

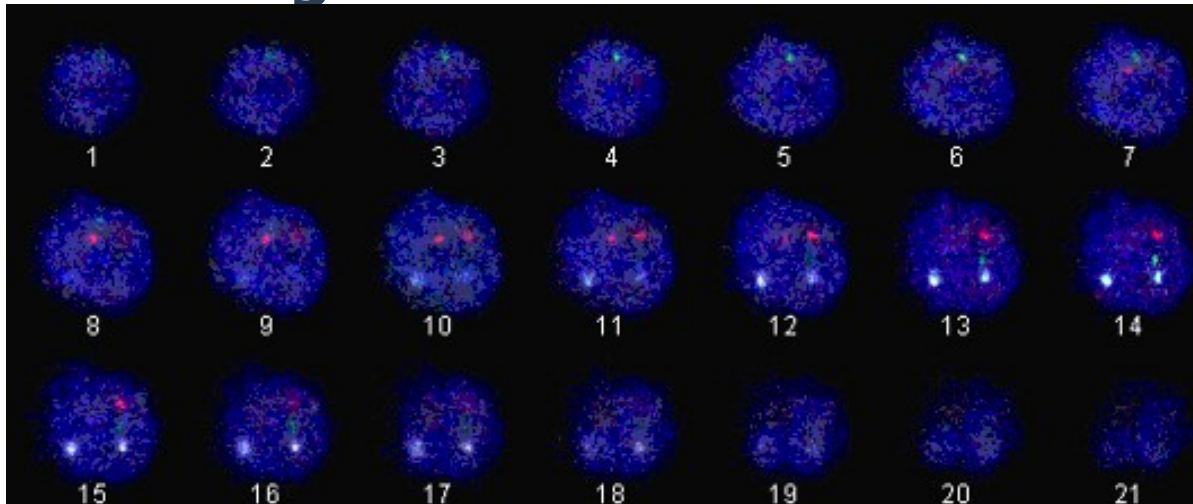
Introduction to 3D Analysis With the 3D ImageJ Suite

Thomas Boudier,
Assoc. Prof., Sorbonne Université, Paris
Visiting Scholar, Academia Sinica, Taipei

NEUBIAS Academy @Home

Why 3D ImageJ Suite

- **Set of algorithms and tools for 3D Analysis**
- **Started in 2006, to analyse distances between gene loci in fluorescence images**
 - Gue *et al.*, BMC Cancer 6 (2006)
- **Need 3D data to get accurate measurements**
- **Need robust algorithms and tools for automation**



What is 3D ImageJ Suite

- **Set of algorithms and tools for 3D Analysis**
- **A core library "mcib3d-core"**
 - 3D Images and related processing
 - 3D Objects and related analysis
 - 3D Objects population and related analysis
- **A set of plugins calling core algorithms, "mcib3d-plugins"**
 - Processing, segmentation, analysis, utils
- **Open-source**
 - <https://github.com/mcib3d>

Who developed 3D Suite

- **Dr Cedric Messaoudi, first version**
- **Dr Jean Ollion, second version**
- **Me, supervision and maintenance**
- **Acknowledgment : Dr Philippe Andrey**
- **PhD Students : Cédric, Seb, Jean, Jaza, Hoa, Lamees, Afshin**

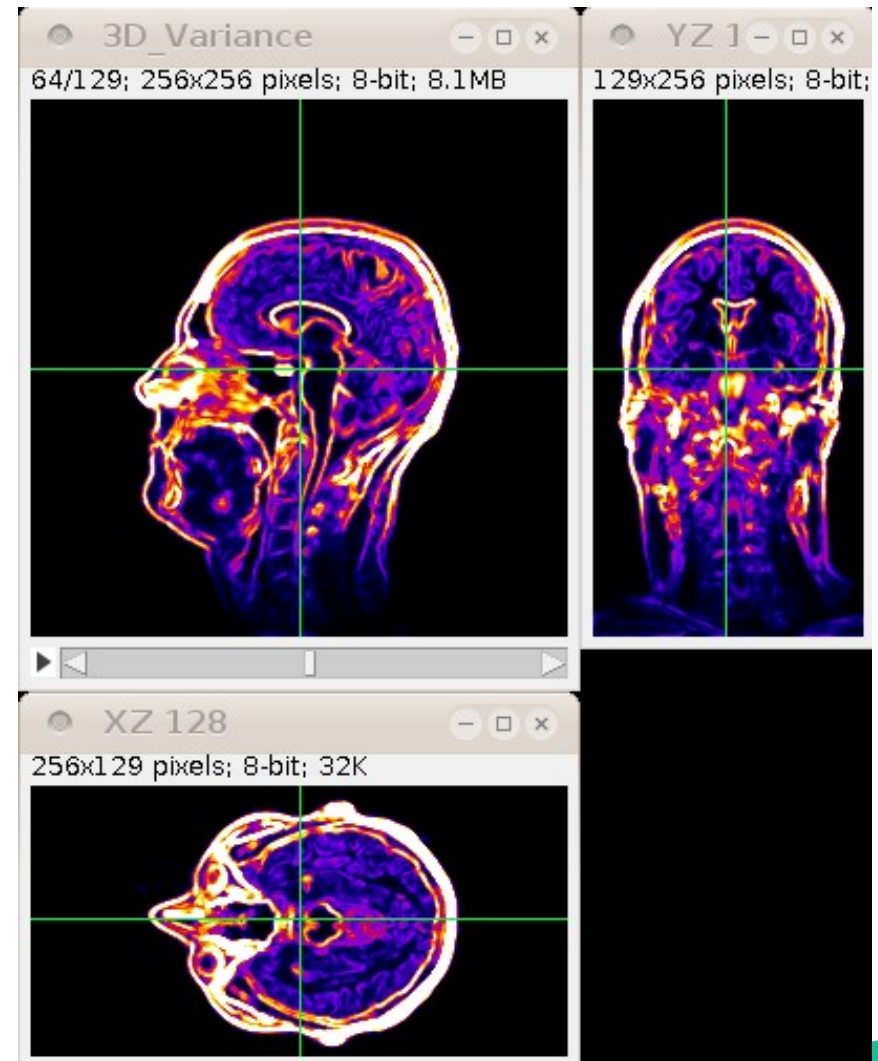
- **Extensive (but not complete) documentation**
 - https://imagej.net/3D_ImageJ_Suite

What difference in 3D

- **3D Image analysis :**
 - Anisotropy in Z
 - More noise
 - More complex shape in 3D
 - More pixels/voxels to process
- **Other sets for 3D analysis :**
 - BoneJ, MorphoLibJ, ICY, 3D Object Counter, ...

Pre-processing - filters

- **Reduce noise in images**
 - 3D Filters : mean, **median**
- **Enhance contrast of objects**
 - For spots : topHat
- **3D Filters :**
 - multi-threaded (CPU)
 - Ellipsoid neighbourhood (anisotropy)
 - Implemented in ImageJ in Filters menu
- **For faster versions :**
 - JNI (CPU), CLIJ (GPU)

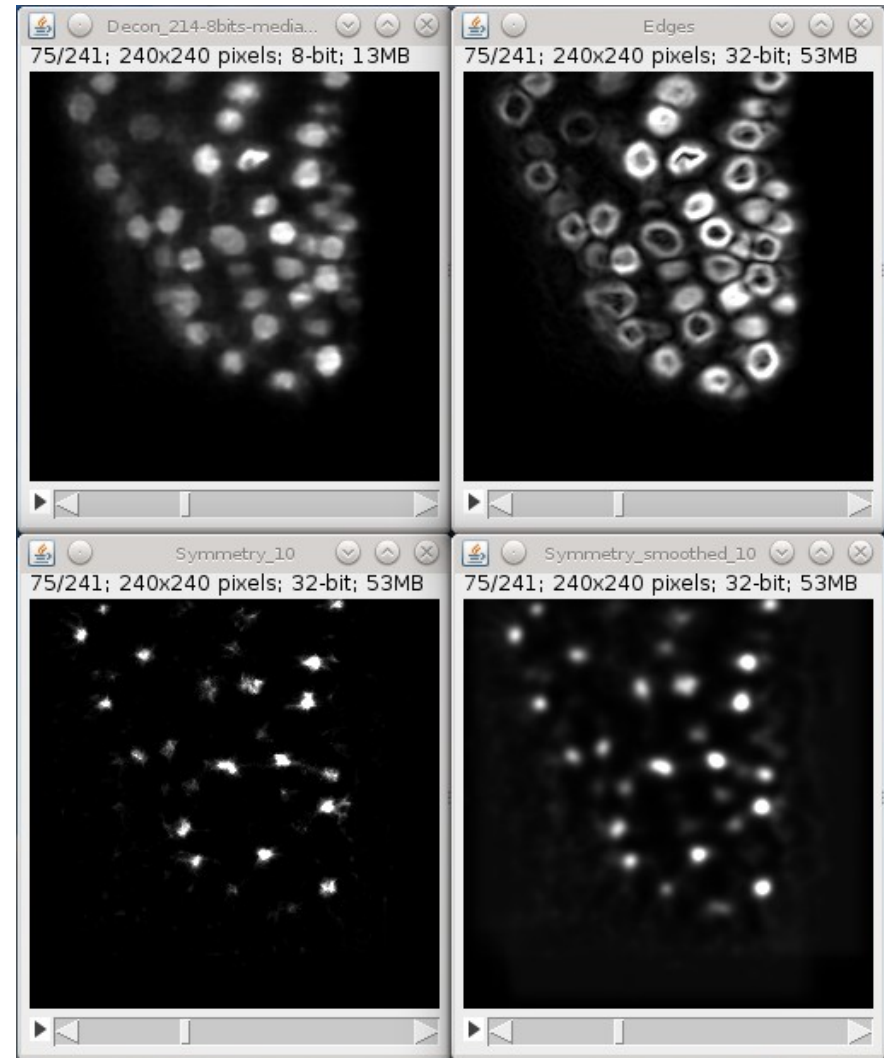


Pre-processing - edge symmetry

- **Edge and symmetry filter**

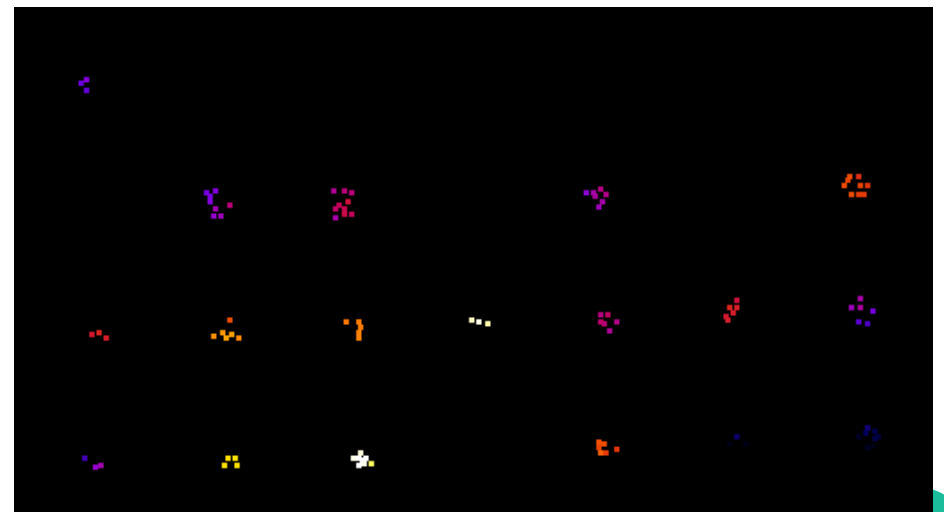
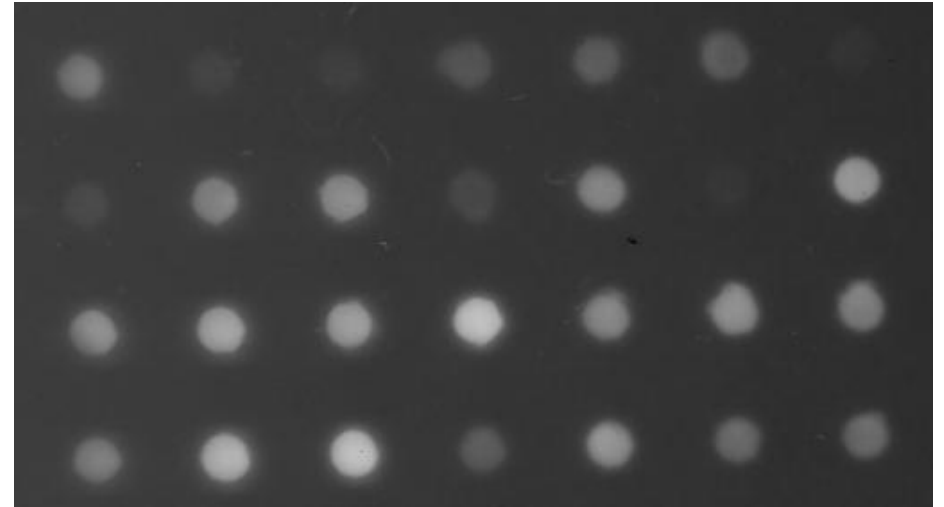
- Compute edges in X, Y and Z
- Compute “magnitude” of edge
- Rays converging towards centres of objects

- **A. Gertych *et al.*,
Computers in Biology and
Medecine, 2015**



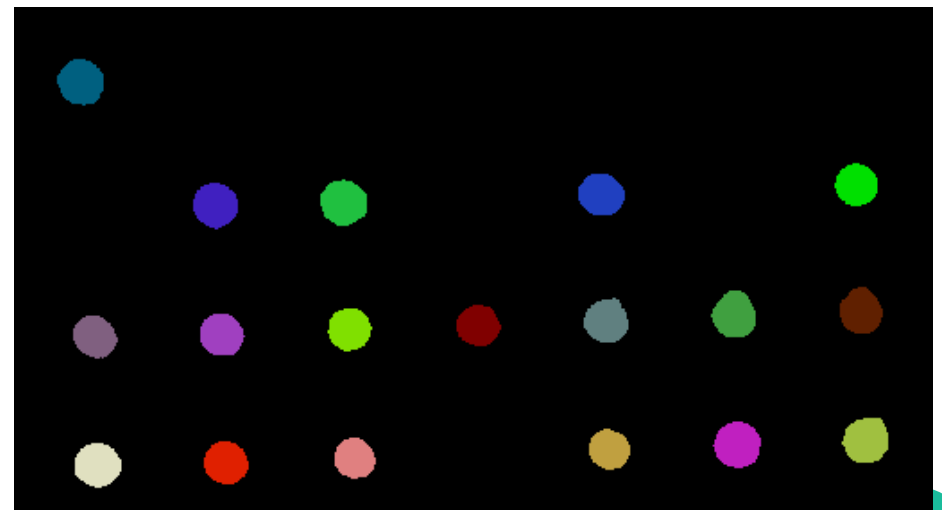
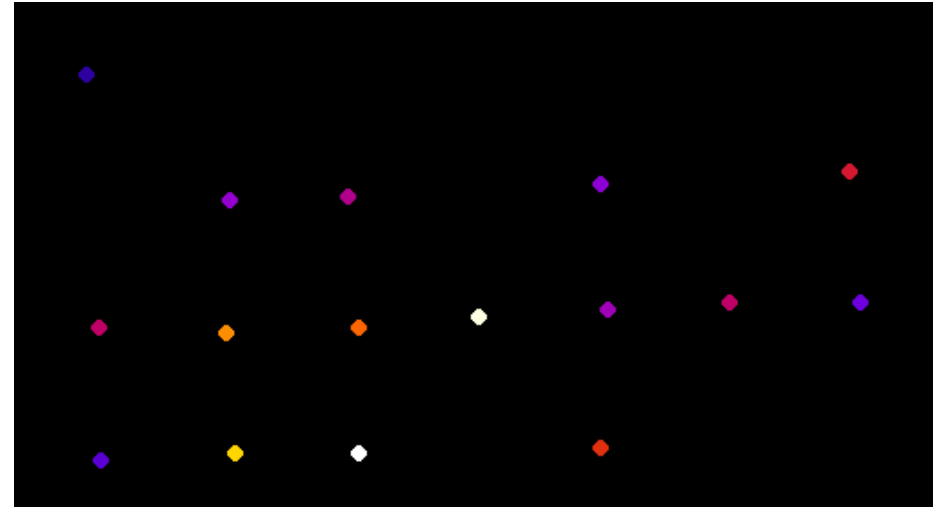
Pre-processing - seeds

- **Finding seeds is essential for segmentation**
 - 1 seed = 1 object
- **3D Local Maxima**
- **3D Maxima Finder**
 - Similar to *Find Maxima* (but slower)



