A continuous on-chip droplet washing platform with high bead recovery by acoustofluidics

Zhenhua Liu, Anna Fornell, and Maria Tenje

Dept. Engineering Sciences, Science for Life Laboratory, Uppsala University, Uppsala, Sweden
E-mail: zhenhua.liu@angstrom.uu.se, URL: https://www.teknik.uu.se/mikrosystemteknik/embla/

Introduction

Acoustofluidics is a promising technology for manipulation of fluids and particles in microchannels [1], and the technology has the ability to sort beads and cells in continuous flow with very high efficiency [2]. Recently acoustofluidics has also been applied in segmental flow for positioning beads inside droplets [3]. Compared with single-phase systems, droplet microfluidics has the advantages of faster reactions, lower cross-contamination and higher throughput. Moreover, the small size of the droplets makes them ideal as cultivation and reaction vials for single cell analysis [4]. However, as the droplets are so small one challenge is to wash the droplets before image analysis. P. Mary *et al.* developed a microfluidic platform for droplet wash, which is based on electrocoalescence and droplet break-ups with equal volume [5]. The background noise was decreased significantly, however the recovery of the encapsulated cells was low. Alternative solutions have been presented by H. Lee *et al.* [6] and S.R. Doonan *et al.* [7] but as the bead recovery is controlled via magnetophoresis, the technology is only applicable to magnetic samples. Here we present a droplet microfluidic platform that enables background dilution with high bead recovery in a label-free manner using acoustophoresis.

Methods

Figure 1 shows the design of the microfluidic chip, which contains a droplet generation channel, a bead focusing channel, two droplet splitting channels, a pico-injection channel and a serpentine channel for mixing. The microfluidic system was fabricated on a silicon wafer by standard photolithography and dry etching. The channels were closed by anodic bonding of a glass wafer. Liquid alloy was injected into the electrode channels for the pico-injector. A piezoelectric element was glued on the silicon side of the chip, and an electrical signal was applied over the piezoelectric element (23 V_{pp} and 1.81 MHz of sine waves) to generate the first harmonic resonance in the channel. The aqueous phase containing 10 µm polystyrene beads was injected into the central channel and mineral oil with 2% surfactant (Span 80) was injected into the two side channels. A fluorescent solution was used as the dispersed phase for the dilution quantification. The concentration of the fluorescent solution was 1 mg·mL^{-1}. Fresh water at different flow rates were injected via the pico-injector by electrocoalescence [8]. The electrical signal applied on the pico-injector electrodes was 180 V_{pp}, 5 kHz of square waves. The volume of the droplets was measured from acquired microscope images by a method presented by Musterd *et al.* [9], and the concentration in the droplets after pico-injection was calculated by the equation: $C_2 = C_1V_1/V_2$.

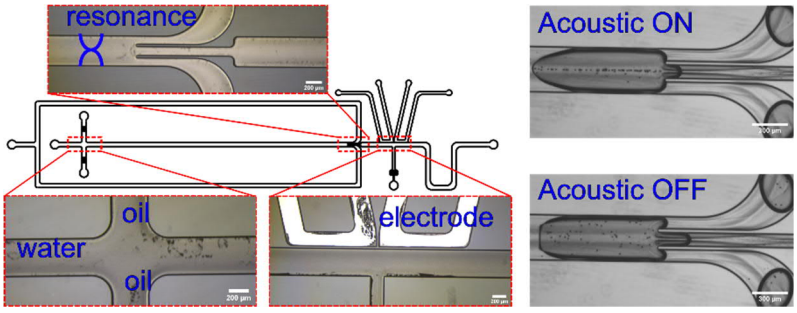

Figure 1: The design of the chip for droplet generation and washing. Droplets with high background noise are generated via flow focusing at the channel junction. A half wavelength acoustic standing wave field is generated in the channel which focuses the beads along the central line of the droplets. The droplets are split into three daughter droplets at the trifurcation. After droplet splitting, the droplets are refilled with fresh fluid to the original volume by the pico-injector.

