Refractive Adaptive Optics for Microscopy

Adaptive optics (AO) refers to a powerful range of image correction techniques with proven benefits for a large range of life-science microscopy methods. However, the additional complexity and cost of conventional AO systems has prohibited widespread adaptation of AO in microscopy so far. Phaseform develops refractive, completely in-line AO systems that can reduce the complexity and cost of many setups.

In this application note, we explore how our refractive AO concept could empower microscopy, and showcase this with a wide-field AO fluorescence microscope that uses our Deformable Phase Plate (DPP) technology.

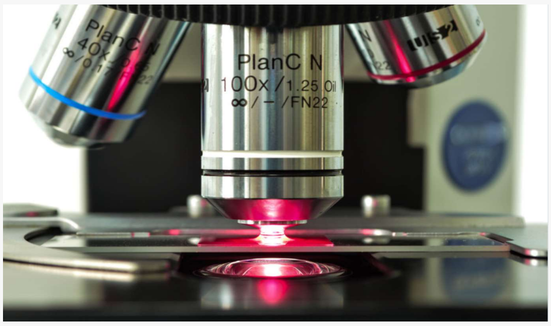

Figure 1: Oil / Water immersion microscopy setup.

Adaptive optics in microscopy research

It is often said that optical imaging systems, from microscopes to cameras and telescopes, are only as good as their optics. This may be true, but the images are also only as good as the intervening medium allows.

Therefore in many cases, the performance of modern microscopes suffers from two major sources of aberrations: refractive index mismatch in the layers between the sample and the objective resulting in spherical aberrations, and variations in the shape and/or refractive index of the samples leading to specimen-dependent complex aberrations. Such challenges are more severe in single molecule and deep tissue imaging. If left uncorrected, they prevent microscopes from reaching their theoretical resolution; reducing the contrast and sharpness of the acquired images [1-2].

Extensive research in use of adaptive optics in microscopy over the last two decades has proven its potency. AO can compensate for aberrations and restore the native performance of a microscope independent of the type of sample and sample holder thereby helping to relax the index matching criteria and cutting down sample preparation time. AO is effective in virtually all advanced microscopy techniques: confocal, wide-field, multi-photon and even super-resolution methods such as STED, SMS, STORM. Particularly for deep tissue microscopy (a key tool that uniquely allows inspection of cells in their natural environment) AO makes it possible to retain best resolution well below the sample surface.

The path to commercialization

Unlike in professional astronomy, where AO sub-systems are ubiquitous, uptake of the technology in microscopes has been slow. This is primarily influenced by the lower cost ceiling of microscopes and the fact that microscopes are generally physically much smaller and composed of lenses that work in refraction.

Nevertheless, state-of-the-art research by the microscopy community has resulted in the first commercial AO solutions for microscopy. They come as attachments to the microscope's extension ports. Inside, they perform wavefront measurement and correction by relaying the pupil-plane to their deformable mirrors (DM), and after correction, redirect the light back to the microscope's detection/imaging path.

These first-generation products however need careful set up, are not universally compatible, and are still relatively bulky.

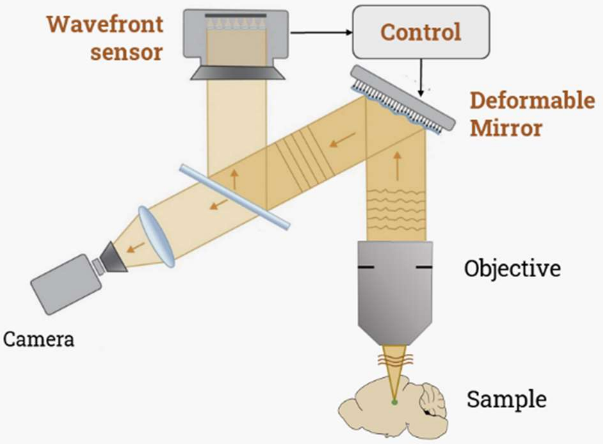

Figure 2: Conventional adaptive optics microscopes use deformable mirror imposes the folding of the optical path.

