ADAPTIVE OPTICS MULTIPHOTON MICROSCOPY OF THE DROSOPHILA BRAIN

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Drosophila melanogaster (fruit fly) is a valuable animal model in developmental biology and genetic studies. Since its entire genome has already been sequenced, there has been an increasing interest in using this attractive model organism in different fields of biomedical research. In particular, the *Drosophila*'s brain is being used in the analysis of degenerative diseases (Alzheimer, Parkinson,…) [1]. Although the brain morphology is an invaluable research structure, it presents complex structures which are often hard to be imaged. Multiphoton (MP) microscopy provides inherent confocality and is able to visualize fine features within highly aberrated/scattering specimens without exogenous markers. However, MP signals (both two-photon excitation fluorescence (TPEF) and second harmonic generation (SHG)) are compromised at deeper layers. To overpass this, adaptive optics (AO) schemes are often used.

Here we use a custom AO MP microscope to improve the images acquired at different depth locations within the *Drosophila*'s brain. A spatial light modulator and a sensorless hill-climbing algorithm were used in the AO operation. For each individual layer within the brain the procedure looked for the appropriate spherical aberration value to get the optimum MP image based on a particular metric. The correction of other Zernike terms was not necessary since their influence was not dominant [3].

Only TPEF signal was provided by the specimens, what indicates the absence of collagen-based stuctures in the brain. This signal decreased up to 80% at deeper layers. The AO procedure was able to improve the quality of the TPEF images independently of the depth location of the imaged plane. Aberrations hardly affected shallower locations, but the amount of aberration required to obtain the optimum image increased with depth. Our results suggest that AO MP microscopy is a powerful tool to examine the *Drosophila*'s brain physiology and may be used in neuro-degenerative experiments that use this animal model.

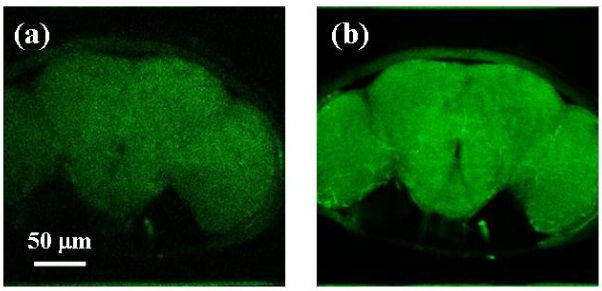

Figure 1: TPEF images of the *Drosophila*'s brain before (a) and after (b) aberration correction..

[1] Jeibmann & Paulus, *Int. J. Mol. Sci.* **10**, 407-440 (2009).
[2] Skorsetz, Artal & Bueno, *J. Microsc.* **261**, 249-358 (2016).
[3] Bueno *et al.*, *J. Biomed. Opt.* **19**, 011007 (2014)

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