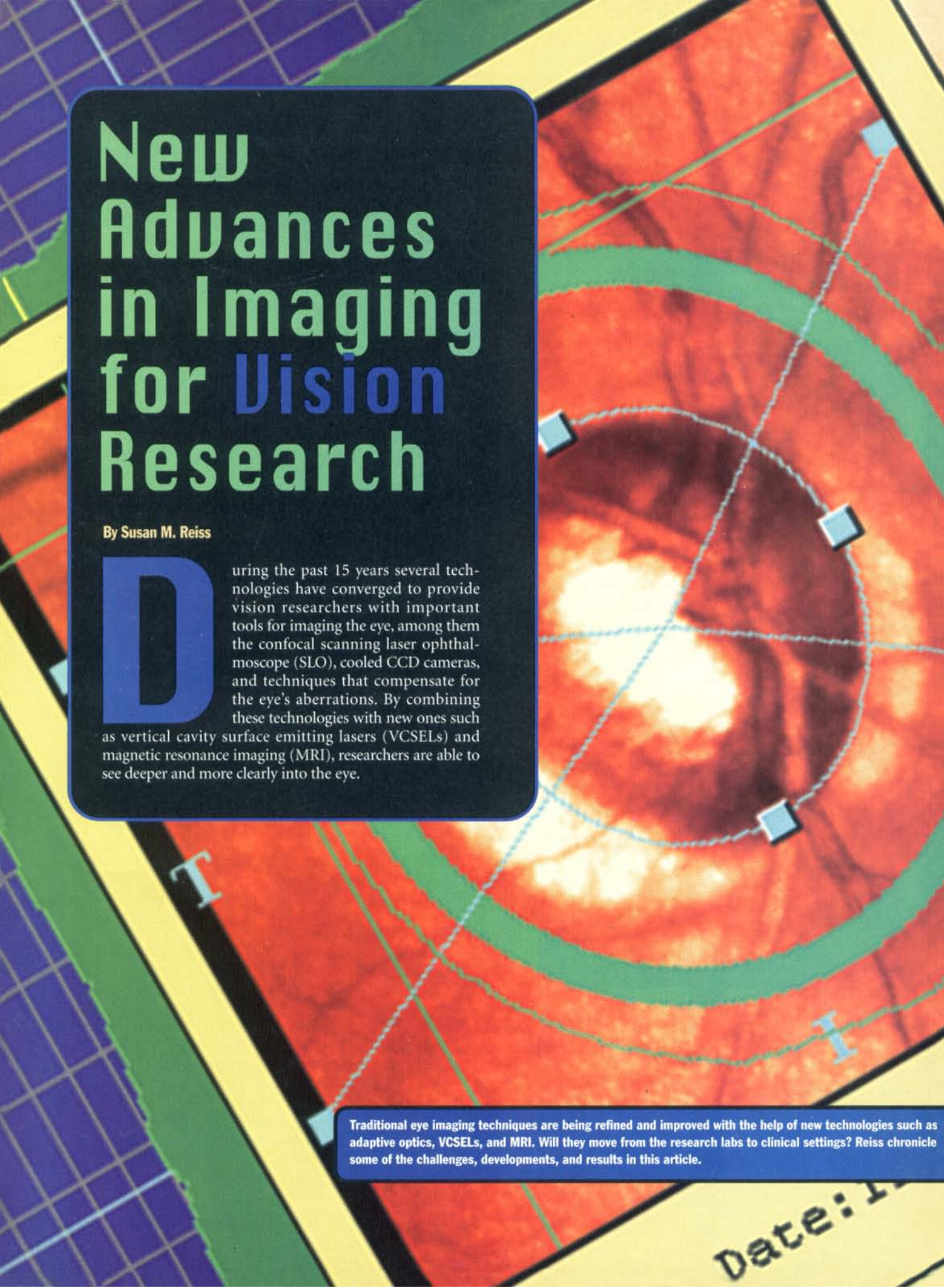
New Advances in Imaging for Vision Research

By Susan M. Reiss

During the past 15 years several technologies have converged to provide vision researchers with important tools for imaging the eye, among them the confocal scanning laser ophthalmoscope (SLO), cooled CCD cameras, and techniques that compensate for the eye's aberrations. By combining these technologies with new ones such as vertical cavity surface emitting lasers (VCSELs) and magnetic resonance imaging (MRI), researchers are able to see deeper and more clearly into the eye.



