SINGLE-BEAM ACOUSTIC TWEEZERS: A NEW TOOL FOR MICROPARTICLE MANIPULATION

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Introduction

It is difficult to move an object of a large volume and mass; however, it is even more difficult to accurately manipulate a tiny object. Currently, very few technologies are available for tweezing micron-sized objects. Among them are optical tweezers[1] and micropipettes[2]. The present article reports primarily on a sequence of studies undertaken in the NIH (National Institutes of Health) Transducer Research Center (NIH TRC) at the University of California that have demonstrated the feasibility of using a highly focused acoustic beam for the noninvasive dynamic control of particles in size ranges from a few to hundreds of micrometers. This kind of device is termed single beam acoustic tweezers or simply acoustic tweezers[3]. These studies[4] have been ongoing for the past eight years. The article does not attempt a comprehensive survey of all the related literature, but reports on the successes of the research program at USC, with the hope that such an account may be of interest to the readers of this magazine. During this year period, several methods, namely a press-focused method, a self-focused method, and a lens-focused method were developed to fabricate high frequency focused transducers for this application. With continuous adjustment and optimization of the fabrication process, the performance of these acoustic tweezers has been gradually improved. Currently, acoustic tweezers fabricated at USC are capable of noninvasively manipulating a single red blood cell and a single micro-particle as small as 1 micron. In this article, the status of this technology as developed at USC is reviewed and its future direction is also discussed. The content covers the theoretical and experimental studies of acoustic tweezing or trapping phenomenon, the fabrication process of acoustic tweezers, and its applications.

"[Recent work] has demonstrated the feasibility of [tweezing micron-sized] objects by a highly-focused acoustic beam."

Acoustic trapping force

It is essential to understand first the physical principle involved in acoustic trapping before the actual device is described. Experimental studies already demonstrated that single beam acoustic tweezers could trap particles of a size either greater or smaller than a wavelength, i.e., what might be termed as Mie particles (larger than a wavelength) or Rayleigh particles (smaller than a wavelength). The acoustic trapping in these two cases has been analytically studied by two different models. The ray acoustics method was applied to calculating the trapping force on Mie particles[3,4]. As shown in Fig.1, the acoustic rays are reflected and refracted by the sphere when they impinge on a sphere. As they travel through and interact with the sphere, momentum transfer occurs and results in a radiation force. The resultant force on the sphere is the integration of the radiation force from all rays. Simulation results show that the reflection plays a critical role in producing the scattering force in the direction of acoustic wave propagation, whereas refraction plays a more important role in producing the trapping force that pushes the particle toward the acoustic beam axis. Therefore, for acoustic trapping to occur it is preferred that the particle is homogeneous and has similar acoustic properties to the surrounding medium so that the refraction could take place more dominantly than reflection. Recently, the trapping force on Rayleigh particle was also investigated analytically by calculating the potential field of the incident acoustic beam[5]. It demonstrated the possibility of manipulating spherical and irregular-shaped Rayleigh particles with different mechanical properties. However, the feasibility of the acoustic trapping in Mie regime was only demonstrated in the case of spherical particles. In other words, there is less restriction in trapping Rayleigh particles than Mie particles.

In both cases, the trapping force could be affected by various parameters, such as frequency, shape and size of particle, beam width, axial position, and the acoustic properties of particles and medium. Although analytical methods are very useful to study the influence of these factors on trapping performance, it is difficult to estimate the absolute value of a trapping force, given the many factors which may affect the trapping force. Moreover, most of current analytical studies assumed ideal Gaussian ultrasonic beams at a single frequency without considering the sensitivity of acoustic tweezers, the effect of medium attenuation, and the possible streaming effect generated by an acoustic beam. Therefore, experimental methods were developed to calibrate the trapping force. The equipartition theorem method[6], power spectrum analysis method[7], and viscous drag force method[8] are the three

classical methods that have been developed to calibrating the trapping force of optical tweezers. However, all of them are only feasible for calibrating the trapping force on spherical objects. Besides, not all of them could apply to acoustic tweezers. So far, only the viscous drag force method where the trapping force and the trap stiffness were calibrated against the known drag force exerted by fluid flows was employed to calibrate the trapping force of acoustic tweezers[5,9]. Here the friction force on microparticles was always neglected for the sake of simplicity[8,10]. In addition the flowing fluid must be calibrated, inducing additive measurement errors. A simpler calibration method was introduced by accelerating the trapped object[11]. As shown in Fig.2, the maximum trapping force of acoustic tweezers estimated by gradually increasing the acceleration of acoustic tweezers until the trapped object can no longer follow the acoustic tweezers. One of advantages of this method is its applicability to irregular shaped objects. Additionally, it measures the effective trapping force, which is the net of the trapping force that overcomes the friction force. Its drawback is that it is incapable of measuring the trap stiffness.

