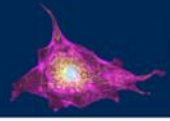
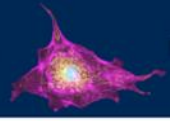


Introduction to image processing and analysis with ImageJ / Fiji. Part 6

Colocalisation, cell tracking, 3D analysis, deconvolution & other software

Course by Dale Moulding



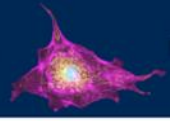
Session 6

30 minute lecture
15 minutes exercise

Learning objectives:

- **Explain the difference between colocalization and co-expression**
- **Track moving objects**
- **Perform segmentation in 3D datasets**

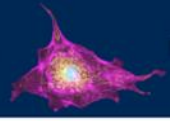
- **Access other useful (free) software**



Colocalisation v co-expression

https://imagej.net/Colocalization_Analysis

- Identifying double stained cells is not measuring colocalisation
- Double stained cells are identified as overlapping objects
- Colocalisation analysis is a measure of the degree of overlap & the relationship in intensity between two channels.
- It is often measured as Pearson's correlation coefficient and Manders split coefficients.

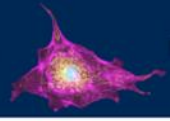


Colocalisation v co-expression

https://imagej.net/Colocalization_Analysis

- Pearson's gives a measure of the intensity relationship between 2 channels.

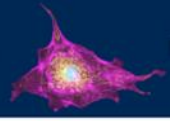
  1 = perfect correlation, 0 = n correlation, -1 = perfect exclusion
- Measured in every pixel (or voxel) of an image. Perfect colocalisation may be expected between 2 subunits of a protein complex.
- SubUnit-A & SubUnit-B are always found together in cells. If there is a lot of A in a particular pixel, there will be an equivalent amount of B. As the intensity of one Subunit rises or falls, the other does so to exactly the same degree.



Colocalisation v co-expression

https://imagej.net/Colocalization_Analysis

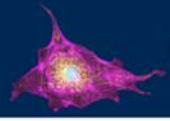
- Manders gives in indication of the amount of fluorescence (above a pre-defined background level) in each of 2 channels that is found in the same place as the other channel.
- It does not measure the relative amounts of each channel, rather it gives a value from 0 to 1 indicating the fraction of Channel A signal that overlaps with Channel B & vice versa. The values for each channel are likely to be different.
- The value given is not a statistical measure. You need to measure multiple images for a statistical analysis.



Colocalisation v co-expression

https://imagej.net/Colocalization_Analysis

- ImageJ / Fiji plugins Coloc 2 & JaCoP can be used for both Manders and Pearsons analysis.
- Great care must be taken in these measurements. Image noise, resolution, background, intensity (too bright / too dim) etc etc can all have a massive impact on the analysis.
- Get expert help!
- Read the link above very carefully.