Traditional eye imaging techniques are being refined and improved with the help of new technologies such as adaptive optics, VCSELs, and MRI. Will they move from the research labs to clinical settings? Reiss chronicle some of the challenges, developments, and results in this article.

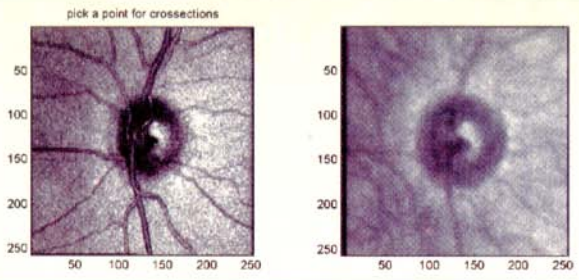


Figure 1. (Left) A confocal tomographic section acquired using the center element of a VCSEL at 850 ns, aligned with a confocal aperture. This image features the optic nerve head, which is the structure in the eye where the nerves exit the eye and travel toward the brain, as well as where retinal blood vessels enter and exit the eye. The section is the 10th of 32, acquired in 0.9 s. (Right) A multiply scattered light section acquired using the surrounding 8 elements of the VCSEL array.

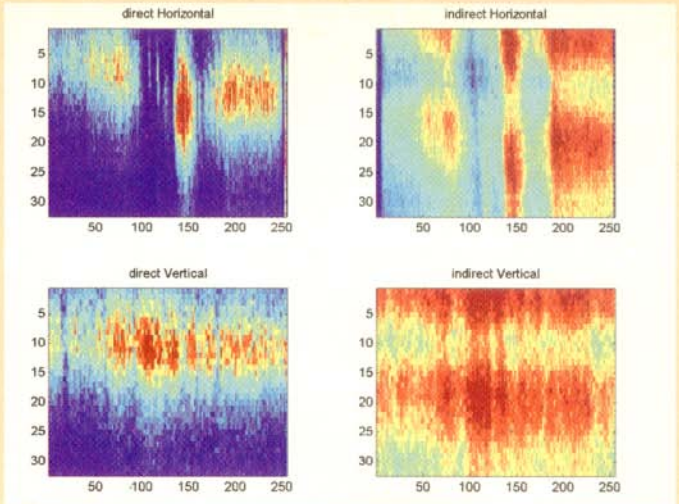


Figure 2. Cross-sections in pseudo-color through the stack of 32 sections, with the top images along a horizontal and the bottom along a vertical dimension, centered at 131, 192 in Figure 1. (Left) Only one region in depth returns a strong signal for the confocal tomographic sections. Little information returns from the deeper layers, with a low signal, shown in blue. (Right) The two bright layers indicate strong signals, shown in red. The borders of the optic nerve head are shown by the wide column at reduced intensity, best seen in the multiply scattered light tomography images.

"Some of these technologies may not end up in the clinic," says Schepen's Eye Institute researcher Robert Webb, who invented the SLO. "But they will lead to an understanding of how disease develops." He maintains that the next level in vision research is trying to "get to perfect vision." Some of the new imaging technologies, such as those using adaptive optics and wavefront sensors, may aid in reaching this level. This is especially true for photorefractive keratotomy (PRK) in which the cornea is shaped to improve vision and lessen the need for eyeglasses (see *OPN*, Nov. 1997, page 18). Though as Webb notes, since no long-term data are available on how PRK affects vision, and since visual losses are still associated with the technique, "none of us [vision researchers] are having this done ourselves."

"From the research that's being done now, wonderful things will occur," Webb predicts. "The use of deformable mirrors and deconvolution methods both compensate for aberrations and offer another step in quality imaging for research purposes. Use of SLOs to look deep under the retina will help us understand how eye diseases such as age-related macular degeneration get started. These techniques help us understand how retinal problems begin and how to prevent them. We're not finding cures, but we can discern how to prevent or slow the progression of eye disease."

