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Acoustic tweezers for the life sciences

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Acoustic tweezers are a versatile set of tools that use sound waves to manipulate bioparticles ranging from nanometer-sized extracellular vesicles to millimeter-sized multicellular organisms. Over the past several decades, the capabilities of acoustic tweezers have expanded from simplistic particle trapping to precise rotation and translation of cells and organisms in three dimensions. Recent advances have led to reconfigured acoustic tweezers that are capable of separating, enriching, and patterning bioparticles in complex solutions. Here, we review the history and fundamentals of acoustic-tweezer technology and summarize recent breakthroughs.

New discoveries are often preceded by technological progress. The development of cell theory, for example, is inextricably linked to advances in microscopy[1]. Just as early advances in the ability to visualize cells resulted in the development of cell theory, recent advances in the ability to manipulate single cells and biomolecules have contributed to breakthroughs in microbiology[2], molecular biology[3], biophysics[4], and bioanalytical chemistry[5].

Acoustic tweezers are an emerging platform for the precise manipulation of bioparticles across a broad size range. Acoustic tweezers spatially and temporally manipulate matter by using the interaction of sound waves with solids, liquids, and gases. The term 'acoustical tweezers' was first coined to describe the linear translation of latex spheres and frog eggs that were trapped in an acoustic field[6]. Since then, a substantial number of acoustic-tweezer configurations have been developed for applications in science and engineering. Many of these acoustic-tweezer devices are modeled after their predecessor, optical tweezers. Optical tweezers, invented in 1986 (ref. [7]), were quickly adopted as an invaluable tool in biology, chemistry, and physics, and have been used to trap viruses, bacteria, and cells[8,9]. Despite being a powerful tool for force spectroscopy and biomolecular manipulation, traditional optical tweezers require complex optics, including high-powered lasers and high-numerical-aperture objectives, and they are potentially damaging to biological samples[10,11]. To improve the accessibility and versatility of contact-free particle-manipulation technology, alternatives to optical tweezers have since been developed.

Additional platforms for contactless particle manipulation rely on different mechanisms, including magnetic[12], optoelectronic[13], plasmonic[14], electrokinetic[15,16], and hydrodynamic forces[17] (overview of the operating parameters and system requirements for these techniques in Table 1). Magnetic and optical tweezers provide the highest degree of spatial resolution; however, manipulating particles smaller than 100 nm is challenging with either technique. Plasmonic tweezers are a variation of optical tweezers that make use of locally enhanced electromagnetic fields on nanostructured substrates. Plasmonic tweezers require lower laser power and are capable of trapping nanometer-sized particles, but the large localized intensities that help to trap particles can also lead to substantial heating of the surrounding fluid[18]. As a result, thermal management of these devices is necessary to prevent sample heating and convective flows. Electrokinetic tweezers, which use both electrophoretic and dielectrophoretic forces, apply an electric field to trap and manipulate particles across the nanometer–to-millimeter size range[15,16]. However, they are dependent on particle or cell polarizability and generally require low-conductivity media, which may disrupt cell physiology. Optoelectronic tweezers are the dynamic counterpart to electrode-based electrokinetic tweezers. Instead of electrodes, a light source and photoconductive substrate induce dielectrophoresis, thus enabling dynamic manipulation at relatively low optical-power intensities[13]. However, they are constrained by the same requirement for low-conductivity media, thus restricting their use in many biological applications. Hydrodynamic tweezers are perhaps the simplest approach for achieving particle manipulation, by using fluid flows to position particles within a microchannel[17]. They are capable of a variety of applications, including trapping, focusing, and sorting, but their controllability is rather poor, and their ability to manipulate nanoparticles is limited.

Acoustic tweezers are a versatile tool that can address many of the limitations of other particle-manipulation techniques. Because acoustic waves with frequencies in the kilohertz-to-megahertz range can be easily generated[19–21], acoustic tweezers can directly manipulate particles across a length scale spanning more than five orders of magnitude (10^{-7} to 10^{-2} m). In addition, the applied acoustic power (10^{-2}–10 W/cm^2) and frequencies (1 kHz to 500 MHz) are similar to those used in ultrasonic imaging (2–18 MHz, less than 1 W/cm^2)[22], which has been safely used in diagnostic applications[21,23]. Studies on the biocompatibility of acoustic tweezers have shown that their operating parameters can be optimized to avoid damage in cells[24,25] and small-animal models[26]. For example, red blood cells placed in an acoustic-tweezer device for up to 30 min show no changes in cell viability[25], and zebrafish embryos placed in an acoustic-tweezer device for the same duration do not exhibit developmental impairments or changes in mortality rates[26]. The versatility and biocompatibility of acoustic tweezers should allow current challenges in biology and biomedicine to be addressed, such as the isolation and detection of circulating biomarkers for cancer diagnostics[27]. These biomarkers range in size from nanometer-sized extracellular vesicles[28] to micrometer-sized circulating tumor cells (CTCs)[29]. Moreover, acoustic tweezers are capable of isolating both extracellular vesicles[30] and CTCs[31], capabilities valuable for oncology laboratories. For cell-to-cell and cell-to-environment interaction studies, precise control over the physical position of cells, while preserving normal physiology, is necessary. Acoustic tweezers can form flexible 2D[32] and 3D[33] cell arrays and have been used in intercellular communication studies[34]. Furthermore, noninvasive tools for manipulating organisms are required to investigate internal

