The most transparent research

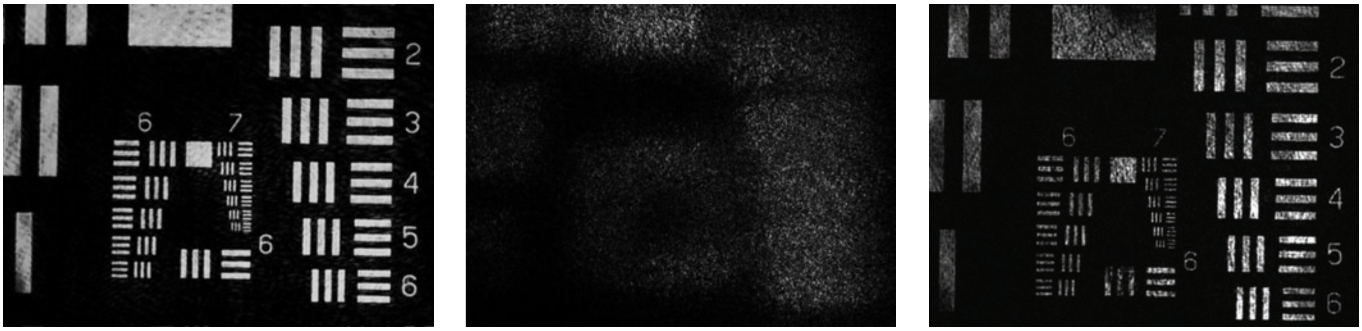
Biomedicine would be a breeze if organisms were transparent. With the ability to see through tissues, scientists could spot the development of tumors more easily in study animals. And biologists could study exactly how an animal's organs develop by observing them as they grow. In effect, the secrets of the body would be out there for everyone to see.

The thought of peering into our tissues may sound like science fiction, but one day it could be science. Using ideas from genetics, electrical engineering, chemistry and solid-state physics, a handful of researchers are working on ways to render biological tissues transparent.

Some have already succeeded: in 2007, Richard White, a biologist at the Dana Farber Cancer Institute in Boston, used careful breeding techniques to create a transparent adult zebrafish named *casper*, evoking a reference to the famous cartoon ghost by the same name. Now, more than 100 labs around the world are using these transparent fish to study cancer pathology and development in real time. "The field of *in vivo* imaging—looking at things that are happening inside an actual organism—is growing rapidly," White says.

Researchers are even making strides toward turning human tissue transparent. The primary reason we can't see what's inside of us is that light scatters when it passes through tissue. The body is densely packed with many types of substances, such as bone and fat, and light travels through them at different speeds because they have what physicists refer to as different refractive indices. The result is that light can't pass through biological tissues in a straight line, much as car headlights don't pass through dense fog. To fix this problem, scientists are working on developing ways to stop tissues from scattering light. Indeed, "if you take away the scattering properties of human tissues, we would look more or less like jellyfish," explains Changhuei Yang, an electrical engineer and bioengineer at the California Institute of Technology.

Though their approaches (described in the following pages) are diverse, these researchers share the common goal of making it one day possible to see what's going on deep inside of the body—a feat that would provide new insights into our biology and help doctors diagnose and treat disease much more easily. "We're trying to push the limits in terms of what can we uncover," says Bernard Choi, a bioengineer at the University of California–Irvine.



Zahid Yaquoob

In Changhuei Yang's experiment, the test pattern is seen through clear agarose gel (left). The test pattern is then obstructed by a 0.5 mm-think piece of chicken tissue (middle). Finally the image of the test pattern could be seen after passing scattered light back through the 0.5 mm-think piece of chicken tissue (right).

Hope in holograms

In 1964, University of Michigan electrical engineer Emmett Leith and his colleagues invented the hologram—a snapshot of how light scatters when it passes through an object. He made his first hologram, an image generated by a toy train and bird, by shining multiple lasers through these objects and recording their interference patterns on a holographic plate. The plate displayed the three-dimensional characteristics of the items on the basis of how the light had traveled through them.

