Nucleus Segmentation
Cell zone estimation
3DSpatialTissueJ plugin

https://github.com/nhuhoa/Spatial3DTissueJ

Hoa Tran

Workflow for tissue spatial organization analysis

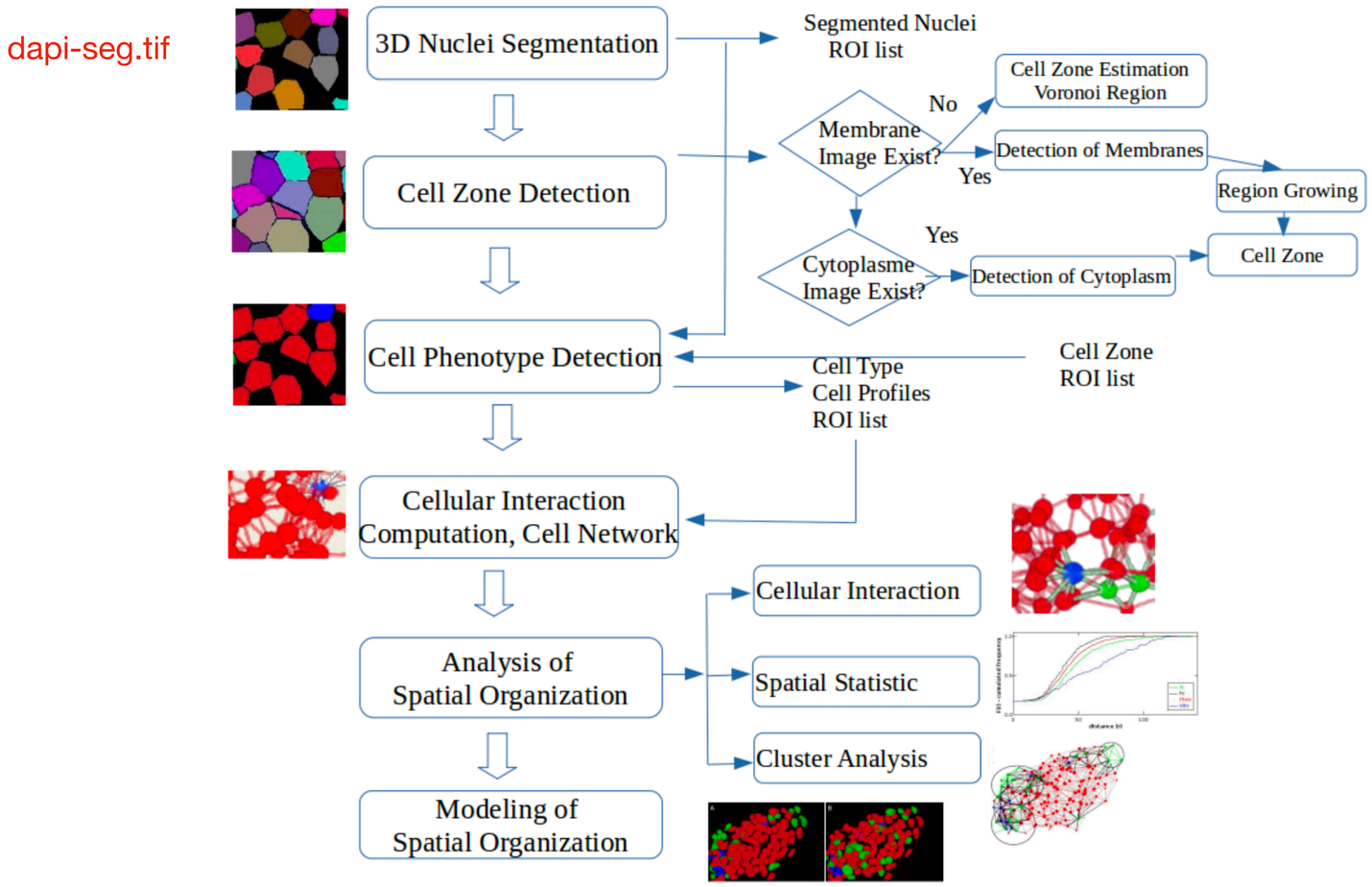


Figure : Workflow: (i) segmentation and cell phenotype detection (ii) spatial organization analysis

Ref [1] "A novel toolbox to investigate tissue spatial organization applied to the study of the islets of Langerhans". Hoa Tran et al.

