ULTRASONIC MICRO-CAGES: A NEW APPROACH FOR MANIPULATION AND MONITORING OF INDIVIDUAL CELLS AND FOR FLUID MIXING

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ABSTRACT

Four designs of ultrasonic microcages are presented together with force field simulations and experimental verification. The microcages enable three-dimensional ultrasonic manipulation of individual microparticles combined with on-line monitoring using high-resolution optical microscopy. The microcages can also be employed as acoustic-streaming-based micromixers. We investigate and compare the force field distributions and streaming patterns in the cages, and we demonstrate concentration, aggregation and positioning of individual particles. The cages can be used for, e.g., studies of interactions between single cells and functionalized particles or pairs of cells in contact only with each other.

KEYWORDS: Ultrasonic standing-wave manipulation, cell handling, micromixing.

INTRODUCTION

Ultrasonic standing-wave (USW) manipulation in microfluidic chips is an emerging tool in lab-on-a-chip systems with applications such as washing, separation, positioning, aggregation and assaying of bio-functionalized particles and/or cells. As a contactless manipulation method, USWs has been shown to be gentle to living cells [1]. However, in comparison to alternative methods such as dielectrophoresis or optical tweezers, USW manipulation has until now been used mostly for flow-through and/or batch handling of microparticles. Furthermore, microsystems for ultrasonic manipulation have often relied on single-frequency actuation and aimed for a uniformly distributed manipulation effect throughout the whole system. We have previously shown spatial confinement of USW fields to predetermined longer sections (several mm) of a microfluidic channel [2], and 2D handling using multi-frequency actuation, giving e.g. alignment of particles in the centerline of the channel [3]. In the present paper, we demonstrate and characterize multi-frequency-actuated discrete manipulation microelements for 3D handling of individual cells – ultrasonic microcages. An ultrasonic microcage is a small section of the channel (typical volume 1-10 pL) geometrically designed to allow for localized and spatially confined ultrasonic manipulation functions such as focusing, retention and levitation of individual particles. Combining these three functions yields 3D contactless caging of microparticles. We also demonstrate pL-volume fluid micromixing by inducing acoustic streaming in the cage.

EXPERIMENTS AND SIMULATIONS

We use a fully transparent glass-silicon-glass chip with exchangeable external transducers with a coupling wedge [4], enabling any kind of high-resolution optical microscopy. Figs. 1a-d show the geometries and force field simulations of four cage designs. The simulations are cut-outs from an entire-chip 2D FEM simulation carried out in Comsol Multiphysics with postprocessing in MATLAB as described in

[4]. The force fields were verified experimentally by seeding the cage with non-fluorescent 5-µm polyamide beads and turning on the actuation voltage. Together with these were mixed 1-µm fluorescent polystyrene beads for streaming visualization. The ability of the cages to handle individual microparticles was investigated using fluorescent 10-µm polystyrene beads, with acoustic properties similar to cells those of cells.

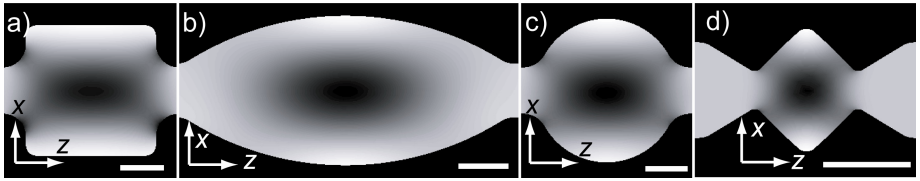

Figure 1: Four microcage designs. The grayscale plots show force potentials, with black as the lowest value: a) "boxtrap" at 2.51 MHz, b) "flat confocal trap" at 2.49 MHz, c) "round confocal trap" at 2.48 MHz and d) "rotated boxtrap" at 6.88 MHz. The scalebars are all 100 µm.

RESULTS AND DISCUSSION

Figs. 1a-d show plots of simulated force potentials in the four microcages. We note that in all four cases, simulations indicate well-confined force fields with both retentive (*z*) and focusing (*x*) components. When combined with USW levitation as described in Refs. 2 and 4, this enables 3D caging of particles. Fig. 2 shows experimental verification of the caging performance using a high concentration of 5-µm beads (dark) for force visualization and 1-µm beads (light) for streaming visualization. We note that the *x* component of the force is stronger than the *z* component, as predicted by the simulations. The discrepancy between the 2D-simulated frequencies and the true resonances in the 3D real structure is discussed, e.g., in Ref. 4.

