Tube Radius Calculation

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Jul 25, 2022

1 Works for me Share

dx.doi.org/10.17504/protocols.io.x54v9y3rqg3e/v1

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DISCLAIMER

ABSTRACT

This protocol details Tube Radius Calculation.

ATTACHMENTS

416-898.pdf

DOI

dx.doi.org/10.17504/protocols.io.x54v9y3rqg3e/v1

PROTOCOL CITATION

Liv Jensen 2022. Tube Radius Calculation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9y3rqg3e/v1

KEYWORDS

Tube Radius Calculation, Fluorescence ratio calibration, ASAPCRN

CREATED

May 03, 2022

LAST MODIFIED

Jul 25, 2022

OWNERSHIP HISTORY

May 03, 2022 maria.s

May 25, 2022 Liv Jensen

PROTOCOL INTEGER ID

61846

MATERIALS TEXT

Materials:

- Image stack of multichannel confocal fluorescence images
- Pressure data over time for GUV aspiration pipette
- Force data over time for bead held in optical trap
- FIJI
- Image segmentation and quantification script (Python)

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Fluorescence ratio calibration:

1 Measure the radius of the GUV and of the aspiration pipette from imaging data.

2 Calculate radius from:

$$R = \frac{F}{4\pi\left(\frac{\Delta P * r_{pip}}{2\left(1-\frac{r_{pip}}{r_{GUV}}\right)}\right)}$$

3 In FIJI, create new image stacks from two rectangular selections: one containing a section of the membrane tube, and one containing the approximately horizontal section of the guv.

4 Save these new stacks as .tif files and paste paths into segmentation and quantification script.

5 Use the output of the segmentation and quantification script to calculate the ratio of fluorescence intensity of the membrane tube and the GUV surface (I_{tub} / I_{GUV}).

6 Plot tube radius as calculated in step 2 vs. the fluorescence ratio calculated in step 5. Perform a linear fit of the data, recording the slope, K_{tub}, for use in subsequent experiments.

Calculating tube radius with fluorescence images and K_{tub}:

7 In FIJI, create new image stacks from two rectangular selections: one containing a section of the membrane tube, and one containing the approximately horizontal section of the guv.

8 Save these new stacks as .tif files and paste paths into segmentation and quantification script.

9 Input K_{tub} as a parameter in the quantification function to normalize fluorescence ratio to tube radius.

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