Sounding out photons

Photoacoustic imaging, using laser light to stimulate the emission of ultrasonic waves from tissue inside the human body, potentially offers a route to far deeper imaging than possible with conventional optical techniques, reports *Duncan Graham-Rowe.*

Bouncing light off biological tissue has become a mainstay of modern medical imaging and microscopy. But most existing techniques are limited in their ability to penetrate the body by more than just a few millimetres. However, a technique called photoacoustics, a marriage of optical and ultrasonic technologies, could be about to change the situation.

The idea behind photoacoustics, which is also known as optoacoustics, is simple: use light to stimulate interior tissue so that it gives off acoustic waves in the ultrasonic range. These waves can be then be detected using wide-band ultrasonic transducers and used to build up high-resolution images of subsurface tissue structure (Fig. 1).

"We want to reach what's called super depth," says Lihong Wang, at Washington University in St Louis, Missouri, USA, and one of the most active researchers in the field of photoacoustics. The hope, he says, is that by using photoacoustics clinicians will be able to carry out safe, high-resolution three-dimensional imaging and microscopy at depths of centimetres rather than millimetres, and without the use of potentially harmful ionizing radiation, such as X-rays.

In addition, photoacoustics should open up new opportunities for diagnosing, monitoring and treating diseases. For example, it could help to guide biopsy needles deep beneath the skin, assist endoscopic techniques for diagnosing gastrointestinal cancer, measure oxygen saturation levels in haemoglobin and study subsurface vascular and lymph nodes to visualize and quantify malignant tumours. It can even be used to probe the brain and to monitor gene expression.

Although the latest photoacoustic apparatus make use of state-of-the-art laser technology, the first examples of using light to stimulate acoustic waves date back to the nineteenth century. As far back as 1880, Alexander Graham Bell discovered that it was possible to make a thin disk emit sound when exposed to a beam of pulsing sunlight. Initially Bell sought to use the effect as a means of communication, converting sound into the light, sending it through free space and then converting it back into sound again. "He called it the photophone," says Wang. Needless to say, his other idea, the telephone, proved a more popular invention, not least because it didn't have issues with line-of-sight.

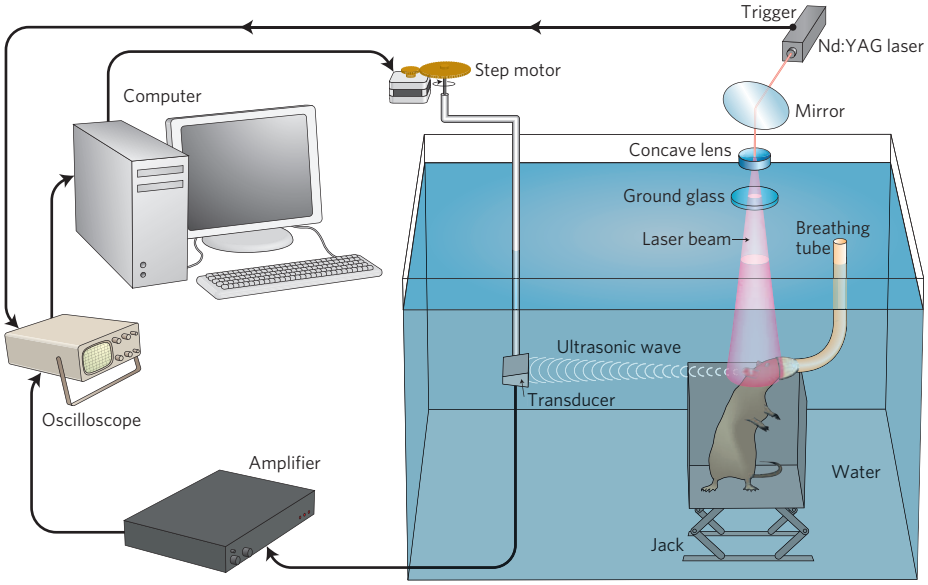

Figure 1 | Schematic of a photoacoustic system for imaging the brain of small animals. Systems can also be equipped with a microscope to allow imaging of smaller target areas. The sample is immersed in water to allow high-quality collection of the ultrasonic acoustic waves. Adapted from ref. 1. © 2003 NPG.

After that, photoacoustics was largely ignored until the 1970s when the development and availability of lasers triggered an interest in its use for non-destructive testing. But it wasn't until the late 1980s that its medical applications started to become apparent. Initially interested in the effects of laser absorption on tissue, Alexander Oraevsky, then at the USSR Academy of Sciences, Moscow, started to look at how the interaction could be used for imaging — a technique he dubbed optoacoustics.

His initial experiments showed that cells produced pulses of ultrasound in response to the pulses of laser light. Oraevsky left Moscow in 1991 to continue his work at the University of Texas, and has since become vice president of research and development with Fairway Medical Technologies, in Houston, Texas.

Fairway, with its commercialization partner, Seno Medical Instruments of San Antonio, Texas, is one of a handful of companies now developing the technology for real-life applications.

