



# 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>

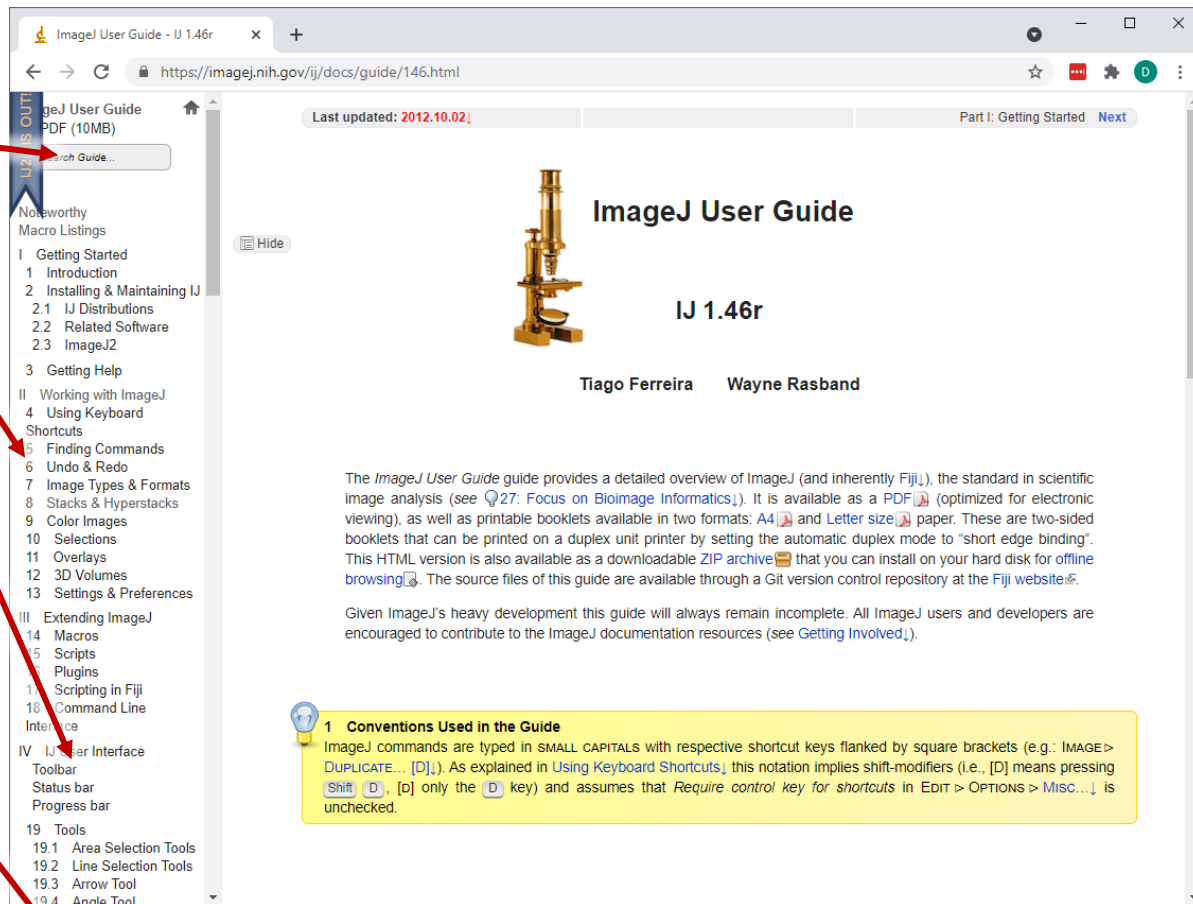
V	Menu Commands
26	File>
26.1	New>
26.2	Open...[o]
26.3	Open Next [O]
26.4	Open Samples>
26.5	Open Recent>
26.6	Import>
26.7	Close [w]
26.8	Close All
26.9	Save [s]
26.10	Save As>
26.11	Revert [r]
26.12	Page Setup...
26.13	Print...[p]
26.14	Quit
27	Edit>
27.1	Undo [z]
27.2	Cut [x]
27.3	Copy [c]
27.4	Copy 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



ImageJ User Guide  
PDF (10MB)

Search Guide...

Noteworthy  
Macro Listings

1 Getting Started  
1 Introduction  
2 Installing & Maintaining IJ  
2.1 IJ Distributions  
2.2 Related Software  
2.3 ImageJ2  
3 Getting Help

II Working with ImageJ  
4 Using Keyboard Shortcuts  
5 Finding Commands  
6 Undo & Redo  
7 Image Types & Formats  
8 Stacks & Hyperstacks  
9 Color Images  
10 Selections  
11 Overlays  
12 3D Volumes  
13 Settings & Preferences

III Extending ImageJ  
14 Macros  
15 Scripts  
16 Plugins  
17 Scripting in Fiji  
18 Command Line Interface

IV User Interface  
19 Toolbars  
19.1 Area Selection Tools  
19.2 Line Selection Tools  
19.3 Arrow Tool  
19.4 Rotate Tool

ImageJ User Guide  
IJ 1.46r  
Tiago Ferreira Wayne Rasband

The *ImageJ User Guide* provides a detailed overview of ImageJ (and inherently *Fiji*), the standard in scientific image analysis (see [Q27: Focus on Bioimage Informatics](#)). It is available as a PDF (optimized for electronic viewing), as well as printable booklets available in two formats: **A4** and **Letter size** paper. These are two-sided booklets that can be printed on a duplex unit printer by setting the automatic duplex mode to "short edge binding". This HTML version is also available as a downloadable **ZIP archive** that you can install on your hard disk for **offline browsing**. The source files of this guide are available through a Git version control repository at the [Fiji website](#).

Given ImageJ's heavy development this guide will always remain incomplete. All ImageJ users and developers are encouraged to contribute to the ImageJ documentation resources (see [Getting Involved](#)).

**1 Conventions Used in the Guide**  
ImageJ commands are typed in **SMALL CAPITALS** with respective shortcut keys flanked by square brackets (e.g.: **IMAGE > DUPLICATE... [D]**). As explained in [Using Keyboard Shortcuts](#), this notation implies shift-modifiers (i.e., [D] means pressing **Shift** + **D**, [D] only the **D** key) and assumes that *Require control key for shortcuts* in **EDIT > OPTIONS > Misc...** is unchecked.



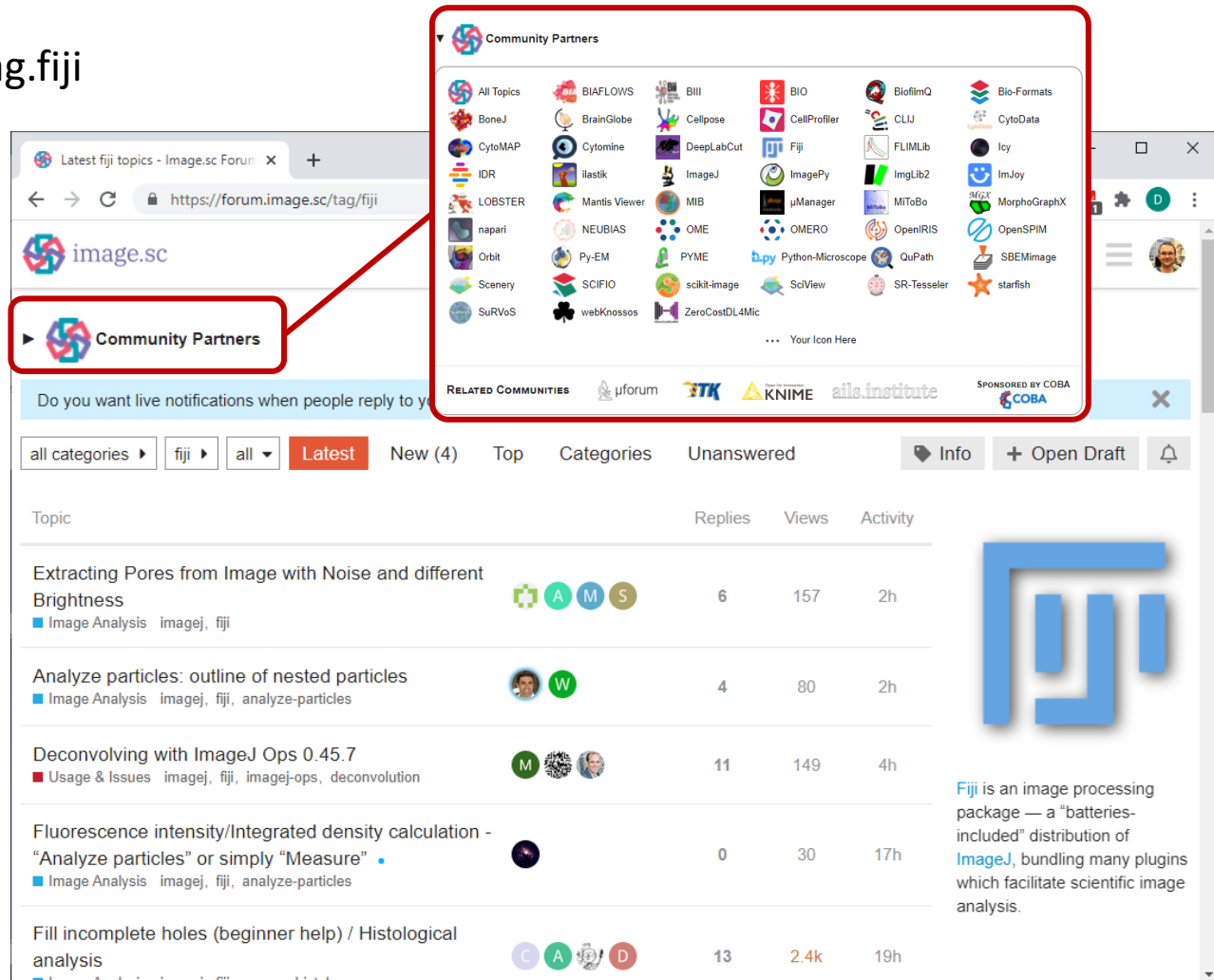
## Fiji on-line support

<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 image analysis tools and organizations. 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

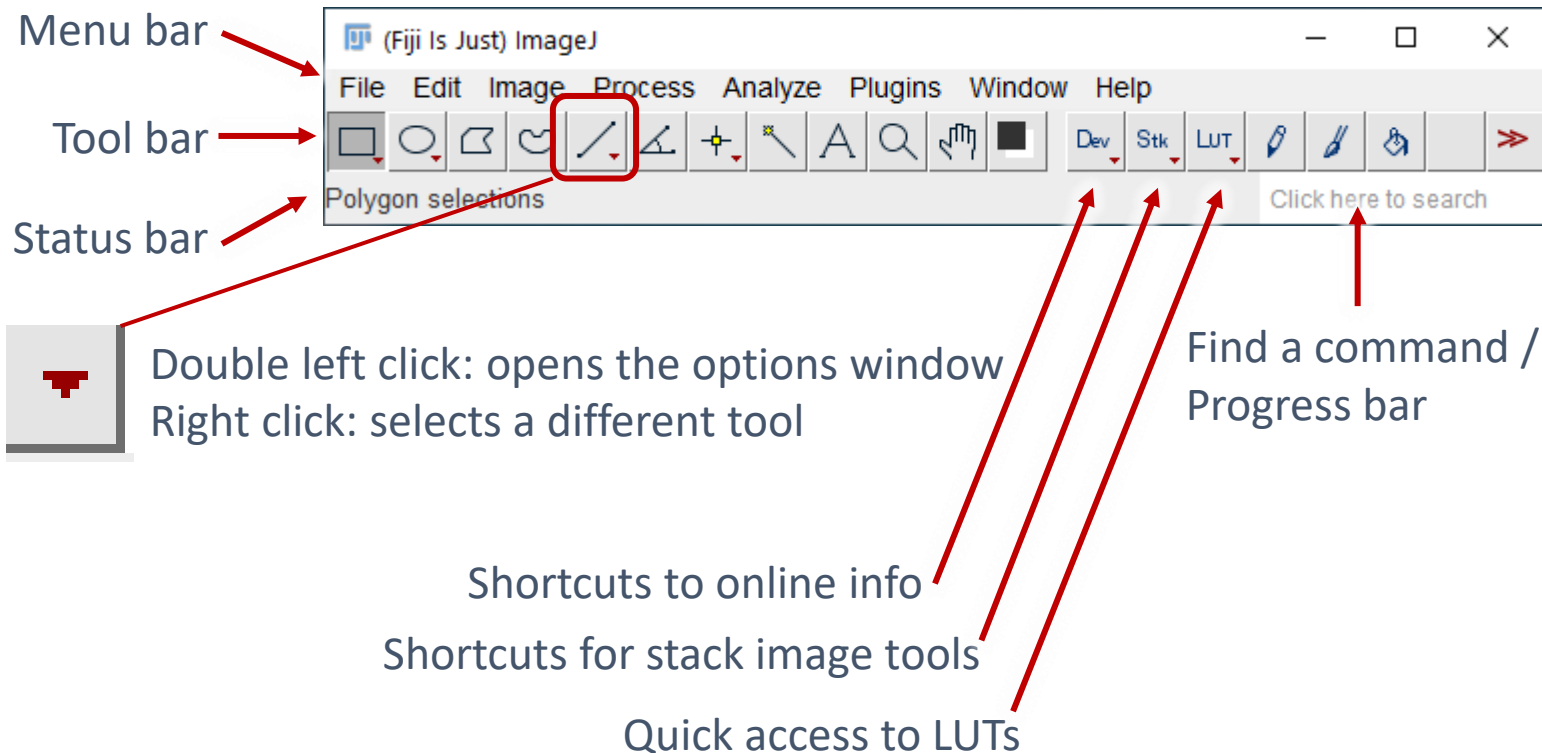
- List of many macros <http://rsbweb.nih.gov/ij/macros/>
- Macro commands <http://rsb.info.nih.gov/ij/developer/macro/functions.html>

### 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

---

- About Plugins
- About ImageJ...

