

# 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.*



Figure 1: Oil / Water immersion microscopy setup.

*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.*

## 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.

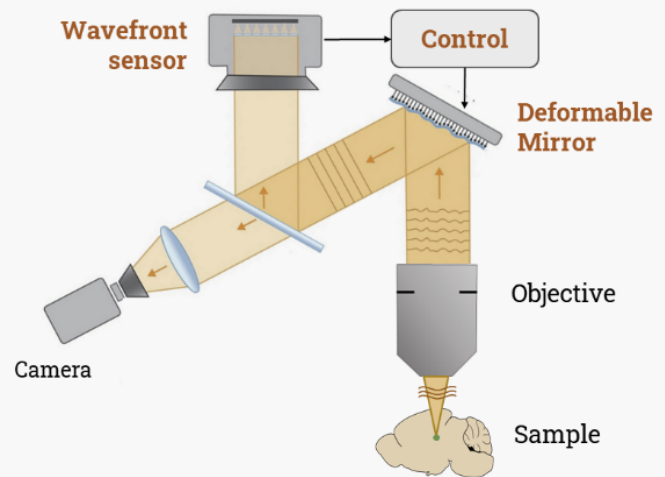


Figure 2: Conventional adaptive optics microscopes use deformable mirrors to impose the folding of the optical path.

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

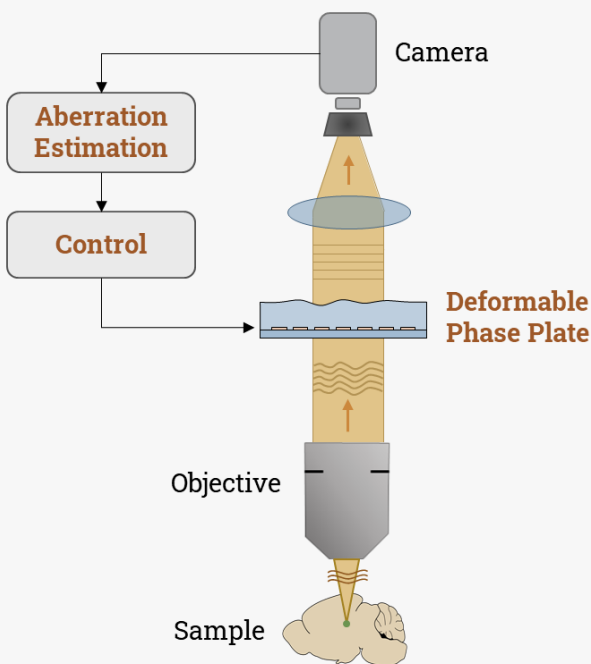


Figure 3: Phaseform develops completely in-line adaptive optics systems for direct integration

## Phaseform's vision for fully refractive AO microscopy

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.

## 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.

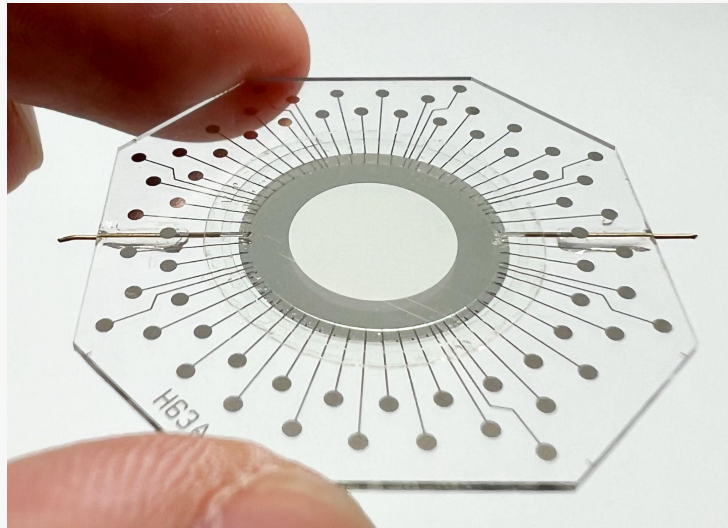


Figure 4: Refractive 63-actuator Deformable Phase Plate, able to correct for 7<sup>th</sup> radial Zernike order.

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 1<sup>st</sup> and 2<sup>nd</sup> 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.

