Recent research in using adaptive-optics techniques for the human eye enables new scientific insights and clinical applications.
Adaptive optics (AO) is a methodology that augments any imaging technique degraded by optical aberrations. First invented and demonstrated in the 1950s to correct distortions caused by atmospheric turbulence in ground-based telescopes, AO has now been used for more than 25 years to achieve cellular- and subcellular-scale visualization of targets for applications in the biological sciences.

Since the imperfect eye serves as the final optic in most in vivo devices targeting the retina, AO has proved a natural fit in ophthalmology and has led to an explosion of advances in vision science and clinical disease diagnosis. In a generally closed-loop operation, the eye's aberrations are measured with a wavefront sensor and corrected with a deformable mirror shaped to flatten out the aberrated phase map from the eye's imperfections, thereby enhancing the contrast and spatial detail of the resulting retinal images.

This article highlights recent advances with AO in three areas: imaging retinal cells and structures in healthy and diseased human eyes; probing cellular function and dysfunction; and using visual simulators to better understand the spatial limits of vision and simulate visual corrections before prescription of contact lenses and intraocular or corneal surgery.

Visualizing cellular structures in human eyes

While the anatomy, physiology and function of retinal cells and structures are well known from decades of studies in excised eyes, the AO-enabled ability to resolve these cells in live human eyes has allowed the exploration of variability in human retinal structure and function. This provides the real potential for personalized diagnosis and treatment, especially when combined with an increased understanding of inherited retinal diseases.

Since the first application of AO to sense and correct for ocular aberrations, nearly every major type of retinal cell has been visualized in vivo. In view of their high contrast and their significance to visual function, cone photoreceptors, which act as waveguides, were the first and most examined retinal neuron. Since then, additional meticulous and ingenious investigation has overcome technical challenges in imaging other structures. For example, high-fidelity AO systems have resolved small-diameter, densely packed rod photoreceptors, and the micro-scale depth-sectioning capabilities of optical coherence tomography (OCT) have been used to visualize the retinal pigment epithelium (RPE) monolayer that lies directly below the layer of bright photoreceptors.

AO-enhanced devices have followed the developmental trends of more general biomedical imaging technologies—higher speeds, better sources and detectors, more automation and an exponential increase in the use of artificial intelligence and machine learning (AI/ML). AI/ML has been applied to rich AO datasets in ways similar to its use for more conventional image analysis approaches, such as for feature extraction or generation of objective metrics and biomarkers, but on a cellular scale—for example, for automated retinal cell segmentation and quantification.

There have also been advances in technology specific to AO, including better deformable mirrors with higher stroke and actuator count, improved wavefront sensing and correction approaches, and, in particular, new techniques to manipulate the light scattering field (split field, dark field, etc.) and phase of the back-reflected light signal for better contrast or delineation of structural features. Further, AI/ML has been used to tackle problems unique to AO—for example, to improve image quality from sparse, unaveraged datasets with low pixel density that were previously collected in line with historical AO imaging constraints, like low contrast and small fields of view.
Recent trends in retinal imaging

One recent development has been the use of new AO techniques to resolve the more-transparent inner retinal cells, including ganglion cells, horizontal cells, monocytes, microglia and macrophages, among others. Another current trend is a focus on noncellular structures, particularly the retinal vascular plexuses and complexes, which supply the inner retinal neurons, and the choriocapillaris, one of the densest vascular networks in the human body, which nourishes the photoreceptors. Along with the RPE, the choriocapillaris is centrally important to visual metabolic health.

The cellular-level detail that AO provides has also been exploited clinically to better understand and diagnose retinal diseases, from increasingly prevalent conditions like age-related macular degeneration and diabetic retinopathy, to rarer inherited genetic diseases like the retinitis pigmentosa variants and cone-rod dystrophies, to ocular diseases that primarily start in the inner retina like glaucoma. AO-enhanced retinal imaging is also increasingly being used to study neurodegenerative diseases, like multiple sclerosis and Alzheimer’s disease.

As a result of these advances, AO is slowly but surely being recognized as an indispensable tool in retinal disease diagnosis and treatment assessment. It is particularly valuable for outer retinal diseases where higher-order cortical visual processing renders coarse measures like visual acuity—assessed with eye chart tests—insufficient to detect diseases early to track their progression. AO gives texture to retinal disease visualization that conventional fundus photography lacks, and it provides cellular detail and highly sensitive quantification of disease changes that clinical OCT captures only imprecisely.

The application of AO in clinical settings may accelerate in future years as its use becomes more widespread in disease prediction (with AI/ML support) and...
especially in the development of new therapeutic interventions. The barrier to increased uptake has been a chicken-and-egg situation between clinical availability and AO-dependent applications that could drive market conditions toward more successful clinical translation. The use of AO-based clinical endpoints, or outcome measures, in new drug trials requires standardized and repeatable clinical AO devices. However, successfully translating AO technology into such devices requires a large investment that an application related to a novel drug treatment would provide. Whether it occurs slowly or rapidly, increased use of AO in the clinic will benefit patients who suffer from ocular diseases.

