Title	**CW STED nanoscopy with a Ti:Sapphire oscillator**
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Abstract	Fluorescence microscopy has become an essential tool to study biological molecules, pathways and events in living cells, tissues and animals. Meanwhile, the conventional optical microscopy is limited by the wavelength of the light. Even the most advanced confocal microscopy or multiphoton microscopy can only yield optical resolution approaching the diffraction limit of ~200 nm. This is still larger than many subcellular structures, which are too small to be resolved in detail. These limitations have driven the development of super-resolution optical imaging methodologies over the past decade. The stimulated emission depletion (STED) microscopy was the first and most direct approach to overcoming the diffraction limit for far-field nanoscopy. Typically, the excitation focus is overlapped by an intense doughnut-shaped spot to instantly de-excite markers from their fluorescent state to the ground state by stimulated emission. This effectively eliminates the periphery of the Point Spread Function (PSF), resulting in a narrower focal region, or super-resolution. Scanning a sharpened spot through the specimen renders images with sub-diffraction resolution. Multi-color STED imaging can present important structural and functional information for protein-protein interaction. In this work, we presented a dual color, synchronization-free STED stimulated emission depletion (STED) microscopy with a Ti:Sapphire oscillator. The excitation wavelengths were 532nm and 635nm, respectively. With pump power of 4.6 W and sample irradiance of 310 mW, we achieved super-resolution as high as 71 nm. We also imaged 200 nm nanospheres as well as all three cytoskeletal elements (microtubules, intermediate filaments, and actin filaments), clearly demonstrating the super-resolution resolving power over conventional diffraction limited imaging. It also allowed us to discover that, Dylight 650, exhibits improved performance over ATTO647N, a fluorophore frequently used in STED. Furthermore, we applied synchronization-free STED to image fluorescently-labeled intracellular viral RNA granules, which otherwise cannot be differentiated by confocal microscopy. Thanks to the widely available Ti:Sapphire oscillators in multiphoton imaging system, this work suggests easier access to setup super-resolution microscope via the synchronization-free STED A series of biological specimens were imaged with our dual-color STED.
Laser Quantum Product	Finesse 6W
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