Fluorescence correlation spectroscopy in the light sheet microscope

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The single plane illumination microscope (SPIM) is gaining popularity due to the high speed of data acquisition, the simplicity and relatively low cost of the setup and the reduced photobleaching. Yet, for fluctuation correlation spectroscopy, the camera detector which is used in the SPIM microscope has a relatively longer pixel exposure time and larger noise compared to the photon counting detectors usually employed in the laser scanning confocal microscope. To overcome some of these limitations we have developed methods to detect molecular fluctuations based on spatial correlation functions rather than temporal correlation functions. We show that the spatial correlation approach has several benefits for the description of molecular diffusion and flow that accounts for the actual path followed by molecules to reach their targets. The determination of the spatial auto correlation and cross-correlation opens the possibility to determine the full diffusion tensor at each point in an image thereby providing a detailed description of the volume where molecules diffuse in different cellular compartments. However the noise of the camera detectors requires special care since the noise is pixel dependent and it could also be spatially correlated among pixels. For the determination of spatial correlation functions we need a full characterization of the performance of the camera detector of the SPIM microscope, especially for the sCMOS cameras which are now common in the SPIM microscope..

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