
Supplementary information

Harmonic acoustics for dynamic and selective particle manipulation

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Supplementary Information

Harmonic acoustics for dynamic and selective particle manipulation

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Supplementary Note 1. Multi-harmonic SAW generation with segmented interdigital transducers

The controllable generation of complex SAW potential fields with interdigital transducers (IDTs) is often limited by the narrow passband (approximately within $\pm 10\%$ of the resonant frequency)^{41,42} of the conventional IDT or the so-called single-electrode-type transducer. Additionally, the conventional IDT design has zero response at some specific harmonic frequency orders⁴³⁻⁴⁶, which are determined by the number of electrodes per period (denoted as S_e). This is because the elemental charge density is zero⁴⁶ at harmonic frequency orders that are multiples of S_e . Moreover, the metallization ratio a/p , the ratio of the width (a) to the pitch (p) of an electrode, can influence the charge density. To overcome these limitations, split-electrode-type IDTs^{27,28,47} have been designed and demonstrated for multi-harmonic SAW generation. However, the harmonic components that can be generated are still quite limited. For example, the Split-4 design can only provide odd (f_1, f_3, f_5) harmonic components, and the Split-52 design can suppress the fifth component (f_5)²⁷.

Here, we demonstrated a segmented transducer that can generate multi-harmonic SAWs, including all passbands with frequencies $[f_n]_N$ ($N=8$). As shown in Supplementary Fig. 1a, a segmented IDT is composed of N individual sections with a finger (i.e., digit) spacings $d_n = d_1/n$ ($d_1 = \lambda_1/4, n=1, 2, \dots, N$). The spacings provide N distinct passbands with wavenumbers $[k_n]_N$ at the resonant frequencies $[f_n]_N$. By programming the frequency sequence of the applied signal, we can activate different harmonic interdigital transducers accordingly. Another advantage of this segmented transducer is the flexibility in response intensity design. For a given split-electrode-type IDT design, the SAW intensity of an individual harmonic component cannot be tuned due to the fixed IDT configuration^{27,28}. For the segmented IDT, however, the intensity response of each harmonic component can be individually controlled with the modulation of electrode finger-pair numbers. Specifically, we can strengthen the response intensity of higher-order harmonic components by simply increasing the finger-pair numbers. This enables us to easily separate an adherent cell-cell pair without largely increasing the amplitudes of the excited electronic signals, which may cause considerable heating and decrease the cell viability.

Supplementary Note 2. Time-division multiplexing-based generation of multi-harmonic SAWs

We proposed a time-division multiplexing-based method for generating SAWs at different harmonic orders and programming the sequence of the harmonic orders. Over a time period T , as shown in Supplementary Fig. 1b, the time-division multiplexed signal contains a total of N time divisions with each division denoted as Δt_n . The excitation frequency in each division Δt_n can be selected from the resonant frequencies $[f_n]_N$. Therefore, by programming the frequency sequence of the time-division multiplexed signal, we can activate different harmonic interdigital transducers accordingly. Considering the contributions of all the segmented IDTs, the resultant SAWs present as a temporally modulated acoustic field. And the frequency of this field can be dynamically altered according to the programmed frequency sequence of the time-division multiplexed signal.

We utilized a customized MATLAB program to control a dual-channel function generator (AFG3102C, Tektronix) for programmable time-division multiplexing-based generation of 2D SAW fields. Compared with the SAW generation scheme in the field of quantum technologies²⁶⁻²⁸, our method can significantly decrease the number of function generators needed for the synthesis of harmonic SAWs. We only need one dual-channel function generator, instead of 12

function generators needed in quantum applications, to generate the six-component harmonic SAW waveforms (e.g., f_1 to f_6) in both x- and y-directions. Additionally, due to the time-division multiplexing method, we do not need to use a power combiner for adding output signals, which further decreases the complexity of SAW waveforms generation. The system set up to drive the HANDS device is illustrated in Supplementary Fig. 2.

The HANDS platform enables various functions by only modulating the applied harmonic signals (Supplementary Fig. 2). Specifically, we assembled colloidal crystal monolayers using two five-component harmonic SAWs (f_2 to f_6) in both the x- and y-directions. With amplitude modulation of these multi-harmonic SAWs, colloidal clusters can be focused into a monolayer, as shown in Fig. 2b. By further modulating the phases between harmonic SAWs in the x- and y-directions, spin dynamics of the colloidal crystal monolayers can be studied (Fig. 2d-f).

Notably, we conducted both high throughput and selective colloid/cell pairing and separation with six-component harmonic SAWs (f_3 to f_8). The high throughput cell-cell pairing and separation can be achieved with 2-component harmonic SAWs in both the x- and y-directions (Fig. 3a, b). However, the current SAW-based method can only trap and manipulate cells as a group. The selective cell-cell pairing and separation are extremely challenging and have never been demonstrated before with acoustic methods. Here, we achieved selective cell-cell pairing and separation while keeping neighboring cells intact by the time-effective Fourier synthesis of harmonic SAWs (Fig. 1d). By further modulating the pulsing duration of each harmonic component of SAWs, precise cell-cell distance control for a selected cell pair can be achieved.

We can also generate 2-D customized particle patterns with five-component harmonic SAWs. We demonstrated the 2-D customized particle patterns by simply modulating two five-component harmonic SAWs (f_2 to f_6) in both the x- and y-directions, as shown in Fig. 3c. The resolution of the particle patterns achieved with the multi-harmonic SAWs method is 100 times finer than the acoustic holography methods^{16,17}.