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Table 1 | Summary of different particle-manipulation platforms

Technique	Size range	Input power[a] (W/cm^2)	Spatial resolution	Labeling required	Additional system requirements
Acoustic tweezers	100 nm–10 mm	10^{-2}–10	1–10 μm	No	Acoustic source
Optical tweezers[7–9]	100 nm–1 mm	10^6–10^7	0.1–1 nm	Required for smaller particles	High-powered laser, high-numerical-aperture lens
Magnetic tweezers[12]	1 μm–10 μm	1–10 tesla	1–10 nm	Yes	Permanent magnet, superparamagnetic beads
Optoelectronic tweezers[13]	100 nm–10 μm	10^{-2}–10	1–10 μm	No	Photoconductive substrate, low-conductivity media
Plasmonic tweezers[14]	10 nm–1 μm	10^2–10^4	10–100 nm	No	Plasmonic substrate, heat sink
Electrokinetic tweezers[15,16]	1 nm–1 mm	10^4–10^7 V/m	0.1–1 μm	Yes	Prepatterned electrodes, Low-conductivity media
Hydrodynamic tweezers[17]	100 nm–1 mm	N/A	1–10 μm	No	Multiple pressure regulators, flow-control algorithm

[a] The minimum field strength is reported for magnetic and electrokinetic tweezers.

processes, such as the neuronal activity in *Caenorhabditis elegans*[35]. Acoustic tweezers have been used to manipulate and rotate *C. elegans*[36] as well as larger organisms, such as zebrafish embryos[26], with no adverse effects.

Although acoustic tweezers have been used in various biological studies, the versatility of acoustic tweezers has proven to be a double-edged sword. Currently, many different acoustic-tweezer platforms are available, each with advantages and shortcomings; however, for researchers who are not technical experts in the field, identifying the acoustic-tweezer technology best suited for a particular application is difficult. For example, for manipulating nanometer-sized objects, should an acoustic-tweezer device based on surface acoustic waves (SAWs) or bulk acoustic waves (BAWs) be used? Which acoustic-tweezer platform is best for handling large volumes of biofluids? What if precise control over a particle's position in three dimensions is required? In this review, we hope to answer these questions by categorizing the different types of acoustic tweezers and identifying their strengths and weaknesses. We review recent advances in the field and conclude with an outlook for future development.

Operating principles of acoustic tweezers

The three primary types of acoustic tweezers are standing-wave tweezers, traveling-wave tweezers, and acoustic-streaming tweezers. Both standing-wave and traveling-wave tweezers manipulate particles or fluids directly via an applied acoustic radiation force, whereas acoustic-streaming tweezers indirectly manipulate particles via acoustically induced fluid flows. The characteristics of each type of acoustic tweezers, including advantages, disadvantages, and suitable applications, are listed in Table 2.

Standing-wave tweezers. Standing-wave tweezers can be divided into two subtypes, BAWs and SAWs, according to their method of acoustic-wave generation. BAWs use piezoelectric transducers to convert an electrical signal into mechanical waves. They are widely used for particle and cell manipulation by forming resonance patterns inside channels[37] (Fig. 1a). Acoustic waves reflected from the reflection layer form standing waves and establish a pressure distribution in the fluid. Through adjustment of the frequency with respect to the dimensions of the channel geometry, the number of pressure nodes and antinodes in the channel can be tailored[38]. The periodic distribution of pressure nodes produces acoustic radiation forces that determine the trajectories and positions of particles inside these resonators. SAWs, in contrast, are commonly generated by interdigitated transducers (IDTs) patterned on a piezoelectric surface[39]. 1D and 2D interference patterns can be achieved by using sets of two and four IDTs, respectively[39,40] (Fig. 1b). Suspended particles in a standing SAW field move to pressure nodes or antinodes according to their physical properties[41]. In addition to 2D in-plane manipulation, standing SAWs are used to achieve 3D manipulation by exploiting the modulation of acoustic parameters (for example, phase shifts and amplitude modulation), thus enabling the trapping position to be changed in real time[33]. Owing to their compact size, SAW-based tweezers can be conveniently integrated with microfluidic systems enabling versatile lab-on-a-chip tools[40].

Standing-wave tweezers are mainly used for separating and patterning different types of particles and cells. Whereas BAW-based standing-wave tweezers have the advantage of handling higher volumes of fluids in a shorter time, as is desirable for blood processing in transfusion applications, SAW-based tweezers have higher precision, owing to the higher frequencies used[42], thus rendering them more suitable for nanoparticle manipulation and tissue-engineering applications.

