

RESEARCH ARTICLE | OCTOBER 08 2026

Acoustic planar concentration of suspended micro-objects for rapid microscopy

Yiwen Zhang; Xin Li ; Xian Zou; Haoyue Deng; Yali Liu; Wenwu Ling; Yan Wu ; Liuchao Jin  



Biomicrofluidics 20, 054109 (2026)

<https://doi.org/10.1063/5.0351769>



Articles You May Be Interested In

AVS Quantum Science publishes original research and more in 2021

AVS Quantum Sci. (March 2021)

Seven nonstandard models coupling quantum matter and gravity

AVS Quantum Sci. (May 2022)

Acoustic planar concentration of suspended micro-objects for rapid microscopy

Cite as: Biomicrofluidics 20, 054109 (2026); doi: 10.1063/5.0351769

Submitted: 9 July 2026 · Accepted: 26 September 2026 ·

Published Online: 8 October 2026



Yiwen Zhang,¹ Xin Li,² Xian Zou,³ Haoyue Deng,⁴ Yali Liu,⁵ Wenwu Ling,⁶ Yan Wu,^{1,a)} and Liuchao Jin^{7,a)}

AFFILIATIONS

¹Department of Postgraduate Students, West China Medicine School, Sichuan University, Chengdu 610041, China

²Zhejiang Key Laboratory of Advanced Equipment Manufacturing and Measurement Technology, School of Mechanical Engineering, Zhejiang University, Hangzhou 310058, China

³Research Center of Anesthesiology, West China Hospital, Sichuan University, Chengdu 610041, China

⁴West China Biomedical Big Data Center, West China Hospital, Sichuan University, Chengdu 610041, China

⁵Shenzhen Maternity and Child Healthcare Hospital, Women and Children's Medical Center, Southern Medical University, Shenzhen, Guangdong, China

⁶Department of Medical Ultrasound, West China Hospital, Sichuan University, Chengdu 610041, China

⁷Department of Mechanical and Automation Engineering, The Chinese University of Hong Kong, Hong Kong, China

^{a)}Authors to whom correspondence should be addressed: wuyan@wchscu.cn and liuchao.jin@link.cuhk.edu.hk

ABSTRACT

Rapid microscopic examination of suspended microorganisms, cells, and micrometer-scale particles is often limited by the time required for samples to reach a stable and observable distribution. Conventional counting chambers rely mainly on passive sedimentation, which can require tens of minutes before reliable imaging and counting, whereas automated imaging or flow-based systems improve throughput at the cost of increased equipment complexity. Here, we introduce acoustic planar concentration (APC), a compact pulsed bulk-acoustic strategy for improving the single-plane observability of suspended micro-objects. APC is implemented in a conventional platelet-counting chamber with a 100 μm -high observation cavity, without redesigning the chamber into a microfluidic resonator. An approximately 4 MHz pulsed acoustic field is coupled into the standard slide-based chamber, where acoustically induced particle redistribution improves single-plane observation. Pulsed excitation was used to avoid prolonged uninterrupted acoustic driving, while an external acoustic waveguide defines the actuation region. Using 5 and 2 μm polystyrene fluorescent particles as model objects, we demonstrate improved single-plane observability within seconds under the tested conditions. For 2 μm particles, the fraction of detected particle images classified as in focus increased from $76.1 \pm 6.5\%$ to $90.6 \pm 2.5\%$ within approximately 5 s. APC was further demonstrated using *Chlorella sorokiniana* cells of comparable size, for which the detected cell count within the selected observation plane increased by approximately 2.6-fold after actuation. This approach provides a compact, contact-free route compatible with conventional counting chambers and an inverted fluorescence microscopy setup.

Published under an exclusive license by AIP Publishing. <https://doi.org/10.1063/5.0351769>

I. INTRODUCTION

Microscopic examination and enumeration of suspended microorganisms, cells, and micrometer-scale particles are widely used in environmental monitoring, biological laboratories, and practical teaching.^{1,2} However, the limiting step in many microscopy-based workflows is not optical magnification itself, but the conversion of a three-dimensionally suspended sample into a stable, microscope-observable, and countable planar

distribution.^{3,4} Conventional chamber-based methods usually rely on passive sedimentation to bring suspended objects close to the observation plane, introducing waiting periods that can range from tens of minutes to hours depending on the counting chamber, sample type, and protocol.^{5,6} More rigorous sedimentation-based methods further extend this delay, making them poorly suited for time-sensitive inspection.⁷ Automated microscopy,⁸ image-based counters,⁹ and flow cytometry^{10,11} can improve throughput and

reproducibility; however, these approaches often rely on motorized scanning, autofocus modules, dedicated optical or fluidic systems, staining or fluorescence procedures, trained operation, and relatively instrument-intensive platforms.¹² Existing approaches, therefore, involve a trade-off between procedural simplicity and analytical speed. This trade-off is particularly restrictive for routine microscopy workflows in laboratory and teaching settings, where recent reports^{13,14} have emphasized that emerging technologies must be not only effective, but also easy to operate, workflow-compatible, and practical for real-world implementation.

Several active strategies have been developed to shorten the preparation time of suspended samples before microscopy. Mechanical approaches, such as slide centrifugation or cytocentrifugation, can rapidly concentrate and deposit cells onto glass substrates,¹⁵ but they often require dedicated centrifugal devices, holders, cassettes, or filter assemblies and may perturb cell attachment, spatial distribution, or morphology depending on the sample type and operating conditions.^{16,17} Filtration, magnetic enrichment, and electric-field-based manipulation can also accelerate sample concentration, yet they often involve membranes, labels, electrodes, or specific microstructured devices, which increase procedural complexity and reduce compatibility with routine slide-based microscopy.^{18–20}

Acoustic manipulation provides an attractive alternative because acoustic radiation forces can act remotely on suspended micro-objects in a contact-free and label-free manner.^{21–24} Rode *et al.* demonstrated acoustic tethering of freely swimming microorganisms, enabling individual organisms to be maintained near the focal plane for behavioral observation with a standard microscope.²⁵ Hammarström *et al.* used acoustic focusing for sedimentation-free flow-through imaging of microalgae, improving image quality and throughput for automated identification.²⁶ Zmijan *et al.* further developed a high-throughput imaging cytometer using acoustic focusing to position suspended objects for optical analysis.²⁷ These studies demonstrate the capability of acoustic fields to improve microscopic observation by regulating the axial position of suspended objects. However, they mainly rely on purpose-built resonant chambers,^{28–30} continuous flow operation,^{31,32} bonded chip structures^{33–35} or synchronized imaging systems, and are, therefore, not directly compatible with simple static counting chambers used in routine microscopy. For conventional counting chambers and simple slide-based microscopy, this creates a practical need for an acoustic strategy that can regulate particles axially within a standard shallow observation chamber, without requiring a purpose-built acoustic resonator or microfluidic device. Moreover, while many acoustofluidic studies have demonstrated lateral focusing, node-based aggregation, or localized trapping,^{22,36} less attention has been paid to rapidly concentrating particles into a broad planar layer that can be directly brought into focus for microscopic observation and counting.

In this paper, we introduce acoustic planar concentration (APC), a compact pulsed bulk-acoustic strategy for improving the single-plane observability of suspended micro-objects. APC is implemented in a conventional platelet-counting chamber with a 100 μm -high observation cavity, rather than in a chip-defined microfluidic resonator. A miniaturized actuation module couples a 4.745 MHz pulsed bulk acoustic field into the standard slide-based

chamber, where acoustic excitation induces particle redistribution toward a reproducible observation layer that can be brought into focus for observation and analysis. Pulsed excitation was used to avoid prolonged uninterrupted acoustic streaming or interference, while retaining sufficient particle redistribution under the tested conditions. An external acoustic waveguide formed from ultrasound coupling gel, without altering the counting cavity itself, defines the actuation region and may improve the spatial selectivity of particle regulation. Using 5 and 2 μm particles as model suspended micro-objects, we demonstrate an increase in the number of detected particle images within a selected focal plane. The 2 μm particles were evaluated as a smaller-particle test case. APC was further demonstrated using *Chlorella sorokiniana* cells of comparable size, for which the detected cell count within the selected observation plane increased by approximately 2.6-fold after actuation. By actively redistributing suspended objects within seconds while retaining the conventional counting-chamber format, APC is intended to reduce the waiting time associated with passive sedimentation without introducing the instrumentation complexity of automated or flow-based analysis.

