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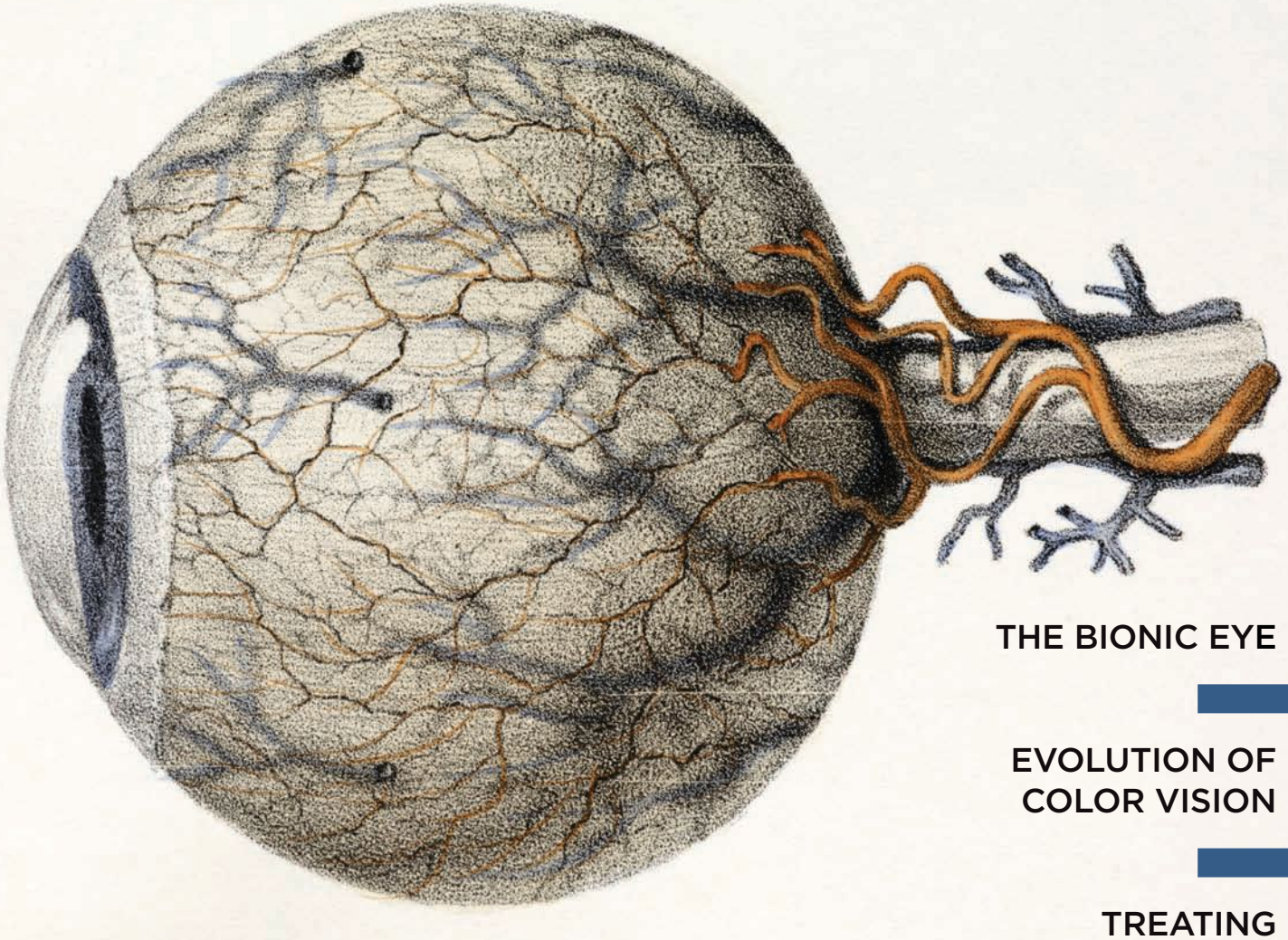

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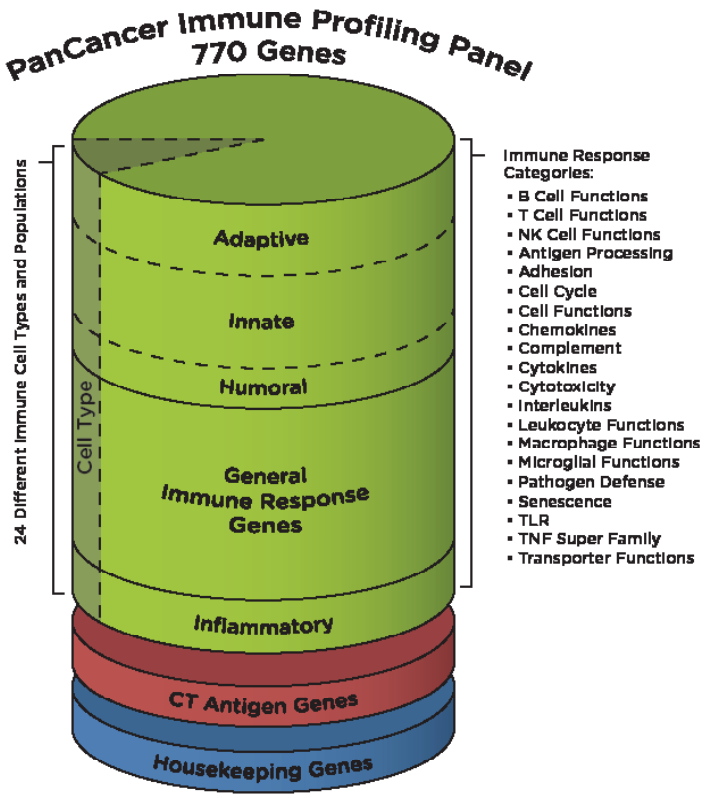

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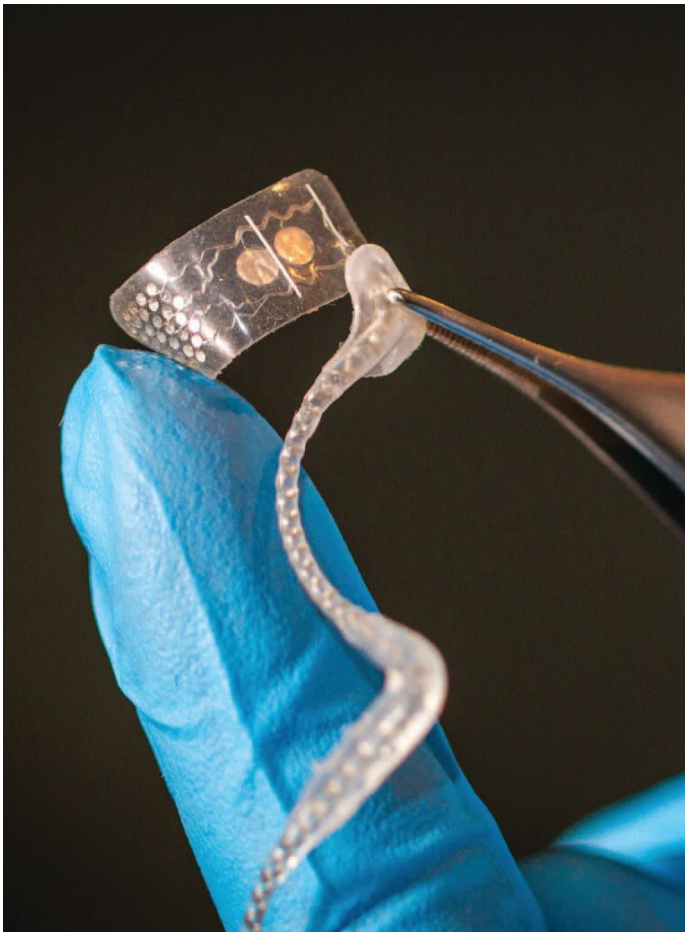

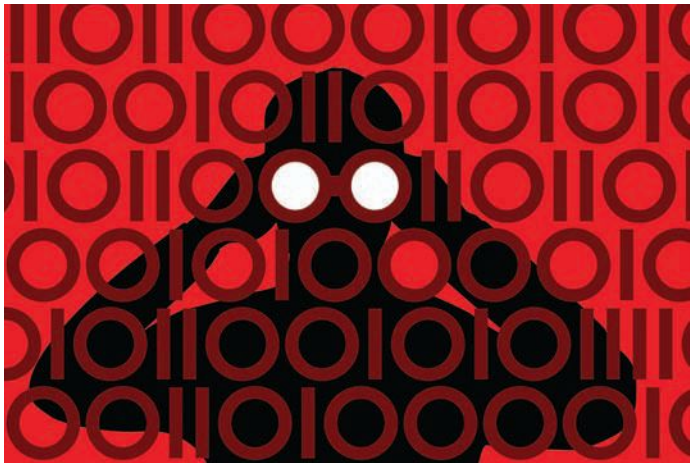

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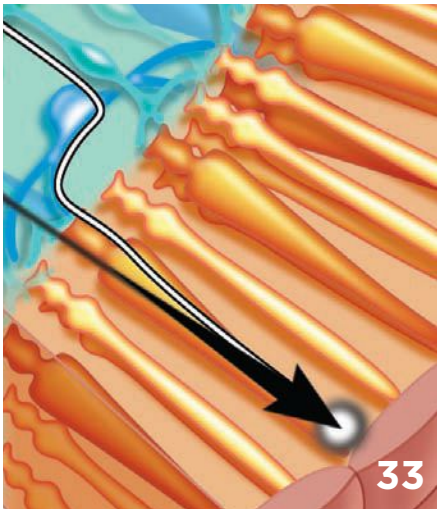

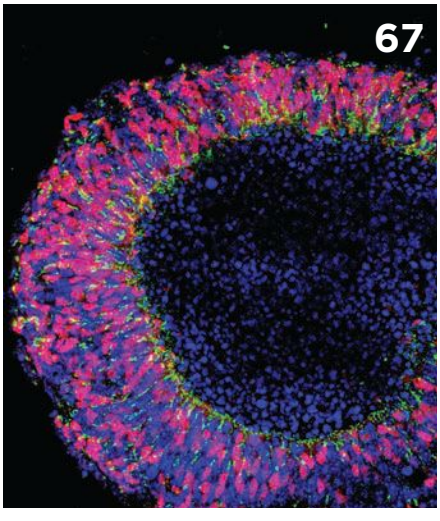

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CORRECTIONS:

In "Chagas Watchdogs" (*The Scientist*, September 2014), a credit is missing for the right-hand photograph. The credit should read Gabriel L. Hamer.

The fish pictured on page 40 ("On the Other Hand," *The Scientist*, September 2014) is *Arapaima gigas*. The correct spelling of the lungfish species mentioned is *Neoceratodus forsteri*.

The Scientist regrets the errors.

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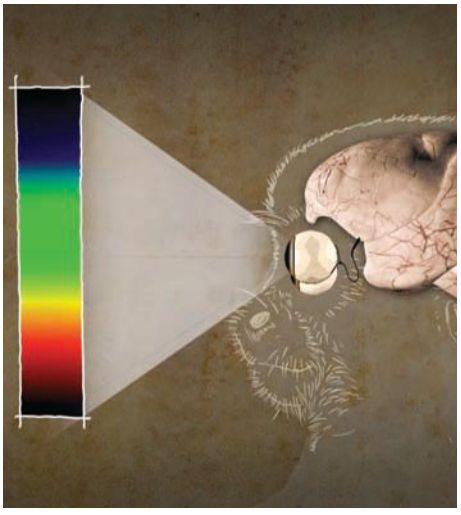

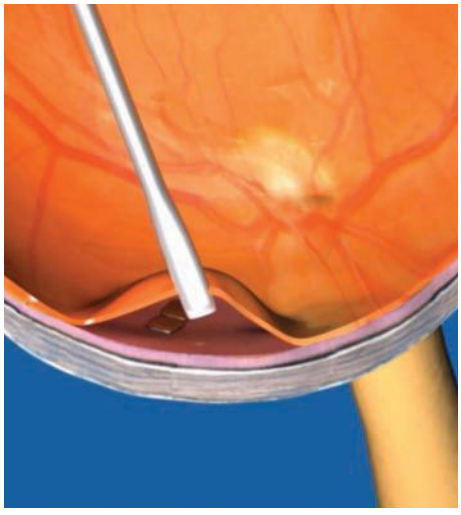

THIS MONTH AT WWW.THE-SCIENTIST.COM:

VIDEO
The Human Color Palette
Journey to the lab of Jay Neitz, whose work on the evolution of color vision is featured in Kerry Grens's feature article "The Rainbow Connection."

VIDEO
Flashes of Light
Hear Australian Diane Ashworth, one of the world's first recipients of a bionic eye implant, describe the experience.

VIDEO
Pulses of Light
Learn about the photovoltaic retinal prosthesis from one of its developers, Daniel Palanker, who coauthored a feature article on the bionic eye.

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HERE'S WHAT YOU'LL FIND IN NEXT MONTH'S ISSUE:

- Annual Salary Survey
- Neuroprosthetics
- The neuroscience of face perception
- Circadian synapses
- A profile of Marcus E. Raichle

AND MUCH MORE

DIANE ASHWORTH PHOTO COURTESY OF BIONIC VISION AUSTRALIA'S BIONIC EYE PROJECT

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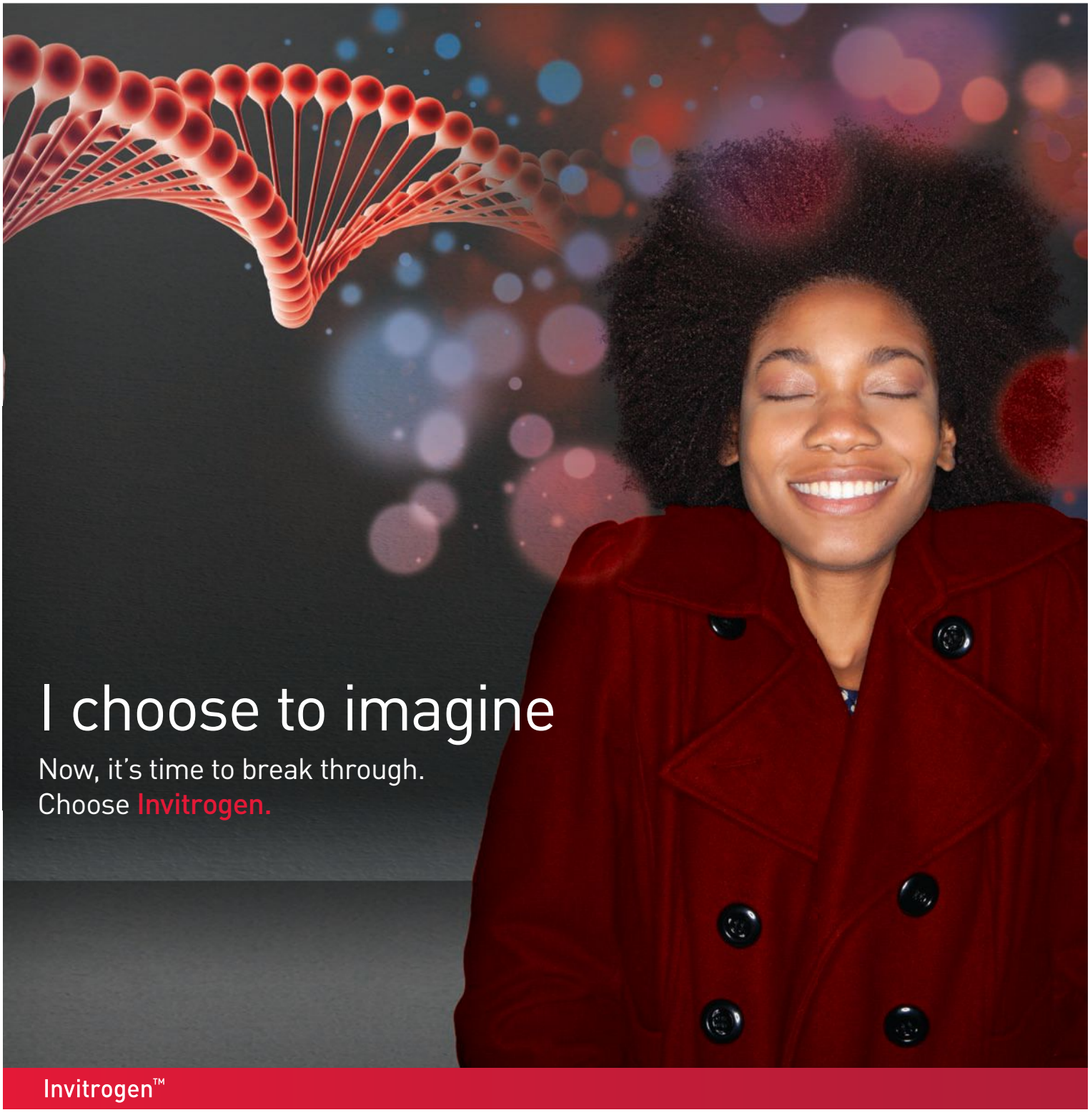

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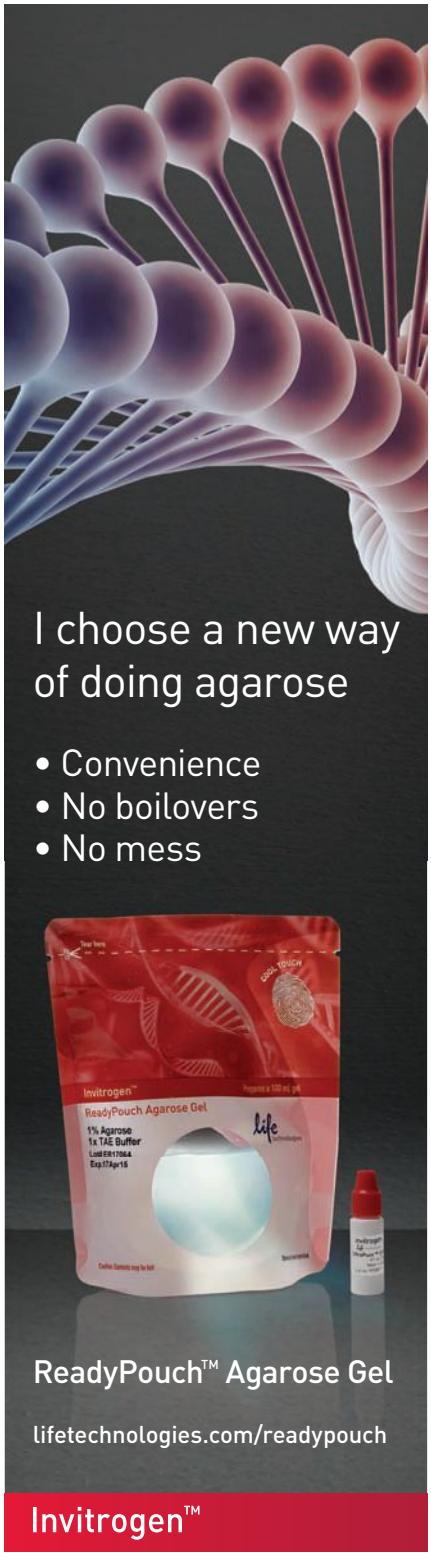

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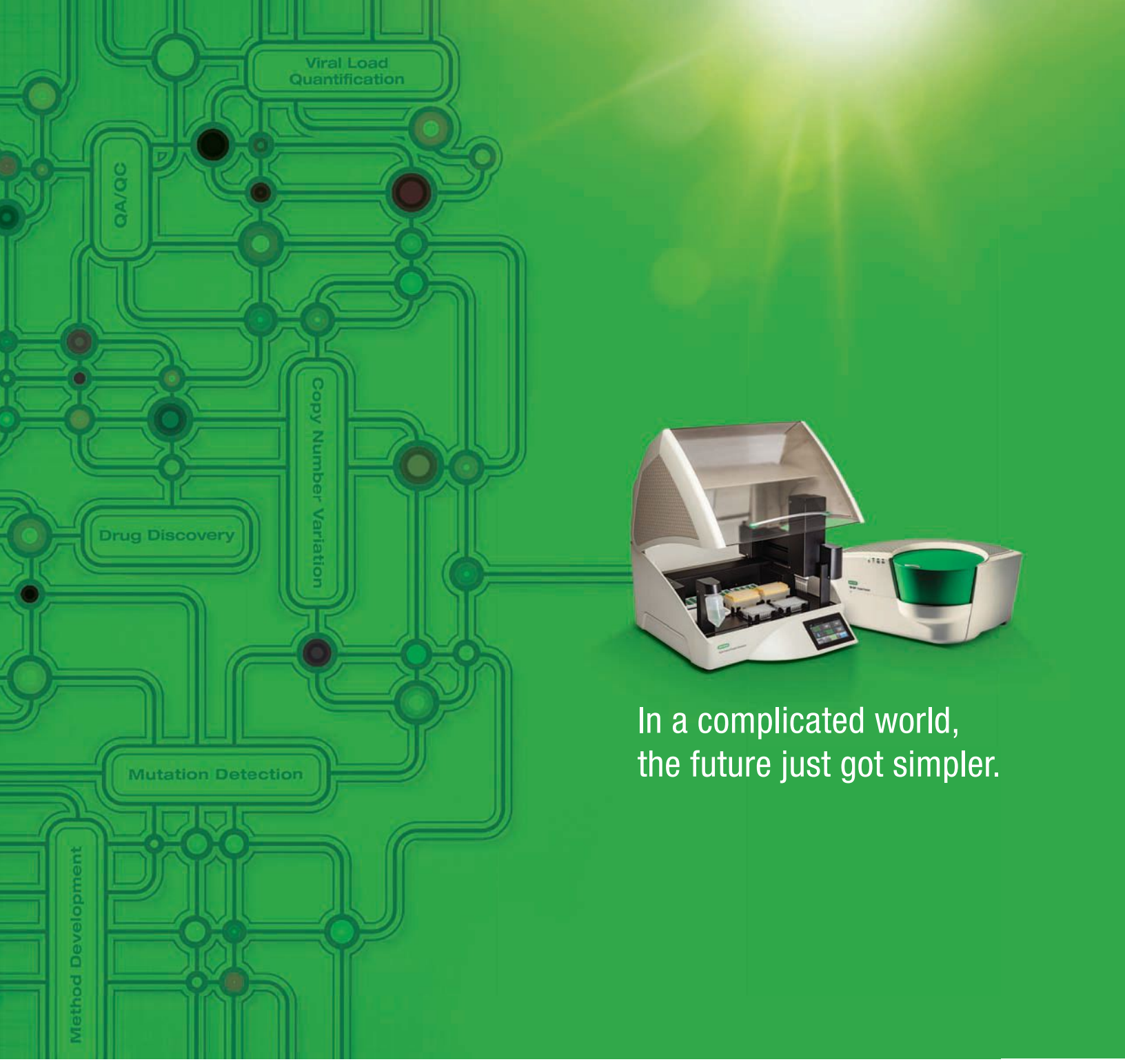

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Contributors

Emily Monosson began exploring chemistry under her mother's sink, mixing home cleaning liquids together to fashion "insect killers" and mystery solutions. "I was always fascinated by these chemicals that could kill you," she recalls. Since then, her childhood love of chemicals has blossomed into a career as an environmental toxicologist.

Monosson studied biology as an undergraduate at Union College in Schenectady, New York, and earned a PhD in biochemical toxicology at Cornell University. She describes herself as "a scientific vagabond," as balancing her scientific interests and family has led her to juggle writing, teaching at local colleges, and consulting as an independent toxicologist. Monosson began blogging about chemicals that were in the news at *The Neighborhood Toxicologist*, a process that eventually led to her first book, *Evolution in a Toxic World*. A chapter in that book sparked her second book *Unnatural Selection: How We Are Changing Life, Gene by Gene*, which focuses on how quickly evolution can occur in response to antibiotics, pesticides, herbicides, and even chemotherapeutics. In "Sleep Tight" on page 71, Monosson describes the resurgence of pesticide-resistant bed bugs. "It's important to think about how the chemicals we use are having these insidious effects on other life around us," she says.

It wasn't until *TS* correspondent **Ruth Williams** had completed her postdoctoral studies that she finally accepted her lot in life as a science writer. "All the evidence was there, from my A levels in biology, certainly in university, and then beyond," she says. "I just kept masochistically ignoring the signs." Even during difficult times, Williams did not desert the bench. When her PhD advisor at King's College London quit unexpectedly, Williams found a new lab. When nuclear-transfer experiments kept failing during her postdoc at the Medical Research Council's Clinical Sciences Centre in London, she repeated them again, and again, and again. But writing always tugged at her, and she eventually listened, taking a position as an editor at *Nature Reviews Neuroscience* after completing her postdoc. "Two weeks into the job, I was like, 'This is the job for me.' It was like I came home." Shortly after, she moved to New York to become a news editor for two journals, and now enjoys the flexibility of freelancing. Her tenacity and love of science is evident in her contributions to *The Scientist*, tracking down breaking science news for the-scientist.com each week and digesting cutting-edge techniques in her entertaining and informative Modus Operandi articles every month (page 31).

Anna Azvolinsky published her first story about science while working as a full-time consultant. Trained as a biologist, she earned a BA from the University of Pennsylvania, and during graduate research at Princeton studied how cells ensure DNA replication occurs accurately, using baker's yeast as a model system. After earning her PhD in molecular biology, Azvolinsky moved on to a postdoctoral position at Memorial Sloan Kettering Cancer Center and then to consulting with Goldman Sachs and other firms, before turning to full-time science writing.

In her work as a correspondent for *The Scientist*, both in print and online, Azvolinsky particularly enjoys talking to scientists about the significance of their findings, whether it's breaking news or the arc of a long and fruitful research career. "It's a way of looking behind the process, at how people who are super-successful have got to that point," she says. "It's not always clear to the lay public how science is done. I strive to make that process more transparent."

Azvolinsky's work has also appeared in *Nature Medicine*, *LiveScience*, and the *Journal of the National Cancer Institute*. In this issue, she profiles vision researcher John Dowling in "An Eye for Detail" on page 56.

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Eye Spies

An issue highlighting advances in vision research

BY MARY BETH ABERLIN

To give a blind man an image / is to give something so tenuous it can be infinite / something so vague it can be the universe." Literary giant Jorge Luis Borges penned these lines in a poem toward the end of his life, and he knew whereof he spoke, having gone completely blind when he was 55 years old after suffering from progressive vision loss beginning in his 30s. Borges lived for 31 years in total darkness.

Devoted to vision and the researchers who study it, this issue continues our annual in-depth consideration of one of the senses. So far, we've covered taste (2011), touch (2012), and smell (2013). Several of the articles deal with research aimed at actually giving "a blind man an image." In "The Bionic Eye" (page 34), four different groups of scientists explain how, from tenuous beginnings, they are refining prostheses for implantation at different locations, from particular layers in and around the retina to the brain. The descriptions of the devices, a number of them already approved or in clinical trials, are fascinating, and ongoing progress in miniaturization and design offer new hope for restoring some level of sight to the blind.

In "Eyes on the Prize" (page 67), Jeffrey Perkel reports on stem cell therapies for treating conditions such as retinitis pigmentosa and macular degeneration, the severe form of which currently afflicts some two million Americans. Early clinical trials look promising, helped by the fact that the eye is small and immune-privileged as well as amenable to observation with noninvasive methods.

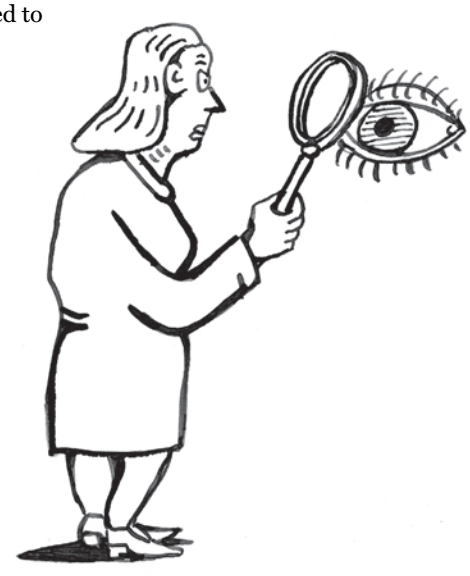
This month's Modus Operandi (page 31) describes an easier, cheaper method for obtaining ex vivo retinal recordings, which should make retinal function easier to study. And a recent publication covered in The Literature section highlights new findings on how Müller cells—glial cells in the retina painstakingly detailed by Cajal more than 100 years ago—guide incoming light, which must travel through a forest of cells before reaching the photoreceptors at the back of the retina. Our short literature reports include a story on research into how sounds activate the visual cortex and one on the role of microRNAs in the maintenance of cone cells, which modulate color vision. And in Online First (page 29), you can revisit an article detailing what is currently known about the eye's unique microbiome.

The collective brainpower devoted to giving "a blind man an image" and to understanding the mechanisms of vision on myriad levels is opening up a whole new universe.

Associate Editor Kerry Grens dissects the evolution of human color vision in "The Rainbow Connection" (page 42). It's a fascinating story and one that can be told "from beginning to end, in exquisite genetic and molecular detail." Interestingly, one researcher used his own DNA to clone a human cone opsin for the first time, and another muses about "curing" himself of the trichromatic vision humans possess by self-administering the same gene therapy he employs to give monkeys additional opsins.

This month's Notebooks (page 16) are all vision-related: What does it mean that blind cavefish can tell larger from smaller quantities of objects? That ancient sea predators had crappy vision? New image-tracking programs extract a unique fingerprint for each individual in an animal swarm. And, most mind-boggling of all, video recordings of the vibrations in plants and potato-chip bags can be processed to decode the sounds they "hear."

The collective brainpower devoted to giving "a blind man an image" and to understanding the mechanisms of vision on myriad levels is beginning to open up a whole new universe. As vision researcher and eye spy Jay Neitz puts it, "It's a brave new world." ■



ANDRZEJ KRAUZE

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QUOTES

Vision
Speaking of ^ Science

The Bishop goes on to [write about] the human eye, asking rhetorically, and with the implication that there is no answer, "How could an organ so complex evolve?" This is not an argument, it is simply an affirmation of incredulity.

—University of Oxford evolutionary biologist **Richard Dawkins** writing about *The Probability of God*, the 1985 book by Bishop Hugh Montefiore, in *The Blind Watchmaker: Why the Evidence of Evolution Reveals a Universe Without Design* (1986)

Self-organization is so mysterious. We still can't explain why the cells come together to make an eye. There must be more principles that we still don't understand yet. It's something that makes me completely in awe of life.

—The late **Yoshiki Sasai** of Japan's RIKEN Center for Developmental Biology, who grew retinal tissue from mouse embryonic stem cells, speaking with *Mosaic* in January, 2014 (Published August 26)

A lot of people with measurable vision impairment are unaware of their affliction because their brain fills in the missing pieces.

—Vision scientist **Peter Bex** of Northeastern University, on the fact that one-third of the human brain is devoted to visual processing (news@Northeastern, September 4)

When we're asked "What do the words 'red', 'blue', 'black', 'white' mean?" we can, of course, immediately point to things which have these colours—but our ability to explain the meanings of these words goes no further! For the rest, we have either no idea at all of their use, or a very rough and to some extent false one.

—Philosopher **Ludwig Wittgenstein**, in *Remarks on Colour* (1950–51)

EYE SEE!: The structure, function, and evolution of the eye has provoked careful thought and wild conjecture throughout scientific history.

In short, there is nothing about my job that makes it unsuitable for a blind person. Of course, there are inherent risks in the fieldwork; I have been stung by rays, struck down by stomach cramps, and detained by police who mistook me for an operative trying to overthrow the government of their African country. All field scientists have similar experiences. The blind, no more than the sighted, must act sensibly and with appropriate caution.

—**Geerat Vermeij**, an evolutionary biologist at the University of California, Davis, in a speech delivered at the convention of the National Federation of the Blind in Chicago about the challenges of doing biology field work (July, 1988)

Ninety percent of the blind people on our planet are in the poorest developing countries. And eighty-five percent of that could be prevented or easily treated.

—University of Utah ophthalmologist **Geoff Tabin**, who has performed thousands of cataract surgeries and trained surgeons in Nepal and Africa, in a NOVA video profile

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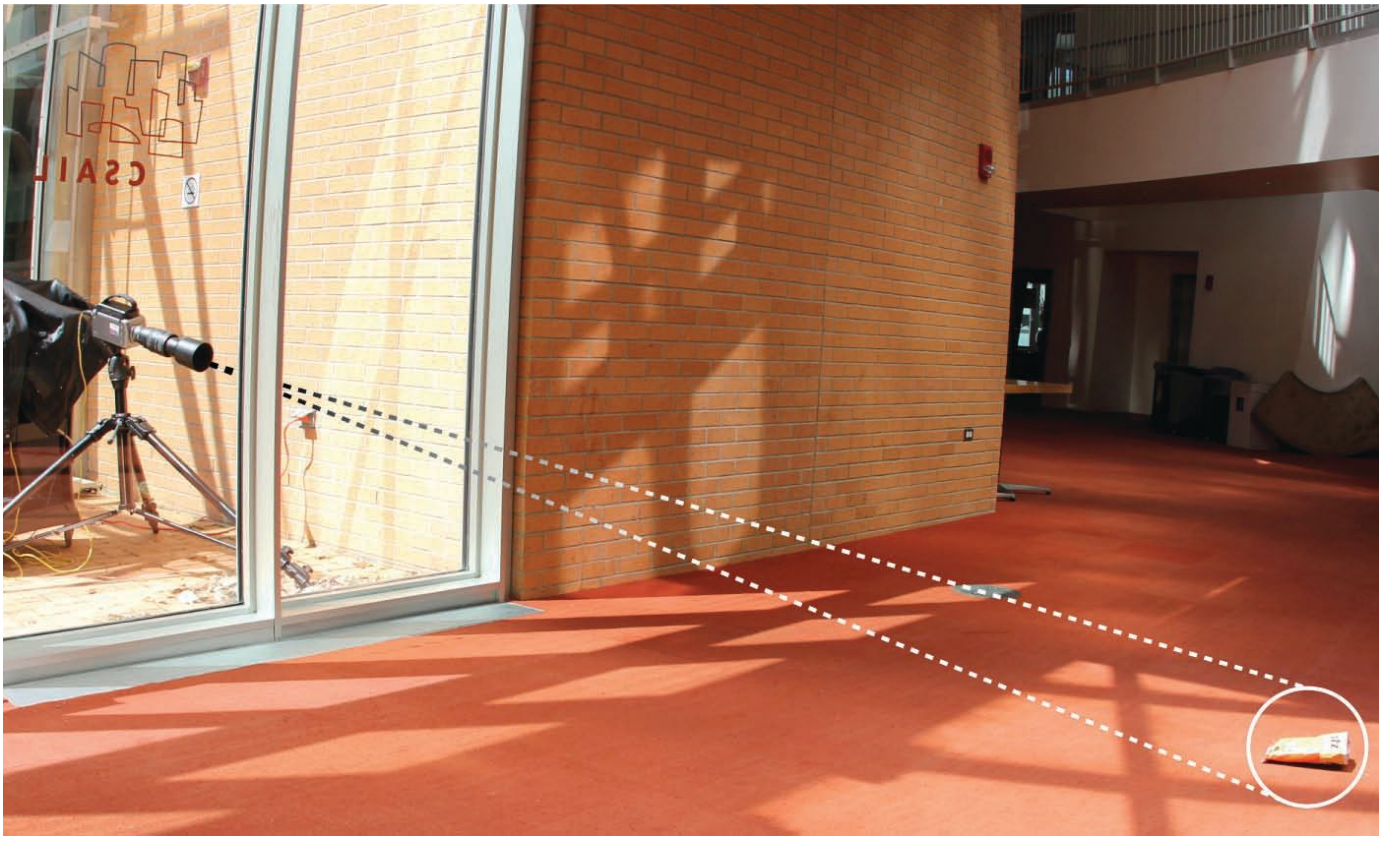
Notebook

OCTOBER 2014



ABE DAVIS

Visualizing the Vibe

Watch what you say. Nearly everything around you—from potted plants to a bag of chips—is catching your vibes. Sound waves pinging off the surfaces of these objects cause tiny vibrations invisible to the naked eye. But a group of MIT researchers led by William Freeman has devised a way to spot these movements on a high-speed video recording and use them to reconstruct the sound that triggered them. Their technique, presented at a meeting in August, effectively turns a variety of everyday objects into visual microphones.

