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Frontiers in Fluctuation Spectroscopy: Measuring protein dynamics and protein spatio-temporal connectivity[1]

MICHELLE DIGMAN, University of California Irvine

Fluorescence fluctuation spectroscopy has evolved from single point detection of molecular diffusion to a family of microscopy imaging correlation tools (i.e. ICS, RICS, STICS, and kICS) useful in deriving spatial-temporal dynamics of proteins in living cells The advantage of the imaging techniques is the simultaneous measurement of all points in an image with a frame rate that is increasingly becoming faster with better sensitivity cameras and new microscopy modalities such as the sheet illumination technique. A new frontier in this area is now emerging towards a high level of mapping diffusion rates and protein dynamics in the 2 and 3 dimensions. In this talk, I will discuss the evolution of fluctuation analysis from the single point source to mapping diffusion in whole cells and the technology behind this technique. In particular, new methods of analysis exploit correlation of molecular fluctuations originating from measurement of fluctuation correlations at distant points (pair correlation analysis) and methods that exploit spatial averaging of fluctuations in small regions (iMSD). For example the pair correlation fluctuation (pCF) analyses done between adjacent pixels in all possible radial directions provide a window into anisotropic molecular diffusion. Similar to the connectivity atlas of neuronal connections from the MRI diffusion tensor imaging these new tools will be used to map the connectome of protein diffusion in living cells. For biological reaction-diffusion systems, live single cell spatial-temporal analysis of protein dynamics provides a mean to observe stochastic biochemical signaling in the context of the intracellular environment which may lead to better understanding of cancer cell invasion, stem cell differentiation and other fundamental biological processes.

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