



# Introduction to image processing and analysis with ImageJ / Fiji.

## Part 2

### Viewing & manipulating images in Fiji

Course by Dale Moulding



# Session 2

1 hour

30 minute lecture

30 minutes exercises

## Learning objectives:

- Open images in Fiji
- Check bit depth, channel number, dimensionality (2D / 3D / 4D / 5D)
- Adjust brightness and contrast
- Adjust image display without changing the image data
- Separate images by channel, time, z-position
- Present images as 3D projections
- Make montages for presentations



## Fiji (ImageJ) User guide

<https://imagej.nih.gov/ij/docs/guide/index.html>

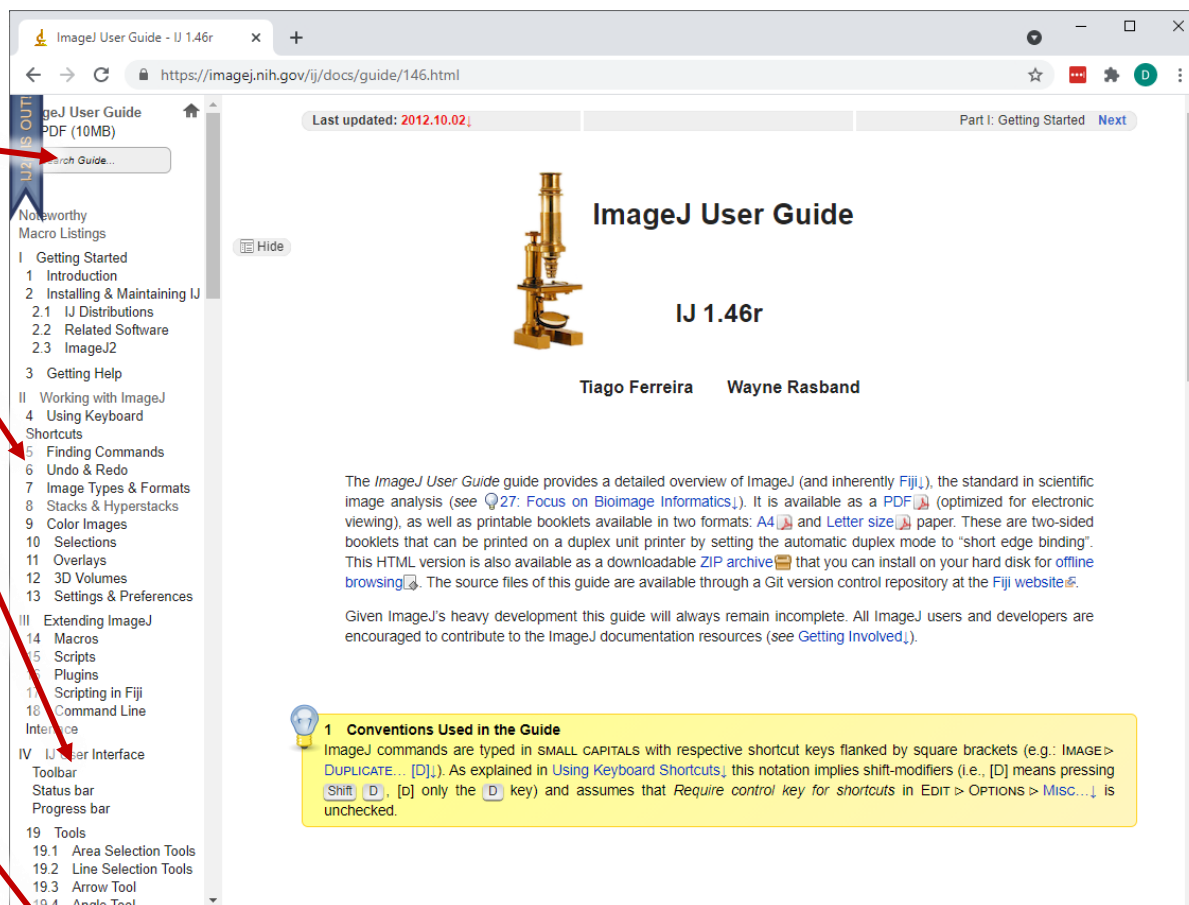
V	Menu Commands
26	File>
26.1	New>
26.2	Open...[o]
26.3	Open Next [O]
26.4	Open Samples>
26.5	Open Recent>
26.6	Import>
26.7	Close [w]
26.8	Close All
26.9	Save [s]
26.10	Save As>
26.11	Revert [r]
26.12	Page Setup...
26.13	Print...[p]
26.14	Quit
27	Edit>
27.1	Undo [z]
27.2	Cut [x]
27.3	Copy [c]
27.4	Copy to System
27.5	Paste [v]
27.6	Paste Control...
27.7	Clear
27.8	Clear Outside
27.9	Fill [f]
27.10	Draw [d]
27.11	Invert [I]
27.12	Selection>
27.13	Options>
28	Image>
28.1	Type>
28.2	Adjust>
28.3	Show Info...[i]

Searchable

Description of  
image types

Toolbars

Every Menu  
command and  
all sub  
commands



The screenshot shows the ImageJ User Guide website. The browser address bar displays <https://imagej.nih.gov/ij/docs/guide/146.html>. The page title is "ImageJ User Guide" and it indicates it is a PDF (10MB). The page is dated "Last updated: 2012.10.02". The main heading is "ImageJ User Guide" with the version "IJ 1.46r" and authors "Tiago Ferreira" and "Wayne Rasband". A search bar is visible at the top left. A sidebar on the left contains a table of contents with sections like "Getting Started", "Working with ImageJ", "Extending ImageJ", and "User Interface". A yellow box at the bottom right contains a section titled "1 Conventions Used in the Guide" explaining the notation for ImageJ commands and shortcuts.



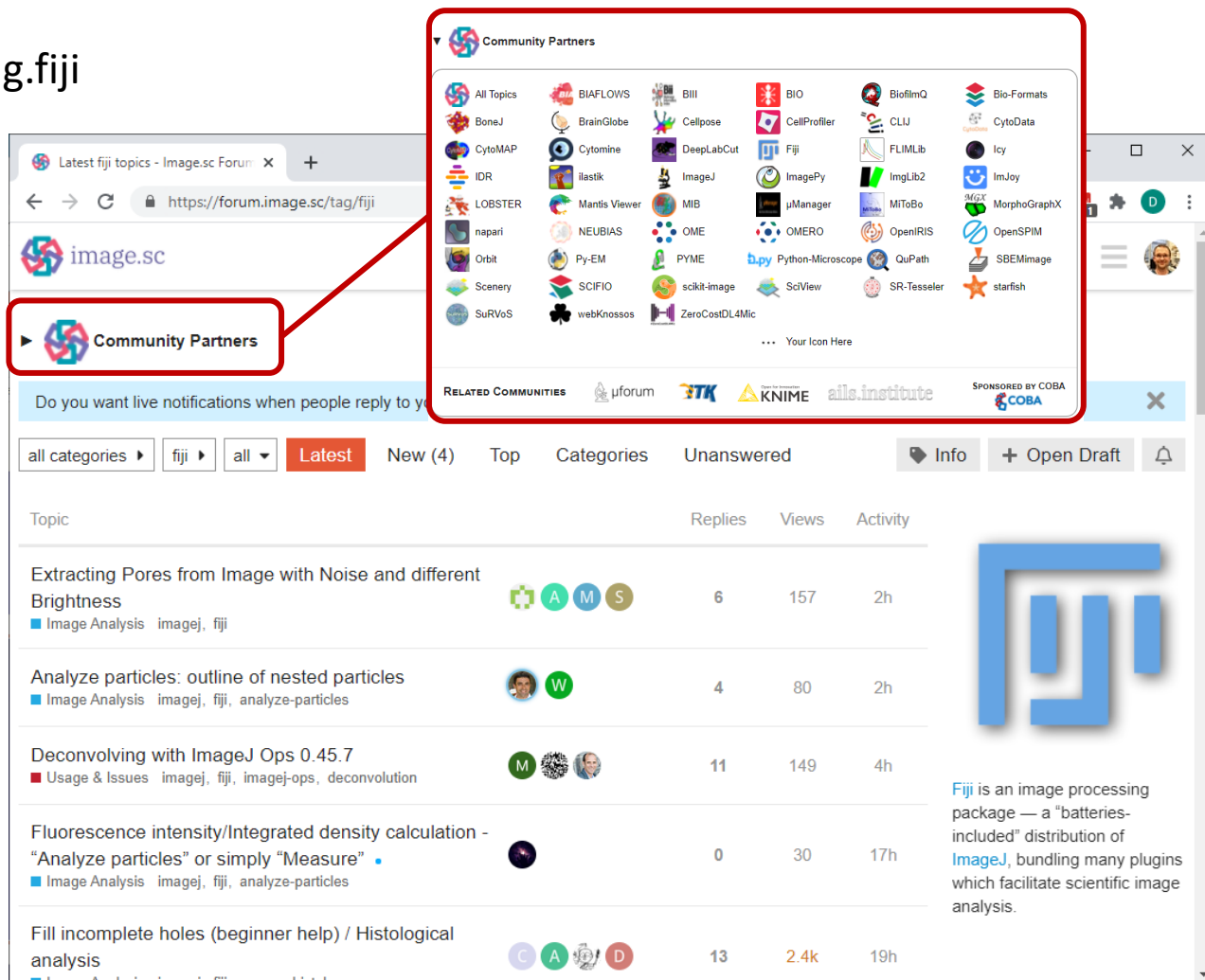
## Fiji on-line support

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

Just google:  
Image.sc

Fully searchable forum.

Access to Image  
analysis experts,  
including developers of  
Fiji, plugins and other IA  
software.



The screenshot shows the Image.sc forum interface. A red box highlights the 'Community Partners' section, which lists various software and services. Below this, the forum topics are listed with columns for Topic, Replies, Views, and Activity.

**Community Partners:**

- All Topics, BIAFlows, Bill, Bio, BiofilmQ, Bio-Formats
- BoneJ, BrainGlobe, Cellpose, CellProfiler, CLUJ, CytoData
- CytoMAP, Cytomine, DeepLabCut, Fiji, FLIMLib, Icy
- IDR, Ilastik, ImageJ, ImagePy, ImgLib2, ImJoy
- LOBSTER, Mantis Viewer, MIB, µManager, MIToBo, MorphoGraphX
- napari, NEUBIAS, OME, OMERO, OpenIris, OpenSPIM
- Orbit, Py-EM, PYME, Python-Microscope, QuPath, SBEMImage
- Scenery, SCIFIO, scikit-image, ScView, SR-Tesseler, starfish
- SuRVoS, webKnossos, ZeroCostDL4Mic

**Forum Topics:**

Topic	Replies	Views	Activity
Extracting Pores from Image with Noise and different Brightness ■ Image Analysis imagej, fiji	6	157	2h
Analyze particles: outline of nested particles ■ Image Analysis imagej, fiji, analyze-particles	4	80	2h
Deconvolving with ImageJ Ops 0.45.7 ■ Usage & Issues imagej, fiji, imagej-ops, deconvolution	11	149	4h
Fluorescence intensity/Integrated density calculation - "Analyze particles" or simply "Measure" ■ Image Analysis imagej, fiji, analyze-particles	0	30	17h
Fill incomplete holes (beginner help) / Histological analysis ■ Image Analysis imagej, fiji, macro, histology	13	2.4k	19h

Fiji is an image processing package — a "batteries-included" distribution of ImageJ, bundling many plugins which facilitate scientific image analysis.



