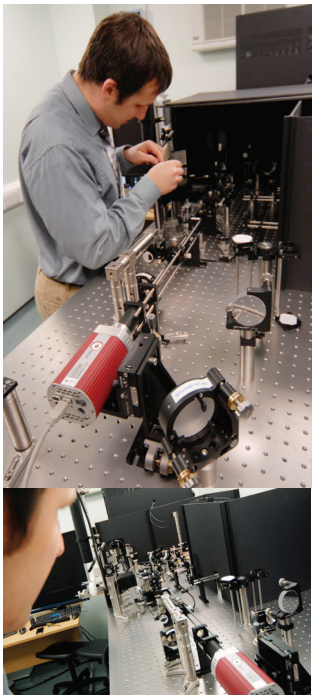
Adaptive optics imaging: resolving single cells in the living eye

BY M MICHAELIDES AND A DUBIS

The human retina is unique in the central nervous system (CNS) in that it can be directly visualised non-invasively. Technological advances of several imaging modalities, including optical coherence tomography (OCT), multichannel scanning laser ophthalmoscopy (SLO) and fundus photography, have afforded views of the retina previously unimaginable. OCT has revolutionised clinical practice and vision research, because it non-invasively generates cross-sectional images of the living retina, with software available to reconstruct 3-dimensional images. The high axial resolution of OCT allows discrimination of the distinct retinal layers that are affected differentially in different diseases. Incorporation of new light sources and imaging filters has allowed functional testing of gross retinal anatomy in the form of fundus autofluorescence and multispectral imaging. While these views have greatly improved our understanding, by the time retinal pathology is evident using standard techniques, significant cellular damage has already occurred.

In vivo imaging resolution has been limited by the relatively poor optical quality of the human eye. Helmholtz, the famous optical physicist and physician said that if he were to purchase an optical system with the quality of the human eye, he would surely return it for it would be worthless. These imperfections are classified as types of aberrations, and result in the scattering of light energy over a larger area. Many people are familiar with the greatest magnitude aberrations, sphere and astigmatism. Lesser magnitude aberrations arise every time light is diffracted moving from media of one refractive index to an area of different refractive index [1]. These localised changes in refractive index are induced by the alignment of the crystallins in the lens and corneal stromal cells [1].

Astronomers have long dealt with similar aberrations, induced by refractive index changes going from the vacuum of space and through the earth's atmosphere. To mitigate these aberrations, astronomers developed a system to measure the aberrations or distortions in the wavefront. They then coupled these measurements with adaptable mirrors to correct for the aberrated wavefront and to refocus the scattered light waves – resulting in a much higher resolution image. The same is true of light passing through the media of the eye, such as from the lens to the vitreous humour. The result of this wavefront distortion is a lower resolution image. In ophthalmology a wavefront sensor measures the ocular aberrations and a deformable mirror corrects for these aberrations (Figure 1). These components can be added to any existing ophthalmic imaging system. Current research grade adaptive optics (AO) equipped systems provide exquisite visualisation of single cell neuroanatomy in the human living eye to interrogate retinal structure and function at the cellular level [2]. Such advanced imaging will improve understanding of disease mechanisms, progression and diagnosis.



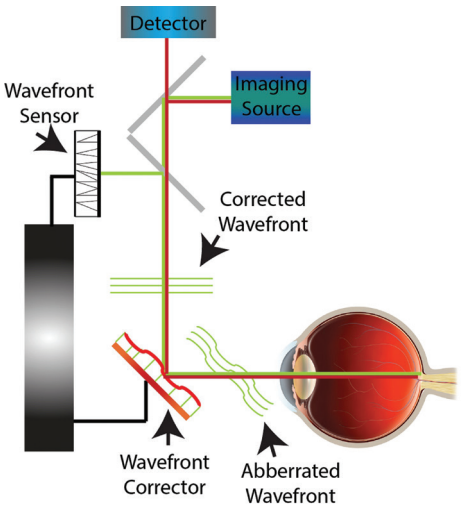


Figure 1: Adaptive optics allows the aberrations of the eye to be measured by a Shack-Hartmann wavefront sensor. The aberration measurements can then be applied to a wavefront corrector, which is able to correct for the aberrations and allow near diffraction limited resolution of the retina. In an adaptive optics imaging system, light is reflected out of the eye in a distorted or aberrated wavefront. The aberrations are measured by the wavefront sensor. A correction is then applied to the wavefront corrector, resulting in an undistorted wavefront being imaged with the detector.

Commercial and custom-built AO devices are available in ophthalmology with significant differences between them including, in terms of ease of use, cost, maintenance, and the depth and breadth of their capabilities [2]. The custom devices use a superior optical design that nulls out the aberrations of the imaging system. The result is a diffraction-limited system, with consequent higher resolution. Many of the parts are custom optics, so they are more expensive, but give better performance. The highest resolution devices to date are custom-built Adaptive Optics Scanning Laser Ophthalmoscopes (AOSLOs), which are able to exquisitely visualise retinal anatomy including microvasculature, including cellular blood flow, cone, rod and retinal pigment epithelium mosaics, and the retinal nerve fibre layer [2-5] (Figure 2).

With the support of a Multi-User Equipment Grant from the Wellcome Trust and a collaboration with Professors Dubra and Carroll at the Medical College of Wisconsin, a custom-built AOSLO has been established at Moorfields Eye Hospital and UCL Institute of Ophthalmology. An

additional £100,000 raised through the Moorfields Eye Charity has helped to further develop its capacity and capabilities.

