CONTINUOUS RARE CELL EXTRACTION USING SELF-RELEASING VORTEX IN AN INERTIAL MICROFLUIDIC DEVICE

Xiao Wang, Jian Zhou and Ian Papautsky
BioMicroSystems Lab, School of Electronic and Computing Systems
University of Cincinnati, Cincinnati, OH USA

ABSTRACT

This work presents an inertial microfluidic device for size-dependent continuous extraction of rare cells. The design takes advantage of the inertial microfluidics concept to order cells in a high-aspect ratio rectangular channel followed by chambers for capture of large cells from cell mixture. Captured cells exit from the side-outlet located at the corner of the chamber which enables continuous releasing. Continuous particle and cell extraction were showed experimentally. We expect this inertial microfluidic device could achieve continuous cell extraction with high selectivity, high throughput and without limitation on capture capacity.

KEYWORDS: Inertial microfluidics, continuous rare cell extraction, self-releasing vortex

INTRODUCTION

Cell separation, such as extraction of leukocytes or circulating tumor cells (CTCs) from blood, is a critical sample preparation step in cell biology and clinical diagnostics [1]. A variety of techniques for cell separation have been developed based on microfluidics, which can be divided into active or passive techniques. Active techniques utilize external force, such as magnetic force [2], to separate cells providing high separation efficiency but requiring complicated external setups. Passive techniques depend on the intrinsic properties such as hydrodynamic force [3] that are much simpler than active techniques.

Among passive techniques, inertial microfluidics has been developing rapidly in recent years. In this approach, inertial force is exploited for separating cells or microparticles purely based on their size. Inertial microfluidic devices with various channel geometries, including spiral [4], serpentine [5], and straight channels, have been demonstrated for particle/cell ordering and size-based sorting. The spiral inertial microfluidic devices reported by our group [4] offer high throughput and separation efficiency. But restrictions on selectivity and sensitivity limit their use for rare cell extraction. DiCarlo and colleagues [6] recently reported a rare cell extraction approach based on vortex trapping that is highly selective. However, the system faces a number of shortcomings stemming from the need to switch flows to release trapped cells, which increases operation complexity. The extraction capacity and efficiency are also both limited by the vortex dimensions. Herein, we present a simple inertial microfluidic device using a self-releasing vortex that can achieve continuous rare-cell extraction with high selectivity and no limitation on capture capacity.

DESIGN PRINCIPLE

Our system utilizes the inertial microfluidics concept to order cells in a rectangular microchannel, followed by a chamber to trap large cells. In the channel, cells experience shear lift force and wall lift force which balance each other so that the cells are focused into two streams. As cells arrive at the chamber region, the absence of the channel wall disrupts the force balance. Larger cells are pushed into the chamber due to the larger shear lift force on them while small cells pass through the chamber. Instead of intact chamber structure, side outlets are added for continuous cell release from vortices (Fig.1a). The concept of a self-releasing vortex was predicted by our CFD-ACE+ numerical model (Fig.1b). The simulation indicates that side-outlet at optimized position does not inhibit vortex formation. The vortex, while forming in the chamber, was released simultaneously from the side-outlets forming dynamic steady

