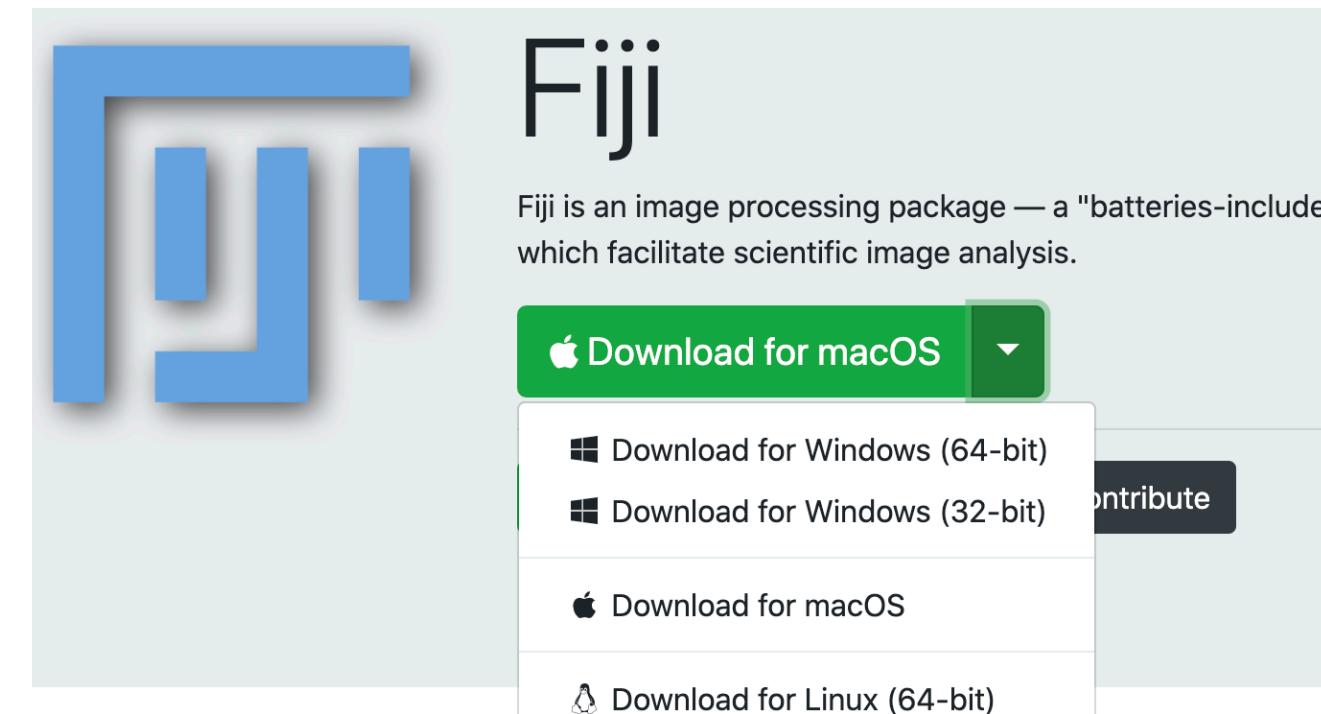


Install and Update Fiji

Download Fiji

<https://fiji.sc/>



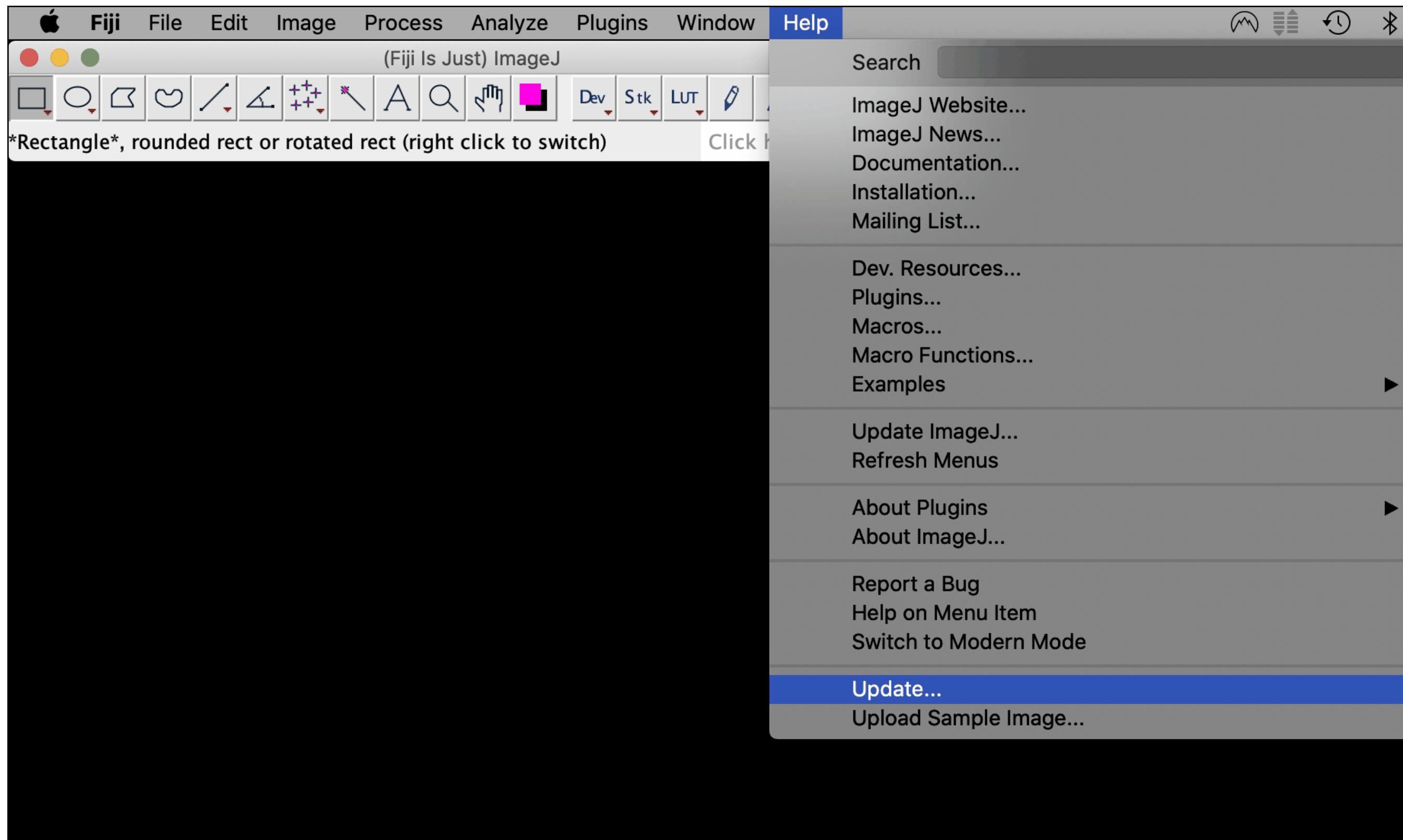
<https://imagej.net/Fiji>



Fiji is distributed as a **portable application**,
which means that you do not have to run an installer.
Just download (**zip file**), **unpack** and start it.

Update Fiji

(and install/uninstall plugins)

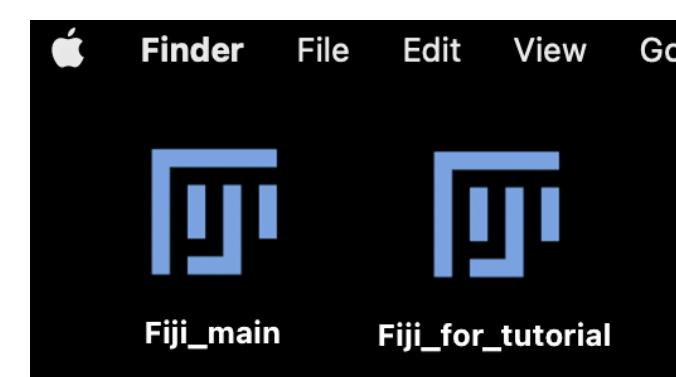


Help > Update...

To update Fiji and/or the plugins.

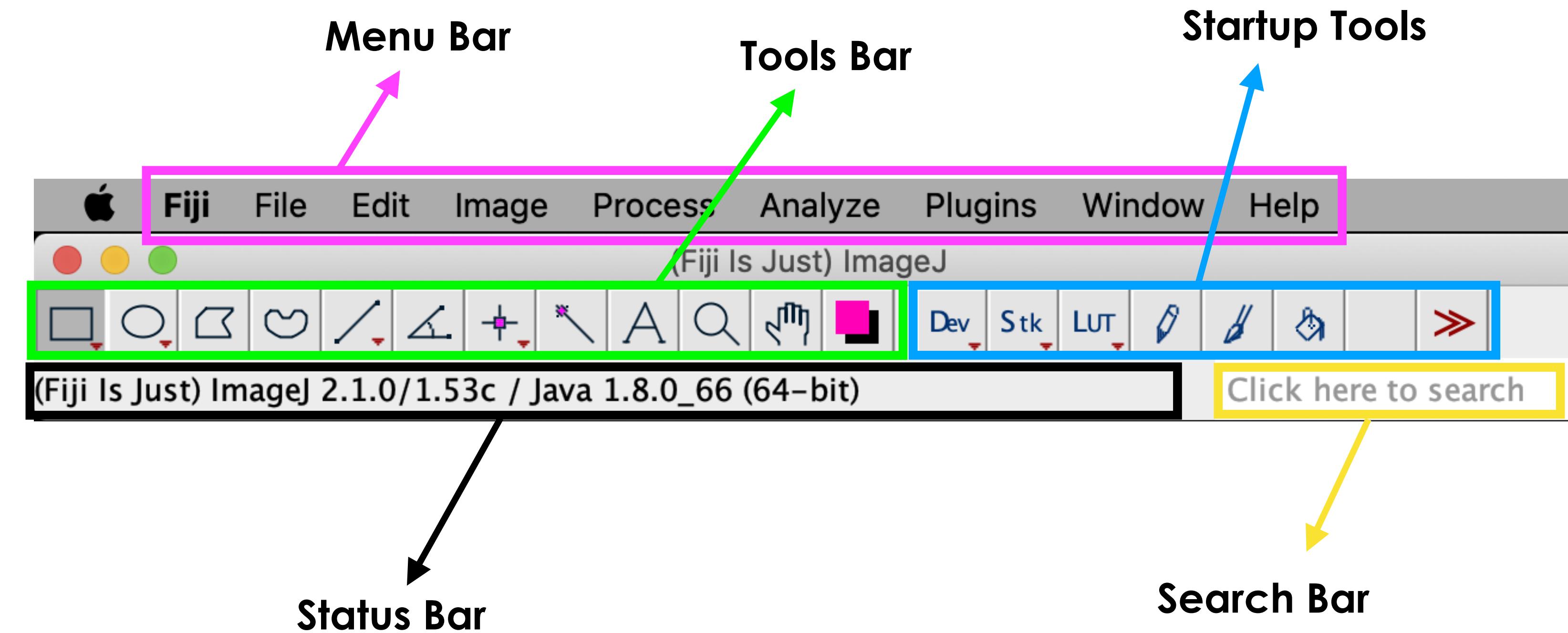
Apply Changes: Install/Update the listed plugins

Manage Update Sites: opens a list of plugins, you can select which one(s) to install in Fiji

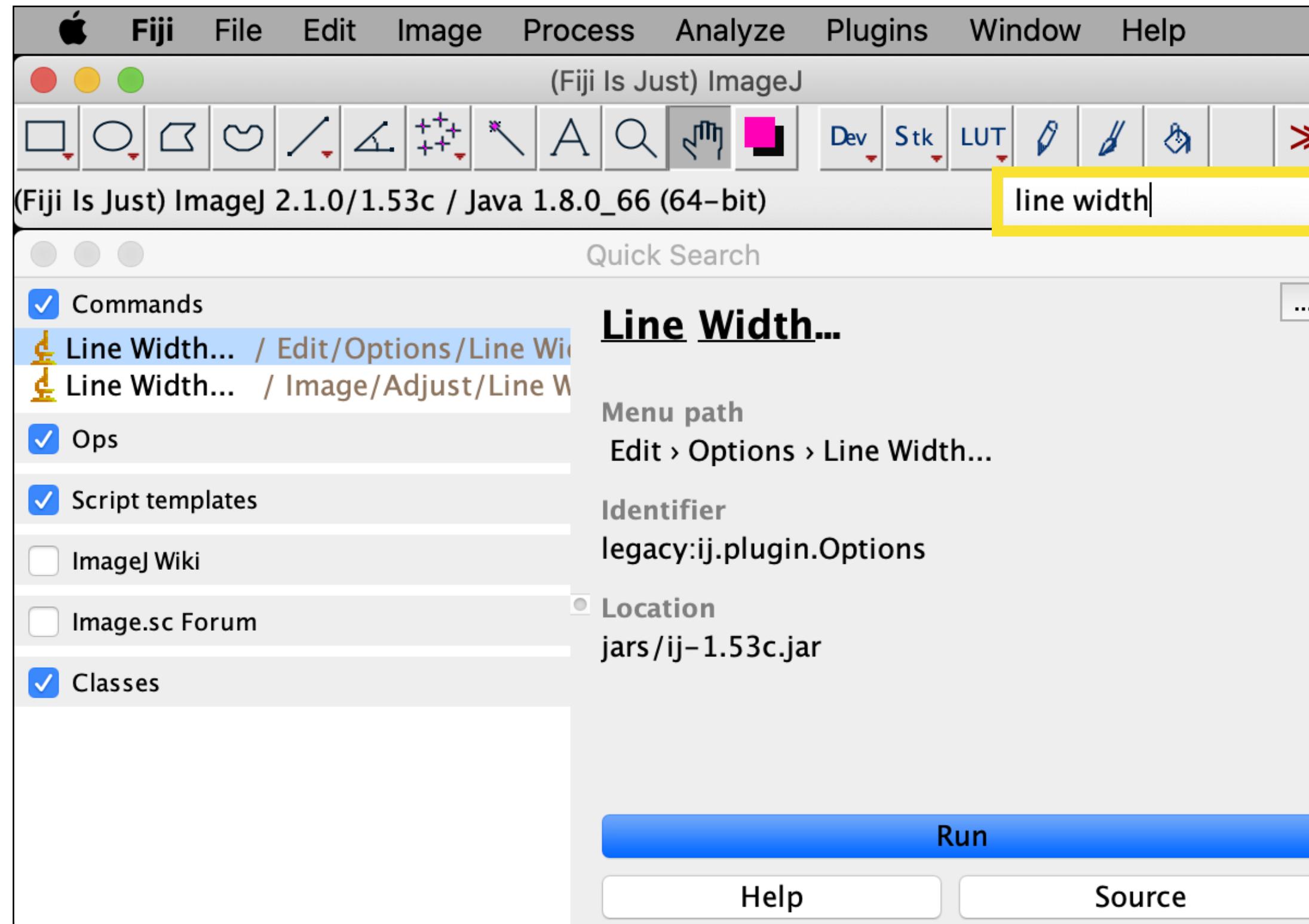


You can have more than one Fiji app!

Graphic User Interface (GUI)



Graphic User Interface (GUI)



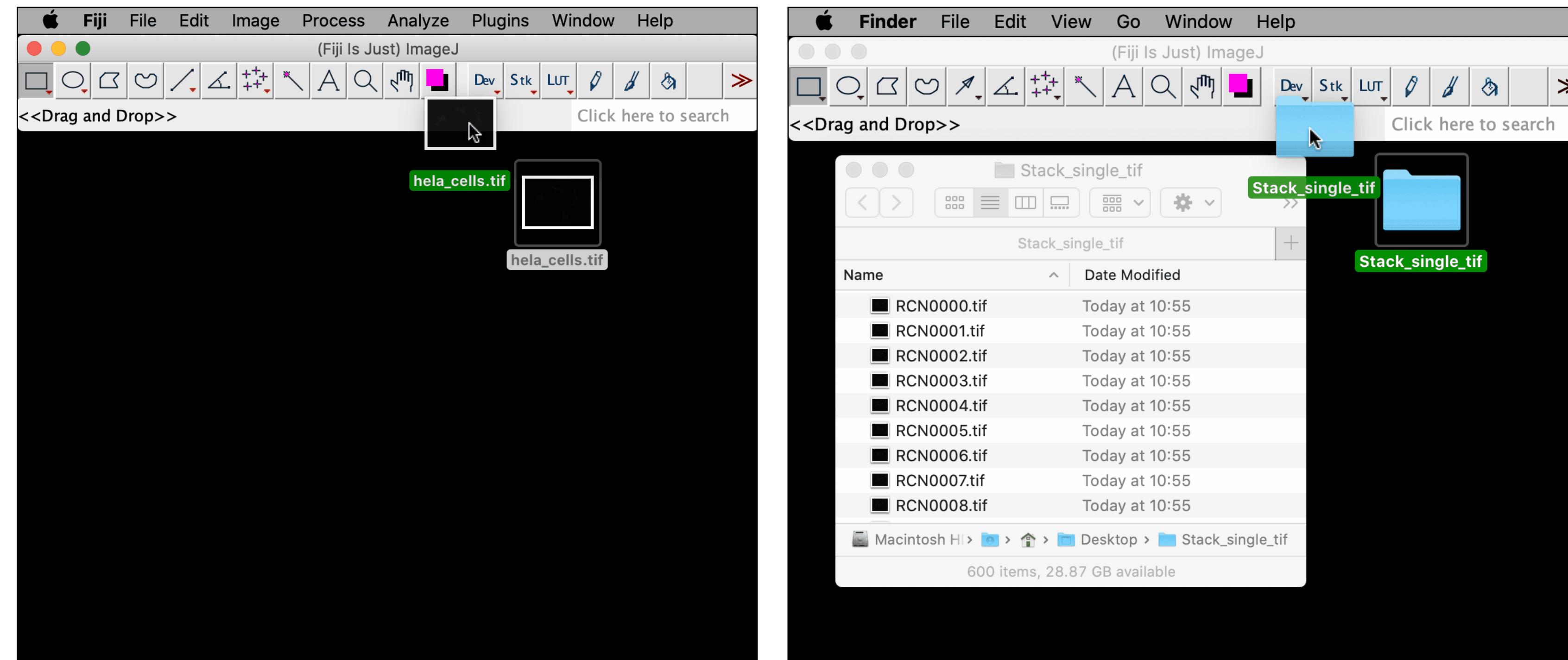
Search Bar
(L key shortcut)

***Plugins > Shortcuts > List Shortcuts - list of the default shortcuts**

****Plugins > Utilities > Find Commands - search for Fiji Commands**

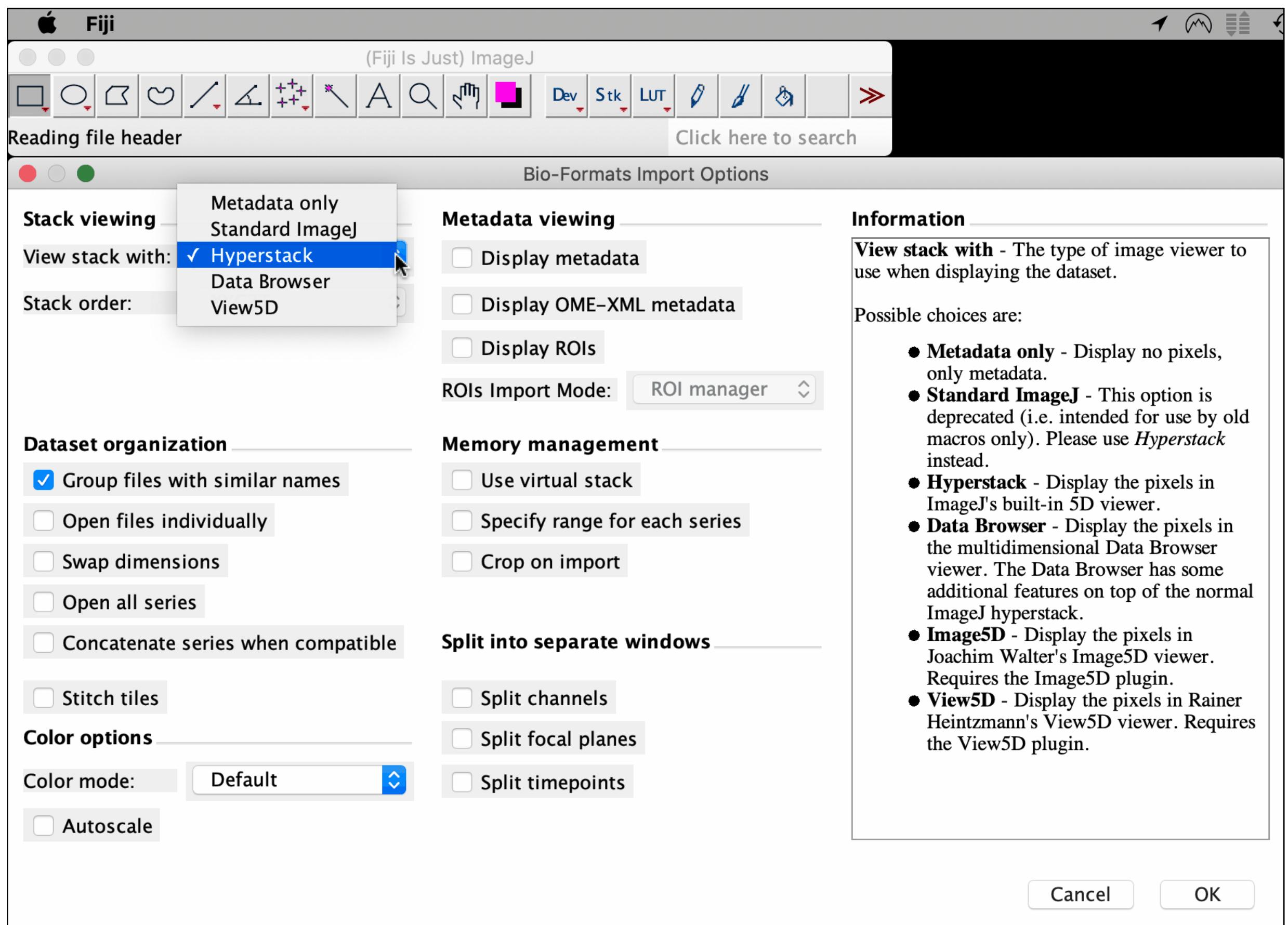
Open a file in Fiji

Drag and Drop the file you want to open onto the Status Bar.