## Documentation & Guides

### User guides

- online manual <http://rsb.info.nih.gov/ij/docs/guide/index.html>
- pdf manual <http://rsbweb.nih.gov/ij/docs/user-guide.pdf>

### Tutorials

- ImageJ Wiki <http://imagejdocu.tudor.lu/>
- EMBL course notes and PDF textbook <http://cmci.embl.de/documents/ijcourses>
- New EMBL / Olympus textbook <http://cmci.embl.de/>

### Macros

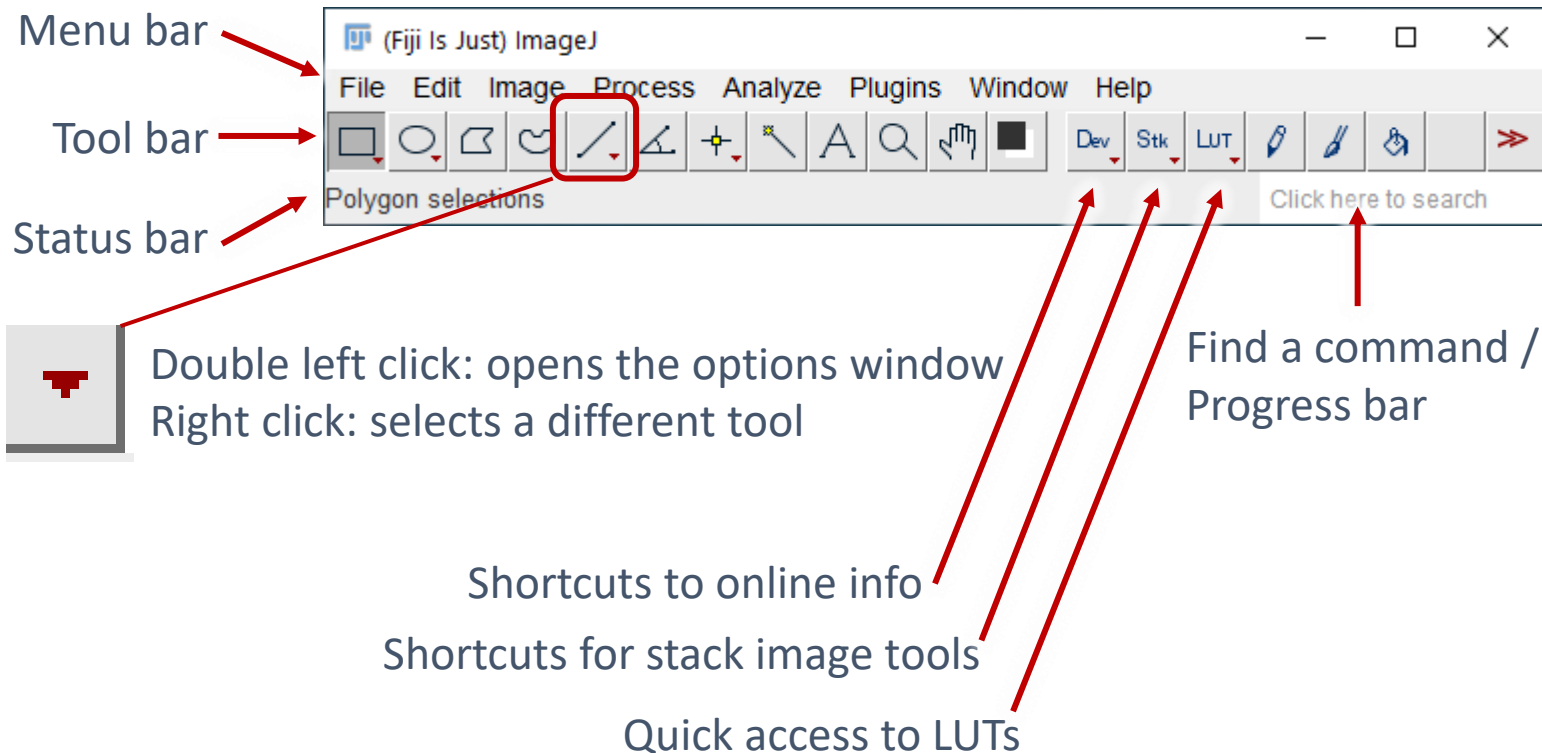
- List of many macros <http://rsbweb.nih.gov/ij/macros/>
- Macro commands <http://rsb.info.nih.gov/ij/developer/macro/functions.html>

### Plugins

- Lists of many plugins <http://rsbweb.nih.gov/ij/plugins/index.html>  
<http://fiji.sc/wiki/index.php/Category:Plugins>



## Fiji GUI





## Updating and upgrading Fiji (adding plugins)

Help BAR

- ImageJ Website...
- ImageJ News...
- Documentation...
- Installation...
- Mailing List...

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- Dev. Resources...
- Plugins...
- Macros...
- Macro Functions...
- Examples ▶

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- Update ImageJ...
- Refresh Menus

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- About Plugins ▶
- About ImageJ...

---

- Report a Bug
- Help on Menu Item
- Switch to Modern Mode

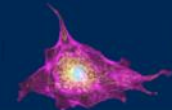
---

- Update...**
- Upload Sample Image...

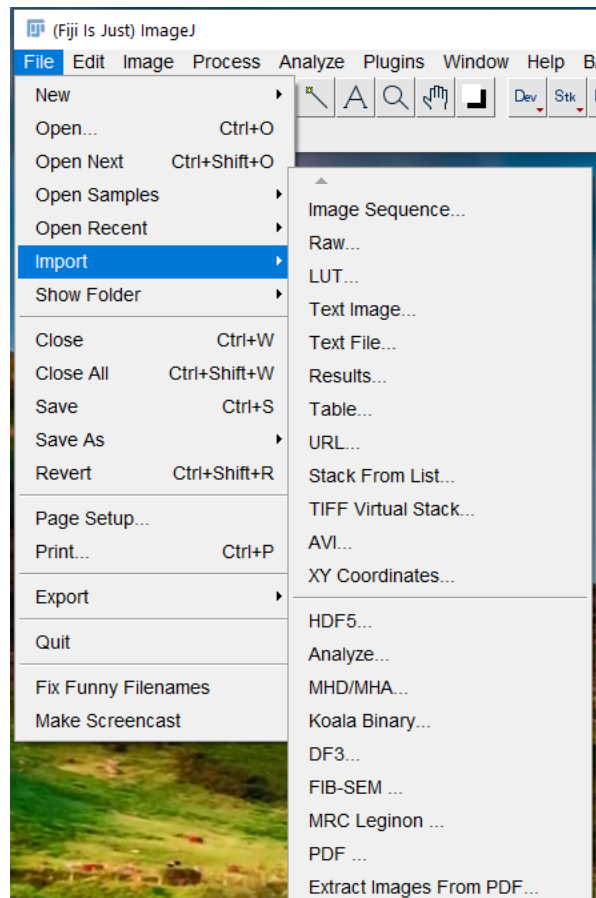
ImageJ Updater

Manage update sites

A...	Name	URL	Host	Directory on Hos
<input checked="" type="checkbox"/>	ImageJ	https://update.imagej.net/		
<input checked="" type="checkbox"/>	Fiji	https://update.fiji.sc/		
<input checked="" type="checkbox"/>	Fiji-Legacy	https://sites.imagej.net/Fiji-Legacy/		
<input checked="" type="checkbox"/>	Java-8	https://sites.imagej.net/Java-8/		
<input type="checkbox"/>	2015-Conference	https://sites.imagej.net/2015-Conference/		
<input checked="" type="checkbox"/>	3D ImageJ Suite	https://sites.imagej.net/Tboudier/		
<input type="checkbox"/>	3Dscript	https://romulus.oice.uni-erlangen.de/updatesite/		
<input type="checkbox"/>	ActogramJ	https://romulus.oice.uni-erlangen.de/imagej/upd...		
<input type="checkbox"/>	AIC Janelia - Course	https://sites.imagej.net/AICjanelia-course/		
<input checked="" type="checkbox"/>	Angiogenesis	https://sites.imagej.net/Angiogenesis/		
<input type="checkbox"/>	AngioTool	https://sites.imagej.net/AngioTool/		
<input type="checkbox"/>	Archipelago	https://sites.imagej.net/Lindsey/		
<input type="checkbox"/>	AxoNet	https://sites.imagej.net/AxoNet/		
<input type="checkbox"/>	BACMMAN	https://sites.imagej.net/Ljp/		
<input checked="" type="checkbox"/>	BAR	https://sites.imagej.net/Tiago/		
<input type="checkbox"/>	BaSiC	https://sites.imagej.net/BaSiC/		
<input type="checkbox"/>	BigDataProcessor	https://sites.imagej.net/BigDataProcessor/		
<input type="checkbox"/>	BigDataViewer-Playground	https://biop.epfl.ch/Fiji-Bdv-Playground/		
<input checked="" type="checkbox"/>	BIG-EPFL	https://sites.imagej.net/BIG-EPFL/		
<input type="checkbox"/>	BigStitcher	https://sites.imagej.net/BigStitcher/		
<input type="checkbox"/>	BigVolumeViewer Demo	https://sites.imagej.net/BigVolumeViewer/		
<input checked="" type="checkbox"/>	Bio-Formats	https://sites.imagej.net/Bio-Formats/		
<input type="checkbox"/>	Biomat	https://sites.imagej.net/Biomat/		
<input checked="" type="checkbox"/>	Biomedgroup	https://sites.imagej.net/Biomedgroup/		



## Opening Images in Fiji

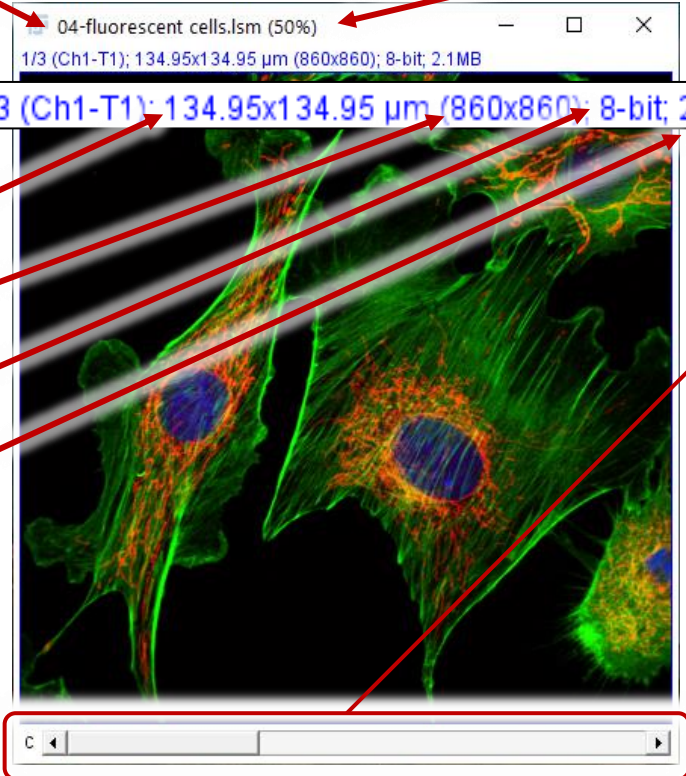


- ***File / Open...***
- ***File / Import >***
- Drag & drop  
Single file, multiple files, whole folders
- ***Plugins / BioFormats / Importer***





## Fiji image window



**Filename** → 04-fluorescent cells.lsm (50%)

**Multi channel image** → 1/3 (Ch1-T1); 134.95x134.95 µm (860x860); 8-bit; 2.1MB

**Image zoom: adjust with + /- keys on keyboard** → [Zoom controls]

**Image Info:**

- Channels (active / total)** → 1/3 (Ch1-T1)
- Total size (if scale known)** → 134.95x134.95 µm
- Size in pixels** → (860x860)
- Bit depth** → 8-bit
- File size** → 2.1MB

**Image dimensions control bar** → [c] [Slide bar]

c = Channels (slide to set active channel)  
Can also have:  
z (3d images),  
▶ (time series)



## Fiji image window

Multi dimensional image

Filename (Image zoom) →

Image Info: →

Channels (active / total) →

z slices (3d image) →

t points (time series) →

Total size (if scale known) →

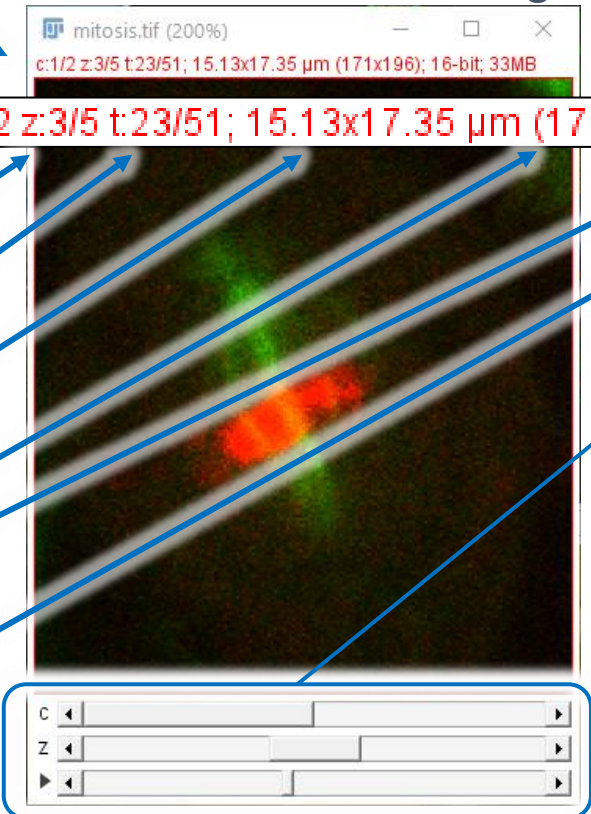
Size in pixels →

Bit depth →

File size →

Image dimensions control bar

c = Channels  
z (3d images),  
▶ (time series)



mitosis.tif (200%)

