HUNDRED-FOLD VOLUME CONCENTRATION OF CELLS AND PARTICLES USING CONTINUOUS FLOW MULTISTAGE ACOUSTOPHORESIS

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ABSTRACT
We present a microfluidic chip capable of two hundred-fold volume concentration of cells or particles in continuous flow with high throughput and minimal losses. Cells or particles are focused in the node of an ultrasound standing wave in two dimensions, enabling dramatically higher concentration than obtained in standard one dimension acoustophoretic focusing. The system is developed for concentrating rare cell events, such as circulating tumor cells isolated from blood samples after a rare event separation step, or for chip integrated cell sorting devices which commonly deliver target populations in a large void volume.

KEYWORDS
Ultrasound, concentration, 2-D focusing, acoustophoresis

INTRODUCTION
Concentrating cell samples is a critical step when handling rare events or dilute samples, to enable cell culturing or cell-based assays. Detection and identification of microorganisms or rare cells associated with various diseases, such as circulating tumor cells, has an important diagnostic and prognostic value in disease treatment. Assessment of water or food quality, can also pose challenges in terms of concentration.

Traditional centrifugation as a means of sample concentration suffers from drawbacks in dealing with rare cell events and in combination with lab-on-a-chip applications, where highly diluted sample or small volumes need to be handled. Microfluidic systems make it possible to handle samples in a continuous mode, without restricting sample volumes. They also offer the possibility to handle samples in a closed system, allowing concentration of cells for transplantation or cell culture. Previous reports on cell concentrating microsystems have mostly been done in batch systems, using traps that have a limited capacity [1].

This paper presents a continuous flow, high throughput acoustophoresis device capable of concentrating cells and particles up to two hundred times with minimal losses. Cells and particles are concentrated in two dimensions by ultrasound standing wave forces, according to their intrinsic properties size, density and compressibility [2]. The method is label free and has been shown to be gentle to the cells [3]. Furthermore, the continuous flow mode makes it possible to achieve concentration factors of several orders of magnitude and the method can be used independently of suspending fluid.

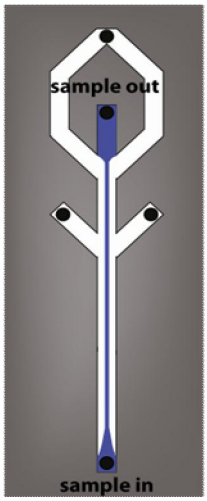

Figure 1. Chip design.

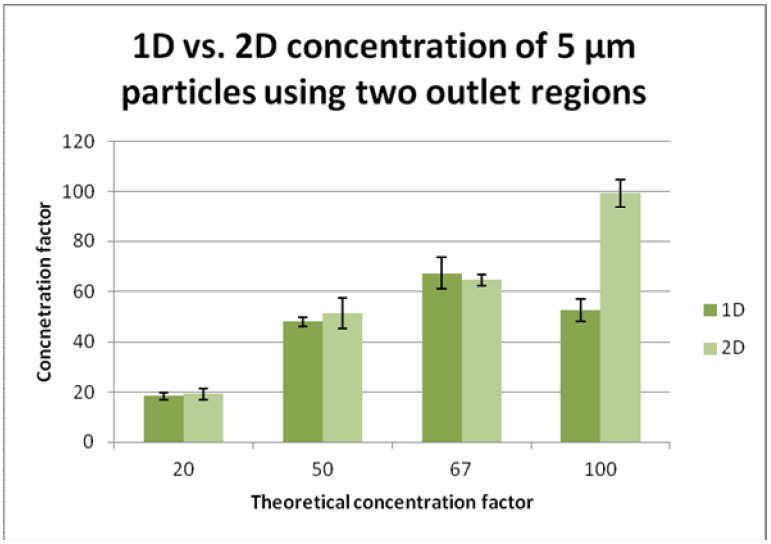

Figure 2. 1D vs. 2D concentration of 5 µm particles.The theoretical concentration assuming full recovery of the particles are indicated at the x-axis. (n=3). Inflow rate 100 µL/min.

EXPERIMENT
The presented system, shown in Figure 1, was fabricated in silicon by anisotropic wet etching and sealed with a glass lid. The chip was actuated using one or two ultrasonic transducers resonant at 2 or 5MHz, corresponding to half a wavelength ultrasound standing wave along the width or height or the channel, respectively. Cells or particles were focused in the microchannel centre and clarified fluid was withdrawn from the side outlets along the acoustophoresis channel and the sample was extracted through the center outlet at a vastly reduced volume flow rate.

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RESULTS AND DISCUSSION

A comparison of the system performance using either one or two outlet regions together with one-dimensional or two-dimensional acoustic focusing was done. 5 µm diameter polystyrene particles were used as a model system showing that two outlet regions outperformed chips with one outlet region (data not shown) and that two-dimensional focusing outperformed one-dimensional focusing (Figure 2).