Bio-Format Plugin

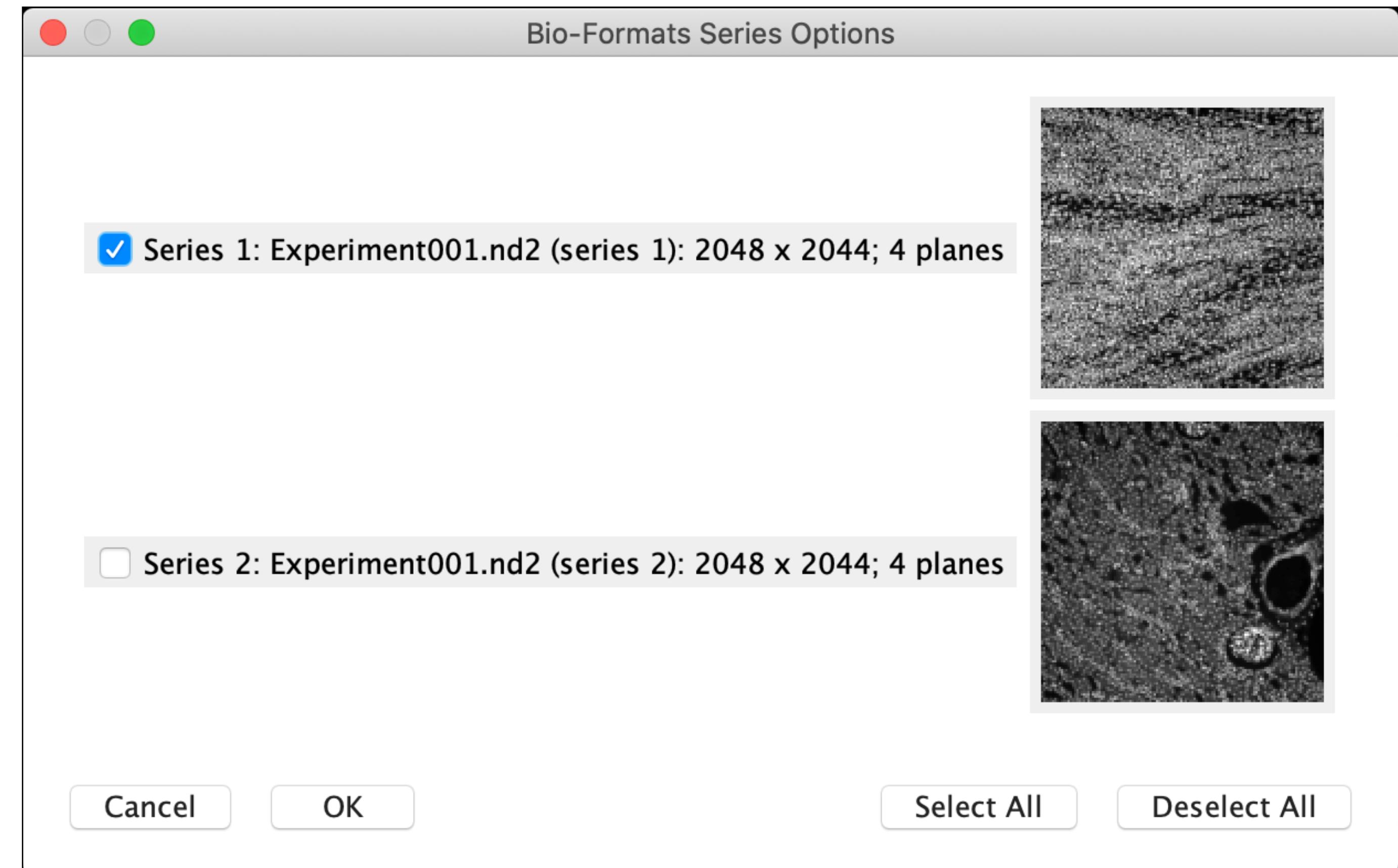
import a file - options



Import data from many life sciences file formats (e.g. @NIC .nd2 format)

Bio-Format Plugin

import a file - options

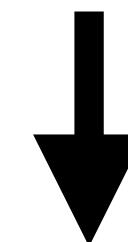


Bio-Format Plugin

import a file

Drag and Drop

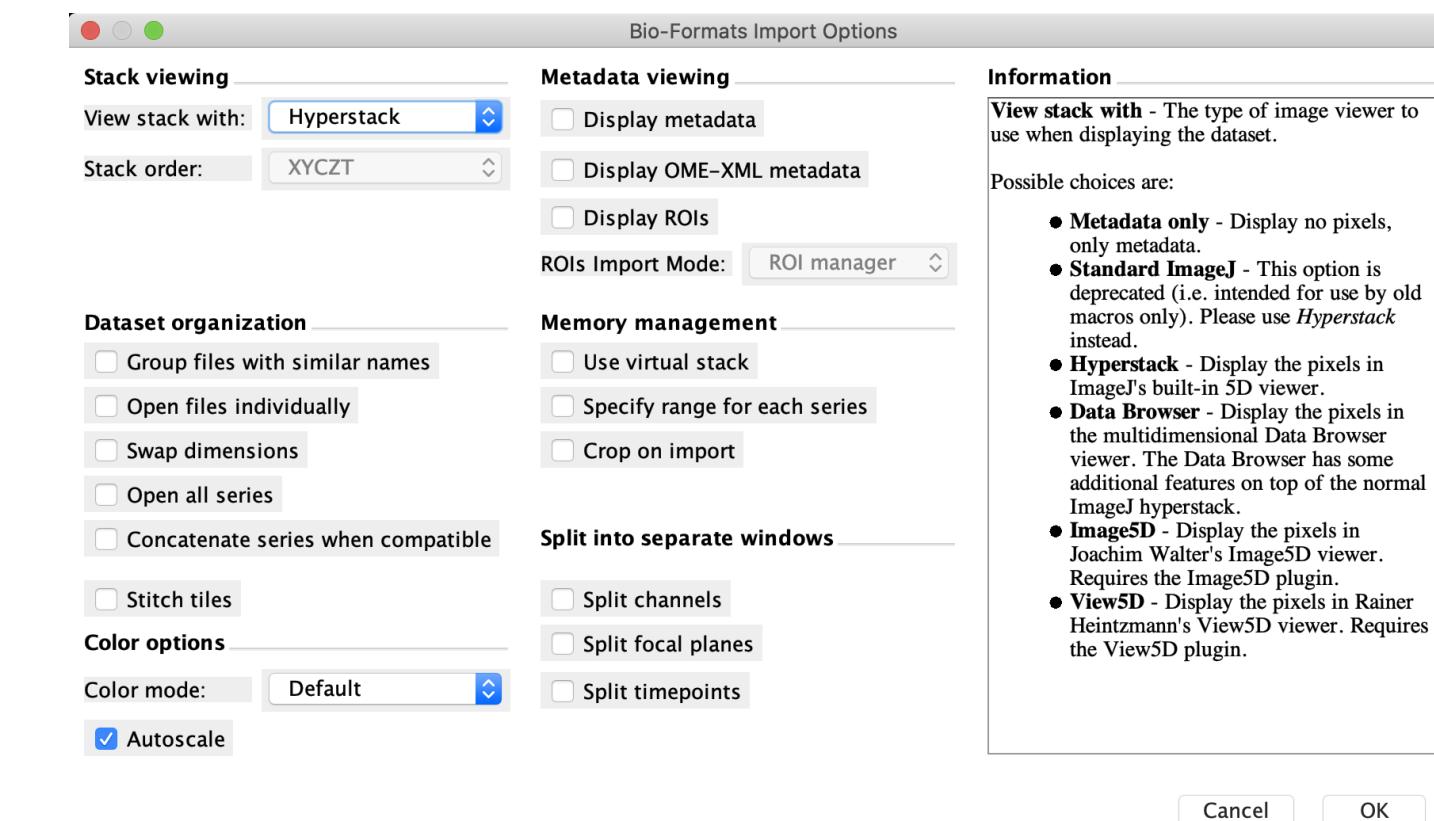
If you have a **tiff** file, “**Drag and Drop**”
does **not open Bio-Format Importer**.



File > Import > Bio-Formats

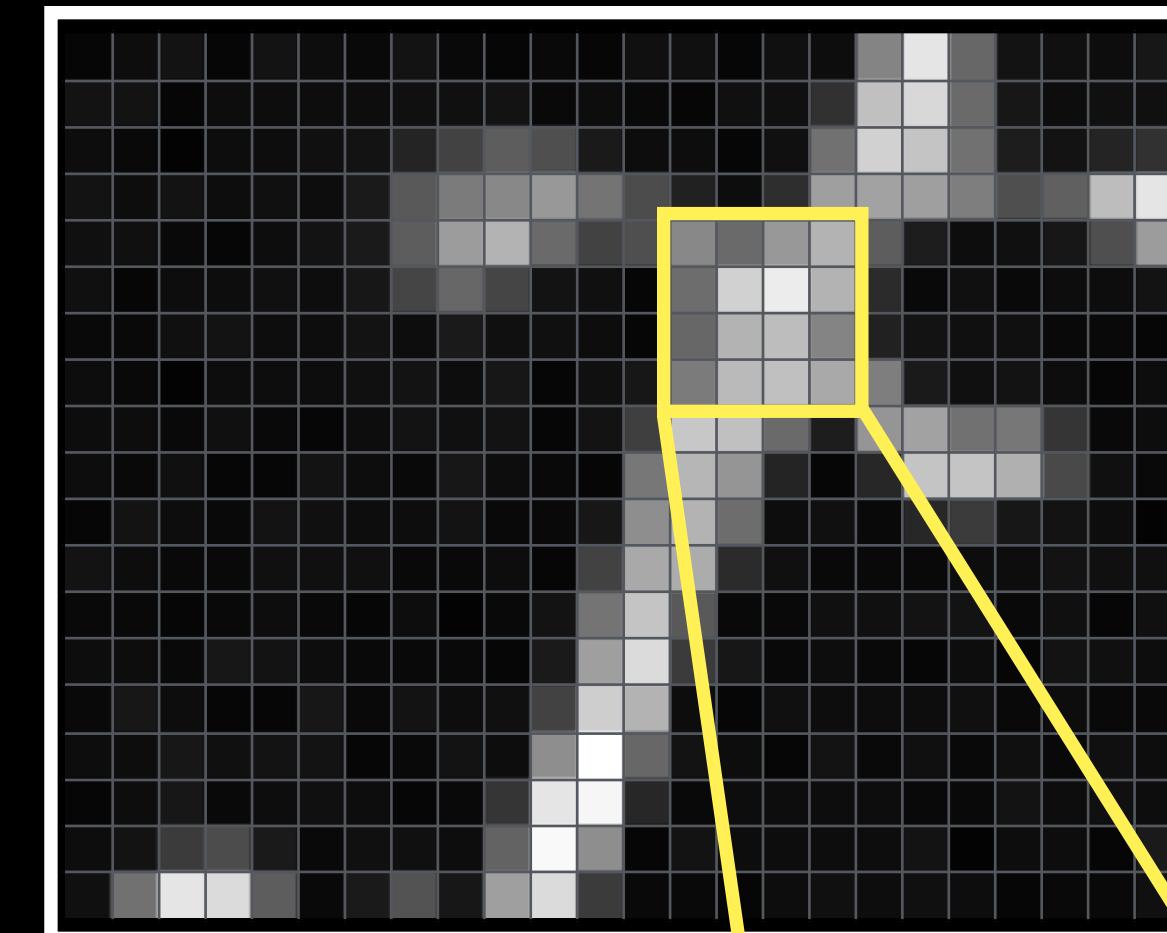
or

Plugins > Bio-Formats > Bio-
Formats Importer



what is an image?

A digital image is a matrix of numbers!



=

6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9
19	19	6	13	13	13	13	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	36	49	36	33
19	13	19	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	96	189	229	226	212
16	16	9	6	13	19	26	93	156	179	106	66	79	136	106	152	179	93	29	13	16	23	79	156	123	49
16	6	13	13	16	13	23	69	103	69	19	16	6	109	209	236	179	43	9	16	9	13	13	19	13	13
9	9	16	19	13	13	19	13	26	16	16	13	6	103	179	189	132	33	19	16	16	9	9	6	6	6
13	9	4	13	13	13	16	19	13	23	6	16	23	123	186	192	169	126	26	16	19	13	6	13	16	13
13	13	9	16	9	6	13	19	16	19	6	19	63	199	192	106	29	149	162	113	119	53	9	13	6	13
13	9	16	6	6	19	13	9	23	13	9	6	119	182	149	36	6	39	196	196	176	73	16	9	9	9
6	19	13	9	19	16	13	13	19	9	9	23	142	179	109	13	16	9	39	59	23	19	13	4	9	9
19	13	9	9	16	16	16	9	9	13	6	66	169	172	43	16	9	9	9	13	13	19	16	16	16	9
9	9	6	9	13	9	6	13	4	9	19	116	196	89	9	9	16	16	19	19	9	16	6	16	9	9
13	13	9	23	19	13	9	9	9	6	26	159	219	59	23	9	13	9	6	13	6	19	16	13	16	13
9	23	13	6	6	23	9	19	13	16	66	206	179	13	6	16	13	13	13	16	9	13	9	9	16	13
13	13	23	16	19	19	6	9	19	13	142	255	103	19	13	6	19	9	16	9	16	9	16	13	23	9
6	13	23	9	13	16	13	6	9	53	229	246	39	9	13	13	13	13	9	9	19	13	16	13	13	13
13	19	59	76	26	9	16	16	13	99	249	142	6	19	13	13	13	13	19	4	13	13	6	26	9	13
16	113	229	219	93	9	26	83	23	159	219	59	9	9	6	13	16	13	16	13	6	9	9	16	23	9

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136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

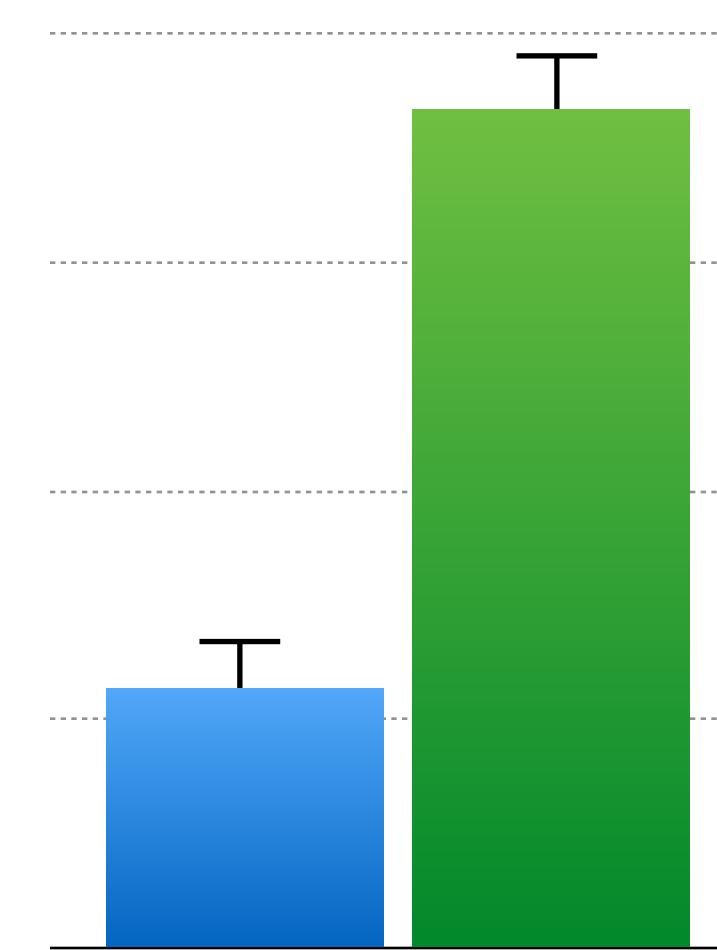
Pixel = Picture Element

Images in publications and presentations
should be used to **communicate** a finding...
not **be** the finding

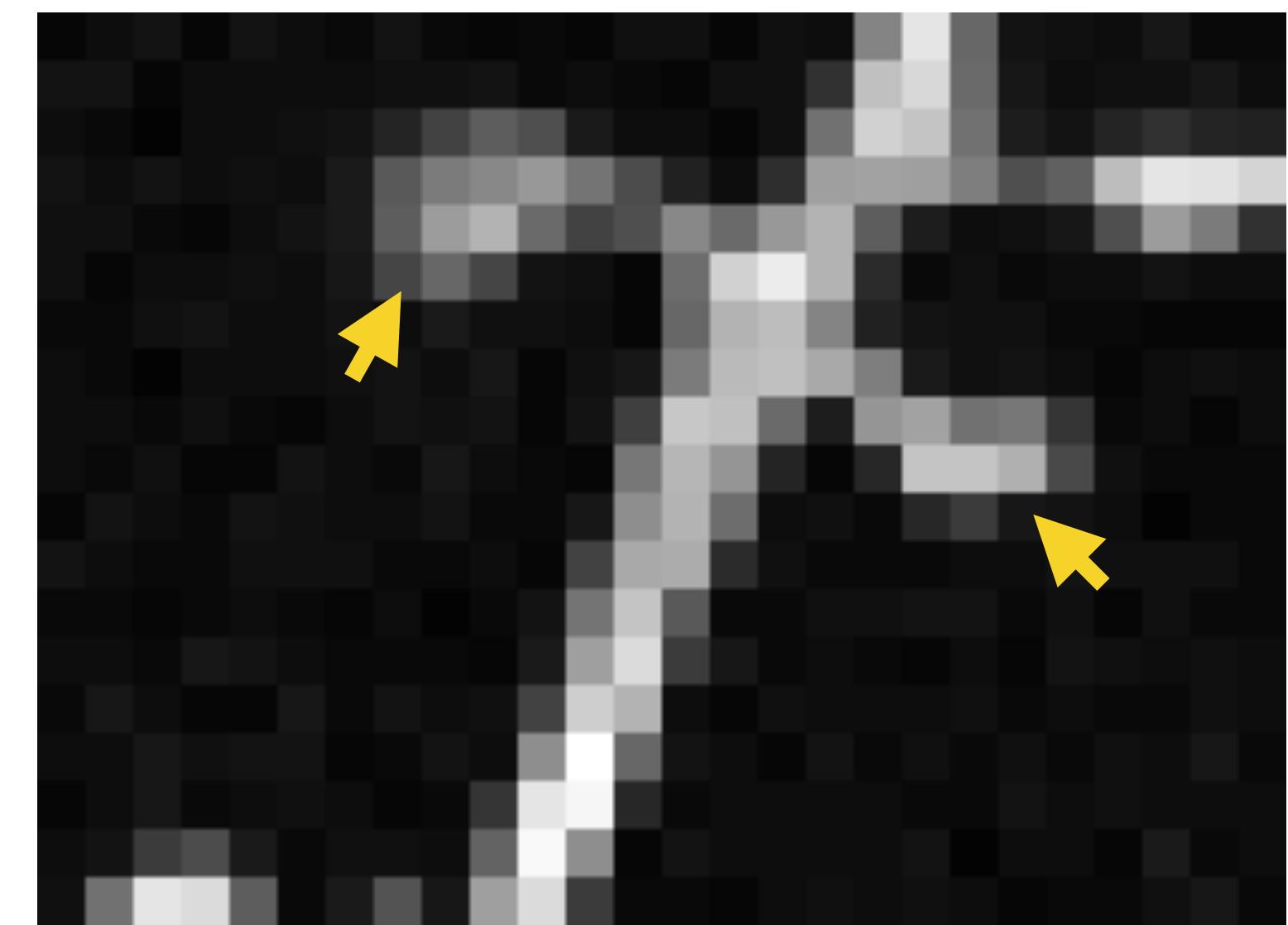
this is your **data**

6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9	
19	19	6	13	13	13	13	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13	
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	16	49	36	33	
19	13	19	13	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	96	189	229	226	212
16	16	9	6	13	19	26	93	156	179	106	66	79	136	106	152	179	93	29	13	16	23	79	156	123	49	
16	6	13	13	16	13	23	69	103	69	19	16	6	109	209	236	179	43	9	16	9	13	13	19	13	13	
9	9	16	19	13	13	19	13	26	16	16	13	6	103	179	189	132	33	19	16	16	9	9	6	6	6	
13	9	4	13	13	13	16	19	13	23	6	16	23	123	186	192	169	126	26	16	19	13	6	13	16	13	
13	13	9	16	9	6	13	19	16	19	6	19	63	199	192	106	29	149	162	113	119	53	9	13	6	13	
13	9	16	6	6	19	13	9	23	13	9	6	119	182	149	36	6	39	196	196	176	73	16	9	9	9	
6	19	13	9	19	16	13	13	19	9	9	23	142	179	109	13	16	9	39	59	23	19	13	4	9	9	
19	13	9	9	16	16	16	9	9	13	6	66	169	172	43	16	9	9	9	13	13	19	16	16	16	9	
9	9	6	9	13	9	6	13	4	9	19	116	196	89	9	9	16	16	19	19	9	16	6	16	9	9	
13	13	9	23	19	13	9	9	6	26	159	219	59	23	9	13	9	6	13	6	19	16	13	16	13	13	
9	23	13	6	6	23	9	19	13	16	66	206	179	13	6	16	13	13	16	9	13	9	9	16	13	13	
13	13	23	16	19	19	6	9	19	13	142	255	103	19	13	6	19	9	16	9	16	9	16	13	23	9	
6	13	23	9	13	16	13	6	9	53	229	246	39	9	13	13	13	13	9	9	19	13	16	13	13	13	
13	19	59	76	26	9	16	16	13	99	249	142	6	19	13	13	13	13	19	4	13	13	6	26	9	13	
16	113	229	219	93	9	26	83	23	159	219	59	9	9	6	13	16	13	6	9	9	16	23	9			

