THE UNIVERSITY OF MELBOURNE

FIJI for Beginners Workshop

Dr Paul McMillan

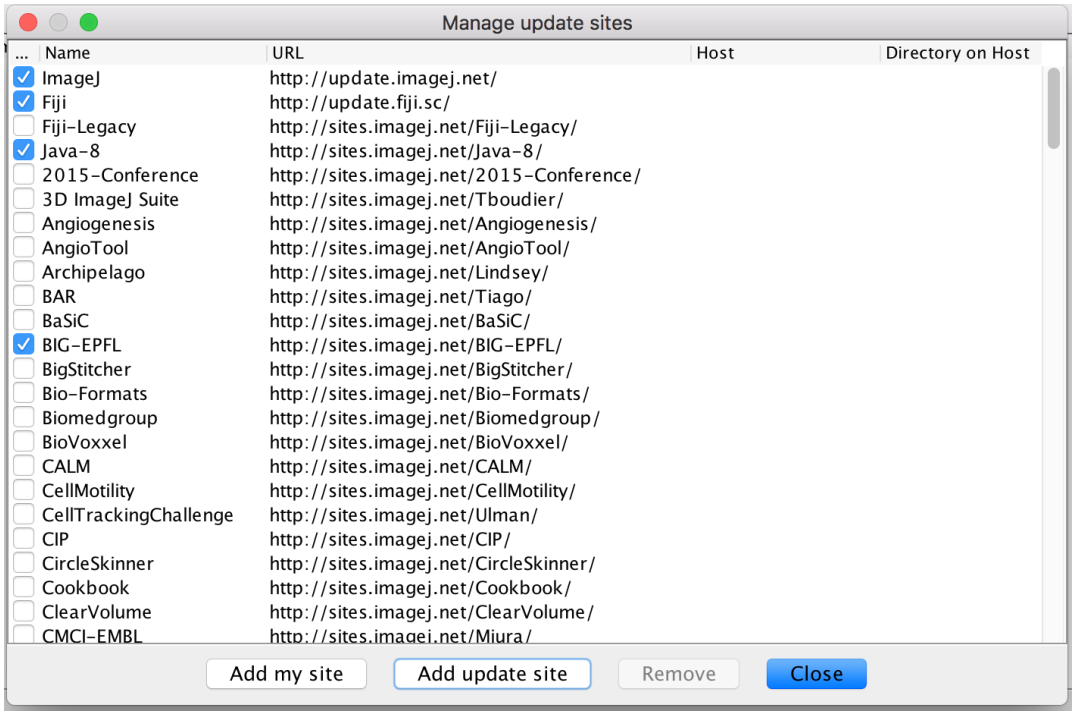
Biological Optical Microscopy Platform

Hands on Demonstrations

- Installation
- Image Import
- Image manipulation
  - Duplicating
  - Colour tools
  - ROI tools
  - Cropping
  - Brightness & Contrast
  - Look Up Tables (LUTs)
- Annotation
  - Calibrating images
  - Adding a scale bar
  - Calibration bar
- 2D data
  - Image panel
- 3D data
  - Z-projection
  - Orthogonal viewer
  - Montage
  - Projection movie
  - Rotation Movie
- Timelapse data
  - Timelapse movie
  - Annotating movies
  - Kymogram

Installation

- Download from http://fiji.sc/#download
- Install on a drive you have admin rights over
- Plugins
- Update sites (FIJI/Help/Update



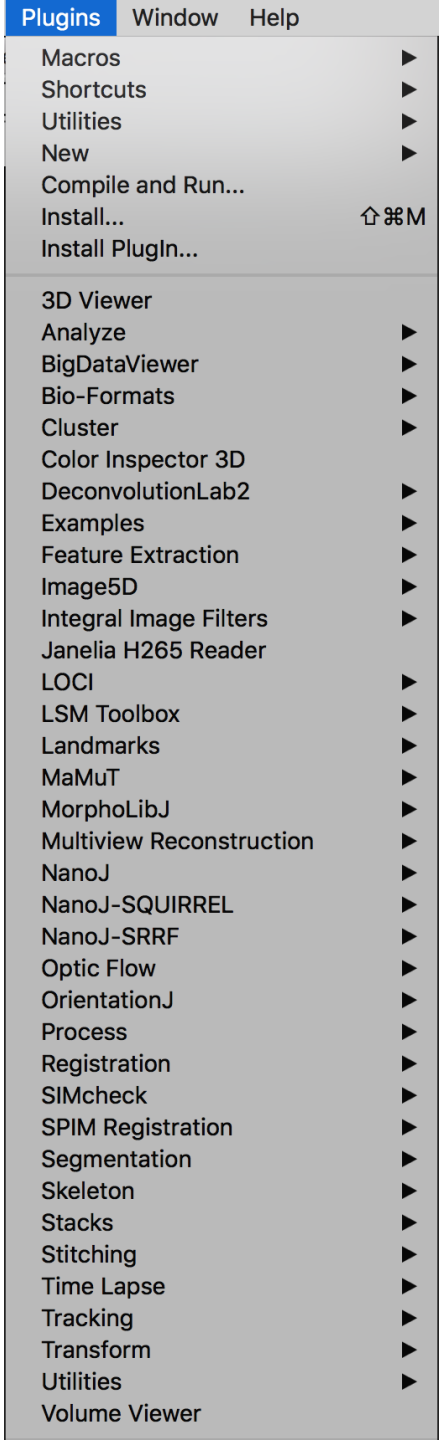


Image Import

1. FIJI/File/open
2. Drag & drop files
3. FIJI/Plugins/bio-formats/bioformats importer

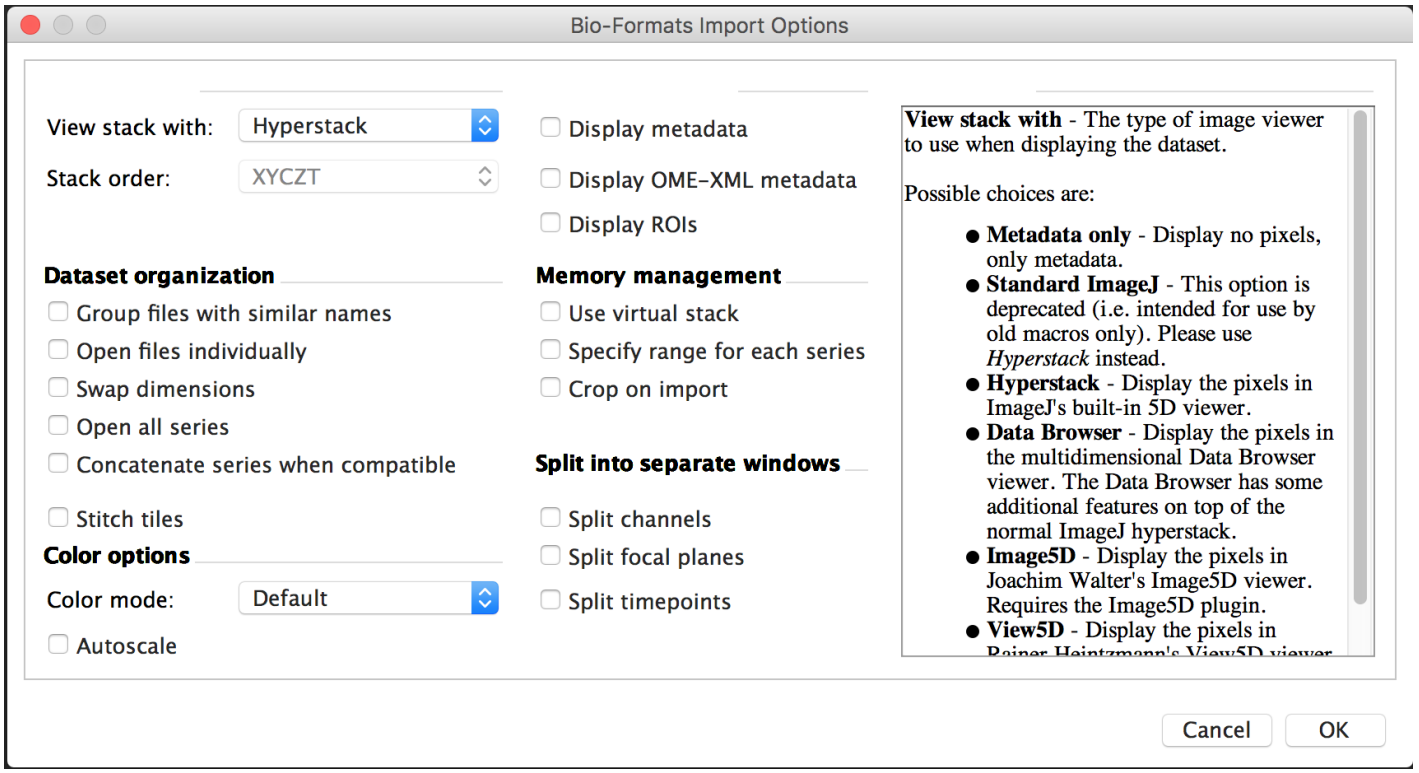


Image Information

Top of images gives us information about the image

The below example tells us:

c: 1/2 There are two channels in this images. We are looking at channel 1 at the moment.