VCSELs see beneath the retina

Although techniques such as infrared imaging and indocyanine green angiography reveal retinal vascular anomalies associated with diseases such as age-related macular degeneration and thinning of the retina's nerve fiber layer resulting from glaucoma, the techniques fail to image structures beneath the retina. To view those layers, referred to as the fundus, a team of researchers from the Schepen's Eye Research Institute and Laser Diagnostic Technologies Inc. (LDT) has developed a technique known as scattered light tomography.

Previous tomography techniques, including optical coherence tomography (OCT), captured less light from the deeper eye layers because the sampled light is limited to light directly backscattered from the retinal and subretinal tissues according to Schepen's researcher Ann Elsner. "OCT is great when it comes to measuring axial dimensions," she explains. "However, you can't easily see deep structures because they don't produce strong interference signals. Structures in the superficial layers of the retina are imaged with good contrast, while structures lying beneath the retina appear dark." Elsner notes that infrared light can be used to image structures beneath the retina, but "the problem with that is you must control illumination or else you don't get any contrast."

To overcome these challenges, scattered light tomography generates multiply scattered light at 850 nm with a VCSEL array (see Fig. 1). The array, which replaces a near-infrared diode laser as the illumination source in a topographic scanning system (TOPSS by LDT), is arranged in a 3 × 3 square matrix with 125-μm spacing between neighboring elements. A lens group immediately after the VCSEL collimates and reduces the spacing between lasers. A pinhole aperture of 50 μm is aligned to the light returning from the center laser to produce a confocal image.

An image taken in multiply scattered light uses the light returning through this pinhole aperture from the surrounding eight lasers, since the directly backscattered light from each of these is blocked. Dedicated software permits images to be taken using the center laser, the eight surrounding lasers, or an alternating sequence of lasers.

The topographic scanning system, which combines confocal imaging and optical sectioning, takes a series of 32 sequential cross-sectional images in different depth planes (see Fig. 2). After image alignment, the intensity of the light returning from a given position on the fundus is plotted over each of the 32 images. "The advantage of this system is that it has no moving parts that operators must adjust and you don't lose depth comparisons," says Elsner. Applications for

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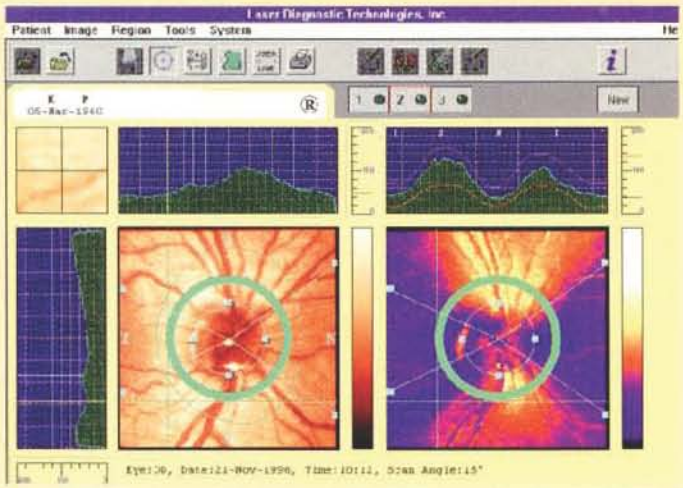

Figure 3a. GDx displays an image of the patient's optic nerve (left) and a thickness map (right). On the thickness map, the bright colors (yellows and reds) indicate areas where the nerve fiber layer is thickest; dark colors (blues) indicate thinner areas of the nerve fiber layer. In a normal, healthy person, the GDx shows bright colors on the top and bottom of the image, where the nerve fiber layer is thickest.

