RECENT DEVELOPMENTS IN IMAGING FLUORESCENCE CORRELATION SPECTROSCOPY

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The ultimate goal of quantitative bioimaging is to collect the maximum information from a sample with a single measurement. For this purpose, the measurements require high spatiotemporal resolution with single molecule sensitivity. To date, most techniques reach either good spatial or good temporal resolution but rarely both. Here we explore the possibility to use imaging Fluorescence Correlation Spectroscopy (imaging FCS) to obtain a maximum of information from a sample and use combinations with other techniques to obtain super-resolution.

Imaging FCS is a quantitative imaging modality that explores the spatiotemporal structure in images to obtain information on molecular mobilities, interactions and sample structure and organization [1]. We address the lack of spatial resolution by combining imaging FCS with Super Resolution Radial Fluctuations (SRRF) [2]. We demonstrate that we can use the exact same data to obtain super-resolution images on static structures and evaluate the molecular dynamics of a mobile transmembrane protein, thus providing super-resolution on the order of < 80 nm and time resolution on the order of milliseconds on live biological samples. Lastly we address the issue of the limited signal to noise ratio to explore long distance correlations [3]. To date long distance correlations have been limited to ~ 1 μm. By using the ability in imaging FCS to bin pixels in space and time we show that the distance over which correlations can be explored and thus sample organization can be probed can be extended almost and order of magnitude. The combination of these techniques provides a quantitative bioimaging modality that can maximize the information that is obtainable from a sample.