Acoustofluidics

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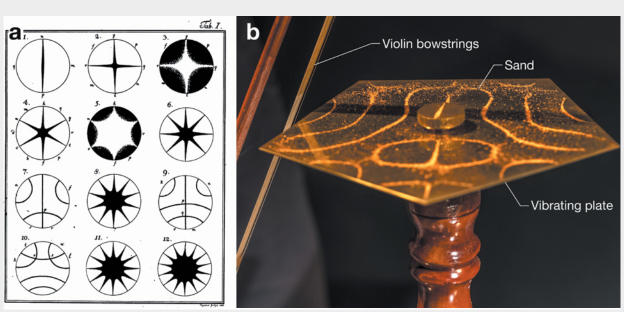
Introduction

From one of the first formally published works, Chladni (1787) became very well-known for his discovery of the patterns that now bear his name: sand organized into patterns on vibrating membranes (**Figure 1**). He toured European venues in the late 1700s to the early 1800s, demonstrating the curious phenomena to laypeople and trained scientists alike. The acoustic wave present in the membrane interacted with the sand, causing it to move away from regions that were vibrating, the antinodes, and to collect in regions that were quiescent, the nodes.

Figure 1. *In one of the first physical demonstrations of acoustics, sand spread on metal plates and membranes forms fascinating patterns called Chladni figures (**a**). Taken directly from Chladni's text (1787), it is composed of drawings of the patterns formed by the sand (**black**) on vibrating circular membranes at different frequencies. By mounting the plates and drawing a violin bow across the edge of the plate, the sand moves in response to the driven vibration into patterns (**b**). Chladni is often described as the father of acoustics for these figures and the work that arose from them by many researchers. It would likely have surprised him to learn that questions on how these patterns formed were still being asked in the past decade and that the discrepancies Savart found in Chladni's work would become so useful today.* ***a*** *from Chladni (1787);* ***b*** *licensed under CC-BY-SA 4.0 from Matemateca (IME/USP)/Rodrigo Tetsuo Argenton. Available at bit.ly/3JfsrFS.*



Sound formed diaphanous pictures on the vibrating surface, changing with the sound before the audience's eyes.

Félix Savart was among the attendees at one of these events. He was an acclaimed scientist in his own right who came to be known for his work with the acoustics of violins. He later sought to replicate Chladni's work (1787) and found that not only would the sand sometimes collect at the nodes, but it would also occasionally collect at the antinodes. This phenomenon was not explained by Chladni's efforts. Savart hypothesized that air currents adjacent to the membrane were being driven by the vibration in the membrane and that these currents were responsible for the particle motion. The airflow and the transport of these particles were the genesis of the field of *acoustofluidics*, the physical effect of a passing acoustic wave on a fluid and particles suspended within it. Little did they know that the discrepancy Savart identified alongside Chladni's discovery would together eventually grow to define acoustofluidics as a new research discipline, let alone produce a crucial solution to one of the most vexing problems in modern medical diagnostics today.

The discrepancy in particle collection phenomena was one of several between the two men that sadly grew to become a rather vindictive relationship (Bell, 1991). It also attracted the interest of prominent scientists over the years and decades to follow. Thirty years later, Faraday (1831) conducted several simple experiments to clearly identify the existence of acoustic streaming, one of the key contributors to fluid transport and suspended particle manipulation responsible for the disagreement between Chladni and Savart.

Thirty years after Faraday's work, Kundt (1866) devised a simple hollow tube that became a popular platform to explain particle manipulation via acoustic streaming. Open on both ends and with a sound source at one end, Kundt's tube was originally devised to determine

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the speed of sound in gases. It was a simplification of Chladni's vibrating plates (1787) because the tube itself remained completely stationary. The particles in the tube were moved about solely due to airflow, which no doubt would have pleased Savart.