Retrieving speech or song from footage of a shiny piece of foil may seem like the stuff of spy movies, but by magnifying minuscule movements, researchers could do some surprising, far-fetched things. "Our labs have been doing this work on amplifying and visualizing small motions in video for a while," explains graduate student Abe Davis, who participated in the recent study.

Sounds cause ripples in air pressure that can make surrounding objects move. "Sound is just a motion that travels in the fluid of air," says David Stoker of SRI International (until 1977 the Stanford Research Institute) in California who was not involved with the study. "Being able to see vibrations is a fundamental tool in doing science."

To identify the original sound that made objects vibrate, Davis and his colleagues analyzed the video recordings and calculated the amount of motion at every pixel, orientation, and scale of the image. The researchers aligned these signals to create a single, global picture of the object's motion, and finally, filtered this vibration to recover audio. Although they tested the technique on things ranging from bricks to roses, teapots to crumpled-up foil, the method worked best on well-lit, thin surfaces that provided plenty of contrast.

"I talked at a lot of inanimate objects. Chips, plates, cups, hair . . . [it was] actually pretty embarrassing," says Davis.

SEEING SOUND: Researchers recovered human speech by analyzing high-speed video recordings of a bag of chips vibrating in response to speech from a cell phone in the same room.

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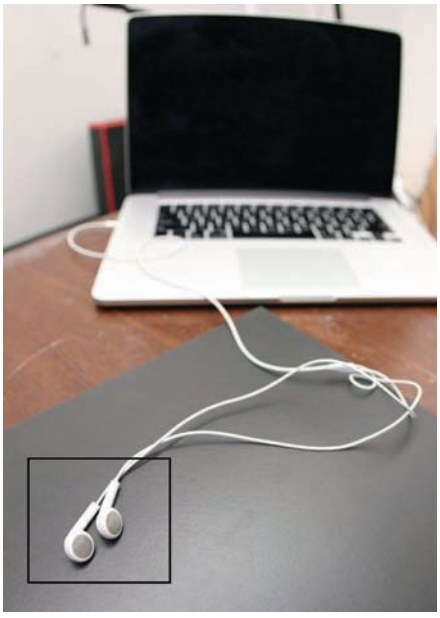

TINY MICS: Researchers were also able to use silent high-speed video recorded from a distance to identify music playing through a pair of earbuds simply by analyzing minuscule vibrations of the buds.

Refining these early results enabled the group to apply its techniques to less contrived situations. Eventually, the team reproduced the notes of "Mary Had a Little Lamb," played on a simple speaker, from the barely noticeable movements of a potted plant in the same room.

In previous work, Freeman and his colleagues extracted heart-rate data from color changes in a person's face caused by blood flow, finding that asymmetries in facial blood circulation may reveal deeper arterial malfunctions. The group also demonstrated how newborn babies' vital signs could be detected remotely by filming and amplifying the movement of blood under their skin. These techniques offer the unique advantage of being completely passive, according to Davis. Whether monitoring ambient sound or a newborn's heartbeat, all that's required is a video recording of a leaf or a cheek (*ACM Transactions on Graphics,* doi: 10.1145/2185520.2185561, 2012).

The new work is "a neat paper," says computer-vision researcher Jon Barron of Google[x] who was not involved with the study. "It's really exciting [that they] discovered and solved a problem that no one knew existed."

It's less certain whether the visual-microphone method has the diagnostic potential possessed by Freeman's earlier studies. "From a scientific perspective it's brilliant," says medical acoustics researcher Tyrone Porter of Boston University who was not involved with this project. He adds, however, that it was hard to "think of where it would be used that's more efficient than [other methods]."

Porter suggests the technique may help researchers studying physical processes in cultured cells, both microbial and human. Hints that physical vibrations—including sound—may serve as a means of intercellular microbial communication have emerged in recent years. The ideas stem from experiments that found sound waves might stimulate bacterial growth; one early study suggested that *Bacillus subtilis* produced reproducible sound vibrations. Others have suggested that electrical or electromagnetic currents may also play a role in single-cell communications. Pending confirmation, many of these hypotheses remain controversial (*Trends Microbiol*, 19:105-13, 2011).

Sound is just a motion that travels in the fluid of air. Being able to see vibrations is a fundamental tool in doing science.

—David Stoker, SRI International

"The notion of sound waves propagating between cells and that being a form of communication between cells is very unique and different," says Porter. "This [filming technique] could have applications there because trying to capture sound waves with traditional pressure transducers would just be really difficult."

Whether the visual-microphone method will allow researchers to eavesdrop on microbial chatter is still unknown. The technique is one that hadn't been seriously considered before, according to Barron. "Now that we know [retrieving sound in this way] is possible, there's a lot of excitement about what we can do in this space," he says. The most interesting applications, Barron adds, are likely to be "in the ideas it spawns that aren't necessarily obvious to us yet."

—Jyoti Madhusoodanan

One Fish, Two Fish

For two million years, *Phreatichthys andruzzii*, a species of cavefish found in Somalia, has been swimming around in utter darkness. Whatever visual abilities its ancestors possessed, useless in such a habitat, have since atrophied, making the fish a very dubious subject for studying visual tasks. But for Christian Agrillo, a researcher at the University of Padova in Italy, the blind cavefish was the perfect species to challenge whether numerical assessment abilities among fish required visual cues.

Agrillo and his colleagues had been studying the numerical skills of fish whose vision functions just fine. It turns out that fish—like many other animals—can, if not exactly count "one, two, three," at least distinguish one from three. And Agrillo's group is not the only one to observe this.

Several years ago, Gil Rosenthal, who studies fish behavior at Texas A&M University, tested whether female green swordtails (*Xiphophorus helleri*) could discriminate between shoal sizes. In his experimental setup, an individual would swim in the middle compartment of a tank. At each end of the tank was a separate compartment in which a group of green swordtails swam—one group larger than the other. The subject fish preferred to spend time near the larger shoal, but her ability to distinguish differences in number was limited. It turned out, Rosenthal found, that the ratio of the two shoals mattered immensely. At a ratio of 2:1, the fish had no problem picking out the bigger shoal—whether it was two vs. four fish or eight vs. sixteen fish. But drop that ratio down to 1.5:1 and she could no longer tell the difference (*Behaviour*, 144:1333-46, 2007). Her "assessment breaks down, and she can't

ABE DAVIS

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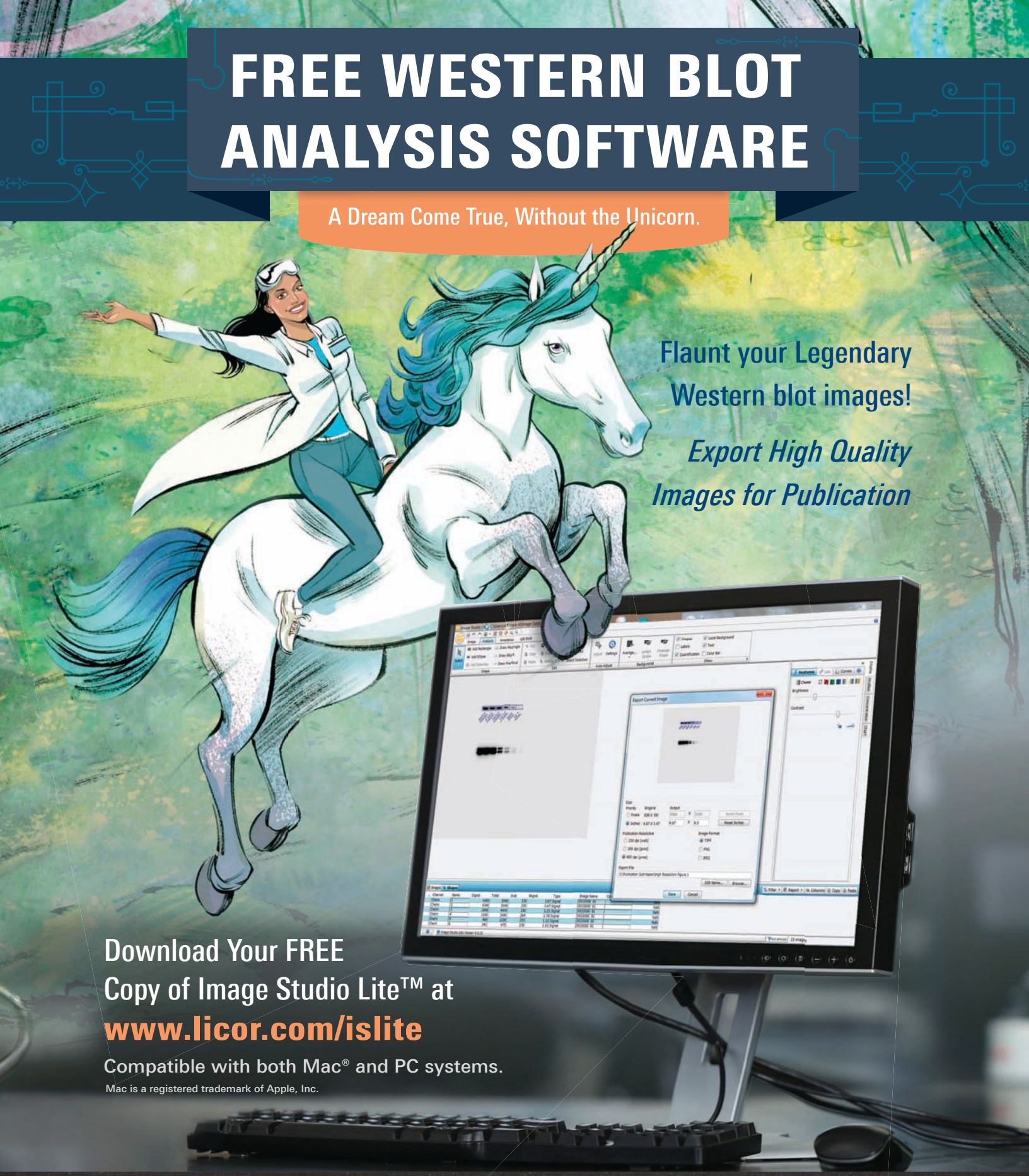

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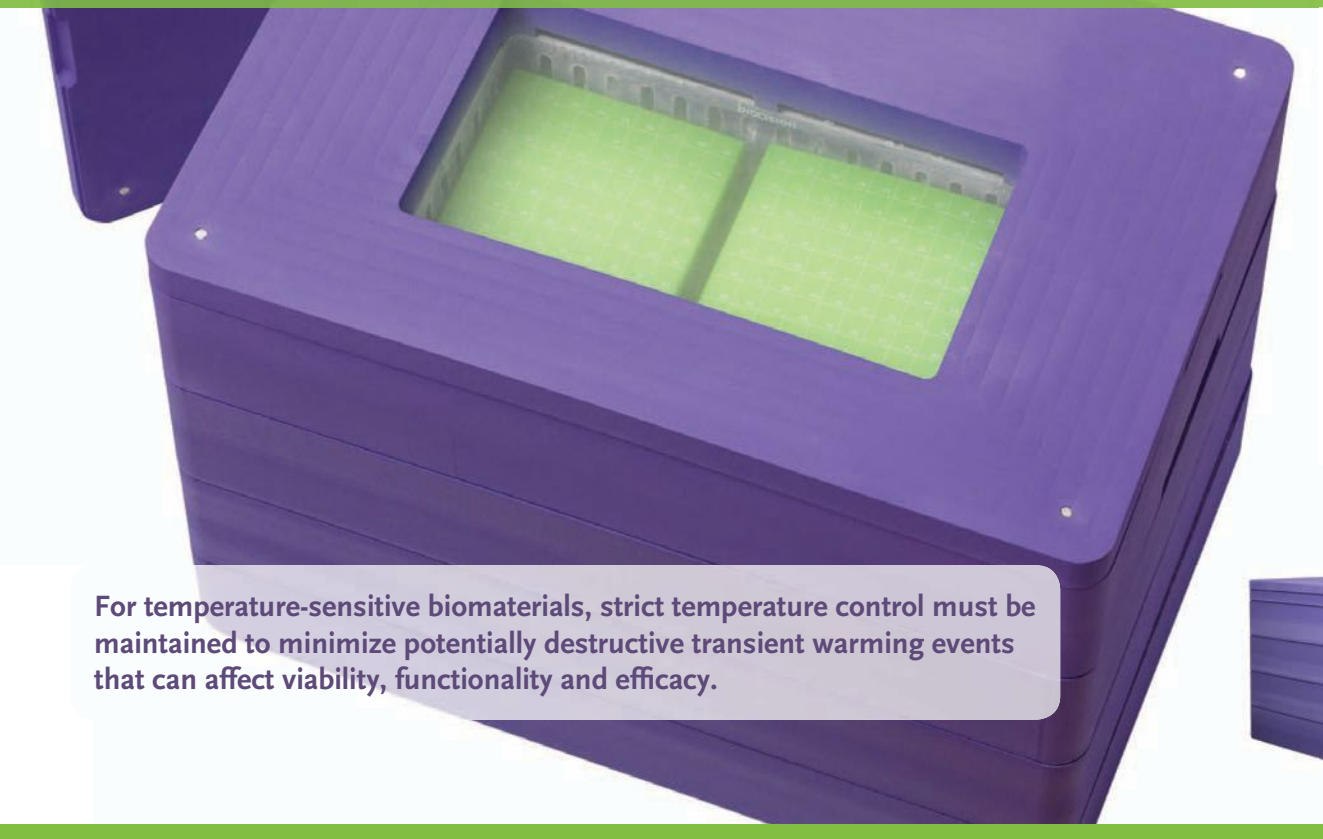

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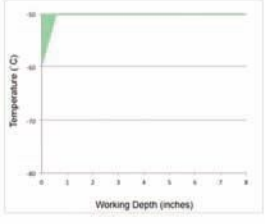

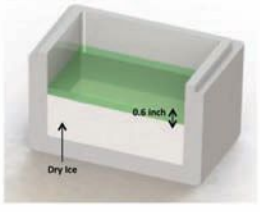

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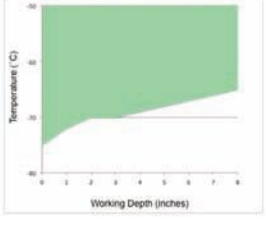

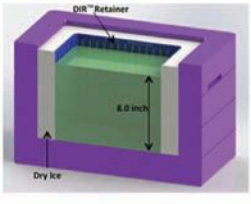

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discriminate between the two," says Rosenthal. "Where it might be beneficial to join a shoal of twelve vs. eight, they can't do it."

Robert Gerlai, a behavioral geneticist at the University of Toronto at Mississauga, and his collaborator, Luis Laplaza-Gómez of the University of Oviedo, have found similar abilities among freshwater angelfish (*Pterophyllum scalare*) (*Animal Cognition*, 14:1-9, 2011). "Always, the test case chooses the larger shoal [because] the larger shoal gives some adaptive advantage," he says, such as avoiding predators and finding mates.

It's presumed that the fish are making their decision based on visual information. Because the compartments used in such experiments are separated, olfaction and lateral line detection (a pressure-based sense conferred by pores along the sides of fishes' bodies) can be ruled out. Auditory signals are possible, but they'd have to travel through glass and air to reach the chamber where the test fish is swimming, Gerlai says.

So if the fish are calculating size from visual cues, can blind cavefish still perform this important task? To find out, Agrillo set up a circular tank with two clusters of sticks opposite one another. He trained the cavefish to swim toward the larger cluster to receive a food reward. Then he tested the animals by placing them in a tank without any food and watched where they would swim. Over and over, the fish tended to hang out by the cluster where they had been trained to find food (*J Exp Biol*, 217:1902-09, 2014). "Then we did two versus four sticks, and they solved the task," says Agrillo. "Now we know from this study that the lateral line can be used to solve quantity," he says.

The blind fish offered another opportunity: to study whether the fish are relying on volume or are actually assessing the numerical value of the group. In another set of experiments, Agrillo controlled for volume and surface area. Remarkably, the fish could still tell two objects from four objects, even if they occupied the same amount of space.

There's now evidence that a great many different animals can do some numerical tasks with varying degrees of competence.

—Brian Butterworth,
University College London

Given that the fish didn't need visual information to assess quantity, Rosenthal says, the study suggests that determining numerical value is not dependent on how the animal gets the information. "There's

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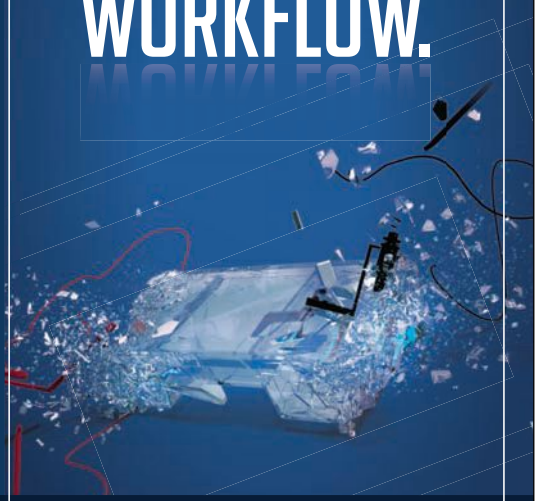

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something downstream of sensory processing where this is happening," he says. Perhaps information from the different senses is processed first by their respective brain regions, such as the visual cortex or auditory cortex, and then moves up to a higher, numerical processing center where those streams of information converge. Agrillo says he wasn't terribly surprised that sensing number is such a robust skill. "Even invertebrates with smaller brains can discriminate," he says.

Recent studies have shown, for example, that ants, spiders, and bees can "count." "There's now evidence that a great many different animals can do some numerical tasks with varying degrees of competence," says Brian Butterworth, a professor emeritus at University College London who has collaborated with Agrillo in the past. "It's not associated with the type or size of the brain or where they are in the phylogenetic tree." And scientists have shown that animals can use their other senses to do the job, says Butterworth; toads and lions, for instance, use their hearing to size up mates or intruders, respectively.

Not only is this grasp of quantity robust in its capacity to gather numerical information from different senses, but the memory of it appears to be retained. Gerlai and Gómez-Laplaza recently conducted a study in which they presented a fish with two shoals at either end of a tank. They then concealed the compartments and removed all but one fish of each shoal. When the fish was again allowed to view the compartments, it appeared to remember where the larger shoal was located and hung out at that end of the tank (*J Comp Physiol*, in press). "In fish you don't expect that kind of complexity, yet what we did was show these fish can make a decision based on their short-term memory," he says. "They don't have to see the items."

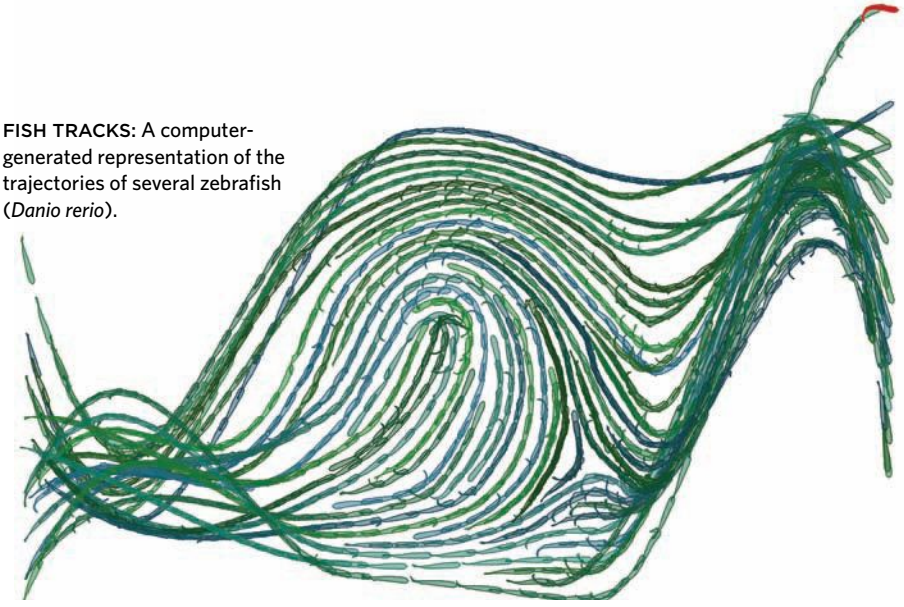
The neural circuitry responsible for numerical abilities in the fish brain is unknown. Gerlai and Butterworth say they'd like to take advantage of zebrafish's known genome and well-characterized anatomy to learn more about how numerosity—the value of a set—is computed. Butterworth says they want to identify the genes responsible for constructing the neural mechanisms. Such information might help uncover the basis of human dyscalculia—learning disabilities in math. "Can we identify this gene or these genes in order to tell which individuals are going to have problems doing this task?" asks Butterworth. "We might be able to get to the bottom of it by looking for genes in other species." —Kerry Grens

Keeping Track

Mackerel shoaling in silvery spheres, flocks of blackbirds billowing like dark clouds, and ant colonies carpeting forest floors—nature boasts some spectacular examples of individual animals coming together to form coordinated hordes. The question of how they accomplish such collective behavior has occupied biologists for decades. But although the majesty of swarms is clear for all to see, the mechanisms that explain how starlings coordinate their speed and direction, say, or how honeybees decide where to make a new hive are far too subtle to be detected by the naked eye. (See "Crowd Control," *The Scientist*, July 2013.)

"We need to see the fine-scale trajectories of every individual in a group at the same time so we can know precisely where they're moving with respect to everybody else," says Andrew King, who studies collective behavior in fish, birds, and mammals at Swansea University in the United Kingdom.

One option is to physically mark the animals and track their movements using video footage. But attaching labels is labor intensive for researchers and may disrupt the natural behaviors of the target animals. Tracking unmarked animals is a better bet, but existing automated image-tracking software has its limitations. When two individuals cross paths, for example, the software works out which is which by calculating the most likely identity based on their trajectories before the two animals overlapped. Sometimes it mixes up the animals, and the errors are propagated across the rest of video, which means researchers, usually grad students, have to spend hours painstakingly checking each crossing incident by eye.



FISH TRACKS: A computer-generated representation of the trajectories of several zebrafish (*Danio rerio*).

ALFONSO PÉREZ-ESCUDERO AND SARA ARGANDA

Not any more. Now, a team of researchers at the Cajal Institute in Madrid, led by Gonzalo de Polavieja, has launched idTracker—an image-tracking program that maintains the correct identities of hundreds of individuals in a video with almost 100 percent accuracy, regardless of how similar they look and how many times they cross each other's paths.

"It's something that a lot of people wanted to be able to do, but they were the first to come up with a method that actually works," said Simon Garnier, who runs the Swarm Lab at the New Jersey Institute of Technology in Newark. "It will ease the tedium burden and make it easier for us tease apart swarm intelligence."

Princeton University's Iain Couzin, who studies collective animal behavior, is even more impressed. "I was frankly stunned to see such a brilliant solution to this long-lasting problem," he wrote in an e-mail to *The Scientist*. "Previous methods did not work. At all. This method works near-flawlessly."

The study of animal behavior has been stuck in traditional methodology for far too long and is stagnating as a result. This type of technology will revitalize this field.

—Iain Couzin, Princeton University

De Polavieja's team didn't originally set out to make a tracking system. Back in 2008 the group wanted to create software that could distinguish between identical-looking fish in video footage. After a couple of years, though, the recognition software worked so well that the researchers realized it was capable of recognizing many individuals over the course of a video. "That's when we knew we could make a tracking system," says de Polavieja, who had noticed while reading the literature on collective behavior that there was nothing out there like what they had in mind. De Polavieja and graduate student Alfonso Pérez-Escudero conceived the program, and Pérez-Escudero wrote the software.

After several years' tweaking and polishing, de Polavieja's team described idTracker in *Nature Methods* earlier this year (11:743-48, 2014). In that paper, the researchers explain how the program extracts a unique visual fingerprint for every individual—a signature that humans cannot see. Analyzing short segments of footage for each animal in isolation, the software compares differences in gray-scale intensity and distance between hundreds of pairs of pixels to generate a set of data points that is unique to that particular individual. That signature can then be recognized and tracked regardless of the animal's position or posture.

The fingerprints are used as references to identify individuals in each frame of video, so the correct identities are kept even when animals cross over and over again. "Even if the system makes some mistakes, they will not propagate because you're constantly identifying each individual in each frame," says de Polavieja. "If it's incorrect in one frame, it can be corrected in the next frame."

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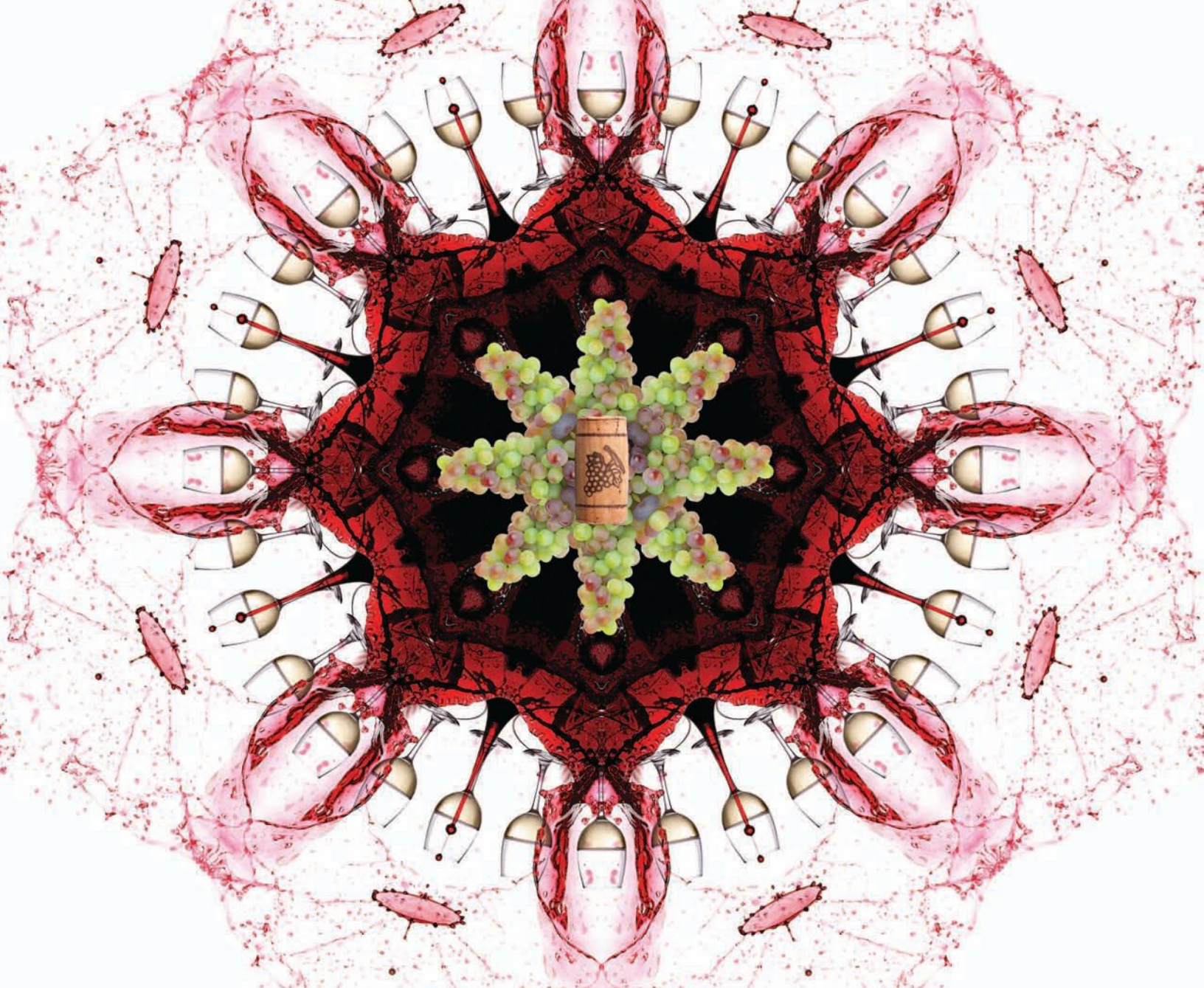

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Finally, the software stitches together the tracks of each individual to produce a multicolored map of the movements of every animal in the group. It works indefinitely, so researchers can study collectives over long periods. And it reidentifies individuals when they're put into different groups, meaning it should help reveal how individual differences contribute to collective behavior.

"What's fantastic is the level of precision and accuracy you get," says Garnier. Indeed, when de Polavieja and his colleagues tested their software on 23 videos of five different species—including mice, fruit flies, zebrafish, and ants—it achieved 99.7 percent accuracy on average. "Now, for the first time, there is no need for graduate students to go back and check footage frame by frame," says de Polavieja.

Better still, idTracker is free to download for noncommercial purposes (www.idtracker.es) and, according to Andrew King, is "pretty easy to use." It's also open-source, meaning code-savvy researchers are free to alter it to suit their specific requirements.

Garnier plans to use the new software to study how ants organize themselves to locate food sources or new nest sites. King wants to apply it to explore how fish with different personalities or experiences can affect group dynamics. "I can now mix up shoals, and the system will subsequently reidentify individuals I've already been working with," he says. "That was much harder to do before, so it's going to be really useful for us."

Couzin concurs: "The study of animal behavior has been stuck in traditional methodology for far too long and is stagnating as a result. This type of technology will revitalize this field." —Daniel Cossins

Predator Demoted

Eurypterids, or sea scorpions, immediately caught paleontologist Richard Laub's attention when he became a curator at the Buffalo Museum of Science in 1973. Browsing the museum's fossil collection, he was impressed with the formidable clawlike mouthparts of the largest of this extinct group of arthropods, the pterygotids. Reaching lengths of more than 2 meters, the aquatic animals hold the title as the largest arthropods to ever live, and Laub didn't have much doubt about the utility of their giant pincers. "I thought they were a combination fishing spear and can opener," says Laub, now retired. "It seemed obvious."

I discovered, as I had earlier in my career, as you enter scientific research, check your ego at the door.

—Richard Laub, Buffalo Museum of Science

But a few years ago, when Laub finally got around to testing his hypothesis, he found out that the claws were simply not strong enough to stab and crush armored prey without breaking. Rather, it seemed, the structures were more adapted for scavenging or pulling and tearing at soft-bodied prey (*Bull Buffalo Soc Nat Sci*, 39:29-42, 2010). "I discovered, as I had earlier in my career, as you enter scientific research, check your ego at the door," he says. "My assumptions had been wrong."

They weren't just Laub's assumptions. For years, researchers had presumed pterygotids to be a top predator of the world's Paleozoic waterways. But Laub and colleagues' work suggests that this was not the case. And this year, a team at Yale University uncovered more evidence that these large arthropods, which lived between 450 million and 400 million years ago, were not so scary after all.

For his final project in Yale paleontologist Derek Briggs's class on exceptionally preserved fossils, graduate student Ross Anderson closely examined a collection of eurypterid fossils housed at the Yale Peabody Museum of Natural History. These particular specimens fit with the theme of the class in that the eyes—a soft part of body that typically doesn't fossilize—were well preserved. Taking inspiration from a technique recently used by John Paterson's group at the University of New Eng-

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CRACKING CRABS: Laub's colleague used this apparatus to measure the force needed to penetrate the shell of a horseshoe crab.

land in New South Wales, Australia, to examine the eye of another extinct predator (*Nature*, 480:237-40, 2011), Anderson, Briggs, and their colleagues measured the number, size, shape, and angle of the lenses of the pterygotid species *Acutiramus cummingsi*.

"When we get fossils like the ones we have, where some of the soft parts are preserved," says Anderson, "then you can start to, in much more detail, discover how this organism lived, more about its morphology, and, in this case, anatomical detail about how the eye actually functioned."

The researchers used high-resolution photographs and a light microscope to examine the fossilized bands of cuticle that run between the lenses that make up the large *A. cummingsi*'s compound eye. They then compared their results with similar features in smaller eurypterids, as imaged by a scanning electron microscope, and in modern-day organisms such as the horseshoe crab, eurypterids' closest living marine relative. "The problem with doing any kind of biomechanics or functional analysis of a fossil is that, given the distance in time and the number of unknowns, it's pretty much impossible to produce meaningful numerical values," says Briggs. "So what's much more powerful is if you can compare two things."

They found that *A. cummingsi* had a relatively small number of fairly large lenses, and that the angles between the lenses were greater than those of modern arthropod predators (*Biol Letters*, 10:20140412, 2014). The data suggested that this large eurypterid did not have the sharp eyesight one might expect of a finely tuned killer. "The larger predator doesn't have the kinds of attributes in the eye that you'd expect by comparison with modern arthropods," says Briggs. Rather, he observes, the eye anatomy suggests the animal may have seen well in low light, a quality that would have been advantageous for a bottom-dweller or night feeder. "Then our hunch was . . . maybe it was feeding on slow-moving, soft-bodied things that we don't have any record of."

"Derek Briggs and his colleagues' conclusion correlates well with our conclusion," says Laub. "Instead of these creatures being *T. rex*es of the past, actually I think a better model is long-necked sauropod dinosaurs, which developed, if you will, a strategy of gigantism to make themselves less accessible to predators."

"The old vision of *Acutiramus* . . . swimming around rapidly and terrorizing other eurypterids, I think is almost certainly an exaggeration," Briggs agrees.

