Two-photon Excitation STED Microscopy for Deep-Tissue Super-Resolution Imaging

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Stimulated emission depletion (STED) microscopy is a powerful technique that allows the three-dimensional (3D) visualization of nanoscale structures in living cells [1]. When combined with two-photon excitation (2PE) [2], STED microscopy can be used to image deep in optically scattering samples like thick biological tissue sections [3]. Nevertheless, 2PE-STED microscopy has yet to become a mainstream imaging modality for deep-tissue super-resolution imaging.

Here, we present a custom-built 2PE-STED microscope that addresses the main issues limiting the widespread application of 2PE-STED microscopy to deep-tissue imaging. One of the most important issues is that the depletion profile used for 3D resolution enhancement is easily compromised by optical aberrations [4], therefore imposing a practical limit on the maximum achievable imaging depth in aberrating tissue. Thus, for fast, user-friendly aberration-correction, we have adapted the non-linear-guidestar-based wavefront sensing approach reported by Wang *et al.* [5], and use a deformable mirror to correct the measured aberrations. Moreover, we have optimized the system for imaging in the red to far-red emission range to improve imaging depth and allow the use of 2PE-STED-compatible organic dyes [6] that are generally brighter and more photostable than their red fluorescent protein counterparts. We will present the technical realization of our instrument and present images that demonstrate the feasibility of using 2PE-STED microscopy for deep-tissue super-resolution imaging experiments.

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