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NOVEL APPROACHES TO CELL ISOLATION IN SIMPLE INERTIAL MICROFLUIDIC DEVICES

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NOVEL APPROACHES TO CELL ISOLATION IN SIMPLE INERTIAL MICROFLUIDIC DEVICES

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ABSTRACT

Cell sorting is essential in many biological and biomedical applications. One example is isolation of rare cells such as circulating tumor cells (CTCs) from blood, which are of great clinical significance as early indicator of cancer. Yet, isolation of CTCs is remarkably challenging, primarily due to their incredible rarity, and thus need for high separation selectivity, efficiency, and purity. Currently, the prevalent approach is based on immunodetection, which is effective but relies on availability and homogeneous expression of biomarkers. This work describes alternative approaches based on inertial microfluidics, which permits size-based, highly-selective separation of cells with high efficiency and purity. In these devices, cells experience inertial forces which are strongly size-dependent and cause them to migrate laterally to equilibrium positions within microchannel cross-section. The result is cell separation based on size only, leading to an effective high-throughput ($10^4$ cells per min) alternative to immunodetection. A new model of particle/cell migration was developed to explain lateral migration behavior and address limitations of the current understanding. Lift coefficients acting on neutrally-buoyant particles and cells were experimentally measured to confirm the new model, and to allow accurate prediction of particle/cell behavior. With guideline from the new model, three designs were developed to realize superior performance in cell separation and isolation. In the first design, highly-sensitive isolation (as low as 1 particle per mL) was demonstrated by suppression of wall induced lift force. This approach also showed very high selectivity ($1:10^5$) of separating different sized species. The second design modulated the shear force to achieve continuous filtration with extremely high efficiency (~100%) and capability of precise re-
concentration. The third more advanced design, based on modulation of rotation induced forces, manipulated equilibrium positions of particles/cells for complete separation with ~100% efficiency and >90% purity. Blood spiked with human prostate cancer cells was used to characterize cell separation performance. A ~83% viability was achieved, with proliferation results showing preservation of functionality. All the three designs outperform the existing immunodetection-based and other separation platforms. The approaches demonstrated in this work offer promising alternatives to cell sorting, potentially including isolation of CTCs. Due to the planar structures and simple geometries, the devices should be easy to integrate with existing lab-on-a-chip systems.
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LIST OF SYMBOLS

\[ a = \text{particle diameter} \]
\[ AR = \text{Aspect ratio} \quad \text{where} \quad AR = \frac{H}{W} \]
\[ C_L = \text{Lift coefficient} \]
\[ C_L^- = \text{Negative lift coefficient} \]
\[ C_L^+ = \text{Positive lift coefficient} \]
\[ D = \text{Diameter of tube} \]
\[ D_h = \text{Hydraulic diameter} \quad \text{where} \quad D_h = 2 \times W \times H/(W + H) \]
\[ F_D = \text{Drag force} \]
\[ F_L = \text{Lift force} \]
\[ F_L^- = \text{Negative lift force} \]
\[ F_L^+ = \text{Positive lift force} \]
\[ F_{SL} = \text{Shear-induced lift force} \]
\[ F_{WL} = \text{Wall-induced lift force} \]
\[ G = \text{Average shear rate} \]
\[ H = \text{Height} \]
\( L = \) Focusing length, channel length required for focusing

\( L^{-} = \) Focusing length dominated by \( C_{L}^{-} \)

\( L^{+} = \) Focusing length dominated by \( C_{L}^{+} \)

\( L_{m} = \) Migration distance

\( L_{o} = \) Length of chamber opening

\( L_{c} = \) Length of chamber width

\( Re = \) Reynold’s number \( Re = \rho U_{f} D_{h} / \mu \)

\( Re_{p} = \) Particle Reynold’s number \( Re_{p} = Re(a/D_{h})^{2} \)

\( Re_{th} = \) Threshold Reynold’s number

\( U_{f} = \) Average flow velocity

\( U_{L} = \) Lateral migration velocity

\( U_{m} = \) Maximum flow velocity

\( W = \) Width

\( \rho = \) Density

\( \mu = \) Dynamic viscosity

\( V = \) Velocity vector

\( \Omega = \) Rotation vector
CHAPTER 1

INTRODUCTION

Circulating tumor cells

Cell sorting is a critical yet very challenging sample preparation step in many biological applications.\textsuperscript{1-4} No example illustrates this better than isolation of circulating tumor cells (CTCs) from peripheral blood. Isolation of these rare cells has been identified as great challenge ever since their discovery in 1869.\textsuperscript{2, 5-11} CTCs are the cells detached from primary tumor and shed into bloodstreams; they act as seeds to disseminate the disease to distant organs.\textsuperscript{9, 11-14} CTCs possess the phenotype of cancer that may represent risk of metastasis,\textsuperscript{5, 6, 9, 11, 13-24} and thus have attracted considerable attention recently for their clinical significance.\textsuperscript{17, 19, 21, 25-31} Since invasion can start very early during tumor development\textsuperscript{2}, CTCs are very promising for early detection of malignancy and prediction of the metastatic risk.\textsuperscript{2, 6, 12, 15-17, 19, 23, 32-38} Moreover, they provide a powerful tool for assessing prognosis, monitoring response to treatment, informing treatment-decision making and realizing personalized therapy.\textsuperscript{2, 6, 11, 27, 31, 35, 37, 39-45, 45, 46}

The detection rate and the number of CTCs are strongly correlated with the disease stages and can be used for risk-stratification.\textsuperscript{2, 6, 10, 45} Development of CTC evaluation technologies could allow early disease detection, prognostic assessment, treatment response monitoring and personalized targeted therapy.\textsuperscript{2, 6, 11, 27, 31, 35, 37, 39-45, 45, 46} In addition, drug development could also

1
benefit from molecular analysis of CTC.\textsuperscript{41} However, their rarity (<1 cell/mL) of whole blood and lack of robust clinical methods for their isolation is a critical challenge.\textsuperscript{2, 5, 6, 8-10, 28, 47}

A number of emerging techniques have been utilized in rare cell isolation. One, the so-called indirect molecular method, takes the advantage of RT-PCR.\textsuperscript{48} While more sensitive than other methods, this approach destroys cells and CTCs and loses morphological information which is critical for follow-up analysis.\textsuperscript{33} In addition, the choice of the marker RNAs is limited. In 2000, Giovanna Vona et al. reported an alternative approach to isolate CTCs, named as isolation by size of epithelial tumor (ISET) cells.\textsuperscript{33} However, it inevitably suffers from clogging issue due to the membrane filtration-based mechanism. Moreover the sensitivity and yield also depend on the uniformity of filter pore size and the sample flow conditions.\textsuperscript{2} Noticing the density difference between CTCs and other cells in blood, density gradient separation has been demonstrated.\textsuperscript{49, 50} Unfortunately, whole blood mixes with the density gradient fast which leads to low and variable sensitivity.\textsuperscript{2} Today the most common way to isolate and enrich CTCs is based on immunoselection, an approach used in the only commercialized system. “Cell Search” utilizes immunomagnetic beads to extract target cells.\textsuperscript{44} Although the high specificity due to selectivity of biomarker (EpCAM), this prevalent immunoselection approach suffers several critical drawbacks: 1) some CTCs lack expression of the marker and 2) the same antigen expresses on non-tumor cell which gives false positive.\textsuperscript{2, 51} Other approaches, such as microscopic scanning\textsuperscript{52}, laser scanning cytometry\textsuperscript{53}, conductivity-based\textsuperscript{54}, and dielectric-based\textsuperscript{55} methods, have been proposed but have not exhibited significant improvement.\textsuperscript{12} While flow cytometry shows specificity the sensitivity is poor.\textsuperscript{28} Therefore, despite intensive effort in recent years, the implementation of CTC detection remains challenging.
Microfluidics for cell handling

Recent development in microfluidics has the potential providing a solutions to effective isolation of CTCs. Microfluidics is the science and technology of systems that process or manipulate small amounts of fluids. It is also a multi-disciplinary field involving subjects of engineering and life science. Its capability of precise manipulation of cells within fluid has attracted considerable attention for cell sorting applications including isolation of CTCs. A number of microfluidic platforms have been developed attempting to overcome the aforementioned drawbacks of the current systems.

