FILM Macros

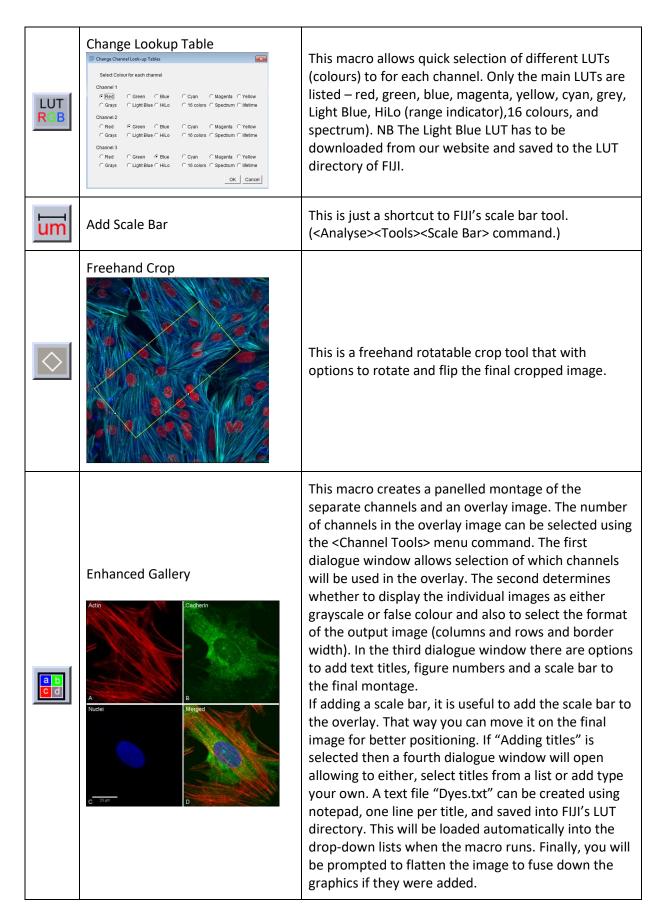
Uses this to select the macros below:

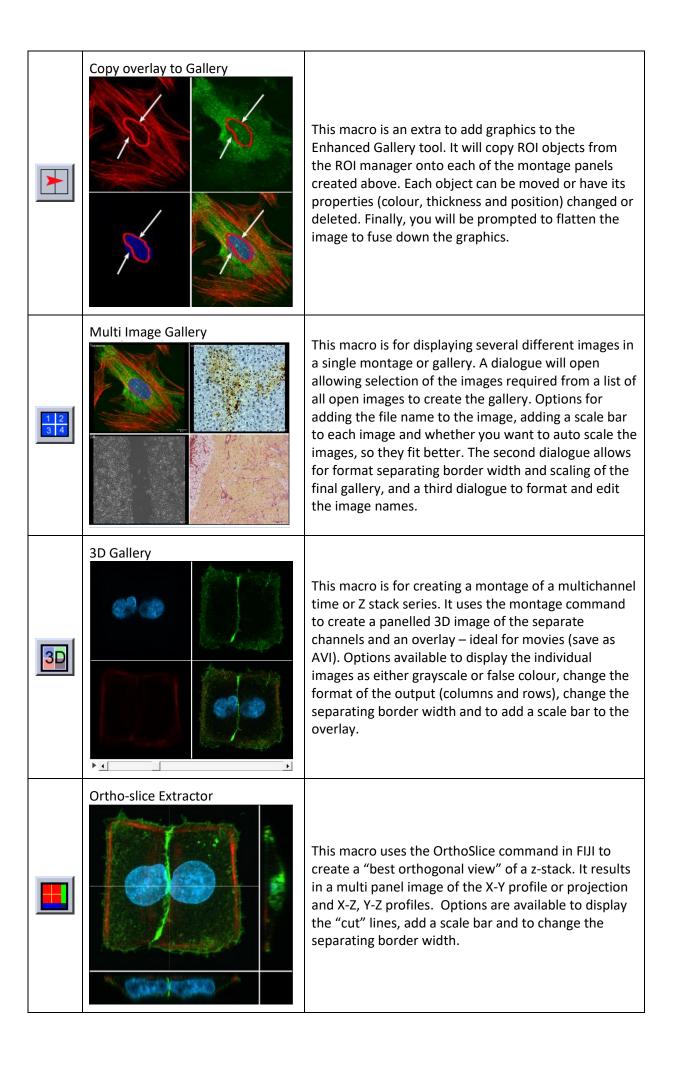
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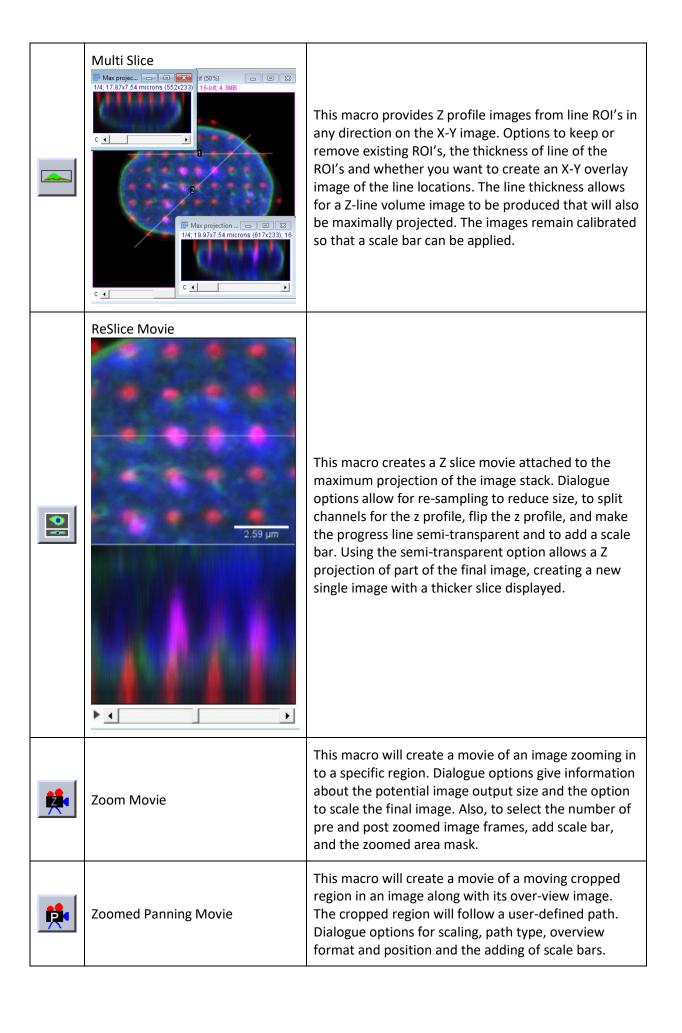


