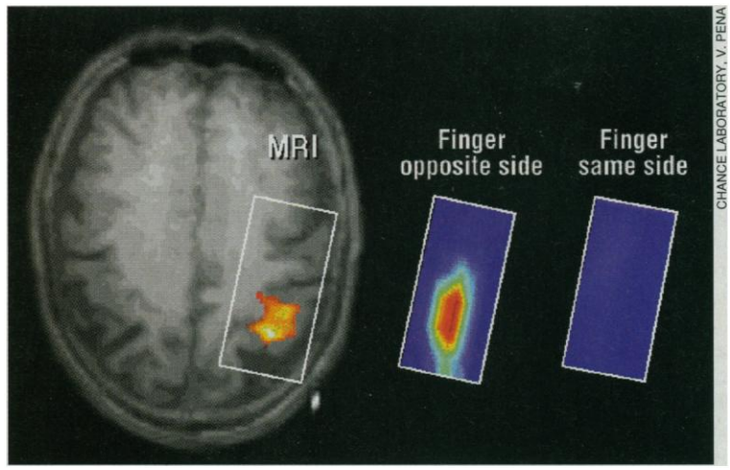
MEDICINE

Play of Light Opens a New Window Into the Body

A light bulb or a laser beam is not the first tool you'd think of using to get a look inside an opaque object such as a brain or a breast. That may be why the idea seems to attract researchers for unusual reasons. City University of New York physicist Robert Alfano, for instance, says he entered the field because one of his students sent a light beam through a glass of milk and saw the shadow of a bead suspended in the milk. Britton Chance, a biochemist at the University of Pennsylvania, got started because his synchrotron broke. With little to do, he had the curious thought that it might be possible to propagate laser light through his own brain, so he tried it. Enrico Gratton of the University of Illinois then took to the research because Chance came to him wondering why lasers took so much longer to go through his students' brains than they did his own. "As a physicist," says Gratton, "I never thought a laser would go through a head. But it does, and when it goes, you can learn something about what is inside."



The brain lights up. Finger movement on the opposite side of the body causes a change in oxygen content, detected by conventional MRI *(left)* and by pulses of light that diffuse through the brain and reveal changes in absorption *(right)*.

The fact is you can learn a lot about what is inside, and so these unorthodox beginnings have created a field of research in which light at optical and near-infrared wavelengths is used to image and probe inside human tissue. Some techniques haven't made it out of the lab. But others, such as ways of mapping the oxygenation of the brain and other organs with light, are already in clinical tests. Ultimately, researchers hope light will complement x-rays in mammography and maybe even eliminate the surgeon's knife for biopsies, tagging a tumor as benign or malignant simply by its optical properties. While some of these goals may be distant ones, light's advantages for imaging and diagnosis make it worth pursuing, says Stanford University engineer and physician David Benaron.

"Most imaging modalities are not only expensive but they're potentially harmful," says Benaron. "And those who need imaging are often critically ill people who can't easily be transported. They want the imaging modality to come to them. Optics is perfect: Light bulbs are small; they don't emit x-rays; and they're low power." Perhaps light's greatest advantage is its colors. Other imaging techniques rely on contrast agents—such as chemical or radioactive dyes—to make them sensitive to different structures or tissues. With light, "every wavelength you use is a new contrast agent," says Benaron. "You have the ability to gain contrast chemically, to see whether you're looking at hemoglobin, or bilirubin, or to analyze the water and fat content of tissues."

The catch to imaging with optical wavelengths is the obvious one. Shine a light bulb or a laser pulse through a breast, and very little of it will go straight through. Most of the light, considerably more than 99.9999% of it, will be absorbed by molecules or will scatter off cells and cell organelles and at best may end up lighting up the tissue like a dim street light illuminating a dense fog. The challenge of optical imaging is either to eke a signal out of the few photons, known as coherent or ballistic photons, that make it straight through the tissue without scattering, or to reconstruct an image from the deluge of photons that scatter hundreds or thousands of times in the course of a few centimeters. Better yet, says General Electric physicist Deva Pattanayak, is combining the two, "and doing it at multiple wavelengths."

First light. In the late 1980s, Alfano was among the first to try to capture ballistic photons when he sent light pulses through a highly scattering medium—in his case, a glass full of polystyrene beads—and looked for photons that sneaked through without scattering. Alfano assumed that any photons that get a clear shot would arrive earlier than the photons that scattered along the way. And photons that "snake their way through the matter" with only a little scattering, he says, will follow shortly thereafter. "If you capture the early portion of the light, then you get a clear image," he says. The trick was to catch those early photons, which is what Alfano has spent the last 7 years doing.