Leith then took the hologram one step further. He decided to try to use it to reverse the effects of light scattering. He knew that light scatters in a deterministic fashion—it always passes through a static object the same way—and thus must be reversible. Leith made a hologram that recorded how light scattered through a piece of glass with a ground matte surface, and then he sent light back through the hologram from the other side so that it retraced its steps through the glass. The scattered light beams converged when they passed through, exiting the other side as a single beam.

Now, decades later, Caltech's Changhuei Yang is attempting to use this technique to eliminate scattering in biological tissues. It's a tall order, as biological tissues are not static—cellular constituents move around constantly, so light scatters differently over time. But still, "I thought it was worth a shot," Yang says.

In an experiment published last year, Yang and his colleagues shone a green laser through a sliver of chicken breast 0.69 mm thick and used a holographic crystal to record how the light scattered (*Nat. Photonics* 2, 110–115; 2008). (Although the chicken tissue is dead, its cell constituents still move around slightly.) He then passed the light back through the hologram on the other side, so that it retraced its original path back through the tissue.

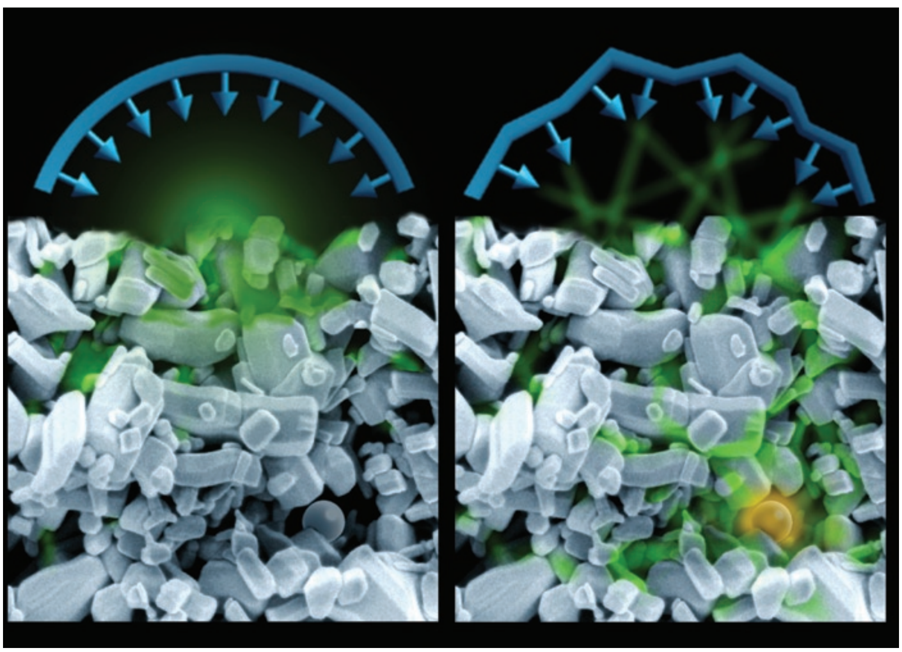
"I didn't expect to get anything out of it," Yang says—so he was shocked that his technique boosted the amount of light transmission through the breast back to the eye from 5% up to 20%. In other words, the end result of this light manipulation was that the chicken sliver appeared partially transparent, making it possible for a person to see images displayed behind it.

Yang now plans to attempt the experiment with thicker pieces of chicken tissue (chicken meat works well because it is relatively homogeneous). One way to do this is to use longer wavelengths of light, which do not scatter as much.

Researchers say that this approach might be applied to allow microscopes to see beneath the first few layers of cells. Yang also hopes that by adjusting the position of the hologram he could one day see through surface of skin. This could open up possibilities to detect biochemicals, such as glucose, in the blood, he claims. And Yang adds that, by using this technique to reverse light scattering, scientists might one day help improve existing medical therapies. For example, in photodynamic therapy, doctors tag cancerous tumors with compounds that release poisons when illuminated with light. Currently, photodynamic therapy is primarily used on superficial tissues, because the light cannot penetrate far enough to reach deep tumors; Yang's hologram approach could change that.