Travelling-wave tweezers. Travelling-wave tweezers, which consist of two subgroups, active and passive methods, are able to form arbitrary pressure nodes in 3D space by controlling the phase patterns of the acoustic waves. Active traveling-wave tweezers make use of a single acoustic-transducer element or an array of elements[43–45]. By selectively controlling each individual element in an array, active methods can produce complex acoustic beams that result in dynamic manipulation capabilities (Fig. 1c). Passive methods use structures with features that are smaller than the acoustic wavelength, such as acoustic metamaterials and phononic crystals, to manipulate the acoustic waves[46–48]. Passive methods are an inexpensive approach for modulating acoustic waves and forming complex beam patterns (Fig. 1d). SAW-based traveling-wave tweezers featuring a single IDT are mainly used for on-chip cell and particle manipulation in sorting applications. Compared with standing-wave tweezers, traveling-wave tweezers can more easily be modulated in real time and are better suited for applications requiring arbitrary patterning or single object handling (e.g., cell printing or single-cell analysis).

Acoustic-streaming tweezers. The steady flow generated by the absorption of acoustic energy by the liquid can also be used to indirectly manipulate particles in a solution[49,50]. This flow, termed acoustic streaming, is most commonly generated via oscillating microbubbles or oscillating solid structures. Oscillating microbubbles can produce sufficient acoustic radiation forces to trap

Table 2 | Different types of acoustic tweezers

Type	Subtype	Advantages	Disadvantages	Applications
Standing-wave tweezers	Surface acoustic waves[40]	Precision (for example, the ability to manipulate nanoparticles);simple, compact, inexpensive devices and accessories	Low throughput (<1 mL/min); limited acoustic-field pattern	Nanoparticle manipulation, cell separation, cell patterning, cell concentration, 3D translation and rotation
	Bulk acoustic waves[71]	High throughput (e.g., 10 mL/min)	Limited precision; excessive heat generated due to high power	Cell separation, sample preparation, levitation of cells and small organisms
Traveling-wave tweezers	Active[43]	Flexibility (i.e., the ability to rewrite the acoustic field in real time)	Typically multiple transducers needed; multiplexed transmission system needed	Cell sorting, real-time cell patterning for bioprinting and tissue engineering, 3D translation and rotation of cells and droplets
	Passive[46]	Simple, easily fabricated structures;simple electronic control scheme	Generation of only a few acoustic-field patterns with one structure; complex simulation and calculations needed	Cell patterning, levitation of droplets, high-resolution ultrasonic imaging
Acoustic-streaming tweezers	Bubble based[36,52,72]	Selective frequency actuation	Unstable bubble size; limited reproducibility	Fluid mixing and pumping, 3D rotation of cells and small organisms, neural stimulation
	Solid structure based[53,54]	Stability and reproducibility; ability to handle highly viscous fluids (for example, blood and sputum)	Limited vibration patterns	Fluid mixing and pumping, 3D rotation of cells and small organisms

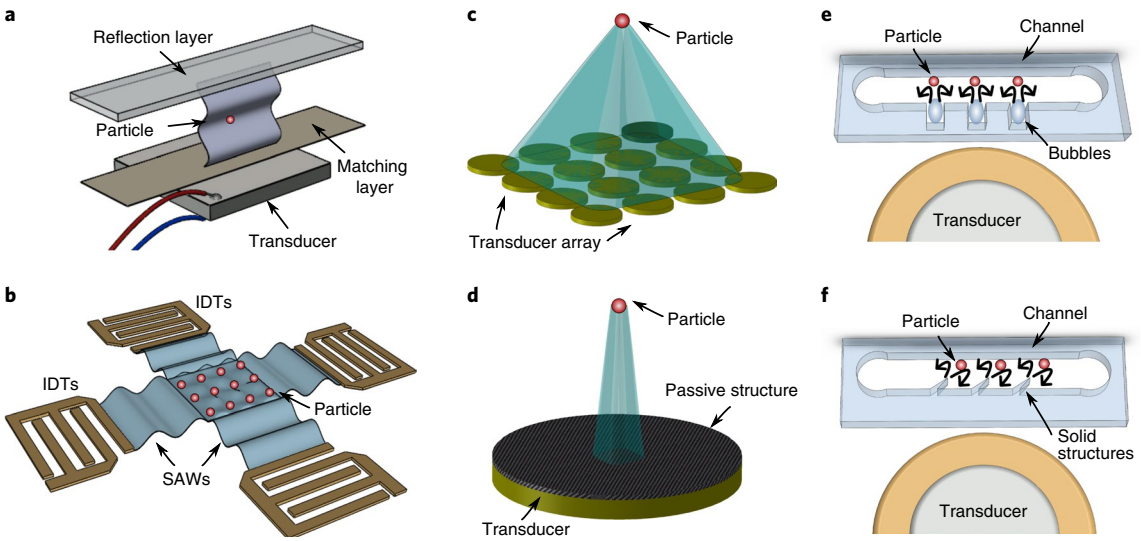

Fig. 1 | Illustrations of various acoustic-tweezer technologies. a, A typical BAW-based standing-wave tweezer device. The number of pressure nodes and antinodes inside the channel is determined by adjusting the applied acoustic wave frequency with respect to the distance between the matching layer and the reflection layer. **b**, SAW-based standing-wave tweezers use IDTs to generate mechanical waves. Four sets of IDTs are used to generate a 2D pressure-node field that traps and patterns particles. **c**, Active traveling-wave tweezers with a transducer array to manipulate particles. By controlling the relative phase of the acoustic wave from each transducer, flexible pressure nodes can be formed to achieve dynamic patterning. **d**, Passive traveling-wave tweezers with a single transducer to achieve complex acoustic distributions and control over particles. **e**, Acoustic-streaming tweezers use oscillating microbubbles inside a microfluidic channel to generate out-of-plane acoustic microstreaming flows. **f**, Solid-structure-based acoustic-streaming tweezers generate a directional fluid flow under acoustic excitation.

