A flat-field TIRF-SIM for combining live cell super-resolution with quantitative single molecule analysis.

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Abstract

Total internal reflection fluorescence microscopy (TIRFM) is the method of choice for single molecule imaging at the plasma membrane. By tracking individual molecules, spatiotemporal analysis of trajectories reveals single protein dynamics and interactions involved in important cellular processes such as signaling, endocytosis or trafficking. A simple objective-based TIRF microscope is based on focusing a single Gaussian laser beam into the TIR ring of the objective's back focal plane. This results in a totally reflected laser beam at the glass/sample medium interface and a 100-200 nm thin evanescent field for fluorescence excitation directly above the cover slip. Unfortunately, the evanescent field of such a microscope shows a Gaussian intensity profile which is typically deteriorated by overlaying interference fringes which hampers a precise quantification of single molecule intensities - an important requisite, e.g., for stoichiometry or single molecule FRET analysis. Here, we combined flat-field illumination by using a πShaper with multi-angular TIR illumination by incorporating a spatial light modulator into our system as it is used for super-resolution structured illumination microscopy (SIM). This unique combination enables quantitative multi-color single molecule analysis with a perfect homogenous illumination. By using a dual camera setup with optimized image splitting optics, we demonstrate high-speed single molecule tracking and FRET analysis which can be correlated with super-resolution SIM.