TRANSLATION AND ELECTRICALLY CONTROLLED ROTATION OF LARGE ZEBRAFISH EMBRYO BY ACOUSTIC TWEEZERS

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ABSTRACT

This paper presents trapping, micromanipulation and electrically controlled rotation of a large inhomogeneous/nonsymmetrical late-stage zebrafish embryo underwater using two sets of MEMS acoustic tweezers. The trapping and manipulation of such large living organisms in liquid is studied with two different acoustic tweezers operating at frequencies of 0.524 and 2.10 MHz.

The two different acoustic tweezers can provide complementary features; one to achieve stable rotation-less trapping (as a trapped embryo is translated in 3-dimensional space) and the other to electrically control the rotation of a trapped embryo while changing applied voltage, pulse repetition frequency, and duty cycle for an achievable rotation speed ranging from 0.2 revolution per second (rps) to 1 full rotation per second.

The translation capability of the acoustic tweezers has been characterized through the maximum translation speed before losing trapping (up to 12mm/s), corresponding to an estimated lateral trapping force of 80 and 60nN for the 0.524 and 2.1MHz tweezers, respectively. The comparison of the embryo's longitudinal and transverse stability exhibits a more enclosed trapping zone for the 2.1MHz tweezers with an average vertical shaking of 10µm compared to 20µm for the 0.524MHz (and a longitudinal shaking of 60 and 120µm for the 2.1 and 0.524MHz tweezers, respectively).

KEYWORDS

Acoustic tweezers, micromanipulation, zebrafish embryo, electrically controlled rotation

INTRODUCTION

Manipulating living organisms in liquid environments has long been required for fundamental studies in various scientific fields including biology, biophysics, and medicine. Understanding the complex behaviors and interactions of living organisms at the cellular and molecular levels requires strong and fine control over their spatial position without the use of external support, such as gel, which can disturb the organism's natural growth.

Compared to other trapping mechanisms, acoustic trapping utilizes generated acoustic waves to create forces that can immobilize and manipulate large, heavy objects, including living organisms, in a liquid environment. Unlike optical tweezers or microfluidic-based devices, acoustic trapping offers strong contactless trapping with low heat generation for a safer manipulation of sensitive living organisms. Precise sample manipulation and orientation control are particularly important to acquire fine imaging [1]. Among the various acoustic tweezers studied, quasi-Bessel-beam-based acoustic tweezers has shown to provide a stable trapping of large and heavy objects (without the need of additional component such as reflector), thus facilitating its integration in an existing biological experimental setup [2-5]. The quasi-Bessel beam is generated using multiple consecutive focal points. Such a desired pressure pattern can be achieved using active or passive lens design that focuses generated acoustic waves at the focal points. Active lens is based on the electrodes (sandwiching a piezoelectric substrate) being driven with different phases in order to achieve a desired pressure pattern with trapping capabilities. A pressure pattern is achieved typically by sequentially driving various electrodes, which results in a relatively poor capability in trapping large particles like zebrafish embryos.

Passive lens, however, provides a specific pressure distribution pattern based on a designed lens through which a particle can be trapped. Since all electrodes are derived simultaneously in passive lens configuration, passive lens tends to be more effective in trapping large and heavy particles than an active lens with sequential driving of the electrodes.

Here, two sets of passive-lens quasi-Bessel beam acoustic tweezers are used to trap and manipulate zebrafish embryos. The two devices presented here operate at 524kHz and 2.1MHz.

Zebrafish embryos are largely studied in biology due to their vertebrate developmental biology, as they are optically transparent and have a short growth cycle for external development. They are also studied for their genetic properties, which allow them to regenerate damaged cardiovascular cells. Among vertebrates, zebrafish embryos exhibit common genetic underpinnings with humans [6].

ACOUSTIC TWEEZERS

The two sets of tweezers are designed to operate in the fundamental thickness resonance mode on a 1- and a 4-mm thick Lead Zirconate Titanate (PZT) substrate sandwiched between 200 nm thick Nickel top and bottom electrodes corresponding to the working (fundamental thickness-mode resonance) frequencies of 2.1 and 0.524 MHz, respectively. The two fabricated acoustic tweezers use Parylene-based (2.1MHz device) and PDMS-based (0.524MHz device) air cavities [7]. Due to the high acoustic impedance mismatch between water and air, the air cavities are used to block the waves that destructively interfere at the desired focal point. The passive air-cavity lens is patterned into annular Fresnel-Half-Wavelength Bands (FHWB) for focusing at three different focal points to create a trapping zone within the three focal points (Fig. 1). The annular air-cavity FHWB lens is divided into three sets of six 20° pie-shaped sectors with the three sets designed to have focal lengths of 1.96, 2.32, and 2.72mm for the 2.1 MHz tweezers; and 9, 10.8, and 12.5 mm for the 524 kHz tweezers (Fig. 1).