In summary, both theoretical and measurement results to date show that it is possible to trap particles either greater or smaller than the ultrasound wavelength and the magnitude of the forces produced by acoustic tweezers are on the order of a few tens of picoNewtons to a few hundreds of nanoNewtons, which are substantially greater than the forces produced by optical tweezers without inducing damaging effects to the particles or cells.

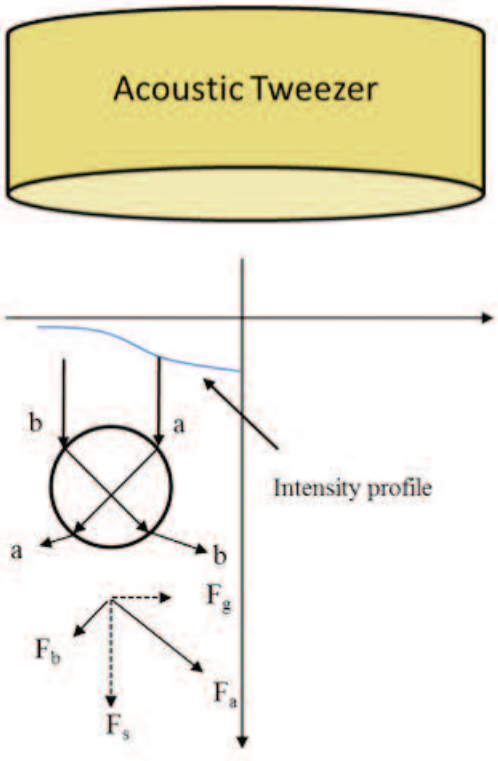

Fig.1 The radiation force on a sphere generated by momentum exchange between a pair of rays and the objects; Fa is the net force produced by a corresponding ray "a" and Fb is the net force produced by a corresponding ray "b". Two forces were resolved in the direction of the incident ray and the direction of the intensity gradient, the component forces are scattering force Fs and gradient force Fg, respectively.

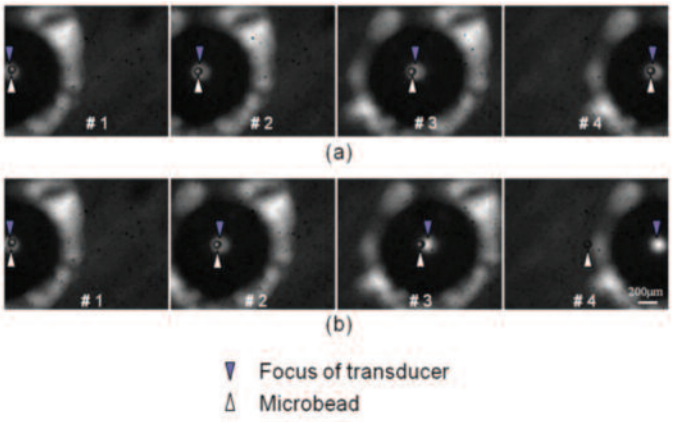

Fig.2. Demonstration of a polystyrene microsphere (90 m mean diameter) manipulated by a 70 MHz acoustic tweezer. (a) The acoustic tweezer was moving at a relative low acceleration (a =5,000 m/s2), the polystyrene microsphere could follow the motion of the acoustic tweezer. (b) The acoustic tweezer was moving at a relative high acceleration (a =5,500 m/s2), the polystyrene microsphere failed to follow the motion of the acoustic tweezer.

Fabrication of acoustic tweezers

A few criteria must be met for a high quality acoustic tweezer, i.e., high sensitivity, low f-number, and the acoustic beam being cylindrically symmetrical about the vertical axis. A critical recognition was that in order to accurately trap a single microparticle or a cell, the wavelength of the acoustic tweezer must be short. As a result of the progress of ultrasonic transducer technology that has been made, recently an acoustic tweezer platform at frequency higher than 200 MHz was developed at the NIC TRC. The minimum size of particle that may be stably trapped by acoustic tweezers was down to 1μm.