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

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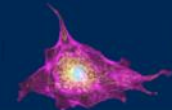
Update...

Upload Sample Image...

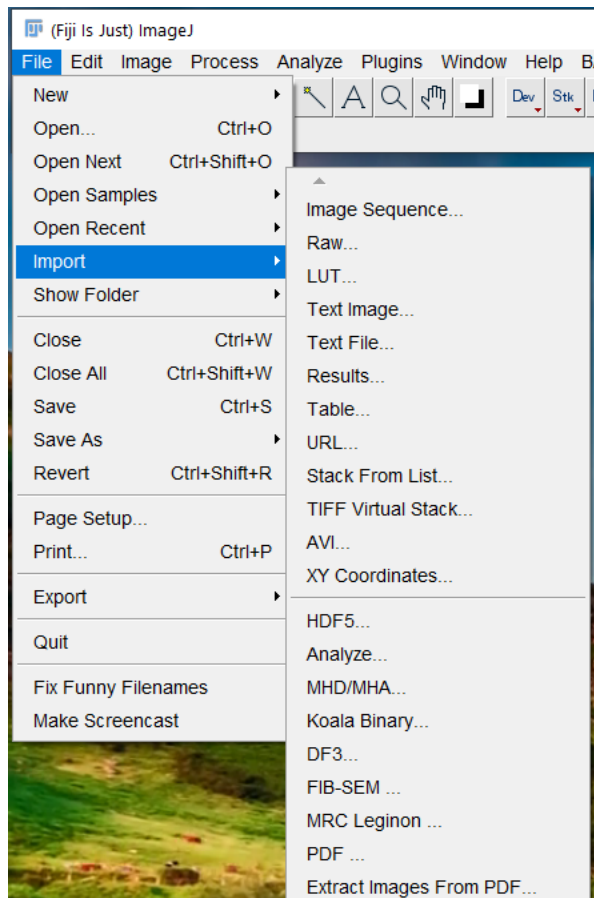
ImageJ Updater

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

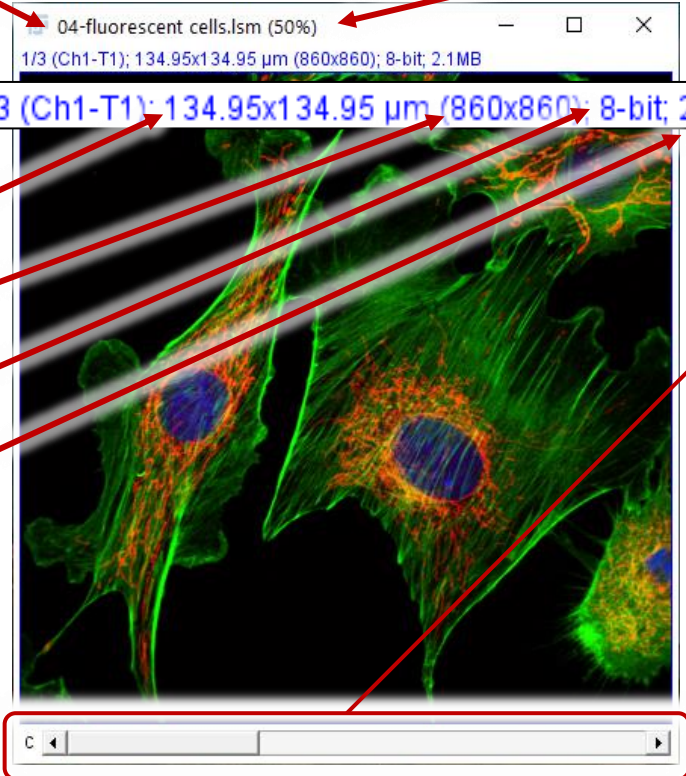


- ***File / Open...***
- ***File / Import >***
- Drag & drop  
Single file, multiple files, whole folders
- ***Plugins / BioFormats / Importer***





## Fiji image window



Filename

Multi channel image

Image zoom: adjust with + /- keys on keyboard

Image Info:

Channels (active / total)

Total size (if scale known)

Size in pixels

Bit depth

File size

Image dimensions control bar

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

04-fluorescent cells.lsm (50%)  
1/3 (Ch1-T1); 134.95x134.95 µm (860x860); 8-bit; 2.1MB

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

c



## Fiji image window

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

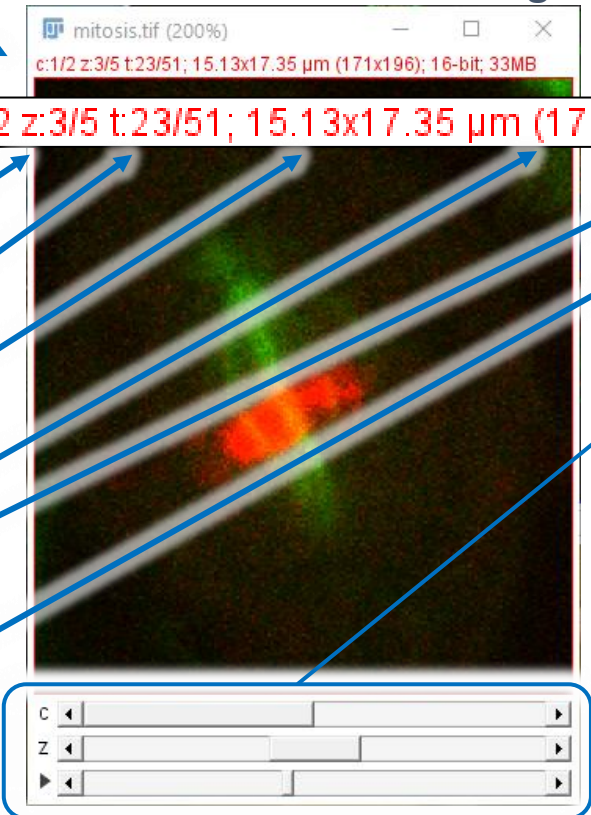
File size

Multi dimensional image

c:1/2 z:3/5 t:23/51; 15.13x17.35  $\mu\text{m}$  (171x196); 16-bit; 33MB

Image dimensions control bar

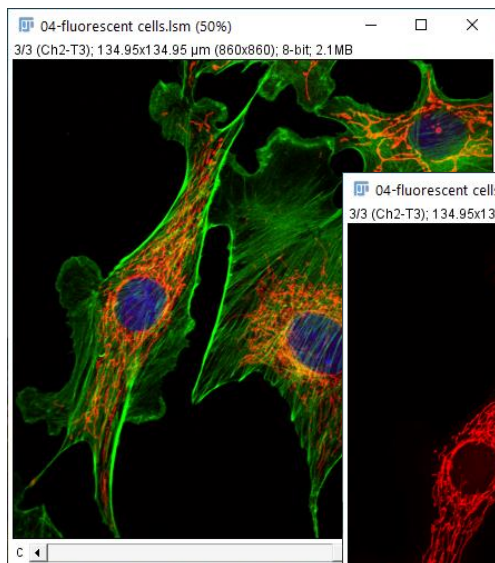
c = Channels  
z (3d images),  
t (time series)



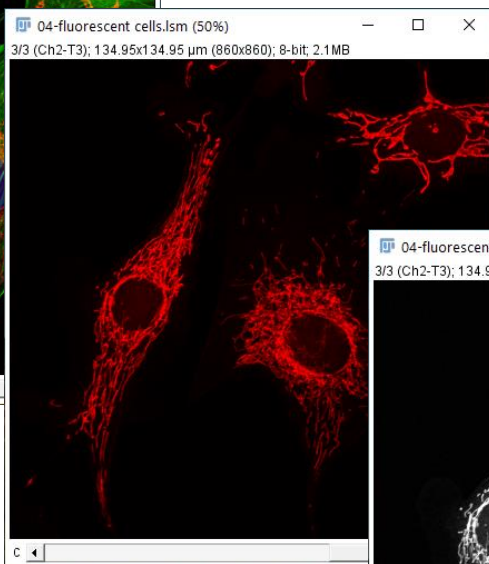


## Channels tool (Ctrl H)

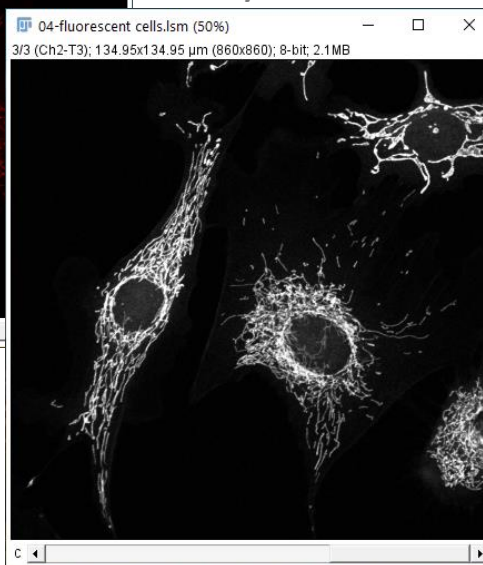
Composite



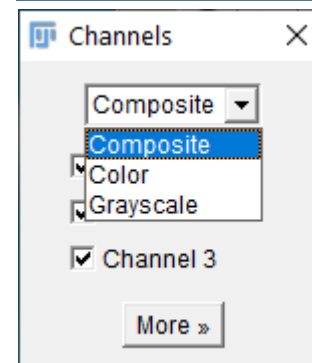
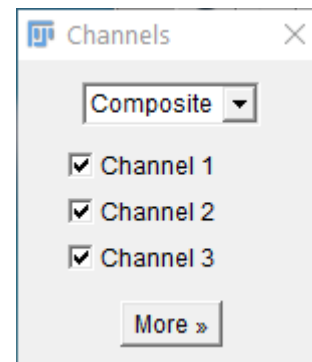
Color



