New Sight for Old Eyes

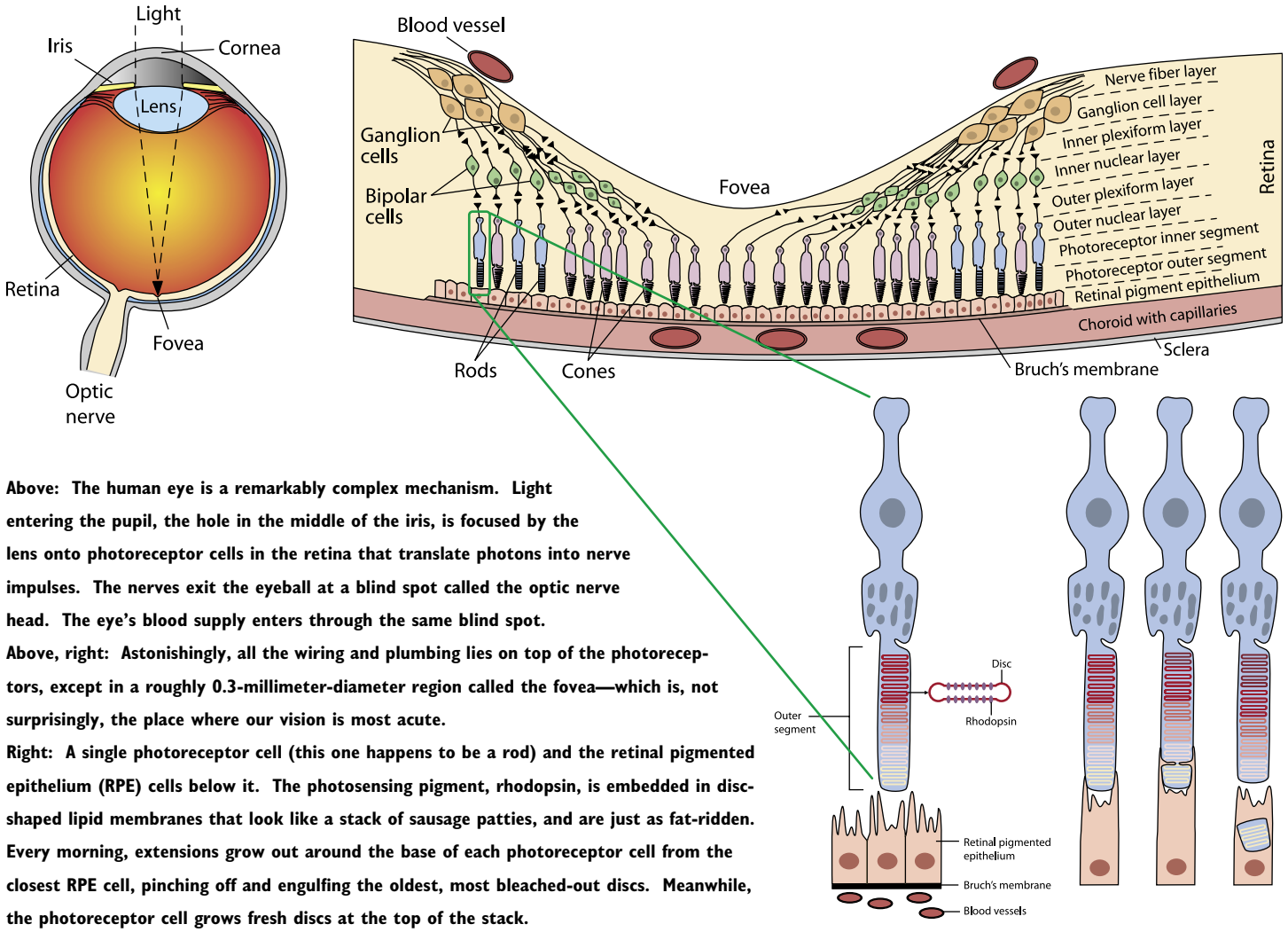
by Scott E. Fraser

Our research team as we might appear through the eyes of someone with advanced age-related macular degeneration, or AMD. Back row, from left: Assistant Professor of Electrical Engineering and Bioengineering Changhuei Yang, biology staff member Changjun Yu, Professor of Chemical Engineering Julia Kornfield (BS '83, MS '84), and grad student Jeff Fingler (MS '03). Front row: Member of the Professional Staff Mike Tyszka, biology staff member Jon Williams, Fraser, and Dan Schwartz, an associate professor of ophthalmology at UC San Francisco. Obscured in the AMD fog: Nobel Laureate Bob Grubbs, the Atkins Professor of Chemistry.

