TWO-COLOR FLUORESCENCE (CROSS-)CORRELATION SPECTROSCOPY ON A SELECTIVE PLANE ILLUMINATION MICROSCOPE

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ABSTRACT: Fluorescence correlation spectroscopy (FCS) is a useful technology to characterize the mobility of molecules inside living cells and the accessibility of cellular compartments. Typically confocal microscopy based FCS is used. This gives us high time-resolution but only for a single-position measurement. To extend this approach to imaging, we built a selective plane illumination microscope (SPIM) equipped with a high-speed image acquisition device (an EMCCD camera) and high-NA detection optics, offering high temporal and spatial resolution.

We recently extended our setup with dual-view optics and an additional laser which allows us to observe two distinct dyes simultaneously with the same camera. Using this system we performed imaging fluorescence cross-correlation spectroscopy (FCCS) on a SPIM. We will present a characterization of the setup to show its range of applicability in vitro and in biological samples. Amongst others we observed cells expressing oligomeric fluorescent proteins (FP), as well as two-colored FP dimers and the transcription factor system AP-1. We will present spatially resolved measurements of protein-protein interaction maps from these systems, showing e.g. the AP-1 binding state.

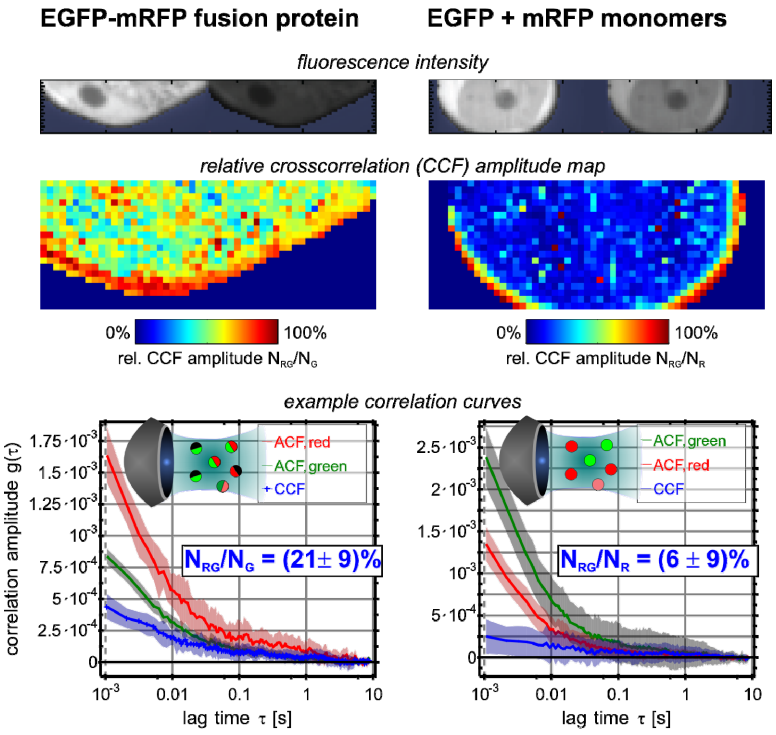

Figure 1: Imaging FCCS measurement in a SPIM, showing HeLa cells expressing EGFP-mRFP dimers (lhs) and EGFP + mRFP monomers (rhs)