Like many topics, it drew the interest of the polymath Lord Rayleigh (Strutt, 1883) who offered the first explanation of the acoustic streaming phenomena. As acoustic waves passed through the air, they compressed and rarefied in the air. The viscosity of the air caused its velocity to be slightly out of phase with the density, leading to slightly denser air as it moved along the tube away from the source rather than in the other direction. Over time, this caused the air to flow slowly. Lord Rayleigh devised and solved a set of equations that explained why, the first of many attempts to explain the phenomena of acoustofluidics.

Over 60 years would then pass before acoustic streaming would be rediscovered in a significant way, this time in a completely different discipline: materials science. A class of materials, piezoelectric materials, was discovered to have the ability to usefully transform energy between mechanical and electrical forms. Vaguely known since ancient times, piezoelectric materials were vitally important with the advent of submarines in World War I. The submarines used sonar transducers formed from these materials to detect ships. The ships used similar transducers to detect the noise of submerged submarines. From the early 1900s, researchers sought to identify and improve piezoelectric materials from the easily dissolved and modestly performing Rochelle salt to quartz and, much later, ceramics like lead zirconate titanate and single crystal piezoelectric materials like lithium niobate.

Quartz is still found today as accurate oscillator crystals in watches, and lithium niobate is present in many telecommunication devices to handle signal-processing tasks, especially mobile phones. As the materials improved, so did the number of occasions that researchers observed odd air currents emanating from the surfaces of plates made with these new materials. Application of an oscillating electric field to the plates would produce vibrations in and on them, much like those observed by Chladni two centuries before (1787). However, this time there was no need for an external sound source because the piezoelectric plates themselves vibrated directly from applied electrical signals. Moreover, the air currents occurred without audible sound at all; the vibrations causing them were ultrasound waves at frequencies of 20 kHz and beyond. The ethereal air currents, "quartz wind," was another example of acoustic streaming reported by Eckart (1948), accompanying an analysis of the phenomenon.

Acoustic streaming had more secrets to proffer. Westervelt (1957) explained the most peculiar phenomenon of producing two completely different results from an analysis of Kundt's tube (1866). In one treatment, the analysis would suggest that there is no flow at all along the length of the tube. In another treatment, the analysis would suggest that there was a net flow after all toward the sound source. Neither prediction compared with experimental data that showed that the flow was away from the sound source. This odd result came to be named Westervelt's paradox and took some two decades and the discovery of a rare mistake made by Lord Rayleigh back in 1866 to properly explain.

In all that time, particles were used to track the flow of fluids driven by the passage of acoustic waves. In Chladni's original experiments (1787), the fluid was ignored as particles bounced about on vibrating surfaces to form the namesake patterns. Savart had demonstrated that the fluid's motion mattered, and in the years since, the question of what drives particles on vibrating surfaces to collect at the nodes or antinodes has been answered in many different contexts (Doinikov, 1996; Dorrestijn et al., 2007), producing a rich tapestry of individual solutions that together have produced a complex story and some interesting applications.

Indeed, in the modern era, Chaldni's techniques were used to verify the presence of vibrations in structures from aircraft wings to microdevices. In one example, the surface of an acoustic wave device was formed from a single crystal piezoelectric plate with an electrode deposited on it in the shape of interlaced metal fingers. These fingers form an interdigital transducer (IDT; see **Figure 2a**) that when driven with an oscillating electrical signal produces an acoustic wave in the plate that propagated across its surface. That wave is known as a Rayleigh wave, named after the same Lord Rayleigh who provided the early explanation of acoustic streaming. The wave formed patterns across its surface while being operated from 20 to 500 MHz, many orders of magnitude greater in frequency than in Chaldni's demonstrations.

