From microtubules tracking to cell nuclear volume evaluation: swapping from T axis to Z axis in confocal microscopy

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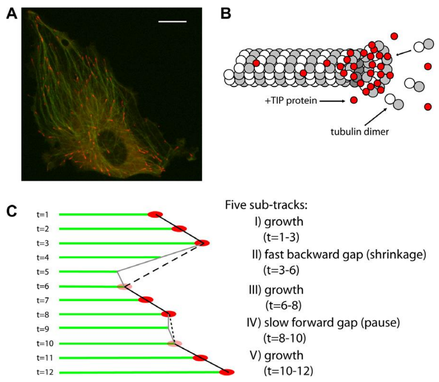
Introduction

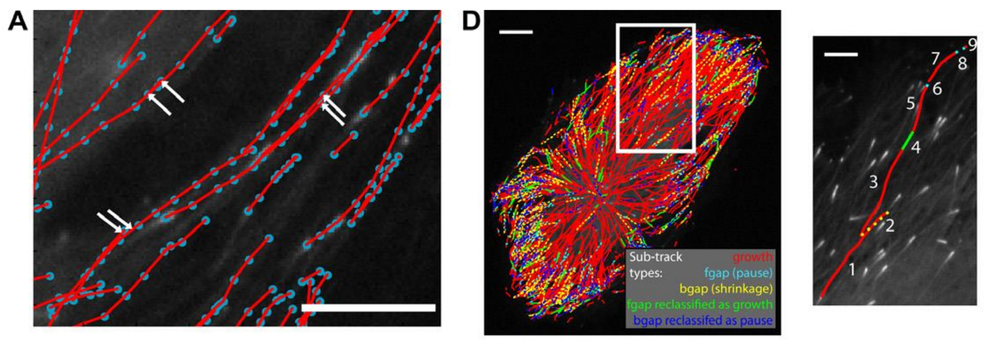
Image post processing with Matlab and ImageJ: microtubules dynamics, endoreduplicated nuclei size

- Microtubules dynamics measurements: growth rate, shrinkage. Time series images in confocal microscopy, post processing imaging with Matlab PlusTipTracker
- Endoreduplication: size of the nuclei which double DNA content without dividing. Z series images in confocal microscopy, post processing imaging with three compared methods: Our designed plugin in ImageJ, 3D Object counter in ImageJ, TrackMate in ImageJ.

T Axis

Microtubules dynamics can be evaluated analyzing time-series images of fluorescently labeled microtubule plus end binding proteins.

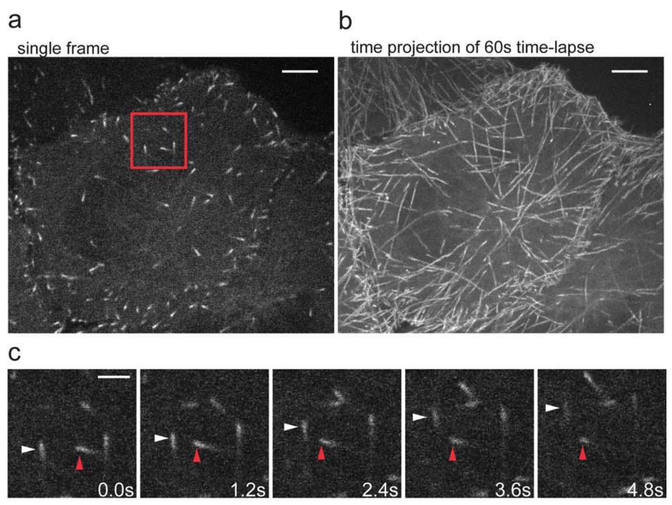




Kathryn T. Applegate, Sebastien Besson, Alexandre Matov, Maria H. Bagonis, Khuloud Jaqaman, Gaudenz Danuser (2011) plusTipTracker: Quantitative image analysis software for the measurement of microtubule dynamics Journal of Structural Biology 176, 168-184

Matlab

PlusTipTracker



Anne Straube (2011) How to measure microtubule dynamics? Anne Straube (ed.), *Microtubule Dynamics: Methods and Protocols*, Methods in Molecular Biology, vol. 777, 2011, pp 1-14