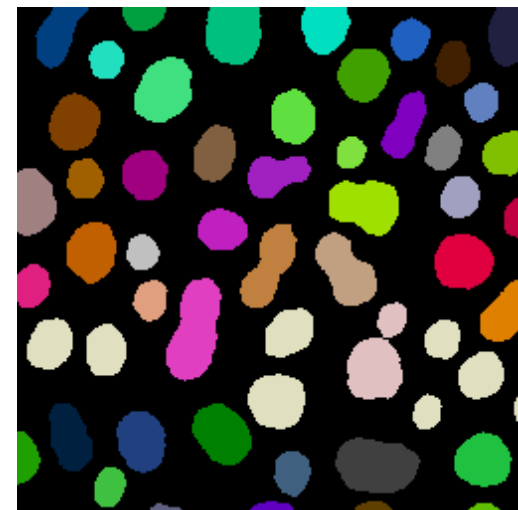
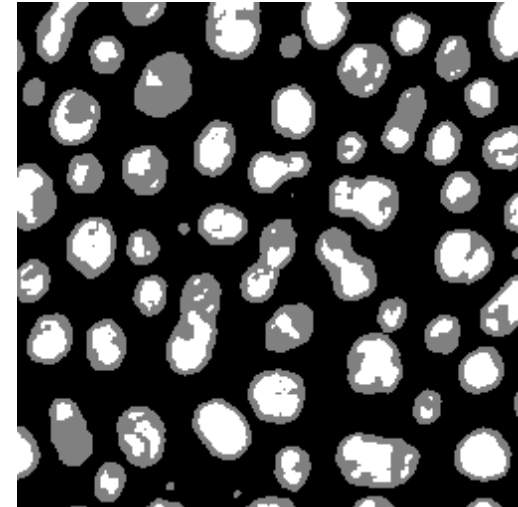
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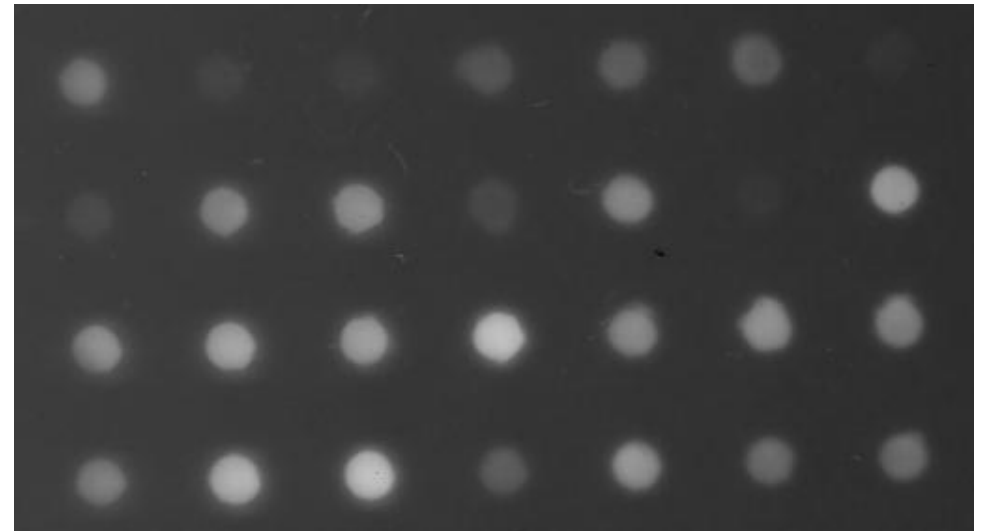
Thresholding - hysteresis

- **Global 2D thresholding for 3D images**
 - Check "stack histogram"
 - Check threshold for all slices
- **Hysteresis 2 thresholds**
 - 3 areas : Background, undetermined, signal
 - Undetermined connected to signal becomes signal
 - Signal act as seed in objects
 - Remove patches of noise



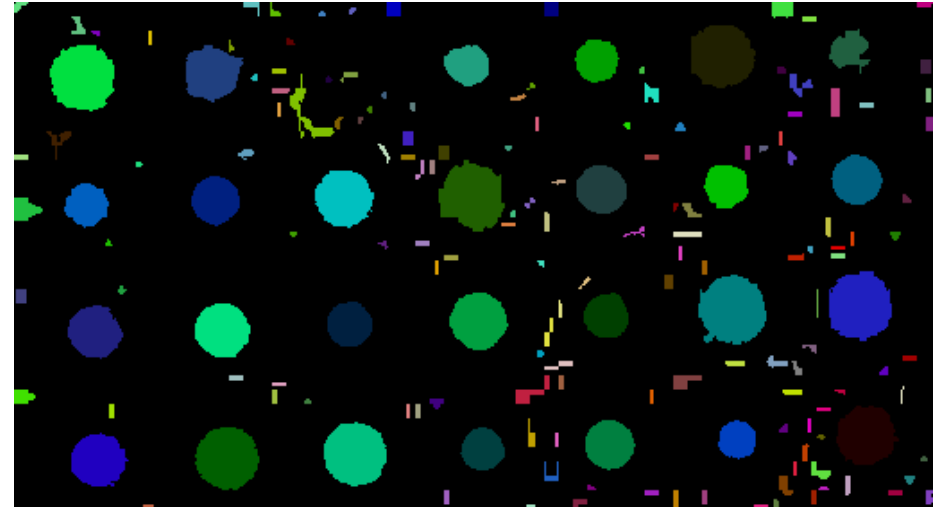
Thresholding - iterative

- **Iterative : check all threshold and select best threshold for each object**
 - Shape criteria (most round, largest)
 - Edge criteria
- **Can separate touching objects**
 - Two separated objects have better criteria than the merged one
- **Slow for 16-bits images**



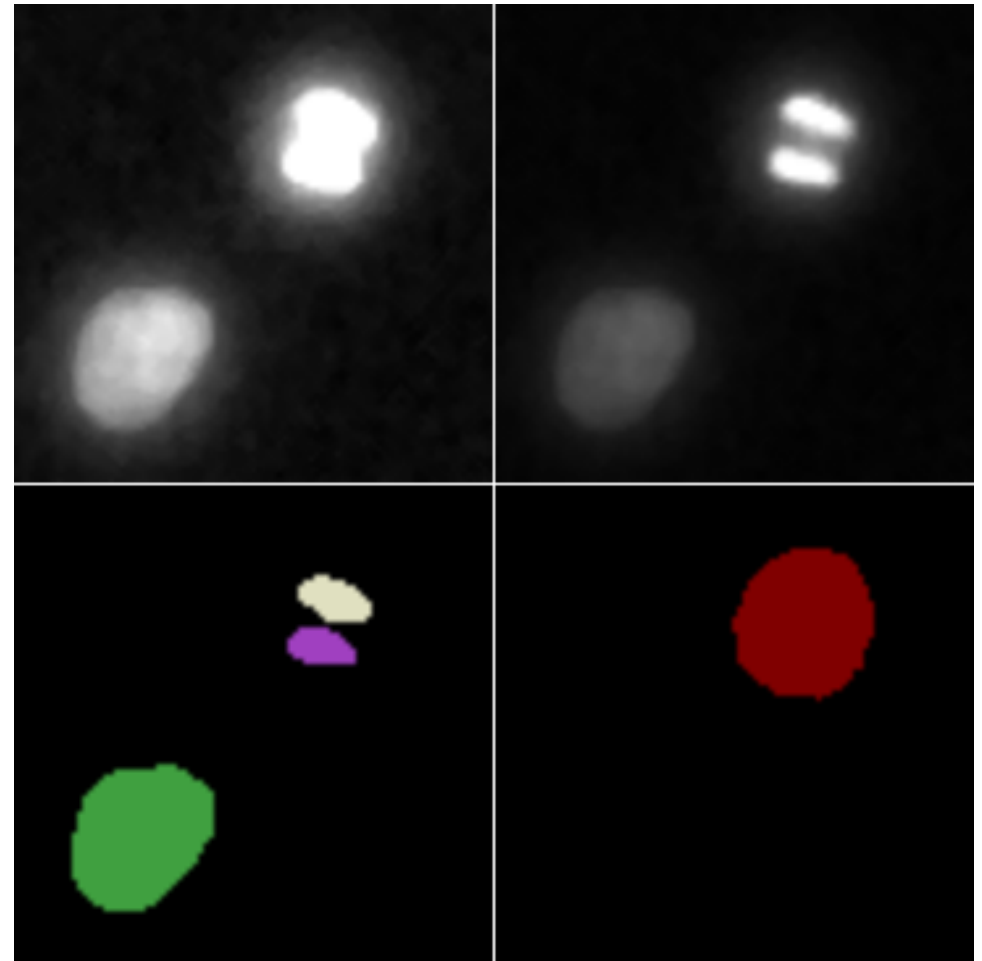
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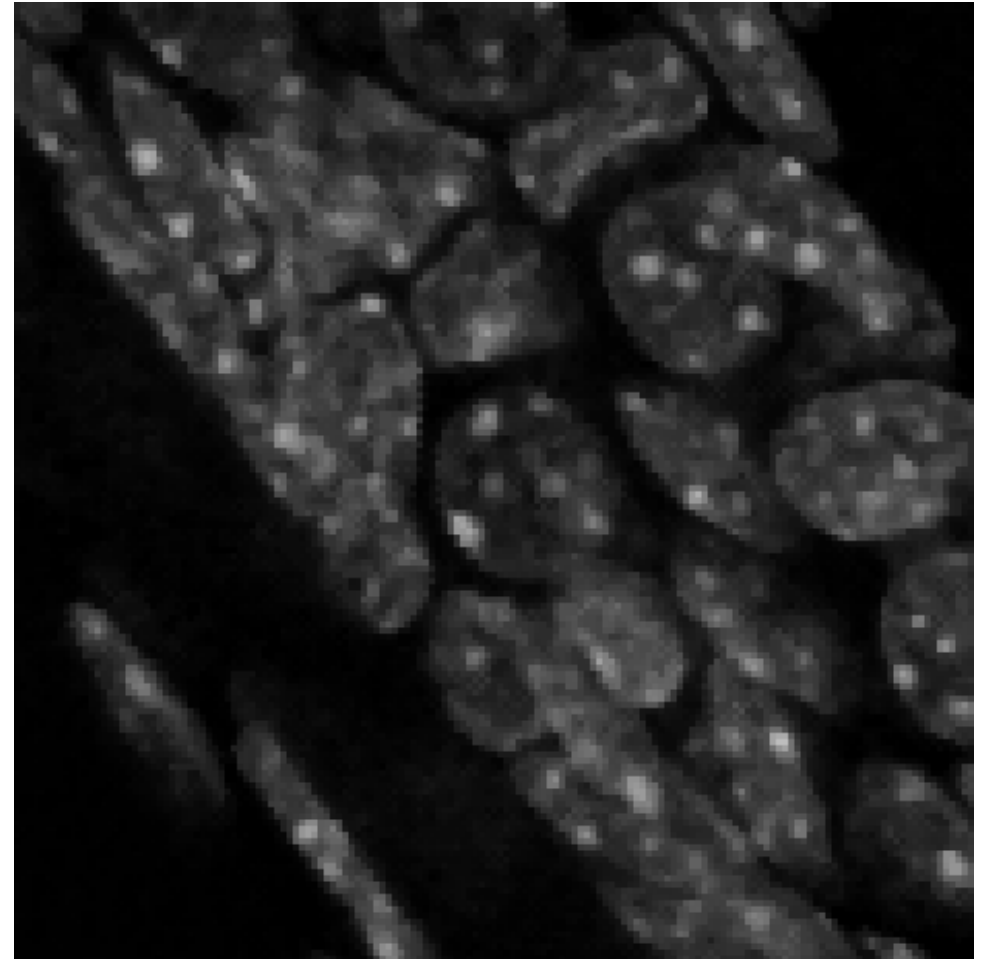
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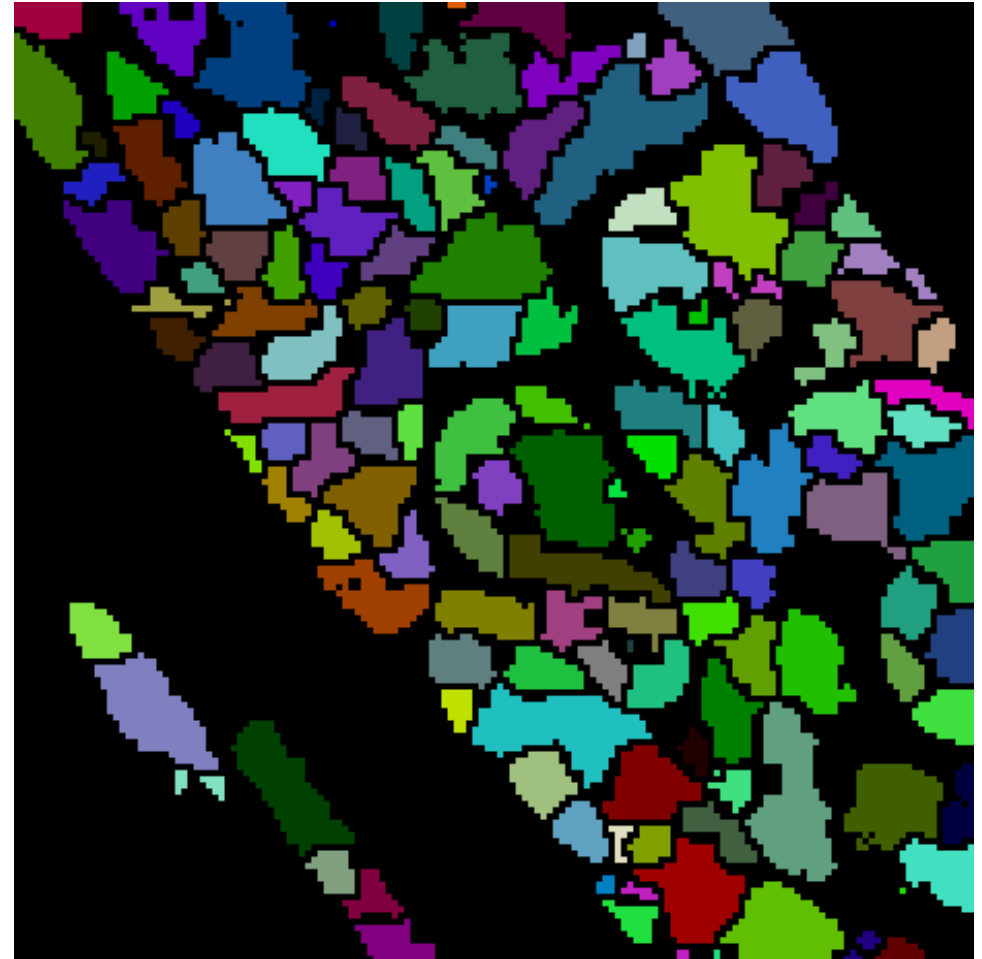
Segmentation - watershed

- **Watershed**
- **Detect seeds then cluster voxels around by decreasing intensity**
 - Used in 2D ImageJ to separate objects
 - Can separate 3D objects based on seeds
 - Will do segmentation
- J. Visvader, WEHI



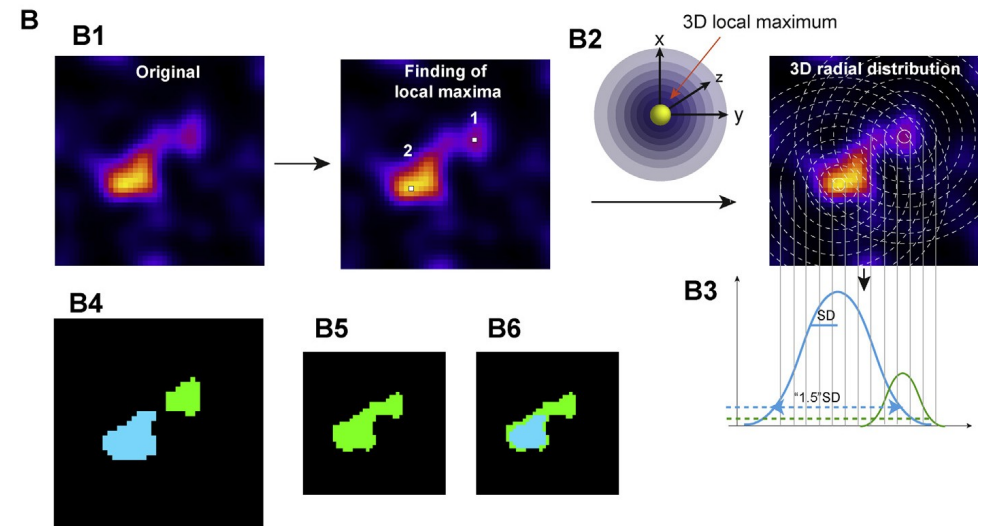
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Segmentation - spots and nuclei

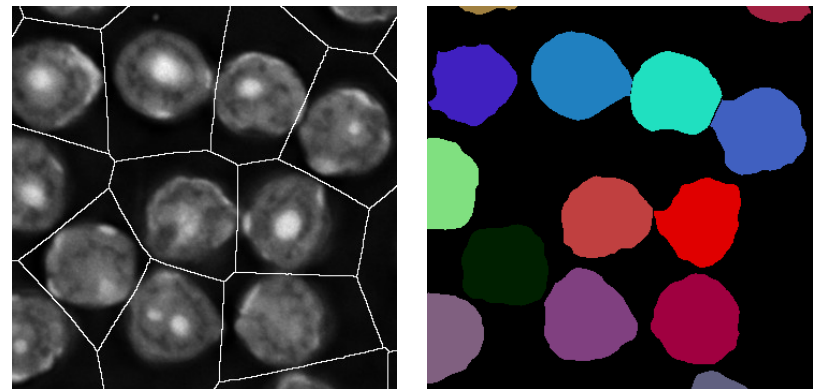
- **Spots segmentation**
 - Find seeds : local maxima, maxima finder
 - Local threshold around seeds
 - Gaussian fitting
- **Nuclei segmentation (culture cells)**
 - Z project, thresholding + 2D watershed separation
 - 3D extension
- **Tissue and more complex : ML and DL**
 - Weka, StarDist, CellPose, ...