II. MATERIALS AND METHODS

A. Working principle of acoustic planar concentration

Acoustic planar concentration was developed to rapidly transform suspended micro-objects from a three-dimensionally dispersed state into a microscope-observable planar layer. As schematically illustrated in Fig. 1(a), the APC platform consists of a bulk acoustic wave (BAW) actuator, an ultrasound coupling-gel acoustic waveguide, a glass slide or counting chamber, a liquid sample layer, and a coverslip. The liquid sample is confined within the shallow chamber between the lower glass substrate and the upper coverslip. Acoustic energy generated by the BAW actuator is coupled into the sample region through the ultrasound coupling-gel waveguide, enabling acoustic actuation within the observation area without redesigning the chamber into a microfluidic resonator.³⁷ The waveguide is positioned beneath the liquid chamber and is laterally confined such that its in-plane boundaries are smaller than and fully contained within those of the chamber, thereby avoiding acoustic diffraction from gas-liquid or solid-liquid interfaces at the chamber periphery.

The working principle of APC is shown in Fig. 1(b). In the absence of acoustic excitation, suspended particles are distributed at different heights within the liquid layer. As the depth of field of the microscope is limited, only a fraction of the suspended particles can be clearly observed within a single focused imaging plane, while out-of-plane particles appear blurred or are excluded from reliable image-based analysis. When acoustic excitation is applied, the acoustic field generates axial acoustic radiation forces that regulate the vertical distribution of suspended particles. The observed enrichment is consistent with acoustically induced axial particle redistribution toward a reproducible observation layer, which can then be brought into focus for microscopic observation and quantitative analysis.

By improving particle observability within a selected focal plane, APC can facilitate subsequent particle counting and morphology-based analysis [Fig. 1(c)]. Importantly, the acoustic

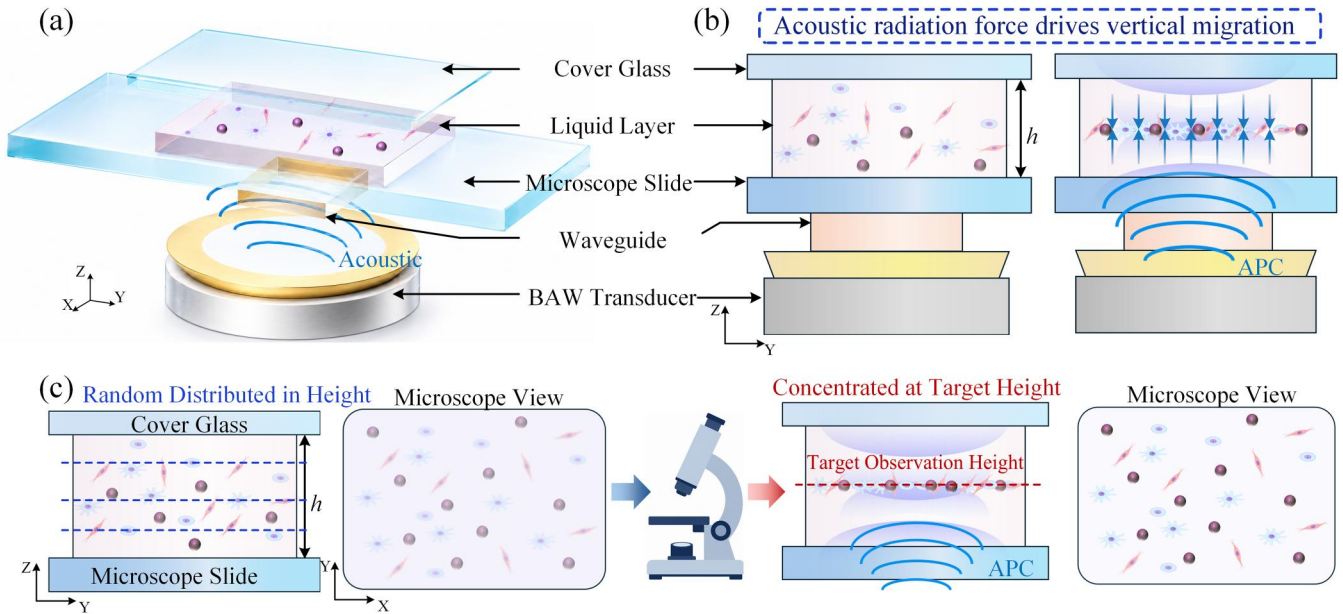


FIG. 1. Schematic illustration of acoustic planar concentration (APC). (a) APC platform consisting of a bulk acoustic wave (BAW) actuator, an ultrasound coupling-gel acoustic waveguide, a slide-based observation chamber, a liquid sample layer, and a coverslip. (b) Before acoustic actuation, suspended particles are distributed over different axial positions and only partially overlap with the focused imaging plane. After actuation, acoustically induced axial particle redistribution produces a reproducible observation focal plane. (c) APC increases the number of particle images detectable within a selected focal plane, enabling more efficient microscopic observation and counting in conventional slide-based chambers.

actuation is introduced externally through the BAW actuator and the ultrasound coupling-gel waveguide, while the counting chamber itself remains structurally simple. This design preserves compatibility with conventional counting chambers and an inverted fluorescence microscopy setup, making the method suitable for laboratory and teaching demonstrations.

B. First-order interpretation of acoustic radiation force

The axial particle redistribution observed in APC was interpreted using the primary acoustic radiation force acting on small spherical particles in an ultrasound field. According to the acoustic radiation force theory, the time-averaged radiation force is related to the gradient of the acoustic radiation potential; therefore, the axial component in the shallow chamber can be expressed as $F_{\text{rad},z} = -\partial U_{\text{rad}}/\partial z$. For a first-order qualitative interpretation, a simplified one-dimensional standing-wave approximation along the chamber height was considered. Under this approximation, the axial radiation force is given by³⁸

$$F_{\text{rad},z} = 4\pi\phi ka^3 E_{\text{ac}} \sin(2kz), \quad (1)$$

where a is the particle radius, $k = 2\pi/\lambda$ is the acoustic wavenumber, z is the axial position, ϕ is the acoustic contrast factor, and E_{ac} is the acoustic energy density. The acoustic contrast factor and

acoustic energy density are defined as

$$\phi = \frac{1}{3} \left(\frac{5\tilde{\rho} - 2}{2\tilde{\rho} + 1} - \tilde{\kappa} \right), \quad (2)$$

$$E_{\text{ac}} = \frac{p_a^2}{4\rho_0 c_0^2}, \quad (3)$$

where $\tilde{\rho} = \rho_p/\rho_0$, $\tilde{\kappa} = \kappa_p/\kappa_0$, ρ_p and ρ_0 are the densities of the particle and suspending medium, respectively, κ_p and κ_0 are their compressibilities, p_a is the acoustic pressure amplitude, and c_0 is the sound speed in the medium. This relation indicates that the axial acoustic radiation force depends on acoustic energy density, particle size, acoustic contrast, and particle position within the standing-wave field. For polystyrene particles suspended in water, the acoustic contrast factor is positive, so a one-dimensional model predicts migration toward stable axial equilibrium positions. Accordingly, the one-dimensional standing-wave model was used as a first-order framework to interpret the qualitative dependence of acoustic radiation force on particle size and axial position. As the experimental platform comprises multiple acoustic layers, including the PZT actuator, coupling gel, glass substrate, shallow liquid cavity, and coverslip, the model is not intended to reconstruct the exact three-dimensional field or determine the absolute equilibrium height of the particle layer. The observed

enrichment is consistent with acoustically induced axial particle redistribution toward a reproducible observation layer.

The primary acoustic radiation-force model provides a first-order interpretation of the observed axial redistribution but does not exclude contributions from acoustic streaming or secondary acoustic radiation forces. In the Rayleigh regime, the primary acoustic radiation force scales approximately with particle volume $F_{\text{rad}} \propto a^3$, whereas the viscous drag associated with acoustic streaming scales approximately with particle radius $F_{\text{str}} \propto a$. Therefore, the relative contribution of streaming is expected to increase as the particle size decreases, and may be more significant for the $2\text{ }\mu\text{m}$ particles than for the $5\text{ }\mu\text{m}$ particles. In the present study, pulsed acoustic excitation was employed to avoid prolonged continuous actuation and may reduce the buildup of streaming compared with continuous excitation, while still enabling effective particle redistribution.³⁹ Nevertheless, acoustic streaming cannot be excluded and was not independently quantified. Secondary acoustic radiation forces may also promote particle-particle attraction when particles approach one another,^{40,41} although no pronounced or persistent clustering that hindered individual-particle identification was observed under the present conditions. These additional effects may become more important for smaller particles, higher particle concentrations, or prolonged acoustic actuation.

