

13 JOBS and HCA

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Note: This is a part of a larger document. Chapter numbering may not start from 1.

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(requires: JOBS Editor)

13.1 Introduction to the JOBS Module

JOBS is an environment aiming at complex acquisition and data analysis with easy to use and comprehensive user interface.

Features highlights:

- Well-plate, dish and slide support
- Conditional acquisition
- Set of ready-to-use templates
- Wizard for Operators
- Visual Programming Style
- Integrated Analysis
- Asset Management via Database
- Data Mining
- Remote Access and Sharing
- NIS-Elements platform

Jobs are automated procedures for complex image acquisition and data analysis. Typically it is a conditional acquisition on well plates, dishes or slides for long times. It may include Confocal and wide-field camera acquisition combined with photoactivation or bleaching.

The strength of Jobs is their flexibility. They rely on building blocks that can be freely assembled into arbitrary procedures in order to fulfill any particular needs. As all this is done visually using Drag-drop it is called Visual Programming. This approach combines advantages of programming - flexibility - and limits the drawbacks: having to learn how to write a programming source code.

When such procedures - Jobs - are executed, images and analysis data are produced. The images are stored in ND2 files. Metadata and analysis results are stored in the database together with links to the ND2 files.

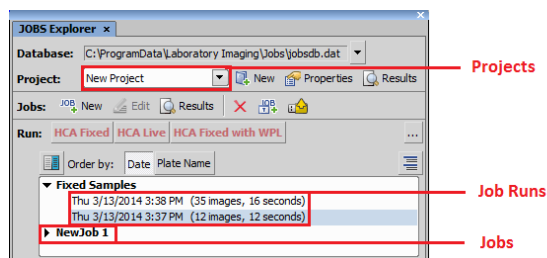
The complete acquisition history is stored in the database as well as the whole organization and structure of Jobs and Projects.

13.2 Jobs Explorer

13.2.1 Introduction to Jobs Explorer

Jobs Explorer is the heart of the HC Template and JOBS Editor modules. Run the *View > Analysis Controls > JOBS Explorer* `JOBS` command to display it. All jobs, job runs and all acquired data are viewed and managed throughout this window.

Figure 12.290. JOBS Explorer with the JOBS Editor module



Database Name of the currently connected job database is displayed in this row. The pull-down menu provides tools for managing job databases. See 13.2.3 Database context menu.

Project Jobs are organized into *Projects* - virtual containers which help you organize your jobs. Create your own project or use the default one. You can have projects named e.g. “cell proliferation”, “translocation” etc. or name it by its author e.g. “Catherine”, “John”, or for example use dates as project names.

However, since you can display results of the whole project in a single result view, it may be a good idea to keep similar jobs together. For instance it may be useful to have a 5hour and a 10 hour proliferation jobs

together in one project, but it may produce some complications to put 6-well-plate data together with a 96-well-plates.


Jobs  *Results* open Results of the selected Job.


Run This area aggregates predefine job templates which are executed by clicking on their button.

List of job runs Jobs Explorer shows the contents of one project at a time as a list of jobs. Each job can be unfolded to disclose all instances of when it was run. Single jobs (highlighted in bold) represent the definition which can be viewed and modified in the job editor, wizard or run time. The run instances can be opened to reveal a view on acquired images and data.


Jobs Explorer shows the contents of one project at a time as a list of jobs. Each job can be unfolded to display all instances of when it was run - so called *Job Runs*. Single jobs (in bold type) represent the definition which can be viewed and modified in the job editor, wizard or run time. The run instances can be opened to reveal a view on acquired images and data.


13.2.2 Jobs Explorer Options

 **New** Adds a new project. The *Project* window appears. Enter name of the new project and/or a custom description.


 **Properties** Enables changing the information of the active project which were set in the *Project* window. Projects (if not active) can be deleted here by clicking **Delete**.


 **Results (Project)**,  **Results (Jobs)** Opens the results window of the selected project, job or job run.

 **New** (requires: *JOBS Editor*) Adds new job to the active project. The *Job Definition* window appears. See 13.5 Job Definition Window for details about creating new jobs.


 **Edit** (requires: *JOBS Editor*) Opens the job definition window and displays the selected job to be edited.


 **Delete Selected Job** Removes the selected job.


 **Add New Job from Template** (requires: *JOBS Editor*) *NIS-Elements AR* comes with a set of job templates. This button opens the *Job Templates* window where you can select one of the templates and load it to the *JOBS Explorer*. The predefined template cannot be overwritten however if changed, it can be saved to your new *Job* or exported to a *.BIN* file.


 **Import from File** (requires: *JOBS Editor*) This button enables to import a previously saved job definition into your current project.

HCA Fixed, **HCA Live**, **HCA Fixed with WPL** Executes a job optimized for high content analysis of fixed/live samples. A simple wizard appears. See 13.6.3 Using Job Definition Wizard.

 **Order by** The jobs in the list are ordered by *Date* by default. Within this button, you can add a second field for job sorting.

 **Show/Hide Jobrun Details** This button displays/hides job details such as the *Jobrun Description* defined in the *Job Run Properties* window accessible from the context menu over the job run.

 **Run Job** This icon appears next to a selected job and is used for its execution.

 This button opens the *Quick Start Toolbar Configuration* enabling to adjust the *JOBS Explorer* panel. System templates can be inserted into the buttons area by checking their check box. Also *Templates*, *Existing Jobs* or *Jobs* from a file can be inserted and arranged in the window by dragging&dropping. To remove a button, drag it below into the white area.

13.2.3 Database context menu

Next to the path where the database is set, there is a pull down menu marked with ▼ containing following options.


Create New Empty... Click this button to create a new database. Specify where it will be stored and enter its name.

Create New Based on Current... Enables to select which items will be copied into the newly created database.

Open Existing... Opens an existing database from a .dat file.

Rename... Renames the current database file.

Quick Open This option is used for fast switching between the databases checked in the *Manage Quick Databases* window. To edit the list of quick open databases, click **Manage** and add/remove/move up/move down your databases, check which of them will be shown and specify the maximal number of the recently used databases.

 **Refresh** This function enables refreshing the database.

Cleanup Frequent database inserts, updates, and deletes can cause the database file to become fragmented. This function rebuilds the entire database to improve the speed when working with the database.

Edit JavaScript... Opens the *Run Script* dialog window enabling to edit and run the selected JavaScript code. *Manage...* opens the *Manage JavaScript files* dialog window aggregating the recently used JavaScript files. Please see: 13.14 JavaScript.

Run JavaScript... Executes the selected JavaScript code. *Manage...* opens the *Manage JavaScript files* dialog window aggregating the recently used JavaScript files. Please see: 13.14 JavaScript.

Backup... Specify the folder where you want to backup your current database and select whether to include its images.

Restore... Click **...** and search for the .bin file to load your backed up database. Specify whether you want to restore images (*Include Images* check box) and click **Restore**.


Upload... This window opens the *Upload* dialog. Please see: 13.11.6 Database Upload/Download. Click **...** and specify where the *Remote database* .dat file is present. Use the check boxes next to each job to include/exclude them into/from the uploading process. The *Uploading Rules* table below shows the compared data status of both the local and the remote databases. Adjust the *Action to perform* combo boxes to set the merging rules and then click **Upload** to start the uploading process.

Download... This window opens the *Download* dialog. Please see: 13.11.6 Database Upload/Download. Click **...** and specify where the *Remote database* .dat file is present. Use the check boxes next to each job to include/exclude them into/from the downloading process. The *Downloading Rules* table below shows the compared data status of both the local and the remote databases. Adjust the *Action to perform* combo boxes to set the merging rules and then click **Download** to start the downloading process.

Import Files... Opens the *Import Files into Current Project* dialog window enabling to import selected .nd2, .tif and .tiff files into the current job.

Advanced Import Files... Opens the *Advanced Import Files into Current Project* dialog window used for advanced image importing into the current job. Dimension, its parsing and loop can be defined before the import.

Import BioStation CT Files... Opens the *Import Biostation CT files into Current Project* dialog window enabling to import selected biostation .CT files into the current job.

 **Properties...** Enables setting the database root folder, sharing properties together with image storage properties. For more information about database properties, please see: 13.11.3 Database Properties.

13.2.4 Context menu on a job

Run Job/Wizard Runs the selected job definition in normal/wizard mode.

Edit Job Opens the 13.5 Job Definition Window containing the selected job.

View Job Results... Opens the Job Results window (see 13.8 Running a Job, Viewing Results and Graphs).

Run Analysis Runs analysis (13.9 Analysis) on the job run results contained in the selected job.

Rename Job Definition Enables renaming your job definition.

Duplicate Job Definition Duplicates the selected job.

Delete Job Definition Deletes the selected job definition.

Lock Job Definition Locks your job definition so that it cannot be changed in the 13.5 Job Definition Window.

Copy Job into another Project... Copies the selected job into the selected destination project

Export Job Definition... Saves the definition of the selected job as a .bin file into the specified folder under a specified filename.

Export to TIFF Files... (*requires: Local Option*) Can be used to export job results to TIFF.

Show in JOBS Toolbar control Places the selected job on the 13.3 Jobs Toolbar as a button.

13.2.5 Context menu on a job run

View Job Results... Opens the Job Results window (see 13.8 Running a Job, Viewing Results and Graphs).

View Job Results in a New Window... Opens the Job Results in a new window.

Run Analysis... Runs analysis (13.9 Analysis) on the selected job results.

Open Containing Folder Opens a folder on your hard drive containing your result image data.

Copy/Move Selected Jobrun Enables to copy/move selected job runs into a new or already existing job.

Delete Job Run Deletes the selected job run.

Stitch Points into Large Image Opens the *Image > ND Processing > Stitch Multipoint to Large Image* dialog window enabling to stitch captured images into a large image. This function works on any Job or HCA with any Point loop if image overlapping exists. Resulting new (and modified) Job runs are put under the same job into a separate group called *Stitched Large Images*. These stitched images are shown stitched in the Preview area inside *Job Results* window.

Maximum Intensity Projection Performs maximum intensity projection on the selected dimension (Time or Z-stack) of the selected job run.


Import Job Definition This button enables to import a previously saved job definition into your current project.

Save this Job Definition As... Saves your job as a new Job Definition (see 13.5 Job Definition Window).

Revert to this Job Definition Assigns the job settings of the selected run to its Job Definition. By default the Job Definition uses the settings of the latest run.

Job Run Properties Enables assigning short text notes to each of your job runs.

13.3 Jobs Toolbar

Jobs Toolbar (*View > Analysis Controls > JOBS Toolbar* ) has some common features with 13.2 Jobs Explorer. It enables organizing, editing and running single Jobs, Job Wizards or Test Runs.


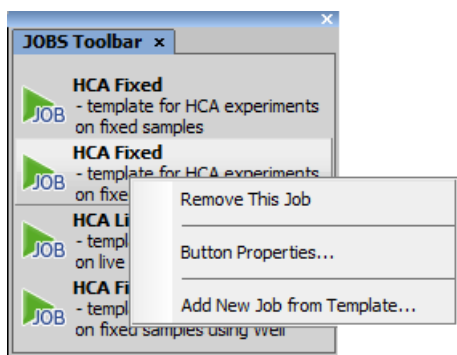
A job can be added to the toolbar from within the *View > Analysis Controls > JOBS Explorer*  window: right click on the job name and select *Show on Toolbar* or just drag and drop a Job from *Jobs Explorer*.

Figure 12.291. Jobs Toolbar with HCA templates



13.4 Basic Job Rules

Introduction

Jobs are automated procedures for image acquisition and analysis. When a job is executed, images and analysis data are produced. The images are stored in ND2 files while metadata and analysis results are stored in a database including links to the corresponding ND2 files. A complete acquisition history is stored in the database as well.

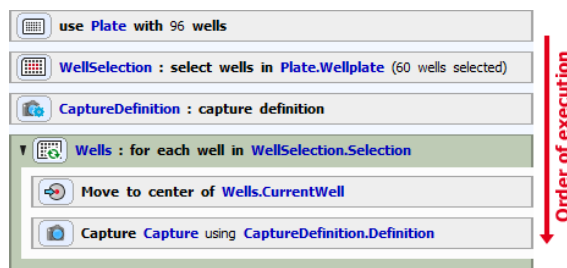
Order of execution

A *job* is composed of *tasks*. The order of tasks in a job is essential because they are executed from top to bottom. There are two kinds of tasks: simple tasks and

tasks which have blocks of other tasks nested inside them (e.g. loops and conditions). If there were only simple tasks, the job would be a mere chain of operations, very easy to assemble. On the other hand, it would be too difficult to create a simple time-lapse with 100 repetitions - having to copy the *Capture* task 100 times in a row. In order to avoid such copying, JOBS provide the concept of loops. Loops can embed other tasks. If a loop is run, it repeatedly executes the whole inner block of tasks until the defined number of repetitions is achieved.

Another task which embeds other tasks is the *Condition* (`IF` Condition `IF`, `IF_ELSE` Condition (If-Else)). Conditions are used for branching the job (deciding within the job flow). The embedded tasks are executed only if the specified condition is satisfied. For example: a condition in a job which scans a well plate can go like this: “capture a multi-point sequence on a well only if there is a fluorescence signal detected, otherwise proceed to the next well”.



Figure 12.292. A simple job capturing an image on each well center of the well plate selection

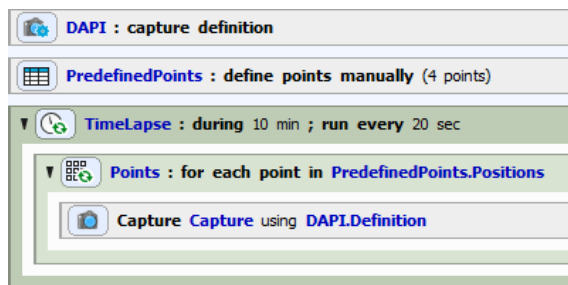




Rules

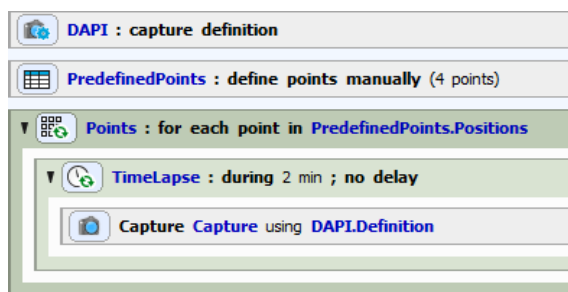
- Tasks are executed in order (from top to bottom).
- If a task contains child tasks, these child tasks are executed in order (conditions) or repeatedly (loops).
- Tasks inside a condition are executed only if this condition is fulfilled.

Task position

Position of each task in the job definition is substantial. In the following example, *Time Lapse*  and *Loop over Points*  tasks are swapped, bringing completely different results.



Herein, points on the sample are created manually. *Time Lapse* loop is then started for 10 minutes, executing embedded tasks every 20 seconds. Therefore a *Loop over Points*  (containing *Capture* ) is executed. Hence, an image is captured for each predefined point one after another. This capturing takes place every 20 seconds during the 10 minute *Time Lapse* interval.

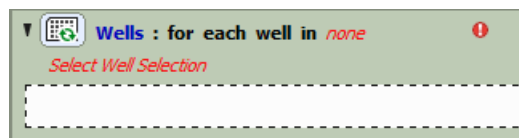


If the *Time Lapse* task is placed inside the *Loop over Points* task, the job works differently. Images are captured (for the duration defined in the *Time Lapse* task - 2 min in our example) on the first point, then for 2 minutes on the second point, etc. When all 2 min time

lapses of all points we are looping over are captured, the job is finished.


Task dependency

Some of the tasks require other tasks to be present in the job - they depend on them. For instance, a well-loop which goes over wells requires a selection of wells on a well plate in order to loop over it (otherwise the job wouldn't know which wells to loop through). Moreover, the well selection task requires a well plate definition in order to know the dimensions of the well plate, how many wells are present and their exact position.





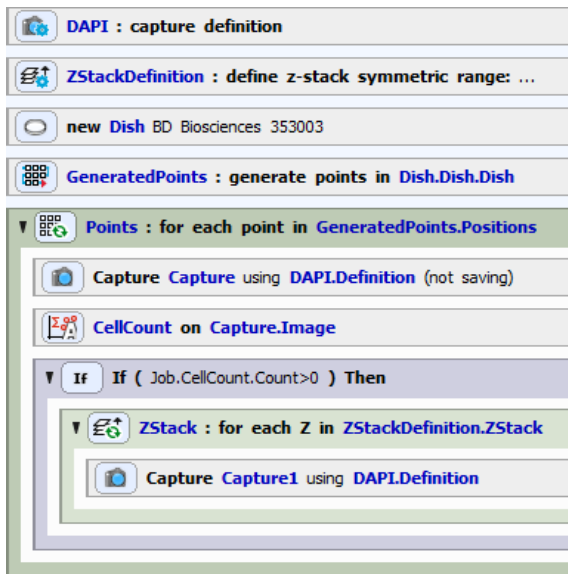
Note

If a task is not set-up properly (e.g.: missing dependent task, wrong task order in the definition, wrong task setting,...) a red exclamation mark

 appears in the caption of the concerned task. The job cannot be executed until all such errors are resolved.

Definition-Execution type tasks

Some operations consist of two separate tasks - one for *definition* (e.g. *Capture Definition* ) and one for *execution* (e.g.: *Capture* ). This is often useful in situations where a single definition is to be executed in different places of the job. If you wish to use such a task, always place the definition task before the execution task.



In this example, DAPI optical configuration is used in the *Capture Definition* task and *Define Z-Stack* parameters are defined. Points are generated on a dish and a loop over these points is created. On every point an image is captured (not saved) and used for the *Cell Count Analysis*. Information about the cell quantity at each point of the dish is used in a condition. If *Cell Count* is bigger than 0, all the tasks inside this *Condition IF* task are executed (*Define Z-Stack* containing *Capture*). Hence, image on each plane of the Z-Stack is captured (and saved). Notice that *Capture* uses the same definition in both places. If the user decides to switch the Optical Configuration used (e.g. from DAPI to FITC), he can simply change it in the *Capture Definition* and all *Capture* tasks linked with this definition will use the new setting.

Rules

- The *Definition* task (usually containing the word Define, Definition,...) has to be placed above its *Execution* task.

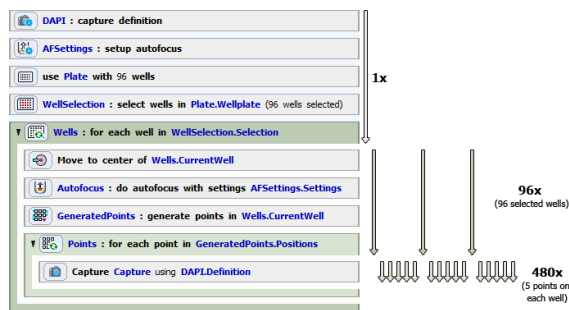
Loops

We can consider a well plate as a set of many wells. To sequentially pass through all the wells in Jobs we use Loops. Each processes (tasks) which we want to execute on the well level have to be placed inside this loop.

Note

Loop Over Wells does not move between the wells on a well plate by itself. It is therefore impossible to expect pictures of single wells by placing just *Capture* inside the *Loop Over Wells*. To do so, you have to place the *Move to Well Center* task (*Move to Well Center*) inside the loop right before capture. This task tells the loop to move to the appropriate well.

Figure 12.297. Loop over Wells and Loop over Points



In this job, stage moves to the center of the first well of the well selection, auto focus is performed and random points are generated (first level loop). Then the job encounters the embedded second level loop. Still on the same well these random points are looped

through and image is captured for each point. First level loop was now completed for the first well. Stage moves to the second well and the inside of the loops is executed repeatedly until all the wells of the well selection are run through.

Rules

- Tasks contained in a loop task are executed repeatedly and from top to bottom.

Adjusting the Task Settings

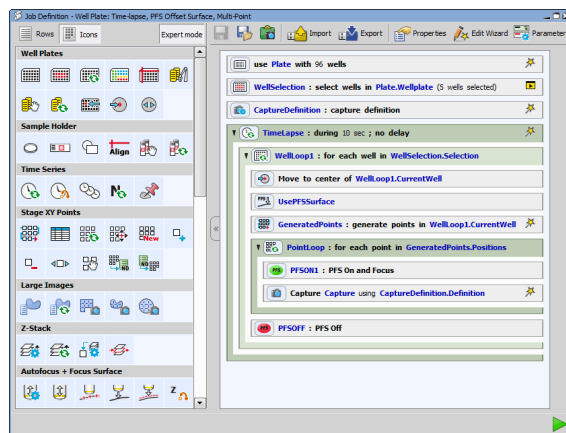
Tasks have different parameters which need to be set. These parameters are usually set at job-design time by double-clicking the caption of the task. However, crucial tasks can be selected and displayed within a wizard out of the main job body, so that the person running the job is not distracted by the whole job structure (see 13.6.3 Using Job Definition Wizard). Similarly, the same tasks can be displayed at runtime which enables the user to interact with the job or make decisions. Complex jobs can be parametrized (13.6.7 Defining Parameters) to be more user friendly.

Effective job building

To avoid errors when building a job, all of the rules mentioned above have to be followed. The best practice is to think about the results we want to acquire and then go backwards. Which devices are necessary for our procedure? Is the procedure done only once or shall it repeat? Do I need to change sample holders (slides, well plates) during the job? Are there any decision-making points? What images exactly do we want to capture? Which device operations need to be done before an image is captured? Also think about the user interaction. Will the job be fully automatic or do you want to adjust the parameters during the job run? Try to answer all of these questions to make the job as simple and effective as possible. After that, you can start creating the job (see 13.6 Creating a Job).

13.5 Job Definition Window

Once an empty job is created or an existing job is opened, the job definition window appears.



This window is the main place for the Job program flow and for changing task parameters. Apart from the main toolbar there are two main areas available:

The leftmost task palette All tasks available are displayed here in the form of icons (or icon+description). *Expert mode* button switches between the simple (basic tasks) and advance view mode (all tasks). Tasks are organized into logical groups. Some tasks depend on the presence of some devices. They may not appear in the palette until that device is added into the device manager (*Devices > Manage Devices*). To insert a task to the job, drag its icon into the main area which forms the job definition. Width and thus the number of the icons(tasks) in every row in the task list can be set to any number of icons by sliding the right border of the task list.

The main program area containing the job definition This pane holds the tasks that form the program. Each task has an icon and text that describes what the task will do when executed. Every task has different parameters which are revealed by double-clicking on its

caption. Tasks can be marked as to be shown in the Wizard or at runtime. All tasks which were dragged here will be run one after another until the job is finished.

Before thinking about single tasks you have to decide whether you want to modify a predefined template (13.6.1 New Job from Template), use the assistance of the definition wizard (13.6.3 Using Job Definition Wizard) or create a new Job from scratch (13.6.2 New Blank Job).

Operation

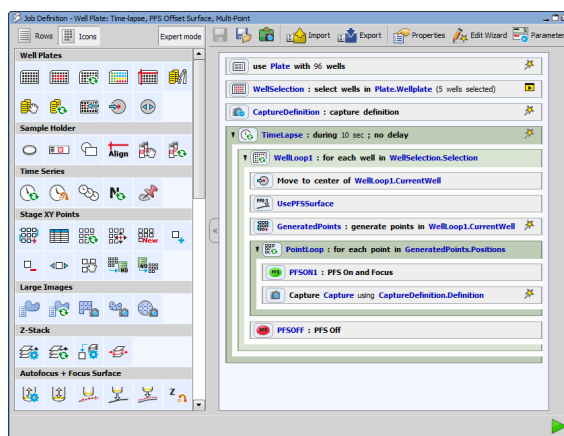
Most of the editing operations are done by moving the Tasks around. By simple dragging and dropping the tasks from the left to the right you create your complete Job sequence in a matter of minutes.

- To insert a task into the job program, drag the icon to the main program area which contains the job definition.
- To move a single task or multiple tasks to different place, hold the task in the main area and drag it to another place in the program.
- To delete a task from the program area, simply drag it out of the job main program area.
- To set parameters of each task, double-click its caption.

Warning

Some tasks depend on other and their order of execution needs to be correct. Please see 13.4 Basic Job Rules to avoid error warnings when setting up your Job.

Figure 12.299. Job Definition window



Window Options


Rows, Icons, Hidden These buttons change the appearance of the task panel. It is possible to switch between the *Rows* + description view mode and *Icons* view mode. In the *Icons* view mode it is possible to change the width of the task panel by dragging the right border line of the scroll bar. The task panel can also be fully hidden by clicking on the *Hide* button in the middle of the 13.5 Job Definition Window.

Expert mode Expert mode in JOBS displays all available tasks. By default, this mode is off and the Job Definition window shows only the basic tasks.


Save Changes Automatically saves any changes in the *Job Definition*.

Save As Saves the *Job Definition* with desired name into a Project that could be then selected from the 13.2 Jobs Explorer.

Print Job Definition to Clipboard Copies a screen shot of the current Job including key parameters into the clipboard.

 **Import** Imports existing Job .BIN files into the *Job Definition* window.

 **Export** Exports current Job to a single .BIN file.

 **Properties** This button opens the *Job Properties* dialog window where following features related to the current job can be set.


In the *Job description* edit box a description of each job can be made and saved together with the Job. This description is displayed in the caption of the *Job Wizard* and *Job Execution Progress* window.


Progress window section enables to Show/Hide Progress Window during the Job run or to preview image in the Progress window after the first capture or on the job start.

When Job definition changes section defines what is done when changes in the job definition are made. These changes can be saved to the current Job definition, saved as a new definition or the user is asked to specify the location where to save them. *Treat adjustments in Wizard as Job change* should be checked if it is requested to apply the changes made in wizard directly to the job definition.

Wizard section specifies when the Run button is shown in wizard - *always* or *only if all tasks are visited* (highlighted green).

Advanced features section groups advanced features such as enabling the *Test Run*, *Parameters* or *Programmable Actions in Wizard*.

 **Edit Wizard** This button opens the Job Wizard edit dialog window. Please see: Edit Wizard.

 **Parameters** Each job can be parametrized on the task level. This button opens the Job Parameters dialog window where you can define the name of your parameter, type, values it takes and whether it requires another parameter from the list to be set to a certain value. You can also mark each parameter to be shown in wizard/runtime. For more information about using parameters, please see: 13.6.7 Defining Parameters.

Note

This button is visible only if Parameters are enabled inside Job Properties.

▶ **Run Job** Executes the current Job.

13.6 Creating a Job

13.6.1 New Job from Template


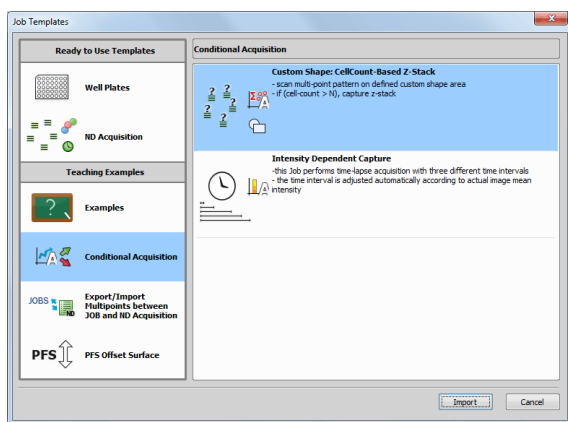
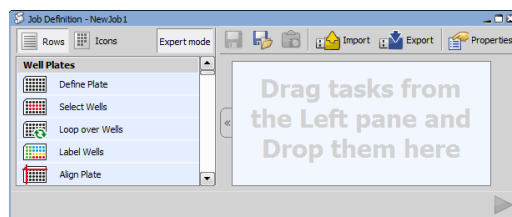
Easiest way to start using Jobs is to choose from pre-existing Job templates created for most laboratory tasks. By clicking the  *Add New Job from Template* button in 13.2 Jobs Explorer you can access the Job Templates. A template is a predefined set of tasks (see 13.15 Task reference) which you simply insert into your current job definition. The template can be used either “out of the box” or you can select a template closest to your objective and edit some of its tasks or add other tasks into it. Double-click on the desired template or press the *Import* button. The *Job Wizard* window appears. You can now set up the parameters step by step (see: 13.6.3 Using Job Definition Wizard). To see the job definition and edit the imported template, click and then click inside 13.2 Jobs Explorer.

Figure 12.300. Job Templates



AR. Just drag and drop tasks from the left into the main area of the *Job Definition* window.



- 5) The tasks inside a job definition can be reorganized by dragging them up or down. Tasks which are not needed can be deleted by dragging the task out of the job window.
- 6) To set the task parameters, double click the caption of the appropriate task.

Note

Name field (if available) can be used to alter the default name of the task, however some tasks do not allow numbers at the beginning of the name. Therefore letters may have to be entered first.

- 7) Don't forget to save your job before closing the *Job Definition* window.

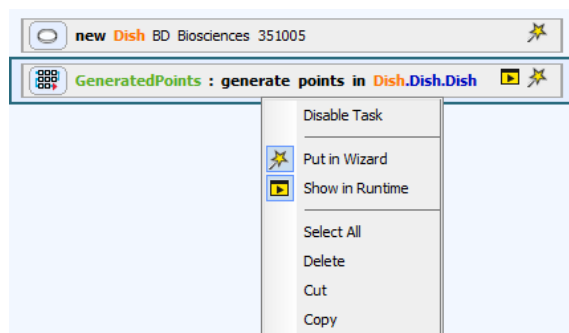
13.6.3 Using Job Definition Wizard

A Job is usually run multiple times to acquire data in different conditions. It happens that few parameters have to be changed before each run (e.g. length and interval of a time-lapse, well-plate metadata, like drug concentrations or labels, or even threshold intensity level). For this reason it is possible to mark some tasks for reviewing in a Wizard. In this case it is possible to run a Wizard where only the tasks that were marked are presented for definition. To put a task into wizard right-click the task and choose *Put in Wizard*.

