Effective and robust implementation strategies for adaptive optics in sectioning microscopy

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Purpose

Microscopy is an essential tool for research in cellular and molecular biology. The optical quality of the instruments as well as the properties of the sample, like diffusion, absorption and optical aberrations, tend to limit the quality of the image. This is an important constraint, especially in sectioning and super resolution techniques.

Adaptive Optics (AO), originally developed to compensate for atmospheric perturbation in astronomy application, is a technique based on an active element able to minimize refractive distortions induced while propagation of light in a media. Since then, it has been implemented in ophthalmology and microscopy, and it demonstrated important improvements in both image quality [1] and system efficiency.

We discuss about several AO strategies based on MicAO^{tm}, a plug and play AO solution [2] and the mirao^{tm} 52-e Deformable Mirror (DM). We present the implementation of these strategies in different sectioning techniques, the analysis of their benefits and drawbacks and some of the obtained results.

Methods

The implementation of AO can be divided in three groups: i.- optimization of the excitation path (for non-linear techniques such as Two-Photon Excitation Fluorescence (TPEF), second or third harmonic generation microscopy), ii.- optimization of emission path (for wide field or standard epi-fluorescence, PALM/STORM microscopy) and iii.- optimization of both paths (for confocal, spinning disk, Structured Illumination Microscopy), see Fig. 1.

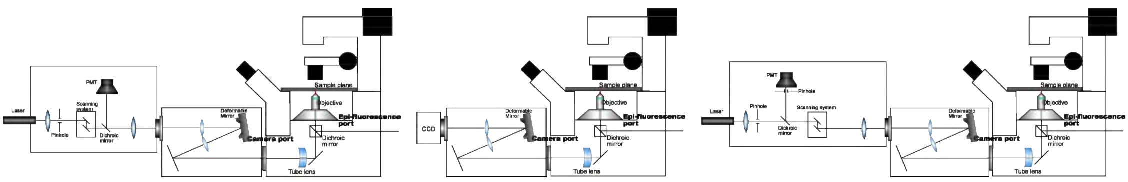

Fig. 1. Adaptive Optics hardware implementation. AO compensating the excitation light path (left). AO compensating the emission light path (middle). AO compensating both light paths (right).

Moreover, there are also several correction strategies to control the active element of an AO solution, a DM in our case, like: i.- a closed-loop configuration that uses a wavefront sensor to measure optical aberrations and shape the DM [3,4], ii.- iterative algorithms based on trial and error to converge to a best optimization within user-defined criteria [5] and iii.- a mathematical model that compensates wavefront distortions based on sample parameters such as index refraction and depth of penetration.

We developed specific designs for the implementation configurations and tested it with the different correction strategies on both artificial and biological samples.

Results

We present the results obtained with the three correction strategies. In epi-fluorescence microscopy, we demonstrated that we can compensate the decrease of intensity due to depth penetration and recover, at 120um depth, up to approximately 50% of its maximum value. We were also able to precompensate for the spherical aberration corresponding to 60um of glass, as shown in Fig. 2. We obtained important improvement of TPEF signal using genetic algorithm on brain tissue sample.

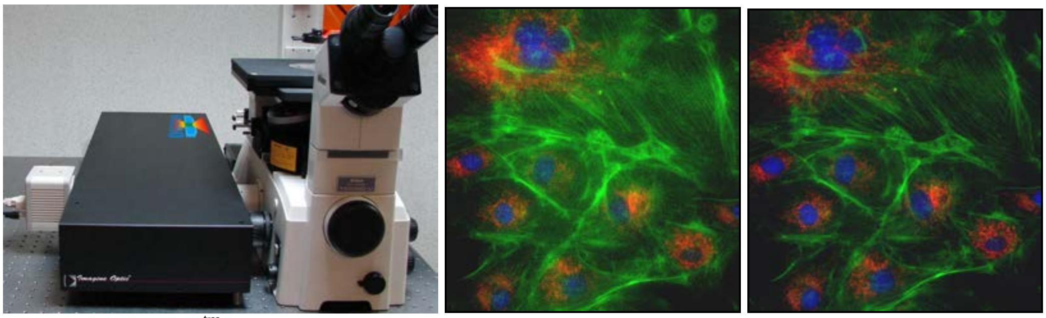

Fig. 2. MicAO^{tm} installed in the emission path of a microscope (left) and aberration correction of BPAE cell sample, imaged with 40x 0,95NA, at 60um depth with air glass index refraction mismatch. Before optimization (middle), after optimization (right).

Conclusions

Adaptive Optics is a powerful technique that enhances the capabilities of sectioning optical microscopes to a great extend. Users have to carefully select the implementation strategy that best suits their microscopy technique and samples to get the best out of it. We presented several alternative strategies that have their own characteristics and can be used in individual cases. This allows microscopists to choose among them to optimize their images in an effective way.

References

1. M. J. Booth, M. A. A. Neil, R. Juškaitis and T. Wilson, “Adaptive aberration correction in a confocal microscope” , Proc. Natl. Acad. Sci. 99 (9), 5788-5792 (2002)

2. J. Andilla and X. Levecq, “MICAO: first universal all-in-the-box adaptive optics plug in accessory for standard high resolution microscopy”, Proc. SPIE 7568, 75680 (2010)

3. M. Zacharria, B. Lamory and N. Chateau, “Biomedical imaging: New view of the eye”, Nat. Photonics, 5, 24–26 (2011)

4. O. Azucena, J. Crest, J. Cao, W. Sullivan, P. Kner, D. Gavel, D. Dillon, S. Olivier and J. Kubby, “Wavefront aberration measurements and corrections through thick tissue using fluorescent microsphere reference beacons”, Opt. Express 18, 17521-17532 (2010)

5. N. Olivier, D. Débarre and E. Beaurepaire, “Dynamic aberration correction for multiharmonic microscopy”, Opt. Lett. 34, 3145-3147 (2009)

6. D. Débarre, M.J. Booth and T. Wilson, “Image based adaptive optics through optimisation of low spatial frequencies”, Opt. Express 15, 8176-8190 (2007)

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