3D STED MICROSCOPY FOR SUPER-RESOLUTION WITH QUANTUM DOTS AND PERMEABLE DYES

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

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The family of super-resolution microscopy methods based on the idea of STED is ever growing. In our lab, we are developing a new STED microscope that combines previous 2D and 3D-based methods, multi-color approaches and adaptive optics implementations, all into one microscope that operates in the near-infrared part of the spectrum. A spatial light modulator is used to generate two separate and complementary depletion patterns that simultaneously deplete the fluorescence. Moreover, a single STED color is used to deplete multiple dyes, which has been made possible through recent breakthroughs concerning suitable combinations of STED-compatible and cell permeable dyes that allow for high resolution in two colors simultaneously. To achieve the best depletion, minimize photo-damage and facilitate deeper tissue imaging all colors are in the red part of the spectrum. Together, this allows for functional nanoscale imaging with a three-dimensional spatial accuracy in the range of 30x30x70 nm. The approach is being developed to allow for live cell imaging and aim to study organelles and macromolecular complexes in living neuronal cells of rodent brain tissues.

Pushing the performance of super-resolution methods is often achieved by optimizing the choice of fluorescent labels. While dyes and fluorescent proteins are the most commonly used labels for STED microscopy today, quantum dots (QDs) have recently been shown to be a viable option in the near-infrared part of the spectrum. The unique properties of QDs, such as outstanding brightness and long-term photostability, are intriguing in the search for better fluorophores and may open new questions to be investigated by STED microscopy. We have been able to extend STED imaging with QDs to both blue and red-shifted parts of the spectrum. Moreover, through time-correlated single photon counting we measured how the QDs lifetime depends on excitation as well as STED illumination. This agrees with a previously published theoretical model about altering exciton energy level interactions in QDs through varying excitation intensity. These results show that QDs has potential to be more widely used as fluorescent labels for established super-resolution methods such as STED microscopy, and that novel methods based on excitation intensity manipulation and lifetime separation are viable.