3D PALM IMAGING DEEP IN THE SAMPLE

Grégory Clouvel¹, Ignacio Izeddin², Audrius Jasaitis¹, James Sillibourne³,
Mohamed El-Beheiry⁴, Xavier Levecq¹, Maxime Dahan⁴, Michel Bornens³, Xavier Darzacq²

¹Imagine Optic, 18 rue Charles de Gaulle, 91400 Orsay, France
²Institute de Biologie de l’Ecole Normale Supérieure, CNRS UMR8197, 46 rue d’Ulm 75230
Paris Cedex 05, France
³Institut Curie, CNRS UMR144, 26 rue d’Ulm, 75248 Paris Cedex 05, France
⁴Institut Curie, CNRS UMR 168, 26 rue d’Ulm, 75248 Paris Cedex 05, France

The determination of three-dimensional arrangement of cellular structures has become a necessary requirement in cellular biology. Unfortunately, the size of such structures usually lies beyond the diffraction limit and therefore they cannot be visualized in studies using today’s widely popular fluorescence microscopy techniques. Photoactivation localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) enables us to locate fluorescent molecules with nanometric resolution. Unfortunately in current implementations, these techniques are efficient only in the vicinity of the coverslip, like in total internal reflection (TIRF) or in the first few micrometers inside the sample. Imaging deeper is highly perturbed by the presence of the spherical aberration, caused by the refractive index mismatch between the sample and the immersion oil of the objective. This aberration drastically reduces the amount of detected photons and ruins the shape of the point spread function (PSF) which is extremely important in the case of the astigmatic approach for three-dimensional imaging.

Aberrations can be efficiently corrected using adaptive optics. In this work for the correction of aberrations we used MicAO 3DSR – and adaptive optics device containing the Shack-Hartman type wavefront sensor and continuous membrane deformable mirror, as previously described in [1, 2]. By correcting the spherical aberration we can obtain perfectly symmetrical PSF along the Z axis up to the depth of 25µm in the sample. After the correction, also using the deformable mirror, we apply various amounts of astigmatism for three-dimensional imaging. The calibration curves, obtained using fluorescent beads embedded in agar, were symmetrical above and below the focus at any depth until 25µm.

To test the performance of this system for PALM imaging at depth we used fixed HeLa cells, stably expressing the centrosomal protein centrin-1. We suspended those cells in agar so they would be evenly dispersed in the whole volume of the sample. For the optimization of the PSF with adaptive optics and for the drift correction to the sample we also added 100nm fluorescent beads. Our results show that single molecule imaging can be done at depths in the sample of up to 25µm.

References