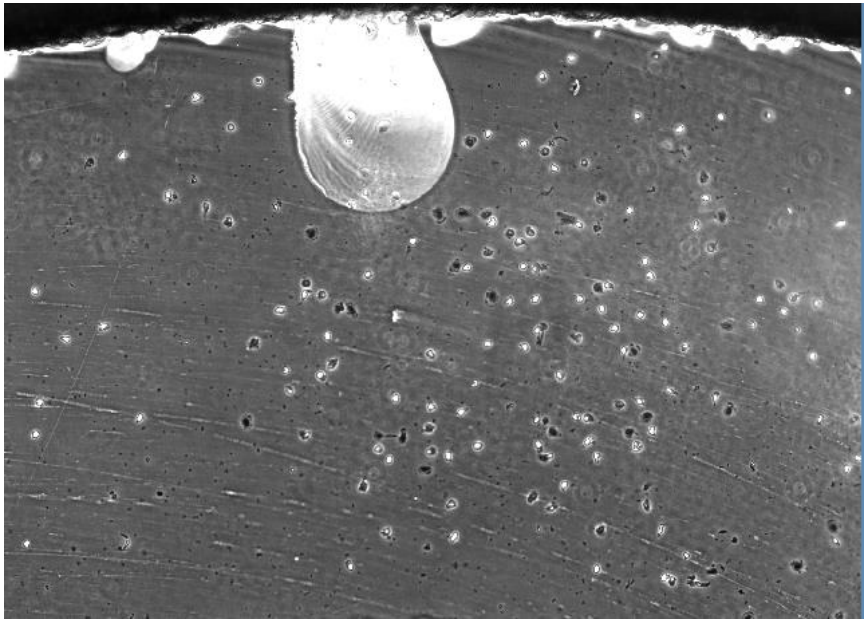


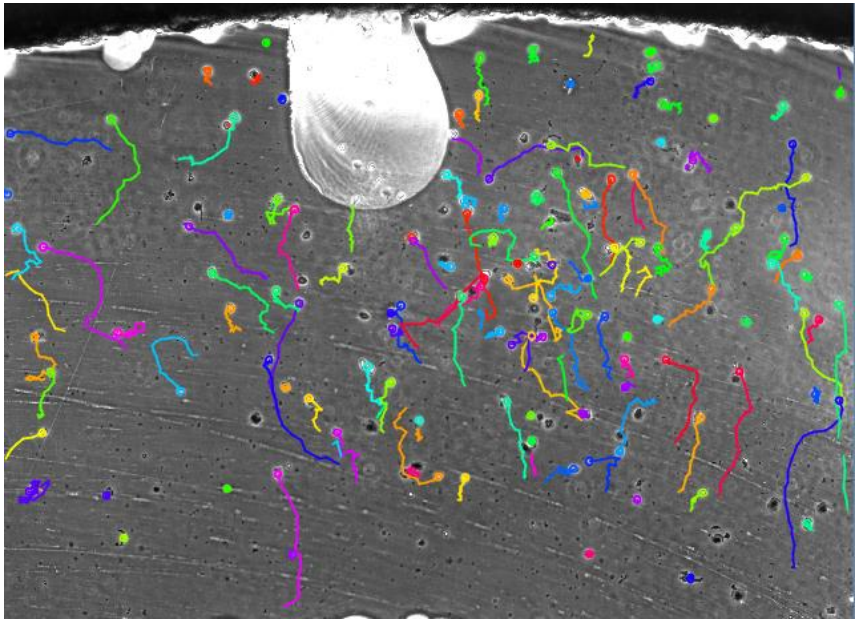
Colocalisation v co-expression

https://imagej.net/Colocalization_Analysis

- Co-expression: you get a count of the proportion of objects that overlap between two different channels
- Colocalisation: you get an indication of the interaction between two channels, either as the degree of agreement between the intensity in each channel (Pearson's) or the degree of overlap in signal between two channels (Manders).

Object tracking in time-lapse imaging





Objects (cells, organelles etc) can be tracked over time, to measure their movement.

- Speed – steady? variable?
- Direction
- Tortuosity

Object tracking in time-lapse imaging – Image registration

- Multi-point time-lapse imaging may have image drift. The stage / sample may move slightly between positions.
- This can be corrected: Image registration

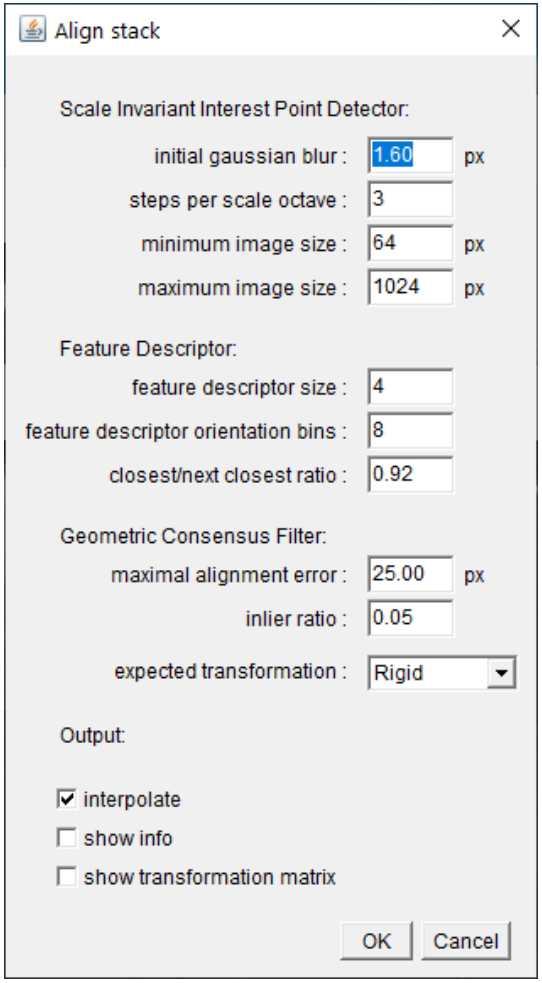
Plugins / registration >

Linear Stack Alignment with SIFT

Or

Plugins / registration > StackReg

(Big-EPFL update site)



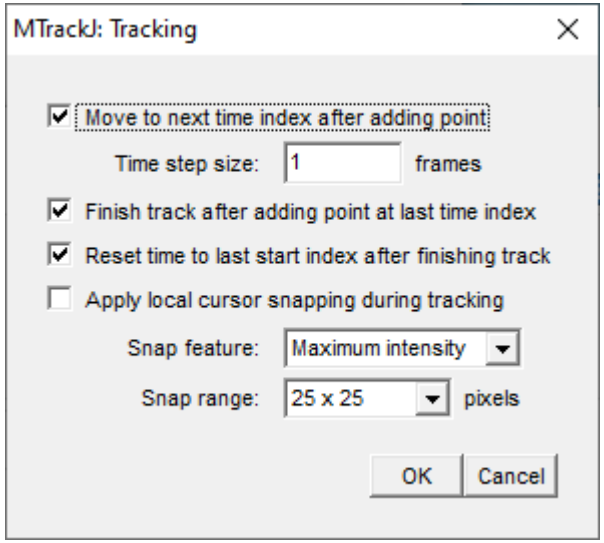
Object tracking in time-lapse imaging

https://imagescience.org/meijering/software/mtrackj/

Plugins / MTrackJ

(requires ImageScience update site in Fiji)

Extremely well documented on the website.



Manual tracking. Click an object, the image advances one frame click again, etc etc…