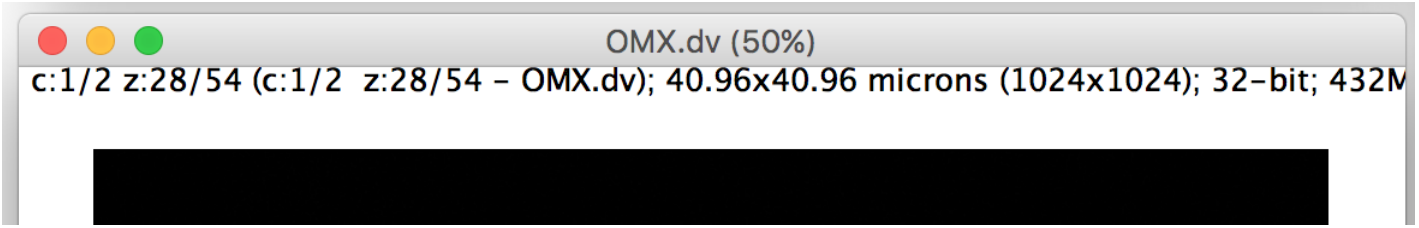
z: 28/54 There are 54 z slices in this image. We are looking at slice number 28 at the moment.

Size: Our image is 40.96 microns in length/height

Pixels: The image is made up of 1024 x 1024 pixels

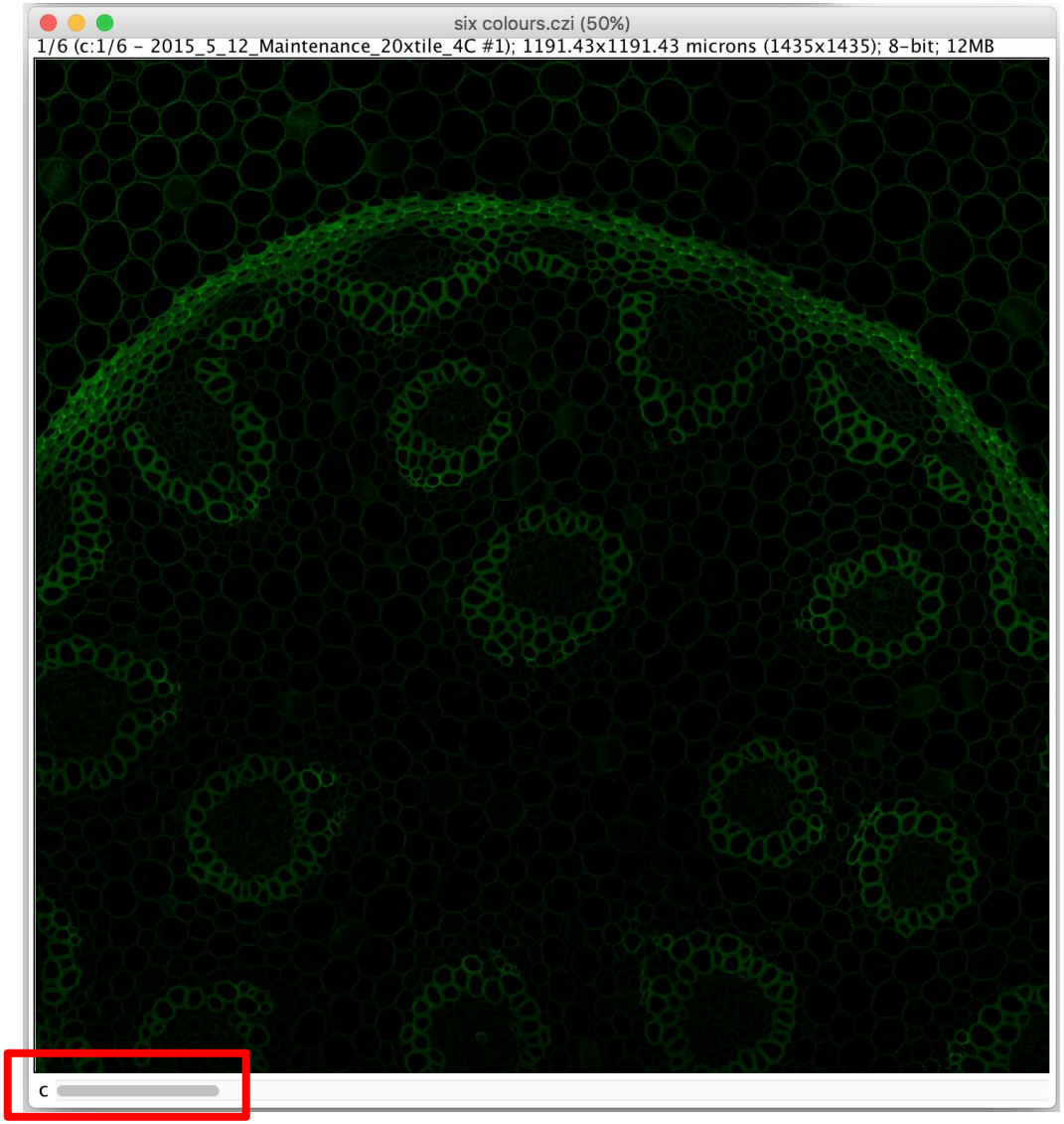
Bit-depth: The image is 32-bit

File size: The Image is 432 Mb



Stacks

- Visualises a single parameter
- Open sixcolours.czi image
- Scroll through channels at the bottom left



Hyperstacks

- Visualises Multi-parameter data
- Open OMX.dv image
- Scroll through channels & Z positions
- We will modify this image for our visualisation

FIJI Functions

Command Finder: If you are unsure where a function is located

Shortcuts

- Control + Shift L (PC)
- Command + Shift L (Mac)
- Help/Search (Mac)

Close All Images

Use File/Close All

Shortcut

- Control + Shift W (PC)
- Command + Shift W (Mac)

FIJI Functions

Image Type:

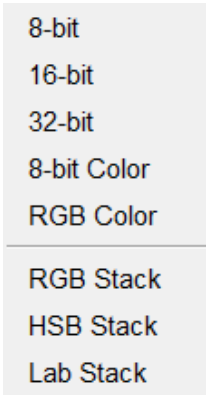
FIJI/Image/Type

e.g. Convert images to RGB format for use in office etc

Stacks:

