INTEGRATING IMAGING FCS WITH SRRF/SOFI ANALYSES FOR SPATIALLY MULTIPLEXED PROTEIN DYNAMICS AND NANOSCALE ORGANIZATION IN LIVE CELLS

Xue Wen Ng[1,2], George Barbastathis[2,3], Thorsten Wohland[1,4]
[1]Department of Chemistry and NUS Centre for Bioimaging Sciences, National University of Singapore, Singapore
[2]Singapore-MIT Alliance for Research and Technology (SMART) Centre, Singapore
[3]Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America
[4]Department of Biological Sciences, National University of Singapore, Singapore
E-mail: a0054184@u.nus.edu

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The introduction of fluorescence-based super-resolution microscopy in the past decade revolutionized the study of biological systems at spatial scales beyond the diffraction limit. Several super-resolution microscopy techniques cleverly exploit the photophysics of fluorophores to achieve nanometer spatial resolution. Of particular interest are computational super-resolution techniques such as super-resolution optical fluctuation imaging (SOFI) and, more recently, super-resolution radial fluctuations (SRRF) analysis which are compatible with time-lapse diffraction-limited microscopy imaging of live samples and do not require the use of any specialized fluorophores or sophisticated optical systems, unlike conventional super-resolution techniques. Consequently, SOFI/SRRF can be combined with fluorescence correlation spectroscopy (FCS) to achieve superior spatial and temporal resolutions. In SOFI, higher-order temporal correlation analysis is conducted on fluorescence fluctuations originating from the stochastic switching between a bright and dark state of the fluorophores on a diffraction-limited image stack to achieve super-resolution [1]. SRRF extends SOFI by conducting a spatial analysis on the image stack to generate radiality maps prior to performing a similar temporal correlation analysis as SOFI on the radiality image stack to produce a super-resolved image [2]. Furthermore, one can perform camera-based Imaging fluorescence correlation spectroscopy (Imaging FCS) analysis on the same raw image stack to generate diffraction-limited maps of diffusion when acquired at sufficiently fast rates [3]. In this study, we apply Imaging FCS and SOFI/SRRF analyses on total internal reflection fluorescence microscopy (TIRFM) image stacks to obtain complementary information on the diffusion dynamics and super-resolved structures of proteins in live cell membranes.

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