"We have developed various ways of selecting the earliest portion of the light, the first couple hundred picoseconds [trillionths of a second]," he says. One is a blindingly fast shutter consisting of two pieces of oppositely polarized glass—a combination that ordinarily blocks any light—separated by what's known as a Kerr medium, which reacts to light by changing its optical properties.

To trip this shutter, Alfano sends two pulses of light through the material to be studied. The first is a trigger pulse: It passes through the first polarizer and then hits the Kerr medium, making it birefringent. The result is that for the next few picoseconds, the medium will flip the polarization of any light that passes through it, allowing the light to slip through the second polarizer and in effect opening the shutter. The first part of the next pulse makes it through, but by the time the bulk of the pulse gets to the Kerr medium, it will have stopped being birefringent and the second polarizer will block the light. "We carve out a section of the scatter profile by using this system," says Alfano.

By converting the first few photons of the second pulse into an image, Alfano says he has been able to see droplets of water floating inside a glass of milk with millimeter resolution. To image actual tissue that is thicker than a centimeter or so, he will have to use scattered light as well, which is what he is working on now.

Irving Bigio and his collaborators at the Los Alamos National Laboratory in New Mexico, however, believe they can see deeper into tissues by replacing the pulsed light with a continuous beam, which delivers more photons. The Los Alamos technique sorts out the ballistic photons by splitting the light into two matching beams. One goes through the sample while the other, the reference beam, is routed around it; then the two are recombined and allowed to interfere. "We time the reference beam so that it has

the same path length as the ballistic photons in the probe beam," says Bigio. Only the ballistic photons interfere with it. "Then the scattered photons will have a longer path length, so they will no longer be in phase with the reference beam and therefore will not be able to produce an interference pattern." The interference pattern produced is literally a hologram, which can be read out by a third laser and will show "a shadow image of whatever is inside that scattering medium."

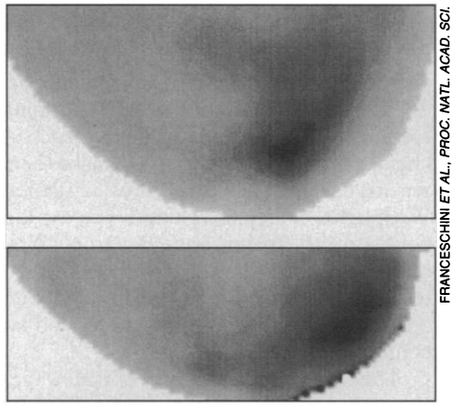
Bigio says that so far he and his colleagues have "generated fairly sharp images of cross hairs, 300 micrometers in diameter, embedded in a scattering medium that is 2 centimeters long and has half the scattering power of real tissue." They hope to optimize the method until they can image a millimeter-sized object through 4 or 5 centimeters of real tissue.

Until then, however, the use of ballistic light is likely to be limited to surface or near-surface imaging. Indeed, one system based on roughly the same principle—but limited to extremely thin slices of tissue—is already in the clinic for biopsies of the retina. James Fujimoto of the Massachusetts Institute of Technology and his colleagues shine light on tissue with a fiber-optic source and gather the photons reflected from the first few hundred micrometers, looking for light that comes back without significant scattering. Like Bigio, he knows he has minimally scattered light when it interferes with a reference beam. The results, says Benaron, are "images of amazing resolution in living tissue, although the depth is limited to probably less than 2 millimeters."

As Fujimoto and his colleagues describe in this issue of *Science*, they now have expanded the concept to general optical biopsy and have figured out a way to use the system in catheters, endoscopes, and surgical microscopes (see Report on p. 2037 and Perspective on p. 1999). "In principle," he says, "you can image at micrometer-scale resolution any part of the body you can access optically through instruments."

Fog light. Getting even coarse resolution at depths of more than a couple of centimeters, however, means exploiting photons that have been scattered so many times that they form a diffuse glow. But this diffusive light can supply surprising amounts of information. The proportion of light absorbed at different wavelengths reflects the chemical makeup of the tissue—fat, water, or blood content, for example—while the deflection or scattering of the light depends on how the cells are organized. For example, the regularly ordered cells in healthy tissues will scatter light differently than will the tumultuous explosion of cells in a tumor. "These different effects can be used to deduce the structure of the tissue you're looking at," says Benaron. "That is the crux of it."

