Biophysics: using sound to move cells

Vivien Marx

Moving and sorting cells with sound are a few of the possible applications for this no-contact technique.

Sound is music to the ears of some bioengineers and device developers. Sound waves can be put to work as acoustic tweezers to trap, move, sort and manipulate cells and particles in fluidic environments[1]. But those are not the only types of lab applications possible with sound.

"Acoustic tweezers can be used way beyond cell sorting," says Pennsylvania State University bioengineer Tony Jun Huang. Potentially, acoustic methods could help to create 'smart' Petri dishes with specially patterned substrates for tissue engineering or single-cell assays to allow researchers to better study cell-cell interaction and signaling. Sound pulses can transfect cells or move nanoparticles *in vivo* in model organisms. The marriage of acoustics and fluid dynamics promises acoustofluidic devices.

"One's imagination is the limit," says K. Kirk Shung, a biomedical engineer at the University of Southern California, about potential biomedical applications of acoustic tools. There are several types of acoustic tweezers (**Table 1**). These are nascent but maturing technologies, and academics and company researchers believe that advanced acoustics-based applications are on the way.

The force that drives this trapping, patterning or tweezing is technically known as acoustic radiation force. "It's all down to the momentum carried by the sound waves, like that of a breaking water wave that you can surf," says Bruce Drinkwater, a mechanical engineer at the University of Bristol.

An advantage is that these forces work on any material: cells of all types, beads, microbubbles or tags, says Drinkwater. This is not true for optical tweezers because objects to be moved in that case have to be transparent. Optical tweezers also require a complex optical bench and special laser-safe rooms, and the deployed lasers can damage the objects being moved. Magnetic or electric fields are easier to apply for moving or manipulating cells, but the former require magnetically labeled objects, and the latter must be used in low-salt conditions, a nonphysiological environment for cells.

Amplified idea

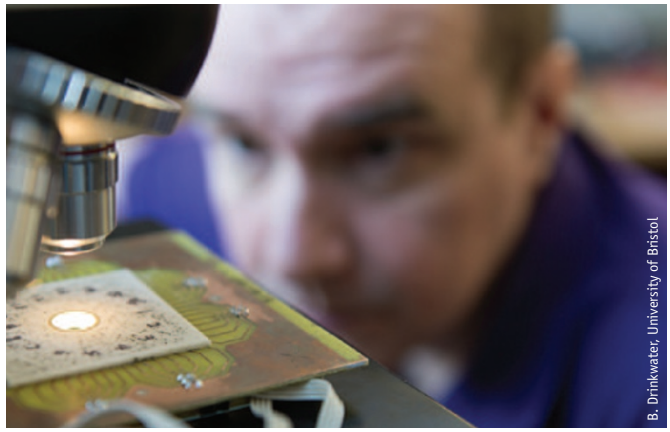
Captivated by the way optical tweezers[2,3] use light to hold objects in place or move them, Drinkwater decided he wanted to build an ultrasonic equivalent. With his colleague Sandy Cochran of the University of Dundee, in a café next to the town hall of Northampton, UK, he hatched a plan that led to a public-private consortium, called Sonotweezers, devoted to helping life scientists use sound to manipulate microparticles. Drinkwater and Cochran reached out to University of Southampton bioengineer Martyn Hill, who works on lung tissue and cartilage, and to Mathis Riehle at the University of Glasgow, whose research is on neurons and cell patterning.

With a budget of £4 million (6.3 million US$), the Sonotweezers initiative ran from 2009 to 2013 and included labs at the Universities of Bristol, Dundee, Glasgow and Southampton in the UK; the UK's Defence Science and Technology Laboratory; the Fraunhofer Institute for Ceramic Technologies and Systems in Germany; and six companies.

Sonotweezers kicked off the field in the UK, says Drinkwater. The sizeable grant and industry involvement were important for the project to gain traction and credibility. Although the program has ended, collaborations and projects continue. "This is important as we pretty much are the UK acoustic tweezing scene," he says. He is also happy that five of the eight postdoctoral fellows involved in Sonotweezers have landed faculty posts in the UK, which bodes well for the field.

Central to the Sonotweezers project was the design and development of tiny piezotransducers and the generation of new geometries, such as array-based acoustic tweezers. These arrays can arrange particles, such as cells or beads, in patterns and move the particles independently of one another.

