STED ADD-ON FOR A STANDARD TIME-RESOLVED CONFOCAL MICROSCOPE

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Superresolution microscopy is about to evolve into a standard tool for biological research. Overcoming the diffraction limit for fluorescence imaging has been shown to be crucial for addressing various relevant biological questions. Here, we show how superresolution, namely Stimulated Emission Depletion (STED), can be easily added to a standard confocal time-resolved fluorescence microscope, the MicroTime 200.

An EASYDOnut phaseplate converts the STED laser beam into the required donut-shaped focal spot while leaving the excitation beam unaffected [1]. An alignment of the STED donut in respect to the excitation spot is not necessary since both, STED and excitation beam are delivered by the same optical single mode fiber. A resolution below 50 nm FWHM is achieved.

Externally triggered pulsed lasers and confocal detection with time-correlated single photon counting (TCSPC) allow this system to take advantage of various excitation schemes like bunched excitation, pulsed interleaved excitation (PIE) and detection modalities such as gated STED. This inherent flexibility makes it easy to modify experiments to address highly specific biological questions. Combinations of STED with other time-resolved techniques such as FLIM (Fluorescence Lifetime Imaging Microscopy) or FLCS (Fluorescence Lifetime Correlation Spectroscopy) as well as investigations down to the single molecule level are feasible.

Multi-label STED imaging is possible using one STED depletion wavelength. The fluorophores are excited by nearby excitation wavelengths in PIE mode. Using this technique highly accurate co-localization measurements are possible. Small differences in absorption and emission spectra as well as in the fluorescence lifetimes can be utilized to create fluorescence patterns which act like a fingerprint. The labels are distinguished by applying fluorescence Pattern Matching analysis which takes into account the full spectral and temporal information. The principle will be shown on double labeled biological cells.