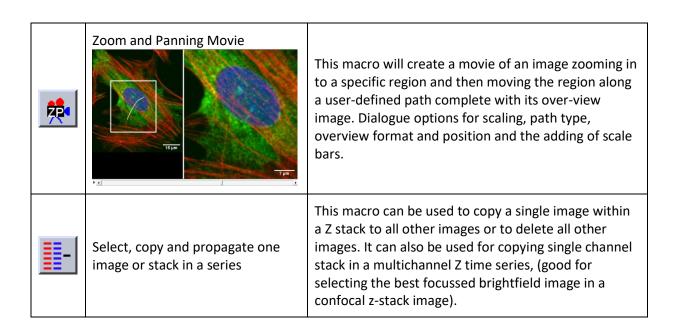
Presentation Macros





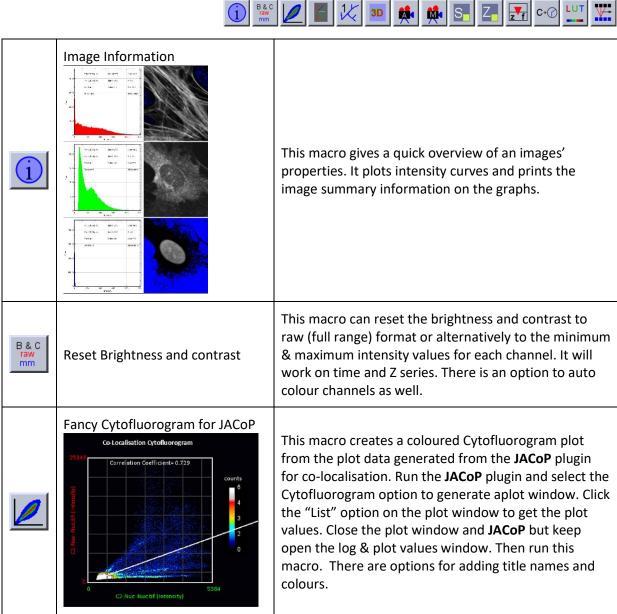


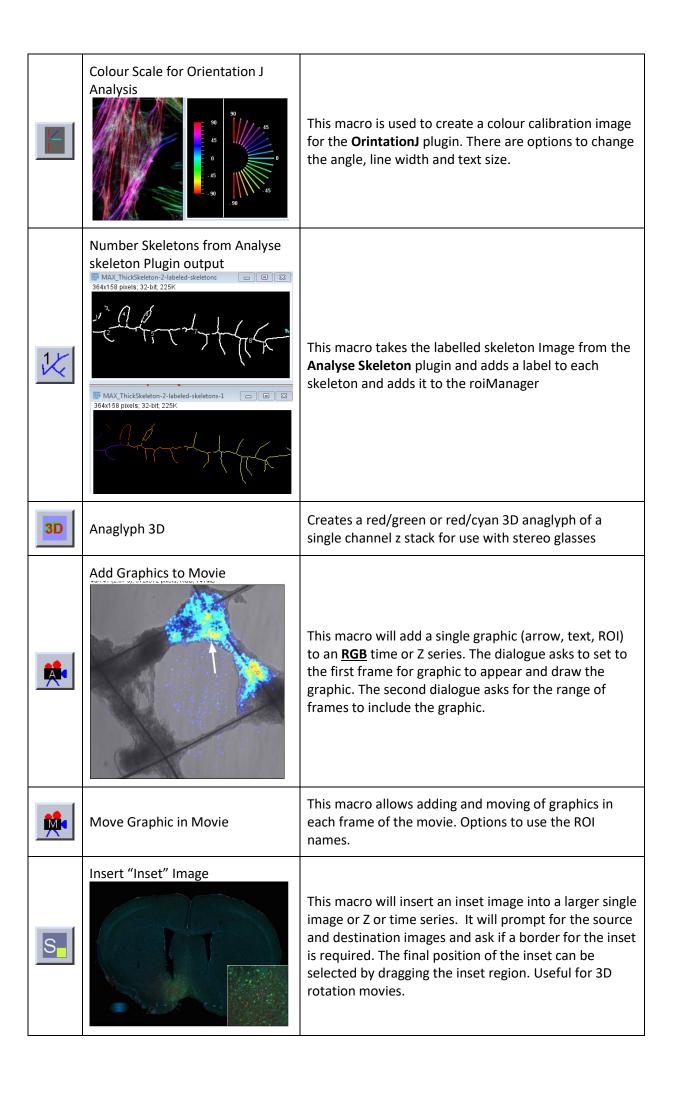




Presentation 2 Macros







Z	Insert a Z Stack	Inserts an inset Z-stack into another Z-stack. Images must be of the same type and number of Z-slices.
z f	Copy ROI's to time or Z series	This macro copies a list of ROI's in the ROI manager to each image in a Z or time series and flattens the final result. The dialogue will ask if you want to keep any existing ROI's and to add new ones. The image is then duplicated, converted to RGB before ROI's are added and flattened
C+	Join a single channel Image to a time series	This macro adds a single fixed channel image to a time series. It will ask for selection of the time series and then the fixed image. Will work on single or multichannel time series
LUT	Set LUT to wavelength colour	This macro allows setting the look-up table colour of non RGB images to the colour represented by wavelengths
	Rolling & Sequential projections	This macro will create a new enhanced stack either by grouping a rolling projection around each image slice/frame eg 1-5, 2-6, 3-7 or by grouping sequential projections eg 1-5, 6-10, 11-15 (It currently only works on a time or Z series, not both)

