Fast Adaptive Optics in optically sectioned fluorescence microscopes for functional neuroimaging

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Abstract. We demonstrate how a novel approach for closed-loop Adaptive Optics (AO) specifically adapted to microscopy enables straightforward integration in Light-Sheet and Multiphoton microscopes, as well as fast aberration correction. We present corresponding experimental setups as well as first demonstrations of the benefits of the correction of sample-induced aberrations in zebrafish and mouse brain tissue, with the ultimate goal to enable high-speed, high-sensitivity functional imaging at large depths.

1. INTRODUCTION AND CONTEXT

Optical microscopy has emerged as a major tool in neuroscience as it allows to image the architecture of neuronal networks and to record their functional activity in several animal models as zebrafish, rodents, ferrets or primates through different methods including widefield fluorescence microscopy, confocal, light-sheet and non-linear scanning microscopy.

When targeting 3D imaging, modern optical sectioning methods such as confocal or light-sheet microscopy can image a large part of the entire brain of relatively transparent animal models such as zebrafish or C-Elegans. In mammalian brains, in-depth imaging can only be achieved using non-linear microscopy. But image quality deep inside biological tissues remains limited by scattering and optical aberrations due to the refractive index inhomogeneity, which limit both resolution and sensitivity in all microscopy modalities.

2. A NOVEL APPROACH FOR FAST AO IN FLUORESCENCE MICROSCOPY

To overcome this difficulty, in particular regarding aberrations, AO has been implemented on several linear and non-linear optical sectioning microscopy setups and currently provides a reliable live correction of the aberrations, enabling functional imaging of synaptic boutons, axons and spines and soma in infragranular layers of the mouse cortex in 2-photon microscopy, or enabling subcellular imaging of organelle dynamics in the early zebrafish brain in light-sheet microscopy. Significant gain in spatial resolution and signal intensity was demonstrated, but the two approaches based on a direct wavefront sensing or a sensorless process still suffer from several limitations, linked to the complex generation or the invasive introduction of a guide star inside the sample, or a time-consuming iterative approach to reach a good correction.

We proposed a few years ago an innovative strategy of adaptive optics for neuroimaging, by adapting pioneer work from astronomy to the constraints of fluorescence microscopy. By using an extended-source Shack-Hartmann wavefront sensor (ESSH), our approach allows to preserve speed and accuracy of the direct wavefront sensing method and takes advantage of existing labelling methods of biological samples [1]. It relies on the cross-correlation of images of an extended source obtained through a microlens array and requires to be coupled to an optical sectioning method in order to provide a guide plane: it is thus interestingly compatible with various fluorescence imaging techniques already implemented for 3D imaging such as two-photon microscopy (TPEF) [2] or light-sheet fluorescence microscopy (LSFM) [3].

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3 AO-ENHANCED LIGHT-SHEET MICROSCOPY IN THE ZEBRAFISH BRAIN

We performed ESSH-based AO integration in LSFM setups, in order to achieve a first quantitative demonstration of the performance of the new, fast closed-loop approach. We developed 2 versions of an AO-LSFM lab setup: 1. An AO-DSLM setup (DSLM for Digitally Scanned Light-sheet fluorescence microscopy), including AO at the emission path, in order to correct sample-induced aberrations, and 2. An AO-ASLM setup (ASLM for Axially-Swept Light-Sheet Microscopy), also with AO at emission, for even thinner light-sheet <1µm).Based on dual-color labelling of the sample, which is routinely used in biology to enable structural and functional imaging, we then demonstrated ESSH-based AO running at 10Hz with an optimal photon budget, enabling enhanced imaging of GCaMP7b labelled neurons in depth in the live adult drosophila brain using AO-DSLM [3]. Recently, we demonstrated that ESSH-based AO-ASLM maintains diffraction-limited 3D resolution deep (>300µm) into the Zebra Fish (ZF) brain, by correcting sample-induced aberrations, along with a x2 signal increase on large cells. Figure 1 (depths 160-300µm), shows that, even at large depths, AO maintains the capability to distinguish individual cells. This is key to many studies, since a major biomarker consists of quantifying the amount/density of cells (segmentation) in multiple fields (developmental biology, neuro, cardio).

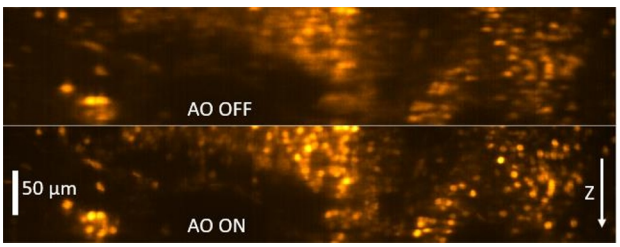

Fig. 1. LSFM images of the live ZebraFish brain, without (top) and with (bottom) AO. Depths 160-300µm.

4. AO-ENHANCED TWO-PHOTON MICROSCOPY OF THE MOUSE BRAIN

We performed ESSH-based AO integration in a 2-photon microscopy lab setup, in order to demonstrate fast closed-loop AO deep in scattering media, and in particular in brain tissue. We imaged fixed, cortical brain slices of GAD-GFP mice, with inhibitory neurons labelled. We demonstrated the capability of the device to correct aberrations up to 350µm deep in fixed tissue, with large gain in contrast and resolution, and with a closed-loop speed of 2Hz. Since scattering length measured in-vivo are 2 to 3 times higher than the ones realized on fixed tissues, we expect the method to provide a strong benefit from AO down to significant depths (≥ 700µm in the mouse brain). Figure2 is an XZ reslice of a 150x150x350µm stack, demonstrating that our AO approach enables to maintain resolution deep in brain tissue, while significantly improving signal (up to a factor of 5 for smallest structures, close to the diffraction limit).

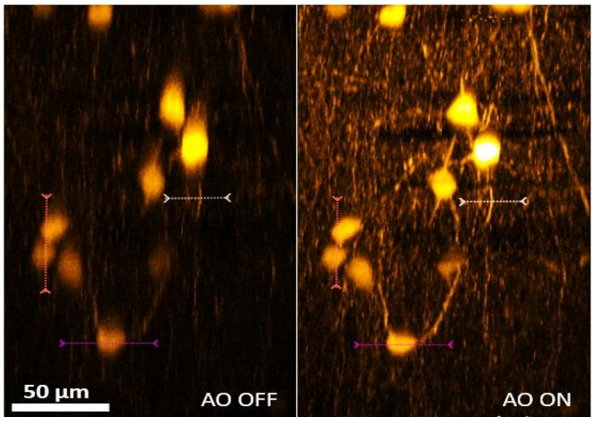

Fig. 2. Two-photon imaging of a fixed mouse brain slice, without (left) and with (right) AO. Images are XZ reslice of a volumetric stack.

References

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