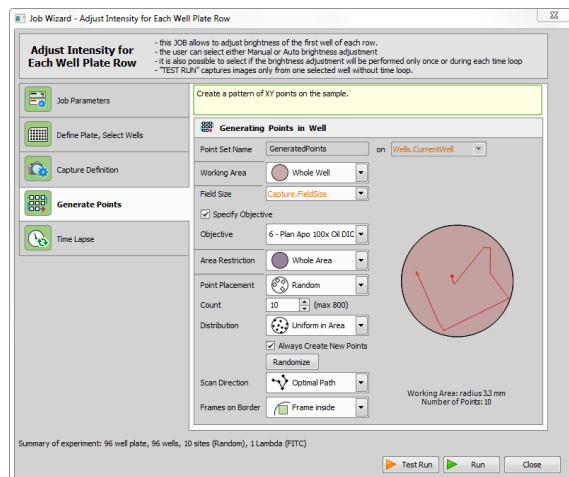
13.6.2 New Blank Job

- 1) Go to *View > Analysis Controls > JOBS Explorer JOBS* window.
- 2) Here you can select an existing project or create a new one by clicking the *Add New Project* button.
- 3) After choosing a project click on the *Add New Job* button.
- 4) The *Job Definition* window opens. Here you can combine all different tasks to create a new job fulfilling your research needs together with all available devices compatible with *NIS-Elements*

Figure 12.302. Putting tasks into Wizard

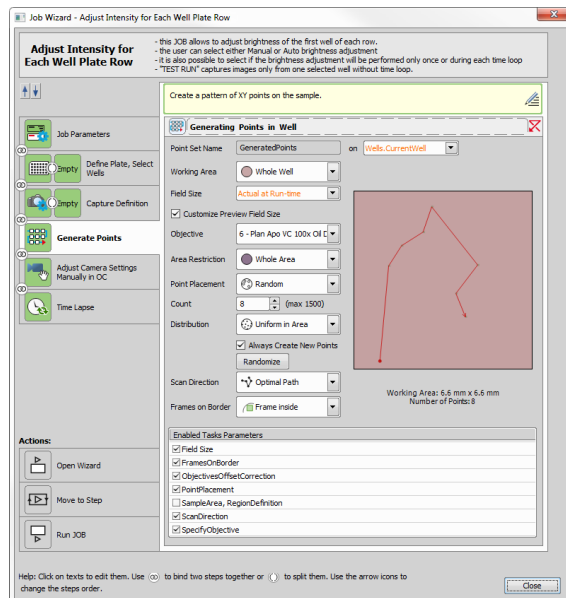








Definition Wizard is also useful for specialist / operator role division. The specialist creates complex Jobs with decisions and analyses. He does not want the operator to see all the complexity of the task but still needs to let him change a few parameters. The wizard enables this.

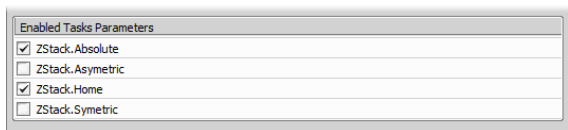


Edit Wizard

Figure 12.304. Job Wizard dialog window in the edit mode



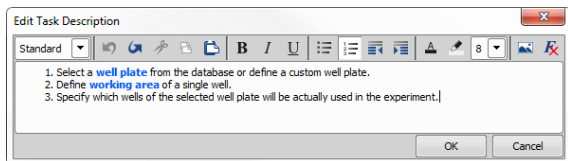
The  **Edit Wizard** button opens the Job Wizard dialog window in the edit mode. Wizard steps (tasks that were marked with the  wizard icon) can be freely sorted using the  up and  down arrows, except the *Parameters* step which has to stay always first. Wizard steps can be bound together by clicking on the  Connect or  Disconnect buttons. For each task, only selected options can be enabled for the operator, and others can be disabled. Thus the user working with the wizard can use only the options checked in the list under each task (*Enabled Tasks Parameters*).



Three programmable *actions* (events) can be used to validate the user's settings during the wizard run and to adjust them pragmatically or to display warnings. Tasks at the top of the Job Wizard edit window can be used to create a “mini job” which is then performed before the wizard opens - *Open Wizard*, between each step/grouped steps - *Move to Step* or after the Run button is clicked - *Run JOB*.

Texts of all used tasks can be changed (by clicking on the task name) and their description can be edited after clicking on the *Edit Task Description* icon.

Figure 12.306. Edit Task Description window



Font Style Select one of the predefined styles which will be applied to the whole paragraph or the selected text.

Undo Moves back through the history of changes.

Redo Moves forward through the history of changes.

Cut Cuts the selected text into clipboard.

Copy Copies the selected text into clipboard.

Paste Pastes the selected text from clipboard to the cursor position.

B Bold Applies the bold font style.

I Italic Applies the italic font style.

U Underline Applies the underlined font style.

Bulleted list Applies a bulleted list to the paragraph. **Ctrl** + **-** turns this function on/off.

Ordered list Applies a numbered list to the paragraph. **Ctrl** + **=** turns this function on/off.

Decrease indentation Decreases the paragraph offset.

Increase indentation Increases the paragraph offset.

Text foreground color Changes color of the selected text.

Text background color Changes background color of the selected text.

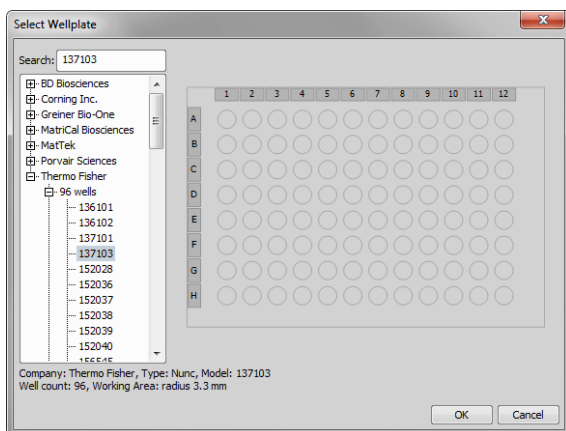
Font Size Changes size of the selected text.




Insert image Inserts an image (jpg, gif, png, bmp) from a file.

Remove character formatting Removes any character formatting from the selected text.


13.6.4 Defining Samples


First step of any job procedure is to define your samples. You can choose between well plates, slides or stage areas (free shapes). If defining standardized sample holders (well plates, slides), it is recommended to select the specific product model you are using from the database. This can be done by clicking Select from DB....





- For custom well plate definition, please see Custom Well Plate Options.
- Slide definition is closely described here: *Define Slide* .
- Any other free shapes on a stage can be defined using *Define Stage Area* . Please see *Define Stage Area* .

Note

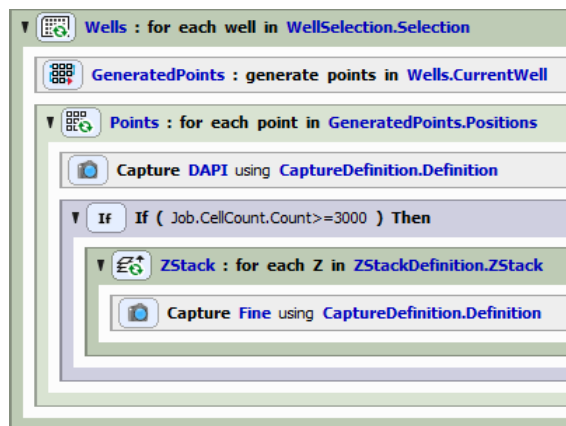
Any sample holder that was selected/defined has to be properly aligned. Well plates are aligned using *Align Plate*  inside Jobs or globally using HCA/JOBS > Well Plate > Align Well Plate.

Slides are aligned using *Align Slide*  in Jobs or globally using HCA/JOBS > Slide Holder > Align Slide. Alignment of well plates and slides is stored in the database. User can see not only absolute stage coordinates but also coordinates relative to the plate/slide. Relative coordinates are helpful when later revisiting locations on the same or different system and when doing analyses outside NIS-Elements (e.g. doing statistics in MS Excel).

13.6.5 Defining Capture

Before capturing any images using the *Capture*  task you have to define the capture settings in the *Capture Definition*  task window, where you select your optical configuration to be used to capture each of the channels. Typically, laboratory computer image analysis systems consist of a computer, a camera, and a microscope equipped with certain accessories (objectives, filters, shutters, illumination, rotary changers, etc). Most of the mentioned microscopic hardware is often motorized and therefore can be controlled via *NIS-Elements*. In addition, it is possible to integrate single settings of all these devices into one compact set called Optical Configuration. It is recommended to create several optical configurations containing particular devices settings. Then a single click can completely change the current hardware configuration. For more information about setting up the Optical Configuration please see: .

13.6.6 Defining Loops








Loops are special repetitive tasks which contain an inner block. When you fill this block with tasks and run the loop, it repeatedly executes the whole inner block of tasks one after another. You can then define

whenever you want to stop the repetition (after a certain amount of time, number of loops, after a condition is true, etc.). You can also loop tasks inside another loop to make building of repeated processes even more effective.

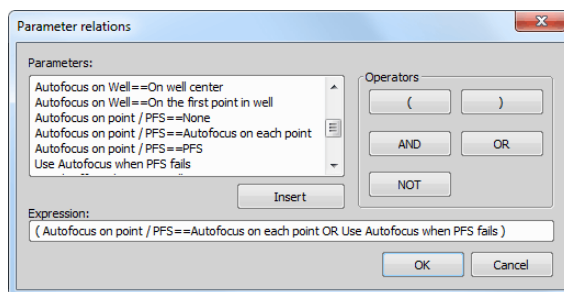
13.6.7 Defining Parameters




Parameters represent “conditions” for single tasks. Multiple experimental situations can be prepared and organized into a single job so that the operator does not have to switch between many jobs.

A single task can carry many parameters. If all values of its parameters are met at the beginning of the job run, the task is executed. If one parameter value is different than the initial parameter selection, the task will be skipped.

To start using parameters, click the  *Properties* button inside 13.5 Job Definition Window and check the *Enable Parameters* check box. Now click to confirm the selection and open the *Job Parameters* window by clicking the  *Parameters* button. Click the  *Add New Parameter* button to add the first parameter. Give it a name, choose its type (On/Off, choice from a list of options or a numeric value) and define the values it takes clicking . If multiple parameters are used one can require another one to be executed. This conditional parameter can be selected from the *Requires parameter* combo box. Don't forget to mark where each parameter will be shown and adjusted - in  *Wizard*,  *Run Time* or both. When done with defining all your parameters, click .

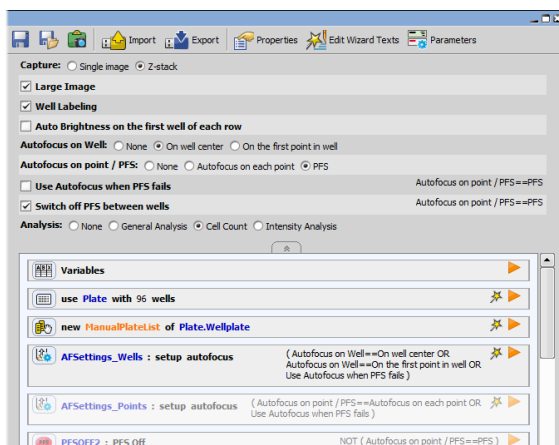
Now you need to assign prepared parameters to single tasks. This can be done in the 13.5 Job Definition Window by right-clicking on the desired task and choosing *Requires parameter...* Parameter relations dialog window opens up.



Here you can choose from all parameter values defined previously and *Insert* them separately or use them with *Operators* defining an expression (e.g. *Capture==Z-Stack AND Analysis==Cell Count*; task will run only if the user selects to *Capture*  a *Define Z-Stack*  and to use the *Cell Count Analysis*  at the beginning of a job run). After confirming the expression, it is displayed in the caption of the appropriate task.

Before executing the job it is important to define the initial parameter values at the top of the 13.5 Job Definition Window.

Figure 12.310. Job Definition window showing the initial parameter values



After executing the job, a parameter table will be shown. There you have to set the values you wish to use in the current experiment and click **[Next]**. All the subsequent steps of your job depend on this selection.

Figure 12.311. Parameter table in Job Wizard

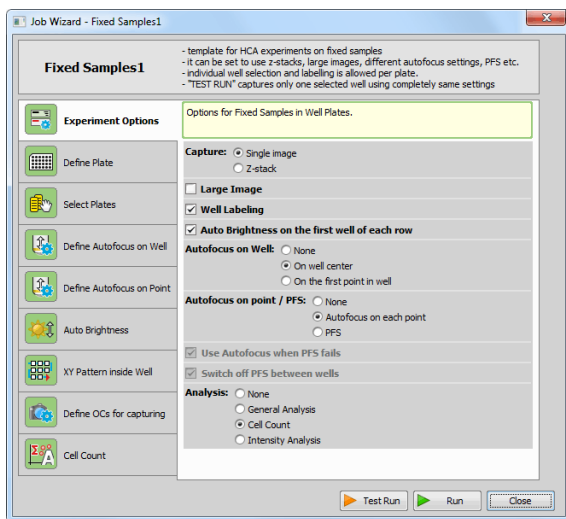
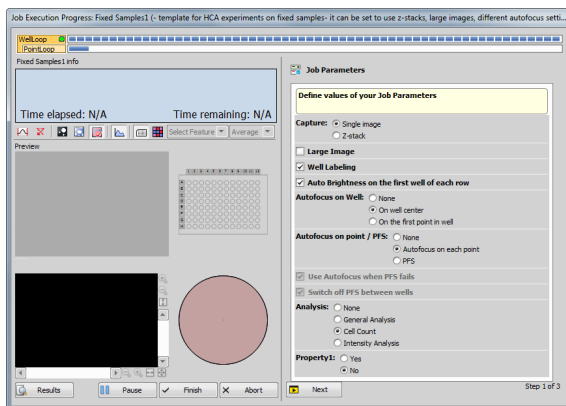








Figure 12.312. Parameter table in Job Execution Progress window



13.7 Merging Different Acquisition Types

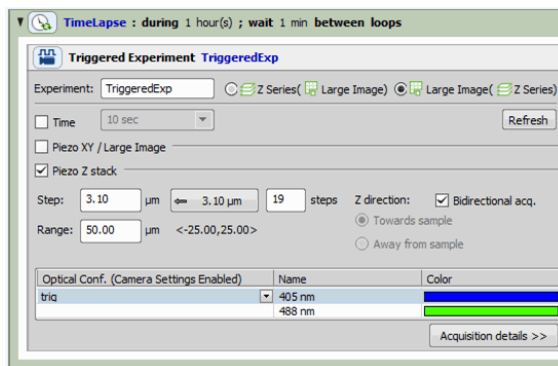
From version 5.0, different acquisition types use a unified storage system which enables the user to nest different acquisition tasks while the result is a single ND2 data set. See the examples below. The following tasks are concerned:

- *ND Acquisition* 
- *Fast Timelapse* 
- *Sequential Stimulation* 
- *Simultaneous Stimulation* 
- *Illumination Sequence* 
- *Triggered Experiment* 

13.7.1 Simple Acquisition Tasks Inside a Parent Loop

Triggered Experiment  inside *Time Lapse* .

Figure 12.313. Result: TZ file



Illumination Sequence  inside *Loop Over Wells* .

Figure 12.314. Result: MT file

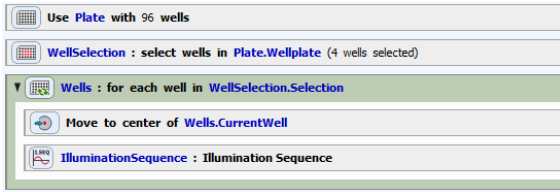
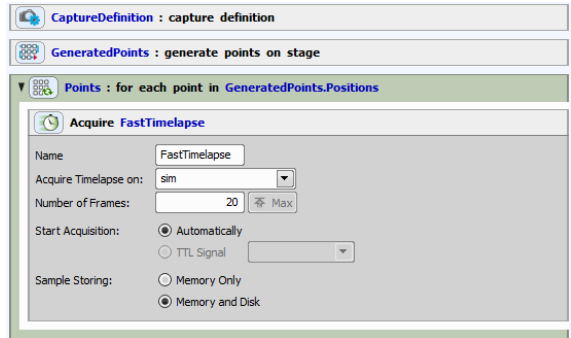


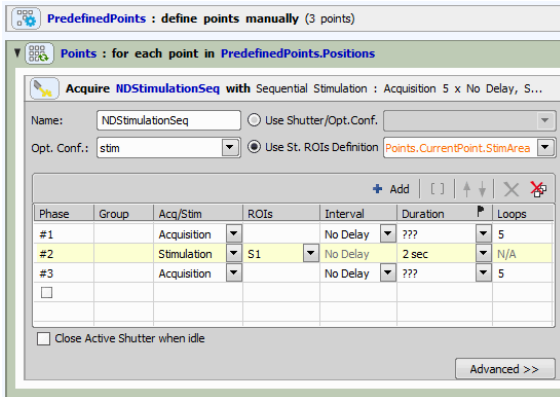
Figure 12.316. Result: MT file



Sequential Stimulation  inside Loop over Points



Figure 12.315. Result: MT file




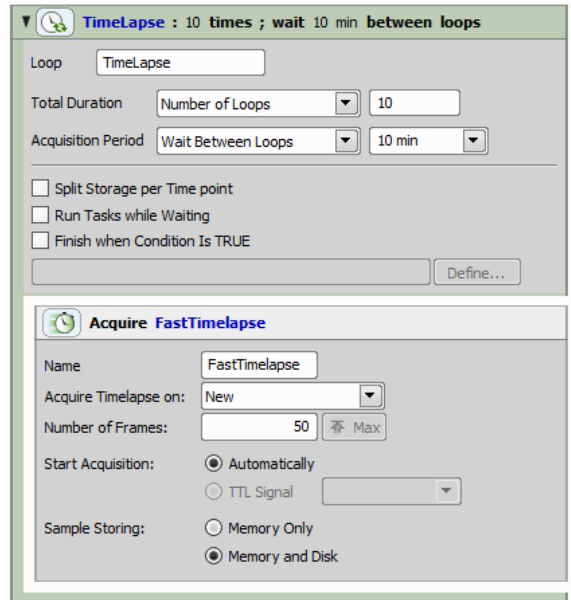





Fast Timelapse  inside Time Lapse  :

Figure 12.317. Result: T file



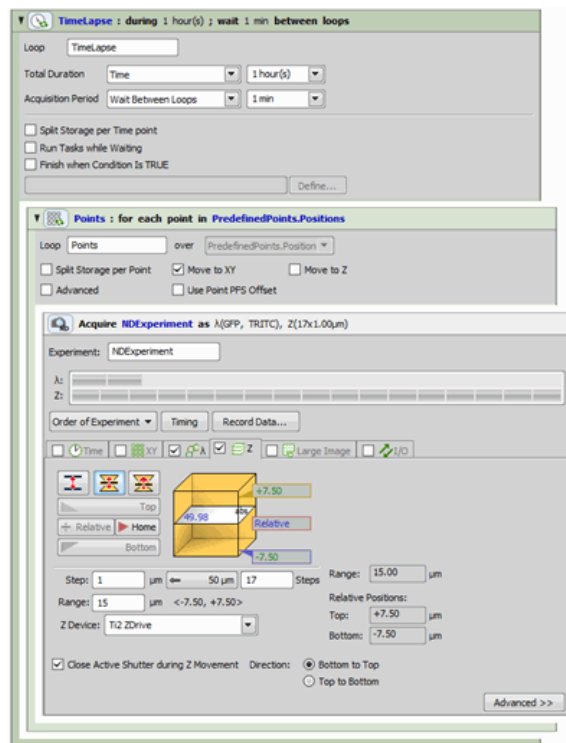
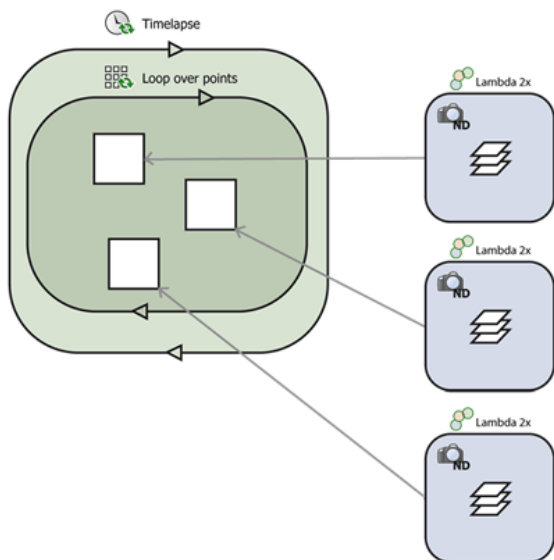
Fast Timelapse  inside Loop over Points  :

13.7.2 Merging Matching Z-Stacks




ND Acquisition  inside Loop over Points  inside Time Lapse .

- Result: single TMPZ file
- The same Z-stack is captured for each Lambda channel




Figure 12.318. TMPZ Experiment Scheme





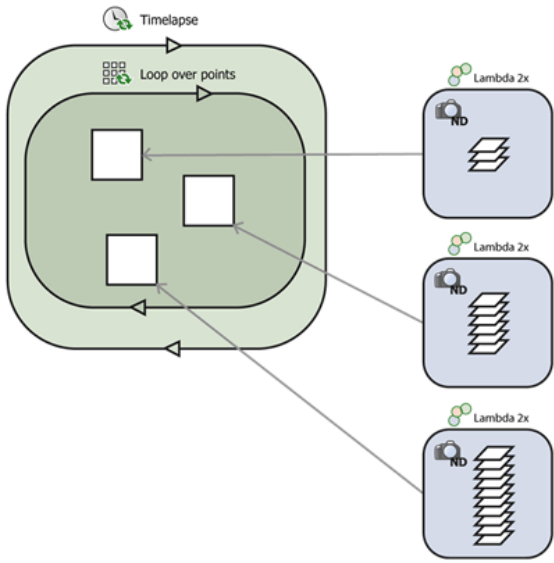
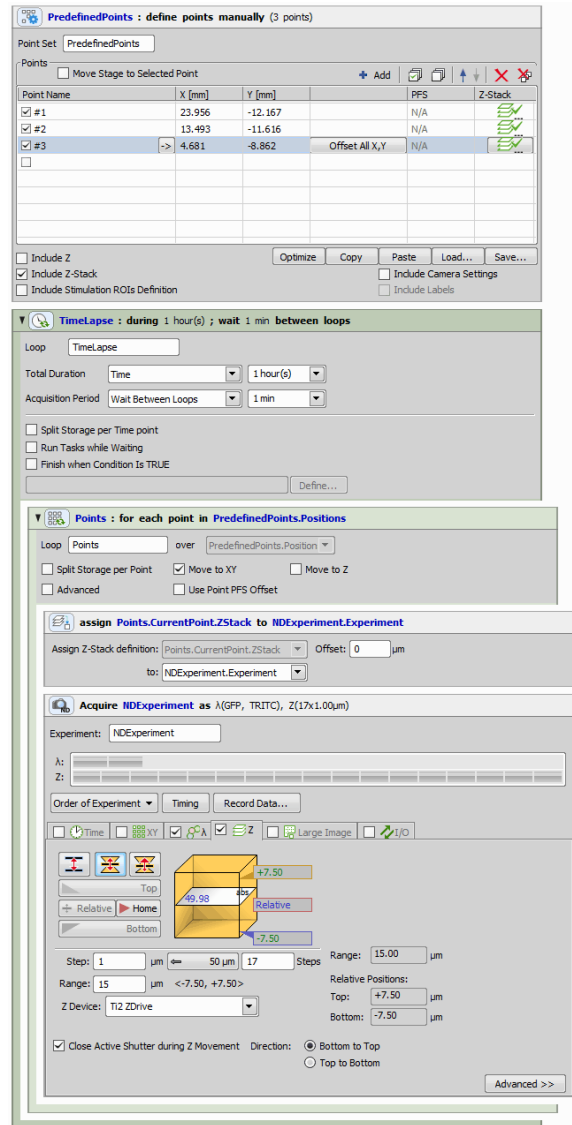
Note

Instead of the ND Acquisition  task, you could also use the Z-Stack Loop  task containing a (multichannel) Capture , however, the arrangement used in the example eliminates unwanted delays which occur when the filter-changer is slower than the Z drive (e.g. if a piezo Z is engaged).




13.7.3 (Not) Merging Z-Stacks Different for Each Point

ND Acquisition  inside Loop over Points  inside Time Lapse .

- Assign Z-Stack to Point/Well/ND Acquisition  and Predefined Points  are used to define different Z-stacks for each XY point.
- Result: multiple TZ files, different Z-Stacks cannot be merged in one ND2 file.






The screenshot shows the software interface for defining points and a timelapse loop. The top window is titled 'PredefinedPoints : define points manually (3 points)'. It contains a table with the following data:

| Point Name | X [mm] | Y [mm] | PFS | Z-Stack |
|------------|--------|---------|----------------|-------------------------------------------------------------------------------------|
| #1 | 23.956 | -12.167 | N/A |  |
| #2 | 13.493 | -11.616 | N/A |  |
| #3 | 4.681 | -8.862 | Offset All X,Y |  |

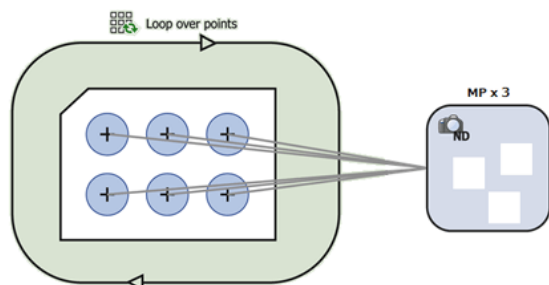
Below the table are checkboxes for 'Include Z', 'Include Z-Stack', and 'Include Stimulation ROIs Definition'. The bottom window is titled 'TimeLapse : during 1 hour(s) ; wait 1 min between loops'. It shows settings for 'Total Duration' (1 hour(s)), 'Acquisition Period' (Wait Between Loops, 1 min), and 'Points : for each point in PredefinedPoints.Positions'. The 'Points' section includes an 'assign Points.CurrentPoint.ZStack to NDExperiment.Experiment' block and an 'Acquire NDExperiment as M(GFP, TRITC), Z(17x1.00um)' block. The 'Acquire' block shows a 3D Z-stack diagram with 'Step: 1 um', 'Range: 15 um', and 'Z Device: TI2 ZDrive'.

13.7.4 Multi-point inside Multi-Point Loop

ND Acquisition  (XY) inside Loop over Points .

- All XY positions (points) are merged together.

- Result: single MP file



New Slide

Align Slide.Slide

GeneratedPoints : generate points in Slide.Slide.Slide

Point Set Name: GeneratedPoints on Slide.Slide.Slide

Working Area: Whole Slide

Field Size: Actual at Run-time

Customize Preview Field Size

Objective: 1 - Achromat LWD 20x Ph

Area Restriction: Whole Area

Point Placement: Manual

Scan Direction: Optimal Path

Working Area: 76.2 mm x 25.0 mm
Number of Points: 9

Points : for each point in GeneratedPoints.Positions

Acquire NDExperiment as XY(3)

Experiment: NDExperiment

M: _____

Order of Experiment | Timing | Record Data...

Time XY Z Lambda Large Image

Move Stage to Selected Point

| Point Name | X [mm] | Y [mm] | |
|----------------------------------------|-----------|--------|----------------|
| <input checked="" type="checkbox"/> #1 | +0.000 | +0.000 | |
| <input checked="" type="checkbox"/> #2 | +1.035 | -1.167 | |
| <input checked="" type="checkbox"/> #3 | -> -2.343 | +3.299 | Offset All X,Y |
| <input type="checkbox"/> | | | |

Include Z Relative XY

Optimize Load... Save... Custom...

Close Active Shutter during Stage Movement

Advanced >>

13.8 Running a Job, Viewing Results and Graphs

13.8.1 Job Execution Progress window

Once the job is run (after clicking the **Run Job** button) a window is displayed showing the progress. Similarly to ND2 acquisition blue rectangles representing dimensions or frames turn green when captured. The window shows the analysis results immediately during the job after the analysis is done. Some tasks require user interaction during the job progress (e.g. *Add/Edit Points Manually*) and other tasks can be forced to do it as well if you check *Show in runtime* (13.6.7 Defining Parameters). Once the task definition dialog shows up, the Job execution is paused until user confirms settings modification by pressing Next button.

The following picture shows a well plate selection of 6x10 wells being scanned. Green color indicates which wells have been already captured, yellow indicates the well being scanned at the moment (C9).

Figure 12.324. Job Progress Visualisation

Job Execution Progress: Fixed Samples1

PlateLoop WellLoop PointLoop

Fixed Samples1 info


Plate(1, 1): a
Loop(13, 60): WellLoop

Time elapsed: 0:02:52 Time remaining: 0:08:57


Preview


New


Results


 **Auto Contrast** Automatically adjusts contrast value in the Preview image.

 **Reset Contrast** Resets the contrast value.


 **View Binary** Displays the binary layer in the preview.


 **View Color** Displays the color layer in the preview.




 **View Overlay** Displays the color layer and the binary layer in overlay.


 **Hide Histogram** Hides/Shows the histogram below the preview.


 **Show Well Plate** Displays the Well plate view.

 **Show Heat Map** Displays the Heat map view. *Average* or *Last* values of the *Feature* selected from the first combo box can be shown above the heat map. (Choose a feature that is displayed during the job progress and select the last or average value - it displays a number of the Runtime steps.)


 **Results** Opens the Job Results dialog window (see: 13.8 Running a Job, Viewing Results and Graphs).

 **Pause** Enables to pause the experiment - then the button changes to  **Continue**. For example: if your job does not perform auto-focus before each frame but you see that the preview image is getting blurry, you may pause the acquisition, re-focus and then start it again by clicking  **Continue**.

 **Finish** When clicked, only images captured so far will be saved, the experiment will be ended.

 **Abort** Click this button to end the experiment without saving any data.

Note

Right-clicking on the image preview enables to move the stage to keep an object in view manually using the  Move this Point to Center com-

mand. Context menu over the well plate enables to display a Schematic View of the well plate, show a scale bar (Show Scale) or move the stage to the selected well, to a cursor position or to the stage center. Context menu over the well preview enables to display a scale bar.

13.8.2 Result View

(requires: JOBS Viewer)


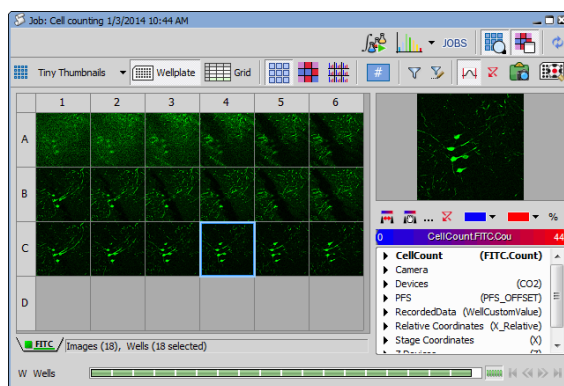
Once the job run is finished, results are saved automatically and the *Job results* window opens. To open some other saved job results, select the particular job/job-run in the 13.2 Jobs Explorer and click  **Results**. The Job result window shows captured images and all associated captured data of a single job run.

Figure 12.325. Job Results window



This picture shows a result of a well-plate experiment displaying preview of the captured images in each well. If multiple images are captured in a single well, they are automatically stitched into a single large image.

Basic dialog areas

Basic navigation is done using the ND navigation bars in the bottom part of the dialog window. Right side

shows a preview of the selected well/frame with heat-map controls and acquired data browser.

The Job results window enables to view the results from “various angles”. There are three basic views:

- Thumbnail view
- Wellplate view (if wellplate was used during acquisition)
- Grid view

In *Thumbnail* and *Plate* view you can view Images, Heat-map of selected data values (Quantities, Acquisition metadata and Analysis results) and labels (in case of wellplates). *Grid* view displays all results in a tabular form.