Supplementary Note 3. Analytical model of 1-D harmonic standing SAWs

For a single spherical particle in an inviscid fluid, the acoustic radiation force⁴⁸⁻⁵¹ applied on the particle by the n^{th} -order harmonic standing acoustic wave can be derived from:

$$\mathbf{F}_n = -\nabla U_n. \quad (\text{S1})$$

Here, U_n is the Gor'kov acoustic potential generated by the n^{th} order harmonic standing SAWs, which can be calculated by:

$$U_n = \frac{4\pi r^3}{3} \left[\frac{1}{2} \beta_f \alpha_1 \langle p_n^2 \rangle - \frac{3}{4} \rho_f \alpha_2 \langle v_n^2 \rangle \right], \quad (\text{S2})$$

where,

$$\alpha_1 = 1 - \frac{\beta_p}{\beta_f}, \quad (\text{S3})$$

$$\alpha_2 = \frac{2(\rho_p - \rho_f)}{2\rho_p + \rho_f}, \quad (\text{S4})$$

r is the radius of a single spherical particle; $\langle p_n^2 \rangle$ and $\langle v_n^2 \rangle$ are the time-averaged squares of the acoustic pressure and velocity, for the n^{th} -order harmonic standing acoustic wave; $\beta_f (= 1/\rho_f c_f^2)$ and ρ_f are the compressibility and density of the inviscid fluid; β_p and ρ_p are the compressibility and density of the spherical particle.

For 1-D harmonic standing SAWs, the acoustic pressure in the x-direction can be defined as:

$$p_n^x = A_n \cos(k_n^x x) \cos(\omega_n^x t), \quad (\text{S5})$$

where A_n is the acoustic pressure amplitude, $\omega_n^x = 2\pi f_n^x$ is the angular frequency, and k_n^x is the wavenumber of the n^{th} order harmonic standing SAWs in the x-direction. k_n^x is given by:

$$k_n^x = \frac{\omega_n^x}{c_s^x} = nk_1^x, \quad (n = 1, 2, \dots, N), \quad (\text{S6})$$

where c_s^x ($3,589 \text{ m}\cdot\text{s}^{-1}$) is the SAW phase velocity in the x-direction and k_1^x is the wavenumber of the SAW at the base frequency f_1^x . The velocity v_n^x in the x-direction can be determined through the substitution of the pressure field p_n^x into the equation $\frac{\partial p_n^x}{\partial x} e_x = -\rho_f \partial v_n^x / \partial t$:

$$v_n^x = \frac{A_n}{\rho_f c_s^x} \sin(k_n^x x) \sin(\omega_n^x t) e_x, \quad (\text{S7})$$

e_x is the directivity vector in the x-direction.

The time-effective Gor'kov acoustic potential was generated by the HANDS device using a time division multiplexed signal with N divisions $[\Delta t_n]_N$ in a period T . To characterize this acoustic potential, we leveraged a time-weighted expression:

$$U_E = \sum_{n=1}^N \frac{\Delta t_n}{T} U_n, \quad (\text{S8})$$

where U_n is obtained with equation (S2). Here, U_E is the Fourier-synthesized acoustic potential which can be interpreted as the summation of sine or cosine wave harmonics by substituting (S2-7) into (S8). Each Fourier coefficient, which is proportional to the time-division Δt_n , can be modulated to generate arbitrary wave functions. The corresponding time-effective acoustic radiation force can be expressed as

$$\mathbf{F}_E = -\nabla U_E. \quad (\text{S9})$$

Notably, the acoustic radiation forces that are generated by the leaky SAWs are studied with a time-averaged basis over a complete oscillation cycle (microsecond scale⁴⁸). Considering the microparticle's relatively low velocity ($20 \text{ nm}/\mu\text{s}$) when applying an acoustic radiation force, the time-averaged cycle can be set as $10 \mu\text{s}$ but still sufficient to achieve a high spatial resolution (200 nm) for manipulating a $10 \mu\text{m}$ -diameter particle. Based on this consideration, signals in a wide frequency band (e.g., $10 \text{ MHz} - 500 \text{ MHz}$) can be sequentially applied, and the resultant distance change of a micrometer particle is less than 200 nm during a $10 \mu\text{s}$ cycle. In theory, a $10 \mu\text{s}$ cycle can enable more than 100 frequency components for multi-harmonic waves generation with a base frequency of 10 MHz . With the modulation of frequency, amplitude, phase, and pulsing period for each harmonic component, a variety of acoustic potential and radiation forces can be Fourier synthesized in a time-effective manner as indicated by equations (S8) and (S9).

Supplementary Note 4. Analytical model of 2-D harmonic standing SAWs

The activation of two orthogonal pairs of harmonic interdigital transducers produces harmonic acoustic wells with a 2-D grid-like shape. For a general case where two harmonic standing SAWs are excited, the time-averaged square of the acoustic pressure is described as:

$$\langle p_{i,j}^2 \rangle = \langle (p_i^x + p_j^y)^2 \rangle, \quad (S10)$$

where p_i^x is acoustic pressure of i^{th} harmonic standing SAW in the x direction and p_j^y is the acoustic pressure of j^{th} harmonic standing SAW in the y-direction.

By substituting (S5) into (S10), we can derive the analytical equation for the time-averaged squared acoustic pressure for 2-D harmonic standing SAWs.

