

Fiji for Beginners

A slow paced, hands on seminar for the uninitiated. Fiji is a powerful tool with a lot of flexibility. Let's start with the basics!

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Getting Started. Fiji or Image J?

- Fiji is ImageJ!
- It downloads with all the plugins
- It will update regularly (if you approve)
- Updates (plugins) can be broken
- Fixing broken updates (plugins) can be difficult
- You can always uninstall and re-install (this fixes almost everything)
- Extract zip folder to your desktop (works best when not in program files)

Fiji Download

<https://fiji.sc/>

ImageJ Download

<https://imagej.nih.gov/ij/download.html>

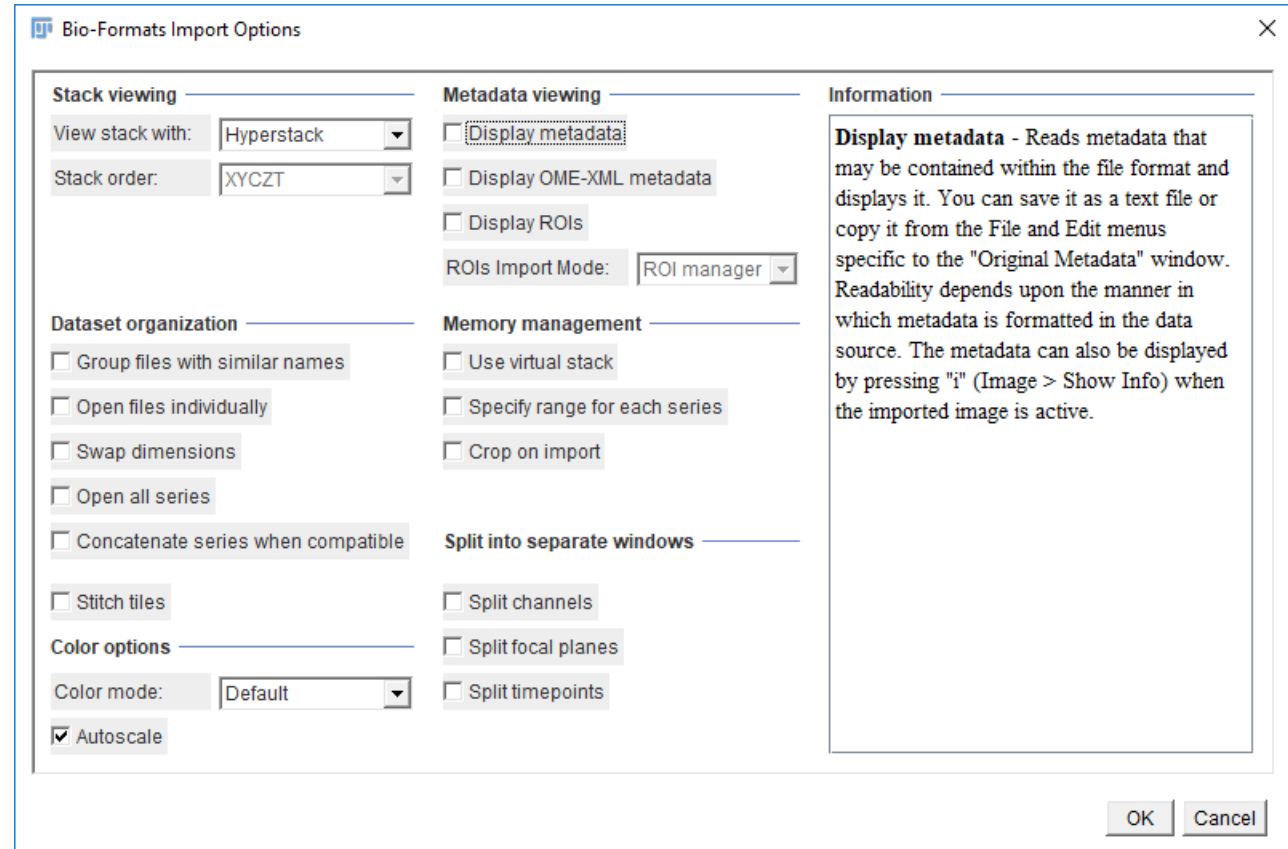
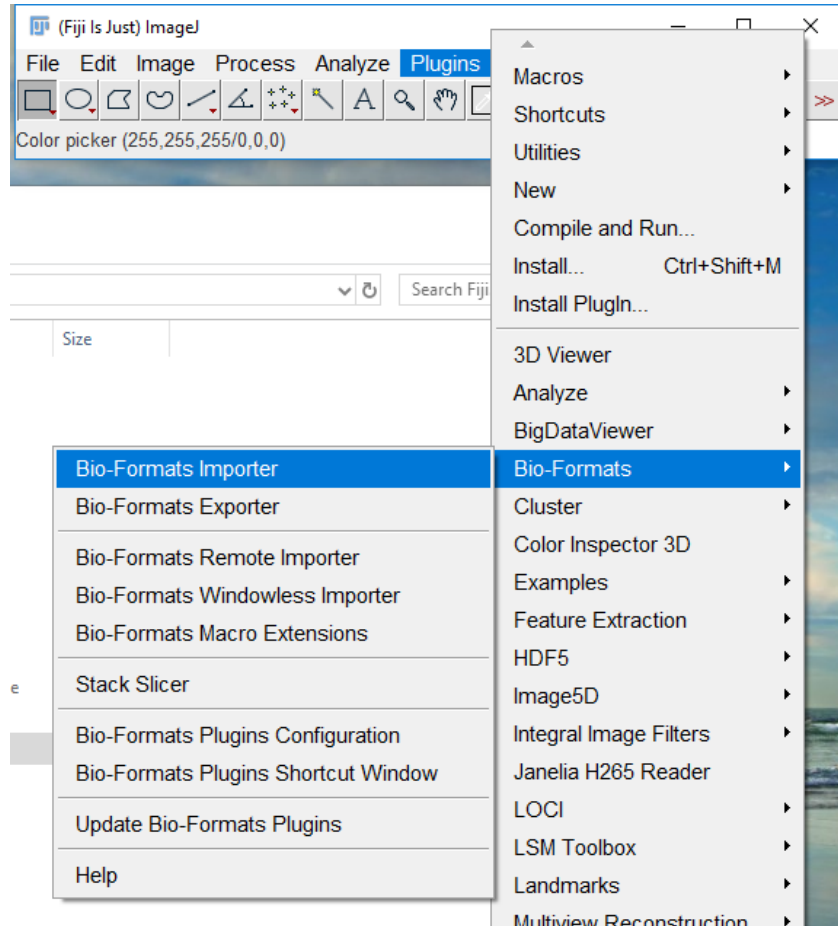
Bioformats Importer Opens All File Formats

- Found in plugins
- Opens all image formats
- Updated regularly to include new formats
- Gives flexibility in how you open your file

