



FIJI/Image J for beginners

Hands on session

Dr Paul McMillan

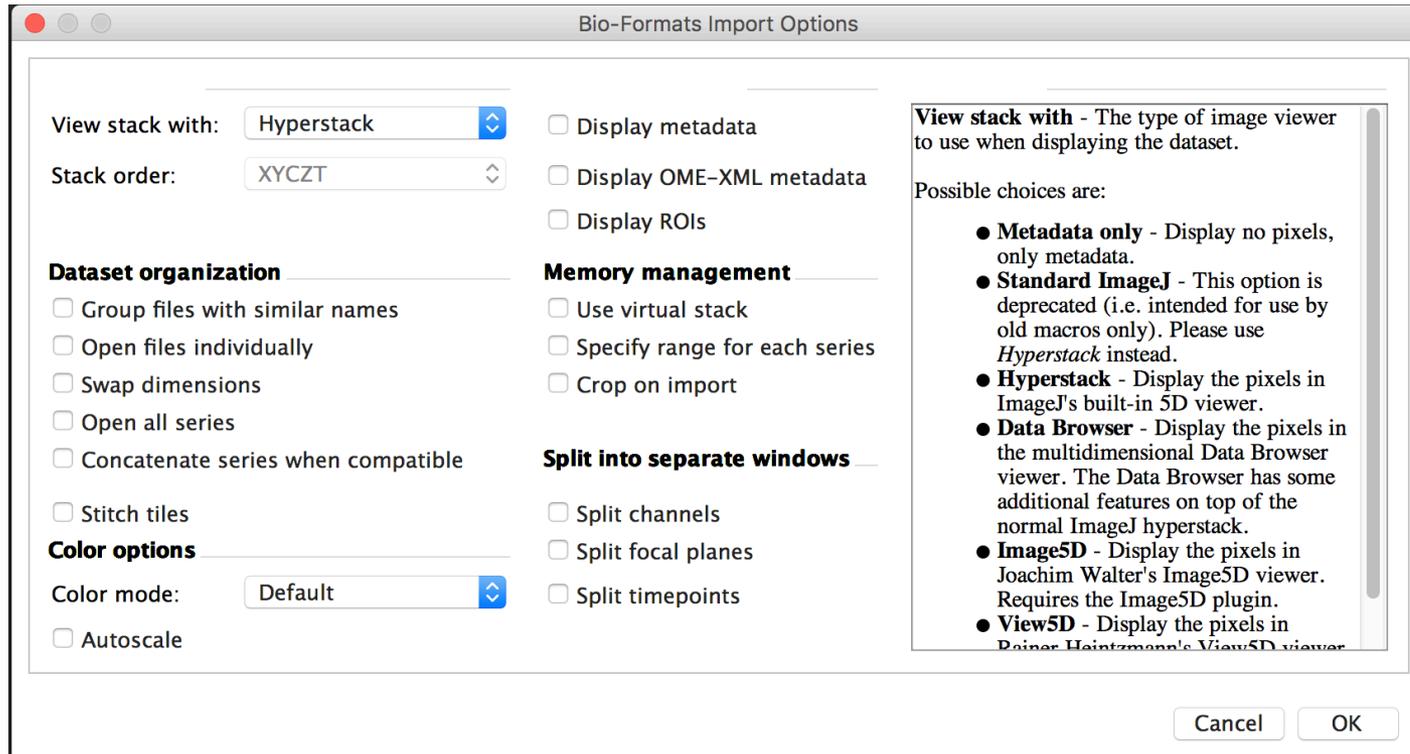
Biological Optical Microscopy Platform



- 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
- 3D Data
 - Z- projection
 - Orthogonal viewer
 - Montage
 - Projection movie
 - Rotation Movie
- Timelapse data
 - Timelapse movie
 - Annotating movies

