Jacqueline Ross,
jacqui.ross@auckland.ac.nz

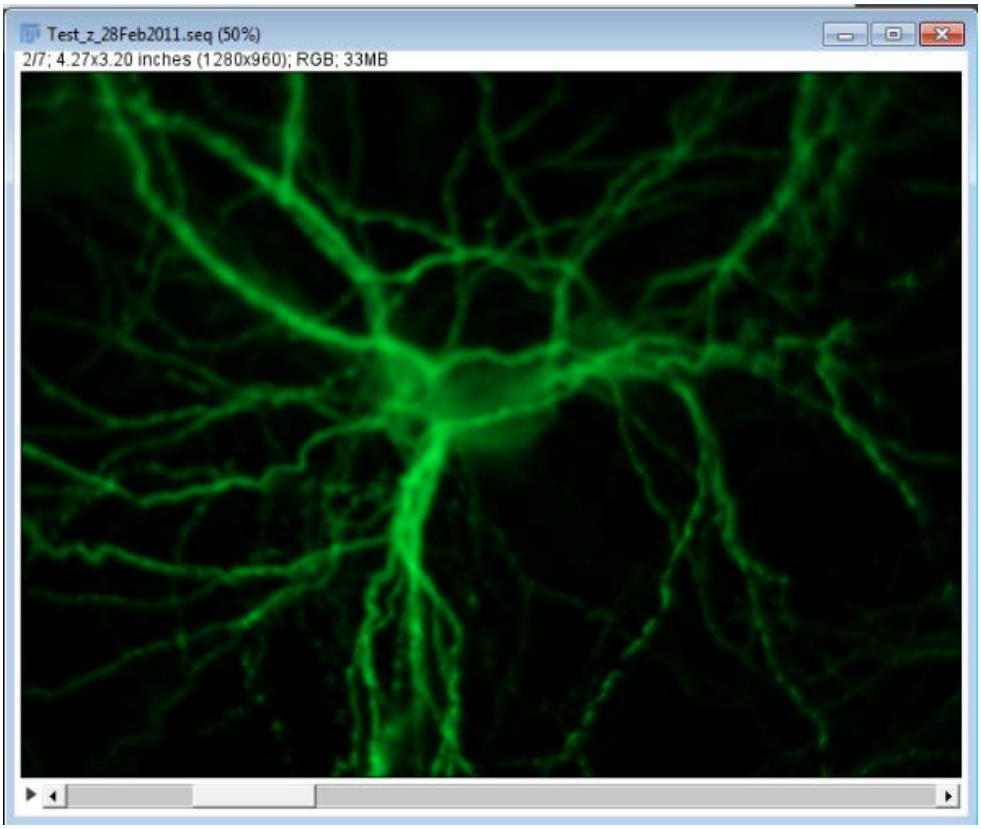
MICROSCOPY NEW ZEALAND INC. CONFERENCE WORKSHOP 2017

Working with stacks using Fiji 31 January 2017

1. Image – Stacks menu

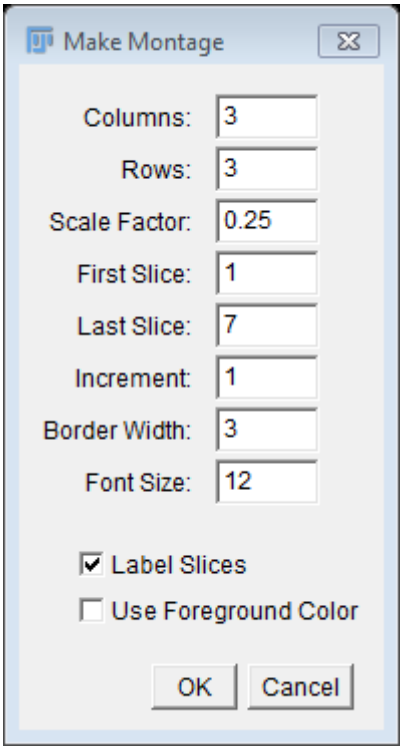
Example 1 – Widefield fluorescence microscopy

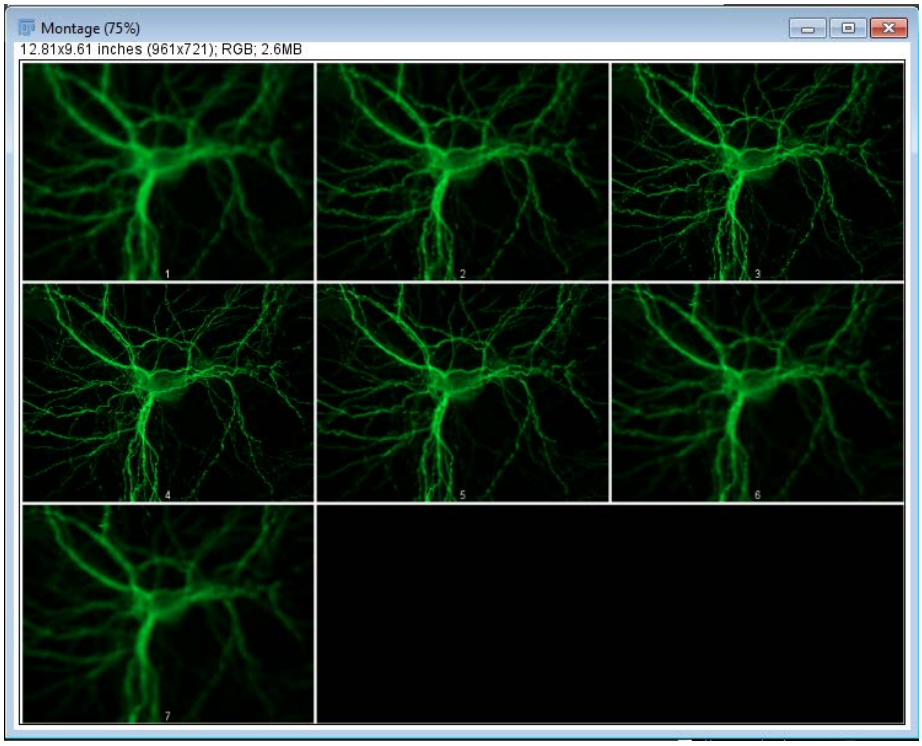
1. Open the file called **Test_z_28Feb2011.seq** (inside the **Light Microscopy-Neuron folder**);



2. Use the scroll bar at the bottom of the image to look through the images. Click the arrow head to make the series play automatically. Or use the left/right arrows on the keyboard.
3. Go to ***Image – Stacks – Make montage*** to create a gallery image of the stack. This can be saved as an image file. This can be used for any stack of images and is also useful for creating thumbnails from a set of images.

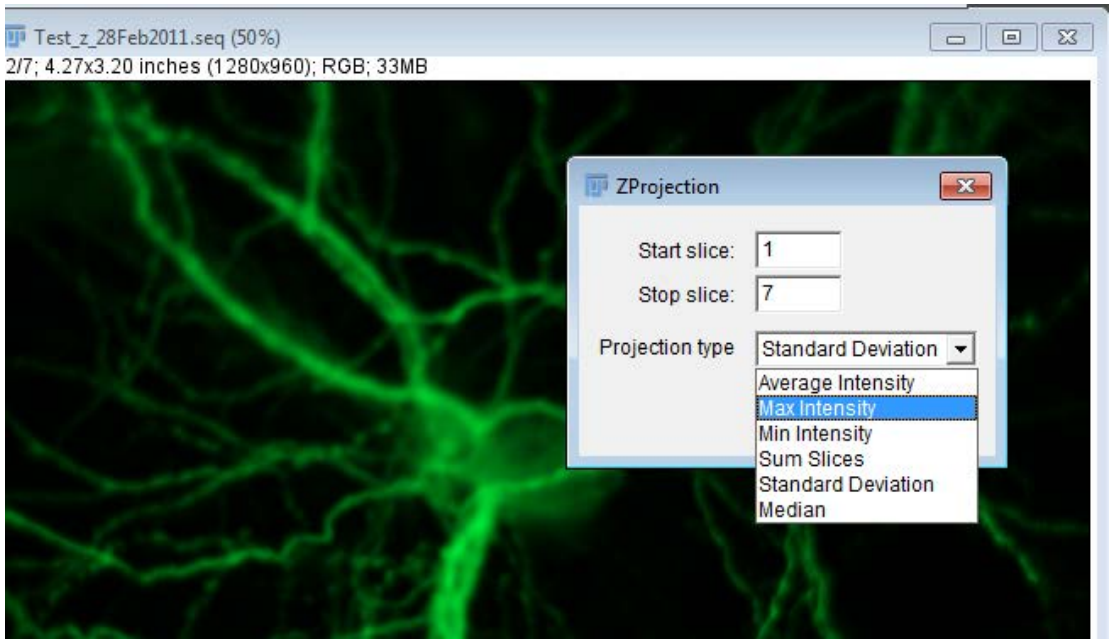
4. Select the options you want and click **OK** to create the montage.





