ADAPTIVE OPTICS LIGHT-SHEET MICROSCOPY OF THE DROSOPHILA BRAIN USING DIRECT WAVEFRONT SENSING WITHOUT ANY GUIDE STAR

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When targeting neuroimaging of neuronal networks in live specimens with high temporal and spatial resolution, Light-Sheet Fluorescence Microscopy (LSFM) has demonstrated its capability to provide enhanced signal to noise ratio (SNR) while decreasing toxicity and photobleaching. However, imaging neuronal networks is still a challenge since fluorescence variations induced by neuronal activity is weak and imaging depth is still limited. Adaptive Optics (AO) has shown its ability to increase signal, resolution and imaging depth in LSFM, with best in-vivo performance achieved through direct wavefront (WF) sensing typically using a Hartmann-Shack WF sensor [1]. However, a couple of parameters, such as the availability of a guide star or limited scattering along the optical path, either drive optimal AO performance (e.g. regarding speed or gain in image quality) or impose complex AO implementation, including the use of fluorescent beads inside the sample [2].

We propose here a new WF sensing approach optimized for LSFM, based on the use of a Hartmann-Shack sensor specifically designed for extended scenes, providing $\lambda/50$ accuracy

Figure 1: Living HeLa cell labeled with MitoTrackerGreen (MTG), (left) without AO (right) with AO

without the need for a guide star. Such devices have been proposed for astronomy [3] and recently for LSFM [4], with no demonstration of closed-loop performance or image quality enhancement. Parameters driving the design and WF measurement accuracy will be presented and discussed. For the first time, we present closed-loop AO-LSFM imaging of living cells and drosophila brain, demonstrating significant SNR improvement with minimal instrumental complexity. We will also discuss the adaptability of the approach to various microscopy modalities, as well as strategies to enlarge the corrected field-of-view.
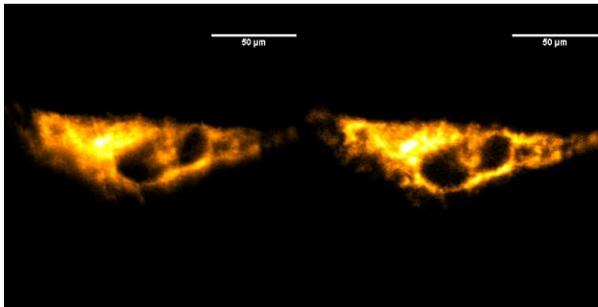
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