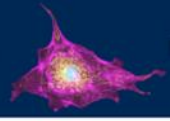
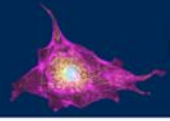


Introduction to image processing and analysis with ImageJ / Fiji. Part 2

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

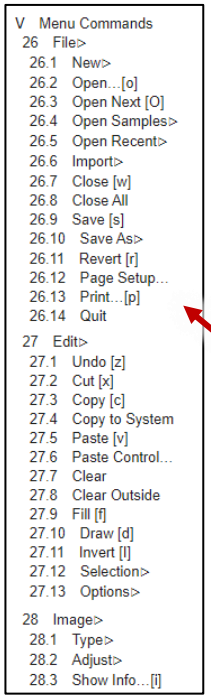
https://imagej.nih.gov/ij/docs/guide/index.html

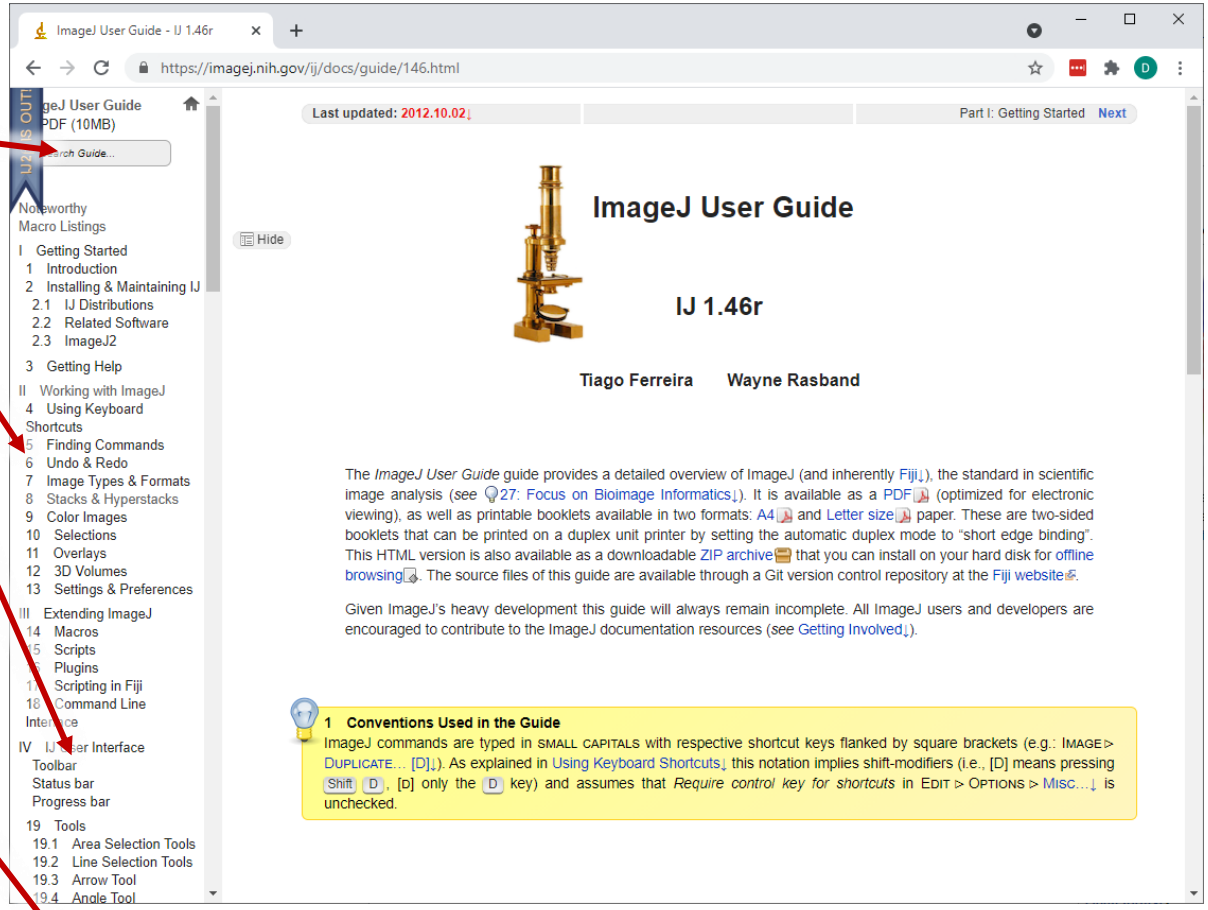
Searchable

Description of image types

Toolbars

Every Menu command and all sub commands





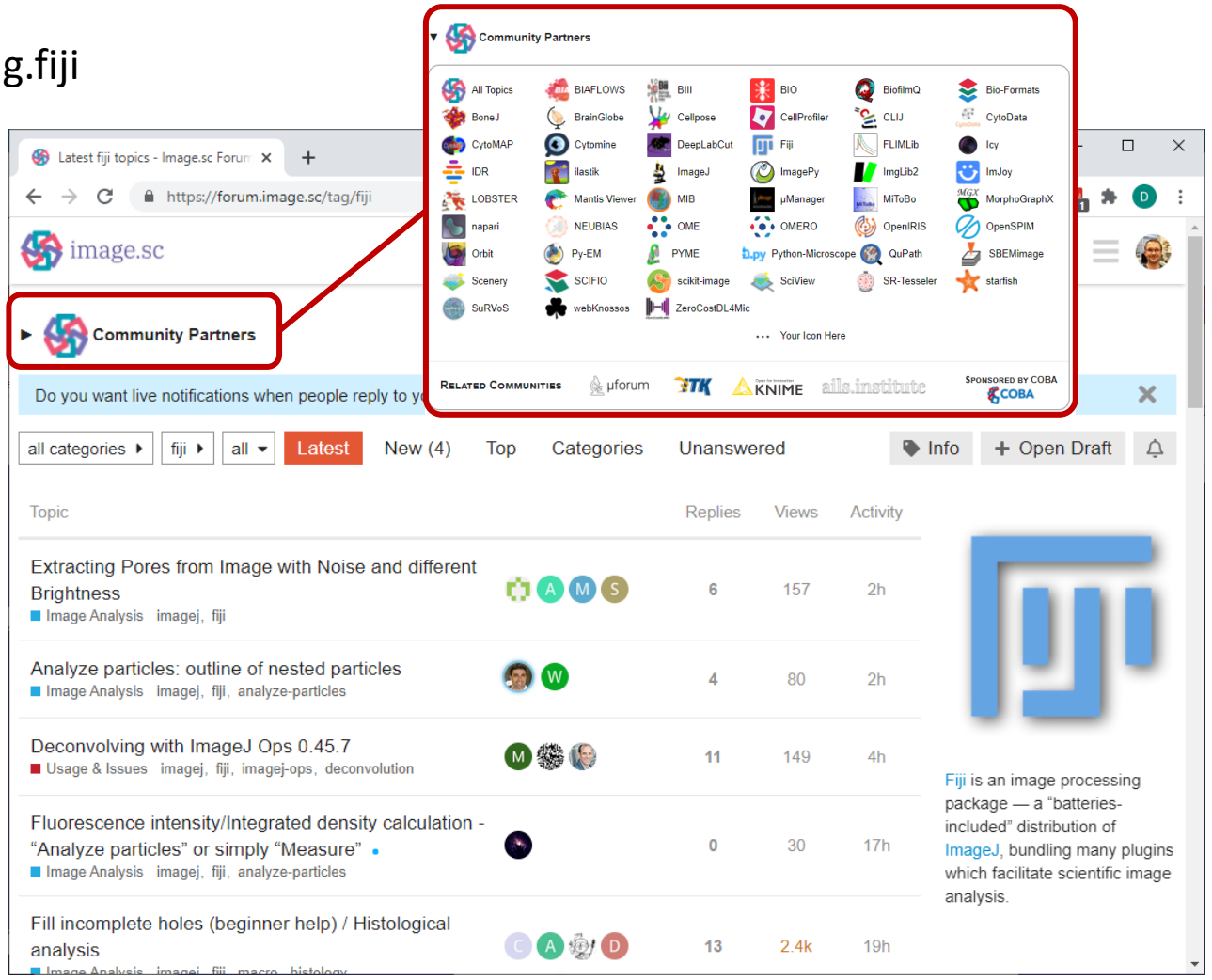
Fiji on-line support

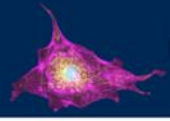
https://forum.image.sc/tag.fiji

Just google:
Image.sc

Fully searchable forum.