Using only one-dimensional focusing a considerable amount of particles or cells was lost into the side outlets (Figure 3a). However, when employing two-dimensional focusing significantly higher concentration factors with minimal losses were obtained, (Figure 3b). The reason for the improved performance is based on two factors, where 1) the negative influence of an irregular acoustic resonance pattern in the outlet region is minimized and 2) the influence of flow disturbances is reduced, using multiple acoustic focusing steps. When trying to achieve high concentration factors of particles without losses, in chips with one outlet region, the critical flow fraction that must contain all the particles is only a few micrometers wide. Even minor fluctuations in the outlet flow balance can easily disturb the minute central flow fraction to the side outlets. Using several outlet regions, on the other hand, will increase the width of the critical flow fraction. Thereby, only modest concentration factors will be achieved in each step but will in total multiply into higher concentration factors.

a) **b)**

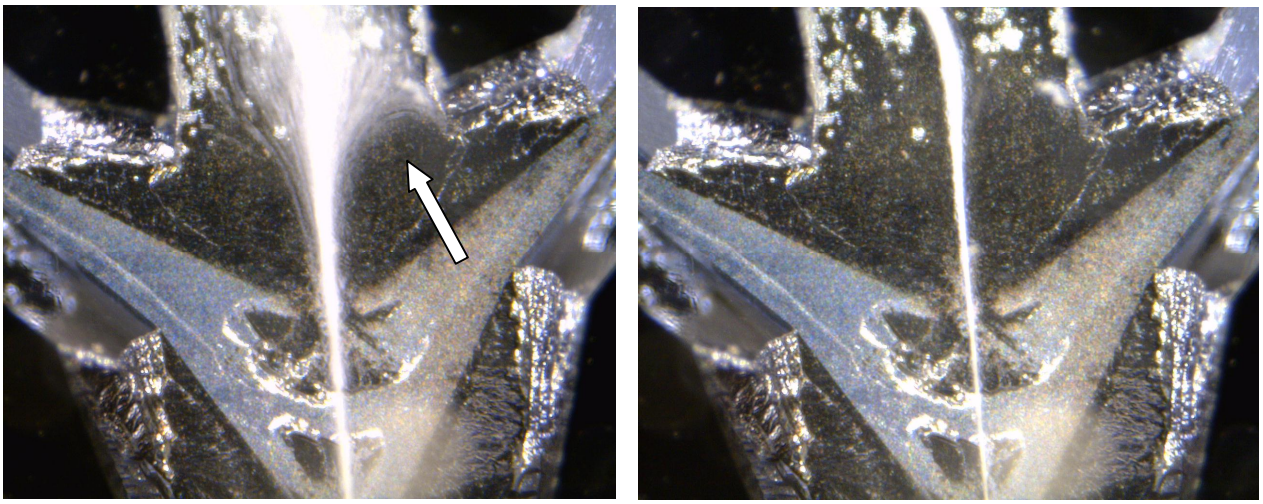


Figure 3. a) Particles focused in one dimension entering the last outlet region with at total flow rate of 100 µL/min where 2 µL/min is taken out in the centre outlet. The white arrow indicates particles escaping to the side outlet, deflected by the undefined acoustic field present in the outlet region. b) Particles focused in two dimensions. Particles escape is no longer present.

In the outlet regions where the channel widens and the width no longer matches the half wavelength ultrasound standing wave, an undefined acoustic field of higher resonance modes is present with nodes no longer located in the channel centre. When the particles enter these regions they can be deflected from their original trajectory and fail to enter the centre outlet. The retention time of most of the particles when passing the outlet regions is sufficiently short not to be affected by this irregular ultrasonic field. However, the particles situated in the top and bottom of the channel, where the flow rate approaches zero, will spend sufficient time in the outlet regions to be deflected from their original trajectory. As the minute flow fraction taken out in centre outlet does not allow for deflections from the original trajectory of more than a few micrometers, this easily leads to particles escaping through the side outlets instead. However, when the particles are focused in two dimensions, there will be no particles situated in the slowest moving flow regime and thus very few particles can escape to the side outlets (Figure 4).

