Improvement and automatization of an image analysis tool to characterize the plant 3D nucleus

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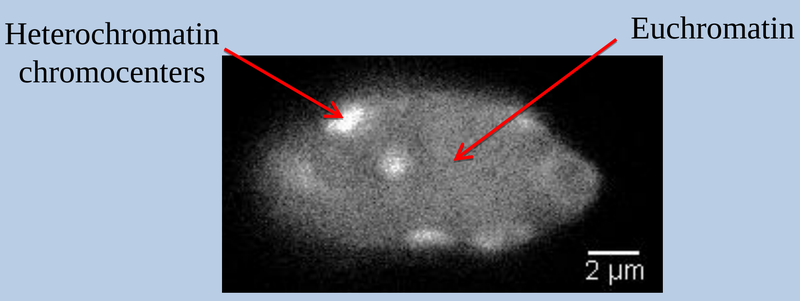
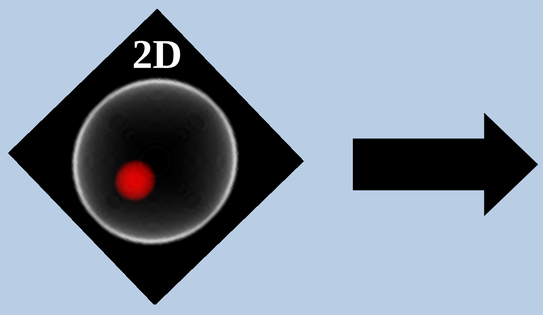
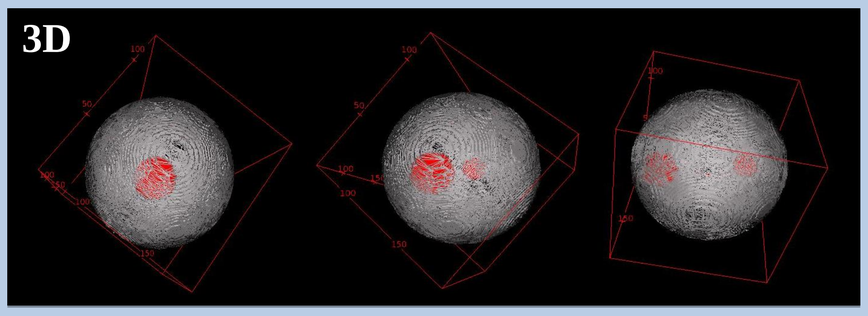
I. Objective: study nuclear organization (nuclear morphology and chromatin organization) in 3D



Parameters to characterize a nucleus?
Volume, shape, heterochromatin density & position,…



2D: only 1 chromocentre (red object) can be detected in the center of a nucleus (white circle)

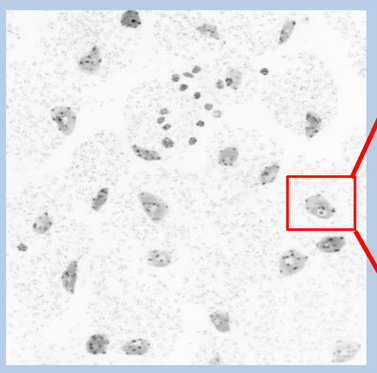


3D: reveals a **second chromocentre** behind the first one, and both are **close to the nuclear periphery.**

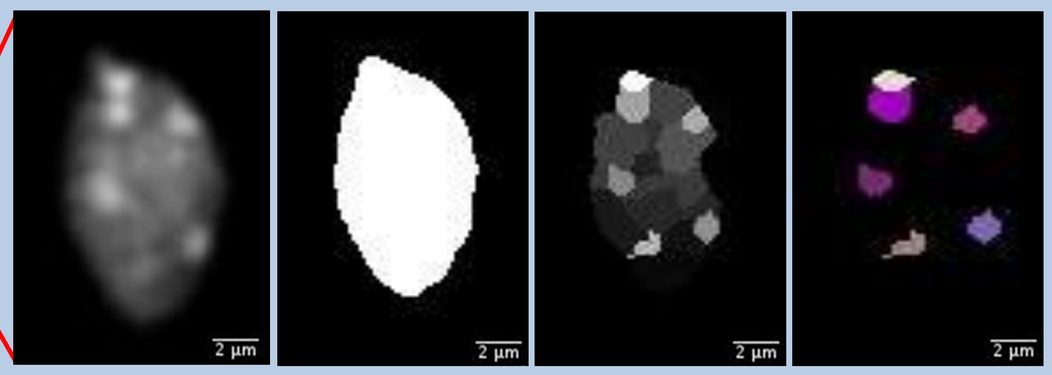
Aim : implement a workflow to compute 3D morphometric from Plant nuclei

II. Method: Wide field images acquisition and general image process analyses through the *NucleusJ* plugin

Whole-mount preparation
Fixed-tissue + DNA dye (*DAPI*)



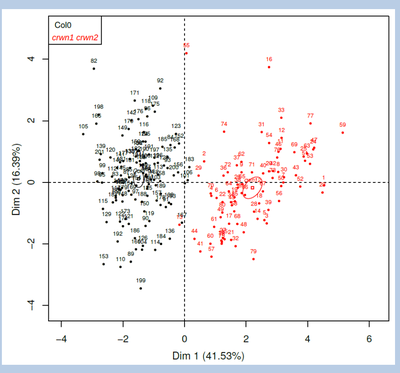
Wide field microscopy
3D image stack
(*MMAF+ optigrid*)



Automated isolation of nuclei (*Autocrop*)