Presentation 3 Macros







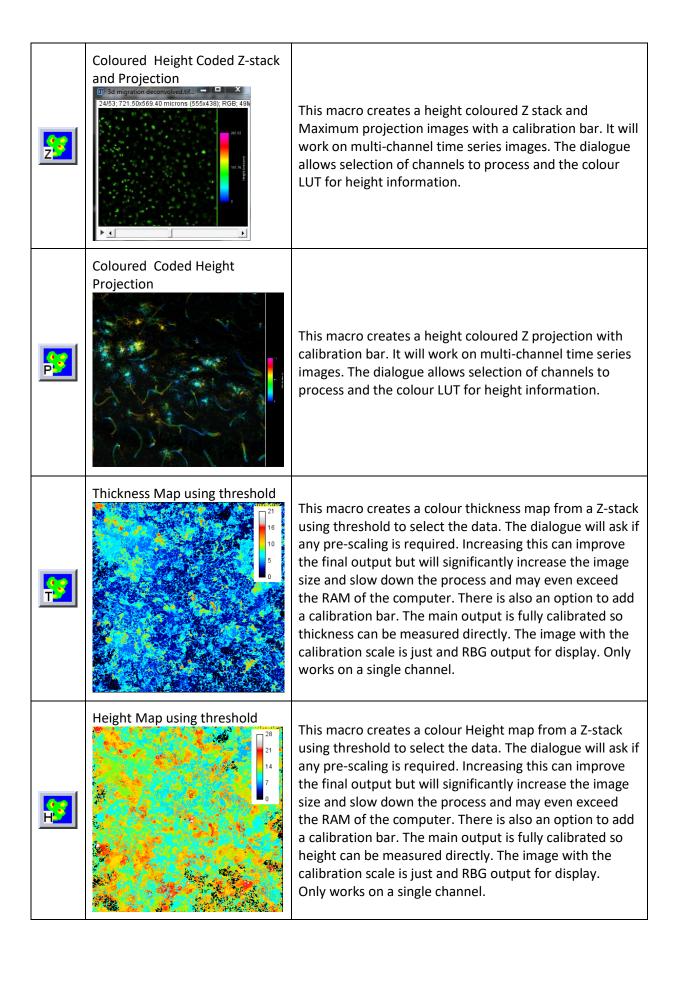




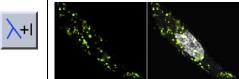




8	Tilted Rotation Movie	This macro creates a movie, of first a tilt series in the X direction, then secondly rotates this tilted view 360 degrees. Options for tilted angle and steps
3D tilt	3D Tilt Movie	This macro creates a movie of tilted views from different rotations. Options for tilted angle and steps
3D	Multi 3D Rotational	This macro creates a series of rotations using the 3D projection command which are then concatenated (joined) into one movie series. The first dialogue will ask for how many rotations you want. Then the second dialogue has the options for each rotation step (type, axis, direction, start, range, step, etc).



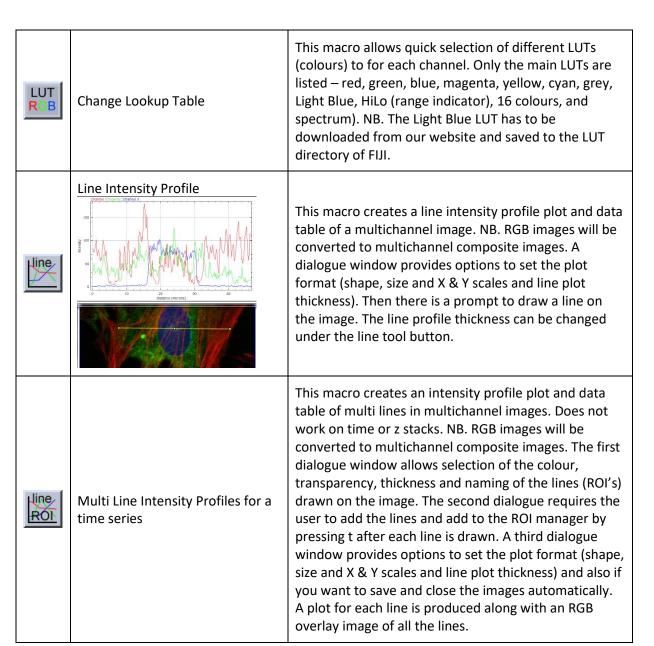
Add a Fixed Image to Lambda Colour Coded Series