Transducers, which are made of piezoelectric material containing positively and negatively charged molecules, convert electrical to mechanical energy and vice versa, enabling the creation of sound waves needed for acoustic tweezers. When an electric field is applied across a piezoelectric material, the piezo's shape changes. Temperature, too, can change a piezoelectric material's traits. When a high-frequency alternating voltage is applied, the piezo vibrates like a piston, says



Acoustic devices can be slotted into a microscope for experiments in which sound waves move particles or cells in almost any medium.

Drinkwater. This piston 'forces' the fluid; in other words, the movement forms acoustic waves that propagate through the fluid. In acoustic tweezers, a cell or a particle is trapped in an essentially quiet zone between acoustic beams. To manipulate particles or cells, scientists generate an acoustic energy landscape.

Often, piezoelectric ceramics such as lead zirconate titanate (PZT) are used in transducers because of their good piezoelectric efficiency. Piezos made of some materials used in the past, such as quartz, were hard to fabricate, inefficient and expensive, says Drinkwater. PZT is currently the most commonly used material for this application, and it balances piezoelectric characteristics, good temperature resistance and low cost, he says. And PZT-based piezos deliver much displacement for each volt applied.

For the manipulation of biological samples, temperature control in transducers is key. The piezos heat up as some energy dissipates in the conversion of electrical energy to mechanical energy. When the temperature gets too high, it can damage the cells, and the piezos become inefficient and eventually stop working altogether. The researchers therefore added heating and cooling devices to provide temperature control. For the cooling, small water chambers were situated behind the piezos, allowing the temperature to be adjusted to 'just right' for cell viability and transducer function.

The Sonotweezers teams built a device with an array of 64 transducers[4] that can be controlled electronically without moving parts, says Drinkwater. Particles can be manipulated in two dimensions with the help of transducers placed orthogonally or in a ringed array. The device can readily slot into a microscope. The array tweezers are mounted onto a printed circuit board and are only a few millimeters thick. "Yet it has capabilities approaching that of optical tweezers," he says. One day, Drinkwater hopes the device will be no larger than a credit card and can be positioned into a standard microscope, lending researchers what he calls "microscope hands."

Using the array of transducers, the Sonotweezers team also built a levitation device[5], with which particles a few millimeters in size can be moved through a fluid medium with sound. It was applied in a small reaction vessel, or bioreactor, in which human cartilage cells were grown for cartilage engineering. Acoustic waves were used to generate three-dimensional (3D) clusters of cells, helping to promote the growth of cartilage without artificial scaffold materials. Separately, the team built a device to seed rat neurons at precise spots in culture and used acoustic forces in the piconewton range to guide neurite outgrowth.

Table 1 | Some types of acoustic tweezers

Method	Advantages	Disadvantages
Bulk acoustic wave (BAW) devices and arrays	No moving parts; good ability to steer and focus the acoustic beam; many patterns achievable	Cost; requires larger traps than single-beam method, so better for larger cells or cell clusters; needs more power than SAW-based systems
Surface acoustic wave (SAW) devices and arrays	No moving parts; can manipulate single cells or clusters; easy to integrate onto electronic systems such as for lab-on-a-chip	Waves confined to the surface, but this can also be an advantage; limited variety of patterns thus far
Single-beam acoustic devices	Easy to fabricate; can accurately manipulate single cells	Use of high frequencies can lead to attenuation of sound-wave signal and to heating

Sources: B. Drinkwater, T.J. Huang, Y. Li, K.K. Shung

Among the post-Sonotweezers projects, Drinkwater has a grant from the UK's Engineering and Physical Sciences Research Council to integrate acoustic tweezers with 3D printing. He and colleagues at the Bristol Heart Institute would like to use acoustic tweezers to seed heart stem cells on a printed scaffold, such as a structured grid, to try to develop heart-repair patches.

Agilent Technologies joined Sonotweezers at the start, which encouraged other companies to join, says Drinkwater. Although there is no immediate intent to use the acoustic technology in products, Agilent and its recent spin-out Keysight Technologies maintain close relationships with the Sonotweezers labs. The company has a strong interest in acoustic technologies for biology and medicine, says Agilent engineer Gerry Owen, who was part of Sonotweezers with two other Agilent colleagues. (Owen's comments were relayed to *Nature Methods* via a company spokesperson.)

Joining the project helped the company do longer-range research and benefit from the interaction with approximately 30 researchers, says Owen. It was a way to explore the design and troubleshooting of acoustic devices that manipulate cells and biological particles in single-cell experiments.

