

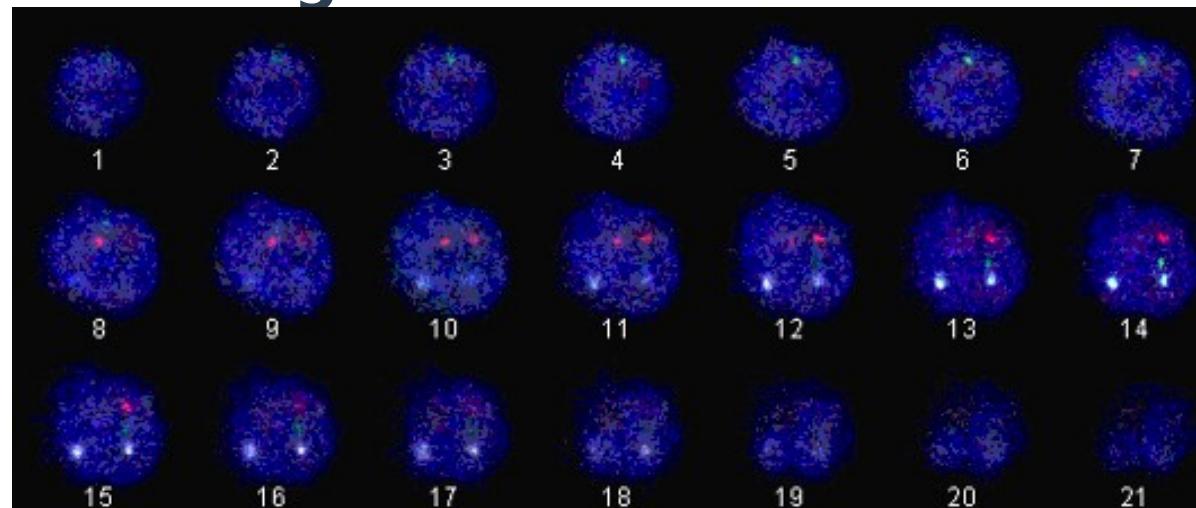
Introduction to 3D Analysis With the 3D ImageJ Suite

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

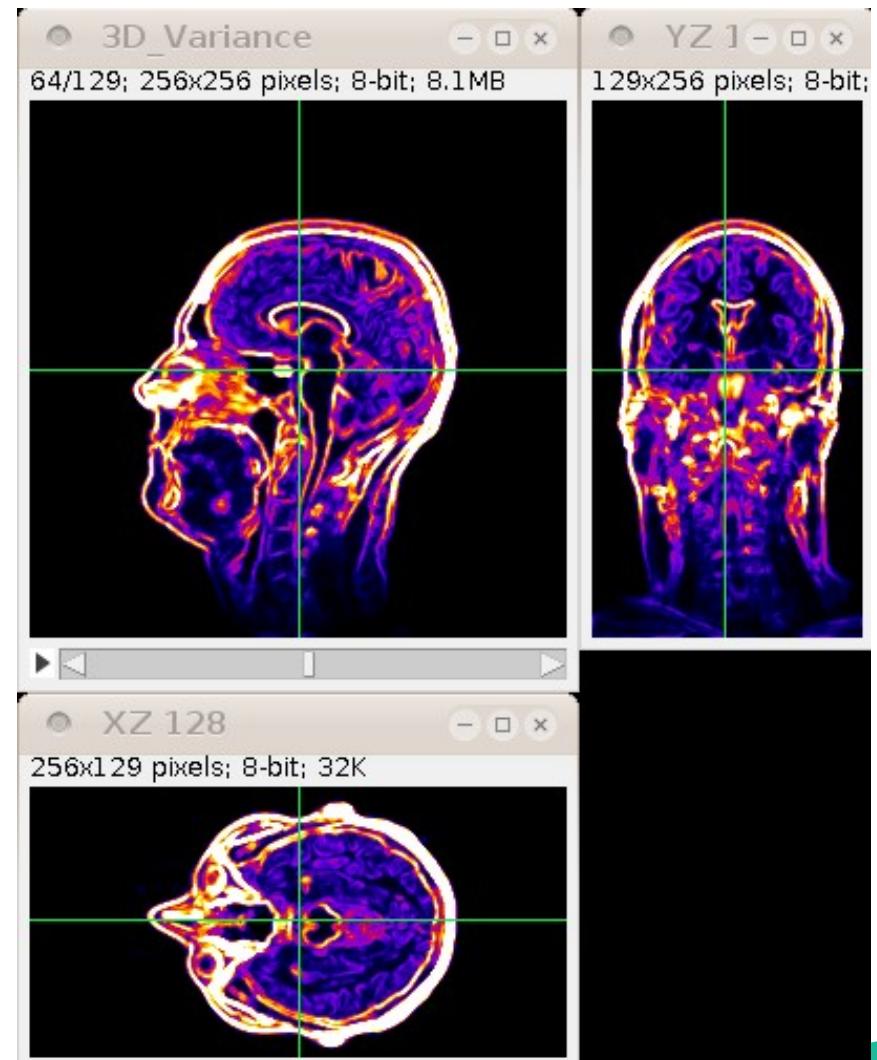
- **Dr Cedric Messaoudi, first version**
- **Dr Jean Ollion, second version**
- **Me, supervision and maintenance**
- **Acknowledgment : Dr Philippe Andrey**
- **PhD Students : Cédric, Seb, Jean, Jaza, Hoa, Lamees, Afshin**
- **Extensive (but not complete) documentation**
 - https://imagej.net/3D_ImageJ_Suite

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)

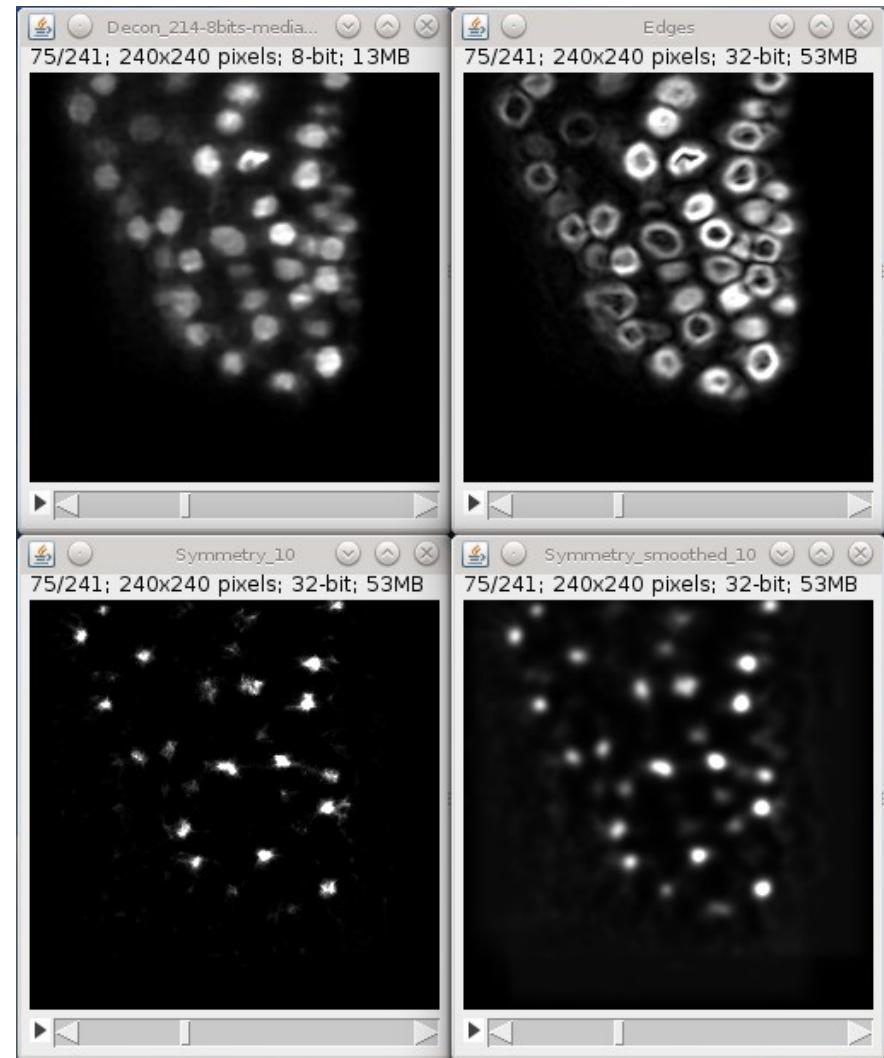


Pre-processing - edge symmetry

- **Edge and symmetry filter**

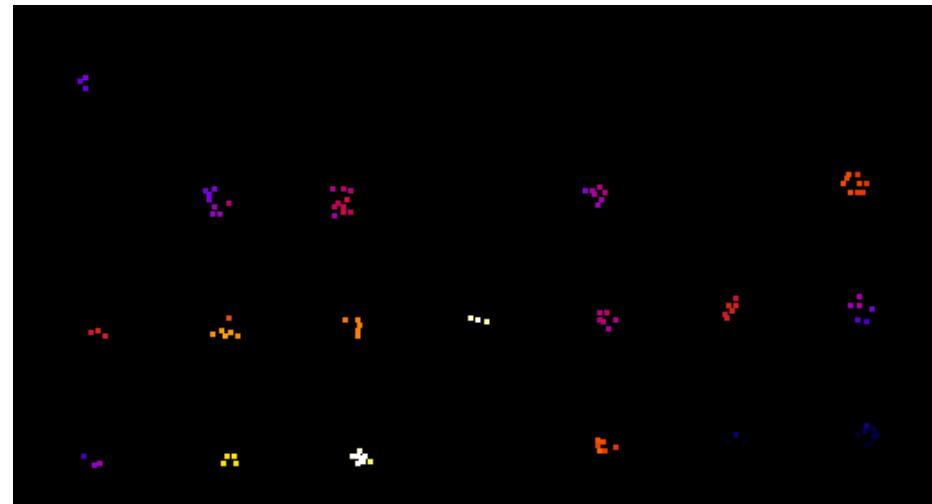
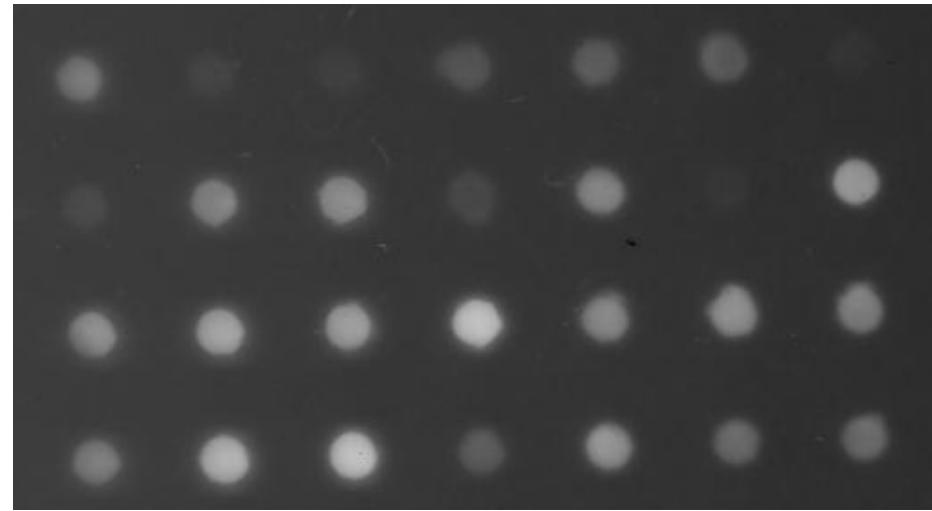
- Compute edges in X, Y and Z
 - Compute “magnitude” of edge
 - Rays converging towards centres of objects

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



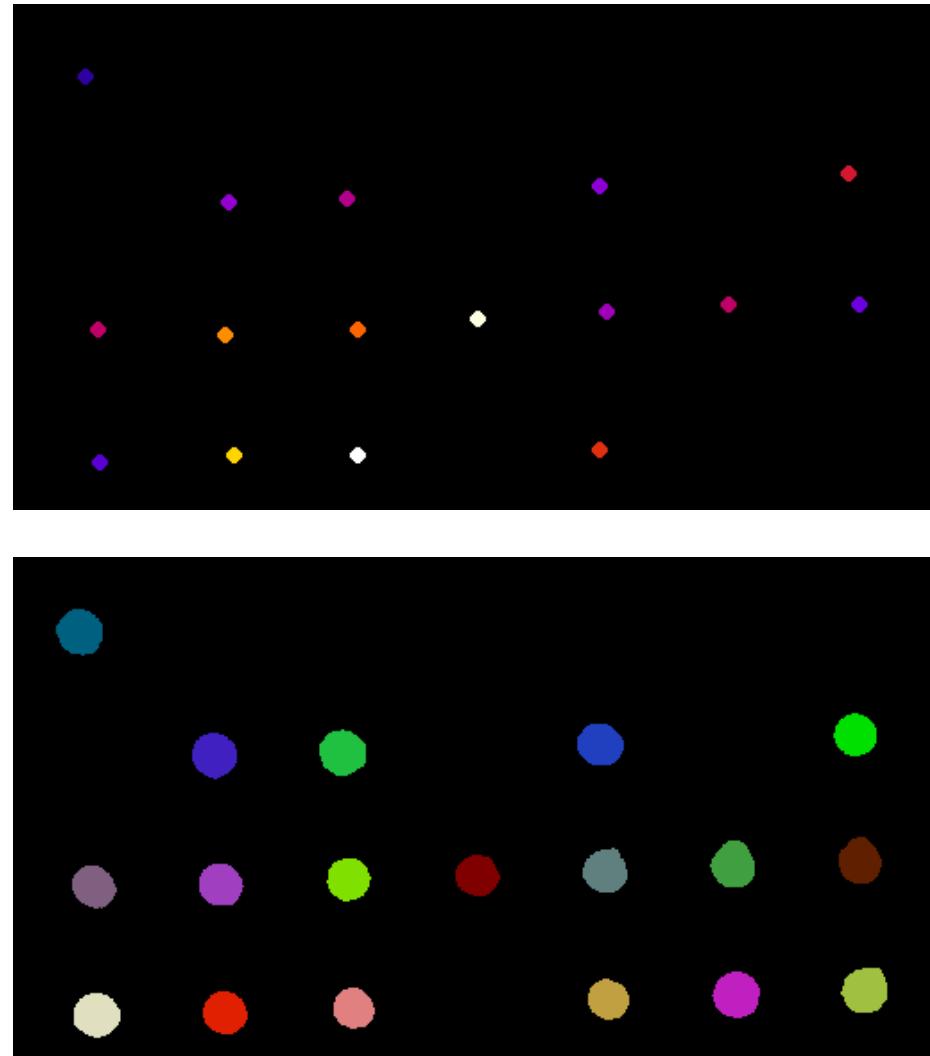
Pre-processing - seeds

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



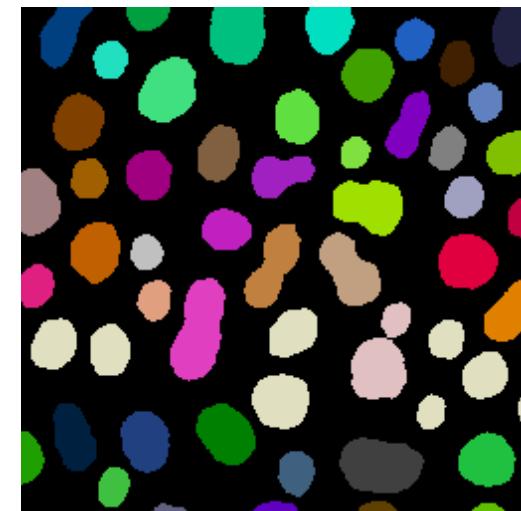
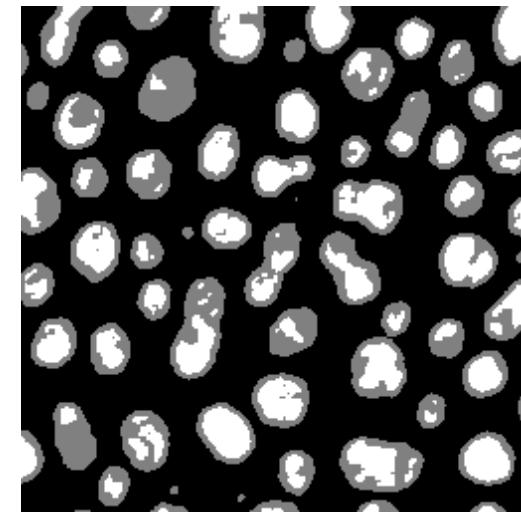
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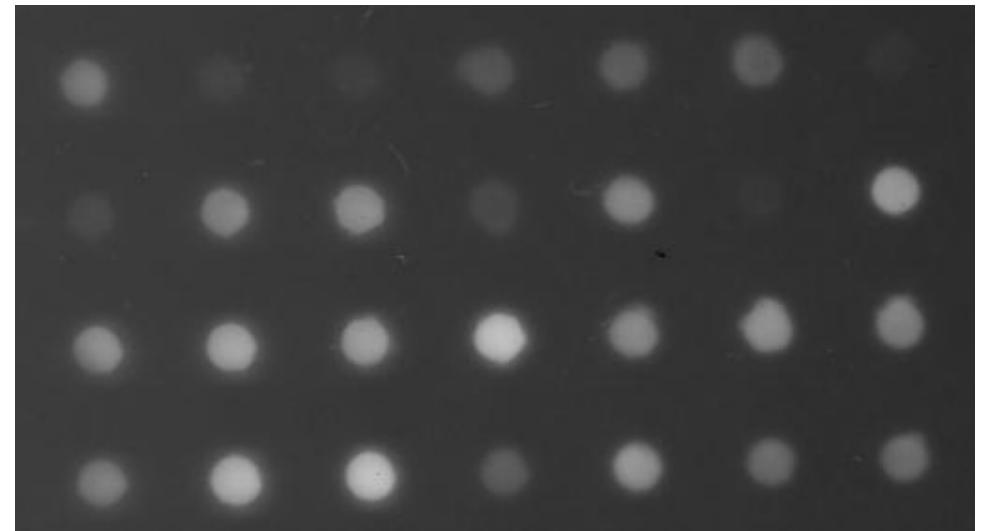
Thresholding - hysteresis

