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The ability of surface acoustic waves to trap and manipulate micrometer-scale particles and biological cells has led to many applications involving “acoustic tweezers” in biology, chemistry, engineering, and medicine. Here, we present 3D acoustic tweezers, which use surface acoustic waves to create 3D trapping nodes for the capture and manipulation of microparticles and cells along three mutually orthogonal axes. In this method, we use standing-wave phase shifts to move particles or cells in-plane, whereas the amplitude of acoustic vibrations is used to control particle motion along an orthogonal plane. We demonstrate, through controlled experiments guided by simulations, how acoustic vibrations result in micromanipulations in a microfluidic chamber by invoking physical principles that underlie the formation and regulation of complex, volumetric trapping nodes of particles and biological cells. We further show how 3D acoustic tweezers can be used to pick up, translate, and print single cells and cell assemblies to create 2D and 3D structures in a precise, noninvasive, label-free, and contact-free manner.

Significance

We present 3D acoustic tweezers, which can trap and manipulate single cells and particles along three mutually orthogonal axes of motion by recourse to surface acoustic waves. We use 3D acoustic tweezers to pick up single cells, or entire cell assemblies, and deliver them to desired locations to create 2D and 3D cell patterns, or print the cells into complex shapes. This technology is thus shown to offer better performance over prior cell manipulation techniques in terms of both accurate and precise motion in a noninvasive, label-free, and contactless manner. This method offers the potential to accurately print 3D multicellular architectures for applications in biomannufacturing, tissue engineering, regenerative medicine, neuroscience, and cancer metastasis research.


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resulting streaming in a microfluidic chamber by recourse to both modeling and controlled experimental validation. Unlike Rayleigh streaming in bulk acoustic wave devices, the unique acoustic streaming (i.e., streaming motion caused by acoustic oscillation) pattern in a SSAW device determines how objects are lifted from the substrate surface. By regulating the 3D distributed acoustic field and acoustic streaming, induced by two superimposed pairs of orthogonally placed SSAWs, we achieved an array of 3D trapping nodes in a microfluidic chamber. By independently tuning the relative phase angle of each SSAW or by varying the input power, the position of these 3D trapping nodes can be precisely controlled in a 3D environment. Using this concept, we demonstrate 3D trapping and three-axis manipulation of single microparticles and single biological cells. Finally, we illustrate how this 3D acoustic tweezers technique can be used for printing with live cells and for producing prescribed cell culture patterns.

Results

Working Mechanism of Three-Dimensional Acoustic Tweezers. To manipulate suspended objects along three orthogonal axes with surface acoustic waves (SAWs), it is necessary to form 3D trapping nodes and to move these nodes precisely in three dimensions. To achieve this objective, we created a 2D displacement field on a lithium niobate (LiNbO₃) piezoelectric substrate by superimposing two mutually orthogonal pairs of interdigital transducers (IDTs) that produce SSAWs. The propagation of these waves through a microfluidic chamber produces an acoustic field distributed in three dimensions and induces acoustic streaming which creates stable 3D trapping nodes within the fluid-filled chamber. The positions of these 3D trapping nodes were precisely manipulated in the transverse (x), longitudinal (y), or vertical (z) directions by adjusting the phase angle of each individual IDT pair (x or y axis motion control) or the input acoustic power (z axis motion control), respectively.

Two orthogonal SSAWs were used to perform particle/cell manipulation in a microfluidic chamber. Two pairs of IDTs were deposited onto a 128° YX-cut LiNbO₃ substrate, which were positioned along the x and y axes. The IDTs were made up of 40 pairs of electrodes with a 75-μm width of each finger electrode and a 75-μm spacing between fingers, and a 1-cm aperture. A polydimethylsiloxane (PDMS) layer with a 1.8 mm × 1.8 mm × 100 μm fluidic chamber was bonded to the substrate, at the center of the two orthogonal pairs of IDTs. Fig. L4 shows a schematic diagram of the device. Each pair of IDTs was individually connected to a double-channel radio-frequency (RF) signal generator and two amplifiers, which generated SSAWs with different frequencies and independent SSAW phase angle control. Once the pairs of IDTs were activated, a 2D displacement field (including both longitudinal and transverse vibrations) was produced on the surface of the LiNbO₃ substrate (18). The acoustic waves induced by these surface vibrations propagated in the fluid, reflected by the chamber walls, and established a 3D, differential Gor'kov potential field (19). Meanwhile, these surface vibrations also induced 3D acoustic streaming in the microfluidic chamber. The interaction of the fluidic and acoustic fields produced 3D trapping nodes within the chamber (Fig. 1B). Along the vertical direction, suspended microobjects were pushed and levitated to a stable trapping node because of the competitive interaction of the acoustic radiation force, the gravitational force, the buoyancy force, and the Stokes drag force induced by acoustic streaming. After increasing the input acoustic power, the vertical trapping position was raised because of a rebalancing of these forces. Along the horizontal plane (x-y plane), the objects were pushed toward the substrate (Fig. 1D) at the 3D trapping node. These trapping positions can be independently manipulated along the transverse (x) or longitudinal (y) directions by relocating the 2D displacements via changing the input phase angle. As a result, microobjects were trapped into a 3D node and manipulated along three axes within a microfluidic chamber (Fig. I4).