For 2-D harmonic standing SAWs with the same excitation angular frequency ($\omega_i^x = \omega_j^y$) in the x and y directions, the time-averaged acoustic pressure squared is given by:

$$\langle p_{i,j}^2 \rangle = \frac{1}{2} \left[A_i^2 \cos^2(k_i^x x) + A_j^2 \cos^2(k_j^y y) + 2A_i A_j \cos(k_i^x x) \cos(k_j^y y) \right]. \quad (S11)$$

For 2-D harmonic standing SAWs with different excitation frequencies ($\omega_i^x \neq \omega_j^y$) in the x and y directions, the time-averaged acoustic pressure squared is given by:

$$\langle p_{i,j}^2 \rangle = \frac{1}{2} \left[A_i^2 \cos^2(k_i^x x) + A_j^2 \cos^2(k_j^y y) \right]. \quad (S12)$$

The time-averaged velocity squared for a general case with 2-D harmonic standing SAWs is described as:

$$\langle v_{i,j}^2 \rangle = \langle (v_i^x)^2 + (v_j^y)^2 \rangle, \quad (S13)$$

where v_i^x is velocity of i^{th} harmonic standing SAWs in the x direction and v_j^y is the velocity of j^{th} harmonic standing SAWs in the y-direction.

By substituting (S7) into (S13), we can derive the analytical equation for the time-averaged velocity squared for 2-D harmonic standing SAWs. In both cases (2-D harmonic standing SAWs with the same ($\omega_i^x = \omega_j^y$) or different ($\omega_i^x \neq \omega_j^y$) excitation angular frequencies), the time-averaged velocity squared for 2-D harmonic standing SAWs can be described as:

$$\langle v_{i,j}^2 \rangle = \frac{1}{2} \left\{ \frac{A_i^2}{\rho_f^2 c_s^{x2}} \sin^2(k_i^x x) + \frac{A_j^2}{\rho_f^2 c_s^{y2}} \sin^2(k_j^y y) \right\}. \quad (S14)$$

By substituting the squared values of time-averaged acoustic pressure and velocity into the acoustic potential equation (S8), the time-effective 2-D acoustic potential can be calculated. Furthermore, with equation (S9) analytical solutions of force distribution for 2-D harmonic acoustic wells can be solved.

Supplementary Note 5. Subwavelength manipulation resolution achieved by the Fourier-synthesized harmonic SAWs

In the acoustofluidic field, the smallest distance between two particles or cells that can be trapped with current available acoustic tweezers^{43,48-51} is half of the wavelength of the applied acoustic standing waves. Herein, we demonstrated particle manipulation within the half-wavelength with a time-effective Fourier-synthesized acoustic potential field, which is realized sequentially using nanosecond pulsing of multi-harmonic SAWs. Specifically, for acoustic wells (Fig. 4a, left) established by a standing acoustic potential field with a frequency $f = 80.0$ MHz, the distance, d , between two adjacent wells is fixed as the half-wavelength of the applied wave (i.e., $d = \lambda/2 = 22.5$ μm). However, for trapping wells (Fig. 4a, right) established by Fourier-synthesized acoustic potential fields with Ψ_1 and Ψ_2 (base frequency $f_1 = 40.0$ MHz, 2nd harmonic frequency $f_2 = 80.0$ MHz), the distance, d , between two adjacent harmonic acoustic wells can be fine-tuned below the minimum half-wavelength of the applied harmonics (i.e., $d < \lambda_{\min}/2 = 22.5$ μm). By sequentially applying nanosecond pulsing SAWs, f_1 and f_2 , the time-effective Fourier-synthesized acoustic potential field is realized; spectrum trapping can be achieved by modulating the time-division Δt_1 , Δt_2 , and their ratio κ (Supplementary Fig. 1c), for positional tuning to manipulate objects with subwavelength resolution. Based on our analytical simulations, increasing the order of the harmonics, as shown in Supplementary Fig. 1e, results in the ability to arbitrarily tune the shape and position of the harmonic acoustic wells.

Supplementary Note 6. Single-particle and cell force analysis

To characterize the forces applied on a single particle, we carried out single particle position tracking to obtain distance, velocity, and acceleration information. Initially, single-particle manipulation videos were obtained with a CMOS camera. Then, position tracking of multiple particles was conducted simultaneously with the assistance of a tracking function in the NIS-Elements AR software (Nikon, Japan). To precisely calculate the force acting on a given particle, we utilized a fast camera with a frame rate of 1,000 fps to obtain videos. We measured the tracking distance, velocity, and acceleration data. Correlating the measured displacement with the acceleration of the particle, we can extrapolate the force utilizing fundamental kinetics. For a single particle, the force is calculated through Newton's second law:

$$\mathbf{F}_{\text{net}} = \rho \frac{4\pi(d/2)^3}{3} \mathbf{a}. \quad (\text{S15})$$

Here, $d = 9.51$ μm and $\rho = 1060$ kg/m^3 correspond with the diameter and density of the non-functionalized polystyrene beads (Bangs Laboratories, USA). The acceleration $\mathbf{a}$ can be directly derived from the measured displacement. Thus, the net force $\mathbf{F}_{\text{net}}$ applied to the particle can be calculated.