ND2 Interconnection

Double-clicking an image in the result view opens the underlying ND2 file with that particular image frame displayed. If the job consists of multiple ND files, just a single image window stays open. To display each ND2 file in a separate window, use **alt + double click**. Both options are available also on the context menu.


Reusing a Job Definition


ND2 files created in Jobs are saved together with their Job Definition. To display or reuse this definition, simply right-click on the opened image and choose *Reuse Job Definition*.


Top Toolbar options


Huge Thumbnails, Large Thumbnails, etc.


Thumbnail view displays all acquired images in the order of acquisition. Channel displayed in the thumbnails can be selected in the tabs below the thumbnail view.

 **Wellplate** Real layout of the plate is displayed.

 **Grid** Images along with metadata are displayed in a grid (see below). Filtering and grouping is supported (you can sort and group by any column). Statistics can be turned on. See Figure 12.329, “Job Result: Grid View”.

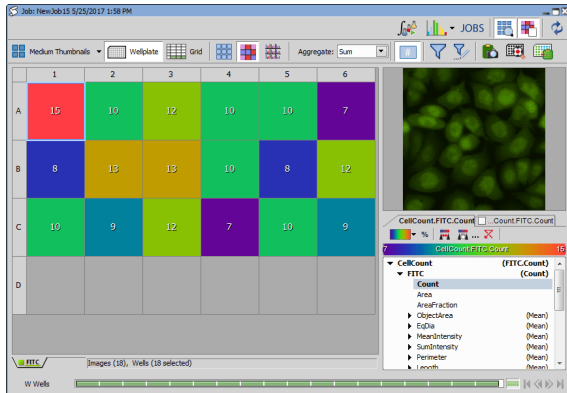
 **Show Images** Displays all acquired results as images.

 **Show Labeling** In the wellplate or thumbnail view, this button displays color labels for each well if the labels were defined (please see: 13.10 Labels and Metadata for more information about labeling).

 **Show Heatmap** Independently on the current view (thumbnails, well plate, grid), you can select a heatmap which represents one of the meta-data values (e.g. analysis results, gas concentration, temperature, etc.). In the heat-map view you see the selected colored squares instead of images. The color is based on the value and can be tuned. If multiple data are acquired in one well/frame (e.g. when multi points were captured in each well of the well plate), it is possible to *aggregate* these data (calculate min, max, mean, sum, SD, etc.) in order to get a single value per well. If one of the aggregating features is selected from the combo box, entire multi point bar is selected and the results show the aggregated values. If one multi point is selected in the multi point control bar, *Aggregate* combo box is automatically switched to *None*, displaying only non-aggregated results of the currently selected multi point.

An example of a heatmap is displayed in the following picture. Displayed as thumbnails, each image is overlaid by a color and a value both of which carry information about the meta-data selected in the bottom-right corner of the window. By default the color range of the selected feature uses minimal and maximal values found in the entire job run. The color scale range and colors can be modified by the user.

Figure 12.326. Job Result: Thumbnail View (Heatmap)



Note

Select the meta-data to be displayed in the heatmap in the Quantities panel (bottom-right area of the window).

Displaying two features in a heatmap

Heatmap mode enables to view two features at once. Open the *Quantities* panel and select a feature to be displayed from the first tab. Then check the second tab and select a feature. Both features are now displayed in the heatmap. Use the *Properties Compare Mode* button to switch to the circle diameter mode or back to the divided mode.

Figure 12.327. Circle diameter mode

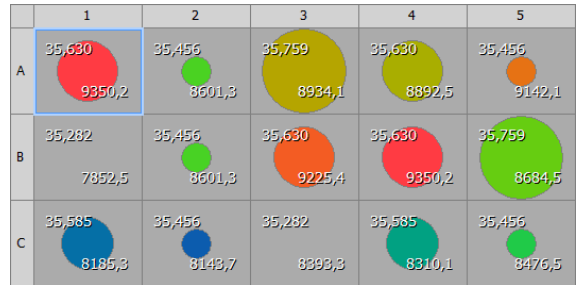
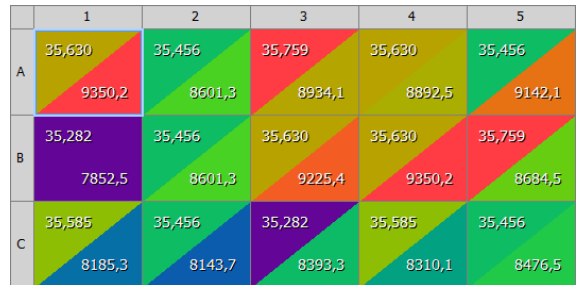


Figure 12.328. Divided mode



Show Histogram Data of each image are represented by a histogram.

Aggregate Aggregates data by the selected feature.

Time Trend Time trend represented by arrows for each image can be displayed using this button.

Show Feature Values Displays a numeric value of the selected feature over each well.

Use Filter Activate this button to filter the results. The filter can be defined by clicking the **Define Filter** button.

Define Filter A filter may be applied to images displayed in the result view. Click the **Define Filter**

button to display a table with the filter definition. Toggle the **Use Filter** button to turn the filter ON and OFF.

Figure 12.329. Job Result: Grid View

| Thumbnail | Frame Index | File | CellCount | CellCount_FFTC | CellCount_FFTC_ObjectsArea | CellCount_FFTC_Mean |
|-----------|-------------|--------------------------------|-----------|----------------|----------------------------|---------------------|
| | | Name | Count | Area [µm²] | Area/Fraction | Mean [µm²] |
| 1 | 0 | WellA1_ChannelFFTC_Sep2000.nd2 | 15 | 1,727 | 0,0100 | 0,115 |
| 2 | 0 | WellA2_ChannelFFTC_Sep2001.nd2 | 10 | 3,341 | 0,0193 | 0,334 |
| 3 | 0 | WellA3_ChannelFFTC_Sep2002.nd2 | 12 | 1,951 | 0,0113 | 0,163 |
| 4 | 0 | WellA4_ChannelFFTC_Sep2003.nd2 | 10 | 3,295 | 0,0190 | 0,329 |
| 5 | 0 | WellA5_ChannelFFTC_Sep2004.nd2 | 10 | 1,539 | 0,0089 | 0,154 |

Auto Scale Intensity Performs automatic setting of LUTs on the image thumbnails. Click on the pull-down menu to reveal LUTs options. Scaling can be based on a selected image (*Scale From Selection*) or all images (*Scale From All Loaded*). To adjust the LUTs settings, select *Modify LUTs...*

Export View Exports the well plate overview.

Show Selected Well/Jump to Wellplate Switches between single well view and well plate view.

View Graph Click the black arrow next to this button to choose a graph view from the list. Then click the button to open the selected graph view. For more information about viewing results in different graph modes, please see: 13.8.3 Graph View.

Run Analysis This button runs an analysis on images captured using a job definition. See 13.9 Analysis.

JOBS View Definition (requires: Local Option)

Click this button to display the job definition window. See 13.5 Job Definition Window.

Grid Settings Shows a table with all acquired metadata. Each feature can be displayed or hidden in the *Grid View* using the check boxes.

Show Preview Display or hide a small image preview window.

Show Labels Display or hide the labels toolbar. Labels of the wells selected in the wellplate layout are highlighted here. Any label can be temporarily turned off by unchecking the check box next to its name.

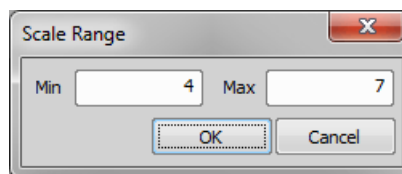
Show Quantities Display or hide the Quantities panel showing image metadata.


Refresh Contents Hit this button to refresh the currently displayed results.

Quantities toolbar

Set range from current view This button assigns the left color to the lowest value and the right color to the highest value present in the current data results view.

Set manual range Applies the custom minimal/maximal scale range. Click the **...** button and enter the minimum and maximum values to be used instead of the default ones.




Click  to apply the manual scale range.

Set maximum range Any manual scale changes are turned off and the default (widest) range is applied.

% Feature values are represented by their percentage. 100% is assigned to the maximal and 0% to the minimal value.

13.8.3 Graph View

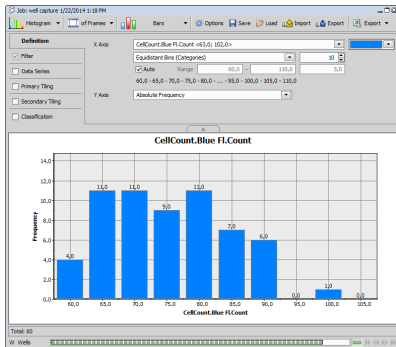
(requires: JOBS Viewer)

Clicking on the  View Graph icon in the result view opens the graph view.


Note




To display different graphs of your results, click the graph button to expand a combo box containing more options.







Figure 12.331. Histogram graph view




Top toolbar options


 **Graph selection** The first button is used for selecting the graph type.


   **of Frames/of Objects/of Tracks** Select whether your graph results are related to image *Frames*, binary *Objects* or *Tracks*.


 **Graph sub-type** Specifies the graph sub-type. Following types are available:  *Histogram*,  *Scatterplot*,  *Barchart*,  *XY Line*,  *Timechart*.


 **Options** Opens the Graph Options dialog window. Color and line features of the graph can be adjusted



in the *Appearance* tab. *X Axis* and *Y Axis Left* modify the axes.



 **Save** Opens the *Save Graph Settings* window enabling to save the current graph settings into the database.

 **Load** Opens the *Load Graph Settings* window used for loading graphs settings from the database.

 **Import** Imports the graph settings from a *.graph* file.

 **Export** Exports the current graph settings into a *.graph* file.

 **Export** This button enables to export the resulting graphs into raster files or Clipboard. Data can be exported into MS Excel or copied to Clipboard as well. Clicking the black arrow next to this button reveals a menu which specifies further export settings ( *Settings*) and which graph features will be exported.

  **Zoom in/out** Zoom in/out inside the graph area.

Tabs on the left represent steps of setting-up your graph visualization. Each tab has a check box, which determines whether the tab parameters are influencing the resulting graph or not.

Definition tab

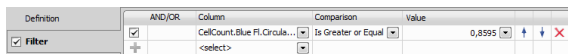
In this tab, the user defines main properties of each graph type. Settings are not the same for each graph type. Generally features shown on the X and Y axis are selected and their range, bins trend lines and other variables can be adjusted.

Filter tab

Filter tab enables filtering results based on selected features. These can be selected from the combo box in the *Column* column. After choosing the feature for filtering, select the *Comparison* type. Click the combo box in the *Value* column to reveal a histogram suitable

for quick value estimation or enter the numeric value directly into the edit box. If you add multiple filtering features, you can move them up/down in the list using these \uparrow \downarrow arrows. You can also delete each filter clicking on this \times icon.

Figure 12.332. Filter tab



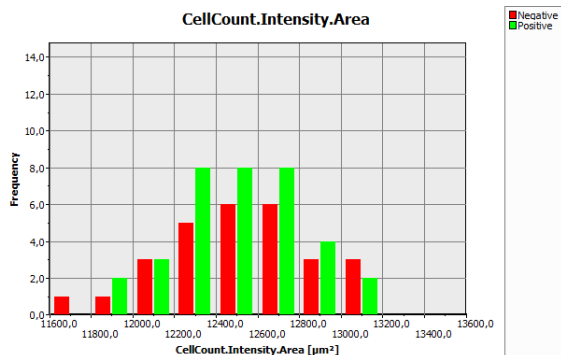
Note

Only filters with the check box marked as checked are used for result filtering.

Data Series tab

Series enable to view different groups of data in the same graph. Each data series can be displayed in a different color. Select one feature from the combo box (label, quantity, class or any measurement result) and adjust its starting and ending color. Bin types, their ranges and limits, legend and other parameters can be adjusted.

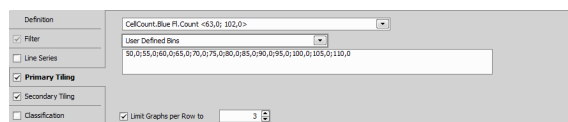
Figure 12.333. Data Series graph



Primary/Secondary Tiling tabs

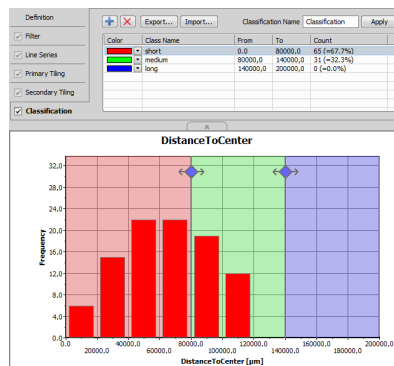
Both define another two groupings enabling the user to see more instances of the same graph populated with different group data. *Primary Tiling* defines the groups to be displayed in graph tiles horizontally whereas *Secondary Tiling* defines them to be displayed vertically. Grouping is defined as in the *Line Series* tab except the colors.

Figure 12.334. Primary Tiling tab



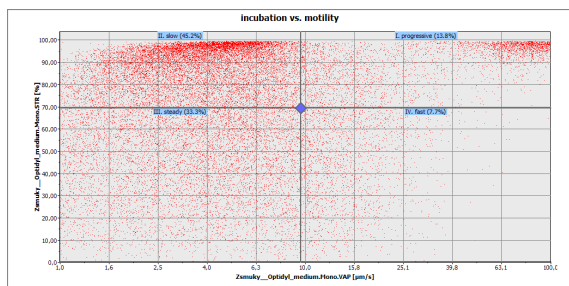
Classification (requires: Local Option)

This tab is used to create classes over the resulting graphs. These classes are easily defined by selecting the class color, entering its name and specifying its range (by editing from and to columns or by moving the rhomb symbol directly inside the graph).

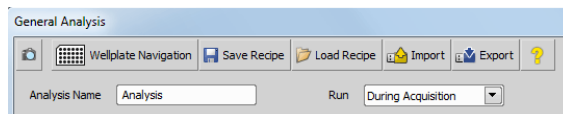


Histogram and Scatterplot allow manual classification (scatterplot allows also gating).

Figure 12.336. Four group scatterplot classification based on the VAP and STR features



you choose, the top part of the definition window is the same for all of them:



Capture This button acquires a single image according to the current Capture definition settings. Use this button to preview results of your analysis setting. By altering your parameters (such as preprocessing, thresholding,...) a binary image is modified within the captured image.

Wellplate Navigation Opens the *Wellplate Navigation* window where you can overview your well plate and navigate to a desired well stage on a selected well plate.

Save Recipe This button saves your recipe to the recipe database. The recipes can then be managed via the *HCA/JOBS > Presets > Manage Recipes* command.

Load Recipe This button loads your recipe from the recipe database.

Import This button opens a dialog for importing recipe files from your hard drive.

Export This button opens a dialog for exporting recipe files to your hard drive.

Load Opens the Load Job Recipe dialog window used for loading job recipes.

Help Opens the HTML Help about the analysis being set.

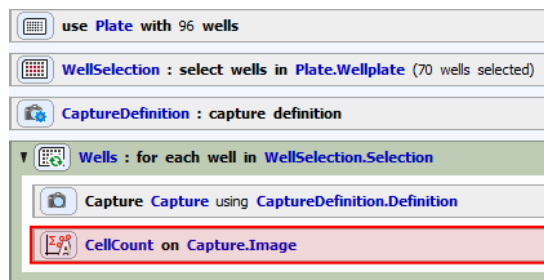
Analysis Name Analysis name will denote the particular analysis in the result view. Analyses with the same name are considered to be the same and thus available for aggregation.

13.9 Analysis

A simple analysis (or a complex one) can be performed either:

- Inside a job using analysis tasks (13.15.14 Analysis). E.g. to make a decision what to do next based on the analysis results - see: 13.13.2 Using Conditions.

Figure 12.337. Analysis task (Cell Count) used inside a job



- On data acquired by a job (see: Analysis performed on job results).
- On any opened image (see: 2.1 Analysis Explorer).

There are several predefined analysis types which are listed here: 10.3 HCA Analysis. No matter what type

13.10 Labels and Metadata

Any color/text labels and any metadata can be assigned to each well of your well plate. Label definition/assigning and metadata assigning can be made inside the HCA template or directly in the *Label Wells* task (see: *Label Wells* task).

Figure 12.339. Sample Labeling tab inside a HCA template

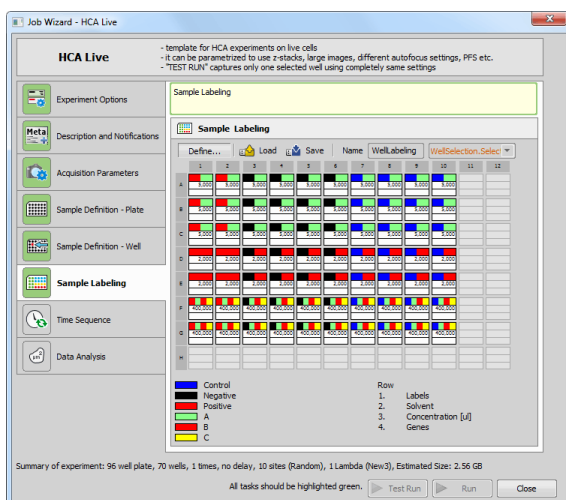
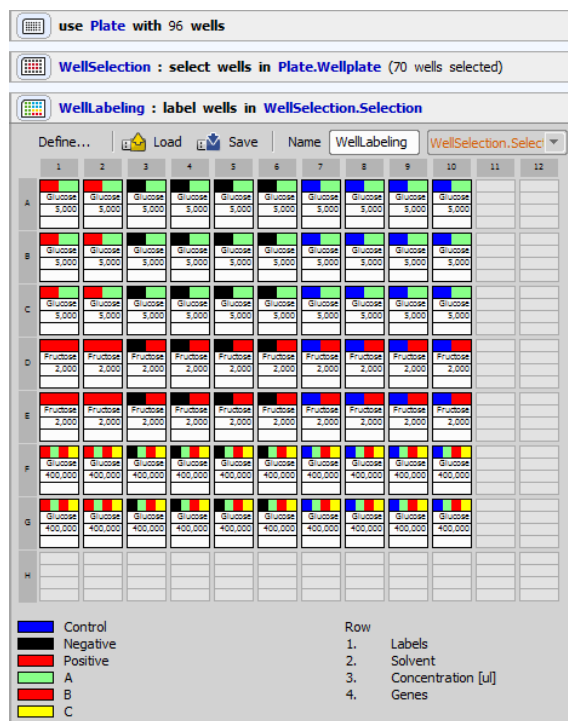
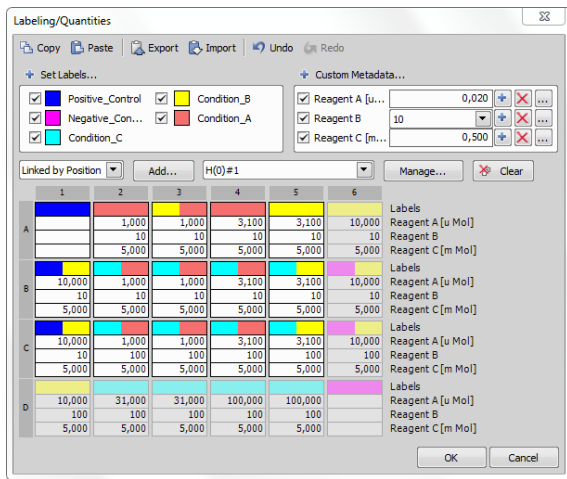


Figure 12.340. Label Wells task used inside a custom job









13.10.1 Labeling/Quantities window

This window is the initial place for defining and assigning labels (see *Defining and Setting Labels*) and for assigning predefined metadata (see *Defining and Setting Metadata*) to cells shown in the well plate overview.



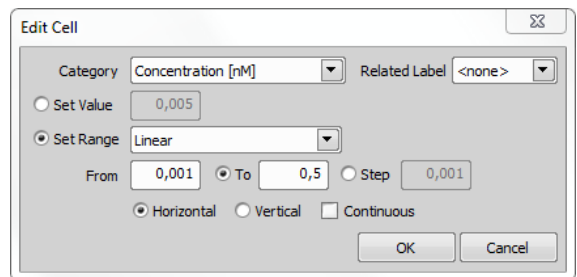
Labeling/Quantities window options

-  **Copy** Copies selected labels/quantities into clipboard.
-  **Paste** Pastes the labels/quantities from clipboard into your *Labeling/Quantities* well plate overview.
-  **Export** Exports all labels and quantities for each well plate preset into your clipboard.
-  **Import** Imports all data from clipboard. Two formats for importing labels and quantities are available. Please see: 13.10.5 Importing predefined presets from spreadsheet applications.
-  **Undo** Standard Undo functionality.
-  **Redo** Standard Redo functionality.
- + Set Labels...** Opens the Labeling/Tags window (see *Defining and Setting Labels*).
- + Custom Metadata...** This button opens the *Select Custom Metadata* dialog window, where you can choose predefined metadata. For their definition, please see: *Defining and Setting Metadata*.

+ To label wells, make a well selection, choose the metadata from the top right list and click the **+** button next to the selected/entered value or right-click on the selected wells and choose *Set Custom Metadata*.



X Deletes the selected value from all selected wells.



... Opens the *Edit Cell* dialog window.



This window enables to adjust the value/range of each metadata and link a label directly to it.


Not Linked/Linked by Position/Linked by Barcode

If you are using a *Plate Loader*  or a *Manual Plate List*  with multiple plates distinguished by a position or a barcode, a new preset linked to this plate can be created. Choose the link type from the combo box and click **Add...** to confirm/adjust the preset name.

Add... Creates a new preset (map of the currently defined well plate labeling and metadata of your well plate) and saves it with a custom name. Define as many presets as you like and switch in between them using the combo box. If you mark the *Label Wells*  task to be shown during  *runtime*, you can choose the preset for the current job run from the *Presets* combo box.

Manage Opens the *Preset Manager* containing a list with all of your presets and links to the particular plates. Here each preset can be renamed, removed and its link can be altered.

13.10.2 Defining and Setting Labels

Open *HCA/JOBS > Presets > Manage Labels* or click **Define...** in the *Label Wells*  task or *Sample Labeling* tab of the HCA template. Click **+ Set Labels...** task to create new labels. *Labeling Tags* window opens enabling to define your labels by clicking **+**, entering a name and choosing a color label or optional description. When done with defining all of your color labels, click **OK**.

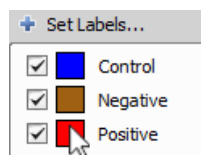
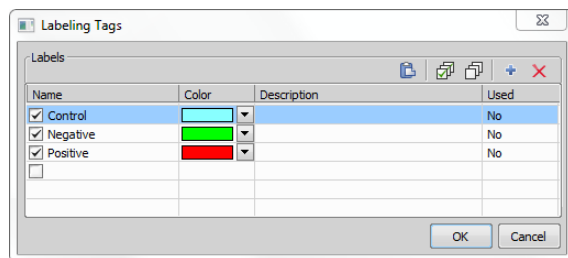



Figure 12.343. Labeling Tags window used for defining labels

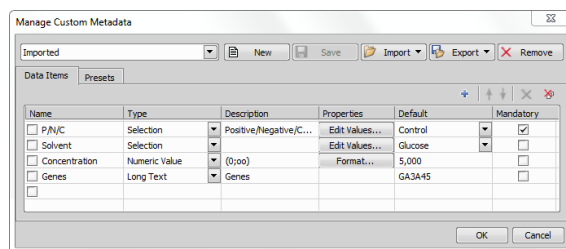


Click **Define...** in the *Label Wells*  task or in the *Sample Labeling* tab of the HCA template to open the *Labeling/Quantities* window (closely described here: *Labeling/Quantities* window). Click and drag over the table with your wells to select those you want to label. You can use the shift key to select a range of cells or the control key to select remote parts of the well plate. By right-clicking on any cell, you get more selecting options - i.e.: select all, invert all, select row, invert row, select column and invert column. When you are satisfied with your well selection, right-click on one of the selected wells and choose the desired labeling tag to be assigned to these wells.






Labels can also be added/removed by selecting well(s) and clicking on the labeling color.

13.10.3 Defining and Setting Metadata

Open the *Manage Custom Metadata* window (*HCA/JOBS > Presets > Manage Custom Metadata* to create new metadata.




Data Items tab



-  **New** Creates new custom metadata set.
-  **Save** Saves the current custom metadata definition using the name on the left.
-  **Import** Enables to import custom metadata definition from a .xml file or reuse them from an existing image file.
-  **Export** Enables to export current or all metadata definitions to a .xml file.
-  **Remove** Removes the current metadata.

Presets tab


This tab is used for setting up metadata sets (grouped metadata of different types). In the left list, single sets are created, arranged, renamed or deleted. On the right side of the dialog window, values of individual



items are set. Predefined presets can be used in the *Custom Metadata*  task.





Defining metadata

Create a new metadata set - click on  **New** and give it a name. Click  **Add New** and name your first metadata, choose its *type*, enter a text *description*, edit its *properties*, choose the *default* value and select whether it is *mandatory* or not. Add as many metadata as you like and confirm their definition by clicking **OK**.


Setting metadata


Click **Define...** in the *Label Wells*  task or in the *Sample Labeling* tab of the HCA template to open the *Labeling/Quantities* window (closely described here:

Labeling/Quantities window). Click  **Custom Metadata...** to open the *Select Custom Metadata* window where you can choose the metadata set from the first combo box and select (by a check mark) which metadata will be used in the current job. Make your selection and click **OK**. In the wellplate table make a well selection of wells to which you want to assign the chosen metadata. To assign metadata to selected well(s), right-click on the selection and choose *Set Custom Metadata* and confirm your metadata selection or click  on the right side of the metadata name listed in the top right corner of the window.

Once you are done with adding labels/metadata, click **OK** to get back to the *Label Wells*  task or *Sample/Labeling* tab of the HCA template. You can save your label as a XML file by clicking the  **Save to file** button. You can also import your saved labels by clicking the  **Load from File** button. Clear your labeling any time by clicking the  **Clear** button.

13.10.4 Labels and Metadata in Result View

After the HCA/Job containing labels/metadata is executed and finished, the Job Results window opens. Click  **Show Labeling** to display labels over wells.

Labels of the wells selected in the wellplate area are highlighted in the labels toolbar (invoked by clicking  **Show Labels**). Any label can be temporarily turned off by unchecking the check box next to its name.


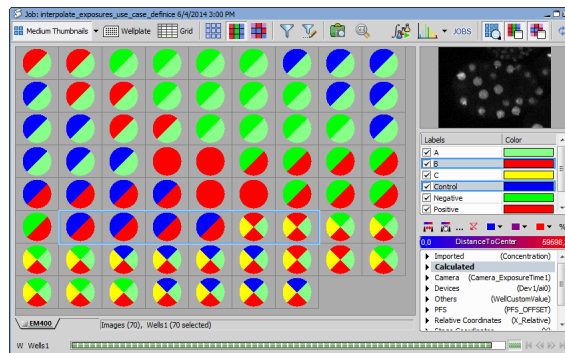
To assign prepared labels and metadata to the wells displayed in the result view, copy the metadata inside your spreadsheet application and click  **Import Metadata** in the results view. Please see 13.10.5 Importing predefined presets from spreadsheet applications for importing prerequisites.

Figure 12.346. Job Results View displaying well labels



13.10.5 Importing predefined presets from spreadsheet applications

Two types of formats are supported for importing custom presets into the *Labeling/Quantities* dialog window. Labels and quantities have to be defined similarly to one of the examples below.

1. Plate View

Figure 12.347. Plate View identification and definition

| Plate | Plate Name | Plate name meaning [1", Barcode, Position] | Hotel ID (optional) |
|------------|------------------------------|--------------------------------------------|---------------------|
| Value Type | Value Name (empty for Label) | Unit (empty for Label) | |

First row defines the plate (its name, name meaning, hotel). Next row contains the *value type* (Label, Numeric Value, Short Text, Long Text, Selection, Date), *value name* (empty for Label) and *Unit* (empty for Label). The values are then typed in the following rows as they appear in the well plate.

Figure 12.348. Example of a Plate View table format

| Plate | 1 | Position | 1 |
|---------------|---------------|---------------|-------------|
| Label | | | |
| Condition A | Condition B | Condition A | condition B |
| Condition A | Condition B | Condition A | Condition B |
| Condition A | Condition B | Condition A | Condition B |
| Condition A | Condition B | Condition A | Condition B |
| Plate | 1 | Position | 1 |
| Selection | Treatment | | |
| taurine | GABA | strychnine | tropisetron |
| taurine | GABA | strychnine | tropisetron |
| taurine | GABA | strychnine | tropisetron |
| taurine | GABA | strychnine | tropisetron |
| Plate | 1 | Position | 1 |
| Numeric Value | Concentration | μM | |
| 10 | 5 | 20 | 1 |
| 50 | 25 | 40 | 2 |
| 100 | 50 | 60 | 3 |
| 200 | 100 | 80 | 4 |
| Plate | 2 | Position | 1 |
| Date | Date | | |
| 28.5.2014 | 28.5.2014 | 28.5.2014 | 28.5.2014 |
| 8.5.2014 | 9.5.2014 | 10.5.2014 | 11.5.2014 |
| 11.5.2014 | 11.5.2014 | 11.5.2014 | 11.5.2014 |
| 11.5.2014 | 11.5.2014 | 11.5.2014 | 11.5.2014 |

2. List View

This view is used to label and quantity features of each well by a single row record. First four rows (Variant A) or three rows (Variant B,C) specify the hotel position (optional), identify the plate, position of each well on a plate, *value types* (Label, Numeric Value, Short Text, Long Text, Selection, Date) with *value names* and *units*. If the *Plate name meaning* value contains an empty string “ ”, then the name of the plate is used for plate identification. *WellIndex*

and Plate Position identifiers are considered to start at 1.

