ADAPTIVE OPTICS FOR ABERRATION-CORRECTED 4D PSF ENGINEERING

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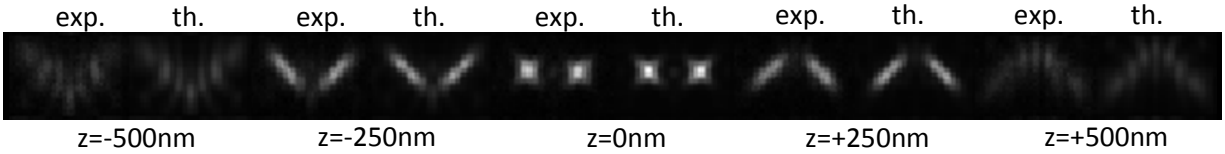
Recently we proposed a method for simultaneous measurement of the 3D position and the emission color of single molecules [1]. This is accomplished by creating an emission Point Spread Function (PSF) with sufficiently low Cramer-Rao Lower Bound (CRLB) in all four relevant parameters using a diffractive optical element in the microscope's emission light path. In particular, we have demonstrated a PSF in which the emission PSF is split in two sub-spots that are astigmatically aberrated with equal magnitude but with opposite sign. This PSF is subsequently fitted using the exact vectorial PSF model, taking all effects of high NA and polarization into account, as opposed to popular Gaussian spot fitting [2]. It appears that the method is quite sensitive to aberrations, which should be controlled to well within the diffraction limit in order to approach the theoretical CRLB. To that end we have set up an adaptive optics system, in which the Spatial Light Modulator (SLM) is calibrated using a separate optical branch. Aberration values are retrieved from a through-focus bead scan and a fit with the exact vectorial PSF model. Subsequent correction with the SLM produced wavefront errors down to 20 mλ. With this well-calibrated and corrected system "4D"-PSFs can be created that conform well to the vectorial PSF model and fits for the four parameters can be obtained close to the CRLB with precisions below 10 nm in all four parameters xyz+λ over an axial range of 1 μm with 5000 signal photons and 12 background photons. In the presentation we will provide an outlook on application of the technique to distinguishing multiple fluorescent species from images acquired with a single camera microscope.



Through-focus images of the double astigmatic engineered PSF measured with our adaptive optics aberration corrected microscope on green 175 nm diameter beads and corresponding fits with the vectorial PSF model, showing excellent correspondence.

[1] C.S. Smith et al., Optics Express Vol. 24, 4996, 2016.
[2] S. Stallinga and B. Rieger, Optics Express Vol. 18, 24461, 2010.