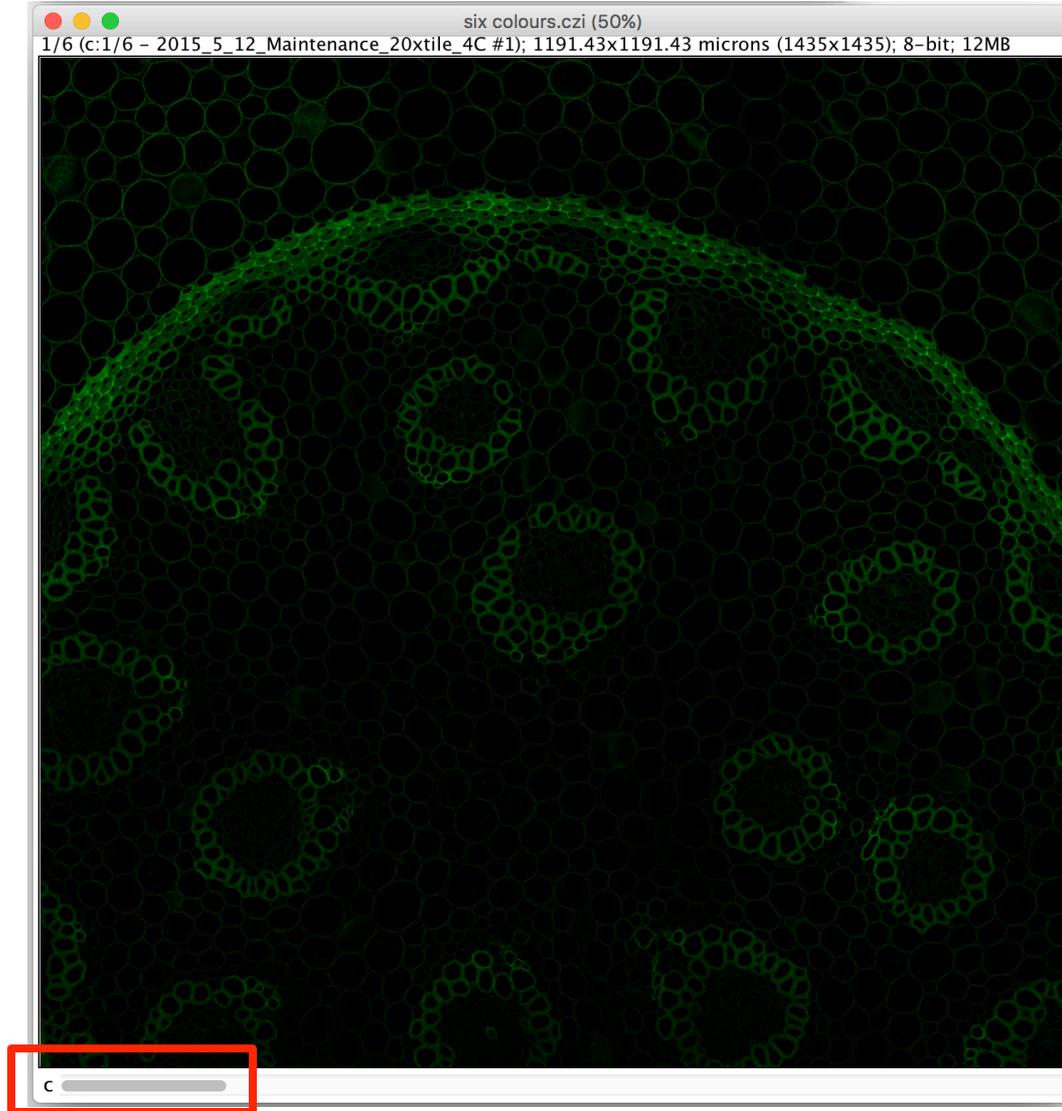


1. FIJI/File/open/select demo images
2. Drag & drop files
3. FIJI/Plugins/bio-formats/bioformats importer





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



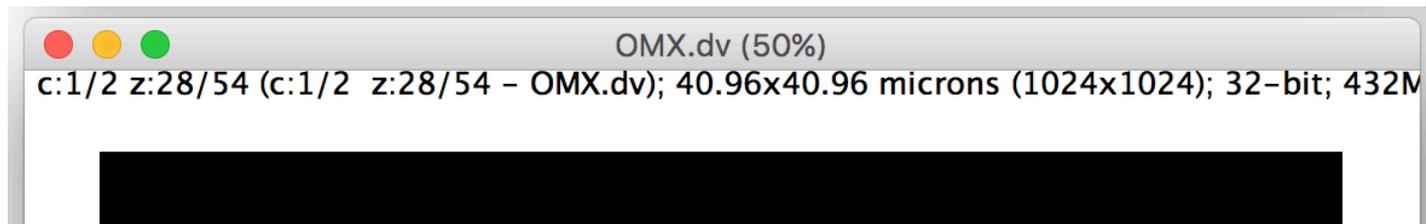


- Visualises Multi-parameter data
- Open OMX.dv image
- Scroll through channels & Z positions
- We will modify this image for our visualisation





- Top of images gives us information about the image
- The below example tells us:
 - $c = 2$. There are two channels in this images. We are looking at channel 1 at the moment.
 - $Z = 54$. 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





If you are unsure where a function is located, then use the Command Finder option

- Shortcut
 - 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)

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

Image Duplication: Always duplicate an image before making any modifications

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



FIJI/Image/Colour

Split Channels

Splits RGB images into 3 images

Merge Channels

Merge up to 7 images

Arrange Channels

Re-arrange order in a stack

Channels tool

Reassign LUTs within a stack,
make a merge

Split Channels

Merge Channels...

Arrange Channels...

Channels Tool...

⇧ ⌘ Z

Stack to RGB

Make Composite

Show LUT

Display LUTs

Edit LUT...

Color Picker...

