

SINGLE-MOLECULE PHOTOSWITCHING MICROSCOPY USING ONLY A SINGLE EXCITATION WAVELENGTH

Volker Buschmann, Ben Krämer, Mike Heilemann (*), Sebastian van der Linde (), Markus Sauer (**), Steve Wolter (**), Sandra Orthaus, Rainer Erdmann**

**PicoQuant GmbH, Rudower Chaussee 29, 12489 Berlin, Germany
www.picoquant.com, info@picoquant.com**

**(*) Bielefeld Institute for Biophysics and Nanoscience, Bielefeld University,
Universitätsstrasse 25, 33615, Bielefeld, Germany**

() Würzburg University, Biozentrum, Am Hubland, 97974 Würzburg, Germany**

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Super-resolution microscopy techniques offer unprecedented insight into cellular structures [1]. In Stochastic Optical Reconstruction Microscopy (STORM) and Photo-Activation Light Microscopy (PALM), the dye-sensor-molecules are switched using two different excitation wavelengths. Recently, it has been shown that certain dye labels exhibit intrinsic dark states with a lifetime tunable by adjusting the concentration of oxidants and reductants in the buffer [2,3]. We have built an instrument for high resolution imaging based on a standard inverse microscope using TIRF excitation and ultrasensitive EMCCD camera detection which exploits redox-level adjusted photoswitching. We achieved a lateral resolution well below 100 nm when imaging ATTO655 molecules, which have been immobilized via a Biotin/Streptavidin tag. Since the method uses only a single excitation wavelength, it provides the potential to image several fluorescent labels with different absorption properties in parallel.

References:

- [1] N. Blow, "New ways to see a smaller world," *Nature*, **456**, 825-828 (2008)
- [2] J. Vogelsang, T. Cordes, C. Forthmann, C. Steinhauser, and P. Tinnefeld, "Controlling the fluorescence of ordinary oxazine dyes for single-molecule switching and superresolution microscopy," *PNAS*, **106**, 08107-08112 (2009)
- [3] M. Heilemann, S. van de Linde, A. Mukherjee, and M. Sauer, "Super-resolution imaging with small organic fluorophores," *Angew. Chem. Int. Ed. Engl.* **48** (37), 6903-6908 (2009)