imaris Program Files path and you will have access to new plugins within Imaris.

The following is the example configuration file.

```
// <CustomTools>  
// <Menu name="Fiji">
```

```
// <Submenu name="submenu name (e.g. Process)"> -This line is optional (embedding the plugin in the
submenu)
// <Item name="plugin name" icon="Fiji">
// <Command>Fiji::submenu name_plugin name </Command> - Please make sure that the plugin
name is the same as the configuration file name
// </Item>
// </Submenu>
// </Menu>
// </CustomTools>
```

```
call("Imaris_Bridge.In", getArgument());
run("plugin name");
call("Imaris_Bridge.Out", getArgument());
call("Imaris_Bridge.Terminate", getArgument());
```

Save the file, and copy it to the folder:"

**Windows:** C:\Program Files\Bitplane\Imaris x64 X.X.X\XT\fiji

**MAC:** /Applications/Imaris\ 9.2.0.app/Contents/SharedSupport/XT/fiji/

**Please note: The Imaris version cannot be set differently than the currently running version**

Restart Imaris and the newly inserted plugin will appear in the Fiji menu.

**Please note: Imaris Fiji compatible plugins are plugins that provide image data as a result.**

## 10.4 Image From Fiji

Transfer the image to Imaris by selecting the Image From Fiji option.

## 10.5 Image To Fiji

Transfer the image to Fiji by selecting the Image to Fiji option.

## 10.6 Initially Installed Plugins

### File

Open

Save

### Process

Find Edges

Morphology

Gray Morphology

### Sharpen

If you would like to find out more about these plugins, additional information is available in the form the Fiji website.

## Analyze

Color inspector 3D

## Plugins

### LOCI

Bio-Formats Exporter

Bio-Formats Importer

Plugins are not only used to implement new processing functions, they are also used to support reading and writing file formats, which are not normally supported by Imaris.

## Process

Dilate (3D)

Distance Transform (3D)

Erode 3D

Exact Euclidean Distance Transform (3D)

Exact Signed Euclidean Distance Transform (3D)

Gradient (3D)

IFT (3D)

Laplace (3D)

Maximum (3D)

Median (3D)

Minimum (3D)

Particle Analyzer (3D)

Smooth (3D)

For the detail description it is recommend to visit the Fiji website.

There are a lot of examples and tutorials, which could help you to understand the features of these plugins.

[http://pacific.mpi-cbg.de/wiki/index.php/Fiji's\\_Menu](http://pacific.mpi-cbg.de/wiki/index.php/Fiji's_Menu)

## Registration

Correct 3D Drift

Linear Stack Alignment with SIFT

## Skeleton

Analyze Skeleton

<http://imagejdocu.tudor.lu/doku.php?id=plugin:analysis:analyzeskeleton:start>

Skeletonize (2D/3D)

<http://imagejdocu.tudor.lu/doku.php?id=plugin:morphology:skeletonize3d:start>

## 10.7 Options...

### Custom Tools

In Custom Tools you can specify the Fiji folder. Either type in the respective path or use the button **Browse**.

## 10.8 Fiji Help

You can find further Fiji information by clicking on the following link:

<http://fiji.sc/wiki/index.php/Fiji>

## Section 11 3D View

	<b>Please refer to Section:</b>
Cells	3D View - <a href="#">Cells</a>
Clipping Plane	3D View - <a href="#">Clipping Plane</a>
External Object	3D View - <a href="#">External Object</a>
Filament	3D View - <a href="#">Filament</a>
Frame	3D View - <a href="#">Frame</a>
Surpass Group	3D View - <a href="#">Surpass Group</a>
Light Source	3D View - <a href="#">Light Source</a>
Measurement Point	3D View - <a href="#">Measurement Point</a>
Oblique Slicer	3D View - <a href="#">Oblique Slicer</a>
Ortho Slicer	3D View - <a href="#">Ortho Slicer</a>
Spots	3D View - <a href="#">Spots</a>
Surfaces	3D View - <a href="#">Surfaces</a>
Volume	3D View - <a href="#">Volume</a>

[Export Selected Objects...](#)

[Delete Selected Objects...](#)

Del

[Switch View Mode](#)

Esc

InMotion

[InMotion](#)

### 11.1 Cells

ImarisCell is an Imaris module designed specifically to identify, segment, track, measure and analyze Cell, Nucleus and Vesicles in 2D and 3D time-resolved images. ImarisCell provides simultaneous detection, measurement and analysis of cell objects combined with the interactive identification in the viewing area.

ImarisCell calculates and displays detailed statistics values for all the cell's components and their tracks. Interactive image exploration, simultaneous measurement and analysis of the many features in the image provide quick, accurate, quantitative and reproducible results.

#### How to Add new Cells?

- Select menu 3D - Cells.

The Surpass Tree list is automatically updated and the first window of the Creation Wizard displays in the Cells - Properties.

For information on cells statistics calculations used refer to [Cells](#).

#### 11.1.1 Creation Wizard

In the first window of the Cell Creation Wizard, you find the algorithm settings. Here, you have to decide, if you want to load a set of previously stored creation parameters, or to create a new one.

The most convenient way to apply the same creation parameters to different data sets is to use the [Load Parameters](#) option.

**Skip automatic creation, edit manually** Button

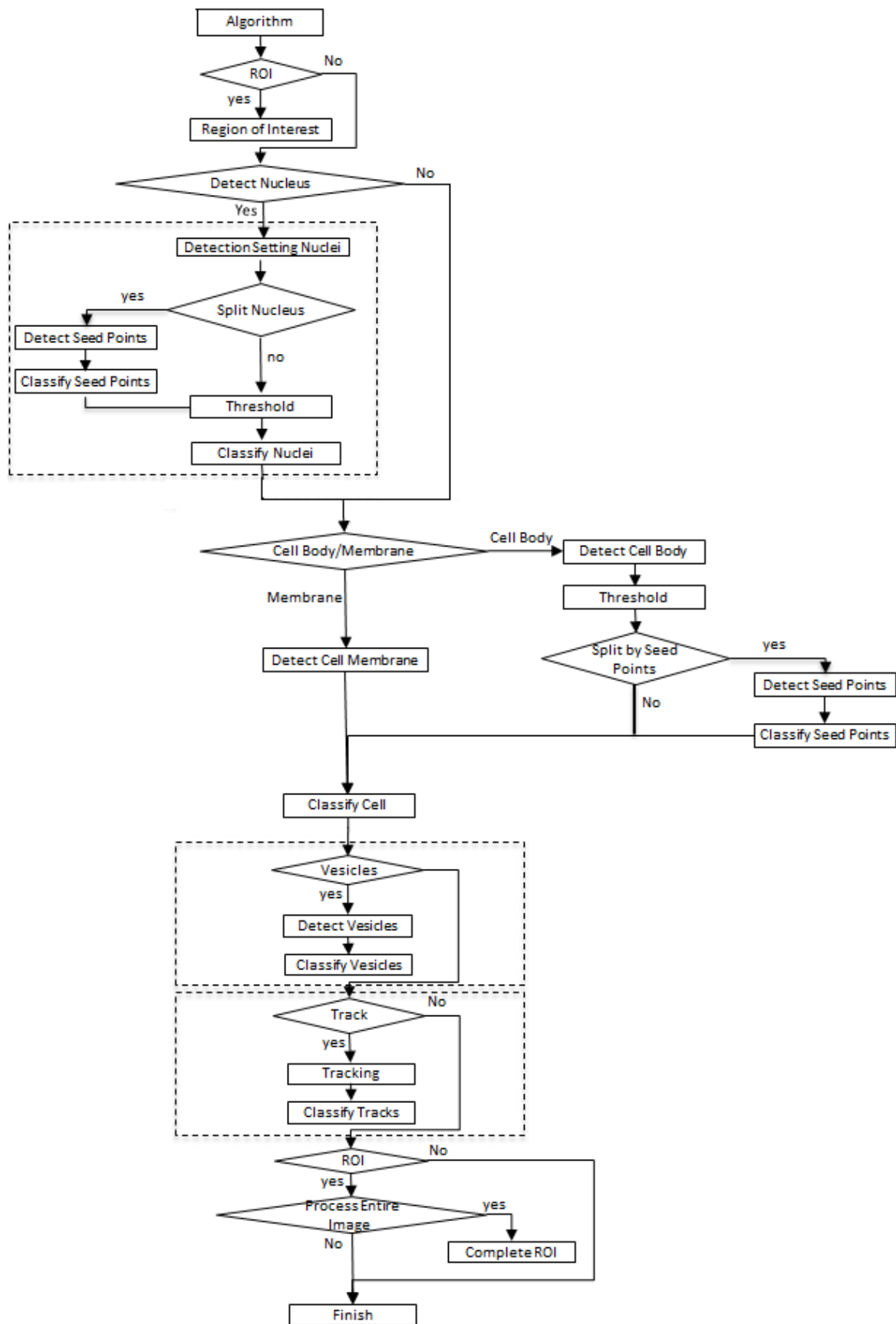
Terminates the creation wizard and activates the manual Cells creation.

If the image data is for a [multi-image scene](#) an option to select which source image to be analyzed is available



from the Align to: drop down box.

Depending on the chosen algorithm setting the Creation Wizard could consists of the following major steps:



### 11.1.1.1 Algorithm Settings

Note: If you do not want to use the automatic Cell creation wizard select the **Skip automatic creation, edit manually** button.

#### Skip automatic creation, edit manually Button

Terminates the creation wizard and activates the Edit options.

#### Favourite Creation Parameters

This option lets you use the identical creation parameters for multiple data sets.

The Favourite Creation Parameters option is only available if the set of the creation parameters has already been saved under the [Creation tab](#) using the Remember Parameters button.





Then you can choose to segment a **Region of Interest (ROI)** only, to create **Tracks** after objects detection or to **Skip automatic creation**.

When the wizard is started, all stored creation parameters, corresponding to the chosen object wizard, are automatically loaded. The stored parameters contain all of the settings, options, and parameters required for image segmentation.

Expand the Favourite Creation Parameters drop-down list by clicking on it. The list shows all the creation parameters sets that have been previously saved, appropriate to the chosen creation wizard. Simply load your chosen creation parameters set, by clicking on it, and run the wizard as you have preset, without defining all of the options. On the other hand, you can customize each procedure to your own specific needs by modifying each part separately - e.g. the same channel settings, with a slightly different threshold.

#### Algorithm Settings

Depending on the used cell staining techniques and sample preparation Imaris Cell lets you choose from four different detection types.

You can select to detect **Nucleus, Cell and Vesicles** , detect **Nucleus and Cell** , detect **Cell and Vesicles**  or just to detect **Cell** .

#### Segment only a Region of Interest

A Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.

Check this box if you want to segment only a [Region of Interest](#). If option Segment only a Region of Interest is selected, in the last step of the Creation Wizard only the region of interest is processed.

#### Process entire Image finally

This option appears only, if Segment only a Region of Interest is checked.

Check this box and in the last step of the Creation Wizard the entire image is processed with the adjusted parameters ([Complete ROI](#)). Un-check this box and only the region of interest is processed.

#### Track Cells (over Time)

Check this box if you want to track the resulting Cells over time, for details please refer to the [Tracking](#) Section.

#### Cells Wizard Buttons

## Button Next

Press **Next** to proceed with the next window of the Creation Wizard.

## Button Finish

Click on **Finish** to calculate the desired Cells and display the Cells objects in the viewing area.

## Button Cancel

If you do not want to create the Cell objects click on **Cancel**.

### 11.1.1.2 Region of Interest

Here you define the region of interest (ROI). Parameters such as width, height and location of the ROI are used to directly specify the area to work on.

#### X, Y, Z, Time

#### Min, Max, Size

**Minimum, maximum** and **Size** of ROI coordinate values can be specified either by entering the value in the value fields or by changing the values by clicking on the up-and down arrows on the right hand side of the value fields.

Alternatively the location and size of the ROI can be adjusted directly in the viewing area. Size of the ROI:

1. Click on the ROI preview rectangle arrows (change to the pointer mode Select)
2. Drag the arrows to define new coordinates for X, Y and Z.

Location of the ROI:

1. Click inside the ROI preview rectangle
2. Drag the entire rectangle (with mouse button held) to the chosen image location.

The numbers in the value fields are updated automatically.

#### Memory Usage:

Display of the memory usage for the image size within the ROI.

## Regions of Interest

To select a particular ROI, left-click on its name in the list and the selected ROI will be highlighted within an image and its properties can be adjusted.

#### Button **Add**

Click on this button and a new region of interest appears in the list. To adjust this ROI you have to select the ROI with a left mouse click.

#### Button **Delete**

Select a ROI in the list with a left mouse click and click on this button to delete the ROI.

#### Total Memory Usage:

Display of the memory usage for the image size within the ROI.

## Cells Wizard Buttons

#### Button **Back**

With Back you return to the previous window of the Creation Wizard.

#### Button **Next**



Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**

If you do not want to create Cells click on **Cancel**.

### 11.1.1.3 Nuclei Detection

#### Source Channel

Select the respective source channel, from which the Nuclei are to be computed.

#### Nucleus Diameter

The image is processed based on the specified minimum of the object diameter that you have entered. Use either the automatically given value or set a new measured value.

#### Advanced Options

##### Smooth

Check the Smooth option to set up the smoothness of the resulting area. Smoothing applies a Gaussian filter to the data set.

Smoothing may require a great deal of memory if the data set is very large. Uncheck the check-box or use smaller ROI if your system does not have enough memory.

If the option Smooth is selected then the Filter Width value should be determined. Use either the automatically given value or type in a new value in the data field.

##### Filter Width

The value of the Filter Width determines the Gaussian filter width.

The value is either automatically calculated from the voxel sizes (oriented at the largest voxel dimension) or it can be directly entered into the numerical field. The value is given by image units and not by the amount of voxels.

##### Background Subtraction

Background Subtraction applies a Gaussian filter to estimate the background intensity value of each voxel. This variable background is then subtracted from every voxel in the image; Baseline Subtraction is performed.

The width of the Gaussian filter is calculated by subtracting the diameter of the **largest sphere that fits into the Object** from the intensity of the original channel.

If the option Background Subtraction is selected then the **Sphere Diameter** should be determined.

##### Sphere Diameter

The image is processed based on the specified minimum of the object diameter that you have entered. Use either the automatically given value or set a new measured value. The value of **Sphere Diameter** should be slightly larger than the diameter of the largest object expected to be found in the data set.

If the option Background Subtraction is not selected, the Absolute Intensity threshold is performed.

##### Split Nuclei by Seed Points

Select this option to apply filters to the seed points of the nuclei. Refer to the topic [Classify Nuclei Seed Points](#) for more information on this step.

## Cells Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

### Button **Cancel**

If you do not want to create Cells click on **Cancel**.

## 11.1.1.4 Classify Nuclei Seed Points

The Seed Points filtering is incorporated into the creation process. This tab only appears, if the Split Nuclei by Seed Points option has been selected.

Filters may allow objects that would interfere with interpretation of results to be removed.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

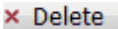
**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.**

## Filters

### Add Button

To add a new filter, click on the button Add  and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

### Delete Button





Select a filter in the list with a left mouse click and click on this button  to delete the filter.

## Filter Type

Select the filter you want to apply from the drop-down list.

## Compact Lower and Upper Threshold









Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking the histogram or by entering the value directly in the data field. Either type in the value or click the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking the histogram or by entering the value directly in the data field. Either type in the value or click the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left-click the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected is displayed at the bottom of the threshold histogram.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram**

display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.



## Cells Wizard Buttons

Button **Back**

With Back you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**





If you do not want to create Cells click on **Cancel**.

### 11.1.1.5 Nuclei Threshold

#### Nucleus Threshold (Absolute Intensity)

In this creation step the Nucleus objects are detected and segmented.




#### Threshold




Activating/deactivating the Threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

#### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the threshold value is not activated (indicated by the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the threshold option is activated (indicated by the green button ) , the corresponding A/M button turns black.

As a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.



## Manual

Define the requested threshold by clicking the histogram or by entering the value directly in the data field.

The image's regions with intensity higher than the set threshold value are Cell objects to be created.

## Lower and Upper Threshold

In Imaris you can set two threshold values, **Lower** and **Upper**.

The two threshold values separate the data into three regions: above the higher threshold, below the lower threshold, and the region in between the two values.

Object with values below the Lower Threshold and objects with values exceeding the Higher Threshold will be excluded and only those objects with quality inside the interval defined by the two threshold values are considered.

Please note if the value of Lower threshold is set to be higher than the value of Higher threshold the selected objects will be with outside the interval defined by the two threshold values.

To change the upper and/or lower threshold value(s) you can drag the line(s).

Alternatively, left click in the histogram to set the lower value of the threshold range and right click to set the upper value of the threshold range.

## Cells Wizard Buttons

Button **Back**

With Back you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**

If you do not want to create Cells click on **Cancel**.

### 11.1.1.6 Classify Nuclei

The Nuclei classification is incorporated into the creation process under Nuclei Detection Advanced options.

This step allows the removal of objects that would interfere with interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area.

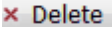
By using multiple filter types, more than one criterion can be specified for the data analyses.

## Filters

### Add Button

To add a new filter, click on the button Add  and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

### Delete Button

Select a filter in the list with a left mouse click and click on this button  to delete the filter.

















## Filter Type

Select in the drop down list the filter you want to apply.

## Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.





In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.




## Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected is displayed at the bottom of the threshold histogram.

## Cells Wizard Buttons

### Button **Back**

With Back you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**

If you do not want to create Cells click on **Cancel**.

### 11.1.1.7 Cells Detection

Depending on the cell staining techniques and sample preparation used, you can select either to detect:

1. Cell Boundary from Cytoplasm



2. Cell Boundary from Cell Membrane



## Cell Body Detection

### Source Channel

Select the respective source channel, from which the Cells are to be computed.

### Smooth

Check the Smooth option to set up the smoothness of the resulting area. Smoothing applies a Gaussian filter to the data set.

Smoothing may require a great deal of memory if the data set is very large. Uncheck the check-box or use smaller ROI if your system does not have enough memory.

If the option Smooth is selected then the Filter Width value should be determined. Use either the automatically given value or type in a new value in the data field.

### Filter Width

The value of the Filter Width determines the Gaussian filter width.

The value is either automatically calculated from the voxel sizes (oriented at the largest voxel dimension) or it can be directly entered into the numerical field. The value is given by image units - not by the amount of voxels.

### Background Subtraction

Background Subtraction applies a Gaussian filter to estimate the background intensity value of each voxel. This variable background is then subtracted from every voxel in the image; Baseline Subtraction is performed.

The width of the Gaussian filter is calculated by subtracting the diameter of the largest sphere that fits into the Object from the intensity of the original channel.

If the option Background Subtraction is selected then the Sphere Diameter should be determined.

### Sphere Diameter

The image is processed based on the specified minimum of the object diameter that you have entered. Use either the automatically given value or set a new measured value. The value of Sphere Diameter should be slightly larger than the diameter of the largest object expected to be found in the data set.

If the option Background Subtraction is not selected, the Absolute Intensity threshold is performed.

## Cell Membrane Detection

### Source Channel

Select the respective source channel, from which the Cells are to be computed.

### Cell Smallest Diameter

Enter a minimum value for the cell diameter (um).

## Membrane Detail

Set a membrane detail value for the membrane thickness present in the image dataset.

## Filter Type





Select the filter type used, either Local contrast (default) or Smooth. Smoothing applies a Gaussian filter to the data set. A lot of small regions will be detected after this step. These small sub-regions can be fused (merged) together by adjusting cell threshold values to generate the correct cell detection regions in the Cell Membrane Threshold step.

### 11.1.1.8 Cells Threshold

#### Cell Body Detection Threshold (Absolute Intensity)

When Cell Body Detection has been selected the following options are available in this creation step in which the Cell objects are detected and segmented.




#### Threshold




Activating/deactivating the Threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

#### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the threshold value is not activated (indicated by the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the threshold option is activated (indicated by the green button ) , the corresponding A/M button turns black.

As a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

## Manual

Define the requested threshold by clicking into the histogram or by entering the value directly in the data field.

The image's regions with intensity higher than the set threshold value are Cell objects to be created.

## Lower and Upper Threshold

In Imaris you can set two threshold values, **Lower** and **Upper**.

The two threshold values separate the data into three regions: above the higher threshold, below the lower threshold, and the region in between the two values.

Object with values below the Lower Threshold and objects with values exceeding the Higher Threshold will be excluded and only those objects with quality inside the interval defined by the two threshold values are considered.

Please note if the value of Lower threshold is set to be higher than the value of Higher threshold the selected objects will be with outside the interval defined by the two threshold values.

To change the upper and/or lower threshold value(s) you can drag the line(s).

Alternatively, left click in the histogram to set the lower value of the threshold range and right click to set the upper value of the threshold range.

## Split Touching Cells

### Don't Split

When this option is selected the Split Cells' algorithms are **not** activated.

### One Nucleus per Cell

When the automatic algorithm does not properly segment the cells, creating cell objects containing two or more nuclei, this option can be used. When activated, this option performs a separation of cells, resulting in every single cell containing only one Nucleus. The algorithm used for this separation is the **Marker Controlled Watershed**.

### Split by Seed Points

This option supports the separation of two or more of the Cells objects, that have been identified as one, in the Threshold step.

The Split Cells method for segmentation in Imaris starts with defined seed points. You use the Cells as seed points for the Cells segmentation that will be performed in the next steps. During the process, the region around each seed point grows until it reaches the border.

### Seed Points Diameter

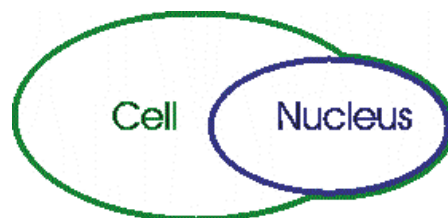
Type in the estimated diameter of a seed point.

## Nuclei Options

### Expand Cell on Nucleus

This option defines the voxel set which is assigned, in the segmentation step, as a boundary of the Cell object. Areas of the image that have been initially segmented as Cells are expanded outside the original borders, to include a set of voxels which are defined in the segmentation step as Nuclei.

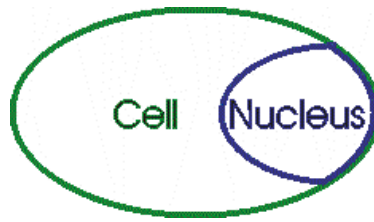
Resulting in a union between the set of voxels defining the Cells and the set of voxels initially defined as Nuclei.



### Erase Nucleus outside Cell

This option defines the voxel set which is assigned as a boundary for the segmented Nucleus.

Any parts of Nuclei that are segmented outside the Cell object is removed from the preview area, leaving only set of voxel assigned as Nucleus within the Cell object. This means that the segmented Nuclei boundary will be reduced to the voxel set defined as the Cell object.



## Cells Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

### Button **Cancel**

If you do not want to create Cells click on **Cancel**.

## 11.1.1.9 Cell Membrane Threshold

### Cell Membrane Threshold

When the Cell Membrane Detection type has been selected the following options are available for Cell Membrane Threshold Detection:

In the previous Cells Detection step, there are two filter options "Local Contrast" and "Smooth" that set the available cell membrane threshold settings.

For the "Local Contrast" filter, two threshold values can be adjusted to fuse the basic regions. The first value is Intensity which is the average intensity value of the border segment of the regions on the smooth image. The histogram of the Intensity represents the values of all border segments. By adjusting the Intensity value, the border segment whose value is below the threshold is removed so that its connected two basic regions are fused together. By adjusting the Intensity value threshold, the small sub-regions which are located on the background region can be easily fused together to generate a better background region. The second value is Quality which is the value of the border segment of the fused regions which are generated by adjusting the first value Intensity on the filtered image. So the Quality value is based on the fused regions generated by adjusting the Intensity.

For the "Smooth" filter, one threshold value can be adjusted to fuse sub-regions. The Quality is the value of the border segment of the basic regions on the smooth image. By adjusting the Quality value threshold, the basic regions can be fused together to generate the better results.

## 11.1.1.10 Classify Cells

The Cell object classification is incorporated into the creation process.

Filters may allow objects that would interfere with interpretation of results to be removed.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.


**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for data analysis.**

## Filters

### Add Button

To add a new filter, click on the button Add  and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.





### Delete Button

Select a filter in the list with a left mouse click and click on this button  to delete the filter.

## Filter Type

Select the filter you want to apply from the drop-down list.




## Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.



## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected is displayed at the bottom of the threshold histogram.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.**

## Cells Wizard Buttons

### Button **Back**

With Back you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

### Button **Cancel**

If you do not want to create Cells click on **Cancel**.

### 11.1.1.11 Vesicles Detection

Select this option to start the automatic creation process for Vesicles.

The drop-down list shows the types of Vesicles that will be detected in the creation process.

To detect the additional vesicles types

To add a new Vesicles type, click on the button and choose the name. The new Vesicles type name appears in the list.

1. Click on the **Add New Type** Button
2. Enter a Vesicle name in the **Vesicles name** field
3. The new Vesicles type name appears in the list
4. Select the Source Channel
5. Define the Estimated Diameter
6. Decide on the Background Subtraction

### Vesicle Name

This field allows you:

1. To enter the name for a newly added vesicle type or
2. To edit a name already defined

### Source Channel

Select the respective source channel, from which the Vesicles are to be computed.

### Estimated Diameter

Objects with size smaller than the specified **Estimated Diameter** are not detected.

### Background Subtraction

If checked, the background will be removed prior to Vesicle detection. Technically the background subtraction is used to smooth the image. The intensity equals the Gaussian filtered channel (Gaussian filtered by 3/4) minus the intensity of the original channel Gaussian filtered by 8/9 of sphere radius.

### Different Vesicles Sizes (Region Growing)

The following options only appears, when the **Different Vesicles Sizes (Region Growing)** box is checked.

The region method for segmentation in Imaris starts with defined seed points. You use Vesicles as seed points for the region growing that is performed. During the region growing process around each seed point the region grows until a border stops the growing.

## Vesicles Regions from

### Absolute Intensity

#### Local Contrast

You can set the border using the **Absolute Intensity** or the **Local Contrast** as criteria. In both cases as next step the selected channel is displayed in the viewing area.

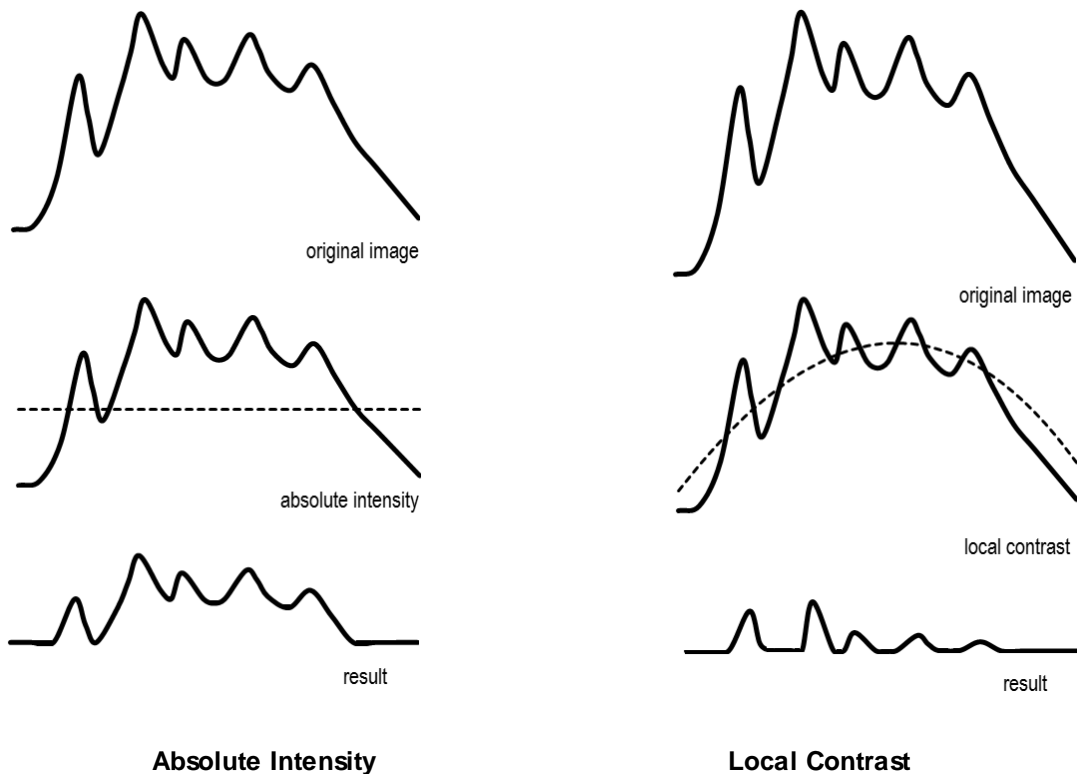
#### Local Contrast

This option should only be used when the **Absolute Intensity** method does not properly segment all objects. Please note: Local Contrast applies a Gaussian filter in order to estimate the intensity value of each voxel. Baseline Subtraction is then performed by subtracting the variable background from every voxel in the image. As a result, it uses more memory than the Absolute Intensity method.

Gaussian Smoothing replaces the voxel intensity value with a newly calculated value. The new value is a weighted sum, defined using a Gaussian function, of the voxel and its neighbouring voxels.

The degree of the smoothing is directly dependent on the shape of this kernel, which in turn is a function of the

standard deviation. A Gaussian kernel is a symmetric bell shaped curve.



**Absolute Intensity**

**Local Contrast**

#### **Add New Type Button**

To add a new vesicles types, click on the button Add and choose the appropriate settings.

#### **Delete Button**

Select a Vesicles type in the list and click on this button to delete it.

### **Cells Wizard Buttons**

#### **Button Back**

With Back you return to the previous window of the Creation Wizard.

#### **Button Next**

Press **Next** to proceed with the next window of the Creation Wizard.

#### **Button Finish**

Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

#### **Button Cancel**

If you do not want to create Cells click on **Cancel**.

#### **11.1.1.12 Classify Vesicles**

Vesicle filtering is incorporated into the creation process.

Click on the drop-down list and select the required Vesicle type to be classified.

Filters may allow objects that would interfere with interpretation of results to be removed.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria.

First select the Vesicle type. Then select the filter type by selecting a variable from the drop-down list, and finally define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.


**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analysis.**

## Filters

### Add Button

To add a new filter, click on the button Add  Add  and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

### Delete Button

Select a filter in the list with a left mouse click and click on this button  Delete to delete the filter.












## Filter Type

Select the filter you want to apply from the drop-down list.

## Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .





These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.

## Compact Lower and Upper Threshold









Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected is displayed at the bottom of the threshold histogram.

**Tip:** You can use the **Zoom** function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.

## Cells Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**

If you do not want to create Cells click on **Cancel**.

### 11.1.1.13 Vesicle Regions

This tab only appears, if **Different Vesicle Sizes (Region Growing)** is checked.




## Vesicle Regions

The working channel is visualized in the viewing area, the histogram is displayed in the properties area. Regions above the threshold are displayed in white, regions below threshold are displayed in black. Depending on the previously selected detection method (Absolute Intensity or Local Contrast) refer to Absolute Intensity or Local Contrast.

## Threshold

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

## Absolute Intensity

### Automatic

In the working channel a copy of the intensity histogram of the source channel is displayed. Regions above the threshold are displayed in white. Now you adjust the threshold of the working channel. Edit the data field or adjust the threshold manually using the mouse (click & drag the line in the histogram). In the next step the regions will grow until they fill the white regions and reach the border.

## Local Contrast



### Automatic

In the selected channel the regions with a local contrast brighter than the background are displayed in white. Now you adjust the threshold value of the local contrast channel. Edit the data field or adjust the threshold manually using the mouse (click & drag the line in the histogram). In the next step the regions will grow until they fill the white regions and reach the border.

### Diameter from

#### Region Volume

The Vesicles radius is calculated from the region volume (the region volume and the Vesicles volume are equal).

#### Create Color Coded Region Channel

Additionally the Vesicles the Region Channel is displayed. Each region has a different color.

#### Region Border

The radius of the calculated Vesicles is the smallest distance from each Vesicles center to the region border.

### Cells Wizard Buttons

#### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

#### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to calculate the desired Cells and display the Cells objects in the viewing area.

#### Button **Cancel**

If you do not want to create Cells objects click on **Cancel**.

### 11.1.1.14 Tracking Cells

Tracking allows objects to be traced throughout sequential frames of a time data set, so each object maintains a unique identity in the output measurements.

Tracking the Nucleus and Vesicle objects with respect to the inertial image coordinates is less significant than tracking their relative motion within the cell.

In order to cope with the spatial position of Nuclei and Vesicles within the Cell, the concepts of relative tracking are required.

Tracking of Nuclei and Vesicles objects is presented in relative terms of their parent Cell objects, rather than their real coordinates on the screen.

Since the Cell object itself can be moving (relative to the inertial image reference frame), the tracking of Nuclei and Vesicle is **relative** to the parent Cell of a specified reference point.

The position of the Nuclei and Vesicle objects is specified as a relative distance from a reference point. This reference point is the Cell object center of mass.

The position of the individual Nuclei and Vesicles objects is calculated using two types of information. One is the absolute position of the selected object in terms of the inertial image reference coordinates and the other is the absolute position of the parent Cell object center. The **relative** Nuclei and Vesicles object's **position** is calculated by subtracting these two positional differences and is then tracked.

This concept of relative tracking allows direct measurements and statistical data comparison between moving

cells to be calculated.

## Algorithm

### Brownian Motion

This algorithm models the motion of each object (Nuclei or Vesicles) as a Brownian motion. This model is appropriate if the object performs similar to a Brownian motion (the random movement of objects with very frequent changes in direction and no discernible pattern).

The only prediction applied is that the object does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

### Autoregressive Motion

This algorithm models the motion of each cell object (Nuclei or Vesicles) as an autoregressive AR1 process. This model is appropriate if the object performs any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the object will move again the same distance and in the same direction. The user-specified **Maximum Distance** value is the distance in which the object is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

### Autoregressive Motion Expert

This algorithm models the motion of each cell object (Nuclei or Vesicles) as an autoregressive AR1 process (see above). It is suitable for tracing of multiple objects with adjacent or crossing paths, especially if the neighboring objects have a stable (but different) intensity. In Autoregressive Motion Expert the algorithm called Total Cost determines the most appropriate connections for tracks by seeking the lowest Total Cost. In this case, the Total Cost algorithm combines Intensity Cost and Distance Cost functions (see Intensity Weight below).

### Connected Components

For this algorithm, objects in adjacent time-points are considered connected if the object spheres occupy some of the same space (the spheres would overlap if two time-points were fused into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. All the object with the overlap will be assigned the same track ID.

Connected Components and Lineage are the only algorithms that automatically handle lineage (objects that fuse or fragment). It will only work with images that have excellent time resolution, because the object cannot ever jump a distance larger than its own size.

### Lineage

The Lineage model is intended for events such as cell division in which the splitting of objects is to be analyzed. This algorithm models the motion of each object (Nuclei or Vesicles) as an autoregressive AR1 process (refer to Autoregressive Motion above). The object data is then compared so that only the objects that undergo splitting are connected, and assigned the same track ID. Objects that pass in close proximity, or overlap, are not associated. This is in contrast to connected components, where any objects that overlap are determined and considered connected.

### Maximum Overlapping

For a cell at time point  $t$  the overlap with all cells at the previous time point  $t-1$  is computed and the cell is linked to that cell from the previous time point with which it has the largest overlap. The link is only created if the ratio of the volume of the smaller of the two linked cells to the larger is greater than the "maximum overlapping ratio".

## Parameters





### Max Distance Cell

The value of the Max Distance specifies the maximum distance that a track Cell object could possibly move between two consecutive time points. This parameter disallows the connection between a Cell and a candidate match if the distance between the predicted future position of the Cell and the candidate position exceeds the maximum distance.

### Max Relative Distance Nucleus

The value of the Max Distance specifies the maximum distance that a track Nucleus could possibly move between two consecutive time points, relative to the parenting Cell center of mass.

### Max Relative Distance Vesicles

The value of the Max Distance specifies the maximum distance that a track Vesicles could possibly move between two consecutive time points, relative to the parenting Cell center of mass.

## Options

It can happen that the object segmentation fails for some time points. This can break a Track apart into two Tracks. A gap-closing algorithm links track segment ends to track segment starts in order to recover tracks that were interrupted by the temporary disappearance of particles.

### Close Gap

Select this option and the gap-closing algorithm is implemented.

## Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

## Fill the gaps with all detected objects

This algorithm allows tracks to be continued, even if the object was not detected in periods of up to two consecutive time points along the trajectory. If this option is selected the algorithm performs the optimization of the object expected position for the particular time point. The track is generated by connecting objects assuming their optimized expected positions.

## Track Nuclei and Vesicles

Select this option to track nuclei and vesicles. Refer to [Tracking Nuclei and Vesicles](#) for information (This option is only available if the relevant detection algorithm has been selected).

## Cells Wizard Buttons

Button **Back**

With Back you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

Button **Cancel**

If you do not want to create Cells click on **Cancel**.

## Track

Select track to select a complete track. Refer to [Tab Edit Tracks](#) for editing information.

### 11.1.1.15 Classify Cells Tracks

Tracks filtering is incorporated into the creation process. This tab only appears, if **Track Cells (over Time)** is checked ([Algorithm](#)).

Filters may allow objects that would interfere with interpretation of results to be removed.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for data analysis.**

## Filters

### Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

### Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.










### Filter Type

Select the filter you want to apply from the drop-down list.

### Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.





In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.




### Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

### Lower Threshold

#### Automatic

The initial threshold value is automatically computed.

#### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

### Higher Threshold

#### Automatic

The initial threshold value is automatically computed.

#### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type

in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within the threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Tracks) is displayed at the bottom of the threshold histogram.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.**

## Surfaces Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

### Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

### 11.1.1.16 Tracking Nuclei and Vesicles

Tracking allows objects to be traced throughout sequential frames of a time data set, so each object maintains a unique identity in the output measurements.

Tracking the Nucleus and Vesicle objects with respect to the inertial image coordinates is less significant than tracking their relative motion within the cell.

In order to cope with the spatial position of Nuclei and Vesicles within the Cell, the concepts of relative tracking are required.

Tracking of Nuclei and Vesicles objects is presented in relative terms of their parent Cell objects, rather than their real coordinates on the screen.

Since the Cell object itself can be moving (relative to the inertial image reference frame), the tracking of Nuclei and Vesicle is **relative** to the parent Cell of a specified reference point.

The position of the Nuclei and Vesicle objects is specified as a relative distance from a reference point. This reference point is the Cell object center of mass.

The position of the individual Nuclei and Vesicles objects is calculated using two types of information. One is the absolute position of the selected object in terms of the inertial image reference coordinates and the other is the absolute position of the parent Cell object center. The **relative** Nuclei and Vesicles object's **position** is calculated by subtracting these two positional differences and is then tracked.

This concept of relative tracking allows direct measurements and statistical data comparison between moving

cells to be calculated.

## Track

### Track Nuclei

Select this option to apply the required algorithm and parameters to Nuclei.

### Track Vesicles

Select this option to apply the required algorithm and parameters to Vesicles.

## Algorithm

### Brownian Motion

This algorithm models the motion of each object (Nuclei or Vesicles) as a Brownian motion. This model is appropriate if the object performs similar to a Brownian motion (the random movement of objects with very frequent changes in direction and no discernible pattern).

The only prediction applied is that the object does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

### Autoregressive Motion

This algorithm models the motion of each cell object (Nuclei or Vesicles) as an autoregressive AR1 process. This model is appropriate if the object performs any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the object will move again the same distance and in the same direction. The user-specified **Maximum Distance** value is the distance in which the object is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

### Autoregressive Motion Expert

This algorithm models the motion of each cell object (Nuclei or Vesicles) as an autoregressive AR1 process (see above). It is suitable for tracing of multiple objects with adjacent or crossing paths, especially if the neighboring objects have a stable (but different) intensity. In Autoregressive Motion Expert the algorithm called Total Cost determines the most appropriate connections for tracks by seeking the lowest Total Cost. In this case, the Total Cost algorithm combines Intensity Cost and Distance Cost functions (see Intensity Weight below).

### Connected Components

For this algorithm, objects in adjacent time-points are considered connected if the object spheres occupy some of the same space (the spheres would overlap if two time-points were merged into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. All the object with the overlap will be assigned the same track ID.

Connected Components and Lineage are the only algorithms that automatically handle lineage (objects that diverge). It will only work with images that have excellent time resolution, because the object cannot ever jump a distance larger than its own size.

### Lineage

The Lineage model is intended for events such as cell division in which the splitting of objects is to be analyzed. This algorithm models the motion of each object (Nuclei or Vesicles) as an autoregressive AR1 process (refer to Autoregressive Motion above). The object data is then compared so that only the objects that undergo splitting are connected, and assigned the same track ID. Objects that pass in close proximity, or overlap, are not associated. This is in contrast to connected components, where any objects that overlap are determined and considered connected.

## Parameters

### Max Distance Cell

The value of the Max Distance specifies the maximum distance that a track Cell object could possibly move between two consecutive time points. This parameter disallows the connection between a Cell and a candidate match if the distance between the predicted future position of the Cell and the candidate position exceeds the maximum distance.

### Max Relative Distance Nucleus

The value of the Max Distance specifies the maximum distance that a track Nucleus could possibly move between two consecutive time points, relative to the parenting Cell center of mass.

### Max Relative Distance Vesicles

The value of the Max Distance specifies the maximum distance that a track Vesicles could possibly move between two consecutive time points, relative to the parenting Cell center of mass.

## Options

It can happen that the object segmentation fails for some time points. This can break a Track apart into two Tracks. A gap-closing algorithm links track segment ends to track segment starts in order to recover tracks that were interrupted by the temporary disappearance of particles.

### Close Gap

Select this option and the gap-closing algorithm is implemented.

## Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

## Fill the gaps with all detected objects

This algorithm allows tracks to be continued, even if the object was not detected in periods of up to two consecutive time points along the trajectory. If this option is selected the algorithm performs the optimization of the object expected position for the particular time point. The track is generated by connecting objects assuming their optimized expected positions.

## Cells Wizard Buttons

### Button Back

With Back you return to the previous window of the Creation Wizard.

### Button Next



Press **Next** to proceed with the next window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to calculate the desired Cells and display the Cell in the viewing area.

#### Button **Cancel**

If you do not want to create Cells click on **Cancel**.

### 11.1.1.17 Classify Nuclei and Vesicles Tracks

Tracks filtering is incorporated into the creation process. This tab only appears, if **Track Cells (over Time)** is checked ([Algorithm](#)).

Filters may allow objects that would interfere with interpretation of results to be removed.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for data analysis.**

## Filters

### Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

### Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.


## Filter Type

Select the filter you want to apply from the drop-down list.

## Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks 







These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.




## Compact Lower and Upper Threshold


Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.



In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode.

Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram





In the histogram the regions above and below the threshold values are displayed in white, while the region within the threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Tracks) is displayed at the bottom of the threshold histogram.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.**

### Surfaces Wizard Buttons

#### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

#### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

#### Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

### 11.1.1.18 Complete ROI

This tab only appears, if **Process entire Image finally** is checked ([Algorithm](#)).

### Region of Interest

The parameters you have set up will now be used to compute Spots (and Tracks) for the entire data set. Depending on the size of the data set this may take a while.

### Surfaces Wizard Buttons

#### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to end the calculation and display the results in the viewing area.

#### Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

## 11.1.2 Tab Settings

On Tab Settings you can choose the rendering methods and adjust the rendering quality to produce faster performance of the display.

### Object Type

A choice of Object types is presented. For time series images the choice of the Object Type is in the drop-down list. After choosing the Object type, a selection of the Setting Tab is listed.

[Cell/Nuclei](#)

[Cell Tracks](#)

[Nuclei Tracks](#)

[Vesicle Type and Tracks](#)

### Volume and Slicer Display

The option **Volume Display** is used to identify the different objects and distinguish them from the surrounding by visualizing the volume around a created object. It extracts a subset of the volume data set by using the specified axis-aligned limits.

The border around each object is defined independently and corresponds to the regions equal to bounding box of the created object.

### MIP

In the Maximum Intensity Projection mode only the point with maximum intensity of all layers along the viewing direction displayed. If your data set has more than one channel, the combination color is displayed.

### Shaded Volume

This option displays the subvolume around a certain object in the shaded volume mode. In this mode, all values along the viewing direction including their transparency are used for the calculation.

Slicer display options are described in the [Cell/Nuclei](#) section.

#### 11.1.2.1 Cell/Nuclei

### Cell and Nuclei Style

#### Cell visible

This option shows or hides the Cell objects in the viewing area.

#### Nucleus visible

This option shows or hides the Nucleus objects in the viewing area.

### Slicer Display

#### Slicer Direction

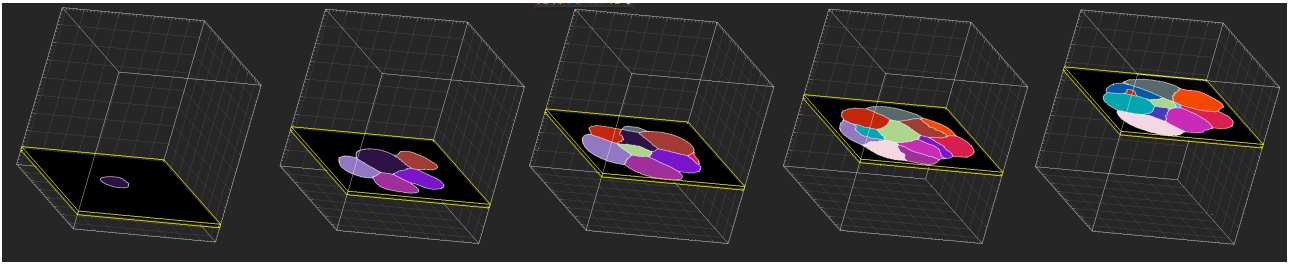
The Slicer display represents an orthogonal plane (orthogonal to the coordinate axis of the data set) that can be moved within the data set. Three orthoslicer planes show their intersection with a plane perpendicular to two of the coordinate axes

Change the slice direction if required by selecting the appropriate XYZ plane option.

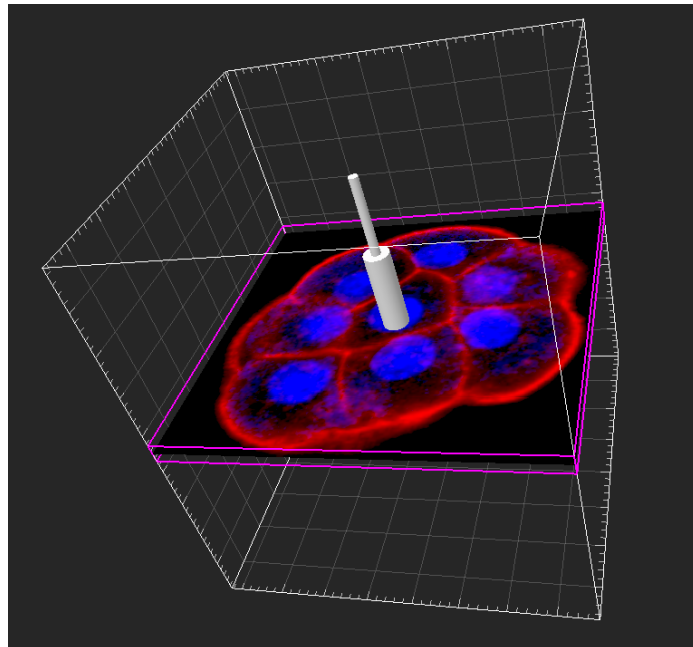
The position of slices can be changed in the viewing area. Switch the pointer to the Select mode and then click on the plane and drag it to a new position. A series of slices is shown below for XY Slicer direction, Border



rendering and Solid color region rendering.



