Potential of the acoustic micromanipulation technologies for biomedical research

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ABSTRACT
Acoustic micromanipulation technologies are a set of versatile tools enabling unparalleled micromanipulation capabilities. Several characteristics put the acoustic micromanipulation technologies ahead of most of the other tweezing methods. For example, acoustic tweezers can be adapted as non-invasive platforms to handle single cells gently or as probes to stimulate or damage tissues. Besides, the nature of the interactions of acoustic waves with solids and liquids eliminates labeling requirements. Considering the importance of highly functional tools in biomedical research for empowering important discoveries, acoustic micromanipulation can be valuable for researchers in biology and medicine. Herein, we discuss the potential of acoustic micromanipulation technologies from technical and application points of view in biomedical research.

I. INTRODUCTION

Technological advancements have been historically tied to new scientific discoveries. There has been a constant quest to invent new engineering tools to facilitate research efforts for emerging and more challenging problems in biology and medicine. Pioneered by the early work of Spallanzani on non-audible sound waves inspired by bats, ultrasonic waves have been used in medical imaging since the early 1900s. Analogous to optical tweezers, acoustic waves were also employed to spatially maneuver microscopic samples, and hence the term “acoustical tweezers” emerged. Since then, acoustic tweezers have been widely applied in numerous biomedical applications within the last two decades.

Acoustic micromanipulation technologies (AMTs) represent a group of tools that apply acoustic waves to control the behavior of solid particles and fluids on a scale ranging from nanometers to millimeters. In various types of AMTs, acoustic waves are tailored in many ways to achieve specific manipulation capabilities intended for a particular application. For example, when requiring precision, and working with small sample volumes, surface acoustic waves (SAWs) from MHz to GHz frequencies are applied in microfluidics to exert nanonewtons to femtonewtons forces on suspended particles. On the other hand, bulk acoustic waves (BAWs) from kHz to MHz frequencies are implemented in high-throughput continuous-flow cell separation and sorting applications.

Optical tweezers employ focused laser beams to achieve micromanipulation with better spatial resolutions and single cell/particle selectivity compared to AMTs. However, complexity of the optical setups and potential damage to manipulated cells are the main limitations of optical tweezers. Besides, AMTs with similar single-cell selectivity and maneuvering were recently realized with new technical advancements in wave generation and modulation capabilities. Compared to other methods including optical, electrical, magnetic, and hydrodynamic tweezing, AMTs acquire a set of prominent characteristics arising from the nature of the sound waves and their interactions with the environment. One of the most significant attributes of AMTs arises from the fact that sound waves can penetrate and propagate within matter without significant adversity. This capability is an important precursor for any method to be applied for in vivo biological studies. While AMTs can be tuned to interact and alter cell functions for therapeutic applications, they can also be used for gentle handling of single cells for cell isolation and manipulation studies. This dexterity qualifies AMTs as a group flexible tools for applications in fundamental biology and clinical therapies. A general comparison of the major micromanipulation methods is given in Table I.

In various micromanipulation methods, intrinsic properties of samples are critical to permit precise maneuvering. For example, dielectric constant, electrical conductivity, and magnetism properties of particles are important for their manipulation capacity in optical, electrical, and magnetic tweezers. In the absence of these inherited material properties, labeling is needed for the mentioned tweezing methods to provide spatial control of a particle.
on the other hand, can be used for micromanipulation without any label requirements. For example, in cell separation studies, different cells can be diverted to different streams by exploiting their physical differences. Similarly, in two-dimensional AMTs, cells or particles can be moved in any desired path irrespective of their inherent properties. This is a considerable advantage of AMTs compared to most of the other micromanipulation methods. Overall, the AMT stands as a set of versatile tools for biomedical research and medical applications including single-cell biology, neurostimulation, and cancer cell isolation.