cells, particles, or small organisms on the bubble surface[52] (e.g., the magnitude of the acoustic radiation forces to move red blood cells is approximately 2 pN (ref. [51])) (Fig. 1e). Streaming vortices created by oscillating bubbles can also rotate particles at a fixed position[36] and enable fluidic actuation by enhancing mass transport across laminar flows in confined microchannels[52]. Similarly to microbubbles, acoustically driven sharp-edge structures or thin membranes oscillate in a liquid (Fig. 1f), thus resulting in acoustic streaming,

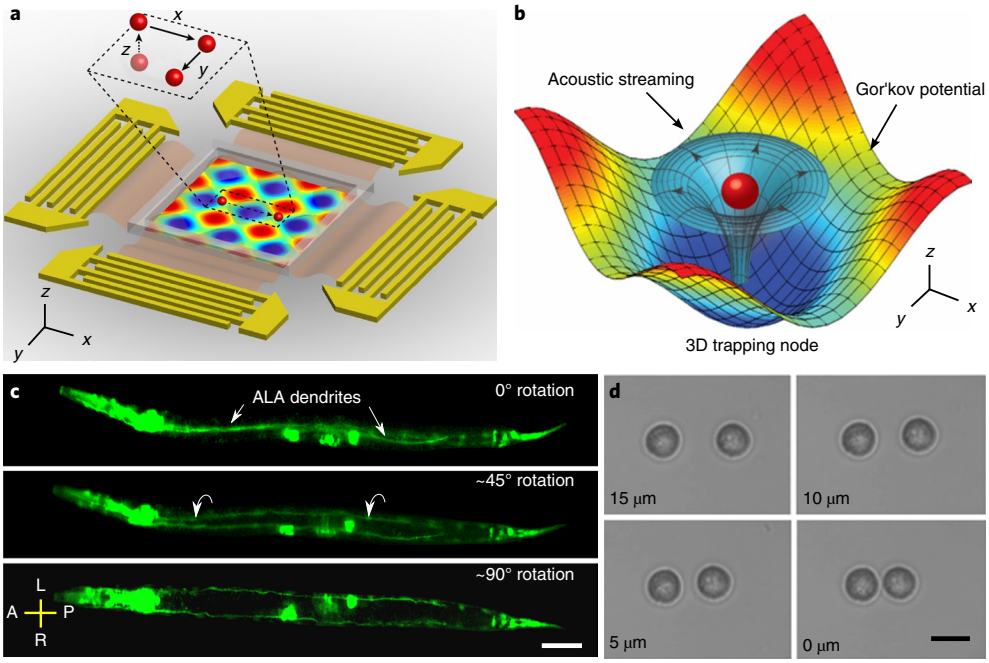

Fig. 2 | Acoustic manipulation of various sample sizes and types. a, Two pairs of IDTs are configured to generate a planar standing-wave field. The inset demonstrates the path of a single particle in 3D[33]. **b**, Numerical simulation results show the mapping of the acoustic field around a single particle that demonstrates the operating principle for 3D manipulation with standing-wave tweezers[33]. **c**, Acoustically driven microbubbles are used to trap and rotationally manipulate *C. elegans* under a fluorescence microscope to visualize ALA-neuron dendrites that are overlapping in the dorsoventral view[36]. A, anterior; P, posterior; L, left; R, right. Scale bar, 40 μm. **d**, Two HEK 293T cells are manipulated toward each other and brought into contact for intercellular-communication applications[34]. Scale bar, 20 μm. **a**, **b**, and **d** are reprinted with permission from refs [33,34], respectively, National Academy of Sciences. **c** is reprinted with permission from ref. [36], Springer Nature.

owing to viscous attenuation. These streaming flows generate regions of recirculation or pressure gradients that can be used in particle manipulation, fluid mixing, and pumping applications[53,54]. Acoustic-streaming tweezers tend to be simple devices that are easy to operate; however—in contrast to traveling-wave tweezers, which can be used in liquids and in air—acoustic-streaming tweezers can operate only in liquids. In addition, acoustic-streaming tweezers offer a lower degree of spatial resolution, because microbubble- and microstructure-based phenomena are nonlinear. These tweezers are primarily used for fluid handling[55], such as pumping or mixing of highly viscous fluids, or rotational manipulation applications (Table 2).

Versatility of acoustic tweezers

The primary advantage of acoustic tweezers stems from their ability to perform a diverse set of particle and fluid manipulations. Although other platforms, such as optical and magnetic tweezers, offer superior spatial resolution (Table 1), acoustic tweezers provide a versatile, noninvasive, and highly scalable approach for performing complex manipulations of different biological targets.

