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

This is the author's manuscript

Original Citation:

Availability:
This version is available http://hdl.handle.net/2318/1674691 since 2018-08-24T16:12:46Z

Terms of use:
Open Access
Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)
From microtubules tracking to cell nuclear volume evaluation: swapping from T to Z axis in confocal microscopy

Sciascia I.A., Carotenuto G., Di Cunto F., Gai M., Geno A.
1Department of Life Sciences and Systems Biology Università di Torino
2Department of Molecular Biotechnology and Health Sciences Università di Torino
ivan.sciascia@unito.it

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.

Matlab
PlusTipTracker

Table 1: Endoreduplication and nuclear size analysis (µm3 calculated

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mesophyll</th>
<th>Mesophyll 2</th>
<th>Demi64A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>115</td>
<td>49</td>
<td>292</td>
</tr>
<tr>
<td>Std</td>
<td>6.06</td>
<td>9.81</td>
<td>7.09</td>
</tr>
</tbody>
</table>

Z Axis

ImageJ

Designed detection macro

3D Object counter

Trackmate

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

Anne Straube (2011) How to measure microtubule dynamics?

Endoreduplication of root nuclei

Double DNA content without dividing

Table 2: Extracted Images and Nuclear size analysis (% calculated

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mesophyll</th>
<th>Mesophyll 2</th>
<th>Demi64A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4.1±0.2</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
</tr>
</tbody>
</table>

Table 3: Extracted Images and Nuclear size analysis (% calculated

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mesophyll</th>
<th>Mesophyll 2</th>
<th>Demi64A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4.1±0.2</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
</tr>
</tbody>
</table>

Table 4: Extracted Images and Nuclear size analysis (% calculated

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mesophyll</th>
<th>Mesophyll 2</th>
<th>Demi64A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4.1±0.2</td>
<td>0.1±0.1</td>
<td>0.1±0.1</td>
</tr>
</tbody>
</table>


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.