DIRECT CAMERA READ-OUT FOR LONG TERM SAMPLE OBSERVATION IN IMAGING FLUORESCENCE CORRELATION SPECTROSCOPY

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Camera-based measurements allow fast acquisitions of a large number of frames in fluorescence spectroscopy in general and fluorescence correlation spectroscopy (FCS) in particular. Most of these imaging fluorescence experiments require further post-processing to extract information from its spatial and temporal details. It is especially true in fluorescence correlation spectroscopy which requires 10s or 100s of thousands of frames acquired with millisecond time resolution, resulting in GB of data collected in a single measurement.

This put forward three problems. First, the large amount of data requires for off-line data treatment making experiments time consuming as the experimenter can decide on the quality and success of an experiment only after the acquisition. Second, the alignment of the system is challenging without real-time data representation. And third, the illumination of large amounts of the sample can lead to photobleaching effects that need to be corrected. We solve this problem by introducing a GPU supported direct camera read-out approach that simultaneously depicts the data from all pixels of an image stack. This allows displaying of correlation function during the measurement providing immediate feedback to the experimenter. It will enable alignment of critical angle and focusing of the total internal reflection (TIRF) system or alignment of the detection and illumination objective in single plane illumination microscopy (SPIM) by optimizing the correlation functions for each pixel. In multi-color experiments, it allows aligning the pixels in the different wavelength channels.

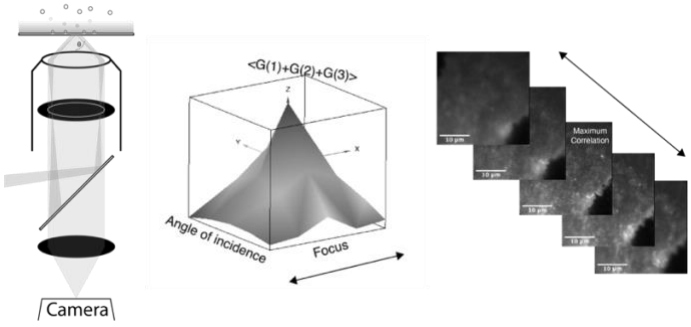

Fig. 1: FCS-based focus finder

The issue of photobleaching is solved by using a bleach correction approach which models the depletion of fluorophore by a number of different correction approaches (exponential and polynomial corrections) and a transformation of the original trace into one of constant mean values without changing the variance of the signal. We provide a proof-of-principle on the power of the combination of the alignment and bleach correction procedures on supported lipid bilayers (SLBs). In our system, we acquire 1 million frames at 1ms time resolution (~17 min) and demonstrate constant recovered parameters of molecular mobility over the full time of the experiment irrespective of photobleaching.

This proposed workflow with real-time FCS functionality is available as an ImageJ Plugin that operate with selected EMCCD and sCMOS cameras from Andor, Hamamatsu, and Photometrics independent of the particular TIRF or SPIM system used.