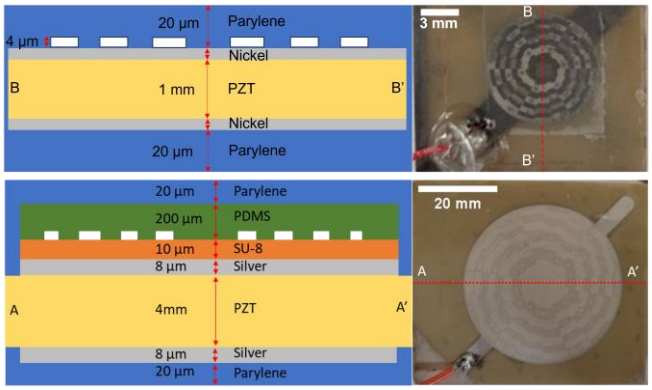

Figure 1: Cross-sectional schematics (left) and top view photos (right) of the 2.1MHz tweezers (top row) and 524 kHz tweezers (bottom row).

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For precise on-demand manipulation of a trapped embryo over a large distance in 3-dimensional space, it is necessary to limit the acoustic reflection from the water-air interface. Hence, an acoustic absorber (APT-Flex F48) is placed above the acoustic tweezers.

To explain the trapping mechanism of the fabricated tweezers, 3D Finite Element Method (FEM) simulations are performed to obtain the time-averaged second-order pressure pattern created in the presence of a particle in the quasi-Bessel beam (Fig. 2b). The trapping force generated by this specific pressure wave pattern is based on the pressure gradient force as well as the momentum flux transfer between the incident wave and the trapped object. With such acoustic tweezers, the particle or object of interest is shown to be fully trapped in 3 dimensions.

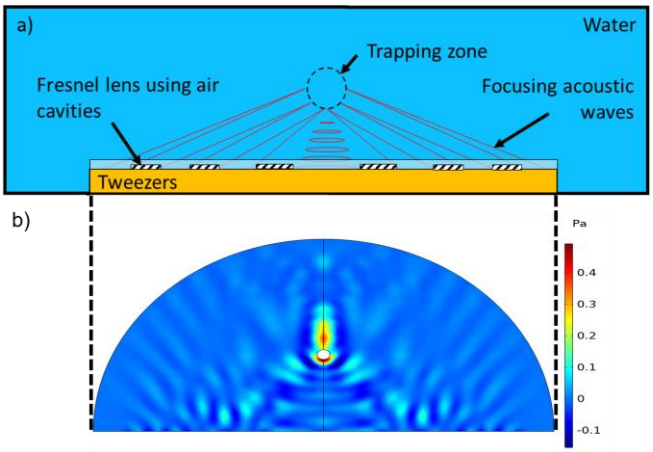

Figure 2: (a) Cross-sectional schematic of forming a trapping zone with three focal points from the acoustic transducer (or tweezers). (b) Time-averaged second-order pressure depicting the mechanism of particle trapping.

EMBRYO MANIPULATION

Both of the fabricated acoustic tweezers are able to trap zebrafish embryos as well as other similar size objects in liquid. The tweezers' manipulation capabilities are studied and compared by observing and measuring the embryo motion while being trapped in still and moving states.

Embryo rotation

The 2.1 and 0.524 MHz acoustic tweezers can trap an 18-hour post-fertilization (hpf) zebrafish embryo (about 1 mm in diameter) at the particular designed location. The 2.1MHz tweezers has the trapped embryo almost completely still, with a measured wiggling of 10-15µm, showing a small, enclosed trapping zone. The embryo orientation may be modified on-demand by manually rotating the tweezers around the rotation axis positioned in the center of the acoustic tweezers. A 90° rotation of the tweezers induces an exact 90° rotation of the embryo (Fig. 3).

