IMAGING FCS AS A TOOL TO STUDY MEMBRANE ORGANIZATION

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KEY WORDS: FCS, Cross-Correlation, Diffusion law, Heterogeneity, Membrane Organization

The cell membrane is characterized by a complex composition of molecules organized into various domains. However, direct visualization of these domains is hindered by the fact that the size of these is smaller than the optical diffraction limit. Therefore, there is a need to develop new biophysical tools which can infer the existence of domains within membranes. Imaging FCS performed using EMCCD or sCMOS cameras is one such tool which allows the measurement of mobility at a large number of (up to ~1 million) contiguous locations on cell membranes of live cells [1]. The spatial information from the cameras can be used to obtain information on the structure and organization of the membranes. Two different approaches allow us to infer the organization of those membranes: \( \Delta \)CCF distributions [2] and FCS diffusion laws [3]. \( \Delta \)CCF distributions are obtained by calculating differences between the forward and backward cross-correlations between adjacent pixels A and B. These distributions are characterized by the mean and the width. The mean serves as a discriminant to differentiate flow and diffusion processes in the sample. The width is influenced by both the mobility and heterogeneity in diffusion of the sample. In the case of pure diffusion, the distribution is expected to be Gaussian in nature. Any deviations from normality can be quantified as a metric for heterogeneity independent of the mobility. The software binning of pixels allows one to calculate the transit time of molecules through areas of various sizes from a single imaging FCS measurement. According to the diffusion laws, the transit time is linearly proportional to the observed area for free diffusion with a zero intercept. However, for probes which preferentially partition into domains, the intercept of this function is positive. In this work, we perform simulations to demonstrate how \( \Delta \)CCF distributions change characteristically with increasing complexity. Simulations demonstrate that domains as small as 50 nm yield non-normal \( \Delta \)CCF distributions and positive intercepts as well. The \( \Delta \)CCF distributions which are calculated from adjacent pixels serve to quantitate the local heterogeneity in the sample whereas diffusion laws provide one with the global heterogeneity of the sample. Thus we demonstrate that unlike single point FCS which yields only mobility, imaging FCS provides not only mobility but also other metrics to characterize the heterogeneity of membranes.