5. Close the montage and select the stack again.
6. Go to ***Image – Stacks – Z Project*** *to* create a z series projection file. Try out the different options. Note that you can choose to delete any slice from the stack by ***Image – Stacks – Delete Slice*** if you think that will improve the projection***.***

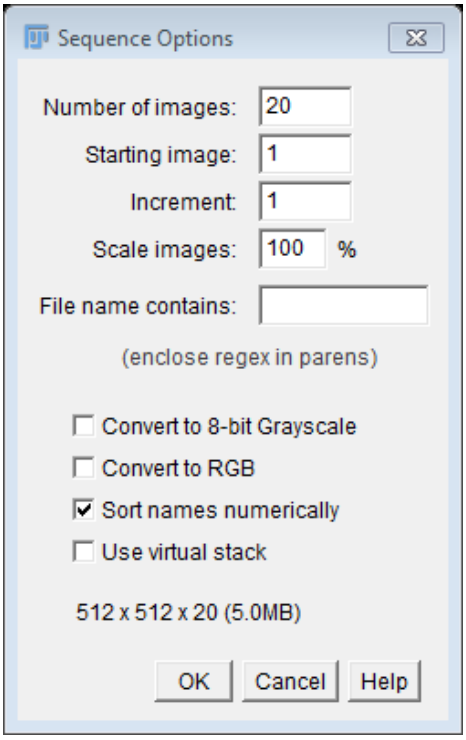
   Which one works best for this data set?



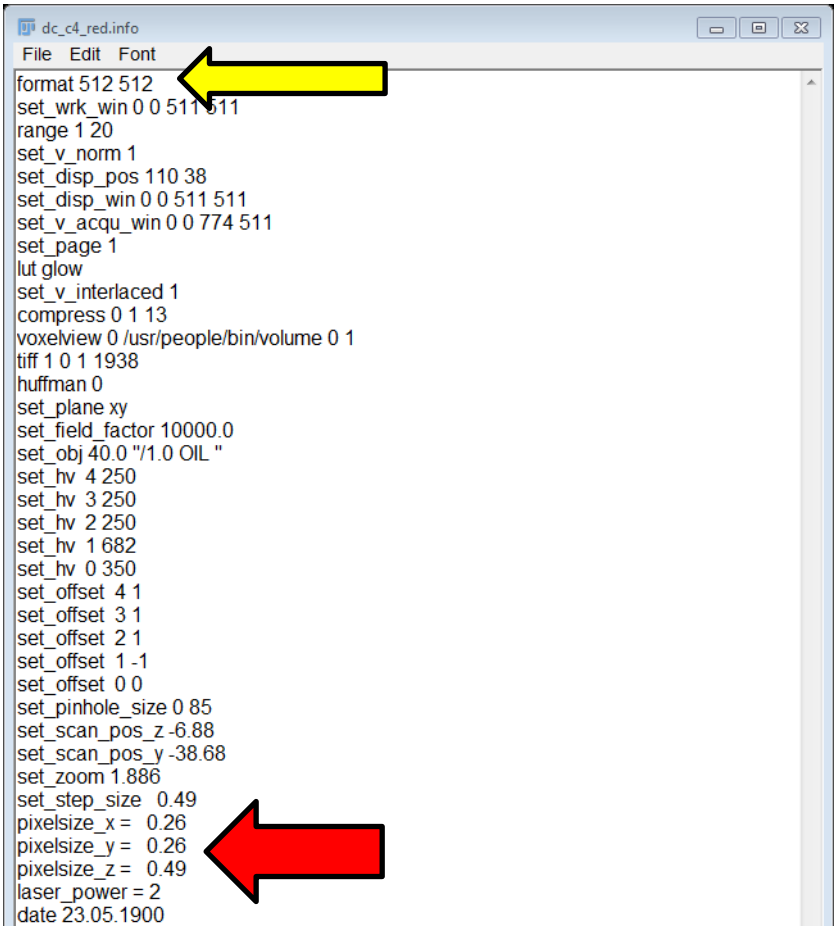
7. Now try out the **Extended Depth of Field** macro. You will find it under the **Plugins** menu. How does it compare with the Z Projections?

Example 2 – Confocal microscopy

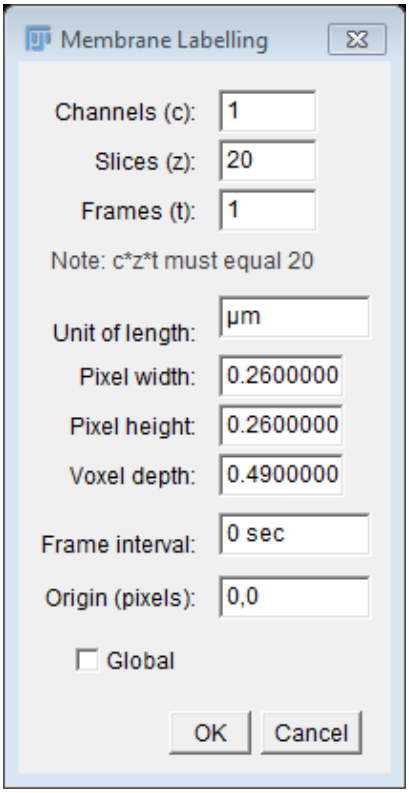
1. Go to ***File – Import Image Sequence***, select the first TIFF file of the data set inside the **Membrane Labelling** folder/dataset (=**dc_c4_red01.tif**) and click **Open**;
2. Make sure the number of images in the stack (**20**) is correct and click **OK**.



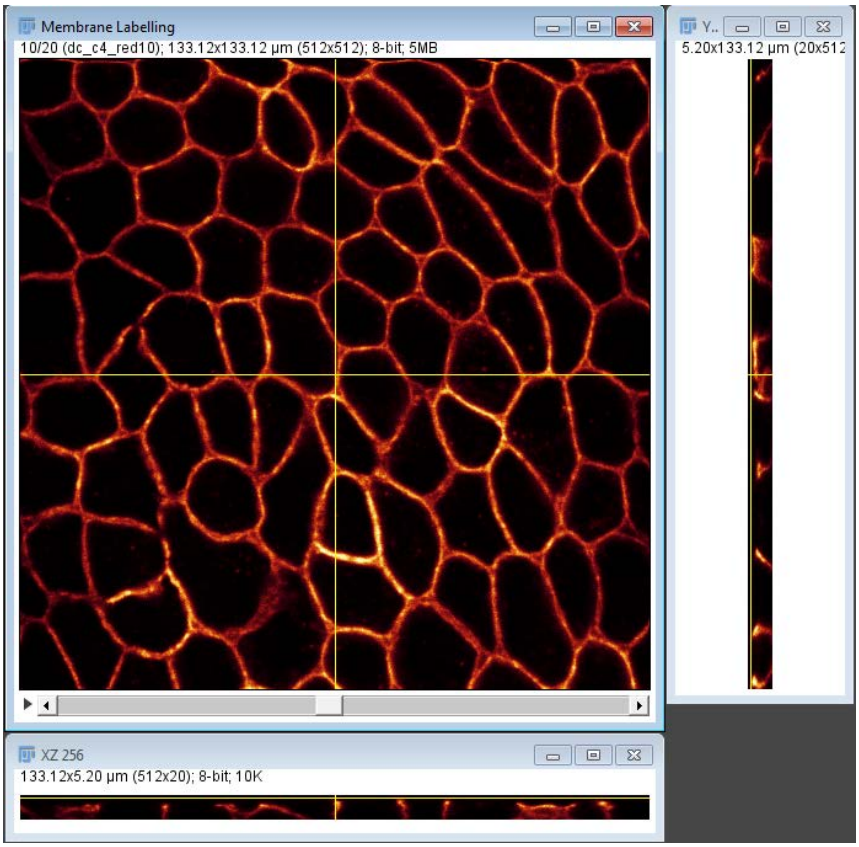
3. Open the file called **dc_c4_red.info.** This information file is used to calibrate the stack. Note that the format is 512 x 512 pixels and the pixel sizes are x = 0.26; y = 0.26; z = 0.49. These values are in microns.