The Stokes drag force acting on a particle can be expressed as:

$$\mathbf{F}_d = -6\pi\mu r \mathbf{v}_r, \quad (\text{S16})$$

where μ is the fluid viscosity, r is the radius of a single spherical particle, and $\mathbf{v}_r$ is the particle velocity. When a particle suspended in a quiescent fluid translates near a flat surface, the Stokes drag force acting on the particle increases due to the alteration of the hydrodynamic interaction between the particle and the fluid generated by the nearby surface⁵². Thus, the Stokes drag force needs to be corrected with

$$\mathbf{F}'_d = -6\pi\mu r \mathbf{v}_r \chi, \quad (\text{S17})$$

where χ is the correction factor due to parallel translation along surfaces.

According to Faxén's correction^{52,53}, the correction factor, for a particle that moves in a direction parallel to one surface, can be expressed with power series expansions to fifth-order as:

$$\chi_1 = \frac{1}{1 - \frac{9}{16}\left(\frac{r}{z}\right) + \frac{1}{8}\left(\frac{r}{z}\right)^3 - \frac{45}{256}\left(\frac{r}{z}\right)^4 - \frac{1}{16}\left(\frac{r}{z}\right)^5}, \quad (S18)$$

where z is the distance between the particle and a surface. If the particle is precisely located in the middle plane between two surfaces (i.e., $z=h/2$), the correction factor due to parallel translation along these two surfaces can be expressed as:

$$\chi_2 = \frac{1}{1 - 1.004\left(\frac{r}{z}\right) + 0.418\left(\frac{r}{z}\right)^3 + 0.21\left(\frac{r}{z}\right)^4 - 0.169\left(\frac{r}{z}\right)^5}, \quad (S19)$$

For a 9.51 μm -diameter particle that parallelly translates along one surface or in the middle plane between two surfaces, the correction factors as a function of z/r are plotted in Supplementary Fig. 6a. We could find that the correction factor χ_2 (caused by two-surfaces) is higher than χ_1 (caused by only one surface). In our experiments, the dimension of the smallest device that we utilized is $400 \times 400 \times 20 \mu\text{m}^3$. For this 20 μm height microfluidic chamber, the value of the correction factor χ_2 for a 9.51 μm -diameter particle is 1.74. Additionally, χ_2 decreases as the chamber height h increases, as shown in Supplementary Fig. 6a. For a z/r ratio that is larger than 40, the Stokes drag force error could be neglected since the calculated error ($(\chi_2 - 1) \times 100\%$) is smaller than 3% with the presence of two surfaces. This is also true when investigating the presence of side walls of the microfluidic chamber. Based on this discussion, we need to calculate the chamber height induced χ_2 to correct the Stokes drag force error when a single particle is in the middle of a microfluidic chamber.

Next, we investigate the influence of the variation of particle positions along with the chamber height direction (z -direction) on the correction factor χ_2 . With equation (S19), we can only calculate the correction factor in the middle plane with the presence of two surfaces. We seek to use the linear superposition approximation method⁵⁴ to calculate χ_2 with varied particle positions along z -direction between two surfaces to solve this issue. The correction factor χ_2 can be expressed as:

$$\chi_2 = \chi_1(z) + \chi_1(h - z) - 1. \quad (S20)$$

By inserting equation (S18) to (S20), the correction factor χ_2 at various z -positions can be calculated. As shown in Supplementary Fig. 6b, the correction factor ($\chi_2=1.72$) matches the value calculated by equation (S19) when the 9.51 μm -diameter particle is in the middle plane of a 20 μm height chamber. More importantly, the minimum value of the correction factor is achieved when the particle is close to the middle plane, and the value of χ_2 increases when the particle approaches one of the surfaces. For example, for a 9.51 μm -diameter particle which deviates 2 μm from the middle plane along with the height direction, the value of χ_2 changes from 1.72 to 1.78, with an increase of 3.4%.

Accordingly, the momentum equation for the particle can be expressed as:

$$m \frac{dv_r}{dt} = F_r + F'_d, \quad (S21)$$

where m is the mass of the particle. By substituting the corrected Stokes drag force equation (S17) into (S21), the acoustic radiation force in the HDNDS device can be described as:

$$F_r = \frac{4\pi r^3 \rho_p}{3} \frac{dv_r}{dt} + 6\pi \mu r v_r \chi_2. \quad (S22)$$

Therefore, we can determine the acoustic radiation force using equation (S22) with the velocity measured from a recorded video of particle position tracking.

In Fig. 3e, applying a 2nd harmonic standing SAW excitation with an amplitude of 7 Vpp to the interdigital transducers generates the maximum separation force $F_{\text{net}}^{\text{max}}$ of 12.2 pN and a velocity v_r^{max} of 5.6 mm/s (both in the x direction). Using equation (S22), the peak acoustic radiation force F_r^{max} in the x direction is 876 pN for a 9.51 μm polystyrene particle. By substituting this peak acoustic radiation force and particle parameters into equation (S1), the calculated peak acoustic pressure amplitude A_2 is 479 kPa, when applying a SAW excitation with an amplitude of 7 Vpp. With this peak acoustic pressure amplitude, we can then calculate the maximum separation force for the cells.

