IMAGING FLUORESCENCE CORRELATION: NOVEL RESULTS ON NEW IMAGE SENSORS (SPAD ARRAYS) AND A COMPREHENSIVE NEW SOFTWARE PACKAGE (QUICKFIT 3.0)

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ABSTRACT: Fluorescence (cross-)correlation spectroscopy (FCS/FCCS) is a useful technology to characterize the mobility of molecules inside living cells and the accessibility of cellular compartments. The typically used confocal microscopy based FCS gives us high time-resolution but only for a single-position. To extend this approach to imaging, we typically use a selective plane illumination microscope (SPIM) equipped with high-NA detection optics. With this setup we could demonstrate the feasibility of imaging FCS and FCCS with several fluorescent proteins in all compartments (cytosol, nucleoplasm, membrane) of a living cell [1].
On this microscope we normally use a high-speed, high-sensitivity commercial EMCCD camera (128x128 pixels), which offers high spatial and moderate, but for many samples (e.g. large proteins) sufficient temporal resolution (~500µs temporal resoluation for 128x20 pixels) [1,2]. Here we demonstrate new *in vitro* and first *in vivo* results on the use of an alternative, experimental image sensor: an array of single-photon sensitive avalanche diodes (SPAD array, 512x128 pixels) [3]. This novel type of image sensor improves the temporal resolution to 6.4µs for full frames (!), while retaining acceptable photo-sensitivity. It is therefore applicable also to small fluorescent particles, such as single chemical dye molecules. For this sensor, we also developed advanced data evaluation techniques, that can perform the autocorrelation of data from the full sensor in real-time or faster.
We also developed a comprehensive open-source (GPL3) software package QuickFit 3.0, which implements all necessary computational methods to perform confocal and imaging FCS/FCCS. It also contains modules for advanced analysis methods, such as global fits, maximum entropy (MaxEnt) or mean squared displacement (MSD) evaluations of FCS data. QuickFit can be extended on several levels (fit functions/algorithms, raw data types, evaluations, general extensions) by plugins, written in C++. In addition, this software is also used to control our home-built lightsheet microscope. QuickFit 3.0 is available from: http://www.dkfz.de/Macromol/quickfit/.

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