3D Nuclei Segmentation

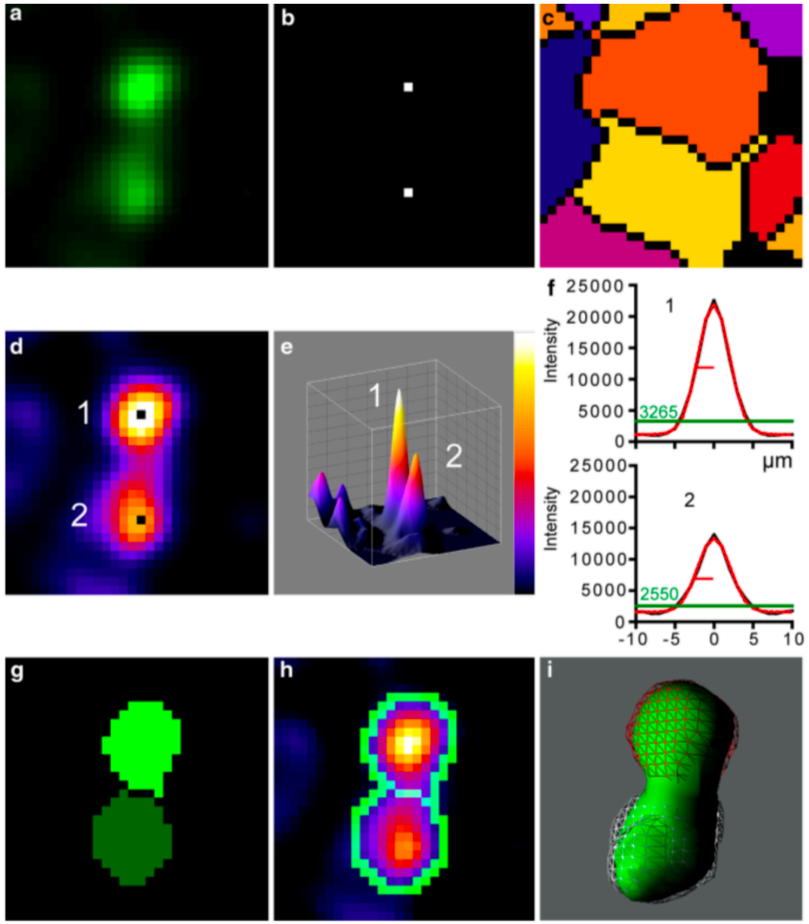


Figure : Steps of nuclei segmentation.

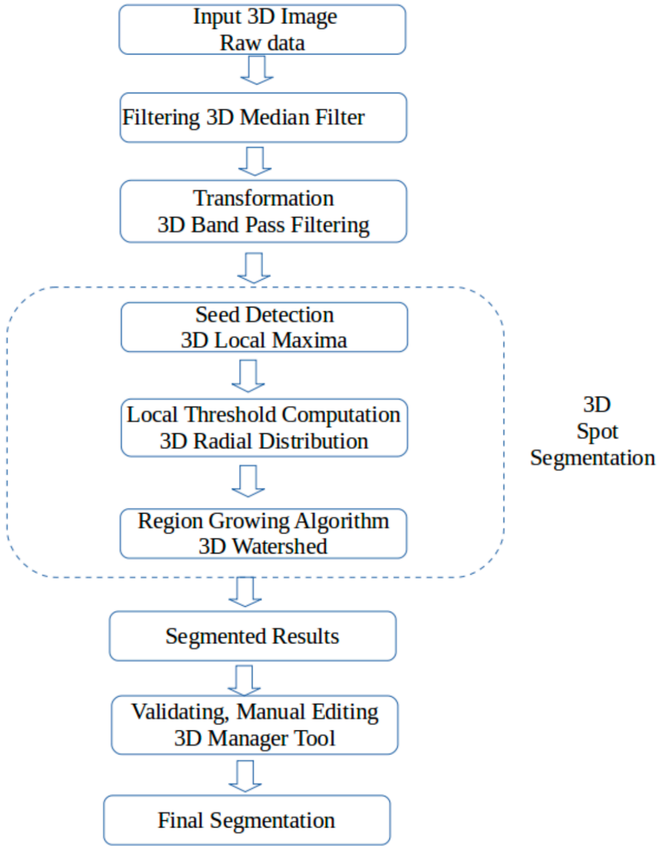


Figure : Diagram of nuclei segmentation [1].

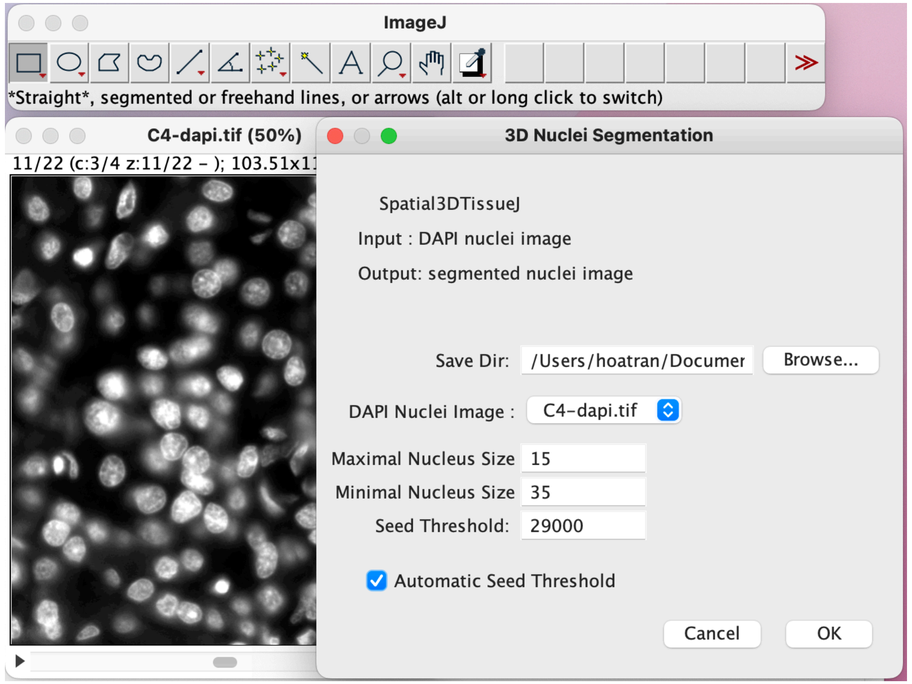
Ref [1] "A novel toolbox to investigate tissue spatial organization applied to the study of the islets of Langerhans". Hoa Tran et al.

