Refractive Adaptive Optics for Microscopy

Adaptive optics (AO) refers to a powerful range of image-correction techniques with proven benefits for a wide variety of life-science microscopy methods. However, the additional complexity and cost of conventional AO systems have so far limited their widespread adoption in microscopy. Phaseform develops refractive, fully in-line AO systems that significantly simplify integration into diverse setups.

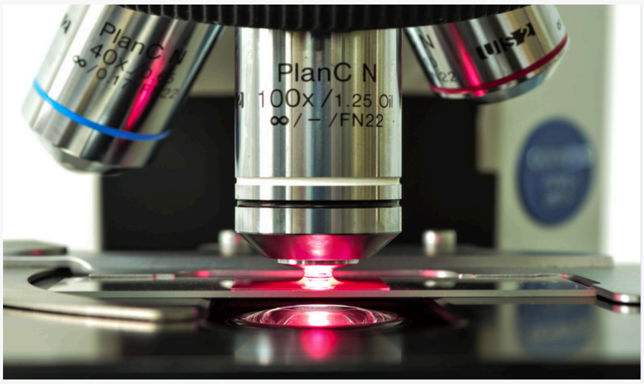

Figure 1: Oil / Water immersion microscopy setup.

In this application note, we explore how our refractive AO concept can benefit microscopy, along with several practical implementations that use 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. Although this is true, the imaging medium itself also matters. In many modern microscopes, performance suffers from two main sources of aberration: 1) Refractive index mismatch among the layers between the sample and the objective, resulting in spherical aberrations 2) Variations in specimen shape/refractive index, leading to complex, sample-dependent aberrations. These issues are especially severe in single-molecule and deep-tissue imaging. If left uncorrected, they prevent microscopes from achieving their theoretical resolution, reducing both contrast and sharpness in acquired images [1,2].

Over the past two decades, extensive research has shown that adaptive optics can compensate for these aberrations and restore the native microscope performance, regardless of the sample type or holder. By easing matching criteria for refractive indices and reducing sample preparation times, AO has become integral in advanced microscopy methods such as confocal, wide-field, multi-photon, STED, SMS, and STORM. Notably for deep-tissue imaging (which uniquely allows observation of living cells in their native environment), AO makes it possible to reach the best resolution well below the sample surface.

The path to commercialization

Unlike professional astronomy—where AO subsystems are ubiquitous—the uptake of this technology in microscopy has been relatively slow. Microscopes are typically designed for refraction and use lens-based optics in a form factor with tight cost and space constraints. Nonetheless, recent advances by the microscopy community have led to the first commercially available AO solutions for microscopy. These typically attach to a microscope's extension ports, relay the pupil plane to a deformable mirror (DM) for wavefront measurement and correction, and then redirect light back into the detection/imaging path (Figure 2).

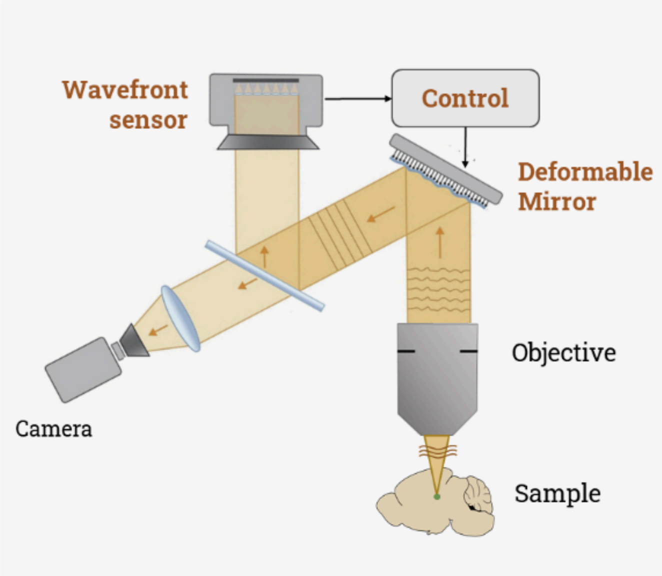

Figure 2: Conventional adaptive optics microscopes use deformable mirrors to impose the folding of the optical path.