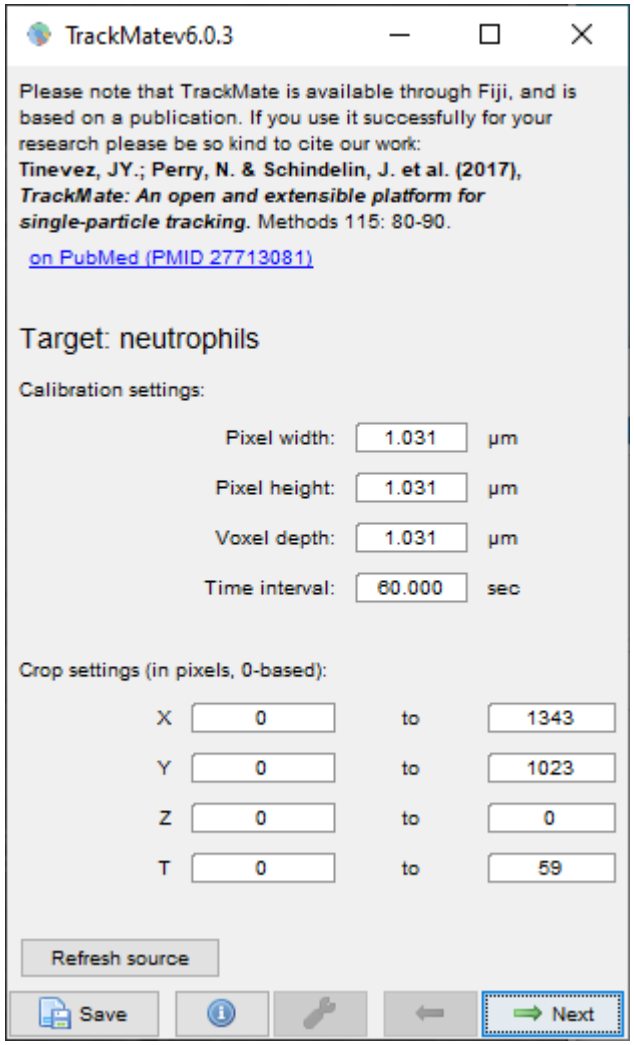
Object tracking in time-lapse imaging

https://imagej.net/TrackMate

Plugins / Tracking / TrackMate

Extremely well documented on the website.

Automatic tracking. Follow the step by step interface, fine tune the parameters to detect objects.

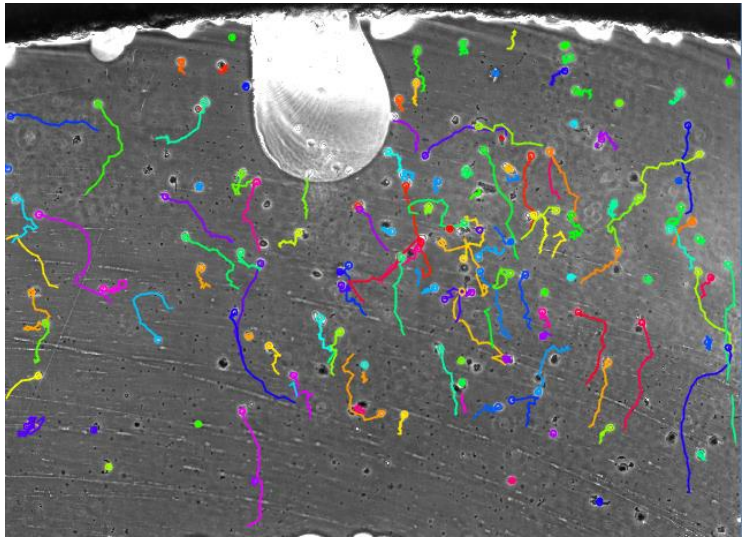


Object tracking in time-lapse imaging

http://icy.bioimageanalysis.org/plugin/spot-tracking/

Automatic tracking.

Well documented on the website.



Other open source, free Image Analysis software

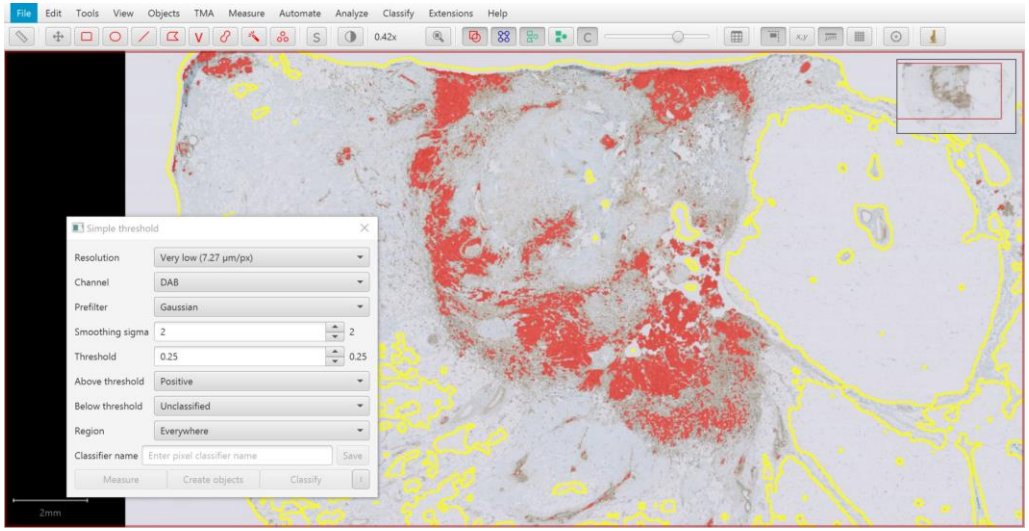
http://icy.bioimageanalysis.org/

- Great companion to Fiji
- 3D image analysis
- 3D visualisation
- Object tracking
- Segmentation etc
- Automation via scripts & protocols

Other open source, free Image Analysis software

https://qupath.github.io/

- Fantastic histopathology analysis
- H&E, DAB etc
- Segmentation, counting, machine learning
- Fluorescence
- User guides, videos etc make learning the software very easy



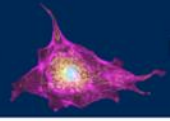
Other open source, free Image Analysis software

https://cellprofiler.org/

CellProfiler™
cell image analysis software

- High content screen
- Designed to analyse massive data sets
- Well documented

- Templates for many standard analysis protocols
- Templates relatively easily adapted to your own analysis



3D image analysis

- ImageJ can perform most functions in 3D
  (filters, segmentation, binary functions)
- 3D Segmentation is relatively simple
- 3D find maxima is possible but computationally complex, therefore slow
- Fiji has a 3D ROI manager