Nucleus Segmentation Plugin

Noted: in windows system, your path will be:
ex: C::\yourdir\inputfolder\
Using '\' instead of '/' as in linux sys
Browser button automatically take care of directory path syntax

- Example macro
- 3 parameters need to defined:

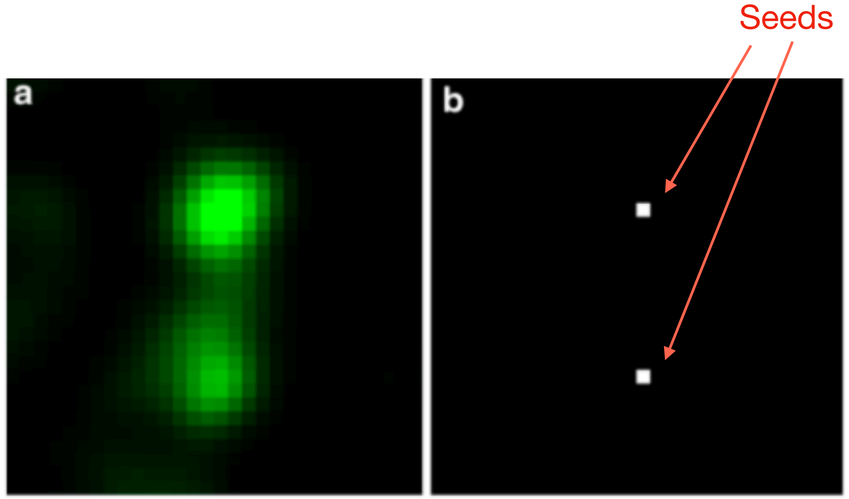
- *dir="C:/Users/SALAB VR/Documents/Hoa/Spatial3DTissueJ-master/H1536_islet1/";*
- ***small_nucleus_diameter_thrs=15;***
- ***large_nucleus_diameter_thrs=36;***
- *open(dir+"C4-dapi.tif");*
- *//// Using automatic seeds selection mode*
- *run("NUCLEI SEGMENTATION", "save_dir=[+dir+] dapi=C4-dapi.tif maximal="+large_nucleus_diameter_thrs+" minimal="+small_nucleus_diameter_thrs+" seed=29000* ***automatic****");*

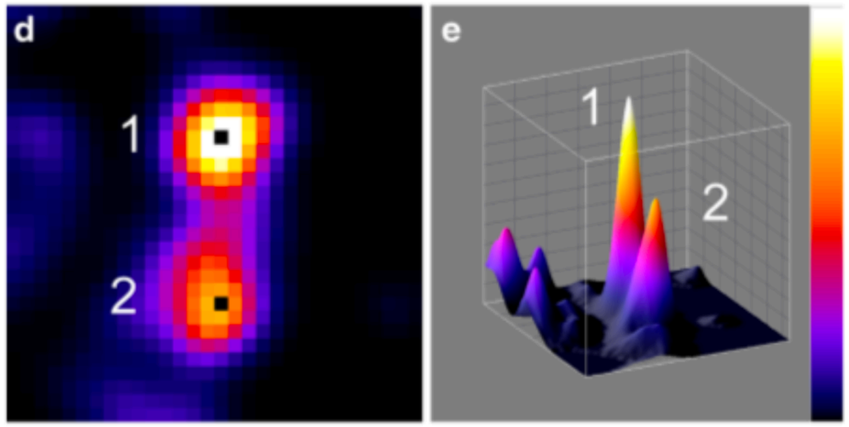


Ref [1] "A novel toolbox to investigate tissue spatial organization applied to the study of the islets of Langerhans". Hoa Tran et al.

Parameter 1: seed threshold

- Seeds: local maximal, pixels with intensity higher than intensity of the neighbourhood pixels.
- Manually define seed threshold value: ex: 29000, 30000 for 16 bits (similar to ex:120 /255 for 8 bits image)
- Automatic define seed threshold: (program will generate a seed threshold value)
  - Get mean value of all pixels in image
  - Seed = mean + sd, ex: sd=500, a pixel is considered as a seed if its intensity is higher than mean value of all pixels.





Ref [1] "A novel toolbox to investigate tissue spatial organization applied to the study of the islets of Langerhans". Hoa Tran et al.

Parameter 2: nucleus diameters

- Example macro
- *dir="C:/Users/SALAB VR/Documents/Hoa/Spatial3DTissueJ-master/H1536_islet1/";*
- ***small_nucleus_diameter_thrs=15;***
- ***large_nucleus_diameter_thrs=36;***
- *open(dir+"C4-dapi.tif");*
- *//// Using automatic seeds selection mode*
- *run("NUCLEI SEGMENTATION", "save_dir=[+dir+"] dapi=C4-dapi.tif maximal="+large_nucleus_diameter_thrs+" minimal="+small_nucleus_diameter_thrs+" seed=29000* ***automatic****");*

Key idea: Fourier transform as the transformation function for seed detection.