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





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/

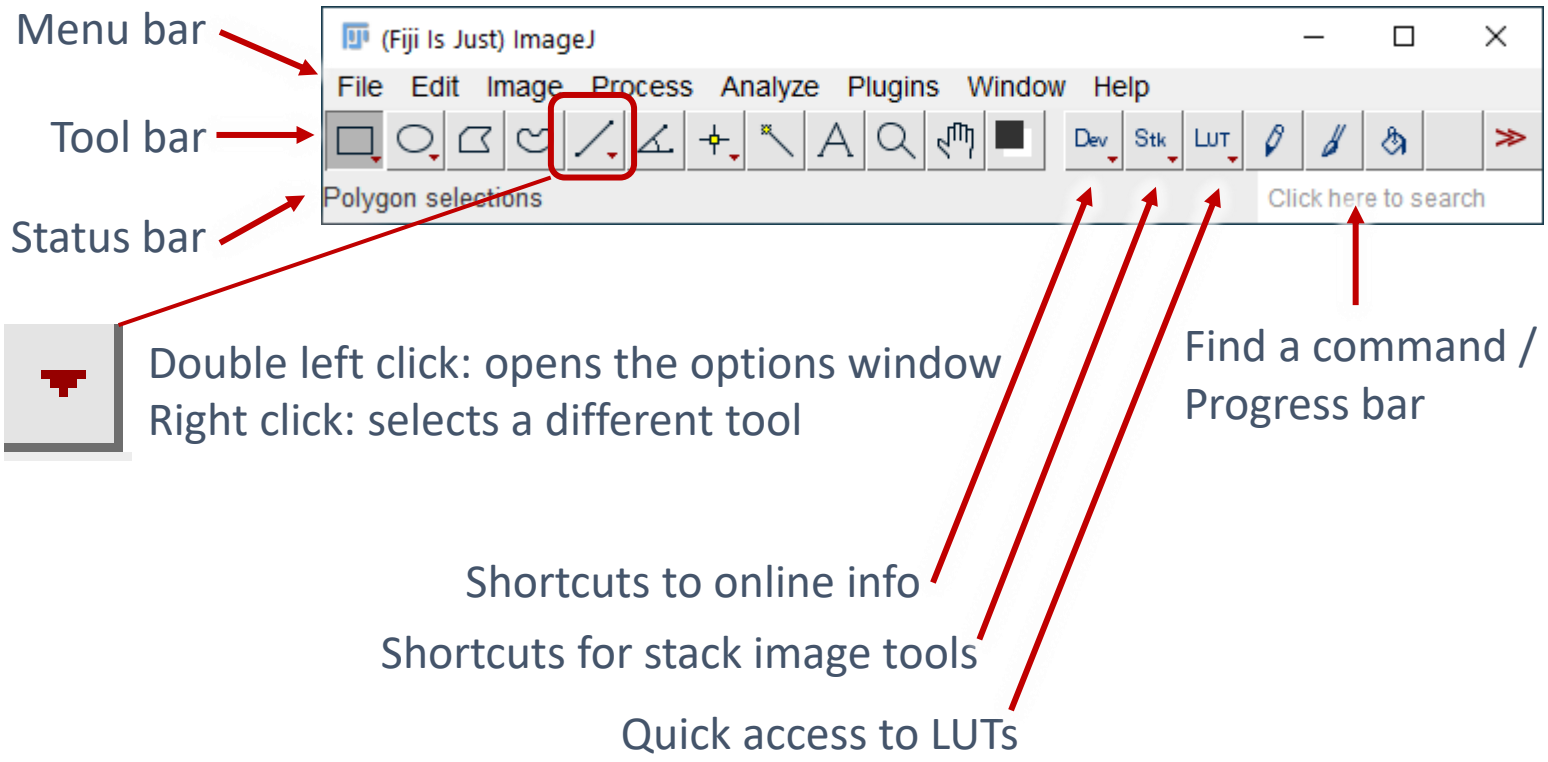
Macros

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

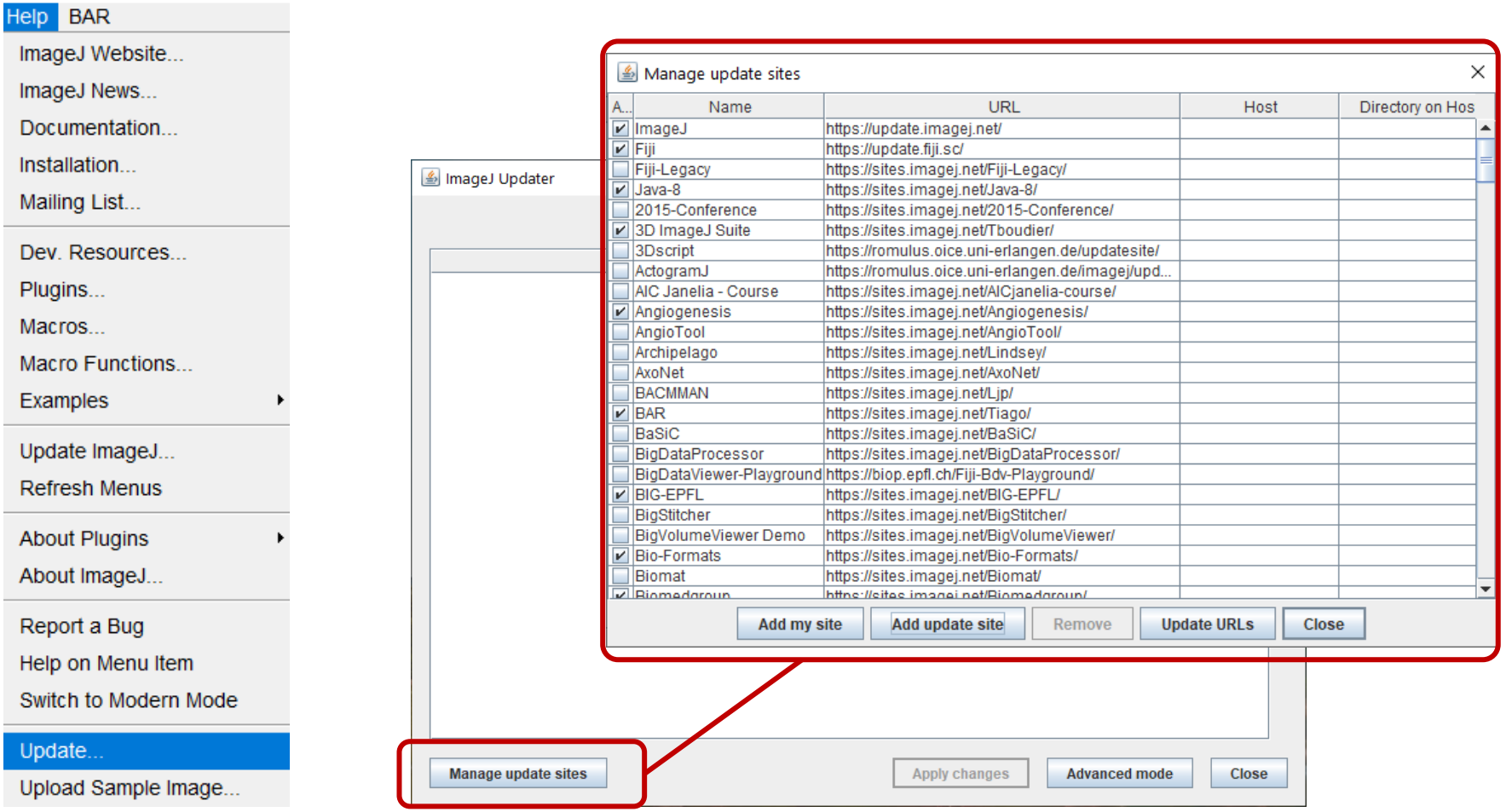
Plugins

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

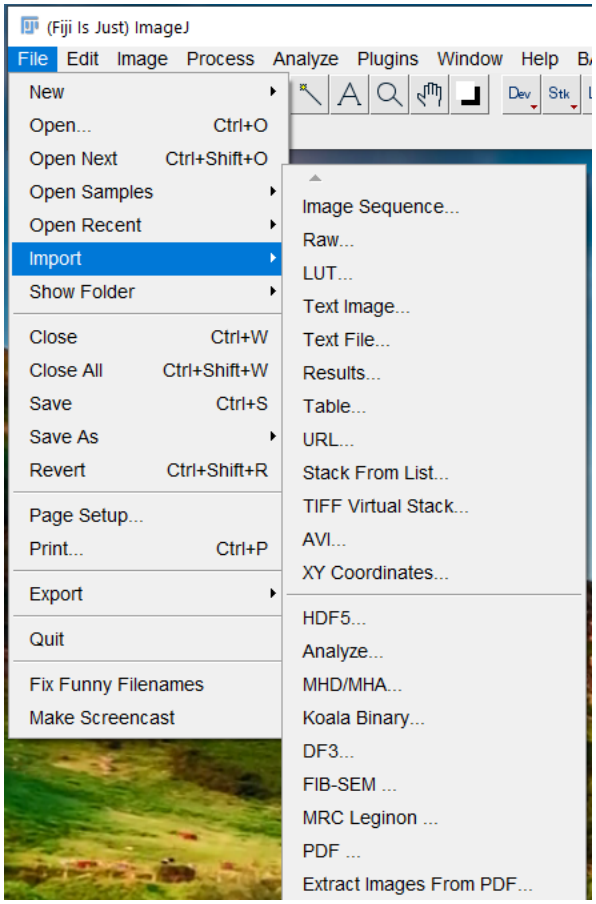
Fiji GUI


Menu bar
Tool bar
Status bar
(Fiji Is Just) ImageJ
File Edit Image Process Analyze Plugins Window Help
Dev Stk LUT
Polygon selections
Click here to search
Double left click: opens the options window
Right click: selects a different tool
Find a command /
Progress bar
Shortcuts to online info
Shortcuts for stack image tools
Quick access to LUTs

Updating and upgrading Fiji (adding plugins)

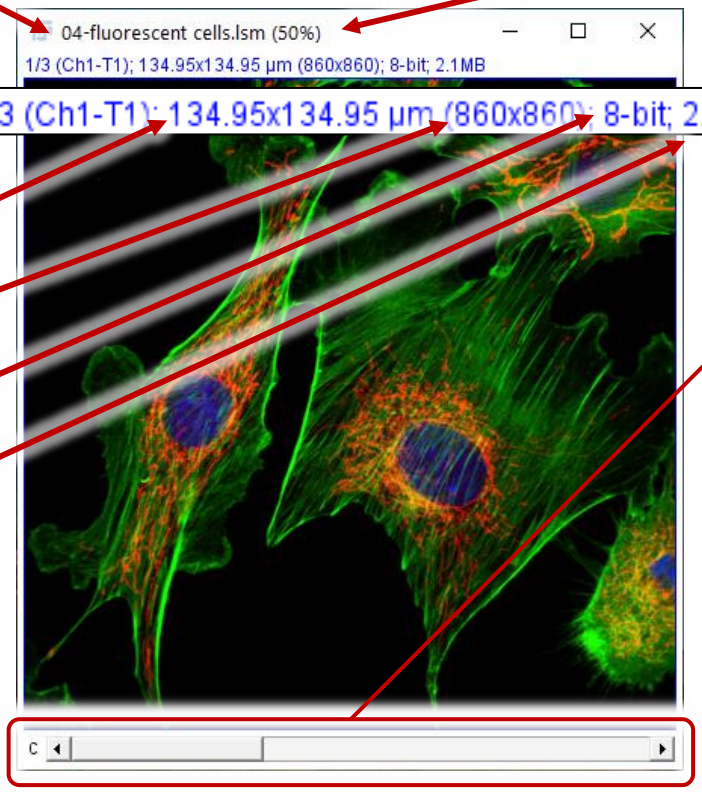


Opening Images in Fiji

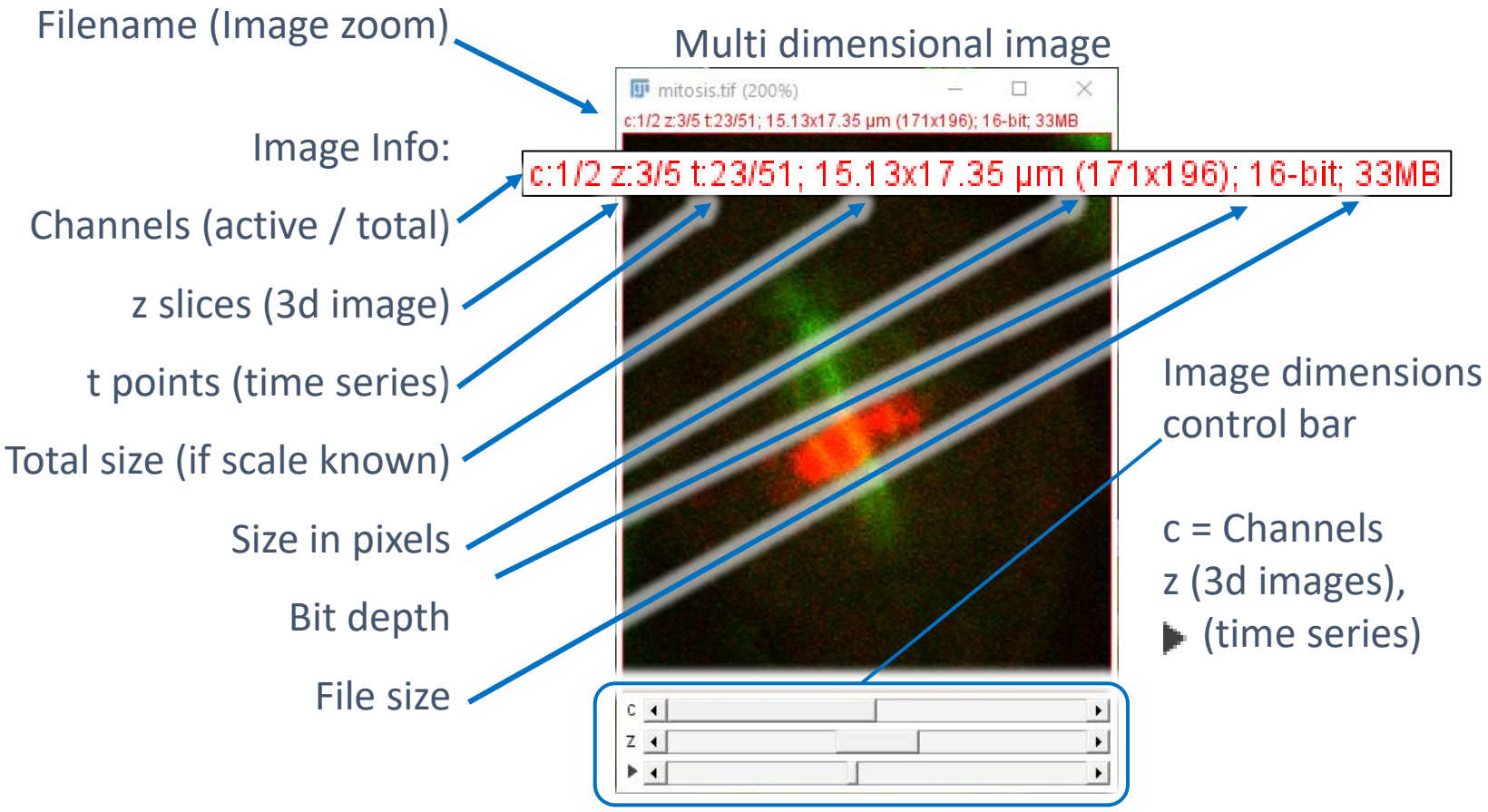


- ***File / Open…***
- ***File / Import >***
- Drag & drop
  Single file, multiple files, whole folders
- ***Plugins / BioFormats / Importer***

Fiji image window



Fiji image window



Channels tool (Shift + Ctrl + Z)