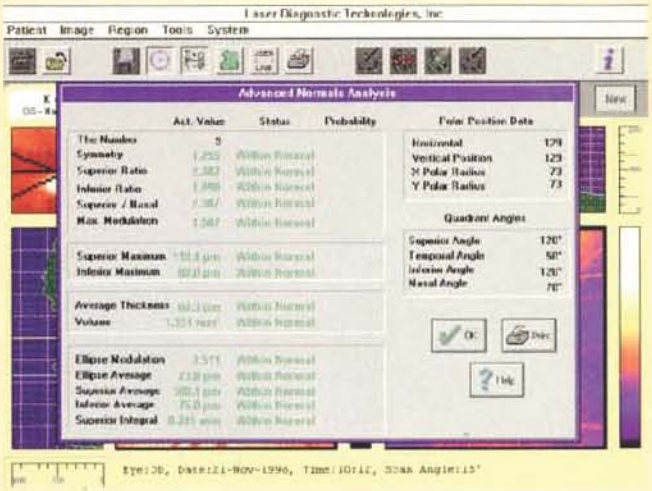

Figure 3b. GDx automatically compares each patient's measurements to a database of age- and race-matched normative values. For this healthy person, the comparison shows that all parameters are within normal limits. The first parameter, "the number," indicates the likelihood that a person has glaucoma. For this person, the likelihood is very low—9 on a scale of 0–100.

scattered light tomography include monitoring the progression of glaucoma and age-related macular degeneration, the leading cause of blindness among adults in the U.S.

Glaucoma detection and real-time imaging from the field

The eye's nerve fiber layer (NFL) is the first structure damaged by glaucoma. "Since this layer is about 30 to 120-μm thick, it's difficult to image, but we are able to take advantage of the fact that the NFL is a polarizing structure," says Andreas Dreher, a developer of the GDx™ Nerve Fiber Analyzer and one of the founders of LDT. "By using polarized light to penetrate through the NFL, we can measure its thickness with nanometer resolution." Dreher notes that the proprietary polarimetry technique found in the GDx analyzer is similar to those used to measure thin films on optical components and semiconductor wafers.

The system is composed of a confocal scanning laser ophthalmoscope with an integrated polarimeter. "Because the system doesn't use optical imaging techniques, its measurements are not affected by refractive errors or opacities within the eye," Dreher says. Within the past two and a half years, he and his colleagues have collected normative data for retinal nerve fiber layer thickness and incorporated this information into the GDx system (see Figs. 3a and 3b). "Image acquisition takes less than a second, and with the normative data, the exam provides immediate information to assist in making a diagnosis," explains Dreher (see Figs. 4a and 4b). In addition to screening for disease, Dreher notes that this tool will be useful in determining the effectiveness of drugs designed specifically to protect ocular structures from the effects of glaucoma.

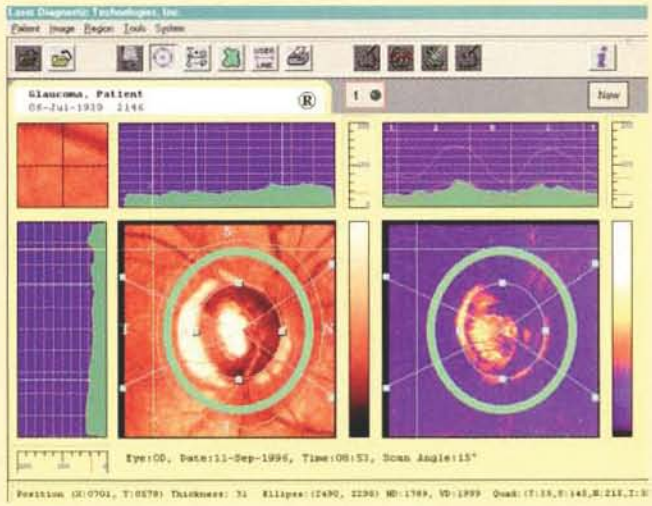

Figure 4a. Patient with glaucoma. Notice the totally dark thickness map, indicating that the nerve fiber layer is thin throughout.

Figure 4b. Comparison to the database shows that this person is outside normal limits for most parameters and "the number"—94 out of a possible 100—indicates a high likelihood of glaucoma.

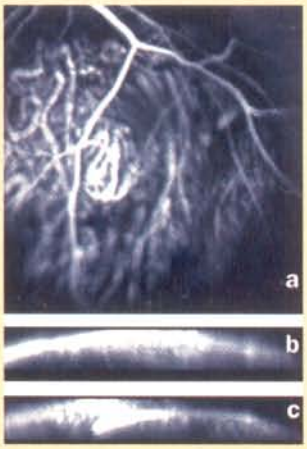

Figure 5. Angiogram of a patient with ocular melanoma. A stack of 32 confocal angiograms of the retinal circulation viewed from the front (a), the right side (b), and the top (c). The retinal surface is curved, due to the elevation of the tumor. No detail can be seen inside the tumor.