C. Acoustic-field confinement and chamber-height design

To analyze the acoustic field responsible for APC, we examined the wave propagation and field distribution in the shallow observation chamber. As illustrated in Fig. 2(a), the BAW generated by the actuator propagates upward through the acoustic waveguide and enters the liquid sample layer. After reaching the upper glass boundary, part of the acoustic wave is reflected by the coverslip. The interaction between the incident and reflected waves forms a time-

averaged acoustic-field distribution within the chamber, giving rise to the axial acoustic radiation force that drives particle migration.

The lateral range of acoustic actuation is defined by the ultrasound coupling-gel waveguide. As shown in Fig. 2(b), the acoustic pressure amplitude is mainly concentrated within the waveguide-covered region and decreases rapidly outside this region. This localized pressure distribution is consistent with an actuation region that is primarily defined by the waveguide footprint. Under the simulated conditions, the waveguide may contribute to spatially selective APC without modifying the counting chamber itself. The position of the waveguide-defined actuation region should also be carefully selected. In particular, the effective actuation area was kept away from the liquid boundary and meniscus region, where ultrasound-induced interface oscillations may generate parasitic capillary-wave modes, including harmonic and higher-order responses.^{42,43} Such boundary-related disturbances can perturb the local acoustic field and lead to unstable particle motion. Therefore, avoiding actuation near the liquid boundary helps maintain a more stable and spatially confined APC effect.

Regarding biological compatibility, acoustic manipulation has been widely demonstrated to be compatible with living cells under appropriately controlled operating conditions, while acoustothermal heating remains an important consideration for maintaining cell viability during acoustic actuation.^{44,45} We, therefore, additionally evaluated the thermal exposure associated with APC. A pulsed excitation scheme was used to avoid prolonged uninterrupted acoustic driving while retaining sufficient particle redistribution under the tested conditions. As shown in Fig. 2(c), the actuator was driven by a sinusoidal burst signal consisting of a 30-cycle acoustic burst followed by a 70-cycle off interval, corresponding to a duty cycle of 30%. Acoustic streaming was not independently quantified, and particle-redistribution performance under pulsed and continuous-wave excitation was not directly compared. Temperature measurements were performed at a room temperature of approximately

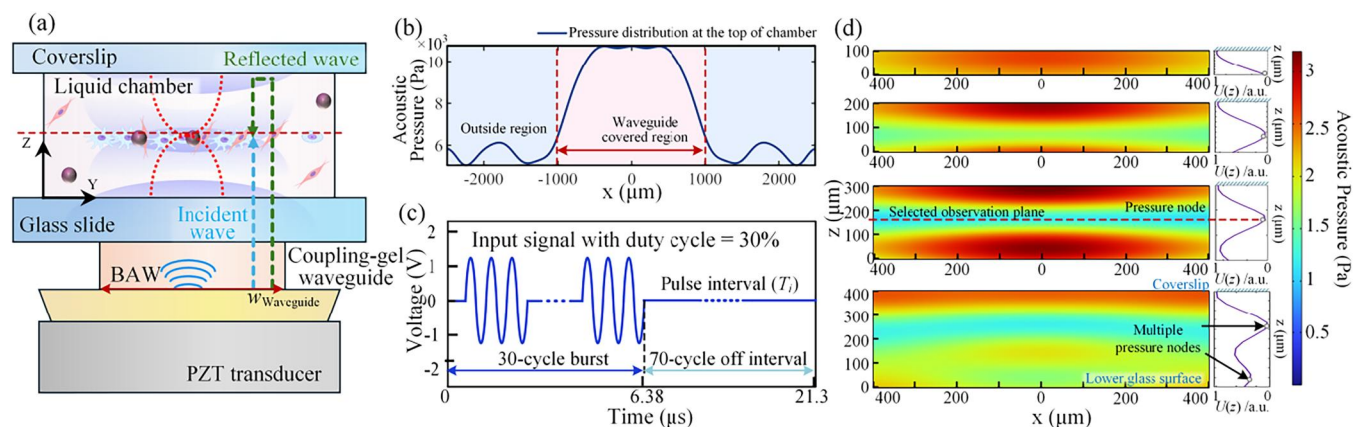


FIG. 2. Acoustic-field confinement and chamber-height design for APC. (a) BAW propagation and reflection in the shallow observation chamber, forming an acoustic field associated with particle migration. (b) Simulated acoustic pressure distribution showing a localized pressure field beneath the ultrasound coupling-gel waveguide. (c) Pulsed excitation waveform consisting of a 30-cycle sinusoidal burst followed by a 70-cycle off interval, corresponding to a 30% duty cycle. (d) Simulated acoustic pressure distributions for chamber heights of 100–400 μm . The axial pressure profiles along the waveguide centerline suggest that the 100 μm chamber used in the standard counting chamber can support an observation layer near the lower portion of the chamber under the modelled conditions.

25 °C. This duty cycle was selected to limit cumulative acoustothermal effects, as verified by the temperature measurements shown in Fig. S2 in the [supplementary material](#). Under the 30% duty-cycle condition, the mean temperature increase did not exceed approximately 1.6 °C during the first 30 s, substantially longer than the 2–10 s actuation required for APC.

As chamber height can influence the axial distribution of the simulated acoustic field, we used COMSOL finite-element analysis to evaluate chambers with heights ranging from 100 to 400 μm under near-operating-frequency excitation. The acoustic response was analyzed in the time domain using the same pulsed excitation condition. As shown in Fig. 2(d), the pressure–amplitude profiles along the vertical centerline of the waveguide reveal distinct height-dependent field distributions. For the 100 μm -high chamber corresponding to the standard counting chamber used in this study, the simulated field is consistent with the formation of an observation plane near the lower portion of the chamber. These results further suggest that, to maintain a relatively simple axial field distribution suitable for APC, the chamber height should preferably be kept below approximately one acoustic wavelength in water. At 4.745 MHz, the acoustic wavelength in water is approximately 310 μm . Therefore, both the nominal chamber depth of 100 μm and the experimentally measured assembled height of approximately 110–120 μm remain well below one wavelength. The modest difference between the nominal and assembled chamber heights is, therefore, not expected to change the qualitative axial-field regime identified in the present simulations. This simulation was used as a qualitative design assessment and does not establish the exact equilibrium height of the particle layer.

D. Materials and experimental setup

A PZT-5 piezoelectric ceramic plate with a resonance frequency of 4.745 MHz and a size of 1.5 cm $\times$ 1.5 cm was used as the BAW actuator. The actuator was operated at 4.745 MHz, selected from the measured electromechanical resonance in the S_{11} spectrum of the PZT actuator (Fig. S1 in the [supplementary material](#)). The operating frequency was, therefore, determined from the actuator response rather than from an idealized resonance condition of the nominal chamber depth. The excitation signal was generated by a function generator (AFG3022C, Tektronix) and amplified using a radio frequency power amplifier (ZHL-1-2W+, Mini-Circuits) before being applied to the PZT actuator. The function generator was set to 1 V_{pp} into 50 Ω , corresponding to an RF input power of approximately 2.5 mW (+4.0 dBm) at the amplifier input. Fluorescence images and videos of PS particles, together with bright-field images and videos of *C. sorokiniana*, were acquired using an inverted fluorescence microscope (CKX53, Olympus) equipped with an industrial camera (INFINITY3, Lumenera).

The acoustic waveguide was formed from medical ultrasound coupling gel (HYNAUT, China), which coupled acoustic energy from the BAW actuator to the sample region. During assembly, the axial thickness of the gel layer was mechanically constrained by the surrounding edge structure of the actuator and was, therefore, maintained approximately constant, although its lateral contour could vary slightly between preparations. A commercially available improved Neubauer hemocytometer and its matched

cover glass were used to form the observation chamber. The nominal chamber depth specified for the hemocytometer is 0.1 mm (100 μm). As the actual liquid-layer height can vary with cover-glass seating and capillary loading, the assembled chamber height was measured after filling at four corner regions. Under the standardized loading procedure used in this study, the measured heights were approximately 110–120 μm , with observable corner-to-corner variation. To improve chamber reproducibility and minimize cover-glass inclination, approximately equal amounts of wetting liquid were applied to the two cover-glass support rails using a micropipette before positioning the matched cover glass. Excess liquid, asymmetric wetting, visible air bubbles, and incomplete filling were avoided, as asymmetric chamber assembly could introduce a lateral height gradient and was experimentally associated with noticeable lateral particle motion during acoustic actuation. The grid area used for counting was 0.0025 mm².