However, it has been quite a challenge in the development of high frequency acoustic tweezers with acceptable trapping performance. The thickness of piezoelectric layers of the transducers at frequencies higher than 100MHz, is usually only a few tens of microns, which cannot be easily achieved with conventional approaches, e.g. lapping and grinding. It is an even greater challenge to produce a highly focused configuration with such thin piezoelectric layers. Several methods, namely a press-focused method, a self-focused method[12] and a lens-focused method were undertaken to develop highly focused transducers for acoustic tweezer applications. Details of fabrication process were described elsewhere[12-14]. Each method that has been applied to fabricating acoustic tweezers has its advantages and disadvantages. For instance, the press-focused method has an advantage of simplicity, but the mechanical pressing process could easily break the piezoelectric material, therefore affecting the performance of the transducer. Self-focused method is relative easy to fabricate transducers with low f-number (~ 1) and consistent quality, but the sensitivity of the self-focused transducers is usually poor. Lens-focused transducers would incur extra attenuation caused by the lens especially in the ultrahigh frequency range (>100 MHz).

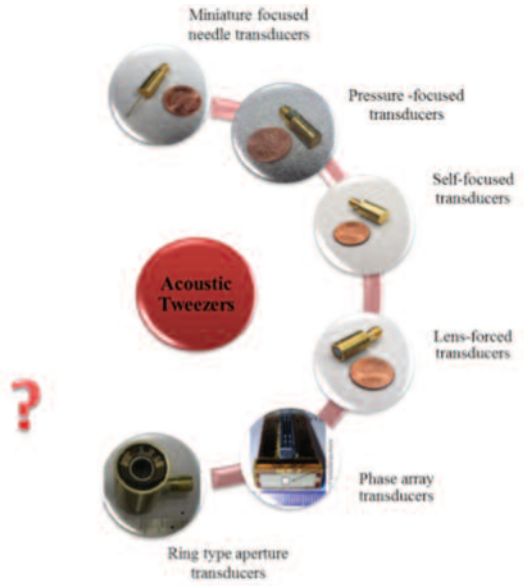

Fig.3. Different types of ultrasonic transducers for the acoustic tweezers application.

In addition, needle-type[14], ring-type[15] and phased-array transducers[16] were also fabricated for acoustic tweezers applications. Those transducers may have advantages in certain applications compared to traditional acoustic tweezers. For example, the size of the transducer is crucial in a number of applications, and there is a need to miniaturize the transducer size. Phased array transducers could focus and steer the acoustic beam; therefore no mechanical movement of transducer is required. However, phased array transducers are still limited to the frequency range less than 50 MHz and further improvement is needed. Meanwhile, the present authors are also exploring new types of transducers, which may generate the acoustic beam with better cylindrical symmetry and higher intensity, consequently yielding better trapping performance and deeper penetration depth.

Biomedical applications of acoustic tweezers

The most common technology for micro-particulate or cellular manipulation is micropipettes, but it has intrinsic shortcomings, such as time consuming and low control accuracy. Optical tweezers which could overcome those problems, has become a powerful tool with broad applications in biology, medicine, and physics. It has been used for noninvasive dynamic control of particles in the size ranged from tens to hundreds of nanometers, including bacteria, viruses and living cells. There are however a few disadvantages of optical tweezers as well. First of all, its application has been limited to optically purified samples or media. Second, the high energy of focused lasers may induce local heating and photo-damage. Third, the maximum trapping force of optical tweezers is limited to a few hundred picoNewtons. Furthermore, the system of optical tweezers is complex and expensive.

Acoustic tweezers may alleviate these problems. On one hand, it has comparable time efficiency and control accuracy to optical tweezers, and on the other hand, it has lower cost and stronger trapping force than optical tweezers. Moreover, it has deeper penetration depth in the light opaque objects such as tissues and less biological damages, which brings a wide variety of biomedical applications for this technology. It has been applied to study the deformability of red blood cells with two different approaches.