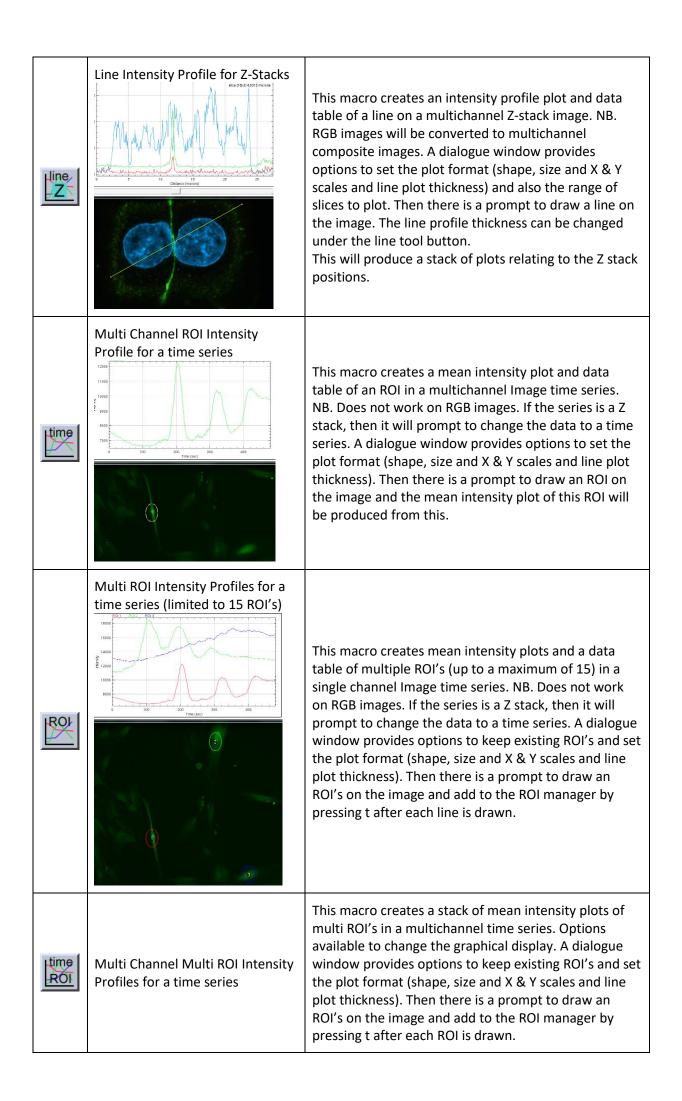


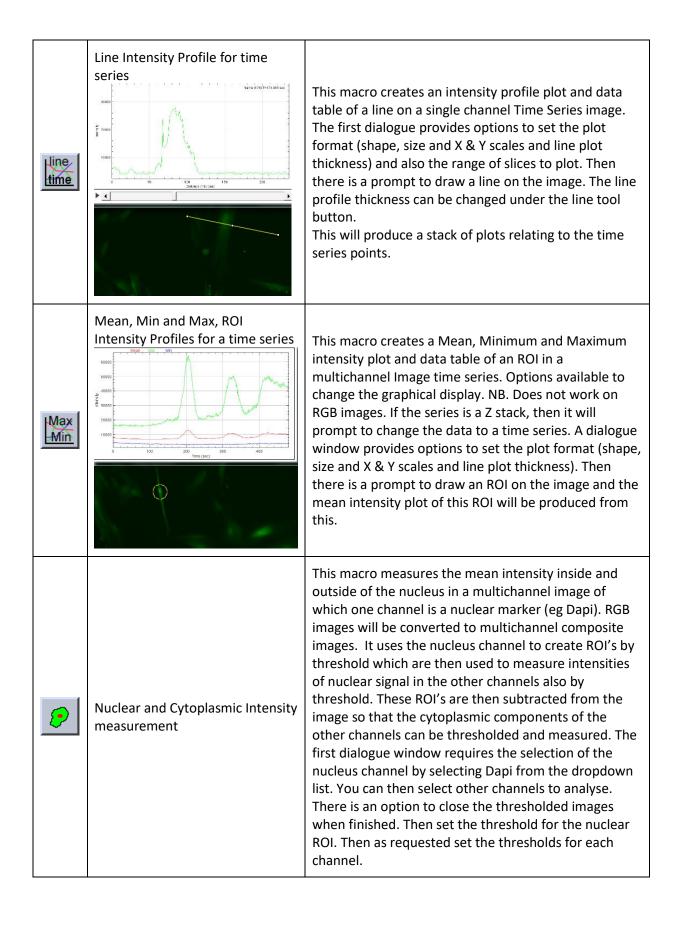
The coloured coded image is a multichannel image and the single image to add must be 8-Bit. Adjust the colour, brightness and contrast of the insert image and the lambda image (NB when changing the brightness and contrast of the lambda image select brightest channel and adjust settings, then use the SET button to propagate to all other channels to maintain relative brightness). The macro asks to select the required images then joins them creating a new RGB image series.

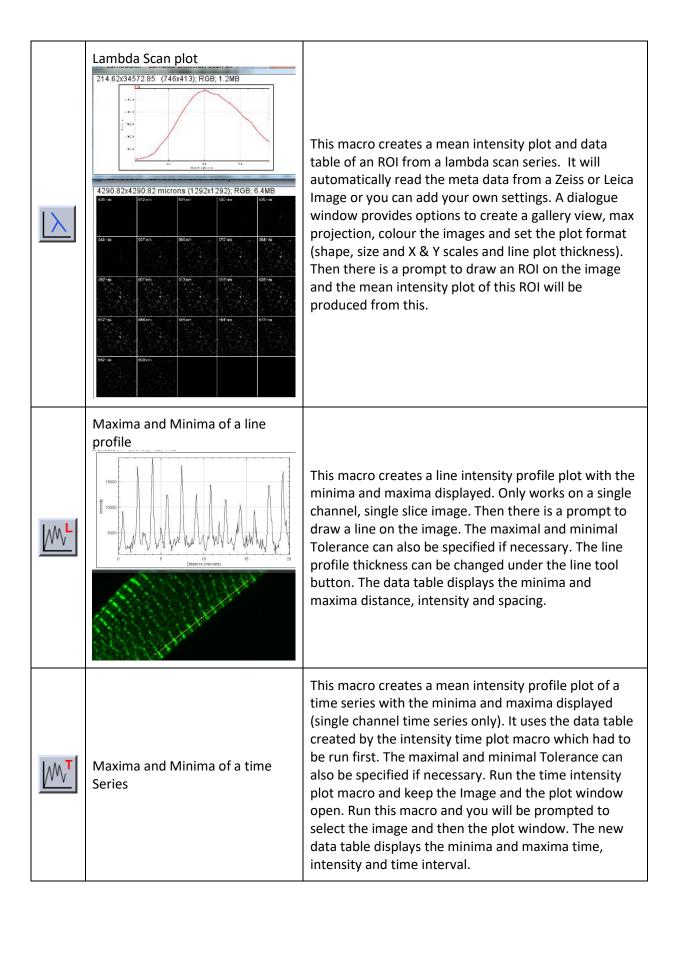
Intensity Macros



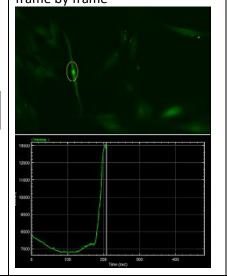








Multichannel channel single ROI time Intensity Plot displayed frame by frame



This macro creates a progressing (frame by frame) mean intensity time plot and data table of an ROI in a multichannel Image time series. NB. Does not work on RGB images. If the series is a Z stack, then it will prompt to change the data to a time series. A dialogue window provides options to set the plot format (shape, size and X & Y scales & line plot thickness), and options to Invert the plot (white on black background) and also to attach the graph onto a copy of the original image series (to the right or below) to create a movie. Then there is a prompt to draw an ROI on the image and the mean intensity plot of this ROI will be produced from this.

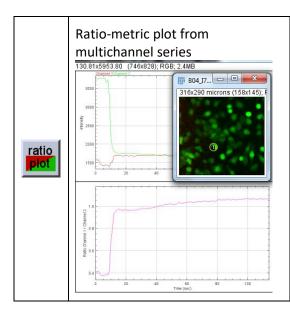
Single channel multi ROI (max 10) time Intensity Plot displayed frame by frame

(2)

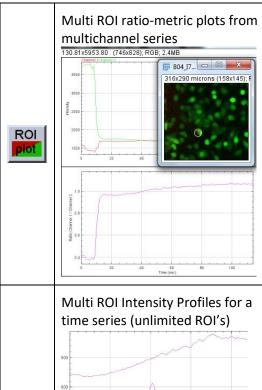
This macro creates a progressing (frame by frame) mean intensity time plot and data table of multiple ROI's (up to a max of 10) in a single channel Image time series. NB. Does not work on RGB images. If the series is a Z stack, then it will prompt to change the data to a time series. A dialogue window provides options to set the plot format (shape, size and X & Y scales & line plot thickness), and options to Invert the plot (white on black background) and also to attach the graph onto a copy of the original image series (to the right or below) to create a movie. Then there is a prompt to draw an ROI on the image and the mean intensity plot of this ROI will be produced from this.