Although these first-generation AO products offer significant benefits, they often require careful setup, are not universally compatible across microscope models, and tend to be relatively bulky.

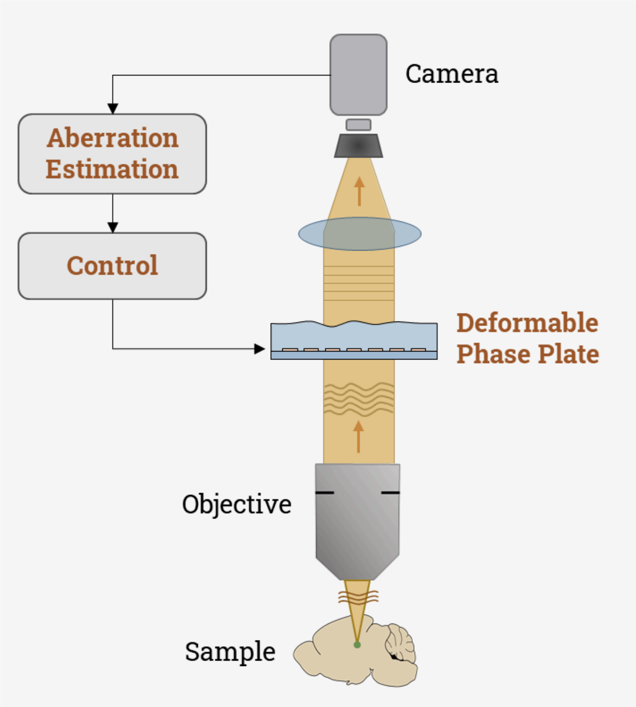

Figure 3: Phaseform develops completely in-line adaptive optics systems for direct integration

Phaseform's vision for fully refractive AO microscopy

Phaseform's goal is to make adaptive optics technology accessible to most microscopy users. To provide more compact, fully integrated AO solutions without compromising performance, we believe a technological shift from reflective to refractive wavefront modulation is necessary.

Our novel in-line AO system for microscopy (Figure 3) replaces the deformable mirror with a refractive element and eliminates wavefront sensors in favor of an aberration estimation algorithm. This approach drastically simplifies both hardware and alignment, making AO far easier to integrate into existing microscopes.

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. The term “phase plate” traditionally refers to a thin slab of transparent material with a fixed surface relief for compensating specific aberrations in advanced microscopy. By contrast, the DPP’s surface can be dynamically shaped into arbitrary forms via an array of actuators spanning the clear aperture [6]. Thus, it is a refractive alternative to deformable mirrors.

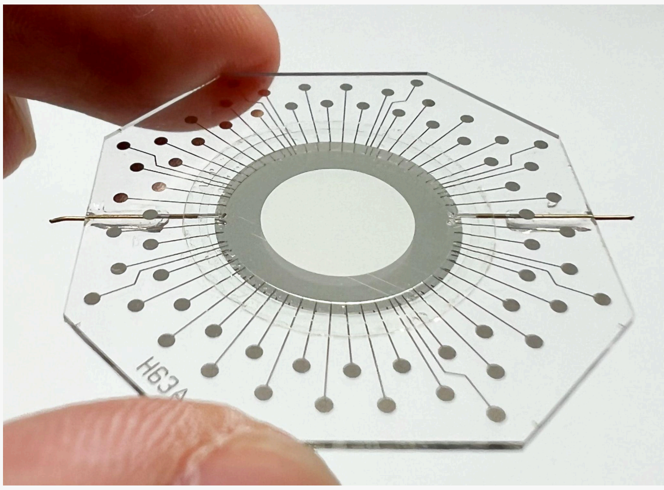

Figure 4: Refractive 63-actuator Deformable Phase Plate, able to correct for 7th radial order of Zernike modes.