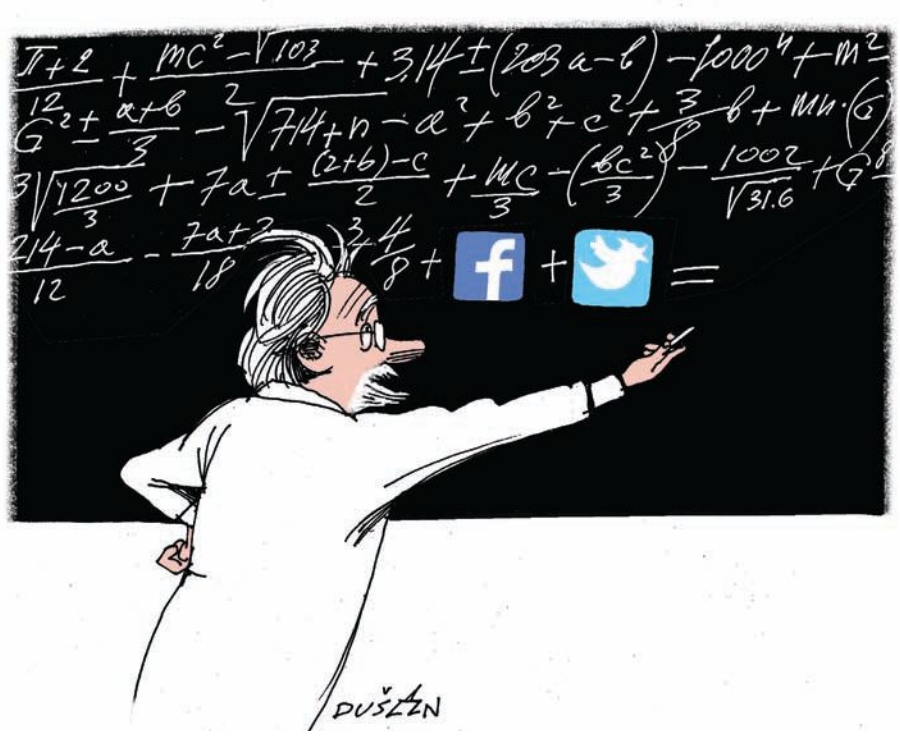
—Jef Akst

RICHARD S. BERKOF

Science Gone Social

Scientists are beginning to embrace social media as a viable means of communicating with public audiences.

BY SARA K. YEO, MICHAEL A. CACCIATORE, DOMINIQUE BROSSARD, DIETRAM A. SCHEUFELE, AND MICHAEL A. XENOS



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On March 20, 2013, Senator Tom Coburn's (R-Oklahoma) proposed amendment to block federal funding for political science passed in the US House of Representatives. Around the same time, in a letter to the National Science Foundation (NSF), the senator urged that research ranging from robotics to ecology, among others, be ineligible for federal funding. Additionally, the High Quality Research Act, proposed by Congressman Lamar Smith (R-Texas) in April of last year, was designed to ensure that NSF only supports projects addressing problems "that are of the utmost importance to society at large." The ability to communicate the societal value of basic research to nonacademic audiences is therefore morphing from an optional soft skill to a crucial tool for scientists who are competing over finite or shrinking resources for research.

National Academy of Sciences President Ralph Cicerone argued as early as 2006 that "scientists themselves must do a better job of communicating directly to the public," taking advantage of "new, non-traditional outlets" on the Internet (*In Focus*, 6, 2006). In 2011, Laura Van Eperen of the strategic communications company Van Eperen & Company, along with National Institutes of Health researcher Francesco Marincola, called for scientists to use social media, such as Facebook or Twitter, to "communicate to the masses" (*J Transl Med*, 9:199, 2011).

So have scientists heeded this call? To find out, we conducted a survey of tenure-track scientists at the University of Wisconsin–Madison, polling them on their use of social media for science-related purposes, their attitudes toward such use, and their political ideology. Not surprisingly, politics matters, even for scientists. The stronger scientists' political beliefs—regardless of their leaning—the more likely they were to use Facebook or Twitter to talk about their work. Liberals tended to use Facebook more than conservatives, consistent with charges from the political right in the U.S. that Facebook has a liberal bias and is an echo chamber for left-leaning thinkers. Aside from political ideology, the perceived effectiveness and barriers to use of social media for science-related purposes predicted use of Twitter, but not Facebook. Scientists who perceived social media as effective communication tools were more likely to use Twitter. Moreover, greater interest in actively seeking new ways to share science significantly predicted use of Twitter, but not Facebook.

One potential explanation for why Twitter seems to be the social medium of choice for scientists is that it appears to be viewed as a more professional outlet, while Facebook is more often perceived as a space for personal information. Scientists may also avoid Facebook as a tool for sharing research because of the emergence of a host of other social networks specifically tailored to researchers. Online communities, such as ResearchGate or Academia.edu, are Facebook-like networks designed specifically for scientists and researchers to share their work. In a recent *Nature* survey, more than half of the roughly 3,500 scientists polled visited ResearchGate or Academia.edu regularly. Our survey yielded a similar result, with 49 percent of our sample reporting that they visited science-related social networks.

Scientists may also have adopted Twitter more readily than Facebook because it

is unnecessary for a researcher to "friend" or even "follow" specific individuals for one's tweets to reach them. Rather, users can search for content or set up Twitter to funnel relevant tweets their way.

Regardless of the reasons, our findings suggest that scientists have begun to embrace Twitter as a viable tool for communicating research and keeping abreast of advancements in their fields. Perhaps more importantly, science topics have "trended" on Twitter, earning popularity among users. Trending topics are listed on Twitter's website, increasing the likelihood that they will be viewed by large audiences. In recent years, both the general meeting of the American Society for Microbiology and the rumors surrounding the discovery of the Higgs boson particle have trended on Twitter. In fact, the latter issue topped the list of trending topics for June 20, 2012.

In most cases, however, the Twitter users most likely to encounter information about science are those who are already interested in science and related topics. Social media hold great promise for science communication, and use of these tools may even correlate to a researcher's standing in her own field. In fact, Twitter has been found to amplify the positive effects of scientists' interactions with more traditional forms of media, thus increasing a scientist's prominence. In other words, engagement with social media may bring rewards in and outside of the ivory tower.

The ability to communicate the societal value of basic research to nonacademic audiences is morphing from an optional soft skill to a crucial tool for scientists who are competing over finite or shrinking resources for research.

But as our data show, scientists are only beginning to get their feet wet in this new communication world. Given the controversial nature of many recent scientific debates, researchers will have to do much more to connect directly with public audiences. (See "Science Speak," *The Scientist*, August 2014.) ■

Sara K. Yeo is an assistant professor of communication at the University of Utah. Michael A. Cacciatore is an assistant professor in the advertising and public relations department at the University of Georgia. Dominique Brossard is chair of the Department of Life Sciences Communication at the University of Wisconsin–Madison, where Dietram A. Scheufele is John E. Ross professor. Michael A. Xenos is chair of the Department of Communication Arts at the University of Wisconsin–Madison.

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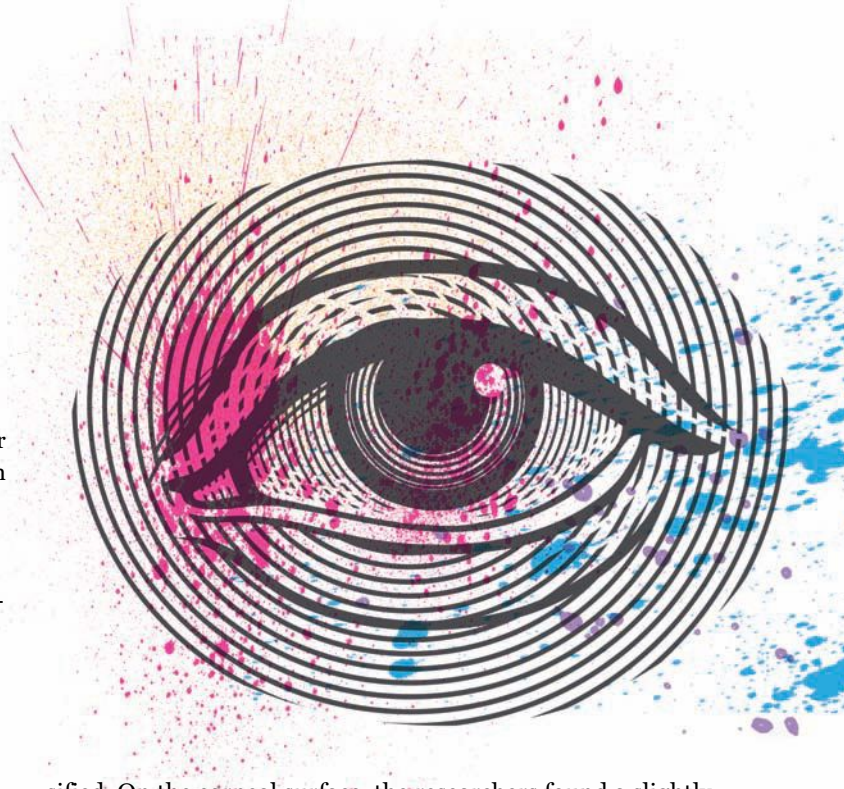
The Ocular Microbiome

Researchers are beginning to study in depth the largely uncharted territory of the eye's microbial composition.

BY RINA SHAIKH-LESKO



When researchers started using modern molecular diagnostic tools such as PCR and genome sequencing to study the microbes living on and in the human body, they found much more complex ecosystems than previous generations had imagined. The Human Microbiome Project undertook a massive effort to characterize microbial communities from five sites—the gut, mouth, nose, skin, and urogenital tract. But it did not include many areas of the body that harbor microbial life, including the surface of the eye.

Ophthalmologists have treated pathogenic eye infections for several decades, and the advent of contact lenses has made such infections more common. But little is known about the bacteria that live on the surface of a healthy human eye, and how this microbial makeup differs when a pathogenic strain takes over. Many bacteria known to live on the eye are difficult to culture, making them virtually invisible to researchers. Adapting sequencing technologies to study the ocular microbiome has opened up new avenues for understanding what's really happening under the eyelids.

About five years ago, Valery Shestopalov of the Bascom Palmer Eye Institute at the University of Miami was speaking with his microbiology colleagues about the bacteria found on healthy eyes. Conventional wisdom at that time held that healthy eyes don't harbor much microbial life—tears and blinking tend to clear away foreign objects, including bacteria. But Shestopalov's early tests revealed something different. "The tests ran positive. All exposed mucosal epithelium are populated densely," he said. In 2009, Shestopalov began the Ocular Microbiome Project with funds from his institution. Eventually, he secured a grant from the National Eye Institute and began collaborating with Russell Van Gelder at the University of Washington, who had been developing PCR-based diagnostic tests to identify bacteria and fungi on the eye. The project now has a dozen collaborators at five universities.

In May, Shestopalov presented preliminary ocular microbiome data at the Association for Vision Research and Ophthalmology annual meeting held in Orlando, Florida. His team sequenced samples from healthy corneas, contact lenses, and conjunctiva—the inner surface of the eyelids—using 16S ribosomal RNA sequencing, along with a new method Van Gelder developed called biome representational in silico karyotyping (BRiSK), which uses high-throughput sequencing to identify bacteria at the species level.

The team found that about a dozen bacterial genera dominated the eye's conjunctiva, a third of which could not be classified. On the corneal surface, the researchers found a slightly different community. Again, about a dozen genera dominated. And everywhere they've looked, the researchers have found more than just bacteria. "We haven't published on this yet, but I have been surprised by how often we find phage or viruses on the normal ocular surface," Van Gelder told *The Scientist* in an e-mail.

"People can have a huge variation in microflora and still have healthy eyes, making our job difficult, but really amazing," Shestopalov said.

The researchers also found that during keratitis infections—infections of the cornea—only about half as many bacterial varieties were present, most prominently *Pseudomonas* strains. The changes typically occurred well before a diagnosis of an eye infection, suggesting the ocular microbiome could inform future diagnostics, Shestopalov noted. His team is refining the algorithm for predicting infection based on the dynamics of these changes in bacterial composition.

One factor that may be expected to impact the composition of ocular microbiota is the use of contact lenses. Contact lens wear is one of the biggest factors leading to corneal infection. Common bacterial infections that can cause irritation and redness affect an estimated 7 percent to 25 percent of contact lens–wearers, and much rarer keratitis infections can even cause

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blindness. Researchers believe contact lenses make it easier for pathogens to colonize the surface of the eye by giving the bacteria something to adhere to. Sequencing biofilms from used contact lenses, Shestopalov's team found evidence of microbial communities that were different from the ocular microbiomes of people who don't use contacts. On the lenses themselves, the researchers have found much less diversity—many of the bacterial genera that dominate the conjunctiva and cornea were depleted. In their place, *Staphylococcus* dominated.

To tackle the potential-infection problem, Mark Willcox, a medical microbiologist at the University of New South Wales in Australia, has developed antimicrobial contact lenses. Together with colleagues Debarun Dutta and Jerome Ozkan of the Brien Holden Vision Institute in Sydney, Willcox bonded the naturally occurring antimicrobial peptide melimine to the surface of normal contact lenses. The researchers reported on preclinical studies on rabbits, and in April, on the first phase of human trials, which included 17 volunteers. They found that the antimicrobial lenses appeared as safe as regular lenses and maintained their antimicrobial activity against two major pathogens, *P. aeruginosa* and *S. aureus*. The researchers next plan to test the lenses in a larger sample of about 100 to 200 people, but it will be some time before antimicrobial lenses are available on the market.

People can have a huge variation in microflora and still have healthy eyes, making our job difficult, but really amazing.

—Valery Shestopalov, Bascom Palmer Eye Institute, University of Miami

The lenses are not likely to harm normal, commensal bacteria on the eye. "As the peptide is bound to the surface of the lens we believe it will only affect the growth of those microbes that attempt to bind to the lens surface and not those cultured from the surface of the eye," Willcox told *The Scientist* in an e-mail. "But large-scale clinical trials are needed to prove this hypothesis."

Whether the bacteria identified living on the surface of the eye are permanent residents or transient colonizers remains to be seen. The work of deconstructing the ocular microbiome is just getting started, but preliminary results have suggested it is distinct from the rest of the bacterial community that inhabits our bodies. "It stands apart," Shestopalov said. "There's statistical evidence of its difference from any other human microbiome." ■

A version of this article appeared on www.the-scientist.com in May 2014.

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Retina Recordings in a Dish

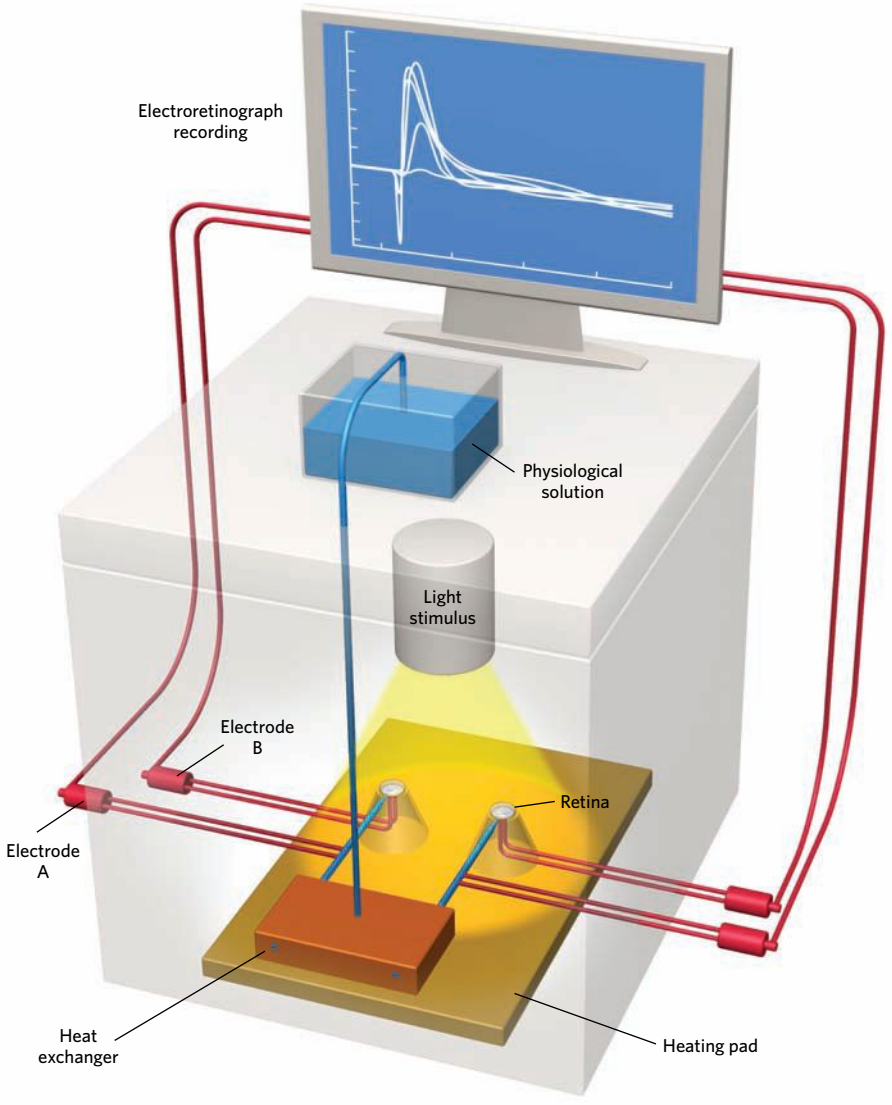
Scientists adapt an in vivo retina recorder for ex vivo use.

BY RUTH WILLIAMS

To record the activity of retinal cells in live organisms, researchers use in vivo electroretinography (ERG) systems. Essentially, these consist of contact lenses with attached electrodes that are placed on the eyes and used to detect responses to different intensities and wavelengths of light. Such in vivo analysis has limited potential for experimentation, however, so some researchers choose to study dissected retinas in culture. Surprisingly, "there was no system on the market" for ex vivo ERG, says Vladimir Kefalov of Washington University School of Medicine in St. Louis. "You had to build a whole system from scratch," which he estimates would cost between $60,000 and $100,000. Indeed, Kefalov himself had built such a system.

To trim the expenses and complications of a custom ex vivo system, Kefalov's team built an ex vivo adapter, based on in vivo ERGs available in most ophthalmology departments. The adapter, which Kefalov has also made available for purchase, holds two retinal specimens, maintains them at body temperature, perfuses them with physiological buffer, and connects the buffer to electrodes. In essence, the adapter replaces the contact lenses of in vivo systems, but retains the software, electronics, and illumination of the in vivo ERG—and all for just a few thousand dollars.

One benefit of ex vivo ERG is that "you have a level of experimental control that allows you to know uniquely what cell type you're recording from," says Alapakkam Sampath of the University of California, Los Angeles. Using the new adapter, Kefalov showed that the same intensity of light produces larger electrical signals from rod photoreceptors than from cone photoreceptors—perhaps explaining how rod cells enable us to see in low light conditions. (*Vision Research,* 101:108-17, 2014)



OUT OF BODY EXPERIENCE: Two dissected retinas, photoreceptor side up, are mounted to two domes on a heated ex vivo recording rig. A buffer solution flows through a heat exchanger and runs over the retinas, which are illuminated from above. The perfusate can carry drugs or change temperature depending on the experiment. Two sets of electrodes send retinal activity information to an amplifier. Electrode A is connected to the buffer that runs above the retinas, and electrode B records from below on the ganglion side of the retina.

AT A GLANCE

EX VIVO METHODS	HOW IT WORKS	RECORDING TIME	DRUG TESTING	CONE RECORDING
Suction electrode recording	A glass electrode records the activity of a single cell.	Max 20–30 minutes until cell is exhausted	Not possible due to short recording time	Difficult due to the low number of cones in mammalian retinas (three percent of cells in the mouse retina)
ERG	An electrode records from a whole, dissected retina.	Many hours	Possible by simply adding drug to perfusate	Yes, by saturating rods so only cones will respond to light, or by using genetically engineered animals lacking functional rods

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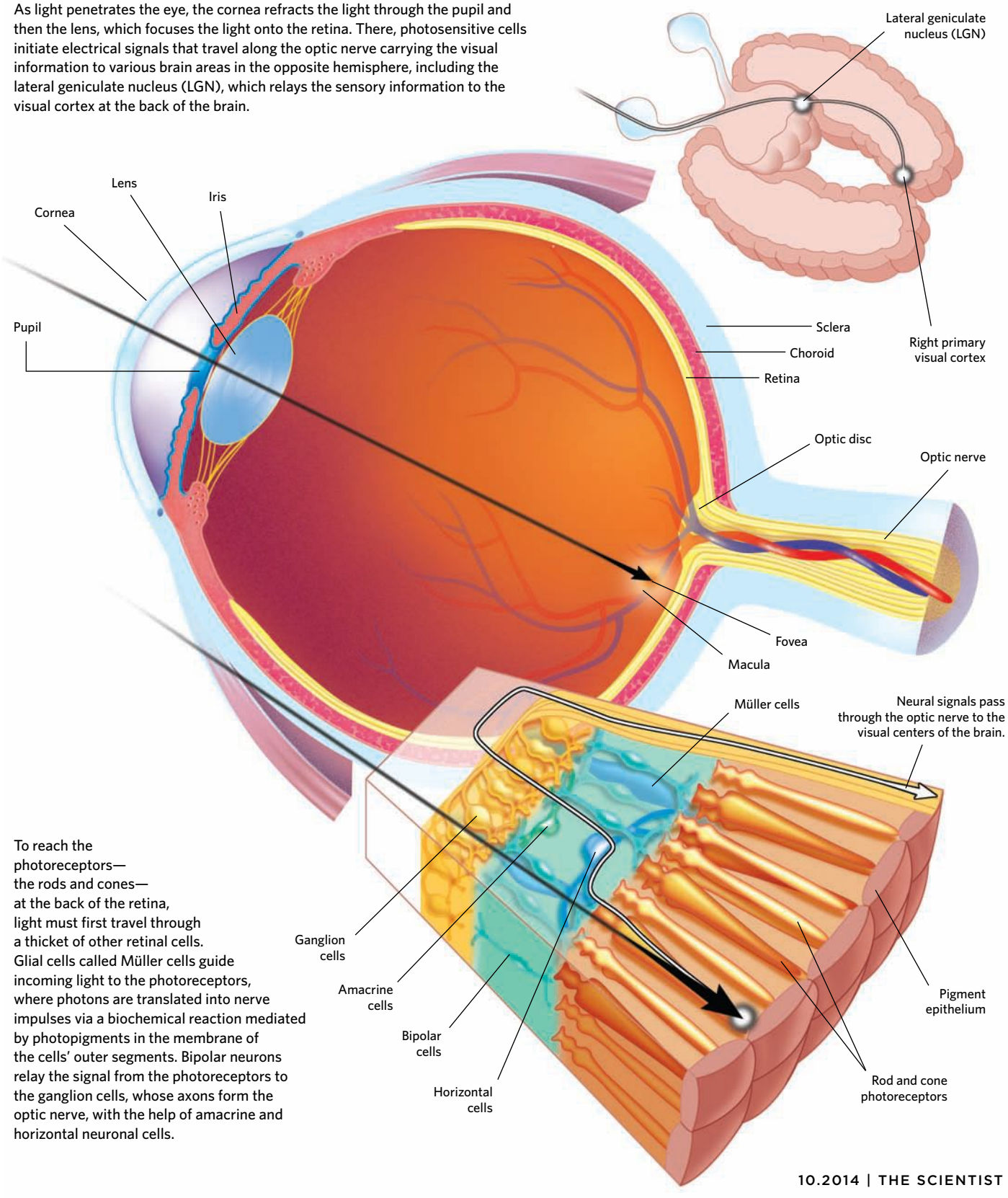
The Eye: An Overview

As light penetrates the eye, the cornea refracts the light through the pupil and then the lens, which focuses the light onto the retina. There, photosensitive cells initiate electrical signals that travel along the optic nerve carrying the visual information to various brain areas in the opposite hemisphere, including the lateral geniculate nucleus (LGN), which relays the sensory information to the visual cortex at the back of the brain.



To reach the photoreceptors—the rods and cones—at the back of the retina, light must first travel through a thicket of other retinal cells. Glial cells called Müller cells guide incoming light to the photoreceptors, where photons are translated into nerve impulses via a biochemical reaction mediated by photopigments in the membrane of the cells' outer segments. Bipolar neurons relay the signal from the photoreceptors to the ganglion cells, whose axons form the optic nerve, with the help of amacrine and horizontal neuronal cells.

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THE BIONIC EYE

Using the latest technologies, researchers are constructing novel prosthetic devices to restore vision in the blind.

INTRODUCTION
BY HENRI LORACH

In 1755, French physician and scientist Charles Leroy discharged the static electricity from a Leyden jar—a precursor of modern-day capacitors—into a blind patient's body using two wires, one tightened around the head just above the eyes and the other around the leg. The patient, who had been blind for three months as a result of a high fever, described the experience like a flame passing downwards in front of his eyes. This was the first time an electrical device—serving as a rudimentary prosthesis—successfully restored even a flicker of visual perception.

More than 250 years later, blindness is still one of the most debilitating sensory impairments, affecting close to 40 million people worldwide. Many of these patients can be efficiently treated with surgery or medication, but some pathologies cannot be corrected with existing treatments. In particular, when light-receiving photoreceptor cells degenerate, as is the case in retinitis pigmentosa, or when the optic nerve is damaged as a result of glaucoma or head trauma, no surgery or medicine can restore the lost vision. In such cases, a visual prosthesis may be the only option. Similar to cochlear implants, which stimulate auditory nerve fibers downstream of damaged sensory hair cells to restore hearing, visual prostheses aim to provide patients with visual information by stimulating neurons in the retina, in the optic nerve, or in the brain's visual areas.

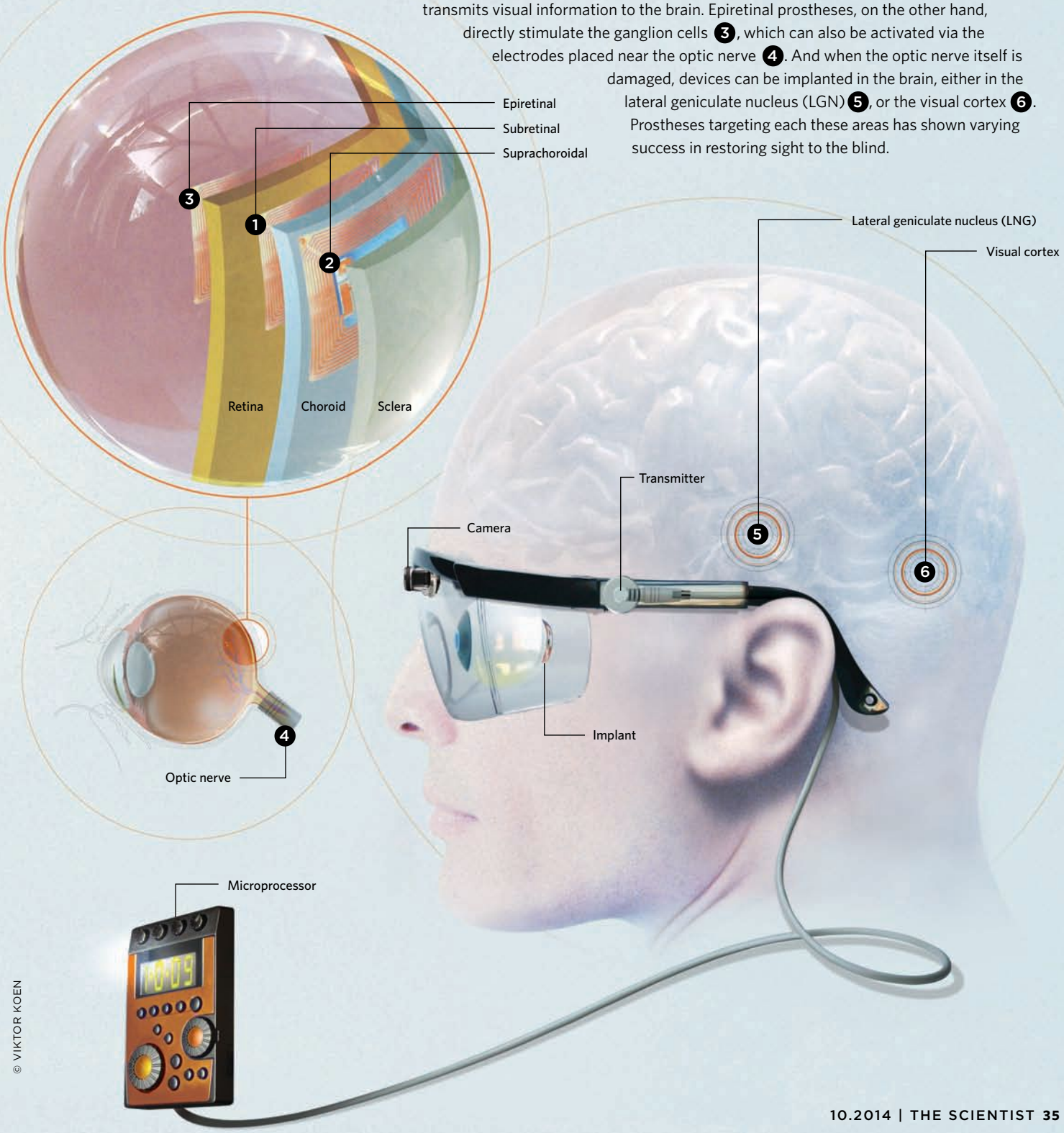
In a healthy retina, photoreceptor cells—the rods and cones—convert light into electrical and chemical signals that propagate through the network of retinal neurons down to the ganglion cells, whose axons form the optic nerve and transmit the visual signal to the brain. (See illustration on page 33.) Prosthetic devices work at different levels downstream from the initial reception and biochemical conversion of incoming light photons by the pigments of photoreceptor rods and cones at the back of the retina. Implants can stimulate the bipolar cells directly downstream of the photoreceptors, for example, or the ganglion cells that form the optic nerve. Alternatively, for pathologies such as glaucoma or head trauma that compromise the optic nerve's ability to link the retina to the visual centers of the brain, prostheses have been designed to stimulate the visual system at the level of the brain itself. (See illustration on opposite page.)

While brain prostheses have yet to be tested in people, clinical results with retinal prostheses are demonstrating that the implants can enable blind patients to locate and recognize objects, orient themselves in an unfamiliar environment, and even perform some reading tasks. But the field is young, and major improvements are still necessary to enable highly functional restoration of sight.

Henri Lorach is currently a visiting researcher at Stanford University, where he focuses on prosthetic vision and retinal signal processing.

STIMULATING VISION

Prostheses can be placed anywhere along the visual pathway, from just behind the photoreceptor cells—the rods and cones—to the brain itself. Subretinal devices stimulate the bipolar cells and other neurons downstream of the eye's rods and cones from between the retina and the pigment epithelium 1, while suprachoroidal prostheses stimulate these same cells from between the choroid, the vascular layer that supports the retina, and the sclera, the protective outer layer of the eye 2. Both types of devices take advantage of the remaining intact retinal neural network that leads to the ganglion cells, whose axons form the optic nerve that transmits visual information to the brain. Epiretinal prostheses, on the other hand, directly stimulate the ganglion cells 3, which can also be activated via the electrodes placed near the optic nerve 4. And when the optic nerve itself is damaged, devices can be implanted in the brain, either in the lateral geniculate nucleus (LGN) 5, or the visual cortex 6. Prostheses targeting each these areas has shown varying success in restoring sight to the blind.



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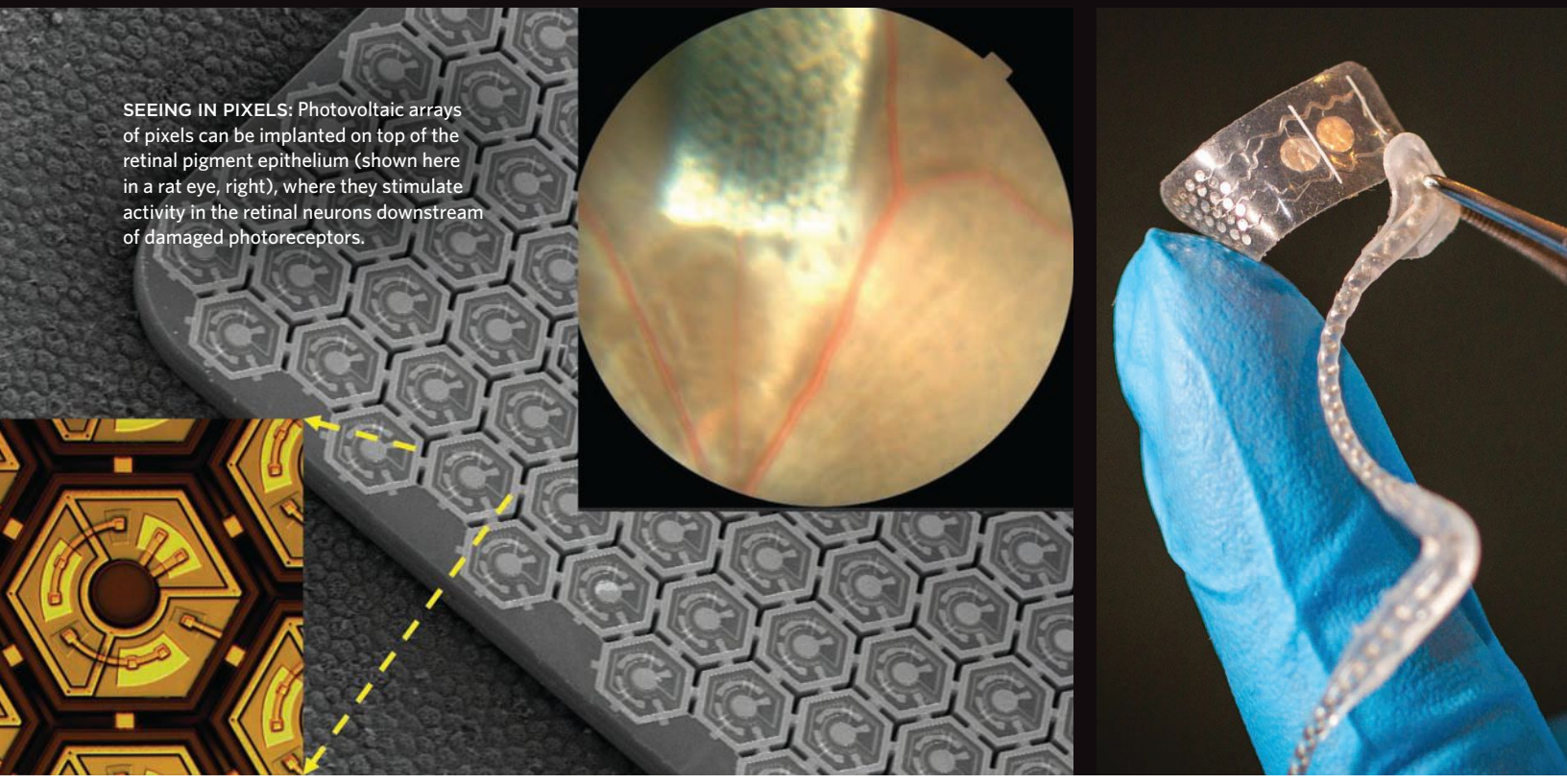

SEEING IN PIXELS: Photovoltaic arrays of pixels can be implanted on top of the retinal pigment epithelium (shown here in a rat eye, right), where they stimulate activity in the retinal neurons downstream of damaged photoreceptors.

