SUPER-RESOLUTION OPTICAL FLUCTUATION IMAGING ANALYSIS OF PROTEIN DENSITIES AND NANOSCALE ORGANIZATION

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Patterns and densities of surface proteins heterogeneously distributed on the surface of living cells influence the function of the cell. Characterization of their nanoscale organization is important for better understanding of cell membrane related processes. This nanoscale organization of proteins have been investigated by super-resolution optical fluctuation imaging (SOFI). SOFI microscopy achieves resolution beyond the diffraction limit by computing higher order statistics (cumulants) of time series of stochastically blinking fluorophores [1]. SOFI resolution improvement is based on properties of spatio-temporal cross cumulants calculated from the entire image sequence at once. By combining SOFI higher order cumulants, molecular parameters like fluorophore blinking rates and localization density can be estimated [2], [3]. We present a model-free SOFI based method for quantitative evaluation of local densities and distribution of proteins in the plasma membrane. Using SOFI localization density maps, the method is robust to multiple-blinking of fluorescent proteins and compatible with wide range of blinking conditions and high labelling densities. We demonstrate the method by investigating the impact of CD4 palmitoylation on nanoscale distribution of CD4 fluorescent-protein fusion variants in the plasma membrane of resting T cells.

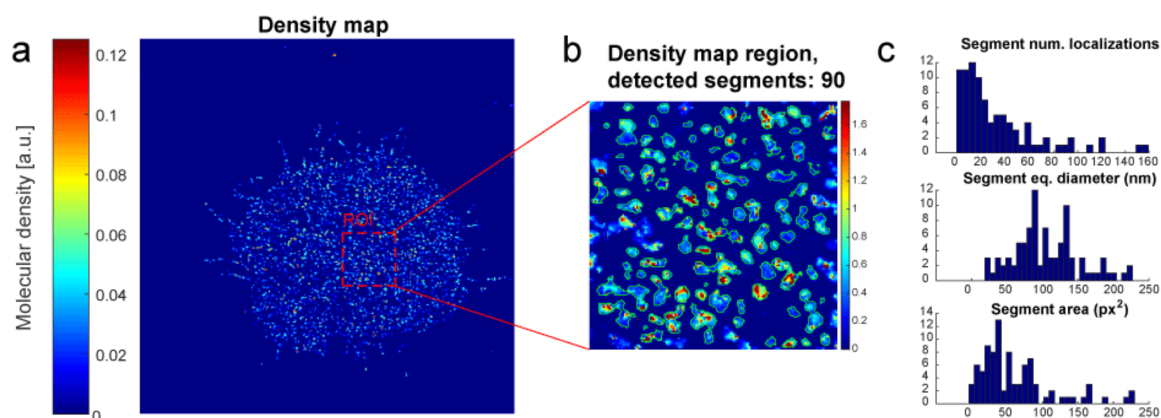

Figure 1: Representative image of data processing for a single cell wild-type CD4 labelled with mEos2 fluorescent protein. (a) Molecular density map estimated from SOFI images after background removal. (b) An example of segmentation of the 3 x 3 µm region of interest indicated in (a) by the dashed red line. (c) Properties of the segmented region in (b).

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