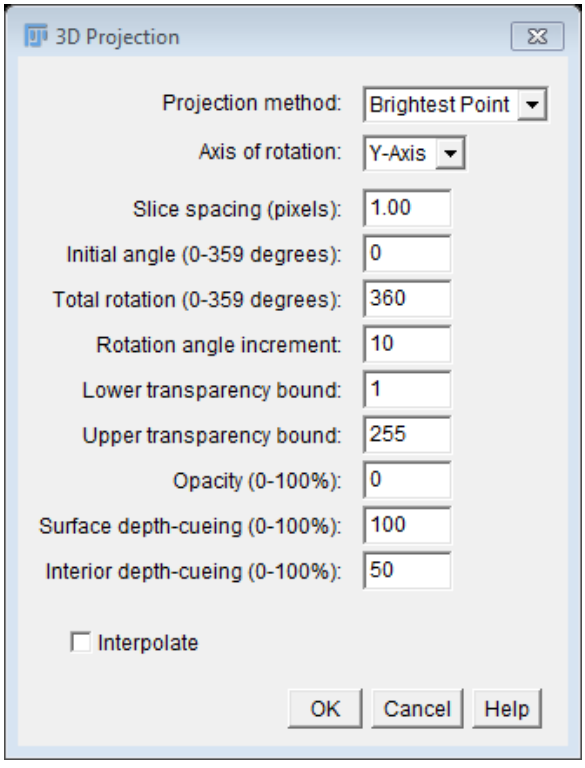
4. To calibrate the stack, go to ***Image – Properties*** and enter the **Pixel width**, **Pixel height** and **Voxel depth** 0.49um. Then click **OK**.

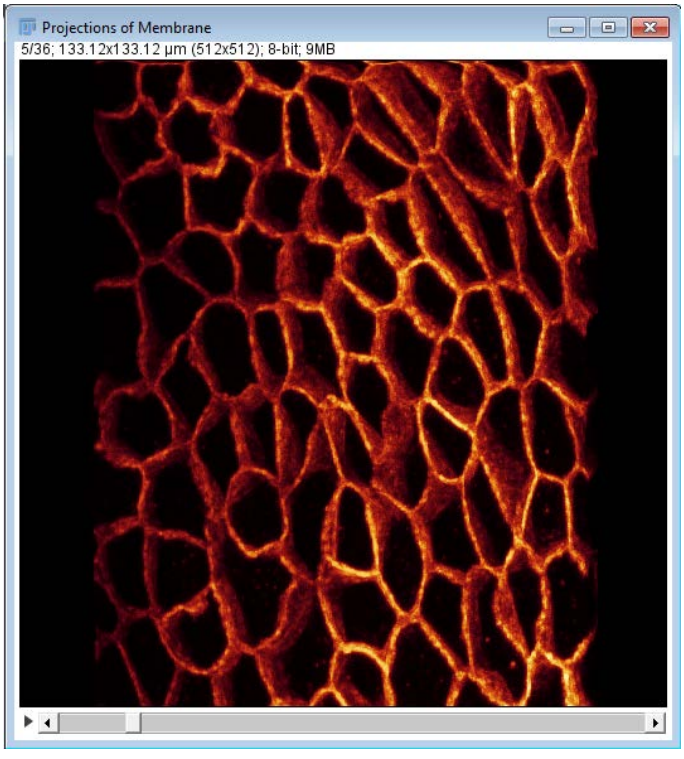


5. Go to ***Image – Stack – Orthogonal Views*** to get a cross-section view of the stack (XZ and YZ views). You can move the position of the cross and also move through the XY stack to get different views through the stack. This option is good for looking for structure/distribution/colocalisation.

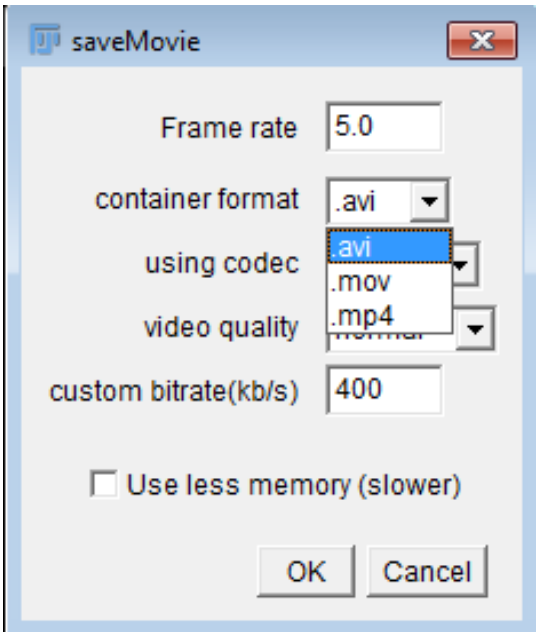


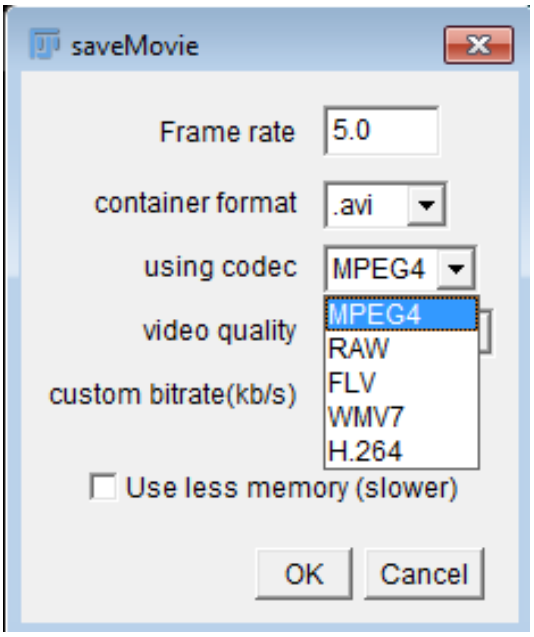
6. Close the XZ and YZ windows.
7. Try out the z projection options for this stack. Which works best?
8. Go to ***Image – Stacks – 3D Project***. Use the settings below. Click **OK** to create the animation.





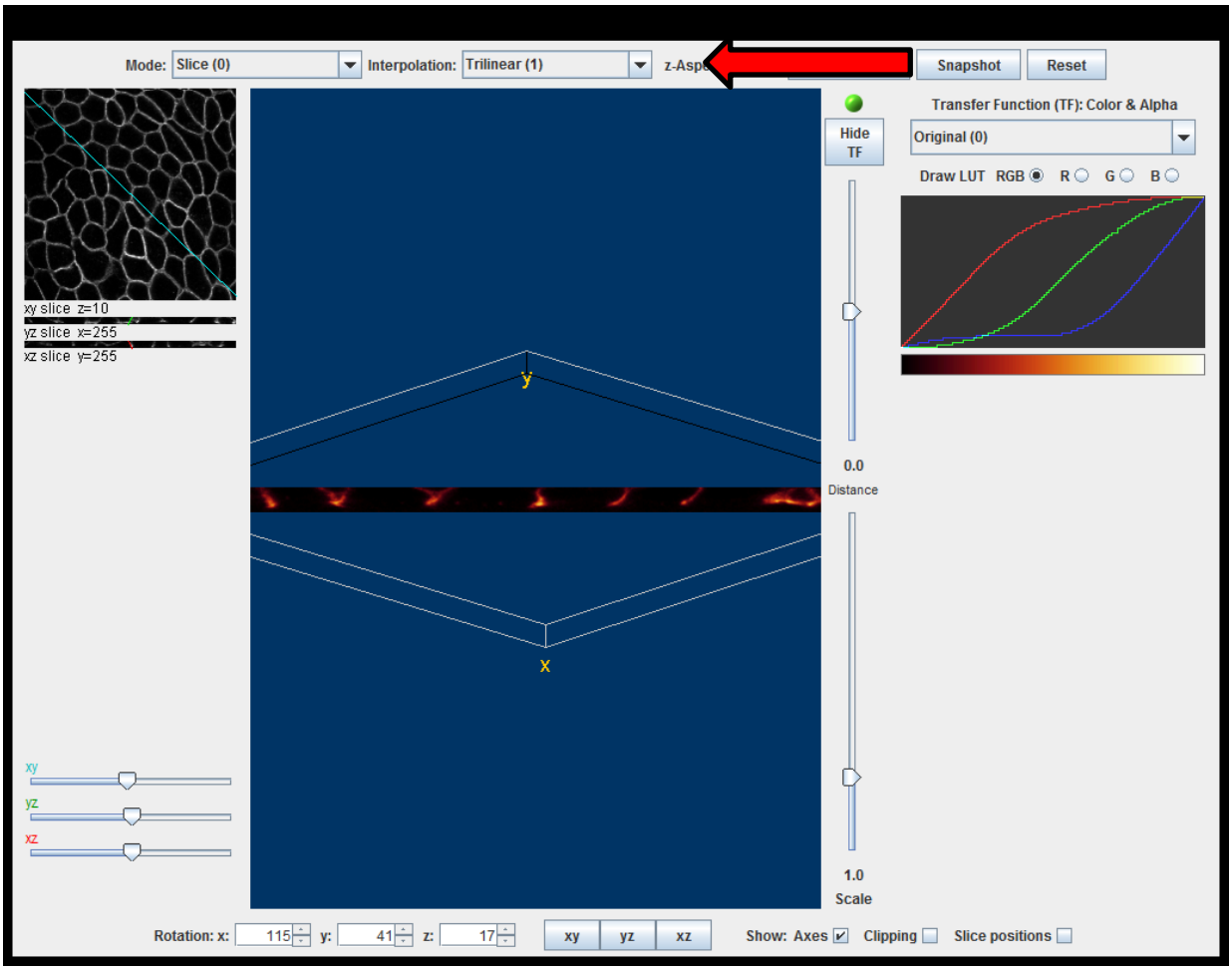
9. Click the **Arrow head** to play through the animation. If you want to change the speed, go to ***Image – Stacks – Tools – Animation Options*****.** If you want to save the animation as a movie, go to ***File – Save As AVI***.
10. Or go to ***File – Save As Movie***. This provides additional output options, AVI, MOV or MP4. It also has compression options and allows you to specify the frame rate (speed) and quality for the movie.