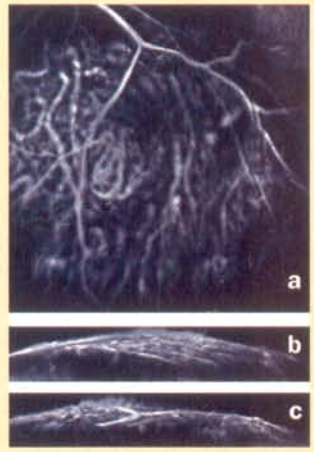

Figure 6. Composition angiogram of a patient with ocular melanoma after digital signal processing. A stack of 32 confocal angiograms of the retinal circulation viewed from the front (a), the right side (b), and the top (c). The blood vessels in all three pictures can be seen in greater detail than in Figure 5.

Of Light Sources and New Imaging Systems

Light sources for imaging technology in vision research will move away from gas lasers toward solid-state laser systems, predicts Schepen's Ann Elsner. However, the solid-state systems must be low noise and able to maintain any existing noise over the entire spectrum to avoid Moire patterns. "Most of our needs are in the low power range," she says. "We need compact, well-behaved lasers." Access to stable lasers that produce good quality beam shapes would open up "a range of new research," Elsner notes.

In the next five years, adaptive optics will continue to produce new ways of seeing. "A major effort in the future will be to use real-time correction of the eye's wave aberration in conjunction with confocal imaging. No one has done this yet," notes the University of Rochester's David Williams. With confocal SLO, one plane is selected and light from all other planes is rejected. "You want as compact a point of light as is possible imaged on the retina, and to achieve this you need to get rid of the eye's aberrations. This work is a very natural extension of adaptive optics."

Adaptive optics "will make confocal SLO more valuable because once adaptive optics are applied, we'll be able to distinguish microscopic structures at different depths in the retina," says Williams. "By combining adaptive optics and confocal imaging of the retina, a new class of imaging systems will emerge," Williams predicts.

In clinical trials in Holland, the nerve fiber analyzer detected glaucoma damage with a specificity of 93%. The widely used "air-puff" glaucoma test to measure intraocular pressure has a 30% specificity.

Portable digital ophthalmoscope

In late August 1997, LDT, which specializes in inexpensive, compact, user-friendly scanning laser ophthalmoscopes, received a $750,000 Small Business Innovation Research grant from the U.S. Department of Defense to continue developing a prototype for a portable digital ophthalmoscope for the U.S. Army. Prior to the grant, LDT demonstrated that the device could acquire digital images of the fundus taken through undilated pupils, assess visual acuity, and take external ocular photos in real time. Although the main application of the device is to transmit images and information from a field location to a remote station during combat, Dreher points out that the technology could offer high quality eye care to individuals in remote rural or urban settings that lack access to such care.

In Vivo tumor assessment

"In vision research, people have lived with the same resolution since Helmholtz," says Dirk Uwe Bartsch, director of the Retinal Imaging Laboratory at the University of California at San Diego's Shiley Eye Center. "The main criticism of new imaging techniques has been their limited resolution, due to field size, when compared to film."

To overcome this limitation, Bartsch and colleagues from Rensselaer Polytechnic Institute transferred and adapted software for 3-D microscopy used in biology to vision imaging. The result is a technique that combines indocyanine green angiography (IGA) with confocal SLO and offers ophthalmologists a noninvasive way to study choroidal melanomas, and in general, the microvasculature of the retina. Prior to this technique, pathologists needed to remove an eye to determine how advanced the melanoma was and its likelihood of metastasis.

"What we've done is refresh an old idea with a new instrument and provide an *in vivo* method to examine these tumors," says Bartsch. He notes that because more than 80% of the blood supply to the eye is delivered through the choroidal circulation, detecting abnormalities in the choroidal vessels is critical. By adjusting the confocal SLO's focal plane in one diopter increments, Bartsch and his colleagues can obtain serial optical sections of choroidal tumors, which are used to calculate tumor height. Nine microvasculature patterns are known to occur in choroidal melanomas: some indicate aggressive tumors, while others indicate a more benign condition (see Figs. 5 and 6).