c:1/2 z:3/5 t:23/51; 15.13x17.35 µm (171x196); 16-bit; 33MB

C

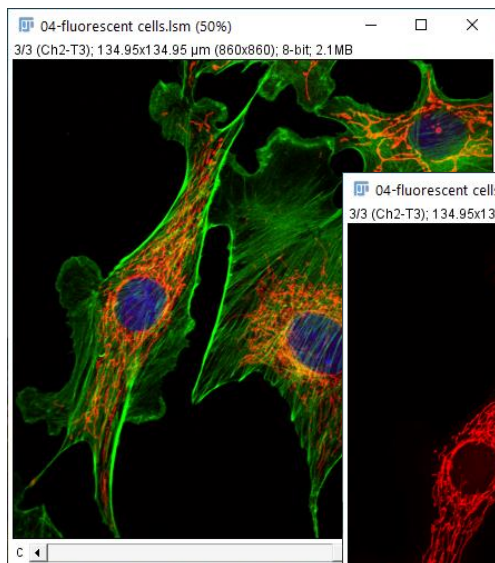
Z

T

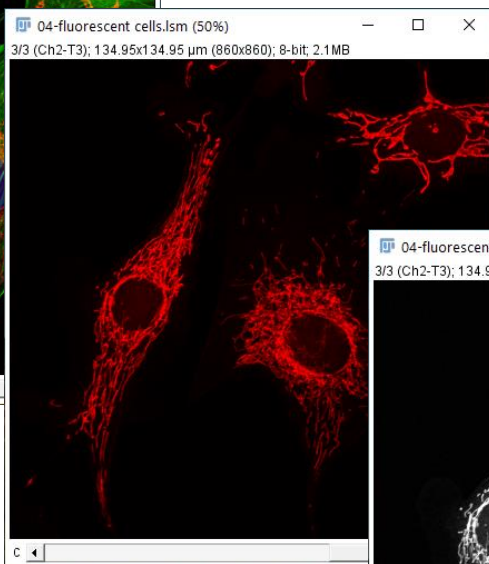


## Channels tool (Shift + Ctrl + Z)

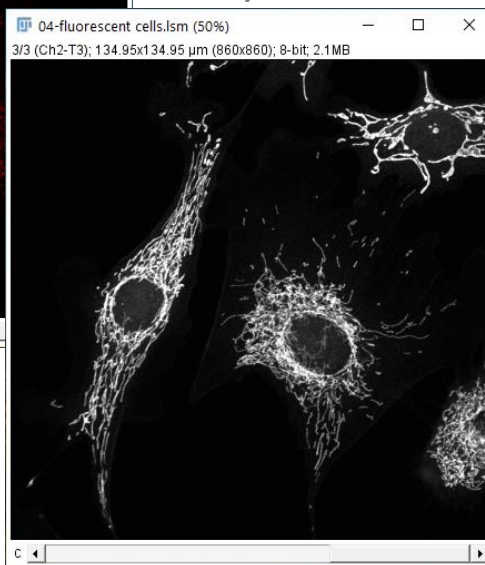
Composite



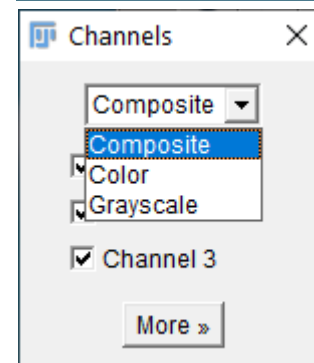
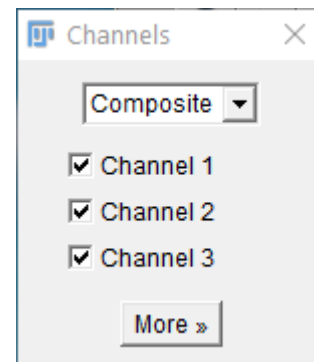
Color



Grayscale



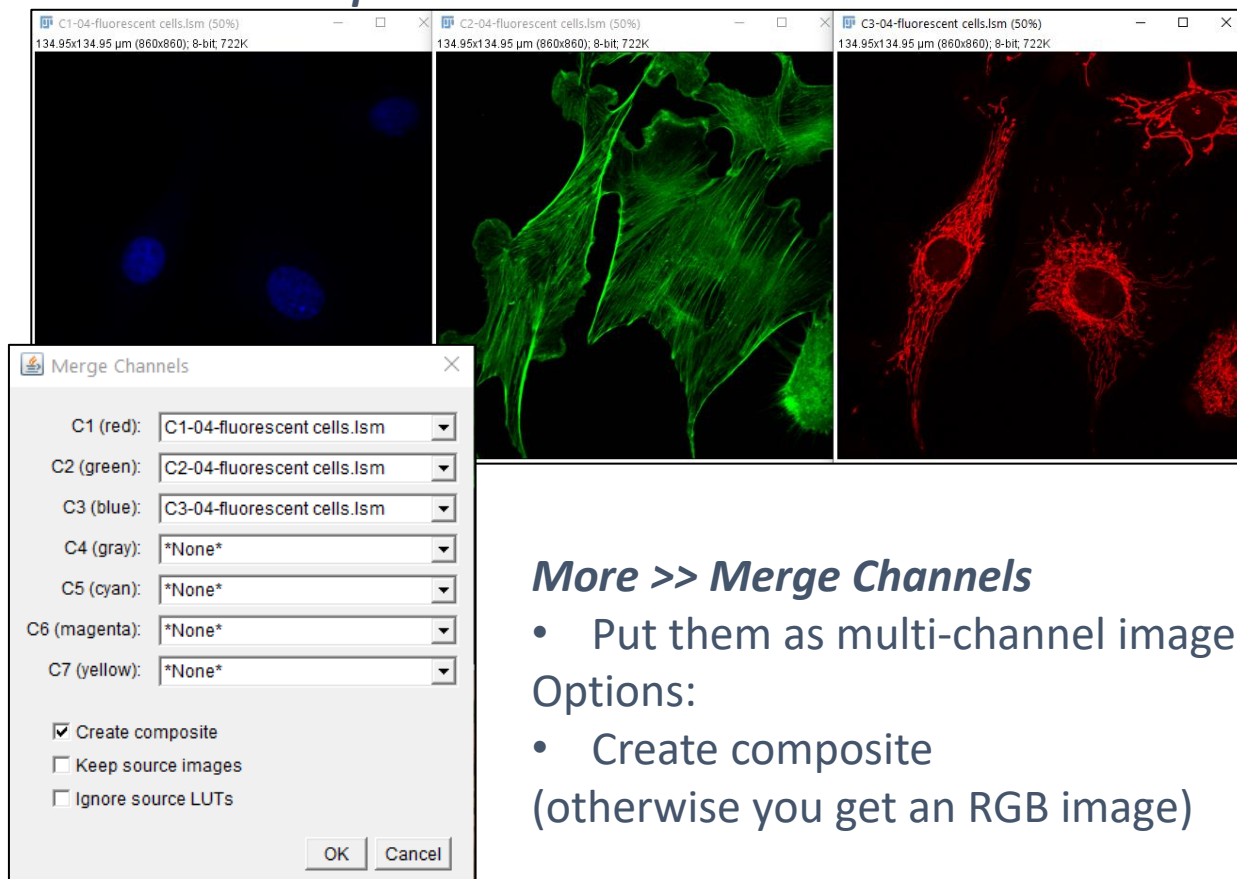
*Image / Color > Channels tool*





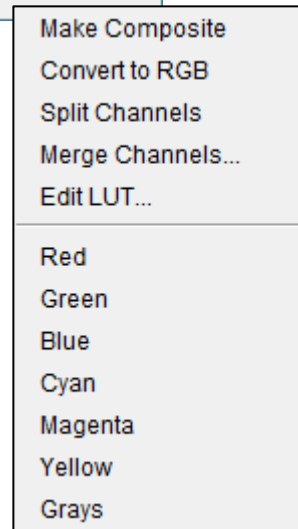
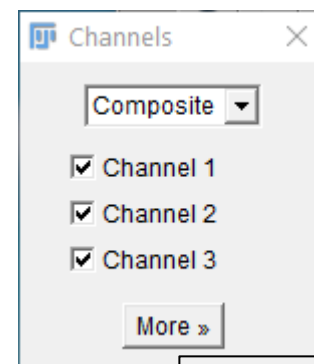
## Channels tool (Shift + Ctrl + Z)

**More >> Split Channels**



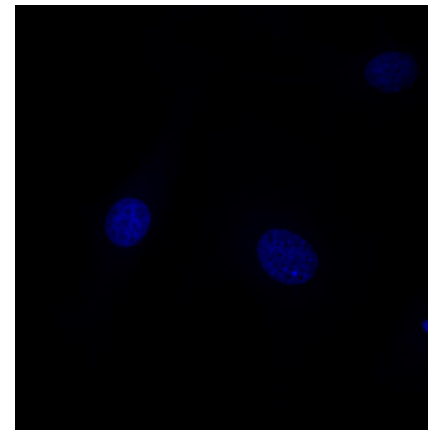
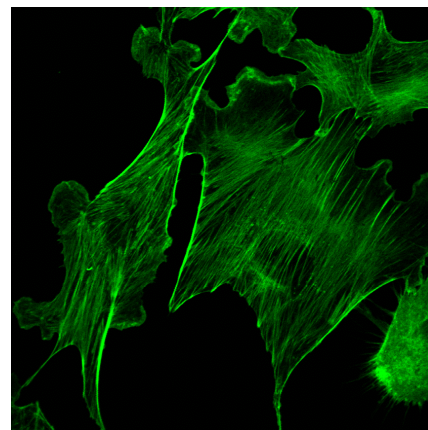
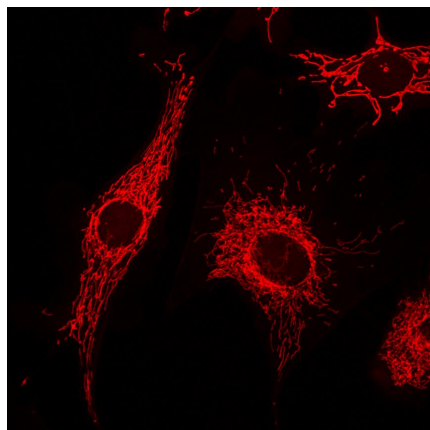
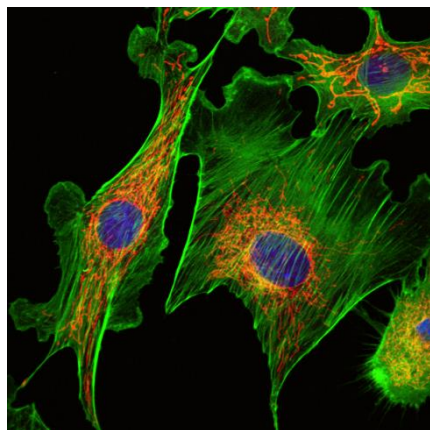
**More >> Merge Channels**

- Put them as multi-channel image
- Options:
- Create composite  
(otherwise you get an RGB image)

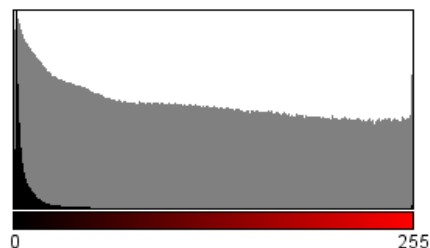




## Checking image brightness & bit depth – Image Histograms (Ctrl+H)



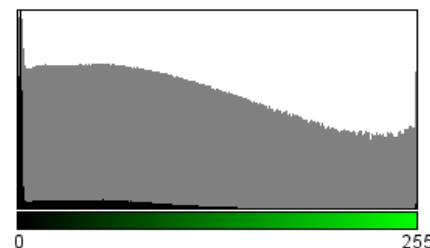
3 channels  
8-bits per channel



Count: 739600  
Mean: 21.371  
StdDev: 45.341

Min: 0  
Max: 255  
Mode: 1 (143778)

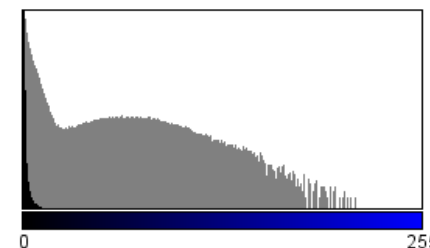
Min: 0  
Max: 255  
Mode: 1 (143778)



Count: 739600  
Mean: 35.707  
StdDev: 46.293

Min: 0  
Max: 255  
Mode: 1 (224321)

Min: 0  
Max: 255  
Mode: 1 (224321)

