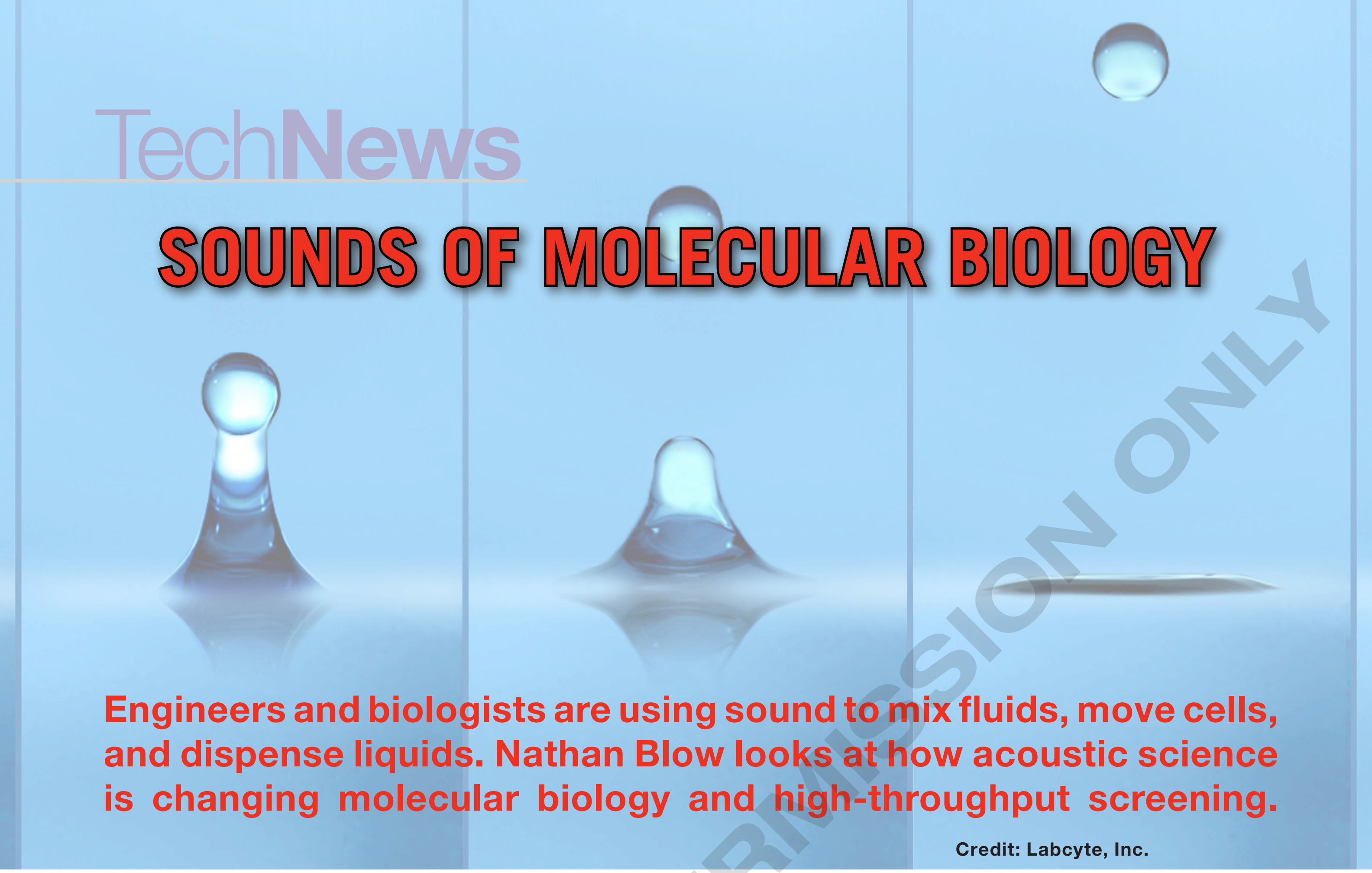
TechNews

SOUNDS OF MOLECULAR BIOLOGY



Engineers and biologists are using sound to mix fluids, move cells, and dispense liquids. Nathan Blow looks at how acoustic science is changing molecular biology and high-throughput screening.

Credit: Labcyte, Inc.

Tony Huang, Professor and The Huck Distinguished Chair in Bioengineering Science and Mechanics at Pennsylvania State University, remembers when doctors used ultrasound to monitor his wife and child during her pregnancy. “I quickly realized that it was a safe and gentle technique,” says Huang.

