

Introduction to Image Processing & Analysis with Fiji



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Where to find the workshop documentation ?

USB Keys

Ateliers Fiji 2019

Sample Images from ImageJ

Fiji Installers

Online:

https://github.com/bvernay/Fiji_Workshop

Where to find help after the workshop ?

- ImageJ Website <https://imagej.net/>
- Fiji Website <https://fiji.sc/>
- Forum <https://forum.image.sc>
- Microscopy Facility groupe-mic-photon@igbmc.fr
- Your colleagues

Workshop rules

Ask many questions,
...
And ask more questions

Digital Image

Image Acquisition Mode



Widefield



Spinning Disk

Confocal



Lamp & Camera

Lasers & Camera

Laser & Photomultiplier

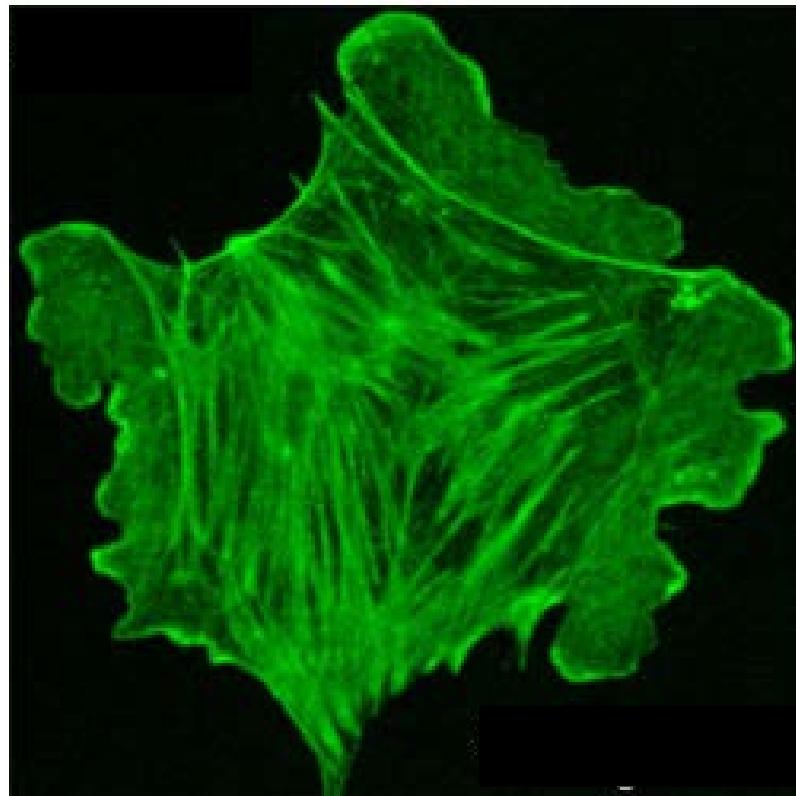
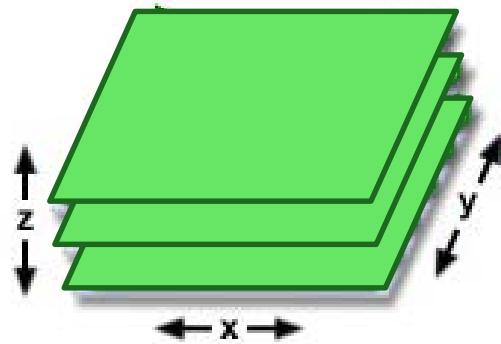
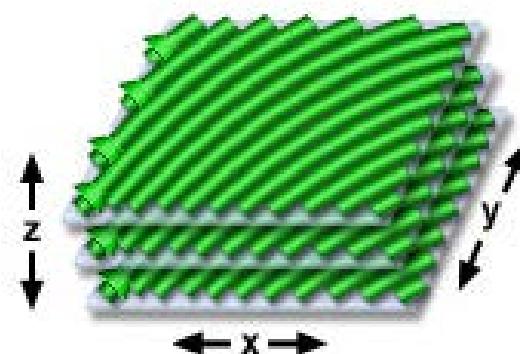


Image Acquisition Mode

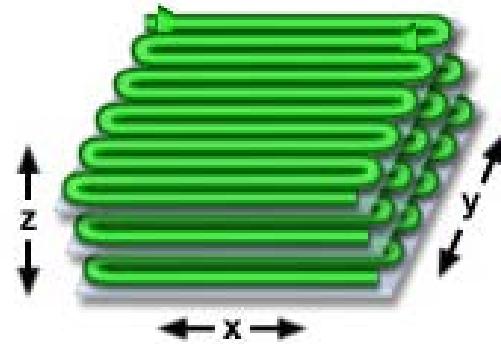
Widefield



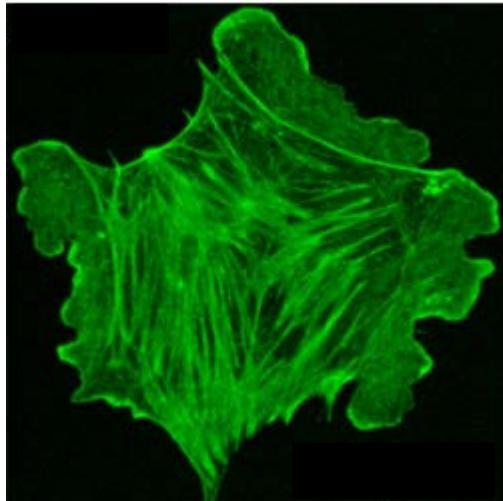
Spinning Disk



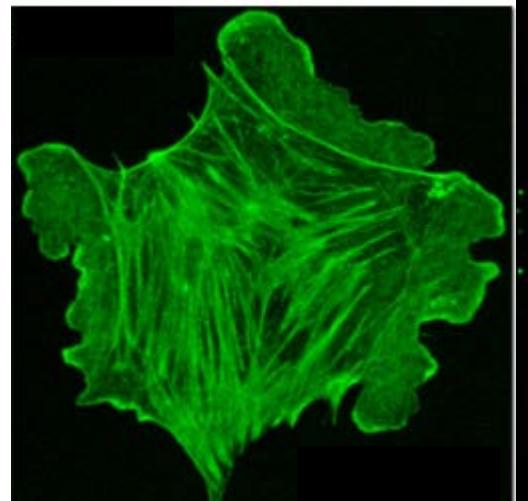
Confocal



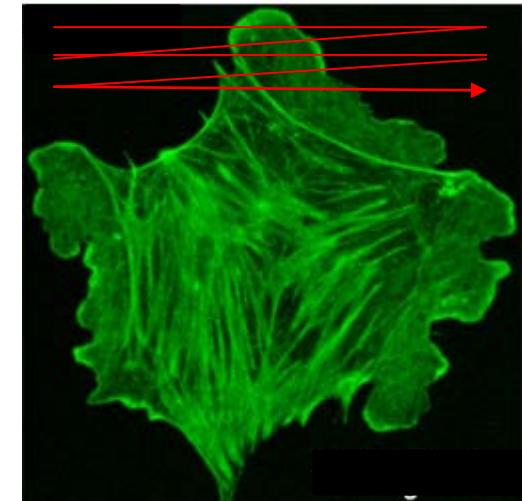
Full frame grabbing



Multiple points scanning

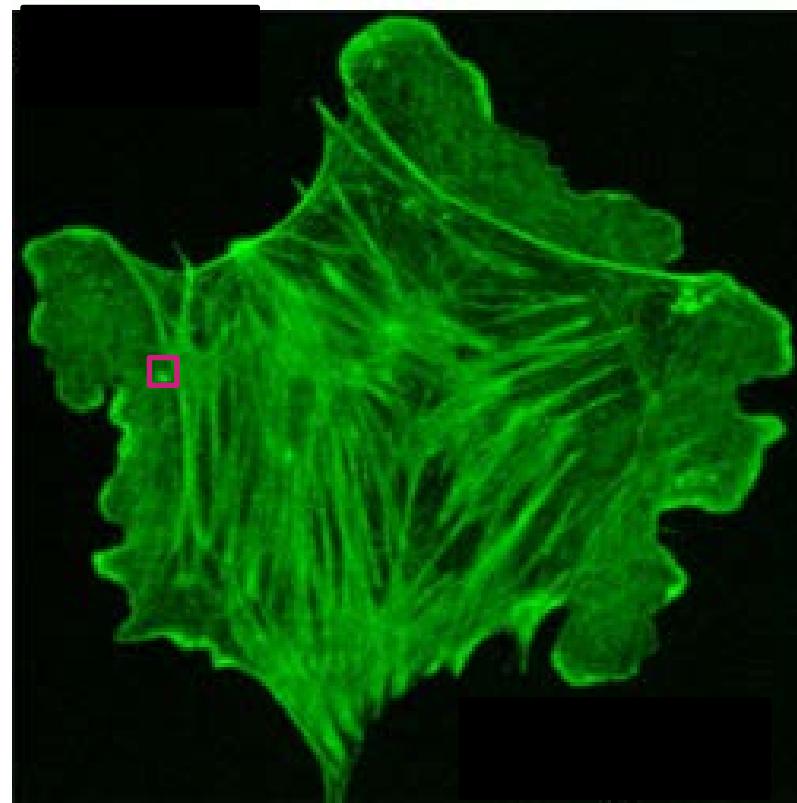


Single point scanning



Pixels

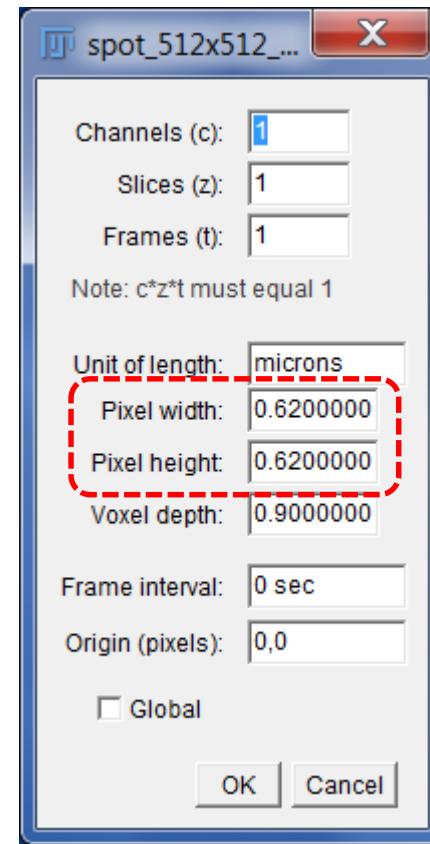
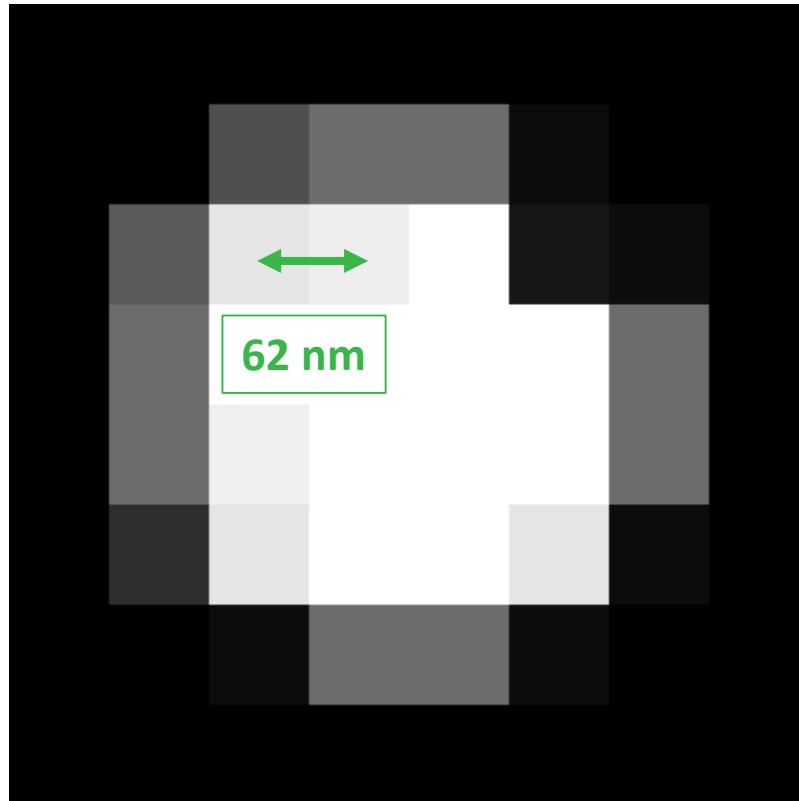
An image is recorded as a numerical array of pixels



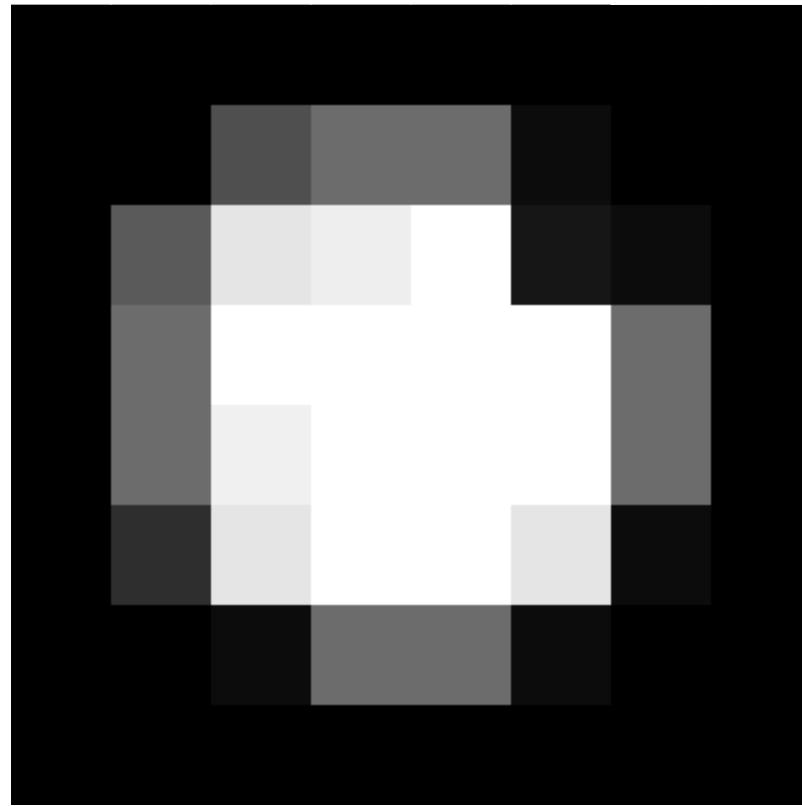
Each pixel has its defined

- size depending on the sampling resolution
- Intensity value with a range defined by the bit depth

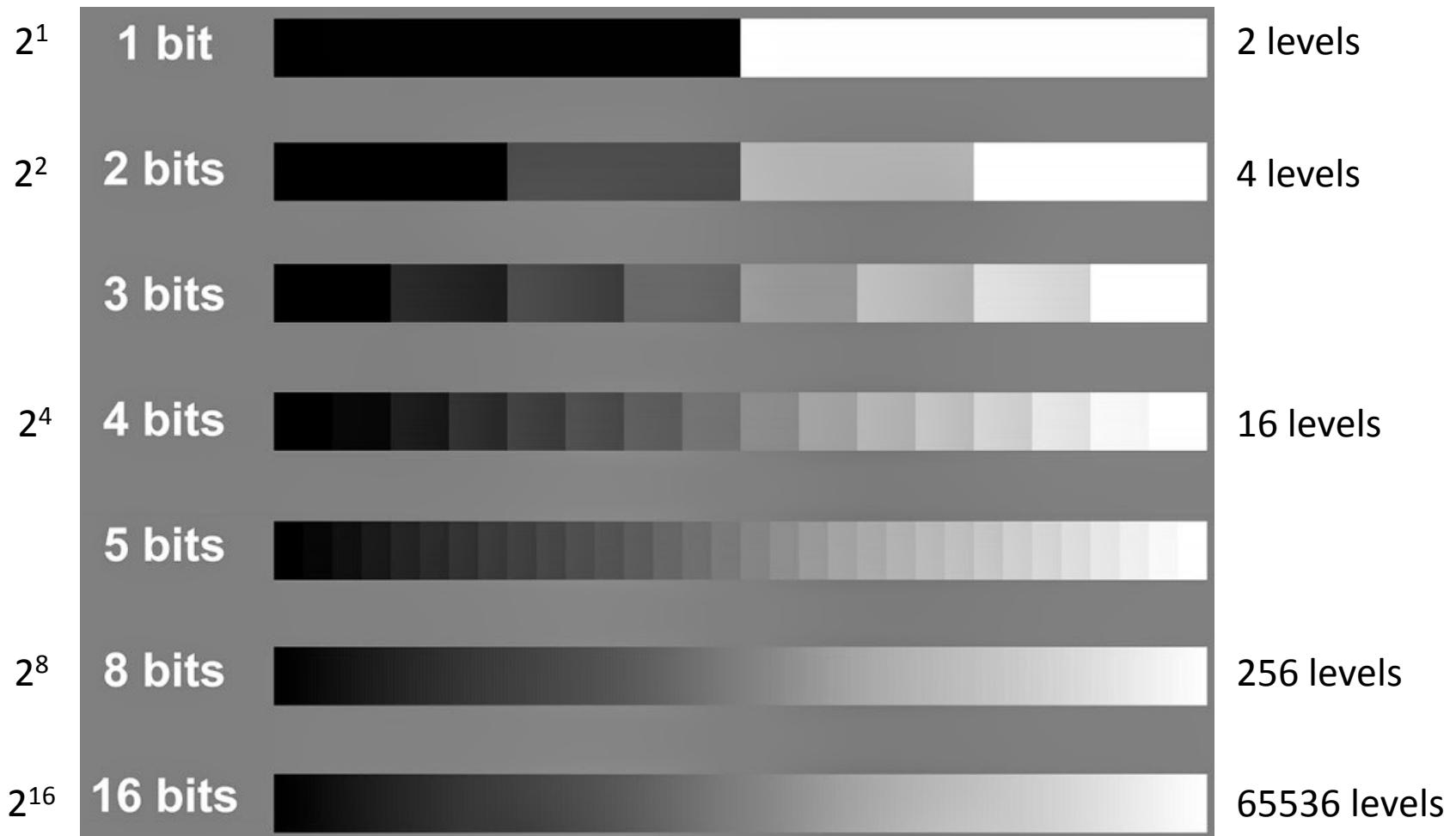
Size



Pixel Intensity



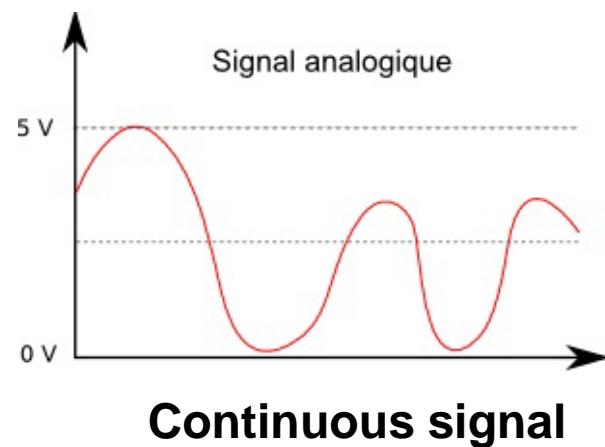
Bit Depth



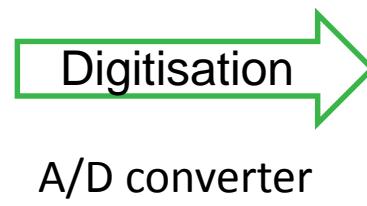
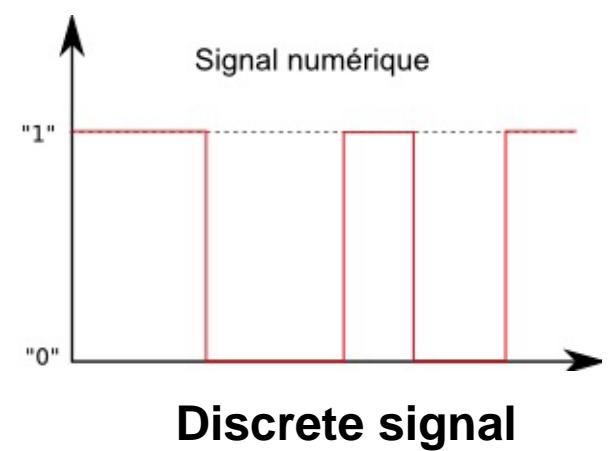
32-bits (2^{32}) format for image processing 4 294 967 296 levels

Digitisation

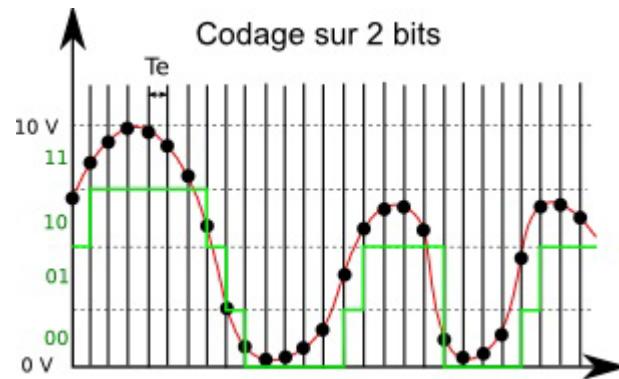
Detector



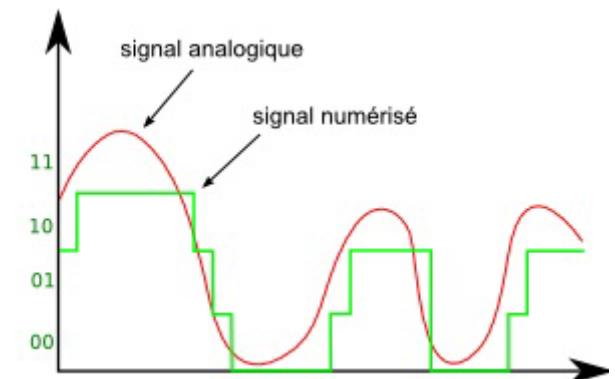
Computer



Digitisation

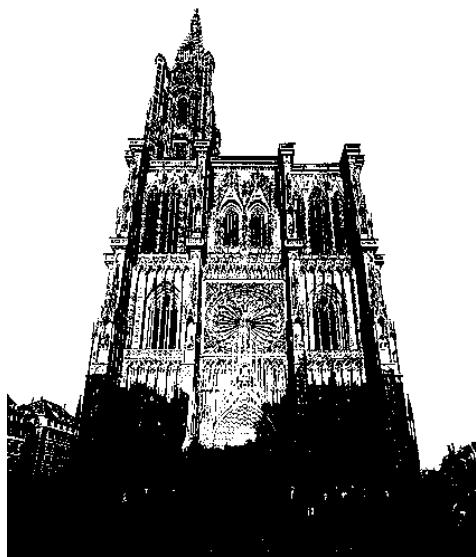


2-bits
4 values

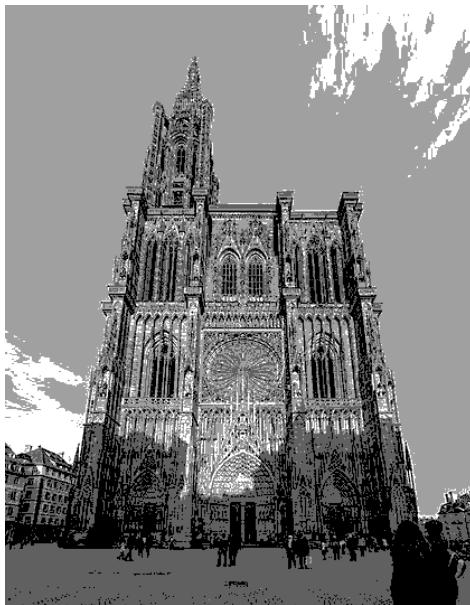


→ Convert analog signal into a digital (i.e. computer-readable) format , in which the information is organized into bits

Bit Depth



1-bit
2 values



2-bits
4 values



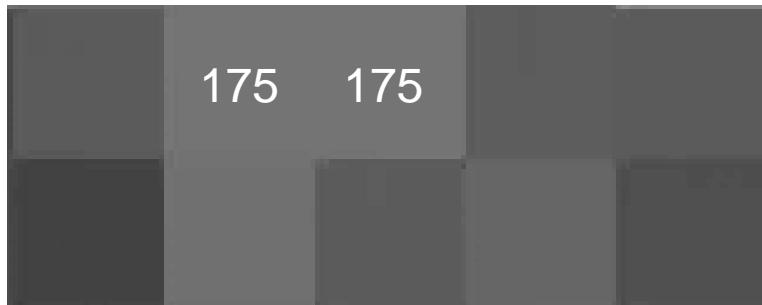
4-bits
16 values



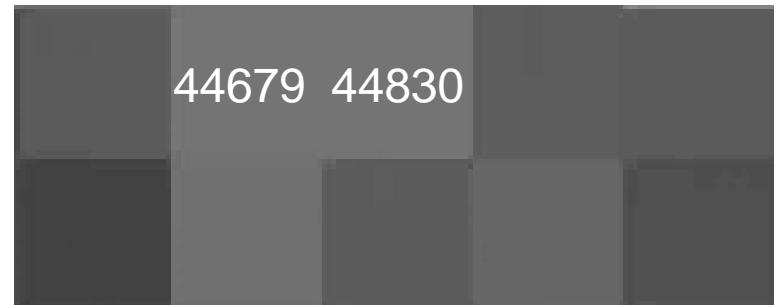
8-bits
256 values

Bit Depth : 8-bits versus 16-bits

8-bits, range 0-255



16-bits, range 0-65535



- Look the same on the screen
- Pixel values are different
- Screen bit-depth = 8-bits
- human bit-depth = ~ 8-bits
- 16-bits finer intensity steps but not distinguishable by human eyes only by using tools (profile, histogram, ...)

Using the Right Bit Depth

Bit depth	Gray levels	Use	ICL Equipment
1	2	Binary masks for image analysis	FIJI, Imaris, Metamorph
8	256	Scientific cameras, PMT, HyD	Widefield, Confocal, SD
12	4096	Scientific cameras, PMT, HyD	Confocal
16	65,536	Scientific cameras, PMT, HyD	Widefield, Confocal, SD
32	4,294,967,296	Image analysis	FIJI, Imaris, Metamorph

Visualisation = 8-bits, Quantification = 12- or 16-bits

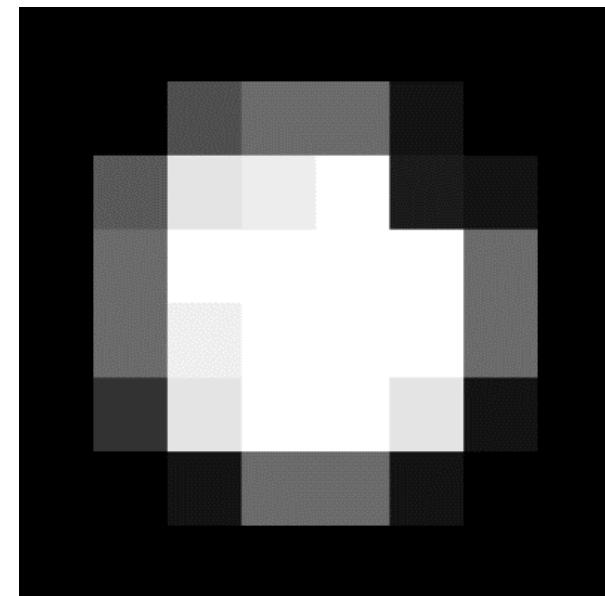
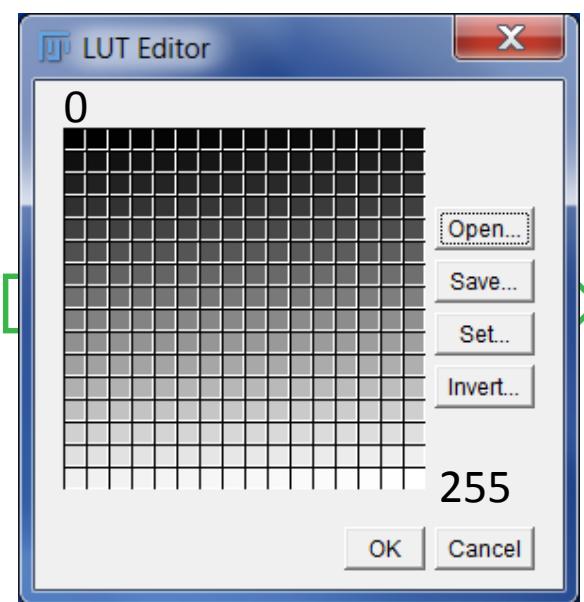
Look-up Table (LUT)

0	0	0	0	0	0	0	0
0	0	80	108	108	20	0	0
0	90	228	237	255	28	20	0
0	108	255	255	255	255	108	0
0	108	240	255	255	255	108	0
0	50	228	255	255	228	20	0
0	0	20	108	108	20	0	0
0	0	0	0	0	0	0	0

8-bits image, value range from 0 to 255

Look-up Table (LUT)

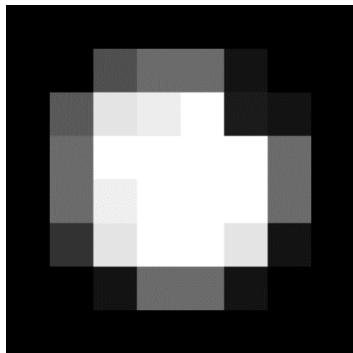
0	0	0	0	0	0	0	0
0	0	80	108	108	20	0	0
0	90	228	237	255	28	20	0
0	108	255	255	255	255	108	0
0	108	240	255	255	255	108	0
0	50	228	255	255	228	20	0
0	0	20	108	108	20	0	0
0	0	0	0	0	0	0	0



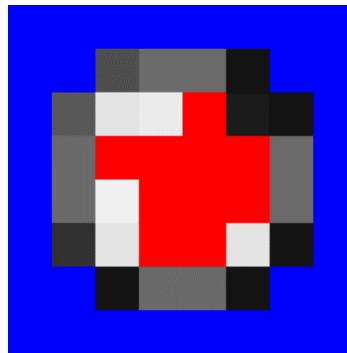
8-bits image, value range from 0 to 255

Look-up Table (LUT)

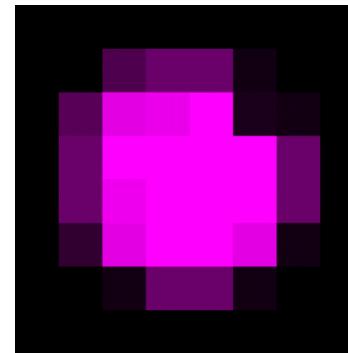
Grays



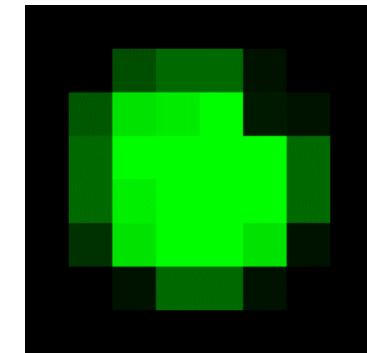
HiLo



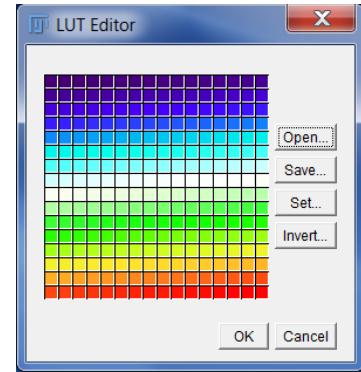
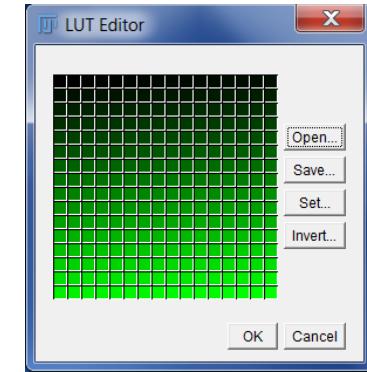
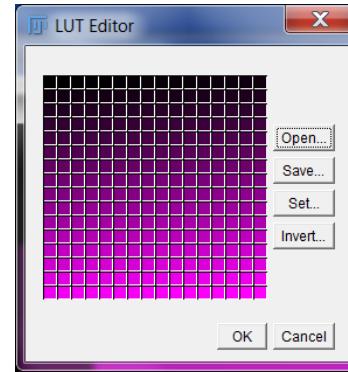
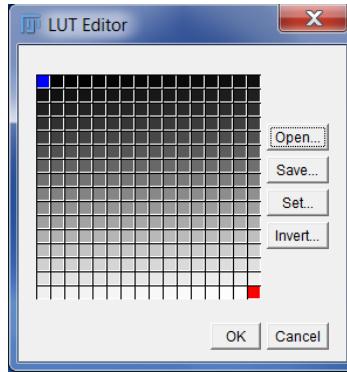
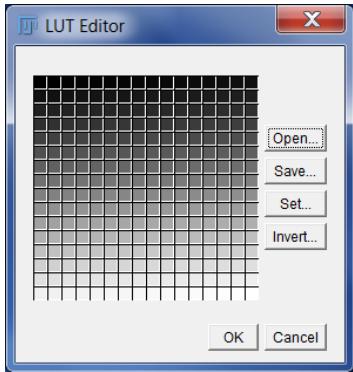
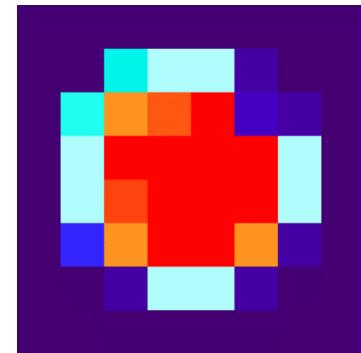
Magenta



Green

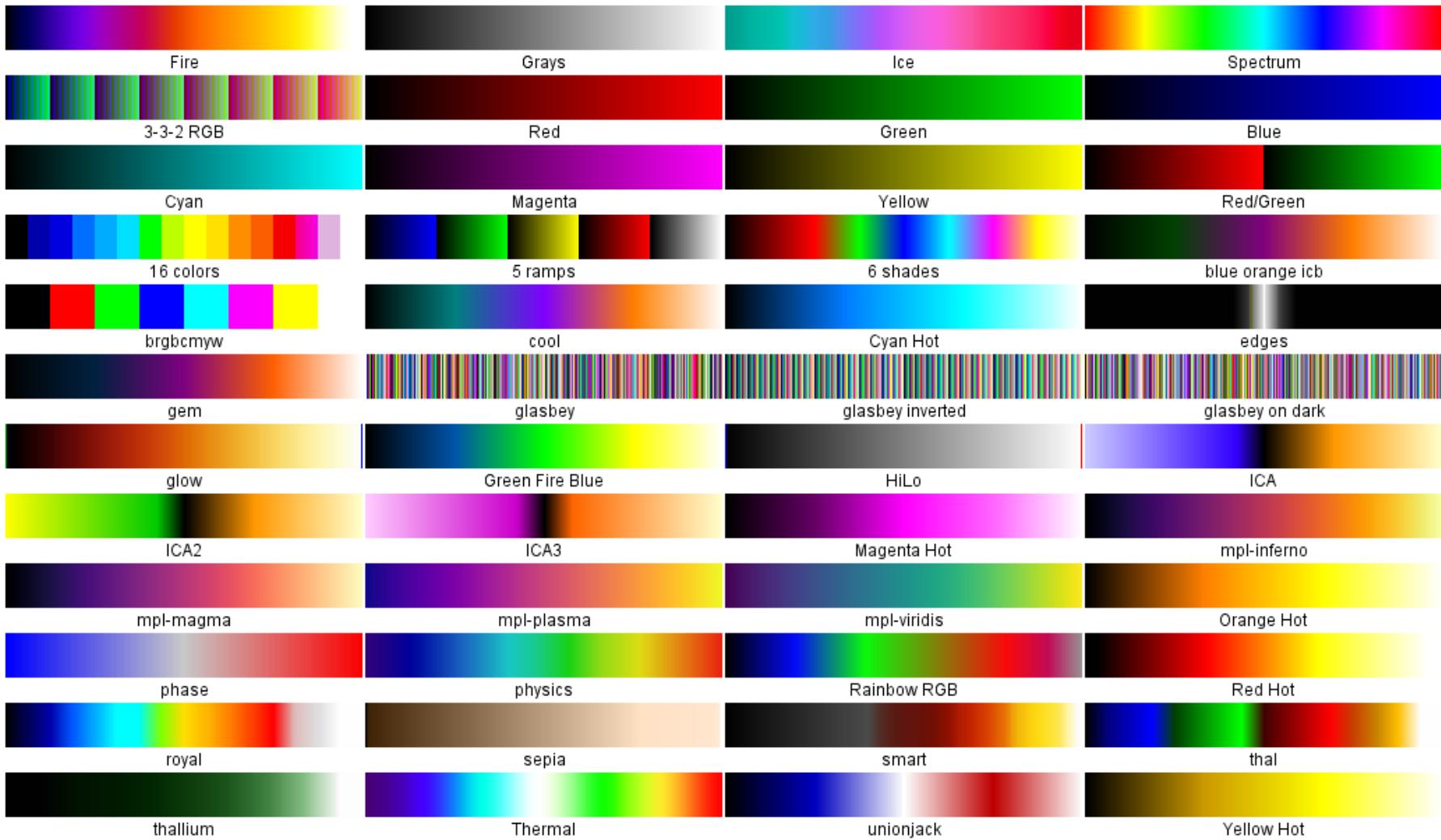


Thermal



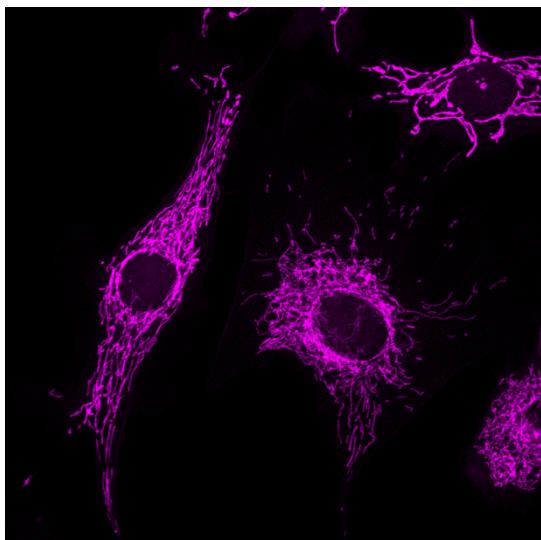
Picking the right LUT for the job helps to visualise, interpret and present your data

Fiji's LUT

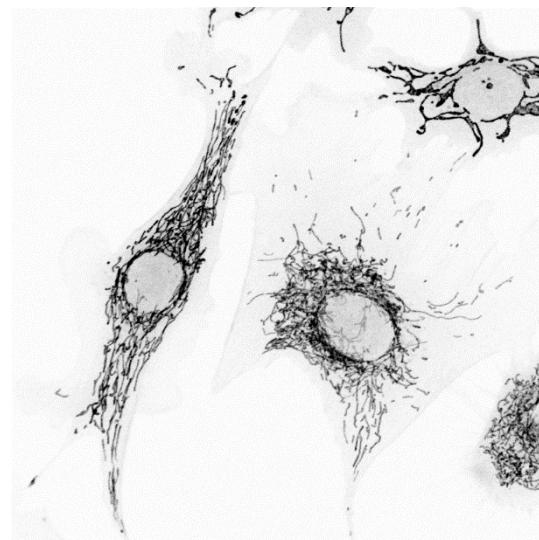


Look-up Table (LUT)

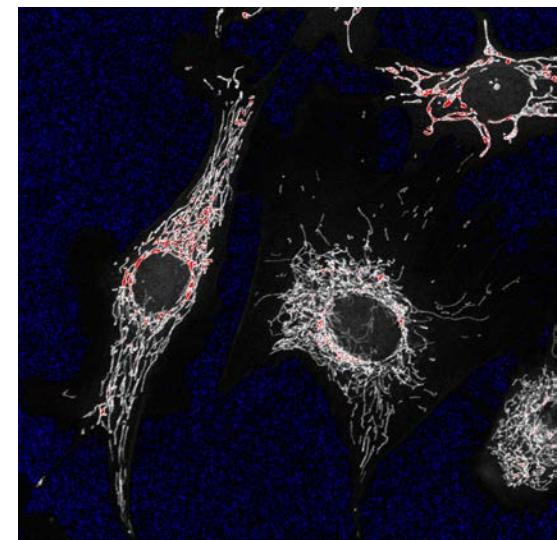
Colour LUT



Grays LUT

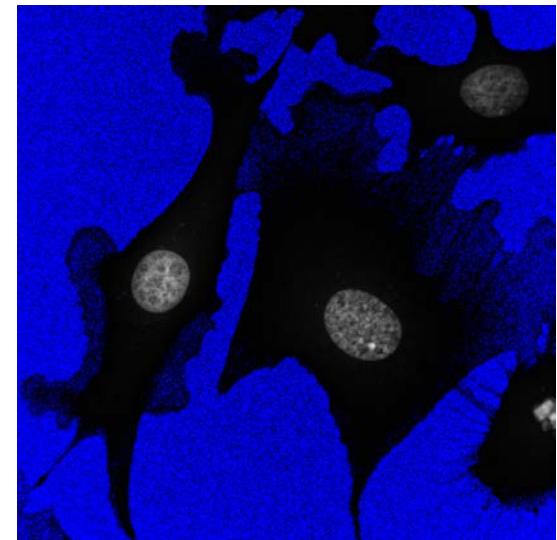
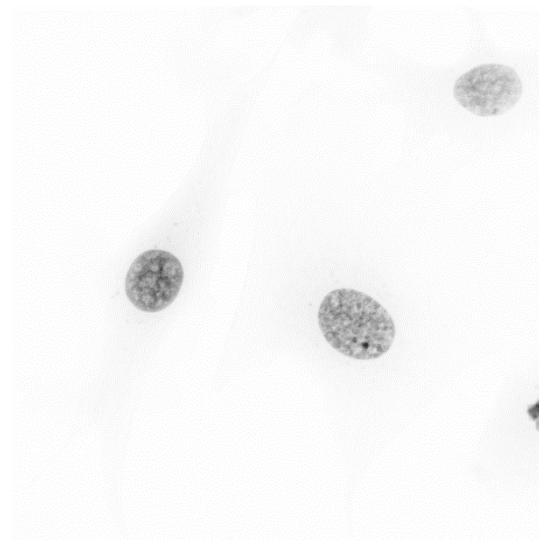
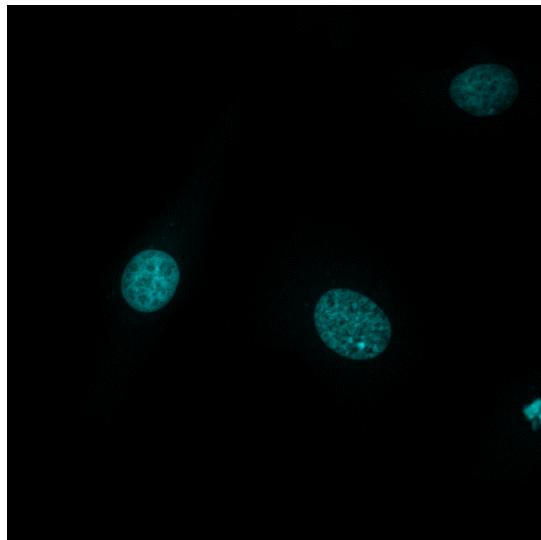


HiLo LUT



MitoTracker

DAPI

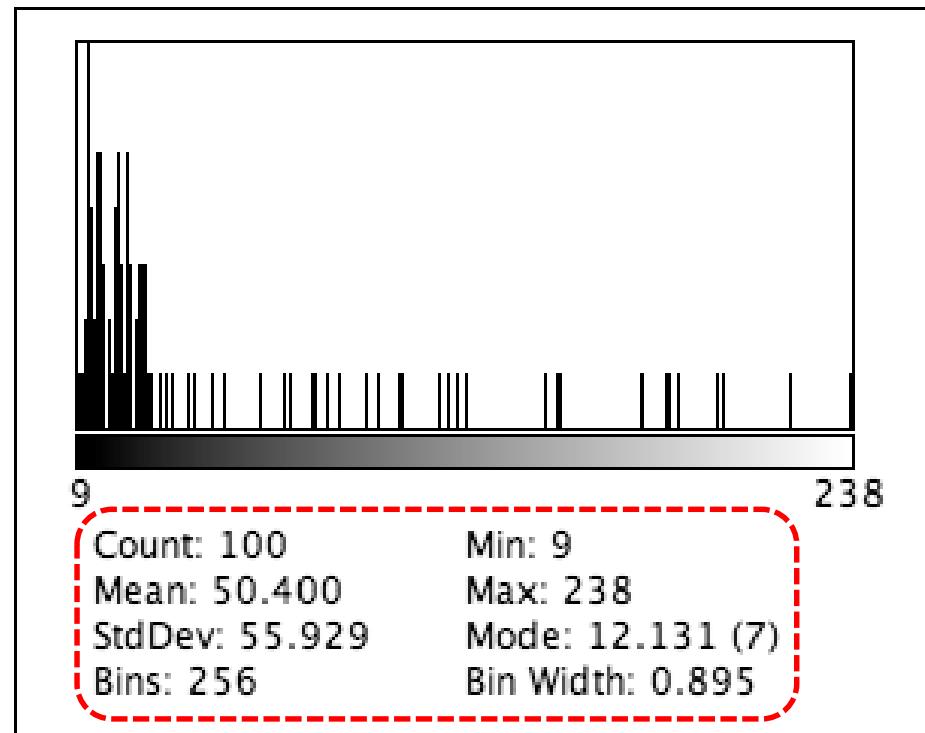
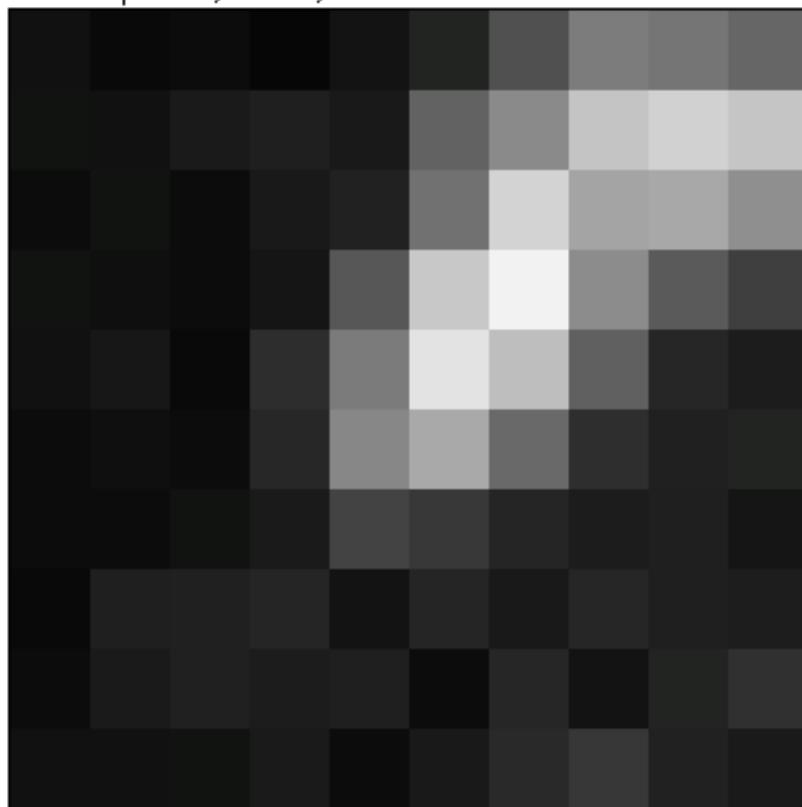


Range Indicator

Histogram

Distribution of pixels intensity (x axis) plotted against the number of pixel (Y axis) independently of their localisation within the picture

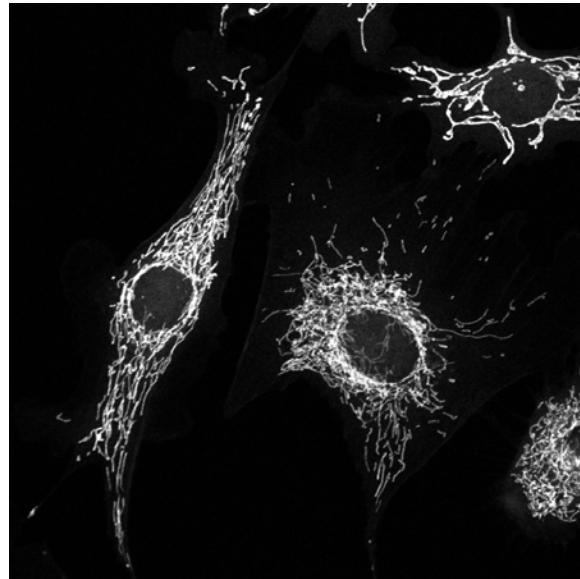
10x10 pixels; 8-bit; 0K



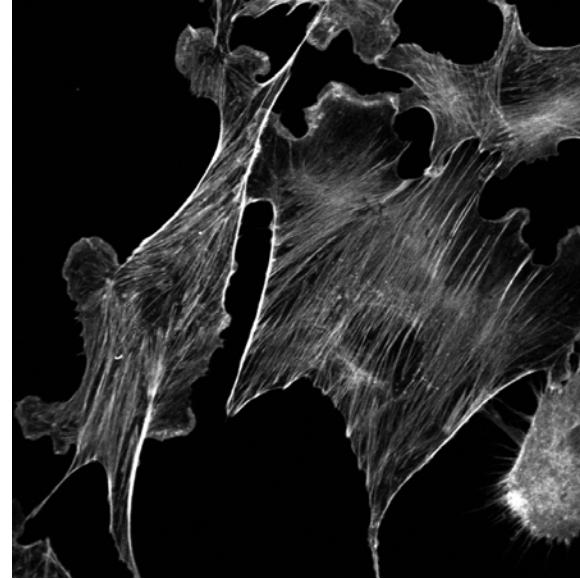
Pixel Statistics

Histogram

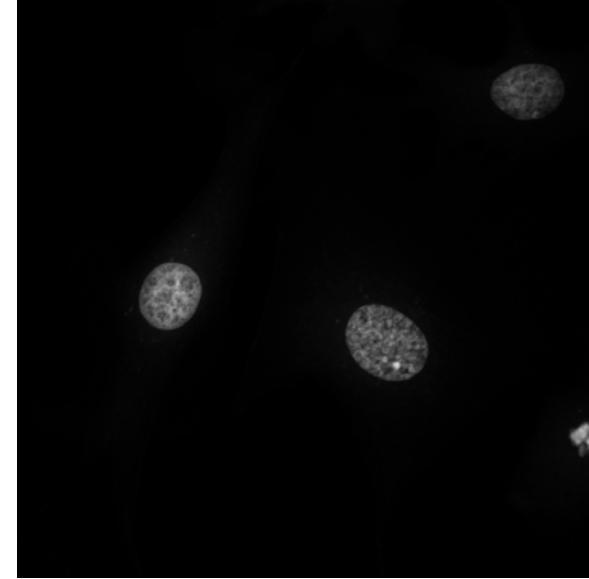
MitoTracker



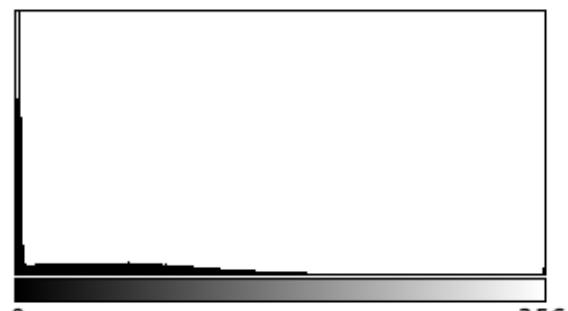
Actin



DAPI



Count: 739600 Min: 0
Mean: 21.371 Max: 255
StdDev: 45.341 Mode: 1 (143778)



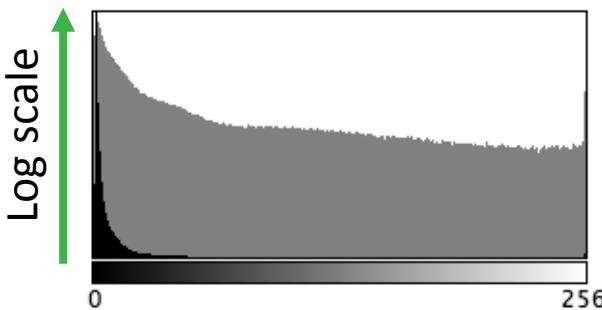
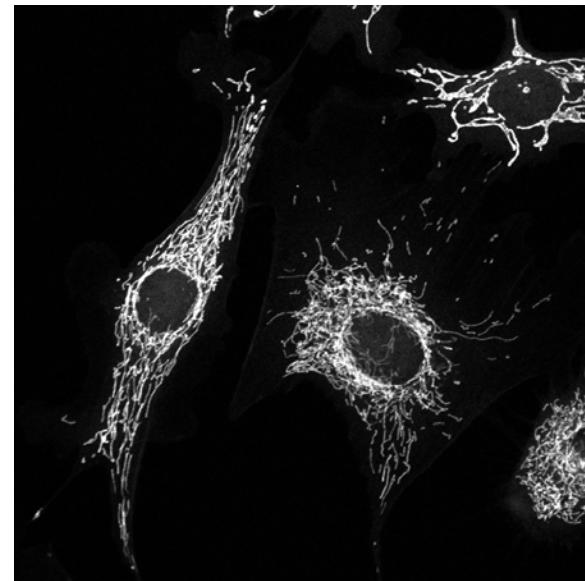
Count: 739600 Min: 0
Mean: 35.707 Max: 255
StdDev: 46.293 Mode: 1 (224321)



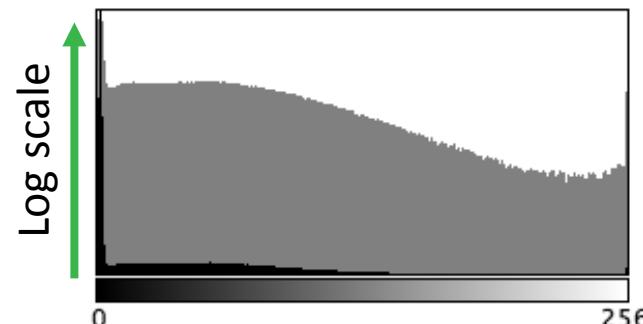
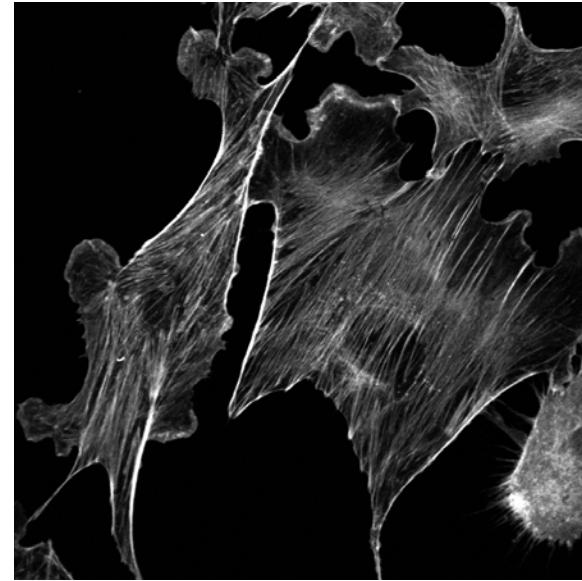
Count: 739600 Min: 0
Mean: 4.209 Max: 218
StdDev: 15.016 Mode: 0 (312533)

Histogram

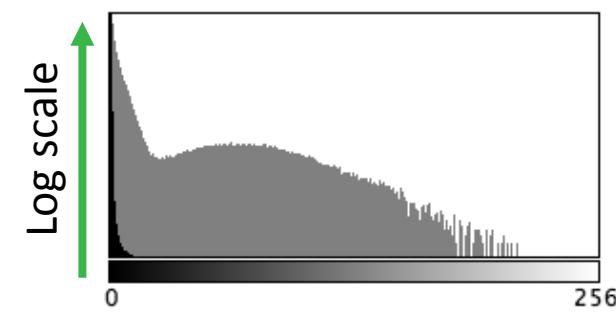
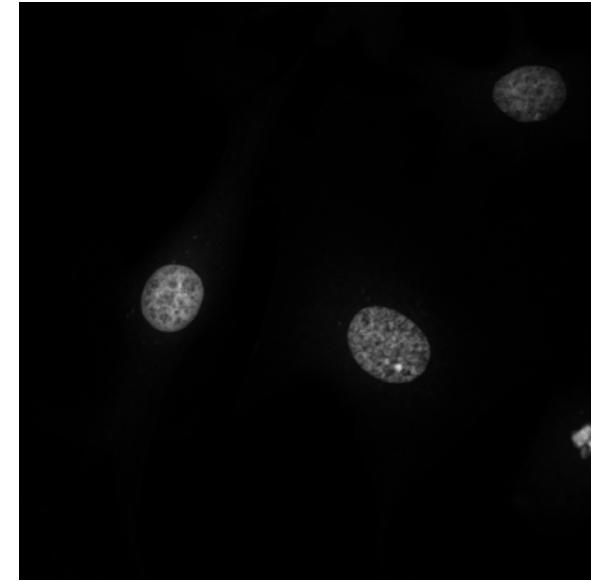
MitoTracker



Actin

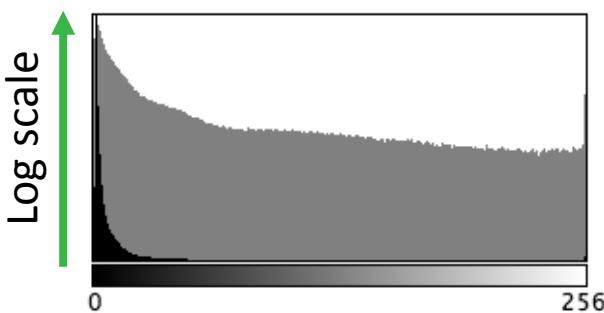
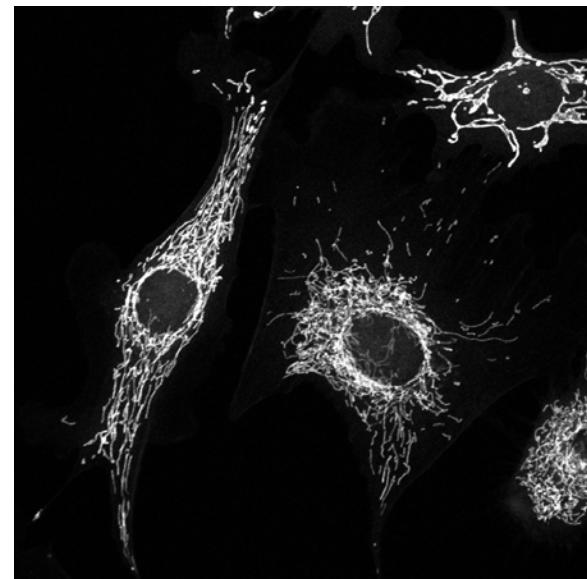


DAPI



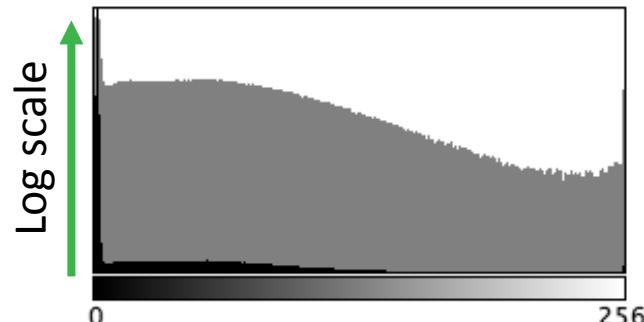
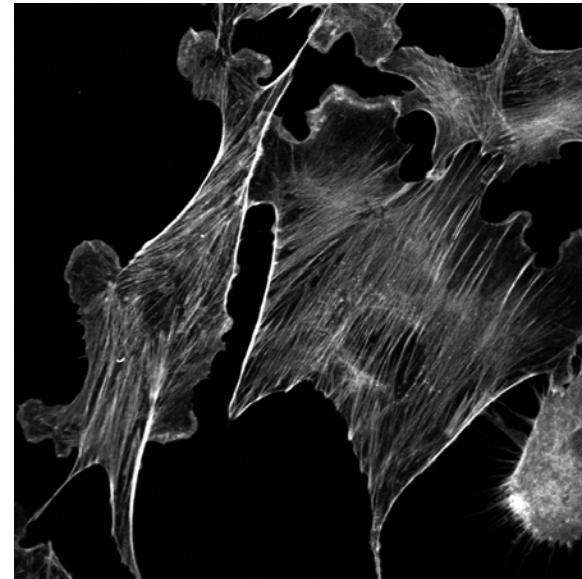
Histogram