There are several recent reviews highlighting different aspects and applications of AMTs. For example, Chen et al. presented a comprehensive review of micromanipulation capabilities enabled by vibration-induced acoustic waves and acoustic streaming. In their work, different mechanisms of vibration-induced acoustic streaming flows and their applications were discussed in detail. Mohanty et al. reported an extensive review of AMTs with an emphasis on their technical details. They discussed various acoustically powered autonomous agents and categorized the acoustic manipulation approaches as active and passive based on their working principles. Kolesnik et al. introduced a critical review of specifically tailored acoustic approaches to achieve localized and purpose-driven acoustic micromanipulation abilities, and their applications in cell manipulation, tissue engineering, and medical diagnostics. New AMTs have been constantly developed and the existing ones improved but they are mostly applied in proof of concept applications. There are a substantial number of research papers and patents on these technologies, yet there is only a limited number of successfully launched commercial AMTs that are available to biomedical researchers. Although there is a tremendous potential of AMTs, it is not fully implemented to achieve widespread adoption in the life sciences community. It is imperative to resolve existing limitations and challenges of AMTs to increase the number of viable commercial acoustic micromanipulation tools. The existing reviews generally focus on technical details and applications of acoustic systems. In the current perspective, limitations, challenges, and versatility of AMTs are discussed, and a future perspective is presented for biomedical research.

II. PRINCIPLES OF ACOUSTIC MICROMANIPULATION

A. Acoustic transducers and wave generation

Herein, the term “acoustics” is used to represent the entire spectrum of devices employing mechanical vibrations from a few kHz up to tens of GHz frequencies. The fundamental mechanism of acoustic wave generation mainly relies on the generation of elastic deformations in a medium. For this, piezoelectric materials are fabricated into acoustic transducers to generate acoustic waves. Lasers and oscillating electric fields are generally applied as means of excitation sources to generate mechanical vibrations within a piezoelectric material. A basic acoustic transducer architecture involves either a piezoelectric material sandwiched between two metal electrodes or two arrays of comb-like metal electrodes fabricated on a piezoelectric surface to form interdigitated transducers (IDTs). It is convenient to group acoustic waves into bulk and surface acoustic waves (BAW and SAW) based on the nature of acoustic wave generation and propagation. The shape of the wavefront and the frequency of acoustic waves are defined by the geometry of the transducers. The resonance frequency of an acoustic transducer is defined by dimensions of the piezoelectric material and pitch of metal electrodes in BAW- and SAW-based systems, respectively. Acoustic waves can propagate in a medium as traveling waves, or two counter-propagating acoustic waves can interfere and form standing acoustic waves. Interactions of acoustic waves with fluids media and suspended inclusions generate acoustic radiation forces, which are employed in AMTs to trap and maneuver objects. For example, when SAWs leak into a liquid, a pressure distribution emerges with minimum and maximum pressure regions, which attracts or repels particles based on their relative density compared to that of the medium. In general, the acoustic radiation forces can be categorized as primary and secondary radiation forces resulting from the direct and indirect interactions with the acoustic field.

New concepts and innovations in fabricating acoustic transducers have yielded novel capabilities for AMTs. For example, the concept of semi-circular IDTs was applied in forming propagating SAWs that were focused to a small region enabling localization of acoustic energy for high precision sorting applications. Similarly, chirped IDTs enabled a band of active frequencies to spatially maneuver objects by tuning the applied frequency and exiting the corresponding region of the IDTs. In a more recent example, a configuration of multiple IDT pairs was introduced as a wave-number spiral to permit dynamic reformatting of a SAW field to actively control acoustic wave interactions. In this design, the driving frequency of selected IDT pairs was tuned to spatially control the acoustic wave distribution using a spiral of IDTs with gradually changing the wavenumbers. Discoveries of new modes of acoustic transducers can incite new capabilities of wave generation and manipulation.
modulation functions to further advance the versatility of AMTs. While significant progress has been shown in the design and fabrication of new types of acoustic transducers, there is still room for improvements and innovation. For example, IDTs of existing SAW-based AMTs require extensive use of cleanroom facilities and multiple steps of lithography and metal depositions. New approaches to the fabrication of acoustic transducers can permit the development of simple AMTs suitable for low-cost production. There have been some attempts to alleviate the complexity of the IDT fabrication process. In these approaches, low-melting-temperature metals were injected in a network of microfluidic channels to form IDTs, and acceptable acoustic tweezing performances were reported compared to the droplet and liquid manipulation efficiency of the traditional IDTs. Despite the efforts to reduce the complexity of cleanroom processes in these works, there are still considerable fabrication requirements for microfluidic channels that were used to form the liquid metal electrodes. As an alternative solution, the advancement of direct printing technologies could be exploited to directly print IDTs on piezoelectric substrates. This could be a feasible solution to circumvent costly and multi-step IDT fabrication requirements.