Composite

Color

Grayscale

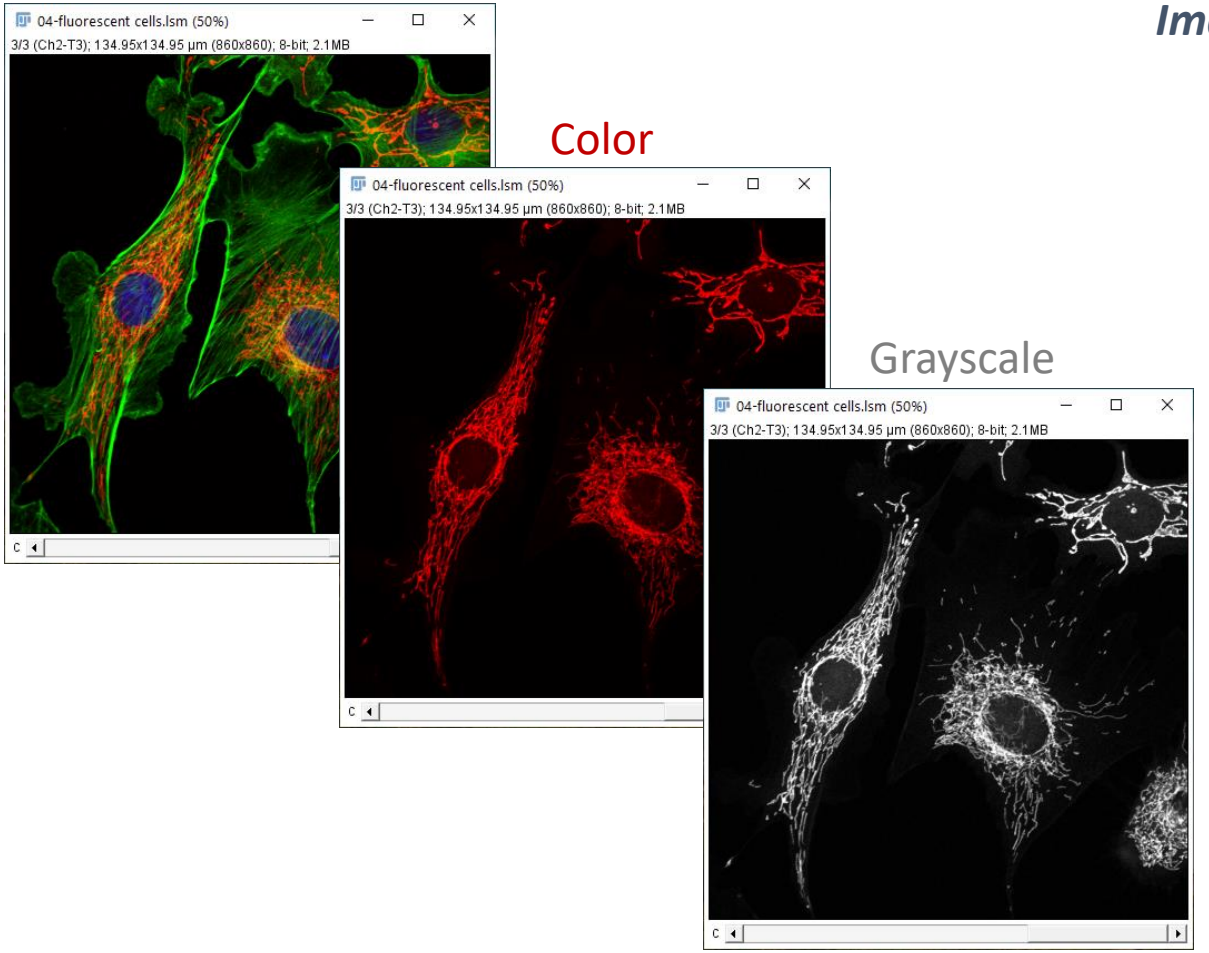
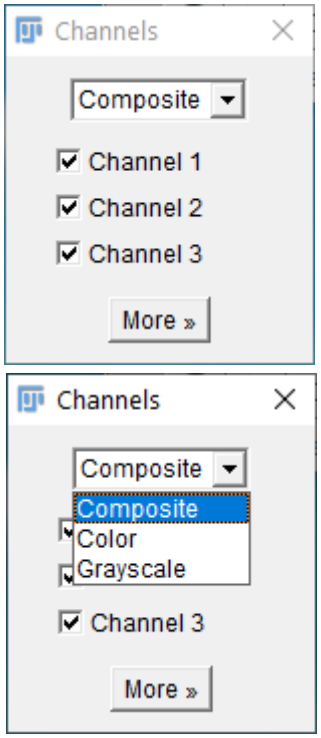
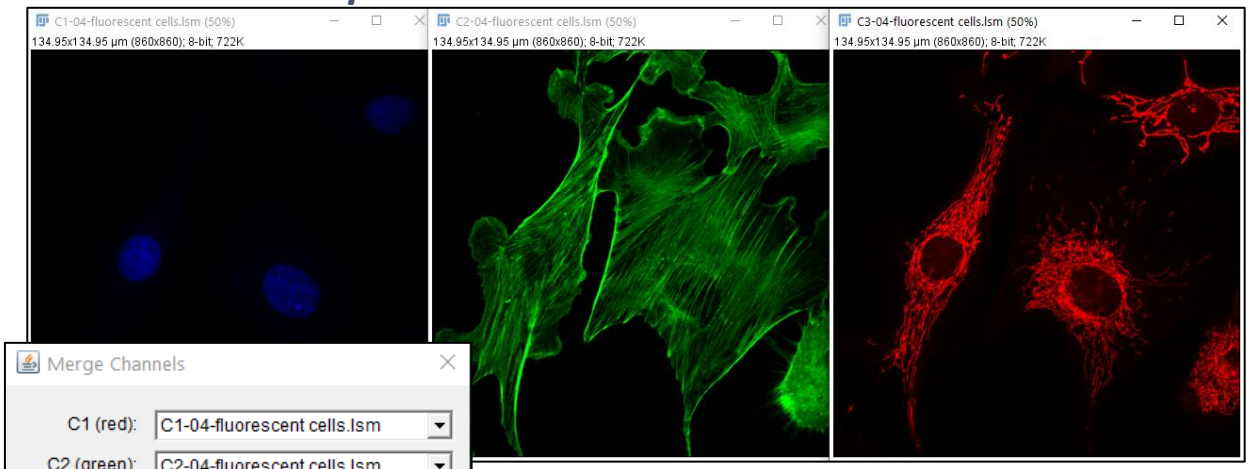


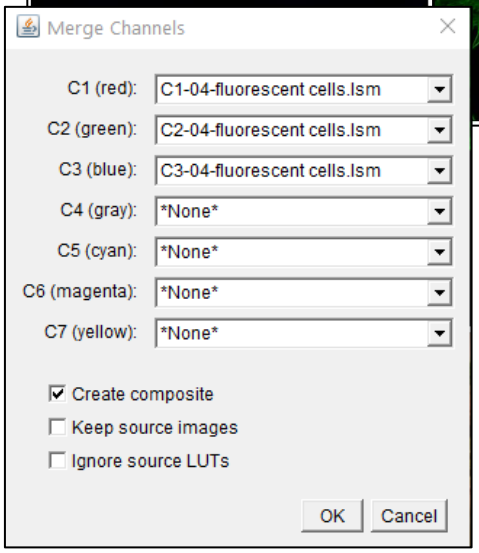
Image / Color > Channels tool



Channels tool (Shift + Ctrl + Z)

More >> Split Channels





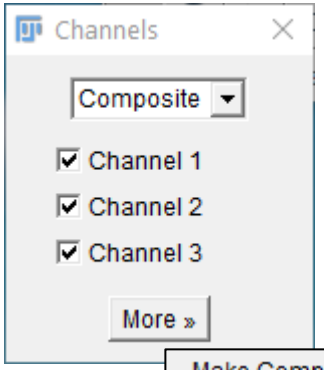
More >> Merge Channels

- Put them as multi-channel image

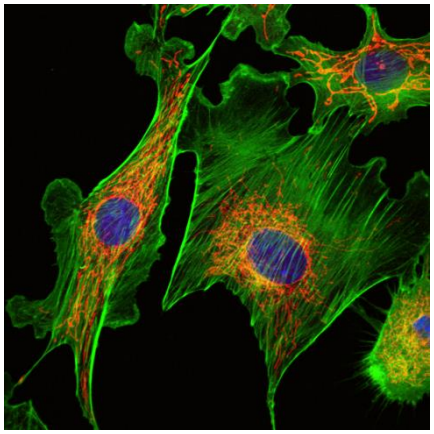
Options:

- Create composite

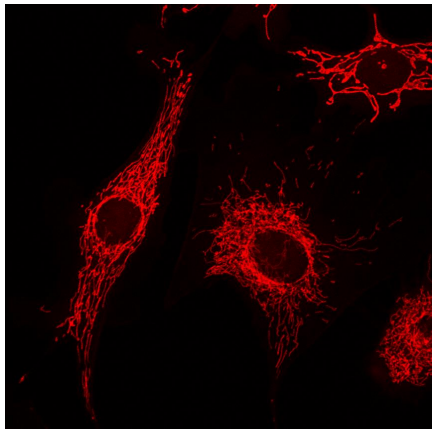
(otherwise you get an RGB image)

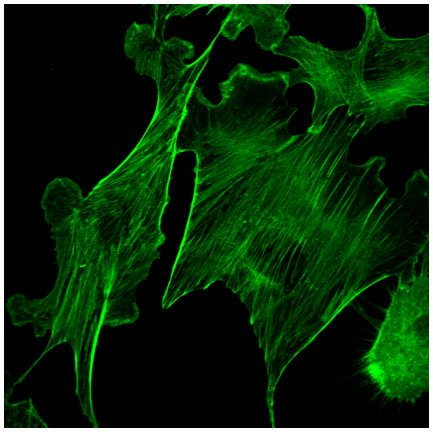


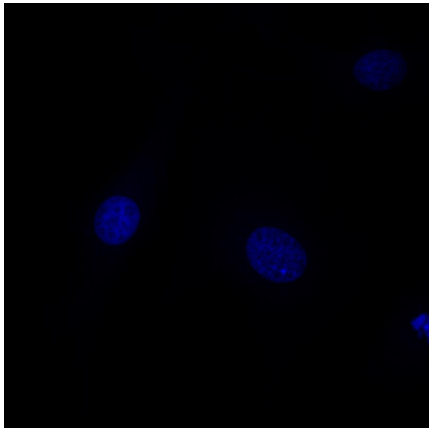
Checking image brightness & bit depth – Image Histograms (Ctrl+H)

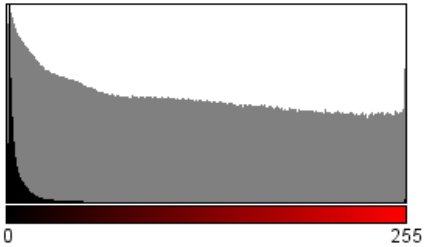


3 channels
8-bits per channel



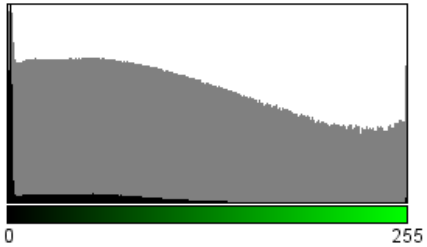






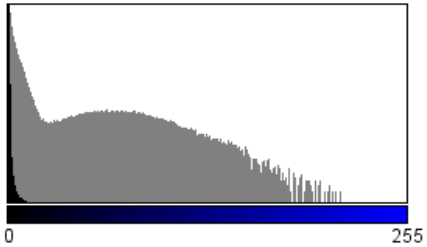
Count: 739600
Mean: 21.371
StdDev: 45.341

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



Count: 739600
Mean: 35.707
StdDev: 46.293

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



Count: 739600
Mean: 4.209
StdDev: 15.016

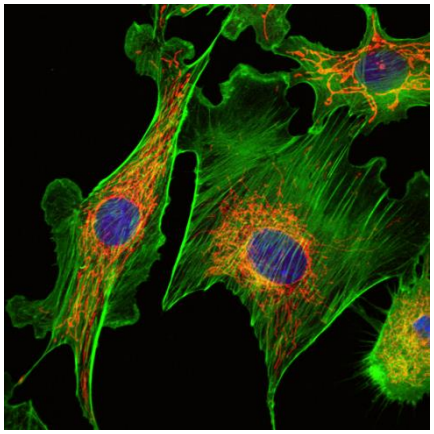
Min: 0
Max: 218
Mode: 0 (312533)

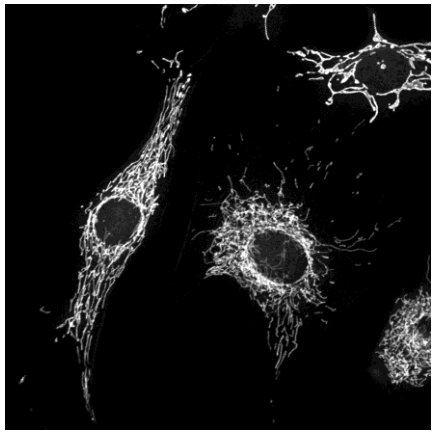
Min: 0
Max: 255
Mode: 1 (143778)

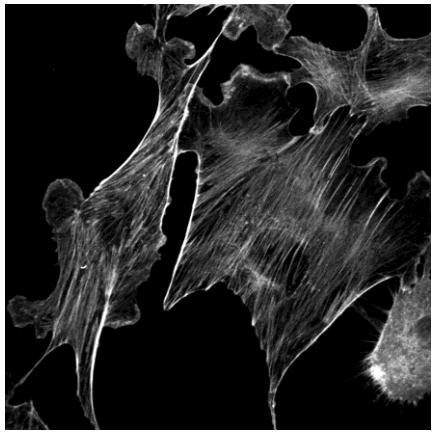
Min: 0
Max: 255
Mode: 1 (224321)

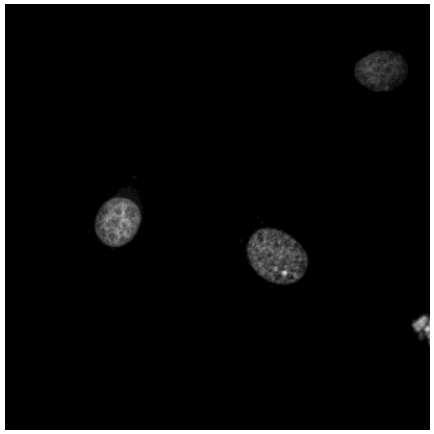
Min: 0
Max: 218
Mode: 0 (312533)

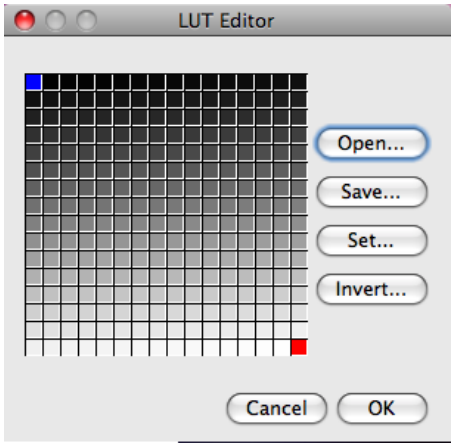
Checking image brightness – using a Hi-Lo LUT