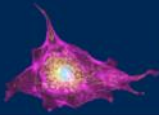


Count: 739600  
Mean: 4.209  
StdDev: 15.016

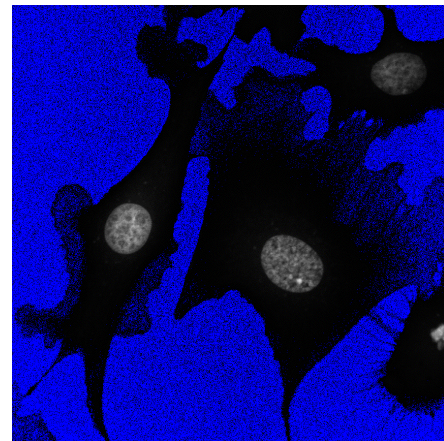
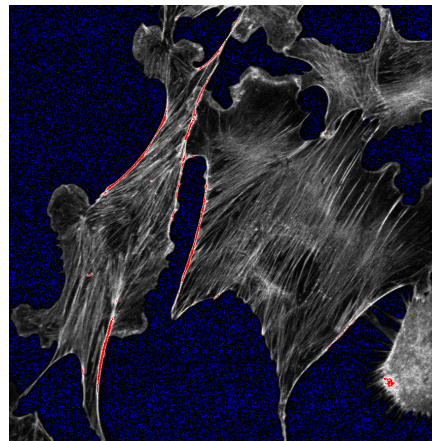
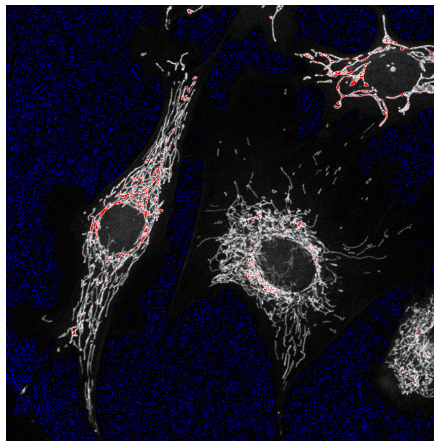
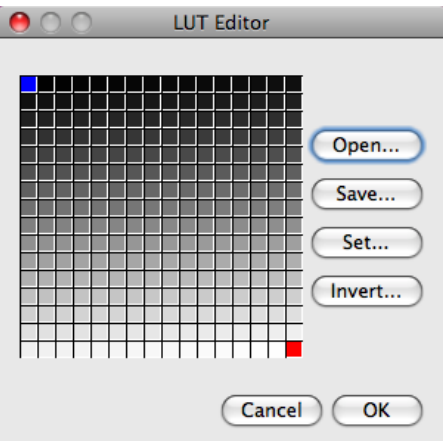
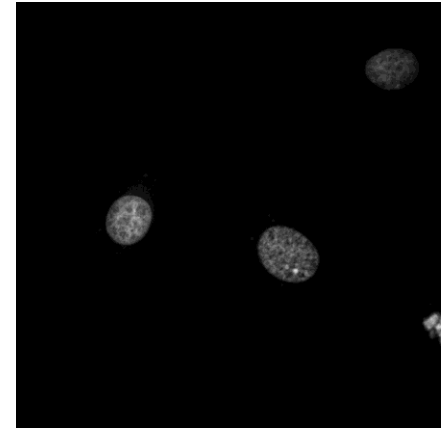
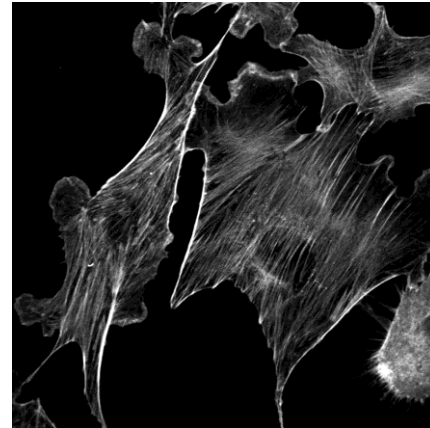
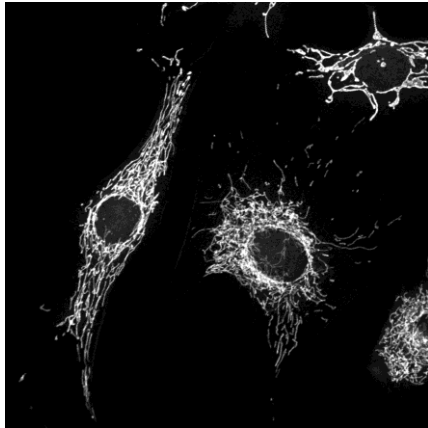
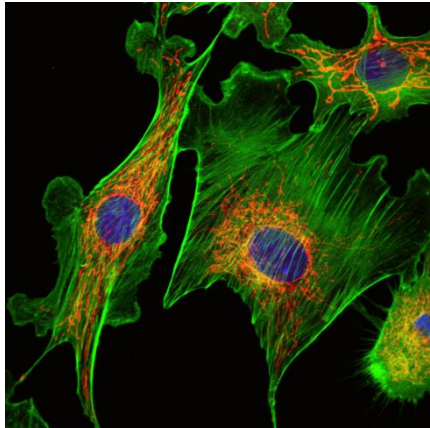
Min: 0  
Max: 218  
Mode: 0 (312533)

Min: 0  
Max: 218  
Mode: 0 (312533)



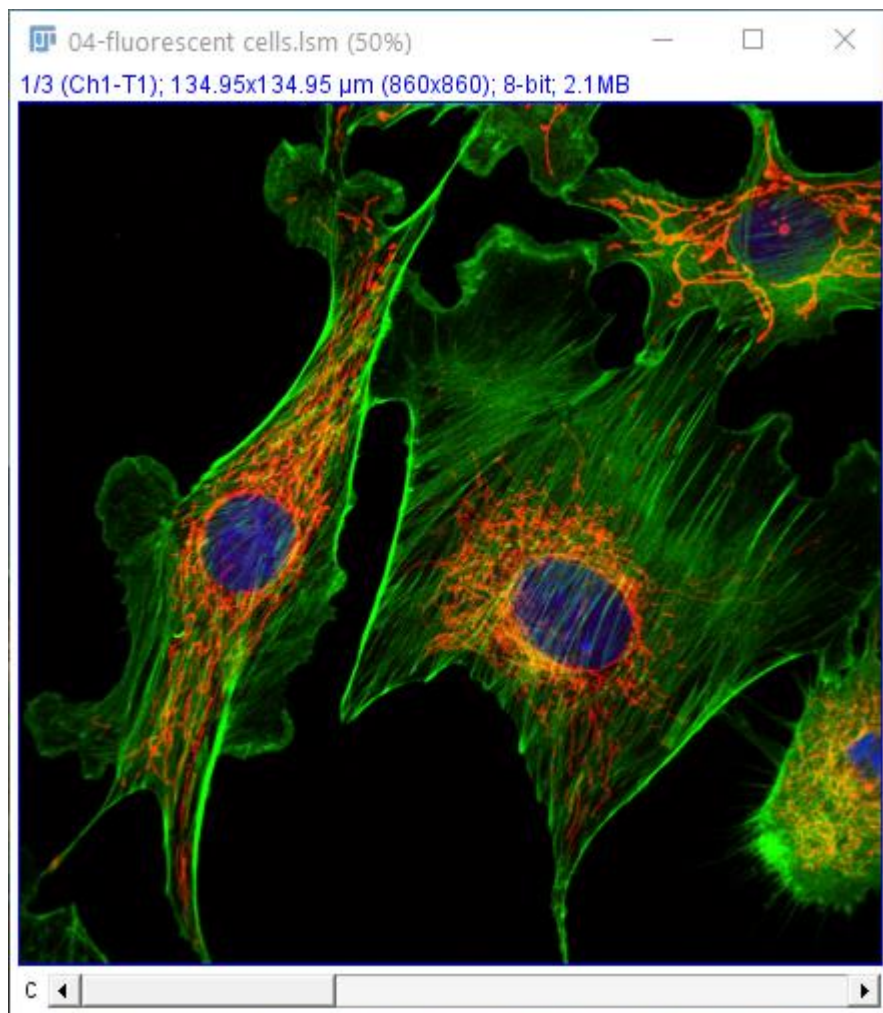


## Checking image brightness – using a Hi-Lo LUT

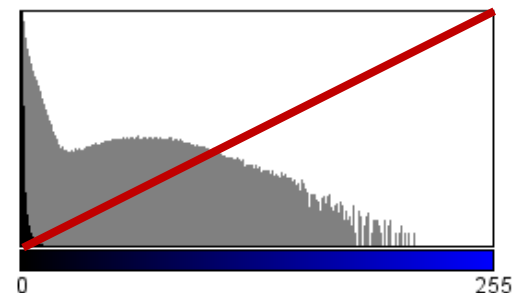
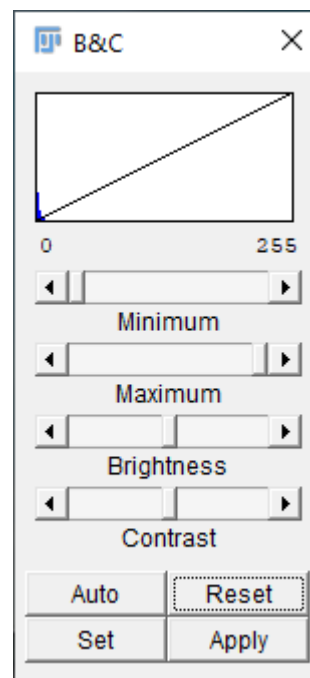




## Changing image brightness



***Image / Adjust >  
Brightness/Contrast...***

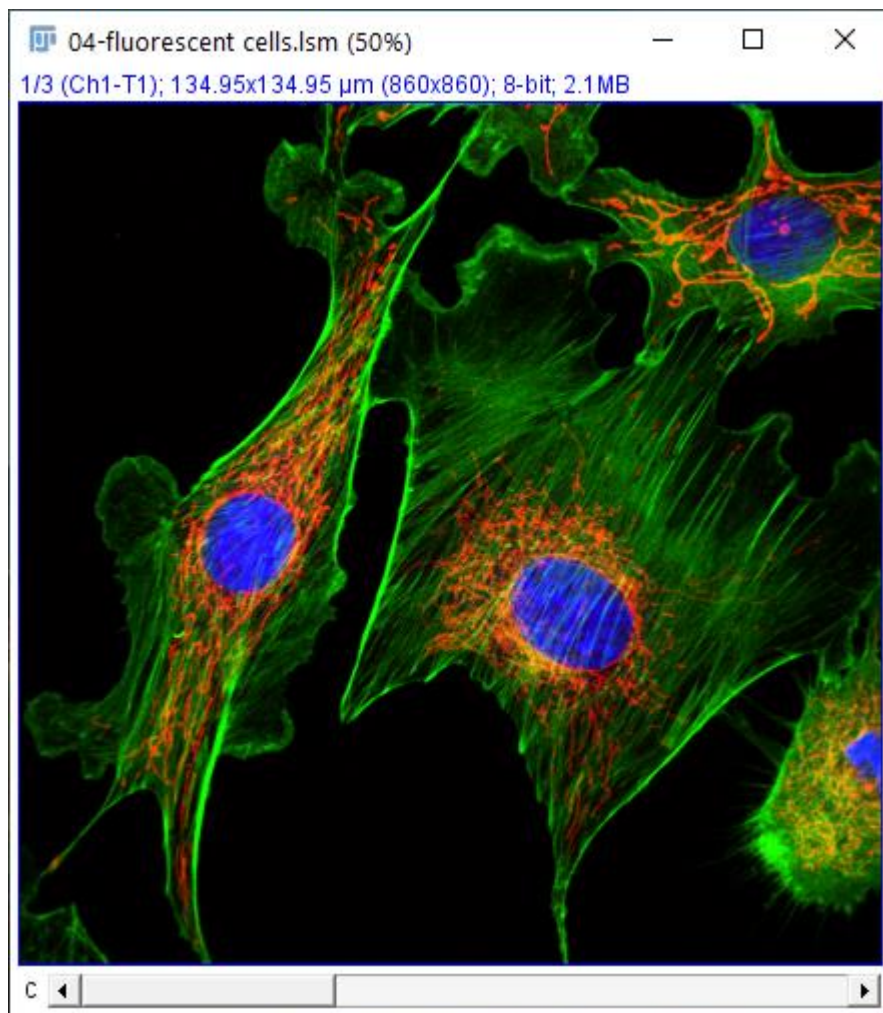


Count: 739600  
Mean: 4.209  
StdDev: 15.016

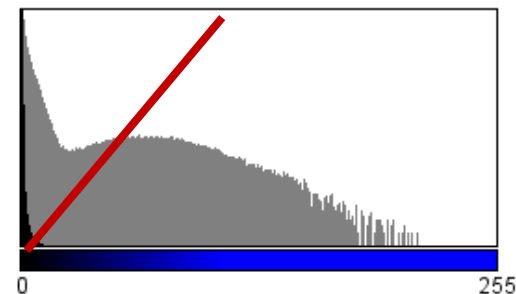
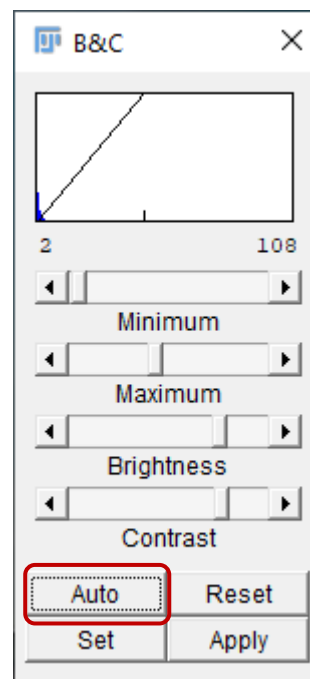
Min: 0  
Max: 218  
Mode: 0 (312533)