Using Bioformats

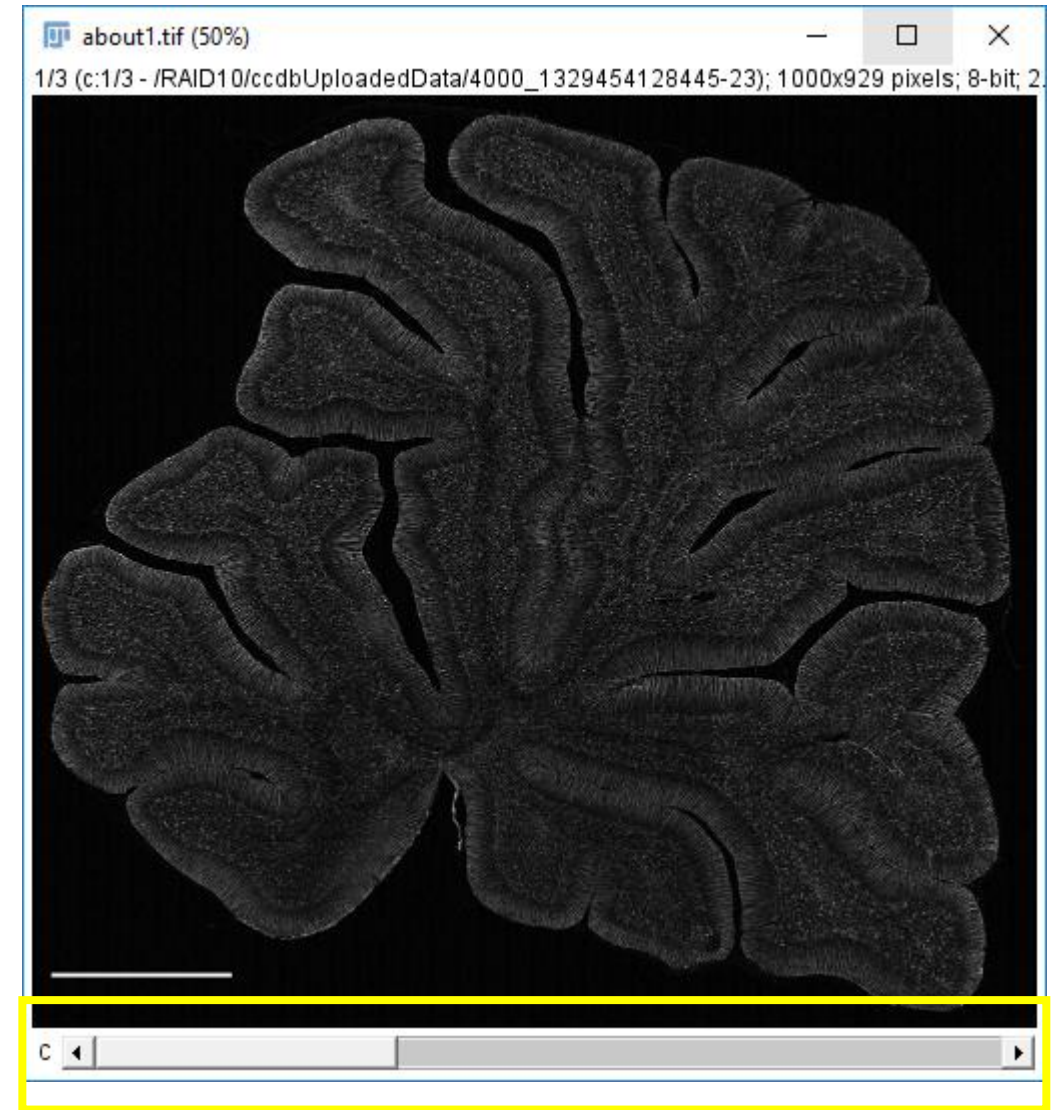
- Click on Plugins
- Choose Bioformats > Bioformats Importer
- Choose you file
- Choose how to open it-description of choices on the right
 - Hyperstack
 - Metadata Only
 - Split channels
 - Composite (colors together as an RGB)/default
 - Stitch files (will open sequential files as one)

Open file in ImageJ > Images > about1.tif

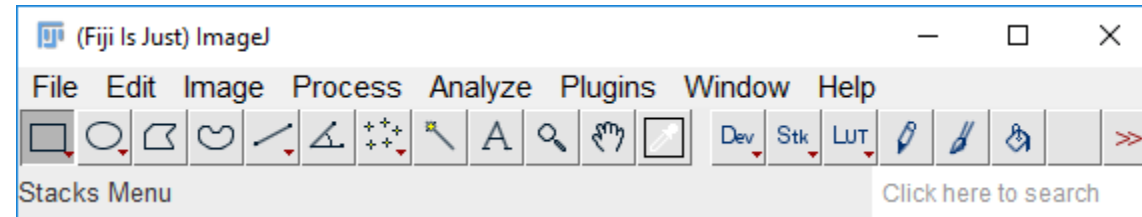


What Just Happened?

- The image opened in monochrome
- Image stack
- The each color is found by using the slider at the bottom
- Fiji opens the image in order of acquisition or save order from the imaging system *(usually lowest wavelength is color 0 or 1 (C0))