Cell/particle separations in microfluidics are generally achieved by amplifying the lateral deflections of different particles based on their intrinsic or labeled properties, such as size, density, surface charge and magnetic label. After obtaining certain lateral distances among those particles, they can be separated by taking advantage of laminar flow. In other words, they can be collected in different outlets downstream. The challenge then is to amplify lateral deflections which may be accomplished by manipulating hydrodynamic forces within the flow or introducing external forces like electrostatic force. Though dissimilar forces might be used based on different mechanisms, the underlying configurations of the forces are generally the same – the introduced forces are always perpendicular to the flow directions.

Depending on the separation mechanism/forces, microfluidic particle/cell separators can be classified as active or passive devices. Active devices utilize external power source to generate the lateral deflection while the latter ones are taking the advantages of hydrodynamic forces which can be manipulated by channel geometry, flow rate and so on. Electrical field is very commonly used in active separators. For example electrophoresis (based on charge to size ratio), dielectrophoresis (based on polarizability), isoelectric focusing (based on isoelectric
point) \(^{60}\) have been demonstrated.\(^{61}\) Magnetic and acoustic forces can also be used for separating particles/cells. Active devices have many evident advantages: 1) high separation efficiency (up to 100%), 2) high resolution (hundred nanometer in size difference, 3) Be tunable. However, not every device has the same advantages, which often depends on particle properties, channel dimensions, or some other factors.

In contrast to the active devices, passive counterparts (inertial microfluidic devices) take advantage of hydrodynamic forces and require no external power source. These systems commonly depend on particle size and microchannel geometry. Therefore, their footprints can be much smaller and the fabrication processes are easier. Typical advantages of passive separation devices include 1) no power consumption, 2) small footprint, 3) label-free, 4) high throughput, 5) Low cost and disposable (depending on material), 6) easily paralleled (depending on geometry). Although the resolution might not be comparable to their active counterparts and the analysis time might be longer, passive platforms have been gaining popularity for cell handling due to lower external impact is introduced and thus better preservation of cell biological properties.

In deed several recent works \(^{57,62-65}\) reported the label-free and membrane-less cell separation based on inertial microfluidic platforms. Di Carlo et al. reported on a simple straight channel for high throughput filtration of red blood cells (RBCs) from bacteria. Their results show \(\sim 80\%\) removal of bacteria after two-stage operation.\(^{63}\) Our previous work with spiral microchannels demonstrated separation of neuroblastoma and glioma cells at high efficiency and relative cell viability.\(^{64}\) Other investigators also reported cell sorting by combining the effect of cell deformability and inertial focusing in microchannel.\(^{66,67}\) High enrichment ratio was achieved in these works. Integrating immunoselection component, capture of CTCs in patient blood has
been demonstrated in a passive microchannel with thousands of posts coated with antibody. More recently Han et al. demonstrated separation of CTCs in a pinched inertial microfluidic channel using MCF cell spiked blood. All these works has envisioned the promising and active role of inertial microfluidics in the case of rare cell isolation.

Motivation

The clinical significance of CTCs has triggered a considerable interest and demand for their identification, enumeration and characterization for both oncological research and diagnostics. However, detection of CTCs has not been implemented in clinical routine due to the excessive challenges lying on the very first step—isolation. CTCs are rare events in blood stream and the concentration could be less than 1 cell per mL blood containing 50 billion RBCs. As discussed previously, the three main approaches of CTC separation suffer from a number of critical issues such as heterogeneous expression of biomarkers. Several inertial microfluidic platforms have been reported aiming at isolation of CTCs, which have shown encouraging perspectives. However, many essential limitations are yet to be addressed, such as low sensitivity, moderate efficiency and untested purity which are the requirements of CTC extraction. In fact isolation of CTCs has been recently identified as Grand Challenge facing microfluidic community.

This work proposes alternative novel approaches incorporating inertial microfluidics to isolate and enrich CTCs. Considering the inertial nature, these methods are expected to expedite development of low cost, easy to use, portable automated CTC detection and analysis systems in clinical setting. These purely size-based methods take the advantage of inertial microfluidics, and they are capable of accomplishing highly sensitive, selective isolation of rare cells as well as providing high efficiency, purity and preservation of cell viability. Cell separation in the
proposed platforms in this work relies only on the intrinsic inertial forces acting on cells flowing through geometrically-confined microchannels.

The concept of inertial microfluidics is based on the fact that cells (or solid microparticles) flowing in microchannel experience mainly two forces—drag force and lift force.\textsuperscript{70, 71} The former is responsible for particle entrainment of cells or particles within streamlines. The later causes lateral migration. There are two main lift forces, as shown in Fig. 1. Shear-induced lift force is responsible for particle migration toward the walls while the wall-induced lift force push particle away from walls. The interaction of these two lifts, combined with the drag force, lead to cell or particle focusing within microchannel cross-section. These inertial forces are strongly dependent on cell/particle size, which results in the differentiated lateral migration and the size-sensitive separation.

![Fig. 1 Current model of particle migration in microchannel. Due to parabolic velocity nature, particles experience shear-induced lift force ($F_{SL}$) which drives them away from channel center toward the walls. When they are close to wall, the force is balanced with a wall-induced lift force ($F_{WL}$) which directs particles away from wall. As a result, particles stop migration and reach their equilibrium positions near walls.](image-url)
Scope of work

In this work, three simple inertial microfluidic designs for size-selective cell filtration and isolation are reported. These approaches are demonstrated for effective and sensitive separation of CTCs using blood samples spiked with prostate cancer cells. While the concept of inertial focusing has been reported in literature, the actual mechanism of cell/particle migration remains unclear. This presents great challenges in developing and exploring inertial microfluidic devices for separation of cells such as CTCs. Hence prior to development of separation devices, fundamental of inertial migration within microchannel are explored. Three other than two inertial lift forces are found to be responsible for migration and equilibration behavior of microspheres. The interactions of these forces, coupled with drag force, are associated with the distinct observations of transportation and with the two-stage focusing procedure which is proposed as a new model in this work. These investigations are expected to improve the understanding of the working mechanisms in inertial microfluidic systems, and thus serve as design rules in the field.

With the improved knowledge on inertial migration, three methods were developed for different cell sorting applications. The first design consists of simple straight rectangular microchannel and trapping cavities symmetrically located at sidewalls. Due to the cavities, force balance is disrupted and thus cells/particles are trapped based on size. This approach shows multiple advantages, such as enhanced throughput, high sensitivity and selectivity and capability of enrichment. The second design which is a simple low aspect ratio microchannel exhibits extreme efficiency and the capability of precise re-concentration during filtration. By modulating channel aspect ratio, another device is developed to manipulating particle/cell equilibrium positions. The reform of these positions in the same channel leads to a complete separation with
high efficiency and purity. Ultimately, human prostate cancer cells are used to demonstrate and characterize these approaches to cell separation, such as isolation of CTCs.

**Chapter summaries**

Following this introduction, Chapter 2 will provide the basic materials and methods used in this research. First of all, device fabrication process will be described in detail. In addition, Other than SU-8 which is a conventional negative photoresist used by soft lithography, here the dry film (PerMX) will be utilized due to its superior height uniformity and shorter process time. Polystyrene particles (fluorescently labeled) are widely employed to demonstrate and validate the designs in this work. Since cancer cells were employed for demonstration of cell isolation, the sample preparation and protocols will be discussed. Finally the methods of characterization, data acquisition and analysis will be discussed in this chapter.