Variant A

Figure 12.349. Variant A: list view identification and definition

| Hotel (optional) | Plate | Row | Column | Value Type |
|------------------|--------------------------------------------|----------------|-------------------|------------------------------|
| | Plate name meaning (“”, Barcode, Position) | | | Value Name (empty for Label) |
| | | | | Unit (empty for Label) |
| Hotel ID | Plate Name | Row Identifier | Column Identifier | Well Data |

Figure 12.350. Variant A: example of a list view table format

| Hotel | Plate | Row | Column | Label | Numeric Value | Selection | Numeric Value |
|-------|----------|-----|--------|--------------------------|---------------|-----------|---------------|
| | Position | | | | Reagent A | Reagent B | Reagent C |
| | | | | | u Mol | u Mol | m Mol |
| 0 | 1 | A | 1 | Positive Control | | | |
| 0 | 1 | A | 2 | Condition A | 1 | 10 | 5 |
| 0 | 1 | A | 3 | Condition A, Condition B | 1 | 10 | 5 |
| 0 | 1 | A | 4 | Condition A | 3,1 | 10 | 5 |
| 0 | 1 | B | 1 | Positive Control | | | |
| 0 | 1 | B | 2 | Condition A | 1 | 10 | 5 |
| 0 | 1 | B | 3 | Condition A | 1 | 10 | 5 |
| 0 | 1 | B | 4 | Condition A | 3,1 | 10 | 5 |
| 0 | 1 | C | 1 | Positive Control | | | |
| 0 | 1 | C | 2 | Condition A | 1 | 100 | 5 |
| 0 | 1 | C | 3 | Condition A | 1 | 100 | 5 |
| 0 | 1 | C | 4 | Condition A | 3,1 | 100 | 5 |

Variant B

Figure 12.351. Variant B: list view identification and definition

| Hotel (optional) | Plate | WellIndex | Value Type |
|------------------|--------------------------------------------|-----------------------|------------------------------|
| | Plate name meaning (“”, Barcode, Position) | | Value Name (empty for label) |
| | | | Unit (empty for label) |
| Hotel ID | Plate Name | Well Index Identifier | Well Data |

Figure 12.352. Variant B: example of a list view table format

| Hotel | Plate | WellIndex | Label | Numeric Value | Selection |
|-------|----------|-----------|--------------------------|---------------|-----------|
| | Position | | | Reagent A | Reagent B |
| | | | | u Mol | u Mol |
| 0 | 1 | 1 | Positive Control | | |
| 0 | 1 | 2 | Condition A | 1 | 10 |
| 0 | 1 | 3 | Condition A, Condition B | 1 | 10 |
| 0 | 1 | 4 | Condition B | 3,1 | 10 |
| 0 | 1 | 5 | Positive Control | | |
| 0 | 1 | 6 | Condition A | 1 | 10 |
| 0 | 1 | 7 | Condition A | 1 | 10 |
| 0 | 1 | 8 | Condition A | 3,1 | 10 |

Variant C

Figure 12.353. Variant C: list view identification and definition

| Hotel (optional) | Plate | WellName | Value Type |
|------------------|-------------------------------------------|----------------------|--------------------------------------------------------|
| | Plate name meaning [™, Barcode, Position] | | Value Name (empty for label) Unit (empty for label) |
| Hotel ID | Plate Name | Well Name Identifier | Well Data |


Figure 12.354. Variant C: example of a list view table format

| Hotel | Plate Position | WellName | Label | Numeric Value | |
|-------|-------------------|----------|--------------------------|--------------------|--------------------|
| | | | | Reagent A u Mol | Reagent B u Mol |
| 0 | 1 | A1 | Positive Control | | |
| 0 | 1 | A2 | Condition A | 1 | 10 |
| 0 | 1 | A3 | Condition A, Condition B | 1 | 10 |
| 0 | 1 | A4 | Condition B | 3,1 | 10 |
| 0 | 1 | B1 | Positive Control | | |
| 0 | 1 | B2 | Condition A | 1 | 10 |
| 0 | 1 | B3 | Condition A | 1 | 10 |
| 0 | 1 | B4 | Condition A | 3,1 | 10 |





Note

Both row and column well localisation can be described using numbers starting from one (1,2,3, ...) or by letters (A, B, C, ...). For example well "B3" can also be described as well "23", "2C" or "BC".

Linking with Plate Loader

Imported labels/quantities can be linked based on position of the well plate in a hotel (*Linked by Position*) or using a barcode (*Linked by Barcode*). Plate # selection among multiple hotels has to be unique, otherwise the linking is not performed. E.g. if plates #1 and #2 from hotel 1 and plates #3 and #4 from hotel 2 are selected in the *Plate Loader* , labels marked with hotel number 1 to 4 are linked correctly. Whereas if e.g. plate #1 is selected in both hotels the link for plate 1 is not created and a new preset named #1 is created instead.

13.10.6 Labeling during runtime (requires: Local Option)

Single wells or single points can be easily labeled with a color and a name during runtime. Use tasks *Create Labels*  (see: *Create Labels* ) and *Assign Label*  (see: *Assign Label* ).

13.11 Job Database

(requires: *JOBS Editor*) or (requires: *JOBS Viewer*)

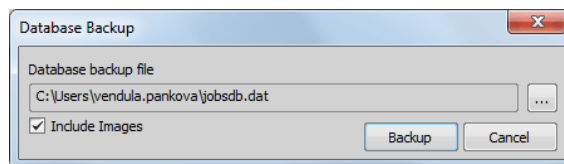
13.11.1 Database Backup

Warning

Always use the *HCA/JOBS > Database > Backup* command when doing a backup. Copying the "jobsdb.dat" file manually could corrupt the database structure and the backup will be inaccessible.

Run the *HCA/JOBS > Database > Backup* command, a dialog box appears. The backup routine copies the database file to a specified folder. If image files are also backed up (recommended) they are copied as well. The backup folder is a regular database bundle and can be accessed from *NIS-Elements*. If the backup folder already contains a previous backup, it is merged in the following way:

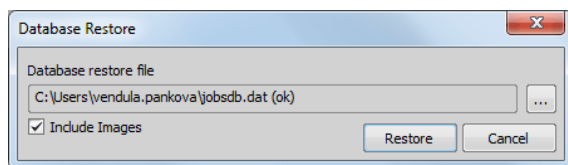
- The previous database file is renamed (current date is appended) and a new file is created.
- New image files are added to the backup folder.



Please see 13.11.3 Database Properties chapter on how to set the database.

13.11.2 Database Restore


This operation replaces the content of the current database folder with the content of the backup folder. Restoring the database destroys the original content of the current database.

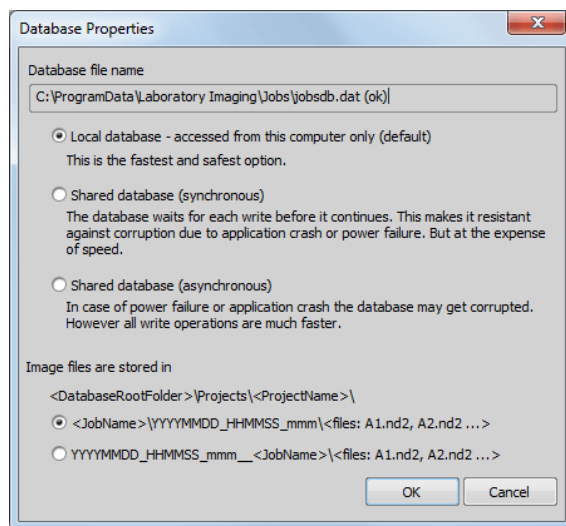


Press the ... button to browse to your database folder. After selecting it, click **OK** and *Restore* to finish the restoration process. You can uncheck the *Include Images* check box in case you don't want to restore image files.

13.11.3 Database Properties

Projects, jobs and job results are stored in a database. This database contains all metadata except images acquired by jobs. Images are not stored in the database file. This is for two reasons: efficiency and data security. As images are ordinary nd2 files they are independent of the database and can be opened by *NIS-Elements* directly. Images are marked as read-only and should not be modified in *NIS-Elements*. On the other hand, the database can be opened and accessed without the images. Therefore images are stored in folders and the database contains relative paths to them. Images and database files exist in a folder and make a bundle. This bundle can be copied as a whole. However it is not safe to copy the folder while it is accessed by *NIS-Elements*. Use the 13.11.1 Database Backup / 13.11.2 Database Restore functions instead.

You can adjust the following database settings after you click the  **Database Properties** button:



Database root folder Define the path to your database files and select the type of access to the data. You can choose between:

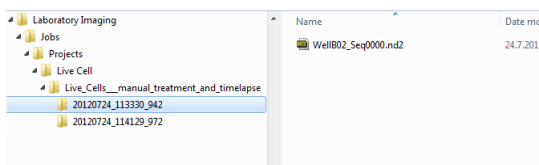
- *Local database* - recommended, fast, safe.
- *Shared database (synchronous)* - opt this if access from multiple PC's is required. This setup is safe and sufficient for long-lasting experiments (e.g. 1 frame per minute).
- *Shared database (asynchronous)* - if access from multiple PC's is required along with the speed of access. Regular back-ups are absolutely necessary with this setup.

Warning

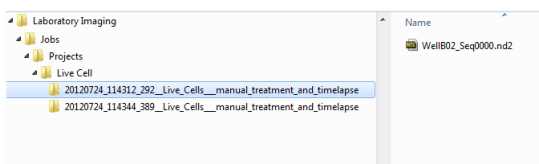
If an unexpected error such as power failure occurs while the database is being accessed, the database may get corrupted (lost).

Image files are stored in Choose in which form you want to save your image files. The following options are available:

- <JobName>\YYYYMMDD_HHMMSS_mmm\<files: A1.nd2, A2.nd2 ...>



- YYYYMMDD_HHMMSS_mmm__<JobName>\<files: A1.nd2, A2.nd2 ...>



Y = Year, M = month, D = day, H =hour, M = minute, S = second, m = millisecond

13.11.4 Database Files

The jobs database is contained in the `jobsdb.dat` file in the database folder. It contains the project and the job hierarchy, all the definitions (jobs, analysis recipes, labels, tags, etc...), acquisition data like plate or well information, loop indexes, time analysis results, links to the image files and much more. All this information is stored in a single file. The database file is usually much smaller than the size of an image file.

Other temporary files may also appear in this folder (typically `jobsdb.dat-shm`, `jobsdb.dat-wal` or `jobsdb.dat-journal`). These files indicate that the database is in use. All temporary files should disappear after all *NIS-Elements* applications accessing that particular database are closed. If *NIS-Elements* did not finish properly, these files may remain. They must not be deleted as they contain a valid database

state. In this case *NIS-Elements* should be launched, database connected and *NIS-Elements* closed again. Note that it is not safe to copy the `.db` file at that moment. *NIS-Elements* is connected to a single Jobs database at any given time - it is the current database. The current database can be changed in *Database Properties* (see: 13.11.3 Database Properties). *NIS-Elements* stores the current database folder on per user basis which means that every user can have his own database.

Database size

To avoid deposit and upgrade difficulties caused by huge databases, please follow the basic database rules.

- Make smaller, more manageable databases (use *HCA/JOBS > Database > Create New Empty*).
- Measure features per object only where it makes sense - e.g. in many HCS analyses on a well-plate, statistics per frame (not per object) may be enough.
- Cut off irrelevant data before merging databases.

If the database is still huge, it makes sense to put the `.db` file on an SSD disk (must be big enough) and mount a normal drive to "Projects". Navigate to the folder containing your database file and execute the following command (from the command line): `mklink /J Projects "C:\Temp"`, where "C:\Temp" is the folder of your choice.

Note

If you experience any other problems with the database (slow performance, crashing, ...), try to clean up the local and remote database (HCA/JOBS > Database > Cleanup).

13.11.5 NIS-Elements Upgrades

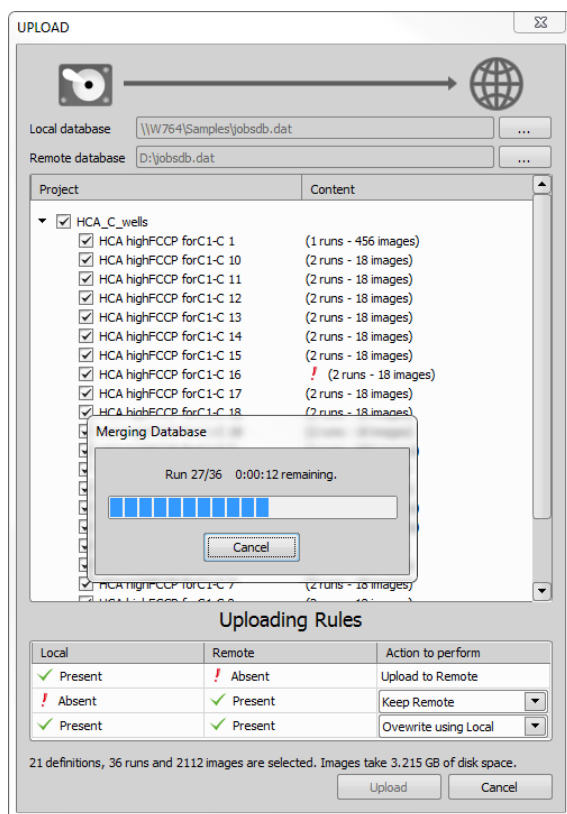
With new versions of *NIS-Elements* the database file format may change to support new features. An upgrade of the database is required before it can be used

by a new version of *NIS-Elements*. However, the upgrade renders the database inaccessible from any previous version of *NIS-Elements*. It is a good practice to make a backup of the database before an upgrade of *NIS-Elements* is made, although

<name>_backup_<date>_<time>.dat backup file is created automatically before the upgrade is performed. When the database is accessed by multiple *NIS-Elements* instances, they all must be working with the same database version.

13.11.6 Database Upload/Download

(requires: *JOBS Remote Database*)



Downloading a remote database or uploading a local database can result in database merging. Parameters and rules of database merging are specified in the *Upload/Download* dialog window.

Dialog window options

Local Database If necessary, click and specify where the *Local database* .dat file is present.

Remote Database Click and specify where the *Remote database* .dat file is present.

Project Use the check boxes next to each job to include/exclude them into/from the uploading/downloading process.

Content Displays the number of job runs and acquired images per each job definition. If the content displays this **!** symbol, some files are missing in the source database.

Uploading Rules This table shows the compared data status of both the local and the remote databases. Adjust the *Action to perform* combo boxes to set the merging rules and then click / to start the uploading/downloading process.

Note



New data of runs/analyses/tags are transferred only if Overwrite is selected. New projects and job definitions are transferred always (selected only).

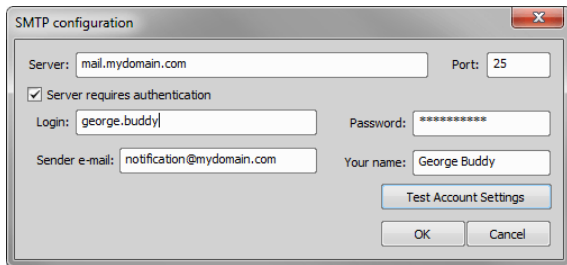
Invalid actions

- downloading/uploading into the same database
- downloading/uploading into a database created using a different version of *NIS-Elements*
- downloading/uploading into a local database on a network drive
- downloading/uploading into a network database folder on a network drive with read-only permission

13.12 Advanced Settings

13.12.1 SMTP Configuration for Sending Email/SMS Messages

You can make a running job send you messages via email or SMS (see *Send E-mail Notification*  and *Send SMS Notification*  tasks). To do this successfully, SMTP server and email account settings must be defined correctly. Run the *HCA/JOBS > Presets > Email Setup* command to display the following window and fill all its fields.



Server Enter the name of your server (e.g. “smtp.mydomainname.com” or “mail.mydomainname.com”).

Port Enter the server's port number.

Server requires authentication Check if your server requires authentication.

Login If the SMTP server requires authentication, fill in the appropriate user account name (e.g. “first-name.surname”).

Password If the SMTP server requires authentication, fill in the password for the “SMTPLogin” account.

Sender e-mail Type the email address which will be displayed in the “from” field (and also used as the “reply-to” address).

Your name “Alias” of the sender email - usually a human-readable equivalent of the email address. E.g. “Peter Smith” for an address such as ps001@company.com

Test Account Settings Click this button in order to test your SMTP configuration.

13.13 Use Cases

13.13.1 Analysis Use Cases

- 2.5.4 General Analysis in NIS-Elements - Model Use Case
- 2.5.5 General Analysis in JOBS Module - Appended Point Set Use Case
- 2.5.6 General Analysis in JOBS Module - Replaced Point Set Use Case

13.13.2 Using Conditions


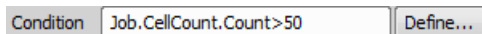

Condition (*Condition IF* ) is a great tool useful in all sorts of laboratory situations where we define which parameters influence the task execution. Condition tasks need to be defined using expressions (see 13.13.3 Using Expressions). For example: *If Expression is TRUE, run the contained tasks...*

Figure 12.362. Example of an expression




Deciding whether the condition is TRUE or FALSE is called “evaluation” and it is computed by *NIS-Elements* on job runtime.

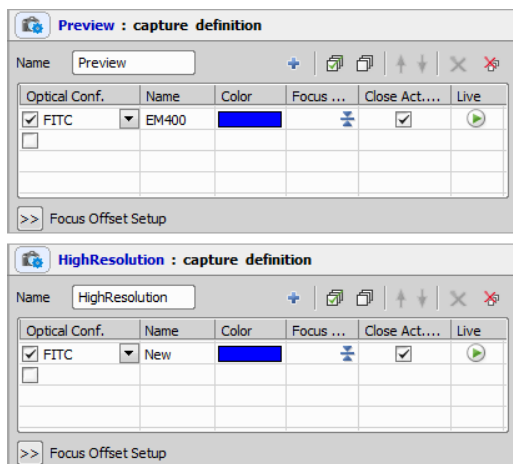
Almost every task produces number of variables which are then available within the *Expression*  task.

Condition use case


Let us show you the usage of an condition on a simple job. We will capture a large image in low resolution and measure its mean intensity. Only if the mean intensity is above the defined threshold (which may indicate that there is something interesting on the scene), we will capture the large image again this time using a high magnification objective.

1) Create two capture definitions

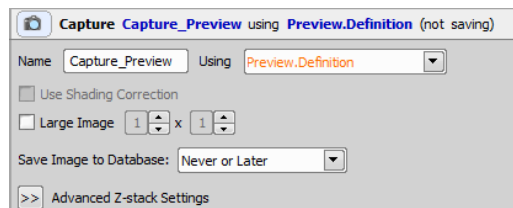
Define two *Capture Definition*  tasks, each using a different optical configuration. Assign some simple names to the tasks.




2) Capture the preview image

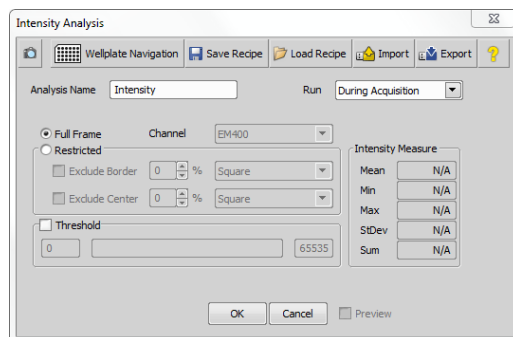
Insert the first *Capture*  task and set it so that it will use the low magnification capture definition - choose the Preview Capture definition from the *Using* combo box. Since purpose of this task is to decide whether to capture the high-magnification image or not, there is no need to

save the images, so leave the *Save Image to Database* pull-down menu set to *Never or Later*.



3) Analysis Definition

Insert the *Intensity Analysis*  task and double-click on it to specify its parameters.

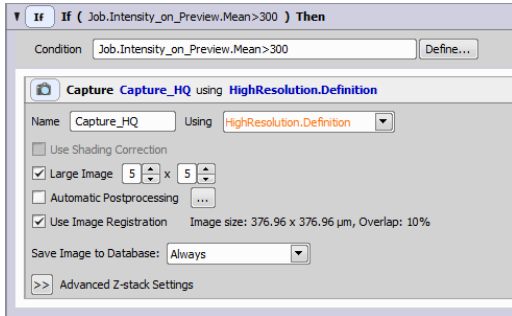


Because intensity of the whole frame will be measured, just select the *Full frame* option and click **OK**.

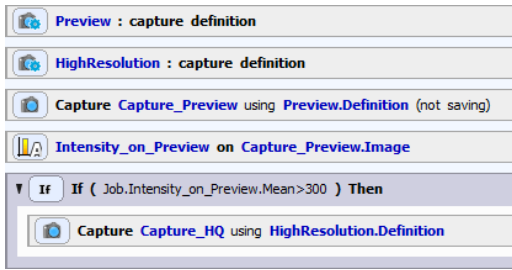
4) Conditional Capture

By now, everything is prepared to make the decision whether to capture the high-resolution image or not. We captured a preview image, measured its intensity and now we can set the resulting

value into the *Condition IF* task and add another *Capture* task within.



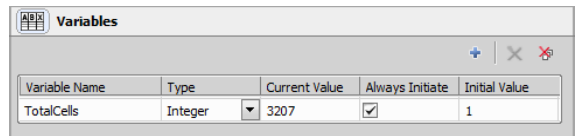
Specify the conditional expression (Condition). If evaluation (result) of this expression is TRUE (i.e. 1), the capture will be performed. Use the **Define** button to specify the conditional expression as described in (13.13.3 Using Expressions). The resulting job could look like this:



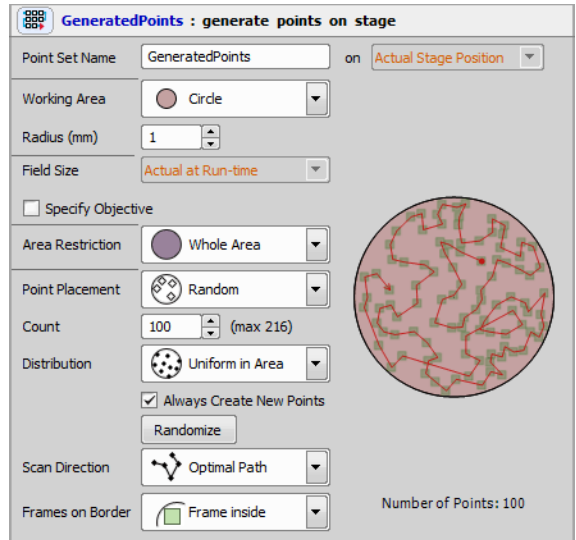
Expression use case

In the following text we show an example which uses an expression. Imagine that we want to capture random positions on a dish. We want to finish capturing when we gather 3000 objects.

First of all we have to define our capture using the *Capture Definition* task. We choose the optical configuration and insert the *Variables* task which we set as follows:








Then we insert the *Generate Points* task, define the dish area on the stage and adjust point generating parameters as shown in the following picture.





13.13.3 Using Expressions

An expression in Jobs is a combination of explicit values, variables, operators and functions (see: Wikipedia [[http://en.wikipedia.org/wiki/Expression_\(programming\)](http://en.wikipedia.org/wiki/Expression_(programming))]). It is typically in the form of an assignment (variable = expression) whereby a value is computed (by evaluating the expression on the right side) and assigned to a variable (on the left side). Expression is evaluated and assigned to the variable when the task is executed.

Finally we insert the point loop (*Loop over Points* ) and fill it with *Capture*  , *Cell Count Analysis*  , *Expression*  and *Condition IF*  .

Double-clicking on the analysis task we can further define its parameters (see 2.2 Cell Count Analysis).

In the task expression we enter the following expression: `TotalCells = TotalCells + Job.CellCount.Count`

Into the *Condition IF*  task we define the following condition: `TotalCells > 3000`. Inside the condition we insert a *Break* (*Break* ). Thus if the condition is true (we receive 3000 objects), the whole job stops.

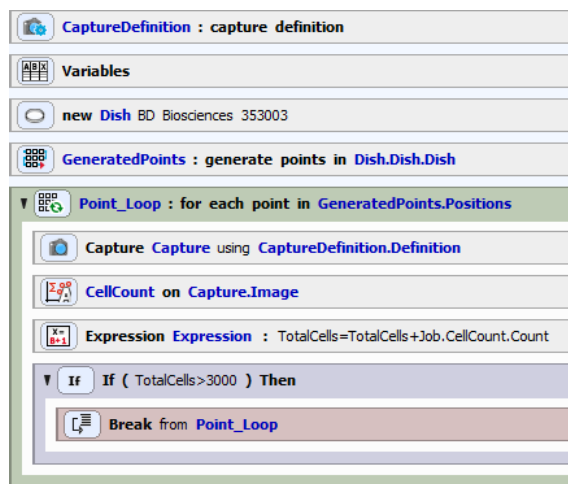


Figure 12.370. Loop and its containing tasks

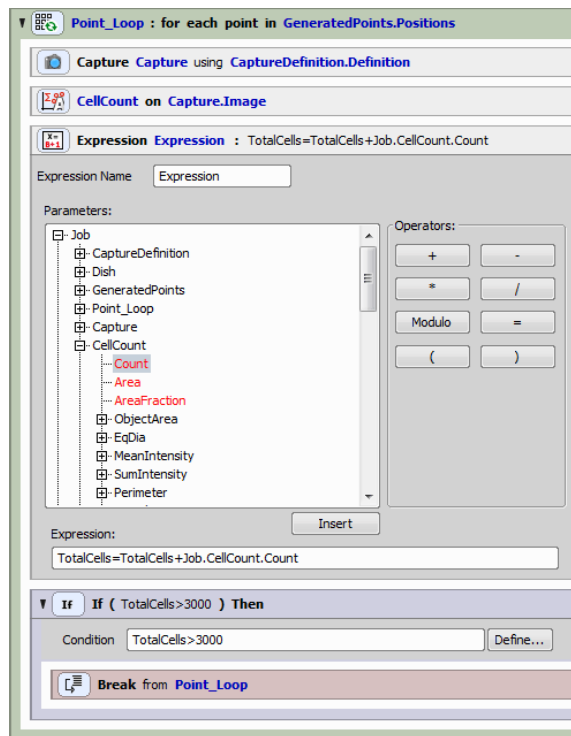
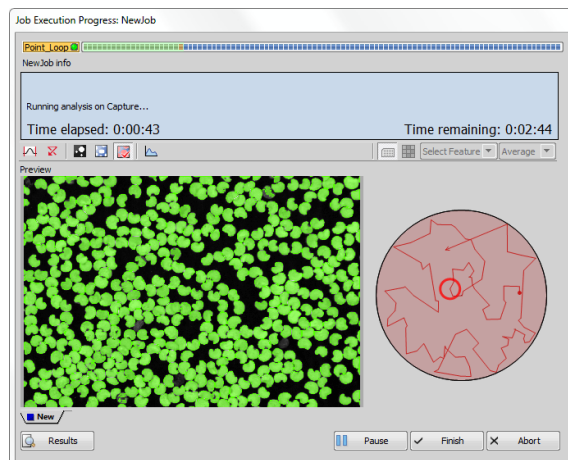


Figure 12.372. Job Execution Progress window



The final job looks as follows:

Figure 12.373. Final results

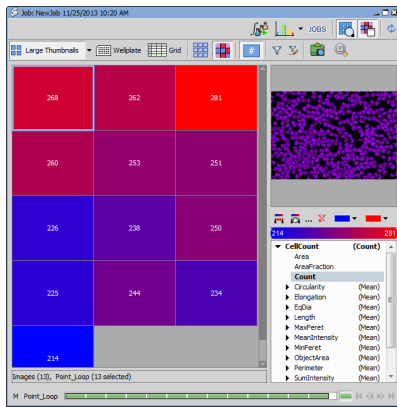
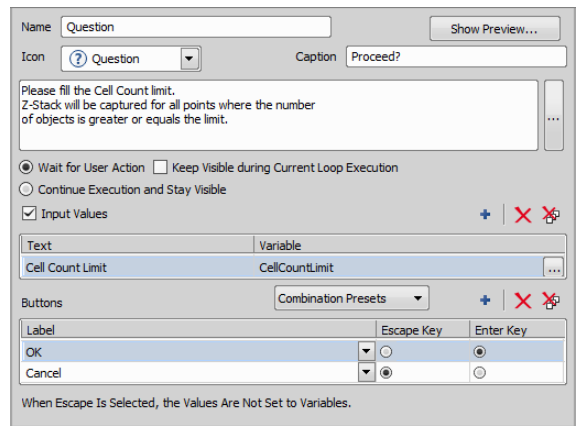


Figure 12.374. Question task.



For more information about Jobs Progress and Results window, please see 13.8.1 Job Execution Progress window and 13.8 Running a Job, Viewing Results and Graphs.

13.13.4 Using Questions

Question (*Question*) is a useful task if you want to stop your job in a specified part of the Job sequence and let the user decide what to do next. Start by dragging the *Question* task into the main area of the Job Definition window (see: 13.5 Job Definition Window). Then double click the task to maximize it and set up your question.

Name your Question task, choose an icon which will be displayed together with your question and type a text into the Caption box to be used in the dialog window heading. Then type in the text of your question.

Wait for user action If checked, the question dialog shows at the bottom of the Job Execution window (13.8.1 Job Execution Progress window) and pauses the job until the question is answered by user. After that, the job continues or terminates depending on the question settings. If solely this option is checked, the question dialog disappears until the loop meets the question again (e.g. until the next Capture).

Keep visible during current loop execution Displays the question dialog during the whole Job run. The answering buttons are disabled (gray) until the loop meets the question again.

Continue execution and stay visible Proceeds the job even without user interaction (answering the question). The question dialog stays visible until the job run is over.

If any variables are available, check *Input values*. Click the *Add New* button, modify the Text or change your variables by clicking the ... button. During the Job exe-

cution the question task asks you to input these variables manually. Finally define your question buttons. You can use basic predefined buttons by clicking the *Predefine Combination* button or add your custom buttons and captions.

Tip

Assign the *Escape* and *Enter* key to two of your buttons for faster work with questions during the *Job* progress.

13.13.5 Using Time Lapse




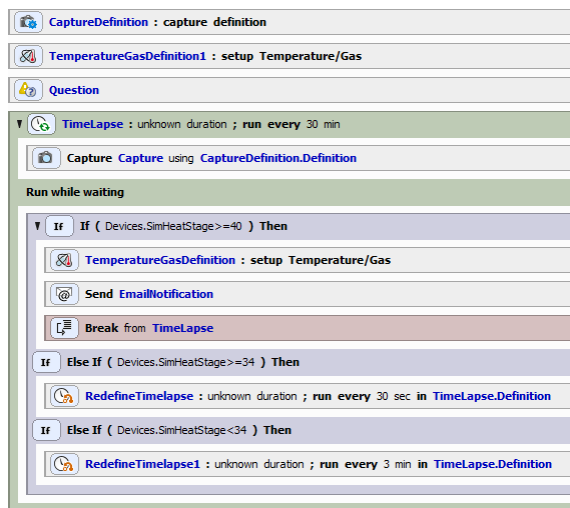


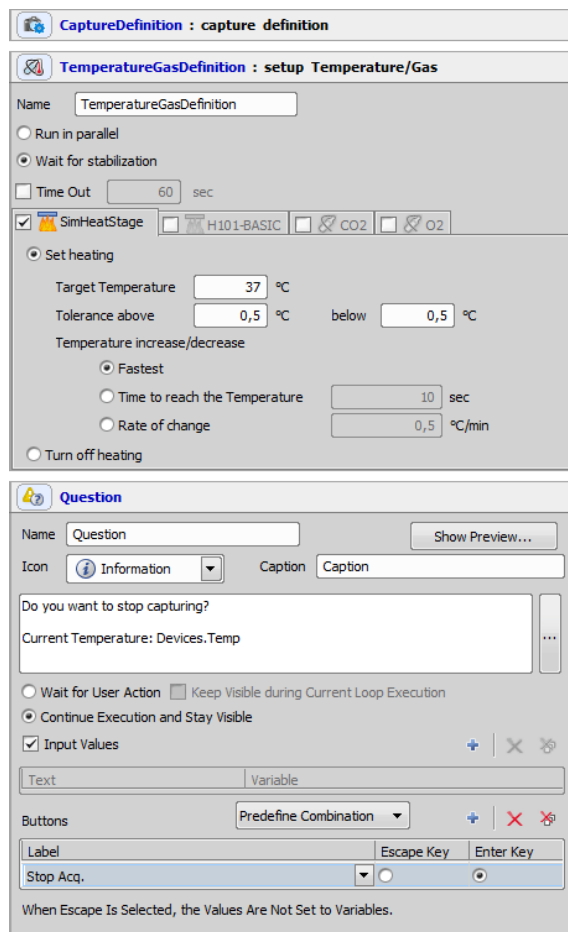
In the following example we will build a job benefiting from the *Time Lapse* , *Time Lapse*  and *Condition IF*  tasks. This job will guard the temperature of our device and will alter the image capture in compliance with its current temperature. This type of job is advisable especially when working with living objects (e.g. cells).