Key benefits of the DPP for microscopy:

- **Transmissive:** Can be placed into virtually any optical beam path without requiring beam folding, re-imaging, or complex optical recalculations.
- **Compact:** As an ultrathin transmissive element, the DPP saves space from a system standpoint; its small footprint also makes it easy to integrate or to stack multiple units.
- **Efficient:** Operates in a polarization-independent mode and shows minimal diffractive losses.
- **Versatile:** Corrects a broad range of aberrations (e.g., spherical, astigmatism, coma). For deep-tissue imaging with significant index mismatches, it can provide higher-order corrections comparable to those of a deformable mirror.
- **Dynamic:** Supports real-time control and operation in high-resolution microscopy settings.

Sensorless Wavefront Estimation

Typically, foreknowledge of the optical aberration is necessary for correction. Classical AO systems use a Shack-Hartmann wavefront sensor or interferometer to measure dynamic aberrations. However, wavefront sensors add complexity and cost—often prohibitive in microscopy—and can be unwieldy if the microscope setup or specimen type is not conducive to direct wavefront measurement.

Sensorless wavefront estimation (SWE) offers an alternative by substituting the wavefront sensor with algorithms that optimize an “image quality” metric. In AO microscopy, where aberration changes are relatively slow or small, SWE methods merely require:

1) A suitable figure of merit, such as image sharpness or contrast, for quantifying aberration.
2) A predictable and robust control scheme for the wavefront modulator. The modulator must accept multiple precisely defined configurations, while the algorithm measures image quality in each configuration to converge on an optimal correction.

The electrostatic actuation principle of Phaseform’s DPP—combined with its deterministic, hysteresis-free response—makes it especially well-suited to these SWE methods [4,5].

Although SWE generally increases computational load and image acquisition times, it greatly reduces hardware complexity (as demonstrated in the Case Study section below). Multiple variants of SWE have been shown in confocal, multi-photon fluorescence, structured illumination, lightsheet, STED, and SMS microscopy [1,2]. Several published AO systems using a DPP in a microscope employ sensorless methods [5].

Case Study: Plug-and-Play Adaptive Optics for Microscopy

The transmissive nature of the DPP, combined with SWE, enables plug-and-play AO systems. Much like a lens mounted in a standard optical cage, the DPP can be inserted into the microscope’s optical path with minimal disruption, providing dynamic correction of system and sample-induced aberrations in real time.

Figure 5 shows four examples of a DPP (Phaseform’s Delta 7) integrated into both commercial (Figures 5a and 5b) and custom-built (Figures 5c and 5d) microscopes. Applications range from fluorescence wide-field imaging to high-end two-photon microscopy for deep imaging. The DPP’s transmissive design allows easy refractive, in-line integration.

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

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 the results of imaging more than 150 µm 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 µm 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).

