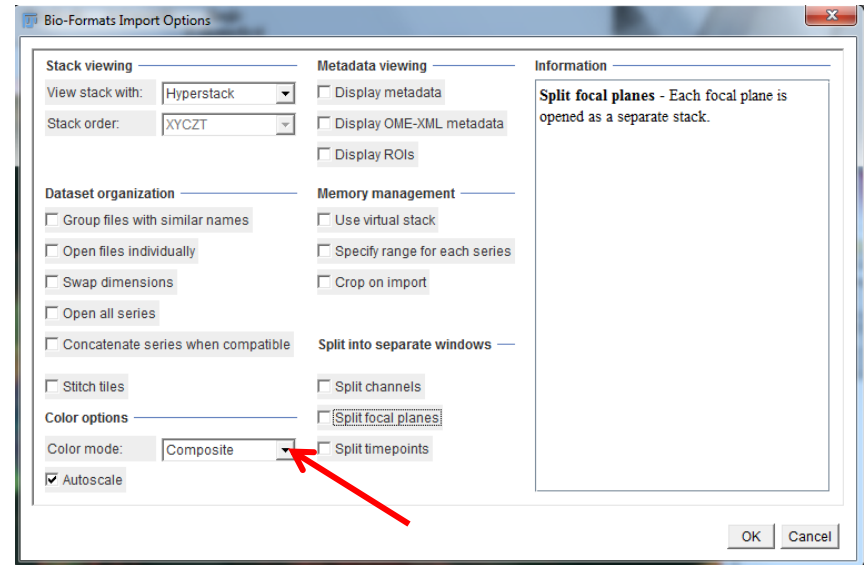
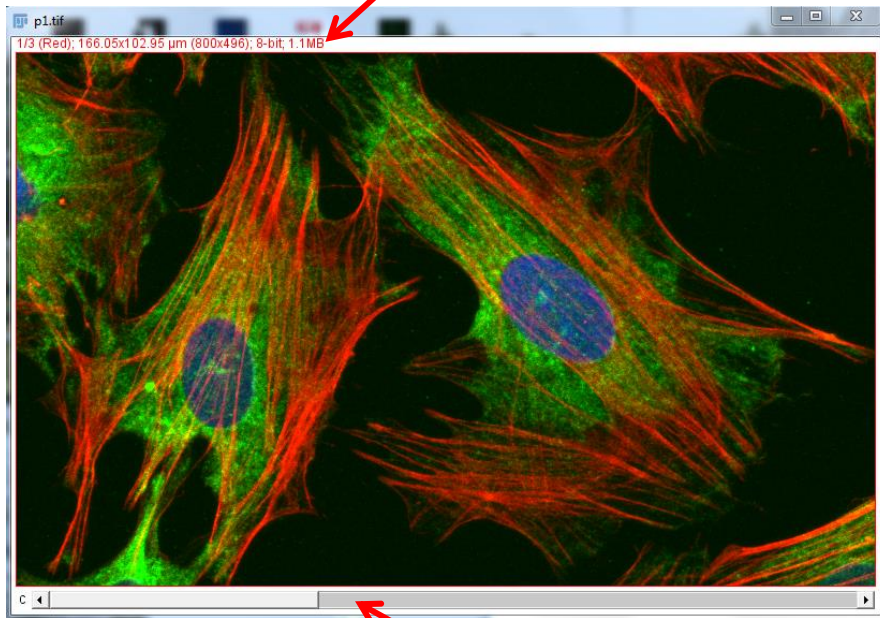


Multi-Channel Image

To load an image select - “File” – “Open”
- If the bio-formats importer window opens then select “composite” to see the channels over-layed or “default” if you want the channels displayed separately (this can be changed after loading in “Channel Tools”)

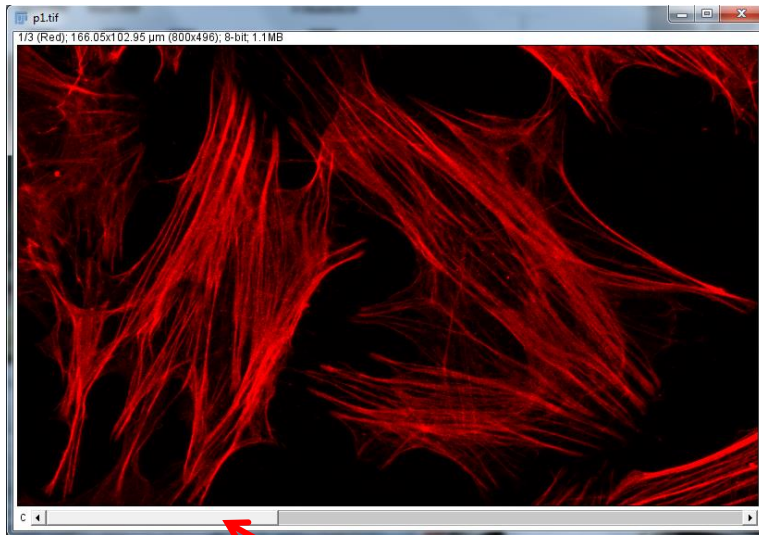


Colour of channel



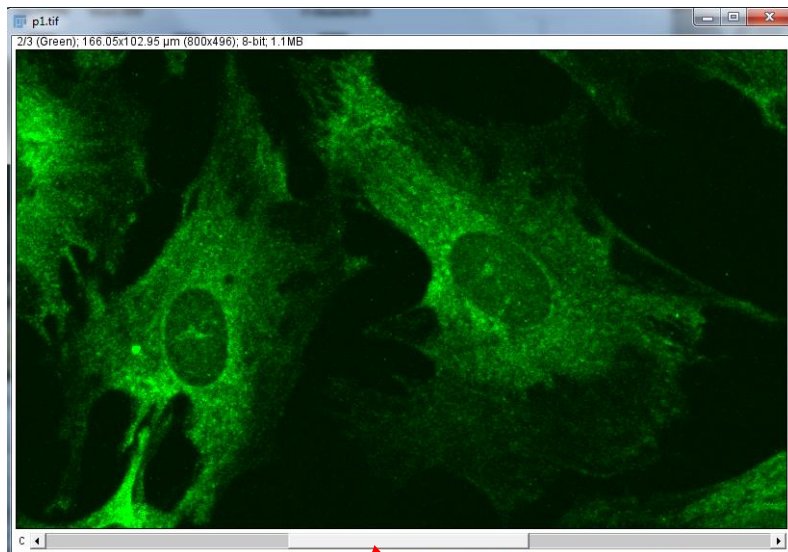
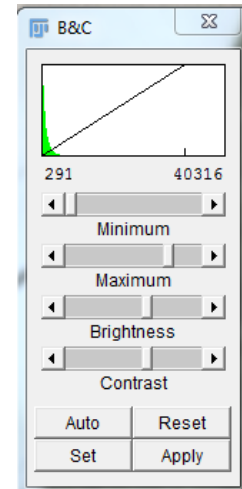
Channel select

The image will open with the channel bar along the bottom. In this image (displayed as composite mode) there are 3 positions, one for each channel with the colour displayed under the title. This image is composite so all channels are displayed together



Channel 1 selected

Here the image is displayed as colour or default mode. There are still 3 positions, one for each channel along the bottom. However, this time only the selected channel will be displayed. The brightness and contrast can be changed by selecting the channel then run “Image” – “Adjust” - “Brightness & Contrast” for that channel



Channel 2 selected

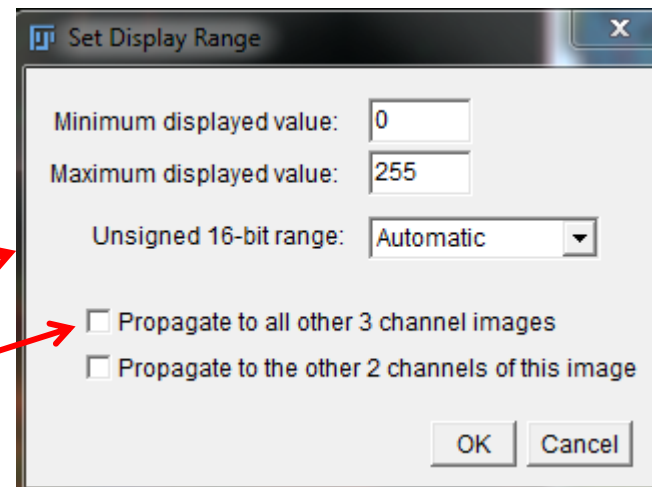
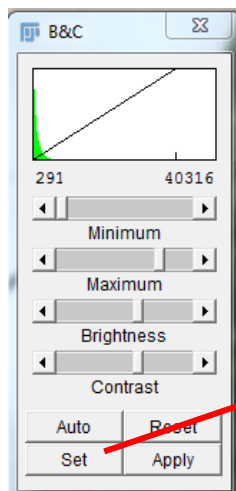


Channel 3 selected

Setting Brightness and Contrast

Brightness and contrast can be set the same for multiple images so they can be compared visually.

