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”)

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
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.
Setting Brightness and Contrast

Brightness and contrast can be set the same for multiple images so the cam 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.

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 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
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

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

Channel select

Z- position select

Time or Frame select

Time Series player
(right click to get options)
Multi Dimensional images - Annotating a series

Use:
- “Image”
- “Stacks”
- “Series Labeler”

Use:
- “Analyse”
- “Tools”
- “Scale Bar”
Z- Stacks

Montage

Z project

Otho-Slice

Z- slice selector

Channel select
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
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
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