Microfluidic manipulation of Caenorhabditis elegans using acoustic radiation forces

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ABSTRACT

The ability to precisely trap, transport and manipulate micrometer-sized objects is very important in microfluidic applications. Caenorhabditis elegans (*C. elegans*) is one of the most ubiquitous lab animals used for biological research; and for experimental studies and observations it needs to be selected, transported or immobilized. In this study we demonstrate the use of acoustic radiation forces, including Primary Radiation Force (PRF) and Secondary Radiation Force (SRF) to trap and manipulate *C. elegans*. PRF in a standing wave field is used to trap multiple *C. elegans*. Simulations are found to be in good agreement with the patterns of the trapped worms. SRF generated around an array of insonated microbubbles is used to trap a single *C. elegans* and precisely direct its motion in a specified path. Theoretical explanation for SRF to trap and manipuate worms via microbubble arrays is also presented. Viability test proves that the two approaches do not pose harm to the biological matter. Due to non-contact and non-electrical-field nature of the two techniques, we believe that these inexpensive methodologies can pave way for novel lab on a chip technologies for manipulating *C. elegans* or potentially any other important biological sample.

Keywords: Microfluidics, Manipulation, Acoustic radiation force, *C. elegans*

1 INTRODUCTION

Particle and cell manipulation, which includes trapping, transporting, seperating and concentrating has been an important task for lab-on-a-chip devices for a long time, and thus precise manipulation of these microparticles and cells is at the heart of most microfluidic devices. Different mechanisms have been employed in the past in an attempt to achieve manipulations in microfluidic systems, e.g. mechanical grippers, electrothermally actuated gripper [1] and dielectrophoretic tweezers [2] have been the most common of all. However, their usage comes with certain disadvantages: mechanical tweezers tend to denature or deform microobjects due to their contact-based nature; and soft matter such as biological membranes are the easiest of victims. On the other hand, the electrical tweezers may result in hydrolysis and can severely affect the content of the cells and living organisms. Since the aforementioned approaches have a latent tendency to affect characteristics, there is a need to explore for novel ways that are more reliable and at the same time inexpensive to implement.

Acoustic radiation forces of the first [3] and the second kind prove to be a viable candidate for achieving the next generation of non-contact, invasive manipulation in Lab-on-a-Chip devices. The advantage of using acoustics for manipulation resides in the fact that the approach does not rely on establishing any physical contact with the particles. Moreover, unlike the electrical tweezers, it does not require particles to possess any special electrical properties, which prevents occurrence of hydrolysis and any potential damage to the cell content or the soft membranes that envelop it.

Particles whether soft or rigid have tendency to respond to sound i.e. mechanical perturbations of a medium wherein they are submerged. The response is either a reaction to a Primary Radiation Force (PRF) or due to the Secondary Radiation Force (SRF) [4] that tend to impart momentum.

PRF is simply the response that a particle exhibits when it is subjected to a standing wave. It can be estimated by:

$$F_{PRF} = V_P k E_{ac} \varphi_{(\beta,\rho)} \sin(2ky) \qquad (1)$$

where F_{PRF} is primary radiation force, V_p is volume of the particle, k is the wave factor, E_{ac} is the acoustic energy density, φ represents the acoustic contrast factor which is a function of compressibilities and densities of the respective particle and its medium, and y denotes the distance of the particle from the nearest pressure node/antinode.

The response of a particle to a PRF can be attractive or repulsive depending upon the sign of the contrast factor. A positive sign would force the particle toward a pressure node and vice versa.

On the other hand, the response of a particle to a SRF is a measure of how a particle behaves to the scattered pressure waves. An acoustically actuated bubble will radiate some of the incoming pressure waves onto a particle in its vicinity. Therefore, a time-average force will act on the particle, either drawing it toward the bubble or repelling it away. For interactions between a small spherical particle and a bubble, the SRF can be described as following [5]:

$$F_{SRF} = 4\pi \frac{\rho_l - \rho_p}{\rho_l + 2\rho_p} \frac{R^4 R_p^3}{d^5} \omega^2 \xi^2 \qquad (2)$$

Where F_{SRF} is the secondary radiation force, ρ is density, ω is the angular frequency, ξ is the amplitude of

the bubble vibration, *R* is the radius and *d* is the distance of separation between the center of the particle and the bubble, and the subscripts '*p*' and '*l*' represent the particle and the liquid respectively.