Intensity 2 Macros

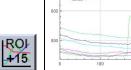




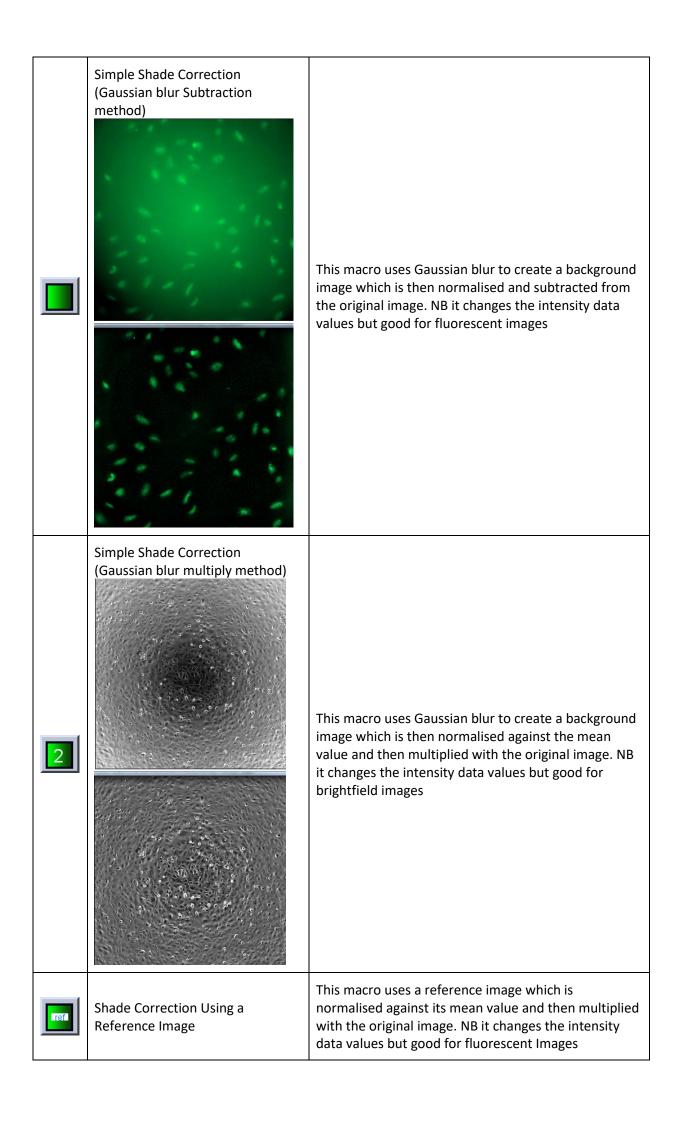
This macro creates a mean intensity time plot, a ratio plot and data table of 2 channels of an ROI in a multichannel Image time series. NB. Does not work on RGB images. If the series is a Z stack, then it will prompt to change the data to a time series. The first dialogue provides options to select which channels to use and to set the ratio order. There is also an option to join the two plots together. A second dialogue window provides options to set the plot format (shape, size and X & Y scales and line plot thickness). Then there is a prompt to draw an ROI on the image and the mean intensity plot of this ROI will be produced from this.



This macro creates a mean intensity time plot, a ratio plot and data table of 2 channels of multiple ROI's in a multichannel Image time series. NB. Does not work on RGB images. If the series is a Z stack, then it will prompt to change the data to a time series. The first dialogue provides options to select which channels to use and to set the ratio order. There is also an option to join the two plots together. A second dialogue window provides options to set the plot format (shape, size and X & Y scales and line plot thickness). Then there is a prompt to draw an ROI's on the image and add to the ROI manager by pressing t after each ROI is drawn.

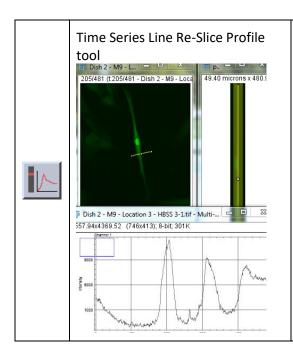


This macro creates a stack of mean intensity plots (one for each ROI), a combined plot window of all ROI's and a data table, for multiple ROI's (no limit) in a single channel Image time series. NB. Does not work on RGB images. If the series is a Z stack, then it will prompt to change the data to a time series. A dialogue window provides options to keep existing ROI's, calculate Maxima and set the plot format (shape, size and X & Y scales and line plot thickness). Then there is a prompt to draw an ROI's on the image and add to the ROI manager by pressing t after each line is drawn.



line 2	Line Intensity Profile with channel selection	This macro creates a line intensity profile plot and data table of a multichannel image. NB. RGB images will be converted to multichannel composite images. The first dialogue allows selection of the channels to analyse. A second dialogue window provides options to set the plot format (shape, size and X & Y scales and line plot thickness). Then there is a prompt to draw a line on the image. The line profile thickness can be changed under the line tool button.
Line RÓ12	Multi Line Intensity Profiles for a time series with channel selection	This macro creates an intensity profile plot and data table of multi lines in multichannel images. Does not work on time or z stacks. NB. RGB images will be converted to multichannel composite images. The first dialogue allows selection of the channels to analyse. A second dialogue window allows selection of the colour, transparency, thickness and naming of the lines (ROI's) drawn on the image. The third dialogue requires the user to add the lines and add to the ROI manager by pressing t after each line is drawn. A third dialogue window provides options to set the plot format (shape, size and X & Y scales and line plot thickness) and also if you want to save and close the images automatically. A plot for each line is produced along with an RGB overlay image of all the lines.
Jine Z2	Line Intensity Profile for Z-Stacks with channel selection	This macro creates an intensity profile plot and data table of a line on a multichannel Z-stack image. NB. RGB images will be converted to multichannel composite images. The first dialogue allows selection of the channels to analyse. A second dialogue window provides options to set the plot format (shape, size and X & Y scales and line plot thickness) and also the range of slices to plot. Then there is a prompt to draw a line on the image. The line profile thickness can be changed under the line tool button. This will produce a stack of plots relating to the Z stack positions.
time 2	ROI Intensity Profile for a time series with channel selection	This macro creates a mean intensity plot and data table of an ROI in a multichannel Image time series. NB. Does not work on RGB images. If the series is a Z stack then it will prompt to change the data to a time series. The first dialogue allows selection of the channels to analyse. A second dialogue window provides options to set the plot format (shape, size and X & Y scales and line plot thickness). Then there is a prompt to draw an ROI on the image and the mean intensity plot of this ROI will be produced from this.