Grayscale



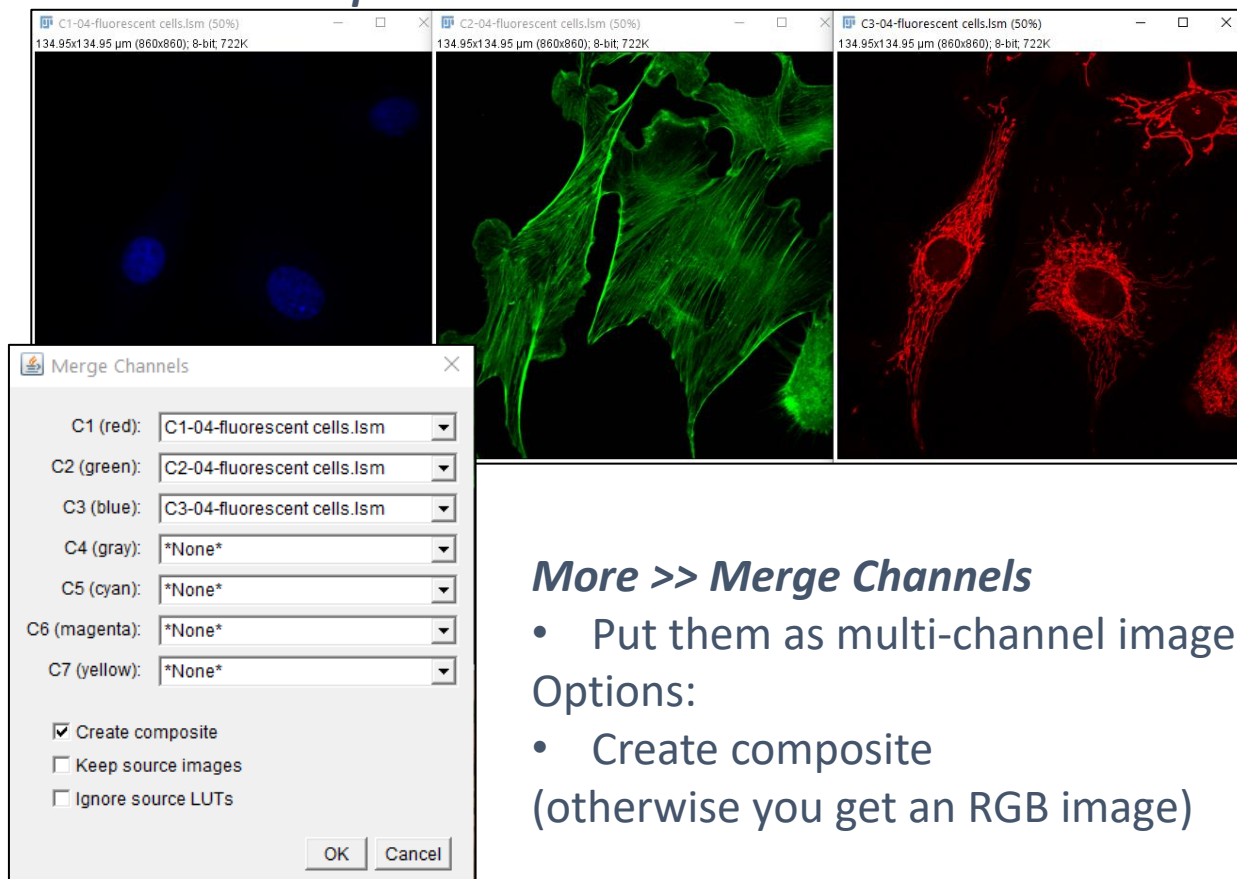
*Image / Color > Channels tool*





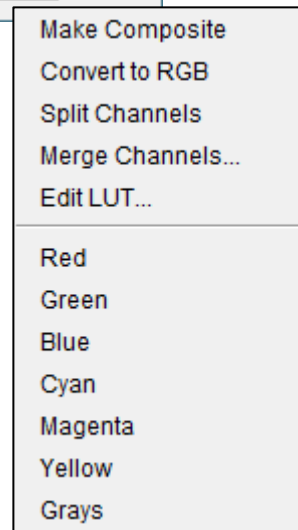
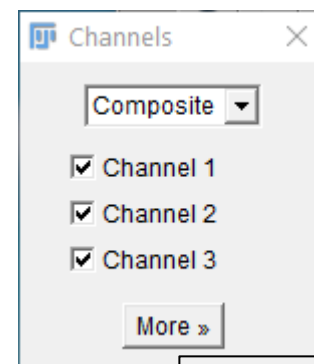
## Channels tool (Ctrl H)

**More >> Split Channels**



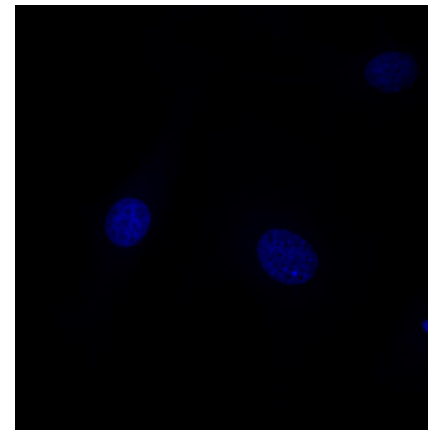
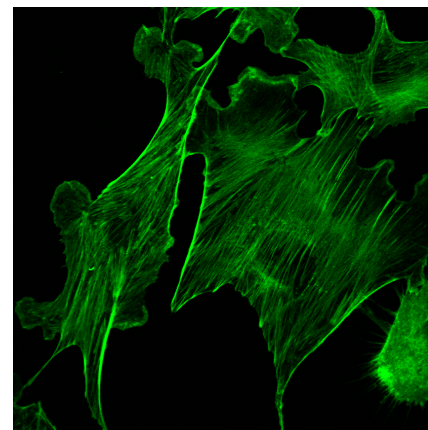
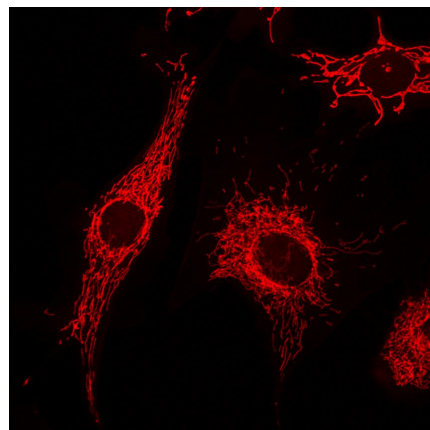
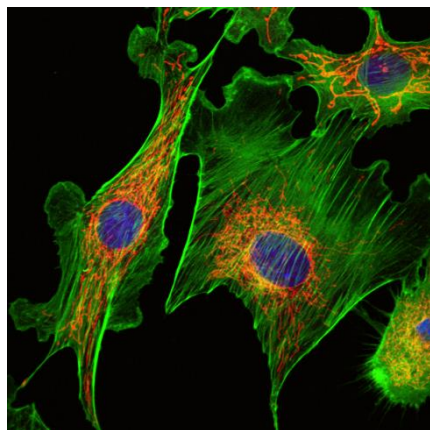
**More >> Merge Channels**

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

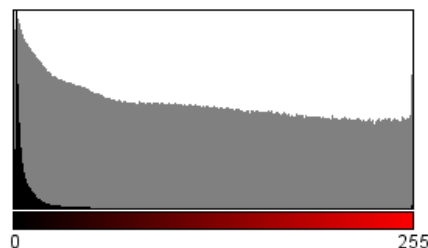




## Checking image brightness & bit depth – Image Histograms



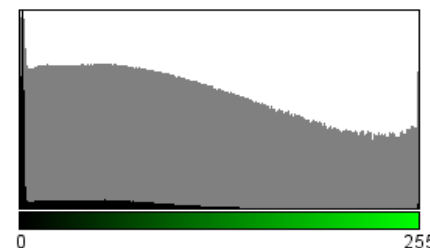
3 channels  
8-bits per channel



Count: 739600  
Mean: 21.371  
StdDev: 45.341

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

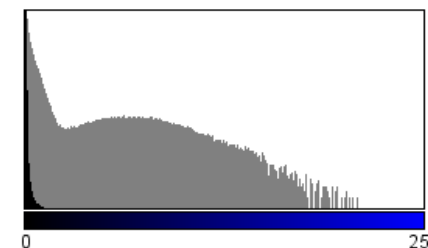
Min: 0  
Max: 255  
Mode: 1 (143778)



Count: 739600  
Mean: 35.707  
StdDev: 46.293

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

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

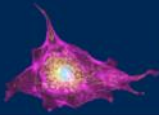


Count: 739600  
Mean: 4.209  
StdDev: 15.016

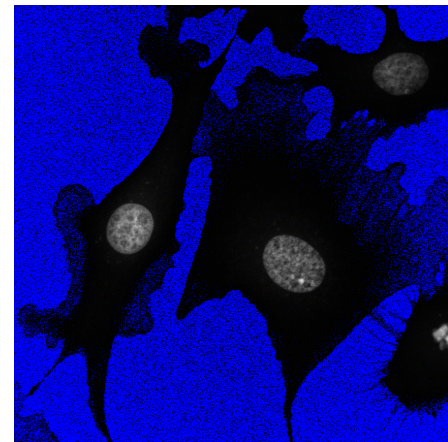
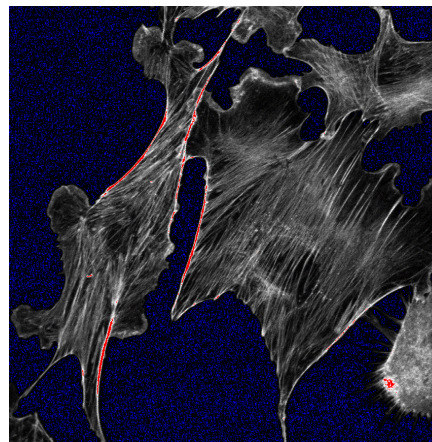
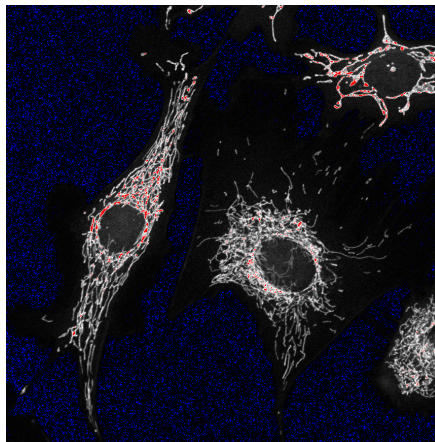
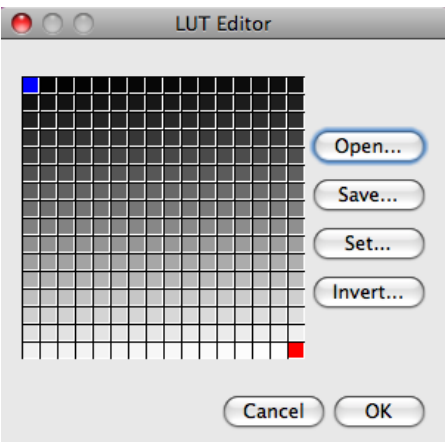
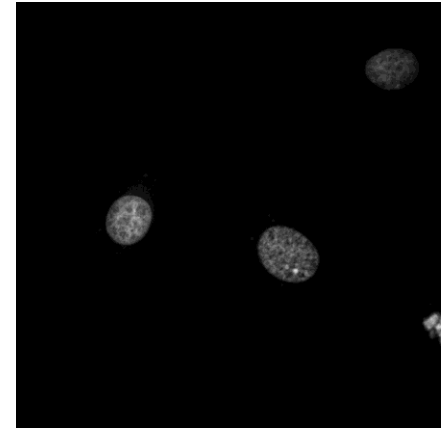
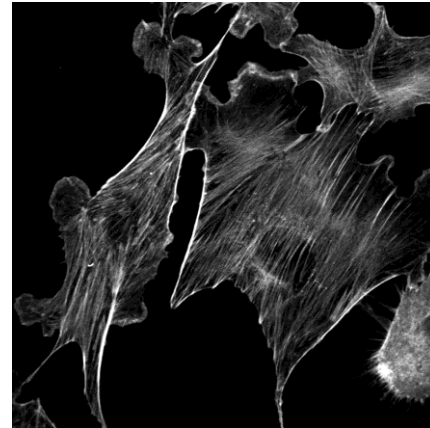
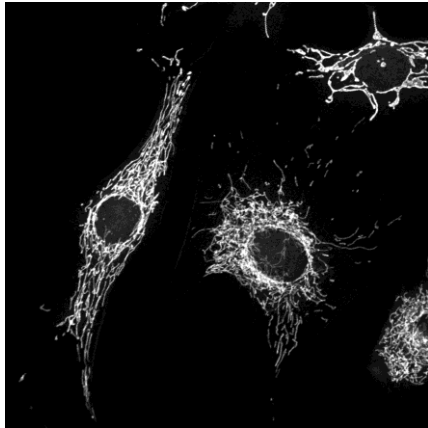
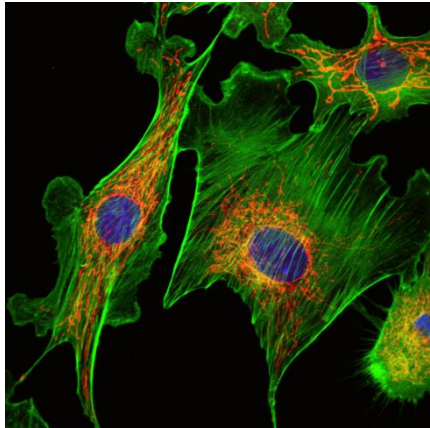
Min: 0  
Max: 218  
Mode: 0 (312533)

