ILLUSTRATION: VIKTOR KOEN

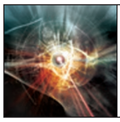
SUPER VISION

USING TECHNIQUES ADAPTED FROM ASTRONOMY, PHYSICISTS ARE FINDING WAYS TO SEE THROUGH OPAQUE MATERIALS SUCH AS LIVING TISSUE.

by Zeeya Merali

It seemed too good to be true, says Allard Mosk. It was 2007, and he was working with Ivo Vellekoop, a student in his group at the University of Twente in Enschede, the Netherlands, to shine a beam of visible light through a 'solid wall' — a glass slide covered with white paint — and then focus it on the other side. They did not have a particular application in mind. "I really just wanted to try this because it had never been done before," Mosk says. And in truth, the two researchers did not expect to pick up much more than a faint blur.

But as it turned out, their very first attempt[1] produced a sharp pinprick of light a hundred times brighter than they had hoped for. "This just doesn't happen on the first day of your experiment," exclaims Mosk. "We thought we'd made a mistake and there must be a hole in our slide letting the light through!"



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But there was no hole. Instead, their experiment became the first of two independent studies[1,2] that were carried out that year pioneering ways to see through opaque barriers. So far it is still a laboratory exercise. But progress has been rapid. Researchers have now managed to obtain good-quality images through thin tissues such as mouse ears[3], and are working on ways to go deeper. And if they can meet the still-daunting challenges, such as dealing with tissues that move or stretch, potential applications abound. Visible-light images obtained from deep within the body might eliminate the need for intrusive biopsies, for example. Or laser light could be focused to treat aneurysms in the brain or target inoperable tumours without the need for surgery.

"Just ten years ago, we couldn't imagine high-resolution imaging down to even 1 centimetre in the body with optical light, but now that has now become a reality," says Lihong Wang, a biomedical engineer at Washington University in St. Louis, Missouri. "Call me crazy, but I believe that we will eventually be doing whole-body imaging with optical light."

RICH SOURCE

It is already possible to peer inside the body with X-rays and ultrasound. But the images produced by such tools are crude compared with those that should be possible with visible light. Partly this is because visible-light images tend to have higher resolution, says Wang. But it is also because optical wavelengths interact strongly with organic molecules, so the reflected light is packed with information about biochemical changes, cellular anomalies and glucose and oxygen levels in the blood.

However, those interactions also make visible light prone to scattering and absorption. Absorption will scupper any imaging attempt: the information the photons pick up is lost as they are absorbed into the material. Scattering, however, preserves a ray of hope. Many materials, such as skin, white paint or fog, are 'opaque' only because photons passing through them ricochet until they are thoroughly scrambled. But they are not lost — so in principle, the scrambling can be reversed.

Astronomers have already solved a version of this scattering problem using a technology called adaptive optics, which allows them to undo the distortions imposed on images of stars, planets and galaxies by the scattering of light in the atmosphere (see *Nature* **517,** 430–432; 2015). The basic idea is to collect light from a bright reference star and use an algorithm to calculate how the atmosphere has smeared and blurred its point-like image. The algorithm then controls a special 'deformable' mirror that cancels out the atmospheric distortions, turns the guide-star image into a point, and at the same time brings other distant objects into sharp focus.

Unfortunately, this technique is tough to use in the body. Targets deep inside biological tissues do not shine the way that stars do — they have to be illuminated from the outside — and the scatterers are much more densely packed than those that scatter light in the atmosphere. "You'd need the equivalent of a deformable mirror with billions of moving parts to compensate for the scattering caused by an egg shell," says Ori Katz, an optical physicist at the Langevin Institute in Paris. That is why Mosk and Vellekoop were not too hopeful of success when they started. Still, the pair took heart from the advance of technology. "Until recently it had been preposterous to think you could control a million pixels, but, by 2007, every smartphone could do it," says Mosk.

They therefore made use of a 'spatial light modulator': a device similar to an LCD smartphone display that can control the transmission of different parts of a laser beam by delaying one part relative to another. They fired their laser through the modulator towards the painted glass slide, placed a detector beyond the slide and used a computer to monitor how much light the detector picked up. The computer then added and subtracted delays at each pixel of the modulator, going through a process of trial and error to see what changes minimized the scattering of the laser light as it passed through the slide. In effect, it was trying to give the incoming light a distortion that the opaque barrier would exactly cancel out. Mosk and Vellekoop ran the algorithm for more than an hour, and when it was done they had a result that beat all their expectations: a focus that was a thousand times more intense than the background signal[1].

"The Mosk experiment was an eye-opener," says Katz. "It changed the paradigm of what could be done with optical light."