Figure 12.375. Overview of the job.



We start by defining our capture (selecting Optical Configuration) and inserting the first *Temperature and*

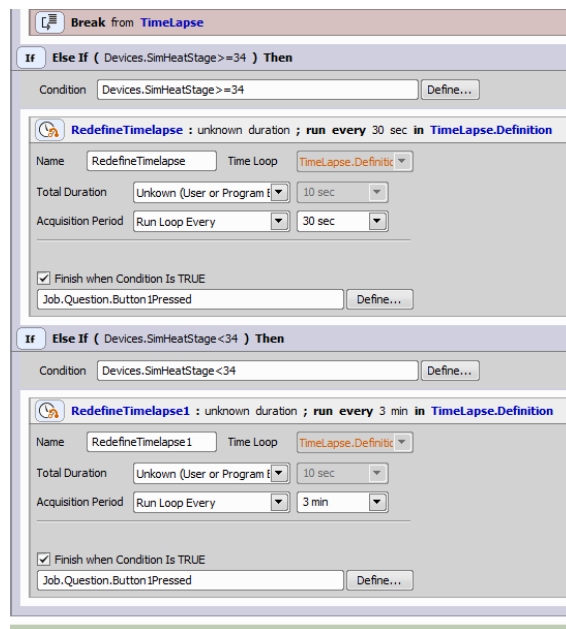
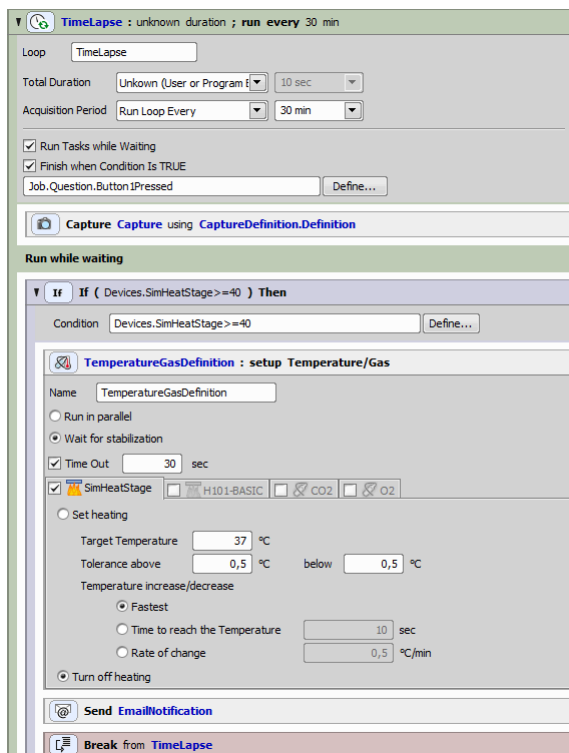
Gas Control  task where we set the target temperature of our device to 37 °C. We go on with inserting a *Question*  task. We want the user to have an option to stop the job progress at any time, so we define a *Stop Acquisition* button and check *Continue execution and stay visible*. Task setting details are shown in the following picture:



During normal conditions we want to capture every 30 minutes and we want the *Time Lapse*  to enable quitting at any time. Therefore we check the *Finish* when condition is TRUE and Define the *Stop Acquisition*

button from our question. We continue by inserting our *Capture* task followed by an *Condition IF* task containing another *Temperature and Gas Control*, *Send E-mail Notification* and a *Break*. If the temperature of our device exceeds 40°C, the *Time Lapse* stops and an e-mail is sent to the user with custom warnings about the job termination.

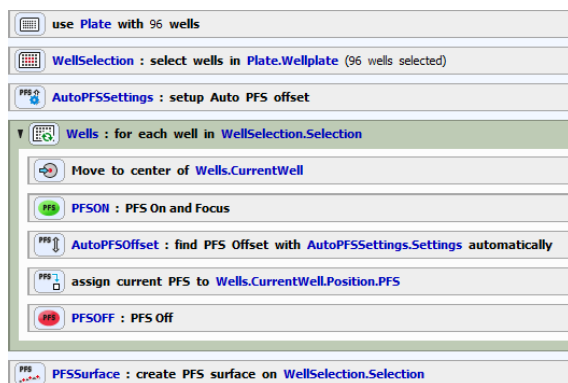
clicking the If task and selecting Add “If Else”. Accordingly if the temperature is lower than 34 °C we want to augment the capture interval to 3 minutes (see the following settings).













13.13.6 Creating PFS Surface

Perfect Focus System Surface, displayed in the XYZ Overview (see: *View > Acquisition Controls > XYZ Overview*) can be defined using a job. Following example shows PFS Surface definition on a well plate.

Our live sample behaves differently under cold and warm temperatures, therefore we right-click on the frame of our *Condition IF* task and choose Add “If Else”. If the temperature of our device is greater and equal than 34 °C we want to capture every 30 seconds. Because our specimen moves slower under colder temperatures we define another condition (“Devices.Temp<34”) as in the first case by right-



Auto PFS Offset Settings  task defines settings (Optical configuration) to be used by the *Find Auto PFS Offset*  task. On each well (*Loop Over Wells* ) of the well selection (*Select Wells* ), the stage moves to the center of a well (*Move to Well Center* ), turns on PFS (*PFS On and Focus* ), automatically finds the PFS offset value (*Find Auto PFS Offset* ), assigns it to the each well (*Assign PFS to Point/Well* ) and turns PFS off (*PFS Off* ). Once all wells undergo this procedure, the PFS Surface is finally created on the well selection using the *Create PFS Surface*  task.

13.14 JavaScript

Standard (ECMA-262) JavaScript syntax is supported in *NIS-Elements*.

13.14.1 Constructors

There are three table constructors for each data type:

- new *FrameTable*(columnList, whereClause, orderClause)
- new *ObjectTable*(columnList, whereClause, orderClause)

- new *TraceTable*(columnList, whereClause, orderClause)

where:

```
columnList: ["columnName1", "columnName2", ..., "columnNameN"]
```

The parameter specifies column names of the columns which will be accessible in created table (as columns). Columns are ordered as they appear in the list. To learn how to name the columns see section "The format of column names".

```
whereClause: "columnName1 = value  
AND/OR
```

```
AND/OR columnName2 = value AND/OR ..."
```

The parameter defines which jobruns will be included in the table (as rows). Currently, following patterns are recognized (and can be combined using AND or OR operators):

- only specified jobrun will be included


```
"Jobrun.ID = value"  
"Jobrun.name = 'value'"
```
- all jobruns contained in specified job will be included


```
"Jobdef.ID = value"  
"Jobdef.name = 'value'"
```
- all jobruns contained in specified project will be included


```
"Project.ID = value"  
"Project.name = 'value'"
```

```
orderClause: "columnName1 ASC/DESC,  
columnName2 ASC/DESC, ..."
```

This parameter is optional. If specified it defines the row order.

13.14.2 Member Methods

Following methods (function properties) are defined on a table object:

- `length()`
Returns the number of rows in the table.
- `column(columnName)`
Returns the index of given column (same as in constructor).
- `addColumn(columnName)`
Creates (or modifies) a column and returns its index. Column is added to "Others" group.
- `commit(columnList)`
Writes modified data into database. Only columns mentioned in `addColumn()` method are stored. The parameter `columnList` is optional if omitted all columns added using `addColumn()` are saved. Any modification to any other data is temporary.

13.14.3 Indexing

Data are accessed using indexing operators:

- `table[rowIndex][colIndex]`

13.14.4 Format of Column Name

The column name is formed as seen in JobsViewer column tree (red frame in the picture).

| Thumbnail | ID | Name | Mean [µm] | CellCountAnalysis.DAPI.EqDia |
|-----------|----|------------------|-----------|------------------------------|
| 1 | S1 | MetadetaOrniVels | 2,42 | |
| 2 | S1 | MetadetaOrniVels | 2,41 | |
| 3 | S1 | MetadetaOrniVels | 2,43 | |
| 4 | S1 | MetadetaOrniVels | 2,40 | |
| 5 | S1 | MetadetaOrniVels | 2,41 | |
| 6 | S1 | MetadetaOrniVels | 2,41 | |
| 7 | S1 | MetadetaOrniVels | 2,42 | |
| 8 | S1 | MetadetaOrniVels | 2,42 | |

Items appearing inside another one (parent) is named as follows: `parent.child`. The matching is case-insensitive.

Sometimes (in the case of analyses) the item has a grandparent group: `analysis.channel.feature`.

For convenience where the Parent and an item starts with the same word it can be omitted:

Jobdef -> Jobdef Name can be addressed by both "Jobdef.name" or "jobdef.jobdef name".

In some cases the aggregation part (Mean, Min, Max, ...) of column name is needed, for example: "CellCountAnalysis.DAPI.EqDia.Mean".

Expressions "ID" and "Key" are equivalent (except while using with "Object" or "Trace"):

```
"Jobrun.Key" = "Jobrun.ID", "Jobdef.Key" = "Jobdef.ID", but "Object.Key" ≠ "Object.ID".
```

13.14.5 Code Example

```
var table = new FrameTable(["Image.Calibration", "Image.Width", "Image.Height", "Mono.Count"], "jobrun.id = 116");
```

```
var columnCalibration = table.column("Image.Calibration");
```

```
var columnWidth = table.column("Image.Width");
```

```
var columnHeight = table.column("Image.Height");
```

```
var columnCount = table.column("Mono.Count");
```

```
var columnConcentration = table.addColumn("Concentration");
```

```
for (row in table) {
```



```

var volume = table[row][columnWidth]
* table[row][columnHeight];

volume *= Math.pow(table[row][columnCal-
ibration], 2.0);

volume *= 10 * 1000;

table[row][columnConcentration] =
table[row][columnCount] / volume;
}

table.commit(columnConcentration);

```

The same example using column indices:

```

var table = new FrameTable(["Image.Cal-
ibration", "Image.Width", "Im-
age.Height", "Mono.Count"], "jobrun.id
= 116");

var columnConcentration = table.ad-
dColumn("Concentration");

for (row in table)
{
var volume = table[row][1] *
table[row][2];

volume *= Math.pow(table[row][0], 2.0);
volume *= 10 * 1000; table[row][column-
Concentration] = table[row][3] /
volume;
}

table.commit(columnConcentration);

```

13.14.6 Helper Classes and Functions

CsvWriter

Constructor

- new CsvWriter(filename [, truncate])

Opens the file for writing. Takes filename as parameter and an optional bool value if the file should be truncated.

Member Methods Following methods (function properties) are defined on a CsvWriter object:

- separator (property)
Column separator (by default “\t” - TAB)
- endOfLine (property)
End Of Line (by default “\r\n” - Windows style)
- close()
Closes the file.
- writeValue(value)
Writes the value (nothing more).
- writeSeparator([sep])
Writes Column separator. If not supplied the separator property is used.
- writeValues(array [, sep])
Writes whole line using the values in the array separated by sep or separator property and followed by the End Of Line.
- writeEndOfLine()
Writes End Of Line.
- writeTextLine(text)
Writes the text followed by End Of Line.

Example:

```

var f = new CsvWriter("c:\\data.csv",
true)

f.writeValues([1.1, 2.1, 3.1])
f.writeValues([1.2, 2.2, 3.2])
f.writeValues([1.3, 2.3, 3.3])
f.writeValues([1, 2.0, "abc"])
f.close()

```

CsvReader

Constructor

- `new CsvReader(filename)`
Opens the file for reading. Takes filename as parameter.

Member methods Following methods (function properties) are defined on a `CsvReader` object:

- `separator` (property)
Column separator (by default “\t” - TAB)
- `endOfLine` (property)
End Of Line (by default “\r\n” - Windows style)
- `close()`
Closes the file.
- `bool = isAtEnd()`
Returns true if there are more data to be read.
- `text = readTextLine()`
Reads the whole line.
- `array = readValues([sep])`
Reads the whole line separated by `sep` or separator to the array.

```
var f = new CsvReader("c:\\data.csv")
while (!f.isAtEnd())
{
r = f.readValues() trace(r.toString())
}
```

Macro functions

`int execProgram(command)`, `int execProgramWait(command)`

Both function take one string parameter with executable command. The second function waits until the

program terminates. See the documentation about macro functions `Int_ExecProgram()` and `Int_ExecProgramWait()`.

`trace (text)`

The function logs the text into debug window and into *NIS-Elements* Log File.

`int messageBox(message [, caption [, buttonFlags]])`

Displays a message box with given message. Optionally caption and button flags may be specified. The values for the `buttonFlags` have the same value as for Windows `MessageBox` function.

`double timeCounter()`

Returns actual time, suitable for measuring execution time (see The code example 1).

Code Example1:

```
var tableObject = new ObjectTable
(["frame.index", "CellCountAnalysis.DAPI.MaxFeret", "CellCountAnalysis.DAPI.MinFeret"], "jobrun.ID = 116", "frame.index ASC");

var tableFrame = new FrameTable
(["frame.index", "CellCountAnalysis.DAPI.Count"], "jobrun.ID = 116", "frame.index ASC");

var columnFeret = tableFrame.addColumn("FeretMean");

var i = 0;
var start = timeCounter();
for (frameRow in tableFrame)
{
tableFrame[frameRow][columnFeret] = 0;
}

for(objRow in tableObject)
{
```

```

if(tableObject[objRow][0] == i)
{
tableFrame[i][columnFeret] += tableObject[objRow][1]/tableObject[objRow][2];
}
else
{
tableFrame[i][columnFeret] /= tableFrame[i][1];
tableFrame[i+1][columnFeret] += tableObject[objRow][1]/tableObject[objRow][2];
i++;
}
}
var end = timeCounter();
tableFrame.commit(columnFeret);
messageBox(end - start);
}

f.close()
execProgram("Notepad.exe c:\\data.csv")
if (messageBox("Do you want to read-back the data?", "Question", 0x24))
{
var i = 0
f = new CsvReader("c:\\data.csv")
while (!f.isAtEnd())
{
r = f.readValues()
trace(r.toString())
if (r.equals(a[i]) == false) // equals must be defined
messageBox("Row does not match")
i++;
}
}
}

```

Code Example 2:

```

var a = [
[1.1, 2.1, 3.1],
[1.2, 2.2, 3.2],
[1.3, 2.3, 3.3],
[1, 2.0, "abc"],
]
var f = new CsvWriter("c:\\data.csv", true)
f.writeValues(a[0])
f.writeValues(a[1])
f.writeValues(a[2])
f.writeValues(a[3])

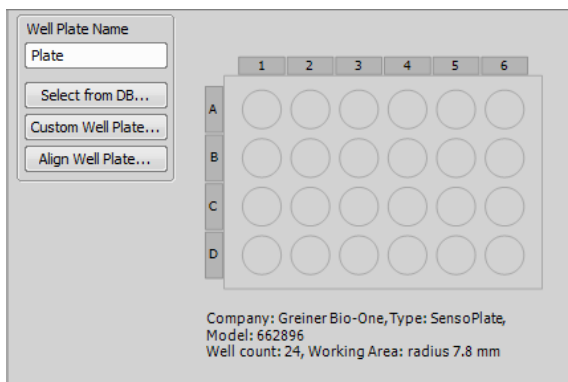
```


13.15 Task reference

A detailed description of each task can be found [here](#).


13.15.1 Well Plates** Define Plate**

This task specifies shape and size of a well plate as well as the working area of single wells.



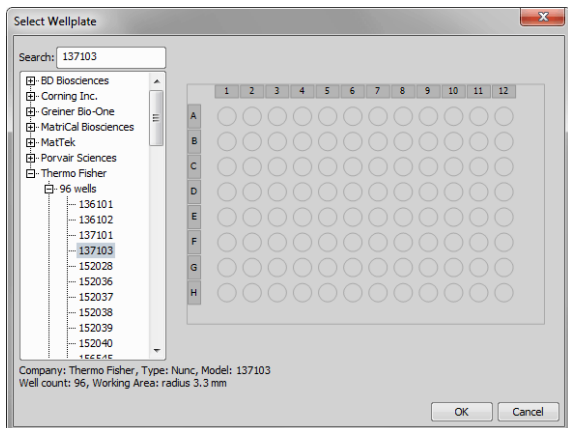
Align Well Plate... Opens the *Align Well Plate* dialog window. Please see *Align Plate*  .

Note

*Well plate alignment can be done inside the job definition using the *Align Plate*  and is job dependent - has to be repeated with each new job. It is also possible to align the plate globally by HCA/JOBS > Well Plate > Align Well Plate. This alignment is saved into the database and applied on new wellplates automatically.*

Well Plate name Type the name of your well plate.

Select from DB... This button opens the well plate database, where you can find many standardised well plates.



Note

Type your well code into the Search field to find your well faster.

Custom Well Plate... This function opens the *Define custom well plate* window where it is possible to adjust all key parameters of nonstandard well plates. Please see *Custom Well Plate Options*.

Custom Well Plate Options

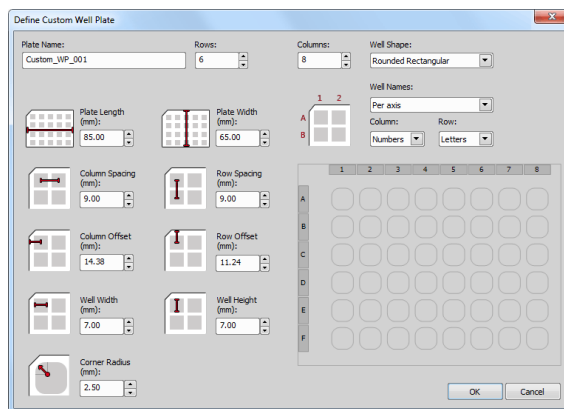


Plate name Type the name of your well plate.

Rows Type how many rows does your well plate have.

Columns Type how many columns does your well plate have.

Well shape Select which shape does your well plate have. Circular, Rectangular and Rounded Rectangular are available.

Well names Here you can choose the single well labeling method. The *Per axis* method enables labeling the wells with letters and numbers, applicable on both axis. The *Per well* method labels the wells from number

one, from the left side to the right side and from top to bottom. Specify your choice in the *Column* and *Row* combo box.

Plate length, Plate width Define size of the wellplate.

Column spacing, Row spacing Define distances between adjacent well rows.

Row offset, Column offset Define distances from the edge of your plate to the center of the A1 well.


Well diameter Define the diameter of a single circular well.

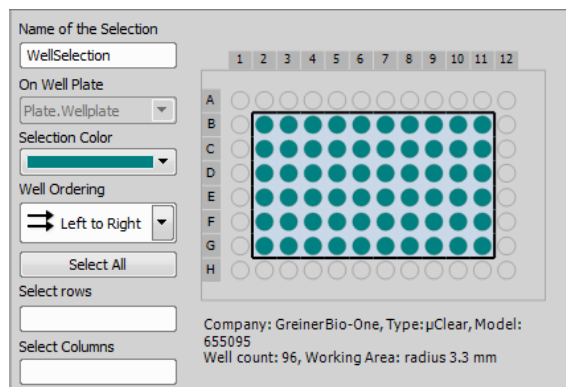
Well height, Well width In case of rectangular well shape, define its height and width.

Corner Radius If you are using a Rounded Rectangular well shape, here you can set the corner radius in [mm].

Column, Row In *Per axis* mode this function assigns either letters or numbers to all columns and rows.

Select Wells

This tool specifies which wells of the selected well plate are actually used in the experiment. Before selecting any wells you need to define the well plate by the *Define Plate*  task. After the plate definition, you can select the wells using the *Select Wells* task. The wells can be easily selected by clicking and dragging over the well board. If you hold down the shift key, you can select remote parts of the well plate. If you hold down the control key and drag over wells already selected, you exclude them from your actual selection. You can also select single rows / columns by clicking on their letter / number. If using mouse for the well selection, the selected values automatically appear in the *Select rows* or *Select columns* fields where you can manually rewrite them using comma (for separating the values) and dash (for entering range values).



Name of the Selection Type the name of your well selection.

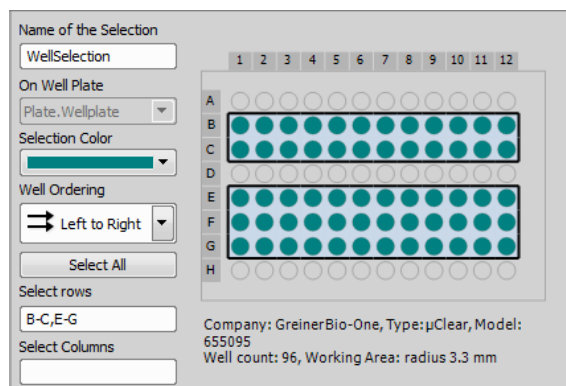
On Well Plate Here you can switch between different well plates created in your actual job.

Selection color Choose any color you wish. This color is then going to be used to visualize the wells selected.

Well Ordering This function defines the order in which the wells are passed (e.g. during a Loop). *Meander* (the most effective way of moving between wells), *Left to Right* and *Top to Bottom* options are available.

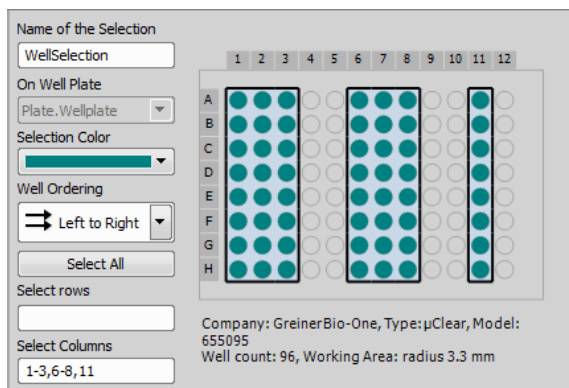
Select all Selects all wells from a chosen well plate.

Select Rows



Enter a single row or a range of rows that you want to select.

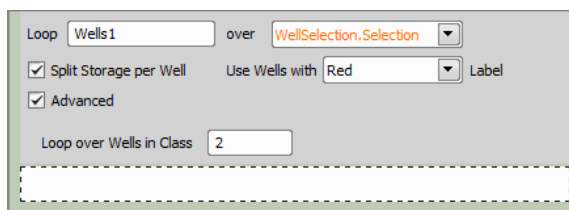
Select Columns



Enter a single column or a range of columns that you want to select.

Loop Over Wells

Runs the contained tasks on each well of the selected well selection.





Loop Type the name of your loop.

over Switch between different well selections available within your job.


Split storage per well This option influences how the system stores the captured data on the disk. If

checked, separate ND2 file will be created for each well.

Use Wells with ... Label If your wells are labeled (by the *Label Wells*  task), you can loop only over wells marked with the selected label.

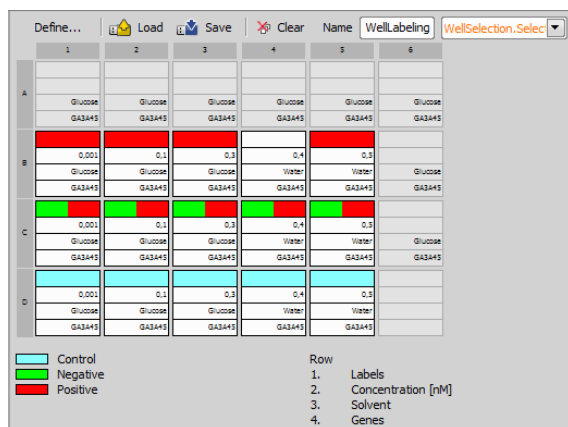
Advanced If you select this option, the *Loop over wells* will be performed only on wells with defined class typed in the *Loop over wells in class* field. The class number can be assigned to each well using an expression. The Class can be defined in the *Expression*  task - find a parameter named "Class" under Job / Name of your Well Loop / CurrentWell / Class. Insert this expression by double-clicking on it and use given operators to define your class (e.g.: Job.WellLoop.CurrentWell.Class=2). In our case "2" is the Class we fill into the *Loop over wells in class* field.


Note

Loop over Wells does not move between the wells on a well plate by itself. It is therefore impossible to expect pictures of single wells by placing just Capture inside the Loop over Wells. To do so, you have to place the Move to Well Center task (Move to Well Center ) inside the loop right before capture. This task tells the loop to move to the appropriate well.

Label Wells

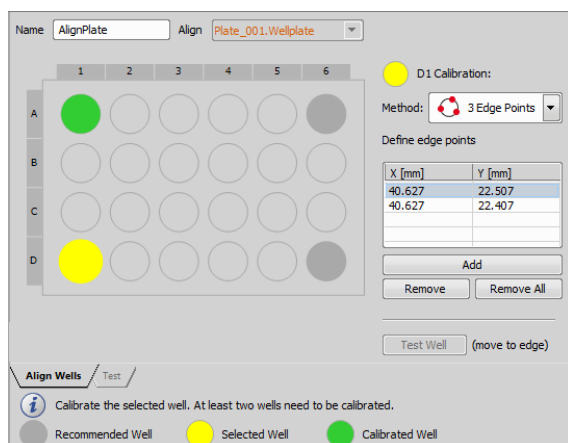
Enables to label, add a description or metadata to each well or a group of wells.



Unfold your *Label Wells*  task inserted into the *Job definition* window by double clicking on its caption and click **Define...** to open the *Labeling/Quantities* window (see: *Labeling/Quantities* window).

Align Plate

This function determines the exact position of a well plate on the XY stage. Typically, this task is executed during runtime.



Correct Wellplate Alignment - Well Calibration

For a precise alignment, at least two wells should be calibrated. Each well is calibrated using one or more points based on its shape and selected calibration method. To align your wellplate correctly follow this procedure:

1. Select a calibrating *Method* from the combo box (defines how many points have to be added for each well).
2. Choose a well in the wellplate preview and click on it (Recommended Wells are coloured in grey).
3. Display live image from the camera. A cross will be displayed in the image.
4. Move the XY stage so that the edge/center/corner (based on the chosen *method*) of the well is in the center of the cross.
5. Click **Add**.
6. Now use the stage to add more edge points on the same well (add as many points as you selected in the *Method* combo box; e.g. for Center Point one is sufficient). If all calibration points of a single well were correctly added, the well is highlighted green.
7. Choose a second well in the wellplate preview and click on it.
8. Add calibration points for this well (repeat procedure from step 3).
9. If done properly, two wells are calibrated (highlighted green) and the well plate is correctly aligned. To increase accuracy of the alignment, calibrate more than two wells.


Note

The more wells you calibrate, the more well plate alignment imperfections are eliminated. Two calibrated wells can rectify the XY position and rotation of the well plate. Three calibrated wells can rectify the XY position and a slight tilt of the well plate. Four calibrated wells can rectify the XY position and a large tilt.

However one well is also enough for rough calibration. If one well is used for calibration it is assumed that the whole well plate is placed orthogonally on the stage with its top-left well oriented to the top-left stage corner.

Align Wells tab

Name Type the name of your align.

Align Choose a well plate defined by the *Define Plate*  which will be aligned.

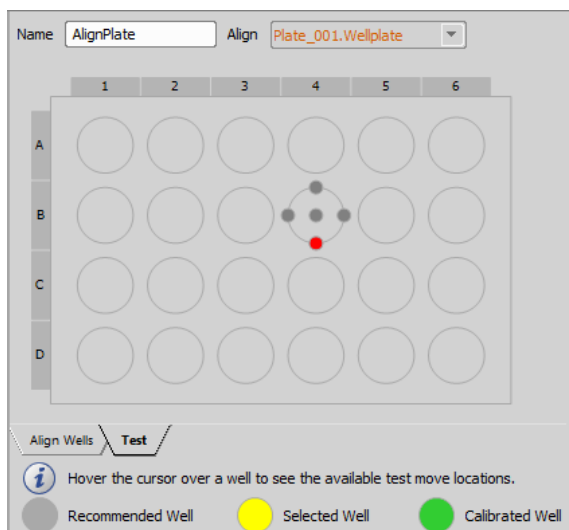
Method Specify the calibration method based on the number and position of the calibration points on the currently calibrated well.

Add Adds point coordinates into the well alignment table.

Remove Removes the selected XY coordinate.

Remove All Removes all points from the alignment table.

Test Well Select a calibrated well and click this button to move to its edge. This way you can check the correct edge calibration.



Test tab

This tab is useful for checking the correct alignment of the whole well plate. Hover the cursor over any well. Available test move locations are displayed. Click on a gray circle to move the stage to this exact position and verify the alignment precision.

Plate Loader

This task communicates with the well plate loader connected to your system.

- 1) Click the **Initialize** button and follow the instructions to initialize your plate loader.
- 2) Scan for the loader hotels by clicking the **Scan Hotels** button.
- 3) Select / deselect the plates and hotels found to be used in your job.
- 4) Add notes to each of your plates if necessary.

Other dialog options are similar to *Manual Plate List*



Wellplate loader name: Reinitialize

Wellplate definition: Scan Hotels

H1 (5) H2 (5)

| Plate # | Name | Barcode |
|-----------------------------------------|---------------|---------|
| <input checked="" type="checkbox"/> #2 | Well Plate 2 | |
| <input checked="" type="checkbox"/> #4 | Well Plate 4 | |
| <input checked="" type="checkbox"/> #6 | Well Plate 6 | |
| <input checked="" type="checkbox"/> #8 | Well Plate 8 | |
| <input checked="" type="checkbox"/> #10 | Well Plate 10 | |
| <input type="checkbox"/> | | |
| <input type="checkbox"/> | | |
| <input type="checkbox"/> | | |
| <input type="checkbox"/> | | |
| <input type="checkbox"/> | | |
| <input type="checkbox"/> | | |

Notes for Well Plate 2

First wellplate in hotel #1.

Manual plate list name:

Wellplate definition:

Use Well Plate Holder

First Plate Is Already on Stage

| Plate # | Name | Barcode | X Offset from Plate #1 | Y Offset from Plate #1 |
|----------------------------------------|-------------|-----------|------------------------|------------------------|
| <input checked="" type="checkbox"/> #1 | Wellplate_1 | 103010302 | 0.00 | 0.00 |
| <input checked="" type="checkbox"/> #2 | Wellplate_2 | 103010303 | 0.10 | 0.20 |
| <input checked="" type="checkbox"/> #3 | Wellplate_3 | 103010304 | 0.20 | 0.40 |
| <input type="checkbox"/> | | | | |
| <input type="checkbox"/> | | | | |
| <input type="checkbox"/> | | | | |
| <input type="checkbox"/> | | | | |
| <input type="checkbox"/> | | | | |
| <input type="checkbox"/> | | | | |

Notes for Wellplate_3 (103010304)

Manual plate list name Name your manual plate list.