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



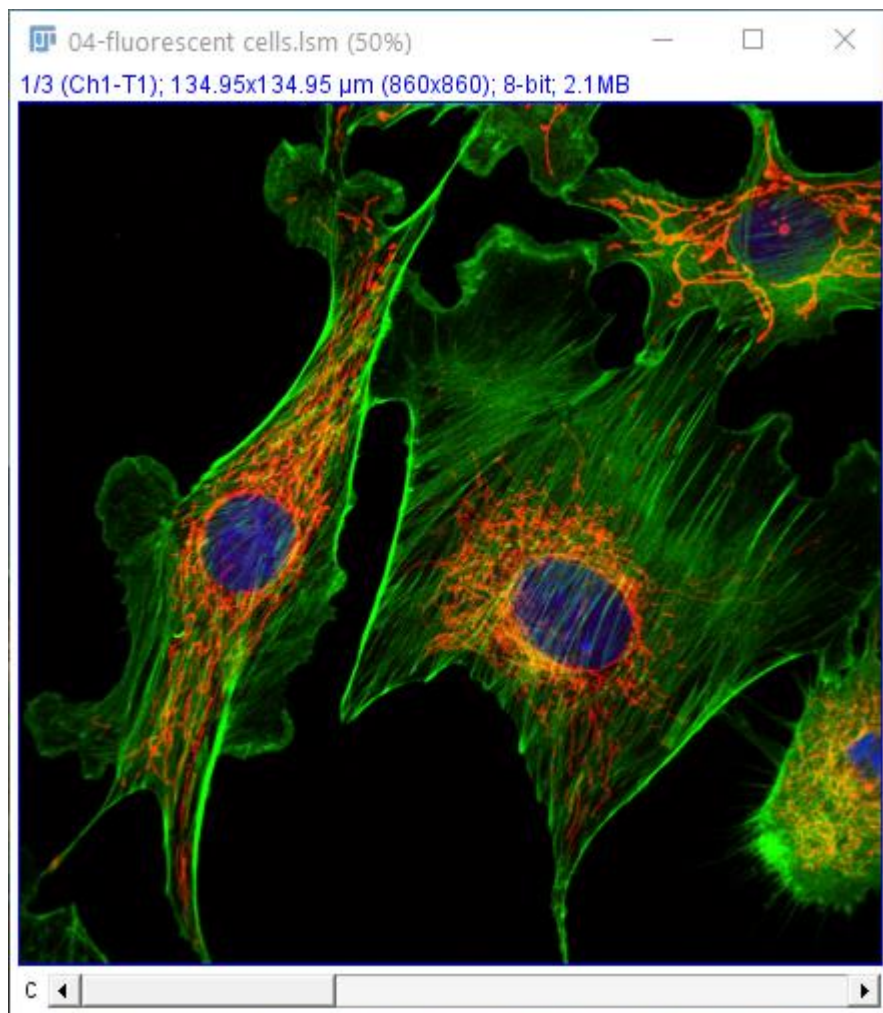


## Checking image brightness – using a Hi-Lo LUT

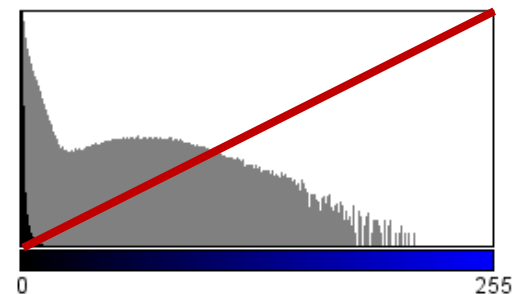
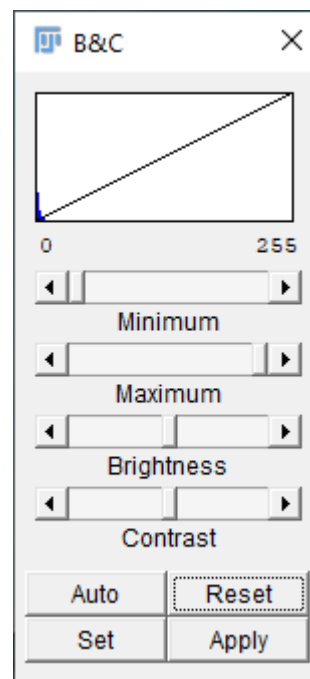




## Changing image brightness



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

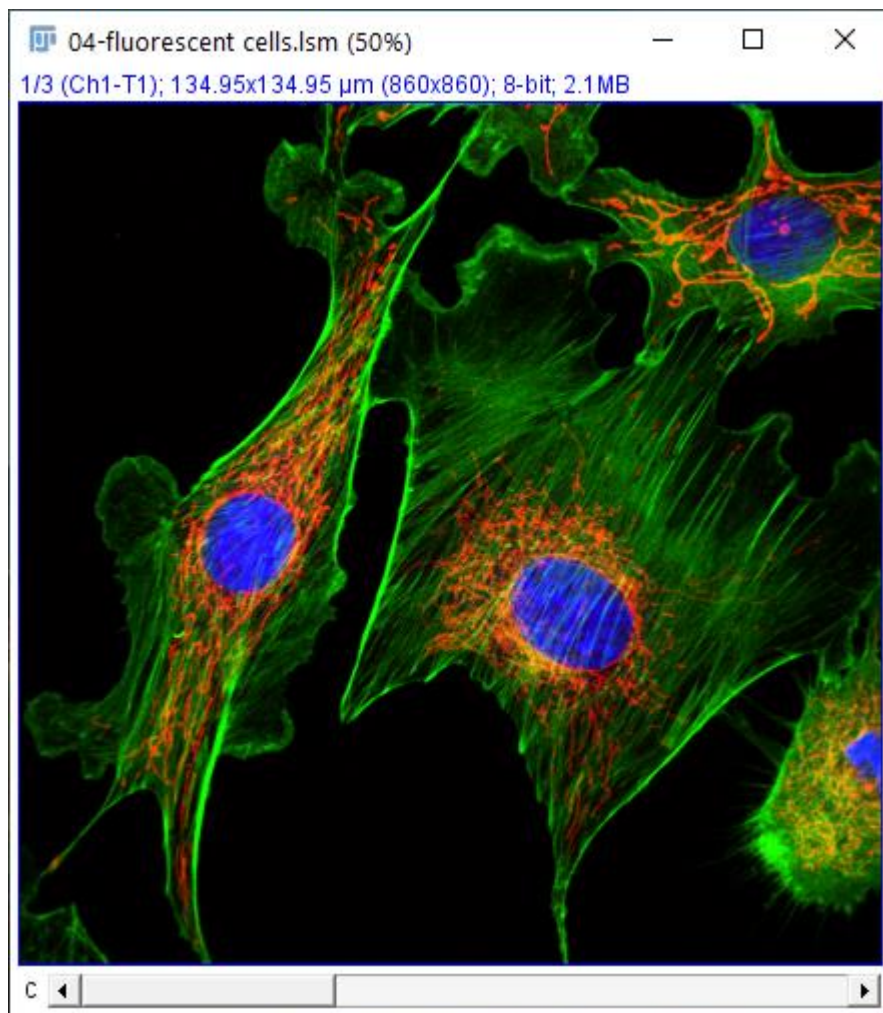


Count: 739600  
Mean: 4.209  
StdDev: 15.016

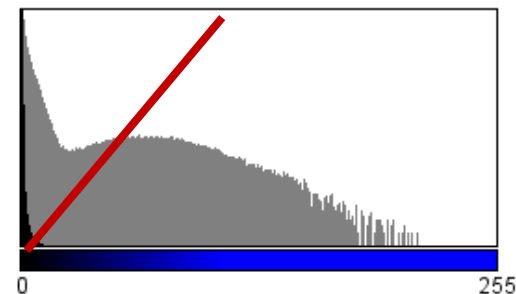
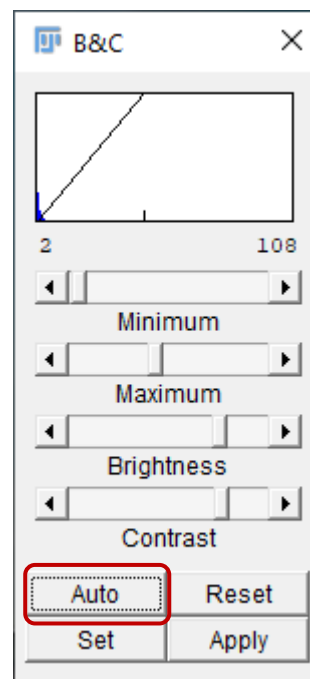
Min: 0  
Max: 218  
Mode: 0 (312533)



## Changing image brightness



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



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

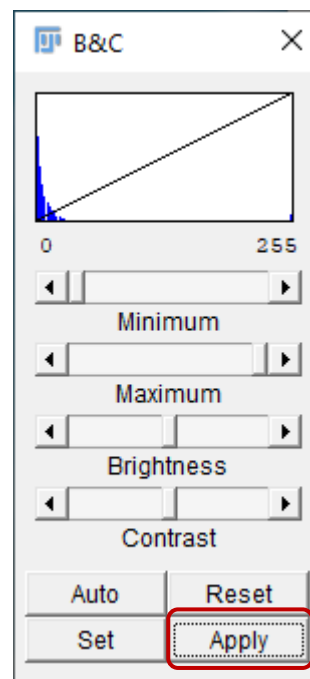




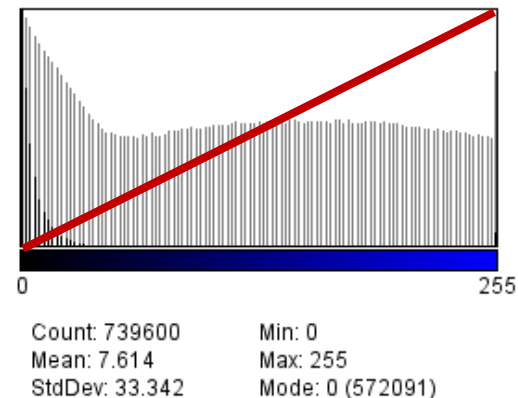
## Changing image brightness



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

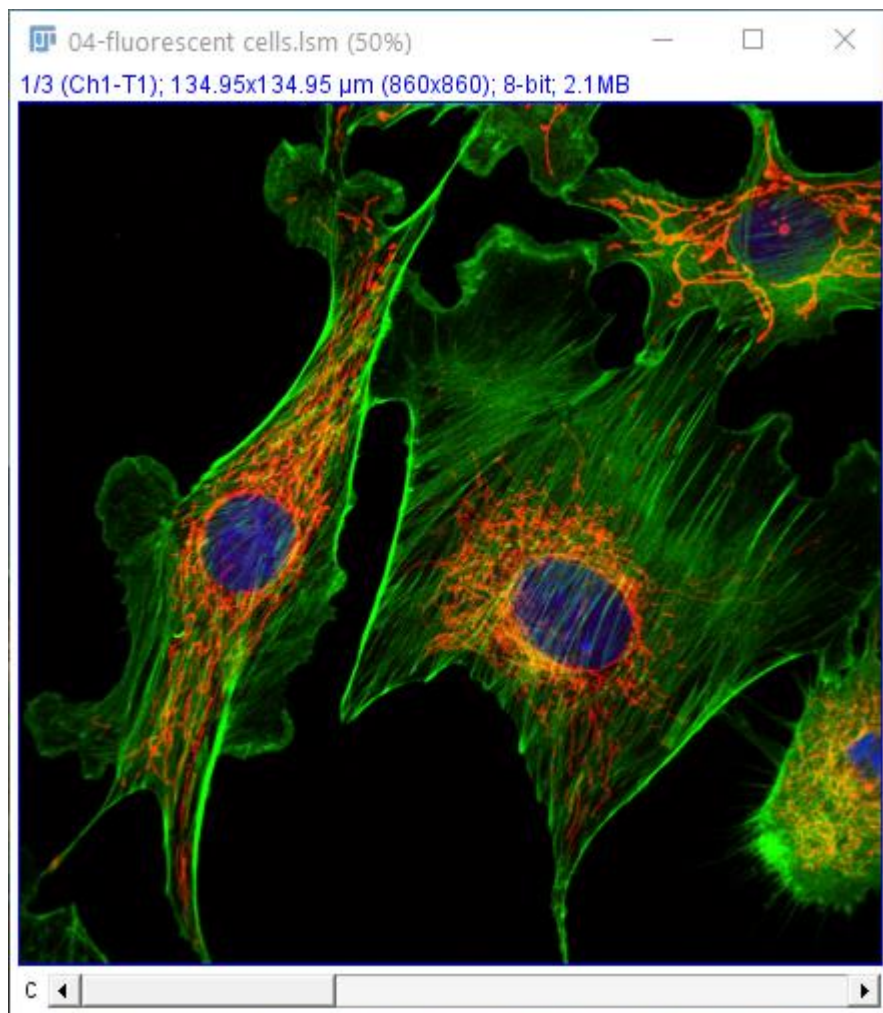


If you press 'Apply' in B&C you change the image data!