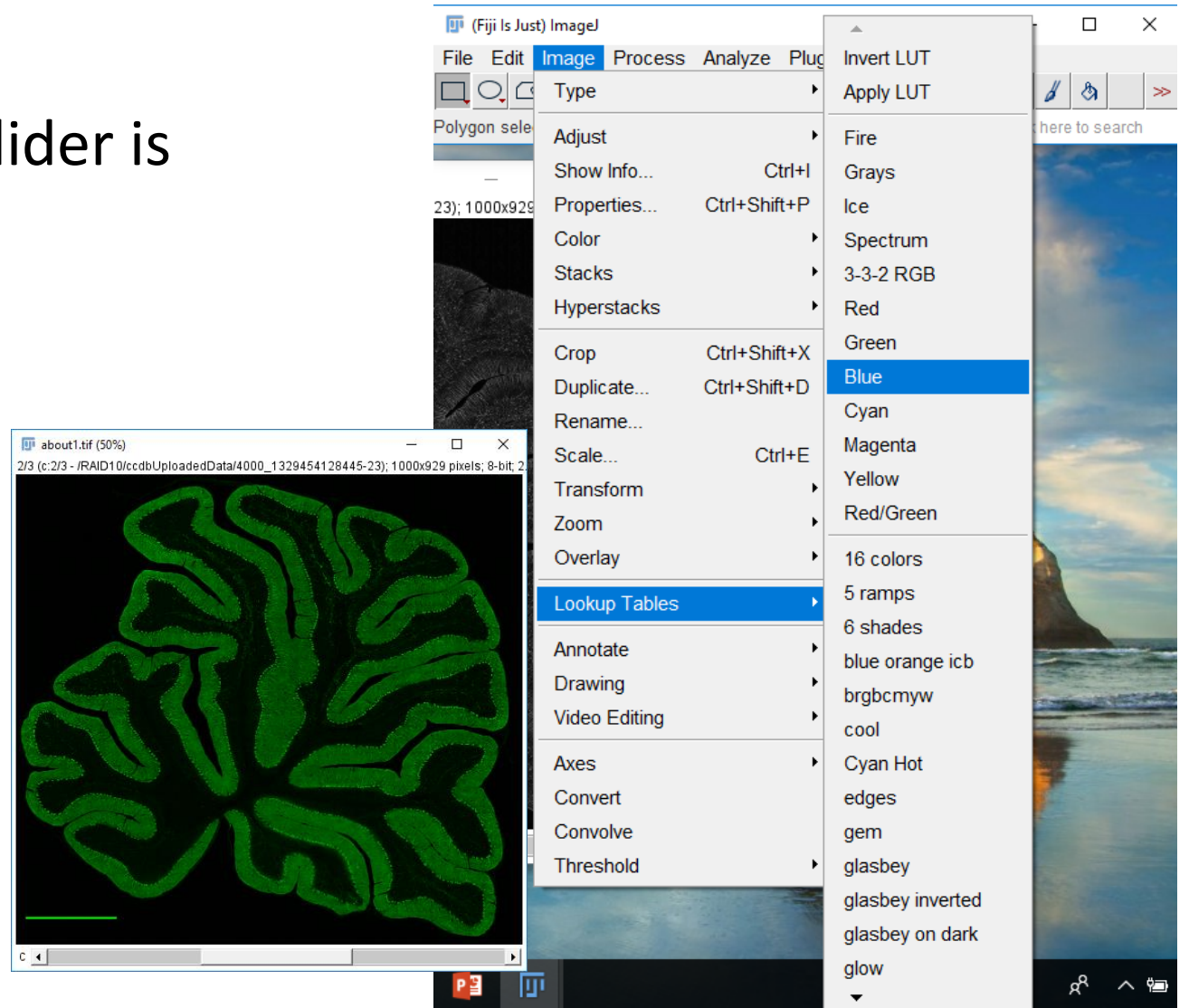
The Menu Bar



- Allows manipulating of the image size and location
- Drawing tools
- Paintbrush and font

Add Color to Your Image

1. Make sure you are on C=1 (slider is on far left)
2. Image>Lookup Table>Blue
3. Move slider to C=2
4. Image>Lookup Table>Green
5. Move slider to C=3
6. Image>Lookup Table>Red

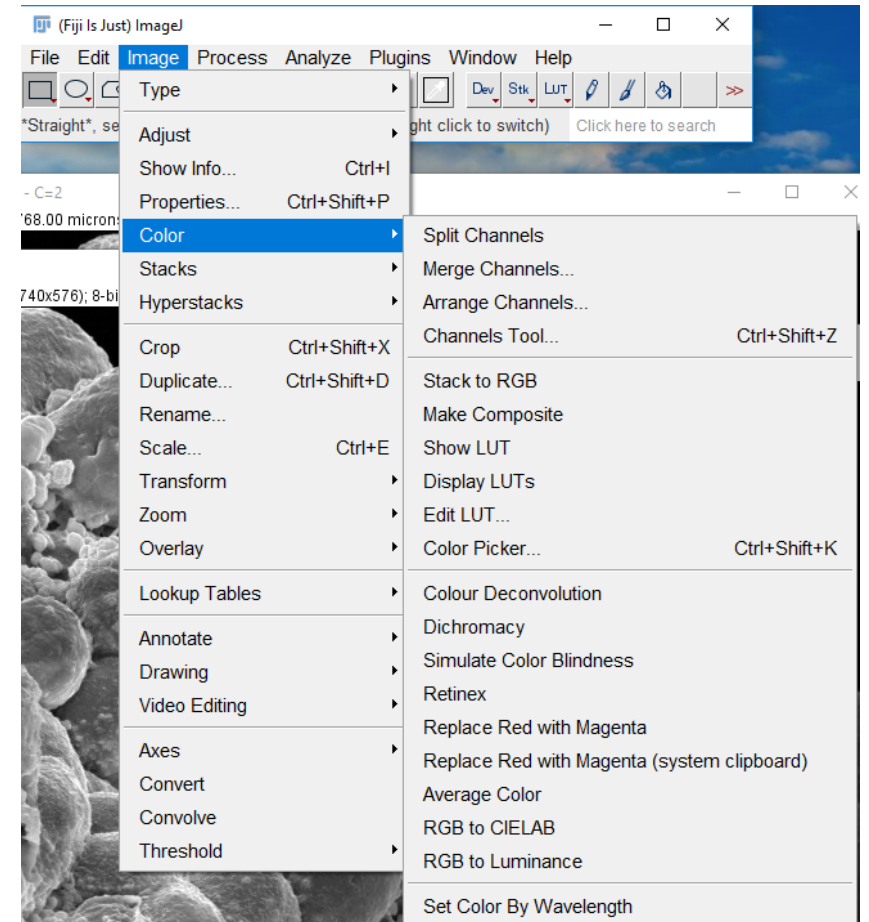


Split Colors

- Almost everything attributed to the image view is in the Image menu
- Choose “Color”
- Choose “Split Channels”

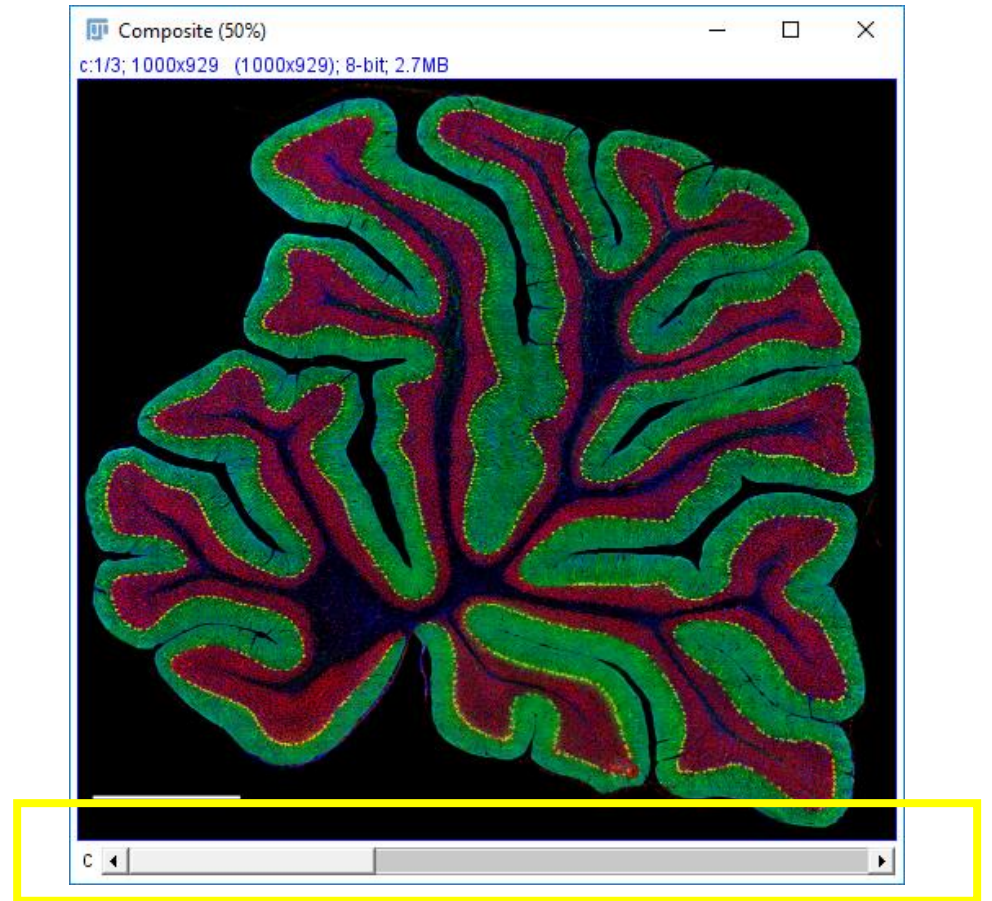
Merge Colors

- Choose “Color”
- Choose “Merge Channels”
- Merge channels C0=Blue, C1=Green, C2=Red



Adjusting Intensity and Contrast. Keep It Separate!