MitoTracker



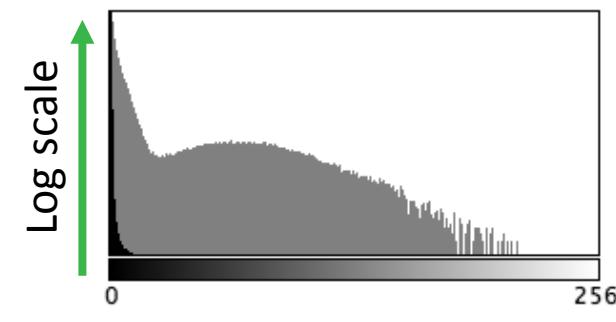
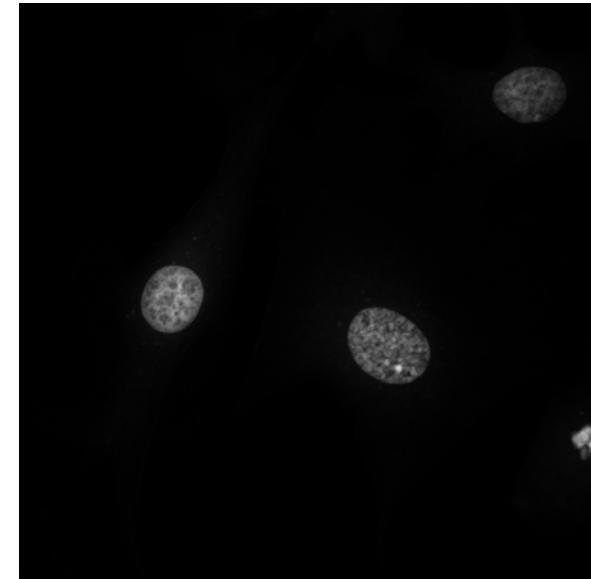
Count: 739600 Min: 0
Mean: 21.371 Max: 255
StdDev: 45.341 Mode: 1 (143778)

Actin



Count: 739600 Min: 0
Mean: 35.707 Max: 255
StdDev: 46.293 Mode: 1 (224321)

DAPI

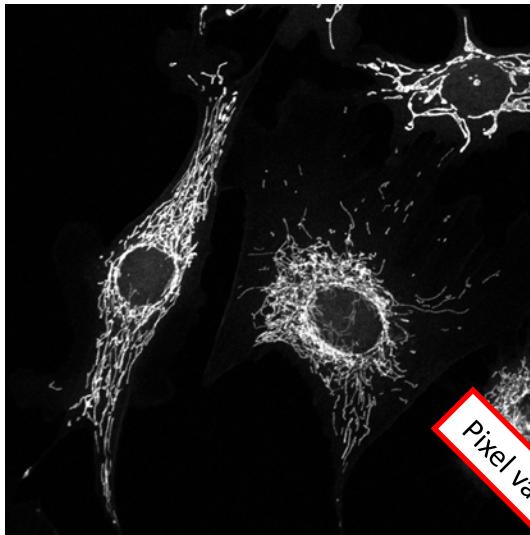


Count: 739600 Min: 0
Mean: 4.209 Max: 218
StdDev: 15.016 Mode: 0 (312533)

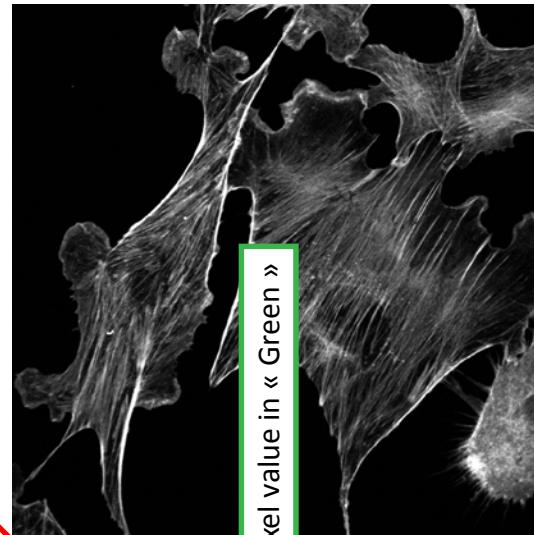
- Fill the dynamic range of the detector
- Do not clip the data (black threshold, saturation)

Merging Channels

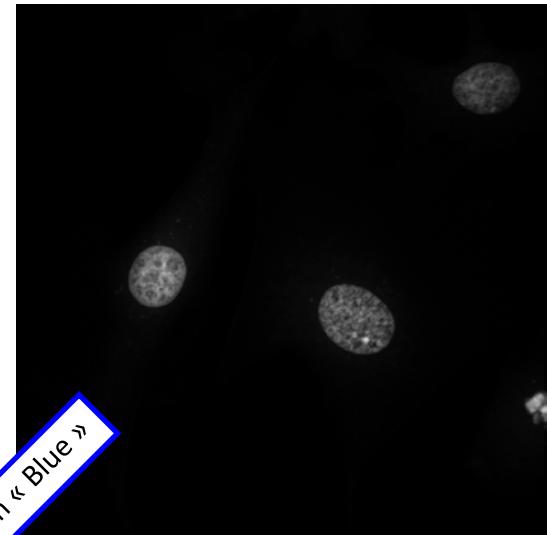
Red



Green



Blue

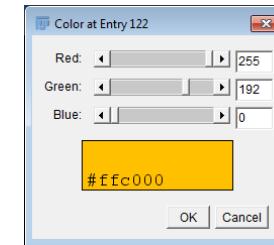
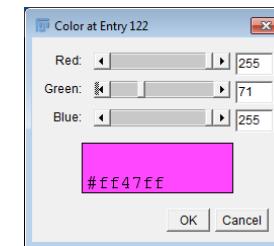
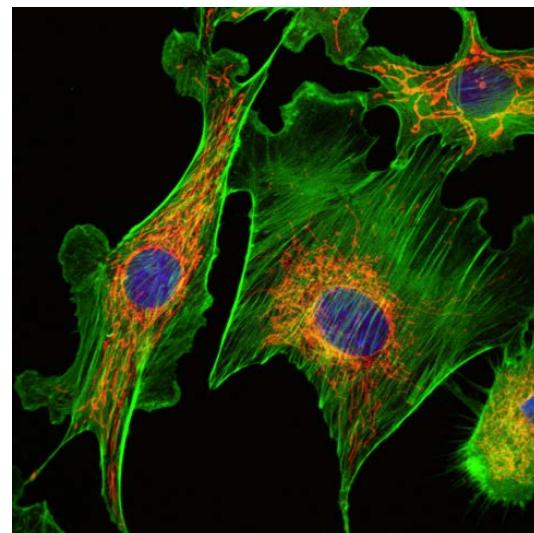


Pixel value in « Red »

Pixel value in « Green »

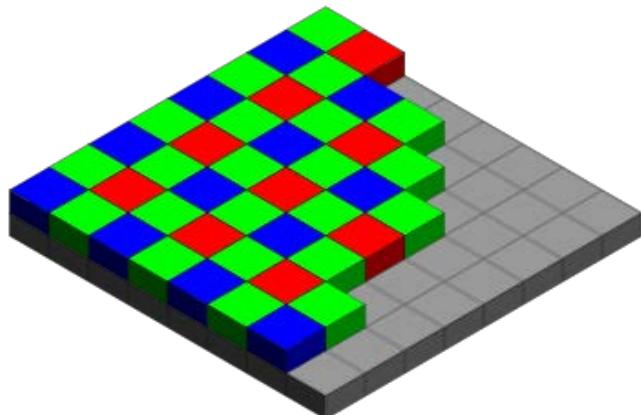
Pixel value in « Blue »

RGB colour

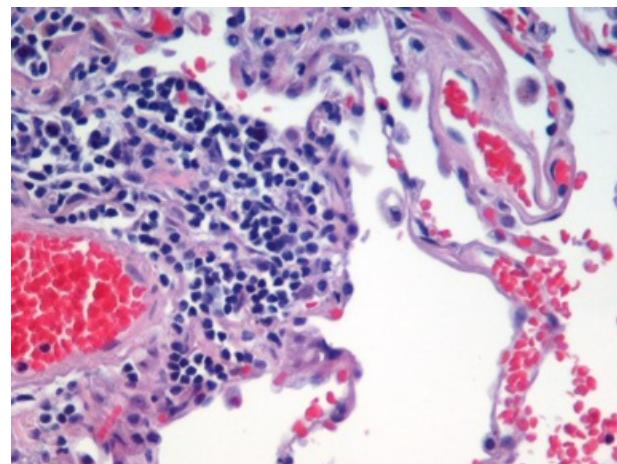
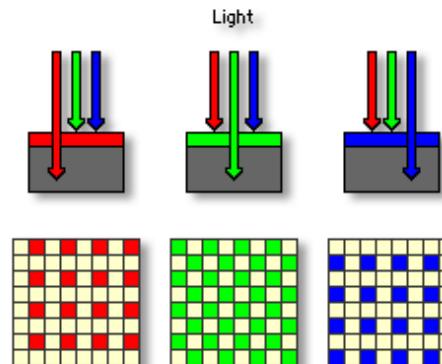


RGB Image: Colour Camera

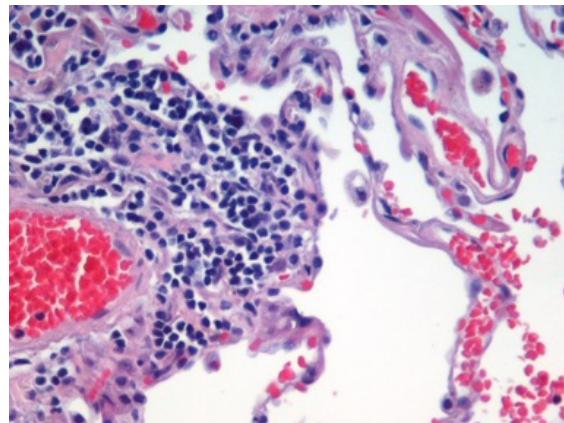
Bayer Matrix Sensor



Bayer Matrix Sensor



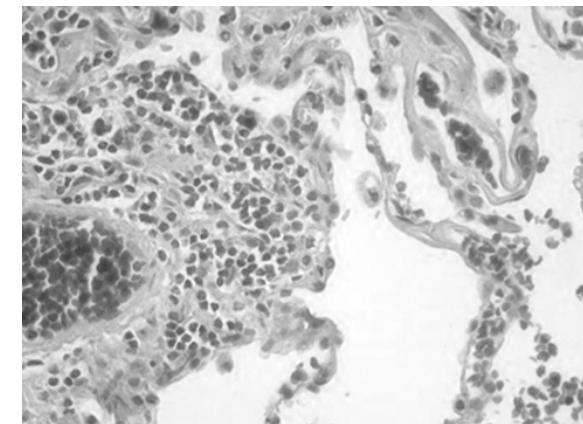
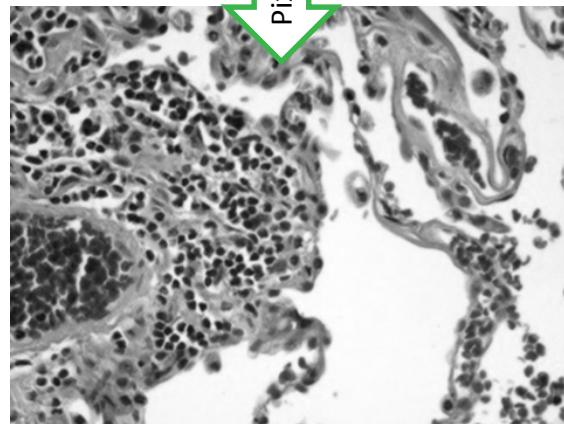
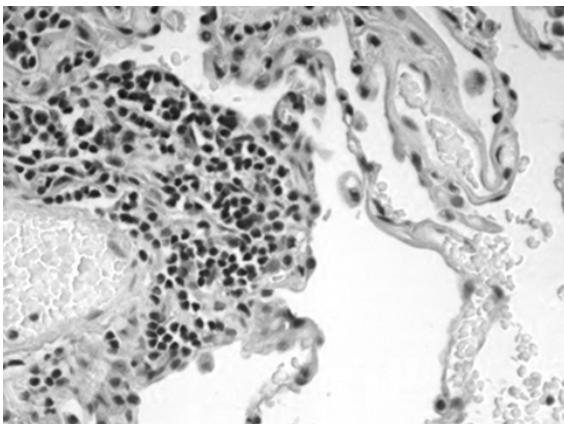
RGB Image: Colour Camera



Pixel value in « Red »

Pixel value in « Green »

Pixel value in « Blue »



TIFF File Format

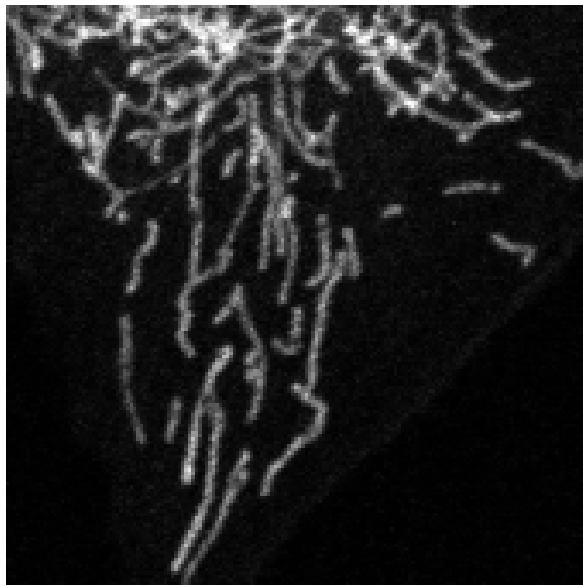
- Digital images can be stored in a variety of file formats
- The most common file format used for digital image is the TIFF format (Tagged Image File Format), file types ending in .tif
- TIFF is a flexible format: grayscale, RGB, layers, stack, image tags, ,....
- Image file header with the image information and description (metadata).
- Universal compatibility with all imaging software
- By default, TIFF format do not apply any compression to the image

Compression

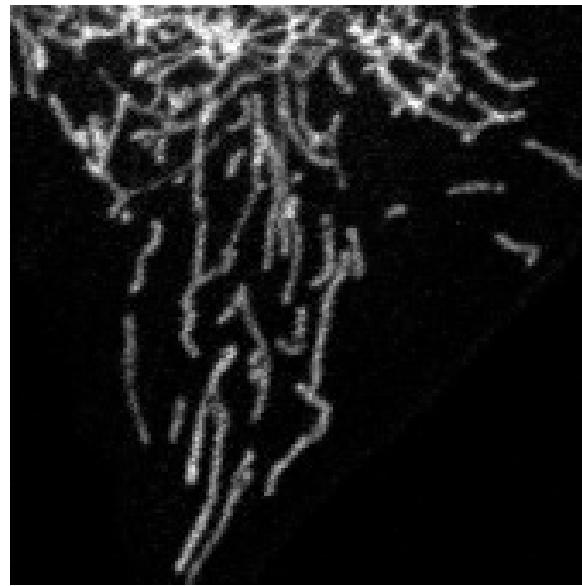
- ***Loss-less compression***
 - Writes EXACTLY the same data in a smaller file
 - ZIP
 - Not that efficient (space/time) for microscopy data
- ***Lossy compression***
 - JPEG format
 - Change the dataset during compression
 - NEVER USE for scientific data
 - Good for web publication and pictures distribution

JPEG is Evil

TIFF

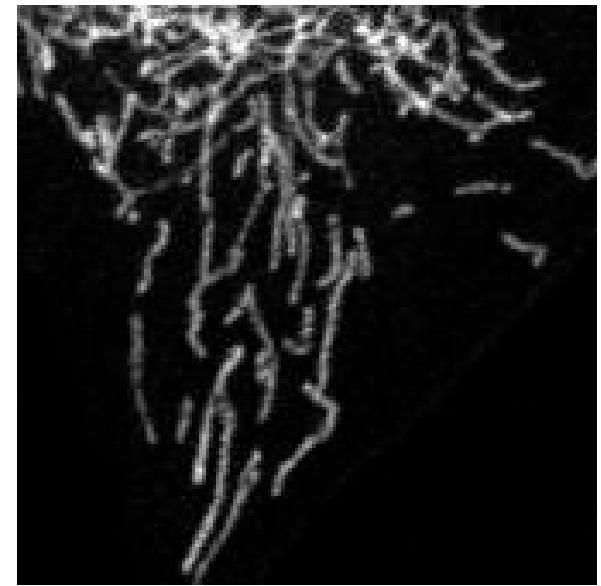


JPEG



Quality 90%

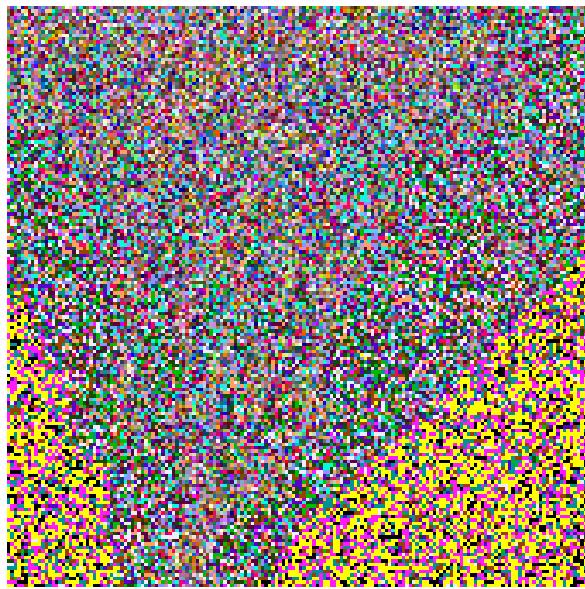
JPEG



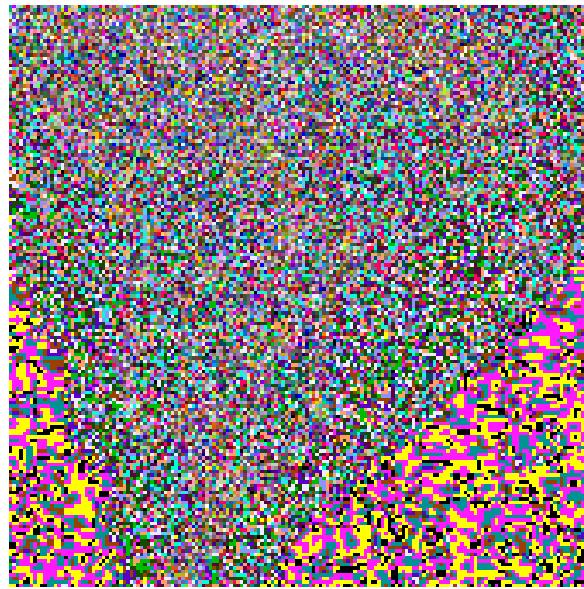
Quality 70%

JPEG is Evil

TIFF



JPEG

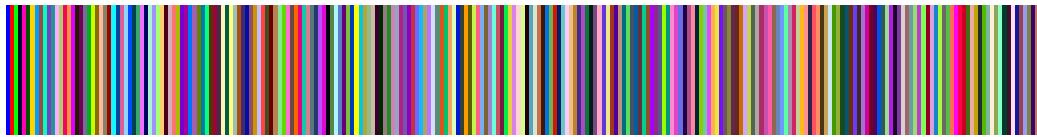


Quality 90%

JPEG



Quality 70%



glasbey

Metadata

Excitation wavelength

Emission wavelength

Dyes

Exposure

Gain

Offset

Time interval

Z-stack

Optical slice thickness

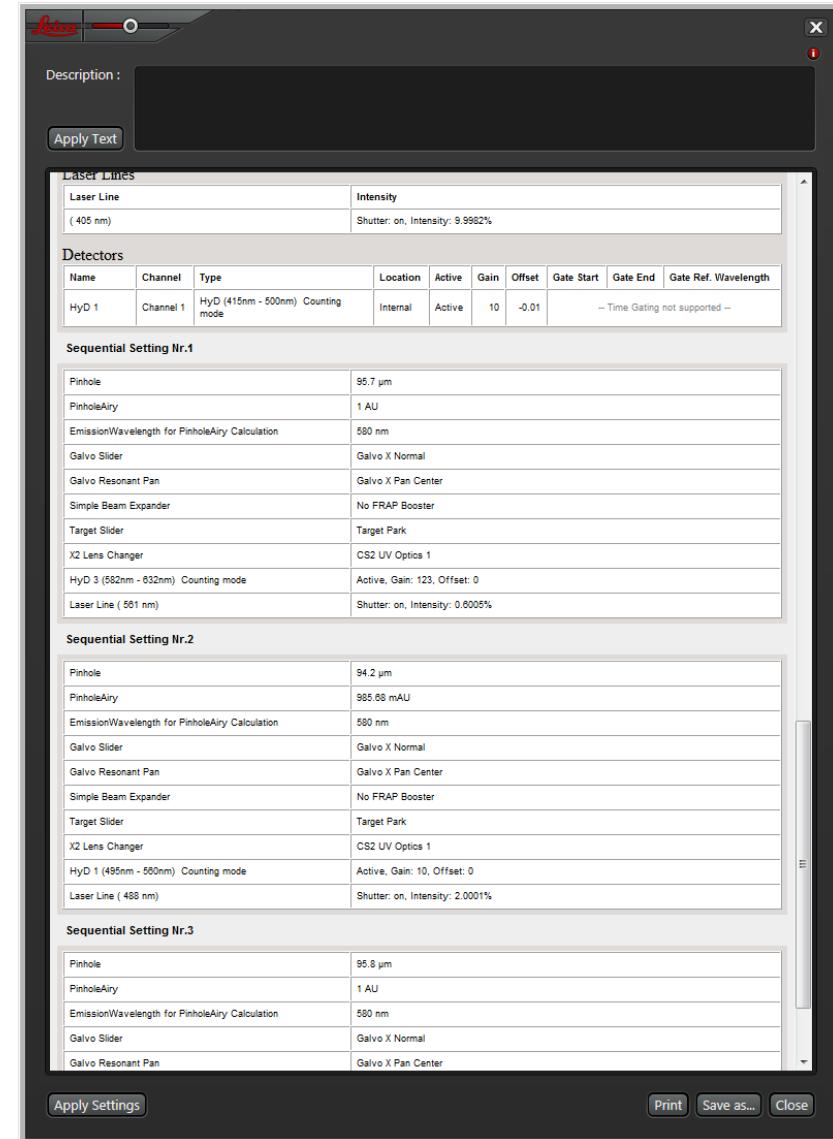
Objectives

Pixel size : scale bar

Personal notes

and more ...

Use the proprietary file format or tiff format to save the essential metadata
(6 months later what is that picture ?!?!?)



Digital Image

Data

- array of pixels
- each pixel has an intensity value

Metadata

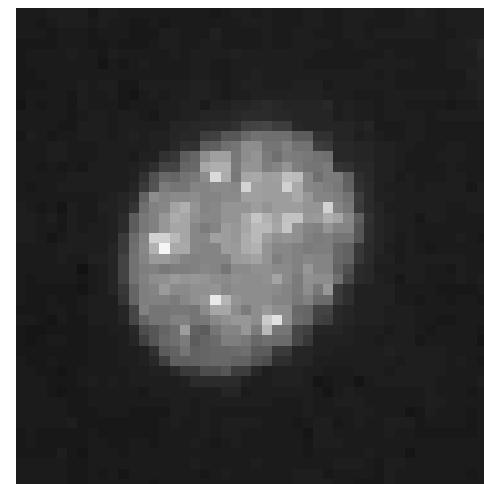
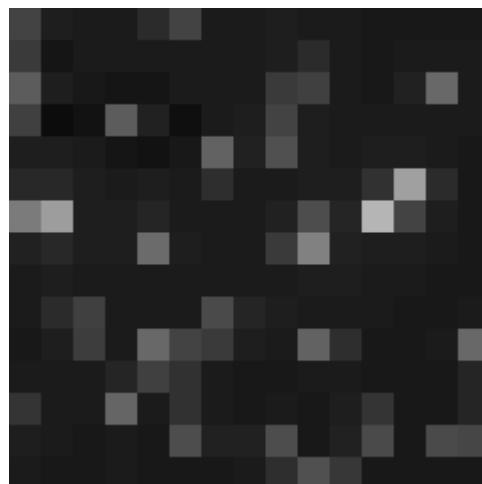
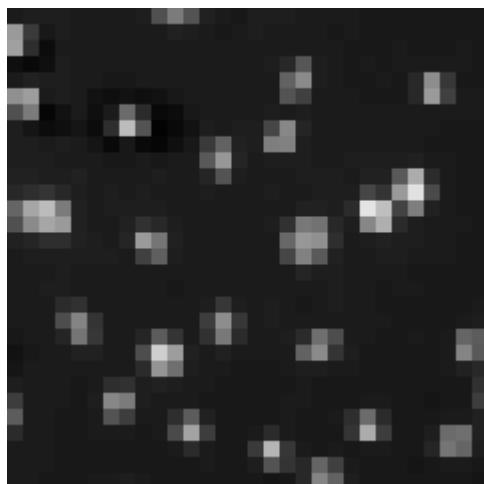
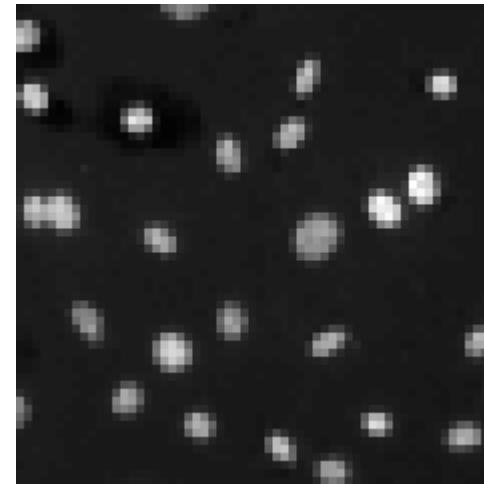
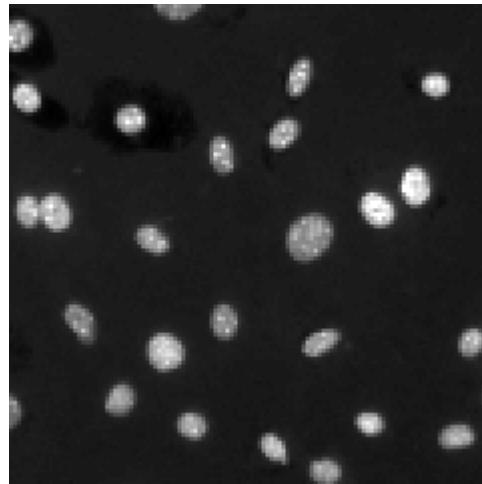
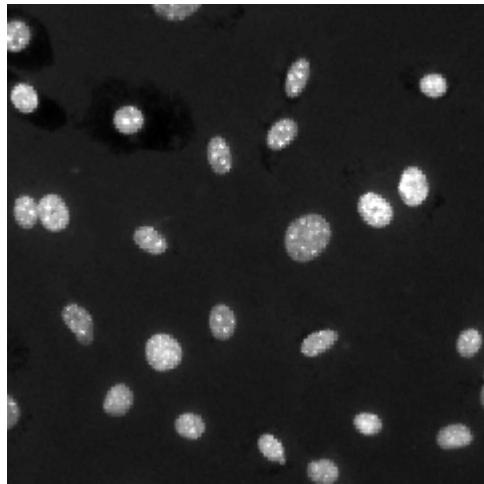
- How
- What
- Where
- When
- Who
- ...

Data

- Table of intensity value
- Picture formed of pixels of different intensity
- Histogram of pixel intensity distribution
(loss of spatial information)

Image Resolution to match object to detect

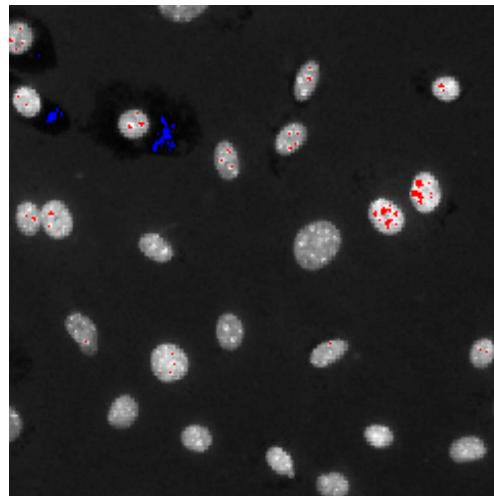
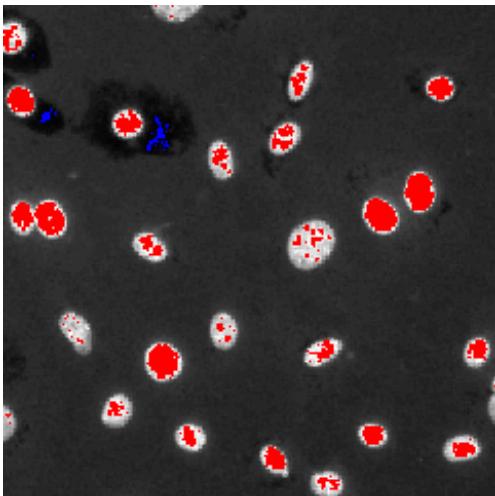
Nyquist sampling rule = object covered by 2,3 pixels



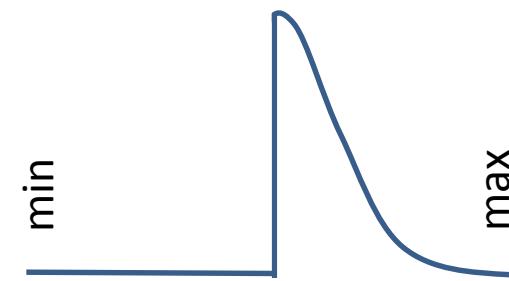
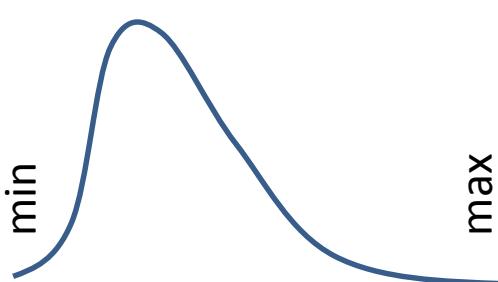
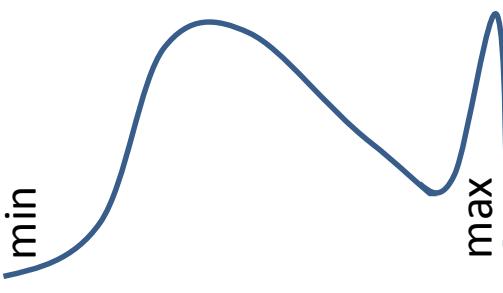
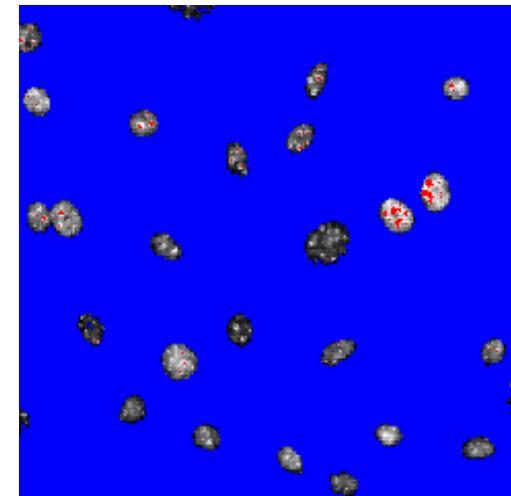
Histogram Clipping

- Do not clip your data (range indicator, histogram)
- Fill the dynamic range

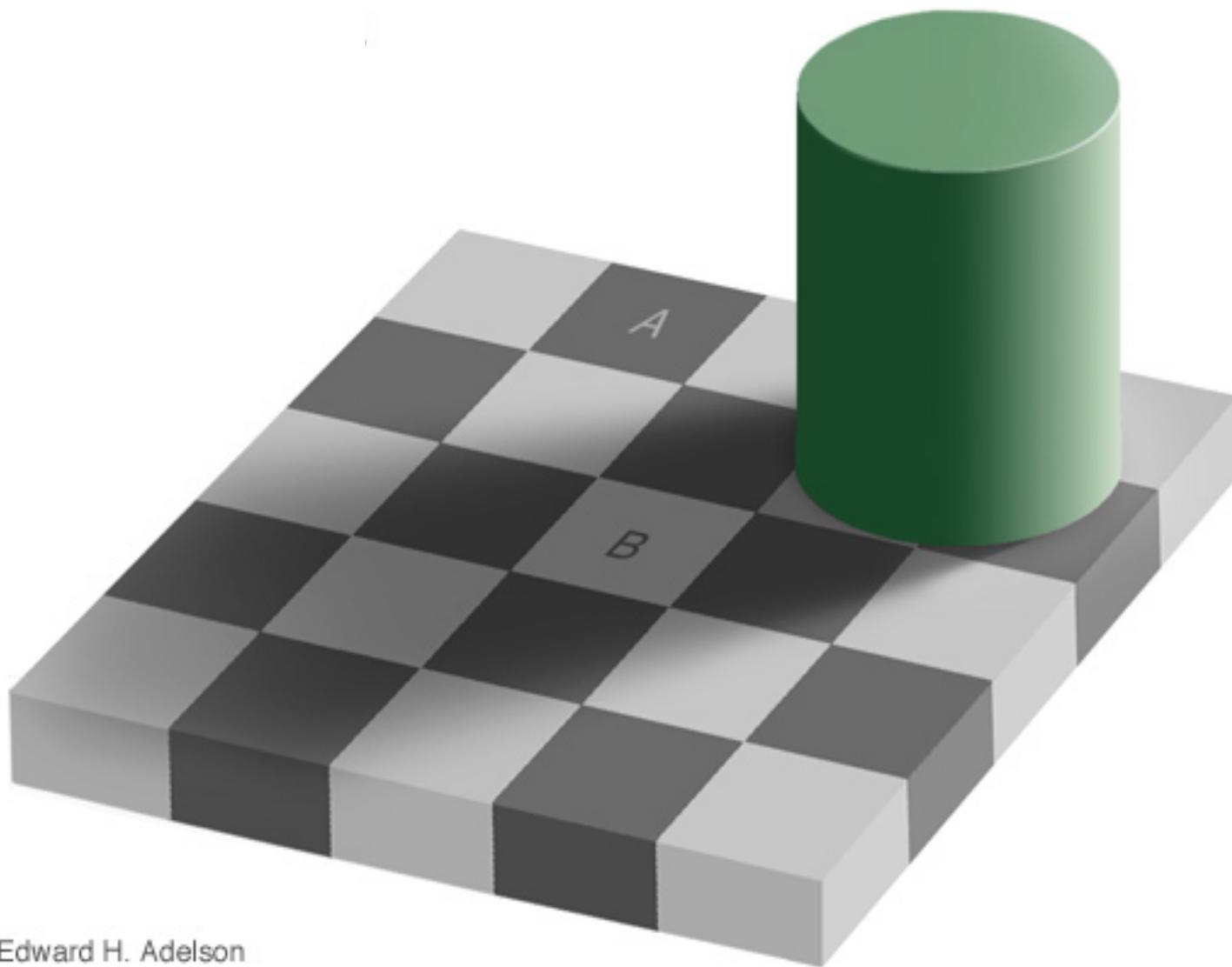
Saturation



Wrong offset



Don't trust your brain

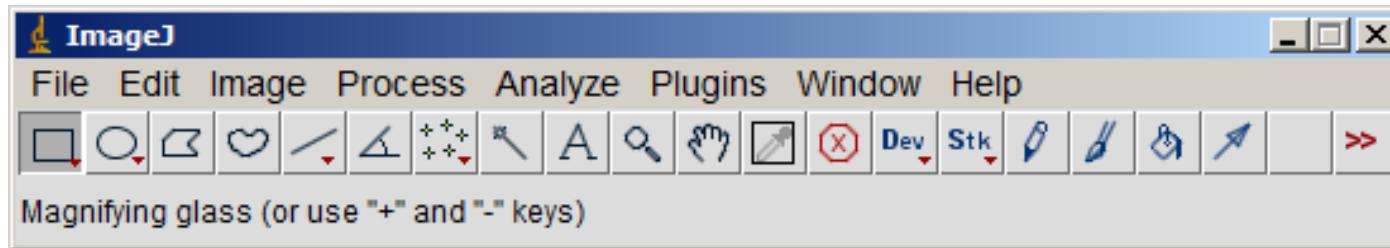


Edward H. Adelson

Fiji Overview

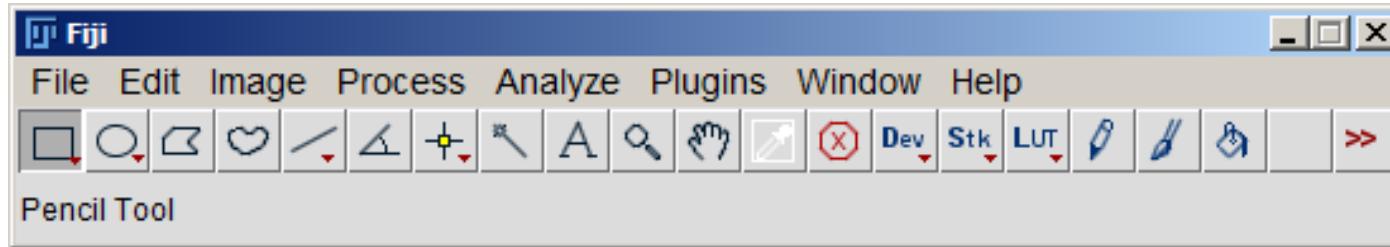
ImageJ Distributions

ImageJ & ImageJ2



<https://imagej.net/>

Fiji



<https://fiji.sc/>

ImageJ General Features

Platform independent written in Java and run on Linux, Mac OS and Windows, in both 32-bit and 64-bit modes. Java has to be installed on your machine.

Open source code are freely available and in the public domain. No license is required = FREE (lab and home usage) and the code can be modified (Java language).

ImageJ has a ***large and knowledgeable user community***
<https://forum.image.sc>

Automate tasks and create custom tools using macros. More than 300 macros are available on the ImageJ Web site.

Extend ImageJ by developing and running *plugins* using ImageJ's built in text editor and Java compiler. More than 500 plugins are available.

Fiji ImageJ distribution

ImageJ is an open source image processing program designed for ***scientific multidimensional images***.

Fiji is a distribution of the free image analysis package ImageJ, Fiji stands for **Fiji Is Just ImageJ**.

Fiji comes with a ***large collections of plugins already installed***. It also has a built in ***auto update*** system to make sure you always have the current versions of the plugins.

Fiji has a ***biology slant*** to most of the plugins provided but they can be used for analysis of a range of samples.

ImageJ/Fiji does ...

Open (almost) all image type

Display (merge, split channels ...)

Edit (R.O.I, scale bar, ...)

Analyse (measurements, cell counting,...)

Process (filters, z-projection, ...)

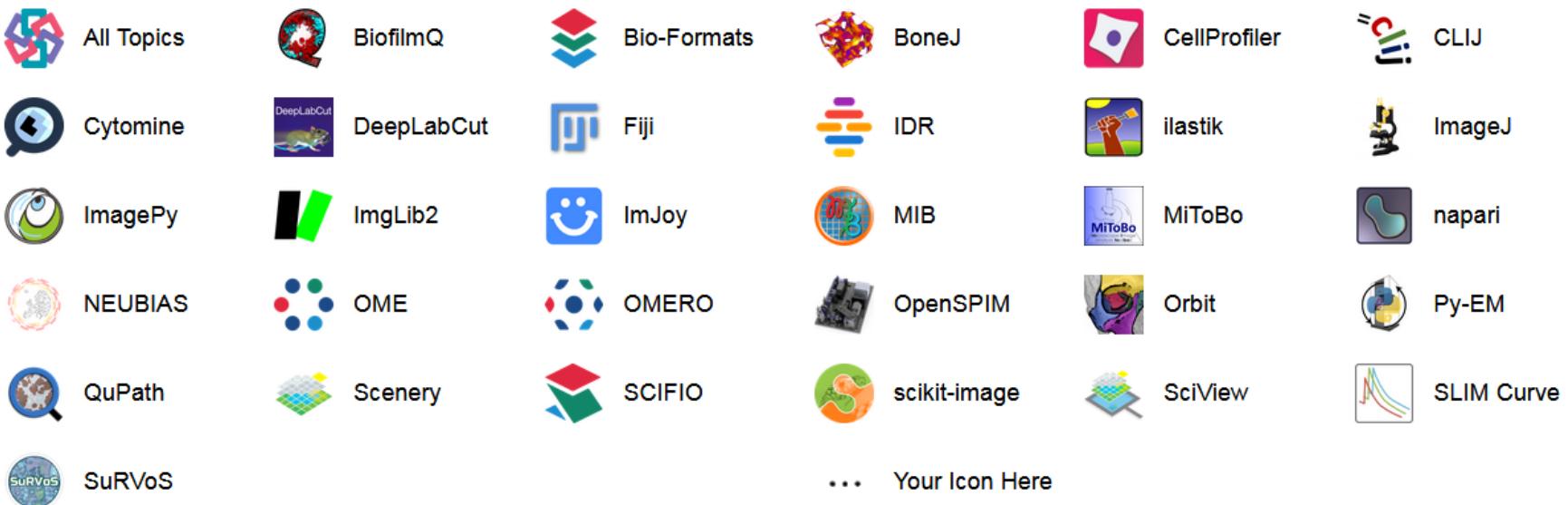
Save and export

Print

And many more advanced functions ...

You don't need Photoshop !

ImageJ and Friends



<https://forum.image.sc/>

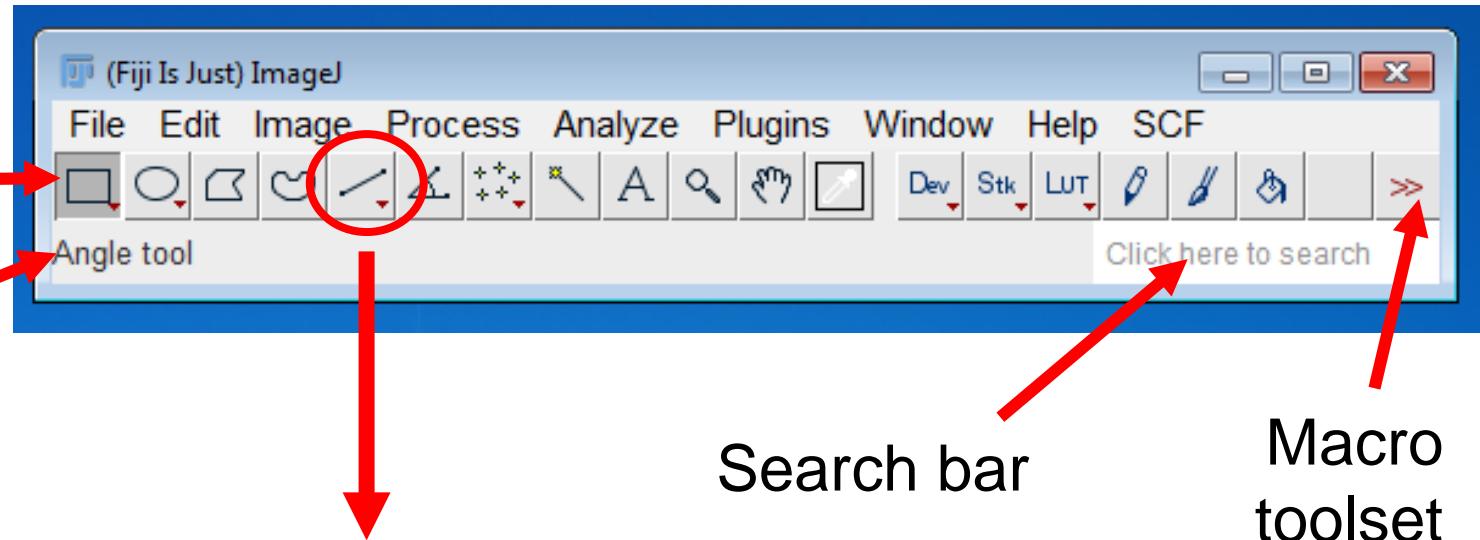
User Interface

Graphical User Interface

Menu Bar

Tool Bar

Status Bar



Double left click : opens the tool options

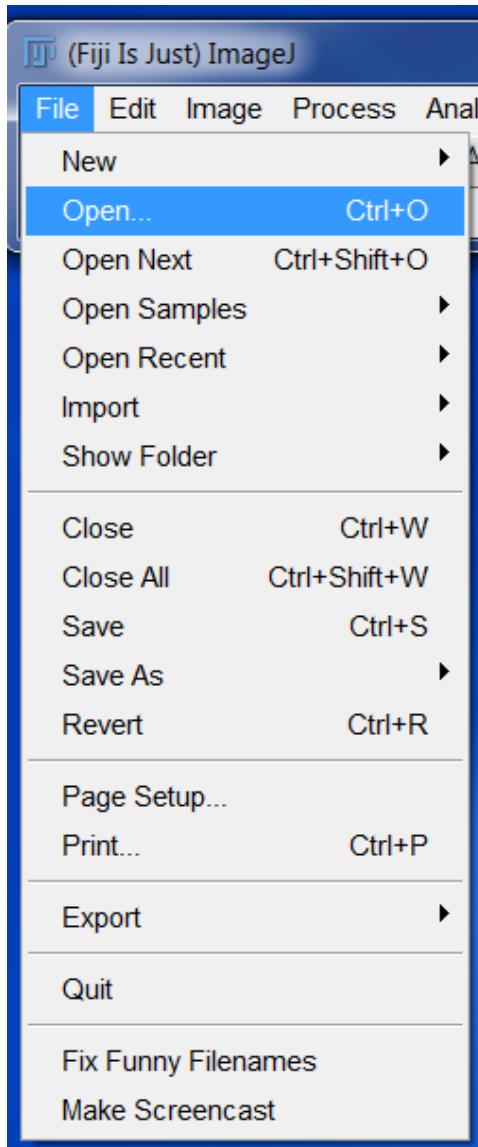
Right click : selects a different tool

Search bar

Macro
toolset
Switcher

Remember ! All operations are performed on the active window
i.e. the front most image with the highlighted title bar.

File



<https://imagej.nih.gov/ij/docs/guide/146-26.html#toc-Section-26>

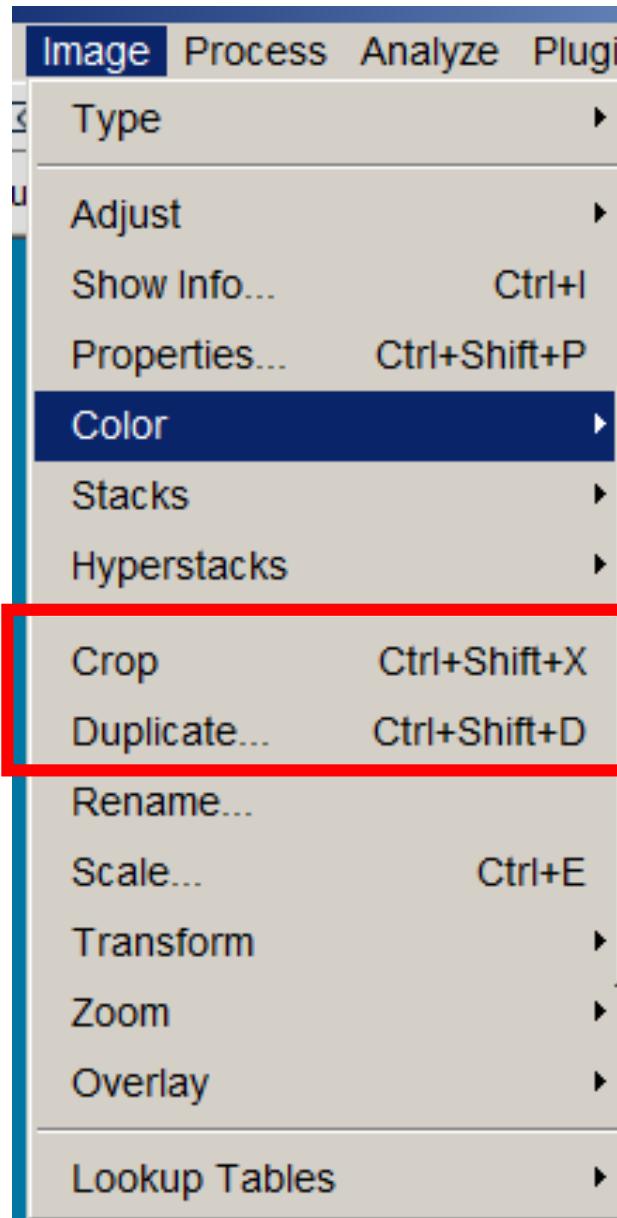
Edit

- reverse the effect of the most recent operation. Not always available
- the current image selection and copy it into the internal clipboard
- the current image selection or the entire active image into the internal clipboard
- the content of the internal clipboard
- more options
 - clear the selection and replace by background colour
 - clear outside the selection and replace by background colour
 - fill the selection with foreground color
 - outlines the selection
 - creates a “negative image”

Edit>Selection

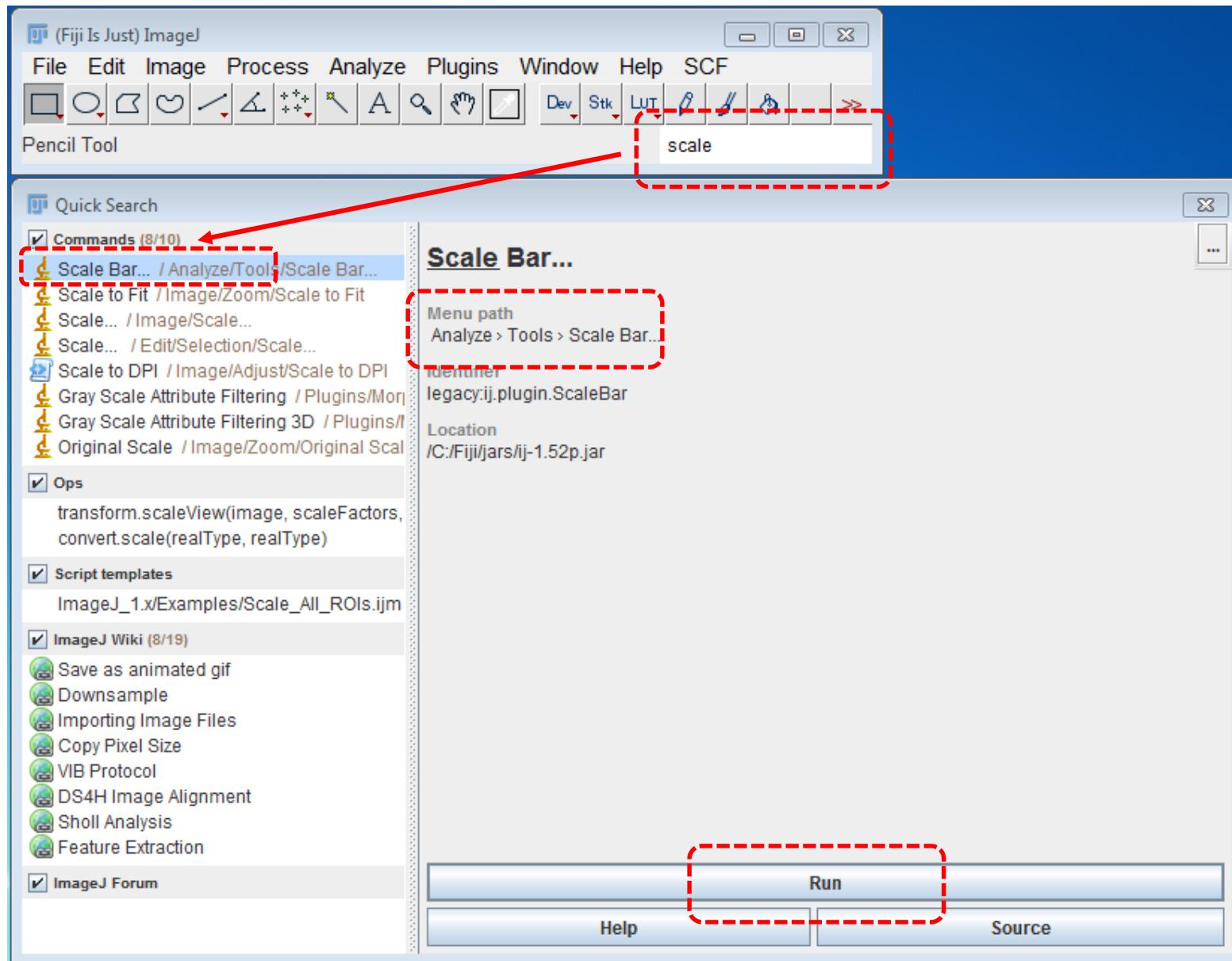
- Select All Ctrl+A
- Select None Ctrl+Shift+A
- Restore Selection Ctrl+Shift+E
- Fit Spline
- Fit Ellipse
- Convex Hull
- Make Inverse
- Create Selection
- Create Mask
- Properties...
- Rotate...
- Enlarge...
- Make Band...
- Specify... Coordinates and size of the selection
- Straighten... Region Of Interest manager
- Add to Manager Ctrl+T

Image

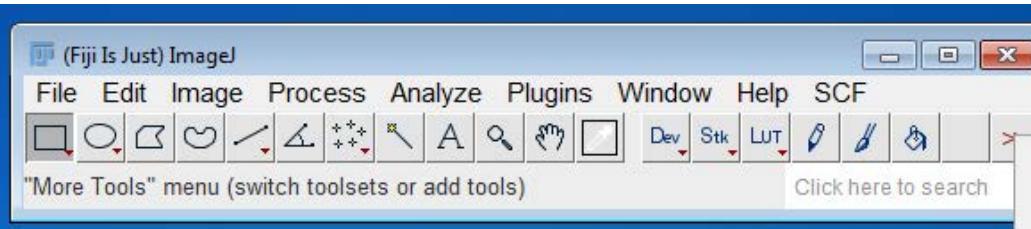


Remember these shortcuts !

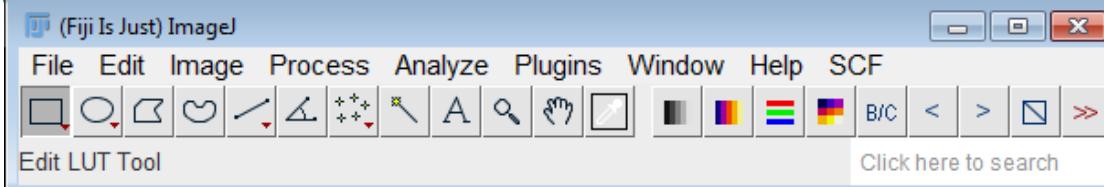
Search bar (L)



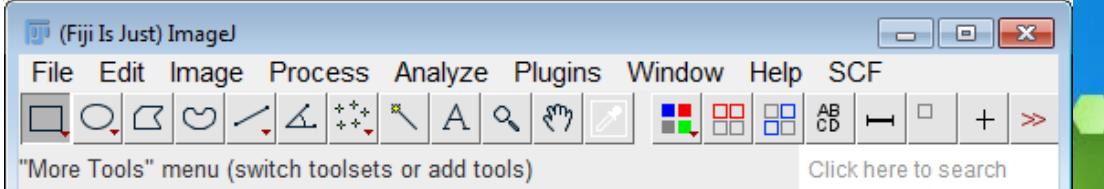
Fiji Macro Toolset Switcher



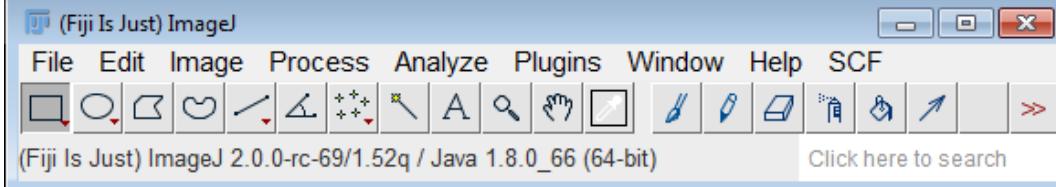
Lookup Tables



Montage Tools



Drawing Tools



- StartupMacros*
- BioVoxel_Toolbox
- Clear Custom Tools
- Drawing Tools ←
- LineAndProfileTool20150413
- Lookup Tables ←
- Montage Tools ←
- Nearest_Neighbor_Indicator

- Arrow
- Brush
- Command Finder
- Developer Menu
- Flood Filler
- LUT Menu
- Overlay Brush
- Pencil
- Pixel Inspector
- Selection Rotator
- Spray Can
- Stacks Menu

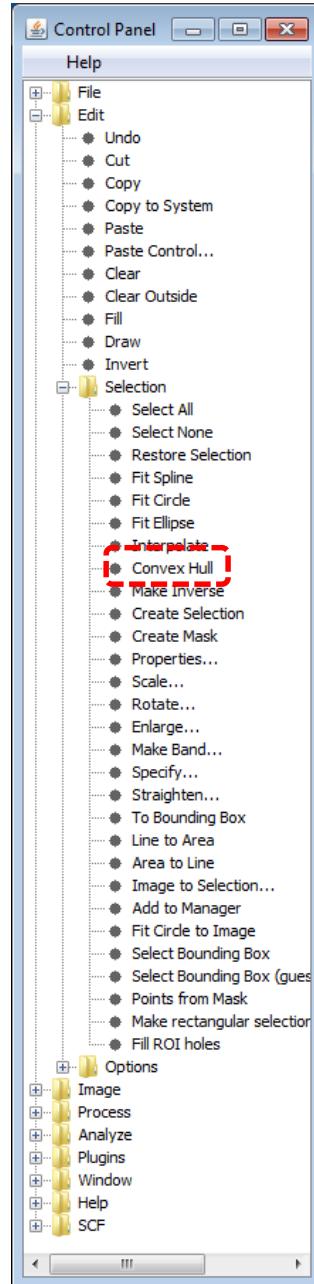
Restore Startup Tools

Remove Custom Tools

Help...

Control Panel

Shift+U



Double left click

Keyboard Shortcuts

Plugins>Shortcuts>List shortcuts...

Windows
Linux



Mac



Keyboard Shortcuts

CROP [X]↑

Shift X

Crop active image or selection

DUPLICATE... [D]↑

Shift D

Duplicate active image or selection

SELECTION>RESTORE SELECTION [E]↑

Shift E

Restore previous selection

SELECTION>ADD TO MANAGER [T]↑

T

Add selection to ROI Manager

MEASURE... [M]↑

M

Display statistics of active image / selection

HISTOGRAM [H]↑

H

Display histogram of active image / selection

PLOT PROFILE [K]↑

K

Display density profile plot of active selection

Open and Save



Opening Your Data

- File>Open...
- Drag and Drop (Files and Folders)
- File>Import>Image Sequence...
- Plugins>Bio-Formats>Bio-Formats Importer

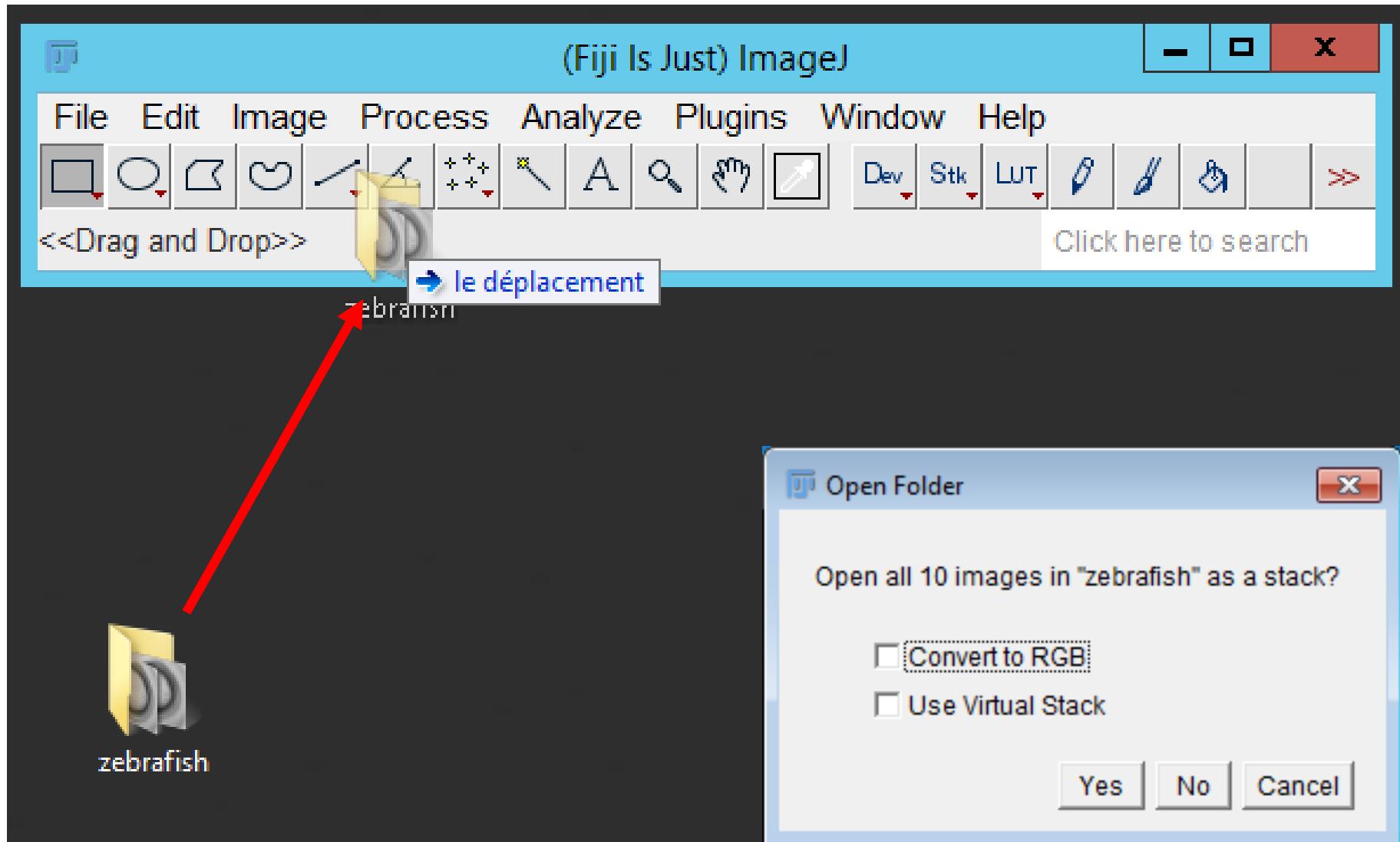
File>Open...