The check buttons labeled as **XY**, **XZ** and **YZ** direction, are used to select the visibility of the corresponding slice. The **Oblique** option represents a plane that can be freely moved and rotated within the data set and allows the user to look inside any object. The Oblique Slicer (shown below) consists of a colored frame with a white double rod in the middle.

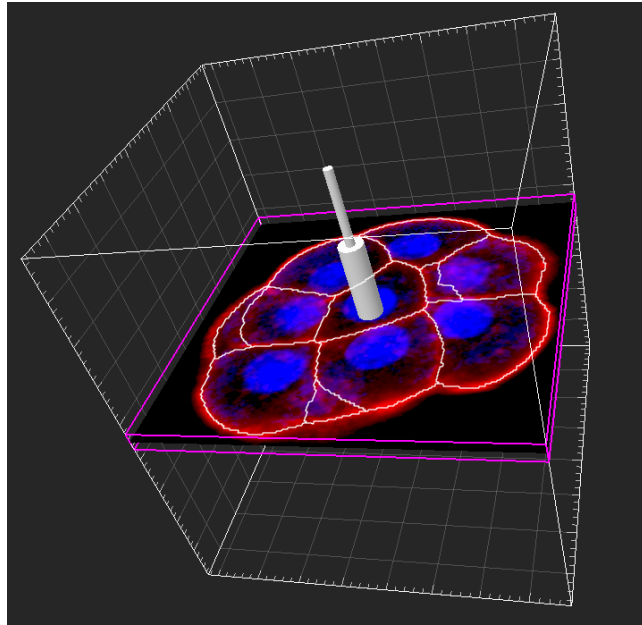


The position of the Oblique Slicer can be changed. Choose the pointer mode **Select**, highlight the Oblique Slicer in the viewing area and click on the bigger diameter rod. It will appear highlighted as it is selected, allowing you to move it and to set the Oblique Slicer to the required position.

The Oblique Slicer can be rotated around the x-, y-, and the z-axes. In the **Select** mode click the smaller diameter rod and it will be highlighted as it is selected. To rotate the Oblique Slicer, move the smaller rod.

### Border Rendering

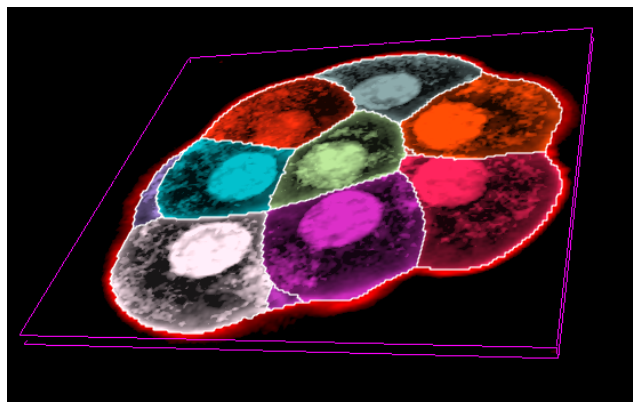
Border rendering can be a useful visualization tool- it displays the cell borders that have been calculated during the cell creation process. This can be seen in the following image compared to the previous image without Border rendering. The width of the border may be changed using the Border width slider bar.



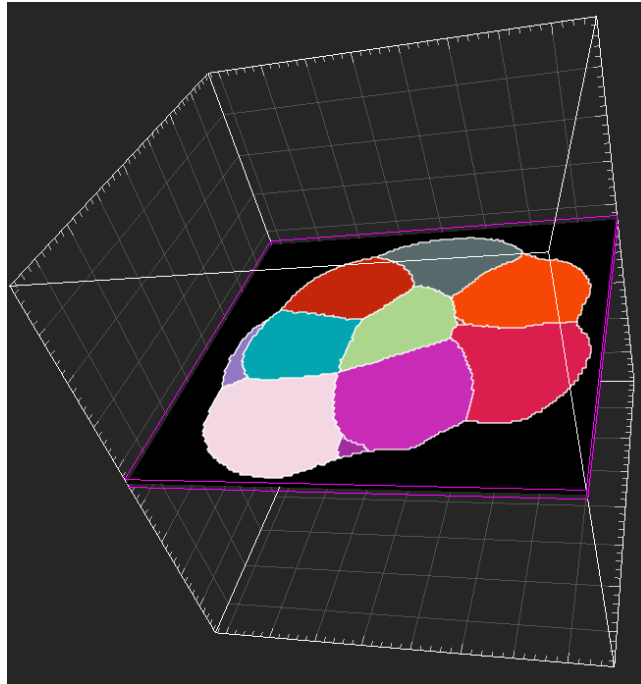
## Region rendering

The region rendering tool enables the individual regions of the cell to be visualized as separate colors. There are two options available: Colored data set and Solid colors.

**Colored data set**- showing the regions of the cell in transparent colors so you may see features of the image.



**Solid colors**- showing each region as a solid color, without cell image detail, so you may view the arrangement and relationship of each region clearly.



### 11.1.2.2 Cell/Nucleus Tracks

#### Cell/Nucleus/Vesicles Tracks

##### Tracks Style/Quality

The box Style/Quality has settings that determine the display of the connections between the Track objects.

##### Cylinder

Select this option to display the tracks connections as cylinders.

##### Line

Select this option to display the tracks connections as lines.

##### Off

Select this option to disable the visualization of the Tracks.

##### Width

##### Diameter/Render quality

Depending on the previous selection you can either adjust the width of the line or the diameter of the cylinder.

##### Displacement

Check the box Displacement to show the displacement of a Track as an arrow. To assign another color, click on the Color symbol to open the Color Editor.

##### Radius Scale

An Edit field for the displacement vector radius.

##### Dragon Tail

Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points. The number of time points is determined with the slider

position.

## Slider

The Dragon Tail length can be adjusted using the slider. The slider determines the number of time points in the length of the Dragon Tail.

### 11.1.2.3 Vesicle Type and Tracks

#### Vesicles Style/Quality

On the tab Settings you can adjust the rendering style or quality.

#### Center Points

This option displays the Vesicles as center points.

#### Off

Select this option to disable the visualization of the Vesicles.

#### Sphere

Select this option to display the Vesicles as spheres.

#### Radius Scale box

The size of the Vesicles can be changed via the Radius Scale box.

#### The Render Quality

The Render Quality is adjustable using the slider. When the number of Vesicles is very large, adjust the Render Quality, to produce a faster display performance.

#### Tracks Style/Quality

The box Style/Quality has settings that determine the display of the connections between the Track objects.

#### Cylinder

Select this option to display the tracks connections as cylinders.

#### Line

Select this option to display the tracks connections as lines.

#### Off

Select this option to disable the visualization of the Tracks.

#### Width

##### Diameter/Render quality

Depending on the previous selection you can either adjust the width of the line or the diameter of the cylinder.

#### Displacement

Check the box Displacement to show the displacement of a Track as an arrow. To assign another color, click on the Color symbol to open the Color Editor.

#### Radius Scale

An edit field for the displacement vector radius.



### Dragon Tail

Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points. The number of time points is determined with the slider position.

### Slider

The Dragon Tail length can be adjusted using the slider. The slider determines the number of time points in the length of the Dragon Tail.

### 11.1.3 Tab Rebuild

#### Rebuild

Imaris stores the list of operations and the mapping of parameters. Select from the list, which step of the Cell Creation Wizard you want to re-enter.

For details please refer to Section:

Menu 3D View - Cells - [Algorithm](#)

Menu 3D View - Cells - [Tracking](#)

### Rebuild Objects Button

Click on this button to start the rebuild.

#### Creation Parameters

The Creation Wizard saves parameters and processing instructions so that the Cell computation can be repeated for the same region, a different region, or the entire image. The Creation Parameters are listed as the table.

### Store Parameters for the Batch

The Store Parameters for the Batch button allows you to save the complete set of the creation parameters and all relevant settings shown in the list. In the Store Creation Parameter window, enter a descriptive name for your creation parameters and select the required store location.

There are two options to store your creation parameters:

- Arena
- Favorite Creation Parameters

### Arena

When you select this option a new Arena item is automatically created within the Arena view. The Arena view is updated, a new item, [Creation Parameters](#), is created and displayed.

Once stored, Imaris [Batch](#) applies the creation parameters set to all images within the selected Group.

### Favorite Creation Parameters

To help you organize and find your most used creation parameters more easily, we advise you to store them by selecting the Favorite Creation Parameters option.

When you select this option the Creation Parameter file is automatically added to the list that is displayed under the Edit/Preference/ Favorite Creation Parameters. This option allows you to use the creation parameters in future on another data set. These saved parameters can then be imported and re-used on any new data set should you wish to perform an identical analysis sequence. This option is also useful for sharing the parameters with other users to allow them to perform the same analysis on their own data sets.

Within the 3D View view storing allows the parameters to be reused or modified. In addition, this option is useful to create and share the creation parameters with other people and allow them to perform their own analysis on it.

Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the creation wizard.

When you save the Creation Parameter file, it is automatically added to the list which is displayed under the [Edit/Preference/Creation Parameters](#).

Click on the Store Parameters for the Batch button opens the Store Creation Parameter window.

## Store Creation Parameter

Refer to [Store Creation Parameters](#)

### 11.1.4 Tab Edit

Once the Cell creation wizard is completed, use these buttons for manual editing of the Cell objects. It allows you:

1. To import Surfaces or Spots and incorporate them in the Cells analysis.
2. To export objects from the Cells.
3. To clear the selection or delete all selected component of the cell object.

#### [Import](#)

#### Import Surface to Cells

To import the Surface as the Cell or the Nuclei Objects, click on this button. The window, **Import to Cells Objects**, will appear.

#### Import Spots to Vesicles

To import the Spots as the Vesicles, click on this button. The window, **Import to Cell Object**, will appear.

## Import to Cell Object window

Choose one of the objects from the drop-down list. Choose how the selected object type is to be imported and specify the selection and the time.

The Surface can be imported either as the Cell or the Nuclei.

The Spots can be imported only as the Vesicles.

#### Import as

Choose to import the object either as a new Vesicle type or as a previously detected Vesicle type.

To import the Spots object as an already segmented Vesicle type, please select the correct name from the drop-down list.

#### Selection

The user can decide either to import the **Selection** or the **All** objects of the specified component.



## Time

The **time point** of the object selected can be imported either as the **Current time point** or the **All time points**.

## Export


### Selection

The **Selection** checkbox is used to export only the currently selected object.


### All Timepoints

The **All Timepoints** checkbox is used to export the object for all time points.


### Export Cells to Surface

With a click on this button you export the Cell objects to a Surface object. The Surpass Tree list is automatically updated with the new item with the automatically generated name  **Cells n Cell Export**. The exported objects can be then manually edited.

### Export Nuclei to Surface

With a click on this button you export the Nuclei objects to a Surface object. The Surpass Tree list is automatically updated with the new item with the automatically generated name  **Cells n Nucleus Export**. The exported objects can be then manually edited.

### Export Vesicles to Spots

With a click on this button you export the Vesicles to a Spots object. The Surpass Tree list is automatically updated with the new item with the automatically generated name  **Cells n Vesicles Export**. The exported objects can be then manually edited.

### Full Export

With a click on this button you export all the Cell objects. The **Cell** and **Nuclei** objects are exported to **Surface**, while the **Vesicles** objects are exported to **Spots**.

The Surpass Tree list is updated and three new surpass objects are generated (two Surface items and one Spots item) with the automatically generated names **Cells n object-name Export**. Pressing this button has the same effect as pressing the three buttons above.

## Selection

### Clear Button

Click this button to clear the current object selection.

### Delete Button

Click on this button to delete all the currently selected components.

All actions in the Draw , Edit  and Edit Tracks  tabs can be Undone/Redone by selecting the [Undo/Redo](#) command or by pressing Ctrl+Z/Ctrl+Y.

## Selection Visible Time Point

Fuse and Fragment are available only when cells were detected using **Detect Cell Boundary from Cell Membrane** option.

## Fuse Button

Select this to fuse two or more selected cell objects within the visible time point.

## Fragment Button

Select this to split selected cell objects within the visible time point. When fragment is selected it will return all previously fused objects back to their original dimensions.

### 11.1.4.1 Import

#### Import Surface to Cell

To import the Surface as the Cell or the Nuclei Objects, click on this button. The window, **Import to Cell Object**, will appear.

#### Import Spots to Vesicles

To import the Spots as the Vesicles, click on this button. The window, **Import to Cell Object**, will appear.

#### Import to Cell Object window

Choose one of the objects from the drop-down list. Choose how the selected object type is to be imported and specify the selection and the time.

The Surface can be imported either as the Cell or the Nuclei.

The Spots can be imported only as the Vesicles.

#### Import as

Choose to import the object either as a new Vesicle type or as a previously detected Vesicle type.

To import the Spots object as an already segmented Vesicle type, please select the correct name from the drop-down list.

#### Selection

The user can decide either to import the **Selection** or the **All** objects of the specified component.

#### Time

The **time point** of the object selected can be imported either as the **Current time point** or the **All time points**.

### 11.1.5 Tab Edit Tracks

#### Mouse Selects

This option allows you to select an object within an image according to its object type by a single mouse click.

To edit the properties of multiple objects simultaneously, select the required object type.

There are the following options:

#### Object

#### Segment

#### Branch

#### Pivot

#### Track

The differences between these options is described below.



## Table Layout

In the track editor a horizontal line represents each track. Each horizontal line is composed of circles, representing objects, and line segments which connect a sequence of objects.

The first row of the track-editing table displays the timeline. Each number represents a time point in the series.

The second row of the track-editing table displays the **object name**.

Multiple object types can be tracked easily since every object type appears in different colors.

## Interactive Selection

When you click on an individual row, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (row in table) is highlighted.

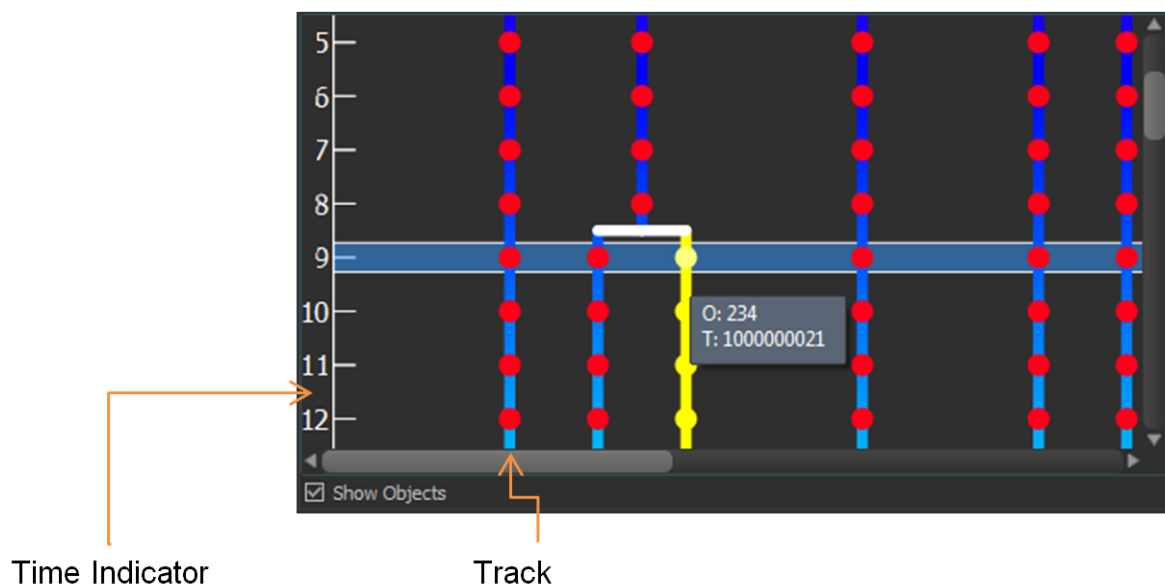
In addition, the Statistical and Filter Tab can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.

For multiple selections press Ctrl+click (Mac: Command+click).

## Using the Track Editor

### Table Layout

The track editor table:



### Zoom

Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table.

Using the mouse wheel will have the same affect.

### Pan

To move the track editing table click and hold the right mouse button while dragging the mouse. Release the right mouse button to place the table on the chosen location.

### Time indicator

The axis on the left displays the frame number.

## Track

Each track is displayed in a vertical format by default. If connected components or Lineage algorithms are used there may be a number of branch points. The branch points indicate where objects fuse or fragment, e.g. cells undergoing division.

## Object

Each data-point is displayed as an object in the track with a unique Object ID. The object will retain its unique ID number- even if, for example, it is connected manually to another track.

If the mouse pointer is on an object, a tooltip appears that identifies the Object ID number (O) and track ID number (T). You can untick the Show Objects checkbox to hide objects from the display.

## Show Objects

Use this option to select to show or hide each object in the tracks from being visible.

## Camera icon

Use the camera icon to save an image of the currently displayed tracks (either .tif or .svg formats) to a specified location. The image will automatically open in the default graphics applicatin after saving is complete.

## Mouse Selects

This option allows you to select objects or tracks as follows:

### Object

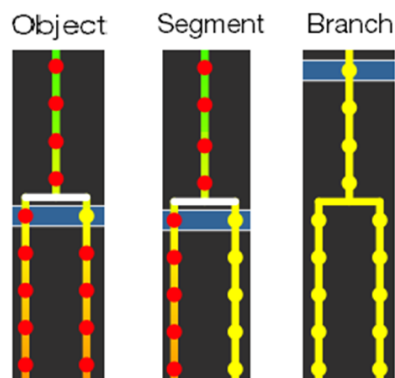
Click Object to select an individual object, or number of specific objects. To connect two unconnected objects, simply select both objects and click the Connect button. To disconnect two connected objects, select both objects and click the Disconnect button.

### Segment

Click Segment to select a specific segment (between branch points).

### Branch

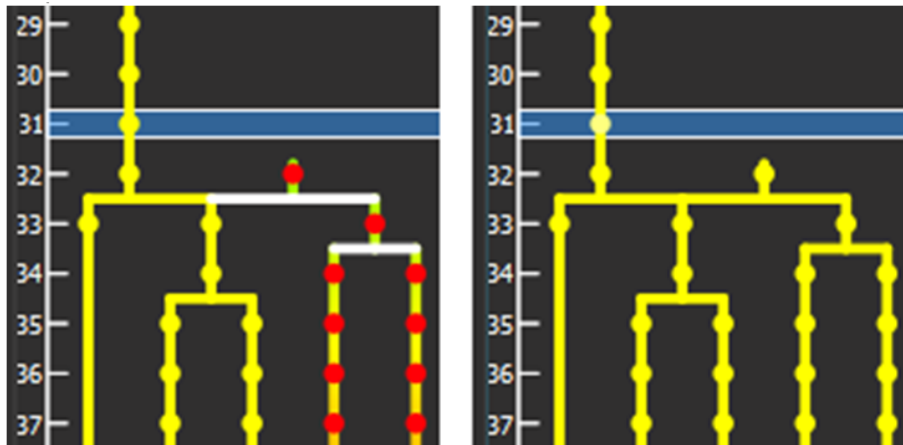
Select Branch to select the segment and the branched segments and objects that appear later in time only.



### Pivot

Pivot enables selection of a segment and all segments and objects that are linked afterwards. This includes objects later in time, and also backwards in time.

For example, selecting branch (left) selects only the connected segments and objects that are later in time. Selecting Pivot (right) all connected segments and objects to be selected.



### Track

Select track to select a complete track.

**Please Note** you can change between mouse selects options using the first letter of each option as a keyboard shortcut i.e. Object: O, Segment: S, Branch: B, Pivot: P and Track: T.

### Focus Tracks Mode

The focus tracks mode can be a useful when you want to focus the view to selected tracks so you may focus on editing them (describes in the following section).

#### Focus Tracks Checkbox

Activate focus tracks mode.

#### Rebuild around Selection button

This feature updates the track editor display around the selected track selection when focus tracks mode is on. This allows you to edit tracks focused in one region, and then move on to another region.

#### Select All button

Select all tracks.

### Editing

Track Editor enables you to modify your tracks.

#### Connect Button

Click this button to connect track components such as objects or segments as selected under Mouse Selects. For example, select objects in different tracks at the subsequent time points (just one object per time point). The connect and disconnect buttons will update to show the number of objects selected. Confirm the selection, then click on the button Connect (you can also press the "c" key as a shortcut). The connection is automatically created between all selected objects.

#### Disconnect Button

Use this option to disconnect track components such as objects or segments as selected under Mouse Selects. For example, select object(s) to be disconnected. The connect and disconnect buttons will update to show the number of objects selected. Confirm the selection, then click on the button Disconnect (you can also press the "d" key as a shortcut). The button will update to show the number of objects selected. The connection between the selected objects in the time sequence is broken.

## < Button

Click this button to move a selected track left. When axis is inverted (horizontal) the selected track moves up.

## > Button

Click this button to move a selected track right. When axis is inverted (horizontal) the selected track moves down.

## << Button

Click this button to move a selected track all the way to the left. When axis is inverted (horizontal) the selected track moves to the top.

## >> Button

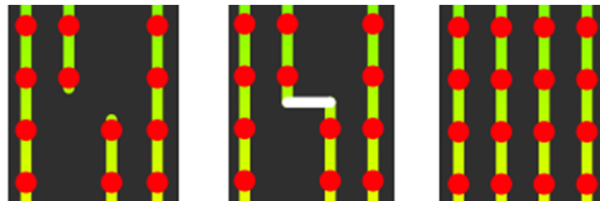
Click this button to move a selected track all the way to the right. When axis is inverted (horizontal) the selected track moves to the bottom.

## Flip

The Flip button may be used (with Pivot mode selected) to flip the order that the connected branched segments and objects are displayed in about the pivot point.

## Reorganize

The Reorganize button can be used after editing of tracks is complete so that tracks are displayed in a clear and logical format without affecting the track arrangement during the editing process.



Editing tracks. Connection added. Tracks reorganized.

## Invert Axis

Click the Invert Axis button to switch between displaying the tracks vertically (default) and horizontally.

## Correct Drift

Refer to [Drift Correction](#).

## Algorithms

The Drift Correction is calculated based on the two algorithms, translational drift and translational and rotational drift.

### Translation

Upon translation, the object position is transformed to a straight line of identical orientation. Within the same time point the relative distance of the objects is preserved.

### Translation and rotation

Upon translation and rotation, the object position is transformed based on the image polar coordinates. Within the same time point the relative distance of the objects is preserved.

**Please note: The number of the selected tracks affects the algorithm calculation.**

Selection of a single track gives a complete description of a translation.

Selection of two tracks gives a minimum description of a translation and rotation algorithm (planar rotation).

A better fit between the time points is achieved when three or more tracks are selected.

### **Result DataSet size**

After completion of the drift correction, the algorithm uses the final position of the initial and resulting image to create a final image. The new image coordinates are calculated in accordance with the size of the original and new image.

Select the Result data set option to determine which new image part will be displayed.

### **Crop largest common region**

The final image will be the size of the common overlap between the original and the drift corrected image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

### **New size equal to current size**

The final image will be the same size as the original image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

### **Include entire result**

The final image will be the size of the combined area of the original and corrected image.

Selecting the appropriate Result data set size may speed up the data visualization. This is especially important with (with large data sets.)

## **Correct Cells positions**

Activate this parameter and the Cells are automatically re-positioned after the drift correction. If this option is not activated only the image volume is aligned.

**All actions in the Draw , Edit  and Edit Tracks  tabs can be Undone/Redone by selecting the [Undo/Redo](#) command or by pressing Ctrl+Z/Ctrl+Y.**

### **11.1.6 Tab Filter**

**Please note: Before using you have to add a filter to the list (see below: button Add)!**

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple**

filter types, more than one criterion can be specified for the data analyses.

## Filters

### Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

### Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.



















## Filter Type

Select the filter you want to apply from the drop-down list.

## Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.





In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determine the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects the Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.




## Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.



### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated  . Clicking on the



Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold. Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click the histogram to move the colored line and then a reduced/enlarged set of objects are visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click the histogram to move the colored line and then a reduced/enlarged set of objects are visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within the threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold and to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality) and the number and percentage of objects that fall into the particular filtering class are displayed at the bottom of the threshold histogram.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.**

### Duplicate Selection to New Surfaces Button

Click on this button and all selected objects are copied to a new Surpass Group. The new group displays in the Surpass Tree.

## 11.1.7 Tab Statistics

Cells statistics are automatically computed. For the statistical values please refer to the Section Menu Edit - Preferences... - Statistics - [Cells](#). The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different data sets through summarized statistical values and graphical data presentations.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data.

The Statistics View is configured as follows:

- a) Overall
- b) Detailed
- c) Selection

In the statistical data set the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

To sort the columns click on the arrow in column heading. Click again to reverse the sort order.



















## Overall Tab

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

## Category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are the following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.

## Detailed Tab

Tables in the Detailed Tab are showing a statistical data collected from all of the objects within the image. A pull down list offers the following types of **Detailed** statistical calculations:

- Specific Values**
- Average Values**

### Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.


### Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).



### Specific Values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Object [Statistical Annotation](#)  option is available for Specific values parameters.

### Average values

The Average table contains values determined on the basis of all values within a set of the selected variable.

Columns are labeled as follows:

Min (Minimum)- the smallest value within a set of values of the selected variable

Max (Maximum)- the largest value within a set of values of the selected variable

Mean – value of the sum of all values divided by number of values within a set

Std Dev (Standard deviation)- a measure of the values distribution around the mean values

Median- the middle value of the sorted set of variables

Sum- the sum of all values within a variable set

Count- number of values within a set

Unit- variable unit

Level/Distance


The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

### Statistical variable classification:

1. Statistical variables associated with objects (Cells, Nucleus, Vesicles -volume, area, intensity values, position, speed, displacement) have values at every time point. Therefore for these variables the Average values are displayed per time point. Changing the time points, statistical variable values and images are consequently changed.
2. Statistical values with data associated with Tracks (track duration, track volume mean, track displacement length, track intensity) are not time dependent.

### All values

If the option All values is selected for the detailed tab, all values will be displayed. Even though this option is always readily available, it is discouraged to use it in order to avoid data clutter.

Object [Statistical Annotation](#) option  is available for Specific values parameters.

### Selection Tab

Statistical tables for a selected object will be displayed. Change to the pointer mode Select and click on an image element that has been detected/segmented. A possibility of multiple selections is available by Ctrl+click (Mac: Command-click).

A pull down list offers two types of statistical calculations from a Selection:

#### Specific Values

#### Average Values

### Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

### Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

### Specific values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Object [Statistical Annotation](#) option is available for Specific values parameters.

### Average values

The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

Object [Statistical Annotation](#) option is available for Specific values parameters.

### Buttons Overview

	PC:	Mac:
Select Cells	Left-click	Click
Select multiple Cells	Ctrl + left-click	Command + click

### Buttons at the bottom of the Statistics tab

Button **Settings** -Configure a List of visible statistics values.

Click on the button **Settings** and you switch to the window Preferences - Statistics - Cells.

Button **Search** - Search by object ID.

Type in the object ID in the data field. Click on the button **Search**.

- Highlights the object in the viewing area
- Highlights the object on the Detailed Tab
- Displays of the values on the Selection Tab

Button **Cells** - Duplicate Selection to new Cells.

Click on this button and all selected elements are copied to a new Cell. The new Cell appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Statistical Annotation** 

Click this button and an object statistical annotation will be automatically generated in the viewing area. This option is available only when **Specific** is selected

Button **Export** - Export Statistics on Tab Display to File.



Only the statistics that are shown within the current tab will be exported.

Button **Export** - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.

All of the statistics (that are enabled in the statistics settings) will be exported for Overall and Detailed tab data. When the Selection tab is selected, pressing the Export all Statistics to File button, will export All the statistics (that are enabled in the statistics settings) of all the selected objects, not all the statistics of all the objects.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).



See also:

Menu Edit - Preferences... - Statistics - [Cells](#)

### 11.1.7.1 Statistical Annotations

Statistical annotations provides the opportunity to view and annotate image objects with the statistical data values. Using this function, the individual objects in an image are labeled with the specified statistical values and the statistical data is displayed directly in the viewing area

Imaris objects can be labeled with a set of any number of the user-defined statistical values.

The Statistical Annotation button  provides a convenient interface to label the object with the statistical data. Click this button  and an object statistical annotation will be automatically generated in the viewing area. The selected object is annotated for all time points with the specified statistical values.

#### How to add Statistical Annotation

In the Statistical tab choose the **Detailed** or **Selection** Tab.

In the viewing area select the object or group of objects. Change to the pointer mode Select and click on an image object that has been detected/segmented. For multiple selections press Ctrl+click (Mac: Command-click)

In the **Detailed** or **Selection Tab** a pull down list offers **Specific Values** or **Average Values** as types of statistical parameters.

Select the statistical value associated with the particular object type you are working with.

Press the Statistical Annotation button .

In the viewing area the object label appears showing selected statistical values as a statistical annotation.

The statistical annotation shows by default the name of the selected statistical parameter and its value.


The color white displays the text of annotations associated with statistical values.

For a single object, all selected statistical parameters are placed in the same annotation box. Once the object is labeled, selecting the additional statistical parameter updates the object statistical annotation; the new parameter and its value is added in the viewing area.

If the image is a time series, the statistical annotation will be visible in the viewing area for all time points. Selecting a statistical variable that changes over time creates an statistical annotation that consequently changes with every time point.

The statistical annotation displays values always for the current time point.

The [Annotation](#) options will allow you to modify and specify the contents and format of the object labels.

To modify and adjust the statistical label click on the icon [Annotation](#) , press Ctrl + Shift + P, or in the menu bar select File – Annotation.

## 11.1.8 Tab Color

Choice of the Object type is presented in a drop-down window. Select Cell, Cell Tracks, Nucleus, Nucleus Tracks, Vesicle (multiple types) or Vesicle Tracks.

Depending on the chosen object type selection of the variables is listed.

The following color types are available:

Labels

[Base](#)

[Statistics Coded](#)

[Time Mapped](#)

[Object ID](#)

Track ID

### 11.1.8.1 Tab Color-Labels

The labels associated with the data set is displayed as a list.

Click **Only show labelled objects** to show only data which have labels associated with them.

Note that if there is more than one label associated with an object, the object will be displayed as a combined color. Non-labelled objects are displayed in the base color.

## Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### 11.1.8.2 Tab Color-Base

#### RGB

The RGB color model is an additive color model in which Red, Green and Blue color information are combined together to reproduce the required color. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

## Light reflection

Quality of the object material is further characterized by reflection properties.

- Diffusion- diffuses reflection of a matte, uneven material.
- Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.
- Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

## Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).



## Palette

Palette- this option provides a system of preset colors organized into color schemes.

In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, Artdeco, Autumn, Glass, Metal, Neon, Rococo, Santafe, Sheen, Silky, Spring, Summer, Tropical, Winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

### 11.1.8.3 Tab Color-Statistics Coded

Depending on the chosen object type the Statistical variable selection is listed in the drop-down window. For example, if Cell/Nuclei/Vesicle is chosen as the Object type, available Statistical data will be the Cell/Nuclei/Vesicle linked variable. Similarly, variables for Tracks will be listed, if the object type is selected to be Tracks.

## Statistics Colorbar Properties

Statistics colorbar are displayed in the viewing area. The upper one is the colorbar range for Cel/Nucleus/Vesicles and the lower one is the colorbar for the Tracks.

### Show Colorbar

Check the box to see the statistics colorbar in the viewing area.

### Show Title

Check this box to see the title in the statistics colorbar.

### Show Range

Check this box to see the range in the statistics colorbar.

Font color in colorbar is adjustable by selecting a color within the color map.

## Colormap

### Button Reset

Click the **Reset** button to set the image back to the original values.

### Colormap

Click on the button Colormap to display a large number of predefined colormaps. If the default colormap (Spectrum) is not appropriate, you can change it by clicking on the drop-down list. The list gives a preview of what different colormaps look like.

## Colormap range

Colormap range-specify the limits for the colorbar.

**Min** - lower color limit

**Max** - upper color limit

### Button Auto

Click the button **Auto** and the system detects the real min and max values and sets the Max. and Min. limits automatically to these values.

## Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### 11.1.8.4 Tab Color-Time Mapped

This option is only available when Tracks (see above) is selected as an Object Type. The color range for the Tracks goes from blue to white to the range of time points.


## Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### 11.1.8.5 Tab Color-Object ID

Object ID assigns a random color to every object. This is available for all surpass components. For Cells, this is the default coloring.

### 11.1.8.6 Tab Color-Track ID

Track ID assigns a fixed, random color to each object. This mode is useful for validating tracks as an object will have the same color through the track duration. Erroneous behavior can easily be detected and corrected using the Edit Tracks  functions. Refer to the [Tab Edit Tracks](#) section.

## Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

## 11.2 Clipping Plane

The Clipping Plane cuts away objects on one side of the plane. It can be freely moved and rotated in the scene and allows you to look inside any object. The Clipping Plane consists of a colored frame with a white double rod in the middle.

To change the position and orientation of the Clipping Plane in the View Area the Pointer must be in Select mode.

### How to Add a new Clipping Plane?

- Select menu 3D View - Clipping Plane.

The Surpass Tree list is automatically updated and in the Clipping Plane - Properties the Settings parameter are displayed. The Clipping Plane is displayed in the viewing area.

To clip another object, this object must be placed behind the clipping plane within the Surpass Tree. It must be in the same group as the Clipping Plane and it must be placed below the Clipping Plane.

### Change Position of Clipping Plane

The position of the Clipping Plane can be changed. Choose the pointer mode Select, highlight the Clipping Plane in the viewing area and click on the bigger diameter rod. It will turn colored as it is selected, move it to set the Clipping Plane to the required position.





## Rotate Clipping Plane around Axes

The Clipping Plane can be rotated around the x-, y-, and the z-axes. In Select mode click the smaller diameter rod. It will turn colored as it is selected, move it to rotate the Clipping Plane.

See also:

Surpass View - Overview - [Surpass Tree](#)

Surpass View - Overview - Camera Toolbar - [Pointer](#) (Mode Select)

### 11.2.1 Tab Settings

#### Position

Button **Recenter**

The Clipping Plane center is moved to the data set center.

#### Orientation

Button **YZ Plane**

Button **XZ Plane**

Button **XY Plane**

The Clipping Plane is oriented parallel to either the yz-plane, the xz-plane or the xy-plane.

Button **Flip**

Click on the **Flip** button to flip the orientation of the clipping plane.

#### Configuration

Button **Store**

Button **Recall**

After changing the position and/or orientation of the Clipping Plane, the new configuration can be stored by clicking on the **Store** button. The position previously stored can be recalled by selecting the **Recall** button.

#### Camera

Button **Orthogonal View**

The camera is set to a position which is perpendicular to the Clipping Plane.

Button **Show Frame**

To make the frame visible, check this box.

#### Perpendicular Movement

The pointer must be in the pointer mode Select. Place the mouse within the image. To change the orientation of the rod press the Ctrl button. The Clipping Plane is moved in the directions parallel to the plane.

See also:

Surpass View - Overview - Camera Toolbar - [Pointer](#) (Mode Select)

## 11.2.2 Mouse & Keyboard PC

### Clipping Plane Mouse & Keyboard Functions

Left-click on plane & drag	Move plane to desired position
Left-click on the bigger diameter rod & drag	Move plane to desired position
Left-click on the smaller diameter rod & drag	Rotate plane to desired position

See also:

Addendum - [Mouse & Keyboard PC](#)

## 11.2.3 Mouse & Keyboard Mac

### Clipping Plane Mouse & Keyboard Functions

Click on plane & drag	Move plane to desired position
Click on the bigger diameter rod & drag	Move plane to desired position
Click on the smaller diameter rod & drag	Rotate plane to desired position

See also:

Addendum - [Mouse & Keyboard Mac](#)

## 11.3 External Object

Surpass provides the ability to import External objects (both, non-Imaris and Imaris objects) and display them concurrently with a Surpass object for comparison.

Only the External objects with the extensions \*.iv (Inventor file) or with extensions \*.vrmf (VRML2 files) or .wrl (WRL files) can be imported.

The imported External objects can be rotated, scaled and translated.

For example, previously calculated Surfaces, which have been exported as Inventor files, can be imported to another data set.

### How to Add a new External Object?

- Select menu 3D View - External Object.

The Surpass Tree list is automatically updated and in the External object - Properties the Settings parameter are displayed.

See also:

Menu 3D View - [Export Selected Objects ...](#)

### 11.3.1 Tab Settings

The object properties area displays a number of parameters for the positioning and display of the External object. The following display/handling options can be checked:

#### Box Handle

The object shows a surrounding box with handles. These handles allow you to transform/distort the object in the x-, y-, and z-direction.



### Trackball

The object shows surrounding circular lines. These lines allow rotation of the External object in any direction.

### File Path Field

Display of the file path (to load the object, see below).

### Button Load...

To select and load a data set as a External object click on the button **Load...** and the Load External Object File window is displayed. Select the file to be loaded as External object. The External object will be only loaded if the selected files have the extension **\*.iv** (Inventor file), **\*.vrmf** (VRML2 files) or **.wrl** (WRL files). Click on the button Open.

### Button Reset Size and Location

Clicking this button sets the External object back to its original size and position before any translational, scaling or rotational operations have been performed.

### Fix Ratio

If this option is checked and if scaling is performed on the External object by entering a value, the same scaling factor is applied to all three dimensions.

## Position/Scaling

The following positional/scaling options are available:

### Translation

Use these fields to move the External object in the x-, y-, or z-direction.

### Rotation

Use this field to rotate the External object around the x-, y-, or z-axis.

### Scaling

Use this field to scale the External object in one of the dimensions (or all together if Fix Ratio, see above, is checked). Fix Ratio is only valid with this method of scaling.

## 11.3.2 Tab Color

Color Tab edit, manage and assign material colors of an Object.

### RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

## Light reflection

The quality of the object material is further characterized by reflection properties.

- Diffusion- diffuses reflection of a matte, uneven material.
- Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting is for no specular intensity.
- Emission- light emitted from the material defines the self-illumination. Default setting is for no emission of

light.

## Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

## Palette

Palette- this option provides a system of preset colors organized into color schemes.

In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, Artdeco, Autumn, Glass, Metal, Neon, Rococo, Santafe, Sheen, Silky, Spring, Summer, Tropical, Winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

### 11.3.3 Mouse & Keyboard PC

#### External Object Mouse & Keyboard Functions

Left-click white box & drag	Scale object from center
Left-click white box + Ctrl & drag	Scale object from opposite corner
Left-click trackball & drag	Rotate object
Shift + left-click trackball & drag	Set up a rotation axis

See also:

Addendum - [Mouse & Keyboard PC](#)

### 11.3.4 Mouse & Keyboard Mac

#### External Object Mouse & Keyboard Functions

Click white box & drag	Scale object from center
Click white box + Ctrl & drag	Scale object from opposite corner
Click trackball & drag	Rotate object
Shift + click trackball & drag	Set up a rotation axis

See also:

Addendum - [Mouse & Keyboard Mac](#)

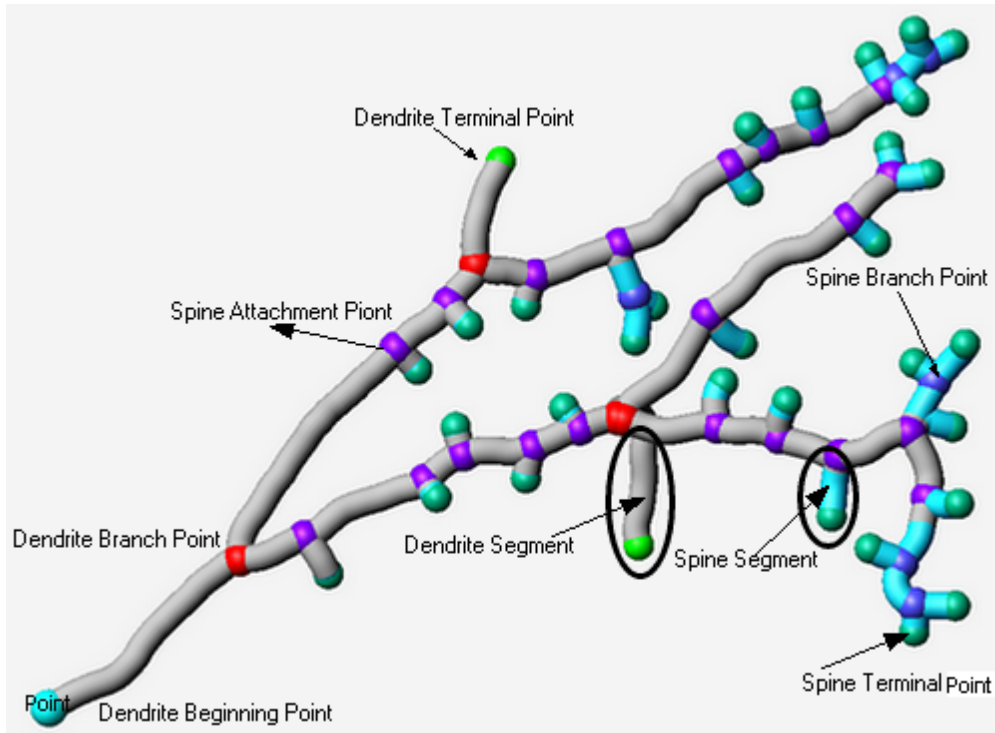
## 11.4 Filament

In Imaris dendritic trees are represented by a set of **vertices** and **edges**. The **Vertex** is drawn as a node or a dot and defines the center axis of the dendrites. An **edge** is presented as a line connecting two vertices. The entity of vertices and edges is referred to as filament graph.

Parts of the filament graph can be classified as spines either collectively, based on some statistical values, or individually by the user, by determined specifications.

**Starting, ending and branching vertices** are classified into **dendrite beginning point, dendrite branch point, dendrite terminal point, spine attachment point, spine branch point, and spine terminal point** depending on their position in the graph (refer to the following image).

Segments are defined as the collection of edges between two starting, ending, or branching vertices. Segments are classified either as **dendrite segments** or **spine segments**. In a dendrite the edge is called a **dendrite edge**, in a spine it is called a **spine edge**.



The Imaris FilamentTracer offers features to segment, track, edit, draw, display, measure and annotate Filaments. The FilamentTracer processes one channel (color) at a time and creates objects that qualify as Filaments (e.g length and average thickness comply with criteria).

After automatic Filament creation (see [Creation Wizard](#)) you can launch an editor operation to delete Filaments, connect Filaments, or draw new Filaments. You may also skip the automatic Filament generation and process the data with semi-automatic or manual segmentation and Filament generation (see the [Tab Draw](#)).

Generated Filament objects will be visualized in the context of Surpass view. This will allow multiple viewing combinations in which the Filaments are displayed individually or together with other cellular components that may have been imaged in other channels.

Properties and measures of entire filament graph or the selected parts (Points, Edges, Dendrite, and Spines) are automatically generated in the [Tab Statistics](#) and it can be exported in tabular format or directly to MS Excel.

### How to Add a new Filament?

- Select menu 3D View - Filament.

The Surpass Tree list is automatically updated and you start in the first window of the [Creation Wizard](#) (see below).

See also:

Surpass View - Overview - [Surpass Tree](#)

3D View - Filament - [Creation Wizard](#) (for automatic Filament creation)

3D View - Filament - [Tab Draw](#) (for interactive Filament tracing method)

For information on statistics calculations used refer to [Filament](#)

## 11.4.1 Data Input

The FilamentTracer is designed to operate on 3D time-resolved images. These images can be multi-channel (multiple colors) but only one channel will be traced at a time. The following input images are acceptable:

- The ideal input is a fluorescently labeled confocal image. Such images generally have good contrast (due to selective labeling) and good resolution in all three spatial dimensions.
- 3D images can also be acquired using a standard microscope (non-confocal) equipped with a CCD camera and a stepper motor. Non-confocal optical arrangements do not provide resolution along the optical axis which is why every single plane consists of in-focus components and out-of-focus components. These images require deconvolution prior to tracing. The technique of deconvolution uses information about the imaging process (the point spread function) to remove the out-of-focus components from every section.
- Finally there are 3D images taken in transmission light microscopy. Staining is usually less specific than for fluorescence techniques and it will therefore be more difficult to automatically segment the Filament. Deconvolution can also be applied to transmission images although - theoretically - its performance is weaker than for fluorescence methods.

## 11.4.2 Creation Wizard

The automatic filament graph compilation (creation) is guided by a wizard-like user interface and consists essentially of five steps.

- Select the appropriate algorithm (or quit the automatic filament creation and draw the filaments interactively).
- Define a Region of Interest
- Select source channel and do some pre-processing.
- Segmentation to specify filamentous and non filamentous parts.
- Graph compilation and finishing.

The Creation Wizard allows you to move forward or backwards until the optimal parameters are found.

If the image data is for a [multi-image scene](#) an option to select which source image to be analyzed is available from the Align to: drop down box.

In the first window of the Filament Creation Wizard, you find the algorithm settings. Here, you have to decide, if you want to load a set of previously stored creation parameters or to create a new one.

The most convenient way to apply the same creation parameters to different data sets is to use the [Load Parameters](#) option.

See also:

3D View - Filament - Creation Wizard - [Algorithm](#)

3D View - Filament - Creation Wizard - [Preprocessing](#)

3D View - Filament - [Tab Draw](#) (for interactive filament tracing method)

### 11.4.2.1 Algorithm

In this first window of the Creation Wizard you decide, if you want to go for an fully automated filament creation using one of the two predefined algorithms (AutoPath-no loops or Threshold-loops, see below); or if you want to draw the filaments interactively.

Note: If you do not want to use the automatic filament creation wizard select the **Skip automatic creation, edit manually** button.

#### **Skip automatic creation, edit manually** Button

Terminates the creation wizard and activates the interactive filament tracing method.

#### **Favourite Creation Parameters**

This option lets you use the identical creation parameters for multiple data sets.

The Favourite Creation Parameters option is only available if the set of the creation parameters has already been saved under the [Creation tab](#) using the Remember Parameters button.

Then you can choose to segment a **Region of Interest (ROI)** only, to create **Tracks** after the objects detection or to **Skip automatic creation**.

When the wizard is started, all stored creation parameters, corresponding to the chosen object wizard, are automatically loaded. The stored parameters contain all of the settings, options, and parameters required for image segmentation.

Expand the Favourite Creation Parameters drop-down list by clicking on it. The list shows all the creation parameters sets that have been previously saved, appropriate to the chosen creation wizard. Simply load your chosen creation parameters set, by clicking on it, and run the wizard as you have preset, without defining all of the options. On the other hand, you can customize each procedure to your own specific needs by modifying each part separately - e.g. the same channel settings, with a slightly different threshold.