FIJI/Image/Stacks

e.g. Stack to images = Turns stack into individual images



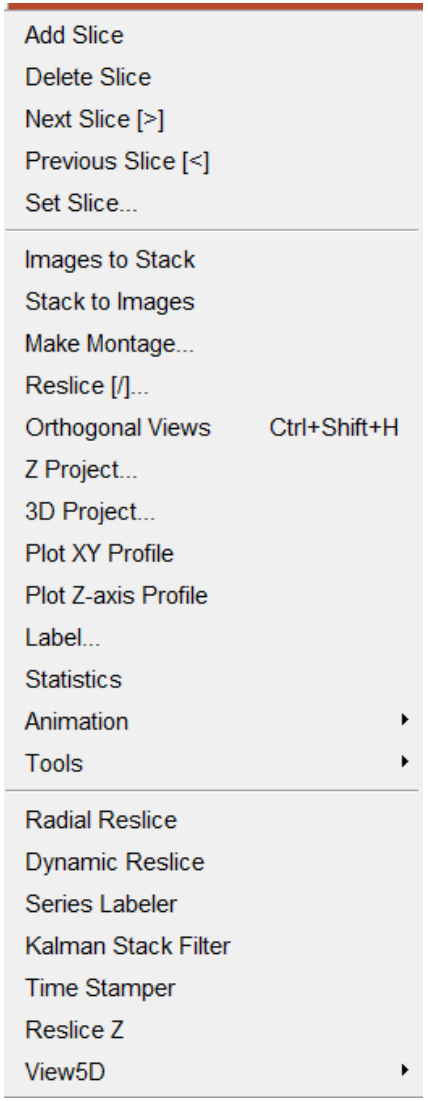


Image Resizing & Duplication

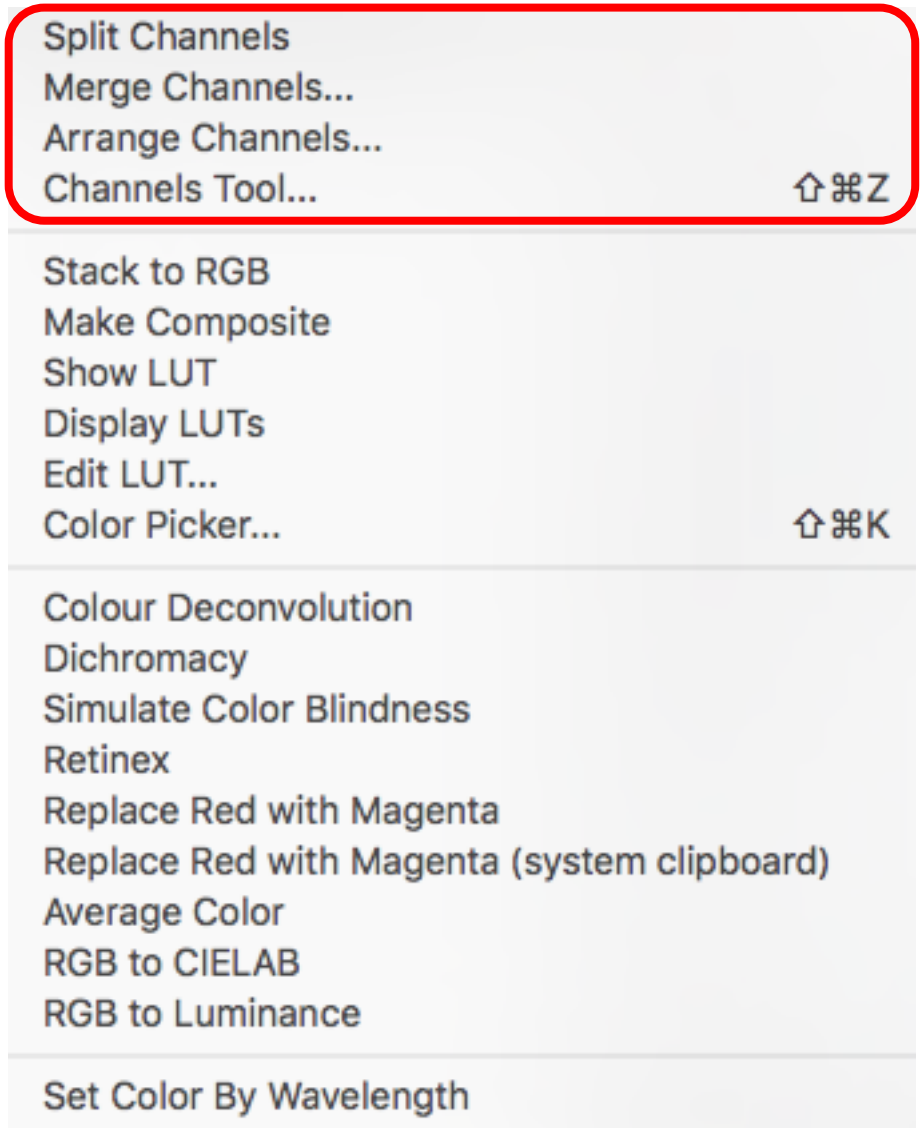
Resizing an image: Use the + or - buttons on your keyboard

Image Duplication: Always duplicate an image before making any modifications

- Select open image (or tab to select)
- FIJI/Image/duplicate
- Shortcut
  - Command + Shift D (Mac)
  - Control + Shift D (PC)

Colour Tools

FIJI/Image/Colour



Colour Tools: Split Channels

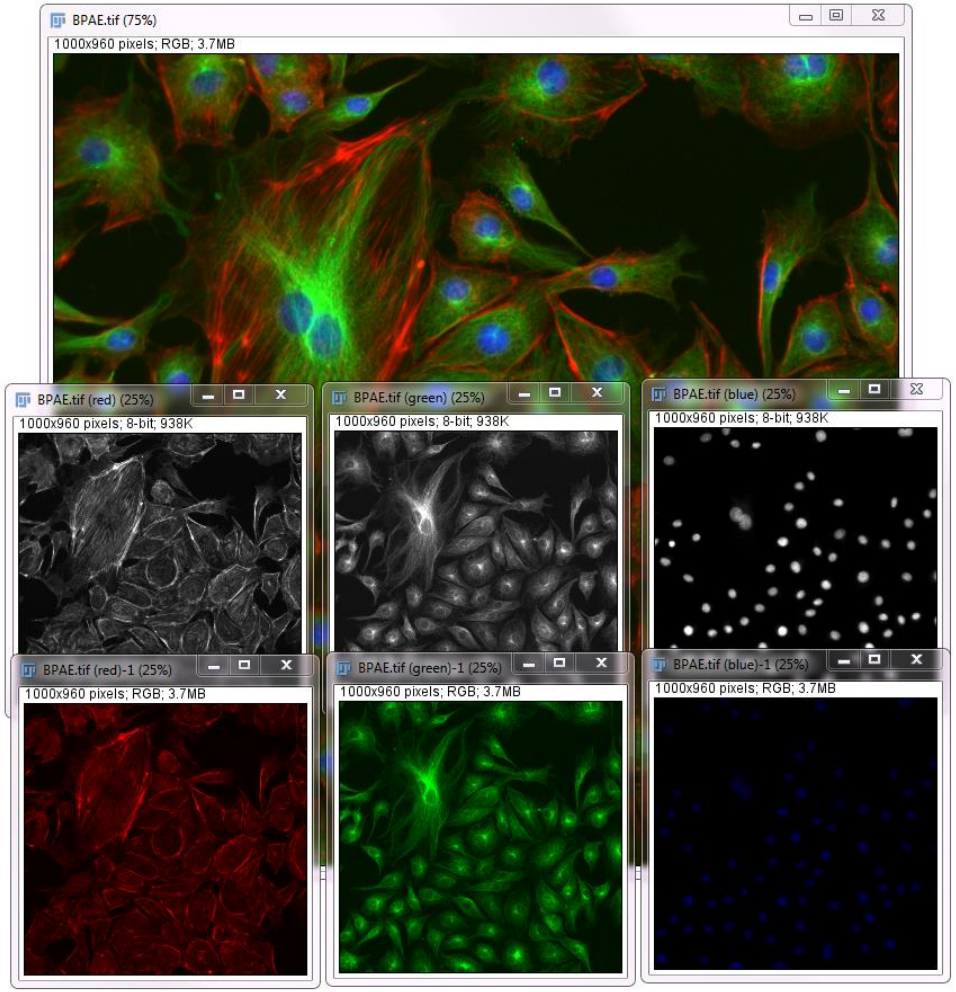
FIJI/Image/Colour/Split Channels

Activity:

1) Open BPAE.tif
2) Split the channels

Optional:

1) Duplicate the images (keep the split images for next activity)
2) Change LUTs
3) Convert to RBG
4) Save as .tiff

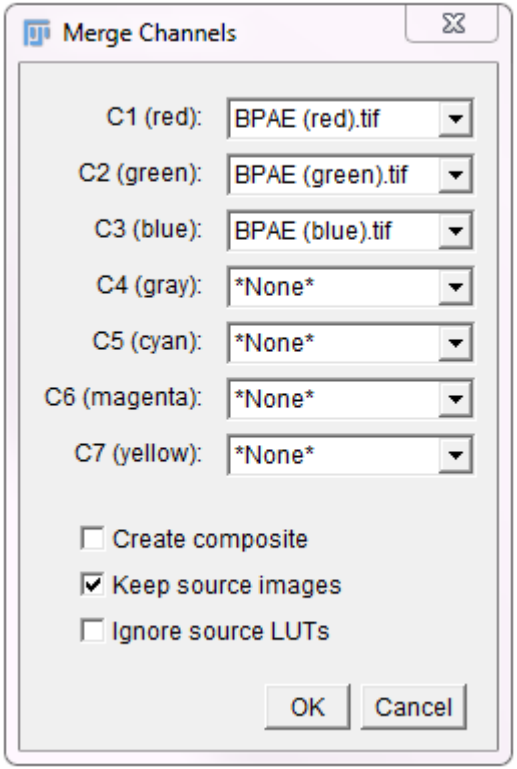


Colour Tools: Merge Channels

FIJI/Image/Colour/Merge Channels

Activity:

1) Select BPAE folder
2) Open BPAE (blue).tif, BPAE (green).tif and BPAE (red).tif in FIJI
3) Select which colour you want to assign for each image
4) Click OK and save as .tif

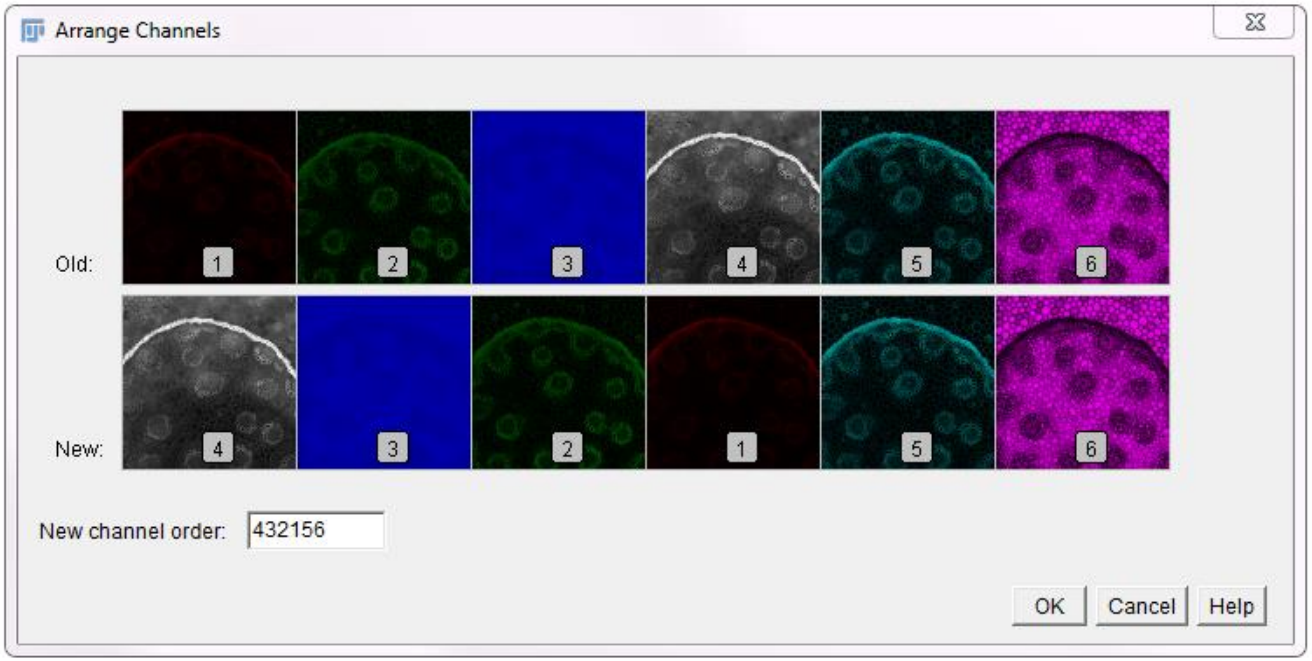


Colour Tools: Arrange Channels

FIJI/Image/Colour/Arrange Channels

Activity:

1) Open 6 colours.czi file
2) Rearrange the order of the channels (as per your choice)



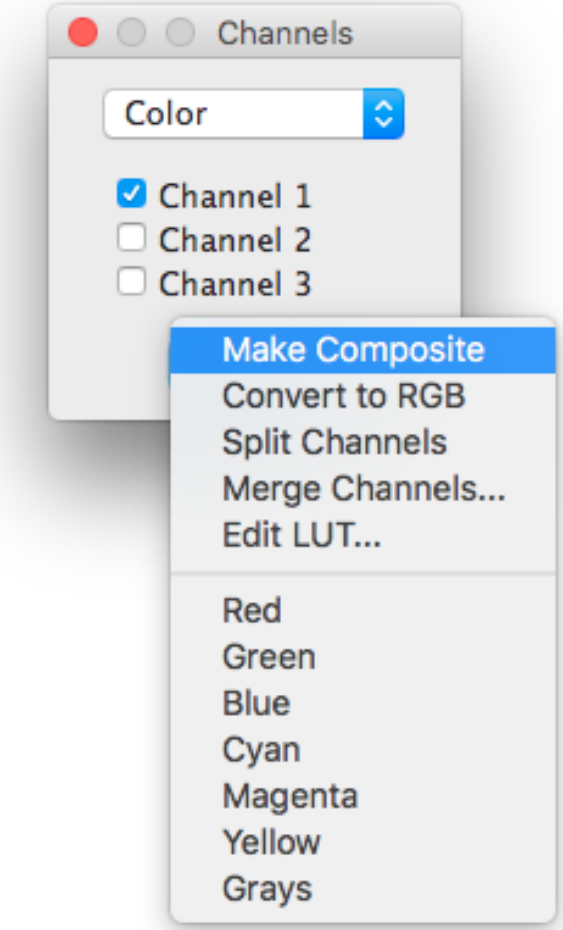
Colour Tools: Channels tool

FIJI/Image/Colour/Channels tool

Shortcut: Control + Shift Z

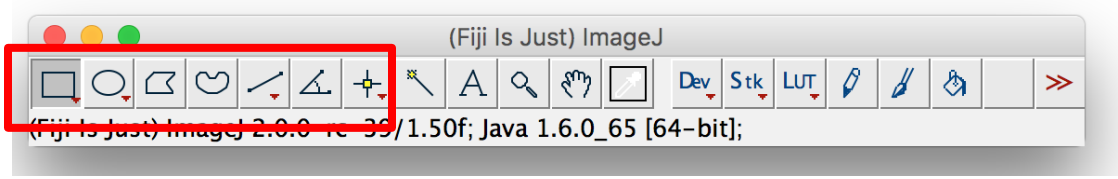
Activity:

1) Open Zeiss.lsm in FIJI
2) Open Channels tool dialog
3) Select colour option, select channel, click more and assign a LUT
4) Do this for all channels
5) Make Composite
6) Convert to RGB
7) Save as tif



ROI tools

- Select open image
- Select rectangle tool
- Draw on image



Resizing ROIs (combinations can be used)

Shift – resizes symmetrically (same dimensions in x & y)

Control (PC), Command (Mac) - Free resize are centre of ROI

Alt – maintains aspect ratio on existing ROI

Define ROI size

FIJI/Edit/Selection/Specify

ROI Tools

Making same ROI of multiple images

- Draw ROI on first image
- Select next image, FIJI/Edit/Selection/Restore Selection
- Shortcuts
  - Command + Shift E (Mac)
  - Control + Shift E (PC)

ROI Manager (more than one ROI on an single image)

- Select open image
- FIJI/Analyse/Tools/ROI manager
- Draw on ROI & click “Add”
- Click “Show All” view all selected ROI

Image Cropping

FIJI/Image/Crop

Shortcuts: Control + Shift X (PC) or Command + Shift X (Mac)

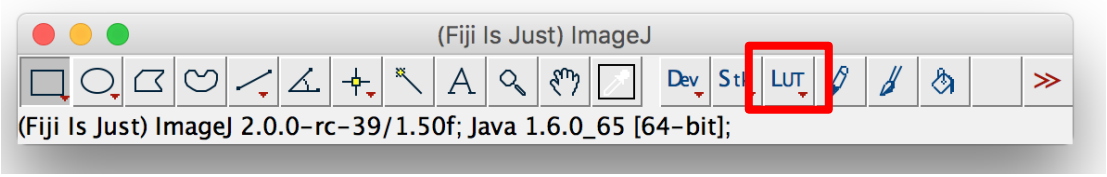
Activity:

1) Select open image
2) Duplicate the image
3) Draw an ROI (left click and hold)
4) FIJI/Image/Crop

- Can use Restore Selection (FIJI/edit/Selection/Restore Selection) to crop same area on multiple images

Look Up Tables (LUTs)

