Whitepaper

STED Microscopy- A Nobel prize winning technique

***Abstract**: STED microscopy has enabled super resolution imaging to resolve structures of proteins and viruses to below 10nm – several orders below the diffraction limit. Laser Quantum's **opus 660** laser has proved ideal for this imaging technique, with the high power 660nm beam able to create the STED de-excitation needed to achieve these high-resolution images.*

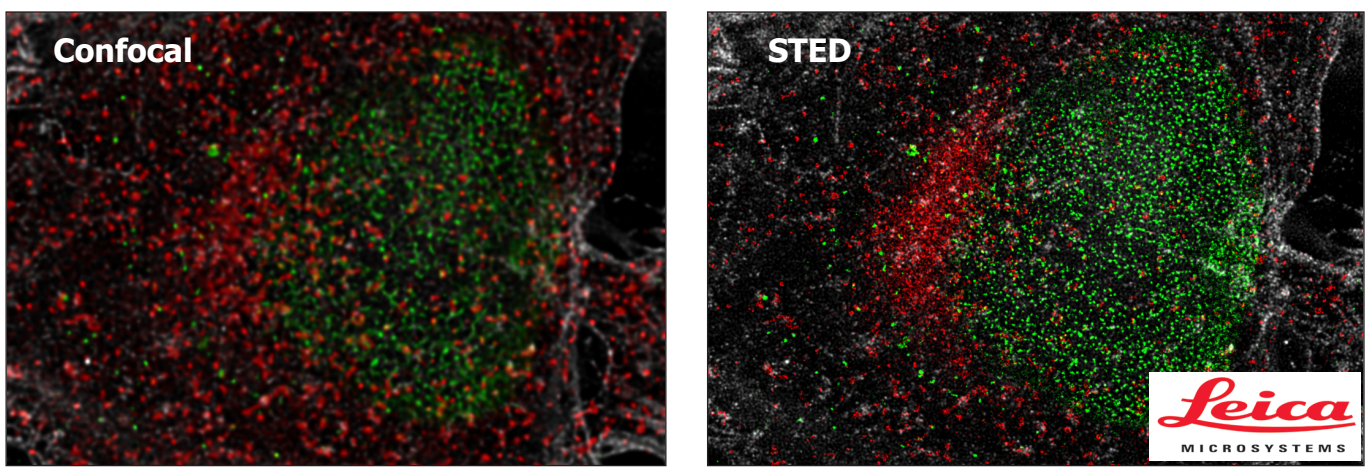

Figure 2: Confocal microscopy compared to the resolution achieved with STED and the **660nm opus** laser. Triplecolour confocal and STED images were acquired with Leica TCS SP8 STED 3X. Courtesy of Leica Microsystems.

Introduction

Microscopy plays an important role in many disciplines of science including the study of biological specimens with far-field microscopy being the most commonly used. This is due to the high specificity of fluorescent labelling at a molecular level and the non-invasiveness of the focussed light. The most important factor in microscopy is image resolution: the ability to distinguish between two closely positioned points. Until recently, far-field light microscopy had a limited resolution and a physical size limit of 200nm, determined by the diffraction limit of the visible radiation used. Many viruses, proteins and small molecules are less than 100nm in size and could not be easy studied, limiting the advances in the field. STimulated Emission Depletion microscopy (or STED Microscopy) is a process that provides super resolution imaging (Figure 1), developed by Hell and Wichmann, 1994 and demonstrated by Hell and Klar, 1999. Hell was awarded the 2014 Nobel Prize in Chemistry for its development.