- Reads an image and displays it in a separate window.
- If multiple images are present in the file, open as a stack (3D) or hyperstack (4D and more).

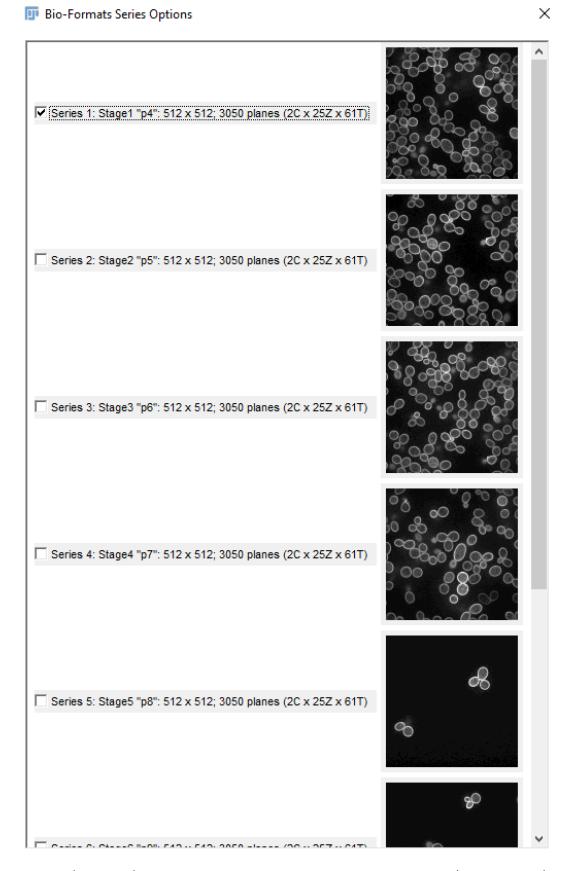
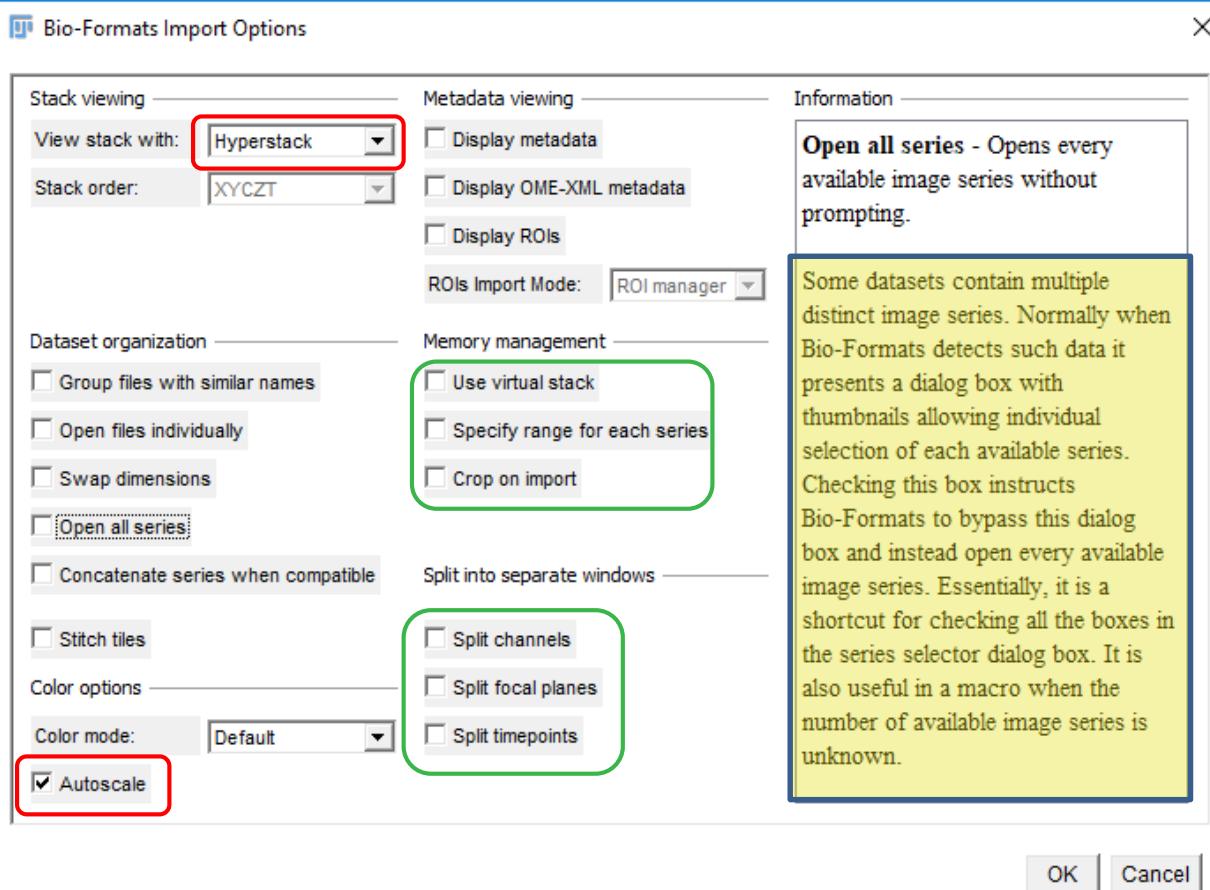
Drag and Drop

Works with single files, multiple files, folder



Bio-Formats

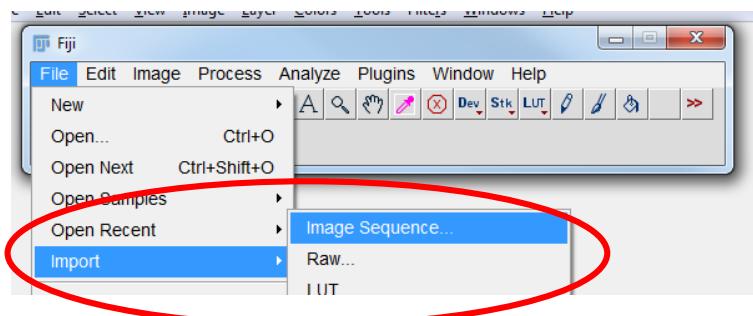
Plugins>Bio-Formats>Bio-Formats Importer



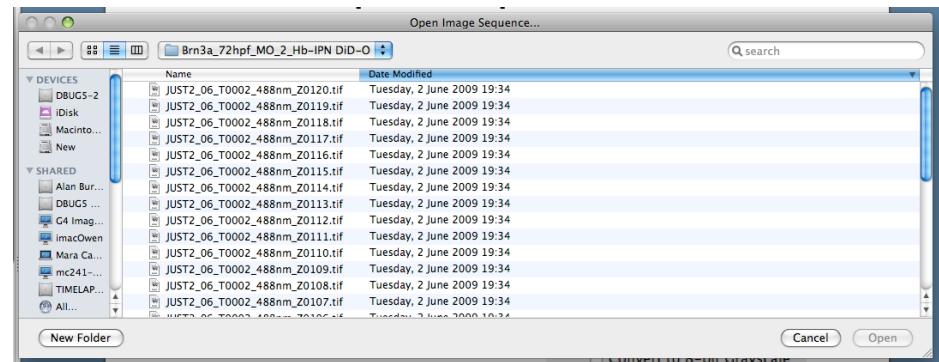
Supported formats

<https://docs.openmicroscopy.org/bio-formats/6.2.1/supported-formats.html>

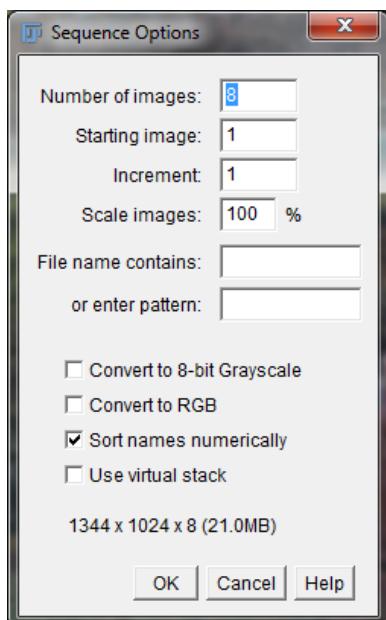
File>Import>Image Sequence...



1- Select folder and one file

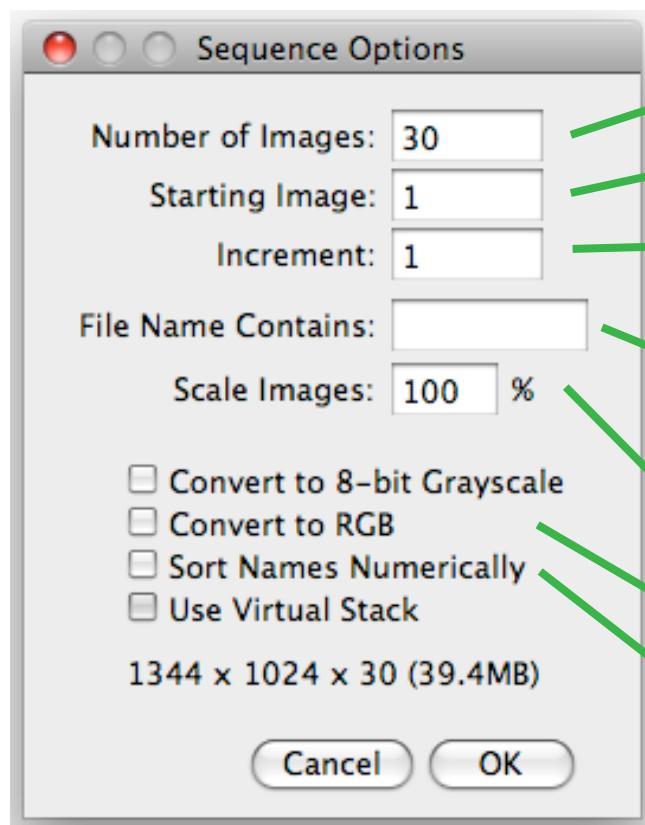


2- Specify options



Opens a series of images in a folder as a stack. The images must all be the same size and type.

File>Import>Image Sequence...



- How many images to open
- To start with the nth image in the folder
- To set the increment : n=2 open every other image, n=3 to open every third image
- Open only the file whose names contains that string

- Reduces memory requirement but change image size
- Allows a mixture of RGB and grayscale to be open
- Order : 1 2 10 . Alphanumeric default : 1.tif 10.tif
2.tif

Virtual Mode

- Virtual stacks are disk resident (as opposed to RAM resident) and are the only way to load image sequences that do not fit in RAM
- Virtual stacks are read-only
- Several ways to open data in virtual mode
 - *Plugins>Bio-Formats>Bio-Formats Importer*
 - *File>Import>TIFF Virtual Stack*
 - *Drag and Drop on the Macro Toolset Switcher*

<https://imagej.nih.gov/ij/docs/guide/146-8.html#sub:Virtual-Stacks>

Virtual Mode

- ImageJ appends a '(V)' to the window title



<https://imagej.nih.gov/ij/docs/guide/146-8.html#sub:Virtual-Stacks>

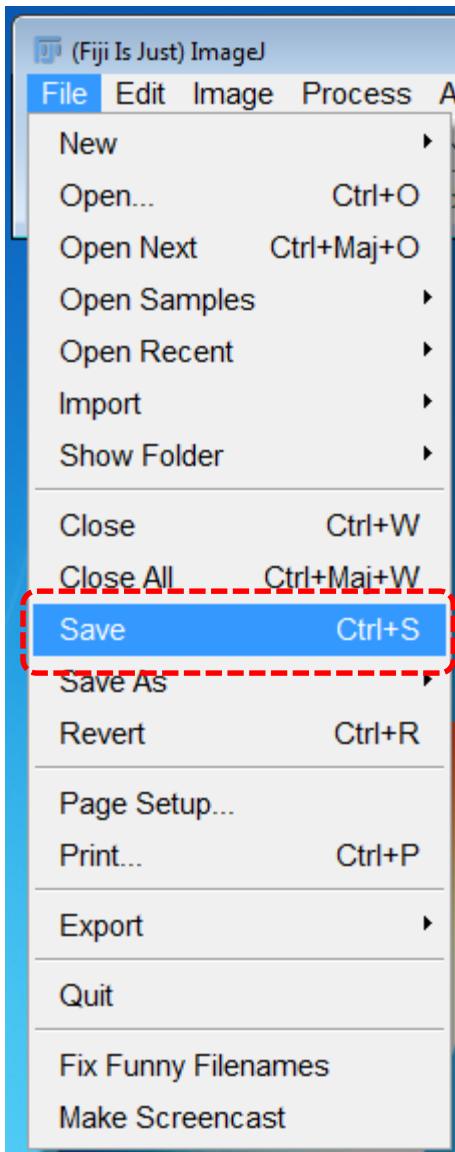
Close Image(s)

- File>Close
- File>Close All

ctrl W

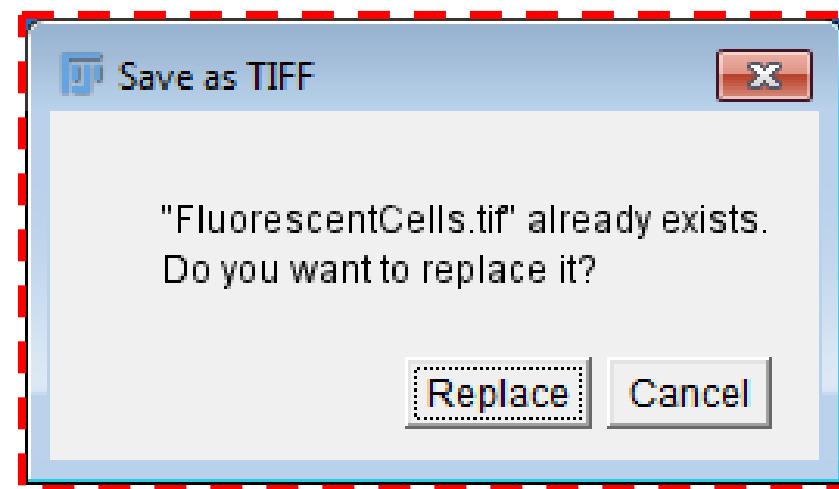
ctrl shift W

Save



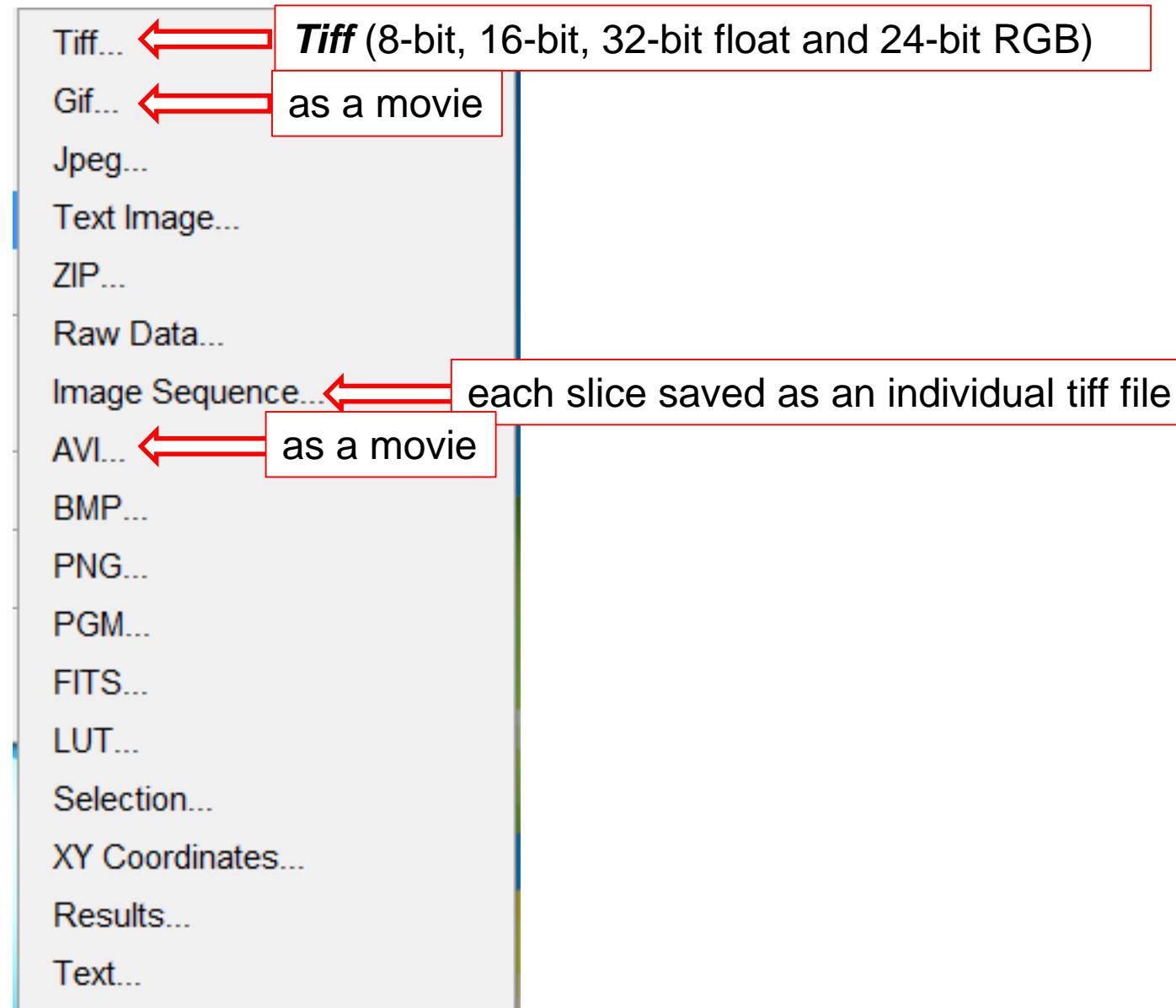
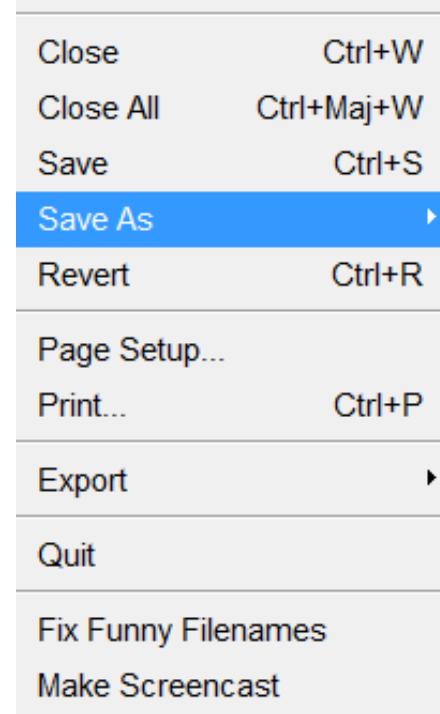
File>Save (as Tiff the active window)

Caution : If the original file is already a Tiff file, it will be replaced by the new file !

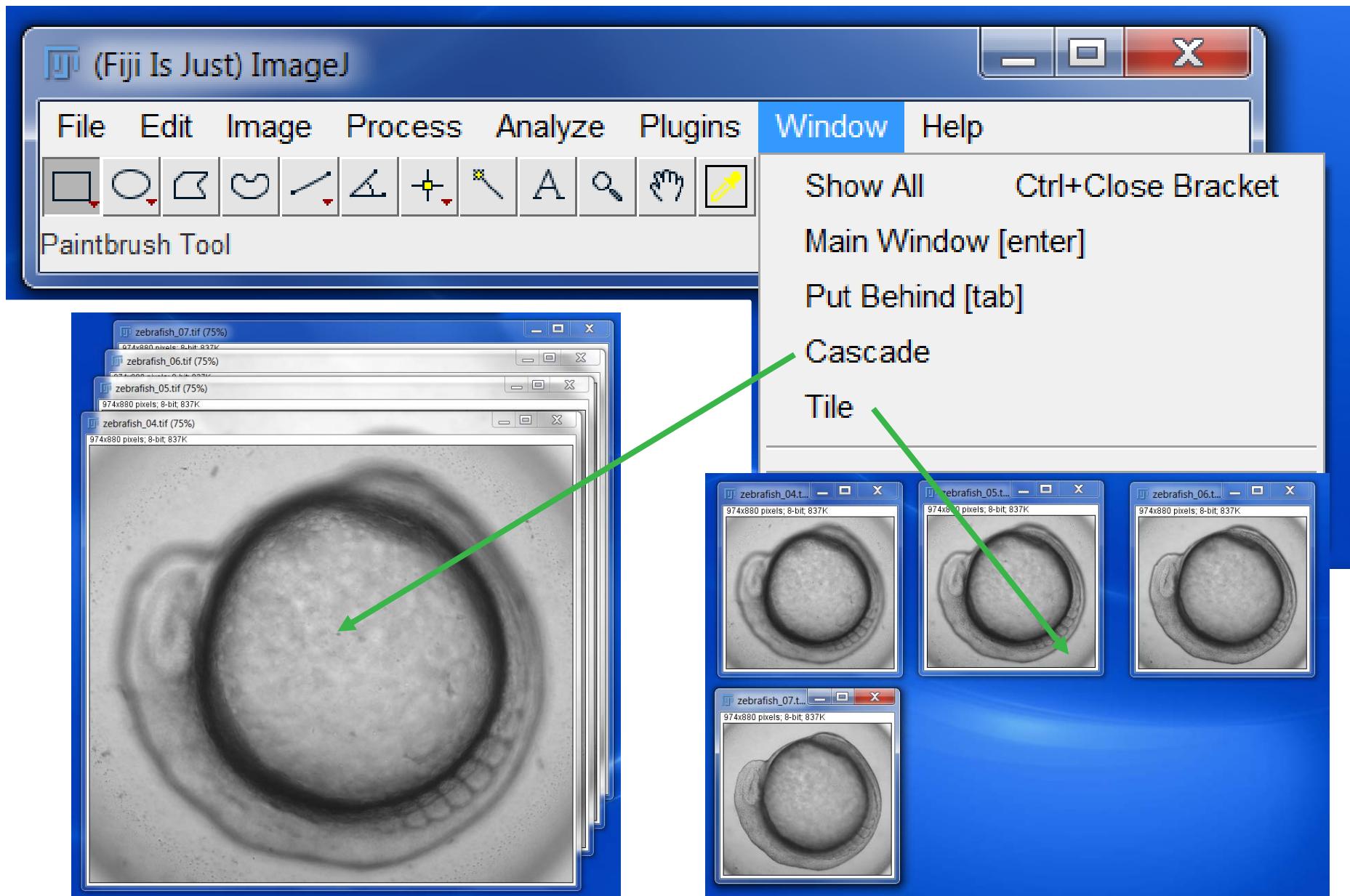


Save as

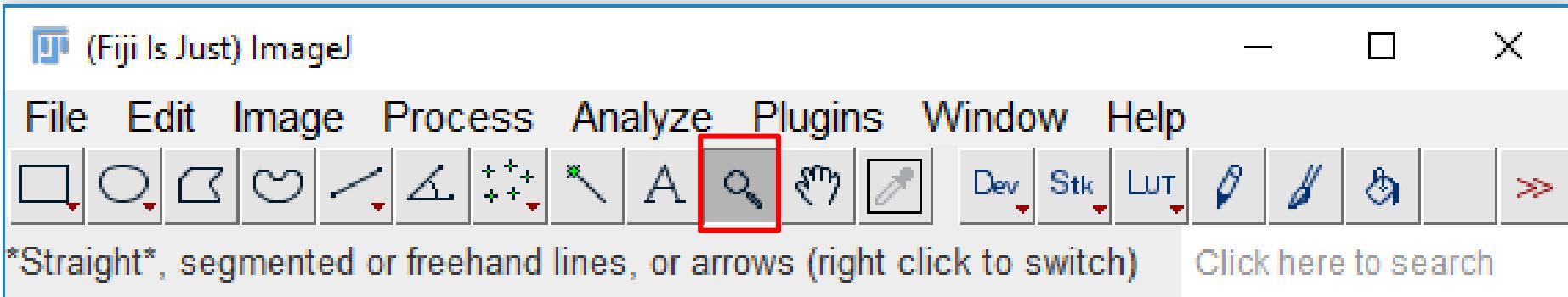
File>Save as...



Organising the Windows



Navigation: Zooming



Menu Image>Zoom

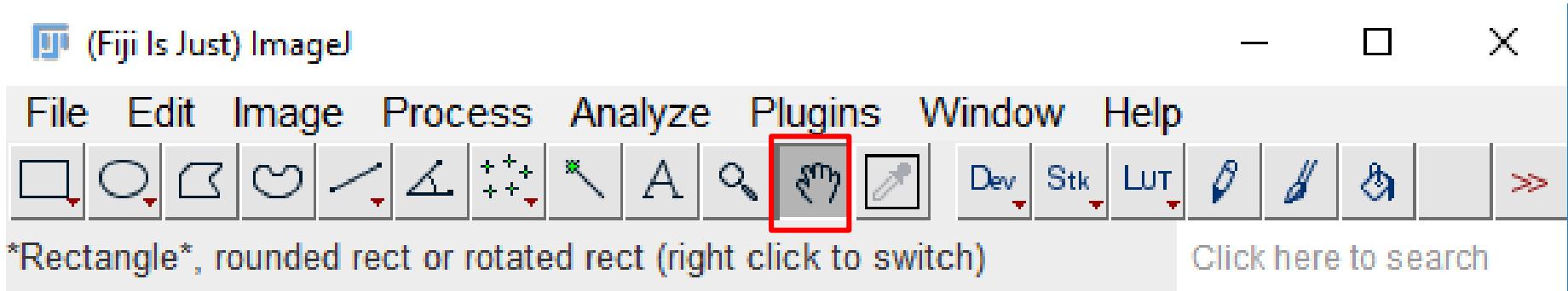
→ **Zoom In** with left click
Zoom Out with right click

→ **crtl + mouse wheel**

→ **+ and – keys**

→ **↑ and ↓**

Navigation: Panning



Select the **Hand Tool** and hold down the left button on your image and move the mouse to pan around. You can also **hold down the spacebar** to activate the hand tool anytime.

Navigation

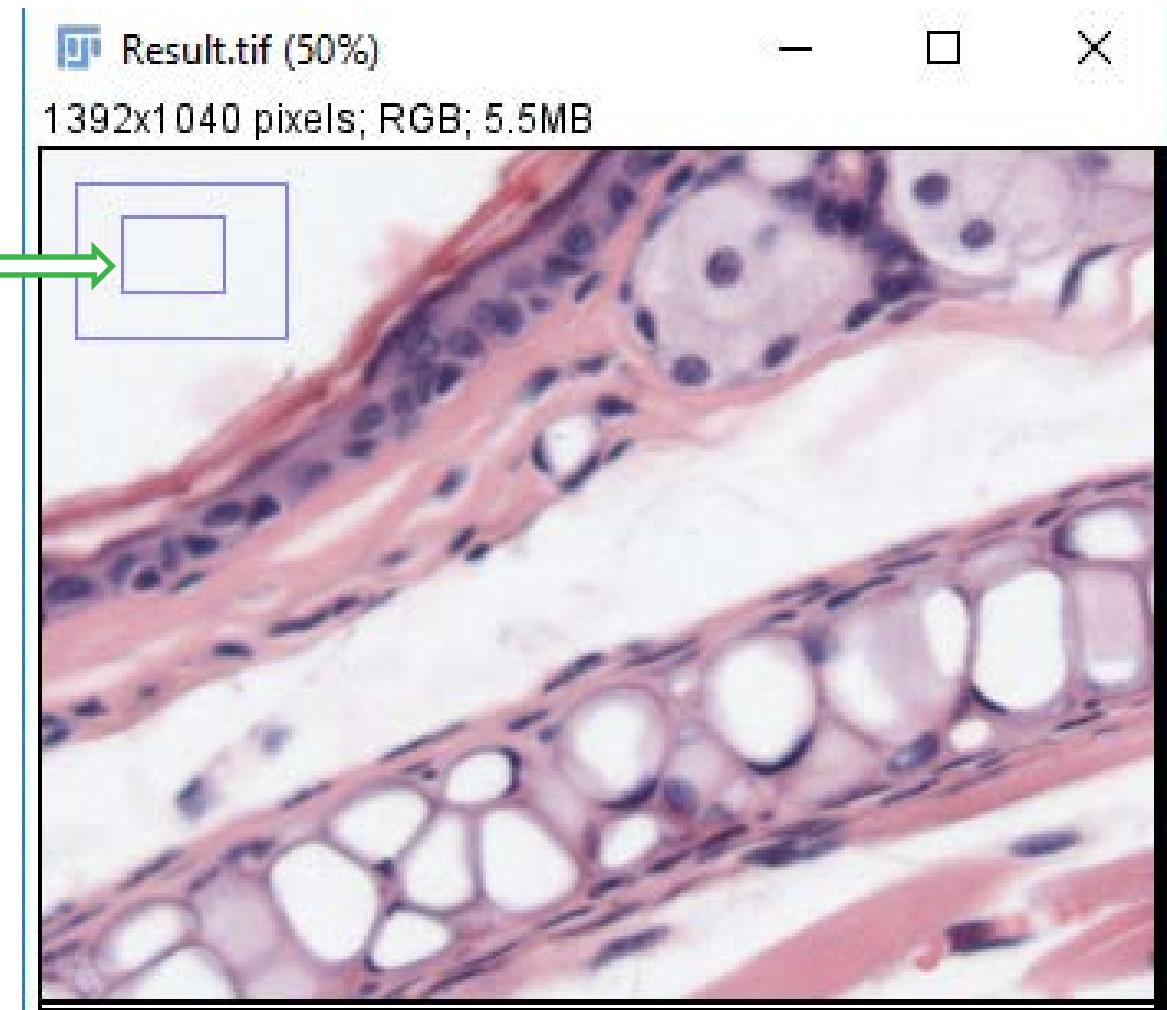
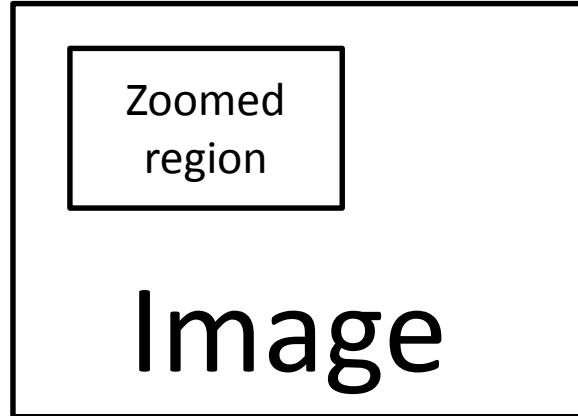


Image Properties

& Info

Mouse Pointer

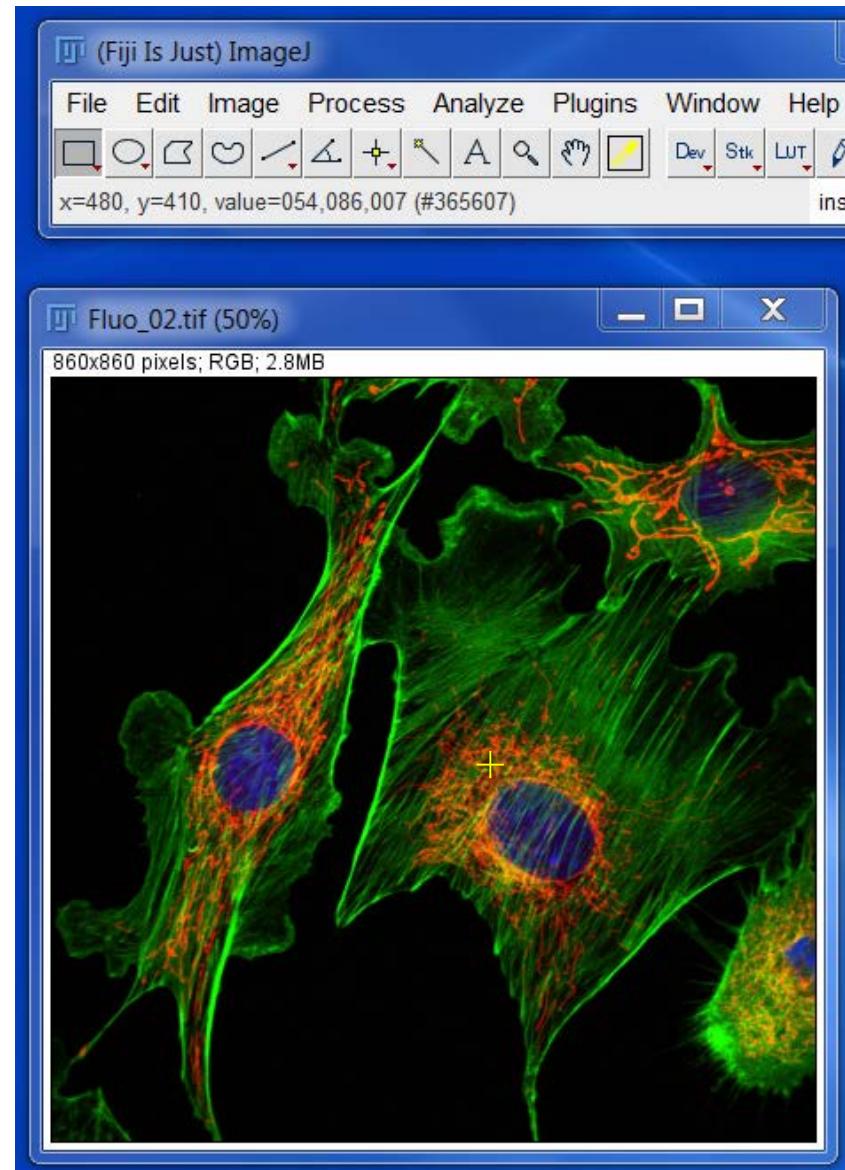
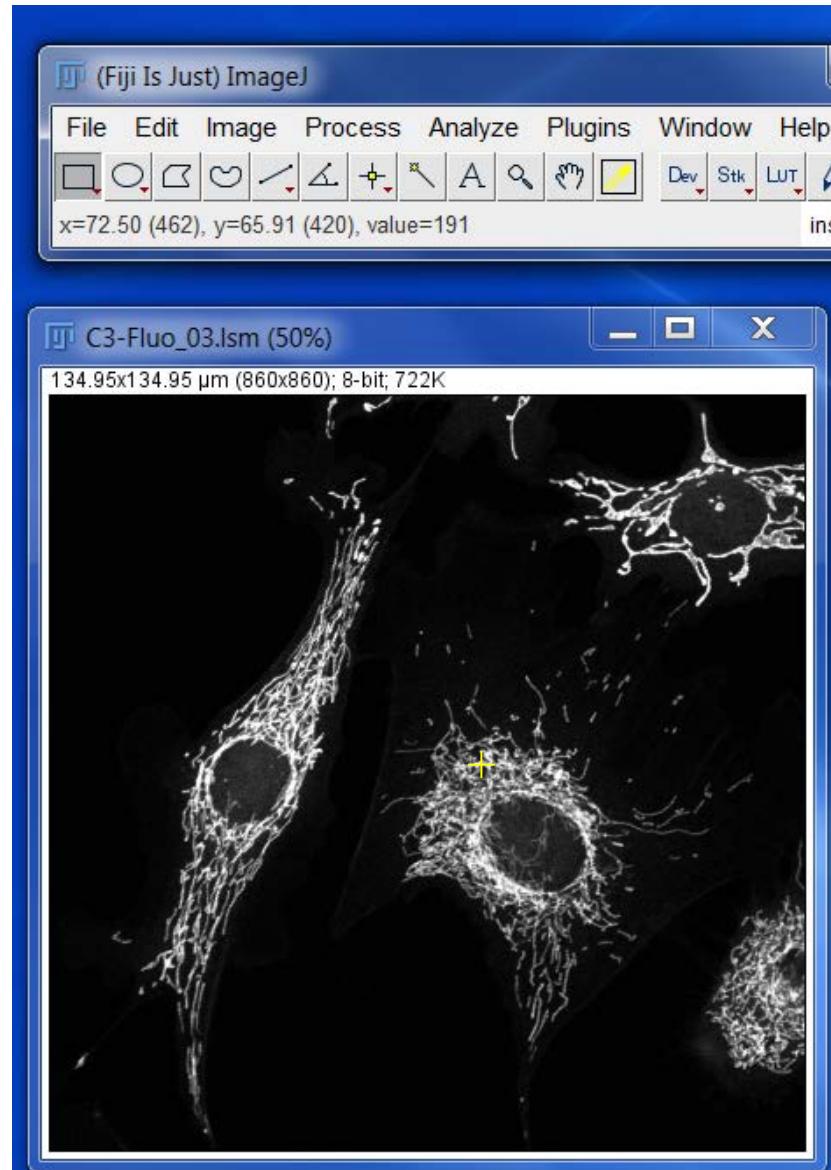


Image : example 1 - Grayscale

- File name
- Frame size (width & height)
- Image type (bit depth) in pixel
- File size



Image : example 2 – Grayscale Stack

- File name
- Z Slices
- Frame size (width & height)
- Image type (bit depth) in pixel
- File size

Z slices

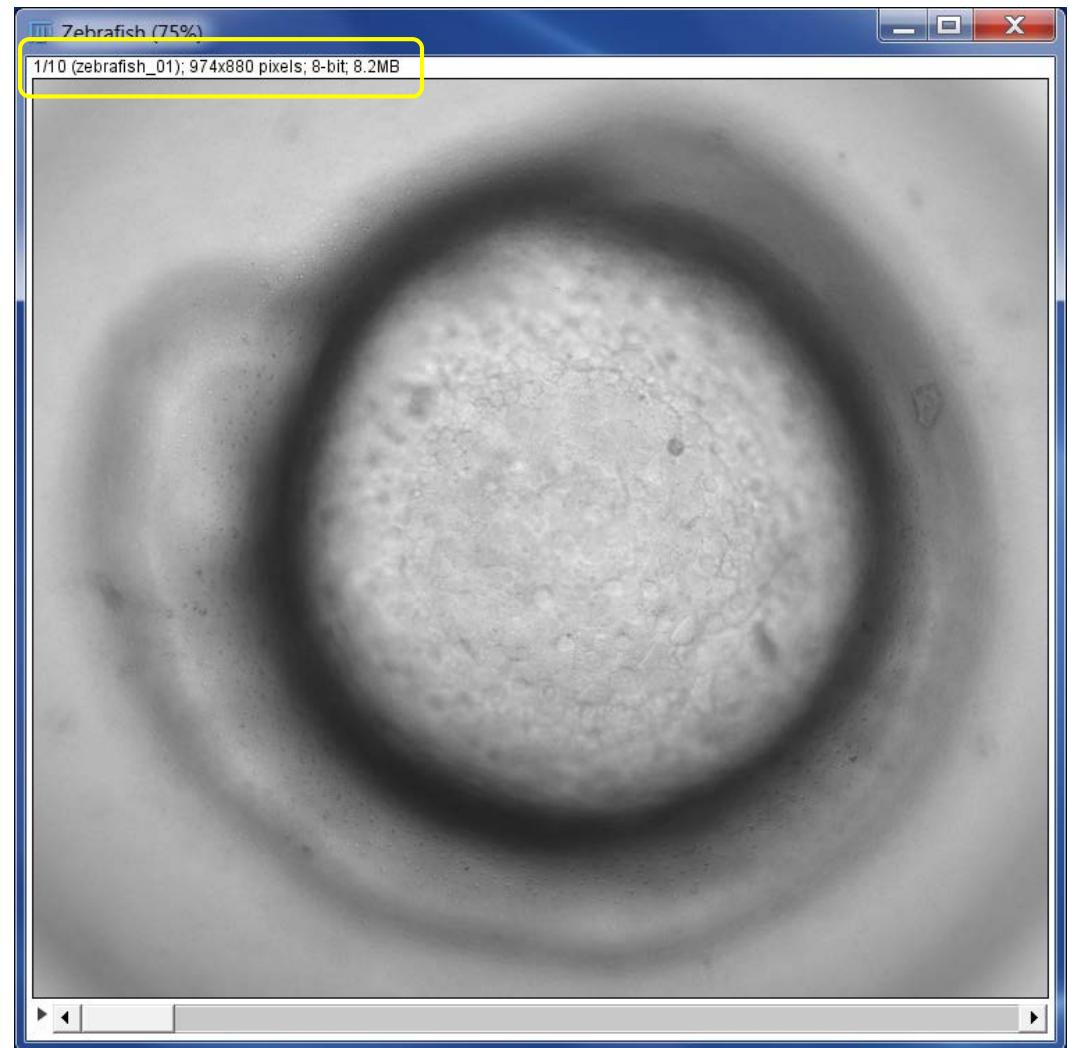


Image : example 3 - RGB

- File name
- Frame size (width & height)
- Image type (bit depth) in pixel
- File size

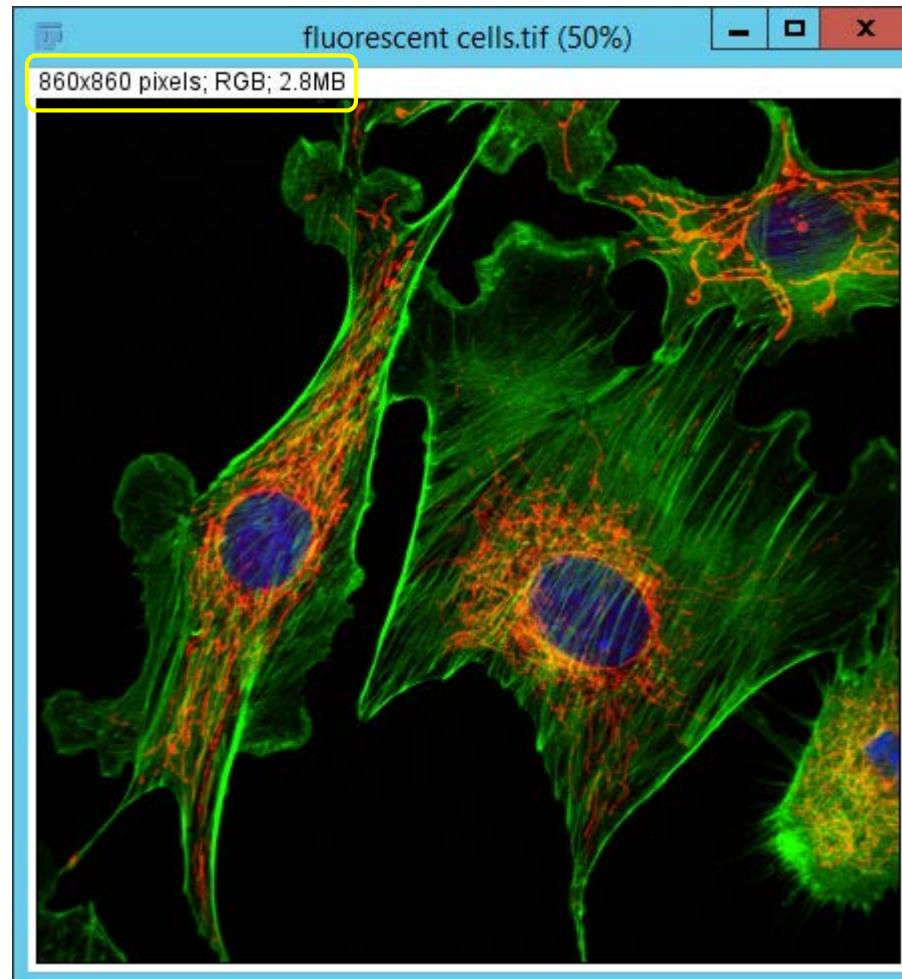


Image : example 4 - Composite

- File name
- Channels
- Frame size (width & height) in μm (pixel)
- Image type (bit depth)

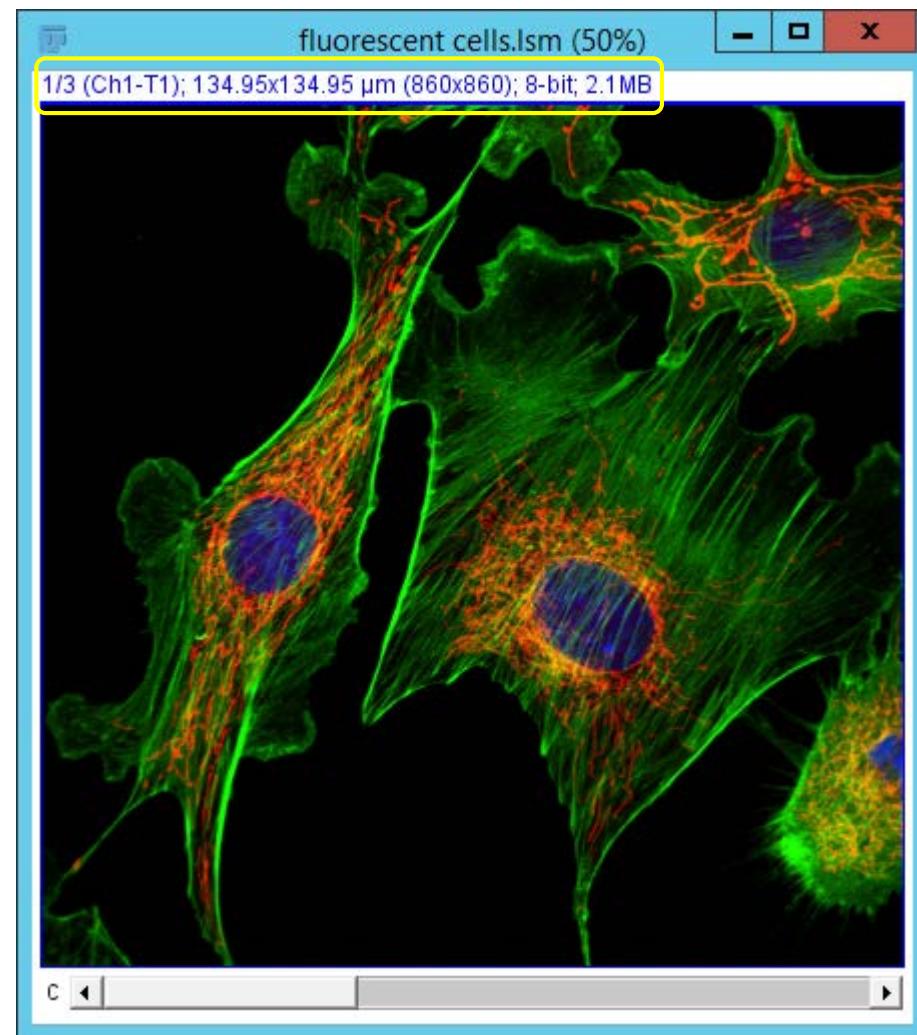


Image : example 5 - Hyperstack

- File name
- Channels
- Z Slices
- Time points
- Frame size (width & height) in μm (pixel)
- Image type (bit depth)
- File size

Channel

Z Slices

Time

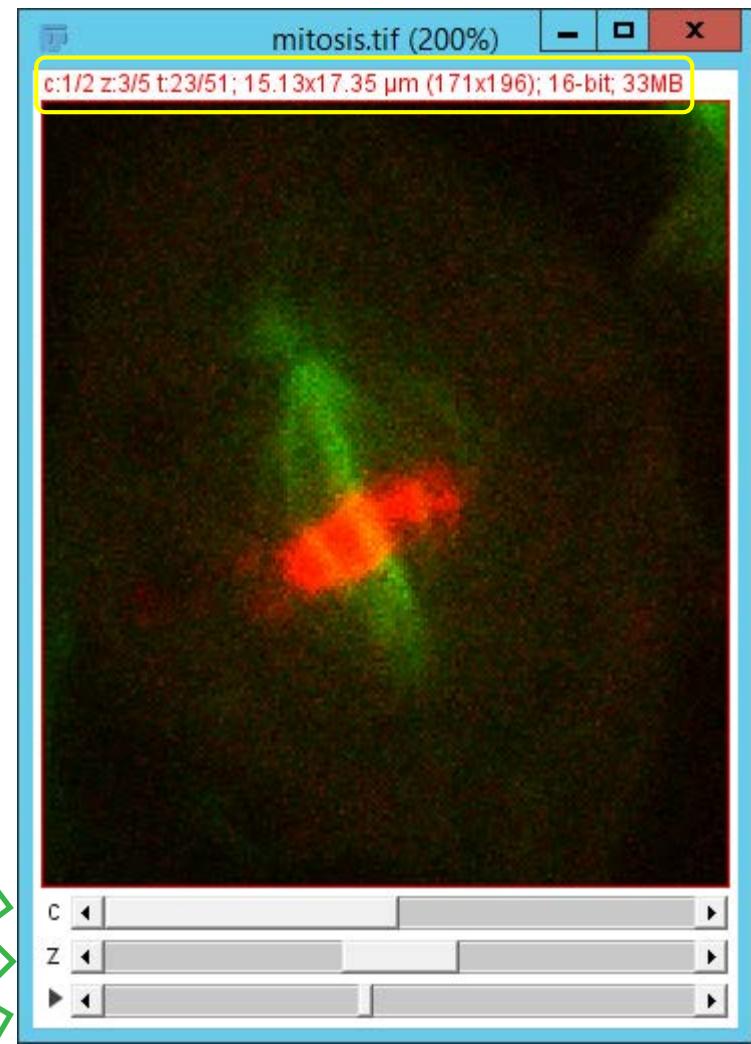


Image Properties and Info

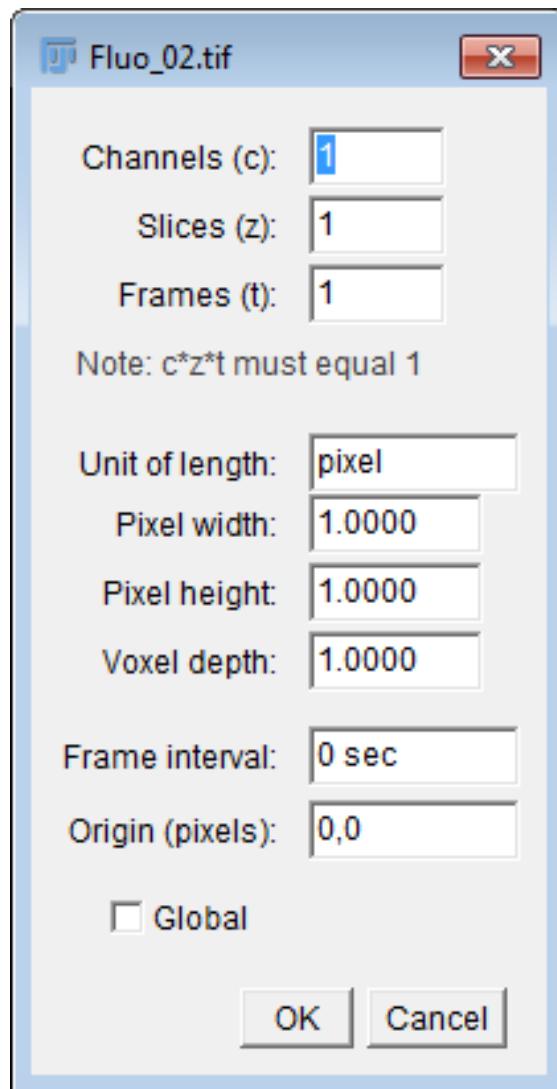
1. Open *Fluo_02.tif*
2. Image>Properties... (ctrl+shift+P)
3. Image>Show Info...

The image shows two windows from the Fiji software interface. The left window is titled "Fluo_02.tif Properties" and contains fields for Channels (c), Slices (z), and Frames (t), all set to 1. It also includes fields for Unit of length (pixel), Pixel width (1.0000), Pixel height (1.0000), Voxel depth (1.0000), Frame interval (0 sec), and Origin (pixels) (0,0). A note at the bottom states "Note: c*z*t must equal 1". The right window is titled "Info for Fluo_02.tif" and displays various metadata: Title: Fluo_02.tif, Width: 860 pixels, Height: 860 pixels, Size: 2.8MB, Pixel size: 1x1 pixel², ID: -47, Bits per pixel: 32 (RGB), No threshold, ScaleToFit: false, Uncalibrated, Path: L:\common\01-formation analyse d'image\IGBMC\En_cours\Ateliers Fiji 2019\Images\Input-Ouput\Fluo_02.tif, Screen location: 984,209 (1920x1200), No overlay, and No selection.

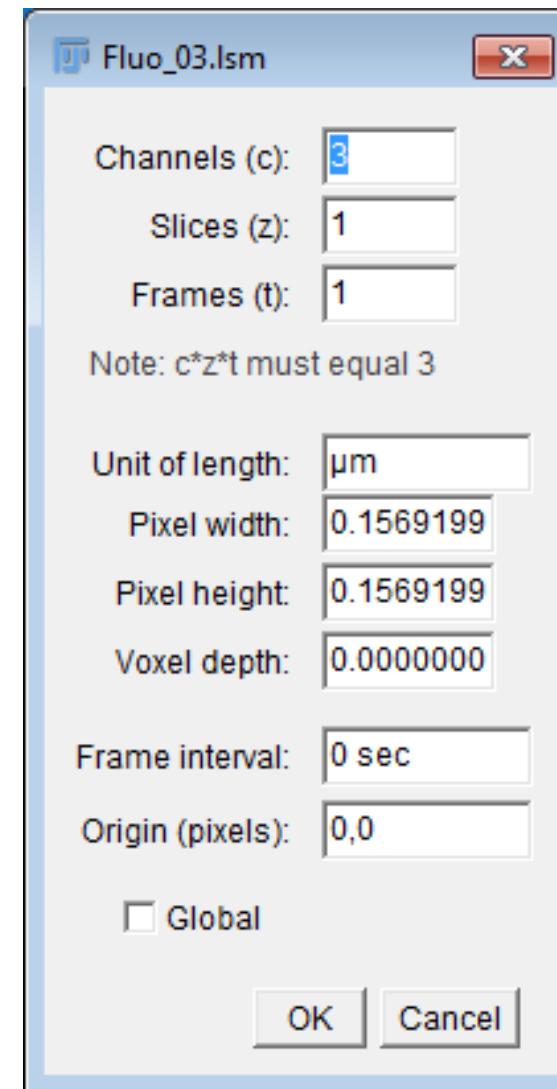
1. Now with *Fluo_03.lsm*
2. Spotting some differences ?

Calibrating and Adding a Scale Bar

Uncalibrated

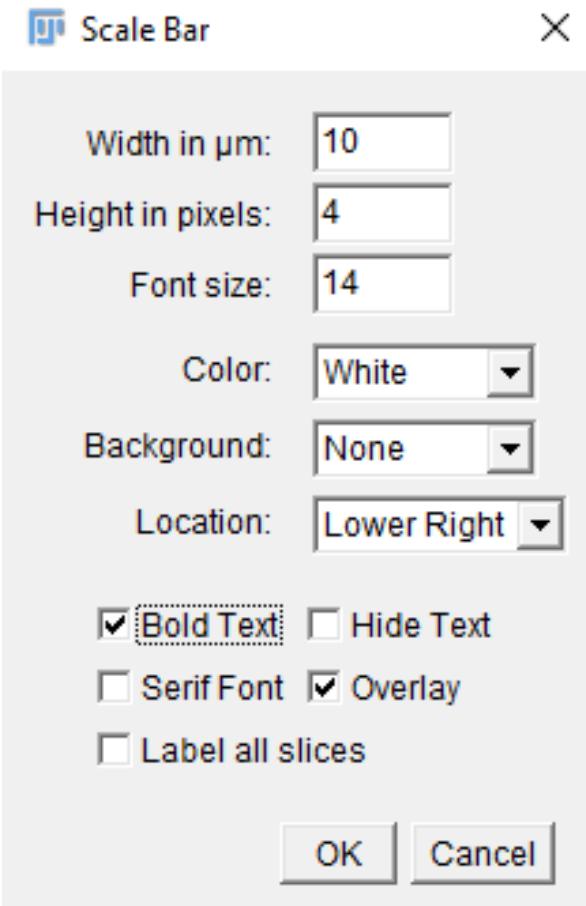


Calibrated



Adding a Scale Bar

Analyze>Tools>Scale Bar...

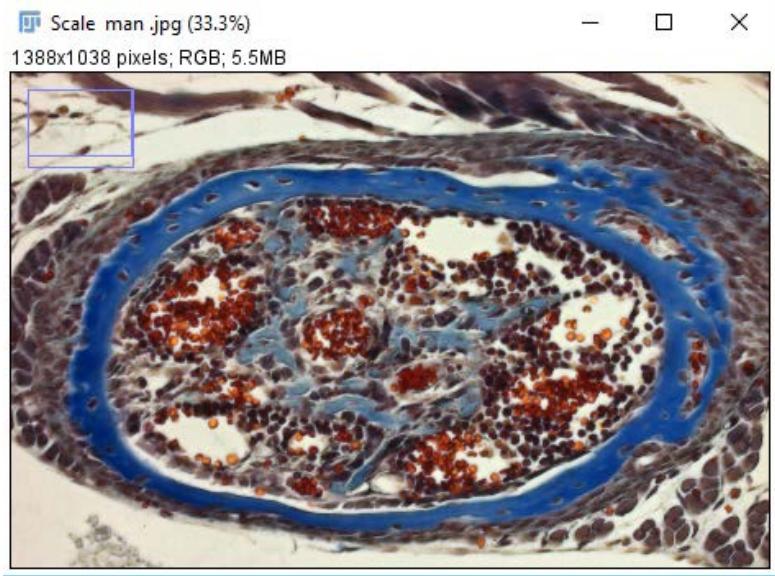


Calibrating and Adding a Scale Bar

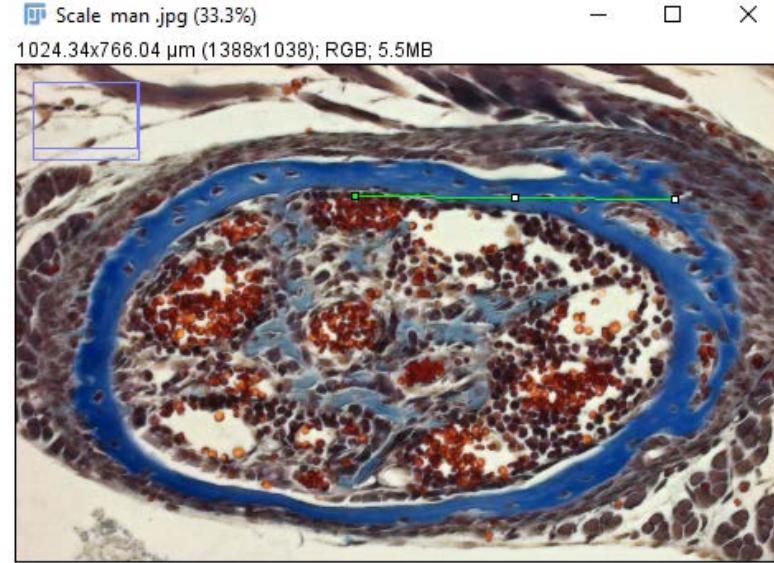
How to calibrate ?