FIJI/Image/Lookup Tables or LUT shortcut

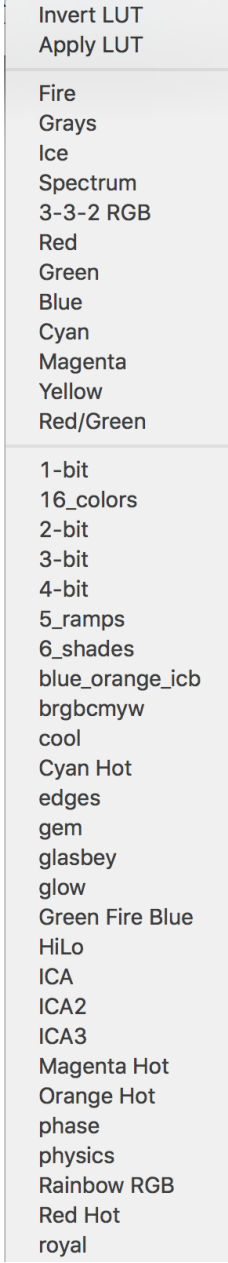


Activity:

1) Open an image from demo images (e.g. BPAE green.tif)
2) Click LUT
3) Select a LUT of your choice
4) Convert to RBG (Image/Type/RGB color)
5) Save image as tif

To visualize LUTs available

Image/Color/Display LUTs

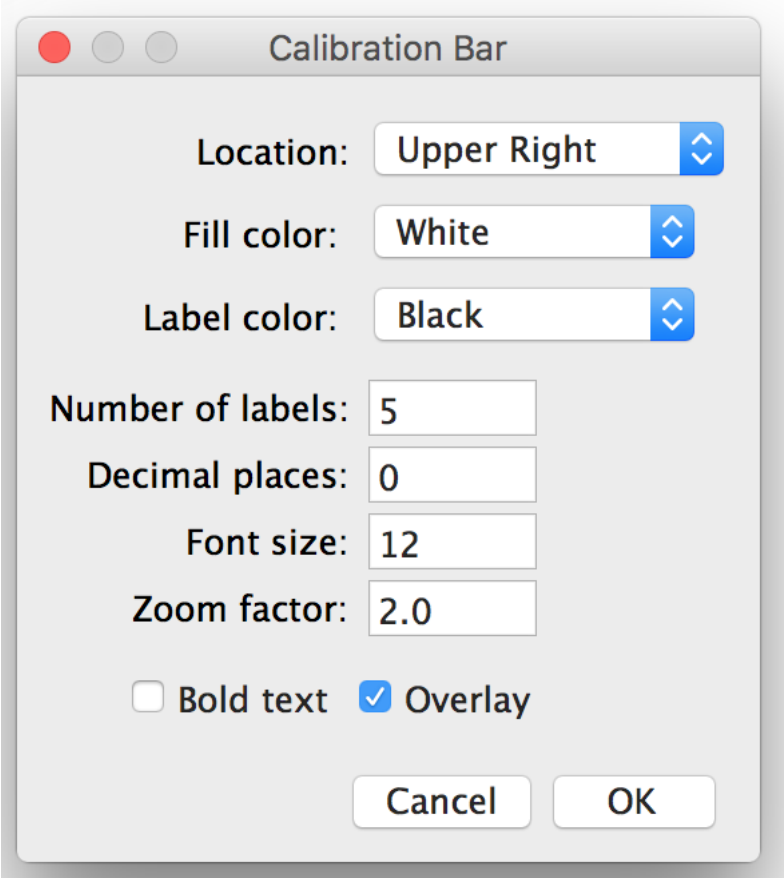


Calibration Bar

Enables people looking at your image to interpret what the different colours in the LUT mean

Activity:

1) Select the open file
2) Change LUT to fire
3) FIJI/Analyze/Tools/Calibration Bar
4) Select various options
5) Untick Overlay
6) Save as tif



Brightness & Contrast

Adjust for Visualisation purposes only (mention this in materials & methods)

Activity:

1) Select Zeiss.lsm image
2) Convert LUT to HiLo (Used to detect saturated (red) or clipped (Blue) pixels)
3) Move maximum and minimum only to (and not past) the data
4) FIJI/Image/Adjust/Brightness & Contrast
5) Shortcut
   - Command + Shift C (Mac)
   - Control + Shift C (PC)

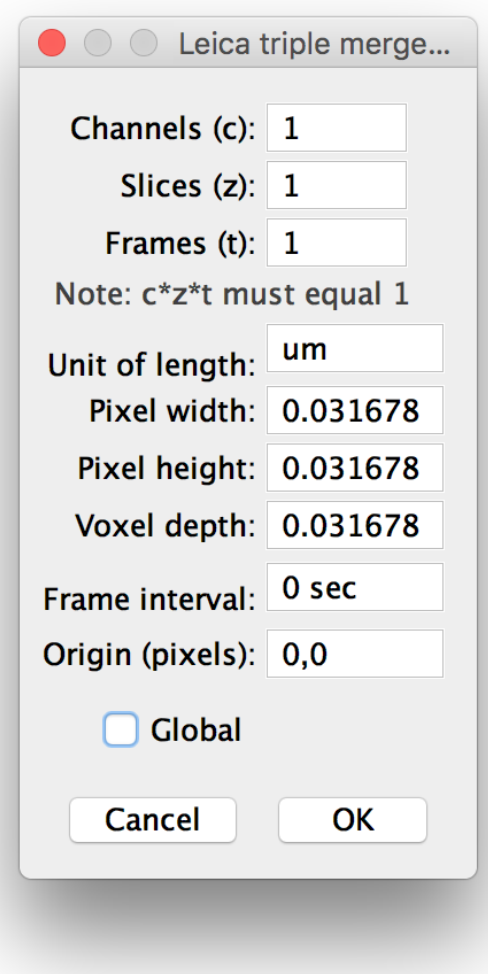
Click Apply once you have changed the settings (original data is LOST!!)

Calibrating images

Activity:

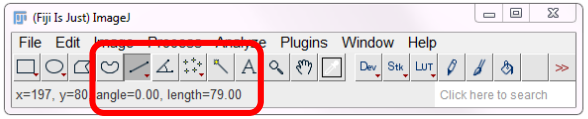
- Open “Leica triple merge (uncalibrated).tif” from demo images folder
- FIJI/Images/Properties
- Shortcuts
  - Command + Shift P (Mac)
  - Control + Shift P (PC)
- Enter the below numbers
  - Unit of length = um
  - Pixel width = 0.031678

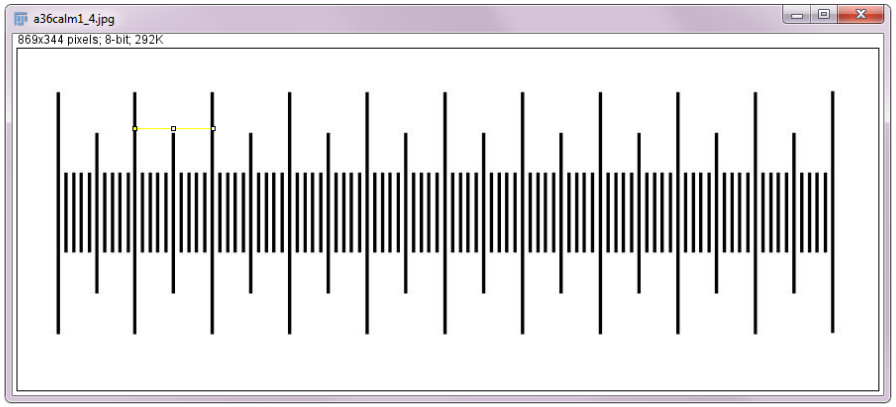
We can assist you to measure your pixel sizes e.g.Calibration slide or Hemocytometer grid

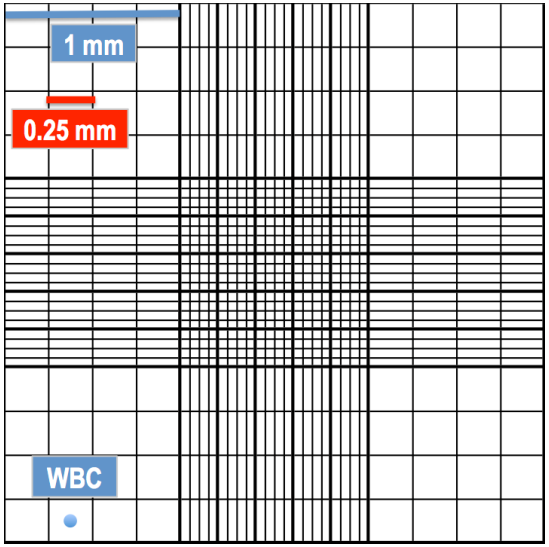


Calibrating images

- Measure the pixel length over a known distance
- Images must be acquired EXACLTY the same way as your data (camera, magnification, pixel number, binning)
- Use the Line ROI to measure the distance in pixels

