Adaptive Optics Two-photon Excitation STED Nanoscopy for Sub-diffraction 3D Imaging in Scattering Samples

Mary Grace M. Velasco[1,2], Xiang Hao[2], Jacopo Antonello[3], Martin J. Booth[3,4], and Joerg Bewersdorf[1,2]

[1] Dept. of Biomedical Engineering, Yale School of Engineering & Applied Science, CT, USA
[2] Dept. of Cell Biology, Yale School of Medicine, CT, USA
[3]Centre for Neural Circuits and Behaviour, University of Oxford, UK
[4]Dept. of Engineering Science, University of Oxford, UK
Email: marygrace.velasco@yale.edu

KEY WORDS: 2-photon, STED, adaptive optics

Stimulated emission depletion (STED) nanoscopy, when combined with two-photon excitation (TPE), can achieve sub-diffraction-limit resolution more than 100 µm deep in thick, scattering specimens [1-5]. Extending the super-resolution capabilities of this technique to three dimensions (3D) can be realized using an annular phase mask. However, the quality of the resulting depletion profile is easily compromised by specimen-induced optical aberrations [6]. As a result, imaging with TPE-STED nanoscopy in 3D, deep in aberrating tissue can prove challenging.

Here we present our implementation of a TPE-STED nanoscope, optimized for super-resolution imaging in optically dense specimens. To correct for specimen-induced aberrations, our system incorporates a 140-actuator deformable mirror (DM) that can introduce phase distortions to negate those induced by the specimen. To determine the phase profile that the DM must generate, we have adapted an approach by Wang *et al*. [7] and installed a custom Shack-Hartmann wavefront sensor that can measure the aberrated wavefront directly, using the inherently confined TPE focus in the sample as a "guide star" or wavefront source. We will present the technical realization of our instrument and present images that demonstrate the feasibility of using TPE-STED for biological studies requiring super-resolution in thick tissue samples.

References

1. G. Moneron, and S. W. Hell, Opt. Express **17**, 14567-14573 (2009).
2. J. B. Ding, K. T. Takasaki, and B. L. Sabatini, Neuron **63**, 429-437 (2009).
3. Q. Li, S. S. H. Wu, and K. C. Chou, Biophys. J. **97**, 3224-3228 (2009).
4. P. Bianchini, B. Harke, S. Galiani, G. Vicidomini, and A. Diaspro, Proc. Natl. Acad. Sci. U.S.A. **109**, 6390-6393 (2012).
5. P. Bethge, R. Chereau, E. Avignone, G. Marsicano, and U. V. Nagerl, Biophys. J. **104**, 778-785 (2013).
6. T. J. Gould, D. Burke, J. Bewersdorf, and M. J. Booth, Opt. Express **20**, 20998-21009 (2012).
7. K. Wang, D. E. Milkie, A. Saxena, P. Engerer, T. Misgeld, M. E. Bronner, J. Mumm, and E. Betzig, Nat. Methods **11**, 625-628 (2014).