## Changing image brightness



*Image / Adjust >  
Brightness/Contrast...*



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

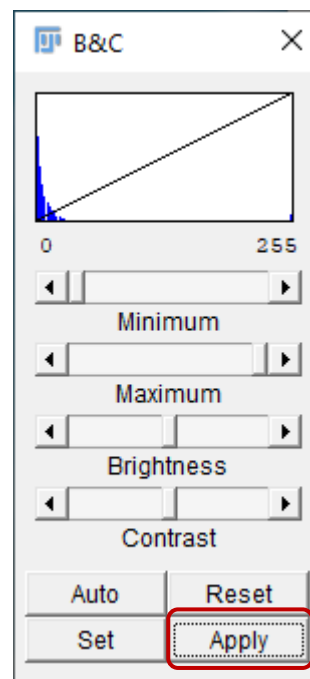




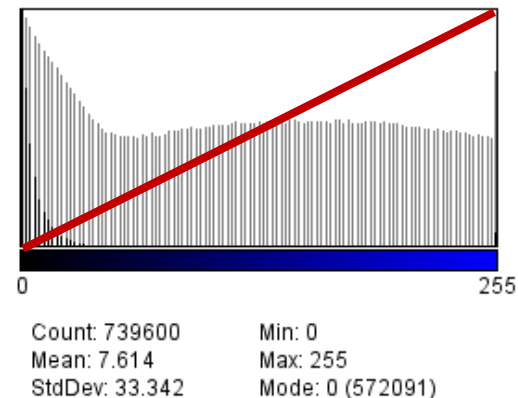
## Changing image brightness



### *Image / Adjust > Brightness/Contrast...*

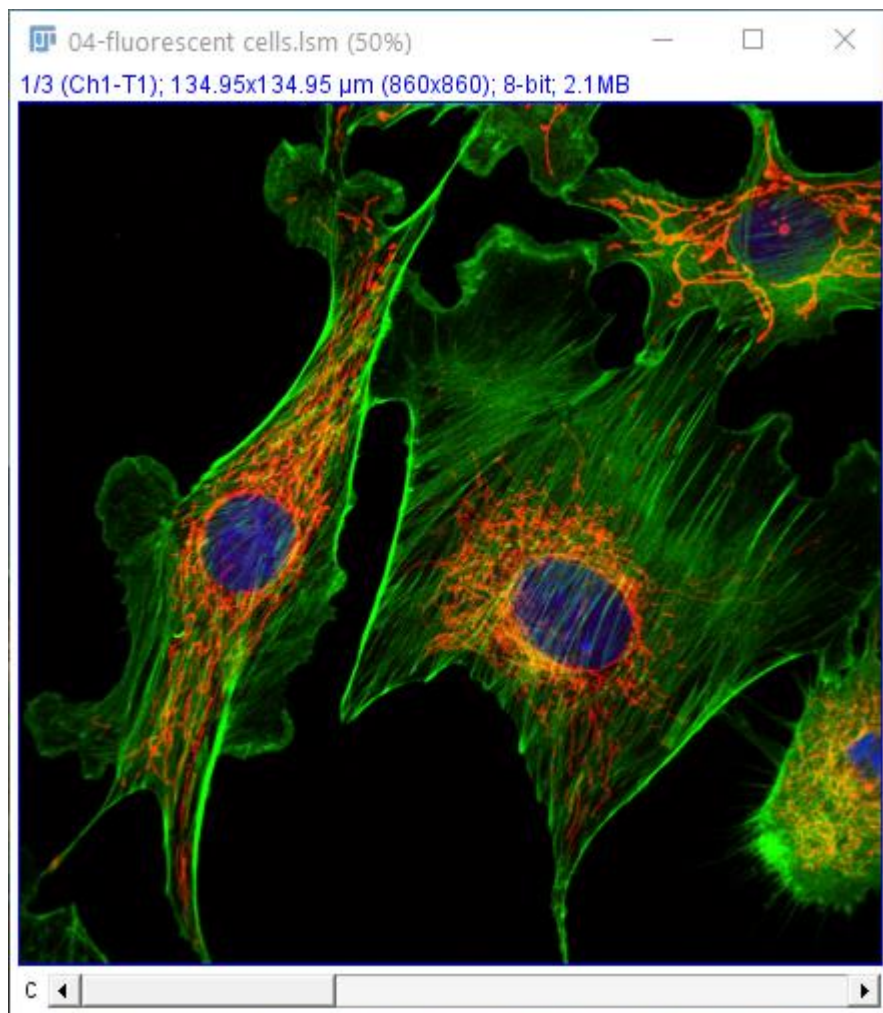


If you press 'Apply' in B&C you change the image data!

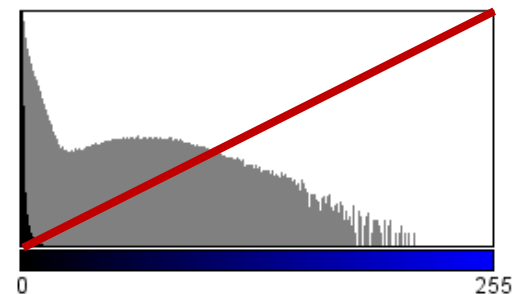
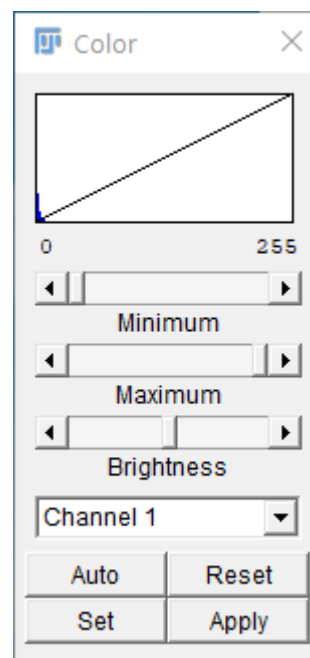




## Changing image brightness



*Image / Adjust >  
Color Balance...*

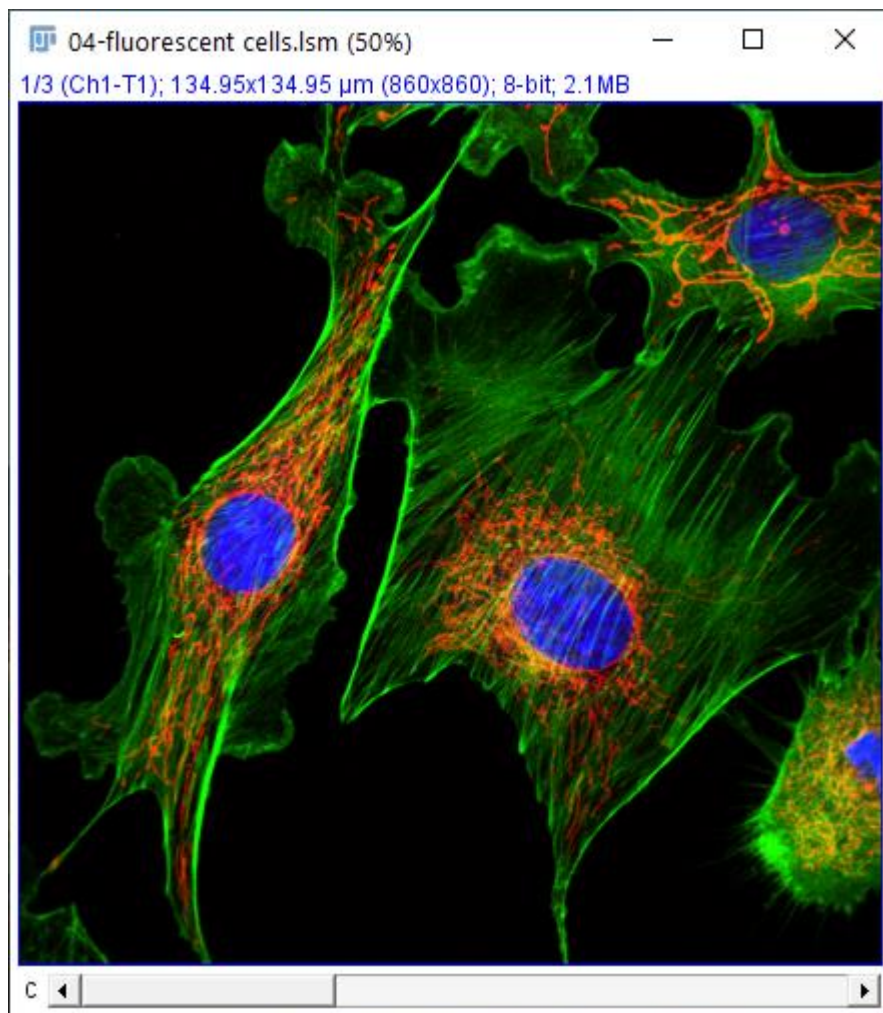


Count: 739600  
Mean: 4.209  
StdDev: 15.016

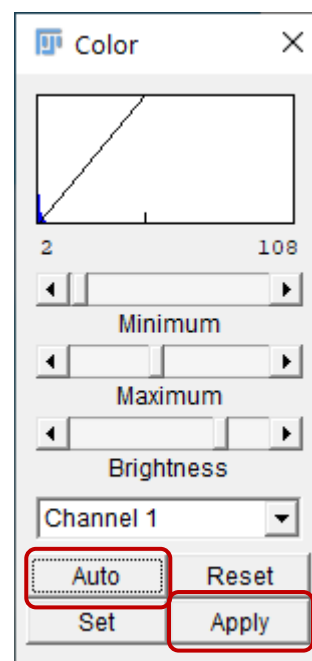
Min: 0  
Max: 218  
Mode: 0 (312533)



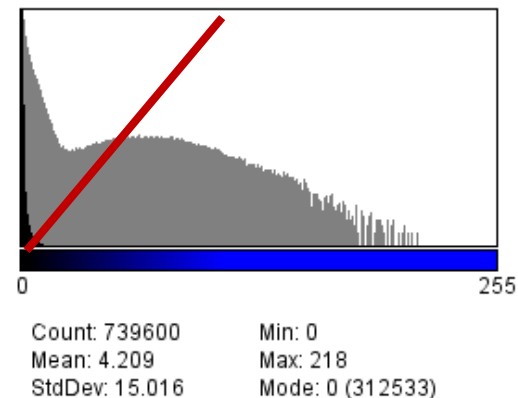
## Changing image brightness



### *Image / Adjust > Color Balance...*



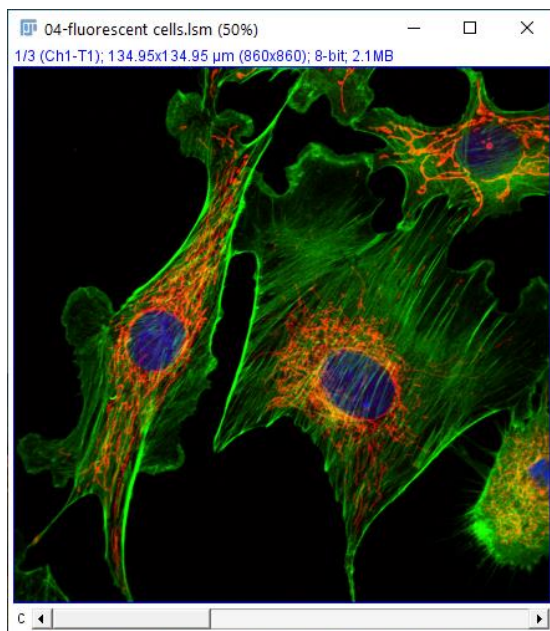
If you press 'Apply' in Color Balance you only change the view.



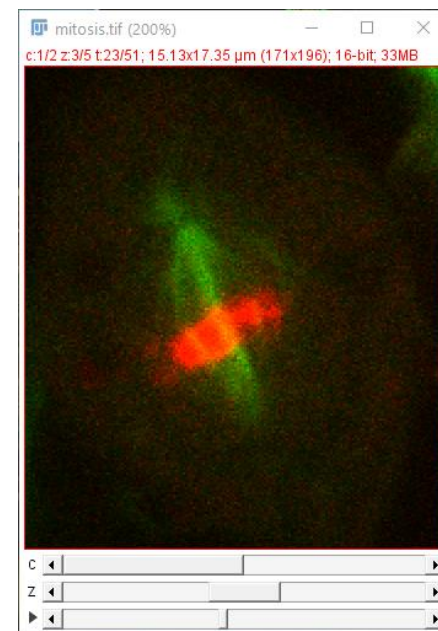
If you save the image as a .tif & re-open in Fiji, the image is displayed as you set it here.



