Non-contact multi-particle annular patterning and manipulation with ultrasound microbeam

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Multiparticle-trapping offers diverse opportunities and applications in biotechnology. It can be applied to creating various functional materials or organizing reactive particles. In this paper, we demonstrate that it is possible to trap and manipulate multi-particles in an annular pattern with a 24 MHz focused ring-type single element ultrasound transducer. Acoustic ring trap can be useful in undertaking biotopism studies due to an equal-distance condition from the center. Also, this ring trap could serve as a force shield to protect analysis area from other cells. The experimental results showed the capability of the proposed method as a multi-cell manipulator in formatting specific patterns of small cells like sperms. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4884938>]

Single particle manipulation using focused laser beam was proposed and demonstrated by Ashkin.^{1,2} Optical trapping (or tweezers) and non-invasive control of micro-particles have typically been used as an important tool in studying biophysical processes including cell adhesion,³ targeted drug delivery,⁴ gene transfection,⁵ etc. In contrast to these existing methods aimed at single-particle manipulation, it has been shown that it is feasible to simultaneously trap and handle multiple particles with optical tweezers by exploiting laser beam interference^{6–8} and fast laser-scanning.^{9,10} Such multi-trapping optical systems offer experimental platforms for creating various functional materials or organizing reactive particles.¹¹ Despite the popular use of these systems, however, they are all bulky and often require complicated optical instruments for beam patterning and scanning.

Single beam acoustic trapping has been developed as an alternative method for optical tweezers in using focused ultrasound beams whose frequency ranges from 30 MHz to 200 MHz.^{12,13} In our previous works, single element focused ultrasound transducers were employed to laterally immobilize individual lipid droplets in confined microfluidic channels¹⁴ or to induce targeted cell membrane deformation.¹⁵ It was also shown that ultrasound might be applied to cell patterning at pressure nodes arising from surface standing acoustic waves.¹⁶ In this paper, the feasibility of trapping of multi-particles in an annular pattern is experimentally demonstrated via a focused ring-type single element transducer.

A 24 MHz ring type transducer of f-number = 1 was designed and fabricated for multi-target trapping experiments. The outer- and inner diameters of the transducer's aperture are 4 mm and 2 mm, respectively. The transducer was press-focused to 4 mm, and its acoustic pressure field

was then probed by a needle hydrophone whose active element diameter is 40 μm (Precision Acoustics, UK). An example of measured temporal waveform and its corresponding spectrum and the actual view of the transducer are shown in Fig. 1. Oleic acid (or lipid) droplets of 50 μm were synthesized in poly (dimethyl) siloxane (PDMS) microfluidic channels for the trapping test.

The transducer was mounted on a three-axis motorized stage (LMG26 T50MM; OptoSigma, Santa Ana, CA, USA) and positioned perpendicularly to a thin mylar membrane at the focus. Lipid droplets were loaded underneath the membrane in deionized water. Due to their buoyancy, the membrane was used to restrict the droplet motion in the field of view of the microscope. The distance between the transducer and the membrane remained at 4 mm. The experimental setup is shown in Fig. 2. Trapped droplet pattern was monitored via both a microscope (SMZ1500; Nikon, Tokyo, Japan) and a CCD camera (Infinity X; Lumenera, Atkinson, NH, USA). The transducer was translated on the focal plane by a LabVIEW program, as being driven with a 24 MHz sinusoidal burst signal of 1000 cycles. The peak-to-peak voltage input to the transducer was 31.6 V_{pp} and the pulse repetition frequency (PRF) was 1 kHz.

After the burst signal was supplied to the transducer, randomly positioned droplets were slowly formed into a circle, while one droplet was isolated at the center as shown in Figs. 3(a)–3(f). As the transducer was continuously moved, these trapped droplets were observed to move at once while retaining their shape in Figs. 3(g)–3(l). Fig. 4 indicates that the diameter of the circle is 160 μm and the distance between the neighboring droplets is 80 μm .

It is possible to elucidate the reason how this annular pattern is formed from the hydrophone measurement data of the acoustic pressure field produced by the transducer. According to the lateral beam profile on the focal plane, the measured –6 dB beam width is 70 μm and the negative peak

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FIG. 1. (a) Temporal waveform measured by a hydrophone at the focus of 24 MHz transducer and its frequency response (b) actual image of the transducer used in this paper.

