CONTROLLING ACOUSTIC STREAMING IN A MULTI-WELL MICROPLATE FOR IMPROVING LIVE CELL ASSAYS

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ABSTRACT

Acoustic streaming in a multi-well microplate is investigated using two different ultrasonic actuation frequency-schemes: Single-frequency and frequency-modulation. The streaming is tracked by the use of 1 μm fluorescent polymer beads and micro-particle image velocimetry. The suspension also contained human B cells for studying the acoustic trapping and aggregation performance simultaneously with the acoustic streaming. Our results show a significant difference in the acoustic streaming between the two ultrasonic actuation schemes. A rotational fluid flow speed decreased a factor of 30 when frequency-modulation was applied compared to single-frequency actuation without apparently interfering with the acoustic cell trapping function.

KEYWORDS: Ultrasound, Acoustic Streaming, Mixing, Multi-Well, Microplate, Particle Image Velocimetry

INTRODUCTION

Acoustic streaming and acoustic particle manipulation are two related phenomena that have been implemented in lab-on-a-chip devices. Applications include fluid mixing and stirring (using acoustic streaming), and trapping, separation, aggregation and positioning of particles and cells (using acoustic particle manipulation). However, although both phenomena are generated by the same technology there are few reports focused on combining the two effects.

This paper presents a novel method for controlling acoustic streaming in a multi-well microplate designed for parallelized ultrasonic aggregation and positioning of individual live cells. By switching between single-frequency and frequency-modulated ultrasonic actuation, we demonstrate that a rotational fluid flow can be turned on and off, respectively, around a cell or cell aggregate trapped at the bottom in one well of the microplate. The work is an extension of our study of the trapping and positioning performance of individual cells in a 100-well microplate with square-shaped wells [1].

In the present study, we characterize the acoustic streaming with micro-particle image velocimetry (μPIV) in a 100-well microplate with concave-shaped wells by the use of a novel ultrasonic broadband transducer (see Fig. 1). Furthermore, we compare the pattern and magnitude of the acoustic streaming for two different frequency-schemes of actuation in order to control the acoustic streaming independently on the acoustic cell trapping function (see Fig. 2). The goal is to use the device in live cell assays for studying cell-cell interactions and cellular response to fluid-based stimuli at the level of single cells, in particular in studies involving the dynamics and heterogeneity of the immune cell function [2].

THEORY

In bulk-acoustic-wave devices based on hard materials (such as silicon and glass), both acoustic streaming and acoustic particle manipulation take advantage of the steep gradients obtained in resonant acoustic fields. As a consequence, the streaming and manipulation effects are strongly frequency-dependent in terms of the patterns and magnitudes of the acoustic force fields acting on the fluid or the particles. In order to circumvent the problem of predicting and controlling the resonant acoustic fields we have previously demonstrated an ultrasonic actuation frequency-scheme based on frequency-modulation [1]. By this technique, it is possible to average out the differences in acoustic force fields in different wells in the multi-well microplate, as well as for different actuation frequencies. As a result, particles or cells are trapped and positioned uniformly in the center of each well.

Here, we utilize a similar ultrasonic actuation scheme for controlling acoustic streaming in the microplate, but now by modulating the frequency within a larger band (>100 kHz). The modulation consists of cycling linear frequency sweeps within this band around a center frequency ~2 MHz and a rate ~1 kHz. The idea of suppressing acoustic streaming is based on averaging the different fluid velocity fields obtained for the different frequencies within the modulation band; for some frequencies the streaming has a clockwise direction and for others a counterclockwise direction.

EXPERIMENTAL

The device, shown in Fig. 1, consists of an open 22×22×0.5 mm^3 glass-silicon microplate with 100 concave-shaped wells, each with size ~0.35×0.35×0.30 mm^3 with a 0.1 mm wall thickness. The cell suspension is loaded from the top and stored within a polydimethylsiloxane (PDMS) frame bonded to the silicon layer using corona discharging. The ultrasound is generated by a novel in-house built broadband-type wedge transducer operated using two different frequency-schemes: Single-frequency actuation using 2.13 MHz and frequency-modulation using 2.13 MHz as a center frequency with linearly sweeps at a rate of 1 kHz width a bandwidth of 200 kHz. The backing layer on the wedge transducer was fabricated using a tungsten-enriched epoxy mixture. The device (microplate with PDMS frame and glued wedge transducer) is securely fixated in a stainless steel holder for rigidity and improved microscope compatibility (Fig. 1a).

