

ADAPTIVE OPTICS LIGHT-SHEET MICROSCOPY FOR FUNCTIONAL NEUROIMAGING

Antoine Hubert^{1,2*}, Fabrice Harms¹, Sophia Imperato^{2,4}, Vincent Loriette², Cynthia Veilly¹, Xavier Levecq¹, Georges Farkouh³, François Rouyer³, Alexandra Fragola²

¹Imagine Optic, 18 rue Charles de Gaulle, 91400 Orsay, France

²ESPCI – LPEM, 10, Rue Vauquelin, 75005 Paris, France

³Institut des Neurosciences Paris-Saclay, 91190 Gif-sur-Yvette, France

*antoine.hubert@espci.fr

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Light-sheet fluorescence microscopy (LSFM) has successfully demonstrated its ability to increase the signal-to-background ratio (SBR) and to limit the phototoxicity for live samples imaging thanks to its intrinsic optical sectioning [1]. Although many developments have contributed to the optimization of axial and lateral resolution, the image quality is degraded in depth by the optical aberrations due to the refractive index inhomogeneities. Adaptive Optics (AO) has been reported in LSFM [2-4] as an efficient method to compensate for aberrations. However, reported implementations, which are based on a sensorless strategy or a direct wavefront measurement imply respectively a time-consuming iterative approach or a complex method of creating a guide star.

We recently reported a new AO-LSFM approach based on an extended-source Shack-Hartmann wavefront sensor [5] with minimal requirements, in particular without the need for a guide star. Even though this approach provides significant image improvement, its applicability to fast, 3D functional imaging of neuronal networks has not yet been reported.

We present here an AO-LSFM setup based on this approach, including an optimized photon budget regarding AO vs. imaging for improved temporal performance, as well as a quantitative analysis of key AO parameters such as the number of corrected modes and the influence of the SBR on the accuracy of the measurement.

We also report first AO-enhanced functional images of GCaMP neurons involved in sleeping behavior in a freshly dissected, uncleared drosophila brain at high depths (30 to 80 μ m).

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