Results

At actuation of the transducer the beads in the droplets were focused to the center of the channel by the acoustic radiation force (Figure 1). Next, the original droplet (with high fluorescent intensity) was split in the trifurcation and the beads were directed into the central channel. In this experiment the flow rate of each inlet to generate droplets was 2 µL·min^{-1} and the pulling flow rate to split the droplets was 3 µL·min^{-1}. The side daughter droplets without beads flowed into the side channels and the central daughter droplet containing beads

was refilled to the same volume as the original droplets by the pico-injector. Figure 2 (a) shows the volume of the droplets before and after the splitting, and after pico-injection. With the system it was possible to obtain up to 35% dilution.

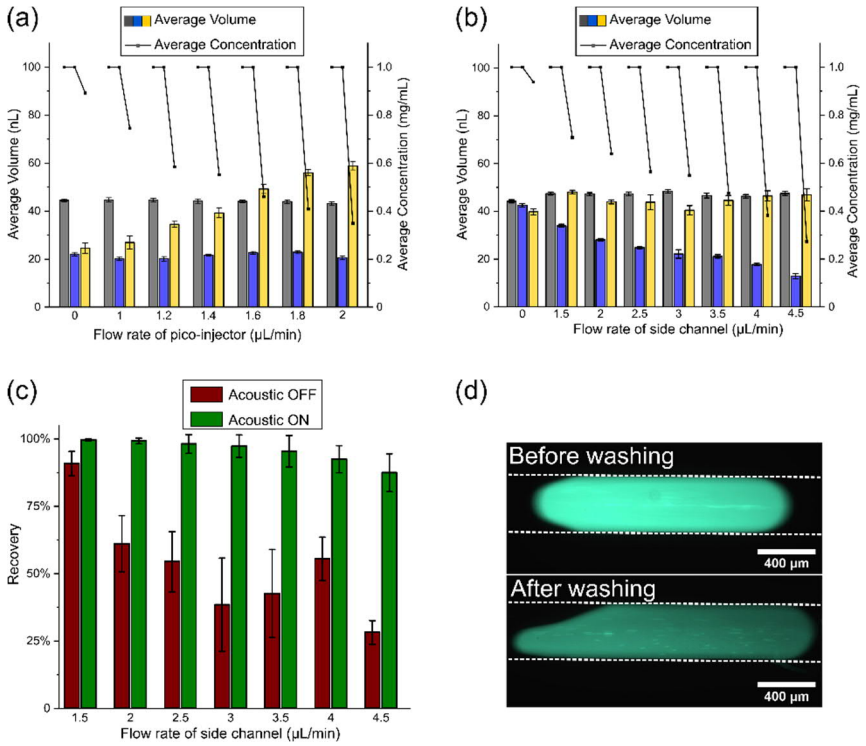

Figure 2: (a) Characterization of the pico-injection: the grey bar presents the average volume of the original droplets, the blue bar presents the average volume of the droplets after splitting, the yellow bar presents the average volume of the droplets after pico-injection. The line presents the calculated concentration in the droplets; (b) the volume of the droplets and the concentration of the background noise in droplets as the droplets are split and pico-injected; (d) the bead recovery at different splitting flow rates; (d) fluorescent microscope images of droplet washing.

An experiment to study the droplet splitting was performed (Figure 2 (b)). In this experiment, the flow rates of the droplet generation were constant (2 $\mu L \cdot min^{-1}$) and the flow rate of side channels was varied to obtain different splitting ratios. Figure 2 (c) shows the bead recovery with the acoustics and without the acoustics. As seen in the figure a high bead recovery was achieved when the acoustics was on, while much lower recovery was obtained when the acoustics was off. When the flow rate of side channel was 4.5 $\mu L \cdot min^{-1}$, the dilution reached 27% and the bead recovery was 87%. Figure 2 (d) shows the high concentration of fluorescent solution before pico-injection, and the low concentration of fluorescent solution after pico-injection with a maintained high bead number. The results showed a significant decrease in the background noise and a high bead recovery, which together means high-efficient droplet washing.

Conclusion

We have designed and fabricated an acoustofluidic platform to wash droplets continuously on-chip. The washing ability and the bead recovery were characterized. We demonstrated a bead recovery of 87% when the droplet content was diluted to 27% of its original concentration.

References

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