From 1D to 3D translation. Acoustic tweezers enable three degrees of freedom in manipulating samples. Although optical, magnetic, and electrokinetic tweezers can also achieve 3D manipulation, acoustic tweezers provide a versatile label-free approach that is independent of the dielectric or magnetic properties of samples and media[19,21,56–58]. The simplest mode of acoustic tweezing is to push inclusions to pressure nodes or antinodes depending on their relative densities with respect to the medium. This mode of manipulation occurs in 1D, by using one set of parallel IDTs, and is commonly used to focus[59], sort[60,61], and separate[41] particles and cells. By controlling the position of the pressure nodes in a standing-wave tweezer by using two sets of orthogonally positioned IDTs, the inclusions inside the liquid are manipulated along any user-defined path in a 2D plane[33] (Fig. 2a). Furthermore, the position along the *z* axis can be controlled by exploiting SAW-generated streaming, which enables complete 3D-manipulation capabilities inside a liquid domain[33] (Fig. 2b). SAW-based standing-wave tweezers can be used for dynamically printing complex patterns of cells[33,34] and for heterogeneous layer-by-layer tissue engineering[62]. Off-chip manipulation capabilities of standing-wave tweezers through use of ceramic piezo transducers have been applied to in vivo cell manipulation inside blood vessels[59]. This approach can be adapted for in vivo flow cytometry applications, especially for studying human diseases in animal models.

From translational to rotational motions. Acoustic tweezers enable rotational manipulation of cells, microstructures, droplets, and model organisms[36,44,63–65]. For example, SAW-based traveling-wave tweezers achieve a fast rotation of liquid droplets that can be used for cell lysis and real-time polymerase chain reaction in a miniaturized setting[63]. Microstreaming flows generated by acoustic-streaming tweezers enable rotational manipulation of cells and organisms for 3D optical imaging applications. By gradually rotating *C. elegans* via acoustic-streaming tweezers[36] (Fig. 2c), green fluorescent protein–expressing cells that appear to overlap in a single view can be resolved and clearly imaged.

From millimeter to micrometer to nanometer scales. Acoustic tweezers enable manipulation of samples with sizes from 100 nm up to 10 mm, a range that no other manipulation method is capable of (Table 1). Generally, acoustic tweezers with lower frequencies are better suited for samples with millimeter sizes, owing to the larger forces and spot sizes achievable[43,66,67]. Cells and nanoparticles are

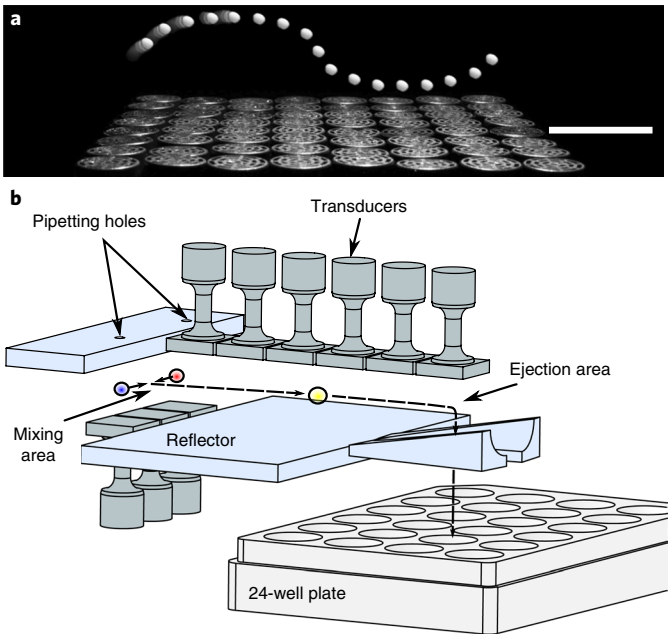

Fig. 3 | Acoustic manipulation of single particles and droplets. a, A polystyrene particle is levitated and moved in 3D by controlling the phase difference in active traveling-wave tweezers[43]. Scale bar, 20 mm. **b**, Acoustic-based droplet manipulation in an open system is demonstrated. Two droplets that are pipetted from the holes are transported, mixed, and ejected into a 24-well plate[66]. **a** and **b** are reprinted with permission from refs [43,66], respectively, Springer Nature.

better handled by SAW-based acoustic tweezers, which provide higher frequencies, smaller active regions, and better precision[30,68]. Acoustic tweezers are commonly used to manipulate millimeter-sized objects, such as *C. elegans*[36,69] (Fig. 2c), and micrometer-sized objects, such as cells[34] (Fig. 2d), because the forces generated by acoustic tweezers scale well across micro- to millimeter length scales. In addition, isolation of ~100-nm exosomes from whole blood[30] has been achieved.

Although acoustic tweezers are commonly integrated into microfluidics to achieve high precision in a miniaturized platform, they can also be scaled up into macrofluidic applications. This feature enables various biomedical applications such as blood transfusions, tissue engineering, and drug discovery, in which high-throughput handling of a large number of particles is needed. Acoustic separation of platelets from whole blood with a throughput of 10 mL/min and a greater than 80% removal rate of red and white blood cells, and recovery rate of platelets, has been achieved[70].

