STED-fluorescence correlation spectroscopy and -microscopy using adaptive optics

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Spot-variation fluorescence correlation spectroscopy (svFCS) with variable observation areas reveals the different lateral diffusion modes of molecules on cell plasma membranes, and proved to reveal nanodomain structural features [1,2]. It is also interesting to combine svFCS with STED microscopy. This allows to push the excitation volume bellow the diffraction limit and gives new insight into fast nanoscopic dynamical processes and inhomogeneity occurring at the plasma membranes [3].

Here, we have implemented a home-made STED illumination path on a commercial fluorescence correlation microscope (ALBA FCSTM, from ISS, Inc., Champaign, America). In our system, the depletion point spread function (PSF) is shaped through a spatial light modulator (SLM), with which we compensate for system and sample-induced aberrations using adaptive optics methods and also for the fine beam alignment, in three dimensions.

Using nanometric fluorescent beads, we demonstrate a 7-fold improvement in lateral or axial resolution as compared to a classical confocal volume. Preliminary STED-FCS observations will be reported. The presence of the STED beam allows for an active tuning for the spot size well into the nanometric domain, allowing for svFCS to be performed at regimes not accessible by conventional microscopy. We will also present our aim to use this tool to explore molecular interactions in live cell plasma membranes.

References

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