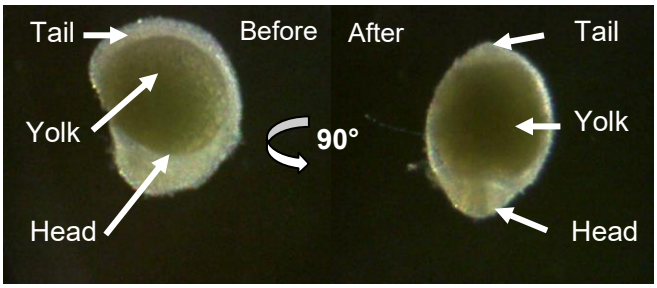

Figure 3: Photos of an 18-hour-post-fertilization (hpf) zebrafish embryo trapped with the 2.1MHz tweezers before and after rotating it (to change the orientation of the embryo) through mechanically rotating the tweezers.

The 524kHz tweezers has the trapped embryo rotating, likely due to its relatively large trapping zone because of its larger wavelength compared to the zebrafish embryo size and the embryo's non-symmetrical shape composed of inhomogeneous components (Fig. 3). However, the rotation can be electrically controlled and is observed to be at 1 rotation per second (rps) when 40V_{pp} continuous sinusoidal wave is applied to the tweezers; the rotational speed reduces to 0.33 rps when the amplitude is reduced to 30V_{pp} (Table. 1).

When the 524kHz tweezers is driven by a pulsed sinusoidal signal with its amplitude fixed at 50V_{pp}, the rotational speed can be reduced to 0.2 rps (i.e., 1 full rotation per 6 seconds) with a pulse repetition frequency (PRF) of 200Hz and a duty cycle of 6.5% (or 1 kHz PRF and 8% duty cycle) (Table 1). When decreasing the voltage or reducing the duty cycle below 6%, the trapped embryo's wobbling increases until the trapping force is no longer sufficient, and the embryo drops. These results also show that the PRF has negligible influence on the trapping and rotation of the embryo as long as the off-time period, when the tweezers is not generating acoustic waves, is small compared to the time needed for the embryo to drop out of the trapping zone which is estimated at 100ms.

Table 1. Electrical control of the rotational speed of a trapped 18hpf embryo (by the 524kHz tweezers) through the voltage magnitude (with continuous wave) and pulse repetition frequency and duty cycle.

524kHz Acoustic tweezers					
Continuous signal		Pulsed signal			
Applied voltage (Peak-peak)	Revolution per second	Applied voltage (Peak-peak)	Pulse repetition frequency	Duty cycle	Revolution per second
40	1	50	200Hz	6.5%	0.167
34	0.4	50	1kHz	8%	0.167
30	0.33	50	5kHz	8.6%	0.25

Embryo translation

Both acoustic tweezers exhibit the capability to trap and move a large and heavy 18 hpf zebrafish embryo over a large range in a 3-dimensional (3D) space (Fig. 4) by when the tweezers itself is translated manually in liquid medium (Fig. 5).

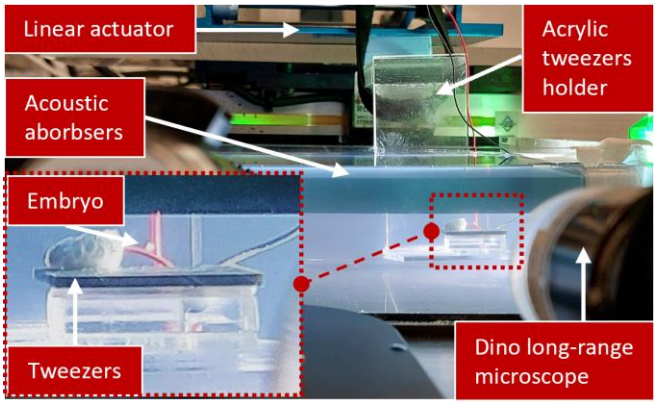

Figure 4: Photo of the experimental setup used for the translation study of the trapped embryo underwater. Two Dino long-range microscopes in front of the chamber capture the motion, as the tweezers (mounted on an acrylic holder attached to a linear actuator) is moved linearly.