One technique for exploiting diffusive light relies on the time it takes photons in a pulse of light to penetrate the tissue. Fire in a flash of light at a single wavelength, and "what you detect is a pulse of light with a delay over time," explains Benaron. "It's like an echo. It peaks after a period of time and then decays. That curve allows you to separate out how much absorption there is in your sample and where it is, and how much scattering there is in the sample and where that is." The higher the absorption, for example, the faster the signal decays, while the average time the light takes to diffuse through the tissue depends more heavily on scattering. Probing the tissue with multiple pulses at different wavelengths can reveal chemical composition. And by moving the sources and detectors around, the system can gather enough information to reconstruct a three-dimensional image.



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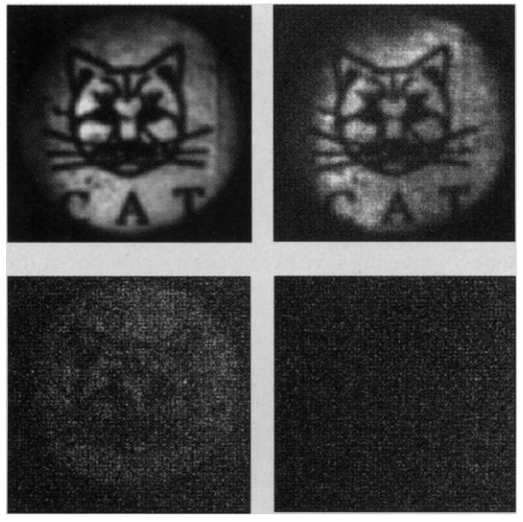
Optical mammograms. Two views of the same breast, derived from pulses of light that diffused through the tissue, reveal a tumor.



Ballistic cat. Capturing the first, "ballistic" photons from a light pulse sent through a turbid medium reveals the outline of a cat, embedded in the material. The image fades when later photons are captured.

Benaron and his Stanford colleagues have created a fiber optic–based head band that allows light to be emitted and detected at 32 locations on the skull. This, he says, is enough to provide a "unique solution that allows you to solve for an image." Because oxygen-starved brain tissue absorbs light at different wavelengths compared to healthy brain tissue, this kind of imaging could allow physicians to watch a patient for a possible stroke during surgery or monitor the effectiveness of drugs designed to restore blood flow after a stroke.

Another diffusion technique also uses light at one or a few frequencies, but modulated in a smooth sine wave. In this case, the key is to watch how the shape of the wave changes as it passes through tissue, explains Arjun Yodh, a Penn physicist who collaborates with Britton Chance: "None of the photons themselves travel more than about a millimeter before they get their direction completely randomized." But the overall pattern is preserved, he says. "If you look at the photon number density, the number of photons per unit volume in this tissue, it is going to vary as a function of position and time."

By measuring the amplitude and the phase of the photon-density wave as it reaches different points on the tissue surface, the researchers can figure out how much scattering and absorption the photons experienced. Mathematically, the analysis is no different from analyzing the scattering of any waves. It's analogous, says Yodh, to watching a wave on a lake scattering from piers and rocks, and then reconstructing the position and the size of the objects from the scattering pattern.

This technique, too, can map tissue oxygenation, based on differences in the absorption patterns of density waves at several different wavelengths. Chance says he and his collaborators can now "make pictures, with a resolution of about a half-centimeter, of regions of the brain, breast, or legs that don't have oxygen and therefore don't function well." They can also detect hemorrhage, because the oxygenation of leaking blood is different from that of blood flowing normally through veins and arteries.

Chance is collaborating with researchers at Baylor University in Waco, Texas, in a clinical study that compares the diffusive-light method with x-ray and computerized tomography (CT) scans on subjects brought into the emergency room with potential brain damage. In Chance's technique, a light source and detector are placed against the

skull and the transmission of light at two wavelengths is measured. The procedure, which takes a few seconds per measurement, is then repeated on the other side of the brain. "What they find is a huge change in differential absorption at those two wavelengths when there's a stroke, or bleeding." says Yodh. The light-absorption signal, measured with a cheap and portable system, could serve as an early warning telling physicians when a CT scan is urgently needed.

The Holy Grail in this field, as Chance puts it, is developing light-based systems that could detect breast tumors and even determine whether a tumor is malignant or benign based solely on its response to light. Many researchers are skeptical that this will ever be done, if for no other reason than because of the near impossibility of getting sufficient resolution out of tissue more than a few centimeters thick.