In a preliminary experiment, fresh blood samples were obtained from a healthy adult male. Red blood cells (RBC) were diluted in phosphate-buffered saline (PBS) and then washed three times by centrifuging. All preparations were made at room temperature. The experiment process was captured by a CMOS (complementary metal-oxide-semiconductor) camera (ORCA-Flash2.8, Hamamatsu, Japan) connected to the microscope. RBCs were suspended in a specially designed chamber filled with PBS. The chamber was open at the top and had a thin mylar membrane as its bottom. After 2 hours, the suspended RBCs would sink down to the bottom. A cell stuck to the chamber was chosen as the object of study. A selected RBC was stretched directly or indirectly by an acoustic beam. Two 200 MHz focused transducers were employed in this study. One is a 200 MHz lithium niobate (LiNbO3) single crystal pressed focus (PF) transducer and the other is a 200 MHz Zinc oxide lens focus (LF) transducer.

Method #1: The PF transducer was employed to trap a 5μm polystyrene microsphere. The trapped polystyrene microsphere was attached to a selected RBC and the RBC could be stretched by an acoustic beam through the trapped microbead. As displayed in the Fig.4 (PF), the RBC was stretched to left and right and deformed to different degrees.

Method #2: The LF transducer was used to deform a RBC (red blood cell) directly. A RBC near the acoustic beam was selected. As the voltage input to the transducer was increased from 200 mV_{pp} to 800 mV_{pp} followed by 50 dB amplification, the RBC was observed to start to elongate after the input voltage was increased to above 200mV_{pp}. The degree of elongation was observed to increase with the increase of input voltage.

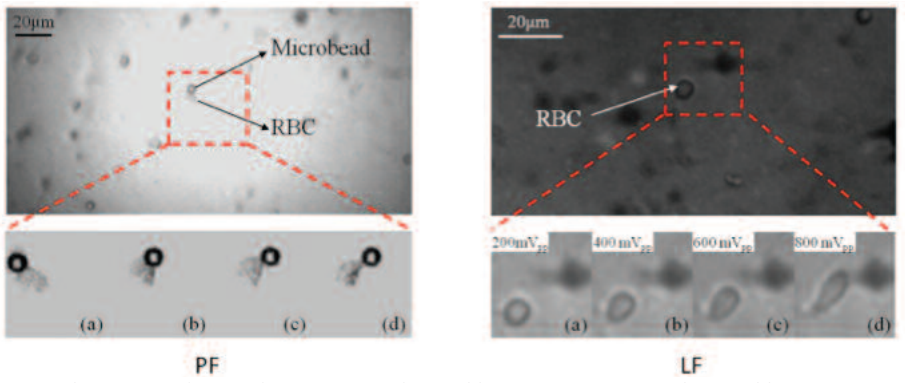

Fig. 4. Video sequence showing that a RBC was elongated by a 200 MHz PF transducer and by a 200MHz LF transducer

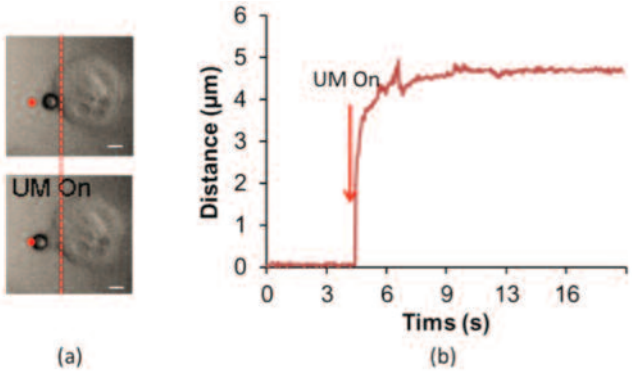

Fig. 5. Cell stretching images and analyzed data associated with the stretched length. (a) The images indicate that the stretching test is carried out at various voltage inputs to the transducer; 16 V_{pp} ((b) Temporal displacement change at the indicated voltage.

The membrane properties of cancer cells were also studied with an acoustic beam with the similar method[17]. The 5μm fibronectin-coated microbead was trapped in a 200 MHz acoustic trap and successfully attached to the membrane of a single breast cancer cell (MCF-7). The fibronectin proteins here allowed the microbead to be firmly attached to cell membrane. The cell membrane was then stretched by trapping the microbead in the acoustic beam. Fig. 5 shows that the cell membrane is stretched through the trapped microbead when the transducer is on. The stretched distance of the cell membrane was found to be ~4.7 μm at the input voltage of 16 V_{pp}.