## Multi dimensional images



**Stack.** Each image is a 'slice'  
It can be a set of:  
Channels **or**  
Z slices **or**  
Timepoints

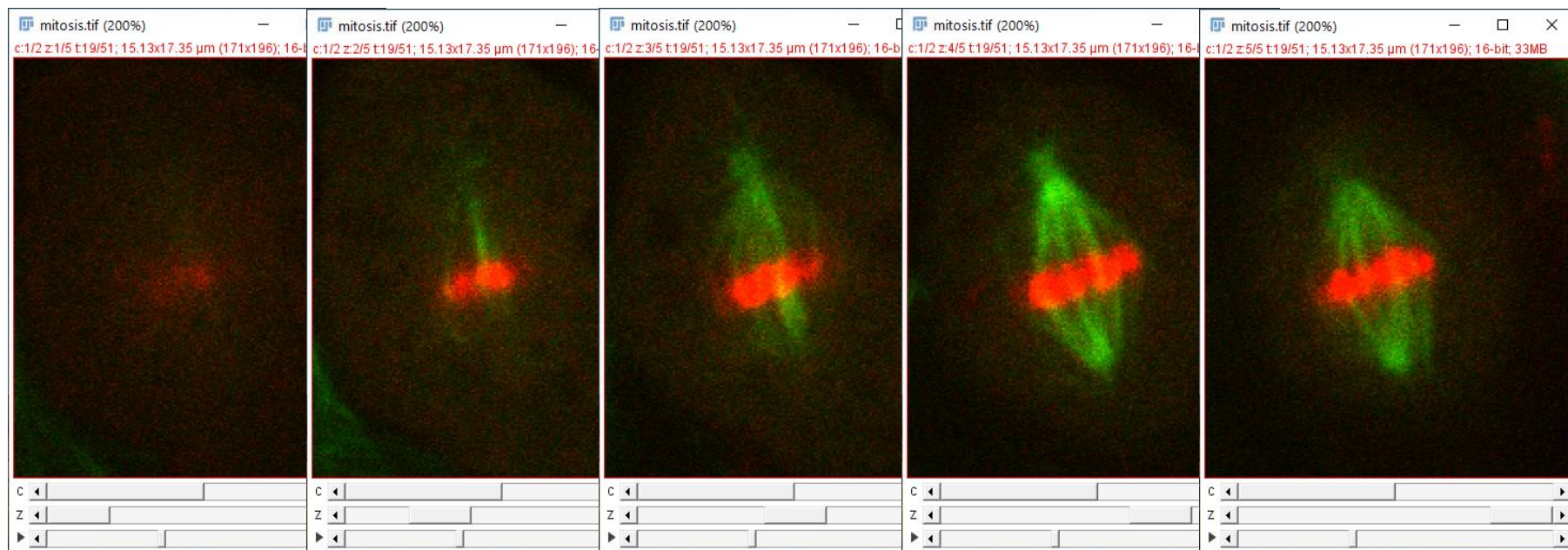


**Hyperstack.** Each image is a 'slice'  
It can be a set of:  
Channels **&/or**  
Z slices **&/or**  
Timepoints





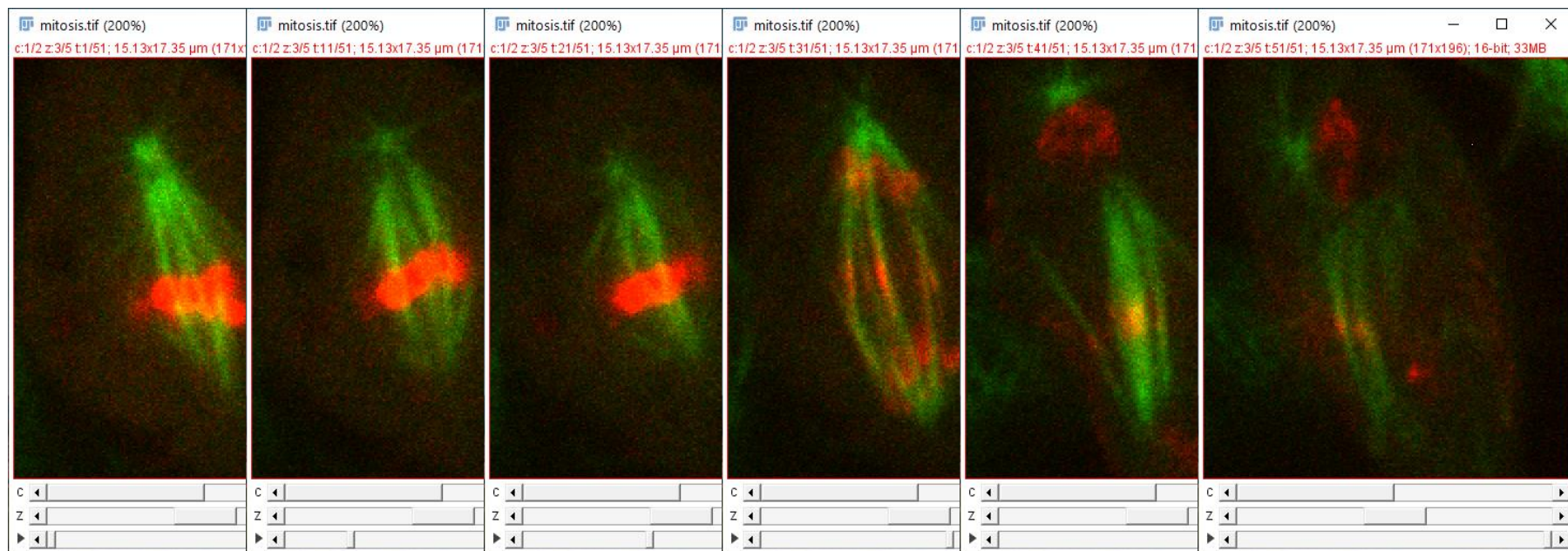
## Multi dimensional images



5 z slices



## Multi dimensional images

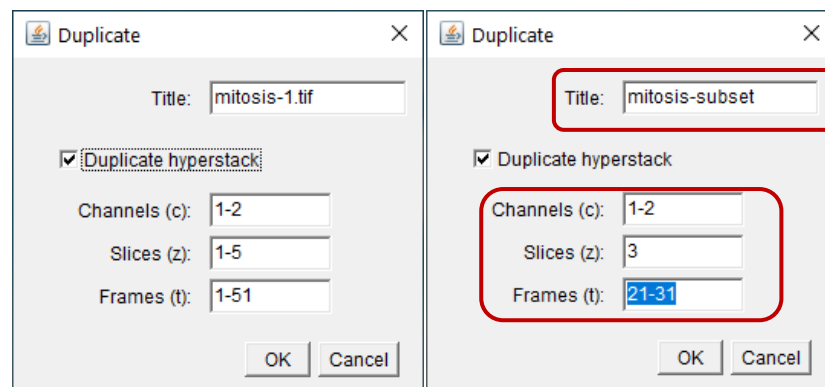
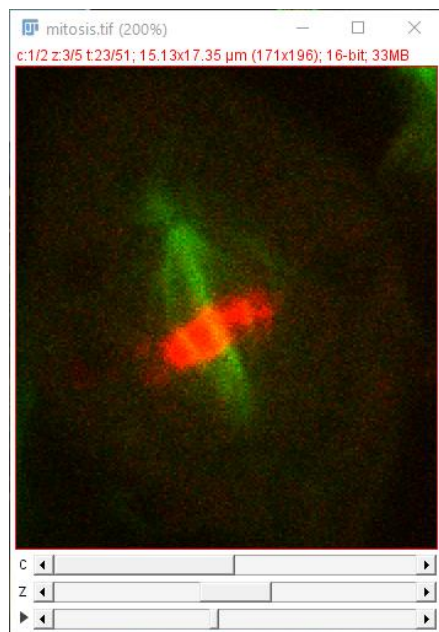


51 time points



## Extract parts of multi dimensional images

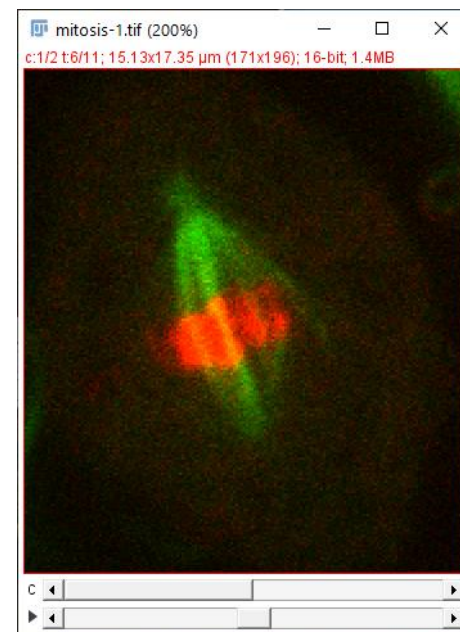
### *Image / Duplicate... (Ctrl + Shift + D)*



Make a copy of  
the entire  
hyperstack

A subset of  
some or all of  
the channels,  
slices or  
timepoints.

You can also change the title

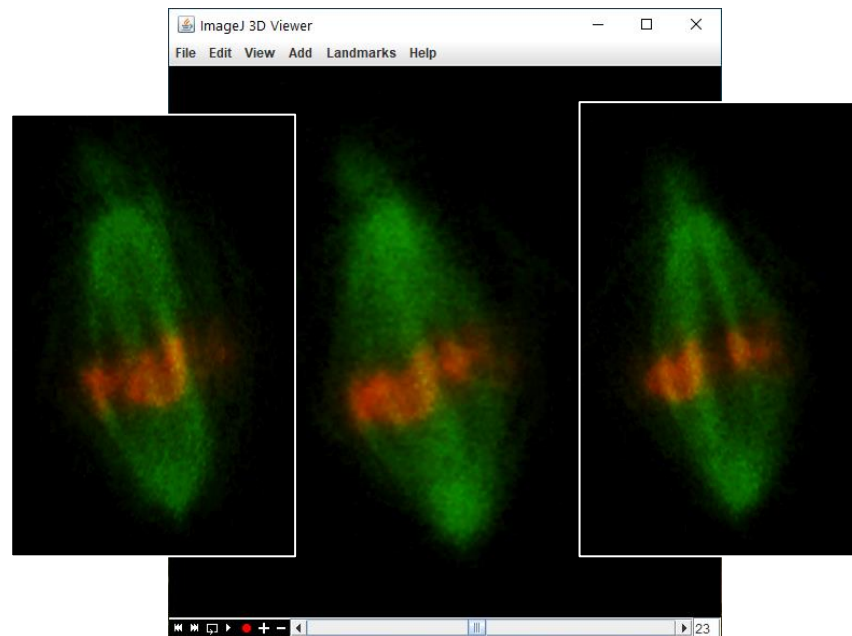
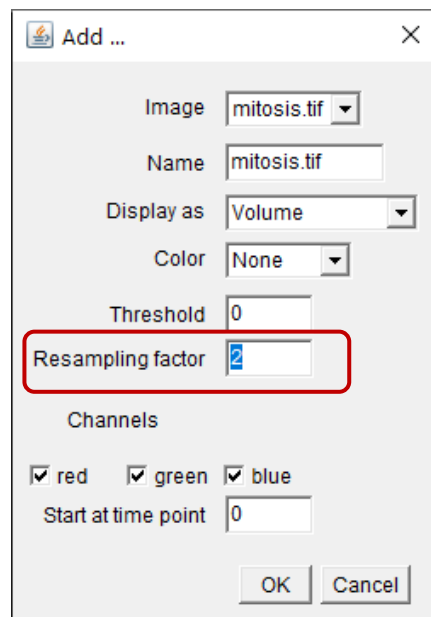
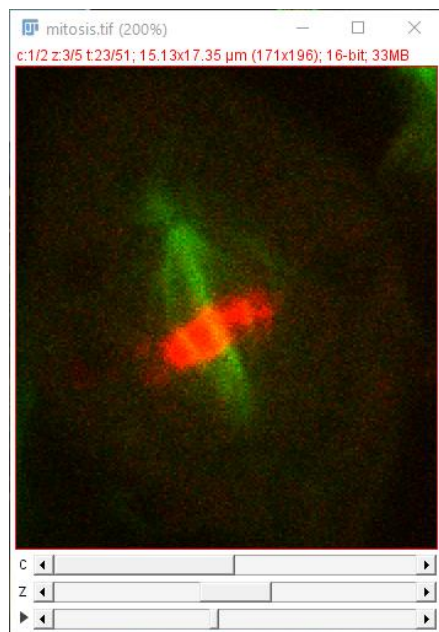






## Make a 3D projection

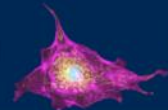
### *Plugins / 3D Viewer*



Will keep a time series (plays as a movie) if present

You can view the 3D projection from any angle...

