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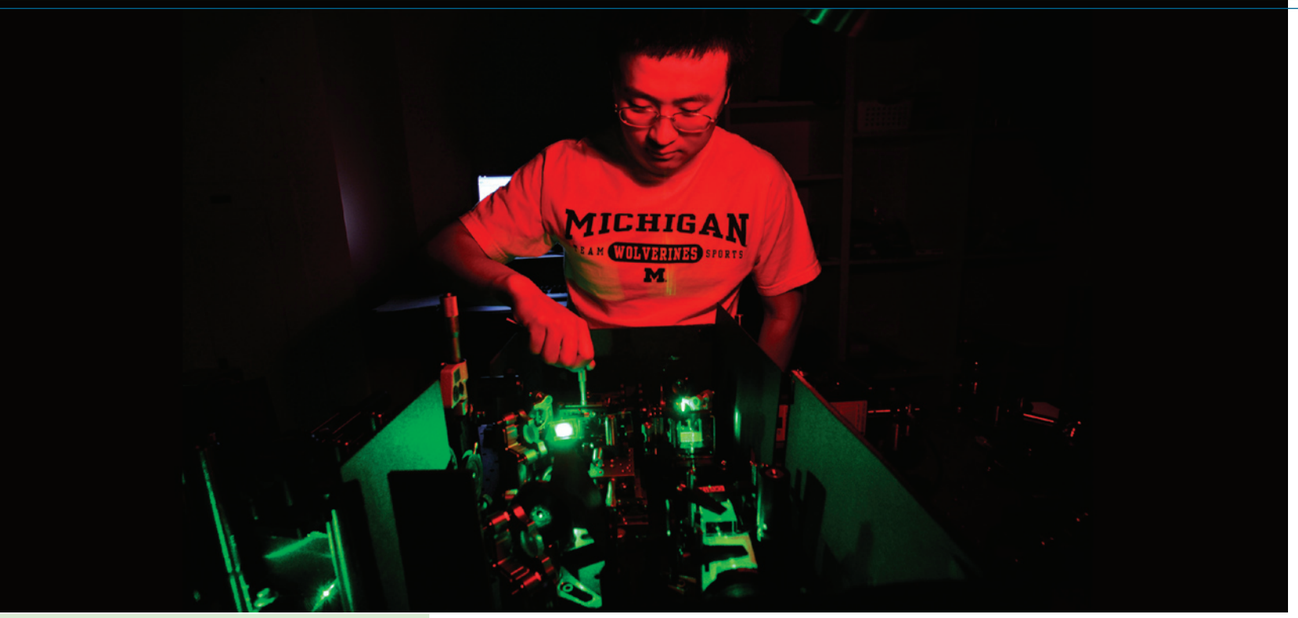
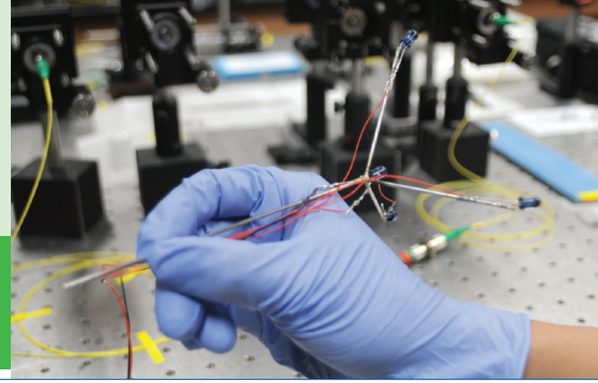
A Toymaker's Lab

By Marcus Y. Woo



Left: Christmas in the lab? Postdoc Meng Cui shows off an experiment that would allow you to see through skin and flesh.
Right: The four LEDs at the base of the new probe shine in infrared light. A camera connected to the computer follows the LEDs as the probe moves.



Changhuei Yang's lab is filled with clever projects: a system to see through skin and flesh, new kinds of microscopes, and even a device inspired by a video game. But his work's not just fun and games—it may change the way diseases are diagnosed and treated.

Changhuei Yang shuttles between workbenches littered with lasers and optics equipment. Yang, an assistant professor of electrical engineering and bioengineering, runs the biophotonics lab at Caltech, and he's showing off one of his latest gadgets—a thin, five-inch-long rod with three prongs that could eventually aid doctors in everything from eye surgery to biopsies. This tool, he explains with delight, was inspired by nothing other than the Nintendo Wii, whose remote controller is a motion-sensitive wand. To hit a baseball, for example, you would swing the remote as if you were swinging a bat. Yang's tool doesn't control anything, but uses the same tracking technology so that its precise position is known at all times. The technology could lead to valuable instruments for procedures like prostate exams, in which it's a good idea for doctors to know exactly where they're prodding.