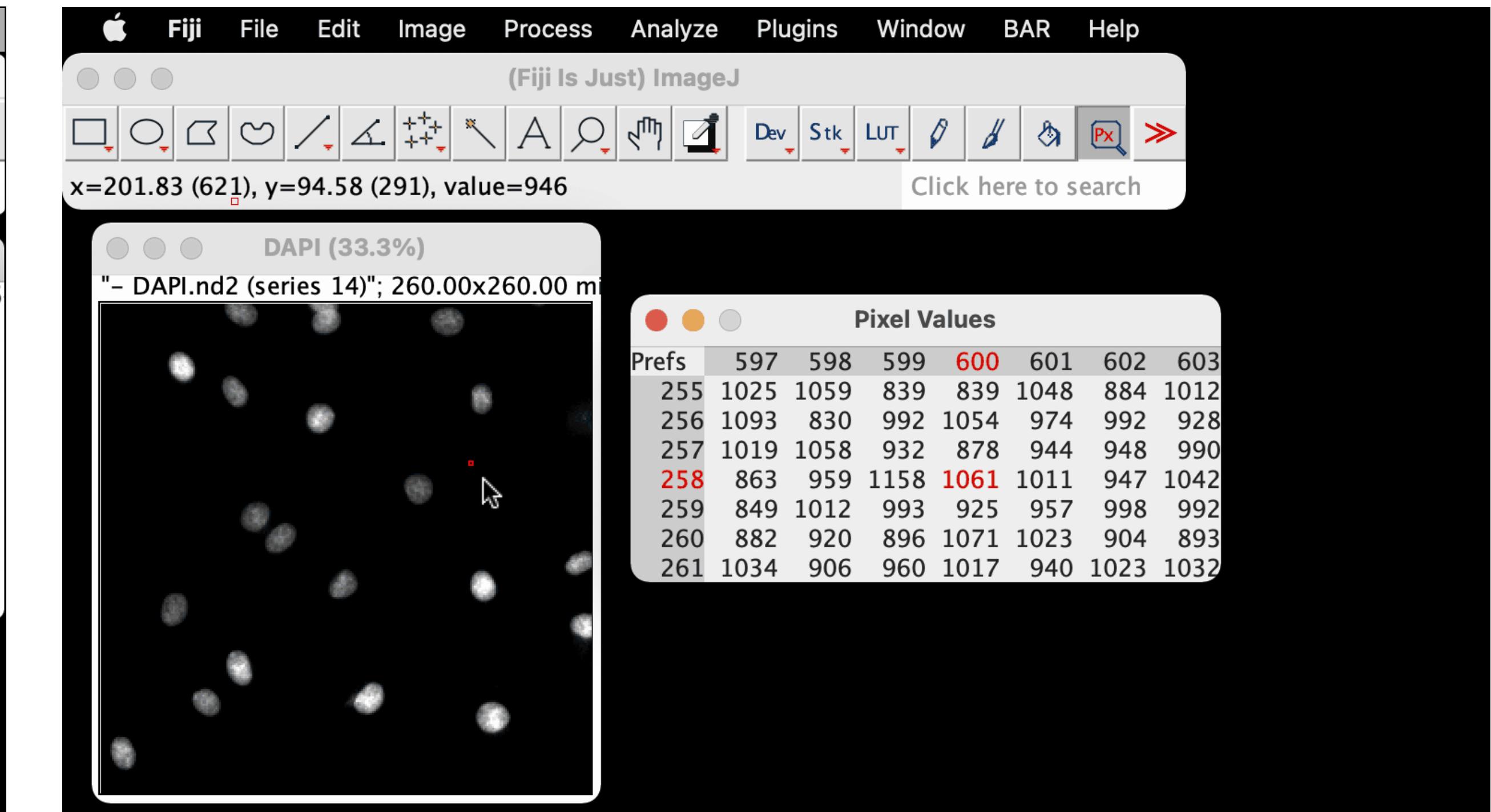
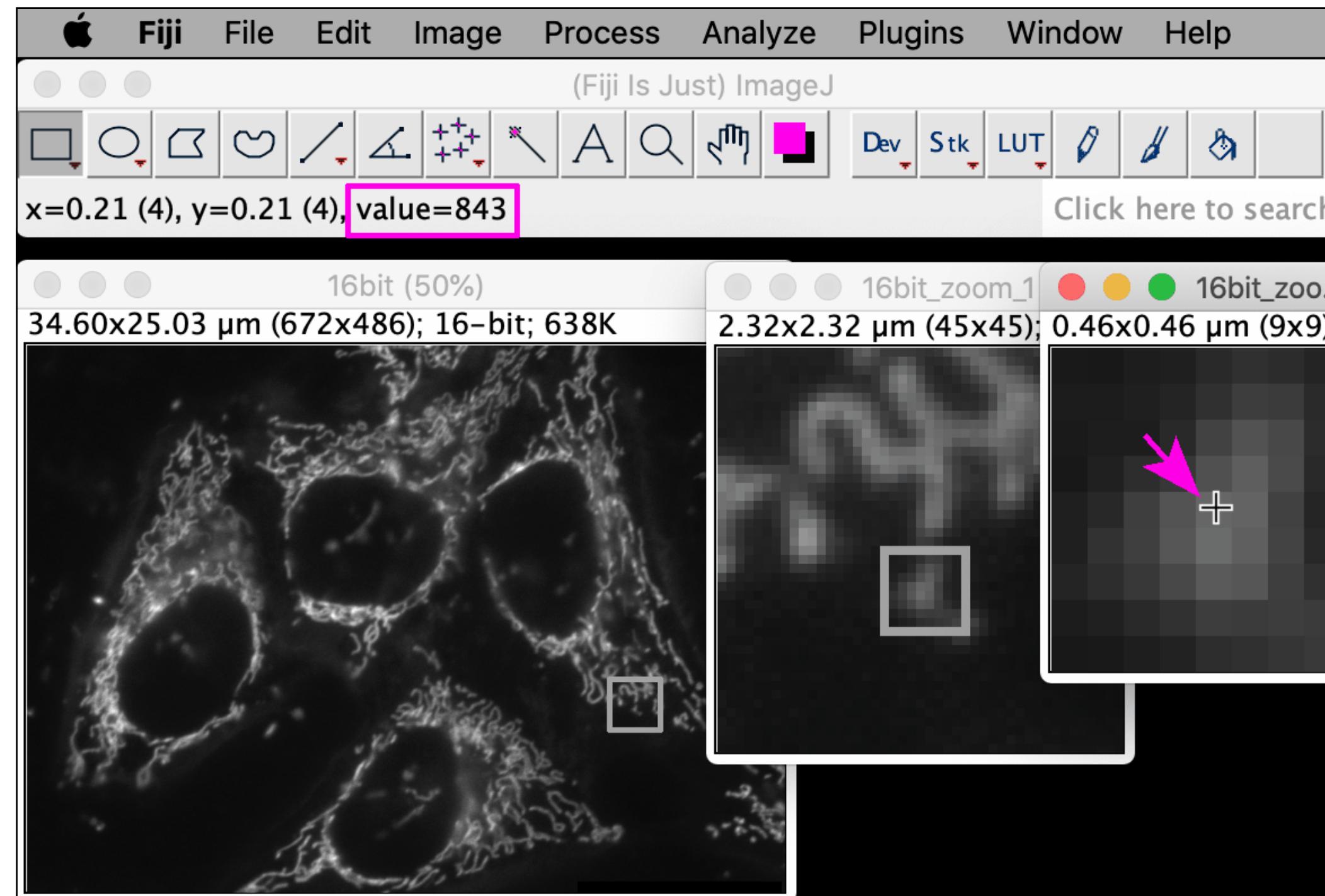
this is your **result**



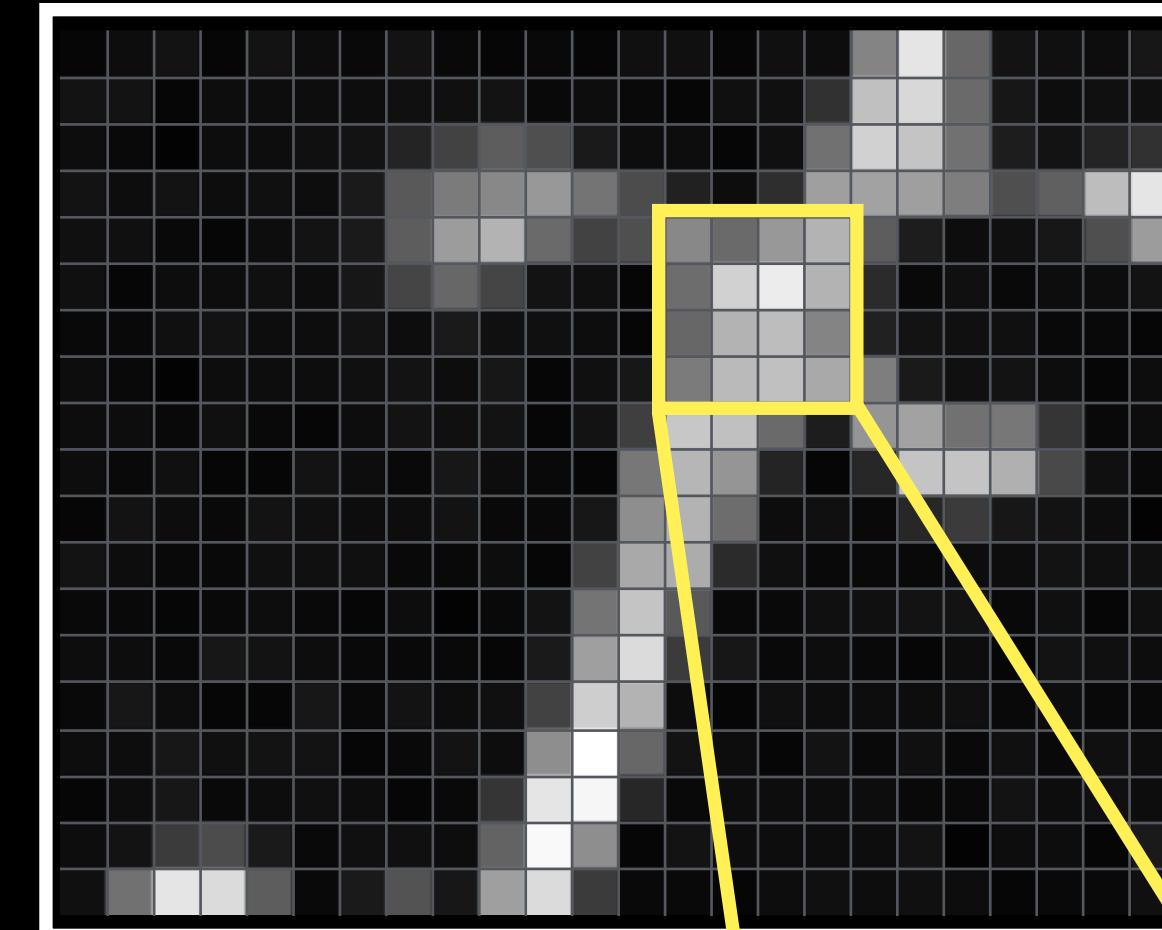
this just helps to
communicate the result



Individual Pixel Values in Fiji



A digital image is a matrix of numbers!



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6	13	19	6	19	13	9	19	9	6	9	6	16	16	6	16	13	132	229	103	19	16	13	23	9	9
19	19	6	13	13	13	13	16	16	19	9	13	9	6	16	16	49	192	216	106	23	13	16	16	23	13
13	9	4	13	13	16	19	36	66	93	79	26	13	13	6	16	113	209	196	113	29	19	36	49	36	33
19	13	19	13	16	13	26	89	123	136	152	116	76	33	13	46	159	162	159	126	79	96	189	229	226	212
16	16	9	6	13	19	26	93	156	179	106	66	79	136	106	152	179	93	29	13	16	23	79	156	123	49
16	6	13	13	16	13	23	69	103	69	19	16	6	109	209	236	179	43	9	16	9	13	13	19	13	13
9	9	16	19	13	13	19	13	26	16	16	13	6	103	179	189	132	33	19	16	16	9	9	6	6	6
13	9	4	13	13	13	16	19	13	23	6	16	23	123	186	192	169	126	26	16	19	13	6	13	16	13
13	13	9	16	9	6	13	19	16	19	6	19	63	199	192	106	29	149	162	113	119	53	9	13	6	13
13	9	16	6	6	19	13	9	23	13	9	6	119	182	149	36	6	39	196	196	176	73	16	9	9	9
6	19	13	9	19	16	13	13	19	9	9	23	142	179	109	13	16	9	39	59	23	19	13	4	9	9
19	13	9	9	16	16	16	9	9	13	6	66	169	172	43	16	9	9	9	13	13	19	16	16	16	9
9	9	6	9	13	9	6	13	4	9	19	116	196	89	9	9	16	16	19	19	9	16	6	16	9	9
13	13	9	23	19	13	9	9	9	6	26	159	219	59	23	9	13	9	6	13	6	19	16	13	16	13
9	23	13	6	6	23	9	19	13	16	66	206	179	13	6	16	13	13	13	16	9	13	9	9	16	13
13	13	23	16	19	19	6	9	19	13	142	255	103	19	13	6	19	9	16	9	16	9	16	13	23	9
6	13	23	9	13	16	13	6	9	53	229	246	39	9	13	13	13	13	9	9	19	13	16	13	13	13
13	19	59	76	26	9	16	16	13	99	249	142	6	19	13	13	13	13	19	4	13	13	6	26	9	13
16	113	229	219	93	9	26	83	23	159	219	59	9	9	6	13	16	13	16	13	6	9	9	16	23	9

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136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

Where do these numbers come from?

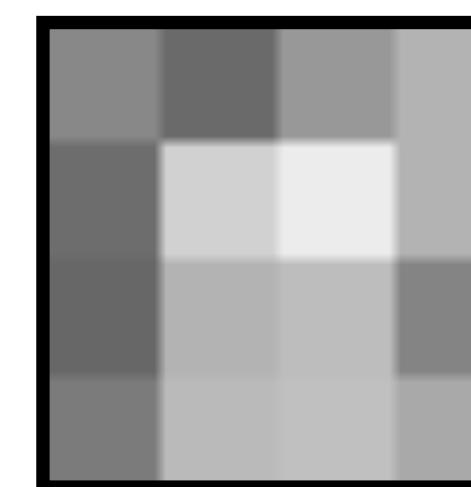
Bit depth

Detectors in Fluorescence Microscopy

The detectors used in fluorescence microscopy are **monochromatic**.

Cameras or PMTs are **not able to distinguish between different wavelengths** (they just collect photons), you need **fluorescence filters** to separate your fluorophores.

The detector converts photons in digital numbers (linear relation).



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136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

Each pixel in the digital image has **one digital value** that **depends on** the **intensity** of the signal emitted by the **sample**.

Digital Values = Pixel Intensity Value

The **range** of possible **digital values** is defined by the **bit depth**.

Bit Depth

The **bit depth** defines the range of possible **digital values** that each pixel can have, usually **8, 12 or 16 bit**.

The **bit depth** is expressed in **grey values**.

bit depth of the image = bit depth of the detector

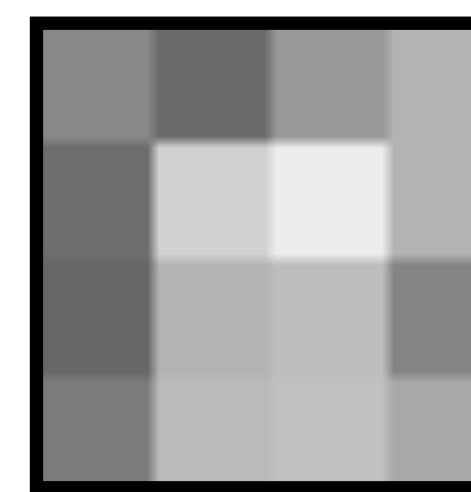
(Unless you change that during acquisition)

x bit = a range of 2^x grey values

8 bit image = **each pixel** can have 2^8 **grey values** = 256 grey values = **range 0-255**

12 bit image = **each pixel** can have 2^{12} **grey values** = 4096 grey values = **range 0-4095**

16 bit image = **each pixel** can have 2^{16} **grey values** = 65536 grey values = **range 0-65535**



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136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

Digital Value = Pixel Intensity Value = Grey Value

Image Metadata

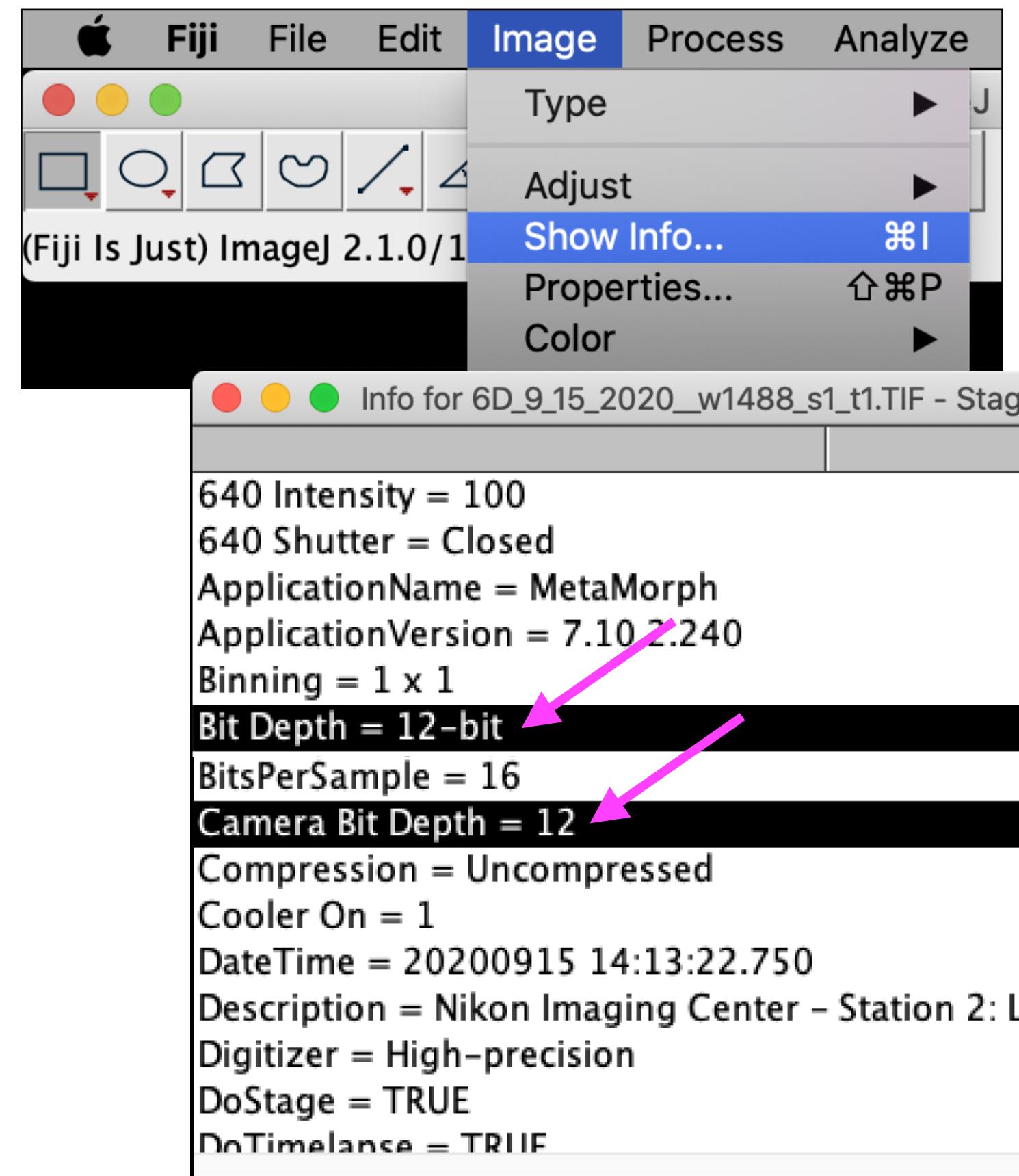
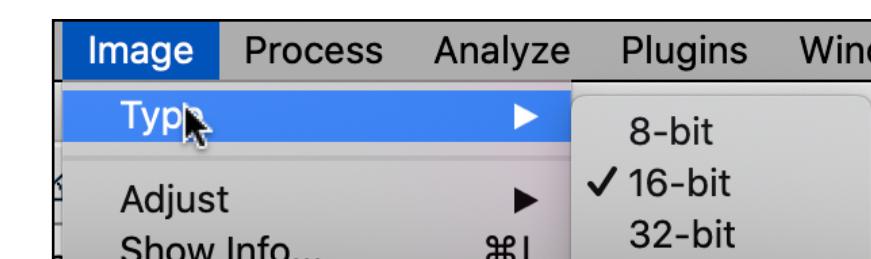
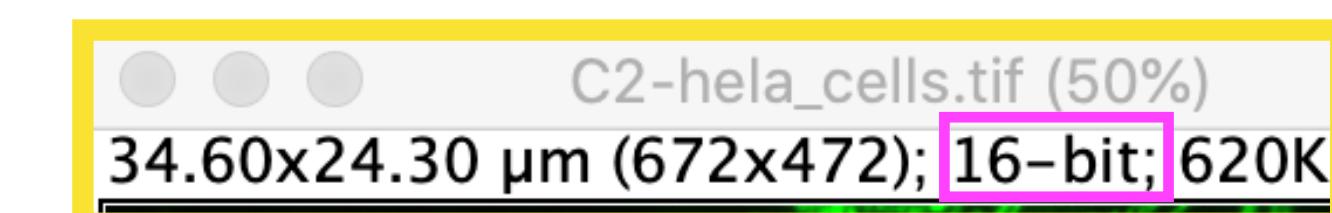


Image > Show Info...

