



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





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.



https://imagej.net/Colocalization_Analysis

- Manders gives in indication of the amount of fluorescence (above a predefined 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.





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.





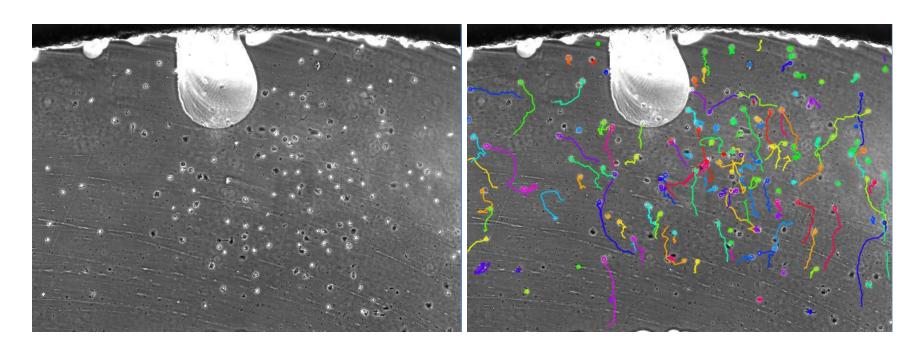
https://imagej.net/Colocalization Analysis

- 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

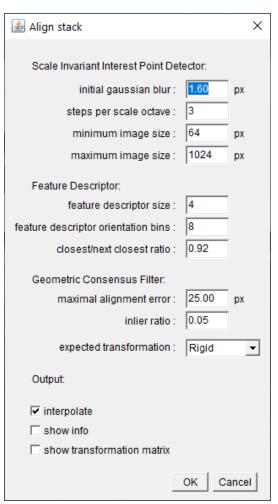
Plugins / registration >

Linear Stack Alignment with SIFT

Or

Plugins / registration > StackReg

(Big-EPFL update site)







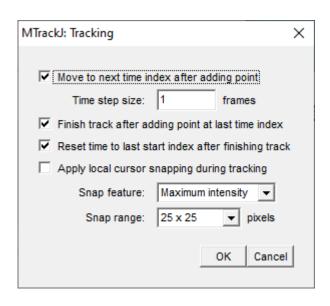
Object tracking in time-lapse imaging

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

Plugins / MTrackJ

(requires ImageScience update site in Fiji)

Extremely well documented on the website.



MTrackJ ·	- 🗆 ×			
Clear	Load			
Import	Save			
Add	Cluster			
Hide	Color			
Delete	Move			
Merge	Split			
Refer	ID			
Measure	Movie			
Tracking	Displaying			
Options	Help			

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.

TrackMatev6.0.3	_	□ ×			
Please note that TrackMate is available through Fiji, and is based on a publication. If you use it successfully for your research please be so kind to cite our work: Tinevez, JY.; Perry, N. & Schindelin, J. et al. (2017), TrackMate: An open and extensible platform for single-particle tracking. Methods 115: 80-90. on PubMed (PMID 27713081)					
Target: neutrophils					
Calibration settings:					
Pixel width:	1.031	μm			
Pixel height:	1.031	μm			
Voxel depth:	1.031	μm			
Time interval:	60.000	sec			
Crop settings (in pixels, 0-based):					
X 0	to	1343			
Υ 0	to	1023			
Z 0	to	0			
T 0	to	59			
Refresh source					
Save (1)	-	⇒ Next			



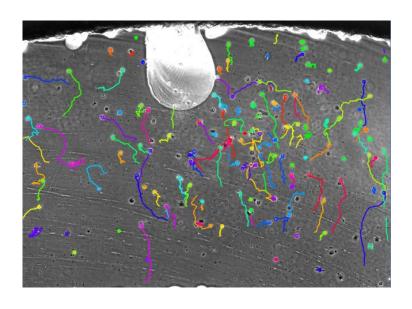


Object tracking in time-lapse imaging

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

Automatic tracking.

Well documented on the website.



