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.

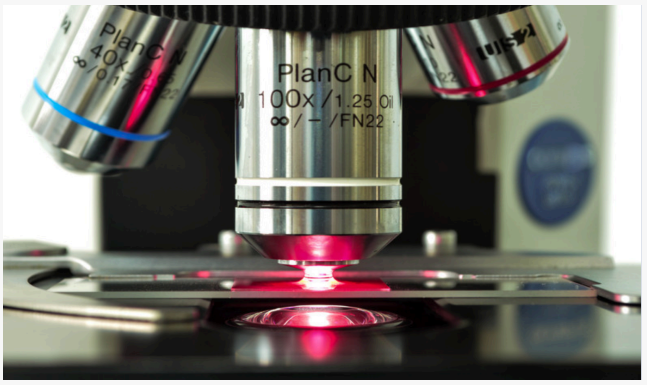

Figure 1: Oil / Water immersion microscopy setup.

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 wide-field, confocal, multi-photon, light-sheet, 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).

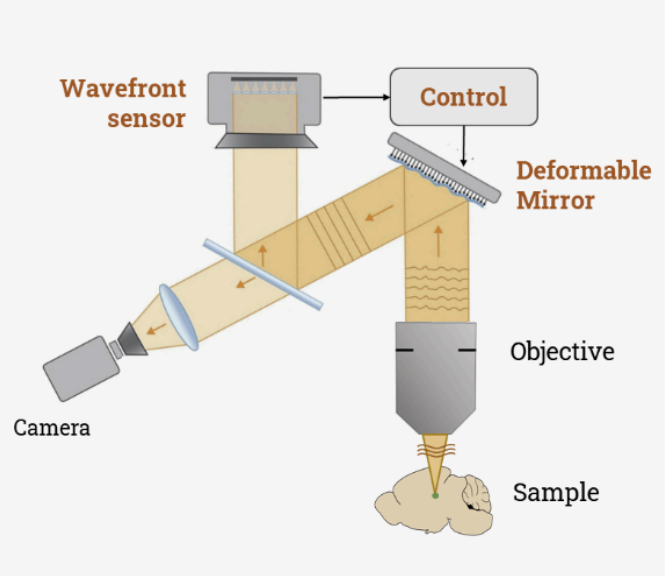

Figure 2: Conventional adaptive optics microscopes use deformable mirrors to impose the folding of the optical path.

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.

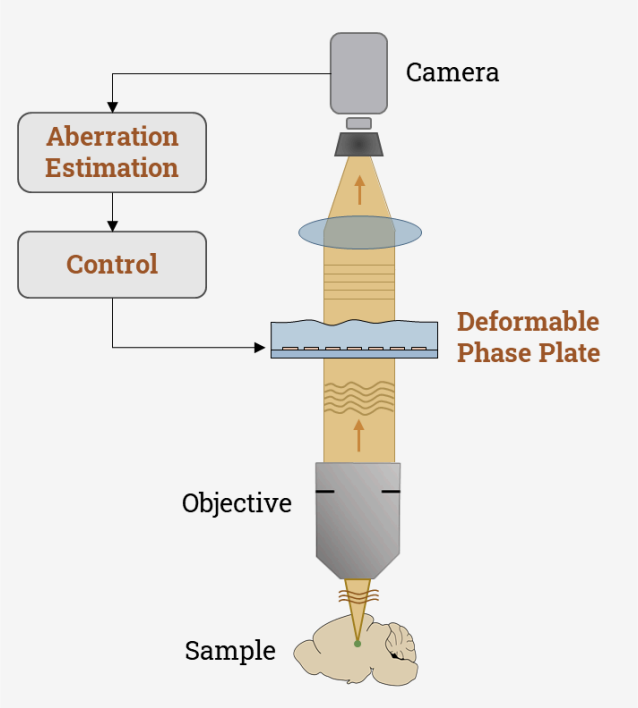

Figure 3: Phaseform develops completely in-line adaptive optics systems for direct integration

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 (DPP), shown in Figure 4 top left, 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. The DPP is available in two product series from Phaseform—the Delta 7 series with a 10 mm or 20 mm clear aperture DPP variants, and the PHI series, the plug-and-play adaptive optics system for microscopes in the form of a camera port attachment—each built on the same underlying DPP technology.