(cmd) + i

Show the **Metadata stored with the file**

e.g. **bit depth, camera bit depth**

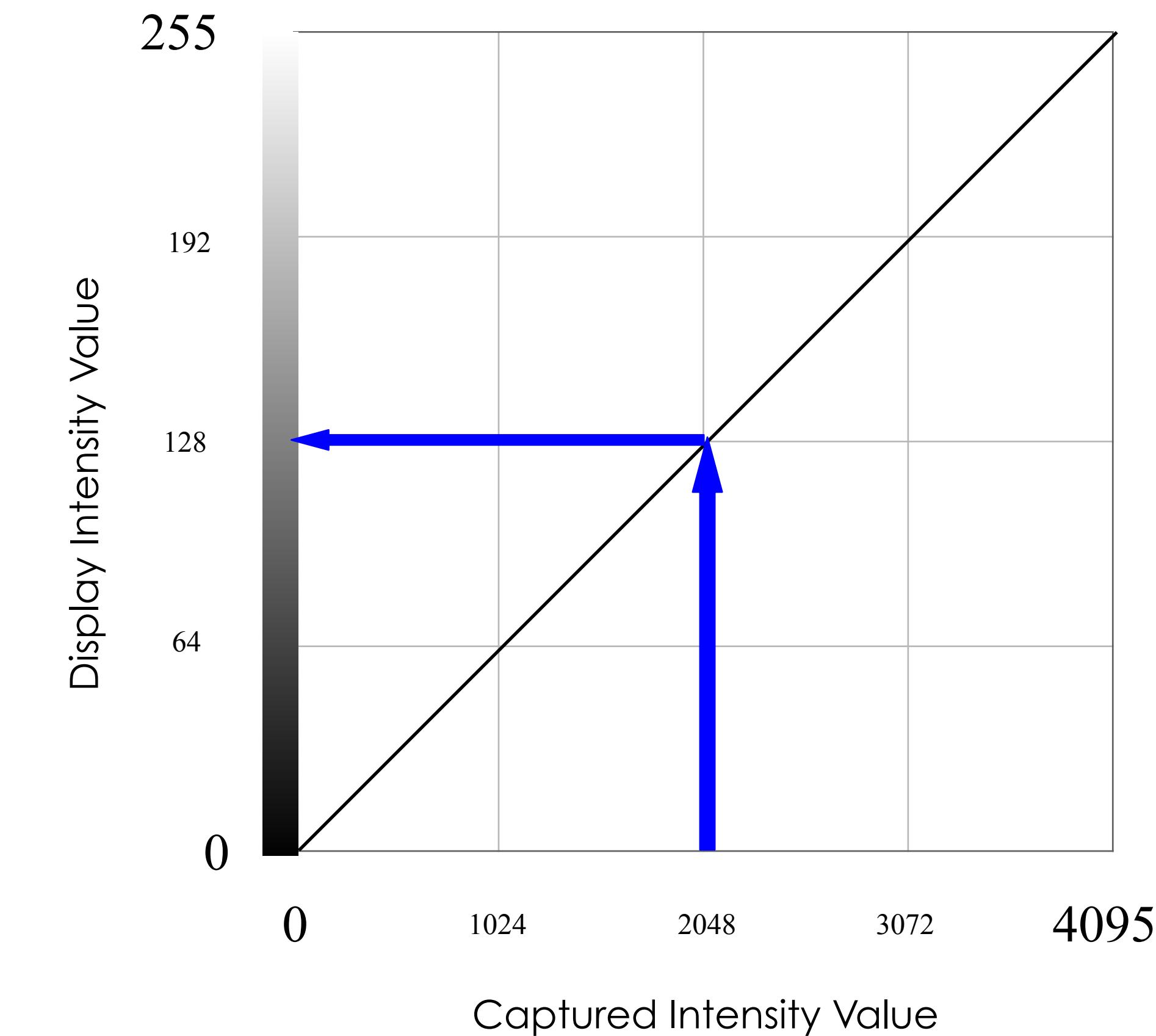
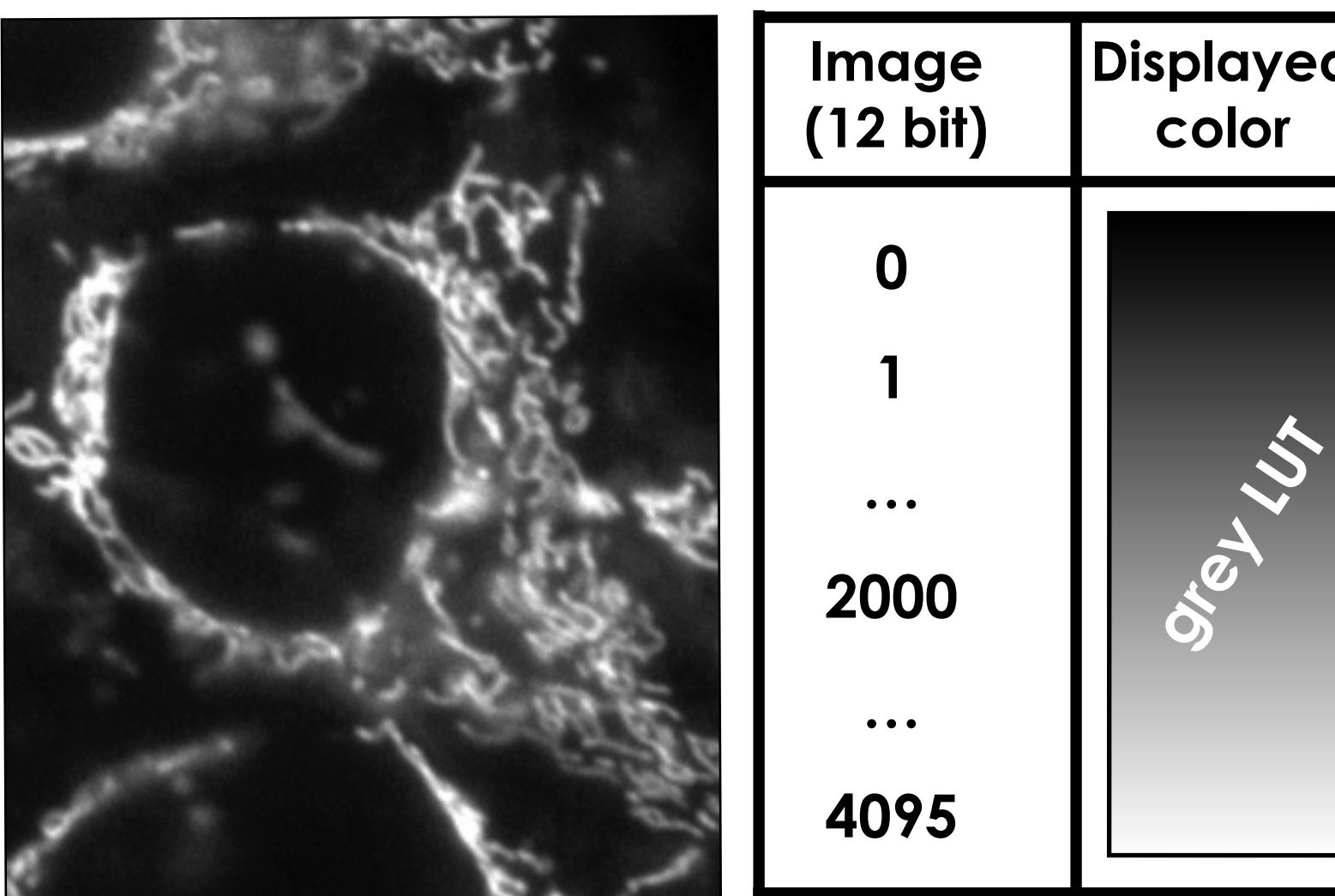
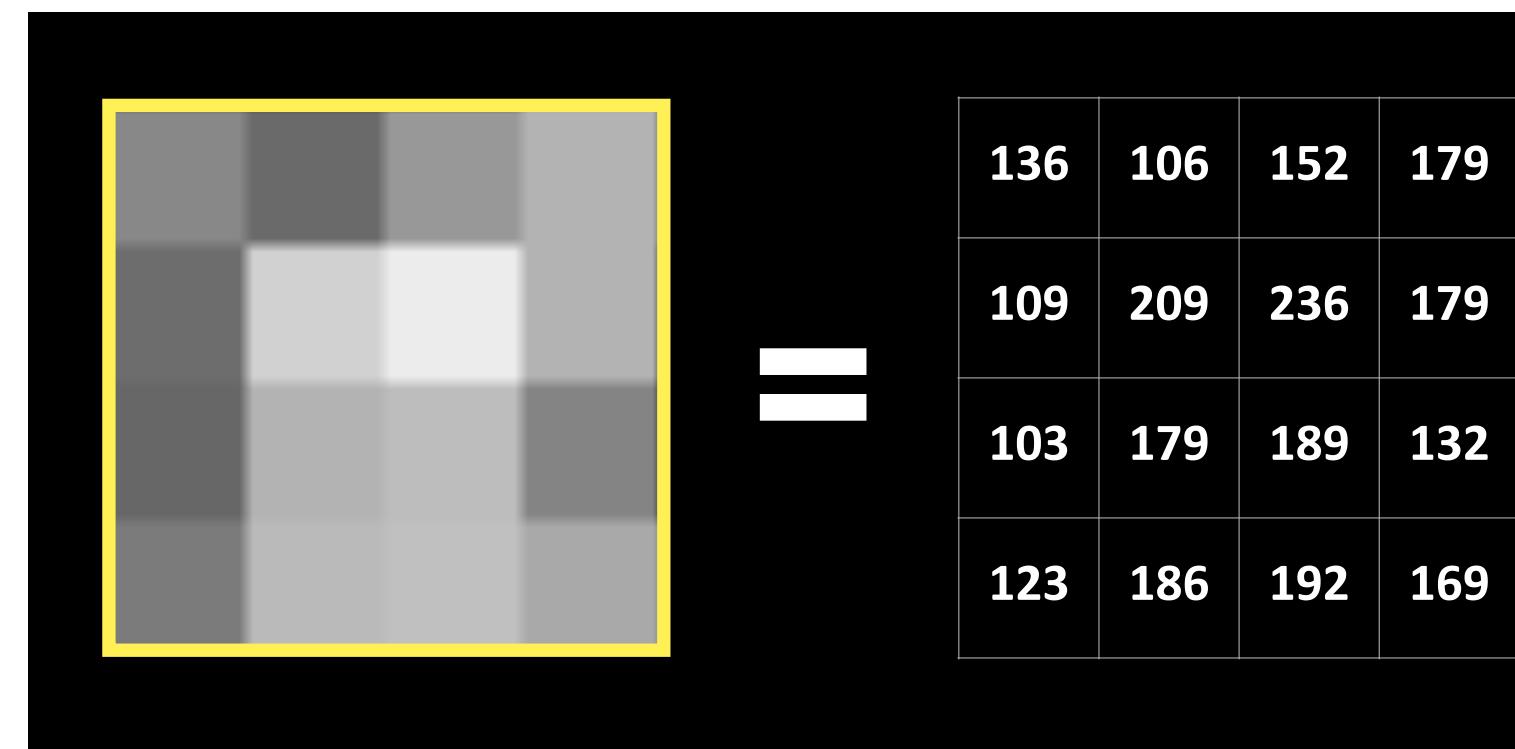


display your images

Mapping Image Intensity to Monitor Intensity (LookUp Tables)

LUT = how the grey values are displayed

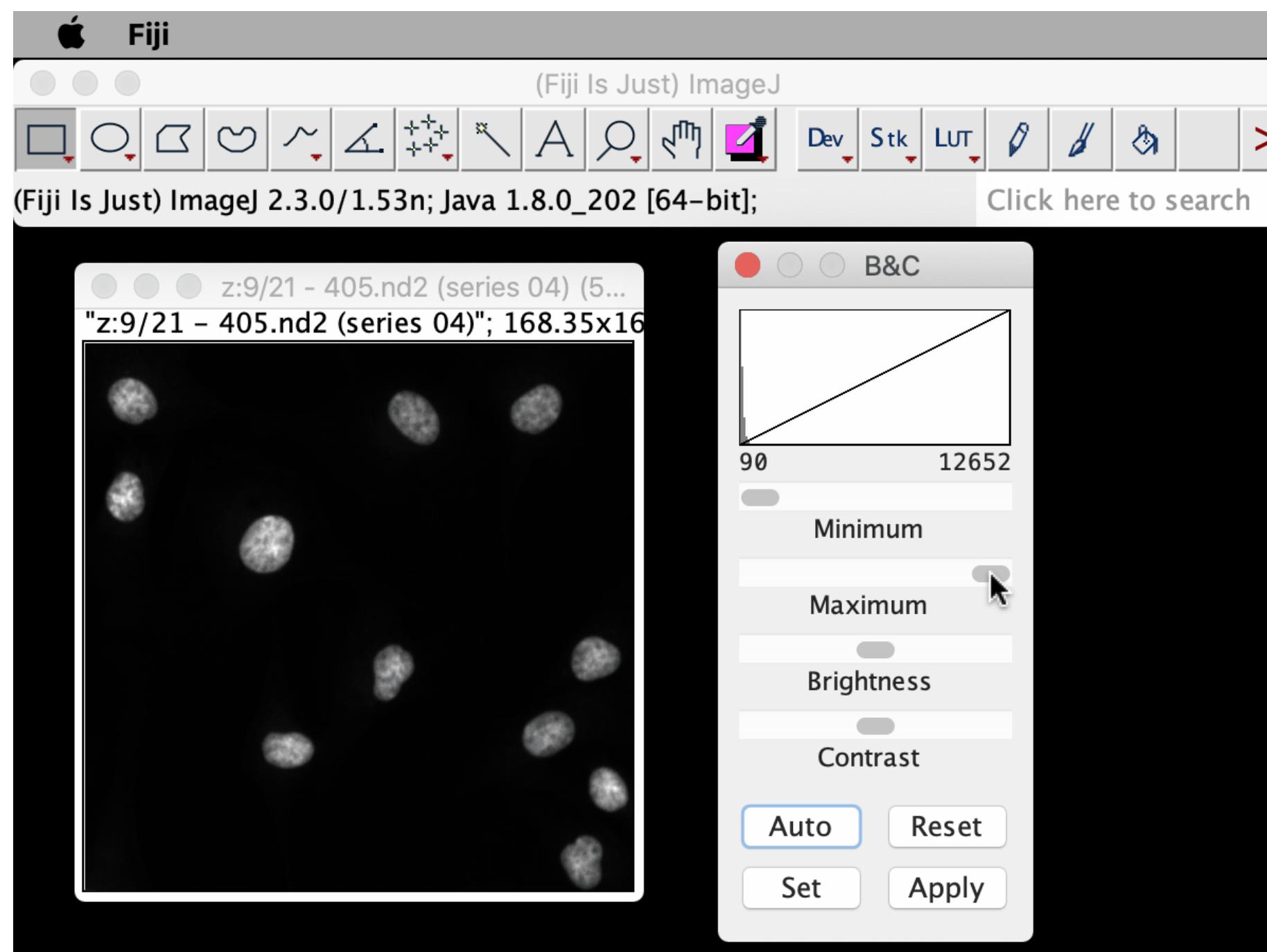
LUTs do not change the pixel values



Display a file: Brightness & Contrast

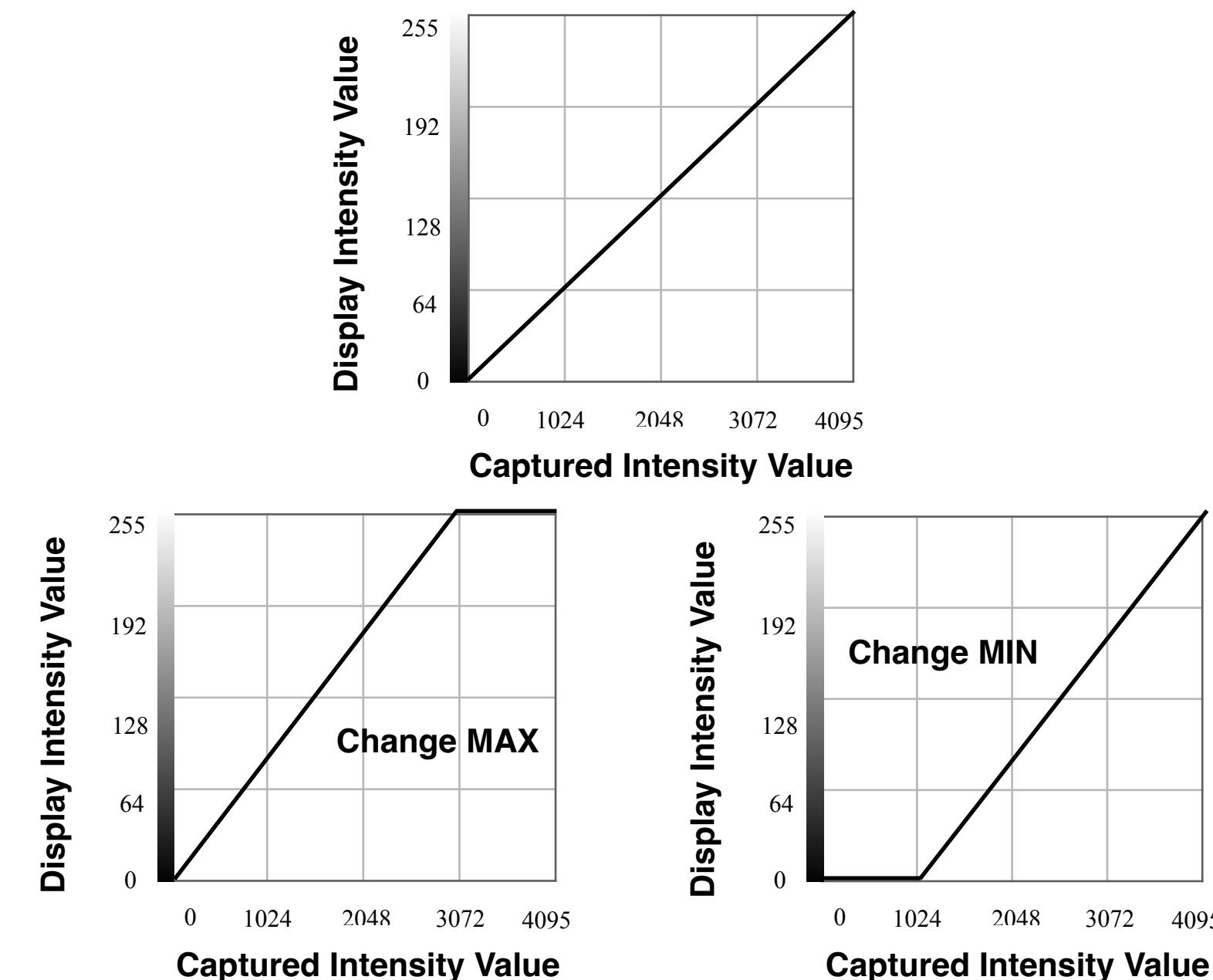
Image > Adjust > Brightness/Contrast

Interactively change the displayed brightness and contrast of the active image.



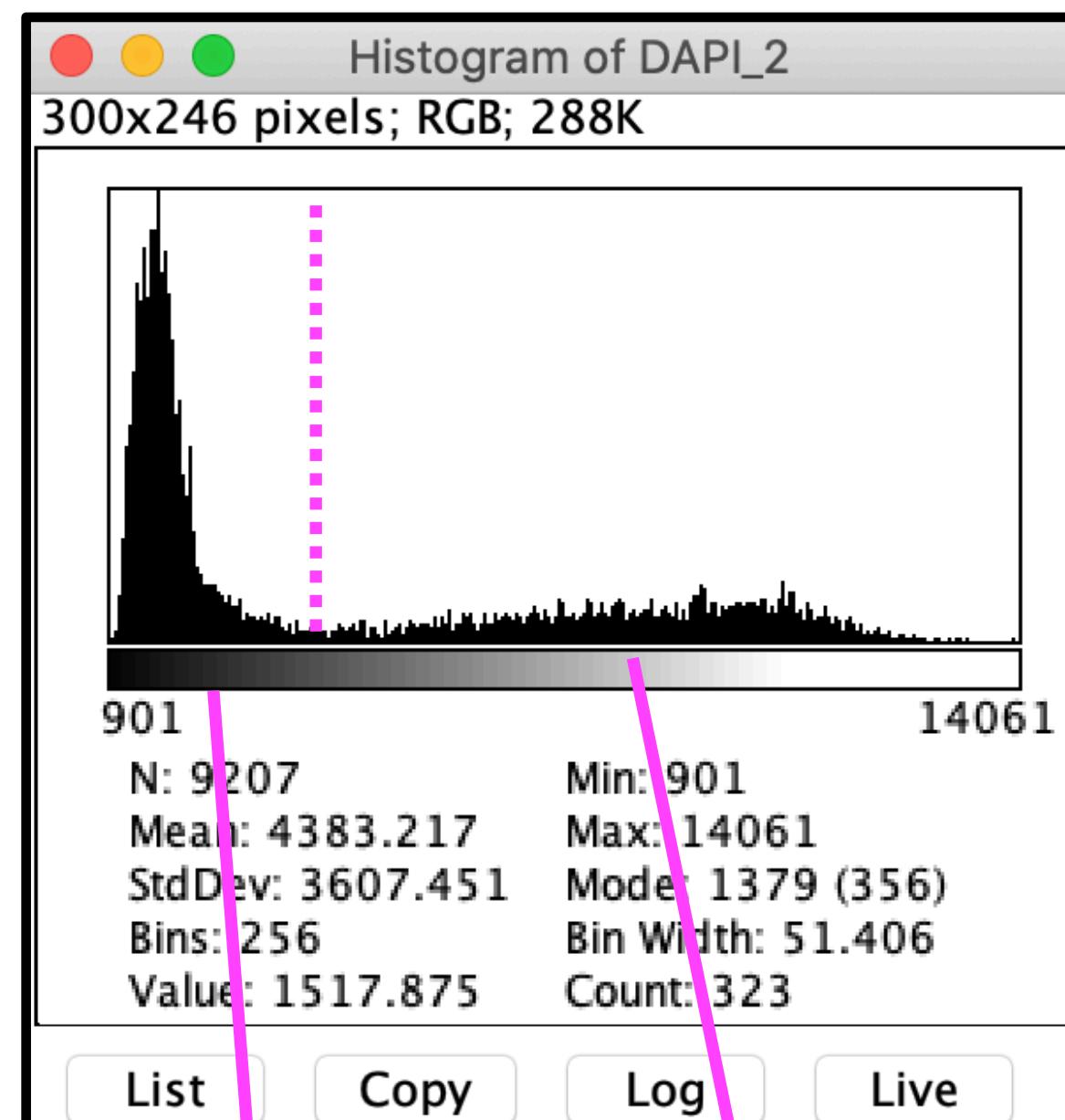
(cmd) + shift + c

*You are **NOT changing the pixels values, you are just changing how the image is displayed (unless you click on the “Apply” button).**