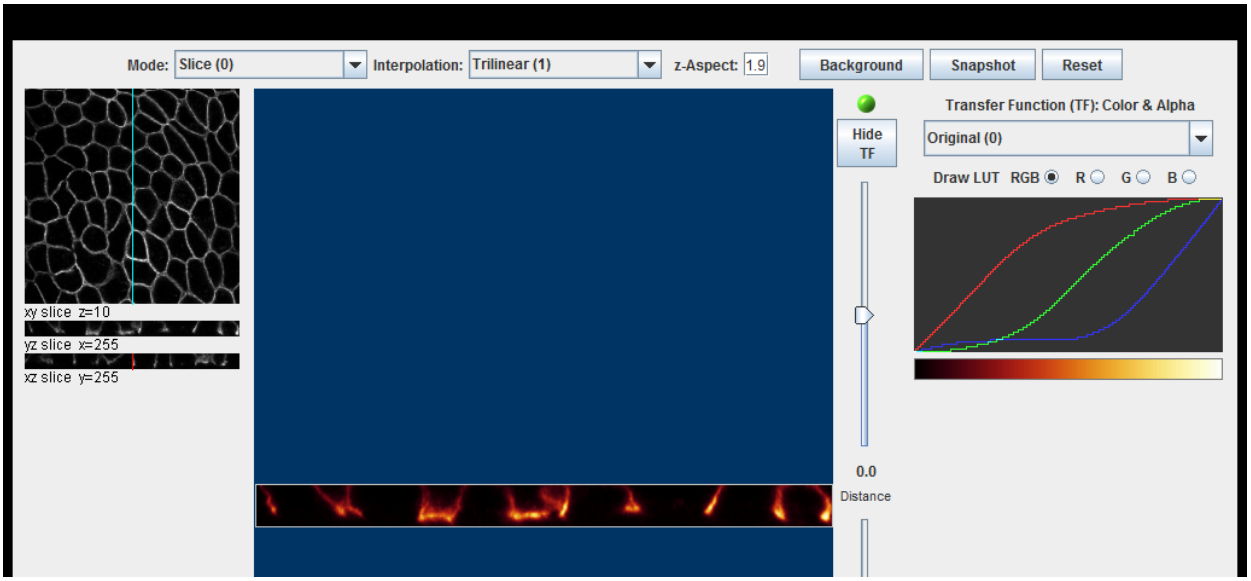


1. Plugin – Volume Viewer

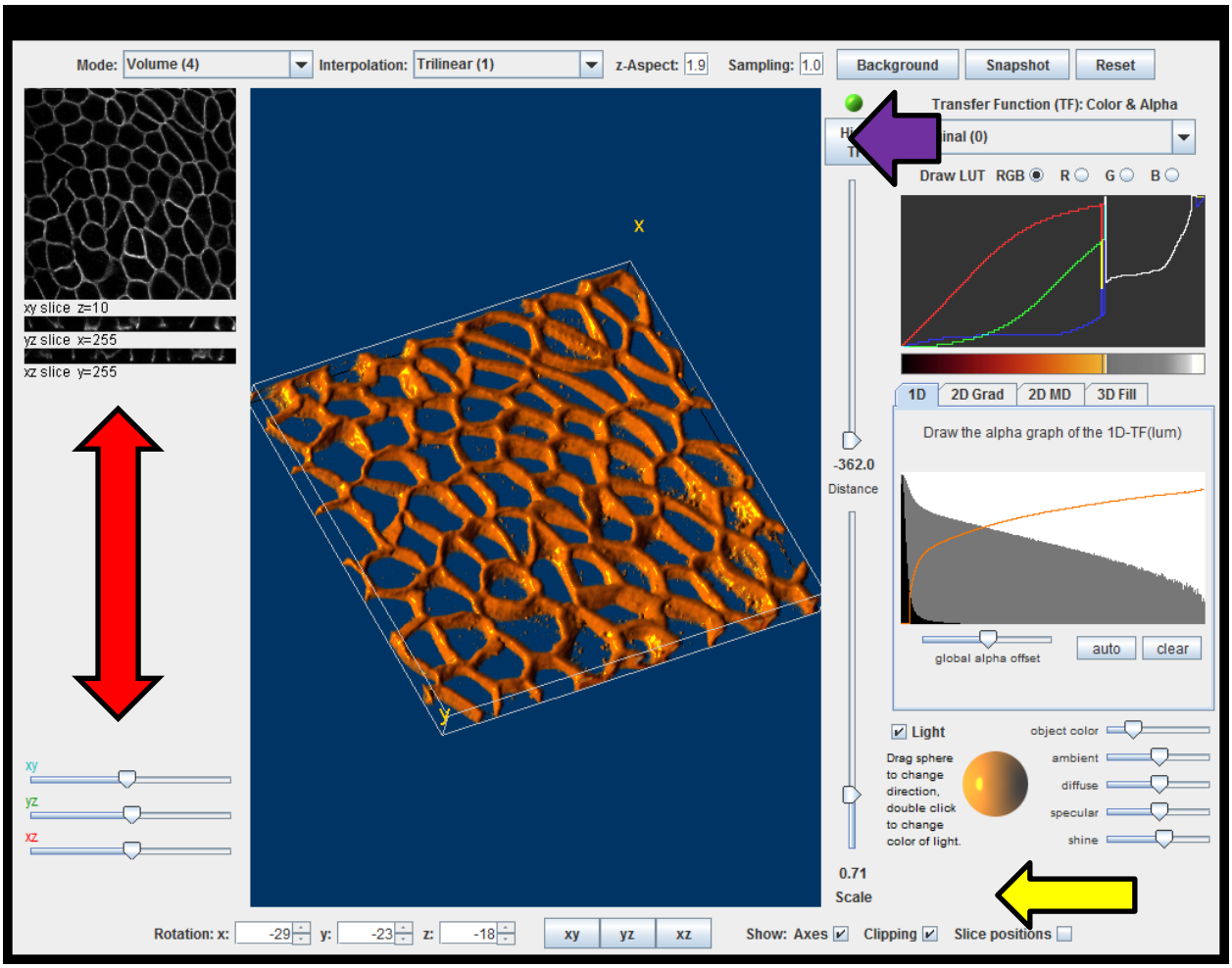
1. Go to ***Plugins – Volume Viewer***;



2. Choose the view by clicking on the buttons at the bottom – XY, YZ, XZ.
3. Make sure that the **Z-aspect** is correctly displayed (1.88). If not, change the **Z-Aspect** to 1.9 (X, Y pixel size = 0.26um, Z = 0.49um) so that the correct depth is being displayed.