PALANKER LAB, STANFORD UNIVERSITY; COURTESY OF NESTLAIR PHOTOGRAPHY

SUBSTITUTES FOR LOST PHOTORECEPTORS

BY DANIEL PALANKER

In the subretinal approach to visual prosthetics, electrodes are placed between the retinal pigment epithelium (RPE) and the retina. (See illustration on previous page.) There, they stimulate the nonspiking inner retinal neurons—bipolar, horizontal, and amacrine cells—which then transmit neural signals down the retinal network to the retinal ganglion cells (RGCs) that propagate to the brain via the optic nerve. Stimulating the retinal network helps preserve some aspects of the retina's natural signal processing, such as the "flicker fusion" that allows us to see video as a smooth motion, even though it is composed of frames with static images; adaptation to constant stimulation; and the nonlinear integration of signals as they flow through the retinal network, a key aspect of high spatial resolution. Electrical pulses lasting several milliseconds provide selective stimulation of the inner retinal neurons and avoid direct activation of the ganglion cells and their axons, which would otherwise considerably limit patients' ability to interpret the spatial layout of a visual scene.

The Boston Retinal Implant Project, a multidisciplinary team of scientists, engineers, and clinicians at research institutions across the U.S., is developing a retinal prosthesis that transmits information from a camera mounted on eyeglasses to a receiving antenna implanted under the skin around the eye using radiofrequency telemetry—technology similar to radio broadcast. The decoded signal is then delivered to an implanted subretinal electrode array via a cable that penetrates into the eye. The information delivered to the retina by this device is not related to direction of gaze, so to survey a scene a patient must move his head, instead of just his eyes.

The Alpha IMS subretinal implant, developed by Retina Implant AG in Reutlingen, Germany, rectifies this problem by including a subretinal camera, which converts light in each pixel into electrical currents. This device has been successfully tested in patients with advanced retinitis pigmentosa and was recently approved for experimental clinical use in Europe. Visual acuity with this system is rather limited: most patients test no better than 20/1000, except for one patient who reached 20/550.[1] The Alpha IMS system also needs a bulky implanted power supply with cables that cross the sclera and requires complex surgery, with associated risk of complications.

In the subretinal approach to visual prosthetics, electrodes are placed between the retinal pigment epithelium and the retina, where they stimulate the nonspiking inner retinal neurons.

To overcome these challenges, my colleagues and I have developed a wireless photovoltaic subretinal prosthesis, powered by pulsed light. Our system includes a pocket computer that processes the images captured by a miniature video

STIMULATING ARRAY: This prototype suprachoroidal array, which is implanted behind the choroid, can be larger than prostheses inserted in front of or behind the retina.

FOLLOW THE LIGHT: A blind patient navigates an obstacle course without the assistance of her guide-dog, thanks to a head-mounted camera and a backpack computer, which gather and process visual information before delivering a representation of the visual scene via her suprachoroidal retinal prosthesis.

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camera mounted on video goggles, which project these images into the eye and onto a subretinally implanted photodiode array. Photodiodes in each pixel convert this light into pulsed current to stimulate the nearby inner retinal neurons. This method for delivering the visual information is completely wireless, and it preserves the natural link between ocular movement and image perception.

Our system uses invisible near-infrared (NIR, 880–915 nm) wavelengths to avoid the perception of bright light by the remaining functional photoreceptors. It has been shown to safely elicit and modulate retinal responses in normally sighted rats and in animals blinded by retinal degeneration.[2] Arrays with 70 micrometer pixels restored visual acuity in blind rats to half the natural level, corresponding to 20/250 acuity in human. Based on stimulation thresholds observed in these studies, we anticipate that pixel size could be reduced by a factor of two, improving visual acuity even further. Ease of implantation and tiling of these wireless arrays to cover a wide visual field, combined with their high resolution, opens the door to highly functional restoration of sight. We are commercially developing this system in collaboration with the French company Pixium Vision, and clinical trials are slated to commence in 2016.

Fabio Benfenati of the Italian Institute of Technology in Genoa and Guglielmo Lanzani at the institute's Center for Nanoscience and Technology in Milan are also pursuing the subretinal approach to visual prostheses, developing a device based on organic polymers that could simplify implant fabrication.[3] So far, subretinal light-sensitive implants appear to be a promising approach to restoring sight to the blind.

Daniel Palanker is a professor in the Department of Ophthalmology and Hansen Experimental Physics Laboratory at Stanford University.

References

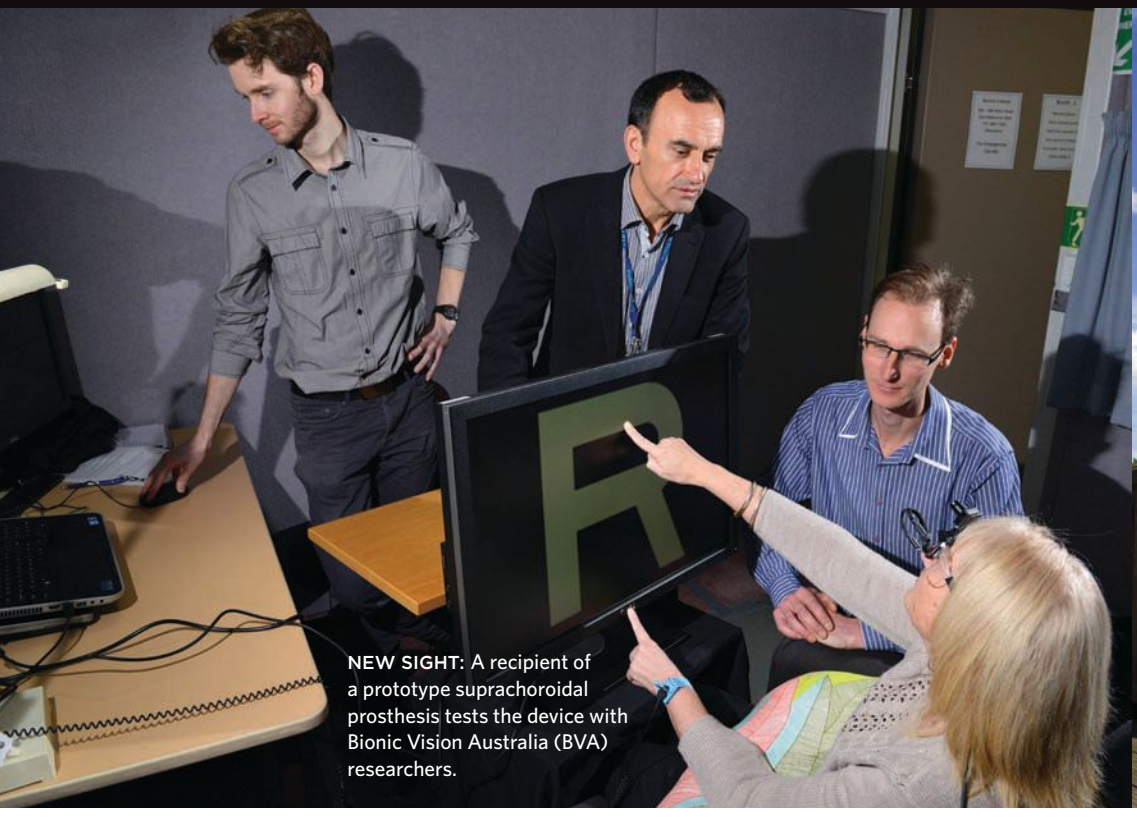
1. K. Stingl et al., "Functional outcome in subretinal electronic implants depends on foveal eccentricity," *Invest Ophthalmol Vis Sci*, 54:7658-65, 2013.
2. Y. Mandel et al., "Cortical responses elicited by photovoltaic subretinal prostheses exhibit similarities to visually evoked potentials," *Nat Commun*, 4:1980, 2013.
3. D. Ghezzi et al., "A polymer optoelectronic interface restores light sensitivity in blind rat retinas," *Nat Photonics*, 7:400-06, 2013.

BEHIND THE EYE

BY LAUREN AYTON AND DAVID NAYAGAM

Subretinal prostheses implanted between the retina and the RPE, along with epiretinal implants that sit on the surface of the retina (see below), have shown good results in restoring some visual perception to patients with profound vision loss. However, such devices require technically challenging surgeries, and the site of implantation limits the potential size of these devices. Epiretinal and subretinal prostheses also face challenges with stability and the occurrence of adverse intraocular events, such as infection or retinal detachment. Due to these issues, researchers have been investigating a less invasive and more stable implant location: between the vascular choroid and the outer sclera. (See illustration on page 35.)

Like subretinal prostheses, suprachoroidal implants utilize the bipolar cells and the retinal network down to the ganglion cells, which process the visual information before relaying it to the brain. But devices



NEW SIGHT: A recipient of a prototype suprachoroidal prosthesis tests the device with Bionic Vision Australia (BVA) researchers.

implanted in this suprachoroidal location can be larger than those implanted directly above or below the retina, allowing them to cover a wider visual field, ideal for navigation purposes. In addition, suprachoroidal electrode arrays do not breach the retina, making for a simpler surgical procedure that should reduce the chance of adverse events and can even permit the device to be removed or replaced with minimal damage to the surrounding tissues.

Early engineering work on suprachoroidal device design began in the 1990s with research performed independently at Osaka University in Japan[1] and the Nano Bioelectronics and Systems Research Center of Seoul National University in South Korea.[2] Both these groups have shown proof of concept in bench testing and preclinical work, and the Japanese group has gone on to human clinical trials with promising results.[3] Subsequently, a South Korean collaboration with the University of New South Wales in Australia continued suprachoroidal device development.

More recently, our groups, the Bionics Institute and the Centre for Eye Research Australia, working as part of the Bionic Vision Australia (BVA) partnership, ran a series of preclinical studies between 2009 and 2012.[4] These studies demonstrated the safety and efficacy of a prototype suprachoroidal implant, made up of a silicone carrier with 33 platinum disc-shaped electrodes that can be activated in various combinations to elicit the perception of rudimentary patterns, much like pixels on a screen. Two years ago, BVA commenced a pilot trial, in which researchers implanted the prototype in the suprachoroidal space of three end-stage retinitis pigmentosa patients who were barely able to perceive light. The electrode array was joined to a titanium connector affixed to the skull behind the ear, permitting neurostimulation and electrode monitoring without the need for any implanted electronics.[5] In all three patients, the device proved stable and effective, providing enough visual perception to better localize light, recognize basic shapes, orient in a room, and walk through mobility mazes with reduced collisions.[6] Preparation is underway for future clinical trials, which will provide subjects with a fully implantable device with twice the number of electrodes.

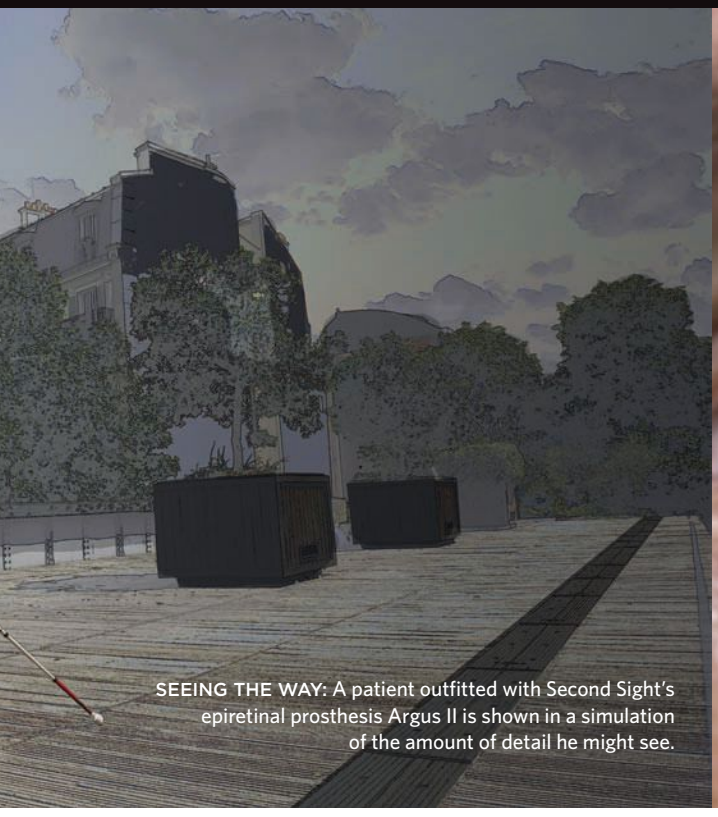
Suprachoroidal prostheses can be larger than those implanted directly above or below the retina, allowing them to cover a wider visual field, ideal for navigation purposes.

Meanwhile, the Osaka University group, working with the Japanese company NIDEK, has been developing an intrascleral prosthetic device, which, unlike the Korean and BVA devices, is implanted in between the layers of the sclera rather than in the suprachoroidal space. In a clinical trial of this device, often referred to as suprachoroidal-transretinal stimulation (STS), two patients with advanced retinitis pigmentosa showed improvement in spatial resolution and visual acuity over a four-week period following implantation.[3]

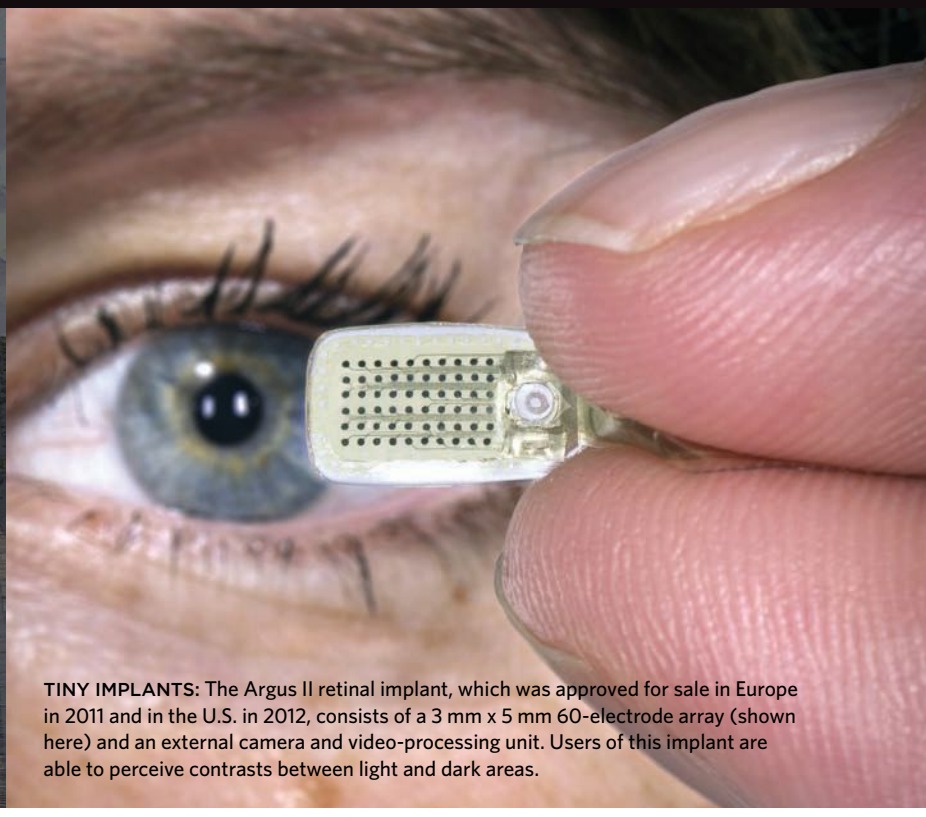
Future work will be required to fully investigate the difference in visual perception provided by devices implanted in the various locations in the eye, but the initial signs are promising that suprachoroidal stimulation is a safe and viable clinical option for patients with certain degenerative retinal diseases.

Lauren Ayton is a research fellow and the bionic eye clinical program leader

COURTESY OF BIONIC VISION AUSTRALIA'S BIONIC EYE PROJECT; © PHILIPPE PSAILA/SCIENCE SOURCE



SEEING THE WAY: A patient outfitted with Second Sight's epiretinal prosthesis Argus II is shown in a simulation of the amount of detail he might see.



TINY IMPLANTS: The Argus II retinal implant, which was approved for sale in Europe in 2011 and in the U.S. in 2012, consists of a 3 mm x 5 mm 60-electrode array (shown here) and an external camera and video-processing unit. Users of this implant are able to perceive contrasts between light and dark areas.

at the University of Melbourne's Centre for Eye Research Australia. David Nayagam is a research fellow and the bionic eye chronic preclinical study leader at the Bionics Institute in East Melbourne and an honorary research fellow at the University of Melbourne.

References

1. H. Sakaguchi et al., "Transretinal electrical stimulation with a suprachoroidal multichannel electrode in rabbit eyes," *Jpn J Ophthalmol*, 48:256-61, 2004.
2. J.A. Zhou et al., "A suprachoroidal electrical retinal stimulator design for long-term animal experiments and in vivo assessment of its feasibility and biocompatibility in rabbits," *J Biomed Biotechnol*, 2008:547428, 2008.
3. T. Fujikado et al., "Testing of semichronically implanted retinal prosthesis by suprachoroidal-transretinal stimulation in patients with retinitis pigmentosa," *Invest Ophthalmol Vis Sci*, 52:4726-33, 2011.
4. D.A.X. Nayagam et al., "Chronic electrical stimulation with a suprachoroidal retinal prosthesis: a preclinical safety and efficacy study," *PLOS ONE*, 9:e97182, 2014.
5. A.L. Saunders et al., "Development of a surgical procedure for implantation of a prototype suprachoroidal retinal prosthesis," *Clin & Exp Ophthalmol*, 10.1111/ceo.12287, 2014
6. M.N. Shivdasani et al., "Factors affecting perceptual thresholds in a suprachoroidal retinal prosthesis," *Invest Ophthalmol Vis Sci*, in press.

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SHORTCUTTING THE RETINA

BY MARK HUMAYUN, JAMES WEILAND, AND STEVEN WALSTON

Bypassing upstream retinal processing, researchers have developed so-called epiretinal devices that are placed on the anterior surface of the retina, where they stimulate the ganglion cells that are the output neurons of the eye. This strategy targets the last cell layer of the retinal network, so it works regardless of the state of the upstream neurons. (See illustration on page 33.)

In 2011, Second Sight obtained approval from the European Union to market its epiretinal device, the Argus II Visual Prosthesis System, which allowed clinical trial subjects who had been blind for several years to recover some visual perception such as basic shape recognition and, occasionally, reading ability. The following year, the FDA approved the device, which uses a glasses-mounted camera to capture visual scenes and wirelessly transmits this information as electrical stimulation patterns to a 6 x 10 microelectrode array. The array is surgically placed in the macular region, responsible in a healthy retina for high-acuity vision, and covers an area of approximately 20° of visual space.

A clinical trial showed that 30 patients receiving the device are able to more accurately locate a high-contrast square on a computer monitor, and when asked to track a moving high-contrast bar, roughly half are able to discriminate the direction of the bar's movement better than without the system.[1] The increased visual acuity has also enabled patients to read large letters, albeit at a slow rate, and has improved the patients' mobility.[2] With the availability of the Argus II, patients with severe retinitis pigmentosa have the first treatment that can actually improve vision. To date, the system has

been commercially implanted in more than 50 patients.

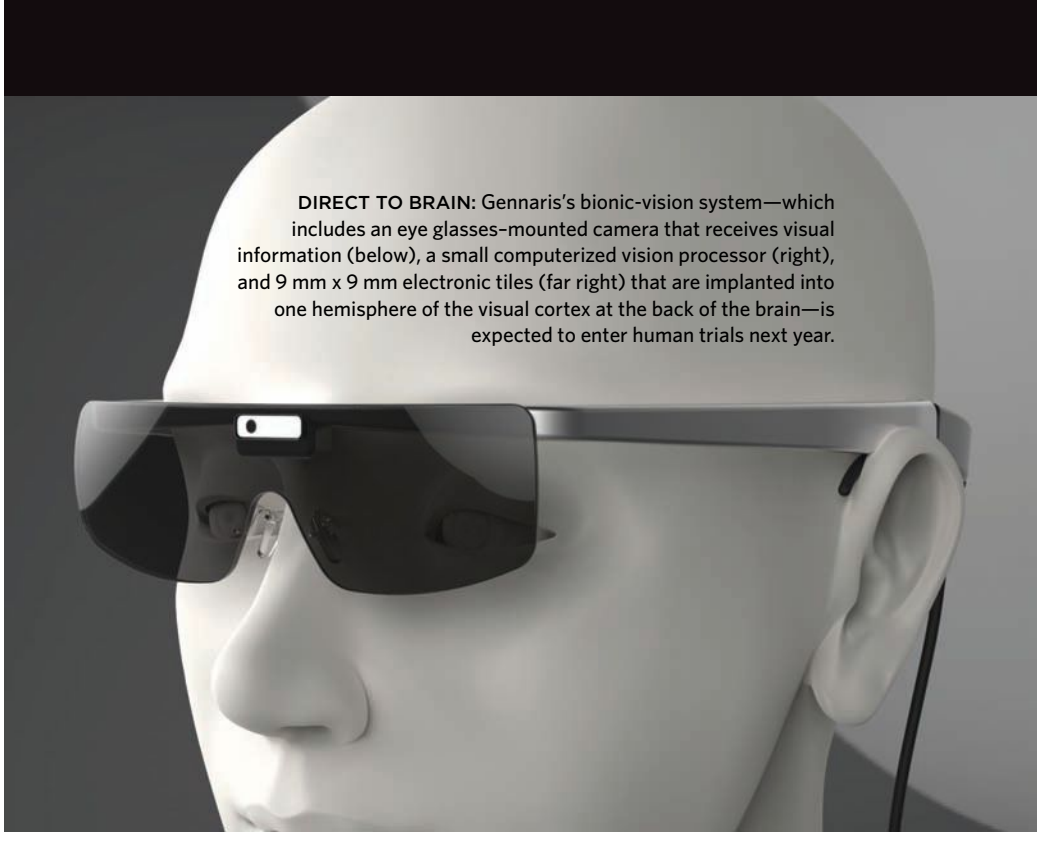
Several other epiretinal prostheses have shown promise, though none have received regulatory approval. Between 2003 and 2007, Intelligent Medical Implants tested a temporarily implanted, 49-electrode prototype device in eight patients, who reported seeing spots of light when electrodes were activated. Most of these prototype devices were only implanted for a few months, however, and with no integrated camera, patients could not activate the device outside the clinic, limiting the evaluation of the prosthesis's efficacy. This group has reformed as Pixium Vision, the company currently collaborating with Daniel Palanker's group at Stanford to develop a subretinal device, and has now developed a permanent epiretinal implant that is in clinical trials. The group is also planning trials of a 150-electrode device that it hopes will further improve visual resolution.

Future developments in this area will aim to improve the spatial resolution of the stimulated vision; increase the field of view that can be perceived; and increase the number of electrodes. Smaller electrodes would activate fewer retinal ganglion cells, which would result in higher resolution. These strategies will be rigorously tested, and, if successful, may enable retinal prostheses that provide an even better view of the world.

Mark Humayun is Cornelius J. Pings Chair in Biomedical Sciences at the University of Southern California, where James Weiland is a professor of ophthalmology and biomedical engineering. Steven Walston is a graduate student in the Bioelectronic Research Lab at the university.

References

1. M.S. Humayun et al., "Interim results from the international trial of Second Sight's visual prosthesis," *Ophthalmology*, 119:779-88, 2012.
2. L. da Cruz et al., "The Argus II epiretinal prosthesis system allows letter and word reading and long-term function in patients with profound vision loss," *Br J Ophthalmol*, 97:632-36, 2013.



DIRECT TO BRAIN: Gennaris's bionic-vision system—which includes an eye glasses–mounted camera that receives visual information (below), a small computerized vision processor (right), and 9 mm x 9 mm electronic tiles (far right) that are implanted into one hemisphere of the visual cortex at the back of the brain—is expected to enter human trials next year.

COURTESY OF MVG

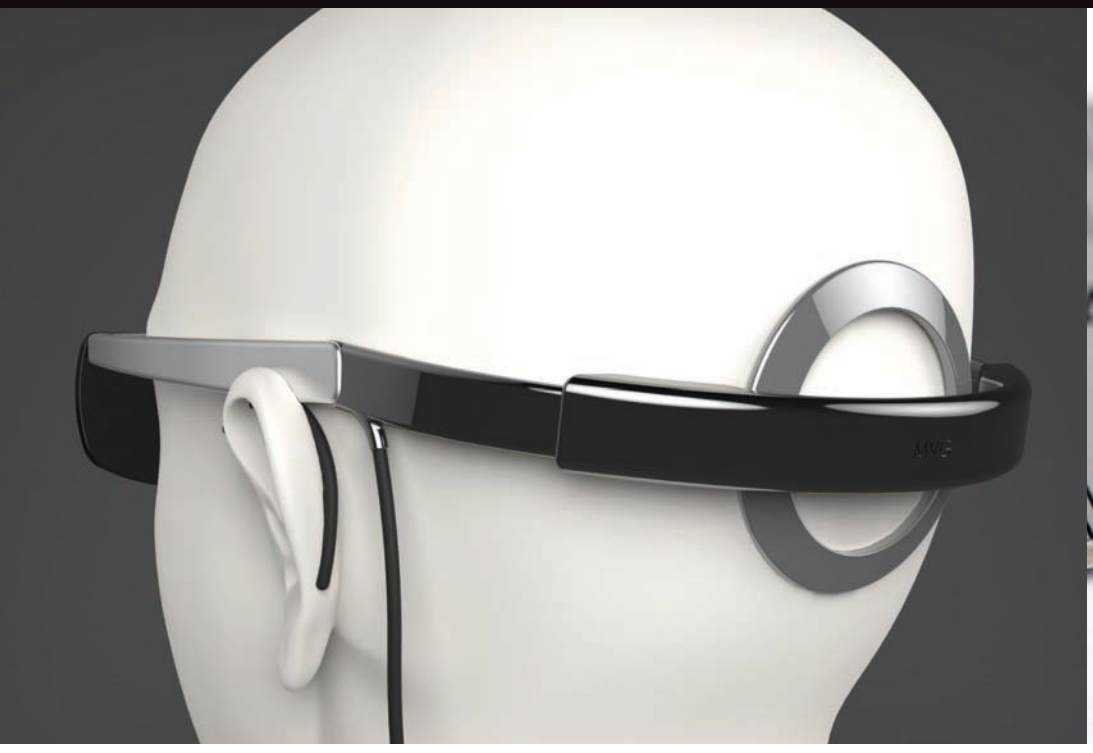
INTO THE BRAIN

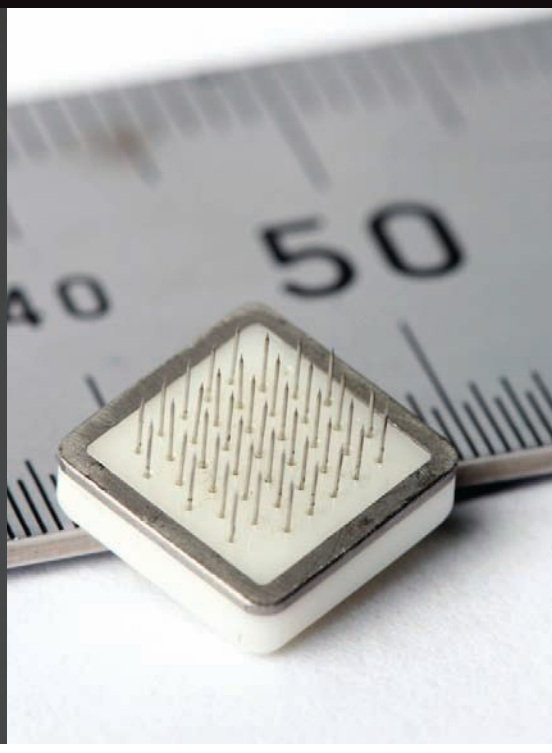
BY COLLETTE MANN, JEFFREY V. ROSENFELD, AND ARTHUR LOWERY

In addition to the neurons of the eye, researchers have also targeted the brain to stimulate artificial vision in humans. Early experimentation in epileptic patients with persistent seizures by German neurologists and neurosurgeons Otfrid Förster in 1929 and Fedor Krause and Heinrich Schum in 1931, showed that electrical stimulation of an occipital pole, the most posterior part of each brain hemisphere, resulted in sensations of light flashes, termed phosphenes. By the mid-1950s, Americans John C. Button, an osteopath and later MD, and Tracy Putnam, then Chief of Neurosurgery at Cedars-Sinai Hospital in Los Angeles, had implanted stainless steel wires connected to a simple stimulator into the cortices of four people who were blind, and the patients subsequently reported seeing flashes of light.

The first functional cortical visual prosthesis was produced in England in 1968, when Giles Brindley, a physiologist, and Walpole Lewin, a neurosurgeon, both at Cambridge University, implanted 80 surface electrodes embedded in a silicone cap in the right occipital cortex of a patient. Each electrode connected to one of 80 corresponding extracranial radio receivers, which generated simple, distinctly located phosphene shapes. The patient could point with her hand to their location in her visual field. When more than one electrode at a time was stimulated, simple patterns emerged.

The subsequent aim of the late William H. Dobelle was to provide patients with visual images comprising discrete sets of phosphenes—in other words, artificial vision. Dobelle had begun studying electrical stimulation of the visual cortex in the late 1960s with sighted patients undergoing surgery to remove occipital lobe tumors. He subsequently implanted surface-electrode arrays, first temporarily, then permanently, in the visual corti-





ces of several blind volunteers. However, it was not until the early 2000s that the technology became available to connect a miniature portable camera and computer to the electrodes for practical conversion of real-world sights into electrical signals. With the resultant cortical stimulation, a patient was able to recognize large-print letters and the outline of images.

To elicit phosphenes, however, the surface electrodes used in these early cortical prostheses required large electrical currents (~3 mA–12 mA), which risked triggering epileptic seizures or debilitating migraines. The devices also required external cables that penetrated the skull, risking infection. Today, with the use of wireless technology, a number of groups are aiming to improve cortical vision prostheses, hoping to provide benefit to millions of people with currently incurable blindness.

One promising device from our group is the Gennaris bionic-vision system, which comprises a digital camera on a glasses frame. Images are transmitted into a small computerized vision processor that converts the picture into waveform patterns, which are then transmitted wirelessly to small electronic tiles that are implanted into the visual cortex located in the back of the brain. (See photographs on this page.) Each tile houses 43 penetrating electrodes, and each electrode may generate a phosphene. The patterns of phosphenes will create 2-D outlines of relevant shapes in the central visual field. The device is in the preclinical stage, with the first human trials planned for next year, when we hope to implant four to six tiles per patient to stimulate patterns of several hundred phosphenes that patients can use to navigate the environment, identify objects in front of them, detect movement, and possibly read large print.

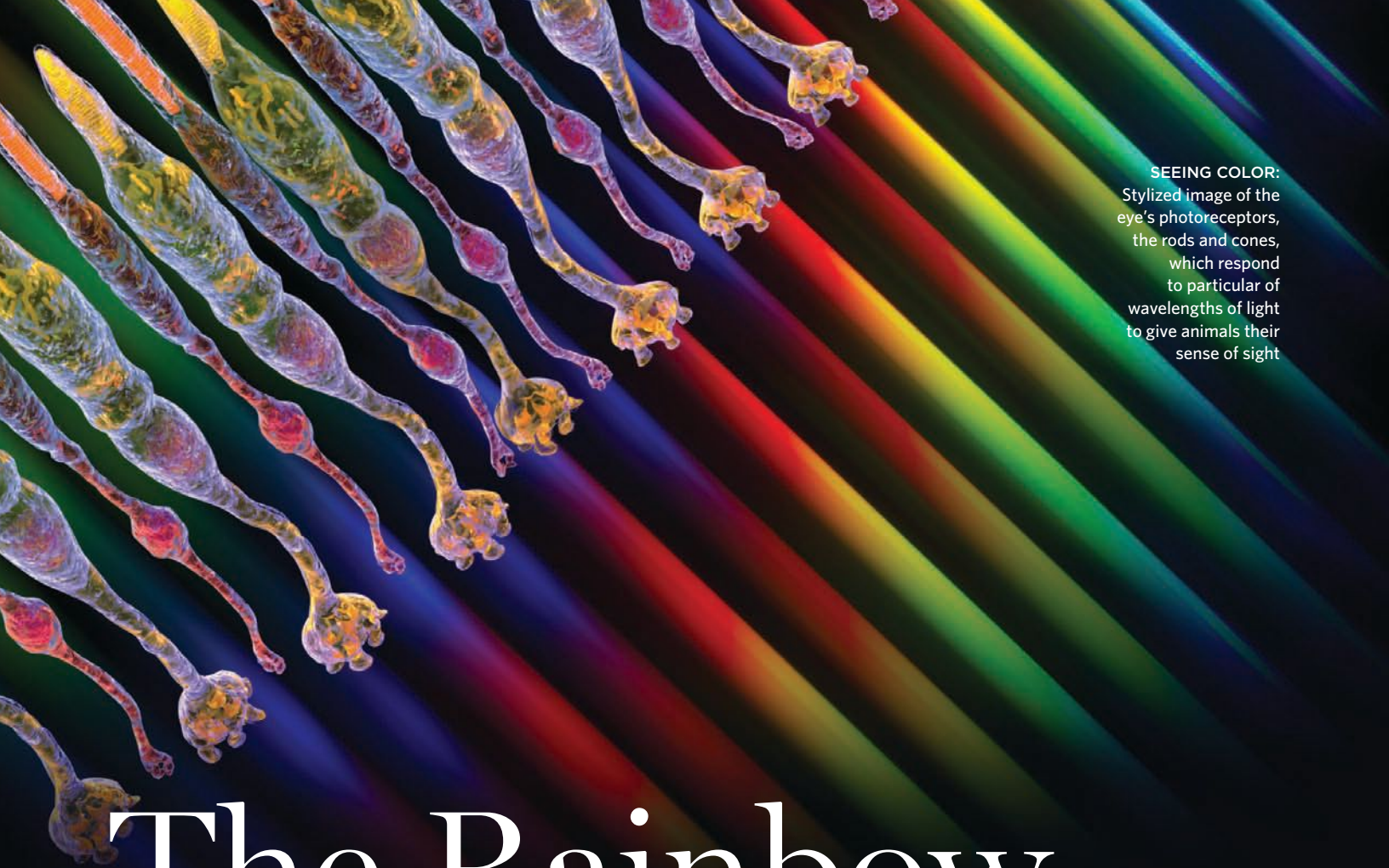
The development in bionic vision devices is accelerating rapidly due to collaborative efforts using the latest silicon chip and electrode design, computer vision processing algorithms, and wireless technologies.

