ANALYSIS OF TRAPPING AND STREAMING IN AN ULTRASOUND-ACTUATED MULTI-WELL MICROPLATE FOR SINGLE-CELL STUDIES

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ABSTRACT

The dynamics of the acoustic streaming and the acoustic positioning performance in an ultrasound-actuated multi-well microplate are investigated by two different ultrasonic frequency actuation schemes: Frequency-modulation and single-frequency actuation. Our results show a significant decrease in size of the field of view when using frequency-modulation compared to single-frequency actuation, which can be used for improving the scanning time for 3D high-resolution confocal microscopy by almost one order of magnitude. Furthermore, in the ultrasound-actuated multi-well microplate the high-voltage acoustic streaming show a complex time and temperature dependence and could gain stability by the use of temperature control.

KEYWORDS

Ultrasound, acoustic streaming, positioning, microscopy, multi-well, microplate.

INTRODUCTION

We analyze the dynamics of acoustic streaming in a novel ultrasound ring-transducer microplate designed for parallel aggregation and positioning of cells. Furthermore, we quantify the positioning accuracy of cell and particle clusters at single-frequency and frequency-modulation actuation.

We have previously described a gentle method for measuring individual cell-cell interactions in a highly parallel manner, based on ultrasonic trapping of cells in a multi-well microplate [1]. This device has been used for imaging the immune synapses formed between natural killer cells and target (cancer) cells [2]. Moreover, we have demonstrated that acoustic streaming – an effect that often limits the trapping performance – could be suppressed by frequency-modulation ultrasonic actuation (without affecting the cell trapping performance), compared with single-frequency actuation [3]. However, when our device is operated at higher amplitudes the repeatability and robustness of our method is reduced.

Therefore, in this paper we analyze the dynamics of cell or particle trapping and fluid streaming for various actuation frequencies and voltages using the novel planar ring-transducer. The purpose is to improve the robustness and efficiency of the device, and to calibrate our method for small microscopy field-of-view (FOV). This is done by studying the particle and fluid tracks at high-voltage actuation (up to 200 V_{pp}), and by measuring the positioning accuracy of particle clusters for actuation frequencies between 2.2 and 2.4 MHz. In addition, we measure the dependence of acoustic streaming on temperature and actuation time.

THEORY

Manipulation of cells or particles using ultrasound is based on the acoustic radiation force, F^{rad}. In the simple case of a one-dimensional standing-wave system, the acoustic radiation force can be written as [4]:

$$F_z^{rad} = 4\pi\Phi k a^3 E_{ac} \sin(2kz). \quad (1)$$

Here, z is the propagation direction, Φ is the acoustic contrast factor dependent on the density ratio between particle (or cell) and suspension medium and the compressibility ratio between particle (or cell) and the suspension medium. Furthermore, k is the wavenumber, a is the particle or cell radius and E_{ac} is the acoustic energy density proportional to the acoustic pressure amplitude squared. For example, in the ultrasound actuated multi-well microplate the maximum acoustic radiation forces vary from 10 to 60 pN.

EXPERIMENTAL

A schematic view of the device is shown in Fig. 1 a). The device consists of the concave-shaped multi-well microplate being fixated to the ring-shaped PZT piezo crystal by the variable spring-loaded holder. To visualize the acoustic trapping, 10 µm-sized green-fluorescent polystyrene particles were used and the acoustic streaming was visualized by 1 µm-sized red-fluorescent polystyrene particles. Both particle types were mixed with Milli-Q water and 0.01% Tween-20, yielding a concentration of 9.1×10^4 beads per mL and 3.6×10^8 beads per mL for the 10 µm and 1 µm-sized particle, respectively. Two types of microscope systems were used: an inverted bright-field microscope in epi-fluorescent mode combined with a video camera for acoustic streaming imaging, and an inverted laser-scanning confocal microscope for acoustic trapping performance imaging. The acquired images and videos from the microscopes were processed and analyzed with Matlab image processing toolbox and the free particle image velocimetry (PIV) toolbox [5].