Chapter 3 will describe the underlying mechanism of particle/cell inertial migration in microchannels and the proposed new model for prediction and explanation of particle/cell behaviors dominated by inertial forces. Three forces, including rotation-induced lift force, will be introduced for elucidation of microsphere behavior and the formation of equilibrium positions within channel cross-section. Two focusing positions in rectangular microchannel under proper condition will be identified, and employed in investigation of lift coefficients which characterize the inertial forces. Characterizing channel length needed for full focusing, the lift coefficients will be for the first time measured experimentally. A generalized equation that precisely predicts channel length required for final equilibration will be developed as a guideline for microchannel design.

Under the developed guidelines, Chapter 4 will introduce a simple device for sensitive size-selective isolation and enrichment of particle/cells by modulating wall-induced lift force.
The expansions after a high aspect ratio straight channel substantially suppress the wall induced lift force and disrupt the streamlines for vortex formation. Numerical model using ESI CFD-ACE+ program will demonstrate the micro-vortices in the expansions. A sensitivity of extraction at particle concentrations as low as 1 particle per mL and an enrichment on the order of $10^5$ will be demonstrated. Characterization of design will be carried out in multiple ways, such as expansion dimension and particle concentration effects. These detailed \textsuperscript{63, 71} investigations serve to be practical guideline for designing specific devices suitable of various applications.

In Chapter 5, a low-aspect-ratio straight channel will be utilized for particle/cell purification and re-concentration. The membrane-free method exhibits extremely high filtration efficiency (~100\%). The purification and concentration factors are also much better than high aspect ratio alternative proposed in previous works. A mixture of 20 $\mu$m and 7.32 $\mu$m particles will be introduced into the device to demonstrate the purification and concentration. Particle spiked blood will be used for verification of the practical implementation of cell filtration. Then cancer cells will be run through the device to test the filtration efficiency, cell viability and proliferation. Demonstration of cell filtration will be realized using blood spiked with cancer cell.

An advanced version of design in Chapter 5, which is capable of complete separation with ~100\% efficiency and >90\% purity, will be present in Chapter 6. The outstanding performance of this approach is based on modulation of channel aspect ratio in straight rectangular channel. The switch of channel aspect ratio enables of manipulation of particle/cell equilibrium positions within the channel cross-section. A mixture of 20 $\mu$m and 9.94 $\mu$m particles will be utilized for demonstration and characterization of the complete separation. Subsequent experiments using blood spiked with particles will show the feasibility of the application for cell separation.
Finally, Chapter 7 will conclude and summarize this work and will provide prospective on the remaining challenges and future directions.
CHAPTER 2

MATERIALS AND METHODS

The approaches of cell separation in this work were based on inertial microfluidics and developed in microchannels. Devices were designed and fabricated in polymer by soft lithography and characterized primarily using optical imaging. Before demonstration of cell isolation, fluorescent particles were injected into the channels to characterize the device. Fluorescent images were captured to explore the particle migration and determine the channel length required for complete focusing. To quantitate the performance, hemocytometry was used to count particles and cells. As isolation of CTCs was proposed, it was demonstrated using cancer cell spiked blood. Cells were fluorescently labeled for visualization. Cell viability and proliferation was also determined to show the preservation of cell biological properties.

Device fabrication

Microfluidic channels in this work were fabricated using standard soft lithography in polydimethylsiloxane (PDMS). It is probably one of the most popular approaches in microfluidics research today, and it was selected due to simplicity, low cost and fast prototyping. PDMS is optically transparent for wavelength above ~230 nm and its autofluorescence is very low, which permit characterization of microfluidic devices using optical detention including bright field and fluorescent imaging. Secondly, PDMS is biocompatible, enabling processing of biological sample in both biomedical research and diagnostics. In addition, the surface
properties of PDMS allows it to be sealed to other polymers and glass without additional adhesive. 76, 78 Herein, PDMS soft lithography was used to replicate all the microfluidic devices.

The soft lithography process used in this work is schematically shown in Fig. 2. Initially a clean 3 inch silicon wafer was coated with dry film negative resist (PerMX 3050, Dupont) using a rolling laminator heated to 85 °C at < 1 RPM. Multiple layers can be laminated sequentially to achieve greater height. Following a 10 min cool-down, the wafer was coated with Glycerin and exposed to UV at 175 mJ/cm² (~5 mW/cm²) using I-line 365 nm long pass filter. The exposure time for single layer of 50 µm dry film was ~150 sec. Additional layer will require long exposure time, for example ~270 sec was used for two-layer 100 µm. After exposure, wafer was washed with DI water to remove Glycerin, dried by N₂ gun, and placed on a hotplate at

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**Fig. 2** Schematic of the soft lithography. (a) Dry film negative resist (PerMX, DuPont) is coated on a silicon wafer with a laminator and (b) exposed to UV light modulated by mask. (c) After photoresist development, a master is formed. (d) Next, a PDMS mixture is poured onto the master and allowed to cure. (e) The cured PDMS is then peeled off, resulting in a replicated pattern. (f) An enclosed channel is formed by bonding with a glass slide.
100 °C for 10 min to relax and improve resist adhesion. Once the wafer was cooled to room temperature, the photoresist was spray developed in PGMEA (Kanto Corporation). Periodic inspection using isopropyl alcohol (IPA) was used to judge complete removal of unexposed resist as undeveloped resist turns milky white. A 10 min development for a 50 μm film was typical. After a rinse with DI water, and N₂ dry, O₂ plasma (CS-1701 RIE, March Plasma System, CA) was used to de-scum the surface. At this point, the device master was complete and could be used for replication of multiple devices in PDMS (Fig. 2c).

Before casting PDMS, the master was salinized to improve mold release. Simply, the entire master was covered by Sigmacote® (Sigma Aldrich) and allowed to dry typically for 30 min. To form replicas, liquid PDMS prepolymer and curing agent were thoroughly mixed in a 10:1 (w/w) ratio. After degasing under vacuum, the mixture was poured onto the salinized mold and degassed again. PDMS was cured on a leveled hotplate at 80 °C for about 2 hours. The pattern was then cut and peeled off the master and bonded to a glass slide (Fisher Scientific Inc.) with a corona discharge treater to form closed microchannels (Fig. 2f).

Dry resist PerMX instead of conventional SU-8 was used in this work to fabricate the master due to several critical advantages. Since the film is produced with fixed height, the height of channels replicated is highly uniform, which tremendously improves reproducibility of experiments. Since the conventional SU-8 process requires the spin-coating, it intrinsically generates height variations of the thin film on wafer. Fig. 3 shows the channels fabricated by SU-8 process. Although the channel widths are approximately the same, the channel height in Fig. 3b is 13 μm larger than that in Fig. 3a. This is ~25% variation. In the contrast, the height of channel fabricated using PerMX film only showed a 2% variation. Furthermore, it is difficult to develop a process of spin coating for specific channel height. Even following the same, process, the
height can depend on factors such as room humidity other than the spin coater speed. However, using PerMX it is easy to maintain the channel height and reproducibly fabricate the channel regardless environmental change. To achieve different height, simple stack of the films will make it as shown Fig. 4. Single layer offered 50 μm was given in Fig. 4a. Fig. 4c and d demonstrated the two and three layer process, which are 100 μm and 150 μm in height respectively. Different aspect ratios ($AR=height/width$) of channel cross-section have also been demonstrated by single and multiple layer process. Fig. 5a shows the cross-section of low aspect ratio channel ($AR=0.15$) and high aspect ratio ($AR=3$) channel has been achieved by 3 layer-stack as in Fig. 5b and Fig. 4d. In addition, the channel sidewalls were very straight which were comparable or even better than those fabricated by SU-8 process. Because of the absence of spin-coating step, the fabrication using dry film reduces the processing time in a considerable way. Considering the short developing process, patterning single wafer can be done approximately in
**Fig. 4** Images of a multi-layer process. (a) A 51.2 μm channel height using single layer of 50 μm thick film. (b) A 79.7 μm channel height obtained by stacking of two layers of 14 μm films and one layer of 50 μm film. (c) A 98.5 μm channel height obtained by stacking of two 50 μm layers. (d) A 150.2 μm channel height obtained by stacking of three 50 μm layers.