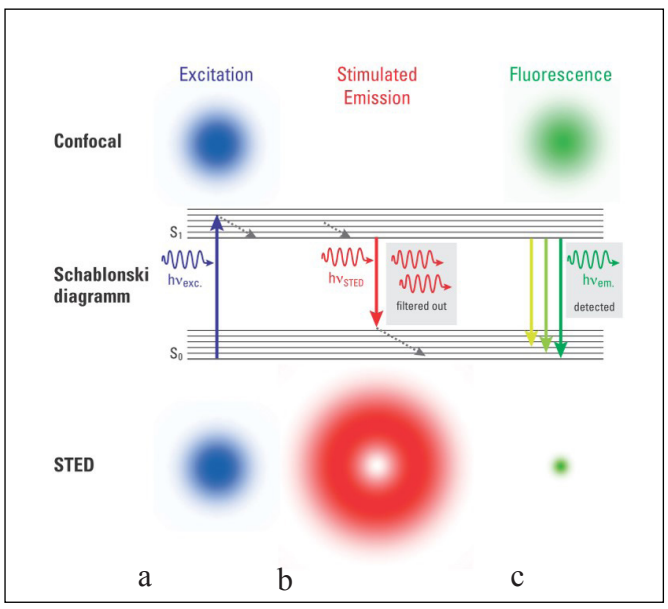

Figure 1: a- Excitation spot (2D, a-left), doughnut-shape de-excitation spot (b-centre) and remaining area allowing fluorescence (c-right) Courtesy of Leica Microsystems.

The STED microscopy process is achieved by depleting fluorescence in specific regions of a sample, whilst leaving a central focal spot active to fluoresce. This process achieves a far better resolution than traditional confocal microscopy. The STED microscopy process requires incident photons to strike a fluorophore attached to the sample of interest, and generate spontaneous emission photons. This incident excitation beam is usually configured to have a Gaussian profile and arranged in a confocal architecture. A second, much higher intensity, laser beam is then added at a different wavelength and this beam combination leads to a quenching of 'unwanted' fluorescence and a much higher image resolution. Previously, optical microscopes were limited to a resolution of approximately half the wavelength of light used to observe the sample, based on Abbe's diffraction limit. Using STED techniques, these limitations are swept aside.

Scientists are now able to peer into the nanoscopic world without the need to sacrifice samples in a vacuum, as is the case with Transmission Electron Spectroscopy. Previously, the detailed studies of structural and functional relationships of biological samples were near-impossible using diffraction-limited microscopy. Further to this, multicolour super-resolution STED imaging enables the interactions between biological and engineered nanostructures to be studied in detail which can be applied to life sciences and nanotechnologies.

STED microscopy uses a TEMoo laser, such as Laser Quantum's **ventus 473** or **ventus 532**, focused to a diffraction limited ~200nm spot, to excite fluorescent markers in the sample in a typical confocal geometry (Figure 1a). Continuous wave laser sources require no pulse length optimisation, synchronisation or timing between the excitation and STED beams. A second, collinear red laser beam shaped into a 'doughnut' then quenches the fluorescence (Figure 1b) in all but the central 'hole' (Figure 1c). The **opus 660** from Laser Quantum is the ideal STED de-excitation red laser, as the 1.5W of 660nm light creates a very sharp central hole. By controlling the shape of the red STED laser, resolution can be greatly increased over the standard light techniques, and details below 40nm can now be visualised. The size of the unquenched spot decreases with increasing power from the STED de-excitation laser and Laser Quantum's **opus 660** laser is ideal for this application as it has an M^2 value of close to unity, allowing accurate focusing of the beam into the required shapes.

STED microscopy has cast a sharper light on various topics in cell biology and materials science, making nanoscale materials and components of the cell accessible for fluorescence-based investigations. The application is transforming the studies on the analysis of nerve endings and brain synapses, also developing those studies on proteins involved in Huntington's disease and tracking cell division in embryos, with real benefits to medical research.

Figure 3: Laser Quantum **opus 660.**

Acknowledgements: Dr. Jochen Sieber and Dr. Arnold Giske of Leica Microsystems, Jennifer Szczepaniak Sloane and Dr. Gregor Klatt from Laser Quantum

References:
[1] T. Muller, C. Schumann &A. Kraegeoh, STED microscopy and its applications: new insights into cellular processes on the nanoscale (June 4, 2012). Chemphyschem, Vol.13(8)
[2] S. Hell & J. Wichmann, Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy (June 1, 1994). Optic Letters, Vol.19(11)

For further information please contact Niccola Seddon, Marketing Coordinator, e: nseddon@laserquantum.com