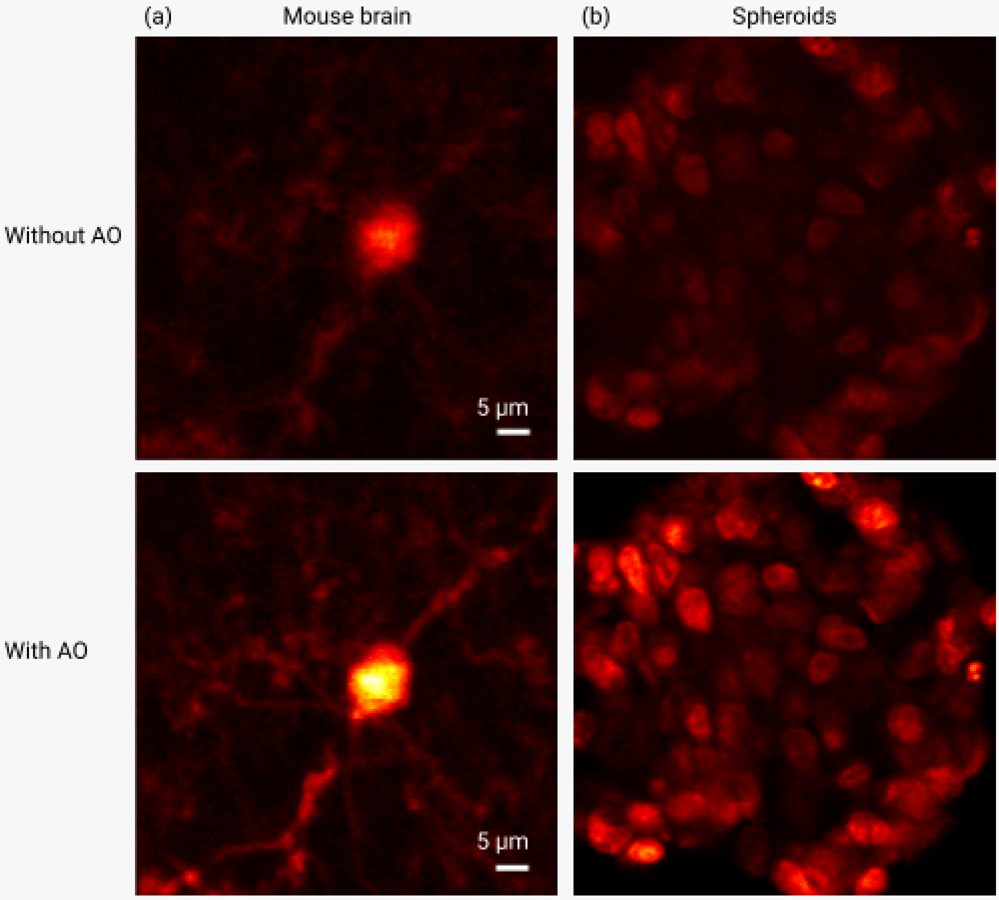

Figure 6: Images of various samples acquired with custom-built (a) and commercial (b) two-photon microscopes (MPX-1040, Prospective Instruments), with and without wavefront sensorless AO correction using the DPP. Scale bars: 5 µm.

A study from the University of Freiburg further demonstrates the effectiveness of DPP-based AO in advanced microscopy applications. In their setup, a DPP was combined with an isoplanatic patch estimation and field segmentation approach, enabling plug-and-play AO for commercial wide-field microscopes. They empirically identified the isoplanatic patch size and used full-field correction by stitching together individually corrected segments. Placing the DPP directly between the objective and turret (similar to Figure 5a) minimized the system's footprint while improving the overall correction efficiency [7].

Conclusion

Phaseform believes the latest technological advances in refractive wavefront modulators and aberration-estimation algorithms will transform adaptive optics microscopy. Just as AO revolutionized astronomy, it is likely to become the default in self-built and commercial microscopes. This future may be closer than we think.

DPP as a General-Use Wavefront Modulator

The first commercial DPP product is the Phaseform Delta 7 Transmissive Wavefront Modulator. This continuous-sheet, refractive, optofluidic device features 63 electrodes and can replicate Zernike modes up to the 7th radial order. Its 10 mm aperture is compatible with 30 mm optical cage systems. The Delta 7 includes dedicated drive electronics, control software, and simulation tools. Applications include: Vision Science and Ophthalmology, Life Science & Microscopy, Material Science & Semiconductor Inspection, 3D Micro and Nano Printing, and AR/VR.

Delta 7 Transmissive Wavefront Modulator

About the company

Phaseform GmbH is a deep-tech spin-off from the Department of Microsystems Engineering (IMTEK) at the University of Freiburg in Germany. Our goal is to make Adaptive Optics affordable and practical, translating decades of cutting-edge research into innovative products and technologies. Phaseform aspires to lead the adaptive optics market with a clear vision of continuous innovation in a “New Era of Adaptive Optics.”

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