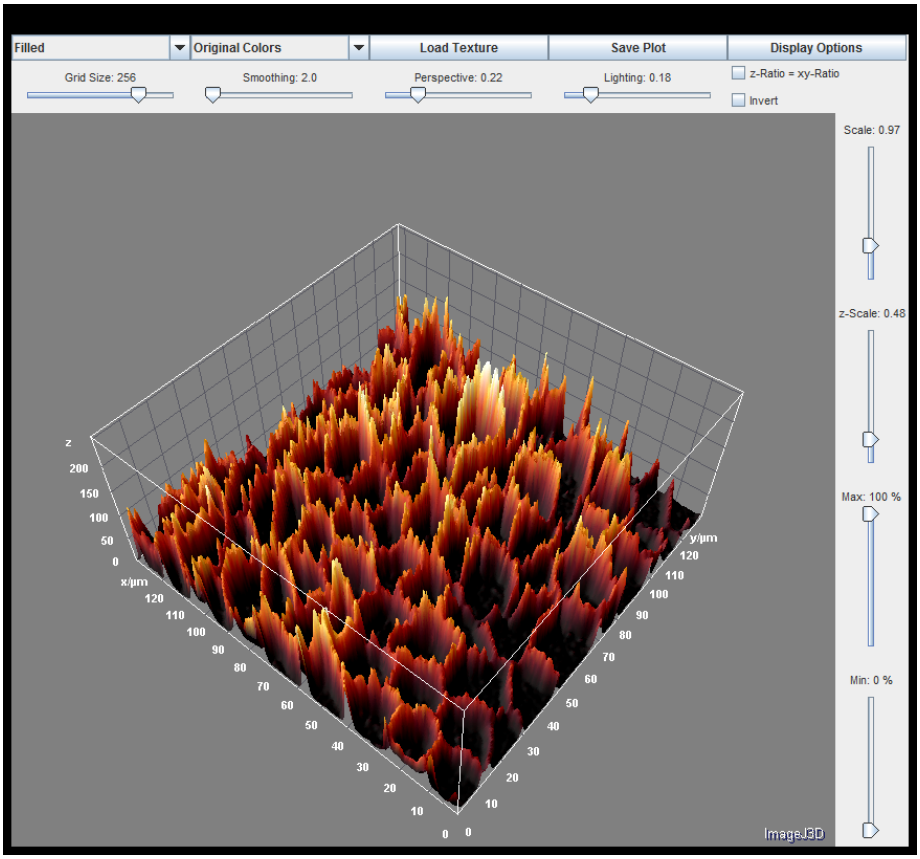
4. Try out the different **Modes** and **Interpolation** methods. You can also use different LUTs and change the **Background** colour. Lighting effects are available in the bottom right-hand corner (yellow arrow).
5. Click **Hide TF** to hide the options on the right-hand side. Click **Snapshot** to create an image.
6. The bottom left-hand side controls (red arrows) are for navigation through the stack, shown by the grayscale images at the top.



7. Close the **Volume Viewer** window.

2. 3D Surface Plot

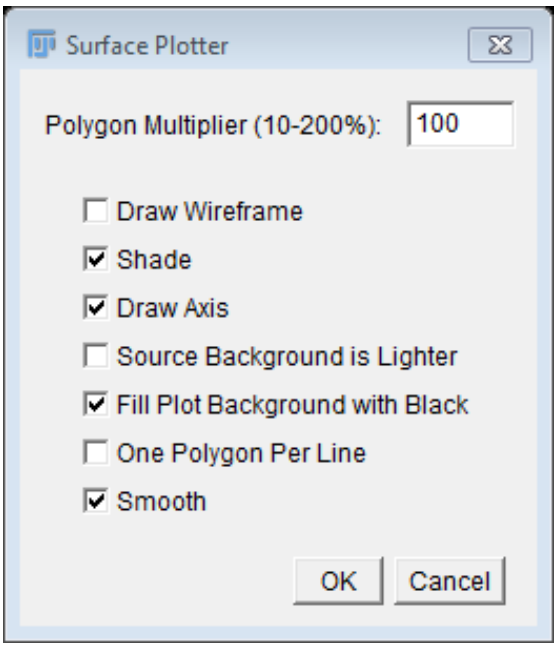
1. Go back to the original dataset. Move through the slices until you are at slice 9.
2. Go to ***Analyze – 3D Surface Plot***. There are lots of options here to optimize the display of your dataset, such as different LUTs, smoothing, perspective and lighting.
3. If you want to save an image, click **Save Plot.** Try out the different options with this dataset.



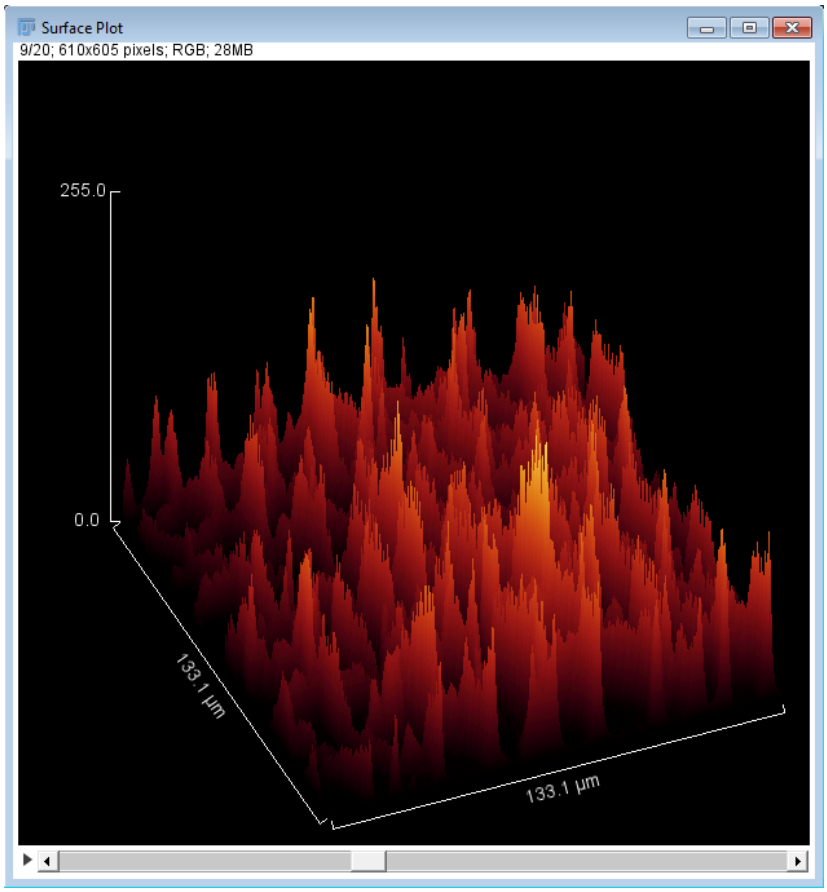
4. Close the **3D Surface Plot** window.

3. Surface Plot

1. Using the same data set, go to ***Analyze – Surface Plot.*** This creates a series of surface distribution images of your labelling.



2. The **Surface Plot** can be saved as an image sequence or a movie. You can also save individual images.



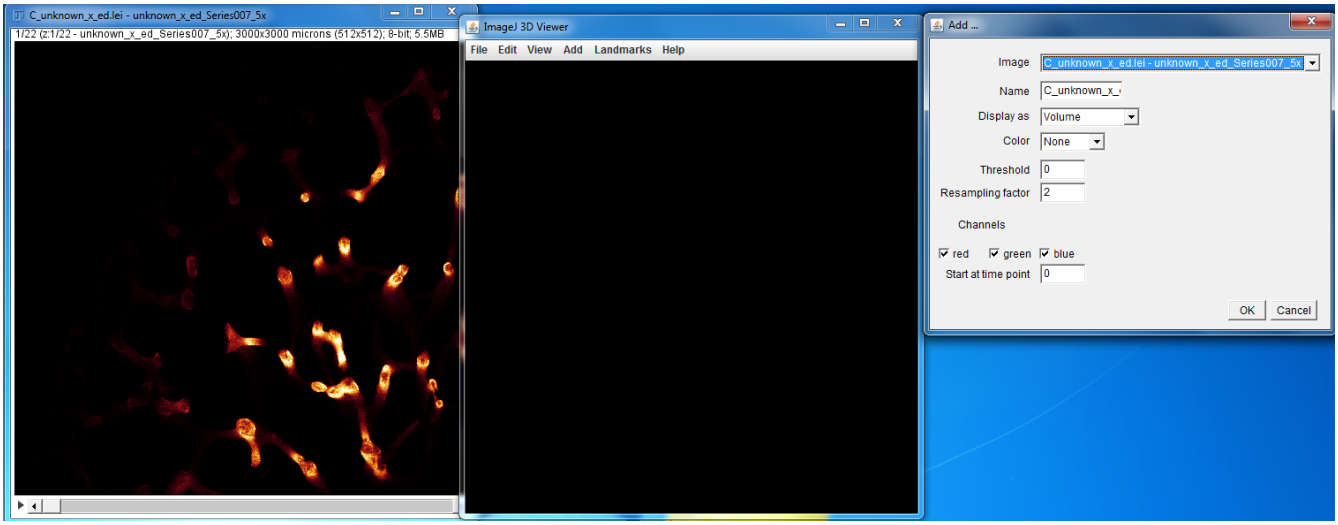
3. Close the **Surface Plot** window.