⇧ ⌘ K

Colour Deconvolution

Dichromacy

Simulate Color Blindness

Retinex

Replace Red with Magenta

Replace Red with Magenta (system clipboard)

Average Color

RGB to CIELAB

RGB to Luminance

Set Color By Wavelength

Change LUT within a hyperstack and create a merge

Open Leica.lif (Only first image)

FIJI/Image/Colour/Channel Tool

(Shortcut = Control/Shift Z)

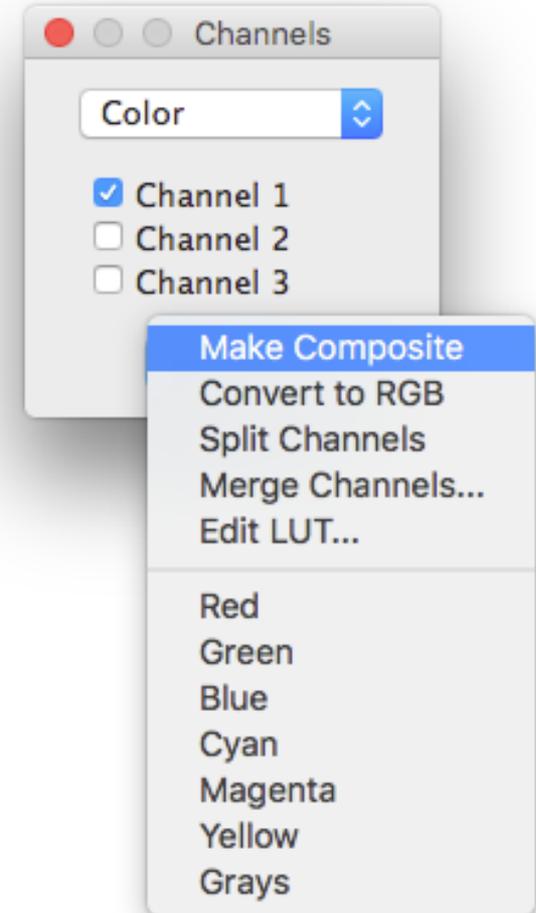
Select color option

Change LUT for each channel

Make Composite

Convert to RGB

Save as tif



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



Resizing ROIs (combinations can be used)

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

Control - Free resize are centre of ROI

Alt – maintains aspect ratio

Define ROI size

- FIJI/Edit/Selection/Specify



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)

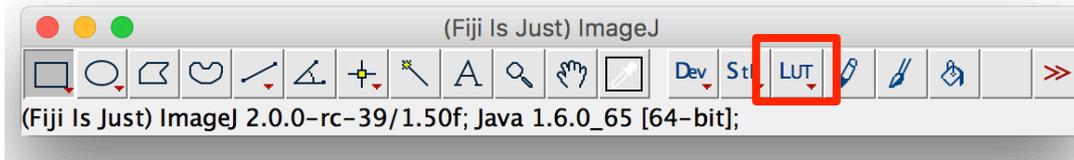
ROI Manager (more than one ROI on an single image)

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



- Select open image
- Duplicate the image
- Draw an ROI (left click and hold)
- FIJI/Image/Crop
- Shortcuts
 - Command + Shift X (Mac)
 - Control + Shift X (PC)

1. FIJI/Image/Lookup Tables
2. LUT shortcut



- Open an image from demo images
- Click LUT
- Select a LUT of your choice
- Convert to RGB (Image/Type/RGB color)
- Save image into desktop
 - FIJI/File/Save As/Tiff

To visualize LUTs available

- Image/Color/Display LUTs

Invert LUT
Apply LUT

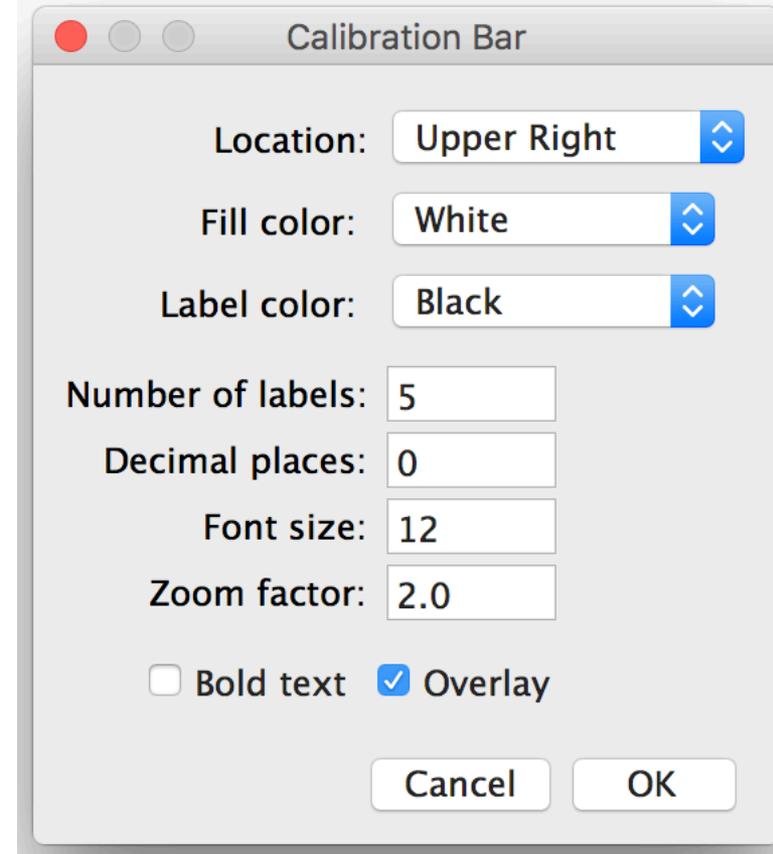
Fire
Grays
Ice
Spectrum
3-3-2 RGB
Red
Green
Blue
Cyan
Magenta
Yellow
Red/Green