Soon after his succcess, Mosk learned of similar work being done by bioengineer Changhuei Yang and his team at the California Institute of Technology in Pasadena.

These researchers had used a different technique to focus scattered optical light, and a different opaque substance: a thin slice of chicken breast[2]. But they, too, were surprised by how easy it was to do. "I had thought 'we'll spend six months on this, and when it doesn't work, we'll chalk it up as a learning experience'," says Yang. "But actually it wasn't that hard."

Soon after the two papers were published, the field exploded as other physicists rushed to join in. One of them was optical physicist Jacopo Bertolotti, who came to work with Mosk in 2010. Bertolotti, now at the University of Exeter, UK, says that he was drawn both by the "beauty of the experiment" and by the potential it offered for medical imaging. But he could see that that goal was still a long way off.

> "CALL ME CRAZY, BUT I BELIEVE THAT WE WILL EVENTUALLY BE DOING WHOLE-BODY IMAGING WITH OPTICAL LIGHT."

The first issue that Bertolotti faced was that Mosk's original set-up required a camera to be placed behind the opaque surface. That is a problem for medical applications because placing a camera under the skin would involve surgery, which would be invasive, dangerous and rarely worth the risk. In 2012, however, Bertolotti, Mosk and their colleagues devised a way to put both the laser light source and the detector in front of the surface[4].

Their target was a fluorescent Greek letter π just 50 micrometres across hidden behind a thin opaque screen. As such, the target was roughly the same size as a cell and analogous with medical techniques that involved injecting fluorescent dyes into living tissue to aid in imaging. When the laser was switched on, the photons would bounce their way through the screen and produce a diffuse illumination of the fluorescent π. The light reflected from the letter would then make its way back through the screen and produce a blurry speckled pattern on the other side. It was like trying to see the symbol through a shower curtain.

Yet the shape of the letter was still encoded in the scattered light. To retrieve that shape, the team recorded the speckle pattern, moved the laser to shine at a different angle, then recorded the new speckle pattern[4]. By repeating this many times and comparing the patterns point by point, a computer could work out how the patterns were correlated — and from that, work backwards to reconstruct the hidden letter π.

That was progress, says Bertolotti, but it still was not good enough. "It only works if the object to be imaged is on the other side of the scattering medium," he says. For many medical applications, such as seeing inside the brain, or within a blood vessel, the target is buried within tissue.

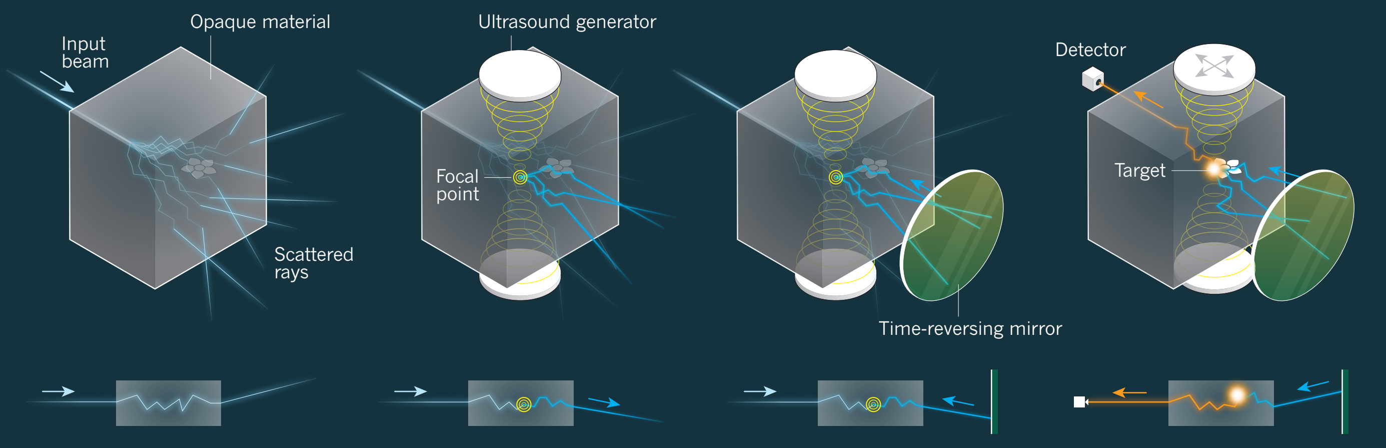
INSIDE OUT

The challenge of imaging inside the scattering medium has been taken up by a number of groups, including Yang's and Wang's. In 2013, for instance, Yang's team achieved this feat with unprecedented resolution by picking out a fluorescent bead just one micrometre across sandwiched between two artificial opaque layers[5].