Between January 1994 and April 1996, 18 patients suspected of choroidal melanoma took part in a study at the Shiley Eye Center to evaluate the ability of IGA performed with the new confocal SLO to image microcirculatory patterns in choroidal melanomas; "Tumor circulation in choroidal melanomas is often difficult to recognize and the absence of this finding does not indi-

cate lack of extensive tumor vasculature," says Bartsch.

In 16 of the 18 patients, deep tumor vessels were visible and could be identified clearly within the first 30 seconds after dye injection. In 11 patients, complete serial optical sections provided tumor height measurements. This work was reported in *Archives of Ophthalmology*, December 1997.

CCDs monitor photoreceptors

The retina contains six to seven million cones of which about 10% are concentrated on the fovea and the remainder are distributed uniformly over the rest of the retina. The retina also contains about 120 million rods. Together cones and rods compose the photoreceptor layer of the retina. "When a photoreceptor collects light, it acts as a fiber optic element guiding light along the length of the cell. The waveguide properties of the photoreceptors increase the chances for a photon to be absorbed, and thus improves our efficiency at detecting light," explains Stephen Burns, a researcher at the Schepen's Eye Institute. "The photoreceptor also guides the light back out of the eye toward the pupil." Using cooled CCD cameras, Burns and his colleagues image the light distribution returning out of the pupil to determine how well the photoreceptors are functioning. "The photoreceptor layer of the retina is the most metabolic layer in the body," Burns says. "Images of this layer further our understanding not only of how this layer functions, but also of how the body reacts to disease."

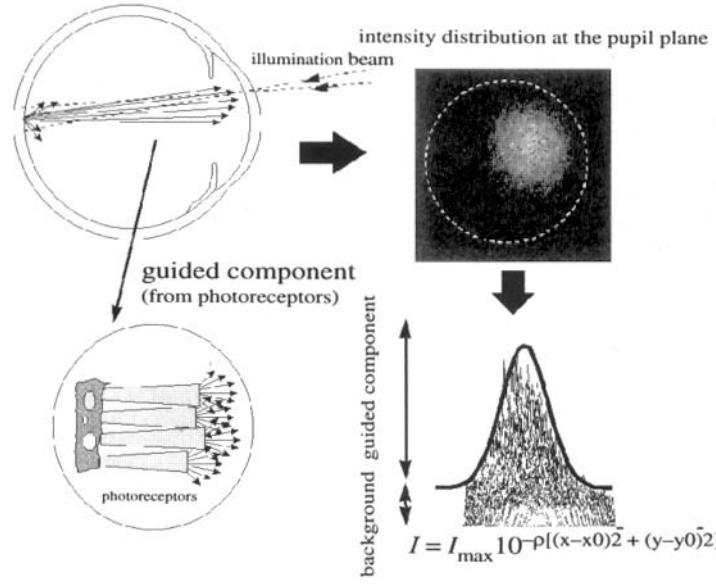

Figure drawn by Susana Marcos.

Figure 7. Waveguide properties of the photoreceptors as measured in the living human eye. A small region of the retina is illuminated with light from a single position in the pupil (top left). Light scattered or reflected from the back of the eye through the pupil has two components. One component is widely scattered and fills the pupil with a uniform background. Another component is guided toward the pupil by the waveguide properties of the cone photoreceptors (bottom left). The total distribution of light in the pupil is measured with a cooled, scientific grade CCD camera (top right). The resulting intensity distribution is then fit with a model to extract a parametric description of the waveguide properties of the cones (bottom right).

In recent work, Burns has focused on the waveguide properties of photoreceptors (see Fig. 7). For the cones to function normally, they must have the proper shape and index of refraction, as well as a full amount of photopigment, and they must be aligned with the pupil. Because normal alignment toward the pupil results from a dynamic process that can be altered by a number of retinal diseases or trauma, understanding the factors that control alignment provides a key to understanding the disease process and healing.