#### **Select Algorithm**

The automatic filament creation provides two algorithms to choose from:

##### **AutoPath (no loops)**

##### **Threshold (loops)**

##### **AutoPath (no loops)**

##### **The automated AutoPath Algorithm:**

- Produces a tree-like filament
- Is based on local intensity contrast
- Connects large start- and small end-points

If you select this algorithm please refer to Section: 3D View - Filament - Creation Wizard - [Autopath Algorithm \(no loops\)](#)

##### **Threshold (loops)**

##### **The Threshold based Algorithm:**

- Produces a filament with loops
- Is based on an absolute intensity threshold
- Thinning to a skeleton

If you select this algorithm please refer to Section 3D View - Filament - Creation Wizard - [Threshold Algorithm \(loops\)](#)

## Calculate Diameter of Filament from Image

Check this box and the Filament diameter is calculated based on the image data.  
Uncheck the box and the Filament diameter will taper smoothly from the starting point to the end points.

## Segment a Region of Interest-ROI

A Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.

## Track (over time)

Check this box if you want to track Filament over time, for details please refer to the tab Tracking.

## Filament Wizard Buttons

### Button **Next**

Press **Next** to proceed with the next of the Creation Wizard.

### Button **Finish**

Press **Finish** and based on the selected algorithm (see above) Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the Filaments are displayed in the viewing area.

### Button **Cancel**

Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Please refer to Section 3D View - Filament - [Tab Draw](#) to adjust the parameters.

### 11.4.2.2 Autopath Algorithm (no loops)

The Autopath (no loops) algorithm consists of the following steps:

[Region of Interest](#)

[Dendrite Points Diameter](#)

[Classify Dendrite Points](#)

[Dendrite Diameter](#)

[Spine Points Diameter](#)

[Classify Spine Points](#)

[Spine Diameter](#)

[Tracking](#)

[Finish](#)

### 11.4.2.3 ROI

Here you define the region of interest (ROI). Parameters such as width, height and location of the ROI are used to directly specify the area to work on.

**X, Y, Z, Time**

**Min, Max, Size**





**Minimum, maximum** and **Size** of ROI coordinate values, can be specified either by entering the value in the value fields or changing the values by clicking on up-and down arrows at the right hand side of the value fields. Alternatively the location and size of the ROI can be adjusted directly in the viewing area.

Size of the ROI:

1. Click on the ROI preview rectangle arrows (change to the pointer mode Select)
2. Drag the arrows to define new coordinates for X, Y and Z.

Location of the ROI:

1. Click inside the ROI preview rectangle
2. Drag the entire rectangle (with mouse button held) to the chosen image location.

The numbers in the value fields are updated automatically.

### Memory Usage:

Display of the memory usage for the image size within the ROI.

## Regions of Interest

To select a particular ROI, left-click on its name in the list and the selected ROI will be highlighted within an image and its properties can be adjusted.

### Button **Add**

Click on this button and a new region of interest appears in the list. To adjust this ROI you have to select the ROI with a left mouse click.

### Button **Delete**

Select a ROI in the list with a left mouse click and click on this button to delete the ROI.

### Total Memory Usage:

Display of the memory usage for the image size within the ROI.

## Filament Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

### Button **Cancel**

If you do not want to create Filament automatically click on **Cancel**.

## 11.4.2.4 Dendrite Points Diameter

### Select Source Channel

Select the respective source channel, from which the Dendrite structure is to be created.

### Starting Points

**Detect a new starting point** -a new starting point of the filament will be determined, based on the value given

for the largest diameter.

### **(largest Diameter: e.g. Dendrite Beginning)**

Enter a value of the maximum (largest) diameter of dendrite to specify the Starting point diameter.

### **Seed Points**

### **(Thinnest Diameter e.g. Dendrite Ending)**

Enter a value of the minimum (smallest) diameter of dendrite to specify the Seed points diameter.

### **Rebuild Options**

As part of the rebuilding process in step three of the Creation wizard – Dendrite Points Diameter, a new option exists for selecting a **Starting Point**.

### **Use Existing Dendrite**

**Use Existing Dendrite**- the previously determined dendrite points will be used in the creation of new starting point. Therefore, in the next creation step (Starting point threshold) an option for Manual Start point editing in (shift+right click) will not be available.

### **Filament Wizard Buttons**

#### **Button Back**

With **Back** you return to the previous window of the Creation Wizard.

#### **Button Next**

Press **Next** to proceed with the next window of the Creation Wizard.

#### **Button Finish**

Press **Finish** and Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the Filaments are displayed in the viewing area.

#### **Button Cancel**

Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Switch to the tab Draw and please refer to Section 3D View - Filament - [Tab Draw](#) to adjust the parameters.

By re-entering the Creation Wizard, either after selecting [Rebuilding Algorithm](#) or for [Processing additional ROI\(s\)](#) a new option exists for selecting a Starting Point.

See also:

3D View - Filament - [Tab Settings](#)

3D View - Filament - [Tab Draw](#) (for interactive filament tracing method)

### **11.4.2.5 Classify Dendrite Points**





Thresholds are used to classify the detected dendrite's starting points and seed points.

The detected dendrite starting point(s) are visualized in the image by different colors. The respective histograms are displayed.

The threshold can be set manually or automatically.






## Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower and Upper Threshold

In Imaris you can set two threshold values, **Lower** and **Upper**.

The two threshold values separate the data into three regions: above the higher threshold, below the lower threshold, and the region in between the two values.

Object with values below the Lower Threshold and objects with values exceeding the Higher Threshold will be excluded and only those objects with quality inside the interval defined by the two threshold values are considered.

Please note if the value of Lower threshold is set to be higher than the value of Higher threshold the selected objects will be with outside the interval defined by the two threshold values.

To change the upper and/or lower threshold value(s) you can drag the line(s).

Alternatively, left click in the histogram to set the lower value of the threshold range and right click to set the upper value of the threshold range.

## Starting Points Threshold

Display of the starting points threshold and the respective histogram. The threshold can be adjusted by entering a new value in the data field or by moving the colored threshold line (click & drag) in the histogram. The updated points are visualized in the viewing area.

## Manual Starting Points addition/deletion in Creation Wizard

Manual addition of starting points in the filament structure is available by pressing Shift+ right-click. Shift+ right-click on an existing filament start point (created either automatically or manually added) and the selected starting point will be deleted.

## Seed Points Threshold

Display of the end points threshold and the respective histogram. The threshold can be adjusted by entering a new value in the data field or by moving the colored threshold line (click & drag) in the histogram. The updated points will be visualized in the viewing area.

## Manual Seed Points Addition/Deletion in Creation Wizard

Manual Addition of Seed points in the filament structure is available by Shift+ left-click. Shift+ left-click on an existing point, (created either automatically or manually added) and the selected point will be deleted.

## Remove Seed Points Around Starting Point(s)

Check the box and all seed points located within the radius of the sphere centred at the starting point are removed. The boundary of the sphere is defined by the value Diameter of Sphere Region(s).

### Diameter of Sphere Region(s)

Enter the diameter of the sphere centred at the beginning point. Default value set to twofold of the starting points diameter.

## Remove Disconnected Segments

These parameters allow the user to adjust and improve the accuracy of the interactive filament tracing. This feature will help eliminate misdirected filament branching due to labeling that is not continuous, contains low signal regions, and for images that contain high levels of noise.

By selecting the Remove Disconnected Segments, it enables the algorithm to continue filament tracing for a distance defined in the **Max Gap Length** field.

This parameter determines which branches are identified as disconnected based on gap length and excludes them from the filament.

When this option is enabled, you first set parameters to generate threshold mask using smoothing and/or background subtraction. These smoothing and/or background subtraction parameters are identical to those used in Surface rendering.

A connection between disconnected branches will be created only when the maximum spacing between two disconnected masks does not exceed the defined length in the **Max Gap Length** field.

### Smooth With

Set **Smooth With** value as you would define the parameter for a surfaces object. This define the Guassian filter width used when background subtraction is enabled.

The value is either automatically calculated from the voxel sizes or it can be directly entered into the numerical field.

### Background Subtraction

Background Subtraction applies a Gaussian filter to estimate the background intensity value of each voxel. This background value is then subtracted from every voxel in the image; Baseline Subtraction is performed.

### Max Gap Length

The distance in which the objects connection is automatically established is based on the value defined in the Max Gap Length value.

The Max Gap Length defines the maximum distance, which is allowed to be missing in order to create a

connection between two disconnected segments. If the distance is longer than the defined gap distance, then the segments will not be considered part of the filament.

### Filament Wizard Buttons

#### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

#### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

#### Button **Cancel**

If you do not want to create a Filament automatically click on **Cancel**.





### 11.4.2.6 Dendrite Diameter

The option is only available if in the step [Algorithm](#) the Calculate Diameter form the image is selected.

The threshold value is based on the local contrast and the respective histogram is displayed.

The defined threshold value is used for the dendrite diameter calculation.




### Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

The effect of changing the threshold value is directly visible in the viewing area.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.**

## Algorithm

The Algorithm provides two methods to calculate dendrite diameter.

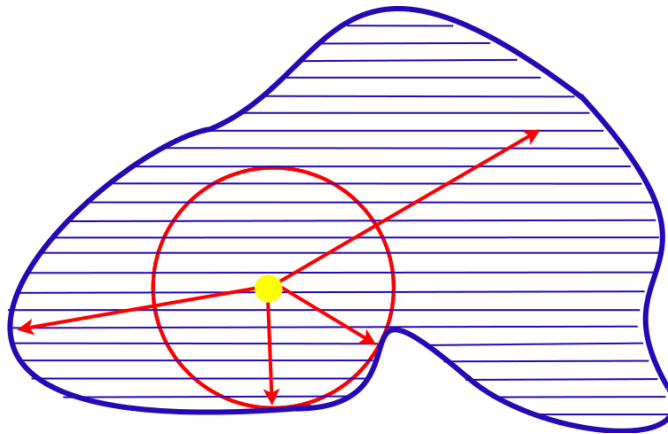
The method of diameter calculation has no effect on the position of the filament centre axis, located at the local intensity peak (yellow disk)

For cases where the centre axis is closer to the centre of diameter mask (blue hatched area), the difference between the options is smaller.

### Shortest Distance from distance map

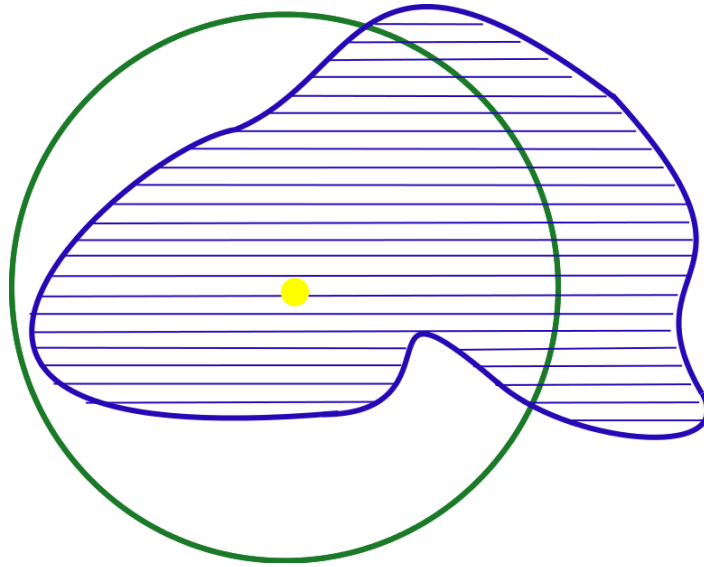
The Shortest Distance from Distance Map option considers the radius as the shortest distance from the seed point (yellow disk) to the edge of the filament mask (blue line), in any (x,y,z) direction

This method is typically less sensitive to diameter overestimation due to axial (z) blur. It is recommended when the centre axis is overall well centred on the structure and the real structure has a roughly circular cross-section.



### Approximate Circle of cross section area

The Approximate Circle of cross section Area option is recommended when the centre axis position is not well centred in a large diameter structure e.g large irregular spine heads. The area of the green circle is equal to the cross-section area determined in the threshold step.



### Filament Wizard Buttons

#### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

#### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

#### Button **Cancel**

If you do not want to create a Filament automatically click on **Cancel**.

### 11.4.2.7 Spine Points Diameter

#### Spine

##### Detect Spines

Check this box to Optimize the Creation wizard for Spine Detection; creating the highly branched filament structure.

Uncheck the box Detect Spines and the Spine channel selection and the Seed Points diameter are disabled. Spines detection and creation are not performed in the following creation procedure.

#### Select Source Channel

Select the respective source channel, from which the spines are to be computed. The default channel is the same as for the dendrite detection.

#### Seed Points

##### (Thinnest Diameter e.g. Small Spine Head)

Enter a value of the minimum (smallest) diameter of spine head to specify the Seed points diameter.

## Maximum Length

The Maximum Length is estimated spine length, the length from the dendrite center to the furthest Spine seed point.

### Allow Branch Spines

Check the **Allow Branch Spines** option to allow formation of the spines's bifurcation points. To form bifurcation point the two spine's vertices have to be in the vicinity of 1 voxel.

## Filament Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

### Button **Cancel**

If you do not want to create a Filament automatically click on **Cancel**.

## 11.4.2.8 Classify Spine Points

The option is only available if in the step [Spine Points Diameter](#) the Detect Spine option is selected.





## Spine

### Seed Points Threshold

Thresholds are used to classify the spines seed points. The detected Spine seed points are visualized in the image. The respective histogram is displayed.

The threshold can be set manually or automatically.




### Compact Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.



### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated  . Clicking on the



Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold. Clicking on the histogram automatically activates a Manual threshold adjustment.

The threshold can be adjusted by entering a new value in the data field or by moving the colored threshold line (click & drag) in the histogram. The updated points are visualized in the viewing area. The effect of changing the threshold value is directly visible in the viewing area.

**Tip:** You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.



## Filament Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.





### Button **Cancel**

If you do not want to create a Filament automatically click on **Cancel**.

## 11.4.2.9 Spine Diameter

The option is only available if in the step [Spine Points Diameter](#) the Detect Spine option is selected. The threshold value is based on the local contrast and the respective histogram is displayed. The defined threshold value is used for the Spine diameter calculation.




## Compact Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

## Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated  . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

The effect of changing the threshold value is directly visible in the viewing area.

## Algorithm

The Algorithm provides two methods to calculate Spine diameter.

### Shortest Distance From Distance Map

The Spine diameter is calculated as the shortest distance between spine center point and spine surface.

### Approximate Circle of Cross Section Area

The diameter of the approximate circle of cross-sectional area is determined as a value of the spine diameter.

## Filament Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

### Button **Cancel**

If you do not want to create a Filament automatically click on **Cancel**.

### 11.4.2.10 Tracking

The filament object consists of a set of the dendrites, the spine points and the connection between them. Tracking allows objects to be traced throughout sequential frames of time data set. Each object maintains a unique identity in the output measurements.

This tab only appears, if the Track (over Time) is checked ([Algorithm Settings](#)).

The Filament tracking tab provides Autoregressive Motion algorithms, which model the filament movement.

The algorithm utilizes the object's direction and speed information from one single time point to predict the best potential object position at the next time point.

Based on the tracking of the entire Filament graph, the algorithm predicts the future positions of the Dendrite Beginning Point, Branching and Terminal Points as well as the Spine Attachments, Spine Terminal Points

Based on these predicted positions, the best matches to the candidate points can be determined. The algorithms use a Linear Assignment algorithm and a Total Cost function to find the best track connections. The total cost computation is based on the change in distance.

### Autoregressive Motion

This algorithm models the motion of the filament graph's center of gravity as an autoregressive AR1 process. The AR1 model looks back to one time-point, and predicts that the object will move again the same distance and in the same direction. The user-specified **Maximum Distance** value is the distance in which the object is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the value of the maximum distance parameter, and because the AR1 algorithm performs correlations only to one previous time-point.

### Track Filaments Center of Gravity

#### Max Distance

The value of the Max Distance specifies the maximum distance between a predicted object location and its actual location for two consecutive time points. This parameter does not allow connections between a Filament graph and a candidate match when the distance between the predicted future position of the Filament graph and the candidate position exceeds the maximum distance.

It can happen that the object segmentation fails for some time points, which can break a Track apart into two Tracks. The gap-closing algorithm tries to continue the movement of a disappearing object and connects the Tracks if they reappear.

#### Close Gap

Select this option and the gap-closing algorithm will be implemented.

#### Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points which are allowed to be missing in order to a track to be continues.

### Track Dendrites

Select this option and the Dendrite points will be tracked.

When the Track Dendrite option is not selected the drop-down list remains inactive.

From the drop-down list you can select the type of Dendrite points to be tracked. There are two options:

- Dendrite Branch Points
- Dendrite Terminal Points

**Please note: The tracking of the Dendrite Beginning point is automatically computed based on the tracking of the entire Filament structure.**

#### Enable

Check the box to enable the selected Dendrite points to be traced

## Max Distance

The value of the Max Distance specifies the maximum distance between a predicted object location and its actual location for two consecutive time points. This parameter does not allow connections between a Filament graph and a candidate match when the distance between the predicted future position of the Filament graph and the candidate position exceeds the maximum distance.

It can happen that the object segmentation fails for some time points, which can break a Track apart into two Tracks. The gap-closing algorithm tries to continue the movement of a disappearing object and connects the Tracks if they reappear.

## Close Gap

Select this option and the gap-closing algorithm will be implemented.

## Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

**Please note: The spine tracking options are only available if the spines have been previously created.**

## Track Spines

Select this option and the Spine points will be tracked.

From the drop-down list select the type of Spine points to be tracked. There are three options:

- Spine Attachment Points
- Spine Branch Points
- Spine Terminal Points

## Enable track

Check the box to enable the selected Spine points to be traced.

## Close Gap

Select this option and the gap-closing algorithm will be implemented.

## Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates



tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

**Please note: The spine tracking options are only available if the spines have been previously created.**

### Filament Wizard Buttons

#### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

#### Button **Cancel**

If you do not want to create a Filament automatically click on **Cancel**.

### 11.4.2.11 Finish ROI Processing

The selected parameters will be used to complete the computation of a Filament structure in an image. By selecting one of six finishing options a Filament structure will be created in the following ways:

1. **Additional Regions** - re-enters creation process (in step two-[ROI](#)) and uses the same set of parameters for a new ROI to create the filament structure. This option is only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for all time points with the same set of parameters.
2. **Entire Image in All Time Points**- the entire image is processed with the same parameters used for the ROI processing and the filament structure is created for the entire image and all time points. Only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for each time point with the same set of parameters.
3. **All Done**- creates a filament structure only within an ROI.

Please refer to [Tab Rebuild](#). In step three of the Creation wizard – [Point Diameter](#) an option exists for selecting a Starting Point (Use existing filament or Detect a new starting point).

**Note: The Use Existing Filament option requires that the new ROI overlaps with some portion of the existing filament.**

### Filament Wizard Buttons

#### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

#### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

Button **Cancel**

If you do not want to create a Filament automatically click on **Cancel**.

## 11.4.2.12 Threshold Algorithm (loops)

The threshold (loops) algorithm consists of the following steps:

[Preprocessing](#)

[Segmentation](#)

[Graph Compilation](#)

[Finish](#)

## 11.4.2.13 Preprocessing

### Source Channel

Select the respective source channel, from which the Filament structure is to be computed.

### Feature Preprocessing

#### Enable Feature Preprocessing

Check the box and the following parameters are adjustable. Uncheck the box and the following parameters are grayed. Preprocessing is not necessary if the data is already deconvolved or if the noise has already been removed by a Gaussian filter. The preprocessing will be applied to the source channel and the result will be added as new Filament Working channel in the Display Adjustments.

#### Appr. Filament Diameter

An approximate minimal Filament diameter has to be provided.

#### Preserve Edges

You can also enable an edge preserving preprocessing to maintain filamentous structures when the Threshold (loops) algorithm is selected. It is not available if the Autopath (no loops algorithm is selected).

Before continuing you can inspect the result of the previous step with any viewer of Imaris: Slice, Section, Gallery, Easy3D, Surfaces etc.

### Filament Wizard Buttons

Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

Button **Finish**

Press **Finish** and Imaris runs through all parameters (coming on the following windows of the Creation Wizard) using the default settings. At the end of the calculation the Filaments are displayed in the viewing area.

Button **Cancel**

Click on this button if you do not want to use the automatic graph compilation. Use instead the interactive filament tracing method. The parameters on the tab Settings are displayed in the properties area. Switch to the tab Draw and please refer to Section 3D View - Filament - [Tab Draw](#) to adjust the parameters.



See also:

Menu Edit - [Show Display Adjustment](#)

Menu Image Processing - Thresholding - [Connective Baseline](#)

3D View - Filament - [Tab Draw](#) (for interactive filament tracing method).

#### 11.4.2.14 Segmentation

##### Segmentation

##### Fill Cavities (fully enclosed volumes)

Check this box to avoid hollow regions. These regions would be enclosed by a Filament mesh, which is not desired in most cases.

**Note:** Fill Cavities option is not available in Imaris version 7.7 onwards.

##### Connective Baseline

Type in two values for the lower threshold (colored line) and upper threshold (red line). You can also click into the histogram. Left mouse button for lower, right mouse button for upper threshold or you can click & drag the respective line.

Simple thresholding methods classify voxels purely based on a single intensity value. This approach often does not lead to good results because the staining, the illumination, and the detection operate within a precise range. The connective thresholding scheme takes care of this and searches for object voxels using an intensity range rather than a single intensity.

You must provide two threshold values. The lower threshold (colored line) is the intensity below which a voxel is recognized as part of the background, the upper threshold (red line) is the minimal intensity for a voxel to be part of the Filament object. Voxels with an intensity value between the two thresholds are treated as part of the Filament object if and only if they are connected to a voxel within intensity above the upper threshold ("double thresholding" with "feature-AND").

The result of the segmentation process described above is a clean image where the non-zero voxels are describing the objects (Filaments and some non-filamentous structures). Because such a description of the data is not suitable for morphometric analysis the structures are converted. Graph compilation is a process involving several image processing operations such as skeletonization, erosion, dilation and the creation of a vector definition for the Filaments. The result of the process is a database (kept in memory) describing the Filaments as a set of segments that may contain branches.

##### Filament Wizard Buttons

##### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

##### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

##### Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

##### Button **Cancel**

If you do not want to create a Filament click on **Cancel**.

See also:

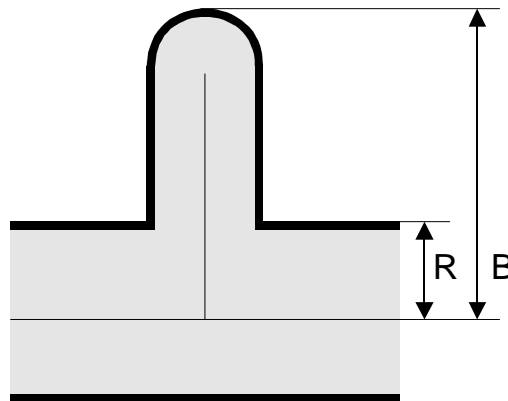
Menu Image Processing - Thresholding - [Connective Baseline](#)

## 11.4.2.15 Graph Compilation

### Graph compilation

#### Minimal Ratio of Branch Length to Trunk Radius

Enter a minimal ratio of branch length to trunk radius (see figure below). Branches shorter than this value will be removed.



*Ratio of branch length ( $B$ ) to trunk radius ( $R$ ).*

#### Find Dendrite Beginning Point

A dendrite beginning point has to be specified for specific statistic values (e.g. tree order). The point is selected from the trunk position with the biggest radius.

### Filament Wizard Buttons

#### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

#### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to calculate the desired Filament and display the Filament in the viewing area.

#### Button **Cancel**

If you do not want to create a Filament click on **Cancel**.

## 11.4.2.16 Finish

### Finish

To validate the automatically generated graph and the prior segmentation, you can inspect the result together with numerous Surpass modules: Ortho Slicer, Volume, Surfaces etc. You can optionally superimpose the segments with the original slices of the image stack using Imaris OrthoSlicers. OrthoSlicers are planes, which are perpendicular to any of the three spatial axes and extend throughout the entire image volume.



### Build Filaments for all Time Points

Only available if you work with a time data set. When the data set contains multiple time points, the Filament Creation Wizard can create Filament objects for each time point with the same set of parameters. They will be visible within the Surpass Tree. However the 3D Filament graph is only visible according to its time point.

### Delete Working Channel

The Filament Working channel can be kept for another use. Principally it is not needed any more when finishing the Filament graph.

## Filament Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

This button is grayed in the last window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to end the calculation and display the results in the viewing area.

### Button **Cancel**

If you do not want to create Filaments click on **Cancel**.

## 11.4.3 Filament Properties

When the creation process is finished, the [Tab Rebuild](#) disappears while some other property Tabs appear. They allow the manipulation of the filament graph:

### Tab **Rebuild**

Only appears when you Add a new Filament.

### Tab **Settings**

Change Filament appearance and inspect selection statistics.

### Tab **Rebuild**

Re-enter the creation process.

### Tab **Draw**

Manually draw filaments and connections.

### Tab **Edit**

Select automatically filament parts and delete or smooth them.

### Tab **Statistics**

Inspect all statistic values.

### Tab **Color**

Change the color appearance (material) of the filament.

See also:

3D View - [Filament](#) (Add a new Filament)

3D View - Filament - [Tab Settings](#)

3D View - Filament - [Tab Rebuild](#)

3D View - Filament - [Tab Draw](#)

3D View - Filament - [Tab Edit](#)

3D View - Filament - [Tab Statistics](#)

3D View - Filament - [Tab Color](#)

## 11.4.4 Tab Settings

On the tab Settings you can edit and adjust the display options.

Select the [Filament](#) option to adjust quality and style of the filament graph.

Select the [Filament\\_Tracks](#) option and you can edit and adjust settings that determine the display of connections between the Track filament graphs.

Select the [Point Tracks](#) option and you can edit and adjust settings that determine the display of connections between the **dendrite beginning, branch and terminal points, as well as spine attachment, branch and terminal points** of the filament graphs.

### 11.4.4.1 Filament

#### Style

##### Line

##### Cylinder

##### Cone

The visualization of the graph is done as lines with a specified thickness (pixel), as cylinders (with a defined diameter), or cones (using the measured graph diameter).

#### Show Dendrites

Check the box in the heading to highlight dendrite points in the filament. The key points are highlighted with color-coded spheres.

##### Beginning Point

Check this box to highlight the dendrite beginning points.

##### Branch Points

Check this box to highlight the dendrite branch points.

##### Terminal Points

Check this box to highlight the dendrite terminal points.

#### Show Spines

Check the box in the heading to highlight spine points in the filament. The key points are highlighted with color-coded spheres.

##### Attachment Points

Check this box to highlight the spine attachment points.

##### Branch Points

Check this box to highlight the spine branch points.



**Terminal Points**

Check this box to highlight the spine terminal points.

**Render Quality**

With the slider you can adjust the render quality of the filament. Moving the slider to the left reduces the render quality, moving it to the right increases render quality.

**11.4.4.2 Filament Track****Tracks Style/Quality**

The box Style/Quality has settings that determine the display of connections between the Track objects.

**Cylinder**

This option displays the tracks connections drawn as cylinders.

**Line**

This option displays the tracks connections drawn as lines.

**Off**

Select this option to disable the visualization of the Tracks.

**Width****Diameter**

Depending on the previous selection you adjust the width of the line or diameter of the cylinder.

**Render Quality**

When you select Cylinder (see above) the Render Quality is adjustable using the slider.

**Filament Tracks****Displacement**

Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

**Radius Scale**

Edit field for the displacement vector radius.

**Color Editor**

Select a desired color for the displacement arrow.

**Dragon Tail**

Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points.

**Slider**

Adjust the number of time points using the slider.

## 11.4.4.3 Point Tracks

### Tracks Style/Quality

The box Style/Quality has settings that determine the display of connections between the Track objects.

#### Cylinder

This option displays the tracks connections drawn as cylinders.

#### Line

This option displays the tracks connections drawn as lines.

#### Off

Select this option to disable the visualization of the Tracks.

#### Width

##### Diameter

Depending on the previous selection you adjust the width of the line or diameter of the cylinder.

#### Render Quality

When you select Cylinder (see above) the Render Quality is adjustable using the slider.

### Filament Tracks

#### Displacement

Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

#### Radius Scale

Edit field for the displacement vector radius.

#### Color Editor

Select a desired color for the displacement arrow.

#### Dragon Tail

Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points.

#### Slider

Adjust the number of time points using the slider.

## 11.4.5 Tab Rebuild

### Rebuild

Imaris stores the list of operations and the mapping of parameters.

Click on drop-down window and six choices are available for re-entering the Filament Creation Wizard.

[Rebuild All](#)

[Rebuild Dendrite](#)

[Rebuild Dendrite Diameter](#)

[Rebuild Spine](#)

[Rebuild Spine Diameter](#)

## Rebuild Tracks

Select the rebuild algorithm to be applied from the list. As you select the rebuild algorithm, the creation wizard opens at the appropriate step.

The wizard retains all the prior parameters allowing you either to reuse or edit them while re-creating the filament structure.

If you choose the rebuild option, the window Rebuild Diameter opens which performs the diameter calculation for either the Rebuild Dendrite Diameter or the Rebuild Spine Diameter.

### Rebuild Diameter

Select the Specific Source channel, from which the Dendrite/Spine structure is to be computed. To specify the channel, enter the channel number in the field.

**The Creation Wizard is using previous parameter settings as initial values. You can easily change just a few parameters without going in detail through all the parameters.**

## Spine Rebuilding

The spines can be created at any later time by re-entering the creation wizard, since the spine detection and creation is the default last step of the Filament Creation Wizard.

**Note that spine rebuilding options are available if spines were not previously created.**

## Keep Data

The option **Keep Data** should be selected depending on whether the existing Filament graph should be **discarded**, or **re-used** in the Filament rebuild process.

If this option is selected, the previously created filament structure is kept. The Wizard creates a newly added filament that is merged with the existing one.

If this option is not selected, the previously created filament structure is discarded and the Wizard creates a new filament structure.

## All Time Points

The option **All Time Points** should be selected depending on whether the parameters used for creating the Filament structure should be applied to the selected time point only or to all time points.

If this option is selected, the same set of creation parameters used for selected time point is reused to the create filament structure for all time points.

If the option is not selected, the creation parameters are used only for the selected time point.

**All Time Point is an option available only with time resolved data sets.**

In the rebuild process the following structure of the filament creation is applied:

1. According to the setting of the **Keep Data** option the existing Filament structure is discarded or re-used
2. The selected Rebuild algorithm is completed
3. In the case of the time resolved data sets, according to the setting of **All Time Points** options, the parameters are either used to create the filament structure for the selected time point only or to create the filament structure for all time points.

## Button **Rebuild Objects**

Click on this button to start the rebuild.

## Creation Parameters

The Creation Wizard saves parameters and processing instructions so the Filament computation can be repeated for the same region, a different region, or the entire image. The Creation Parameters values are listed as a table.

In step three of the Creation wizard – [Dendrite Points Diameter](#), a new option exists for selecting a **Starting Point**.

### Use existing filament

#### Detect a new starting point

**Use existing filament**- the previously determined starting point will be used in the new filament structure. Therefore, in the next creation step (Starting point threshold) an option for Manual Start point editing in (shift +right click) will not be available.

**Detect a new starting point** -a new starting point of the filament will be determined, based on the value given for the largest diameter.

## Store Parameters for the Batch

The Store Parameters for the Batch button allows you to save the complete set of the creation parameters and all relevant settings shown in the list. In the Store Creation Parameter window, enter a descriptive name for your creation parameters and select the required store location.

There are two options to store your creation parameters

### Arena

#### Favorite Creation Parameters

### Arena

When you select this option a new Arena item is automatically created within the Arena view. The Arena view is updated, a new item, [Creation Parameters](#), is created and displayed.

Once stored, Imaris [Batch](#) applies the creation parameters set to all images within the selected Group.

### Favorite Creation Parameters

To help you organize and find your most used creation parameters more easily, we advise you to store them by selecting the Favorite Creation Parameters option.

When you select this option the Creation Parameter file is automatically added to the list that is displayed under the Edit/Preference/[Creation Parameters](#). This option allows you to use the creation parameters in future on another data set. These saved parameters can then be imported and re-used on any new data set should you wish to perform an identical analysis sequence. This option is also useful for sharing the parameters with other users to allow them to perform the same analysis on their own data sets.

Within the Surpass view storing allows the parameters to be reused or modified. In addition, this option is useful to create and share the creation parameters with other people and allow them to perform their own analysis on it.

Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings

and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the creation wizard.

When you save the Creation Parameter file, it is automatically added to the list which is displayed under the [Edit/Preference/Creation Parameters](#).

Click on the Store Parameters for the Batch button to open the Store Creation Parameter window.

### Store Creation Parameter

Refer to [Store Creation Parameters](#)

#### 11.4.6 Tab Draw

### Method

When it is not possible to use Imaris automatic filament creation algorithms, either semi-automatic or manual creation will allow you to create filament objects by manually marking the object location in using method AutoPath, AutoDepth or Manual.

### AutoPath

The AutoPath function automatically computes all the paths from a user-defined starting point to the end of the structure. If you select AutoPath the button **Set Starting Point** is available. If you select this method please refer to Section [AutoPath](#) (3D View - Filament - Draw - AutoPath).

### AutoNetwork

The Auto Network is an additional option that is disabled by default, but it can be enabled in Advanced Preferences by setting the Preference SurfaceAppFilament/EnableAutoNetwork to true. The AutoNetwork function automatically detects and creates all connections according to a user-defined diameter value. The results of the Auto Network function are visualized as lines. From the created network, the user can decide which part of the structure will be connected.

To set the starting point press **Shift + right-click** on the preferred location of the structure.

Place the mouse at the ends of the structure and the path is displayed.

To draw the displayed path press Shift + left click.

Please note: To create "way-points" press Shift+left-click at the location of the waypoint, and simultaneously press Shift-right-click (at the same location) in order to move forward toward the real end-point.

### Network

#### Select Source Channel

Select the respective source channel, from which the Network structure is to be created.

#### Diameter

Enter a value of the minimum (smallest) diameter to be detected.

#### Create Network Button

Click on this button to start the Network structure creation.

#### Show Network

Select this box to visualize the created network connections.

## AutoDepth

The AutoDepth function adds the automatic computation of the depth while doing manual segmentation (e.g. if the manual segmentation is done while looking at xy-plane, z-coordinate will be automatically computed). If you select this method please refer to Section [AutoDepth](#) (3D View - Filament - Draw - AutoDepth).

## Manual

The manual segmentation feature is used to edit automatically traced Filaments or to build the Filaments manually from the image template. If you select this method please refer to Section [Manual](#) (3D View - Filament - Draw - Manual).

## Setting

### Diameter

Set the Diameter for the cursor. The size of the cursor determines the resolution at which the tracing is calculated (larger cursor provides lower resolution but is a faster calculation and less precision- but often these low resolution paths are effective).

### Type

Select between Dendrite and spine options

## Select Source Channel

Select the source channel you will use to draw the filament.

### Button **Set Selection as Starting Point(s)**

If you select as Method **AutoPath** (see above, selection of the drawing Method) the button **Set Selection as Starting Point(s)** is available.

Select a point and click on this button to define the starting point of the filament.

## Correction

### AutoCenter

This option runs an automatic centering when the path is added (after pressing shift+left-click).

### AutoDiameter

Select this option to run an automatic diameter calculation when the path is added (after pressing shift+left-click). The calculation takes the mouse cursor size on shift+left-click as an input parameter (initial guess of diameter). The mouse cursor size should roughly correspond to the diameter of the filament for the calculation to succeed.

## View

Select between the two viewing options:

### Torch View

Provides a highlighted area to help allow you to focus on an area of interest. The size of the area illuminated by the torch can be adjusted using the mouse wheel.



## Full View

Provides a normal view through the full image area.

Both **Torch** and **Full View** options are described and compared in the [AutoPath](#) and [AutoDepth](#) sections along with the **Depth Visibility** controls.

## Slice

If you select the Method as **Manual**, see above (and also the section [Manual](#)) for further information the Slice parameter settings are available.

### YZ Plane

### XZ Plane

### XY Plane

Select the plane orientation of the drawing board: yz, xz, xy.

## Automatic Placement

When this parameter is activated, the position is set automatically to the location of the highest data intensity. In order to avoid unwanted horizontal or vertical drawing offsets, the camera is set to a perpendicular position with an orthogonal projection. In this case the camera can only zoom or pan but not rotate.

### x ... um

Display of the Slice the position.

## Slider

The position of the drawing board can be altered in perpendicular direction by the Slice slider. The same is achieved using the Arrow-keys of the keyboard (Arrow up, Arrow down).

**All actions in the Draw , Edit  and Edit Tracks  tabs can be Undone/Redone by selecting the [Undo/Redo](#) command or by pressing Ctrl+Z/Ctrl+Y.**

See also:

Menu Edit - [Show Display Adjustment](#)

3D View - Overview - Camera Toolbar - [Pointer](#)

3D View - Filament - Draw - [AutoPath](#)

3D View - Filament - Draw - [AutoDepth](#)

3D View - Filament - Draw - [Manual](#)

### 11.4.6.1 AutoPath

The AutoPath function automatically computes all the paths from a user-defined starting point to the end of the structure. If you select AutoPath the button **Set Starting Point** is available.

- Use the Channel drop-down options to specify the channel on which the calculation runs.
- Placing a new start point cancels the previous calculation and starts again from the new start point.
- When a start point is placed close to an end point the path will automatically connect.
- The most recently drawn filament is automatically highlighted.
- Press Shift+T to switch between Torch View and Full View modes.
- Pressing Ctrl+8 is useful immediately after adding a path to turn on InMotion for validation of the tracing.
- Autopath mode respects clipping planes: It chooses the start and end points only within the visible part of the volume. (Note that the clipping plane frames and handles may be turned off so they do not selected by mistake when making other adjustments to the image).

## Setting

### Diameter

Set the Diameter for the cursor. The size of the cursor determines the resolution at which the tracing is calculated (larger cursor provides lower resolution but is a faster calculation and less precision- but often these low resolution paths are effective).

### Type

Select between Dendrite and spine options

## Correction

### AutoCenter

This option runs an automatic centering when the path is added (after pressing shift+left-click).

### AutoDiameter

Select this option to run an automatic diameter calculation when the path is added (after pressing shift+left-click). The calculation takes the mouse cursor size on shift+left-click as an input parameter (initial guess of diameter). The mouse cursor size should roughly correspond to the diameter of the filament for the calculation to succeed.

## View

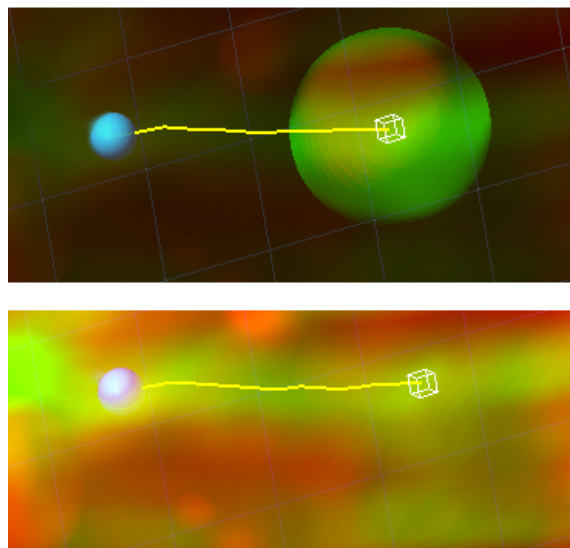
Select between the two viewing options:

### Torch View

Provides a highlighted area to help allow you to focus on an area of interest (top image). The size of the area illuminated by the torch can be adjusted using the mouse wheel.

### Full View

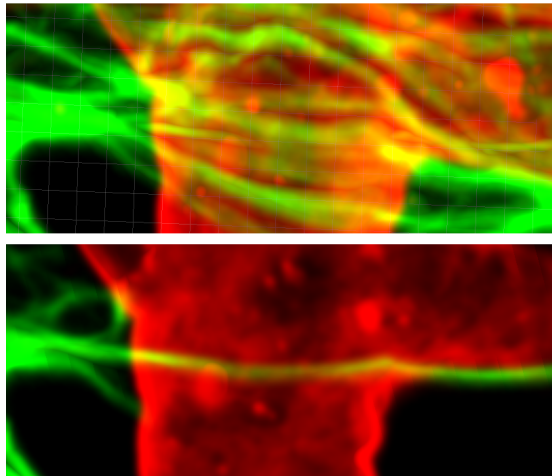
Provides a normal view through the full image area (bottom image).



### Depth Visibility

When working through an image with depth, it may have features that can obscure an area or structures of interest. You may enable Depth Visibility and then use the sliders to adjust the area of visibility between Near and Far and between a Narrow and Wide field to help obtain a suitable view as shown in the following image.

Unchecking the Depth Visibility will return the image to the normal view.



### Drawing in AutoPath Mode on a PC:

1. Select AutoPath and Shift + right-click on the desired starting point of your structure (e.g. in a neuron picture it would typically be the nucleus). The automatic computation of all the possible paths is then completed.
2. Place the mouse cursor at the ends of the structure. The computed path is displayed.
3. Shift + click and the displayed path is drawn.

If some false paths appear you can change the Starting Point at any time by repeating the procedure or switch to AutoDepth/Manual mode to make corrections. It is very useful to use a Volume object to help drawing the desired Filaments.

### PC Mouse & Keyboard Function Overview in the AutoPath Mode

Shift + right-click	Set a start point
Set starting point, then move mouse	Display the path to the end point
Shift + Ctrl + right-click	Connect and restart.
Shift + left-click	Add the path
Shift + Ctrl + left-click	Add path and restart
Hold Shift	Pan the image
Mouse wheel	Change the "pencil" diameter for drawing the filament

### Drawing in AutoPath Mode on a Mac:

1. Select AutoPath and Shift + right click on the desired starting point of your structure (e.g. in a neuron picture it would typically be the nucleus). The automatic computation of all the possible paths is then completed.
2. Place the mouse cursor at the ends of the structure. The computed path is displayed.
3. Shift + click and the displayed path is drawn.

If some false paths appear you can change the Starting Point at any time by repeating the procedure or switch to AutoDepth/Manual mode to make corrections. It is very useful to use a Volume object to help drawing the desired Filaments.

### Mac Mouse & Keyboard Function Overview in the AutoPath Mode

Shift + right-click	Set a start point
Set starting point, then move mouse	Display the path to the end point
Shift + Command + right-click	Connect and restart.
Shift + left-click	Add the path
Shift + Command + left-click	Add path and restart
Hold Shift	Pan the image
Mouse wheel	Change the "pencil" diameter for drawing the filament

All actions in the Draw , Edit  and Edit Tracks  tabs can be Undone/Redone by selecting the [Undo/Redo](#) command or by pressing Ctrl+Z/Ctrl+Y.

## Notes

There is no visual indication of the progress of the autopath calculation. One way to find this out indirectly is by moving the mouse pointer to different positions in the image. For those positions where the calculation has already arrived a tracing will be shown. For those positions where the calculation has not yet arrived no tracing is shown.

Since it is very easy to trace a small piece and then continue the tracing from there users can work without interrupt.

## 11.4.6.2 AutoDepth

The AutoDepth function adds the automatic computation of the depth while doing manual segmentation (e.g. if the manual segmentation is done while looking at xy-plane, z-coordinate will be automatically computed).

- An autosnap feature enables easy drawing along the filament structure as you do not need to click in the exact centre. When ambiguities arise, autodepth keeps track of multiple paths until the ambiguities resolve. (Only the best path is displayed to the user).
- Press Shift+T to switch between Torch View and Full View modes.
- Pressing Ctrl+8 is useful immediately after adding a path to turn on InMotion for validation of the tracing.
- Autodepth mode respects clipping planes: It chooses start and end points only within the visible part of the volume. (Note that the clipping plane frames and handles may be turned off so they do not selected by mistake when making other adjustments to the image).

## Setting

### Diameter

Set the Diameter for the cursor. The size of the cursor determines the resolution at which the tracing is calculated (larger cursor provides lower resolution but is a faster calculation and less precision- but often these low resolution paths are effective).

### Type

Select between Dendrite and Spine options

## Correction

### AutoCenter

This option runs an automatic centering when the path is added (after pressing shift+left-click).

### AutoDiameter

Select this option to run an automatic diameter calculation when the path is added (after pressing shift+left-click). The calculation takes the mouse cursor size on shift+left-click as an input parameter (initial guess of diameter). The mouse cursor size should roughly correspond to the diameter of the filament for the calculation to succeed.



## View

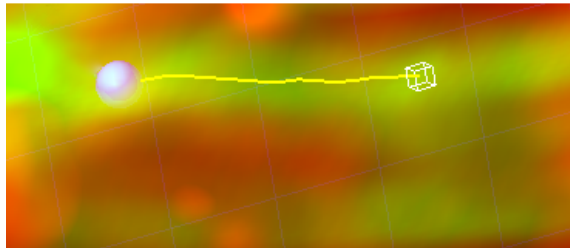
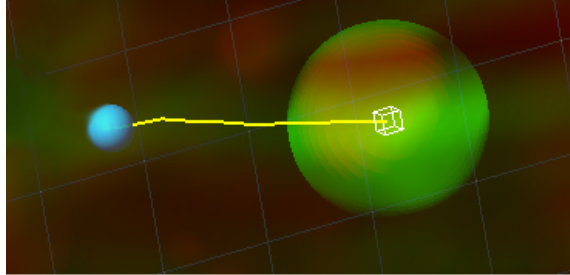
Select between the two viewing options:

### Torch View

Provides a highlighted area to help allow you to focus on an area of interest (top image). The size of the area illuminated by the torch can be adjusted using the mouse wheel.

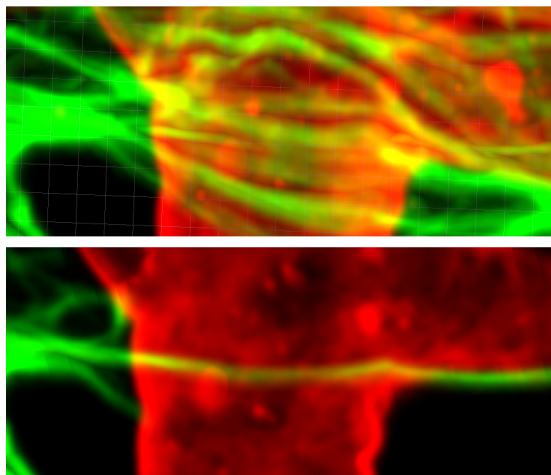
### Full View

Provides a normal view through the full image area (bottom image).



### Depth Visibility

When working through an image with depth, it may have features that can obscure an area or structures of interest. You may enable Depth Visibility and then use the sliders to adjust the area of visibility between Near and Far and between a Narrow and Wide field to help obtain a suitable view as shown in the following image. Unchecking the Depth Visibility will return the image to the normal view.



### Drawing in AutoDepth Mode on a PC:

Select AutoDepth, press the Shift-key and hold down the left mouse button to draw a Filament. The depth is automatically computed by comparing local intensities.

Drawing in AutoDepth mode is then similar to drawing in Manual mode. In order to fully use the potential of the AutoDepth mode, the pictures should not be too noisy. If this is the case, pre-filtering would be then necessary.