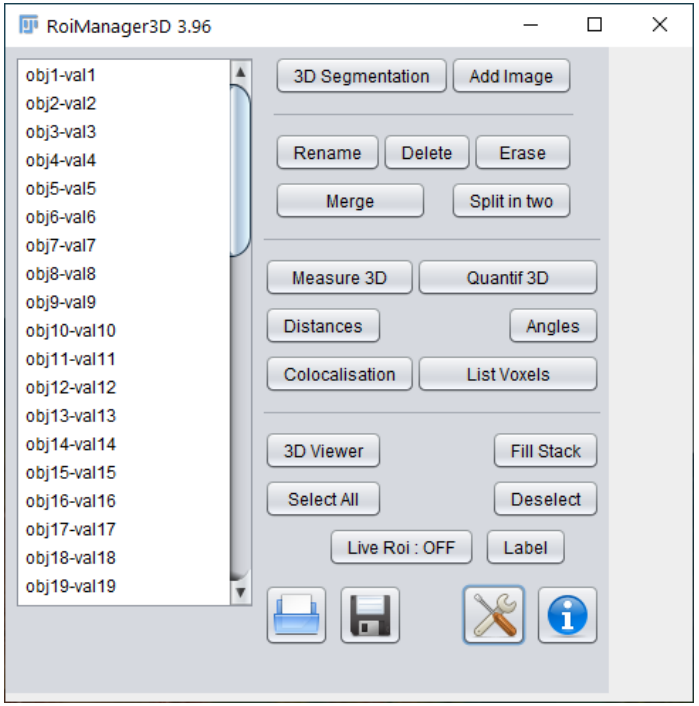
3D image analysis – 3D suite & ROI manager

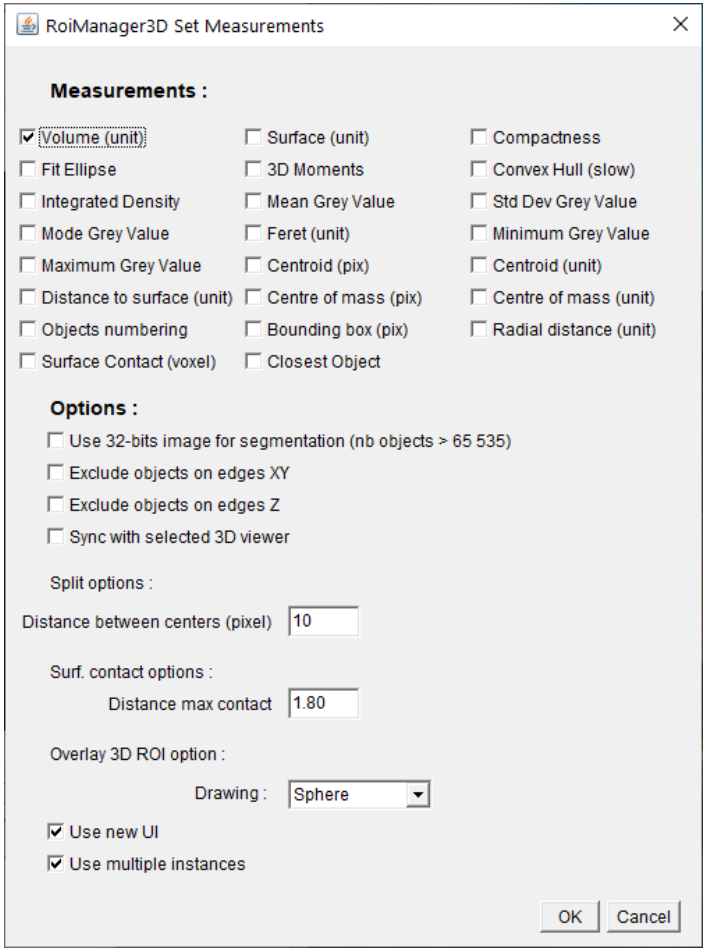
TANGO: a generic tool for high-throughput 3D image analysis for studying nuclear organization

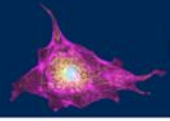
Jean Ollion, Julien Cochennec, François Loll, Christophe Escudé, Thomas Boudier

Bioinformatics, Volume 29, Issue 14, 15 July 2013, Pages 1840–1841,
https://doi.org/10.1093/bioinformatics/btt276

Plugins / 3D
(need the update site: 3D ImageJ suite)







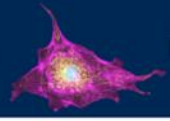
3D image analysis – 3D suite & ROI manager

Macros can use the 3D suite…

```
//Run the 3Dmanager to 3D segement cells, delete small 3D ROIs and save the ROIs
        selectWindow("Cells Mask");
        run("3D Manager");
        Ext.Manager3D_Segment(128, 255);
        Ext.Manager3D_AddImage();
        Ext.Manager3D_Measure();
        Ext.Manager3D_Count(nb);
        print("Number = "+nb+" cells");
    // loop to find and delete small volume ROIs
            n=0;
            for(j=0;j<nb;j++) {
                Ext.Manager3D_Measure3D(n,"Vol",V);
                    if(V<10) {
                    Ext.Manager3D_Select(n);
                    Ext.Manager3D_Delete();
                    }
                if(V>10) n++;
            }
```

- Add 3D objects as ROIs
- Filter by size (remove small stuff)
- Measure overlaps in 3D

```
//Run the 3Dmanager to Open the Cells ROIs and Synapses ROIS. Measure The colocalisation of
//all the ROIs and save the results.
        run("3D Manager");
        Ext.Manager3D_Load(dir2+ImageSet+"-3DCells.zip");
        Ext.Manager3D_Load(dir2+ImageSet+"-3DSynapesInCells.zip");
        Ext.Manager3D_Coloc();
        Ext.Manager3D_SaveResult("Coloc", dir2+ImageSet+"-coloc.txt");
        Ext.Manager3D_CloseResult("Coloc");
        Ext.Manager3D_Close();
```