Ultrasound technology relies on sound waves to generate images of soft tissues. By sending high-frequency waves using a probe, ultrasound machines record the echoes off tissues in the body for imaging; different tissues and structures reflect sound at different degrees. Huang, an engineer by training, was thinking more and more about the unique properties of sound around this time—and not just for imaging applications. His thoughts were moving towards the possibility of applying acoustics to some of the more basic biomedical research questions and experiments that he was working on at the time.

There are actually a number of advantages to using sound in the lab. Unlike light-based approaches to imaging, for example, systems and instruments that rely on sound can be smaller (there is no need for large detectors and optics) and significantly cheaper, making sound-based devices more portable and applicable in point-of-care settings. And the gentle nature of techniques based on sound, what Huang witnessed in the hospital, means less potential for damage to the biological samples under investigation. For Huang, whose lab had a major interest in developing novel microfluidics solutions, applying sound in molecular biology applications, rather than conventional mechanical pumps or light-based optics, seemed like the right direction to follow—even if it was a little non-traditional.

“At the time, so many people were working on photonics and not acoustics,” says Huang. But that, he says, also presented him with the opportunity to get in on the ground floor of what he viewed as a very significant developing technology. “And,” he quickly adds, “it was clear acoustics has inherent advantages that the other methods do not.”

This was a few years back. Today, Huang and his co-workers are founding members of what was once seen as an odd corner of the molecular biology world, where sound waves are used to manipulate and move individual cells, and fluids and biomolecules are mixed and dispensed into tubes and plates using acoustics. He is no longer alone, though, in his interest in applying acoustics in the biology lab—many methods developers and researchers are coming to the same realization, learning how sound provides unique solutions to different microfluidics challenges and how sound-based approaches might even provide unique ways to extend the capabilities of high-throughput drug and gene screening systems.

Sounding a new era

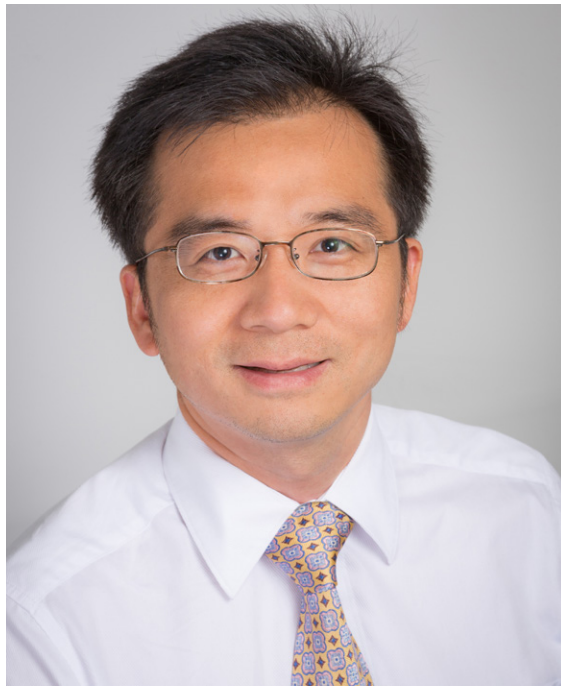
When speaking, Huang refers back to the word “acoustofluidics” often when describing the engineering efforts within his lab. “We are working at the interface of acoustics, fluidics, and micro/nano technologies,” he explains, “adapting and applying these fields to biomedical problems.” Thus acoustofluidics does seem appropriate terminology.

Huang’s lab is currently divided into three different areas of acoustofluidics development projects; two of these projects involve using sound to move cells from place to place.

Cell sorting is an important application in cell biology research. This is one of the areas where Huang believes acoustics-based techniques could make a large impact. "The flow is very high-speed in current FACS systems, and the voltage is high," notes Huang. Sound is a gentler option for manipulating cells, and Huang's lab is taking advantage of this property by developing acoustic cell-sorting devices. In their most recent work, Huang and his colleagues were able to use sound to separate circulating tumor cells (CTCs) in peripheral blood samples from cancer patients (1). The CTC separation method uses "tilted-angle" standing surface acoustic waves. Here, the waves are induced at specific angles compared to the flow direction, instead of being parallel to the flow, which means cells experience both acoustic radiation forces and laminar drag forces, enabling more accurate sorting.

But the key for Huang with his new CTC sorter is still the gentle effects of sound on the cells, since the integrity of the sorted CTC cells can be persevered. "This is label-free sorting where the cell's properties are preserved, which can give us more information on cancer and basic biology, and provide better diagnostics and therapy," explains Huang.

However, the Huang lab isn't just using sound for cell sorting; sound can also be used to move cells into specific locations. "By carefully controlling the sound field, we can position cells from a distance into cell assemblies," explains Huang. This positioning can enable tissue engineering applications as well as the analysis of cell-to-cell communications in 2-D or 3-D structures. Huang's team has even figured out how sound can be used to move and position cells in suspensions or when in contact with a surface.



