IMPROVED OPTICAL SETUP FOR LOCALIZATION MICROSCOPY

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KEY WORDS: Super-resolution microscopy, localization microscopy, SPDM, photoblinking, Shack–Hartmann wavefront sensor, thermal stability, DNA origami

Recent advances in optical microscopy open up new possibilities to circumvent Abbe’s diffraction limit in conventional far-field microscopy and thus allow us to study tiny structures such as viral particles or membranes at the nanoscale level.

Here, we present a unique and improved optical super-resolution set-up which is based on previous localization techniques such as SPDM, dSTORM, or GSDIM. These methods utilize a switching mechanism which evolves from a light induced long-lived dark state followed by stochastic recovery of single molecules to the fluorescent state. Fast time-lapse recording of sparse blinking events allows for the optical isolation of single molecules with high spatial localization precision. The superposition of these events finally results in a super-resolution image. Due to the required acquisition time, this method is generally limited to the observation of fixed specimens.

Although the general principle of localization microscopy seems simple in theory, current methods are limited by certain issues and pitfalls, which we have addressed in our improved optical set-up. Specific sample preparation and the limitation to certain fluorochromes is one of them. In contrast to other stochastic methods (e.g. PALM or STORM), a broad variety of commercially available standard fluorochromes (e.g. Alexa Fluor, Cyanines, Atto, fluorescent proteins) can be used in our set-up. Furthermore, our method does not require UV irradiation for switching the spectral properties of fluorochromes. Photoactivation in wide-field mode is also not restricted to structures located in close proximity to the cover slip surface (such as plasma membranes) but allows the observation of general intracellular structures.

In the current system, we established a novel beam-shaping procedure and a high-precision optical alignment using a Shack–Hartmann wavefront sensor. We also improved the thermal and mechanical stability of the entire optical system. The high localization accuracy was validated by DNA origami standards. Self-developed LabView and MATLAB software was used to record and reconstruct localization images from a time series and to analyze the distribution and clustering of different molecules of interest. Due to the extremely stable construction of our microscope, we can record images for extended time periods significantly improving spatial resolution in comparison to previous SPDM set-ups.