- **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



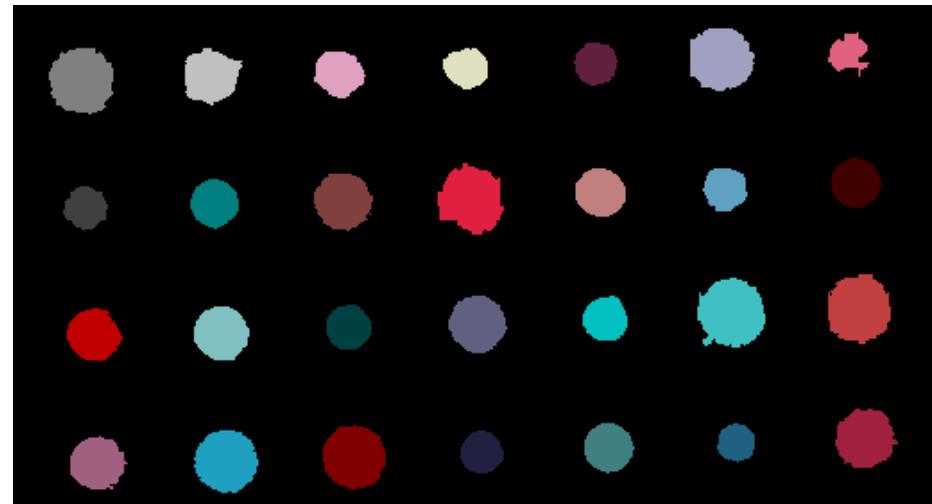
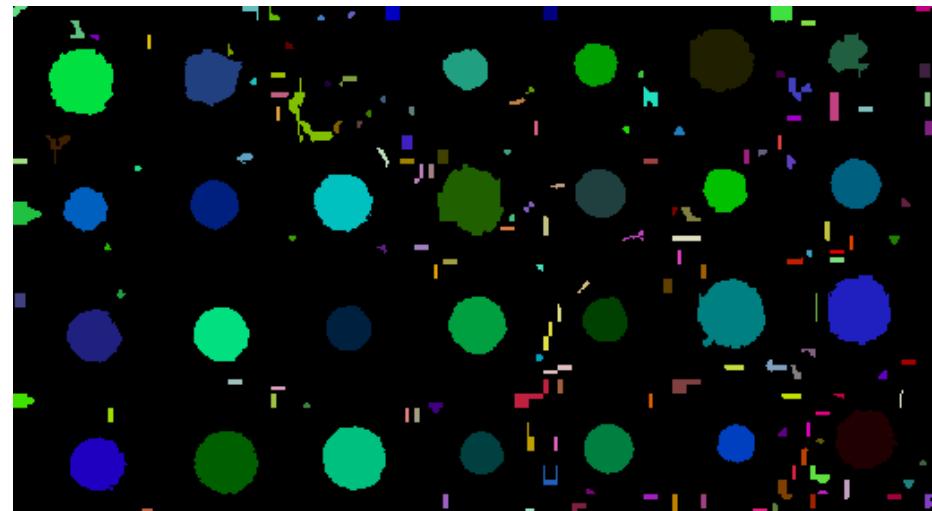
Thresholding - iterative

- **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**



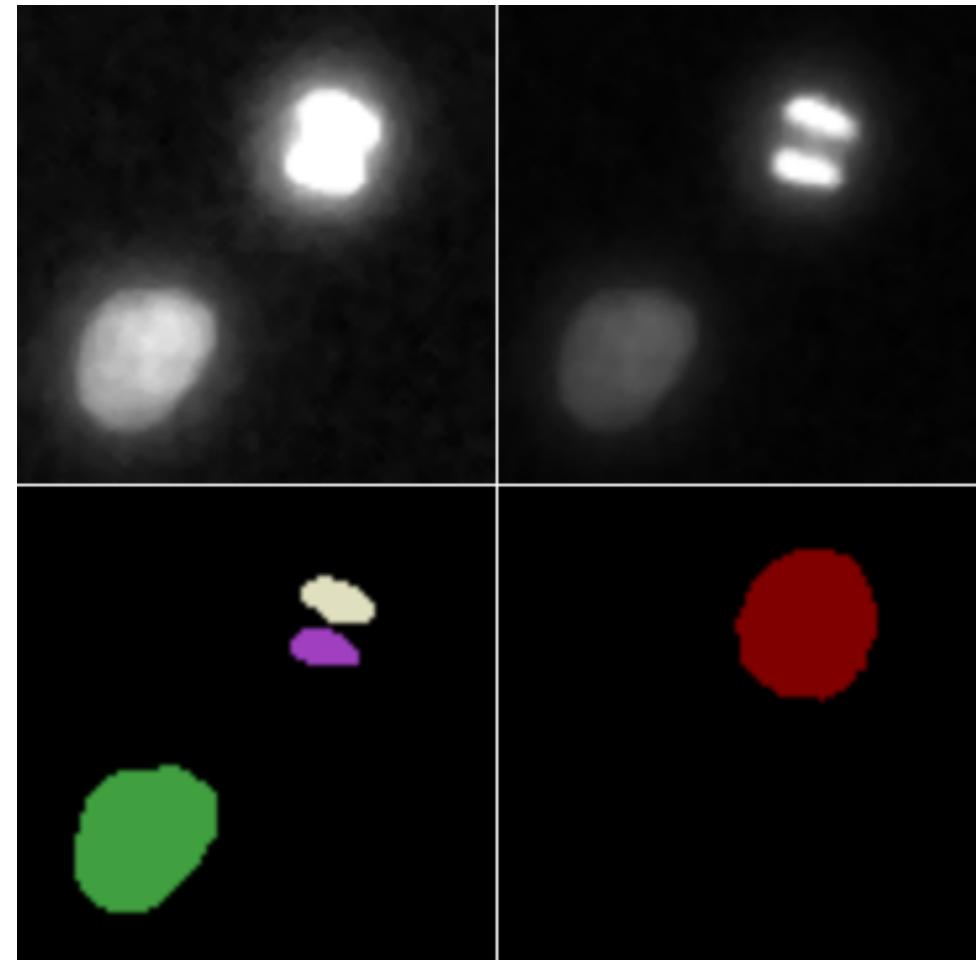
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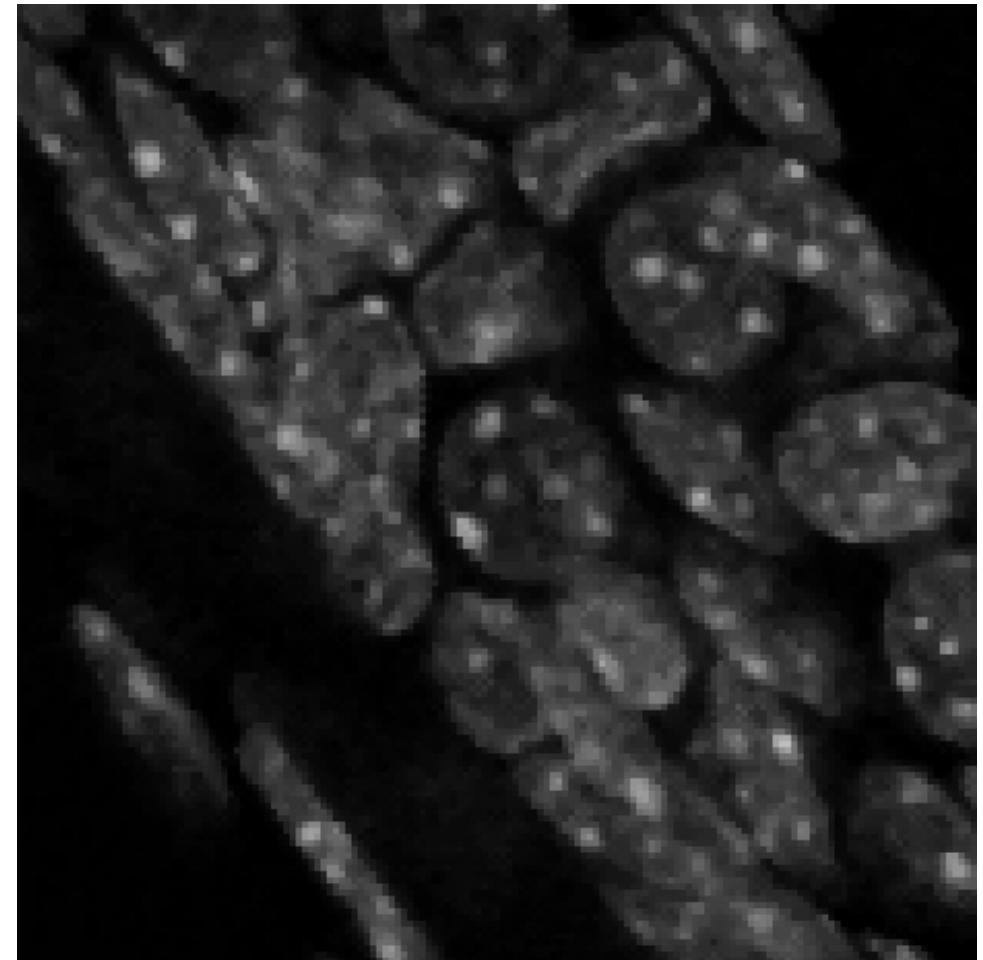
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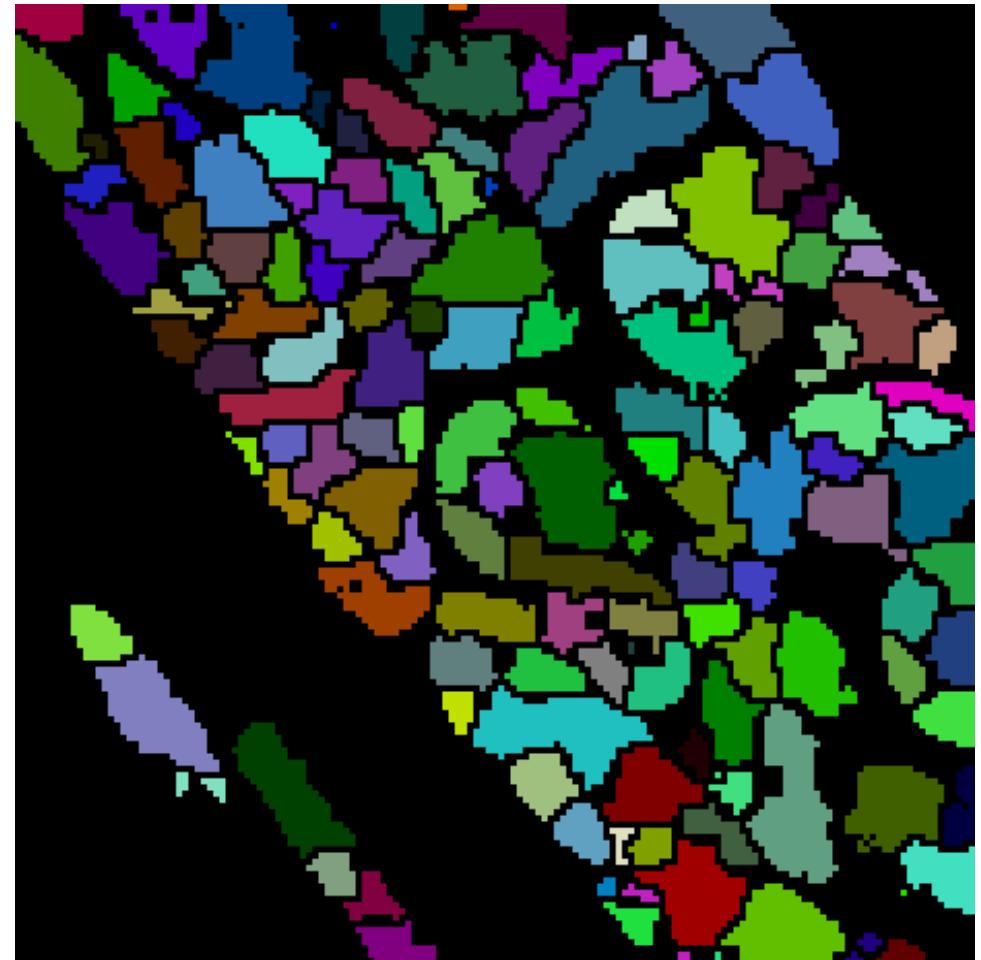
Segmentation - watershed

- **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**



Segmentation - watershed

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Segmentation - spots and nuclei

- **Spots segmentation**

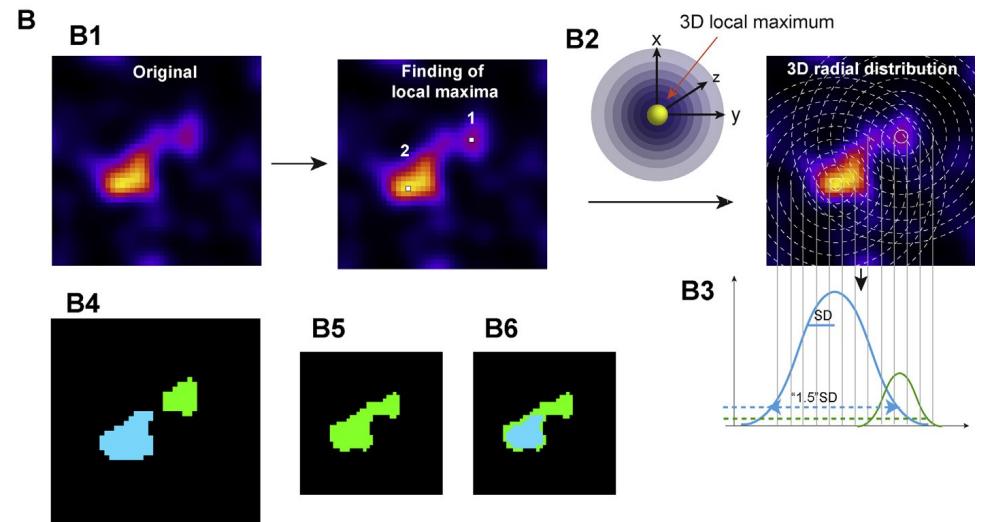
- Find seeds : local maxima, maxima finder
- Local threshold around seeds
 - Gaussian fitting

- **Nuclei segmentation (culture cells)**

- Z project, thresholding + 2D watershed separation
- 3D extension

- **Tissue and more complex : ML and DL**

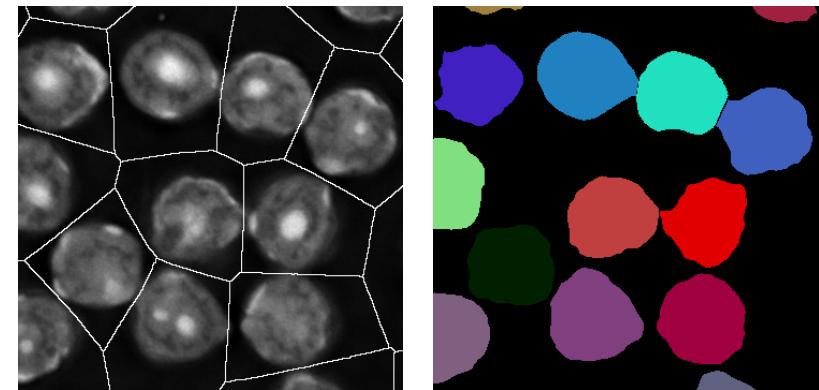
- Weka, StarDist, CellPose, ...