**Fig. 5** Low and high aspect ratio of channels fabricated by PerMX dry resist. The scale bars represent 100 μm.
one hour. In contrast, it requires at least 4 hours to process SU-8. Hence, dry film process is superior in terms of fast prototyping compared to the traditional SU-8 process.

Although dry film method outperformed SU-8 in a number of ways, it should be noted that it also suffers from some limitations. For example the height can only be the sum of multiple layers. Arbitrary height is not possible since the height of each layer is fixed. Another critical trick of using PerMX is to minimize the bubbles trapped in the film. Although bubbles are normally very small, it becomes disaster if it is comparable to the feature size of channel. Fig. 6a shows the surface roughness of channel was compromised and the channel geometry was modified due to the bubbles near side walls (Fig. 6b). Fortunately, the bubbles can be minimized or even avoided by using fresh materials and proper baking condition. Therefore dry film process is better and was widely used in this work.

**Sample and protocols**

Fluorescent micro particles were used to perform the experiments and measurements to enhance the quality of visualization and imaging. Particles used in this work are listed in Table 1.
To minimize the particle-particle interaction we diluted particle suspensions in deionized (DI) water to reach the volume fraction ($VF$) of 0.025%. A small drop of Tween-20 (Fisher Scientific Inc.) was applied to particle suspensions to avoid the clogging issue. To exploit and characterize the designs for cell separation, particles were spiked into diluted human blood (Hoxworth blood center) in Saline solution (0.9% NaCl) for demonstration.

The LNCaP and HPET cell line were obtained from American Type Culture Collection and was cultured as described by Pitkänen-Arsiola et al. LNCaP cells were grown in 10cm plates and when they are 80% confluence, the cells were stained with the green fluorescent chloromethyl derivatives of fluorescein diacetate (CMFDA). The cells were stained following manufacturer’s protocol (MP02925 – Invitrogen). Briefly, 5μM CMFDA diluted in serum free medium and added to cells. After 15 minutes, aspirate the medium and add fresh medium and incubate for 45 minutes. The cells can be trypsinized and used for the assay. Cell viability and growth was determined by counting cell number using trypan blue staining (Sigma, St Louis, MO) and hemocytometer. Five thousand cells were plated in triplicates in 24-well plate and treated with +/- androgen (DHT) at concentration of $10^{-8}$M and/or anti-androgens (OH-). The cells were trypsinized and counted by staining with trypsin blue at different time points.

**Setup and characterization**

Samples were injected into device with a syringe pump (NE-1000, New Era Pump Systems, Inc.) to sustain stable flow rate. The loaded syringe was connected to 1/16” Peek tubing (Upchurch Scientific) and secured to the device inlet. High-speed images were captured using an inverted epi-fluorescence microscope (Olympus IX71) equipped with a 12-bit CCD camera (Retiga EXi, QImaging) for characterization. At least 100 frames were obtained for every measurement. To achieve better signal processing and stream visualization, multiple frames at
the same position might be stacked using ImageJ®. Fluorescent intensity data were obtained
from line scan for quantitative assessment of focusing. Fluorescent images in this work were
pseudo-colored by ImageJ®. When the mixture of particles was tested, we took images
alternatively using different filter cubes (FITC, TRITC and DAPI). Then the grey scale images
from different filter cubes but identical location were merged forming RGB composite picture to
show the separation in the channels. To characterize efficiency and purity, mixture of particle
suspension were injected into channels and the samples were collected at each outlet. The
samples were then counted using hemocytometry to determine the particle population. At least
three counts were carried out for the experiments to ensure the reproducibility.
Table 1. Summary of particles used in this work.

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<th>Color</th>
<th>Excitation</th>
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CHAPTER 3

NEW MODEL OF INERTIAL FOCUSING

Particle inertial migration has been widely applied in development of separation systems on the microscale due to their promising capabilities. However the underlying mechanism in terms of governing forces remains unclear which posts substantial difficulty in development and design of precise systems for improved performance to compete with their macroscale counterparts. Although shear and wall induced lift forces have been employed to explain particle behavior of lateral migration and aggregation, they are unable to decode particle focusing into specific positions in quadrangular channels (e.g. square and rectangular channels). In this work by re-examining the particle motion in rectangular channel, we are able to provide extended map of particle migration and its corresponding dominated forces. Our results have shown that particle focusing in microchannel is completed in two stages subjected to distinct forces. Shear induced lift remains valid for particle migration from channel center to walls indicating a negative sign of this force. This negative lift is eventually balanced with wall induced force resulting in particle aggregation near walls. Next particles are found to translate from corners along walls toward their centers upward velocity gradient. This motion has been identified under the control of positive lift which is induced by particle rotation. To improve the understanding of the two lifts and suggest guideline for development in aforementioned applications, we have managed to characterize them in terms of negative and positive lift coefficients, which is a conventional method. Interesting results have revealed their dependence on particle size and
Reynolds’ number as well as the relation to channel dimensions, which are in good accordance to existing numerical outcomes. Based on results and analysis our theory is able to predict particle motion in other channels including square channel and round duct where no corner exists. We believe it help improve the understanding of inertial migration of particles in microchannels.

**Introduction**

Nearly half a century ago Segré and Silberberg first observed that rigid particles flowing in a pipe aggregated in the form of annulus at ~0.6R away from the paper central axis (R is the radius of the pipe).\(^8^0\) Due to the neglected inertial effect this finding was not able to be explained at that time. Later after a number of experiments and theoretical studies they found that the inertial lift due to moving fluid was mainly responsible for the radial migration of particles.\(^8^1\) Their works have also implied that increasing flow rate (Re) shifts particle equilibrium position further closer to pipe wall, which have been observed in later works.\(^8^2-8^5\) In 1965, Saffman showed that a lift force acting on a spinning sphere in a linear unbounded flow. His work also shows that when applied to the radial migration phenomenon the effect of rotation which is the higher order in his expression is in significant compared the lift due to shear when Reynolds number (Re) is small. Therefore the lift due to shear is the mainly force that drives particle away the pipe central axis. Then three years later another work presented by Cox & Brenner analyzed particle migration in a flow field bounded by wall.\(^8^6\) They utilized Green’s function to obtain the general expressions for the lift force by assuming the particle is very far from the wall. Leighton & Acrivos analyzed the case of stationary sphere in a shear flow when the particle touches the wall.\(^8^7\) According to their results the lift directs particles away from the wall and varies with particle size and velocity gradient. Hence there is lift acting on the particle in the opposite direction of shear force. Experiments aimed on wall induced lift were performed by Cherukat &
They showed that particles were expelled away from the wall as they sedimented. In this case particle rotation was observed near wall. Then in 1989 Schonberg & Hinch explored the inertial migration velocity of a neutrally buoyant sphere in a plane Poiseuille flow. They found that the equilibrium position moves toward the wall as $Re$ increases.82

Researches in the past two decades have significantly improved the understanding of particle lateral migration in the sheared flow. Mclaughlin evaluated the inertial lift on a sphere translating in a shear flow bounded by a single wall.89 Feng et al explained that the inertial lift force acting on the rigid sphere in Poiseuille plow is induced by the parabolic nature of the velocity profile.90, 91, 91 Asmolov further explored the inertial lift on a sphere in plane poiseuille flow for both unbounded and bounded cases and he generated an expression for estimating the lift forces on a neutrally buoyant particle. His results also show that particles migrate away from wall.92 More recently Matas et al. investigated the inertial migration of particles at high $Re$ experimentally.93, 94 Their results indicated that the lift force exerted on particles varies with particle position in channel flow which matches Asmolov’s outcomes. Particle lateral migration in square duct was numerically investigated by Chun et al.95 Different focusing behavior from Segré annulus was found. Eight and four equilibrium positions were proposed corresponding to different $Re$ regime.

