IMPROVEMENT OF 3D LOCALIZATION IN PALM, STORM AND SINGLE PARTICLE TRACKING BY USING ADAPTIVE OPTICS

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Key words: Adaptive optics, super resolution microscopy, 3D, SPT, PALM, STORM

Fluorescent microscopy is widely being used in biology as a basic tool to investigate cellular and molecular processes. Nowadays, super-resolution microscopy is unveiling new information beyond the diffraction limit. However, super-resolution techniques, as all the other optical techniques, are affected by the optical aberrations of the system which reduce the performance of its point spread function. In PALM/STORM or single particle tracking techniques the accuracy of the system is directly related with the number of collected photons by the detector. Because of this, the optical aberrations of the system degrade the precision of the detection. In microscopy techniques like two photon microscopy or confocal microscopy, adaptive optics has already been demonstrated as a suitable technique to compensate the effects of the optical aberrations improving the obtained signal and optimizing the systems.

In this communication we present the application of adaptive optics in pointillist super-resolution techniques (PALM/STORM/SPT). We will show how the adaptive optics system is installed in the emission path of the microscope in a very simple way. We will also show the improvement obtained after correcting the optical aberrations. Finally, we will demonstrate the capability of the adaptive optics system to modify the wave-front and introduce the third dimension information as in the PALM3D/STORM3D/3D-SPT techniques (see fig. 1). We will discuss about the accuracy of using these capabilities in comparison with previous strategies. We will also show how dynamical properties of this approach allow the arbitrary adaptation of the shape as a function of the particular experimental conditions.

Fig. 1. A) Three-dimensional PALM images of actin bundles in fibroblasts transfected with ABP-EOS. Inset : diffraction limited 2D image of the same region. B) Three-dimensional trajectory of quantum dot bound to a transmembrane protein diffusing in the plasma membrane of a cultured HeLa cell. The color bar corresponds to the z position. Scale bars: 1 µm.