Wellplate definition Select a well plate to be looped.

Use Well Plate Holder If using a well plate holder, you can adjust the well plate offset from the first well plate in the table.

First Plate is Already on Stage Check this option if your first plate is already on stage.

+ Add New Plate Adds a new plate to the table.

✗ Remove Removes the selected plate.

✗ Remove All Removes all plates in the table.

Plate # Automatic sequence number of your well plates.

Name Name of your well plate.

Barcode Here you can enter your own barcoding for each well plate.

X Offset from Plate #1 X axis offset from plate 1.



Y Offset from Plate #1 Y axis offset from plate 1.

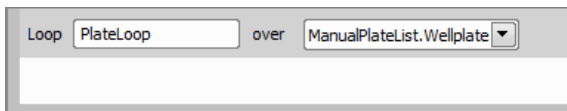
Notes for ... In the blank window below the well plate ordered list you can fill out any other information about each of your currently selected well plates.

Manual Plate List

Specifies the list of well plates to be used in a loop over well plates. These well plates are changed manually.

Loop over Plates

Runs the contained tasks on each well plate of the specified list of plates. The list of plates is defined by the *Manual Plate List*  or *Plate Loader*  tasks.

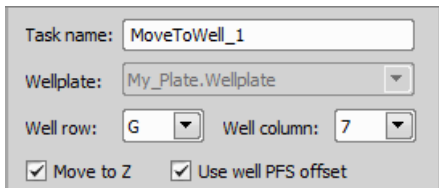


Loop Name your plate loop.

over Choose a plate list from your current job.

Move to Well

Moves the XY stage to a particular well of the specified well plate.





Task name Name your move task.

Wellplate Select a well plate for the move definition.




Well row Define a row position where to be moved.

Well column Define a column position where to be moved.




Move to Z If checked, the Z-drive moves to the Z position assigned to the current well (e.g. Z value found by *Autofocus*  during the first *Loop Over Wells* ).

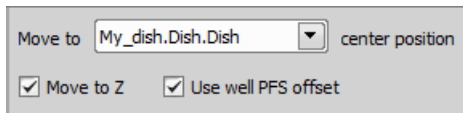
Use well PFS offset These options tell the system to set the Z drive to a position previously assigned to the current well.

Note



*Particular Z drive position can be assigned to each well by the *Assign current Z to Point/Well*  or *Assign PFS to Point/Well*  tasks used within *Loop Over Wells* . The check boxes refer to this Z coordinate.*

Move to Well Center

Moves the XY stage to the center of the current well. This task can be used with a slide (*Define Slide* ), stage area (*Define Stage Area* ) or inside a *Loop Over Wells*  .






Move to Select a sample holder used in your current job.

Move to Z If checked, the Z-drive moves to the Z position assigned to the current well (e.g. Z value found by *Autofocus*  during the first *Loop Over Wells* ).

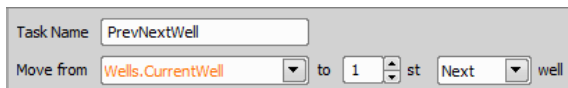
Use well PFS offset These options tell the system to set the Z drive to a position previously assigned to the current well.

Note

*Particular Z drive position can be assigned to each well by the *Assign current Z to Point/Well*  or *Assign PFS to Point/Well*  tasks used within *Loop Over Wells* . The check boxes refer to this Z coordinate.*

Move to Previous/Next Well

Moves the XY stage to the previous/next well. This task can be used with a condition during runtime, e.g. when there is a need to get to the previously treated well.



Task name Name of your task.

Move from Select the well from which you want to move.

to Specify the moving distance to the previous/next well.

Previous/Next well Choose the direction you want to move.

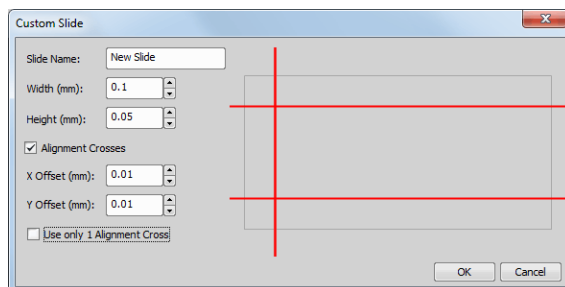
13.15.2 Sample Holder

Define Slide

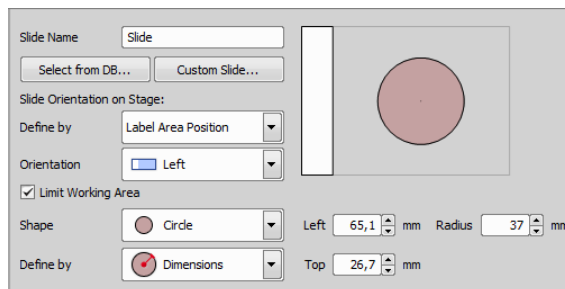
Specifies the dimensions of a slide including its working area.

If you use a standardized slide, click **Select from DB...** and search for your slide in the slide database.


For non-standard slides, click **Custom Slide...** and define its properties (width, height). If you use slides with alignment crosses, it is recommended to check **Alignment Crosses** and set their X and Y Offset for later precise alignment.



Once you have selected/defined your slide, you have to specify its orientation on the stage. The orientation can be defined by **Label Area Position**, **Smear Direction** or **Crosses Position**. Select the real **Orientation** of the slide from the combo box.



At last it is possible to *limit working area* of the slide. This area can be a *Rectangle* or a *Circle* defined by *Dimensions* or using the *Interactive* feature which requires to *Add XY* points lying on the border of the selected shape (3 corner points for the rectangle or 3 edge points for the circle).

After defining the slide it is ready for alignment. Please see: **Align Slide** .

Holder

This task defines the slides contained in a slide holder. Start by clicking **Redefine holder** or go to **HCA/JOBS > Slide Holder > Align Slide**, check **Allow Multiple Slides**

and specify their count. Click **Next** and follow the alignment steps until all slides are aligned. Then click **Select...** on each row of the table to specify the slides being used.

| Redefine holder | | | | | |
|-----------------|-----------|---|----------|---------|-----------|
| | | | Model | Name | Barcode |
| 1 | Select... | ✗ | 76x26 mm | Slide A | 150456213 |
| 2 | Select... | ✗ | 76x26 mm | Slide B | 156516567 |
| 3 | Select... | ✗ | 75x25 mm | Slide C | 154654656 |

Redefine holder Enables to redefine and realign the slide holder.

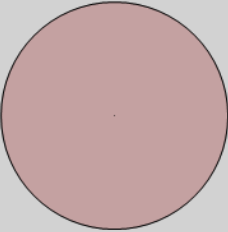
Select Opens the *Select Slide* dialog window enabling to add a custom/system slide.

✗ Deletes the selected slide from the list.

Define Stage Area

In this task you can specify the area of interest on your stage holder.

| | | |
|-----------|---------------|------------|
| Name | StageArea | |
| Shape | Circle | |
| Define by | 3 Edge Points | |
| X [mm] | Y [mm] | |
| 1.720 | 28.000 | |
| 5.720 | 32.000 | |
| 9.720 | 27.000 | |
| Add | | |
| Remove | | Remove All |



Name Type the name of your stage area task.

Shape Select the shape of your stage area.

Define by Define the selected shape. If using a *Circle*, it can be defined by **Setting Center Position** and **Radius** or by **Adding 3 Edge Points**. If using a *Rectangle*,

you have to **Add** two opposite corners or three corner points..

Align Slide

This task determines exact position of the selected slide on the XY stage. Select a slide from the combo box, move your stage to one corner of your slide and click **Add**. Move the stage to the Opposite Corner and click **Add**. Alignment is now finished. Changes to the alignment can be done by removing the XY coordinates clicking *Remove* or *Remove All*.

| | | | |
|----------------------------------------------------|-------------|-----------|---------|
| Name | Align | X [mm] | Y [mm] |
| Slide | Slide.Slide | 49.000 | -30.400 |
| Set stage position for two opposite slide corners. | | 55.000 | -27.400 |
| Set | | | |
| Clear | | Clear All | |

Name Name of the Align Slide task.

Slide Select slide for alignment.

Add Uses the stage coordinates as corner points used for alignment of the slide.

Remove Removes the selected coordinate.



Remove All Removes all coordinates used for slide alignment.

Note

Slides can also be aligned globally using HCA/JOBS > Slide Holder > Align Slide. This alignment is saved into the database and applied on new slides automatically.

Slides with covered corners

If your slide is mounted to the stage in a manner that its corner(s) are not visible in the Live View, it is impossible to align it using the predefined slides from the database. In this scenario there are two options:

- Create a custom slide (using *Define Slide* ) with *Alignment Crosses* and use these crosses to target corners of the coverslip, visible corners of the slide or any other reference points.
- Use *Define Stage Area*  with a rectangular shape and find corners of the coverslip, visible corners of the slide or any other visible reference points.

Manual Slide List





Specifies the list of slides to be used in a loop over slides. These slides have to be changed manually.

Manual Slide List Name:

Slide Definition:

Use Slide Holder


First Slide is Already on Stage

+    

| Sli... | Name | Barcode | X Offset fr... | Y Offset fr... |
|-------------------------------------|------------|---------|----------------|----------------|
| <input checked="" type="checkbox"/> | #1 Slide 1 | | 0.00 | 0.00 |
| <input checked="" type="checkbox"/> | #2 Slide 2 | | 0.00 | 0.00 |
| <input type="checkbox"/> | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

Notes for Slide 2

Manual slide list name Name of your manual slide list.

Slide definition Select the slide definition (*Define Slide* ).

Use slide holder If using a slide holder, you can adjust the slide XY offset from the first slide in the table.

First slide is already on Stage Check this option if your first slide is already on stage.

+ **Add New Slide** Adds a new slide into the table.

✗ **Remove** Removes the selected slides.

✗ **Remove All** Removes all slides from the table.

Slide # Automatic sequence number of your slides.

Name Name of your single slides.

Barcode Here you can enter your own barcoding for each slide.

X Offset from Slide #1 X axis offset from slide 1.

Y Offset from Slide #1 Y axis offset from slide 1.

Notes for ... In the blank window below the slide ordered list you can fill out any other information about each of your currently selected slides.

Slide Loader

(requires: Local Option)

This task communicates with the slide loader connected to your system.

- 1) Click the **Initialize** button and follow the instructions to initialize your slide loader.
- 2) Scan for the loader hotels by clicking the **Scan Hotels** button.
- 3) Select or deselect the found slides and hotels to be used in your job.
- 4) Add notes to each of your slides if necessary.

Slide loader name: Initialize

Slide definition: Scan Hotels

H1 (1) H2 (1) H3 (3) H4 (18)

| Slide # | Name | Barcode |
|-----------------------------------------|------|---------|
| <input checked="" type="checkbox"/> #2 | | |
| <input checked="" type="checkbox"/> #8 | | |
| <input checked="" type="checkbox"/> #10 | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

Notes for #2

Loop over Slides

Runs the contained tasks on each slide of the specified slide list. The list of slides is defined by the *Manual Slide List* or *Slide Loader* tasks.

Loop over

Loop Name your slide loop.

over Choose a slide list from your current job.

Loop over Holder

Runs the contained tasks on each slide in the specified *Holder* .

Name

Loop over

Name Name of the *Loop over Holder* task.

Loop over Select the *Holder* over which the loop will be performed.

13.15.3 Time Series

Time Lapse

Repeats the contained tasks according to the specified parameters (total duration / number of loops, interval...). This task typically contains just the *Capture* task, but can contain any meaningful task as well.

Loop

Total Duration

Acquisition Period

Split Storage per Time point

Run Tasks while Waiting

Finish when Condition Is TRUE

Run while waiting

Loop Type the name of your loop.

Total Duration Select one of the following time lapse modes:

Time Set a precise amount of time to be used as the *Timelapse* duration [msec / sec / min / hour(s)].

Number of Loops Define the number of loops (how many times will the containing tasks executed).

Unknown (User or Program Break) Use this function if you don't know when to stop the time lapse. With this setting it can be stopped manually or automatically after a program break.

Acquisition Period Select *No-delay* to let the task run as fast as possible. Two other options specify a time interval between two adjacent repetitions of the task. *Run Loop Every* option specifies how fast the tasks are started. *Wait Between Loops* defines a time pause between the end of one loop and the beginning of the next one.


Split Storage per Time point If checked, each time loop is stored in a separate nd2 file.

Run Tasks while Waiting Other tasks can be performed repeatedly if there is a spare time between two adjacent loops. When you check this option, additional space named *Run while waiting* appears at the bottom of this task. Place the tasks which you want to run while waiting for the next loop into this area.

Finish when condition is TRUE Especially if a time lapse with *Unknown total duration* is defined, you can specify a conditional expression which will cause the loop to be discontinued. Click *Define* to specify the conditional expression (Please see 13.13.3 Using Expressions).

Time Lapse


Modifies an existing *Time Lapse*. This task is usually used within a *Condition* - if something happens, re-

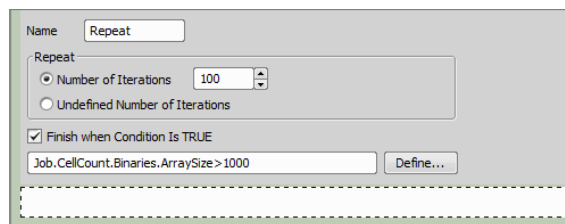
define *Time Lapse* (e.g. increase frame rate). See *Time Lapse*  for description of the task options.

Time Sequence

Chains acquisition time-phases and phases of different types (temperature / gas control, perfusion control). Please see the 5 Time-lapse Acquisition chapter for description of the task options.

Repeat N times


Repeats the contained tasks for the specified number of iterations or indefinitely until *Break*  .



Name Type the name of your repeat task.

Repeat Here you can choose from the following options:

Number of iterations Insert a number to define how many times the tasks will be repeated.

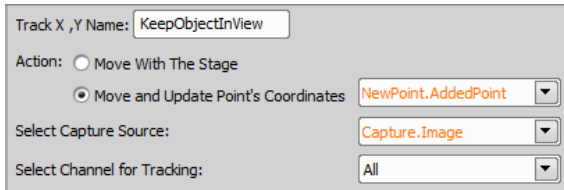
Undefined number of iterations Use this function if the loop will be exited either by the *Break*  task or by the *Finish when condition is TRUE* option.

Finish when condition is TRUE You can specify a conditional expression which will cause the loop to be exited. Click *Define* to specify the conditional expression. See also 13.13.3 Using Expressions.

Keep Object In View

(requires: Local Option)

This function finds the object of interest based on the correlation between the previous and the next frame and always keeps it in the center of view using the microscope stage to compensate the object's motion.





Track X ,Y Name: KeepObjectInView

Action: Move With The Stage
 Move and Update Point's Coordinates NewPoint.AddedPoint

Select Capture Source: Capture.Image

Select Channel for Tracking: All

Action: Move With The Stage Moves the stage to keep the object in view.

Action: Move and Update Point's Coordinates Moves the stage to the current center of the object and updates coordinates of your points defined in different tasks (e.g. *Loop over Points*  defined by *Generate Points* ).

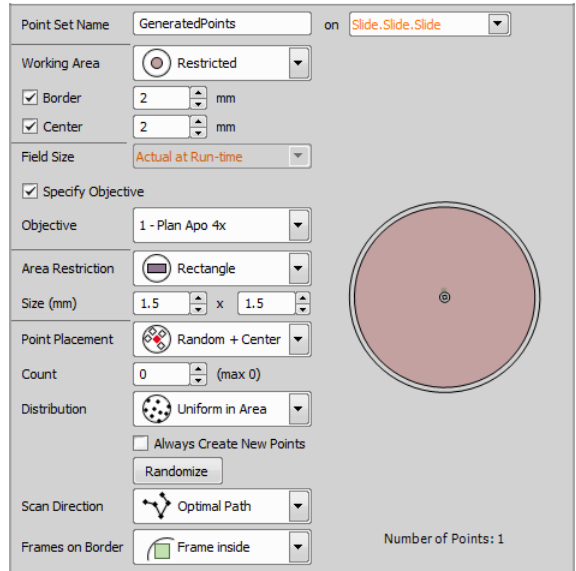
Select Capture Source: Select the source from which the image will be taken.

Select Channel for Tracking: Select the channel which will be used for following the object.

13.15.4 Stage XY Points

Generate Points

Creates a pattern of XY points on the selected sample.



Point Set Name: GeneratedPoints on Slide.Slide.Slide

Working Area: Restricted

Border: 2 mm
 Center: 2 mm

Field Size: Actual at Run-time

Specify Objective
Objective: 1 - Plan Apo 4x

Area Restriction: Rectangle

Size (mm): 1.5 x 1.5

Point Placement: Random + Center

Count: 0 (max 0)

Distribution: Uniform in Area
 Always Create New Points
Randomize

Scan Direction: Optimal Path

Frames on Border: Frame inside

Number of Points: 1

Main settings of this task are described below, however some settings enable advanced adjustments (e.g. size, distance, count,...). These functions are self-explanatory and are clearly depicted in the preview area.

Point Set Name Type the name of your point set.

On Select on which sample holder to generate the points.

Working area Define the working area of the sample holder.

Field Size *Actual at Run-Time* uses the field of the currently used objective. Points are generated using the objective set right before the job run. *Custom* can be used to select a specific objective from the combo box which is then always used for the point generation.

Camera If multiple cameras are connected, select the one to be used with this task.

Objective Objective to be used for generating points.

Area Restriction Specify the restriction inside the working area.

Point Placement Choose one of the point placement methods listed. *Manual* method enables to add points by clicking into the preview area. Created points can be moved using the primary mouse button. Right-clicking deletes the point below the cursor.

Scan Direction If Point Placement is set to *Random*, *Random+Center* or *Manual*, *Random* scan direction scans in the randomized order whereas *Optimal Path* scans points in the most effective order.

If *Covering* or *Regular Pattern* point placement is selected, *Meander* scans in a winding manner and *Left to Right* scans row by row from left to right.

If Point Placement is set to *Spiral*, options in the Scan Direction combo box specify the direction of rotation (*ClockWise/CounterClockWise*) and heading from/to the center (*Outwards/Inwards*).

Frames on Border Specify whether the frames on the border of the restriction area are touching the border, completely inside or having a center inside.

Overlap In *Covering* mode you can select the frame *Overlap* amount in percent.

Count Set the number of points you want to generate. The maximum number is indicated in the bracket.


Distribution *Uniform* places points evenly and *Prefer Center* places most of the points into the center of the restriction area.



Always Create New Points There is always a new point set generated whenever the task is performed.


Randomize Regenerates your random points.


Predefined Points

Using this task, you can specify a list of XY points on the selected sample manually in a tabular way. Please see 6 Multi-point Acquisition for details about points predefining. There are some additional options available within the *JOBS* module:



Include Z-Stack Displays the Z-Stack definition column with the  Z-stack definition button enabling to define a Z-Stack for the currently selected point.

Include Camera Settings Displays the Camera column with the  Redefine Camera Setting button enabling to adjust the camera settings (please see: *Adjust Camera Settings Manually in OC* ).

Include Stimulation ROIs Definition Displays the *ROIs* column with the  button. Click this button and draw stimulation ROI(s) for the selected multi-point.

Include Labels Labels defined in *Label Wells*  task can be assigned to each multi-point. Select a label from the pull-down menu in the *Label* column.

New Point Set

Creates an empty point set. Points shall be added to it later either by *Add Point to Point Set*  or *Add/Edit Points Manually* .

Point Set

Point Set Name your point set.

Loop over Points

Runs the contained tasks on each XY point of the selected point set.




Loop over

Split Storage per Point Move to XY Move to Z

Advanced Use Point PFS Offset Use Points with Label



Loop over Points in Class

Loop Name your point loop.

over Select one of your active point tasks to be looped (*Generate Points*  , *Predefined Points*  , *New Point Set* ).


Split storage per point This option influences how the system stores the captured data on the disk. If checked, separate ND2 file will be created for each point.


Move to XY If checked, the stage is moved to the XY points.

Move to Z If checked, the Z-drive moves to the Z position assigned to the current well (e.g. Z value found by *Autofocus*  during the first *Loop Over Wells* ).


Use well PFS offset These options tells the system to set Z drive to a position previously assigned to the current point.

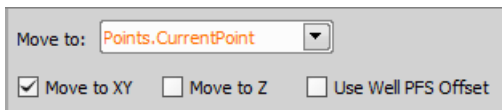
Advanced Each point can be assigned to a class (by an expression). If you select this option, the *Loop over wells in class* field appears. Type the class number to which the current loop will be applied.

Use Points with Label If you select a label defined by the *Create Labels*  task, the tasks inside *Loop over Points* will be executed only on points marked with this label.

 **Move to Point**

(requires: *Local Option*)

This task can be used inside *Loop over Points*  to move to a XY/Z position of a point.




Move to Defines in which point loop the movement will be performed.

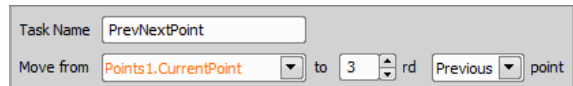
Move to XY Moves to the XY coordinates of the point.

Move to Z Moves to the Z coordinate of the point.

Use Well PFS Offset PFS Offset of the well is incorporated into the movement.

 **Move to Previous/Next Point**

Moves the XY stage to the previous/next well.




Task name Name of your task.

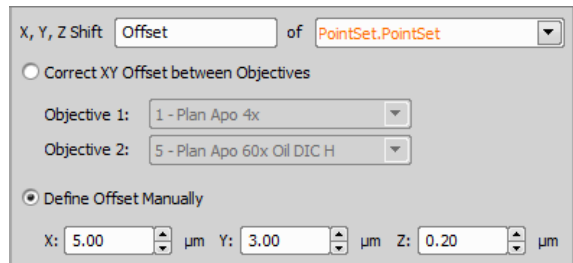
Move from Select the point from which you want to move.

to Specify the moving distance to the previous/next point.




Previous/Next point Choose the direction you want to move.

 **Offset Point Set**

This task can correct the XY offset between objectives and can be used to offset all XYZ coordinates of the current stage position or of the selected point set.



X, Y, Z shift Name of the task.

of Is used to select a point set (*Generate Points* , *Predefined Points* , *New Point Set* ) or the *Actual Stage Position* to be shifted.

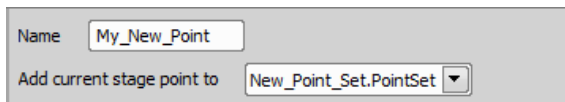
X, Y, Z Specify distances of the shift in all three axes.

Correct XY Offset between Macro and Scanning Objective If checked, XY correction between objectives is performed using the two objectives specified below. Please see *Devices > Objectives XY Offset Setup* for more information.

Define Offset Manually Specify distances of the manual shift in all three axes (X, Y, Z).

Add Point to Point Set

Adds a named XY point to the selected point set. Current XY position is used.



Name Name your new point.

Add current stage point to Select a point set to which you want to add a new stage point.

Remove Point from Point Set

Removes the current XY point from the selected point set which is used in a point loop. For example you can use this task in an XY point loop to remove points which are not useful for further analysis (e.g. do not produce any image signal).



Remove Point Select an active point loop from which your point will be removed.

Add/Edit Points Manually

Waits for the user to add or edit points to the selected point set manually during the runtime.



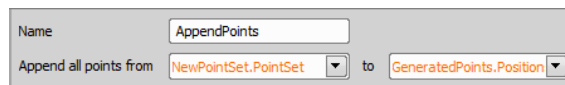
Name Enter the name of your manually defined points.

Embed to progress window Check this function if you want to embed the *Edit Points* window into the bottom of the *Job Execution Progress* window.

Point set Select a point set which you want to edit manually.

Append Point Set to Point Set

Appends points from a selected point list into another selected point list.



Name Name of your task.

Append all points from All points from this list will be appended.

to Defines to which point list will the points selected from the first combo box be appended.

Clear Point Set

Removes all points from the selected point list.

Name Name of your task.

Remove all points from Select the plate list which will be cleared.

Export Point Set to ND

Exports the selected point set definition to the *ND Acquisition* window.

Point set to export Select the point set you want to export to the XY tab of the *ND Acquisition* window.

Append points Adds new generated points to the the XY tab of the *ND Acquisition* window right behind already existing points.

Replace points Replaces all existing points in the XY tab of the *ND Acquisition* window by newly generated points.

Import Point Set from ND

Imports the multi point definition from the *ND Acquisition* window to the selected point set. The existing point set settings will be overwritten.

Import to point set Select a point set to which data from the *ND Acquisition* window will be imported.

Append points Adds newly generated points to your selected point set behind already existing points.


Replace points Replaces all existing points in your point set by newly generated points.

13.15.5 Large Images

Scan Large Image

Task enabling to scan large images using precise parameters.

Name Name of your task.

Scan using Select a *Capture Definition*  and specify its objective from the combo box (*with ... objective*).

Save Image Choose whether or not to save the acquired images.


Fields Define the field dimensions of the large image.


Placement *Around the Current Position* places the corners of the fields directly next to the current stage position. *Current Position is at Top-Left Corner* uses the stage position as a position of the top-left field.

Use Captured Shading Correction Shading correction saved with the Optical Configuration can be used. Select your Optical configuration, select *Acquire > Shading Correction > Shading per Optical Configuration* and then go to *Acquire > Shading Correction > Capture Correction Image* to capture the shading correction. Now you can check the *Use Captured Shading Correction* check box.

Use Automatic Shading Correction Automatic shading correction can be used checking this function. Choose the type of correction which best represents your sample - Brightfield, DIC like or Fluorescence with *offset* enabling to heighten brightness level of the corrected image to make objects in dark areas more visible. *Remove dust* compares all frames, searches for a repeating pattern (e.g. dust, objective scratches) and removes it.

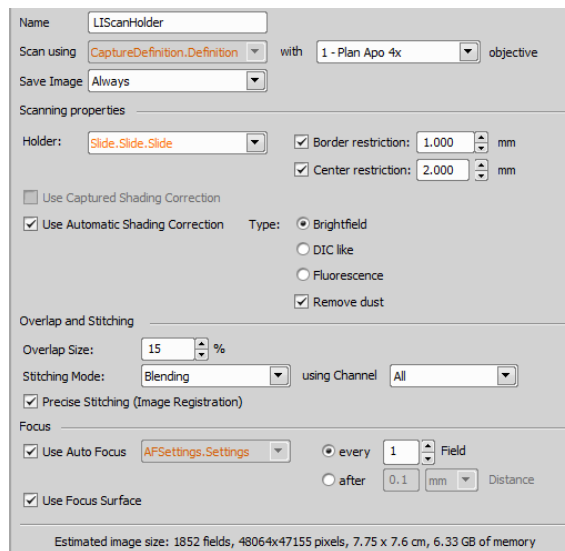
Overlap and Stitching This area contains parameters defining the *overlap size*, stitching mode (*blending* or *optimal path*) and a channel which is used for the stitching. *Precise stitching (Image Registration)* can be checked but be aware that it is more computationally demanding.

Focus Autofocus can be used checking this function. Select a *Autofocus Settings*  and choose where in the large image it will be performed (*every Nth field* or *after a specified stage distance*). Autofocusing on *Frames without sample* can be skipped.

Use Focus Surface Focuses using the surface defined in the *Focus Surface* tab of the *View > Acquisition Controls > XYZ Overview*  panel.

Scan Large Image in Slide/Well/Area


This task scans large images on a sample holder (slide, stage area or well).





Holder Specifies your sample holder.

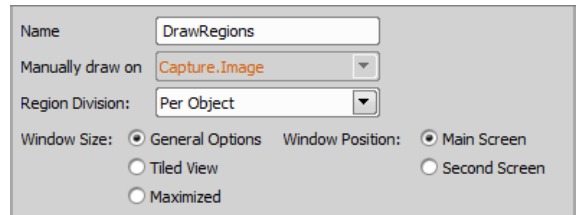
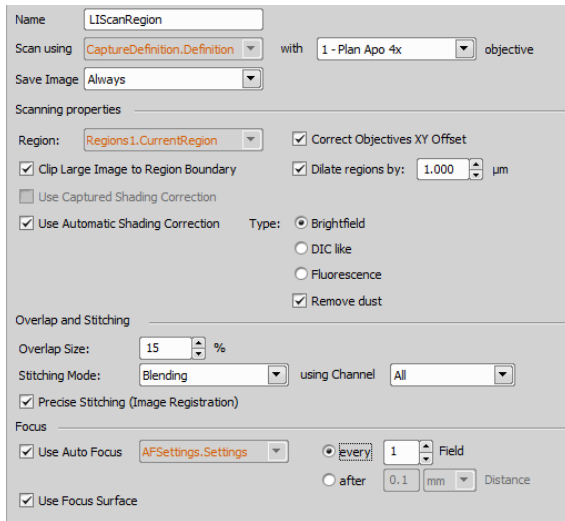
Border restriction Enter an optional border restriction on your sample holder.

Center restriction Enter an optional center restriction on your sample holder.

Please see *Scan Large Image*  describing other parameters which are similar to this task.

Scan Large Image in Region

This task is used to scan large images on a predefined set of regions. Regions can be generated automatically using *General Analysis*  or drawn manually on a captured image or a large image. Place this task inside *Loop over Regions* .



Region Loop over *Regions* in which this task is placed.

Correct Objectives XY Offset This feature corrects the XY offset previously set in *Devices > Objectives XY Offset Setup*.

Please see *Scan Large Image* describing other parameters which are similar to this task.

Draw Regions

This task is used to manually draw regions during acquisition. Once the capture is made, the job is paused and Simple ROI Editor is shown over the *Draw Regions* window. Please see *ROI > Simple ROI Editor* for more details about drawing regions. Once you finish the drawing, click **Finish** to continue the job.

Name Name of your *Draw Regions* task.

Manually draw on Select a capture on which the regions will be drawn.

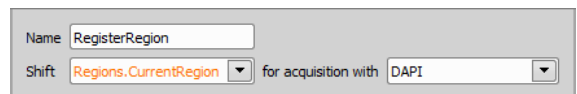
Region Division Regions can be treated *Per Frame* - all drawn regions are considered as one area in the particular frame, or *Per Object* - each region is treated as a single object.

Window size Depending on your preference, choose one of the three window size options: *General options* (exerts the last used window size), *Tiled view* (tiles the live window alongside with other windows) or *Maximized* (Maximizes the live window).

Window position If using multiple monitors, this function can put your *Draw Regions* window onto your *Second screen*, keep it on the *Main screen* or embed it directly into the *Progress dialog*.

Shift Region

When *Multimodal Image Registration (Image > Channel Alignment and Registration > Multimodal Image Registration)* is used (e.g. for comparing images from SIM and Confocal), shift of the regions can be made using this task.