Fluorescently labeled monodisperse polystyrene (PS) particle suspensions (Tianjin BaseLine, China) with a mass fraction of 1% were used. Two particle diameters, 2 and 5 μm , were evaluated as well-defined model suspended objects. The density and compressibility of PS were taken as approximately 1050 kg m⁻³ and 2.49×10^{-10} Pa⁻¹, respectively, based on values commonly used in acoustofluidic studies.^{46,47} Before experiments, the 2 and 5 μm particle suspensions were diluted separately with de-ionized water at volume ratios of 1:20 and 1:4, respectively. *C. sorokiniana* was additionally used as a biological test sample. The commercially obtained live culture (Fengbiao Algae Industry, China) contained approximately spherical cells with diameters of 2–5 μm , comparable to the PS particles used in this study. The culture was gently mixed and used directly without further dilution for the APC experiments.

E. Image acquisition and quantitative focus analysis

To quantify single-plane particle observability, PS-particle fluorescence images were analyzed using a unified image-processing workflow. For each experiment, images were acquired before acoustic actuation at different focal positions and after acoustic actuation at the selected observation layer. The green fluorescence channel was extracted, corrected for uneven background, and used for particle detection.

For each detected particle, a local focus index was calculated from two image features: edge sharpness and spot compactness. Sharply focused particles show stronger local intensity gradients and more compact fluorescent spots, whereas out-of-focus particles appear blurred and spatially spread. A single global threshold was then applied to classify detected particle images as either in focus or out of focus. The in-focus particle fraction was calculated as the number of detected particle images classified as in focus divided by the total number of detected particle images in each image. The focus analysis was used as an image-based metric of single-plane observability rather than as a direct measurement of the absolute axial position of individual particles. Accordingly, the reported in-focus fraction does not provide a quantitative measurement of the complete particle z-distribution or the physical thickness of the acoustically redistributed particle layer. A detected particle image was classified as in focus only when its predefined focus index exceeded the selected threshold. Therefore, the

reported in-focus fraction represents the fraction of detected particle images satisfying the image-quality criterion. In-focus particles are marked in cyan and out-of-focus particles are marked in red.

C. sorokiniana cells were analyzed separately using bright-field images. Red, green, and blue (RGB) images were converted to grayscale, and local dark-cell contrast was calculated as the Gaussian-smoothed background minus the grayscale image. Candidate cells were identified by intensity thresholding and filtered by area and circularity. Detections near image boundaries were excluded. Merged candidates containing multiple spatially separated dark peaks were split where individual cells could be resolved. Detected cells were marked with green circles and counted within each image.

III. RESULTS AND DISCUSSION

A. Rapid acoustic planar concentration of micrometer particles

The APC experiments in Secs. III A and III B were performed at 4.745 MHz with a function-generator setting of 1 V_{pp} into 50 Ω (approximately 2.5 mW at the amplifier input) and a 30% duty cycle (30 cycles ON and 70 cycles OFF). We first evaluated the

ability of APC to improve single-plane particle observability in a vertically dispersed suspension using 5 μ m fluorescent polystyrene particles. As illustrated in Fig. 3(a), three focal conditions were compared: the upper region of the chamber before acoustic actuation, the lower region of the chamber before acoustic actuation, and the selected observation layer after actuation. This comparison was designed to assess the difference between pre-actuation axial dispersion and post-actuation single-plane observability. Before actuation, particles were distributed over different heights in the 100 μ m chamber; therefore, focusing near either the upper or lower region brought only part of the particle population into clear focus. After acoustic actuation, particle images were more readily detected within a selected observation layer that could be brought into focus for observation. Under these conditions, with the microscope focal position kept unchanged, most initially defocused 5 μ m particle images became sharply observable within approximately 2 s of acoustic actuation, consistent with axial redistribution toward the selected observation plane, as shown in Fig. 3 (Multimedia available online).

Representative fluorescence images confirmed a change in single-plane particle observability. Before actuation, the upper focal plane showed 403 detected particle images, of which 45.9%

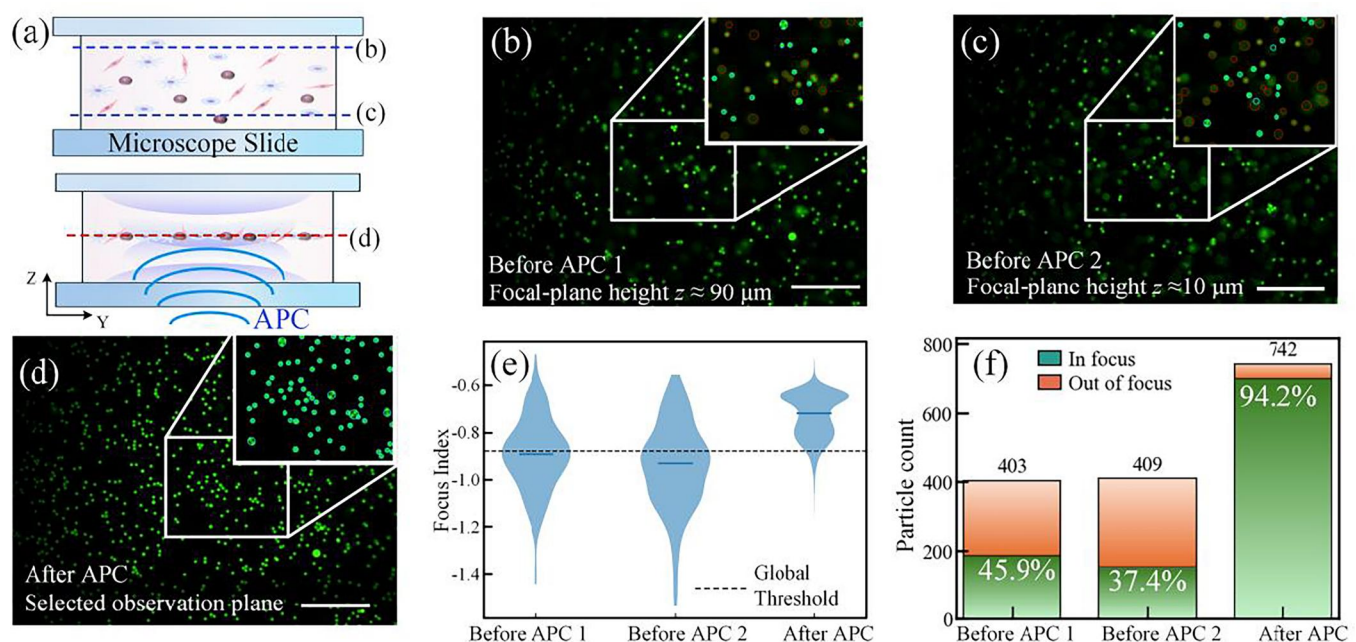


FIG. 3. Acoustic planar concentration of 5 μ m fluorescent particles. (a) Schematic showing the three focal conditions used for image comparison: the upper region of the chamber before acoustic actuation, the lower region before acoustic actuation, and the selected observation layer after actuation. (b) Representative fluorescence image acquired at the upper focal plane before acoustic actuation. Detected particle images are classified by image-based focus analysis, with in-focus particle images marked in cyan and out-of-focus particle images marked in red. (c) Representative fluorescence image acquired at the lower focal plane before acoustic actuation. (d) Representative fluorescence image after acoustic actuation, acquired at the selected observation layer. (e) Distribution of log-transformed focus-index values for the three imaging conditions. The focus index combines local edge sharpness and fluorescent-spot compactness; higher values indicate sharper and more compact particle images. The dashed line indicates the global threshold used to classify particle images as in focus or out of focus. (f) Quantitative comparison of detected-particle count and in-focus fraction before and after acoustic actuation. APC increased the number of detected particle images in the selected focal plane and the fraction classified as in focus. Scale bars: 100 μ m (Multimedia available online).

were classified as in focus [Fig. 3(b)]. The lower focal plane showed a similar detected-particle count of 409, but only 37.4% were classified as in focus [Fig. 3(c)]. These two focal planes indicate that, without APC, no single focal plane contained most particle images in a clearly observable state. After acoustic actuation, the selected observation layer contained 742 detected particle images, most of which were classified as in focus [Fig. 3(d)]. This result shows that APC increased the number of detected particle images within the selected focal plane and the proportion classified as in focus. To classify particle images objectively, we used a focus index based on local image sharpness and spot compactness. The focus index increases when a particle image exhibits sharper edges and a more compact fluorescent spot, whereas blurred particle images have lower focus-index values. The distributions of log-transformed focus-index values before and after acoustic actuation are shown in Fig. 3(e), together with the global threshold used to

separate in-focus and out-of-focus particle images. This analysis provides an objective image-based definition of whether a detected particle image satisfied the predefined in-focus criterion. The complete focal scanning and refocusing process for $5\text{ }\mu\text{m}$ particles is provided in Fig. 3 (Multimedia available online) and shows the initial axial dispersion of particles, the APC actuation process, and the final refocusing to the selected observation plane.