To measure photoreceptor directionality, a beam of light at 543 nm—strong enough to bleach more than 95% of the cone photopigments—is directed into the pupil. A cooled, 16-bit CCD camera then measures the intensity distribution of light in the plane of the pupil.[1]

"The main challenge with this work is bringing all of the technologies together," Burns says. "Building a body of knowledge to pull together the cooled CCD technology and confocal imaging takes time. In addition, more powerful computers reduce processing time." Before Burns' work, researchers relied on psychophysical techniques to study the eye's waveguide properties. "This was highly subjective and labor intensive," notes Burns. The technique he and his colleagues at Schepen's developed reduces several hours of work to about 15 minutes.

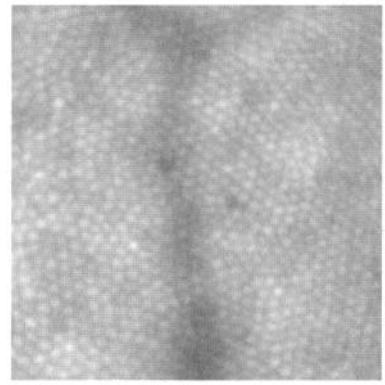

Figure 8. The retina of a living human eye taken after adaptive optical correction of the eye's optical system. The image, taken with 550 nm light, is a registered sum of 61 images taken over four days. Image size is 0.5 X 0.5 degrees (146 X 146 μm). The bright spots in the image are cone photoreceptors, each on the order of 5 μm. The darker vertical band down the center of the image is a shadow of a capillary that when focused on, is about 5 μm in diameter. The retinal location of the image is about 1° from the central fovea. The only filtering used was histogram equalization to enhance contrast.

Adaptive optics add another dimension

Historically associated with astronomy, adaptive optics offers a double benefit to vision research: sharper vision—even for individuals who do not wear glasses—and better retinal imaging. "With adaptive optics we can remove the optical blur from the eye," says David Williams, William G. Allyn Professor of Medial Optics at the University of Rochester's Center for Visual Science. "A new method of measuring this blur will help us develop a new kind of contact lens that corrects aberrations not remedied by conventional lenses." Better intraocular lens designs and improved monitoring of patients following refractive surgery will also result, Williams predicts.

In addition, by removing aberrations for light that leaves the eye, Williams and his colleagues are able to resolve single retinal cells such as cone photoreceptors. "This is the first demonstration of using adaptive

optics to resolve microscopic features of the retina, and we still have a long way to go," notes Williams. "So far we've achieved a two-fold improvement in resolution over current methods to image the fundus" (see Fig. 8, page 27). Williams and his team report on their work in the November 1997 issue of *JOSA A*.[2]

Two technologies critical to the success of adaptive optics are wavefront sensors and deformable mirrors. Joseph Bille and his colleagues at the University of Heidelberg first demonstrated that a Hartmann-Shack wavefront sensor could be used to measure the eye's wave aberration. The sensor delivers a small spot of laser light to the retina and the light returning from the retina is used to determine the eye's aberrations. "If the eye's optics were perfect, then all of the light rays leaving the pupil from a point on the retina would be parallel," says Williams. "Aberrations bend rays as they pass out of the eye so that they are no longer parallel, and this bending can be measured at each point in the pupil with the wavefront sensor." Thirty-seven pistons attached to the back of a deformable mirror move independently from one another and provide a total range of motion of about 4 μm. "If the mirror is shaped in just the right way, the bent rays leaving the pupil reflect from the mirror so that they are parallel again and the aberrations are corrected," says Williams.

"Concerning the practical clinical use of these new technologies, one can only be encouraged by what we have learned thus far."

To improve the wavefront sensing/adaptive optics compensation system, two elements are needed: a real-time wavefront sensor and a less expensive deformable mirror. "Aberrations change over time," says Williams. "With the current setup, 20 minutes can pass during the iterative processes making it difficult to study aberrations over time. If we have a wavefront sensor that operates at 30 Hz, the aberrations don't have a chance to change."

As for the deformable mirrors, their current cost of $45,000 makes them impractical for widespread use in clinical settings. "It's not likely that a $100,000 ophthalmic instrument that contains such an expensive element would make it in the commercial market," notes Williams. Several groups, including Meadowlark Optics and SY Technology, are developing less expensive adaptive optics technologies that rely on liquid crystals and micro-electro mechanical systems (MEMS) mirrors, respectively.

