3D STED DEEP TISSUE IMAGING USING ADAPTIVE OPTICS

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Stimulated emission depletion (STED) microscopy has proven to be a powerful and increasingly popular microscopy technique that enables imaging of structures at the molecular level. Its resolution is not limited by diffraction and imaging optics but rather the level of noise within the acquired image. In order to obtain super-resolution images with resolution that surpasses the diffraction limit, a vortex phase mask is used to suppress fluorescence in the in-focus plane. To obtain axially super-resolved images, a bottle-beam phase mask is applied (3D STED), creating an optical-void along optical ais [1]. When imaging through a thick and aberrating specimen, the noise level increases due to the local variations of the refractive index introducing strong spherical aberrations. These aberrations significantly degrade the shape of the depletion beam, especially when imaging with the bottle-beam phase mask [2]. As a result, imaging of strongly aberrating samples with the 3D STED depletion beam is challenging and non-trivial.

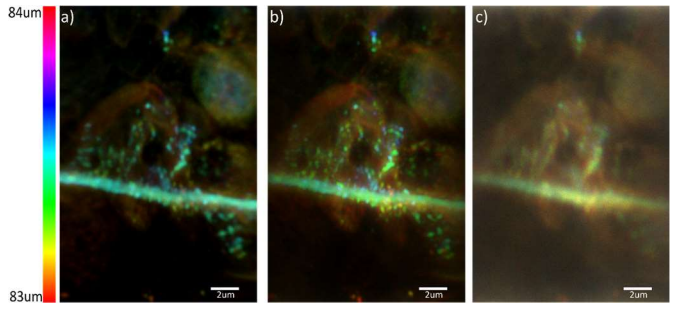

Fig. 1. *Colorcoded Z projections comparison of the confocal (a), aberration corrected 3D STED (b) and uncorrected 3D STED (c) images.*

In this contribution we show, that using adaptive optics (AO) for aberration correction enables high quality 3D STED imaging of iPS cell axons located 80µm below the surface of the tissue. We use Fourier Ring Correlation [3] (FRC) for the evaluation of the resolution of STED images. Using our custom-built AO 3D STED microscope setup we were able to obtain a 256nm lateral and 300nm axial resolution of the acquired images. Figure 1 shows the comparison of the quality of images acquired using confocal, 3D STED with aberration correction and 3D STED without corrections. The corrected images show more detail and less out of focus light represented by higher variation of the colors when compared with the aberration corrected confocal image, while the non-corrected image is significantly degraded.

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