1-bit
16_colors
2-bit
3-bit
4-bit
5_ramps
6_shades
blue_orange_ichb
brgbcmyw
cool
Cyan Hot
edges
gem
glasbey
glow
Green Fire Blue
HiLo
ICA
ICA2
ICA3
Magenta Hot
Orange Hot
phase
physics
Rainbow RGB
Red Hot
royal



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

- Select the open file
- Change LUT to fire
- FIJI/Analyze/Tools/Calibration Bar
- Untick Overlay
- Save as tif

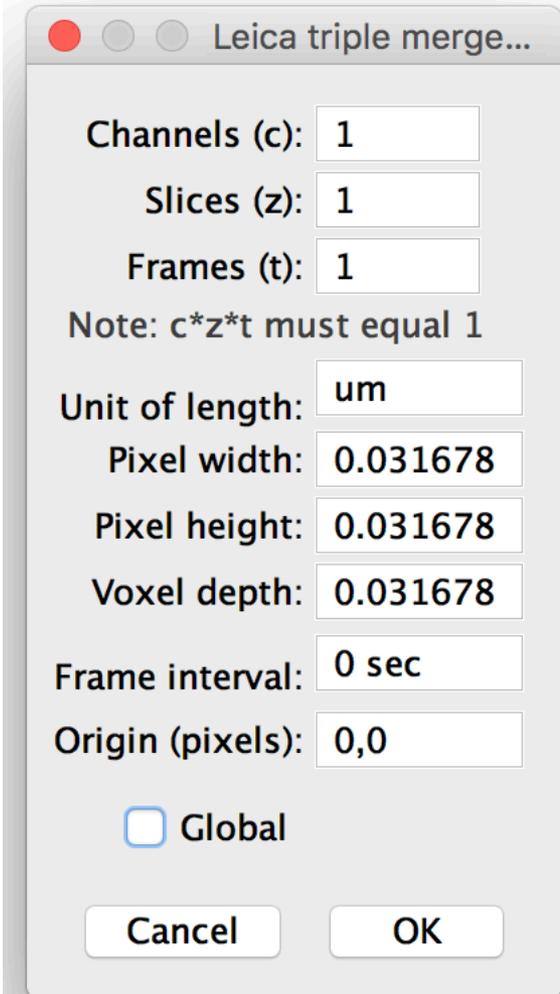


Adjust for Visualisation purposes only (mention this in materials & methods)

- Select Zeiss.lsm image
- Convert LUT to HiLo
- FIJI/Image/Adjust/Brightness & Contrast
- Shortcut
 - Command + Shift C (Mac)
 - Control + Shift C (PC)
- Move maximum and minimum only to (and not past) the data
 - Can use a LUT to detect saturated (red) or clipped (Blue) pixels
- Click Apply once you have changed the settings (original data is LOST!!)