My mother leads a very active life, and she has near-perfect vision. In a few years, however, she may be forced to give up driving, and eventually even reading, as what is now just sort of a fuzzy zone in the middle of her field of view becomes a black hole. Like many of her friends, my mother has age-related macular degeneration, or AMD, a progressive eye disease affecting the area of the retina that gives us our clearest, most detailed vision. AMD begins by distorting and blurring what we see, and can lead to the loss of sight in the center of the field of vision. While none of us will die *of* AMD, a huge fraction of us will die *with* it. It's the leading cause of vision loss in people over 50 in the Western world. As many as 10 million Americans have AMD now, but the numbers will reach staggering proportions as the baby boomers reach their 60s and 70s. So a couple of years ago a group of us here at Caltech, and Dan Schwartz, an ophthalmologist at UC San Francisco, assembled a team to tackle this problem.

AMD affects a region of the retina called the macula, the "bulls-eye" where the lens focuses the light rays. The macula is densely packed with light-receptor cells in order to give very sharp vision. It seems odd, but the photoreceptors are actually at the back of the retina, so before any light reaches them it has to first pass through several layers of nerve cells, blood vessels, and other tissues. To minimize the effects of the intervening cells on our highest-resolution vision, there is a small sunken area in the center of the macula where many of the nerve cells are pushed to the side and there are no overlying blood vessels. This area is called the fovea, and it is packed tight with "cones," the photoreceptors that sense colors. But, as you can see in the diagram below, there is a complication to this arrangement—the fovea's blood supply must come from the back of the retina, and that's perhaps the key to understanding what goes wrong in macular degeneration.

The photoreceptors are the retina's most metabolically active cells. Their outer segment is basically a stack of thousands of discs containing a pigmented protein called rhodopsin, and when this pigment absorbs light, it sends a signal through the inner segment and then, via other nerve cells, to the optic nerve that leads to the brain. Being a photoreceptor is rough, because it's bombarded with light all day long—each rhodopsin molecule absorbs count-



Above: The human eye is a remarkably complex mechanism. Light entering the pupil, the hole in the middle of the iris, is focused by the lens onto photoreceptor cells in the retina that translate photons into nerve impulses. The nerves exit the eyeball at a blind spot called the optic nerve head. The eye's blood supply enters through the same blind spot.

Above, right: Astonishingly, all the wiring and plumbing lies on top of the photoreceptors, except in a roughly 0.3-millimeter-diameter region called the fovea—which is, not surprisingly, the place where our vision is most acute.

Right: A single photoreceptor cell (this one happens to be a rod) and the retinal pigmented epithelium (RPE) cells below it. The photosensing pigment, rhodopsin, is embedded in disc-shaped lipid membranes that look like a stack of sausage patties, and are just as fat-ridden. Every morning, extensions grow out around the base of each photoreceptor cell from the closest RPE cell, pinching off and engulfing the oldest, most bleached-out discs. Meanwhile, the photoreceptor cell grows fresh discs at the top of the stack.

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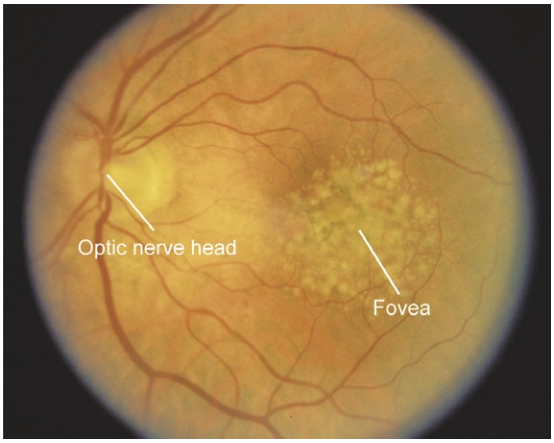
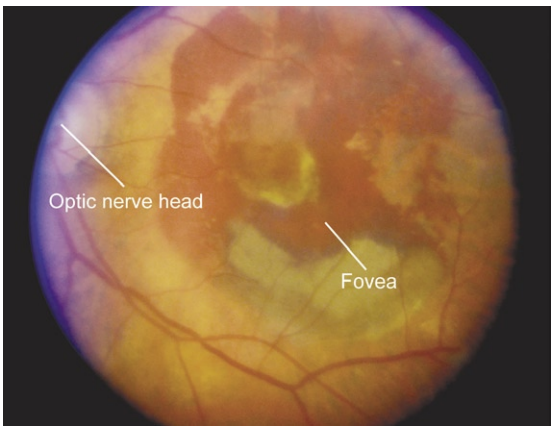