Phaseform's vision for fully refractive AO microscopy

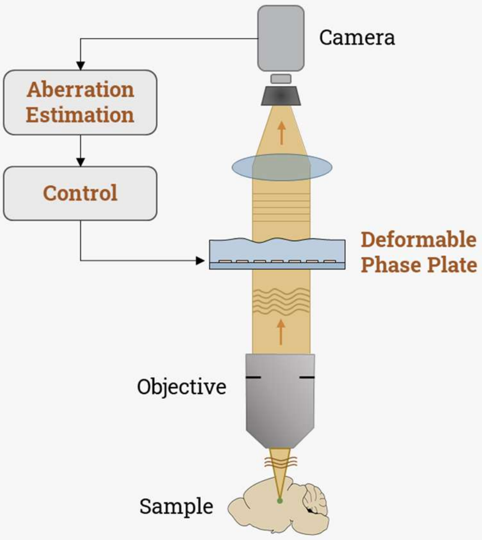

Figure 3: Phaseform develops completely in-line adaptive optics systems for direct integration

Phaseform's vision is to make adaptive optics technology accessible to most microscopy users. To provide integrated, more compact AO solutions with uncompromised performance, we believe, a technological shift is necessary - from reflective to refractive wavefront modulation. We therefore present a novel completely in-line AO system (Figure 3) tailored for microscopy.

This is enabled by modifying the classical AO systems as follows: (A) the deformable mirrors are replaced with a refractive element and (B) the wavefront sensors are omitted in favor of an aberration estimation algorithm.

A. Refractive DPPs - A key enabling technology for AO microscopy

The Phaseform Deformable Phase Plate, shown in Figure 4, is a novel type of dynamic optical component. Its name comes from the conventional phase plate - a thin slab of transparent material with a surface relief used to compensate for fixed aberrations in advanced microscopy applications. Unlike those, however, the surface of the DPP can be shaped dynamically into any arbitrary form by an array of actuators spanning the clear aperture [3]. Therefore, it is a refractive alternative to deformable mirrors.

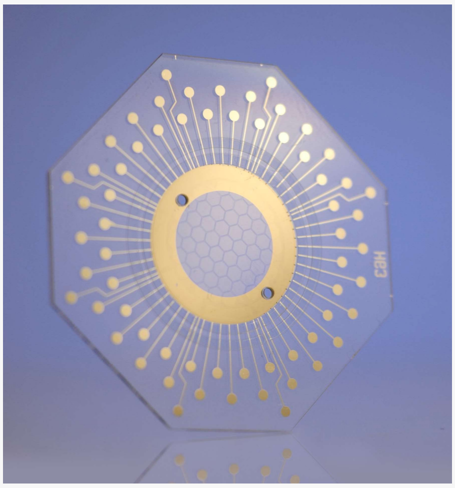

Figure 4: Refractive 63-actuator Deformable Phase Plate, able to correct for 7th radial Zernike order.

Key benefits of the DPP for microscopy are:

- **Transmissive**: Can be slotted into any optical beam path without the requirements for re-calculation, re-imaging, or folded optical beam paths.
- **Compact**: As an ultra-thin transmissive element, the DPP already provides space savings from a system point-of-view, but its small footprint makes it particularly suitable for integration and it can even be stacked multiple times in a row.
- **Efficient**: Its operation is polarization independent and shows limited diffractive losses.
- **Versatile**: Automatically correcting 1st and 2nd order spherical aberrations and astigmatism is particularly useful for microscopy applications. More complex aberrations, such as those resulting from regions of different index of refraction in deep-tissue imaging, however, need higher-order correction, which the DPP can provide, similar to a DM.
- **Dynamic**: the DPP can be controlled and operated in real-time in high resolution imaging and microscopy settings.

B. Sensorless Wavefront Estimation

Foreknowledge of the optical aberration is essential for its correction. A wavefront sensor, such as a Shack-Hartmann sensor or interferometer, is generally used in classical AO systems to measure the dynamic aberrations. However, using a wavefront sensor on the one hand adds to the complexity and cost of AO systems, and on the other hand is not always a practical solution especially for microscopy applications. The latter may be simply because of the constraints of the microscopy setup, or even due to the nature of the specimen under study.