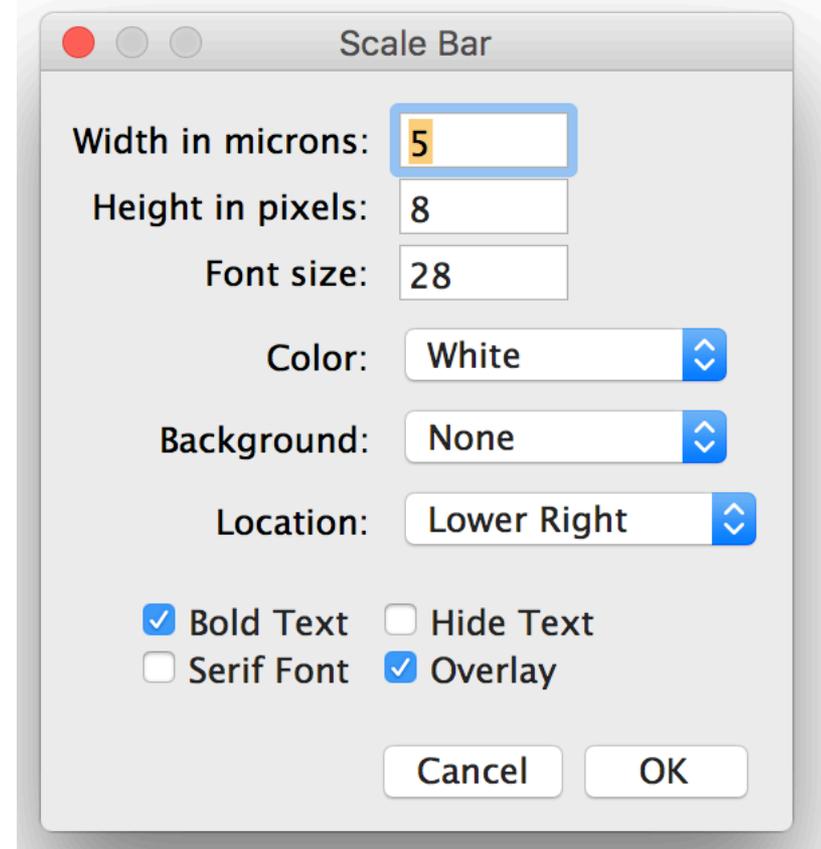


- Open “Leica triple merge (uncalibrated).tif” from demo images folder
- FIJI/Images/Properties
- Shortcuts
 - Command + Shift P (Mac)
 - Control + Shift P (PC)
- Enter the below
 - Unit of length = um
 - Pixel width = 0.031678
- We can assist you to measure your pixel sizes e.g. Hemocytometer grid



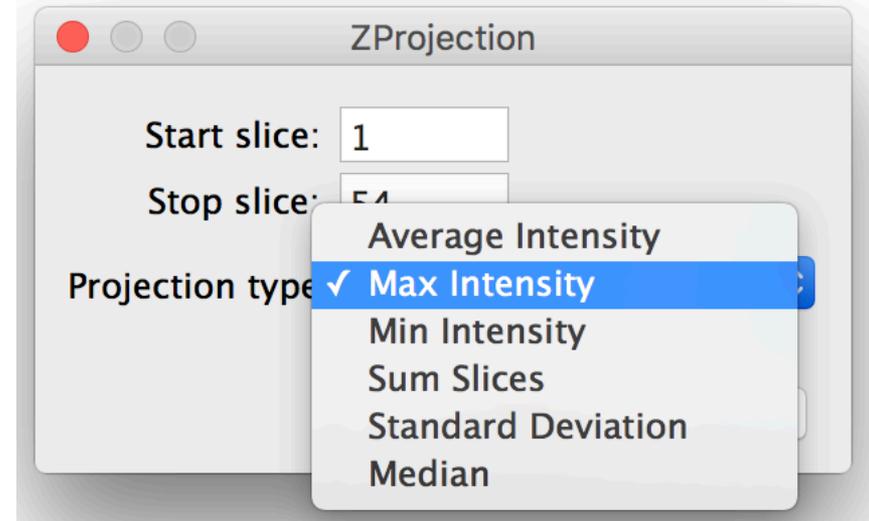
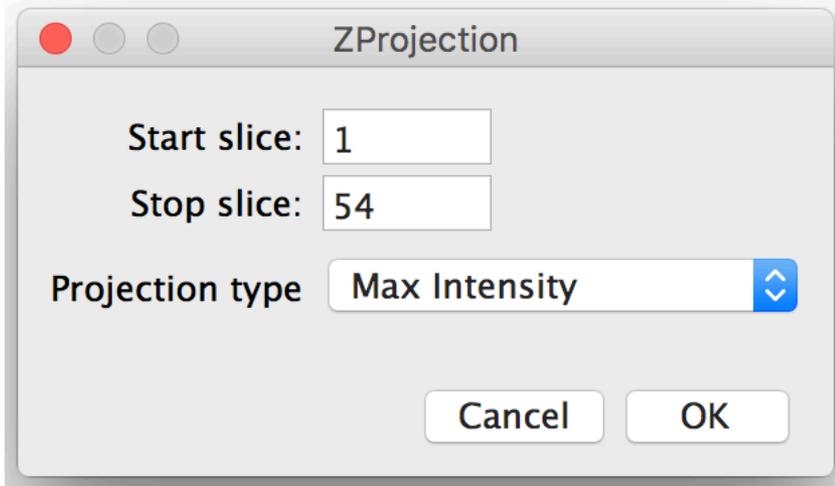


- Select the open file
- FIJI/Analyse/Tools/Scale Bar
- Modify the options to suit