There is a third critical force also, but it arises only as a result of a particle's motion in the fluid. The resistive force, also known as Stokes Drag, is experienced by a particle by virtue of its relative motion within a fluid. For spherical particles, the drag force can be computed by the expression:

$$F_{Drag} = 6\pi\mu R_p U$$

where μ is the dynamic viscosity and U is the relative velocity between the particle and the fluid

It is the interplay between the Stokes drag and radiation forces of the first and second kind that essentially enables particles to be manipulated according to size and densities.

In our study, we have used action-at-a-distance principle for manipulating *C.elegans* by utilizing radiation forces of the first and the second kind. Using a simple experimental setup, we demonstrate for the first time the trapping of a living microorganism with a standing wave and an array of oscillating bubbles. By merely tuning the input frequency of the piezoelectric transducer, and switching it on and off, we trap *C.elega*ns and manage to change the course of its motion. Although trapping of organisms e.g. water flea has already been demonstrated in an earlier study using millimeter sized acoustically actuated bubbles [6], it is the first time that we demonstrate manipulation of *C. elegans* – a worm of great biological importance – and we achieve it in a more controlable way. The techniques presented in our study can be improved upon further and used as a viable alternative to manipulating cells and living organisms both inexpensively and with ease.

2 EXPERIMENTAL SETUP

Our experimental system is sketched in Figure 1. A disk-shaped piezoelectric actuator (HF-28/2MC, Huifeng Piezoelectric Co. Ltd) was attached to the side of an aluminium block (27 mm x 27 mm x 50 mm) using an ultrasonic transmission gel (Aquasonic 100, Parker Laboratories, Inc). To generate PRF, water droplet is deposited over the plain face of the aluminium block and covered by a glass slide on the top for acquiring a 2D disk shape. This two-dimensional water droplet behaves as an acoustic resonator, thus generating the standing waves. To generate SRF, an array of holes sized 200 μm is drilled onto the surface of the aluminium block for trapping air bubbles upon deposition of a water droplet. A function generator (DG1022, Rigol) and an amplifier (7602M, Krohn-Hite) were used to input sinusoidal wave signals to the piezoelectric transducer at different frequencies to actuate the bubble array. For our experiments, the top view of the droplet is captured via a high-speed camera (Monochrome Machine Vision Camera, PIxeLINK, PLB771U).

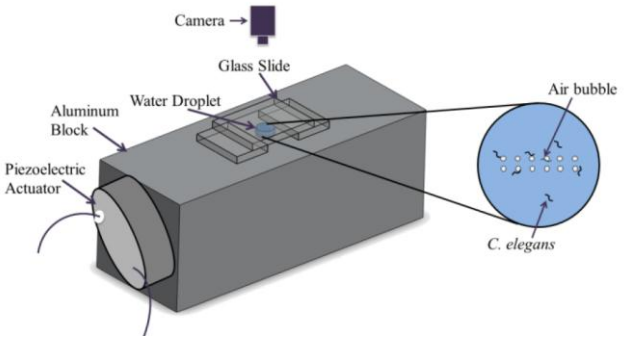

Figure 1. Experimental setup

The selected aluminium block is much larger than the droplet size to ensure that the temperature of the droplet does not increase during experiments due to the resistive heating from piezoelectic transducer.

