SINGLE-SHOT CHARACTERIZATION OF OPTICAL ABERRATIONS AND SCATTERING PROPERTIES OF THE MOUSE CORTEX USING AN EXTENDED SOURCE SHACK-HARTMANN WAVEFRONT SENSOR

S. Imperato[1,2]*, F. Harms[3], A. Hubert[1,3], M. Mercier[1], T. Pons[1], L. Bourdieu[2], and A. Fragola[1]
[1] **ESPCI – LPEM, 10, Rue Vauquelin, 75005 Paris, France**, [2] **Institut de Biologie de l'Ecole Normale Supérieure, 75005 Paris, France[3], Imagine Optic, 18 rue Charles de Gaulle, 91400 Orsay, France**
*sophia.imperato@espci.fr

Optical microscopy has emerged as a major tool in neuroscience as it allows to image the structure and the activity within large volume of tissue with cellular and sub-cellular resolution, in particular in non-linear fluorescence microscopy. Measurement of neuronal network activity in awake behaving animals is thus possible but remains limited in depth because resolution and signal intensity are strongly deteriorated by the refraction index inhomogeneity of the biological tissues. Thus, as the imaging depth increases, non-negligible aberrations are observed on the imaging path, which affect significantly the microscope performances and the image quality. To overcome this difficulty, adaptive optics has been implemented on several microscopy set ups, for *in vivo* imaging, and currently provides a reliable live correction of the aberrations, enabling e.g. high resolution two-photon imaging in infragranular layers of dendritic spines, synaptic boutons and axons in the mouse cortex. The use of direct wavefront sensing using Shack-Hartmann sensors [1] was a key step to evaluate and correct aberrations *in vivo*. However, this method fails at large depths because of the strong scattering inducing a poor signal to background ratio of the wavefront measurement. A method for direct wavefront sensing more resilient to scattering would therefore improve the use of adaptive optics in optical microscopy.

This project* proposes an alternative method of wavefront measurement that takes advantage of existing labelling methods of the biological samples and that relies on the cross-correlation of images of an extended source obtained through a micro-lens array. This wavefront sensing approach already demonstrated its efficiency in Light Sheet Fluorescence Microscopy for neuroimaging in drosophila [2]. We show here that our Shack-Hartmann sensor based on extended scene wavefront measurement is also relevant to assess the optical properties of mouse brain tissues.

In particular, we present the following results obtained using fixed brain slices of the mouse cortex: (1) such a sensor is able to provide, in a single measurement, a characterization of both optical aberrations and the scattering length of the tissue; (2) it allows us to measure the aberrations introduced by the slices as a function of their thickness and of the wavelength; (3) by comparing our extended-scene wavefront measurement approach to the classical Shack-Hartmann method based on centroid calculation, we show the benefit of the former in the case of scattering samples. These measures lead us to targeting an optimal aberrations correction strategy for an adaptive optics two-photon excitation microscope to be built based on an extended source Shack-Hartmann sensor.

[1] Wang K, Sun W, Richie CT, Harvey BK, Betzig E, Ji N. Direct wavefront sensing for high-resolution in vivo imaging in scattering tissue. Nat. Commun. **6,** 7276 (2015)

[2] Hubert, A. et al. Adaptive optics light-sheet microscopy based on direct wavefront sensing without any guide star. Opt. Lett **44**(10) 2514-2517 (2019)

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