## PC Mouse & Keyboard Function Overview in the AutoDepth Mode

Shift + left-click & drag	Draw a filament into the volume
Hold Shift	Pan the image
Mouse wheel	Change the "pencil" diameter for drawing the filament

### Drawing in AutoDepth Mode on a Mac:

Select AutoDepth, press the Shift-key and hold down the mouse button to draw a Filament. The depth is automatically computed by comparing local intensities.

Drawing in AutoDepth mode is then similar to drawing in Manual mode. In order to fully use the potential of the AutoDepth mode, the pictures should not be too noisy. If this is the case, pre-filtering would then be necessary.

All actions in the Draw 🖋️, Edit ✏️ and Edit Tracks 🗑️ tabs can be Undone/Redone by selecting the [Undo/Redo](#) command or by pressing Ctrl+Z/Ctrl+Y.

## Mac Mouse & Keyboard Function Overview in the AutoDepth Mode

Shift + click & drag	Draw a filament into the volume
Hold Shift	Pan the image
Mouse wheel	Change the "pencil" diameter for drawing the filament

### Notes

Pressing the d-key on the keyboard while drawing enables an eraser so you can work backwards and redraw from an earlier point. This works only on those parts of a filament that were drawn right before pressing the d-key.

### 11.4.6.3 Manual

The manual segmentation feature is used to edit automatically traced Filaments or to build the Filaments manually from the image template. Essentially you draw the Filaments onto the image, which serves as a visual reference (template). Drawing is done in the xy-plane. For z-positioning in the image you can choose to change planes manually (Arrow-keys or mouse) or to have the system change the planes automatically in such a way that the center of the Filament is kept in focus.

An orthogonal section plane appears within the Surpass view. Use the channel visibility and the Display Adjustments to optimize the viewing. In order to draw Filaments onto the drawing plane switch the mode for the mouse pointer from Navigate to Select.

If you select Manual the Slice parameter settings are available.

### Drawing in Manual Mode on a PC:

Select Manual in the Draw box and press the Shift-key and hold down the left mouse button to begin drawing. The circle on the drawing plane indicates the Filament diameter.

The diameter can also be changed using the mouse wheel. To make a connection to an existing Filament, start painting on this Filament. If the filamentous structure runs perpendicular to the drawing plane, use the right



mouse button to alter the plane orientation.

All actions in the Draw , Edit  and Edit Tracks  tabs can be Undone/Redone by selecting the [Undo/Redo](#) command or by pressing Ctrl+Z/Ctrl+Y.

### PC Mouse & Keyboard Function Overview in the Manual Mode

Left-click plane & drag	Change plane position perpendicular to its orientation
Shift + left-click	Set a filament vertex
Shift + left-click plane & drag	Draw a filament
Right-click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the "pencil" diameter for drawing the filament
PageUp	Increase the "pencil" diameter for drawing the filament
PageDown	Decrease the "pencil" diameter for drawing the filament
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
Arrow up	Change slice position up
Arrow down	Change slice position down
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

### Drawing in Manual Mode on a Mac:

- Select Manual in the Draw box and press the Shift-key and hold down the mouse button to begin drawing. The circle on the drawing plane indicates the Filament diameter.

The diameter can also be changed using the mouse wheel. To make a connection to an existing Filament, start painting on this Filament. If the filamentous structure runs perpendicular to the drawing plane, use the right mouse button to alter the plane orientation.

### Mac Mouse & Keyboard Function Overview in the Manual Mode

Click plane & drag	Change plane position perpendicular to its orientation
Shift + click	Set a filament vertex
Shift + click plane & drag	Draw a filament
ctrl + click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the "pencil" diameter for drawing the filament
PageUp	Increase the "pencil" diameter for drawing the filament
PageDown	Decrease the "pencil" diameter for drawing the filament
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

## 11.4.7 Tab Edit

Up to this point in the analysis process, most steps have been carried out automatically. You have influenced the tracking process by specifying a set of parameters. The Tab Edit allows you to make changes to the graph topology and the number of nodes and segments in the filament graph. You make these changes directly by processing part or all of the existing filament graph, or by operating on the 3D view of the graph.

### Mouse Selection

Selecting a tool will change how the mouse interacts with the objects within the viewing area to match the properties of that selection. Specify how to select parts of the Filament by a single mouse click on the Filament graph.

**Please Note: The selection of the filament type can be very different depending on which selection is chosen in the [Track Edit](#) tab. The selection is governed by the selection mode under both the [Edit](#) and [Track Edit](#) tab.** The basis for the selection is the Mouse Selection mode found under in the Edit tab.

### Expand selection on full Spines

Expand the initial selection set outwards from the original selected filament element until all attached spine objects are included in the selection.

### Point

Point selects the smallest part of the segmented filament graph, only the position nearest to the mouse pointer.

### Segment

Segment selects the graph section between two key vertices (branch points, beginning point, or ending points) of the same class, either dendrites or spines.

- Between the dendrite beginning point and dendrite branch point
- Between the dendrite branch points
- Between the dendrite branch point and dendrite terminal point
- Between the spine attachment point and spine terminal point
- Between the spine attachment point and spine branch point
- Between the spine branch point and spine terminal point

### Branch

Branch selects the graph starting from the selected point to all the connected terminal points of the same class (a beginning point should be defined first for this mode).

Between the Dendrite Attachment or Dendrite Branch point to the Dendrite Terminal point for Dendrites.

Between the Spine Attachment point or Spine Branching point to the Spine Terminal point for Spines.

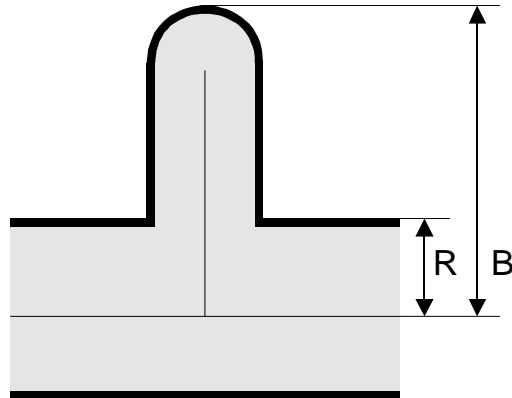
### Filament

The Filament option selects the entire Filament graph.

### Select Parts from Filament

There are various methods to make automatic selections on the whole Filament graph.





Button **Rel. Branch...**

**Rel. Branch** selects short parts of the Filament defined by the ratio of the branch length to trunk radius  $B/R$ .

Button **Loops...**

**Loops** finds circular closed Filaments limited by a maximum length.

Button **Invert**

**Invert** selects all unselected parts of the graph and de-selects all selected parts.

Button **Abs. Branch**

**Abs Branch** selects all branches shorter than an absolute length  $B$ .

Button **Path**

**Path** requires at least two selected points from the Filament graph. The shortest path between these two points will be selected.

Button **Clear**

**Clear** removes the selection.

## Selection

The following buttons apply only to the previously selected parts (see above) of the Filament.

Button **Assign as Dendrite**

Click on this button to assign the selection as dendrite.

Button **Assign as Spine**

Click on this button to assign the selection as spine.

Button **Assign as Dendrite Beginning Point**

Click on this button to assign the selection as dendrite beginning point.

Button **Smooth**

Removes the roughness of the current selection. It affects the position and the diameter of all segments except the branch points and end points.

Button **Duplicate**

Click on this button to create a duplicate of the current selection. The new Filament item appears in the

Surpass Tree.

## Button **Delete**

Click on this button to remove the current selection.

## Filaments

The following buttons apply to the entire Filament.

### Clear Dendrite Beginning Point

Click on this button to remove the dendrite beginning point.

### Button **Smooth**

Smooth removes roughness from the Filament path. It affects the position and the diameter of all segments except the branch points and end points.

### Button **Split**

Unconnected parts of the Filament can be split into separate Filament objects. The new objects will appear as new Items in the Surpass Tree. This allows individual coloring and individual statistics.

### Button **Merge...**

In Imaris it is possible to merge Filament objects into one Filament objects. This button is only available if you have two or more Filament items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Filament X" displays.

Check the boxes in front of the desired Filaments or use the button **Select All**.

### Button **Clear All**

Use this button to clear the Filaments selection.

### Button **OK**

If you want to merge the Filaments click on **OK**.

### Button **Cancel**

If you do not want to merge the Filaments click on **Cancel**.

### Connect & Translate selected Edges (Vertices)

Check this box to connect and translate selected edges.

### Button **Export...**

The whole Filament graph can be stored in the Neuron file format (\*.hoc). The Neuron application is free and is able to do electrical simulations.

## Intensity-based

### Source Channel

Select the respective source channel, from which the structure is to be created.

### Diameter...

Enter a value for the the minimum (small) diameter to be detected. The maximum (large) diameter is set in Imaris to be the small diameter x 10. The threshold value has a default value of 3.0- however this can be



adjusted in Advanced Preferences. Under SurfaceAppFilament set the value of DiameterContrastThreshold from 3.000 to the required value.

## Selection

### Button **Center**

Click on this button to re-center the selection after e.g. adjusting the diameter, according to the image intensity gradient.

### Button **Diameter**

Click on this button to recalculate the filament diameter of the selection to the value set in the Diameter field.

### Button **Join**

Select the objects to be joined in the image by Ctrl + left-click (Mac: Command+ left-click). The selected point or segment will be highlighted, then click Join. A new filament structure will be created structurally joining previously unconnected points or segments.

All actions in the Draw , Edit  and Edit Tracks  tabs can be Undone/Redone by selecting the [Undo/Redo](#) command or by pressing Ctrl+Z/Ctrl+Y.

## 11.4.8 Tab Edit Tracks

### Mouse Selects

This option allows you to select an object within an image according to its object type by a single mouse click. To edit the properties of multiple objects simultaneously, select the required object type. There are the following options:

**Object**  
**Segment**  
**Branch**  
**Pivot**  
**Track**

**Please Note:** The selection of the filament type can be very different depending on which selection is chosen in the Track Edit tab. Selection is governed by the selection mode under both the [Edit](#) and Track Edit tab.

**Note also you can change between mouse selects options using the first letter of each option as a keyboard shortcut i.e. Object: O, Segment: S, Branch: B, Pivot: P and Track: T.**

How to use the Selection Tools

Selection of different filament elements and tracks can be made within the [Edit](#) and **Track Edit** Tabs.

To make a suitable selection for time resolved filament images, you may need to make the object selection by clicking on the Mouse Selection options both under the **Edit** and **Track Edit** tabs.

Once you have selected the Filament graph element under the **Edit** tab you can also use the selection options under the Track Edit tab to decide whether the object to be selected is just for a **single time point** or for **all time points**.

Under the Track Edit tab you can decide either to select objects for a **single time point** by choosing the

option **Filament** or for **all time points** along the selected track by selecting the option **Track**.

For example, if under the Edit tab the mouse pointer is the Segment option and under the Edit Track tab the mouse selection is the Filament option, then the Segment will be selected for a single time point.

However, if you alter the selection under the Track Edit tab to the Track option, the particular Segment will be selected for all time points along that track.

## Table Layout

In the track editor a horizontal line represents each track. Each horizontal line is composed of circles, representing objects, and line segments which connect a sequence of objects.

The first row of the track-editing table displays the timeline. Each number represents a time point in the series.

The second row of the track-editing table displays the **object name**.

Multiple object types can be tracked easily since every object type appears in different colors.

## Interactive Selection

When you click on an individual row, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (row in table) is highlighted.

In addition, the [Statistical Tab](#) and [Filter Tab](#) can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.

For multiple selections press Ctrl+click (Mac: Command+click).

## Visualization options

### Zoom

Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table.

Using the mouse wheel will have the same affect.

### Pan

To move the track editing table click and hold the right mouse button while dragging the mouse. Release the right mouse button to place the table on the chosen location.

### Time indicator

The first row of the track-editing table displays the timeline. Each number represents a time point in the series.

The current time point is indicated by a vertical line highlighted with the current selection color.

To modify the time point, do one of the following:

1. Moving the slider in the Time bar modifies its position in time and changes the time point in the track editor.
2. Clicking on the time point within the track-editing table automatically moves the time line indicator to the corresponding point.

Double-clicking the time indicator, fixes the position of the current time point at the center of the track-editing table, regardless of the selected zoom or pan options)

## Editing

Track Editor has several features that help you modify your tracks

**Connect Button**

Click this button to connect track components such as objects or segments as selected under Mouse Selects. For example, select objects in different tracks at the subsequent time points (just one object per time point). The connect and disconnect buttons will update to show the number of objects selected. Confirm the selection, then click on the button Connect (you can also press the "c" key as a shortcut). The connection is automatically created between all selected objects.

**Disconnect Button**

Use this option to disconnect track components such as objects or segments as selected under Mouse Selects. For example, select object(s) to be disconnected. The connect and disconnect buttons will update to show the number of objects selected. Confirm the selection, then click on the button Disconnect (you can also press the "d" key as a shortcut). The button will update to show the number of objects selected. The connection between the selected objects in the time sequence is broken.

**< Button**

Click this button to move a selected track left. When axis is inverted (horizontal) the selected track moves up.

**> Button**

Click this button to move a selected track right. When axis is inverted (horizontal) the selected track moves down.

**<< Button**

Click this button to move a selected track all the way to the left. When axis is inverted (horizontal) the selected track moves to the top.

**>> Button**

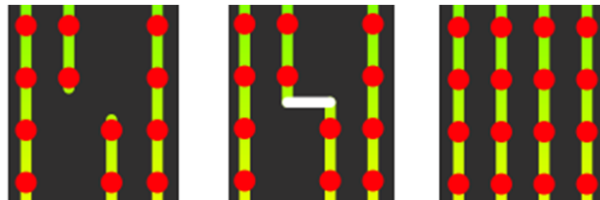
Click this button to move a selected track all the way to the right. When axis is inverted (horizontal) the selected track moves to the bottom.

**Flip**

The Flip button may be used (with Pivot mode selected) to flip the order that the connected branched segments and objects are displayed in about the pivot point.

**Reorganize**

The Reorganize button can be used after editing of tracks is complete so that tracks are displayed in a clear and logical format without affecting the track arrangement during the editing process.



Editing tracks. Connection added. Tracks reorganized.

**Invert Axis**

Click the Invert Axis button to switch between displaying the tracks vertically (default) and horizontally.

**Correct Drift**

Refer to [Drift Correction](#).

Focus tracks mode may be useful when editing tracks- refer to the [Cells Edit Track](#) section.

## Algorithms

The Drift Correction is calculated based on the two algorithms, translational drift and translational and rotational drift.

### Translation

Upon translation, the object position is transformed to a straight line of identical orientation. Within the same time point the relative distance of the objects is preserved.

### Translation and rotation

Upon translation and rotation, the object position is transformed based on the image polar coordinates. Within the same time point the relative distance of the objects is preserved.

**Please note: The number of the selected tracks affects the algorithm calculation.**

Selection of a single track gives a complete description of a translation.

Selection of two tracks gives a minimum description of a translation and rotation algorithm (planar rotation).

A better fit between the time points is achieved when three or more tracks are selected.

### Result DataSet size

After completion of the drift correction, the algorithm uses the final position of the initial and resulting image to create a final image. The new image coordinates are calculated in accordance with the size of the original and new image.

Select the Result data set option to determine which new image part will be displayed.

### Crop largest common region

The final image will be the size of the common overlap between the original and the drift corrected image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

### New size equal to current size

The final image will be the same size as the original image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

### Include entire result

The final image will be the size of the combined area of the original and corrected image.

Selecting the appropriate Result data set size may speed up the data visualization. This is especially important with (with large data sets.)

### Correct Filaments positions

Activate this parameter and the filaments are automatically re-positioned after the drift correction. If this option is not activated only the image volume is aligned.

**All actions in the Draw , Edit  and Edit Tracks  tabs can be Undone/Redone by selecting the [Undo/Redo](#) command or by pressing Ctrl+Z/Ctrl+Y.**

### 11.4.9 Tab Filter

**Please note: Before using, you have to add a filter to the list (see below: button Add)!**

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for data analysis.**

#### Filters

##### Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

##### Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.







#### Filter Type

Select the filter you want to apply from the drop-down list.

#### Category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking the histogram you move the colored line and a reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Dendrite) is displayed at the bottom of the threshold histogram.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.**

### Duplicate Selection to New Filament Button

Click on this button and all selected objects are copied to a new Surpass Group. The new group displays in the Surpass Tree.

#### 11.4.10 Tab Statistics

Filament statistics are automatically computed. For the statistical values please refer to the Section Menu Edit - Preferences... - Statistics - [Filament](#). The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different data sets through summarized statistical values and graphical data presentations.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:

##### a) Overall

##### b) Detailed

##### c) Selection

In the statistical data set the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

To sort the columns click on the arrow in column heading. Click again to reverse the sort.





















## Overall Tab

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

## Category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.

## Detailed Tab

A pull down list offers the following types of **Detailed** statistical calculations:

### Specific Values

### Average Values

### Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

### Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

### Specific Values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

### Average values

The Average table contains values determined on the basis of all values within a set of the selected variable. Columns are labeled as follows:

Min (Minimum)- the smallest value within a set of values of the selected variable  
Max (Maximum)- the largest value within a set of values of the selected variable  
Mean – value of the sum of all values divided by number of values within a set  
Std Dev (Standard deviation)- a measure of the values distribution around the mean values  
Median- the middle value of the sorted set of variables  
Sum- the sum of all values within a variable set  
Count- number of values within a set  
Unit- variable unit  
Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

## Selection Tab

Statistical tables for a selected object will be displayed. Change to the pointer mode Select and click on an image element that has been detected/segmented. A possibility of multiple selections is available by Ctrl+click (Mac: Command-click).

A pull down list offers two types of statistical calculations from a Selection:

### Specific Values

### Average Values

## Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

## Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

## Specific values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

## Average values

The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

## Buttons at the bottom of the Statistics tab

Button **Settings** -Configure a List of visible statistics values.

Click on the button **Settings** and you switch to the window Preferences - Statistics - [Filament](#).

Button **Search** - Search by object ID.

Type in the object ID in the data field. Click on the button **Search**.

- Highlights the object in the viewing area
- Highlights the object on the Detailed Tab
- Displays of the values on the Selection Tab

Button **Filament** - Duplicate Selection to new Filament.


Click on this button and all selected elements are copied to a new Filament object. The new Filament appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Statistical Annotation** 

Click this button and an object statistical annotation will be automatically generated in the viewing area. This option is available only when **Specific** is selected

Button **Export** - Export Statistics on Tab Display to File.

Only the statistics that are shown within the current tab will be exported.

Button **Export**  - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.

All of the statistics (that are enabled in the statistics settings) will be exported for Overall and Detailed tab data. When the Selection tab is selected, pressing the Export all Statistics to File button, will export All the statistics (that are enabled in the statistics settings) of all the selected objects, not all the statistics of all the objects.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

See also:

Menu Edit - Preferences... - Statistics - [Filament](#)

### 11.4.11 Tab Color

In Tab Color you can edit, manage and assign colors material to the Filament object.

## Object Type

The choice of Object type is presented in drop-down window Dendrite, Spine, Dendrite Beginning Point, Dendrite Branch Point, Dendrite Special Branch Point (more than three segments attached to one point), Dendrite Terminal Point, Spine Attachment Point, Spine Branch Point, and Spine Terminal Point.

Choose one of the objects from the drop-down list and depending on the chosen object type selection of the variables is listed.

The following color types are available:

Labels (default)

[Base](#)

[Statistics Coded](#)

### 11.4.11.1 Color Type- Labels

The labels associated with the data set are displayed as a list.

Click **Only show labeled objects** to show only data which have labels associated with them.

Note that if there is more than one label associated with an object, the object will be displayed as a combined color. Non-labeled objects are displayed in the base color.

## Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### 11.4.11.2 Color Type-Base

#### RGB

The RGB color model is an additive color model in which Red, Green and Blue color information is combined together to reproduce the required color. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

## Light reflection

The quality of the object material is further characterized by reflection properties.

- Diffusion- diffuses reflection of a matte, uneven material.
- Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. The default setting is for no specular intensity.
- Emission- light emitted from the material defines the self-illumination. The default setting is for no emission of light.

## Transparency


Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

## Palette

Palette- this option provides a system of preset colors organized into color schemes.

In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, Artdeco, Autumn, Glass, Metal, Neon, Rococo, Santafe, Sheen, Silky, Spring, Summer, Tropical, Winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

### 11.4.11.3 Color Type-Track ID

Track ID assigns a fixed, random color to each object. This mode is useful for validating tracks as an object will have the same color through the track duration. Erroneous behavior can easily be detected and corrected using the Edit Tracks  functions. Refer to the [Tab Edit Tracks](#) section.

#### Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image)

### 11.4.11.4 Color Type-Statistics Coded

#### Statistics Coded

Depending on the object type selection the Statistical variable selection is listed in the drop-down window. For example, if Dendrite is chosen as the Object type, the available Statistical types will be the Dendrite linked variables.

#### Statistics Colorbar Properties

Statistics colorbar are displayed in the viewing area.

##### Show Colorbar

Check the box to see the statistics colorbar in the viewing area.

##### Show Title

Check this box to see the title in the statistics colorbar.

##### Show Range

Check this box to see the range in the statistics colorbar.

Font color in colorbar is adjustable by selecting a color within the color map.

#### Colormap

##### Button **Reset**

Click the **Reset** button to set the image back to the original values.

##### Colormap

Click on the button Colormap to display a large number of predefined colormaps. If the default colormap (Spectrum) is not appropriate, you can change it by clicking on the drop-down list. The list gives a preview of what different colormaps look like.

#### Colormap range

Colormap range-specify the limits for colorbar.

**Min** - lower color limit

**Max** - upper color limit

##### Button **Auto**

Click the button **Auto** to detect the system the real min and max values and sets the Max. and Min. limits automatically to these values.

## Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### 11.4.12 Mouse & Keyboard PC

#### Filament Mouse & Keyboard Functions on the Tab Draw

##### AutoPath Mode

Shift + right-click	Set a starting point into volume. Multiple paths/branches can be drawn from this start point.
Set starting point, then move mouse	Display the path to the end point
Shift + left-click	Draw the path visible on screen to the cursor. Does not change the start point or calculation thread from the start point.
Shift + Control + Left Click	Draw the path visible on screen to the cursor. Starts a new calculation from the end of a drawn path. Continues on same filament path.
Shift + Control + Right Click	Start a new calculation from an existing point on a drawn segment. This will create a new branch point, if it is in the vicinity of a drawn segment.
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

##### AutoDepth Mode

Shift + left-click & drag	Draw a filament into the volume
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

##### Manual Mode

Left-click plane & drag	Change plane position perpendicular to its orientation
Shift + left-click	Set a filament vertex
Shift + left-click plane & drag	Draw a filament
Right-click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)
PageUp	Increase the filament diameter (to visualize change to Style Cone - Tab Settings)
PageDown	Decrease the filament diameter (to visualize change to Style Cone - Tab Settings)
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
Arrow up	Change slice position up
Arrow down	Change slice position down
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

See also:

Addendum - [Mouse & Keyboard PC](#)



### 11.4.13 Mouse & Keyboard Mac

#### Filament Mouse & Keyboard Functions on the Tab Draw

##### AutoPath Mode

Shift + right-click	Set a start point
Set starting point, then move mouse	Display the path to the end point
Shift + left-click	Draw the path visible on screen to the cursor. Does not change the start point or calculation thread from the start point.
Shift + Command + Left Click	Add the path.
Shift + Command + RightClick	Restart.
Click	
Hold Shift	Pan the image
Mouse wheel	Change the "pencil" diameter for drawing the filament

##### AutoDepth Mode

Shift + click & drag	Draw a filament into the volume
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)

##### Manual Mode

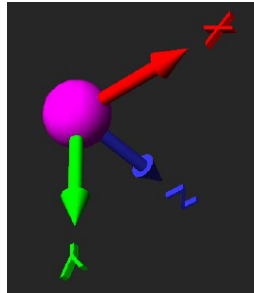
Click plane & drag	Change plane position perpendicular to its orientation
Shift + click	Set a filament vertex
Shift + click plane & drag	Draw a filament
Command + click plane	Change the plane orientation (from XY to YZ to XZ)
Mouse wheel	Change the filament diameter (to visualize change to Style Cone - Tab Settings)
PageUp	Increase the filament diameter (to visualize change to Style Cone - Tab Settings)
PageDown	Decrease the filament diameter (to visualize change to Style Cone - Tab Settings)
Num plus	Increase the extended section (of the drawing plane)
Num minus	Decrease the extended section (of the drawing plane)
X	Set the plane orientation to YZ
Y	Set the plane orientation to XZ
Z	Set the plane orientation to XY

See also:

Addendum - [Mouse & Keyboard Mac](#)

## 11.5 Reference Frame

Reference frames can be used for image alignment. When time series need to be rotated or shifted for better alignment this transformation can be achieved using reference frames. Reference frames are orthogonal coordinate systems that can be positioned manually. To facilitate reporting of Object Statistics in the reference frame coordinate systems all positional and vectorial statistics values are automatically reported both in image coordinates and in reference frame coordinates.



A time play mode lets you play through a movie so that reference frames stay still.

## The Reference Frame Coordinate System

A reference coordinate system consisting of three orthogonal axes can be positioned anywhere within the image and oriented in any direction by the user. The reference coordinate system can be positioned independently for each time point. A user can for example, place a reference coordinate system so that one axis aligns with the anterior-posterior axis of a Zebrafish.

Reference frames are positioned manually by clicking into the 3D View.

### To adjust orientation of the axes

- Click on arrow of an axis and hold the left mouse button to adjust the orientation of the axes.

### To move position of the axes

- Click on the central sphere to translate/move the position of the axes within the image.

For a time series it is not necessary to place a reference frame at every time point. It is possible to position a few frames at a few time points and all frames in between will be interpolated.

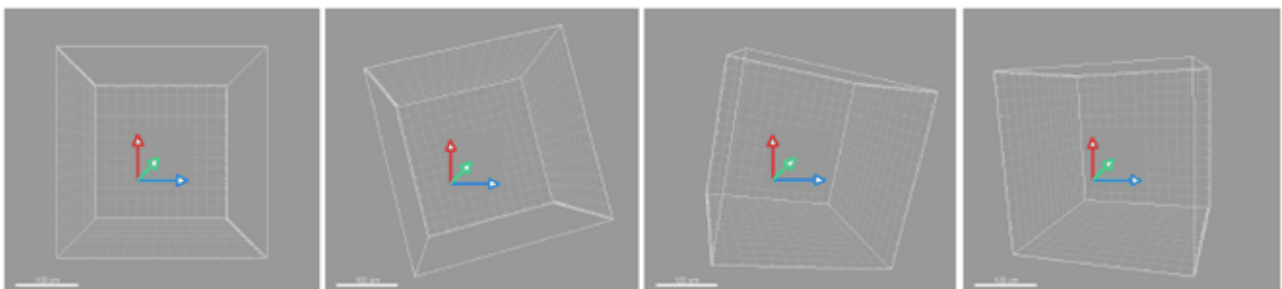
It is possible to create multiple reference frames within one image.

## Statistics Values reported in Reference Frame Coordinates

All statistical values of surpass objects (spots, surfaces, cells, filaments) are computed both in world coordinates and in reference coordinates. In the Zebrafish example Imaris would report track-velocity vectors in the reference coordinate system such that it is easy to filter for objects that move toward the head of the fish for example.

## Time Series with Fixed Reference Frame

When reference frames have been set for different time points of a time series it is possible to play the movie in such a way that the reference frames stay still. This is depicted in the following sequence:



## ImarisXT Interface





Reference Frames can also be used and set through the ImarisXT interface.

## Statistics Values

Reference frame specific statistics values for each surpass object are:

- Distance from origin of reference frame.
- Velocity angles with axes of reference frame.

These statistics values allow you to color objects by their distance to a reference frame, or by their direction of motion relative to the reference frame.

### 11.5.1 Settings Tab

#### Key Frame

Use the **Previous** and **Next** buttons to move between the key frames. The default option is interpolated (displayed in the Key Frame Type field. To set a translational key frame enable the **T** option, or to set a rotational key frame enable **R** option. Activating these options will update the description in the Key Frame Type field.

#### Planes

Set the frame plane options between **XY**, **XZ** and **YZ** planes.

Tick the **Visible** checkbox to toggle the visibility of the Reference frame on or off.

Use the **fix** checkbox to fix the plane in position.

Set the **extended section** for each plane by using the up and down arrows to increase or decrease the value incrementally through each plane, or enter the required value directly.

#### Interpolation mode

Select between **No Interpolation** and **Linear Interpolation**.

No Interpolation: the translational and rotational positions will remain fixed between each reference frame. They may therefore appear to "jump" between reference frames.

Linear Interpolation the translation and rotation positions between reference frames will be calculated in linearly interpolated increments. This will provide a smooth transition between reference frames.

#### Resample Image to align Reference Frames

Press this button to resample the image in the reference frame coordinates.

For information on drift correction please refer to the section [Drift Correction](#).

## 11.6 Correct Drift

If there is object drift (e.g. a cell drift) during the image acquisition this can be corrected in Imaris using the Correct Drift function.

- Drift correction can be applied to images with multiple time points, or to tracked objects.
- The result of the drift correction is either a translational and rotational correction of image time points or a reference frame object.

## Drift correction on an image

To apply drift correction to an image:

1. Load an image with multiple time points into Imaris.
2. Choose Image Processing / Image Alignment / Align Image, or Image Processing / Image Alignment / Set Reference Frame.

Drift correction on an image is a parameter free method. It performs an automatic spots detection and tracking on the image and then applies drift correction on these points. The spots are never visible in Imaris and there is no way to adjust the spots detection. If this procedure does not produce adequate results, detect and track objects using e.g. the Spots wizard and use the Correct Drift functionality on the Track Editor Tab of the tracked objects.

## Drift correction based on tracked objects

To perform drift correction based on tracked objects, load an image with multiple time points in Imaris, create and track a surpass object, e.g. Spots. Switch to the Edit Tracks Tab and press the Correct Drift button. Then configure the Drift Correction Options dialog to produce a new dataset or reference frames.

### Drift Correction Options Dialog

Depending on the context, the Drift Correction Options dialog displays a subset of the following options.

#### Objects options

This section controls which objects should be affected by the drift correction.

##### Correct image only

Only the loaded image will be corrected, surpass objects that have already been detected remain unchanged.

##### Correct image and selected object

The image and those surpass objects that are selected in the surpass tree will be corrected.

##### Correct image and all objects

The image and all surpass objects will be corrected.

#### Reference Frame correction

The image and selected objects don't change, but a new Reference Frame will be added to the surpass scene.

#### Algorithm

This section controls how the drift correction should be calculated.

##### Translational drift

The image/objects will be corrected by only shifting the objects.

##### Translational and rotational drift

The image/objects will be corrected by shifting and rotating the objects.

#### Result dataset size

This section controls the size of the corrected image. After drift correction, a resampled image is created.

**Crop largest common region**

Only those parts of the image that overlap after drift correction will be present in the new image.

This produces the smallest result image.

**New size equal to current size**

The image after drift correction will have the same size as the original image.

**Include entire result**

The image after drift correction will contain all data from the original image.

This option produces the biggest image.

A Preview image at the bottom of the dialog illustrates how the result will look like.

## 11.7 Frame

The Frame serves to outline the object in the viewing area. In the Surpass Tree check the check-box Frame to display the frame in the viewing area. Uncheck the box to make the frame invisible.

**How to Add a new Frame?**

- Select menu 3D View - Frame.

The Surpass Tree list is automatically updated and in the Frame - Properties the Settings parameter are displayed.

Surpass View - Overview - [Surpass Tree](#)

### 11.7.1 Tab Settings

Highlight Frame in the Surpass Tree and click on the tab Settings. The Frame Settings display in the properties area.

#### Box and Time Settings

**Box**

Check the parameter to display a box around the Frame.

**Line Width**

Adjust the box line width with the respective slider. Moving the slider to the left reduces the line width, moving it to the right will increase line width.

**Shading**

Check this parameter to display a shading. Use the slider to adjust the shade contrast.

**Shadow**

The entire Scene can produce shadows on the three planes of the Frame object. Check this box to display the shadows. Use the Shading slider (see above) to adjust the contrast. The rendering is done in real-time, and requires a high performance graphics card (depending on the complexity of the scene).

**Time Colorbar**

Only available if you work with a time data set. Check this box and the Time Colorbar displays in the lower left corner of the viewing area.

## Grid and Tickmarks Settings

### Grid

Check the box Grid to display a grid. Use the slider to adjust the grid width.

Scale is shown using a grid behind the object. The frame moves to stay behind the object as it is rotated. The distance represented by each square in the grid, the [scale\\_bar](#), is shown in the bottom right corner of the screen.

### Tickmarks

Check the box Tickmarks to display tickmarks on the edges of the frame.

### Spacing

Specify the grid and tickmark spacing.

## Labels Settings

### Axis Labels

Check the box Axis Labels to display the x, y, and z axes to clearly show the orientation of the object. If this box is checked the parameter Label Color and Label Font is available (see below).

### Label Color

Only available if the parameter Axis Labels (see above) is checked. Click on the icon to open the Color Editor window to change the color of the label on the coordinate axes.

### Label Font

Only available if the parameter Axis Labels (see above) is checked. Click on the icon to open the Font Editor window to change the font to label the coordinate axes.

### Fix Decoration to Data Set

Check Fix Decoration to Data Set to fix the Frame to the same side when navigating the object.

## 11.7.2 Tab Color

Tab Color edit, manage and assign colors material to the Frame.

## 11.8 Surpass Group

Surpass Groups are folders in the Surpass Tree that contain other Surpass objects. They are visible in the objects area and allow you to create a treelike hierarchy of Surpass objects. When the first Surpass object is created, it is automatically placed in a Group called Scene. Additional Groups can be created in order to sort objects of the scene.

### How to Add a new Group?

- Select menu Surpass - Surpass Group.

The Surpass Tree list is automatically updated and in the Group - Properties the Settings parameter are displayed.

### How to Add Items in a new Group?

- Click on an Item in the Surpass Tree list and drag it onto the Group object. Once released, the Item is added to the Group.

Like all other objects, Groups have a visibility check-box. This box affects the visibility of all objects in the Group.

See also:

3D View - Overview - [Surpass Tree](#)

## 11.8.1 Tab Settings

### Number of Objects

Displays the number of objects in the Group.

### Button Show All

If you click this button the check-box(es) of the Item(s) in the Group are checked.

### Button Hide All

If you click this button the check-box(es) of the Item(s) in the Group are unchecked.

## 11.8.2 Tab Color- Base

Color Tab edit, manage and assign material colors of an Object.

### RGB

The RGB color model is an additive color model in which Red, Green and Blue are combined together to reproduce an array of colors. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

### Light reflection

Quality of the object material is further characterized by reflection properties.

- Diffusion- diffuses reflection of a matte, uneven material.
- Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.
- Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

### Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### Palette

Palette- this option provides a system of preset colors organized into color schemes.

In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, Artdeco, Autumn, Glass, Metal, Neon, Rococo, Santafe, Sheen, Silky, Spring, Summer, Tropical, Winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

### 11.8.3 Tab Statistics

The Statistics tab enables us to understand and compare different data sets through summarized statistical values and graphical data presentations. To perform further analysis of large amounts of statistical data, use the Group Level Statistic. The Statistics tab collects and displays the Surpass Group's statistical values, which are lists of the statistical value summaries of all created objects contained in the specified Surpass Group. For further analysis select the Export button and the statistic values of all objects are exported as a single file.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:

- a) Overall
- b) Detailed

In the statistical data set the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

#### Overall Tab

The output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

#### Detailed Tab

A pull down list offers the following types of Detailed statistical calculations:

- Specific Values
- Average Values

#### Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

#### Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

#### Specific Values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical

values. Select the variable and a list of values will be displayed.

### Average values

The Average table contains values determined on the basis of all values within a set of the selected variable.

Columns are labeled as follows:

Min (Minimum)- the smallest value within a set of values of the selected variable

Max (Maximum)- the largest value within a set of values of the selected variable

Mean – value of the sum of all values divided by number of values within a set

Std Dev (Standard deviation)- a measure of the values distribution around the mean values

Median- the middle value of the sorted set of variables

Sum- the sum of all values within a variable set

Count- number of values within a set

Unit- variable unit

Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

**Settings Button** -Configure a List of visible statistics values

Click on the button Settings and switch to the window Preferences

**Search Button** - Search by object ID

Type in the object ID in the data field. Click on the button Search.


Highlights the object in the viewing area

Highlights the object on the Detailed Tab

Displays of the values on the Selection Tab

**Export Button** - Export Statistics on Tab Display to File

Only the statistics that are shown within the current tab will be exported.

Button **Export**  - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm). If you select MS Excel as file type, the program starts automatically.

**Export Button** - Export All Statistics to File

All of the statistics (that are enabled in the statistics settings) will be exported for Overall and Detailed tab data. When the Selection tab is selected, pressing the Export all Statistics to File button, will export All the statistics (that are enabled in the statistics settings) of all the selected objects, not all the statistics of all the objects.

With a click on the button Export the window Export Statistics opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

## 11.9 Light Source

A Light Source serves to illuminate objects in the viewing area. A light source node serves as the location and orientation of a light source.

## How to Add a new Light Source?

- Select menu 3D View - Light Source.

The Surpass Tree list is automatically updated and in the Light Source - Properties the Settings parameter are displayed. Multiple light sources can be added in the Surpass Tree.

## Move Light Source

To change the Light Source position and direction, choose the pointer mode Select and drag the cone or line.

## Light Intensity

Click on the cylinder above the cone and move it closer or further away from the object to change the light intensity.

### 11.9.1 Tab Settings

#### Fix to Data Set

Check the box to fix the Light Source to the same side when navigating the object. When the box is unchecked, the light source is fixed to a particular position in space and remains in place when the object moves or rotates.

### 11.9.2 Tab Color

Color Tab edit, manage and assign material colors of an Object.

#### RGB

The RGB color model is an additive color model in which Red, Green and Blue color information is combined together to reproduce a selected color. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

#### Palette

Palette- this option provides a system of preset colors organized into color schemes.

In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, Artdeco, Autumn, Glass, Metal, Neon, Rococo, Santafe, Sheen, Silky, Spring, Summer, Tropical, Winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

### 11.9.3 Mouse & Keyboard PC

#### Light Source Mouse & Keyboard Functions

Left-click cone (or line) & drag	Change direction
Left-click cylinder & drag	Change intensity

See also:

Addendum - [Mouse & Keyboard PC](#)



## 11.9.4 Mouse & Keyboard Mac

### Light Source Mouse & Keyboard Functions

Click cone (or line) & drag      Change direction  
Click cylinder & drag              Change intensity

See also:

Addendum - [Mouse & Keyboard Mac](#)

## 11.10 Measurement Point

### How to Add a new Measurement Point?

- Select menu 3D View - Measurement Point.

The Surpass Tree list is automatically updated and in the Measurement Point - Properties the Edit parameters are displayed.

### 11.10.1 Tab Settings

#### Point Shape

**Tetrahedron**

**Cube**

**Sphere**

Here you can choose from three different shapes for the Measurement Points (tetrahedron, cube, or sphere).

**Filled**

Check the box and the point shape is solid.

#### Point Diameter

Here you can define the Measurement Point diameter.

#### Point Labels

**Name**

Check the check-box to display the name of a Measurement Point in the viewing area.

**Intensity**

Check the check-box to display the intensity of a Measurement Point in the viewing area. The intensity of the channel is displayed in brackets.

**Angle-Polygon**

This option is only available when Line Mode Polygon is selected in Settings. In the Pairs mode this option becomes inactive.

For each pair of adjacent measurement points, the angle between them at their common point is computed and displayed next to the angle arc.

## Line Mode

### Pairs (AB, CD, ...)

Any two consecutive points are connected by a line.

### Polygon (ABCD ...)

All points in the Group are connected consecutively (P1-P2-P3-P4, etc.).

## Line Width

Here you define the line width.

## Line Labels

### Distance

The measured distance between two points is displayed next to the line connecting the two Measurement Points. Check the check-box to display the distances in the viewing area.

### Velocity

Only accessible if you have a time data set. Check the box to display the speediness between two Measurement Points.

**Tip: If you do not have the module ImarisTrack you can easily use the velocity function to track single objects over several time points and measure the velocity.**

Button **Color**

Button **Font**

### Label Color and Font

To change the color or font of the label (display of the line length) click on the respective icon.

**Tip: You can find the parameters to change the color of the Measurement Points and connecting lines on the tab Colors.**

### Visible for all Time Points

Only accessible if you have a time data set. Check the box to display the inserted Measurement Points, lines, and annotations at all time points.

See also:

3D View - Measurement Point - [Tab Color](#)

## 11.10.2 Tab Edit

### AutoDepth Position

In the mouse pointer mode Select:

**Shift-click with the left mouse button to add a new point. Left-click to select an existing point. Shift-left-click to reposition a selected point.**

### Intersect with

The first two parameters are relevant if you want to insert Measurement Points in a volume rendered data set.



**all visible Channel**

Select this parameter and all visible channel are relevant for the placement of the Measurement Points.

Please note: In the Display Adjustments you can decide, whether a channel is visible or not in the viewing area.

**specific Channels**

Select this option for the placement of the Measurement Points and choose the relevant channel directly from the drop-down list.

Please note: In the Display Adjustments you decide, whether a channel is visible or not in the viewing area.

**Surface of Object**

Select this option and the Measurement Points are placed on the surface of an object. Uncheck the box and you can also place Measurement Points in an object.

**Center of Object**

Select this option and the Measurement Points are placed in the center of the object.

Please note the difference with the different objects types.

For Spots objects (also Vesicles and dendrite/spines beginning, branching, attaching, terminal points): Select this option and a new measurement point is placed at the center of the sphere.

For Surfaces objects: Select this option and a new measurement point is placed at the center of the object homogeneous mass

For Cells objects (Cell and Nucleus): Select this option and a new measurement point is placed at the center of the object homogeneous mass.

For Filament objects (Dendrite and Spines): Select this option and a new measurement point is placed at the dendrite/spine center axis. (For more information please refer to the [Filament](#) description).

**Button Delete All Points**

Click on this button to delete all Measurement Points. Alternatively click on the Delete-key on the keyboard.

**Selected Point**

Click to select an existing point. If no point is selected the following parameters are grayed. Re-click on a selected point to un-select the point.

**Button Delete**

Click on this button to delete the selected Measurement Point.

**Name**

Text field to edit the Measurement Point name.

**Position**

X

Y

Z

T

Data field of the x-, y-, z- and t-coordinates.

**11.10.3 Tab Intensity**

Imaris reads out the intensity profile of each voxel against the distance. Here you select the channels that should be displayed in the histogram and you choose the desired connecting lines between the Measurement

Points.

## Histogram

The histogram displays the intensity profile along the lines. The labeled lines represent the Measurement Points. The x-axis represents the length of the profile. On the left hand side of the histogram you find the display of the channel(s) intensity range. The channel selection is located below the histogram. Here you can switch channels in the intensity profile on or off. Below the channel selection you find the profile selection. In the drop-down list select the desired line and the histogram updates accordingly.

### Button **Snapshot**

Click on this button to do a Snapshot of the histogram. The window Save Snapshot as... opens.

See also:

Imaris Menu - Export... [Snapshot](#)

## 11.10.4 Tab Statistics

Measurement Point statistics are automatically computed. For the statistical values please refer to the Section Menu Edit - Preferences... - Statistics - [Measurement Points](#). The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different data sets through summarized statistical values and graphical data presentations.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:

### a) Overall

### b) Detailed

### c) Selection

In the statistical data set the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

To sort the columns click on the arrow in column heading. Click again to reverse the sort.

















## Overall Tab

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

## Category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical variables from the list (turns the category button into a

light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.

## Detailed Tab

A pull down list offers two types of **Detailed** statistical calculations:

### Specific Values

### Average Values

### Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

### Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

### Specific Values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

### Average values

The Average table contains values determined on the basis of all values within a set of the selected variable.

Columns are labeled as follows:

Min (Minimum)- the smallest value within a set of values of the selected variable

Max (Maximum)- the largest value within a set of values of the selected variable

Mean – value of the sum of all values divided by number of values within a set

Std Dev (Standard deviation)- a measure of the values distribution around the mean values

Median- the middle value of the sorted set of variables

Sum- the sum of all values within a variable set

Count- number of values within a set

Unit- variable unit

Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

## Selection Tab

Statistical tables for a selected object will be displayed. Change to the pointer mode Select and click on an image element that has been detected/segmented. A possibility of multiple selections is available by Ctrl+click (Mac: Command-click).

A pull down list offers three types of statistical calculations from a Selection:

## Specific Values Average Values

### Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

### Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

### Specific values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

### Average values

The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

### Buttons at the bottom of the Statistics tab

Button **Settings** - Configure a List of visible statistics values.

Click on the button **Settings** and you switch to the window Preferences - Statistics - [Measurement Points](#).

Button **Search** - Search by object ID.

Type in the object ID in the data field. Click on the button **Search**.

- Highlights the object in the viewing area
- Highlights the object on the Detailed Tab
- Displays of the values on the Selection Tab

Button **Measurement Points** - Duplicate Selection to new Measurement Points.

Click on this button and all selected elements are copied to a new object. The new Measurement Points appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Export** - Export Statistics on Tab Display to File.

Only the statistics that are shown within the current tab will be exported.

Button **Export**  - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.

All of the statistics (that are enabled in the statistics settings) will be exported for Overall and Detailed tab data. When the Selection tab is selected, pressing the Export all Statistics to File button, will export All the statistics (that are enabled in the statistics settings) of all the selected objects, not all the statistics of all the objects.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).

### Buttons Overview

	PC:	Mac:
Select spot	Left-click	Click
Select multiple spots	Ctrl + left-click	Command + click

See also:

Menu Edit - Preferences... - Statistics - [Measurement Points](#)

### 11.10.5 Tab Color

Color Tab edit, manage and assign material colors of an Object.

#### RGB

The RGB color model is an additive color model in which Red, Green and Blue color information is combined together to reproduce an a selected color. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

#### Light reflection

Quality of the object material is further characterized by reflection properties.

Diffusion- diffuses reflection of a matte, uneven material.

Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. Default setting has no specular intensity.

Emission- light emitted from the material defines the self-illumination. Default setting emits no light.

#### Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

#### Palette

Palette- this option provides a system of preset colors organized into color schemes.

In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, Artdeco, Autumn, Glass, Metal, Neon, Rococo, Santafe, Sheen, Silky, Spring, Summer, Tropical, Winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

## 11.10.6 Mouse & Keyboard PC

### Measurement Point Mouse & Keyboard Functions on the Tab Edit

Shift + left-click	Add a new measurement point
Shift + left-click	Reposition selected measurement point
Left-click on existing point	Select measurement point
Mouse wheel in Add mode	Turn wheel up: Increase size of next measurement point Turn wheel down: Decrease size of next measurement point

Depending on the depth of the structure where you want to put the next Measurement Point, the size of the shape around the mouse pointer changes accordingly. This helps when handling 3D data sets.

See also:

Menu Edit - Preferences ... - [3D Cursor](#) (adjust 3D Cursor Shape)

Addendum - [Mouse & Keyboard PC](#)

## 11.10.7 Mouse & Keyboard Mac

### Measurement Point Mouse & Keyboard Functions on the Tab Edit

Shift + click	Add a new measurement point
Shift + click	Reposition selected measurement point
Click on existing point	Select measurement point

Depending on the depth of the structure where you want to put the next Measurement Point, the size of shape around the mouse pointer changes accordingly. This helps when handling 3D data sets.