Small nuc diameter threshold

Large nuc diameter threshold

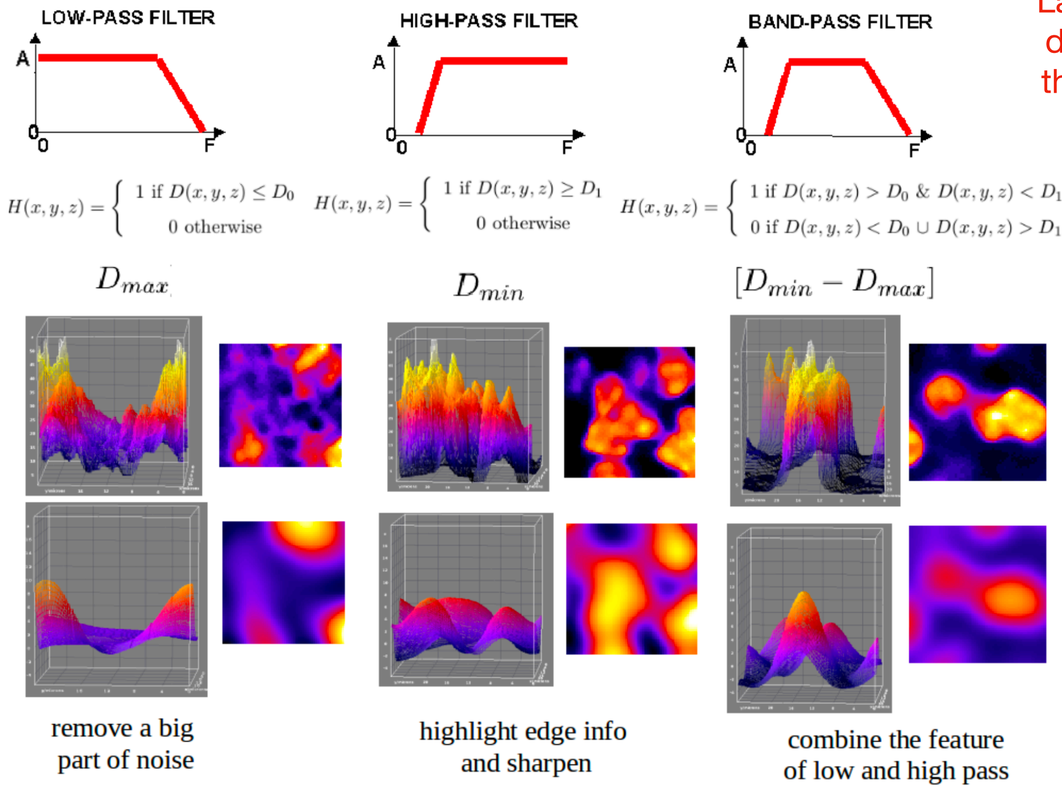
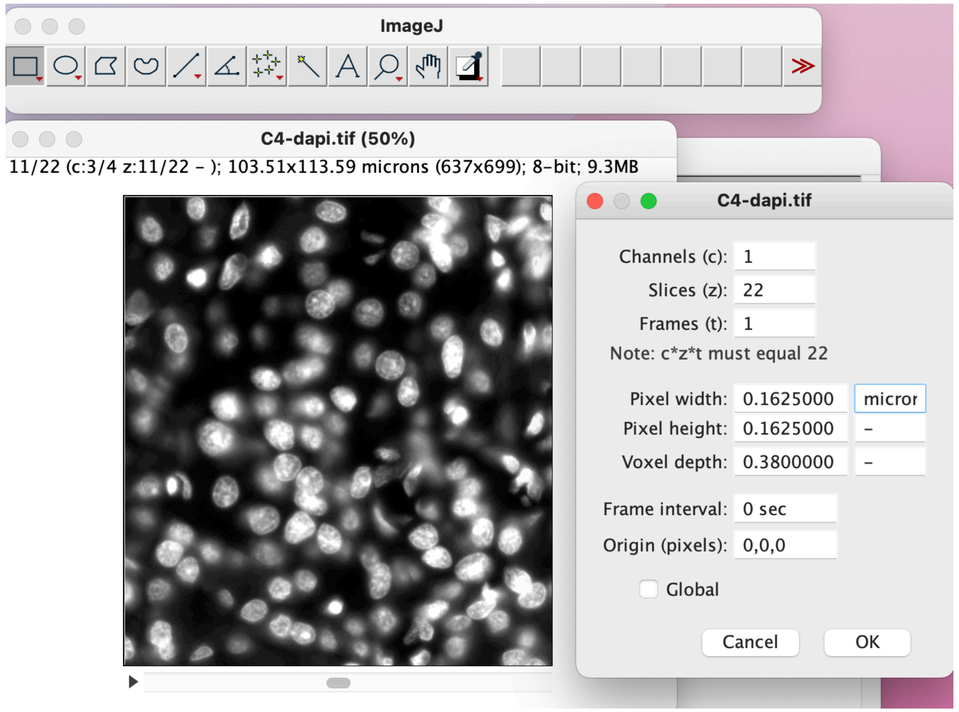
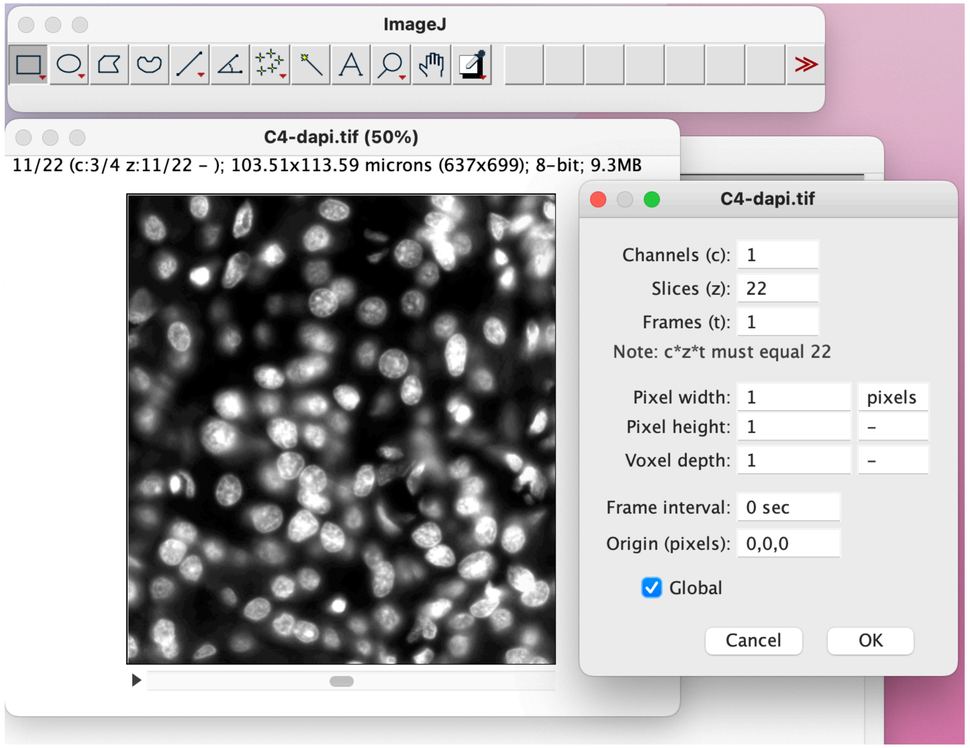


Figure : Band pass filtering and implementation in our 3D nuclei segmentation application.