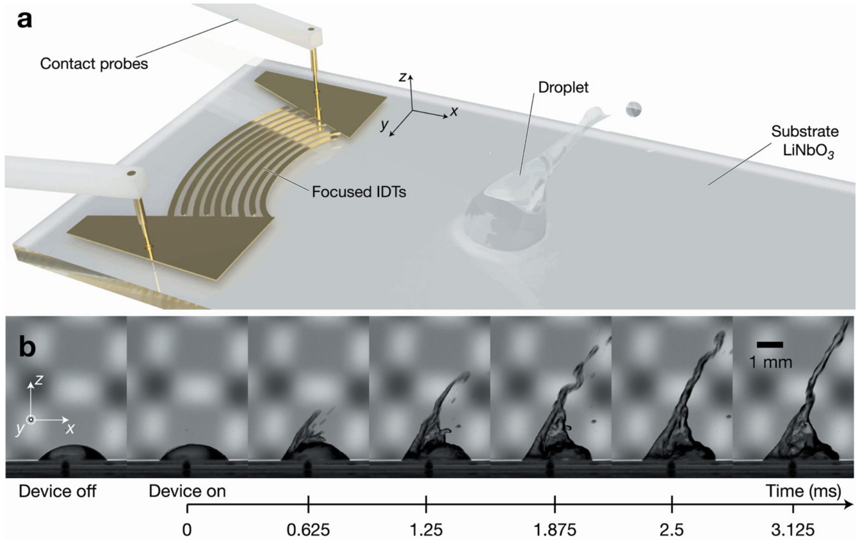


Figure 2. ***a:*** *Acoustic device made of single crystal piezoelectric lithium niobate ($LiNbO_3$) as a substrate, with an interdigital transducer (IDT) formed from a thin layer of gold deposited on it. Passing an oscillating electric field to the IDT via the contact probes (**left**), in this case at 40 MHz, causes an acoustic wave to be produced on the substrate. This Rayleigh wave propagates rightward along* x *and under a 6-µL fluid (water) droplet sitting on the surface. The fluid absorbs some of the energy from the passing Rayleigh wave, causing sound at the same frequency to be generated in the fluid. The sound propagates at a characteristic angle up and to the right in the* x-z *plane. This angle is the Rayleigh angle, 23° in this system.* ***b:*** *As the propagating sound is attenuated in the fluid, the fluid itself is being driven into motion, resulting in acoustic streaming. The velocity of the fluid is sufficient to cause it to jet from the surface. This result, first demonstrated by Shiokawa (1989), is one of the first demonstrations of acoustofluidics.*

The gateway to modern acoustofluidics was driven by research on these surface acoustic wave (SAW) devices by a seemingly minor discovery after swapping the particles with fluid. Shiokawa discovered how 20-MHz SAW devices the size of one's thumbnail would eject fluids nearly a meter away (Shiokawa et al., 1989), generating the Rayleigh wave across its surface (**Figure 2b**). Shiokawa, much like Chladni, toured laboratories in Japan and other locations overseas in the early 1990s demonstrating the curious physical phenomena of an ejecting droplet from a SAW device but with a limited explanation of what was happening and no connection to what would eventually become its principal application.

Microfluidics in Health Care

Acoustofluidics would have likely remained little more than a curiosity had there not been a profound need for fluid and particle manipulation methods in microfluidics for medical diagnostics. After Manz' demonstration of microfluidics in 1990 (Manz et al., 1990), its application drew the excitement of many researchers who recognized the potential of shrinking the laboratory to a single, handheld chip, a *lab-on-a-chip* (Stone et al., 2004). In their minds, microfluidics enabled the lab-on-a-chip to revolutionize the future of personal health care with rapid at-home point-of-care diagnostic tests that would lessen the burden on labs and physicians.

Indeed, microfluidics offered many advantages thanks to its short processing time, dynamic control, lower costs, and portability. From the beginning, however, the dual challenges of viscous drag and surface-dominated forces in microfluidics were seen as potential problems. The viscosity of the fluid in small scales resisted flow and made mixing difficult. Filling a microchannel with a fluid would stop at an intersection, forming a meniscus. To continue filling the channel required overwhelming this meniscus and the capillary forces responsible for it by coaxing the fluid along with extreme pressures. At the time, researchers were confident that these issues would eventually be overcome. Applications emerged from capillary electrophoresis (Swerdlow and Gesteland,

1990) for efficient DNA sequencing to polymerase chain reaction devices (Northrup, 1993), all of which needed effective methods to transport fluids at these small scales. Researchers searched for effective methods to transport fluids at these small scales (Laser and Santiago, 2004), using electric fields, large pressures, chemical and thermal gradients, and capillary forces. The trend continued even after the initial purpose of sequencing the human genome was completed in 2003, with the adoption of polydimethylsiloxane, a soft silicone polymer material closely related to the caulk used to seal windows and bathtubs, to produce devices (Friend and Yeo, 2010) at much lower costs, facilitating broad advances in the scope and utility of microfluidic devices.

