SINGLE SAMPLE CALIBRATION AND ALIGNMENT OF CLSM AND (3D) STED

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In the past decades, confocal laser scanning microscopy (CLSM) has proven to be a powerful tool in the field of life sciences [1,2]. More recently, the advent of super-resolution techniques, such as STED, PALM and STORM, has enabled the study of features well below the diffraction limit [3]. This development, in combination with image restoration techniques [4], has given the ability to image in much greater detail.

The increased resolutions also require a higher precision in calibration and alignment of the microscopes. Currently, a multitude of samples is used for the calibration and alignment of (STED) confocal microscopes. For the calibration of lateral distances, stage micrometers are available, while for axial distances, different methods exist in literature [5]. To check the alignment of the excitation and depletion lasers, gold beads are typically used. For the imaging of the resulting point spread function (PSF), a different sample containing small fluorescent beads is used [6].

Here, we present a single sample for the alignment, calibration (lateral and axial) and measurement of the PSF. The sample is composed of silica colloids, with (sub-diffraction limit sized) gold and (multi-wavelength) fluorescent cores, in a colloidal crystal. The ordered structure of the crystal can be used as a ruler in both the lateral and the axial directions. The alignment of the excitation and depletion lasers can be checked using the gold cores. The small sized fluorescent cores enable one to also measure the PSF of the imaging system.

We will demonstrate the improved quality of our acquired images, resulting from the proper calibration and alignment using the sample. In addition, we will show preliminary results on studying the effects of refractive index mismatches on 3D CLSM stacks, and which effective medium model best corrects for these effects.

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