4. 3D Viewer

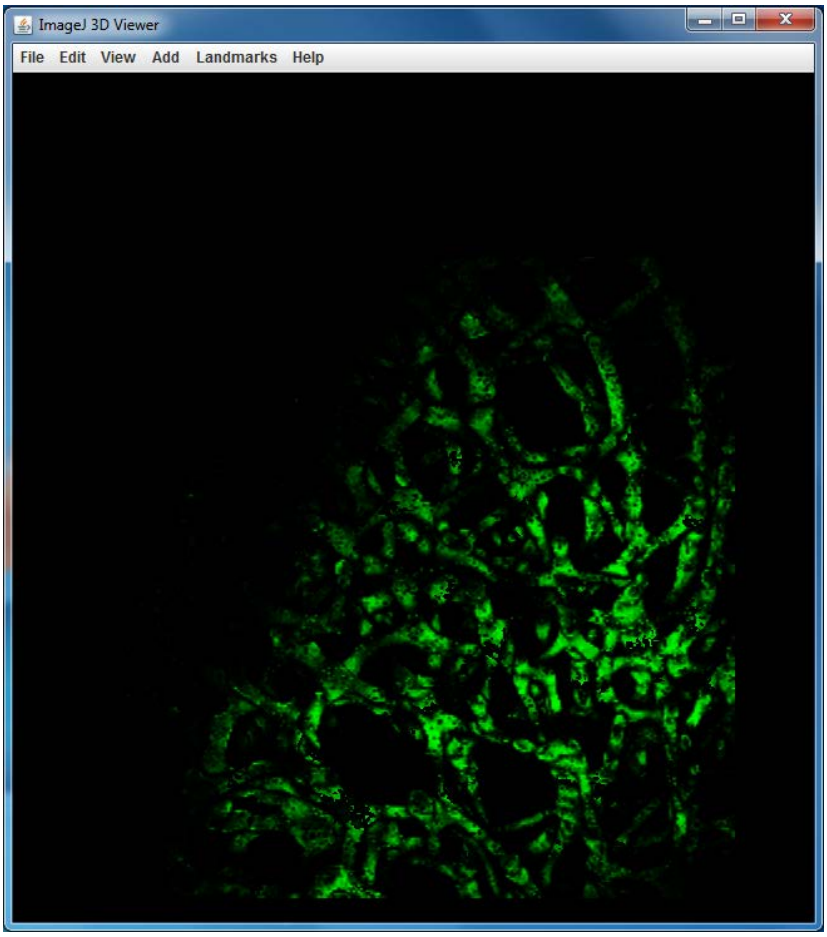
- Published article and instructions on USB stick.
- Used for visualization, volume measurements, etc.

- Located under **Plugins** menu.

Open the data set called **C_unknown_x_ed.lei.** Select the highest resolution option. Then launch the **3D Viewer** (**Plugins – 3D Viewer**);

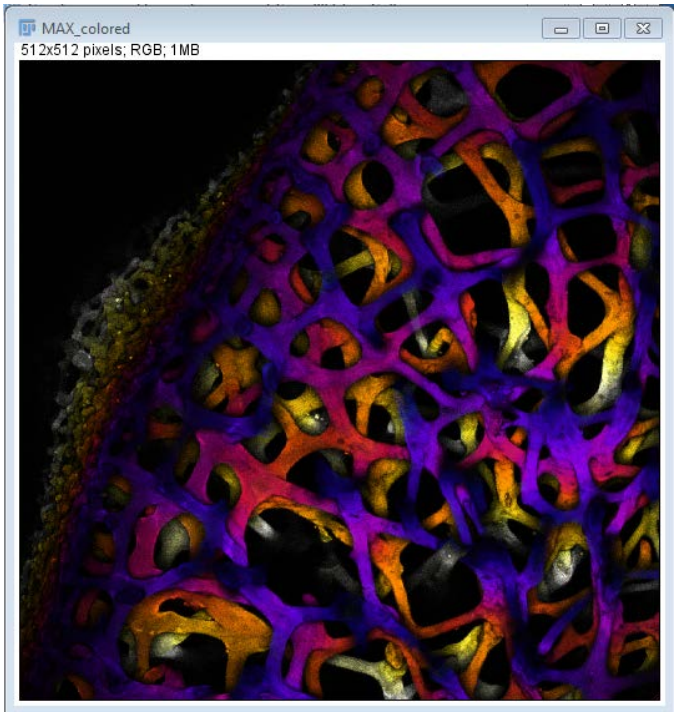


Select the options you want and click **OK**.



5. Temporal Colour Coding

1. Go to ***Image – Hyperstacks – Temporal Color Code*** and choose from the **Look Up Tables** listed, e.g. Fire. This will treat the z stack as a time series so you get different colours for different depths = depth-coded projection.



6. Extended Depth of Field plugins

Two versions – **Complex wavelet-based** method (Easy and Expert) and **Model-based** method

Plugins available here: http://bigwww.epfl.ch/demo/edf/index.html

7. Extended Depth of Field Macro

Author – Richard Wheeler

Available here

Creates a focused image from a widefield fluorescence stack.

Installation – place into **Plugins** folder

8. Stack Contrast Adjustment plugin

Authors – Jan Michalek, Martin Capek, Jiri Janacek

Adjusts for drop off in signal through a z stack

Available here

Documentation and demo stacks also available on the website

Installation – place into **Plugins** folder

9. Plane Brightness Adjustment Plugin

Authors – Jan Michalek, Martin Capek, Jiri Janacek

Available here

Adjusts for uneven intensity in XY planes, e.g. darker corners.

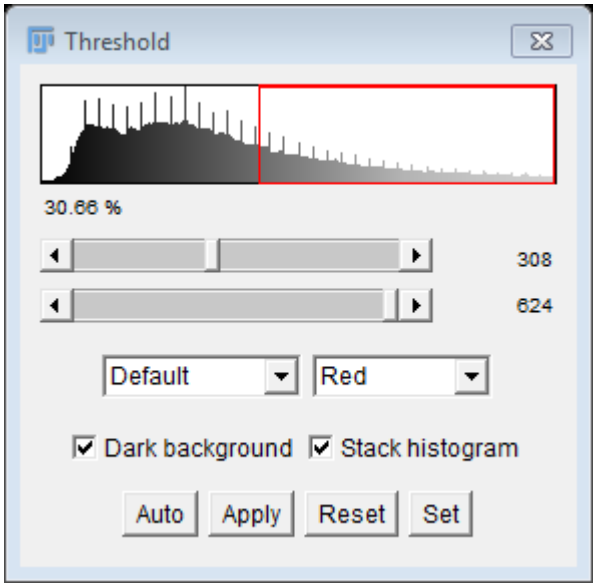
Documentation and demo stacks also available on the website