SAW Vibrations Induce Three-Dimensional Acoustic and Fluidic Fields. To create a 3D trapping node in a microfluidic chamber, the mechanism by which SSAWs manipulate objects within a microfluidic chamber must be understood. A simple acoustic tweezers device, consisting of a PDMS chamber and a pair of IDTs (positioned along the x axis of a 128° YX LiNbO₃ substrate), was used to investigate this mechanism. Two sets of SAWs, traveling toward each other, were produced after applying a RF signal to the IDTs. A SSAW was formed via the superposition of these SAWs. This type of resulting wave is considered a Rayleigh wave. These waves confine most of the energy to the surface because of the exponential decay of their amplitude with the depth of the substrate. In addition, these waves include both longitudinal and transverse vibrations with a phase lag on the substrate. Once the SSAWs interfere with the liquid in the microfluidic chamber, periodically distributed vibrations are created which lead to periodically distributed acoustic fields and acoustic streaming in the microfluidic chamber (Fig. 2A). We developed a numerical model that could account for the Gor'kov potential and acoustic streaming in a lateral plane (x-z plane) as well as the acoustic radiation force acting on suspended particles. The model considers the effects of the transverse and longitudinal vibrations on the liquid, and the acoustic radiation transmission and reflection between the parallel PDMS and the PDMS chamber. A detailed description of the numerical model can be found in SI Text, Theoretical Framework and Model Setup (see also Fig. S1 and Table S1).

The periodically distributed transverse vibrations are considered as the primary perturbation source for generating an acoustic field in a liquid chamber, whereas the longitudinal vibrations decay very fast and have little impact on the acoustic field. Fig. 2B predicts the distribution of the Gor'kov potential along the x-z plane. The regions of maximum Gor'kov potential (in red color), known as pressure antinodes (ANs), are located at places with a displacement antinode of the transverse vibrations (DATVs), whereas minimum regions (in blue color), known as pressure nodes (PNs), occur atop a displacement node of transverse vibrations (DNTVs). The distribution of PNs and ANs coincide with the location of DNTVs and DATVs on the substrate's surface; therefore, the distance between adjacent PNs or ANs is half-wavelength of a SAW. Because of the gradient of the Gor'kov potential, an acoustic radiation force was generated to push the suspended cells or microparticles from ANs to PNs. The experimental results show that all of the suspended 10.1-μm diameter polystyrene also particles were pushed to the parallel PNs by the acoustic radiation force (Fig. 2C). The dependence of the acoustic radiation force on input power was investigated, and the results were plotted (Fig. 2D and SI Text, Data Analysis).

In addition to the aforementioned acoustic field, the vibrations also induced acoustic streaming. Both the transverse and
longitudinal vibrations attenuate in a thin boundary layer close to the substrate and result in a particular streaming pattern in the microfluidic chamber (Fig. 2A). Our numerical results describe the streaming vortices and the periodic distribution of the acoustic streaming in the $x$-$z$ plane, as shown in Fig. 3A. The streaming flows rise up from DNTVs on the substrate, rotate clockwise or counter clockwise to the two nearby DATVs, and then rise up again from the original DNTVs. In contrast to Rayleigh streaming (18, 20), which is driven by standing bulk acoustic waves, this streaming, which is induced by SAWs, has a reversed direction with respect to the streaming vortices. However, these streaming processes share the same spatial distribution with four vortices in one wavelength. This particular streaming plays an important role in the vertical manipulation of microparticles/cells. Fig. 3B plots the numerical streaming pattern over Gor’kov potential in the $x$-$z$ plane. The streaming flows rise vertically from DNTVs on the substrate toward the PNs where particles are trapped.