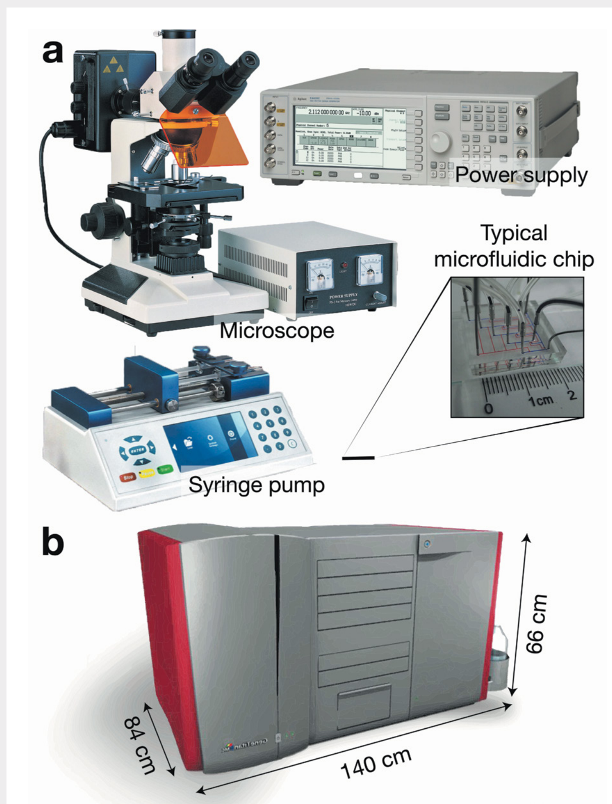
In some ways, microfluidics technology was devised and used many years before, using paper as the fluid transport medium. As anyone who has placed a coffee cup on a piece of paper and left a growing ring of coffee behind on the paper knows, liquid will absorb into and flow within the paper. The capillary forces responsible for flow of the fluid along the paper was used in everything from paper chromatography in the 1940s to pregnancy tests in the 1970s (Chard, 1992), but they also prevented anything more than simple device designs. It thus became clear that a combination of this method with fluid handling and chemical sensors devised in micro/nanofluidics research efforts would make the method nearly universally relevant for many diagnostics applications (Koczula and Gallotta, 2016). It has certainly made lateral flow assays instantly recognizable, with the nearly ubiquitous "Covid home test" enabled by engineered antibodies and having been used at one time or another over the past three years by so many of us around the world (Chu et al., 2022).

Chip in a Lab

The problem of fluid and suspended particle handling remained, particularly for more the complex processes that required more intricate control of fluids and particles. Some devices required continuous flow through a device to look for rare target cells or molecules in larger fluid samples. Other devices required fluid mixing; the manipulation of suspended cells; or the production of separations, mixtures, or suspended immiscible droplets. Researchers searched, somewhat fruitlessly, looking for methods to propel fluids, cells, particles, and mixtures through their microfluidics devices without the burden of all the equipment that kept them in the laboratory (Laser and Santiago, 2004). Many researchers and companies gave up on the heralded lab-on-a-chip concept, the idea that an entire laboratory could be shrunk to fit on a chip and used as a portable device. Instead, they returned their tiny microfluidics chips to the laboratory, surrounded again by large pumps and other equipment needed to make them work. The limitations in microfluidics technology remained, earning the discipline the derisive moniker "chip-in-a-lab" (**Figure 3**).