See also:

Menu Edit - Preferences ... - [3D Cursor](#) (adjust 3D Cursor Shape)

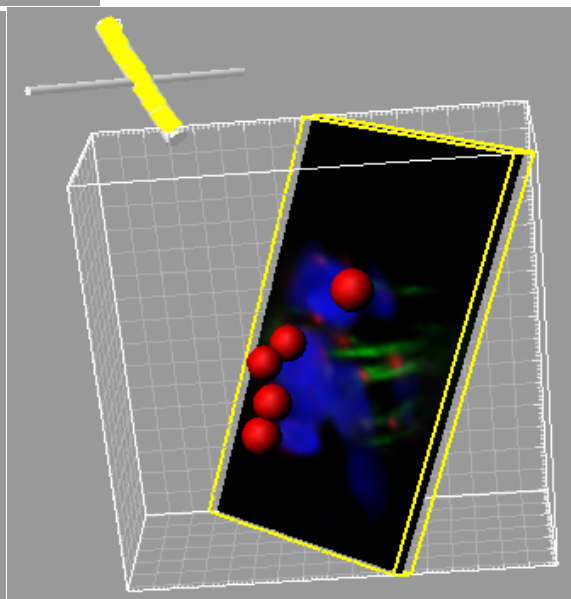
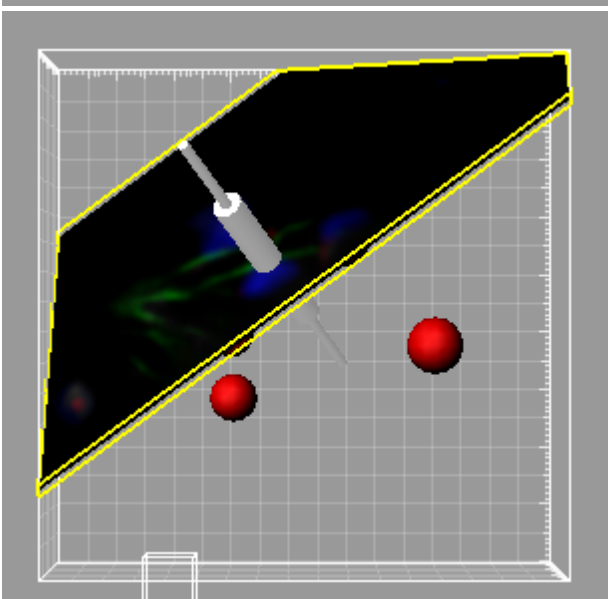
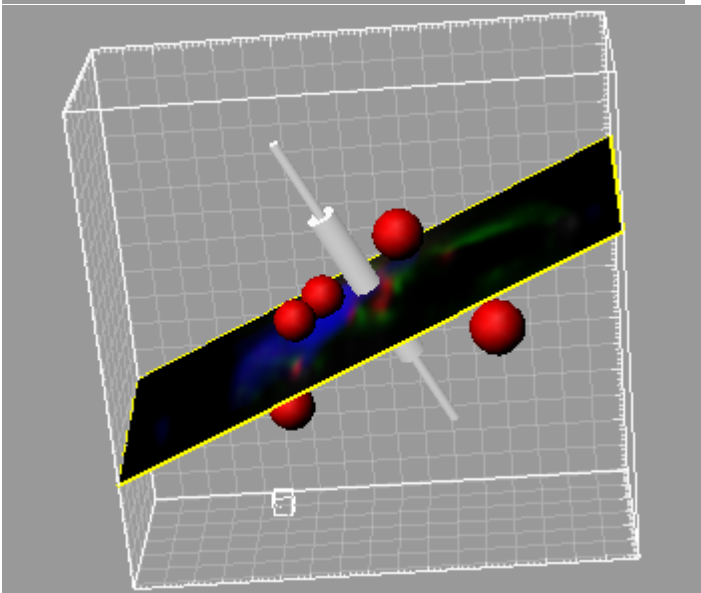
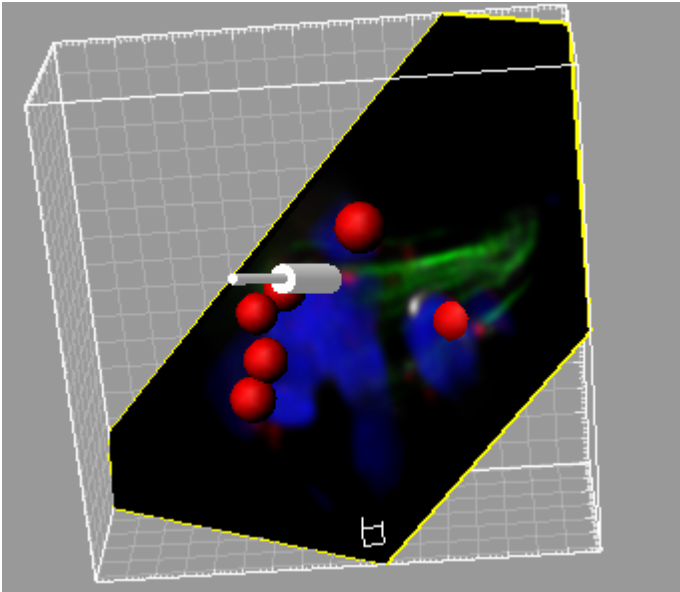
Addendum - [Mouse & Keyboard Mac](#)

## 11.11 Oblique Slicer

The Oblique Slicer represents a plane on which original data are projected. It can be moved and rotated freely within the data set. The Oblique Slicer consists of a colored frame with a white double rod in the middle.

The Oblique Slicer, and related tools, allows you to re-slice your data sets in any chosen rotation and position.





## How to Add a new Oblique Slicer?

- Select menu 3D View - Oblique Slicer.

The Surpass Tree list is automatically updated and in the Oblique Slicer - Properties the Settings parameter are displayed. The Oblique Slicer is displayed in the viewing area (depending on the default parameter on the tab Settings).

## Change Position of the Oblique Slicer

The Oblique Slicer can be freely moved and rotated to any angle and depth, allowing you complete control over which portion of the data set is visible and which portion is hidden.

To move and rotate the data set within the viewing area, the pointer must be in the Select mode.

To move the Oblique Slicer in the viewing area and click on the bigger diameter rod. It will turn colored as it is selected, move it to set the Oblique Slicer to the required position.

## Rotate Oblique Slicer around Axes

The Oblique Slicer can be rotated around the x-, y-, and the z-axes. In Select mode click the smaller diameter rod. It will be highlighted as it is selected. To rotate the Oblique Slicer, move the smaller rod.

### 11.11.1 Tab Settings

Under the Setting tab you can adjust the position and orientation of the Oblique Slicer, adjust the slice thickness and control the visibility of the Frame.

## Orientation

Button **YZ Plane**

Button **XZ Plane**

Button **XY Plane**

Change the Slicer direction, if required, by selecting the adequate plane.

## Position

Button **Recenter**

Click on this button to re-center the Slicer.

## Extended Section

The section is a single cut through the data set, the highlighted lines indicate the frame around the active slice. In the data field you can adjust the distance between the two lines.

## Show Frame

Check the box to display a colored frame around the Slicer.

## Camera

Button **Orthogonal View**

The camera is set to a position which is perpendicular to the Slicer.

### 11.11.2 Mouse & Keyboard PC

#### Oblique Slicer Mouse & Keyboard Functions

Left-click on the bigger diameter rod & drag      Move plane to desired position  
 Left-click on the smaller diameter rod & drag      Rotate plane to desired position

See also:

Addendum - [Mouse & Keyboard PC](#)

### 11.11.3 Mouse & Keyboard Mac

#### Oblique Slicer Mouse & Keyboard Functions

Click on the bigger diameter rod & drag      Move plane to desired position  
 Click on the smaller diameter rod & drag      Rotate plane to desired position

See also:

Addendum - [Mouse & Keyboard Mac](#)

## 11.12 Ortho Slicer

Ortho Slicer represent an orthogonal plane (orthogonal to the coordinate axis of the data set) that can be moved within the data set. When adding slices, within the Surpass view per default suggested are the directions z, x, and y as sequence for the first three and all subsequent slices.

#### How to Add a new Ortho Slicer?

- Select menu 3D View - Ortho Slicer.

The Surpass Tree list is automatically updated and in the Ortho Slicer - Properties the Settings parameter are displayed. The first Slice is displayed in the viewing area (depending on the default parameter on the tab Settings).

### 11.12.1 Tab Settings

#### Slice Orientation

**YZ Plane**

**XZ Plane**

**XY Plane**

The check XY, XZ and YZ plane, to select the visibility of the corresponding slice.

#### Extended Section

The section is a single cut through the data set, the colored lines indicate the frame around the active slice. In the data field you can adjust the distance between the two lines.

#### Show Frame

Check the box to display a colored frame around the active slice.

## Slice Position

### Slider

Choose the relative position of the Slice in the data set by means of the slider or a direct entry of the slice position given as the offset distance from the data set origin (equals the absolute coordinates).

### 11.12.2 Mouse & Keyboard PC

#### Ortho Slicer Mouse & Keyboard Functions

Left-click plane & drag	Change slice position
Right-click plane	Change slice orientation
Num plus	Increase the extended section
Num minus	Decrease the extended section
Arrow up	Change slice position up
Arrow down	Change slice position down

See also:

Addendum - [Mouse & Keyboard PC](#)

### 11.12.3 Mouse & Keyboard Mac

#### Ortho Slicer Mouse & Keyboard Functions

Click plane & drag	Change slice position
Ctrl + click plane	Change slice orientation
Num plus	Increase the extended section
Num minus	Decrease the extended section

See also:

Addendum - [Mouse & Keyboard Mac](#)

## 11.13 Spots

The Spots object is available to model point-like structures in the data. It provides a procedure to automatically detect point-like structures, an editor to manually correct detection errors, a viewer to visualize the point-like structures as spheres, and statistics output.

#### How to Add new Spots?

- Select menu 3D View - Spots.

The Surpass Tree list is automatically updated and the first window of the Creation Wizard displays in the Spots - Properties.

See also:

Surpass View - Overview - [Surpass Tree](#)

For information on statistics calculations used refer to [Spots](#)

### 11.13.1 Creation Wizard

The Creation Wizard allows you to move forward or backwards until the optimal parameters are found.

If the image data is for a [multi-image scene](#) an option to select which source image to be analyzed is available from the Align to: drop down box.

In the first window of the Spots Creation Wizard, you will find the algorithm settings. Here, you have to decide, if you want to load a set of previously stored creation parameters or to create a new one.

The most convenient way to apply the same creation parameters to different data sets is to use the [Load Parameters](#) option.

In the first window of the Creation Wizard you find the algorithm settings. Here you have to decide, if you want to segment only a **Region of Interest (ROI)**, if you want to use the **Region Growing** for the Spots detection, and if you want to create **Tracks** after the **Spots** detection.

For further details please refer to Section [Algorithm](#), [Region of Interest](#), [Source Channel](#), [Classify Spots](#), [Spots Region Type](#), [Spots Regions](#), [Edit Spots](#), [Tracking](#), and [Classify Tracks](#).

#### 11.13.1.1 Algorithm

##### Algorithm Settings

**Note:** If you do not want to use the automatic spots creation wizard select the **Skip automatic creation, edit manually** button.

##### **Skip automatic creation, edit manually** Button

Terminates the creation wizard and activates the manual Spots creation.

##### Favourite Creation Parameters

This option lets you use the identical creation parameters for multiple data sets.

The Favourite Creation Parameters option is only available if the set of creation parameters has already been saved under the [Creation Tab](#) using the Remember Parameters button.

Then you can choose to segment a **Region of Interest (ROI)** only, to create **Tracks** after the objects detection or to **Skip automatic creation**.

When the wizard is started, all stored creation parameters, corresponding to the chosen object wizard, are automatically loaded. The stored parameters contain all of the settings, options, and parameters required for image segmentation.

Expand the Favourite Creation Parameters drop-down list by clicking on it. The list shows all the creation parameters sets that have been previously saved, appropriate to the chosen creation wizard. Simply load your chosen creation parameters set, by clicking on it, and run the wizard as you have preset, without defining all of the options. On the other hand, you can customize each procedure to your own specific needs by modifying each part separately - e.g. the same channel settings, with a slightly different threshold.

##### **Segment only a Region of Interest**

A Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.

Check this box if you want to segment only a [Region of Interest](#). If option Segment only a Region of Interest is selected, in the last step of the Creation Wizard only the region of interest is processed.

### Process entire Image finally

Process entire Image finally this option appears only, if Segment only a Region of Interest is checked. Check this box and in the last step of the Creation Wizard the entire image is processed with the adjusted parameters ([Complete ROI](#)). Uncheck this box and only the region of interest is processed.

### Different Spots Sizes (Region Growing)

Check this box if you want to apply the region growing, starting on the tab [Spots Region Type](#).

### Track Spots (over Time)

Check this box if you want to track Spots over time, for details please refer to tab [Tracking](#).

## Spots Wizard Buttons

### Button **Back**

This button is grayed in the first window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

### Button **Cancel**

If you do not want to create Spots click on **Cancel**.

## 11.13.1.2 Region of Interest

### Region of Interest 1

Here you define the region of interest (ROI). Parameters such as width, height and location of the ROI are used to directly specify the area to work on.

### X, Y, Z, Time

### Min, Max, Size

**Minimum, maximum** and **Size** of ROI coordinate values, can be specified either by entering the value in the value fields or changing the values by clicking on up-and down arrows at the right hand side of the value fields. Alternatively the location and size of the ROI can be adjusted directly in the viewing area.

Size of the ROI:

1. Click on the ROI preview rectangle arrows (change to the pointer mode Select)
2. Drag the arrows to define new coordinates for X, Y and Z.

Location of the ROI:

1. Click inside the ROI preview rectangle
2. Drag the entire rectangle (with mouse button held) to the chosen image location.

The numbers in the value fields are updated automatically.

### Memory Usage:

Display of the memory usage for the image size within the ROI.



## Regions of Interest

To select a particular ROI, left-click on its name in the list and the selected ROI will be highlighted within an image and its properties can be adjusted.

### Button **Add**

Click on this button and a new region of interest appears in the list. To adjust this ROI you have to select the ROI with a left mouse click.

### Button **Delete**

Select a ROI in the list with a left mouse click and click on this button to delete the ROI.

### Total Memory Usage:

Display of the memory usage for the image size within the ROI.

## Spots Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

### Button **Cancel**

If you do not want to create Spots automatically click on **Cancel**.

See also:

Surpass View - Overview - [Camera Toolbar](#) (change to pointer mode Select)

### 11.13.1.3 Source Channel

## Source Channel

Select the respective source channel, from which the Spots are to be computed.

## Spots Detection

### Estimated XY Diameter

This parameter specifies the dimension of the Spots object in the X and Y direction. Objects with size smaller than the specified **Estimated Diameter** are not detected. The values are reported under the statistics tab as separate variables.

By default the radius X and Y are equal.

### Detect Ellipsoids

To change the detection and creation of the spherical Spots objects to ellipsoid shape objects select the **Detect Ellipsoids** box.

Check the Detect Ellipsoids box to activate the Estimate Z Diameter field.

If both the **Detect Ellipsoids** and **Different Spots Sizes (Region Growing)** options are selected, the value entered in the estimated diameter field is used only to determine the position of the spots.

The spot sizes are finally determined by the region growing parameters (Region Growing type and Regions threshold).

## Estimate Z Diameter

This parameter specifies the dimension of the Spots object in the Z direction. Objects with size smaller than specified **Estimated Diameter** are not detected. This value is reported under the Statistics tab as a separate variable.

The value of the specified Z diameter in comparison to the XY diameter determines the overall object shape.

If  $Z < X, Y$  the created shape is an oblate spheroid

If  $Z > X, Y$  then the created shape is a prolate spheroid

If  $Z = X, Y$  then the diameter the created shape is a perfect sphere

## Background Object Subtraction

If checked, the background will be removed prior to Spots detection. Technically background subtraction smoothes the image. The intensity is the one of a Gaussian filtered channel (Gaussian filtered by 3/4) minus the intensity of the original channel Gaussian filtered by 8/9 of sphere radius.

## Spots Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

### Button **Cancel**

If you do not want to create Spots click on **Cancel**.

## 11.13.1.4 Classify Spots

Spots filtering is incorporated into the creation process.

Filters allow the removal of objects that would interfere with interpretation of results.

**Quality: The 'Quality' is the intensity at the center of the spot in the channel the Spots was detected.**

**If 'Background Subtraction' was not checked this is the intensity of the channel Gaussian filtered by**





3/4 of spot radius.

If 'Background Subtraction' was active the intensity is the one of a Gaussian filtered channel from above minus the intensity of the original channel Gaussian filtered by 8/9 of spot radius.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

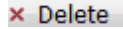
**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.**

## Filters

### Add Button

To add a new filter, click on the button Add  and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

### Delete Button

Select a filter in the list with a left mouse click and click on this button  to delete the filter.



















## Filter Type

Select in the drop-down list the filter you want to apply.

## Filter category

A Category identifies, gathers and summaries a set of statistical values calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.





In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.




## Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly

visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

**Tip:** You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.



### Spots Wizard Buttons

#### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

#### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

#### Button **Cancel**

If you do not want to create Spots click on **Cancel**.

### 11.13.1.5 Spot Region Type

This tab only appears, if **Different Spot Sizes (Region Growing)** is checked ([Algorithm Settings](#)).

The region method for segmentation in Imaris starts with defined seed points. You use Spots as seed points for the region growing that is performed in the next steps. During the region growing process around each seed point the region grows until a border stops the growing.

### Spots Regions from

#### **Absolute Intensity**

#### **Local Contrast**

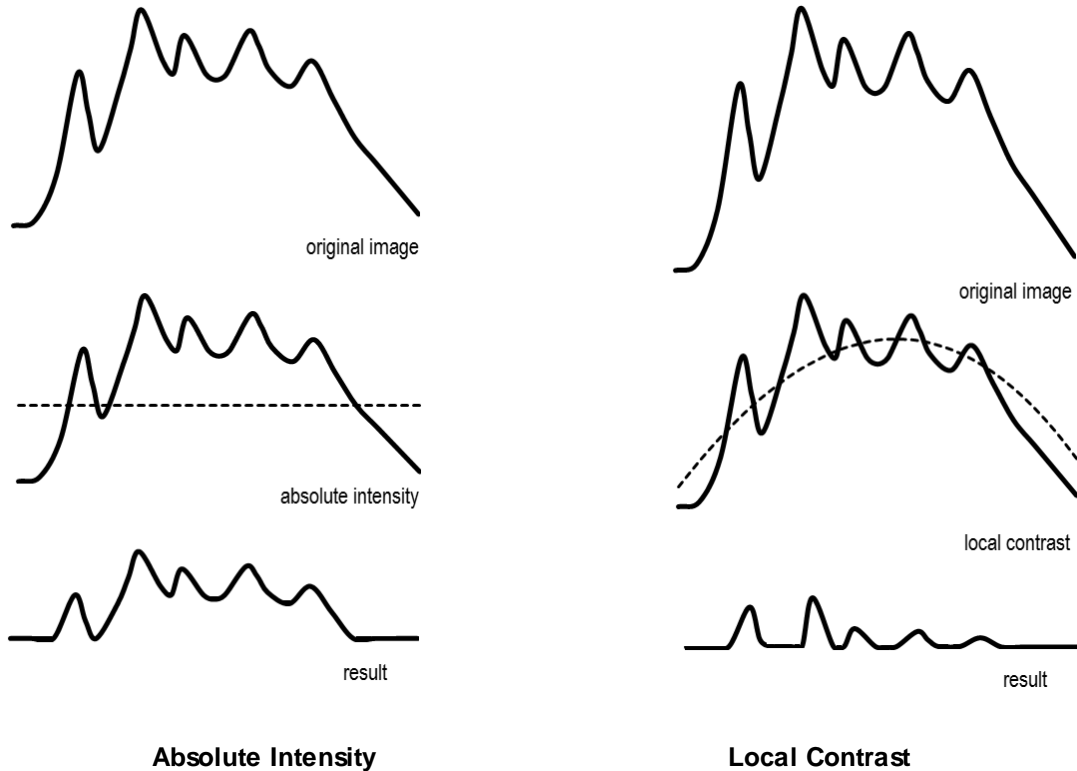
You can set the border using the Absolute Intensity or the Local Contrast as criteria. In both cases as next step the selected channel is displayed in the viewing area.

#### **Local Contrast**

This option should only be used when the **Absolute Intensity** method does not properly segment all objects. Please note: Local Contrast applies a Gaussian filter in order to estimate the intensity value of each voxel. Baseline Subtraction is then performed by subtracting the variable background from every voxel in the image. As a result, it uses more memory than the Absolute Intensity method.

Gaussian Smoothing replaces the voxel intensity value with a newly calculated value. The new value is a

weighted sum, defined using a Gaussian function, of the voxel and its neighbouring voxels. The degree of the smoothing is directly dependent on the shape of this kernel, which in turn is a function of the standard deviation. A Gaussian kernel is a symmetric bell-shaped curve.



## Spots Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

### Button **Cancel**

If you do not want to create Spots click on **Cancel**.

## 11.13.1.6 Regions Threshold

This tab only appears, if **Different Spots Sizes (Region Growing)** is checked ([Algorithm Settings](#)).

## Spots Regions




The working channel is visualized in the viewing area, the histogram is displayed in the properties area. Regions above the threshold are displayed in white, regions below threshold are displayed in black. Depending on the previously selected detection method (Absolute Intensity or Local Contrast) refer to Absolute Intensity or Local

Contrast.

## Threshold

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.**

## Absolute Intensity

### Automatic

In the working channel a copy of the intensity histogram of the source channel is displayed. Regions above the threshold are displayed in white. Now you adjust the threshold of the working channel. Edit the data field or adjust the threshold manually using the mouse (click & drag the line in the histogram). In the next step the regions will grow until they fill the white regions and reach the border.

## Local Contrast

### Automatic

In the selected channel the regions with a local contrast brighter than the background are displayed in white. Now you adjust the threshold value of the local contrast channel. Edit the data field or adjust the threshold manually using the mouse (click & drag the line in the histogram). In the next step the regions will grow until they fill the white regions and reach the border.

## Diameter from

### Region Volume

The Spot(s) radius is calculated from the region volume (the region volume and the Spots volume are equal).

### Region Border

The radius of the calculated Spot(s) is the smallest distance from each Spot center to the region border.

### Create Color Coded Region Channel

Additionally the Spots the Region Channel is displayed. Each region has a different color.

## Spots Wizard Buttons

### Button Back

With **Back** you return to the previous window of the Creation Wizard.

## Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

## Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

## Button **Cancel**

If you do not want to create Spots click on **Cancel**.

### 11.13.1.7 Edit Spots

**Tip:** In the Surpass view be sure that the mouse is in the pointer mode **Navigate**. Switch between the two pointer modes **Select/Navigate** with the **ESC**-key.

#### AutoDepth Draw

##### Intersect with

The auto depth function will automatically calculate the Spots depth. Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Spots.

##### all visible Channel

**specific Channel** - selected channel will be used for Spots depth computation

##### solid Objects

Select as reference for the Spots depth the first visible channel, all visible channels, or other solid objects.

#### Process Selection

	<b>PC:</b>	<b>Mac:</b>
Select spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click
Add new spot	Shift + left-click	Shift + click
Delete spot	Left-click to select, then click on the button Delete or Shift + left-click on a selected spot	Click to select, then click on the button Delete or Shift + click on a selected spot
Change spot diameter	Mouse wheel	Mouse wheel

## Button **Duplicate**

Click this button and all selected Spots appear in a new Surpass Group in the Surpass Tree (Spots Selection).

## Button **Recenter**

Click on the Recenter button results in change of the selected Spots position. The new center is placed in position of the local intensity maximum in the vicinity of the initial spot center.

## Button **Delete**

Click on this button to delete the selected Spot(s).

**Button Clear**

Click this button to clear the Spots selection.

**No Spots Selected/Selected Spots****Position X: Y: Z: [um]**

Display of the Spots position.

**Diameter [um]**

The initial Spots diameter of a new Spot is the minimum diameter in the creation process. With this parameter you can change the size.

**Time Index**

The number indicates the current time point in the series for the selected object.

**Spots**

In Imaris it is possible to merge two or several Spots items into one Spots item.

**Button Merge ...**

This button is only available if you have two or more Spots items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Spots X" displays (see below).

**Window: Merge selected Objects to "Spots X"**

Check the boxes in front of the desired Spots items or use the button **Select All**.

**Button Clear All**

Use this button to clear the Spots selection.

**Button OK**

If you want to merge the Spots click on **OK**.

**Button Cancel**

If you do not want to merge the Spots click on **Cancel**.

**Mask Properties**

Sometimes it is useful to apply a certain function not on the entire image but only in a region of interest (ROI). As region of interest you can choose Spots.

**Mask All... Button****Mask Selection ... Button**

Here you decide, if the masking is based on selected objects or on all objects. The window Mask Channel displays (see below).

**Window: Mask Channel****Channel Selection**

Select the channel (destination channel) you want to mask with the Surfaces (ROI).

### **Duplicate Channel before applying Mask**

When this option is selected, the channel will be duplicated before applying Mask.

## **Mask Settings**

### **Random Color Map**

#### **Constant Inside/Outside**

Define, if you want to use a random color map or if you want to set a constant inside/outside.

### **Set Voxels outside Surface to:**

The default value is 0.000, no voxels outside the region of interest are displayed.

### **Set Voxels inside Surface to:**

If you leave the box unchecked the original channel intensities inside the region of interest are displayed. If you check the box and type in 0.000, no voxels inside the region of interest are displayed (no masking!).

### **Apply to all Time Points**

Only available if you work with a time data set. Check the box if you want to apply the mask to all time points. If the Surfaces changes size and position through the time points the applied mask changes size and position, respectively.

## **Spots Wizard Buttons**

### **Button Back**

With **Back** you return to the previous window of the Creation Wizard.

### **Button Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### **Button Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

### **Button Cancel**

If you do not want to create Tracks click on **Cancel**.

### **11.13.1.8 Tracking**

Tracking allows objects to be traced throughout sequential frames of a time data sets, so each object maintains a unique identity in the output measurements.

This tab only appears, if **Track Spots (over Time)** is checked ([Algorithm Settings](#)).

## **Algorithm**

The Tracking tab provides several tracking algorithms to choose from. The motion models are used to predict the future positions of Spots. Based on these predicted positions, the best matches to candidate Spots can be determined. All of the algorithms except for Connected Components use a Linear Assignment algorithm and Total Cost function to find the best track connections. The first two algorithms compute cost based on change in position (distance), while Autoregressive Expert mode combines changes in position and intensity to compute total cost.



### Brownian Motion

This algorithm models the motion of each Spot as a Brownian motion. This model is appropriate if your Spots perform similar to a Brownian motion (the random movement of Spots with very frequent changes in direction and no discernible pattern).

The only prediction applied is that the Spot does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

### Autoregressive Motion

This algorithm models the motion of each Spot as an autoregressive AR1 process. This model is appropriate if your Spots perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the Spot will move again the same distance and in the same direction. The user-specified **Maximum Distance** value is the distance in which the Spot is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

### Autoregressive Motion Expert

This algorithm models the motion of each Spot as an autoregressive AR1 process (see above). It is suitable for tracing of multiple objects with adjacent or crossing paths, especially if the neighboring objects have a stable (but different) intensity. In Autoregressive Motion Expert the algorithm called Total Cost determines the most appropriate connections for tracks by seeking the lowest Total Cost. In this case, the Total Cost algorithm combines Intensity Cost and Distance Cost functions (see Intensity Weight below).

### Connected Components

For this algorithm, Spots in adjacent time-points are considered connected if the Spot spheres occupy some of the same space (the spheres would overlap if two time-points were merged into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. All the object with the overlap will be assigned the same track ID.

Connected Components and Lineage are the only algorithms that automatically handle lineage (spots that diverge). It will only work with images that have excellent time resolution, because the Spot cannot ever jump a distance larger than its own size.

### Lineage

The Lineage model is intended for events such as cell division in which the splitting of objects is to be analyzed. This algorithm models the motion of each object as an autoregressive AR1 process (refer to Autoregressive Motion above). The object data is then compared so that only the objects that undergo splitting are connected, and assigned the same track ID. Objects that pass in close proximity, or overlap, are not associated. This is in contrast to connected components, where any objects that overlap are determined and considered connected.

## Parameters

### Max Distance

A maximum distance disallows the connection between a spot and a candidate match if the distance between the predicted future position of the spot and the candidate position exceeds the defined maximum distance.

The parameter is only available if you select Brownian Motion, Autoregressive Motion, or Autoregressive Motion Expert (see above).

### Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Track fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order for a track to continue.

This parameter is only available if you select Autoregressive Motion or Autoregressive Motion Expert (see above).

## Fill the gaps with all detected objects

This algorithm allows tracks to be continued, even if the object was not detected in periods of up to two consecutive time points along the trajectory. If this option is selected the algorithm performs the optimization of the object expected position for the particular time point. The track is generated by connecting objects assuming their optimized expected positions.

## Intensity Weight

Based on the information obtained from the measured Spots intensity and position, the Intensity Weight function is used to balance the relative influence of the Intensity and Distance costs. The Total Cost is determined by combining the Intensity term and Distance term, and minimization of the Total Cost is used to determine the best track from all feasible ones.

$$\text{TotalCost} = \sum(\text{all connections}) \text{DistanceCost} + \text{IntensityWeight} * \text{IntensityCost}$$

The parameter is only available if you select Autoregressive Motion Expert (see above).

## Spots Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

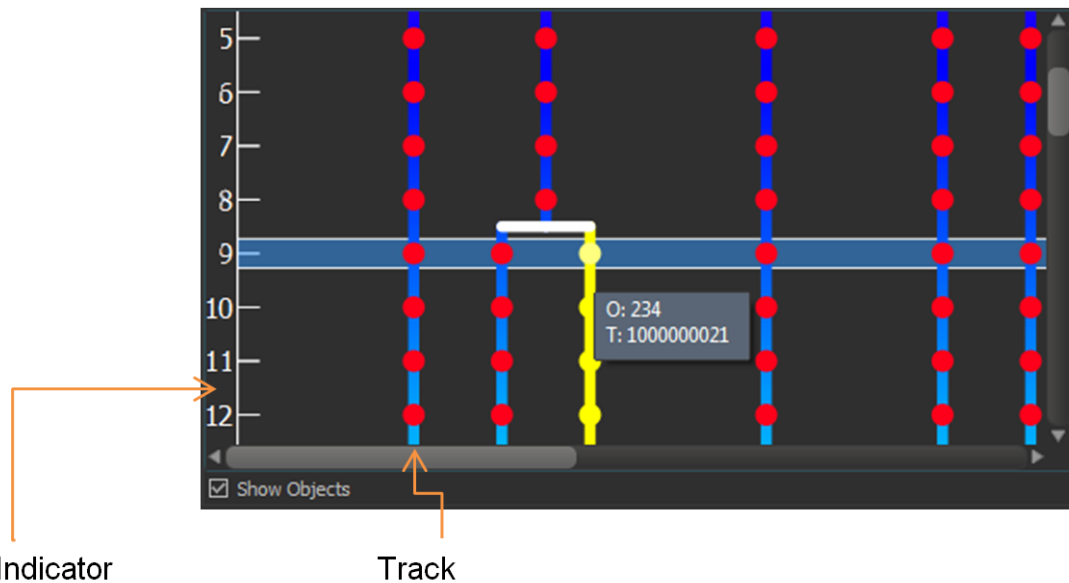
### Button **Cancel**

If you do not want to create Tracks automatically click on **Cancel**.

## Lineage

## Table Layout

The track editor table:



Time Indicator

Track

**Time indicator**

The axis on the left displays the frame number.

**Track**

Each track is displayed in a vertical format by default. If connected components or Lineage algorithms are used there may be a number of branch points. The branch points indicate where objects diverge, e.g. cells undergoing division.

**Object**

Each data-point is displayed as an object in the track with a unique Object ID. The object will retain its unique ID number- even if, for example, it is connected manually to another track.

If the mouse pointer is on an object, a tooltip appears that identifies the Object ID number (O) and track ID number (T). You can untick the Show Objects checkbox to hide objects from the display.

**Interactive Selection**

When you click on an individual column, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (column in the table) is highlighted.

In addition, the Statistical Tab and Filter Tab can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.

For multiple selections press Ctrl+click (Mac: Command+click).

**Visualization options****Zoom**

Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table. Using the mouse wheel will have the same effect.

**Pan**

To move the track editing table click and hold the right mouse button while dragging the mouse. Release the right mouse button to place the table on the chosen location.

## Mouse Selects

This option allows you to select objects or tracks as follows:

### Object

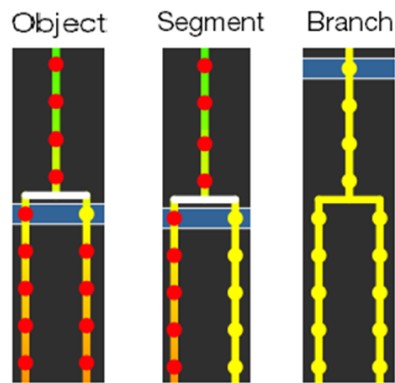
Click Object to select an individual object, or number of specific objects. To connect two unconnected objects, simply select both objects and click the Connect button. To disconnect two connected objects, select both objects and click the Disconnect button.

### Segment

Click Segment to select a specific segment (between branch points).

### Branch

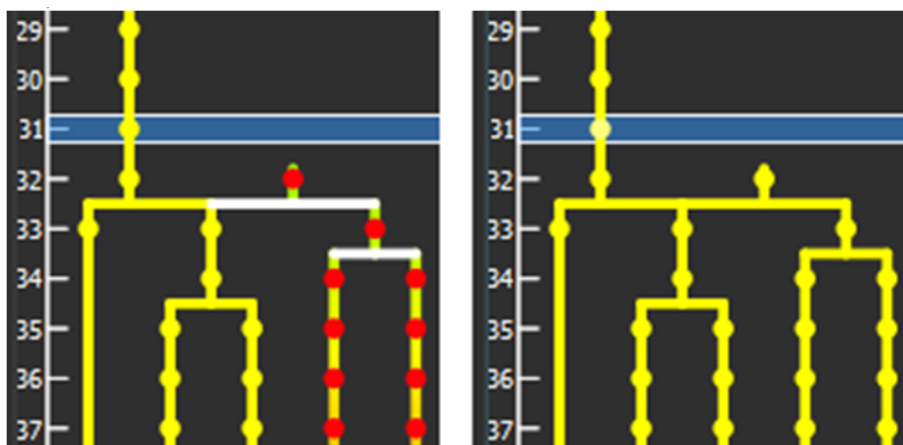
Select Branch to select the segment and the branched segments and objects that appear later in time only.



### Pivot

Pivot enables selection of a segment and all segments and objects that are linked afterwards. This includes objects later in time, and also backwards in time.

For example, selecting branch (left) selects only the connected segments and objects that are later in time. Selecting Pivot (right) all connected segments and objects to be selected.



### Track

Select track to select a complete track.

## Editing

Track Editor enables you to modify your tracks.

**Connect Button**

Click this button to connect two existing objects. Select two objects in two different tracks at the subsequent time points (just one object per time point) and click on the button Connect. The connection is automatically created between all selected objects.

**Disconnect Button**

Select an object and click on the button Disconnect. The connection between the two selected objects in the time sequence is broken.

**< Button**

Click this button to move a selected track left. When axis is inverted (horizontal) the selected track moves up.

**> Button**

Click this button to move a selected track right. When axis is inverted (horizontal) the selected track moves down.

**<< Button**

Click this button to move a selected track all the way to the left. When axis is inverted (horizontal) the selected track moves to the top.

**>> Button**

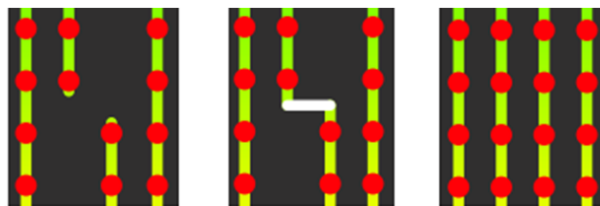
Click this button to move a selected track all the way to the right. When axis is inverted (horizontal) the selected track moves to the bottom.

**Flip**

The Flip button may be used (with Pivot mode selected) to flip the order that the connected branched segments and objects are displayed in about the pivot point.

**Reorganize**

The Reorganize button can be used after editing of tracks is complete so that tracks are displayed in a clear and logical format without affecting the track arrangement during the editing process.



Editing tracks. Connection added. Tracks reorganized.

**Invert Axis**

Click the Invert Axis button to switch between displaying the tracks vertically (default) and horizontally.

**11.13.1.9 Classify Tracks**

Track filtering is incorporated into the creation process. This tab only appears, if **Track Spots (over Time)** is checked ([Algorithm Settings](#)).

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up

criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

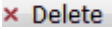
**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.**

## Filters

### Add Button

To add a new filter, click on the button Add  and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

### Delete Button

Select a filter in the list with a left mouse click and click on this button  to delete the filter.



















## Filter Type

Select in the drop-down list the filter you want to apply.

## Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.





In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.

## Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.




In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green

circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.**

## Spots Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Spots and display the Spots in the viewing area.

### Button **Cancel**

If you do not want to create Tracks click on **Cancel**.

## 11.13.1.10 Complete ROI

This tab only appears, if **Process entire Image finally** is checked ([Algorithm Settings](#)).

## Region of Interest

The parameters you have set up will now be used to compute Spots (and Tracks) for the entire data set. Depending on the size of the data set this may take a while.

## Spots Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

This button is grayed in the last window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to end the calculation and display the results in the viewing area.

### Button **Cancel**

If you do not want to create Spots/Tracks click on **Cancel**.

## 11.13.2 Tab Settings

On the tab Settings you can adjust the rendering quality to produce faster performance of the display when the number of Spots is very large. The size of the Spots can be changed via the Radius Scale box.

## Spots Style/Quality

### **Off**

Select this option to disable the visualization of the Spots.

### **Sphere**

This option displays the Spots as as spheres.



**Center Point**

This option displays the Spots as center points.

**Please note: The computation of Spheres is time consuming. If you have many Spots in your data set Imaris automatically switches to the display mode Center Points. If you would like to switch to the Spheres mode again select Sphere and confirm the changes.**

**Tracks Style/Quality**

The box Style/Quality has settings that determine the display of connections between the Track objects.

**Off**

Select this option to disable the visualization of the Tracks.

**Line**

This option displays the tracks connections dawn as lines.

**Cylinder**

This option displays the tracks connections dawn as cylinders.

**Width****Diameter**

Depending on the previous selection you adjust the width of the line or diameter of the cylinder.

**Render Quality**

When you select Cylinder (see above) the Render Quality is adjustable using the slider.

**Tracks Path****Color Editor**

Select a desired color for the Track display.

**Displacement**

Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

**Radius Scale**

Edit field for the displacement vector radius.

**Color Editor**

Select a desired color for the displacement arrow.

**Dragon Tail**

Check the box Dragon Tail and instead of showing a Track in its entire length on all time points, only parts of it are shown depending on the selected time points.

**Slider**

Adjust the number of time points using the slider.

**Subvolume**

The option Subvolume is used to identify the different objects and distinguish them from the surrounding by visualizing the volume around a created object. It extracts a subset of the volume data set by using the specified axis-aligned limits.

The border around each object is defined independently and corresponds to the regions equal to bounding box of the created object.

## MIP

In the Maximum Intensity Projection mode only the point with maximum intensity of all layers along the viewing direction displayed. If your data set has more than one channel, the combination color is displayed.

## Blend

This option displays the subvolume around a certain object in the Blend mode. In the blend mode, all values along the viewing direction including their transparency are used for the calculation.

### 11.13.3 Tab Rebuild

#### Rebuild

Imaris stores the list of operations and the mapping of parameters. Select from the list on which step of the [Spots Creation Wizard](#) you want to re-enter the creation process.

For details please refer to Section:

Menu 3D View - Spots - Creation Wizard - [Algorithm](#)

Menu 3D View - Spots - Creation Wizard - [Region Growing](#)

Menu 3D View - Spots - Creation Wizard - [Tracking](#)

#### Button **Rebuild Objects**

Click on this button to start the rebuild.

#### Creation Parameters

The Creation Wizard saves parameters and processing instructions so the Spots computation can be repeated for the same region, a different region, or the entire image. Creation Parameters commands are listed.

#### Store Parameters for the Batch

The Store Parameters for the Batch button allows you to save the complete set of the creation parameters and all relevant settings shown in the list. In the Store Creation Parameter window, enter a descriptive name for your creation parameters and select the required store location.

There are two options to store your creation parameters

- **Arena**
- **Favorite Creation Parameters**

#### Arena

When you select this option a new Arena item is automatically created within the Arena view. The Arena view is updated, a new item, [Creation Parameters](#), is created and displayed.

Once stored, Imaris [Batch](#) applies the creation parameters set to all images within the selected Group.

#### Favorite Creation Parameters

To help you organize and find your most used creation parameters more easily, we advise you to store them by selecting the Favorite Creation Parameters option.



When you select this option the Creation Parameter file is automatically added to the list that is displayed under the Edit/Preference/[Creation Parameters](#). This option allows you to use the creation parameters in future on another data set. These saved parameters can then be imported and re-used on any new data set should you wish to perform an identical analysis sequence. This option is also useful for sharing the parameters with other users to allow them to perform the same analysis on their own data sets.

Within the Surpass view storing allows the parameters to be reused or modified. In addition, this option is useful to create and share the creation parameters with other people and allow them to perform their own analysis on it.

Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the creation wizard.

When you save the Creation Parameter file, it is automatically added to the list which is displayed under the [Edit/Preference/Creation Parameters](#).

Click on the Store Parameters for the Batch button opens the Store Creation Parameter window.

### Store Creation Parameter

Refer to [Store Creation Parameters](#)

#### 11.13.4 Tab Edit

Once the Spots creation wizard is completed, use these buttons for manual editing of the Spot objects. It allows you:

1. To add Spots and incorporate them into analysis.
2. To delete selected Spots.
3. Create tracks from Selection
4. Merge selected spots.

**Tip: In the Surpass view be sure that the mouse is in the pointer mode Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.**

### Add/Delete (Cursor intersects with):

#### All visible Channels

The All-visible channel option places a spot at the cursor position while the depth through the volume is determined by the most intense voxel of all the channels

#### Specific Channel

Select the required channel from the drop-down list and selected channel will be used for Spots computation. A new spot will be placed at the most intense voxel of the selected channel, even if that channel is overlaid with other channels of higher intensity.

#### Surface of Object

Select this option and a new spot will be placed on the surface of an object.

#### Center of Object

Select this option and a spot will be placed in the center of the object.

For Spots object (also Vesicles and dendrite/spines beginning, branching, attaching, terminal points): Select this option and a new measurement point is placed at the center of the sphere.

## Selection

Select between a pointer, circle or box shaped selection.

The pointer mode provides the normal pointer function making it easy to select specific individual spots,

In circle mode a 2D circle appears around the mouse pointer on the screen. Press left-click to select all the spots that have center points within the circle. Press Ctrl+left-click will add spots to the existing selection, if some unselected spots are within the circle, otherwise it will deselect all spots within the circle. Use the mouse wheel to adjust the size of the on-screen selection circle.

In box mode a 3D box appears on the screen around the mouse pointer. Use left-click or Ctrl+left-click the spots that have center points within the 3D box to add or remove them from the selection.

	PC:	Mac:
Select Spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click
Add new Spot	Shift + left-click	Shift + click
Delete Spot	Left-click to select, then click on the button Delete or Shift + left-click on a selected Spot	Click to select, then click on the button Delete or Shift + click on a selected Spot
Change Spot diameter	Mouse wheel	Mouse wheel

### Clear Button

Click this button to clear the Spot selection.

### Delete Button

Click on this button to delete the selected Spot(s).

### Duplicate Button

Click this button and all selected Spots appear in a new Surpass Group in the Surpass Tree (Spots Selection).

### Recenter Button

Clicking on the Recenter button changes the position of the selected Spots. The new center is placed in the position of the local intensity maximum, which is in the vicinity of the initial spot center.

### Mask button

Click this button to define the mask functions for the current selection- see [Mask Channel](#) section for more details.

### Create Track from Selection Button

Clicking this button creates a track for the selected Spot(s) - see the [Create Track from Selection](#) section for more details

## No Spot Selected/Selected Spot

### Position X: Y: Z: [um]

Display of the Spot position.

### Diameter [um]

The initial Spots diameter of a new Spot is the minimum diameter in the creation process. With this parameter you can change the size.

### Time Index

The number indicates the current time point in the series for the selected object.

## Edit All

### Merge selected Objects to "Spots X" Button

Click this button to combine multiple spot objects into a single spot item. See the [Merge selected Objects to "Spots X"](#) section for more details.

### Mask... button

Click this button to define the mask functions for the image- see [Mask Channel](#) section for more details.

## Manual Tracking

The Manual Tracking option can either be used when automatic tracking algorithms require modifications and adjustments, or to create an entire track from scratch.

The manual tracking option allows you to track objects within a time-resolved data set by manually specifying the object location in each time point. Essentially, the manual tracking is done by clicking on the structure in the image, which serves as a visual reference (template).

This option can be activated by clicking in the box **Auto-connect to selected spot**.

To start manual tracking, move the time slider to the first time point of the feature you want to track. Then use the pointer to Shift and then left click on the feature in the data set.

After each mouse click, the time slider advances one frame, allowing you to see the subsequent image of the temporal stack. To continue manual tracking, keep clicking on the structure on the image. As you add more manual tracking points by clicking within the viewing area, a track is created allowing you to follow the moving features, frame by frame and from point to point, until you've built a complete motion path.

**Please note: Manual tracking behaviour is governed by the Selection mode under [Track Edit tab](#).**

### Auto-connect to the selected spot box

Select the option to add all selected or added objects to one track and advance to next time point when a new object is added or an existing is selected.

**Please note: Objects are always added at the current time point (t).**

The result of Manual tracking can be very different depending on which selection is chosen in the [Track Edit](#)

tab.

If the option **Track** is selected, a connection is established between added object at time point  $t$  and the last added object of the selected track at time point  $t-1$ .

If the option **Spots** is selected, a connection is established between the selected object at the immediately preceding time point ( $t-1$ ) and the newly added object at time point  $t$ .

**Please note: If there is no the immediately preceding time point, a new track will be constructed.**

The box on the drawing plane represents the created object diameter. The diameter of the object can be changed, during the manual tracking, by using the mouse wheel. Use the mouse wheel to increase/decrease the radius of the created object.

### **Enable Delay before auto-advancing**

The Enable Delay before auto advancing option sets a delay before proceeding to the next time point. When this option is selected, the created object will be displayed for the duration of the delay in the viewing area. The visual feedback of the created object gives you an opportunity to adjust it.

The delay duration is adjustable under the Edit menu/Preference/Surpass. You can specify the value between 0.05 and 5 seconds.

**All actions in the Draw , Edit  and Edit Tracks  tabs can be Undone/Redone by selecting the [Undo/Redo](#) command or by pressing **Ctrl+Z/Ctrl+Y**.**

### **11.13.4.1 Mask Channel**

#### **Channel Selection**

Select the channel (destination channel) you want to mask with the Surfaces (ROI).

