Abberior STED Workshops

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Abstract

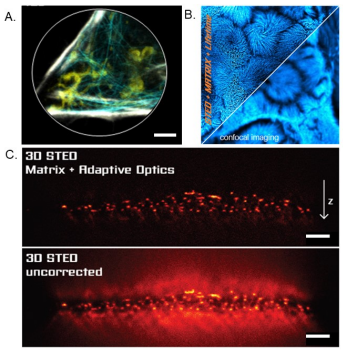
STED microscopy is a powerful microscopy technique which has contributed to numerous studies in the life sciences [1,2]. Current state of the art STED microscopes as the abberior STEDYCON and abberior FACILITY line microscopes combine confocal and STED imaging functionalities.

(I) Improved Multi-color STED microscopy. Until recently STED microscopy was rarely used for multi-channel imaging due to the lack of suitable fluorophores and imaging routines [3]. Multichannel imaging fluorophores are typically separated using their spectral characteristics. In addition, the fluorescence lifetime can be used for multi-channel imaging and to receive information about the fluorophore's environment such as ion concentration or pH. In STED microscopy, fluorescence lifetime information is particularly beneficial, as it can be additionally harnessed to increase resolution - especially when imaging at reduced STED laser powers to protect sensitive samples (Fig 1B).

In this workshop we will show how multi-color super-resolution imaging can be performed with the STEDYCON by combining novel long-Stokes shift dyes with fluorophores in the red and far-red spectra using only a single depletion laser in the infra-red region (Fig 1A). This allows for precise analysis of structures, positions, and interactions of multiple proteins.

Further, we will demonstrate, with the FACILITY LINE and our TIMEBOW toolbox, how fluorescent lifetime separation experiments are planned, imaged, and analyzed for confocal and STED.

(II) Adaptive Optics 3D STED imaging of thick samples. Until recently super-resolution microscopy was mainly applied to study single cells and thin sections, because imaging of specimen as tissue sections is often hampered in thick and inhomogeneous samples. Refractive index-mismatches often result in low-resolution and poor-quality images. abberior FACILITY LINE microscopes offer Adaptive Optics [4] and the MATRIX detector to correct aberrations and remove haze to allow for high-quality super-resolution imaging in thick tissue samples (Fig 1C). In a workshop participants will learn about how to use the MATRIX detector and Adaptive Optics to improve STED imaging of different thick tissue samples.



A. Multi-color live cell STED imaging. Mammalian cells were stained with abberior STAR 460L to visualize mitochondria (yellow), LIVE 610 to show tubulin (cyan) and LIVE 550 to stain actin (grey). Scale bar 2 µm.

B. MATRIX STED and lifetime imaging. Differentiated Caco-2 cells were stained for Actin with Star Red (provided by D.Günzel, Charité Berlin, Germany).

C. 3D STED with MATRIX and ADAPTIVE OPTICS. Wheat cell labelled with abberior STAR RED to visualize chromosomes. Scale bar 2 µm.

References

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