- Open up the images you want to set the brightness & contrast on.
- Select the brightest image and change the brightness and contrast for each channel by selecting the channel using “Image” – “Adjust” - “Brightness & Contrast” .
- Then click “Set” and there is an option to select to propagate the settings to all other open images

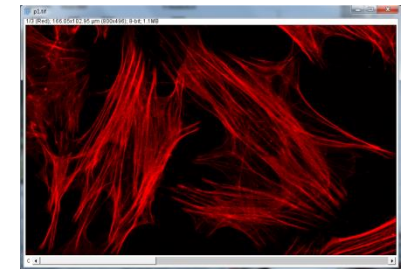
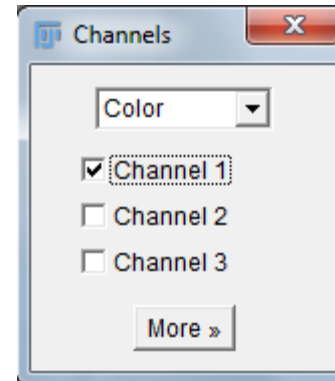


Channel Tools

This tool allows you to change the way the image is displayed

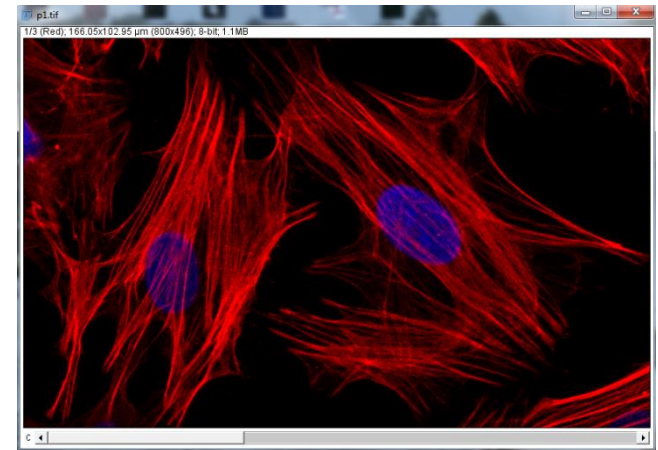
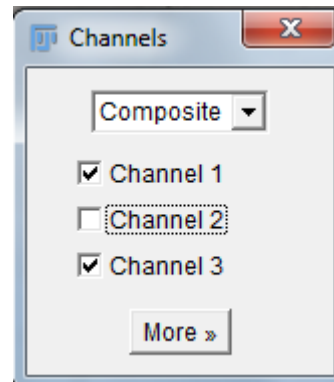
To activate, select - “Image” – “Colour” – “Channel Tools”

In “Colour” mode – it can be used to select the channel



Channel 1 selected

In “Composite” mode it does NOT select the channel but allows the display of selected channels
- The channel is only selected by the bar at the bottom of the image



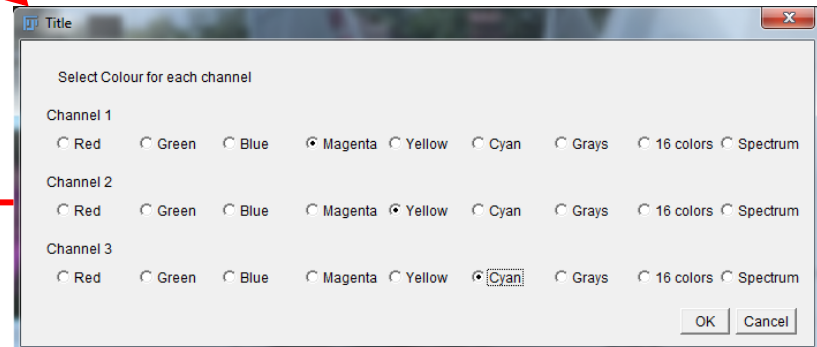
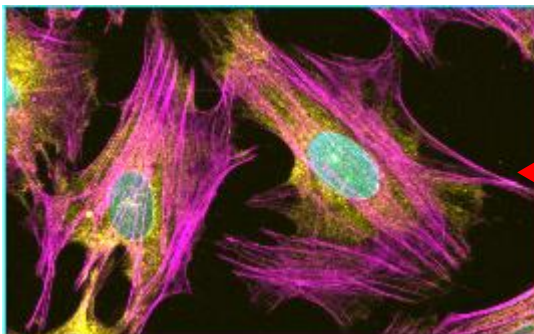
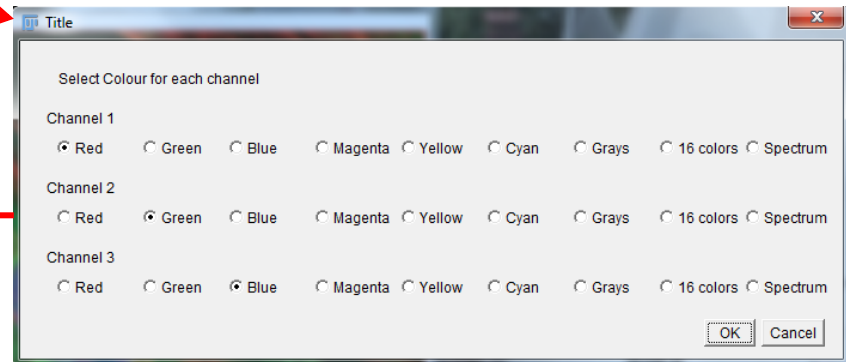
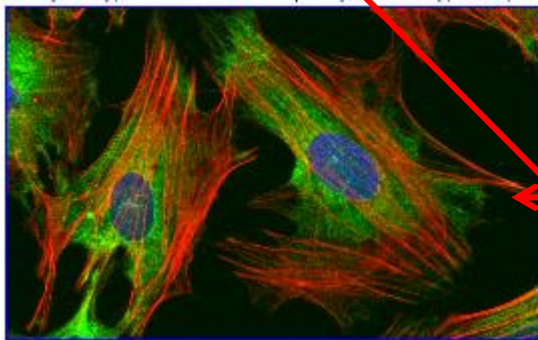
Channels 1 & 3 displayed
But only channel 1 selected

Lookup Tables

Each channel can be assigned different colours (or a range of colours if you want to study intensities.)
Either select the channel to change then select “Image” – “Lookup tables” – select colour
or for a multichannel use the LUT macro

>> Presentation Tool Set

LUT macro

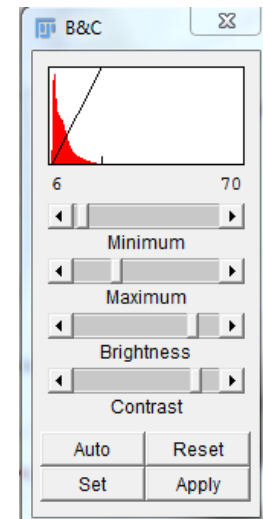
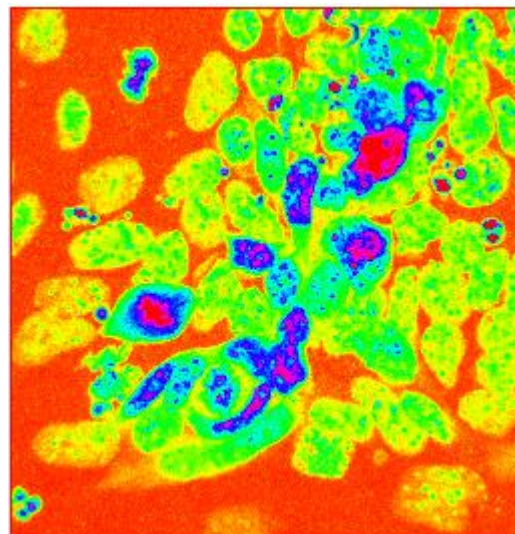
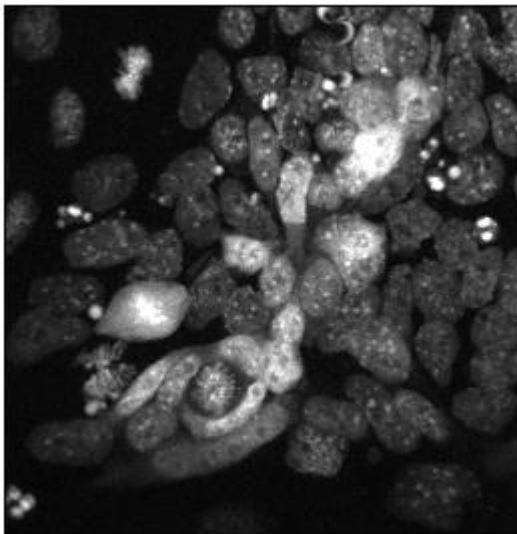


LUT Spectrum – for single channel intensity display

select “Image” – “Lookup tables” – “Spectrum”

Adjust the brightness and contrast to change the colour range displayed

(“Image” – “Adjust” - “Brightness & Contrast”)



Multi channel images cannot be imported into PowerPoint, Word, Illustrator etc.