#### **Duplicate Channel before applying Mask**

When this option is selected, the channel will be duplicated before applying Mask.

#### **Mask Settings**

##### **Random Color Map**

##### **Constant Inside/Outside**

Define, if you want to use a random color map or if you want to set a constant inside/outside.

##### **Set Voxels outside Surface to:**

The default value is 0.000, no voxels outside the region of interest are displayed.

##### **Set Voxels inside Surface to:**

If you leave the box unchecked the original channel intensities inside the region of interest are displayed. If you check the box and type in 0.000, no voxels inside the region of interest are displayed (no masking!).

##### **Apply to all Time Points**

Only available if you work with a time data set. Check the box if you want to apply the mask to all time points. If the Surfaces changes size and position through the time points the applied mask changes size and position, respectively.

Button **OK**



If you want to apply the mask click on **OK**.

Button **Cancel**

If you do not want to apply the mask click on **Cancel**.

#### 11.13.4.2 Merge selected Objects to "Spots X" Function

Check the boxes in front of the desired Spots items or use the button **Select All**.

Button **OK**

If you want to merge the selected spot objects click on **OK**.

Button **Cancel**

If you do not want to merge the selected spot objects click on **Cancel**.

#### 11.13.4.3 Create Track from Selection

##### 1. Select the Source channel

##### 2. Spot Detection

###### Estimated Diameter

This is the diameter of the inserted Spots. Spots with a diameter smaller than this are not detected.

There are two options for a Surface creation Threshold:

###### Background Subtraction

If checked, the background will be removed prior to spot detection.

##### 3. Tracking algorithm

The Tracking tab provides several tracking algorithms to choose from. The motion models are used to predict the future positions of Spots. Based on these predicted positions the best matches to candidate Spots can be determined.

There are two algorithms to choose from:

###### Brownian Motion

This algorithm models the motion of each Spot as a Brownian motion. This model is appropriate if your Spots perform similar to a Brownian motion (the random movement of Spots with very frequent changes in direction and no discernible pattern).

The only prediction applied is that the Spot does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

###### Autoregressive Motion

This algorithm models the motion of each Spot as an autoregressive AR1 process. This model is appropriate if your Spots perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the Spot will move again the same distance and in the same direction. The user-specified **Maximum Distance** value is the distance in which the Spot is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

##### Lineage

##### 4. Max Distance

The maximum search distance that a tracked object are looked for is specified with the Max Distance value. The parameter Maximum Distance disallows connections between a Spot and a candidate match if the distance between the predicted future position of the Spot and the candidate position exceeds the maximum distance.

## 5. Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

## 6. End Time Point

The parameter specifies the last time point for the tracking algorithm implementation.

**OK** Button

**Cancel** Button

## 11.13.5 Tab Edit Tracks

### Mouse Selects

This option allows you to select an object within an image according to its object type by a single mouse click. To edit the properties of multiple objects simultaneously, select the required object type.

There are the following options:

**Object**

**Segment**

**Branch**

**Pivot**

**Track**

The differences between these options is described in [Tracking](#).

**Please Note you can change between mouse selects options using the first letter of each option as a keyboard shortcut i.e. Object: O, Segment: S, Branch: B, Pivot: P and Track: T.**

### Table Layout

In the track editor a horizontal line represents each track. Each horizontal line is composed of circles, representing objects, and line segments which connect a sequence of objects.

The first row of the track-editing table displays the timeline. Each number represents a time point in the series.

The second row of the track-editing table displays the **object name**.

Multiple object types can be tracked easily since every object type appears in different colors.

### Object ID

If the mouse pointer is on the object, a tooltip appears that identifies the object ID. The ID numbers of the objects are sequentially ordered column-wise from the top to the bottom of the table. The object maintains its unique ID number independent of the track being allocated to.

If the mouse pointer is on the connection, a tooltip appears that identifies the track ID number.



## Interactive Selection

When you click on an individual row, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (row in table) is highlighted.

In addition, the [Statistical Tab](#) and [Filter Tab](#) can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.

For multiple selections press Ctrl+click (Mac: Command+click).

## Visualization options

### Zoom

Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table.

Using the mouse wheel will have the same affect.

### Pan

To move the track editing table click and hold the right mouse button while dragging the mouse. Release the right mouse button to place the table on the chosen location.

### Time indicator

The first row of the track-editing table displays the timeline. Each number represents a time point in the series. The current time point is indicated by vertical line highlighted with in current selection color.

To modify the time point, do one of the following:

1. Moving the slider in the Time bar modifies its position in time and changes the time point in the track editor.
2. Clicking on the time point within the track-editing table automatically moves the time line indicator to the corresponding point.

Double-clicking on the time indicator, fixes the position of the current time point at the center of the track-editing table, regardless of the selected zoom or pan options)

## Editing

Track Editor has several features that help you modify your tracks.

### Connect Button

Click this button to connect track components such as objects or segments as selected under Mouse Selects. For example, select objects in different tracks at the subsequent time points (just one object per time point). The connect and disconnect buttons will update to show the number of objects selected. Confirm the selection, then click on the button Connect (you can also press the "c" key as a shortcut). The connection is automatically created between all selected objects.

### Disconnect Button

Use this option to disconnect track components such as objects or segments as selected under Mouse Selects. For example, select object(s) to be disconnected. The connect and disconnect buttons will update to show the number of objects selected. Confirm the selection, then click on the button Disconnect (you can also press the "d" key as a shortcut). The button will update to show the number of objects selected. The connection between the selected objects in the time sequence is broken.

### < Button

Click this button to move a selected track left. When axis is inverted (horizontal) the selected track moves up.

## > Button

Click this button to move a selected track right. When axis is inverted (horizontal) the selected track moves down.

## << Button

Click this button to move a selected track all the way to the left. When axis is inverted (horizontal) the selected track moves to the top.

## >> Button

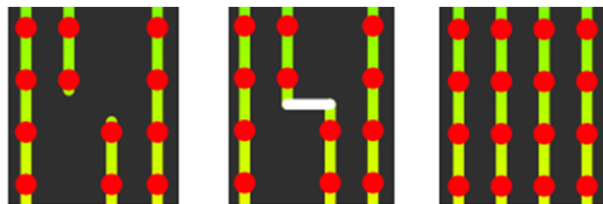
Click this button to move a selected track all the way to the right. When axis is inverted (horizontal) the selected track moves to the bottom.

## Flip

The Flip button may be used (with Pivot mode selected) to flip the order that the connected branched segments and objects are displayed in about the pivot point.

## Reorganize

The Reorganize button can be used after editing of tracks is complete so that tracks are displayed in a clear and logical format without affecting the track arrangement during the editing process.



Editing tracks. Connection added. Tracks reorganized.

## Invert Axis

Click the Invert Axis button to switch between displaying the tracks vertically (default) and horizontally.

## Correct Drift

Refer to [Drift Correction](#).

Focus tracks mode may be useful when editing tracks- refer to the [Cells Edit Track](#) section.

## Algorithms

The Drift Correction is calculated based on the two algorithms, translational drift and translational and rotational drift.

## Translation

Upon translation, the object position is transformed to a straight line of identical orientation. Within the same time point the relative distance of the objects is preserved.

## Translation and rotation

Upon translation and rotation, the object position is transformed based on the image polar coordinates. Within the same time point the relative distance of the objects is preserved.

**Please note: The number of the selected tracks affects the algorithm calculation.**

Selection of a single track gives a complete description of a translation.

Selection of two tracks gives a minimum description of a translation and rotation algorithm (planar rotation).

A better fit between the time points is achieved when three or more tracks are selected.

### Result DataSet size

After completion of the drift correction, the algorithm uses the final position of the initial and resulting image to create a final image. The new image coordinates are calculated in accordance with the size of the original and new image.

Select the Result data set option to determine which new image part will be displayed.

### Crop largest common region

The final image will be the size of the common overlap between the original and the drift corrected image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

### New size equal to current size

The final image will be the same size as the original image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

### Include entire result

The final image will be the size of the combined area of the original and corrected image.

Selecting the appropriate Result data set size may speed up the data visualization. This is especially important with (with large data sets.)

### Correct spots positions

Activate this parameter and the spots are automatically re-positioned after the drift correction. If this option is not activated only the image volume is aligned.

**All actions in the Draw , Edit  and Edit Tracks  tabs can be Undone/Redone by selecting the [Undo/Redo](#) command or by pressing Ctrl+Z/Ctrl+Y.**

### 11.13.6 Tab Filter

**Please note: Before using you have to add a filter to the list (see below: button Add)!**

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the

defined interval will be considered.

**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.**

## Filters

### Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

### Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.



















## Filter Type

Select in the drop-down list the filter you want to apply.

## Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.





In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.

## Lower and Upper Threshold




Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.




In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

## Automatic/Manual A/M button



The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within the threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and a reduced/enlarged set of the selected objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Spots) is displayed at the bottom of the threshold histogram.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.**

### Button Duplicate Selection to New Spots

Click on this button and all selected objects are copied to a new Surpass Group. The new group displays in the Surpass Tree.

## 11.13.7 Tab Statistics

Spots statistics are automatically computed. For the statistical values please refer to the Section Menu Edit - Preferences... - Statistics - [Spots](#). The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different data sets through summarized statistical values and graphical data presentations.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:

### a) Overall

### b) Detailed

### c) Selection

In the statistical data set the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

To sort the columns click on the arrow in column heading. Click again to reverse the sort.

















## Overall Tab

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

## Category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.

## Detailed Tab

A pull down list offers three types of **Detailed** statistical calculations:

### Specific Values

### Average Values

### Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.


Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

### Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

### Specific Values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Object [Statistical Annotation](#) option  is available for Specific values parameters.

### Average values

The Average table contains values determined on the basis of all values within a set of the selected variable.

Columns are labeled as follows:

Min (Minimum)- the smallest value within a set of values of the selected variable

Max (Maximum)- the largest value within a set of values of the selected variable

Mean – value of the sum of all values divided by number of values within a set

Std Dev (Standard deviation)- a measure of the values distribution around the mean values

Median- the middle value of the sorted set of variables

Sum- the sum of all values within a variable set

Count- number of values within a set

Unit- variable unit

Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

### Statistical variable classification:

1. Statistical variables associated with objects (Cells, Nucleus, Vesicles -volume, area, intensity values, position, speed, displacement) have values at every time point. Therefore for these variables the Average values are displayed per time point. The changing the time points, statistical variable values and images are consequently changed.
2. Statistical values with data associated with Tracks (track duration, track volume mean, track displacement length, track intensity) are not time dependent.

### Selection Tab

A pull down list offers three types of statistical calculations from a Selection:

**Specific Values**

**Average Values**

### Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time

point. Altering the time point changes the variable value and the image.

Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

### Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

### Specific values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Object [Statistical Annotation](#) option is available for Specific values parameters.

### Average values

The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

### Buttons Overview

	PC:	Mac:
Select Spot	Left-click	Click
Select multiple Spots	Ctrl + left-click	Command + click

### Buttons at the bottom of the Statistics tab

Button **Settings** -Configure a List of visible statistics values.

Click on the button **Settings** and you switch to the window Preferences - Statistics - [Spots](#).

Button **Search** - Search by object ID.

Type in the object ID in the data field. Click on the button **Search**.

- Highlights the object in the viewing area
- Highlights the object on the Detailed Tab
- Displays of the values on the Selection Tab

Button **Spots** - Duplicate Selection to new Spots.

Click on this button and all selected elements are copied to a new Spots object. The new Spots appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Statistical Annotation** 


Click this button and an object statistical annotation will be automatically generated in the viewing area. This option is available only when **Specific** is selected.

Button **Export** - Export Statistics on Tab Display to File.

Only the statistics that are shown within the current tab will be exported.





Button **Export**  - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.

All of the statistics (that are enabled in the statistics settings) will be exported for Overall and Detailed tab data. When the Selection tab is selected, pressing the Export all Statistics to File button, will export all the statistics (that are enabled in the statistics settings) of all the selected objects, not all the statistics of all the objects.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).



See also:

Menu Edit - Preferences... - Statistics - [Spots](#)

### 11.13.7.1 Statistical Annotations

Statistical annotations provides the opportunity to view and annotate image objects with the statistical data values. Using this function, the individual objects in an image are labeled with the specified statistical values and the statistical data is displayed directly in the viewing area

Imaris objects can be labeled with a set of any number of the user-defined statistical values.


The Statistical Annotation button  provides a convenient interface in order to label the object with the statistical data. Click this button  and an object statistical annotation will be automatically generated in the viewing area. The selected object is annotated for all time points with the specified statistical values.

#### How to add Statistical Annotation

In the Statistical tab choose the **Detailed** or **Selection** Tab.

In the viewing area select the object or group of objects. Change to the pointer mode Select and click on an image object that has been detected/segmented. For multiple selections press Ctrl+click (Mac: Command-click)

In the **Detailed** or **Selection Tab** a pull down list offers **Specific Values** as the type of statistical parameters. Select the statistical value associated with the particular object type you are working with.

Press the Statistical Annotation button .

In the viewing area the object label appears showing selected statistical values as a statistical annotation.

The statistical annotation shows by default the name of the selected statistical parameter and its value.


The color white displays the text of annotations associated with statistical values.

For a single object, all selected statistical parameters are placed in the same annotation box. Once the object is labeled, selecting the additional statistical parameter updates the object statistical annotation; the new parameter and its value is added in the viewing area.

If the image is a time series, the statistical annotation will be visible in the viewing area for all time points. Selecting a statistical variable that changes over time creates an statistical annotation that consequently changes with every time point.

The statistical annotation displays values always for the current time point.

The [Annotation](#) options will allow you to modify and specify the contents and format of the object labels.

To modify and adjust the statistical label click on the Icon [Annotation](#) , press Ctrl + Shift + P, or in the menu bar select File – Annotation.

## 11.13.8 Tab Color

Tab Color edit, manage and assign colors material to the Spots or Tracks.

### Object Type

Choice of the Object type is presented in a drop-down window. Select either Point (for Spots) or Tracks and depending on the chosen object type selection of the variables is listed.

For **Object Type Points** two color types are available:

[Base](#)

[Statistics Coded](#)

For **Object Type Tracks** the following color types are available:

Labels- non-labelled objects are displayed in the Base color.

[Base](#)

[Statistics Coded](#)

Track ID

[Time Mapped](#)

[Color Type-Object ID](#)

### 11.13.8.1 Color Type- Labels

The labels associated with the data set is displayed as a list.

Click **Only show labelled objects** to show only data which have labels associated with them.

### Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### 11.13.8.2 Color Type-Base

#### Base

Assign the Spots/Track color via the Color Editor. Tab Color edit, manage and assign material colors of a Spots/Track.

#### RGB

The RGB color model is an additive color model in which Red, Green and Blue color information is combined together to reproduce a selected color. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

### Light reflection

The quality of the object material is further characterized by reflection properties.

Diffusion- diffuses reflection of a matte, uneven material.

Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is



applied to the object. The default setting is no specular intensity.

Emission- light emitted from the material defines the self-illumination. The default setting is for no emission of light.

## Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

## Palette

Palette- this option provides a system of preset colors organized into color schemes.

In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, Artdeco, Autumn, Glass, Metal, Neon, Rococo, Santafe, Sheen, Silky, Spring, Summer, Tropical, Winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

### 11.13.8.3 Color Type-Statistics Coded

#### Statistics Coded

Depending on the chosen object type the Statistical variable selection is listed in the drop-down window. For example, if Point is chosen as the object type, available Statistical data will be Point linked variable. Similarly, variables for Tracks will be listed, if the object type is selected to be Tracks.

#### Statistics Colorbar Properties

Statistics colorbar are displayed in the viewing area. The upper one is the colorbar range for Points and lower one is the colorbar for the Tracks.

#### Show Colorbar

Check the box to see the statistics colorbar in the viewing area.

#### Show Title

Check this box to see the title in the statistics colorbar.

#### Show Range

Check this box to see the range in the statistics colorbar.

Font color in colorbar is adjustable by selecting a color within the color map.

## Colormap

#### Button Reset

Click the **Reset** button to set the image back to the original values.

#### Colormap

Click on the button Colormap to display a large number of predefined colormaps. If the default colormap (Spectrum) is not appropriate, you can change it by clicking on the drop-down list. The list gives a preview of what different colormaps look like.

## Colormap range

Colormap range-specify the limits for the colorbar.

**Min** - lower color limit

**Max** - upper color limit

Button **Auto**

Click the button **Auto** and the system detects the real min and max values and sets the Max. and Min. limits automatically to these values.

## Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### 11.13.8.4 Color Type-Time Mapped

#### Time Mapped

This option is only available when Tracks (see above) is selected as Object Type. The color range for the Tracks goes from blue to white to the range of time points.


#### Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### 11.13.8.5 Color Type-Object ID

Assigns a random color to every object. This is available for all surpass components.

### 11.13.8.6 Color Type-Track ID

Track ID assigns a fixed, random color to each object. This mode is useful for validating tracks as an object will have the same color through the track duration. Erroneous behavior can easily be detected and corrected using the Edit Tracks  functions. Refer to the [Tab Edit Tracks](#) section.

#### Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### 11.13.9 Mouse & Keyboard PC

#### Spots Mouse & Keyboard Functions on the Tab Edit

Select Spot	Left-click
Select multiple Spots	Ctrl + left-click
Add new Spot	Shift + left-click
Delete Spot	Left-click to select, then click on the button Delete
Change Spot diameter	Mouse wheel

See also:

Addendum - [Mouse & Keyboard PC](#)

### 11.13.10 Mouse & Keyboard Mac

#### Spots Mouse & Keyboard Functions on the Tab Edit

Select Spot	Click
Select multiple Spots	Command + click
Add new Spot	Shift + click
Delete Spot	Click to select, then click on the button Delete
Change Spot diameter	Mouse wheel

See also:

Addendum - [Mouse & Keyboard Mac](#)

### 11.14 Surfaces

The Surfaces visualization is a computer-generated representation of a specified gray value range in the data set. It creates an artificial solid object in order to visualize the range of interest of a volume object.

#### How to Add a new item Surfaces?

- Select menu 3D View - Surfaces.

The Surpass Tree list is automatically updated and the first window of the Creation Wizard displays in the Surfaces - Properties.

For information on statistics calculations used refer to [Surfaces](#)

#### 11.14.1 Creation Wizard

The Creation Wizard allows you to move forward or backwards until the optimal parameters are found.

If the image data is for a [multi-image scene](#) an option to select which source image to be analyzed is available from the Align to: drop down box.

In the first window of the Surface Creation Wizard, you find the algorithm settings. Here, you have to decide, if you want to load a set of previously stored creation parameters or to create a new one.

The most convenient way to apply the same creation parameters to different data sets is to use the [Load Parameters](#) option.

In the first window of the Creation Wizard you find the algorithm settings. Here you can choose to segment a **Region of Interest (ROI)** only, to create **Tracks** after the objects detection, use the **Seeded Region Growing** for the Surfaces detection or to **Skip automatic creation** and start [Contour Surface](#) drawing .

If you do not want to use the automatic Surface creation wizard select the **Skip automatic creation, edit manually** button.

#### **Skip automatic creation, edit manually** Button

Terminates the creation wizard and activates the manual Surface creation.

For further details please refer to Section [Algorithm](#), [Region of Interest](#), [Source Channel](#), [Threshold](#), [Classify Seed Points](#), [Classify Surfaces](#), [Tracking](#), [Edit Surfaces](#), [Classify Tracks](#) and [Complete ROI](#).

## 11.14.1.1 Algorithm

**Note:** If you do not want to use the automatic surface creation wizard select the **Skip automatic creation, edit manually** button.

### **Skip automatic creation, edit manually** Button

Terminates the creation wizard and activates the manual Surface creation.

### **Favourite Creation Parameters**

This option lets you use the identical creation parameters for multiple data sets.

The Favourite Creation Parameters option is only available if the set of the creation parameters has already been saved under the [Creation tab](#) using the Remember Parameters button.

Then you can choose to segment a **Region of Interest (ROI)** only, to create **Tracks** after the objects detection or to **Skip automatic creation**.

When the wizard is started, all stored creation parameters, corresponding to the chosen object wizard, are automatically loaded. The stored parameters contain all of the settings, options, and parameters required for image segmentation.

Expand the Favourite Creation Parameters drop-down list by clicking on it. The list shows all the creation parameters sets that have been previously saved, appropriate to the chosen creation wizard. Simply load your chosen creation parameters set, by clicking on it, and run the wizard as you have preset, without defining all of the options. On the other hand, you can customize each procedure to your own specific needs by modifying each part separately - e.g. the same channel settings, with a slightly different threshold.

### **Algorithm Settings**

#### **Segment only a Region of Interest**

A Region of Interest (ROI) is a selected sub-region of an image identified for particular processes and analyzes. Defining and processing ROI allows other parts of an image to be unchanged.

Check this box if you want to segment only a [Region of Interest](#). If option Segment only a Region of Interest is selected, in the last step of the Creation Wizard only the region of interest is processed.

#### **Process entire Image finally**

Process entire Image finally this option appears only, if Segment only a Region of Interest is checked.

Check this box and in the last step of the Creation Wizard the entire image is processed with the adjusted parameters ([Complete ROI](#)). Uncheck this box and only the region of interest is processed.

#### **Track Surfaces (over Time)**

Check this box if you want to track the resulting Surfaces over time, for details please refer to tab [Tracking](#).

### **Surfaces Wizard Buttons**

#### **Button Back**

This button is grayed in the first window of the Creation Wizard.

#### **Button Next**



Press **Next** to proceed with the next window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

#### Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

### 11.14.1.2 Region of Interest

#### Region of Interest 1

Here you define the region of interest (ROI). Parameters such as width, height and location of the ROI are used to directly specify the area to work on.

#### **X, Y, Z, Time**

#### **Min, Max, Size**

**Minimum, maximum** and **Size** of ROI coordinate values, can be specified either by entering the value in the value fields or changing the values by clicking on up-and down arrows at the right hand side of the value fields. Alternatively the location and size of the ROI can be adjusted directly in the viewing area.

Size of the ROI:

1. Click on the ROI preview rectangle arrows (change to the pointer mode Select)
2. Drag the arrows to define new coordinates for X, Y and Z.

Location of the ROI:

1. Click inside the ROI preview rectangle
  2. Drag the entire rectangle (with mouse button held) to the chosen image location.
- The numbers in the value fields are updated automatically.

#### **Memory Usage:**

Display of the memory usage for the image size within the ROI.

#### Regions of Interest

To select a particular ROI, left-click on its name in the list and the selected ROI will be highlighted within an image and its properties can be adjusted.

#### Button **Add**

Click on this button and a new region of interest appears in the list. To adjust this ROI you have to select the ROI with a left mouse click.

#### Button **Delete**

Select a ROI in the list with a left mouse click and click on this button to delete the ROI.

#### **Total Memory Usage:**

Display of the memory usage for the image size within the ROI.

#### Button **Cancel**

If you do not want to create Surface click on **Cancel**.

#### Surfaces Wizard Buttons

## Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

## Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

## Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

## Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

### 11.14.1.3 Source Channel

#### Source Channel

Select the respective source channel, from which the Surfaces are to be computed.

#### Smooth

Check the Smooth option to set up the smoothness of the resulting area. Smoothing applies a Gaussian filter to the data set.

Smoothing may require a great deal of memory if the data set is very large. Uncheck the check-box or use smaller ROI if your system does not have enough memory.

If the option Smooth is selected then the **Surface Area Detailed Level** value should be determined. Use either the automatically given value or type in a new value in the data field.

#### Threshold

There are two options for Surface creation Threshold:

##### **Absolute Intensity**

**Background Subtraction** (Local Contrast)

#### Background Subtraction

Background Subtraction applies a Gaussian filter to estimate the background intensity value of each voxel. This variable background is then subtracted from every voxel in the image; Baseline Subtraction is performed.

The width of the Gaussian filter is calculated by subtracting the diameter of the **largest sphere that fits into the Object** from the intensity of the original channel.

If the option Background Subtraction is selected then the **Sphere Diameter** should be determined.

#### Sphere Diameter

The image is processed based on the specified minimum of the object diameter that you have entered. Use either the automatically given value or set a new measured value. The value of **Sphere Diameter** should be slightly larger than the diameter of the largest object expected to be found in the data set.

If the option Background Subtraction is not selected, the Absolute Intensity threshold is performed.

#### Surfaces Wizard Buttons





**Button Back**

With **Back** you return to the previous window of the Creation Wizard.

**Button Next**

Press **Next** to proceed with the next window of the Creation Wizard.





**Button Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

**Button Cancel**

If you do not want to create Surfaces click on **Cancel**.




**11.14.1.4 Threshold****Threshold**




Activating/deactivating the threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.

In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

**Automatic/Manual A/M button**

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

**Lower and Upper Threshold**

In Imaris you can set two threshold values, **Lower** and **Upper**.

The two threshold values separate the data into three regions: above the higher threshold, below the lower threshold, and the region in between the two values.

Object with values below the Lower Threshold and objects with values exceeding the Higher Threshold will be excluded and only those objects with quality inside the interval defined by the two threshold values are considered.

Please note if the value of the Lower threshold is set higher than the value of Higher threshold the selected objects will be with outside the interval defined by the two threshold values.

To change the upper and/or lower threshold value(s) you can drag the line(s).

Alternatively, left click in the histogram to set the lower value of the threshold range and right click to set the upper value of the threshold range.

## General

### Automatic

The initial threshold value is automatically computed using an algorithm based on [T.W. Ridler and S. Calvard, "Picture thresholding using an iterative selection method", IEEE Trans. System, Man and Cybernetics, SMC-8 (1978) 630-632].

### Manual

Define the requested threshold by clicking into the histogram or by entering the value directly in the data field.

## Split touching Objects (Region Growing)

This option supports the separation of two or more objects that are identified as one. The region method for segmentation in Imaris starts with defined seed points. During the region growing process around each seed point the region grows until a border stops the growing.

If you want to apply the seeded region growing for the Surfaces creation check the respective option. The threshold you set above is the border of the region growing.

## Seed Points Diameter

Type in the estimated diameter of a seed point.

## Preview Region Info

For large data sets that exceed a certain memory limit, a Surface preview is only created for a centred sub-region for which the volume is highlighted. The size of the sub-region matches a memory limit defined in the advanced preferences, see Preferences -> Advanced, the parameter `bpSurfacesModelViewer->3DPreviewMemoryLimitMB` for 3D volumes and `bpSurfacesModelViewer->4DPreviewMemoryLimitMB` for time resolved data. For placing the Surface preview region manually, a ROI needs to be defined.

## Surfaces Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

### Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

### 11.14.1.5 Classify Seed Points

The Seed Points filtering is incorporated into the creation process. This tab only appears, if **Split touching Objects (Region Growing)** is checked ([Algorithm Settings](#)).

Filtering allows the removal of objects that would otherwise interfere with the interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.


**Please note that if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.**

#### Filters

##### Add Button

To add a new filter, click on the button Add  Add  and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

##### Delete Button

Select a filter in the list with a left mouse click and click on this button  Delete to delete the filter.



















#### Filter Type

Select in the drop-down list the filter you want to apply.

#### Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .





These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a

light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.




## Lower and Upper Threshold


Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.



In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode.

Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram

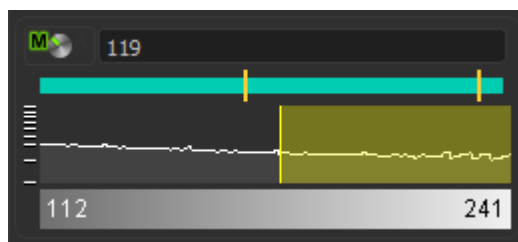
In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change

the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

**Tip:** You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.



### Surfaces Wizard Buttons

#### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

#### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

#### Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

### 11.14.1.6 Classify Surfaces

The Surfaces filtering is incorporated into the creation process.

Filters allow the removal of objects that would interfere with interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting the required variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.


The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple**

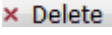
filter types, more than one criterion can be specified for the data analyses.

## Filters

### Add Button

To add a new filter, click on the button Add  and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

### Delete Button

Select a filter in the list with a left mouse click and click on this button  to delete the filter.












## Filter Type

Select in the drop-down list the filter you want to apply.

## Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.





In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.




## Lower and Upper Threshold

Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.



In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the

Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold. Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.**

## Surfaces Wizard Buttons

### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

### Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

## Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

### 11.14.1.7 Edit Surfaces

Here you find the manual editing tools for the Surfaces.

**Tip: In the Surpass view be sure that the mouse is in the pointer mode Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.**

## Edit Mode

### Add/Delete

This activates a semi-automatic surface segmentation (see below). Single objects can be added by one mouse click. If you select **Add/Delete** as edit mode, the Add/Delete Properties are available (see below).

### Cut

This activates the Surface Scissors function (see below). You can manually split two touching surface objects. If you select **Cut** as edit mode, the Cut Properties are available (see below).

## AutoDepth Draw

The auto depth function will automatically calculate the Surface depth. Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Surface.

### Intersect with:

**all visible Channels**

**specific Channel**

**solid Objects**

Select as reference for the Spot depth the first visible channel, all visible channels, or other solid objects.

## Add/Delete Properties

The Add/Delete Properties are only available, if you select **Add/Delete** as edit mode (see above).

There are two different techniques available:

### Marching Cubes

Optimized for objects with a complex rough surface. It runs a marching cube type surface generation process in the bounding box around the mouse (automatic threshold).

- Position the autodepth cursor to the center of the object.
- Adjust the size of the box to the approximate size of the object (use the mouse wheel).
- Shift + click to create the new object.

### Magic Wand

Optimized for single, complex objects, which are located over a larger region of the image. The Magic Wand algorithm is ideal for the global detection of arbitrarily-shaped objects. It reads the threshold from the cursor position and creates a surface (same as marching cube but on entire image).

- Position the autodepth cursor to the border of the object (the size of the box does not influence the result).





- Shift + click to create the new object.

### Process Selection

#### Button **Clear**

Click this button to clear the Surfaces selection.

#### Button **Duplicate**

Click this button and all selected Surfaces appear in a new group in the Surpass Tree (Surfaces Selection).

#### Button **Delete**

Select a Surface and click on this button to delete the Surfaces.

#### **Duplicate to all Timepoints**

If you check this box the selection is not only active for the current time point but for all time points in which the object appears.

	<b>PC:</b>	<b>Mac:</b>
Select Surfaces	Left-click	Click
Select multiple Surfaces	Ctrl + left-click	Command + click
Add new Surfaces	Shift + left-click	Shift + click
Delete Surfaces	Left-click to select, then click on the button Delete or Shift + left-click on a selected Surface	Click to select, then click on the button Delete or Shift + click on a selected Surface
Change Surfaces diameter	Mouse wheel	Mouse wheel

### Scissors Properties

Use the Surface Scissors to manually split two touching Surface objects.

**Tip: You can easily switch between the two pointer modes using the ESC-key. The effect is directly visible on screen by the altered mouse pointer display.**

- Change to the pointer mode Select.
- Select as Edit Mode the mode Cut (see above).
- Shift + click on the Surface object.
- A colored line indicates the cut in the viewing area (the scissors tool cuts the Surface object perpendicular).
- Click on the button **Cut Surface** (see below) and the object splits into two individual Surface objects with two different colors.

#### Button **Cut**

Click on this button to cut the Surface object along the line. The object splits into two individual Surface objects. This is not directly visible on the screen. To check the cutting select/highlight the new object.

#### Button **Clear**

Click on this button to clear the selection.

## Close Borders

Check this box to add a surface along the cutting line and close the surface.

## Mask Properties

Sometimes it is useful to apply a certain function not on the entire image but only in a region of interest (ROI). As region of interest you can choose Surfaces.

Button **Mask All...**

Button **Mask Selection ...**

Here you decide, if the masking is based on selected objects or on all objects. The window Mask Channel displays (see below).

## Surfaces

In Imaris it is possible to merge two or several Surfaces items into one Surfaces item.

Button **Merge ...**

This button is only available if you have two or more Surface items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Surfaces X" displays (see below).

## Window: Mask Channel

### Channel Selection

Select the channel (destination channel) you want to mask with the Surfaces (ROI).

### Duplicate Channel before applying Mask

When this option is selected, the channel will be duplicated before applying Mask.

### Mask Settings

#### Random Color Map

#### Constant Inside/Outside

Define, if you want to use a random color map or if you want to set a constant inside/outside.

#### Set Voxels outside Surface to:

The default value is 0.000, no voxels outside the region of interest are displayed.

#### Set Voxels inside Surface to:

If you leave the box unchecked the original channel intensities inside the region of interest are displayed. If you check the box and type in 0.000, no voxels inside the region of interest are displayed (no masking!).

#### Apply to all Time Points

Only available if you work with a time data set. Check the box if you want to apply the mask to all time points. If the Surfaces changes size and position through the time points the applied mask changes size and position, respectively.

Button **OK**

If you want to apply the mask click on **OK**.

**Button Cancel**

If you do not want to apply the mask click on **Cancel**.

**Window: Merge selected Objects to "Surfaces X"**

Check the boxes in front of the desired Surfaces or use the button **Select All**.

**Button Clear All**

Use this button to clear the Surfaces selection.

**Button OK**

If you want to merge the Surfaces click on **OK**.

**Button Cancel**

If you do not want to merge the Surfaces click on **Cancel**.

**Surfaces Wizard Buttons****Button Back**

With **Back** you return to the previous window of the Creation Wizard.

**Button Next**

Press **Next** to proceed with the next window of the Creation Wizard.

**Button Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

**Button Cancel**

If you do not want to create Surfaces click on **Cancel**.

**11.14.1.8 Tracking**

Tracking allows objects to be traced throughout sequential frames of a time data sets, so each object maintains a unique identity in the output measurements.

This tab only appears, if **Track Surfaces (over Time)** is checked ([Algorithm Settings](#)).

**Algorithm**

The Tracking tab provides several tracking algorithms to choose from. The motion models are used to predict the future positions of Surfaces. Based on these predicted positions the best matches to candidate Surfaces can be determined. All of the algorithms except for Connected Components use a Linear Assignment algorithm and Total Cost function to find the best track connections. The first two algorithms compute cost based on change in position (distance), while Autoregressive Expert mode combines changes in position and intensity to compute total cost.

**Brownian Motion**

This algorithm models the motion of each surface as a Brownian motion. This model is appropriate if your Surface performs similar to a Brownian motion (the random movement of Surface with very frequent changes in direction and no discernible pattern).

The only prediction applied is that the Surface does not move further than the specified speed allows, so the maximum speed constrains the distance from one time-point to the next.

## Autoregressive Motion

This algorithm models the motion of each Surface as an autoregressive AR1 process. This model is appropriate if your Surface perform any kind of continuous motion. The AR1 model looks back to one time-point, and predicts that the Surface will move again the same distance and in the same direction. The user-specified **Maximum Distance** value is the distance in which the Surface is allowed to deviate from the predicted position. Some changes in direction are tolerated because of the maximum distance parameter, and because AR1 does not remember farther back than one time-point.

## Autoregressive Motion Expert

This algorithm models the motion of each Surface as an autoregressive process. This model is appropriate if your Surfaces perform any kind of continuous motion. In the expert mode the tracks are predicted by the additionally established algorithm called Intensity Weight (see below).

## Connected Components

For this algorithm, Surface objects in adjacent time-points are considered connected if the Surface spheres occupy some of the same space (the spheres would overlap if two time-points were merged into one). It compares the amount of overlaps between identified objects in the previous frame with those in the current frame. All the object with the overlap will be assigned the same track ID. Connected Components and Lineage are the only algorithms that automatically handle lineage (objects that diverge). It will only work with images that have excellent time resolution, because the object cannot ever jump a distance larger than its own size.

## Lineage

The Lineage model is intended for events such as cell division in which the splitting of objects is to be analyzed. This algorithm models the motion of each object as an autoregressive AR1 process (refer to Autoregressive Motion above). The object data is then compared so that only the objects that undergo splitting are connected, and assigned the same track ID. Objects that pass in close proximity, or overlap, are not associated. This is in contrast to connected components, where any objects that overlap are determined and considered connected.

## Parameters

### Max Distance

The maximum search distance that a tracked object are looked for is specified with the Max Distance value. This parameter disallows connections between a Surface and a candidate match if the distance between the predicted future position of the Surface and the candidate position exceeds the maximum distance.

The parameter is only available if you select Brownian Motion, Autoregressive Motion, or Autoregressive Motion Expert (see above).

### Max Gap Size

An object might fail to be segmented from some time points, which could lead to a Track breaking apart and the creation of Tracks fractions.

Instead of creating track fragments, with gaps between undetected objects, the gap-closing algorithm creates tracks by linearly connecting objects associated with the same track. The time period in which the objects connection is automatically established is based on the value defined in the Max Gap Size value.

The **Max Gap Size** defines the maximum number of the consecutive time points, which are allowed to be missing in order to a track to be continues.

The parameter is only available if you select Autoregressive Motion or Autoregressive Motion Expert (see



above).

### Fill the gaps with all detected objects

This algorithm allows tracks to be continued, even if the object was not detected in periods of up to two consecutive time points along the trajectory. If this option is selected, the algorithm performs the optimization of the object expected position for the particular time point. The track is generated by connecting objects assuming their optimized expected positions.

### Intensity Weight

Tracks are computed by minimization of a cost function that combines distance based and intensity based costs. "Intensity Weight" is the factor by which IntensityCost is taken into account:

$$\text{TotalCost} = \sum_{\text{all connections}} \text{DistanceCost} + \text{IntensityWeight} * \text{IntensityCost}$$

The parameter is only available if you select Autoregressive Motion Expert (see above).

### Surfaces Wizard Buttons

#### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

#### Button **Next**

Press **Next** to proceed with the next window of the Creation Wizard.

#### Button **Finish**

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

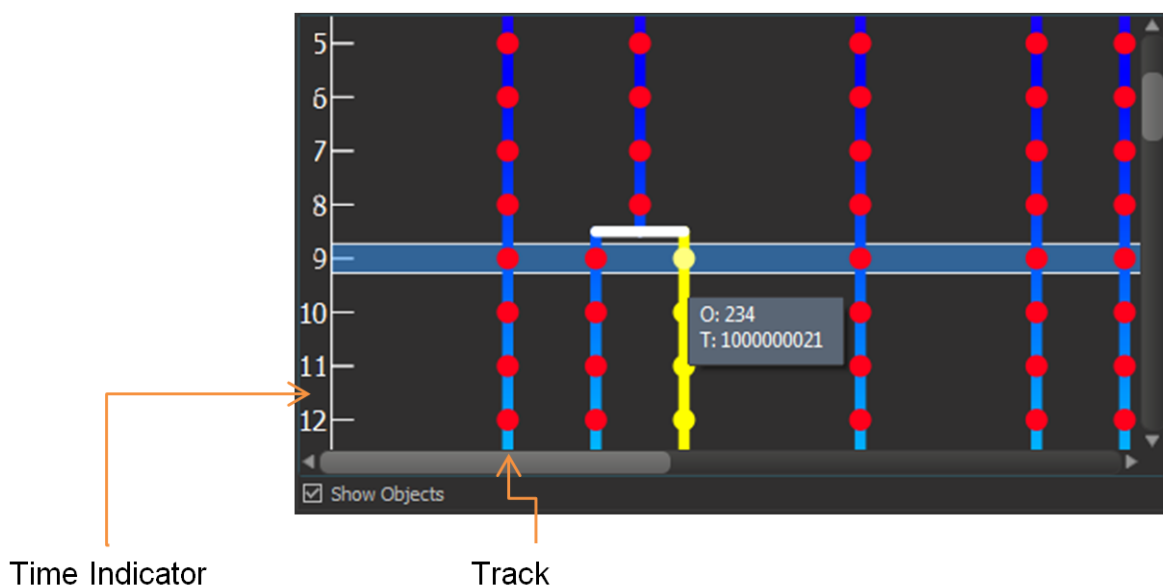
#### Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

### Lineage

### Table Layout

The track editor table:



## Time indicator

The axis on the left displays the frame number.

## Track

Each track is displayed in a vertical format by default. If connected components or Lineage algorithms are used there may be a number of branch points. The branch points indicate where objects diverge, e.g. cells undergoing division.

## Object

Each data-point is displayed as an object in the track with a unique Object ID. The object will retain its unique ID number- even if, for example, it is connected manually to another track.

If the mouse pointer is on an object, a tooltip appears that identifies the Object ID number (O) and track ID number (T). You can untick the Show Objects checkbox to hide objects from the display.

## Interactive Selection

When you click on an individual column, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (column in the table) is highlighted.

In addition, the Statistical Tab and Filter Tab can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.

For multiple selections press Ctrl+click (Mac: Command+click).

## Visualization options

### Zoom

Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table. Using the mouse wheel will have the same effect.

### Pan

To move the track editing table click and hold the right mouse button while dragging the mouse. Release the right mouse button to place the table on the chosen location.

## Mouse Selects

This option allows you to select objects or tracks as follows:

### Object

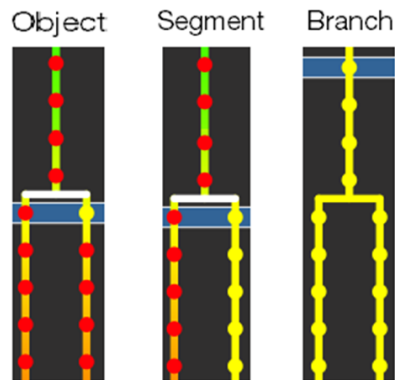
Click Object to select an individual object, or number of specific objects. To connect two unconnected objects, simply select both objects and click the Connect button. To disconnect two connected objects, select both objects and click the Disconnect button.

### Segment

Click Segment to select a specific segment (between branch points).

### Branch

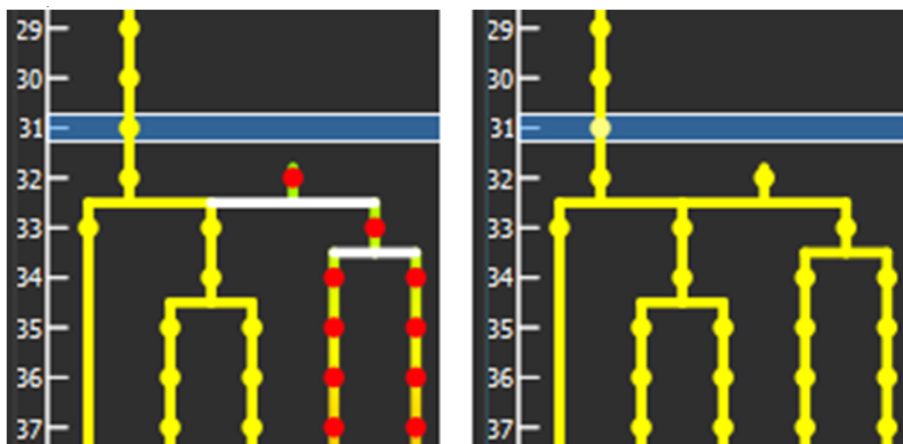
Select Branch to select the segment and the branched segments and objects that appear later in time only.



### Pivot

Pivot enables selection of a segment and all segments and objects that are linked afterwards. This includes objects later in time, and also backwards in time.

For example, selecting branch (left) selects only the connected segments and objects that are later in time. Selecting Pivot (right) all connected segments and objects to be selected.



### Track

Select track to select a complete track.

## Editing

Track Editor enables you to modify your tracks.

### Connect Button

Click this button to connect two existing objects. Select two objects in two different tracks at the subsequent time points (just one object per time point) and click on the button Connect. The connection is automatically created between all selected objects.

### Disconnect Button

Select the two adjacent objects you want to disconnect (using Ctrl) and then click on the button Disconnect. The connection between the two selected objects in the time sequence is broken.

### < Button

Click this button to move a selected track left. When axis is inverted (horizontal) the selected track moves up.

### > Button

Click this button to move a selected track right. When axis is inverted (horizontal) the selected track moves

down.

## << Button

Click this button to move a selected track all the way to the left. When axis is inverted (horizontal) the selected track moves to the top.

## >> Button

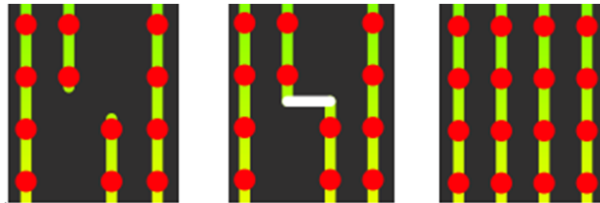
Click this button to move a selected track all the way to the right. When axis is inverted (horizontal) the selected track moves to the bottom.

## Flip

The Flip button may be used (with Pivot mode selected) to flip the order that the connected branched segments and objects are displayed in about the pivot point.

## Reorganize

The Reorganize button can be used after editing of tracks is complete so that tracks are displayed in a clear and logical format without affecting the track arrangement during the editing process.



Editing tracks. Connection added. Tracks reorganized.

## Invert Axis

Click the Invert Axis button to switch between displaying the tracks vertically (default) and horizontally.

### 11.14.1.9 Classify Tracks

Track filtering is incorporated into the creation process. This tab only appears, if **Track Surfaces (over Time)** is checked ([Algorithm Settings](#)).

Filters allow the removal of objects that would otherwise interfere with the interpretation of results.

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting the required variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.**

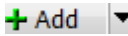
## Filters

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**Add Button**

To add a new filter, click on the button  and choose one of the Filter Types from the drop-down menu. The new filter appears in the list.

**Delete Button**

Select a filter in the list with a left mouse click and click on this button  to delete the filter.

**Filter Type**

Select in the drop-down list the filter you want to apply.

**Filter category**

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.





In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button de-selects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.




**Lower and Upper Threshold**



Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.


In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

**Automatic/Manual A/M button**

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode. Clicking on the A/M button changes the appearance of the A/M button form  indicating an activated

Automatic threshold to  indicating a Manual threshold.  
Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and a reduced/enlarged set of objects is visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while region within threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right to increase the threshold, while to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality), the number and percentage of objects selected (e.g. Surfaces) is displayed at the bottom of the threshold histogram.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.**

## Surfaces Wizard Buttons

### Button Back

With **Back** you return to the previous window of the Creation Wizard.

### Button Next

Press **Next** to proceed with the next window of the Creation Wizard.

### Button Finish

Click on **Finish** to calculate the desired Surfaces and display the Surfaces in the viewing area.

### Button Cancel



If you do not want to create Surfaces click on **Cancel**.

#### 11.14.1.10 Complete ROI

This tab only appears, if **Process entire Image finally** is checked ([Algorithm Settings](#)).

#### Region of Interest

The parameters you have set up will now be used to compute Surfaces (and Tracks) for the entire data set. Depending on the size of the data set this may take a while.

#### Surfaces Wizard Buttons

##### Button **Back**

With **Back** you return to the previous window of the Creation Wizard.

##### Button **Next**

This button is grayed in the last window of the Creation Wizard.

##### Button **Finish**

Click on **Finish** to end the calculation and display the results in the viewing area.

##### Button **Cancel**

If you do not want to create Surfaces click on **Cancel**.

#### 11.14.2 Tab Settings

#### Surfaces Style/Quality

##### **Surface**

Select this option and the created Surfaces is displayed in the viewing area.

##### **Slicer View**

This presents the created Surfaces in a rendered slice view (requires OpenGL 4.0 support). Select the slicer orientation from the drop-down box.

##### **Center Point**

This option displays the Surfaces as center points.