Figure 4: (Top) a 63-actuator DPP. (Middle) Delta 7 10 mm and Delta 7 20 mm. (Bottom) PHI, plug&play adaptive optics system for microscopes.

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 - Phinden

Typically, correcting optical aberrations requires some prior knowledge of what those aberrations are. In classical AO, a Shack–Hartmann wavefront sensor or an interferometer measures distortions directly. However, wavefront sensing adds complexity and cost—often prohibitively so for microscopy—and in many microscope configurations it is not even feasible: there may be no suitable reference or "guide-star" light at the sample plane, or the specimen itself may not provide a stable wavefront for measurement. As a result, direct wavefront sensing can be impractical or impossible in real microscopy systems.

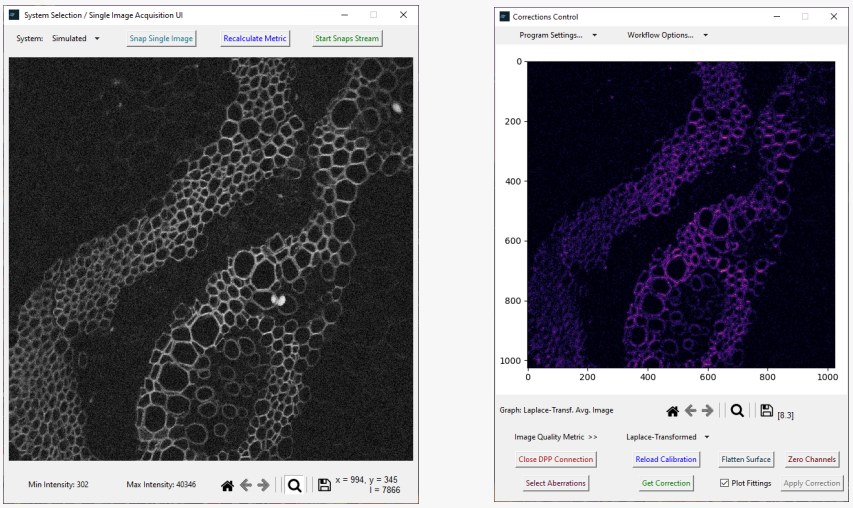

Figure 5: Phaseform's sensorless wavefront estimation software Phinden. Image-acquisition window on the left and correction control window on the right.

Figure 5 shows Phaseform's sensorless wavefront estimation (SWE) software *Phinden,* which 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 the image quality.
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 Phinden 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 6 shows four examples of a DPP (Phaseform's Delta 7) integrated into commercial (Figure 6 top left),custom-built (Figures 6 top right and bottom left) microscopes and with the camera attachment system PHI (Figure 6 bottom right). Applications range from Brightfield imaging to high-end two-photon microscopy for deep tissue imaging. The DPP's transmissive design allows easy refractive, in-line integration.