Gilles et al., DiAna

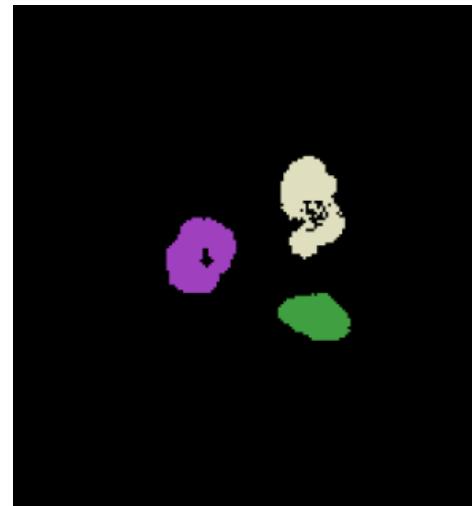
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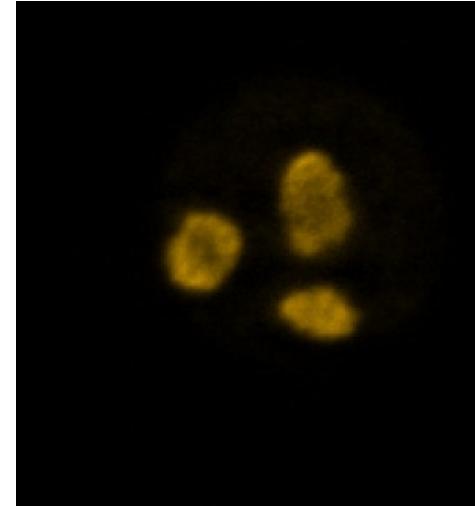
Post-processing

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



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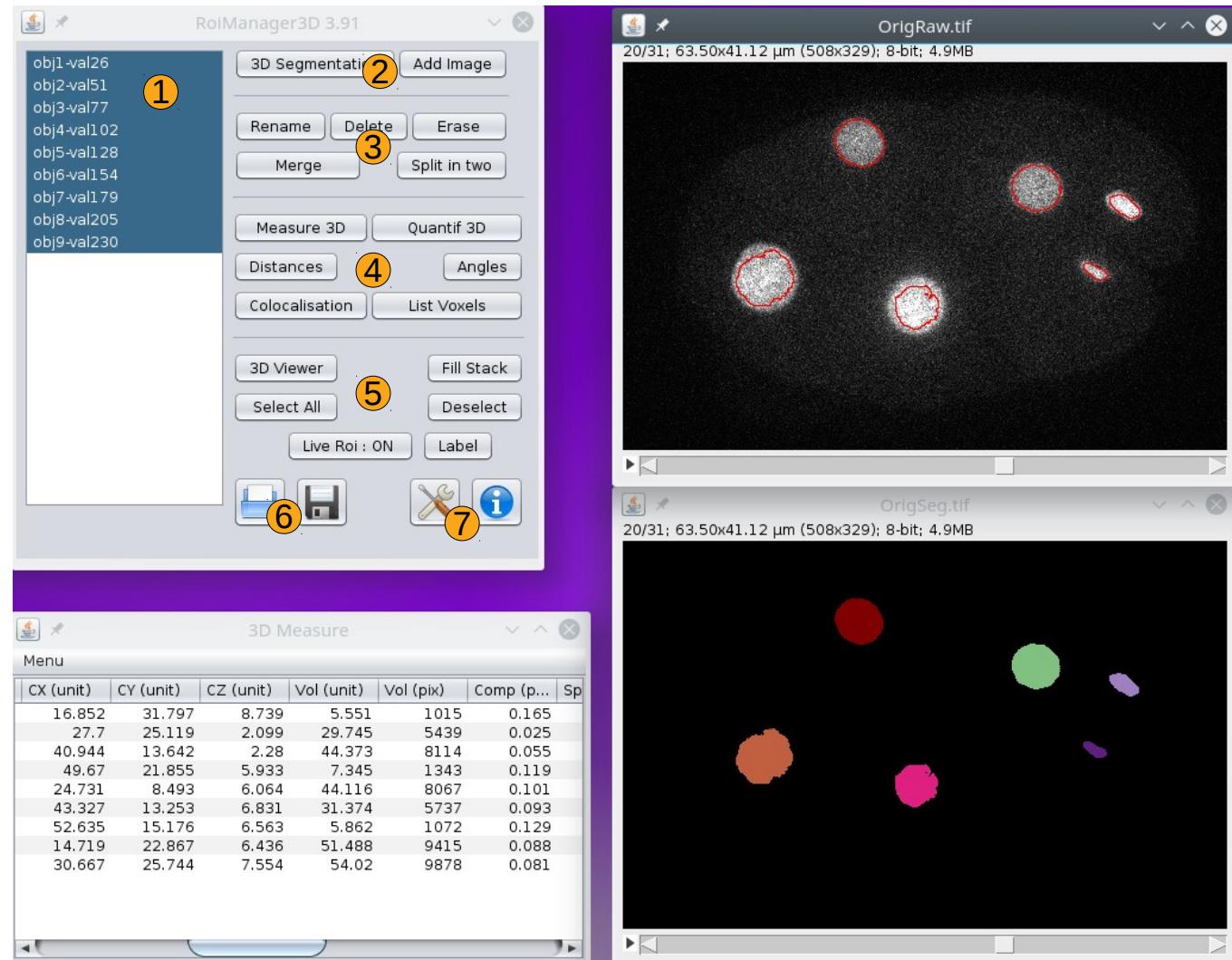


3D Manager

- **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

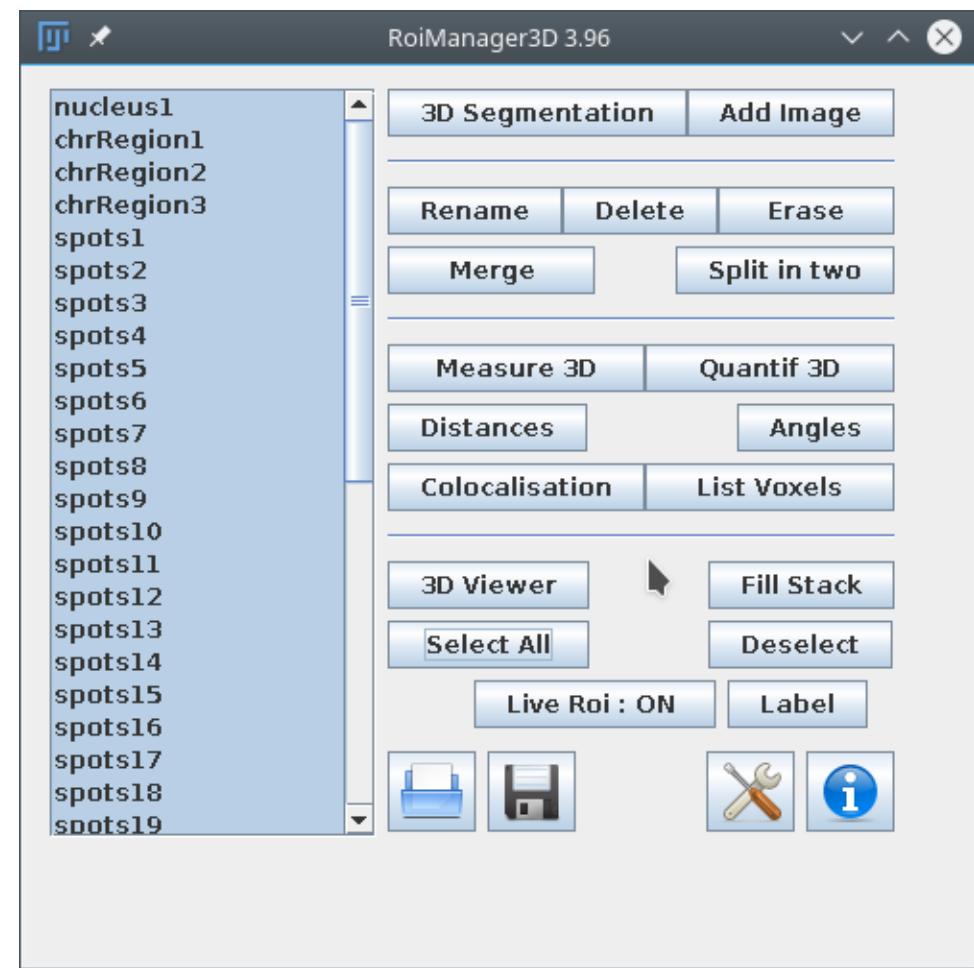
1. 3D Rois list
2. Segmentation + add
3. Edit
4. Measurements
5. Visualisation
6. Load / Save
7. Options / About



3D Manager

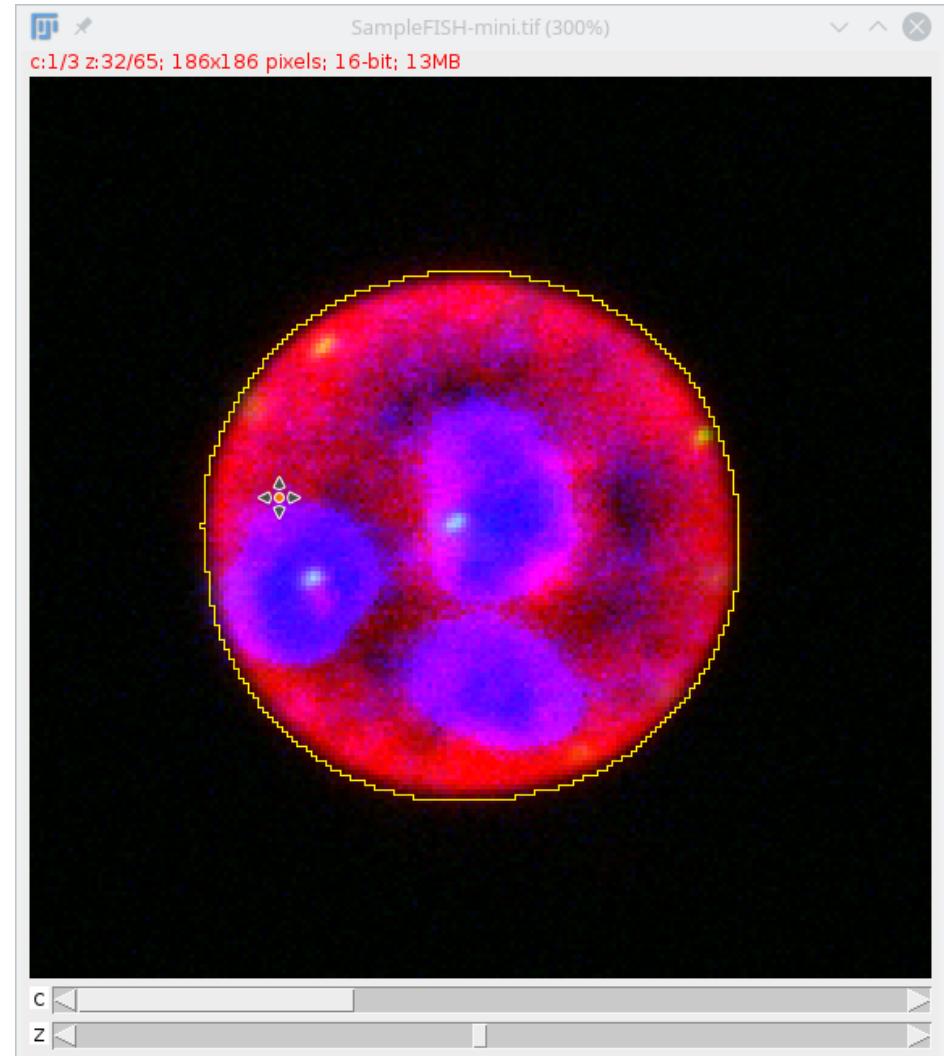
- **Visualisation**

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



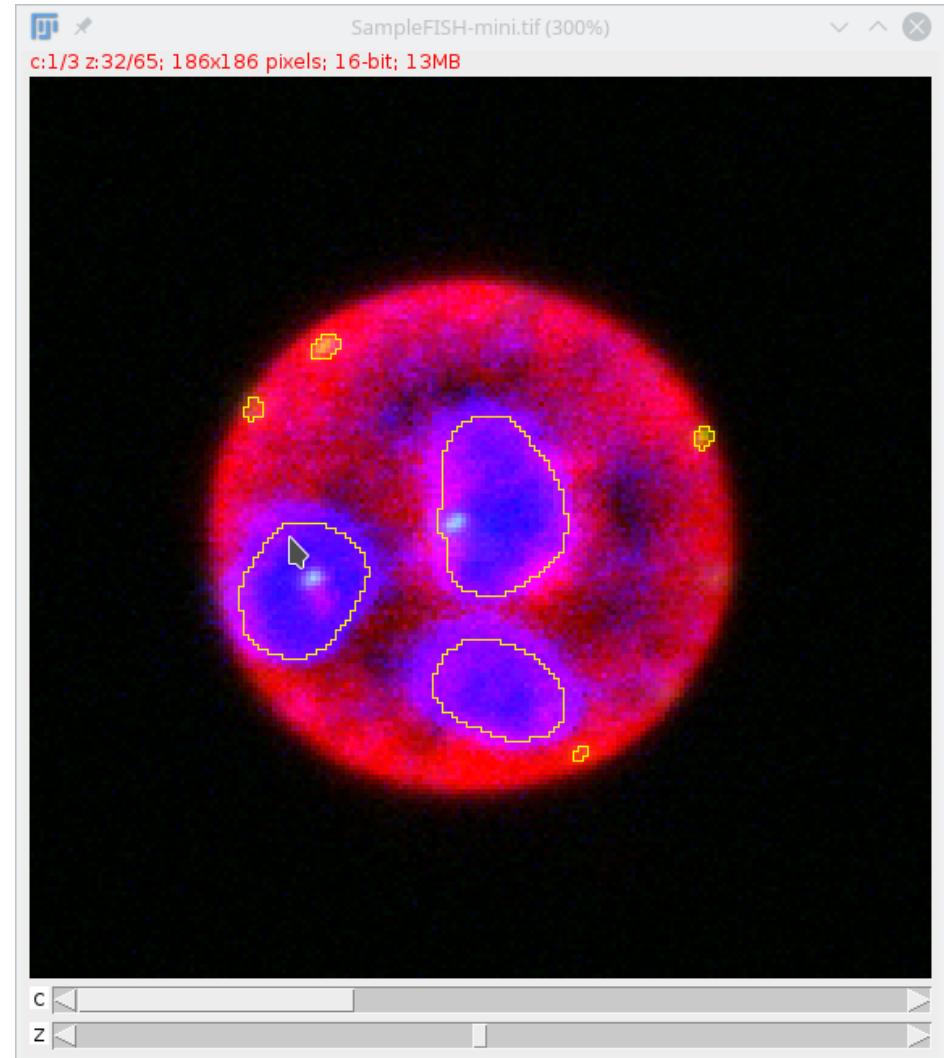
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3D Manager

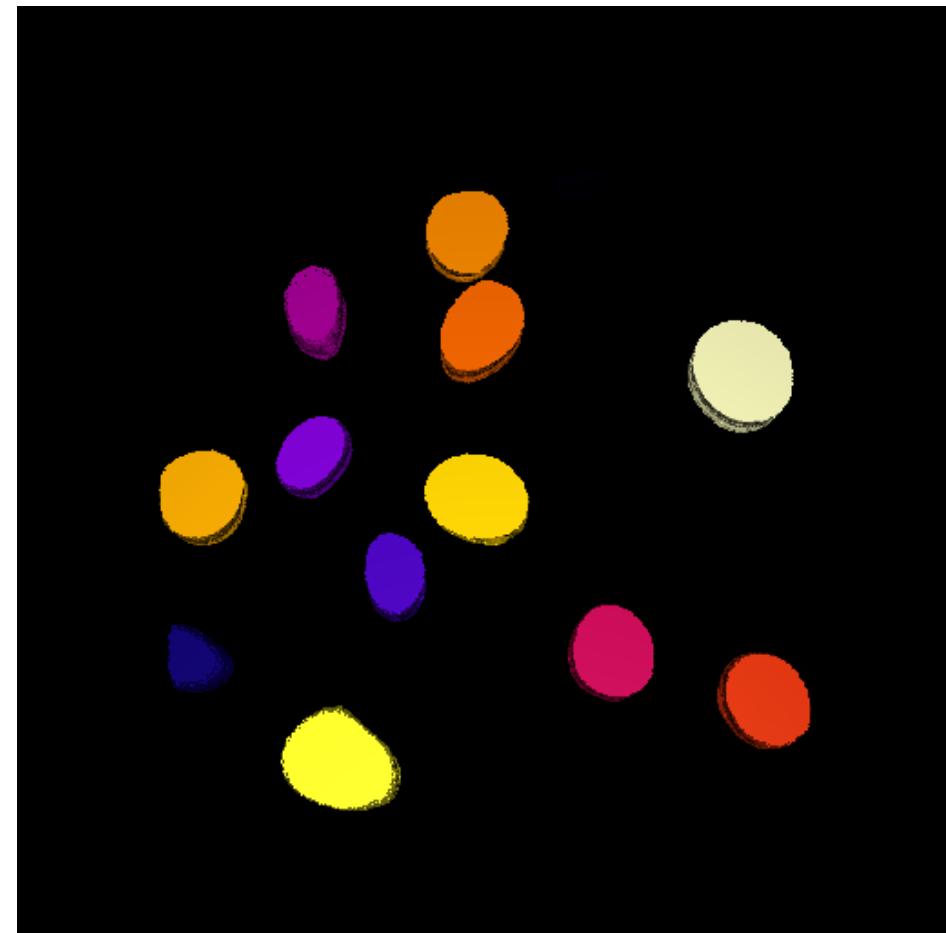
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3D Manager