Other groups currently developing cortical visual prostheses include the Illinois Institute of Technology, the University of Utah, the École Polytechnique de Montréal in Canada, and Miguel Hernández University in Spain. All these devices follow the same principal of inducing phosphenes that can be visualized by the patient. Many technical challenges must be overcome before such devices can be brought to the clinic, however, including the need to improve implantation techniques. In addition to the need for patient safety, accuracy and repeatability when inserting the device are important for maximum results.

Development of bionic vision devices is accelerating rapidly due to collaborative efforts using the latest silicon chip and electrode design, computer vision processing algorithms, and wireless technologies. We are optimistic that a range of practical, safe, and effective bionic vision devices will be available over the next decade and that blind individuals will have the ability to "see" their world once again.

Collette Mann is the clinical program coordinator of the Monash Vision Group in Melbourne, Australia, where Arthur Lowery, a professor of electrical engineering, is the director. Jeffrey V. Rosenfeld is head of the Division of Clinical Sciences & Department of Surgery at the Central Clinical School at Monash University and director of the Department of Neurosurgery at Alfred Hospital, which is also in Melbourne.

COURTESY OF MVG



SEEING COLOR: Stylized image of the eye's photoreceptors, the rods and cones, which respond to particular of wavelengths of light to give animals their sense of sight

The Rainbow Connection

Color vision as we know it resulted from one fortuitous genetic event after another.

BY KERRY GRENS

In a steamy Eocene jungle, a newborn monkey opens its eyes for the first time. The world it sees is unlike any other known to its primate kin. A smear of red blood shines against a green nest of leaves. Unbeknownst to its mother, this baby is special, and its eyes will shape the human experience tens of millions of years in the future. Were it not for this little monkey and the series of genetic events that created it, we might not have the color vision we do: Monet's palette would be flattened; the ripeness of a raspberry would be hidden among the leaves; traffic lights? They likely would never have been invented.

Like most other mammals, monkeys that lived 30 million to 60 million years ago had just two opsin genes encoding the photopigment proteins that tune cone photoreceptor cells in the retina to absorb light in a range of wavelengths. Then, an allele of one of the opsin genes mutated, producing a pigment protein that responded to previously unseen wavelengths of light. Later, a region of the allele duplicated and inserted, creating a third opsin gene and solidifying the transition from a landscape of blues and either reds or greens (it's not certain which opsin came first) into the rich color spectrum that humans and many other primates see today.

Adding a third opsin gene doesn't simply introduce 50 percent more colors; its effect is multiplicative. If a single opsin gives an animal the ability to distinguish 100 shades, say, the addition of a second opsin, "amazingly, multiplies that by 100," says color vision researcher Jay Neitz of the University of Washington in Seattle. "Adding a third photopigment has been the greatest invention of all, because it multiplies color vision by another 100 times."

Such a profound expansion of our visual experience actually required very minor genetic alteration. In 1991, Neitz, working with his wife Maureen and their postdoc advisor Jerry Jacobs of the University of California, Santa Barbara, demonstrated that just three amino acid substitutions account for the 30 nm difference in peak absorption between the modern-day red and green cones in humans, with each change shifting the photopigment's color spectrum by 5 nm to 15 nm.[1] "It's absolutely stunning," says Jacobs. "A single nucleotide change can change your color vision." (See illustration on following page.) Yet, despite this simplicity, the evolutionary circumstances that allowed our primate ancestors to adopt trichromacy—the three-cone system that gives humans and some other primates the ability to see the world in full-spectrum color—are remarkably intricate.

To understand how that first trichromatic monkey and its similarly equipped primate descendants responded to their heightened sense of sight remains an ongoing quest. But experiments by the Neitzes and others that provide dichromatic animals, such as mice or squirrel monkeys, with an extra opsin are helping to fill in the story of the evolution of human color vision. The results suggest that the first trichromatic monkey may have been able to respond immediately to its new, more vibrant world—see the ripe fruit among the green buds; the red ants on the leaves. The work may also point the way to a future in which scientists could treat color blindness by replacing malfunctioning opsin genes, and perhaps, one day, even supercharge humans' color perception to reveal a new rainbow altogether.

A colorful duplication

It's 1980. Jeremy Nathans, then a graduate student at Stanford University, is driving back to campus after visiting a slaughterhouse in San Jose. Beside him jiggles a bucket of cow eyeballs on ice.

He's heading to the lab of his advisor, David Hogness, where he plans to use the eyeballs, along with a revolutionary new tool called recombinant DNA, to answer a question that had been posed decades before: What is the molecular basis of color vision? "It seemed clear to me that the way to solve these problems was not to study light-absorbing proteins, which are extremely rare, hard to work with, and intermixed with far more abundant proteins," says Nathans. "This was a problem that was going to be solved by going directly to the genes."

At the time, very few human genes had been cloned, and recombinant DNA methods were crude. "It was hard," recalls Nathans, now a professor at Johns Hopkins University School of Medicine. "We went up quite a few wrong paths." But four years of labor eventually paid off. In 1983, Nathans and Hogness published the amino acid sequence of bovine rhodopsin, and a year later they published the human rhodopsin sequence.[2]

Rhodopsin, expressed in rod photoreceptor cells, enables animals to see in dim light. To understand color vision, Nathans and his colleagues had to track down the three opsins embedded in the cell membranes of the three varieties of cones, which absorb short (blue), medium (green), or long (red) wavelengths of light. Fortunately, despite about a billion years of divergence between them, rhodopsin and the cone opsin genes shared enough sequence homology for the known sequence to serve as a probe for the unknown genes.

What makes the tale of primate color vision so special is that it can be told, from beginning to end, in exquisite genetic and molecular detail.

A couple of years later, using his own DNA, Nathans and colleagues cloned the cone opsins. Two of them—the red and the green—reside on the X chromosome and are 96 percent similar in their amino acid sequence. The results provided support for the idea that an ancient X-linked opsin gene underwent a single duplication event and that subsequent mutations in the copy shifted the absorbance spectrum of the photopigment.[3]

"The work illuminates not only the physiology of color vision, but basic mechanisms of evolution," geneticist David Botstein, now at Princeton, wrote in a commentary accompanying Nathans's paper in *Science*. "It has been thought for some time that a major theme of evolution is duplication followed by divergence."

But, as it would turn out, duplication and divergence are not the whole story.

Around the same time that Nathans was cloning human opsin genes, the Neitzes were working on squirrel monkeys in Jacobs's Santa Barbara lab. Previously, Jacobs had found that these New World monkeys do not have the same color vision as Old World primates and humans. In particular, squirrel monkey color vision is highly polymorphic—some females see much larger ranges of shades than males or other females.

Digging into the genetics of this unusual variation, Jay Neitz and his colleagues discovered that while squirrel monkeys have just two opsin genes (one on chromosome 7 and one on the X chromosome), they have several opsin alleles. Three alleles, which resemble the human red and green opsins, are present in the same locus on the X chromosome. With just one X chromosome, all males are dichromats, but because females carry two Xs, they can carry two different alleles for the X-linked opsin gene, granting such heterozygotes trichromatic vision.[4]

"The first step then to getting to trichromacy [in primates] was to just get diversity, polymorphism, in the one gene [they] have," says Neitz. Then, somewhere along the line a genetic translocation likely plucked an opsin allele from one X chromosome and plunked it next to a different opsin allele on the other X chromosome, giving that animal two opsin genes adjacent to one another, as humans have today.

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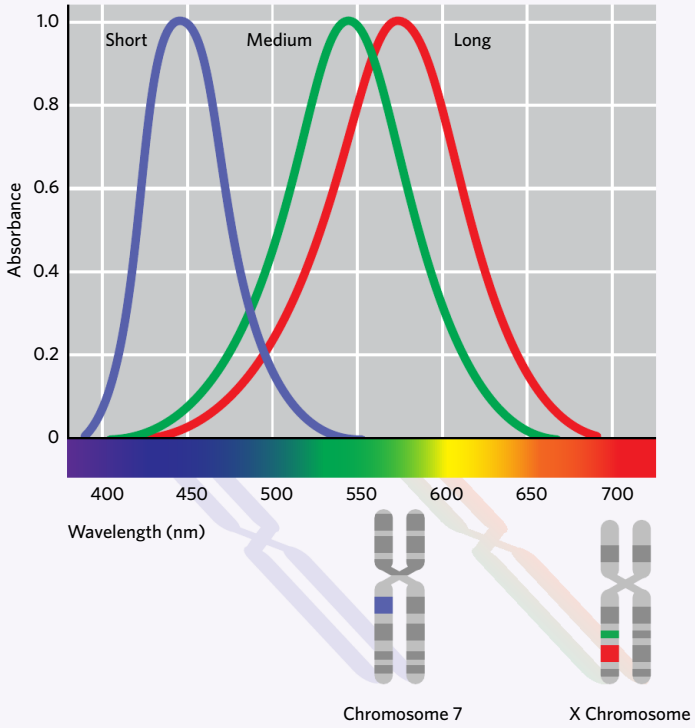
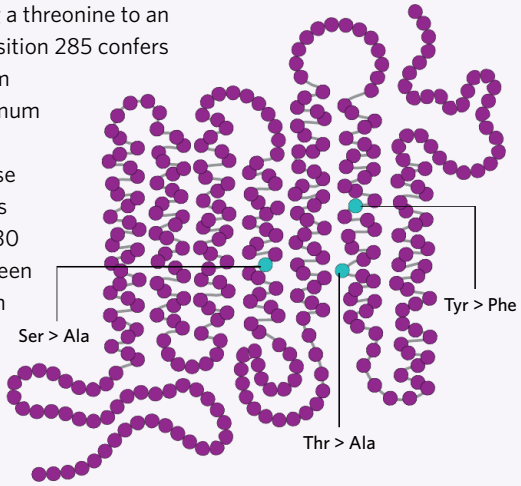
IN LIVING COLOR

Human color vision is based on the different wavelengths of light absorbed by three cone opsin proteins, which are responsible for the spectral tuning of the cone cells in the retina. The red and green opsins, whose genes reside on the X chromosome, are thought to have evolved from an ancestral cone opsin gene that duplicated itself.



Three amino acid substitutions in the red opsin protein account for the spectral tuning of the green opsin. At position 180, swapping serine for alanine produces a 6 nm shift of the absorption spectrum; tyrosine to phenylalanine at position 277 provides a 9 nm shift; and changing a threonine to an alanine at position 285 confers another 15 nm shift in maximum absorption. Together these three changes produce the 30 nm gap between the maximum absorption of the red and green opsins.



"The fact that we have the same few amino-acid substitutions as New World monkeys argues there was a single ancestral variation that gave rise to [the cone opsins of] both Old and New World primates," Nathans says. "It leads to an interesting twist on the evolutionary dogma of gene duplication."

X marks the spot

While a gene duplication of the X-linked opsin was necessary to grant all the animals in a group, including the males, trichromatic vision, this part of the color vision story is, once again, not so simple. There needed to be some mechanism to ensure that both genes on the X chromosome were not coexpressed in the same cone cell.

The prevailing model for how the brain discriminates colors is that it assigns cone classes—green, red, or blue—to each cell by comparing how it and its neighbors respond to various wavelengths. For instance, if red light hits the eye and one cone activates while an adjacent cone stays silent, then the brain figures out that those two are in different cone classes. But cone cells are only useful in discriminating colors in this way if each cell expresses only one type of opsin. If an individual cone carried two different opsins and responded to the absorption spectra of each, its firing wouldn't be very informative. So how do cone cells, which carry an organism's full genome and thus the genes for all three opsins, limit the expression of two of them?

Researchers are still unsure how the blue opsin gene, positioned on an autosomal chromosome, is not coexpressed with either of the opsins on the X chromosome, but Nathans's work has yielded clues regarding the mechanism that allows only one of the two X-linked opsins to be expressed in a given cell. Studying people who have only one functional opsin that absorbs short wavelengths of light and can thus discriminate only among blue hues, Nathans discovered that some of these so-called blue-cone monochromats had deletions about 4,000 base pairs upstream of the red and green opsins on the X chromosome.[5] "It smelled like an enhancer," says Nathans, referring to short genetic sequences adjacent to promoters that help initiate transcription. He later showed in transgenic mice that this enhancer sequence is required for the expression of red and green opsins and that it selects which one will be transcribed.[6]

Importantly, the opsin gene duplication on the X chromosome did not include the enhancer, resulting in a single enhancer being responsible for turning on both genes. But it acts on only one opsin gene in any given cell—likely chosen at random—meaning that the enhancer will lead to the expression of the red opsin in one cone and the green opsin in another. Without this mechanism, cones would likely express a gobbledygook of green and red opsins, and our perception of color would be drastically different.

Brain power

Whether that first trichromatic monkey could actually take advantage of the expression of all three opsin alleles—whether it could see the blood on the leaves as a different color—is not entirely clear. And in fact, a basic quandary in evolutionary biology is how animals process new sensory input. "When she got this new cone, did she say, 'Great, this is a nicely colored world,'" says Jacobs, "or did she say,

THE SCIENTIST STAFF; OPSIN PROTEIN ADAPTED FROM FIGURE COURTESY OF JAY NEITZ AND *SCIENCE*, 252:971–74, 1991.

'Oh, now I suppose I have to redesign my nervous system'?" Around 2000, he and Nathans got the opportunity to answer that question.

The researchers replaced one of the medium-wavelength opsin alleles on the mouse X chromosome with a human long-wavelength opsin gene to create a line of trichromatic mice. They then trained the mice to select the color in a panel of three that differed from the other two. Next, the mice were tested for their ability to perform this task across a range of shades, including those within the absorption spectrum of the new opsin. The newly trichromatic animals excelled at the task. In 2007, the team published data showing that heterozygous female mice, which carried the long-wavelength gene on one X chromosome and the medium-wavelength gene on the other, plus their short-wavelength gene on chromosome 6, were able to discriminate additional colors compared to animals with only medium- and short-wavelength alleles. This suggested that the animals' brains were making use of the new opsin.[7] "I think that's the cool part of it, that [plasticity] is just an intrinsic property of a sophisticated nervous system like a mammal's," says Nathans.

A couple of years later, the Neitzes and their colleagues tried something similar with adult squirrel monkeys, using viral-vector gene therapy to introduce a third cone opsin into full-grown males. In this case, the animals were not immediately able to discriminate colors, but after about four months, the monkeys showed marked improvement, detecting previously indistinguishable colors in blue-green and red-violet hues.[8] The delay corresponded to the timing of robust transgene expression, as if having the new visual pigment was all it took. "Marvelously, the monkeys gained what looks like full trichromatic vision," says Jay Neitz, adding that the effects remained stable for a few years.

Both studies suggest the possibility that the primate brain was primed to accept the new stimulus offered by a third cone opsin—no major rewiring required. Such an ability may reflect our far-distant ancestors' perception of even more colors than we see today. "If you go back to the vertebrate ancestor, they used to have five different kinds of pigments," says Shozo Yokoyama, who studies vertebrate opsins at Emory University. Mammalian ancestors presumably lost some of these opsin genes along the evolutionary way, but their brains may have retained the capability to interpret the activity of additional opsins. (See "Animals' Diverse Palettes" on following page.)

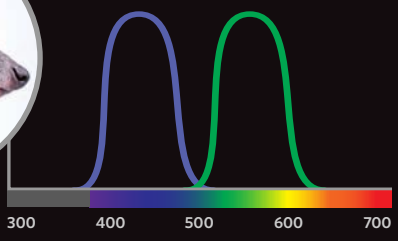
But others in the field aren't convinced that the animals were able to process new colors as soon as the retinal hardware was in place. Shortly after Nathans's mouse study came out, Walter Makous, a vision science researcher at the University of Rochester, commented in *Science* that the mice might not be discriminating colors with their new opsin. Rather, they could have detected blotchiness in the color presented as a result of the human and mouse cones responding differently to the same stimulus, as if they were detecting different luminosities of the same color.[9] It's possible then that true color vision does not explain the mice's improved performance.

DICHROMACY VERSUS TRICHROMACY: Like squirrel monkeys, female capuchins (*Cebus capucinus*) can express either two opsins, giving them dichromatic vision (approximated visible spectrum generated via a computer program in left column), or three, giving them trichromatic vision (approximated visible spectrum in right column). These color vision phenotypes affect perception of relevant objects in the natural environment, including other primates (*Ateles geoffroyi*, top row), ripe fruits (*Ficus ovalis*, middle row), and predators (*Puma concolor*, bottom row).

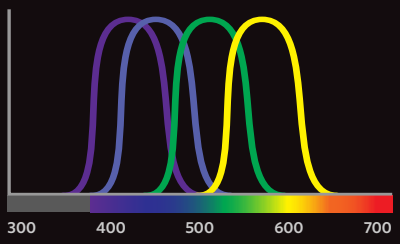
PLOS ONE, DOI:10.1371/JOURNAL.PONE.0084872.G001, 2014. PHOTO CREDITS PRIMATES: F. CAMPOS; FRUITS: A. MELIN; PUMA: N. PARR

ANIMALS' DIVERSE PALETTES

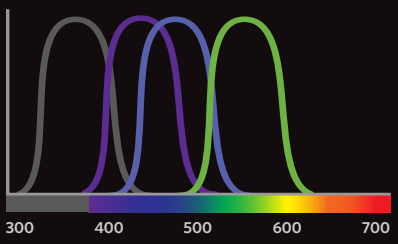
Most mammals, such as dogs, express just two types of opsins in the distal ends of their eyes' cone cells, which are responsible for color vision. Humans and some primates have three. Other animals, including birds, fish, and insects, have even more opsins, although insects don't have cones, but instead use other types of cells to detect color. Such diversity yields whole new worlds of color, with each opsin adding an order of magnitude more hues. Reconstructing the evolution of opsin genes, Shozo Yokoyama of Emory University and his colleagues have found that substitutions at only a couple dozen amino acid sites in opsin proteins account for this diversity of spectral tuning found among vertebrates.



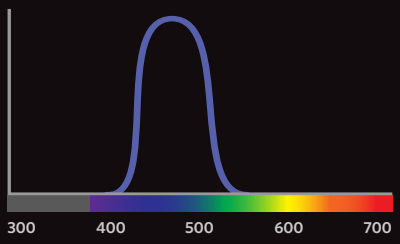
Like most mammals, dogs (*Canis familiaris*) see in color, just far fewer colors than other animals. From a behavioral study of two Italian greyhounds and a toy poodle, researchers figured out their limited color discrimination is due to dichromatic color vision (*Visual Neuroscience*, 3:119-25, 1989).



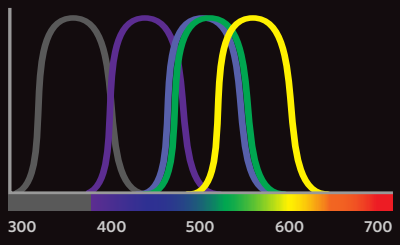
Chickens have four types of cone opsins (*PNAS*, 89:5932-36, 1992), and in some birds, the short-wavelength opsin is shifted to absorb in the ultraviolet. Bird cone cells also have an oil droplet that serves to filter or concentrate particular wavelengths of light.



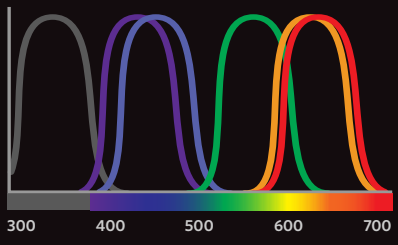
The eye of the American chameleon (*Anolis carolinensis*) has no rods and uses multiple cone opsins to detect color. The peaks here show the maximum absorption of the photopigments reconstituted in vitro (*Vision Research*, 38:37-44, 1998).



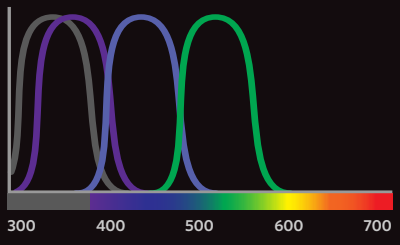
In its ocean habitat, the coelacanth (*Latimeria chalumnae*) receives only blue light. Correspondingly, its rod-enriched eyes absorb light in this range. The peak here represents the absorption maximum of the visual pigment in vitro (*PNAS*, 96:6279–84, 1999).



Like many fish, goldfish can see in the ultraviolet, thanks to a shift in their short-wavelength opsin. Using their long-wavelength opsin (yellow peak), they can also see red, likely an adaptation to their shallow aquatic environment, in which red light is not filtered out (*Genetics*, 153:919–32, 1999).



The small white butterfly (*Pieris rapae*) expresses four types of opsins but has at least six types of photoreceptors (*PLOS ONE*, 5:e15015, 2010). Filters in the eye adjust the spectral sensitivity of the photoreceptor cells. In males, the violet receptor is modified into a second blue one (not shown).



Repeated florets called ommatidia in the compound eye of the fruit fly (*Drosophila melanogaster*) are made of a central color-vision cell surrounded by six blue-light absorbing cells. Shown here are the absorption maximums for the opsins expressed in the central cell of the ommatidium (*J Neurosci*, 19:10716-26, 1999).

DOG: © MALEXEUM; CHICKEN: © WIKTOR RZEŹUCHOWSK; CHAMELEON: © LEEKRIS; COELACANTH: © LOONGER; GOLDFISH: © IROCHKA_T; BUTTERFLY: DIDIER DESCOUENS/WIKIMEDIA COMMONS; FRUIT FLY: © NANCY NEHRING

Instead, the brain "could see changes in color as essentially changes in texture," says David Brainard, a vision scientist at the University of Pennsylvania. "Or it could be the brain is primed to figure this out and gives you color vision. I don't think we know."

A recent modeling study by Brainard's group points to the latter scenario, at least for primates. The research team used computer learning to simulate human color vision and found that the configuration of the human retina—its particular mosaic of cone types, the ratio of long- to medium-wavelength cones, and the differences in their maximum absorption spectra—allows for such learning.[10] These variables present the brain with enough information to determine the class (red, green, or blue) of each cone based on the wavelengths that each cell responds to.

It's absolutely stunning. A single nucleotide change can change your color vision.

—Jerry Jacobs, University of California, Santa Barbara

"It was possible for the information-processing system to observe the signal of a cone and successfully assign labels for each cone class that are highly accurate," Brainard says. Whether that was the case when trichromacy first appeared among primates is not certain, but Brainard's simulation demonstrates that the modern human retina can make use of three opsins. Given the similarities among primate retinas, perhaps the first trichromatic monkey could do the same.

Colors with benefits

At whatever point primates were able to perceive additional color, the advantages would have spurred trichromacy's quick spread through the population, researchers presume. It's been thought that the monkeys would have been able to better distinguish between ripe and unripe fruit, for example, and to spot reddish young leaves among less protein-rich older ones, allowing them to forage more efficiently and improve the nutritional quality of their meals. (See photographs on page 45.) But demonstrating the existence of such benefits has proven difficult.

Amanda Melin of Washington University in St. Louis has spent years traveling to the forests of Costa Rica to observe capuchin monkeys in their natural habitat. Like squirrel monkeys, these New World primates have dichromatic males and either dichromatic or trichromatic females. Melin spends days at a time watching the animals forage, walking kilometers through the forest as the monkeys move from tree to tree, and collecting DNA from fecal samples, in an effort to determine which colors each animal can see. Much to her surprise, she's found that fruit feeding rates between dichromats and trichromats are the same, initially suggesting that color vision doesn't offer an advantage for foraging.[11]

Digging deeper into the data, however, Melin uncovered a subtler effect. "Where we see the difference is in accuracy," she says. "Trichromats are making way fewer mistakes, but foraging at a more leisurely pace." Dichromats, on the other hand, appear more frantic, touching, sniffing, and biting more fruits, including unripe or inedible ones.[12] The question Melin is trying to answer now is whether that sloppier foraging behavior has any nutritional impact on the animals.

For people, of course, the importance of color vision is immeasurable, and Neitz is hopeful that the gene therapy he has used in monkeys could translate to a therapy for patients with color blindness. If successful, this would not only bring color acuity into the lives of those whose color experience is limited, it would allow researchers to explore how humans experience the leap from dichromacy to trichromacy.

But why stop there? Neitz wonders if it might also be possible to expand the range of normal human vision. This summer, he used gene therapy to give two monkeys a fourth cone opsin, such as birds have. Its absorption peak sits between that of the short- and medium-wavelength opsins, somewhere between violet and green. As this article goes to press, it's too soon to know whether the supplemental gene has given the monkeys added sensitivity at that end of the rainbow, but if it works, the monkeys may soon be able to see 100 times more colors. It is enticing to think of how such a therapy could enrich human vision. Would a forest no longer look homogeneously green, but as diverse in hue as it is in individual trees? What beauty might we experience in art if we are able to perceive an order of magnitude more colors?

"Sometimes when I'm driving around I ask myself if the IRB [institutional review board] would let me do that to myself," says Neitz. Why not try it out, he muses, to see if he can "cure" himself of trichromacy? Indeed, whether it happens by the hand of evolution or by human intervention, perhaps the story of human color vision's progression is not over, Neitz says. "It's a brave new world." ■

References

1. M. Neitz et al., "Spectral tuning of pigments underlying red-green color vision," *Science,* 252:971-74, 1991.
2. J. Nathans, D.S. Hogness, "Isolation and nucleotide sequence of the gene encoding human rhodopsin," *PNAS,* 81:4851-55, 1984.
3. J. Nathans et al., "Molecular genetics of human color vision: The genes encoding blue, green, and red pigments," *Science,* 232:193-202, 1986.
4. G.H. Jacobs, J. Neitz, "Inheritance of color vision in a New World monkey (*Saimiri sciureus*)," *PNAS,* 84:2545-49, 1987.
5. J. Nathans et al., "Molecular genetics of human blue cone monochromacy," *Science,* 245:831-38, 1989.
6. Y. Wang et al., "A locus control region adjacent to the human red and green visual pigment genes," *Neuron,* 9:429-40, 1992.
7. G.H. Jacobs et al., "Emergence of novel color vision in mice engineered to express a human cone photopigment," *Science,* 315:1723-25, 2007.
8. K. Mancuso et al., "Gene therapy for red-green colour blindness in adult primates," *Nature,* 461:784-87, 2009.
9. W. Makous, "Comment on 'Emergence of novel color vision in mice engineered to express a human cone photopigment,'" *Science,* 318:196b, 2007.
10. N.C. Benson et al., "Unsupervised learning of cone spectral classes from natural images," *PLOS Computational Biology,* 10:e1003652, 2014.
11. A.D. Melin et al., "Fig foraging by dichromatic and trichromatic *Cebus capucinus* in a tropical dry forest," *International Journal of Primatology,* 30:753-75, 2009.
12. A.D. Melin et al., "Food search through the eyes of a monkey: a functional substitution approach for assessing the ecology of primate color vision," *Vision Research,* 86:87-96, 2013.

SETTING THE RECORD STRAIGHT

Scientists are taking to social media to challenge weak research, share replication attempts in real time, and counteract hype. Will this online discourse enrich the scientific process?

BY DANIEL COSSINS

Sometimes even the best-known stories have hidden subplots. This January, *Nature* published two papers describing an astonishing new way to make stem cells: simply grow blood cells from adult mice in acidic media.[1,2] The researchers behind the work—a team from the RIKEN Center for Developmental Biology in Japan and Harvard Medical School—called it stimulus-triggered acquisition of pluripotency, or STAP. These stress-induced stem cells were even more malleable than induced pluripotent stem cells (iPSCs), and, even better, they could be produced without the addition of transcription factors. Naturally, the press was abuzz with the promise of STAP to accelerate stem cell research. But in the less well-lit corners of the Web, some were already raising doubts.

Leading the way was Paul Knoepfler, a stem cell researcher at the University of California, Davis. "I quickly had the feeling this might be entirely wrong," he says, "and that's pretty unsettling when it's in *Nature*." On January 29, the day both papers went up online, Knoepfler posted a review of the research on his blog. "It just seems too good and too simple of a method to be true," he wrote. He followed it up a week later with another post outlining five reasons to doubt the STAP results. Meanwhile, a team led by Ken Lee, a stem cell researcher at the Chinese University of Hong Kong, was failing to replicate the results. "We tried it on various cells and none worked," Lee recalls. He posted his results on ResearchGate, a social networking site for scientists, and continued trying to produce STAP cells to no avail. "Now I was starting to get pissed off."

Lee and Knoepfler weren't the only ones with concerns. Knoepfler ran an online poll to gauge opinion, revealing rapidly dwindling confidence in STAP, and created a Web page where people could post results from their replication attempts. Lee penned a review detailing his results for ResearchGate's brand-new Open Review site, and a Japanese blogger discussed specific problems with the papers' figures. Commenters on PubPeer, a postpublication peer review website, raised further concerns. On February 14, RIKEN initiated its own investigation and, six weeks later, announced that Haruko Obokata, first author on both studies, was guilty of scientific misconduct. On July 2, *Nature* retracted the papers.

Knoepfler says social media played an influential role in righting the literature. "The momentum started on blogs and Twitter, and it took off from there," he says. "I believe that without social media, right now the STAP papers wouldn't have been retracted."

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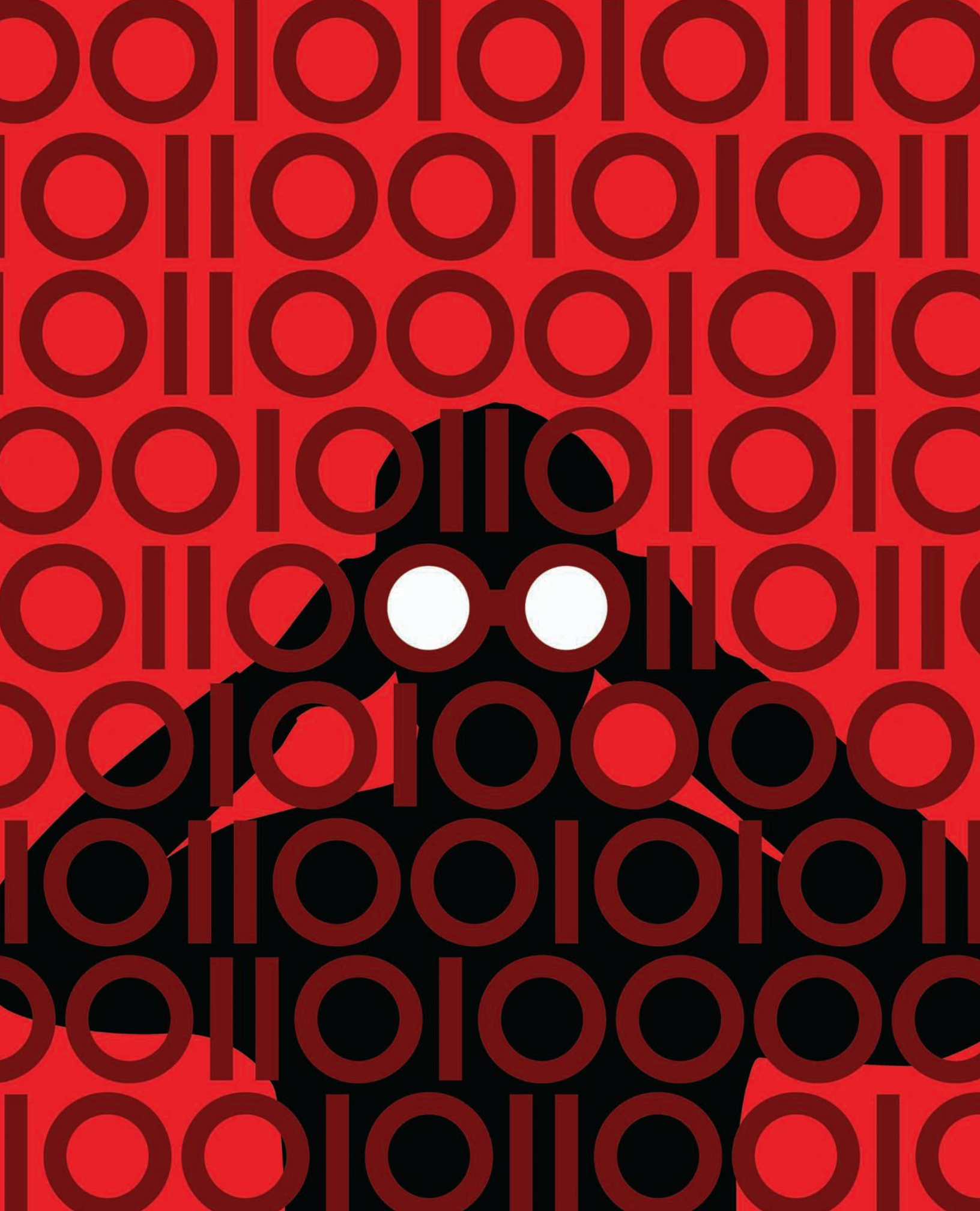

The STAP saga—which took a tragic twist when Obokata's supervisor at RIKEN, Yoshiki Sasai, committed suicide in August—is just the freshest example of scientists turning to blogs and social media to question and refute published findings. Back in 2011, University of British Columbia microbiologist Rosie Redfield made a splash when she live-blogged her attempts to replicate a study reporting the discovery of bacteria that could incorporate arsenic in place of phosphorus into their DNA. Beyond such high-profile cases, a small but growing band of scientist bloggers are hoping to accelerate research evaluation and make science more transparent. Ultimately, they argue, rapid-fire open critiques will enrich the scientific process.

"[Social media is] introducing a robust culture of community-driven postpublication peer review, and that's hugely valuable," says Chris Chambers, a neuroscientist at Cardiff University in the U.K. and a blogger for *The Guardian*. "It chips away at this idea that something must be true because it's in a peer-reviewed journal and replaces it with the idea that your work is out there to be poked and prodded."

Not all scientists are so enthusiastic. Many are apathetic about social media, and some are cautious of new pitfalls, not least the potential for undeserved reputational damage. But as the scientific generations turn over, social media is on track to become a central part of research evaluation.

"Whether you like it or not, this is an unstoppable trend," says Knoepfler. "It's the new reality for today's researchers. Your papers, particularly high-impact ones, are going to be subject to continuous feedback in real time."