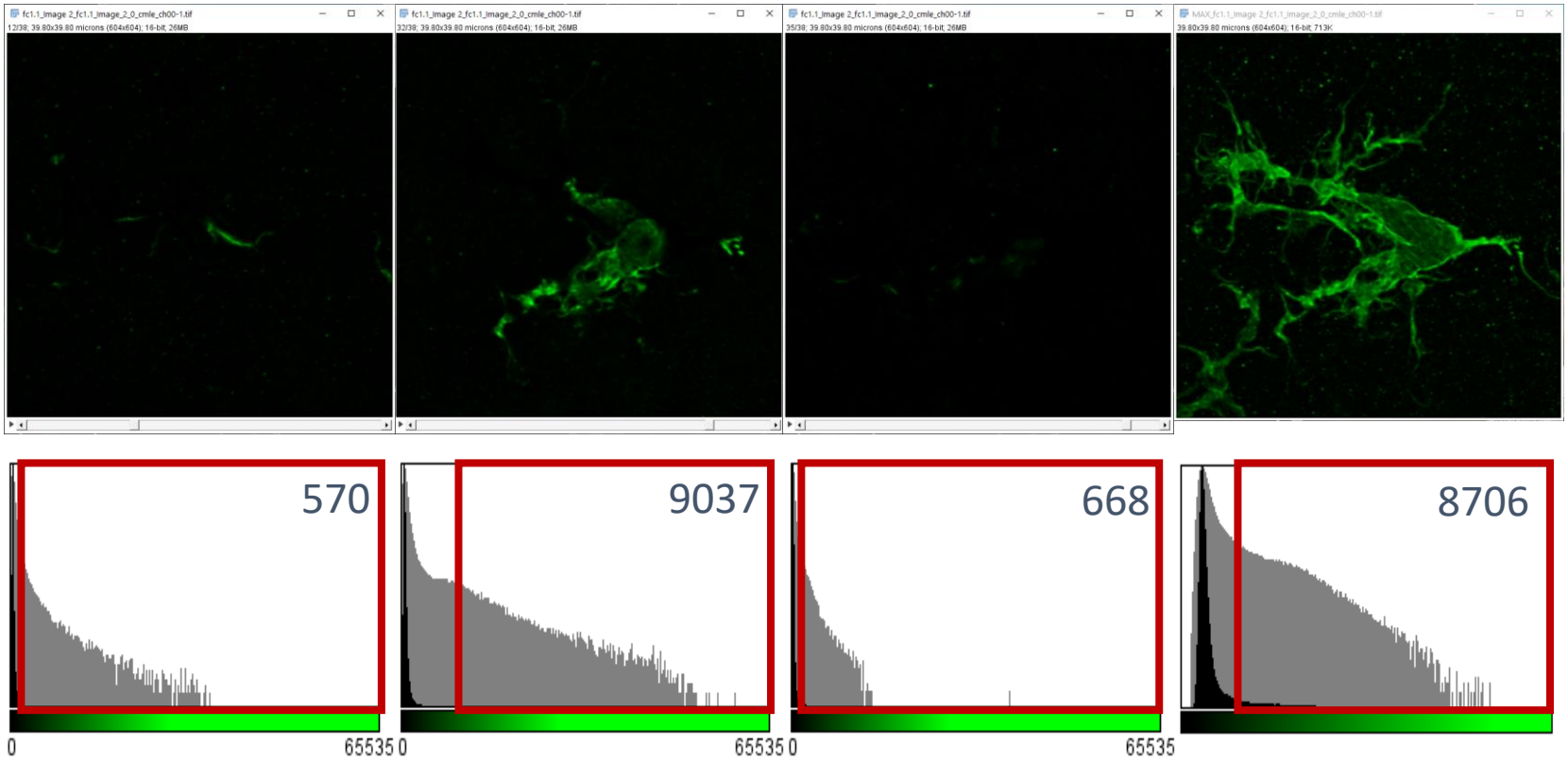


3D image analysis – 3D suite & ROI manager

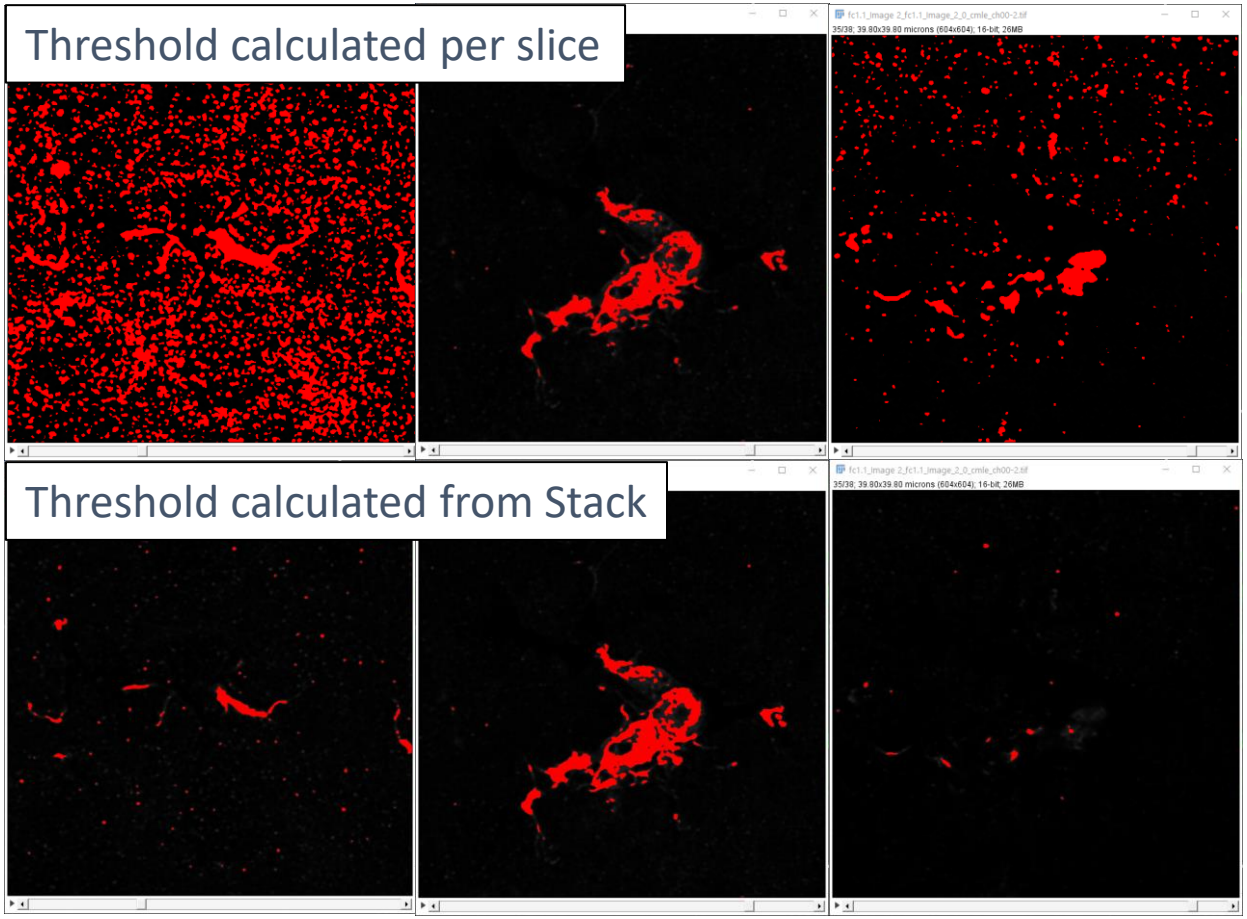
- 3D segmentation follows the same steps as 2D
- Background correction if needed
- Filter the image – clean up noise
- Threshold (Stack histogram – next slide)
- Add to 3D ROI manager
- Measure

