

**Optical sectioning in 3D far-field fluorescence nanoscopy
based on single molecule switching**

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Far field optical microscopy and especially confocal fluorescence microscopy are well established methods for the non-invasive 3D-investigation of cellular structures. However, the resolution of conventional light microscopy is limited by diffraction to ~200nm in the focal plane and ~600nm along the optic axis. In order to discern identical labels which are much closer than this, one has to overcome the diffraction barrier.

The utilization of optical switching events allows one to circumvent Abbe's diffraction limit: The switching of the fluorescence ability of fluorophores that are closer than the diffraction limit allows one to record these objects sequentially and hence to assemble a sub-diffraction image. Stimulated Emission Depletion (STED) microscopy was the first concept following this insight by. The fluorescence ability of neighboring markers is here sequentially switched by stimulated emission. This concept records fluorophore ensembles and has been extended to photoswitching of proteins and fluorophores. Another method utilizing molecular switching events for achieving nanoscale resolution in microscopy uses a single molecule approach: Single molecules which are initially in a dark state are sequentially switched on (activated), computationally located and switched off (deactivated). Although the resolution increase provided by single molecule switching concepts can be extended to the z-axis, they inherently lack 3D optical sectioning. Here, we present such a single molecule photoswitching based microscope where this shortcoming has been surmounted by advantageously combining two photon activation and 3D-localization of individual marker molecules.

References:

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