MULTIPLEXING STED NANOSCOPY WITH FAR RED-SHIFTED PROBES

Jonatan Alvelid, Ilaria Testa
Science for Life Laboratory (SciLifeLab)
KTH Royal Institute of Technology
Tomtebodavägen 23A, 17165, Solna, Sweden
E-mail: jonatan.alvelid@scilifelab.se

KEY WORDS: STED, red-shifted, quantum dots, multiplexing, multicolor, single particle.

Since its invention, STED is one of the most used nanoscopy techniques. Developments of the technique have been done in various directions, including novel acquisition schemes, multicolor and improved scanning speed, but much is still left unexplored. Here we present multiplexing of STED nanoscopy, mainly focused on improving multicolor abilities and introducing smarter and semi-automated acquisition to enable higher-throughput microscopy.

STED has entered the multicolor regime in various ways, and spectrally separated two-color STED is now an established approach to study co-localization of proteins. Additional super-resolved channels in the resulting image have so far been limited to alignment- or post-processing-demanding methods such as additional STED beams, lifetime separation or spectral unmixing. Instead, here we present a straightforward way to expand the multicolor scheme into three colors by using far red-shifted quantum dots (QDs) in combination with far-red dyes, a method that is directly translatable to existing commercial systems. The excitation and emission wavelengths of the QDs fill gaps previously unused in the spectra of the technique, with emission peaking at 740 nm and still readily depleted with a STED wavelength of 775 nm. Additionally, we utilize other beneficial properties of the QDs such as extremely low photobleaching and the possibility to do single particle counting directly in the recorded images.

Moreover, we present a semi-automated recording scheme for STED, also applicable to other scanning microscopy techniques. Based on a custom-developed open-source software to perform sequential recording we can image field-of-views with technically unlimited size. This allows us to do whole-neuronal imaging with highly increased throughput, thus shedding new light on the differences of the nanoscale organization of proteins on a whole-cell level without compromising the fine spatial resolution.