- **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

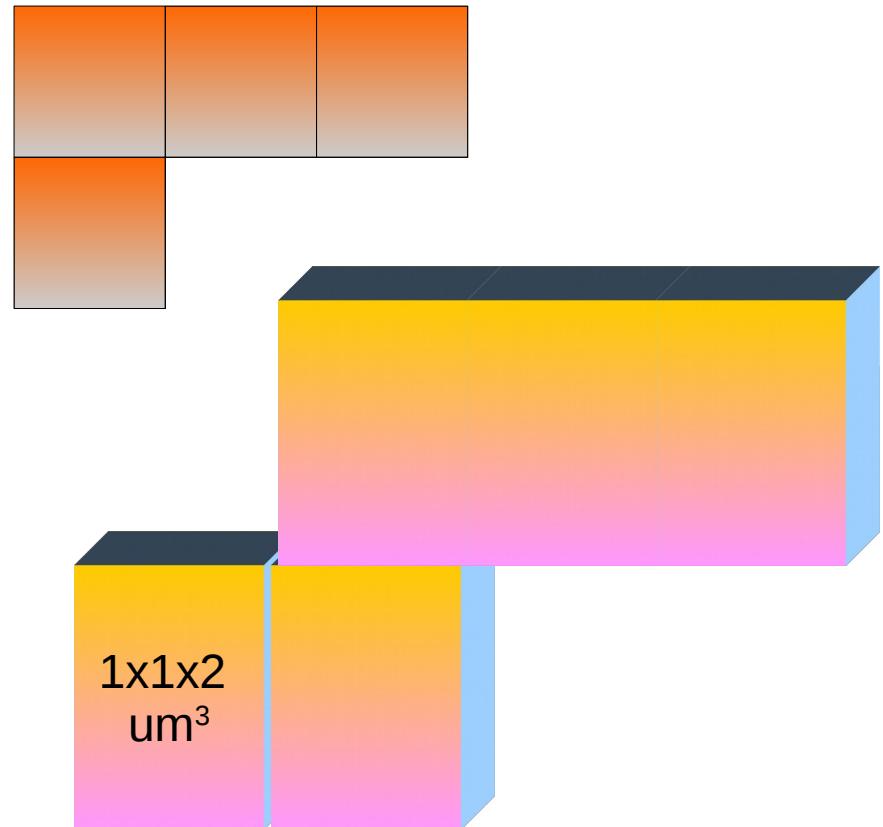
- **Centroid**

- **Volume**

- Nb of voxels (5 vox)
 - Nb of “unit cubes” (10 um^3)

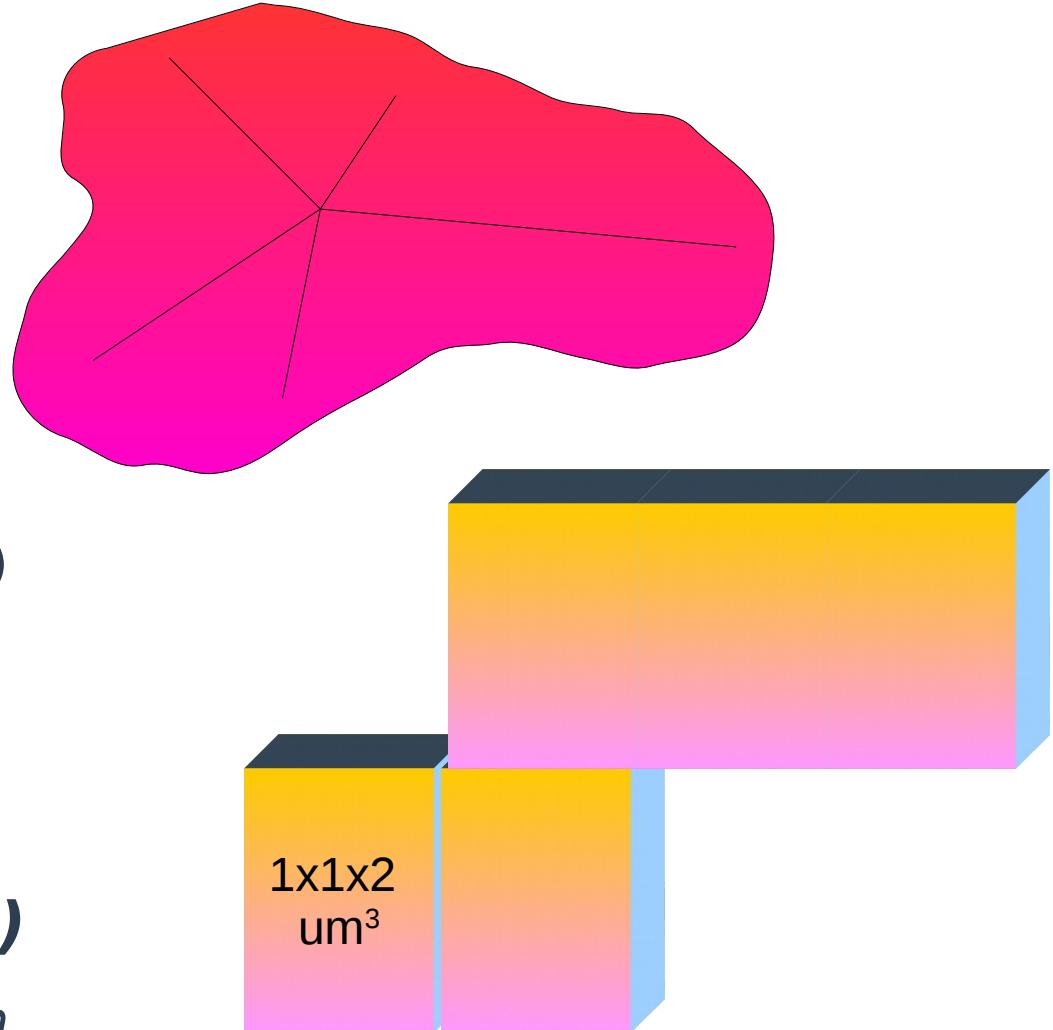
- **Surface**

- 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

- **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)*
- **Feret (3.6 um)**
- **Distances to Center (um)**
 - *Min (0.89), max (1.84), mean (1.36), SD (0.44)*

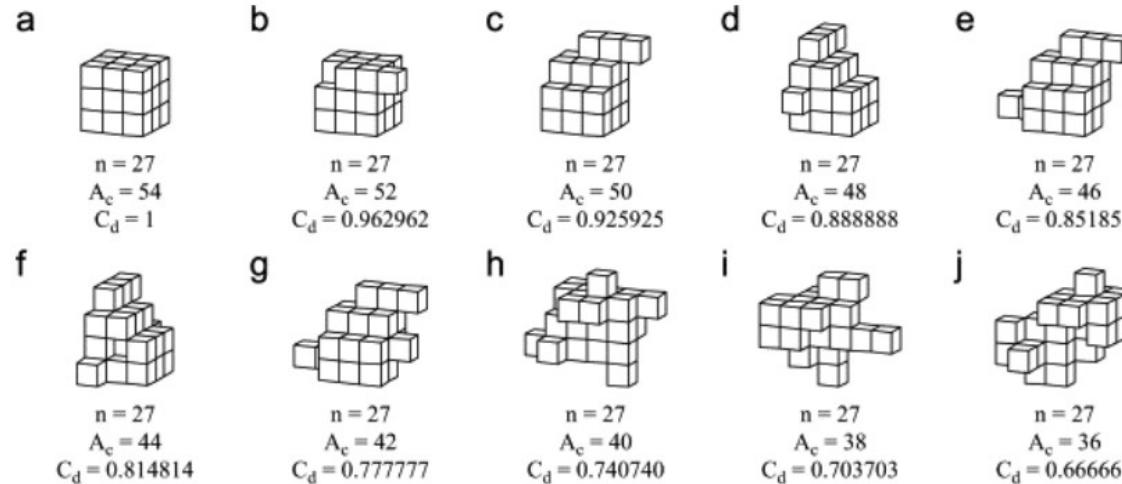


Shape

- **Compactness and sphericity**

- Ratio between volume and surface
 - In voxels or units
- Maximal compaction for sphere (1)
- Compactness discrete
 - *An easy measure of compactness for 2D and 3D shapes, Bribiesca 2008*

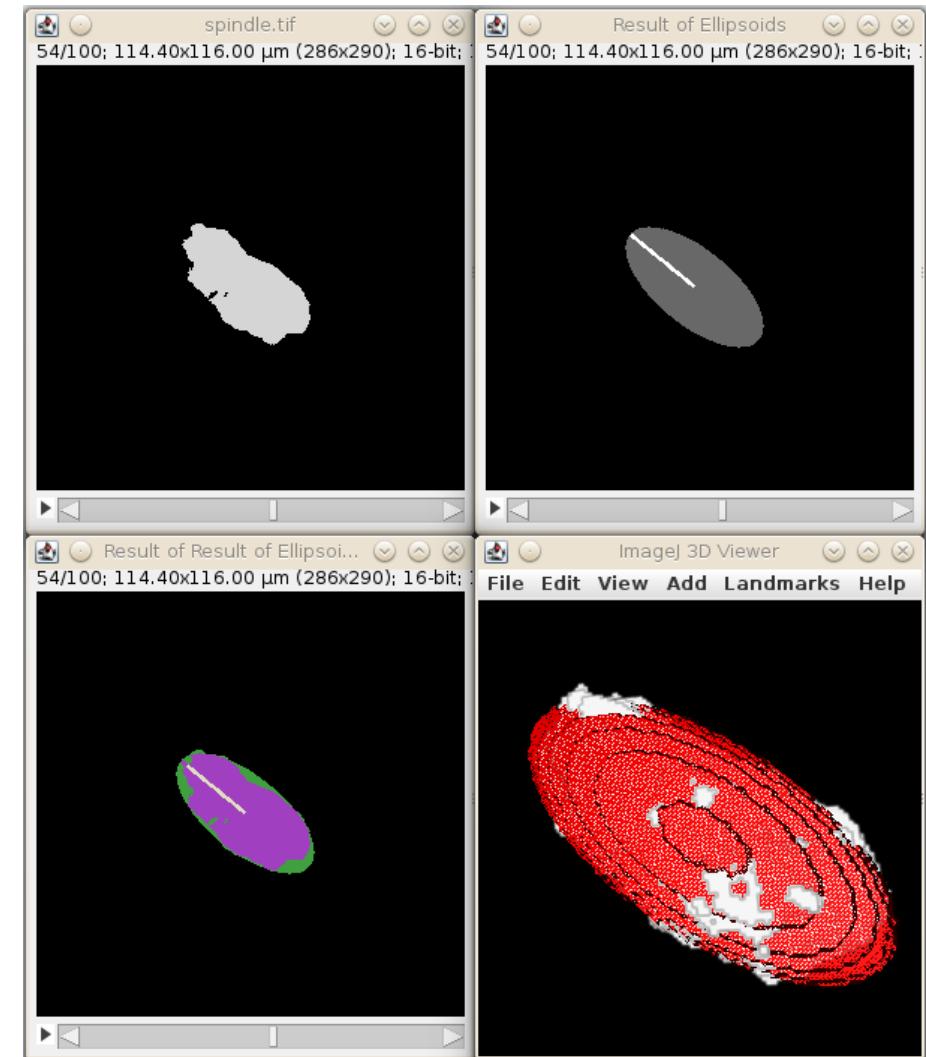
$$C = \frac{36 \cdot \pi \cdot V^2}{A^3}; S = C^{1/3}$$



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

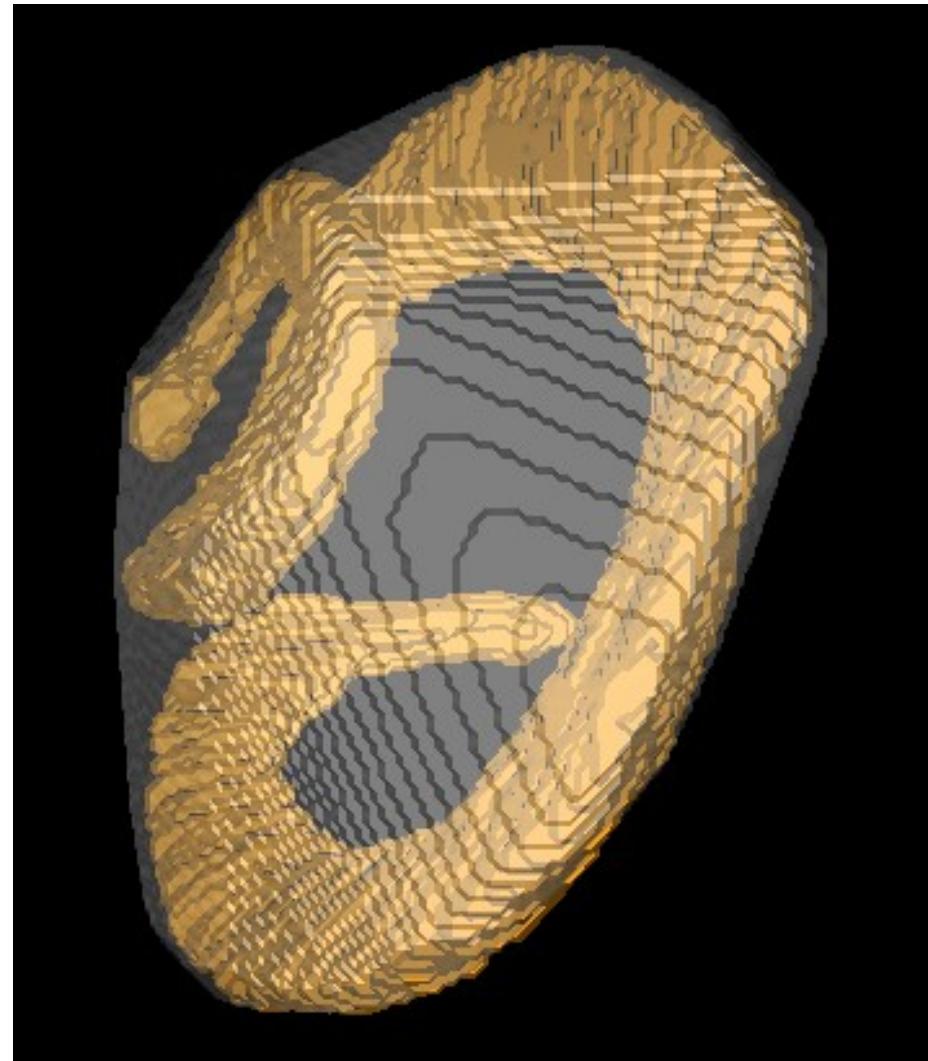
Shape

- **Ellipsoid fitting**
 - Best fitting ellipsoid
 - Radii = $\sqrt{5.eigen}$
 - 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>



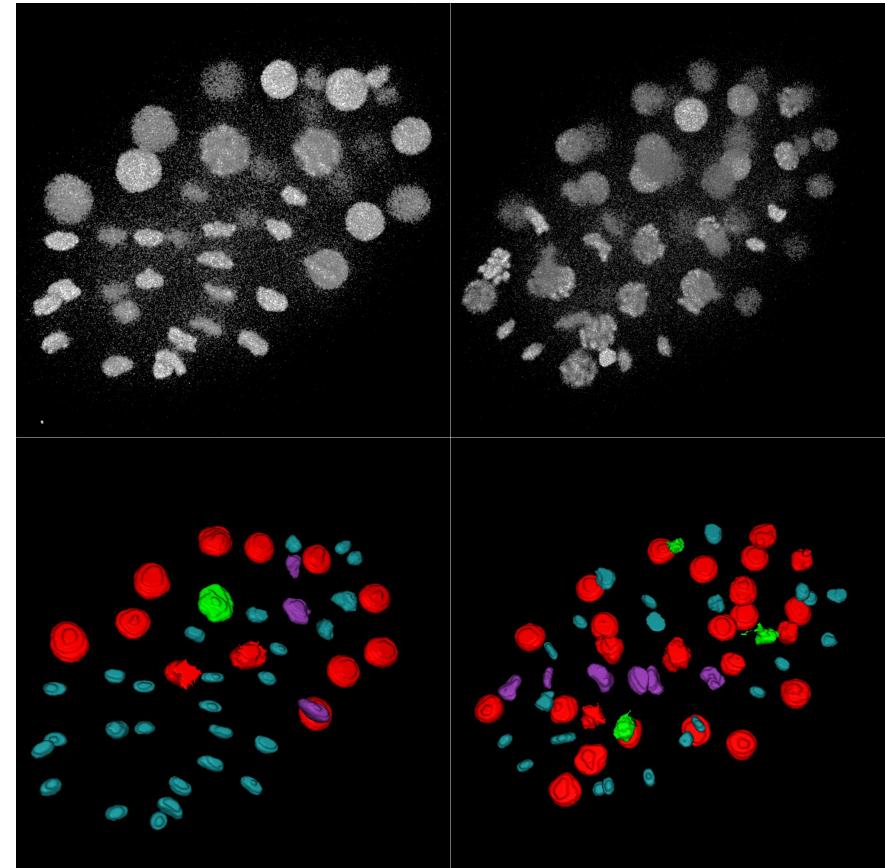
Shape

- **3D Moments**

- Based on ellipsoid computation
- $J_1 = S_{XX} + S_{YY} + S_{ZZ}$
- $J_2 = \dots$