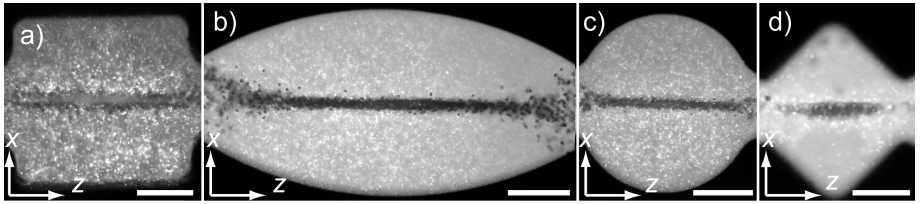

Figure 2: Experimental verification of force fields in the x-z *plane. The scalebars are all 100 µm. The frequencies used are a) 2.53 MHz, b) 2.55 MHz, c) 6.75 MHz d) 2.55 MHz. The levitation frequency was 6.92 MHz.*

In Fig. 3, 3D microcaging of individual 10-µm beads is shown. The beads are kept levitated, focused and can be retained against a medium flow. This experiment is a proof-of-principle demonstration of, e.g., monitoring cell-particle or cell-cell interactions on an individual level. Note that the magnification and resolution is kept low enough to show the entire cage, but can be greatly increased. The forces in the "rotated boxtrap" were too small to cage single particles at our actuation voltages (<10 V peak-to-peak) and flow rate (>0.1 µL/min).

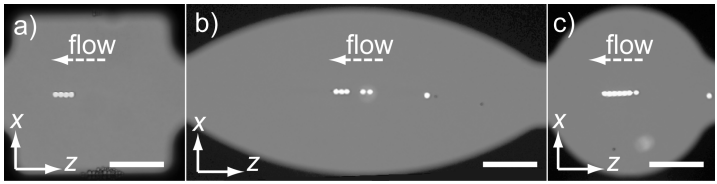

Figure 3: 3D Microcaging of 10-µm PS beads, acoustically similar to biological cells. The frequencies are the same as in Fig. 2. The flow rates are all >0.1 µL/min.

Fig. 4 shows the ultrasound-induced streaming patterns with (a) and without (b) a simultaneously trapped aggregate of 5-µm beads in the "boxtrap". Here, 1-µm beads were used for streaming visualization, as these are small enough to give a viscous drag force dominating over the ultrasonic radiation force in this arrangement at 10 V_{pp} actuation. We note that the streaming pattern in Fig. 4b is asymmetric in *x*, which can be used for mixing of parallel laminar flows. Note also that the streaming pattern in an *x*-*z* plane depends on *y*, so that refocusing would reveal a different pattern. The streaming pattern in the two rounded cages is more symmetrical, and also slower for a given actuation voltage (slowest in the "flat confocal"), while the rotated boxtrap produces patterns similar to that in Fig. 4 but of higher symmetry.

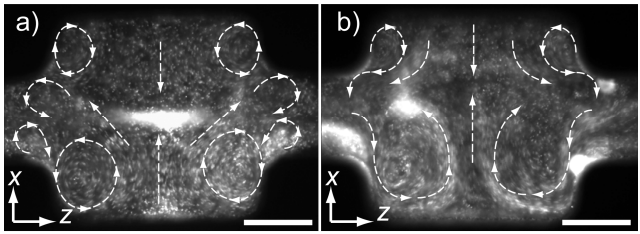

Figure 4: Ultrasound-induced streaming with (a) and without (b) a trapped bead aggregate. Arrows indicate main streaming directions in the current focal plane.

CONCLUSIONS

An ultrasonic microcage is a flexible acoustofluidic concept that can be used for 3D caging of one to hundreds of microparticles as well as micromixing with or without simultaneous caging. Future applications include monitoring of behavior of cell-cell or cell-bead interactions without wall contact, and the possibility to serialize USW manipulation functions in a microfluidic chip.

REFERENCES

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