Real-time replication

Rapid-fire feedback is not new to science. In the 17th and 18th centuries, gentleman scientists shared their results at scientific societies and faced criticisms on the spot. That still happens at conferences today to some extent, but the modern scientific process came to be dominated by private and anonymous peer review. Once published, data and conclusions were rarely questioned or discussed outside of the formal confines of academic journals, says Chambers. "If you saw a paper you thought was bullshit, you would probably discuss it with colleagues and leave it at that," he says. "You could try to send a letter to the journal, but that's very slow, and there's no guarantee they'll publish it anyway."

Online publishing and social media has changed all that. Now anyone can share their opinions with the world. For scientists, that provides an unprecedented opportunity to accelerate discussions once mediated by journal editors. It's early days, but some researchers have embraced digital discourse with open arms—and quick-typing fingers.

Chambers, who uses brain-imaging techniques and transcranial magnetic stimulation (TMS) to study cognitive control in the human brain, started blogging in 2011. He reviews new papers, offers thoughts on how to improve research practice, and occasionally shares sharp criticisms of other people's work. In March 2012, for example, Chambers posted a detailed critique of a study from the University of Sydney's Richard Chi and Alan Snyder, who concluded that a form of electrical brain stimulation helps people solve tricky puzzles. "I've read their paper several times now, back to front, even sideways a couple of times," he wrote. "And I still can't find any evidence to support this claim. Instead all I found was a long list of flaws."

A dozen or so other researchers have joined Chambers among the ranks of dedicated scientist bloggers, and on several occasions their posts have made news. Redfield rocketed to relative fame in 2011, when she publicly refuted the NASA-funded study apparently demonstrating that bacteria from California's Mono Lake could survive without phosphorus, instead incorporating arsenic into their DNA.[3] The finding, published online in *Science* in December 2010, would have profound consequences for astrobiology, suggesting that environments lacking phosphorus, an element thought to be essential to all organisms, might support life after all. NASA teased the paper for a few days before it was released, touting "an astrobiology finding that will impact the search for evidence of extraterrestrial life," and the press was all over it. Redfield, on the other hand, was not impressed.

"I thought it was garbage," she recalls. She posted a critique on her blog *RRResearch* detailing potential flaws. The authors had not ruled out the possibility that phosphorus had contaminated the medium on which the bacterium, called

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GFAJ-1, was grown, argued Redfield, and the arsenic they detected may have come from something other than DNA. "Basically, it doesn't present ANY convincing evidence that arsenic has been incorporated into DNA," she wrote. "Lots of flim-flam, but very little reliable information."

The post went viral, kick-starting an online orgy of criticism and counter-criticism. Six months later, *Science* published eight "technical comments" about the paper, including one from Redfield, and a response to the comments from first author Felisa Wolfe-Simon, now at Lawrence Berkeley National Laboratory in California. By that time, Redfield had begun live-blogging about her attempts to replicate the results in her lab. "It was a great chance to do open science while people were actually watching," she says.

In the end, Redfield and colleagues from Princeton University, who had reached out to Redfield via her blog, failed to replicate Wolfe-Simon's results. In February 2012, Redfield posted data demonstrating that there was no arsenic present in the DNA of GFAJ-1 bacteria taken from Mono Lake and grown in a low-phosphate medium. They uploaded their report to the preprint server arXiv immediately and waited for a response from *Science*.

Despite the new results, Wolfe-Simon and her colleagues stood by their conclusions and even denounced Redfield's approach. "We do not fully understand the key details of the website experiments and conditions," Wolfe-Simon told *Nature News* at the time. "So we hope to see this work published in a peer-reviewed journal, as this is how science best proceeds." They got their wish when *Science* published Redfield's paper in July 2012.[4] A few months later, it was followed up with a *Nature* paper from another group demonstrating that GFAJ-1 has a high preference for phosphorus over arsenic.[5] Scientists in the field have all but dismissed the original results, though the paper has not been retracted.

The episode serves as a dramatic example of how social media can speed up science's oft-boasted ability to self-correct. It also shows how scientist bloggers can set the record straight in a highly visible

DON'T BELIEVE THE HYPE

In addition to scrutinizing each other's work, researchers who are active online are also taking aim at exaggerated or inaccurate science reporting. "This is an area where social media can be incredibly powerful if scientists do it right," says Jonathan Eisen of the University of California, Davis, who regularly takes down dodgy science reporting on his *Tree of Life* blog. Many scientist bloggers first engaged in the activity specifically to counter misleading information being peddled by the media.

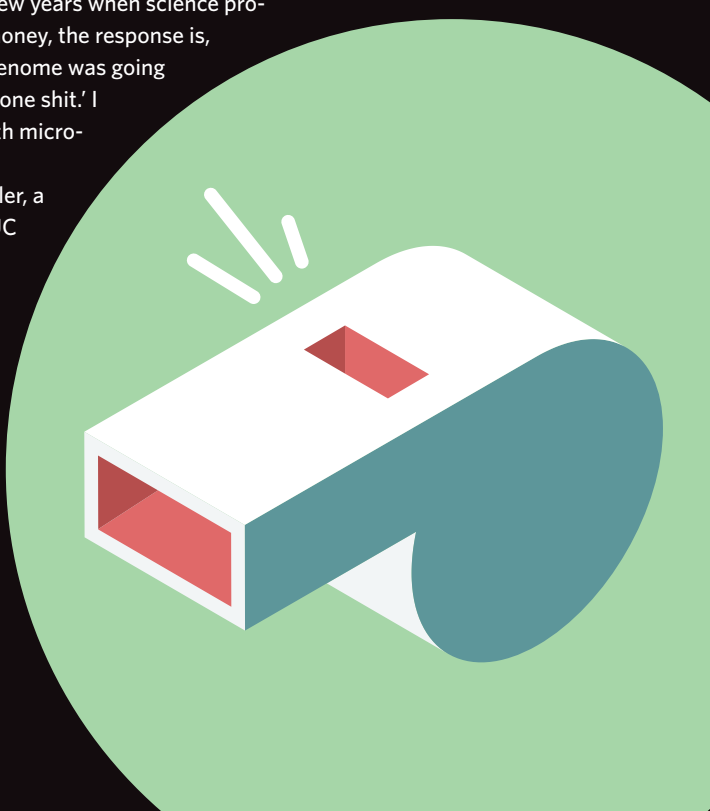
Over the past couple of years, Eisen has trained his sights on coverage of microbiome research. In August 2010, incensed by reports claiming that each new study would lead to a cure for this disease or that, he started dishing out the "Overselling the Microbiome" award to offending journalists and PR departments. It's kept him busy. Eisen has blogged about 23 of these awards and has given out even more on Twitter, without going into as much detail. (He also doles out awards for overselling genomics.)

In May this year, Eisen launched a forensic dissection of the reporting from *Science* and *The New York Times* on a paper that characterized the microbial community living in the placentas of 320 pregnant women. His main gripe was the claim that the study suggests a causal link between oral health, the placental microbiome, and premature birth. "I see no evidence presented anywhere of the importance of oral health or any causal connection between oral health and the placental microbiome or risks to pregnancies," wrote Eisen. "The claims made about this here in this news story are irresponsible." It's speculation, he said, and that must be made clear.

Although the vast majority of the people who read the original stories will not have seen the corrective, Eisen insists it's a worthwhile exercise. "Reporters have told me they're more careful because they don't want one of my awards," he says. And it's not just reporters, he adds. PR departments at Cedars-Sinai Medical Center in Los Angeles and the University of Bern have received Eisen's microbiome-hype awards.

But Eisen says he wants to do more than make sure the public isn't misled; he also strives to protect the reputations of scientists, who can suffer the consequences when a field inevitably doesn't deliver on trumped-up promises. "I remember seeing [President Bill] Clinton talking about how sequencing the human genome is going to cure cancer, and it was just completely overselling it," says Eisen. "Now, in the last few years when science programs have asked for more money, the response is, 'Oh yeah, well, you said the genome was going to cure cancer, and it hasn't done shit.' I don't want that to happen with microbiome research."

According to Paul Knoepfler, a stem cell researcher also at UC Davis and a dedicated blogger, scientists have a responsibility to skewer hype. "It's particularly important in a field like stem cells, where the potential clinical applications are huge and the public is very engaged," he says. "There is a lot of misinformation out there, but we can offer a dose of reality on our blogs. Who else is going to do it, if not us?"



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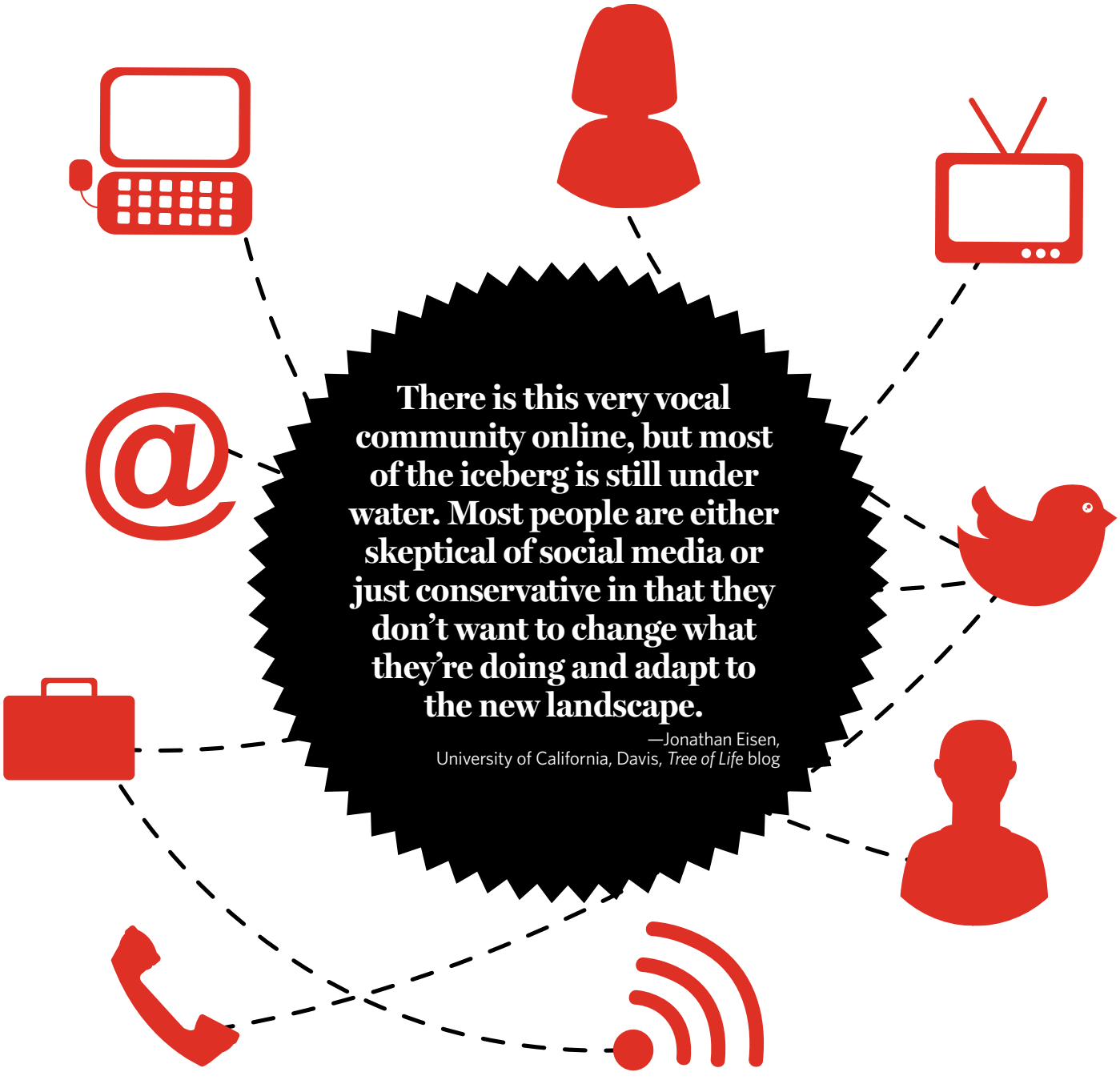

way. "If you Google 'arsenic DNA,' most of the top hits are about the refutation rather than the original result," says Redfield. "If we've got Google serving up the truth, then I think that represents some level of success for the approach."

Rethinking review

Open-science enthusiasts will point to the rising numbers of retractions, cases of misconduct, and problems with reproducibility as evidence that research must be critically examined even after publication. "There is the wrong impression that [peer review is] infallible," says Jonathan Eisen, an evolutionary biologist at the University of California, Davis, who writes the *Tree of Life* blog. "That's not how science should work. We should be evaluating things continuously, and I believe dynamic online discussion is the best way."

But while online communication has the potential to accelerate postpublication review and open a public window on the scientific process, the speed and reach of social media also harbors dangers for scientists. In an editorial published in June 2013, *Current Biology* editor Geoff North pointed out that "[online] critics are less accountable than in the more 'traditional' system of peer-reviewed journals," and that hastily posted criticisms, often penned in a fit of pique, can cause unwarranted reputational damage. "And once a scientific reputation has been tainted, it can be hard to restore confidence," he wrote.

Aware of such risks, scientist bloggers emphasize the importance of self-control. "Things that feel cathartic to write often don't feel good to read," says Chambers. "You have to be very careful with tone." In the discussions of Mono Lake's

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arsenic-DNA bacteria, for example, refutation by blog spilled over into personal attacks on Wolfe-Simon. On that occasion, Eisen took to his own blog to call for commentators to focus on the data. He should know; in 2009 Eisen himself posted a missive against another scientist for not citing a nearly identical study from his own group. "On reflection, I was way too aggressive, and I retracted the post," he says. "The lesson was to stick to commenting on the work, rather than speculating about motivations."

If kept professional, though, open conversation can be a shortcut to clarification. Following public questioning of the new STAP method, Harvard's Charles Vacanti, a senior researcher on one of the papers who has since announced that he will take a one-year sabbatical, published a new protocol stipulating that the cells should be squeezed through tiny pipettes before being dunked in acid. Having been nudged on Twitter by Knoepfler, Chinese University of Hong Kong's Lee again took up the challenge. This time he live-blogged his efforts, posting daily updates and photos on ResearchGate.

Again, he could not replicate the results, but his efforts did yield some interesting findings—and highlighted another possible pitfall of the online approach. Lee noticed the negative control cells he'd squeezed through his narrow pipettes, but had not dunked in acid, did show some expression of the genes associated with pluripotency. Lee was cautious, of course; he knew he'd need to repeat the experiment to validate what he'd seen, and he wrote as much. But several journalists saw the post and, without speaking to Lee, reported that he'd validated the STAP technique. "These things can quickly take on a life of their own," he says.

So how is the scientific community as a whole handling the double-edged sword of instantaneous research evaluation? Many are ignoring it altogether, according to several scientist bloggers, and among researchers who do acknowledge it, the majority holds firmly to the belief that all scientific debate should take place in the pages of scientific journals. "There is this very vocal community online, but most of the iceberg is still under water," says Eisen. "Most people are either skeptical of social media or just conservative in that they don't want to change what they're doing and adapt to the new landscape."

Going mainstream

Although open online discussion of peer-reviewed work remains the exception rather than the rule, websites dedicated to postpublication peer review are beginning to sprout, typically tended by younger scientists who have grown up with the Web.

Among the most popular is PubPeer, launched in October 2013 by anonymous researchers who describe themselves as "early-stage scientists." The site allows people to comment on any scientific article with a DOI or those published as preprints in arXiv. "Many people write blogs, but even in the Google age it is quite difficult to search for comments in any systematic way," a spokesperson for PubPeer told *The Scientist* in an e-mail. Indeed, while some journals allow comments on online articles, researchers have to post and view them on a journal-by-journal basis. PubPeer aims to change that by providing a centralized repository for comments, which are kept anonymous to assuage researchers concerned that critical reviews could damage their careers.

Others are taking a more open approach. PubMed Commons, launched by the National Center for Biotechnology Information (NCBI) database in December 2013, invites PubMed-indexed scientists to post comments, along with their name and institution, at the bottom of the 23 million (and counting) papers in the literature repository. And in March 2014, in the midst of the STAP debacle, ResearchGate joined the postpublication peer review movement by launching Open Review, which asks authors to provide slightly more formal evaluations of published studies—and to put their names to their comments. That's important, says Ijad Madisch, a Berlin-based physician with a PhD in virology who cofounded ResearchGate in 2008. "The main benefit of postpublication peer review using social media is that researchers can engage in discussions about their work and get feedback on it in real-time," he says. "But this only works if the process is open and transparent and researchers use their real names."

The approach certainly seems to be popular: ResearchGate now boasts 5 million members, and researchers have posted more than 12,000 reviews on Open Review. What's more, in a recent *Nature* survey, 88 percent of 3,500 scientists and engineers polled said they were aware of ResearchGate, and 1,589 of those researchers said they visited the site regularly. Still, the survey suggests the number of researchers who actively discuss research remains low, with just 14 percent of regular visitors saying they have posted comments to the site.

To encourage more researchers to post critiques of each other's work, Eisen suggests attaching DOIs to constructive comments, so that each comment can itself be cited. "We probably need to make it more formal and offer rewards if we're to get scientists to really embrace postpublication review," he says.

Most agree that traditional peer review, for all its problems, will retain a central role in science in the 21st century. But at this point it seems almost inevitable that social media will have a big impact on what happens after publication. "Transformative is a strong word," says Knoepfler, "but I think it applies here." ■

Daniel Cossins, a former associate editor of The Scientist, *is a freelance writer living in London.*

References

1. H. Obokata et al., "Stimulus-triggered fate conversion of somatic cells into pluripotency," *Nature,* 505:641-47, 2014.
2. H. Obokata et al., "Bidirectional development potential in reprogrammed cells with acquired pluripotency," *Nature,* 505:676-80, 2014.
3. F. Wolfe-Simon et al., "A bacterium that can grow by using arsenic instead of phosphorus," *Science,* 332:1163-66, 2011.
4. M.L. Reaves et al., "Absence of detectable arsenate in DNA from arsenate-grown GFAJ-1 cell," *Science,* 337:470-73, 2012.
5. M. Elias et al., "The molecular basis of phosphate discrimination in arsenate-rich environments," *Nature,* 491:134-37, 2012.

The Literature

NEUROSCIENCE

Guiding Light

THE PAPER

A.M. Labin et al., "Müller cells separate between wavelengths to improve day vision with minimal effect upon night vision," *Nat Comm*, 5:4319, 2014.

Our eyes, like those of most vertebrates, are layered counterintuitively, with light-receiving rod and cone cells at the back of the retina and neurons and glial cells stacked in front. Theoretically, this inverted structure—five layers deep—should result in blurry vision, given that light must propagate through all the reflecting and scattering cell layers before triggering the photoreceptors. Yet a normal eye forms images clearly.

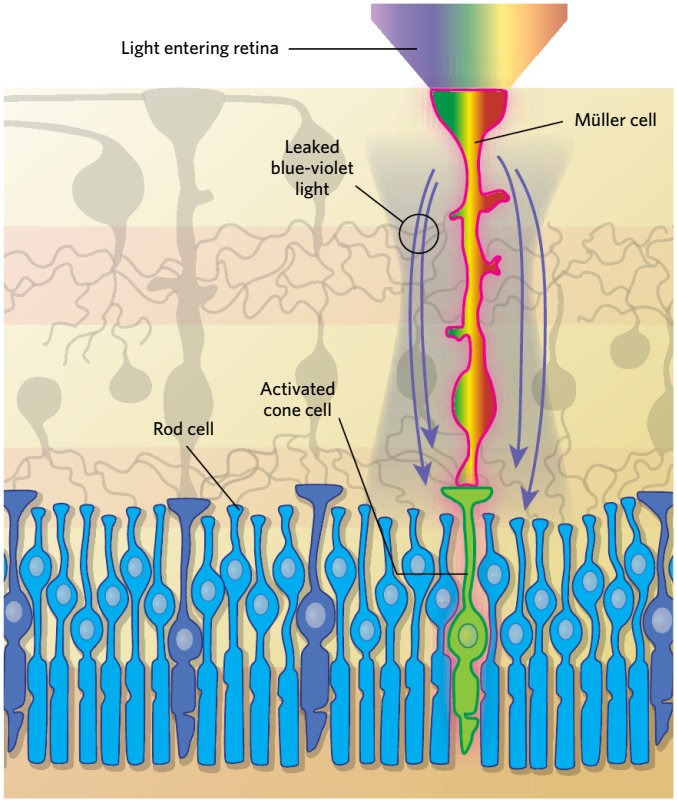
A 2007 study led by Kristian Franze, now of the University of Cambridge, found that one kind of retinal glia, known as Müller cells, resolved the problem by functioning as optical fibers, channeling light to the buried photoreceptors. Still unanswered was: How did these natural optical fibers support two different kinds of photoreceptors—rods, which function in low-light conditions, and cones, which help us see in bright daylight?

Based on Müller cells' refractive index (how much they bend light), diameter, and other properties, Amichai Labin of Technion—Israel Institute of Technology in Haifa and his colleagues simulated how the cells might work as optical fibers. They found that Müller cells struck with white light concentrated wavelengths in the green-red spectrum—a range overlapping greatly with the absorbance spectra of two types of cone cells, and, to a lesser degree, with a third cone type—while blue-violet light leaked out, diffusing through the retina to activate rods.

The Müller cells' maximal light concentration occurred in the green-yellow part of the light spectrum at a wavelength of 560 nm, which happens to be the wavelength one cone cell type is most sensitive to. "The next question was, if they're guiding mainly green light, where are they directing it?" asks Labin.

Zooming in on guinea pig retinas under a confocal microscope, the researchers found that each Müller cell was coupled to an individual cone photoreceptor, and that nearly 90 percent of all cone cells were linked to Müller cells. The optical-fiber effect could increase the number of photons reaching a single cone cell nearly 11-fold at its peak concentrating power, but had only a minimal effect on the light reaching rod cells.

"How optimal light guidance is matched to the absorption spectra of the cone photoreceptors is very surprising," says Franze, who was not involved with this study. Diameter and refractive index are the "two factors [that] determine the color that optical fibers can guide efficiently," says Labin. "Our immediate next step is to understand the exact mechanism that creates this special phenomenon."



SPLIT SPECTRUM: When a rainbow of white light enters the retina, funnel-shape Müller cells guide the beam through layers of cells and cellular processes to the photoreceptors (rods and cones). Müller cells function as optical fibers, directing and concentrating the yellow-green spectrum of light, to which many cone cells are tuned to respond maximally. Blue light seeps out of the Müller cells to activate rods.

Labin suggests his group's data could eventually help design better biomimetic sensors and cameras, or address the clinical implications of Müller cells' dysfunction. For now, he says, these results clear the picture on a long-standing biological question. "We finally understand how our eyes compensate for the strange, upside-down architecture of the retina."

—Jyoti Madhusoodanan

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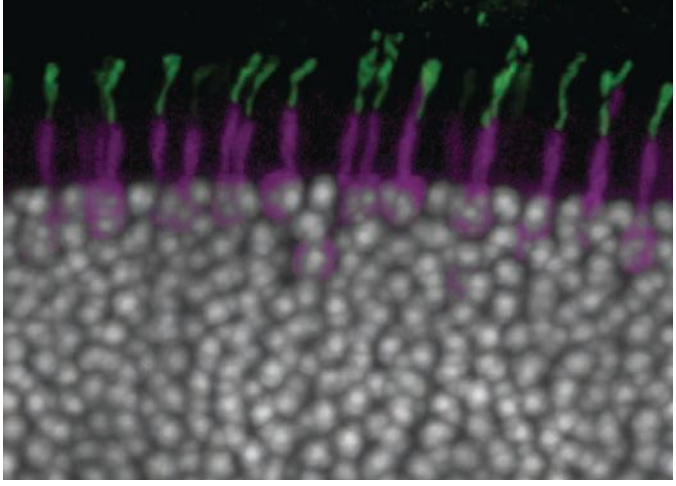


EDGE OF SIGHT: The outer segments (green) of photoreceptors in the mouse retina rely upon a pair of microRNAs for proper structure and function.

MOLECULAR BIOLOGY

Cone Cell Correctors

THE PAPER

V. Busskamp et al., "miRNAs 182 and 183 are necessary to maintain adult cone photoreceptor outer segments and visual function," *Neuron*, 83:586-600, 2014.

THE BACKGROUND

In retinal photoreceptors, the conversion of light to an electrical signal occurs in an organelle known as the outer segment. Malfunctioning outer segments are linked to cone cell diseases and blindness. Previous studies have shown microRNAs (miRNAs)—noncoding RNAs that repress gene expression—are essential to normal cone cell development, but how they operate in adult retinas was unclear.

THE EXPERIMENT

Botond Roska of the Friedrich Miescher Institute for Biomedical Research in Switzerland and his colleagues developed knockout mice and in vitro models in which all miRNAs were depleted in fully formed retinas. Lacking miRNAs, cone cells lost their outer segments and showed reduced responses to light, but the cells did not degenerate. "Finding a phenotype where the cells lose the outer segment but stay absolutely intact was very unexpected," says Roska. Reexpressing two of the most abundant cone miRNAs, miR-182 and miR-183, restored outer segments and normal light responses in cultured retinal cells.

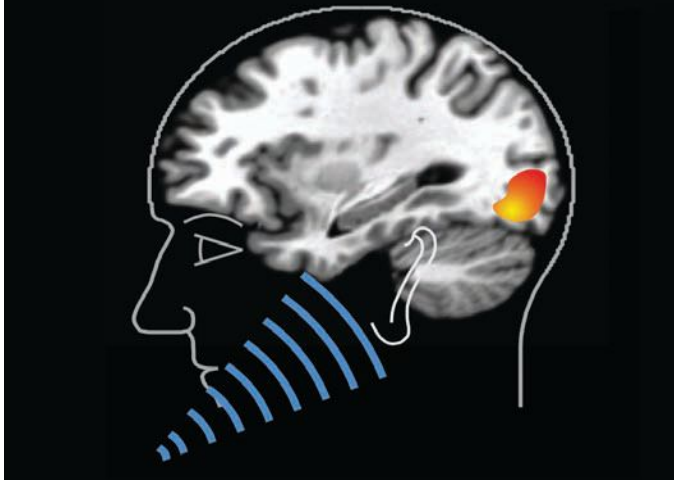
THE FUNCTIONS

These two miRNAs are thought to play a role in lipid metabolism within cells. Roska speculates that they may help regulate a supply of lipids and other molecules to cone cells' apical membranes to renew outer segments.

THE IMPLICATIONS

The outer segments restored by miRNAs 182 and 183 in culture are shorter than those in normal mouse retinas. Nonetheless, these miRNAs could enhance the utility of retina-in-a-dish models. "The field is basically stuck right now because nobody knows how to make a photoreceptor outer segment," says molecular ophthalmologist Jean Bennett of the University of Pennsylvania. "This could be a clue." —Jyoti Madhusoodanan

NEURON, BUSSKAMP ET AL., 2014; WENFENG FENG



SOUND AND VISION: A noise activates the visual cortex (colored spot), which helps a viewer identify an object at the sound's source.

NEUROSCIENCE

Sound and Light Show

THE PAPER

W. Feng et al., "Sounds activate visual cortex and improve visual discrimination," *J Neurosci*, 34:9817-24, 2014.

THE CONTEXT

Noticeable sounds activate neurons in the visual cortex, helping us make out visual targets at the location the sounds came from. Wenfeng Feng of SooChow University in China and colleagues last year observed neural correlates of such sensory tag-teaming in human brain recordings: an activation in the visual cortex of the brain hemisphere opposite the source of the sound, called the auditory-evoked contralateral occipital positivity (ACOP).

THE EXPERIMENT

To figure out how ACOP functions, Feng's group used electroencephalography (EEG) to study the performance of 16 volunteers. Each participant heard a noise from a speaker on the left or right side of a monitor, after which the letter T or L flashed on one side of the screen. The sounds and letters were paired at random, and the participants were asked to identify the image.

THE RESULTS

Sound from one side of the monitor improved the volunteers' discrimination of letters flashed on the same side of the screen. ACOPs before correct answers were stronger than those before incorrect ones, and were localized to Brodmann's area 19, a portion of the visual cortex linked with shape recognition and feature extraction. The researchers "show that the magnitude of ACOP can actually be used to predict participants' subsequent performance on visual discrimination," Philip Tseng, a cognitive neuroscientist at National Central University in Taiwan, says in an e-mail. "It is pretty cool."

MORE QUESTIONS

Feng is now probing deeper into the mechanisms of sound's effect on vision. "What are the brain rhythm activities of the activation of visual cortex by sound? Are they the same as the activities activated by visual cues?" —Tracy Vence

PROFILE

An Eye for Detail

Vision researcher John Dowling has spent a lifetime studying the neural architecture of the retina. He is closing his laboratory after 53 years, opting to extend these studies as a postdoc.

BY ANNA AZVOLINSKY

As a high school student in Providence, Rhode Island, John Dowling was not a good student. "I was doing too many other things, like playing sports, starting a school newspaper, and being a class officer," he says. In tenth grade, he contracted polio and spent months recuperating. Not wanting him to lose the entire school year, his mother requested that Dowling's teachers prepare lessons for him to do at home. "All of my teachers enthusiastically prepared the lessons except for my biology teacher, who wrote my mother that I was so hopeless in biology that I should drop the course." Dowling gladly complied.

Dowling reconsidered his relationship with the subject during his undergraduate days at Harvard University, where he studied how vitamin A deficiency influences vision. He has conducted vision research ever since, working on the functional organization of the retina, studying its synaptic connections, teasing out how the neurons of the retina respond to light, investigating how retinal neurons communicate information, and using a zebrafish model to study the development and genetics of vision.

Here, Dowling discusses how he helped revamp the biology curriculum at Harvard, pursued a PhD without knowing it, fished for laboratory supplies, and how, at age 79, he's finally going to do a postdoctoral fellowship.

DOWLING DEBUTS

Falling in love with biology. Dowling majored in biology at Harvard and planned to attend medical school. During his junior year, he took a biochemistry course taught by vision researcher and future Nobel laureate George Wald. "We first studied glycolysis, respiration, and photosynthesis, material that can be very dry, but George made it lively. He was a marvelous teacher. I can still recall his descriptions of Albert Szent-Györgyi's famous experiments on muscle fibers: if you extract a frog or rabbit muscle with glycerol, you end up with a piece of inert tissue, but when you add back ATP, as long as the major proteins are intact, the muscles will contract. This excited me enormously, and still does. I thought it was really getting to the essence of life."

First vision experiment. After learning about Wald's discoveries of the role of vitamin A in vision, Dowling asked about working in his laboratory. "That's where I fell in love with research," says Dowling, His first research puzzle was to sort out why recovery from vitamin A deficiency is incomplete in humans who are placed on a vitamin A–containing diet. Prior studies in rats had suggested that prolonged vitamin A deficiency might cause degeneration of photoreceptors, so Dowling began with biochemical measurements on vitamin A–deficient rats. He found that levels of the visual pigment protein opsin decreased with time and the photoreceptor cells did indeed degenerate. Dowling learned how to record in vivo electroretinograms from the rats to understand what happens physiologically to their vision on a vitamin A–deficient diet. He observed that light sensitivity decreased logarithmically as the visual pigments in the retina declined. These experiments led to Dowling's first publication—in 1958.

Keeping the lab bench warm. In 1957, Dowling entered Harvard Medical School. "But Wald kept a lab bench for me, and on the odd afternoon during my first year at medical school I would come back and continue doing experiments. Then I spent the following summer working in the lab." During that summer Dowling mapped the exchange of vitamin A and vitamin A aldehyde (retinal) between the retinal pigment epithelium and photoreceptor cells during light and dark adaptation as well as the relation between visual sensitivity and visual pigment levels during adaptation, showing that, just as with vitamin A deficiency, there is a logarithmic relationship between visual pigment levels and light sensitivity.

Permanent leave of absence. The following year, a member of the Wald lab learned of a study showing that when vitamin A–deficient rats consumed vitamin A acid (retinoic acid), the rats no longer showed signs of deficiency, even though no vitamin A could be detected in the animals' tissues. Vitamin A in food is converted to retinol, its alcohol form, which is further metabolized to retinoic acid, but biological tissues have a hard time converting acids to aldehydes. "So we surmised that vitamin A acid could substitute for the somatic functions of vitamin A but not for the visual functions, which require retinal. I repeated the experiment, and the rats grew fine, but they became completely blind."

Dowling decided to explore the functions of vitamin A and vitamin A acid in more detail—and to see if he could get the "research bug" out of his system. "I took a leave of absence from medical school in 1959, and I am still on that leave of absence 55 years later." Dowling showed that while retinoic acid can indeed fulfill somatic tissue functions, it cannot be reduced to retinal, which is essential for vision. "With retinoic acid, we could study vitamin A deficiency confined to the eye—biochemically, electrophysiologically, and anatomically—and we showed that with long-term vitamin A deficiency, photoreceptors may be completely lost."

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JOHN E. DOWLING

Gordon and Llura Gund Professor of Neurosciences
Department of Molecular and Cellular Biology
Harvard University

Greatest Hits

- Showed that as visual pigment levels in photoreceptor cells decrease, in both vitamin A deficiency and light and dark adaptation, light sensitivity decreases logarithmically
- Discovered that retinoic acid (vitamin A acid) can fulfill the essential somatic functions of vitamin A but cannot be converted to vitamin A aldehyde (retinal), which is essential for vision
- Provided some of the first descriptions of the synaptic organization of the vertebrate retina, with Frank Werblin and others, and pioneered the use of intracellular recordings and staining to study the light responses of retinal neurons
- With Harris Ripps, showed that neurotransmitter is continuously released from photoreceptors in the dark, depolarizing horizontal cells
- Studied the role of dopamine in the retina, showing that the neurotransmitter uncouples horizontal cell electrical synapses and alters the sensitivity of horizontal cells to the photoreceptor neurotransmitter glutamate
- Pioneered the use of zebrafish for studies of retinal development, retinal mutations, and color vision

DOWLING DETERMINED

PhD material. "Halfway through my leave of absence, Wald approached me about pursuing a PhD: 'You've taken virtually all of the courses offered at Harvard in biology, have had two years of medical school, and you've done enough research to write a thesis. Why don't you think about getting a PhD?' Well, I never even thought about the possibility. This was long before there were any MD-PhD programs." Dowling entered the PhD program in February 1960 and received his degree the following January.

Back to biology class. "I was bored silly when, as a freshman at Harvard, I had to endure introductory biology, which was taught as botany and then zoology, in two different semesters. Mostly it was just memorizing and had nothing about concepts," says Dowling. So when Wald asked him to help teach a new introductory course that emphasized the commonalities shared by cells and organisms at the molecular level, Dowling jumped at the chance. And instead of going back to medical school as he had intended, Dowling accepted the offer of an assistant professor position at Harvard. "The biology department gave me a lab—I never did a postdoc." Dowling helped teach the course for the three years he remained a junior faculty member at Harvard.