- Image > Adjust > Brightness/Contrast
- Move “Maximum” slider to middle position
- Move “Minimum” past the first peak
- Move “Brightness and Contrast” Slider



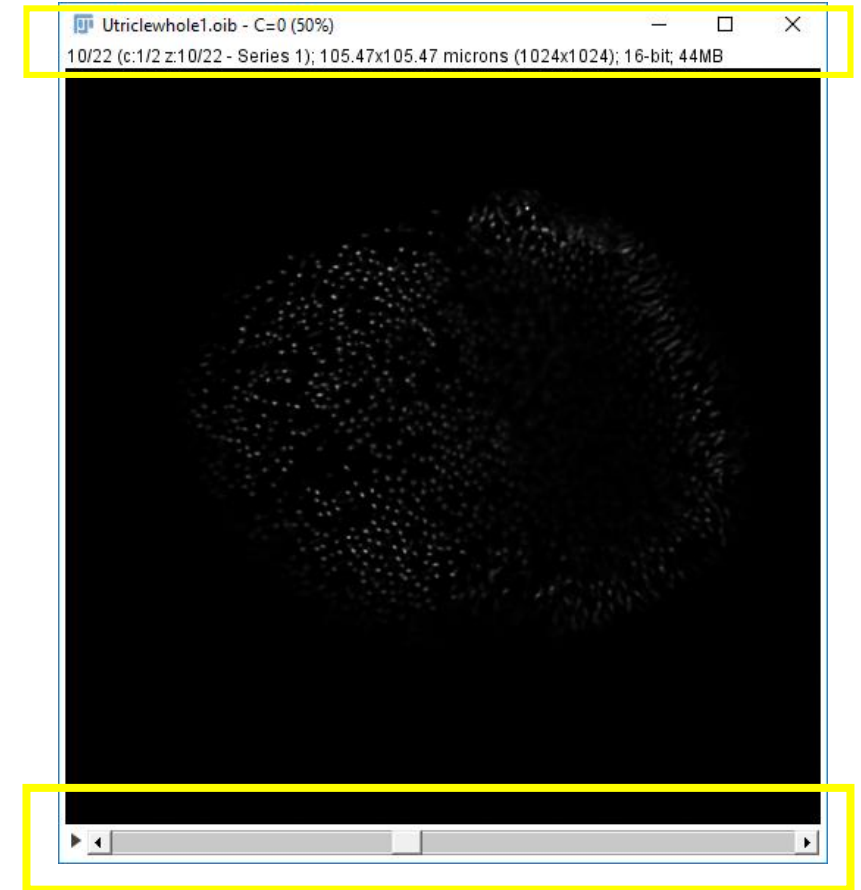
Opening Image Stacks

Image1.oib

- Similar to opening a single image
- Use Bio-formats
- Slider at bottom changes stack number

Image Stacks

- Stack to Images/ Images to Stack
- Slider at bottom changes stack number
- Montage
- Z Project... (Projection)

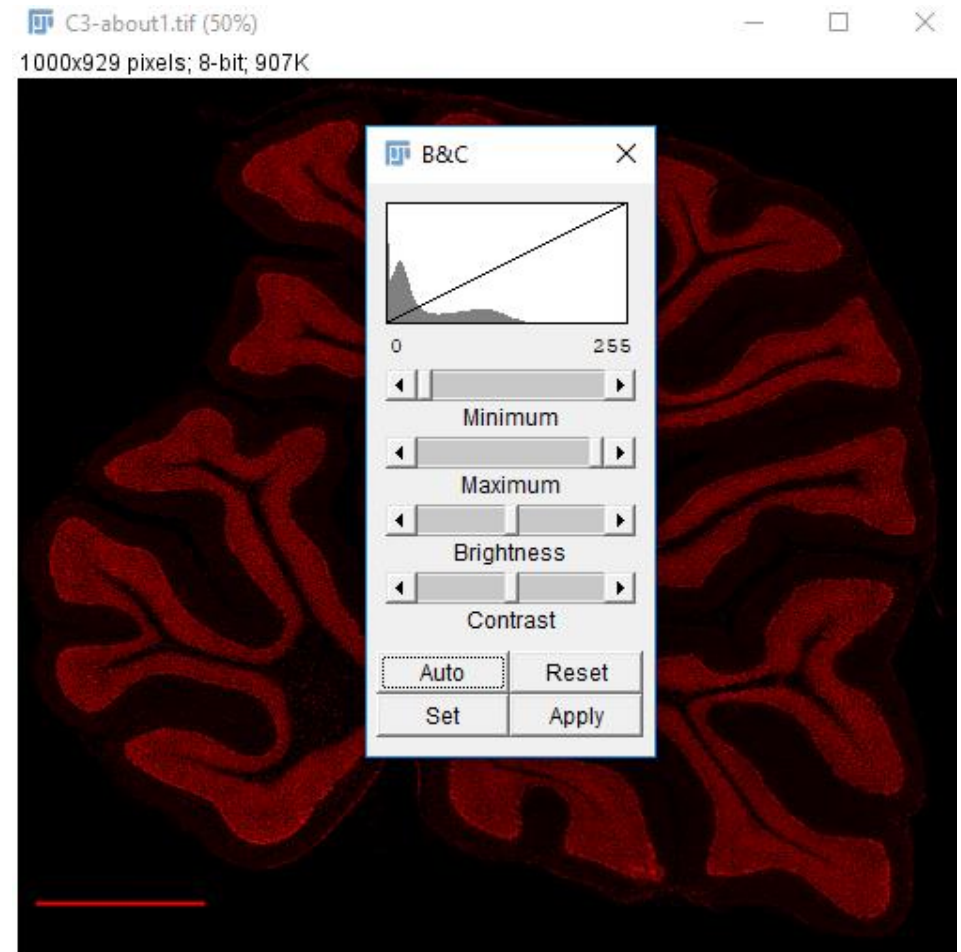


Histograms are Your Secret Weapon

- Histograms are found when adjusting Brightness/Contrast and by pressing cntrl + H
- Histograms reveal the pixel intensity information for your image
- It reveals the bit depth (what the maximum pixel intensity)
 - 2 types of resolution
 1. PHYSICAL- **Optical resolution**: the resolution of the objective (Numerical Aperture and Magnification)
 2. DIGITAL - **Bit Depth**: the degree of intensity range in the image. Each pixel has an intensity value. A high value is bright a low value is dark. The number of intensity points between the lowest and highest intensity number is bit depth.
 - 8 bit = 256
 - 12 bit = 4096
 - 16 bit = 65535

