BIOLOGICAL APPLICATIONS OF SUBDIFFRACTION STED MICROSCOPY

Robert R. Kellner, Katrin I. Willig, Stefan W. Hell
Max-Planck-Institute for Biophysical Chemistry
Am Fassberg 11, 37077 Göttingen, Germany
Department of NanoBiophotonics
E-mail: rkellne@gwdg.de

KEY WORDS: STED microscopy, neuromuscular junction, acetylcholine receptor

The resolution of a standard far-field light microscope is usually limited by diffraction to $\Delta r = \lambda / 2NA$, with λ denoting the wavelength of light and NA the numerical aperture of the lens. A STED microscope however is a capable of attaining diffraction unlimited resolution using regular lenses and is therefore a useful tool in modern cell biology. Recent advances in the STED technique allow the use of visible dyes as well as the green and yellow fluorescent proteins for imaging with improved resolution, forming the basis for new applications of STED microscopy to modern cell biology.

Using STED microcopy we were able to observe that the Drosophila coiled-coil domain protein Bruchpilot forms donut-shaped structures centerd at active zones of neuromuscular synapses [1]. Standard confocal microcopy is not able to resolve this structure.

In another application, we investigated Acteylcholine receptor (AChR) supramolecular aggregates in the Chinese hamster ovary cell line CHO-K1/A5 that stably expresses adult murine AChR. Whereas confocal microscopy displays AChR clusters as diffraction-limited dots of 200 nm diameter, STED microscopy yields nanoclusters with a peak size distribution of 55 nm. Utilizing this resolution, we show that cholesterol depletion of the cell membrane alters the short and long range organization of AChR nanoclusters on the cell surface [2].

[1] R. J. Kittel, C. Wichmann, T. M. Rasse, W. Fouquet, M. Schmidt, A. Schmid, D. A. Wagh, C. Pawlu, R. R. Kellner, K. I. Willig, S. W. Hell, E. Buchner, M. Heckmann, and S. J. Sigrist, „Bruchpilot Promotes Active Zone Assembly, Ca2+ Channel Clustering, and Vesicle Release," *Science*, **312**, 1051-1054 (2006)
[2] R. R. Kellner, C. J. Baier, K. I. Willig, S. W. Hell, and F. J. Barrantes, „Nanoscale organization of nicotinic acetylcholine receptors revealed by stimulated emission depletion microscopy", *Neuroscience*, **144**, 135-143 (2007)