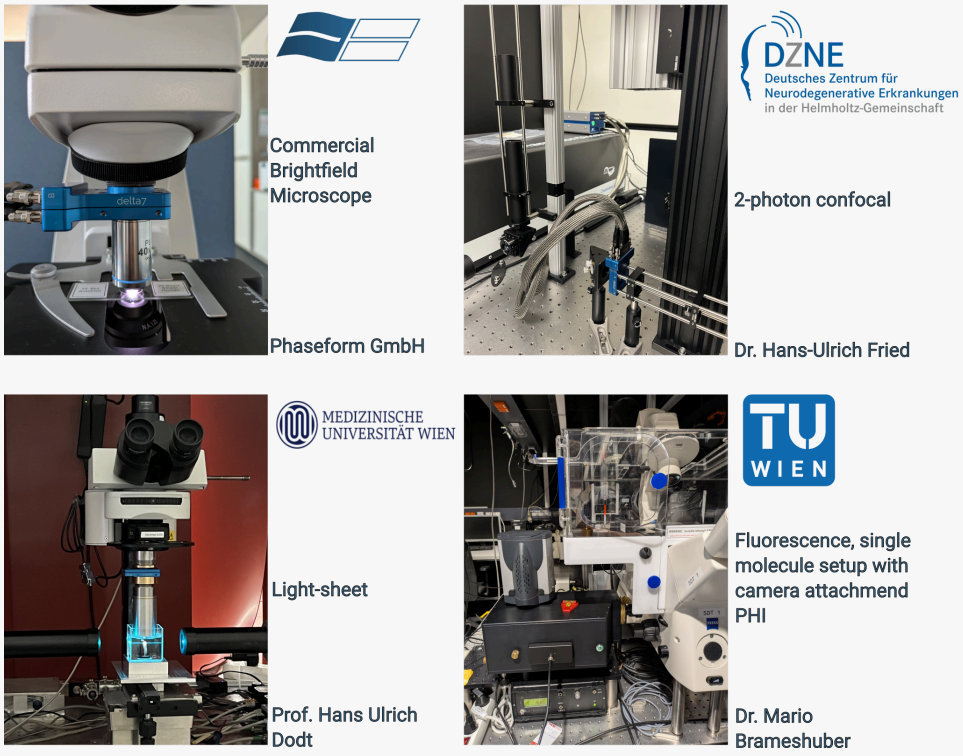

Figure 6: Examples of DPP-based AO systems in commercial and custom-built microscopes. (Top left) DPP between the objective and turret in a commercial microscope. (Top right) DPP in the illumination path of a custom-built two-photon microscope. (Bottom left) DPP integrated into a light-sheet microscope for deep tissue imaging. (Bottom right) DPP integrated into Phaseforms microscope attachment PHI at a single molecule setup.

The benefit of AO in microscopy is shown in Figure 7. 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).