They have to be converted to “RGB” format

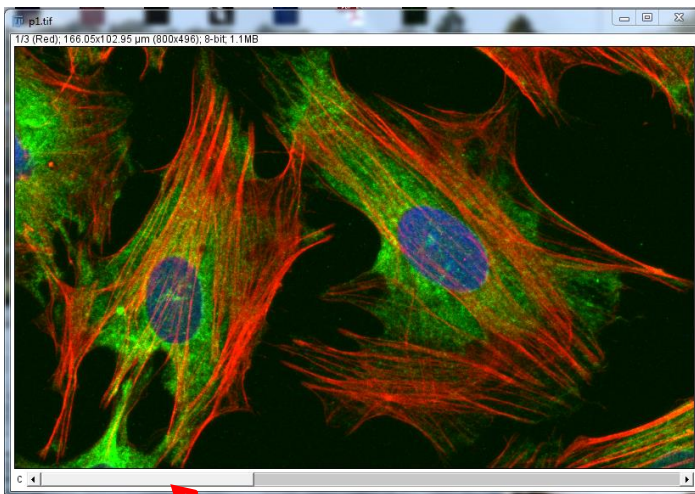
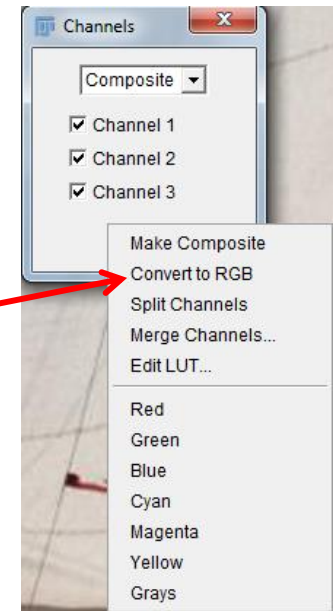
Use the Channel Tools window:

In “Composite” mode, select the channels you want displayed then select “More” – “Convert to RGB”

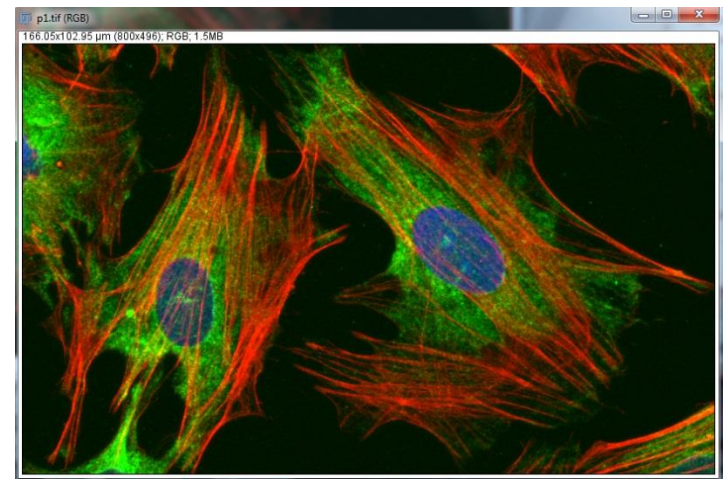
This will create a new window in RGB format that can be used in PowerPoint

Use the “File” – “Save As” commands to export. (TIFF format in preference)

To copy and paste – use “Edit” – “Copy to System” – then paste in PowerPoint



Multi - channels



RGB Image - No channels

Scale Bar

To add a scale bar:

Either

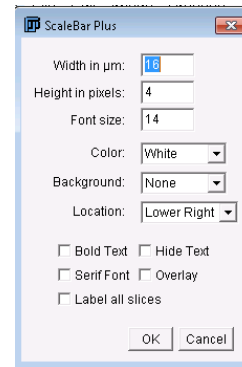
use “Analyse”- “Tools” – “Scale Bar”

or

- Download the FIJI macros from the FILM website
- Open your image
- Select the Presentation Tool Bar >>
- Select Add Scale Bar
- Opening the original file format image will usually bring in the correct scale information so adding a scale bar will have the correct dimensions
- An exported TIFF image when opened may not have the dimensions set. Use “Analyse” – “Set Scale” to add back the dimensions before adding the scale bar

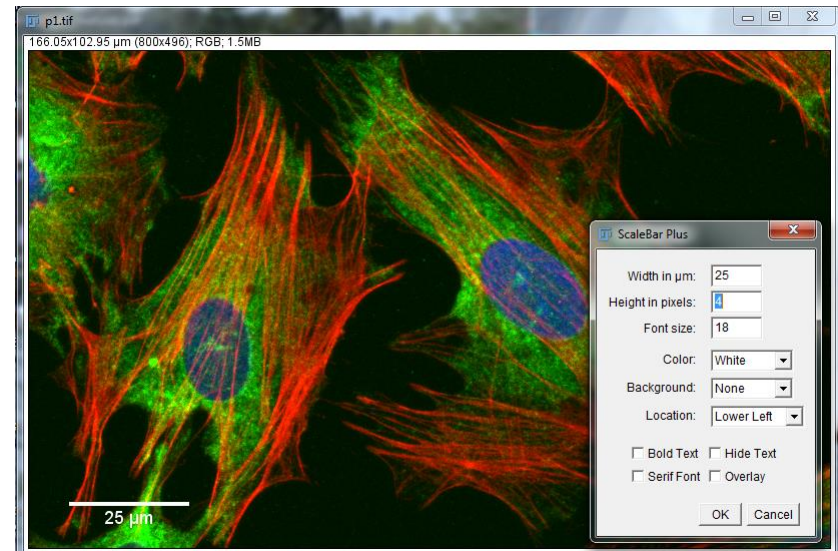


Add scale bar



Scale bar options

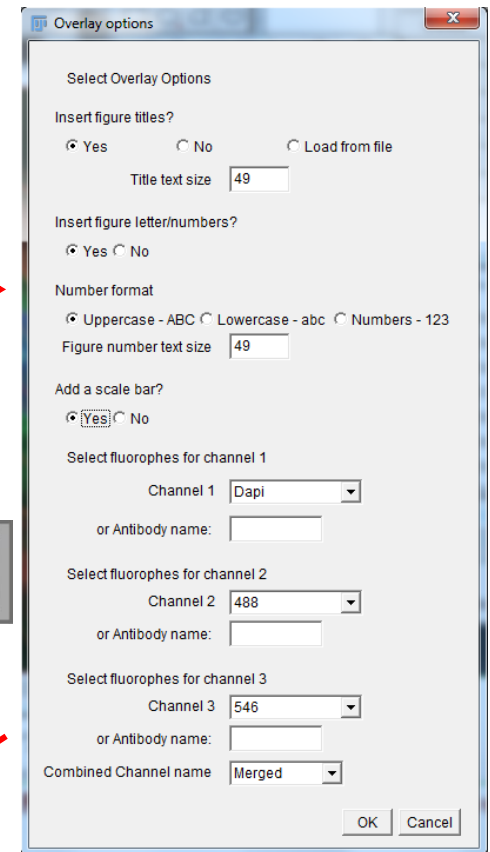
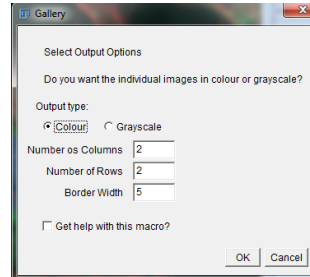
- Length
- Height
- Colour
- With/without Text
- Location



Annotated Gallery View

To create a Gallery view:

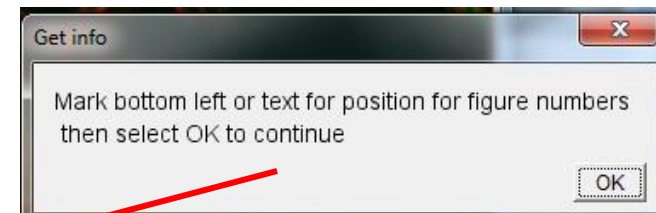
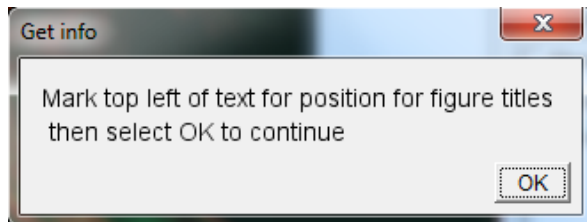
- Download the FIJI macros from the FILM website
- Open your image
- Select the Presentation Tool Bar >>
- Select Annotated Gallery

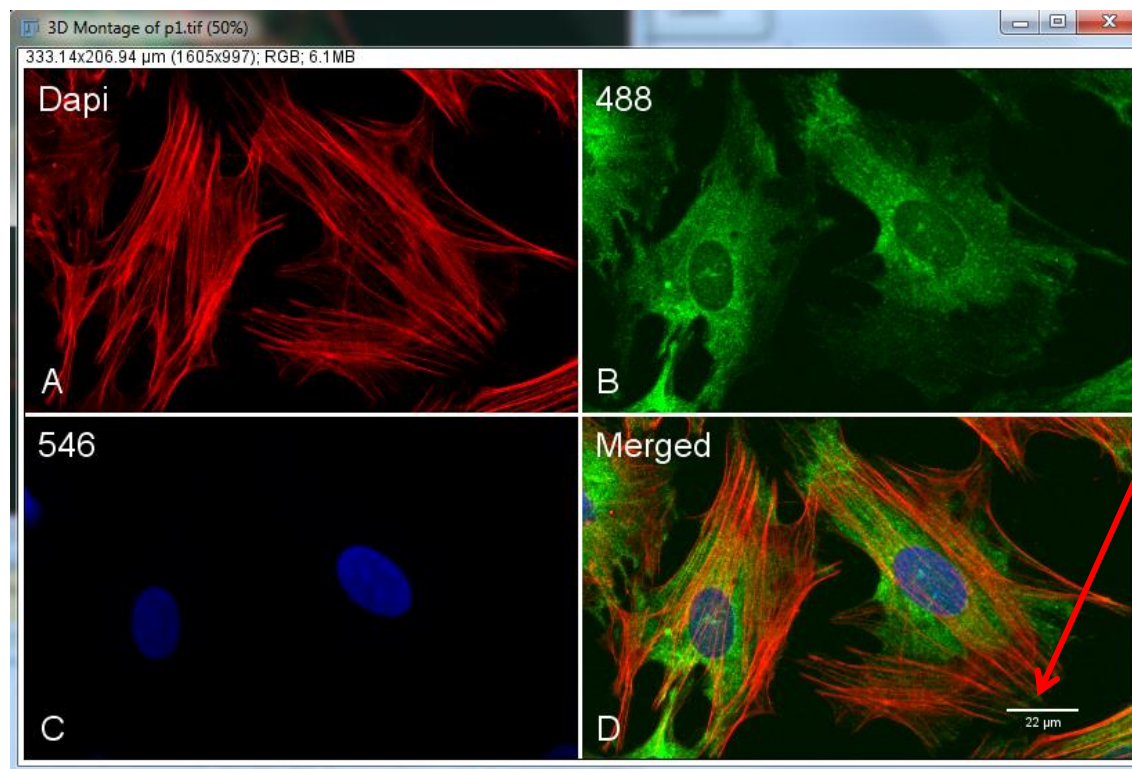
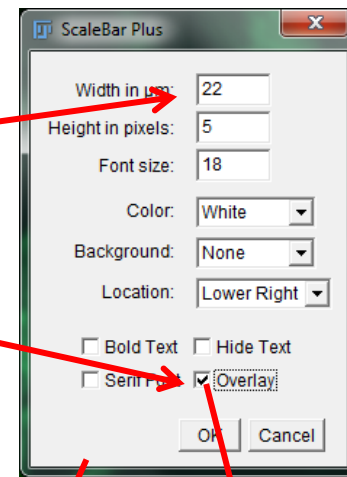
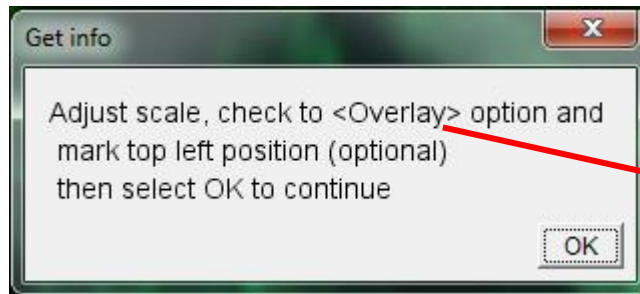


Annotated Gallery

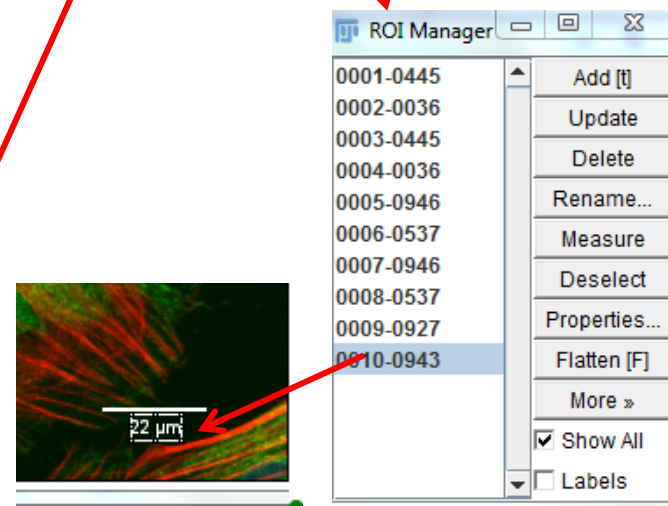
Options to Select:

- Format
- Individual channels in colour or black & white
- Add titles and figure numbers
- Add a scale bar



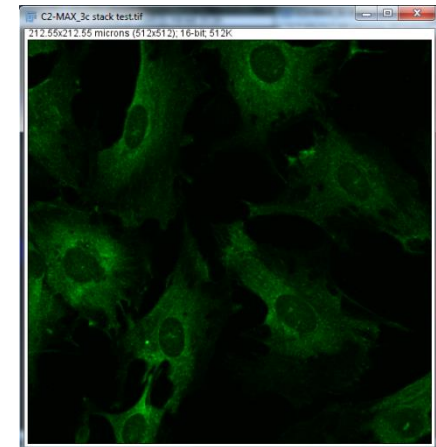
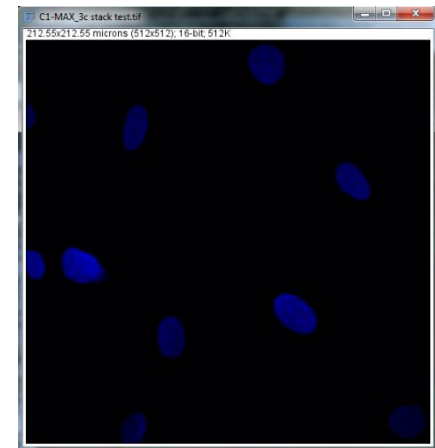
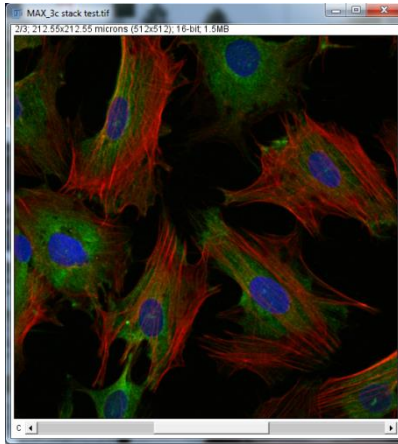