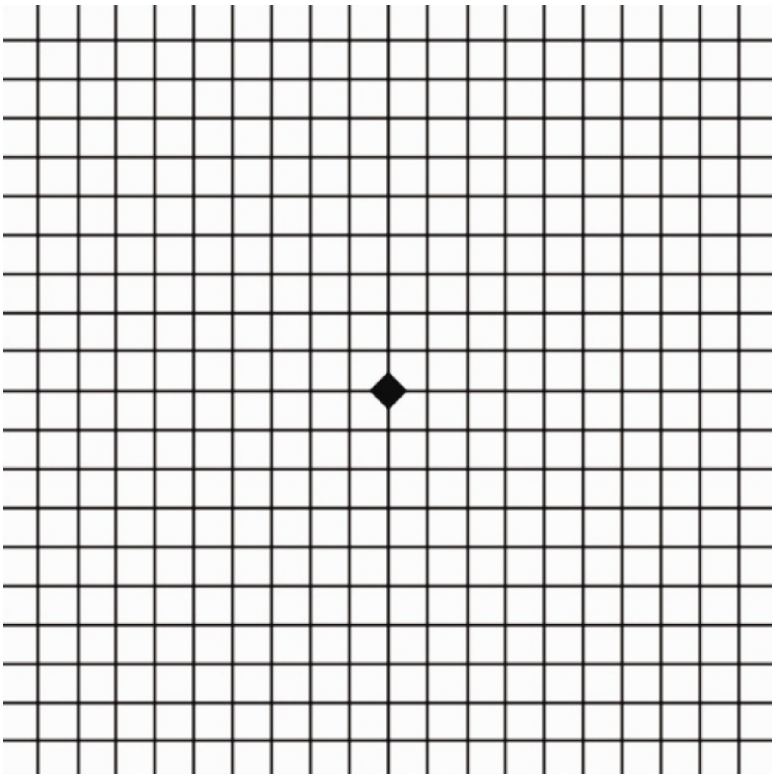


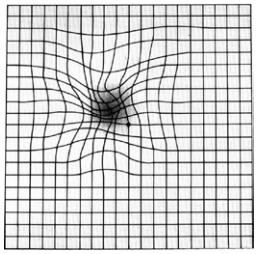
Top: The central part of a retina in the early, or dry, stages of AMD. The yellow spots are fatty deposits called drusen. Bottom: A retina with wet AMD in an advanced stage. The red regions are leaking blood, and it's likely that many of the the photoreceptors therein have died. This person would retain his or her peripheral vision, yet be legally blind—whenever this eye focused on an object, it would disappear into a black hole. These images were made with a fundus camera, which is essentially a low-power microscope designed for looking into the eye.

less photons each hour and must regenerate itself after each photon with fresh retinal, a derivative of vitamin A, before it can absorb another. With time, this huge influx of light bleaches the visual pigment in the same way that a brightly colored piece of fabric laid out in the sun loses its color, so every morning the oldest discs—about 10 percent of the outer segment—are pinched off the back end of the photoreceptor and eaten by the cells of the retinal pigmented epithelium (RPE), while new discs are added to the inner part of the segment. The RPE cells do more than take out the garbage; they also deliver nutrients and the molecules of retinal needed to regenerate the visual pigment, so they're important support cells for the photoreceptors and need to be in close contact with them.

There are two forms of AMD. In the early, or "dry," form, cream-colored plaques called drusen are deposited in the macular region. Drusen are located within Bruch's membrane, a crucial matrix at the base of the RPE cells that separates the retina from the blood vessels. These fatty deposits build up naturally with age, and many people over 50 have some. But if they get out of hand, they can block the diffusion of nutrients, and even potentially oxygen, from the underlying blood supply to the retina. Usually, patients with dry AMD are able to read just fine; however, many have difficulty with night vision and give up driving under dimly lit conditions.

With time, the disease can advance to "wet" AMD, which is much more serious. It begins when new blood vessels start to grow into the retina to try to resupply the starving photoreceptors. To understand where these new blood vessels come from, we have to go back to the early 1970s. Judah Folkman, a surgeon at the Children's Hospital, Boston, realized that when tumors were first forming, they didn't have a very good relationship with the blood vessels around them, and so they grew very slowly. After a while, vessels did grow out to supply the tumor with blood, and as soon as that happened,





Above: An Amsler grid to self-test for AMD. (The small grid at right shows what an AMD sufferer might see.) To use the grid, hold the magazine 12 inches away, and cover one eye with your free hand. Look at the central dot with the other. Repeat the test with the other eye covered. If you cannot see all four corners of the grid, or if some of the lines are blurry, wavy, or missing, call your eye doctor.

the tumor grew explosively. Folkman proposed that the tumor cells' need for oxygen was causing them to give off a growth factor that spoke to the nearby blood vessels and caused them to grow, and he identified it as vascular endothelial growth factor (VEGF), which, as we now know, also plays a major role in wound healing. The new blood vessels that grow in response to VEGF are more fragile, thinner, and less organized than normal ones, and they leak. This is useful if we want to deliver a chemotherapeutic agent into a tumor—the agent is injected into the bloodstream and leaks out around the cancerous cells—but it's very bad when such blood vessels grow up through Bruch's membrane to underlie the RPE cells. These abnormal vessels leak blood and fluid that damage the macula, inevitably replacing it with scar tissue.