Installation – place into **Plugins** folder

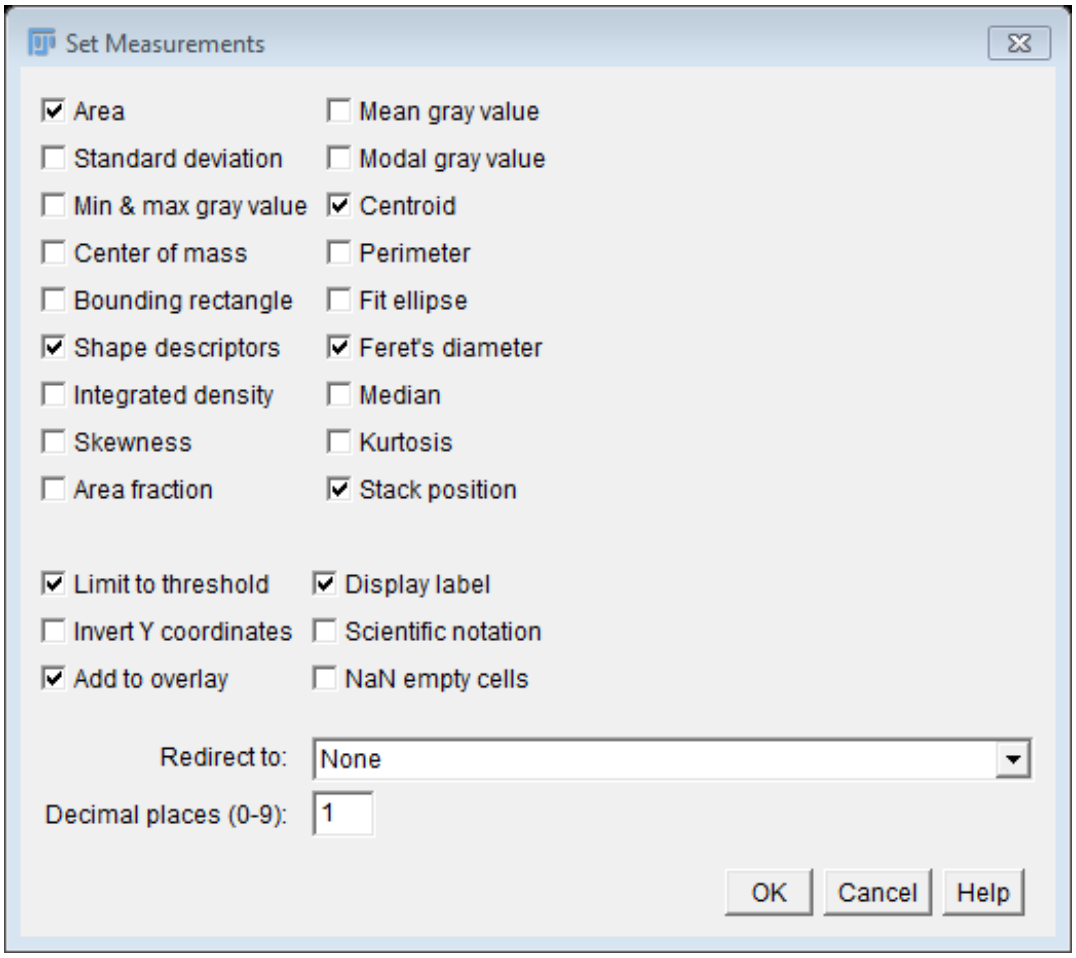
10. Analyzing Stacks as individual slices

- Generally, the same methods are used for measuring stacks as for individual images.

1. When thresholding the images, you select **Stack histogram** as below;



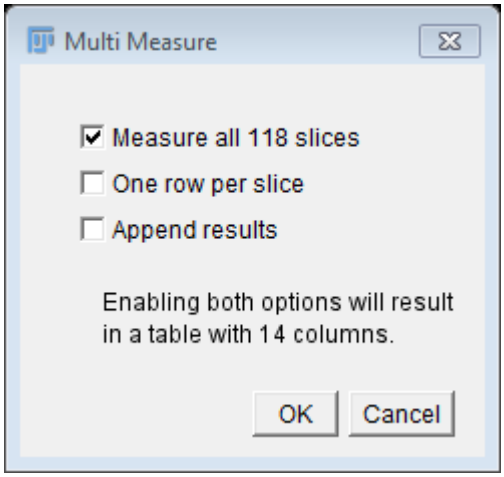
2. The parameters you want to measure are selected under ***Analyze – Set Measurements***. Make sure that you select **Stack position**;



3. When you make measurements of a stack, you use the **Measure Stack** macro instead of ***Analyze – Measure***. This macro can be installed as a plugin in your **Plugins** menu.

4. If you are using the **ROI Manager**, then you go to ***More – Multi Measure*** to measure all of the images. You could also use this option by creating a selection of the entire image and adding it into the **ROI Manager**.

