

Modulating phase for adaptive optics and PSF shaping in bio-imaging: requirements & development of a new deformable mirror tailored to microscopy

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Abstract. Modern bio-imaging techniques such as light-sheet, multiphoton and PALM/STORM are now aiming to image more complex biological samples at larger depth and therefore face larger-amplitude and more complex aberrations. We provide an analysis of key requirements driving optimal implementation of adaptive optics (AO) in microscopy, with a focus on wavefront modulators. We show that some specifications of wavefront modulators such as linearity, hysteresis or actuators performance & layout can end up to better AO performance in microscopy systems, when specifically optimized for such use. We then provide design details and characterization results of a newly developed deformable mirror, and report on experimental images obtained from AO-enhanced microscopes based on the device, for several modalities such as light-sheet, multiphoton or super-resolution single molecule localization systems. Finally, we provide recommendations on how to define the right set of AO components, algorithms and overall method depending on modality, instrument and sample constraints.

1 INTRODUCTION

1.1 AO in microscopy

Modern 3D microscopy techniques such as e.g. light-sheet, multiphoton, or super-resolution PALM/STORM are continuously aiming at deeper imaging with preserved high resolution and image contrast. Among phenomena limiting imaging performance, in particular in depth, optical aberrations induced by the intrinsic inhomogeneity of biological samples play a significant role. Over the last decade, many instrumental implementations of Adaptive Optics (AO) have been reported for most microscopy techniques [1-3], demonstrating a significant improvement of image quality. Basically, by compensating for optical aberrations, AO enables to redirect photons at the right place, i.e. in a diffraction-limited Point Spread Function (PSF) for each point of an image, thus increases image contrast which directly benefits to achievable imaging depth and/or resolution.

1.2 Enabling AO correction in microscopy: requirements and methods

In microscopy, two main methods are currently used to drive AO, depending on how the wavefront is estimated: first, closed-looped AO uses direct wavefront sensing, requiring a wavefront sensor, to drive a feedback loop, and second, sensorless AO uses indirect wavefront sensing, usually based on a merit factor on the image, to

drive iterative algorithms aiming at minimizing such merit factor. On top of these approaches achieving aberration correction, it is often necessary to add a pre-defined amount of a specific aberration, e.g. in order to introduce defocus (for fast 3D imaging) or create a particular PSF shape (for 3D localization based on axially non-symmetric shapes), which is usually achieved without the use of a wavefront sensor. Due to the spatio-temporal characteristics of aberrations encountered in biological samples, and considering the previously described AO methods, the requirements for optimal AO implementation in microscopy strongly differ from its historical implementation in astronomy. For example, when compared to astronomy, aberrations vary slowly, show a larger isoplanetic patch, are measured and corrected on a non-truncated circular pupil and mostly show low-order spatial distribution. For all AO methods, a common, key component is the wavefront modulator, allowing for phase manipulation. Multiple wavefront modulation technologies are currently available, including mostly Deformable Mirrors (DM), Adaptive Lenses (AL), and Spatial Light Modulators (SLMs). An ideal wavefront modulator for microscopy should provide optimal specifications regarding the previously described AO methods and characteristics of aberrations in tissue. Also, other requirements, such as pupil size, temporal stability, footprint & integration constraints, as well as price, are likely to provide better performance and wider adoption of AO in the field.

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2. REQUIREMENTS, DEVELOPMENT AND ACHIEVED PERFORMANCE OF A NEW DM FOR BIO-IMAGING

Here, we first provide an analysis of the capabilities and limitations of each of the various AO methods used in microscopy, and provide recommendations for proper implementation, in particular regarding the required characteristics of a wavefront modulator or sensor, such as e.g. wavefront sampling, speed, photon budget, dynamic range, linearity.

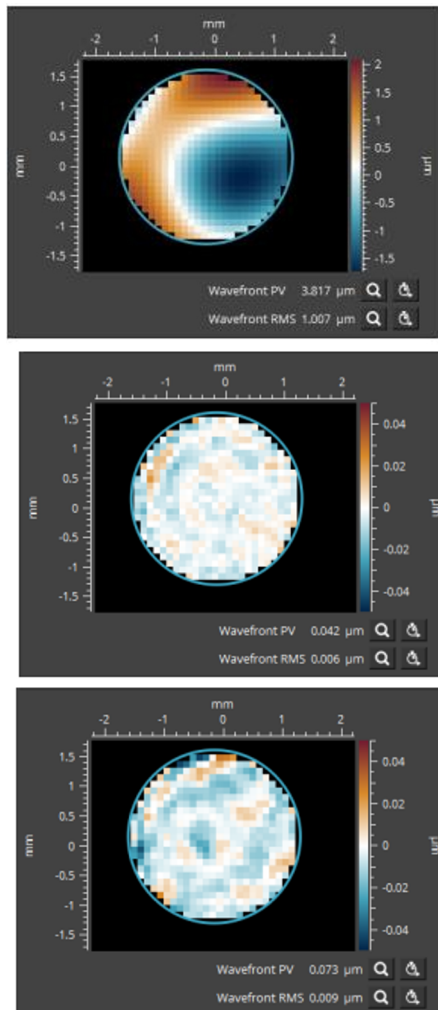


Fig. 1. wavefront measurements illustrating the linearity of the developed DM. In this experiment, an AO setup, including a Shack-Hartmann wavefront sensor, was used to achieve a specific target wavefront. Top: example of a target wavefront to be achieved by the DM (1.007 μm RMS of a combination of Zernike polynomials up to 4th order). Middle: obtained wavefront error after correction achieved in closed-loop by the DM (6 nm RMS). Bottom: obtained wavefront error after correction achieved in open-loop with the DM (9nm RMS).

Then, we report on our achieved development of a new DM optimized for use in microscopy, in an attempt to provide a wavefront modulator optimally fulfilling most of the previous requirements. We show that the design of current wavefront modulators inherits from historical developments in astronomy and from technology

constraints, that often limit optimal use in microscopy. We then describe technical details of the DM related to actuators technology & layout, system design, characterization methods. In particular we focus on some key design parameters and performance of the device that better fit to microscopy requirements, such as linearity, temporal stability, actuators layout, and system integration. We demonstrate that linearity and hysteresis are critical, and most of the time even more important than speed, either for open- or closed-loop AO. We describe experimental results obtained from the characterization of key specifications, such as for example linearity, as illustrated by Figure 1.

3 AO-ENHANCED IMAGING OF BIOLOGICAL MEDIA

Finally, we show examples of achieved AO-enhanced images with the use of such DMs in several microscopy modalities such as Light-Sheet, multiphoton and SMLM.

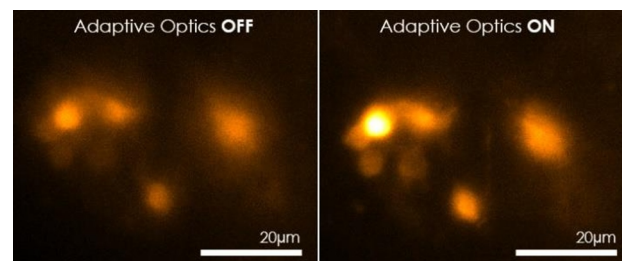


Fig. 2. Enhanced functional imaging of GCaMP7 labelled neurons of the circadian clock network in the live adult drosophila brain ($\sim 50\mu\text{m}$ depth), without (left) and with (right) AO.

References

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