To validate our modeling prediction, we performed an experiment to investigate SAW-induced acoustic streaming within the microfluidic chamber. We started the experiment by pre-marking the location of DNs by patterning 10.1-$\mu$m polystyrene particles along the $x$-$y$ plane without, and then with, an applied standing surface acoustic wave. (D) The experimental results show the dependence of the acoustic radiation force on the input power. (Scale bar: 100 $\mu$m.)
Fig. 4. Vertical (z axis) acoustic manipulation. (A) Calculated trapping node induced by a standing acoustic wave in the x-y plane within the microfluidic chamber and an illustration of the forces along the z direction in the trapping node. (B and C) The theoretical calculations (B) and experimental demonstration (C) of vertical position control of a single 10.1-μm polystyrene particle by varying the input power. The images taken in the x-y plane show the stable trapped single particle was lifted up and through the fixed microscope’s focal point by increasing the input power from 200 to 1,500 mW. (D) Experimental results showing the vertical positions of particles with different sizes (4.2, 7.3, 10.1 μm) under different input powers, which is consistent with the predictions from our numerical model. (Scale bar: 20 μm.)

position of the particle as a function of input power. Fig. 4D shows the experimental results of the trapping position of single particles with a diameter of 4.2, 7.3, and 10.1 μm, respectively, under different input powers (Fig. 4D). The datasets shown in Fig. 4D were obtained by averaging 10 repeated experimental measurements. The model predictions of the vertical position versus input power are seen to match the experimental results. This method can thus levitate single particles to any vertical position within the chamber.

Three-Dimensional Trapping and Manipulation. With an understanding of the actuation mechanism for the 1D SSAW, we investigated 3D trapping and manipulation by using orthogonally arranged 2D SSAWs. Fig. 5A illustrates the 2D distribution of transverse vibrations from the 2D SSAWs on a substrate: the interaction of DATVs (blue lines) formed node points (blue filled circles) with minimum Gor’kov potentials, and the interaction of DNTVs (red lines) formed antinode points (red filled circles) with maximum Gor’kov potentials. Vibrations from 2D SSAWs exhibit characteristics similar to those of 1D SSAW and can induce acoustic fields and streaming into the liquid of the microfluidic chamber. The interaction of these two fields forms an array of 3D trapping nodes in the microfluidic chamber with each node superposed on each displacement node of the transverse vibrations.

Next, we used the acoustic tweezers device with two orthogonal pairs of IDTs to experimentally demonstrate 3D trapping and manipulation. By applying two different RF signals (13 and 12 MHz/810 mW) to the two pairs of IDTs (along the x and y axes, respectively), 3D trapping was achieved as shown in Fig. 5B. The square trapping region (marked with white lines) consists of a displacement node point and four nearby antinode points from the transverse vibrations. The 10.1-μm green fluorescent polystyrene particles were levitated to a 3D trapping node above a displacement node point in the microfluidic chamber. Close to the substrate, the trajectories of the 1-μm red fluorescent polystyrene particles indicate that the streaming flows come from the edges of the square-like region (DATVs) and stream toward the center. There, the flow rises up and rotates back toward the edges. Combining the effects of the acoustic radiation force and acoustic streaming induced by 2D SSAWs, we have demonstrated that a 3D trapping node can be generated in the volumetric space above this cubic region within a microfluidic chamber.

To achieve 3D manipulation, we need to precisely move the trapping nodes along the x, y, and z directions. We have demonstrated that the vertical (z direction) movement of the trapped particles can be achieved by tuning the input power on the IDTs. To move trapped particles in the horizontal plane (x-y plane), we used a phase shift strategy: changing the relative phase angle lag (Δϕ) of the RF signals applied to each pair of IDTs can move the DNTVs on the substrate as well as the PNs in the fluid. The change in distance of the DNs (ΔD) along the x or y direction is given as ΔD = (λ/4π)Δϕ. As a result, the trapped particles in the PNs are moved the same distance along the same direction. For example, at a relative phase angle lag of π/2, the trapped particles move over a distance of λ/8. With our 3D acoustic tweezers, the randomly distributed 10.1-μm-diameter polystyrene particles were first pushed and levitated to 3D trapping nodes in a dot-array configuration. Gradually increasing the relative phase angle lag of the RF signal from 0 to 3π/2 along the y axis, while keeping all other parameters fixed, we found that all of the trapped particles moved along the y direction while in the dot-array formation. By decreasing the input power of the RF signals to zero, all of the trapped particles settled to the bottom but kept the dot-array formation. The entire process was recorded and is shown in Fig. 5C (Movie S3). Focusing and defocusing indicates that our 3D acoustic tweezers are capable of manipulating microparticles along the z direction. The manipulation of particles along the x direction can be achieved in a similar way. With the wide tuning range of the relative-phase-angle lag, we can translocate microobjects to any desired location within the entire
microfluidic chamber and maintain any desired formation of the trapping array, which results in a significant improvement in manipulation dexterity, compared with our previous work (14). By demonstrating a vertical range from the substrate surface to the ceiling of the microfluidic chamber, our approach can manipulate microparticles within the entire volume of a microfluidic chamber. Our 3D acoustic tweezers provide new possibilities for massively parallel and multi-axis manipulation.