Using a linear 1-axis motorized stage with controllable translation velocity, the acoustic tweezers are placed on an acrylic holder immersed in a water-filled chamber, which follows the motion of the linear stage. Due to the configuration of the experimental setup, acoustic absorbers (APT-Flex F48) are needed and are placed above the tweezers to minimize acoustic reflections from external interfaces (such as solid-liquid and liquid-air interfaces) which can create standing waves that disturb the desired pressure pattern. At 524kHz, the acoustic absorbers reduce echo pressure by 18dB (almost 10 times). Because of the relatively low frequency of the 524kHz tweezers, it is necessary to degas the water using a 3M™ Liqui-Cel™ degassing filter to avoid creation of bubbles that act like small reflectors which disturb the trapped embryo. The embryo is initially dropped using a narrow hole machined in the acoustic absorbers. Then, two long-range microscope cameras (Dino reference) are placed outside of the fluidic chamber and are used to record videos of the embryo motion to characterize the translation capabilities of the tweezers. Stability of a trapped embryo, as the trapped embryo is translated in 3D space by moving the tweezers, is measured as a function of the translation or movement velocity of the tweezers. The stage velocity is varied from 2 up to 15 mm/s.

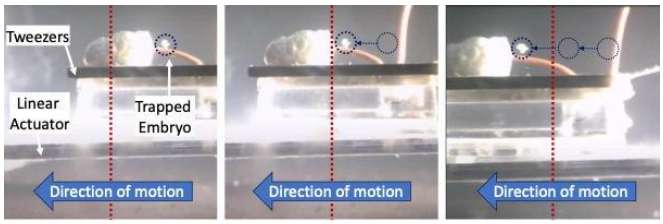

Figure 5: Photos of an 18hpf zebrafish embryo trapped with the 2.1MHz tweezers, as the tweezers is mechanically translated to move the trapped embryo horizontally.

To extract the wobbling of the embryo in motion along the longitudinal and vertical direction obtained via the tracking of the embryo trajectory, the recorded videos are post-processed using ImageJ/Fiji software with two supplementary plugins: FFMPEG plugin is used to open the video as a stack of images, then after converting the stack to 8-bits images, TrackMate plugin is used to detect the outline of the embryo and to follow it through the image stack [8]. This way, the horizontal and vertical coordinates of the embryo trajectory are automatically extracted (Fig. 6). To correct the angle of the camera, the linear regression of the extracted trajectories is subtracted from the trajectory's curves. The standard deviation of the corrected trajectories is calculated and is defined as the longitudinal and vertical shaking of the embryo. For each velocity, the experiments are repeated 6 times.

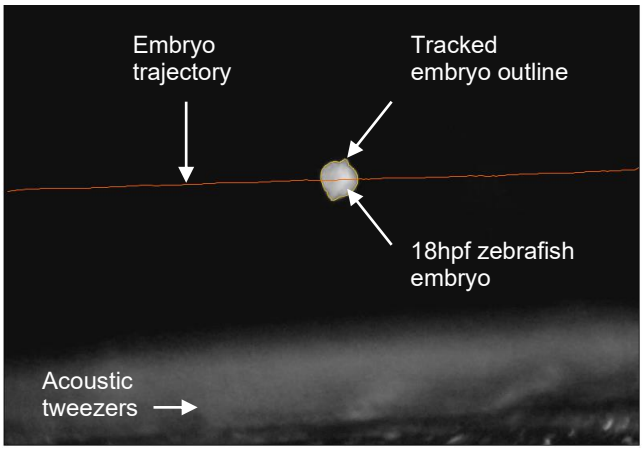

Figure 6: Photo of the 8-bit image showing the tracking of an 18hpf zebrafish embryo trapped with the 524kHz tweezers, as the tweezers is mechanically translated to move the trapped embryo horizontally at 2mm/s.

The software tracking of the embryo shakiness is illustrated with a photo shown in Fig. 6. For almost all the velocities tested, the longitudinal and vertical shaking of the trapped embryo is smaller with the 2.1MHz tweezers than with the 524kHz, proving more enclosed trapping which keeps the trapped embryo more stable (Fig. 7). For both of the tweezers, the longitudinal wobbling is measured to be higher than the vertical shaking; 50 and 10µm, respectively, for the 2.1MHz device. Furthermore, when increasing the stage velocity, the longitudinal shaking also increases due to the induced drag force which perturbs the stable trapping (Fig. 7a). The vertical shaking of the embryo is less affected by the translation speed and remains around 15-20µm and 10-15µm for the 524kHz and 2.1MHz acoustic tweezers, respectively, proving once more their different trapping zone size (Fig. 7b).