- the file's metadata are correctly read by Fiji
- you know the scale
- you calculate it
- you measure it with a graticule

Calculate the Calibration



1388x1038 pixels



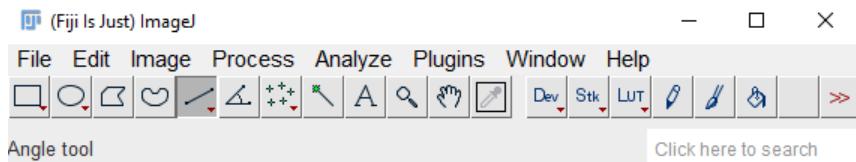
1024,34x766,04μm
(1388x1038 pixels)

Objectif of microscope	10X
Camera Adapter Magnification	0.63X
Pixel size of the camera	4,65μm

Theoretical pixel size of image

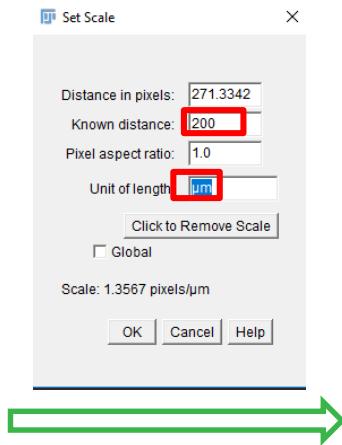
$$4,65 / (10 \times 0,63) = 0,738 \mu\text{m}$$

Set Scale

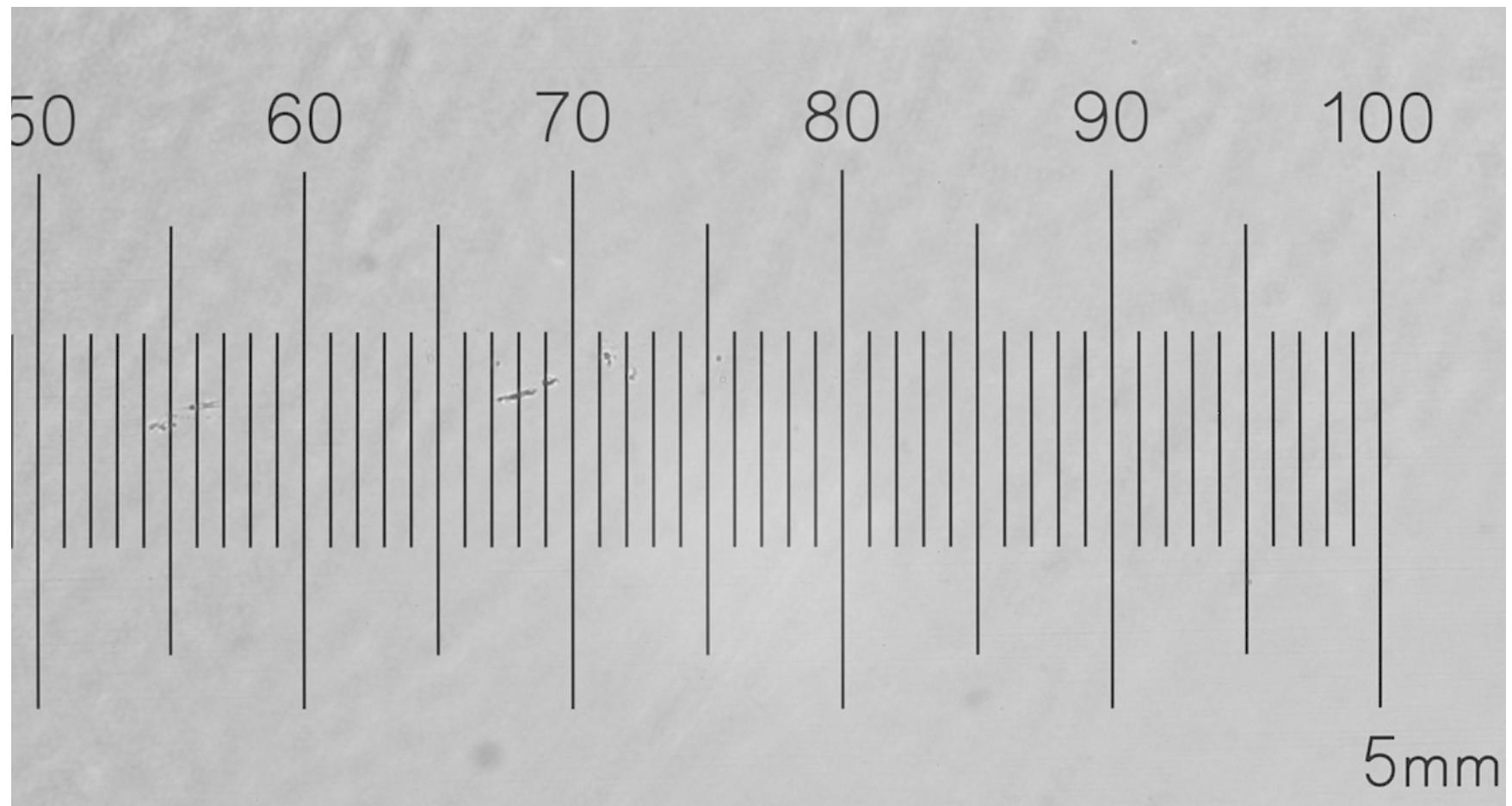


Use the Line Selection tool to draw a selection line a known length. Here we've drawn a 200 μ m line

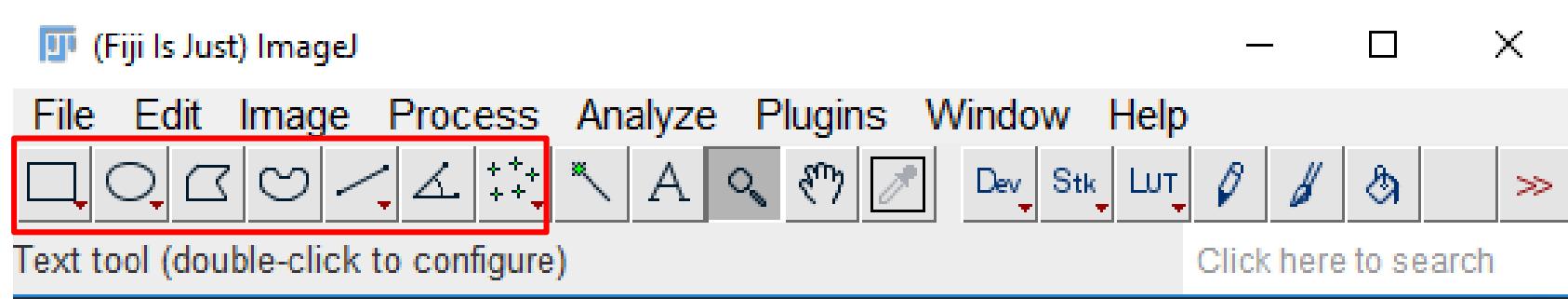
To calibrate the image go to: Analyze>Set Scale...



Use a Graticule



Region of Interest (ROI)

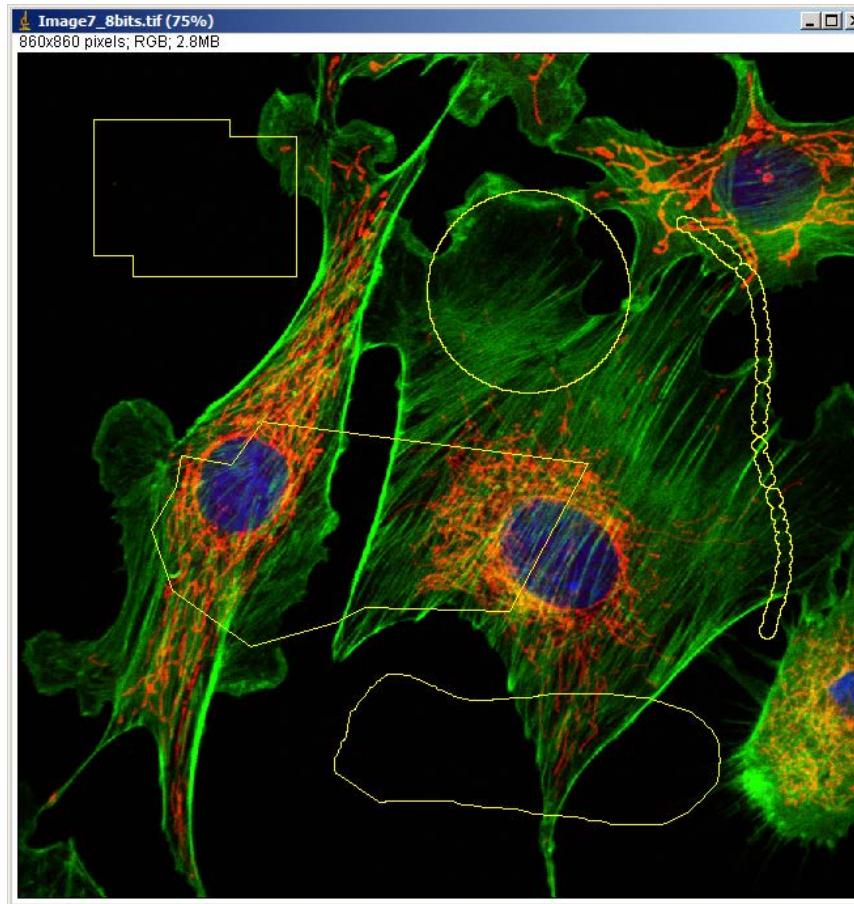


- Crop an image, make a subset
- Duplicate a portion of an image
- Annotate your data
- Highlight a specific area for further analysis
- Measure
- ...

Selection Tools for Creating ROI

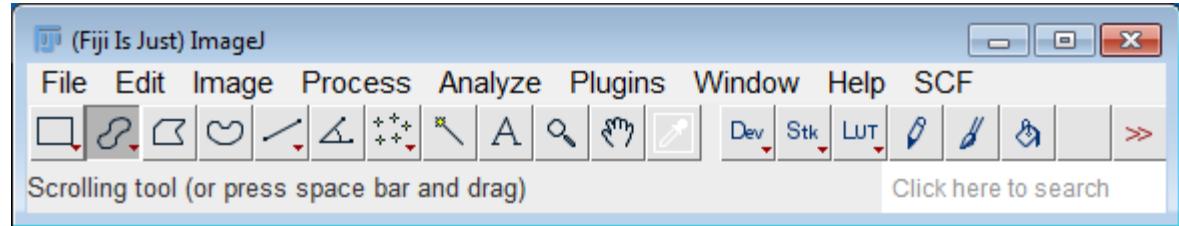
Selection tools
Edit menu

<https://imagej.nih.gov/ij/docs/guide/146-10.html#toc-Section-10>
<https://imagej.nih.gov/ij/docs/guide/146-27.html#toc-Section-27>

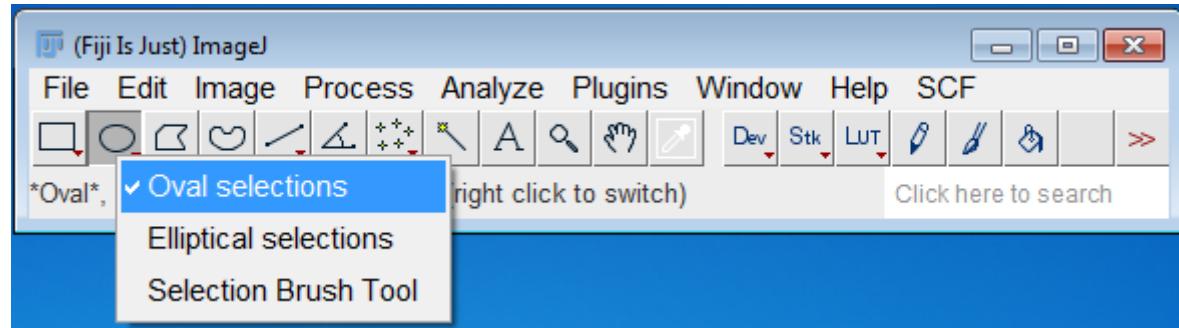


Selection Tools

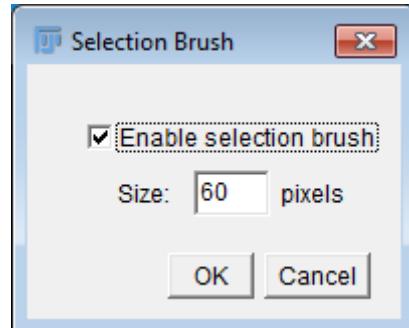
Left click



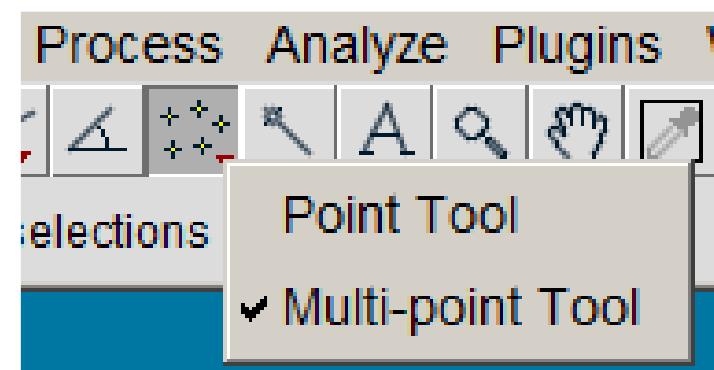
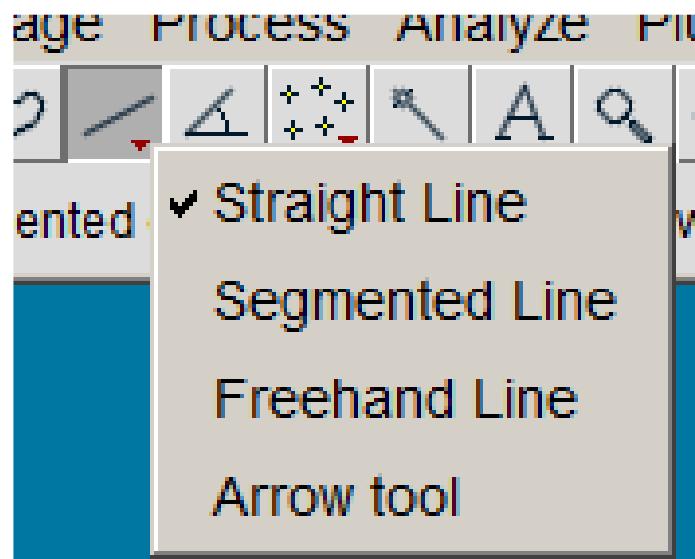
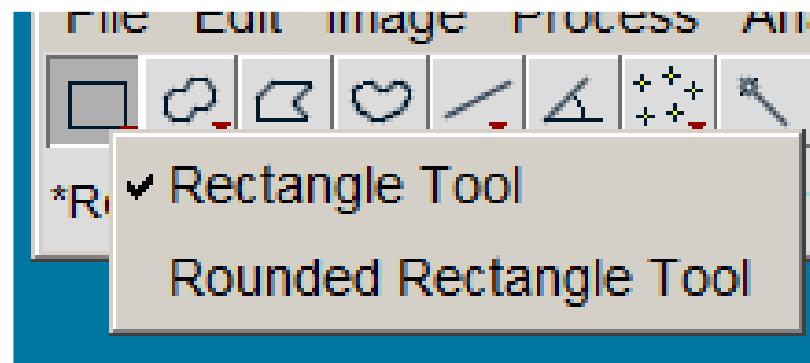
Right click
for more tools



Double left click
for tool options

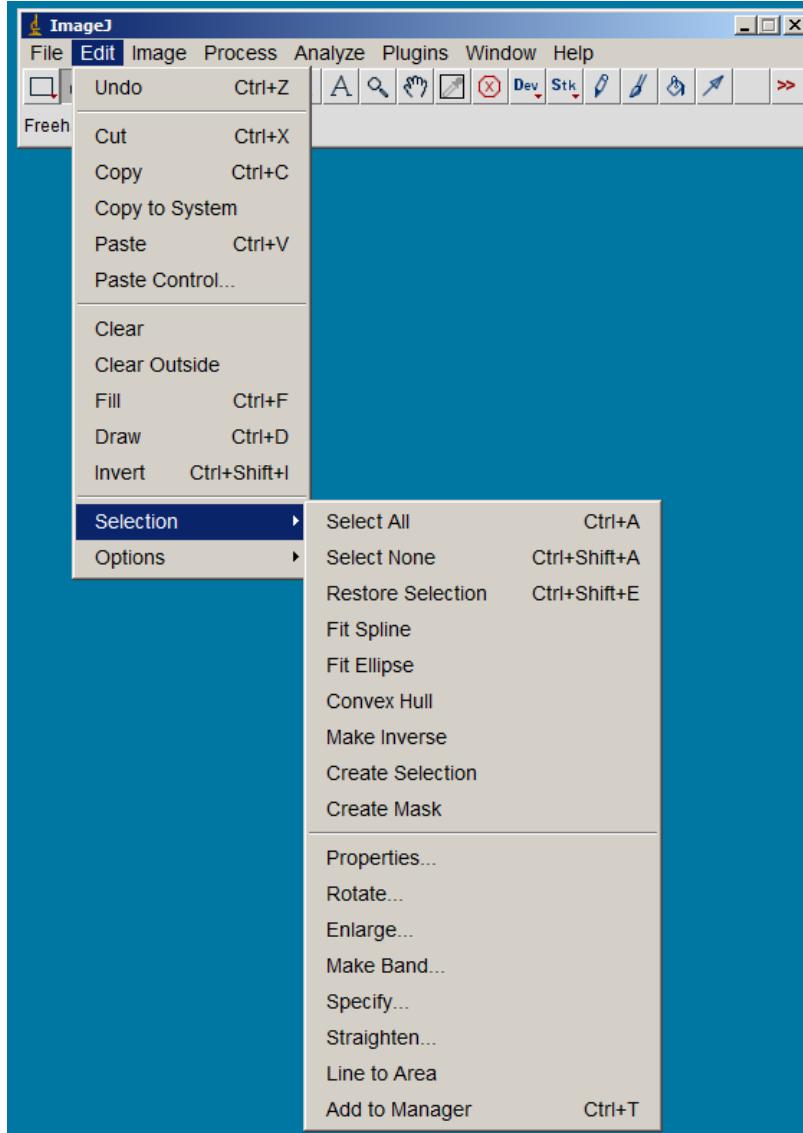


More Selection Tools

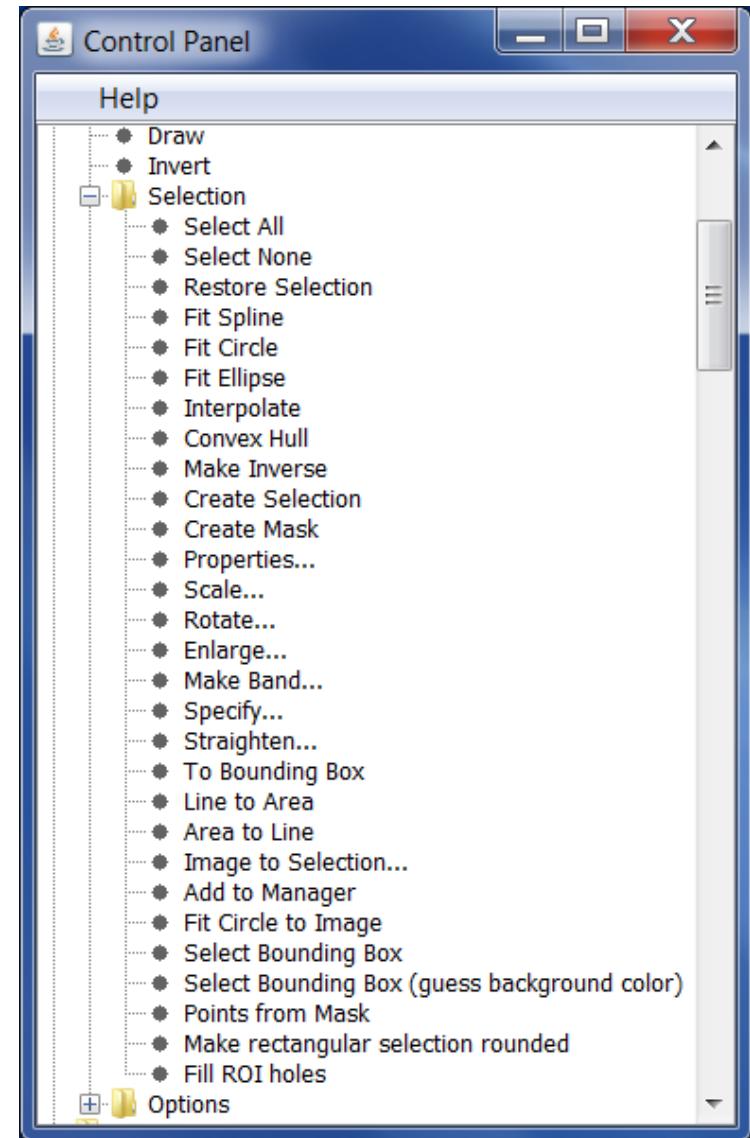


Edit>Selection

Via the menu Edit>Selection

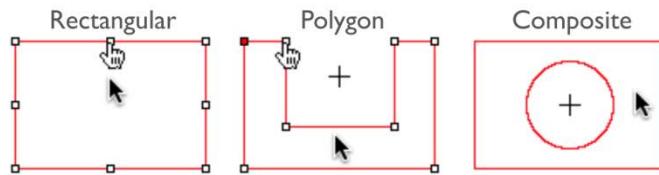


Control Panel shift+U

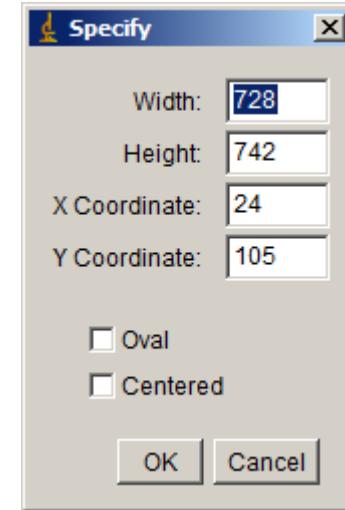
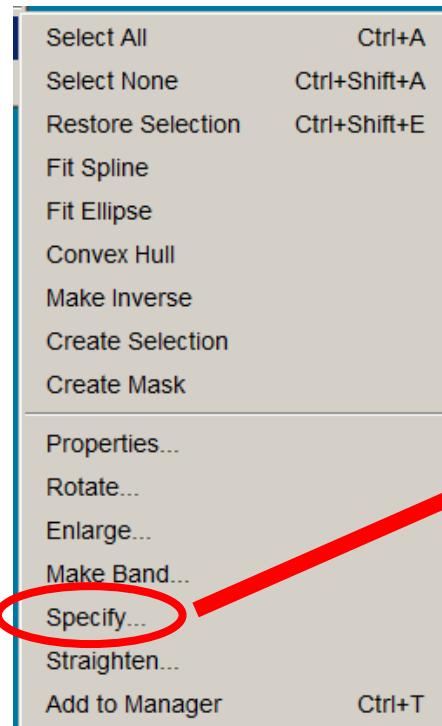


Specify Selection

<https://imagej.nih.gov/ij/docs/guide/146-27.html#toc-Subsection-27.12.>



- + Cursor outside selection
- ← Selection can be moved
- Selection can be resized
- Edge can be moved, deleted or added



Composite Selection

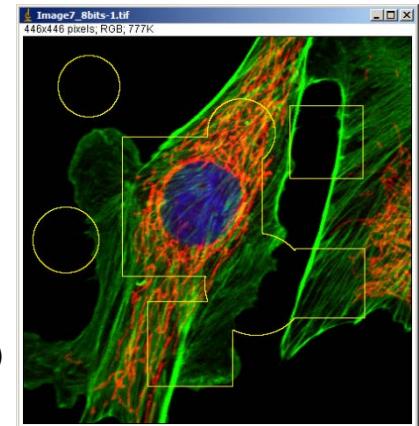
<http://rsbweb.nih.gov/ij/docs/guide/146-10.html#sub:Composite-selections>



+

Add to selection

(release shift key to add non-square selection)

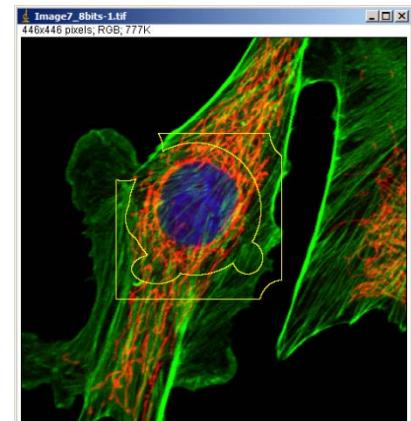


Selection tool

+



Remove from selection



Resize ROIs

Holding down different keys while resizing will have different effects on the resizing.

Shift – create/resize ROI with a 1:1 width:height ratio.
Makes square, circle and horizontal/vertical lines

Ctrl – Free resize around the centre of the ROI

Alt – Resize the ROI while keeping the aspect ratio

Combinations of keys: **Alt** and **Ctrl** to resize an ROI with the same aspect ratio around its centre.

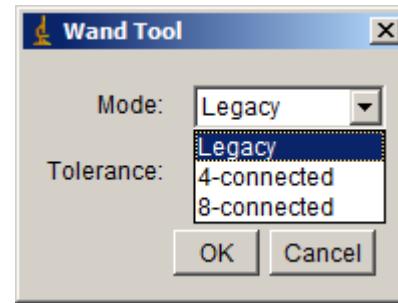
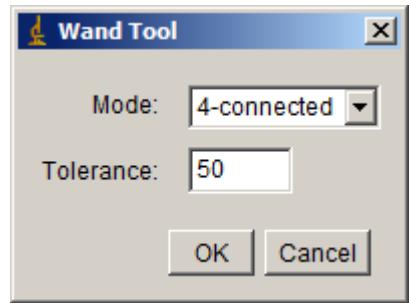
Remove ROIs

To remove the last ROI:

- click outside the ROI's boundaries
- Ctrl + Shift + A

Wand Tool

<https://imagej.nih.gov/ij/docs/guide/146-19.html#toc-Subsection-19.7>



Selection range = from initial value – tolerance to initial value + tolerance

4-connected

	1	
4		2
	3	

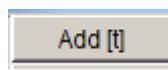
8-connected

1	2	3
4		5
6	7	8

ROI Manager



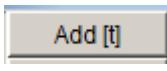
Add a ROI to the manager (slice-X-Y coordinates)



or



Add and draw a R.O.I.



Add and rename a R.O.I.

Region Of Interest (ROI) Manager

<http://rsbweb.nih.gov/ij/docs/guide/146-30.html#fig:The-ROI-Manager>

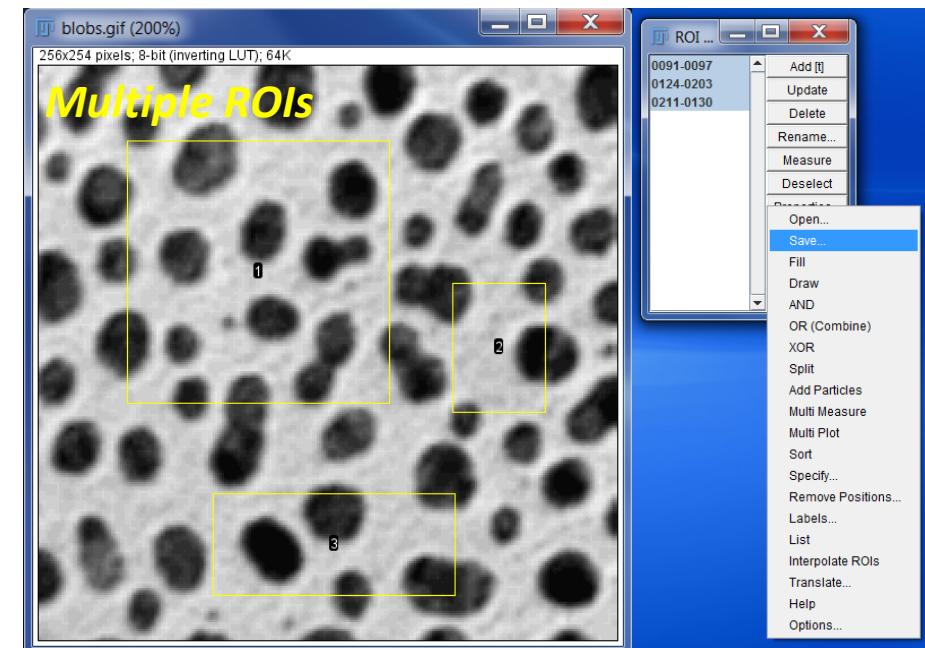
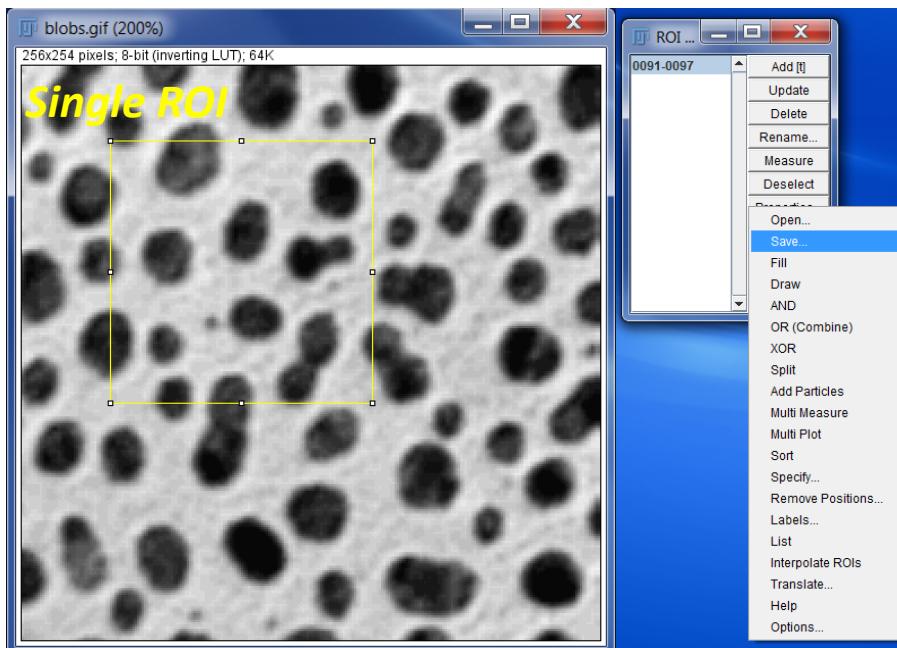
Region Of Interest (ROI) Manager

Single ROI

- save the image as .tiff
- save the roi via the ROI manager as a .roi file

Multiple ROIs

- Save the set of ROIs via the ROI Manager as a Roiset.zip file

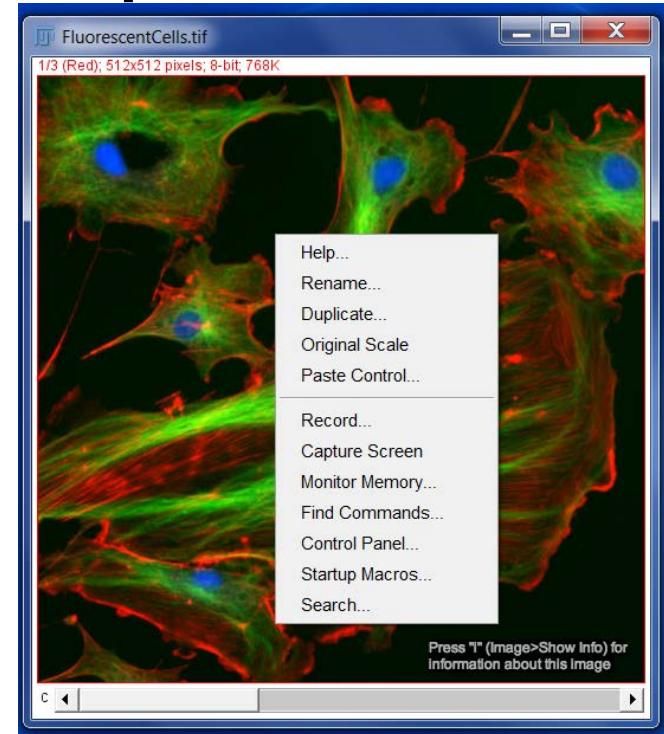


Crop & Duplicate

To duplicate an image:

- right click on the image and select **Duplicate....**
- use the shortchut **Ctrl+Maj+D**
- ***Image>Duplicate...***

AVANTAGES: keep a copy of the data for history and comparison

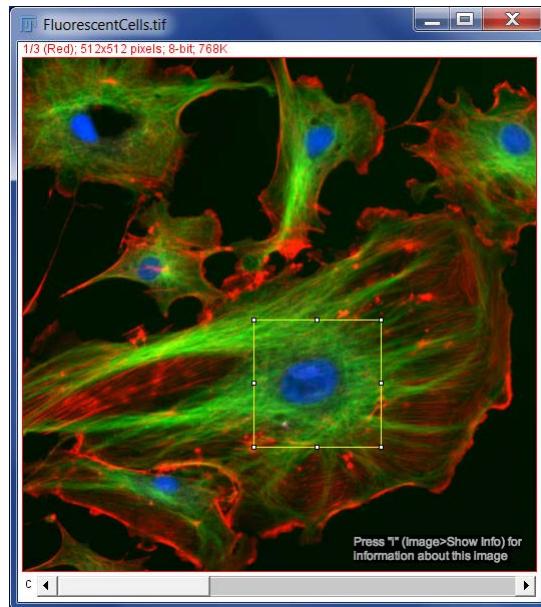


To crop an image:

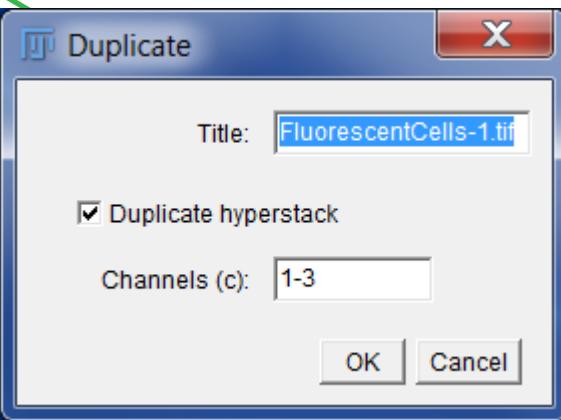
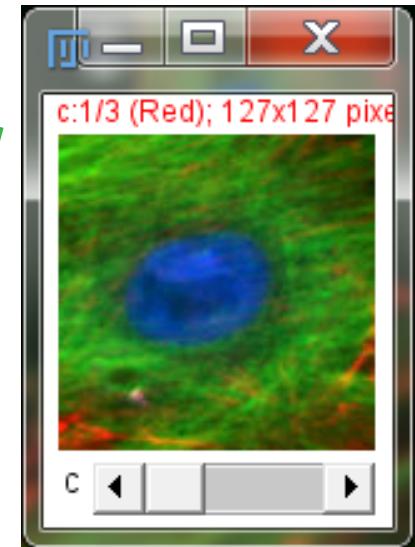
- ***Image>Crop***
- use the shortchut **Ctrl+Maj+X**
- duplicate the ROI (keeps the original image)

ADVANTAGES: save on memory usage

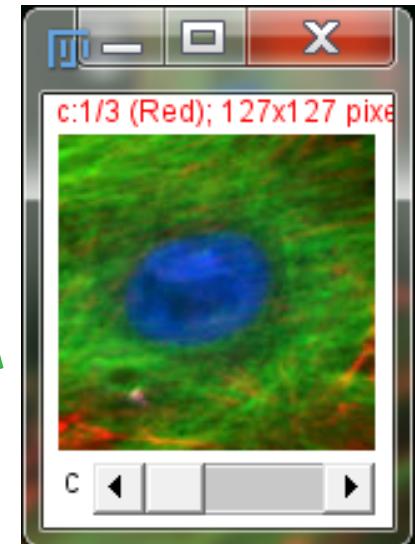
Crop or Duplicate ?



Crop

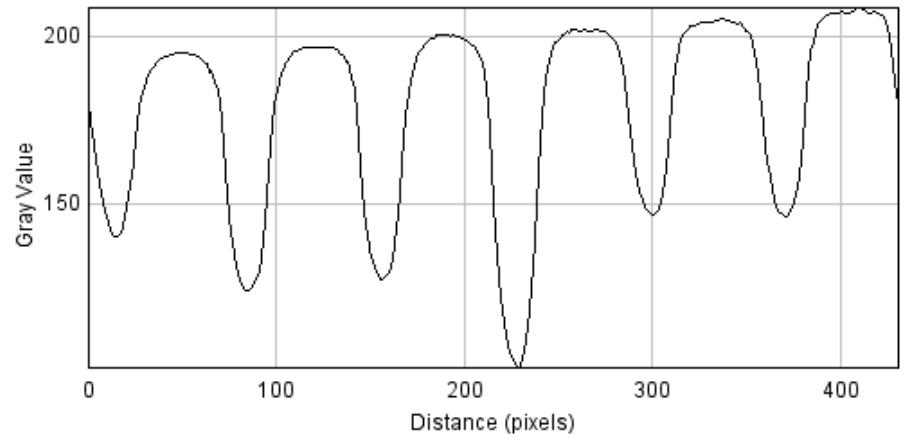
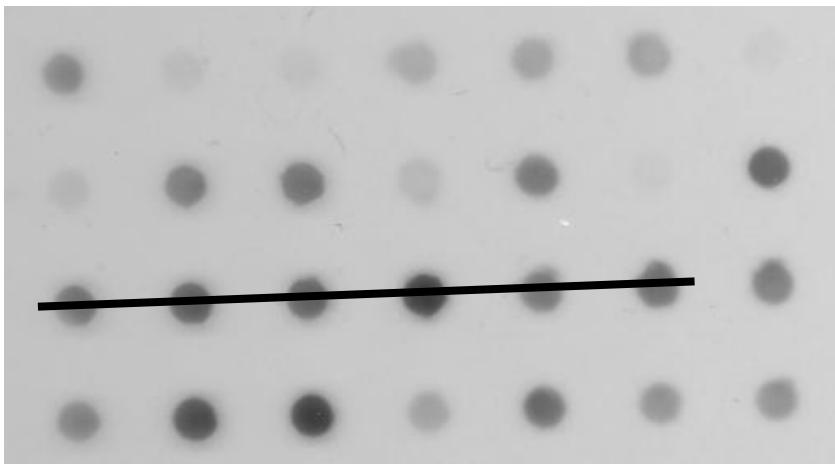


Duplicate



Plot Profile (ctrl+k)

Displays a two-dimensional graph of the intensities of pixels along a line or rectangular selection.

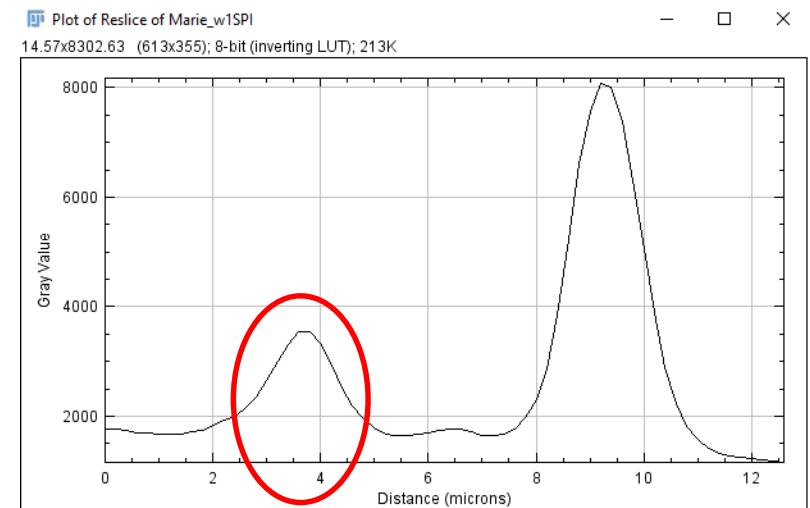
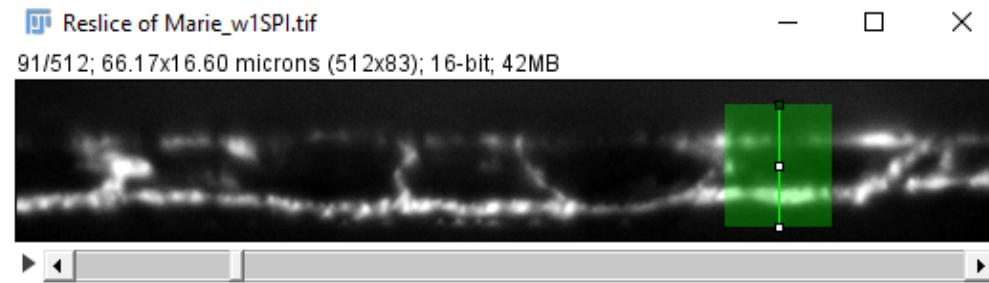
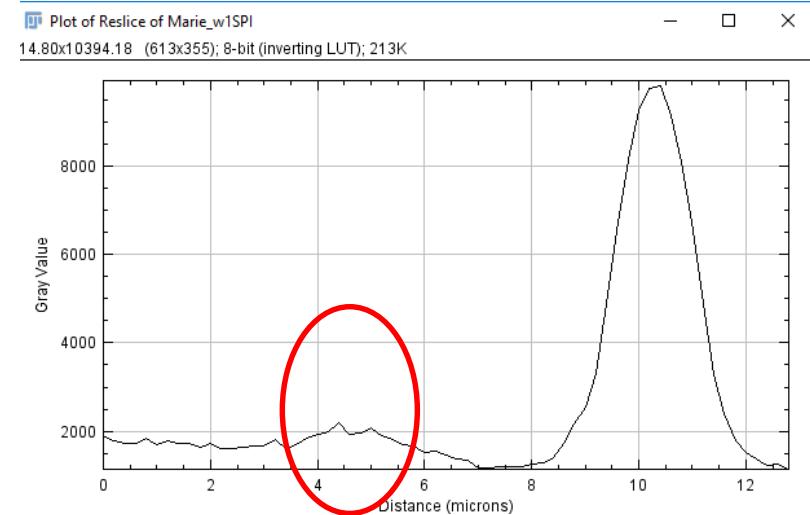
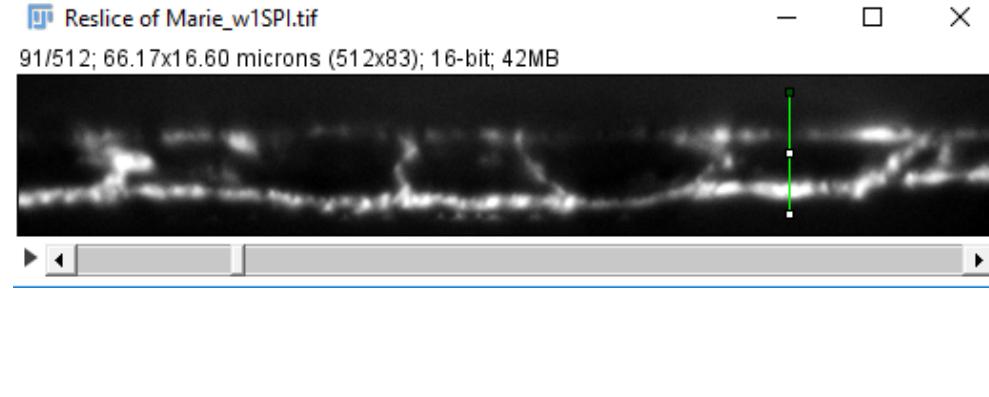


Rectangular selection: “ctrl+k” or “alt+k”

“Multi plot” command

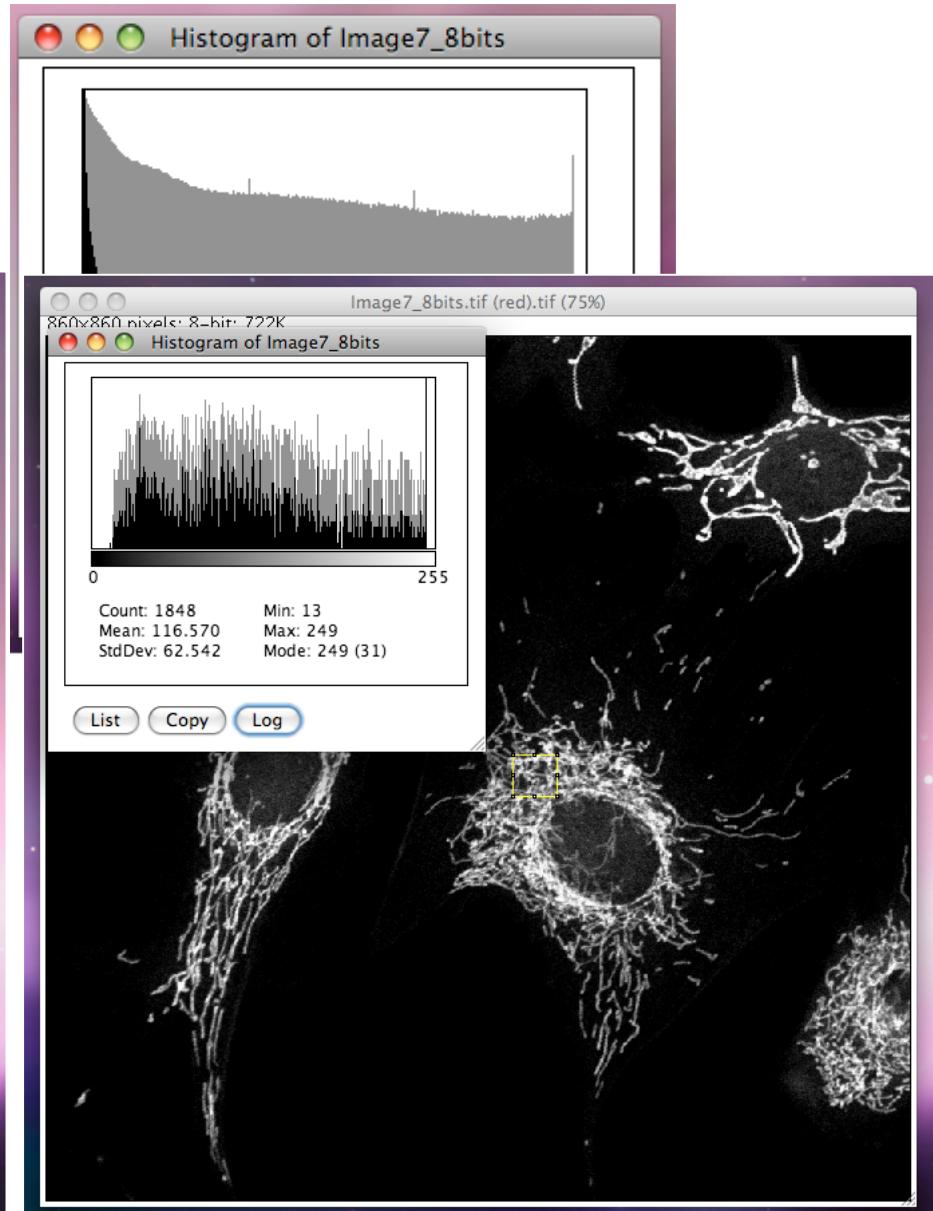
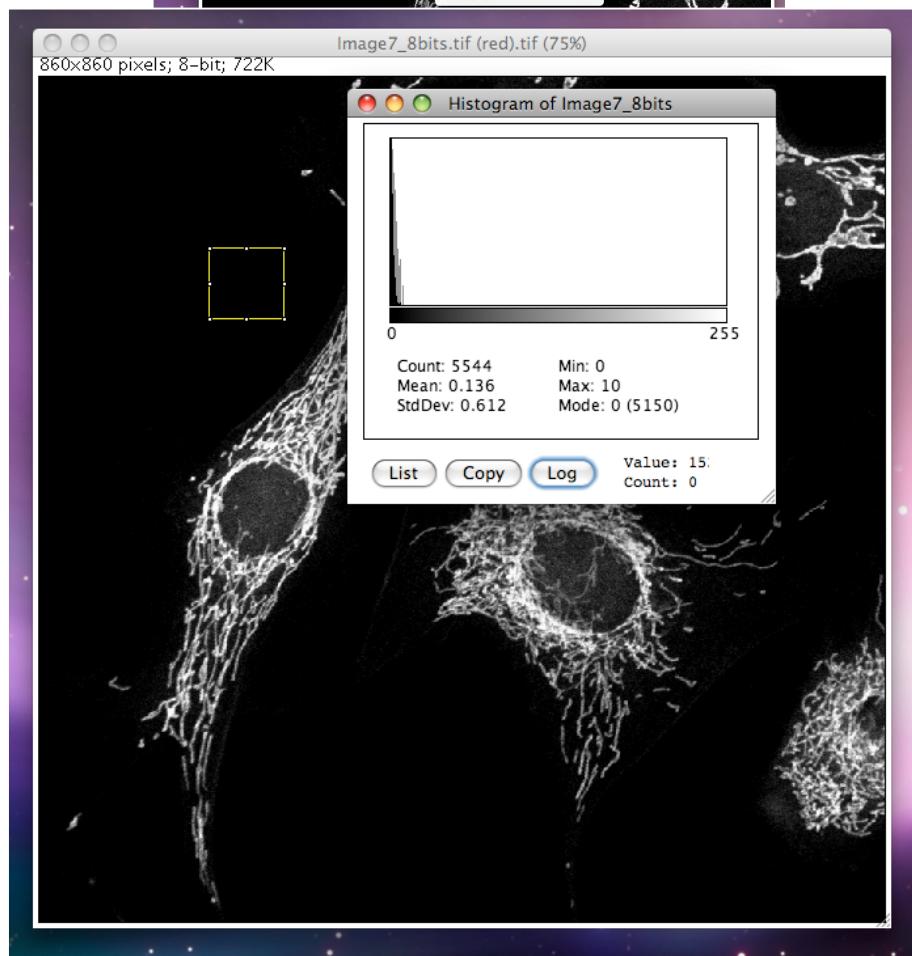
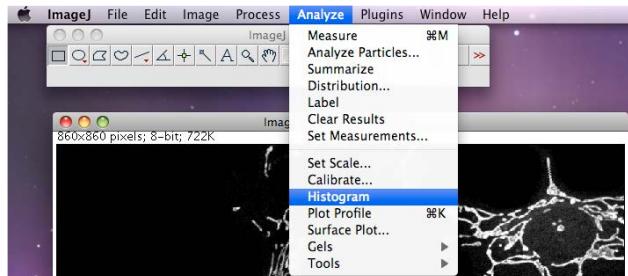
“Live” option

Plot Profile (ctrl+k): thickness of the line



Histogram

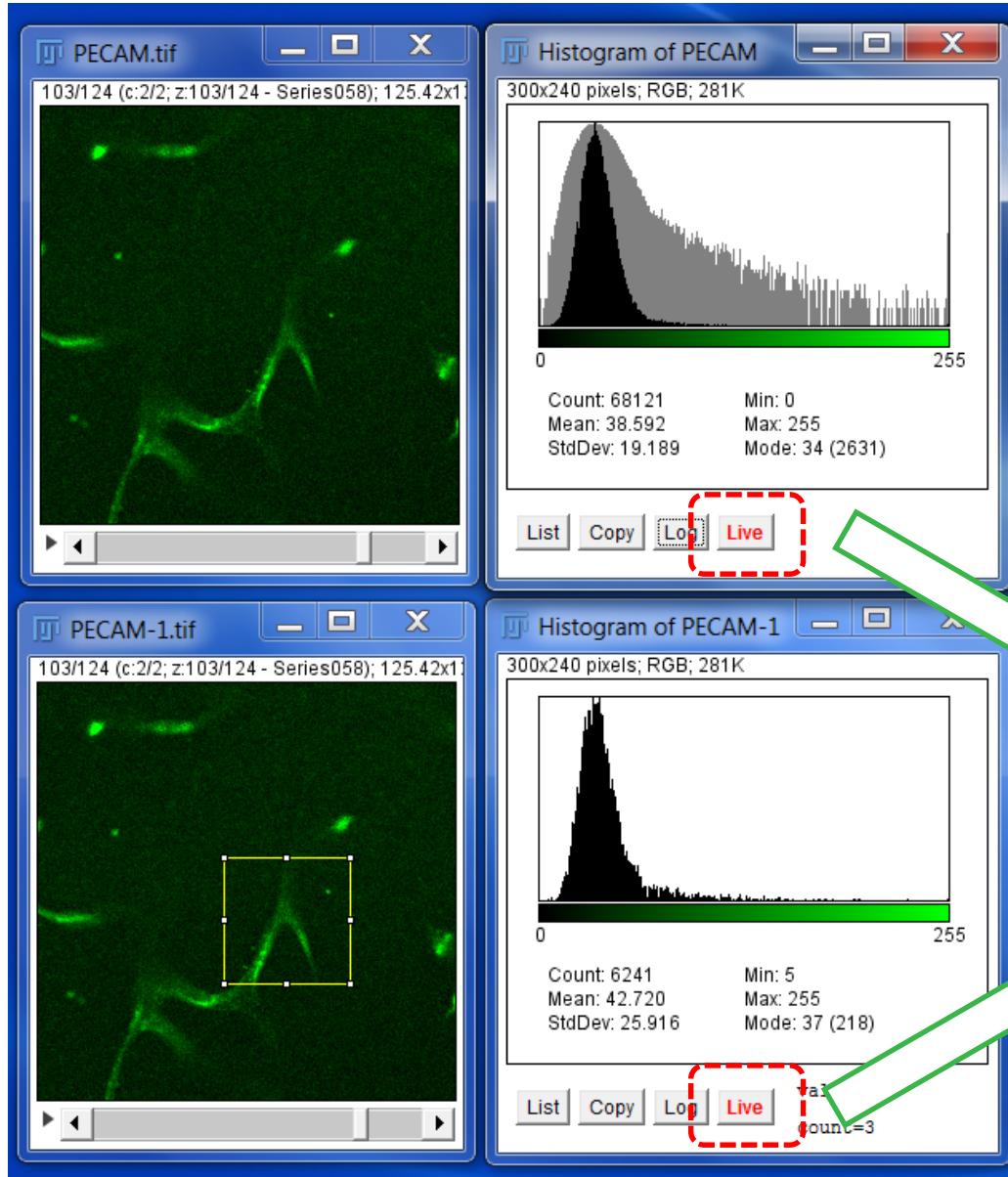
Ctrl H



Histogram

Ctrl

H



Live mode
- slice histogram
- ROI histogram

Measurements

- Based on the selection type calculates and displays on the Results Table either area statistics, line lengths and angles, or point coordinates.

<https://imagej.nih.gov/ij/docs/guide/146-22.html>

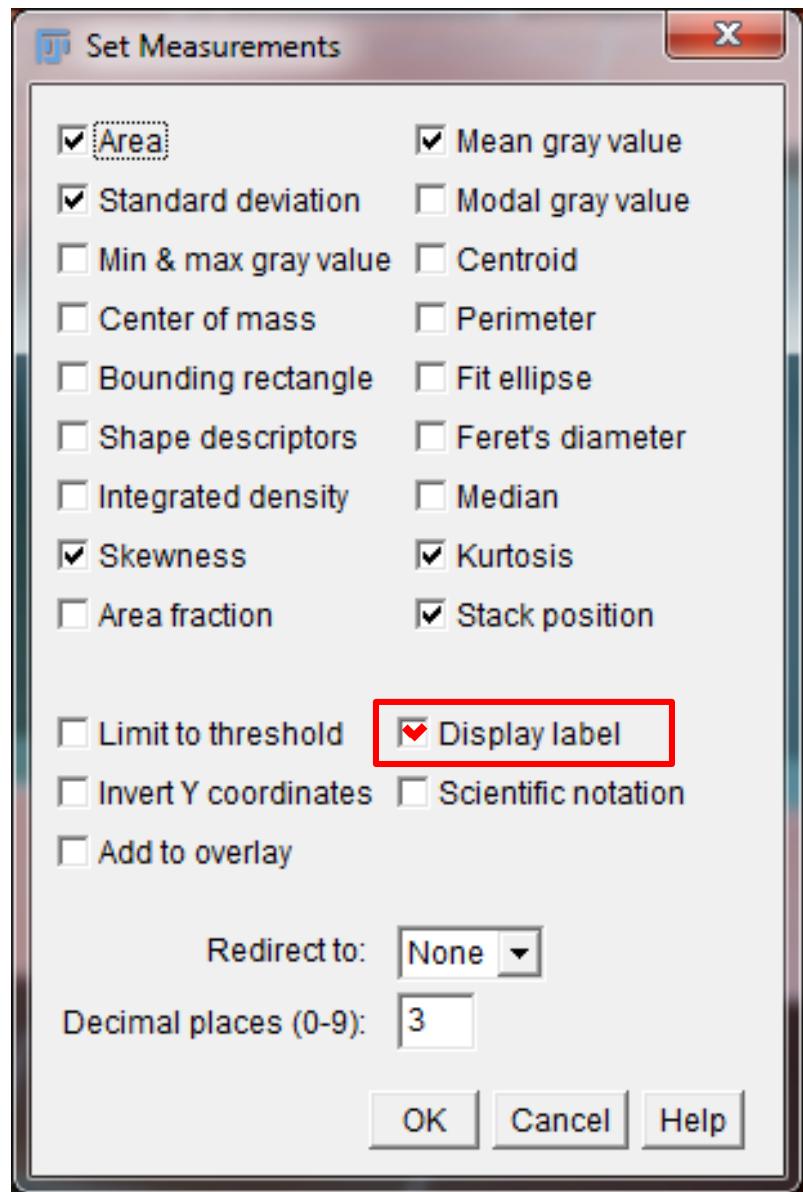
- Performed measurements can be specified in the Set Measurements... dialog box.

<https://imagej.nih.gov/ij/docs/guide/146-30.html>

Measurements

Analyze>Measure (ctrl+M)
Analyze>Set Measurements...