- **Used for more accurate shape description**

- GulMohammed 2014, BMC Bioinformatics

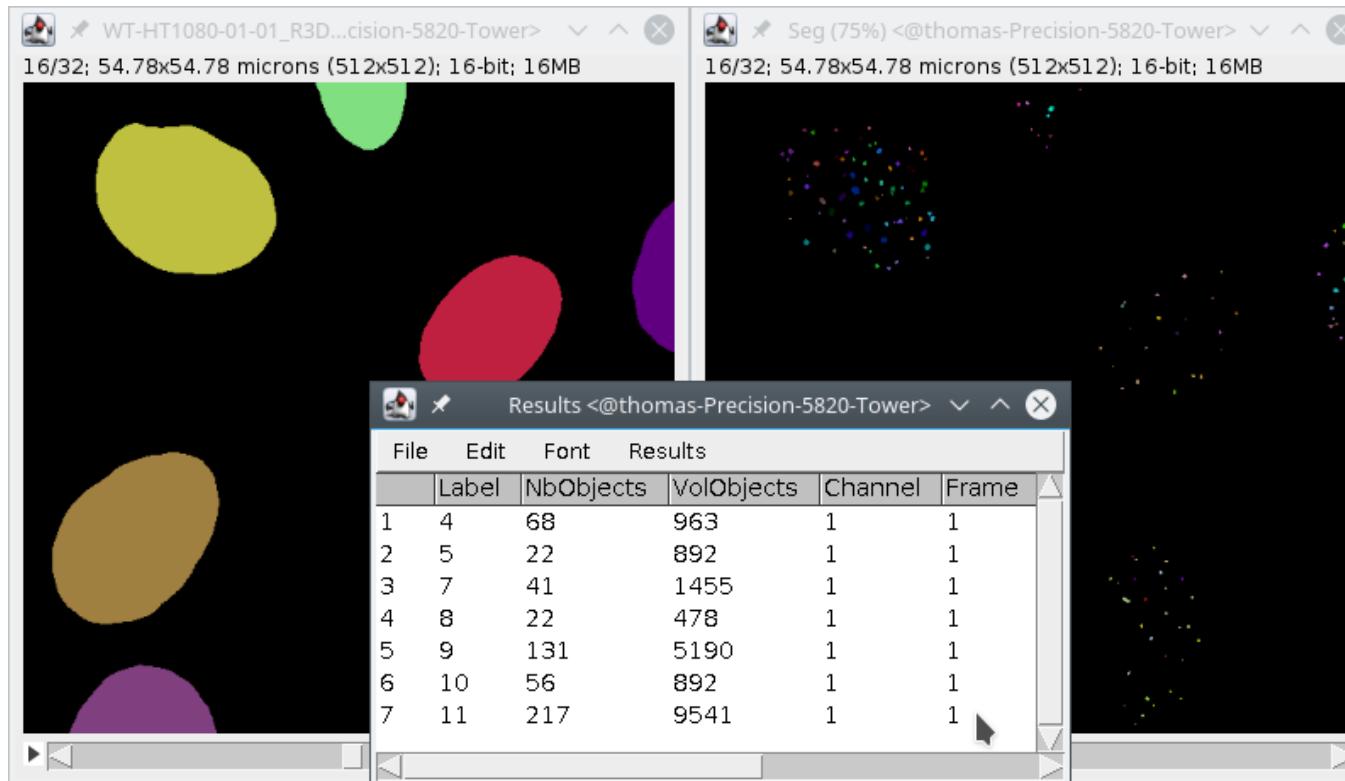


Intensity

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

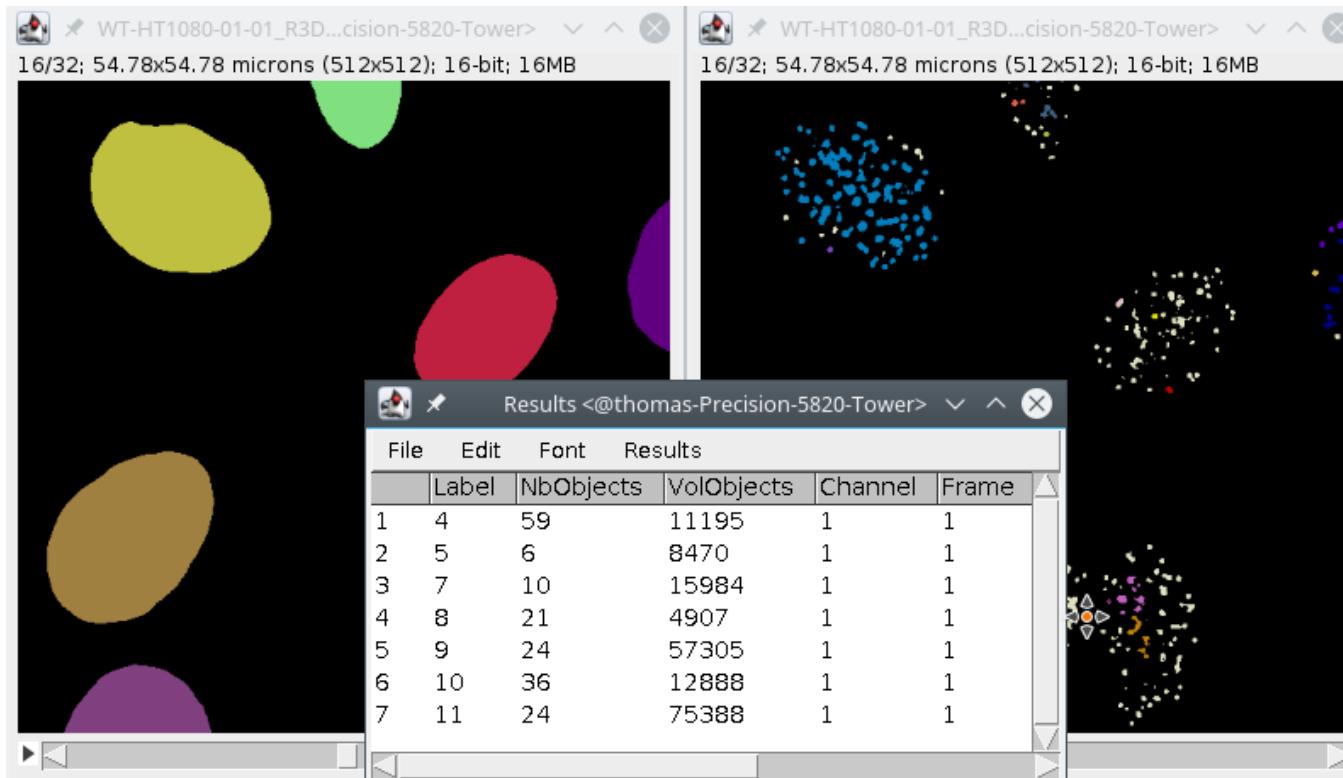
Numbering

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



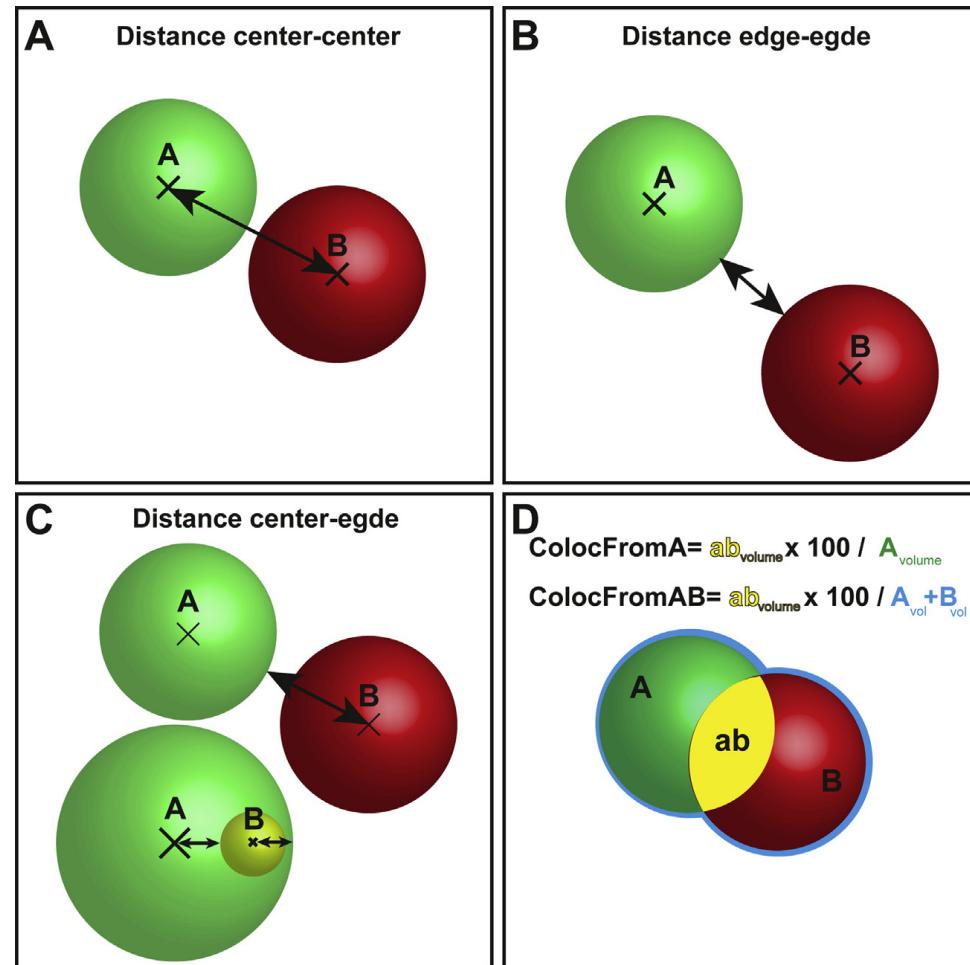
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Distances

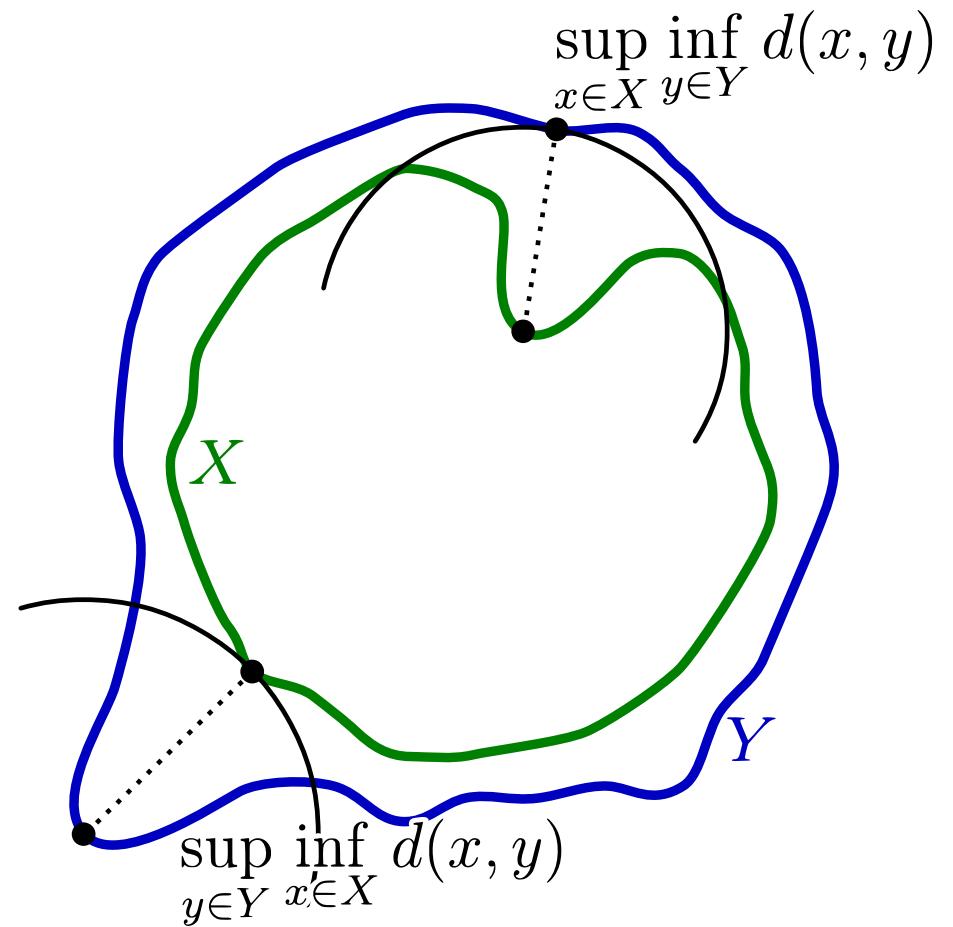
- Centre
- Border
- Hausdorff (plugin)
- Radial distance
- Closest



Gilles et al., DiAna

Distances

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

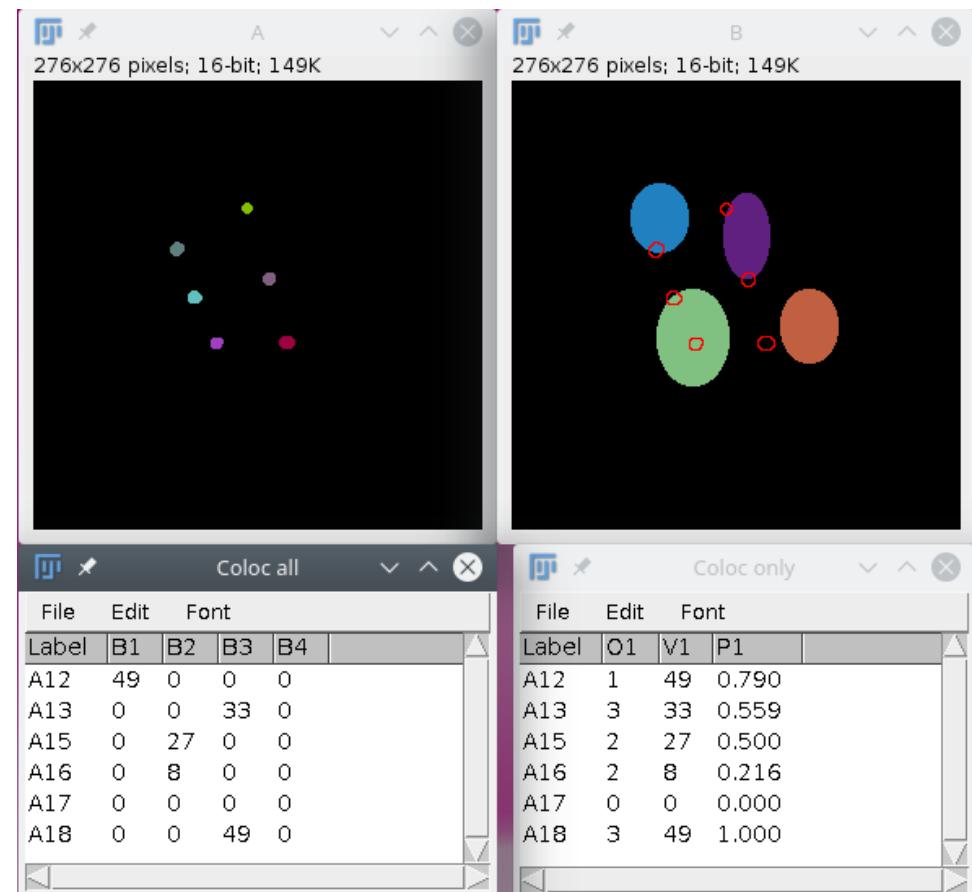


By Rocchini - Own work, CC BY 3.0,
<https://commons.wikimedia.org/w/index.php?curid=2918812>

Colocalisation

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

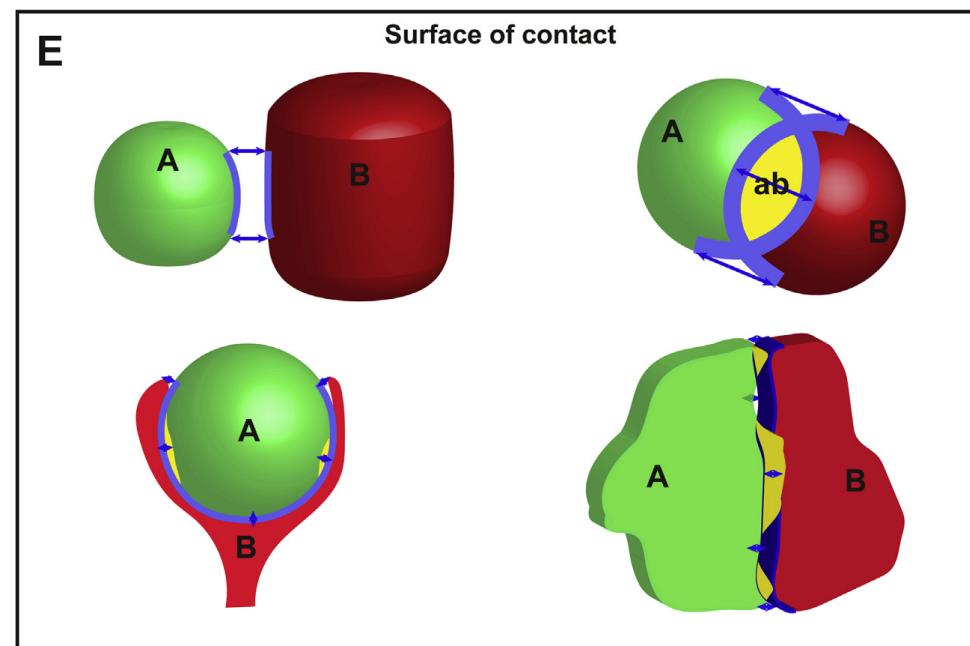
Gilles et al. DiAna, an ImageJ Tool for Object-Based 3D Co-Localization and Distance Analysis, 2017



