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The Pair-Correlation Approach to FCS
Enrico Gratton, Michelle A. Digman.
University of California, Irvine, Irvine, CA, USA.
Molecular diffusion and transport processes are fundamental in physical, chemical, biochemical and biological systems. Current approaches to measure molecular transport in cells and tissues based on perturbation methods like fluorescence recovery after photobleaching are invasive, fluctuation correlation methods are local and single particle tracking requires the observation of isolated particles for relatively long periods of time. We propose to detect molecular transport by measuring the time cross-correlation of fluctuations at a pair of locations in the sample. When the points are further than two times the size of the point spread function, the maximum of the correlation is proportional to the average time a molecule takes to move from a specific location to another. We demonstrate the method with simulations, using beads in solution and by measuring the diffusion of molecules in cellular membranes. The spatial pair cross-correlation method detects barriers to diffusion and heterogeneity of diffusion because the time of the correlation maximum is delayed in the presence of diffusion barriers. This non-invasive sensitive technique follows the same molecule over a large area producing a map of molecular flow and does not require isolated molecules thereby many molecules can be labeled at the same time and within the point spread function. Work supported in part by U54 GM064346 Cell Migration Consortium (MD and EG), NIH-P41 P41-RRO3155 (EG) and P50-GM076516 (EG).