Inserting the peak acoustic pressure amplitude A_2 and monocyte cell parameters, such as the radius, density, and compressibility (Supplementary Table 1), into equation (S1) results in the maximum acoustic radiation force around 270 pN applied on a monocyte cell. This occurred during the cell-cell separation process when applying a SAW excitation with an amplitude of 7 Vpp. Due to cell-cell adhesion, the cell velocity v_r is nearly zero, and the Stokes drag force F_d can be neglected. Thus, the maximum separation force is 270 pN for separating the cell-cell pairs with the given SAW excitation of 7 Vpp. Tuning the amplitude of the applied SAW excitation allows the maximum separation force to be modified accordingly. For example, using a 10 Vpp SAW excitation causes the maximum cell-cell separation force to reach around 2 nN for a monocyte cell with a radius of 7.5 μm . To be noticed, this calculated force is just an estimated value assuming that the cell is located in the middle of a microfluidic chamber. A correction of the drag force is needed if the cell deviates from the middle plane along with the height direction (Supplementary Fig. 6b).

Supplementary Note 7. Cell lysis and Western blot

Before cell lysis, suspended M0 THP-1 cells were counted using a hemocytometer, and adherent M1 THP-1 cells were counted by assessing cell density under a microscope. 5×10^6 cells of each cell line were lysed using 400 μL of RIPA buffer (ThermoFisher, USA) added with a protease inhibitor cocktail (ThermoFisher, USA) and incubated on ice for 30 minutes, respectively. Lysis products were then collected and centrifugated at 12,000 g for 15 minutes and the supernatants were collected. After being mixed with a tricine sample buffer (Bio-rad, USA), the lysis products were incubated in a water bath (Benchmark, USA) at 95 $^{\circ}\text{C}$ for 6 minutes. Samples were then loaded into 12% acrylamide (Bio-rad, USA) for electrophoresis. After electrophoresis, targeted proteins in the acrylamide gels were transported to PVDF membranes (Bio-rad, USA) using a Transblot turbo system (Bio-rad, USA). The membranes were then blocked using a membrane blocking solution (ThermoFisher, USA) at room temperature for 1 hour, followed by incubation with anti-CD11b (Abcam, USA), anti-ICAM-1 (Abcam, USA), and anti-Beta-actin (Abcam, USA) at the recommended dilution rates and incubated at 4 $^{\circ}\text{C}$ for 12 hours. After the first antibody incubation,

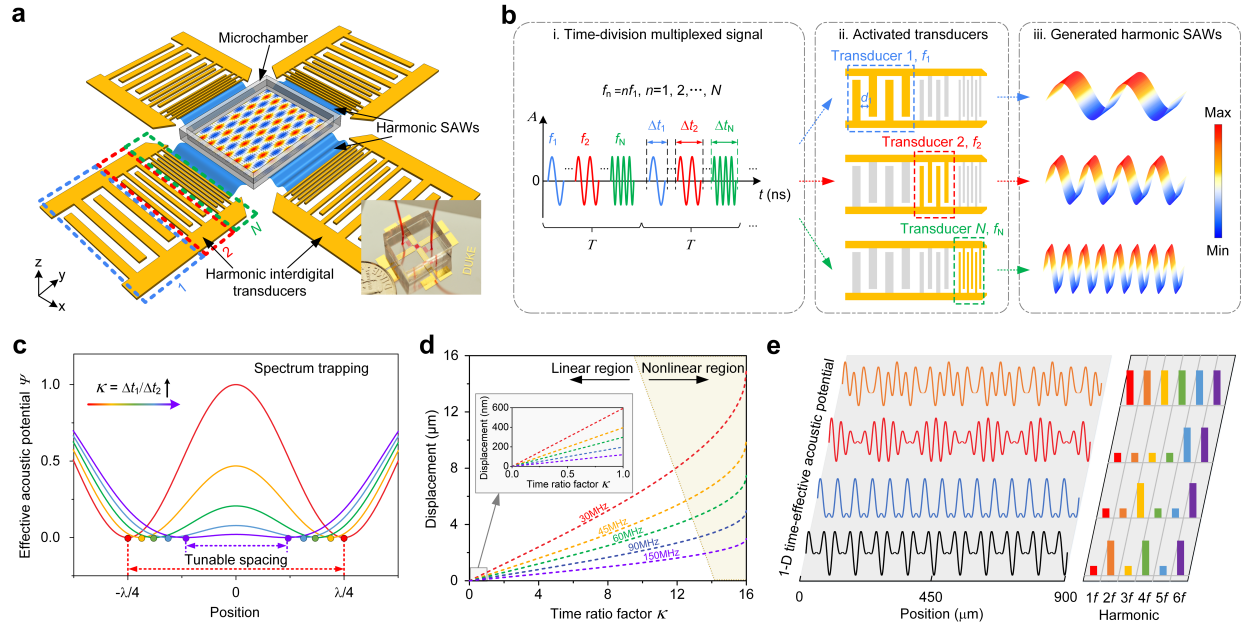
membranes were incubated using HRP conjugated secondary antibodies (Abcam, USA) at room temperature for 1 hour. An ECL substrate (Bio-rad, USA) and ChemiDoc Touch Imaging System (Bio-rad, USA) were used to observe the targeted proteins' bands on the membranes.