Gilles et al., DiAna

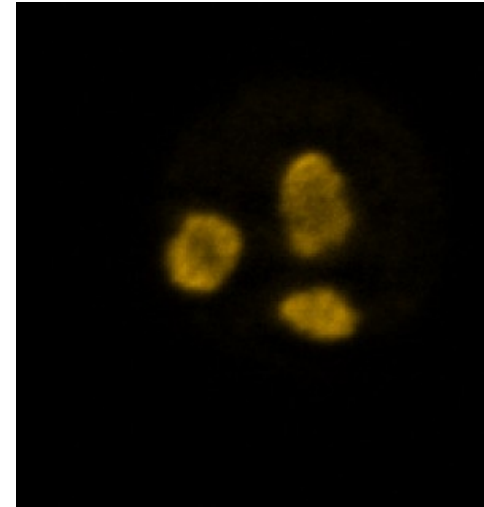
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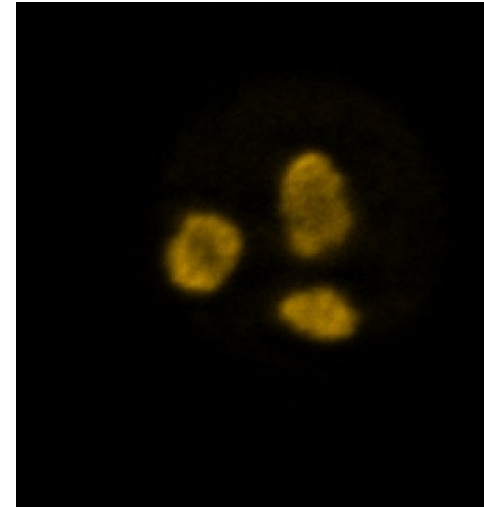
Post-processing

- **Process binary thresholded images**
 - Erode, dilate (min/max)
- **Remove small regions**
 - opening
- **Close small holes**
 - closing
- **Make shapes compact by closing**
 - BinaryCloseLabels



Post-processing

- **Process binary thresholded images**
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3D Manager

- **Manager for 3D objects (ROI)**
 - First *AddImage* with a labelled image
- **Load/save set of 3Drois**
 - Specific format .3droi (zip if multiple)
- **3D visualisation in stacks and 3DViewer**
 - Overlay in each slice (may take time to compute/update)
 - Manual classification (press 0-5)
- **Various measurements available**
 - Check 3DManager options
- **Macro recordable + macros extensions (not detailed here)**

3D Manager

1. 3D Rois list
2. Segmentation + add
3. Edit
4. Measurements
5. Visualisation
6. Load / Save
7. Options / About

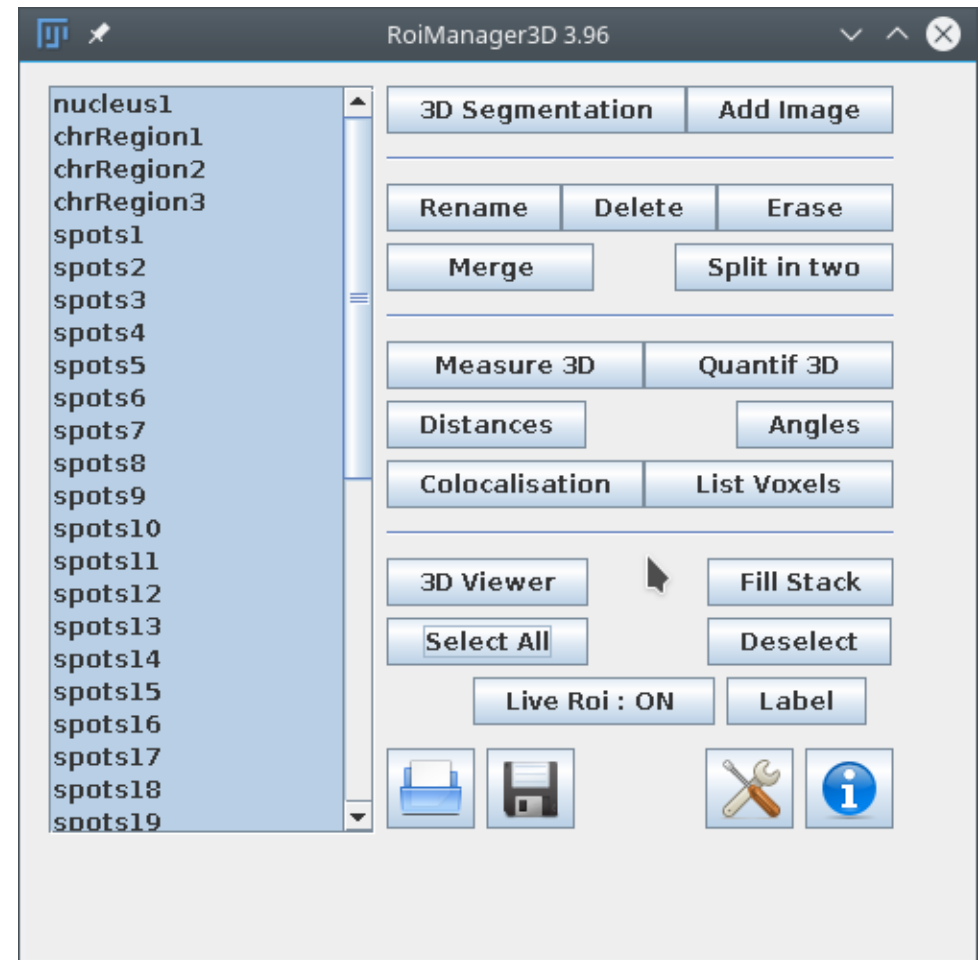
The screenshot displays the 3D Manager software interface, which is divided into several panels. The top-left panel shows a list of 3D objects (Rois) with their IDs. The top-right panel contains controls for segmentation and editing, including buttons for '3D Segmentation', 'Add Image', 'Rename', 'Delete', 'Erase', 'Merge', and 'Split in two'. The middle-right panel has buttons for 'Measure 3D', 'Quantif 3D', 'Distances', 'Angles', 'Colocalisation', and 'List Voxels'. The bottom-right panel features a '3D Viewer' and buttons for 'Fill Stack', 'Select All', 'Deselect', 'Live Roi : ON', and 'Label'. The bottom-left panel shows a '3D Measure' window with a table of measurement data. The right side of the interface shows two image windows: 'OrigRaw.tif' and 'OrigSeg.tif', both displaying a 3D volume with segmented regions.

CX (unit)	CY (unit)	CZ (unit)	Vol (unit)	Vol (pix)	Comp (p...	Sp
16.852	31.797	8.739	5.551	1015	0.165	
27.7	25.119	2.099	29.745	5439	0.025	
40.944	13.642	2.28	44.373	8114	0.055	
49.67	21.855	5.933	7.345	1343	0.119	
24.731	8.493	6.064	44.116	8067	0.101	
43.327	13.253	6.831	31.374	5737	0.093	
52.635	15.176	6.563	5.862	1072	0.129	
14.719	22.867	6.436	51.488	9415	0.088	
30.667	25.744	7.554	54.02	9878	0.081	

3D Manager

- **Visualisation**

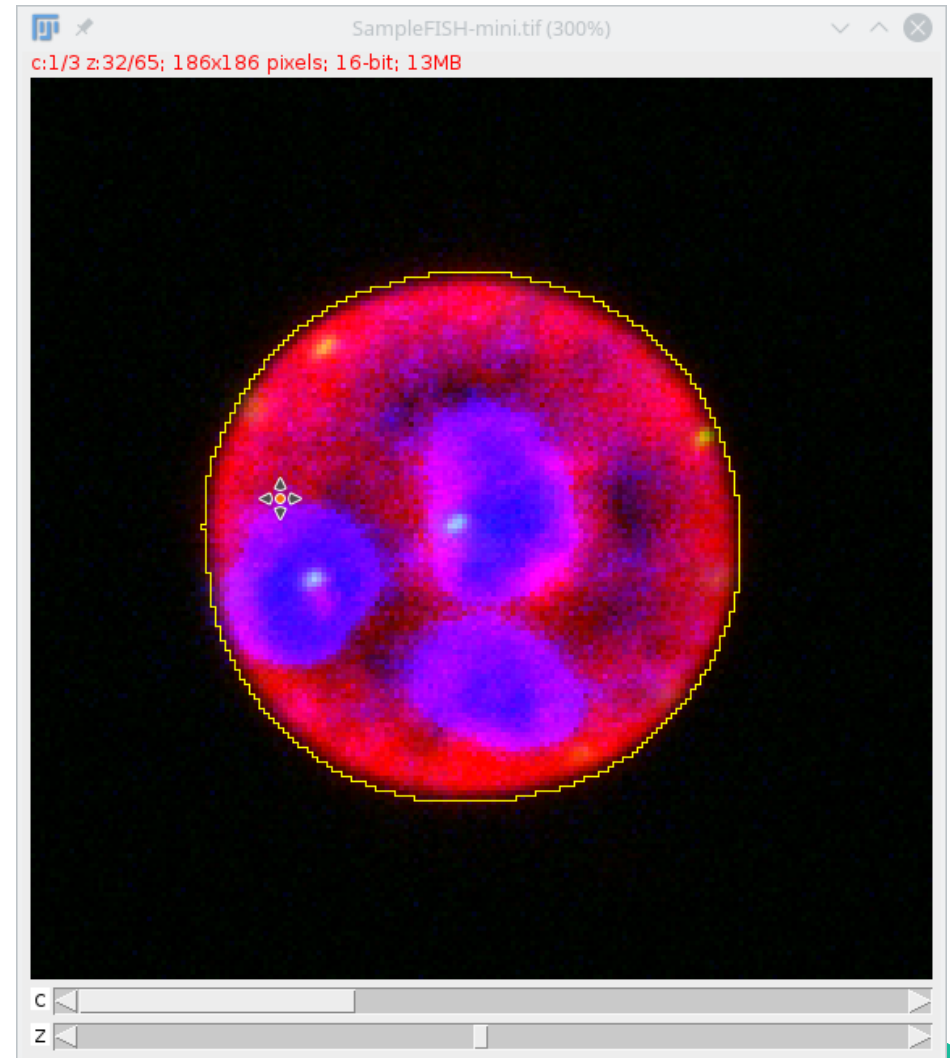
- Selected objects
 - None = all
- Set of Rois displayed on current image/slice
- Contour, centre, sphere or BB
- Do not display inclusions



3D Manager

- **Visualisation**

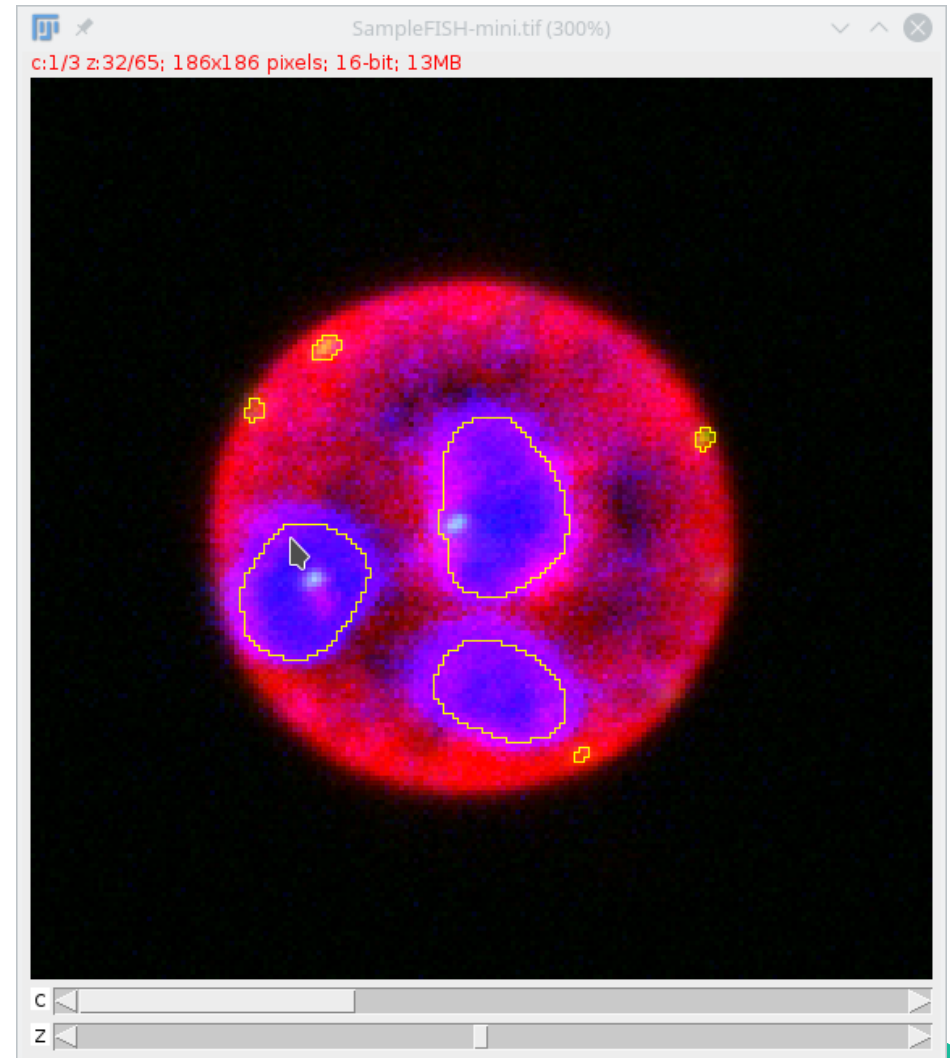
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3D Manager

- **Visualisation**

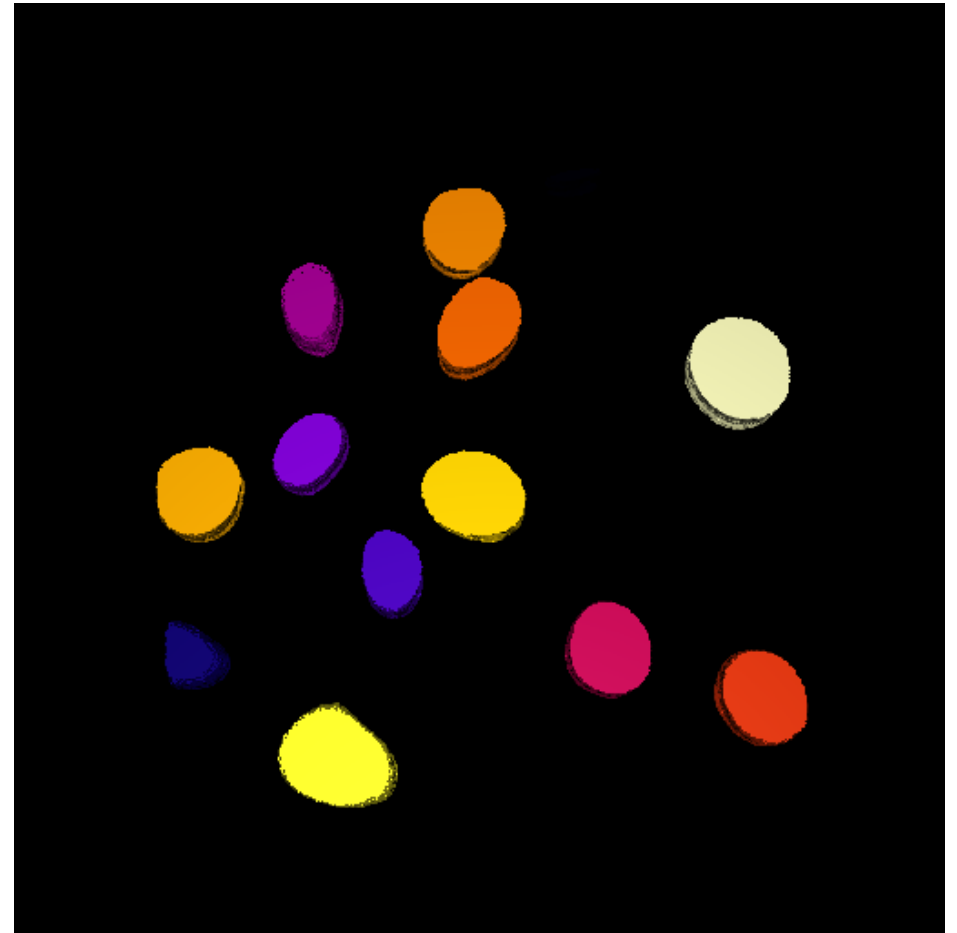
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3D Manager

- **Visualisation**

- Visualisation based on measurement
- Volume, compactness, ...
- Using LUT colors
- 3D Viewer (+smooth mesh) or stack
- Available soon in macro

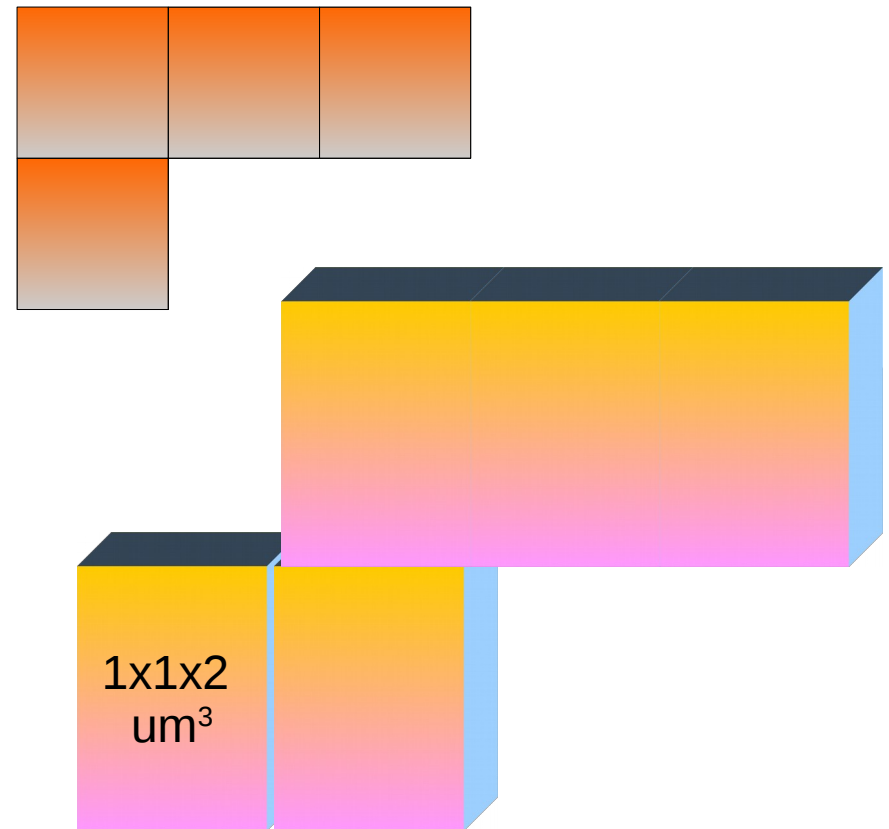


Analysis

- **Measurements available**
 - **Geometrical** measurements of objects
 - **Shape** measurements of objects
 - **Intensity** measurements of objects
 - Objects **numbering**
 - **Relationship** between objects
 - Co-localisation, distances
 - Angle between 3 objects (centres)