From particles to droplets to bulk fluids. Compared with other particle-manipulation technologies, acoustic tweezers can manipulate a wider spectrum of sample types, including particles inside droplets[71], bulk fluids[72], and air[43]. Simple yet functional on-chip fluid actuation applications have also been realized by oscillating microbubbles and sharp-edged solid microstructures[53,73]. As a general guideline, for on-chip[53,73] and on-surface[74,75] fluid-manipulation applications, acoustic-streaming tweezers are more suitable. For open-system fluid and particle manipulation, the levitation capabilities of standing-wave and traveling-wave tweezers can be applied[76]. For instance, a 2-mm polystyrene particle can be levitated and moved along a 3D path by using traveling-wave-based acoustic tweezers[43] (Fig. 3a). Similarly, droplets can also be levitated, moved, and merged in mid-air, thus enabling off-chip fluid handling and sample-preparation applications[66,67] (Fig. 3b). Here, the sorting of droplets into a 24-well plate demonstrates the ease with which acoustic tweezers can be integrated with existing tools in biology and medicine.

Applications of acoustic tweezers in biology and medicine

The versatility of acoustic tweezers enables them to address current challenges in biology and medicine. From the large-scale isolation of CTCs to the manipulation of individual proteins, acoustic tweezers are becoming an attractive alternative to conventional particle- and fluid-manipulation tools in areas ranging from diagnostics to single-molecule studies.

Isolation of circulating biomarkers. Recently, the 'liquid biopsy', a noninvasive means of evaluating patient health through the collection and analysis of circulating biomarkers, has been identified as a potentially transformative technology in biomedical research[77]. Circulating biomarkers, including CTCs[29], cell-free DNA[78], and exosomes[79], are recognized as promising biological targets for the development of liquid biopsies for both diagnostic and prognostic applications. One of the primary obstacles in the development of liquid biopsies is the isolation of circulating biomarkers. The versatility of acoustic tweezers has allowed them to be used for label-free, size-based isolation of both CTCs and exosomes.

SAW-based standing-wave tweezers have been used to successfully isolate CTCs from blood samples taken from patients with metastatic breast cancer[31]. This approach has also been used to isolate exosomes from whole blood[30] (Fig. 4). In this configuration, consecutive acoustic-tweezer modules are integrated onto a single microfluidic chip. The first module removes all blood components larger than 1 μm, including platelets and red and white blood cells; the second module isolates exosomes from other extracellular vesicles (diameter greater than 140 nm). The cell-removal rate of this device exceeds 99.999%, thus producing isolated exosome samples with a purity of ~98% and a yield of ~82%. This ability of acoustic tweezers to isolate exosomes with both high purity and high yield holds promise for future diagnostic applications and studies seeking to uncover new exosome-related biomarkers for different disease states.

Single-cell analysis. The field of single-cell analysis aims to observe complex cellular properties that may be masked by conventional population-averaging assays. In many single-cell-based studies, manipulation techniques are required to position cells before analysis and to ensure identical optical-interrogation conditions for each cell. Owing to their noninvasive nature, acoustic tweezers have been extensively used to conduct cell manipulations for single-cell analysis, particularly in applications in which preserving normal cell physiology after manipulation is desirable.

Trapping and patterning cells in large 2D arrays is one strategy used to observe the behavior of cells over time in response to environmental stimuli. This approach has been used to study topics ranging from cell–cell interactions[34] to the transfer of viruses between cells[42]. However, most acoustic-tweezer platforms trap clusters of cells rather than individual cells when forming 2D arrays, thus limiting their use in true single-cell studies. Recently, gigahertz frequencies of standing SAWs have been used to generate 2D patterns of individual cells (Fig. 5)[42]. In that work, a small number of *Plasmodium falciparum*–infected red blood cells were observed after 2D patterning (Fig. 5d) to study pathogen biology. The ability to trap individual cells in 2D arrays shows promise for the use of acoustic tweezers in future studies of cell-to-cell, cell-to-bacterium, and organism-to-bacterium interactions.

Single-molecule analysis. The study of biomolecules at the individual level can provide insights into the forces and motions associated with biological processes. Conventional tools for single-biomolecule analysis include optical tweezers, magnetic tweezers, and

