TUNABLE TIME-RESOLVED STIMULATED EMISSION DEPLETION MICROSCOPY FOR DYNAMIC IMAGING AT THE SUB CELLULAR SCALE.

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Circumventing the limit imposed by diffraction is a major issue in the instrumental development to realize finer resolutions in biological samples. STED microscopy [1] is an elegant method that has allowed us to break the diffraction barrier with light microscopes and has achieved resolutions of the order of 20 nm (transverse) and 100 nm (axial) [2]. In this technique, we exploit the molecular transitions of the fluorescent marker to overcome the resolution limit due to diffraction. Resolution enhancement is achieved by efficient depletion of the excited state of the marker in the peripheral spatial regions of the focal volume by using depletion beams in addition to the excitation beam. Despite the major resolution improvement demonstrated, the technique is not well spread out, mainly due to its apparent complexity; and the cost and limited tunability of the commercial system.

The idea of our development is to implement a tunable, compact and easy to use STED microscope based on a conventional confocal microscope. The laser lines for the excitation and depletion beams are provided by a supercontinuum source (SC-450 PP-HE, Fianium). This offers high tunability in terms of wavelengths for excitation and depletion, repetition rates, inherent synchronization of the imaging beams and affords compactness to the setup. Suitable filter combinations are used to select the required excitation beam and the two depletion beams. The depletion beams’ intensity profiles are shaped with phase plates which imprint different depletion patterns for the improvement of the lateral and axial resolutions. Incoherent superposition of these appropriately shaped beams results in the simultaneous enhancement of the resolution in all the three dimensions [2]. Various optical elements are introduced in the beam path to ensure precise spatial overlap and temporal synchronization of the beams at the back focal plane of the microscope objective. These beams are coupled to a commercially available confocal microscope (Nikon Ti-E) through a customized scan head (Nikon-C1). Scanning the sample is performed by the galvanometric scanning mirrors providing excellent temporal resolution for dynamic life processes and a nano-positioning stage (E-550, PI Gmbh) for best spatial resolution.

We will present the current state of development and discuss the performance realized so far. Once optimized, this system would be dedicated to the investigation of neurobiological issues associated with Alzheimer’s disease.

REFERENCES