Other types of measurement are available through macros and plugins



<https://imagej.nih.gov/ij/docs/guide/146-30.html#toc-Subsection-30.7>

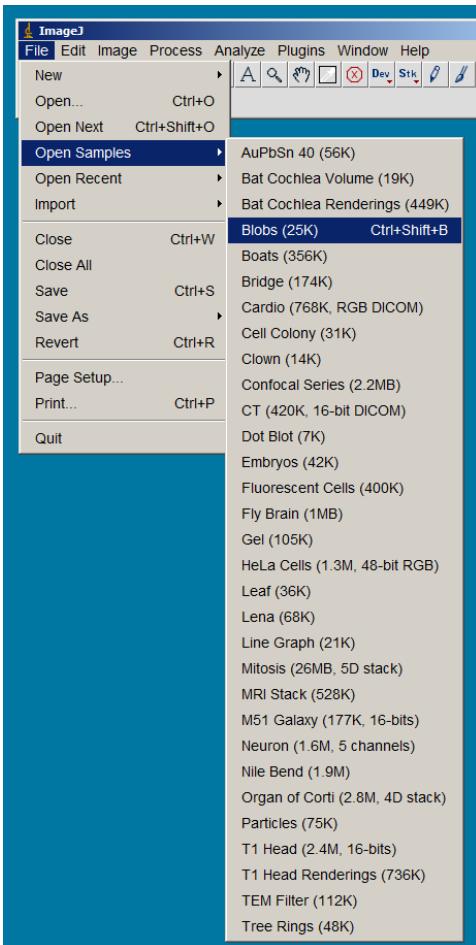
Results Table

	Area	Mean	Min	Max	%Area
1	29680	6.67	0	111	0
2	9408	31.37	6	119	0
3	12312	50.49	0	160	0
4	4836	46.20	8	121	0
5	10780	29.57	2	107	0
6	14820	22.29	0	115	0
7	18200	24.16	0	132	0
8	8400	45.16	8	153	0
9	17360	32.41	0	160	0
10	15860	19.05	0	146	0
11	22120	27.96	0	160	0
12	19608	33.05	0	144	0
13	22176	23.34	0	92	0
14	24568	60.11	0	160	0
15	15624	29.49	2	119	0

Save As...
text file .txt
Excel file .xls

Image Type
and
Channels

Image Type: examples



Blobs

8-bit grayscale

T1 Head

16-bit grayscale

Clown

RGB color

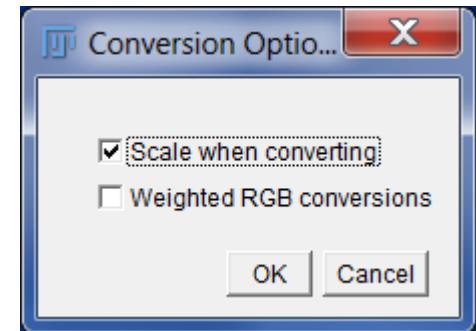
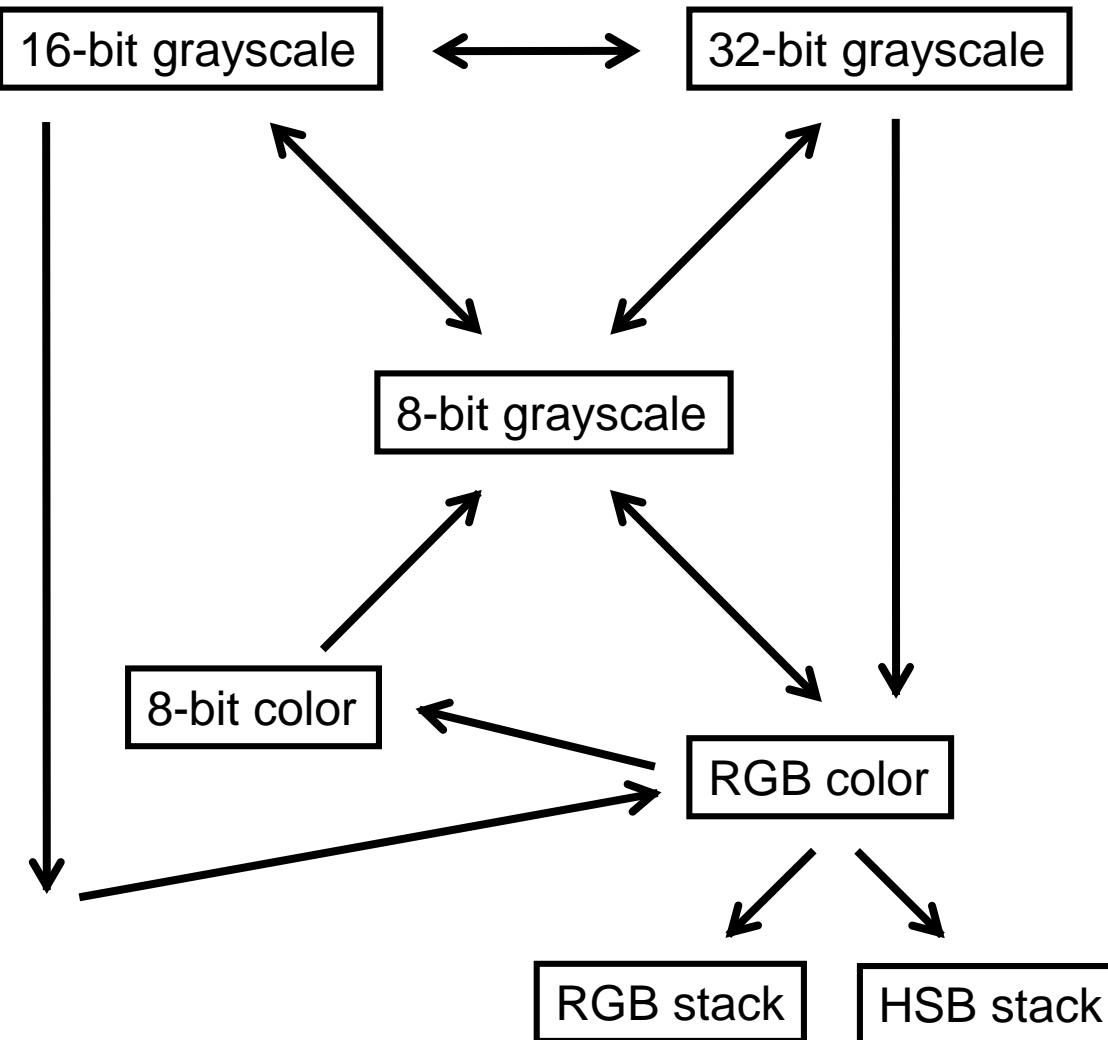
Fluorescent Cells

8-bit composite

Neuron

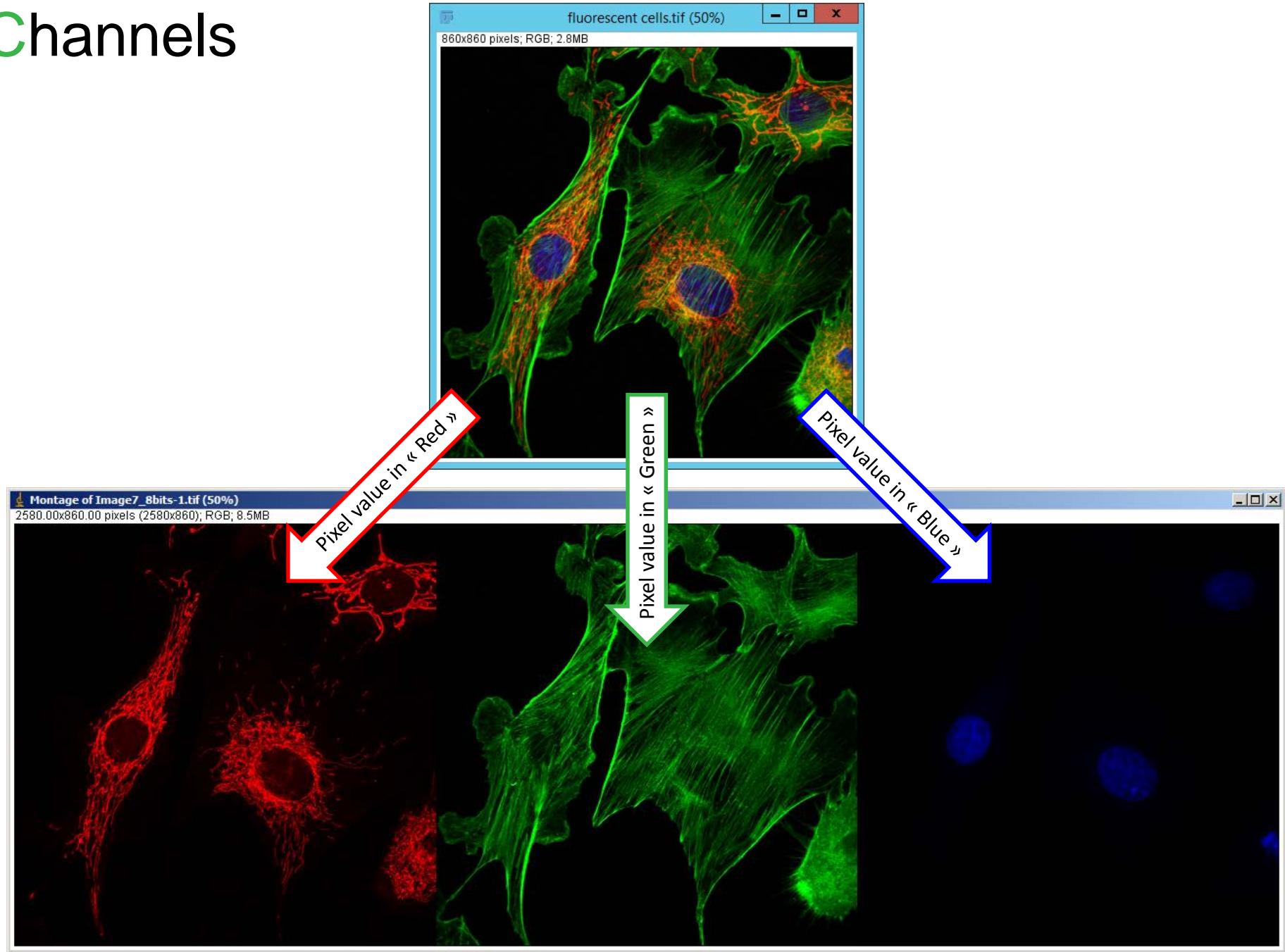
16-bit composite

Image Type Conversion

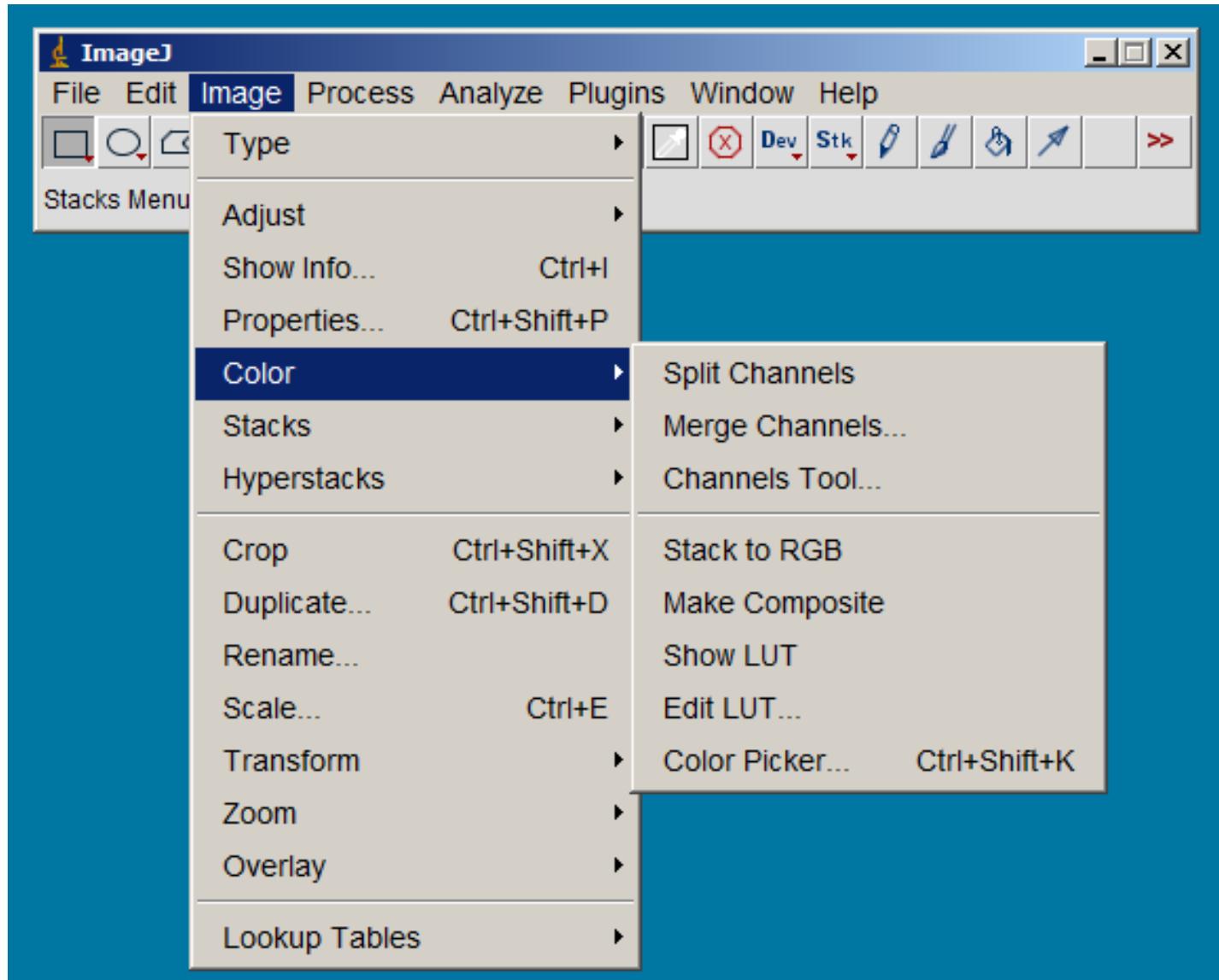


ImageJ will scale from min--max to 0--255 when converting from 16-bit or 32-bit to 8-bit or to scale from min--max to 0--65535 when converting from 32-bit to 16-bit.

Channels



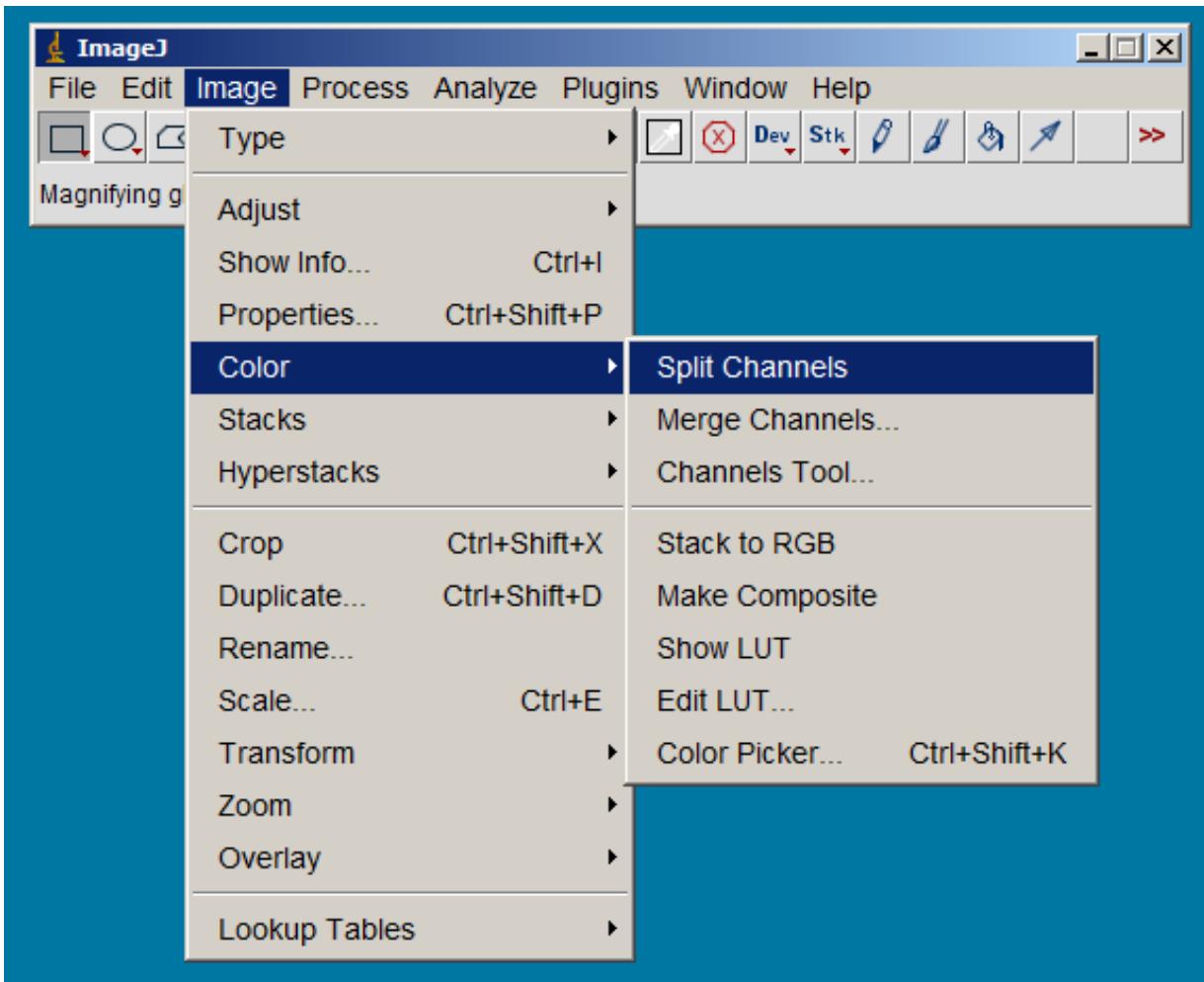
Image>Color



Image>Color>RGB split

Files must be RGB color

Output : 3 files in 8-bits grayscale for Red, Green and Blue
LUTS

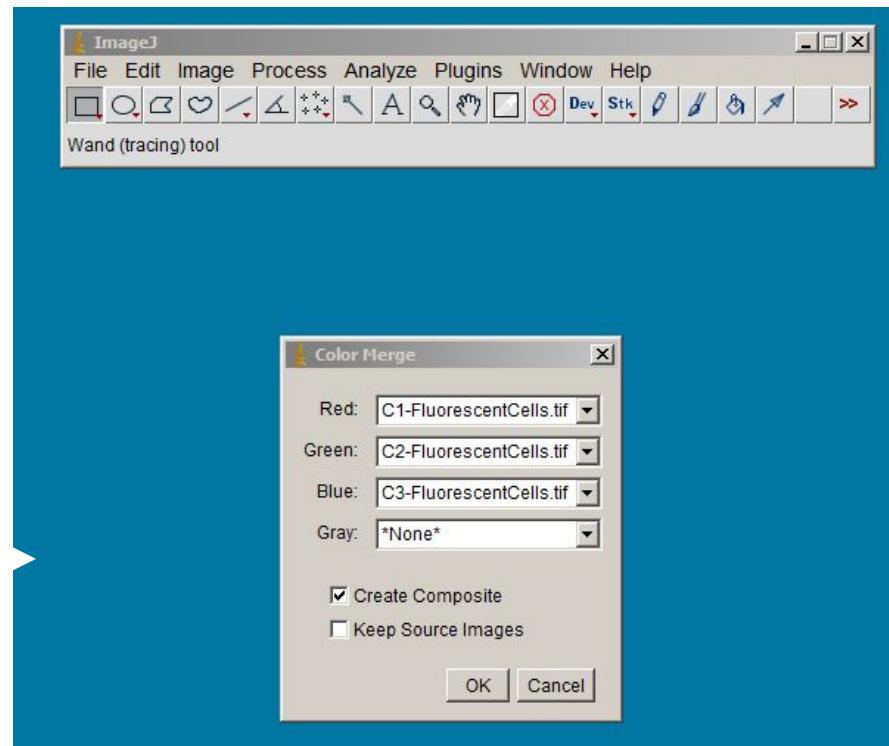
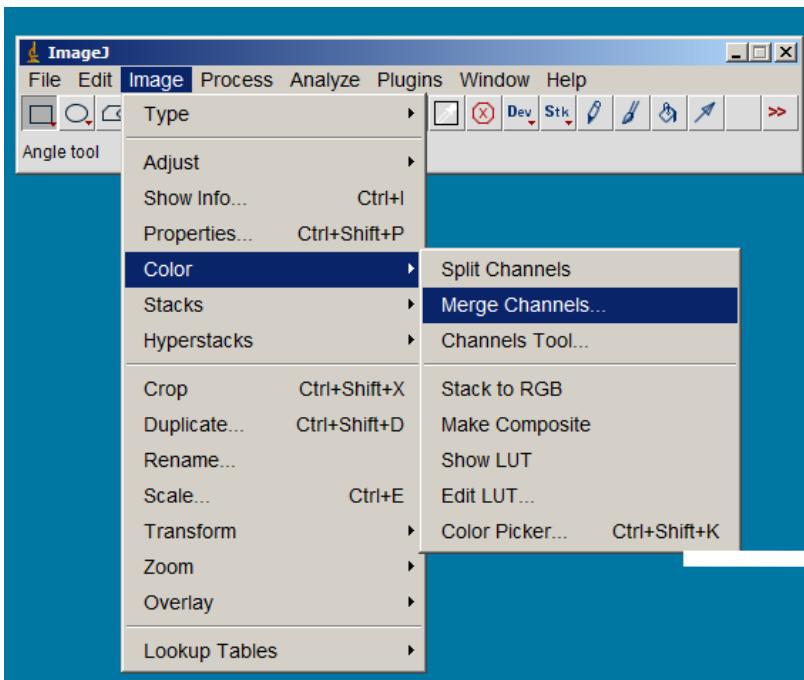


Image>Color>RGB merge

Files must have the same bit-depth and size

Select the file name for each channel (3 maximum)

Tick “keep image source” to keep source file open



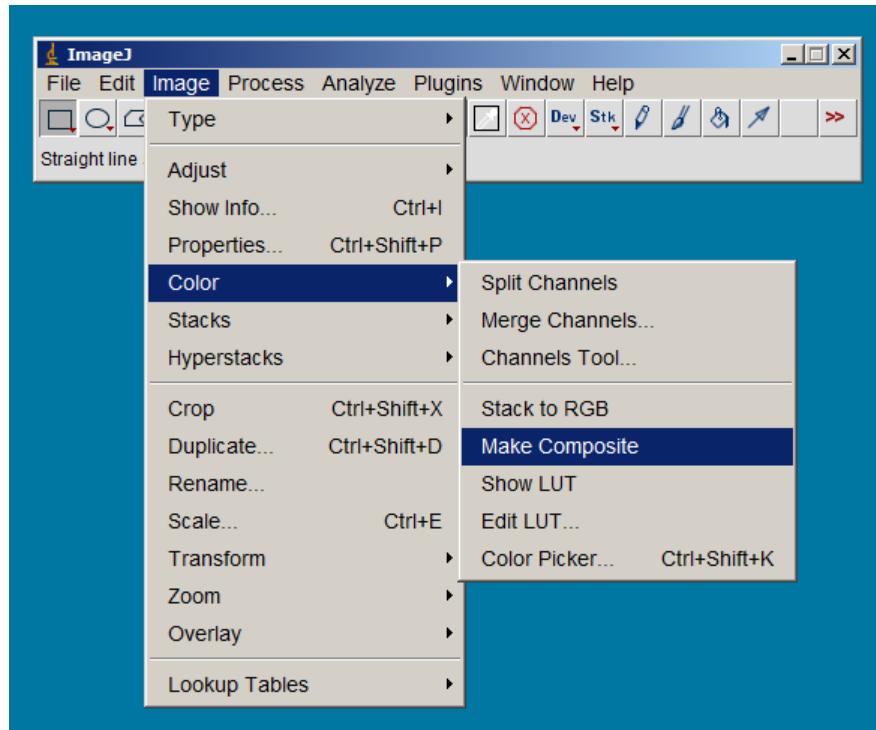
Composite Image

In a composite image, colours are handled through channels. The advantages with this type of image over plain RGB images are:

- each channel is kept separate from the others and can be turned on and off using the ‘Channels’ tool (Image>Color>Channels Tool...). This feature allows, e.g., to perform measurements on a specific channel while visualizing multiple.
- Channels can be 8, 16 or 32-bit and can be displayed with any lookup table
- More than 3 channels can be merged or kept separate

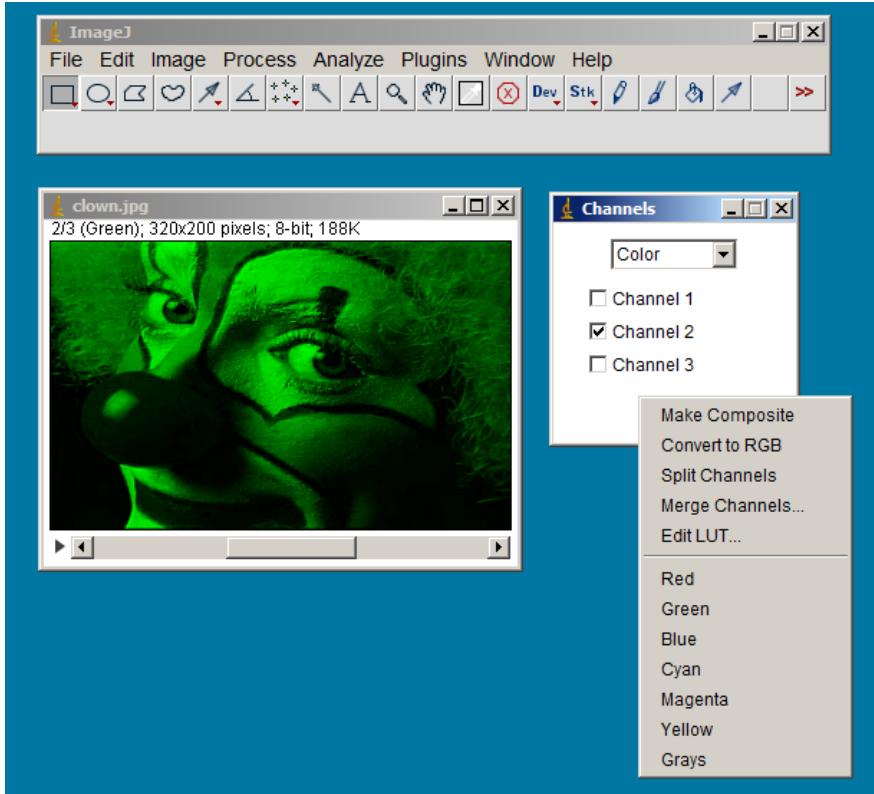
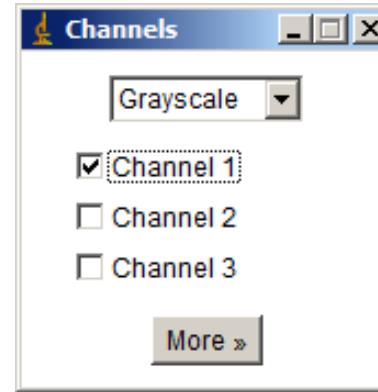
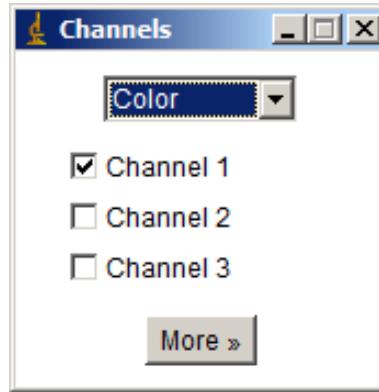
Composite Image

- More than 3 channels can be merged
- Can use 16-bit channel
- Single channel manipulation
- Same tools as RGB image



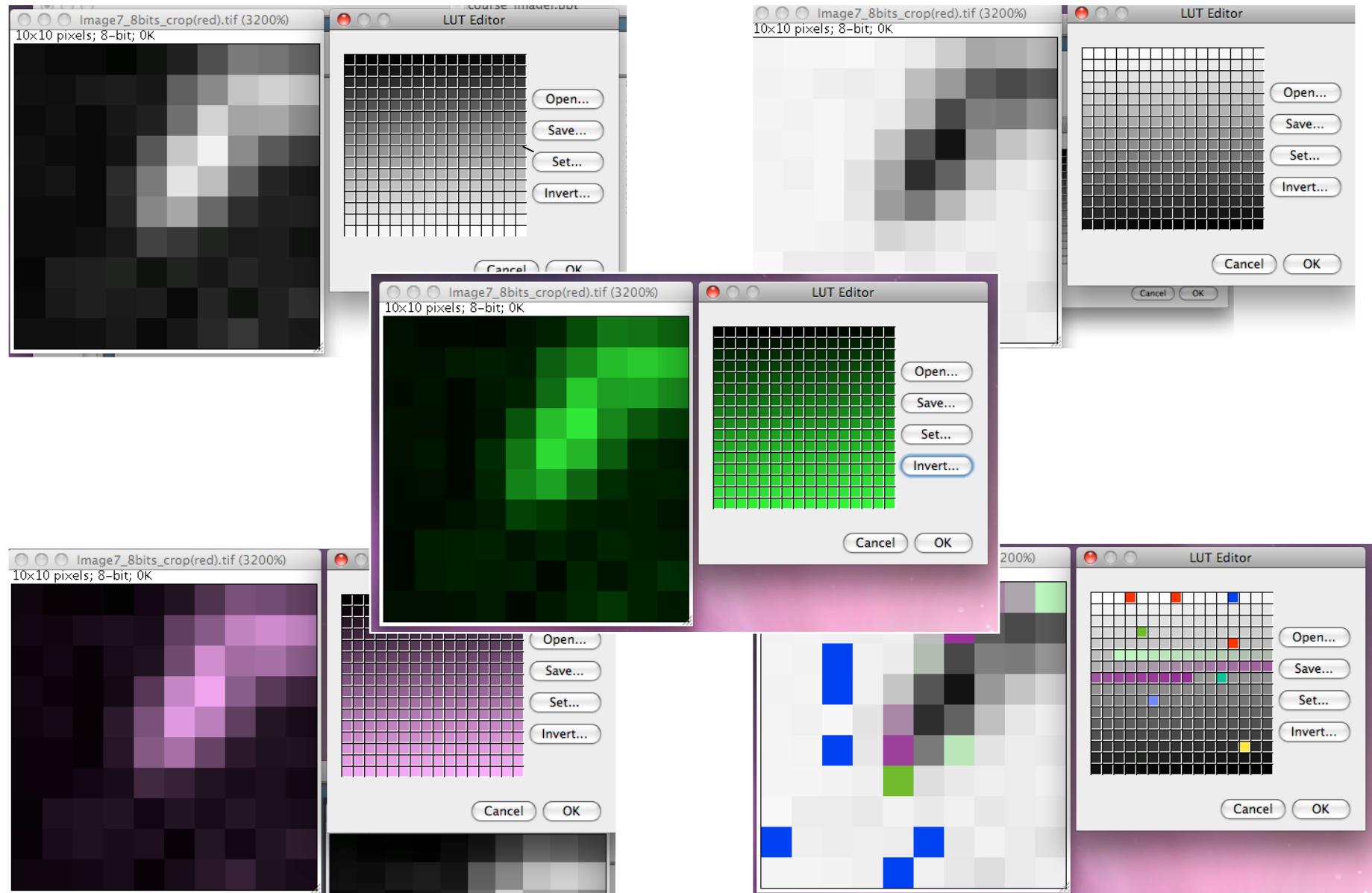
Examples : Open Samples>Neuron

Channels Tool **ctrl+shift+Z**



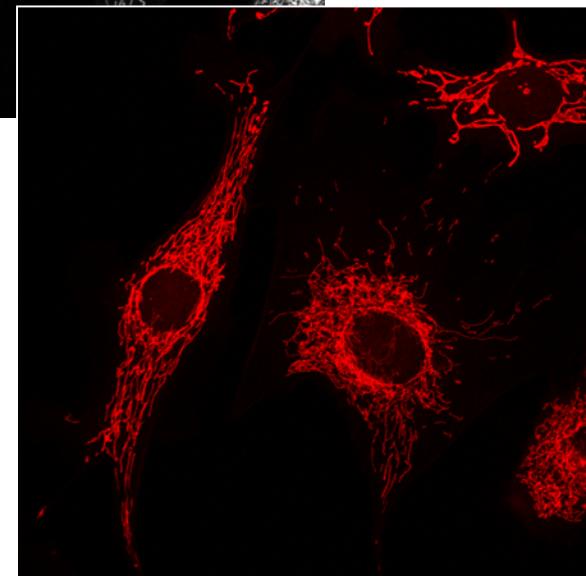
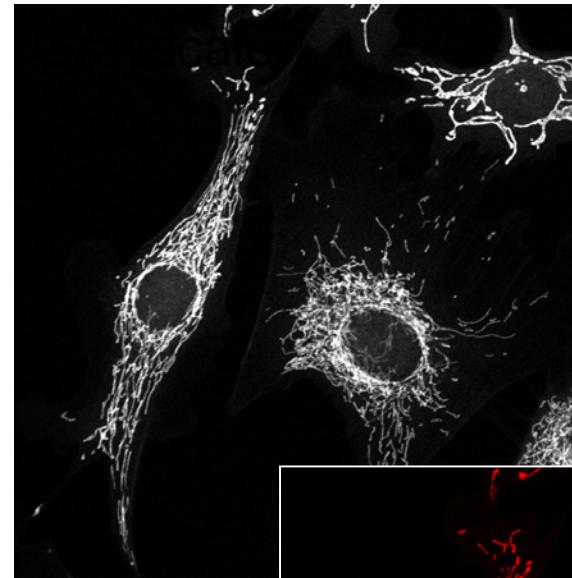
- Multi-channel overlay
- Single channel view
- Colour view
- Grayscale view
- Channels manipulations

Look-up Tables (LUT)

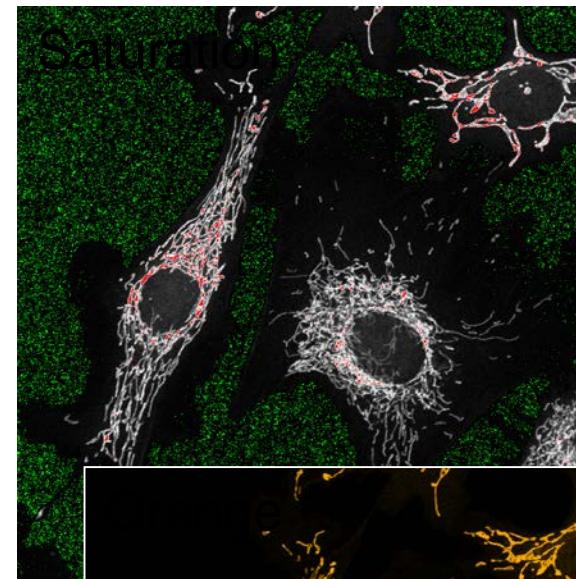
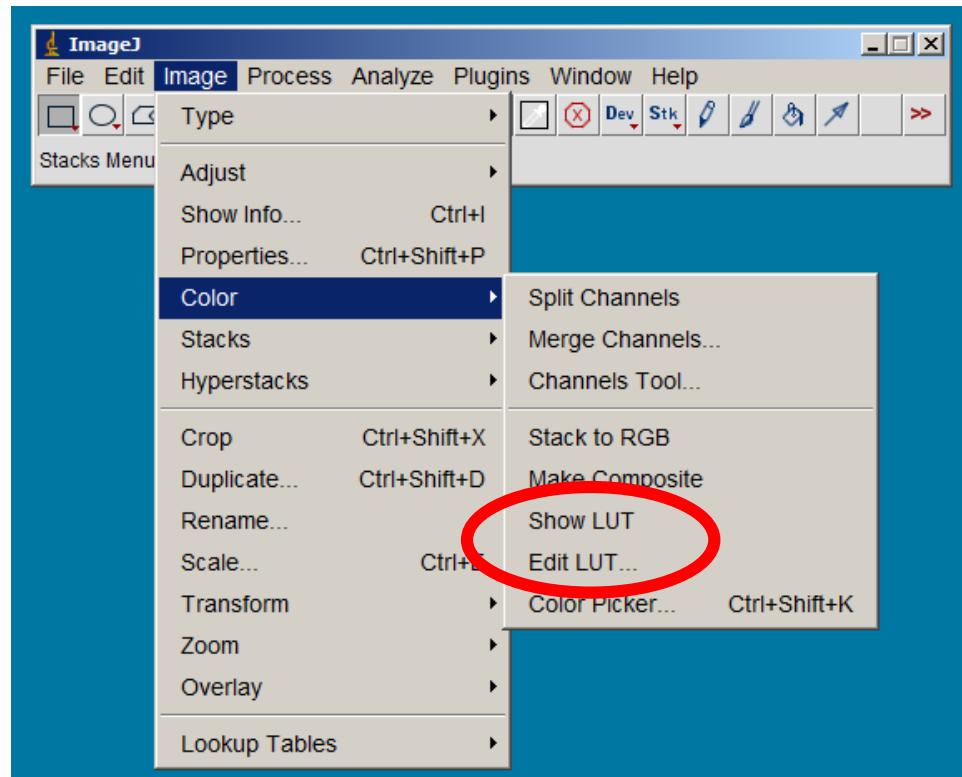


Changing Channel's Colour

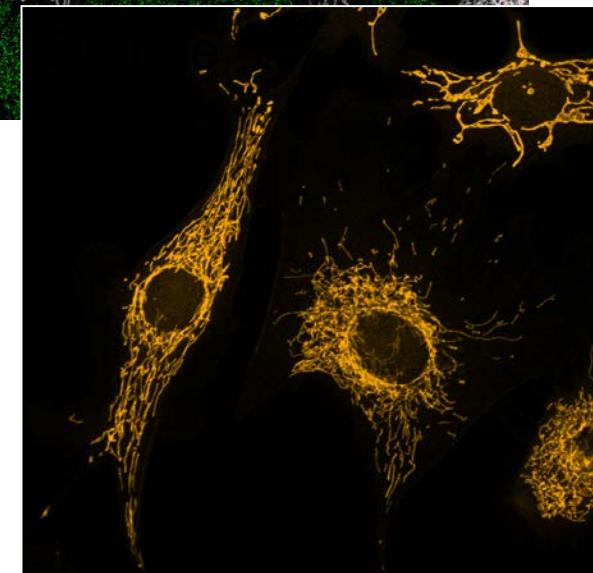
Look up table : Assign a color or shade of a color to a pixel intensity



Creating your own LUT



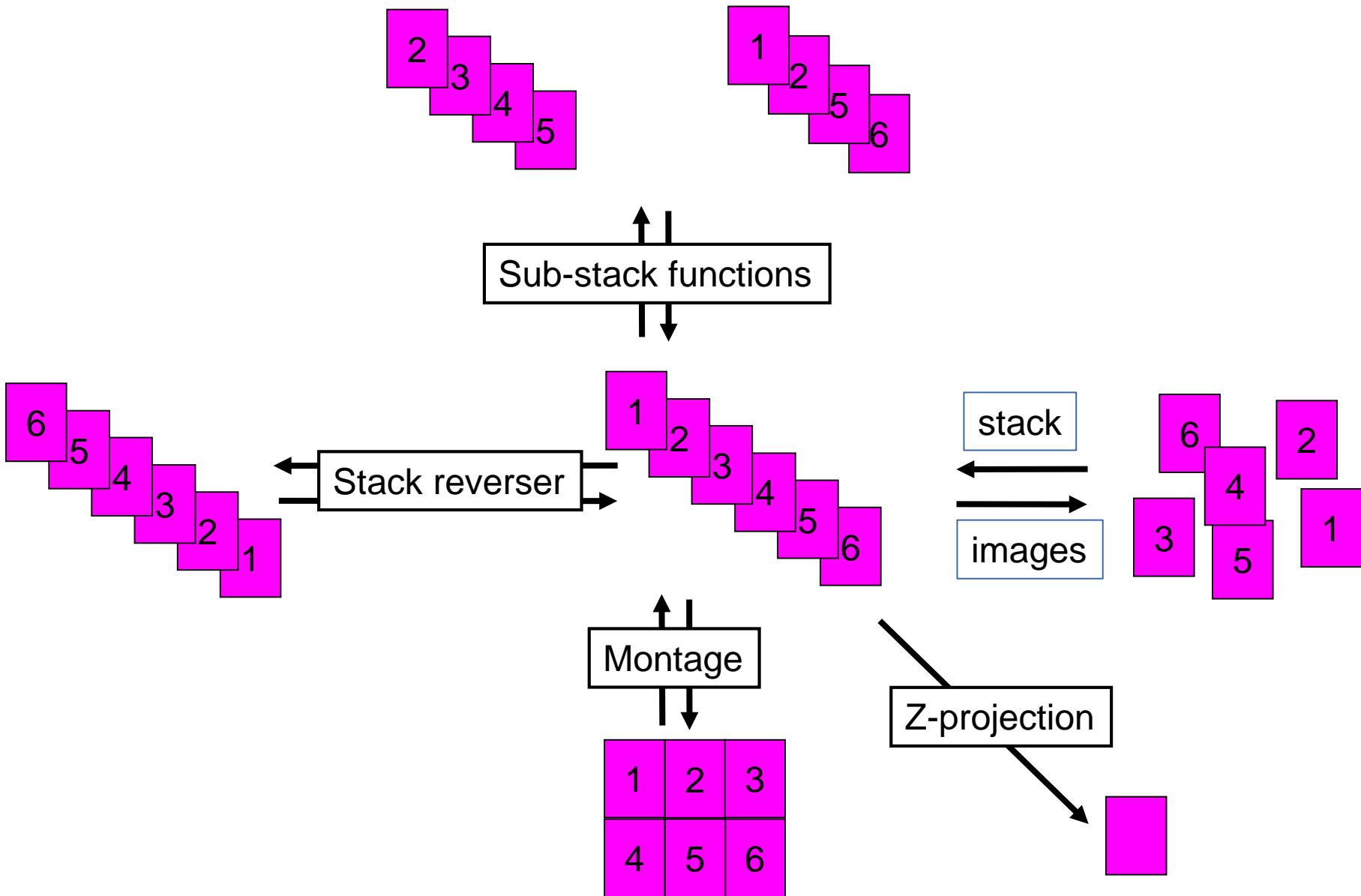
OR



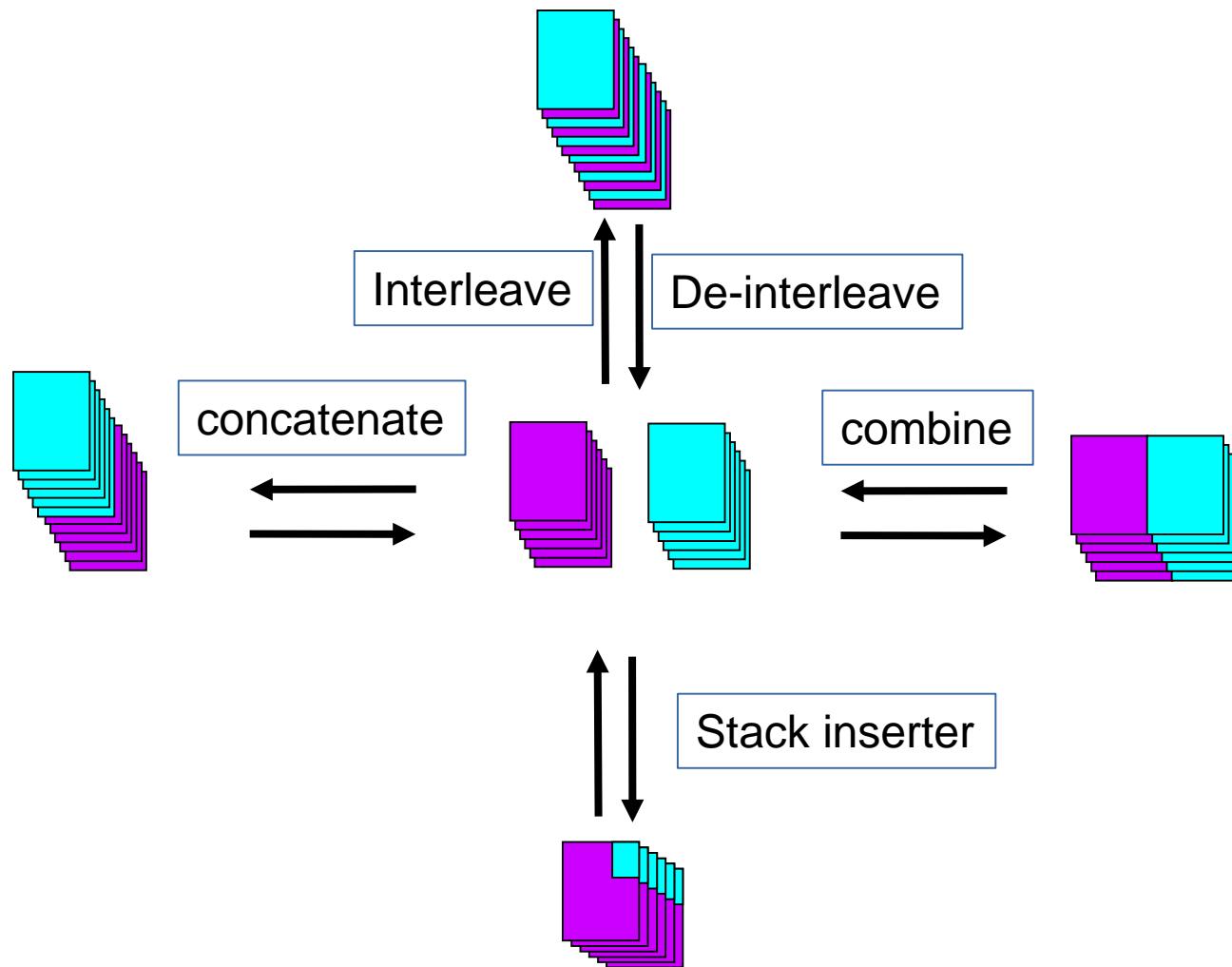
Stacks

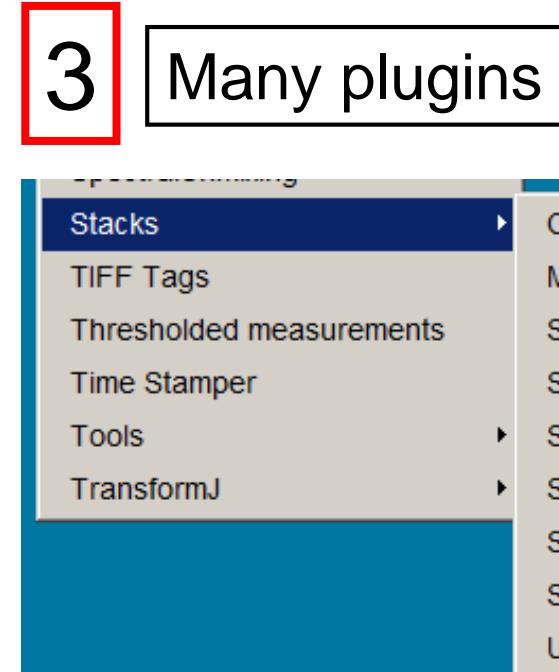
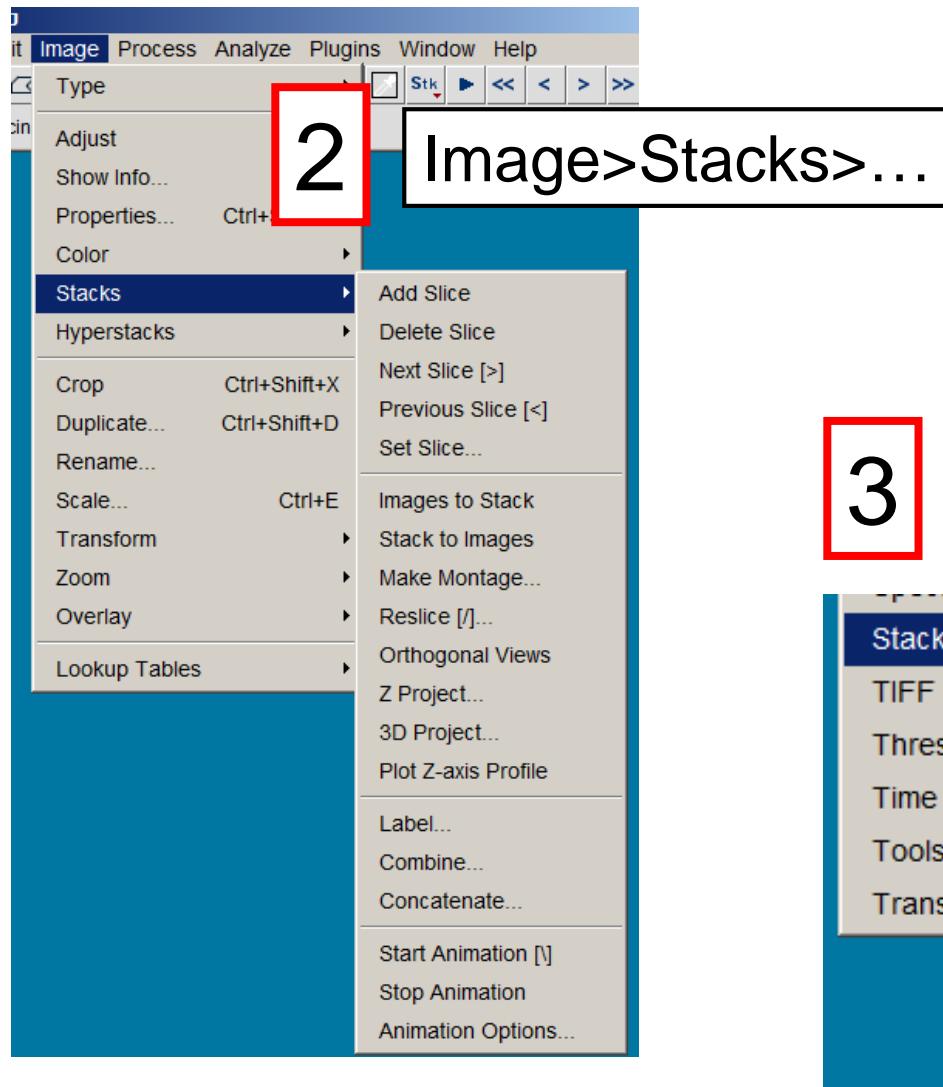
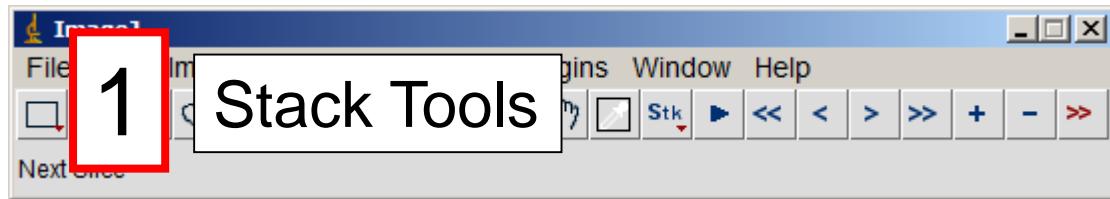
- ImageJ/Fiji can display multiple spatially or temporally related images in a single window
- The image set is called a stack and the images that make up a stack are called slices
- In stacks, a pixel (which represents 2D image data in a bitmap image) becomes a voxel (volumetric pixel), i.e., an intensity value on a regular grid in a three dimensional space.
- All the slices in a stack must be the same size and bit depth.

Stacks Manipulation #1



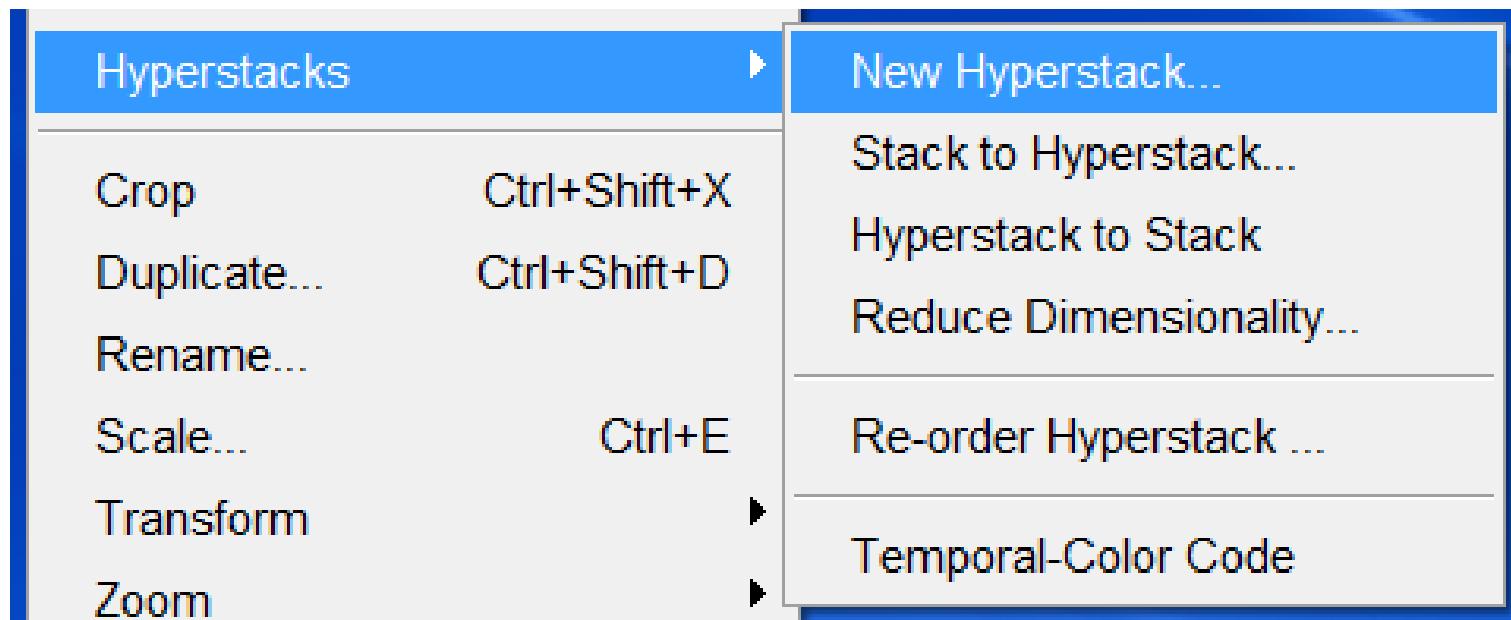
Stacks Manipulation #2



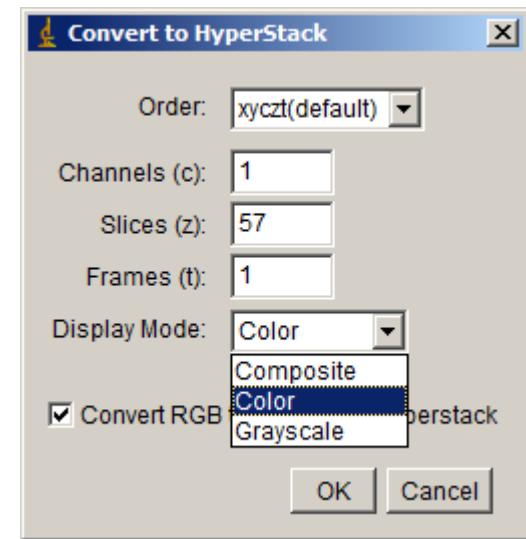
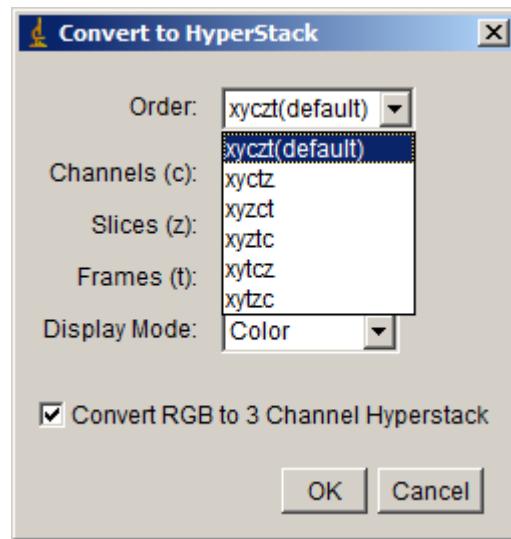
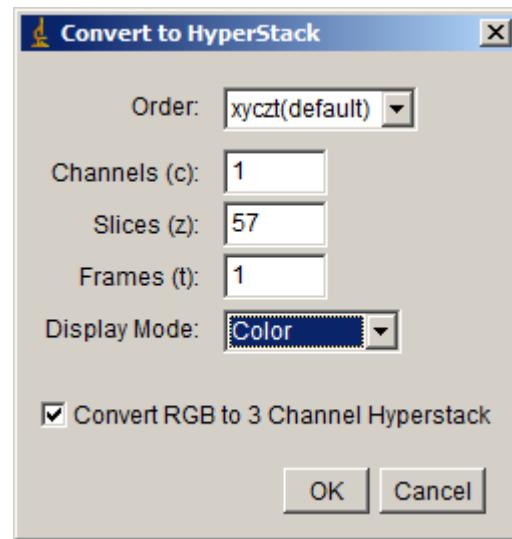


Image>Hyperstacks

Hyperstacks are multidimensional images, extending image stacks to four (4D) or five (5D) dimensions: x (width), y (height), z (slices), c (channels or wavelengths) and t (time frames).

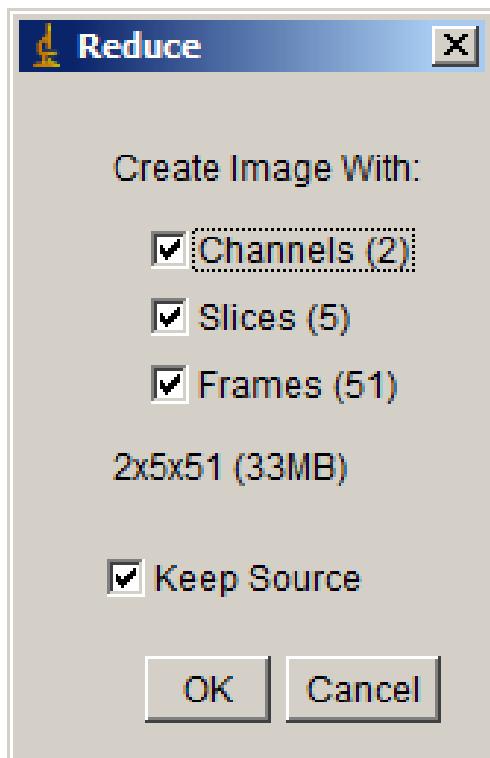


Stack to Hyperstack

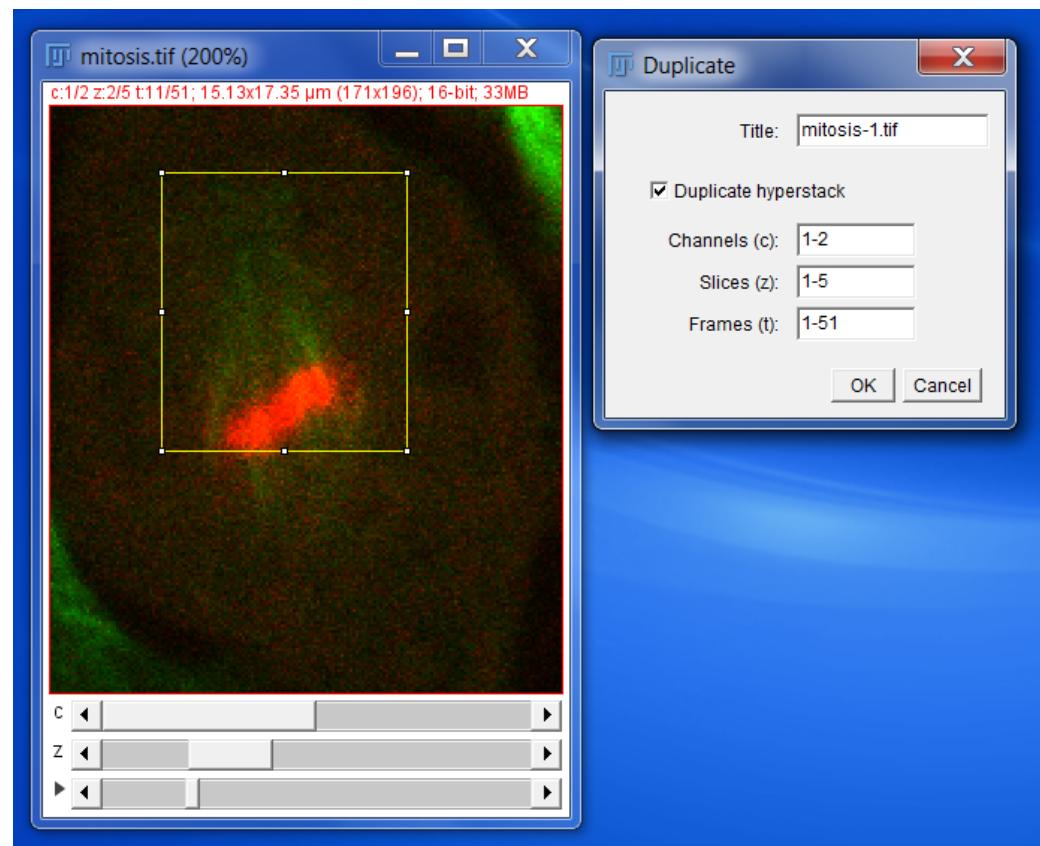


Reduce Dimensions

1- Image>Stacks>Tools>Reduce...



