Motivation

Fluorescence microscopy is a sensitive and non-invasive tool to study biomolecular structure and interaction. However, diffraction limits the spatial resolution to ~200 nm in lateral and ~700 nm in axial direction.

Different methods have been developed that bypass this resolution limit, and many of them rely on the use of photoactivatable or photoswitchable fluorophores [1]. Here, we demonstrate that a large selection of commonly used organic fluorophores can be used as photoswitches for localization-based super-resolution microscopy.

A General Concept for Photoswitching of Organic Fluorophores

Photoswitching microscopy with standard fluorophores can be realized in the presence of a reducing agent, e.g., mercaptoethylamine (MEA), glutathione (GSH), or dithiothreitol (DTT), in an air saturated solution [2]. The excited fluorophore is reduced out of its triplet state into a stable off-state. As expected for a light-induced process, the formation of fluorophore radical anions and secondary products is mainly determined by the irradiation intensity in the presence of adequate concentrations of RS−. Consequently, the off-rate increases linearly with increasing excitation intensity.

Super-Resolution Imaging with dSTORM

A target structure, e.g., the cytoskeleton of a cell, is densely labeled with photoswitchable fluorophores via immunocytochemistry [2-6]. The basic step of photoswitching microscopy is to drive the majority of photoswitchable fluorophores into the off-state. Only a fraction of single fluorophores are switched on stochastically where the position of every single fluorophore is determined with high precision.

After multiple cycles of switching and localizing single fluorophores over time, all localization events are summed up and reconstructed to a super-resolved image with an experimental precision of 20 nm.

A Universal Concept for Photoswitching Microscopy

The principle of dSTORM can be extended to many other fluorophores exploiting photophysical processes. Hereby, the number of organic fluorophores suitable for super-resolution imaging can be largely extended by almost all common rhodamine and oxazine derivatives from the blue to the red part of the electromagnetic spectrum [5]. The underlying mechanism can be described as a remarkably efficient cycling between a fluorescent and non-fluorescent state of the fluorophores in the presence of millimolar concentrations of thiols.

Quantitative Biological Studies with Super-resolution Photoswitching Microscopy

Beyond fluorescence imaging with subdiffraction resolution, we apply molecular photoswitches for quantitative studies on concentration and distribution of proteins in subcellular compartments [6], or mRNA [2]. With an effective localization precision of ~10 nm, it becomes possible to identify several thousands of target molecules per 1 µm² (compared to about a few tens with confocal microscopy).

Video-like Super-Resolution Microscopy

Video-like dSTORM employs fast photoswitching of carbocyanine fluorophores and imaging frame rates of up to 1.5 kHz [3, 7]. Subdiffraction movie sequences are generated with a sliding-window algorithm, and a true temporal resolution of 1 Hz with ~30 nm spatial resolution was applied to study actin motility on a myosin surface. We also studied the interplay of temporal and spatial resolution with respect to on- and off-switching kinetics [8].

References


Acknowledgment

This work was supported by the Systems Biology (FORSYS) and Biophotonics Initiative of the German Ministry of Research and Education (BMBF) (grants No. 0315262 & 13N9234).