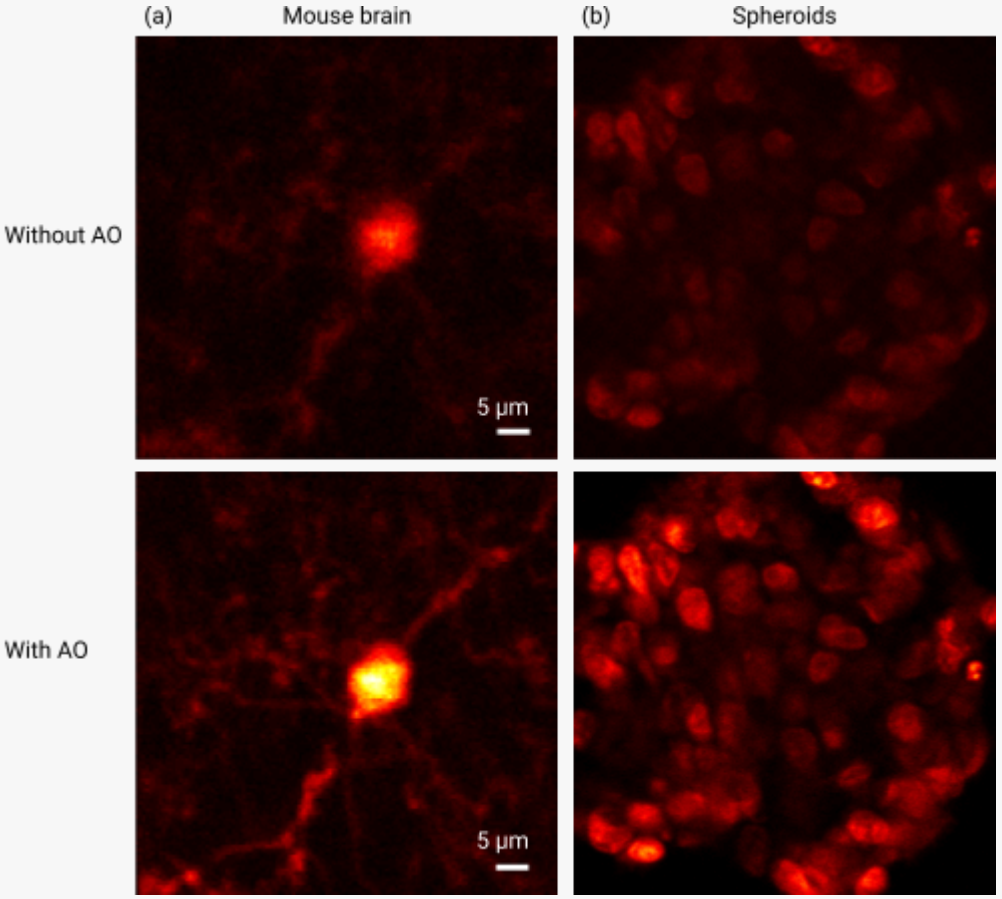

Figure 7: Images of various samples acquired with custom-built (a) and commercial (b) two-photon microscopes (MPX-1040, Prospective Instruments), with and without wavefront sensorless AO correction using the DPP. Scale bars: 5 µm.

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 6a) minimized the system's footprint while improving the overall correction efficiency [7].

Figure 8 (adapted from [7]) illustrates how the wide-field microscope's field of view (FoV) is segmented, with each segment's aberration independently measured and corrected. Comparing before- and after-AO images of 350 nm fluorescent beads shows significant improvement in resolution and uniformity across the entire FoV—demonstrating the system's ability to overcome isoplanatic limitations.

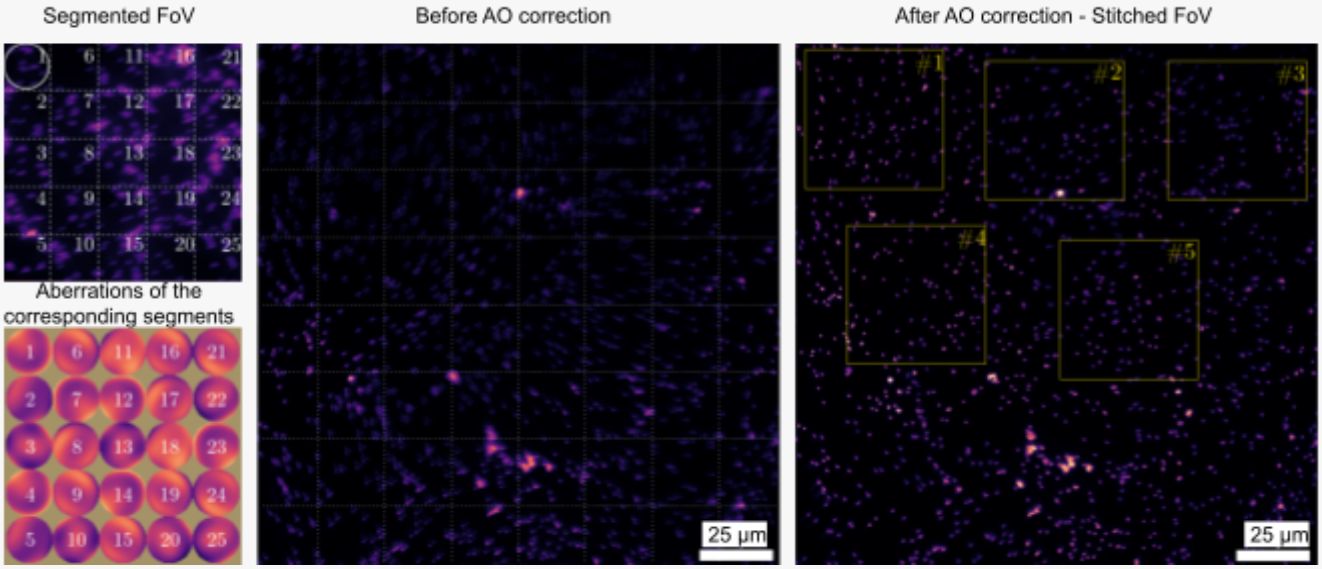

Figure 8: FoV segmentation-based AO correction (adapted from [7]). The widefield microscope's FoV is segmented and each segment's aberration is independently corrected. The stitched result (right) shows uniform image quality improvement across the entire FoV compared to the uncorrected image (center).

