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Microfluidic acoustic trapping force and stiffness measurement using viscous drag effect

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Abstract

It has recently been demonstrated that it was possible to individually trap 70 μm droplets flowing within a 500 μm wide microfluidic channel by a 24 MHz single element piezo-composite focused transducer. In order to further develop this non-invasive approach as a microfluidic particle manipulation tool of high precision, the trapping force needs to be calibrated to a known force, i.e., viscous drag force arising from the fluid flow in the channel. However, few calibration studies based on fluid viscosity have been carried out with focused acoustic beams for moving objects in microfluidic environments.

In this paper, the acoustic trapping force (F_{trapping}) and the trap stiffness (or compliance k) are experimentally determined for a streaming droplet in a microfluidic channel. F_{trapping} is calibrated to viscous drag force produced from syringe pumps. Chebyshev-windowed chirp coded excitation sequences sweeping the frequency range from 18 MHz to 30 MHz is utilized to drive the transducer, enabling the beam transmission through the channel/fluid interface for interrogating the droplets inside the channel. The minimum force ($F_{\text{min, trapping}}$) required for initially immobilizing drifting droplets is determined as a function of pulse repetition frequency (PRF), duty factor (DTF), and input voltage amplitude (V_{in}) to the transducer. At $PRF = 0.1$ kHz and $DTF = 30\%$, $F_{\text{min, trapping}}$ is increased from 2.2 nN for $V_{\text{in}} = 22$ V_{pp} to 3.8 nN for $V_{\text{in}} = 54$ V_{pp}. With a fixed $V_{\text{in}} = 54$ V_{pp} and $DTF = 30\%$, $F_{\text{min, trapping}}$ can be varied from 3.8 nN at $PRF = 0.1$ kHz to 6.7 nN at $PRF = 0.5$ kHz. These findings indicate that both higher driving voltage and more frequent beam transmission yield stronger traps for holding droplets in motion.

The stiffness k can be estimated through linear regression by measuring the trapping force (F_{trapping}) corresponding to the displacement (x) of a droplet from the trap center. By plotting $F_{\text{trapping}} - x$ curves for certain values of V_{in} (22/38/54 V_{pp}) at $DTF = 10\%$ and $PRF = 0.1$ kHz, k is measured to be 0.09, 0.14, and 0.20 nN/ μm , respectively. With variable PRF from 0.1 to 0.5 kHz at $V_{\text{in}} = 54$ V_{pp}, k is increased from 0.20 to 0.42 nN/ μm . It is shown that a higher PRF leads to a more compliant trap formation (or a stronger F_{trapping}) for a given displacement x . Hence the results suggest that this acoustic trapping method has the potential as a noninvasive manipulation tool for individual moving targets in microfluidics by adjusting the transducer's excitation parameters.

Keywords

High frequency focused ultrasound; Microfluidic channel; Trap stiffness; Viscous drag force; Chebyshev-windowed chirp coded; excitation

1. Introduction

Acoustic particle manipulation methods in microfluidic environments have recently been developed to non-invasively control the particle motion via acoustic radiation force. The capability of exact particle positioning at designated locations has been an essential element for performing accurate on-chip analysis of cells or bead assays. Various manipulation techniques based on acoustic actuation have been experimentally demonstrated by exploiting different types of sound fields. Separation of lipid particles from erythrocytes in whole blood suspension was implemented with 2 MHz standing waves in a silicon microfluidic structure, depending on mass density and compressibility of the two biological objects [1]. Surface acoustic waves (SAWs) produced from lithium niobate (LiNbO_3) interdigital ultrasonic transducers were employed to direct either 20 μm droplets in a poly(dimethyl) siloxane (PDMS) channel [2] or 1.9 μm polystyrene beads in a trapezoidal glass channel [3]. 30 MHz Rayleigh standing-SAWs arising from a pair of focused LiNbO_3 interdigital transducers enabled 6 μm particles to be collected on the surface of a 3 μl sessile droplet [4]. Several mathematical models related to particle manipulation have also been proposed. The potential well model [5,6] for trapping polystyrene beads was derived from an acoustic vortex field that forms a null domain of zero intensity on the beam axis. The potential energy of a bead in the vortex profile was simulated by assuming the use of a 1 MHz four-element planar transducer, where a spiral shape of the wavefront was numerically synthesized by exciting adjacent elements with 90° phase difference. The maximum trapping force acted on a 13 μm bead was calculated to be 50 pN. The computational results of Bessel beam particle trapping in the axial direction showed that fluid or solid spheres in inviscid liquid media could be attracted in the reverse direction to the beam propagation [7–9]. More recently, high frequency focused ultrasound emitted from a 24 MHz single element transducer has been utilized to laterally immobilize moving droplets in a PDMS microfluidic channel [10]. The experimental results indicated that each droplet was held near the beam's focus (or the trap center) by a two-dimensional trapping force and routed to a particular outlet at the channel's bifurcation. Trapping force control with precision is thus crucial for accurately directing targeted droplets in certain directions, which can be achieved by measuring the magnitude of the trapping force within laminar flow regions.