The probe is one of several research projects that are under way in Yang's lab. They are hard to put in a single category, but they all have an element of ingenuity. Biophotonics is the science and technology of using light in biology, or as he puts it, "We use optics in clever ways to solve problems." The lab is creating potentially revolutionary new microscopes and developing other methods to see through skin and flesh, possibly changing the way doctors diagnose and treat diseases. All this has landed him on *Discover* magazine's "20 Best Brains Under 40," a list of young, pioneering scientists and engineers from around the country. A colleague once described him as a toymaker, he says, and Yang agrees it's a fair assessment. After all, in how many labs would you find a Nintendo game system on the bench?

With the Wii, a stationary sensor unit tracks the position of two light-emitting diodes (LEDs) embedded in the wand. By measuring the movements of the LEDs and how their sizes change as you move the wand toward or away from the unit, the Wii gauges direction and depth. Yang's probe, being developed by graduate student Jian Ren, looks like a miniature Eiffel Tower with a triangular base and sports four LEDs—on the bottom of the tower and the foot of each leg. Because the LEDs are at the back end of the probe, they would stick out and remain visible to the tracking camera while the tip is inside the body. As you manipulate the device, a computer follows the positions of each LED, showing the real-time movements on a monitor. The present design is just a prototype—the tool a doctor would eventually use would likely be smaller and tailored to the specific medical procedure.

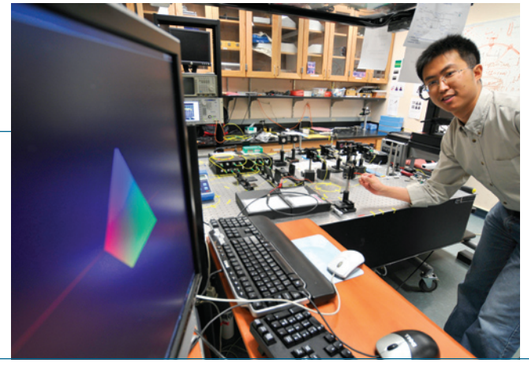
The researchers are now working with doctors to design an instrument for use in eye surgeries. Eye surgeons have to be especially precise, and so must their tools. Many of the probes now have a built-in laser to "see" what's in front of them, Yang says. But these devices have heavy, clunky mechanical parts—not exactly the sort of thing you'd want stuck through your eye. Yang's technology, on the other hand, would make for a light and maneuverable probe. The lab only started developing the device last fall, but the technology is straightforward, and Yang anticipates that doctors will be able to buy the tool in just two to three years.

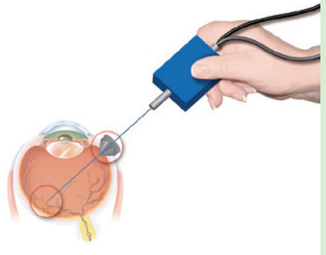
A MICRO-MICROSCOPE

Another tool of Yang's has already made a splash: a lensless microscope small enough to fit on a microchip. The basic design of the optical microscope—a set of lenses magnifies an object and brings it into focus—hasn't changed in more than 400 years. The space between the lenses dictates that microscopes have to be a minimum size, and when it comes to technology, size often does matter—think ever-shrinking iPods and cell phones. To use the most powerful microscopes, which are too big to lug out into the field, you have to take your samples back to the lab. Yang's solution is the optofluidic microscope, a device that's about the size of George Washington's nose on a quarter.