Oraevsky explains that as light passes through the tissue certain wavelengths are preferentially absorbed by cells. The absorbed energy causes a very small amount of heating that makes the cell swell. This so-called thermoelastic expansion produces acoustic pressure waves that can then be detected by placing ultrasonic transducers on the skin.

But to get really useful high-resolution imagery requires lasers capable of emitting nanosecond pulses, says Oraevsky. "You have to use a short enough pulse to ensure that the energy is delivered before it can escape as pressure," he says. Wang agrees. Nanosecond pulsing, along with lasers capable of high spectral purity, is really necessary if you want to obtain very high spatial resolution, says Wang.

What makes photoacoustics different from other three-dimensional imaging techniques — such as optical coherence

Table 1 | Comparison of various forms of medical imaging techniques

Technique	Contrast mechanism	Spatial resolution	Maximum depth penetration
Confocal microscopy	Scatter, fluorescence	~0.2 μm	~0.5 mm
Two-photon microscopy	Fluorescence	~0.2 μm	~0.5 mm
Optical coherence tomography	Scatter, polarization	~10 μm	~1 mm
Magnetic resonance imaging	Proton density	~1 mm	~200 mm
X-ray computed tomography	Electron density	~0.1 mm	~200 mm
Ultrasound	Scatter	~1 mm	~100 mm
Photoacoustic microscopy	Absorption	~2–200 μm	~1–30 mm
Photoacoustic computed tomography	Absorption	~0.8 mm	~50 mm

DATA COURTESY OF LIHONG WANG

tomography (OCT), two-photon microscopy and confocal microscopy (see Table 1) — is that it relies on light being absorbed rather scattered. One of the reasons that these other techniques are so limited in how deep they can delve is that back-scattered light is diffused by the tissue, making it difficult to detect in any meaningful way.

"They rely upon ballistic photonics," says Wang. But typically a photon can only pass through about 0.1 millimetres of tissue before it will be scattered, making it difficult to go much deeper than a millimetre, he says.

The problem is not the source laser, says Wang. If the wavelength is carefully chosen, the laser light has no problem penetrating deep into the tissue; the challenge is getting a signal out without scattering. But for ultrasound waves this is not a problem, he says. "When we convert light into sound we get minimal scattering, so we can penetrate deeper," says Wang. "The deepest we have demonstrated is 5 centimetres, but some colleagues claim to have gone deeper, up to 7 centimetres."

Besides going deeper, it is also much safer than other forms of imaging such as X-rays because it doesn't involve using ionizing radiation, says Wang. And only a minimal and quite safe amount of heating of cells is necessary. For every millidegree Celsius rise in temperature you get around 8 millibars of pressure, he says. Ultrasonic transducers are sensitive down to about 1 millibar or better, so by raising the temperature of tissue by about 100 millidegrees Celsius you can receive a strong signal.

For imaging applications, detection is usually carried out using an array of wide-band ultrasonic transducers. The process of pinpointing the source (origin) of the acoustic wavefronts is performed by measuring the travel time of the ultrasonic waves at multiple positions and then using triangulation.

"But we don't have a single point source, we have a continuous volumetric source, because there is more than one point absorbing the light," says Wang. So within the array are sets of transducers capable of detecting different ranges of acoustic wavelengths. Each transducer typically measures just a few millimetres in size, and 128 or 256 transducers are typically used in total.

The type of tissue molecules from which the acoustic signals can be detected depends strongly on the wavelength of the source laser, so for this reason tunable lasers are commonly used. Different tissue types will absorb different wavelengths of light, making them act like endogenous (natural) contrast agents, says Michael Thornton, Chief Operating Officer of Endra. The firm was set up in 2007 by Boston-based life science research technology company Pure Venture to develop photoacoustic imaging technology and is in the process of moving to Ann Arbor, in Michigan.

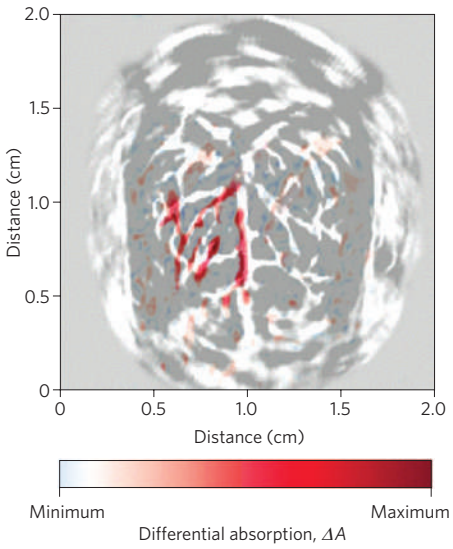

Figure 2 | *In vivo* functional photoacoustic image of the cerebral haemodynamic changes (shown in colour) in response to whisker stimulations acquired non-invasively in a small animal, where the morphology of blood vessels is shown in grey scale. Reproduced with permission from ref. 1. © 2003 NPG.