3 RESULTS AND DISCUSSION

PRF was first used to mainpulate *C.elegans*. In the experiments, a 2-D droplet system (as sketched in Fig. 1) was used, where two pieces of glass spacers were used to support another piece of glass and the droplet was confined within the gap. During the experiment, a small chunk of NGM (Lab Express) with live *C.elegans* (N2 type, requested from Caenorhabditis Genetics Center, University of Minnesota) inside was first picked up and then immersed into 1 or 2 ml of water, depending upon the concentration of worms needed for the experiment. The worms would crawl out of the NGM chunk and swim into the water. After half an hour the water with *C.elegans* inside was collected, and was used to create the droplet resonators as shown in Fig. 1. The piezoelectric transducer was applied with a V_{rms} of 122.6 V, temperature of the living environment of worms was kept at 24^{o}C, and a resonant frequency of 550 kHz was supplied to the piezoelectric transducer. The picture of how the high concentrated *C. elegans* form a pattern consisting of two circles is shown in Fig. 2. The left picture in Fig. 2 shows the randomly distributed *C.elegans* inside the droplet when the power is off. The right picture in Fig. 2 illustrates the distinct circular formations the worms take when the power is turned on. The whole process of rearrangement of the worms i.e. from a haphazard orientation to the circular formation happened instantly (approx. 0.9s). The trapped *C.elegans* could not manage to swim out of the circular patterns as long as the power remained on. The viability of *C. elegans* (Fig. 3) exposed to ultrasound was tested in another experiment with smaller worms; during the 60 s experiment only two out of fifteen worms expired, with possible reason being the strong shear and drag forces exerted on the worm's body. However, further study has to be performed to obtain an in-depth understanding as to whether a short-term exposure to ultrasound is detrimental for the worms .

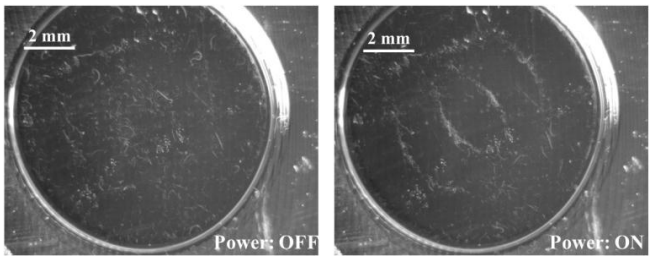

Figure 2. The high concentration of worms (white) forming a pattern inside the 2D droplet. (Left) power off and (right) power on.

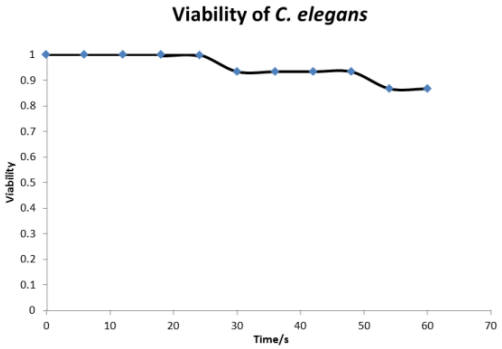

Figure 3. The viability (blue diamonds) of *C.elegans* during 60 s of operation.

To better understand the phenomena, a simulation was conducted using ANSYS. In the simulation, a 2-D acoustic element type 'Fluid29' was employed for an acoustic modal analysis of the pressure field in the simplified droplet chambers. An impedance boundary condition was specified at each air/water interface, with an impedance ratio of water to air given as $Z_{water}/Z_{air}=1.5\times 10^6/4.1\times 10^2 = 3658.5$. The following acoustic properties of water were utilized for our simulation: reference pressure (1×10^6 Pa), density (998 kg m^{-3}) and speed of sound (1483 m s^{-1}). Fig. 4 delineates the simulation results of the acoustic standing wave inside the droplet, the size of which is similar to that used in the experiments. The green areas represent the pressure nodal zones, where pressure oscillations are zero, and the blue/red areas represent the anti-nodal zones where the pressure oscillations are maximal. The simulated pattern at 553 kHz show a good consistency with experimental results in Fig. 2, from which it can be deduced that the worms are captured along the nodes. The relatively high impedence ratio of the water-air interface hinders the ultrasonic pressure waves from escaping past the interface, causing them to reflect, and thus forming a standing wave. The standing waves then exert a strong attraction on the *C. elegans*; since the PRF overcomes the drag force, thus the worms are force alligned along the pressure nodes. Because PRF is dependent on the geometry of the objects as well as the driving parameters, thus much remains to be understood regarding how we can better control the mechanism to get selective worm trapping.

