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New Developments in STED Microscopy

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For more than a century the resolving ability of a lens-based imaging system was believed to be limited by diffraction to approximately several hundreds of nanometers. Recent advances have pushed the spatial resolution in fluorescence microscopy beyond the diffraction barrier [1]. The first concept to demonstrate that diffraction unlimited resolution in the far-field could in fact be achieved was stimulated emission depletion (STED) microscopy [2]. In this approach, a donut-shaped laser focus is superimposed with the conventional excitation focus of a laser-scanning microscope to force fluorophores out of the excited state by stimulated emission. This targeted switching-off of fluorescence results in an effective focal volume of sub-diffraction size which is scanned through the sample to generate images. Using STED microscopy it has been possible to image biological structures with resolution on the order of a few tens of nanometers.

We present our latest results in the development and application of STED microscopy. A novel far-red emitting fluorescent protein [3] that is compatible with STED microscopy presents new prospects for live cell imaging using a commercial STED microscope. Additionally, we demonstrate novel dual-color STED microscopy applications showing the versatility of the technique for biological imaging applications. A dvances in a custom-built STED setup further illustrate the potential of STED microscopy for additional resolution improvement.

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