What can you do if you're in the early-onset stage of dry AMD and want to protect yourself from wet AMD? The best advice is to avoid strong sunlight by wearing sunglasses, and to give up smoking, because heavy smoking has been shown to increase the chance of developing wet AMD. Certain patients with larger drusen, or who are already noticing some visual loss, can benefit from multivitamin tablets containing high levels of antioxidants and zinc, which are available in drug stores. In a 10-year Age-Related Eye Disease Study (AREDS) sponsored by the National Eye Institute, such tablets significantly reduced the risk of advanced AMD and vision loss. A number of other, ongoing, clinical trials are showing promising signs that multivitamin tablets containing the antioxidant lutein may also be effective. Lutein is found in dark-green leafy vegetables such as broccoli, kale, and spinach, but it's not easy to eat enough greens to have any effect on AMD—tablets are better.

Regular self-testing is also a good idea, so that you'll spot differences in vision when they first occur. The brain combines information from both eyes to generate our visual scene, and uses details viewed by one eye to fill in for missing or distorted vision in the other. Thus it's sometimes easy to miss that there's something wrong, so to self-test for the earliest defects, you have to check one eye at a time. A simple test to detect AMD, the Amsler grid, was developed by eye doctors who noticed that people with the undiagnosed condition were coming in and saying that their venetian blinds suddenly looked wavy. We've provided one on this page so that you can do the test right away, but there are also plenty of websites on AMD that feature them. If you do see wavy lines, you might want to book an early appointment with your ophthalmologist.

At present we don't have any foolproof method for preventing the earlierst blood-vessel growth in patients with AMD. The AREDS-formula multivitamins are the only early-stage treatment available, and their effectiveness is limited. Treatment of the later-stage wet form requires repeated injections of drugs that block the VEGF pathway and make the new blood vessels regress. These injections have to go directly into the eye, which

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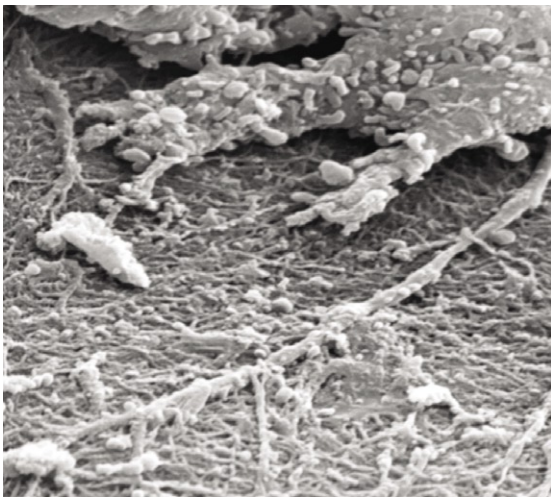
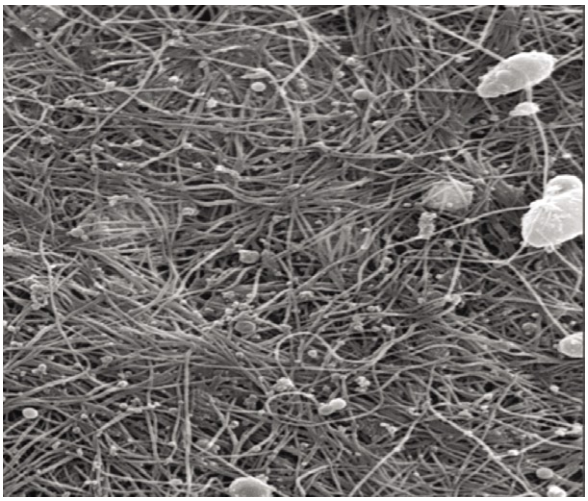
can be painful and may cause complications such as infection, hemorrhaging, and cataracts. The drugs used are either very high-affinity antibodies that bind to the VEGF and remove it, or anti-VEGF aptamers, which are short chains of RNA that grab the VEGF and inactivate it. People are looking at other delivery methods, such as putting little minipumps inside the eye to dose it continuously. But once retinal cells have died, injecting anything is unlikely to bring them back, so these treatments are best thought of as a means to arrest rather than reverse the vision loss. This makes early detection of AMD a critical component of the treatment plan.

Studies of the anti-VEGF drugs shows that they stabilize vision in 90 percent of the cases, and that the earlier the treatment begins, the better the outcome—about 30 percent of patients even see modest improvement. Therefore, if we could catch people in the early stages of wet AMD while their sight is still good, these drugs might save their reading and driving vision. Unfortunately, there are no means for early detection of the abnormal blood vessels, and most people with wet AMD don't see their eye doctors until they've had significant visual loss. So our group is developing an instrument to help find those abnormal vessels in the earliest stages of wet AMD, often before the patients are even aware of any visual problems. I'll return to that subject later.

There's still a lot of dispute about the underlying cause of AMD. Some people argue that the drusen are to blame, citing the similarity of their protein components to the protein plaques seen in the brains of Alzheimer's patients. However, those plaques have not been proven to be a cause of Alzheimer's, and could simply be one of its effects. Other people, including our team, believe that AMD is the result of fat accumulating in Bruch's membrane—the support and filter system I mentioned earlier.