The quantitative comparison is summarized in Fig. 3(f). In the representative experiment, the in-focus fraction increased from 45.9% and 37.4% before actuation to 94.2% after actuation. Meanwhile, the total detected particle number increased from 403 and 409 before actuation to 742 after actuation. Taking the average of the two pre-actuation focal planes as the baseline, the total detected particle number increased by approximately 1.8-fold. More importantly, the number of in-focus particles increased from approximately 165 before actuation to approximately 693 after

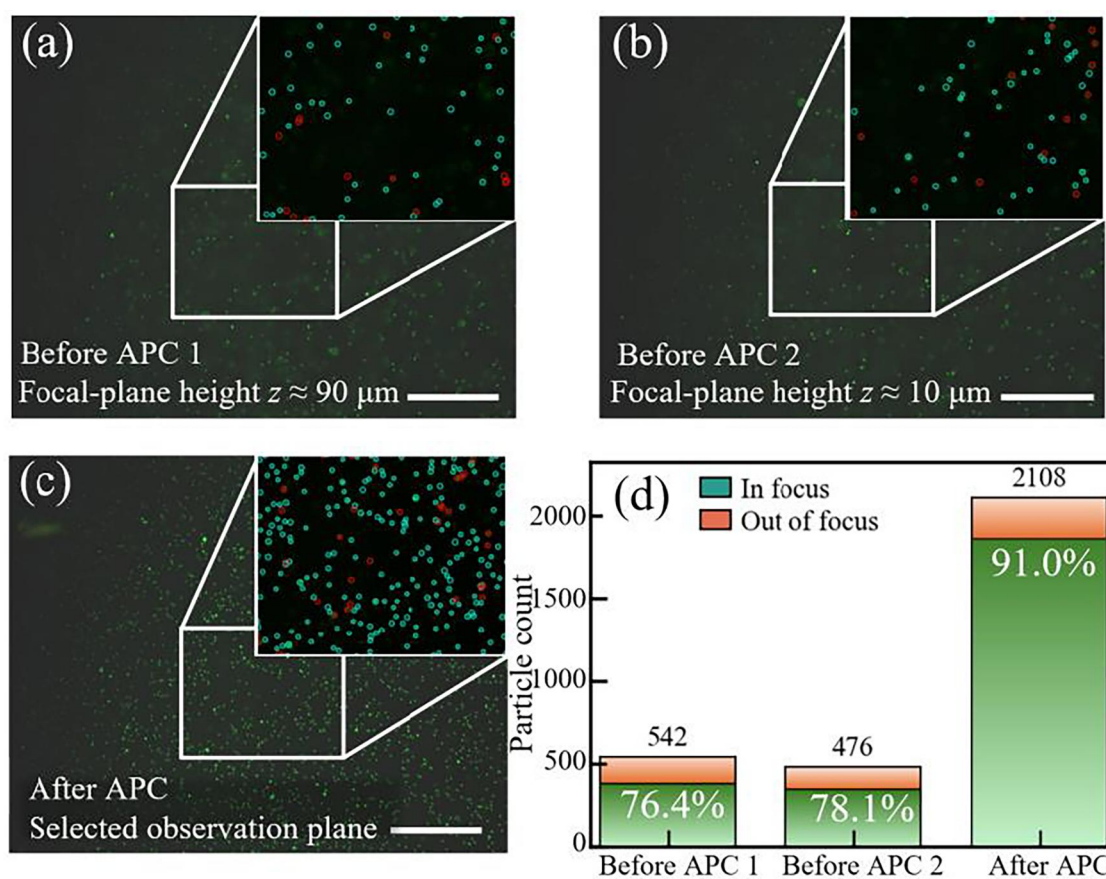


FIG. 4. Acoustic planar concentration of $2\text{ }\mu\text{m}$ fluorescent particles. (a) Representative fluorescence image acquired at the upper focal plane before acoustic actuation. Detected particle images were classified using the same image-based focus analysis as in Fig. 3, with in-focus particle images marked in cyan and out-of-focus particle images marked in red. (b) Representative fluorescence image acquired at the lower focal plane before acoustic actuation. (c) Representative fluorescence image acquired at the selected observation layer after acoustic actuation. (d) Quantitative comparison of detected-particle count and in-focus fraction before and after acoustic actuation. APC increased the number of detected particle images in the selected focal plane and the fraction classified as in focus. Scale bars: $100\text{ }\mu\text{m}$ (Multimedia available online).

actuation, corresponding to an approximately 4.2-fold increase. Repeated experiments further confirmed the rapid and robust enrichment effect, with the in-focus fraction increasing from $40.9 \pm 3.2\%$ before APC to $93.7 \pm 2.3\%$ after APC within approximately 2 s. These results show that APC can enhance single-plane particle observability on a seconds timescale through acoustically induced axial redistribution.

We further evaluated the APC effect using $2\mu\text{m}$ fluorescent polystyrene particles as a smaller-particle test case. For $2\mu\text{m}$ particles, the evaluation considered both the in-focus fraction and the number of detected particle images within a single field of view. Representative images acquired before acoustic actuation showed that particles were distributed across different axial positions in the chamber. At the upper focal plane, 542 particles were detected, with 76.4% classified as in focus [Fig. 4(a)]. At the lower focal plane, 476 particles were detected, with 78.1% classified as in focus [Fig. 4(b)]. Although a substantial fraction of detected particle images was classified as in focus before actuation, each focal plane still captured only part of the suspended particle population. This indicates that the particles remained axially dispersed before APC.

After acoustic actuation, a larger number of particle images was detected within the selected observation layer. The detected-particle count increased to 2108, and 91.0% of the detected particle images were classified as in focus according to the predefined image-based criterion [Fig. 4(c)]. Compared with the average of the two pre-actuation focal planes, the number of detected particle images in the selected focal plane increased by approximately 4.1-fold, while the in-focus fraction increased from 77.3% to 91.0% [Fig. 4(d)]. The estimated number of detected particle images classified as in focus increased from approximately 393 before actuation to approximately 1918 after actuation, corresponding to an approximately 4.9-fold increase within the selected focal plane. This result indicates improved single-plane observability for $2\mu\text{m}$ particles under the tested conditions. Repeated experiments showed that the

in-focus fraction increased from $76.1 \pm 6.5\%$ before APC to $90.6 \pm 2.5\%$ after APC, and the enrichment process was completed within approximately 5 s. In contrast, $2\mu\text{m}$ PS particles typically require about 20 min to sediment naturally in a $100\mu\text{m}$ height chamber. The dynamic focal scanning and refocusing process for the $2\mu\text{m}$ particle experiment is shown in Fig. 4 (Multimedia available online). The result shows that APC can improve single-plane observability for a second particle size under the tested conditions. As the acoustic radiation force decreases rapidly with particle size, as shown in Eq. (1), manipulation of smaller particles may require a more tightly confined acoustic field. One practical route is to increase the acoustic frequency, thereby reducing the acoustic wavelength and strengthening the axial field gradient. In this case, the chamber height should also be reduced proportionally to the acoustic wavelength to maintain an appropriate simulated axial field distribution for planar concentration.

B. Waveguide-associated localized APC effect

After demonstrating improved single-plane observability for both 5 and $2\mu\text{m}$ particles, we assessed whether the APC response was spatially associated with the ultrasound coupling-gel waveguide. This analysis was performed because APC is intended to regulate particles within a prescribed observation region rather than induce uncontrolled particle redistribution across the entire field of view.