Sensorless wavefront estimation (SWE) is an alternative that can substitute a wavefront sensor in AO microscopy, where aberration dynamics are slow and relatively small. The two general requirements for the SWE methods are:

1. An understanding of the sample or imaging target that allows for the creation of an effective figure of merit, such as image sharpness or contrast. This is required for the actual aberration estimation based on quantitatively optimizing a series of captured images for different arrangements of the wavefront modulator.
2. A predictable and robust control scheme for the employed wavefront modulator. The wavefront modulator needs to put in several precise relative configurations to effectively implement any of the SWE methods. The electrostatic actuation principle and the intuitive control algorithm of the Phaseform DPP is especially suited for such tasks [4-5].

Although SWE methods come at the cost of increased computational load and image acquisition time, they drastically reduce the hardware complexities of the AO systems (as we will show in our Case Study). The benefits of different variants of such methods have been demonstrated for many high-end microscopy modalities such as confocal, two-photon fluorescence, structured-illumination, lightsheet, STED and SMS [1-2].

A Case Study: World's Smallest AO Microscope

To showcase Phaseform's vision of "Accessible AO" we constructed a fully refractive, wide-field fluorescence microscope with an AO system based on the DPP and a modal SWE algorithm [5-6].

The microscope, shown in Figure 5, consists of an infinity-corrected 50x objective lens, relay optics to translate the pupil plane from within the objective barrel onto the DPP, and a tube lens to image the specimen onto a camera.

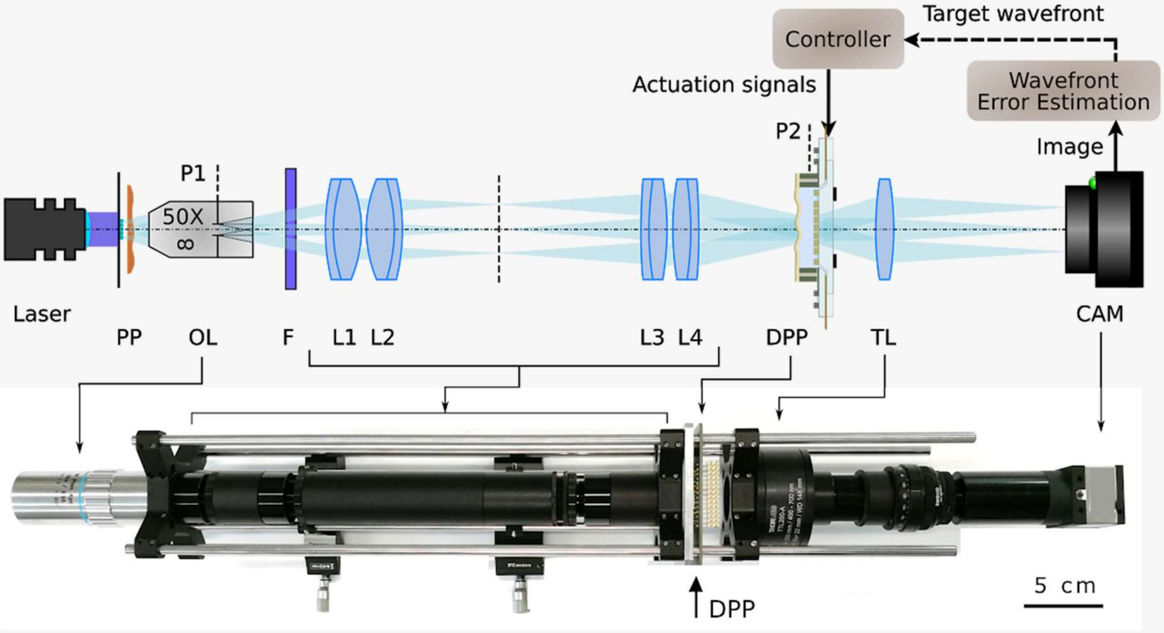

Figure 5: "World's Smallest AO Microscope", made possible by the use of SWE and our DPP as a refractive in-line system.