Making a move. In 1964, Dowling moved to the Wilmer Eye Institute at Johns Hopkins. While still at Harvard, Dowling had expanded from studying the low-light-sensitive rod photoreceptors that predominate in the rat retina to working on the ground squirrel retina, which contains mainly bright-light and color-sensitive cones. Dowling noticed that so-called horizontal cells in the retina received synaptic inputs from the photoreceptor cells. "The horizontal cells in those days were very much a mystery. This observation led to my main theme of research at Hopkins, working out the wiring of the retina and the physiology of the individual retinal neurons."

Ahead of his time. Dowling quickly switched to the better-understood primate retina, working for five years with Brian Boycott to map the wiring of the retina's various nerve cells, to identify its synapses, and to begin to understand the information flow within the eye. "Such mapping is very much in vogue now. The BRAIN Initiative announced by President Obama is exactly this idea—to anatomically reconstruct the nervous system. Well, we were trying to do this back in the 60s in the retina! The retina is a perfect neural structure to do reconstruction even though our methods, back then, were rudimentary compared to the large-scale computer reconstruc-

tions that can be done now." Dowling and colleagues were the first to identify the synapses of the retinal neurons known as amacrine cells and to show how those synapses feed back onto retinal bipolar neuron terminals. Dowling also compared the primate retina to that of the frog, finding that there are many more amacrine synapses in the frog retina. "We thought this was because more complex visual processing happens in the frog retina, including the detection of movement direction, and this has turned out to be correct. Directional selectivity in the retina is mediated by amacrine cells."

From anatomy to physiology. Dowling's first graduate student, Frank Werblin, now a professor of neurobiology at the University of California, Berkeley, characterized the responses of each of the five classes of retinal neurons—photoreceptor, horizontal, bipolar, amacrine, and ganglion cells—using intracellular recordings from mudpuppies. "The mudpuppy is an amphibian with large cells that are ideal for single-cell recordings. Frank showed, for the first time, that bipolar cells have a center-surround organization and that many amacrine cells require constant movement of the light stimulus to keep firing. Frank produced a thesis that is a classic in the field. This got us going on the physiology of the retinal cells and, combined with the anatomy, we began to get a glimpse of the functional organization of the retina."

For the love of teaching. "At Wilmer I was part of a medical school where I only taught medical and graduate students but not undergraduates. So, even though I was happy at Wilmer, I wanted to teach undergraduates, and this was the main reason I moved back to Harvard." There Dowling developed an introductory undergraduate course on behavioral neuroscience that he taught for 31 years.

A new cell type. Returning to Harvard in 1971, Dowling moved from anatomy and physiology to the pharmacology of the retina, first helping to identify retinal cell neurotransmitters. Then, "a Swedish ophthalmologist, Berndt Ehinger, came to my lab to learn electron microscopy. He was interested in dopamine, and initiated my lab's interest in neuromodulators in the retina, of which nothing was really known at the time." Ehinger and Dowling identified special cells in the fish retina called interplexiform cells that carry information from the inner to the outer retina.

Laboratory fishing. "In the early 1980s, we became interested in the effects of neurotransmitters and neuromodulators on neurons maintained in culture. At that time goldfish or carp retinal neurons were used for this purpose, but these cells did not survive well in culture. In 1981, while fishing one day from the dock at the back of our house in Woods Hole, we caught a lot of small fish that turned out to be white perch. The next day, I took one of these fish to the lab and isolated its cells. Well, they were just gorgeous; you could readily identify not only the major classes of retinal neurons but often neuronal subtypes. The neurons also survived in culture for days. In the fall, lab members would drive to Woods Hole, go out on the pond in rowboats, and catch 300 or so fish in a morning. We brought them back in barrels in a U-Haul trailer. That would be enough fish to last us through the winter. From one fish we could isolate enough neurons to last an investigator a week."

"I am finally closing the lab and embedding myself into the labs of Jeffrey Lichtman and Joshua Sanes to do a postdoctoral fellowship."

Trading perch for zebrafish. By the end of the 1980s, the white perch population was diminishing, so Dowling contacted a fishery that raised hybrids of striped bass and white perch. "The results with these fish were astonishingly reproducible, and I realized the advantages of using an organism with a similar genetic background, of the same age, and grown under identical conditions," he says. "This brought me to zebrafish, which were just starting to be appreciated as a tractable model system." Among its first studies with zebrafish, Dowling's lab examined the role retinoic acid plays in retinal development. The team then went on to study the effects of various mutations on retinal structure and function.

Color vision. More recently, Dowling has become interested in zebrafish color vision. "Many fish depend critically on color to identify members of their own species, the opposite sex, and even the age of fish of their species. Juvenile fish often have distinct coloration patterns. Like many fish, zebrafish have four types of cones: red-, blue- and green-sensitive cones, and also ultraviolet (UV) light–sensitive cones." Recently, the lab investigated the role of the UV cones, which are the first to mature during development. "We've found that ultraviolet vision is especially important when the fish are young, so they can avoid the deleterious effects of UV light."

DOWLING DELIBERATES

A postdoc at 79. "I promised my department that when I was 75 I would close my lab. That was three years ago, so I am finally closing the lab and embedding myself into the labs of Jeffrey Lichtman and Joshua Sanes, two colleagues in our department, to do a postdoctoral fellowship. Jeff and Josh are developing automated ways to map neural connections, and it seems an appropriate time to go back and work out in exquisite detail the synaptic wiring of the retina." (See "Critical Connections," a profile of Joshua Sanes, *The Scientist*, December 2011.)

Booming discipline "The number of people working in the vision field has increased enormously. The Association for Research in Vision and Ophthalmology, whose members are a mix of basic and clinical researchers, has an annual meeting each spring. The first time I attended [in 1961] about 150 were there. Last year there were more than 12,000 people in attendance. It's the same in neuroscience. When I was at Hopkins, I was the 178th member of the Society for Neuroscience. Now there are more than 30,000 members." ■

Joeanna Arthur: Charting a Path

Project Scientist, National Geospatial-Intelligence Agency. Age: 32

BY KATE YANDELL

Brooklyn, New York, native Joeanna Arthur grew up thinking she would be a lawyer. A high school advanced-placement psychology class changed her course—the first of many reorientations that would eventually lead her to the National Geospatial-Intelligence Agency (NGA) in Springfield, Virginia.

Arthur had planned to go into clinical practice, but a neuropsychology course during her undergraduate years at Adelphi University on Long Island altered her goals again: she would be a neuroscientist.

As a PhD candidate at George Washington University in Washington, DC, Arthur studied how people use cognitive functions to orient themselves. "I tested healthy college undergrads, spinning them around and blindfolding them," she recalls. "I felt like Dr. Evil."

She showed that allowing people to look at their surroundings before being blindfolded and rotated while seated in a chair helped them to answer more consistently when asked to identify how far they had turned, although the average accuracy of their assessments remained about the same.[1]

"Your memory of the environment before you get the turn is actually playing a very strong role," says her graduate advisor John Philbeck, who is now at the University of Wollongong in Australia.

Arthur explained that being oriented prior to being spun might make people feel more certain about their positions during the spinning process, although they still make systematic errors in determining which direction they are facing.

However, this effect only persisted when the subjects were asked to indicate a target using a pointer fixed a short distance from their bodies, and not when they were allowed to indicate the location of the target relative to their own bodies.[2] This indicates that people are more likely to rely on their spatial memory when they need to judge the relationship between external objects than when judging their own internal motion.

During a postdoc at Johns Hopkins University, Arthur studied patients with reduced function in their vestibular systems, the brain regions and associated inner-ear sensory structures that help people stay oriented. Her research helped confirm that patients' deficits lie in intrinsic pathways for sensing movement.[3]

While Arthur debated remaining in academia, she decided she wanted to be "in a more fast-paced environment where you were able to apply your findings to a larger problem."

Today, Arthur is one of a small group of life scientists working at the NGA, where she is a project scientist for the InnoVision program in the Basic and Applied Research Office. While the agency excels at "mapping out terrain and activities on the Earth" using satellite and other remote-sensing data, it is traditionally less experienced with "mapping underlying psychological processes going on in analysts' heads," says Arthur.

Arthur's first supervisor at NGA, Beth Driver, now retired, recalls that from the get-go Arthur "got some data that rang counter to some self-perception at NGA." The work brought up questions about the limits of human analytic abilities. Arthur was able to present her findings as well as the larger relevant literature calmly and with confidence, Driver adds.

Arthur has also been helping to adapt 3-D visualization tools and ways to navigate through on-screen images using gestures rather than toolbars to enhance the experience of NGA analysts as they examine geospatial data. She says that analysts' experiences with looking at satellite data while standing next to a light table changed when, in the 1990s, they switched to analyzing data while sitting down at a computer—and they found themselves getting disoriented while examining images. Arthur hopes that recreating that earlier feeling of being oriented in an image will increase analysis accuracy and speed.

Arthur goes to conferences and to high schools to talk to younger people, particularly those from minority groups, about careers in the sciences and the intelligence community. "I had awesome mentors," she says.

But Philbeck says that Arthur has also found a unique path. "She creates her own opportunities." ■

KEVIN CLARK, NGA OFFICE OF CORPORATE COMMUNICATIONS

REFERENCES

1. J.C. Arthur et al., "Non-sensory inputs to angular path integration," *J Vestib Res,* 19:111-25, 2009. (Cited 4 times)
2. J.C. Arthur et al., "The role of spatial memory and frames of reference in the precision of angular path integration," *Acta Pscyhol,* 141:112-21, 2012.
3. J.C. Arthur et al., "Linear path integration deficits in patients with abnormal vestibular afference," *Seeing Perceiving,* 25:155-78, 2012. (Cited 1 time)

Capturing Complexes

Techniques for analyzing RNA-protein interactions

BY NICHOLETTE ZELIADT

RNAs are molecules with a wide-ranging repertory, acting in roles that frequently defy dogma and resist being neatly classified into well-defined categories. Once thought of as simply passive intermediates involved in the translation of genes into proteins, RNAs are now known to exist in myriad forms that perform a variety of important biological functions. They can regulate gene expression or catalyze biochemical reactions, jobs once thought to be carried out only by proteins. The job description of some RNAs can also include defending genomes against foreign nucleic acids and controlling genome organization and stability.

However, one thing that all RNAs are believed to do is function through interactions with proteins. Figuring out the details of these interactions, such as when and where they take place, can provide important clues about the roles of coding and noncoding RNAs, which may help to uncover previously unknown functions of the ubiquitous molecules. *The Scientist* spoke with a few of the experts developing and using techniques to probe RNA-protein complexes. Here, we bring you insight into some of the latest advances for studying such interactions.

GETTING A GRIP ON RNA-PROTEIN COMPLEXES

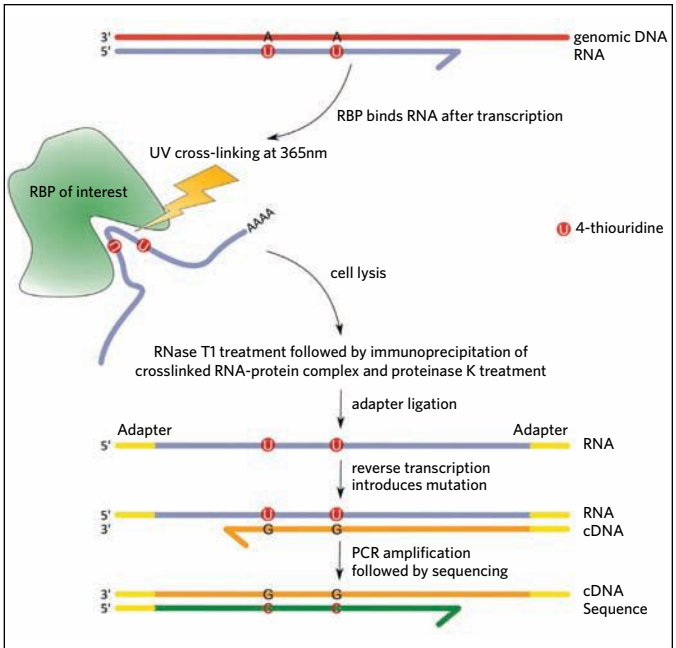
One of the most common methods for studying the RNA-protein interactions that occur in vivo uses antibodies to fish out a protein of interest—along with any RNAs bound to it—from cell or tissue extracts. Following purification, RNAs can then be identified through PCR, sequencing, microarray, or other means.

One form of the technique, known as UV crosslinking and immunoprecipitation (CLIP), involves exposing cells to 254 nm ultraviolet light, which covalently crosslinks RNAs and proteins only at sites of direct contact (*Methods*, 37:376-86, 2005). Following immunoprecipitation, the recovered complexes are digested with a protease. Adaptor sequences are then ligated to both ends of the remaining RNA, one of which serves as a priming site for reverse transcriptase to generate cDNA, while both serve as priming sites for subsequent PCR amplification. However, the RNA ligation steps are inefficient, "which means you have to start with quite a lot of material . . . to get genome-wide coverage," says Jernej Ule, a molecular neuroscientist at University College London who helped to develop the technique.

In addition, the reverse transcriptase has difficulty passing over the crosslink sites on RNAs that contain residual amino acids, yielding cDNAs that are truncated just before the crosslink location. Those truncated sequences cannot be identified by CLIP, because they lack one of the introduced priming sites required for PCR amplification.

What's more, crosslinking at 254 nm is relatively inefficient, and the assay doesn't reveal the precise location of RNA-binding proteins on their targets, though Ule says the binding sites can be inferred in some cases using bioinformatic analysis of the binding sites. "The location where [an RNA-binding protein] binds will give you a good idea of what [that] protein is doing," says Markus Hafner, who studies RNA-protein interactions at the National Institute of Arthritis and Musculoskeletal and Skin Diseases in Bethesda, Maryland. "If it's binding to a coding sequence, or the 3' untranslated region, or within an intron, all of these are very different things, so it's important to really pinpoint it with nucleotide resolution."

To overcome some of these limitations, Ule has since developed a modified protocol called iCLIP (individual-



PROTEIN CLIP: Sites on RNA that interact with an RNA binding protein (RBP) can be identified in complexes fished out of cells using immunoprecipitation, an approach called CLIP. In one variation on this technique, known as PAR-CLIP, cells are incubated with a light-reactive nucleoside analog, 4-thiouridine (U), that becomes incorporated into RNA. Irradiation with UV light crosslinks RNA-protein complexes, which are then isolated from cell lysates using antibodies. RNA located outside the protein binding pocket is degraded, and the remaining sequence is transcribed to DNA, a process that leads to a characteristic T to C mutation wherever the nucleoside analog incorporates.

BRIEFINGS IN FUNCTIONAL GENOMICS, DOI: 10.1093/BFGP/ELU020, 2014

nucleotide-resolution CLIP) that can identify truncated cDNAs and better pinpoint the positions of the RNA-protein crosslink sites (*Nat Struct Mol Biol,* 17:909-15, 2010). The method is similar to CLIP, but differs in the preparation of the cDNA library: rather than ligate adaptor sequences to both ends of the RNA, only the sequence needed for reverse transcriptase priming is added. The primer used for reverse transcription in iCLIP contains a cleavable overhang region with a short barcode sequence and the second priming site for the subsequent PCR reaction. Following reverse transcription, the resulting cDNAs are circularized, effectively attaching the PCR priming site to the opposite end of the cDNA sequence. Then the cDNAs are cut at the cleavage site introduced by the overhang region of the primer, amplified, and sequenced. The nucleotide upstream of the crosslinking site can be readily identified, as it will be the nucleotide in the genome that precedes the matching sequence in the amplicon.

Another method, known as PAR-CLIP (photoactivatable-ribonucleoside-enhanced CLIP), which Hafner helped to develop while a postdoc in Thomas Tuschl's lab at Rockefeller University in New York City, introduces a light-reactive ribonucleoside analog in the crosslinking reaction. After cells incorporate the synthetic nucleoside 4-thiouridine into new RNA, they are irradiated with 365 nm UV light to crosslink the labeled RNA and interacting proteins. When crosslinked, the nucleoside analog typically leads to a T to C mutation in the cDNA sequences that correspond to the sites of interaction (*Cell,* 141:129-41, 2010). This approach makes it possible to distinguish between direct and indirect interactions, Hafner says. "Basically everything that doesn't have this mutation, you can be confident that these were not interacting molecules, so you can set them aside."

PROS

- Captures interactions throughout the transcriptome
- iCLIP and PAR-CLIP can precisely pinpoint interaction sites.

CONS

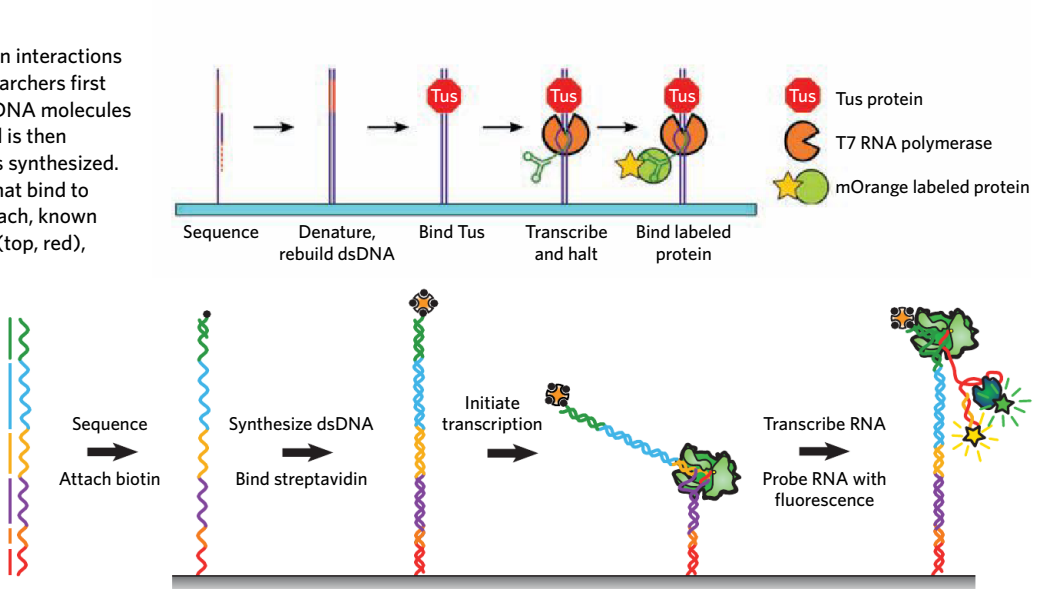
- UV crosslinking tends to be biased towards uridine ribonucleosides.
- Data interpretation can be challenging. "It's really very tricky to properly distinguish between high-affinity functional binding sites and those that are identified because an RNA is highly abundant and accessible in some way that is not relevant to the function of the protein," Ule says.

ANALYSIS BY HIGH-THROUGHPUT SEQUENCING

For researchers interested in making quantitative measurements of RNA-protein affinities, crosslinking methods are not the way to go. Instead, you might consider using one of two new methods that convert an Illumina high-throughput sequencer into a platform for quantifying RNA-protein binding on a large scale.

The techniques harness the optics of the Illumina Genome Analyzer IIx—which is designed to image the sequential incorporation of fluorescent nucleotides into oligos synthesized from immobilized DNA templates—to take pictures of RNA sequences binding to a fluorescently labeled protein of interest. The trick, though, is to convert the immobilized DNA templates into RNA, and retain that RNA at the site where it is made to enable subsequent measurements of protein binding. Each method accomplishes this in a different way.

One approach, known as HiTS-RAP (high-throughput sequencing–RNA affinity profiling), devised in the Cornell University lab of John Lis in collaboration with researchers from the San Diego–based Illumina, Inc., uses a bacterial DNA–binding protein, Tus, which binds to a specific site near the free



IN SEQUENCE: To analyze RNA-protein interactions on a DNA sequencing instrument, researchers first design and then sequence a library of DNA molecules of interest. The sequenced DNA strand is then degraded, and double-stranded DNA is synthesized. Next, researchers add bulky proteins that bind to particular sites in the DNA. One approach, known as HiTS-RAP, employs the protein Tus (top, red), while another method, called RNA-MaP, uses the protein streptavidin (bottom, orange cross). Then RNA polymerase is added, which begins synthesizing RNA but halts when it encounters the protein bound to the DNA, effectively securing the RNA to its template. Finally, a fluorescently labeled RNA-binding protein of interest is added and imaged.

TOP: *NATURE METHODS,* 11:683–88, 2014; BOTTOM: *NATURE BIOTECHNOLOGY,* 32:562–68, 2014

ends of the immobilized DNA templates. RNA polymerase then transcribes the DNA to RNA, but halts when it encounters Tus, effectively tethering the newly transcribed RNA to its template.

In the other approach, called RNA-MaP (RNA on a massively parallel array), developed in the lab of William Greenleaf at Stanford University, the DNA templates terminate with a biotin molecule that is bound by the protein streptavidin, which serves as a roadblock for RNA polymerase and achieves the same tethering of the newly transcribed RNA.

To measure RNA-protein binding affinities, a fluorescently labeled protein of interest is flowed over the RNA in increasing concentrations and imaged to measure the fluorescence intensity of the proteins bound to each RNA. The data can then be used to calculate the dissociation constant for the RNA-protein complex.

PROS

- Quantitative
- High-throughput: can measure interactions between a protein and millions of RNA molecules
- Highly automated: most of the manipulations are performed automatically by the sequencer

CONS

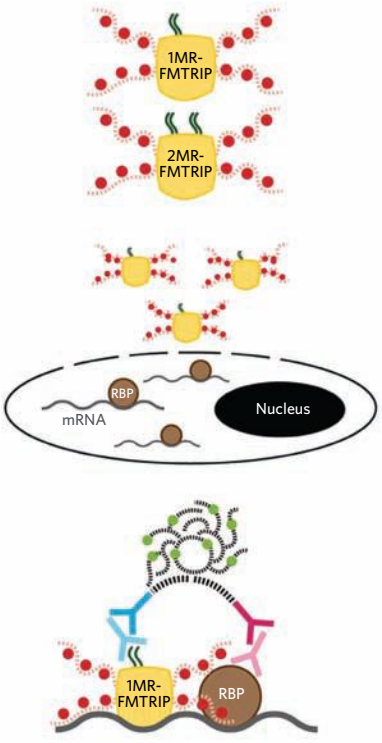
- Illumina has discontinued the Genome Analyzer IIx, but Jacob Tome, a graduate student in Lis's lab who helped to develop HiTS-RAP, says the method is compatible with the newer Illumina sequencing platforms.
- You'll need to have a fluorescently labeled RNA-binding protein that's purified and biochemically active, which can be a challenge, Greenleaf says.

SPOTTING RNA-PROTEIN COMPLEXES IN CELLS

For researchers who'd like to analyze RNAs and proteins in their native cellular context, the previously described techniques won't cut it. To characterize RNA-protein interactions at the cellular level, researchers can image overexpressed, fluorescently labeled versions of their RNA and proteins of interest (see "Live and In Color," *The Scientist,* April 2012). But if your goal is to look at the endogenous, unmodified RNA and protein molecules in the cell, a method recently developed by Philip Santangelo and colleagues at Georgia Institute of Technology might be right for you (*Nucleic Acids Res,* 41:e12, 2013).

The first step is preparation of multiply labeled tetravalent RNA imaging probes (MTRIPs), which Santangelo and colleagues first developed in 2009 (*Nat Methods,* 6:347-49, 2009). The MTRIPs consist of a collection of short strands of fluorescently labeled RNA-DNA chimeras designed to bind a specific RNA target. Each nucleic acid strand in the chimera is labeled with multiple molecules of a single fluorophore and conjugated to a molecule of biotin at one end; these oligos are then mixed with peptide-tagged neutravidin, which binds to biotin and clusters the strands to form an MTRIP.

Once the probes are prepared, they are added to live cells to label the RNA of interest. Santangelo says he usually uses a mixture of three different MTRIPs specific for an RNA to achieve a signal that is bright enough for single-molecule resolution and easily distinguished from any unbound probe. To get the MTRIPs into cells, Santangelo's team employs streptolysin O, a bacterial toxin that reversibly pokes holes in cell membranes. Once the probes have bound their target RNA, the researchers fix the cells and then perform a so-called proximity ligation assay between the RNA probes and the protein. They label the MTRIPs and the RNA binding protein of interest with different primary antibodies. Next, the cells are incubated with two different secondary antibodies, each conjugated to a short DNA oligo. If the RNA and protein of interest are within about 30 nm of each other, the oligos on the antibodies can be ligated to form a circular structure, which can then be amplified using a polymerase and labeled with fluorescent probes. "So in one color, we can see the RNA, and then in a different color, we can see if the interaction has happened," Santangelo says. "That way we get a measure of how much RNA is there, and how many interactions have occurred on a per-cell basis."



SPOTTING COMPLEXES: To visualize RNA-protein interactions inside cells, researchers first prepare a fluorescent nucleic acid probe (yellow with red dots, top). Next, cells are permeabilized to allow uptake of the probe, which binds its target RNA (middle). Then the probe and RNA-binding protein (RBP) are labeled with antibodies (light blue and light pink, bottom). If the RNA and RNA-binding protein of interest are in close proximity, oligos (black) attached to secondary antibodies can be ligated, amplified, and labeled with a differently colored fluorescent probe (green).

PROS

- Reveals the location of RNA-protein interactions
- Captures cell-to-cell variations in RNA-protein interactions
- Enables the quantification of interactions at their native stoichiometries

CONS

- Requires good, narrowly targeted antibodies. "If you have an antibody that binds all over the place, then you could end up with a lot of background," Santangelo says.
- MTRIPs are not commercially available. ■

NUCLEIC ACIDS RES. DOI: 10.1093/NAR/GKS837, 2012

Nuclear Cartography

Techniques for mapping chromosome conformation

BY JEFFREY M. PERKEL

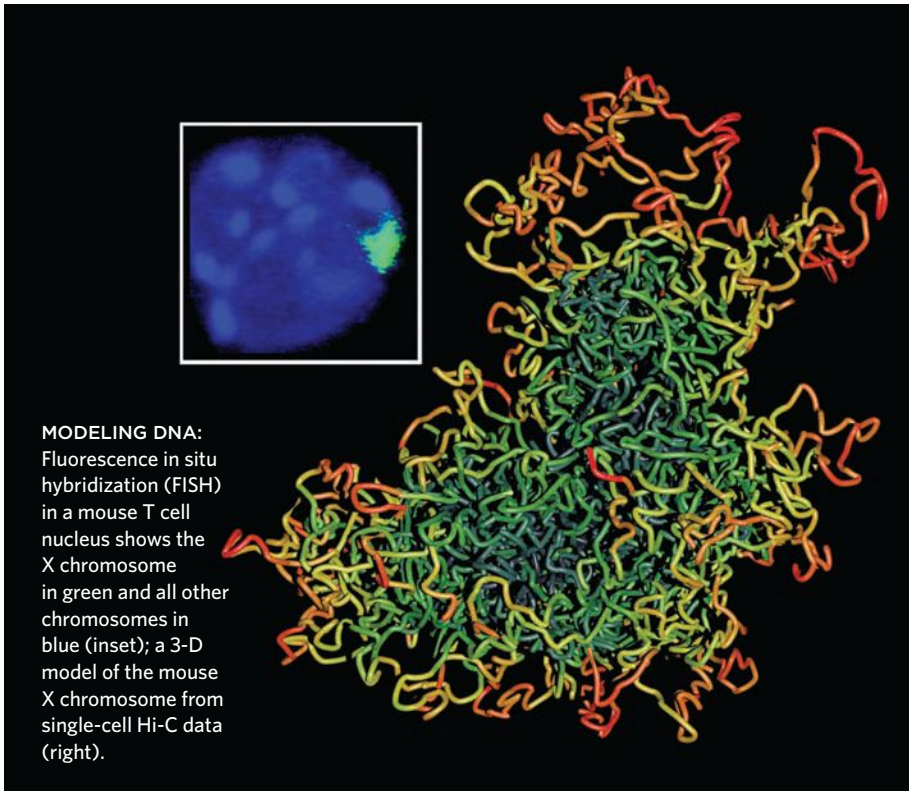
A Google image search for "chromosomes" gets you thousands of pictures showing condensed, X-shaped mitotic chromosomes. There's just one problem with those images, says Peter Fraser, head of the Nuclear Dynamics Programme at the Babraham Institute in Cambridge, U.K.: "That's really not very characteristic of what your genome looks like in your cells."

Most cells, Fraser says, are not dividing, and their genetic material is relatively loosely coiled. But that doesn't mean it's randomly strewn about. The nucleus in general and chromosomes in particular are highly regimented, with DNA domains folding and looping into dynamic structures that vary over time as cellular state changes.

Chromosome structure has a profound effect on cellular biology, with regulatory elements needing to form great genetic arcs to reach promoters located hundreds of kilobases away. On a larger scale, groups of genes and their regulatory elements assemble into domains measuring about a megabase apiece. These domains appear to serve as the structural units of chromosomes, and though physically distinct, they can interact with one another over large distances, leading to even more complex chromosome folding. Ultimately, says Fraser, those different organizational levels impact gene expression itself—a fact that motivates the National Institutes of Health's recently announced 4D Nucleome program, which aims to unravel the spatial organization and temporal dynamics of the nucleus and chromatin and their influence on biology and disease.

For years, imaging-based approaches were the only way to determine chromosome conformation, but those methods are technically demanding and spatially limited. Then, in 2002, Job Dekker, while a postdoc in Nancy Kleckner's lab at Harvard University, figured out a way to study conformation through DNA sequence (*Science,* 295:1306-11). The technique he developed, chromosome conformation capture (3C), uses PCR to determine whether two specific DNA segments which are distantly separated in linear DNA are nonetheless closely associated in space due to chromosome folding.

3C is the grandfather of most conformational-analysis techniques used today. But it and its descendants aren't the only options molecular biologists have in their toolboxes. *The Scientist* interviewed experts on chromosome conformation about the methods they use in their labs. This is what they said.



MODELING DNA: Fluorescence in situ hybridization (FISH) in a mouse T cell nucleus shows the X chromosome in green and all other chromosomes in blue (inset); a 3-D model of the mouse X chromosome from single-cell Hi-C data (right).

COURTESY OF TAKASHI NAGANO AND TIM STEVENS

MANY VS. MANY

RESEARCHER: Job Dekker, Professor and Co-Director, Program in Systems Biology, University of Massachusetts Medical School, Worcester

PROJECT: Mapping long-range DNA interactions as part of the ENCODE project

PROBLEM: 3C is not easily multiplexed, making its application to large genomic surveys impractical.

SOLUTION: 3C is sometimes called a one vs. one technique because it is used to measure individual interactions between selected pairs of genomic segments. In the technique, cellular chromatin structure is frozen in place with a crosslinker such

as formaldehyde. Restriction enzymes cut the DNA and the resulting ends are then ligated together to physically link the two previously separate strands. The crosslink is then reversed to release the now-connected DNA fragments. Finally, researchers probe for specific physical interactions by using PCR to amplify across their ligation junctions.

Although 3C can be used to identify specific long-range contacts, it is difficult to apply to multiple genomic segments at once, as each interaction requires a separate PCR reaction. So Dekker's team developed several variants of the procedure. Hi-C uses next-generation DNA sequencing to probe all possible interactions genome-wide (all vs. all), but it is an expensive and computationally demanding approach.

5C (chromosome conformation capture carbon copy) represents an intermediate approach (many vs. many) that scales 3C up into the range of dozens or even hundreds of interactions. Researchers prepare oligos representing all chromosome regions of interest—typically a couple hundred per megabase, according to Dekker. These oligos are designed to fall precisely on either side of the restriction enzyme cut sites, such that when they anneal to the 3C product they produce a small gap that can be closed with DNA ligase. Amplification and deep sequencing of the resulting ligation products indicates which interactions occurred for all of the selected regions (*Genome Res,* 16:1299-1309, 2006).

As part of the ENCODE project, Dekker's team used this approach to probe interactions between 628 transcription start sites and 4,535 "distal restriction fragments" across 1 percent of the human genome in three cell lines (*Nature*, 489:109-13, 2012). The results, Dekker says, show that, contrary to the conventional model of one enhancer pairing with one gene, genes can interact with multiple enhancers, and enhancers with multiple genes.

"3-D contacts between genes and regulatory elements are really surprisingly abundant," he says.

EASING BOTTLENECKS: With hundreds of oligos required for some studies, 5C oligonucleotide design can be a bottleneck, Dekker concedes. His lab's public My5C website (my5c.umassmed.edu) contains tools for 5C primer design, data upload, and data analysis. An updated Web tool, which will be a one-stop shop for 5C, Hi-C, and other related methods, is slated for release in the near future.

HI-C AT THE CELLULAR LEVEL

RESEARCHER: Peter Fraser, Head, Nuclear Dynamics Programme, Babraham Institute, Cambridge, U.K.

PROJECT: Building 3-D models of chromosome architecture

PROBLEM: 3C-based methods average the data from thousands or millions of cells, obscuring cell-to-cell variation and producing an averaged conformation.

SOLUTION: "The only way to really understand what the chromosome looks like would be to study single cells," Fraser says.

So his team, led by senior research associate Takashi Nagano, worked out a way to take Hi-C to that single-cell level. The major procedural difference, says Fraser, is that Nagano performed most of the Hi-C reaction steps in intact nuclei rather than after cellular lysis, as is typically done. He then isolated individual cells, completed the reaction, and deep-sequenced the resulting material (*Nature*, 502:59-64, 2013). "It was a simple but elegant change," Fraser says.

Fraser and Nagano applied their method to male T cells, which, like all male cells, have only a single X chromosome. They recovered about 2.5 percent of the chromosome, from which they assembled low-resolution models of the molecule's structure, which are vaguely reminiscent of the Everlasting Gobstoppers in *Willy Wonka and the Chocolate Factory*. Subsequent methodological improvements have enabled the team to scale up to model the entire genome of haploid cells, of which they recover about 30 percent at 10-kbp resolution.

NOT JUST EYE CANDY: How do you validate a model of something never previously seen? Fraser says he plans to correlate Hi-C with 3-D imaging and fluorescence in situ hybridization (FISH) data sets to ensure the data conform to reality. So far, though, the models seem grossly accurate. "When we first started making the models, I thought they were really just sort of interesting to look at, maybe a bit of eye candy. But they're actually a bit more robust, and as far as we can tell so far, they seem to reflect the actual structure of chromosomes."