It is hoped that the AOSLO will facilitate earlier disease detection, provide better insight into disease pathogenesis and help to tease out different sub-types of disease. It will have a potential role in all retinal disease (and glaucoma) including inherited retinal disease (now the commonest cause of visual impairment certification in the working age population in England and Wales [6]), diabetic retinopathy, age-related macular degeneration, and even various neuro-degenerative conditions – as a 'window' to the brain, retinal imaging allows study of CNS disease mechanisms and biomarkers for progression – potentially for looking at early signs of disorders such as Alzheimer's and Parkinson's disease.

It will also add to the existing arsenal of diagnostic tools for clinical trials, potentially allowing identification of novel targets for treatment and to more sensitively, reliably and rapidly detect effects of any treatments being investigated. AOSLO imaging will also be invaluable in the identification of patients most likely to benefit from novel therapies, including gene replacement therapy and stem cell approaches.

One of the principal challenges is the vast amount of data produced by AO imaging, with resultant huge processing and analytical demands, both in terms of time and computer processing. Collaborations with software engineers will be needed to address this challenge.

In addition to unparalleled cellular imaging, arguably the next transformational AO application is AO-guided psychophysical testing with effective eyetracking in macular disease for probing subsets of cone photoreceptors in vivo – 'functional imaging'. The ability to accurately and reliably determine retinal sensitivity at defined loci is of critical importance to probe macular function in disease, determine therapeutic effect, and correlate functional with structural data.

Current microperimeters have critically limited dynamic ranges. The stimuli are large, stimulating hundreds of photoreceptors at once, while only one or a few are required to generate a response. In patients with poor fixation or nystagmus, current microperimeters are not able to present repeatable stimuli to given locations and have significant test-retest variability. These issues limit the sensitivity and effectiveness of these tests in people with macular disease.

New eye tracking systems are now coming online to better account for eye movements in these patients. Coupled with AO-guided microperimetry the limitations of stimuli dynamic range and repeatability can be overcome. Significant challenges remain, however, in delivering the stimuli to a desired retinal location, to allow for systematic testing of desired retinal loci.

Despite these challenges we are in a new era of advanced deep phenotyping and better-directed therapies.

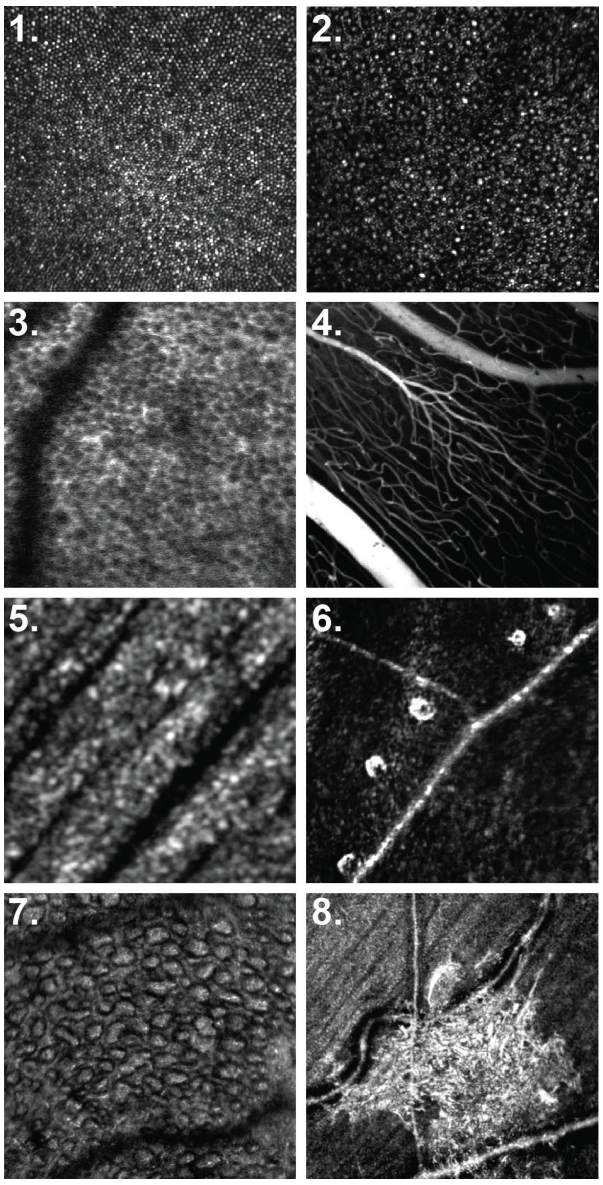


Figure 2: In vivo images acquired with Adaptive Optics Scanning Laser Ophthalmoscope (AOSLO). 1) Macular cone photoreceptor mosaic. 2) Rod and cone photoreceptor mosaic. 3) Retinal pigmented epithelium mosaic. 4) Contrast aided angiography. 5) Retinal nerve fiber layer (RNFL) 6) Inner retinal blood vessels without contrast. 7) A cross-sectional view of micro-retinal cysts in a patient with retinitis pigmentosa. 8) Hyper-reflective area in the RNFL in a patient with glaucoma. These patches are not observable with conventional imaging and studies are ongoing with regard to their exact aetiology. Courtesy of Professors Carroll and Dubra.

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