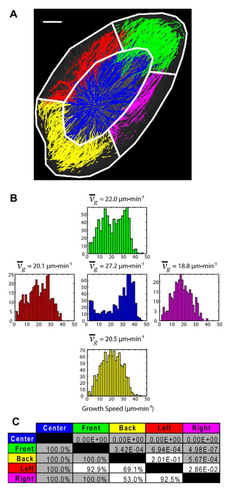


Table 2. Detected comets and velocities automatically calculated

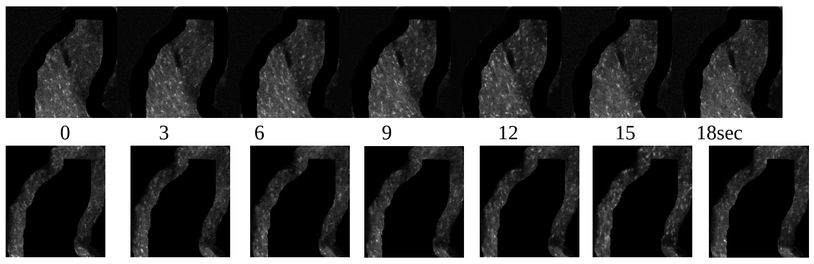
Movie	Minimize1	Minimize2	Series124
Boundary	515	417	282
Inside	578	484	280

Table 3. *t* Student test on two samples H0: does not exist differences between velocities on boundary and inside cell

Movie	Minimize1	Minimize2	Series124
P *t*-test	0.0058	0.55	0.08

Table 4. *t* Student test: population vs samples of comets really near to cell boundary