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Gilles et al. DiAna, an ImageJ Tool
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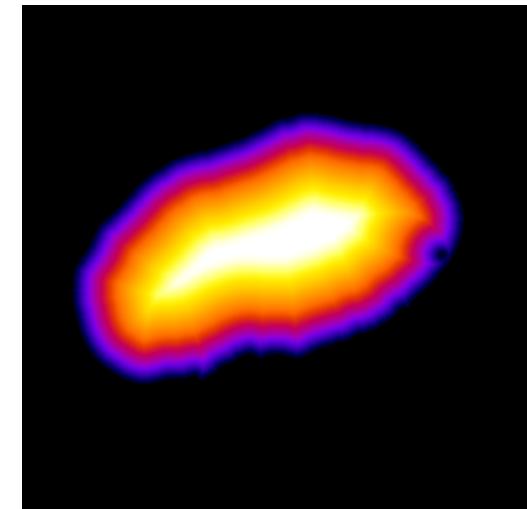
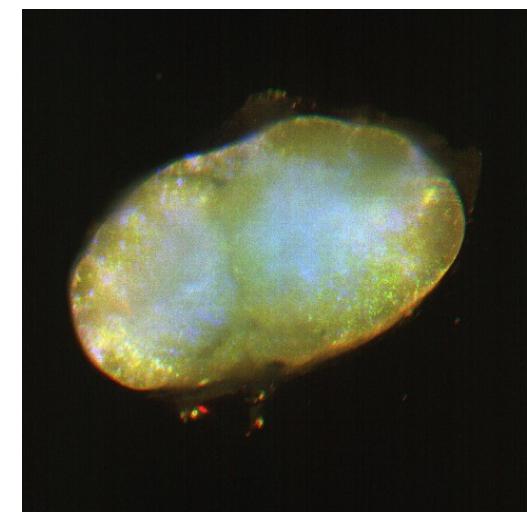
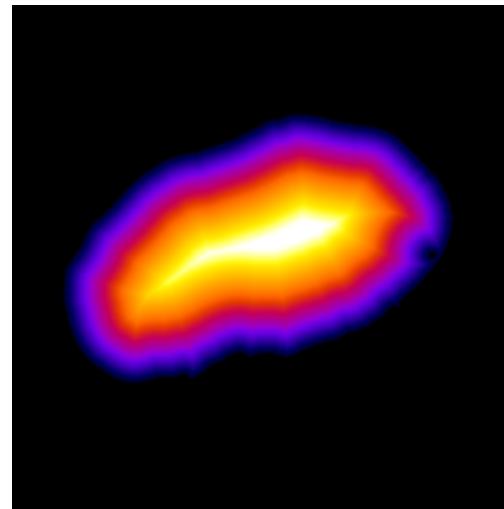
Gilles et al., DiAna

Analysis

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

EDT - EVF

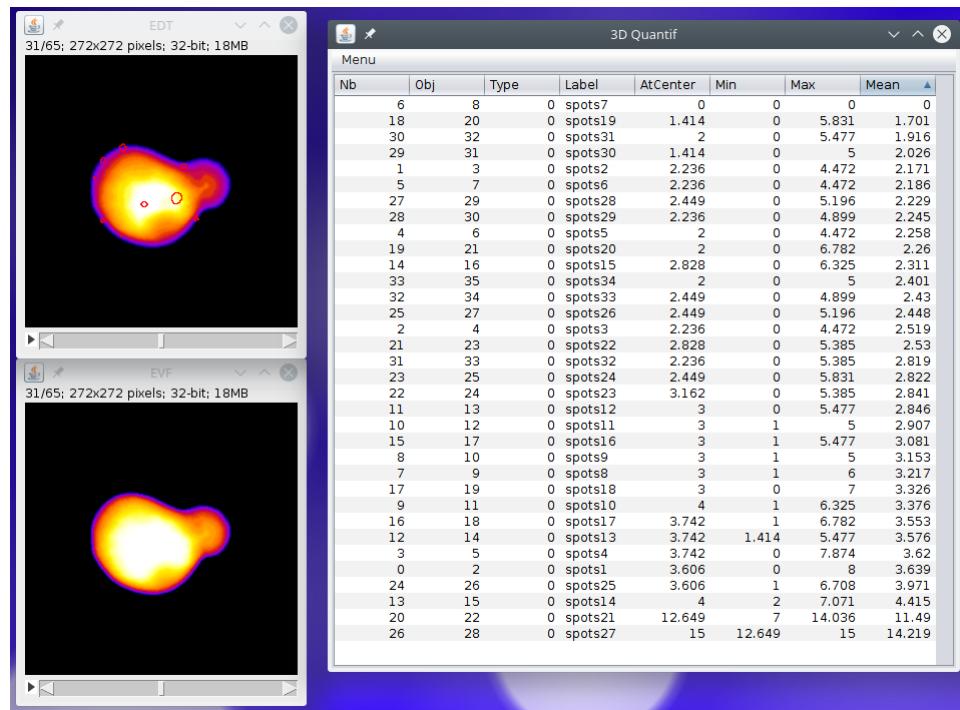
- **EDT :**
**Euclidean
Distance
Map**
- **EVF :**
**Eroded
Volume
Fraction**