Channeling light

While Yang seeks to reverse the effects of light scattering, Allard Mosk, a physicist at the



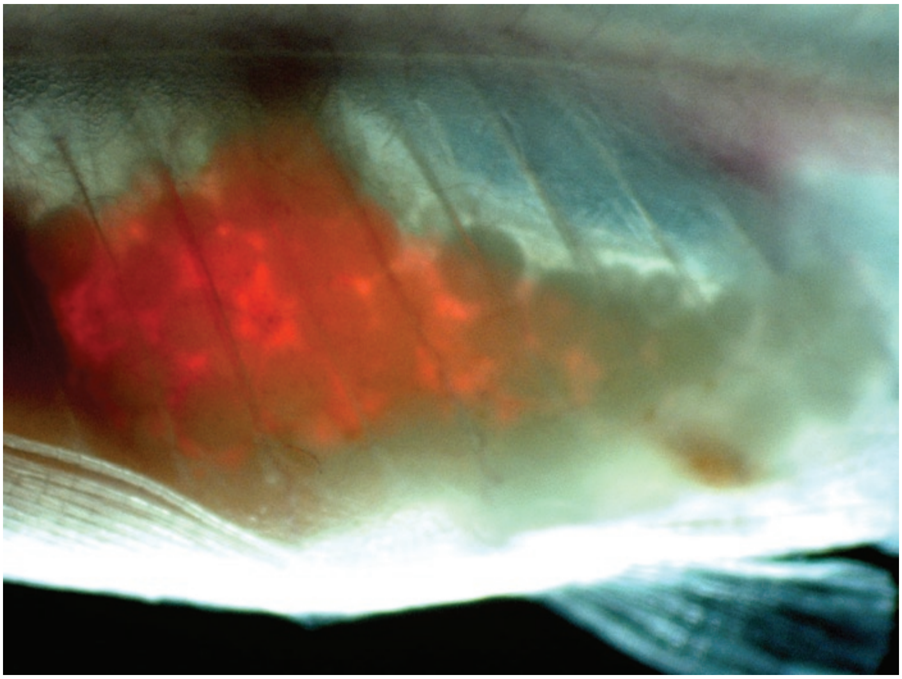
Ivo Vellekoop

A cartoon depiction of how modifying the speed of light waves can sync them up to make fluorescent probe visible beneath an opaque surface, as shown by Allard Mosk's work.

University of Twente's MESA+ Institute, is trying to prevent scattering from occurring in the first place. His approach stems from a theory in solid-state physics known as random matrix theory. It predicts that although most light waves will not pass through opaque materials, some miraculously manage to "pass through completely unhindered" through what are called open channels, he explains. It should theoretically be possible, he says, to manipulate additional light waves to find their way through these open channels too.

Biological tissue is comprised of a conglomeration of materials with various refractive indices, so individual light waves take varied paths and come out the other side of the tissue—if they ever do—at different times. Mosk records the information about each transmitted wave's path and then mathematically figures out how to compensate for it, modifying each wave's starting speed by modulating the light emitted by specific pixels in a liquid-crystal display (LCD) so that they all arrive together. It's as if he observes a track race, records when each contestant crosses the finishes line, and then re-starts the race after staggering each person's starting point—so that they all end up crossing at the same time.

In a recent study, Mosk used this approach to pass light through an extremely opaque material; this made it possible to see a fluorescent probe embedded 9.7 micrometers underneath the opaque layer, increasing the amount of light that hit it by 21-fold as compared with unmanipulated light (*Opt. Express*. **16**, 67–80; 2008). Mosk, who has so far tested his technique in chicken breast tissue, emphasizes that his technique still remains in the arena of physics, not biomedicine. He envisions that it, too, could improve photodynamic therapy by allowing light to pass through the skin more easily. But, to use his technique in living tissue, which is not static, Mosk anticipates he would need to speed up the light-modification process he uses by a factor of 100 to 1,000.



Richard White

A close-up of the *casper* zebrafish

Ghostly fish

When Richard White, a biologist at the Dana Farber Cancer Institute in Boston, first laid eyes on the mutant zebrafish *roy orbison*, he knew it would be valuable—and not just because of its namesake. The fish, which has big black eyes—"somebody thought it looked like Roy Orbison wearing his big black sunglasses," White says—was also partially translucent. "We said, if we cross that to other pigment mutants, maybe we could make one that's markedly more translucent," he recalls.