Histogram

Analyze > Histogram

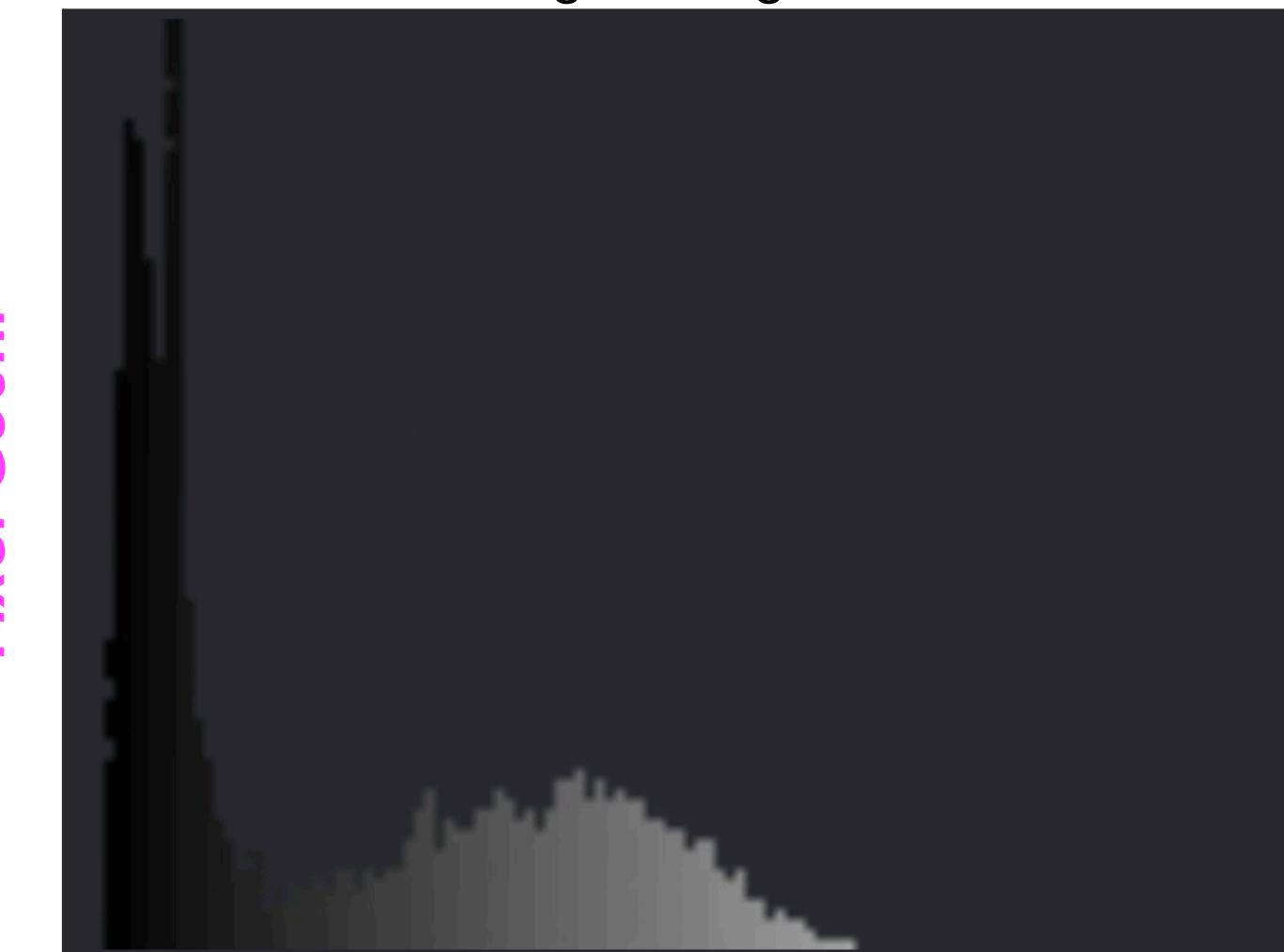


background

foreground

h

jaehyuk-lee: <https://jaehyuk-lee.com/animated-image-histogram/>

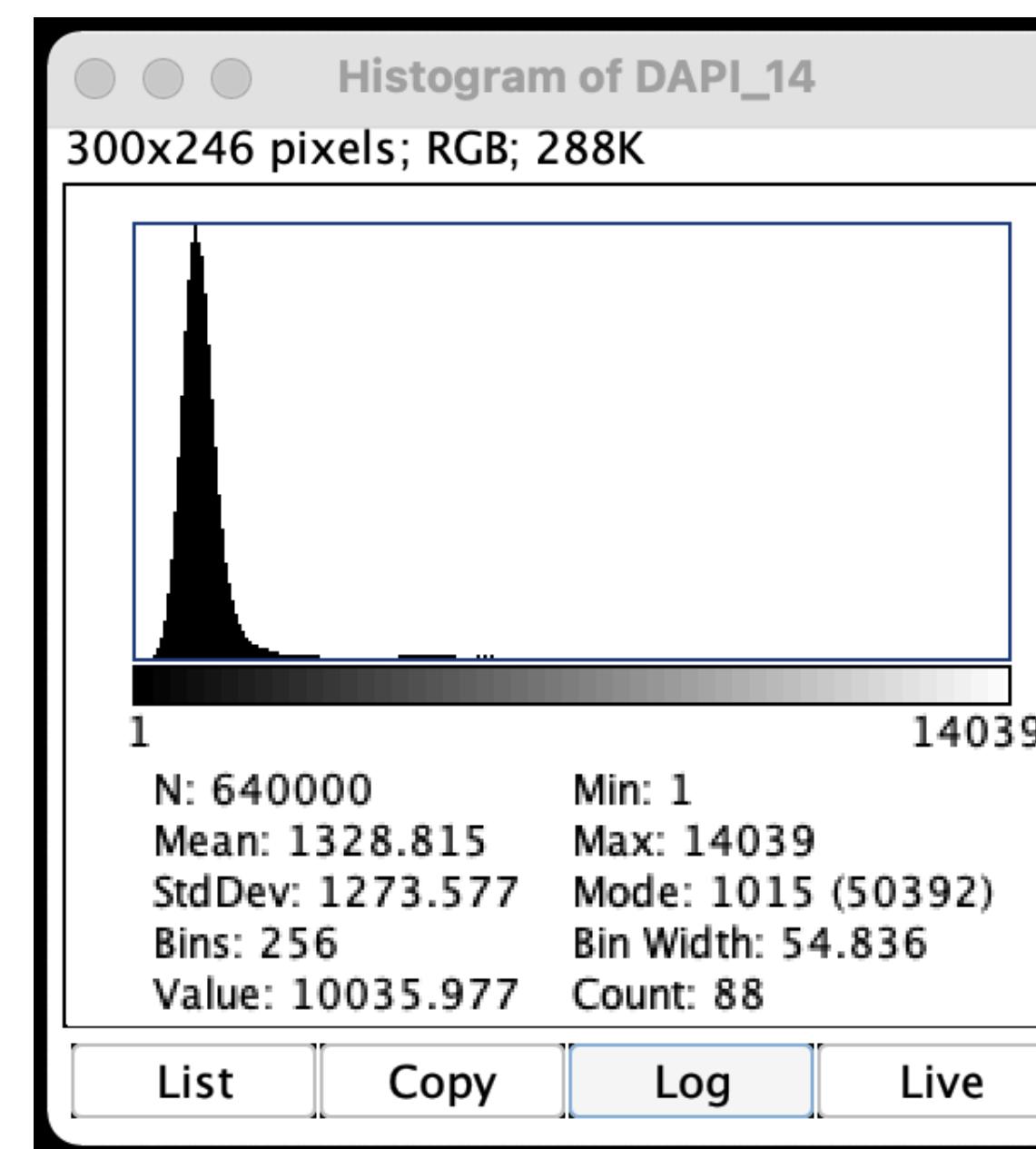


Pixel Values

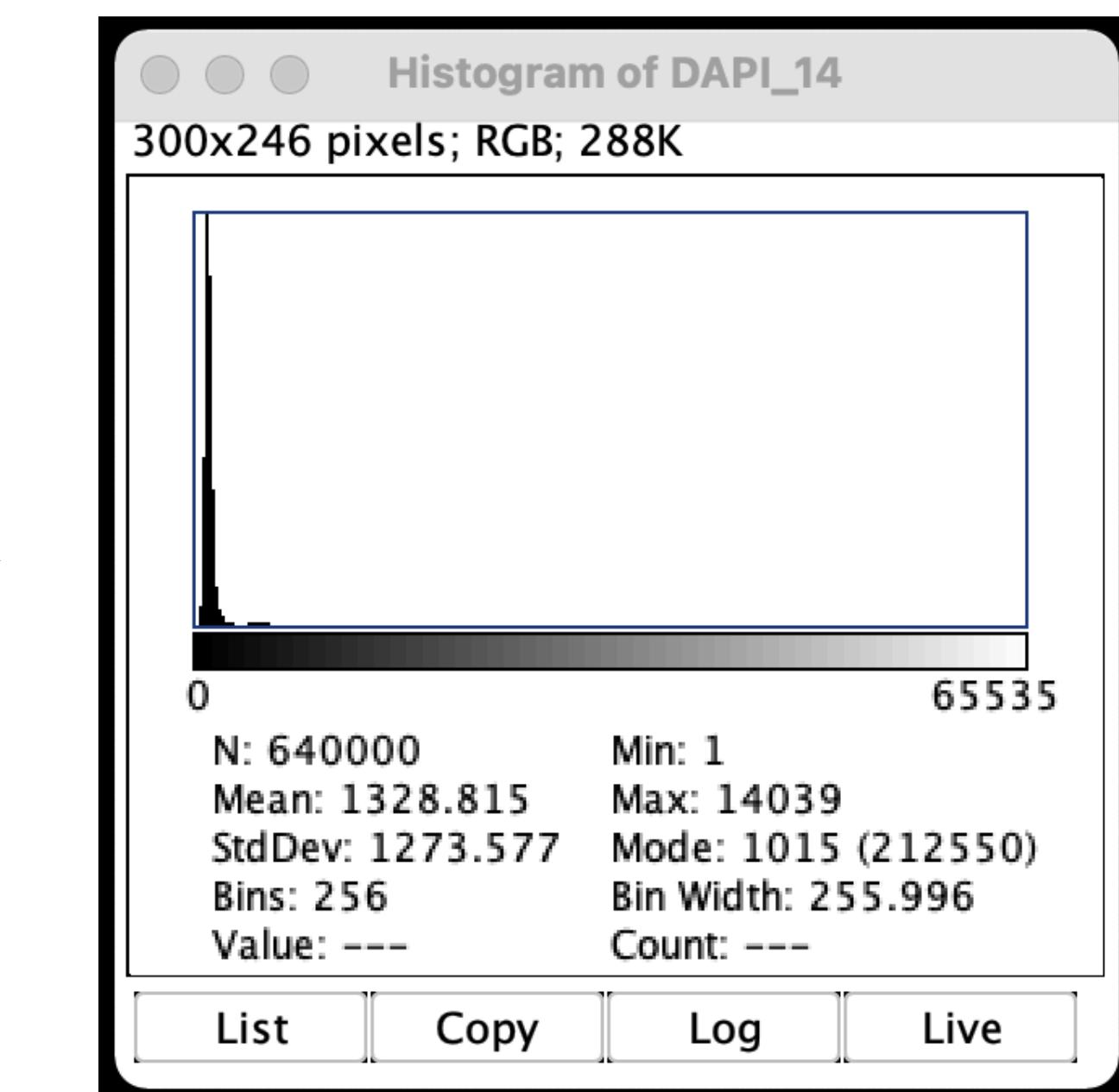
Histogram

Analyze > Histogram

h



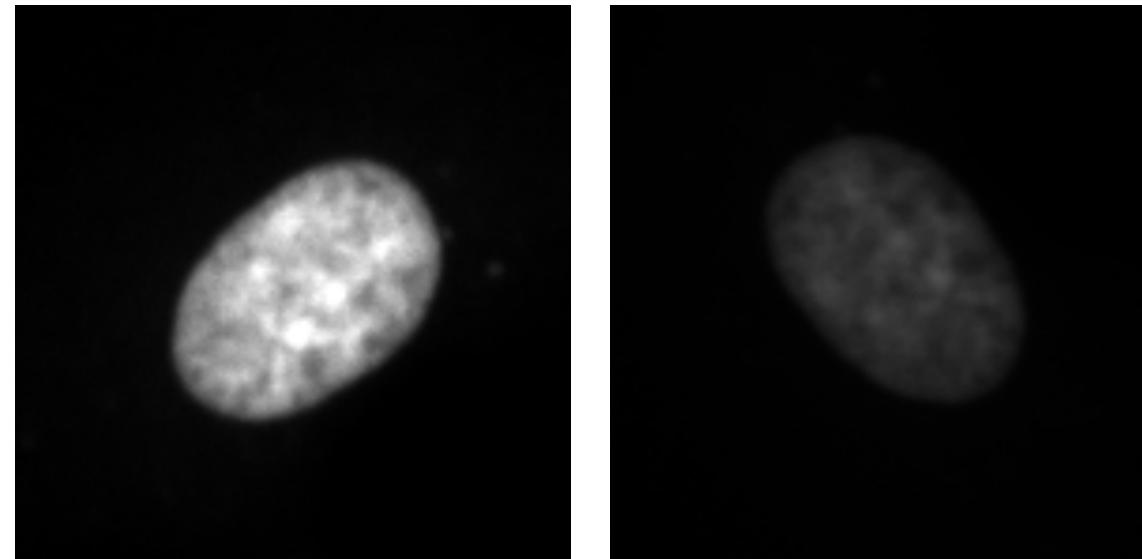
Fiji auto-adjust the range (default option)



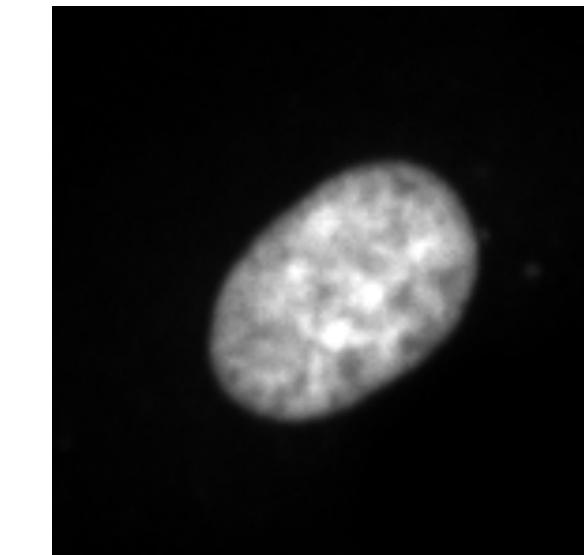
Bit depth

Display a file: Brightness & Contrast

Which image has more fluorescence?



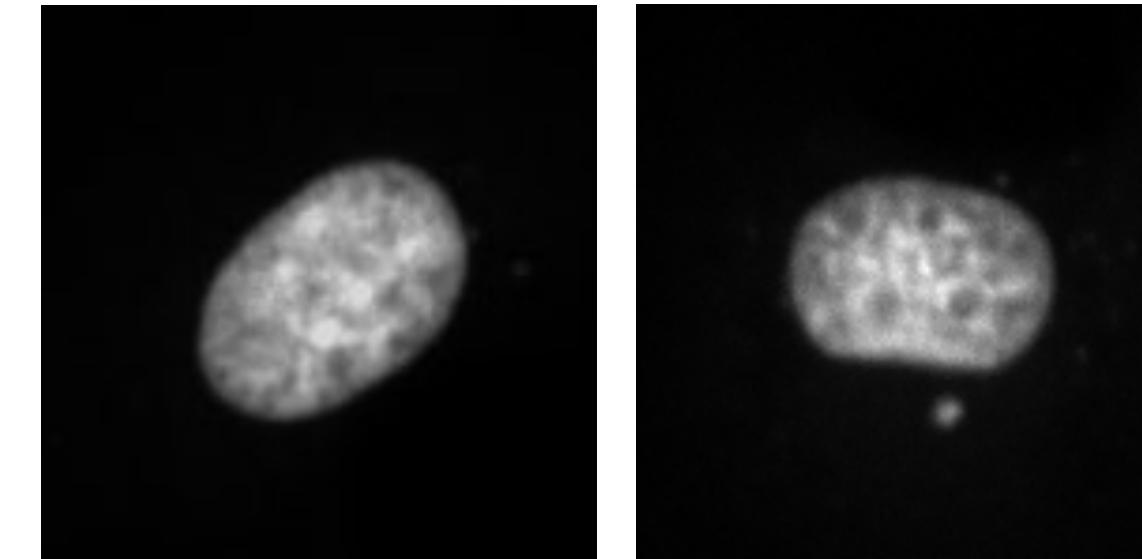
Mean:	4803	4803
Display range:	188- 16828	188- 45514



Mean:	4803	4803
Display range:	188- 16828	188- 16828

Display a file: Brightness & Contrast

Which image has more fluorescence?



Mean:	4803	2074
Display range:	188- 19540	112- 7768

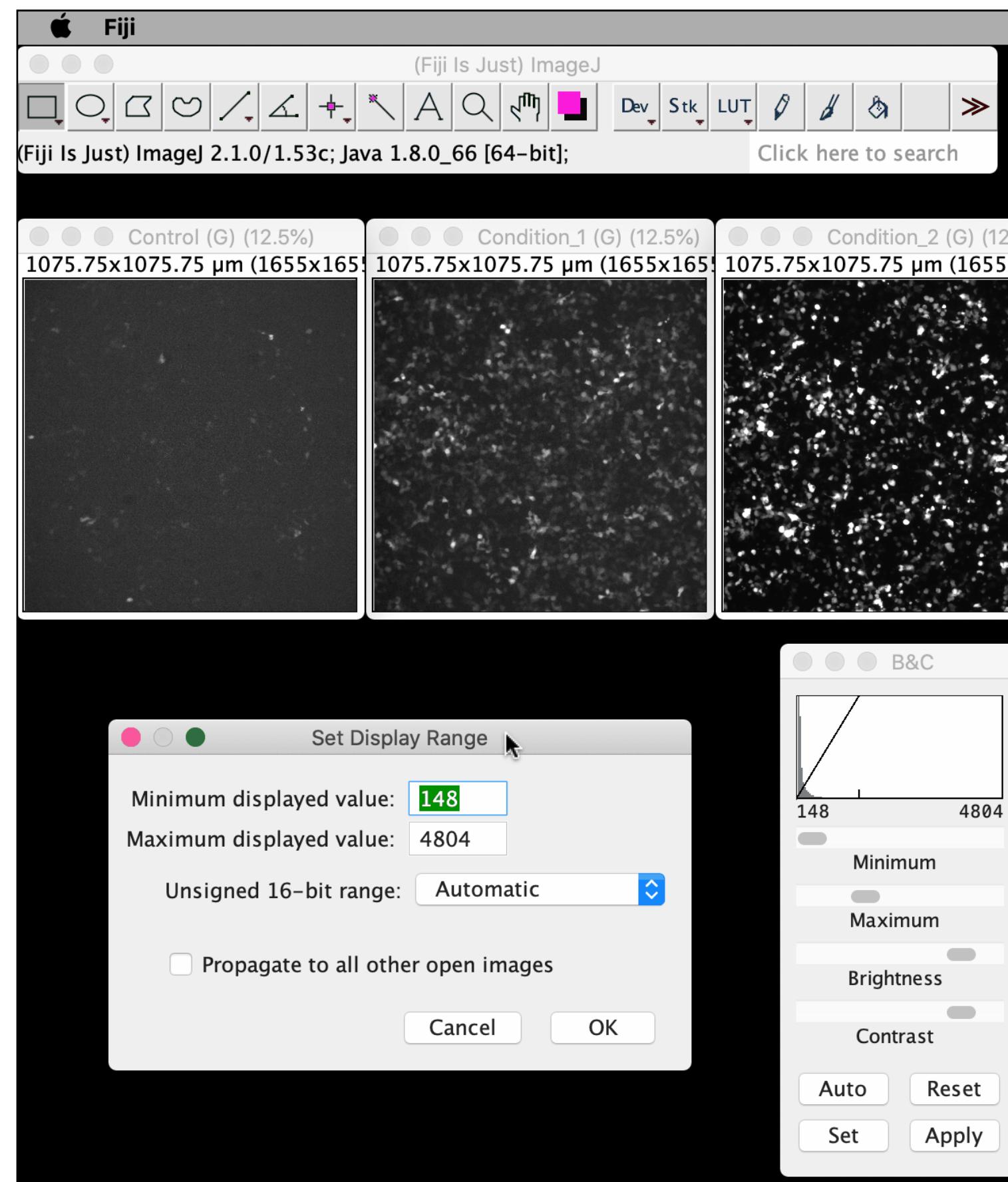
Do NOT trust your eyes,
rely on numbers!



Mean:	4803	2074
Display range:	188- 19540	188- 19540

Display a file: Brightness & Contrast

“Set” button - visually compare images



**Use the same acquisition parameters
(per channel) if you want to compare images!!!
(e.g. same exposure time, illumination intensity,...)**

Example	Exposure time Condition1	Exposure time Condition2
Channel 1	100 ms	100 ms
Channel 2	200 ms	200 ms

For a meaningful comparison, you have to extract numbers from your images (analysis). Use the images to support your results.

If you save the images as jpeg/png/tiff, they will maintain the display range you set.



1.1 image inspection

1.2 adjust brightness/contrast of all open images

Images and Colors

Lookup Tables (LUTs)

LUT = how the grey values are displayed

LUTs do not change the pixel values

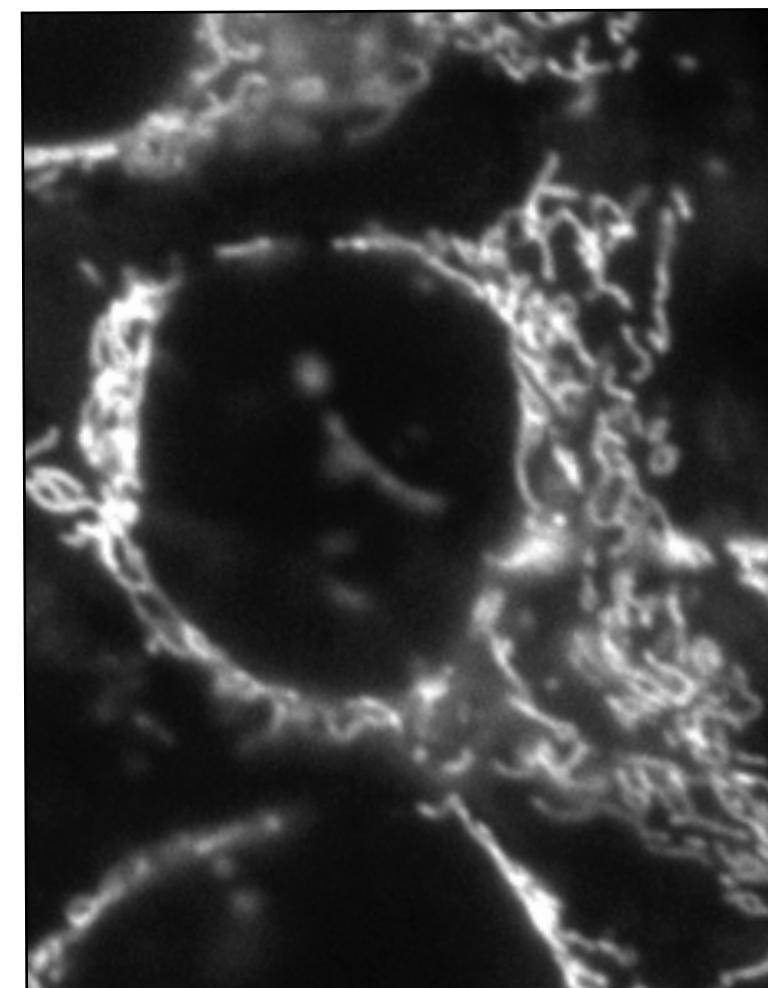


Image (8 bit)	Displayed color
0	
1	
...	
100	
...	
255	

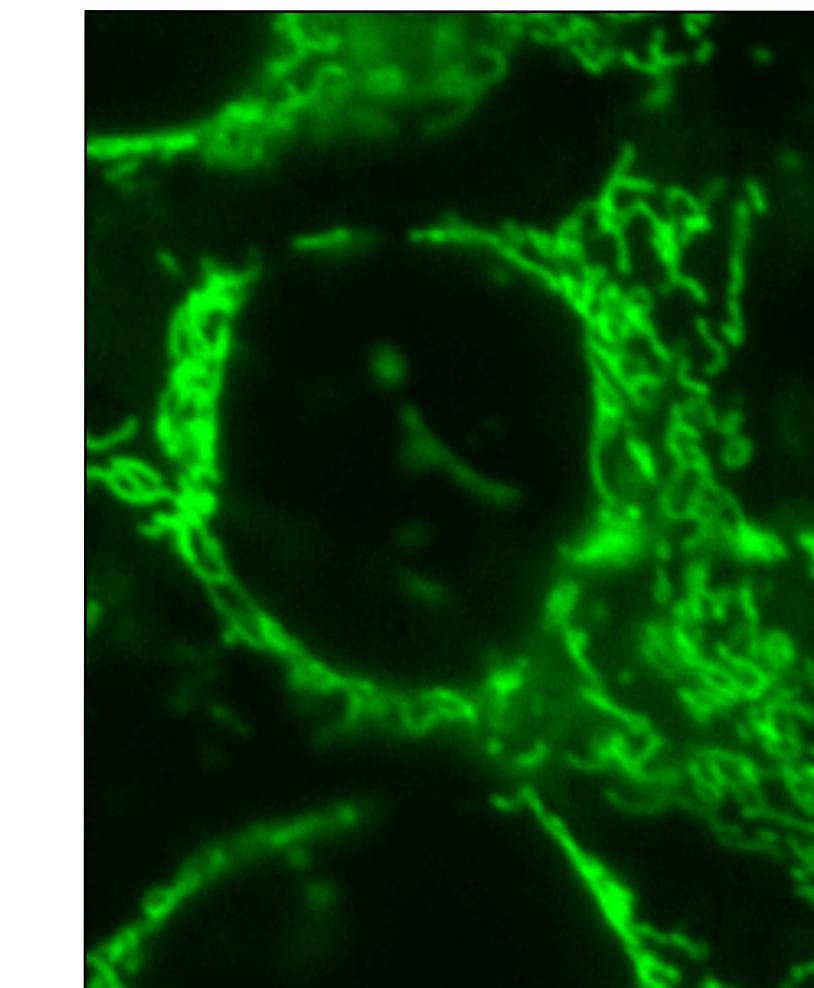
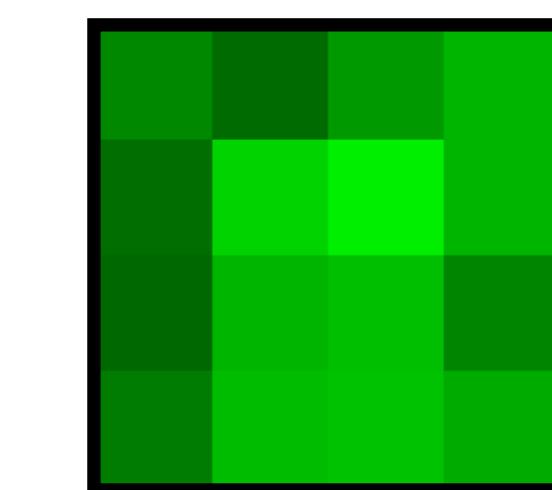


