

Deep nanoscopy in cleared tissue

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Achieving the best resolution at maximal depths inside the specimen is a general desire in microscopy. Refractive index mismatch as well as scattering are two big challenges to overcome. This is especially true when a resolution significantly better than the diffraction limit is aimed for. Offering a solution to minimize scattering as well as refractive index mismatch, clearing techniques are becoming more and more popular.

STED (STimulated Emission Depletion) microscopy [1] provides fast, intuitive, and purely optical access to study subcellular architecture and dynamics at the nanoscale free from post-processing artefacts. Significant improvement of penetration depths for 3D STED in kidney tissue have been achieved with oil objective lenses by tissue clearing [2]. Standard fixed samples are easily imaged with dedicated STED oil objectives (HC PL APO 100x/1.40 OIL) delivering highest NA and resolution as well as access to the full spectral range for multi-color imaging.

With the refractive index of cells and tissue being closer to glycerin than oil a high NA glycerin lens would be the choice for more complex experiments like deep nanoscopy as well as live cell 3D STED imaging. Performance at different penetration depths as well as temperatures can be optimized and most specimen inhomogeneity compensated for by a motorized correction collar.

This talk will focus on deep nanoscopy achieved combining an adopted tissue clearing technique with a dedicated objective lens as well as on live cell STED imaging. 3D STED data 100 μm inside kidney tissue enabled by an adopted clearing protocol and the new STED WHITE Glycerin objective (HC PL APO 93X/1.30 GLYC motCORR) as well as live cell STED data will be shown.

[1] S.W. Hell SW, J. Wichmann, "Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy", *Opt Lett.*, **19**, 780-2 (1994).

[2] D Unnerjö-Jess, L. Scott, H. Blom, H. Brismar, " Super-resolution stimulated emission depletion imaging of slit diaphragm proteins in optically cleared kidney tissue.", *Kidney Int.*, **89**(1), 243-7