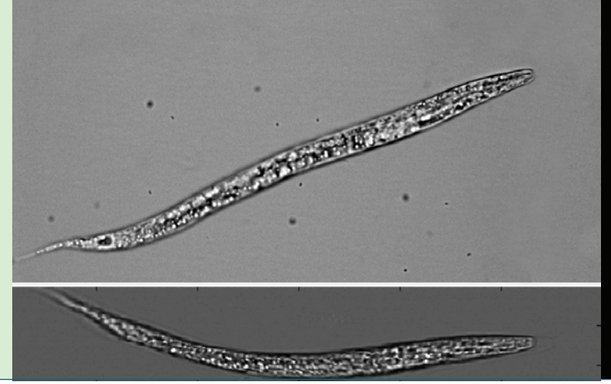
The principle behind the optofluidic microscope is the same as that of the "floaters" in your eye. When you look at a clear, blue sky, you may notice small shapes floating around, seemingly in midair. Those shapes are shadows cast onto your retinas by debris floating around in the vitreous humor, the gel that fills your eyeballs behind the lens. You see floaters without the aid of lenses—in fact, floaters are just as much in focus whether or not you wear glasses. The particles are so close to the retina that they always appear sharp. In Yang's microscope, the specimen is suspended in a liquid and is drawn through a small channel either with an electric charge or by gravity. The object passes in front of a charge-coupled device (CCD), the "retina" that's the basis for all digital cameras. Some uniform light source—like the sun—then casts the object's shadow onto the CCD, which records the image. However, the resolution is limited by the pixel size, which is about five microns—not much better than conventional microscopes.

Yang's team solved that problem by stacking a metal sheet on top of the

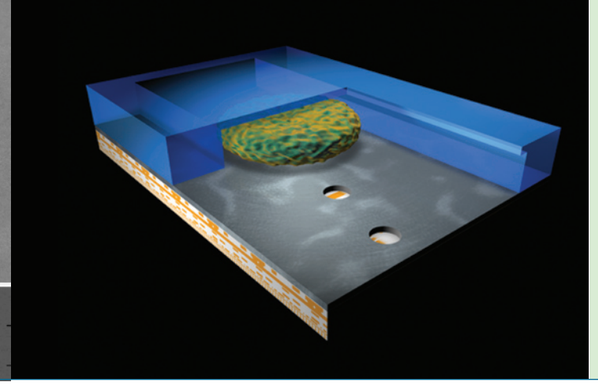




Far left: Graduate student Jian Ren demonstrates the biomedical probe based on the Nintendo Wii. The colorful image on the monitor represents the device, and as Ren moves the probe, the image mirrors the motion.
Left: Yang's probe technology could be used in surgical devices, like this one for the eye.



Images of the roundworm *C. elegans* taken with the optofluidic microscope (bottom) are comparable in quality to those taken with a conventional microscope (top).



An up-close representation of the optofluidic microscope. The green specimen flows across the gray metal sheet. The detector underneath captures images of the specimen through the holes.

CCD. The researchers punched a row of 600-nanometer-diameter holes in a diagonal line across the sheet. The specimen flows across the front of the holes, which operate like pinhole cameras. The holes are spaced five micrometers apart—the same as the pixel size—so that there is one hole per pixel, ensuring that each pixel receives a unique set of photons at a given time. But the key aspect is that the holes are diagonally offset, allowing them to cover the entire specimen while still making sure that each hole corresponds to one pixel. Each hole captures a different part of the object as it flows by, and software then puts the pieces back together to complete the picture. With this approach, the resolution is no longer limited by pixel size; the researchers can increase the resolution just by cutting a row of smaller holes closer together.

While conventional lab microscopes can cost thousands of dollars, the optofluidic microscope can eventually be mass-produced for only $10 a pop. Since it's small enough to put onto iPod-like devices, the microscope could have the biggest impact in developing countries, Yang says. Clinics in rural areas could use the microscopes to diagnose diseases like malaria, test blood and urine samples, and even test for harmful pathogens in food and water. In April, Yang and graduate students Lap Man Lee and Xiquan Cui published a report in *Biomedical Microdevices* showing that the device could take high-quality pictures of *Giardia lamblia*, a common water-borne parasite that wreaks havoc on the digestive system. With some minor tweaking of their microscope, their images of the single-celled bug were comparable with those from a conventional microscope, even resolving the organism's flagella.

Additionally, medics could keep the optofluidic microscope as part of their tool kits when treating soldiers in the battlefield. Future cell phones might even feature tiny microscopes as a way to protect against bioterrorism by continuously monitoring for harmful substances in the air. The device could also be a boon for biology. Imagine tens or hundreds of tiny scopes all on a small semiconductor chip, working in parallel, boosting the efficiency of researchers who have to make a large number of observations at once. For example, Morgan Professor of Biology Paul Sternberg and postdoc Weiwei Zhong, who are collaborating on the project, study genetic variations in a tiny worm called *Caenorhabditis elegans*—a process that involves gathering lots of data by watching dozens of individual worms for hours.