B. Different acoustic micromanipulation technologies

Acoustic resonators are generally fabricated by piezoceramics and glass-like hard materials. By matching half-integer multiples of acoustic wavelength to a certain dimension of a microfluidic channel, resonance condition can be obtained, and minimum and maximum pressure regions (nodes and antinodes) are established. In these resonators, larger volumes of sample fluids can be rapidly processed, and usually higher throughputs for cell separation can be achieved at the cost of lower purity compared to SAW-based tweezers. A commonly practiced acoustic resonator fabrication scheme involves etching a silicon wafer and applying anodic bonding to realize the microfluidic channel sealed with a glass cover. Then, appropriate matching and reflection layers are stacked along with a piezotransducer and the microfluidic channel to obtain a complete resonator. The entire process is fairly complex, and due to the associated costs of fabrication and the materials, these types of acoustic resonators cannot be considered disposable, which is one of the traits of lab-on-a-chip systems that aim to prevent contamination and enable low-cost sample processing. An alternative method is to use disposable glass capillaries to form the resonators. This method can eliminate the intricacy of the microfluidic device fabrication steps. With the advancement of 3D printing and increasing number of available materials to print, acoustic resonators can be even more reachable and easy to fabricate by incorporating 3D printed, detachable, and disposable fluidic channels. With this approach, a variety of resonance conditions can be met within the same microfluidic channel by printing more complex structures for different purposes. For example, a single piezotransducer can be used to achieve both cell focusing and separation simultaneously, rather than applying multiple transducers driven at different frequencies.

SAW-based tweezers constitute an important group of AMTs that employ surface acoustic waves in various means to effectively manipulate single cells, particles, fluids, and organisms. It is possible to group SAW-based tweezers into traveling and standing wave devices depending on the application of acoustic waves. In traveling SAW devices, acoustic waves emanating from a source travel on a piezoelectric substrate and interact with fluids and suspended particles. This approach is frequently implemented in cell sorting applications to deflect single cells rapidly. In standing SAW devices, counter-propagating waves interact with each other to form pressure nodes. Particles or cells can be trapped at these nodes and manipulated by moving the position of the nodes through phase modulation. Piezoelectric substrates such as lithium niobate wafers are widely used to fabricate IDTs in SAW devices. Due to the cost of the substrate and the fabrication, removable microfluidic channels and glass capillaries have been adopted in SAW-based tweezers. Several innovative technologies of SAW-based tweezers have emerged by novel designs and implementation of IDTs resulting in new functions including tunable resonance behavior, wave-front focusing, tilted pressure nodal lines, multitoned excitations, and digital acoustofluidics. Owing to their higher frequencies, SAW-based tweezers provide greater precision by focusing acoustic energy in a narrower region. Smaller wavelengths associated with up to GHz frequencies enable single-cell level manipulation and patterning capabilities. In addition, nanoparticles and nano-sized biological species such as extracellular vesicles and exosomes could be maneuvered for enrichment and isolation purposes. SAW-based tweezers hold great potential for intracellular manipulation capability that can enable sub-cellular level resolution for single cell-related research.

Acoustic levitation is a method to suspend solid particles or liquid droplets in mid-air by applying sound waves. A single acoustic source (with a reflector) or two counter-positioned sources are used to establish pressure nodes to trap and suspend objects in air. Acoustic tractor beams are also demonstrated by using acoustic metamaterials and bending sound fields to enable single-beam levitation. Through the modulation of acoustic waves, the trapped samples can be linearly or rotationally manipulated. Another exciting application of acoustic levitation and metamaterials is generating acoustic holograms. In this mode of acoustic levitation, a complex pattern of particles can be formed instantly, which can enable high-throughput and complex patterning of cells for cell printing and tissue engineering applications. Even though it stands as a versatile non-contact manipulation method, there are not many biological applications done by acoustic levitation. Especially for in vivo applications, acoustic levitation holds great potential, which may even permit the manipulation of cells flowing through our veins. One major reason for the scarcity of biomedical applications of acoustic levitation is that many of the demonstrations are done in air. It is vital to understand the interaction of acoustic fields with tissues and bone-like structures and levitate samples in liquid and gel-like mediums to enable the application of acoustic levitation in biological studies including in vivo manipulation capabilities.