The adaptive optics imaging techniques also promise to extend the ability to study disease. "Before you had to develop an animal model, sacrifice the animal, and then piece together what was occurring," explains Williams. "Now, we can view the progression of disease on a microscopic spatial scale in the living eye."

MRI maps visual cortex

"Functional magnetic resonance imaging (MRI) offers a way to see things we've never seen before," says Brian Wandell, a researcher at Stanford University's neuroscience program. Previous methods to map the brain such as evoked potentials, in which electrical activity in the brain is measured, and positron emission tomography (PET), which tracks the flow of oxygenated blood in the brain, broke new ground in their day, but also suffer from several drawbacks. Evoked potentials provide good temporal resolution but poor spatial localization, while PET imaging is limited because an experiment on one individual can only be run once. "Generally, one must average the results over a population of brains," explains Wandell. "Because brains are very different shapes, the averaging process cuts the already modest spatial resolution significantly."

With MRI, the same brain can be examined time and again. "You get a better signal-to-noise ratio and can do types of studies that are impossible with PET," says Wandell. The improved signal-to-noise can be used to acquire MRI images at a spatial resolution of 10 mm^3, a 100-fold improvement over conventional PET's 1-cm^3 resolution. The MR scanner tube that patients lie in doesn't hinder the use of strong stimuli, says Wandell. "We can't use a TV because of the high magnetic field strength, but liquid crystal displays that project images inside the bore of the scanner or binoculars to look at displays beyond the tube are helpful."

The visual cortex, the part of the brain responsible for vision, can be described at different spatial resolutions: individual neurons, clusters of neurons that form columns, and collections of columns called areas. Researchers have identified 30 visual areas in the brains of monkeys. "The visual areas in the human brain are not well mapped," says Wandell. "Using functional MRI, the first four to five have been identified and more will be shortly. After that, the trend over the next five years will be to improve resolution so that the columns can be measured."

Wandell and his colleagues have reported on the use of functional MRI to determine how color is represented in the brain.[3] They measured the stimulation response function for color in a known part of the brain. "The surprise was that the response was very powerful in the early part of the brain," notes Wandell. To continue this work, Wandell and his group are using functional MRI to examine the effect of variables such as ambient light and motion on how the brain processes color information.

Looking ahead

"Concerning the practical clinical use of these new technologies, one can only be encouraged by what we have learned thus far," asserts Robert Weinreb, a glaucoma specialist and chairman of the University of California at San Diego's Department of Ophthalmology. "Not only will we be able to diagnose disease at an earlier stage, but these techniques will allow us to intervene earlier and prevent disease progression." Weinreb cautions, however, that "unless new techniques are used in clinical research studies to validate measurements and compare new results with gold standards, one should not use them to make clinical decisions." So far, confocal SLO is the only technique being tested in a longitudinal long-term study. The Ocular Hypertensive Treatment study, supported by the National Eye Institute, is assessing the effectiveness of a confocal SLO to diagnose glaucoma at the earliest possible stage. The study includes several hundred patients at leading glaucoma centers in the U.S.

Despite the best designs and most innovative technologies, some imaging techniques may never get beyond the laboratory. "The greatest challenge to the new imaging techniques is acceptance by insurance carriers," contends Andreas Dreher. "The technical issues are all solvable. The greatest hurdle is getting insurance companies to reimburse for the tests. They have to be able to determine if the results that the test offers are worth paying for."

Editor's note

The OSA topical meeting, Vision Science and Its Applications, will be held February 6–9, 1998, at the Eldorado Hotel in Santa Fe, N.M. For details contact 202/416-1980; confserv@osa.org.

References

1. S.A. Burns *et al.*, "Variations on photoreceptor directionality across the central retina," J. Opt. Soc. Am. A **14**, 2033–2040 (1997).
2. J. Liang and D.R. Williams, "Aberrations and retinal image quality of the normal human eye," J. Opt. Soc. Am. A **14**, 2873–2883 (1997); Liang *et al.*, "Supernormal vision and high-resolution retinal imaging through adaptive optics," J. Opt. Soc. Am. A **14**, 2884–2892 (1997).
3. S. Engel *et al.*, "Color tuning in human visual cortex measured using functional magnetic resonance imaging," Nature **388**, 6637 (1997).

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