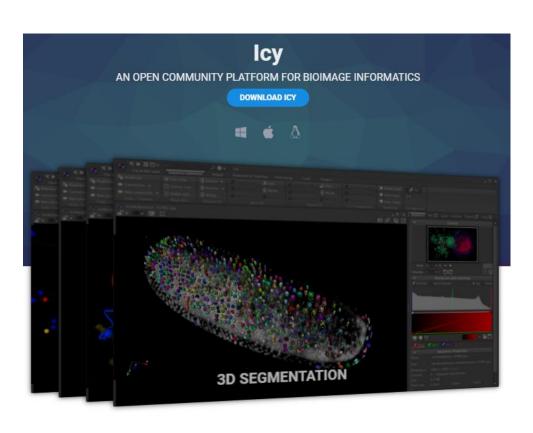






Other open source, free Image Analysis software

http://icy.bioimageanalysis.org/



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



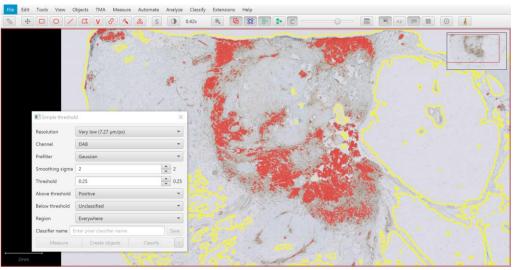


Other open source, free Image Analysis software

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



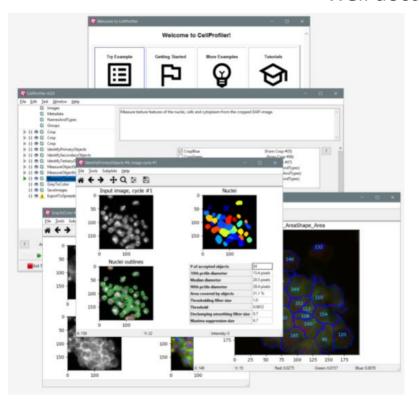


Other open source, free Image Analysis software

https://cellprofiler.org/



- 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



Light Microscopy Core Facility UCL Great Ormond St. Institute of Child Health



3D image analysis – 3D suite & ROI manager

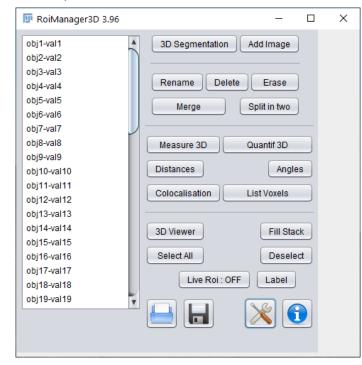
TANGO: a generic tool for high-throughput 3D image analysis for studying nuclear organization

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)



RoiManager3D Set Mea	asurements		×
Measurements:			
✓ Volume (unit)	Surface (unit)	☐ Compactness	
☐ Fit Ellipse	□ 3D Moments	Convex Hull (slow)	
☐ Integrated Density	Mean Grey Value	Std Dev Grey Value	
☐ Mode Grey Value	Feret (unit)	Minimum Grey Value	
Maximum Grey Value	Centroid (pix)	Centroid (unit)	
☐ Distance to surface (unit)	Centre of mass (pix)	Centre of mass (unit)	
Objects numbering	☐ Bounding box (pix)	Radial distance (unit)	
Surface Contact (voxel)	Closest Object		
Options :			
	segmentation (nb objects > 6	E E2E)	
Exclude objects on ed		3 333)	
Exclude objects on ed	_		
Sync with selected 3D	_		
Split options :			
Distance between centers (p	oixel) 10		
Surf. contact options :			
Distance max co	ntact 1.80		
Diotalios max so			
Overlay 3D ROI option :			
Draw	ing: Sphere 🔻		
✓ Use new UI			
✓ Use multiple instance	S		
			. 1
		OK Cance	1