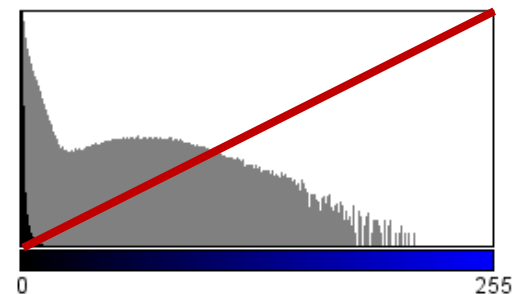
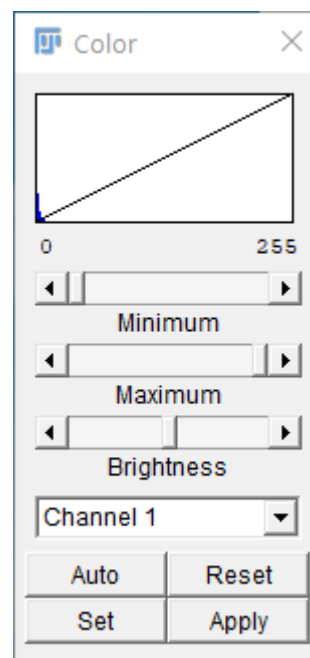




## Changing image brightness



*Image / Adjust >  
Color Balance...*

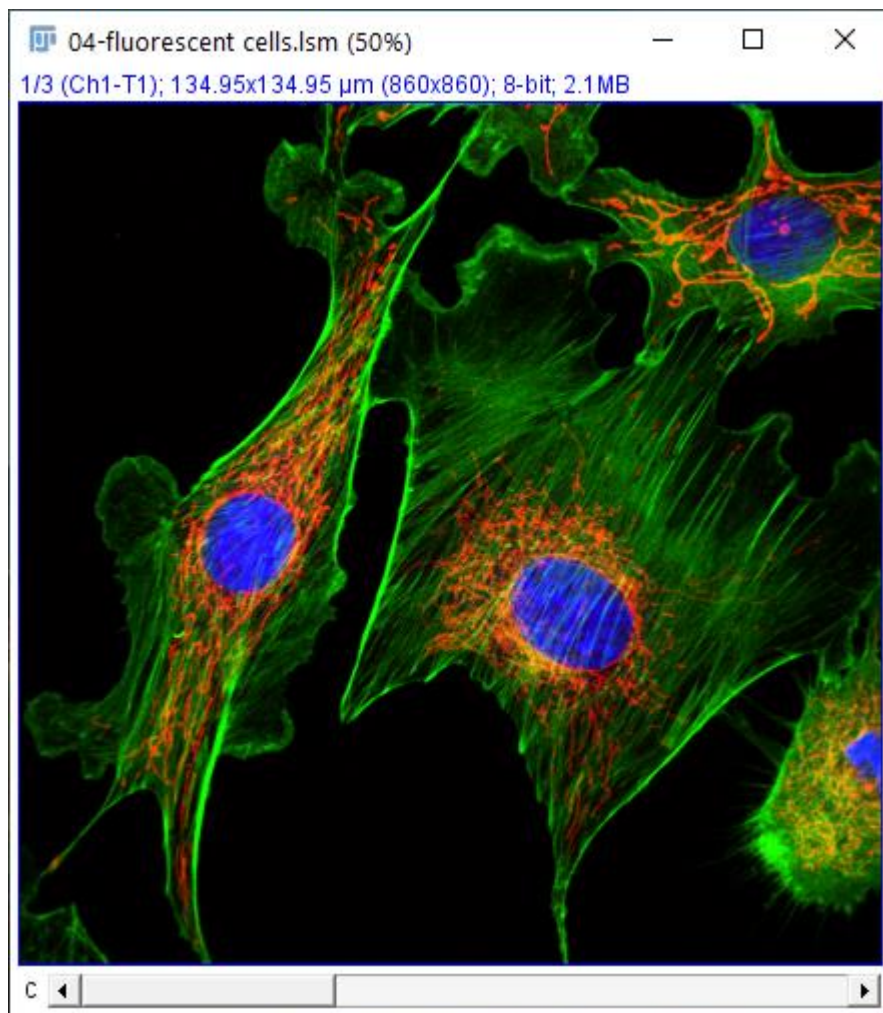


Count: 739600  
Mean: 4.209  
StdDev: 15.016

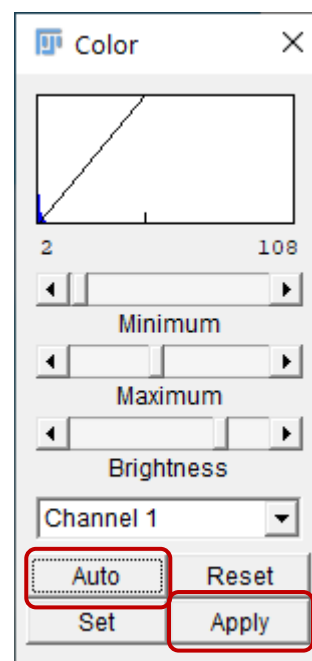
Min: 0  
Max: 218  
Mode: 0 (312533)



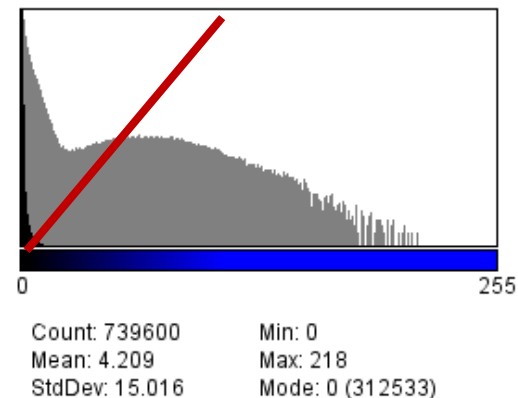
## Changing image brightness



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



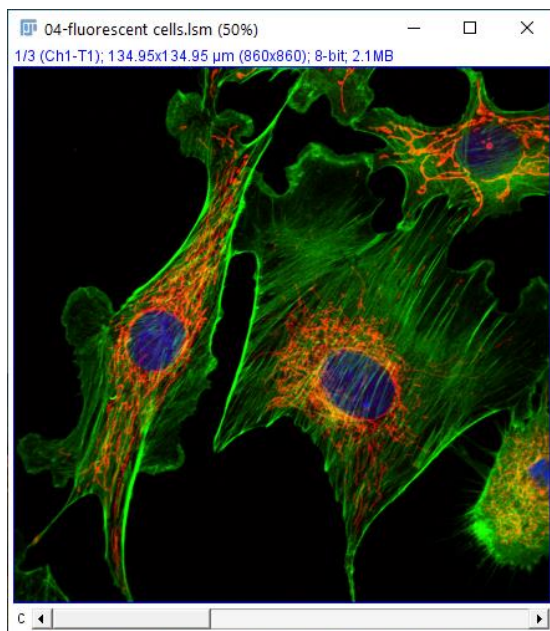
If you press 'Apply' in Color Balance you only change the view.



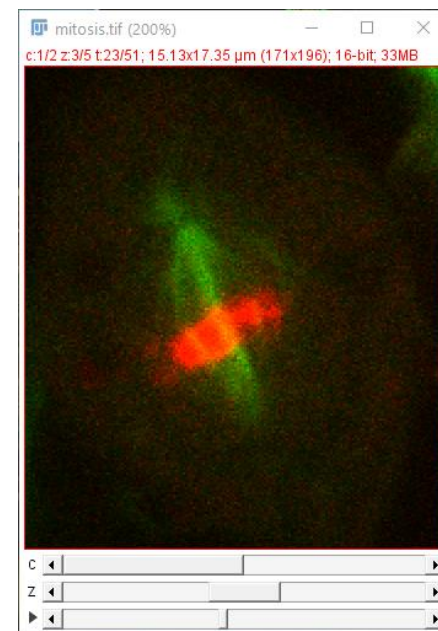
If you save the image as a .tif & re-open in Fiji, the image is displayed as you set it here.