Supplementary Note 8. Confocal imaging

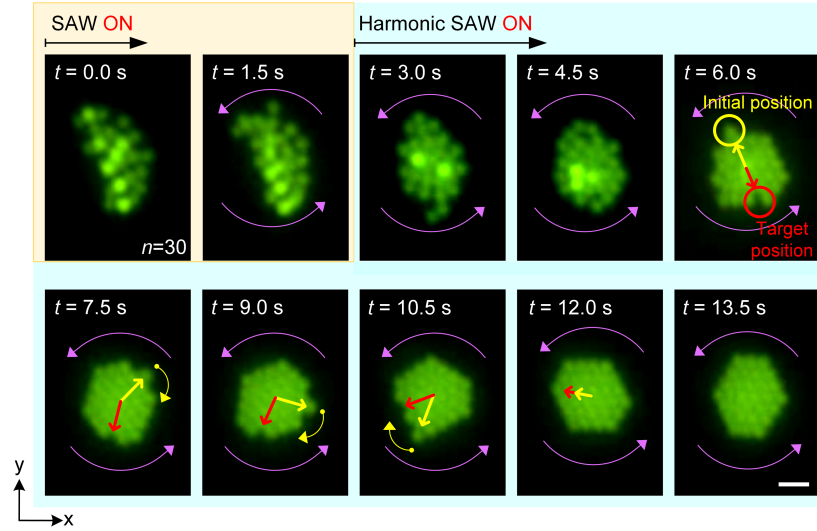
MCF-7 cells, with an appropriate density, were first stained using a SiR-actin kit (Cytoskeleton, USA) previously diluted in a DMEM culture medium with 10% FBS then incubated at 37 °C and 5% CO₂ for 3 hours. The culture medium containing SiR-actin dyes was discarded, and cells were digested by TrypLE (Thermofisher, USA) at 37 °C for 12 minutes or kept attached. Both attached MCF-7 and floating MCF-7 cells were separated into control groups (CytoD-) and Cytochalasin D treated groups (CytoD+), which had Cytochalasin D added a final concentration of 10 µg/mL in DMEM with 10% FBS. Both Cytochalasin D treated groups and control groups were cultured at 37 °C and 5% CO₂ for 1 hour. An inverted confocal microscope (Zeiss 880 Airyscan, Zeiss, Germany), with a 63 × oil-immersion objective, was then used for observing actin structures in the Cytochalasin D treated and control groups for both adherent and floating MCF-7 cells (Supplementary Fig. 5h).