Recently the analysis based numerical data acquired from simulations has unveiled the mechanism of particle lateral migration in a more clear way. Zeng et al. have shown in their works that rigid sphere moving near a wall experiences wall-induced, shear-induced and rotation-induced lift forces.96, 97 The wall-induced lift force is found to be primarily due to the vorticity generated on the particle surface which is not symmetrically distributed in the wake due to the presence of the wall.98 The wall effect on drag force is also found to be very strong. This
effect of wall decays very fast as the distance between particle wall increases.\textsuperscript{99} Hence the interaction of these forces contributes to the particle equilibrium near wall.

The increasing understanding of particle lateral migration has been applied to microscale flow which has been attracting considerable attention due to its promising and operable applications. Recent works have demonstrated that geometrical confinement of flow in microfluidic devices modifies the particle equilibrium positions.\textsuperscript{65, 70, 71, 84, 85, 100-105} More specifically, particles flowing in a square are found to migrate toward the walls and occupy either eight or four positions depending on flow condition and channel dimension.\textsuperscript{70, 71, 95, 100, 106} Four stable equilibrium positions were observed by confocal microscope.\textsuperscript{100, 106} In the other case, particles entrained themselves near long wall in a rectangular microchannel.\textsuperscript{70, 71} By curving the channel walls, the particle ordering in the center were also observed such as in serpentine channel.\textsuperscript{100} Many microfluidic platforms based on manipulating particle focusing behavior have been developed for wide applications in various fields, for instance, membrane-free filtration\textsuperscript{63, 71, 107}, cell isolation\textsuperscript{57, 62, 65, 108, 109} and flow cytometry\textsuperscript{102, 110, 111}. They have shown not only outstanding performance but also practical implementation in reality. Furthermore many unique advantages of this approach have been exposed to its community. For example, our previous work has demonstrated the high throughput label-free filtration of particles and bacteria as well in a simple high aspect ratio straight channel.\textsuperscript{70, 71}

More and more new concepts and designs based on manipulating inertial forces are under developing for distinct applications. However the fundamentals specific for particle positioning in microfluidics have not yet been well established.\textsuperscript{84} The absence of underlying principle could result in substantial difficulty for device design and impede the development eventually. Limited studies on the mechanisms have imposed lots of controversies in the microfluidic field. For
example, both eight positions and four positions of particle focusing in square channel have been observed.\textsuperscript{70, 71, 95, 100} In a rectangular channel particle behaviors are found distinguishable to each in different works.\textsuperscript{70, 71, 105, 106} Even in the same work, different results may be present.\textsuperscript{105} Based on current theory, the equilibrium position in microchannel is as a consequence of the balance between two counteracting forces. The shear-induced lift force is responsible for particle migration toward the channel walls. Once it is balanced with wall-induced lift force particles are considered to be stable. However this explanation suffers from incompleteness. Because it is unable to explain either particle focusing centered the faces in square channel or the two position focusing observed in rectangular channel.

Thus the mechanism for particle migration in microchannel remains unclear. This is mainly due to the difficulty of investigating forces on particles. Even in macroscale, most of works are done by numerical simulation. In this work, we experimentally explored the forces acting on neutral buoyant particles flowing through microchannel. We re-examine the possible forces which are responsible for particle stabilization and describe the experimental results showing particle positioning in rectangular microchannels. Due to the specific equilibrium positions in rectangular channel, we designed experiments to characterize the lifts exerting on particles in the form of lift coefficient. Firstly two-step migration has been demonstrated. And then we have studied the effects of flow condition, particle size and channel dimensions on lift coefficient. Based on the results we have identified two lifts associated to migration process, which are responsible for particle equilibration in the rectangular channel and is also applicable to square channel. We believe our work help improve the understanding of mechanisms for particle migration and can be a general guideline for developing inertial microfluidic systems.
Theoretical analysis

Motion of particles in fluid is complex. Neutrally buoyant particles flowing in microchannel are subjected to the control of two dominant forces. One is the viscous drag ($F_D$) which is responsible for entraining particles along streamlines.\textsuperscript{70, 71} It is related to fluid properties (viscosity and velocity) and particle size. The other predominant force acting on particles is the net lift force ($F_L$) which leads to particle migration across the streamlines. The parabolic nature of the laminar velocity profile in Poiseuille flow and its associated shear gradient produce lift forces pushing particle away from the channel centerline toward to the channel wall.

As particles migrate close to channel wall, there arises a wall induced lift force ($F_w$) directing particle away from the channel wall. Wall lift is found to substantially increase as the distance between particle and wall shortened.\textsuperscript{71, 96, 99} At certain position ($\sim 0.2W$ from wall \textsuperscript{70, 71}) wall lift is sufficient strong that it balances with shear-induced lift force, creating equilibrium positions for particles. As a result of the force cancellation, particles become entrained near channel walls. Both early experiments in macro scale pipe and recent results in microchannel have shown that in a round duct, particles were found to focus like a bracelet close to the wall.\textsuperscript{80, 84, 93} While the force balance of wall induced lift and shear induced lift successfully explains the particle focusing in round duct, square and rectangular channels present more complex situations due to radial asymmetry. Since the shear lift drives particles away from the channel center down the shear gradient toward wall, particles should finally equilibrate along the perimeter and in the corners to achieve balance of the two forces. However, recent work has identified four focusing positions centered in each face in square microchannel.\textsuperscript{85, 100} Our previous work \textsuperscript{70, 71} have also shown that particles were ordered into two bands along the long length in rectangular channel, which is further demonstrated to be two positions in this work (Fig. 7c). The absence of particles
Fig. 7 Schematic of forces and lateral migration of particle flowing in rectangular microchannel. (a) Case (i), particle close to the center experiences a negative net force directing to the long channel wall, which drives particle toward the long edge; case (ii), particle in the corner is push away from the short edge due to a positive net lift force and causing the migration to the middle of the long length; case (iii) the net force is zero and particle occupies its equilibrium position. (b) Topview and cross-section illustration of lateral migration in rectangular channel. Randomly suspended particles migrate fast forming two bands along the long edge in sideview and then slowly translate to the central position resulting of single stream focusing looking from top. (c) Fluorescent image showing 20 μm particle migrating toward to channel center at $Re=30$. The channel is 100 μm wide and 27 μm in height.
in corners both in square and rectangular channels has suggested another lateral migration takes place which transports particles to the center of walls. In other words, particles migrate up the shear gradient in the parabolic velocity profile near walls, which is conflicting to the theory described in early works.  

These discrepancies led us to re-examine forces acting on spheres in microchannel. Rigid particles flowing in the fluid mainly experience drag force and lift force. While drag force is not responsible for particle transverse motion, the lift counts for the migration across streamlines. However lift is one of the more difficult forces to properly model as there are multiple physical phenomena that lead to the generation of lift. The two primary mechanisms are continuous-phase vorticity and the rotation of the particle. In addition in bounded condition, due to the presence of wall, there is a wall-induced lift force normal to the wall.  

Saffman lift is the main type of the first mechanism, which is shear-induced lift force. In the context of an unbound linear shear flow, Saffman showed that rotation-induced lift is insignificant compared with shear-induced force. Numerical simulation has also pointed out that in most flows particles are experiencing more of a shearing behavior. Thus in a channel the motion of particles flow far away from walls is dominated by shear-induced lift, which directs particles away from the channel center toward the wall. This force is found to be mainly dependent on .

