GATED CW-STED MICROSCOPY WITH A SUPERCONTINUUM SOURCE

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In a typical STED microscope [1], a laser beam inducing stimulated emission, the so called STED beam, is overlaid with a regularly focused excitation beam. The wavefront of the STED beam is modified such that a doughnut-shaped focal spot is produced. When the STED intensity at the doughnut crest is significantly beyond the threshold at which the probability to stimulated de-excitation equals that of spontaneous emission, all fluorophores are virtually turned off expected those in close proximity to the doughnut minimum. Thereby, the spatial coordinate from where fluorophores can fluoresce are confined in a 3D sub-diffraction volume. Scanning the co-aligned beams through the sample sequentially turns off the signaling of fluorophores/features located at subdiffraction distance and, thus, allows the recording of subdiffraction image. STED microscopy can be implemented with both continuous wave (CW) and pulsed STED lasers. The implementation with CW laser is probably the most straightforward version, but, for a given number of stimulating photons, pulsed STED implementation yields to a stronger confinement of the effective fluorescent volume. As result the CW-STED implementation requires higher average STED power to reach the same performance of the pulsed counterpart, which in some circumstances can be a drawback in terms of live-cell imaging and laser availability.

Recently, two reports [2-3] have tackled this problem by implementing CW-STED in conjunction with pulsed excitation and time-gated detection. Collecting photons after a time-delay from the excitation pulse, allows better confining the effective fluorescent volume. This improvement follows from the fact that the probability to turn off a fluorophore increases not only with the STED intensity but also with its time of action.

Here we implemented time-gated CW-STED using a supercontinuum source [4] (also referred as white light sources) as excitation beam. The 80 MHz repetition rate and the possibility to cover all wavelengths of the visible range make this supercontinuum implementation one of the most straightforward yet powerful and fast-recording version of multi-color STED microscopy.