Acoustic streaming can be simply defined as a steady flow resulting from the dissipation of acoustic oscillations in a viscous fluid. Various mechanisms can generate acoustic streaming at different length scales such as boundary-layer induced streaming, oscillating solid structure-driven streaming, microbubble-driven streaming, Eckart streaming, and Gedeon streaming. In many
instances, acoustic streaming is characterized as recirculating symmetric streaming vortices. Acoustic streaming-based tweezers are generally used in controlling fluid flows within microfluidic platforms.65 In particular, microbubble and sharp-edge-based devices have been employed in fluid mixing and pumping studies.66,67 While fluid mixing only requires breaking laminar flow interfaces and enabling rapid mass transfer, fluid pumping in microfluidics is more demanding, which can be achieved by generating directional net fluid flows through acoustic streaming. Several different designs have been applied in providing controllable fluid pumping platforms using directional acoustic streaming.78–80 For example, SAW-based platforms are implemented in fluid pumping applications by generating acoustic streaming flows through applying frequencies between 100 and 800 MHz.71,72 In addition, the circulatory nature of acoustic streaming has been applied in providing microcentrifugation, particle separation, and aggregation within a droplet or a microenvironment.73–75 The application of acoustic streaming flows in applying torque to suspended samples also enabled particle manipulation abilities.76,77 While it is possible to control the scale, shape, and speed of acoustic streaming by adjusting the applied frequency and the voltage,78–79 the resolution of acoustic streaming tweezers can be further improved by better localization of acoustic energy through employing acoustic metamaterials.80,81

III. ACOUSTIC MICROMANIPULATION TECHNOLOGIES APPLIED IN BIOLOGY AND MEDICINE

Both BAW and SAW-based acoustic tweezers are frequently incorporated into lab-on-a-chip platforms to achieve continuous-flow cell focusing, sorting, separation, and patterning applications.9 Isolating a target cell type from a heterogeneous population is a crucial step in medical diagnostics and fundamental research.9 Having advantages of miniaturization, AMTs have been used to accomplish highly capable on-chip flow cytometers providing rapid analysis of cell samples by applying acoustic focusing followed by isolation of different cells through acoustic cell sorting.9,82 More recently, acoustic cell sorting was fused with intelligent image-based analysis by implementing deep neural networks to attain molecular specificity using the deformability of individual cells.84 With this method, neutrophils were successfully isolated from whole blood using traveling SAW sorting with no labeling. Acoustic cell sorting requires a means of detection and triggering to isolate cells of interest for downstream analysis. Another approach to acoustic cell isolation is called acoustic cell separation, which depends on the scaling of acoustic radiation forces experienced by cells based on physical and mechanical differences such as cell size, density, and stiffness.85 While some degree of focusing is desired to generate a sufficient amount of deflection, cells are not required to be in a single-file format in acoustic cell separation. Acoustic cell separation has been extensively applied in the isolation of cancer cells from blood samples by exploiting mechanical differences of cancer cells from healthy cells for early cancer diagnostics.86–88 So far, AMTs revealed an important potential for non-invasive disease detection by enabling liquid biopsy,89 which is a promising approach for improving conventional medical diagnostics.

In addition to using isolated cancer cells as biomarkers for disease detection, exosomes were also separated and implemented in medical diagnostics using AMTs. In a recent report, an AMT was applied to isolate exosomes from plasma samples, and enriched exosomes were used to detect biomarkers for traumatic brain injury.90 According to this work, analysis of the untreated plasma sample did not reveal any changes after the injury, and it was only possible by isolation and enrichment of exosomes to detect elevated levels of the traumatic brain injury biomarkers. In another example, an AMT was employed to separate exosomes from saliva samples for a diagnostic and prognostic biomarker for human papilloma viral-associated oropharyngeal cancer.91 Regardless of the variations in viscosity and concentrations of the sample fluid, an AMT provided successful exosome isolation with high purity and yield and maintained the integrity of the isolated exosomes. Using a different approach, extracellular vesicles from blood plasma were trapped and captured by using seeded polystyrene particles in an acoustic tweezer platform.92 Isolated extracellular vesicles were used for biomarker detection in allogeneic hematopoietic stem cell transplantation. Based on these pioneering studies, it is safe to say that an AMT carries a great potential to become very effective clinical tools for high yield and purity, rapid, and simple isolation, and the enrichment of biomarkers for diagnostics and prognostics in near future.