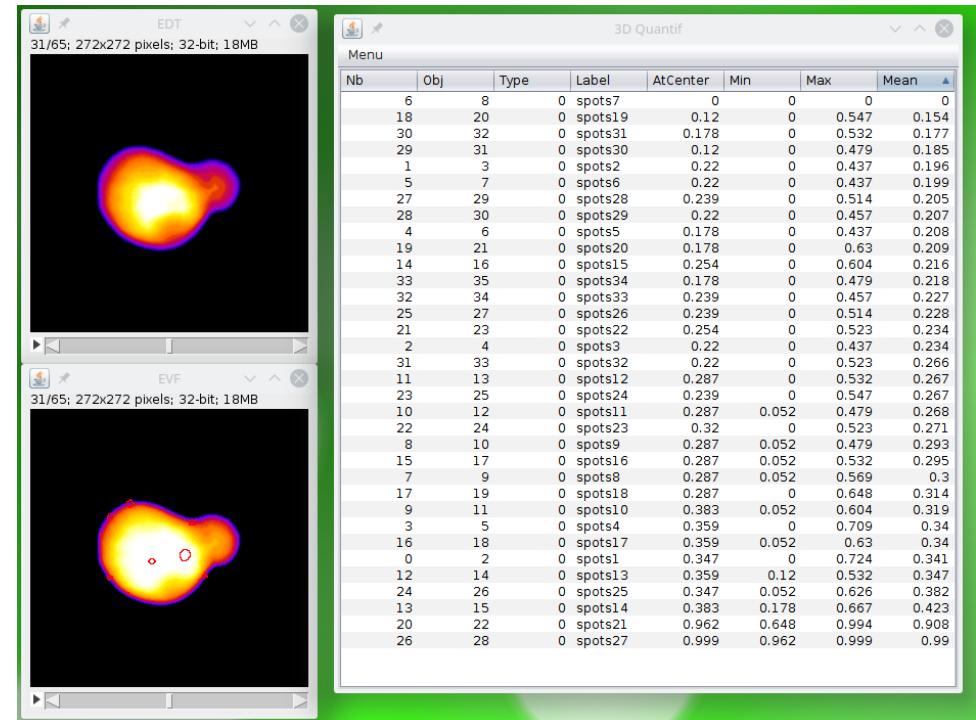
J. Groom, WEHI

EDT - EVF

Values are calibrated distances from closest border

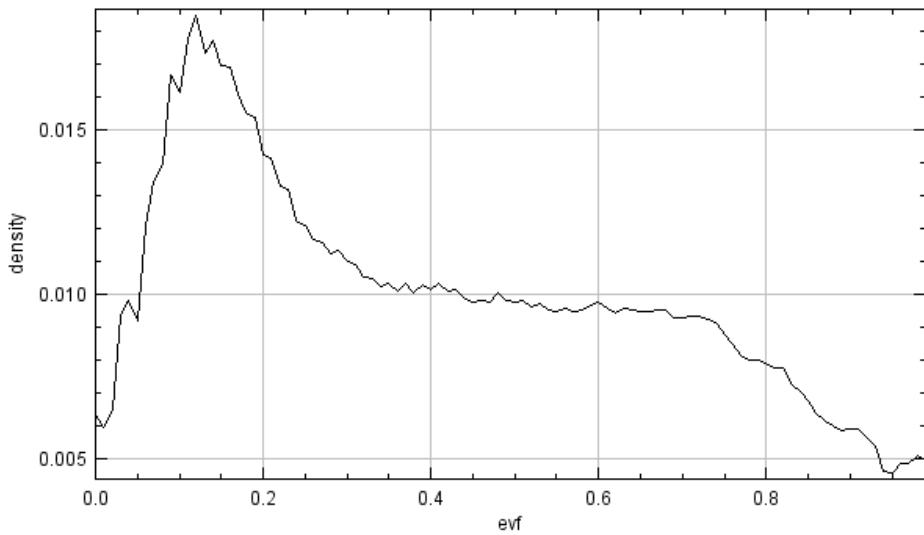


Values are normalised between 0 and 1, from closest to furthest

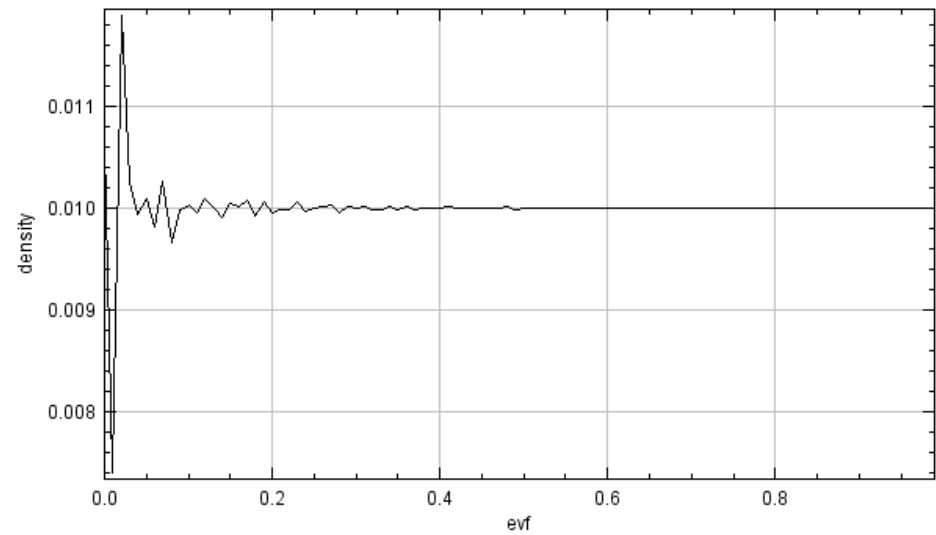


EDT - EVF

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

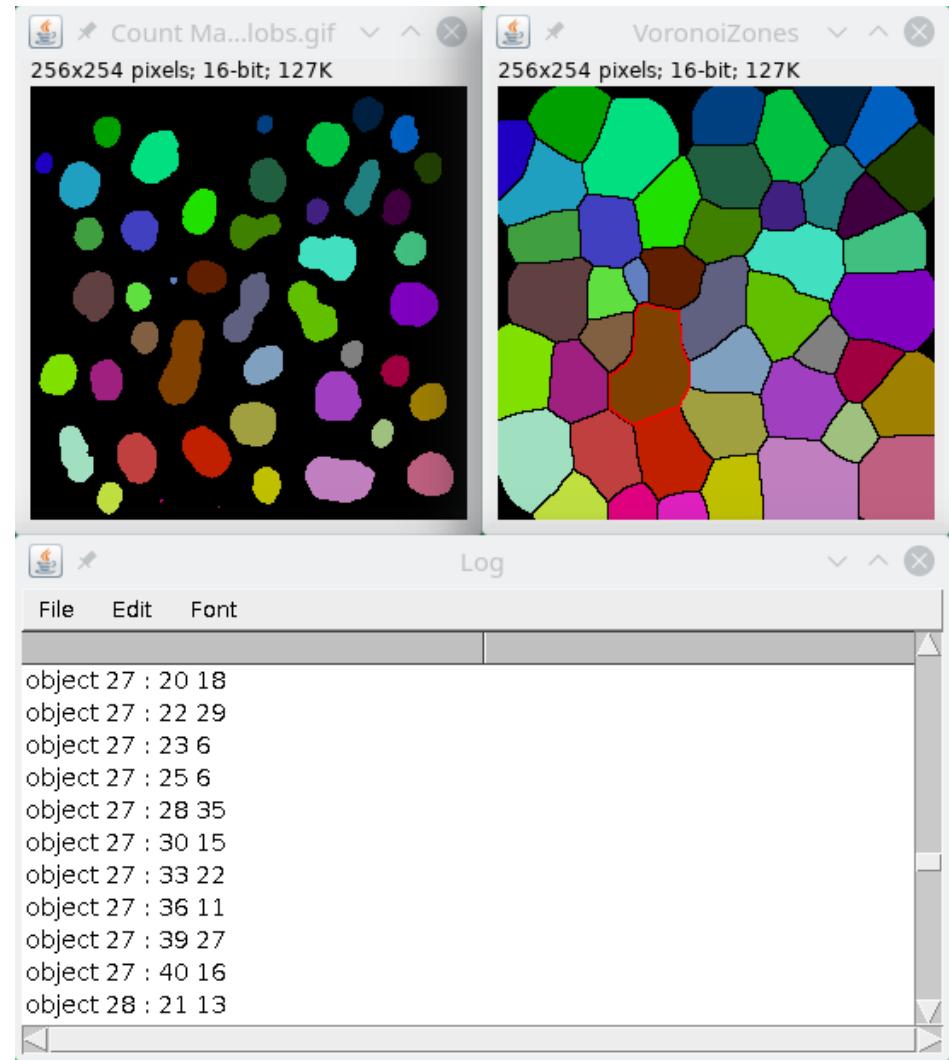


Compute volume inside 100 layers (0-0.01, ...),

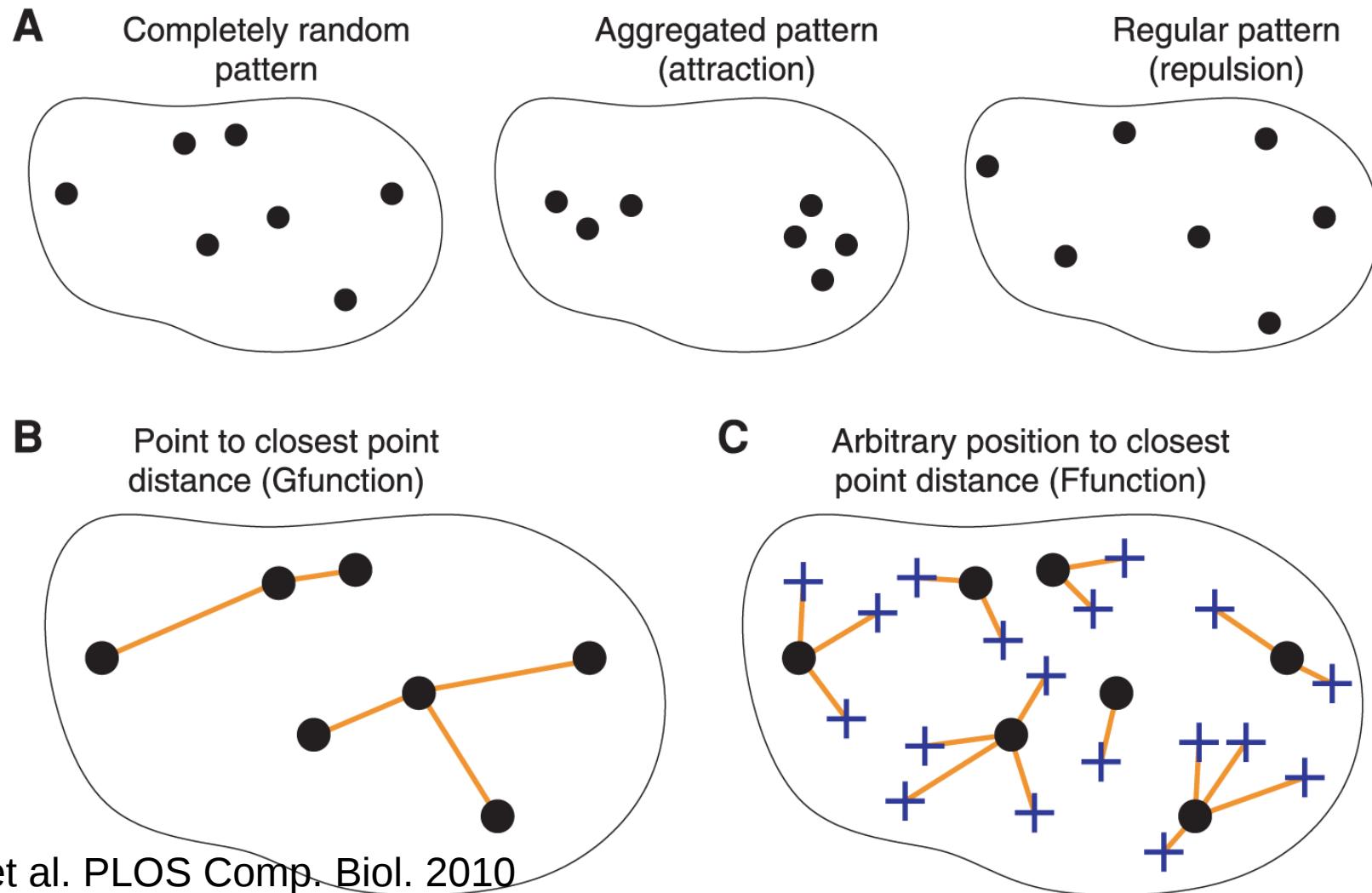


Interactions

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



Spatial statistics



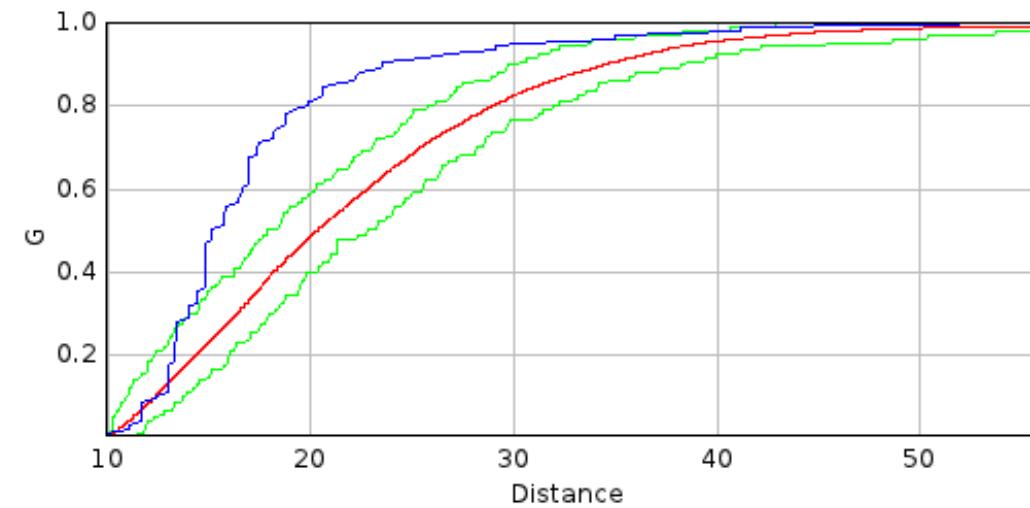
Andrey et al. PLOS Comp. Biol. 2010

Spatial organization

Clusters :

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

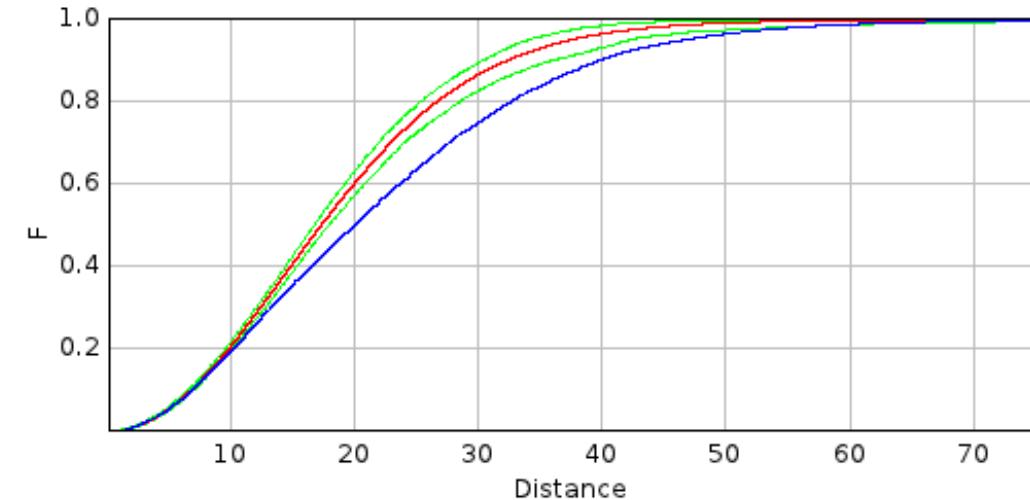
A lot of « voids » in the structure, large distances between reference points and spots → F below curve of random organisation



Uniform :

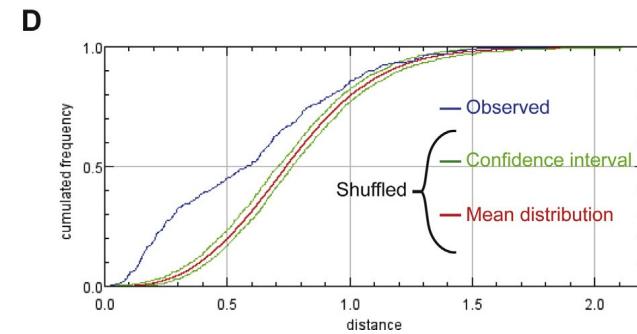
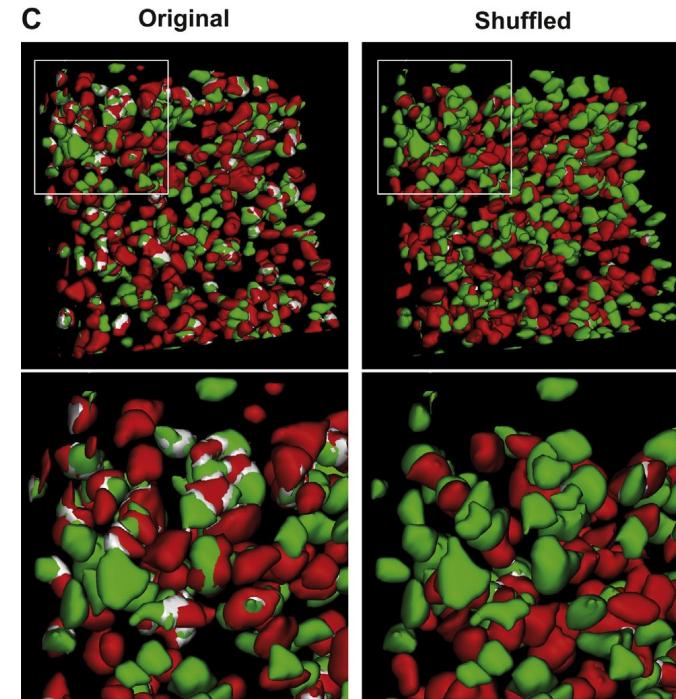
A lot of large similar distances between spots → G below curve of random organisation

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



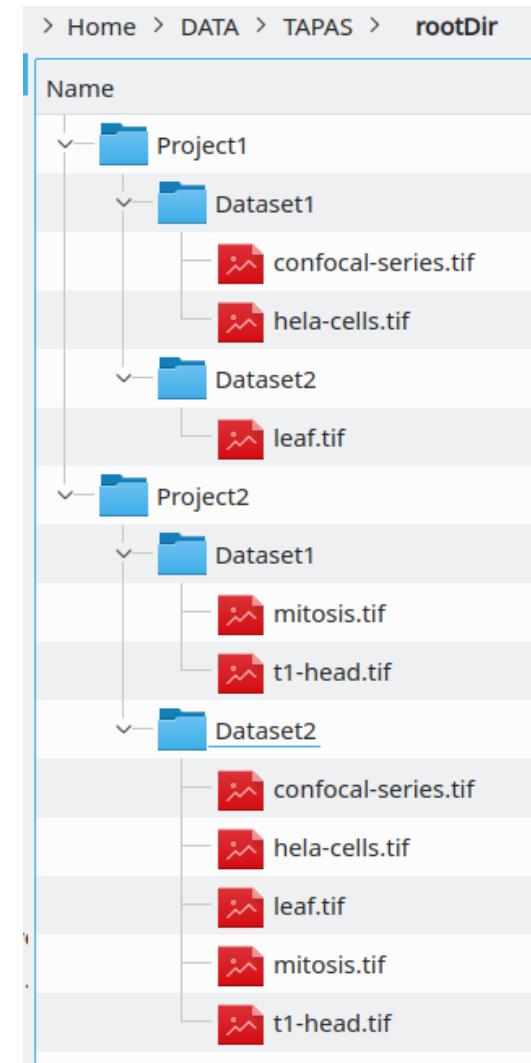
Spatial organization

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

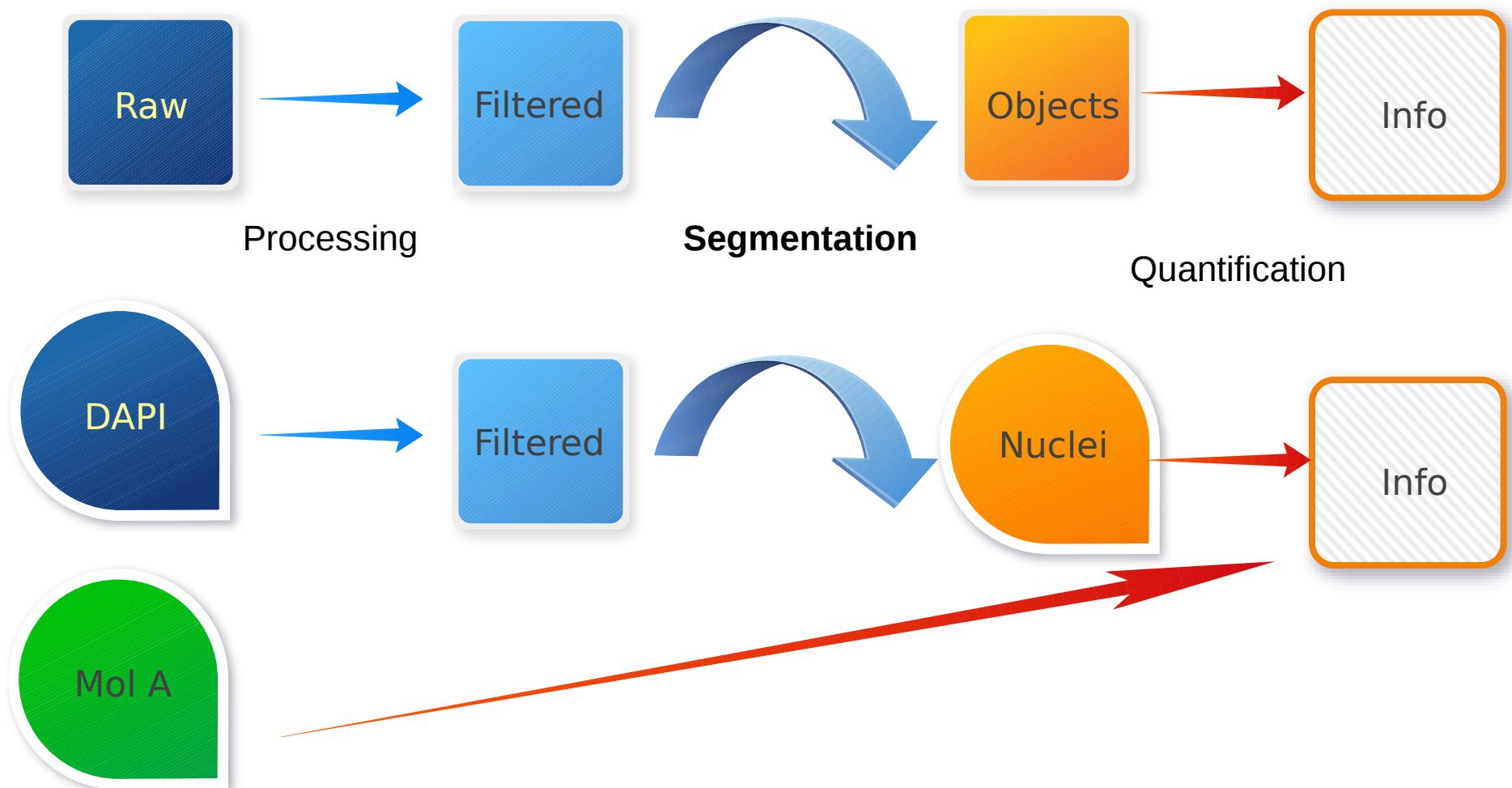


DATA organisation

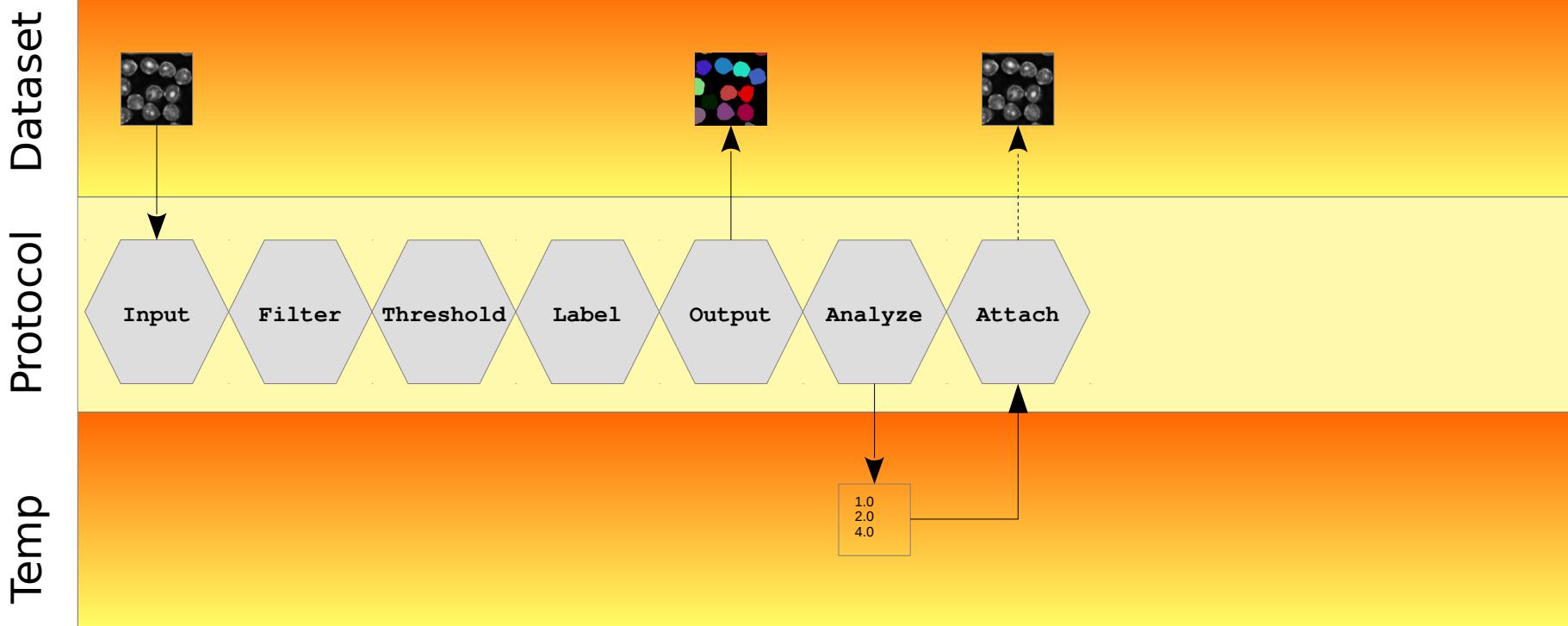
- **Projects / Datasets**
 - Raw data
 - *Filtered data*
 - Segmented data
 - *Analysed data*
 - Results
- **On Disk or DB (OMERO)**



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
radxy:4
radz:2
filter:median