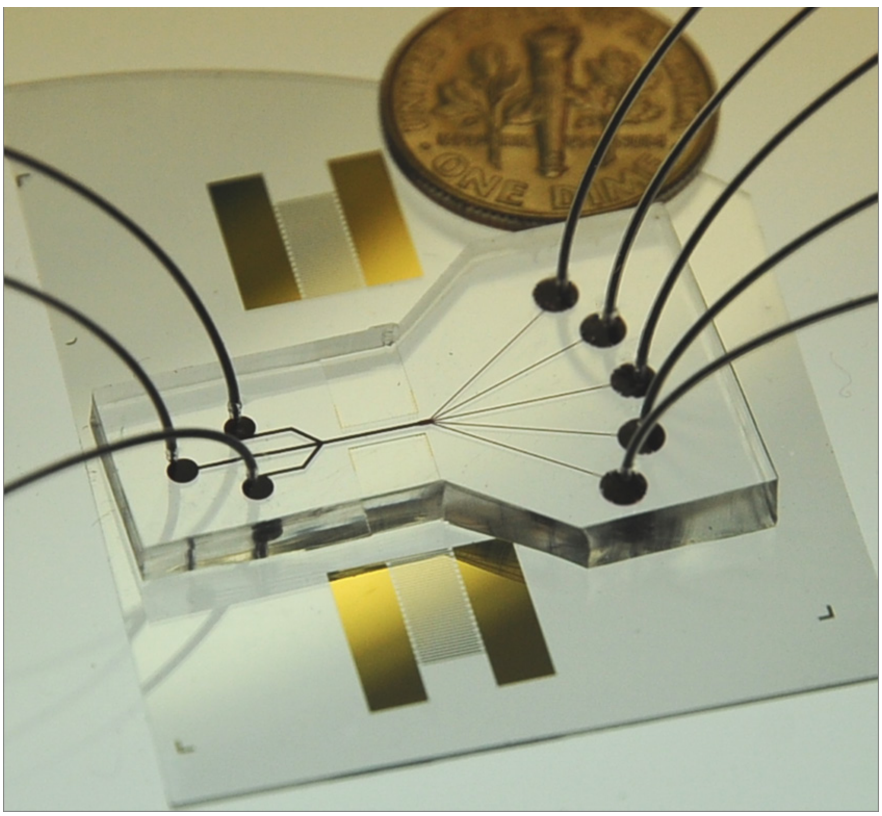
Tony Huang, Professor and The Huck Distinguished Chair in Bioengineering Science and Mechanics at Pennsylvania State University, has been working on acoustofluidics solutions for a number of biological applications. Credit: T. Huang.

Sounding the warning bell

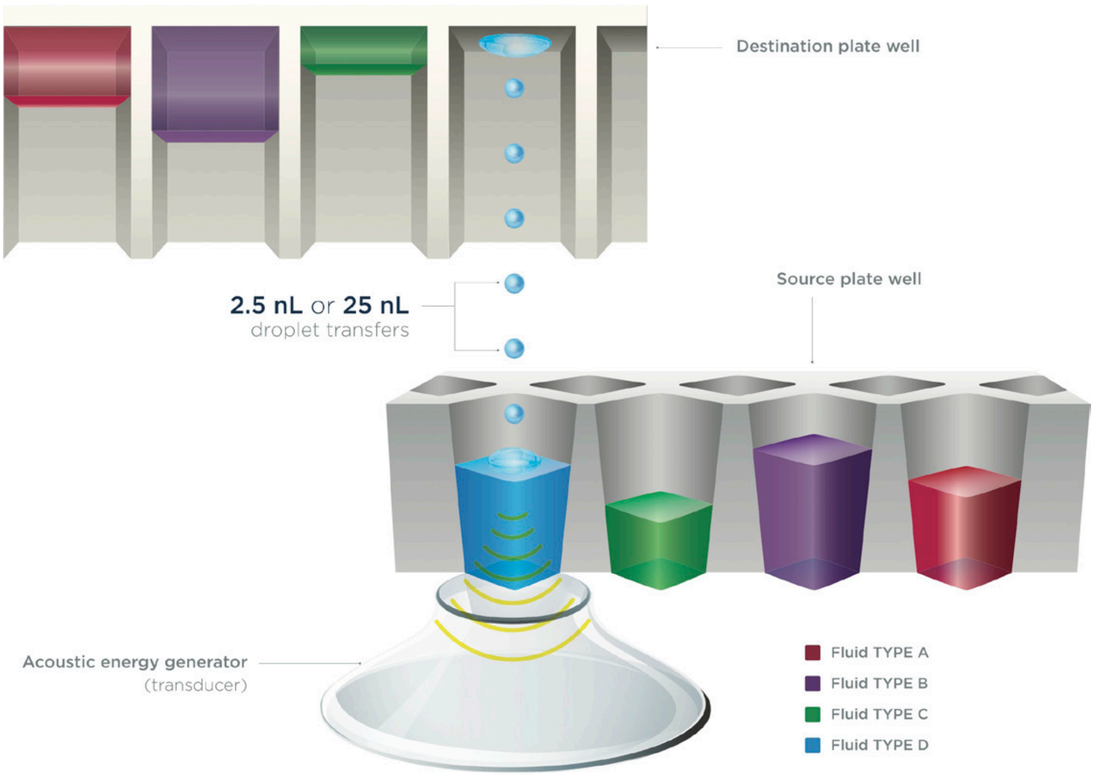
In addition to cell manipulation, acoustics is also being used now for mixing and pumping or transferring of solutions. In fact, it could be argued that this is a more traditional application for sound in the modern molecular biology lab, and it is one that Huang sees as being able to provide some key solutions to the field of microfluidics. "Some people have been a little disappointed with microfluidics over the years," Huang notes, likely a feeling born out of the lack of available microfluidics-based commercial products.