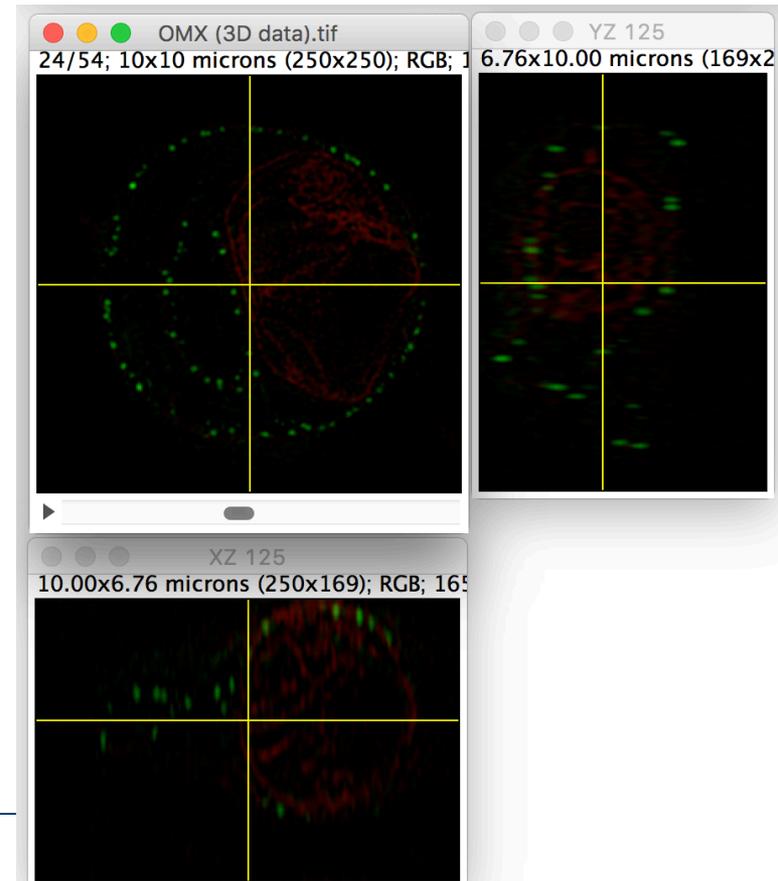


- Open “OMX (3D data).tif”
- FIJI/Image/Stack/Z Project (or via shortcut above)
- Select first & last slices to be used
- Select projection type
 - Maximum intensity = Brightest pixel (in Z) at each xy position
 - Average intensity = Average intensity (in Z) at each xy position



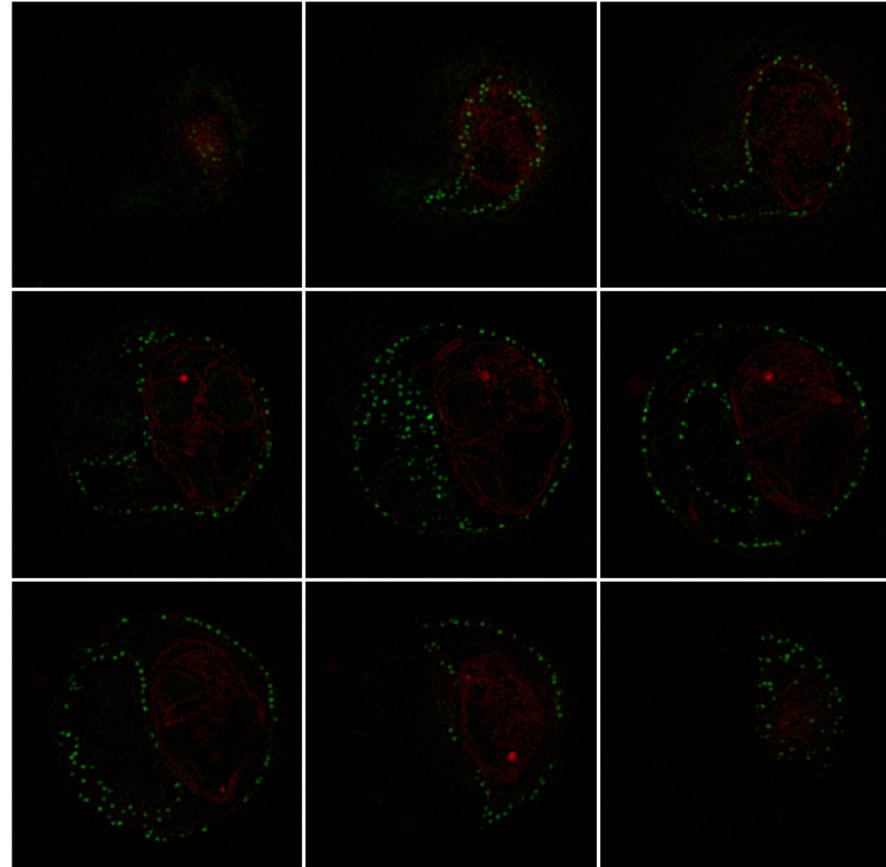


- Open “OMX (3D data).tif”
- FIJI/Image/Stack/Orthogonal views
 - Via STK shortcut
 - Control + Shift H (PC)
 - Command + Shift H (Mac)
- Move cross hairs
- Change focus
- Save individual panels