As particles approach channel wall, two effects take place. On one hand, the vorticity generated at the sphere surface interacting with the wall resulting in a net lift force driving particles way from the wall and thus counteracting with shear-induced lift force. On the other hand, according to parabolic nature of velocity profile in channel, the shear rate increases significantly when particle closer to wall. Cherukat and Mclaughlin have shown that the effect of
rotation is very small but becomes important only when the shear is large and sphere is close to wall. As a result, the effect of rotation increases substantially near wall. Rubinow and Keller derived the analytic solution for rotation induced lift which is scaled by $a^3$. The direction of this force is determined by the cross production of rotation and relative velocity vectors ($\Omega \times V$). Since the rotation is caused by shear rate and the neutrally buoyant particles always lag behind flow velocity in Poiseuille flow, rotation induced lift is thus upward the flow velocity toward middle point of wall. Although it is unclear if the simple superposition of the three lift is valid in the presence of an ambient shear flow, it is possible that particle motion near wall is dominated by spinning lift according to Zeng’s numerical results. If in this case, then particles near wall would further migrate to the center of the wall which is in agreement with experimental observations in square and rectangular channel (Fig. 7c). Indeed, our results in this work also suggest that particle spinning is dominant in case of wall region where shear and wall forces are cancelled. In addition the drag force increases logarithmically as particles are closer to channel wall which can be justified in terms of the added viscous effect arising from the presence of a nearby wall. This enlarged drag force is responsible for preserving particle stably in their equilibrium positions.

As a result of the shear and rotation lifts particles close to channel center first migrate to walls and then translate in parallel to the center of wall, which is two-stage migration. Finally they stay in the middle position undergoing the drag force (Fig. 7a). The schematic of the topview and cross-section view is also present in Fig 1b. The lateral migration along the wall has been demonstrated in low aspect ratio channel in Fig. 7c. However according to the condition of substantial effect of rotation lift particles in the corner can migrate along either of the two nearby walls depending on the shear rate, which further implies that both channel aspect ratio
$(AR=$height/width$)$ and $Re$ can modify the focusing positions in rectangular channel since both of them can alter the magnitude of local shear rate close to walls. More specifically if the closer to 1 of $AR$ or the higher $Re$, the more possible of four focusing positions even in rectangular channel. Indeed this has occasional presented in recent works.$^{105,108}$

The magnitude of the lift is conventionally expressed as dimensionless lift coefficient ($C_L$). The direction of lift is in cross production of vorticity and particle relative velocity vectors for vorticity (shear in this work) induced lift and the cross production of particle spinning and relative velocity vectors.$^{112}$ In the case of particles flowing in microchannel, the sign of lift coefficient for shear force is negative which means particles migrate away the channel center.$^{71,92}$ And the coefficient of rotational lift is always positive which means a lift upward velocity profile.$^{115}$ Since shear lift balances with wall force, the up lift drives particles toward the center of wall where employs maximum local velocity but little particle spinning. In this paper, for convenience, we denote the two coefficients as $C_L^-$ and $C_L^+$ and the corresponding forces are $F_L^-$ and $F_L^+$ respectively.

Characterization of lift coefficients is critical yet challenging. Fortunately, Asmolve derived an equation which estimates the net lift forces exerted on particle moving in a planar poiseuille flow.$^{92}$ This expression is as a function of particle position as

$$F_L = \rho G^2 C_L a^4$$  \hspace{1cm} (1)

where $\rho$ is fluid density, $G$ is shear rate, $C_L$ is the lift coefficient and $a$ is the particle diameter. Given channel width $W$ and height $H$, the shear rate in microchannel is $G = 2U_f/D_h$, where the hydraulic diameter is given by $D_h = 2WH/(W+H)$ and $U_f$ is the average flow velocity. Therefore, equation (1) can be rewritten as

$$F_L = \frac{4\rho C_L U_f^2 a^4}{D_h^2}$$  \hspace{1cm} (2)
Assuming that $F_L$ balance with Stoke’s drag expressed as

$$F_D = 3\pi \mu a U_f$$  \hspace{1cm} (3)

where $\mu$ is the fluid viscosity. Substituting $U_f$ with lateral migration velocity ($U_L$) in equation (3), we arrive an expression as

$$U_L = \frac{4\rho C_L U_f^3 a^3}{3\pi \mu R_H^2}$$  \hspace{1cm} (4)

Therefore the channel length required for full focus of particles can be written as

$$L = \frac{U_m L_m}{U_L} = \frac{3\pi \mu R_H^2 L_m}{2\rho U_f C_L a^3}$$  \hspace{1cm} (5)

where $U_m$ is the maximum flow velocity ($U_m = 2U_f$) and $L_m$ is the particle migration distance.

Thus the lift coefficient is given as

$$C_L = \frac{3\pi \mu R_H^2}{2\rho U_f a^3} \times \frac{L_m}{L}$$  \hspace{1cm} (6)

Therefore as long as we are able to determine particle migration distance ($L_m$) and focusing channel length ($L$), lift coefficient can be obtained. A rectangular cross-section microchannel provides a great opportunity for experimental determination of the two unknown parameters because of the two specific focusing positions. Particles in the center of cross-section will migrate to the long length undergoing the negative lift ($F_L^-$) (Fig 1a) and thus the migration distance is about half of the short length ($H/2$). Similarly particles in the corner are directed toward the center of long length by positive lift ($F_L^+$) and the transportation length is approximately half of the long length ($W/2$).

In terms of focusing length ($L$), we have to find it experimentally. Since particles in rectangular channel in first stage migrate (vertically in Fig 1b) to the long walls forming two bands (Fig 1b.i $\rightarrow$ ii), the focusing length $L^-$ for $C_L^-$ is then the channel length required for particle arrangement near walls. Once particles fully arrange near walls, positive lift ($F_L^+$) takes the
control causing the horizontal migration to stable positions in Fig 1b (ii→iv). This downstream length \( L^+ \) is the focusing length for \( C_L^+ \). Since the lift coefficient is as a function of particle position within the sheared flow, the measurements in this work are the average along the migration direction. Therefore it carries the major information of lift coefficient, such as relation to flow condition.

To find the focusing length, our general approach was to visualize flow of fluorescently labeled microparticles in microchannels using an inverted microscope, an approach analogous to microparticle streak velocimetry (μ-PSV). We imaged various downstream positions. Flowing particles generated streaks across each frame, and we analyzed fluorescent intensities and locations of particle streaks. We used standard soft lithography to fabricate devices in this chapter. Fluorescently labeled, neutrally buoyant polystyrene particles 7.32 to 20 \( \mu \)m in diameter were used to prepare suspensions in this chapter. Particles were mixed at 0.025% volume fraction in deionized (DI) water to minimize the particle-particle interactions.

**Determination of the negative lift coefficient**

Particles in high \( AR \) channel focus near sidewalls as long as proper ratio of particle size to channel hydraulic diameter \( a/D_h > 0.07 \) is maintained, as we have shown previously \(^71\). We first used small particles \( a/D_h = 0.11 \) as their focusing length is supposed to be longer which can potentially minimize the measurement error. Fig. 8a shows a suspension of particles at the inlet (0 mm), spanning the entire channel width. At 9 mm downstream, particles order into two streams near sidewalls, generating a particle-free region in the center. This is in agreement with our previous work \(^70,71\).

