STED-Microscopy

Flexible Supercontinuum Excitation simply paired with a Synchronized Depletion beam

Fluorescence Microscopy allows highly specific imaging of cellular compartments in a minimal invasive fashion while obtaining the highest contrast. However, conventional microscopes are limited in their resolution due to the diffraction barrier. In the past decades several different approaches have shown the ability to circumvent the diffraction limit by either switching on and off single fluorophores, utilizing structured illumination, optical fluctuation or alternatively depleting excited chromophores from the excitation volume. This document provides a quick overview of the STED technique introduced by Stefan W. Hell in 1994. NKT Photonics' SuperK and Onefive KATANA HP lasers turn out to be the perfect combination to realize flexible pulsed excitation as well as synchronized depletion within the visible and near infrared range.

Introduction

STED microscopy is widely used to study luminescent samples with a high spatial resolution far below the diffraction barrier in the fields of biology, medicine as well as materials science.
Therefore, in a confocal laser scanning microscope the sample is excited with a diffraction limited, pulsed laser, followed by a doughnut shaped second laser pulse which is red-shifted with regard to the emission spectrum of the chromophore.

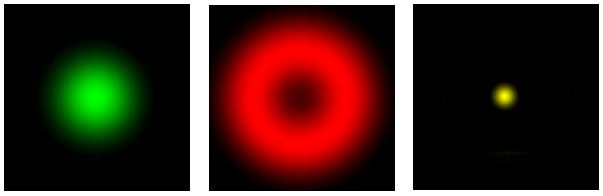

Fig. 1: STED principal, left: diffraction limited excitation from SuperK laser, middle: doughnut shaped depletion from Katana-HP laser, right: super resolved fluorescence signal after depletion.

This leads to a depletion of the outer ring of the confocal excitation volume. The remaining fluorescence after the depletion pulse is therefore only emitted from a shrinked region in the center of the excitation volume.

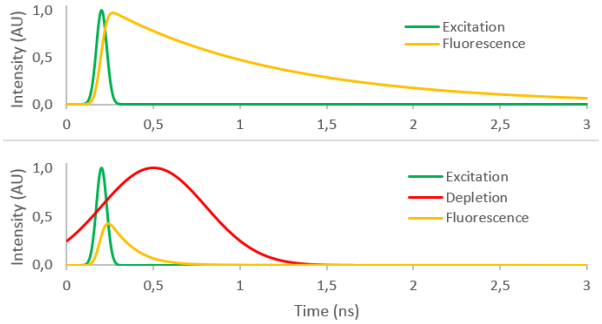

Fig. 2: Temporal behavior of the fluorescence signal, above: from the central minimum of the doughnut, below: within the depleted region in the doughnut.

The SuperK Supercontinuum lasers deliver a continuous spectrum over the visible (Vis) and near infrared (nIR) range, with excellent single mode beam profile ($M^2 < 1.1$) and picosecond (ps) pulse duration. In combination with our filter technology it can be transformed into a tunable laser source, allowing optimized excitation of every chromophore absorbing in the Vis and nIR regions.

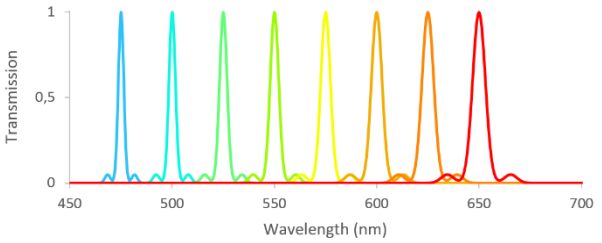

Fig. 3: Multiline filter transmission of Supercontinuum light from a SuperK laser through SuperK SELECT.

The Onefive KATANA HP lasers are available at various wavelength within the Vis-nIR and offer high pulse energy at ps pulse duration making them ideal for STED depletion.