Cell Printing with Three-Dimensional Acoustic Tweezers. To explore potential practical applications of this technology, we performed 3D printing of living cells onto a substrate with customized cell patterns using our 3D acoustic tweezers. Our earlier work (14, 15) demonstrated that the use of acoustic tweezers had no discernible effect on cell viability, functionality, and gene expression (14, 15). When an acoustic field was applied, living single cells were captured into the 3D trapping nodes. Then, cells were transported along the x or y directions in a horizontal plane by tuning the phase angle of each IDT pair accordingly. After the delivery of the cells above the target locations, the trapped cells were lowered onto the substrate or onto other cells that were already in place, by tuning the input power of the acoustic field. As a result, the cells were printed with precise control of the cell number, spacing, and configuration. To demonstrate 3D printing of live cells, we captured a single suspended 3T3 mouse fibroblast and transported it to a desired location on the substrate after injecting a cell suspension into the microfluidic chamber (Fig. 6A, indicated with red arrow). Following placement via our 3D acoustic tweezers, the cells started to adhere and then spread on to the substrate. In this way, a linear cell array was created by depositing cells one by one (Fig. 6A). We could also position another 3T3 cell on top of the previously adhered cells by using the precise control of 3D acoustic tweezers (Fig. 6A, indicated with blue arrow) to form a 3D cell assembly. To further demonstrate the capabilities of the 3D acoustic tweezers, we applied our technology to print living cells into complicated configurations, such as numbers and letters. Through single-cell seeding of HeLa S3 cells using acoustic control, we printed cells into the following patterns: “3” “D” “A” “T,” as an acronym for “Three-Dimensional Acoustic Tweezers” (Fig. 6B). Cells were able to adhere to the surface and then spread along the surface (Fig. 6B). We also demonstrated that the adhered cells were able to split and proliferate in the prescribed morphological patterns (Fig. 5E). Hence, we have demonstrated a versatile acoustic cell printing technique that is capable of single cell resolution and has the ability to manipulate multiple cell types without affecting cell viability.

Fig. 6. Printing living cells with 3D acoustic tweezers. (A) Single-cell printing. After previously deposited 3T3 cells were attached to the substrate, another single cell was picked up, transported, and dropped at the desired location on the substrate (indicated by red arrow) or on top of another cell (indicated by blue arrow). The single cell adhered and spread along the surface. (B) Formation of arbitrary cell culture patterns forming a “3” “D” “A” “T” by printing of single HeLa S3 cells via 3D acoustic tweezers. (Scale bar: 20 μm.)

Discussion
In this work, we have demonstrated 3D manipulation of microparticles and biological cells in a microfluidic chamber with 2D SSAWs. We investigated, through modeling and experiment, the mechanism behind 3D trapping with acoustic tweezers. We showed that 3D trapping originates from the interactions between induced acoustic fields and acoustic streaming in a microfluidic chamber via acoustic vibrations of the substrate. The transverse vibrations of SSAWs induce longitudinal waves into the liquid and establish a Gor’kov potential distribution within the microfluidic chamber. Both the longitudinal and transverse vibrations of SSAWs contribute to the acoustic streaming in the microfluidic chamber; this SSAW-induced streaming differs from Rayleigh streaming associated with bulk acoustic waves, which are only induced by longitudinal vibrations. The vibration amplitude regulates the transverse, out-of-plane position of the trapped objects by rebalancing the acoustic radiation force, the Stokes drag force, the gravitational force, and the buoyancy force. The predictions of the model are validated by our experimental results. This model accounts for the spatial distribution of the Gor’kov potential and acoustic streaming and rationalizes the dependence of acoustic manipulation on vibration parameters. We created 3D trapping node arrays in the microfluidic chamber by superimposing two orthogonal displacement nodes and antinodes. These 3D trapping nodes were then translated horizontally or vertically by tuning the location of the displacement nodes or vibration amplitudes. We further demonstrated 3D manipulation by lifting and pushing microparticles into 3D trapping node arrays, translating them horizontally, and finally allowing them to sink to the surface. In our current IDT design, 3D trapping nodes are created and manipulated in a massively parallel formation. However, the independent operation of a 3D node could be realized with dynamic regulation of the vibrations on the substrate through IDT design and RF signal control. Because it uses gentle acoustic vibrations, our method offers several advantages such as dexterous 3D manipulation, digitally programmable, and potentially automatable operation, as well as contactless and label-free handling of cells. Finally, these processes are all done in a simple, low-cost device without any moving parts.

