

## Cobolt Flamenco™ 660 nm for Stimulated Emission Depletion (STED) microscopy

Light diffraction and the limits in spatial resolution that it creates have been for a long time an obstacle in optical microscopy. The major problem encountered by researchers has been the difficulty in obtaining clear images of the fabric of living cells.

Starting in the mid of the 90's with STED (Stimulated Emission Depletion) and GSD (ground-state depletion) a branch of far-field optical fluorescence microscopy, so called fluorescence nanoscopy, has been developed that goes beyond the spatial diffraction-limit. Advanced nanoscopy techniques such as STED, GSD(IM), PALM or STORM use the capabilities of modulating the fluorescence signal and allow the capture of images of (living) cells with a spatial resolution down to the molecular scale, i.e., far better than conventional (confocal) microscopy (Figure 1).

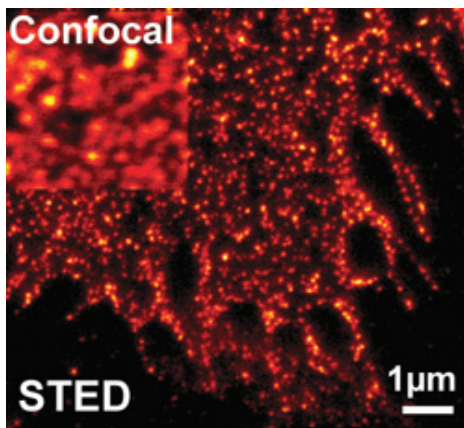


Figure 1.

In this application note we present the results of an experiment conducted by using a Cobolt Flamenco™ 660 nm in a STED nanoscopy setup. The STED nanoscopy technique has been developed by Stefan Hell at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany. In a typical STED microscope, a fluorescence excitation laser beam is coaligned with a doughnut-shaped STED beam to transiently inhibit fluorescence emission by stimulated emission everywhere but at the focal center (Figure 2). Scanning the reduced focal spot over the sample produces images of sub-diffraction resolution.

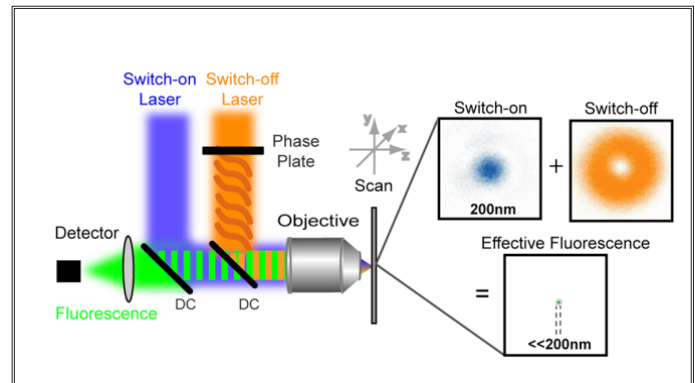


Figure 2.

Recently, STED nanoscopy has been realized with continuous-wave (CW) lasers (Willig et al., Nature Methods 2007, 4: 915-918). The combination with gated detection even allows the capture of images with nanoscale spatial resolution using compact CW lasers with <500 mW power (Vicidomini et al., Nature Methods 2011, 8: 571-573).

Figure 3 shows fluorescence scanning images of labeled microtubuli of fixed mammalian cells for diffraction-limited (left), the CW-STED (right) and gated CW-STED (lower left and upper right corners) recordings using 180 mW CW light of a Cobolt Flamenco™ 660 nm laser. The increase in spatial resolution becomes obvious for the STED recordings.

The use of the Cobolt Flamenco™ 660 nm DPSS laser allows the design of a compact and powerful STED nanoscope, potentially replacing larger and more complex and expensive laser sources.

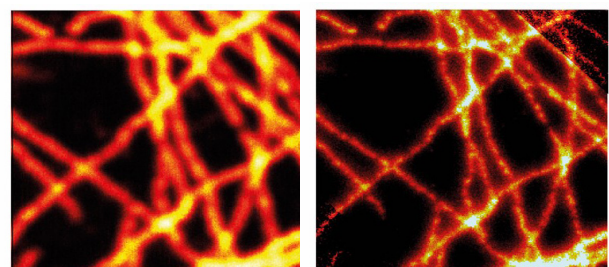


Figure 3.

## Cobolt Flamenco™ 660 nm

The Cobolt Flamenco™ is a continuous-wave single frequency DPSS laser operating at 660 nm with up to 300 mW output power in a perfect quality TEM<sub>00</sub> beam ( $M^2 < 1.1$ ). The laser is manufactured in a compact and hermetically sealed package using Cobolt's proprietary HTCure™ technology which provides a very high level of insensitivity to varying environmental conditions and unparalleled reliability.



Figure 4. Cobolt Flamenco™ 660 nm

Typical noise performance of the Cobolt Flamenco™ shows peak to peak noise <1% and rms noise <0.1%. This kind of single-mode DPSS laser is particularly attractive for use in demanding applications such as fluorescence nanoscopy (STED, GSD(IM), PALM and STORM), DNA sequencing and Raman spectroscopy, as it provides extremely good power stability, and a nearly perfect TEM<sub>00</sub>-mode low-divergent beam ( $M^2 < 1.1$ ). These are all performance characteristics that are required for good results.

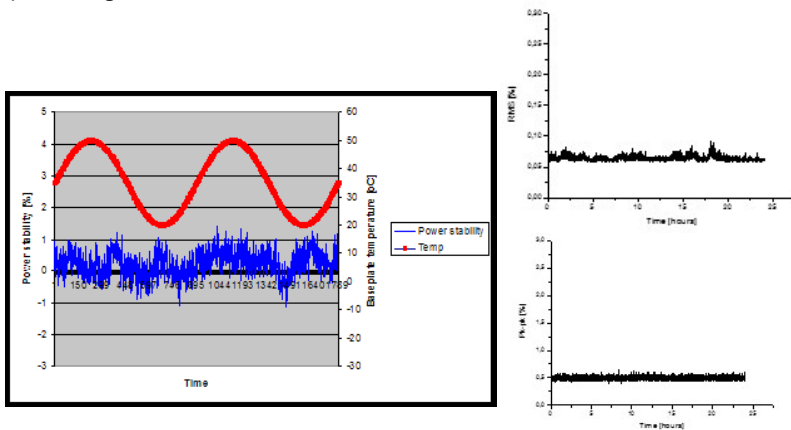


Figure 5. Typical noise performance