In the present work, the utilized sample contains non-adherent human B cells mixed with 1 µm fluorescent polymer beads. Due to the acoustic radiation force's strong size-dependence the small 1 µm beads are much more affected by the acoustic streaming than the ~10× larger B cells making these beads optimal as flow trackers. Fluid velocity vector plots were generated by treating sequential image pairs from high resolution fluorescent microscopy in a free PIV toolbox for MATLAB [3], see Fig. 2.

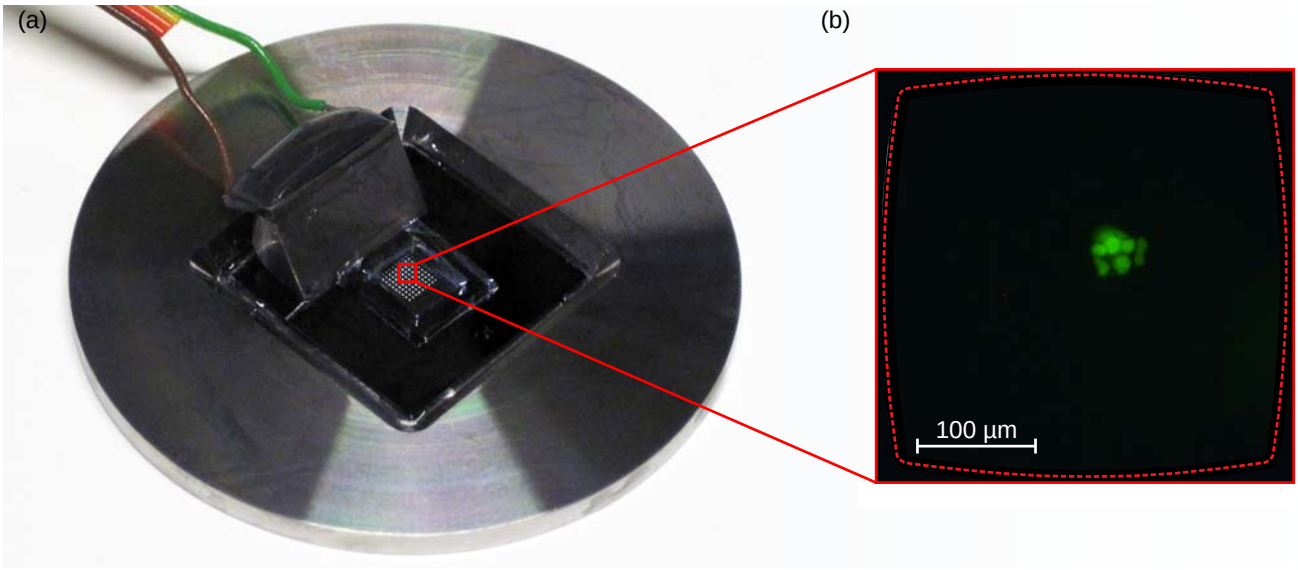

Figure 1: (a) Photograph of the multi-well microplate with a bonded PDMS frame and the broadband transducer attached, altogether mounted in a stainless steel holder. (b) Microscope image showing the aggregation and positioning effect of cells in one of the 100 concave-shaped wells during frequency-modulated ultrasonic actuation.

RESULTS AND DISCUSSION

Generally, acoustic streaming is considered as the limiting factor for the maximum performance of an ultrasonic standing-wave particle manipulation system [4]. For example, it defines the lower limit of particle size that can be manipulated by ultrasound. Our results show that it is possible to design an efficient ultrasonic manipulation system where the acoustic streaming is heavily suppressed or possibly even eliminated. As can be seen in Fig. 2 the acoustic streaming is strongly suppressed by the use of frequency-modulated actuation (Fig. 2a) compared to the use of single-frequency actuation (Fig. 2b). With regard to the rotational fluid flow speed it is almost a factor ~30 lower when using frequency-modulation, however, still maintaining the cell trapping function.