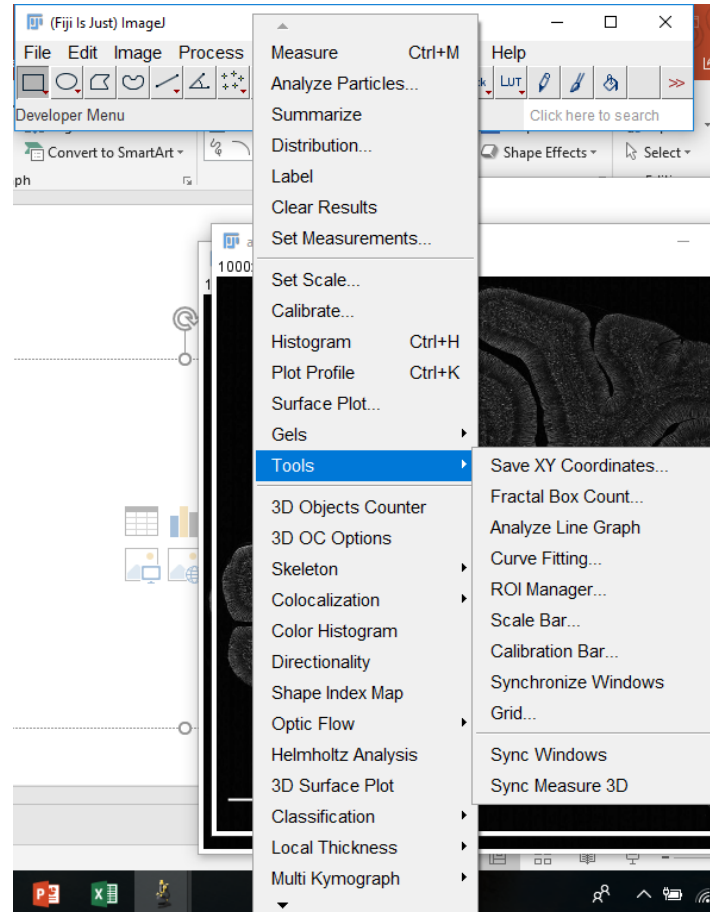
What I know from looking at the Histogram

- This image is 8 bit (intensity values range from 0-256)
- This image has a lot of dark (low signal) pixels
- This image has few or no bright pixels (pixels reaching 256)
- If I click “Auto” I can see that this image has a number of pixels at intensity value 254



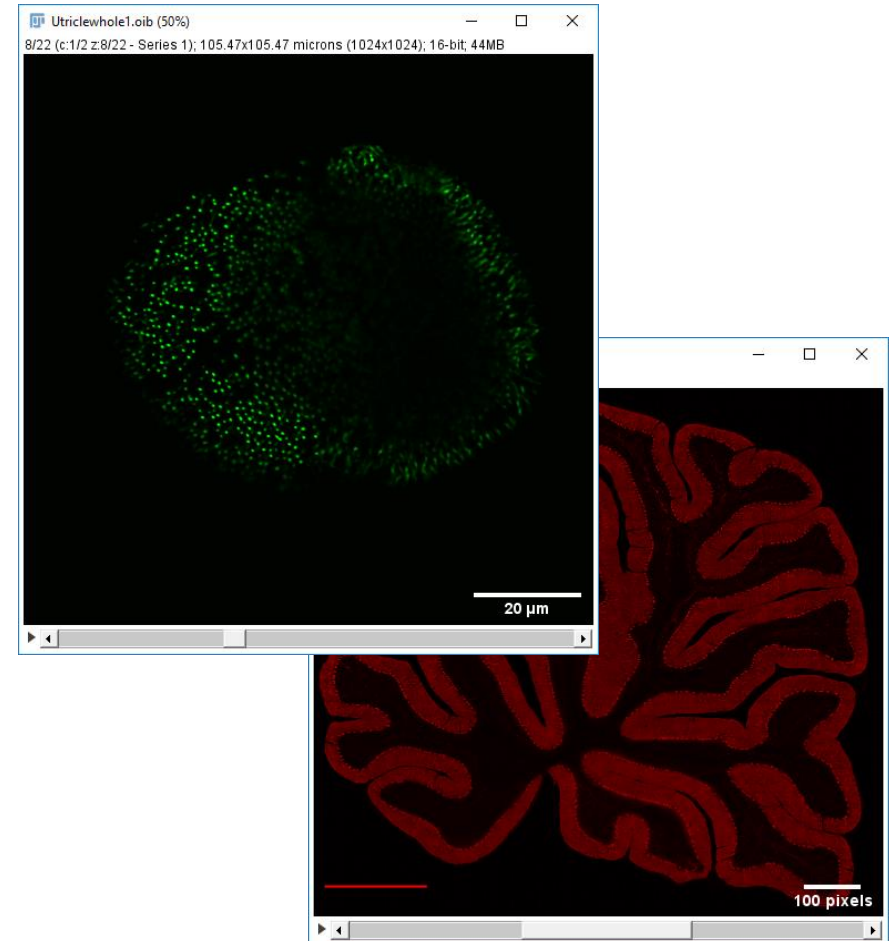
Adding a Scale Bar

- Scale bars are located under Analyze
- It's a little hidden Analyze > Tools > Scale Bar



Scale Bar Continued

- If the image is displaying the scale in pixels and not microns then resolution was not imported with the image
- Go to Image > Image Info to take a look at the information collected.
- Go to Image > Properties to see the pixel information that has (or has not) been saved with your image.
- Saving images as the native format is best