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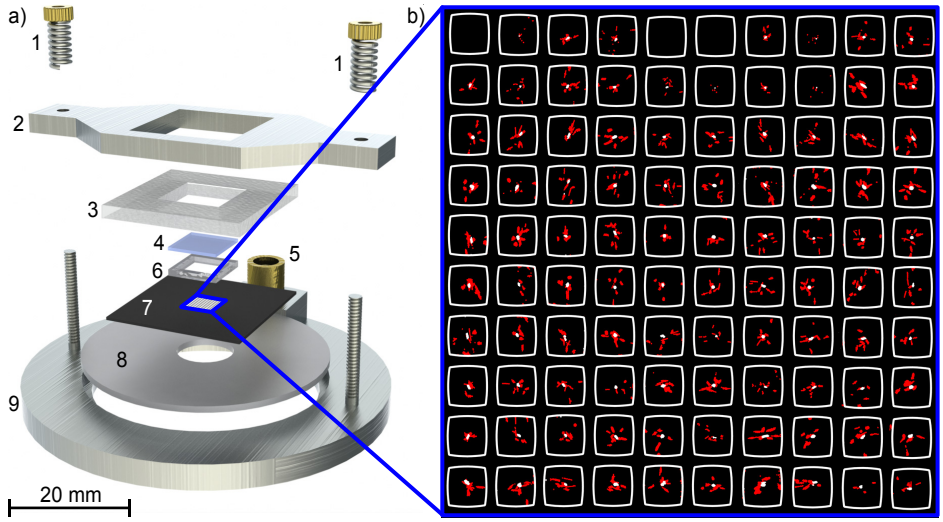

Figure 1: (a) Schematic view of the device (ultrasound transducer and multiwell microplate). (1) Springs. (2) Aluminum clip. (3) Polymethyl methacrylate (PMMA) spacer. (4) Cover glass. (5) Micro coaxial (MCX) connector (6) PDMS frame. (7) Multiwell microplate. (8) Ring-shaped PZT piezo crystal. (9) Aluminum baseplate. (b) Trapping result with 10 µm-sized tracer particles in all 100 wells for frequency-modulation (2.30 MHz ± 100 kHz @ 1 kHz, 50 V_{pp}, white) and single-frequency (2.20, 2.23, 2.26, 2.29, 2.30, 2.33, 2.36, 2.39, and 2.40 MHz, 50 Vpp, superposition of all frequencies shown in red) actuation.

RESULTS AND DISCUSSION

The microscope image in Fig. 1 b) show the trapping result of single-frequency (SF) actuation compared to frequency-modulation (FM) actuation. The device utilizes a new planar ring-transducer of simpler and more uniform geometry (*cf.* Fig. 1), compared to our previously used wedge transducer [1-3]. A more detailed analysis of the shape and the position of few-particle clusters trapped at different single frequencies, as well as at frequency-modulation actuation, are shown in Fig. 2. On average, FM actuation decreases the FOV of the microscope to merely 13 % of the corresponding FOVs when using SF actuation. Fig. 3 shows the initial (first second) streaming and trapping pattern for 1 µm and 10 µm particles, respectively, at 206 V_{pp} FM actuation. Fig. 4 shows the mean streaming speed and the temperature during the first 5 min of constant 206 V_{pp} FM actuation as well as the acoustic streaming above and at the bottom of 10 different wells. In contrast to the direct trapping performance at FM actuation, the diagrams reveal a complex dependence of the acoustic streaming on the temperature. Further complexity is added because of the slow clustering of the 1 µm particles (see Fig. 4 (b-c)) used for fluid tracking, an effect that may affect the acoustic streaming and, in turn, the positioning performance of cells or 10 µm particles. Thus, care must be taken when interpreting PIV-data generated from 1 µm particles.

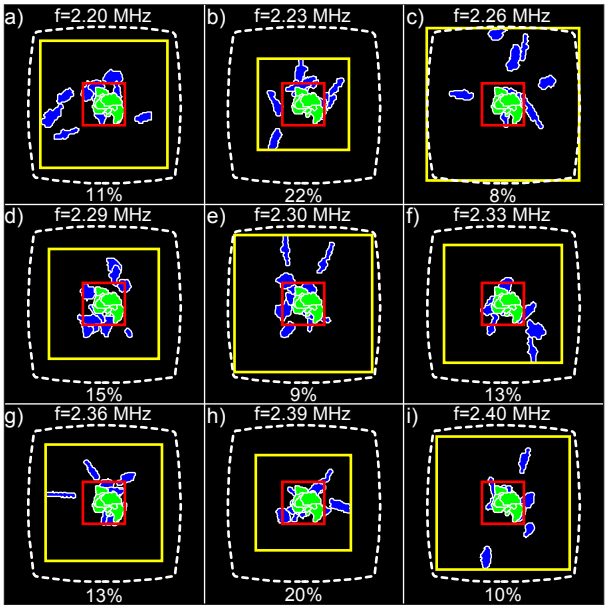

Figure 2: Positioning accuracy as a function of field of view for SF actuation (a-i, blue clusters) and for FM actuation (2.30 MHz ± 100 kHz @ 1 kHz, 50 Vpp, green clusters). For each frequency (a-i), 10 selected wells are superimposed into a single well. The two squares in each frequency (a-i) indicate the minimum well-centered FOV needed to cover all clusters under FM actuation (red) and SF actuation (yellow). Under each well the FOV ratio for FM and SF actuation is given in percent.

