AUROX LASER FREE CONFOCAL

Sensorless adaptive optics with a laser free spinning disk confocal microscope

Application News Report

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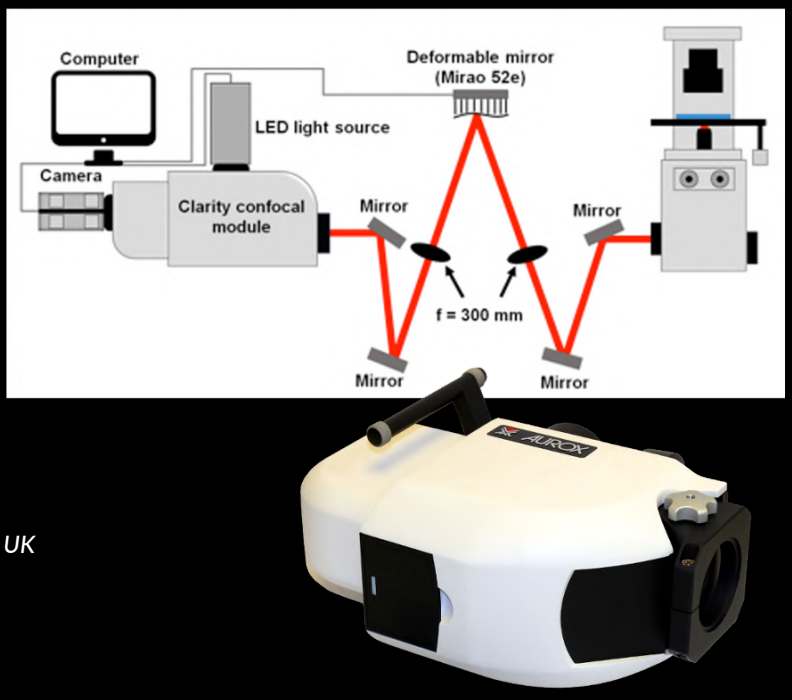


Figure 1. Experimental set-up used in the study. Clarity (inset).

Foreword

Herein we report on the combined work of teams from the Department of Engineering Science and the Micron Advanced Bio-Imaging Unit, University of Oxford and the Department of Applied Physics, Osaka University.[1]

This work was presented at Photonics West, 2019 and at the online Aurox Conference on Microscopy, April 2020. A video replay of the session and talk is available at: https://www.youtube.com/watch?v=0L7tDQ2vDsM .

Introduction

Difficulties arise when imaging at depth inside biological samples as differences in refractive indices and the existence of other biological structures give rise to unavoidable aberrations. Specimen induced aberrations can have detrimental effects in all types of high-resolution microscope. In this study, the authors recorded optically sectioned 3D images using a microscope fitted with the Aurox Clarity laser-free confocal system (Clarity LFC) in partnership with a sensorless adaptive optics (AO) technique that uses a deformable mirror (DM) to correct aberrations of both the system and sample to obtain improved quality images when focusing deep into thick specimens.

Experimental

In normal operation, the Clarity LFC module would be attached directly to the microscope body. For these experiments, the authors created an intermediate pupil plane to insert the DM (Imagine Optic, Mirao, 52e). The DM is sited in the middle of a telescope made from two f=300 mm achromatic doublet (Thorlabs, United States) lenses that provide a conjugate pupil plane for the DM. The optical arrangement of this system (Figure 1) was confirmed by using numerical simulation in Zemax (Zemax, United Kingdom). To test the setup, fluorescence images were acquired using *Drosophila melanogaster* with a 60x (NA=1.4) oil immersion microscope objective (Olympus, Japan). Broadband dielectric mirrors (Thorlabs) were used to steer the He beam between the Clarity laser free confocal module, DM and microscope side port.

Results

Figure 3 (overleaf) shows fluorescence images of *Drosophila melanogaster* larval neuromuscular junction (NMJ) - muscles 6&7, where the system has been used to correct sample-induced and system aberrations. Comparing the two pictures in this case, the picture with AO correction has better contrast and reveals fine details. Moreover, synapses (in red) that were barely visible in the uncorrected image become visible in the corrected image. Figures 3 and 4 show a marked increase in signal to noise ratio and image quality with the correction applied.