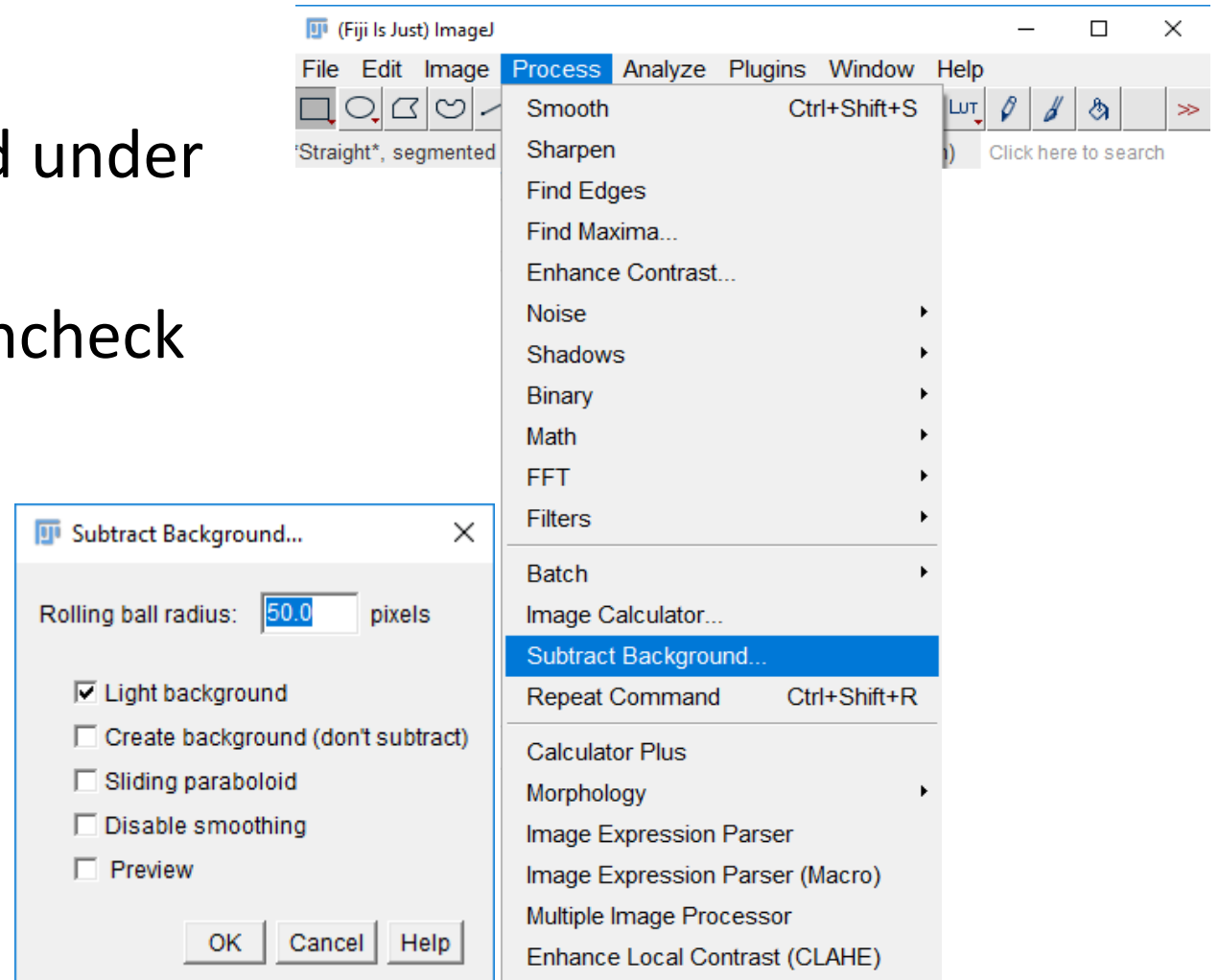
Preparing for Analysis

Removing Noise –Median Filtering

- Single pixel noise can ruin analysis
- Removing noise should be applied prior to segmenting your particles for measurement.
- Median filter = nonlinear digital filtering technique
- Distinguishes out-of-range isolated noise from legitimate image features such as edges and lines.
- Specifically, the median filter replaces a pixel by the median, instead of the average, of all pixels in a neighborhood
- Process > Filters > Median

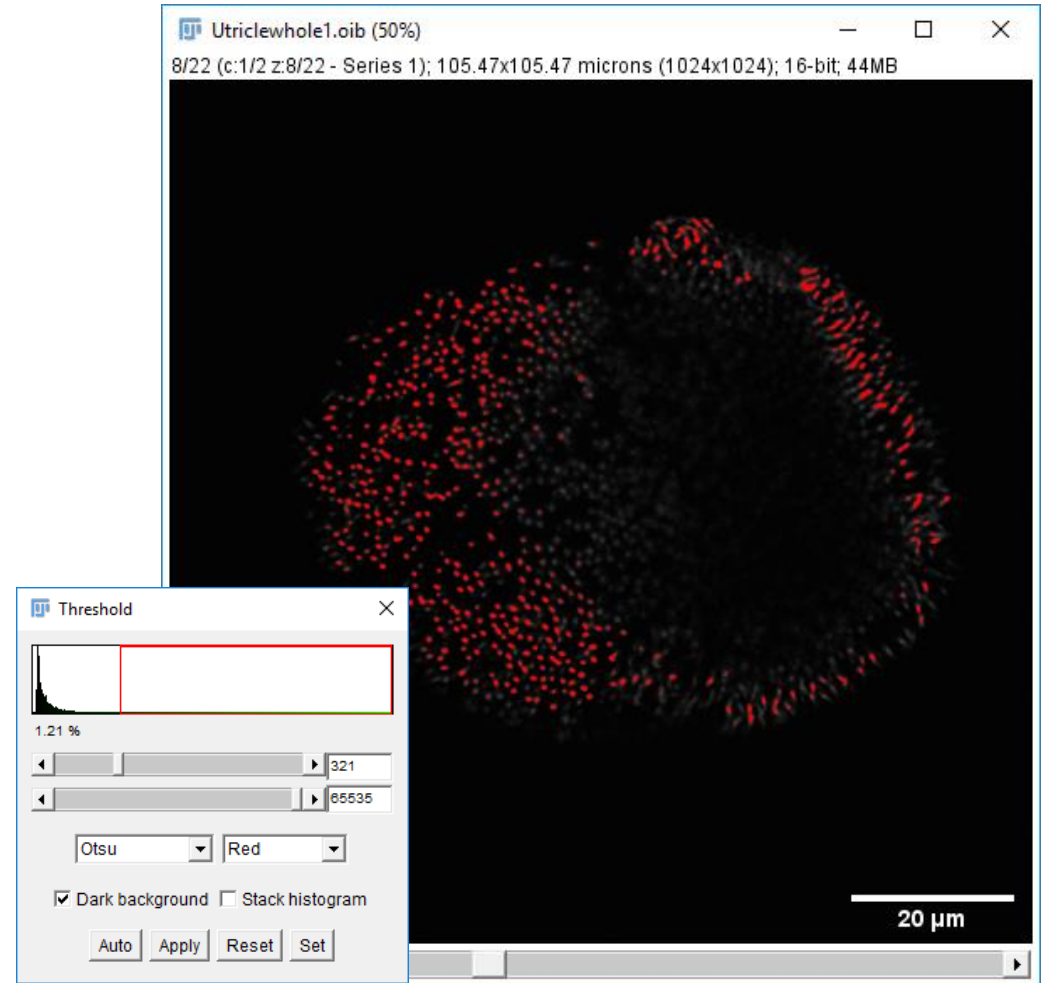
Removing Background

- Background subtraction is found under Process in the menu
- For fluorescent images you uncheck “Light Background”
- Use the “Preview” checkbox to see your progress
- 50 is a good starting point



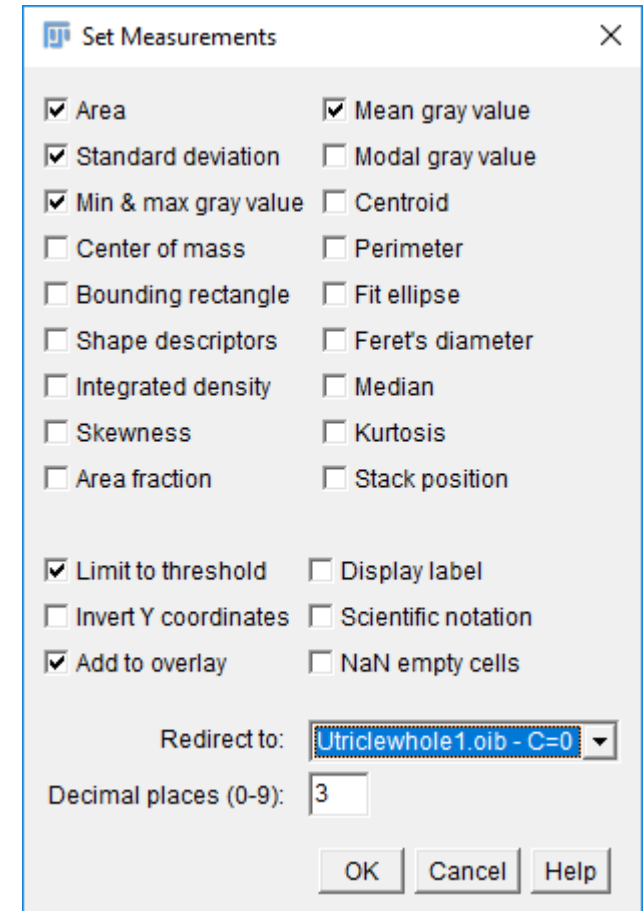
Segmentation/Threshold

- Partitioning a digital image into multiple segments.
- Simplifies the representation of an image into something that is more meaningful and easier to analyze
- Image > Adjust > Threshold