This fat comes from those light-sensing discs in the outer segments of the rods and cones. Each disc has its rhodopsin embedded in a fat-based, or lipid, membrane. When 100 to 200 of those discs are pinched off each photoreceptor every morning,



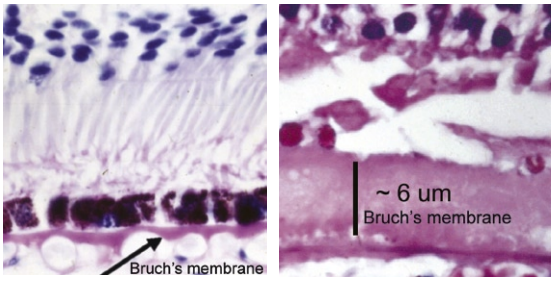


Right: A scanning electron microscope image of an elderly Bruch's membrane. Fat drips off the collagen fibers like rusticles from the *Titanic*'s deck rails. The large RPE cell at the top of the image is pulling its feet away in disgust. Far right: After the membrane has been bathed in alcohol, all the fibers are clean again, forming a nice open mesh for nutrients to pass through. Images courtesy of Ilene Sugino.

the nearest RPE cell has to eat this fatty breakfast. And with thousands of individual photoreceptors in the macula area, it's worse than being on Elvis's bacon diet. The RPE cells then have to clear this fat out into the blood vessels running behind the retina, and they have to do it through Bruch's membrane.

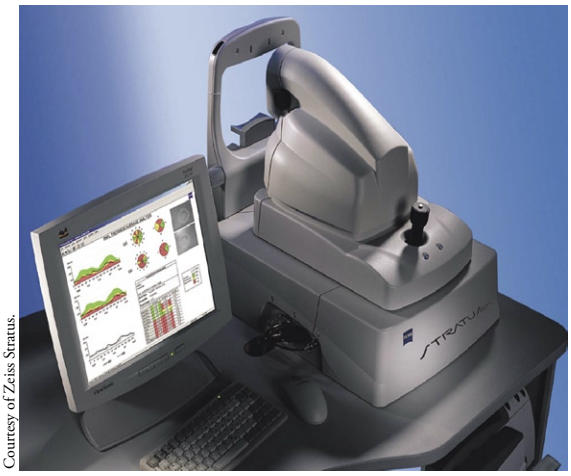
This membrane is a very thin matrix made of the sort of connective tissue that makes skin tough, and in some ways it resembles the stacked layers of multi-ply toilet paper. The theory is that some very small fraction of the lipid molecules get stuck as they pass through it, so that by middle age a good amount of fatty goo will have built up. And that's a problem, because this membrane is the meeting ground for all the retina's nutritional and waste-clearance activities. Once the mat is clogged with fat, it can't do its work as well. In fact, in the photo at far left on the opposite page, you can see that one of the RPE cells is pulling back its processes, as it's not happy touching the membrane any more. As dry AMD progresses, as seen in the photo on this page at far right, Bruch's membrane gets thicker and thicker and creates a barrier between the blood supply and the RPE cells. This fatty barrier may cause the overlying retinal cells to become oxygen-starved, secrete VEGF—triggering wet AMD—and slowly start to die.

When our collaborators, Marco Zarbin and Ilene Sugino of the University of Medicine and Dentistry of New Jersey, dissolved the fat away from Bruch's membrane with ethanol, a healthy-appearing membrane was restored—a nice mat of open fibers that oxygen, water, and all the other nutrients can move through easily. But before you think this is another good reason to drink red wine, you should know that a very high concentration of alcohol was used, and it was done on a cultured membrane from an excised eye. It's not a treatment many people would want. That's why our group is working on a better way of assessing the health of Bruch's membrane and treating the fat deposits.



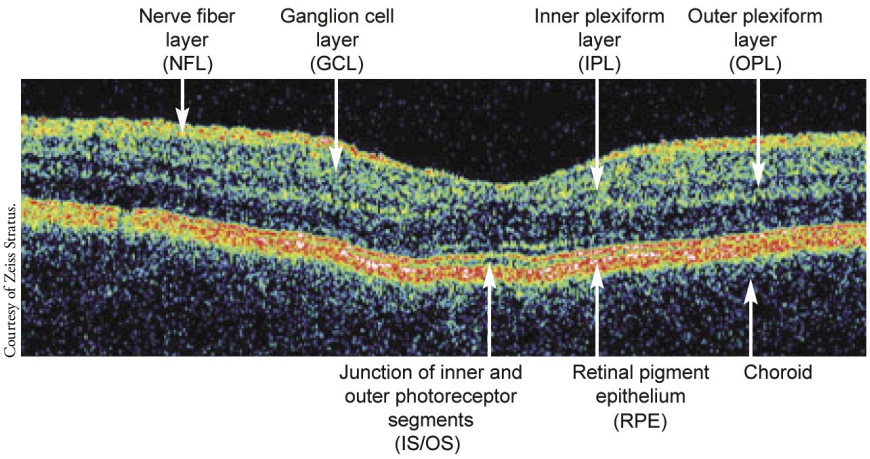
A healthy Bruch's membrane, seen above left as the purple-red layer below the very dark RPE cells. (Another function of the RPE cells is to absorb any light that gets past the photoreceptors. If the RPE cells begin to die, people can actually be bothered by the glare of light bouncing off their own retinas!) This membrane is one or two microns (um, or millionths of a meter) thick. In advanced cases of AMD, the fat-swollen membrane is several times thicker, as seen above right. Images courtesy of Dr. W. Richard Green at the Johns Hopkins Medical Institute.