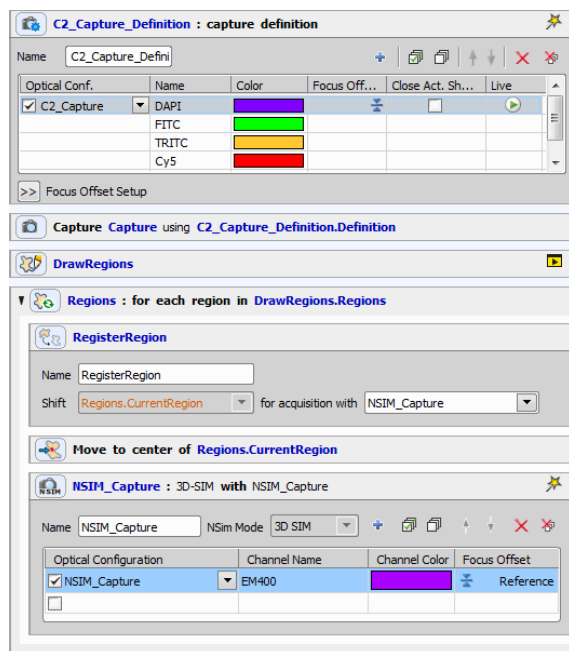


Name Name of your *Shift Region* task.

Shift Select on which regions the shift will be performed.

for acquisition with Select an Optical Configuration from which the shift values are taken.

Figure 12.427. Typical example using *Shift Region* to compensate the shift between C2 and NSIM capture:

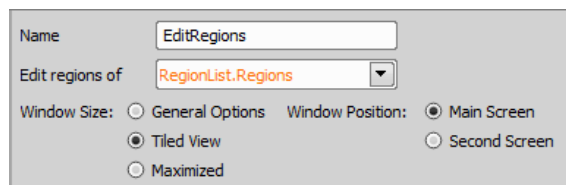


The job defines one *Capture Definition* for C2 which is used to *Capture* an image on which regions are defined using *Draw Regions*. Then the job loops over these regions automatically compensating the shift (*Shift Region*) between two registered systems (stored in *Image > Channel Alignment and Registration > Optical Path Corrections*). *Move to Region Center* moves the stage to the center of each region and captures an image using *NSIM Capture*.

Edit Regions

(requires: *Local Option*)

Enables to edit regions during the job. The job is paused and *Simple ROI Editor* is shown. Please see *ROI > Simple ROI Editor* for more details about drawing and editing regions. Once you finish the edits, click **Finish** to continue the job.



Name Name of your *Edit Regions* task.

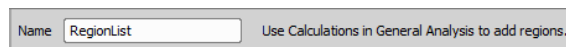
Edit regions of Select which regions are to be edited (e.g. from *General Analysis* or from *Draw Regions*).

Window size Depending on your preference, choose one of the three window size options: *General options* (exerts the last used window size), *Tiled view* (tiles the live window alongside with other windows) or *Maximized* (Maximizes the live window).

Window position If using multiple monitors, this function can put your *Draw Regions* window onto your *Second screen*, keep it on the *Main screen* or embed it directly into the *Progress dialog*.

Region List

This task is used as a blank list which can be filled with regions inside *General Analysis Calculations* tab (please see 2.5 *General Analysis*).



Name Name of your task.

Loop over Regions


This task can be used to execute contained tasks over a set of regions in a loop.




Loop Name of your task.

over Selected *Region List*  task containing regions for the loop.

Move to Region Center

Moves to the center of the region. This task is placed inside *Loop over Regions*  .

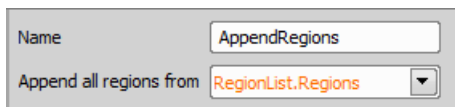



Move to Center Position of Defines in which *Loop over Regions*  the movement to the center of a region will be performed.

Append Region List to Region List

(requires: Local Option)

Adds selected regions to the selected region list.



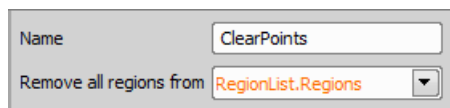
Name Name of your *Append Region List to Region List*  task.

Append all regions from Defines which regions are used for appending the region list.

Clear Region List

(requires: Local Option)

Deletes regions from the selected region list.



Name Name of your *Clear Region List*  task.

Remove all regions from Defines which region list is to be cleared.

13.15.6 Z-Stack

Define Z-Stack

This task defines parameters required for capturing a *Z-Stack* (a sequence of images each having a different focal plane). *Z-Stack* can then be used to create all-in-focus images or 3D models regardless of your sample and depth of field parameters. Please see the 7 *Z-series Acquisition* chapter for details about the *Z-stack* setup.

Figure 12.434. Defined by top bottom mode

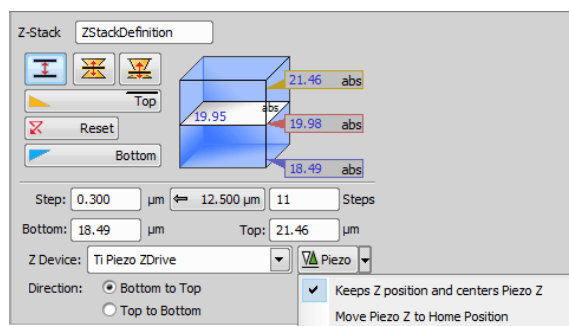


Figure 12.435. Symmetric mode defined by range

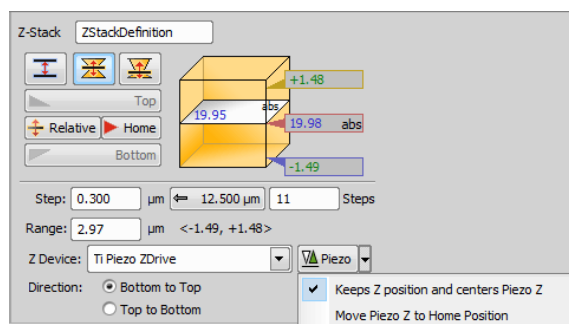
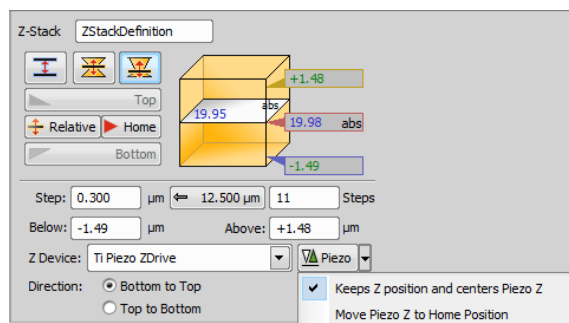




Figure 12.436. Asymmetric mode defined by range



Z-Stack Name of your *Define Z-Stack* task.

Defined by top bottom This mode defines the Z-stack using two parallel planes ( *Top* and  *Bottom*).


Required values: top position, bottom position, step size or number of steps.


Symmetric mode defined by range This mode defines the Z-stack by its center (*Home*) position and the same range above and below the *Home* plane.


Required value: range.


Asymmetric mode defined by range This mode defines the Z-stack by a center (*Home*) position and a custom range above and below the *Home* plane.


Required values: above position, below position.

 **Top** Sets the top Z position.


 **Reset** Discards the Top, Home, and Bottom position settings.

 **Relative** Changes the *Home* (absolute Z) position to *Relative* (current Z position deducted prior to the actual acquisition).

 **Home** In the *Symmetric / Asymmetric mode defined by range* this button sets the position in the middle of your range.

 **Bottom** Sets the bottom Z position.

Step Define the range of a single step.


 **Use suggested step size** Click this button to use the recommended step size.

Steps Insert the number of steps you want to acquire.

Bottom Set the bottom Z position manually.

Top Set the top Z position manually.

Z Device Choose a Z device used for Z-stack acquisition.

 **Piezo** Specifies the role of the Piezo Z drive.


Range Enter the range of the final Z-stack (in-focus portion of your sample).

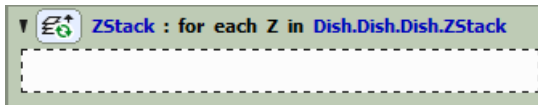
Below Set the bottom position of the Z-Stack manually.

Above Set the top position of the Z-Stack manually.



Direction Enables to set the Z axis direction in which the stage will move during acquisition.

Z-Stack Loop

Repeats the contained tasks for each Z position of the selected Z Stack. This task can be used inside any other loop (time-lapse, point set) with *Capture*  . It captures Z stacks to extend spatial resolution of the image sequence.



Assign Z-Stack to Point/Well/ND Acquisition

Every XY position (well, row of wells, column of wells) can have unique Z stack parameters assigned by combining *Define Z-Stack*  and *Assign Z-Stack to Point/Well/ND Acquisition*  tasks.



Assign Z-Stack definition Select your Z-Stack definition for the assignment.

Offset Add an optional Z offset on top of the Z-Stack definition.

to Select to which the Z-Stack definition will be assigned. If a second combo box appears (e.g. when using well plates) - specify your selection.

Move to Z-Stack home


Moves the Z drive to the home position of the selected Z Stack Definition. When capturing multi-channel Z-stacks, not all the channels are required to contain the Z dimension. These are usually captured in the *home* (center) position.



Move to Home of Choose your Z-stack definition from your current job.

13.15.7 Autofocus + Focus Surface

Autofocus Settings

Defines Z device settings to be used by the *Autofocus*  task. Different AF settings may be defined for different purposes. Settings of this task have many in common with the *Devices > Auto Focus Setup* command.

Select a method you will use:

Step by Step This method moves the Z drive in steps within the defined range, captures images and evaluates their focus criterion. The Z position with the best focus criterion is used as focused.

Continuous (requires Nikon Ni or Ti2-E microscope)

This method is similar to *Step by Step* except that in this case, the step size depends on the camera frame rate. The camera takes pictures as fast as possible while the Z drive is moving within the defined range.

Name Name your autofocus setting.

Z Device Choose the Z drive to be used for focusing. If you choose *<fastest>*, the Piezo Z will be used in as many cases as possible. If its range is not big enough, then the main Z device movement will be added to reach the required absolute Z.

Criterion Defines which focus criterion is used:

- Brightfield - standard contrast based criterion - for brightfield images
- Fluorescence - suitable for fluorescence microscopy

- Max sum intensity - criterion based only on the intensity values. This is useful in confocal microscopy.
- Yeast, Bacteria (Ph) - a criterion optimized for yeasts under phase contrast (Ph) microscope.

Use OC Select the optical configuration to be used for focusing.

Offset Z after AF This option may be useful in fluorescence applications for the cases where the auto focus algorithm produces offset (the system states it is focused but you would like to focus on some other objects within the image, which are blurry). After performing auto focus, the Z drive will move by the distance [μm] defined within this field.

Exclude Border This function enables to exclude the border of the image and use only the central part for focus. Width of the excluded border can be set in the edit box.

Move to Original Z on Failure If autofocus fails, the Z drive moves to the original Z position.

Close active shutter during stage movement The active shutter will close in times between acquisitions phases. Typically, this option is used to reduce the photo-bleaching effect on live cells.


Skip Focusing on Background (requires Background/Specimen detection to be set up) Speeds up the focusing by omitting the background area. Please see: *HCA/JOBS > Background Detection > Teach Background/Specimen Detection*.

AF mode

Auto Automatic focus mode, only the range shall be defined. The system calculates optimal step and whether 1 pass or 2 passes will be performed.

Manual Manual focusing mode enabling further settings. The focusing range and step in the first and second pass can be defined manually.

Range Define the total distance which the Z drive will use for auto focus.

Single pass  performs auto focus in one step. This is a standard auto focus method which can be used.

Two passes This method is suitable when you are far out of focus. It is performed in two steps: Coarse and Fine.


Note

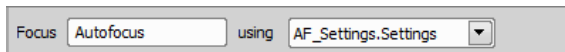
If the Continuous in range two-pass auto focus fails the reason may be:

- Camera exposure time is not compatible (too short or too long) with the speed of your Z drive.
- The automatically calculated coarse step is too large and the specimen is thin so it does not come into focus at all.


Try reducing the auto focus speed to Slow, adjust camera the exposure time, or use the Single pass mode.

Autofocus

Performs autofocus using the selected *Auto Focus* settings defined in *Autofocus Settings*  .




Focus Name your autofocus.

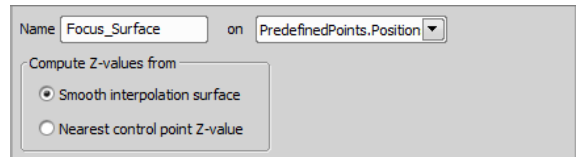
Using Select autofocus setting determined by the *Autofocus Settings*  task.

Note

If Autofocus task fails to focus, captured images will be labeled with Focus Failed flag and will be marked by a red circle in the Labeling view. Focus Fail flag will not be assigned if Z coordinate is modified by 10um or more after unsuccessful autofocus.

Create Focus Surface

Calculates global focus surface from an existing set of Z positions. The Z positions must be defined beforehand e.g. by the *Assign current Z to Point/Well*  task.



Name Name your focus surface.



On Select the source Z point set from which the surface will be calculated.

Compute Z-values from

Smooth interpolation surface Any position on the resulting surface is a result of interpolation.

Nearest control point Z-value This is not really a surface. In any XY position, Z value of the nearest existing XY point is used.

Move to Focus Surface

Moves the Z drive to the position defined by the *Create Focus Surface*  task (or *Offset Focus Surface* ). Use it on each XY position within a point loop to get the image into focus (or pre-focus).

Offset Focus Surface

Modifies an existing Focus Surface by adding / subtracting an offset distance. You can use the focus surface offset on each point for pre-focus and check every N-th point whether the focus has not shifted. If it has, shift (offset) the whole focus surface to match the current focus.

Offset by

Offset by Offset your focus surface by a defined numeric value.

Assign current Z to Point/Well

Assigns the current Z position to the selected XY position of a sample. You can use this task inside a loop after *Autofocus* to pre-define Z positions for fast scanning.

Redefine Z on

Redefine Z on Select a sample holder on which you want to set the current Z position.

Escape Z

This task escapes the Z drive to the lowest position to avoid objective collision.

Refocus Z

This task uses the Z drive to perform refocus of the image.

13.15.8 PFS

PFS On and Focus

Turns the *Perfect Focus System* (PFS) on and tries to find the PFS focal plane. Make sure the Z drive focal plane is not too far from the PFS focal plane otherwise the task may fail.

Name: PFSON

On fail, do the following:

- Auto focus using and retry **PFS ON**
- Move Z and retry **PFS ON**

Move Up by: [µm] Dmax = WD / 2
 Move Down by: [µm] Dmax ... maximum distance allowed
 WD ... working distance of the objective

Current Dmax: 2000 µm Current WD: 4000 µm (Plan Apo 10x DIC)

If you check *On fail, do the following*, more actions can be done when PFS fails. Auto focusing can be done using a selected *Autofocus Settings* task or Z stage can be moved Up/Down by a specified value.

PFS Off

This task switches the PFS Off.

PFS Offset from the current Z position

Finds a PFS offset for the current Z position and sets it as the current offset. It ensure that exactly the same Z level will be kept when PFS is switched on.

Auto PFS Offset Settings

This task defines settings to be used by the *Find Auto PFS Offset* task. Select the optical configuration to be used. Similarly to auto-focus, range can be defined in the automatic mode, range and step for single-pass or two-passes can be defined for the *Manual* mode.

Criterion: AF mode:


Use OC: Range: µm
 PFS units

Exclude Border %

Step: µm
 PFS units


Select the focus criterion based on the nature of your specimen.

Find Auto PFS Offset

The PFS system searches for the most in-focus plane and sets the PFS offset accordingly. This task uses settings defined by the *Auto PFS Offset Settings*  task.

See 13.13.6 Creating PFS Surface for a sample use case.

Assign PFS to Point/Well

Uses the current Z position to specify PFS offset for the selected XY position (e.g. a well). You can use this task inside a loop over XY points after *Find Auto PFS Offset* .

Redefine Z on

Redefine PFS on Select one of your sample holder positions.

Create PFS Surface

Calculates the global PFS surface from an existing set of PFS positions. To XY positions without the PFS position defined, the PFS position of the closest XY point (which includes PFS position) is copied.

See also 13.13.6 Creating PFS Surface.

Name on

Compute PFS Values from

Smooth Interpolation Surface

Nearest Control Point PFS Value

Compute PFS values from

Smooth Interpolation Surface Any position on the resulting surface is a result of interpolation.

Nearest Control Point PFS Value Value of the nearest control point position is taken.

Move to PFS Surface

Changes the current PFS offset value to match the calculated PFS Surface.

Offset PFS Surface

Modifies an existing PFS Surface by adding / subtracting an offset distance.

Offset by

13.15.9 Acquisition

Capture Definition

This task defines the single-channel or multi-channel acquisition settings.

Name

| Optical Conf. | Name | Color | Focus Offset | Close Act. | Shutter | Live |
|-------------------------------------|------|--------------------------------------|--------------|-------------------------------------|---------|----------------------------------|
| <input checked="" type="checkbox"/> | DAPI | ■ | | <input checked="" type="checkbox"/> | | <input type="button" value="▶"/> |
| <input checked="" type="checkbox"/> | FITC | ■ | 6,7 | <input checked="" type="checkbox"/> | | <input type="button" value="▶"/> |
| <input type="checkbox"/> | | | | | | |

<< Focus Offset Setup (for FITC)

Set OC

Live 0.1 1 1 μm Set current Z as

AF

Set 6.7 μm offset from

Select an optical configuration to be used to capture each channel. Please see 8 Multi-channel Acquisition.

Specific options


Focus Offset Sometimes when using multiple channels for acquisition, not all channels have the same Z positions with the best focus. Using one channel as reference, user can specify focus offsets for individual channels. Therefore all channels are captured in focus automatically.

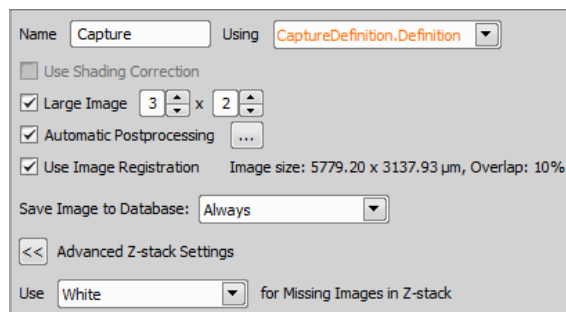
Close Act. Shutter In addition user can control active shutter individually for each channel. Check *Close Act. Shutter* check box to minimize bleaching or uncheck it to leave the shutter opened and thus speed up the acquisition.

Live Opens the live image dialog window.


>> Focus Offset Setup This button reveals the Focus Offset settings. If two or more Optical configurations are used (e.g. when OC's use different objectives) an offset between them can be defined. Click on the first OC, click **Live** to see the live view of the selected OC, focus (you can use **AF** for automatic focus) and set it as **Reference**. Click on the second OC, click **Set OC** to activate it (switch to its live view), use the up/down arrows to focus and click **Offset** to assign the offset distance from the first OC.

Capture

Performs acquisition according to the selected *Capture Definition* . Large images can be acquired and an analysis can be run upon capture.



Name Name of your capture task.

Using Select a *Capture Definition*  task set previously.

Use Shading Correction Check this option to turn the shading correction on.

Automatic postprocessing Click **...** to specify the *Shading Correction Options*. Please see the *Acquire > Shading Correction > Capture Correction Image* command for details on this topic.

Large Image Large image is acquired as a series of overlapping images taken one by one in regular grid of given dimensions. After acquisition, images are stitched together into one big image.

Enter the grid dimensions and check if you want to *Use Image Registration*. This function tries to find the perfect match of the overlapping parts of images, however it takes some time to compute.

Save Image to Database In this part of the *Capture* dialog, you can define the image saving conditions. Two options determining how to save the images to the database are available: *Always* or *Never or Later*.

>> Advanced Z-stack settings When combining multiple captured images together not all frames have to be captured in the image file. For example: Capture Z-stack with *Capture1* and then capture only home position with *Capture2*. Images from *Capture2* will be missing in other than home position of the Z-stack.

Use ... for Missing Images in Z-stack Select *Black* for missing images to be black, similarly select *White*. Select *Duplicate* to duplicate captured image to all missing images in the Z-stack.

ND Acquisition

Acquires an ND2 image according to the definition specified within the task. For more information about ND2 Acquisition please see 4 About ND Acquisition .

Fast Timelapse

This task uses the maximum capture speed of the camera set in 1.2 Optical Configurations to acquire a desired *number of frames*. The acquisition can be triggered by a *TTL Signal* or it can start *automatically*. The frames are then stored into an ND2 file. *Sample Storing* should be faster when the *Memory Only* (RAM memory) option is selected. Use the button to get the maximum number of frames.

Triggered Experiment

(requires: RT Acquisition)

This task aggregates all necessary features used for triggered acquisition.

| Optical Conf. | Name | Color |
|---------------|--------|--------|
| Triaq | 400 nm | Blue |
| | 450 nm | Green |
| | 500 nm | Red |
| | 550 nm | Yellow |

Experiment Name of your Triggered Experiment.

Z Series (Large Image) / Large Image (Z Series)

Specify which procedure is done first and which is done later. First option creates a full Z-Stack and Large Image is created later on each Z plane. Second option creates a Large Image on the first plane, then moves to the second plane, etc.

Time Check this option if you want to specify the duration of your experiment. Number of loops and time per one loop is indicated next to the combo box. Use to update the loop/duration estimate.

Piezo Z stack Check this option if you want to capture a Z stack. Specify the *Step size* and/or a *Range*, use the or enter a number of *steps*. *Z direction* can also be specified. *Bidirectional acq.* moves the stage in both directions from the sample, *Towards sample* scans in the direction to the sample and *Away from sample* scans the Z stack from the sample.

Piezo XY / Large Image (requires: Local Option)

This option can be used for acquiring XY points or Large Images. For Large Images enter the number of *fields* or use the *Full piezo range*. Specify the image *overlap* in percent. You can set whether you want to *Create Large Image* or *XY Multipoint*. *Register* tries to find the perfect match of the overlapping parts of images, however it takes some time to compute. *Point list* can be used for capturing multi points. For more information about XY points, please see 6 Multi-point Acquisition.

Optical Conf. Enables to select which optical configuration is used for the triggered experiment.

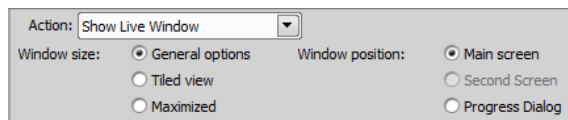
Acquisition details Displays additional information about the adjusted experiment (e.g. used Objective, Camera FOV and Large Image FOV).

Illumination Sequence

This task enables to set camera-to-device triggering patterns. For more information, please see: 12 Illumination Sequence.

Live Window

This task displays, freezes or hides the live-signal window. Use this tool whenever a preview of what is happening under the microscope is needed.



Action Choose one of three live actions: *Show Live Window* (opens a new preview window), *Freeze* (freezes the preview) or *Close Live Window* (closes the preview).

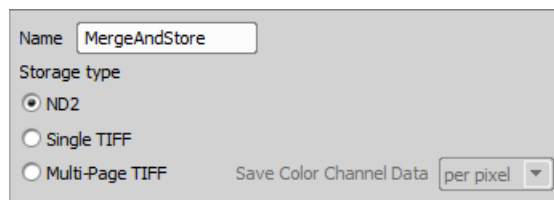
Window size Depending on your preference, choose one of the three window size options: *General options* (exerts the last used window size), *Tiled view* (tiles the

live window alongside with other windows) or *Maximized* (Maximizes the live window).

Window position If using multiple monitors, this function can put your *Live window* onto your *Second screen*, keep it on the *Main screen* or embed it directly into the *Progress dialog*.

Merge and Store Captured Images

Specifies the file-format used to store images created by the *Capture* task. The recommended (and default) setting is ND2 (the system tries to save images from different loops to a single file if possible). If the captured data are to be processed by other software, set the format to TIFF (*Single* or *Multi-page*).




Name Name your storage setting.

Save color channel data Choose a color channel storage method - *Pixel* or *Plane*. The default is per plane.

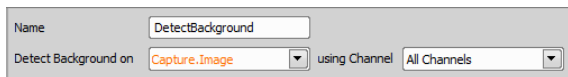
Note

This options may be useful for advanced users who plan to further process the images in other applications (such as MatLab). You can ignore it if you use just NIS-Elements AR.

Detect Background

This task can detect background from captured images. It requires connection to a *Capture*  task

and it sets two properties (IsBackground, IsSpecimen) for later use (e.g. in the *Condition IF* task).



Warning

This task requires Background/Specimen detection to be set up first. Go to: HCA/JOBS > Background Detection > Teach Background/Specimen Detection.

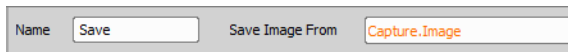
Name Name your detect background task.

Detect Background on Choose the data for the background detection.

using Channel Select on which channel the detection will be performed.

Save Captured Image

If *Save Image to Database* in the *Capture* task is set to *Never or Later*, this task can be placed into the job to decide when you want to save the images (e.g. if a condition is met or when analysis brings satisfying data).



Name Name of the task.

Save Image From Select the *Capture* task from which the acquired images will be taken.

Capture N-SIM

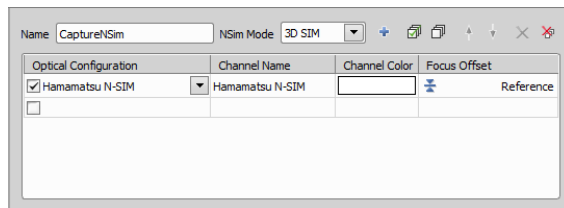
Captures N-SIM image using the selected method. The captured image is composed of several image tiles. A

single N-SIM image can be created within *JOBS Explorer*:

1. Open *JOBS Explorer*
2. Expand the JOB in the list to display job runs.
3. Select and right-click the job run used to capture the N-SIM image.
4. Select *Reconstruct N-SIM Image* from the contextual menu.

Caution

This task is only available with the Nikon N-SIM module installed (it is a separate setup file).



NSim mode Select the mode for capturing the N-SIM image.

Optical Configuration Select an optical configuration which supports N-SIM acquisition.

Focus Offset Sets focus offset - see *Capture Definition* for description.

STORM Acquisition

This task is used for acquisition using the STORM (Stochastic Optical Reconstruction Microscopy) method.

13.15.10 Stimulation

Sequential Stimulation

Chains acquisition time-phases and stimulation / bleaching / waiting phases. For more information about *Sequential Stimulation* please see *Applications > 6D > Define/Run Sequential Stimulation*.

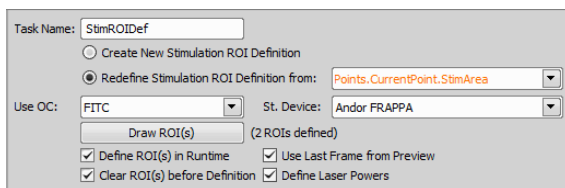
Simultaneous Stimulation

Runs a time-lapse acquisition and stimulation simultaneously. For more information about *Simultaneous Stimulation* please see *Applications > 6D > Define/Run Simultaneous Stimulation*.

Define Stimulation ROI

(requires: Local Option)

This task defines ROIs on the live image using the specified Optical Configuration.



Task Name Name of the Define Stimulation ROI task.

Create New Stimulation ROI Definition Creates a new Stimulation ROI Definition.

Redefine Stimulation ROI Definition from Redefines the existing Stimulation ROI Definition from the selected point/well loop.

Use OC Specify the Optical Configuration which will be used for defining the ROIs.

St. Device Select the device for stimulation.

Draw ROI (s) This button runs the Live view and opens the Simple ROI editor (see: *ROI > Simple ROI Editor*).

Define ROI(s) in Runtime If checked, this feature enables to define ROIs during runtime.

Use Last Frame from Preview If checked, the last frame from the preview is used for the ROI definition.

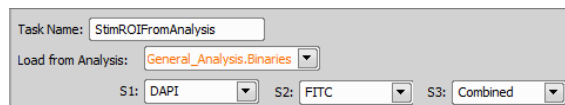
Clear ROI(s) before Definition Any previously drawn stimulation ROIs (drawn by clicking [Draw ROI\(s\)](#) or during *Runtime*) are cleared if this option is checked.

Define Laser Powers If checked, *Laser Power Setting* dialog window appears when drawing stimulation ROIs. This window enables to define power of each laser line on each stimulation group.

Use Stimulation ROI from Analysis

(requires: Local Option)

This task takes binaries created in analyses and applies them to as Stimulation ROIs during stimulation experiments.




Task Name Name of this task.

Load from Analysis Select the analysis task from which the binaries will be taken and used as stimulation ROIs.

S1, S2, S3 These three combo boxes specify three Stimulation groups and the binaries they are using.

Assign Stimulation ROI to Point/Well

(requires: Local Option)

This task assigns the stimulation ROI defined in the *Define Stimulation ROI*  to a Point of a point loop

or Single Well/Row of Wells/Column of Wells of a well loop.

Assign Stimulation ROI definition: to:

Assign Stimulation ROI definition Select the desired *Define Stimulation ROI* definition.

to Choose to which element you want to assign the stimulation ROI.

13.15.11 Optical Configurations

Select Optical Configuration

Switches the system to the selected optical configuration. You can make arbitrary changes on automated devices such as changing the camera settings, switching objectives, etc...

Name:

Optical Configuration: (dropdown menu showing: <undefined>, New 1, New 2)

Optical Configuration Choose one of your optical configurations to be activated.

Auto Brightness

This task can be used to automatically adjust brightness settings (exposure time, gain, ...) based on the parameters of the captured image.

Do Auto Brightness on: (dropdown menu)

Target Maximum Intensity: (dropdown menu)

Overillumination Tolerance:

- Relative: %
- Absolute: pixels

Estimate from single Capture

Do Auto Brightness on Select the *Optical Configuration* on which auto brightness will be performed.

Target Maximum Intensity Determines intensity of the lightest pixel in the resulting image. It is computed as a percentage of the camera bit-depth. For 8-bit cameras, the *Target Maximum Intensity* of 75% would be $255 \times 0.75 \sim 191$.

Overillumination Tolerance Specifies number of pixels which will be ignored when computing the *Target Maximum Intensity*. For example, if your camera has one or more defective pixels which gives constantly the maximum value (white), these pixels shall be excluded from the calculation. Define the tolerance either as percentage of all pixels (*Relative*) or *Absolute* by specifying their number.

Estimate from single Capture Estimates the values needed for *Target Maximum Intensity* from a single capture.

Set Exposure to Optical Configuration

This task assigns desired camera exposure to an optical configuration selected from the combo box.

Name:

Opt. Conf.: (dropdown menu)

Absolute: (dropdown menu)

Multiply by: (dropdown menu)

Name Name of this task.