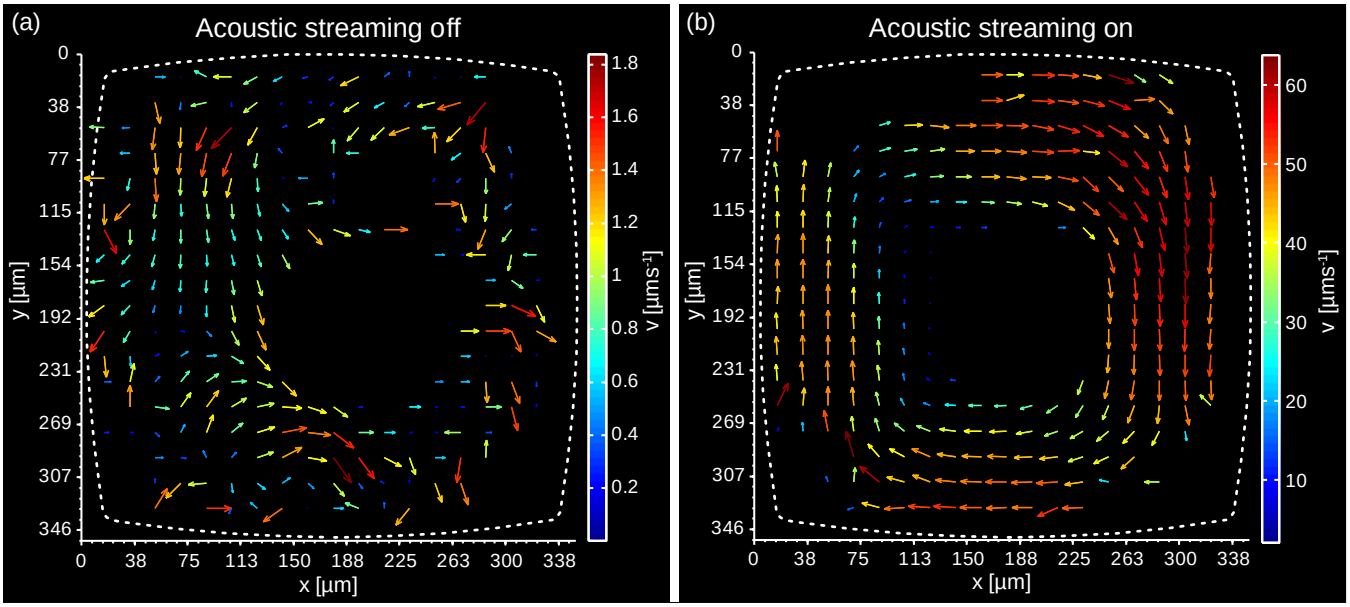

Figure 2: Fluid velocity vector plots generated by micro-particle image velocimetry treatment of microscope images of 1 µm fluorescent polymer beads as flow trackers. (a) The acoustic streaming pattern and magnitude during frequency-modulated ultrasonic actuation, performed by linear frequency sweeps at the rate 1 kHz, center frequency 2.13 MHz, bandwidth 200 kHz, actuation voltage 40 V_{pp}. (b) The corresponding pattern and magnitude during single frequency actuation at 2.13 MHz, same actuation voltage as in (a). The white dashed contours mark the approximate position of the walls in the concave-shaped well.

CONCLUSION

We have shown that acoustic streaming in the wells of a multi-well microplate can be turned on and off by changing the operational mode of a broadband-type ultrasonic transducer. This can be performed without significantly interfering with the main function of the device, which is to aggregate and position individual cells uniformly in the center of each well. Thus, the method provides additional functions (fluid mixing and stirring) to the cell trapping function and can be used for improving live cell assays based on cell-fluid interactions.

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