Use ROI Manager to select and edit overlay

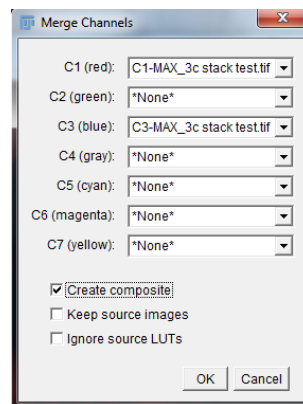
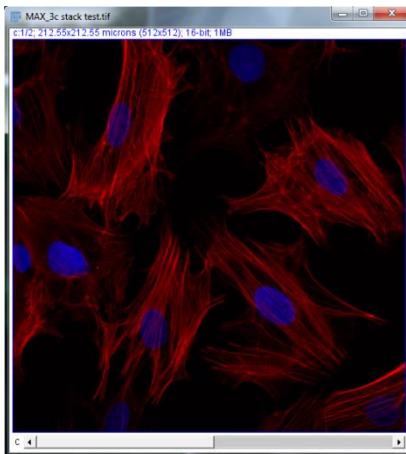


Split Channels

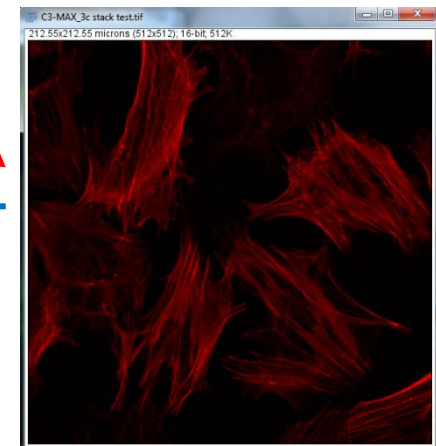
To split apart the separate channels
select - “Image” – “Colour” – “Split
Channels”



To merge channels
select - “Image” – “Colour” –
“Merge Channels”



Select channels
to merge



Multi Dimensional images

The image shows a software interface for viewing multi-dimensional images. The main window displays a dark field with green fluorescent spots. Below the image are three horizontal sliders labeled 'C', 'Z', and 'T'. Red arrows point from text labels to these sliders: 'Channel select' points to the 'C' slider, 'Z- position select' points to the 'Z' slider, and 'Time or Frame select' points to the 'T' slider. A red arrow points from the bottom-left corner of the main window to the text 'Time Series player (right click to get options)'. A blue arrow points from this text to an 'Animation Options' dialog box. The dialog box has a title bar with a close button. It contains a 'Speed (0.1-1000 fps):' label followed by a text input field containing '0.004'. Below this are two checkboxes: 'Loop Back and Forth' (unchecked) and 'Start Animation' (checked). At the bottom are 'OK' and 'Cancel' buttons.

Channel select

Z- position select

Time or Frame select

Time Series player
(right click to get options)

Animation Options

Speed (0.1-1000 fps): 0.004

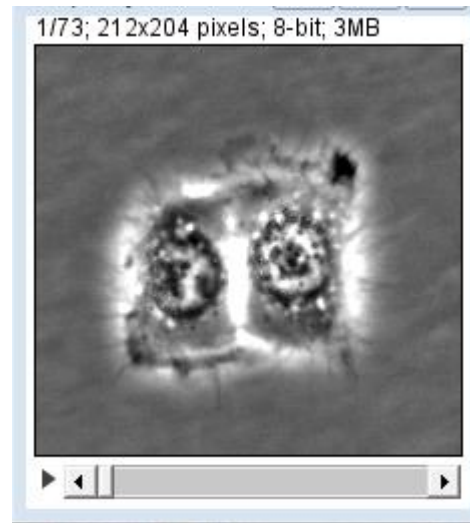
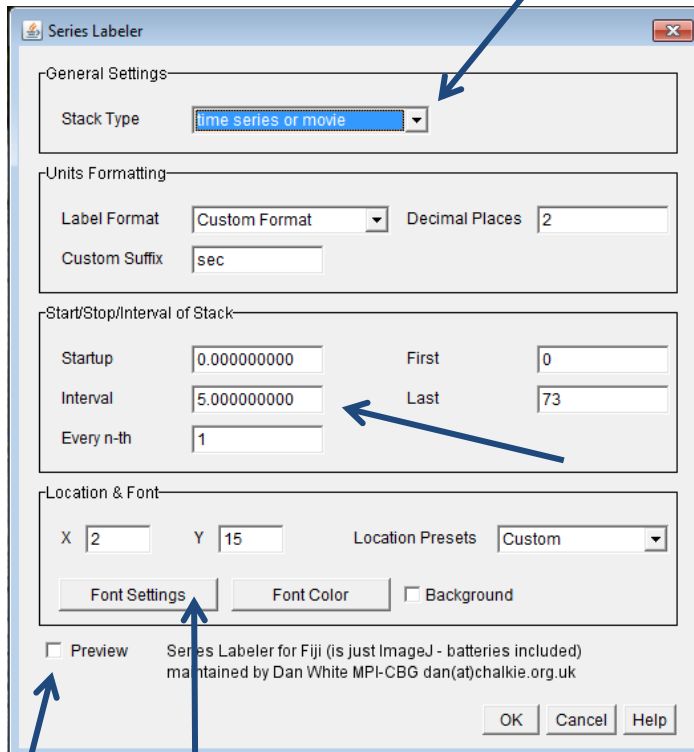
☐ Loop Back and Forth

☒ Start Animation

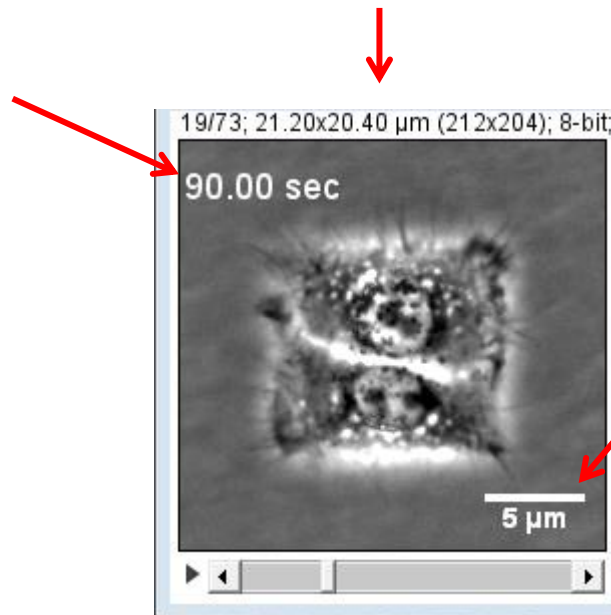
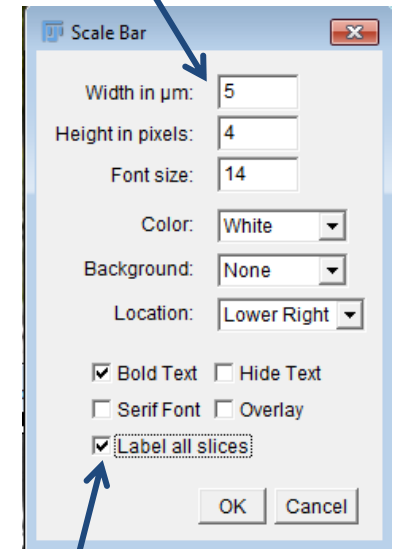
OK Cancel

Multi Dimensional images - Annotating a series

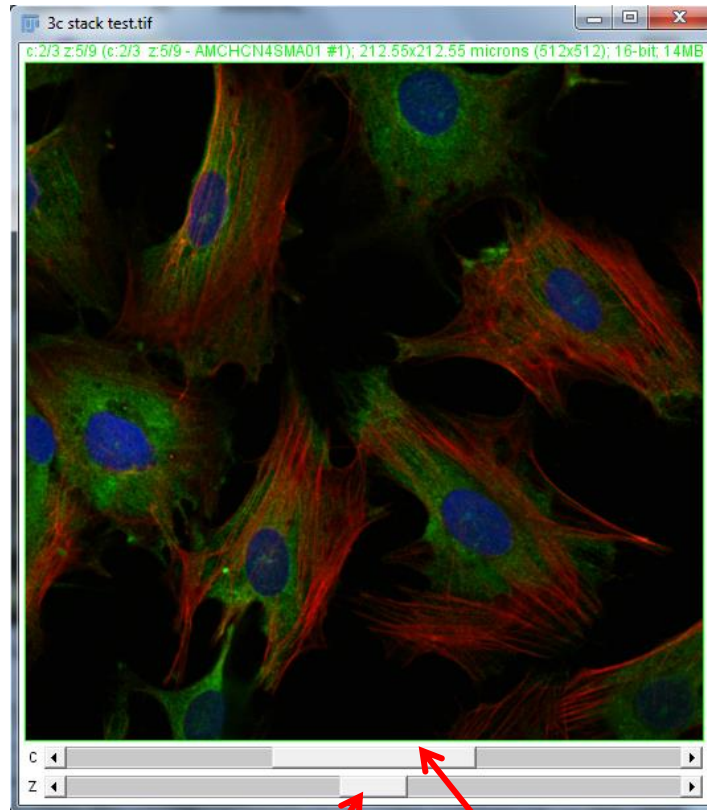
Use:
"Image"
"Stacks"
"Series Labeler"