As shown in Fig. 5(a), the analyzed field of view was divided into two equal-area regions. The upper region corresponded to the waveguide-defined actuation area, whereas the lower region was outside the effective waveguide region. Particles classified as within the focused planar layer were marked with green circles, while particles classified as out of focus were marked with red circles. A clear difference was observed between the two regions: $2\mu\text{m}$ particles inside the waveguide-defined region were more frequently classified as in focus, whereas a larger fraction of particles

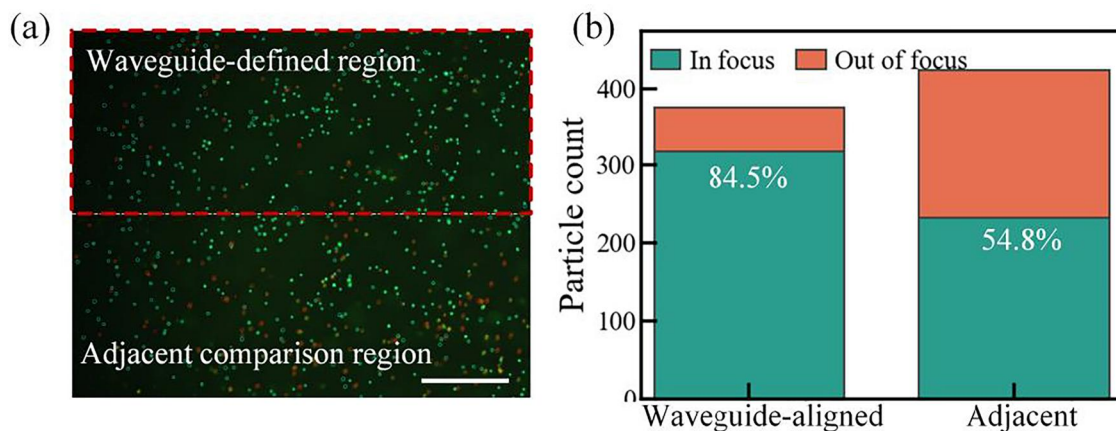


FIG. 5. Waveguide-associated localized APC response. (a) Representative image-based focus analysis comparing regions inside and outside the waveguide-defined actuation area. The upper half of the analyzed field corresponds to the waveguide-defined region, whereas the lower half is an adjacent comparison region. Detected particle images classified as in focus are marked with green circles, and those classified as out of focus are marked with red circles. (b) The representative within-field comparison shows a higher in-focus fraction in the waveguide-defined region than in the adjacent comparison region, consistent with a localized enhancement beneath the waveguide-defined region. Scale bars: $100\mu\text{m}$.

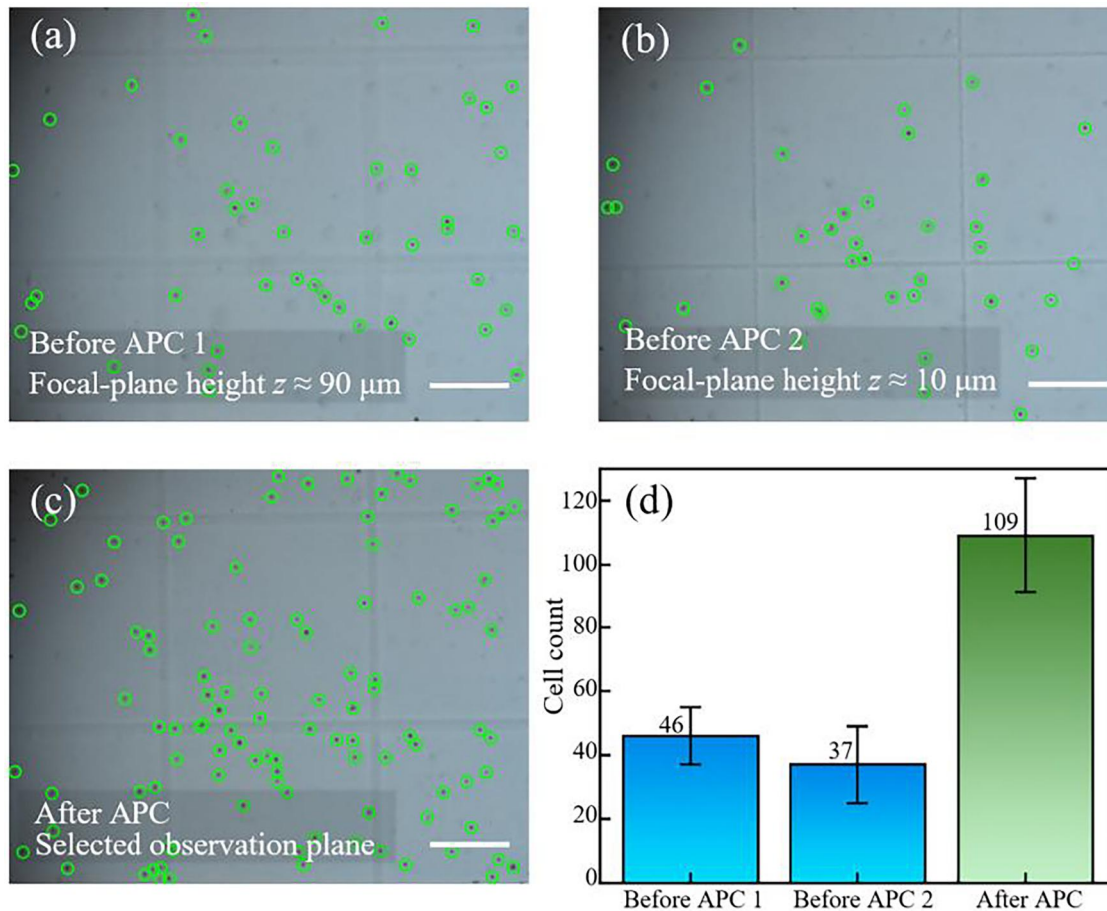


FIG. 6. Acoustic planar concentration of *C. sorokiniana*. (a) and (b) Representative images acquired before acoustic actuation at focal-plane heights of approximately $z = 90 \mu\text{m}$ and $z = 10 \mu\text{m}$, respectively. Detected cells are indicated by green circles. (c) Representative image acquired after APC at the selected observation plane. (d) Quantitative comparison of the detected cell count before and after APC. Scale bars: $100 \mu\text{m}$ (Multimedia available online).

outside the effective actuation area remained out of focus. The quantitative comparison is shown in Fig. 5(b). Within the waveguide-defined actuation region, the in-focus fraction reached 84.5%, whereas the region outside the effective actuation area showed an in-focus fraction of 54.8%. The in-focus fraction was 29.7 percentage points higher inside the waveguide-defined region, corresponding to 1.54 times the value outside the region. Conversely, the out-of-focus fraction increased from 15.5% inside the actuation region to 45.2% outside it. These results are consistent with a localized enhancement of single-plane particle observability beneath the waveguide-defined region, rather than a uniform effect throughout the observation field.⁴⁸ Accordingly, the waveguide may support spatially selective APC while preserving the simple structure of the standard counting chamber.

C. APC of a biological sample

To evaluate the applicability of APC to biological samples, we further performed experiments using *C. sorokiniana*. Before acoustic

actuation, the cells were distributed over different axial positions within the counting chamber. Representative images acquired at focal-plane heights of approximately $z = 90 \mu\text{m}$ and $z = 10 \mu\text{m}$ contained 46 and 37 detected cells, respectively [Figs. 6(a) and 6(b)].

After acoustic actuation, substantially more cells became observable within a single selected observation plane. A total of 109 cells were detected in the corresponding field of view after APC [Figs. 6(c) and 6(d)]. The dynamic redistribution process was completed within approximately 10 s and is shown in Fig. 6 (Multimedia available online). These results extend the APC demonstration from well-defined PS model particles to a biological sample and show that acoustic actuation can increase the number of cells observable within a single focal plane.

Under the 30% duty-cycle condition used in these experiments, the measured mean temperature increase did not exceed approximately 1.6°C during the first 30 s (Fig. S2 in the supplementary material), which is substantially longer than the approximately 10 s actuation required for the biological sample.

This thermal measurement indicates limited acoustothermal exposure during the APC process, although a dedicated viability assay was not performed in the present study.