2- Use the Duplicate Image function



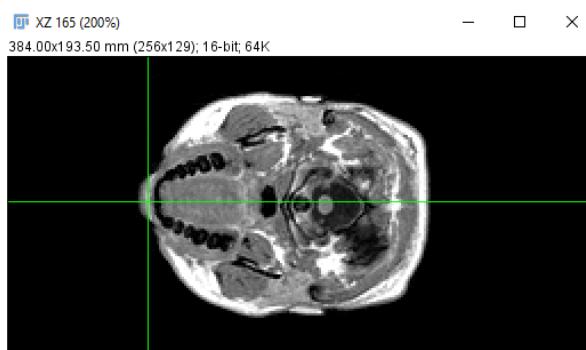
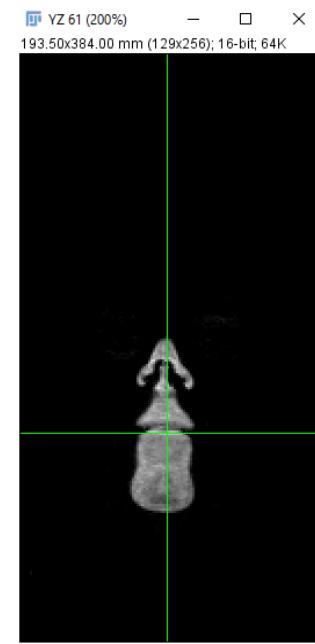
File>Open Samples>Mitosis

Orthogonal View

Image>Stacks>Orthogonal Views

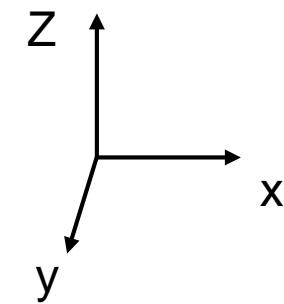
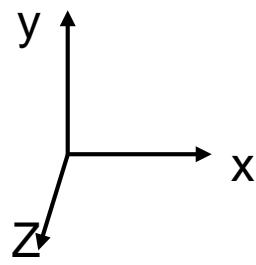
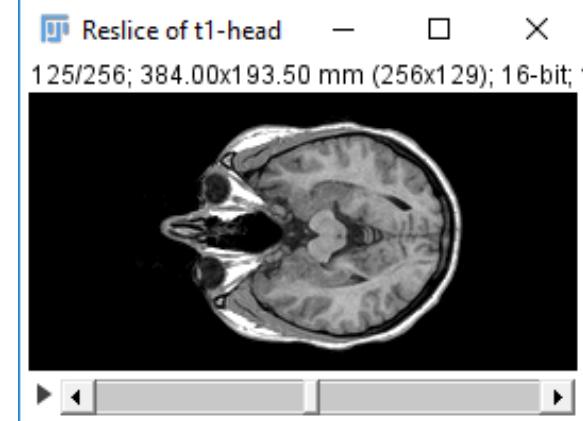
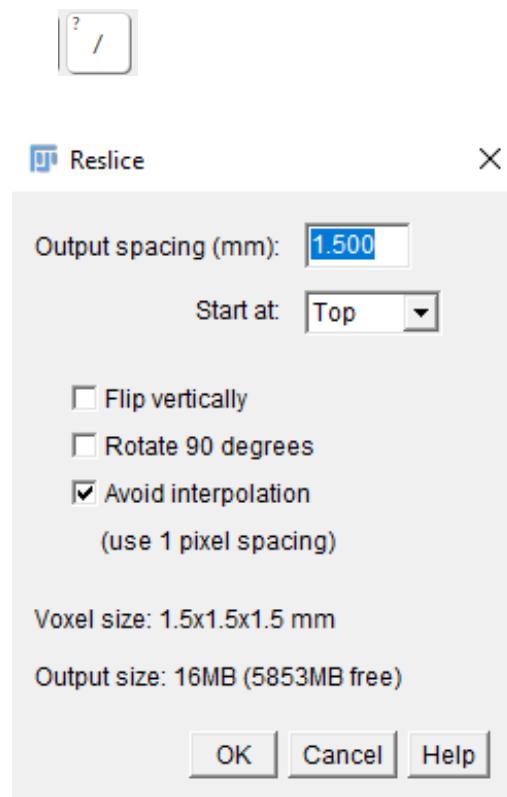
ctrl shift H

Data: Images>3D>T1-head.tif



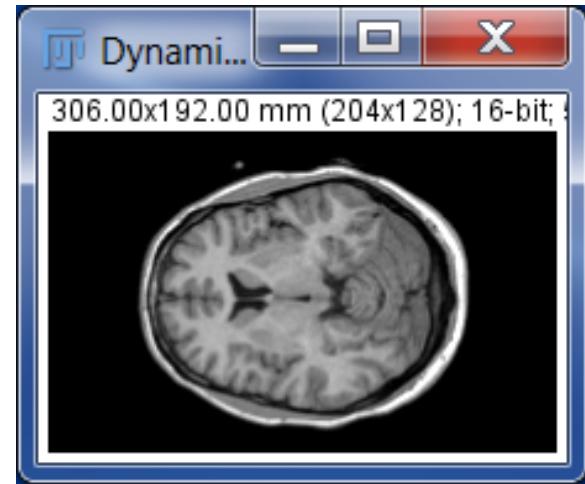
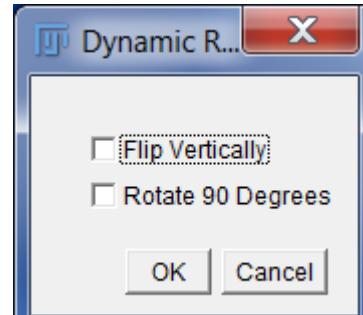
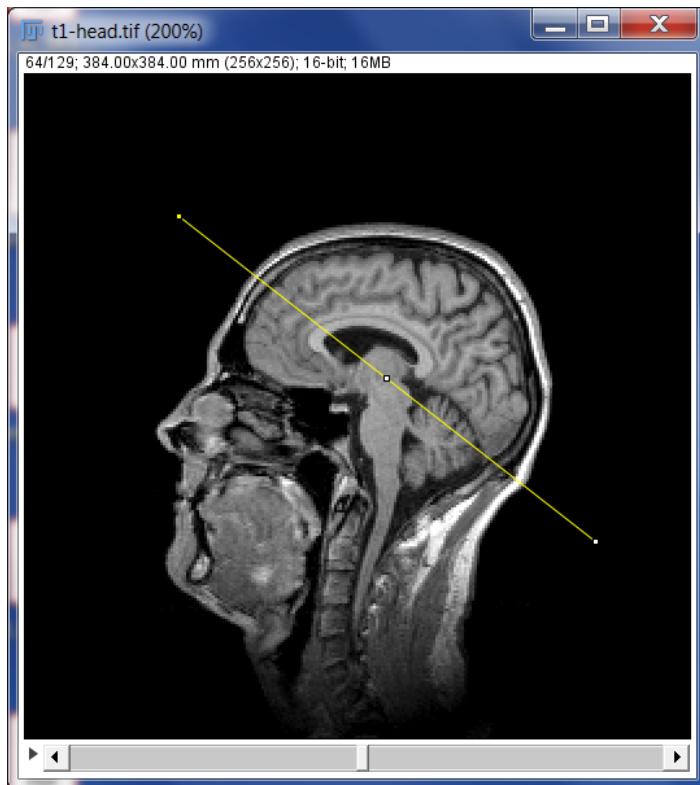
Reslice

Image>Stacks>Reslice(/)...



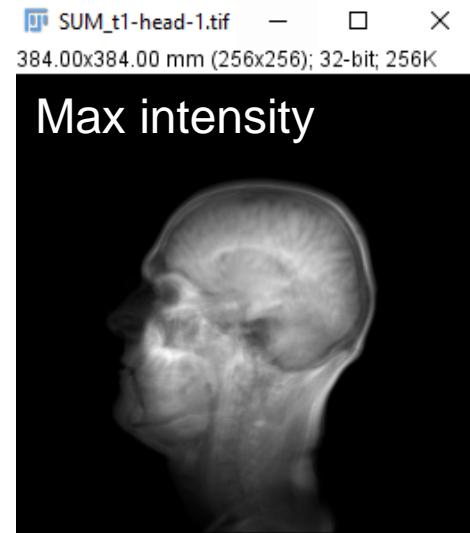
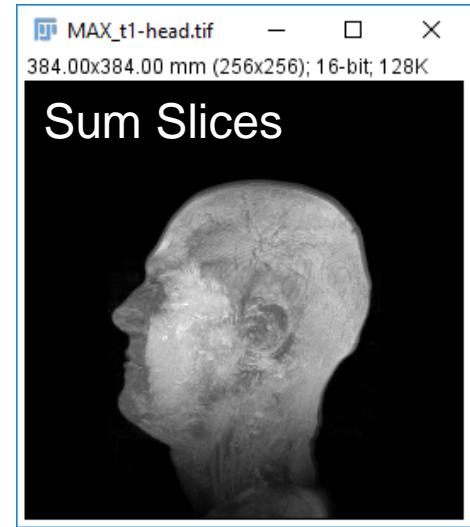
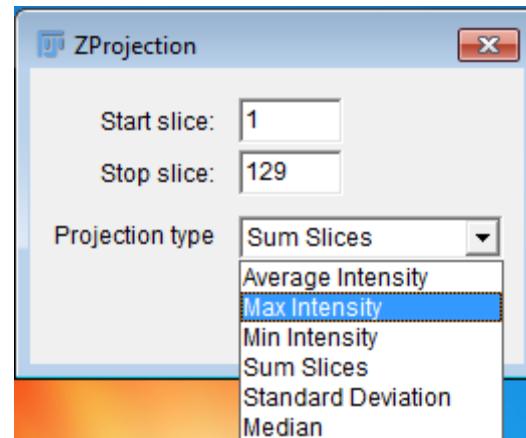
Dynamic Reslice

Image>Stacks>Dynamic Reslice



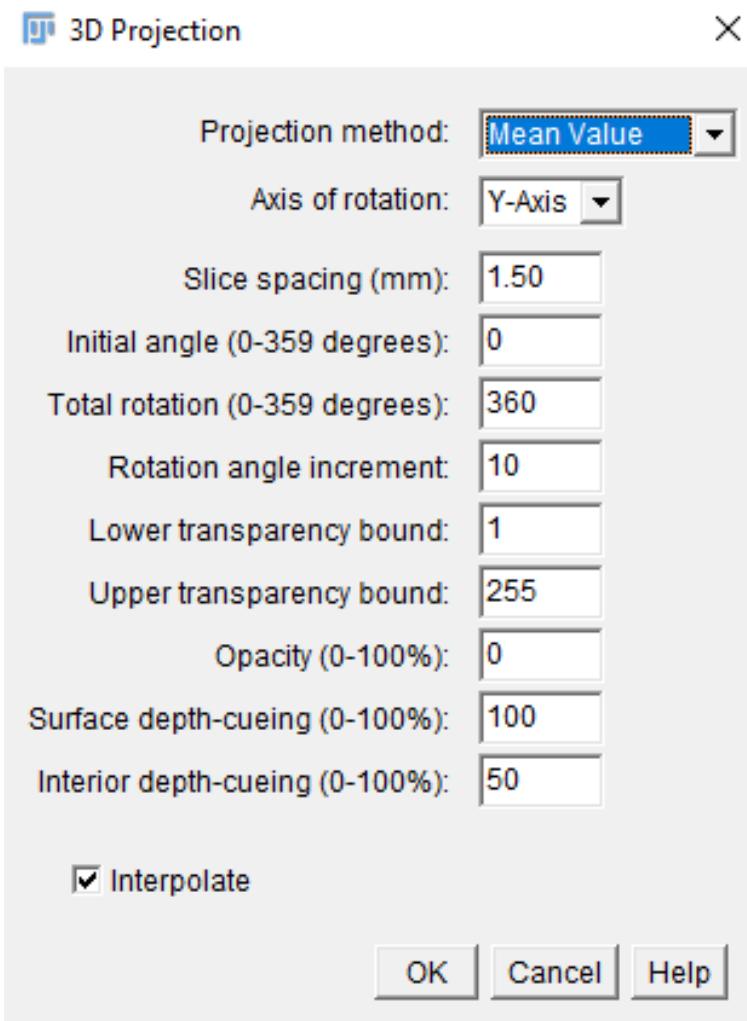
Z-projection

Image>Stacks>Z Project...



Volume Renderer

Image>Stacks>3D Project

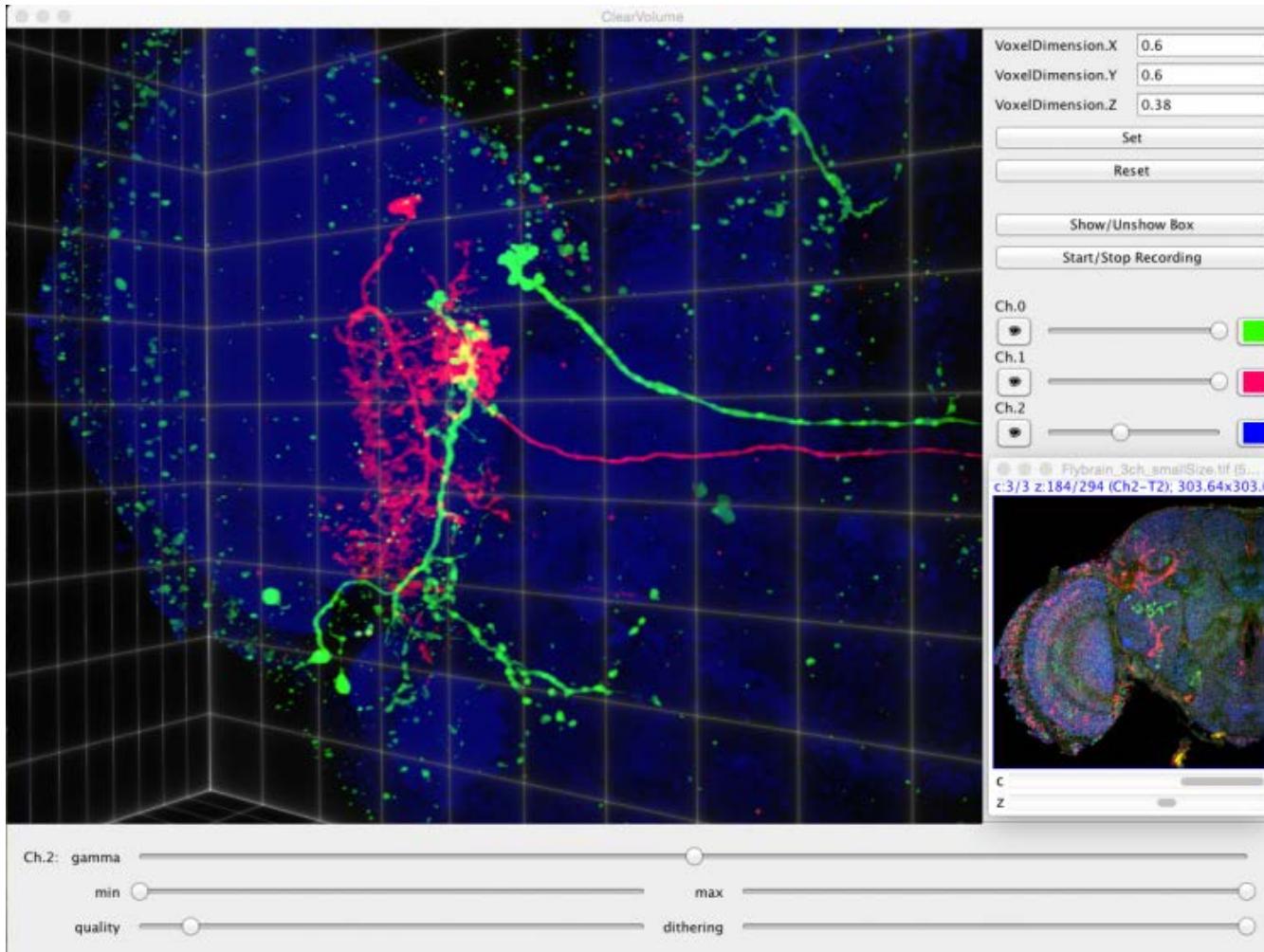


Interactive Volume Renderer

Plugins>3D Viewer

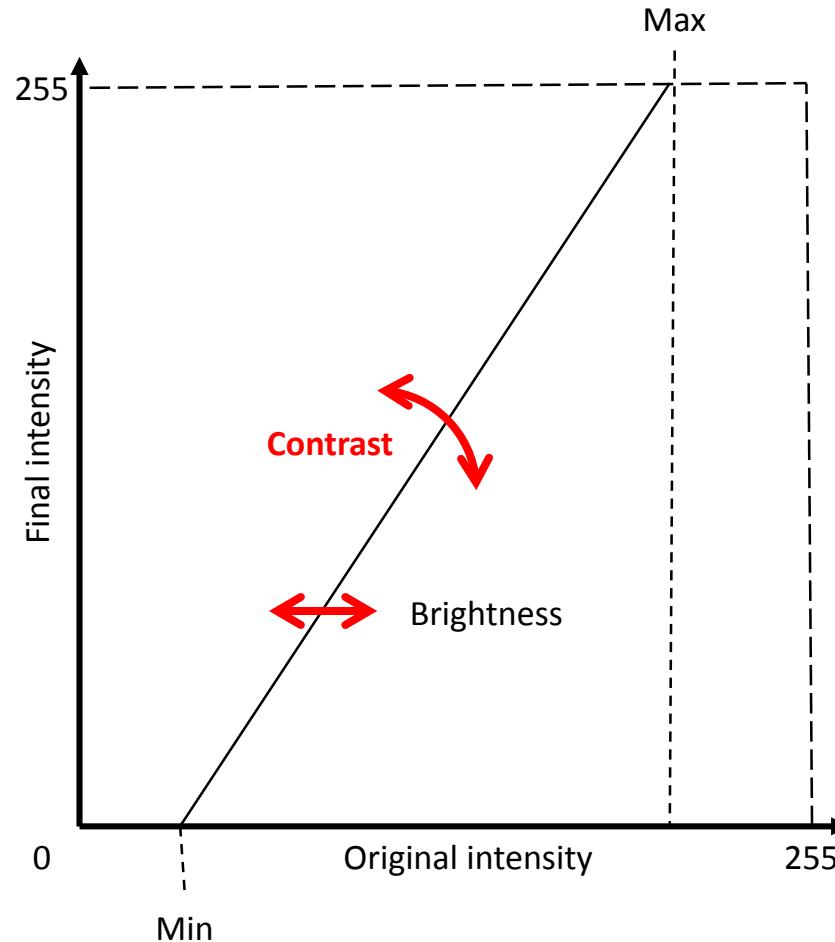
very basic, installed by default with Fiji

Plugin to install: ClearVolume

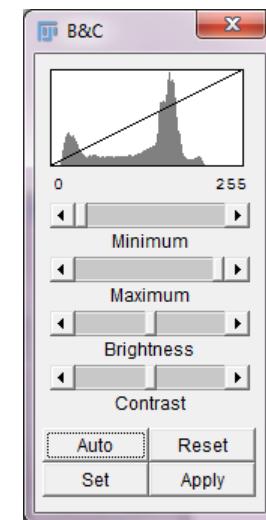
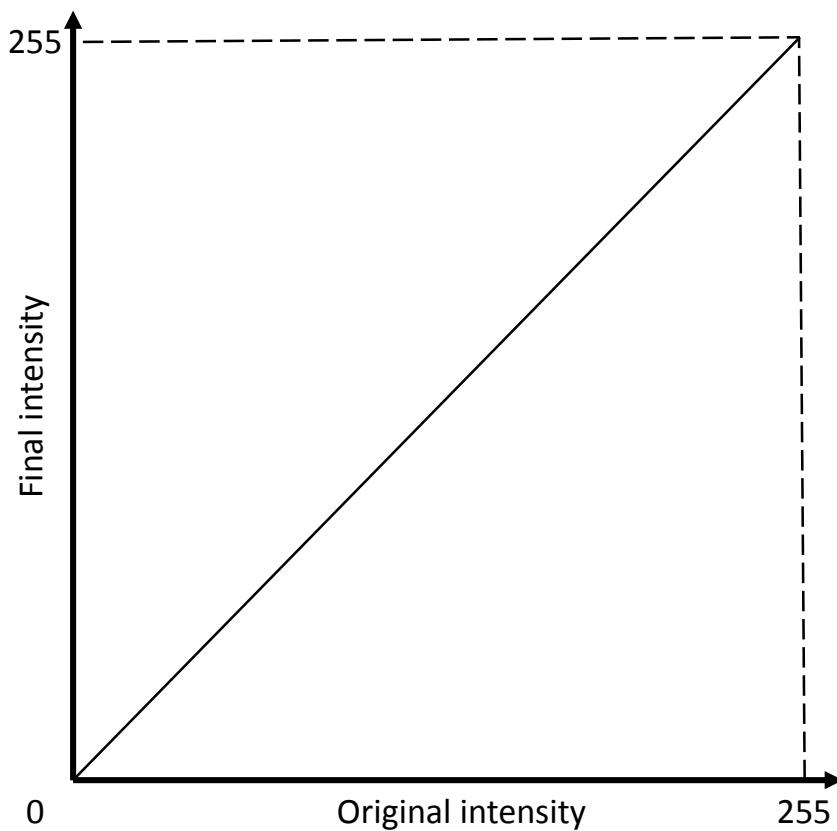


<https://imagej.net/ClearVolume>

Intensity Scaling – 8-bits image

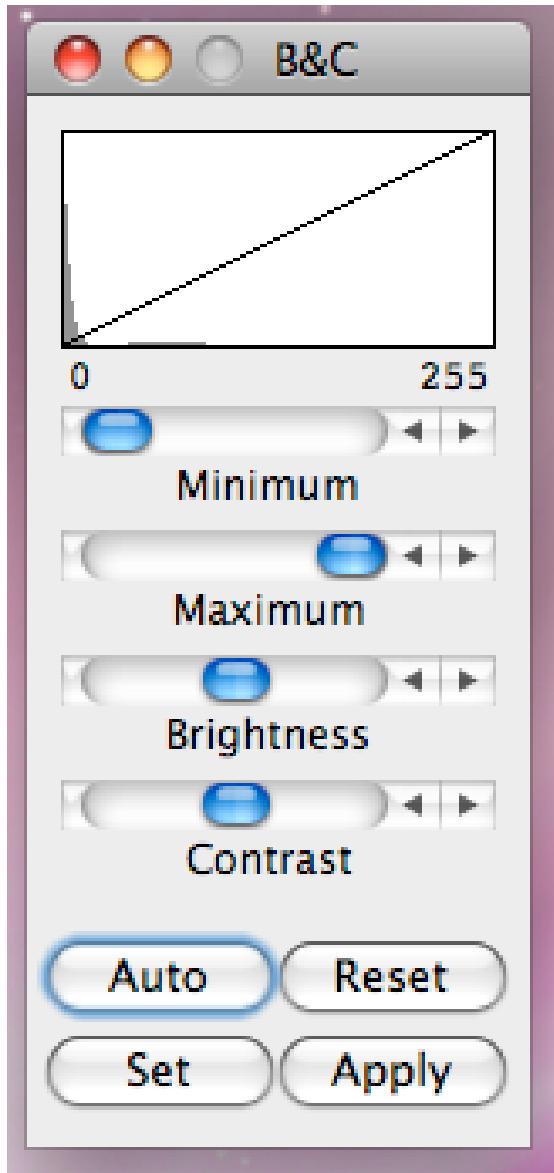


Intensity Scaling – Brightness/Contrast in Fiji



Brightness & Contrast

Ctrl C



Fill the full dynamic range of the display
Eliminate “background” (with caution)

Image histogram
Pixel values mapping to 8-bits (0-255)

Lower limit of the display range : <min = black

Upper limit of the display range : >max = white

display range position

display range width

Auto : automatic histogram stretch

Reset : restore original brightness and contrast values

Set : manually set min/max values of the display range

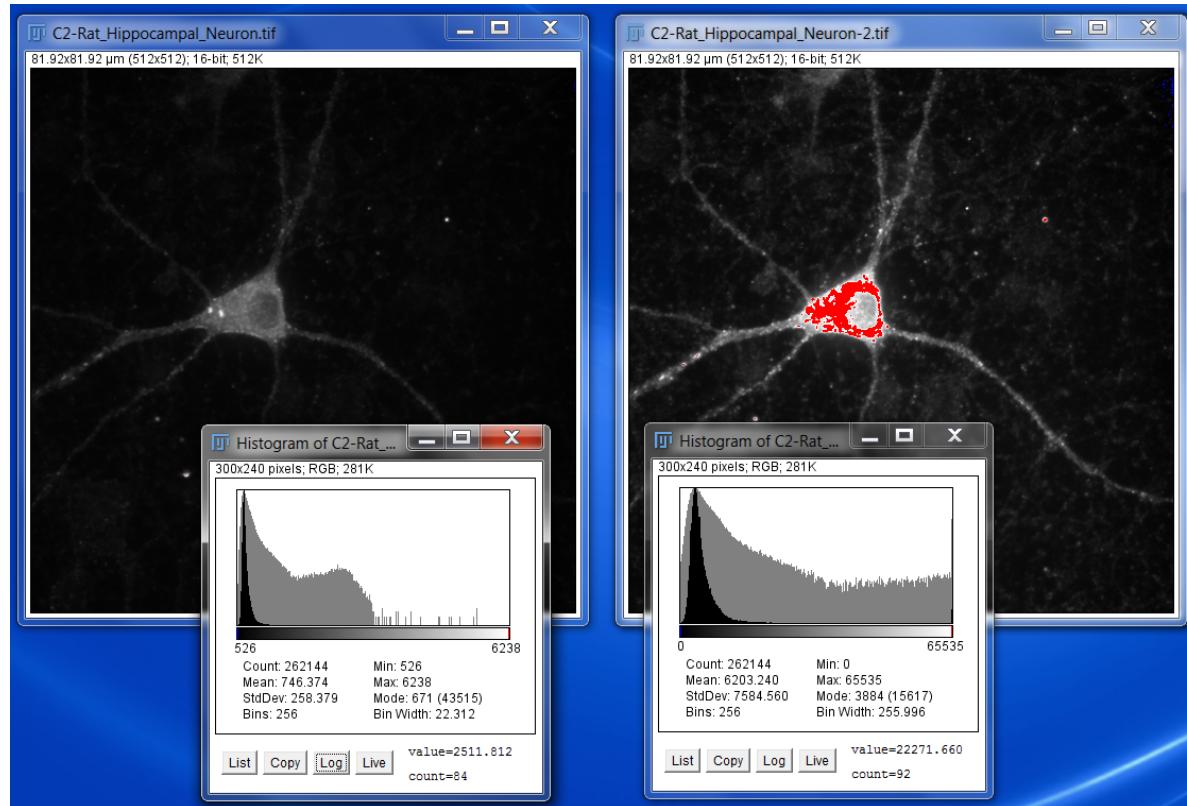
Apply : apply the settings to the whole image or to the selection

Example : Fluorescent cells blue channel

Apply the Intensity Scaling Settings

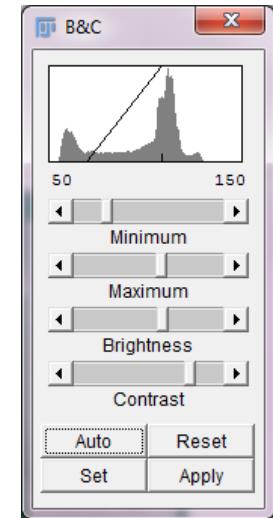
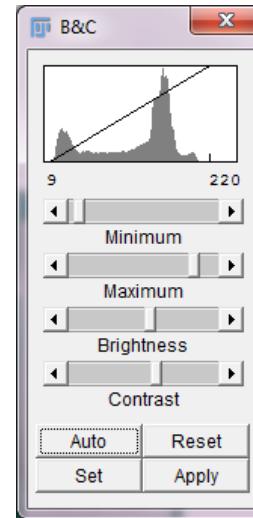
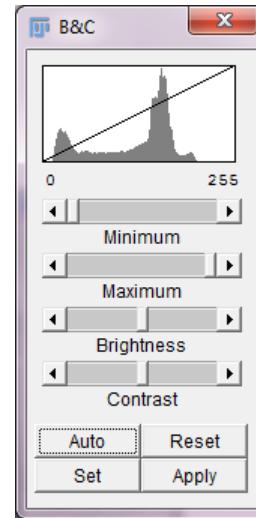
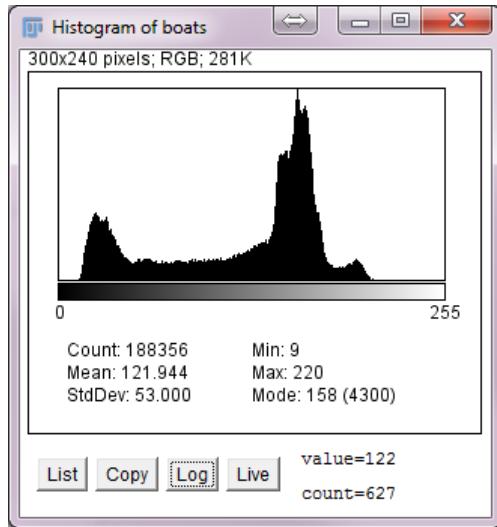
Apply

Applies the current display range mapping function to the pixel data. If there is a selection, only pixels within the selection are modified.



The pixels values are changed when saving the image !!!

Brightness & Contrast adjustments



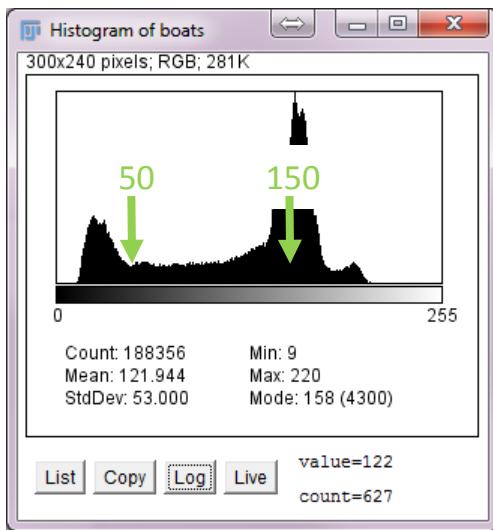
0-255

9-220

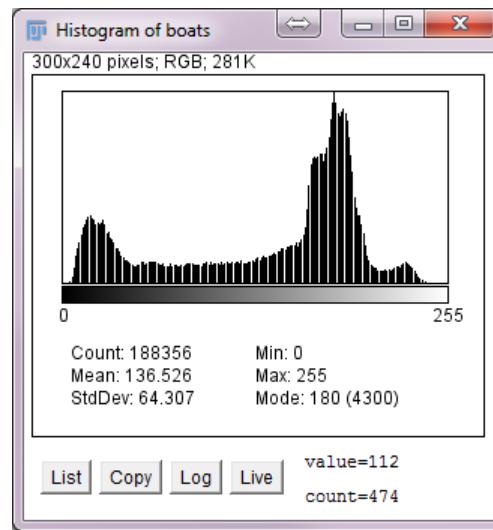
50-150



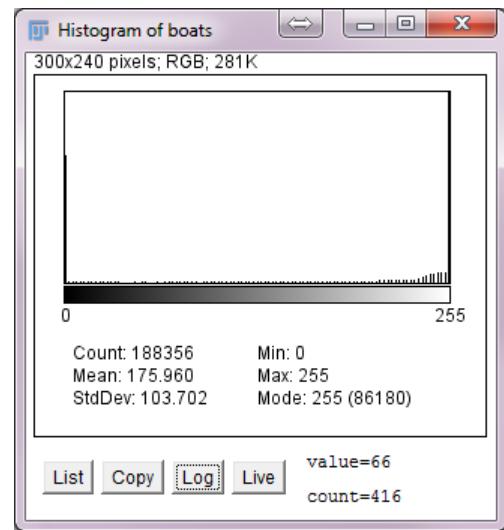
Brightness & Contrast adjustments



0-255



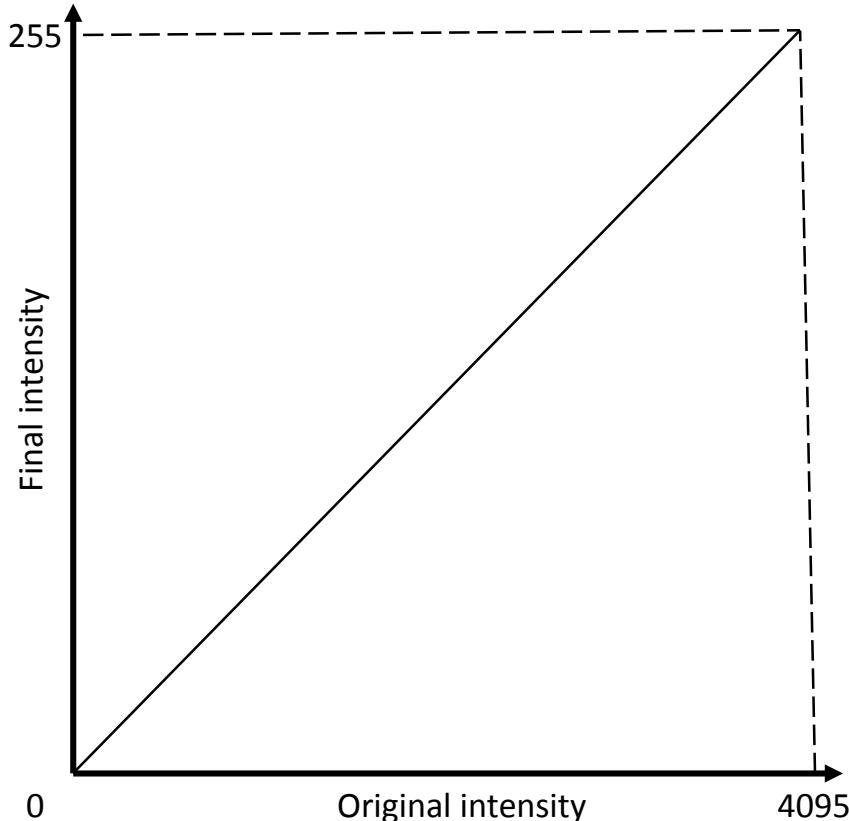
9-220



50-150



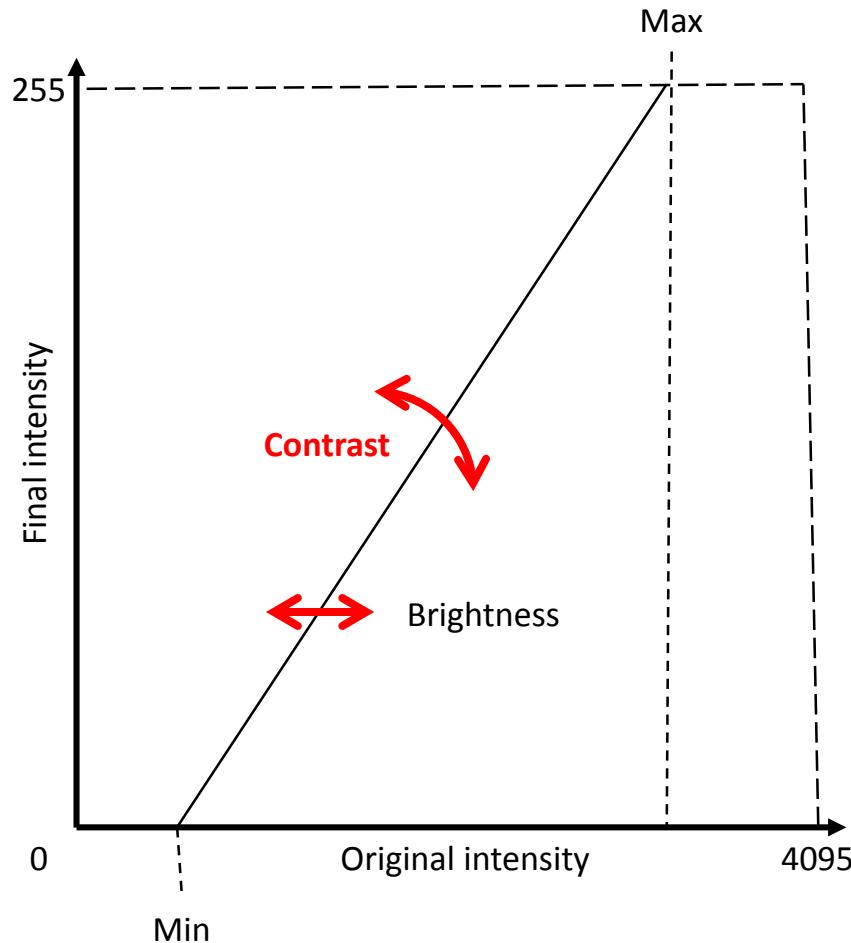
Intensity Scaling – 12-bits data on 8-bits display



- Computer screen are 8-bit
- Publishers want 8-bit files

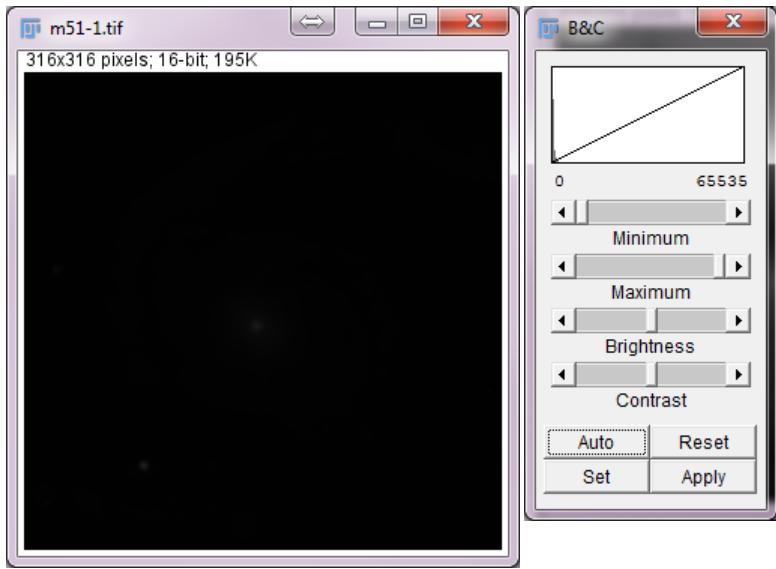
Intensity scaling results in information loss:
For example: Values 4080-4095 are all re-mapped to 255
It should be the last step in your analysis pipeline

Intensity Scaling – 12-bits data on 8-bits display

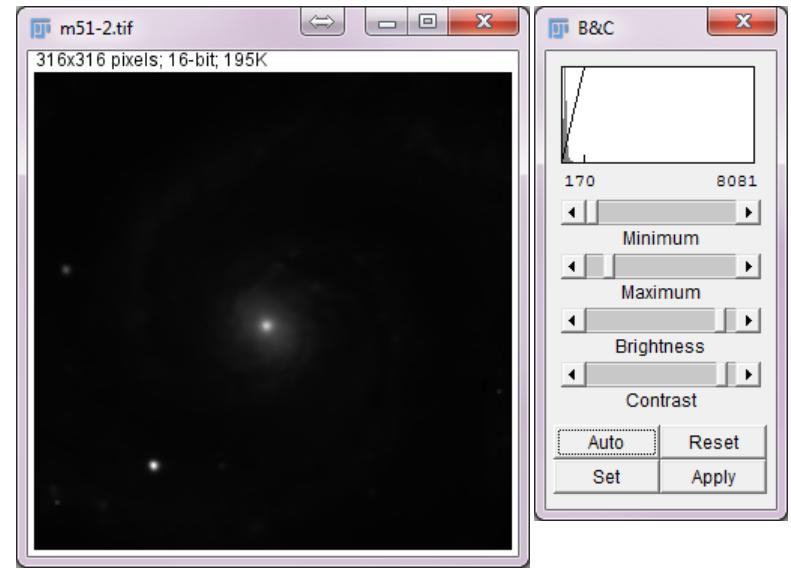


Brightness & Contrast: software default scaling?

Full range



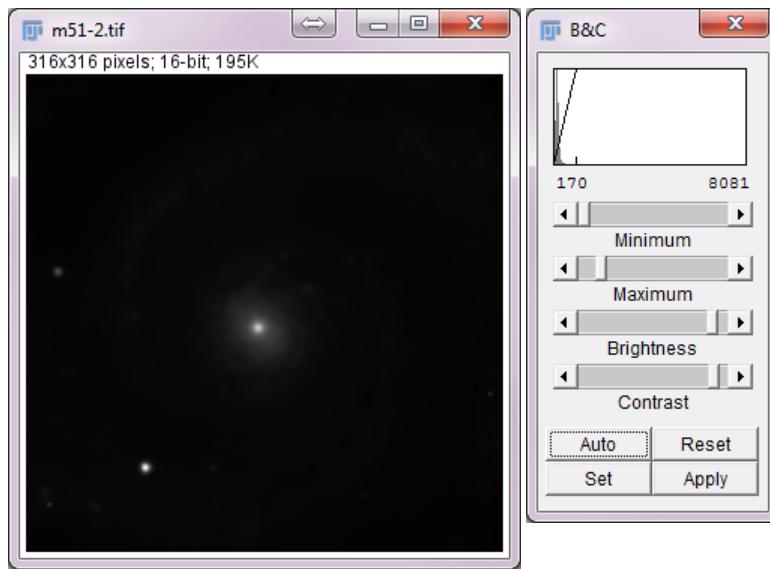
Auto-Scale on Min-Max



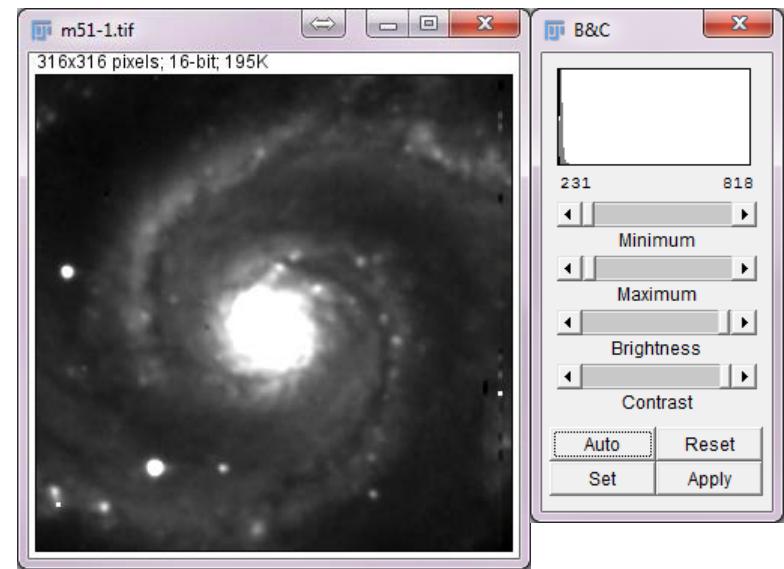
!!! Cannot directly compare
intensity between two images on
auto-scaling

Brightness & Contrast: software default scaling?

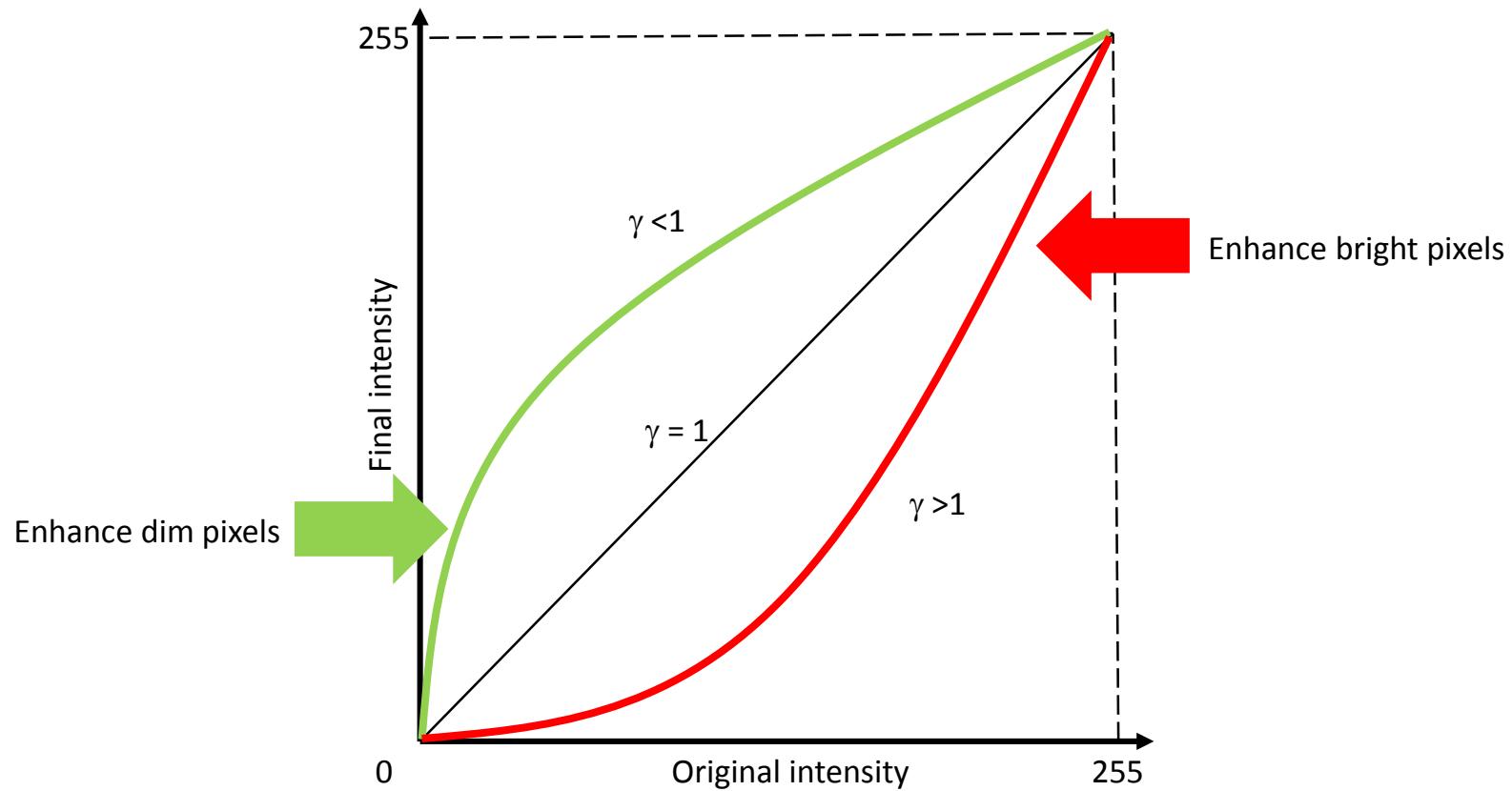
Auto-Scale on Min-Max



High Brightness Contrast



Gamma Correction (exponential mapping)



Process>Math>Gamma

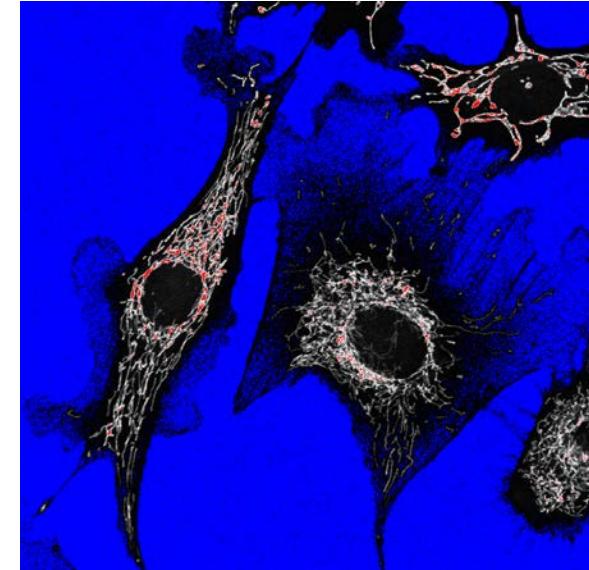
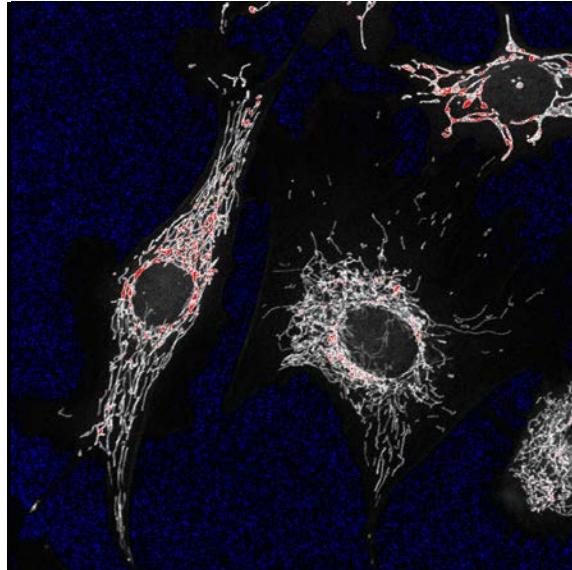
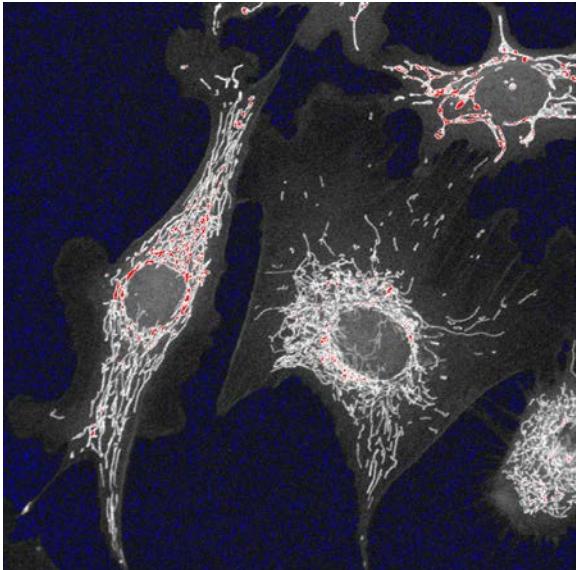
Built-In function: <https://imagej.nih.gov/ij/docs/guide/146-29.html#toc-Subsection-29.9>

Gamma

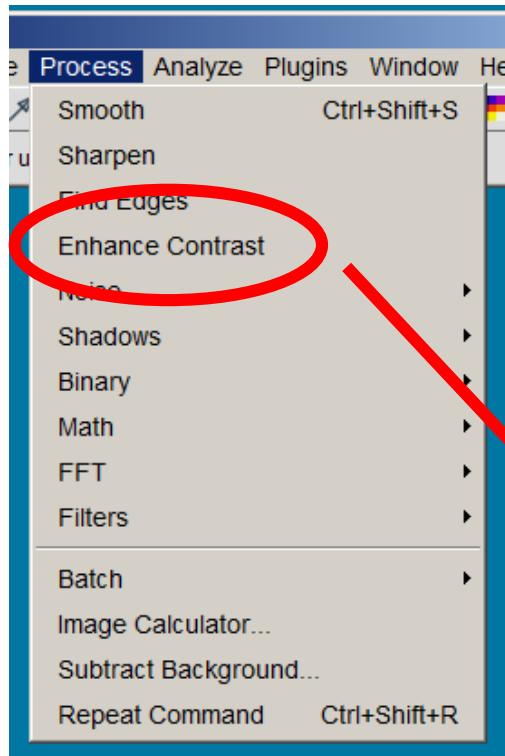
Faint objects can be made more intense without saturating bright objects ($\gamma < 1$).

Medium-intensity objects can be made fainter without dimming the bright objects ($\gamma > 1$).

$$\text{New intensity} = 255 \times [(\text{old intensity} \div 255)^{\gamma}]$$

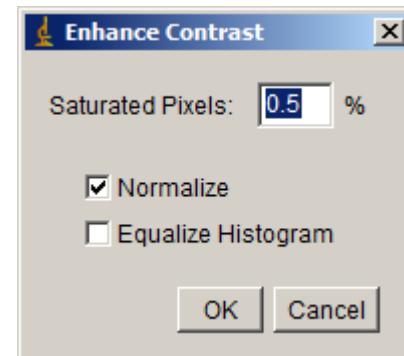


Process>Enhance contrast



Equalisation : non-linear stretch of the histogram
(based on the square root of the intensity see
<http://homepages.inf.ed.ac.uk/rbf/HIPR2/histeq.htm#1>)

Normalisation : linear stretch of the histogram



Example : Fluorescent cells (blue channel)

Gamma Correction (exponential mapping)

$\gamma = 1$



$\gamma = 0.3$



$\gamma = 2.2$

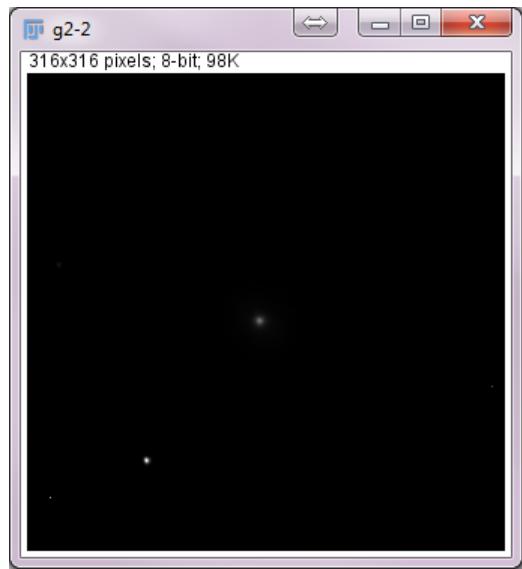


Image Manipulations “Rules”

- Check the *journal guidelines*:
<http://jcb.rupress.org/editorial-policies#data-integrity>
<http://jcb.rupress.org/content/166/1/11>
- Brightness and contrast adjustments are acceptable only if:
 - they are *applied to the whole image*
 - they *do not mask/eliminate* background or other features
- Cutting/pasting *regions of an image is forbidden* (= fraud)
- *Non linear adjustments* (e.g. gamma) must be disclosed
- *Same correction* to all images for comparison
- *Be honest*, detail all image processing in your M&M

Image Processing Rules: No Data Beautification

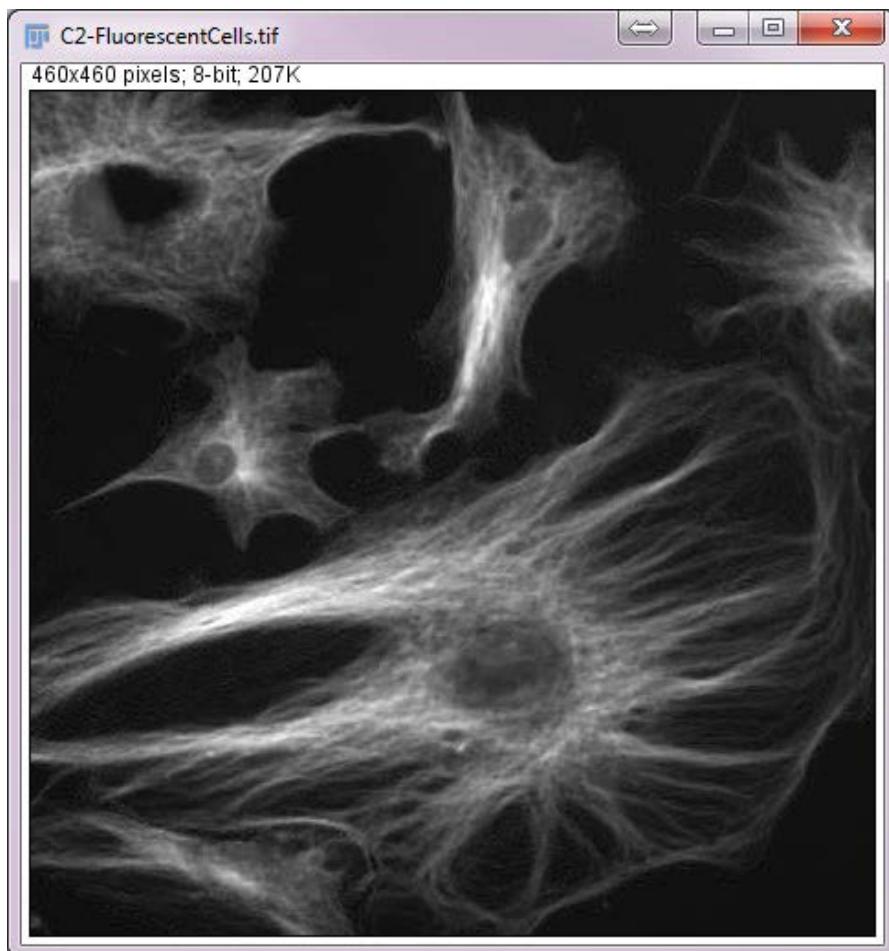
- Manipulation should be done on a copy not the original data
- Small adjustments to the entire image are usually acceptable
- Check journal's guidances
- Be honest, always mention the adjustments made
- Do not hide information (brightness, contrast, level)
- Do not create/destroy information (interpolation, bit-depth conversion)

<http://jcb.rupress.org/cgi/content/full/166/1/11>

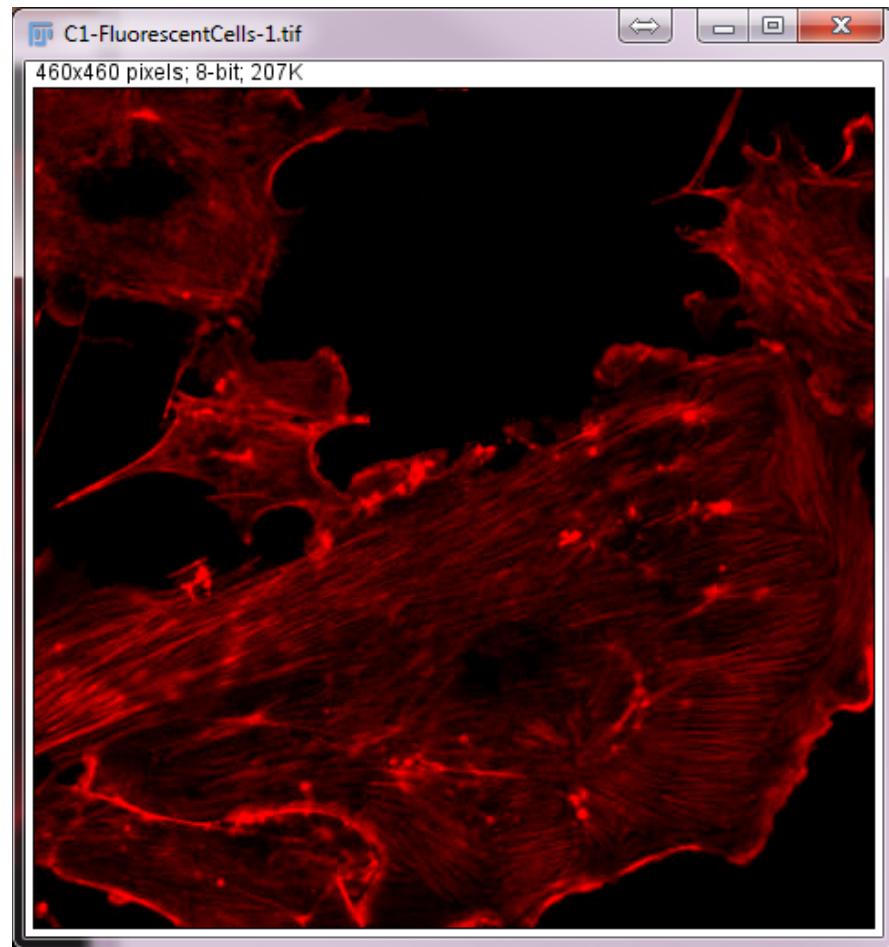
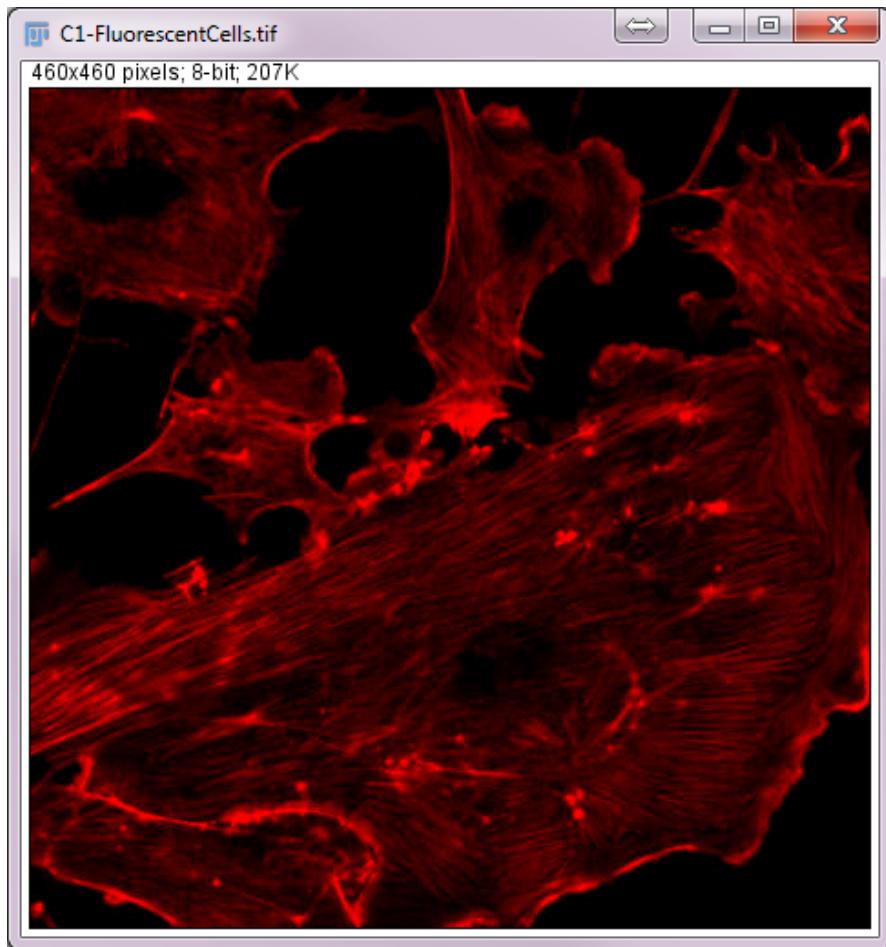
<http://swehsc.pharmacy.arizona.edu/micro/digital-image-ethics>

<http://scienceimageintegrity.org/wp-content/uploads/2010/05/TwistedPixls-Cromey-SEE-Dec2010.pdf>

Spot the Fraud

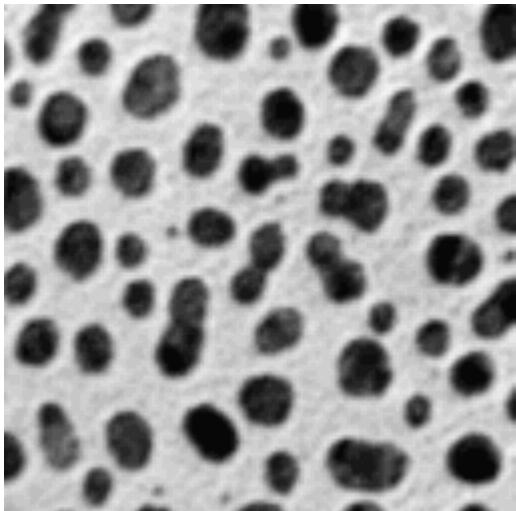


Spot the Fraud



Segmentation

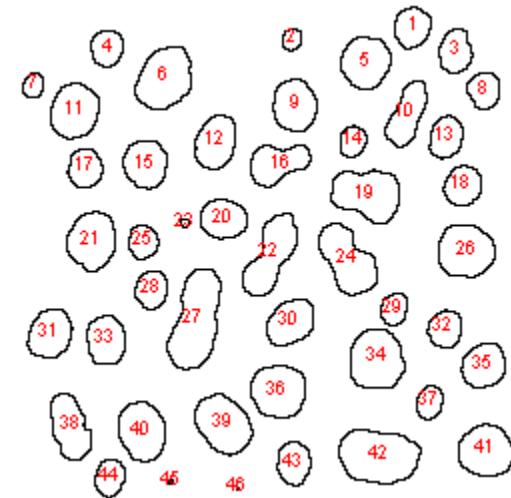
Segmentation



8-bits



Binary Mask



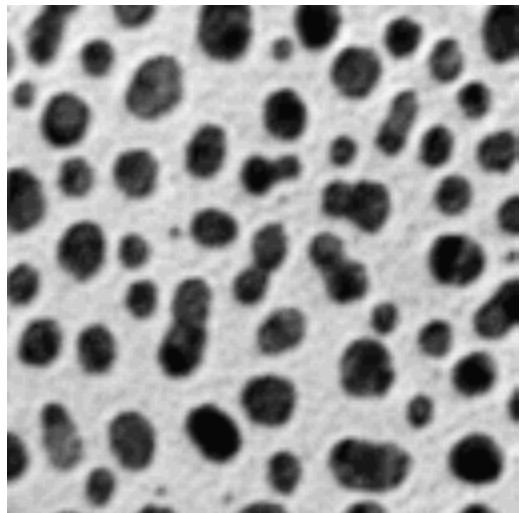
Labelled objects

65024 pixels
Intensity range 0-255
A lot of information !