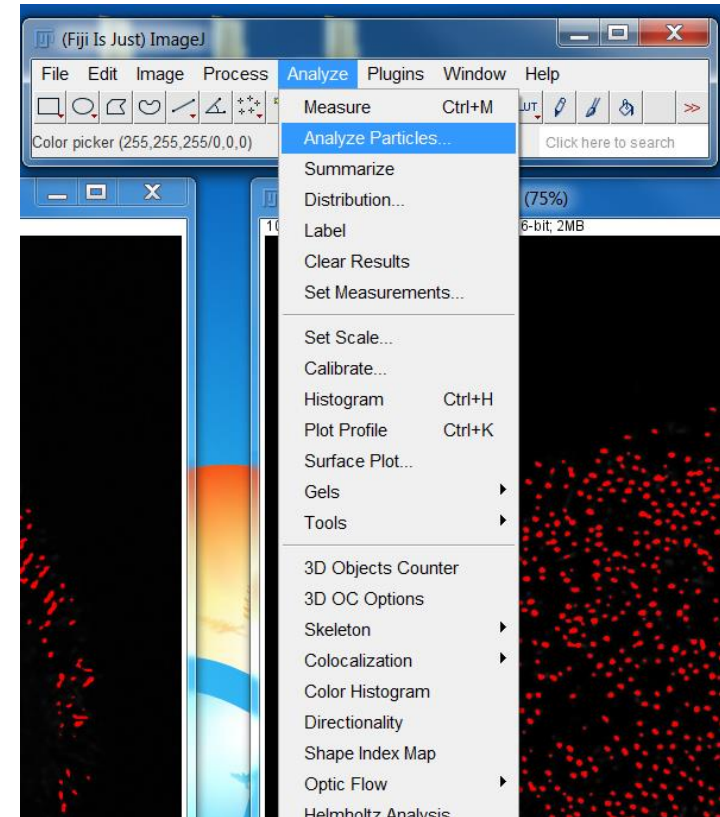
Measuring: Set Measurement

- Basic analysis functions
- Choose what you want to measure and view
- Go to Analyze > Set Measurement
- Area, Mean Gray Value, Standard Deviation, Min/Max



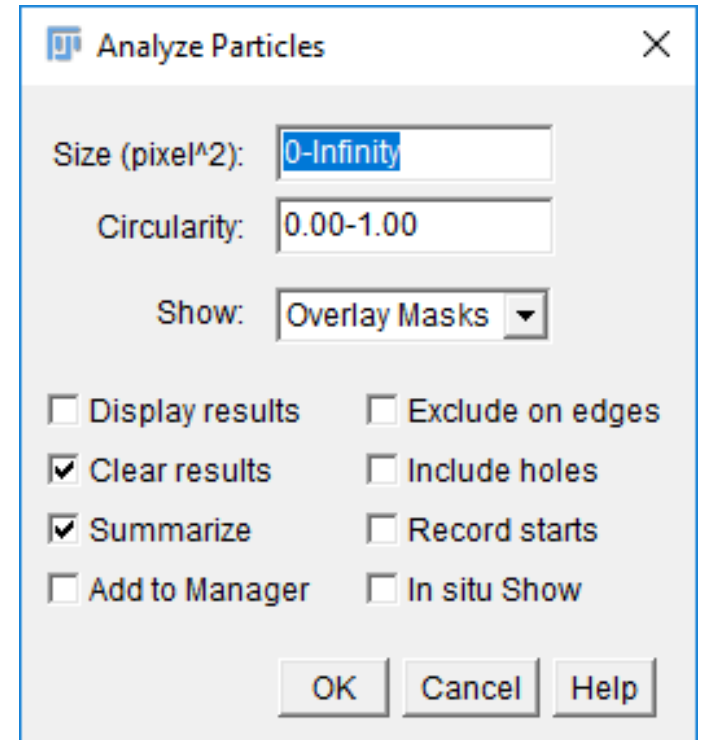
Analyze Particle

- Counts and measures objects in binary or thresholded images
- Finds edges of objects and measures them

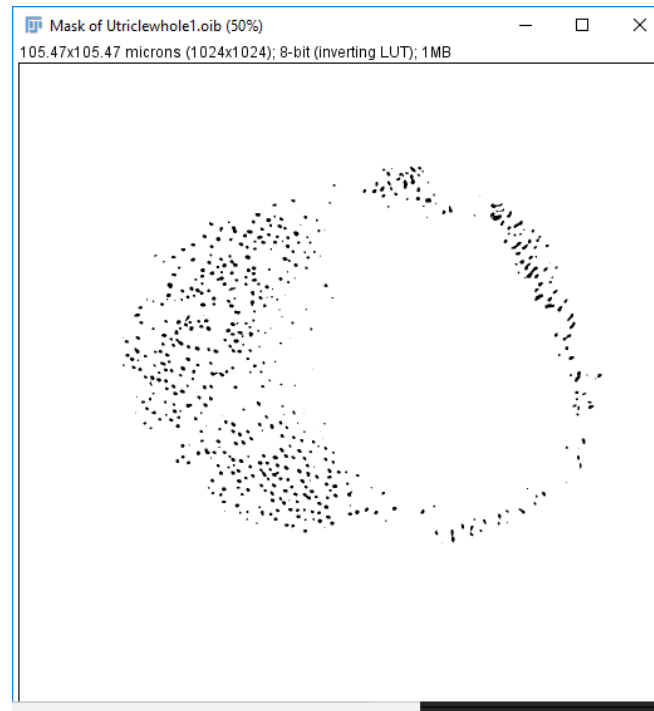
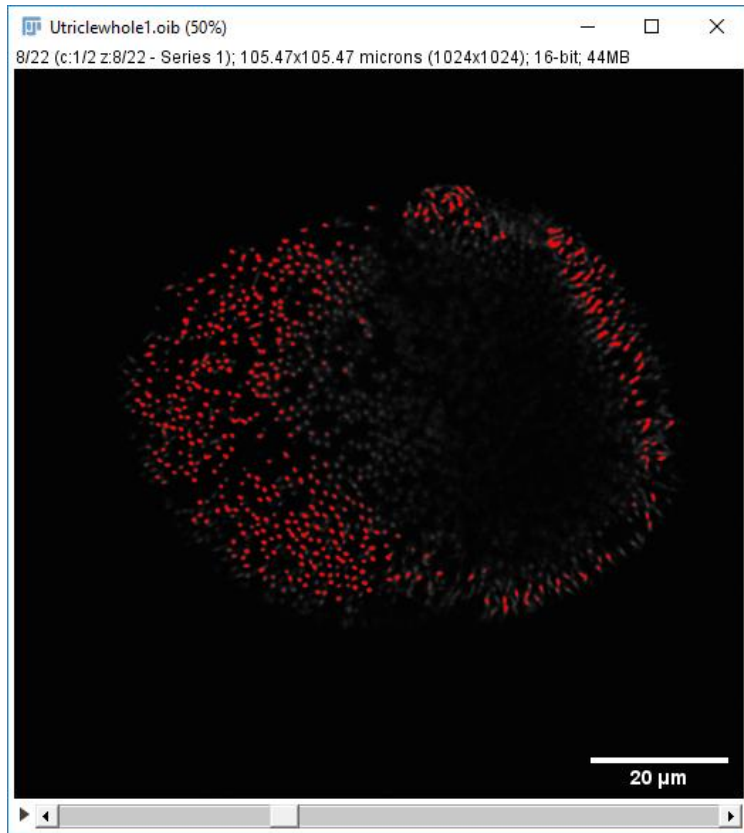


