First In Vivo Images of Rods Resolved

Researchers have used adaptive optics technology to image the contiguous rod photoreceptor mosaic in living retinas for the first time.

BY CALLAN NAVITSKY, ASSISTANT EDITOR

Rod dysfunction is involved in a variety of retinal degenerations. Routine imaging of the rod photoreceptors, however, has been limited due to a lack of tools available to assess the rod structure in the living retina. Alfredo Dubra, PhD, of the Medical College of Wisconsin, and colleagues have recently designed and built a device that can provide enough resolution to image individual rod cells. In doing so, the investigators have presented the first in vivo images of the contiguous rod photoreceptor mosaic in living eyes—a development that will advance the future of ophthalmic imaging and the treatment of retinal diseases.

BROADBAND ADAPTIVE OPTICS SCANNING OPHTHALMOSCOPE

The device designed by Dr. Dubra and colleagues employs adaptive optics technology, which was proposed nearly 60 years ago for use in astronomy. This technique compensates for distortions in the atmosphere, allowing astronomical telescopes to produce sharper images of the stars. Similarly, ophthalmic adaptive optics instruments allow in vivo visualization of microscopic retinal features by compensating for the monochromatic aberrations of the eye. "Adaptive optics technology uses a wavefront sensor, which details how to change a mirror's shape to make the images of the retina appear sharper," Dr. Dubra said.

Dr. Dubra and colleagues designed a broadband adaptive optics scanning ophthalmoscope consisting of 4 afocal telescopes, formed by pairs of off-axis spherical mirrors in a nonplanar arrangement. By folding the spherical mirrors into a 3D structure, astigmatism in the pupil and image planes was reduced, allowing improved wavefront correction and imaging. With the new design, Dr. Dubra and colleagues were able to increase the device's resolution to its optical limit of 2 µm—the approximate diameter of a single rod.

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PRESENTING THE FIRST IN VIVO IMAGES

In a study published in *Biomedical Optics Express,*[1] Dr. Dubra and colleagues presented the first in vivo images of the contiguous rod photoreceptor mosaic in 9 healthy subjects. The images were collected with three confocal adaptive optics scanning ophthalmoscopes at 2 institutions, using 680 nm and 775 nm superluminescent diodes for illumination. "The benefit of using superluminescent diodes instead of lasers is that they produce a lot less speckle," Dr. Dubra explained.

In the study, estimates of photoreceptor density and rod:cone ratios in the 5° to 15° eccentricity range were consistent with histologic findings, which further confirmed their ability to resolve the rod mosaic. In 1 patient, Dr. Dubra and colleagues were able to identify the emergence of the first rods at approximately 190 µm from the foveal center. The investigators also determined that the rod and cone photoreceptor mosaics appear in focus at different retinal depths, with the rod mosaic best focus (brightest and sharpest) being at least 10 µm shallower than the cones at retinal eccentricities larger than 8°.

APPLICATIONS OF IN VIVO ROD IMAGING

Understanding the Mechanism of Eye Disease

According to Dr. Dubra, these images can first—and immediately—be used to increase the understanding of some of the mechanisms of eye disease. "In a condition as important as age-related macular degeneration (AMD), which is the leading cause of blindness in the United States,

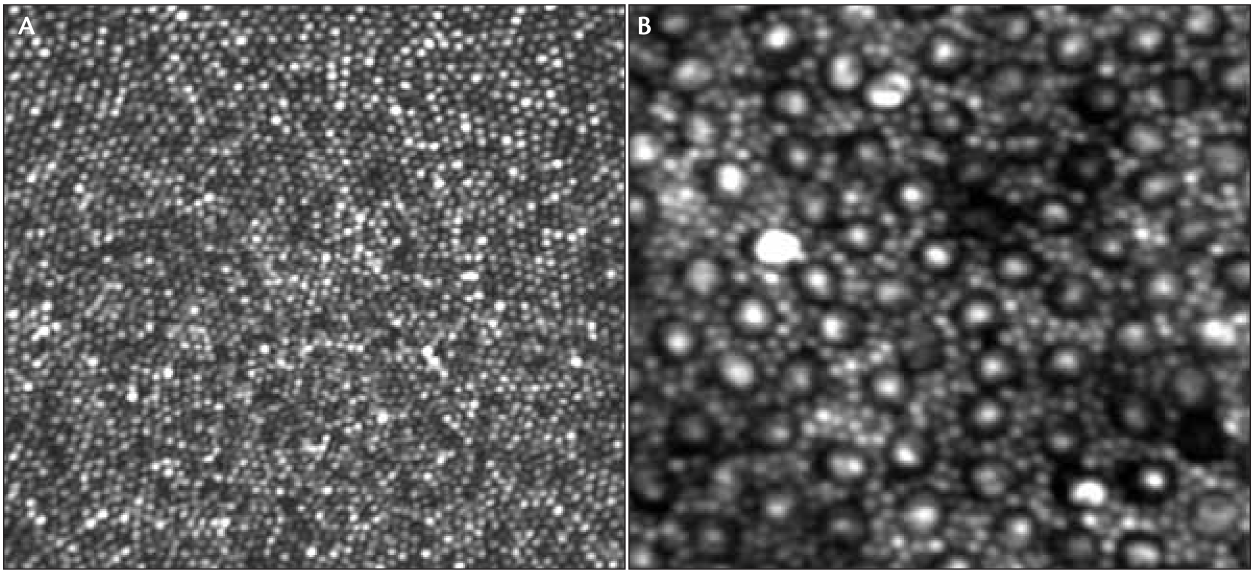

Figure 1. The smallest cone photoreceptors at the center of the fovea (A); a more eccentric retinal location, in which the large bright dots with dark rings around them are cone photoreceptors and the surrounding (and far more numerous) smaller white dots are the rod photoreceptors (B).