// threshold
process:autoThreshold
method:Otsu

// label
process:label
minVolume:100

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

TAPAS

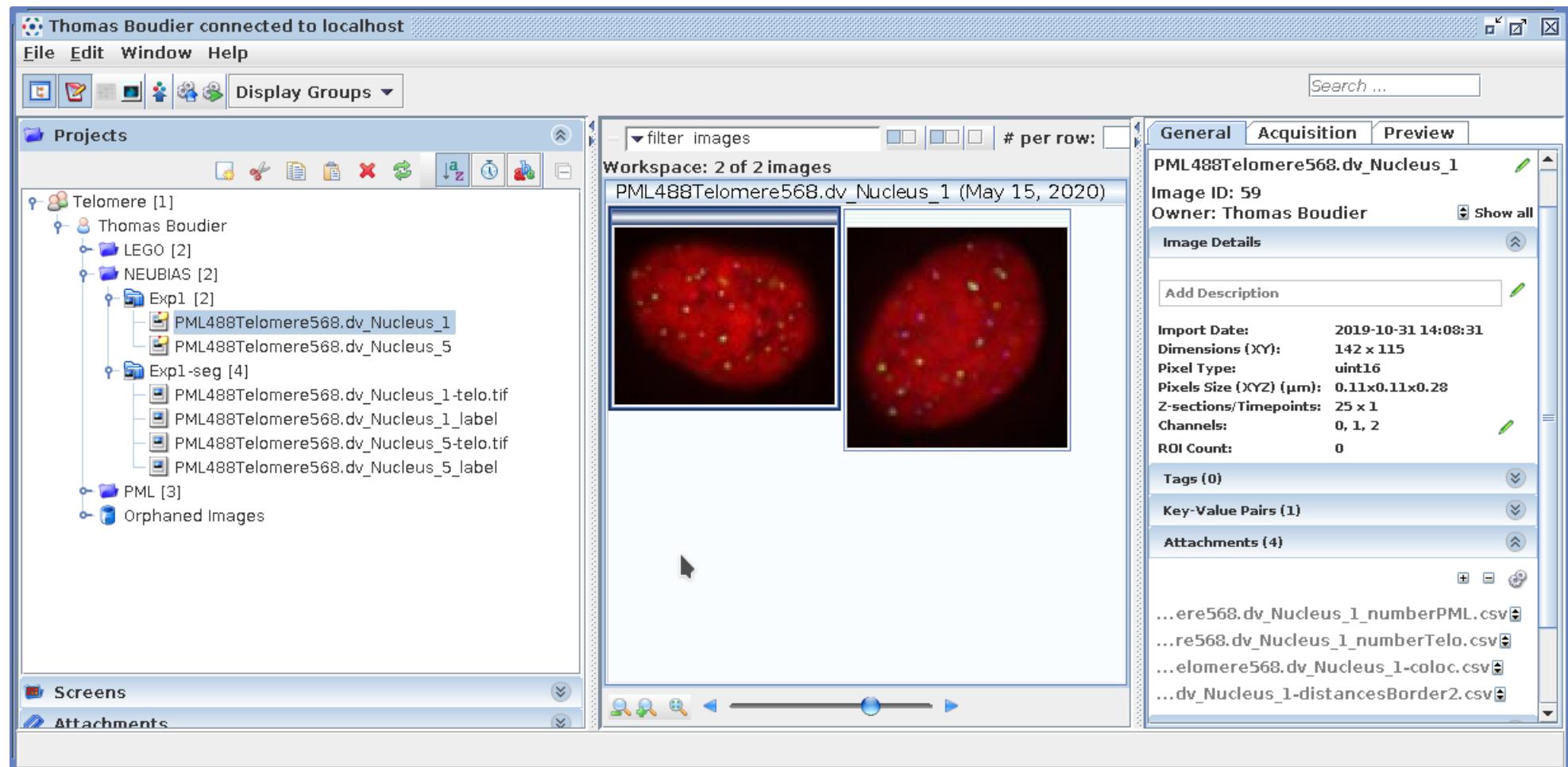
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 - *The parameters*
 - *radiusxy:4*
- **On which datasets to apply the protocol**
 - Either on disk or OMERO

```
// 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

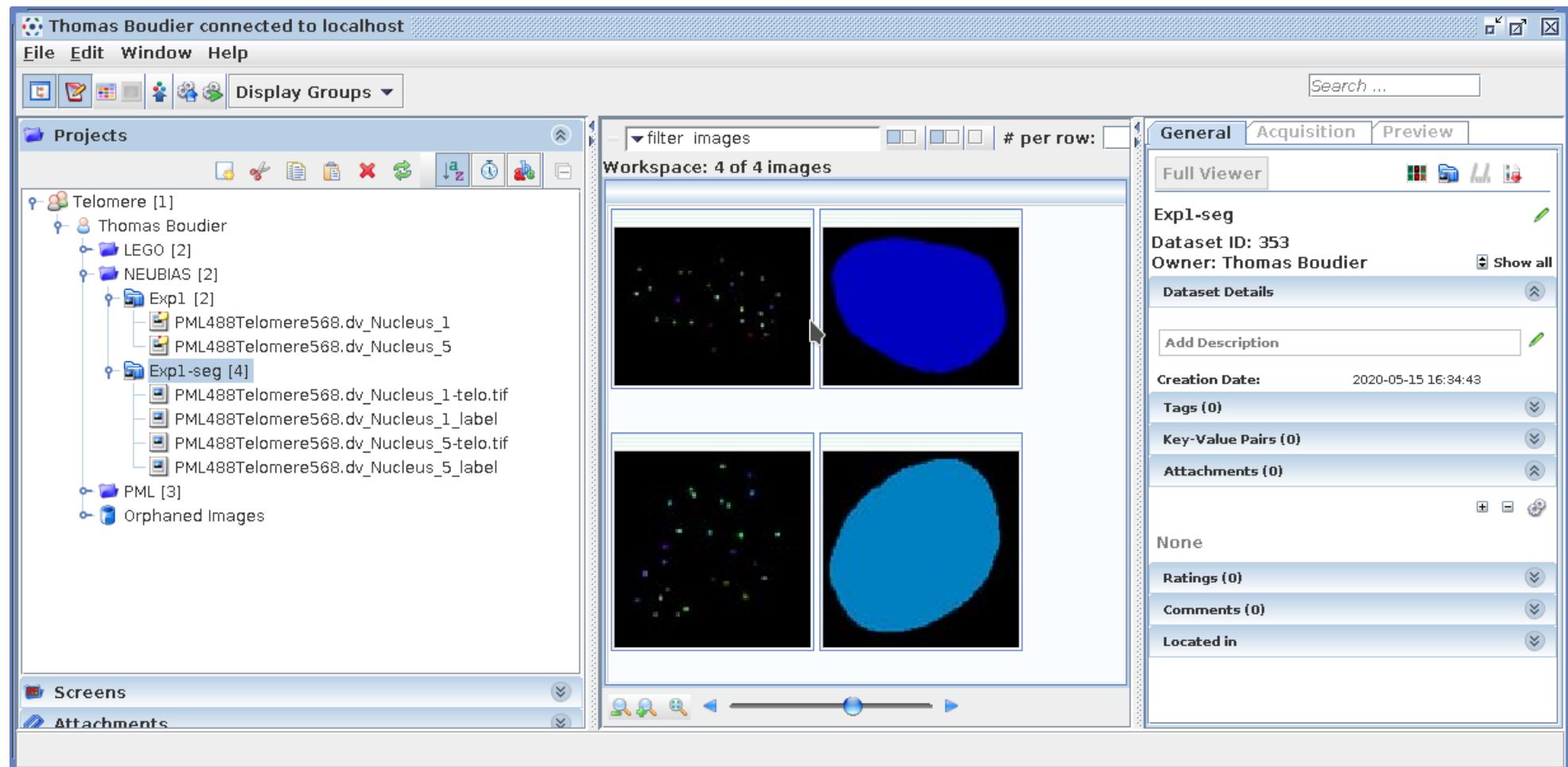


L. Chen, IMB, AS

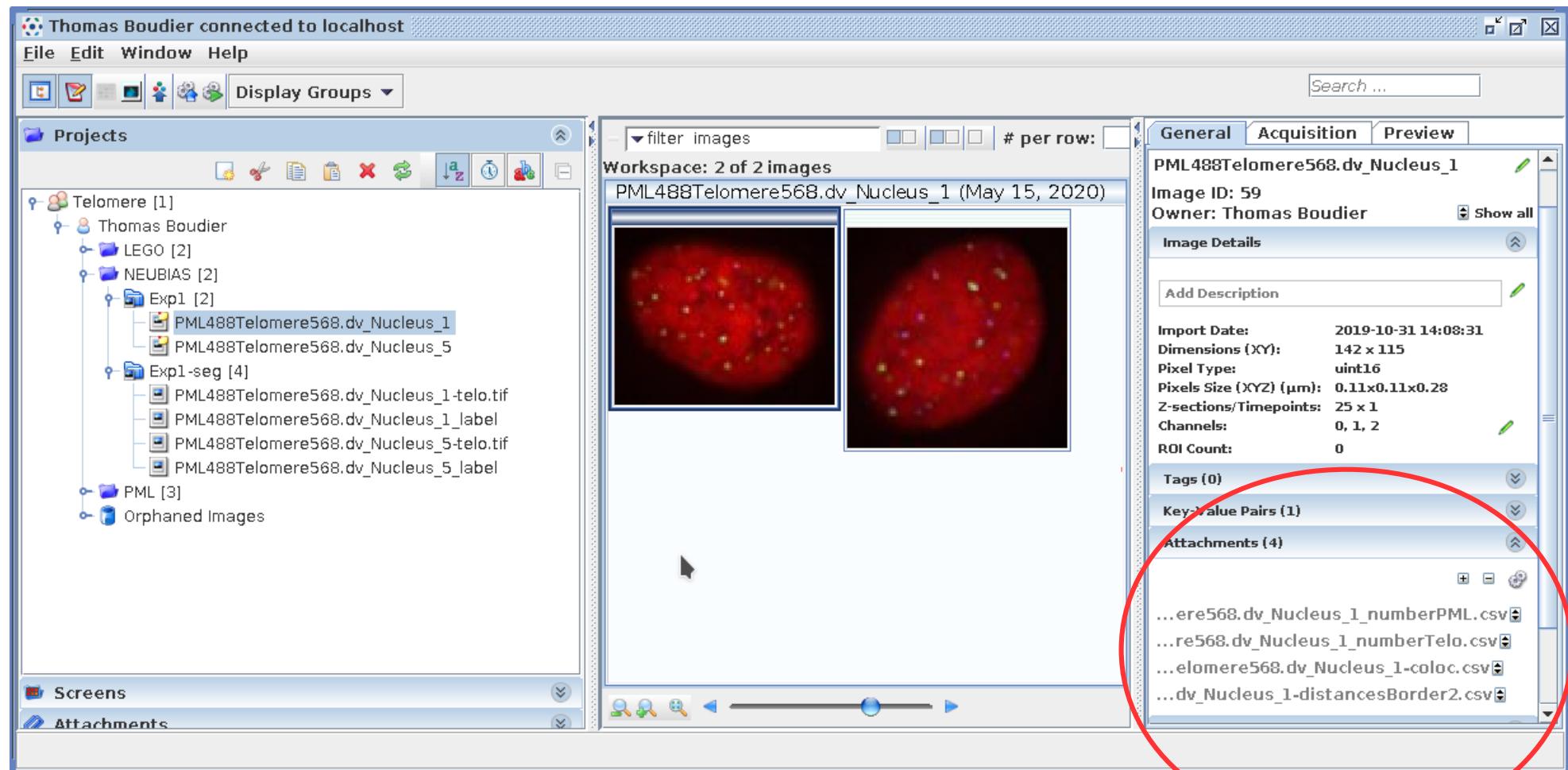
NEUBIAS Academy

T. Boudier - 3D ImageJSuite

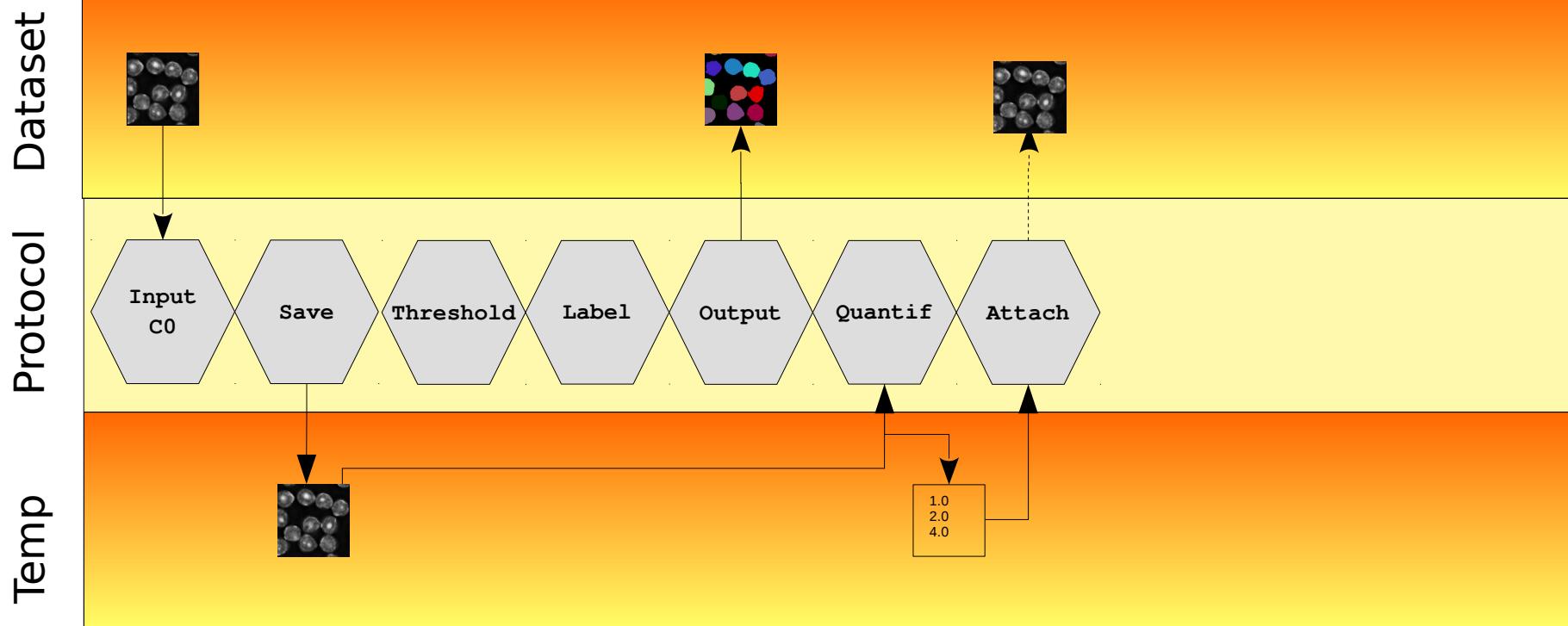
TAPAS



TAPAS

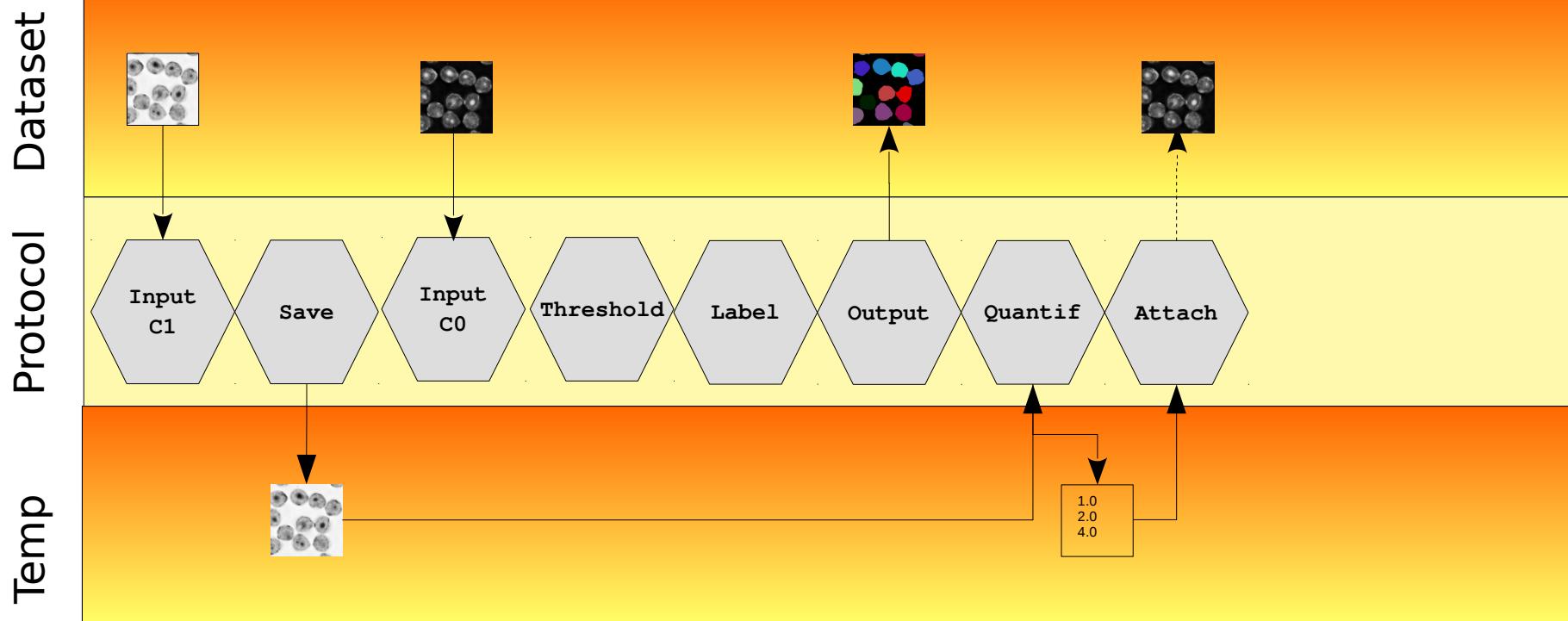


TAPAS



<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 ?