Use:
"Analyse"
"Tools"
"Scale Bar"



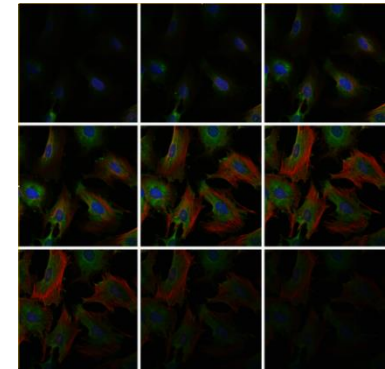
Z- Stacks



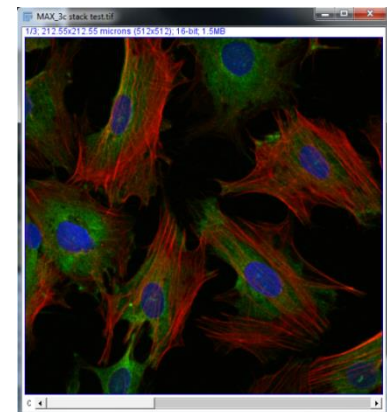
Z- slice selector

Channel select

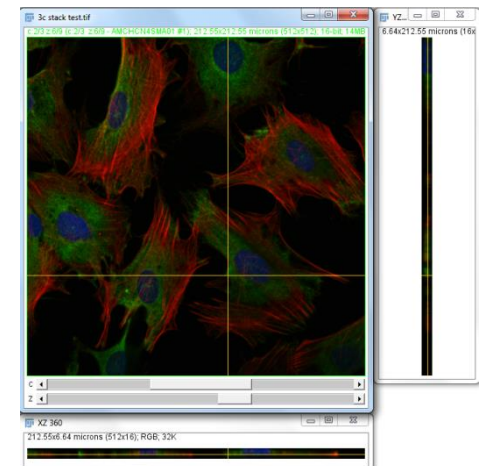
Montage



Z project



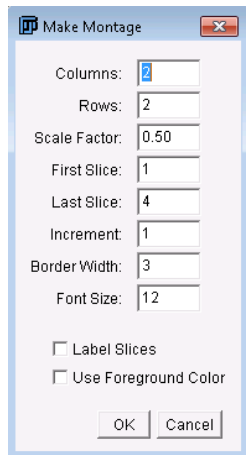
Otho-Slice



Montage View

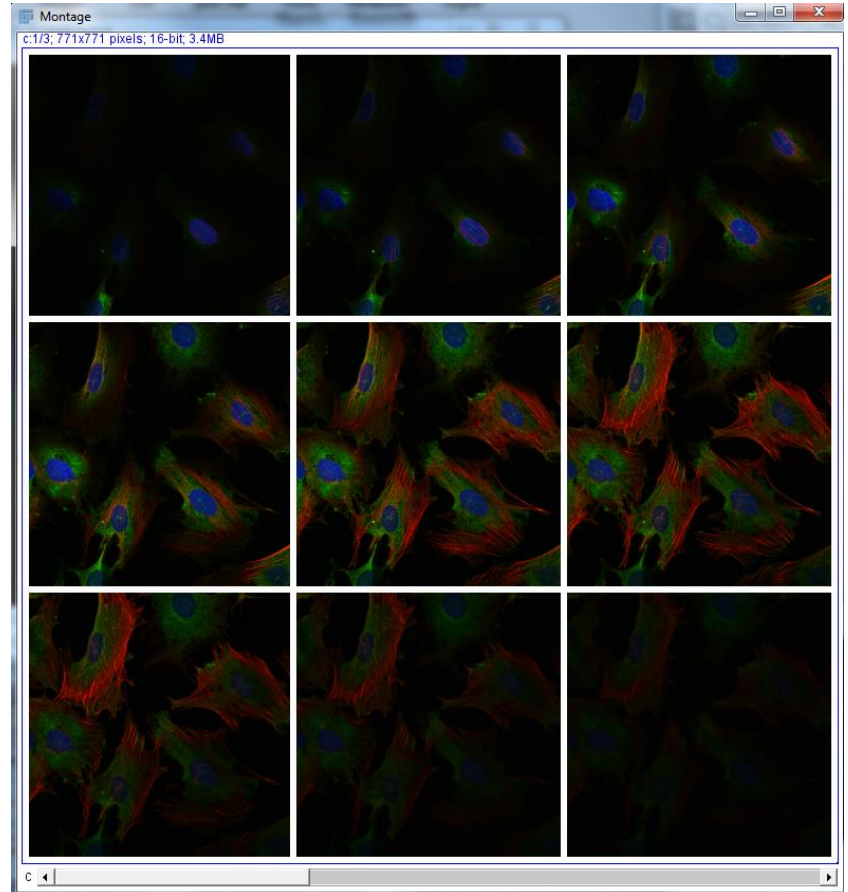
To create a montage view:

- Open your image
- Select “Image” – “Stacks” – “Make Montage”
- Change options if required
- Convert to “RGB” using “Channel Tools” for PowerPoint.



Output options

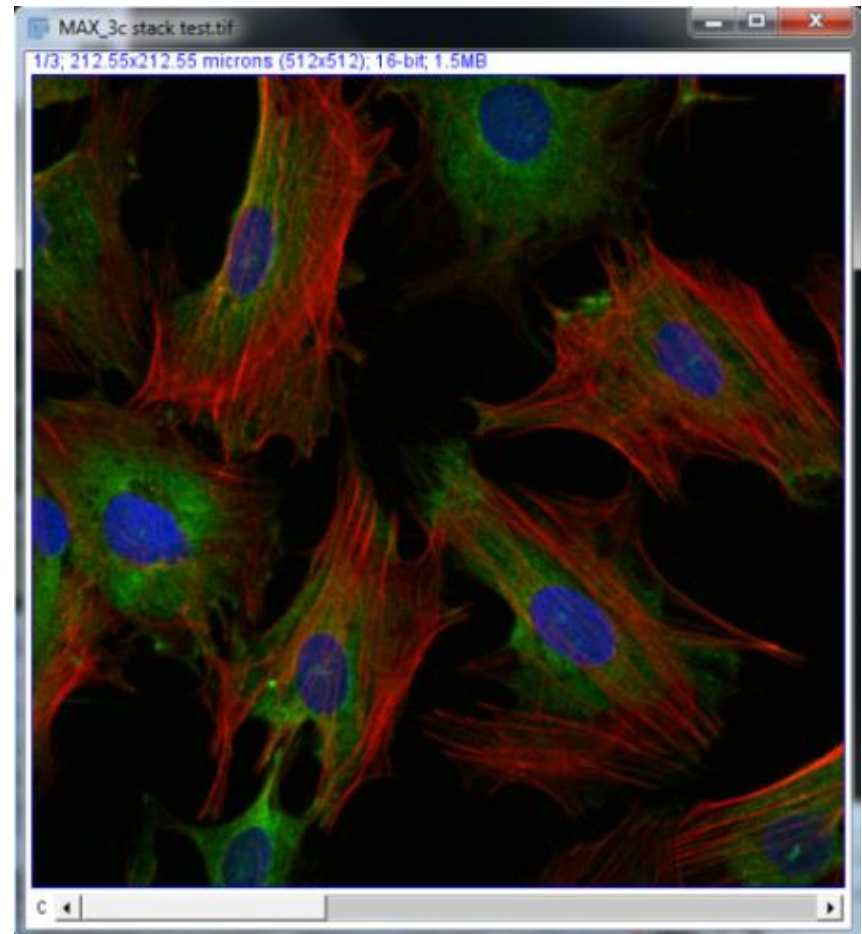
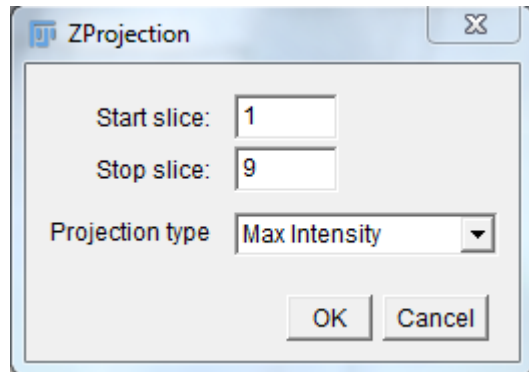
- Number of rows/columns
- Scale factor
- Border width (size of separating line)



Maximum Projection View

To create a maximum projection view:

- Open your image
- Select “Image” – “Stacks” – “Z Project”
- Change the option from Average Intensity to Max Intensity
- Convert to “RGB” using “Channel Tools” for PowerPoint.



