BUFFER CONTROLLED PHOTOSWITCHING MICROSCOPY USING STANDARD ORGANIC FLUOROPHORES

Volker Buschmann¹, Marcelle Koenig¹, Sebastian van de Linde², Markus Sauer³, Steve Wolter², Mike Heilemann³, Felix Koberling¹, B. Krämer¹, Rainer Erdmann¹

¹PicoQuant GmbH, Rudower Chaussee 29, 12489 Berlin, Germany, email: info@picoquant.com
²Biocenter, Department of Biotechnology & Biophysics, Am Hubland, 97074 Würzburg, Germany
³Appl. Laser Physics and Laser Spectroscopy, Bielefeld University, Universitätsstr. 25, 33615 Bielefeld, Germany

KEY WORDS: Super Resolution Imaging, Fluorescence, Single Molecule Spectroscopy, Photoswitching Microscopy

The interest in super-resolution microscopy techniques has dramatically increased in the last years due to the unprecedented insight into cellular structure which has become possible [1]. In all camera-based techniques, such as Stochastical Optical Reconstruction Microscopy (STORM), direct STORM (dSTORM) and Photo-activation localization microscopy (PALM), the dye-sensor-molecules are switched between a bright and a dark state, which is generally achieved using 2 different wavelengths for excitation and photoswitching. Many organic fluophores exhibit intrinsic dark states with a lifetime which can be tuned by adjusting the level of oxidants and reductants in the buffer. This technology was originally applied to increase the photostability of fluorescentophores (Neverfade-technology) and is known in the literature as reducing and oxidizing system (ROXS) [2]. In superresolution microscopy, reversibly applications, it can be used to switch individual fluorophores between an on- and off-state using just a single excitation wavelength [3].

We exploited this redox-level adjusted photoswitching behaviour for high-resolution imaging on a setup based on an inverse microscope coupled with ultrasensitive CCD camera detection. In order to quickly control the quality of the measurement, we used real-time computation of the subdiffraction-resolution image [4]. This greatly increases the applicability of the method, as image analysis times are greatly reduced.