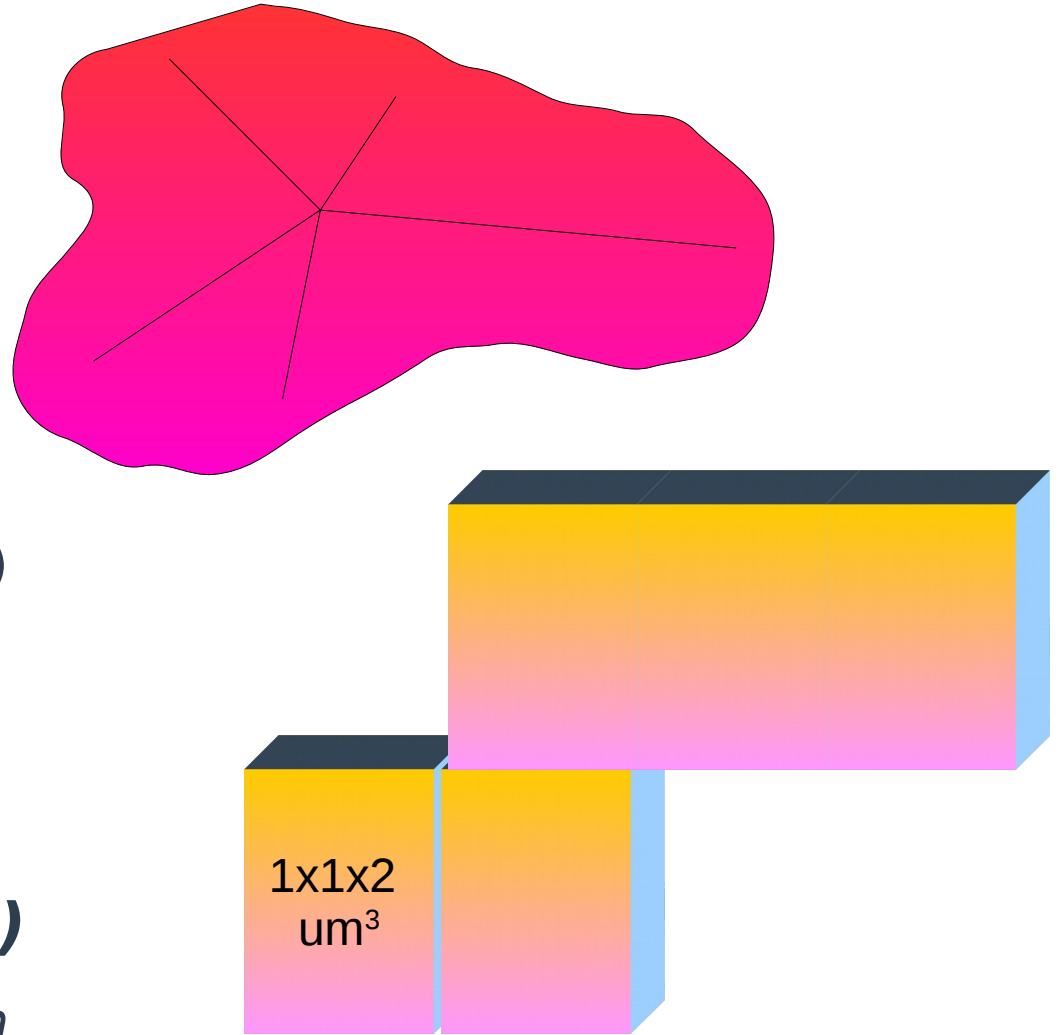
Geometry

- **Centroid**
- **Volume**
 - Nb of voxels (5 vox)
 - Nb of “unit cubes” (10 um^3)
- **Surface**
 - Nb of border “faces” (22 vox)
 - Nb of “unit faces” (36 um^2)
 - *Corrected surface (14.7 vox)*
 - *Surface area estimation of digitized 3D objects using weighted local configurations (Lindblad 2005)*



Geometry

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- **Surface**
 - Nb of border “faces” (22 vox)
 - Nb of “unit faces” (36 um^2)
 - *Corrected surface* (14.7 vox)
- **Feret (3.6 um)**
- **Distances to Center (um)**
 - *Min* (0.89), *max* (1.84), *mean* (1.36), *SD* (0.44)



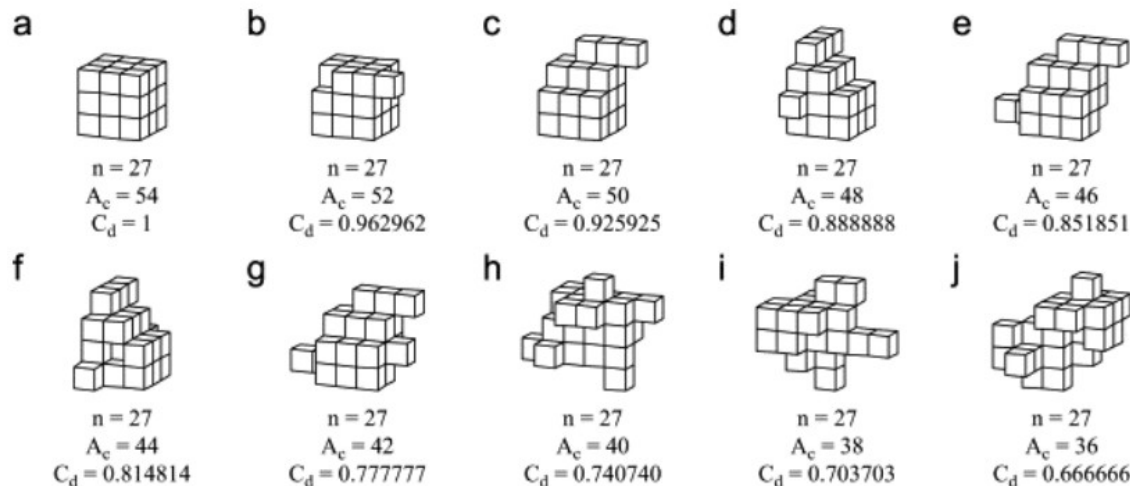
Shape

- **Compactness and sphericity**

- Ratio between volume and surface
 - In voxels or units
- Maximal compaction for sphere (1)
- Compactness discrete

$$C = \frac{36 \cdot \pi \cdot V^2}{A^3}; S = C^{1/3}$$

- *An easy measure of compactness for 2D and 3D shapes, Bribiesca 2008*



$$C_d = \frac{n - A/6}{n - (\sqrt[3]{n})^2}$$

Shape

- **Ellipsoid fitting**

- Best fitting ellipsoid

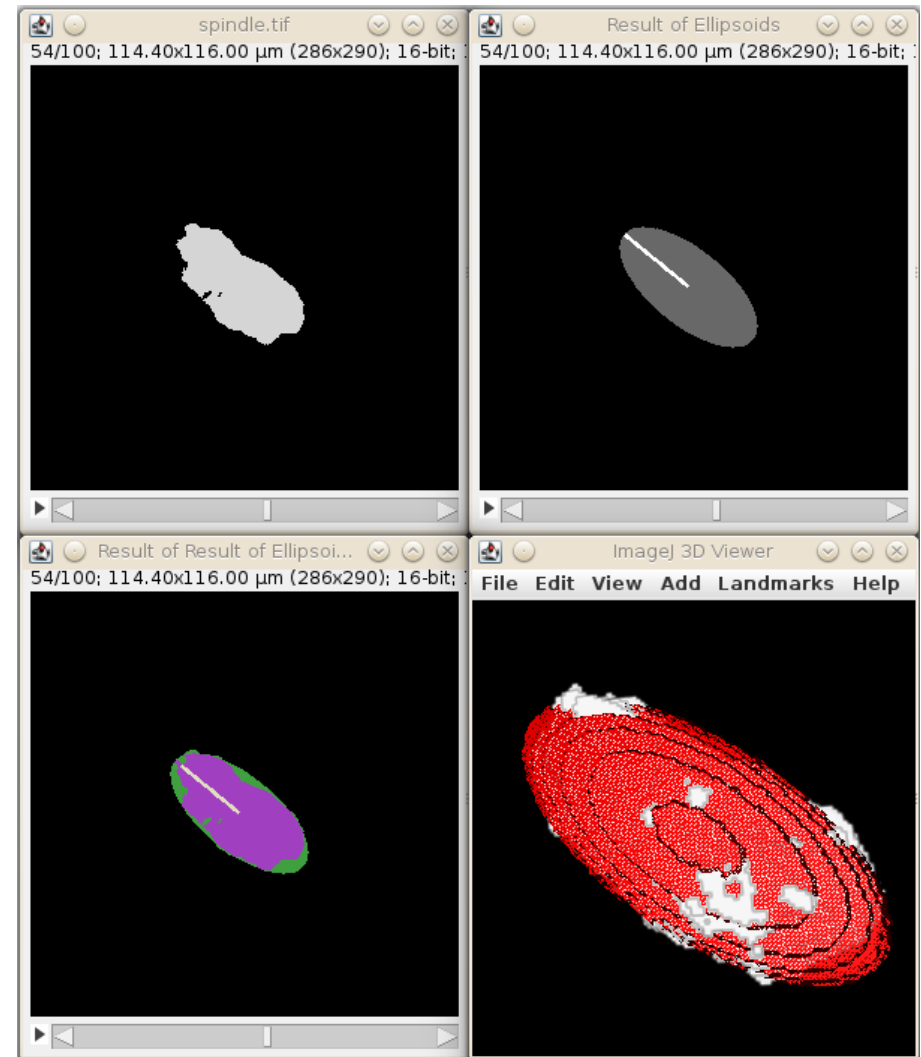
$$sxx = \sum \frac{(x - C_x)^2}{Volume}; syy = \sum \frac{(y - C_y)^2}{Volume}; szz = \sum \frac{(z - C_z)^2}{Volume}$$

$$sxy = \sum \frac{(x - C_x)(y - C_y)}{Volume}$$

$$sxz = \sum \frac{(x - C_x)(z - C_z)}{Volume}$$

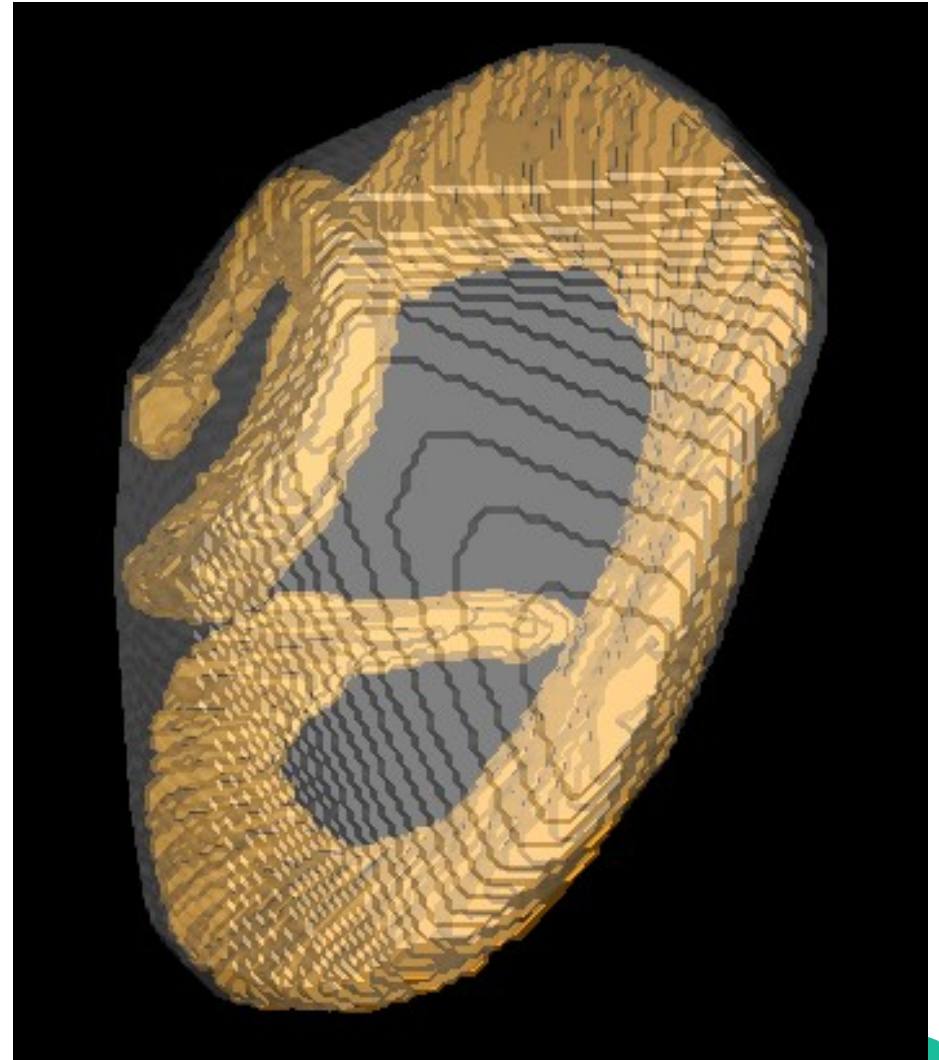
$$syz = \sum \frac{(y - C_y)(z - C_z)}{Volume}$$

- Radii = $\sqrt{(5 \cdot eigen)}$
- Elongation = R1/R2
- Flatness = R2/R3
- Ratio V_ell / V_object



Shape

- **Convex Hull**
 - Minimal enclosing convex shape
 - Binary to mesh
 - Convex hull
 - Mesh to binary
 - Ratio V_{Hull} / V_{obj}
- <https://imagej.nih.gov/ij/plugins/3d-convex-hull/index.html>



