A device for the characterisation of aberrations in thick samples to improve image resolution in STED microscopy

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Imaging in thick biological samples is a challenging task for confocal microscopy and in order to achieve high resolution in depth, beam wavefront corrections need to be applied. Refractive index mismatch between sample fluid and immersion media of the objective induce aberrations that dratically decrease image quality of STED in imaging depths of more than 10 μm.[1] However, these aberration errors can be accounted for and signal to noise ratio (SNR) and resolution preserved, when the influence on the point-spread-function of the media was analysed before the acquisition.

Therefore, we present an easy to fabricate PDMS device which allows to measure and characterize aberrations as function of depth in any arbitrary sample media for confocal microscopy techniques. We manufacture micron sized steps of known height in PDMS via soft lithography [2] and functionalise these surfaces with fluorescent beads and gold nanoparticles. Multiple individual beads are localised in the imaging plane at a specificic depth and used to effectively measure the point spread function over the whole FOV. Aberrations and defocus occurring in common biological cell media e.g phosphate buffer solution (PBS) and water are investigated and their influence on STED in-depth imaging and the procedure of characterisation discussed. High-order Zernike polynomials [3] are fitted to individual point spread functions to analyse an optical system's spherical aberrations, astigmatism and coma. The methodology can be adapted to different step heights and media if required and is easy to reproduce in biological laboratories with access to basic photolithographic equipment.

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[3] Jacopo Antonello, Emil B. Kromann, Daniel Burke, Joerg Bewersdorf, and Martin J. Booth, "Coma aberrations in combined two- and three-dimensional STED nanoscopy," Opt. Lett. 41, 3631-3634 (2016) .