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Yan Chen, Peter T C So, and Enrico Gratton.

Study of molecular aggregation by analysis of photon histograms of single molecules.

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Abstract

Molecular aggregation is a crucial regulatory mechanism in biological systems. However, most experimental techniques require relatively high concentrations (from mM to gM). Since many interesting physiological events happen at concentrations below micromolar, we have explored the possibility to detect molecular aggregation in the low concentration range (from nM to pM) using two-photon fluorescence microscopy. Two-photon fluorescence microscopy has an inherent 3-D resolution and using high numerical aperture objectives the excitation volume can be made as small as 0.1 fl., which only contains 0.06 molecules at 1 nM concentration. A small excitation volume is essential for detecting fluorescence fluctuations among molecules. By analyzing the histogram of photons bursts, one can obtain the degrees of aggregation from their distribution (H. Qian and E. Elson, 1990, PNAS, 87, 5479). We model our photon histograms with a simple two species system and compare experimental results to the theoretical predictions. This method of analysis is intrinsically simpler than the technique of fluctuation correlation spectroscopy, which requires the calculation of higher order correlation functions for the multiple species system. (This work was supported by NIH RRO3155.)