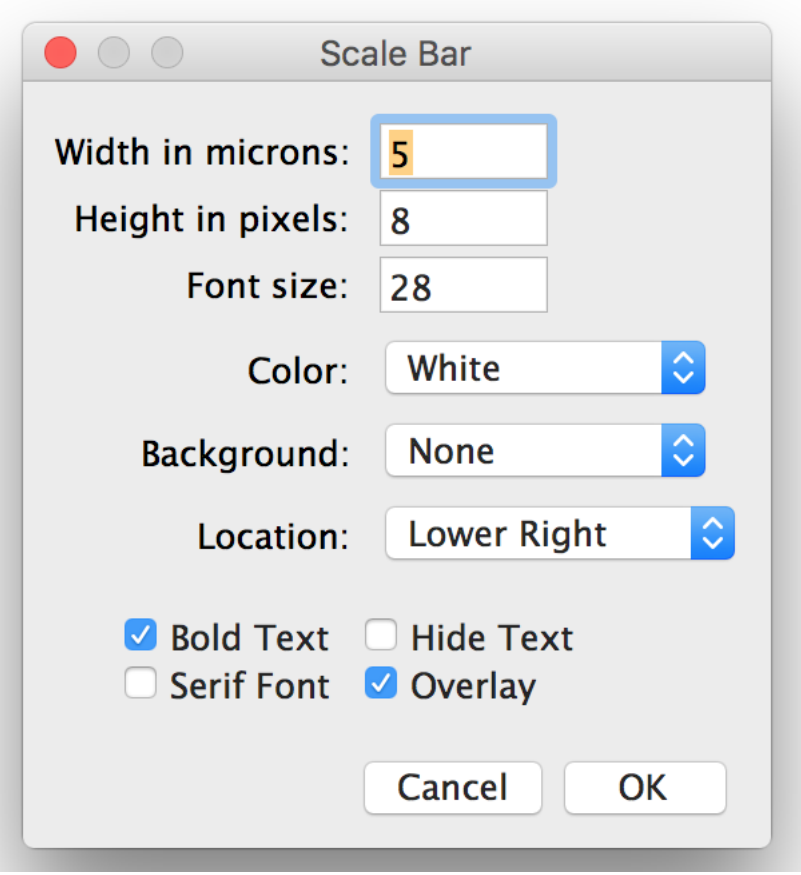






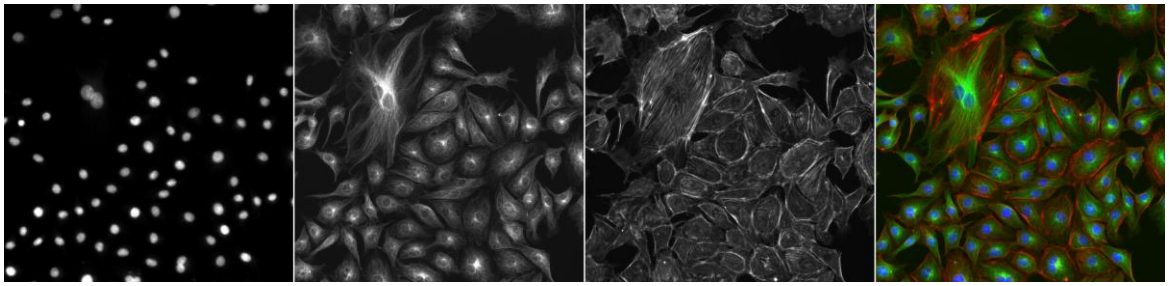
Adding a scale bar

- Select the open file
- FIJI/Analyse/Tools/Scale Bar
- Modify the options to suit



2D Data presentation

Use all you now know to make a panel for a figure



Activity:

1) Open BPAE.tif
2) Duplicate image
3) Split the channels on one image
4) Assign the correct LUTs (if required)
5) Convert Images to stacks (Image/stacks/Images to Stacks, all images should be the same size)
6) Stack sorter (Image/Stacks/Stack Sorter) to re-order stacks
7) Make Montage (Image/Stack/Make Montage)

2D Data presentation

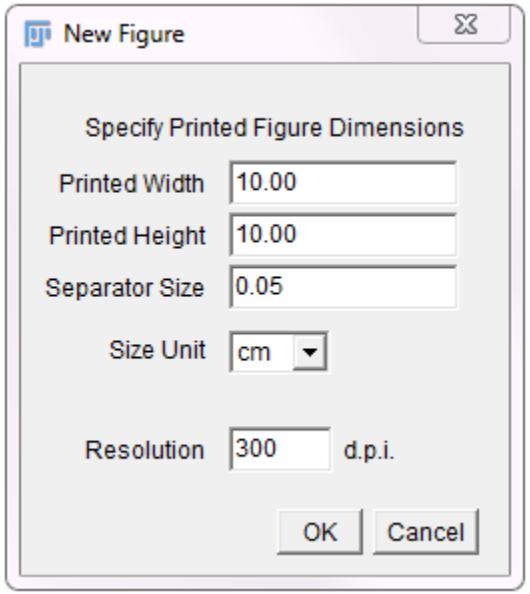
Figure J plugin (download via below update sites)

IBMP-CNRS

ImageScience

BioFormats

http://imagejdocu.tudor.lu/doku.php?id=plugin:utilities:figurej:start)

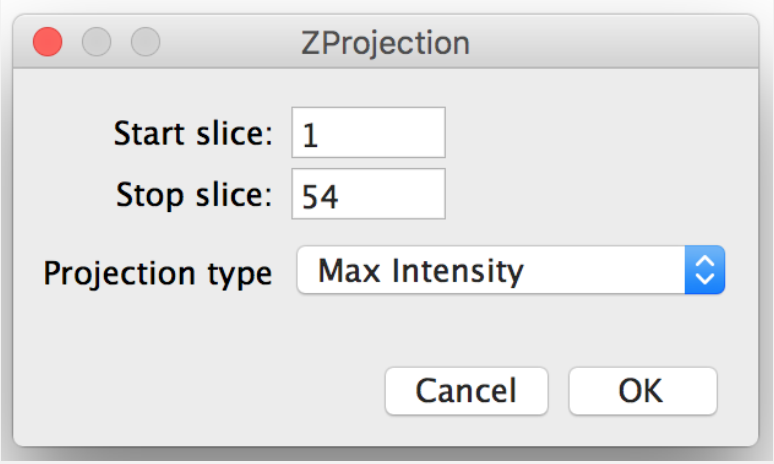


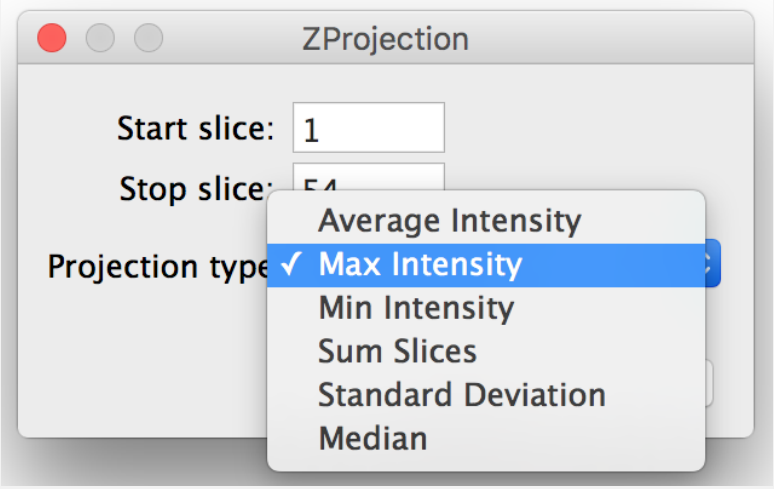
3D data: Z projection

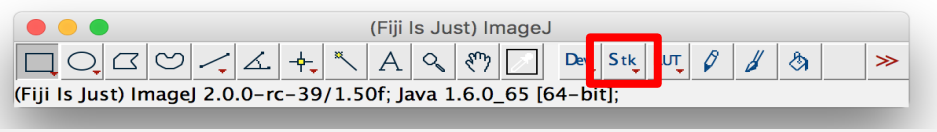
Activity:

1) Open “OMX (3D data).tif”
2) FIJI/Image/Stack/Z Project (or via shortcut above)
3) Select first & last slices to be used
4) Select projection type

- Maximum intensity = Brightest pixel (in Z) at each xy position
- Average intensity = Average intensity (in Z) at each xy position







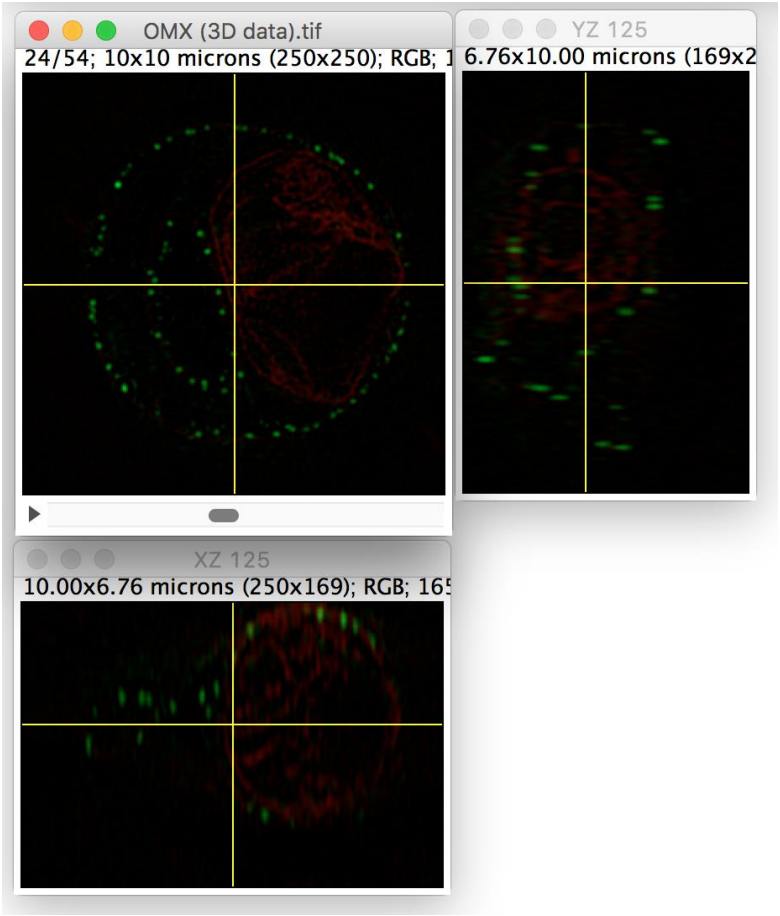
3D data: Orthogonol viewer

Activity:

1) Open “OMX (3D data).tif”
2) FIJI/Image/Stack/Orthogonal views

- Via STK shortcut
- Control + Shift H (PC)
- Command + Shift H (Mac)

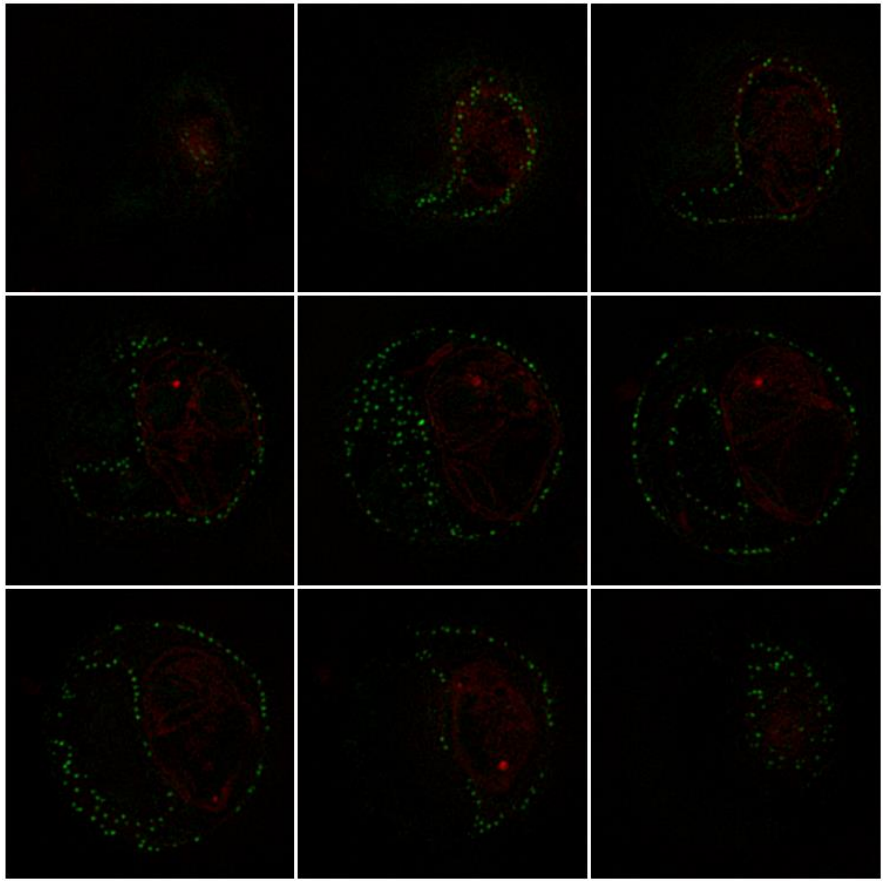
3) Move cross hairs
4) Change focus
5) Save individual panels



3D data: Montage

Activity:

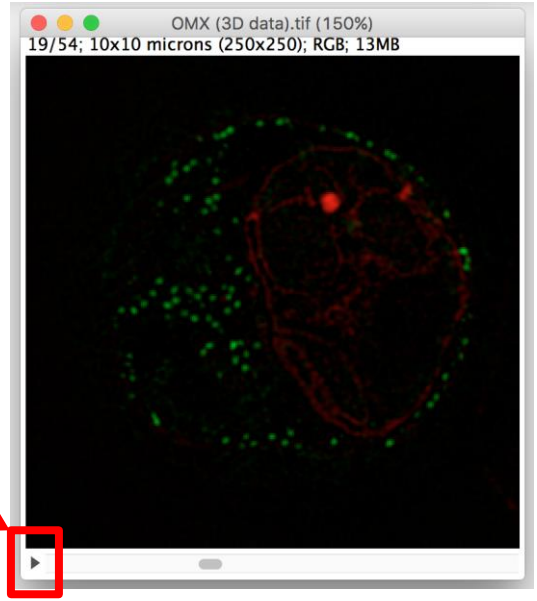
- Open “OMX (3D data)”
- FIJI/Image/Stack/Make Montage
- Update settings below
  - Columns = 3
  - Rows = 3
  - Scale = 1
  - First = 1
  - Last = 54
  - Increment = 5
  - Border width = 3

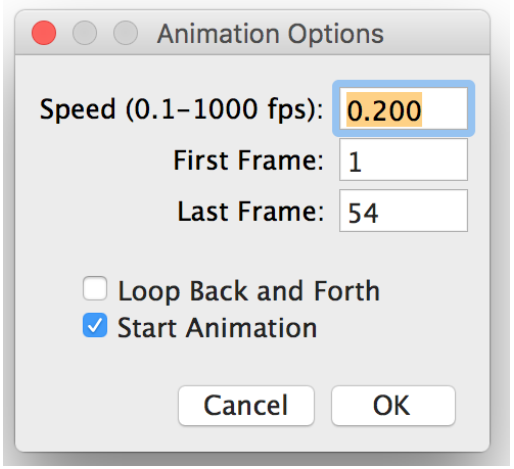


3D data: Z translation

Activity:

1) Open “OMX (3D data).tif”
2) FIJI/Image/Stack/Animation Options or Right click on play button
3) Select the option you require
4) FIJI/Save As/avi
5) Set fps and select uncompressed if you want to convert to a different file type