Nuclei segmentation (*Otsu modified*)

Chromocenters segmentation with manual thresholding (*3D watershed + thresholding*)

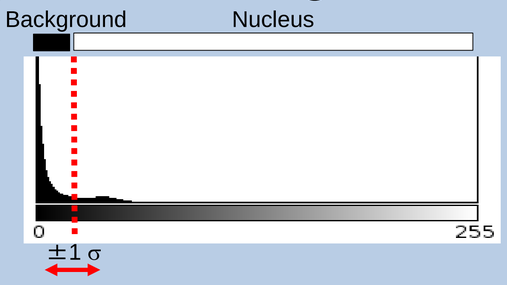


Output: 15 parameters (sphericity, distance, volume…)

NucleusJ is functional (see Poulet *et al.*, *Bioinformatics* 2017, *J. Cell Science* 2017) but needs automated segmentation processes

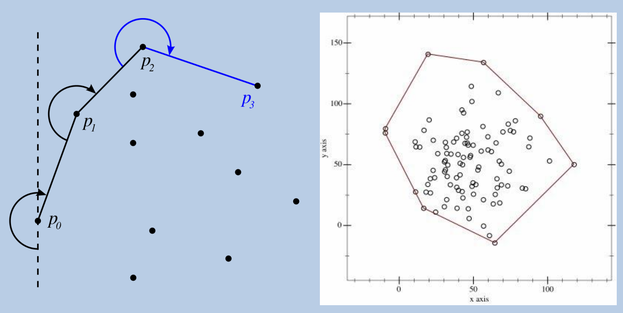
III. Improvement of NucleusJ

A) Modified OSTU segmentation



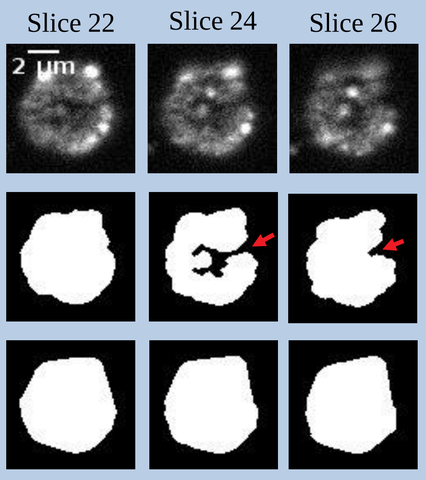
Optimized Otsu threshold maximized sphericity

B) Gift wrapping segmentation



Implementation of Jarvis march algorithm

C) Application on nuclei



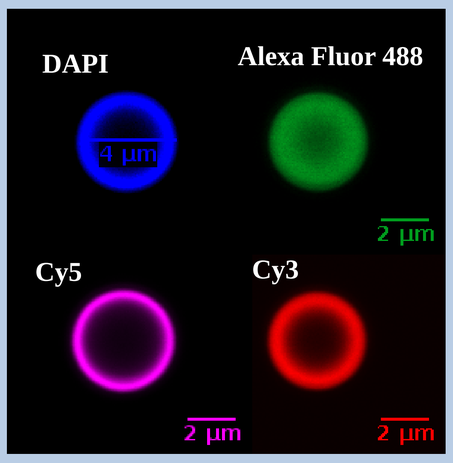
Nucleolus (no DNA = no staining)
Boundaries: Irregularities due to poor DAPI staining

Nucleolus (hole)
Indentations & irregularities at boundaries

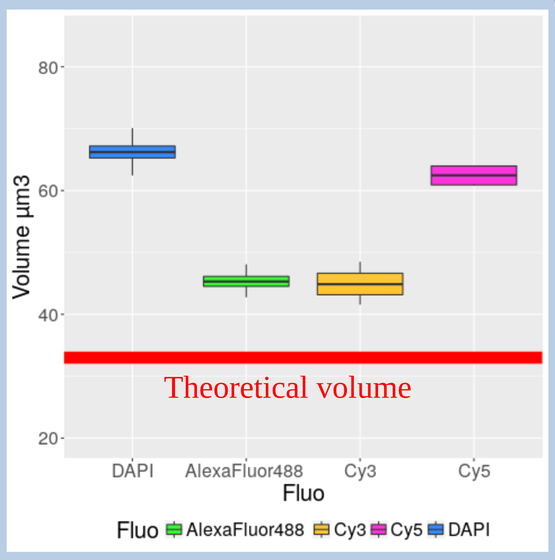
Nucleolus is filled (no hole)
Reduction of indentations & irregularities at boundaries
BUT
Over estimation of the volume

IV. Validation

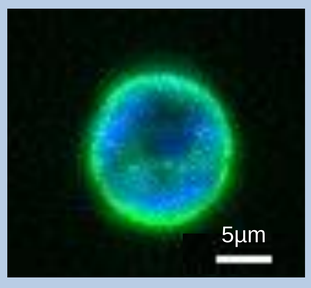
A) Using polychrome beads 4µm Ø



Segmentation → Volume computation



B) Using mutant with periphery marker (in progress)



SUN2-GFP marker : protein localized at the Nuclear periphery (nuclear envelope marker)

DAPI : DNA binding molecule

V- Conclusion & Perspectives

We select a set of segmentation methods to assess **quantitative parameters to describe** nuclear organization.

Future directions:

- Correction of the segmentation methods (theoretical objects, marked mutant …)
- Use of other model species (ex: spermatozoid nucleus compaction)
- Exploration of new method to automatize chromocenters segmentation (3D watershed -> Deep learning, machine learning ?)
- Adaptation to 3D DNA-FISH analyses