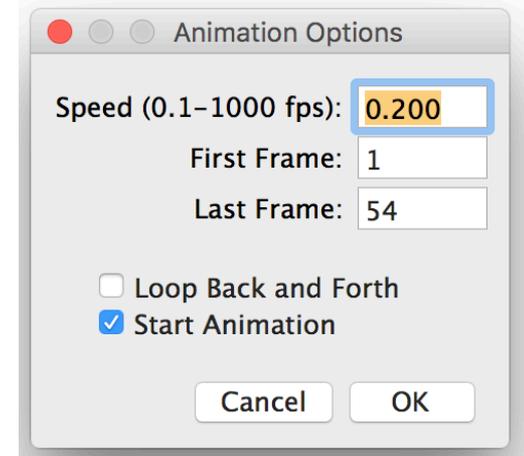
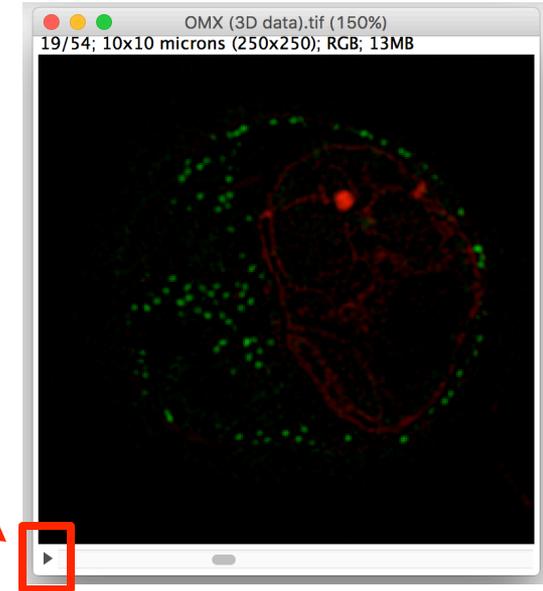
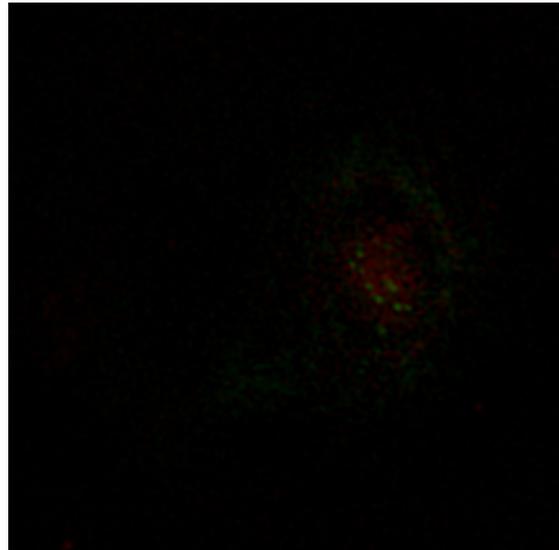


- Open “OMX (3D data)”
- FIJI/Image/Stack/Make Montage
- Update settings below
 - Columns = 3
 - Rows = 3
 - Scale = 1
 - First = 1
 - Last = 54
 - Increment = 5
 - Border width = 3



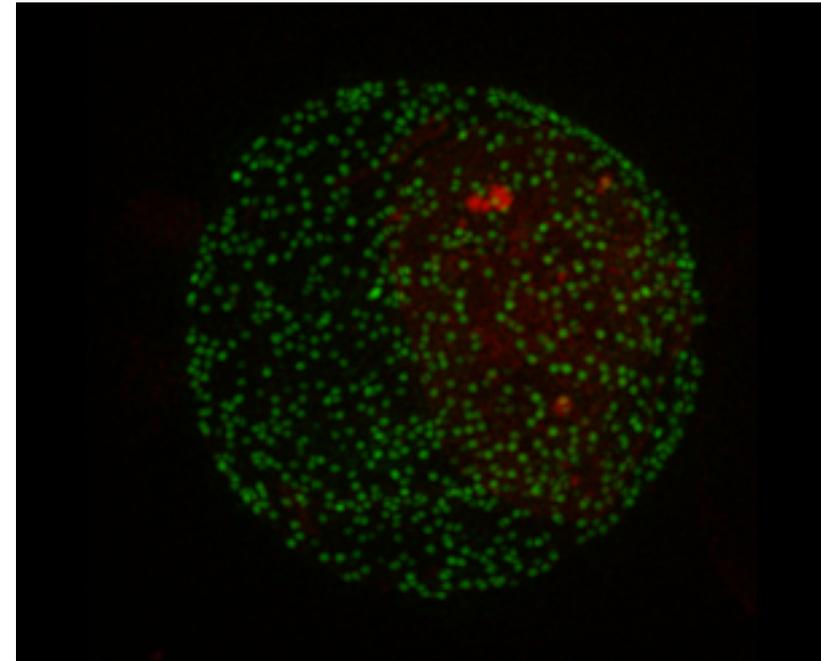
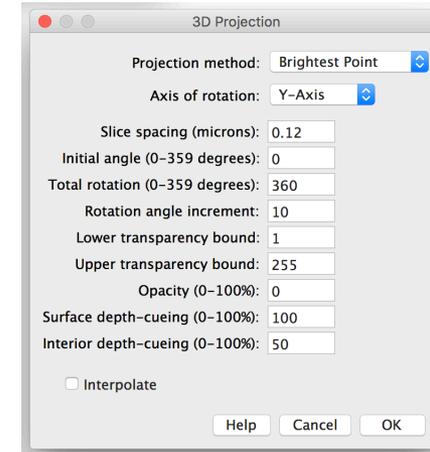


- Open “OMX (3D data).tif”
- FIJI/Image/Stack/Animation Options or Right click on play button
- Select the option you require
- FIJI/Save As/avi
- Set fps and select uncompressed if you want to convert to a different file type