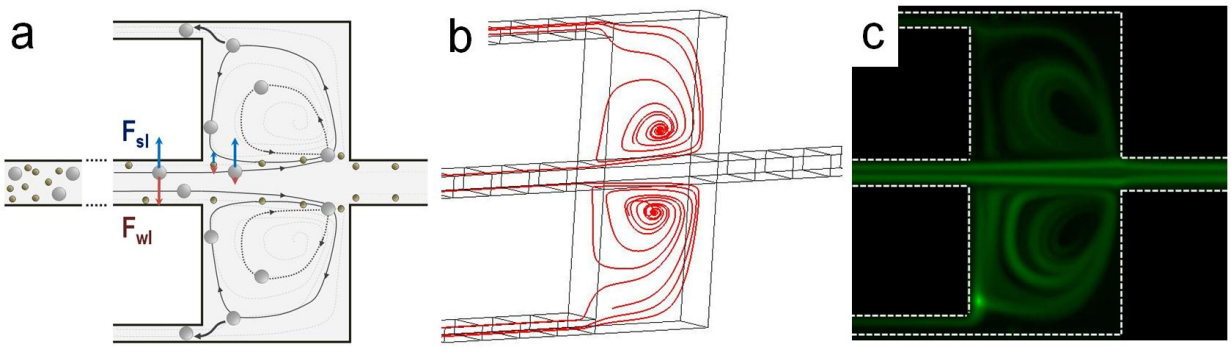

Figure 1: *Schematic of device principle (a). Balanced by the shear lift force and wall lift force, particle of the same size focuses at an equilibrium position. The absence of the channel wall at the chamber region disturbs the force balance leading to the trapping of the particle into the vortex. The side outlet perturbs the stability of vortex resulting in continuous releasing of particles. (b) ESI CFD-ACE+ simulation result demonstrating vortex formation and draining. (c) Proof of concept with fluorescent particle visualization.*

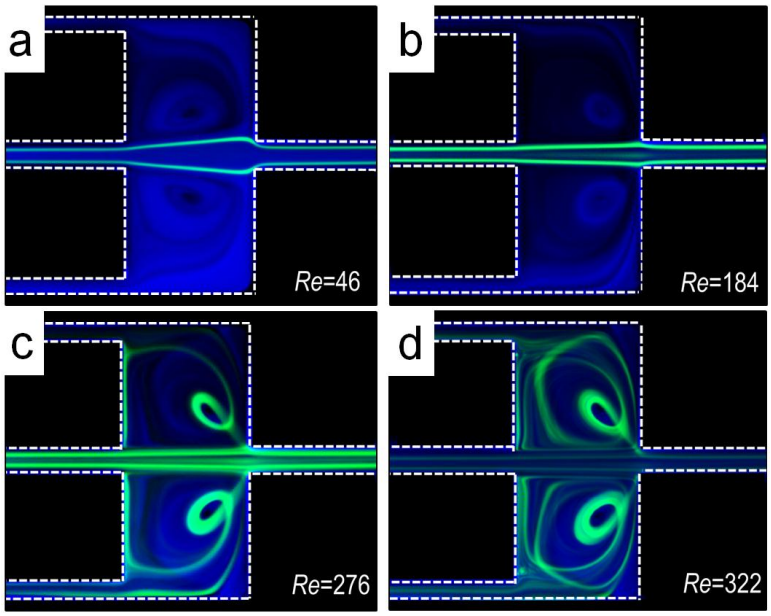

***Figure 2**. Superimposed fluorescent images demonstrating vortex formation with 0.19 µm tracer particles (DAPI) and the behavior of 7.32 µm particles (FITC) at increasing Reynolds number.*

state. Experiments with 20 µm fluorescent particles confirm the mechanism by trajectory visualization (Fig.1c).

EXPERIMENTAL

We used basic soft-lithography to fabricate the polydimethylsiloxane (PDMS) - based inertial microfluidic devices. In all the experiments, we injected fluorescent particles (Polyscience inc.) into the devices using a syringe pump (NE-1000, New Era Pump Systems, Inc.). We captured fluorescent images or bright-field images with an inverted epi-fluorescence microscope (Olympus IX71) equipped with a 12-bit CCD camera (Retiga EXi, QImaging). We added pseudo-color to the fluorescent images and superimposed the images in ImageJ.

RESULTS AND DISCUSSION

Continuous particle and cell extraction were demonstrated experimentally. Evolution of the vortex was visualized by fluorescent tracers (0.19 µm diameter, DAPI) as shown in Fig.2. Vortex dimensions gradually increased and the vortex eye shifted right at increasing Reynolds number (*Re*). Superimposed fluorescent images of 7.32 µm particles illustrate motion induced by the vortex. At low *Re* (Fig.2a,b), particles passed through the chamber region directly and there were no trapping happened in chamber region. As *Re* increased (Fig.2c,d), particles experienced larger lift force leading to the trapping inside the vortex. This trapping behavior is similar to the behavior reported by DiCarlo and colleagues [6]. In addition to the expansion for particle-trapping, two side-outlets were added to enable