a) **b)**

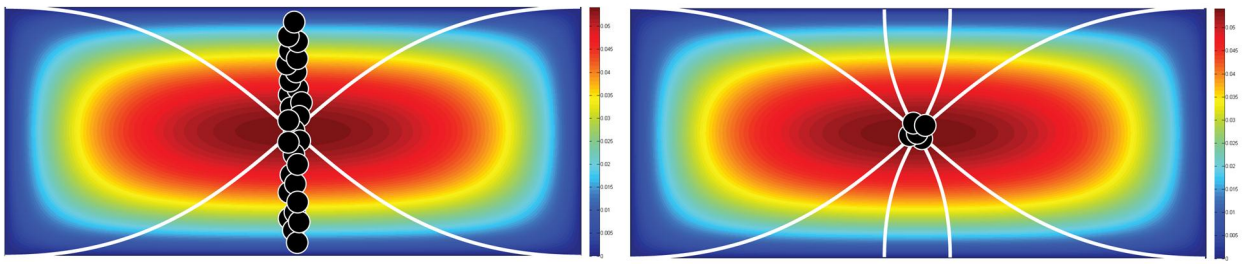
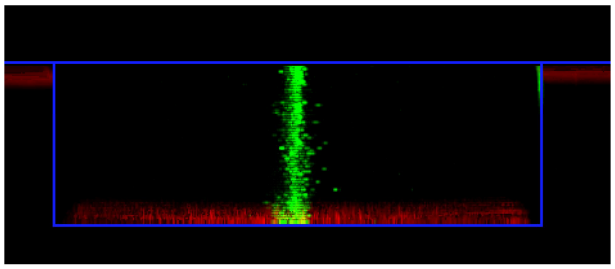


Figure 4. Channel cross-section showing the flow velocity profile,(where red is highest velocity and blue is lowest) with; a) one-dimensionally focused particles and b) two-dimensionally focused particles.

a)



b)

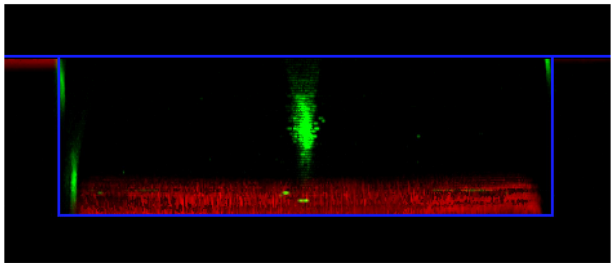


Figure 5. Confocal images. a) Particles focused (green) in one dimension vertically. The channel bottom is viewed in red. b) Particles focused in two dimensions.

Confocal microscopy was used to validate the two-dimensional focusing (Figure 5). In order to allow imaging of the entire channel cross section the magnification was limited to x20. The magnification limit resulted in a confocal depth of about 20 µm, hence causing features in the images to appear enlarged mostly in the height direction of the channel. This resulted in the two-dimensionally focused particle cluster to appear more elongated than round (Figure 5b).

Red blood cells were used as model cells and were concentrated 145.8 ± 5 times with a recovery of 97.2 ± 3.3 at a sample input flow rate of 150 µL/min (Figure 6, blue bars). Since the system is intended to be used for rare cell concentration, circulating tumor cells are of interest. DU145 cells, prostate cancer cells, were used as a model system and we demonstrate a concentration factor of 195.7 ± 36.2 times with a recovery of 97.9 ± 18.1 at sample input flow rate of 200 µL/min, with the potential of reaching even higher numbers (Figure 6, red bars).

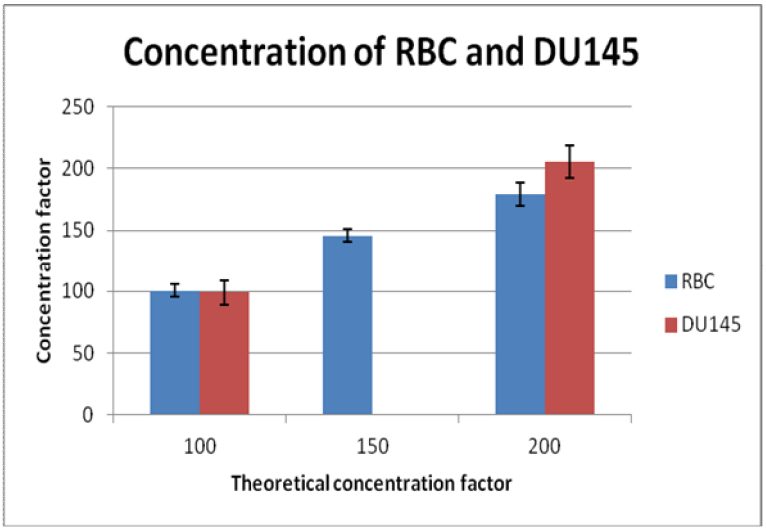


Figure 6. RBC and DU145 concentration usign 2-D focusing. (n=3) Inflow rate of 100, 150 and 200 µL/min, respectively.

CONCLUSIONS

In conclusion, acoustophoresis has been used to concentrate particles and cells up to 200 times in continuous flow with up to 200 µL/min sample inlet flow rate, using two-dimensional acoustic focusing. It has been shown that two-dimensional focusing outperforms one-dimensional focusing and that the strategy of implementing several acoustic focusing regions and sequential outlet regions where excess fluid can be removed opened the route to concentration factors beyond 100 times. The system will fill a need in rare cell event handling, and integration with separation and detection devices is possible.

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