To develop better imaging instruments for eye doctors to use, Member of the Professional Staff Mike Tyszka, biology staff member Changjun Yu, and I began by looking into the eye of a mouse with our magnetic resonance imaging (MRI) machine. MRI detects the nuclei of hydrogen atoms, so we could see the water moving in and out of the retina, and we could see the fluid in the eyeball because of its molecules' random movements. We also showed that with the right sort of MRI machine, it's possible to look into the eye and tell if there's a defect in retinal permeability. But since the chances of an eye doctor having a $3-million machine in the office to screen for AMD are zero, we decided to use a different approach and adapt a machine that's already used to look for retinal



Courtesy of Zeiss Stratus.

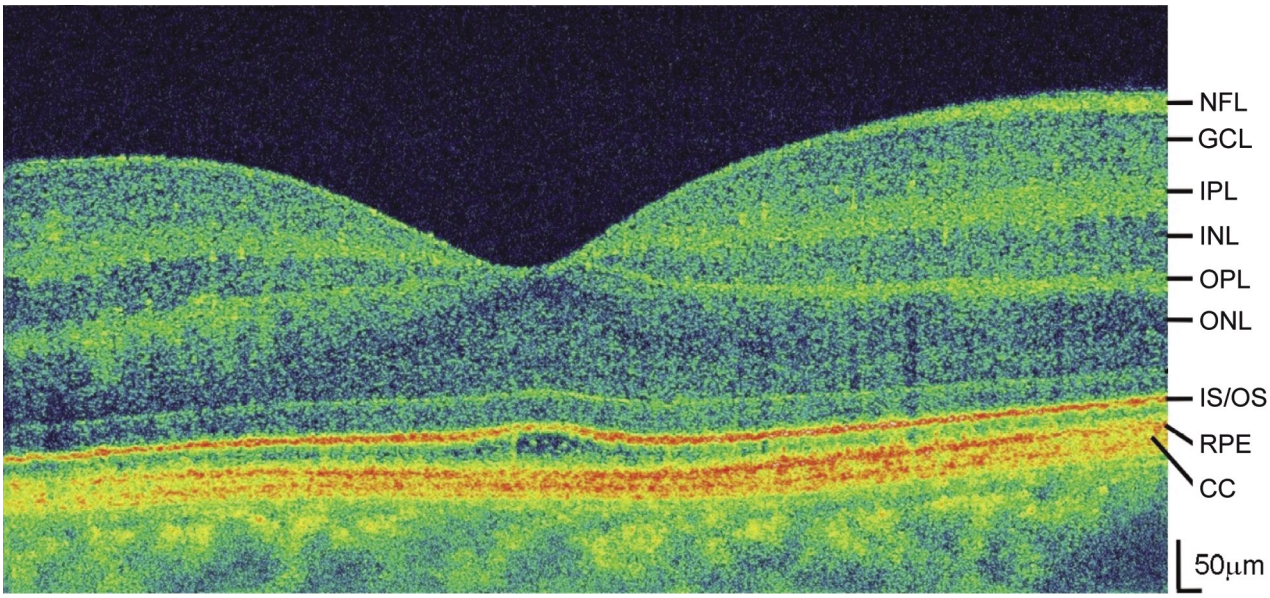
The Stratus optical coherence tomography (OCT) machine, above, produces images like the one below, in which the retinal layers with the highest reflectivity appear in red. (The central dimple is the fovea.) An OCT image from a half-million-dollar machine built at MIT gives much better resolution, as seen at bottom. INL stands for inner nuclear layer, ONL is outer nuclear layer, and CC is the choriocapillaris, a layer the Stratus could not distinguish from the RPE cell layer.



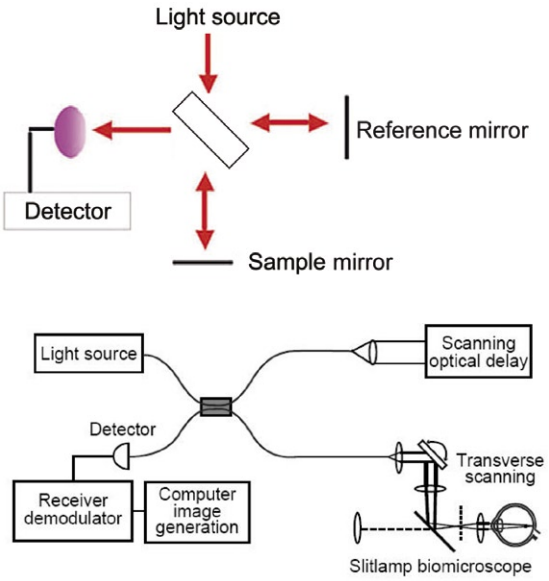
Courtesy of Zeiss Stratus.

holes, swelling, and detachment—the Stratus OCT. Made by Carl Zeiss Meditec, this machine uses optical coherence tomography (hence the OCT) to image the retina.