3D image analysis – 3D suite & ROI manager

Macros can use the 3D suite...

```
85 //Run the 3Dmanager to 3D segement cells, delete small 3D ROIs and save the ROIs
           selectWindow("Cells Mask");
86
           run("3D Manager");
88
           Ext.Manager3D_Segment(128, 255);
           Ext.Manager3D AddImage();
89
                                                                        Add 3D objects as ROIs
 90
           Ext.Manager3D_Measure();
91
           Ext.Manager3D Count(nb);
 92
           print("Number = "+nb+" cells");
                                                                        Filter by size (remove small
 93
       // loop to find and delete small volume ROIs
94
               n=0;
                                                                        stuff)
 95
               for(j=0;j<nb;j++) {
                   Ext.Manager3D_Measure3D(n,"Vol",V);
 96
97
                       if(V<10) {
98
                       Ext.Manager3D Select(n);
                                                                        Measure overlaps in 3D
99
                        Ext.Manager3D Delete();
100
101
                   if(V>10) n++;
102
                                     122 //Run the 3Dmanager to Open the Cells ROIs and Synapses ROIS. Measure The colocalisation of
                                     123 //all the ROIs and save the results.
                                     124
                                                 run("3D Manager");
                                     125
                                                 Ext.Manager3D_Load(dir2+ImageSet+"-3DCells.zip");
                                     126
                                                 Ext.Manager3D Load(dir2+ImageSet+"-3DSynapesInCells.zip");
                                     127
                                                 Ext.Manager3D Coloc();
                                     128
                                                 Ext.Manager3D SaveResult("Coloc", dir2+ImageSet+"-coloc.txt");
                                     129
                                                 Ext.Manager3D CloseResult("Coloc");
                                     130
                                                 Ext.Manager3D Close();
```





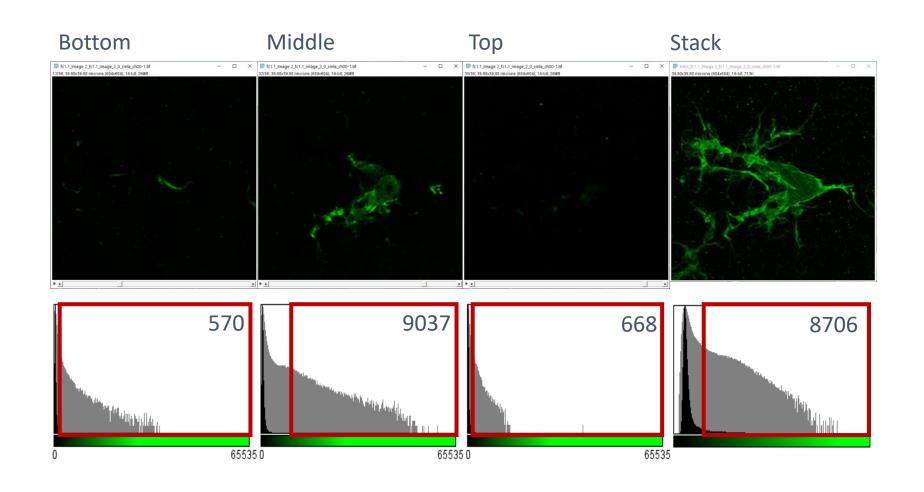
3D image analysis – 3D suite & ROI manager