Yang's lab is now working with a semiconductor company to get the microscope mass-produced, and the scope should be commercially available within a couple of years. But although the microscope attracted a lot of media attention when the design was published last summer, Yang says many of his colleagues seem even more excited about a different project of his: finding a way to see through skin and flesh.

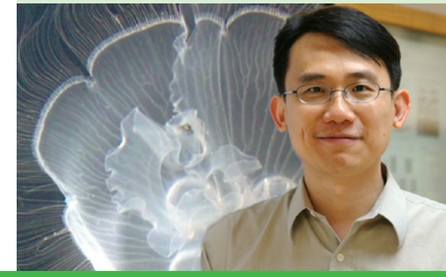
THE NO-SCATTERING ZONE

When a photon enters your skin, it bounces around the organelles in your cells, taking a meandering path like a drunkard getting lost in a dense forest. This "scattered" photon never returns to your eyes, so your body appears opaque. "If we can remove the scattering component," Yang says, "I should appear more or less like a jellyfish." But the ability to see through tissue isn't just some weird form of biological voyeurism—it would make treating ailments much less invasive. For instance, diabetics could check their sugar levels without having to prick a finger to test their blood with a glucose monitor—something they have to do as often as several times a day. "Optical approaches have been getting tantalizingly close to being able to detect glucose noninvasively," Yang says. Many researchers have been trying to pick out the few unscattered photons lucky enough to have penetrated the tissue and returned, but this method is limited—you can't go much deeper than a millimeter before the number of unscattered photons dwindles to zero. Yang wants to eliminate scattering altogether.

Hundreds of ricochets may seem hopelessly complex and random. But it turns out that scattering is what physicists call deterministic. Every bounce can be described by the laws of physics, so if you know some basic information—the initial velocity of the photon and the characteristics of the tissue, for example—you can, in principle, recreate the photon's path. Returning to the analogy of the inebriated wanderer, this means you can deduce a map of the lost drunkard's route. And by retracing his steps, he can find his way back out of the forest. If another photon could follow the same, exact path back out of the tissue, then it could enter your eyes, allowing you to see into the tissue. Of course, sitting around and calculating every photon's path would be impossible in practice. Instead, you can reproduce light paths with a hologram.

A hologram is a two-dimensional record of how light has traveled from an object. Analogous to a sound recording, a hologram allows you to "play back" a three-dimensional image. To make a hologram, you combine laser light passing through the object with another beam called the reference beam. The two light waves combine, making an alternating pattern of light and dark called an interference pattern. The pattern is recorded on a holographic plate, which then undergoes chemical processing to make the recording permanent—similar to developing a photo. Embedded in the interference pattern is information detailing the path of every photon. When a new laser light shines on the plate, the photons retrace the paths of their predecessors, recreating the three-dimensional image of the object.

More than 40 years ago, Emmett Leith, one of the pioneers of holography, used holograms to map the paths of photons scattered by a piece of ground glass. He then sent other photons backward from behind the glass to retrace those paths. Normally, a light beam shone through the glass would appear blurry. But those photons retraced the scattering path and made it through the glass, resulting in a sharp light beam. Leith had successfully cancelled out the scattering. Yang had wanted to try this with tissue since he was a graduate student at MIT, he says, and when he came to Caltech, he got his chance.

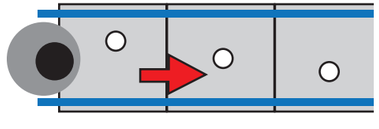


Changhuei Yang's skin and flesh scatters light, rendering him opaque. The jellyfish behind him does not, so it appears transparent.

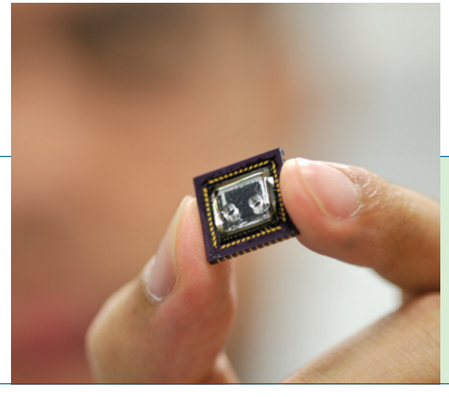
"If we can remove the scattering component," Yang says, "I should appear more or less like a jellyfish."