IV. CONCLUSIONS

In this work, we introduced acoustic planar concentration (APC) as a compact strategy for improving the single-plane observability of suspended micro-objects in a conventional counting chamber with a nominal depth of $100\text{ }\mu\text{m}$. Using $5\text{ }\mu\text{m}$ and $2\text{ }\mu\text{m}$ fluorescent polystyrene particles, APC enabled seconds-scale redistribution and markedly increased the number of detectable particles within a selected focal plane. For $2\text{ }\mu\text{m}$ particles, the in-focus fraction increased from $76.1 \pm 6.5\%$ to $90.6 \pm 2.5\%$ within approximately 5 s. APC was further demonstrated using *C. sorokiniana*, for which the detected cell count increased from 46 ± 9 and 37 ± 12 at two pre-actuation focal planes to 109 ± 18 after APC. Together with the limited temperature rise observed under the 30% duty-cycle condition, these results support the applicability of APC to rapid microscopy of suspended particles and biological cells. This second-scale redistribution highlights the practical advantage of APC over passive chamber-based observation. The hemocytometer retains its conventional counting function, while APC actively accelerates the preparation of a microscope-observable particle distribution. Future work should further investigate three-dimensional particle redistribution, acoustic streaming, and biological viability.

SUPPLEMENTARY MATERIAL

The [supplementary material](#) provides experimental characterization supporting the operating conditions used for acoustic planar concentration. Figure S1 presents the measured reflection coefficient (S11) spectrum of the PZT actuator over 4–6 MHz and identifies the pronounced resonance near 4.745 MHz that was selected as the operating frequency. Figure S2 presents the temperature evolution during 120 s of acoustic excitation at duty cycles of 30%, 50%, and 100%, while maintaining the operating frequency and excitation amplitude at 4.745 MHz and 1 V_{pp} , respectively. These data support the selection of the actuation frequency and the 30% duty-cycle condition used in the experiments.

ACKNOWLEDGMENTS

This work was supported by the Research Start-Up Fund for Young Faculty of Sichuan University (No. sksz202522), and the 1-3-5 Project for Hospital Management Research Program of West China Hospital, Sichuan University (No. ZYYG24010). The authors acknowledge the “Future Medicine+” Innovation Open Course Series (organized by West China School of Medicine, Sichuan University), for fostering the cross-disciplinary exchange between clinical medicine and engineering that informed this work. Liuchao Jin acknowledges support from the Hong Kong Research Grants Council under the Hong Kong Ph.D. Fellowship Scheme (No. PF21-60853).

AUTHOR DECLARATIONS

Conflict of Interest

The authors have no conflicts to disclose.

Author Contributions

Yiwen Zhang: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Validation (equal); Visualization (equal); Writing – original draft (equal). **Xin Li:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Visualization (equal); Writing – original draft (equal). **Xian Zou:** Data curation (equal); Formal analysis (equal); Investigation (equal); Visualization (equal); Writing – original draft (equal). **Haoyue Deng:** Data curation (equal); Visualization (equal); Writing – original draft (equal). **Yali Liu:** Conceptualization (equal); Data curation (equal); Formal analysis (equal); Writing – review & editing (equal). **Wenwu ling:** Project administration (equal); Resources (equal); Supervision (equal); Writing – review & editing (equal). **Yan Wu:** Conceptualization (equal); Funding acquisition (equal); Project administration (equal); Resources (equal); Supervision (equal); Writing – review & editing (equal). **Liuchao Jin:** Conceptualization (equal); Formal analysis (equal); Methodology (equal); Project administration (equal); Supervision (equal); Visualization (equal); Writing – review & editing (equal).

DATA AVAILABILITY

The data that support the findings of this study are available within the article and its [supplementary material](#).

REFERENCES

- 1A. Vembadi, A. Menachery, and M. A. Qasaimeh, “Cell cytometry: Review and perspective on biotechnological advances,” *Front. Bioeng. Biotechnol.* **7**, 147 (2019).
- 2M. Boutros, F. Heigwer, and C. Laufer, “Microscopy-based high-content screening,” *Cell* **163**(6), 1314–1325 (2015).
- 3N. Opatovski, E. Nehme, N. Zoref, I. Barzilai, R. Orange Kedem, B. Ferdman, P. Keselman, O. Alalouf, and Y. Shechtman, “Depth-enhanced high-throughput microscopy by compact PSF engineering,” *Nat. Commun.* **15**(1), 4861 (2024).
- 4A. Bäckström, L. Kugel, C. Gnann, H. Xu, J. E. Aslan, E. Lundberg, and C. Stadler, “A sample preparation protocol for high throughput immunofluorescence of suspension cells on an adherent surface,” *J. Histochem. Cytochem.* **68** (7), 473–489 (2020).
- 5A. Beghin, G. Grenici, G. Sahni, S. Guo, H. Rajendiran, T. Delaire, S. B. Mohamad Raffi, D. Blanc, R. de Mets, H. T. Ong, X. Galindo, A. Monet, V. Acharya, V. Racine, F. Levet, R. Galland, J.-B. Sibarita, and V. Viasnoff, “Automated high-speed 3D imaging of organoid cultures with multi-scale phenotypic quantification,” *Nat. Methods* **19**(7), 881–892 (2022).
- 6T. Zhu, J. Nie, T. Yu, D. Zhu, Y. Huang, Z. Chen, Z. Gu, J. Tang, D. Li, and P. Fei, “Large-scale high-throughput 3D culture, imaging, and analysis of cell spheroids using microchip-enhanced light-sheet microscopy,” *Biomed. Opt. Express* **14**(4), 1659–1669 (2023).
- 7T. Trombetta, F. Vidussi, C. Roques, M. Scotti, and B. Mostajir, “Marine microbial food web networks during phytoplankton bloom and non-bloom periods: Warming favors smaller organism interactions and intensifies trophic cascade,” *Front. Microbiol.* **11**, 502336 (2020).