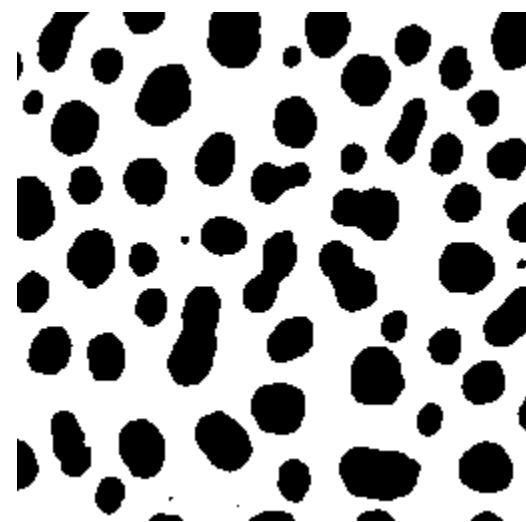


Less information
But simpler to interpret:
46 objects with different properties
(size, shape, intensity, ...)

Segmentation: Intensity

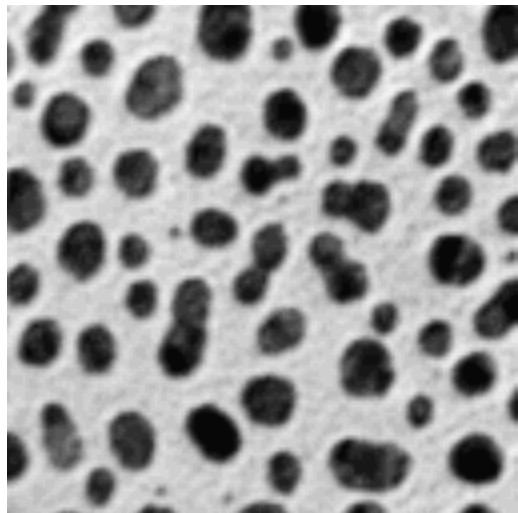


Grey scale image

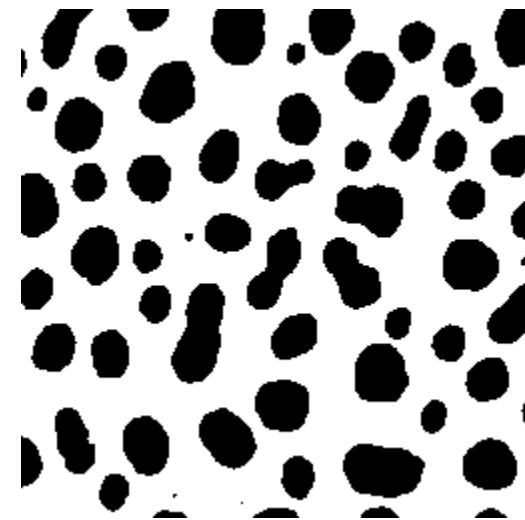


Binary image/mask

Segmentation: Intensity



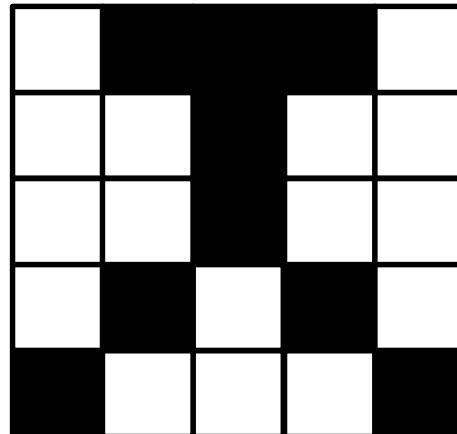
Grey scale image



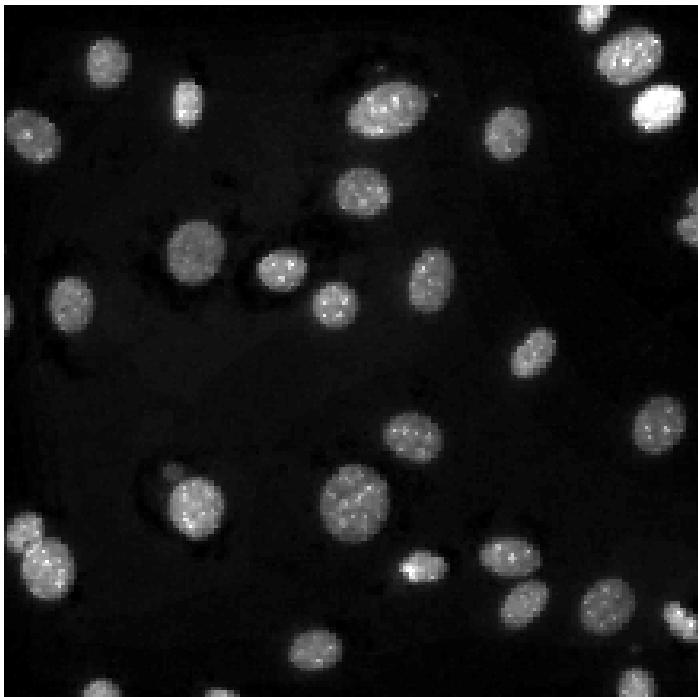
Binary image/mask

1	65	13	55	2
2	3	34	2	1
4	0	31	1	2
1	33	3	54	3
56	3	2	1	34

Threshold above
10

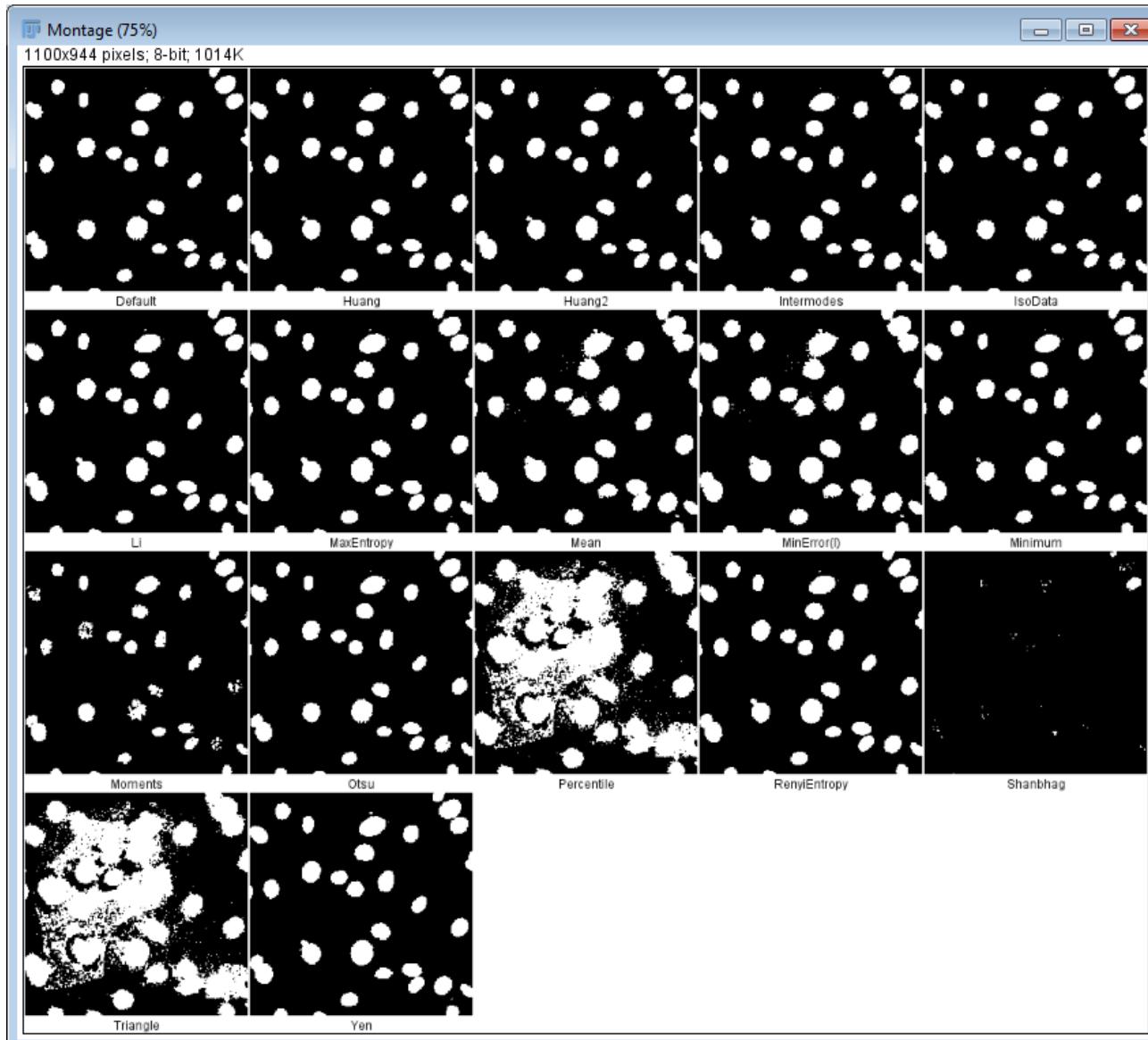


How to Determine the Threshold ?



- Manually (user's bias)
- Automatically (reproducible and automation)
 - Default, Huang, Huang2, Intermodes, IsoData, Li, MaxEntropy, Mean, MinError(l), Minimum, Moments, Otsu, Percentile, RenyiEntropy, Shanbhag, Triangle, Yen.

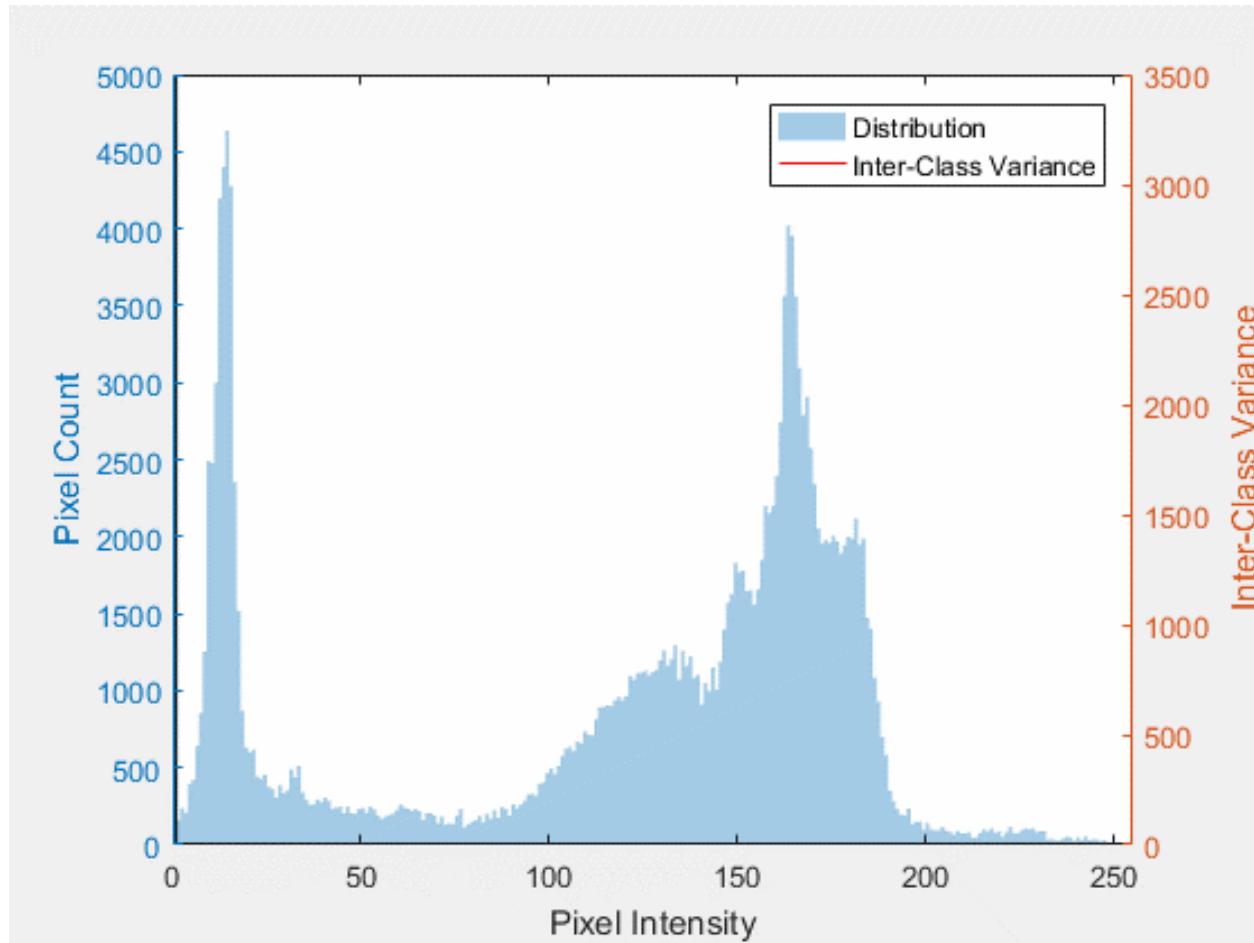
How to Determine the Threshold ?



https://imagej.net/Auto_Threshold

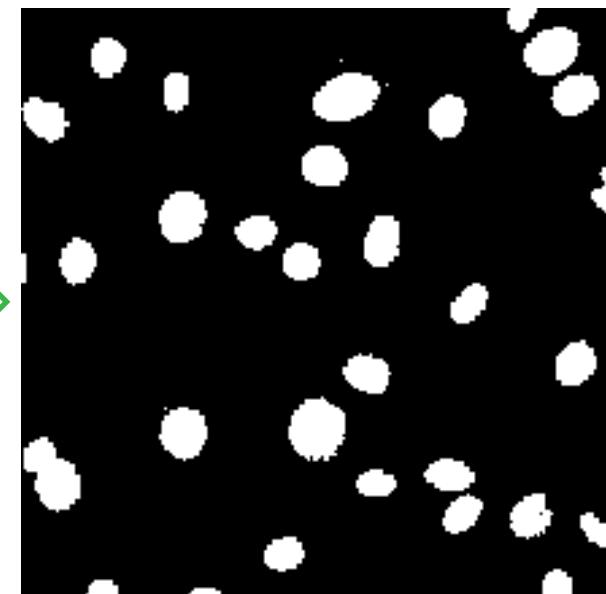
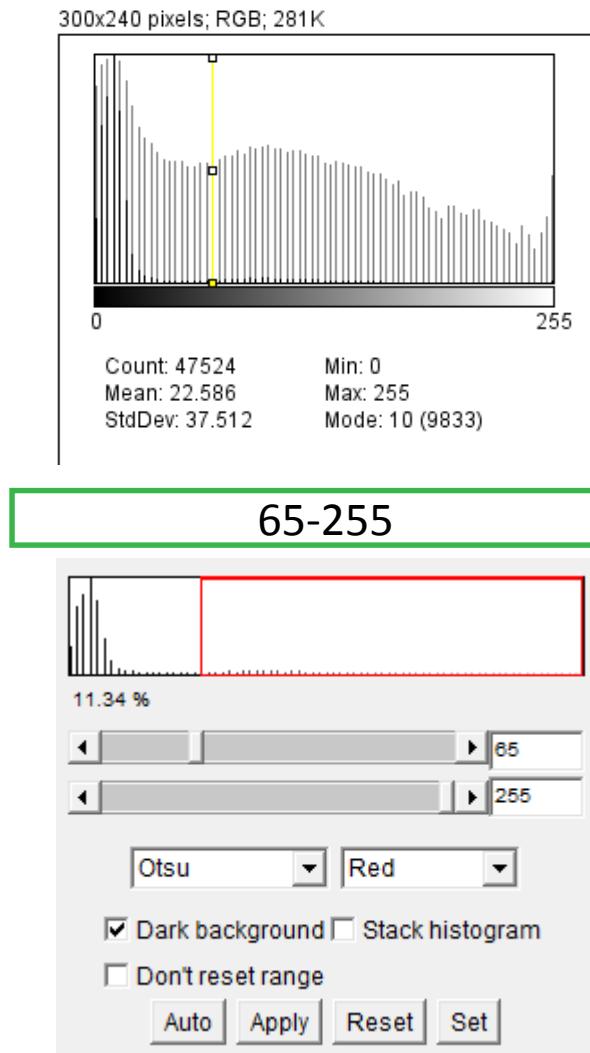
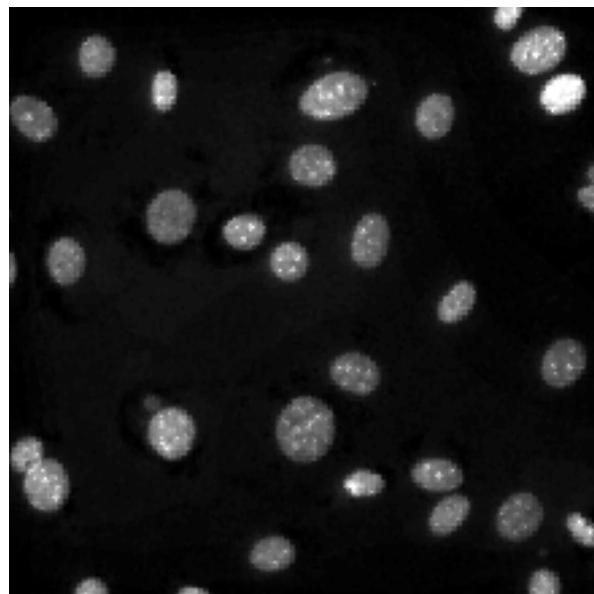
Example: Otsu Thresholding

The algorithm returns a single intensity threshold that separate pixels into two classes, foreground and background by minimizing intra-class intensity variance, or equivalently, by maximizing inter-class variance.



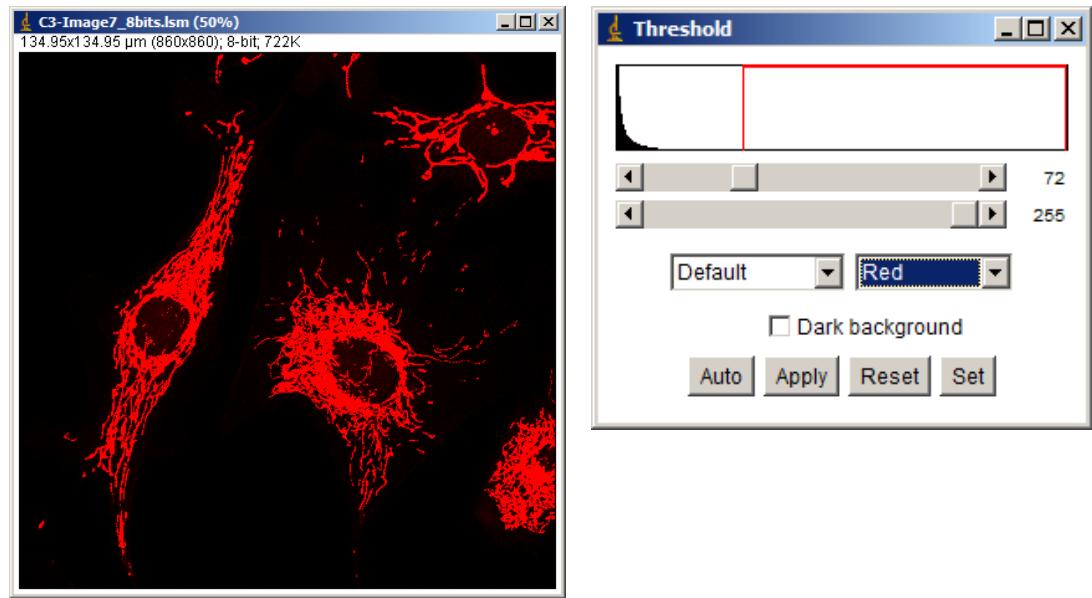
Example: Otsu Thresholding

Works well to segment nuclei in cells monolayer image



Threshold (ctrl+shift+T)

Segment the image into features of interest and background by creating a binary mask



Set the lower threshold value

Set the upper threshold value

Auto : automatically sets the threshold (histogram-based)

Apply : set thresholded pixels to black, the other to white

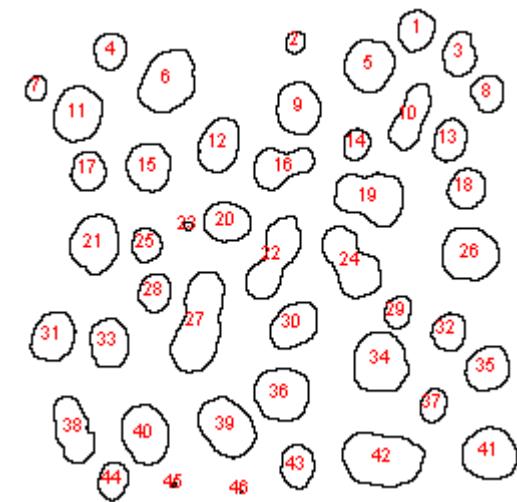
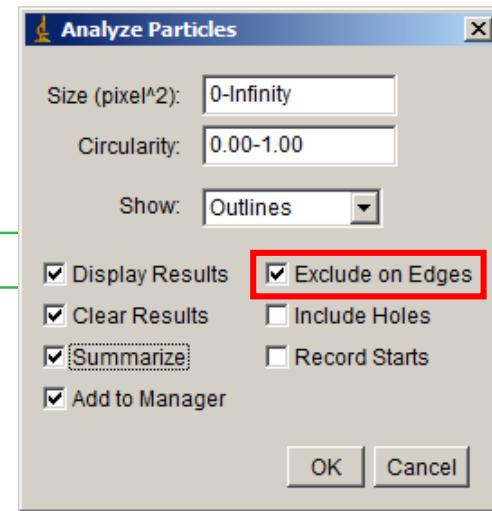
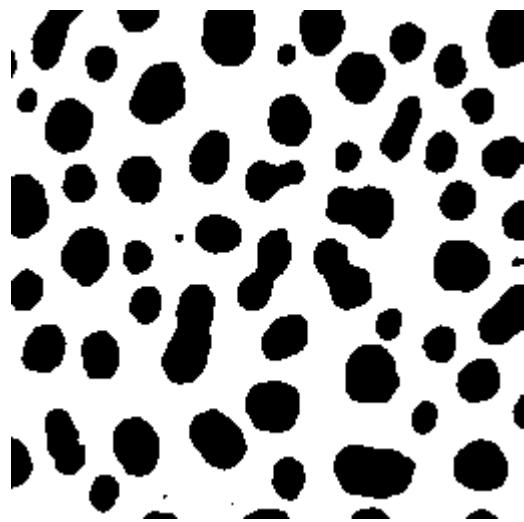
Reset : disable thresholding

Set : manually set the min/max values

Auto threshold options : https://imagej.net/Auto_Threshold

Analyze Particles

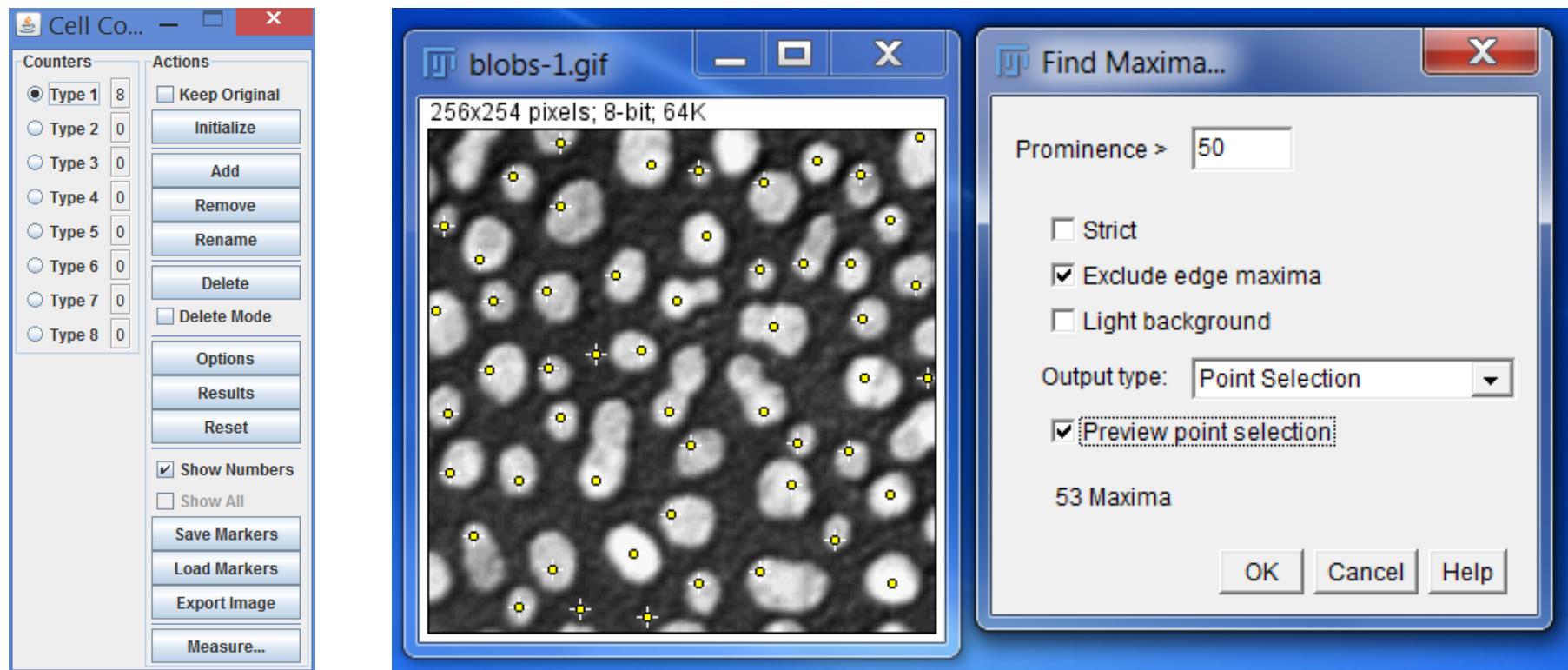
Analyze>Analyze Particles



- Add to manager for further analysis
- Exclude on Edges to eliminates incomplete objects

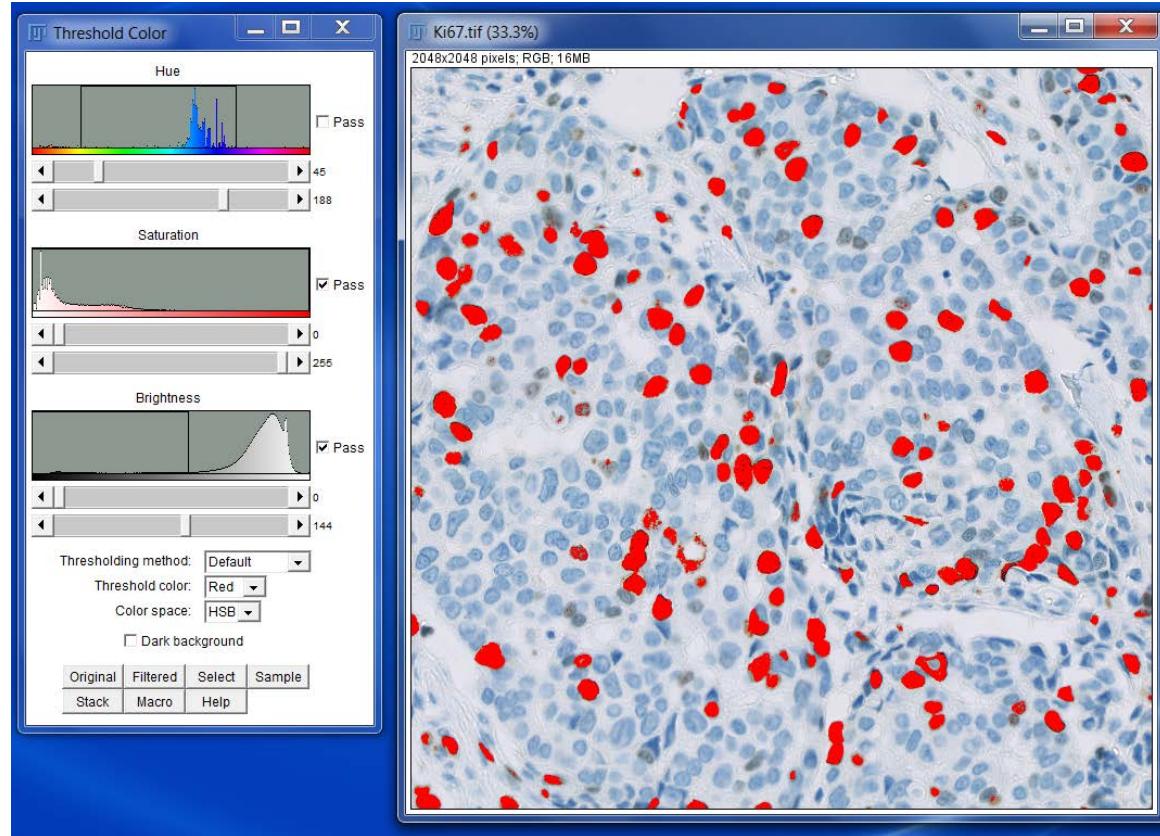
Many other Ways Count Particles

- 1 - Manual counting Plugins › Analyze › Cell Counter › Cell Counter
- 2 - Process › Find Maxima...



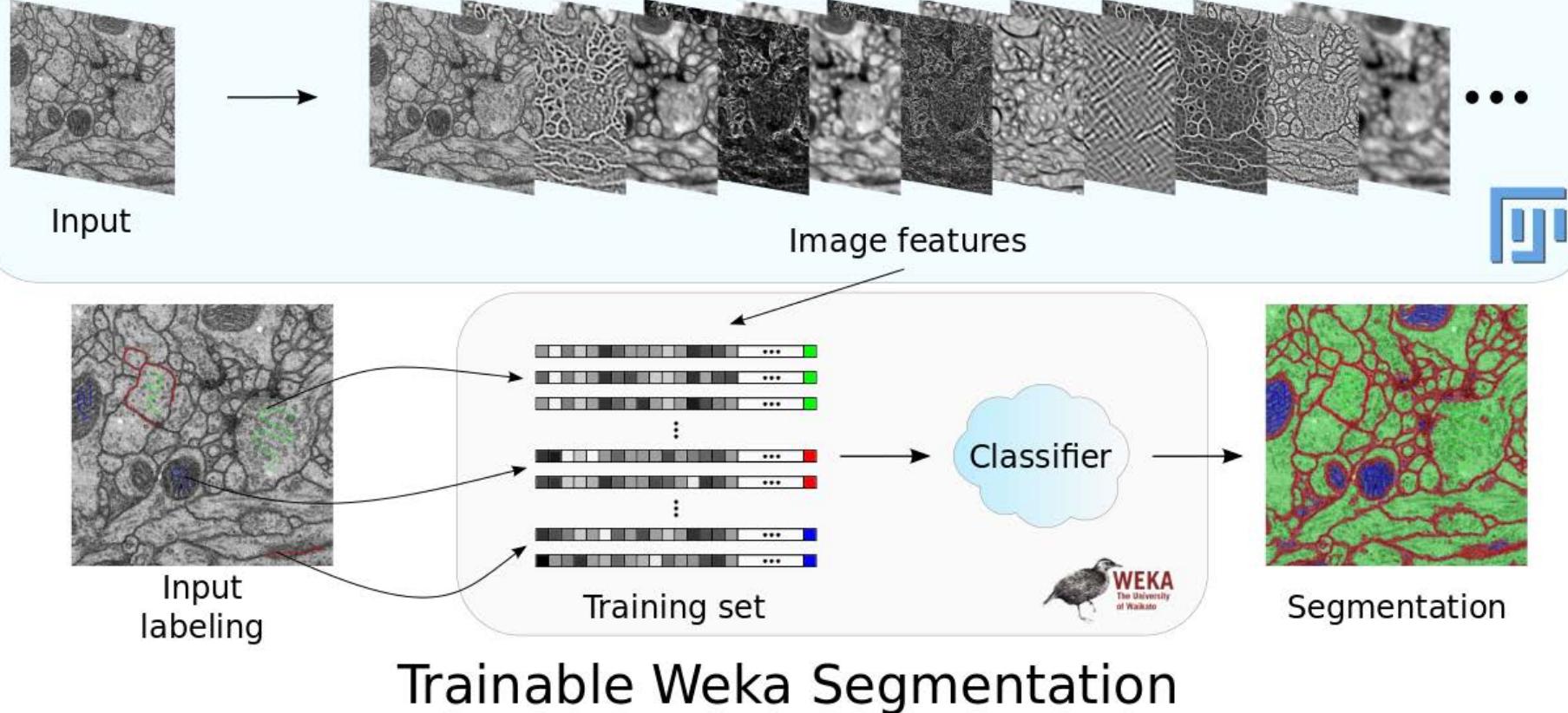
Colour Thresholding

- Segments RGB images based on Hue Saturation and Brightness (HSB), Red Green and Blue (RGB), CIE Lab or YUV components.
- Good method to extract DAB signal from histological stains
- Image>Adjust>Color Thresholding



Segmentation: Machine Learning

- Plugins>Segmentation>Trainable Weka Segmentation
- Plugins>Segmentation>Trainable Weka Segmentation 3D

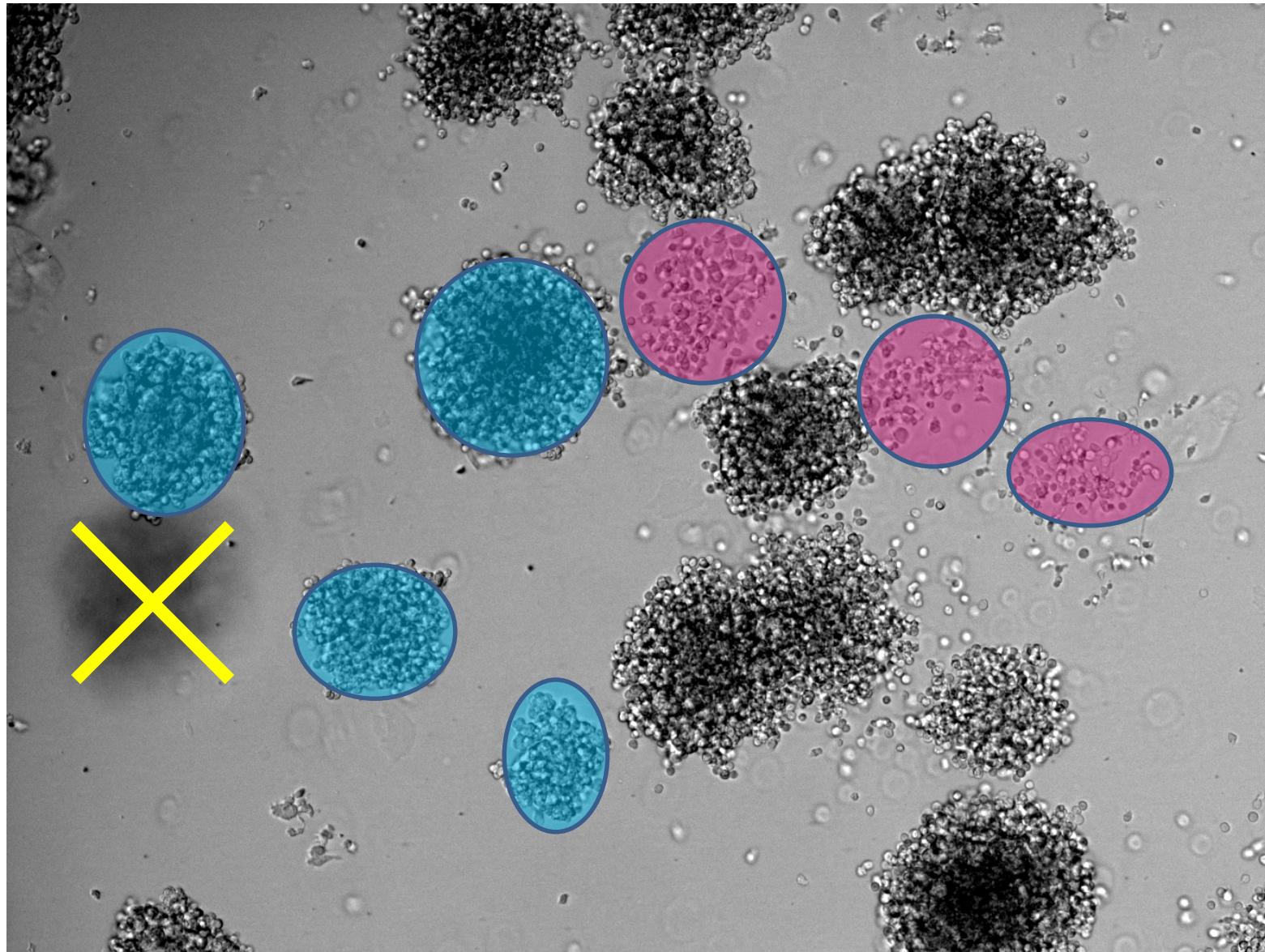


Features: Gaussian Blur, Hessian, Membrane projections, Mean, Maximum, Anisotropic Diffusion, Lipschitz, Gabor, Laplacian, Entropy, Sobel Filter, Difference of Gaussians, Variance, Minimum, Median, Bilateral, Kuwahara, Derivatives, Structure, Neighbors

Exercise: Segmentation

Exercise_04_segmentation

identify both type of colonies (dense & loose)



Advanced Tools for Segmentation

Advanced segmentation tool

- HK-Means (Icy)
- Active Contours (Icy)
- MorphoLiBJ Plugins (Fiji)
- Interactive H-Watershed (Fiji)
- Local environment (Fiji, CellProfiler)
- Machine Learning (Weka, iLastik)

**Don't blindly trust your
microscope**

Don't blindly trust your microscope

1- Imaging artefacts

Illumination pattern

- LUT Glasbey
- Line profile

Chromatic shift between channels

2- Quantification: Excitation, Emission & Detection Linearity

3- Photobleaching correction

4- And more ...

Image Processing and Analysis Workflow

1 - Image Acquisition (Range Indicator, Histogram)

2 - Flat-field correction & Chromatic aberration correction

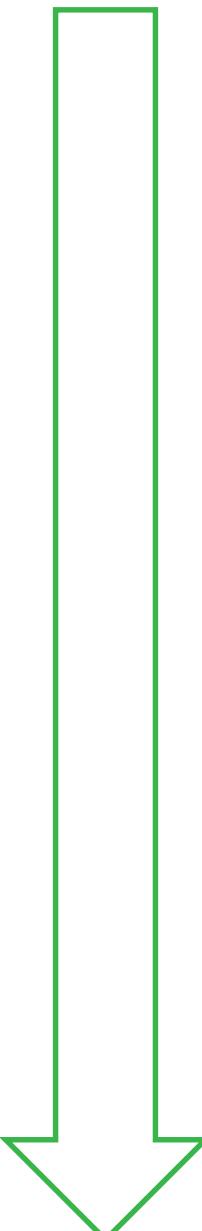
3 - Pre-segmentation processing (filters, histogram stretch)

4 - Segmentation (intensity, machine learning)

5 - Post-segmentation processing (binary operations)

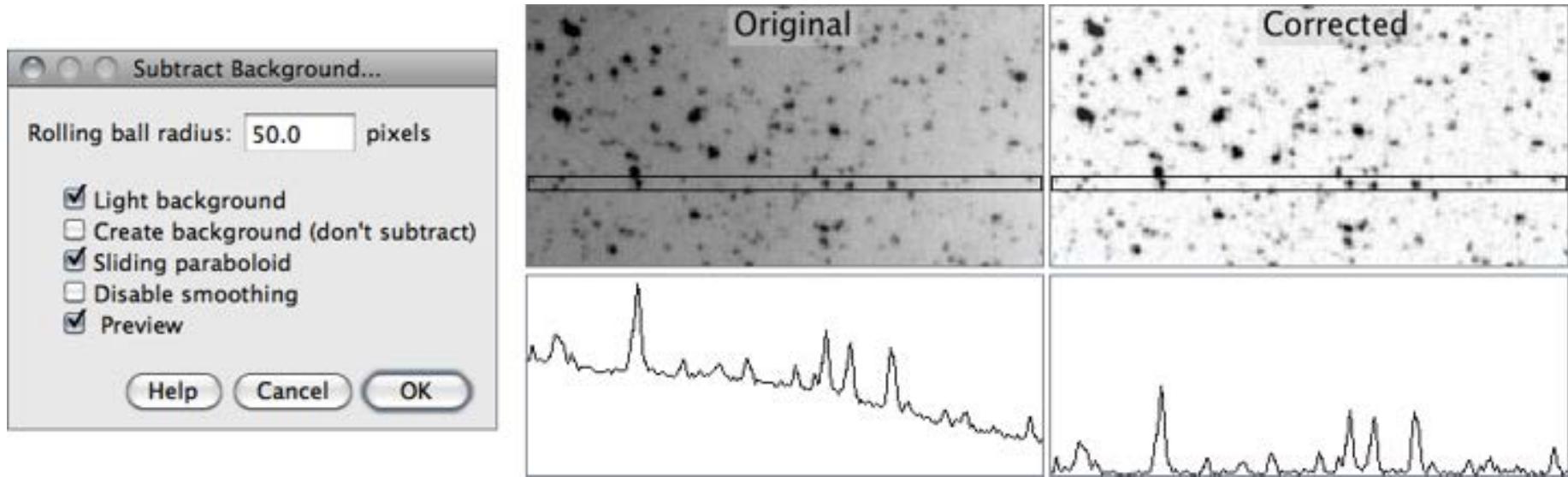
6 - Objects detection and tagging (analyse particles)

7 - Measure objects



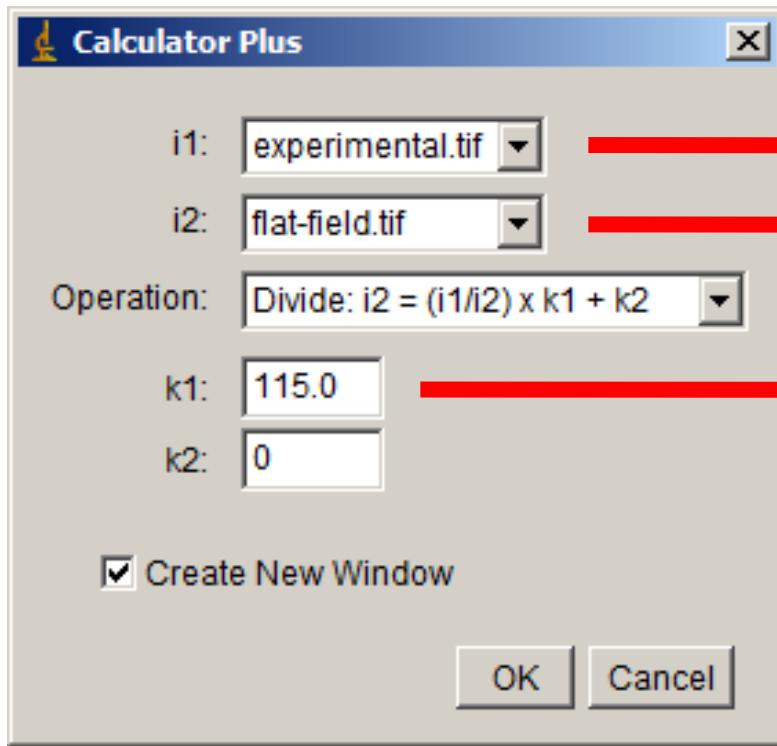
Subtract Background: Rolling Ball

<https://imagej.nih.gov/ij/docs/guide/146-29.html#toc-Subsection-29.14>



- Radius should be set to at least the size of the largest object that is *not* part of the background
- Several iteration may improve the results

Flat-field Correction



i1 : experimental image

i2 : flat-field image

Average intensity of the flat field image

<http://rsbweb.nih.gov/ij/plugins/calculator-plus.html>

Flat-field Correction

Image



Flat-Field

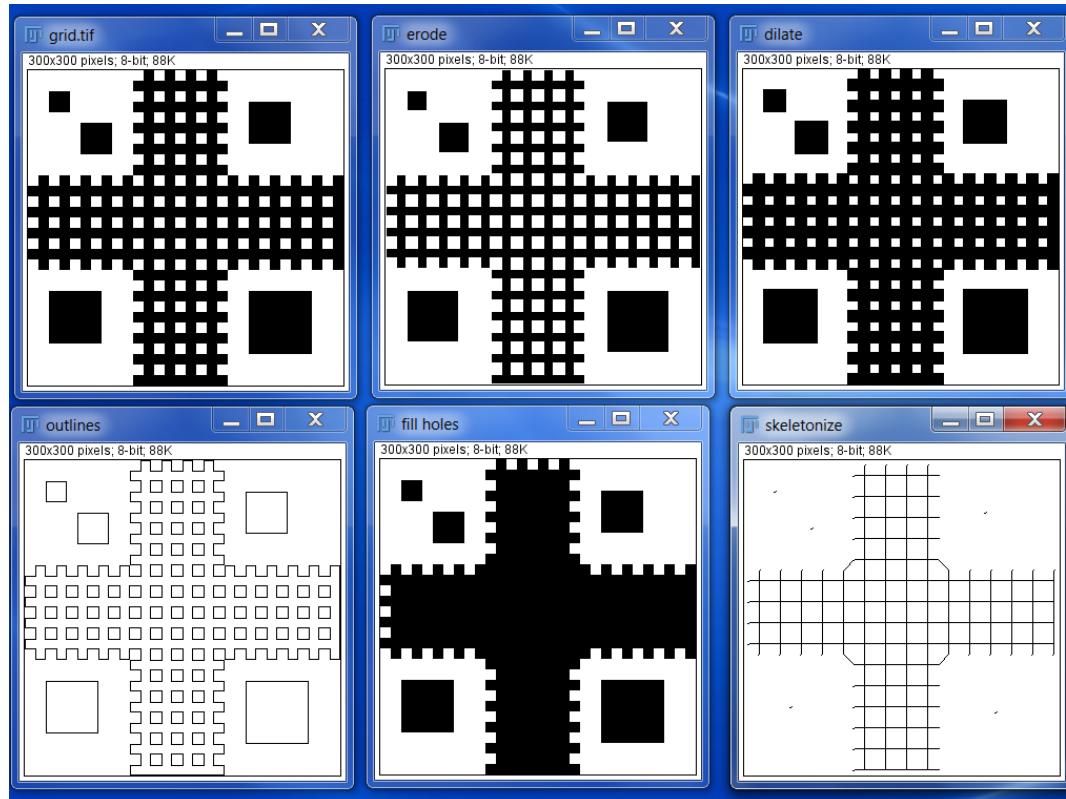


Corrected Image



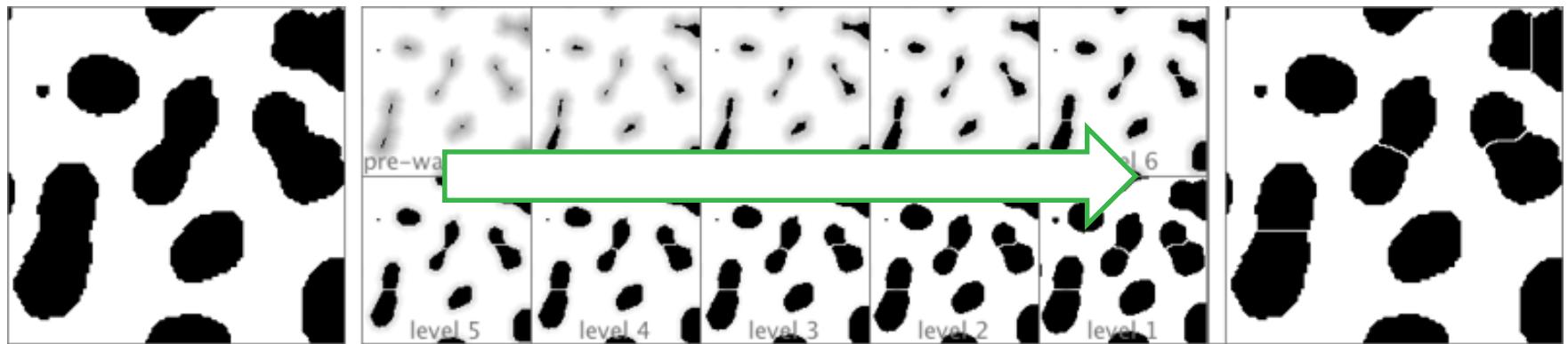
Binary Operations

- Objects are black and background is white unless *Black Background* is checked in the Process>Binary>[Options...](#)
- Useful Functions: erode, dilate, open, close, fill holes, outlines, skeletonize, ...



Watershed

- Automatically separate or cut apart touching particles
- Watershed segmentation works best for smooth convex objects that don't overlap too much.

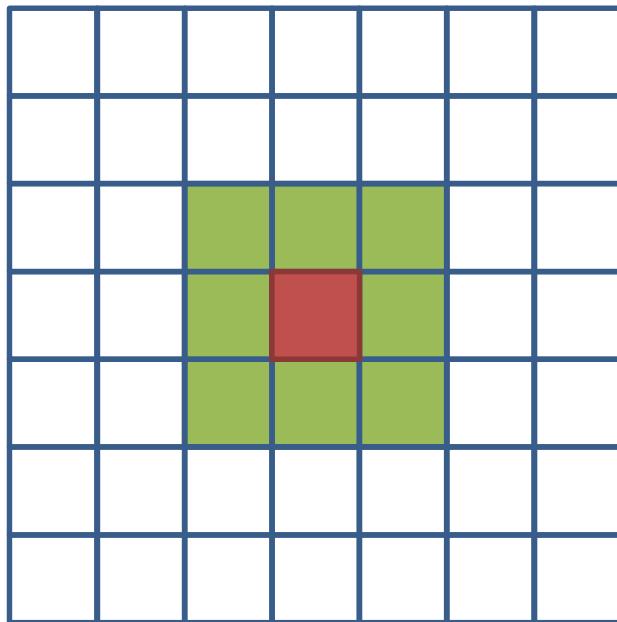


<https://imagej.nih.gov/ij/docs/guide/146-29.html#toc-Subsection-29.8>

Filters

Spatial Filtering

3x3 kernel

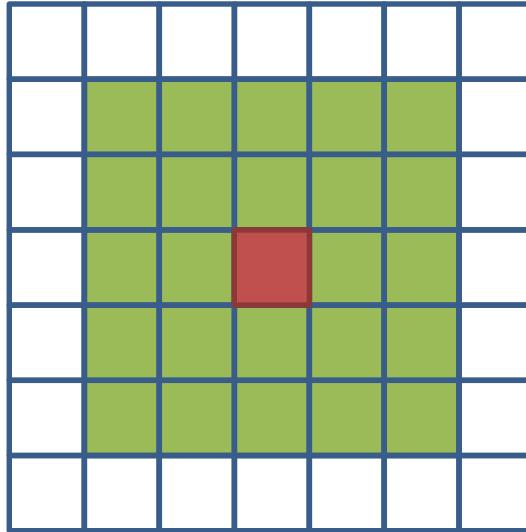


Definition

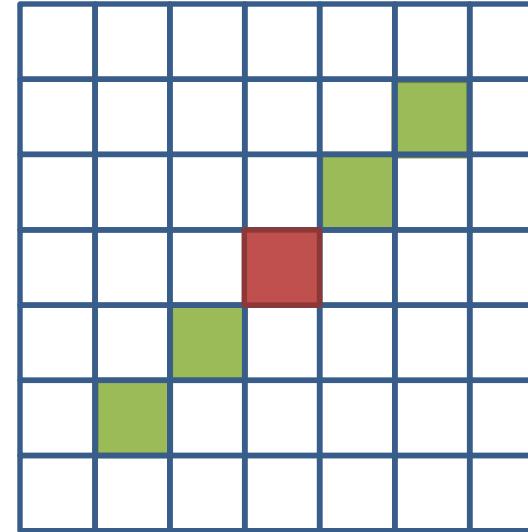
Transformation or set of transformations where a new image is obtained by *neighbourhood operations*

The intensity of a pixel in the new image depends on the intensity values of “neighbour pixels”

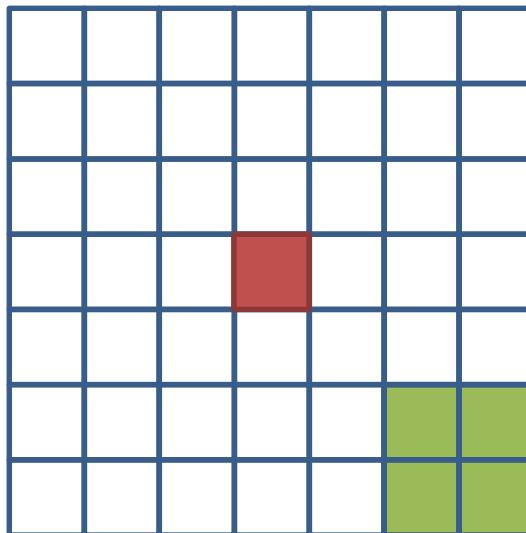
5x5 kernel



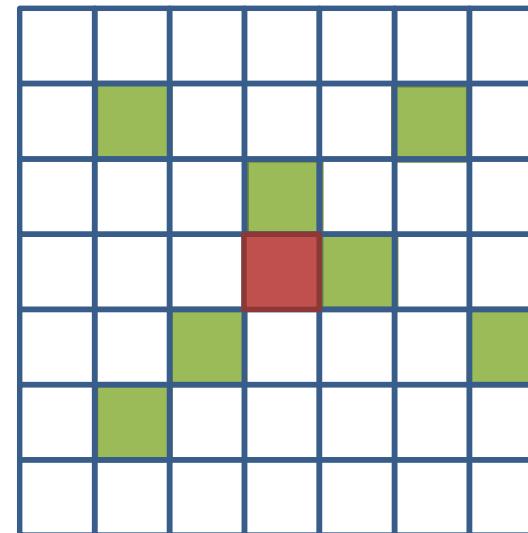
1x5 kernel



2x2 shift



Miscellaneous kernel

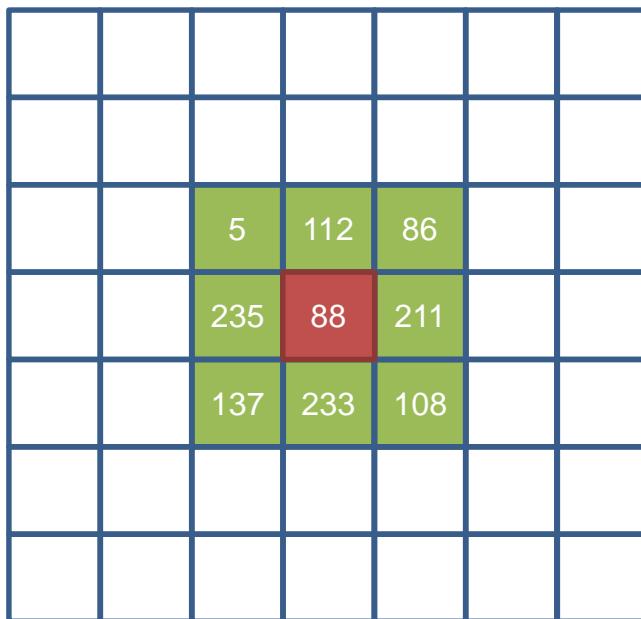


Mean Filter

- Mean filter is a linear filter (“smooth”)
- The new pixel intensity is the intensity mean over the neighbouring pixels
- Average noise (Gaussian/Poisson noise) that appears for weak labelling, short exposure time, confocal,...
- Not good for salt & pepper noise (very weak labelling, high detector gain, ...) -> Median filter
- Low pass filter (smooth small objects & edges)
 - Kernel size influence
 - Number of successive applications

Median Filter

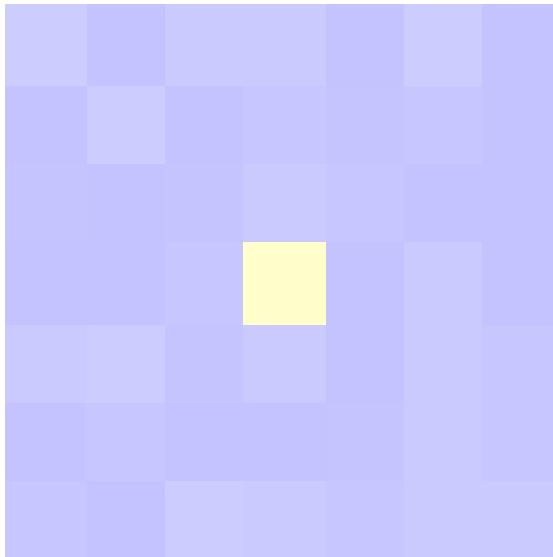
- The new pixel intensity is replaced by the median of pixel intensity over the neighbouring pixels



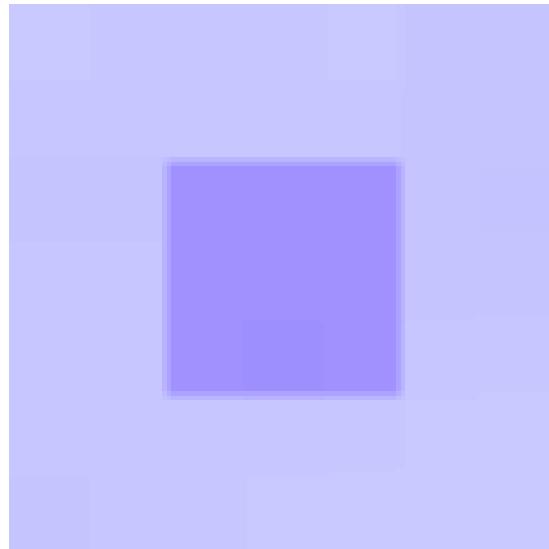
5
86
88
108
112
137
211
233
235

112 = median
135 = mean

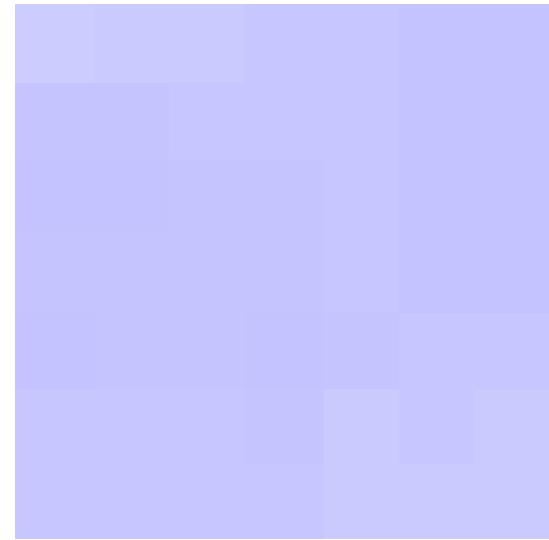
outlier



mean



median



5	9	6	6	9	5	9
9	5	9	7	8	7	9
8	9	8	6	7	9	9
9	9	7	200	9	6	9
6	5	8	6	9	6	7
9	7	9	9	8	6	7
7	9	5	6	7	6	6

2	2	2	3	2	4	4
3	3	3	3	3	4	4
4	4	31	31	31	4	4
3	3	31	31	31	4	4
3	3	31	32	31	3	3
3	3	3	3	2	2	2
4	3	3	2	2	2	1

0	1	1	3	3	5	5
4	4	3	3	3	5	5
5	5	4	4	3	5	5
4	4	4	4	3	5	5
5	4	4	5	4	3	3
3	3	3	4	1	3	1
3	3	3	3	1	1	1

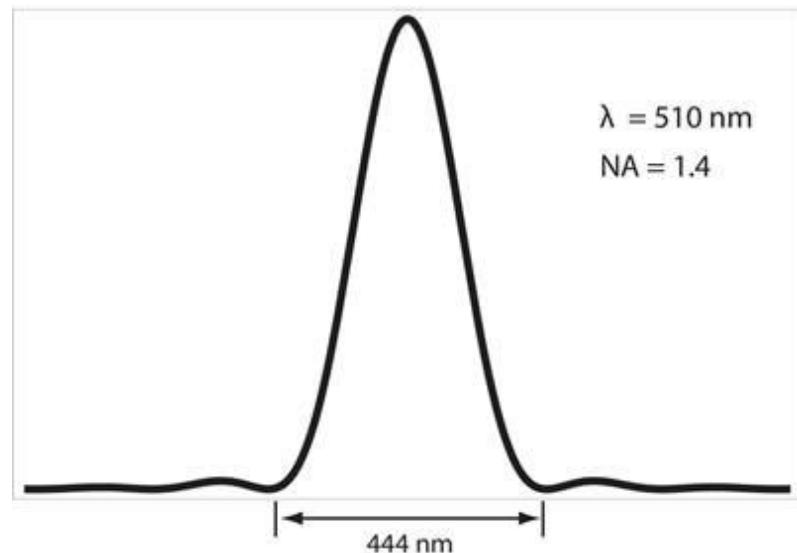
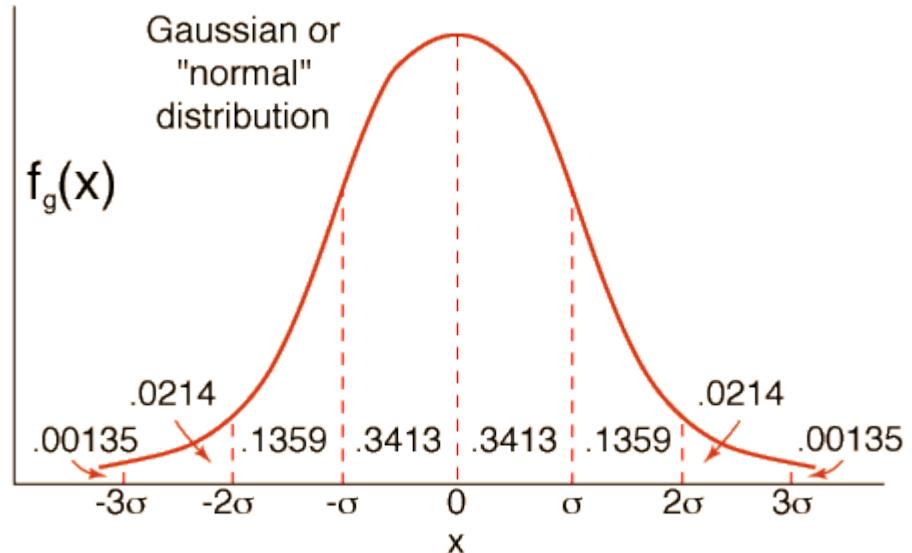
“Salt and pepper” noise removed by median filter

Median Filter

- Good to remove “salt and pepper” noise
- Eliminates noise
- NOT linear !!!
- Edge-preserving

Gaussian Filter

- Gaussian curve
- Smooth Poisson noise
- Linear filter
- Sigma value can be varied: number of pixels to vary the degree of blur
- More mathematically correct than the Mean filter as it looks like the microscope PSF

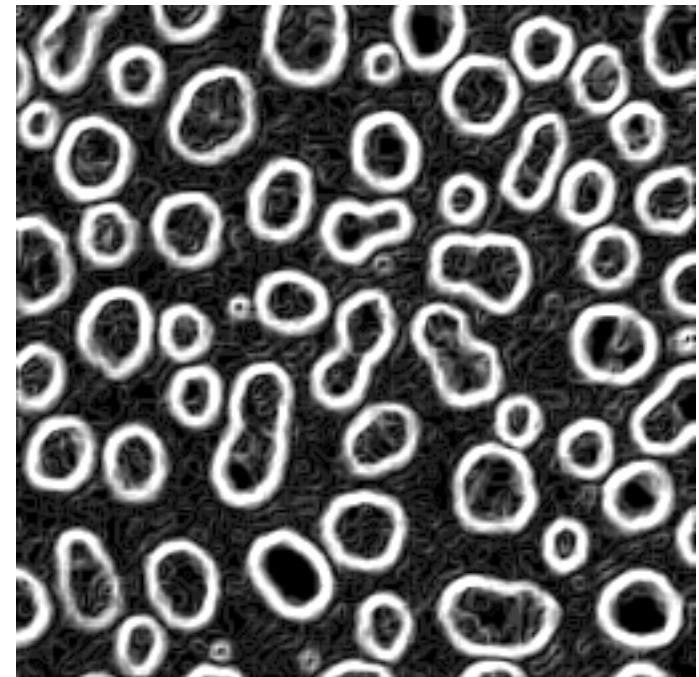
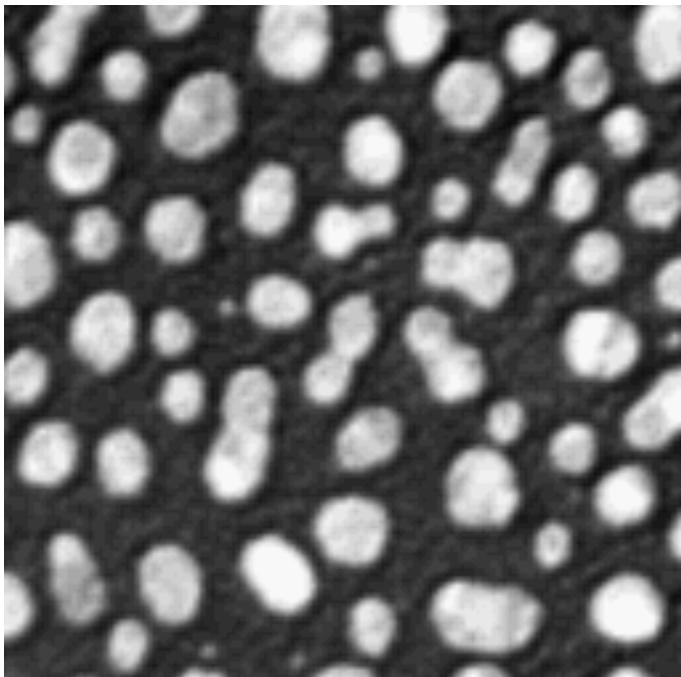


In Fiji

- Process>Smooth
 - Blurs the active image or selection. This filter replaces each pixel with the average of its 3×3 neighborhood.
- Process>Sharpen
 - Increases contrast and accentuates detail in the image or selection, but may also accentuate noise. This filter uses the following weighting factors to replace each pixel with a weighted average of the 3×3 neighborhood: -1,-1,-1/-1,-12,-1/-1,-1,-1
- Process>Filters
 - Gaussian blur
 - Mean
 - Median
- Process>Noise
 - Despeckle (a 3×3 median filter. Median filters are good at removing *salt and pepper* noise.)
 - Remove outliers (Useful for correcting, e.g., hot pixels or dead pixels of a CCD camera.)