This experiment details the measurement of Fluorocells using a 40x, 1.3 NA Oil DIC Plan-Apochromat objective. System aberrations were intentionally introduced by utilizing a sub-optimal immersion medium (Silicon Oil). The Delta 7 (10 mm) is integrated directly into the PHI camera module for real-time wavefront manipulation.

Figure 9 (center) presents the Fluorocells image after correcting static system aberrations. This baseline correction was achieved by running the sensorless aberration estimation software (Phinden) on 200 nm fluorescent beads under the correct oil immersion medium.

Figure 9 (Right) illustrates the subsequent correction of induced spherical aberration and other residual aberrations. Significant improvements in image quality were realized by compensating for high-order spherical aberration terms up to 6th-order spherical Zernike mode, Z (6,0).

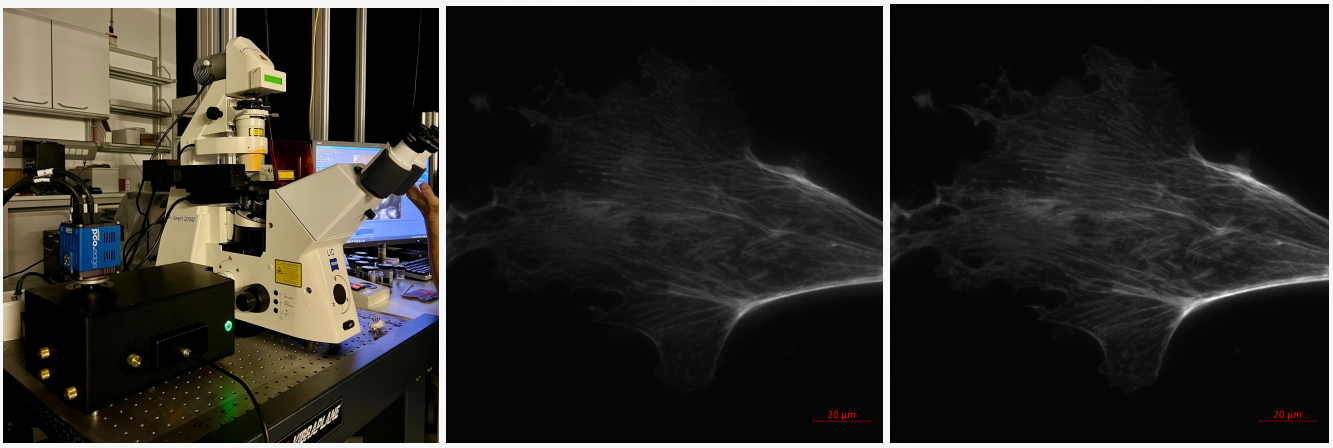

Figure 9: Fluorocell images illustrating the sequential correction of aberrations. The center image shows the Fluorocells after correcting the static system aberrations via the Phinden routine under correct immersion. The right image displays the resulting image quality after applying further correction.

This figure demonstrates the capability of PSF shaping by measuring the point spread function (PSF) of 200 nm fluorescent beads using a 63x, 1.4 NA Oil DIC Plan-Apochromat objective. A coma aberration was intentionally applied to the system via the Delta 7 Adaptive Optics system using its open-loop DPP control software. The resulting 3D PSF was captured through a full Z-scan, clearly revealing the characteristic lateral (x,y) and axial (z) intensity profiles associated with a coma wavefront.

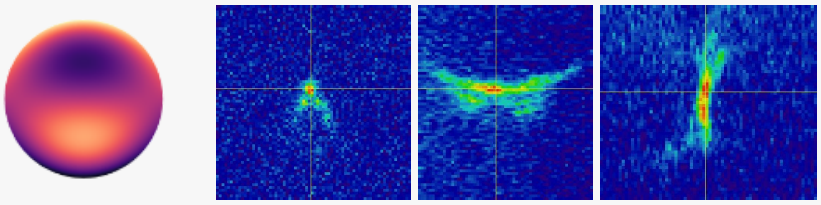

Figure 10: Experimental demonstration of Point Spread Function (PSF) shaping by applying a coma wavefront.

Further DPP implementations in various microscopy applications are discussed in the literature [8–13].

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.

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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