

IMMUNOFLUORESCENCE STED-MICROSCOPY AT ~50 NM RESOLUTION

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We report immunofluorescence imaging with axial and lateral resolution well beyond the diffraction limit. By imaging the microtubular network of mammalian cells we have demonstrated not only that an antibody-labelling is stable enough to be recorded in the STED-microscopy mode, but also that pronounced superresolution can be obtained using a standard immunofluorescence preparation[1].

An axial resolution of ~50 nm is achieved by applying stimulated emission depletion (STED) through two opposing lenses of a 4Pi-microscope. This axial resolution corresponds to 1/16 of the wavelength of 793 nm used for STED. For the first time in immunofluorescence imaging the immunolabeled microtubule to be imaged is larger than the effective size of the focal spot.

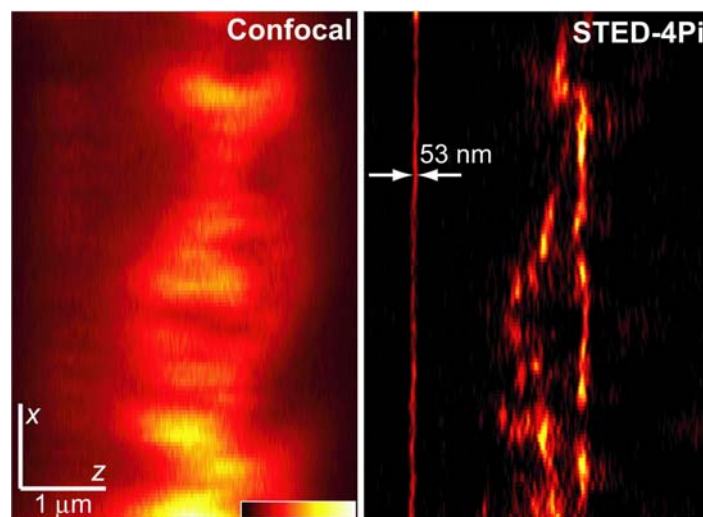


Figure: Superresolution STED-4Pi fluorescence image with ~50 nm axial resolution (right) of the immunolabeled microtubular network of a mammalian cell, compared to an image recorded in the standard confocal mode (left). Both images were recorded with water immersion lenses (NA=1.2) from exactly the same site of the cell. Note the sharp representation in the STED-4Pi mode (sharp vertical line) of a fluorescent monolayer that was coated on the cover slip for resolution measurement purposes. In the confocal image (left) due to the ~15 times poorer axial resolution, the same monolayer is swamped by the signal from the microtubular network.

[1] M. Dyba, S. Jakobs, S. W. Hell, *Nature Biotech.* 21 (2003) 1303