it is not fully clear whether the cone photoreceptors, the rod photoreceptors, or the retinal pigment epithelium cells are the starting point for the disease," Dr. Dubra explained. The researchers hope that adaptive optics imaging will help determine what happens in the early stage of this and other conditions, such as glaucoma. With this ability, individuals who are developing drugs and treatments will be able to narrow their efforts, thus enabling earlier detection and treatment.

"Early diagnosis in eye conditions is of paramount importance because the cells in the retina do not reproduce," Dr. Dubra explained. "Therefore, addressing eye conditions in the early stages is key—we will never be able to do this early enough. Studying the mechanism is the first thing that we can do now."

Accelerating Drug Development and Clinical Trials

The second application of these images, which can also occur immediately, is the acceleration of drug development and clinical trials. Current clinical instruments, such as optical coherence tomography and fundus photography, look at macroscopic changes in the retina; therefore, one has to wait for a change in the retina to occur to enough cells that it can be observed macroscopically. By the time these changes are detectable, thousands or even tens of thousands of cells have likely already died, which typically leads to irreversible vision loss.

With adaptive optics imaging, however, individual cells can be followed over time. "This ability can reduce the number of patients or animals required for a clinical trial," Dr. Dubra said. "It can also accelerate a trial because if you can detect very subtle changes, then you do not have to wait 2, or 3, or 4 years to show that a drug or treatment has made a difference."

Helping Patients With Rare Eye Diseases

For patients with common conditions such as AMD, it is relatively easy for scientists to obtain donor retinas to use in investigations, thus enabling the development of treatments, Dr. Dubra explained. With rare conditions, such as achromatopsia or blue cone monochromacy, it is difficult to obtain donor retinas.

Because adaptive optics imaging enables investigators to observe in vivo images of the rods, they are able to study the retinas of living patients and therefore do not have to wait until a patient dies to study his or her disease. "We are actually working on subjects that have rare conditions such as blue cone monochromacy in collaboration with Joseph Carroll, PhD, of the Medical College of Wisconsin, and we are learning many things that we never could have learned with any other existing type of retinal imaging," Dr. Dubra said.

Creating a System for Interpreting the Images

Despite its advantages, adaptive optics technology will not replace existing imaging devices. For example, fundus photographs, which typically cover 20º, 30º, or even 40º of the retina, are particularly useful because, in just one snapshot, changes in many parts of the retina can be potentially detected at once and very quickly. "Adaptive optics would look at patches of the retina that would individually be 1º x 1º, so you would need to take about 900 pictures to cover a 30º-x-30º image that, with other systems, you can take with just one push of a button," Dr. Dubra said.

Because adaptive optics imaging is therefore not currently practical for diagnostics, it will not replace other systems, but complement them. If an ophthalmologist were to detect a tiny, suspicious detail on a fundus photograph, he or she could take that to the adaptive optics imaging machine and zoom in to see if it is a sign of early disease.

From there, it may be possible to identify the condition and suggest a treatment immediately; this development, however, will take a few years to occur, Dr. Dubra explained. "First of all, the instruments are not commercially available, and second, we do not always know with certainty how to interpret the images," he said. "That is why this is not the end of the research project but really, only the beginning. Now that we can image the cone and rod mosaics, we need to learn how to interpret them."

The investigators are therefore working to develop a way of going from the image to numbers that are useful in the clinic. "A similar example is when you have blood drawn in the hospital," Dr. Dubra said. "Doctors will look at the sample under a microscope to examine your cells, and if you have sickle cell or anemia, for example, that will be determined by counting the number of red and white cells present or by looking at their shape. Then the clinicians will decide what medications or treatment is needed based on that information."

Dr. Dubra maintains that a similar system must be developed for evaluating the photoreceptors in the retina. "We need to come up with a number so that we can say, 'This indicates that you are going to have AMD in 5 years, so you should start treatment.'"

Currently, several research labs in the United States are working on developing meaningful, reliable, and repeatable metrics, according to Dr. Dubra. "It is an exciting time," he said. "Of the 4 applications, there are 3 that we are already trying to do; the fourth is something we will hopefully be able to accomplish in the next 5 or 10 years." ■

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1. Dubra A, Sulai Y, Norris JL, et al. Noninvasive imaging of the human rod photoreceptor mosaic using a confocal adaptive optics scanning ophthalmolscope. *Biomed Opt Express.* 2011;2(7):1864-1876.