Orthogonal View

To create a Orthogonal view:

Either

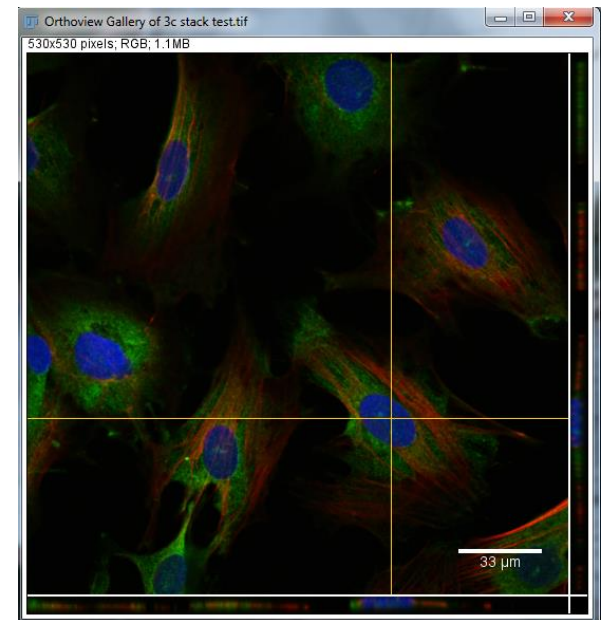
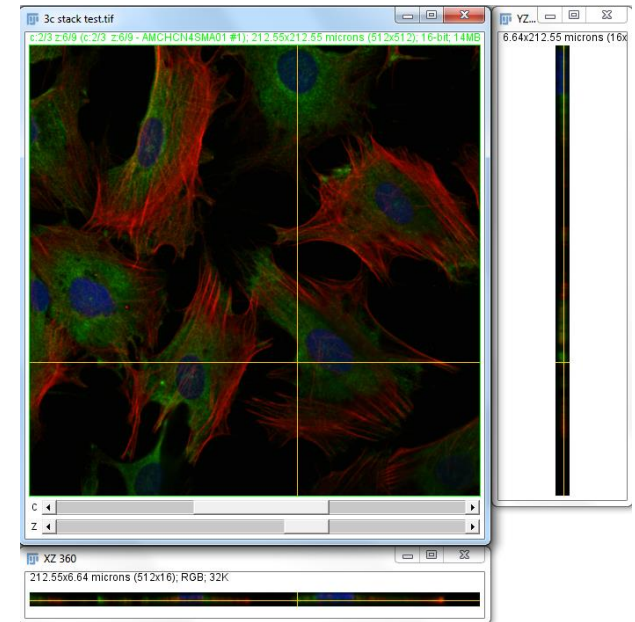
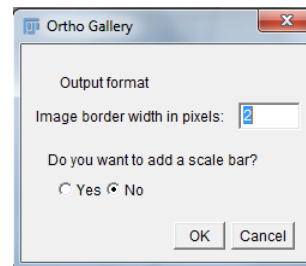
- Open your image
- Select “Image” – “Stacks” – “Orthogonal Views”
- Select the required X,Y and Z positions
- Save the individual images
- Convert to “RGB” using “Channel Tools” for PowerPoint.

Or



Orthoview Gallery

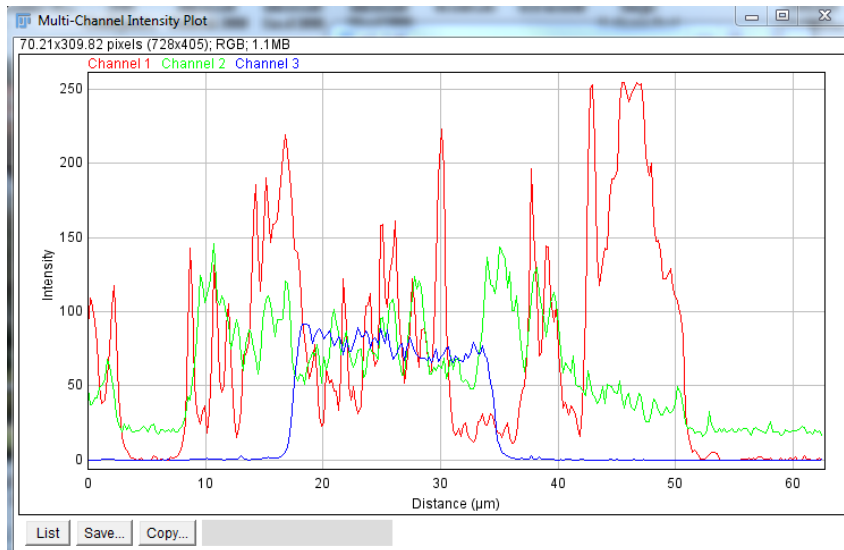
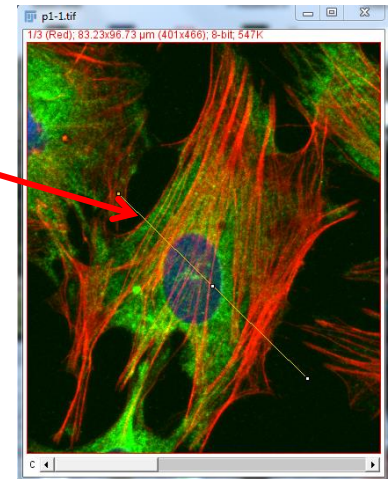
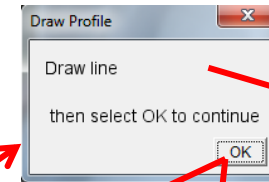
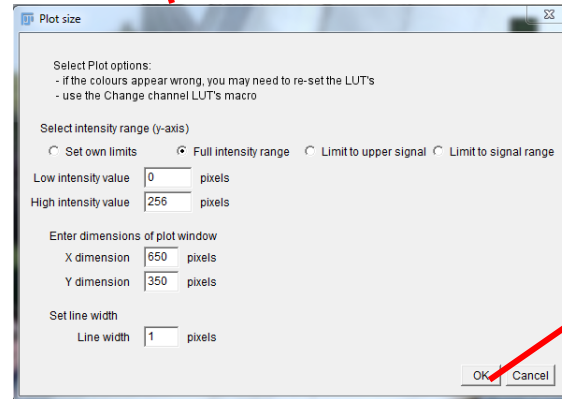
- Open your image
- Select the Presentation Tool Bar >>
- Select Orthoview Gallery tool
- Select border width and scale bar if required
- Select the required X,Y and Z positions



Intensity Plots



- Open your image
- Select the Intensity Tool Bar >>
- Select Intensity Line plot tool
- Select Plot output options
- Draw line when requested
- Result is a plot and data table