Agilent made undisclosed financial contributions to the project and offered practical support. For example, the researchers needed a number of electrical signal generators called arbitrary waveform generators. Owen and his colleagues scrounged up instruments from its labs around the world and loaned them to Sonotweezers teams. Scientists also spent a few weeks in Agilent's labs to test a prototype sonoporation device developed by the Southampton team on rat heart muscle cells and human breast cancer cells.

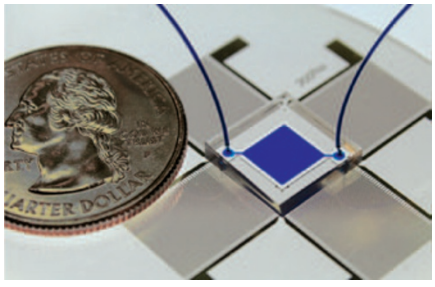
Designing and fabricating large arrays of miniature transducers is both challenging and interesting, says Owen. There may be many tens of transducers, which are each only a few millimeters in size or smaller. The teams needed to figure out how to calculate at high speed the particular customized signal that must be applied to each individual transducer for a particular task, such as moving a particular cell along a particularly shaped path at a given speed.

Sound in action

One idea guiding Sonotweezers efforts was the concept of a smart Petri dish, in which cells are amenable to acoustic manipulation while they are kept viable in a few cubic centimeters of nutrient medium in environments that attempt to model the natural one.

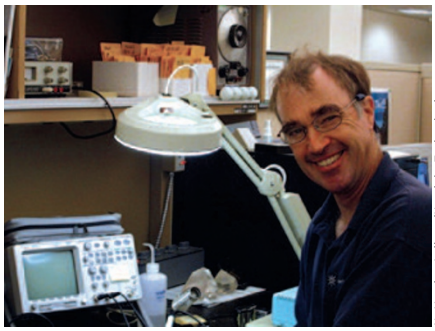
Sonoporation was another technique tested in the Sonotweezers labs. Exposure of live cells to sound allowed molecules as heavy as a few hundred daltons to enter them. "This has intriguing potential uses not only in biological research but also in the testing of small-molecule drugs," says Owen.

Sonoporation has been shown to augment processes such as transfection, says



T.J. Huang, Pennsylvania State University

Penn State researchers developed acoustic tweezers and are currently using them to explore the purinosome, the enzyme complex that controls purine biosynthesis.



N. Martin, Agilent/Keysight Technologies

Agilent engineer Gerry Owen was part of the public-private project Sonotweezers.

Drinkwater, although the exact mechanism through which it does so is not yet known. Some researchers believe that ultrasound punctures cells with tiny holes; others think that sound opens up pores in the cell membrane. It also appears that microbubbles can enhance sonoporation, leading some scientists to position microbubbles near the cells they seek to sonoporate to locally increase the effect, he says.

Acoustics and microfluidics are inherently compatible, says Owen. For example, a rig developed in one of the Sonotweezers labs for sonoporation experiments is an intimate combination of acoustic and microfluidic technologies. Acoustic devices can be made on any scale, but it is appealing to miniaturize them, says Drinkwater. Combining lab-on-a-chip approaches, acoustics and fluidics has led to what has become a field called acoustofluidics, he says. The basic goal is a toolbox so research tasks done on a large scale can be recreated on the microscale.

Some existing acoustics-based instruments exist already and are indicative of the potential, says Drinkwater. For example, in the acoustic focusing cytometer sold by Life Technologies/Thermo Fisher Scientific, an acoustic standing wave makes the particles form a line, which can help to achieve high-throughput measurements.

An example of a miniaturized acoustofluidic device is a microcentrifuge in which a small blood sample can be taken and then drawn into a device where it might be tested as it would be in a classic pathology lab. This area is attractive for medical diagnostics, for example. "But it is still early days for this technology," says Drinkwater.

Manipulating biological particles with acoustic methods may be gentler and therefore more attractive than other methods. For example, magnetic beads—even small ones—that are inserted into cells can make cells somewhat unhappy, says Owen. Also, acoustic instrumentation takes up just a small corner of a lab bench and is inexpensive.

