COMMENTARY

Optoretinography is coming of age

Austin Roorda[a,1]

The arsenal of tools available to today's neuroscientists to study the function and structure of neurons in the brain and retina is impressive. Fluorescent probes that indicate functional activity are becoming faster and more sensitive (1), as are the imaging systems that are being developed to observe them (2). Electrode arrays are becoming increasingly parallelized in three dimensions (3), and combined optoelectrical recording is also possible (4). But as exciting as these technologies are, their translation for human use remains problematic because of ethical and regulatory barriers arising from their invasiveness and potential toxicity. The increased demand for cellular-level recording with comparable sensitivity and resolution noninvasively in live humans has driven invention and innovation for the retina in an emerging area called optoretinography, or ORG. Originally coined by Mulligan et al. (5), ORG generally refers to the recording of optical signals caused by retinal neuronal function. ORG measurements with various names and realizations have been conducted for decades (6–11). A primary goal of ORG research has been to improve the ability to detect and measure loss of function due to disease, and thus Lassoued et al.'s (12) demonstration in PNAS that eye disease causes a change in the ORG represents the method's coming of age. It took a combination of several major developments to reach this point.

One of the major barriers was the eye's natural optics, which have evolved nicely for vision, but are not of sufficient quality to enable microscopic images of the retina. These barriers were overcome in 1997 when a team led by David Williams at the University of Rochester—which also included D. T. Miller, the senior author of the study by Lassoued et al. (12)—used adaptive optics (AO) to measure and correct for aberrations in the eye and recorded retinal images with resolved cone photoreceptor cells in a live human (13). Since that time, AO technology has continued to mature, to the point where near-diffraction-limited optical resolution is now routine (14).

Another major development was optical coherence tomography (OCT), which leverages the interference properties of light to make depth-resolved measurements (15). This technology was perfectly suited for imaging the weakly scattering, mostly transparent, tissue of the retina and allowed cross-sectional and three-dimensional imaging of the human retina (Fig. 1). Its use in clinical eye care is now commonplace (16). The integration of AO into OCT made it possible to resolve retinal cells in three dimensions. Until recently, the axial depth resolution of OCT was primarily obtained through the amplitude of the interference signal only, which already offered axial resolutions that were over an order of magnitude better than confocal optics could ever provide in a human eye (17). More recently, phase-resolved OCT has pushed axial resolution a giant leap further, offering sensitivities to physical changes in neurons on a nanometer scale.

The basic premise of the ORG measurement as reported by Lassoued et al. (12) is this: When a neuron like a photoreceptor is excited, physical changes in the cell take place that lengthen or shorten the optical path length between its scattering surfaces. In phase-resolved OCT, these path length changes are big enough to manifest as measurable changes in the phase of the light waves returned from the eye. The phase is very sensitive to movement in the tissue; for relatively bright structures such as the photoreceptors, displacements smaller than 10 nm are detectable, much smaller than the axial resolution of the system or the wavelength of the imaging light.

What are these changes? Visual transduction is a complex process, involving an interplay of chemical (ion transport) and electrical changes (modulation of membrane potentials). Whereas the electrical and chemical changes are detected with invasive techniques of electrodes and fluorescent dyes, the ORG directly measures the physical changes that are associated with them. The physical changes are thought to result from deformation caused by cell membrane tension changes (18, 19) over short, millisecond time scales followed by osmotic swelling/deswelling (20) on longer time scales. It is these physical changes that Lassoued et al.'s (12) ORG is detecting. Specifically,

[a]Herbert Wertheim School of Optometry and Vision Science, University of California, Berkeley, CA 94720

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[1]Email: aroorda@berkeley.edu.

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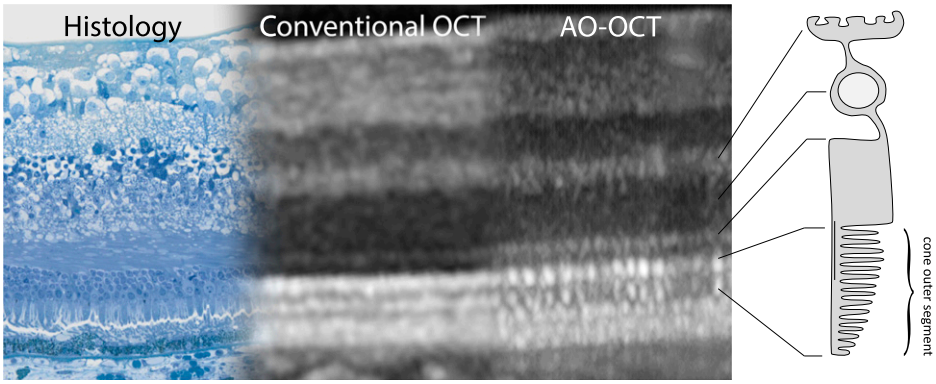

Fig. 1. Three visualizations of a cross-section of human retina. (*Left*) Histology (adapted with permission from projectmacula.org) reveals all cells in the retina. (*Middle*) Conventional OCT can resolve the lamination of the major cell layers, whereas AO-OCT reveals the individual cells that make up these layers, particularity in the cone photoreceptor inner and outer segments. (*Right*) A schematic of a cone cell with lines indicating corresponding locations of the major cone features in the AO-OCT image. The outer segment, which contains the photopigment, is marked by two punctate reflections on either end in the AO-OCT image. Lassoued et al.'s (12) photoreceptor ORG measures the length changes between these two reflections in response to light stimulation.

they measure changes in the optical path length of the outer segment of the photoreceptor, the part of the cell where the cone photopigment resides and where the first stages of visual transduction take place.

The photoreceptor outer segment is particularly suited for ORG measurements. It is long and narrow, it behaves like an optical waveguide (21), and there are relatively strong reflections from each end (the inner-/outer-segment junction and the cone outer-segment tips) that redirect light back out of the eye like a retroreflector. The phase changes between these two reflections are used to quantify the optical path length change of the outer segment. An earlier paper from Miller's group showed that the magnitude of these path length changes was strongly correlated with light-induced activity, and, in an impressive demonstration, they used the photoreceptor ORG signals to classify and generate maps of the three cone classes (22) far more quickly and accurately than previously reported densitometric approaches (23). These cone classifications give confidence that the reductions in photoreceptor ORGs in retinal degeneration patients very likely indicate reductions in function.

In theory, all retinal neurons could be measured by ORG. Indeed, a team from the University of Lübeck in Germany led by Gereon Huttmann used full-field, phase-resolved OCT to show long-term osmotic changes in the retinal ganglion cell layer of living humans following light stimulation (24), but cellular-level measures of more short-term changes remain elusive, at least in vivo. This is because other retinal neurons do not have the ideal structural properties of photoreceptor outer segment mentioned above. A team led by Daniel Palanker at Stanford University used quantitative phase imaging in tandem with multielectrode array recording and showed that the physical changes associated with action potentials in cultured neurons are on the order of 1 nm and last for just 1 ms (25). If, as the authors (25) suggest, these cultured neurons behave anything like retinal ganglion cells, then these tiny, brief changes set a high bar for today's ORG technology—but they might not be out of reach. Smart signal processing and integration of weak signals over time and space might make detection of even single action potentials in a living eye possible. In the meantime, the value of photoreceptor ORGs alone to study the retina in health and disease is reason to be excited.

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