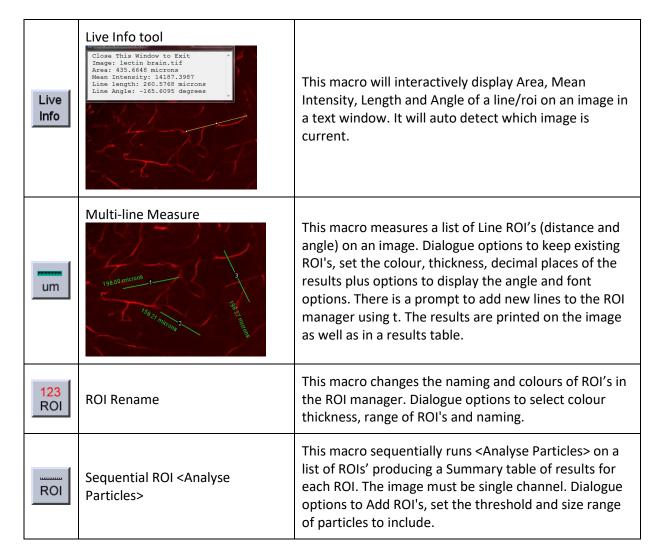
line	Line Intensity Profile for time series with channel selection	This macro creates an intensity profile plot and data table of a line on a multichannel Time Series image. NB. RGB images will be converted to multichannel composite images. The first dialogue allows selection of the channels to analyse and provides options to set the plot format (shape, size and X & Y scales and line plot thickness) and also the range of slices to plot. Then there is a prompt to draw a line on the image. The line profile thickness can be changed under the line tool button. This will produce a stack of plots relating to the time series points.
Var	Variable Time Series tool	This macro can write a time stamp to the meta label using a constant value or from a text file that has variable time intervals or read existing time information. Other options include plotting the intensity data using the meta title time values for time input and drawing the time stamp on the image.
	Profile Intensity Plot in Z of an ROI with Channel select	This macro creates a mean intensity plot in Z and data table of an ROI in multi channels. Options available to change the graphical display. A dialogue window provides options to select which channels to analyse and second dialogue to set the plot format (shape, size and X & Y scales and line plot thickness). Then there is a prompt to draw an ROI on the image and the mean intensity plot of this ROI will be produced from this. NB does not work on RGB images.
	FWHM Intensity Sum Line: 1 Frame: 199 time: 197 3604 sec FWHM Width 52 microns Baseline intensity: 4435.5 15000 10000 500000 500000 500000 500000 500000 500000 500000	This macro calculates the sum of intensities of the full width half maximum of a line profile after subtracting a calculated baseline value. The results table displays the baseline value the FWHM width and sum of intensities. Included is an option to smooth the data.



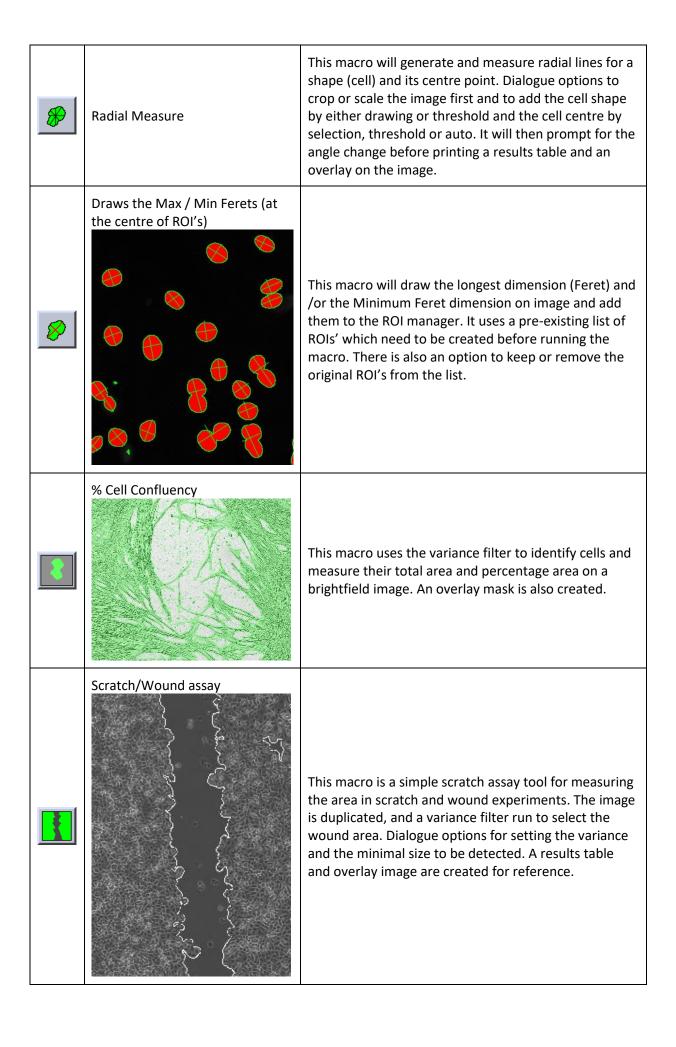
This macro will produce a 2D image from a line drawn on a time series (X- line length, Y – time). A line is then drawn on this new image along the Y-axis and an intensity profile produced. There are options for the second line width.