Parameter 2: nucleus diameters

In current version, I define the nucleus diameters by measuring nucleus using rule tool.
In the future version, I can improve program by automatic estimate nuc radius but need time to test new algo

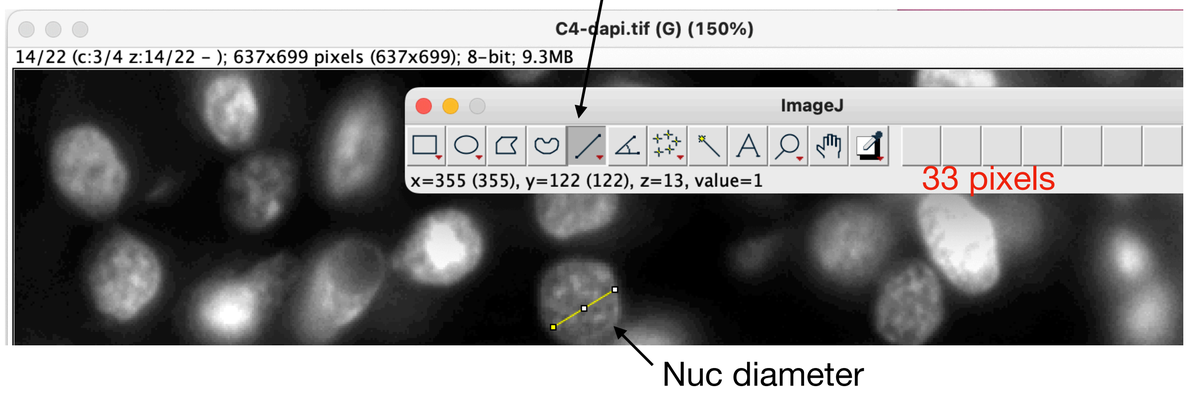


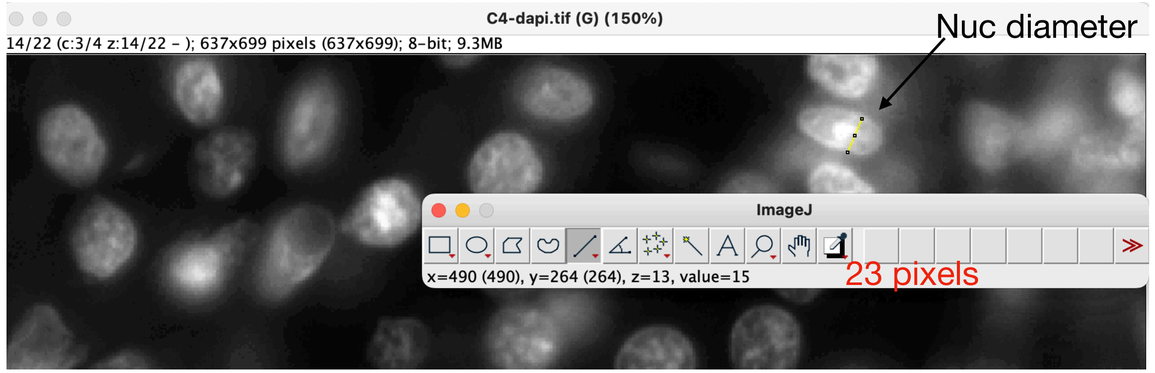


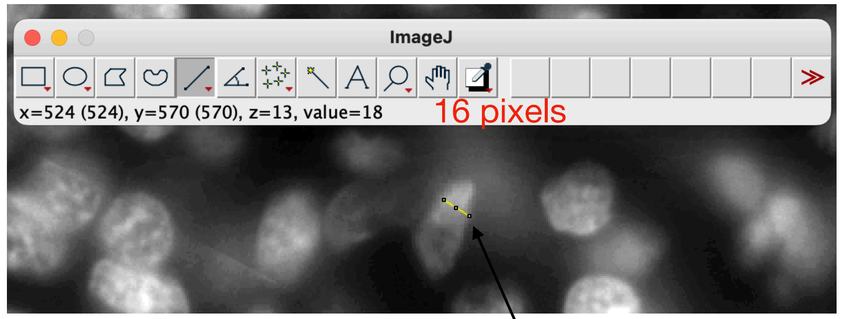
- To measure the small and large nucleus radius diameters: first we convert microns to pixels scale:
- ImageJ menu —> Image —> Properties
- Convert scale from microns to pixel

Parameter 2: nucleus diameters

- Using ruler tool
- Look at several nucleus: a big one, a small one, and check nucleus diameters.
- You can measure one time and use this setting parameter for other islet images in case you use same microscopy setting (ex: same microns to pixels) for all captured images



