Ground-State-Depletion Fluorescence Microscopy provides subdiffraction resolution through triplet state pumping

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The resolution of optical imaging in conventional far-field microscopy is limited by the diffraction of light. We present fluorescence imaging beyond this barrier by forcing the fluorophore into a non-fluorescent state in the outer region of the focus [1], [2]. As an example this non-fluorescent state is realized by the fluorophore’s triplet state [3]. Thus the fluorescence emission in the outer region of the diffraction limited excitation spot is deactivated in a saturated manner, thereby dynamically reducing the effective fluorescence volume down to 50 nm in lateral direction. Applying low power cw-illumination we realized an increased resolution on sub-diffraction scanning images of different fluorescently stained samples including cellular systems. We show that in principle a reversible saturable optical fluorescence transition of a dye molecule can be utilized to achieve optical imaging beyond the diffraction barrier.