## 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: Plug-and-Play Adaptive optics for Microscopy

Transmissive working principle of DPP combined with SWE make plug-and-play AO systems possible. Similar to a lens mounted in a conventional cage, DPP can be inserted into the optical path of microscopes with minimum to zero interruptions to provide dynamic correction of the system and sample-induced aberrations. Figure 5 depicts 4 examples where a DPP in the form of Phaseform's Delta 7 is integrated into commercial (Figure 5a and 5b) and custom-built microscopes (Figure 5c and 5d). Examples include fluorescence wide-field and high-end two-photon microscopes for imaging deep into the biological samples. SWE and the transmissive nature of the DPP enable an easy integration as a refractive in-line system.

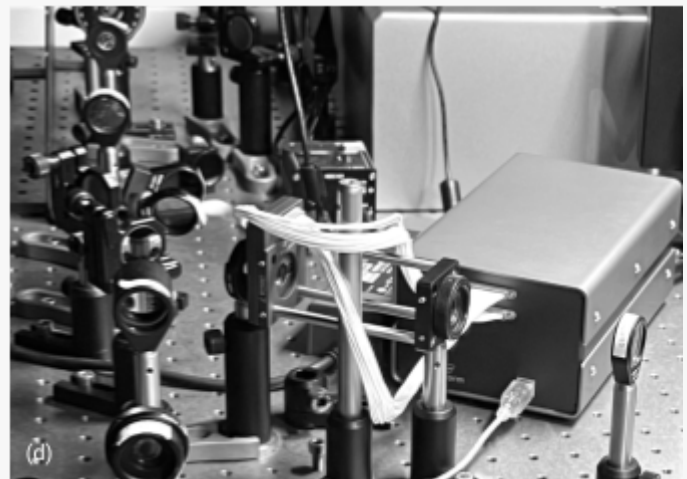
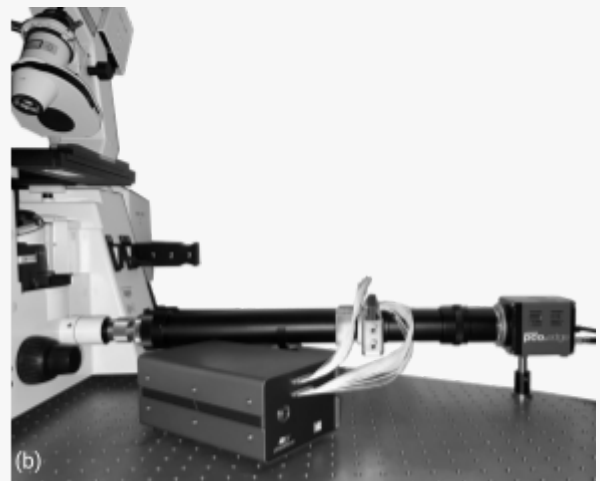
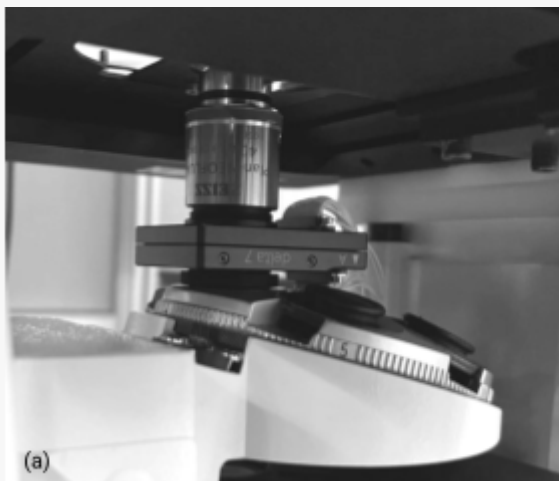


Figure 5: Examples of DPP-based AO systems in commercial and custom-built microscopes. (a) DPP integrated between the objective and turret of a commercial microscope. (b) DPP integrated at the conjugate pupil plane of a commercial microscope using a relay optic attached to the camera port. (c) 'World's smallest AO microscope' for fluorescence wide-field imaging [5]. (d) DPP integrated to the illumination path of a custom-built two photon microscope (in collaboration with the group of Prof. Alexander Jesacher, Medical University of Innsbruck)[10].

The benefit of AO in microscopy is shown in Figure 6. It depicts example results for two-photon imaging deep into the samples and wavefront sensorless compensation of system and sample induced aberrations. Column (a) on the left shows results of imaging more than 150  $\mu\text{m}$  deep into a mouse brain slice for neuronal imaging without and with AO correction (in collaboration with the group of Prof. Alexander Jesacher at the Medical University of Innsbruck). Column (b) shows results for imaging 40  $\mu\text{m}$  deep into a spheroid sample. This experiment was done by installing a DPP in a two-photon microscope (MPX-1040) in collaboration with Prospective Instruments (Dr. Stefanie Kiderlen and Dr. Lukas Krainer).