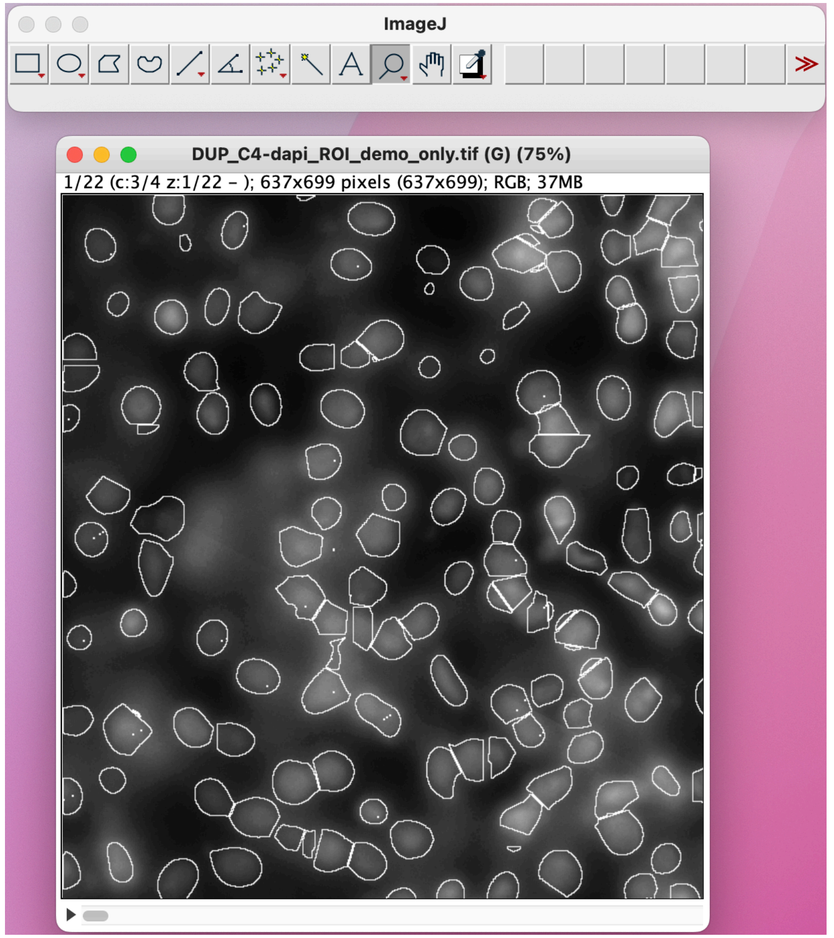




- *Ex: in plugin, I used: small_nucleus_diameter_thrs=15;*
- *large_nucleus_diameter_thrs=36;*

Parameter 2: nucleus diameters

- Look at the demo_only.tif image, if you see the nucleus are detected as your expectation —> good output. Otherwise, you can tune diameters thresholds by increasing or decreasing diameters thresholds.



Workflow for tissue spatial organization analysis

dapi-seg-wat.tif

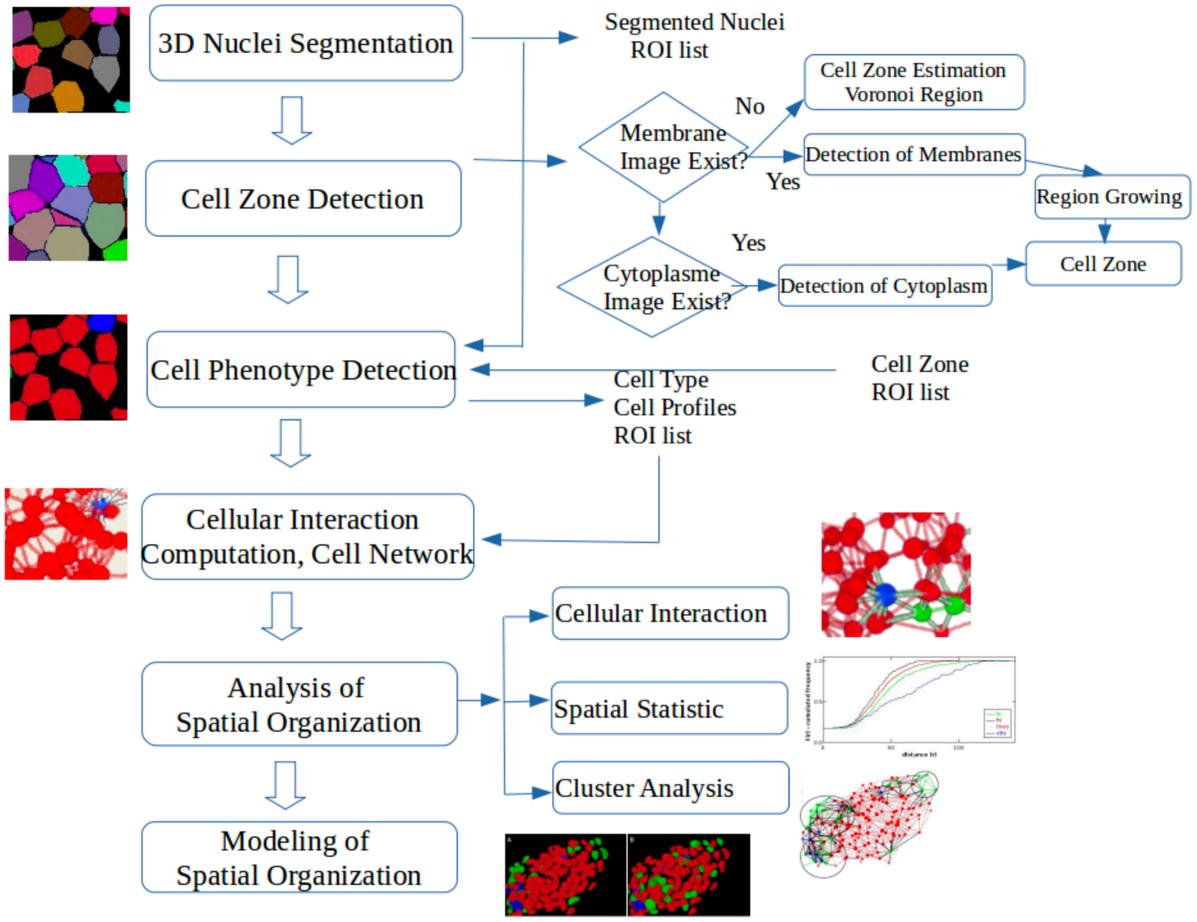


Figure : Workflow: (i) segmentation and cell phenotype detection (ii) spatial organization analysis

Ref [1] "A novel toolbox to investigate tissue spatial organization applied to the study of the islets of Langerhans". Hoa Tran et al.

