STABILITY IN SINGLE NANOMETER SUPERRESOLUTION MICROSCOPY

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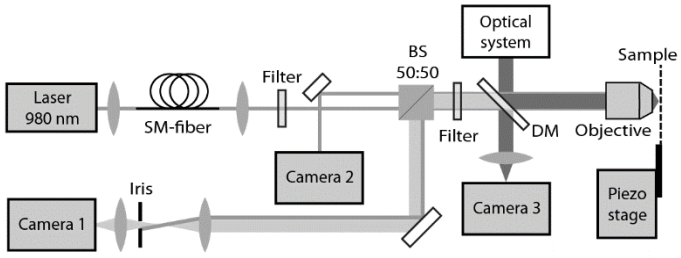
KEY WORDS: superresolution microscopy, stability, localization precision, MINFLUX

Recently, optical microscopy techniques have pushed image resolution to values smaller than 10 nm [1,2]. When performing measurements with this resolution, the stability of the optical setup limits the precision of measurements. Nowadays, superresolution fluorescence microscopy may feature a focus lock but it is less common to control drift of the sample in lateral direction [1,2] and detect the fluctuations in the excitation path. Therefore, we developed a stage lock in all three directions and a beam monitoring unit to determine the beam position, which are powerful tools to increase the stability of the stage and the excitation beam path. The use of these stabilization systems allows MINFLUX microscopy [1] to achieve 1 nm resolution.

The axial sample position is obtained by measuring the displacement of the total internal reflected (TIR) beam on the coverslip-media interface. For this purpose, an infrared laser beam is focused off-center into the back focal plane of the objective lens. A CMOS camera detects the TIR signal and its center of mass is used as a measure of the axial sample position.
For measuring the lateral position of the sample, the evanescent light of the TIR illuminates nanorods on top of the coverslip. Scattered light is detected by a second CMOS camera in a dark-field fashion. The rods' positions are obtained by 2D Gaussian fitting, which reveal the lateral sample position. Both, the axial and lateral positions of the sample were corrected by commanding a xyz piezo stage with a PI feedback loop written in LabView.

By monitoring the beam position, it is possible to determine fluctuations in the excitation beam path of an optical system and to correct systematic errors in the localization of the molecule. To this end, a dielectric mirror with an uncoated backside is placed close to the objective and a small fraction of the excitation beam is transmitted. By focusing the beam on a CMOS camera, its centroid provides a measure for the beam position.



Schematic view of the stabilization systems.

[1] Francisco Balzarotti, et al. "Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes", *Science*, (2016).
[2] Dai, M., et al. "Optical imaging of individual biomolecules in densely packed clusters." *Nature Nanotechnology*, 11(9): 798-807, (2016).