Examples of a natural contrast agent are oxygenated and deoxygenated haemoglobin, says Wang. By using photoacoustic tomography to target these chromophores it is possible to quantify and image oxygen saturation of haemoglobin and its levels of concentration (Fig. 2). This is important because it enables a high-contrast picture containing functional information to be built up showing, for example, the formation of new blood vessels (Fig. 3) and metabolic levels. "These two physical parameters are really important because they are hallmarks for cancer," says Wang.

Because blood is so good at absorbing certain wavelengths it produces correspondingly strong acoustic waves, says Oraevsky. As a result the optical contrast between normal and cancerous tissue (Fig. 4) is substantially greater than with conventional imaging methods, he says.

Indeed, Oraevsky's team have been able to image whole mouse bodies to study blood flow and tumours. They have also performed preliminary clinical work to assess the technology's ability to distinguish between malignant and benign breast cancers, with resolutions down to about 500 micrometres. The kind of laser used depends very much upon what you are imaging, he says. To image breast tissue a 757-nm Alexandrite laser is used with 50-ns pulses, whereas to image the small blood vessels of mice a 1,064-nm Nd:YAG laser emitting 10-ns pulses is needed, he says. Because these pulses are repeated at a rate of 10 a second it is possible to get real-time images that can be played like a movie, says Oraevsky.

Another application being explored for photoacoustic imaging is to detect contrast

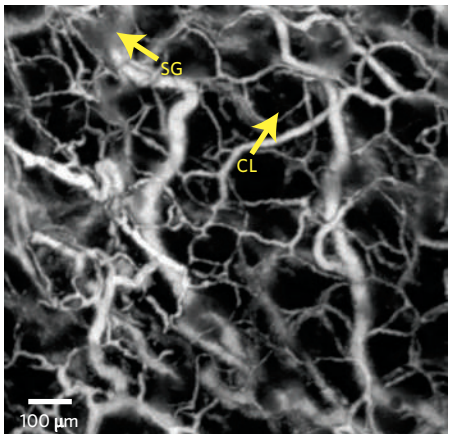

Figure 3 | *In vivo* photoacoustic image of blood vessels including single capillaries in a small animal, acquired non-invasively with an optical-resolution photoacoustic microscope. CL: capillary; SG: sebaceous gland. Reproduced with permission from ref. 2. © 2008 OSA.

agents that are designed to target specific biomarkers, such as for cancer cells. This is one area that Endra is looking at as a means of developing new pharmaceuticals, says Thornton.

Similarly VisualSonics, a leading ultrasonic imaging company based in Toronto, Canada, has been evaluating the technology for detecting cancer targeting contrast agents, says Stuart Foster, founder and Chief Scientific Officer of the company, and professor of medical biophysics at the University of Toronto. Ultrasound is already being used to detect such agents, but there are limitations, he says. Normally these agents are confined to the blood vessels. But VisualSonics is now looking at using the high-contrast properties of photoacoustics to target gold nanorod agents which have the potential to pass through the epithelium of the vessels and bind to specific targets on cells. "We're using this optical approach to get a bit further into the tissue," he says. "It's a natural progression for us, and a way for us to report on a much larger range of molecules."

Indeed, according to Wang a variant of this approach even has great potential to monitor the genetic activity of cells. "We can image gene expression for reporter genes," he says.

But despite its merits photoacoustics is no magic bullet, says David Steinberg, Endra's CEO. Although it is possible to do whole body imaging of mice, for humans this is a bit more challenging. "As the light propagates deeper you get less and less light, and so less pressure," says Wang. Also, the lungs and airways can play havoc with the ultrasound, he says. "Air cavities will give you almost insurmountable problems."

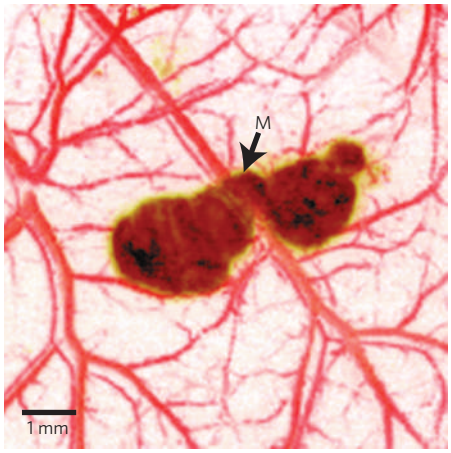

Figure 4 | *In vivo* photoacoustic image of a melanoma and the surrounding blood vessels in a small animal acquired non-invasively with a 50-MHz photoacoustic microscope. Reproduced with permission from ref. 3. © 2007 NPG.

Bone presents similar difficulties, but despite this there is great interest in using photoacoustics for neuro-imaging, says Foster. "The light will pass through the skull," he says. The problem is that the ultrasound becomes aberrated as it passes back out. This is not an insurmountable problem, says Wang, who is now working on a technique to 'de-aberrate' these signals by modelling the skull's properties.

Currently there are no products on the market yet, although Endra plans to make its platform commercially available for clinical research laboratories possibly later this year. But photoacoustic imaging is potentially so cheap and easy to use that eventually it is likely to find its way into clinics.

"It's not a proven technology but it's a technology with phenomenal potential, and that's why it needs to be explored," concludes Foster. ❐

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