CONFOCAL-BASED FLUORESCENCE FLUCTUATION SPECTROSCOPY WITH A SPAD ARRAY DETECTOR

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Fluorescence fluctuation spectroscopy (FFS) is an ensemble of microscopy tools that allow biomolecular dynamics, interactions, and structural changes in living cells to be measured by studying temporal and/or spatial fluctuations in the fluorescence intensity [1]. In particular the combination of FFS and confocal laser-scanning microscopy (CLSM) is a powerful technique to study fast, sub-resolution biomolecular processes. A detector array can further enhance CLSM-based FFS techniques, as it allows the simultaneous acquisition of several samples - essentially images - of the CLSM detection volume. However, the detector arrays that have previously been proposed [2] for this purpose require tedious data corrections and preclude the combination of FFS with single-photon techniques, such as fluorescence lifetime imaging. Here, we solve these limitations by integrating a novel single-photon-avalanche-diode (SPAD) array detector in a CLSM system [3]. We validate this new implementation on a series of FFS analyses: spot-variation fluorescence correlation spectroscopy, pair-correlation function analysis, and image-derived mean squared displacement analysis. We predict that the unique combination of spatial and temporal information provided by our detector will make the proposed architecture the method of choice for CLSM-based FFS.

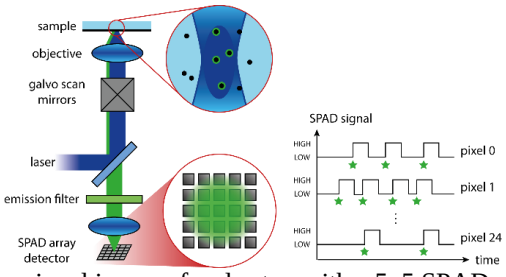

Fig. 1: Imaging the fluorescence signal in a confocal setup with a 5x5 SPAD array detector for FFS analysis.

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