Both Gratton and Bruce Tromberg of the University of California, Irvine, however, are working on systems to do just that. Gratton says they have demonstrated that absorbed and scattered light can reveal tumors, but "the real question is can we see all tumors?" As for the task of distinguishing malignant from benign tumors, he describes it as "another order of magnitude." Blood oxygenation might be one basis for the distinction, he says; it may be lower in malignant tumors because the tissue is growing faster. The cells' mitochondria—which are more abundant in cancers—could also provide a clue, because the density of mitochondria should affect light scattering. "We're trying to understand fundamentally what it is about tissue that changes" in a cancer, says Tromberg, "and why it looks like it does."

In spite of the technical hurdles, researchers persist because they believe optical imaging will be simplicity itself in practice. Benaron describes one vision: "If someone comes into an office and says 'I have this lesion,' you stick a light probe onto it and image the lesion. And the computer, using the absorption and scattering characteristics, can tell you whether this is normal or a cancer. That's more than just a pipe dream."

–Gary Taubes

BIOMEDICINE

Firefly Gene Lights Up Lab Animals From Inside Out

What better way to look inside your resident lab animal than to put a light source inside it and detect the light seeping out? A team of researchers at Stanford University has done just that by genetic engineering.

In a proof of concept, physician and engineer David Benaron, virologist Christopher Contag, and microbiologist Pamela Contag spliced the gene for luciferase, the enzyme that puts the fire in fireflies, into a salmonella bacterium. The photos below, made with no more than a souped-up video camera, show mice infected with the glowing salmonella. Taken 5 hours apart, they trace the course of the infection when untreated (top pair) and when treated with antibiotics (bottom pair).

A transgenic mouse created by John Morrey of Utah State University represents the next step: an animal with the luciferase gene in every cell of its body. The photo at right shows the glow that appears in the ears of this mouse when the gene is turned on. In this mouse, the luciferase gene is tied to a genetic switch that, in human cells, is activated when HIV, the AIDS virus, is replicating. Mice aren't susceptible to HIV infection, but the Stanford researchers simulated its effect with a chemical known as DMSO, which turns on the genetic switch and, with it, the light. "We can image in the intact animal where and when the gene is activated by watching the lights," says Benaron. He adds that with the right animal model for HIV infection—which is still "a huge step," says Contag—the scheme might be used, for instance, to test HIV drug treatments. "We would no longer have to wonder if the drug is effective in vivo; we could watch the virus replicate and see what happens when we give an antiviral," says Benaron.

He adds that the spatial resolution of the technique is limited to roughly 10% of the depth—which means that a glowing cell 5 centimeters deep can be resolved to within a half-centimeter. Even so, Benaron sees unlimited potential. "You can use bioluminescent approaches to study processes in vivo which cannot otherwise be visualized at any resolution," he says. "You could use it to study gene expression in real time. Want to know when a gene turns off and on during development? Add luciferase. Or evaluate genetic therapy. Right now we have no real-time information on genetic therapy. This would give you a way to track genetic therapies in vivo."

–Gary Taubes

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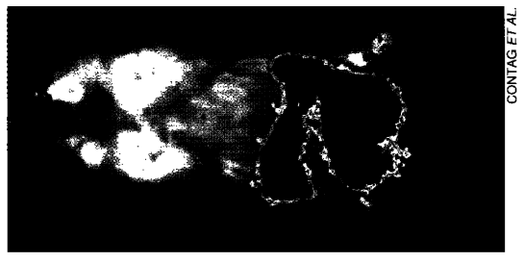

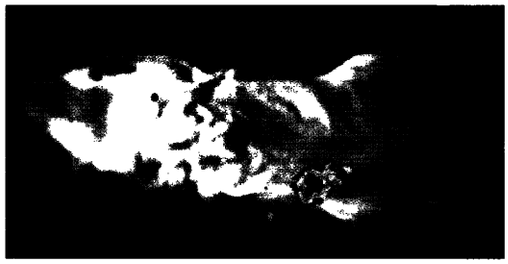

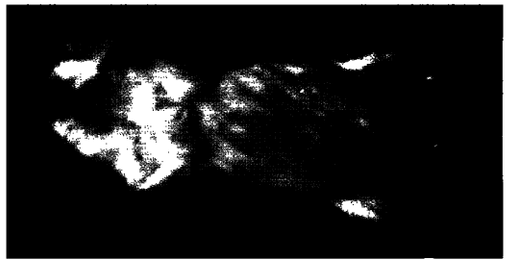

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