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# Surface Density Calculation

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1 Works for me

 Share[dx.doi.org/10.17504/protocols.io.81wgb65nnlpk/v1](https://dx.doi.org/10.17504/protocols.io.81wgb65nnlpk/v1) Liv Jensen

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## ABSTRACT

This protocol details Surface Density Calculation.

## ATTACHMENTS

[416-900.pdf](#)

## DOI

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## PROTOCOL CITATION

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## KEYWORDS

Surface Density Calculation, fluorescence intensity, ASAPCRN

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## CREATED

May 03, 2022

## LAST MODIFIED

Jul 25, 2022

## OWNERSHIP HISTORY

May 03, 2022  maria.s

May 25, 2022  Liv Jensen

## PROTOCOL INTEGER ID

61857

## MATERIALS TEXT

Materials: -

- Multichannel confocal fluorescence images; fluorophores in solution and on GUV at defined densities
- FIJI
- Image segmentation and quantification script (Python)

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## Surface Density Calculation

- 1 Quantify the fluorescence of a serial dilution of membrane fluorophore and of proteintag fluorophore in 1% SDS-containing buffer ([M]**10 micromolar (μM)**, [M]**6 micromolar (μM)**, [M]**2 micromolar (μM)**, [M]**1 micromolar (μM)**, [M]**0.5 micromolar (μM)** of each fluor).
- 2 Compare the slopes of a plot of fluorescence intensity vs. concentration for the membrane dye and the protein dye to account for differences in optical properties.
- 3 Quantify the fluorescence of a series of GUV preparations containing varying mole percentage of membrane fluorophore (0.01%, 0.05%, 0.1%, 0.5%, 1%), using a rectangular selection of an approximately horizontal segment of each GUV.
- 4 Calculate the surface density of membrane dye from known surface area of lipids (~0.7nm<sup>2</sup>/lipid).
- 5 Calculate the slope of a plot of membrane dye surface density vs. fluorescence signal, accounting for intensity from both lipid bilayer leaflets:

$$\phi_{mem\ dye, leaflet} = \frac{AI_{mem\ dye, GUV}}{2}$$

- 6 Relate surface density of lipid to surface density of protein label by normalizing to signal from bulk solution (from step 2):

$$\phi_{prot, GUV} = \frac{AI_{prot, GUV}}{prot\ dye_{bulk} / mem\ dye_{bulk}}$$