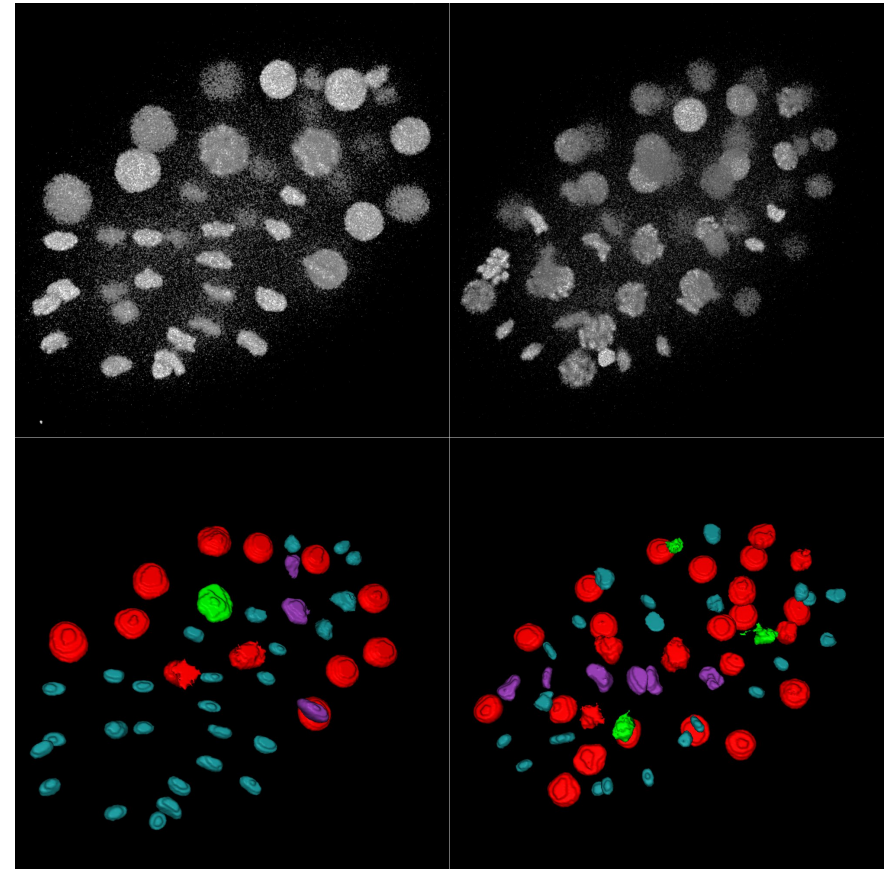
Shape

- **3D Moments**

- Based on ellipsoid computation
- $J1 = sxx + syx + szz$
- $J2 = \dots$

- **Used for more accurate shape description**

- GulMohammed 2014, BMC Bioinformatics

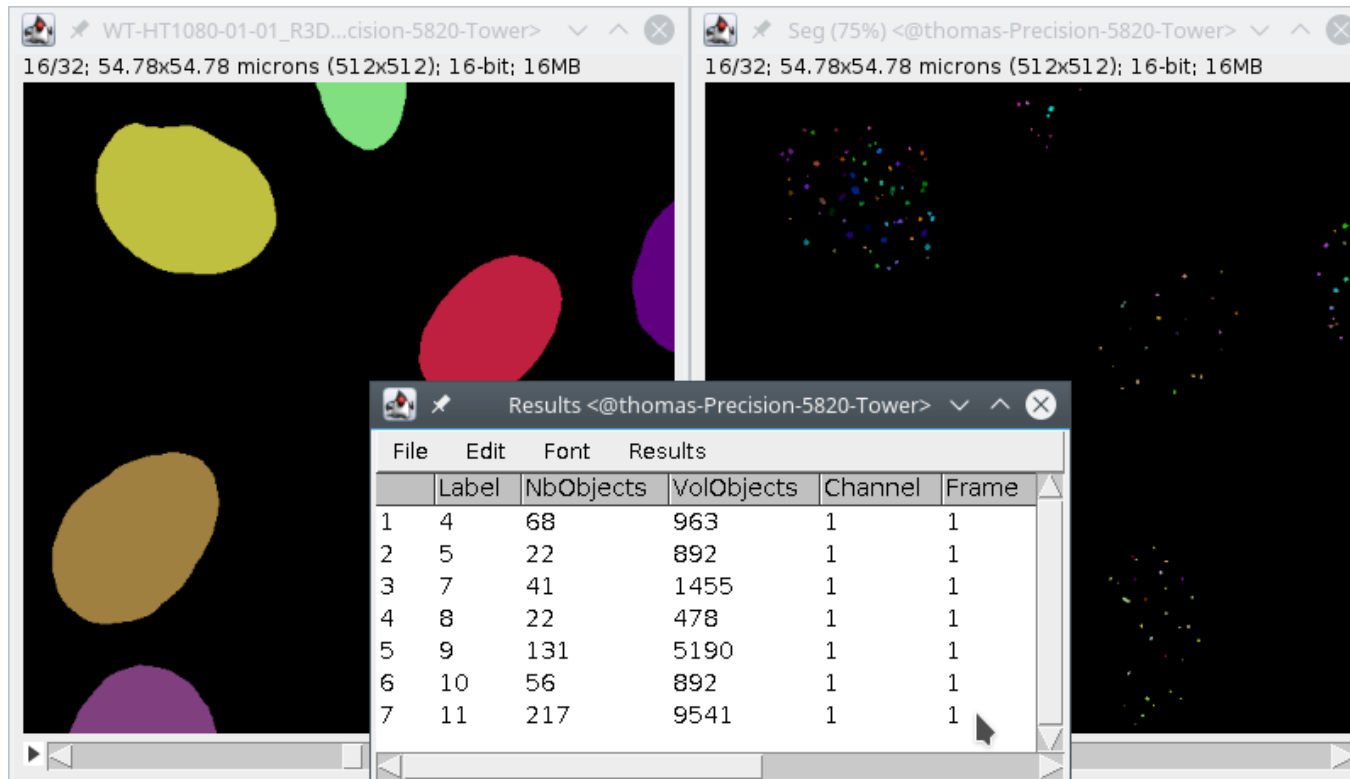


Intensity

- **At Centre**
- **Mean, Min, Max, SD**
- **Mode**
 - Most abundant value
 - Most abundant value > 0
- **Integrated density**
 - Sum of all pixel values
- **List all values**

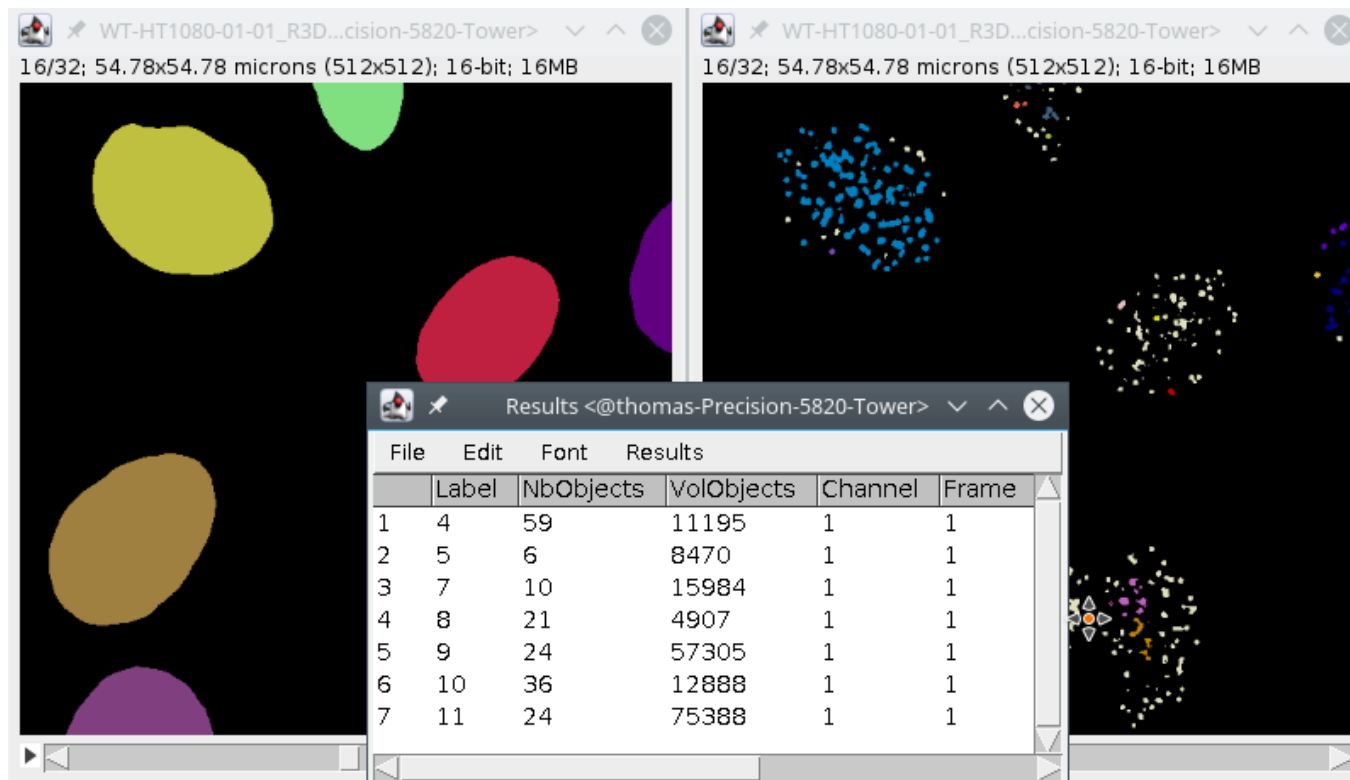
Numbering

- **Number of labels inside an object**
- **Volume occupied by labels**



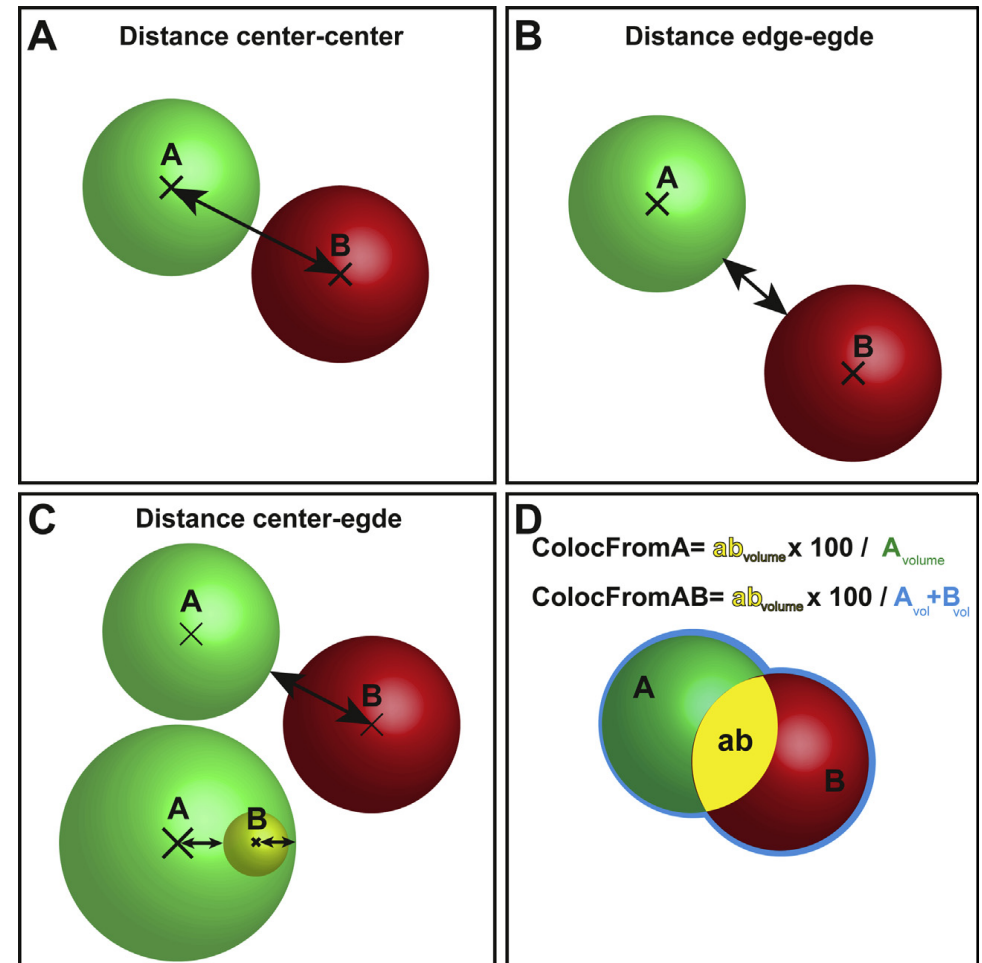
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Distances

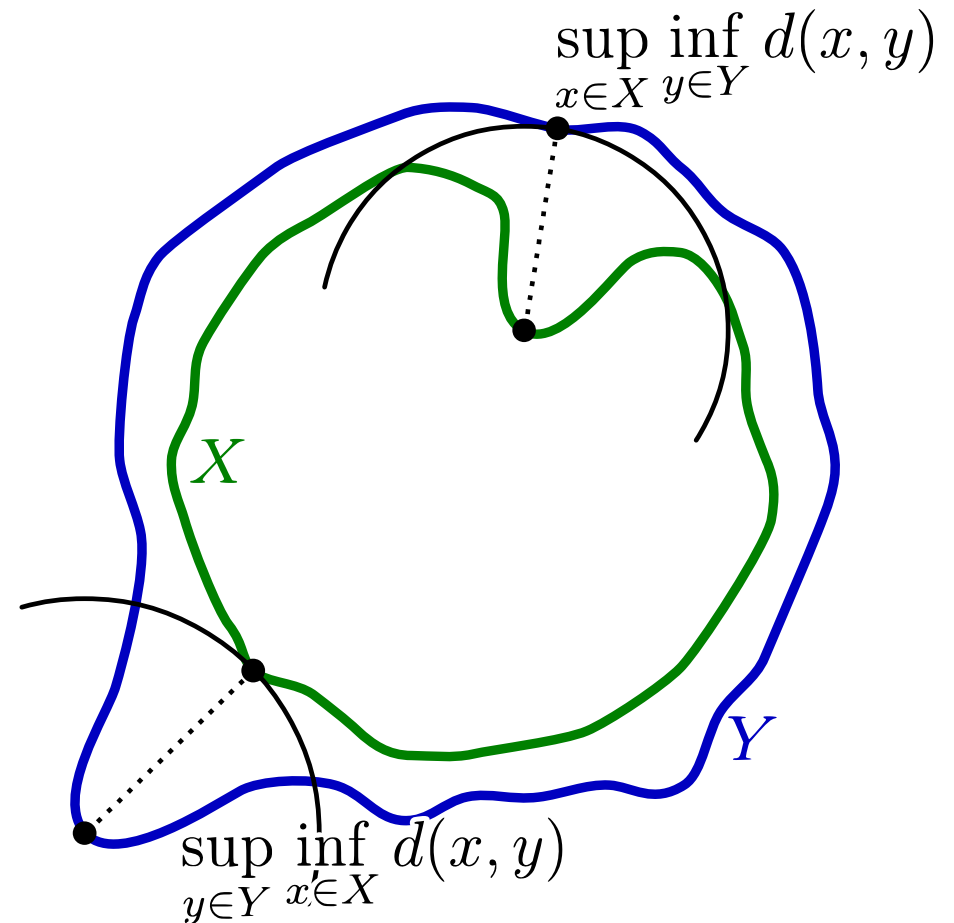
- Centre
- Border
- Hausdorff (plugin)
- Radial distance
- Closest



Gilles et al., DiAna

Distances

- Centre
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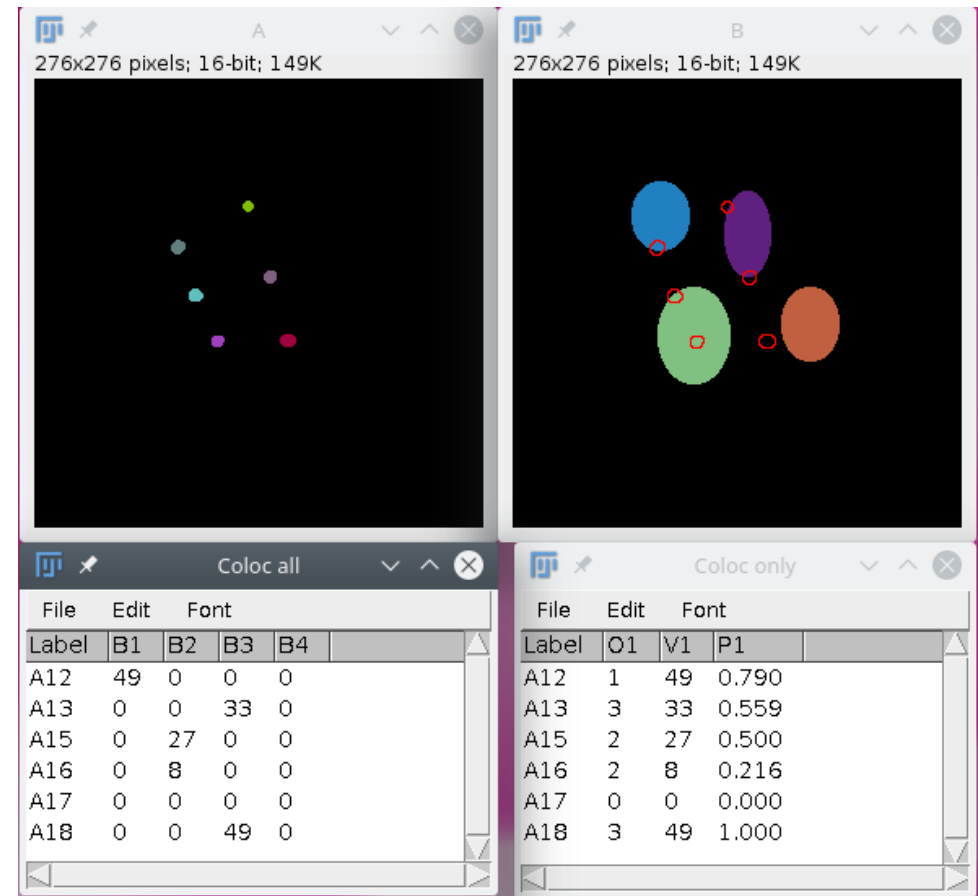


By Rocchini - Own work, CC BY 3.0,
<https://commons.wikimedia.org/w/index.php?curid=2918812>

Colocalisation

- **Coloc (nb voxels)**
- **Percentage coloc**
 - Relative to objects volumes
- **Plugin multiColoc**
- **Surface contact**

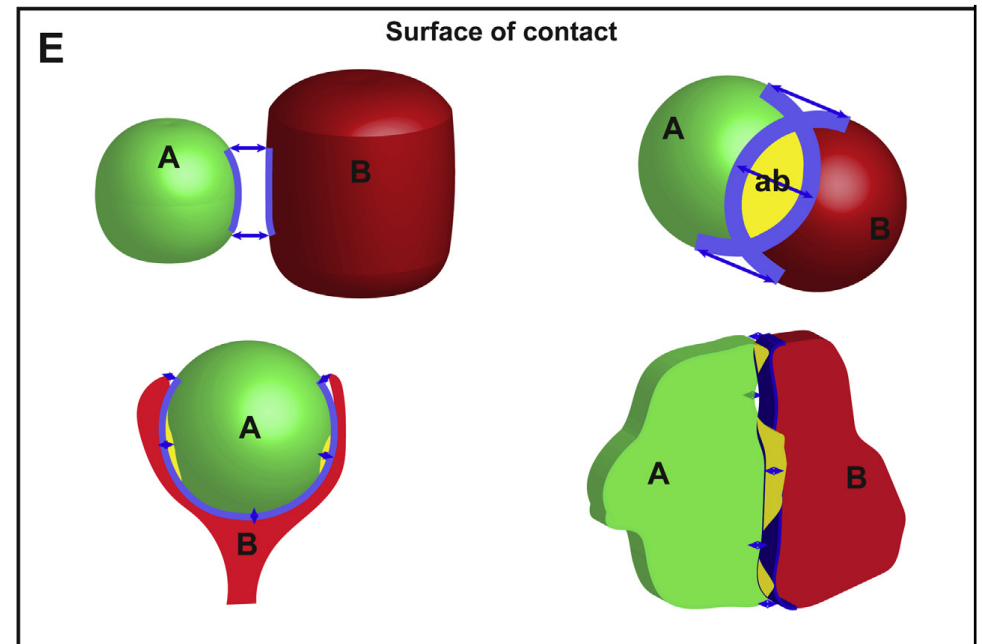
Gilles et al. DiAna, an ImageJ Tool for Object-Based 3D Co-Localization and Distance Analysis, 2017



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Gilles et al. DiAna, an ImageJ Tool for Object-Based 3D Co-Localization and Distance Analysis, 2017



Gilles et al., DiAna

Analysis

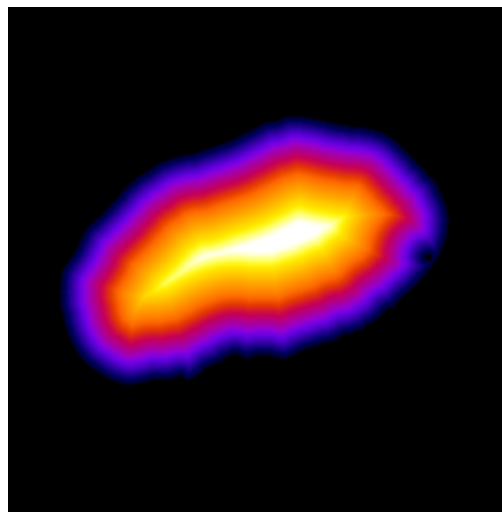
- **Other analysis available in 3D ImageJ Suite**
 - EVF
 - Interactions (Voronoi)
 - Spatial Statistics
 - ...