ROI Tools Macros



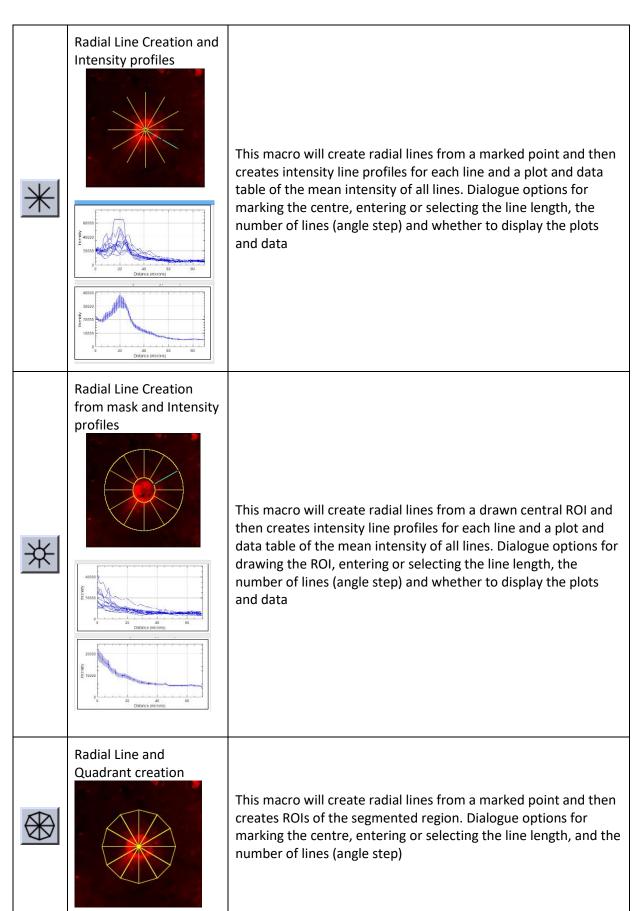


ROI	Move, scale and align Multiple ROI's	This macro allows movement or scaling of all ROI's by the same amount, or the alignment of all or a selection of the ROI's. For moving ROI's, select a single "key" ROI and click OK then move this ROI to its new position and click ok and all other ROI's will move by the same amount. For scaling, just type in the scale factor. For alignment there are options to select left, right, top or bottom and all or a section of the ROI's
ROI	Sequential ROI <analyse particles=""> plus results</analyse>	This macro sequentially runs <analyse particles=""> on a list of ROIs' producing and saving a results and Summary table for each ROI. Dialogue options to Add ROI's, set the threshold and size range of particles to include.</analyse>
\blacksquare	Split into Segments	This macro splits a large image into smaller cropped segments. Dialogue options to set the number of crops or use specific dimension and whether so auto save the results. It results in individual named crop images and an overlay is placed on top of the original image. This overlay can be saved or flattened through the ROI manager.
	Creates a Grid of ROIs' Region 1 Region 2 Region 3 Region 4 Region 5 Region 6 Region 7 Region 8 Region 9 Region 10 Region 11 Region 12 Region 13 Region 14 Region 15 Region 16	This macro creates a continuous grid and adds the regions to the ROI manager. Options for number of grids or grid size and placement
1111	Weibel Grid	This macro generates a Weibel grid (a line grid distribution based on an equilateral triangle). Options available to set the grid size and offset, also to select a region of interest. Prints a summary of area, grid size and number of lines
	Multi Crop	This macro creates cropped images from multiple ROI's – useful for micro-patterns or MEA's. Dialogue options to set the size of the ROI, spacing for the crop or manual selection and how the output is handled (stacking, splitting channels and/or auto saving for the resulting images).
4	Measure Change of Angle	This macro measure angle, changes of direction and accumulative change from a sequential list of ROIs'. Dialogue prompts to add new lines (arrows) with t to the ROI manager before printing the results.



ROI Masks





	Radial Line and Quadrant creation from a mask	This macro will create radial lines from a drawn central ROI and then creates ROIs of the segmented region. Dialogue options for marking the centre, entering or selecting the line length, and the number of lines (angle step)
	Multiple Ring Roi Creation	This macro will create concentric ROI's from a drawn central ROI and then creates ring ROI's. Dialogue options for marking the centre, entering the ring distance, and the total number of rings and an option to delete the intermediate ROI's
	Create Boxes along a line	This macro will create a string of polygonal ROI's from a drawn central line. Dialogue options for the line type, drawing or entering the initial box dimensions, and whether the vertical edges of the polygons should be angled to follow the line.
000	+ + +	This macro can create ROI's from either Maxima, added Multi – points or from a list of ROI's in the RoiManager. There are options for method, ROI shape and size and whether to keep existing ROI's
<i>9</i>	Create a Single Ring ROI	This macro will create a single ring ROI from a drawn ROI Dialogue options to create the ring by entering the distances for enlarging or reducing of the original ROI, or by drawing the second ROI

000	Create Ring ROI's from Multiple Original ROI's	This macro will create ring ROI's from a list of existing ROIs. Dialogue options to create rings by enlarging or reducing or both from the original ROI, entering the distances for enlarging and reducing and options to keep or delete the original and intermediate ROIs
%		This macro will create ring ROIs from a combined list of ROIs. Dialogue options to change the number and width of rings and also whether to keep or delete the original and intermediate ROIs
	Create an array of ROIs	This macro creates an array of ROI's. Dialog options for Roi type (rectangle of oval), size, spacing and array number or an option to use direct input on the image
um	Distance Between source and target ROI's	This macro uses the signed Euclidian distance plugin to get the edge to edge, centre to centre, edge to centre and centre to edge distances between a Source ROI and target ROIS. Options to draw the source and target ROI's, select measurements and draw lines.
um	Distance Between source and target ROI's Multi Channel	This macro uses the signed Euclidian distance plugin to get the edge to edge, centre to centre, edge to centre and centre to edge distances between a Source ROI and target ROIS from two different channels by threshold. Options to select channels, set threshold and analyse particle settings and whether to keep EDT images

um S	Distance Between 2 Nested ROI's	This macro uses the signed Euclidian distance plugin to get the longest and shortest distances between 2 nested ROI's. Options to draw inner and outer ROI's and draw lines
3D	Distance Between source and target ROI's 3D	This macro uses the signed Euclidian distance plugin to get the distances from either the edge or the centre of the source ROI to the centre of the target ROIs in a 3D stack of images. Options to select source and target image channels. The Source channel (a single ROI) is either converted to binary or the "3D Object counter" plugin is used to generate centres and then the "Exact Signed Euclidean Distance Transform (3D)" plugin is run to create a distance map (EDF image). ROI's are generated from the Target channel using the "3D Object counter" plugin to get the X, Y, Z centre values. These are plotted on the EDF image to the nearest slice and then measured to get the distance value. A sub-Z measurement is also calculated from two adjoining slices



LUT RGB	Change Lookup Table	This macro allows quick selection of different LUTs (colours) to for each channel. Only the main LUTs are listed – red, green, blue, magenta, yellow, cyan, grey, Light Blue, HiLo (range indicator), 16 colours, and spectrum). NB. The Light Blue LUT has to be downloaded from our website and saved to the LUT directory of FIJI.
CAL	Calibrate Re-sampled ZEN Tiled Images	This macro can be used to calibrate the re-sampled images from the Bio-Formats importer for tiles where only the full format is calibrated
Са	Calibrate Image	This macro will put back the calibration on an exported tiff format image from the FILM widefield microscopes for both the colour and Flash Cameras. The dialogue prompts for the microscope, camera and format as well as the export setting used
um	Add Scale Bar	This is just a shortcut to FIJI's scale bar tool. (<analyse><tools><scale bar=""> command.)</scale></tools></analyse>