An OCT such as the Stratus is basically a modified Michelson interferometer. A laser's light is split into two beams that are bounced off mirrors and recombined at a detector, where they create interference fringes. Because these fringes depend on the relative distances the two beams have traveled, if one of the mirrors is moved, the fringes change. This exquisitely sensitive method detects a movement by either of the mirrors of even a fraction of the wavelength of light. To turn the interferometer into an OCT system, the laser is replaced by a light source with a broad bandwidth, usually a superluminescent diode. These diodes emit light of many wavelengths, so the "coherence"—the width of the region of space where all



M. Wojtkowski, et al, *Optics Express*, Vol. 12, No. 11, 2004, © 2004 Optical Society of America.



Top: How interferometry works. Light from a single source passes through a partially silvered mirror that reflects some light and transmits the rest. One beam bounces off a reference mirror a fixed distance away, while the other travels a varying distance to the sample mirror. When the two beams are recombined, the interference fringes tell you how much the sample mirror has moved. Bottom: The same idea can be adapted to your eye.

the peaks and troughs can align to make interference fringes—is quite narrow. (Most lasers, by contrast, have a coherence length that can be measured in meters, and so give fringes over a very broad range of mirror movements.) The diode only produces interference if the path difference between the reflected beams is less than half the coherence length—about 15 microns, or 15 millionths of a meter—producing an image with very high depth resolution.

If we replace one of the mirrors with someone's eye, we can record the light that reflects off the different retinal tissues at different depths and build up a 3-D image. It's almost like having an ultrasound machine, but one based on light instead of sound.

The Stratus produces images good enough to see if the retina is swollen or has a shallow retinal detachment, but we're aiming to do better. Some university labs working on OCT can already generate images with much higher resolution, but their usefulness is limited because the instruments cost as much as half a million dollars. We're trying to make a very inexpensive version that can generate images that are just as good, and two members of my lab, grad student Jeff Fingler (MS '03) and biology staff member Jon Williams, working with Assistant Professor of Electrical Engineering and Bioengineering Changhuei Yang, have already created systems that give comparable resolution for one-tenth the money. It's looking very promising.

Most OCT systems take images of slices through the depth of the retina, so if we want to identify blood vessels growing through a particular retinal layer, we have to take a lot of slices and examine each one for a blood vessel. Although the fastest systems can take as little as one second for a scan, the procedure usually has to be repeated many times before getting a well-focused image, because most people aren't very good at keeping their eyes still for that long. It's not their fault—eyes are always darting about and can't fixate on one spot for an entire second. But by turning the slices on their sides and taking an image of an entire retinal layer all at once, we can speed things up. Instead of imaging one small slice of the retina at different depths, then imaging the slice next to it, and so on, we scan from one side of the retina to the other all at one depth and then repeat the process at different depths, which creates an image more than 100 times faster. With colleagues Dan Petersen and Richard Haskell at Harvey Mudd College, we've developed a microscope that can image such constant-depth slices,

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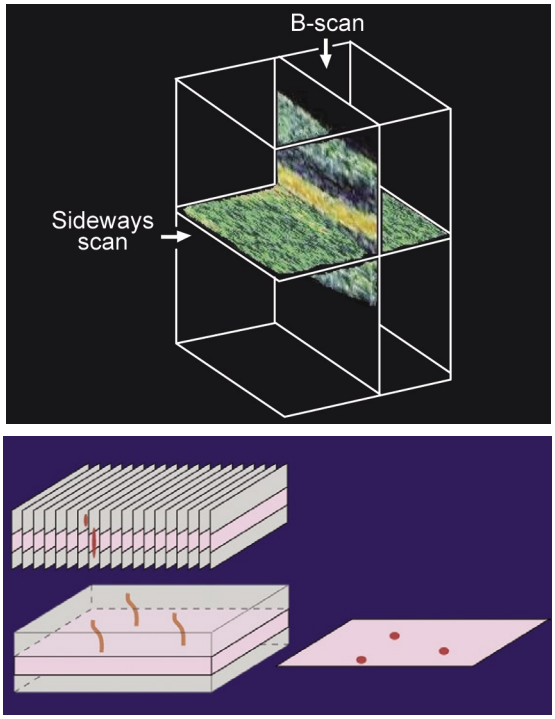


Top: A variable-depth scan, called a B-scan in a term borrowed from ultrasound, versus a constant-depth scan. Bottom: It's much easier to find blood vessels when you are scanning across them rather than along them.