But acoustical engineers still face some inviting challenges. When testing their prototypes, engineers typically use polystyrene spheres. Cells are more fascinating than these spheres and, says Owen, more maddening. With polystyrene spheres in water, scientists can exert relatively large acoustic forces, well into the nanonewton range. Furthermore, polystyrene spheres tend to have consistent diameters, so that when the gradient of the sound field is uniform, they will react to forces in very nearly the same way. But cells consist mainly of water, which means scientists must generate lower forces to avoid damaging them. And cells can vary in size, shape, density, compressibility and other characteristics, which means that each cell feels acoustic force differently from its neighbor. Researchers are puzzling away at these challenges, but it is also what engineers love to do, Owen says.

A gentle nudge

Penn State researcher Huang has cofounded a start-up called Ascent Bio-Nano Technologies to commercialize acoustic tweezers developed in his lab[6,7]. The company has a number of collaborations in place, but confidentiality agreements keep him from naming them.

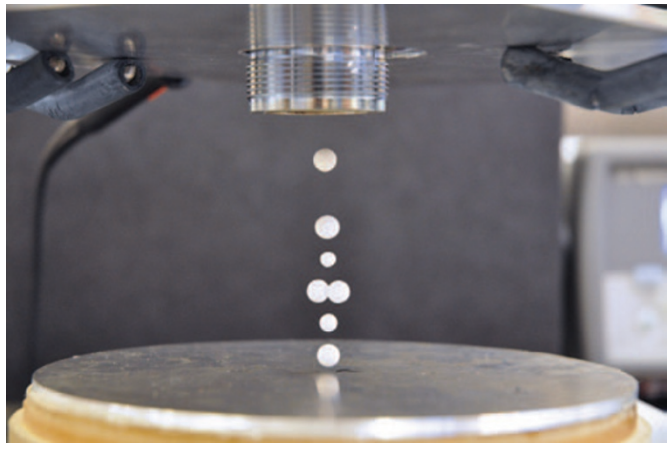
Huang, too, emphasizes the inexpensive, compact and gentle nature of acoustic methods in comparison to, for example, optical tweezers. Acoustic techniques keep cells viable, maintaining normal gene expression and post-translational modifications. The methods can move cells or bioparticles with 1-micrometer precision and in high throughput, he says.

Huang acknowledges that acoustic methods have not been around as long as optical tweezers, fluorescence activated cell sorting or magnetic tweezers and that acoustic tweezers are not as precise as optical ones. But he believes that the technology will mature and reach applications far beyond cell sorting.

For example, researchers studying cell-cell interaction in differentiation and development, in the immune response, or in cancer, all need to do experiments in which they can study intercellular signaling or the effects of regulatory factors in isolation. Manipulating single cells with optical, electrical, magnetic or hydrodynamic methods has shortcomings because of the way these techniques impinge on a cell's native state, Huang says. And combining high throughput and high precision in a single device is hard with such methods.

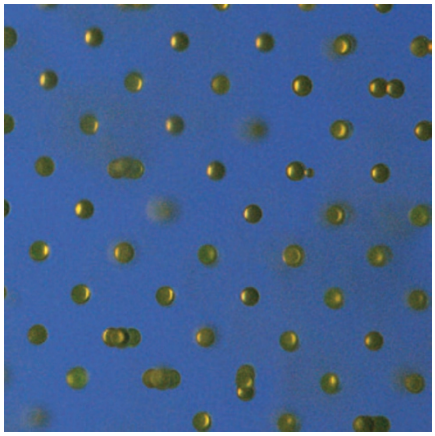
Together with the lab of Penn State colleague Stephen Benkovic, Huang is developing and validating high-throughput acoustic tweezers to study the purinosome, the multiple-enzyme complex required for purine biosynthesis. How purinosomes are assembled and disassembled and how this process is affected by the metabolic status of neighboring cells are hard to study in traditional Petri dish–based assays, given the complex intercellular communication involved. Acoustic tweezers, Huang hopes, will allow the team to precisely characterize an assembly of cells and use spatiotemporal control to capture quantitative data about purine metabolism. This work might lead to an assay for studying how a drug targeting purine metabolism affects cells, he says.

Huang's approach to acoustic tweezers involves surface acoustic waves (SAWs), which lets researchers control the distance between cells and therefore study cell-cell interactions with precision. It is unlike the approach by the Sonotweezers teams, who apply mainly bulk acoustic waves (BAWs), which travel through the bulk of a device substrate. SAWs, by contrast, confine most of their energy to the surface of a device sub-



B. Drinkwater, University of Bristol

Acoustic levitation is a way to move particles in fluids in two, or potentially three, dimensions. Here, a demonstration of the concept with levitation through air.