0	Rotate	This macro will rotate an image by the amount needed to straighten a drawn line on the image. The dialogue prompts to draw a line. The macro enlarges the canvas (maintaining the full image) and rotates the image.
0	Rotate and Crop	This macro will rotate an image by the amount needed to straighten a drawn line on the image. The dialogue prompts to draw a line. The macro enlarges the canvas (maintaining the full image) and rotates the image. Then there is a prompt to crop the rotated image.
Bc	Save, Copy and Paste Brightness and Contrast Settings	This macro allows storing and retrieving the Brightness and Contrast settings for an image so that they can be used for other images later on.
RGB RGB	Merge RGB Images	This macro merges and overlays 2 RGB colour images. Images have to be of the same type.
	White Balance (for RGB images)	This macro is for correcting the white balance background of RGB colour images (histology) where the background has been captured with a colour cast.
INV	Invert Intensities	This macro Inverts the intensity values of the image. Works on all image formats except 32 Bit images. Useful for thresholding some images for tracking.
Dec	Color Deconvolution shortcut	This macro uses the "Color Deconvolution" command to split a histologically stained RGB image into 3 components while maintaining the image calibration. Options to select the vector or User methods, and to run a smoothing filter and add titles to the resulting images. The available vector methods are read from the colourdeconvolution.txt file in the plugins folder and user values can be saved and re-read from the user ROI method or added to the plugin set.
Batch Proj-	Batch Projection	This macro runs the z project function on a directory of images. Dialogue options for input and output directories and type of projection. NB The input directory must only have images to process and the Output directory must not be in the Input directory.
Macro Updat	Update these Macros	This macro updates to the latest macro versions. The computer needs to be a FILM user and also on the College Network (Mac users have to connect to the FILM Documentation server).

Serie	s Macro	EXT Z Area Area Z Blch Corr
LUT RGB	Change Lookup Table	This macro allows quick selection of different LUTs (colours) to for each channel. Only the main LUTs are listed – red, green, blue, magenta, yellow, cyan, grey, Light Blue, HiLo (range indicator), 16 colours, and spectrum). NB. The Light Blue LUT has to be downloaded from our website and saved to the LUT directory of FIJI.
EXT 1	Extract Best Focus	This macro allows manual extraction of the best focal plane from a timed Z series. The dialogue will prompt to sequentially select the best z position for each time point and then create a new single z focussed time series.
EXT	Extract Best Z series	This macro allows manual extraction of the best focal range from a timed Z series. The dialogue will ask for the number of Z slices to retrieve and then prompt to sequentially select the best z position for each time point. It will then create a new z series based on the required number of slices centred on the focal plane.
(iii)	Manually Align Images Over Time	This macro allows manual alignment of images from a time series by using an ROI. It will prompt to draw an ROI and select the first time point and then prompt to sequentially to move the ROI to the correct position at each time point. It will create a new time series based on this alignment.
N	Channel Shift Alignment (pixel based)	This macro will allow the pixel shift of one channel in a multichannel image (single, z and time) to correct for alignment. Options to select channel to shift and number of pixels in X and Y (works with both positive and negative shifts).
-	Splits Library into tiffs	Splits a Leica.lif, velocity or Zen(tile or position) library file into individual images
	Split a stack of images (Z or time)	Splits the stack/time series into separate image sub (stack/time) series. Option to select either z or time to split and the number of splits requires (eg every 3 rd frame – will produce 3 splits).
Area	Plot Change of Area Over Time	Option to run a minimum filter to reduce white and dark edges, select plot options and threshold image
I -	Select, copy and propagate one image or stack in a series	This macro can be used to copy a single image within a Z stack to all other images or to delete all other images. It can also be used for copying single channel stack in a multichannel Z time series (good for selecting the best focussed brightfield image in a z-stack confocal image).
	Manually Align Z-Stack	This macro can be used to manually join and align two Z-Stacks. Images have to be of the same type.
z f	Copy ROI's to time or Z series	This macro copies a list of ROI's in the ROI manager to each image in a Z or time series and flattens the final result. The dialogue will ask if you want to keep any existing ROI's and to add new ones. The image is then duplicated, converted to RGB before ROI's are added and flattened

	Join, set LUT and Reset B&C for Z stacks	This macro can be used to load a related set of images, join them, select the LUTs and also resets brightness and contrast to Minimum/ Maximum levels. The number of images and their names are selected before opening. This is a useful tool for opening de-convolved images saved from Huygens.
	Random Cell Generator	This macro will generate cells in random positions in a Z stack and if the image is a time series then also generate random movement of these cells over time. Options to use the format from an open existing image or to create a new image, control of number and size of cells and the maximum distance allowed for their movement. Additional options to create or use exclusion/inclusion ROI's.
Z↓	Reverse Z stack	This macro reverses the order/direction of a Z-stack in a Hyperstack. (It will work on Multi-channel and time series)
Blch Corr	Bleach Correction	Bleach Correction: This macro will estimate a baseline intensity level in a selected background region (ROI) across the stack or time series. The Curve Fitting Tool using the Exponential with Offset equation (Formula: y = a*exp(-bx) + c) is used to get the value of c from this data. The Bleach Correction plugin using Simple Ratio and using the same ROI is run using this value of c as the background value.

Extras Macro

Live ruler Ω LUT Make INV Map Plot G2 Stack Spark Meta				
<u>(i)</u>	Image Information	This macro gives a quick overview of an images' properties. It plots intensity curves and prints the image summary information on the graphs.		
Live ruler	Live Interactive Ruler	This macro will interactively display ruler length on the selected image. Options to select arrow or line format as well as colour.		
Ω	Add symbols and text to Image	This macro allows adding of non- standard text and symbols to images. Options for font, style, size and colour		
LUT	Set LUT to wavelength colour	This macro allows setting the look-up table colour of non RGB images to the colour represented by wavelengths		
Make LUT	Create a LUT selecting colour from an RGB image	This macro can create and save a lookup table from selecting a colour pixel in an RGB image.		
INV	Invert Intensities	This macro Inverts the intensity values of the image. Works on all image formats except 32 Bit images. Useful for thresholding some images for tracking.		
×	Point Spread Function	This macro uses the OrthoSlice command to create a single "best" orthogonal view of a PSF. It will also create a scaled-up image. Dialogue options are available to add a scale bar on the final X-Y image.		

Heat Map	Heat Map from results table	This macro will generate a custom heat map from a results table. There are dialogue options for format, result parameters and LUT
Plot	Quick Data Plot	This macro will create a graph plot from an open data table. There are options to select the X and Y data, plot type, colour and axis titles as well as general graph options
from data	Frame by Frame plot from data	This macro will create a frame-by-frame plot from a results or data table. There are options to select X and Y data and line colour
G1 G2	Difference of Gaussians (edge detection)	This macro will create 2 Gaussian 32-bit images and then subtract 1 from the other - good for finding edges. Options to set the Sigma value for each image and whether to use scaled units. Output is a 16-bit image.
Stack Save	Save individual images from a stack	This macro will save images from a stack naming them sequentially from 001. Options to select the new name and the location of the saved images
Spark Meta	Time Info Spark Image (LSM format)	This macro will read the time interval and extract the time frame data from a line scan LSM file format image. NB use the plugins link to the Bio-Formats Importer when opening the image not "File Open".