With the current setup and experimental condition used, we can manipulate a single cell or particle and place it at a desired location down to 1-μm accuracy in the x-y plane and 2-μm accuracy in the z direction. Because the acoustic wavelength and input power are both instantaneously tunable during experiments, the spatial accuracy (along x, y, or z direction) of cell/particle placement is in principle only limited by the optical or imaging resolution of the experimental setup. The cell or particle transport time achieved in the present study was in the range of several seconds to a couple of minutes, depending on both the moving velocity and the distance between the cells/particles starting location and the target location. We were able to move a 10-μm particle or a single cell with an average speed of ~2.5 μm/s.

Using these 3D acoustic tweezers, we verified the printing of living cells with acoustic manipulation. We demonstrated that single cells could be picked up, delivered to desired locations, and allowed to adhere and spread on the surface or on top of previously deposited cells (Fig. 6A). We printed cells into prescribed adherent patterns by acoustic-based single cell seeding. Three-dimensional acoustic tweezers were used to pattern cells with control over the number of cells, cell spacing, and the confined geometry, which may offer a unique way to print neuron cells to create artificial neural networks for applications in neuron science and regenerative neuron medicine. For example, during peripheral nervous repair, Schwann cells are naturally aligned into structures, “bands of Büngner”, which guide regenerating neurons to reconnect to their peripheral targets (21). Our method may offer a unique way to control the spatial distribution of Schwann cells for optimal nerve regeneration. With spatial resolution control down to a single cell, this technology shows the potential to enable layer-by-layer positioning of living cells to create 3D tissue-like structures. This technology
could provide new pathways for 3D bioprinting, because the technology addresses the central challenge of replicating tissue structures or even fabricating artificial organs that are made up of multiple cell types and complex geometries, but with single-cell control resolution (4). In addition, we expect this technology to rebuild the 3D architecture of a tumor, which will aid in the investigation of heterogeneous genetic alterations during tumor growth and metastasis process (22). The ability to precisely transport single cells along three axes using our 3D acoustic tweezers may facilitate investigations of a number of challenging problems in biology, particularly those involved in the spatial regulation of cells in 2D or 3D environments (23).

In addition to bioprinting, our 3D acoustic tweezers can aid in the etching or analysis of biological specimens. Taking a confocal microscope as an example, the implementation of our device can provide precise transportation of a target cell or small tissue to the focal area of the microscopic lens. Then, the vertical position of the target object can be moved with respect to the focal plane to generate a confocal image (24). In addition, we demonstrated the rotation of stable trapped objects with the acoustic streaming patterns discussed above (Movie S4), which enables the reconstruction of 3D cells or small tissue images. Furthermore, these 3D acoustic tweezers could serve as building blocks in future integrated imaging and analysis systems including microscopes or fluorescent-activated cell sorter (FACS).

**Materials and Methods**

**Theoretical Model.** We use perturbation theory to model the acoustic fields and second-order acoustic streaming in the microfluidic channel, to assess two main forces acting on the particles: acoustic radiation force and Stokes drag force. With the perturbation theory, continuity and Navier–Stokes equations are asymptotically expanded into two sets of equations in different order based on a small perturbation. The solution to the first-order equations, together with given boundary conditions that contain SAWs vibration and partial acoustic radiative losses at the PDMS/fluid interface, yields the acoustic fields. The first-order equations lead to the determination of the Gor’kov potential and the acoustic radiation force, whereas the solution to the second-order equations identify acoustic streaming. The forces on microparticles are evaluated based on the two solutions. Detailed formulation, model description, force analysis, and parametric assessment can be found in SI Text, Theoretical Framework and Model Setup, Fig. S1, and Table S1.

**Device Fabrication and Experiment Setup.** The procedure for device fabrication was described in our previous work (14, 15). Details of the experimental setup and the operating procedures are given in SI Text, Experimental Setup and SI Text, Calibration of Vibration Amplitude.

**Data Analysis.** The velocity of the microparticles was measured using Nikon NIS Elements Advanced Research (AR) software (Nikon). Details of the analysis steps are described in SI Text, Data Analysis.

**On-Chip Cell Culture.** Cell culture in the acoustic tweezers device was conducted with a customized cell-culture chamber. Details about handling and culturing cells are described in SI Text, Cell Preparation and Culture.

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