Bleaching-insensitive STED microscopy with exchangeable fluorescent probes

Christoph Spahn¹, Mathilda Glaesmann¹, Jonathan B. Grimm², Luke D. Lavis², Hans-Dieter Barth¹, Marko Lampe³ and Mike Heilemann¹

¹ Institute of Physical and Theoretical Chemistry, Goethe-University Frankfurt, Max-von-Laue-Str. 7, 60438 Frankfurt, Germany
² Janelia Research Campus, Howard Hughes Medical Institute, 19700 Helix Drive, Ashburn, Virginia 20147, United States
³ Advanced Light Microscopy Facility, European Molecular Biology Laboratory, Meyerhofstr. 1, 69117 Heidelberg, Germany

Photobleaching affects image quality and resolution in fluorescence microscopy and thus limits the extractable information. This is in particular relevant for super-resolution microscopy where typically high laser intensities are used. In order to minimize photobleaching, we repurposed fluorescent probes that transiently bind to a target, as used in single-molecule localization microscopy methods such as Point Accumulation for Imaging in Nanoscale Topography (PAINT) [1], for STED microscopy. We demonstrate pseudo-permanent labeling of target structures and constant exchange of photobleached fluorophores. We used this labeling concept for whole-cell, 3D, multi-color and live-cell STED microscopy that is insensitive to photobleaching [2]. Using transiently binding hydrophobic dyes [3] and fluorophore-labeled minor groove binders [4], we visualized the nanostructure of chromatin, cell membranes and organelles in bacterial and mammalian cells in 3D [2]. In addition, we employed oligonucleotide-labeled antibodies that transiently bind fluorophore-labeled oligonucleotides for bleaching-insensitive, multi-color STED microscopy [5].

References