Probing cellular function and dysfunction

One of the earliest scientific applications of AO in the eye was imaging the three cone spectral classes—long, middle and short cones, which seed all the sensations that we perceive as colors—in the human retina. No such image had previously existed of the distribution of the three cone types, even from histology, given the lack of histochemical markers that could effectively segregate the chromophores. Since then, AO has ushered in a new era in the study of visual function and retinal physiology by enabling in vivo recordings in human and animal models that were otherwise relegated to ex vivo preparations.

The basic premise for functional imaging in the visual system involves relating an interaction between light and tissue—for instance, absorption, fluorescence, scattering or birefringence—to a known physiological phenomenon, like bleaching, calcium activity within cells, transduction or structural remodeling. The successful history of such measurements and the myriad of tools available for the retina has made it a favorable tissue for this pursuit.

Upon correcting the aberrations of the eye and converting it essentially into a microscope objective, AO has given researchers the ability to see, stimulate, perturb and record individual and collections of retinal cells and study their physiology and role in vision. This has enabled a number of scientific discoveries that capitalize on the increased resolution and contrast provided by AO, and perhaps most importantly on the ability to conduct these studies on living eyes.

Studies that link retinal cells and circuits to perception showcase some of the key benefits of AO for functional imaging. A noteworthy example is in understanding how just three cone spectral types lead humans to experience a rich palette of colors. A simple approach is to explain how just a single cone photoreceptor of a particular type contributes to the perception of hue.

This requires first having knowledge of the cone types in an individual. Second, it requires focusing light to a small area within the retina such that it is captured by only one cone. Third, the eye is incessantly in motion on spatial scales that far exceed the size of individual cells; targeting just a single cone requires technology that tracks the retina in real time on scales commensurate to the size of a photoreceptor and stimulates it with minimal latency and maximum precision. With a combination of AO, real-time eye tracking and correction of chromatic aberration, it has been demonstrated that humans can perceive a small spot of light falling on just one cone and can associate it with a perception ranging from achromatic (or white) to colored. Red and green sensations reported by the subjects were mainly driven by L- and M-cones, respectively, while achromatic sensations were distributed among cone types.

There are a number of exciting new avenues that lie ahead. What will humans see when we stimulate not just one cone, but many of them in parallel with different wavelengths? Will they see new colors that they have never experienced before? Can dichromatic humans experience color vision similar to trichromatic individuals inside an AO system by tricking the brain and labeling a handful of cones as the third missing type? Can trichromatic humans experience tetrachromacy?
Fluorescence and optoretinography

AO has also been central to studies on how information is coded and processed within the retina, and enabled the characterization of different retinal ganglion cells—the primary output neurons of the retina. Signals from the six million cones in the human eye are compressed into just one million nerve fibers leaving the retina, which originate from about 20 different types of ganglion cells.

In a 2018 study, the retinal ganglion cells of macaque monkeys were labeled with a fluorescent marker for the activity of calcium, which caused the cells to fluoresce when activated. By imaging the fluorescence in response to visual stimuli, the spatial receptive fields of the cells could be mapped effectively. AO provided sufficient cellular resolution and improved signal-to-noise to measure the weak fluorescence arising from these individual cells upon activation, offering the first opportunity to link retinal circuits to visual behavior. There currently exist no viable methods to do so in the macula of higher primates.

In addition to exogenous fluorophores like the fluorescent tags described above, AO has enabled imaging of endogenous fluorophores implicated in the visual cycle of macaques and humans using intensity, spectra and lifetime of the fluorescence. Imaging the fluorescent activity provides insight into the biochemistry of various processes involved in the normal and compromised function of photoreceptors and the RPE.

Optoretinography (ORG) is one of the more recent advances in functional imaging of the visual system. In analogy to the more established electroretinogram (ERG), which measures the electrical responses of retinal cells, ORG is the optical imaging of retinal activity induced by a light stimulus. It is based on the sensitive measurement of changes in backscattered light intensity or phase using highly sensitive optical interferometry. This burgeoning field has benefited from the ubiquity of OCT and scanning laser ophthalmoscopy (SLO) systems for retinal imaging, and AO has played an important role in providing access to cellular-scale ORG.

Among other applications, AO-based ORG measurements have been used for rapid and precise classification of cone spectral types and for the early and sensitive detection of changes in photoreceptor function that precede the other macroscopic changes in inherited retinal disease.