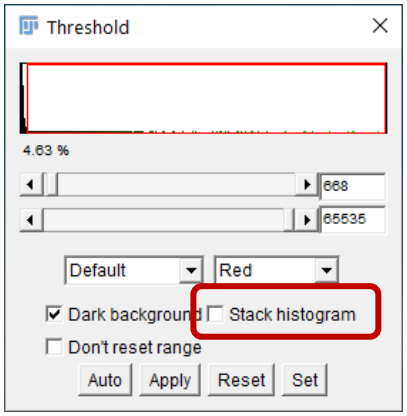
3D image analysis – Thresholding

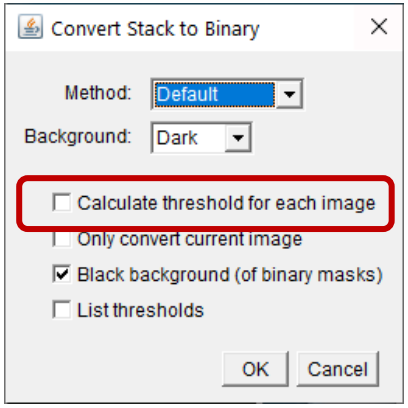
Bottom | Middle | Top | Stack



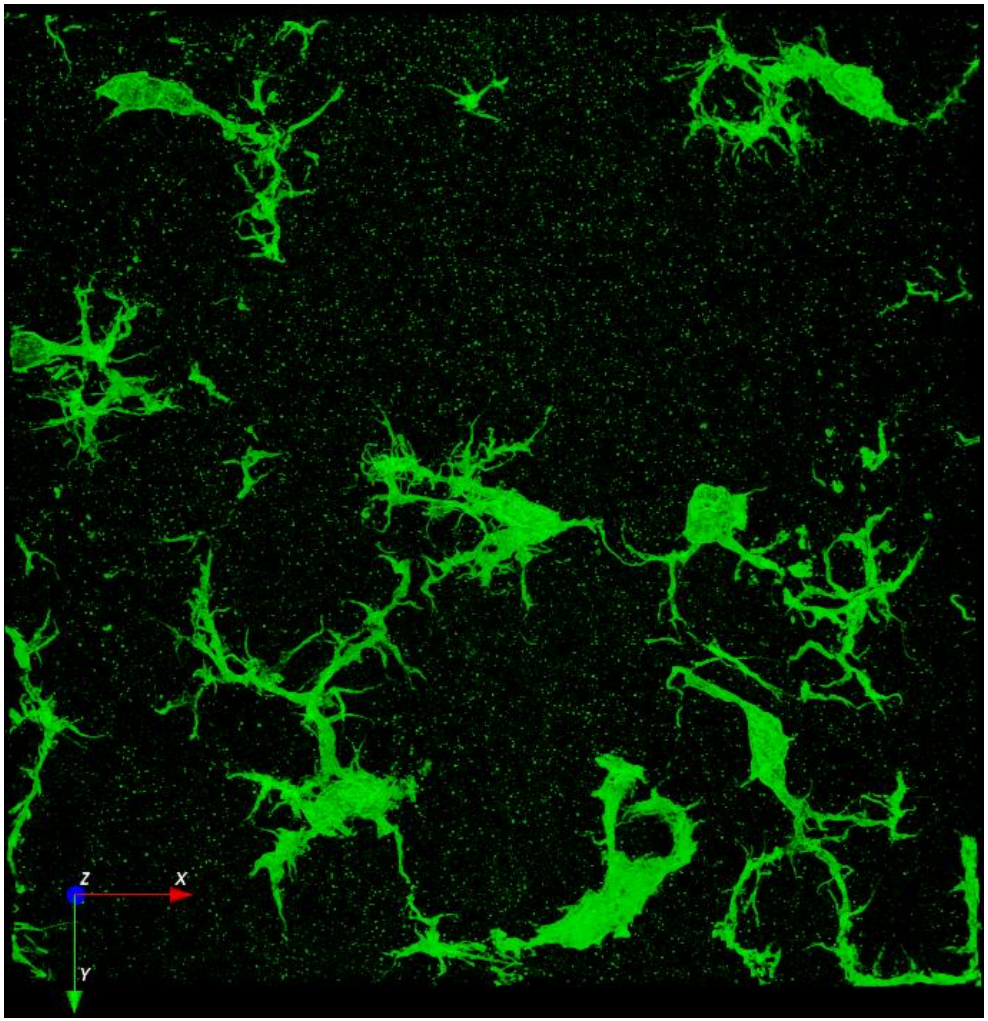
3D image analysis – Thresholding





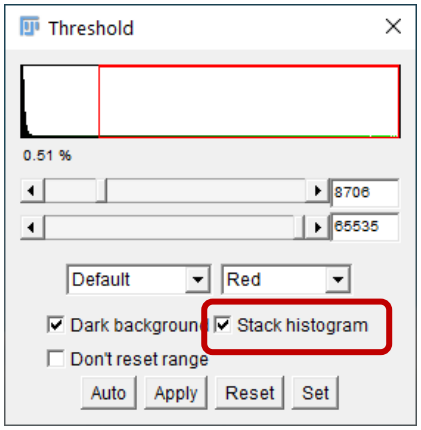


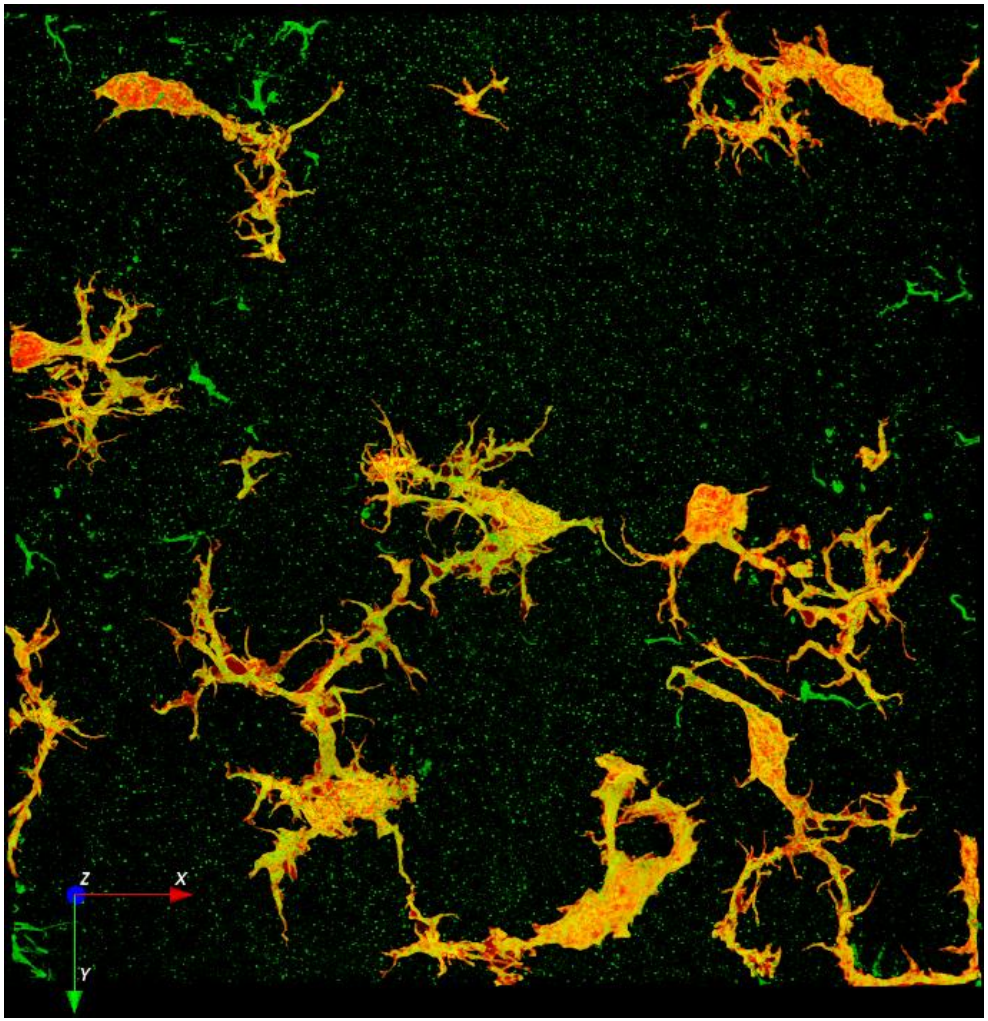
3D image analysis – 3D segmentation