## Multi dimensional images

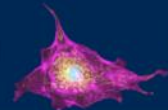


**Stack.** Each image is a 'slice'  
It can be a set of:  
Channels **or**  
Z slices **or**  
Timepoints

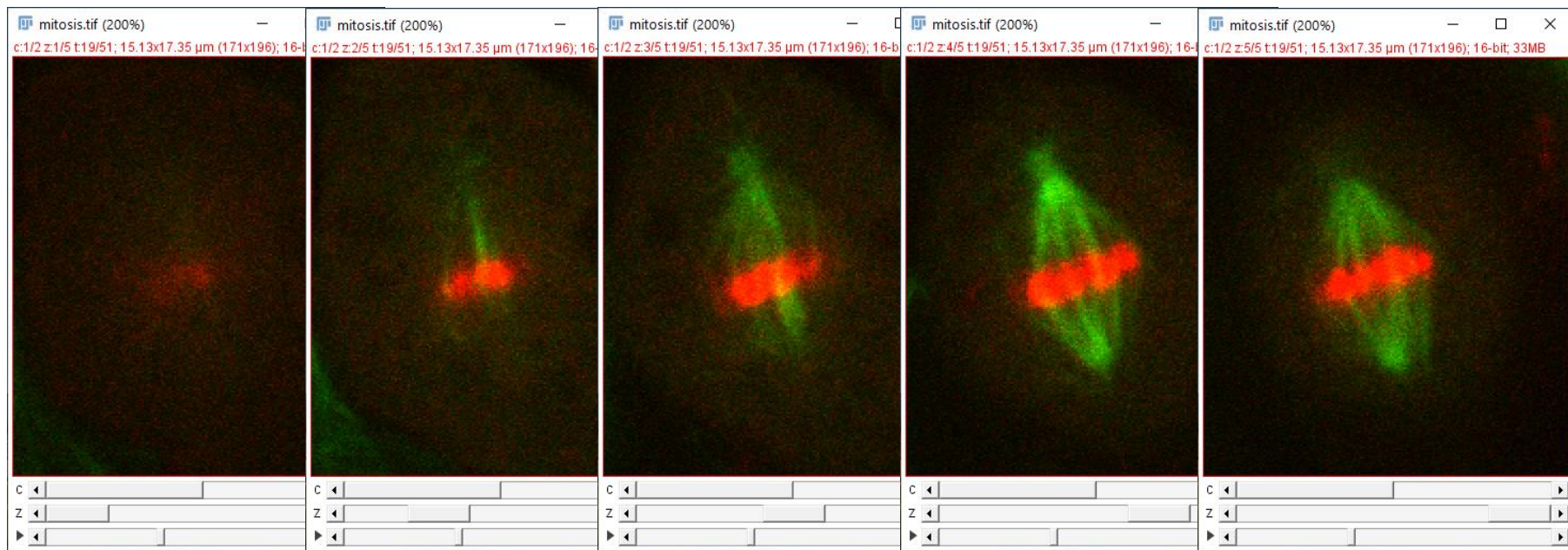


**Hyperstack.** Each image is a 'slice'  
It can be a set of:  
Channels **&/or**  
Z slices **&/or**  
Timepoints





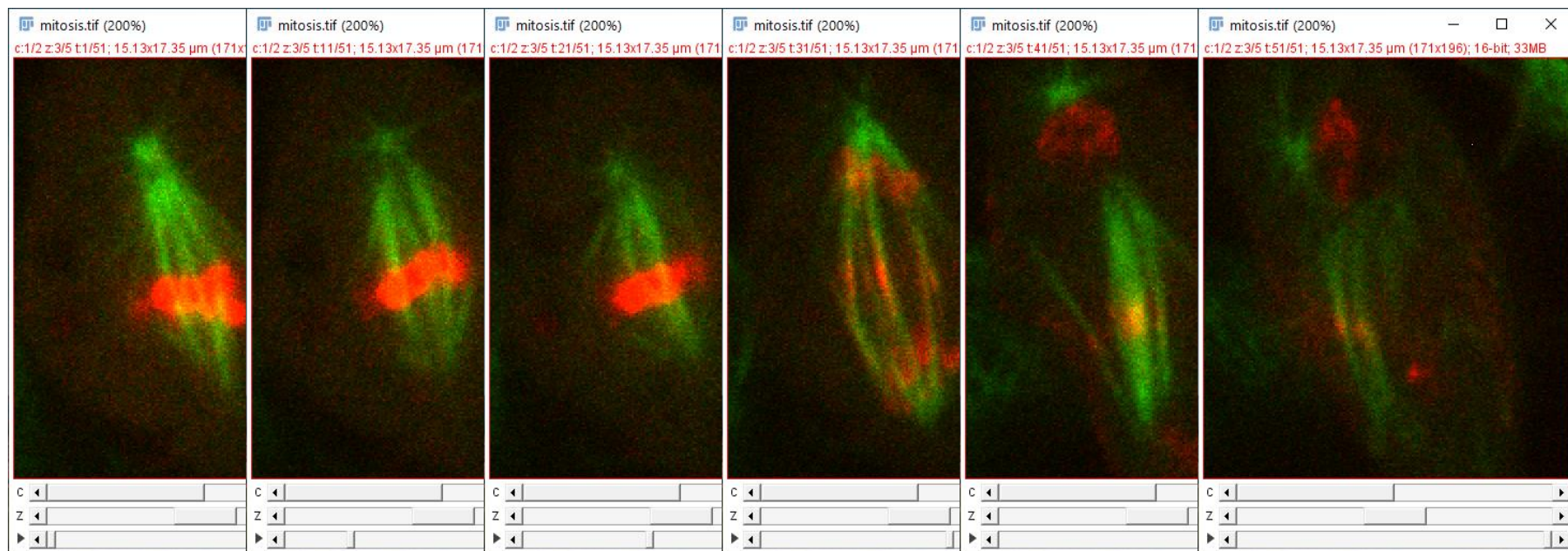
## Multi dimensional images



5 z slices



## Multi dimensional images

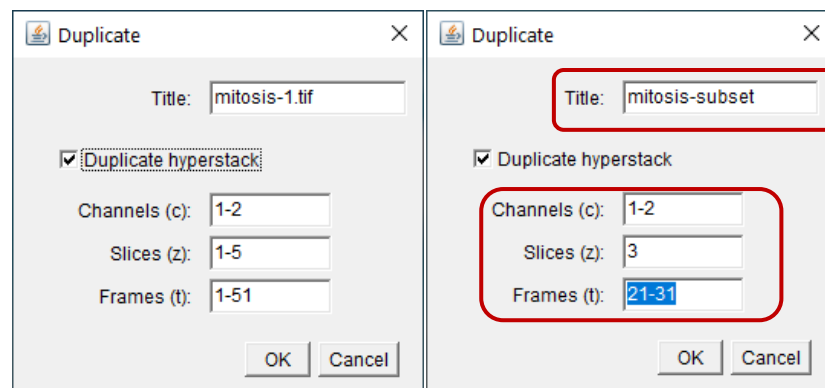
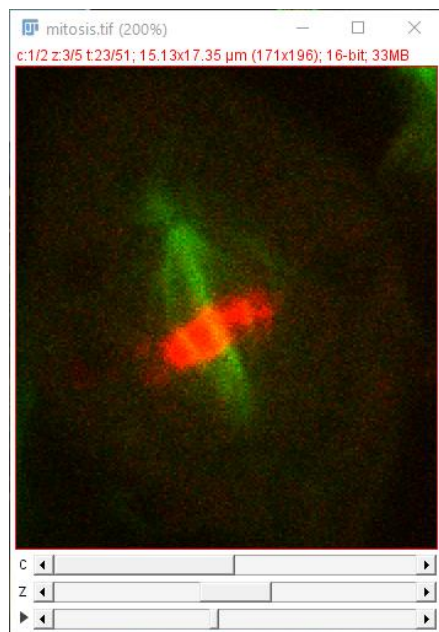


51 time points



## Extract parts of multi dimensional images

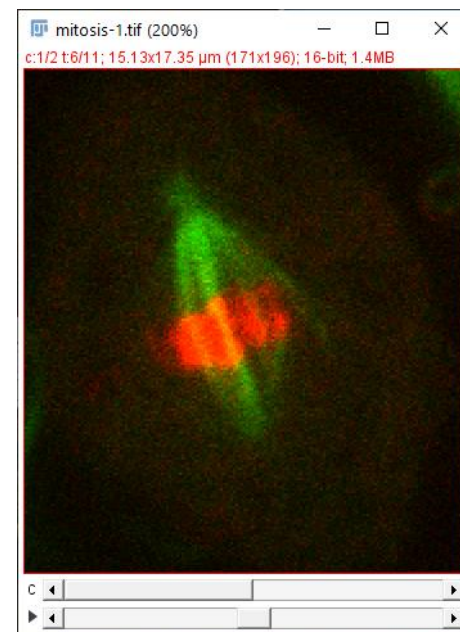
### *Image / Duplicate... (Ctrl + Shift + D)*



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

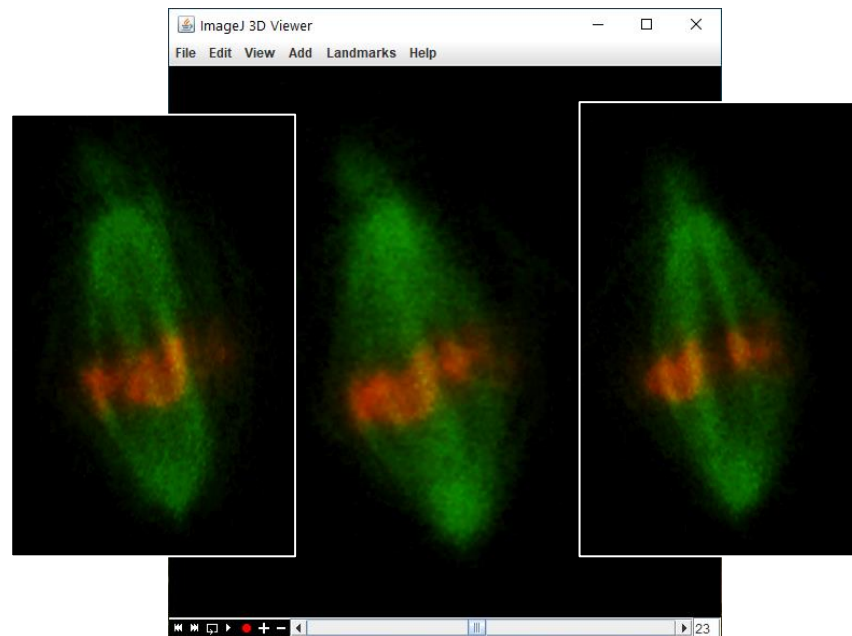
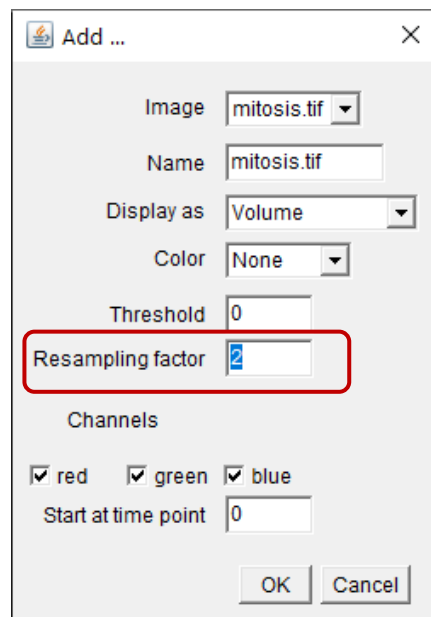
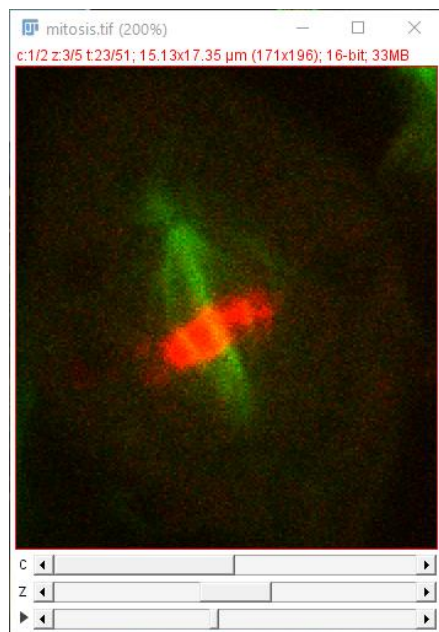






## Make a 3D projection

### *Plugins / 3D Viewer*



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

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

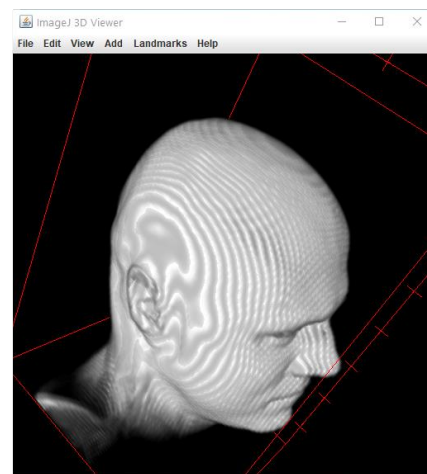
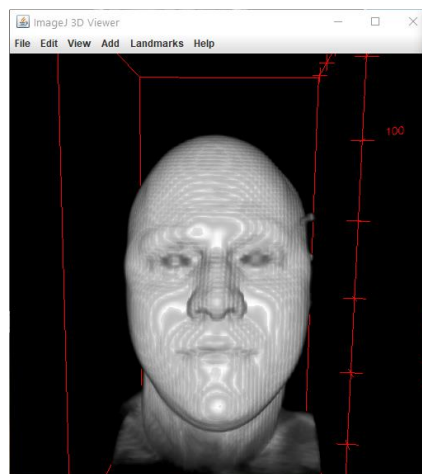
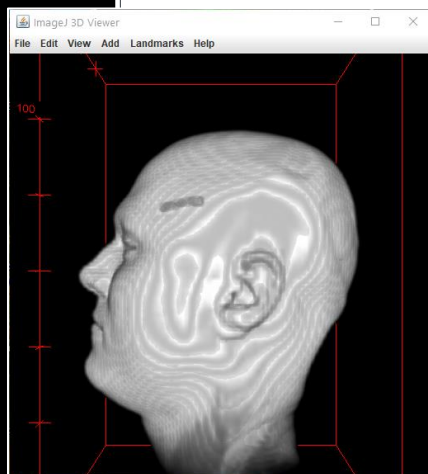
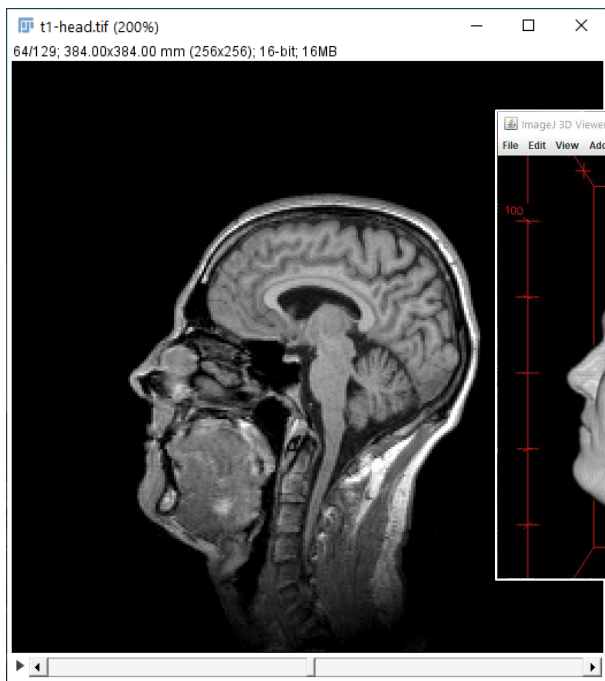