Huang, however, suggests that the lack of microfluidics-based commercial products and the difficulty in implementing past microfluidics solutions might have to do with the fact that many microfluidics researchers do not have commercialization as the end goal. Many innovations in the microfluidics community are far from being practical. He points directly at fluid-handling capabilities as being one of the major issues for previously developed microfluidics platforms. And this, he insists, is where an acoustics-based platform could make all the difference.

The question for some outsiders though, is whether or not acoustics-driven devices are truly the answer to better liquid-handling technologies, and whether they can present new potential applications in the microfluidics field. Pharmaceutical giant AstraZeneca is betting on acoustics



Acoustofluidic cell sorter that has been devised by the Huang lab and validated by sorting circulating tumor cells from blood samples. Credit: T. Huang.



Schematic of the Echo acoustic technology for transferring liquids and samples between wells on two different plates. Credit: Labcyte.

to enhance their drug discovery efforts. The company announced plans in late November to develop a compound management system for their new screening facility that will employ Sunnyvale, California based Labcyte's acoustic liquid-handling system known as Echo.

Echo's technology employs sound waves to eject very small volumes of liquid, on the order of 2.5 nanoliters, very rapidly, at a rate of up to 500 times per second. For plate-to-plate liquid transfer applications, Echo places the source plate on the bottom and a second plate, the plate that will be receiving the liquid, directly above it, only in an inverted position so the wells are in close contact. From there, sound waves are pulsed into the liquid in the wells on the lower plate, resulting in the ejection of small 2.5 nanoliter droplets into the waiting well of the second, inverted, plate. Surface tension holds the small volumes of liquid in the wells of the inverted plate until transfer is completed. This sound-based approach results in less direct contact with the solution and a reduction in consumables costs compared with traditional liquid transferring systems.

In a press release announcing the acoustic compound management system, Clive Green, Director of Sample Management at AstraZeneca said, "Acoustic dispensing will revolutionize compound management by reducing compound volumes by 10-fold. Acoustic tubes represent another significant step forward by enabling a workflow that generates assay-ready plates for high-throughput screening using only acoustic transfers. This should provide an unparalleled level of quality in screening data produced from our biological assays."

The smaller volumes being transferred by the acoustic system are actually critical to efforts to extend the already impressive number of wells and samples that many of today's larger high-throughput robotic systems can process on a regular basis for screening applications. And since the use of pumps and syringes for mixing can lead to fluid loss, as sample sizes decrease, the impact of such losses will be greater when performing high-throughput assays.

A sound future?

Even though Huang says his acoustofluidics approaches offer practical advantages in cost and efficiency, and systems such as Echo from Labcyte are beginning to emerge, he is quick to note that implementing acoustics applications is still in the early phases, and therefore can present challenges.

"The prototypes that we have—the lab versions—are difficult to use without an engineering background." This refrain tends to be common among basic science researchers when discussing early-stage microfluidics-based platforms. Huang is keen to see this barrier to entry reduced in the future and hopes to see more commercialized acoustics-based technologies soon.

With major companies such as AstraZeneca starting to implement acoustics workflows for their liquid-handling needs, and other researchers starting to adopt sound for mixing and cells sorting, it seems that we just might be on the cusp of change as Huang predicted. Only time will tell, but the technology is sounding pretty good at the moment.

References

1. **Li. P. et al.** 2015. Acoustic separation of circulating tumor cells. Proc Natl. Acad. Sci USA. Vol *112*:4970-4975.

Written by Nathan Blow, Ph.D.

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