Adaptive optics module for highest precision 3D localization in super resolution microscopy based on fast and stable PSF engineering

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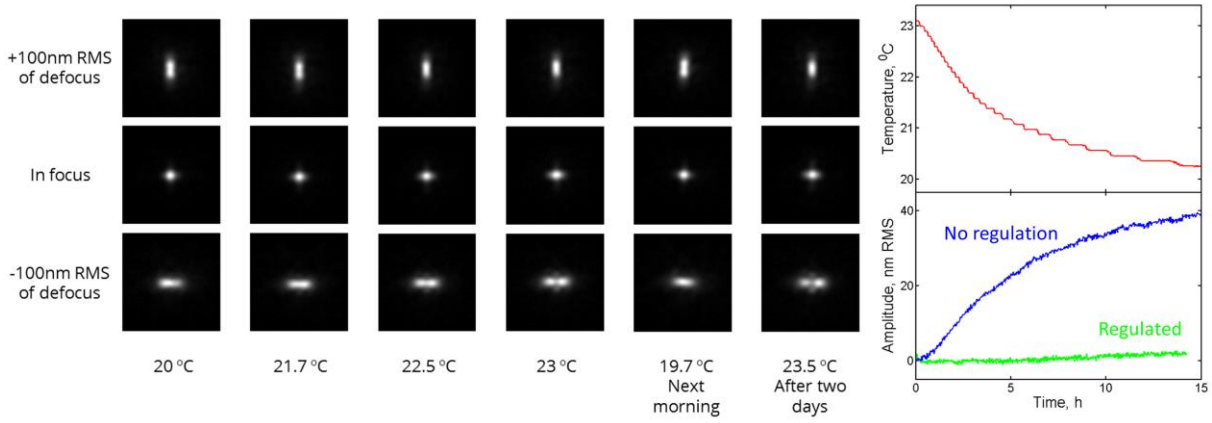
Single molecule localization microscopy (SMLM) methods enable to locate fluorescent molecules with nanometric precision. The quality of the point spread function (PSF) determines the accuracy of single molecule detection in these methods, therefore it is crucial to maintain diffraction-limited PSF. Aberrations induced by the optical setup and biological sample can be compensated by adaptive optics (AO) [1]. Here we propose an add-on AO module for single molecule super resolution microscopy with enhanced performance, compared to previously reported setups. First, the process of aberration detection remains a complex task, since the absence of guide star inside biological samples limits the usability of a direct wavefront sensing. Image-based iterative algorithms are thus used but they are time consuming and induce sample bleaching. To simplify and accelerate the process of aberration detection, we developed a new, automatized method based on phase retrieval. Second, we propose a setup that eliminates temporal drifts of the achieved correction, with a time stability inferior to 5 nm rms over days, providing a significant advantage when considering precise quantitative studies over time. Parameters and performance of these features in super resolution microscopy will be discussed. In order to illustrate this performance, in particular in depth, we built a model sample composed of fixed HeLa cells in agarose gel, stably expressing centrosomal mEOS2-centrin1 conjugate for PALM imaging. We applied AO correction at the vicinity of the coverslip using phase diversity together with additional aberration compensation in depth based on a model. This strategy allowed us to determine the structure of centrin-1 at 50µm depth, with 10-20nm localization precision.



The temporal stability of the PSF. Left panel: Images of the 200nm fluorescent bead in focus and slightly out focus at different temperatures. There is 60nm of astigmatism applied on the deformable mirror. Right panel: the wavefront measurements using the wavefront sensor to illustrate the changes on the left panel picture.

[1] I. Izeddin, M.E. Beheiry, J. Andilla, D. Ciepelewski, X. Darzacq and M. Dahan, *PSF shaping using adaptive optics for three-dimensional single-molecule super-resolution imaging and tracking*, Optics Express, vol. 20, p.4957-4967, 2012.