## Make a 3D projection

### *Plugins / 3D Viewer*

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



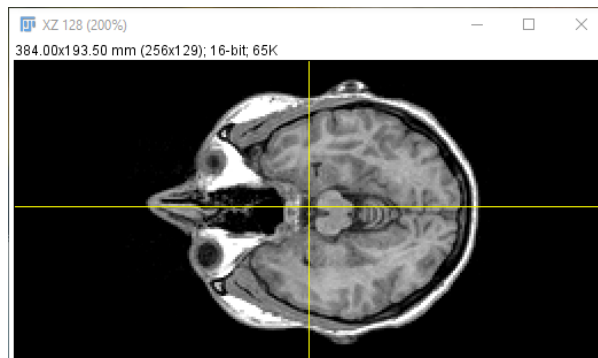
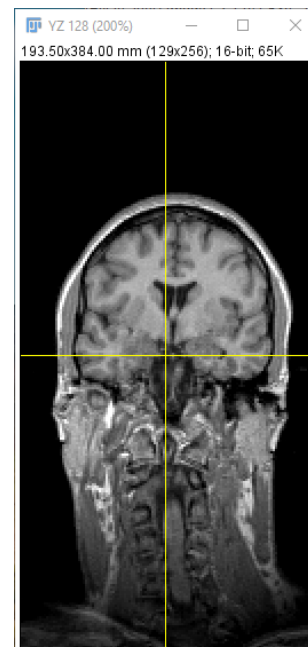


## View from sides (orthogonal views)

### *Image / stacks / Orthogonal Views*

*(Ctrl + Shift + H)*

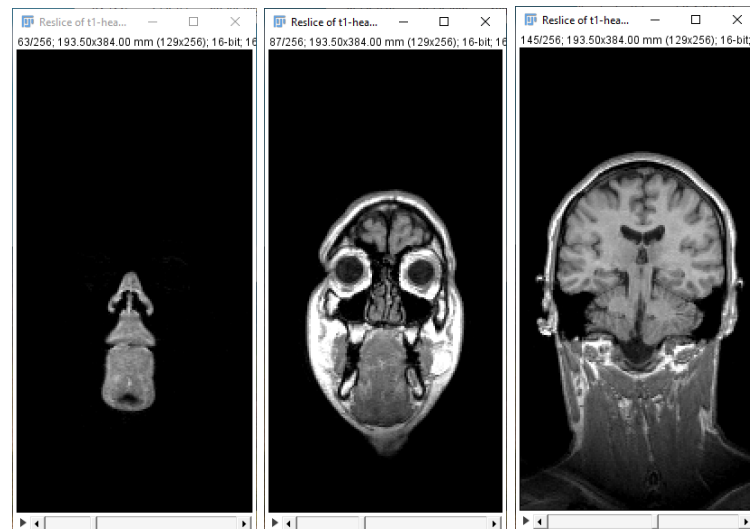
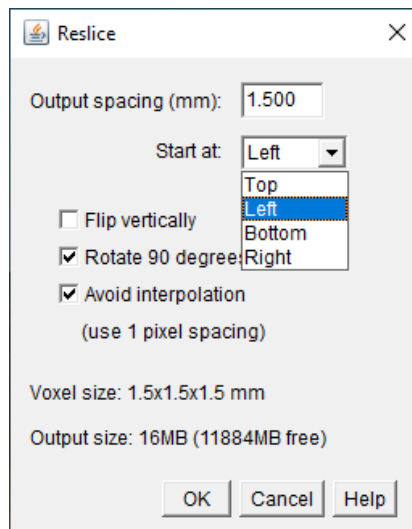
Move the lines  
to view different  
locations





## Reslice to rotate the whole stack

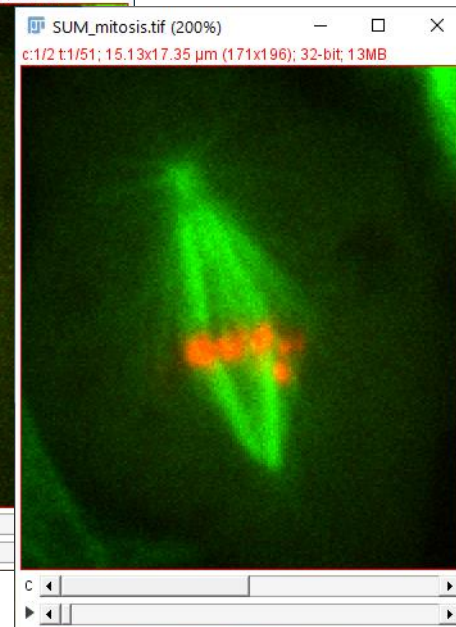
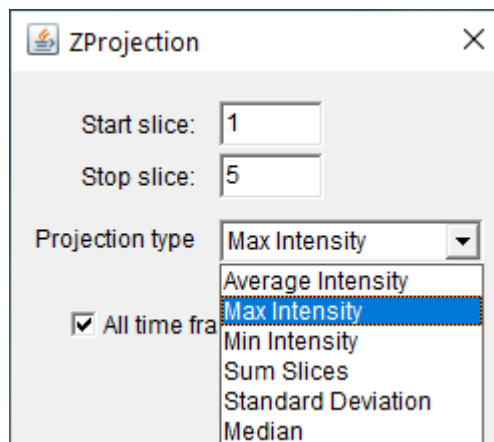
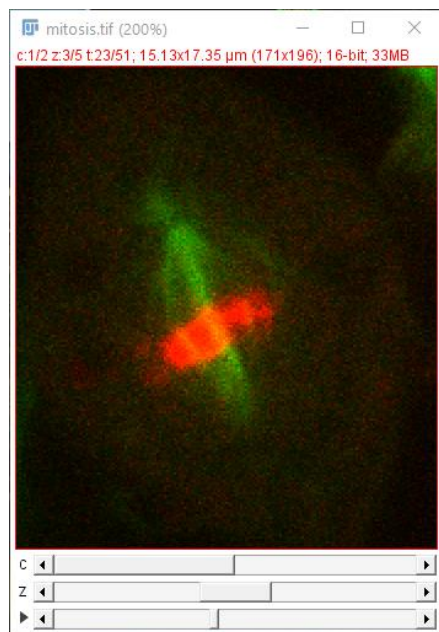
*Image / stacks / Reslice (Ctrl + /)*





Make a projection (flatten) a 3D image

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



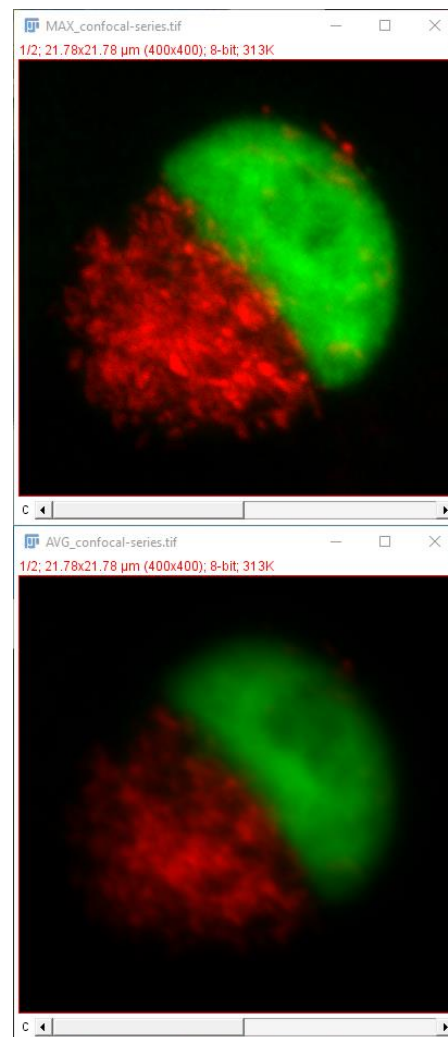
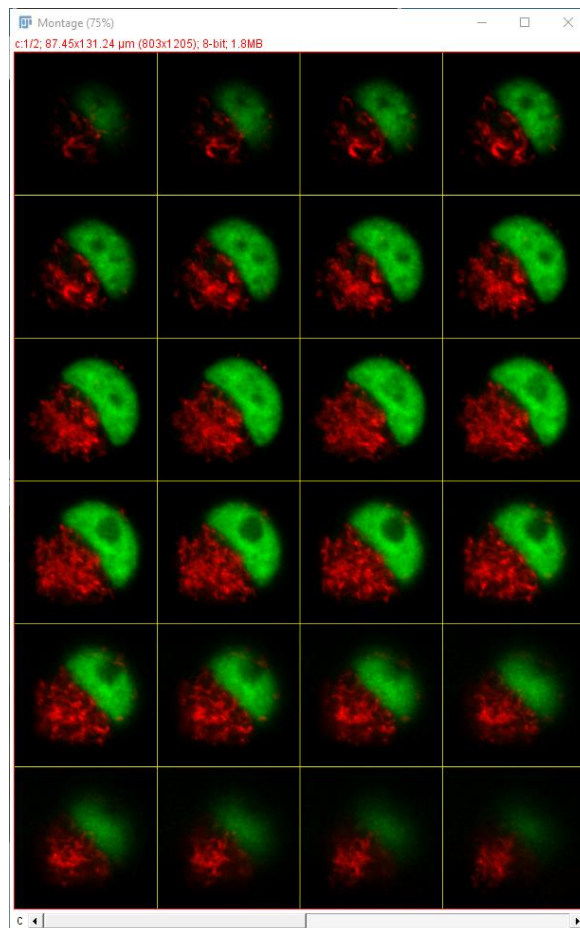
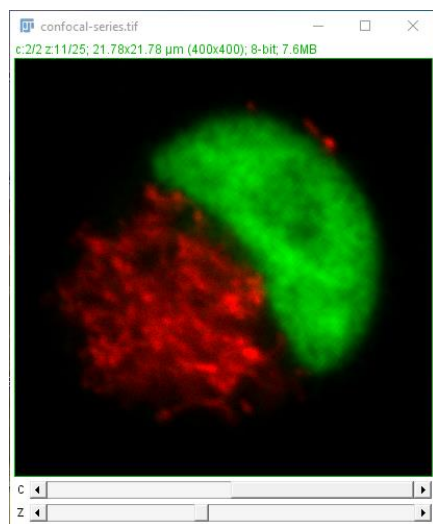
**Max intensity:** for each pixel position (x,y) look through every z slice, and keep only the brightest pixel