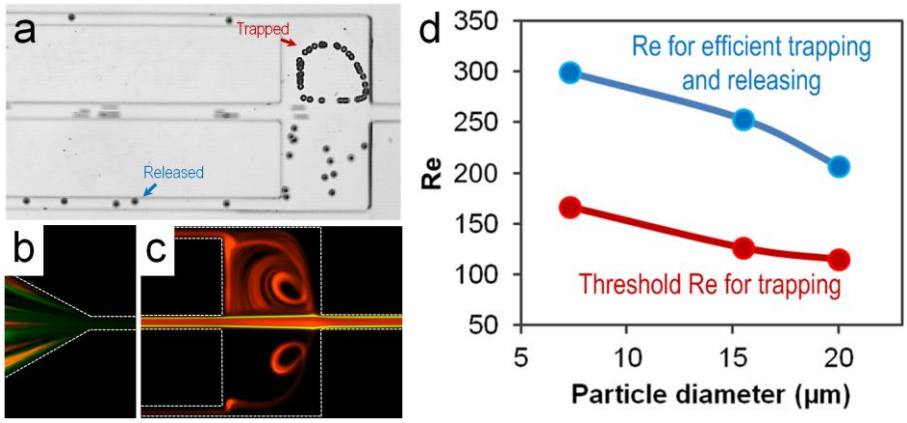

***Figure 3:** (a) stacked images exhibiting the trapping and releasing of 20 µm particles. Fluorescent images at inlet (b) and chamber (c) demonstrated inertial focusing and continuous extraction of 15.5 µm particles (TRITC) from 7.32 µm particles (FITC). (d) The relation between particle size, threshold trapping Re and Re for efficient extraction.*

self-release of trapped particles to eliminate the trapping capability limitations. The clear trajectories in chambers illustrate that particles were released from the side-outlets after travelling within the vortices.

Simultaneous trapping and releasing of 20 μm particles were observed in bright field (Fig.3), clearly indicating the behavior of individual particles. Particle extraction began at a *threshold Re* that decreased from 170 to 100 as particle diameter increases from 7.32 to 20 μm. The *Re* for observation of steady fluorescent trajectory was defined as *Re* for efficient extraction which follows a similar trend as threshold *Re* (Fig.3e). Particle concentration for these quantitative experiments was kept constant at 2×10^4/mL. Continuous extraction of larger particles from a mixture can be achieved under proper *Re* (Fig.3c-d). The 15.5 μm particles were continuously trapped and extracted from the side outlets, while 7.32 μm particles pass through. We also expect a 1:100,000 selectivity and 1 cell/mL sensitivity based on our previous work of intact chamber geometry which utilizes the same trapping mechanism.

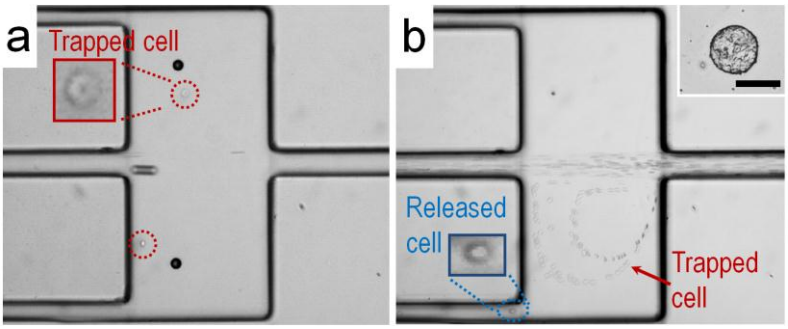

Figure 4: *(a) Blood spiked with 20 μm particles. Both ~15 μm cell and 20 μm particle were trapped into chamber while red blood cells passed through. (b) Blood experiment. Stacked image indicated the orbit of cell in the vortex. Cell exits from the side outlet after circulating in the vortex for certain amount of time. The inset figure shows a monocyte collected from the side outlets. The scale bar is 20 μm.*

In experiments with spiked blood samples, diluted blood (1000×) sample spiked with 20 μm particles was used for demonstration. Both 20 μm particles and WBCs were trapped (Fig.4a) and released from side outlets, while RBCs passed through the chamber region and exited from the main outlet. Continuous cell extraction from blood sample was also demonstrated (Fig.4b) confirming feasibility of cell extraction.

CONCLUSIONS

In conclusion, we have successfully demonstrated a size-based cell separation system that exhibits a number of advantages such as continuous extraction, high throughput (1mL/min), high selectivity and no trapping capacity limitation. The approach has the potential for continuous extraction of either rare cells, e.g. CTCs (1-10/mL) or cells with low concentration, e.g. leukocytes (5,000-10,000/mL), which is critical in cell biology research and clinical diagnostics.

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CONTACT

*Ian Papautsky, tel: +1-513-556-2347, ian.papautsky@uc.edu