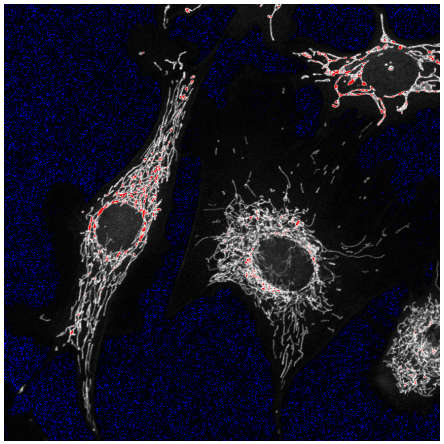


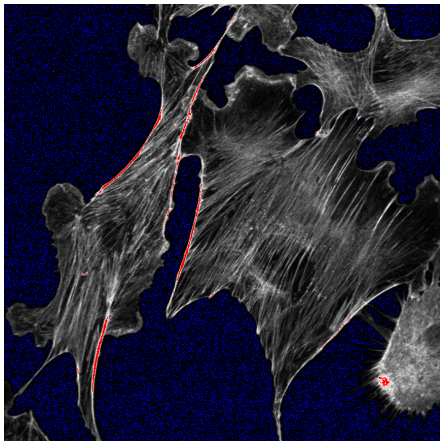


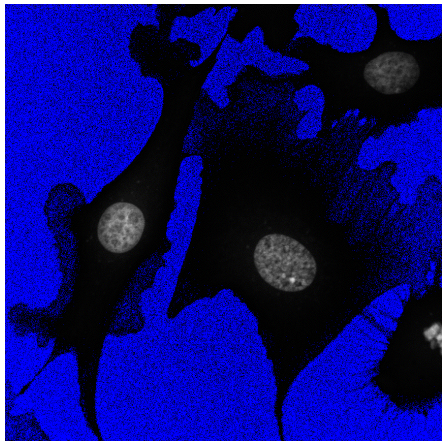












Changing image brightness

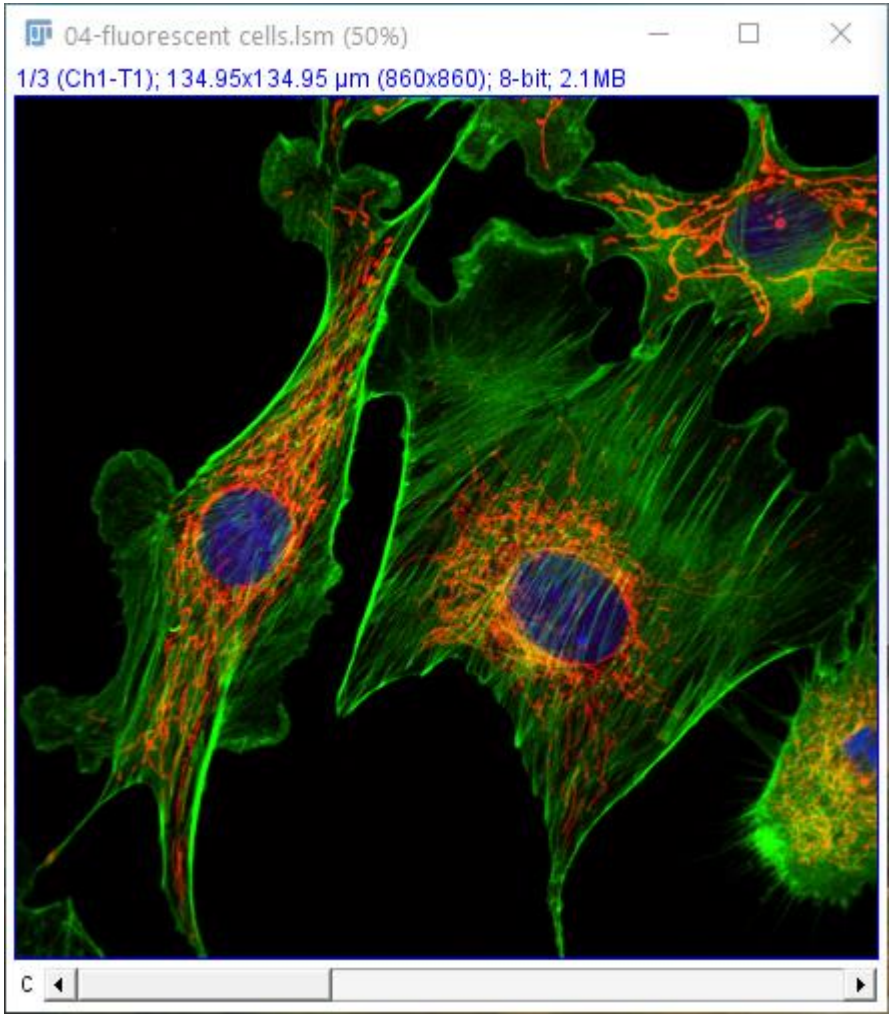
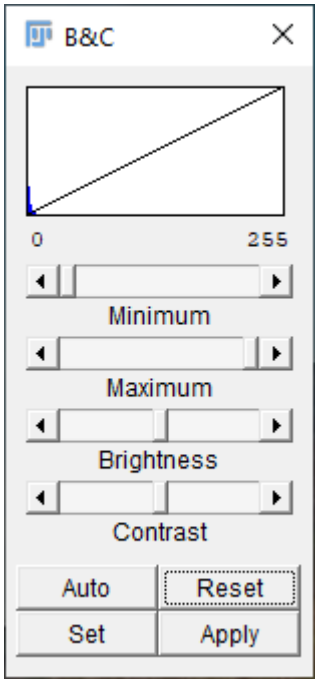
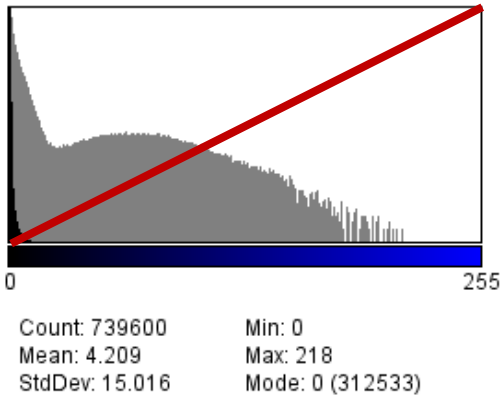


Image / Adjust > Brightness/Contrast…





Changing image brightness

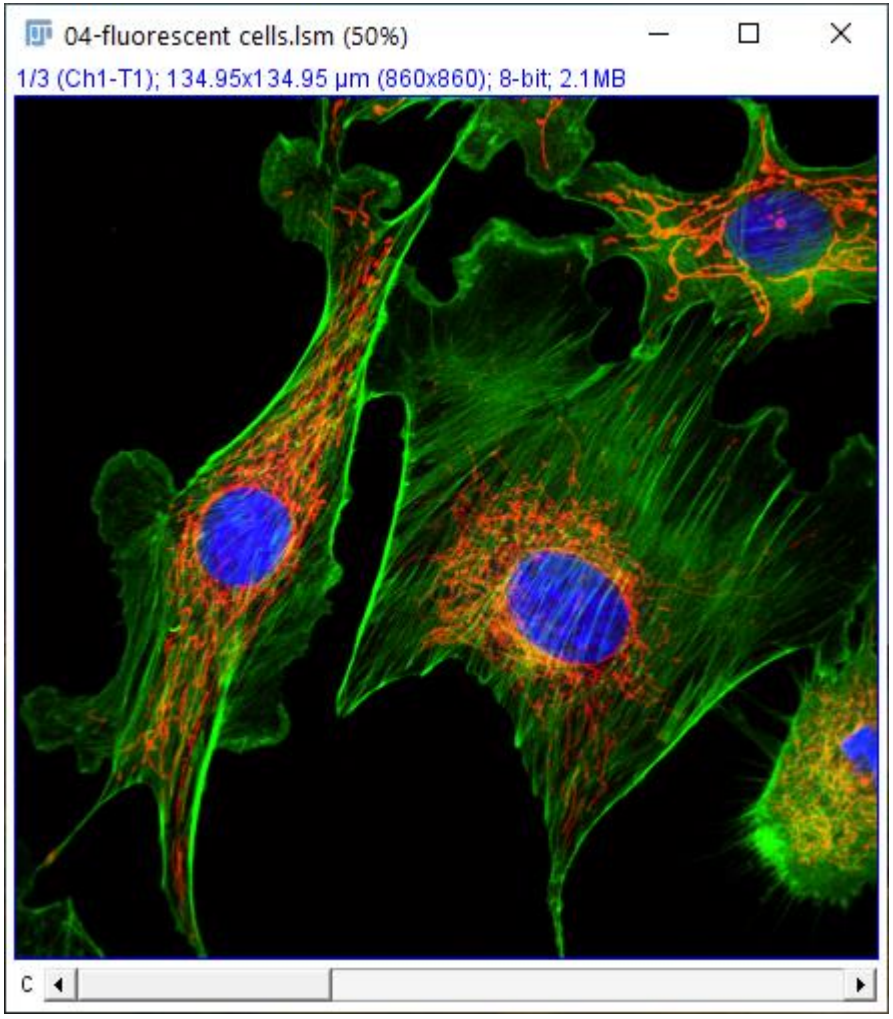
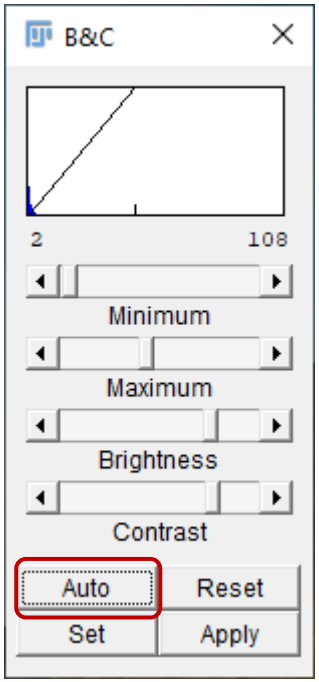
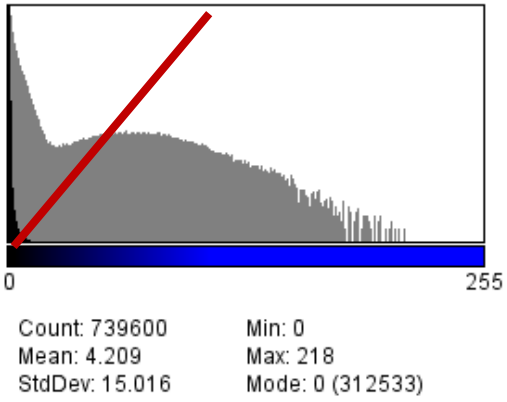


Image / Adjust > Brightness/Contrast…





Changing image brightness

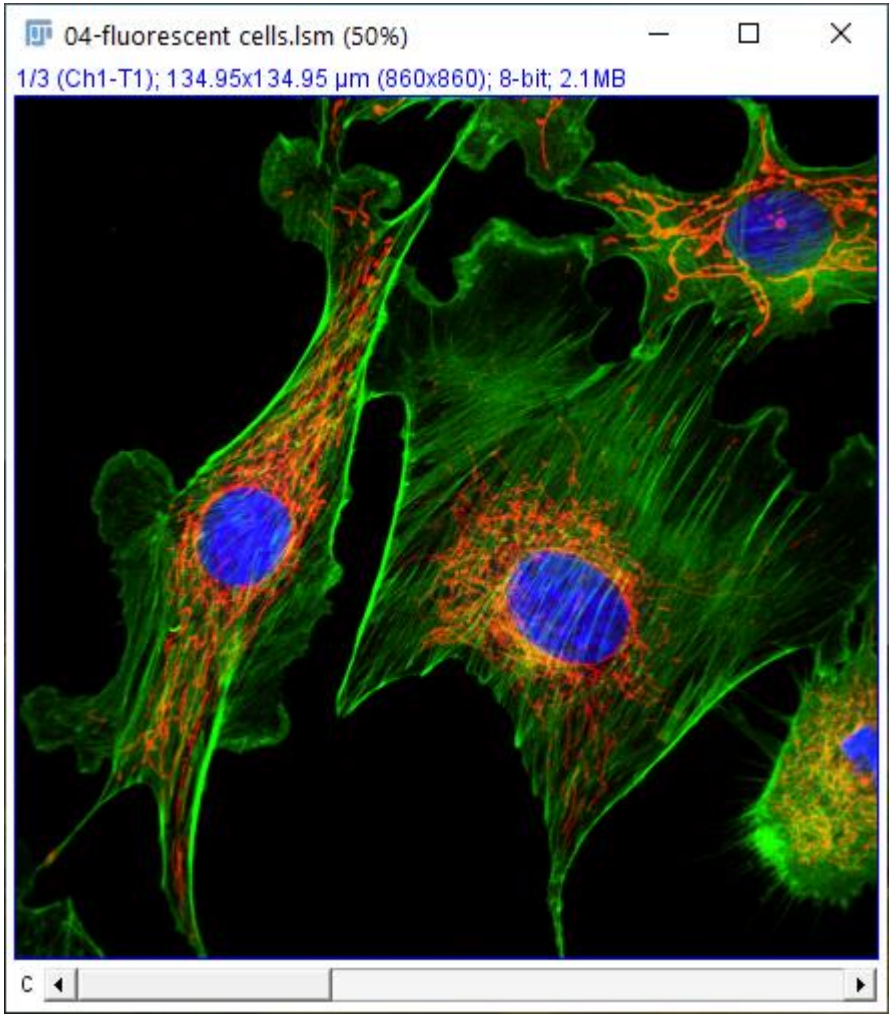
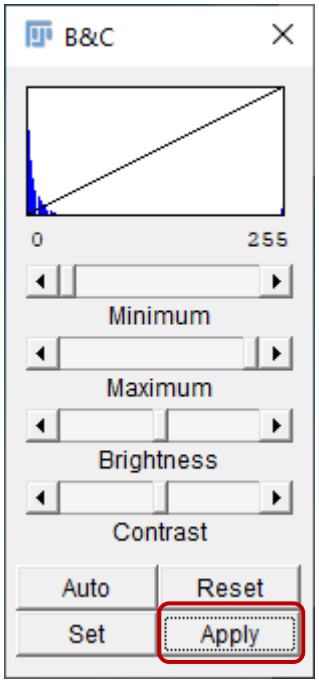
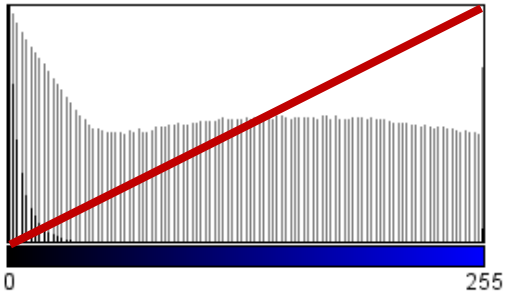


