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Single Molecule Spectroscopy and Imaging IV

**Jörg Enderlein
Zygmunt K. Gryczynski
Rainer Erdmann**
Editors

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Introduction to Nanoscopy and Superresolution Microscopy Sessions:

Nanoscopy with Focused Light

Stefan W. Hell

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For more than a century, it has been generally accepted that the resolution of a lens-based optical microscope is limited to about $d = \lambda/(2 NA) > 200$ nm in the focal plane and > 500 nm along the optic axis, with NA denoting the numerical aperture of the lens and λ the wavelength of light. The discovery in the 1990's that elementary transitions between the states of a fluorophore can be used to eliminate the limiting role of diffraction has led to light microscopy concepts with resolution on the nanometer scale(1, 2). Currently, all existing and successfully applied nanoscopy methods share a common enabling element: they switch fluorescence on or off, so that adjacent features are registered sequentially in time (3, 4).

For example, in a typical Stimulated Emission Depletion (STED) microscope(1), the fluorophores are switched off (=kept dark) by overlapping the excitation beam with a de-exciting (STED) beam which effectively confines the fluorophores to the ground state everywhere in the focal region except at a tiny area where the STED beam is close to zero. Fluorophores that are located in this subdiffraction-sized smaller area are registered. Scanning the beams further in space registers those fluorophores that had been switched off. An image of the whole object is assembled by sequential registration. The resolution is now given by the smaller diameter $d \approx \lambda/(2 NA\sqrt{1+I/I_s})$ of this area in which the fluorophores are still fluorescent. I is the intensity of the STED beam, which, for $I \gg I_s$, entails $d \rightarrow 0$, meaning that the resolution is conceptually no longer limited by λ .

STED microscopy has been used to investigate the fate of synaptic vesicle proteins after exocytosis(5), thus demonstrating the potential of emerging 'fluorescence nanoscopy' for the life sciences. A video-rate STED microscope was used to describe the mobility of vesicles inside the axons of cultured living neurons(6). Live-cell STED microscopy has also been used to image activity-dependent morphological plasticity of

dendritic spines(7), while in another study, it revealed that single sphingolipids, but not phospholipids, are transiently (< 10 ms) and locally (< 20 nm) trapped in a living cell membrane, mediated by cholesterol(8).

The concept of STED microscopy has been expanded to low intensity operation by switching the fluorophore to a long-lived dark (triplet) state or between a 'fluorescence activated' and a 'deactivated' (conformational) state(2) as encountered in switchable fluorescent proteins(9). More recent but seminal nanoscopy schemes such as PALM, STORM and also GSDIM, switch the molecules individually and stochastically to a state that emits $m \gg 1$ detectable photons in a row before returning to a dark state, allowing the calculation of their position. These single fluorophore switching concepts(10-14) require only a single switching cycle(3, 4) per fluorophore, which greatly extends the power of the switching concept for subdiffraction separation. Altogether, lens-based optical nanoscopy is an unexpected and fascinating development in the physical sciences that is poised to impact several areas of science, in particular the life sciences, in the near future.

1. S. W. Hell, J. Wichmann, *Opt. Lett.* **19**, 780 (1994).
2. S. W. Hell, *Nature Biotech.* **21**, 1347 (2003).
3. S. W. Hell, *Science* **316**, 1153 (2007).
4. S. W. Hell, *Nature Meth.* **6**, 24 (2009).
5. K. I. Willig, S. O. Rizzoli, V. Westphal, R. Jahn, S. W. Hell, *Nature* **440**, 935 (2006).
6. V. Westphal *et al.*, *Science* **320**, 246 (2008).
7. U. V. Nagerl, K. I. Willig, B. Hein, S. W. Hell, T. Bonhoeffer, *PNAS* **105**, 18982 (2008).
8. C. Eggeling *et al.*, *Nature* **457**, 1159 (2009).
9. M. Hofmann, C. Eggeling, S. Jakobs, S. W. Hell, *PNAS* **102**, 17565 (2005).
10. E. Betzig *et al.*, *Science* **313**, 1642 (2006).
11. M. J. Rust, M. Bates, X. W. Zhuang, *Nature Meth.* **3**, 793 (2006).
12. S. T. Hess, T. P. K. Girirajan, M. D. Mason, *Biophys. J.* **91**, 4258 (2006).
13. A. Egner *et al.*, *Biophys. J.* **93**, 3285 (2007).
14. J. Fölling *et al.*, *Nature Meth.* **5**, 943 (2008).