Opt. Conf. Select an optical configuration to which the exposure time value will be applied.

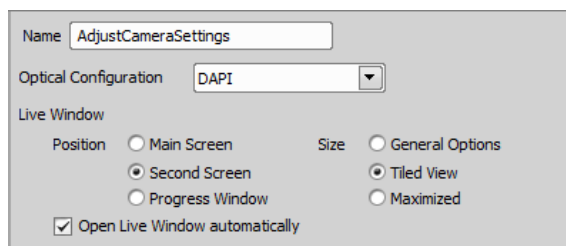
Absolute Absolute exposure value.

Multiply by Relative multiplier of the existing value in an optical configuration.

Variable Specify the exposure value by selecting a global value.

Adjust Camera Settings Manually in OC

This task is useful for changing camera settings during a job run.



Name Name of this task.

Optical Configuration Select optical configuration which will be used during the job run.

Live Window Position Choose where the Live window will be placed. Three options are available: *Main Screen* (main monitor), *Second Screen* (second monitor) and *Progress Window* (embeds into the Progress window).

Live Window Size Select the size of the Live window. *General Options* is the default size set in *NIS-Elements* options. *Tiled View* rearranges your opened windows together with the Live window to form a tiled layout. *Maximized* fully maximizes the Live window.

Open Live Window automatically Check this option if you want to launch the Live window automatically. If


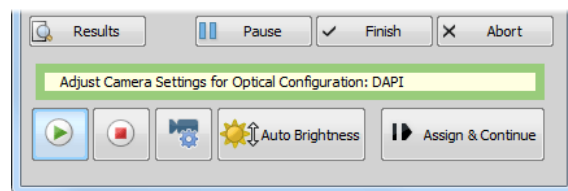


you want to turn this window on manually, leave this check box empty and click the  *Live* button inside the *Job Execution Progress* window after running the job.


Figure 12.467. Bottom part of the *Job Execution Progress* window





When you execute the Job, more options appear at the bottom of the *Job Execution Progress* window:

 **Live window** Turns on the Live window as defined in the *Adjust Camera Settings Manually in OC*  task inside Job Definition.

 **Freeze Live window** Freezes the Live window.

 **Camera settings** This control opens the *Camera Settings* dialog window.

 **Auto Brightness** Enables applying automatic brightness (exposure, gain, ...) on the selected optical configuration.

 **Assign & Continue** Clicking this button assigns the current camera settings to the selected optical configuration and continues in the job progress.

Assign Camera Settings to Point/Well

This task takes camera settings from a selected optical configuration and applies it to a selected row, column, well or point.

Name Name of this task.


Assign Camera Settings from Select optical configuration from which the camera setting is taken for the assignment.

to Select to which you want to assign the camera settings and specify the selection in the last combo box.

Interpolate Exposures between Wells

This task interpolates/copies exposure values from wells with known values (e.g. assigned by *Set Exposure to Optical Configuration* ^{Bmsl}_{OC}) to wells with unknown values.

Name Name of this task.

Well Selection Select the desired *Select Wells*  task.

Optical Configuration Choose the desired Optical Configuration used.

Method Choose one of the methods - *Interpolate* calculates interpolation between two wells with known exposure and applies the new values between these two, whereas *Copy* copies known values to all ongoing unknown values.

Direction Specifies the direction of Interpolating/Copying. *Left to Right* goes from left to right on each well

plate row, *Top to Bottom* goes from top to bottom on each well plate column.

Use case example




When capturing fluorescence images on a well plate using dilute solutions or suspensions, fluorescence intensity may decrease linearly. Exposition and other parameters can be adjusted only on the first and last well of the dilution range - manually using *Adjust Camera Settings Manually in OC*  or automatically using *Auto Brightness* . The middle section can then be interpolated using the *Interpolate Exposures between Wells*  task.

Figure 12.470. Simple use case

Close Shutter

Closes the shutter specified within the task. Select one of the shutters available in the system.

Open Shutter

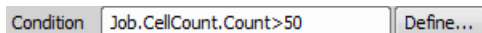
Opens the shutter specified within the task. Select one of the shutters available in the system.

13.15.12 Conditions

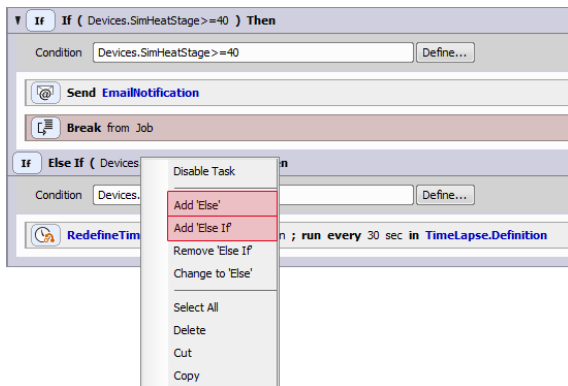
IF Condition IF

If the specified conditional expression is evaluated to TRUE, the contained tasks will be run. Otherwise the contained tasks are skipped.

Figure 12.471. Example of an expression.



After defining your If condition it is possible to add more conditions defined by different parameters. This can be done by right-clicking the task's frame and choosing *Add 'Else'* or *Add 'If Else'*. Else represents all options except those defined by the condition. If Else requires defining another condition in order to run its contained tasks.



See also 13.13.2 Using Conditions.

IF ELSE Condition (If-Else)

If the specified expression is evaluated to TRUE, the contained tasks will run. Otherwise the script proceeds to "Else".

If The same as in the *Condition IF* task.

Else Tasks contained in the *Else* section will be run only if the specified conditional expression is evaluated to FALSE.

See also 13.13.3 Using Expressions.

Every Nth

When using repetitive tasks (e.g.: repeats, well loops,...), you can create a new task sequence that is triggered by every first / last / nth loop using the *Every nth* function. This task changes its options with respect to the type of loop it is placed in.

Figure 12.473. Every Nth task inside a Loop over Wells

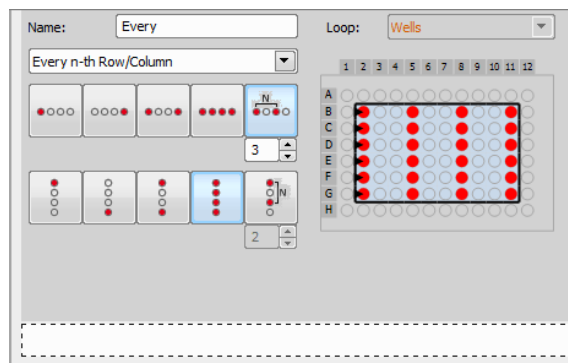


Figure 12.474. Every Nth task inside a Loop over Plates

Figure 12.475. Every Nth task inside a Loop over Points

Figure 12.476. Every Nth task inside a Z-Stack Loop

Figure 12.477. Every Nth task inside a Time Lapse

Loop Choose the loop you would like to modify by this task.

Every Enter the desired number of the repeated task.

First Executes after the first repetitive task selected.

Last Executes after the last repetitive task selected.

Include First Specifies whether the inner tasks will be executed also on the first Plate/Point/Time Loop or not.

Every n-th Row/Column Select this option to execute the inner tasks only on selected wells. These are defined using the icons below, described in the task caption and shown in the preview area as red circles.

Every n-th Well Select this option to execute the inner tasks only on selected wells. These are defined using the icons below, described in the task caption and shown in the preview area as red circles.

Full Z-Stack Executes the inner tasks on each plane of the Z-Stack defined by the *Define Z-Stack* task.

Top/Home/Bottom slices Specifies the part of the Z-Stack and how many slides are taken into account when performing the inner tasks.

Break

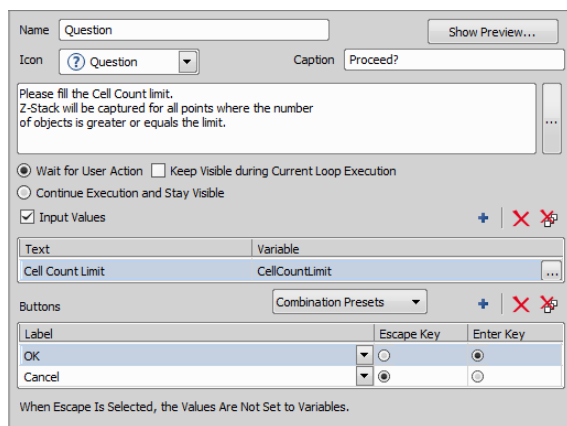
Exits execution of the selected loop and continues to subsequent tasks.

Break from Select one of the parent loops to exit. The job will continue with the task following the selected loop.

13.15.13 System

Question

Displays a pop-up window requiring user interaction. After “answering” by clicking one of the buttons, the job continues.



Name Name your question.



Show Preview... Displays the preview window showing how the defined question will look like.

Icon Choose an icon which will be displayed together with your question. Four graphics are available: Information, Question, Exclamation and Error.

Caption Type a text to be used in the dialog window heading.

Wait for user action Stops the job sequence until the user answers the question. If the *Keep visible during current loop execution* option is selected, the question stays visible during the loop execution even after user input.

Continue execution and stay visible If checking this option, the question shows up and the job continues even without user interaction.

Input Values Check if you want to input any user data (e.g. defined in *Variables* ). Click the **+ Add New** button and select the variable. Name of the selected variable can be set in the Text field. To change the variable, click .

Buttons

Combination Presets Here you can choose some predefined button combinations.

Label Define the button names to be displayed when the question is to be answered.

Escape Key, Enter Key Select which buttons will correspond to **Esc** and **Enter** key-strokes.

+ Add New Adds a new button.

✗ Remove the button Removes the selected button.

✗ Remove All Removes all buttons.

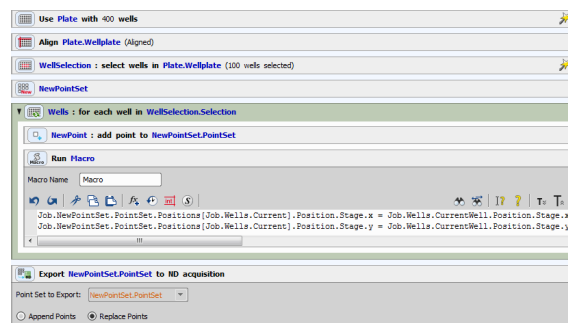
For more information about using Questions please see: 13.13.4 Using Questions.

Macro

This task enables the user to write and run a macro. The whole set of available macro commands can be used. Write sophisticated C-like scripts for tasks which cannot be done by a *Job* or reuse your existing macro. Please see 10. Macros also.

Using Job Parameters within the Macro Task

Figure 12.479. Macro job example 1



This example job generates a point set (pattern of points on a wellplate) without moving the stage. The embedded macro fills the point set with x and y coordinates of the center of each well from the well selection.

Obtained points can be used later in a different job for a fast acquisition.

Figure 12.480. Macro job example 2

function). Global variables declared by this task can be accessed e.g. by a macro.

| Variable Name | Type | Current Value | Always Initi... | Initial Value |
|---------------|---------|---------------|--------------------------|---------------|
| NewVar | Integer | 1 | <input type="checkbox"/> | 1 |

This example job has similar functionality as the *Keep Object In View* task following the object of interest and keeping it in the center of the camera FOV. In this specific job, three manually selected objects are followed for an amount of time set in the *Time Lapse*

task. The first macro defines the variables and declares objective calibration. Then the three points are manually marked using the *Predefined Points* task. *Loop over Points* is performed once and thresholding defined in the *General Analysis* is used for identifying the objects of interest. The second macro sets the objects initial position into the pre-defined variables. The third macro finds the current x and y position of the center of the object and shifts the stage to these coordinates. Then it updates the objects position in the point set with the current position.

Another example of using a macro job can be found here: `Jobs_SetFilenamePart`
`[_includes/call_fr.html#FR_Jobs_SetFilenamePart.html].`

Expression

Assigns values to variables available within the job. It enables to modify settings of any tasks, manipulate motorized devices, etc. For more information about working with expressions please see 13.13.3 Using Expressions.

Variables

Declares global variables within *NIS-Elements*.

Note

In programming, global variable is a variable accessible from anywhere in the application (NIS-Elements in this case). Unlike local variable which is accessible in a limited scope (such as a macro

+ Add new Add a new variable to the *Variables* list.

✗ Remove the variable Removes the selected variable.

✗ Remove All Clears the list of *Variables*.

Variable name Name your variable.

Type Choose the numeric type of your variable. You can choose between: Integer (integral number), Double (real number), String (sequence of characters).

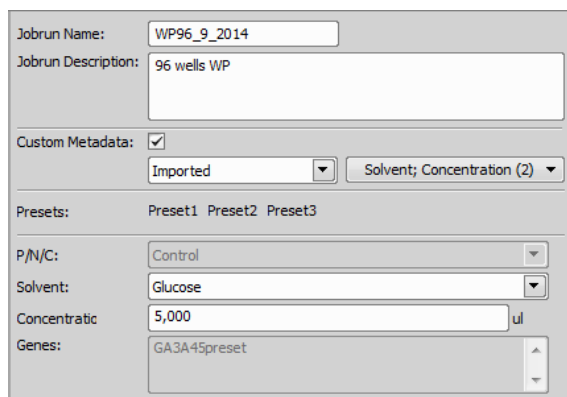
Current value A value can be assigned to the variable within this task or within *Expression*.

Always Initiate Each variable keeps its value after the job is finished. Initialization resets the variable value to *Initial Value* at the beginning of the job.

Initial value Set your initial variable value.

Custom Metadata

Enables to enter custom metadata to be used before (in wizard) or during the job run. Metadata can be created and managed in the *Manage Custom Metadata* dialog window (see *Defining and Setting Metadata*). Defined metadata of different types (e.g. numeric value, text, selection, date,...) are grouped into metadata sets and stored in the database. Metadata information applied by this task during the job are recorded into job results and into corresponding captured images.



Jobrun Name Custom name of the jobrun.

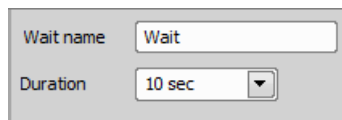
Jobrun Description Custom description of the jobrun.

Custom Metadata If checked, custom metadata will be used. Select a metadata set and choose which metadata from this set will be used from the neighboring combo box. Active metadata values can be adjusted in the edit boxes below depending on the use of this task in your job definition.

Presets Presets defined in the *Presets* tab of *Manage Custom Metadata* (see: *Defining and Setting Metadata*) are displayed here as buttons for quick switching between them.

Wait

This task pauses the job for a specified amount of time.



Wait name Name of your Wait task.

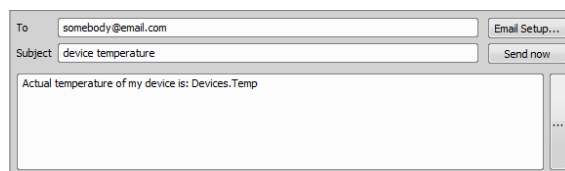
Duration Amount of time for which the job will be paused.

Comment

This task can be used for adding constantly visible notes / comments right into the job sequence. This way you can write down information to easily explain the particular job procedure to a different user seeing the job for the first time.

Send E-mail Notification

Sends an email to the specified address. SMTP server configuration (within the task) must be correct. You can send results of the experiment, messages about the experiment progress (failed, finished, in progress,...), etc.




To Enter the recipient's e-mail address.

E-mail Setup... This function opens the *SMTP Configuration* window (please see *HCA/JOBS > Presets > Email Setup*).

Subject Type the subject of your e-mail.

Send Now This function allows you to send your e-mail immediately.

Text Box Here you can type your message combined with your job *variables*.

... The ... button on the right side adjacent to the text box enables inserting *job variables*. You can also use your custom variables defined in the *Variables* task (see *Variables* ).

Examples: Notification about the Job experiment end, notification to exchange well plates, notification that auto focus failed, etc.

Send SMS Notification

Sends a text message to the specified phone number if the particular mobile phone operator provides such services (sending SMS over email).


Phone Number Type the recipient's phone number.

Carrier Select the local mobile operator of the entered *Phone number*.

Send Now This function allows you to send your SMS immediately.

E-mail Setup This function opens the *SMTP Configuration* window (see *HCA/JOBS > Presets > Email Setup*).


Text Box Here you can type your message combined with your *job variables*.

... The ... button on the right side adjacent to the text box enables inserting *job variables*. You can also use your custom variables defined in the *Variables* task (see *Variables* ).


Create Labels

This task defines labels which can be used for labeling single wells or single points during runtime.

| Label Name | Color |
|------------|-------|
| Good | Green |
| Bad | Red |

Add labels by clicking the **Select Labels...** button or by clicking the **+** *Add New*. Set their name and choose the labeling color. You must use the *Assign Label*  task to assign these settings.

Assign Label

This task assigns labels set in *Create Labels*  to wells or points during runtime. Filtering by these labels inside Well Loops and Point Loops is possible. Select a label from the *Set Label* list and assign it to a well/point loop. Check *Reset other Labels* if you want to clear existing labels.

Tip


Use this task with a *Question*  task inside a well / point loop. During the first loop you can use the question buttons to manually decide which wells / points are suitable / unsuitable e.g. for a precise capture. The high magnification capture can then be made during the second loop only over wells / points specifically marked in the first loop.

Figure 12.488. Label use case

Phase

Some use cases can be logically separated into Phases (preparation, verification, acquisition). This task can be used for holding parts of the job, creating conditions between these parts and running these parts in a custom order.

Example 12.8. Example of a well plate acquisition job separated into 4 phases:

1. Find interesting locations within wells
2. Run Autofocus at each location
3. Verify locations and proper Focus
4. Run Acquisition

Figure 12.490. Phase task

Debug

This task enables to select specific parameters and list them inside the Job Execution Progress window. Values are displayed and the Job is paused until **Continue** is pushed.


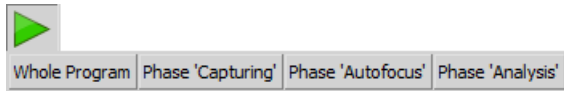
All task contained in the dashed area at the bottom of the task can be executed *Anytime* or *Only after other Phases*. Such condition can be created in the *Expression* area using basic *Operators* and *Insert* for inserting the selected phase. After defining phases and clicking  **Run Job**, options for their direct execution or execution of the whole job are shown below.

Figure 12.491. Job Execution sub-menu



After a phase is executed, this information is stored so that the job can later be executed from the following phase. Each phase can be executed multiple times and the job can still be executed whole at once.

Finish

This task enables to finish the current job run.

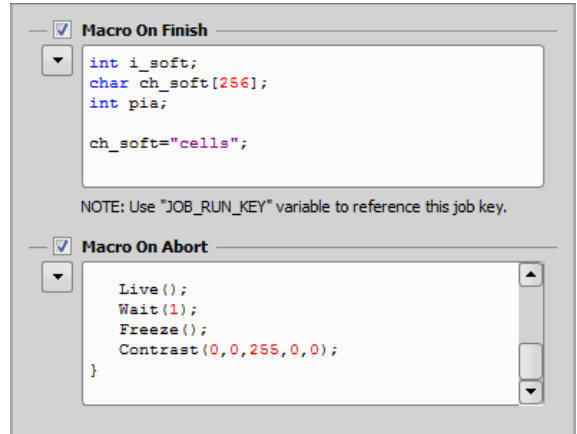
Abort

This task enables to cancel the current job run.

Execute Macro After Run

(requires: *Local Option*)

Enables to enter a macro-command which can be performed on Finish and/or on Abort of the job. See also 10. Macros.



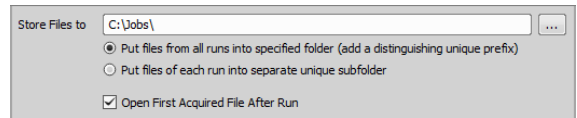
Macro on Finish Executes the specified macro on Job Finish.

Macro on Abort Executes the specified macro on Job Abort.

Drop-down menu Click the arrow button to reveal a menu enabling to *Insert Content of Macro...* from a *.mac* file, *Execute Macro...* from a *.mac* file or to *Append Command...* from the Command list.

Alternative Storage Location

NIS-Elements database is ignored and all acquired files are saved into the specified folder.



Store Files to Defines where the acquired files will be saved.



Put files from all runs into specified folder (add a distinguishing unique prefix) All runs are placed into a single folder. Unique prefix is added.

Put files of each run into separate unique subfolder

Files from each job run are placed into a separate folder.

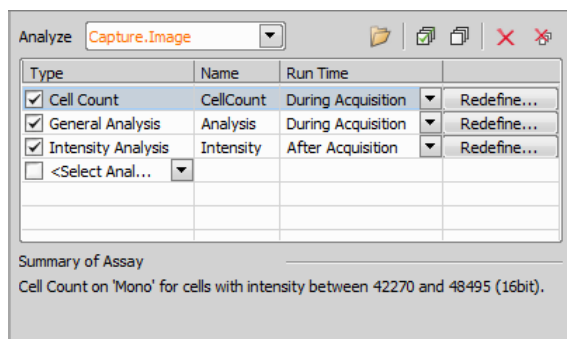
Open First Acquired File After Run First file acquired in the particular job is opened in *NIS-Elements* after the job run.


13.15.14 Analysis

This task section contains other jobs analyses. To be able to execute any analysis in Jobs, your job definition has to contain *Capture Definition*  and *Capture* .



Define Analysis

This task can be used to define multiple analyses which can be run during or after image acquisition.





Link the task to your *Capture*  task displayed in the *Analyze* combo box. Now choose your first analysis from the combo box in the table *<Select Analysis>*. Name it and choose when it will be executed. Click **Define...** and set up the current analysis. Please see 10.3 HCA Analysis for details about each analysis.

JOBS Recipe Manager (requires: *Local Option*)

Clicking on **...** in the *Analysis* task caption opens the *JOBS Recipe Manager* where it is possible to  *Import/*  *Load from NIS...* any previously created analysis recipes. Recipes marked with a check mark are arranged in a new section called *Analysis Recipes* in the *Job Definition Task Palette*.

General Analysis

General analysis in jobs has the same functionality as General analysis from the Image menu, however in Jobs it brings much more versatility. Parameters of the *General Analysis*  task are closely described here: 2.5 General Analysis.

Once you set up a General analysis it can be later used as a custom task. To do so, first you have to save your analysis settings (for General analysis from the Image menu click **Save as...** and name your .ga file, for General analysis in Jobs click **Save Recipe**). Then click **...** and search for your saved settings. Use the  *Import...* or *Load from NIS...* buttons if your analysis is not already listed. Then select the desired recipe and click **OK**. Selected analysis recipe carrying the analysis settings is now represented by a custom task inside the *Analysis Recipes* section of the task palette.

Cell Count Analysis

Parameters of this analysis are closely described here: 2.2 Cell Count Analysis.

Intensity Analysis

(requires: *Local Option*)

Parameters of this analysis are closely described here: 2.6 Image Intensity Analysis.

General Analysis RGB

(requires: Local Option)

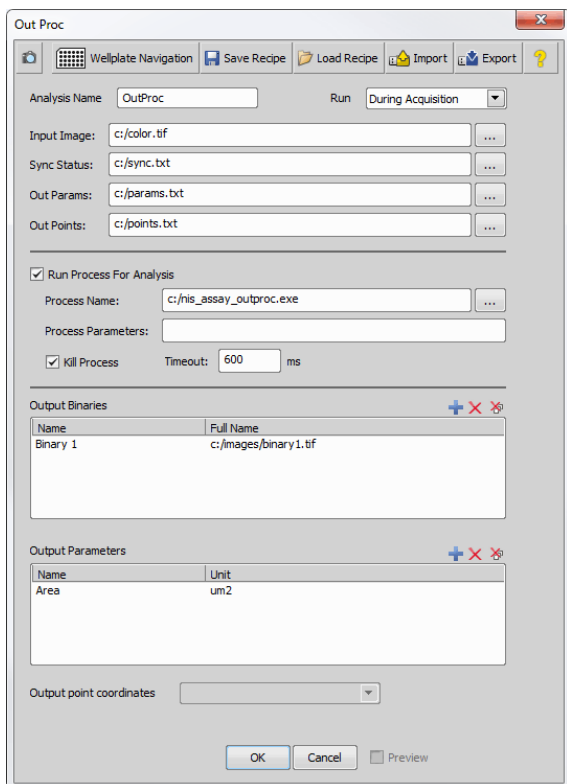
(requires: General Analysis)

Parameters of this analysis are closely described here: *Image > General Analysis RGB*.

Out Proc

(requires: Local Option)

This task enables to use an external program to process/analyze images captured via *JOBS*. If the external program produces output in a form of binary images or an array of values in a text file, *JOBS* load and use them for further analysis.



| Name | Full Name |
|----------|-----------------------|
| Binary 1 | c:/images/binary1.tif |

| Name | Unit |
|------|------|
| Area | um2 |

Analysis Name, Run: The top part of the window is common to all analysis windows are described in the 13.9 Analysis chapter.

Input Image Name and path to the TIF image generated by *NIS-Elements* for analysis. E.g. "C:\color.tif"

Sync Status Name and path to the text file with status of the analysis. E.g. "C:\sync.txt". This file is shared between *NIS-Elements* and the external program. Running processes can communicate due to the sync status saved in this file. Possible values are:

- 0 Not ready
- 1 Ready for analysis
- 2 Analysis is finished
- 3 Cancel execution

Out Params Name and path to the text file where analysis results will be saved. E.g. "C:\output-params.txt". It will contain a list of values specified in the *Output Parameters* section.

Out Points Points are generated into this file by the external application (e.g. points representing centers of binaries).

Run Process for Analysis Select this option if the external program you use runs on demand and needs to be called to process each image. Programs which run independently and are capable of checking the content of the *Sync Status* file do not need this function.

Process Name Name and path of the external program.

Process Parameters Any parameters needed for the external program.

Kill Process, Timeout [ms] Programs which do not end automatically can be killed after the defined *Timeout*.

Output Binaries Definition of binary TIF files generated by the external program which ought to be loaded by *NIS-Elements* after analysis. The first field specifies name of the binary layer, the second field is a name

and path to the TIF file containing the binary image data.

Output Parameters Definition of result values generated by the external program. Supposedly, the external program generates a list of values, each value on a new line. This definition pairs these values to its description under which they will be available within *NIS-Elements*.

Output point coordinates Defines where the points are saved (point list defined by a task).

Preview Runs the analysis on the current image.

Processing with ImageJ

Figure 12.496. Out Proc processing with ImageJ

Out Proc task also enables to process images using ImageJ. Set the path of the *Input Images* generated by *NIS-Elements* and the path to the file containing results generated by ImageJ (*Out Params*). It is import-

ant to let the *Sync Status* field empty. Enable *Run Process For Analysis* and set the *Process Name* as an executable ImageJ file. Specify the macro and other options in the *Process Parameters* field. For example to measure the image area and mean gray value using ImageJ enter the following macro:

```
path = getDirectory("im-
agej")+"NIS"+File.separator;

print (path);

open(path+"input.tif");

run("Input/Output...", "jpeg=85 gif=-
1 file=.txt use_file");

run("Set Measurements...", "area mean
redirect=None decimal=3");

run("Measure");

saveAs("Results", path+"Results.txt");

eval("script", "System.exit(0);");
```

Measured results are connected to the output parameters specified in the *Output Parameters* area of the Out Proc task and are shown in the *Job Results* dialog window.

Wound Healing

(requires: *Local Option*)

This task controls the wound healing analysis used for measuring changes of cell distribution in time.

The top buttons which are common to all analysis windows are described in the 13.9 Analysis chapter.

Analysis Name Name your wound healing analysis.

Run Specify in which phase will the analysis be executed (*During Acquisition* or *After Acquisition*).

Close Holes - Count This command fills holes in detected objects. Specify how many times the command will be run.

Delete Small Objects - Count This command deletes the smallest objects. Set how many times the command will be run. Use the *Preview* button to check your current image settings.

Do Postprocessing Check if you want to use postprocessing.

Preview Check if you want to see a live preview of your current wound healing settings.

13.15.15 Ga3 Processing

Please see 9.4 GA3 in Jobs.

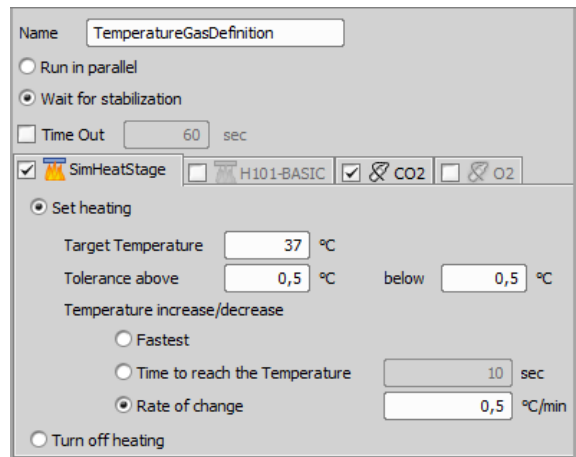
13.15.16 Device Control

(requires: Local Option)

Temperature and Gas Control

(requires: Local Option)

Sets the target temperature and gas concentration.



Name Name your *Temperature and Gas Control* task.

Run asynchronously When selected, the subsequent tasks will be run immediately - the system will not wait till the time-out is reached or the temperature/concentration achieved.

Continue after timeout This option overrides the *Timeout* settings which are ignored. The task waits until the defined temperature/concentration is reached.

Set heating Check this box if you want to adjust the temperature.

Target Temperature Set the target temperature of your selected device.

Tolerance above, Tolerance below Select the tolerance interval above and below your *Target Temperature*. If any temperature within this interval is reached, the system considers the task to be finished successfully.

Temperature increase/decrease If this option is available, you can set the speed of heating. Three options are available: *Fastest*, *Time to reach the Temperature* and *Rate of change* (choose a change rate in degrees per minute). Only some devices support this feature.

Time out Time out specifies the time given to reach the target temperature. If the device does not reach it in time, the job will continue nevertheless. If the temperature is reached before the time-out, the job will continue immediately.

Turn off heating Once the task is used to reach a temperature, the device holds the temperature within the given tolerance. Select this option if you want to turn the heating off in another part of the job.

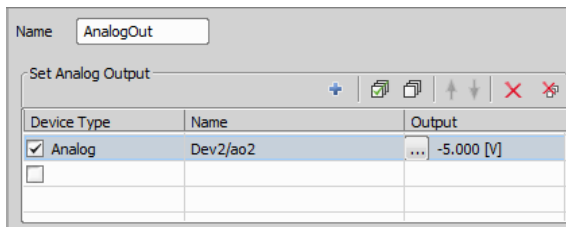
... C02, ... 02

Set Concentration Set the target concentration of the selected gas.

Time out Time given to the task to reach the concentration (see above).

Analog Out

This task controls analog output devices.



| Device Type | Name | Output |
|-------------------------------------|--------|----------|
| <input checked="" type="checkbox"/> | Analog | Dev2/ao2 |
| <input type="checkbox"/> | | |
| | | |

Device Type Type of the output device.

Name Analog output name.

Output Output voltage set by clicking .

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