- 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





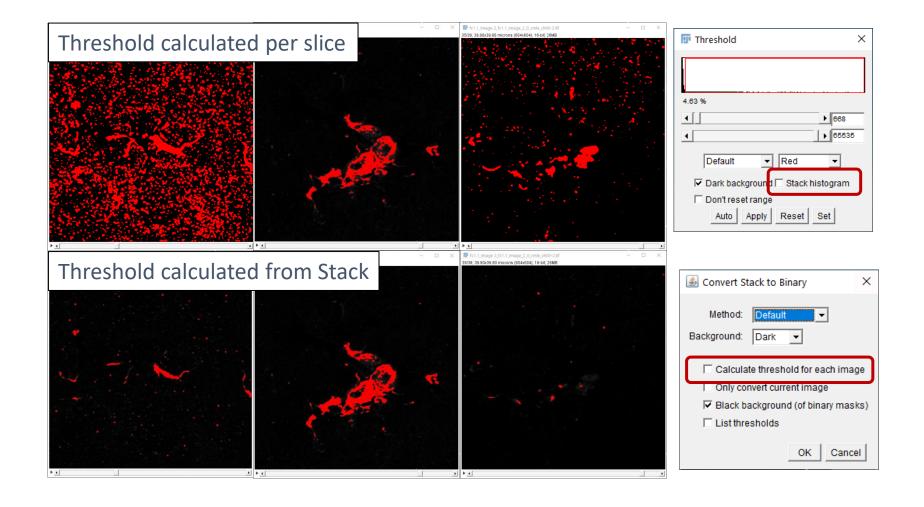
3D image analysis – Thresholding







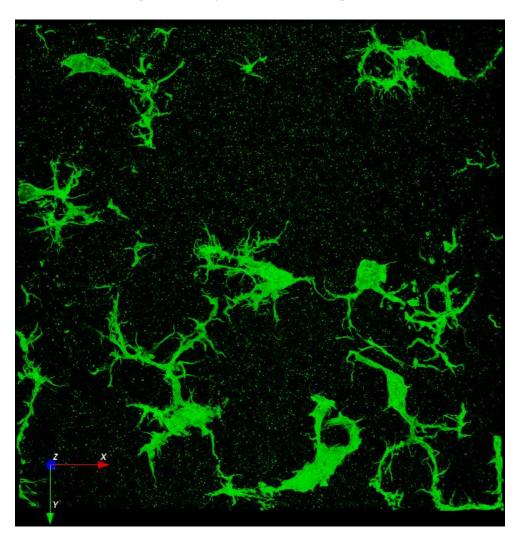
3D image analysis – Thresholding







3D image analysis – 3D segmentation

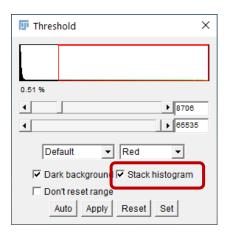


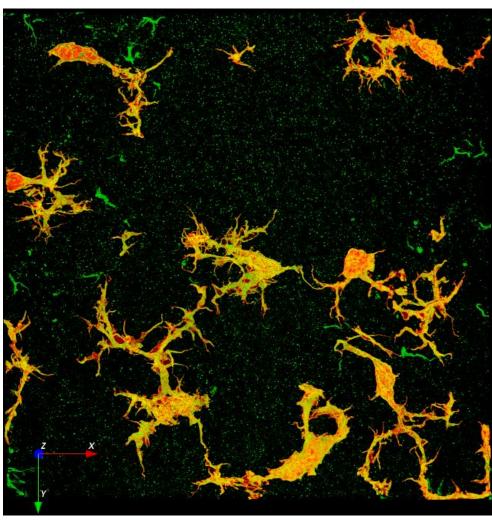




3D image analysis – 3D segmentation

Stack histogram



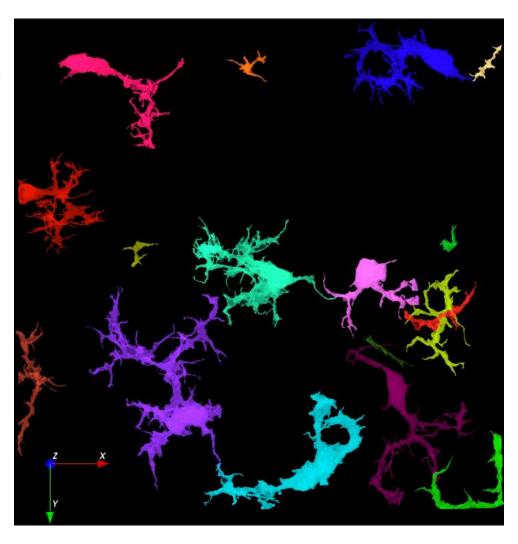






3D image analysis – 3D segmentation

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

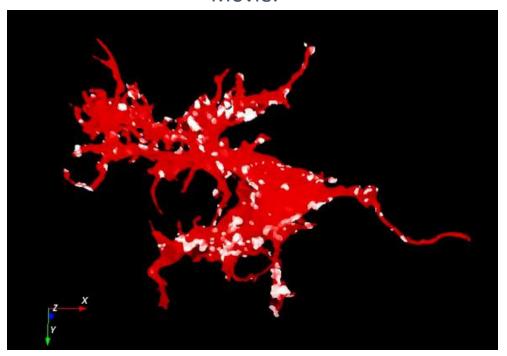






3D image analysis – 3D suite & ROI manager

Movie:



Data collected:

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





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Exercise for 3D segmentation and measurement

18) Segment and analyse the 3D image 'Confocal series'