Paxillin Puncta Measurement

Adapted from Horzum et al. Methods X 2014

Overview

Step by Step Protocol

1. Open Image

2. Process>Subtract Background

3. Plugins>CLAHE

4. Process>Math>Exp
5. Adjust Brightness and Contrast to Improve visibility of the p-paxillin puncta

6. Plugins> LoG 3D

7. Image > Adjust > Threshold
With just background subtract.  

With improved method

8. **Analyze > Analyze Particles**

To measure intensity in a second channel.

1. **Analyze > Analyze Particles**

2. **Open ROI manager, select all of the ROIs**
3. Open second channel of interest, in this case phalloidin
4. Then click the measure button
5. Copy and paste results to excel sheet with p-paxillin measurements
6. You can now compare the p-paxillin measurements with the phalloidin measurements:
Next, protocol for counting nuclei

1. Open Image

2. Subtract background: Process>Subtract Background

3. Threshold the image. Image>Adjust>Threshold

4. Analyze Particles

<table>
<thead>
<tr>
<th>Area</th>
<th>Mean</th>
<th>Perim.</th>
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<tr>
<td>1</td>
<td>1484.2</td>
<td>137.5</td>
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<tr>
<td>2</td>
<td>5905.6</td>
<td>456.3</td>
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<td>181.2</td>
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<td>2068.5</td>
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