Image / Adjust > Brightness/Contrast…



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



Count: 739600
Mean: 7.614
StdDev: 33.342

Min: 0
Max: 255
Mode: 0 (572091)

Changing image brightness

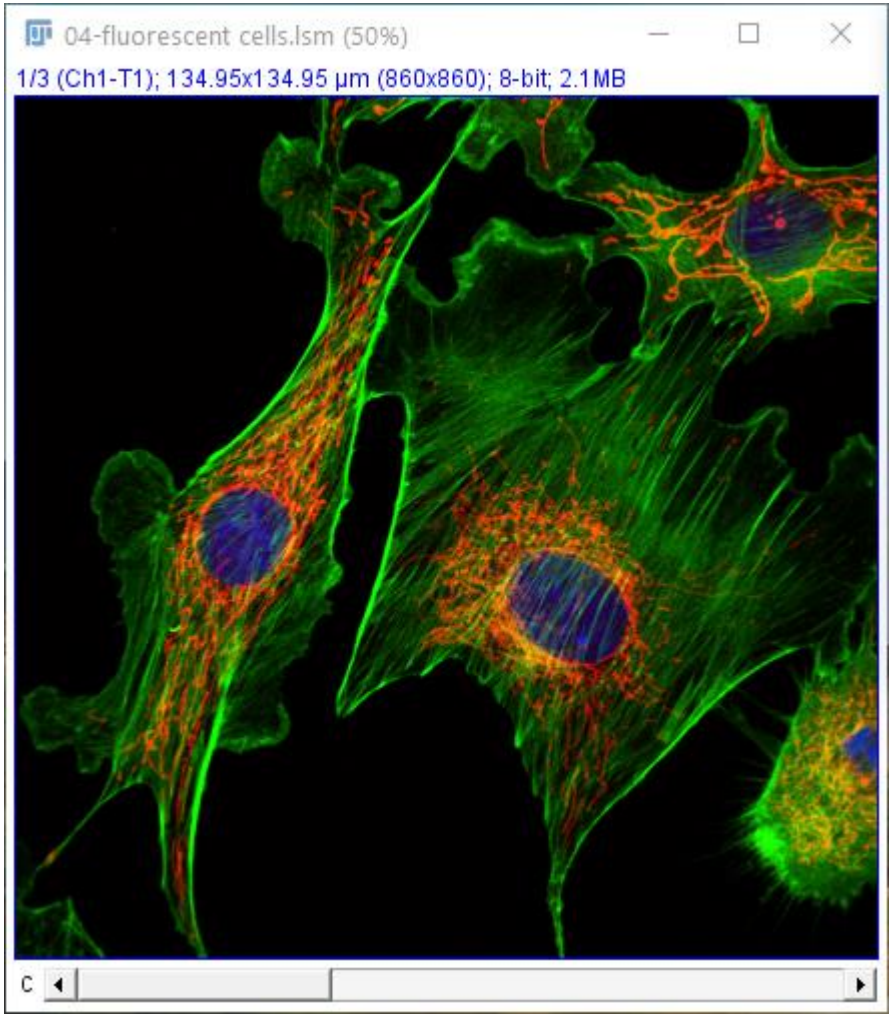
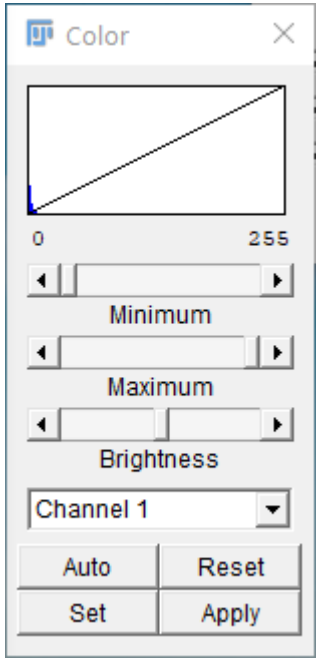
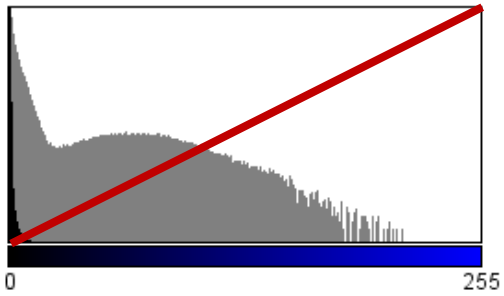


Image / Adjust > Color Balance…





Count: 739600
Mean: 4.209
StdDev: 15.016

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

Changing image brightness

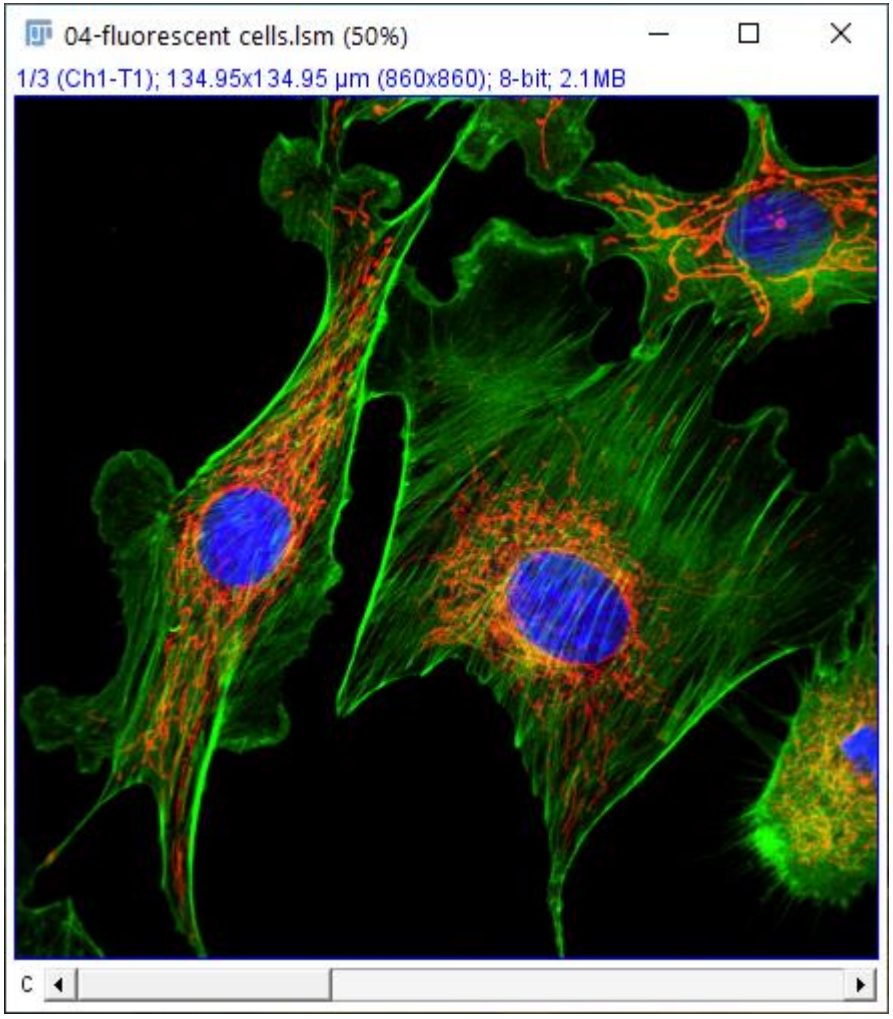
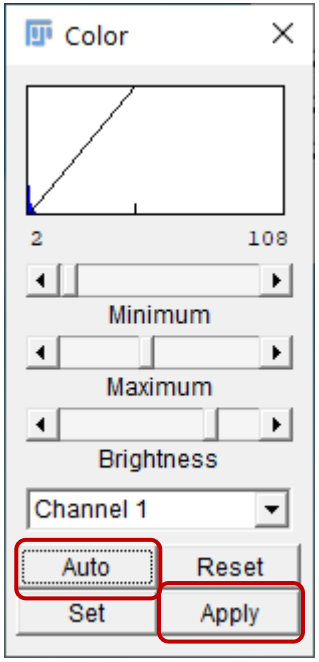
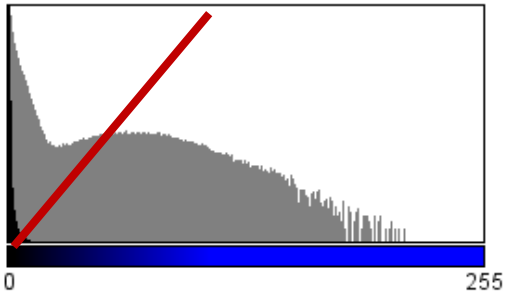


Image / Adjust > Color Balance…

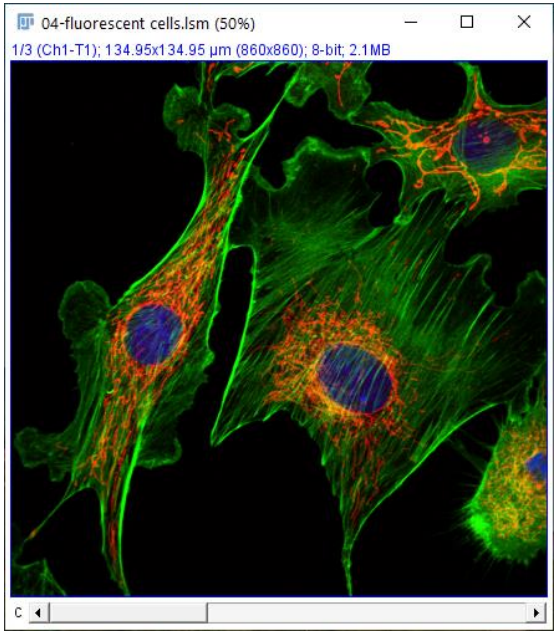


If you press 'Apply' in Color Balance you only change the view.

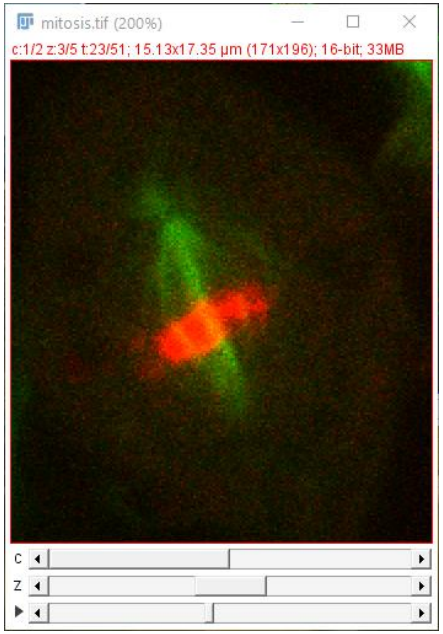


If you save the image as a .tif & re-open in Fiji, the image is displayed as you set it here.