To determine the focusing length, we measured fluorescent intensity line scans across channel width at various downstream positions. The results in Fig. 8b show progressive
Fig. 8 Measurements of downstream length for two-band focusing. (a) Fluorescent images at four downstream positions showing the particle ordering near the sidewalls at $Re=120$. (b) The corresponding line scan plotting normalized fluorescent intensity curves at four channel locations. The decrease of the FWHM of the primary peak (higher peak of each curve) clearly indicates the progressive focusing with respect to downstream length. (c) FWHM as a function of downstream length at various $Re$. This result indicates that higher $Re$ requires longer channel to fully develop the focused streams. (d) The refinement of FWHM of four particles in the same channel at $Re=50$. The channel was 50 $\mu$m wide and 100 high $\mu$m. Particle volume fraction $V_F=0.025\%$. Fluorescent image here is a superposition of 100 frames to improve both the visualization and measurement. Particle size used in a-c was 7.32 $\mu$m. Dot lines in this work represent the approximate positions of channel walls.
entrainment of particles as they flow downstream. The intensity of the middle region decreases, which suggests depletion of particles, while the intensity near sidewalls grows forming two peaks due to migration of particles from channel center. Quality of particle focusing can be quantified by measuring full width at half maximum (FWHM) of the intensity peaks. The reduction in FWHM indicates that particles are focusing tighter and tighter; once the width stops decreasing and remains unchanged, the migration toward the channel wall has completed. The downstream position at which this occurs is the focusing length. Note that particle migration along the vertical sidewall does not affect the peak width in this case.

Repeated experiments at multiple $Re$ ($Re=\rho UfDh/\mu$) lead to an interesting chart of the focusing development with respect to downstream length as in Fig. 8c. First of all, all the curves converge to approximately particle diameter after certain channel length. This result validates our approach of measuring the focusing length. More importantly, increasing $Re$ leads to longer channel length needed for particle focusing, suggesting lift coefficient is not constant in microchannel. Indeed the numerical outcomes in macroscale have revealed similar property.92, 96

Close examination of curves show that particle migration is not uniform, as FWHM first decreases very slowly followed by a sharp drop. This can be explained by the parabolic nature of the velocity profile. Shear rate in the channel center is the smallest and then increases linearly. Since shear force is dependent on the second power of shear rate, as expressed in equation (1), the lateral migration velocity accelerates in a quadratic manner which leads to fast migration. When particles are close to walls, the migration velocity reduces exponentially due to the logarithmic increase of drag force and wall induced lift force counteracting with lift force.98, 99 Thus, the curves converge slowly after sudden drop.
Further experiments were performed to explore effects of particle size which is supposed to modify the focusing length. Four particles ranging from 7.32 to 20 μm in diameter were introduced into the same channel at identical flow conditions and their focusing progress is shown in Fig. 8d. As expected, large particles migrate much faster due to larger lift force according to equation (1). Thus they require shorter channel length for focusing. Decreasing particle diameter, however, does not lead to drastic increase in focusing length but rather acts in a more linear way with respect to reciprocal of particle size.

Measurements of focusing lengths at various Re shown further consolidate the aforementioned hypothesis of non-constant lift coefficient and mild effect of particle size (Fig. 9a). The separation between adjacent curves increases linearly as particle size reduces at various Re. Small particle (e.g. 7.32 μm) is more easily affected by flow condition (Re) as the curve is less flat than that of 20 μm. Apart from the result that high Re moves the curve upward, low flow rate also gives resembling effect which suggests there exists an optimal flow condition in terms of focusing length. While low Re effect is reasonable if referring to equation (5), the results of high Re suggest decrease of lift coefficient. Indeed this trend is explicit when calculated the lift coefficient based on the focusing length as presented in Fig. 9b.

The curves in Fig. 9b decay exponentially. Similar curves of numerical results have been reported in other papers.\(^99,115\) Loth & Dorgan suggested that Saffman lift coefficient is dependent on \(Re_p^{-0.5}\) (particle Re) in his numerical analysis paper.\(^112\) This actually implies the dependence on \(Re^{-0.5}\) as well. One may also infer this relation based on analysis by Asmolov.\(^92\) The lift coefficient \((C_L^-)\) as a function of \(Re^{-0.5}\) is shown in the inset. Although Zeng et al.\(^97\) proposed dependence on \(Re^{-0.44}\), our results exhibit high linearity \((R^2 > 0.99)\) as a function of \(Re^{-0.5}\). In terms of magnitude they are also comparable to Lee’s numerical results in certain case.\(^99\)
Smaller particles exhibit larger coefficient, suggesting an inverse relationship. We calculated the lift coefficient for particles at multiple Re. Fig. 10a presents results as a function of $a^{-2}$. The curves show high linearity ($R^2 > 0.99$). Again, low Re exhibits higher $C_L^-$ which is consistent with previous discussion. From these results, we conclude that $C_L^-$ is proportional to $a^{-2}$ ($C_L^- \sim a^{-2}$). If this relation is used in equation (2), it yields $F_L^- \sim a^2$, which is corresponds to Saffman lift or shear induced lift.\textsuperscript{112, 115} This agreement with numerical results also shows that Asmolov’s equation for parabolic flow condition is applicable in microchannel.

We next investigated the effects of channel dimensions on the negative lift coefficient. First we fixed the channel AR, but scaled the cross-section and then ran the same particle suspensions to determine focusing length. From these measurements we calculated $C_L^-$ data for each channel, as shown in Fig. 10b. As expected, the coefficient increased with increasing
Fig. 10 Negative lift coefficient as a function of particle size and its dimensional effects.

(a) The dependence on -2 power of particle size. Different particles were injected into the channel at four Re to examine the size effect. This chart not only indicates that small particle employs large coefficient but also exhibits its low Re preferred nature. The size of the particle is in the unit of \( \mu m \).

(b) Given fixed aspect ratio, doubling the area of cross-section renders to approximate 4-fold increase of the curve slope. (c) Experimental outcomes of running particle into three channels sharing the same height (~50 \( \mu m \)) but distinct width (W). Comparison of these curves shows a 2 power dependence on the smaller dimension. (d) Minor effect of the long edge when experimented in three channels with controlled short length. The first two widths were both 50\( \mu m \) and the 3rd width was ~45 \( \mu m \). The particles used in (b-d) were 7.32 \( \mu m \) diameter.
channel size. Comparison of the slopes shows that $C_L^-$ scales approximately with cross-sectional area. Additional experiments which fixed channel width showed that the lift coefficient varies with channel width ($C_L^- \sim W^2$) which is the smaller dimension, as data in Fig. 10c show. Plotting data for varying heights (Fig. 10d) shows that the first two channels, which have similar width, are approximately the same but the $C_L^-$ of the third channel deviates from the other data points. This is most likely due to ~10% variation of channel width during fabrication since width contributes significantly as indicated by the first set of results (Fig. 10c). From these results, we conclude that the negative lift coefficient is dominated by the smaller dimension in rectangular channel. This actually has been implied by others previously $^{70,71,110}$ when channel width was used instead of hydraulic diameter as the characteristic dimension in a high AR channel. The predominant effect of width here is related to the coincidence of migration direction and the higher shear rate along small dimension.

Overall, our results show that in high aspect ratio microchannels negative lift coefficient scales with the -$\frac{1}{2}$ power of $Re$ and -2 power of $a$ and 2 power of $W$. These experiments therefore lead us to an expression for the negative lift coefficient as

$$C_L^- \sim \frac{W^2}{a^{2\sqrt{Re}}} \cdot W < H$$  (7)

In combination of this expression, equation (5) and the measurements in this work, it permits accurate prediction of channel length for particle lateral migration. As a result it enables a number of potential applications, such as guidance of designing and improving the performance of filtration platforms in high AR channel. Next we examine the positive lift coefficient…
Determination of the positive lift coefficient

Particle rotation induced lift force (positive lift) has not been described in microchannels, yet it is associated with migration behavior of particles. Unlike the negative lift which directs particles down velocity gradient, the positive lift guides particle migration upward the gradient. It is therefore responsible for particle migration along channel perimeter toward sidewall centers. The result is four equilibrium locations near sidewall centers in a square channel or two stable positions along the middle of the longer sidewalls in a rectangular channel.