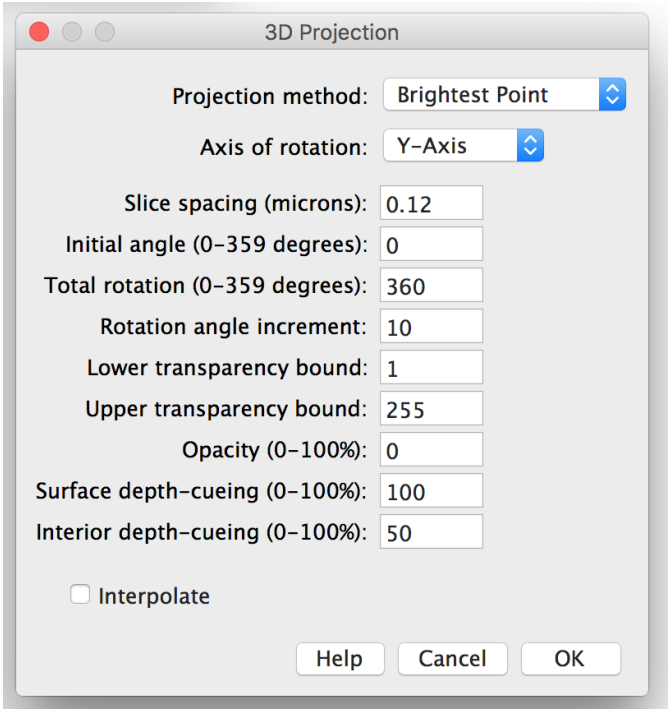




3D data: Rotation Movie

Activity:

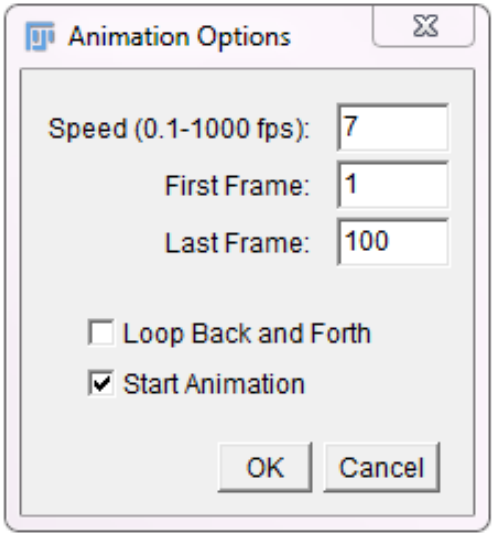
- Open “OMX (3D data).tif”
- FIJI/Image/Stack/3D Project
- Try varying some of these options
  - Projection method
  - Axis of rotation
  - Total rotation
  - Rotation increments
  - Interpolate
- Save as .avi as before



Timelapse data: Movie

Activity:

- Open “ER live.tif”
- Apply green LUT
- FIJI/Image/Stack/Animation Options or Right click on play button
- Select the options you require
- FIJI/Save As/avi
- Set fps and select uncompressed if you want to convert to a different file type

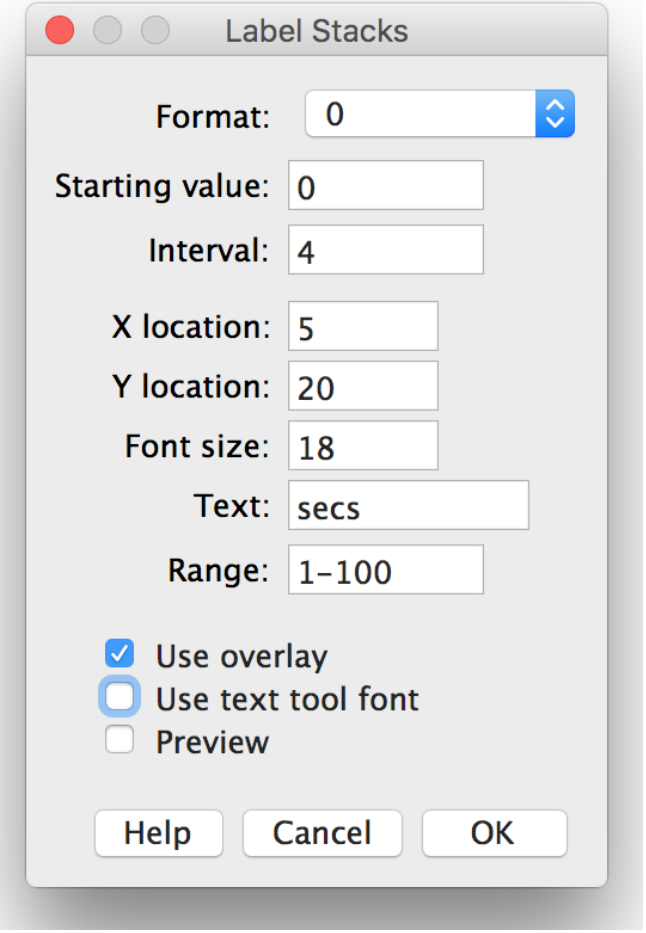


Annotating movies

Images were taken every 4 seconds

Activity:

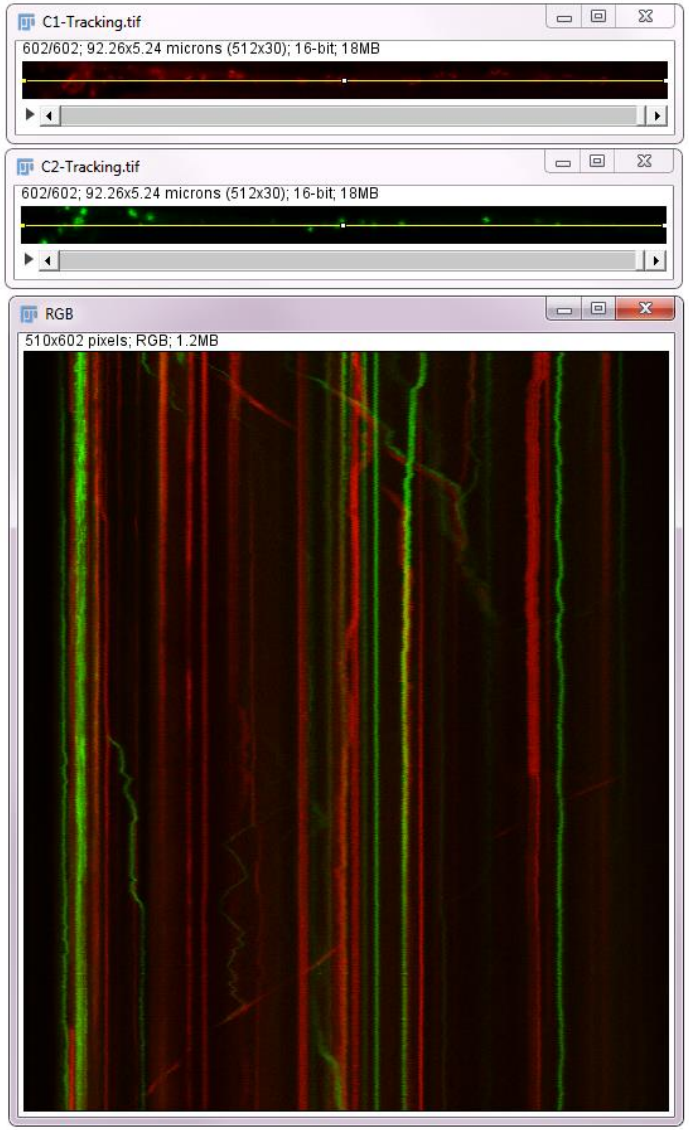
- Open “ER live.tif”
- FIJI/Images/Stacks/Label
- Try changing the below parameters
  - Format
  - Interval
  - XY location
  - Font size
- Text colour can be changed at FIJI/Image/Colours/Colour Picker
- Save as avi as before



Kymogram

Activity:

1) Open tracking.tif from Kymogram
2) Image/Colour/Split channels
3) Draw line form ROI tools (Hold shift to keep it straight)
4) Select channel two and restore selection (Ctrl + Shift E)
5) Select channel 1, Analyze/Multi Kymporaph/Multi Kymograph
6) Set line width to 29 (must be an odd number)
7) Image/Rename, rename channel 1
8) Repeat Kymograph (as above) for Channel 2
9) Merge Channels (Channel 1 = green, Channel 2 = Red)



Hands on Demonstrations

- Installation
- Image Import
- Image manipulation
  - Duplicating
  - Colour tools
  - ROI tools
  - Cropping
  - Brightness & Contrast
  - Look Up Tables (LUTs)
- Annotation
  - Calibrating images
  - Adding a scale bar
  - Calibration bar
- 2D data
  - Image panel
- 3D data
  - Z-projection
  - Orthogonal viewer
  - Montage
  - Projection movie
  - Rotation Movie
- Timelapse data
  - Timelapse movie
  - Annotating movies
  - Kymogram