RESCue STED: LIVE CELL SUPERRESOLUTION MICROSCOPY WITH MINIMAL LIGHT DOSES

Jörn Heine, Andreas Schönle and Christian Wurm
Abberior-Instruments GmbH
Hans-Adolf-Krebs-Weg 1, 37077 Göttingen, Germany
E-mail: J.Heine@abberior-instruments.com

KEY WORDS: superresolution, STED, living cells, signal improvement, multicolor, 3D, volume, time lapse

ABSTRACT

In the recent decade superresolution microscopy techniques as STimulated Emission Depletion (STED) microscopy have evolved to important tools for cell biological research. STED microscopy allows to resolve features in fixed and living specimen which are smaller than the Abbe limit of resolution (~200 nm). Moreover, it facilitates to study processes in living cells with a resolution of some ten nanometers. However, elevated light intensities in the focus, longer pixel dwell times and higher pixel numbers may lead to photobleaching and phototoxicity. This often renders volume and time lapse imaging challenging.

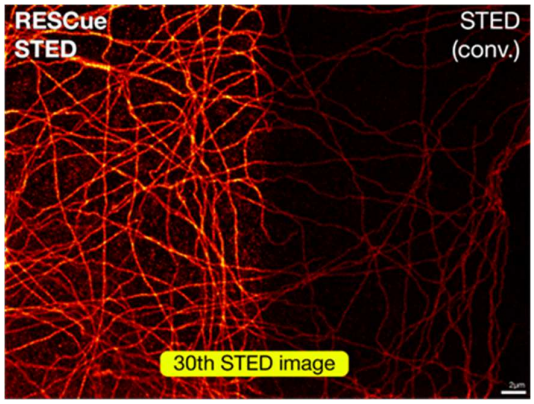

Figure 1: RESCue STED significantly reduces photobleaching compared to conventional imaging.

Here we present an improved scheme for RESCue imaging [1], an adaptive illumination concept for STED and confocal microscopy, which allows to reduce imaging photons and thereby strongly reduces photobleaching and phototoxicity.

The basic idea for this concept is to apply excitation and STED light only to areas where labelled structures are present in the sample. A great potential to save imaging photons exists, because large parts of typical samples are devoid of labelled structures (Fig. 1).

To further minimize the photon flux, illumination is controlled by a multilevel decision making process, which decides if a structure is present at a given spot or not and to switch the lasers depending on the decision.

The presented approach allows to save up to 96% of imaging photons in biological samples, consequently reducing photobleaching and phototoxicity. This enables to do live cell imaging experiments with extended number of time steps, improved multicolor imaging, and 3D-STED volume imaging with higher signal to noise ratios and increased number of sections.

REFERENCES

[1] Thorsten Staudt, Andreas Engler, Eva Rittweger, Benjamin Harke, Johann Engelhardt, and Stefan W. Hell, “Far-Field optical nanoscopy with reduced number of state transition cycles,” *Opt Express*, vol. 19, no. 6, pp. 5644–5657, 2011.