The trapping force (F_{trapping}) can be measured with the trap stiffness (k), an elastic spring constant of the trap. In order to find k , equipartition theorem and power spectrum analysis have been developed by tracking the trajectory of a particle at the trap [11]. Both thermal fluctuation and Brownian random motion of the particle can be quantitatively represented in terms of the particle's displacement (x) from the trap center. The mean square displacement (MSD, $\langle x^2 \rangle$) is inversely proportional to k in equipartition theorem, whereas the corner frequency (f_c) corresponding to a Lorentzian spectrum of the displacement is linearly proportional to k in power spectrum analysis. Note that the corner frequency is defined as a frequency at which the magnitude of the spectrum (-3 dB) is reduced by 3 dB from its peak value (0 dB). According to the Hooke's law, the resultant force is then equal to the product of k and x ($F_{\text{trapping}} = kx$). On the other hand, the frictional drag approach [12] enables a force measurement without the knowledge of k by equalizing the lateral trapping force with the known drag force in viscous fluid media. As a trapped droplet is released from the trap due to flow velocity increase, F_{trapping} is calibrated to an equal and opposite drag force by the Stokes' law. Using this method, k for a given trap can also be deduced

from a linearly fitted curve for $F_{trapping}$ versus x . In the current experimental configuration, the drag approach is more proper for measuring $F_{trapping}$ exerted on moving objects, because the other two methods depend on the result of stochastic position detection.

In this paper, a trapping force calibration technique for droplets in a PDMS channel is thus proposed by taking advantage of viscous drag effect. Few experimental studies under high frequency focused ultrasound have so far been undertaken in microfluidic devices. A 24 MHz lead zirconate titanate-4 (PZT-4) single element transducer is driven by the Chebyshev chirp signals to allow the acoustic energy to penetrate into the channel. The sound beam generated by the transducer exerts the acoustic radiation force on a moving droplet passing through the beam's focus. The trapping force needed for holding the droplet equalizes the drag force arising from the surrounding microfluidics. By finding the escape velocity at which the immobilized droplet escapes from the trap, the frictional drag force is calibrated to the trapping force. The force and the trap stiffness are measured by varying several excitation parameters including duty factor, pulse repetition frequency, and input driving voltage to the transducer. The results suggest that this high frequency trapping mechanism may be utilized in non-invasive microfluidic cell sorting devices by separating specific types of cells from mixed cell population.

2. Methods

2.1. Transducer fabrication and excitation

A focused 24 MHz transducer has been constructed with PZT-4 piezo-composite materials as follows. A PZT-4 wafer (840, APC Company, USA) is first diced to form square-shaped sub-elements, and the gap between those elements (called the kerf) is filled with unloaded epoxy (Epotek 314, Epoxy Tech., USA). The composite wafer is lapped down to the final thickness of 70 μm by fine grit abrasive scheme [13] and then electroplated by sputtering a 1000 Å chrome/gold layer on both sides. Conductive epoxy (E-SOLDER 3022, Von Roll Isola, USA) is applied as a backing layer that is electrically connected with the negative electrode side of the wafer, following the centrifugal casting and curing. A lathe is utilized to turn down the piezo-composite-backing stack to the desired aperture size of 4 mm, before a lead wire is secured to the backing for electrical connection. The stack is concentrically placed in a brass ring, and an insulating epoxy is poured in between. After curing, the brass ring is sputtered with another chrome/gold layer to be electrically connected to the positive electrode side of the wafer. The ring-stack pair is then press-focused at 100 °C by a 8mm diameter chrome/steel ball to obtain a focal length of 4 mm. The completed transducer is housed in a modified SMA connector (Fig. 1a). The pressure distribution emitted from the transducer is obtained by using a calibrated hydrophone (HGL-0085, Onda, USA) whose frequency range lies from 1 MHz to 60 MHz. The transducer moves in the lateral- and axial directions to scan the hydrophone fixed at a certain position. The beam width and the depth of focus (*DOF*) are determined by detecting a spatial point at which the measured pressure drops by 6 dB from its maximum in both directions, respectively. The results indicate that the beam width and the *DOF* are 86 μm and 84 μm , as shown in Fig. 1b and c.