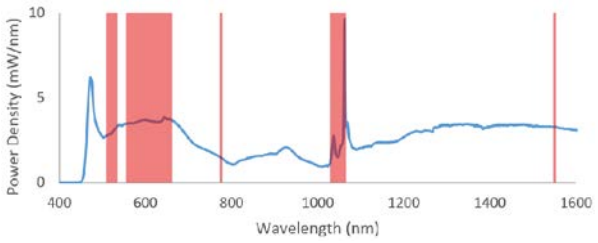

Fig. 4: Spectral power density of the SuperK Extreme EXW-12 (blue), available wavelength for KATANA HP lasers (red).

In addition to using the proper combination of wavelengths and laser power levels, in STED microscopy it is also crucial to precisely adjust the excitation and depletion laser pulses, to synchronize for efficient depletion of the excited chromophores at the beginning of each fluorescence cycle.
NKT Photonics' mode locked Supercontinuum lasers are equipped with a NIM trigger output and built in adjustable trigger delay as a standard feature. The Onefive KATANA HP lasers can run as a slave on external trigger input, which allows for simple software controlled adjustment of the excitation and depletion pulses.

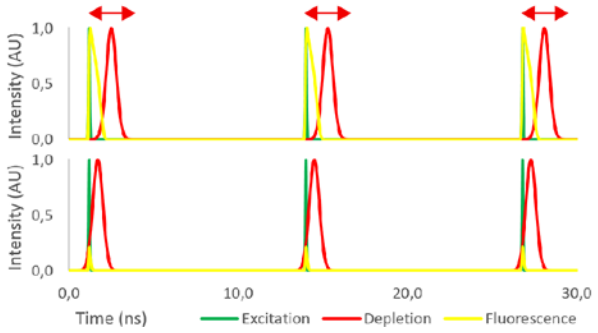

Fig. 5: Timing of excitation and depletion pulses can be easily adjusted to optimize depletion for best resolution.

In the experiment, the resolution is mainly limited by the photo physics of the chromophore used. That is why new fluorescent labels are developed rapidly. Consequently the flexibility of a Supercontinuum laser to freely choose the excitation wavelength helps to prepare for upcoming labels in the Vis-nIR.

The combination of the SuperK platform with the KATANA HP laser family becomes a turnkey solution for the most flexible and modular implementation of STED microscopy on the market successfully proven through multiple installations worldwide.

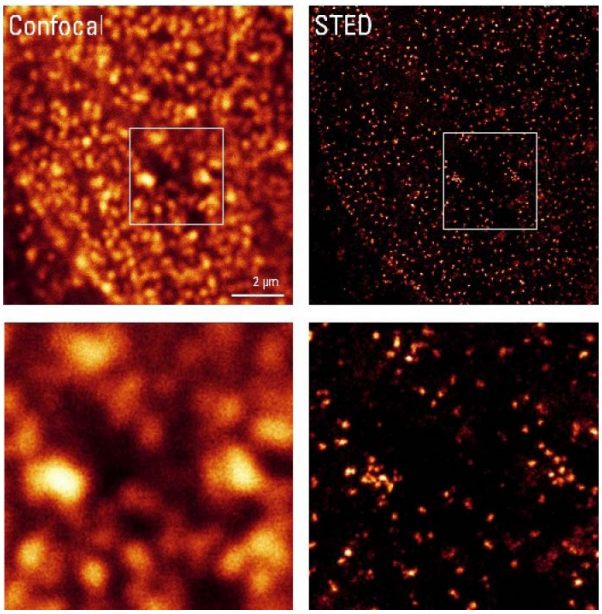

Fig. 6: Typical example of confocal laser scanning image (left) and a super resolved STED image (right). Achieved with Leica TCS SP8 STED 3X microscope. Courtesy of Leica Microsystems.

Suitable Hardware configuration for STED microscopy:

Supercontinuum Excitation:

- SuperK FIANIUM (PP), SuperK EXTREME (PP) or SuperK EVO
- SuperK SELECT or SuperK VARIA
- SuperK PM Fiber Delivery

Pulsed Depletion:

- Onefive KATANA 08 HP (e.g. 775 nm depletion)
- Onefive KATANA 06 HP (e.g. 592 nm depletion)