- ⁸A. Desmaison, L. Guillaume, S. Triclin, P. Weiss, B. Ducommun, and V. Lobjois, "Impact of physical confinement on nuclei geometry and cell division dynamics in 3D spheroids," *Sci. Rep.* **8**(1), 8785 (2018).
- ⁹T. Takahashi, "Applicability of automated cell counter with a chlorophyll detector in routine management of microalgae," *Sci. Rep.* **8**(1), 4967 (2018).
- ¹⁰Y. Han, Y. Gu, A. C. Zhang, and Y.-H. Lo, "Review: Imaging technologies for flow cytometry," *Lab Chip* **16**(24), 4639–4647 (2016).
- ¹¹A. Kleiber, D. Kraus, T. Henkel, and W. Fritzsche, "Review: Tomographic imaging flow cytometry," *Lab Chip* **21**(19), 3655–3666 (2021).
- ¹²J. P. Robinson, R. Ostafe, S. N. Iyengar, B. Rajwa, and R. Fischer, "Flow cytometry: The next revolution," *Cells* **12**(14), 1875 (2023).
- ¹³West China Hospital, Sichuan University, see <https://www.wchscu.cn/comprehensive/72724.html> for "The fourth 'Future Medicine+' innovation open course, 'Medical-engineering integration: Ingenious Chip'" (2023).
- ¹⁴West China Hospital, Sichuan University, see https://www.wchscu.cn/public/teaching_news/79062.html for "The seventh 'Future Medicine+' innovation open course, 'Medical-engineering integration: ingenious calculation'" (2024).
- ¹⁵O. N. Perera, A. P. Sobinoff, E. T. Teber, A. Harman, M. F. Maritz, S. F. Yang, H. A. Pickett, A. J. Cesare, J. W. Arthur, K. L. MacKenzie, and T. M. Bryan, "Telomerase promotes formation of a telomere protective complex in cancer cells," *Sci. Adv.* **5**(10), eaav4409 (2019).
- ¹⁶A. Ligosová and K. Koberna, "New concept and apparatus for cytocentrifugation and cell processing for microscopy analysis," *Int. J. Mol. Sci.* **22**(13), 7098 (2021).
- ¹⁷G. Iacono, A. Abay, J. S. G. Murillo, F. Agliarolo, N. Yagci, E. Varga, T. Bijlsma, J. Sohler, K. Fu, J. A. Reisz, A. Argabright, A. D'Alessandro, A. F. Svendsen, M. von Lindern, and E. van den Akker, "Differentiating erythroblasts adapt to mechanical stimulation by upregulation of cholesterol biosynthesis via S1P/SREBP-induced HMGCR expression," *Sci. Rep.* **14**(1), 30157 (2024).
- ¹⁸R. E. Fernandez, A. Rohani, V. Farmehini, and N. S. Swami, "Review: Microbial analysis in dielectrophoretic microfluidic systems," *Anal. Chim. Acta* **966**, 11–33 (2017).
- ¹⁹L. R. Moore, P. S. Williams, J. J. Chalmers, and M. Zborowski, "Tessellated permanent magnet circuits for flow-through, open gradient separations of weakly magnetic materials," *J. Magn. Magn. Mater.* **427**, 325–330 (2017).
- ²⁰F. Guo, X.-H. Ji, K. Liu, R.-X. He, L.-B. Zhao, Z.-X. Guo, W. Liu, S.-S. Guo, and X.-Z. Zhao, "Droplet electric separator microfluidic device for cell sorting," *Appl. Phys. Lett.* **96**(19), 193701 (2010).
- ²¹X. Hu, J. Zheng, Q. Hu, L. Liang, D. Yang, Y. Cheng, S.-S. Li, L.-J. Chen, and Y. Yang, "Smart acoustic 3D cell construct assembly with high-resolution," *Biofabrication* **14**(4), 045003 (2022).
- ²²S. Yang, J. Rufo, R. Zhong, J. Rich, Z. Wang, L. P. Lee, and T. J. Huang, "Acoustic tweezers for high-throughput single-cell analysis," *Nat. Protoc.* **18**(8), 2441–2458 (2023).
- ²³M. Baudoin, J.-L. Thomas, R. A. Sahely, J.-C. Gerbedoen, Z. Gong, A. Sivery, O. B. Matar, N. Smagin, P. Favreau, and A. Vlandas, "Spatially selective manipulation of cells with single-beam acoustical tweezers," *Nat. Commun.* **11**(1), 4244 (2020).
- ²⁴L. Shen, Z. Tian, K. Yang, J. Rich, J. Xia, N. Upreti, J. Zhang, C. Chen, N. Hao, Z. Pei, and T. J. Huang, "Joint subarray acoustic tweezers enable controllable cell translation, rotation, and deformation," *Nat. Commun.* **15**(1), 9059 (2024).
- ²⁵M. Rode, A. Bioue, F. Miano, H. Bruus, T. Kjørboe, and A. Andersen, "Acoustic tethering of microorganisms," *J. Exp. Biol.* **225**(20), jeb244089 (2022).
- ²⁶B. Hammarström, M. Vassalli, and P. Glynne-Jones, "Acoustic focussing for sedimentation-free high-throughput imaging of microalgae," *J. Appl. Phycol.* **32**(1), 339–347 (2020).
- ²⁷R. Zmijan, U. S. Jonnalagadda, D. Carugo, Y. Kochi, E. Lemm, G. Packham, M. Hill, and P. Glynne-Jones, "High throughput imaging cytometer with acoustic focussing," *RSC Adv.* **5**(101), 83206–83216 (2015).
- ²⁸S. J. Raymond, D. J. Collins, R. O'Rourke, M. Tayebi, Y. Ai, and J. Williams, "A deep learning approach for designed diffraction-based acoustic patterning in microchannels," *Sci. Rep.* **10**(1), 8745 (2020).
- ²⁹E. I. Galanzha, M. G. Viegas, T. I. Malinsky, A. V. Melerzanov, M. A. Juratli, M. Sarimollaoglu, D. A. Nedosekin, and V. P. Zharov, "In vivo acoustic and photoacoustic focusing of circulating cells," *Sci. Rep.* **6**(1), 21531 (2016).
- ³⁰J. P. K. Armstrong, J. L. Puetzer, A. Serio, A. G. Guex, M. Kapnisi, A. Breant, Y. Zong, V. Assal, S. C. Skaalure, O. King, T. Murty, C. Meinert, A. C. Franklin, P. G. Bassindale, M. K. Nichols, C. M. Terracciano, D. W. Huttmacher, B. W. Drinkwater, T. J. Klein, A. W. Perriman, and M. M. Stevens, "Engineering anisotropic muscle tissue using acoustic cell patterning," *Adv. Mater.* **30**(43), 1802649 (2018).
- ³¹Y. Tsuyama, B. Xu, K. Hattori, S. Baek, Y. Yoshioka, R. Kojima, Y. Cho, T. Laurell, S. Kim, S. Ota, and S. Lee, "A 50 μ m acoustic resonator microchannel enables focusing 100 nm polystyrene beads and sub-micron bioparticles," *Sens. Actuators, B* **376**, 132918 (2023).
- ³²C. Devendran, K. Choi, J. Han, Y. Ai, A. Neild, and D. J. Collins, "Diffraction-based acoustic manipulation in microchannels enables continuous particle and bacteria focusing," *Lab Chip* **20**(15), 2674–2688 (2020).
- ³³D. Ahmed, A. Ozcelik, N. Bojanala, N. Nama, A. Upadhyay, Y. Chen, W. Hanna-Rose, and T. J. Huang, "Rotational manipulation of single cells and organisms using acoustic waves," *Nat. Commun.* **7**(1), 11085 (2016).
- ³⁴D. J. Collins, C. Devendran, Z. Ma, J. W. Ng, A. Neild, and Y. Ai, "Acoustic tweezers via sub-time-of-flight regime surface acoustic waves," *Sci. Adv.* **2**(7), e1600089 (2016).
- ³⁵N. Nama, R. Barnkob, Z. Mao, C. J. Kähler, F. Costanzo, and T. J. Huang, "Numerical study of acoustophoretic motion of particles in a PDMS microchannel driven by surface acoustic waves," *Lab Chip* **15**(12), 2700–2709 (2015).
- ³⁶S. Yang, J. Rufo, Y. Chen, C. Chen, B. W. Drinkwater, L. P. Lee, and T. J. Huang, "Acoustic tweezers for advancing precision biology and medicine," *Nat. Rev. Methods Primers* **5**(1), 49 (2025).
- ³⁷Y. Bian, F. Guo, S. Yang, Z. Mao, H. Bachman, S.-Y. Tang, L. Ren, B. Zhang, J. Gong, X. Guo, and T. J. Huang, "Acoustofluidic waveguides for localized control of acoustic wavefront in microfluidics," *Microfluid. Nanofluid.* **21**(8), 132 (2017).
- ³⁸H. Bruus, "Acoustofluidics 7: The acoustic radiation force on small particles," *Lab Chip* **12**(6), 1014–1021 (2012).
- ³⁹L. Li, K. Jia, and K. Yang, "Unsteady time-averaged streaming in microfluidics using traveling surface acoustic waves," *Microfluid. Nanofluid.* **26**(10), 75 (2022).
- ⁴⁰S. Sepehrirahnama, A. Ray Mohapatra, S. Oberst, Y. K. Chiang, D. A. Powell, and K.-M. Lim, "Acoustofluidics 24: Theory and experimental measurements of acoustic interaction force," *Lab Chip* **22**(18), 3290–3313 (2022).
- ⁴¹M. Wu, A. Ozcelik, J. Rufo, Z. Wang, R. Fang, and T. Jun Huang, "Acoustofluidic separation of cells and particles," *Microsyst. Nanoeng.* **5**(1), 32 (2019).
- ⁴²C. Devendran, D. J. Collins, Y. Ai, and A. Neild, "Huygens-Fresnel acoustic interference and the development of robust time-averaged patterns from traveling surface acoustic waves," *Phys. Rev. Lett.* **118**(15), 154501 (2017).
- ⁴³D. J. Collins, R. O'Rourke, C. Devendran, Z. Ma, J. Han, A. Neild, and Y. Ai, "Self-aligned acoustofluidic particle focusing and patterning in microfluidic channels from channel-based acoustic waveguides," *Phys. Rev. Lett.* **120**(7), 074502 (2018).
- ⁴⁴R. Weser, Z. Deng, V. V. Kondalkar, A. N. Darinskii, C. Cierpka, H. Schmidt, and J. König, "Three-dimensional heating and patterning dynamics of particles in microscale acoustic tweezers," *Lab Chip* **22**(15), 2886–2901 (2022).
- ⁴⁵M. Wiklund, "Acoustofluidics 12: Biocompatibility and cell viability in microfluidic acoustic resonators," *Lab Chip* **12**(11), 2018–2028 (2012).
- ⁴⁶A. Edthofer, J. Novotny, A. Lenshof, T. Laurell, and T. Baasch, "Acoustofluidic properties of polystyrene microparticles," *Anal. Chem.* **95**(27), 10346–10352 (2023).
- ⁴⁷V. Vitali, T. Yang, and P. Minzioni, "Separation efficiency maximization in acoustofluidic systems: Study of the sample launch-position," *RSC Adv.* **8**(68), 38955–38964 (2018).
- ⁴⁸H. Bruus, "Acoustofluidics 10: Scaling laws in acoustophoresis," *Lab Chip* **12**(9), 1578–1586 (2012).