**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

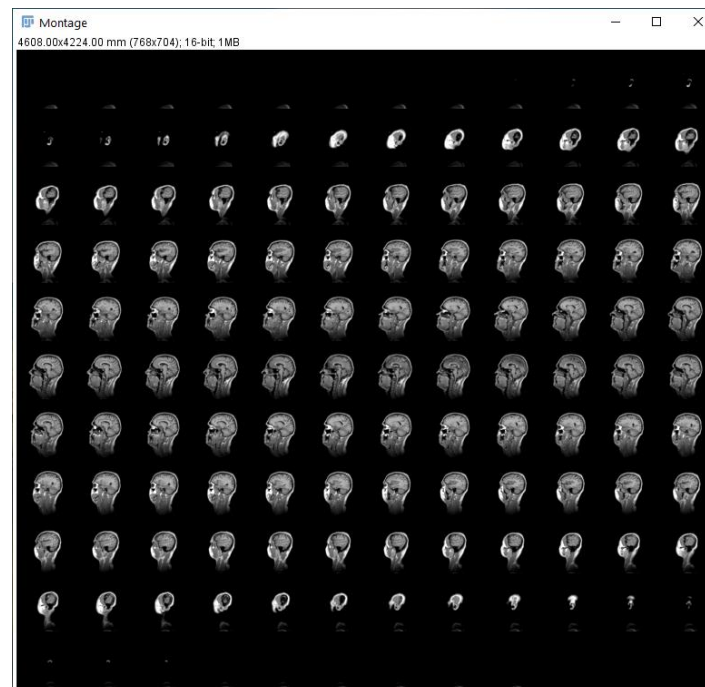
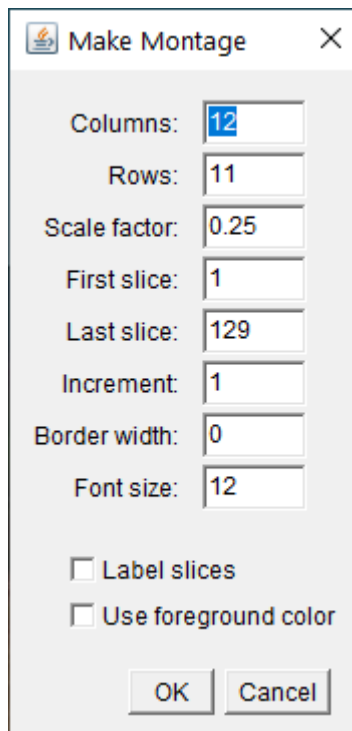
Average or  
Sum for  
measurements





## Make a montage of any stack

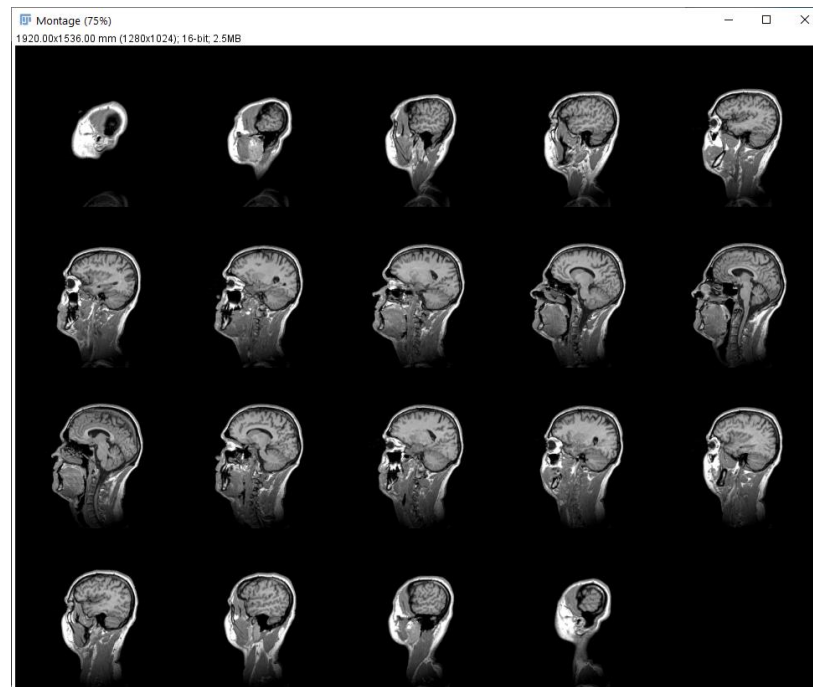
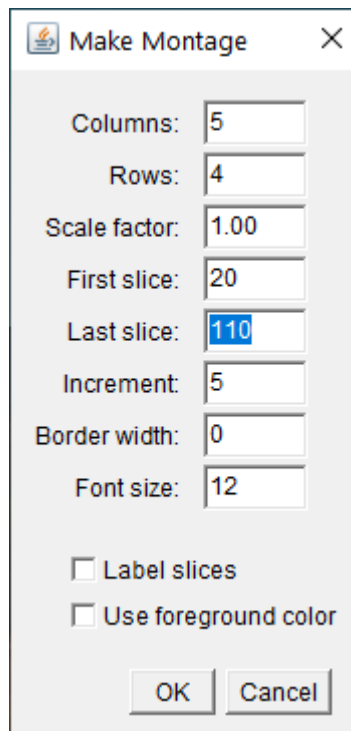
*Image / Stacks / Make montage...*





## Make a montage of any stack

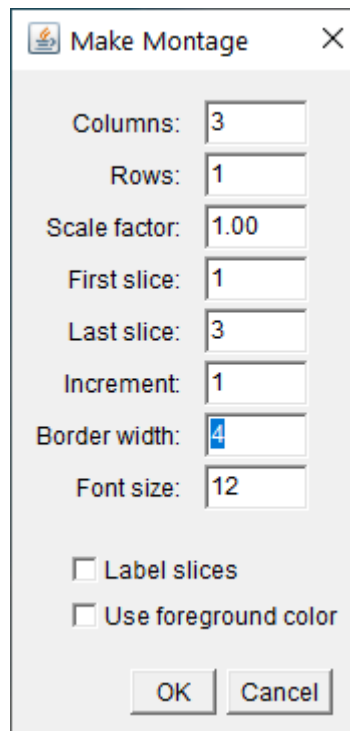
*Image / Stacks / Make montage...*





## Use Make Montage to make a figure

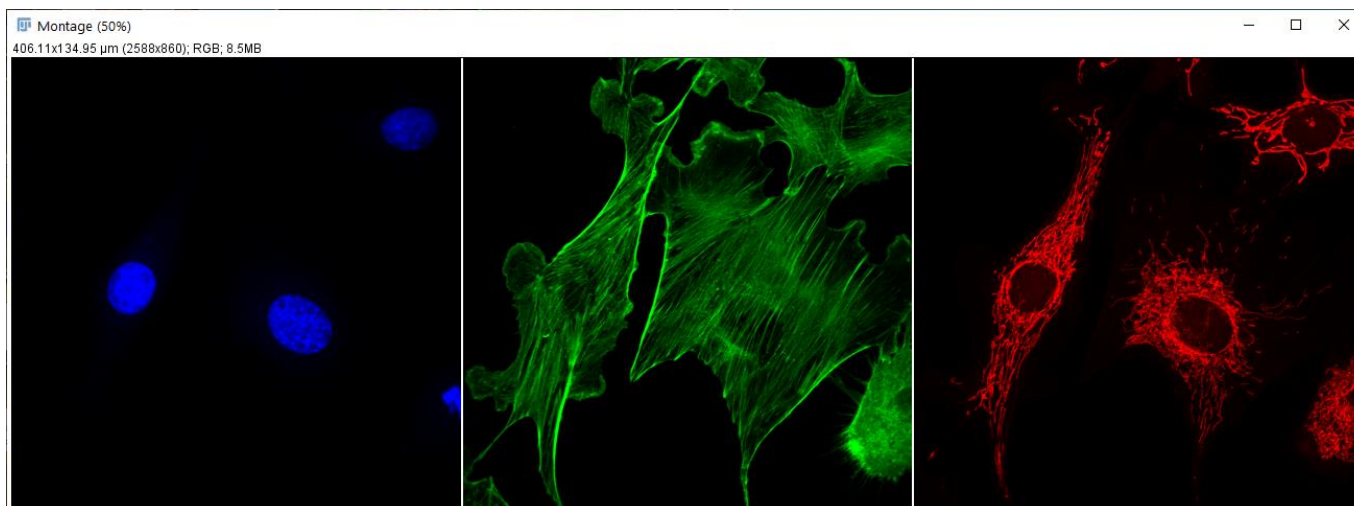
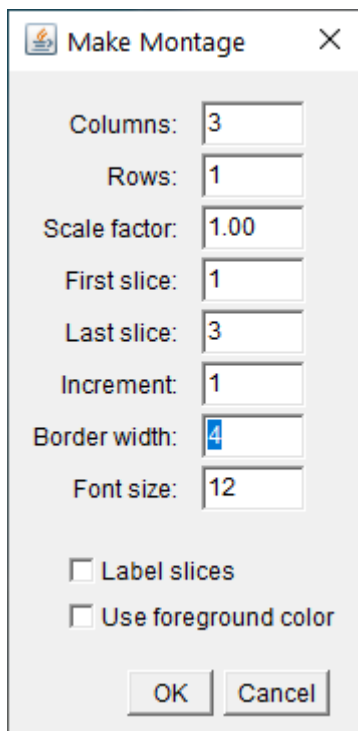
*Image / Stacks / Make montage...*







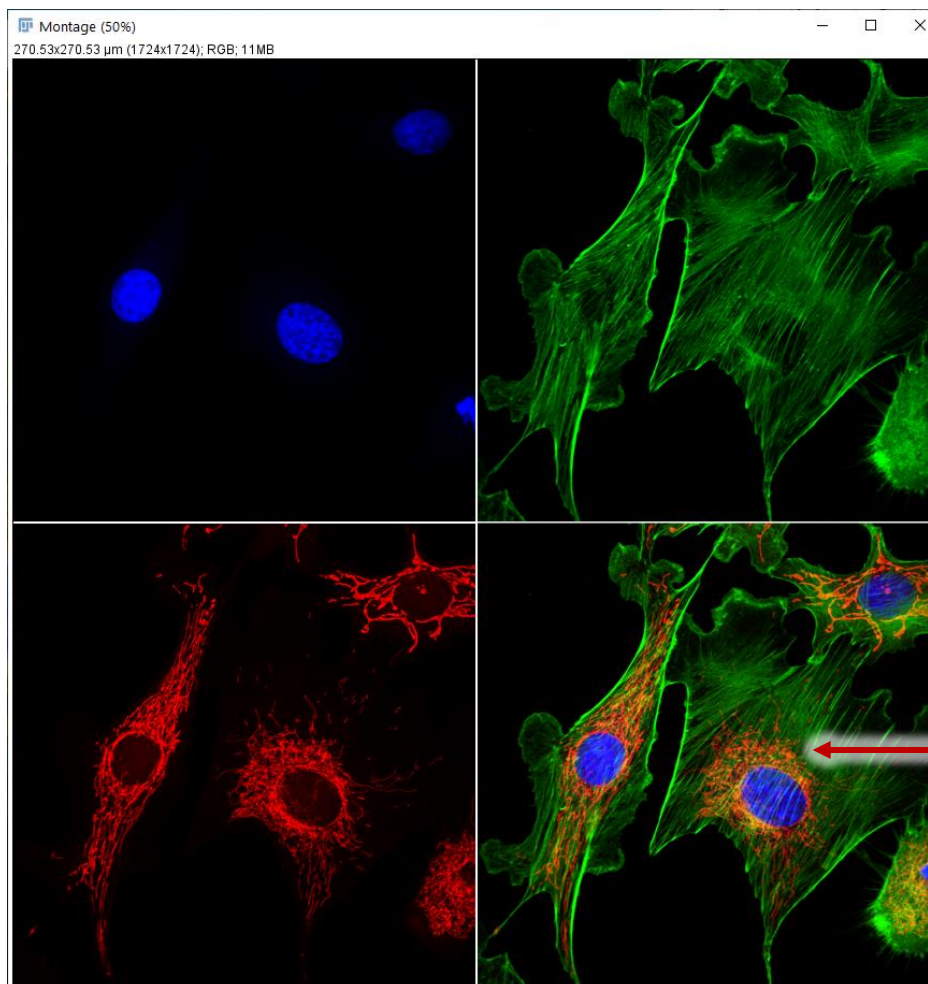
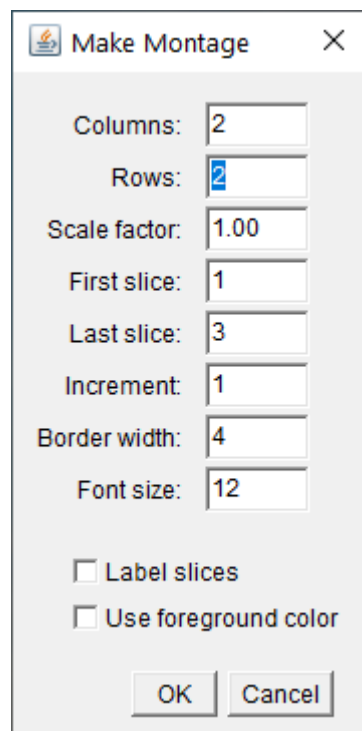
## Use Make Montage to make a figure



- 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