Exploring vision and guiding correction

Ocular aberrations degrade the quality of images projected on the retina, reducing their contrast and filtering out high spatial frequencies—and therefore imposing the first limit to spatial vision. One of the first applications of AO in vision science has been the correction of ocular aberrations to prospectively improve vision. The integration into the AO system of a channel to present visual stimuli has allowed the performing of psychophysical experiments while noninvasively manipulating individual aberrations, taking the exploration of spatial vision limits to a new level.

AO has been used to address fundamental questions about the extent to which visual function (visual acuity, contrast sensitivity) can be improved by correcting the eye’s imperfections and the effect of the optics on perceived visual quality, both with monochromatic and gray-scale stimuli. Optical simulations showed that the presence of monochromatic aberrations attenuated the effects of chromatic aberrations.
SIMULATING VISION

Adaptive-optics visual simulators allow testing the impact of manipulating optics on vision, in both laboratory and clinical settings.

**IN THE LAB**

Aberrations can be corrected or induced with a deformable mirror to investigate the spatial limits of vision and conduct R&D for new types of optical correction. Research has investigated the effect of correcting the natural aberrations of the eye on visual acuity, contrast sensitivity, perceived visual quality, accommodation, and shifts in perceived best focus with corrected or induced astigmatism and high-order aberrations and chromatic defocus.

![Optical table-based visual simulator](image1)

Deformable mirror

Sample image for simulating blurred vision with AO

Blurred vision simulated with AO:

- Defocus + negative astigmatism
- Defocus only
- Defocus + positive astigmatism


**IN THE CLINIC**

Visual simulators can be used to guide correction selection and manage patient expectations by letting patients experience vision with prospective correction options. Phase maps representing a given diffractive or refractive segmented multifocal correction can be mapped on a spatial light modulator to test the effects on vision of contact lens, intraocular lens and refractive-surgery pattern designs, and their coupling with the eye’s optics.

![Portable, see-through binocular visual simulator](image2)

Spatial light modulator

Phase map of SLM-simulated multifocal contact lens

Correction options that can be simulated with AO:

- Lasik
- Intraocular lenses
- Contact lenses

AO visual simulators have also become a great practical tool in the understanding and design of visual corrections for refractive errors and presbyopia.

The beneficial interactions between these aberrations have also been proved visually through the use of AO visual simulators, while assessing the perceived visual quality of blue or green stimuli with natural or corrected aberrations.

The presence of aberrations has often been deemed crucial for the accommodative mechanism—the process by which the eye changes its refractive power to focus on objects at variable distances. Since positive or negative defocus interacts differently with the ocular optics, aberrations could be a signal to dictate the direction of the accommodative response. The selective nature of AO to correct high-order aberrations while monitoring for defocus makes it a very suitable technique to address this issue. AO is also useful to investigate the effect of the interactions of aberrations on the accommodative lag, the difference between accommodative stimulus and response, which is often regarded as a critical factor in the development of myopia.

Some studies using AO have explored differences in the perceived best focus in observers exposed to identical optically degraded images. They revealed that observers rate images that are degraded with their own aberration profile (magnitude and orientation) as better than those degraded by other people’s aberrations or even their own rotated aberration profile, suggesting that humans are adapted to their own optical imperfections. On the other hand, observers can adapt to the aberrations of another individual or scaled versions of their own, indicating that the visual system dynamically recalibrates to a new optical environment.

AO visual simulators have also become a great practical tool in the understanding and design of visual corrections for refractive errors and presbyopia. The phase profile of a contact lens, intraocular lens, or LASIK refractive surgery corneal ablation algorithm are mapped onto a deformable mirror (for smooth surface corrections) or a spatial light modulator (for segmented lenses or diffractive elements). These simulations allow patients to experience vision through those corrections noninvasively before they are fitted or implanted in the eye, or even manufactured. This capability is particularly interesting in lens design, as different profiles can be tested and refined to investigate the interactions of the eye’s optics, lens design and visual neural system in a personalized manner, potentially saving manufacturers costly clinical trials involving surgery or physical lens fitting.

In fact, the possibility of allowing patients to experience the real world through prospective visual corrections has prompted the translation of visual simulators from the lab to the clinic. While optical table–based systems are bulky and impractical in the ophthalmology office, new visual simulators have been miniaturized and even made wearable, binocular and see-through, allowing patients to view the real, unobstructed world though the simulated lenses. Commercial contact lenses, intraocular lenses and presbyLASIK profiles have been programmed in the active element of the system, reproducing with great accuracy the visual performance of the real lenses.

Operated with user-friendly software that allows rapid changes to the lenses mapped onto patients’ right and left eyes, clinicians are incorporating visual simulators into their standard practice. This new tool helps them guide the selection of cataract and presbyopia treatment and manage patients’ expectations, in what has proved one of the most successfully translated uses of AO visual simulators.

References and Resources