3D image analysis – 3D segmentation

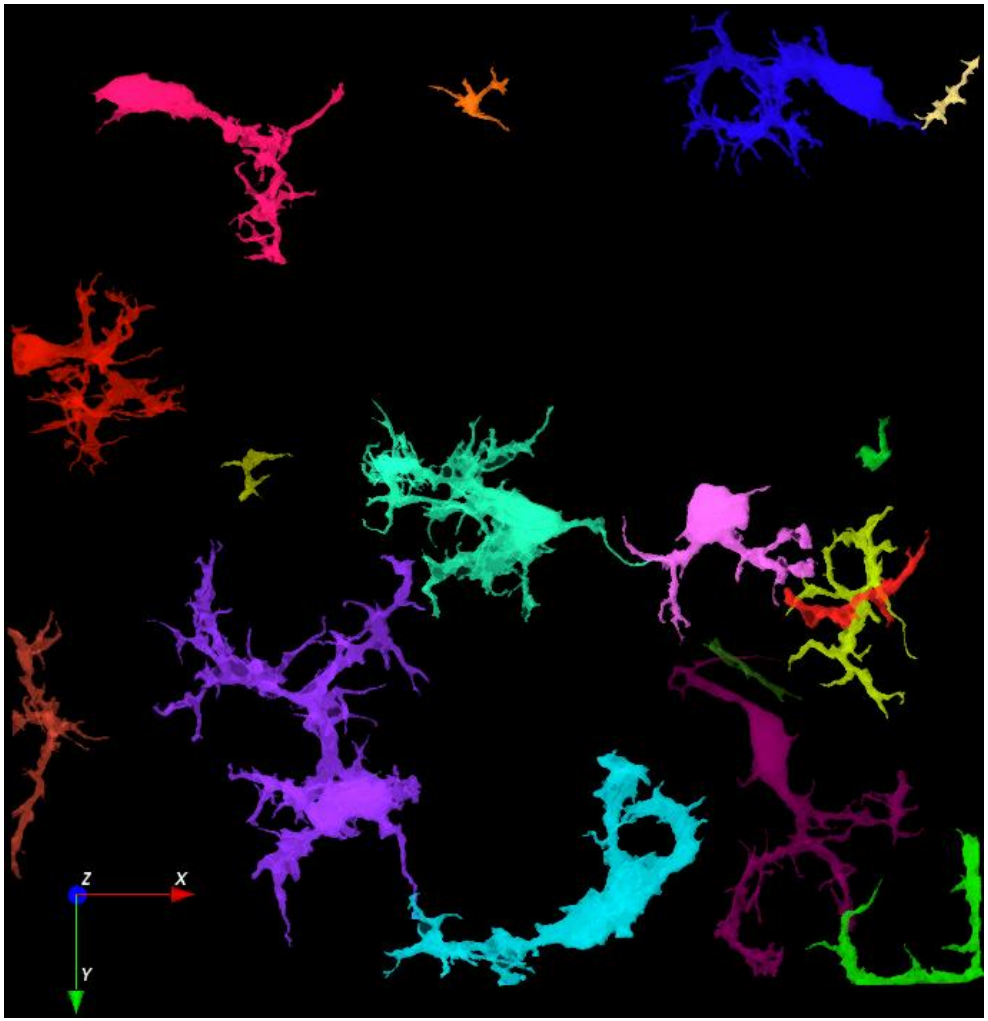
Stack histogram





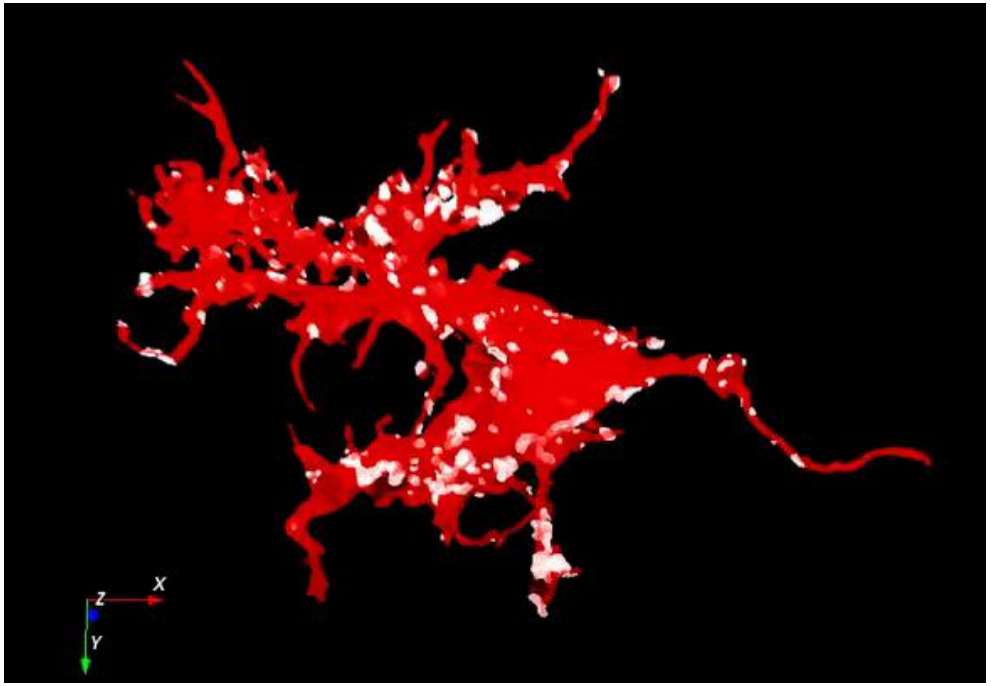
3D image analysis – 3D segmentation

3D segmentation in the 3D ROI manager. LUT = glasbey_on_dark



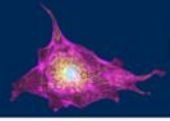
3D image analysis – 3D suite & ROI manager

Movie:



Data collected:

- Number of Glial cells
- Volume of each cell
- Total number synapses
- Number of synapses per cell
- % volume of each cell co-incident with synapses



Introduction to image processing and analysis with ImageJ / Fiji.
Part 6

Exercise for 3D segmentation and measurement

18) Segment and analyse the 3D image ‘Confocal series’