Measuring and Analyzing Particles

- Size (pixel²): ability to exclude a size range
- Circularity: Excludes particles that are less circular (1 = perfect circle)
- Show: How do you want to visualize what was counted
- Checkboxes: Choose what to measure and see



Measure Particle Results



Results

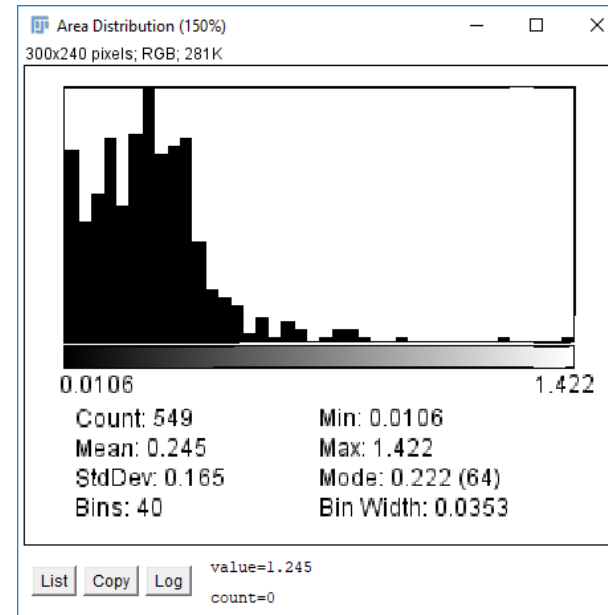
File	Area	Mean	StdDev	Min	Max
537	0.032	329.333	0.577	329	330
538	0.064	362.500	24.288	340	385
539	0.276	490.538	114.511	331	640
540	0.308	408.931	64.523	326	533
541	0.223	446.524	85.744	323	563
542	0.318	404.600	54.327	326	496
543	0.021	332.000	5.657	328	336
544	0.265	475.400	105.246	336	643
545	0.796	427.547	82.964	321	643
546	0.138	357.385	25.438	329	406
547	0.265	405.000	46.232	321	485
548	0.233	354.727	30.485	321	431
549	0.011	324.000	0.000	324	324

Summary of Utriclewhole1.oib

File	Count	Total Area	Average Size	%Area	Mean
c:1/2 z:8/22 - Series 1	549	134.565	0.245	1.210	567.857

Distribution

- Visualize the distribution of particles by your specifications
- Hover cursor over any of the bars to see the size



Distribution

Parameter: Area

549 data points

Automatic binning

or specify bins: 40

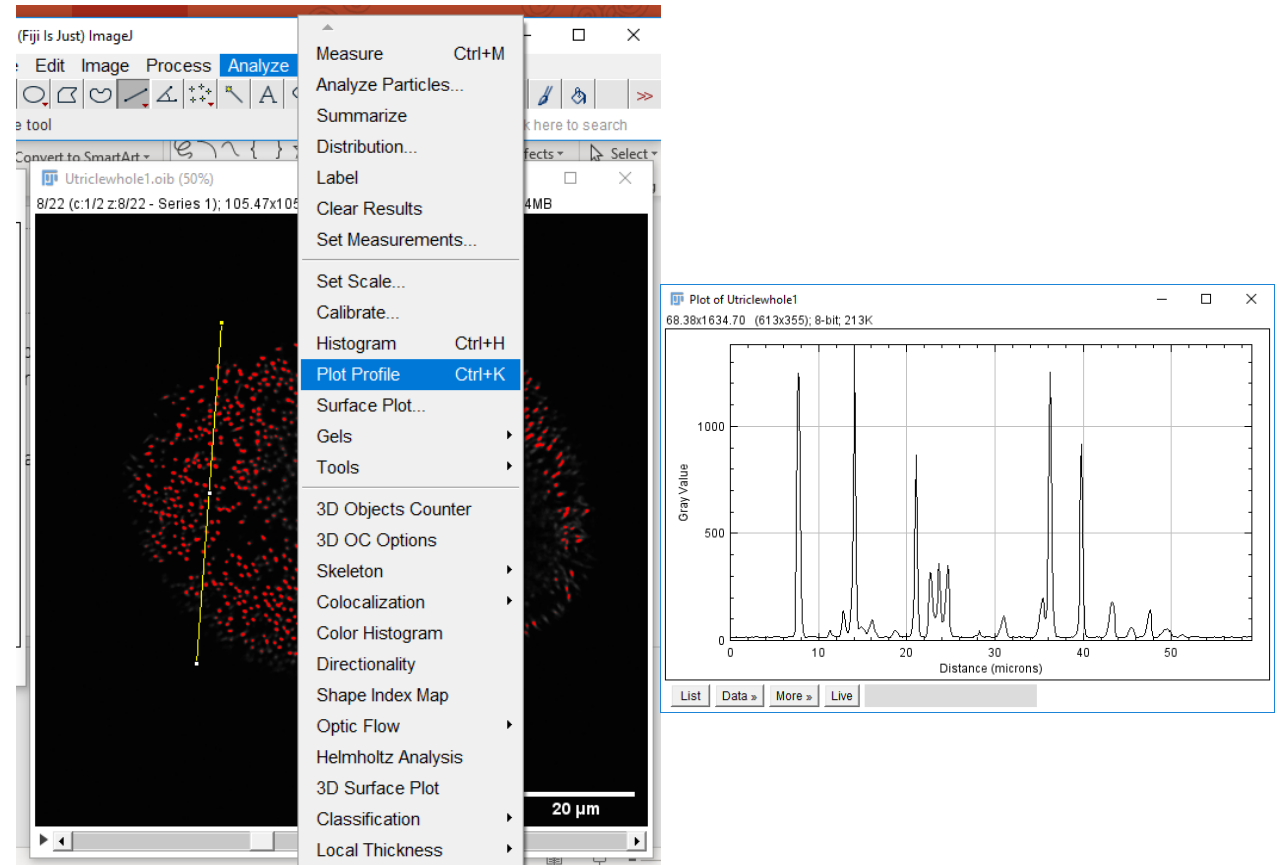
and range: 0-0

OK Cancel

Detailed description: A 'Distribution' dialog box with a close button (X). It contains a 'Parameter' dropdown menu set to 'Area'. Below it, it shows '549 data points'. There is an unchecked checkbox for 'Automatic binning'. Below that, it says 'or specify bins:' followed by a text input field containing '40'. Below that, it says 'and range:' followed by a text input field containing '0-0'. At the bottom, there are 'OK' and 'Cancel' buttons.

Plot Profile

- Intensity across a defined region
- Use with line or square



ROI Manager

- Manages your regions
- Add and measure same region on other image (colocalization)