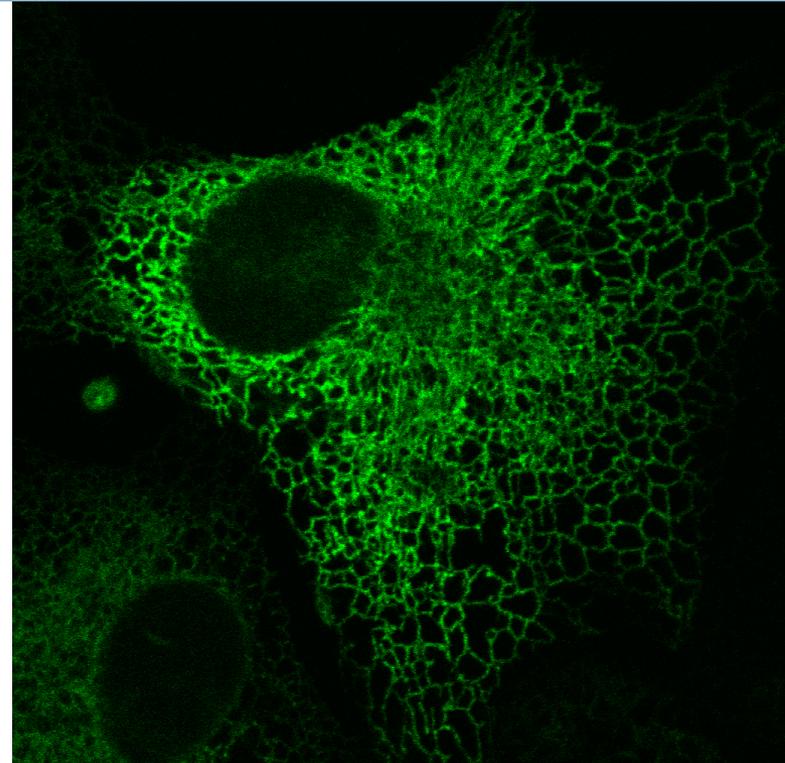


- Open “OMX (3D data).tif”
- FIJI/Image/Stack/3D Project
- Try varying some of these options
 - Projection method
 - Axis of rotation
 - Total rotation
 - Rotation increments
 - Interpolate
- Save as avi as before





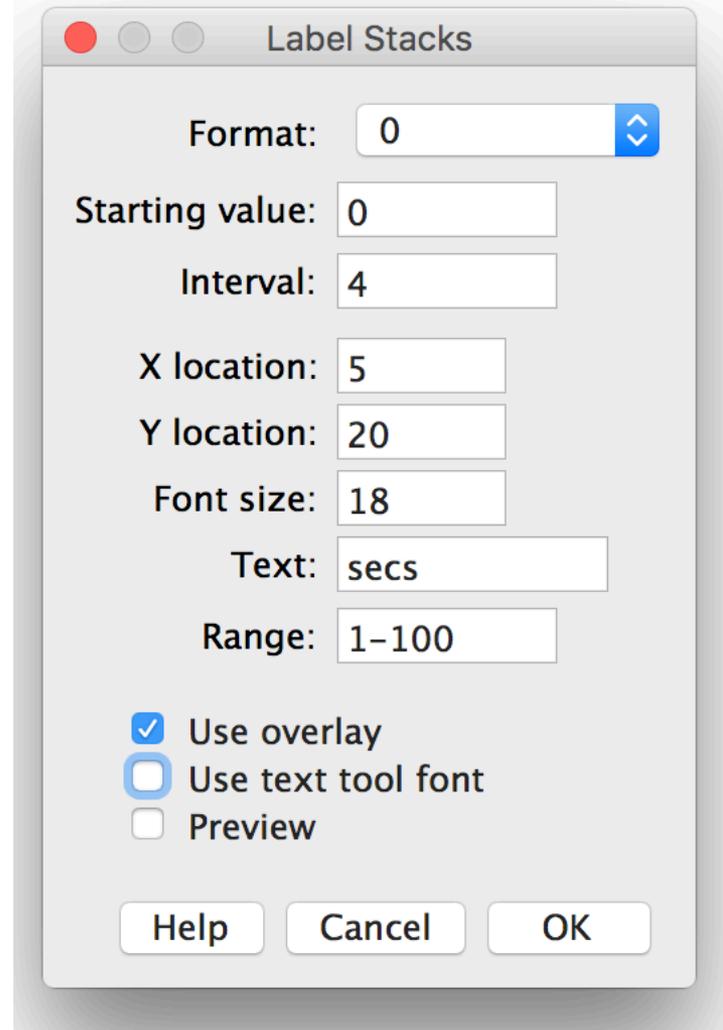
- Open “ER live.tif”
- Apply green LUT
- FIJI/Image/Stack/Animation Options or Right click on play button
- Select the options you require
- FIJI/Save As/avi
- Set fps and select uncompressed if you want to convert to a different file type





Images were taken every 4 seconds

- 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





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