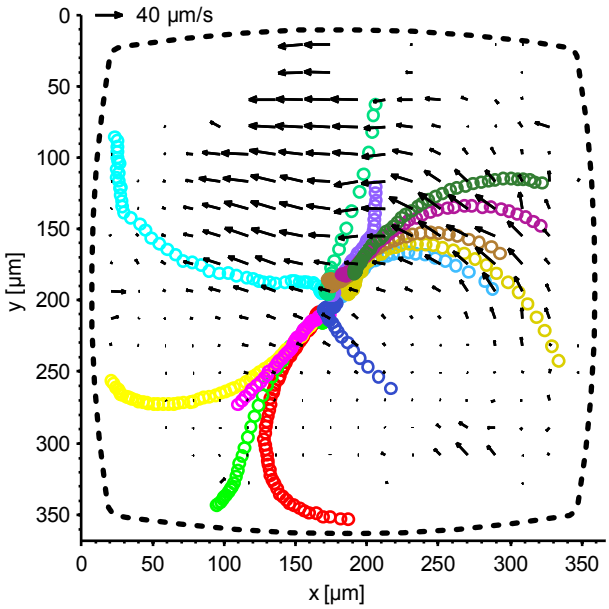

Figure 3: Particle image velocimetry plot (black arrows) in one selected well, showing the initial acoustic streaming during the first second of frequency-modulated actuation. Superimposed onto the PIV plot is the particle-tracking of the 10 µm particles (colored circles) showing the particle paths as they are being trapped by the acoustic radiation force.

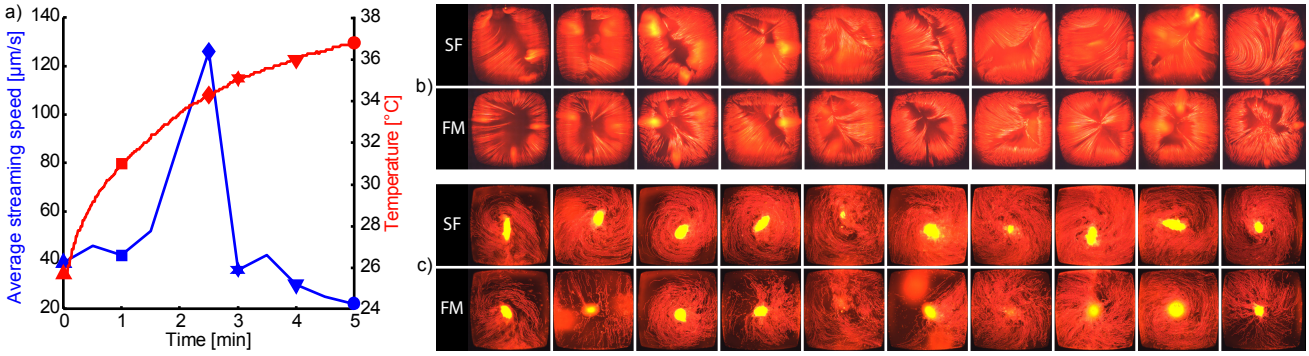

Figure 4: Temperature and time dependence of the acoustic streaming speed under FM actuation (2.36 MHz ± 200 kHz @ 1 kHz, 206 V_{pp}). (a) The PZT piezo surface temperature during the 5 min long experiment is shown in red and the average acoustic streaming speed of 1 µm tracer particles measured by average truncated PIV is shown in blue. (b-c) Overlays showing the acoustic streaming patterns at the entrance (b) and at the bottom (c) for 10 different wells. The yellow areas in (b-c) are clusters of 1 µm particles slowly being aggregated.

CONCLUSION

Our results show that the scanning time of 3D confocal microscopy during high-resolution imaging of the immune synapse (*cf.* Ref. 2) can be decreased almost one order of magnitude with frequency-modulation actuation compared to single-frequency actuation due to significant decrease of the FOV, and that temperature control is important for stabilizing acoustic streaming at high-voltage actuation.

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