Multi dimensional images

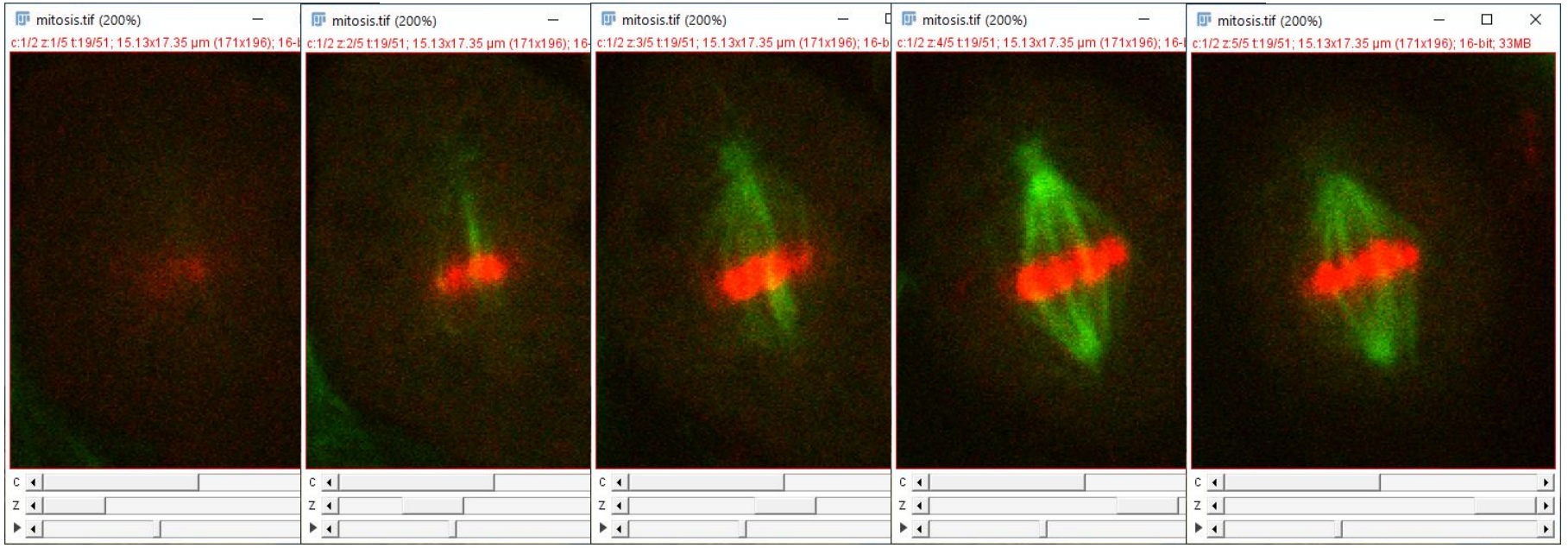


Stack. Each image is a 'slice'
It can be a set of:
Channels **or**
Z slices **or**
Timepoints



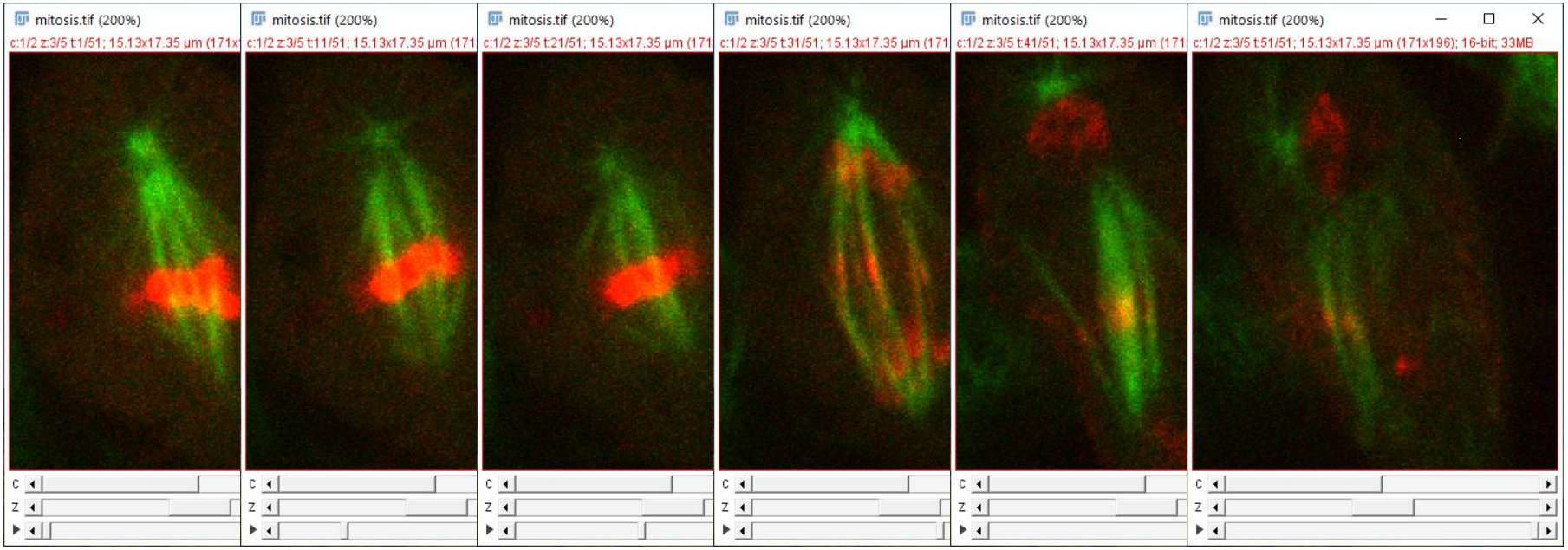
Hyperstack. Each image is a 'slice'
It can be a set of:
Channels **&/or**
Z slices **&/or**
Timepoints

Multi dimensional images



5 z slices

Multi dimensional images



51 time points

Extract parts of multi dimensional images

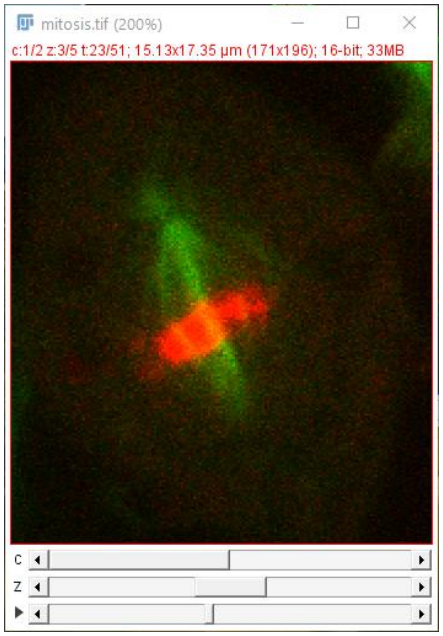
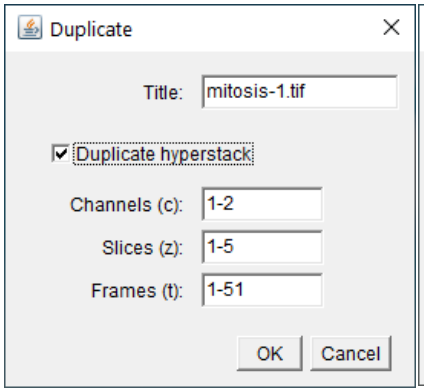
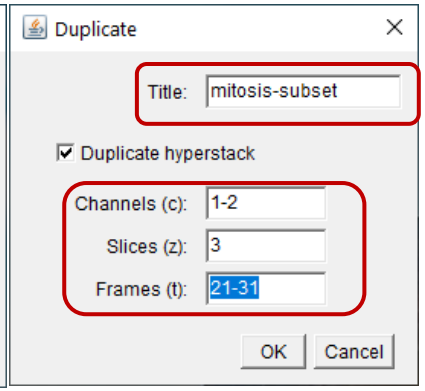


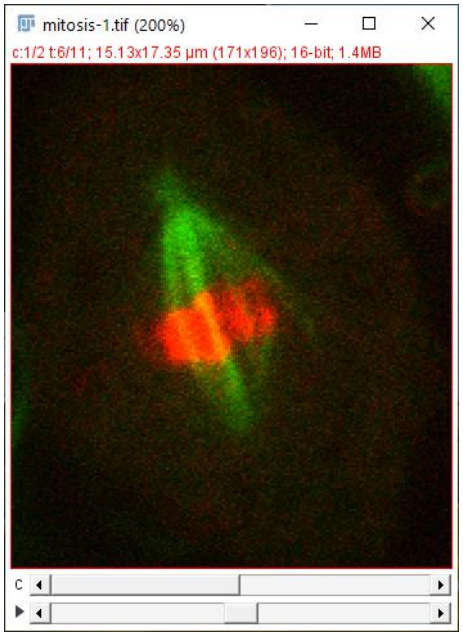
Image / Duplicate… (Ctrl + Shift + D)



Make a copy of the entire hyperstack

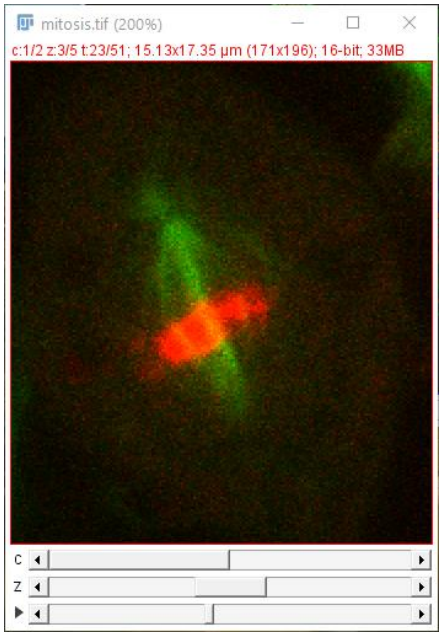


A subset of some or all of the channels, slices or timepoints.

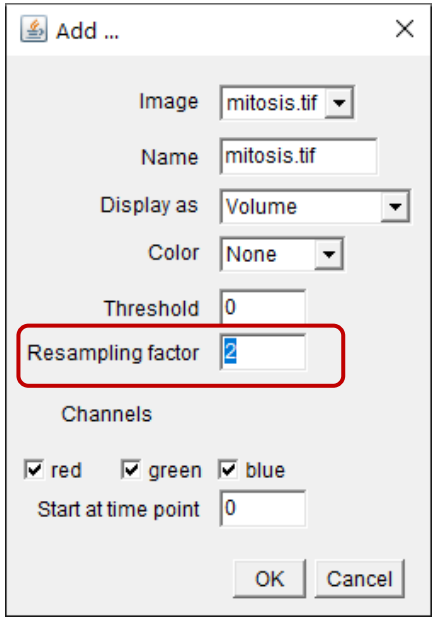


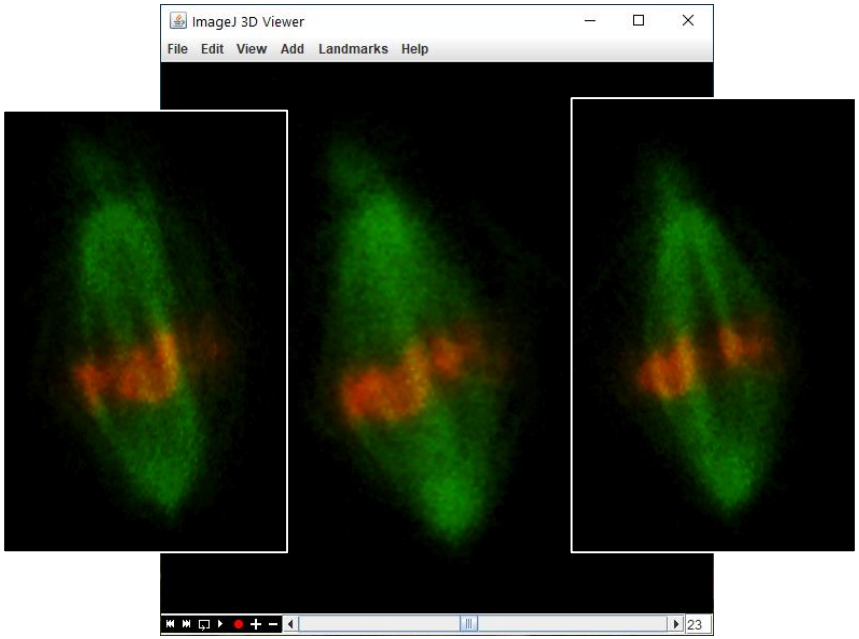
You can also change the title

Make a 3D projection



Plugins / 3D Viewer



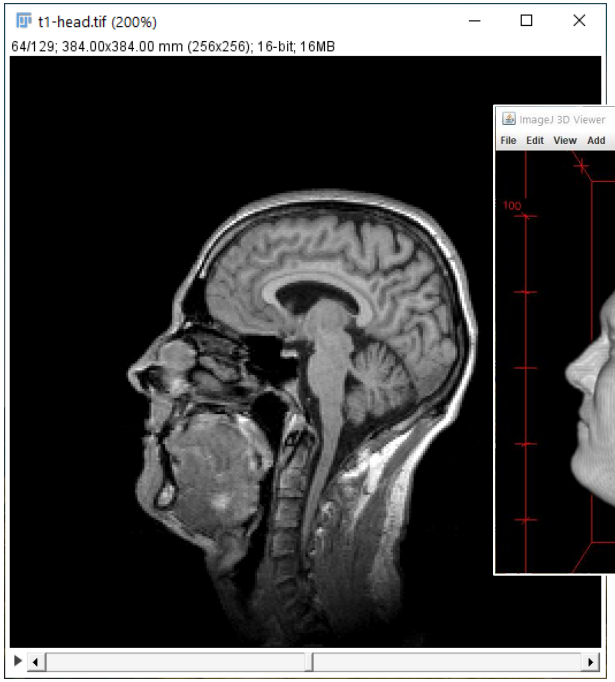


Will keep a time series (plays as a movie) if present

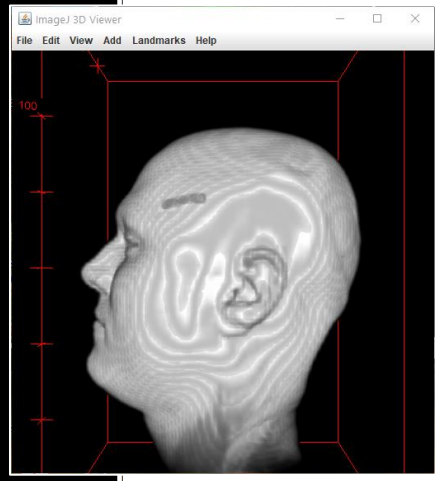
You can view the 3D projection from any angle…