The maximum translation speed supported by the trapped embryo is 9mm/s for the 2.1MHz and 12mm/s for the 524kHz device, exhibiting a different lateral trapping force. The lateral trapping force can be estimated by the induced drag force (F_{lat}) at the maximum velocity when considering an embryo as a 700µm spherical object.

$$F_{lat} = 6\pi r\eta v \qquad (1)$$

with r being the radius of the spherical embryo, η being the viscosity of the water, and v being the relative velocity between the embryo and the liquid. Using equation (1), the lateral trapping force is estimated to be 60nN and 80nN for the 2.1 and 0.524MHz tweezers, respectively. For an embryo with a density of 1,100kg/m^3, the trapping force corresponds to more than two orders of magnitudes larger than the force required to prevent the embryo from falling, thus proving that the quasi-Bessel beam tweezers is perfectly suited for the underwater transportation of large and heavy living organisms.

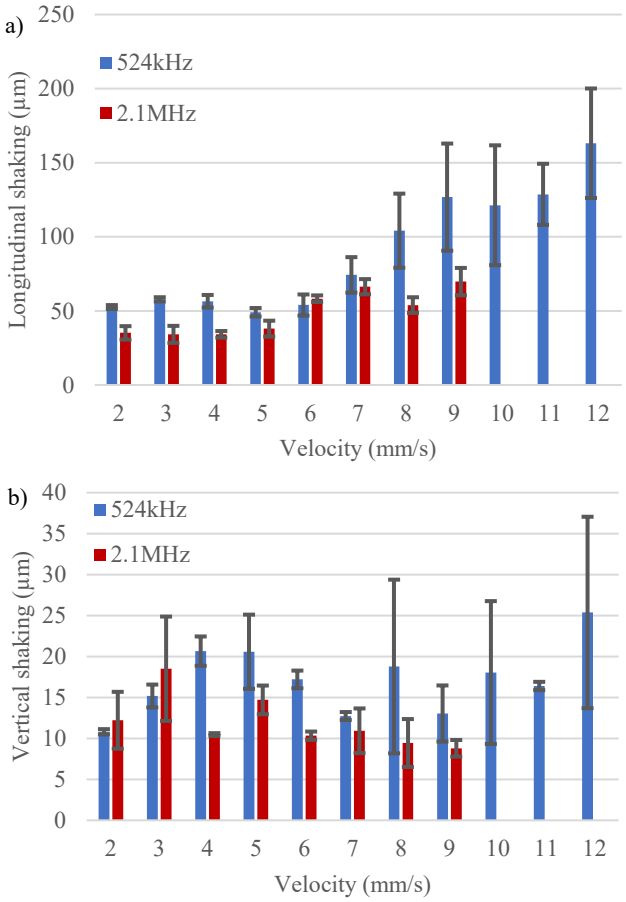

Figure 7: Measured (a) longitudinal and (b) vertical shaking of a 18hpf zebrafish embryo trapped with the 2.1MHz and 0.524MHz tweezers, as the tweezers are mechanically translated horizontally with varying velocities.

The two tweezers with such precise manipulation capability are ideally suited for imaging live organisms in a liquid environment where organisms of interest have to be precisely positioned at the imaging area with minimal shaking. The integration of such device for biological manipulation only requires the tweezers itself and (if needed) an additional acoustic absorber, making the integration very easy in a biological environment.

SUMMARY

We presented the trapping and manipulation of large zebrafish embryo using two sets of acoustic Bessel beam tweezers. These devices present complementary features; (1) one tweezers (operating at 2.1 MHz) able to trap embryo in a rotation-less manner and (2) the other tweezers (operating at 0.524 MHz) exhibiting an intrinsic rotation of the trapped embryo which can be electrically controlled from 1 to 0.2 rotation per second. Both tweezers are able to precisely move the embryo in a fluidic chamber with a measured vertical shaking of 10 and 20µm and longitudinal shaking of 60 and 120µm for the 2.1 and 0.524MHz tweezers, respectively. Shaking results reveal a more enclosed trapping obtained with the 2.1MHz device but the maximum achievable velocity shows a lower lateral trapping force of about 60 nN compared to 80 nN. The 2.1MHz device is best suited for fine, stable positioning of a trapped organism whereas the 524kHz tweezers is a good candidate for experiments and studies which require strong and fast translation motion.

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