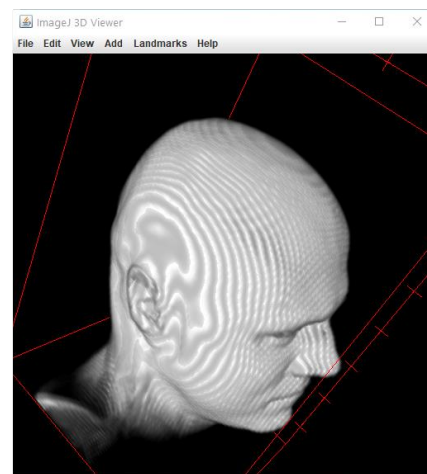
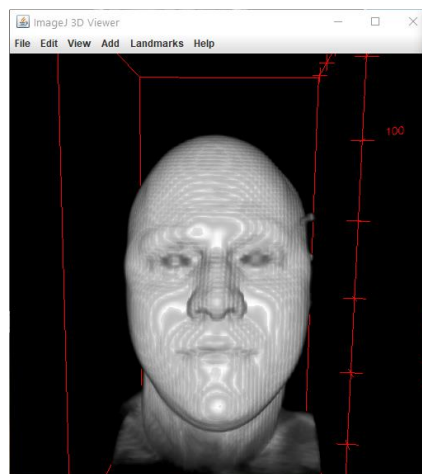
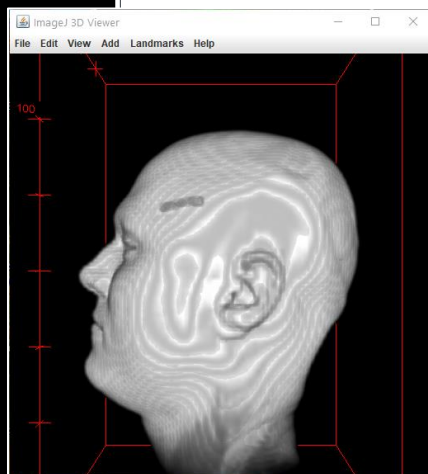
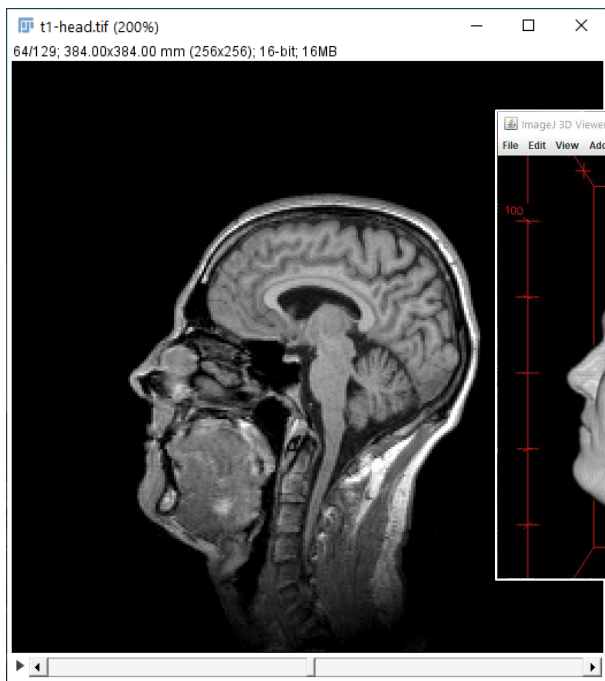




## Make a 3D projection

### *Plugins / 3D Viewer*

You can view the 3D projection from any angle...



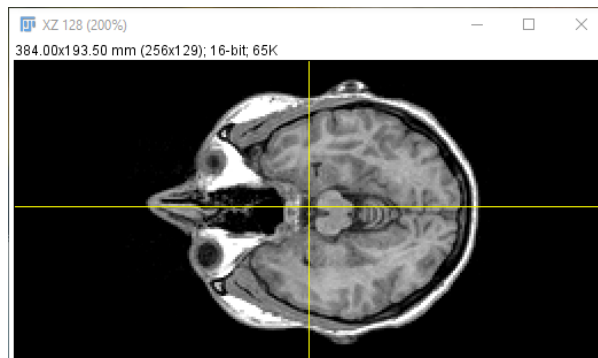
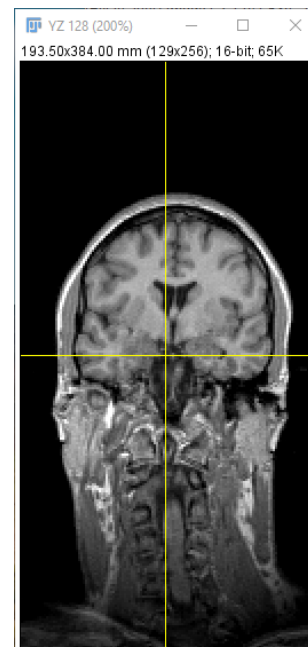


## View from sides (orthogonal views)

### Image / stacks / Orthogonal Views

(Ctrl + Shift + H)

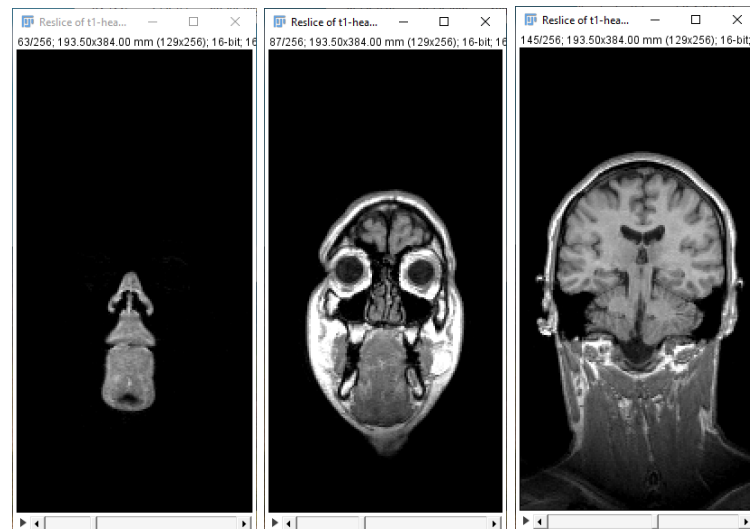
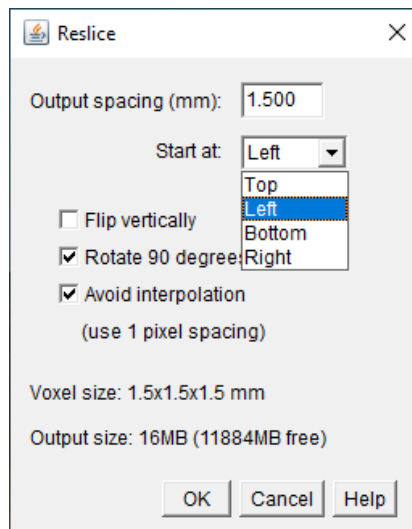
Move the lines  
to view different  
locations





## Reslice to rotate the whole stack

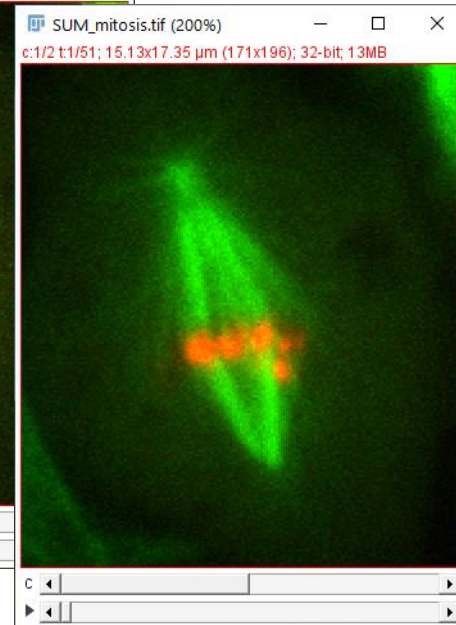
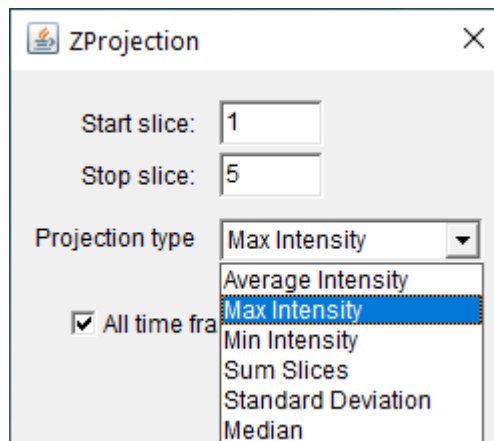
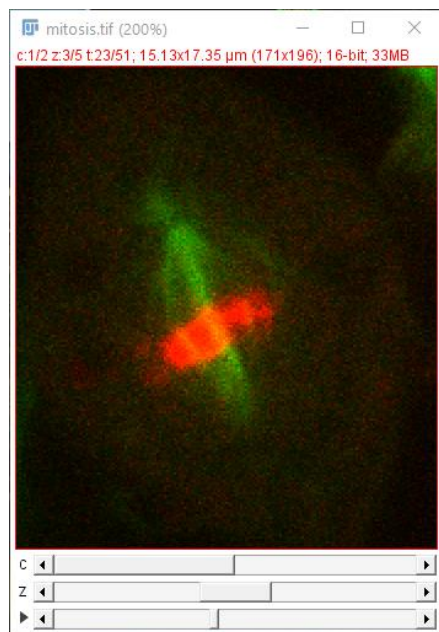
*Image / stacks / Reslice (Ctrl + /)*





Make a projection (flatten) a 3D image

### *Image / Stacks / Z- Project...*



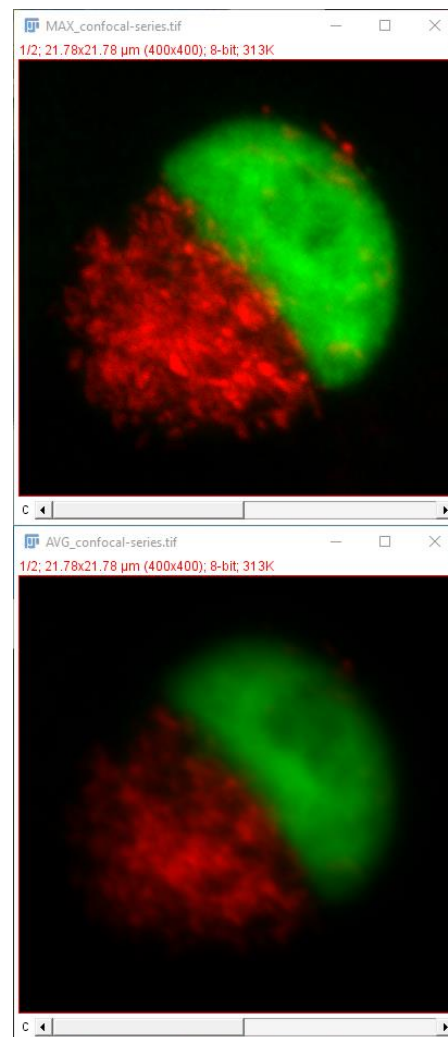
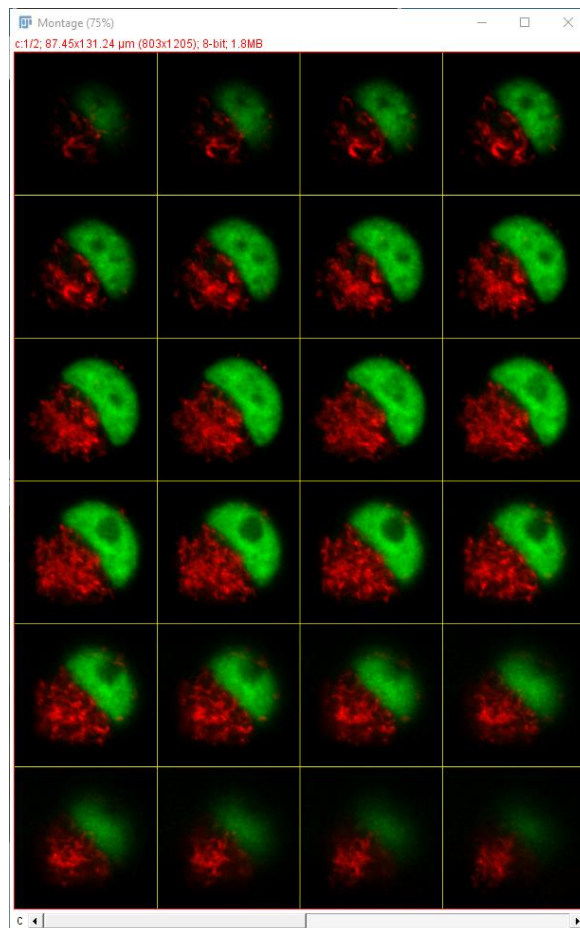
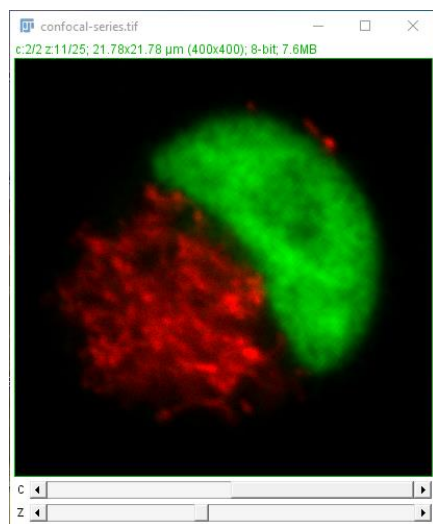
**Max intensity:** for each pixel position (x,y) look through every z slice, and keep only the brightest pixel