5. Select **Measure all slices** and click **OK.**

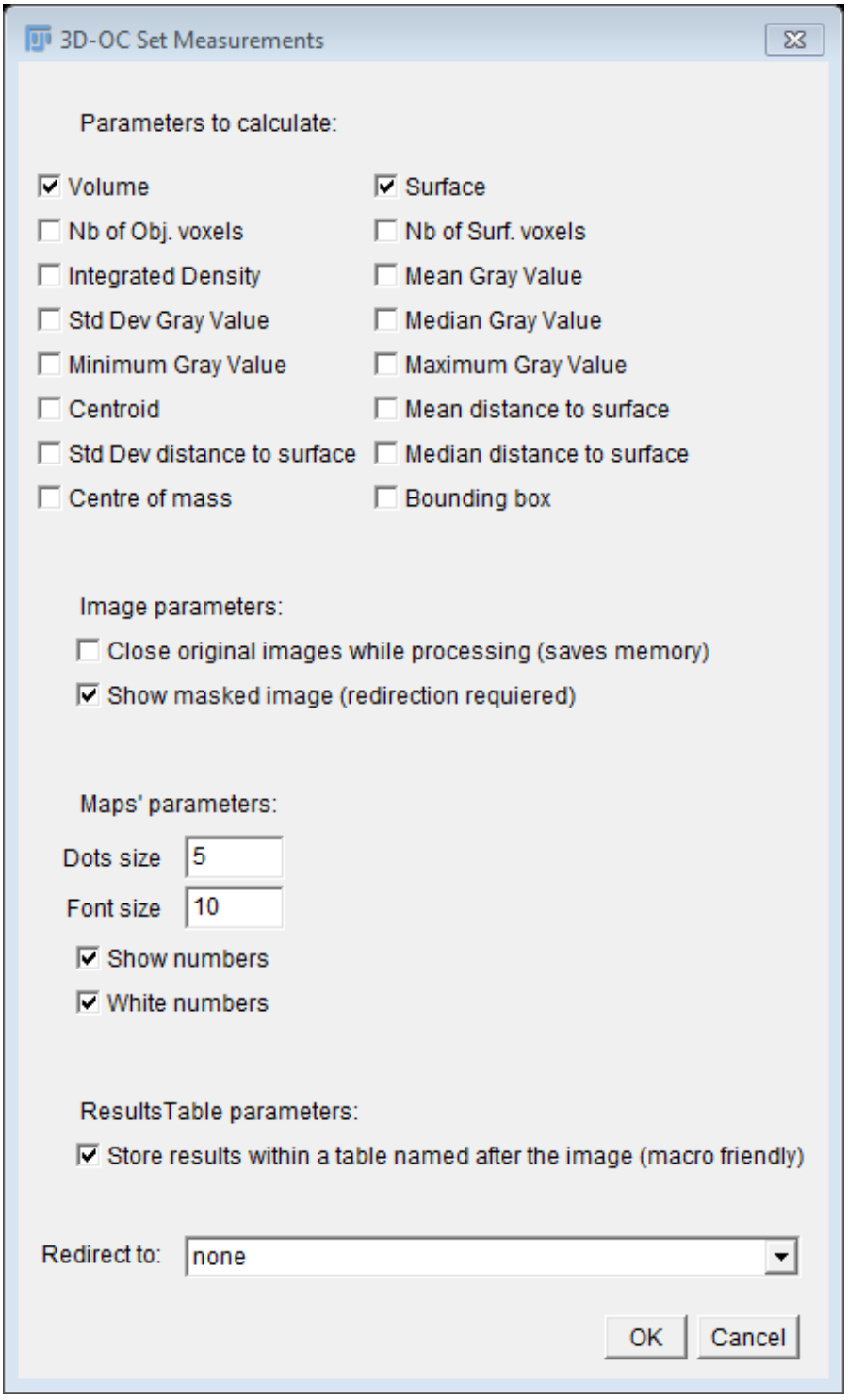


1. The **Label** field will include the name of the stack, the ROI name and the slice name.

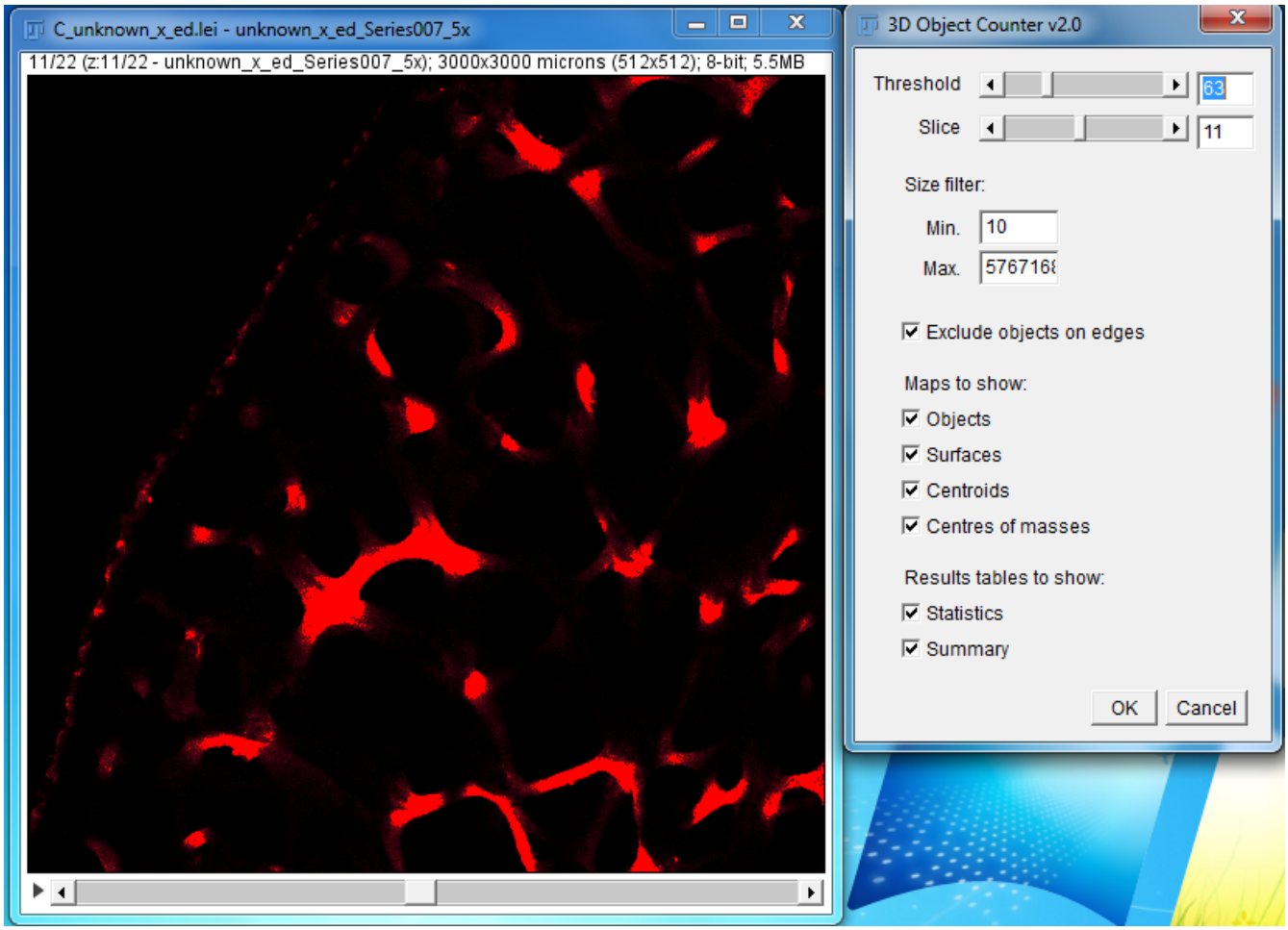
11. 3D Objects Counter

- Located under **Analyze** menu.
- Documentation here

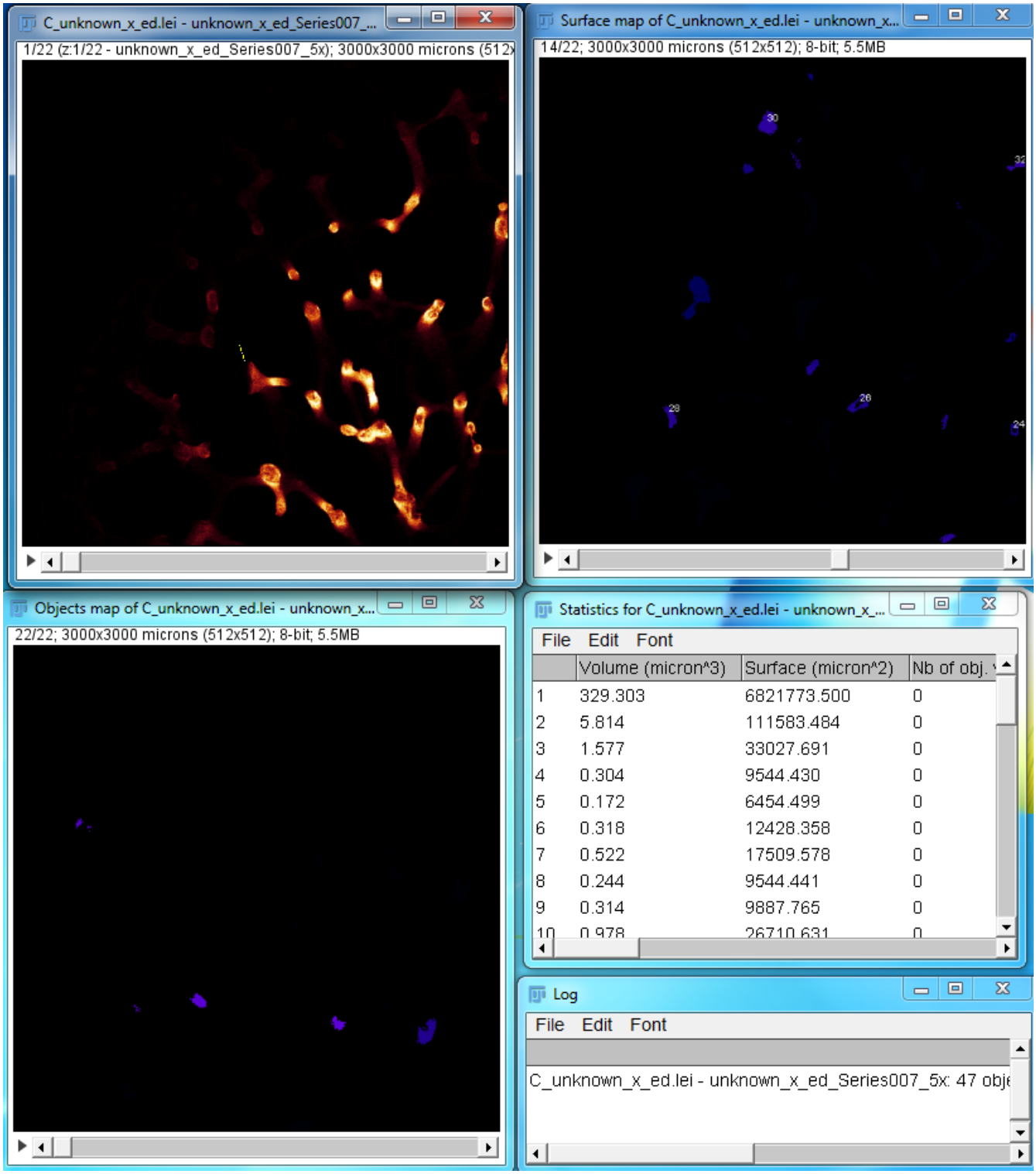
1. Go to ***Analyze - 3D OC Options*** to select the options you want;



2. Then go to ***Analyze – 3D Objects Counter***;
3. Threshold the image and select the options you need;



4. Click **OK** to run the analysis.


C_unknown_x_ed.lei - unknown_x_ed_Series007_...
1/22 (z:1/22 - unknown_x_ed_Series007_5x); 3000x3000 microns (512x
Surface map of C_unknown_x_ed.lei - unknown_x...
14/22; 3000x3000 microns (512x512); 8-bit; 5.5MB
Objects map of C_unknown_x_ed.lei - unknown_x...
22/22; 3000x3000 microns (512x512); 8-bit; 5.5MB
Statistics for C_unknown_x_ed.lei - unknown_x_...
File Edit Font
Volume (micron^3) Surface (micron^2) Nb of obj.
1 329.303 6821773.500 0
2 5.814 111583.484 0
3 1.577 33027.691 0
4 0.304 9544.430 0
5 0.172 6454.499 0
6 0.318 12428.358 0
7 0.522 17509.578 0
8 0.244 9544.441 0
9 0.314 9887.765 0
Log
File Edit Font
C_unknown_x_ed.lei - unknown_x_ed_Series007_5x: 47 obj