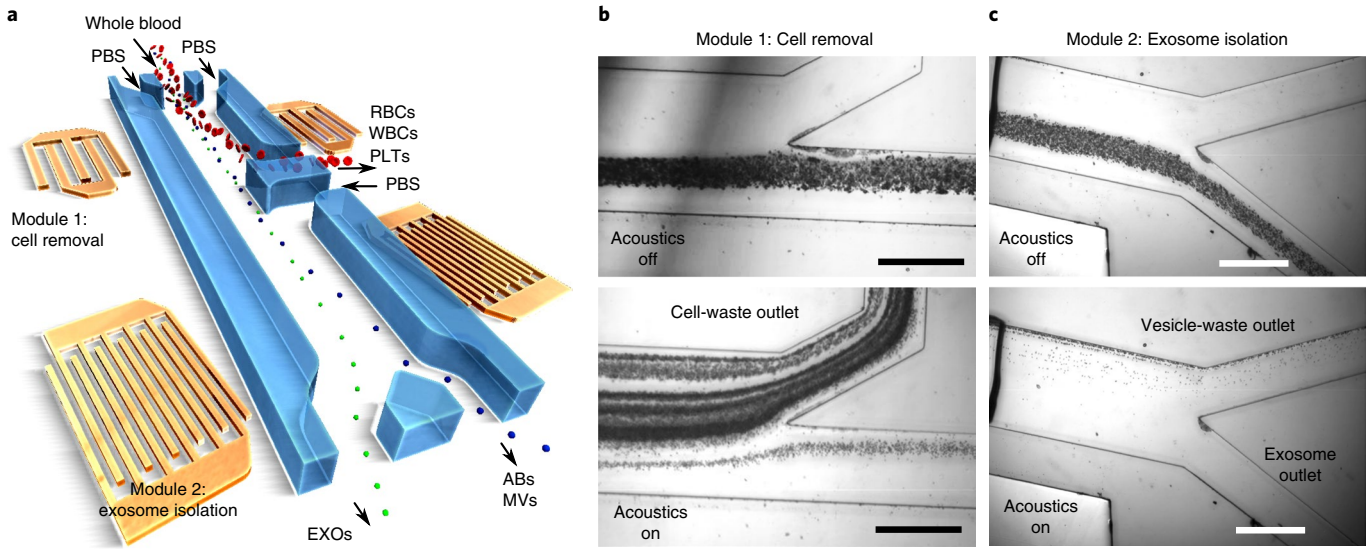

Fig. 4 | Acoustic isolation of exosomes from whole blood[30]. a, A schematic depiction of exosome isolation via standing-wave tweezers. Red blood cells (RBCs), white blood cells (WBCs), and platelets (PLTs) are filtered by the cell-removal module, and then subgroups of extracellular vesicles (ABs, apoptotic bodies; MVs, microvesicles; EXOs, exosomes) are separated by the exosome-isolation module. **b**,**c**, Images were taken under a microscope at the cell-removal module (**b**) and the exosome-isolation module (**c**) of the device. **b**, RBCs, WBCs, and PLTs are shown to be pushed to the cell-waste outlet in the cell-removal module. **c**, Exosomes are separated from microvesicles and apoptotic bodies at the exosome-isolation module. Scale bars, 500 μm. Reprinted with permission from ref. [30], National Academy of Sciences.

atomic force microscopy. However, the complexity of these instruments has largely confined their use to highly specialized laboratories. In addition, most of these tools are inherently low throughput, capable of analyzing only one molecule at a time. Recently, acoustic tweezers have entered the field of single-molecule analysis, thus providing a low-cost, high-throughput alternative for conducting studies on nucleic acid molecules and proteins[80]. In this approach, one end of a molecule is tethered to a glass microchamber, and the other end is attached to a microsphere. When a standing wave is applied to the chamber, the microsphere moves toward well-defined pressure nodes within the chamber and stretches the molecule of interest. By comparing the displacement of the bead with the magnitude of the applied force, insights into the bond strength of the molecule, along with its conformational properties, can be obtained. This approach, termed acoustic force spectroscopy, is capable of applying forces ranging from 0.3 fN to 200 pN (ref. [81]). Magnetic tweezers and atomic force microscopy are slightly more versatile in this regard, being capable of applying forces ranging from 0.01–10^4 pN and 10–10^4 pN, respectively[82]. However, because acoustic force spectroscopy can simultaneously apply forces to thousands of microspheres, it can achieve much higher throughput than its conventional counterparts, which typically manipulate only one particle at a time.

Conclusions and perspectives

There are five main factors contributing to the versatility of acoustic tweezers: (i) the ability to manipulate both fluids and particles in fluids; (ii) the ability to manipulate particles, regardless of geometric, electrical, magnetic, or optical properties, in a variety of different media (for example, air, aqueous solutions, undiluted blood, and sputum); (iii) the ability to manipulate particles, cells, and organisms across a wide range of length scales, from nanometers (for example, exosomes and nanowires) to millimeters (for example, *C. elegans*); (iv) the ability to select and to manipulate a single particle or a large group of particles (for example, billions of cells); and (v) the ability to handle fluidic throughputs ranging from 1 nL/min to 100 mL/min. The simplicity and biocompatibility of acoustic tweezers make them a versatile platform capable of handling a wide range of applications in biology, biophysics, and medicine.

Despite their favorable traits, substantial technological limitations must be addressed before acoustic tweezers can be readily adopted by the scientific and medical communities. For example, one major drawback of current acoustic tweezers is their limited spatial resolution. It is challenging for acoustic tweezers to reach as high a frequency as optical tweezers can, thus limiting the precision of acoustic tweezers. Various research efforts related to metamaterials and phononic crystals are currently being developed that can overcome the diffraction limit and increase the resolution to be smaller than half of the wavelength[46–48]. This improvement can substantially improve the precision of the acoustic tweezers without increasing the frequency. These new concepts could be implemented to enable the manipulation of an individual cell among many others and enable the creation of heterotypic cell assemblies with customized properties (i.e., prescribed cell type, cell number, cell–cell distance, and cell organization).