##### **Off**

Select this option to disable the visualization of the Surfaces.

#### Tracks Display

Select how to visualize the tracks:

##### **Full Tracks**

Select this option to show the complete track.

##### **Dragon Tail**

Select this option to view a dragons tail for the object in motion. The parts of the track ahead and following the object can be adjusted (in seconds) using the Next and Last fields respectively.

## Off

Select this option to disable the visualization of the tracks.

## Tracks Style

Set the display of connections between the Track objects.

## Line

This option displays the tracks connections drawn as lines.

## Cylinder

This option displays the tracks connections drawn as lines.

## Diameter

Depending on the previous selection you can adjust the width of the line (in pixels) or diameter of the cylinder (in um) using the slider.

## Tracks Displacement Display

Check the box Displacement to show the displacement of a Track as an arrow. To assign another color click on the Color symbol to open the Color Editor.

## Arrow Scale

Adjust the thickness of the displacement arrows by changing the value in the Arrow Scale field.

## Color Editor

Select a desired color for the displacement arrow.

## 11.14.3 Tab Rebuild

### Rebuild

Imaris stores the list of operations and the mapping of parameters. Select from the list on which step of the [Surfaces Creation Wizard](#) you want to re-enter the creation process.

For details please refer to Section:

Menu 3D View - Surfaces - [Algorithm](#)

Menu 3D View - Surfaces - [Tracking](#)

### Rebuild Objects Button

Click on this button to start the rebuild.

### Creation Parameters

The Creation Wizard saves parameters and processing instructions so the Surfaces computation can be repeated for the same region, a different region, or the entire image. Creation Parameters commands are listed.

### Store Parameters for the Batch

The Store Parameters for the Batch button allows you to save the complete set of the creation parameters and all relevant settings shown in the list. In the Store Creation Parameter window, enter a descriptive name for your creation parameters and select the required store location.



There are two options to store your creation parameters

## Arena

### Favorite Creation Parameters

#### Arena

When you select this option a new Arena item is automatically created within the Arena view. The Arena view is updated, a new item, [Creation Parameters](#), is created and displayed.

Once stored, Imaris [Batch](#) applies the creation parameters set to all images within the selected Group.

### Favorite Creation Parameters

To help you organize and find your most used creation parameters more easily, we advise you to store them by selecting the Favorite Creation Parameters option.

When you select this option the Creation Parameter file is automatically added to the list that is displayed under the Edit/Preference/[Creation Parameters](#). This option allows you to use the creation parameters in future on another data set. These saved parameters can then be imported and re-used on any new data set should you wish to perform an identical analysis sequence. This option is also useful for sharing the parameters with other users to allow them to perform the same analysis on their own data sets.

Within the Surpass view storing allows the parameters to be reused or modified. In addition, this option is useful to create and share the creation parameters with other people and allow them to perform their own analysis on it.

Once you have saved the creation parameters, you can reuse them in any new creation wizard. All the settings and values required to apply the same parameters to the new data set are automatically loaded and available as a Load Parameter option in the creation wizard.

When you save the Creation Parameter file, it is automatically added to the list which is displayed under the [Edit/Preference/Creation Parameters](#).

Click on the Store Parameters for the Batch button opens the Store Creation Parameter window.

## Store Creation Parameter

Refer to [Store Creation Parameters](#)

### 11.14.4 Tab Draw

The Draw tab allows the user to semi-automatically create further Surface objects.

The Draw tab has two sub tabs:

[Add/Delete](#)

[Contour](#)

All actions in the Draw , Edit  and Edit Tracks  tabs can be Undone/Redone by selecting the [Undo/Redo](#) command or by pressing Ctrl+Z/Ctrl+Y.

#### 11.14.4.1 Add/Delete

##### Add/Delete

This activates a semi-automatic surface segmentation.

Here you select, in which channel or on which objects Imaris calculates the depth position of the inserted Surface.

**Intersect with:**  
**all visible Channels**  
**specific Channel**  
**solid Objects**

Select as reference for the Surface depth the first visible channel, all visible channels, or other solid objects.

## Add Surface using:

There are two different techniques available for adding the Surface Objects:

### Marching Cubes

Optimized for objects with a complex rough surface. It runs a marching cube type surface generation process in the bounding box around the mouse (automatic threshold).

- Position the cursor to the center of the object.
- Adjust the size of the box to the approximate size of the object (use the mouse wheel).
- Shift + click to create the new object.

### Magic Wand

Optimized for single, complex objects, which are located over a larger region of the image. The Magic Wand algorithm is ideal for the global detection of arbitrarily-shaped objects. It reads the threshold from the cursor position and creates a surface (same as marching cube but on entire image).

- Position the cursor to the border of the object (the size of the box does not influence the result).
- Shift + click to create the new object.

## Manual Tracking

The Manual Tracking option can either be used when automatic tracking algorithms require modifications and adjustments, or to create an entire track from scratch.

The manual tracking option allows you to track objects within a time-resolved data set by manually specifying the object location in each time point. Essentially, the manual tracking is done by clicking on the structure in the image, which serves as a visual reference (template).

This option can be activated by clicking in the box **Auto-connect to selected Surface**.

To start manual tracking, move the time slider to the first time point of the feature you want to track. Then use the pointer to Shift and then left click on the feature in the data set.

After each mouse click, the time slider advances one frame, allowing you to see the subsequent image of the temporal stack. To continue manual tracking, keep clicking on the structure on the image. As you add more manual tracking points by clicking within the viewing area, a track is created allowing you to follow the moving features, frame by frame and from point to point, until you've built a complete motion path.

**Please note: Manual tracking behaviour is governed by the Selection mode under [Track Edit tab](#).**

## Auto-connect to the selected Surface box

Select the option to add all selected or added objects to one track and advance to next time point when a new object is added or an existing is selected.

**Please note: Objects are always added at the current time point (t).**

The result of Manual tracking can be very different depending on which selection is chosen in the [Track Edit](#) tab.

If the option **Track** is selected, a connection is established between added object at time point t and the last added object of the selected track at time point t-1.

If the option **Surface** is selected, a connection is established between the selected object at the immediately preceding time point (t-1) and the newly added object at time point t.

**Please note: If there is no the immediately preceding time point, a new track will be constructed.**

The box on the drawing plane represents the created object diameter. The diameter of the object can be changed, during the manual tracking, by using the mouse wheel. Use the mouse wheel to increase/decrease the radius of the created object.

### Enable Delay before auto-advancing

The Enable Delay before auto advancing option sets a delay before proceeding to the next time point. When this option is selected, the created object will be displayed for the duration of the delay in the viewing area. The visual feedback of the created object gives you an opportunity to adjust it.

The delay duration is adjustable under the Edit menu/Preference/Surpass. You can specify the value between 0.05 and 5 seconds.

#### 11.14.4.2 Contour Surface

The Contour Surface allows you to extract a 3D object by manually drawing the object contours on 2D slices. You can employ this method whenever simple threshold does not yield individual structures (e.g. touching cells in confocal microscopy, complex tissue recorded by transmission microscopy).

It is recommended to uncheck any other object in the Surpass Tree before drawing the contour, so that only the contour is visible in the viewing area.

The Contour Tab has three sub-tabs:

[Board](#)

[Mode](#)

[Autofit](#)

##### 11.14.4.2.1 Board

A drawing plane is displayed in the viewing area.

### Orientation

This option defines the orientation of the drawing plane. Once orientation of the drawing plane is selected, the drawing planes of all contours are set in the same position. By default the orientation is set to be in the XY plane-z-direction.

Choose the **YZ**-plane to draw in the x-direction. Choose the **XZ**-plane to draw in the y-direction. Choose the **XY**-plane to draw in the z-direction.

### Visibility

This option defines the Visibility of previously drawn contour lines.

## None

The contour currently being drawn is made visible.

## Next

The contour(s) next to the contour currently drawn is/are shown.

## All

All contours are shown.

## Resolution

This option controls the smoothness and complexity of the surface created by the contour lines. It can be used to reduce the number of triangles in a surface object according to a user-defined value. There are two options:

### Automatic

The Automatic resolution depends on the contour line vertex spacing.

### Manual

The manual resolution is controlled with the slider. Moving the slider to the left reduces the resolution, moving it to the right increase resolution.

Setting the Resolution to 100% corresponds to the data set resolution. Setting the resolution to 1% reduces the voxel resolution to 1% of original data set.

## Preserve feature

If the box Preserve feature is selected, a Surface calculation is then performed using anisotropic Gaussian filtering. This feature aims to reduce image noise without removing significant parts of the image content, ensuring that thin and sharp features are not absent from the created surface. The filter size is calculated adaptively and separately for each plane. It produces a family of contour lines, and each resulting line is a combination between the original image and a filter that depends on the local content of the original image. As a consequence, in the created Surface noise is removed, and significant parts of the image content (like elongated and narrow features) are preserved.

If the Preserve feature is not selected an isotropic Gaussian filter is used for the Surface creation. The smoothing is made along the structures, resulting in consistently smooth Surface.

**Please note: As a result of applying isotropic Gaussian filter, thin and sharp Surface features may fail to be created.**

## Selection

The following button actions apply to the previously created contour lines.

### Button Cut

To cut a contour line in a plane click on the button Copy.

### Button Copy

To copy a contour line in a plane click on the button Copy.

### Button Paste

To paste a contour line in click on the button Paste.

### Button Delete

To delete a contour line click on the button Delete





**Button Smooth**

To smooth a contour line edge click on the button Smooth.

**Slice Position**

The border of the active drawing plane is shown in your selected color, thus clearly indicating the slice currently in use. There are several ways to move between slices and select individual slices to create new contours. You can either:

- Drag the slider handle to the left or right
- Use the up or down arrow keys
- Enter the slice number in the numerical field

**Slice (data field)**

The respective slice number is displayed in the number field.

The position of slices can be alternatively also changed in the viewing area. Switch the pointers to the Select mode and then simply click at the drawing plane and drag it to a new position.

**Lock button**

This option prevents the orthogonal slicer from being accidentally shifted with the mouse during editing or contour line creation.

Selecting the Lock option generally "constrains" movement of the drawing plane in the viewing area, but it still allows changes to be made using either the slider options or up/down arrow key.

The L key allows the switch between locked and unlocked orthogonal slicer.

Please refer to the Section [Shortcuts Overview](#) in the Reference Manual for a detailed list of shortcuts for manual surface creation.

**Draw button**

Click on this button to alter **between the two** Drawing Modes: **Active** and **Inactive**

**Drawing Modes**

In the contour line, creations there are two different drawing modes: **Active** and **Inactive**.

**Active**

The main function of the active drawing mode is to create a contour line. The active drawing mode can also be used to insert new vertices into existing contour lines.

**Inactive**

In the inactive drawing mode, the existing contour lines can be modified or deleted.

You can select the respective mode by using the **Draw button** or **Ctrl+space key**.

Press the **Draw button** or **Ctrl+space key** to alter Drawing mode between **Active** and **Inactive** mode.

The pointer's appearance on the screen depends on the selected drawing mode. The symbol on the screen changes from an **arrow** in the **Inactive** drawing mode to a **cross** in the **Active** mode.

**Create Surface button**

To create surfaces click on the button Create Surfaces. The Surface item appears in the Surpass Tree and is displayed in the viewing area.

## Drawing Modes

In the contour line, creations there are two different drawing modes: **Active** and **Inactive**.

### Active

The main function of the active drawing mode is to create a contour line. The active drawing mode can also be used to insert new vertices into existing contour lines.

### Inactive

In the inactive drawing mode, the existing contour lines can be modified or deleted.

You can select the respective mode by using the **Draw button** or **Ctrl+Space key**.

Press the **Draw button** or **Ctrl+Space key** to alter Drawing mode between **Active** and **Inactive** mode.

The pointer's appearance on the screen depends on the selected drawing mode. The symbol on the screen changes from an **arrow** in the **Inactive** drawing mode to a **cross** in the **Active** mode.

Please refer to the section '[Shortcuts Overview](#)' for a listing of the respective shortcuts.

Several segmentation tools are provided allowing you to select areas in the current slice for contour line drawing.

Select one of the shape object tools in the Drawing Mode. Left clicking on a tool icon activates a drawing tool. Once a drawing style tool is selected you may adjust the specific parameters for that drawing mode.

Once a segmentation tool is activated and adjusted, activate the drawing mode by changing the pointer mode to Select (press the ESC-key) and then once press the **Draw button** or **Ctrl+space key**. The Drawing mode is activated.

By default, contour lines are drawn in a transparent gray color. The color of the contour lines can be modified using a dialog under the Color Tab.

### Click

Clicking within the image inserts a point, a so-called vertex. You can now define subsequent vertices by clicking with the left mouse button. Successive points will be connected with straight-line segments.

### Time

Click once on the contour and then move the mouse along the contour. The number of vertices depends on the time interval (ms), specified in the corresponding field. The interval can be changed by manually specifying the required value. This drawing style allows you to draw a structure that contains fine details.

### Insert a vertex after ms

The number specifies the time interval (in ms) between two inserted vertices.

### Distance

Click once on the contour and then simply move the mouse along the contour. The number of vertices depends on the distance interval (um), which is specified in the corresponding field. To change the interval, specify the required value in the object properties area. This drawing style is recommended if the structure is smooth.

### Vertex spacing um

The number specifies the distance interval (in um) between inserting two inserted vertices.

### Circle

The Circle drawing tool inserts a circular contour line. The Circle tool always draws a contour line from the center; that is, the place you initially click will be the center point of the contour line. To change the circle



radius specify the required value in the object properties area. In the parameters you can also specify the number of contour vertices to be inserted. To create the contour line, move the mouse pointer over the orthogonal slicer plane. A preview outline of the circle is then displayed. To create a contour line, click with the left mouse button on the desired position.

**Radius um**

Defines the radius of the circle in um.

**Tip: Scrolling the mouse wheel can also change the radius of circle.**

**Number of vertices**

Defines the number of vertices in the circle line.

**Isoline**

The Isoline drawing tool generates contours at a fixed iso-value so the geometrical features of the contour line takes into account the image's intensity values. Connecting the points with a constant intensity value forms the contour line.

The Isoline drawing mode selects the maximum intensity iso-value under the mouse pointer and creates a contour line by tracing points of uniform intensity. Clicking with the left mouse button on the orthogonal slicer plane selects the largest connected area that contains the all voxel with the same intensity values. The current iso-value under the mouse pointer is displayed in the parameter field Intensity and is updated automatically as you move the mouse. To create a contour line, click with the left mouse button on the desired position.

**Reduce density to numerical field %**

Defines the density of inserted vertex in relation to the data set.

A density of 100% inserts a contour vertex at each pixel of the data set.

A density of 10% inserts a contour vertex at every tenth pixel along the Isoline.

**Intensity**

Displays the value of the voxel intensity under the mouse pointer.

**Magic wand**

The Magic wand tool creates a contour line by selecting the largest connected area that contains the maximum intensity voxels and all voxels within a user-defined tolerance range. The Tolerance range defines the tool's sensitivity; the higher value you set, than wider image area that is selected. The Tolerance range can be specified in a box in the Parameter panel.

Move the mouse pointer over the orthogonal slicer plane and a preview of the contour line is displayed. The Intensity value in the Parameter panel is updated as the mouse pointer is moved across over the orthogonal slicer plane. To create a contour line, click with the left mouse button on the desired position.

The Intensity with the corresponding Tolerance value is stored in the parameter section of the label field. This makes it possible to easily correct a selection at a later time.

It is possible to modify the range even after the seed voxel has been selected.

**Reduces the density to numerical field %**

Defines the density of the inserted vertex in relation to the data set.

A density of 100% inserts contour vertex at each pixel of the data set.

A density of 10% inserts a contour vertex at every tenth pixel along the Isoline.

**Tolerance**

Defines the range of intensity limits in percentages.

The tolerance 0% selects pixels with exactly the same intensity value as the point under the mouse pointer.

The tolerance value of 100% selects all intensities within the intensity range of the data set. Values in between are interpolated linearly. The tolerance can also be changed incrementally by scrolling the mouse wheel.

## Intensity

Displays the value of the voxel intensity under the mouse pointer and the tolerance range.

## Detects holes box

This option enables you to create a contour line that contains holes of selected areas. If the Detects holes box is selected the line is generated around holes, outlining the hole. If the box is unchecked, the outer line of the selected region is drawn and the holes are not visible.

## Channel Selection

### All Visible Channel

### Specific Channel

Select the respective source channel, from which the Contour surface is to be computed.

## Slice Position

The border of the active drawing plane is shown in your selected color, thus clearly indicating the slice currently in use. There are several ways to move between slices and select individual slices to create new contours. You can either:

- Drag the slider handle to the left or right
- Use the up or down arrow keys
- Enter the slice number in the numerical field

## Slice (data field)

The respective slice number is displayed in the number field.

The position of slices can be alternatively also changed in the viewing area. Switch the pointers to the Select mode and then simply click at the drawing plane and drag it to a new position.

## Lock button

This option prevents the orthogonal slicer from being accidentally shifted with the mouse during editing or contour line creation.

Selecting the Lock option generally "constrains" movement of the drawing plane in the viewing area, but it still allows changes to be made using either the slider options or up/down arrow key.

The L key allows the switch between locked and unlocked orthogonal slicer.

Please refer to the Section Shortcuts Overview in the Reference Manual for a detailed list of shortcuts for manual surface creation.

## Draw button

Click on this button to alter **between the two** Drawing Modes: **Active** and **Inactive**.

## Create Surface button

To create surfaces click on the button Create Surfaces. The Surface item appears in the Surpass Tree and is displayed in the viewing area.

### 11.14.4.2.3 Autofit

Autofit is a method that can help the user automate a manual segmentation.

Autofit is a deformable contour model used to create and optimize the contour line segmentation, so that the



localization and accuracy of the segmented object is improved.

It is a semi-automatic, user interactive detection method that is relatively insensitive to image noise. The contour line creation procedure consists of two steps: initial user segmentation and refinement by the Autofit algorithm.

In the first step the model requires the user input, an initial curve (rough outline) closed to the boundaries of the object to be segmented.

The next step is to perform an Autofit algorithm. The algorithm then tries to deform this initial shape so that it fits onto the contours of the image. The Autofit algorithm performs an iterative optimization process where the sum of external and internal energies along the contour line approach a local minimum.

The external energy is computed from the original image intensity gradients and it should be minimal when the contour line is located at an object boundary.

The internal energy is based on the shape of the contour line. The Autofit algorithm simulates the contour line as an elastic rubber band and the internal energy is defined by the physical constraints that describe the contour. It should be minimal when it meets some regularity and smoothness constraints regarding the shape of the line (curvature is minimized and contour vertices are encouraged to be equidistant).

The outcome of the Autofit algorithm is an optimized line, a good fit between the external forces, which aims at the object boundaries, and deformable internal forces that targets regularity and smoothness.

The method, which is applied by Autofit, is known under the name of active contour model or snakes. Kass, Witkin and Terzopoulos published the seminal work on the method.

M. Kass and A. P. Witkin and D. Terzopoulos (1988), Snakes: Active Contour Models, International Journal of Computer Vision, 1(4): 321-331

The Autofit algorithm in Imaris is based on the following publication:

Donna J. Williams and Mubarak Shah (1992), A Fast Algorithm for Active Contours and Curvature Estimation, CVGIP: Image. Understanding, 55, (1), 14-26.

**Please Note: The Autofit algorithm does not solve the entire problem of finding contours in images, but rather, depends on mechanisms like interaction with a user, interaction with some higher level image understanding process and information from image data. The accuracy and computation time depends on the convergence criteria used in the Autofit. The model is sensitive to the initial contour line estimate and algorithm parameters. The automatically determined parameters provide the optimal values for the Autofit algorithm.**

### Perform Autofit button

Clicking on this button performs an Autofit calculation.

## Presets

### Shape Smooth slider Accurate

This setting affects the shape of the resulting contour line. If the Shape slider is set to be more on one side the resulting contour line can be encouraged either to be **smooth** or more **accurate** with original image intensity gradients.

### Impact Weak slider Strong

The impact signifies how strongly the algorithm will affect the contour line.

If the impact is set to be **Weak**, the algorithm performs only a few iterations and does not move the vertex very far from the initial contour line.

If the impact is set to be **Strong**, the number of iterations will increase and the vertexes can be moved to find edges that are more distant from the initial contour line.

## Advanced

This option activates only if the **Presets** option is not selected.

### Continuity

The Continuity value affects the vertices space regularity and tends to keep the distances between each pair of adjacent vertices equal. Setting a high continuity value enforces the contour vertices to be equally spaced on the line.

### Curvature

The Curvature value constrains the shape of the contour line. Setting a high value for Curvature, results in a circularly shaped smooth contour line.

### Image Energy

The Image Energy value constrains the intensity gradients on the contour. Setting a high value places the contour line at the intensity high gradients.

### Reach Distance um

This parameter controls the maximal distance the contour vertex can move away from the original position during a single iteration.

Setting a high value enables the algorithm to find edges at more distant locations from the initial contour line and therefore decreases the risk of falling into local minima. However, the algorithm then has a tendency of detecting edges different from the desired object.

### Max iteration

This parameter defines the Maximum number of iterations to be performed. The Autofit algorithm terminates if the specified maximum iterations have been performed.

### SSC iterations

Scale space continuation is a method for preventing the Autofit algorithm to place a contour line at the local minima created by noise. First a large Gauss filter is used and then the Autofit algorithm iterates while slowly reducing the size of the Gauss filter. SSC iterations define the number of Autofit algorithm iterations

For example, if the maximum number of iterations is set to 30 and the number of SSC iterations is set to 3, the Autofit algorithm is run three times. The first time with a Gauss filter with three times the specified standard deviation, then for the second time with two times the standard deviation and finally, for the third time with the specified standard deviation. 10 iterations are performed maximally each time.

### Gauss numerical field um

The standard deviation of the Gauss filter applied prior to the algorithm.

## Slice Position

The border of the active drawing plane is shown in your selected color, thus clearly indicating the slice currently in use. There are several ways to move between slices and select individual slices to create new contours. You can either:

- Drag the slider handle to the left or right
- Use the up or down arrow keys
- Enter the slice number in the numerical field

## Slice (data field)



The respective slice number is displayed in the number field.

The position of slices can be alternatively also changed in the viewing area. Switch the pointers to the Select mode and then simply click at the drawing plane and drag it to a new position.

### Lock button

This option prevents the orthogonal slicer from being accidentally shifted with the mouse during editing or contour line creation.

Selecting the Lock option generally "constrains" movement of the drawing plane in the viewing area, but it still allows changes to be made using either the slider options or up/down arrow key.

The L key allows the switch between locked and unlocked orthogonal slicer.

Please refer to the Section [Shortcuts Overview](#) in the Reference Manual for a detailed list of shortcuts for manual surface creation.

### Draw button

Click on this button to alter **between the two** Drawing Modes: **Active** and **Inactive**.

## Drawing Modes

In the contour line creations, there are two different drawing modes: **Active** and **Inactive**.

### Active

The main function of the active drawing mode is to create a contour line. The active drawing mode can also be used to insert new vertices into existing contour lines.

### Inactive

In the inactive drawing mode, the existing contour lines can be modified or deleted.

You can select the respective mode by using the **Draw button** or **Ctrl+Space key**.

Press the **Draw button** or **Ctrl+Space key** to alter Drawing mode between **Active** and **Inactive** mode.

The pointer's appearance depends on the selected drawing mode. The symbol on the screen changes from an **arrow** in the **Inactive** drawing mode to a **cross** in the **Active** mode.

### Create Surface button

To create surfaces click on the button Create Surfaces. The Surface item appears in the Surpass Tree and is displayed in the viewing area.

11.14.4.2.4 Shortcuts Overview

You can select the drawing mode by pressing once the **Ctrl+space** key. The pointer appearance on the screen depends on the selected drawing mode. The symbol on the screen appears as an **arrow** in the **Inactive** drawing mode and as a **cross** in the **Active** drawing mode.

## Contour Line Mouse & Keyboard Functions

up or down arrow keys	Move up / down one slice
left/right arrow keys	Move up / down to next slice which contains contour lines
C key	Copy contour lines from current slice
X key	Cut contour lines on current slice
V key	Paste contour lines to current slice

Del key	Delete all contour lines on current slice
O key	Smooth all contour lines on current slice
A key	Perform Autofit Algorithm on current slice
+ key or .	Increase contour line width
- key or ,	Decrease contour line width
Mouse wheel	Change circle radius or magic wand tolerance

### Drawing mode **Active**

Backspace key	Abort drawing of currently opened contour line
Enter key	Stop drawing currently opened contour line and leave it open
U key	Change to contour line Union mode Union is indicated by a "+" sign next to the mouse cross cursor

### Drawing mode **Inactive**

L key	Toggle lock/unlock orthogonal slicer
T key + Left-click on line (vertex)	Translate contour line or vertex
R key + Left-click on line (vertex)	Rotate contour line
S key + Left-click on line (vertex)	Scale contour line
D key + Left-click on line (vertex)	Delete contour line or vertex

### **Union mode**

The result of Union mode is a single contour line whose area is the minimum superset of the overlapping input contour line areas.

### **11.14.5 Tab Edit**

Here you find the manual editing tools for the Surfaces.

**Tip: In Surpass view ensure that the mouse is in the pointer mode Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.**

### **Edit Mode**

#### **Cut**

This activates the Surface Scissors function for manually splitting two touching surface objects. If you select **Cut** as edit mode, the Cut Properties are available.

### **Scissors Properties**





Use the Surface Scissors to manually split two touching Surface objects.

**Tip: You can easily switch between the two pointer modes using the ESC-key. The effect is directly visible on screen by the altered mouse pointer display.**

- Change to the pointer mode Select.
- Select as Edit Mode the mode Cut.
- Shift + click on the Surface object.
- A colored line indicates the cut in the viewing area (the scissors tool cuts the Surface object perpendicular).
- Click on the button **Cut Surface** and the object splits into two individual Surface objects with two different colors.

#### Button **Cut Surface**

Click on this button to cut the Surface object along the line. The object splits into two individual Surface objects. This is not directly visible on the screen. To check the cutting select/highlight the new object.

#### Button **Clear Scissor**

Click on this button to clear the selection.

#### **Close Borders**

Check this box to add a surface along the cutting line and close the surface.

#### **Mouse selects**

Specify what object is selected by a single mouse click.

#### **Surface**

#### **Track**

#### **Selection**

#### Button **Clear**

Click this button to clear the Surfaces selection.

#### Button **Duplicate**

Click this button and all selected Surfaces appear in a new Surpass Group in the Surpass Tree (Surfaces Selection).

#### Button **Delete**

Select a Surface and click on this button to delete the Surfaces.

#### Button **Duplicate to all Timepoints**

Click this button and the selection is not only active for the current time point but for all time points in which the object appears.

#### Button **Unify**

Click this button to unify (group) the selected Surfaces into one Surface object.

**Please note: Selecting the Unify button does not connect, or bridge, the two objects by adding any new pixels. The new, unified object will consist of a group of disconnected components.**

#### Button **Split**

Click this button and the selected Surfaces are separated into two Surface objects.

	PC:	Mac:
Select Surfaces	Left-click	Click
Select multiple Surfaces	Ctrl + left-click	Command + click
Add new Surfaces	Shift + left-click	Shift + click
Delete Surfaces	Left-click to select, then click on the button Delete or Shift + left-click on a selected Surface	Click to select, then click on the button Delete or Shift + click on a selected Surface
Change Surfaces diameter	Mouse wheel	Mouse wheel

## Mask Properties

Sometimes it is useful to apply a certain function, not to the entire image, but only for a region of interest (ROI). As region of interest you can choose Surfaces.

Button **Mask All...**

Button **Mask Selection ...**

Here you decide, if the masking is based on selected objects or on all objects. The window Mask Channel displays (see below).

## Surfaces

In Imaris it is possible to merge two or several Surfaces items into one Surfaces item.

Button **Merge ...**

This button is only available if you have two or more Surface items in the Surpass Tree. Click on this button and the window Merge selected Objects to "Surfaces X" displays (see below).

## Window: Mask Channel

### Channel Selection

Select the channel (destination channel) you want to mask with the Surfaces (ROI).

### Duplicate Channel before applying Mask

When this option is selected, the channel will be duplicated before applying Mask.

### Mask Settings

#### Random Color Map

#### Constant Inside/Outside

Define, if you want to use a random color map or if you want to set a constant inside/outside.

#### Set Voxels outside Surface to:

The default value is 0.000, no voxels outside the region of interest are displayed.

**Set Voxels inside Surface to:**

If you leave the box unchecked the original channel intensities inside the region of interest are displayed. If you check the box and type in 0.000, no voxels inside the region of interest are displayed (no masking!).

**Apply to all Time Points**

Only available if you work with a time data set. Check the box if you want to apply the mask to all time points. If the Surfaces changes size and position through the time points the applied mask changes size and position, respectively.

**Button OK**

If you want to apply the mask click on **OK**.

**Button Cancel**

If you do not want to apply the mask click on **Cancel**.

**Window: Merge selected Objects to "Surfaces X"**

Check the boxes in front of the desired Surfaces or use the button **Select All**.

**Button Clear All**

Use this button to clear the Surfaces selection.

**Button OK**

If you want to merge the Surfaces click on **OK**.

**Button Cancel**

If you do not want to merge the Surfaces click on **Cancel**.

**All actions in the Draw , Edit  and Edit Tracks  tabs can be Undone/Redone by selecting the [Undo/Redo](#) command or by pressing Ctrl+Z/Ctrl+Y.**

**11.14.6 Tab Edit Tracks****Mouse Selects**

This option allows you to select an object within an image according to its object type by a single mouse click. To edit the properties of multiple objects simultaneously, select the required object type. There are the following options:

**Object**

**Segment**

**Branch**

**Pivot**

**Track**

The differences between these options is described in [Tracking](#)

**Please Note you can change between mouse selects options using the first letter of each option as a keyboard shortcut i.e. Object: O, Segment: S, Branch: B, Pivot: P and Track: T.**

## Table Layout

In the track editor a horizontal line represents each track. Each horizontal line is composed of circles, representing objects, and line segments which connect a sequence of objects.

The first row of the track-editing table displays the timeline. Each number represents a time point in the series.

The second row of the track-editing table displays the **object name**.

Multiple object types can be tracked easily since every object type appears in different colors.

## Object ID

If the mouse pointer is on the object, a tooltip appears that identifies the object ID. The ID numbers of the objects are sequentially ordered column-wise from the top to the bottom of the table. The object maintains its unique ID number independent of the track being allocated to.

If the mouse pointer is on the connection, a tooltip appears that identifies the track ID number.

## Interactive Selection

When you click on an individual row, the editing table highlights the corresponding object in the image. Similarly, by clicking on an object within the image (while the pointer is in Select mode) the corresponding track (row in table) is highlighted.

In addition, the [Statistical Tab](#) and [Filter Tab](#) can be used to select tracks for editing. By clicking on the value in the statistical table, the corresponding object in the viewing area as well as the track in the track-editing table is selected.

For multiple selections press Ctrl+click (Mac: Command+click).

## Visualization options

### Zoom

Moving the mouse up and down while pressing the middle mouse button allows you to see more ("zoom out") or less ("zoom in") of the track-editing table.

Using the mouse wheel will have the same affect.

### Pan

To move the track editing table click and hold the right mouse button while dragging the mouse. Release the right mouse button to place the table on the chosen location.

### Time indicator

The first row of the track-editing table displays the timeline. Each number represents a time point in the series. The current time point is indicated by vertical line highlighted with in current selection color.

To modify the time point, do one of the following:

1. Moving the slider in the Time bar modifies its position in time and changes the time point in the track editor.
2. Clicking on the time point within the track-editing table automatically moves the time line indicator to the corresponding point.

Double-clicking the time indicator, fixes the position of the current time point at the center of the track-editing table, regardless of the selected zoom or pan options)

## Editing

Track Editor has several features that help you modify your tracks

### Connect Button



Click this button to connect track components such as objects or segments as selected under Mouse Selects. For example, select objects in different tracks at the subsequent time points (just one object per time point). The connect and disconnect buttons will update to show the number of objects selected. Confirm the selection, then click on the button Connect (you can also press the "c" key as a shortcut). The connection is automatically created between all selected objects.

### Disconnect Button

Use this option to disconnect track components such as objects or segments as selected under Mouse Selects. For example, select object(s) to be disconnected. The connect and disconnect buttons will update to show the number of objects selected. Confirm the selection, then click on the button Disconnect (you can also press the "d" key as a shortcut). The button will update to show the number of objects selected. The connection between the selected objects in the time sequence is broken.

### < Button

Click this button to move a selected track left. When axis is inverted (horizontal) the selected track moves up.

### > Button

Click this button to move a selected track right. When axis is inverted (horizontal) the selected track moves down.

### << Button

Click this button to move a selected track all the way to the left. When axis is inverted (horizontal) the selected track moves to the top.

### >> Button

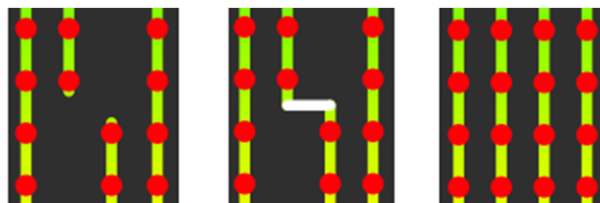
Click this button to move a selected track all the way to the right. When axis is inverted (horizontal) the selected track moves to the bottom.

### Flip

The Flip button may be used (with Pivot mode selected) to flip the order that the connected branched segments and objects are displayed in about the pivot point.

### Reorganize

The Reorganize button can be used after editing of tracks is complete so that tracks are displayed in a clear and logical format without affecting the track arrangement during the editing process.



Editing tracks. Connection added. Tracks reorganized.

### Invert Axis

Click the Invert Axis button to switch between displaying the tracks vertically (default) and horizontally.

### Correct Drift

Refer to [Drift Correction](#).

Focus tracks mode may be useful when editing tracks- refer to the [Cells Edit Track](#) section.

## Algorithms

The Drift Correction is calculated based on the two algorithms, translational drift and translational and rotational drift.

## Translation

Upon translation, the object position is transformed to a straight line of identical orientation. Within the same time point the relative distance of the objects is preserved.

## Translation and rotation

Upon translation and rotation, the object position is transformed based on the image polar coordinates. Within the same time point the relative distance of the objects is preserved.

**Please note: The number of the selected tracks affects the algorithm calculation.**

Selection of a single track gives a complete description of a translation.

Selection of a two tracks gives a minimum description of a translation and rotation algorithm (planar rotation).

A better fit between the time points is achieved when three or more tracks are selected.

## Result DataSet size

After completion of the drift correction, the algorithm uses the final position of the initial and resulting image to create a final image. The new image coordinates are calculated in accordance with the size of the original and new image.

Select the Result data set option to determine which new image part will be displayed.

## Crop largest common region

The final image will be the size of the common overlap between the original and the drift corrected image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

## New size equal to current size

The final image will be the same size as the original image. Areas of the image that have been shifted outside these boundaries (to allow alignment) will be cropped from the final result.

## Include entire result

The final image will be the size of the combined area of the original and corrected image.

Selecting the appropriate Result data set size may speed up the data visualization. This is especially important with (with large data sets.)

## Correct Surfaces positions

Activate this parameter and the surfaces are automatically re-positioned after the drift correction. If this option is not activated only the image volume is aligned.

All actions in the Draw , Edit  and Edit Tracks  tabs can be Undone/Redone by selecting the



[Undo/Redo](#) command or by pressing **Ctrl+Z/Ctrl+Y**.

### 11.14.7 Tab Filter

**Please note: Before using you have to add a filter to the list (see below: button Add)!**

The Filter Type and its values define the section of interest, from this selection, which includes or excludes objects based on a range of criteria. Select the filter type by selecting a variable from the drop-down list, and then define the filter values, which will then limit the selection just to those objects that meet the set up criteria.

In Imaris you can choose two filter values, a Lower and a Higher filter value.

The two filter values separate the data into three regions: above the higher value, below the lower value, and the region in between. Objects with the relevant properties below the Lower filter value and objects with the properties exceeding the Higher filter value will be excluded, and only those objects with qualities inside the defined interval will be considered.

**Please note if the value of the Lower filter value is set to be higher than that of the Higher filter value, the selected objects will be outside the defined interval. Only those objects meeting all the filter criteria are interactively shown and instantly highlighted in the viewing area. By using multiple filter types, more than one criterion can be specified for the data analyses.**

## Filters

### Add Button

To add a new filter, click on the button Add and choose one of the Filter Types from the drop-down menu. Select the filtering type to be used in order to classify objects and specify the measurement of the criteria to be used. The new filter appears in the list.

### Delete Button

Select a filter in the list with a left mouse click and click on this button to delete the filter.



















## Filter Type

Select in the drop-down list the filter you want to apply.

## Filter category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .





These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical variables from the list (turns the category button into a

light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.




## Lower and Upper Threshold


Activating/deactivating the Lower or Upper threshold option is done by clicking on the  or  button. The Red button  indicates that the threshold option is currently inactive, while the green button  indicates an active threshold.



In order to activate the threshold option, click on the red button. To deactivate the threshold, click on the green circle again.

### Automatic/Manual A/M button

The Automatic/Manual A/M button provides a single interaction point for the direct choice of the threshold value adjustment.

When the lower/upper threshold values are not activated (the red button ) the corresponding A/M button is light gray  indicating that threshold adjustment is unavailable. When the lower/upper threshold option is activated (the green button ) , the corresponding A/M button turns black.

After the first threshold activation, as a default, an Automatic threshold value is activated . Clicking on the Automatic/Manual button allows you to switch between an Automatic and a Manual threshold adjustment mode. The A/M button provides a visual feedback as an indication of the selected threshold adjustment mode.

Clicking on the A/M button changes the appearance of the A/M button from  indicating an activated Automatic threshold to  indicating a Manual threshold.

Clicking on the histogram automatically activates a Manual threshold adjustment.

## Lower Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and then a reduced/enlarged set of objects are visualized.

## Higher Threshold

### Automatic

The initial threshold value is automatically computed.

### Manual

Define the threshold by clicking into the histogram or by entering the value directly in the data field. Either type in the value or click into the histogram to move the colored line and then a reduced/enlarged set of objects are visualized.

## Histogram

In the histogram the regions above and below the threshold values are displayed in white, while the region within the threshold is displayed in yellow. To change the automatically set threshold values, select the option Manual. By clicking into the histogram you move the colored line and the reduced/enlarged set of objects. To change the Lower threshold value left click on the histogram and drag the yellow line.

To change the Higher threshold value right click on the histogram and move the line. Move the line to the right



to increase the threshold and to decrease the threshold move the line to the left. These changes are instantly visible in the viewing area.

The numerical range of the statistics value (e.g. Quality) and the number and percentage of objects that fall into the particular filtering class are displayed at the bottom of the threshold histogram.

**Tip: You can use the Zoom function bar above the histogram to zoom in or out of the histogram display. This may be useful to finely adjust the threshold levels. Right click the range limit bars to set the range. Use the mouse scroll button to zoom in or out of the selected range.**

#### Duplicate Selection to New Surfaces Button

Click on this button and all selected objects are copied to a new Surpass Group. The new group displays in the Surpass Tree.

### 11.14.8 Tab Statistics

Surfaces statistics are automatically computed. For the statistical values please refer to the Section Menu Edit - Preferences... - Statistics - [Surfaces](#). The Statistics tab collects and displays the image's statistical values in Imaris. The Statistics tab enables us to understand and compare different data sets through summarized statistical values and graphical data presentations.

Several types of statistical tools enable convenient organization and analysis of heterogeneous statistical data. The Statistics View is configured as follows:

- a) Overall
- b) Detailed
- c) Selection

In the statistical data set the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

To sort the columns click on the arrow in column heading. Click again to reverse the sort.



















#### Overall Tab

Output of the Overall statistics provides an overview of the image's single determining variables. Only statistics that are not measured on a per-object basis are displayed here.

#### Category

A Category identifies, gathers and summaries a set of statistical values or calculations of a particular Object Type.

Statistical values associated with one object type are grouped in one category. Each category has an associated button with graphical indications of Category type.

In Imaris there are following Filter Categories: Surface , Surface Tracks , Spots , Spots Tracks , Dendrites , Dendrites Tracks , Filament , Filament Tracks , Spines , Spine Tracks , Point , Point Tracks , Cell , Cell Tracks , Nucleus , Nucleus Tracks , Vesicle  and Vesicles Tracks .

These buttons allow you to choose the Category and determines the list of displayed statistical values. Clicking the button selects the corresponding category (the category button turns black) and the list of statistical values is displayed according to the selection.

To remove the category from the list, re-click on the Category button. Re-clicking on the category button deselects Category and removes the selected statistical variables from the list (turns the category button into a light gray color).

The exclamation mark sign  indicates that the listed statistical parameter does not contain any value.

## Detailed Tab

A pull down list offers three types of **Detailed** statistical calculations:

### Specific Values

### Average Values

### Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.


Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

### Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

### Specific Values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Object [Statistical Annotation](#) option  is available for Specific values parameters.

### Average values

The Average table contains values determined on the basis of all values within a set of the selected variable.

Columns are labeled as follows:

Min (Minimum)- the smallest value within a set of values of the selected variable

Max (Maximum)- the largest value within a set of values of the selected variable

Mean – value of the sum of all values divided by number of values within a set

Std Dev (Standard deviation)- a measure of the values distribution around the mean values

Median- the middle value of the sorted set of variables

Sum- the sum of all values within a variable set

Count- number of values within a set

Unit- variable unit

Level/Distance

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

### Statistical variable classification:

1. Statistical variables associated with objects (Cells, Nucleus, Vesicles -volume, area, intensity values, position, speed, displacement) have values at every time point. Therefore for these variables the Average values are displayed per time point. The changing the time points, statistical variable values and images are consequently changed.

2. Statistical values with data associated with Tracks (track duration, track volume mean, track displacement length, track intensity) are not time dependent.

## Selection Tab

Statistical tables for a selected object will be displayed. Change to the pointer mode Select and click on an image element that has been detected/segmented. A possibility of multiple selections is available by Ctrl+click (Mac: Command-click).

A pull down list offers three types of statistical calculations from a Selection:

### Specific Values

### Average Values

### Time Plots

The values displayed are always for the current time point. Selecting a variable that changes over time creates a plot of selected values as a time function. The statistical variable value is plotted on the vertical axis and the time is plotted on the horizontal axis. The current time point is indicated with a black vertical line. Each time value is displayed as a point. Click inside the plot and move the time line. Moving the line changes the time point. Altering the time point changes the variable value and the image.


Multiple value selection, available by Ctrl+ click, will plot a superimposed second selected value over the first one.

### Interactive two-way Selection

A click on an individual row of the table highlights the corresponding object in the image. Similarly, by clicking on an element in the image (while the pointer is in Select mode) the corresponding row in table will be highlighted. A possibility of multiple selections is available by pressing Ctrl+click (Mac: Command+click).

### Specific values

In the Specific Values, a second pull down list at the top of the window displays available specific statistical values. Select the variable and a list of values will be displayed.

Object [Statistical Annotation](#) option  is available for Specific values parameters.

### Average values

The Average table contains values determined on the basis of all the values within a set of the selected variables for the selected image element(s). For further explanation see Average values Detailed statistics. This choice only makes sense in the context of the Selection Tab if you have selected more than one element from the image.

The **black** line on the time plot indicates where the **mean** value lies, the **red** indicates the **median**, the **light gray** area shows the **range** between the **min** and **max** values and **dark gray** region labels the **standard deviation range** with error bars.

### Buttons Overview

	PC:	Mac:
Select Surface	Left-click	Click
Select multiple Surfaces	Ctrl + left-click	Command + click

### Buttons at the bottom of the Statistics tab

Button **Settings** -Configure a List of visible statistics values.

Click on the button **Settings** and you switch to the window Preferences - Statistics - [Surfaces](#).

Button **Search** - Search by object ID.

Type in the object ID in the data field. Click on the button **Search**.

- Highlights the object in the viewing area
- Highlights the object on the Detailed Tab
- Displays of the values on the Selection Tab

Button **Surfaces** - Duplicate Selection to new Surfaces.


Click on this button and all selected elements are copied to a new Surface. The new Surface appears in the Surpass Tree, and the old one is hidden, but still available.

Button **Statistical Annotation** 

Click this button and an object statistical annotation will be automatically generated in the viewing area. This option is available only when **Specific** or **All Values** are selected.

Button **Export** - Export Statistics on Tab Display to File.

Only the statistics that are shown within the current tab will be exported.

Button **Export**  - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.

All of the statistics (that are enabled in the statistics settings) will be exported for Overall and Detailed tab data. When the Selection tab is selected, pressing the Export all Statistics to File button, will export all the statistics (that are enabled in the statistics settings) of all the selected objects, not all the statistics of all the objects.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xlm). If you select MS Excel as file type, the program starts automatically and the sheets show up one after another (this will take several seconds).



See also:

Menu Edit - Preferences... - Statistics - [Surfaces](#)

## 11.14.8.1 Statistical Annotations

Statistical annotations provides the opportunity to view and annotate image objects with the statistical data values. Using this function, the individual objects in an image are labeled with the specified statistical values and the statistical data is displayed directly in the viewing area

Imaris objects can be labeled with a set of any number of the user-defined statistical values.

The Statistical Annotation button  provides a convenient interface in order to label the object with the statistical data. Click this button  and an object statistical annotation will be automatically generated in the viewing area. The selected object is annotated for all time points with the specified statistical values.

### How to add Statistical Annotation


In the Statistical tab choose the **Detailed** or **Selection** Tab.

In the viewing area select the object or group of objects. Change to the pointer mode Select and click on an image object that has been detected/segmented. For multiple selections press Ctrl+click (Mac: Command-click)

In the **Detailed** or **Selection Tab** a pull down list offers **Specific Values** or **All values** as types of statistical

parameters.

Select the statistical value associated with the particular object type you are working with.

Press the Statistical Annotation button .

In the viewing area the object label appears showing selected statistical values as a statistical annotation.

The statistical annotation shows by default the name of the selected statistical parameter and its value.


The color white displays the text of annotations associated with statistical values.

For a single object, all selected statistical parameters are placed in the same annotation box. Once the object is labeled, selecting the additional statistical parameter updates the object statistical annotation; the new parameter and its value is added in the viewing area.

If the image is a time series, the statistical annotation will be visible in the viewing area for all time points. Selecting a statistical variable that changes over time creates an statistical annotation that consequently changes with every time point.

The statistical annotation displays values always for the current time point.

The [Annotation](#) options will allow you to modify and specify the contents and format of the object labels.

To modify and adjust the statistical label click on the Icon [Annotation](#) , press Ctrl + Shift + P, or in the menu bar select File – Annotation.

### 11.14.9 Tab Color

Tab Color edit, manage and assign colors material to the Surfaces or Tracks.

#### Object Type

The available Object types are presented in a drop-down window. Select either Surfaces or Tracks and depending on the chosen object type selection for the listed variables.

For **Object Type Surfaces** the following color types are available:

Labels (default)

[Base](#)

[Statistics Coded](#)

For **Object Type Tracks** the following color types are available:

Labels (default)

[Base](#)

[Statistics Coded](#)

[Time Mapped](#)

[Color Type-Object ID](#)

#### 11.14.9.1 Color Type- Labels

The labels associated with the data set is displayed as a list.