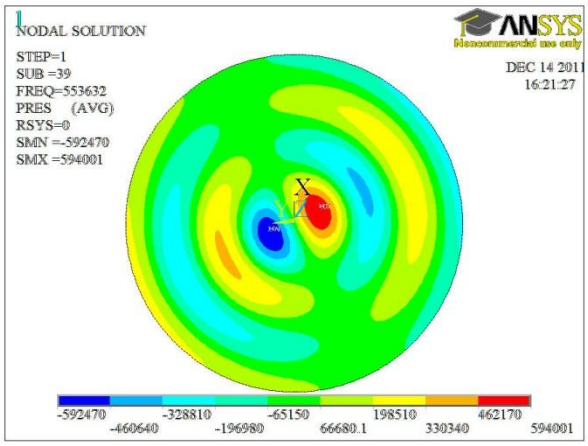

Figure 4. The simulation result of circular droplet at 553 kHz

SRF was also used to achieve even more precise manipulation. Experiments were performed by adding a droplet laden with a live *C. elegan* on top of the microcavities drilled into the aluminium surface. Before turning the piezoelectric transducer on, it was ensured that the air-bubbles were properly trapped into the holes. The piezoelectric transducer was turned on when the worm was in close proximity of the bubble. Upon tuning to a specific frequency it was observed that the microbubble could trap the worm. The worm could only be liberated when the input signal from the frequency generator was turned off. During the period while the worm remained trapped it could only rotate or change its orientation around the bubble. Therefore, by trapping worm and then by turning specific bubbles on and off at defined instances the pathway of the worm was manipulated. Figure 5 shows an isolated worm being forced to traverse in a closed loop via the acoustically actuated microbubbles. Once the piezoelectric transducer was turned on the worm was instantly drawn toward the oscillating bubble where it got trapped. Despite struggling to break free, the secondary radiation force was too strong to bind the worm to the bubble. However, the worm could still rotate around the bubble. Once the worm was in-line with the second bubble the power was turned off allowing it to break free and head in a tagential direction toward the next bubble. Likewise, when that *C.elegans* approached close enough to the second bubble the power was again turned on, which trapped the worm once more. The process was repeated until the worm followed a closed loop. Using an array of microbubbles, we have found that worms could be made to follow both a simple or a complex pathway. For instance, in another experiment (see Figure 6), a horizontal array of bubbles was utilized to make *C.elegans* move in a line without allowing them to stray in a random direction.

In order to explain worm manipulation via bubble arrays we consider the interplay between drag force and secondary radiation force. *C.elegans* getting trapped points to the fact that secondary radiation force dominates drag. Furthermore,

we conclude that the propulsive force of the worm is not strong relative to the secondary radiation force, and thus the worm is unable to escape the attraction of the microbubble. The only time a worm is free to move is when power is off.

Since the secondary radiation force is a function of several parameters including the size of the bubble, the frequency and amplitude of vibration, thus there is a need for developing a mathematical model that can be used for making accurate predictions regarding trapping of matter.

Nevertheless, we have shown that an array of bubbles actuated by ultrasound is an easy and cost effective method to manipulate biological organisms, especially using action-at-a-distance principle without the fear of denaturing them. Therefore, these techniques can be extended to microfluidic devices and lab-on-a-chip applications for manipulation of worms of importance, cells and various other soft-matter.