EDT - EVF

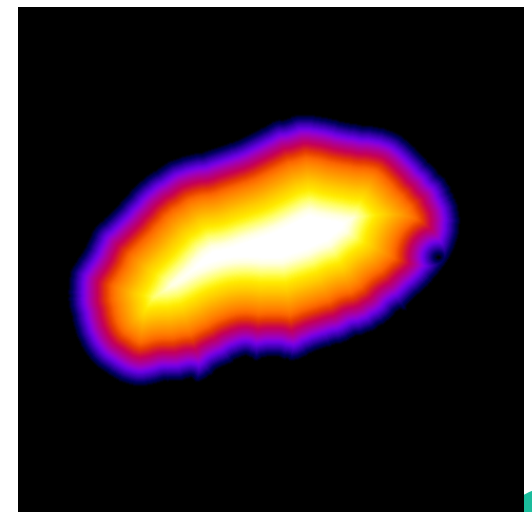
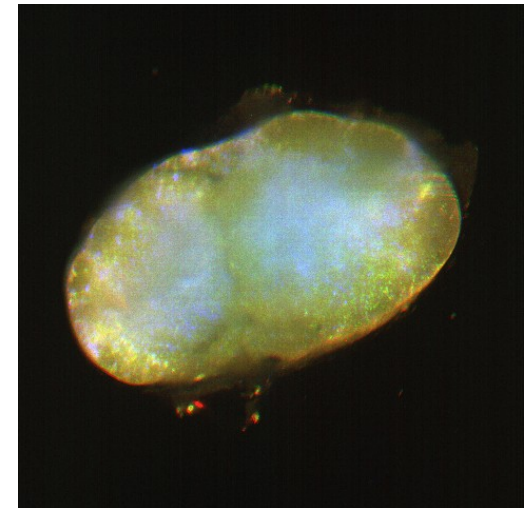
- **EDT :
Euclidean
Distance
Map**



- **EVF :
Eroded
Volume
Fraction**



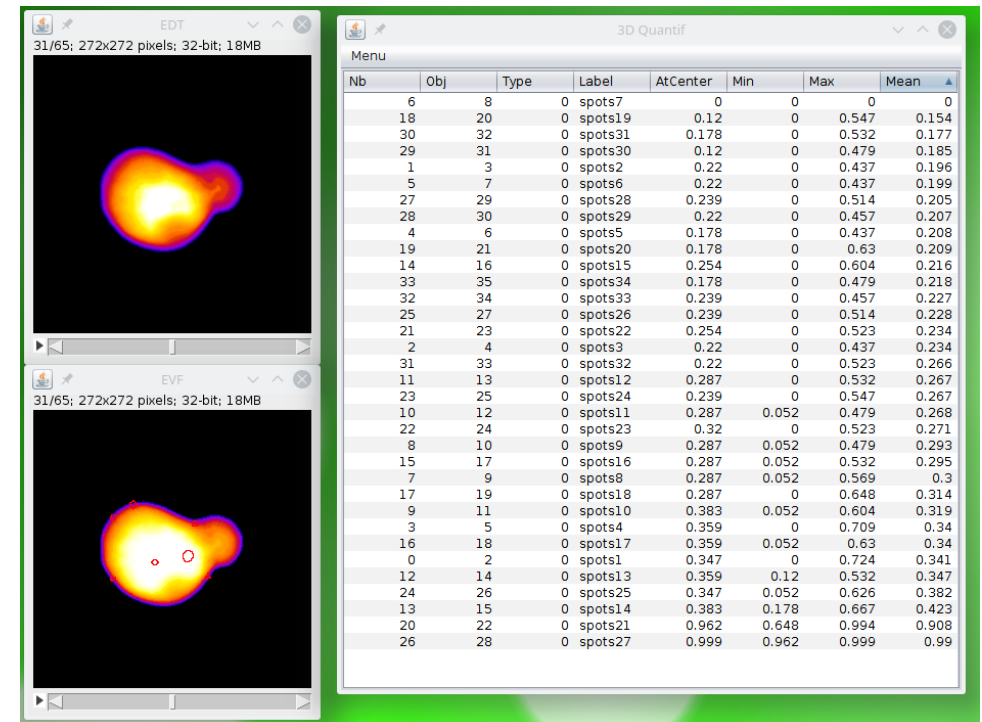
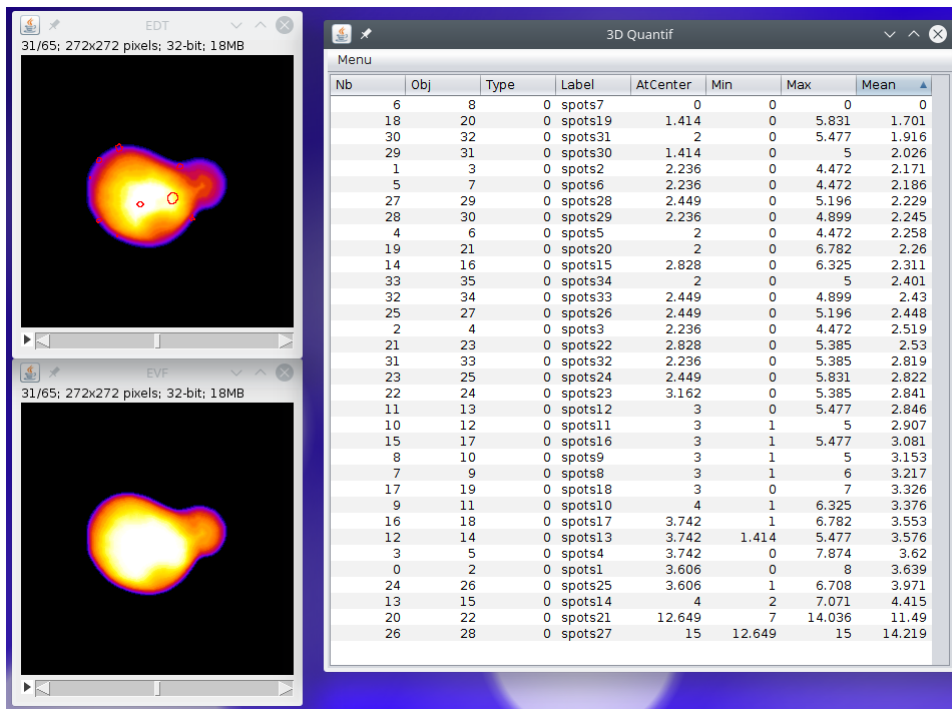
J. Groom, WEHI



EDT - EVF

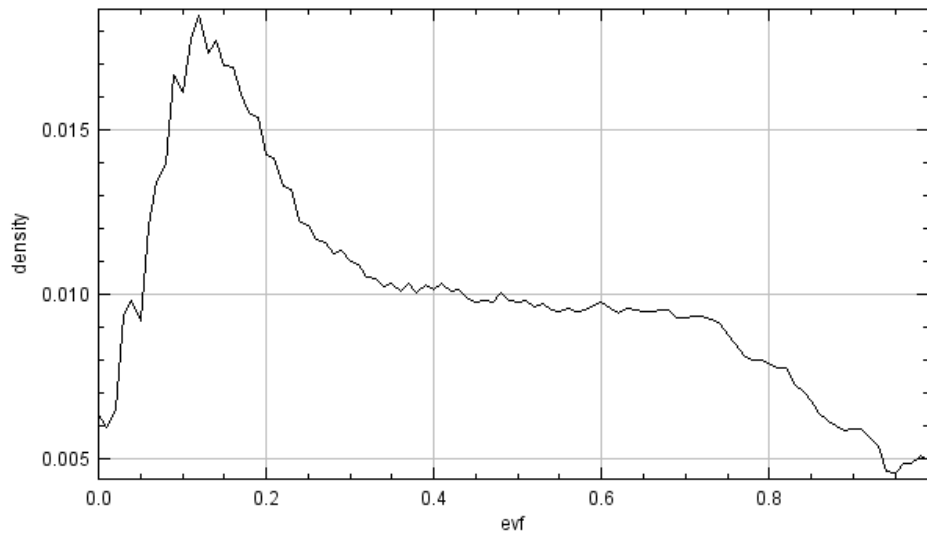
Values are calibrated distances from closest border

Values are normalised between 0 and 1, from closest to furthest

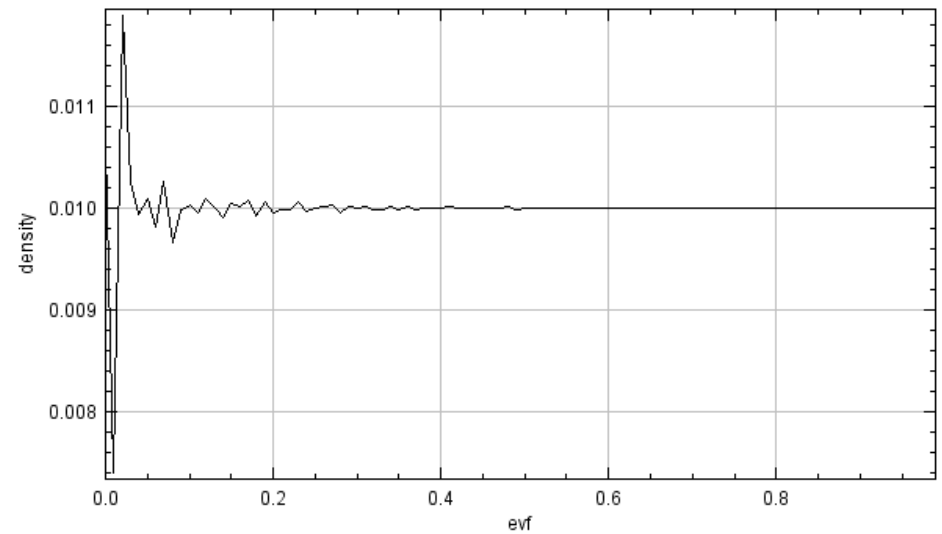


EDT - EVF

Compute number and volume of spots within layers (0-0.01, ..)

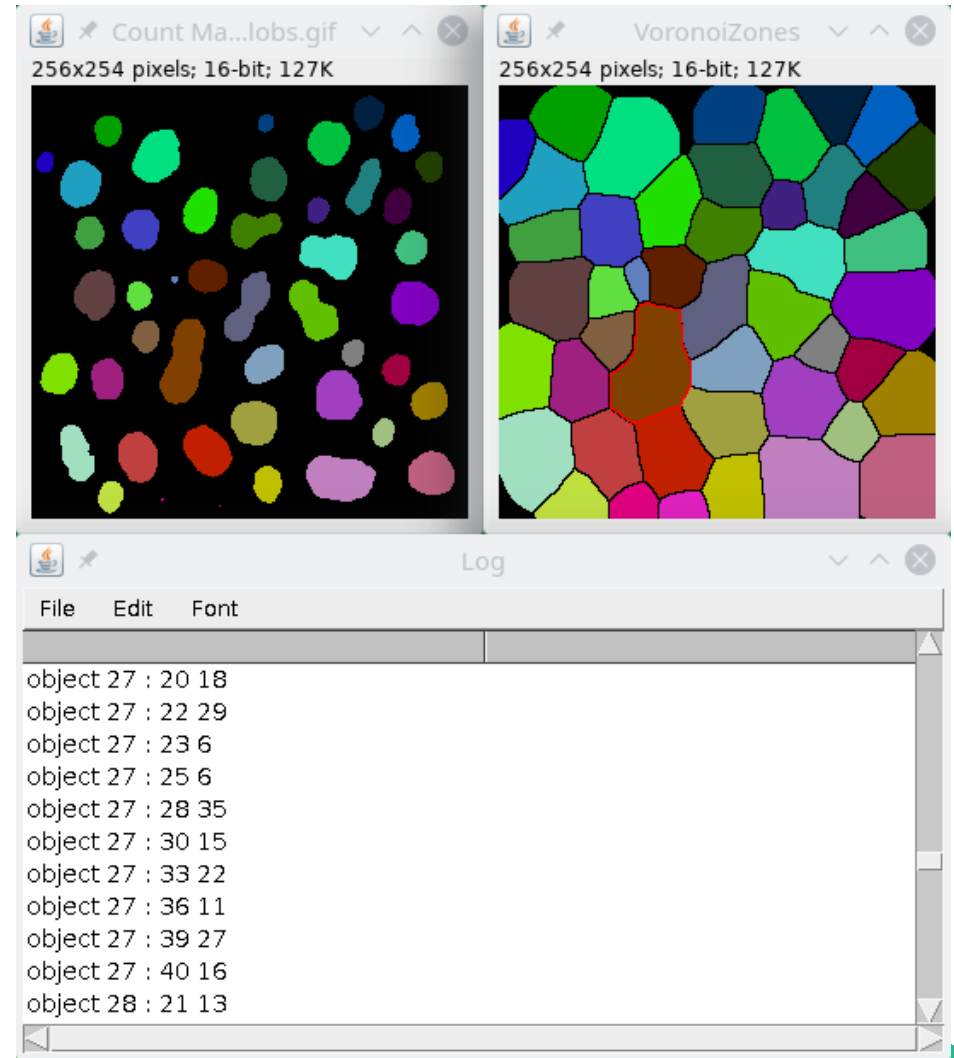


Compute volume inside 100 layers (0-0.01, ...),

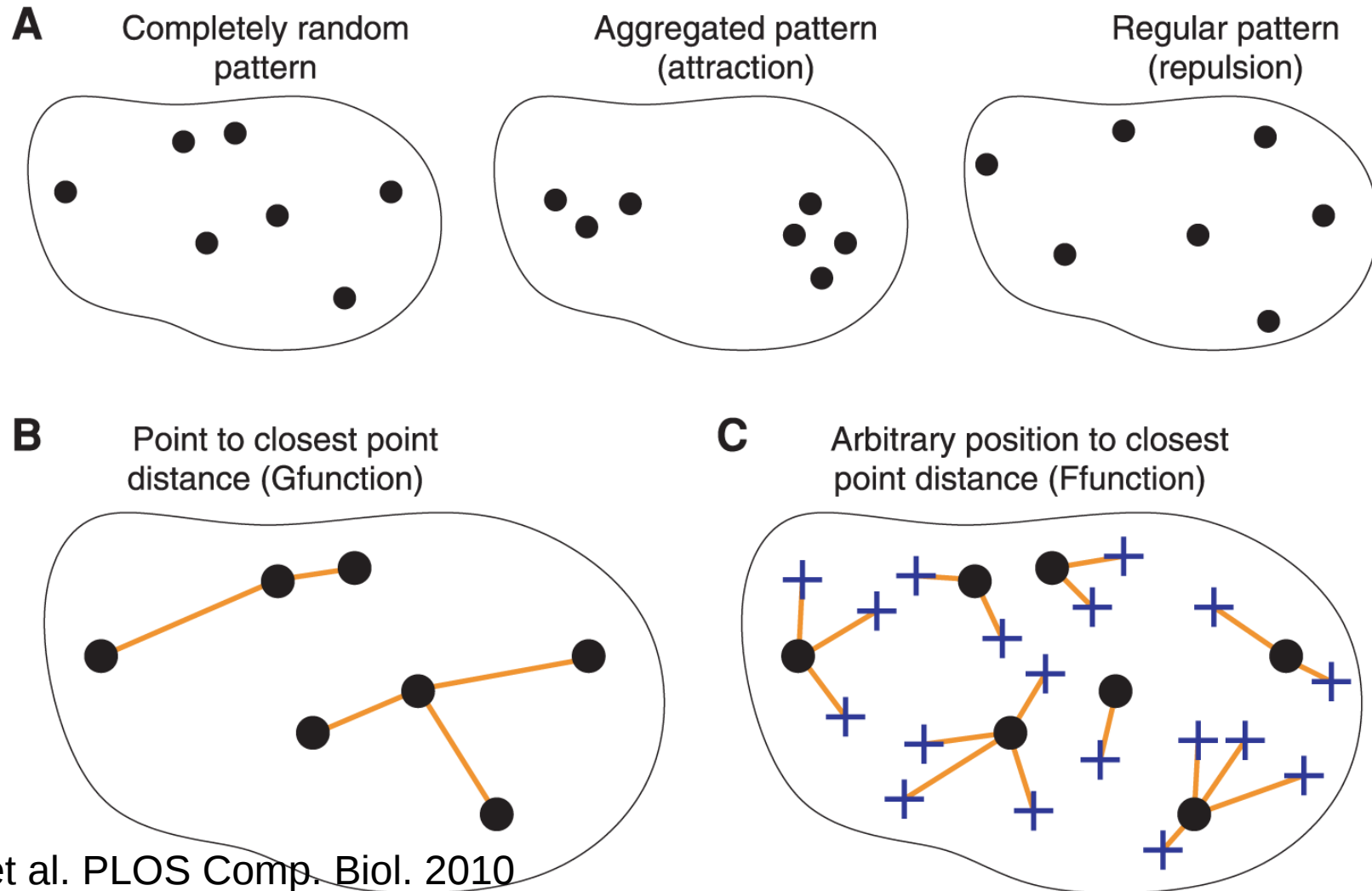


Interactions

- **Compute zones around objects :**
 - Watershed / Voronoi
 - Within fixed radius
- **Dam lines will separate zones**
- **Compute touching**
 - Dam lines, touching, or dilate