Image (8 bit)	Displayed color
0	
1	
...	
100	
...	
255	



=

136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169



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136	106	152	179
109	209	236	179
103	179	189	132
123	186	192	169

Images and Colors

Lookup Tables (LUTs)

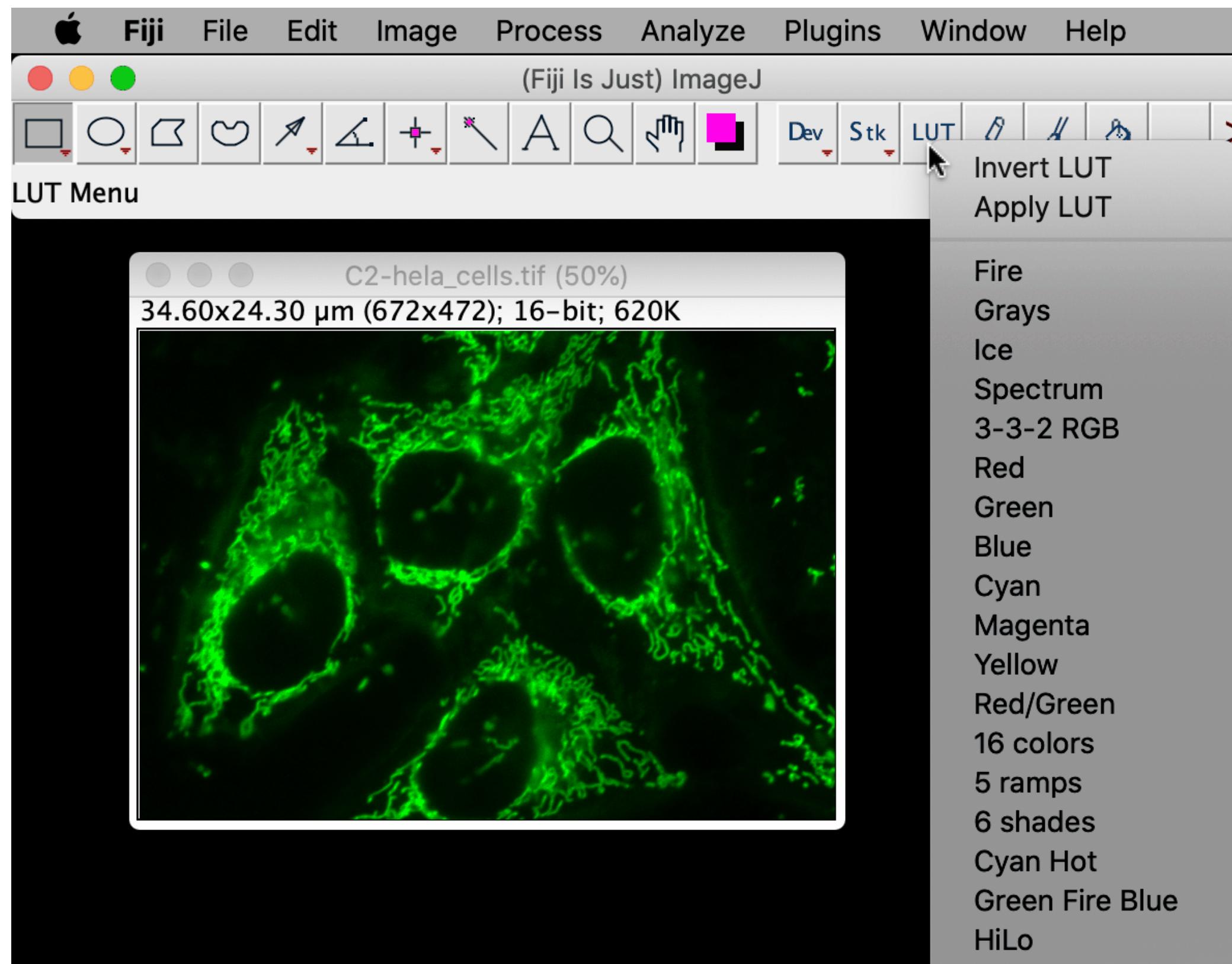


Image > Lookup Tables

or

"LUT" menu in Startup Tools Bar



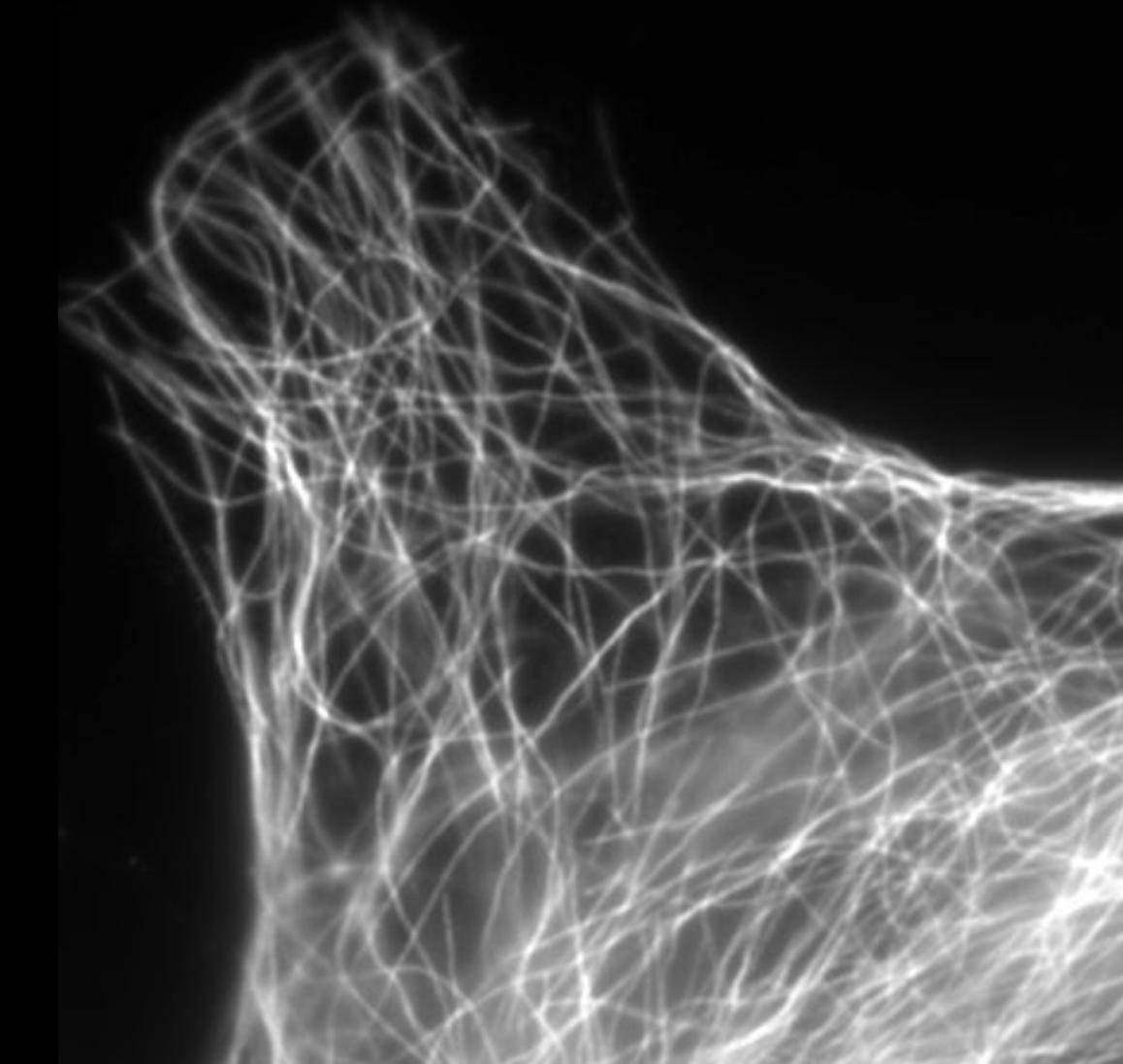
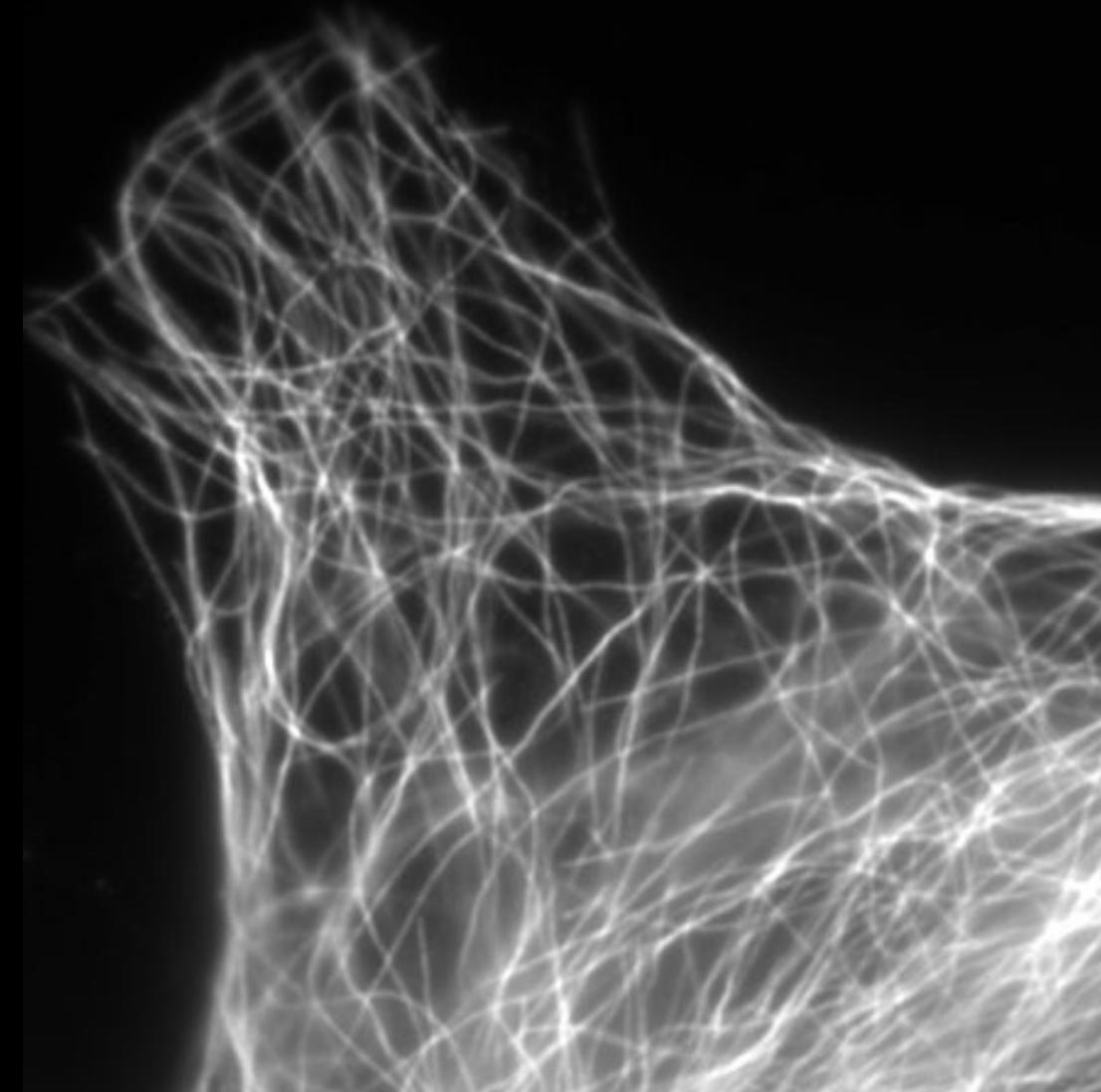
Examples of LUTs

*Image > Color > Display LUTs - to display default LUTs available

Images and Colors in Fiji

Choose the right LUT

Which is brighter?



The human eye evaluates intensity best in grayscale

If you are imaging for example a blue fluorophore, you
are NOT FORCED to display it in blue!

Images and Colors in Fiji

Choose the right LUT

Color blind people don't distinguish some colors

POINTS OF VIEW

Color blindness

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"If a submitted manuscript happens to go to three male reviewers of Northern European descent, the chance that at least one will be color blind is 22 percent"

Images and Colors in Fiji

Choose the right LUT

Color blind people don't distinguish some colors

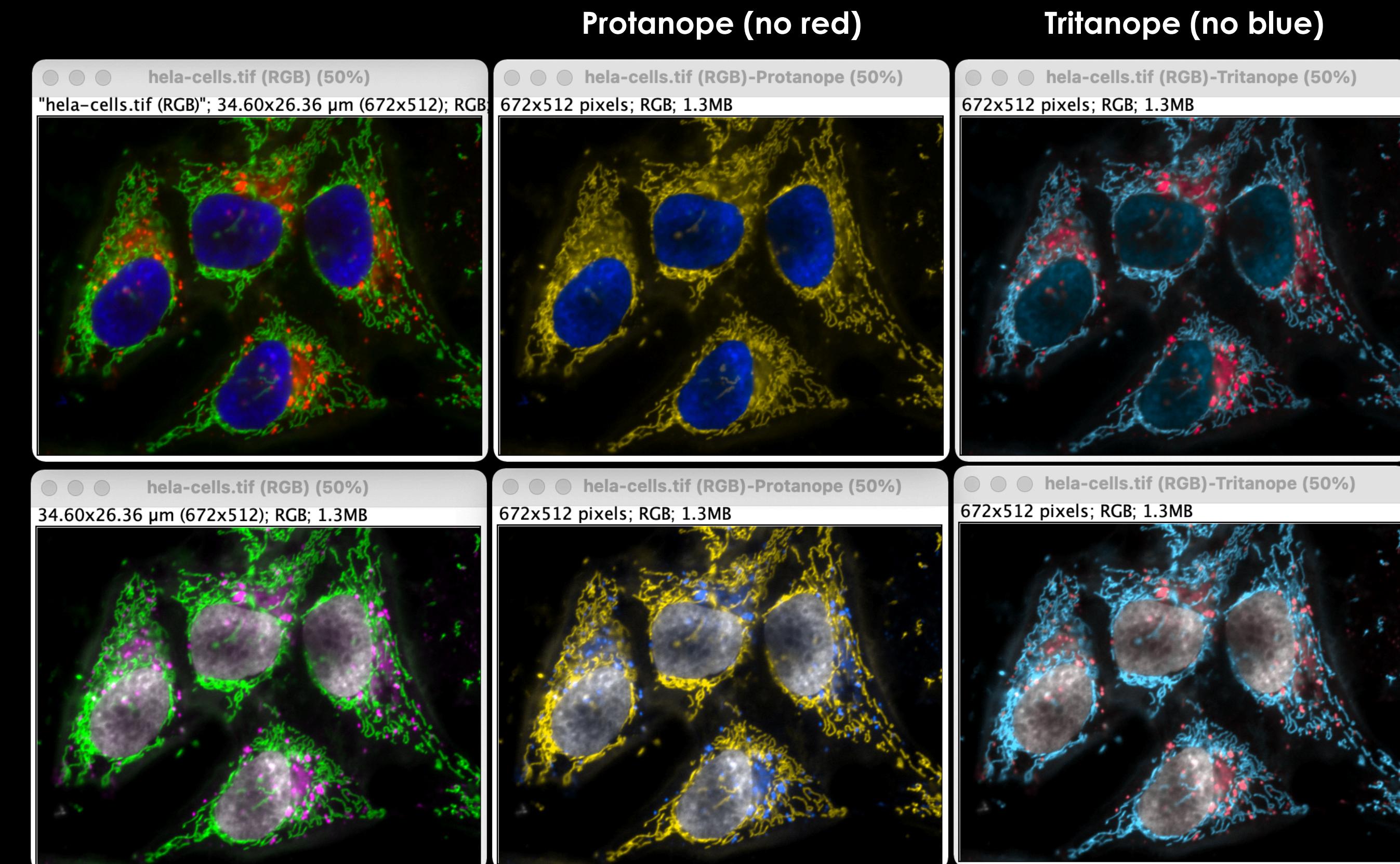
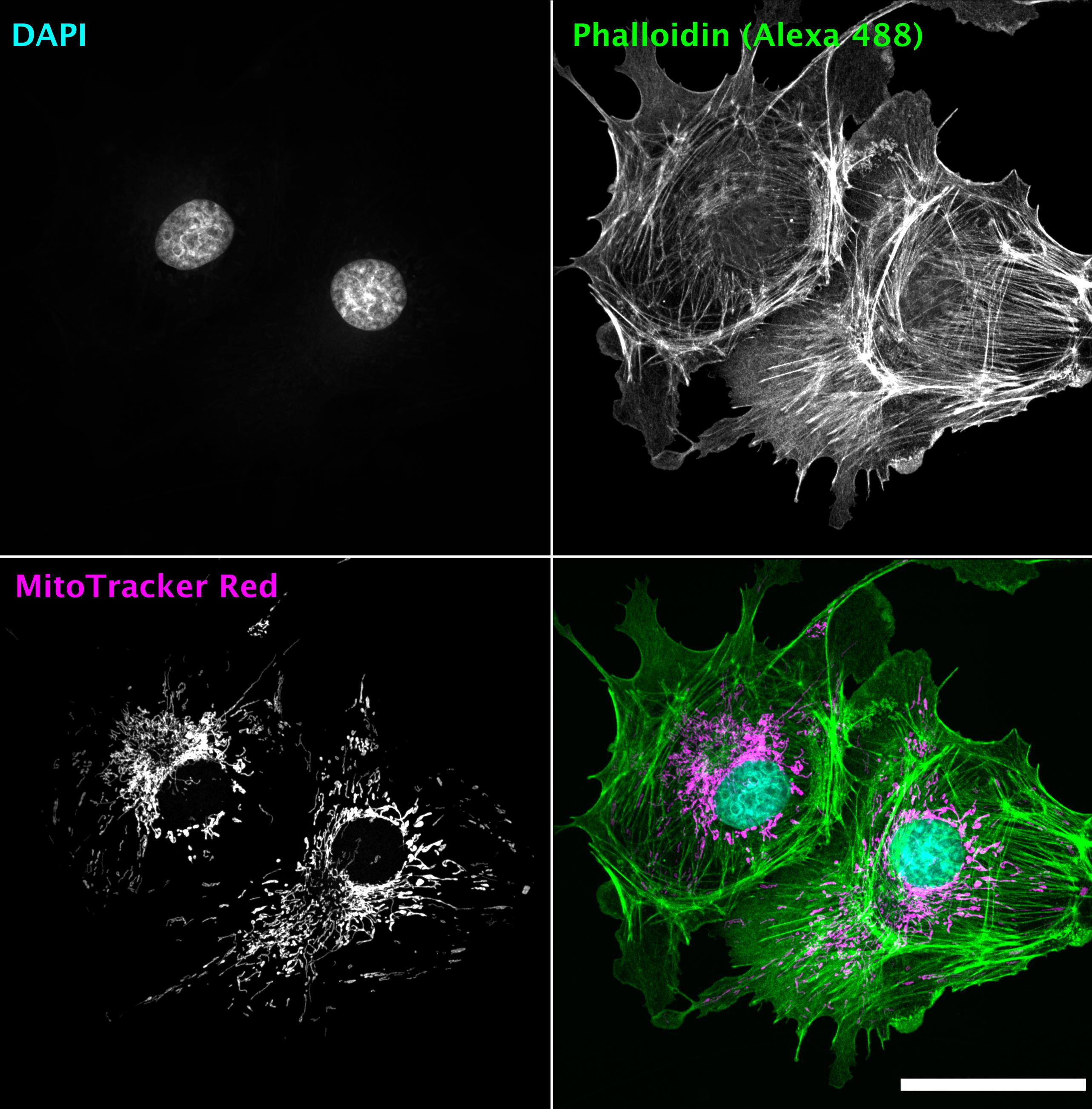
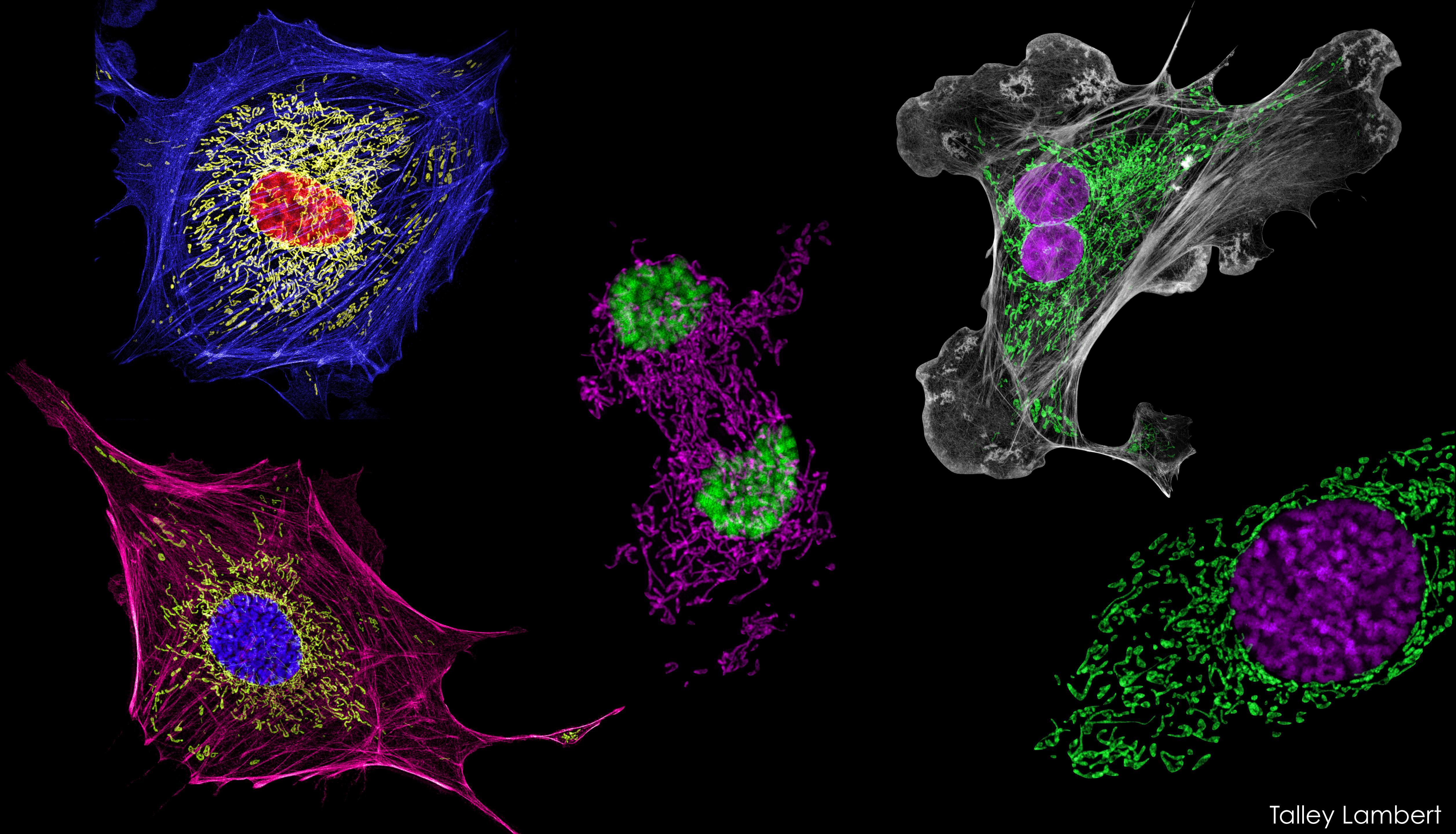