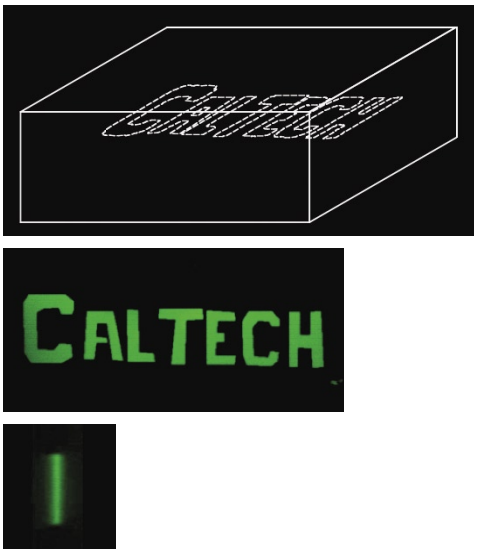
which we're now refining for medical use as part of a Stratus-like instrument. The eye examination will be much quicker, and if there are just a couple of blood vessels coming through, we'll have a good chance of seeing them.

Even so, blood vessels don't really show up well in standard OCT images. We're going to improve that by putting the machine into Doppler mode, which sees moving particles, such as those in the blood. It works very much like a radar gun, analyzing the changes in frequency of the fringes between successive scans. The blood vessels really jump out, and it's even possible to tell the direction of flow.

So we're coming close to developing an inexpensive system that will let doctors see those tiny new blood vessels that are the tell-tale signs that you have an early form of wet AMD and need to get treatment. Better still, while looking at the retina with our Doppler OCT, we've seen that fat-clogged tissue actually moves in a different way than normal. The healthy fibers are in constant motion, buffeted by the water molecules around them, and the entire membrane undulates like a sheet of tissue paper in a light breeze. As fat builds up, the tissue becomes stiffer. So the same machine should be able to tell us whether fat is building up in Bruch's membrane.

Of course, knowing where this fat builds up is only going to be useful if we have a way to get rid of it, and this is something we've been working on with Caltech's latest Nobelist, Atkins Professor of Chemistry Bob Grubbs, and Professor of Chemical Engineering Julia Kornfield (BS '83, MS '84). Simply injecting something that binds to fat and dissolves it won't work. There's a lot of fat in our body that we wouldn't want to be rid of, like the fatty sheaths around our nerve cells. Curing someone of macular degeneration while dissolving their brain is not going to be a great treatment. A better solution would be a targeted drug that only dissolves the "bad" fat in the membrane.

Finding a fat-dissolving chemical that will only



The AMD team put Caltech's name up in lights, albeit very tiny ones. At top is a schematic of the fluorescein-laden epoxy slab showing where the activating laser was focused. At center is the result, as seen from above, and at bottom is a side view showing how thin and uniform the fluorescein uncaging was.

bind to that particular region of the body is proving to be very difficult, so we've chosen another approach, called photoactivation, in which the drug is kept inside a chemical "cage" until hit by a light source that disintegrates the cage and frees the drug. The idea is to inject a patient with a caged molecule, then shine a laser light through the eye onto selected areas of fatty deposits in Bruch's membrane, using our Doppler OCT machine to see exactly where those deposits are. To ensure that no "useful" fat is accidentally dissolved near the target area, we'll use a technique called two-photon activation. To open the cage, two photons have to be absorbed by it simultaneously, something that only happens when a huge number of photons are concentrated in one place. While single-photon activation would uncage the drug anywhere along the beam path, two-photon activation will be localized at the focal depth of the incoming light.

We've tried this technique on a solid slab of epoxy with a caged fluorescein dye in it, and focused the two-photon uncaging laser on different positions at constant depth to spell out "Caltech." As you can see, the shapes of the letters look pretty good. But the impressive—and very important—thing about this is that, when looked at from the side, we've only activated a very, very thin sliver of the dye—in this case approximately five microns thick. Bruch's membrane in older people becomes that thick or more, so this technique can be used to target only the fat in the membrane, and no other tissues above or below it.

By removing the barrier fats, we aren't going to be able to give you the Bruch's membrane of an 18-year-old, but we really don't have to. All we need to do is to restore the membrane's permeability to the point where there's good nutrient and waste flow. If we can change the permeability of a 70-year-old membrane back to the way it was at 50, it will be good enough to nip AMD in the bud. □

Scott Fraser is the Anna L. Rosen Professor of Biology and professor of bioengineering. He is also the director of Caltech's Biological Imaging Center. Fraser earned his BS in physics from Harvey Mudd College in 1976, and his PhD in biophysics from Johns Hopkins University in 1979. He and his hyphenate, Marianne Bronner-Fraser, the Ruddock Professor of Biology, have coauthored two children, much to the delight of his mother, who can see them just fine so far.

This article was adapted by Barbara Ellis from Fraser's Seminar Day talk last May.

Bob Grubbs, the missing member of the macular degeneration team.

PICTURE CREDITS: 22, 31—Bob Paz; 23, 30 — Doug Cummings