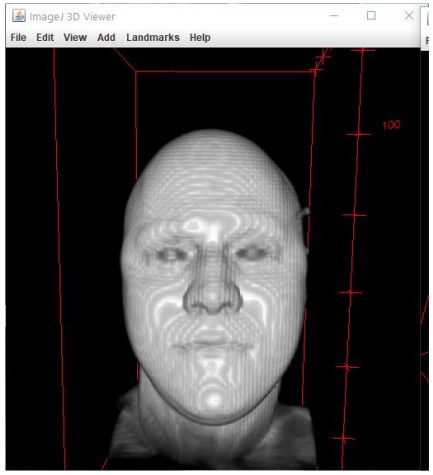
Make a 3D projection

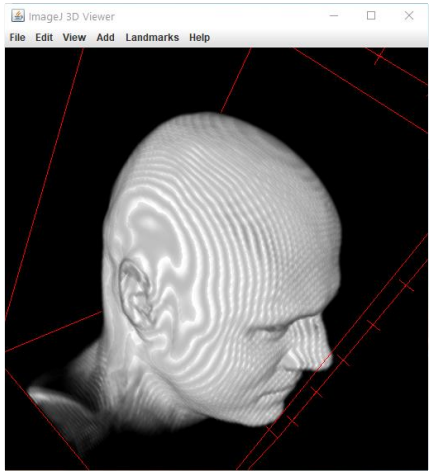
Plugins / 3D Viewer



You can view the 3D projection from any angle…







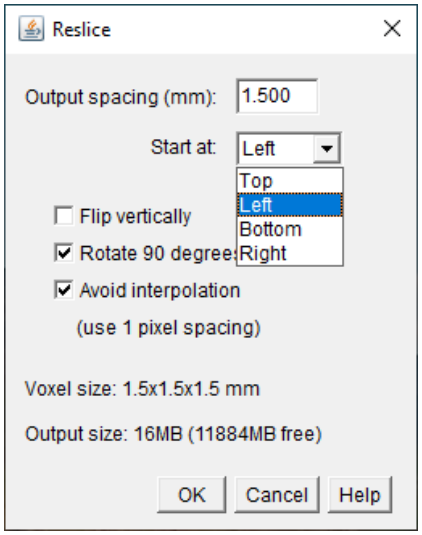
View from sides (orthogonal views)

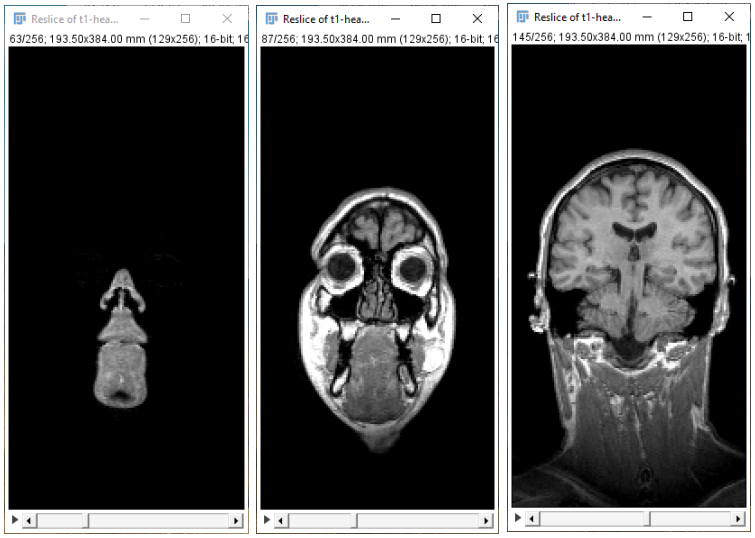
Image / stacks / Orthogonal Views (Ctrl + Shirt + H)

Move the lines to view different locations

Reslice to rotate the whole stack

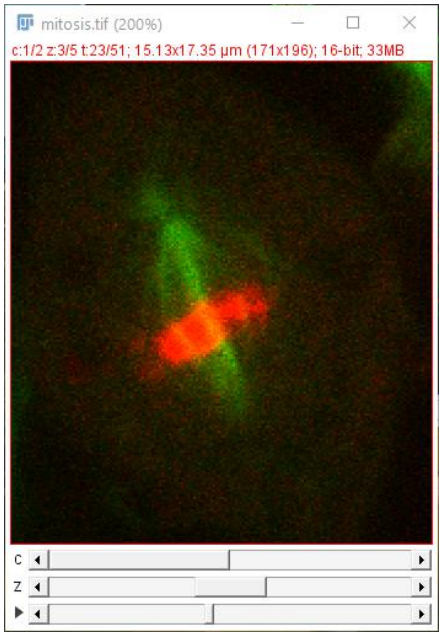
Image / stacks / Reslice (Ctrl + /)

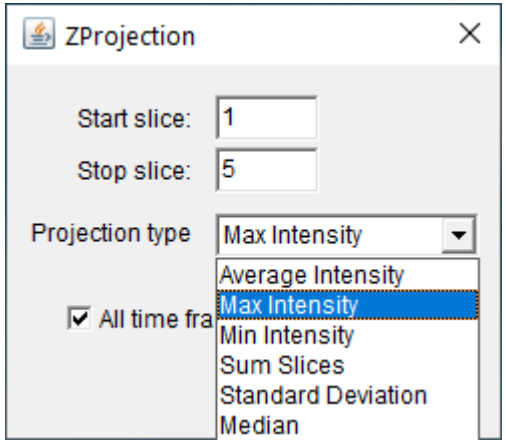


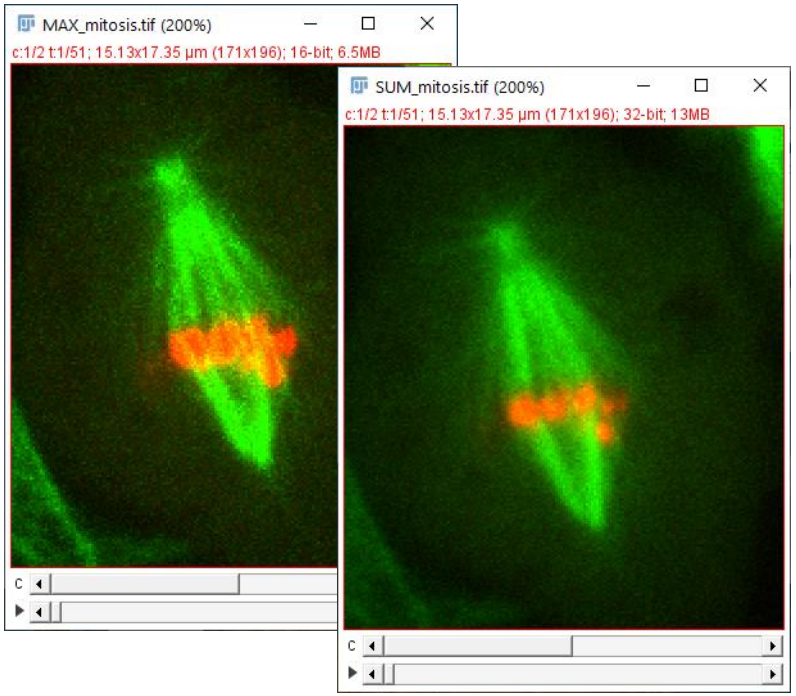


Make a projection (flatten) a 3D image

Image / Stacks / Z- Project…





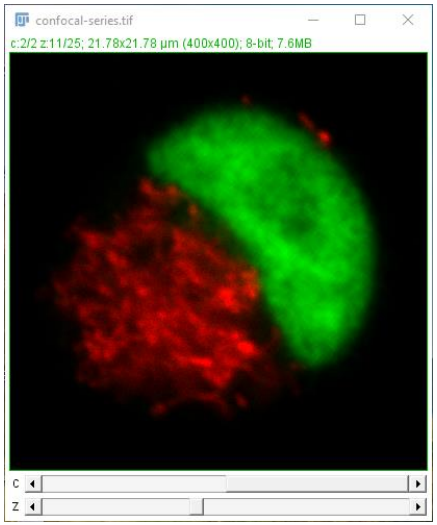


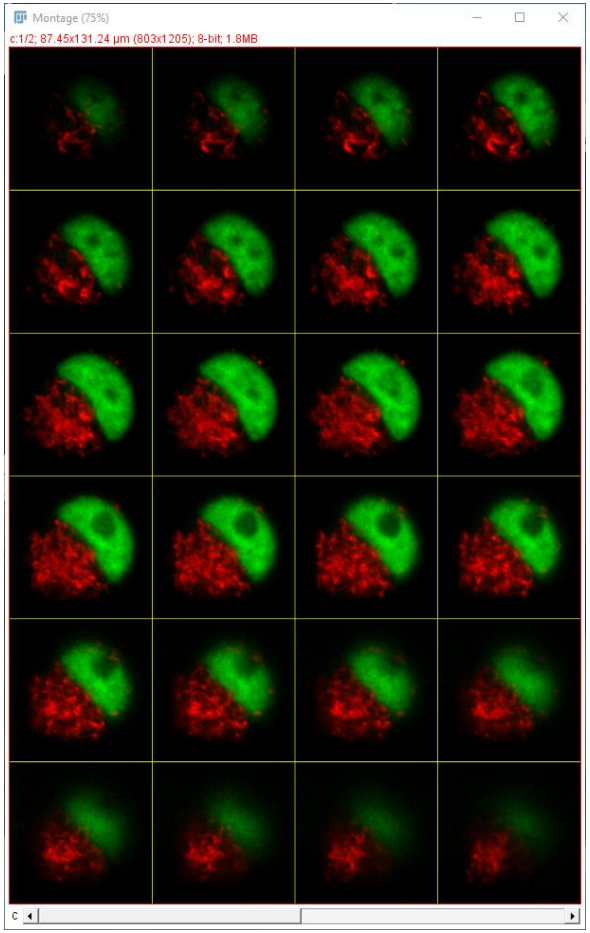
Max intensity: for each pixel position (x,y) look through every z slice, and keep only the brightest pixel

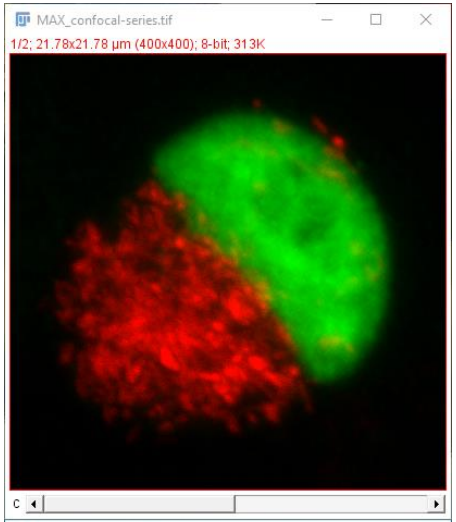
Sum Slices: for each pixel position (x,y) make a new image equal to the sum of every z slice

Make a projection (flatten) a 3D image

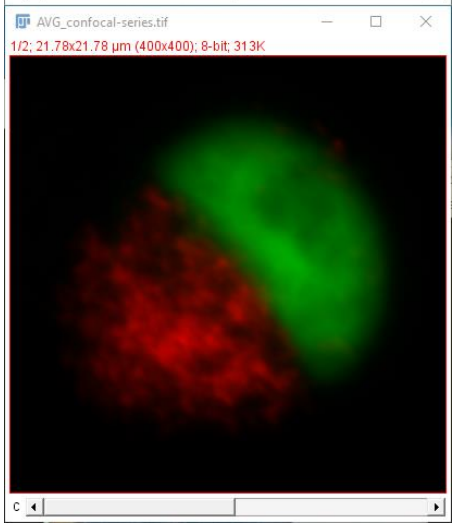
Different projections for different purposes