Edge Detection

- Process>Find edge
 - Uses a Sobel edge detector to highlight sharp changes in intensity in the active image or selection



Making Figures with FigureJ

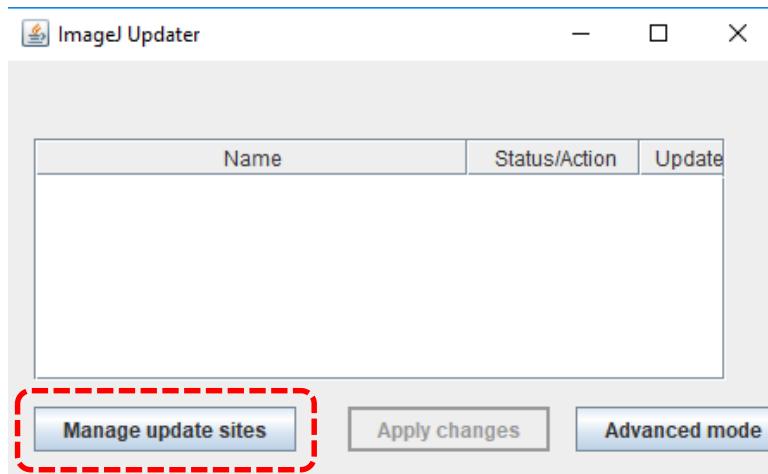
Update FIJI:

Help>Update...

Manage update sites

Select the 3 update sites:

- Bio-Formats
- IBMP CNRS
- ImageScience

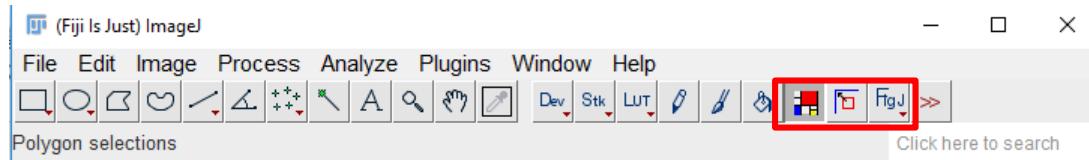


The screenshot shows the 'Manage update sites' dialog box. It lists various update sites with their names and URLs. Two checkboxes are checked: 'IBMP-CNRS' and 'ImageScience'. At the bottom, there are four buttons: 'Add my site', 'Add update site', 'Remove', and 'Close' (highlighted with a red dashed box).

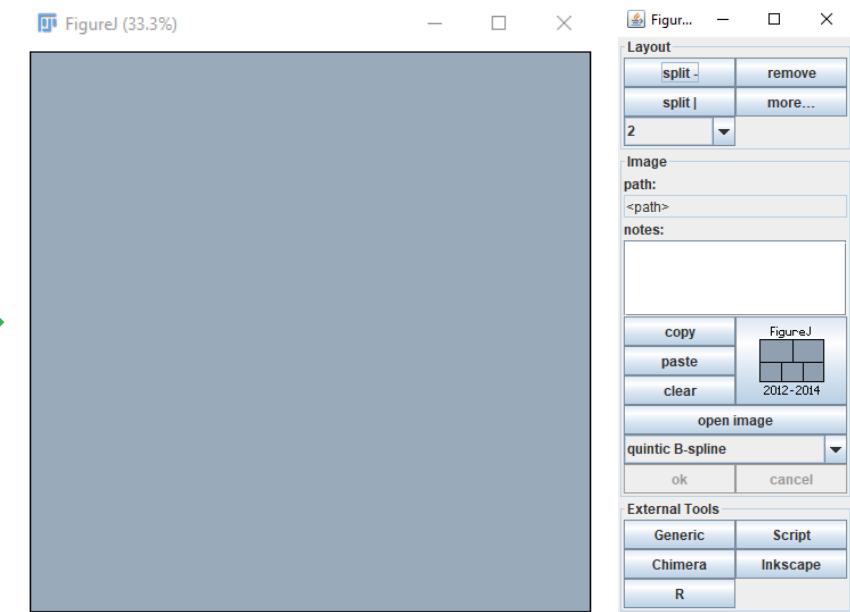
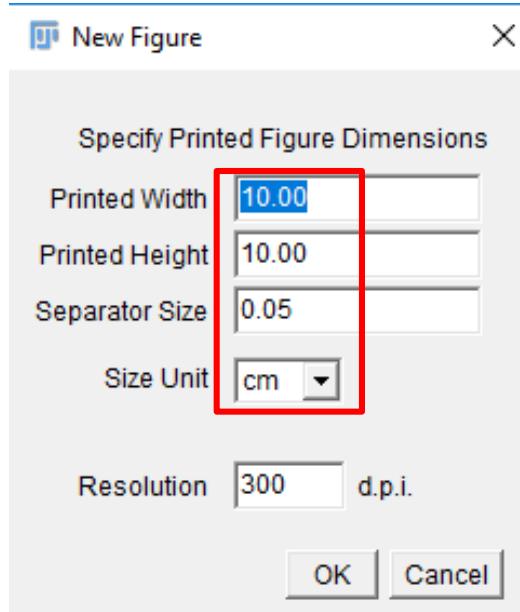
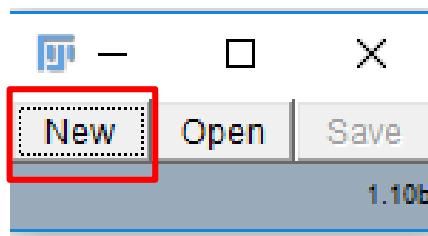
A..	Name	URL	Host	Directory on Host
<input type="checkbox"/>	FiloQuant	http://sites.imagej.net/FiloQuant/		
<input type="checkbox"/>	FPBioimage	http://sites.imagej.net/Fpbioimage/		
<input type="checkbox"/>	FracLac Suite	http://sites.imagej.net/Akarperien/		
<input type="checkbox"/>	FunImageJ	http://sites.imagej.net/FunImageJ/		
<input type="checkbox"/>	Fuzzy logic and artificial n...	http://sites.imagej.net/Astartes91/		
<input type="checkbox"/>	Fuzzy Set	http://sites.imagej.net/Rerger/		
<input type="checkbox"/>	GDSC	http://sites.imagej.net/GDSC/		
<input type="checkbox"/>	GDSC-SMLM	http://sites.imagej.net/GDSC-SMLM/		
<input type="checkbox"/>	Hadim	http://sites.imagej.net/Hadim/		
<input type="checkbox"/>	HDF5	http://sites.imagej.net/Ronneber/		
<input type="checkbox"/>	HistoJ Lite	http://sites.imagej.net/Pathomation/		
<input type="checkbox"/>	IamMM	http://sites.imagej.net/IamMM/		
<input checked="" type="checkbox"/>	IBMP-CNRS	http://sites.imagej.net/Mutterer/		
<input type="checkbox"/>	U-OpenCV-plugins	http://sites.imagej.net/U-OpenCV/		
<input type="checkbox"/>	IJPB-plugins	http://sites.imagej.net/IJPB-plugins/		
<input type="checkbox"/>	ilastik Import Export	http://sites.imagej.net/Ilastik/		
<input type="checkbox"/>	ImageJ Latex	http://sites.imagej.net/Yui.liuyu/		
<input type="checkbox"/>	ImageJ-ITK	http://sites.imagej.net/ImageJ-ITK/		
<input type="checkbox"/>	ImageJ-MATLAB	http://sites.imagej.net/MATLAB/		
<input checked="" type="checkbox"/>	ImageScience	http://sites.imagej.net/ImageScience/		
<input type="checkbox"/>	ImagingBook	http://sites.imagej.net/ImagingBook/		
<input type="checkbox"/>	ImagingBookEn1	http://sites.imagej.net/ImagingBookEn1/		
<input type="checkbox"/>	ImagingBookEn2	http://sites.imagej.net/ImagingBookEn2/		
<input type="checkbox"/>	ImagingBookEn3	http://sites.imagej.net/ImagingBookEn3/		

Making Figures

Plugins>FigureJ>FigureJ



To use figureJ, you have to be sure that the icon is activated



Making Figures

split -

split |

remove

2

more...

Options

Panel Labels

draw	remove
ABC	
TopLeft	A 10 A 10

Panel Scale bars

show value:

height: 10 20 20

Overlay Items

+ T text	+ arrow
add	duplicate
hide	show

Misc.

open color picker
adopt current panel pixels
update separator color
print at actual size

horizontal separation

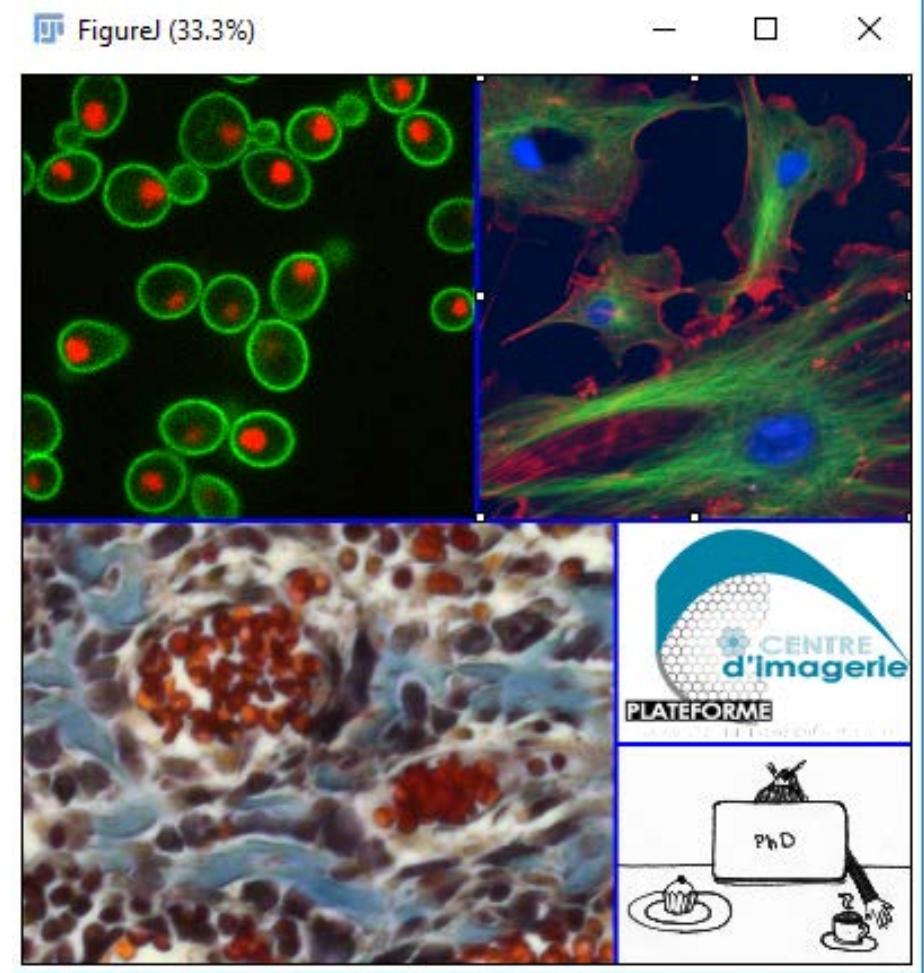
vertical separation

you remove separation

select the number of separation

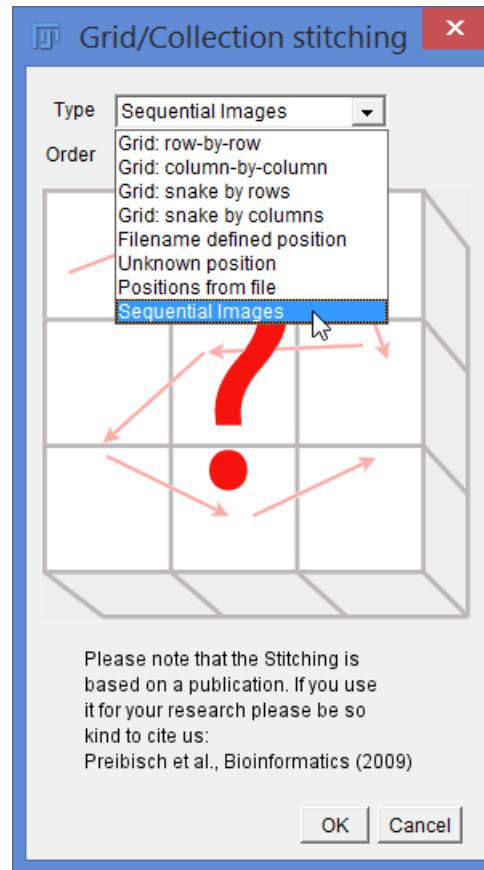
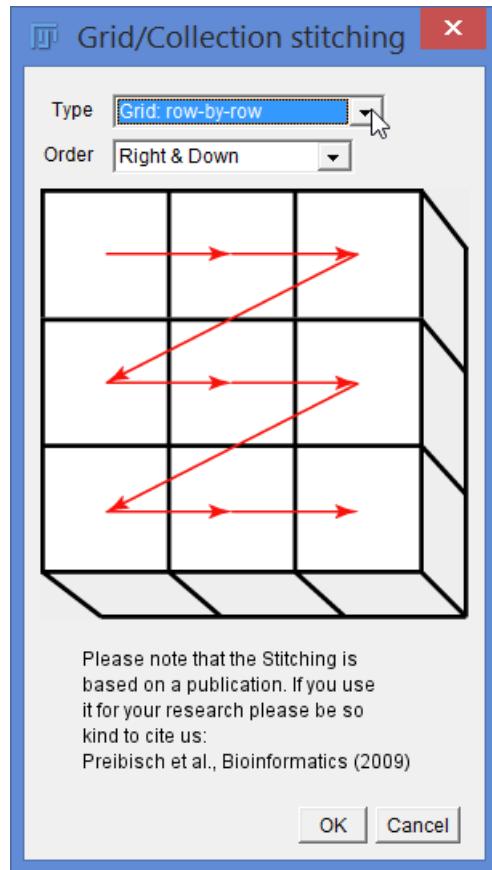
other options

Annotations
Scale bar
Colour
....



Stitching Mosaic

Plugins>Stitching/Grid Collection



Tracking Objects

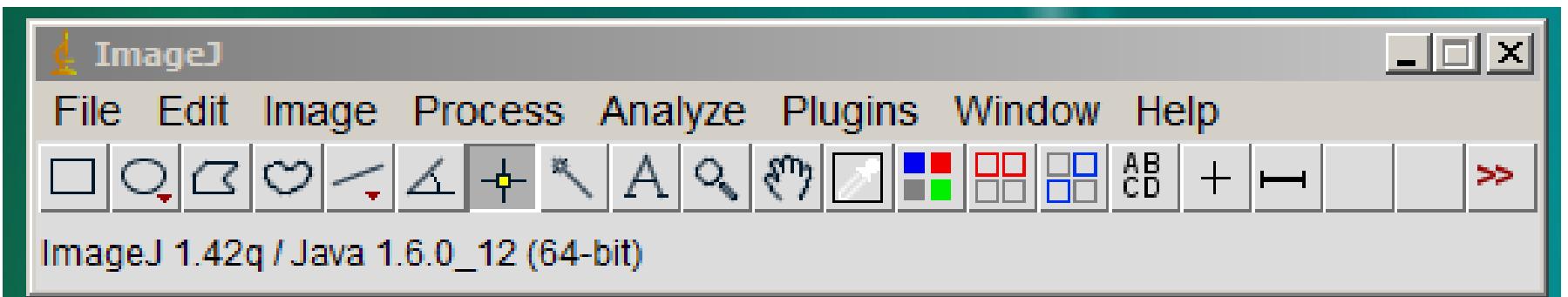
In Fiji Plugins>Tracking

- Manual Tracking
- TrackMate (automatic detection and tracking)

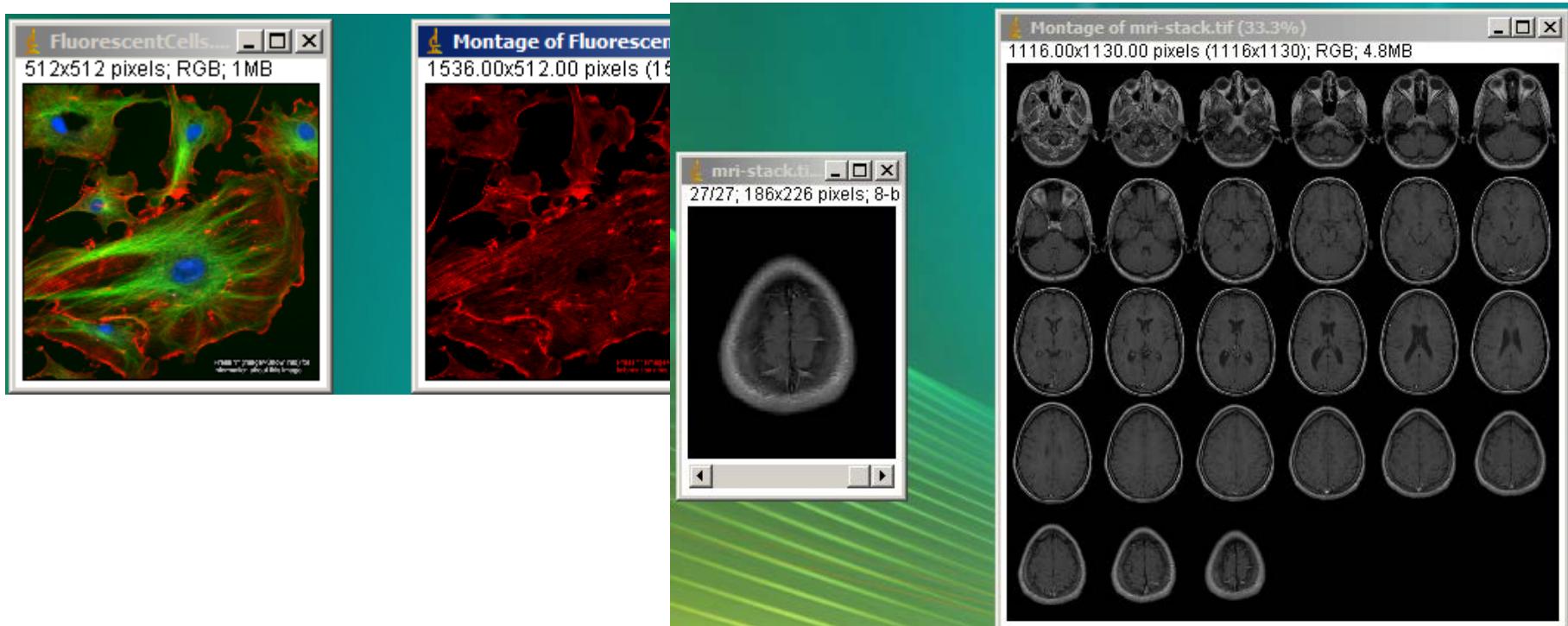
ICY has also some interesting detection & tracking tools

Magic Montage

In ImageJ toolset
To manually install in Fiji



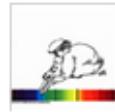
Rapid montage for presentation, lab book pictures



Ethic in Image Processing and Analysis

Guidelines for Best Practices in Image Processing

Please click on each guideline for further details. Also, see the guidelines demonstrated in [Photoshop Videos](#).



Treating Images as Data: Digital scientific images should be treated as data



Saving the Original: Manipulations of digital images should always be done on a copy of the raw image data. The original must be retained.



Making Simple Adjustments: Simple adjustments to the entire image are usually acceptable.



Cropping is usually OK: Cropping an image is usually acceptable.



Comparing Images: Digital images that will be compared to one another should be acquired under identical conditions.



Manipulating the Entire Image: Manipulations that are specific to one area of an image and are not performed on other areas are questionable.



Filters Degrade Data: Use of software filters to improve image quality is usually not recommended for biological images.



Cloning Degrades Data: Cloning objects into an image or from other parts of the image is very questionable.



Making Intensity Measurements: Intensity measurements of digital images should be performed on raw data and the data should be calibrated to a known standard.



Lossy Compression Degrades Data: Avoid the use of lossy compression.



Issues With Magnification: Magnification and resolution issues are important.

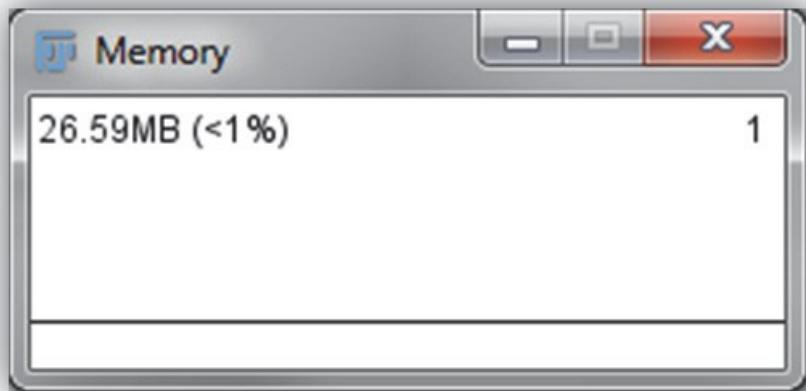


Issues With Pixels: Be careful when changing the size (in pixels) of a digital image.

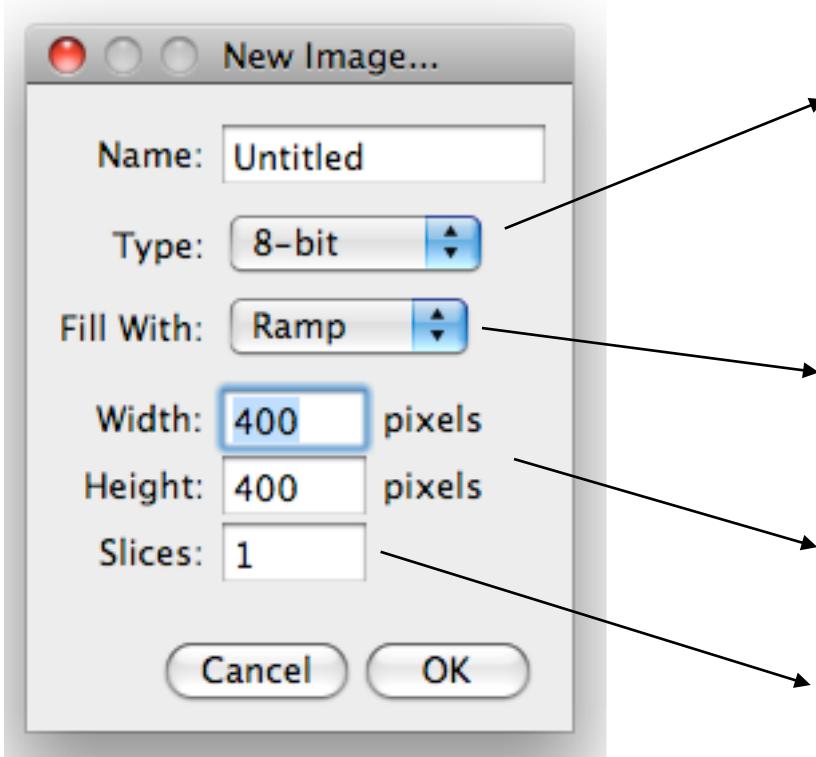
Monitoring Memory

Plugins>Utilities>Monitor Memory...

To monitor the currently used memory and the number of opened images go to



File>New Image...



8-bit grayscale
16-bit grayscale (unsigned)
32-bit grayscale (float)
RGB color

image initialisation

White

Black

Ramp

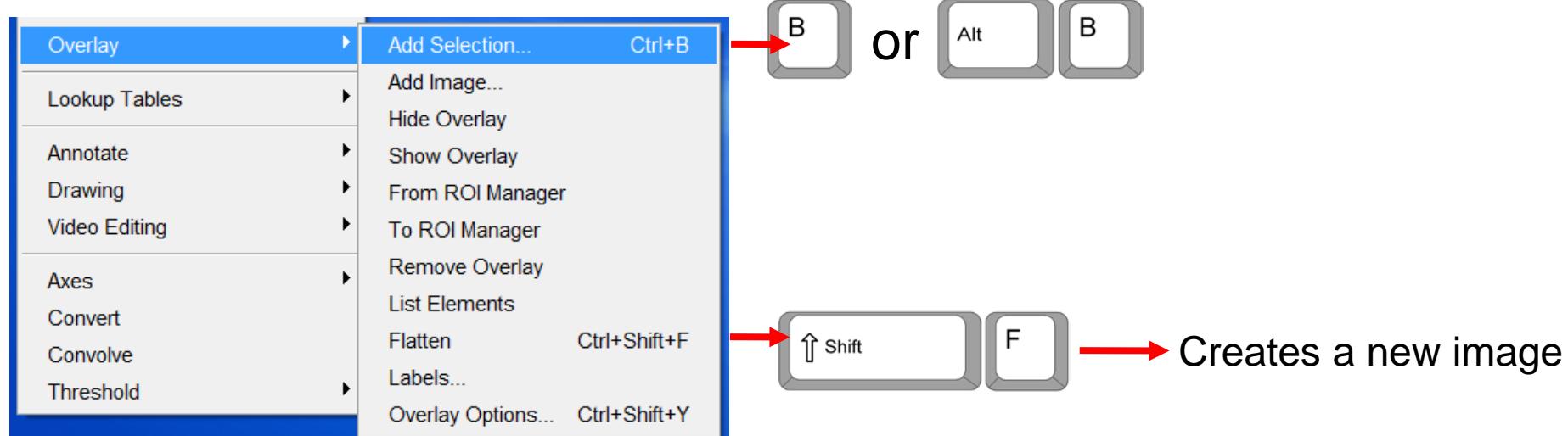
Width x height in pixels

Value >1 creates a stack

Overlay

Image>Overlay

ImageJ/Fiji creates and works with non-destructive overlays. The overlay is preserved when an image is saved in TIFF format.

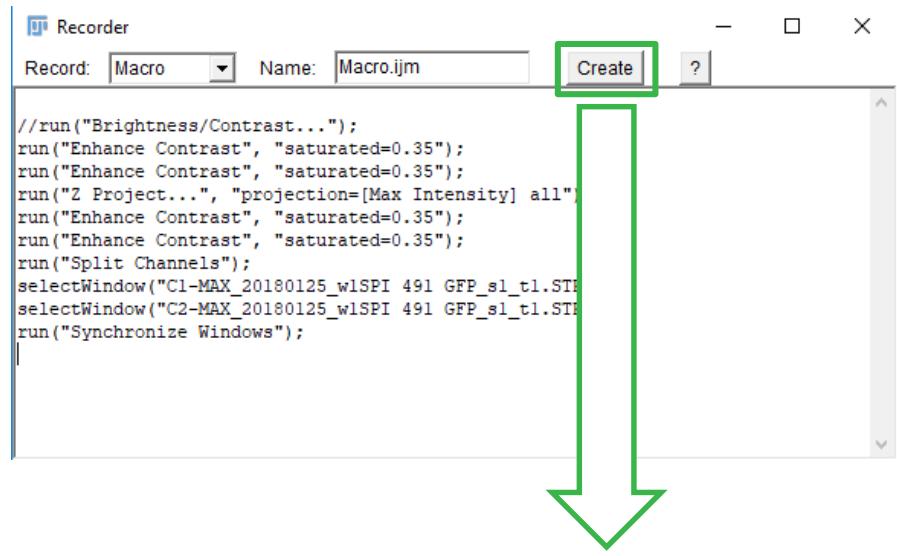


Automation & Scripts

Macros

- Customisable automation
- Written in Macro language
- Macro can be recorded

Macros Recorder



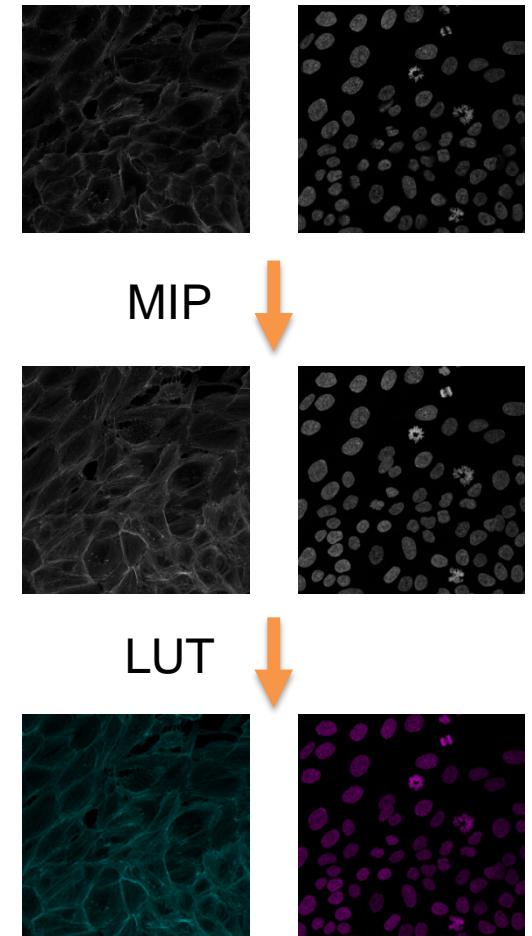
Record your almost all your actions:

- for your lab book
- for automation

Automation

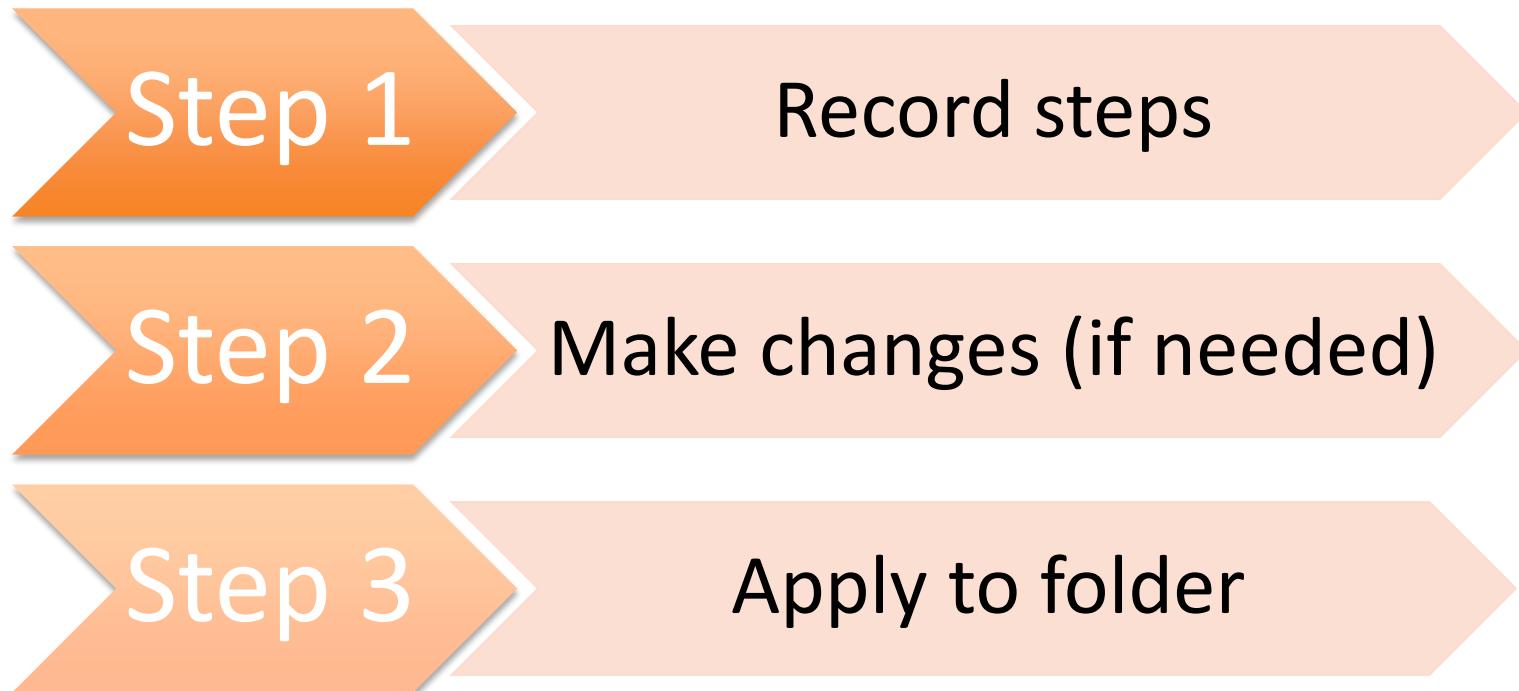
Macros Recorder: Data Set

- Data set:
 - 2 channels hyperstack (DAPI, Phalloidin) in greys
 - Several Z slices
 - multiple images in one folder
- Objective:
 - Creation of easy-to-inspect images.
- Workflow:
 - Make a Max Intensity Projection (MIP)
 - Change Look Up Table (LUT) to Magenta and Cyan
 - Merge channels



/Images/Batch/series_i.tif

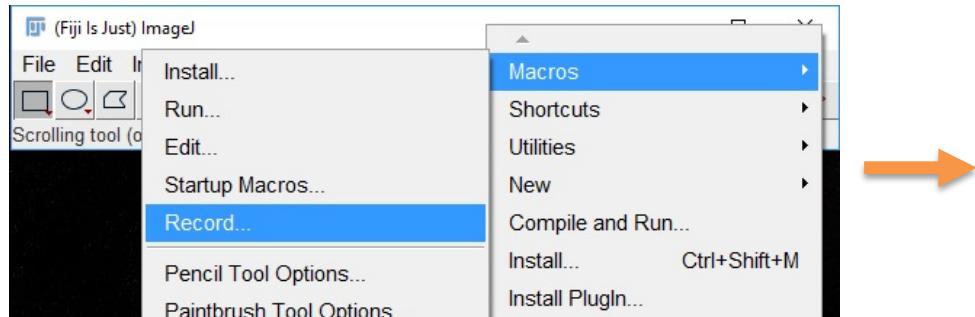
Workflow: Where do We Start



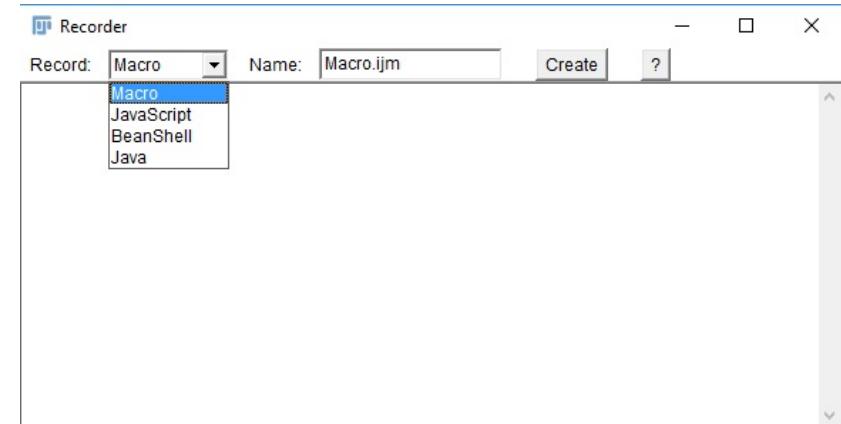
Step #1: the Recorder

- Open the Record window:

Plugins > Macros > Record...

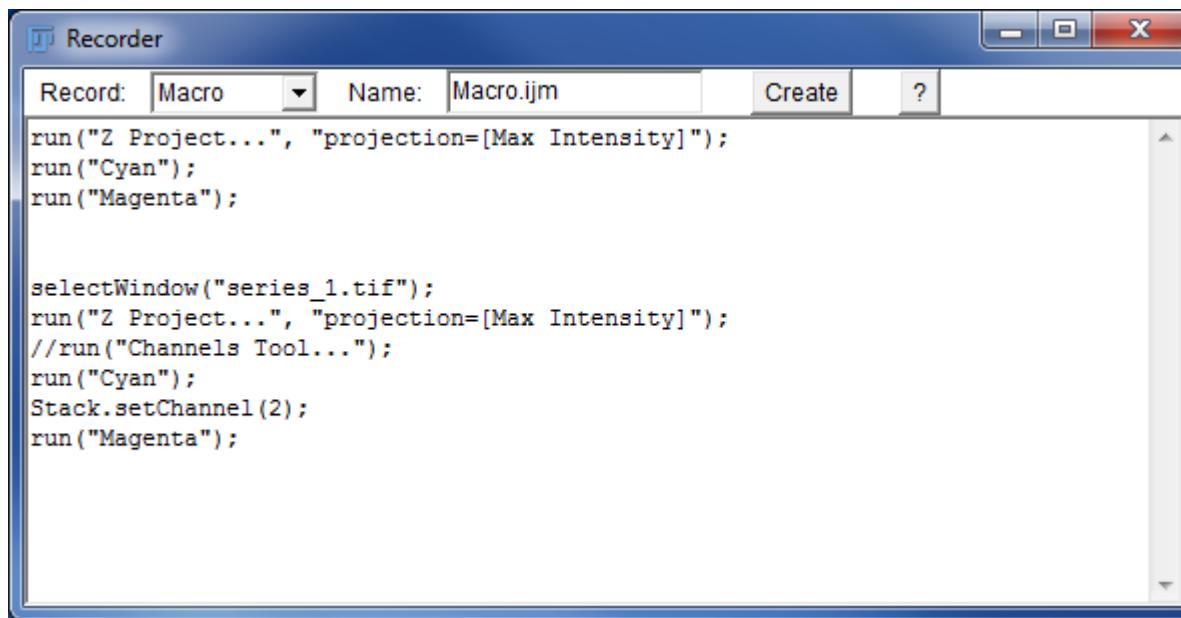


Choose the language : Macro



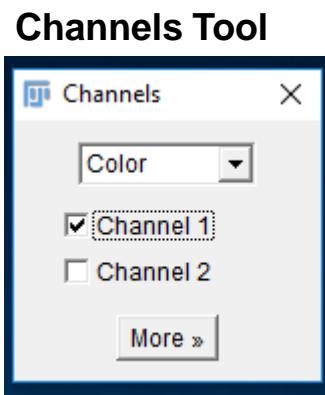
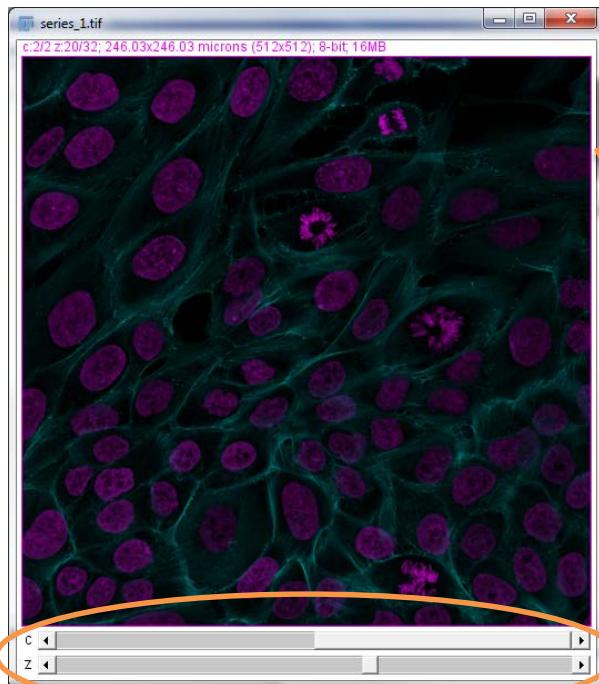
Step #1: the Recorder

- Use the Recorder to:
 - Discover the commands
 - Get arguments for specific functions
 - Define a workflow sketch
 - Write your processing and analysis steps in your lab book
 - The Recorder window can be edited, copied, pasted, cut, etc.



Step #1: the Recorder

- Some commands, functions or plugins are not recorded!
 - Right click on the image
 - Image slider
 - Drag and drop
 - Some plugins
 - Etc.



Recorder

Record: Macro Name: Macro.ijm Create ?

```
run("Z Project...", "projection=[Max Intensity]");
//run("Channels Tool...");
if (cyan);
  Stack.setChannel(2);
run("Magenta");
Stack.setDisplayMode("composite");
```

Channel selection recorded!

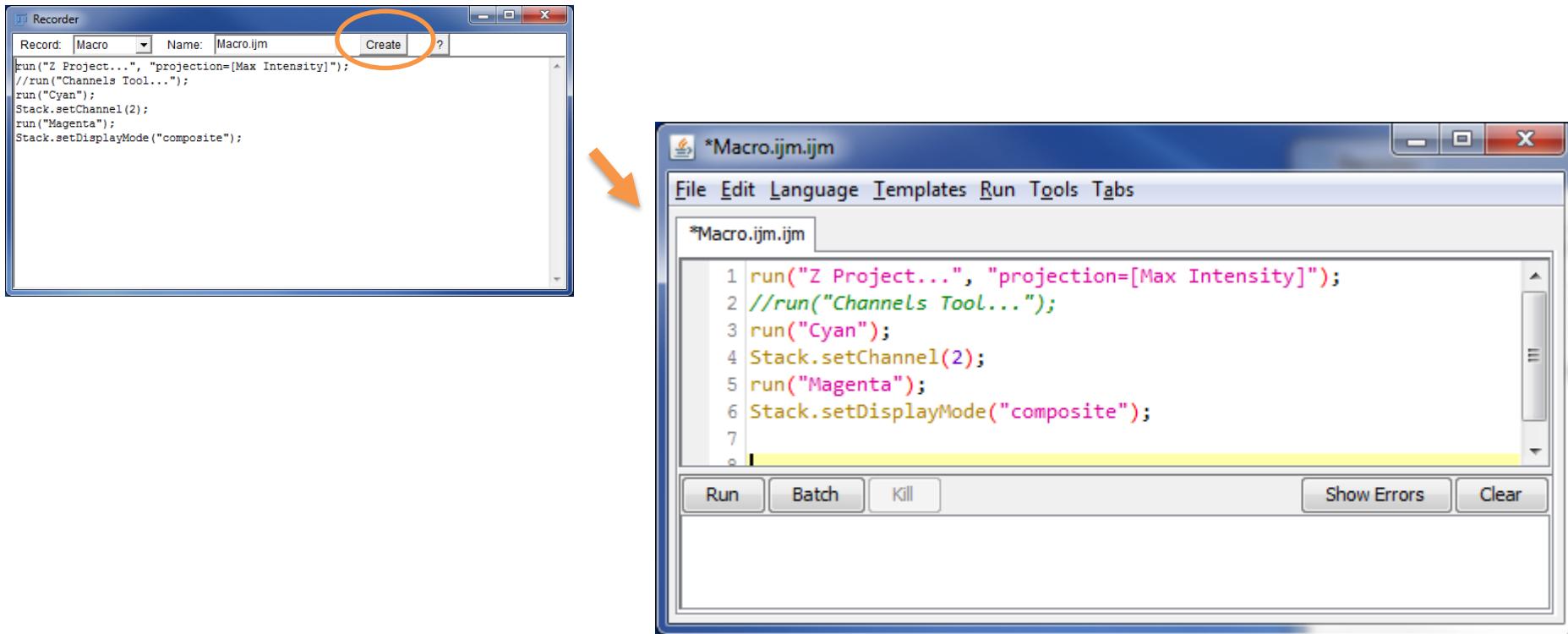
Recorder

Record: Macro Name: Macro.ijm Create ?

```
run("Z Project...", "projection=[Max Intensity]");
run("Cyan");
run("Magenta");
run("Make Composite");
```

Channel selection not recorded!

Step #2: Edit and Test the Recorded Macro



(Choose Language > IJ1 Macro)
Open a new image and press “run”.

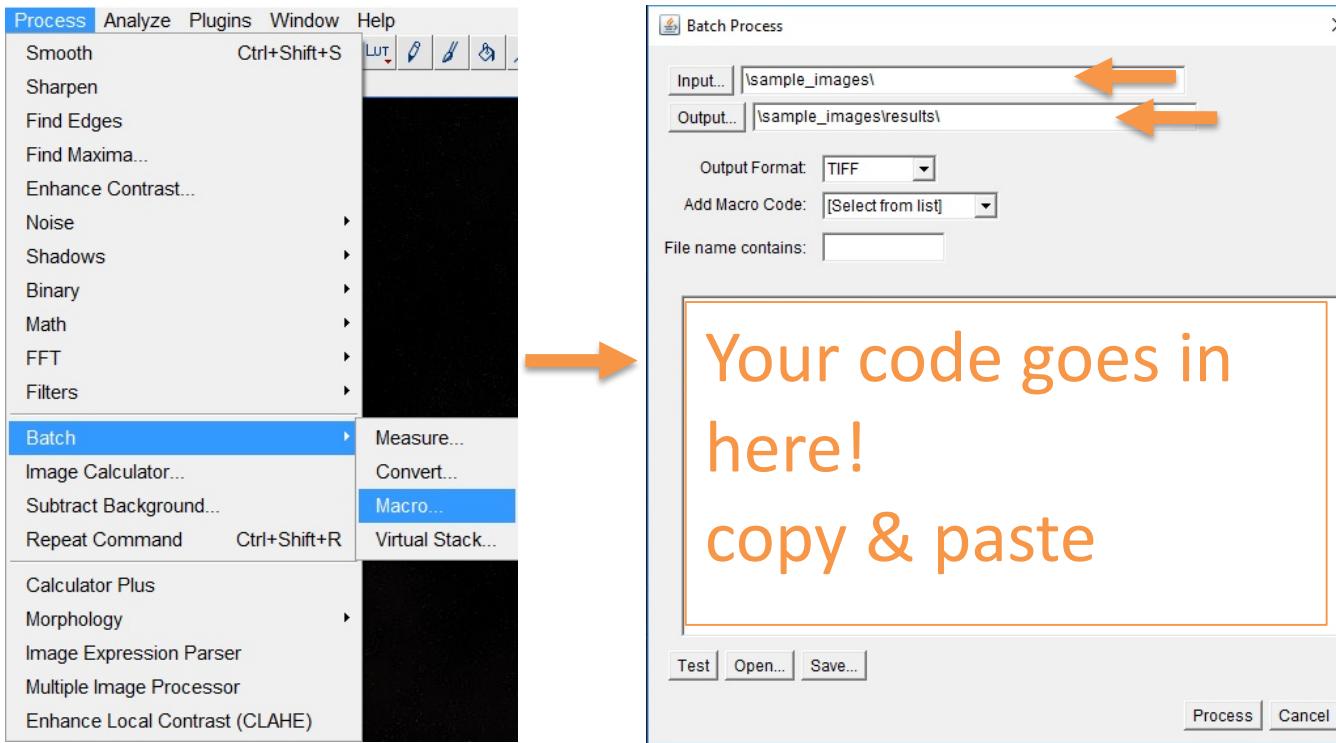
Step #2: Edit and Test the Recorded Macro

```
run("Z Project...", "projection=[Max Intensity]");
//run("Channels Tool...");
run("Cyan");
Stack.setChannel(2);
run("Magenta");
Stack.setDisplayMode("composite");
```



```
run("Z Project...", "projection=[Max Intensity]");
//run("Channels Tool...");
Stack.setChannel(1); //select the first channel
run("Cyan");
Stack.setChannel(2);
run("Yellow"); //Apply the "Yellow" LUT
Stack.setDisplayMode("composite");
```

Step #3: Apply to Folder

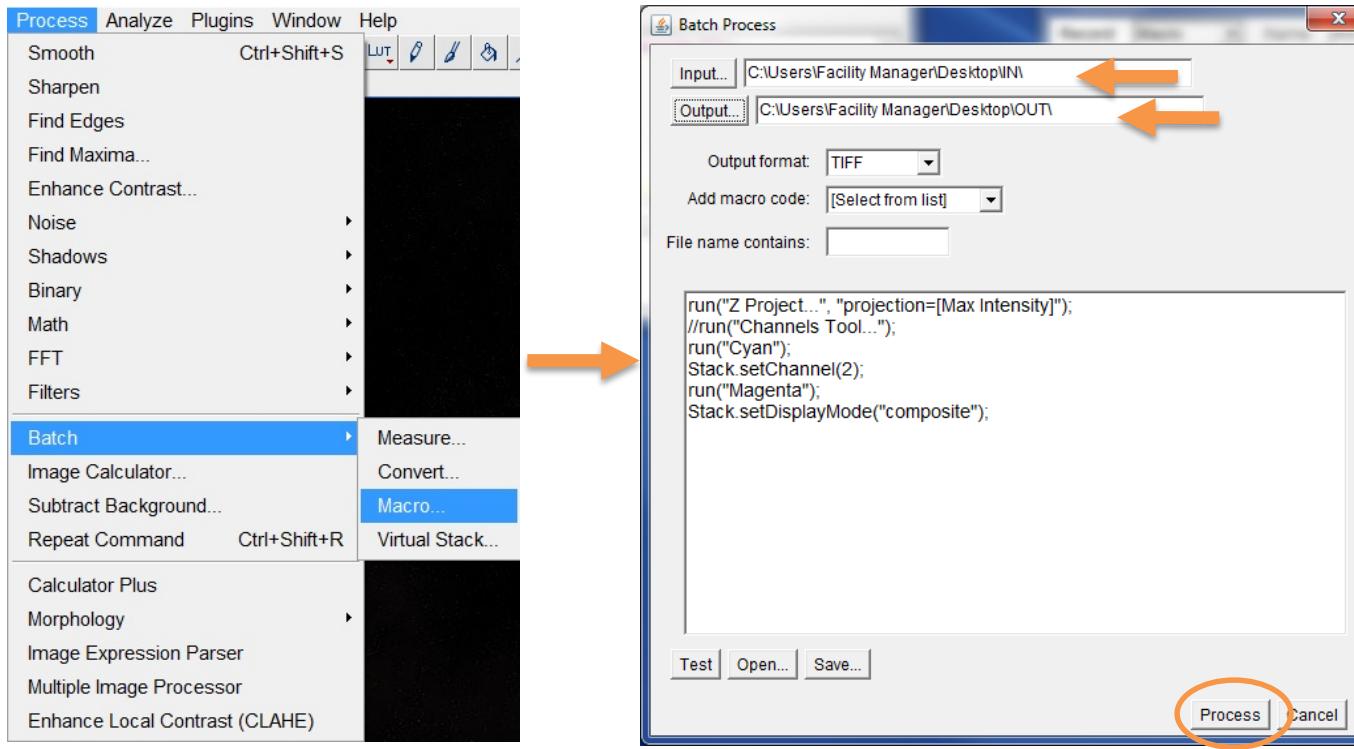


INPUT : Where
are the files to
process ?

OUTPUT : Where
do you want to
save the results ?

Good practice to
use distinct
folders for input
and output

Step #3: Apply to Folder

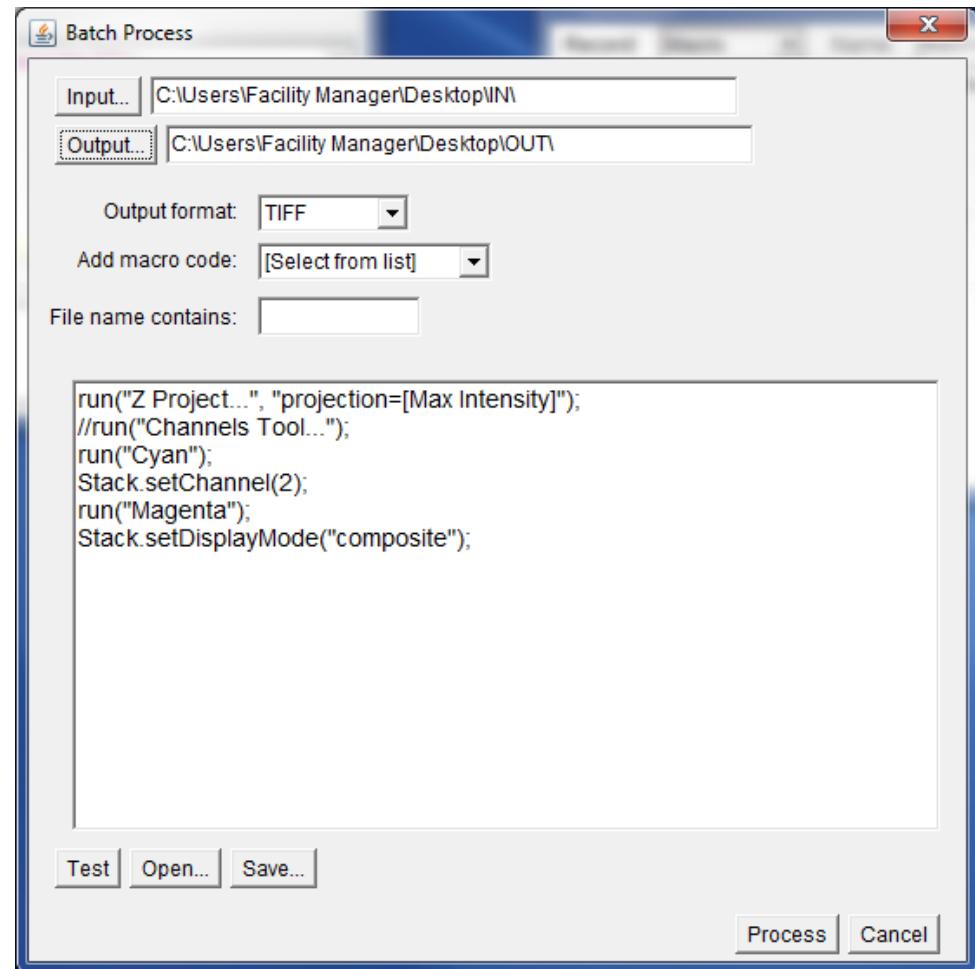


Exercices

- **Workflow:**
 - Start the recorder
 - Open one image
 - Make a Maximum intensity projection
 - Select Channel and change LUT (Image>Colour>Channels Tool)
 - Create a macro
 - Test the macro on a new image
 - Copy and paste it in the Batch Process window
 - run “Process”
 - Check the result in the output folder

Pros & Cons of Batch Processor

- Pros:
 - Easy to do
 - Very fast
- Cons:
 - Only saves in image formats
 - Limited feedback in case of error



Ressources

Pete Bankhead's ***Analyzing fluorescence microscopy images with ImageJ***

<https://petebankhead.gitbooks.io/imagej-intro/content/>



Links

ImageJ core program (as of October 2019 latest 1.52q)

<https://imagej.net/Downloads>

Fiji program

<http://fiji.sc/>

Manual

online manual <https://imagej.nih.gov/ij/docs/guide/index.html> (Last update October 2012, 1.46r)
pdf manual <https://imagej.nih.gov/ij/docs/guide/user-guide.pdf>

Tutorials

ImageJ Wiki http://imagejdocu.tudor.lu/doku.php?id=video:beginner_help:imagej_beginner_s_tutorial
Fiji Wiki <https://imagej.net/Fiji> (Fiji version of ImageJ)

Macros

<http://rsbweb.nih.gov/ij/macros/>
<https://imagej.nih.gov/ij/developer/index.html>

Plugins

<http://rsbweb.nih.gov/ij/plugins/index.html>
<https://imagej.net/Category:Plugins>
many more on the web

Online Courses

http://pcwww.liv.ac.uk/~cci/reveal_ia/ImageAnalysisWithFiji.html

