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Tube Radius Calculation

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details Tube Radius Calculation.

Attachments



[416-898.pdf](#)

42KB

Materials

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)

Fluorescence ratio calibration:

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

2 Calculate radius from:

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

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.