ASSESSING NUCLEAR WALLFLOWERS

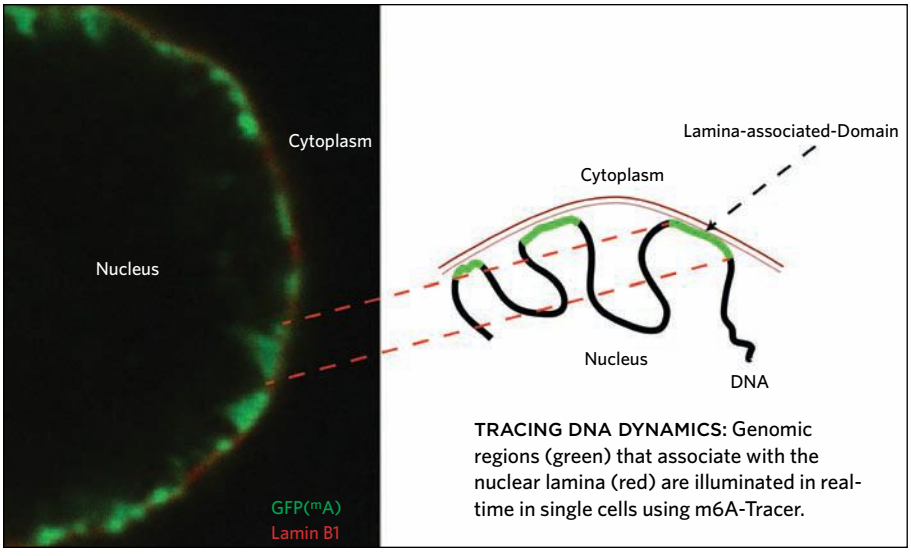
RESEARCHER: Jop Kind, Postdoctoral Fellow, Netherlands Cancer Institute, Amsterdam

PROJECT: Monitoring the dynamics of nuclear organization

PROBLEM: Nuclear organization isn't static, and it isn't random. Kind needed a way to track those segments of DNA that had been near the nuclear envelope (and thus were relatively silent) and then moved away over time.

SOLUTION: Kind is a postdoc in the lab of Bas van Steensel, who developed a method for distinguishing chromatin that was pushed up against the nuclear envelope from chromatin that was not (*Nature*, 453:948–51, 2008). Called DamID, the method relies on a bacterial methyltransferase (Dam), which methylates adenine residues in the context of a GATC sequence—a modification that does not normally occur in eukaryotic cells. By coupling Dam to a protein in the nuclear lamina layer that undergirds the nuclear envelope, DamID covalently marks lamina-associated DNA.

Using this method, van Steensel had demonstrated that a substantial fraction of nuclear DNA is found in so-called lamina-associated domains (LADs), which are highly condensed and relatively silent, while actively tran-



TRACING DNA DYNAMICS: Genomic regions (green) that associate with the nuclear lamina (red) are illuminated in real-time in single cells using m6A-Tracer.

scribed DNA is located more centrally in the nucleus.

It is, Kind says, like "a factory where you would have people working on conveyor belts in the middle of the factory, and anything that you don't need and would just be standing in your way, you could just place it against the wall."

Kind wanted to see if lamina-associated sequences ever swapped places with more active DNA. He built a fluorescent reporter comprised of GFP fused to the methyladenosine-binding domain of a methylation-sensitive restriction enzyme (m6A-Tracer), and coexpressed it in mammalian cells alongside a fusion between Dam and a protein component of nuclear lamina called Lamin B1. He then used a microscope to track fluorescence localization in individual cells as they grew and divided.

The data suggest that 3-D conformation is relatively stable in interphase cells, Kind says, but not in cells that are dividing. "We found that the genome that was localized towards the nuclear lamina in the mother cells, in the daughter cells was pretty much all over the place." As his team wrote in the article detailing these results, the chromosomes were "stochastically reshuffled" (*Cell*, 153:178-92, 2013). That's a surprising observation, Kind says, because it implies that daughter cells don't necessarily inherit the epigenetic state of their parents.

GET THEE TO A BIOINFORMATICIAN: Like most chromosome-conformation methods, DamID and m6A-Tracer require no special tools. "The difficulty comes in the bioinformatics," Kind says. Each experiment requires new software to process and make sense of the data. Kind has now developed a single-cell variant of DamID, which he is using to map the cell-to-cell heterogeneity of nuclear organization, but the technique is still unpublished.

ChIRPING ABOUT RNA

RESEARCHER: Howard Chang, Professor of Dermatology, Stanford University School of Medicine; Early Career Scientist, Howard Hughes Medical Institute

PROJECT: Mapping the architecture of a long noncoding RNA

PROBLEM: Existing methods for probing structure/function relationships in RNA rely either on low-resolution FISH or mutagenesis. Chang wanted a high-resolution method that worked with endogenous, wild-type molecules.

SOLUTION: Long noncoding RNAs may serve as "very general readers or effectors" of chromosome architecture, Chang says. Yet researchers understand little about how they work, including their shape. "It's hard to describe the behavior of something and understand it if you don't know what it looks like."

Chang adapted a method his lab developed called chromatin isolation by RNA purification (ChIRP). In ChIRP, chromatin is crosslinked in situ, just as in 3C, but the isolated sequences are pulled down using biotinylated oligonucleotides complementary to a noncoding RNA of interest. Once isolated, the complexes can be dissociated to reveal associated RNA-binding proteins or genomic DNA.

Chang's team wanted to work out the architecture and modular functionality of a long noncoding RNA called roX1, which is implicated in X chromosome dosage compensation in fruit flies. The team developed a variant of ChIRP called dChIRP (domain-specific ChIRP), which performs the pull-down using pools of oligonucleotides representing distinct functional domains of a given transcript. The resulting material can then be subjected to deep sequencing to identify associated chromatin, western blotting to identify bound proteins, or RT-qPCR to identify bound RNAs (*Nat Biotechnol*, doi:10.1038/nbt.2943, July 6, 2014). Resolution is on the order of tens of bases, Chang says.

dChIRP produced sharper, more intense signals than ChIRP alone, Chang's team found. Based on their analysis, they determined that the roX1 transcript contains three RNA "fingers" that interact with its chromosomal and protein targets, the most 3' of which (D3) can mitigate the male lethality caused by roX1 deletion as efficiently as the full-length transcript itself.

COMING SOON TO A KIT NEAR YOU: Although it's not yet commercially available, Chang says dChIRP technology has been licensed to several companies. But anybody can use the technique, he says. "If you know the sequence of your RNA," he says, "you can just punch it into the computer, order up a bunch of oligos, and you're off to the races." ■

COURTESY OF JOP KIND

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STEM CELL CHARACTERIZATION: CHALLENGES AND STRATEGIES

The identification and isolation of stem cells is dependent on tools and strategies to distinguish stem cells from a heterogeneous cellular population. Stem cells can be characterized by cellular, molecular, and functional assays. Although much progress has been made, technical challenges persist in characterizing stem cell identity, lineage, and purity. *The Scientist* brings together a panel of experts to discuss emerging tools and strategies for stem cell characterization. Attendees can interact with the experts during the live webinar by asking questions and sharing their experiences using stem cells.

TOPICS TO BE COVERED:

- Basic concepts underlying stem cell identification and current industry standards
- Approaches and considerations for stem cell isolation and characterization
- Strategies for incorporating appropriate tools into your workflow

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Eyes on the Prize

A handful of stem cell therapeutics for vision disorders are showing promise in early-stage trials, and still more are in development. But there's a long road to travel before patients see real benefit.

BY JEFFREY M. PERKEL

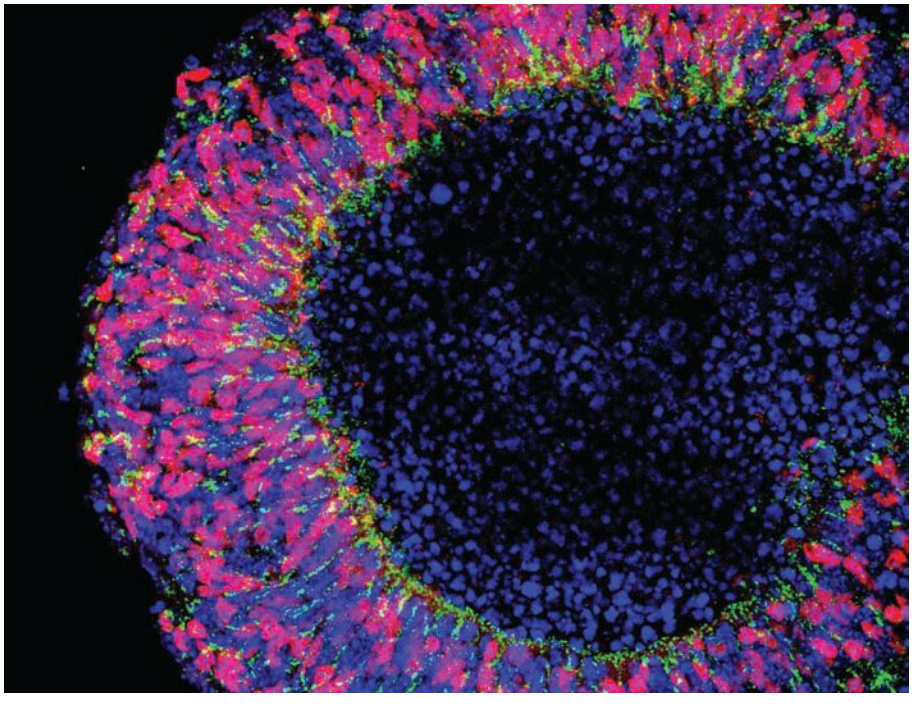
In mid-June, Newark, California–based StemCells, Inc. announced interim results of its ongoing Phase 1/2 trial for the treatment of dry age-related macular degeneration, a form of progressive blindness common in the elderly. Seven patients with advanced disease who had been dosed with the experimental therapeutic—multipotent neural stem cells derived from fetal brain tissue—showed slowed retinal atrophy at one year post-transplant, and four had not just stabilized but improved visual function, the company reported.

"They've actually had gains in their visual ability to sense contrast, which is the difference between light and dark," explains Stephen Huhn, the company's chief medical officer and vice president for central nervous system (CNS) clinical research. "It's very powerful to see that this early in the trial."

StemCells' announcement is the latest in a series of promising developments in the area of cell-based therapeutics for blindness. Advanced Cell Technology (ACT) has several ongoing trials based on differentiated cells derived from human embryonic stem cells (hESCs), and last year, Japanese researchers launched the first clinical study to use induced pluripotent stem cells (iPSCs) derived from adult human cells for the treatment of age-related macular degeneration. Still other strategies are in development, and excitement is high.

"I've been amazed at just how quickly the field has grown and how fast it has progressed toward clinical trials," says David Gamm, an associate professor of ophthalmology and visual sciences and director of the McPherson Eye Research Institute at the University of Wisconsin School of Medicine and Public Health.

It's still early days, he warns. While initial results are promising, that's all they are at the moment. Nobody with blindness has yet been "cured" with a stem-cell therapeutic. And there are substantial safety issues to contend with when implanting live cells in the eye. "We're pushing the boundaries of this technology," Gamm says. "And as such, we expect there to be probably more bumps in the road than smooth parts."



REGROWING RETINAS: By culturing mouse embryonic stem cells, researchers can grow nascent retinas containing photoreceptor precursors that express the visual pigment rhodopsin (green) and the transcription factor Crx (red) and can be isolated and transplanted into adult mice.

IMAGE BY ANAI GONZALEZ-CORDERO

Why the eye?

The eye was not the first organ to receive transplanted stem cells. StemCells tested its cells in the brain and spinal cord before moving to the eye, and Geron, the first company authorized by the US Food and Drug Administration (FDA) to launch an hESC-based trial, targeted the spinal cord as well. (The company has since abandoned the field, selling its stem cell portfolio to BioTime subsidiary Asterias Biotherapeutics, which on August 27 announced it had received FDA approval to launch a new Phase 1/2a trial in 13 patients with spinal cord injury.) Other researchers are targeting the brain and spinal cord as well, not to mention the blood, pancreas, heart, and other nonneural tissues. A search of clinicaltrials.gov for "stem cell transplant" returns some 3,329 hits.

But for many stem-cell researchers and drug developers, the eye is the ideal organ for treatment with stem-cell therapeutics. It is small, and therefore requires relatively few cells for efficacy; and it is

immune-privileged, meaning allogeneic (nonself) transplants may be used with little risk of immune rejection. Function is easily quantified in the eye, and even incremental improvements can yield large benefits for the patients.

As a practical matter, the eye is also the only part of the central nervous system (CNS) that is externally visible and accessible, and researchers can track transplanted cells noninvasively using techniques such as optical coherence tomography (OCT). Like a high-resolution optical version of ultrasound imaging, the technique provides "histological detail down to a micron or so resolution," says Michael Young, associate professor of ophthalmology and codirector of the Ocular Regenerative Medicine Institute at Harvard Medical School. "That turns out to be, from a therapeutic point of view and from an endpoint-analysis point of view, a great tool for us in trying to figure out whether these things work or not, and secondarily, is something wrong."

Another important advantage of targeting the eye, says Young, is safety. The eye is relatively self-contained and, disturbing as it may sound, nonessential. "Imagine a stem-cell transplant for Parkinson's disease, where you inject stem cells into the middle of the brain, and something goes wrong. What do you do? The answer is nothing, you can't do anything. In the eye, if something goes wrong, and in these early stages something can go wrong, you can actually remove the eye and remove the cells," he says.

This is particularly important for therapies derived from hESCs or iPSCs, which, unlike adult stem cells, can divide indefinitely and differentiate into any cell type of the entire body. As such, they also pose a risk of tumorigenesis in transplant recipients should undifferentiated cells accidentally be introduced into a patient.

Providing new support

Though there are hundreds, if not thousands, of diseases that affect the eye, most cell therapeutic programs to date have focused on macular degeneration, "the commonest cause of sight loss in the Western world," says James Bainbridge, a professor of retinal studies at the University College London Institute of Ophthalmology and the chief investigator of ACT's UK-based trials. "We can all expect to develop it if we live long enough." More than 2 million Americans were suffering from age-related macular degeneration in 2010, according to the National Eye Institute.

Macular degeneration involves the loss of retinal pigment epithelium (RPE) cells, which secrete growth factors, remove metabolic waste, and recycle the photopigment retinal that is required for the function of the adjacent rods and cones. "They're basically a support cell for the photoreceptors," says stem-cell biologist Dennis Clegg of the University of California, Santa Barbara (UCSB). As the RPE beneath the macula, or center of the retina, begins to deteriorate, the photoreceptor neurons begin to die as well, and central vision—critical for reading and writing, recognizing faces, and low-light vision, among other functions—is lost. (See illustration on page 33.) One way to halt disease, then, is to replace the RPE cells or provide a substitute to stem the continued degeneration of the photoreceptors.

> I've been amazed at just how quickly the field has grown and how fast it has progressed toward clinical trials.
>
> —David Gamm, McPherson Eye Research Institute, University of Wisconsin School of Medicine and Public Health

StemCells' HuCNS-SC transplantation works by supplementing a patient's remaining RPE cell function with neural progenitor cells not normally found in the eye. Although derived from donated fetal brain tissue—"obtained through a nonprofit tissue procurement agency following an elective abortion," according to Huhn—StemCells' therapeutic is technically an adult stem-cell product, in that the cells have lost the pluripotency that defines embryonic stem cells. Neural stem cells extracted from the fetal brain tissue are expanded and cryopreserved; once thawed and implanted into patients, the cells can differentiate into neurons, astrocytes, and oligodendrocytes. "The broad category of mechanism of action is probably some type of neurotrophic effect," Huhn says.

Janssen Research & Development's CNTO-2476, an allogeneic cell therapy derived from human umbilical cord tissue, is believed to secrete trophic factors that support diseased retinal tissue, according to a company spokesperson. The therapy has been tested in trials for age-related macular degeneration and retinitis pigmentosa, and a large, randomized trial for macular degeneration is being planned to further assess its efficacy and safety.

ACT's strategy is more direct: supply new RPE cells to replace and repair the native RPE layer. ACT has initiated four Phase 1/2 trials testing its hESC-derived RPE therapy for the treatment of the dry form of age-related macular degeneration (dry AMD); a heritable form of the disease called Stargardt's; and myopic macular degeneration, a form of vision loss caused by abnormal elongation of the eyeball. The company described its initial findings for the first dry AMD and Stargardt's disease patients in a 2012 *Lancet* paper, with results pointing to the protocol's safety and hinting at its efficacy (379:713-20). One patient, for instance, improved from 20/500 to 20/320 vision, which corresponded to a modest improvement in ability to read an eye chart, albeit with "mild visual function increases in the fellow [untreated] eye." In 2013, ACT announced that one of the more recently treated dry AMD patients had experienced an improvement from 20/400 to 20/40.

These promising results, along with those from StemCells' Phase 1/2 trial this summer, suggest that restoring or replacing RPE function can not only halt the spread of macular degeneration, but partially reverse it—essentially kick-starting photoreceptors that were dying but not yet dead.

UCSB's Clegg and his colleagues at the nonprofit California Project to Cure Blindness and elsewhere are also pursuing the RPE approach. The team plans to transplant sheets of RPE cells derived from hESCs deposited on an artificial substrate called parylene, which mimics the extracel-

lular matrix layer of the RPE. With $19 million in funding from the California Institute for Regenerative Medicine, the researchers hope to file an investigational new-drug (IND) application for dry AMD with the FDA by the end of the year, Clegg says.

Researchers at the RIKEN Institute in Japan have also announced plans to differentiate and transplant RPE cells for AMD, this time using iPSCs. The team, led by Masayo Takahashi, will generate iPSCs from patients' skin cells, a process RIKEN says will take 10 months to complete. The cells will then be differentiated into 1.3 mm x 3 mm sheets of RPE cells and transplanted back into the patient the cells were taken from. If successful, the therapy would avoid the moral complications that accompany hESCs, which are created from human embryos. "There are a significant number of people who are not comfortable with ES cells," says the University of Wisconsin's Gamm.

Replacing photoreceptors

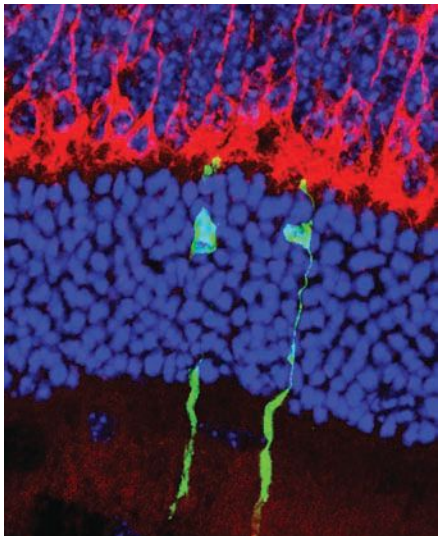
Once rods and cones are dead, however, even an infinite supply of RPE cells cannot help. To more fully restore vision in patients with retinal degeneration, researchers are looking to replace the photoreceptors themselves. This strategy is more complicated than the RPE approach, however. To be functional, photoreceptors must not only implant and survive, but extend neural processes and form synaptic connections with downstream bipolar neurons. (See photograph at right.) Fortunately, it's a relatively short gap to fill, says Matthew Vincent, ACT's Director of Business Development. "If you're going to think about replacing a neuron with a stem cell, that's probably the best one you could imagine doing."

Preclinical work suggests the strategy can work. In 2012, researchers in Robin Ali's group at University College London reported in *Nature* that transplanting murine photoreceptor precursor cells yields functional improvements in mice that lack rods (485:99-103). And while no photoreceptor-based strategy has yet entered clinical trials, several are in development.

Young's group at Harvard Medical School, for example, is working on a strategy for treating retinitis pigmentosa that involves transplanting human fetal retinal progenitor cells, which develop into rods. Retinal progenitor cells, he explains, are proliferative cells that are "one stage less developed" than the precursor cells Ali used in his mouse study and are thus easier to grow. Furthermore, human retinal progenitor cells have been safely banked under good-manufacturing-practice (GMP) conditions and are ready for a future trial, Young says. He and his partners, including UK-based ReNeuron, will meet with the FDA later this year and hope to launch a clinical trial in early 2015.

ACT is also preparing to launch clinical trials based on hESC-derived photoreceptors, says Vincent, possibly also early next year. "I think the photoreceptor progenitors will likely be the next 'IND-able' . . . program for the company."

Gamm, meanwhile, has worked out methods to differentiate iPSCs into photoreceptor precursors and other retinal cells, and is working with Clegg on a strategy for treating dry AMD that involves both RPE and photoreceptor precursors. But rather than deriving such cells from individual patients, as the RIKEN group is doing, Gamm and his colleagues figure that it will be less expensive and faster to bank a wide variety of HLA-typed iPSCs. (See "Banking on iPSCs," *The Scientist*, September 2014.) "Similar to how you might get a close match, but not perfect match, for an organ transplant, we could do the same thing for all cell types derived from certain iPS [cell] lines," Gamm explains.



MIXING OLD AND NEW: When transplanted into the mouse retina, photoreceptors (green) derived from mouse embryonic stem cells integrate into the retinal network and contact the neighboring neuronal layer, the bipolar cells (red).

IMAGE BY DR ANAI GONZALEZ-CORDERO

To the clinic

As cell therapies make their way into the clinic, there's one overriding concern clinicians and regulators have, says Gamm: "Safety, safety, safety." Indeed, Geron, the first company to get an hESC-based therapeutic into clinical trials, submitted an IND application that was reportedly some 22,000 pages long—the largest ever approved by the FDA.

ACT, the second company to win IND approval for an hESC therapy, went to considerable effort to assure the FDA that the risk of tumor formation from its hESC-derived RPE cells was as low as possible—among other measures, developing a new proprietary method for detecting contaminating undifferentiated cells that is some five orders of magnitude more sensitive than PCR, says Vincent. "The first, second, and third issue for the FDA really was safety," he said: "prove to us that there is no risk that these patients are going to develop tumors as a consequence [of] these cells that you're injecting." The company's first IND took a year to get the nod, though subsequent applications were approved in less than one month each, he noted.

As these companies and researchers lay the groundwork, other players should have an easier time. (See "Stem Cells Off the Line," *The Scientist*, April 2014.) Now, says Gamm, the challenge is managing expectations. At the moment, things are looking up for the field, and research is advancing rapidly. But at some point, he says, "we're going to hit something that will take us a while to figure out." Gamm says he tries to make that clear when talking to patients and disease foundations. Riffing on the customary disclaimer accompanying mutual fund literature, he says, "Past performance is not a guarantee of future progress." ■

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- In search of natural products: tools and strategies
- Considerations for the identification, isolation, and testing of natural products and their active compounds

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SPEAKERS

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CHRISTINE SALOMON, PhD, Assistant Professor and Assistant Director, Center for Drug Design, University of Minnesota

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Sleep Tight

Bed bugs are but one example of a species whose populations have evolved in response to human behavior.

BY EMILY MONOSSON

My sister-in-law, the doctor, was losing her mind. She awoke every morning to oozy-itchy bites. At first she'd thought it was fleas, courtesy of the family pets, but she was the only one suffering. The culprits, she eventually realized, were bed bugs. Who knew? For those of us of a certain age, bed bugs belong in old nursery rhymes, killed off decades ago by a cloud of DDT. Yet the critters, which survived in small pockets here in the U.S. and in other countries, are now making a comeback worldwide: in cities, college dorms, even upscale hotels.

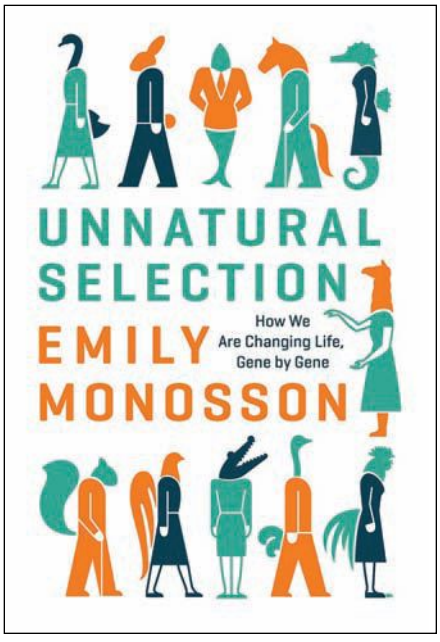
At a recent meeting of environmental toxicologists and chemists, I asked for a show of hands indicating those who had experienced bed bugs personally. While my graying colleagues looked befuddled, several grad students and undergrads timidly raised their hands. Bed bugs have returned, but this time around they are notoriously difficult to eradicate. Populations around the country are resistant not only to DDT, but also to pyrethroid insecticides (both target sodium channel pores in nerve cell membranes), making them even more unmanageable. These crafty pests, along with gonorrhea, mosquitos, pigweeds, killifish, and many other species, are prime examples of chemically induced evolution.

In *Unnatural Selection: How We Are Changing Life, Gene by Gene*, I explore the consequence of evolution in response to antibiotics, pesticides, herbicides, pollutants, and even chemotherapy. What happens when life is faced with synthetic chemicals unlike any found in nature? We know now that hundreds if not thousands of species have undergone microevolution—small changes within a population—in response to industrial-age chemicals. What are the costs of this kind of microevolution? How can the dangers be mitigated? The book touches upon nature's awesome defensive tactics: enzymes tweaked just enough to take the bite out of a chemical attack while maintaining their original function; pumps that act like bouncers at a popular bar; target genes copied tens if not hundreds of times.

That toxic chemicals provide powerful selective pressures should be no surprise. Early last century Alexander Fleming warned that if not careful his precious discovery, penicillin, would lose its punch. Decades later Rachel Carson warned us again, writing of "the chemical barrage [that] has been hurled against the fabric of life—a fabric on the one hand delicate and destructible, on the other miraculously tough and resilient, and capable of striking back in unexpected ways."

Today, bed bugs are striking back; so too are a host of pathogens, including totally drug-resistant tuberculosis and nearly drug-resistant gonorrhea. Many cancer patients live in fear that their errant cells might evolve to evade treatment. Evolution in response to pollutants may also have broad implications, disrupting species less able to respond rapidly.

Some bed bug populations have evolved resistance genes that are most active in their cuticles, warding off pesticides with biochemically active suits of armor. Other organisms employ a variety of mechanisms that are common across taxa (a topic worthy of follow-up study); a few, like plasmid exchange in bacteria, are not. Recent studies indicate that chemicals may also induce epigenetic changes that may affect gene expression across multiple generations. Our evolutionary footprint is large. But even as we tabulate our ecological, material, carbon, and water footprints, so far we have paid little collective attention to how we are influencing evolution.



Island Press, November 2014

Yet as many in the businesses of health care and agriculture have seen firsthand, we can change the dynamic. We can reduce the selection pressure by using less of a particular antibiotic or pesticide. Or we can combine or alternate chemicals; encourage the selection of detrimental traits; or back away from chemical use altogether. Bed bugs, for instance, can be killed by both heat and cold—though these techniques are not always feasible. Fortunately my sister-in-law's uninvited houseguests were not yet resistant to the pyrethroid insecticides: a nonorganic concession made under duress. But the odds are against us. When we challenge life to evolve or die, we don't get to pick and choose the winners. Nature does that for us. ■

Emily Monosson is an independent biochemical toxicologist, writer, consultant, and college instructor. She is an adjunct professor at University of Massachusetts, Amherst. Read an excerpt of Unnatural Selection: How We Are Changing Life, Gene by Gene *at www.the-scientist.com.*

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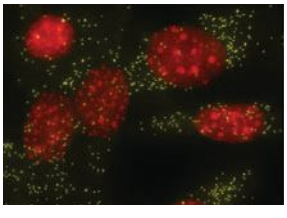

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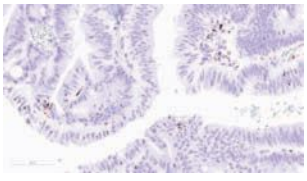

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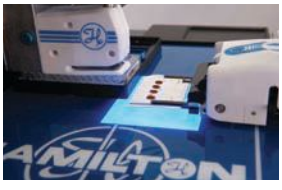

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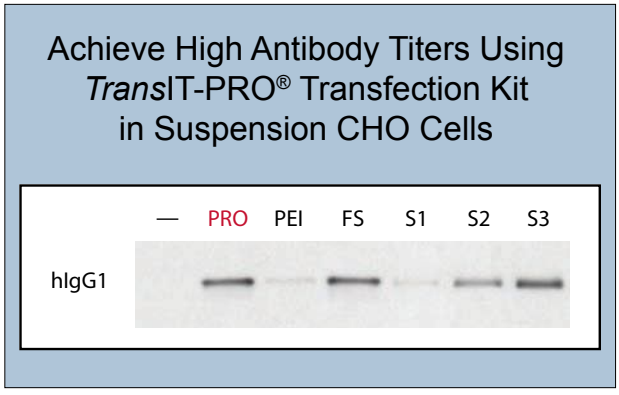

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A Visionary's Poor Vision, 1685

BY JYOTI MADHUSOODANAN

In 1663, budding ophthalmologist William Briggs, just 13 years old, enrolled at Corpus Christi College in Cambridge, England, where he befriended a 21-year-old Isaac Newton. Newton derived much of his anatomical knowledge of the eye from watching Briggs dissect the eyes of various animals, yet Briggs's theories of visual processing were considered little more than rubbish, and his friend's scientific reputation soon eclipsed his own.

As a personal physician to King William III, Briggs was a prominent medical professional in 17th-century England. Despite his reputation as a doctor, he is mostly remembered "as a sort of footnote in Newton's life," says ophthalmologist Daniel Albert of the University of Wisconsin–Madison. "His major contribution was to get Newton thinking about vision."

Briggs's own theories of vision culminated in his second book, *Nova visionis theoria*, "A New Theory of Vision." Published in 1685, the 80-page vellum-bound manuscript included two case studies of patients with different forms of blindness and a 1681 paper, presented to the Royal Society of London, in which Briggs outlined his theory of the basis of binocular vision—how information from a pair of forward-facing eyes combines to form a single image.

Briggs maintained that fibers from the two optic nerves do not cross within the optic chiasm, but instead form two streams that stay within the same cerebral hemisphere of each eye, suggesting that the crossing-over of nerves was not needed for binocular vision. He compared the anatomy of human eyes to that of animals with eyes on either side of their head, writing: "In many fishes the case is clear, where the two nerves are joined only by simple contact, and in the chameleon not at all . . ."

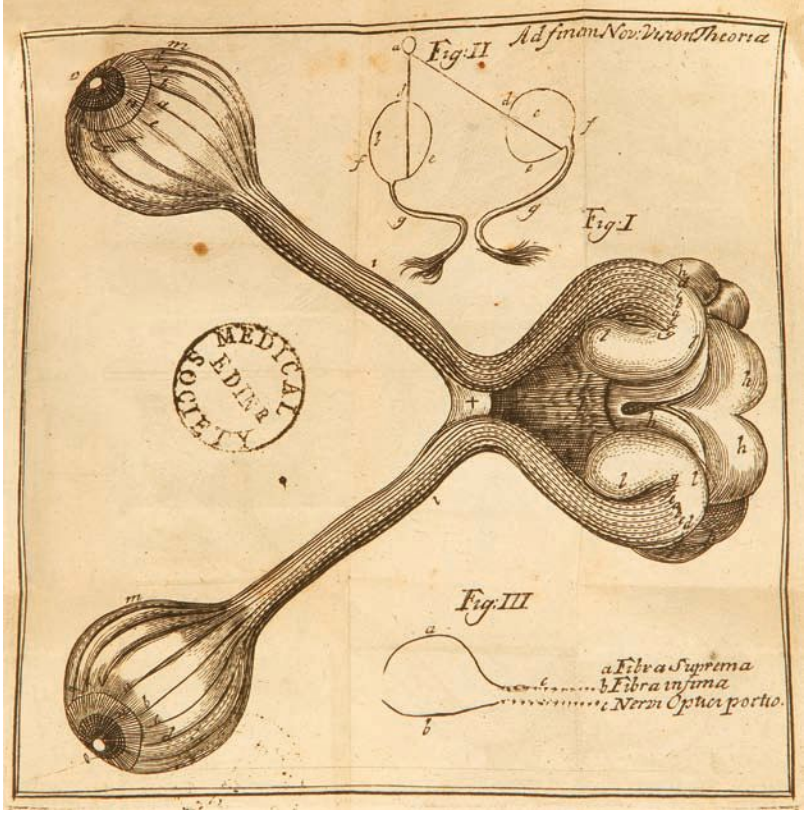
In a letter that prefaced *Nova visionis theoria*, Newton said the book advanced two fields at once, both anatomy and optics. He praised the anatomical work and called Briggs's theory "most ingenious."

"The fact that Newton would endorse the book and think it of sufficient merit to write a very favorable foreword would undoubtedly reflect very positively on the book," says Albert, who translated *Nova visionis theoria* from Latin in 1991 with Jeffrey Wills.

However, Newton disagreed with several of the ideas proposed and "destroyed Briggs's 'theory'" in their personal correspondence, according to the preface Albert penned to introduce his translation. For example, regarding the comparison of fish, chameleon, and human eyes, Newton replied: "In those animals which do not look the same way with both eyes, what wonder if the nerves do not join?" Others voiced harsher opinions, Albert wrote. Scottish physicist David Brewster wrote that Briggs's theory displayed "neither sagacity nor genius"; German ophthalmologist Julius Hirschberg called it "no theory at all."

In 1704, more than 21 years after his exchanges with Briggs, Newton published his own ideas on the origins of binocular vision in *Opticks*, proposing the idea of semidecussation, or a partial crossing-over of the optic nerves.

Despite its inaccuracies, *Nova visionis theoria* "paved the way for Newton's brilliant concept of a semidecussation of the optic nerves," Albert wrote. It "bridged the vague and imprecise descriptions of previous generations of anatomists to more accurate modern concepts." ■



MISSED CONNECTIONS: William Briggs's illustration in *Nova visionis theoria* depicts how he thought vision occurred. As drawn in Fig. II (left, top), he held that the optic nerves from each eye remained distinct, "for that the nerves . . . cross one another . . . is not to be imagined; but those that are in the *thalami optici* on the right side run distinctly to the right eye, and those on the left accordingly." In Fig. III, he depicted how tension in the thalamic fibers conveyed visual input as "vibrations" to the nerves, like vibrations in a spider's web. Both ideas turned out to be incorrect, yet his detailed anatomical surveys provided the foundation for much of the future study of the eye.

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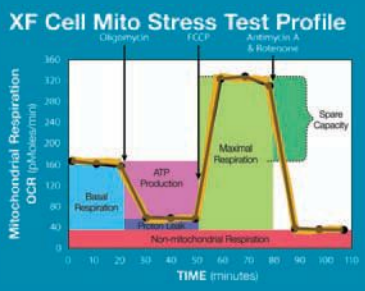

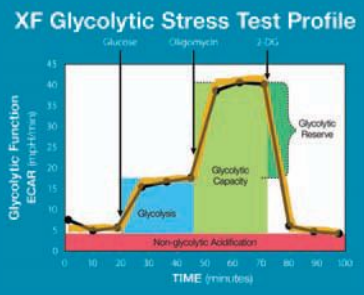

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