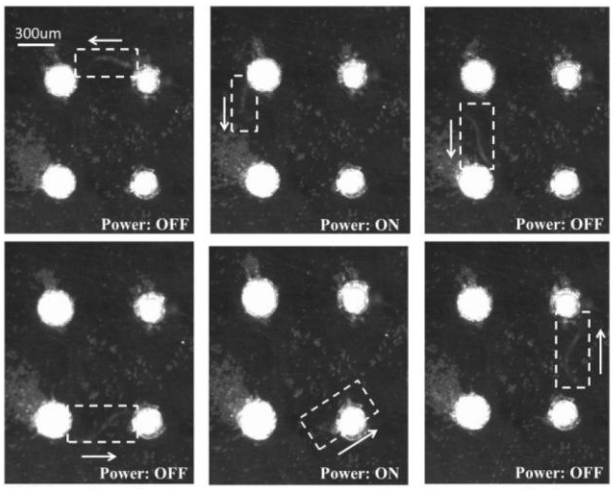

Figure 5. *C. elegans* was manipulated to travel along a loop by turning the acoustic field on and off with proper timing control.

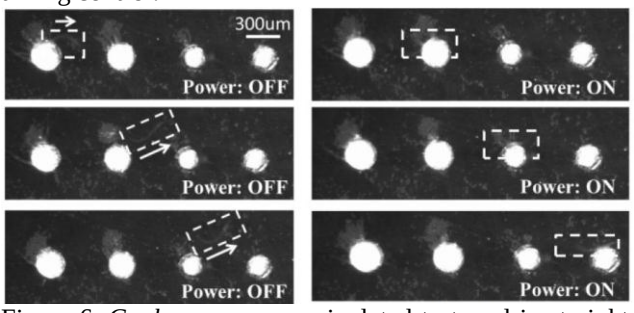

Figure 6. *C. elegans* was manipulated to travel in straight line without letting the worm to stray in random direction.

4 CONCLUSION

In our study, we demonstrated the trapping and manipulation of *C.elegans* using acoustic radiation forces of primary and secondary type. Standing waves produced in a droplet exert a strong attraction on the worms and cause immobilization of the worms along the pressure nodes. The simulation results have been found to agree with the worm-trapping patterns observed in the experiments. The worms remain trapped at nodes as long as the standing wave exists. Viability study reported approximately 87% efficacy of this novel approach to trap worms. Adopting a second technique - using an array of acoustically actuated bubbles - we not only successfully trapped the *C.elegans*, but utilized it to manipulate their motion. The second approach did not render any detrimental effects on the worms and hence can be used to either trap species or manipulate their motion.

In future we believe that the aforementioned techniques can be easily implemented in Lab-on-a-Chip devices for easy and cost-effective manipulation of biological cells, soft matter and even whole organisms. Moreover the approaches do not rely on establishing any contacts; such non-contact based, invasive approach promises to be a superior tweezer. Nevertheless, a detailed investigation is required to develop a quantitative understanding of these trapping mechanisms.

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REFERENCES

[1] Nikolas Chronis and Luke P. Lee, “Electrothermally Activated SU-8 Microgripper for Single Cell Manipulation in Solution”, Journal of Microelectromechanical Systems, 14, 857 – 863, 2005

[2] Han-Sheng Chuang , David M. Raizen, Annesia Lamb, Nooreen Dabbish and Haim H. Bau, “Dielectrophoresis of Caenorhabditis elegans”, Lab on a Chip, 11, 599-604, 2011

[3] Gan Yu, Xiaolin Chen, Jie Xu, “Acoustophoresis in variously shaped liquid droplets”, Soft Matter, 7, 10063-10069, 2011

[4] Bjerknes, “Fields of force”, Columbia University Press, New York, 1906

[5] W. Coakley and W. Nyborg, “Applications in ultrasound: Its applications in medicine and biology”, Elsevier, New York, 1978, ch. Cavitation; Dynamics of gas bubbles; Applications, pp. 77–159.

[6] Chung, S.K. and S.K. Cho, “On-chip manipulation of objects using mobile oscillating bubbles”, Journal of Micromechanics and Microengineering, 18, (12), 2008