Cell zone estimation

- Cell zone estimation: from nucleus - red area, extend into space to obtain cell zone area.
- In general: extend as much as possible —> not good
- Improved here: define the maximum of extension: ex: N pixels from nucleus border

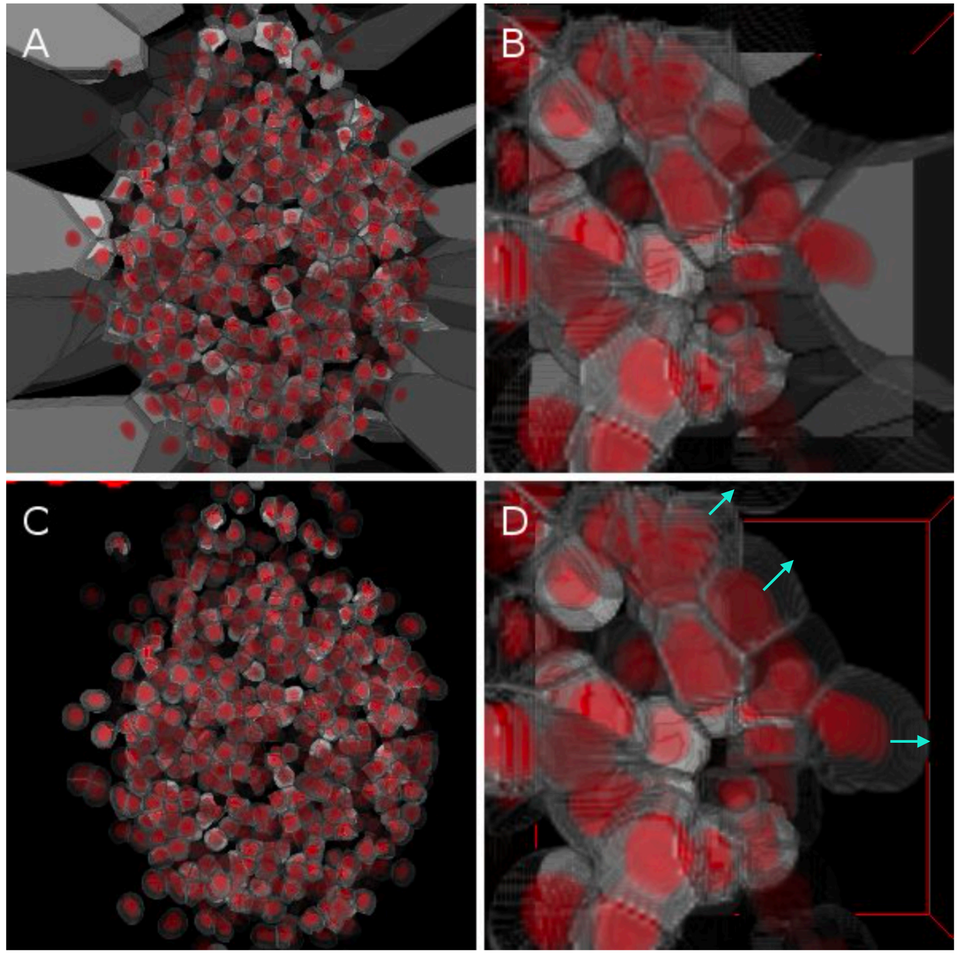
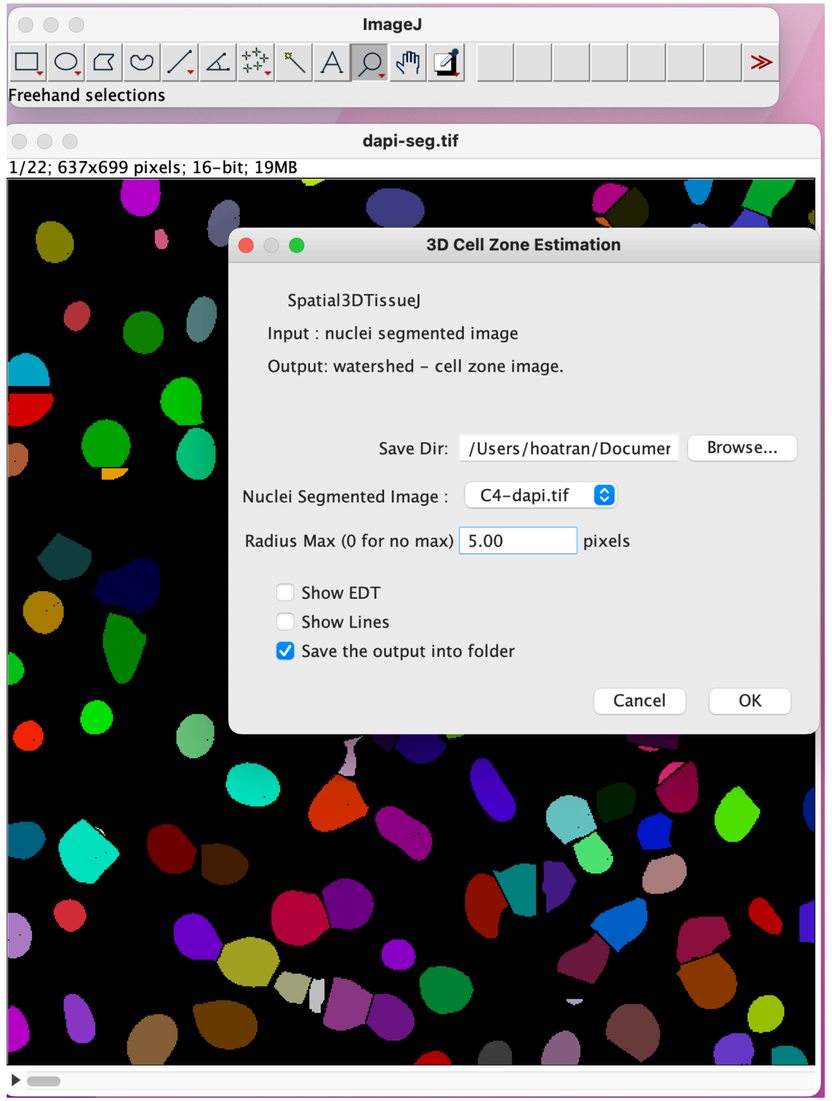


Figure : Cell zone estimation using watershed algorithms.