Traditional holographic plates must be removed from the apparatus for processing. But in order to ensure that the photons retrace the route taken by scattered light, you would have to put the plate back exactly where it had been so that all the light paths would line up—not an easy task. Yang's group uses a material called a photorefractive crystal, which doesn't need to be processed. The crystal takes a temporary snapshot of the interference pattern, which lasts for a few days. Then the team shines a laser through the crystal to retrace the scattered light paths, and a CCD captures the resulting image.

In their initial experiments, the researchers, who include former postdoc Zahid Yaqoob; Demetri Psaltis, the former Myers Professor of Electrical Engineering and now at the Ecole Polytechnique Fédérale de Lausanne in Switzerland; and Michael Feld of MIT, tried their technique with a piece of chicken breast. To test how well they could retrace scattered light, they placed a special glass template in front of the chicken slice, which was less than a millimeter thick. The template—first devised by the U.S. Air Force and used to test the resolution of microscopes and cameras—lets in light through a



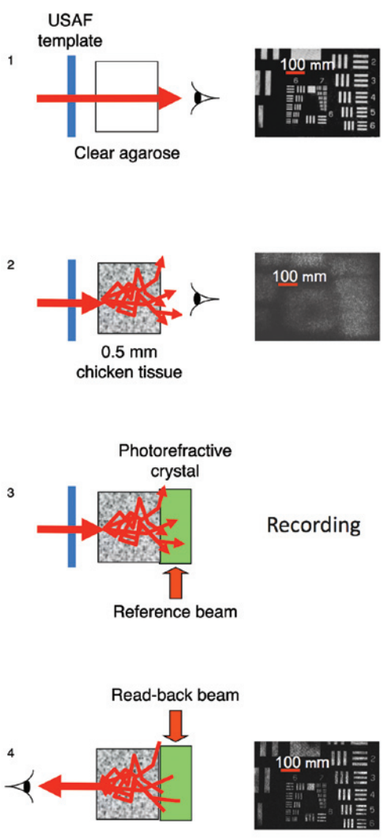
The diagonal arrangement of the holes allows the CCD to take a picture of different parts of the specimen at different times as it flows by. The diagonal arrangement keeps a one-to-one correspondence between each pixel and hole. Computer software puts the image slices together to create a complete picture.



Graduate student Guoan Zheng (MS '08) holds up a microchip that contains the optofluidic microscope.

If you could send light deeply into tissue, then you might be able to stimulate the brain without wires—and without having to drill a hole in the patient's head.

Meng Cui and graduate student Emily McDowell reconstruct scattered light.



simple pattern of rectangular windows. The team shone light through the template and into the piece of chicken. The light scattered, and a photorefractive crystal placed on the opposite side recorded the interference between the scattered light and a reference beam, which was hitting the side of the crystal. (See figure on the left). After removing the template, the researchers fired a different laser beam into the crystal, sending photons back through the chicken, retracing the scattered-light paths. In doing so, the photons recreated a clear image of the template pattern, which was recorded with a CCD. A proof-of-principle experiment, this was the first time anyone had lifted the blurring effect of scattering from biological tissue.

"When we started this, my expectations were fairly low," Yang recalls. Recording the complex process of scattering is a precise task, and he worried that if you couldn't capture every nuance and detail of the light waves, it wouldn't work. But they found the process to be much less sensitive than they had thought—it turns out that you only need to recover a fraction of a percent of the scattered light. The researchers also thought that when the light retraced the paths of the scattered photons, it would lose information along the way. But the team found that the quality of the reconstructed light was just as good as the original. Both results were pleasant surprises, giving Yang confidence that their technique would work well in real-world applications.

The researchers are moving beyond the simple setup of their first experiments, developing a way to see through tissue without having to place a photorefractive crystal on its far side. If you want to look through skin to count glucose levels, for instance, you can't put the crystal underneath your skin and shine a laser from the inside of your body. The new method will involve shining weak light into the front of the tissue. Some of the photons will scatter and come back out—a process called backscattering—and the photorefractive crystal will record their paths. With a map to guide the way, the team can then shoot additional photons to light the way through the tissue.

Light with longer wavelengths goes farther before scattering. To see this, just cup your hand over a flashlight. Only the long-wavelength light makes it through your skin, so any part of your hand that's illuminated appears red—the color that corresponds to the longest wavelength in the visible spectrum. Using light at a wavelength of 500 nanometers—a blue-green hue—the researchers can deliver photons about one centimeter deep into tissue. They can go deeper if they use red or infrared light, but there aren't many photorefractive crystals that work at those wavelengths. One of the lab's goals, then, is finding better materials.

