

# **SINGLE LASER TWO-PHOTON EXCITATION STED MICROSCOPY BASED ON ORBITAL ANGULAR MOMENTUM CONVERSION**

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Two-photon excitation (2PE) microscopy is a powerful technique for imaging thick biological samples. When combined with stimulated emission depletion (STED) microscopy, unprecedented spatial resolution may be achieved at depths far exceeding those possible by confocal approaches. The advent of high-powered femtosecond lasers systems with dual synchronized outputs offers unique opportunities for nanoscopy. Herein, we report super-resolution imaging of red fluorophores through a novel implementation of 2PE-pSTED microscopy using a single dual-output femtosecond laser. The broadening of the absorption cross section in the 2PE regime allows for excitation of several red-emitting fluorophores using the fixed 1045nm output, while depletion may be achieved using the tunable output. Herein, a biological sample labelled with ATTO 565 is excited at 1045 nm, and pulsed depleted at 680 nm following temporal stretching and spatial modulation of the tunable output. A liquid crystal vortex retarder is employed to transform the fundamental Gaussian mode of the depletion beam into a Laguerre-Gaussian “donut-hole” mode. This mode transformation results from conversion of spin to orbital angular momentum. Advantages of orbital angular momentum conversion in STED microscopy are herein further discussed.