Spatial statistics



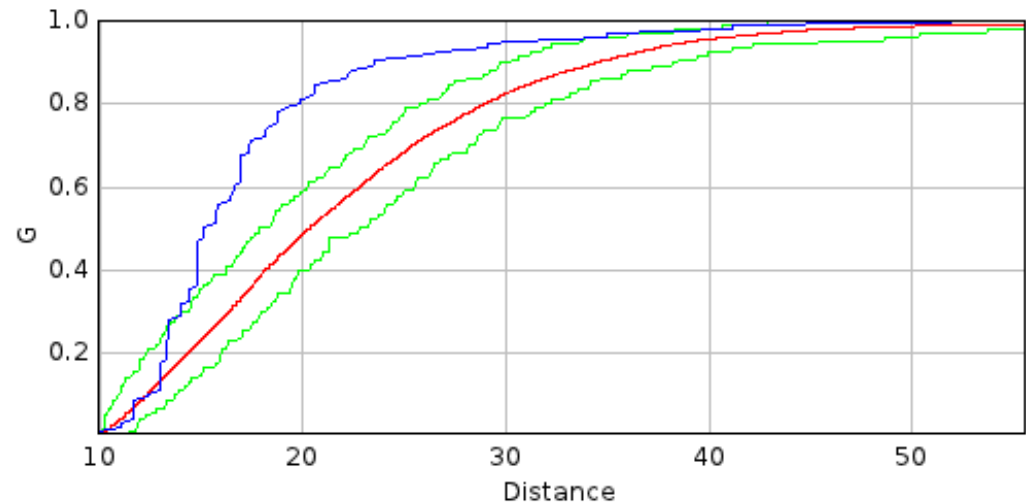
Andrey et al. PLOS Comp. Biol. 2010

Spatial organization

Clusters :

A lot of small distances between spots → G above curve of random organisation

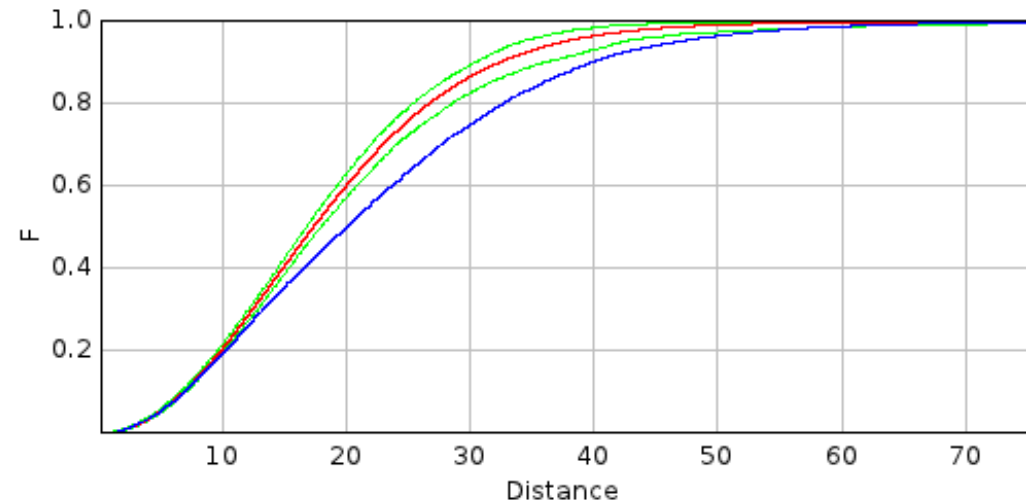
A lot of « voids » in the structure, large distances between reference points and spots → F below curve of random organisation



Uniform :

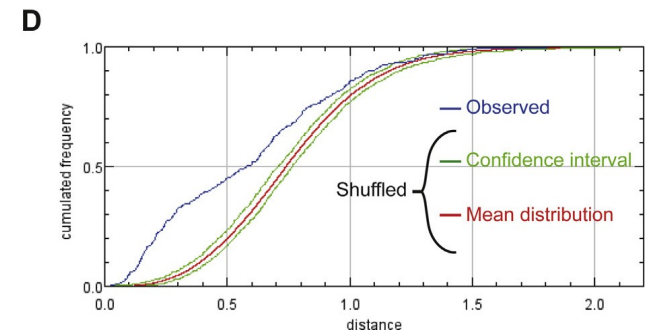
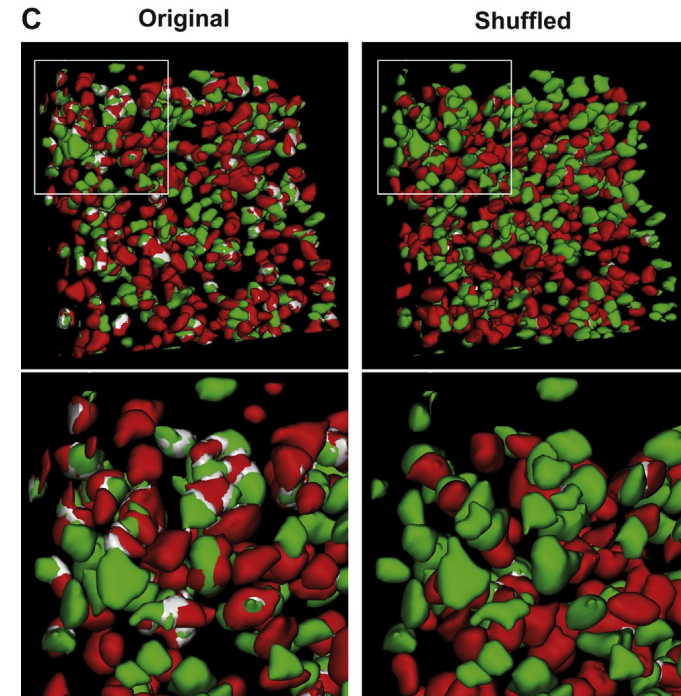
A lot of large similar distances between spots → G below curve of random organisation

No « voids » in the structure, small distances between reference points and spots → F above curve of random organisation



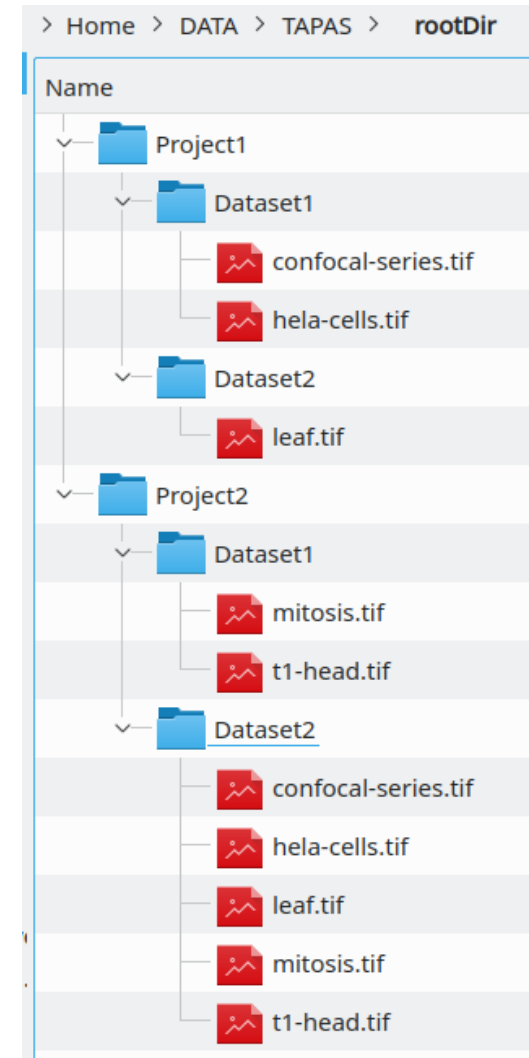
Spatial organization

- **Statistical comparison**
 - original measurements
 - measurements from modelled data
- **Different models**
 - Random
 - Shuffled data

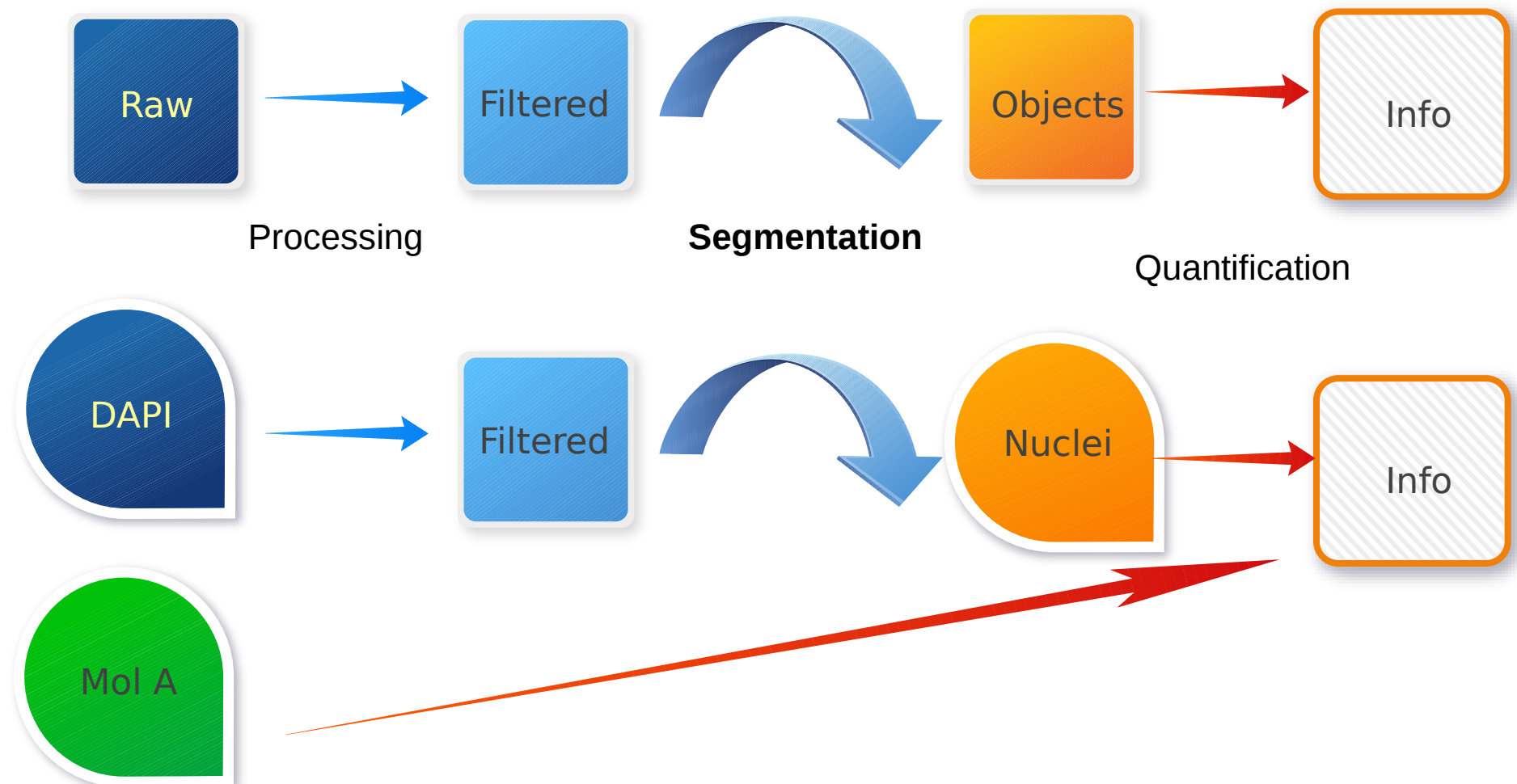


DATA organisation

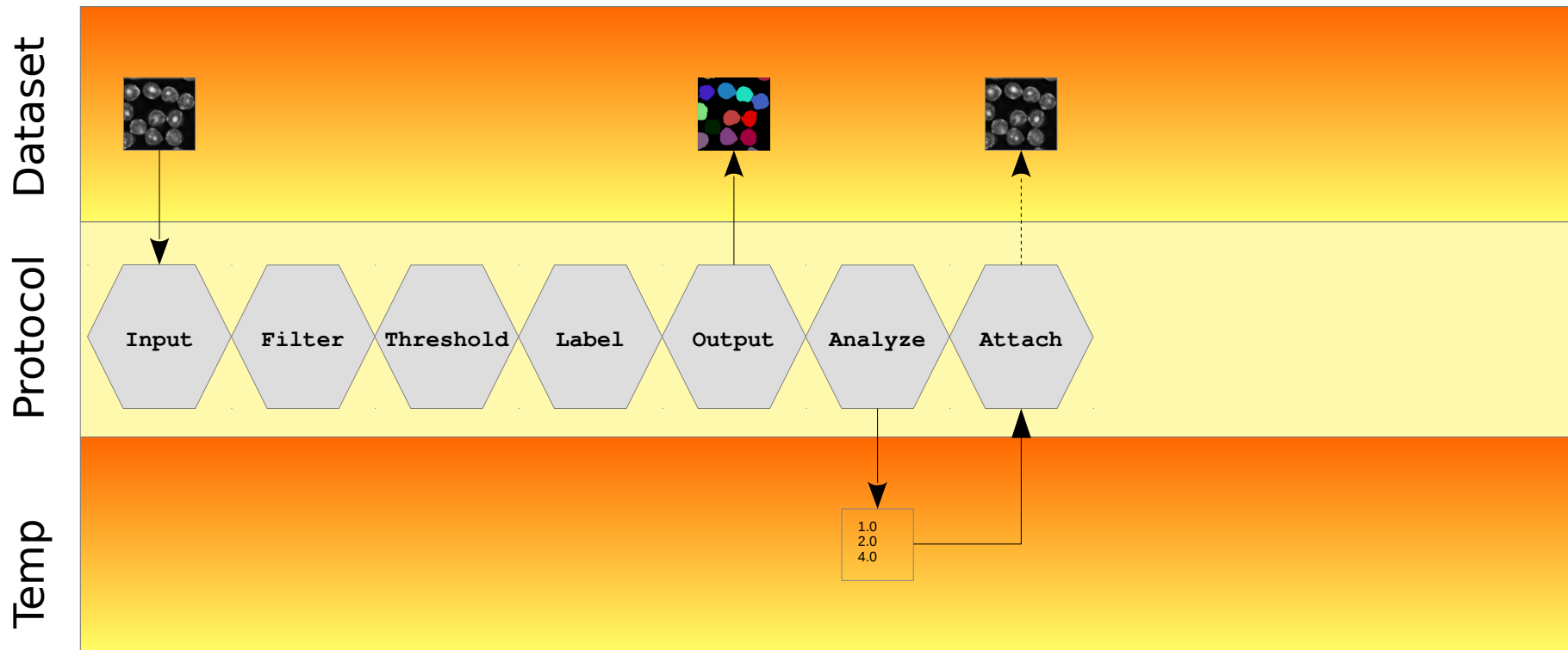
- **Projects / Datasets**
 - Raw data
 - *Filtered data*
 - Segmented data
 - *Analysed data*
 - Results
- **On Disk or DB (OMERO)**



Protocol



TAPAS



<https://imagej.net/TAPAS>

TAPAS

- **A protocol is a list of modules**
- **A module is defined by**
 - Name of the module
 - ***process:filter***
 - *The parameters*
 - *radiusxy:4*
- **On which datasets to apply the protocol**
 - Either on disk or OMERO

```
// read data
process:input

// filter
process:filters
radiusxy:4
radiusz:2
filter:median

// threshold
process:autoThreshold
method:Otsu

// label
process:label
minVolume:100

// output
process:output
name: ?name?-seg
```