It took nearly a decade for researchers skilled in microfluidics and its application to recognize the potential of acoustic waves to solve the problems they faced. They

Figure 3. ***a:*** *The typical components needed in a laboratory benchtop setup to operate a microfluidic chip, alongside the relatively tiny chip itself. The microfluidic dream of "lab-on-a-chip" is tempered by the need for this equipment to operate the microfluidics devices, earning the derisive moniker "chip-in-a-lab." Modern commercial laboratory equipment is similar.* ***b:*** *A recent example is the ThermoFisher GeneTitan instrument, with small "genechips" alongside a very large benchtop instrument to process them (Chen et al., 2023). Adapted from Zhao et al. (2013).*



started to learn and adapt the curious phenomena of acoustic streaming and particle manipulation that had driven the curiosity and consternation of several generations of researchers from Chladni to Shiokawa.

Micro/Nano Acoustofluidics

Micro/nano acoustofluidics is the study of acoustic wave generation, propagation, attenuation, refraction, reflection, and other behaviors in fluids and across interfaces between fluids and solids at extremely small spatial and temporal scales. Although the study of acoustics and its wide range of applications have been around since Chladni's time (Friend and Yeo, 2011), micro/nano acoustofluidics is a relatively new discipline borne from the desire to solve the problems found in microfluidics with new fabrication methods for complex piezoelectric ultrasonic microdevices. Shiokawa's demonstration in the 1990s, discussed in **Introduction**, was but the first hint of the potential of the technology.

Before the 1970s, ultrasonic devices tended to be either large devices for high-power applications in sonar and medicine or very low power devices used in timing and telecommunications, generally operating at 20 kHz to a few hundred kilohertz. With the advent of practical single-crystal lithium niobate and tantalate alongside interdigital fingerlike electrodes in the 1960s (White and Voltmer, 1965), it became practical to generate and use powerful 10 MHz to 10 GHz SAW devices for telecommunications and timing and later for acoustofluidics.

The high frequencies are an important benefit in micro/nano acoustofluidics. First, the wavelength of the propagating acoustic wave needs to be on the order of the micro/nanofluidic channel size for there to be a gradient in forces at that length scale. These force gradients can produce mixing, particle motion, and all the other effects that render the previously laminar flow much more useful. Second, the acoustic wave attenuates as it propagates through the fluid. This causes the generation of fluid flow. The attenuation increases with the square of the frequency, and typically, such attenuation is useful over 5-10 wavelengths of the acoustic wave as it propagates in the fluid. Thus, to "fit" enough wavelengths into the micro/nanofluidic structure, the frequency must be high. Third, and most important, there is a fundamental limitation to the particle velocity that can be generated in the fluid, about one meter per second. Any higher and the acoustic wave will be rapidly attenuated. This also defines the vibration amplitude and acceleration limits; the amplitude is the particle velocity divided by the frequency, whereas the acceleration is the particle velocity multiplied by the frequency. At audible frequencies, one can often see the physical motion of a speaker as it produces sound near its maximum volume, and the acceleration of the speaker cone is perhaps two or three orders of magnitude greater than gravity. But at 2 GHz, for example, in devices reported by Wu et al. (2022), the maximum physical motion is only a few tens of picometers, an order of magnitude smaller than the diameter of a typical atom. However, accelerations over 10 billion m/s^2 are generated. It perhaps is little surprise, then, that the phenomena observed in using these devices is often surprising and new.

A typical example of an acoustofluidics device suitable for the laboratory bench or classroom (**Figure 2**) is similar to the one Shiokawa produced over 30 years ago (Shiokawa et al., 1989). A small fluid droplet placed on a SAW device fabricated from lithium niobate (Mei et al., 2020) can be made to jet from its surface as Shiokawa reported (1989): atomized, forming a mist of tiny droplets (Kurosawa et al., 1995; Collignon et al., 2018), or even driven across the surface to form patterns or thin fluid films that unveil the complex interactions between the acoustic wave in the substrate, the fluid, and the fluid interface (Rezk et al., 2014a). Over the years, other types of waves were rediscovered (Rezk et al., 2014b; Collignon et al., 2018), and the limits of the known useful frequency range continues to be explored all the way up to several gigahertz (He et al., 2021). Some of these fluid manipulations were performed for use in medical diagnostics, but some are finding use in printing, pulmonary drug delivery, producing thin films for coating surfaces, fuel atomization for engines, and many other applications.