Looking Ahead

Although optical tweezers have the advantages in resolution and accurate control of smaller particles, i.e., nanoparticles, the direct exposure of cells to the optical tapping laser beam may cause changes in cellular properties. Those disadvantages may place severe limits in the application of this technology. Acoustic tweezers that do not have these problems may serve as an alternative to optical tweezers in many of these studies. Biomedical applications including measuring white cell adhesive forces are being explored in the NIH TRC at the University of Southern California.AT

References

1 A. Ashkin, "Acceleration and Trapping of Particles by Radiation "Pressure," Physical Review Letters, **24**, p. 156 (19700

2 J. M. Mitchison and M. M. Swann,"The Mechanical Properties of the Cell Surface .1. The Cell Elastimeter," Journal of Experimental Biology, **31**, p. 443 (1954).

3 J. Lee and K. K. Shung, K. K., (**2006**) "Radiation forces exerted on arbitrarily located sphere by acoustic tweezer," J Acoust Soc Am, **120**, pp. 1084-1094 (2006).

4 Lee, J., Ha, K., and Shung, K. K., (**2005**) "A theoretical study of the feasibility of acoustical tweezers: ray acoustics approach," J Acoust Soc Am, 117, pp. 3273-3280.

5 Azarpeyvand, M. and Azarpeyvand, M., (**2013**) "Acoustic radiation force on a rigid cylinder in a focused Gaussian beam," Journal of Sound and Vibration, **332**, pp. 2338-2349.

6 Visscher, K. and Block, S. M., (**1998**) "Versatile optical traps with feedback control," Methods Enzymol, **298**, pp. 460-489.

7 Norrelykke, S. F. and Flyvbjerg, H., (**2010**) "Power spectrum analysis with least-squares fitting: amplitude bias and its elimination, with application to optical tweezers and atomic force microscope cantilevers," Rev Sci Instrum, **81**, p. 075103.

8 Felgner, H., Muller, O., and Schliwa, M., (**1995**) "Calibration of light forces in optical tweezers," Appl Opt, **34**, pp. 977-982.

9 Lee, J., Jeong, J. S., and Shung, K. K., (**2013**) "Microfluidic acoustic trapping force and stiffness measurement using viscous drag effect," Ultrasonics, **53**, pp. 249-254.

10 Lee, J., Lee, C., and Shung, K. K., (**2010**) "Calibration of sound forces in acoustic traps," IEEE Trans Ultrason Ferroelectr Freq Control, **57**, pp. 2305-2310.

11 Li, Y., Lee, C., Lam, K. H., and Shung, K. K., (**2013**) "A simple method for evaluating the trapping performance of acoustic tweezers," Appl Phys Lett, **102**, p. 84102.

12 Lam, K. H., Hsu, H. S., Li, Y., Lee, C., Lin, A., Zhou, Q., Kim, E. S., and Shung, K. K., (**2013**) "Ultrahigh frequency lensless ultrasonic transducers for acoustic tweezers application," Biotechnol Bioeng, **110**, pp. 881-886.

13 Cannata, J. M., Ritter, T. A., Chen, W. H., Silverman, R. H., and Shung, K. K., (2003) "Design of efficient, broadband single-element (20-80 MHz) ultrasonic transducers for medical imaging applications," IEEE Trans Ultrason Ferroelectr Freq Control, 50, pp. 1548-1557.

14 Hsu, H. S., Zheng, F., Li, Y., Lee, C., Zhou, Q., and Shung, K. K., (**2012**) "Focused high frequency needle transducer for ultrasonic imaging and trapping," Appl Phys Lett, **101**, p. 24105.

15 Jeong, J. S. and Shung, K. K., (**2013**) "Improved fabrication of focused single element P(VDF-TrFE) transducer for high frequency ultrasound applications," Ultrasonics, **53**, pp. 455-458.

16 Zheng, F., Li, Y., Hsu, H. S., Liu, C., Tat Chiu, C., Lee, C., Ham Kim, H., and Shung, K. K., (**2012**) "Acoustic trapping with a high frequency linear phased array," Appl Phys Lett, **101**, p. 214104.

17 Hwang, J. Y., Lee, C., and Shung, K. K., (**2013**) "Characterization of Cell Membrane by Acoustic Tweezers," 11th Annual Ultrasonic Transducer Engineering Conference, **2B3**