Image > Color > Dichromacy or Image > Color > Simulate Color Blindness

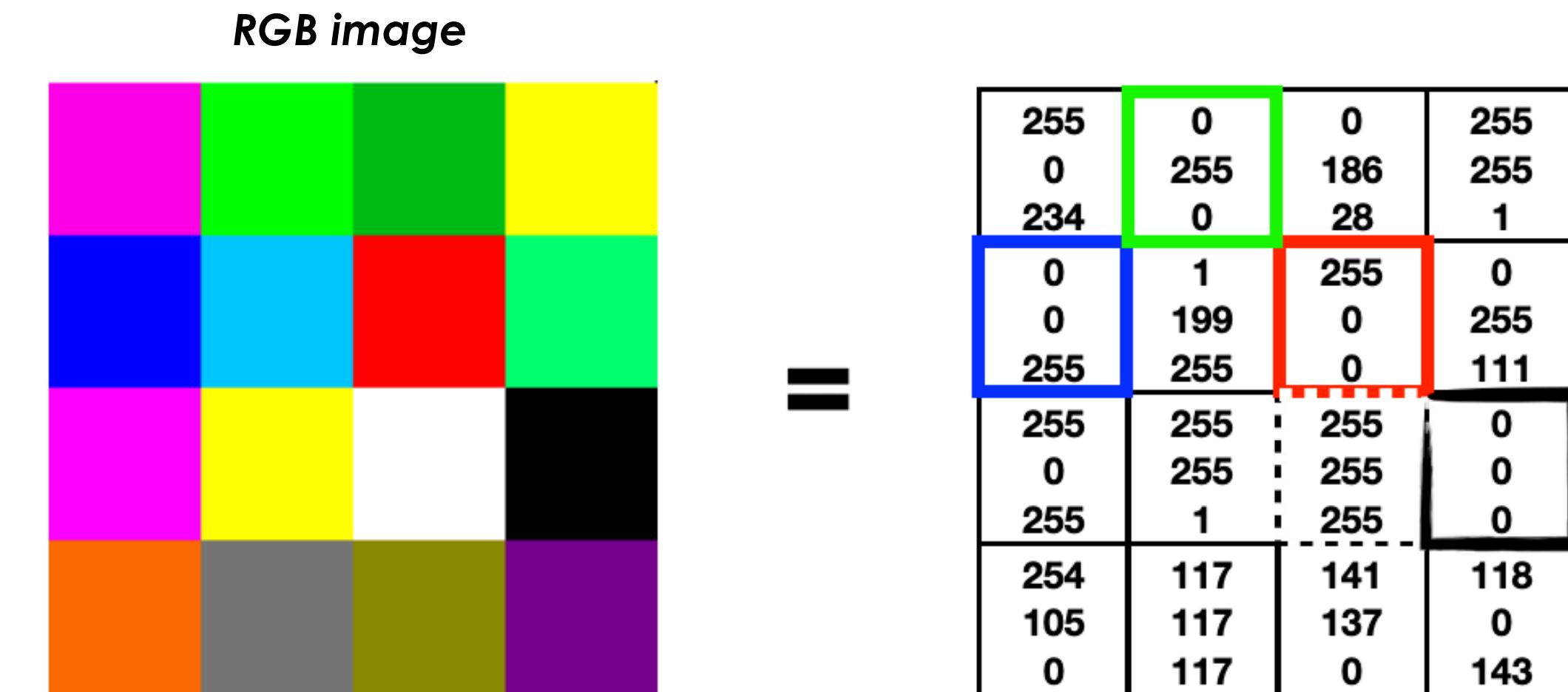




Talley Lambert

RGB Images (still matrix of numbers)

LUTs **cannot** be applied to RGB Images



RGB Color image (e.g. jpeg, png) = **R**ed + **G**reen + **B**lue

RGB Color image = **8 bit Red, 8 bit Green, 8 bit Blue** = **R (0-255), G (0-255), B (0-255)**

Image > Type > RGB Color or Save As...png, jpeg

Images and Colors in Fiji

The Color menu

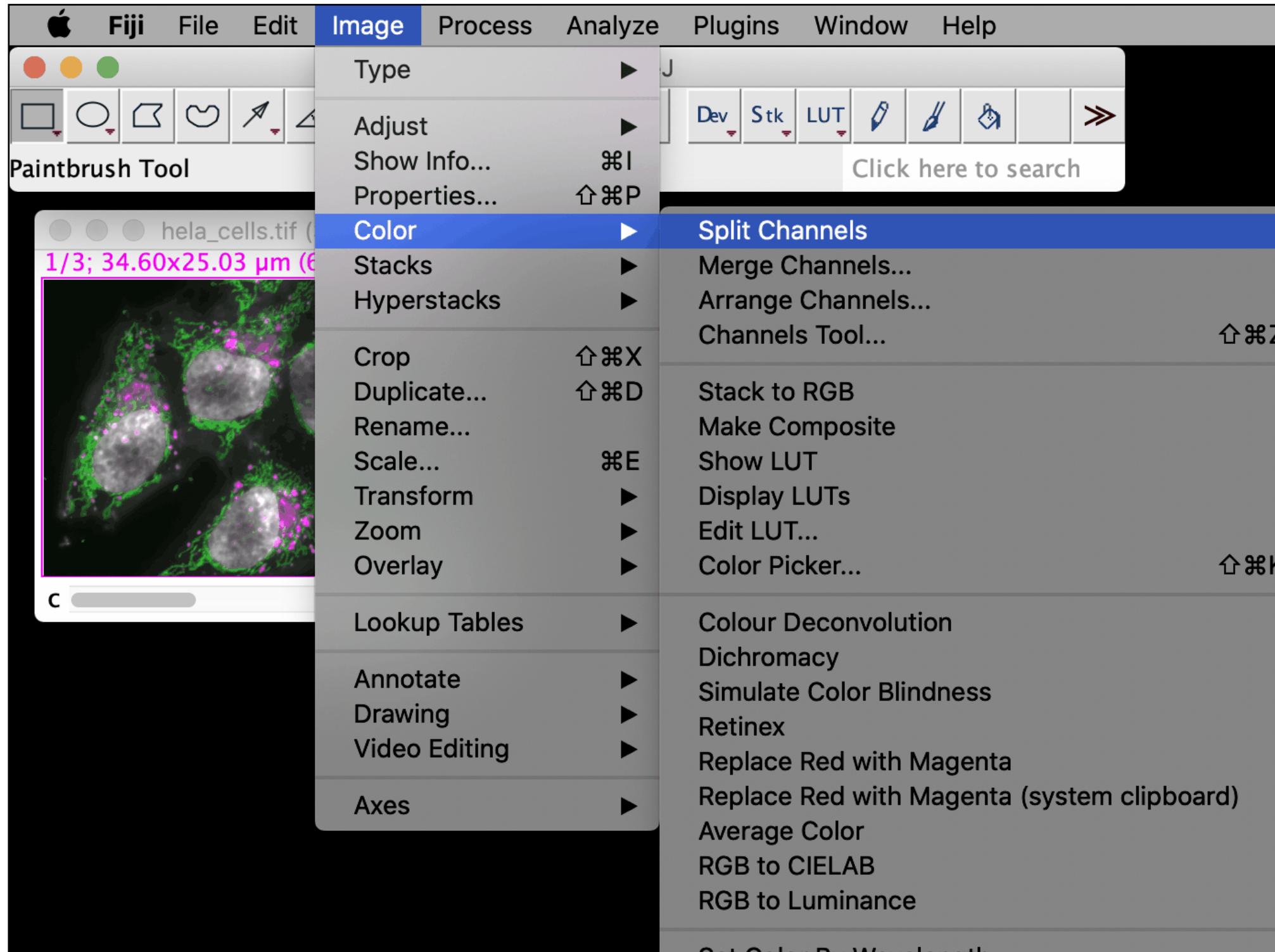


Image > Color > Split Channels

Image > Color > Merge Channels

Split or Merge the channels of the active image

Images and Colors in Fiji

The Color menu

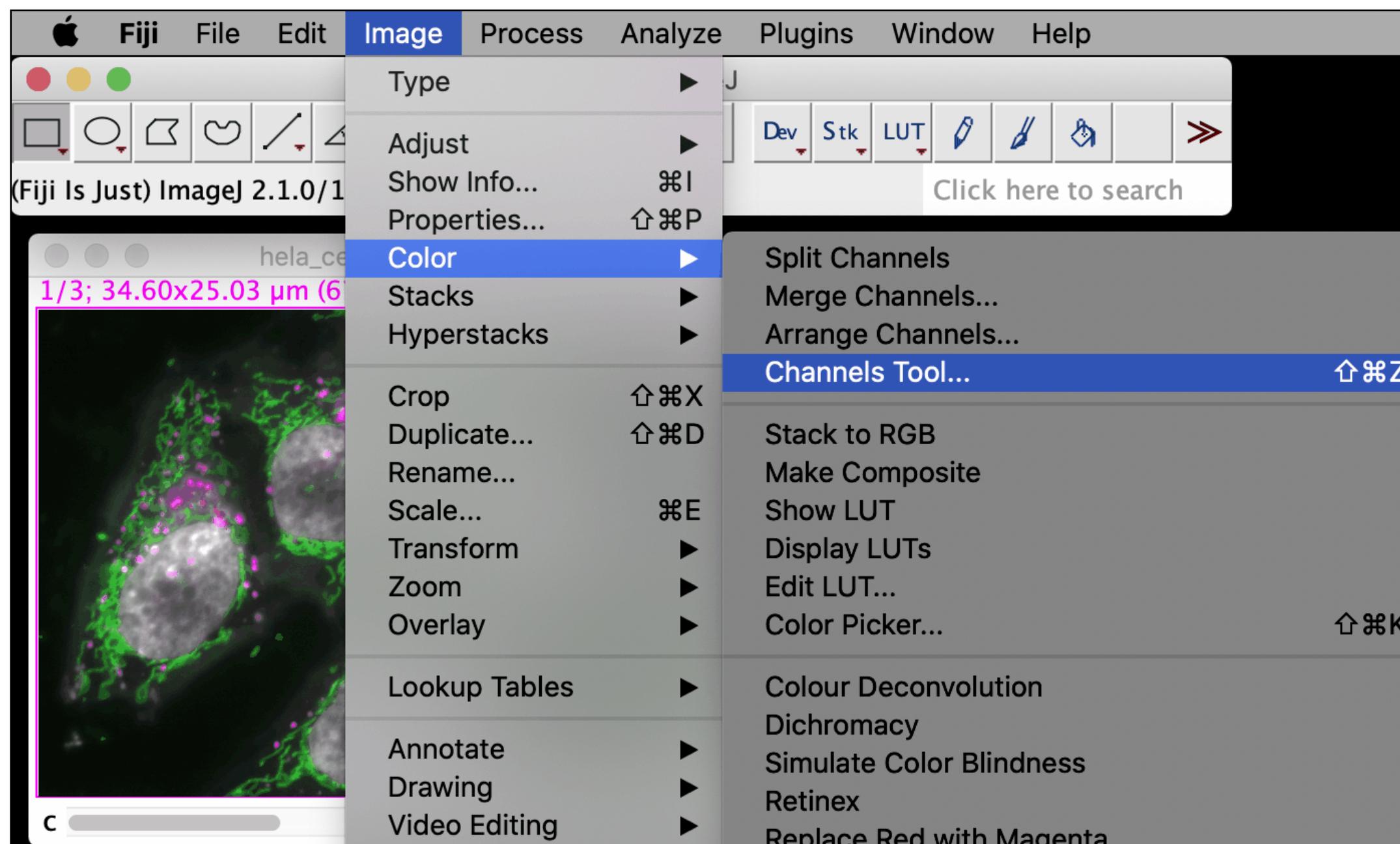


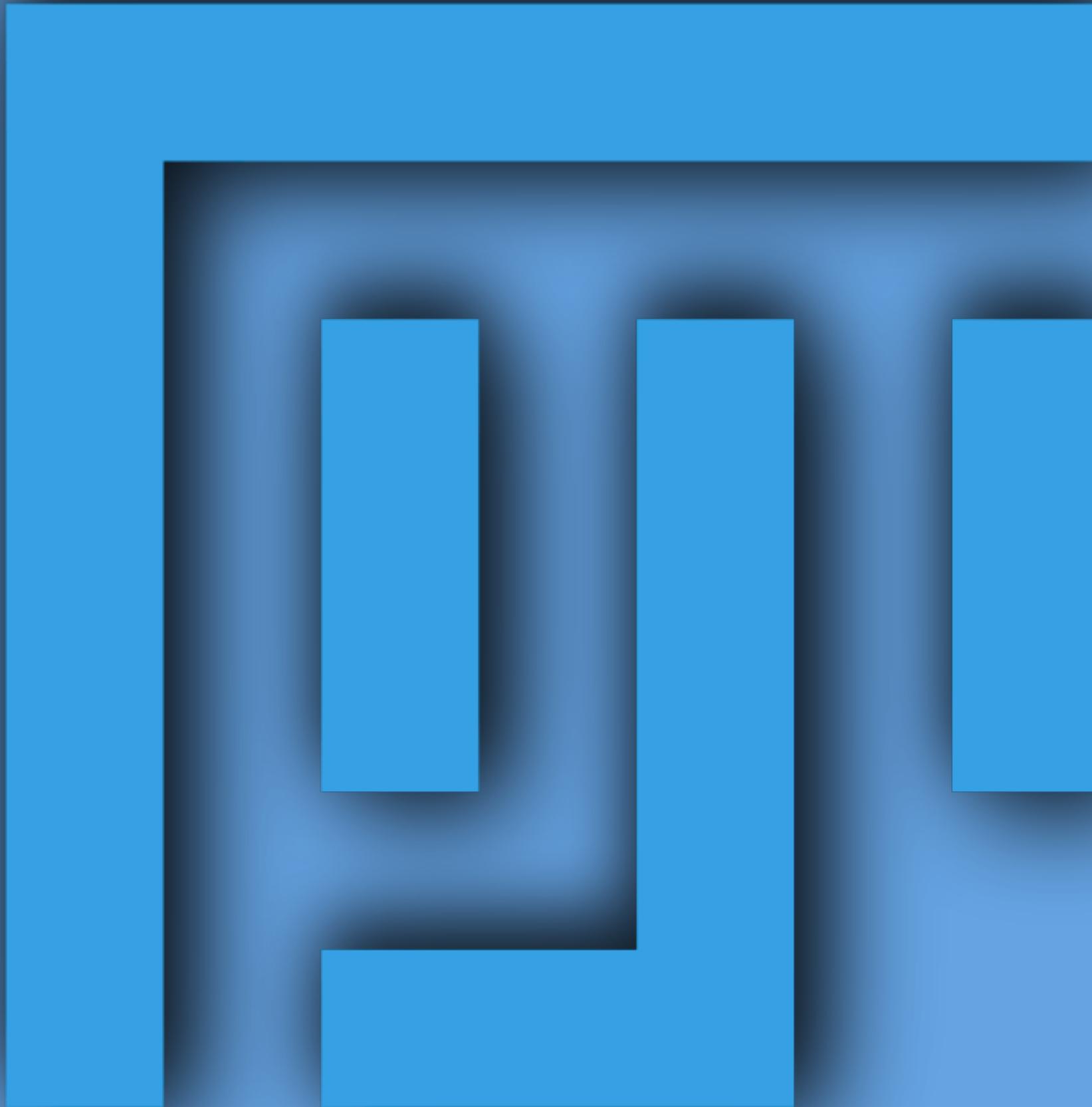
Image > Color > Channels tools

(cmd) + shift + z

Interactive visualization/handling of each image channel.

*Choose how to display your multicolour image: “**Color**” mode (single channel) vs “**Composite**” mode (overlay)*

Segmentation with pixel based classifier—exercises



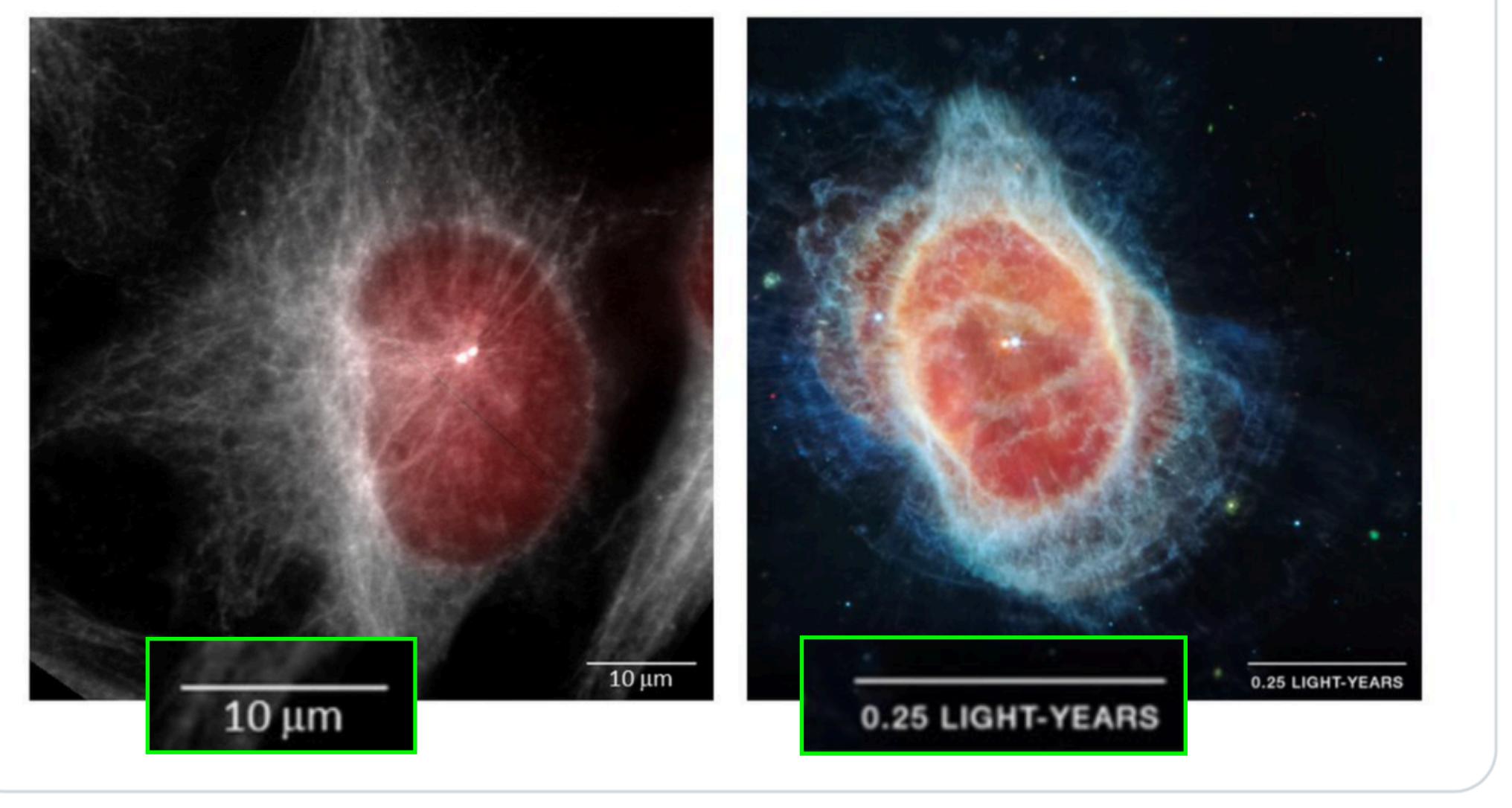
- 2.1 composite images - splitting and merging
- 2.2 RGB images - Replace Red with Magenta

Pixel Size

Scale Bar and Pixel Size

 Laurence Haren
@HarenLaurence ...

never forget the scale bar! [@StearnsLab](#)
when biology meets astronomy: cell vs nebula,
centrosome vs dying star! [@EtienneKlein](#)



The image consists of two panels side-by-side. The left panel is a fluorescence micrograph of a cell showing a prominent red nucleus and surrounding cytoskeletal fibers. A green rectangular box highlights a scale bar at the bottom left, which contains the text "10 μm". The right panel is a photograph of a nebula or dying star, appearing as a bright orange-red central region surrounded by blue and purple wisps against a dark background. A similar green rectangular box highlights a scale bar at the bottom left, which contains the text "0.25 LIGHT-YEARS". Both panels have their respective scale bars located in the bottom-left corner.