The apparent simplicity of the device belies the many phenomena that may arise from acoustic wave propagation within. A combination of acoustic streaming within the fluid bulk and acoustic radiation pressure on the droplet's free interface will arise, and, if there are particles suspended in the fluid, acoustic radiation pressure can also appear on those particles to force them into patterns or rapid motion. These forces can cause selective concentration and separation of cells in microliter droplets stuck on surfaces (Zhang et al., 2021b) and in fluids in channels (Wu et al., 2022). These effects extend into enclosed microfluidic and even nanofluidic systems, with the recent discovery of a new type of acoustic streaming in a nanofluidic channel reliant on the channel's deformation from a passing acoustic wave (Zhang et al., 2021a) and the rapid flows that can be generated around sharp-tipped structures (Ovchinnikov et al., 2014) and bubbles (Doinikov and Bouakaz, 2010; Marin et al., 2015).

These effects are often several orders of magnitude greater than what is possible using electric fields, chemical gradients, thermal effects, or even high pressures in microfluidics and nanofluidics devices. The many ways they may be used to produce useful diagnostic devices is only now being identified (Rufo et al., 2022), and a significant barrier to broader use of acoustics in micro/nanofluidics is the same challenge that faced Chladni so many years ago: the complexity of the underpinning phenomena, particularly acoustic streaming and particle manipulation.

Acoustic Streaming

Acoustic streaming relies on the generation of gradients in the fluid's density and motion that, coupled together, produce momentum acting to transport the fluid in a desired direction. Typical sounds propagating through the air or a fluid are insufficiently powerful to cause the necessary density changes. However, using intense ultrasound, it becomes possible to drive uniform fluid flow as the ultrasound propagates and is attenuated. The analysis of the phenomenon is difficult, attracting interest from researchers since Lord Rayleigh to offer a variety of mathematical analyses to represent it as either a phenomena with the acoustics independent of the acoustic streaming (Strutt, 1883), a one-dimensional phenomenon driven entirely by attenuation (Eckart, 1948), a means to transport vorticity (Nyborg, 1965), a rapid flow within the viscous boundary layer adjacent a vibrating substrate (Schlichting, 1932), much like the phenomena that disrupted Chladni's patterns, or a spatiotemporally varying phenomenon (Orosco and Friend, 2022).

Particle Manipulation

The original discovery of patterns of vibration by Chladni (1787) relied on the presence and manipulation of particles. In the modern context, particles are rarely left to reside on the vibrating surface. There are some notable exceptions, however. It is possible to disassemble carbon nanotube bundles (Miansari et al., 2015) through bouncing the bundles until they split and stick on the vibrating surface to charge and toss out carbon nanotubes. Cigarette smoke has even been allowed to collect on vibrating surfaces to identify the shape of these vibrations and the acoustic streaming they generate (Tan et al., 2007). Typically, however, particles are present as suspensions or colloids and can be anything from cells to bubbles depending on the application.