This microscope can be assembled in relatively short time with little optical alignment and calibration. Furthermore, the system delivers the targeted performance shown with synthesized and biological samples such as 1 µm-diameter fluorescent beads (left), cheek cells (middle) and standard 1951 USAF targets (right).

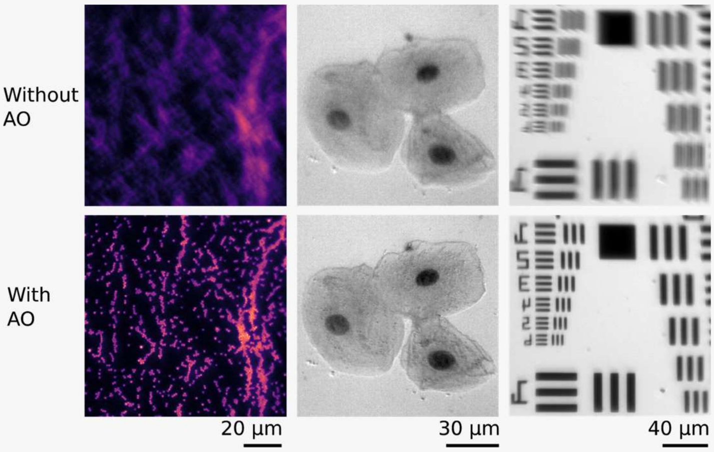

Figure 6: Images of various samples acquired with the AO-microscope with and without AO

Conclusion

We at Phaseform believe that the latest technological advances in refractive wavefront modulators and aberration estimation algorithms will revolutionize adaptive optics microscopy. We envision a future where, just as it happened in astronomy, adaptive optics will become the default in every self-built and every commercial microscope. This future may be closer than we think.

About the company

Phaseform GmbH is a deep-tech spin-off from the Department of Microsystems Engineering (IMTEK) of the University of Freiburg in Germany.

Our goal is to make Adaptive Optics affordable and practical, and to bring decades long cutting-edge research into fruition with innovative products and technologies. Phaseform aspires to become a leading adaptive optics technology company with a clear vision of continuous innovation in a “New Era of Adaptive Optics”.

Phaseform GmbH
Georges-Köhler-Allee 102
79110 Freiburg i.B., Germany

Email: info@phaseform.com
Web: www.phaseform.com
Phone: +49 761 6007 9018

References

1. M. Booth, "Adaptive optical microscopy: the ongoing quest for a perfect image". Light: Science & Applications 3. 4(2014): e165. doi: 10.1038/lsa.2014.46
2. Ji, Na. "Adaptive optical fluorescence microscopy." Nature methods 14.4 (2017): 374-380. doi: 10.1038/nmeth.4218
3. K. Banerjee, et al. "Optofluidic adaptive optics". Applied Optics 57. 22(2018): 6338 – 6344. doi: 10.1364/AO.57.006338
4. P. Rajaeipour, et al. "Optimization-based real-time open-loop control of an optofluidic refractive phase modulator". Applied Optics 58. 4(2019): 1064–1069. doi: 10.1364/AO.58.001064
5. P. Rajaeipour, et al. "Fully refractive adaptive optics fluorescence microscope using an optofluidic wavefront modulator". Optics Express 28. 7(2020): 9944-9956. doi: 10.1364/OE.387734
6. P. Rajaeipour, et al. "Extended field-of-view adaptive optics in microscopy via numerical field segmentation". Applied Optics 59. 12(2020): 3784–8. doi: 10.1364/AO.388000
7. P. Rajaeipour, et al. "Cascading optofluidic phase modulators for performance enhancement in refractive adaptive optics". Advanced Photonics 2. 6(2020): 066005-1-10. doi: 10.1117/1.AP.2.6.066005
8. N. Stockton, “From Cosmology to Biology”. SPIE News, 01 April 2019. tinyurl.com/dtaccya3