OPTIMIZING STED MICROSCOPY OF NUCLEAR DNA STAINING

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Stimulated emission depletion (STED) microscopy is one of the more widely applied superresolution techniques. It combines the advantages of breaking the diffraction barrier with comparatively fast image generation and relative ease of use in commercial systems. STED is currently making the transition to an application that is available to many life science research groups. Thus there is increasing demand for guidance to optimize imaging parameters such as laser intensities and scanning speed.

At our Biomedical Center, many groups are interested in the architecture of chromatin. When we started this study no DNA specific dye was described to work well with STED in mammalian cells. We therefore investigated a number of DNA dyes and labels for their suitability. A good STED dye is bright, stable and can be efficiently depleted by the STED laser. Importantly, the depletion laser wavelength should not excite the dye, or at least only at very low efficiency.

DAPI, Hoechst, SYTO 16, SYTO 21 and SIR-DNA were studied. In addition, we investigated DNA replication labeling with EdU, subsequently coupled to Alexa 488, 555 or 594. After 24 h incubation, most cells in the culture had their chromatin labeled. Alexa 594 was previously described as a good fluorochrome for STED.

On our Leica SP8 STED 3X, each label was imaged first in confocal mode and then imaged with increasing powers of the depletion laser. Depletion at 592, 660, and 775 nm was used if possible with the respective dye. In addition, the intensity of the anti-Stokes signal excited by the respective depletion laser was measured for each dye. Due to the large size of the depletion PSF, anti-Stokes excitation may decrease the resolution, as we have found for the full width half maximum of dot-like nuclear pore stainings at high depletion laser power. This dye dependent anti-stokes excitation fundamentally limits achievable resolution in STED.

For increasing depletion laser intensities, our data show that many dyes have a sweet spot, where depletion is already relatively high, but anti-Stokes excitation yet low. The best results concerning depletion and low anti-Stokes excitation were indeed obtained with EdU-Alexa 594 label. Relatively low bleaching allowed 3D recordings of entire cell nuclei with superresolution.

The outstanding signals of the Alexa 594 replication label were further used to test for optimal image recording parameters with regard to laser intensities, detector saturation and fluorochrome saturation. From our data, some general rules for optimization of recording parameters can be deduced.