Yang, together with biologist Benjamin Judkewitz and the rest of his team did this by illuminating the medium and letting the light bounce its way through to the other side, then reflecting it back with a 'time-reversing' mirror, which effectively forces every light ray to exactly retrace its steps. Time-reversing all the rays would simply undo all the scattering, however. So instead, the team focused an ultrasound beam — which is not easily scattered — at one point in the medium, knowing that any optical light that happened to pass through that point would

LIGHT AND SOUND

One way to see inside opaque materials is to combine ultrasound with a 'time-reversing' mirror system, which forces every light ray to exactly retrace its steps.



SCRAMBLED LIGHT

In many opaque materials — including living tissue, white paint and fog — light is not actually absorbed. It simply bounces around in the material until it is too scrambled to form an image.

ULTRASOUND FOCUS

A beam of ultrasound (yellow rings) is focused at some point within the material. Any light that happens to bounce through this point will undergo a slight shift in its frequency (blue rays).

TIME REVERSAL

A time-reversing mirror sends back only the frequency-shifted light. When the light retraces its steps, it passes through the ultrasound focal point and adds its energy to the light coming through on the first pass.

SCANNING THE MEDIUM

The focal point is scanned through the material. When it passes over targets labelled with a fluorescent dye, the structures emit a detectable glow — and researchers can build up a map of the interior.

JASIEK KRZYSZTOFIAK/NATURE

undergo a slight shift in frequency. Then on the far side, the researchers set up the time-reversing mirror tuned so that it would send back only the light that had experienced that frequency shift. The result was a thin, time-reversed beam that would automatically pass back through the focus and add its energy to the light from the first pass. This turned the ultrasound focus into a spot of comparatively high radiation intensity — "a torch inside the wall", says Judkewitz, who is now at the Charité University Hospital in Berlin. Better still, the ultrasound focus could be moved around within the medium. And when it passed over the bead, the bead fluoresced (See 'Light and sound').

However, the technique was still a long way from seeing into deep layers of tissue, which pose another, much tougher challenge: they tend to move constantly as a result of blood flow and breathing. "We are still not so close to medical applications because these techniques tend to work only if the scattering medium is perfectly frozen in time," says Mathias Fink, a physicist at Langevin who pioneered a version of the time-reversal technique in the 1990s that used ultrasound alone[6]. Most groups have reduced the timing from Mosk's original hour or so to just tens of seconds, says Katz, and that is fine for imaging a bead or a letter π, but not for imaging a tumour in the body.

But last year, a team led by Sylvain Gigan, a physicist at the Kastler Brossel Laboratory in Paris, and including Katz and Fink, demonstrated a way to reconstruct the image of the hidden object in just one camera shot[7]. "It's a bit like magic when you see the algorithm converge on the final image," Gigan says.

Wang agrees that speed is of the essence. "Everything is in motion and we only have a millisecond-scale window to make an image," he says. In a paper published in January[3], Wang and his team managed to get the speed down to 5.6 milliseconds, "which is fast enough for selected *in vivo* imaging", he says. Furthermore, their target was made from ink-stained gelatin and sandwiched between the ear of an anaesthetized mouse and a ground-glass diffuser. Getting success with a live mouse is impressive, says Bertolotti — although he points out that "moving from a mouse ear, which is relatively thin, to imaging human skin and flesh will still take a lot of extra work".

As of today, Bertolotti adds, there is still no imaging approach that stands out above the rest. Each has its advantages and disadvantages. "Rather than developing one technique that's good for everything, I think we'll develop a suite of techniques that could one day all be combined into the same piece of apparatus," he says. "I don't know how quickly that might happen, but this is a young and fast-moving community, so it could be within a few years."

The techniques now being pioneered by bioengineers and physicists for medicine could also be put to a range of other purposes. Mosk, for example, believes that these methods could be a tool for art restoration. "Most painters build up works in several layers, and the layers below can influence the chemical and physical ageing of the painting, so it's of some significance that you know what is in there if you want to preserve it," he says. Methods that in effect unscatter light could also help the telecommunications industry to unscramble the noise in optical fibres that is caused by scattered light.

Another obvious customer is the military, says Fink, who thinks that the technology could be used to allow soldiers to see through a portable shield — either a physical screen or a fogging spray — that obscures them from their enemy's view. "It's not the same as being invisible, but it would allow you to see others while not being seen," he says.

Almost all the scientists in this young field get excited when they start dreaming of applications. But Gigan, for one, is keen to keep the applications above board. "When we tell people what we do, someone always asks if we'll create a phone app to let people look through shower curtains," he says. "This is something that could be done with our technique — but we don't intend to do it." ■

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