The team is also working on ways to reconstruct scattered light in real time, taking videos rather than snapshots. With this approach, doctors can examine moving specimens behind tissue, like blood flowing through veins.

You could put photons to work in other ways, too, such as fighting cancer with a technique called photodynamic therapy, which is used for cancers like skin cancer and certain kinds of lung cancer. A doctor injects a patient with a dye that's absorbed by the body's cells. The dye sticks to cancer cells longer, and when only they still have the dye, the doctor shines a laser onto the cancerous area. With esophageal cancer, for example, doctors insert a fiber-optic cable into the esophagus to deliver the photons. When exposed to the light, the dye produces cell-killing singlet oxygen, a highly reactive substance. The problem, though, is that the light can only penetrate about a centimeter of tissue, making photodynamic therapy only effective for tumors near the surface. "If we have a technique that allows us to deliver light more deeply into tissue, then photodynamic therapy will have a broader range of applications," Yang says.

Another application is in treating Parkinson's disease. Helped into the public consciousness by the likes of Muhammad Ali and Michael J. Fox, this degenerative disorder makes movements slow and rigid, and causes the hands to tremble uncontrollably. Eventually, sufferers can no longer speak or walk. The disorder is caused by the lack of brain cells that make dopamine, an important chemical that relays signals in the nervous system. When drugs don't seem to work, an alternative form of treatment is to directly stimulate the brain with implanted electrodes. But if you could send light deeply into tissue, then you might be able to stimulate the brain without wires—and without having to drill a hole in the patient's head.

Sending photons into the body can also be a way to deliver power to implanted devices, Yang says. A pacemaker, for instance, could be outfitted with photovoltaic cells, and could be recharged by shining light through the chest. The technology isn't quite a solar-powered heart, but without the need for a large battery, smaller pacemakers would be less intrusive and easier to implant.

The lab is still laying the groundwork, and is years away from turning these ideas into marketable devices for doctors, according to Yang, but the work shows a lot of promise. "The problem of scattering is a big one, and there are tricks people can do" to get around it, he says. "But to tackle it head on, there aren't too many tricks. This one works quite well."

A WIDER FIELD

Postdoc Jigang Wu (MS '05, PhD '09) is spearheading yet another project, this one with more short-term goals. Conventional microscopes have a limited field of view, so Wu, who worked on the optofluidic microscope as a graduate student, is developing a new design that could have, in principle, as wide a field as you want without loss of resolution and magnification power. Like the optofluidic microscope, the wide-field design also does not use lenses.

The microscope consists of a holographic plate and a CCD, with the specimen sandwiched in between the two. The holographic plate is made so that when it's hit by a laser beam, it generates a grid of tiny light spots. The network of light spots scans the specimen, casting a shadow that's recorded by the CCD. The size of the holes determines the resolution, and you can enlarge the field of view by expanding the grid.

Yang foresees an immediate application for patients with advanced cancers. When cancer metastasizes, tumor cells leak into the bloodstream, spreading throughout the body. But finding them among a sea of blood cells isn't exactly easy—for every circulating tumor cell, there are 10 billion blood cells, Yang says. A wider field of view would improve the chances of a bad cell being included inside the frame. Yang is collaborating with Richard Cote, a pathologist and professor at USC, and Yu-Chong Tai, professor of electrical engineering and mechanical engineering, whose lab is building a tiny sieve to filter out most of the blood cells. This way, Yang says, the problem is more akin to finding one cell among 10,000. Even though Wu just started on the wide-field microscope at the end of 2008, he should be able to have a working prototype within a year, he says.

From a gadget based on a video game to tiny microscopes and looking through chicken meat, Yang's research may seem playful. Indeed, he says he wants to develop game-changing tools and techniques to improve the way people do biology and medicine. He got into the field because he wanted to make an impact, he says. "I wanted to do something useful, interesting, and tough"—and that's hardly child's play. E&S

1. Light from the template that goes through a clear piece of agarose gel and straight to the eye is clearly visible. 2. When a piece of chicken is put in front of the template, the light scatters and the pattern is blurred. 3. A photorefractive crystal is inserted to record the scattered light. 4. Light sent through the plate retraces the scattered-light paths back into the eye, recreating the image of the templates.

The wide-field microscope. A laser is reflected down through the holographic plate, which is the square of glass seen in the middle. The specimen would go between the plate and the CCD, which is hidden below. Postdoc Jigang Wu looks from behind.

PICTURE CREDITS
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