An AMT offers more than single-cell isolation for biomedical research. For instance, through particular transducer designs and special modes of actuation, selective trapping of cells was demonstrated.92 Here, the suspended polystyrene particles and T cells were trapped and aligned in a localized single and two-column order by applying pulsed excitation signals to concentrically offset focused IDTs. This work illustrates an application of the AMT to achieve selected patterning of single cells. Thus far, mostly two-dimensional (2D) applications are discussed, but the AMT was shown to be prominent platforms to execute 3D manipulations of cells.90,97 3D cell cultures are generally regarded as more effective compared to 2D cell models in terms of physiological relevance.9 AMTs have been proven to be highly practical in rapidly forming 3D cell spheroids and organoids at acoustic pressure nodes by exploiting acoustic radiation forces.95 For example, mesenchymal stem cells were successfully formed into spheroids owing to the gentle nature of the acoustic technologies.94 In a more challenging example, human midbrain organoids and human forebrain organoids were fused together through precise alignment of neuroepithelial buds using AMTs (Fig. 1).95 In addition to being biocompatible, precision and controllable maneuvering characteristics are driving forces enabling the intricate assembly of biological cells and tissues, which mark AMTs as a group of dexterous tools and a strong candidate for contact-free experimentation in cell biology and tissue engineering.

In addition to fulfilling intricate tasks in microfluidic channels, AMTs were also proven to be suitable for more common sample handling environments. In this endeavor, a highly functional acoustic tweezer platform was applied to pattern, trap, enrich, and stimulate cells in Petri dishes.96 Using this capability, 3D tissues were engineered by patterning fibroblasts cells embedded within fibrin gels. Petri dish is a common cell/bacteria handling environment in biology experiments. Adaptation of AMTs to work
with Petri dishes is an important step through more practical instrumentation in biomedical research. Another niche application of AMTs is in bioprinting, which is a promising research field for tissue engineering and organ regeneration. An AMT enables accurate spatial manipulation of different types of cells to form heterogeneous and ordered cell assemblies that mimic the complexity of actual tissues. For example, tumor migration and invasion were studied by acoustically printing tumor spheroids (CAL27) and cancer-associated fibroblasts using low-concentration gelatin methacryloyl as inks.98 More than 94% cell viability was reported for the acoustically printed cells, which was higher compared to 85% for inkjet-printed cells. A key aspect of acoustic bioprinting is size controllable ejection of liquid droplets with various viscosities. Acoustic forces have been successfully adopted into traditional bioprinting to provide the tuneability of droplet sizes.99 Rapid formation of complex cell patterns is also an important capability that can be applied in bioprinting. For example, acoustic holograms were recently implemented in forming cell patterns in biocompatible hydrogels (Fig. 2).100 The holographic formation of cell patterns can be fused with bioprinting to generate custom geometries for tissue engineering.

Model organisms are crucial precursors in advancing our understanding of disease mechanisms, drug discovery, and deciphering biological phenomena. The AMT grants multimodal maneuvering means in studying model organisms. As a commonly used system in developmental biology, Caenorhabditis elegans (C. elegans), which is a nematode roundworm, has been extensively explored using tools enabled by microfluidics. More recently, AMTs have been implemented to provide controllable rotational manipulation that resulted in unobstructed visualization of cell structures from different angles.101 In fundamental biological research, differentiating subtle differences in cell morphologies is critical in understanding the genetic variations and cellular mutations. For example, the vulva ring morphology of C. elegans was studied using AMTs to score worms with abnormally shaped toroids with defective epithelial junctions in nhr-25(RNAi) animals.102 Rotational manipulation yielded a better viewing angle to correctly identify the mutations in cell formations. Zebrafish (Danio rerio) is another important model animal offering higher complexity compared to C. elegans. An effective zebrafish screening platform was accomplished using acoustofluidic rotational tweezing and multispectral imaging (Fig. 3).103 In this work, zebrafish larvae samples were rotated and images from different angles were collected to form a 3D model to identify morphological phenotypes. Leveraging the versatile manipulation capabilities of acoustic technologies, the multi-angle observation of model animals has proven to be very effective for biomedical research. Moreover, AMTs facilitated the sorting of fluorescently tagged C. elegans worms with a throughput of 115 worms per minute with over 90% purity.103 Compared to the costly and bulky commercial flow cytometers for
sorting larger samples such as nematodes, AMT platforms offer an attractive alternative with simpler and cheaper instrumentations.