Supplementary References

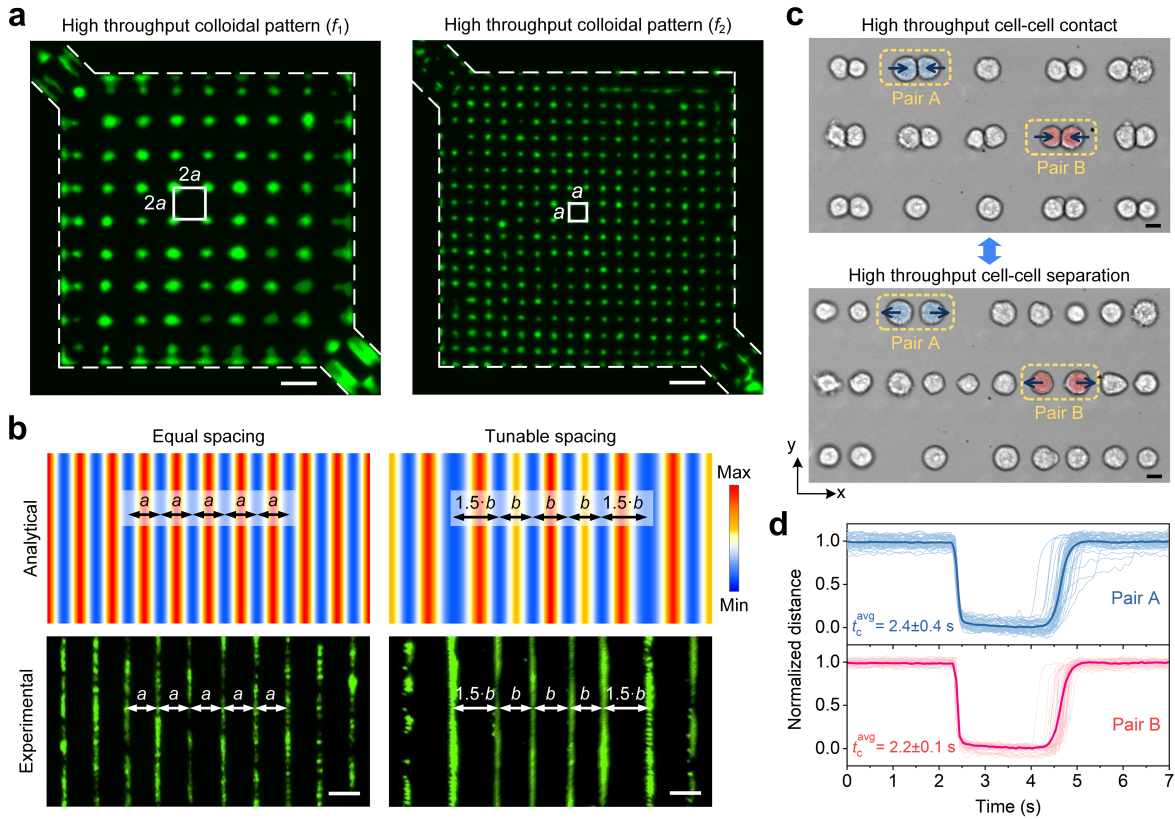
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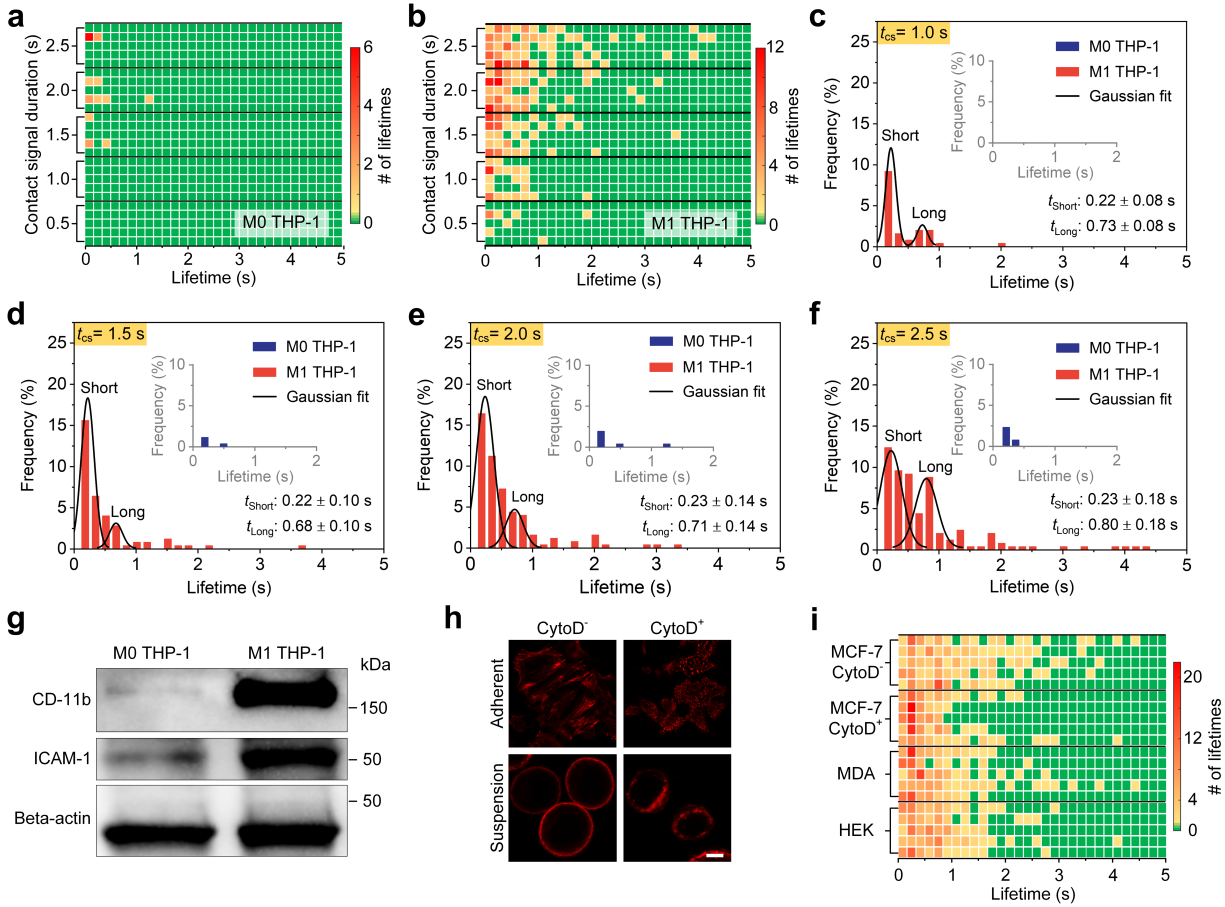
Supplementary Figure 1 | Design of HANDS to compose Fourier synthesis of harmonic waves for creating soft acoustic lattices and colloidal crystals or cell-cell pairing and separation. **a**, Schematic of the HANDS platform with harmonic interdigital transducers composed of N individual transducers for harmonic SAWs generation. Inset: a photo of the assembled HANDS device. **b**, The principle of generating harmonic SAWs with time-division multiplexed excitations. **c**, Analytical simulation of the tunable spacing via spectrum trapping, generated by Fourier-synthesis of the base and 2nd harmonic waves and adjusting corresponding time divisions Δt_1 and Δt_2 . λ is the wavelength of 2nd harmonic wave. **d**, The inter-distance between two neighboring trapping positions can be fine-tuned with nanometer resolution by adjusting the time ratio factor of the applied harmonics. **e**, Four representative 1-D effective acoustic potentials obtained by Fourier-synthesis of the first six harmonics of a base harmonic wave.



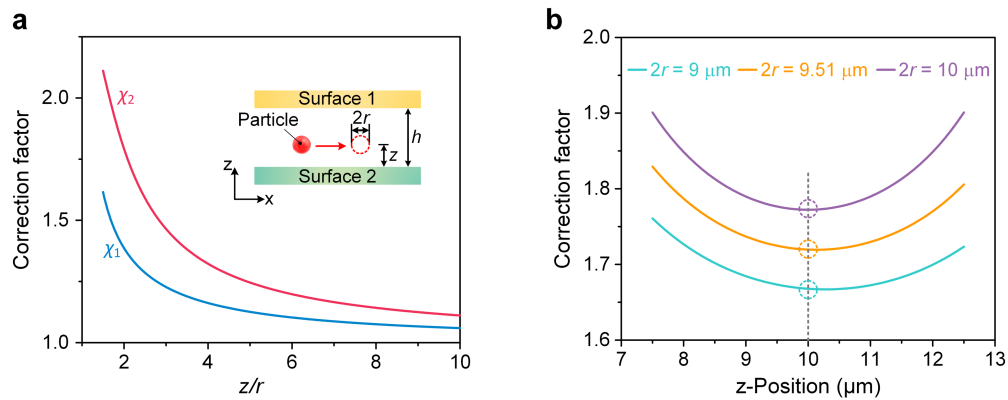
Supplementary Figure 3 | Generation of self-assembly monolayers via HANDS. The SAWs initially generated a 3-D colloidal cluster applied at $t=0.0$ s. When switching from SAW to harmonic SAW at $t=3.0$ s, the colloidal cluster was focused vertically forming a 2-D colloidal crystal monolayer in the x-y plane. By controlling both the direction and speed of the spin motion through phase modulation, the monolayer pattern can be further configured to form regular and close-packed shapes. From $t=6.0$ s, a single colloid (marked with yellow circle and arrow) started to rotate along the monolayer edge to the target position (marked with red circle and arrow), due to the spinning induced hinge-motion. At $t=12.0$ s, the single colloid finally reached the target position, and colloids were firmly trapped to form the close-packed hexagon pattern. Combining the vertical focusing and spinning control induced by HANDS, self-assembly colloidal crystal monolayers with various patterns can be generated. Scale bar: 5 μm .