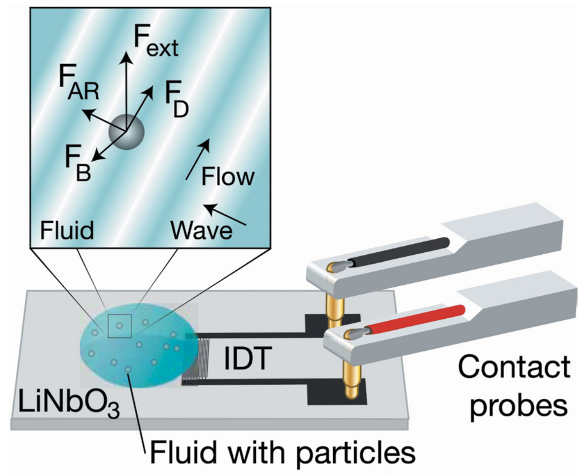


Figure 4. *A simple experiment where a sessile droplet with suspended particles is placed on a surface acoustic wave device. When turning on the device, the acoustic wave generated in the fluid via the Rayleigh wave absorption from the substrate interacts with the suspended particles. Some common forces that particles in a fluid droplet experience from the passage of these acoustic waves include the acoustic radiation (F_{AR}), drag (F_D), Bjerknes (F_B), and externally applied (F_{ext}) forces. Most externally applied forces are insignificant, although some experiments have used buoyancy and electrically applied forces in concert with the three acoustically driven forces to move the particles. All these forces appear on the particles to define their behavior, a complex arrangement that collectively suggests why it has taken over 250 years to begin to explain their motion.*

Particles experience forces from passing acoustic waves in two ways: drag from acoustic streaming and the force imposed directly on the particle from the acoustic wave (Marston, 2006) and any acoustic waves scattered from nearby objects (e.g., **Figure 4**) (Marston and Zhang, 2016). The acoustic wave can interact with a particle by reflecting from it or by being diffracted around it. In most cases, even in modern acoustofludics with its use of ultrasound at 1 MHz and up, the particles tend to be much smaller than the wavelength of the passing wave, becoming so-called Rayleigh particles, implying that the acoustic waves scatter from the particle as a combination of monopole and dipole effects.

Even so, the analysis of the interaction is complicated (Doinikov, 1996) and researchers often resort to remarkable simplifications for the surrounding fluid and the characteristics of the particles to produce tractable approximations. King (1934), in one of the first complete analyses of acoustic forces present on particles, assumed that the particles were not compressible. He also assumed that the surrounding fluid was incompressible and lacked any viscous effects.

The analysis was later improved with relaxation of these assumptions, with compressible particles (Yosioka and Kawasima, 1955), viscous fluids (Zhang and Marston, 2014), symmetrical (Nadal and Lauga, 2016) and asymmetrical (Zhang and Marston, 2014) particles, and the inclusion of the energy conservation equation (Karlsen and Bruus, 2015). Indeed, in Europe USWNet was established to determine how particles could be manipulated, principally via standing acoustic waves in numerous configurations. Many of the modern uses of acoustofluidics in lab-on-a-chip applications rely on live cell manipulation, from sorting (Laurell et al., 2007; Rufo et al., 2022) to separations (Ding et al., 2014; Zhang et al., 2021) and patterning (Melde et al., 2023).

Where the Discipline Is Going

Into the future, acoustofluidics will continue to grow and play an important role in the establishment of true lab-on-a-chip devices and technologies and help establish new disciplines, from batteries (Huang et al., 2022) to drug delivery (Xu et al., 2023) and tissue engineering (Melde et al., 2023), enabled through its unique capabilities of producing large, controlled accelerations at micro- to nanometer scales without contact nor risk of compatibility problems.

However, the burgeoning discipline, as always, relies on a thorough understanding of the underpinning phenomena. From Chaldni's time to Faraday, Rayleigh, Eckart, Westervelt, Shiokawa, and onward to today, the mystery of acoustofluidics has puzzled researchers and the promise it offers, these mysteries are worthy of exploration and elucidation. More questions have been answered in the discipline in the last 10 years than in the past 200. We look hopefully forward to the rapid development of the field over the next few years toward reaching vastly smaller scales, higher powers, and greater utility.

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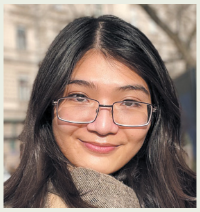

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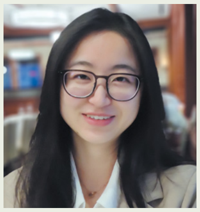

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