In late 2007, White succeeded. He bred *roy* with another mutant zebrafish, *nacre*, and created a nearly transparent fish he named *casper*. Wild-type zebrafish are excellent research models because their genes more closely match ours than mice or yeast genes do—and zebrafish also develop outside of the womb and are transparent as embryos, so scientists can easily use them to study development. *casper*, however, is the world's first line of transparent adult zebrafish.

White uses *casper* to study cancer development. He has been able to observe individual tumor cells as they metastasize or move from their primary tumor to a second location in the body. He has also developed ways to label tumor cells with fluorescent tags such as green fluorescent protein (GFP) so he can watch them move and change in real time under a microscope. He has crossed *casper* with other transgenic zebrafish, too, to incorporate fluorescent markers in various organs. "We have fish where the blood stem cells are green; the vasculature is green; the kidney is green," he says—and since the fish are otherwise transparent, these body parts are all visible to the naked eye.

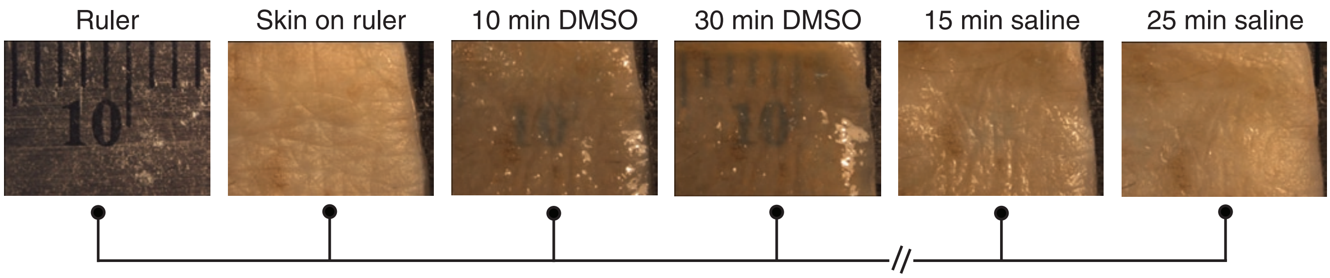
White has sent *casper* embryos out to more than 100 scientists around the world—there is no patent on the fish—who are using it to study various aspects of biomedicine, such as kidney disease, ear development and the effects of small molecules on cancer development. "Word travels fast when there's a pretty hot new tool being developed for the research community," says Richard Klemke, a cancer biologist at the University of California–San Diego, who is using *casper* to study blood vessel formation and cancer progression.

White's next goal is to make *casper* even more transparent and also to engineer other types of transparent organisms, although he has not yet decided what kind. "Studying things inside a living animal is probably as important as, if not more important than, what we learn from tissue culture," he says.

More than skin deep

In 1993, researchers at the University of Texas at Austin noticed that when they dehydrated a piece of human aorta using a hyperosmotic agent—a chemical that pulls water out of tissue—it became partially transparent. Since then, a handful of researchers, including Gracie Vargas—who was a PhD student in the Texas lab at the time and is now a biomedical engineer at the University of Texas Medical Branch—have turned their attention to making other types of tissues transparent using the same approach.

In a landmark study Vargas published in 1999, she added glycerol, a hyperosmotic agent, to a flap of 1.45-mm–thick hamster skin under a microscope and found that it reduced scattering by a factor of four and increased light transmittance through the skin by 50%—to the point where it was possible see through to patterns of lines placed behind it. The effect, she found, was reducible, as the tissue became opaque again—and appeared