FIG. 2. Experimental configuration of multi-particle trapping by using annular transducer.

pressure is 4.98 MPa as displayed in Fig. 5. The position of the first side lobe is found at about $80\ \mu\text{m}$ from the beam center ($y=0$), where those pressure levels are 1.52 MPa and 1.67 MPa, respectively. As a result, the location of multi-trapped particles coincides with the positions of the first lobe and the beam center. This finding is in agreement with our ray acoustics model,^{17,18} suggesting that this type of lateral trapping mechanism can be realized when the acoustic intensity gradient reaches its maximum. Pressure gradient at the beam center is 94.67 MPa/mm, and those of the side lobes are 38.53 MPa/mm and 48.79 MPa/mm. These numbers clearly indicate that the trapped particles forming a ring

shape are resulted from these steep gradients at the corresponding locations.

Acoustic vortices have recently been utilized to develop a two-dimensional manipulation technique for simultaneous trapping of multiple particles.¹⁹ A 64 element ultrasonic array transducer of ring shape was built to immobilize micron-sized polystyrene beads at 2.35 MHz. Over a confined plane enclosed by the transducer aperture, counteracting beams were emitted from two separate array elements facing each other on opposite sides. Higher-order Bessel beams were superimposed to form the pressure maximum within the circular region by varying voltage amplitude and phase delay to each element. The size of a trapping area was controlled as a function of the order of the Bessel function, where a lower order implies a narrower area. It was demonstrated that trapped beads at multiple places were simultaneously moved or rotated with electronically focused beams, as being displaced through a few hundreds of micrometers. Meanwhile, our trapping mechanism depends on whether it is possible to achieve sufficient intensity gradient at the focus, rather than to reach the peak pressure itself. The proposed technique here generates attractive transverse forces by transmitting Gaussian focused beams forward from a single acoustic source, not necessarily using multi-element transducers that often produce a set of opposing beam pairs as in the Bessel beam approach. Desirable target size is determined by the resonance frequency of the transducer in our case, so that the application of a higher frequency results in trapping a smaller particle. Both approaches exhibit a similar capability in transporting a larger particle over a longer distance and demanding lower operating energy than optical tweezers. In comparison to our technique, the array-based

FIG. 3. Demonstration of annular trapping and manipulation of lipid droplets ($50\ \mu\text{m}$ in diameter) (a) randomly positioned droplets, (b)–(f) formation of annular droplet pattern after applying burst signal to transducer, (g)–(l) manipulation of the same pattern without change. A black dot is given as a reference point to show the change in the pattern location. Scale bar is $200\ \mu\text{m}$. (Multimedia view) [URL: <http://dx.doi.org/10.1063/1.4884938.1>]

FIG. 4. Example of an annular pattern (a) Diameter of the pattern is about $160\ \mu\text{m}$. The distance between neighboring trapped droplets on the circle is about $80\ \mu\text{m}$. Scale bar $100\ \mu\text{m}$ (b) Separation of the patterned droplets from others. Scale bar $500\ \mu\text{m}$.

FIG. 5. Lateral acoustic pressure distribution on the focal plane.

method may provide a more versatile way of maneuvering the particle motion at lower frequencies, typically less than 10 MHz. For applications requiring more involved patterns composed of smaller particles than currently achieved, however, the use of higher frequency beams is needed at tens or hundreds of MHz range and its practical trapping implementation may still be a challenging task due to complex focusing scheme. Instead, our proposed method may be more appropriate for those cases by readily adopting single element transducers with fixed focusing at such high frequency range.

This annular trapping method is expected to benefit a variety of self-propelled cell motility and parallel cell sorting studies related to sperms.^{20,21} It is envisioned that sperms in annular trap may freely move along the circumference of the ring, providing a chance to observe the motility of these swimming cells in the field of view. When an attractant is trapped at the center of the ring, furthermore, the trapping force can be adjusted in order to separate certain cells with sufficient motility that have enough energy to escape the trap. Additionally, this unique trapping technique may be useful in conducting biotropy studies that often require an equal-distance condition from the center, or creating a force shield to prevent the neighboring cells from interfering with a specific cell in analysis area.

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