10 μm

0.25 LIGHT-YEARS

Pixel Size and Scale Bar

If you want to make some physical measurements of your sample (length, size, ...),
you need to know the **pixel size** of your **image**.

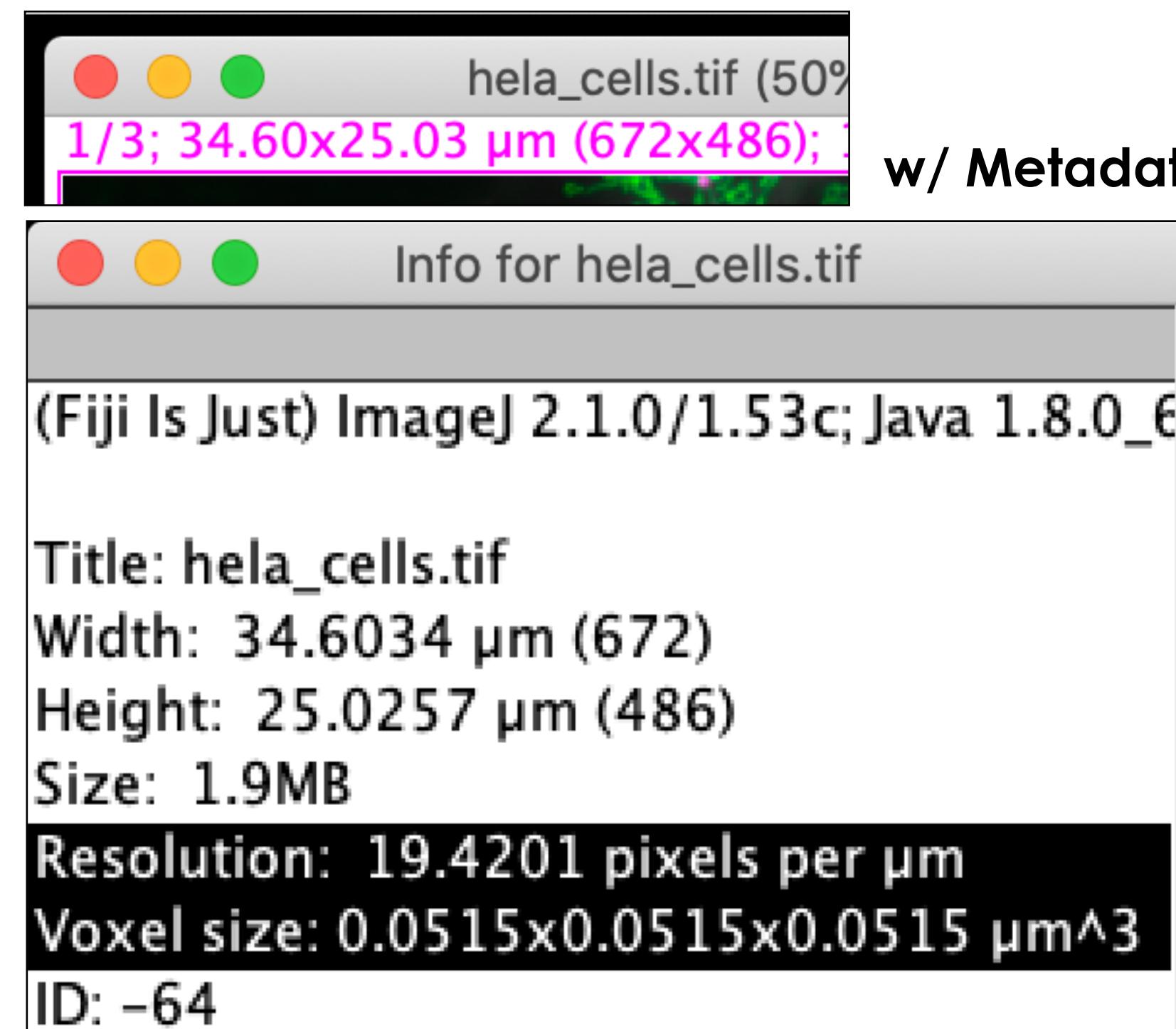


Image > Show Info...

(cmd) + i

Show the **Metadata stored with the file**.

Pixel width (Pixel x) = 0.0515 µm
Pixel height (Pixel y) = 0.0515 µm
Voxel depth (Pixel z) = 0.0515 µm

1 µm = 19.4201 pixel (xy)
(1 µm / 0.0515 µm)

Pixel Size and Scale Bar

If you want to make some physical measurements of your sample (length, size, ...),
you need to know the **pixel size** of your **image**.

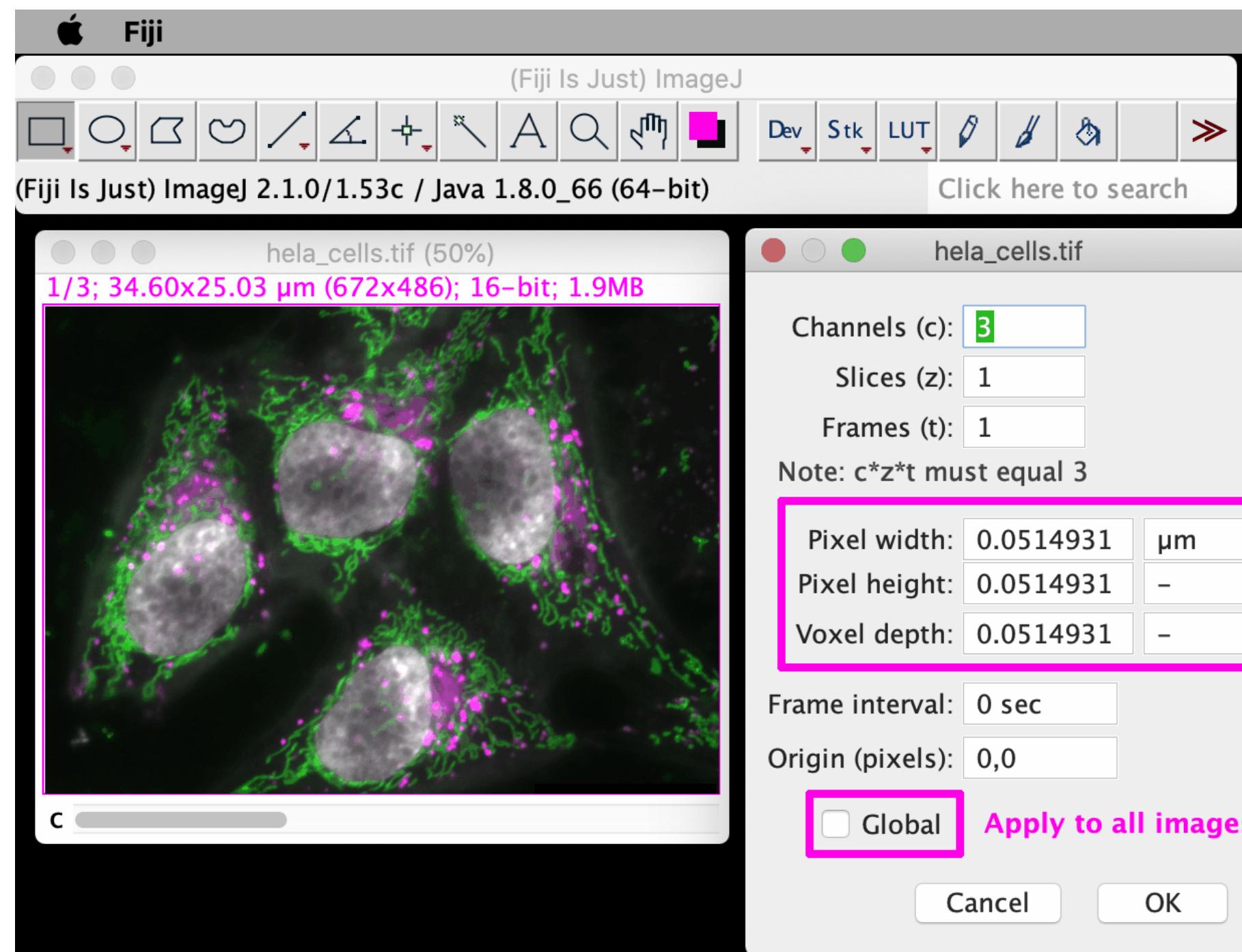


Image > Properties...

(cmd) + shift + p

Open the **image properties** windows
containing pixel size properties (and
multi-dimensional properties)

You can **set/read** the image
pixel size in xyz.

Scale Bar and Pixel Size

What if the pixel size is not stored in the metadata?

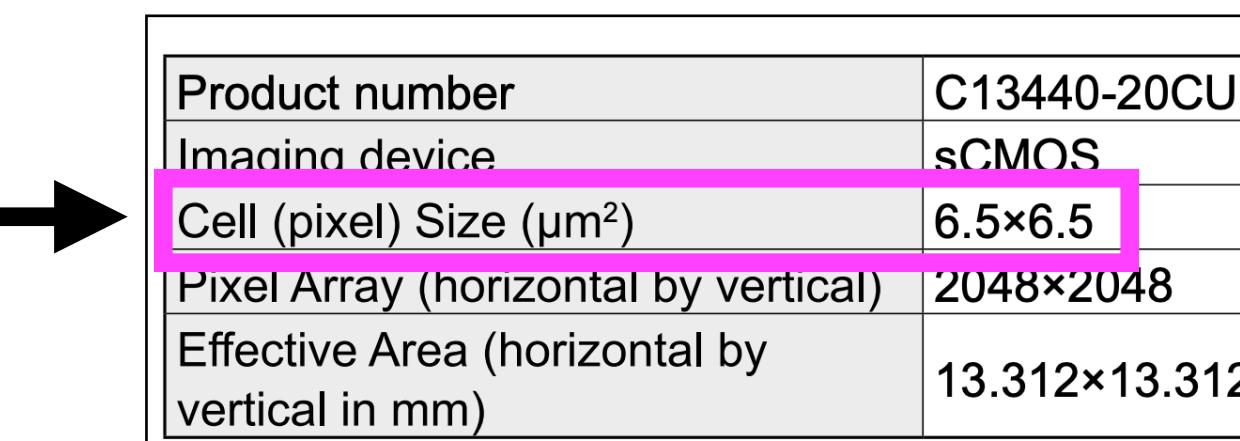
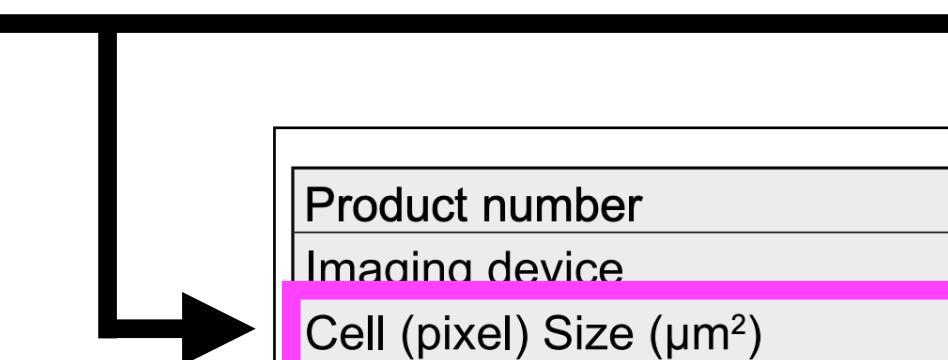
If you know the **magnification** and the **camera** you used for the acquisition, you can estimate the image pixel size.

$$\text{image pixel size} = \text{camera pixel size}/\text{magnification}$$

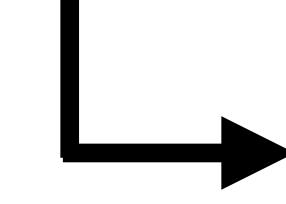
Example

Magnification = 100x Objective

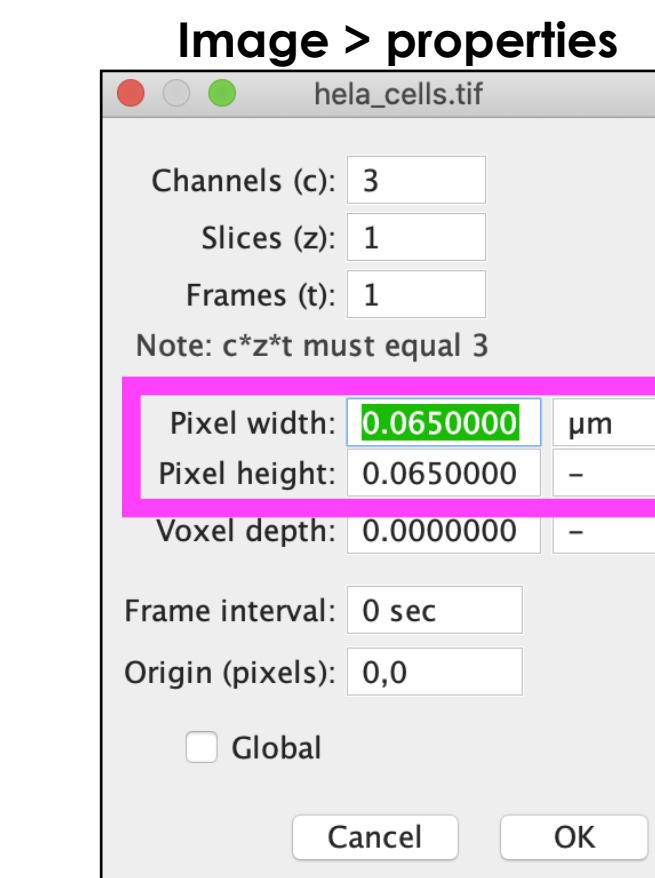
Camera = Hamamatsu Orca Flash 4



Product number	C13440-20CU
Imaging device	sCMOS
Cell (pixel) Size (μm^2)	6.5×6.5
Pixel Array (horizontal by vertical)	2048×2048
Effective Area (horizontal by vertical in mm)	13.312×13.312



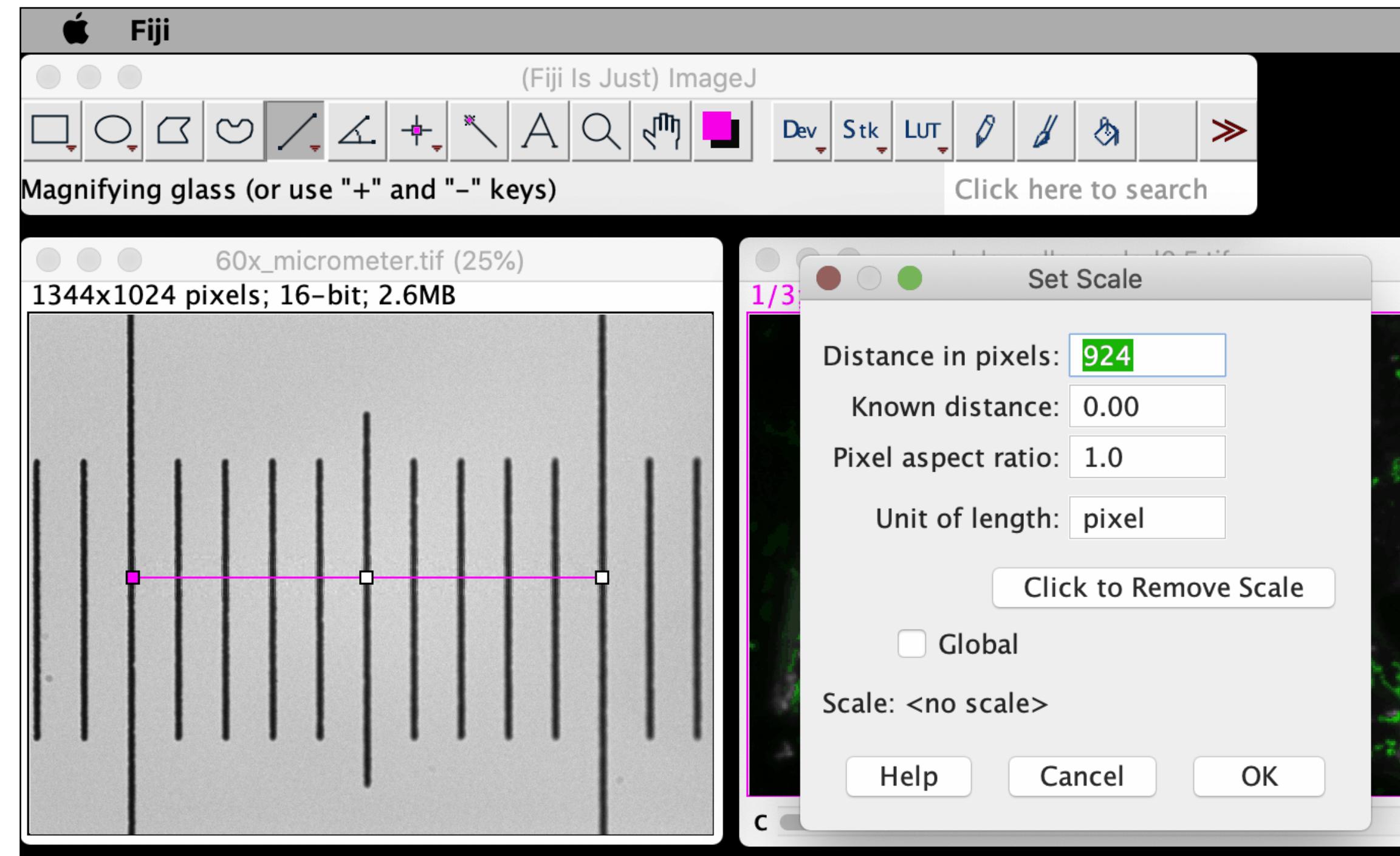
pixel width and height:
 $6.5 \mu\text{m} / 100X = 0.065 \mu\text{m}$



If a z-series was acquired, "Voxel depth" is the z step size choose for the acquisition (cannot estimate).

Scale Bar and Pixel Size

Add pixel information to your images from a reference (same magnification)



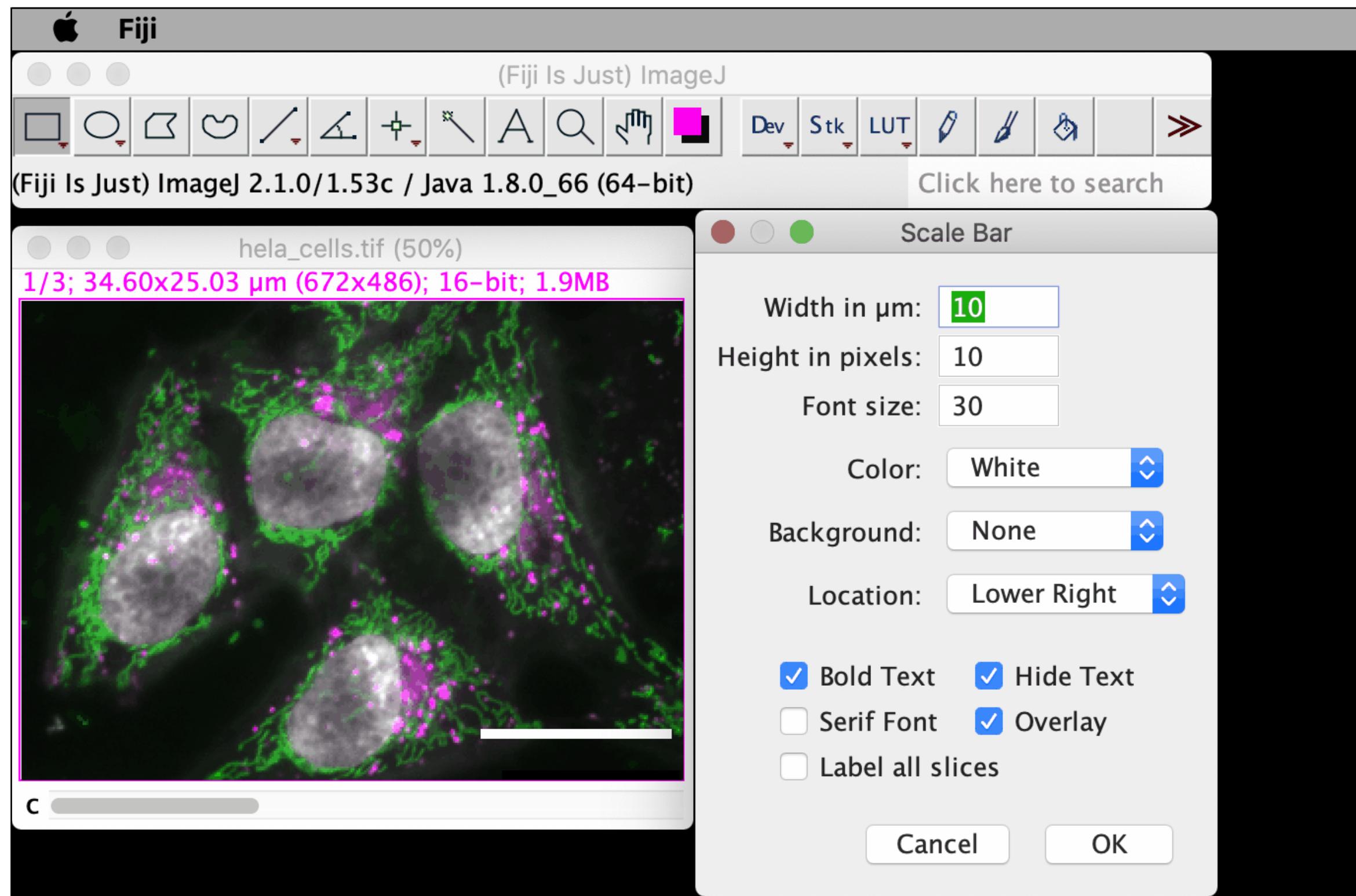
Analyze > Set Scale...

Set the pixel size information starting from an object with a known dimension.

***Perform multiple measures and average them to be more precise**

Scale Bar and Pixel Size

Add scale bar to your images



Analyze > Tools > Scale Bar...

- “Overlay” means that the scale bar is not embedded in your image. You can remove it: “Image > Overlay > Remove Overlay”.
- If you save the image as tiff, the scale bar will be saved as an overlay.
- If you save as jpeg/png, the image will have the scale bar embedded.

Segmentation with pixel based classifier—exercises



- 1.3 file handling and non-invasive editing**
- 1.4 file handling and invasive editing**