Click **Only show labelled objects** to show only data which have labels associated with them.

Note that if there is more than one label associated with an object, the object will be displayed as a combined color. Non-labelled objects are displayed in the base color.

#### Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from

0% (complete opacity) to 100% (complete image transparency).

## 11.14.9.2 Color Type-Base

Tab Color edit, manage and assign material colors of a Surfaces/Track.

### RGB

The RGB color model is an additive color model in which Red, Green and Blue color information is combined together to reproduce a selected color. The color values may be changed either by clicking on the color wheel to select a color or by direct numeric entry for the Red, Blue or Green component (values range from 0 to 1).

### Light reflection

The quality of the object material is further characterized by reflection properties.

- Diffusion- diffuses reflection of a matte, uneven material.
- Specular- defines reflection of highly reflective materials. The brighter a specular map is, the more shine is applied to the object. The default setting is for no specular intensity.
- Emission- light emitted from the material defines the self-illumination. The default setting is for no emission of light.

### Transparency

Transparency- defines the amount of light allowed to pass through an object. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### Palette

Palette- this option provides a system of preset colors organized into color schemes.

In the Palette, colors are labeled and grouped together. The scrolling window contains the 14 color combination groups (Custom, Artdeco, Autumn, Glass, Metal, Neon, Rococo, Santafe, Sheen, Silky, Spring, Summer, Tropical, Winter). Examples of colors in a selected group are displayed in the attached rectangular area. If a custom color has been defined on the RGB tab, it can be added to the Custom palette with the Add to Palette button. After being added, these color choices are stored and can be used with other Imaris objects.

## 11.14.9.3 Color Type-Statistics Coded

### Statistics Coded

The Statistical variable selection is listed in the drop-down window depending on the object type selected (either Surfaces or Tracks). For example, if Surfaces is chosen as the Object type, the available Statistical type will be the Surface linked variable. Similarly, Statistical variables for Tracks will be listed, if the Object type Tracks is selected.

### Statistics Colorbar Properties

The Statistics colorbars are displayed in the viewing area. The upper one is the colorbar range for Surfaces, and lower one is the colorbar for the Tracks.

### Show Colorbar

Check the box to see the statistics colorbar in the viewing area.

### Show Title



Check this box to see the title in the statistics colorbar.

### Show Range

Check this box to see the range in the statistics colorbar.

Font color in colorbar is adjustable by selecting a color within the color map.

## Colormap

### Button **Reset**

Click the **Reset** button to set the image back to the original values.

### Colormap

Click on the button Colormap to display a large number of predefined colormaps. If the default colormap (Spectrum) is not appropriate, you can change it by clicking on the drop-down list. The list gives a preview of what different colormaps look like.

## Colormap range

Colormap range-specify the limits for colorbar.

**Min** - lower color limit

**Max** - upper color limit

### Button **Auto**

Click the button **Auto** to detect the system the real min and max values and sets the Max. and Min. limits automatically to these values.

## Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### 11.14.9.4 Color Type-Time Mapped

#### Time Mapped

This option is only available when Tracks (see above) is selected as Object Type. The color range for the Tracks goes from blue to white to the range of time points.


## Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image transparency).

### 11.14.9.5 Color Type-Object ID

Assigns a random color to every object. This is available for all surpass components.

### 11.14.9.6 Color Type-Track ID

Track ID assigns a fixed, random color to each object. This mode is useful for validating tracks as an object will have the same color through the track duration. Erroneous behavior can easily be detected and corrected using the Edit Tracks  functions. Refer to the [Tab Edit Tracks](#) section.

## Transparency

**Transparency** defining amount of light allowed to pass through. Click this tool to change the percentage from 0% (complete opacity) to 100% (complete image)

### 11.15 Volume

#### How to Add a new Volume?

- Select menu 3D View - Volume.

The Surpass Tree list is automatically updated and in the Volume - Properties the Settings parameter are displayed. A data set can have only one volume.

For information on statistics calculations used refer to [Volume](#)

#### 11.15.1 Display Adjustments

To set the correct Display Adjustments for the Volume rendering of your data set please refer to Section Menu Edit - [Show Display Adjustment](#).

#### 11.15.2 Tab Settings

##### Mode

###### MIP (max)

In the Maximum Intensity Projection mode only the brightest point (the point with maximum intensity) of all layers along the viewing direction displays. If your data set has more than one channel, the combination color displays. A layer in the data set with high red and high green intensity displays yellow.

###### Normal Shading

The result of the normal shading is a depth effect on the screen. The surfaces turned away from the light source appear darker.

###### Blend

The result of the blend mode is a depth effect on the screen. One effect of the blend mode is, that the borders appear darker. In the blend mode all values along the viewing direction including their transparency are used for the calculation.

For further information on **Normal Shading** and **Blend** functionality see [Volume Rendering](#).

###### Shadow Projection

The result of the shadow projection is a depth effect on the screen. An object between light source and the surface you look at projects a shadow.

##### Rendering Quality

If the rendering quality is set to 1.000, then 1 pixel represents 1 voxel. If you decrease the rendering quality the image quality is reduced and becomes coarse-grained.



## View Aligned Planes

Check this box to see the display planes. Examine the planes while rotating the data set in the viewing area.

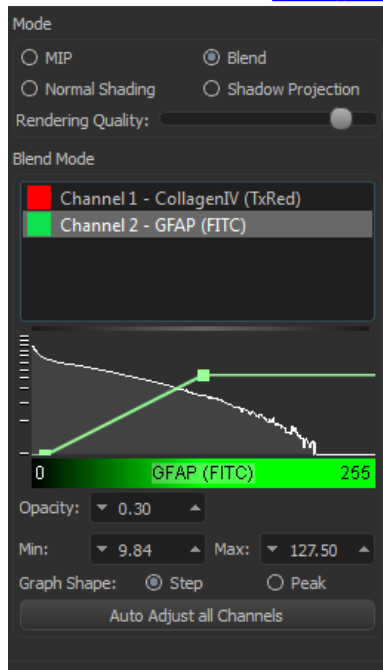
See also:

Preface - [Terminology](#)

### 11.15.3 Volume Rendering

Imaris 9.0.0 onwards introduces new **Blend** and **Normal** shading functionality to make it easier to adjust, and control settings, so you can obtain optimal visualization of your rendered image data under these modes.

The Transfer Function Histogram (shown below) enables you to set the Opacity and the min/max thresholds for the selected channel. Changes to these values are restricted to the selected Blend and Normal mode and channel selected. The Display Adjustment settings allow you to adjust how the rendered data set using the transfer Function Histogram is displayed. Refer to the section [Show Display Adjustment](#).



#### Opacity

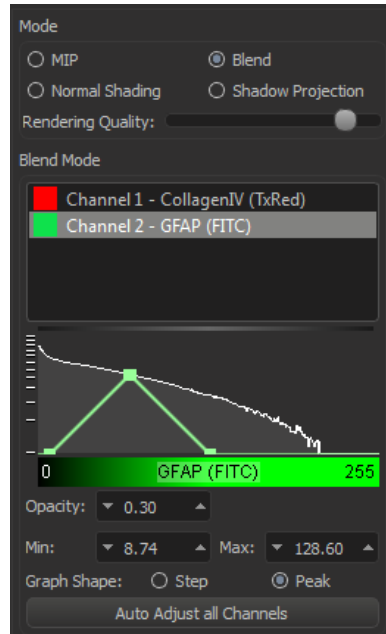
Opacity sets how transparent or solid the rendered data will appear. The value can be set between 0.0 (fully transparent) and 1.0 (solid). The level of opacity can be adjusted by entering the value directly in the Opacity field (or adjusting the value using the up down arrows), or alternatively by clicking and dragging the upper adjustment point in the Transfer Function Histogram in the y-axis.

#### Min/Max

The Min and Max thresholds for the data that is to be rendered using a 0.00 and 255.00 range. Values for Min and Max can be entered directly in the Min and Max fields (or by adjusting the value using the up down arrows). You can also adjust the min and max values from the histogram by clicking and dragging the adjustment points in the x-axis. In the "Step" Graph Shape the two points in the graph may be adjusted independently. For the "Peak" Graph Shape the limits of the peak will increase or decrease as the adjustment points are moved.

#### Graph Shape

Step is the default graph shape and applies a "step" profile so that opacity and Min/max limit can be adjusted using two points (shown in the previous image). Peak is available for Blend Mode only and applies a "peak" profile so that the values can be adjusted using three points (shown below).



### Auto Adjust all Channels Button

Use the AutoAdjust all Channels Button to automatically set all channels to calculated values.

### Notes

- **Display Adjustment settings that map intensities to colors apply to all visualizations. Changing them in MIP changes them for blend and normal shading as well (Control + D).**
- **Zoom in/out on histogram using the mouse wheel**
- **Double right click the histogram to reset zoom level to default**

## 11.15.4 Tab Draw

### Volume Painter

On the tab Draw you can find the settings for the Volume Painter. Use this Volume Painter to adjust the voxel intensities before you do a segmentation based on Surfaces, Spots, or Filaments. (If an automatic algorithm is not able to separate two objects, the intensity between these two objects can be "erased" in advance.) You can draw disks, spheres, or hollow spheres into the selected image channel.

1. First select the desired channel and painter shape.
2. Rotate the view to execute a vertical cut.
3. Then customize the painter settings.
4. Change to the painter mode Select.
5. Move the cursor to the desired position.
6. Hold down the Shift-key and click to add the shape.
7. In the viewing area you see a preview of the painter tool.
8. To re-edit a shape use the point selection.
9. Set intensity to 0 to erase a region, or set the intensity to 255 to fill a region.
10. To fix the shape click on the button **Set Intensity**.

**Please note: The button Set Intensity changes the data set.**

### Selected Channel

Select the respective source channel.



## Painter Shape

Here you select the painter shape.

### Disk

The painter is disk shaped. In the settings selection adjust the diameter, width and intensity of the disk (see below).

### Sphere

The painter is a sphere. In the settings selection adjust the diameter and intensity of the sphere (see below).

### Hollow Sphere

The painter is a hollow sphere. In the settings selection adjust the diameter and intensity of the hollow sphere (see below).

## Selection

### Diameter

Here you select the diameter of a disk, sphere, and hollow sphere. If your mouse has a wheel, you can use the wheel to adjust the diameter.

### Width

Here you select the width of a disk.

### Intensity

Here you select the painter shape intensity. The shape preview in the viewing area changes accordingly. If you want to cut parts out of the channel select 0 as intensity value.

### Button **Set Intensity**

In the last step you click on this button to fix the shapes. The button **Set Intensity** changes the data set and it is not possible to re-edit the shapes again.

### Button **Delete All**

Click on this button to delete all inserted shapes in the preview.

## Selected Point

You can use the following data fields and the button **Delete** to re-edit or delete a selected point.

### Position [um] X, Y, Z

To select a point change to the pointer mode Select and click on a point. The color of the selected point changes. The point position displays in the x, y, and z data field. To move the point click on the new position in the viewing area or edit the x, y, or z data field.

### Button **Delete**

Click on this button to delete the selected point.

## 11.15.5 Tab Statistics

Volume statistics are automatically computed. For the statistical variable values please refer to the Section Menu Edit - Preferences... - Statistics - [Volume](#). The Statistic tab collects and displays image statistical values in Imaris. Statistic tab enable us to understand and compare different data sets through summarized statistical values and graphical data presentations.

Several types of statistical tools enabling convenient organization and analysis of heterogeneous statistical data. Statistical View is configured as follows:

**a) Overall**

**b) Detailed**

**c) Selection**

In a statistical data set the first column contains the name of the variable, followed by columns with calculated statistical data. Corresponding statistical variable values are presented in rows.

To sort columns, click on the arrow in column heading. Click again to reverse the sort.

### Overall Tab

Output of the **Overall** statistics provides an overview of the image single determining variables.

### Buttons at the bottom of the Statistics tab

Button **Settings** - Configure List of visible statistics values.

Click on the button **Settings** and you switch to the window Preferences - Statistics - [Volume](#).

Button **Search** - Search by object ID.


Type in the object ID in the data field. Click on the button **Search**.

- Highlight the object in viewing area
- Highlight the object on the Detailed Tab
- Display of the values on the Selection Tab

Button **Surfaces** - Duplicate Selection to new Surfaces.

Click on this button and all selected objects are copied to a new Surpass Group. The new group displays in the Surpass Tree.

Button **Export** - Export Statistics on Tab Display to File.

Button **Export**  - Export selected Data for plotting

Exports the selected statistical value for plotting. The selected value has to be time dependent.

With a click on the button **Export** the Export Statistics window opens. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xml). If you select MS Excel as file type, the program starts automatically.

Button **Export** - Export All Statistics to File.

With a click on the button **Export** you open the Window Export Statistics. Select the file type (Comma Separated Values \*.csv, Excel file \*.xls, or Excel XML \*.xml). If you select MS Excel, the program starts automatically and the sheets show up one after another (this will take several seconds).

See also:

Menu Edit - Preferences... - Statistics - [Volume](#)

## 11.16 Export Selected Objects...

In the Surpass Tree highlight all Items to be exported. To export all Surfaces, Contours, and External objects, select the Export Selected Objects... .

- Select menu Surpass - Export Selected Objects... .

The Save selection as External Objects box is displayed.

- Select the directory and enter the name for the file to be saved and click **OK**. The object is saved as either as an Inventor file with the extension \*.iv, VRML2 file with extension \*.vrmf or WRL files with the extension .wrl. The saved object can be loaded as External objects to another data set.

## 11.17 Delete Selected Objects ...

In the Surpass Tree highlight all Items to be deleted. To delete all Surfaces, Spots, Contours, and External objects, select the entire Scene folder.

- Select menu Surpass - Delete Selected Objects.

The Delete selection window with a confirmation question is displayed.

## 11.18 Switch Pointer Mode

In the Surpass view there are two pointer modes: Select and Navigate.

### Select

The cursor becomes an arrow. You use the pointer mode Select whenever you want to mark something in the image, e.g. to set Measurement Points on the object surface.

### Navigate

The cursor becomes two turning arrows. You use the pointer mode Navigate to move, rotate or zoom the image in the viewing area.

**Tip: You can easily switch between the two pointer modes using the ESC-Key. The effect is directly visible on screen by the altered mouse pointer display.**

## 11.19 InMotion

The function InMotion is a 3D viewing and precise interaction mode. Imaris produces a real 3D impression with smooth animation of the image. This helps to get an understanding of the image depth, while manipulating with Surpass objects: Spots, Filaments, Measurement Points.

### How to Start the InMotion Mode?

- Click on the icon in the Main toolbar.
- Select View - InMotion.

To switch the mode off, click on the icon Surpass in the Main toolbar.

## Adjust 3D Cursor Settings

To adjust the 3D cursor settings click on menu Edit - Preferences... - [3D Cursor](#).

### 11.20 Store Creation Parameters

Enter a name for the Creation Parameter set. This name identifies the Creation Parameter item in the Arena view and in the Creation Parameter list used in the [Edit/Preference/Creation\\_Parameters](#) and would be used as default name in the subsequent creation wizard algorithms.

## Section 12 Help

<a href="#">Quick Start Tutorials</a>	Ctrl + F1
<a href="#">Keyboard and Mouse</a>	F1
<a href="#">Reference Manual</a>	
<a href="#">Video Tutorials</a>	
<a href="#">Programming Interface</a>	
<a href="#">XTensionDocumentation</a>	
<a href="#">License...</a>	F2
<a href="#">Maintenance Plan</a>	
<a href="#">Check for Updates now...</a>	F3
<a href="#">Bitplane</a>	
<a href="#">About Imaris...</a>	

### How to find the pdf based help for PC Users:

To open the pdf help file in Imaris first select **Start- All Programs – Imaris**.

The pdf version of the **Reference Manual** and **Quick Start Tutorials** are located there.

### How to find the pdf based help for Mac Users:

To open the pdf help file in Imaris first locate and select the Imaris application file.

By default, the file is in the Application folder and the file name is Imaris 8.3.app.

With the right-click select the option **Show Package Contents**.

The new window will open.

Select the **Contents** folder and with a double-click select the **Shared Support** folder.

The pdf version of the **Reference Manual** and **Quick Start Tutorials** are located there.

See also:

Addendum - [Mouse & Keyboard Mac](#)

### 12.1 Quick Start Tutorials

The Quick Start Tutorials provide you with the basic information on how-to-use Imaris but may also show yet unrecognized new features of the software to the advanced user.

The tutorials are designed to be followed sequentially, but if you are already familiar with Imaris the basic lessons may be skipped. The tutorials are cross-referenced by hyperlinks highlighted in blue underlined font. The Table of Contents and the Index provide further support for navigation in the tutorials.

Each tutorial is a hands-on seminar, therefore it is recommended to move Imaris to the left hand side of the screen and adjust the online tutorial on the right hand side of the screen and practice step-by-step.

### 12.2 Keyboard and Mouse

Click on this menu entry and the Reference Manual opens in the actual browser. The page for the global Mouse & Keyboard functions displays.

#### Global Mouse & Keyboard Functions

For PC Users:

Addendum - [Mouse & Keyboard PC](#)

For Mac Users:

Addendum - [Mouse & Keyboard Mac](#)

## 12.3 Reference Manual

The Reference Manual provides you with the information about Imaris: the menu(s), the different Imaris views and the adjustable parameters in the program.

To find a specific parameter use the "Search function" in the online version of the Reference Manual.

### Context Sensitive Help

For PC User:

With a right-click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a left-click on the message the Reference Manual opens in the actual browser and displays the corresponding page.

For Mac User:

With a Ctrl + click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a click on the message the Reference Manual opens in the actual browser and displays the corresponding page.

## 12.4 Video Tutorials

This link opens the Learning Center on the Bitplane website. Use the search facility to find the topics of interest.

## 12.5 Programming Interface

Select this to open the Imaris XT programming Interface.

## 12.6 XTension Documentation

This Section provides a link to descriptions of XTension documentation.

## 12.7 Imaris Open

If your computer is connected to the Internet, Imaris can directly open the Imaris Open webpage (<http://open.bitplane.com/>).

## 12.8 License...

### Overview

#### Node-locked License





Node-locked licenses grant permission to run the licensed software on one computer (node) only. Node-locked licenses are the easiest way to license the software. You can use Imaris Administrator to directly download a node-locked license from the Bitplane server. If your computer is not connected to the Internet it is also possible to download this license from another computer and then copy it to the target machine.

### **Floating License**

Floating licenses grant permission to run the licensed software on several computers. One floating license can activate only one instance of the program at any given time. Floating licenses require the installation of a separate license server that runs on a Windows computer. The server installation requires more technical know how.

The Imaris Administrator application easily let you enable a floating license.

### **Demo License**

A Demo License is a time-limited (usually one month) license. It is not an official licensing scheme and therefore can't be purchased. It's main purpose is for internal Testing and to grant customers with licensing issues an easy and quick workaround so that their productivity can continue until the problem is solved. Simply open Imaris Administrator application and open the Demo License you received from your local support representative.

### **Evaluation License**

Evaluation Licenses are temporary licenses allowing customers to explore all fascinating possibilities Imaris offers before actually purchasing it. The license is only limited in time (10 days), but not in features. Additionally an Evaluation License is issued per user.

To get an Evaluation License you will have to register yourself first at:

<http://www.bitplane.com/go/download/imaris-evaluation>


An automatic mail response should provide you with your personal RegistrationID and its corresponding Passcode. These credentials can then be entered during the application's start up.

### **Imaris Open Developer License**

To get an Imaris Open Developer License you first have to register at:

<http://open.bitplane.com/>

After you entered your OrderID click on the Imaris Open button  to activate the Developer License.

This action requires an internet connection because the licenses are downloaded directly from the Bitplane server. Upon success, the button will turn on .

### **RegistrationID**

Please enter your RegistrationID you received by mail first.

### **First-time Installation**

If you have installed Imaris for the very first time, you have to set up a license before you can use Imaris.

A dialog window appears during the start up of the application.

The same dialog window appears if you have accidentally deleted the license .

You now have two options how to proceed:

- If you have a Node-locked or a Floating License you should use the Imaris Administrator application for the further license setup. This is done by simply clicking on the “Start Imaris Administrator” button. Please note that the Imaris Administrator application needs administrative privileges.

- If you want to evaluate Imaris first, please register yourself first at:

<http://www.bitplane.com/go/download/imaris-evaluation>

The automatic mail response contains your RegistrationID and Passcode.

To enable an Evaluation License enter these values in the corresponding fields and click on the “Use Evaluation License” button.

Please note that a working internet connection is needed to start up Imaris in Evaluation mode.

### Installation of additional Licenses

If you already have set up a license but you want to add other licenses as well, you can do this in the License Preferences dialog.

Once more, you'll have the option either to start the Imaris Administrator application or to use an Evaluation License in addition if you would like to try out a new feature before buying the license.

## Imaris Administrator

Here you can adjust the default license settings.

Imaris Administrator is an application designed to manage the licenses of various Bitplane applications. It provides a simple interface to add or delete any licenses. When a license is added, the license file gets copied to a system folder where it is secure from (accidental) manipulation by standard users. Although it is possible for administrators to modify license files directly in this folder we strongly advise you not to do so. It is recommended to use the Imaris Administrator application instead.

### Imaris Administrator Button

This button starts the Imaris Administrator. This is a tool shipped with Imaris in order to add or modify licenses. Unless you want to use an Evaluation license you need to use the Imaris Administrator to set up your licenses. Please note that you'll need administrative privileges in order to start Imaris Administrator.

Any change of licenses will require Imaris to be re-started.

Except for the Evaluation License which is bound to a single user, all other licenses are bound to the whole system. Therefore any license modifications need [administrative privileges](#).

If you have access to an administrative system account, Imaris Administrator should just start normally.

### Start Imaris Administrator Button

This button starts Imaris Administrator. This is a tool shipped with Imaris in order to add or modify licenses. Unless you want to use an Evaluation license you need to use Imaris Administrator to set up your licenses. Please note that you'll need administrative privileges in order to start Imaris Administrator.

Any change of licenses will require a new start of Imaris.

## Evaluation License

### Use Evaluation License Button

After you entered your RegistrationID and Passcode you can click on this button to enable the Evaluation



License. This action requires an internet connection because the licenses are downloaded directly from the Bitplane server. Upon success, the light bulb will turn on.

You can also disable your Evaluation License by clicking again on this button and the light bulb will turn off. This indicates you aren't using any Evaluation Licenses. Any change of licenses will require a new start of Imaris.

### **RegistrationID**

Please enter your RegistrationID you received by mail.

### **Passcode**

Please enter your Passcode you received by mail.

## **Available Licenses**

The table displays all existing Imaris licenses on the system and additional information for each feature:

### **Version**

The latest Imaris version this license can be used with.

### **Type**

Which type of licensing scheme is used. An overview is given at the very beginning of this page.

### **Status**

Displays the status of a license.

### **Expiration**

Shows the number of days until this license expires.

### **Expiration**

Shows the number of days until this license expires.

### **License colors**

In order to gain a quick overview, the validity of a license is also indicated by different colors:

- Green: License is valid.
- Yellow: License is valid, but all licenses are currently in use.
- Red: License has expired.
- No color: License is not available (e.g. this additional license feature hasn't been purchased).

You can select with which license(s) your Imaris should start per default. Any change will require a new start of Imaris.

### **Check/Uncheck All**

Check this box to enable or disable all available Imaris licenses on the system.

Please note that the Feature "ImarisBase" can't be disabled because this would render Imaris unusable.

The default Imaris Administrator executable can be found here:

Windows 7, 8 and 10:

64-bit: C:\Program Files\Bitplane\Imaris 9.0\ImarisAdministrator.exe

Mac OS X:

/Applications/Imaris 9.0.app/Contents/MacOS/ImarisAdministrator

If you did not have set up a license yet (e.g. first-time usage) this window is empty. In that case, please use

the **Add License Location** button to add a new license location.

## Add License Location Button

Use this button to add new licenses. A description is given in the [Add License](#) Section.

## Delete License Location

If you click this button, the selected license location entry in the table underneath will be deleted. Before using this button, please make sure you selected the right table entry. In case of a node-locked license you are asked if you want to keep the license. It is recommended that you keep all license files.

## License Location Table

This table lists all available license locations. Normally it would be just enough to have one license location defined. But there exist also good reasons to use multiple locations.

For example you might have a floating license server with just some basic features enabled. Maybe later you purchased some additional features in a node-locked licensing scheme. You can now just define both locations and the licensed features are merged on your system automatically.

The table shows also some additional Information:

- Type: This is either a License Server (floating license scheme) or a License File (all other schemes)
- Number of valid / total Licenses: These numbers shows you how many different features your license contains and it lets you easily verify that all licenses are still valid.

If you click on another table entry, the License table underneath gets updated and shows you detailed information about all available features of the current selected license location.

## Licenses Table

In this table you have a detailed overview of all licensed features of a license location. You can switch between different license locations by selecting the corresponding entry in the License Location table.

Each feature provides some information :

### Version, Type, Status, Expiration

The validity of a license is also indicated by different colors.

## Finish Button

This button is only enabled if you added at least one license location. It is an indicator when all necessary steps to set up a license are done. If all features in the above License table are highlighted in green (or only some of them) you successfully managed to add a license. If you click the button, it just closes Imaris Administrator and you can proceed using Imaris.

## Add License Window

First, you have to select your licensing scheme:

### Download Node-locked License:

If you decide to add a Node-locked License, it will check that your computer is connected to the internet. If the connection can be established successfully, the Online-Licensing Wizard is shown; if not, you have the possibility to download the license from another computer which is described in the Offline-Licensing Wizard.

### Online-Licensing Wizard:

This wizard has three steps:



### 1. Select the Network Interface

You need to select the network interface which is bound to the license. If your Host-ID is already known to our server, the available product for installation will be shown in the table below.

### 2. Check your Order-ID for newly ordered Products:

If you have ordered new products, you have to enter the Order-ID in this step. Clicking on the “Check Order-ID” button will add the products to the table below. Once you checked an Order-ID, it is bound to the currently selected network interface.

If your Host-ID is already known to the system, the available products will be shown in the tables below and you could skip that step.

### 3. Select the Licenses for Installation

In this step you just have to mark all products you want to install by checking them in the left table.

The right table is for your information only and shows all currently installed products.

When you click on the “OK” button, the license file is downloaded and the corresponding entry is added to the License Location table.

#### Offline-Licensing Wizard:

If you are not connected to the Internet, this could be due to two reasons:

1. your computer is behind a web proxy server which omits a direct connection or
2. your PC may be unplugged from the internet .

Therefore you have the following choices:

#### A) Set up a Proxy Server

Enter your proxy server's name and its port and click on the **Test Connection** button. If the connection could be established you are automatically redirected to the Online-Licensing Wizard described above.

If not, either the credentials you entered are not correct or the connection is cut at another place in the network. In that case please contact your network administrator or Bitplane Support teams [eusupport@bitplane.com](mailto:eusupport@bitplane.com) or [ussupport@bitplane.com](mailto:ussupport@bitplane.com)

If you want to delete a previously set up proxy server, just delete both fields and click on the button again.

The proxy server settings can also be altered in lmaris' system preferences dialog.

#### B) Download the Node-locked License from another Computer

You can download the license from another computer connected to the internet.

On that machine log on to [http://www.bitplane.com/activation/acti\\_offline\\_ident.html](http://www.bitplane.com/activation/acti_offline_ident.html)

and enter the Host-ID you wish to bind to the license together with your Order-ID into the web form. The download of the license should immediately start and you could transfer the file afterwards to the offline computer (e.g. by using a USB stick).

This instructions can be saved in a file by clicking the **Save Instructions...** button.

When you have downloaded the license you can just open it with the **Open License...** button or enter its path directly into the field on the left of the button.

When you click **OK** the license file is copied to the proper location.

#### Define Floating License Server:

In order to add a Floating License Server, just enter the server's name and its port to the corresponding fields and click on the **OK** button. The default port is 2700.

Install License from Existing File:

If you received a Demo License from your local support representative or you need to add a license file directly you can use this wizard. If you click on the **Open License...** button you can browse to the license you want to add. You can also specify the license file to load by entering its path directly into the field left from the button. If you click on the **OK** button, the license is copied to the right location and it's added to your License Location table.

See also:

Menu Edit - Preferences... - [Calculation](#)

Menu Edit - Preferences... - [Licenses](#)

Menu Edit - Preferences... - [Display](#)

## 12.9 Maintenance Plan

### Imaris Infinity

A message is shown when your Imaris Infinity is about to expire.

If your contract has lapsed, the message indicates when that occurred.

You can renew your software maintenance contract for one or more years at a time. During the validity period of your Imaris Infinity contract, you may install all updates to the program, contact our team for technical support as well as access a wide range of educational and training services/tools.

Click on the Contact Sales button to send an email requesting a renewal of your Imaris Infinity contract.

Click on the **Remind me later** button to be reminded again in seven days.

If you click **No, Thanks** button and later change your mind and do wish to be reminded, change the setting in the the Preferences/[Update](#) dialog.

## 12.10 Check for Updates now ...

If your computer is connected to the Internet, Imaris can directly check, if a software update is available.

If new updates are available, a list of the available software versions is shown.

For a detailed list of new features, improvements and fixed bugs, please refer to the release notes.

## 12.11 Bitplane AG

If your computer is connected to the Internet, Imaris can directly open the Bitplane AG homepage ([www.bitplane.com](http://www.bitplane.com)).

## 12.12 About

The Imaris start screen displays.

**Imaris**

Version number and release date.

Copyright 1993-2018 Bitplane AG  
All rights reserved

European Patent Registry No. 05009677.7  
Patent Pending.

Using XML parser expat: Copyright  
1998, 1999, 2000. Thai Open Source  
Software Center Ltd and Clark Cooper

[www.bitplane.com](http://www.bitplane.com)

## Section 13      Toolbars

In the standard Imaris view there are three different toolbars available. The [Main Toolbar](#), the [Status Bar](#) and the [Time Bar](#).

See also:

Surpass View - Overview - [Camera Toolbar](#)

### 13.1      Main Toolbar

Please refer to Section:

Open	Imaris Menu - <a href="#">Open</a>
Save	Imaris Menu- <a href="#">Save</a>
Export	Imaris Menu - <a href="#">Export</a>
Annotation	Imaris Menu - <a href="#">Annotation</a>

The display modes Slice, Section, Gallery and Easy 3D are in one icon group. Click on the arrow to select the respective mode.

Please refer to Section:

Slice	<a href="#">Slice</a>
Section	<a href="#">Selection</a>
Gallery	<a href="#">Gallery</a>
Easy 3D	<a href="#">Easy 3D</a>
3D View	<a href="#">3D View</a>
Coloc	<a href="#">Coloc</a>
Animation	<a href="#">Animation</a>
Snapshot	<a href="#">Snapshot</a>

Bitplane AG	Menu Help - <a href="#">Bitplane AG</a>
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### 13.2      Status Bar

At the bottom of the screen you find the Status Bar.

#### Zoom %

Imaris lets you enlarge or reduce the viewing size of your data set as is required with the Zoom function. You can zoom from 10% to 1000%. To go to a predefined zoom level, click the percentage of enlargement or reduction you want. To specify a custom level, just type a zoom value. At 100% zoom factor one pixel on screen represents one voxel.

#### Mouse Clicks to Zoom





With a three-button mouse:

- Click with the middle mouse button on the image, hold down the middle mouse button, and drag the mouse up or down.
- Hold the Shift-key and click with the right mouse button on the image, and drag the mouse up or down.

**Tip: In the Surpass view be sure that the mouse is in the pointer mode Navigate. Switch between the two pointer modes Select/Navigate with the ESC-key.**

### Button Fit

Click on this button to pan the position to best fit in the window and adjust the zoom factor.

### Button Reset

Click on this button to reset the image to the original position, center the image in the middle and set the optimal zoom factor.

### Button Full Screen

Click on this button to maximize the viewing area to full size of the monitor. To return to the standard window, click on the button Full Screen in the lower left corner.

### Button Navi

Click on this button to toggle the display of the Navigation window of the data set in the upper right corner of the viewing area. If you move the data set, the overview updates in real-time. In the pointer mode Select you can click & drag the Navigation window to another position in the viewing area. The Navigation window is placed to the nearest corner.

### Change Position of the Navigation Window

- In the pointer mode Select you can click & drag the Navigation window to any desired corner.
- Double-click onto the Navigation window in order to switch off the automatic "snap-to-corner-mode". You can drag it to any desired location. Re-double-click onto the Navigation window to switch the automatic "snap-to-corner-mode" on again.
- Right-click into the Navigation window in order to pan to the clicked position (moves the small yellow window).

In the Section the position of the Navigation window is fixed (lower right corner) and can not be moved.

## 13.3 Time Bar

If a time series is loaded, a Time Bar displays at the bottom of the main window. It contains a slider showing all Time Points in the series, and control buttons (described below).

The Time Bar is visible in the display modes Slice, Section, Gallery 3D Preview, and Surpass. It allows you to view a particular Time Point or to automatically play 4D images. The views change when selecting a new Time Point.

### Play and Record Time Series

Reference Frame button 

Sets the reference frame playmode. Refer to [Reference Frame](#).

When using reference frames, coloured tick marks indicate where the reference frames are located in time as shown below for frames 0, 3, 5, 8 and 10:



The color of the tick mark indicates if the Keyframe is a Translation (green) and/or Rotational (magenta) Reference Frame. In the above example, the reference frame located at frame 3 is both translational and rotational, frame 5 is rotational only.

Time Settings button 

Click on the time settings button to open the time parameter settings.

Jump to previous event and Jump to next event buttons 

Use these options to move between event increments in the playback e.g. frames.

Play button 

Click on this button to play the time series. To pause the continuous display through all the Time Points in the time series re-click on the button.

Record button 

To record the time series as movie click on the record button. The Export Movie window is displayed (see below).

## Slider

To inspect any the Time Point in the time series manually navigate the slider as follows (be sure that the Pointer is in the Select mode):

- Drag the slider handle to the left or right.
- Moving the slider to the left reduces the time point, moving it to the right increase time point.
- Select the slider handle with a left mouse click and then use the Arrow-keys to the left/to the right on the keyboard.
- Select the slider handle with a left mouse click and then press the Home-key or the End-key to display the first respectively last Time Point.

The respective Time Point number is displayed on the right hand side of the slider in the number field.

## Window: Export Movie

### File name

Enter a file name.

**Export type**

Select the desired file type (TIFF series, or AVI).

**Movie Settings****Compression Factor**

Select a compression factor between 0 (High Quality) and 100 (Low Quality), the default setting is 5. Use the slider to set the compression factor between 0 and 100. Moving the slider to the left reduces the compression point, moving it to the right increase compression pint. A lower factor results in a lower compression and therefore a better quality, but also in a larger movie file.

**Frame Rate**

Define how many frames are displayed per second.

**Play Movie with default Media Player when finished**

Check the box to play the movie with the default media player when finished.

See also:

Menu Edit - Preferences ... - [Time](#) (default parameter settings)

Surpass View - Overview - [Camera Toolbar](#) (Pointer Select)

## Section 14 Addendum

In this addendum you find the Imaris global [Mouse & Keyboard PC](#) and [Mouse & Keyboard Mac](#) functions, some tips and tricks on how to customize your [Imaris Interface](#) for your daily routine, and a [Terminology](#) table with frequently used technical terms and specific image processing terms.

### 14.1 Mouse & Keyboard PC

In the following you find the global Mouse & Keyboard functions.

#### Global Mouse & Keyboard Functions

Ctrl + 1	Activates the Slice
Ctrl + 2	Activates the Section
Ctrl + 3	Activates the Gallery
Ctrl + 4	Activates the Easy 3D
Ctrl + 5	Activates the 3D view
Ctrl + 6	Activates the Key Frame Animation
Ctrl + 7	Activates the Coloc
Ctrl + A	Centers the image to the window
Ctrl + B	Adjusts zoom factor and pan position to best fit the image into the window
Ctrl + C	Copies current view to the clipboard
Ctrl + D	Shows the Display Adjustment window
Ctrl + E	Saves Scene file
Ctrl + F	Refreshes the textures of all views
Ctrl + I	Allows to inspect and edit the image properties
Ctrl + L	Opens Scene file
Ctrl + N	Toggles display of Navigation window
Ctrl + O	Opens existing file
Ctrl + P	Allows to edit the settings of Imaris
Ctrl + Q	Exits the application
Ctrl + R	Discards all changes and opens the current data set again
Ctrl + S	Saves the current data set
Ctrl + T	Saves the current view as a *.tif file
Ctrl + W	Crops X, Y, or Z
Ctrl + Z	Revokes the last action
Ctrl + Shift + A	Adds channels of another image
Ctrl + Shift + B	Deletes selected channels
Ctrl + Shift + C	Copies the Imaris version, date and build to the clipboard
Ctrl + Shift + I	Open an additional image without closing the existing image.
Ctrl + Shift + P	Allows to edit figures and annotations
F1	Opens Reference Manual
F2	Displays or updates the license status
F3	Automatic check for updates
F11	Maximizes the viewing area to the full size of your monitor

Ctrl + F1	Opens Quick Start Tutorials
Alt + F4	Exits the application
Del	Deletes selected objects
Right-click	Opens Context Sensitive Help

### Global Mouse & Keyboard Functions to Navigate in All Imaris Views

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image
Left-click & drag	Rotate image

### Context Specific Mouse & Keyboard Functions

In the varying Imaris views there are additional shortcuts, please refer to the respective Section in this Reference Manual for details.

Menu Edit -	Show Display Adjustments - <a href="#">Mouse &amp; Keyboard PC</a>
Slice -	<a href="#">Mouse &amp; Keyboard PC</a>
Section -	<a href="#">Mouse &amp; Keyboard PC</a>
Gallery -	<a href="#">Mouse &amp; Keyboard PC</a>
Easy 3D -	<a href="#">Mouse &amp; Keyboard PC</a>
3D View -	Overview - <a href="#">Mouse &amp; Keyboard PC</a> Clipping Plane - <a href="#">Mouse &amp; Keyboard PC</a> Contour Surface - <a href="#">Shortcuts Overview</a> External Object - <a href="#">Mouse &amp; Keyboard PC</a> Filament - <a href="#">Mouse &amp; Keyboard PC</a> Light Source - <a href="#">Mouse &amp; Keyboard PC</a> Measurement Point - <a href="#">Mouse &amp; Keyboard PC</a> Oblique Slicer - <a href="#">Mouse &amp; Keyboard PC</a> Ortho Slicer - <a href="#">Mouse &amp; Keyboard PC</a> Surface - <a href="#">Shortcuts Overview</a> Spots - <a href="#">Mouse &amp; Keyboard PC</a>
Animation -	<a href="#">Mouse &amp; Keyboard PC</a>
Coloc -	<a href="#">Mouse &amp; Keyboard PC</a>

### Context Sensitive Help

With a right-click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a left-click on the message the Reference Manual opens in the actual browser and displays the corresponding page.

## 14.2 Mouse & Keyboard Mac

In the following you find the global Mouse & Keyboard functions.

### Global Mouse & Keyboard Functions

Command + 1	Activates the Slice
Command + 2	Activates the Section

Command + 3	Activates the Gallery
Command + 4	Activates the Easy 3D
Command + 5	Activates the 3D View
Command + 6	Activates the Key Frame Animation
Command + 7	Activates the Coloc
Command + A	Centers the image to the window
Command + B	Adjusts zoom factor and pan position to best fit the image into the window
Command + C	Copies current view to the clipboard
Command + D	Shows the Display Adjustment window
Command + E	Saves Scene file
Command + F	Refreshes the textures of all views
Command + I	Allows to inspect and edit the image properties
Command + L	Opens Scene file
Command + N	Toggles display of Navigation window
Command + O	Opens existing file
Command + ,	Allows to edit the settings of Imaris
Command + Q	Exits the application
Command + R	Discards all changes and opens the current data set again
Command + S	Saves the current data set
Command + T	Saves the current view as a *.tif file
Command + W	Crops X, Y, or Z
Command + Z	Revokes the last action
Command + Shift + A	Adds channels of another image
Command + Shift + B	Deletes selected channels
Command + Shift + C	Copies the Imaris version, date and build to the clipboard
Command + Shift + P	Allows to edit figures and annotations
Ctrl + Shift + I	Open an additional image without closing the existing image.
F1	Opens Reference Manual
F2	Displays or updates the license status
F3	Automatic check for updates
Command + F11	Maximizes the viewing area to the full size of your monitor
Command + F1	Opens Quick Start Tutorials
Del	Deletes selected objects
Ctrl-click	Opens Context Sensitive Help

## Global Mouse & Keyboard Functions to Navigate in All Imaris Views

### With a one-button mouse:

Shift + Ctrl + click & drag	Move up: zoom out Move down: zoom in
Ctrl + click & drag	Pan image
Click & drag	Rotate image

With a three-button mouse:

To configure a three button mouse on a Mac do the following:

- Open the Apple-menu, select System Preferences... .
- Click on the button Keyboard & Mouse.
- Select the OS X mouse properties.
- Change the middle button to "Button 3".

**Please note: Combined mouse buttons (e.g. left + middle mouse button) do not work in Imaris.**

Middle-click & drag	Move up: zoom out Move down: zoom in
Right-click & drag	Pan image

### Context Specific Mouse & Keyboard Functions

In the varying Imaris views there are additional shortcuts, please refer to the respective Section in this Reference Manual for details.

Edit -	Show Display Adjustments - <a href="#">Mouse &amp; Keyboard Mac</a>
Slice -	<a href="#">Mouse &amp; Keyboard Mac</a>
Section -	<a href="#">Mouse &amp; Keyboard Mac</a>
Gallery -	<a href="#">Mouse &amp; Keyboard Mac</a>
Easy 3D -	<a href="#">Mouse &amp; Keyboard Mac</a>
3D View -	Overview - <a href="#">Mouse &amp; Keyboard Mac</a> Clipping Plane - <a href="#">Mouse &amp; Keyboard Mac</a> Contour Surface - <a href="#">Shortcuts Overview</a> External Object - <a href="#">Mouse &amp; Keyboard Mac</a> Filament - <a href="#">Mouse &amp; Keyboard Mac</a> Light Source - <a href="#">Mouse &amp; Keyboard Mac</a> Measurement Point - <a href="#">Mouse &amp; Keyboard Mac</a> Oblique Slicer - <a href="#">Mouse &amp; Keyboard Mac</a> Ortho Slicer - <a href="#">Mouse &amp; Keyboard Mac</a> Surface- <a href="#">Shortcuts Overview</a> Spots - <a href="#">Mouse &amp; Keyboard Mac</a>
Animation -	<a href="#">Mouse &amp; Keyboard Mac</a>
Coloc -	<a href="#">Mouse &amp; Keyboard Mac</a>

### Context Sensitive Help

With a Ctrl + click on a toolbar, a tab, or a parameter set the message Show Help... appears. With a click on the message the Reference Manual opens in the actual browser and displays the corresponding page.

## 14.3 Customize Interface

In this Section you find a collection of tips and tricks to customize the Imaris interface for your daily routine.

### Histogram

Logarithmic or linear histogram display.

- On the left hand side of a histogram there are horizontal lines representing a linear or logarithmic histogram display.
- Click on these lines to change the display from logarithmic to linear and vice versa.

The default setting is logarithmic.

## Windows

You can plug windows in Imaris in or out.

Example: Key Frame Animation window

Plug out:

- Click on the header of the Key Frame Animation window and drag the window to any free space on the screen.
- On the right hand side of the header click on the little "window" icon. The Key Frame Animation window is plugged out, you can drag it on any free space on the screen.

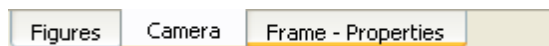
Plug in:

- On the right hand side of the header click on the little "window" icon. The Key Frame Animation window is plugged in on its original place.

## Tabbed Toolbars

Try to undock the toolbars and dock them all at the same side. Especially in combination with Annotation, this saves a lot of screen area.

Example: In the Section you can bring the toolbar Figures (Annotation), Camera, and Properties to the left side at the bottom.



## Objects Toolbar

You can adjust the Objects toolbar to your personal preferences.

- Open the menu Edit - Preferences - [3D\\_View](#) - Object Creation Buttons to adjust your personal Objects toolbar.

## Display Mode Blend and Bland Opacity

You can select the Display Mode Blend in the Surpass view for a Volume object, in the Section (Extended) and in the Easy 3D . Any change in the Display Adjustment window in the parameter Blend Opacity is only visible, if a Blend Mode is selected.

### 14.4 Terminology

Term	Description
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Channel	A channel is a particular piece of information that has been recorded for all voxels. Most of the time in light microscopy, the different channels are intensities that have been measured at different wavelengths. For instance, a two-channel data set contains two intensities for each voxel, one intensity measured in channel 1 (e.g., green light) and one intensity in channel 2 (e.g., red light). Ideally, the intensity values present in different channels are independent of one another.
Coordinates	Any position in the data set can be described either in the voxel grid (e.g., voxel no. 5 in x-direction) or by a coordinate, which corresponds to a m). The distance relative to a defined origin in the real world (e.g. 2 latter) requires the correct calibration of the data set, i.e. that the voxel size is defined appropriately. Generally, in Imaris the origin of the data set is in the left bottom corner. If the data set was cropped from a larger data set, the bottom left corner may have minimum coordinates that are not equal to (0,0,0).
Image stack	A 3D data set is sometimes called an image stack consisting of 2D images stacked on each other.
MIP	A maximum intensity projection (MIP) is a computer visualization method for 3D data that projects in the visualization plane the voxels with maximum intensity that fall in the way of parallel rays traced from the viewpoint to the plane of projection.
Original Data Set	A 3D data set that cannot be derived from any other data set using Bitplane software. Usually "Original data sets" have been acquired using an image sensor and are loaded into Imaris.
Rendering	A technique that visualizes a multi-dimensional data set as a two-dimensional image that can be displayed on a computer screen or printed on a laser printer.
Resultant Image	An image that has been computed using Imaris.
Standard Deviation	<p>In probability and statistics, the standard deviation of a probability distribution, random variable, or population or multiset of values is a measure of the spread of its values. It is defined as the square root of the variance.</p> <p>The standard deviation is the root mean square (RMS) deviation of values from their arithmetic mean. For example, in the population {4, 8}, the mean is 6 and the standard deviation is 2. This may be written: {4, 8} 6. In this case 100% of the values in the population are at one standard deviation of the mean.</p> <p>The standard deviation is the most common measure of statistical dispersion, measuring how widely spread the values in a data set are. If the data points are close to the mean, then the standard deviation is small. Conversely, if many data points are far from the mean, then the standard deviation is large. If all the data values are equal, then the standard deviation is zero.</p> <p>The standard deviation (s) of a population can be estimated by a modified standard deviation (s) of a sample.</p>
Threshold	A threshold in Imaris is a gray value, which acts as a limit. All gray values above (or below) are assigned a particular functionality.
Time Point	A time point is a single 3D image containing one or many channels. The term is used regardless of what data is actually stored in the channels. In particular, the channels could contain data taken at different points in time with a changing object.

Volume Rendering	A rendering technique which operates directly on the voxel data. The input image consisting of a collection of voxels arranged in a regular grid is converted directly into the 2D output image.
Voxels	A volume data set is composed of volume elements called voxels. The voxels are the smallest units within the image about which we have distinct information in the form of a measured intensity. The dimensions of an individual voxel are specified in most image file formats supported by Imaris.