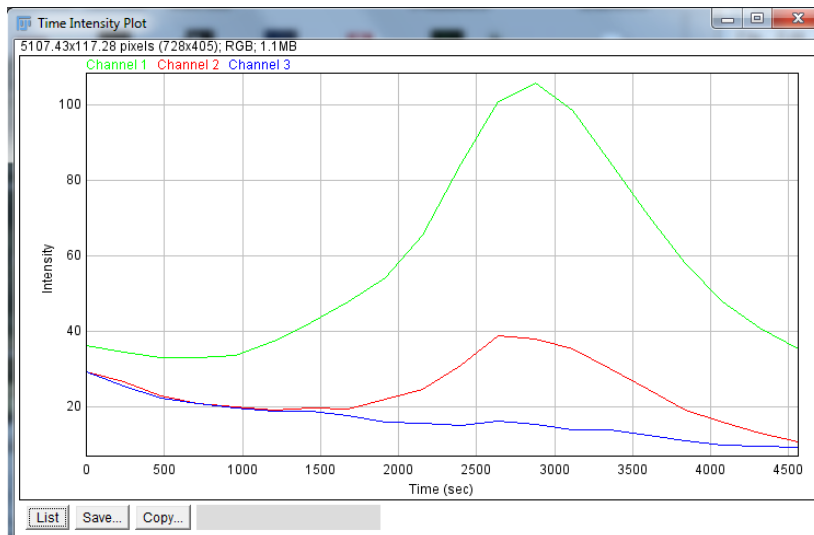
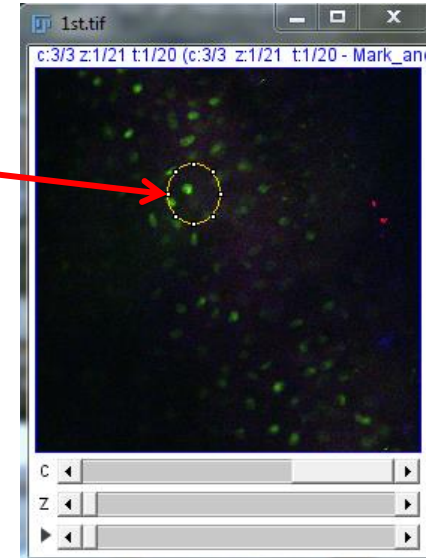
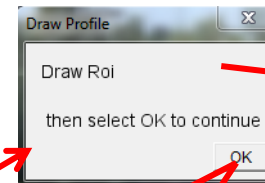
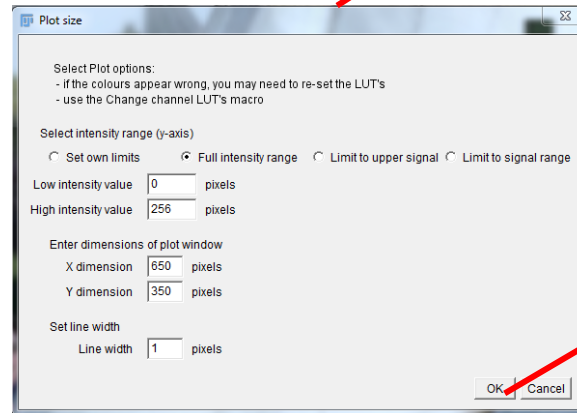


Results				
	Distance (µm)	Channel 1	Channel 2	Channel 3
1	0	91	48	0
2	0.208	109.654	37.568	0.199
3	0.415	103.505	38.296	0.226
4	0.623	93.535	42.203	0.080
5	0.830	78.934	41.870	0.113
6	1.038	49.664	49.379	0.279
7	1.245	38.741	50.568	0.867
8	1.453	40.651	54.953	1.000
9	1.661	56.316	68.060	0.535
10	1.868	75.319	63.359	0.691
11	2.076	102.731	55.542	0.980
12	2.283	117.625	45.635	0.904
13	2.491	84.571	30.621	0.269
14	2.698	43.342	23.468	0
15	2.906	16.465	19.465	0
16	3.113	0.628	24.080	0

Time Series – multi Channel plot



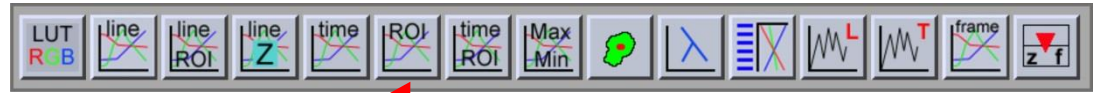
- Open your image
- Select the Intensity Tool Bar >>
- Select Intensity Time plot tool
- Select Plot output options
- Draw ROI when requested
- Result is a plot and data table



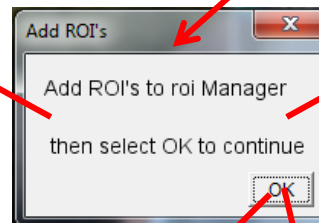
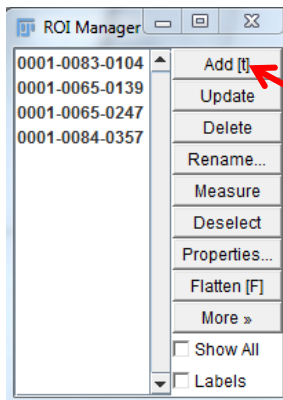
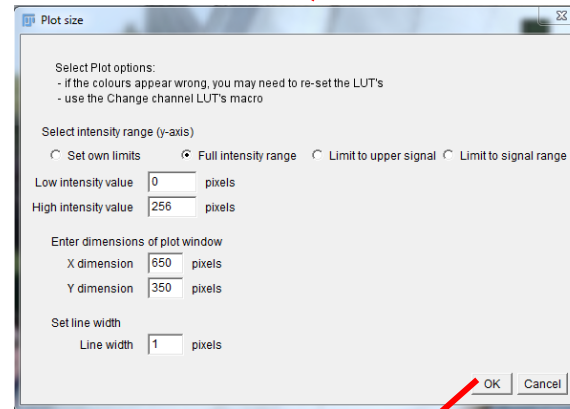
Results

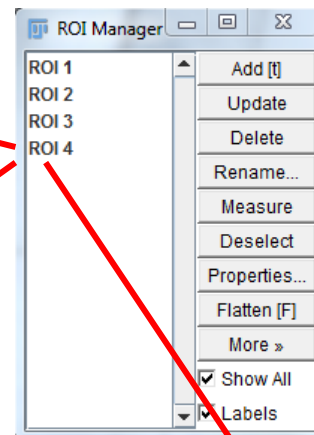
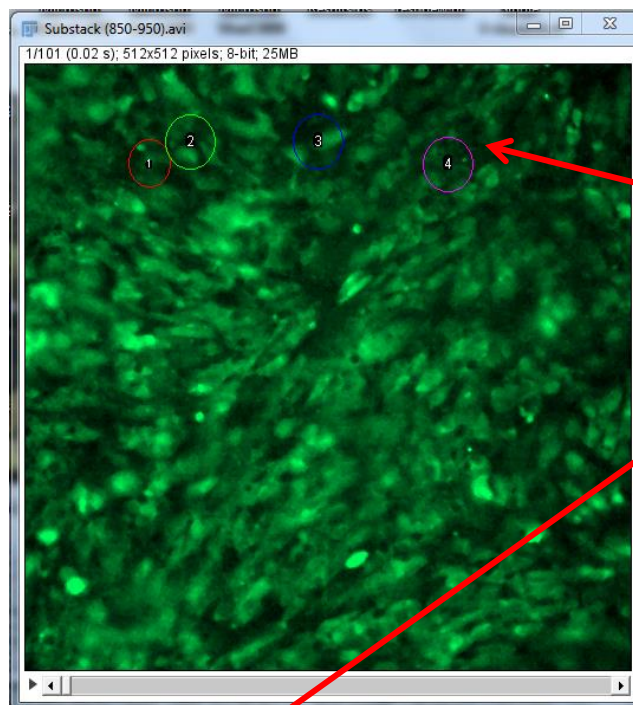
	File	Edit	Font	Results				
					Frame Interval (sec)	Channel 1	Channel 2	Channel 3
1					0	36.280	29.360	29.376
2					240.011	34.515	26.885	25.743
3					480.021	33.044	22.898	22.552
4					720.032	33.147	20.866	20.955
5					960.043	33.625	20.175	19.712
6					1200.053	37.391	19.236	19.018
7					1440.064	42.369	19.853	19.057
8					1680.075	47.862	19.653	17.961
9					1920.085	54.445	22.105	16.149
10					2160.096	65.539	24.816	15.855
11					2400.107	84.535	31.160	15.313
12					2640.117	100.885	38.998	16.264
13					2880.128	105.900	38.231	15.535
14					3120.139	98.690	35.419	14.136
15					3360.150	84.939	30.284	14.186
16					3600.160	70.791	24.765	12.708

Time Series – multi intensity plots



- Open your image
- Select the Intensity Tool Bar >>
- Select Multi ROI Time plot tool
- Select Plot output options
- Draw ROI's and add to Roi manager when requested
- Result is a list of ROI's, which are also displayed on the image, a plot and data table





Results

File	Edit	Font	Results				
	Frame Interval (frame)	Roi 1	Roi 2	Roi 3	Roi 4		
1	0	10.720	10.264	14.188	9.598		
2	1	10.602	10.486	14.057	9.427		
3	2	11.254	10.159	14.414	9.653		
4	3	11.236	10.308	14.300	10.003		
5	4	11.265	10.233	13.886	9.524		
6	5	11.405	10.642	14.284	8.949		
7	6	11.589	10.645	14.163	8.324		
8	7	12.266	10.453	14.250	8.254		
9	8	12.173	10.429	14.638	8.974		
10	9	11.946	10.626	14.853	9.407		
11	10	12.902	10.910	14.538	9.706		
12	11	12.657	10.721	14.703	8.941		
13	12	12.501	10.644	14.734	9.635		
14	13	13.496	10.863	14.785	9.461		
15	14	13.215	10.503	14.674	9.167		
16	15	12.116	10.695	14.312	9.148		