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Angaben zur Veröffentlichung / Publication details:

Willig, Katrin, Birka Hein, U. Valentin Nägerl, and Stefan W. Hell. 2009. "STED nanoscopy in living cells using live cell compatible markers [Abstract]." *Biophysical Journal* 96 (3, supplement 1): 17a. https://doi.org/10.1016/j.bpj.2008.12.988.

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STED Nanoscopy in Living Cells using Live Cell Compatible Markers

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We demonstrate far-field optical imaging with subdiffraction resolution in the interior of living mammalian cells and tissue by applying stimulated emission depletion (STED) microscopy.

Utilizing a yellow fluorescent protein (YFP) to image individual structural elements of the endoplasmatic reticulum (ER) and the tubular network (Fig.1) revealed a focal plane (x,y) resolution < 50 nm inside the living cell, corresponding to a 4-fold improvement over that of a confocal microscope and a 16-fold reduction in the focal spot cross-sectional area. Time lapse STED imaging of dendritic spines of YFP-positive hippocampal neurons in organotypic slices outperforms confocal microscopy in revealing important structural details. As an alternative to the fluorescent protein we employed a genetically encoded protein tag which can be stained in vivo with modified organic dyes. Using a rhodamine dye of high photostability enabled us to image structures in the living cell with a resolution of ~ 40 nm.

Thus nanoscale imaging of structures in the interior of living cells greatly expands the scope of light microscopy in cell biology.

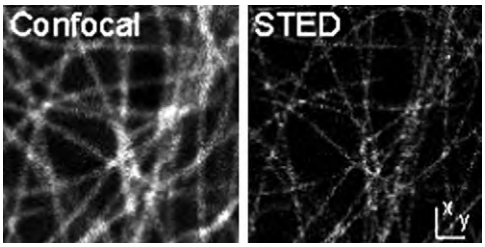

Fig. 1. Citrine labeled microtubules.