IV. ADVANTAGES OF ACOUSTIC MICROMANIPULATION

AMTs are comprised of a diverse set of platforms with a wide range of working frequencies and architectures to accommodate liquid and solid samples. For example, microparticles and cells can be moved along any user-defined path in a microfluidic channel, liquid droplets can be suspended in the air and manipulated in a contactless manner for laboratory automation. While for most of the other micromanipulation methods, intrinsic sample characteristics pose a challenge, samples regardless of their magnetic or dielectric properties can be handled in AMTs. This renders AMT label-free micromanipulation methods.

The range of sample sizes that can be maneuvered in AMTs spans from tens of nanometers to a few millimeters. On the one hand, nanoparticles, extracellular vesicles, and exosomes can be enriched and separated. On the other hand, multicellular organisms including C. elegans and zebrafish larvae can be linearly moved and rotated. Acoustic levitation can further push the envelope by using higher amplitudes of oscillations to suspend and move even larger samples such as insects.

In micromanipulation, throughput is critical for certain applications. For example, separating, sorting, and patterning cells are generally demanded to be high throughput for the practicality of applications. With AMTs, while it is possible to individually manipulate a single cell, patterning hundreds of cells at once or achieving cell sorting with thousands of cells per second is feasible.

For example, BAW-based tweezers can process undiluted whole blood samples with throughputs up to 20 ml/min to separate blood components. Conversely, a very small amount of liquid sample can be sufficient for SAW-based tweezers to isolate rare cells with high precision and purity.

Acoustic waves applied in AMTs can be tailored for different tasks from gentle translation and rotation to cell stimulation and lysis. Proven by more than 50 years of medical imaging, ultrasonic waves can be applied to the most delicate cells even at the early stages of pregnancy without detrimental effects. On the other end of the spectrum, AMTs can be used to induce sonoporation of single cells for intercellular delivery or complete disruption of cell membranes for lysis. Acoustic waves have tissue penetration adequacy, which can be highly beneficial for in vivo applications. For example, focused ultrasound is applied in neurostimulation for studying the peripheral and central nervous systems. Compared to the more traditional methods such as electrical stimulation, AMT platforms offer a non-invasive therapeutic approach for the treatment of neurodegenerative conditions.

V. LIMITATIONS OF ACOUSTIC MICROMANIPULATION

New AMT platforms with novel principles and better capacities have been continuously reported for solving more challenging problems or executing labor-intensive tasks faster compared to the conventional bioanalysis methods. However, the vast number of different devices makes the selection of suitable AMTs confusing for biomedical research. Furthermore, there is a lack of a certain degree of standardization among the existing devices. In some
cases, AMTs can be prohibitively difficult to operate by a non-technical researcher due to the proprietary nature of each device. The absence of standardization also hampers the widespread commercialization of AMTs.

In the majority of AMT platforms, high-level and intricate fabrication processes are required for IDT or microfluidic device fabrication. For example, mask writers, optical lithography equipment, vacuum deposition, a cleanroom environment, and costly consumables are necessary for fabricating IDTs for SAW-based tweezers. This is generally only available in well-funded central laboratories, which are not accessible to many researchers in smaller institutes, especially in developing countries. Aside from the availability of all the required equipment, AMT device fabrication is generally time-consuming and labor-intensive, which impedes their extensive use in the biomedical research community.

Peripheral equipment required to drive acoustic transducers is also an integral part of AMTs. Currently, benchtop function generators and RF power amplifiers are commonly needed to operate SAW and BAW-based tweezers. These benchtop instruments are costly and bulky, which prohibit true mobility and lab-on-chip applications of AMTs. In contrast to SAW-based tweezers, multiple SAW-based microsystems that are implemented in a mobile phone can be operated with an integrated driving circuitry powered by a battery. Acoustic levitation platforms also employ multiple transducer elements, which require elaborate driving units to individually control the transducers for rotational and linear manipulation of suspended samples. There are few reports demonstrating hand-held acoustic devices, which could be model examples for future acoustic technologies. In general, simpler driving units are essential for increasing the practicality and benefits of AMT platforms.

Cell patterning and tissue engineering are frequently performed in microfluidic devices equipped with acoustic transducers. Even though 3D cell structures and complex heterogeneous tissues can be easily formed by employing AMTs, the undisturbed collection of these cell formations is usually not trivial due to the device architectures. Downstream analysis and culturing of 3D cell models...
is important for biomedical research, but picking up an individual spheroid or organoid is not an easy task for the majority of AMT platforms as of yet.

