Stimulated emission depletion (STED) fluorescence is a powerful method in microscopy allowing sub-diffraction-limited resolution. Dominik Wildanger and colleagues at the Department of NanoBiophotonics, Max-Planck Institute, Goettingen, Germany, using a single supercontinuum fibre laser (the SC450-PP-HE* from Fianium) have reported imaging of dense nanoparticles and of the microtubular network of mammalian cells with a spatial resolution of 30-50nm in the focal plane – a factor of 9 times beyond the diffraction limit.

STED microscopy, developed by Prof. Stefan Hell in 1994, has typically been implemented using a large-frame Ti:sapphire oscillator pumping a regenerative amplifier that in turn fed an optical parametric amplifier to offer the range of spectral components required in the visible. Such a system has cost in the region of $500K.

The SC450-PP-HE* is an all-fiber supercontinuum source developed primarily for STED microscopy. It offers a turnkey, compact source for STED and excitation at 1/10th the cost of the prior-art DPSS-Regen-OPA.
Background – STED Microscopy

The basic principle behind scanning STED Microscopy is to confine fluorescence emission of fluorescent markers to a region that is much smaller than the region covered by the diffraction-limited excitation spot.

Typically, a train of high energy (~ 10 nJ) short pulses (~100ps), capable of exciting the fluorophore is focused with an objective lens to a diffraction-limited spot which is overlapped with the focus point of a pulsed “STED” beam, having a donut spatial profile with a central zero intensity point and steep intensity edges in the focal area. The excitation and STED pulses are arranged to reach the focal plane simultaneously such that the STED beam instantly de-excites potentially excited molecules by stimulated emission.

The probability of a dye molecule to emit decreases with the STED pulse intensity, resulting in a sub-diffraction sized region of fluorescence at the centre of the STED donut spot.

The demonstration of STED microscopy using a supercontinuum source for both STED and excitation opens up the opportunity for cost-effective, super-resolution imaging. Moreover, the extreme bandwidth of the supercontinuum now enables tunability of both the STED and excitation beam for optimised excitation and depletion.

The SC450-PP-HE laser is based on Fianium’s patent pending variable repetition rate supercontinuum fibre laser technology. Minor modifications to the core technology of the SC450-PP laser system enables the delivery of supercontinuum with enhanced energy spectral density (>1nJ/nm – 20-fold increase over conventional system) required for STED illumination.

Further developments of the SC450-PP-HE now provide the capability to have STED at wavelengths below 500nm and excitation down to 400nm, enabling further expansion of the method to cover a wider variety of fluorescent dyes. Furthermore, scaling of both the energy spectral density and pulse repetition rate will enable enhanced resolution and scan speeds respectively.
Figure 4: SC450-PP-HE Visible Spectrum