Minimize1	Velocity (µm min⁻¹)	P *t*-test
Population	7.66756	0.030
Sample	4.01092	



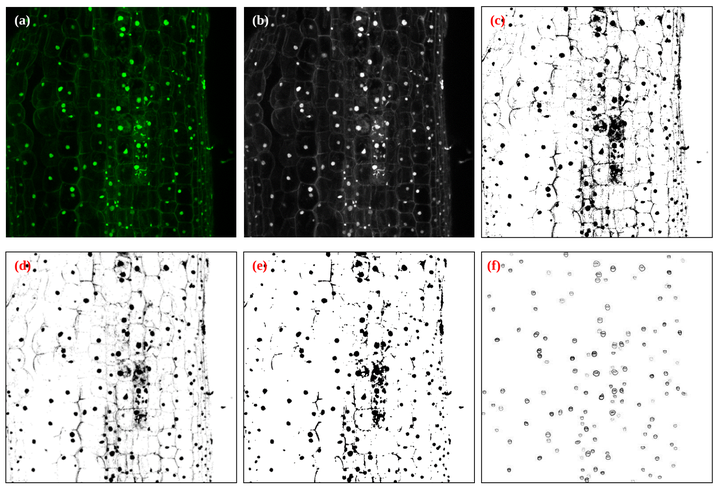
Z Axis

ImageJ

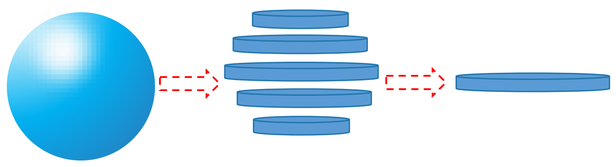
Designed detection macro

3D Object counter

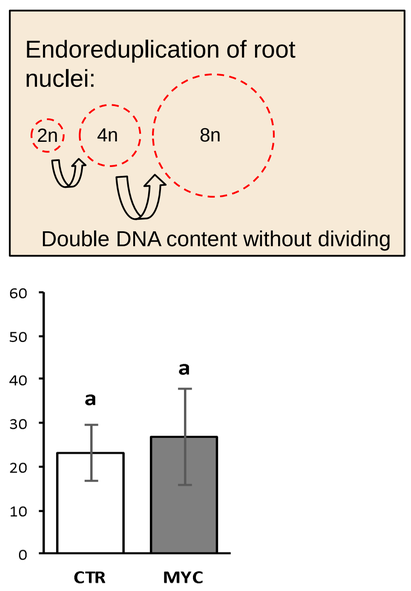
Track mate

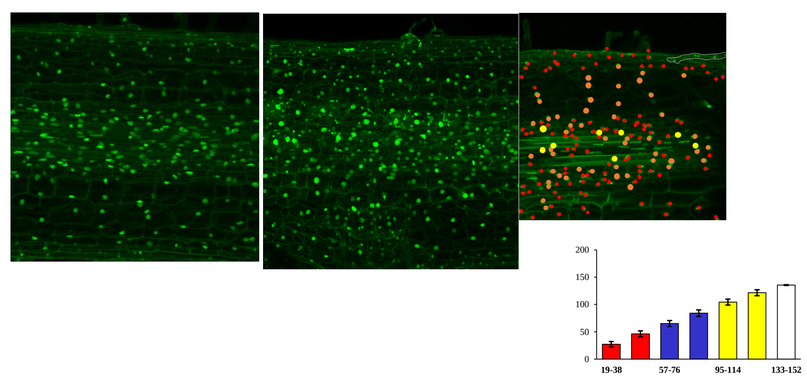


Swapping from T to Z axis we detected and associated all sections from each nucleus

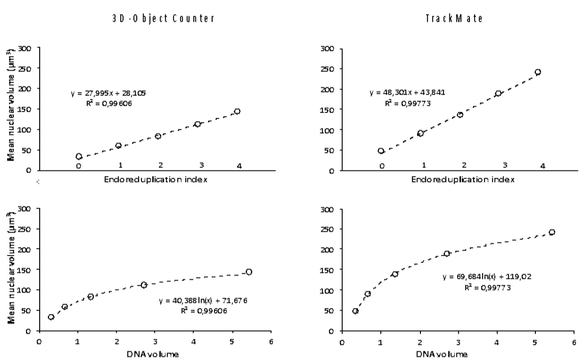


Lingua G, Fusconi A, Berta G. The nucleus of differentiated root plant cells: modifications induced by arbuscular mycorrhizal fungi. Eur J Histochem. 2001;45(1):9-20.

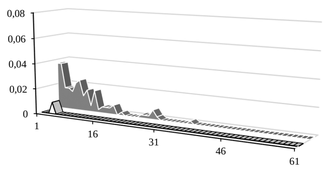


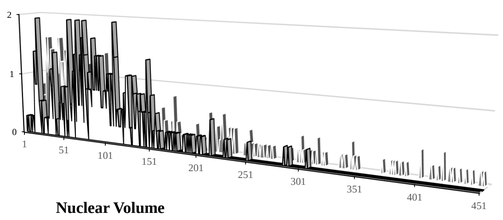


	EI	Mean values (3-OD)	K-means (TrackMate)	DNA (picograms)	DNA volume (µm³)	Volume ratio (VR) DNAvolume /measured nuclear volume (%)
2C	0	30.37 ± 7.35	38.99	1.09	0.34	1.11/0.87
4C	1	55.92 ± 7.09	73.71	2.18	0.68	1.21/0.92
8C	2	82.85 ± 7.15	104.11	4.36	1.36	1.64/1.30
16C	3	108.38 ± 6.06	136.1	8.72	2.72	2.50/1.99
32C	4	134 ± 7.44	167.27	17.44	5.44	4.05/3.25
64C	5	157.63 ± 5.87	199.42	34.88	10.88	6.90/5.45
128C	6	186 ± 9.81	237.26	69.76	21.76	11.69/9.17
256C	7	213 ± 8.08	278.34	139.52	43.52	20.43/15.63



Jovtchev G, Schubert V, Meister A, Barow M, Schubert I.(2006) Nuclear DNA content and nuclear and cell volume are positively correlated in angiosperms. Cytogenet Genome Res. 2006;114(1):77-82.





Results

T-Axis Automatic detection and tracking methods are computational tool useful to follow thousand of particles in each cell: we can compare mean velocities of cellular regions: we divide the ROI in two SUB-ROI: boudary region and inside region to test differences in mean velocities

Z-Axis All volume estimation methods converged on the same conclusion:
- Nuclear size increased in mycorrhizal roots
- Compatible with predicted changes related to different ploidy levels

Full support to flow cytometry and gene expression analyses, with the advantage of localizing endoreduplicated nuclei in the root sections, showing that endoreduplication occurs in the colonized area