VI. FUTURE OUTLOOK

An assortment of different acoustic technologies and a plethora of biomedical applications have been revealed since the first demonstrations of acoustic micromanipulation. Emergent technical discoveries enabled new capabilities and more advanced AMT platforms that led to innovative solutions to biomedical problems. A paradigm shift has taken place in traditional medical diagnostics and prognostics as human interactions change and society evolves. Instead of bulky and expensive equipment in centralized laboratories, modular and smaller footprint devices have become more desired to provide faster, cheaper, and more accessible medical care. An AMT carries an important potential to provide versatile tools for laboratory automation, sample processing, and bioanalysis, which can improve the capabilities of lab-on-a-chip devices. Despite the progress shown in the domain of acoustic technologies, certain limitations need to be addressed. As stated earlier, the classical approach for fabricating AMT devices requires well-equipped cleanroom facilities. On the other hand, 3D printing has become a widely available manufacturing method with an increasingly larger material library. Both metal electrodes and polymer-based device components can be 3D printed for rapid manufacturing. More research efforts should be applied in exploring the feasibility of 3D printing for different AMT platforms. If it is proven to be a viable method, 3D printing can potentially eliminate cleanroom fabrication to provide simpler and faster prototyping of acoustic devices.

Another important aspect for AMT platforms to become prevalent within the biomedical community is the ease of operation. For any commercial product to be popular and widely accepted, device operation must be convenient and easy to use in addition to functionality. However, driving acoustic components of AMT devices generally involves tedious adjustments of input signal characteristics to linearly move cells or organisms. This is usually a highly technical procedure and not user-friendly for the non-technical community. For an ideal AMT setup, interfacing with the micromanipulation procedure should be straightforward considering that end-users will include biologists and medical doctors. For 3D manipulation of single cells or cell clusters, rather than adjusting the phase and amplitude of the acoustic waves through tuning a function generator, users should be able to use joystick-like controls to turn an AMT on and move samples more intuitively and easily. Just like the control of the da Vinci Surgery System, AMT platforms should have more direct and simplified user interfacing.

Even though AMT platforms reached a more advanced technological state compared to the earlier examples, there are still elements to consider for improving their dexterity and capacity. Active control of acoustic wave properties is a highly desired feature. Currently, acoustic transducers are permanent components of AMT platforms. In certain applications, wave-fronts can be reshaped by using acoustic metamaterials to form more complex patterns. Interactions of acoustic waves with these metamaterials reveal a high-level regulation of acoustic waves, but passive nature of the majority of metamaterials only enables static modulation rather than dynamic control. Active metamaterials should be adopted in AMT platforms to provide more flexible micromanipulation capabilities. For example, acoustic bioprinting could be even more functional and faster if complex patterns of cells can be dynamically formed by active holographic structures instead of 3D printed holograms. It is also important to incorporate new developments and trends of science and technology into AMTs. Artificial intelligence (AI) and machine learning have been reshaping industries for more than a decade. While there are few examples of machine learning incorporated in acoustic sorting applications, active metamaterials can further benefit from AI-based technologies for smarter micromanipulation competency. Thus, more research should focus on adopting AI in acoustic systems to improve precision, selectivity, and practicality by assisting human users or autonomously operating AMT platforms.

The frequency of acoustic waves is a critical parameter that usually defines the function and characteristics of AMTs. At present, kHz to MHz frequencies are commonly used in acoustic micromanipulation applications. As the frequency increases to a few hundred MHz, it becomes convenient to handle single cells. Higher frequencies can yield much smaller wavelengths approaching nanorange. For example, 10 GHz acoustic waves result in a 100 nm wavelength in a water medium. At this resolution, subcellular acoustic manipulation or probing can be achieved. It is intriguing to explore the potential and possible effects of GHz acoustic waves in biomedical research.

Fusion of AMTs with magnetic, optical, or mechanical tools can also be considered for providing higher degrees of functionality in biomedical research. For instance, rotational maneuvering of pollen grains and C. elegans by AMTs was merged with a micro-force sensor to enable localized mechanical measurements on a specimen. Here, AMTs provided precise and contactless manipulation to expose desired regions of samples for mechanical characterization. Similar to this example, more practical and advanced solutions can be offered for solving challenging biomedical problems through fruitful combinations of AMTs and other experimental tools.

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AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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