B. Drinkwater, University of Bristol

Acoustic methods can nudge beads or cells into assemblies.

strate. "Surface acoustic wave–based devices require less power than bulk acoustic wave–based devices to achieve the same acoustic effects," Huang says. Their heat dissipation is often smaller, which also reduces the risk of potential damage to cells.

BAW devices are more mature and have shown higher throughput, says Huang, but he prefers the ability, afforded by SAWs, to use a wider range of frequencies. In his view, those traits will make acoustic tweezers more versatile and lend them more precise control when manipulating fluids and particles. SAWs also allow microfluidic devices to be fabricated containing microchannels made of materials that have lower acoustic reflection, such as polymers.

Overall, acoustic tweezers allow biological particles ranging from tens of nanometers to a few millimeters in size to be manipulated in virtually any medium from water-based solutions to body fluids, whether for cell or bioparticle separation, patterning or multichannel sorting, says Huang. Acoustic technology preserves cell integrity and natural states, and cells do not need to be labeled or their surfaces modified.

Calibrated sound

Acoustic approaches come in different configurations, and the resulting devices are built for varying applications. The team of Shung and colleagues at the University of Southern California, also a US National Institutes of Health Ultrasonic Transducer Resource Center, uses single-beam acoustic tweezers at higher frequencies than are traditional, to generate an acoustic gradient and move bioparticles[8]. Shung sees many possibilities for the use of high frequencies, from cell sorting to stem cell manipulation to acoustic transfection. The lab is exploring the biological effects of these frequencies on the particles they want to move.

One project in the Shung lab is using sound to characterize the properties of a single red blood cell and the forces such cells exert on one another. This research can help discover individual differences between the same cell types, says postdoctoral fellow Ying Li. Using a single acoustic beam can be less expensive than a configuration in which a pair or more of transducers are needed, says Li.

In Li's view, all acoustic tweezers need higher precision than they currently have for manipulating particles or cells. One way to achieve that goal is by using higher transducer frequencies to create a sharper acoustic beam. Traditional acoustic tweezers use frequencies around 30 megahertz. The Shung lab has made transducers that use 200-megahertz frequencies. The process of fabricating them is challenging, but, Li says, invoking a Chinese proverb, "A journey of a thousand miles begins with a single step."

He believes that the precision of this technology is also important for potential use for *in vivo* studies in which researchers direct a nanoparticle to a specific site in an lab animal's body. A major effort in his lab, says Shung, is to show the feasibility of such experiments, in which sound is applied transcutaneously to manipulate particles in the blood vessels of mice. This might be a way to deliver a drug or to manipulate a process under study.

One day, says Li, it might even be possible to do so in humans. He knows this application is far off in the future. Li says he meets scientists who call these kinds of acoustic manipulations a sort of 'mission impossible'.

As acoustic methods mature, Shung and his team also want to create and share methods to calibrate acoustic tweezers and gauge trapping performance[9]. There are multiple methods to do so (**Table 2**). A spherical bead is often used to calibrate the trapping force for acoustic tweezers, but, says Li, that approach calculates the force acting on the bead and is difficult to extrapolate to a cell. The lab is also working on ways to calibrate the acoustic force needed for objects that have arbitrary shapes, in order to obtain an effective trapping force for a given experiment. Current methods, says Li, are unsatisfactory and time consuming.

Calibration methods and prototype testing are needed to evaluate the trapping performance of different types of acoustic tweezers as they evolve. These approaches can help compare acoustically driven devices applied in different types of biological studies. Acoustics researchers are hopeful about the potentially wide variety of applications and devices for basic and applied research.

Table 2 | Calibration of acoustic tweezers

Calibration method	Advantages	Disadvantages
Viscous drag force method	Relatively simple to perform; requires only a modest-spec high-speed camera	Can overestimate the trapping force, as it requires a mathematical model of the drag; needs good knowledge of the fluid and particle properties
Equipartition and power-spectrum methods	No information about the particles or the fluid required; can work for any small particle	Requires high-speed position detectors given fast Brownian motion; requires Brownian motion to be observable, so only applicable to small particles (<5 μm)
Optical tweezers	Highly accurate and 3D approach	Requires an integrated optical tweezer and costly optical bench

Sources: B. Drinkwater, Y. Li, K.K. Shung

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Vivien Marx is technology editor for *Nature* and *Nature Methods* (v.marx@us.nature.com).