**Sum Slices:** for each pixel position (x,y) make a new image equal to the sum of every z slice



## Make a projection (flatten) a 3D image

Different projections for  
different purposes



Max  
Projection  
(MaxP or MIP)  
for a nice  
image

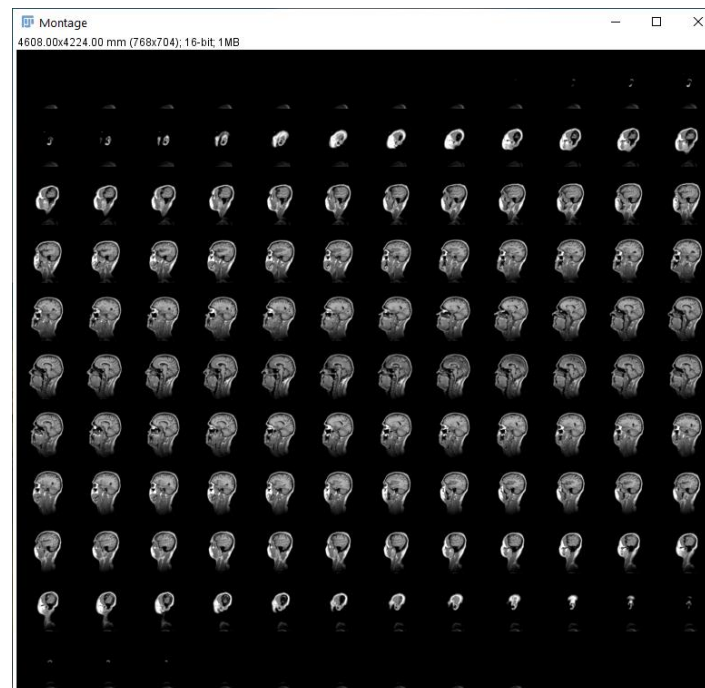
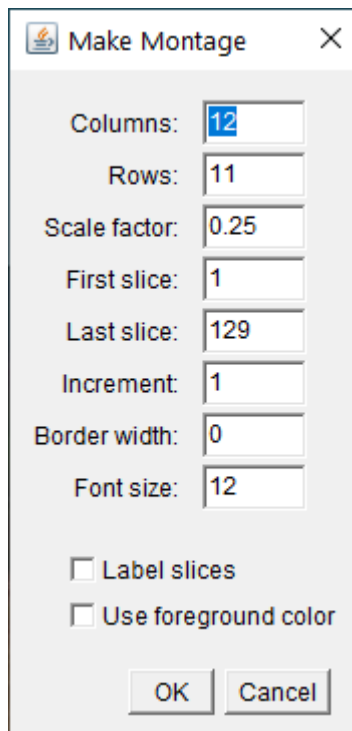
Average or  
Sum for  
measurements





## Make a montage of any stack

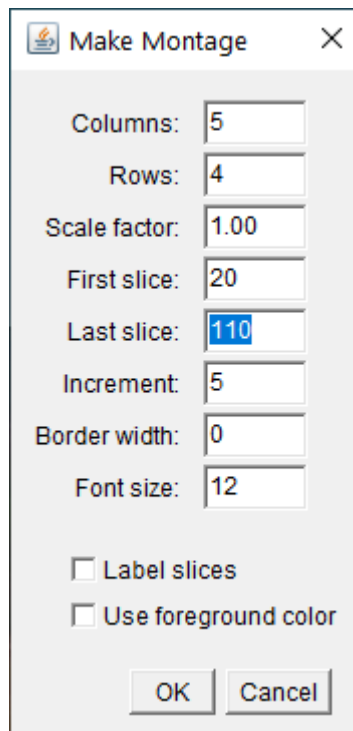
*Image / Stacks / Make montage...*





## Make a montage of any stack

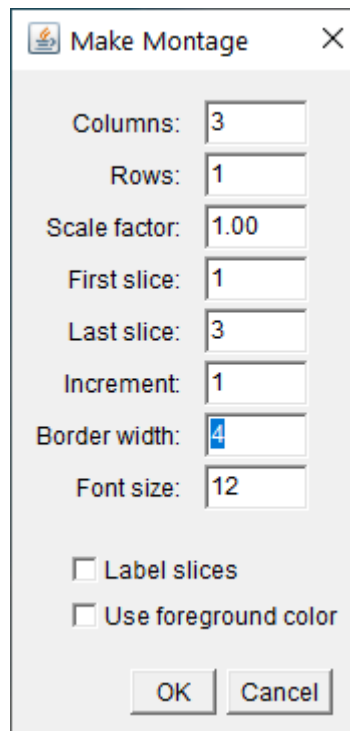
*Image / Stacks / Make montage...*





## Use Make Montage to make a figure

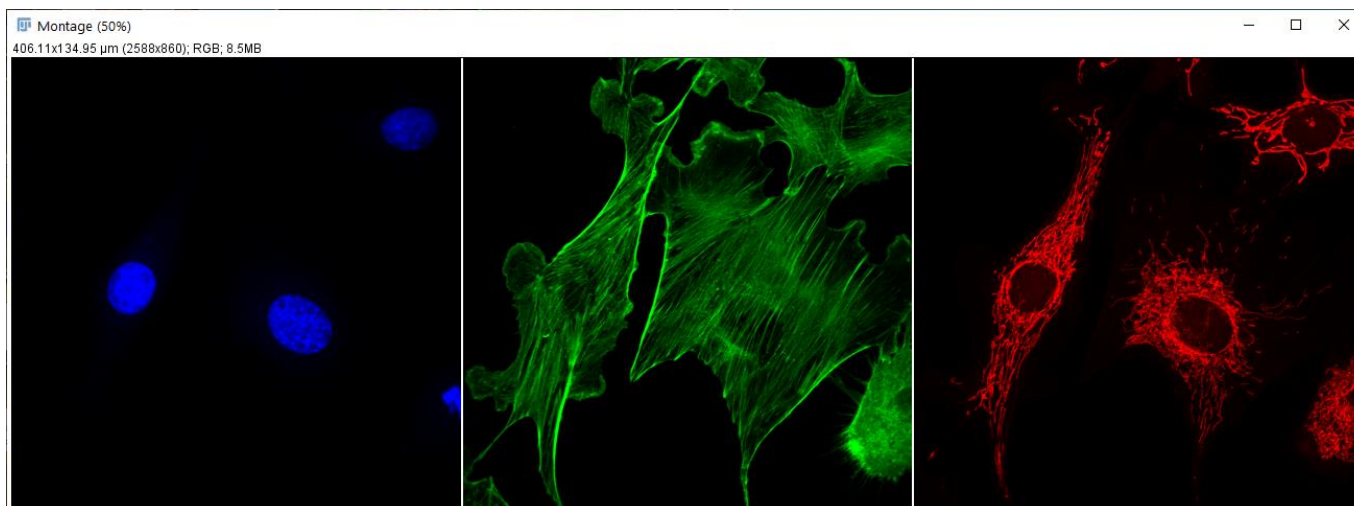
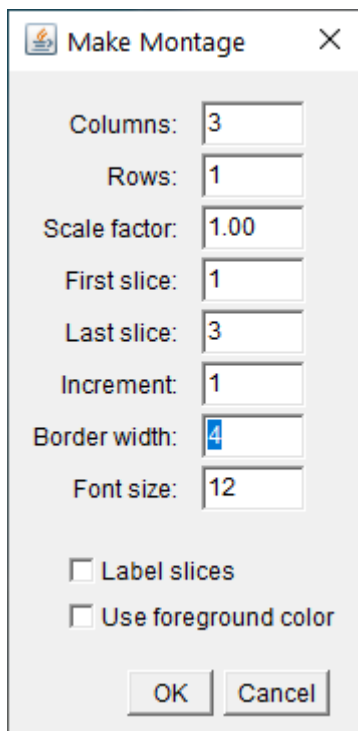
*Image / Stacks / Make montage...*







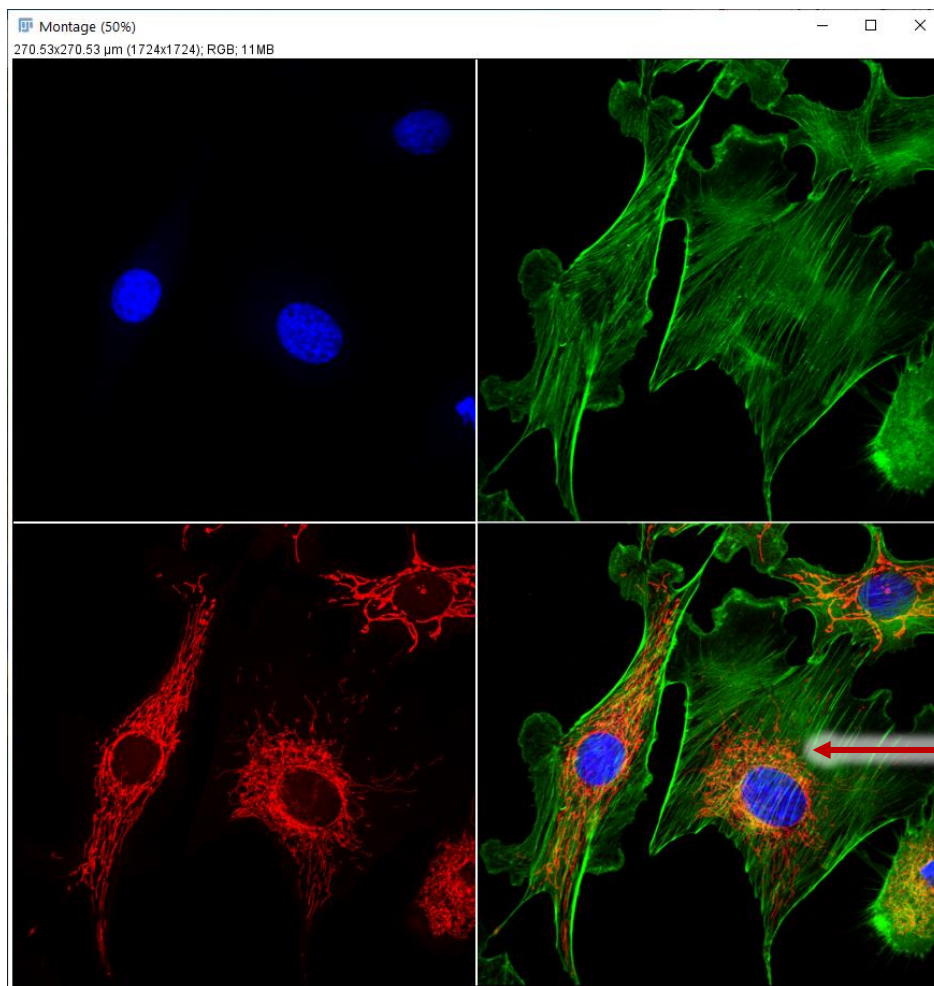
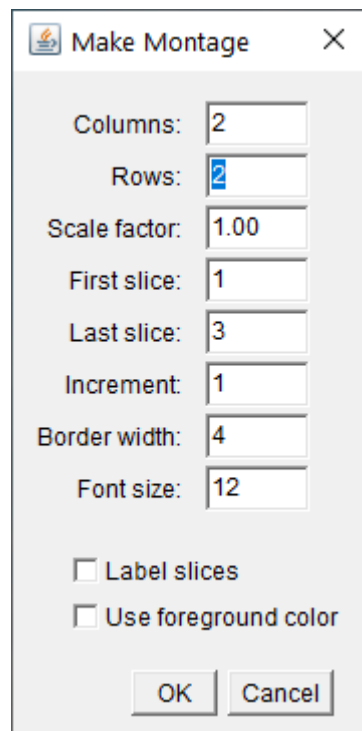
## Use Make Montage to make a figure



- The output is an RGB image (as it is for display only)
- Add borders.
- Change layout (3x1, 2x2, etc)
- Include an overlay (not built in but possible...)



## Use Make Montage to make a figure



This panel was initially empty. Copy / paste the original image (as an RGB) onto the montage.



## Other ImageJ tools to make figures

- **Magic Montage**

Simple interface to make multi panel figures  
(built in to Fiji)

- **FigureJ**

More advanced and well supported

<https://imagejdocu.tudor.lu/plugin/utilities/figurej/start>

- **QuickFigures**

New (2021) plugin available as a Fiji update site

Documentation and video tutorials here:

<https://github.com/grishkam/QuickFigures/blob/master/UserGuide/User%20Guide.md>



## Exercises using Fiji. Session 2- getting to grips with Fiji

### Viewing and manipulating images in Fiji

- 4) Image Histogram. Image brightness. LUTs.
- 5) Make a figure using Montage function