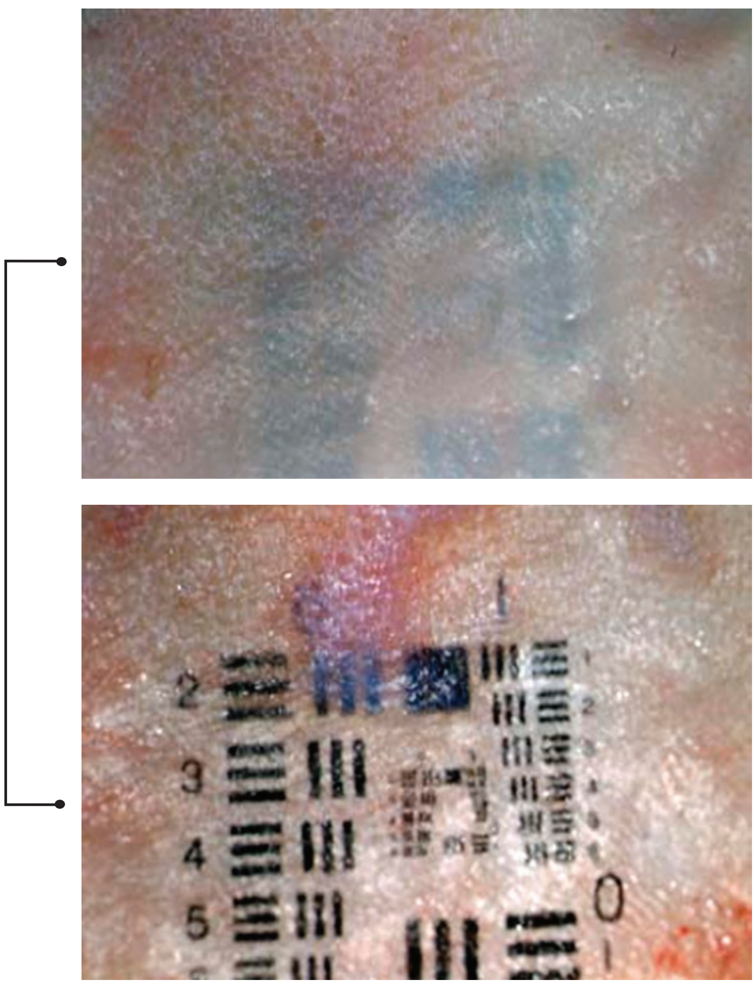


Bernard Choi

This timeline shows DMSO-treated human skin samples from Bernard Choi's lab. The ruler is visible behind the skin after 30min; afterwards the effect is reversed.

to function normally—20 minutes later after they rehydrated it with saline.

No one knows for sure how these agents work their magic, but there is probably a combination of reasons. Water has a very different refractive index than most tissue constituents, so when it is drawn out of the tissue, a type of 'index matching' may occur among the remaining constituents that reduces overall light scattering. In addition, research by Bernard Choi, a biomedical engineer at the University of California–Irvine who focuses on the hyperosmotic solvent dimethyl sulfoxide suggests that the tissue dehydration causes some of the components of collagen, a protein commonly found in biological tissue, to disassociate, which may further reduce scattering.



Gracie Vargas

These images from Gracie Vargas's lab show a hamster skin sample before (top) and after 20 minutes of treatment with glycerol.

This 'optical clearing' technique, as it is called, could perhaps eventually be used to permanently treat birthmarks such as port wine stains, which are caused by raised, discolored capillaries. Normally, doctors treat such blemishes with pulsed laser radiation, which destroy the aberrant blood vessels, but such treatments are often only partially successful, because the laser cannot penetrate deeply enough into the tissue. In a paper Vargas published last year, she found it was possible to treat such lesions using 16 times less incident laser power if the tissue surrounding the birthmark was treated with glycerol first (*J. Biomed. Opt.* doi:10.1117/1.2907327; 2008).

Vargas envisions that optical clearing could also help doctors monitor the biological signatures of various diseases, as tissues often respond to light differently after disease develops. For example, cancer tissues have high levels of porphyrin, a compound that binds iron and absorbs specific wavelengths of light. The idea is that doctors could make the first few layers of skin transparent to more easily visualize deeper tissues.

Ultimately, there are far more questions than there are answers about how to safely and reproducibly render biological tissues transparent. For instance, no one knows how transparency itself might affect biological processes. "It gets to this question of why are things *not* transparent?" White says. Will *casper* lead a different life because it is transparent, or will visualizing our tissues somehow interfere with their biological processes?

No one knows. But if this motley crew of scientists can overcome these hurdles and unknowns—whether through physics, chemistry or biology—then science could one day have an incredible tool at its fingertips. "We are optimistic," Yang says.

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