In order to manipulate the droplets moving inside the channel, ultrasonic beam penetration into the channel is crucial. One immediate solution can be increasing the input voltage amplitude to the transducer, which often results in serious heating or damage to the transducer itself. By extending the excitation signal duration, instead, coded excitation sequences increase the total transmitted energy and yet minimize the transmitted peak power. Unlike conventional sinusoidal waveforms, a depth of penetration in the range of a few centimeters can be achieved with such elongated excitation signals without degrading the transducer's performance [14]. Hence, the Chebyshev-windowed chirp coded excitation

signal $C(t)$ is employed as an input to drive the transducer. The expression (1) describes the frequency modulated chirp signal whose center frequency is linearly increased with time.

$$C(t) = W_{Chebyshev} \times \cos \left[2\pi \left(f_i + \frac{\alpha t}{2} \right) t \right] \quad (1)$$

where f_i denotes the lowest frequency of the signal in MHz within the bandwidth, α the frequency increase rate per unit time ($=4.8 \text{ MHz}/\mu\text{s}$), and $W_{Chebyshev}$ the Chebyshev window function. The Chebyshev window, among other existing gate functions, is chosen for amplitude modulation to minimize heat generation at the focal point. As a result, the chirp signal used here sweeps the frequency range from 18 to 30 MHz for 5 μs .

2.2. Microfluidic channel device

Conventional soft lithography techniques [15,16] have been used to build PDMS microfluidic channel devices. The fabrication process is elaborated in our previous work [9]. Fig. 2 illustrates the schematic diagram of a typical device that has three inlets merged into a 500 μm wide main channel. In the experiment, as droplets (mean radius = 25 μm , BioMINT Lab, UC Irvine, USA) enters the middle inlet, the upper and the lower inlets produce sheath flows that hydrodynamically restrict the droplet flow to the middle of the channel. The channel is then split to form two outlets where the deflected droplets by acoustic traps are collected. The width is 200 μm for each sheath inlet, and 100 μm for the droplet inlet. The outlet widths are designed differently, 150 μm and 300 μm for each. Note that the channel height is 100 μm throughout the device.

2.3. Calibration of trapping force and stiffness

An experimental arrangement for the calibration is shown in Fig. 3. The force applied on a trapped droplet is balanced with the viscous drag force generated by deionized water flow from two syringe pumps (PHD 22/2000, Harvard Apparatus, USA). Its magnitude is proportional to the flow velocity around the droplet. As the velocity increases, the drag force also increases. This frictional fluid force becomes equal to the trapping force before the droplet is released from the trap with an escape velocity v_{esc} . According to Stokes' law, the trapping force $F_{trapping}$ is expressed as:

$$F_{trapping} = 6\pi\eta r v_{esc}, \quad (2)$$

where r is the droplet's radius and η is the dynamic viscosity of water ($=10^{-3} \text{ Pa s}$). This frictional drag force model is particularly valid for laminar flows in microfluidic channels, and can be applied to finding trapping forces in the perpendicular direction to the beam axis. The use of the viscous drag model generally requires both microscope systems for tracking object's motion and flow chambers creating a steady fluid stream inside the channel. The whole experimental apparatus is installed on a three-dimensional motorized micropositioner (LMG26 T50 MM, OptoSigma, USA) immersed in a water bath. The transducer's excitation conditions varied include duty factor, pulse repetition frequency, and input voltage amplitude.

As in optical tweezers, the motion of a trapped droplet can be viewed as a particle tethered to an elastic spring that tends to retract towards the equilibrium point or the trap center [17]. The trap stiffness (or compliance) k is thus estimated by representing $F_{trapping}$ as a function of displacement x relative to the trap center [12]. The displacement is automatically measured with an image analysis program (Infinity Analyze, Lumenera, Canada). The droplet motion is monitored through a stereomicroscope (SMZ1500, Nikon, Japan) equipped with an imaging analysis program. As a trapped droplet is displaced from the trap center (x

= 0), the program displays the droplet motion over vertical and horizontal grid lines. Each line is spaced 5 μm apart. At a certain droplet flow rate, the trapped droplet becomes exactly positioned on one of the grid lines and therefore the displacement can be directly measured from the program by the droplet location. As result, there is no source of artifact involved. While the flow rate R_d is slowly increased with the trap switched on, intermediate values of x are recorded before the droplet escapes from the trap. As a result, k is determined from Hooke's law by finding the ratio ($=F_{\text{trapping}}/x$) via linear regression.

