LONG RANGE SPATIAL CROSS-CORRELATION IN IMAGING FLUORESCENCE CORRELATION SPECTROSCOPY TO STUDY MOLECULAR DIFFUSION DYNAMICS

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Fluorescence correlation spectroscopy (FCS) has been used to quantify molecular diffusion dynamics. Widely used confocal microscope based FCS has often been employed to determine diffusion dynamics on the areal order of utmost one square micron (μm^2). While this allows for the capturing of diffusion dynamics over small, local, diffraction-limited regions with high fidelity, physical limitations of the confocal volume make it impractical to acquire global diffusion dynamics over larger regions of significance.

Here we present an approach of capturing information of the dynamics over larger areas, on the order of about 10-100 times that of a typical confocal area. We use a multiplexed FCS modality named Imaging FCS which allows us to calculate auto- and cross-correlations. By performing long range spatial cross-correlation of molecular fluorescence signals on a stack of continuously acquired images with a set time resolution, we determine the scale-dependence of the diffusion coefficient. The dependence of the maximum distance that can be used on the time resolutions, and on different correlation area geometries is studied using simulations to find optimal signal acquisition parameters. As in a freely diffusing medium the diffusion coefficient is a constant, any trend of the diffusion coefficient with distance over which areas are correlated indirectly implies molecular interactions. The deviation from free diffusion is experimentally tested on various supported lipid bilayers with different composition. This approach allows to extend the amount of information obtainable from imaging FCS recordings and is a further addition to the tools for investigating molecular organization beyond the diffraction limit by the measurement of molecular dynamics.