TAPAS

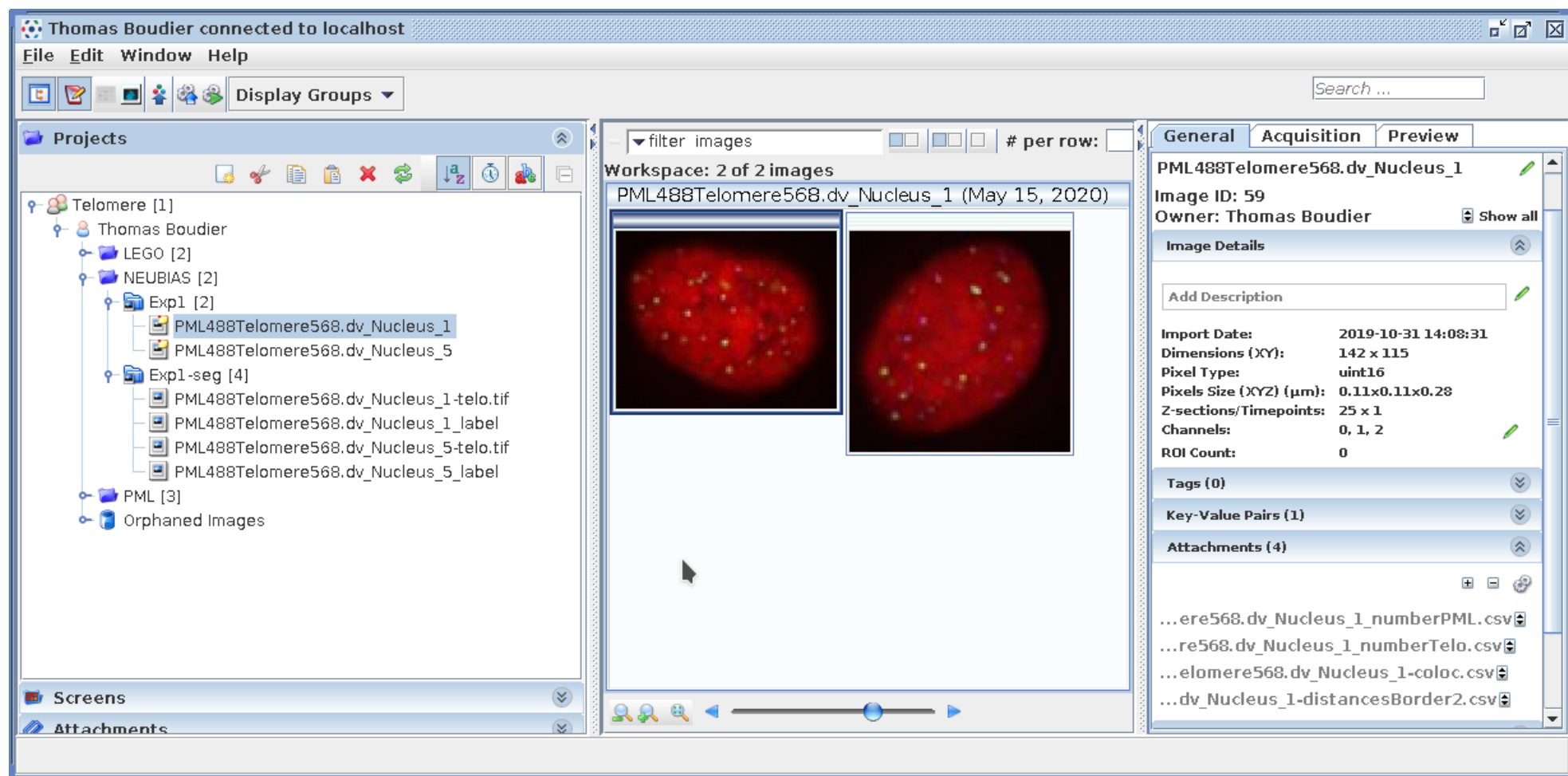
- **A protocol is a list of modules**
- **A module is defined by**
 - Name of the module
 - *process:filter*
 - The parameters
 - *radiusxy:4*
- **On which datasets to apply the protocol**
 - Either on disk or OMERO

```
// analyse
process:measurement
dir:?ij?
file:?name?-results.csv
list:volume, centroid

// attach
process:attach
dir:?ij?
file:?name?-results.csv

// delete
process:delete
dir:?ij?
file:?name?-results.csv
```

TAPAS

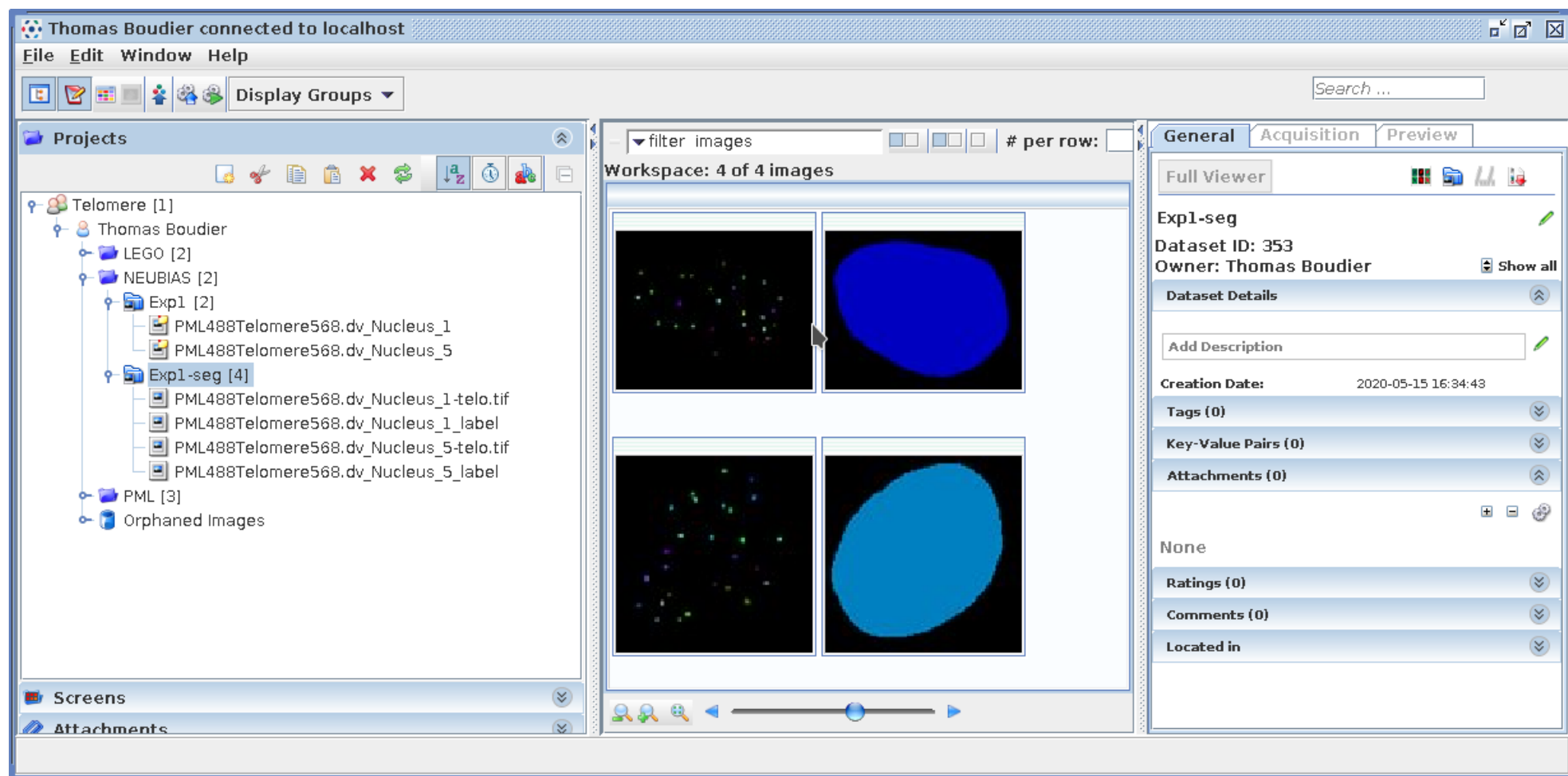


L. Chen, IMB, AS

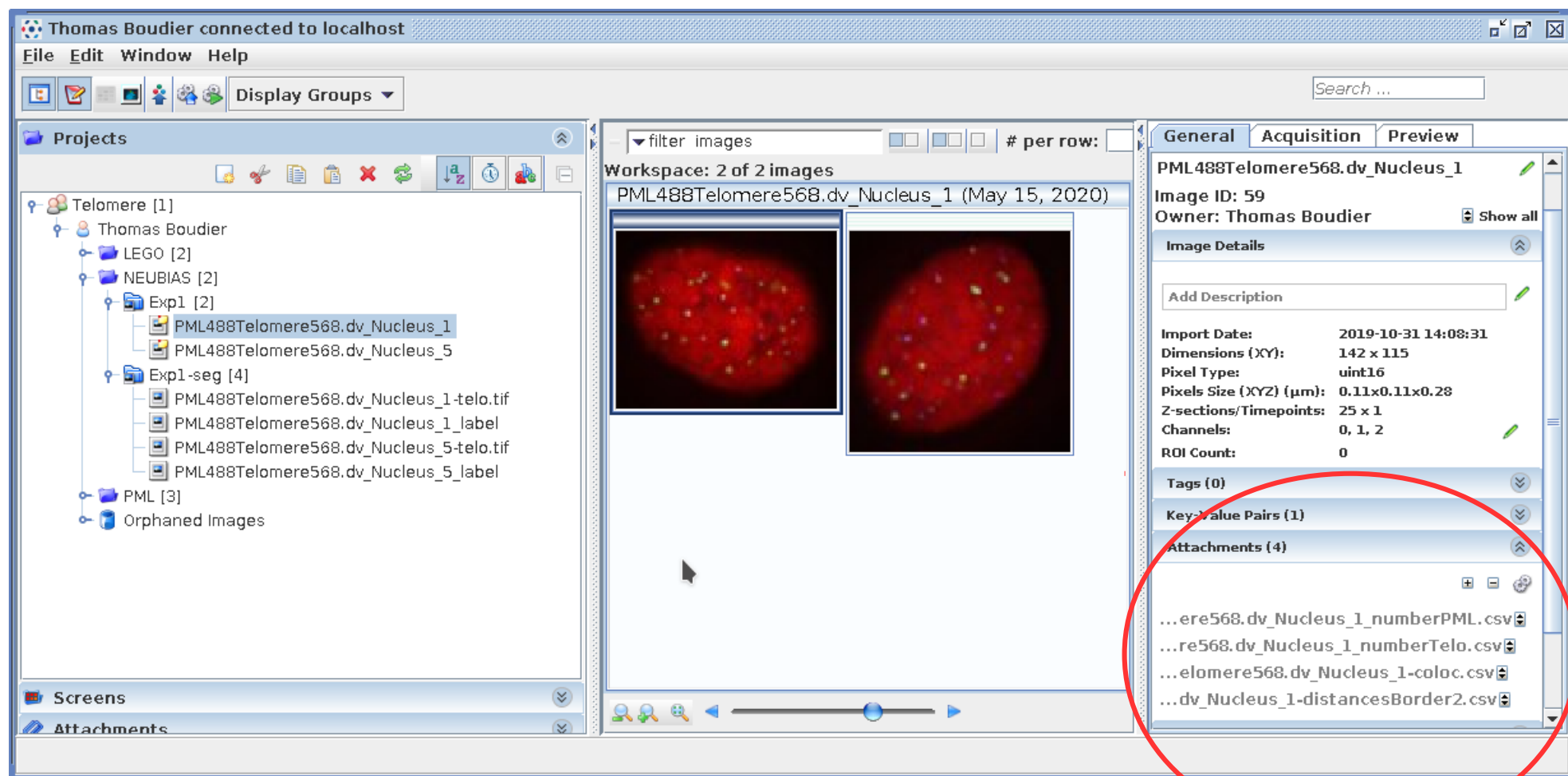
NEUBIAS Academy

T. Boudier - 3D ImageJSuite

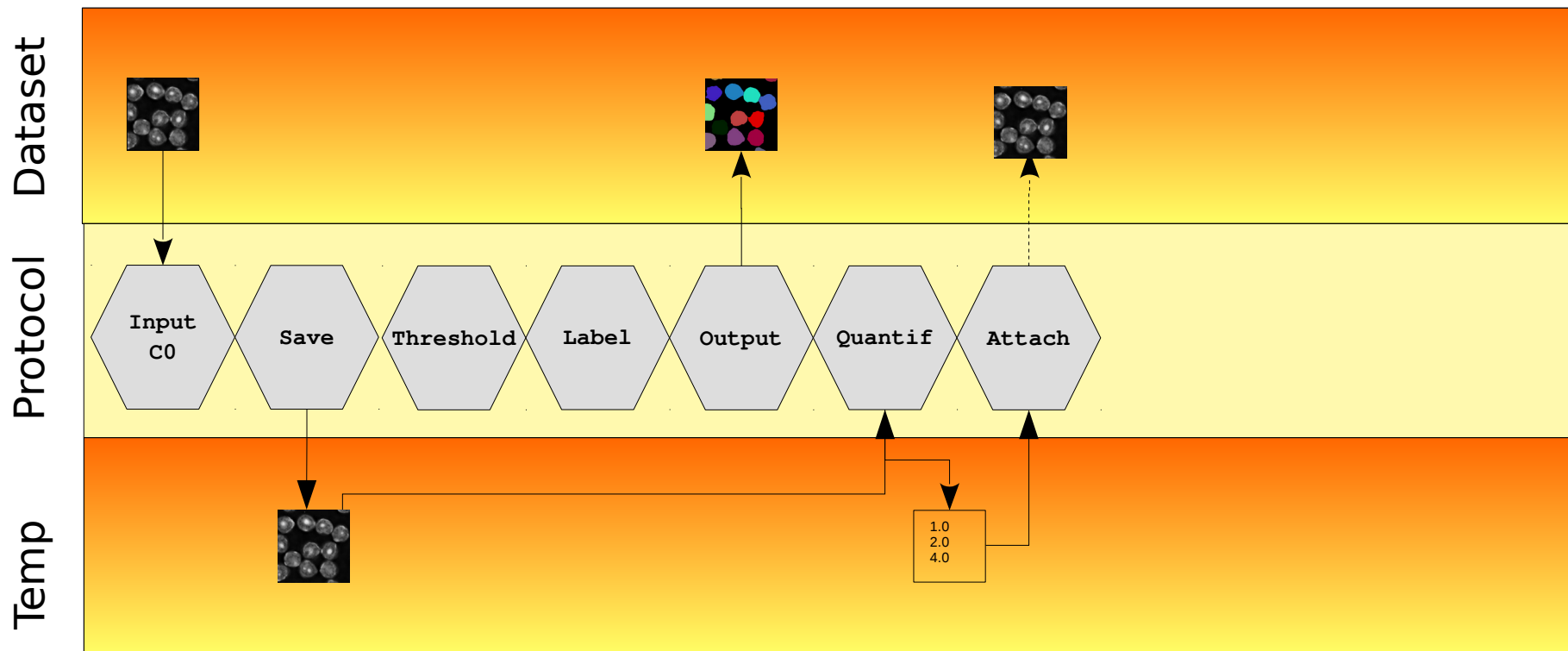
TAPAS



TAPAS

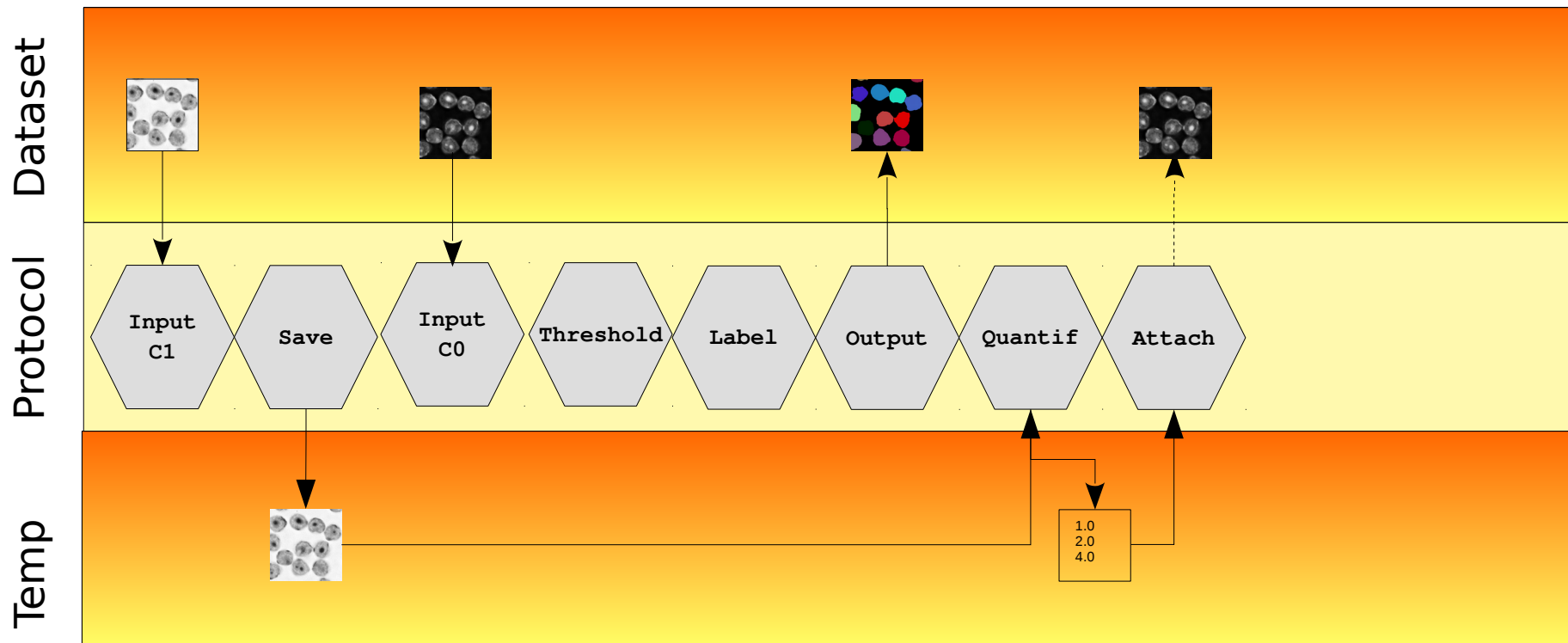


TAPAS



<https://imagej.net/TAPAS>

TAPAS



<https://imagej.net/TAPAS>

General protocol

- **Channels / Structures**
 - One channel → one or many structures
 - One Structure → one or many channels
- **Filter/Segment structures**
- **Geometry/Shape of structures**
- **Intensity of channels within structures**
- **Analysis between structures**
 - Distances, ...

Summary

- **3D ImageJ Suite :**
 - Set of tools for 3D Analysis
 - 3D Manager main graphical interface
 - Set of macros Extension (not detailed here)
- **TAPAS**
 - Framework for automation
 - Agnostic of *where* is the data and *who* is processing

What next ?

- **Q/A in Image.sc Forum**
 - Data and protocols available
- **ImageJ 3D Suite**
 - Better Roi handling in 3D Manager
 - New plugin 1-1 Association (tracking)
- **TAPAS**
 - New modules CLIJ and DeepLearning
 - NEUBIAS webinar ?