The duty factor (DTF) of the coded excitation sequence that drives the transducer is adjusted from 5% to 30% with 5% step increment. The DTF is the time fraction of the transducer's active state within one pulse repetition period (PRP) as shown in Fig. 4. A higher DTF results in a stronger force exerted on a droplet. The pulse repetition frequency ($PRF = 1/PRP$) of the sequence is 0.1 kHz and the sheath flow rate R_{sh} from a syringe pump is set to 10 $\mu\text{l}/\text{min}$. The number of cycles of the chirp signal is 200 at $PRF = 0.1$ kHz and $DTF = 10\%$. The peak-to-peak voltage V_{in} supplied to the transducer is increased every 16 V_{pp} , ranging from 22 to 54 V_{pp} . The minimum force (denoted as $F_{\text{min, trapping}}$) needed to completely capture a flowing droplet at the focus is determined by finding a proper R_d under which the applied F_{trapping} is greater than the drag force. Typically, R_d is decreased from 10 $\mu\text{l}/\text{min}$ to overcome a given F_{trapping} .

3. Results and discussion

Fig. 5 displays the relation between DTF and $F_{\text{min, trapping}}$ as a function of V_{in} at $PRF = 0.1$ kHz. For $V_{in} = 22$ V_{pp} , R_d meeting is found to be from 1.5 to 2.8 $\mu\text{l}/\text{min}$, equivalently to a droplet's moving velocity of 2.5 to 4.7 mm/s. Consequently, the measured $F_{\text{min, trapping}}$ can be reached to 2.2 nN, while it becomes 3.8 nN for $V_{in} = 54$ V_{pp} . The results show that a higher voltage level makes a stronger trap for holding a streaming droplet in the flow. At a fixed peak voltage of $V_{in} = 54$ V_{pp} , the calibrated forces acquired at $PRF = 0.1, 0.2$, and 0.5 kHz are plotted in Fig. 6. At $PRF = 0.1$ kHz, in particular, as R_d is changed from 3.0 to 4.8 $\mu\text{l}/\text{min}$, F_{trapping} varies from 2.4 to 3.8 nN. When PRF becomes 0.5 kHz, F_{trapping} can be increased to 6.7 nN for $DTF = 30\%$. Hence this suggests that more frequent pulse emission from the transducer enables a stronger trap formation at the focus.

Linear regressions are carried out to find the slopes ($k = F_{\text{trapping}}/x$) of linearly fitted $F_{\text{trapping}} - x$ curves. The distance x is read from an image analysis software (Infinity Analyze, Lumenera, USA) that comes with a stereomicroscope (SMZ1500, Nikon, Japan). Fig. 7 shows the measured $F_{\text{trapping}} - x$ for varying V_{in} where DTF and PRF are 10% and 0.1 kHz. When $V_{in} = 22$ V_{pp} , for instance, R_d is increased with the step size of 0.1 $\mu\text{l}/\text{min}$ until each designated x (5 $\mu\text{m}/10$ $\mu\text{m}/15$ $\mu\text{m}/20$ μm) of the trapped droplet from the focus is reached. The droplet held by the trap is finally released at $R_d = 3.6$ $\mu\text{l}/\text{min}$ and $x = 20$ μm . F_{trapping} is varied from 1.5 to 2.8 nN, and the corresponding k is estimated to be 0.09 nN/ μm . For $V_{in} = 54$ V_{pp} and $R_d = 7.5$ $\mu\text{l}/\text{min}$, F_{trapping} and k are further increased up to 3.8 nN and 0.20 nN/ μm , indicating that a higher driving voltage yields a stiffer trap. Similar compliance tests are repeated for three different PRF s with a constant $V_{in} = 54$ V_{pp} , as shown in Fig. 8. When the PRF is adjusted from 0.1 to 0.5 kHz, k is varied from 0.20 to 0.42 nN/ μm . This finding also implies that a faster PRF leads to a stiffer trap formation, therefore generating a stronger F_{trapping} for a given x .