In addition to the technological innovations to improve acoustic tweezers, more in-depth and comprehensive research is needed to characterize their influence on the structures, properties, and functions of the specimens manipulated by acoustic tweezers. Published research efforts have supported the biocompatibility of acoustic tweezers[30,31]. However, these efforts are limited to a specific acoustic system, and the parameters used in those studies cannot be used as a reference for different acoustic-tweezer platforms. To further promote the adoption of acoustic tweezers by the biology and medical communities, more standardized characterization parameters should be examined to quantify their effects on specimens, such as the acoustic pressure and associated fluidic shear stresses on each cell, and the subsequent gene and protein expression after acoustic irradiation. As more device-standardization and specimen-characterization data become available, researchers will gain confidence in using acoustic tweezers to probe more delicate and intriguing biological processes and investigate problems in cancer–immune cell interactions, pathogen–host interactions, and developmental biology.

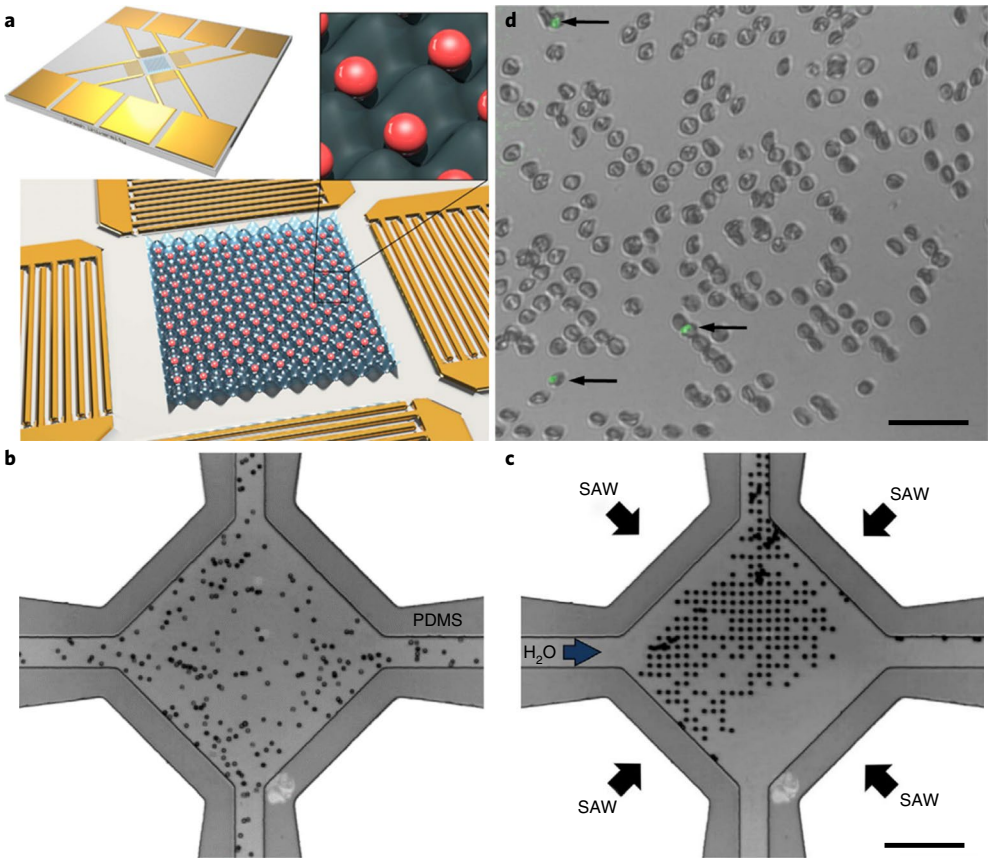

Fig. 5 | Acoustic-based 2D single-cell patterning[42]**. a**, Schematic depiction of a single-cell-patterning device with one cell per pressure node. **b**, 6.1-μm polystyrene particles suspended in water are introduced inside a microchannel. PDMS, polydimethylsiloxane. **c**, After the acoustic field with a frequency of 171 MHz is turned on, particles are patterned as one particle per acoustic well. Scale bar, 100 μm. **d**, A sample of red blood cells patterned in 2D easily revealed cells infected with the green fluorescent protein–expressing malarial parasite *P. falciparum*. Scale bars, 40 μm. Reprinted with permission from ref. [42], Springer Nature.

Although acoustic tweezers have been increasingly used in the manipulation of cells, particles, and organisms, most of the literature has focused only on in vitro applications. In principle, acoustic tweezers have potential for in vivo manipulation of cells or foreign objects, owing to the noninvasive and deep-tissue-penetration characteristics of sound waves. From targeted drug release to neuron activation, acoustic tweezers may have potential effects on in vivo medical research and eventually on clinical applications. The interdisciplinary nature of this field allows scientists from various backgrounds to contribute innovative ideas and solutions. These favorable attributes and emerging applications should enable acoustic tweezers to play critical roles in translating innovations in technology into advances in biology and medicine.

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Competing interests

T.J.H. has four US patents (patent nos. 8,573,060; 9,608,547; 9,606,086; and 9,757,699) related to acoustic tweezers. He also cofounded a start-up company, Ascent Bio-Nano Technologies Inc., to commercialize technologies involving acoustic tweezers.

Additional information

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