DYNAMIC FLOW CYTOMETRY IN AN ACOUSTO-OPTIC MICROFLUIDIC CHIP

J. Svennebring, O. Manneberg, H. Hertz and M. Wiklund
Dept. of Applied Physics, Royal Institute of Technology, SWEDEN

ABSTRACT

A microfluidic ultrasonic manipulation system is used for controlled selection, retention and optical characterization of individual particles or cells. The system is based on a chip-integrated focused ultrasonic resonator that is actuated on either one or both of two different frequencies. Particles are pre-aligned, and either bypassed through or injected and retained in the focused resonator, depending on the actuation mode. Dynamic flow cytometry is demonstrated by real-time optical monitoring of controlled numbers of retained and positioned cells or beads.

KEYWORDS: Ultrasonic manipulation, cell handling, focused resonator, optical microscopy

INTRODUCTION

Ultrasonic standing wave (USW) technology has potential to become a standard method for gentle and contactless cell handling in microfluidic chips. Cells are trapped in the ultrasonic pressure nodal planes by the acoustic radiation force, which may be designed to perform different manipulation functions. In applications aiming for cell aggregation, retention and on-chip cultivation, a stable and fully biocompatible USW system is required to retain all cell-biological functions. We have previously presented a temperature-regulated microfluidic USW manipulation platform for long-term cell handling applications [1], and a particular chip based on an integrated expansion chamber used for cell concentration and positioning [2]. The latter system was designed for operation on a single frequency. In the present paper, we investigate and demonstrate dual-frequency operation of the chip presented in Ref. 2. Additional functions obtained are controlled selection of the injected particle number and bypassing of unwanted or excess particles.

EXPERIMENTAL ARRANGEMENT

Our system, depicted in Fig. 1, consists of a glass-silicon-glass microfluidic chip and two oblique transducers with refractive elements [3], operating at 6.9 and/or 4.6 MHz. The main microchannel is 0.33 mm wide and has a 4.9 mm wide expansion chamber used as a focused ultrasonic resonator in the center of the chip. Three-port inlets and outlets allow for, e.g., hydrodynamic focusing. The system is compatible with any kind of high-resolution optical microscopy, as a result of the thin bottom glass layer (200 μm) and the full chip transparency above and below the channel.

RESULTS AND DISCUSSION

In Fig. 2 the different pre-alignment functions are demonstrated with a high particle concentration during fluid flow. When operating the chip at 4.6 MHz, particles

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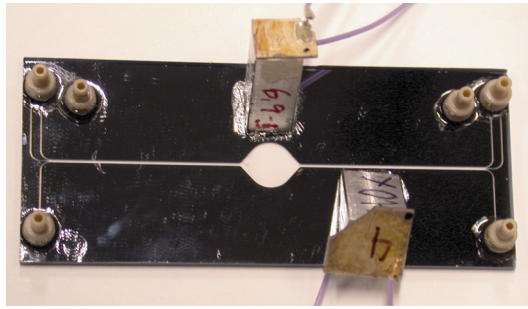

Figure 1. Photograph of the chip with two mounted transducers operating at 4.6 MHz (lower right) and 6.9 MHz (upper left).

are aligned in two parallel bands in the inlet channel, corresponding to the pressure nodes in a full-wavelength resonator. In the expansion chamber, the particles are bypassed through peripheral streamlines and ejected out from the chip (Fig. 2a). By switching actuation frequency to 6.9 MHz, particles are pre-aligned in three parallel bands in the inlet channel (Fig. 2b), corresponding to the pressure nodes in a 3/2-wavelength resonator. The central band can be used for injection of particles to the center of the expansion chamber. By adjusting the time period between on/off mode for the transducers, the number of injected particles or cells can be controlled.

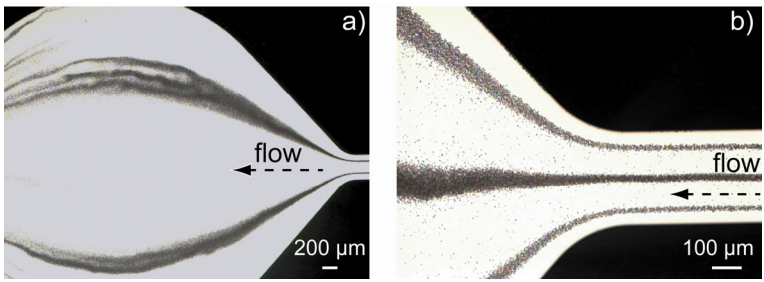

Figure 2. Pre-alignment functions visualized with 5 µm polyamide beads.
a). Sample bypass at 4.6 MHz (without any central node).
b). Sample injection at 6.9 MHz (including a central node).

In Fig. 3 the retention and positioning function of the focused resonator is demonstrated. In Fig. 3a hydrodynamic focusing is combined with 6.9-MHz actuation for injecting particles only to the center of the expantion chamber, thus to eliminate the two outer bands seen in Fig. 2b. When the chip is operated at both frequencies simultaneously, retention and positioning of the injected particles occur at the center of the expension chamber. Furthermore, since the 4.6-MHz resonance dominates over the 6.9-MHz resonance in the inlet channel, excess particles are bypassed around the retained aggregate (cf. Fig. 3b). Figs. 3c-d show the structure of a retained particle aggregate at higher magnification. As seen in Fig. 3d it is possible to

arrange particles or cells in a horizontal monolayer, which makes the system suitable for high-resolution optical microscopy.

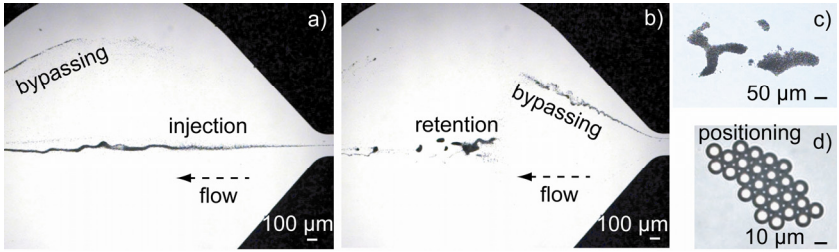

Figure 3. Sample retention and bypassing at 6.9 and 4.6 MHz with 5 µm polyamide beads (a-c) and 10 µm polystyrene beads (d).
a) Single-frequency actuation at 6.9 MHz. Sample injection combined with a hydrodynamic focusing flow for single-node pre-alignment (i.e., without the peripheral nodes seen in Fig. 2b).
b) Dual frequency actuation at 6.9 and 4.6 MHz. When the second transducer is activated (at 4.6 MHz) the retention forces in the middle of the expansion chamber becomes stronger and excess sample is bypassing the trapping region.
c-d) Magnified images of a trapped and retained bead aggregates during flow.

CONCLUSION

The experiment shown in Fig. 3 is a proof-of-concept demonstration of dynamic flow cytometry. Different cellular parameters may be studied in real-time by, e.g., fluorescent probes in a perfusion flow. Examples of future planned applications include ultrasensitive bead-based bioanalytics [4] and dynamic monitoring of immunological synapses for studying immune cell interactions [5].

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