The trapping beam emitted from the transducer is vertically propagated in the direction of the channel height (=100 μm), whereas both droplet and sheath flow are injected from external syringe pumps along the horizontal channel length (=10 mm). Hence, laterally applied trapping force is calibrated to horizontal Stokes drag force, not to vertically induced acoustic streaming force. Acoustic streaming occurs as a result of momentum transfer from

the sound wave to viscous fluid media due to energy attenuation. There are three types of acoustic streaming [18]. Both Eckart- and Rayleigh streaming typically occur in the presence of standing acoustic waves. The length scale of Eckart streaming is much larger than the acoustic wavelength λ , while that of Rayleigh streaming is smaller than λ . Vortices in Rayleigh streaming pattern are transverse to nodal planes, as being spaced $\lambda/4$ apart [19]. In this experiment, no droplet motion near the beam axis seems to be influenced by rotating vortices arising from either Eckart- or Rayleigh streaming. Schlichting streaming is a vortex flow that exists inside the viscous boundary layer whose thickness ($\delta = \sqrt{\frac{\nu}{\pi f}}$) is only $0.12 \mu\text{m}$ for this study. ν is the kinematic viscosity of water ($=10^{-6} \text{ m}^2/\text{s}$) and f is 24 MHz. Given the droplet size and the channel dimension here, this micro-streaming effect can thus be neglected.

No considerable droplet oscillation or deformation has been observed in the lateral direction with the acoustic power of 0.19 mW used for this study. Although there may be a certain amount of deformation or resonance in the droplet, it is difficult to detect such minute change in the axial direction with our experimental setup. The current trapping layout is needed to be significantly re-designed for monitoring axial shape change, so that both transducer and microscope are coaxially aligned on the same side. As far as the particle resonance is concerned, besides, it becomes more pronounced in the Rayleigh regime (the particle size $<$ the acoustic wavelength) than in the ray acoustics regime (the particle size $>$ the acoustic wavelength).

4. Conclusion

The two-dimensional acoustic trapping force exerted on moving droplets in a PDMS microfluidic channel is experimentally calibrated to the fluid drag force of known magnitude in the flow direction. The Chebyshev-windowed chirp coded excitation signal that facilitates the beam penetration into the channel is generated from a 24 MHz piezo-composite focused transducer to form the acoustic trap at the focus. The trapping force is found to be in the nN range, as the transducer's excitation parameters such as duty factor, pulse repetition frequency, and input voltage level are varied. With the droplet being displaced by a known drag force, the trap stiffness is modeled as a linear slope of the force-displacement curve. The results show that higher excitation voltage and more frequent beam emission lead to stronger traps for streaming droplets in the channel. Hence they demonstrate that this proposed approach can be used as an on-chip manipulation technique, where individually captured targets are transported to specific regions within microfluidic devices by remotely applied acoustic forces from ultrasonic transducers.

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Fig. 1. Fabrication of a 24 MHz transducer. (a) Photograph of the final transducer. Normalized pressure distributions are measured in both, (b) the lateral and (c) the axial directions (reprinted from [10]).

Fig. 2.
PDMS microfluidic channel geometry.

Fig. 3.

Calibration of trapping force to viscous drag force in microfluidic channel. (a) A droplet is held at the transducer's focus inside the channel by the trapping beam. Deionized water flow goes from left to right, produced by a syringe pump. (b) As the flow velocity gradually increases, the droplet is pushed away from the trap center by a certain distance. The drag force then equalizes the trapping force before the droplet moves out of the trap. Trapped droplet position relative to the center of the main channel is recorded as the flow rate changes.

Fig. 4.
Illustration of coded excitation sequence for the transducer.

Fig. 5. Dependence of $F_{min, trapping}$ on duty factor (DTF) as a function of V_{in} at pulse repetition frequency (PRF) = 0.1 kHz.

Fig. 6. Dependence of $F_{min, trapping}$ on duty factor (DTF) for variable pulse repetition frequency (PRF) at a fixed $V_{in} = 54 \text{ V}_{pp}$.

Fig. 7. Relation between $F_{trapping}$ and x for different values of V_{in} with pulse repetition frequency (PRF) = 0.1 kHz.

Fig. 8. Relation between $F_{trapping}$ and x for variable pulse repetition frequency (PRF) with a constant $V_{in} = 54 \text{ V}_{pp}$.