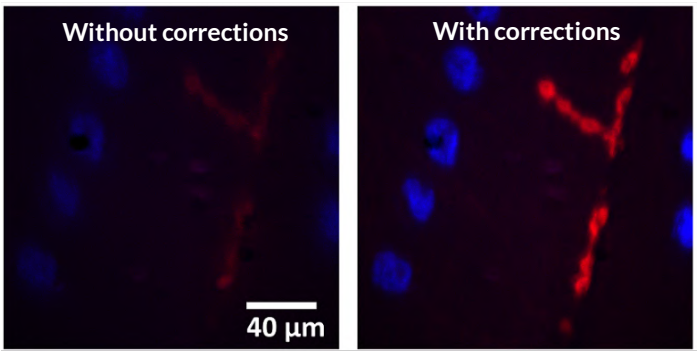
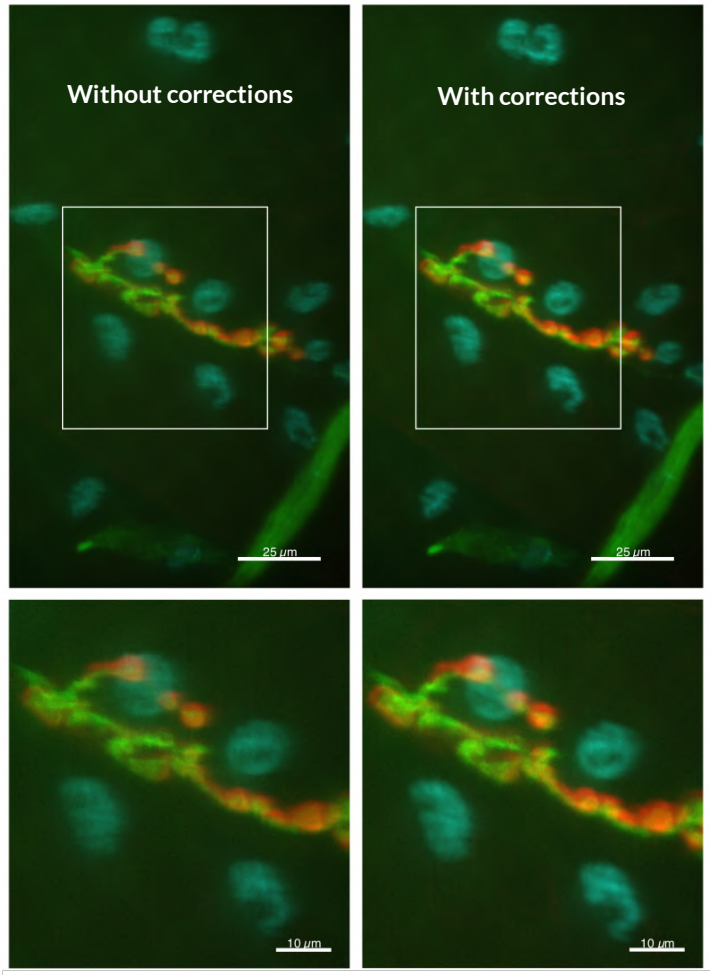
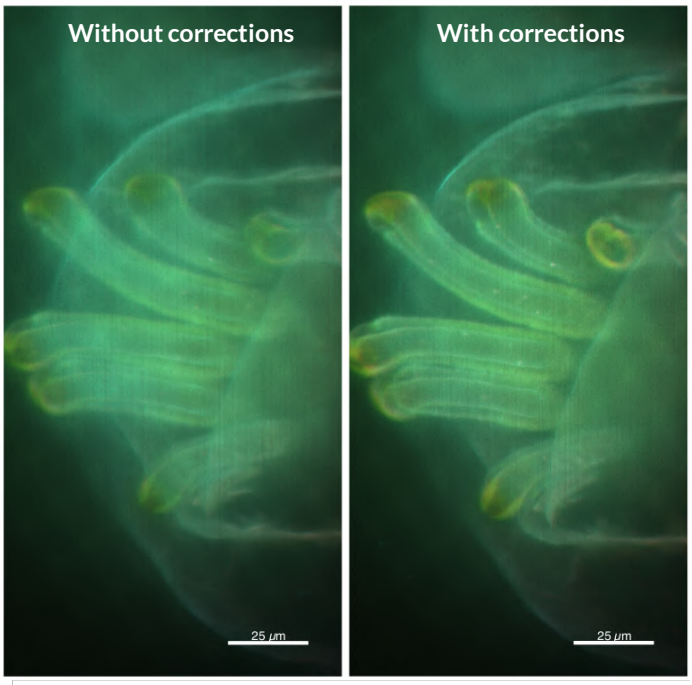


Figure 3. Fluorescent images obtained in the NMJ of Drosophila melanogaster larva. Nuclei (DAPI) are labelled in blue and the synapse (DLG, Alexa Fluor™) in red.



Drosophila NMJ without AO *Drosophila NMJ with AO*

Figure 4. Drosophila melanogaster larval NMJ – chevron muscle. Nuclei (DAPI) – Blue, Axon (DLG, Alexa Fluor 488) – Red and Post-Synaptic Density (FITC-Phalloidin, Alexa Fluor 568) – Green showing increase in signal to noise ratio and image quality.



Drosophila breathing tubes without AO *Drosophila breathing tubes with AO*

Figure 5. Drosophila melanogaster breathing tubes showing improvement in image quality and sharpness.

Conclusions

The compact size and laser free operation of the Clarity LFC make it an ideal sectioning tool for inclusion in complex experimental set-ups such as this.

The results obtained show that by combining the laser free confocal microscope with the adaptive optics the optical aberrations of biological samples used in the study could be corrected and important fine details of the specimens could be observed at depths that were not possible before. It is therefore expected that these systems will help researchers working on various biological systems to obtain improved quality images when focusing deep into thick specimens.

References

[1] *S. A. Hussain, T. Kubo, N. Hall, K. Hampson, M. A. Phillips, D. Gala, I. M. Dobbie, J. Antonello, M. Wincott, I. Davis, M. J. Booth, "Sensorless adaptive optics with a laser free spinning disk confocal microscope," Proc. SPIE 11248, Adaptive Optics and Wavefront Control for Biological Systems VI, 112480W (17 February 2020); https://doi.org/10.1117/12.2542959*