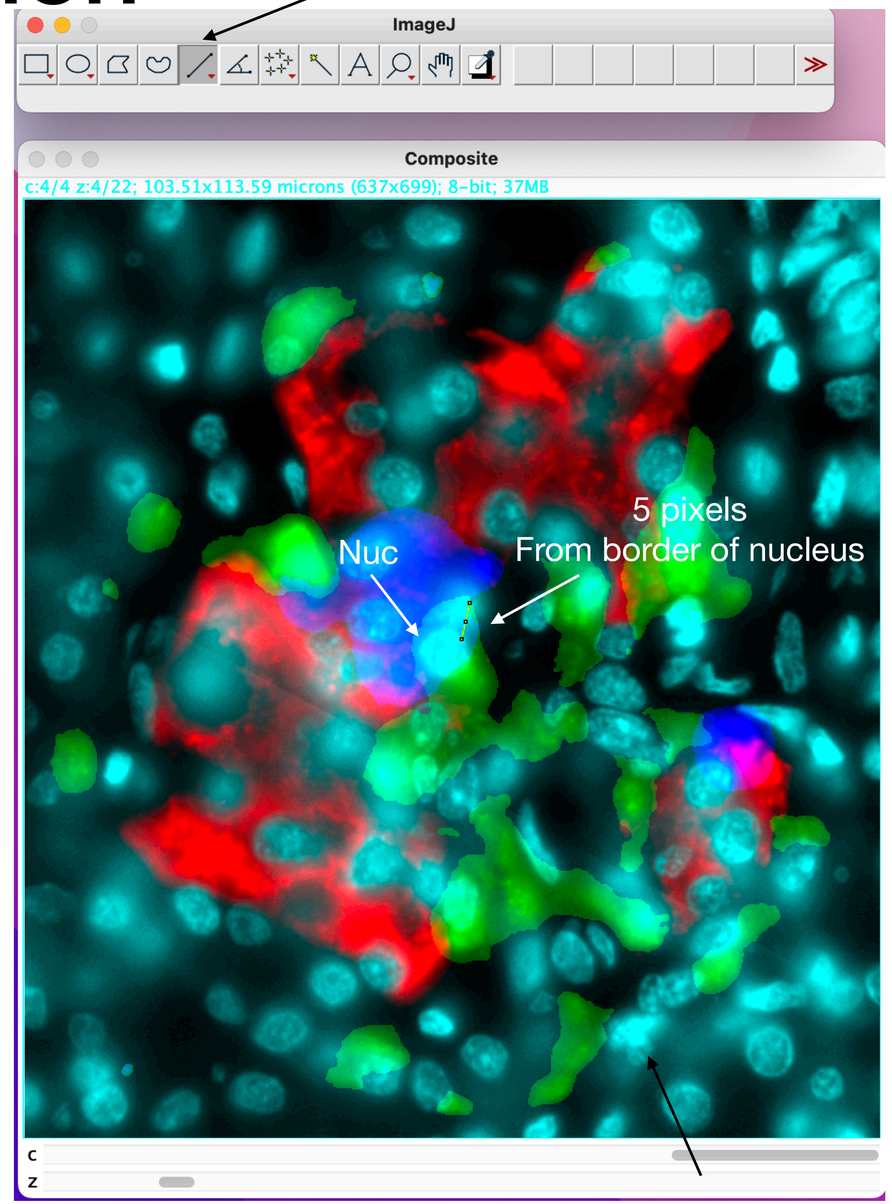
Cell zone estimation

- Example macro:
- dir="C:/Users/SALAB VR/Documents/Hoa/Spatial3DTissueJ-master/H1536_islet1/";
- max_radius_extension_wat=5; //change this threshold if your cell zone area in the image is smaller, depend on image resolution, in general 3 to 5 is a good one.
- open(dir+"C4-dapi.tif");
- open(dir+"dapi-seg.tif");
- run("CELL ZONE ESTIMATION", "save_dir=["+dir+"] nuclei=dapi-seg.tif radius_max="+max_radius_extension_wat+" save");



Parameter: radius of extension

- Using ruler tool
- Look at several nucleus: and check the maximum extension from nucleus border to the cell periphery
- You can measure one time and use this setting parameter for other islet images in case you use same microscopy setting (ex: same microns to pixels) for all captured images
  - ***Ex: in plugin, I used: max_radius_extension_wat=5;***



Acknowledgements

- [1] ”A novel toolbox to investigate tissue spatial organization applied to the study of the islets of Langerhans” Hoa Tran et al.

- [2] J. Ollion, J. Cochennec, F. Loll, C. Escudé, T. Boudier. (**2013**) TANGO: A Generic Tool for High-throughput 3D Image Analysis for Studying Nuclear Organization. *Bioinformatics* 2013 Jul 15;29(14):1840-1.

- [3] Spatial3DTissueJ plugins would like to thank P. Andrey, J.-F. Gilles and the developers of the following plugins :

    - Imagescience
    - LocalThickness
    - ConvexHull3D
    - 3D Object Counter
    - Droplet Counter