Supplementary Figure 4 | High-throughput particle/cell manipulation with HANDS. **a**, High-throughput patterning of 2 μm fluorescent polystyrene particles by applying base standing SAWs (left) and 2nd harmonic standing SAWs (right). The white dashed lines mark the inner boundaries of the microfluidic chamber, which cover an area of $400 \times 400 \mu\text{m}^2$. Each square box (white solid lines) at the center illustrates the area of a primitive cell. Scale bar: 50 μm . **b**, Analytical and experimental comparison of the 1-D pattern with equal trapping spacing and tunable trapping spacing generated by HANDS. Scale bar: 20 μm . **c**, Representative images of 12 pairs of U937 cells being brought into contact and separation reversibly. The blue arrows indicate the directions of acoustic radiation forces. Scale bar: 10 μm . **d**, Trace plots of the changes in the intercellular distance for two representative cell pairs from (c), marked with yellow dashed rectangles. Plots show the individual (light color) and average (bold color) distance traces from 50 cell-cell contact and separation cycles with average contact durations, t_c^{avg} , of 2.4 ± 0.4 s and 2.2 ± 0.1 s, respectively.



Supplementary Figure 5 | An example of HANDS application: Reversible cell-cell pairing with quantification of intercellular adhesion strength. **a,b** Heatmaps of (a) M0 THP-1 and (b) M1 THP-1 cells with various applied contact signal durations ranging from 0.5 s to 2.5 s. Each pixel represents the adhesion events with the measured lifetime for the various contact signal durations, per cell pair. 5 cell pairs, with over 50 contact-separation cycles for each pair and each contact duration, were tested. **c-f**, Histograms of cell adhesion lifetimes for M0 and M1 THP-1 cells were obtained with different contact signal durations from 1.0 s to 2.5 s. **g**, Western blot analysis of CD11b, ICAM-1, and Beta-actin for M0 and M1 THP-1 cells. A high expression CD11b in M1 THP-1 cells indicates successful cell differentiation from M0 THP-1 with PMA stimulation. **h**, Representative single z-layer images of F-actin (SiR-actin) from live MCF-7 cells, with and without CytoD stimulation, for both adherent and suspension cells ($n=8$ fields of view). Scale bar: 10 μm . **i**, Heat map of the distribution of adhesion lifetimes for MCF-7 CytoD-, MCF-7 CytoD+, MDA-MB-231, and HEK-293T cells. Each pixel represents the adhesion events with the measured lifetime for contact signal duration of 2.0 s, per cell pair.



Supplementary Figure 6 | Correction of Stokes drag force for a single particle between two parallel surfaces. a, Correction factors χ_1 (single surface) and χ_2 (two surfaces) as a function of z/r . **b,** Correction factor χ_2 as a function of particle positions along the z -direction for particles with different diameters (dashed circles).

Supplementary Table 1 | Parameters used in the numerical simulations

Material	Parameter	Symbol	Value
Water	Density	ρ_f	$998.2 \text{ kg}\cdot\text{m}^{-3}$
	Speed of sound	c_0	$1,483 \text{ m}\cdot\text{s}^{-1}$
	Viscosity	μ	$1 \times 10^{-3} \text{ Pa}\cdot\text{s}$
	Compressibility	β_f	$4.6 \times 10^{-10} \text{ Pa}^{-1}$
Polystyrene	Density	ρ_p	$1,065 \text{ kg}\cdot\text{m}^{-3}$
	Speed of sound	c_p	$2,066 \text{ m}\cdot\text{s}^{-1}$
	Compressibility	β_p	$2.2 \times 10^{-10} \text{ Pa}^{-1}$
	Radius	r	$4.76 \text{ }\mu\text{m}$
Monocytes	Density ^{54,55}	ρ_p	$1,060 \text{ kg}\cdot\text{m}^{-3}$
	Compressibility ⁵⁵	β_c	$3.9 \times 10^{-10} \text{ Pa}^{-1}$
	Radius ⁵⁴	r_c	$4.75 \text{ }\mu\text{m}$
128° Y-cut LiNbO ₃	Density	ρ_s	$4,650 \text{ kg}\cdot\text{m}^{-3}$
	Phase velocity of SAW along x-direction	c_s	$3,960 \text{ m}\cdot\text{s}^{-1}$
	Phase velocity of SAW (45° offset to the x-direction)	c_s	$3,589 \text{ m}\cdot\text{s}^{-1}$