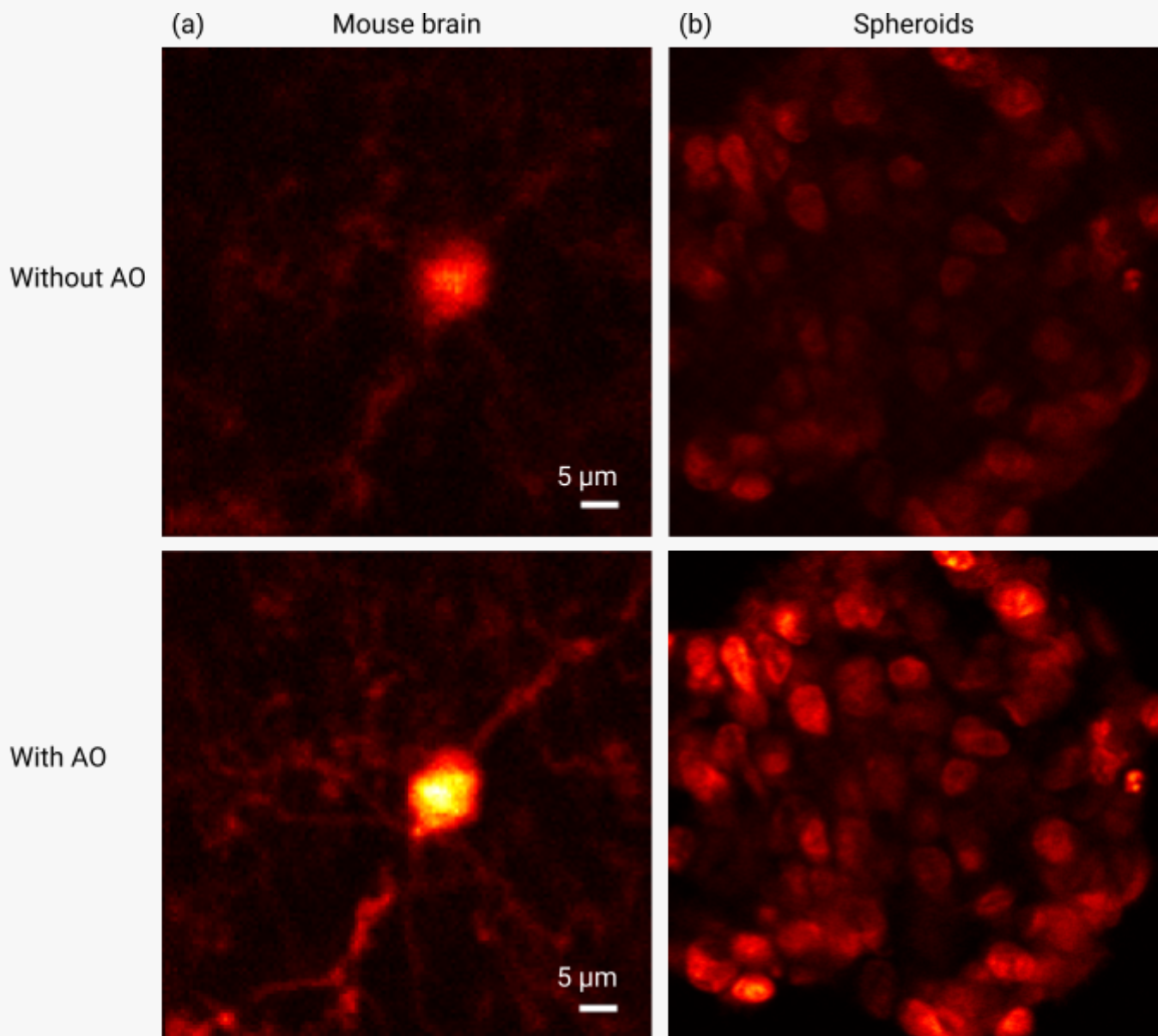


Figure 6: Images of various samples acquired with (a) custom-built and (b) commercial (MPX-1040, Prospective Instruments) two-photon microscopes with and without wavefront sensorless AO correction using DPP.

## Conclusion

Phaseform believes 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”.

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