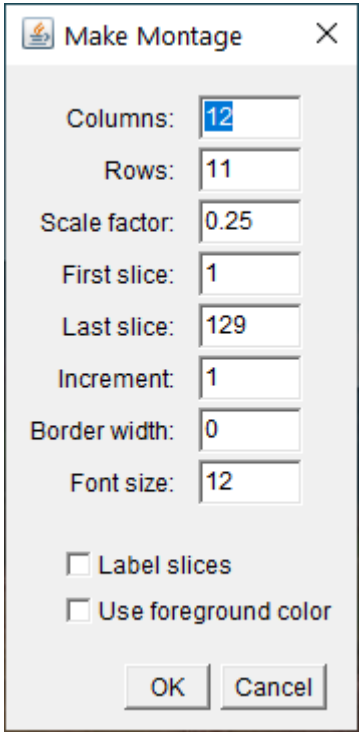
Max Projection (MaxP or MIP) for a nice image

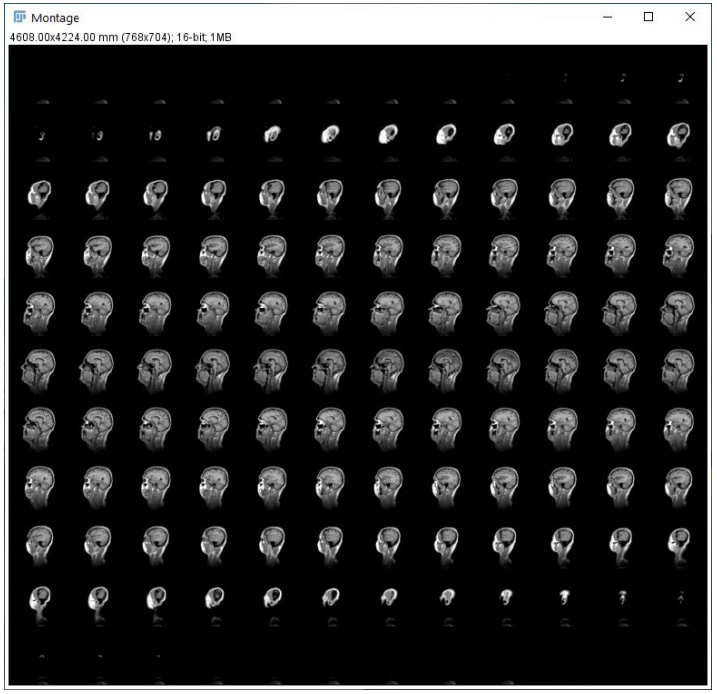


Average or Sum for measurements

Make a montage of any stack

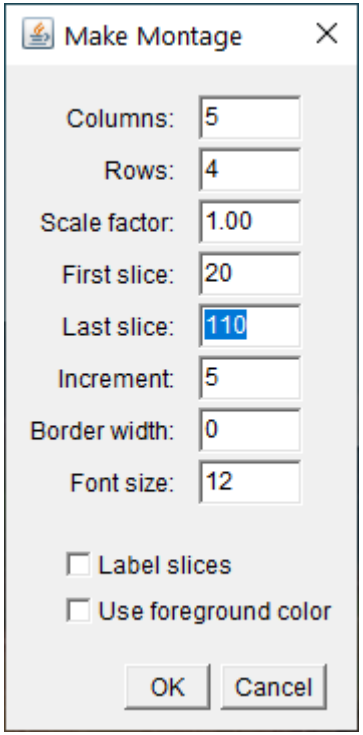
Image / Stacks / Make montage…





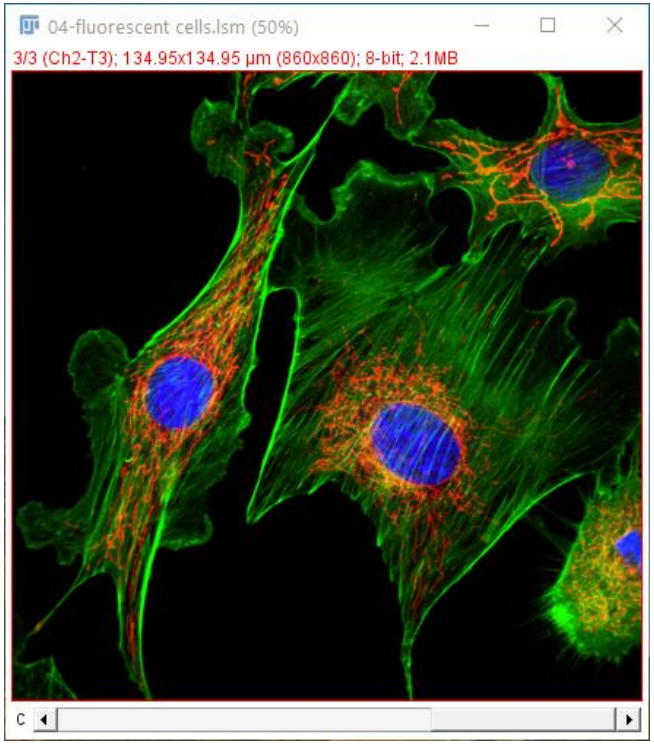
Make a montage of any stack

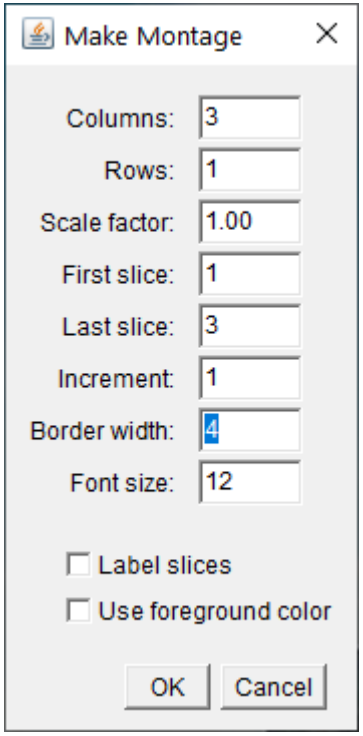
Image / Stacks / Make montage…



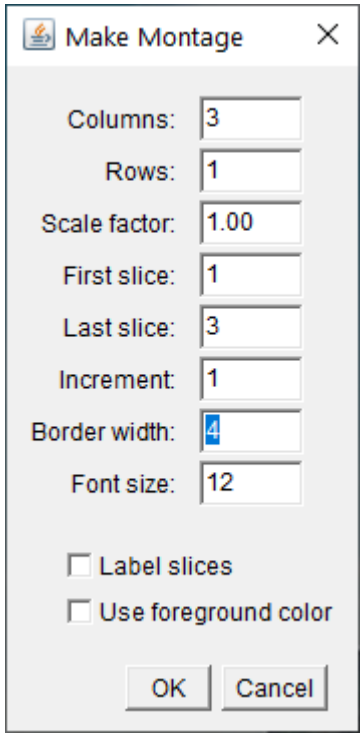
Use Make Montage to make a figure

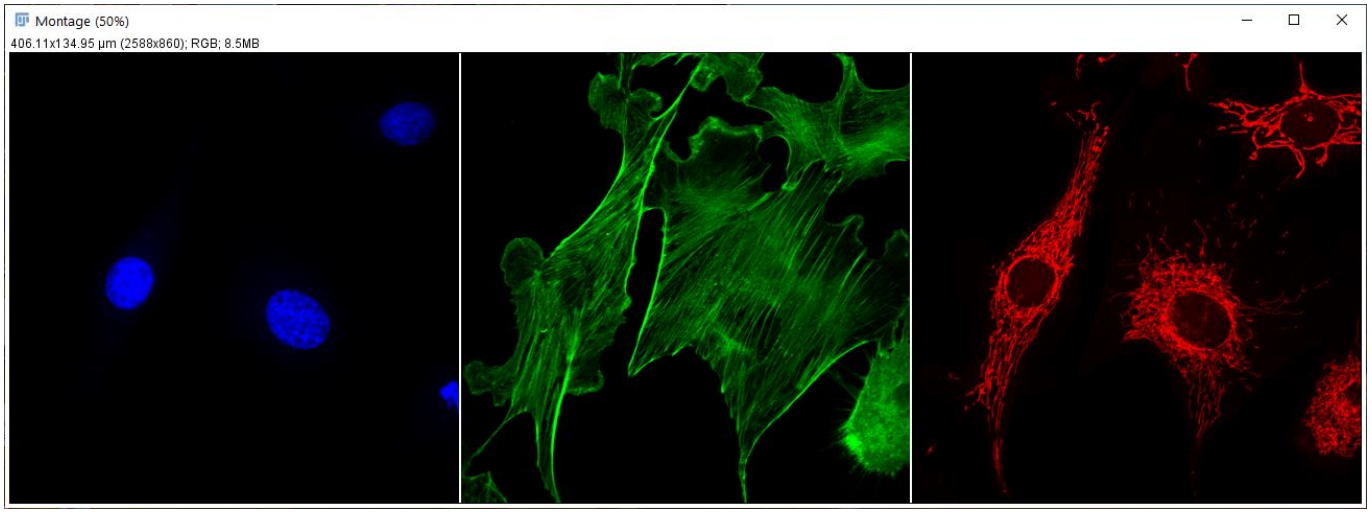
Image / Stacks / Make montage…





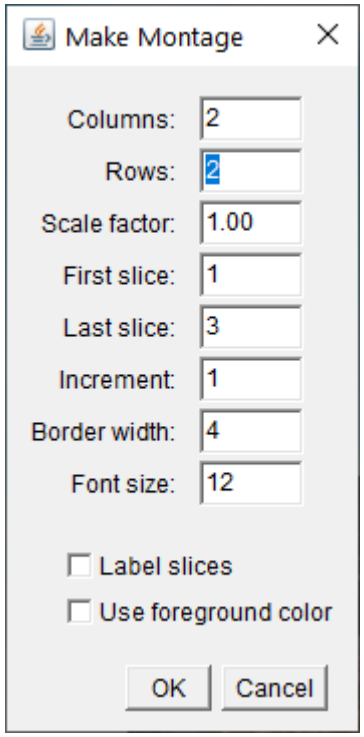
Use Make Montage to make a figure

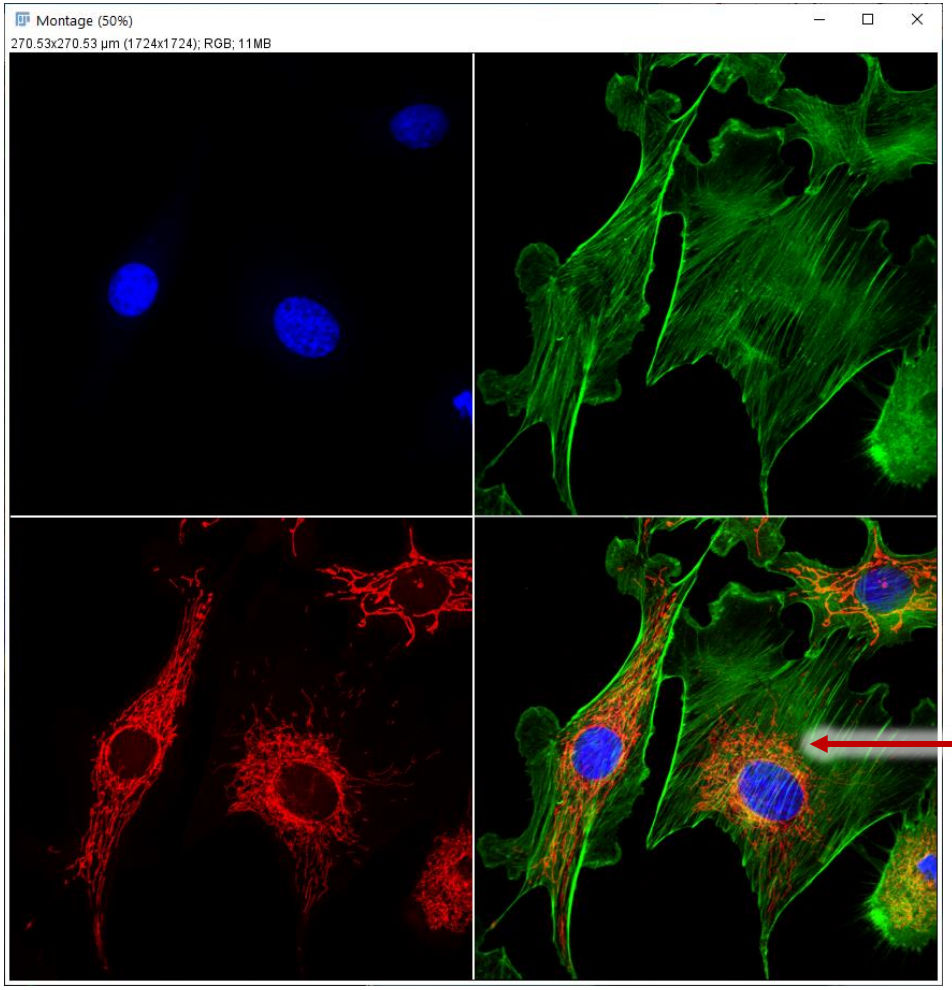




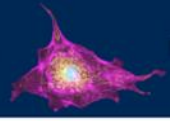
- The output is an RGB image (as it is for display only)
- Add borders.
- Change layout (3x1, 2x2, etc)
- Include an overlay (not built in but possible…)

Use Make Montage to make a figure





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

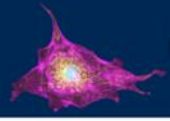


Other ImageJ tools to make figures

- **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