To investigate the effect of positive lift, we performed experiments in low $AR (AR=H/W <1)$ channels. As in previous experiments, we measured fluorescent intensity line scans across channel width at various downstream positions. The results for 7.32 μm diameter particles, Fig. 11a, show three streams – one primary stream in the center and two weak streams near sidewalls, which disappear after sufficient downstream length. The observation of three streams rather and broad bands could be resulted from two facts. On one hand, particles flowing closer to sidewalls undergo doubled wall-added viscous effect due to two adjacent walls, which significantly increases drag force. Hence the net force of drag and rotation-induced lift is much smaller in the corner. On the other hand, the distance from particle to wall significantly affects the drag force. According to Zeng et al.’ results, particle in the intermediate distance to wall experiences smaller drag compared to that of close to wall, which implies larger net force. As a result the migration velocity of particles in the corners is much smaller than those in intermediate positions, which lead to the temporary two side-streams. It is easier to observe two side-streams of small particles than of large ones as small particles are closer to walls and thus stay there for longer period. Increasing $Re$ leads to similar formation of three streams even for large particles since particles are found to be even closer to walls as $Re$ increases. Indeed we have observed
Fig. 11 Single stream development in low aspect ratio channel and its focusing length measurement. (a) Fluorescent images showing the particle ordering at different downstream positions. The $Re$ was 70 and particle size was 7.32 μm with $VF=0.025\%$. Three streams were observed before full stream pattern developed. However only one stream was observed for 20 μm particles. (b) Corresponding line scan illustrating progressive focusing in to single band. (c) FWTM as a function of downstream length at three $Re$ for 7.32 μm particle. After specific length, the curves become flat. The length at turn point is considered as focusing length. Higher $Re$ tends to require longer channel for full focus. (d) The entraining development of four particles in the channel at $Re=50$. Large particle reaches stable equilibrium position in a much fast way compared to small sphere. The device used is 50 μm × 27 μm in cross-section.
two side-streams of 20 μm particles at $Re=180$. Therefore the interaction of drag force and rotation lift could result in different migration behavior depending on particle size and $Re$. We term this as corner effect in this work.

To determine the focusing length, we again measured fluorescent intensity line scans across channel width at consecutive downstream positions. The results in Fig. 11b show progressive migration of particles as they flow downstream. The small peaks representing side streams almost disappear at the focusing length as indicated by the curve at 20 mm. Due to presence of side streams, we used full width at tenth of maximum (FWTM) to quantify the fluorescent linescans. As with FWHM, the reduction in FWTM indicates that particles are focusing tighter and tighter; once the width stops decreasing and remains unchanged, the migration away from the channel sidewalls has completed. The downstream position at which this occurs is the focusing length.

The progressive evolution of FWTM, shown in Fig. 11c, is similar to that of the negative lift presented earlier. The curves exhibit a downward slope, until convergence at focusing length ($L^+$). Again, small shortening of the width followed by a sharp drop is apparent. The rapid decrease indicates that particles leave the control of corner effect. Opposite to the negative lift, shear rate decreases when particles approach the middle of the wall where the local velocity is maximum. This reduced shear rate causes particles to migrate slowly toward the stable equilibrium position, with peak width eventually reaching a constant value. Note that before stabilizing, particles appear to oscillate, as indicated by slight fluctuation of the peak width. This is consistent with observations by others $^{121}$ and is likely due to particle-particle interaction as a result of enhanced local concentration.
Smaller particles were found to require longer downstream length for complete focusing. Fig. 11d shows the results of experiments with particle suspensions of four different sizes. This focusing behavior is similar to the results for the negative lift, and of course is expected since inertial focusing exhibits strong dependence on particle size. One notable difference, however, is that there is no mild decrease (or flat region region) for the 20 and 15.5 μm diameter particles, which was observed for the other two particles. Moreover, the size of the flat region appears to be dependent on particle diameter, with smaller particle exhibiting a larger region, which indicates that smaller particles remain near corners longer. This observation is in agreement with the preceding discussion of corner effect.

To compare the focusing length for particles of different diameter at various Re, we plot the data in Fig. 12a. While the curves resemble the trend for negative lift (Fig. 9a), the focusing length ($L^+$) is much longer than that of $L^-$, indicating slow migration velocity along channel wall. Furthermore, the spacing between the adjacent curves here is increasing faster as particle diameter decreases. The focusing length has exhibited a dependence on $a^{-2}$ rather than $a^{-1}$ in the negative case, which has also been suggested recently by Lee et al. for the square channel.\textsuperscript{85} Therefore, we conclude that particle focusing length in rectangular channels is mainly controlled by the slow process of positive lift.

Smaller particles exhibit a larger positive lift coefficient, suggesting an inverse relationship, analogous to the negative lift. We calculated the positive lift coefficient for particles as a function of Re (Fig. 12b), and found it to scale with $-\frac{1}{2}$ power. This is not surprising considering that both positive and negative lift forces are related to the shear rate.
The magnitude of positive lift is ~10× smaller than that of the negative lift. Regardless of particle position, these results are in agreement with Saffman’s conclusion of ignorable rotational lift which is an order of magnitude smaller as compared to shear induced lift in the unbounded case.\[^{114,115,117}\] Furthermore, the magnitude of the positive lift coefficient is comparable to the numerical results by Kurose and Komori (note that our particle \(Re_p\) is in the range of 0.4-30).\[^{115}\] From our experimental results, we conclude that rotational lift (positive lift) dominates particle motion near walls, which was also suggested by McLaughlin.\[^{89}\]

Presenting the positive lift coefficient as a function of particle diameter reveals a direct inverse relationship (Fig. 13a). Thus, the dependence of positive lift on particle size is not as strong as that of the negative lift, which is a function of the second power (\(C_{L}^{-} \sim a^{-2}\)). Since \(C_{L}^{+} \sim a^{-1}\), plugging it into equation (2), we find that positive lift force is highly dependent on
Fig. 13 Particle size dependence of positive lift coefficient and the dimensional effects. (a) Positive lift coefficient which is inversely proportional to particle diameter. This graph indicates the larger particle the smaller coefficient. The measurements were taken in the same channel with 50 μm width and 27 μm heights in cross-section. (b) Effect of channel size at fixed AR. Enlarging channel increases lift coefficient. (c) Width and AR effects on positive lift coefficient. The channel heights was fixed at ~27 μm. Basically stepping up the channel width first elevates the coefficient and then acts in opposite way as shown in the horizontal curves (AR=H/W). Increasing Re suppresses lift coefficient in all channels. (d) Channel height effect. The widths of the two channels were ~100 μm but one height was ~27 μm and the other was ~50 μm. The slope of high channel is approximately 4× larger. Particle size was 20 μm in (b-d).
particle diameter, as \( F_L^+ \sim \alpha^3 \). This relationship is consistent with both Saffman’s higher order component and Rubinow and Keller’s expression.\(^{112, 114, 115, 118}\) The agreement with previous works again indicates that particle migration near walls is dominated by rotational lift. Considering that shear induced lift (negative lift) is balanced by wall induced lift, this is also reasonable.

We next investigated the effects of channel dimensions on the positive lift coefficient. Increasing channel cross-section led to approximately parabolic increase in slope (Fig. 13b). However it is not clear which dimensional parameter (cross-sectional area, channel height, or channel width) is the major contributor.

Our experiments show that positive lift coefficient exhibits dependence on \( H^2 \) (Fig. 13d). This is analogous to the dependence of the negative lift coefficient on \( W^2 \) (Fig. 10c). In both cases, the lift coefficient is dependent on the smaller channel dimension. In the case of the positive lift coefficient, however, the underlying mechanism is different. The change in positive lift is based on that of the negative lift. While negative lift acts parallel to the smaller dimension, positive force acts perpendicular to the smaller dimension. In the case of negative lift, expanding the smaller dimension renders to an increase of coefficient due to reduced flow velocity at a given \( Re \). But negative lift decreases according to equation (2). Diminished lift force then modifies the focusing position relative to channel walls. More specifically, it causes the position shift to channel center, which has been demonstrated in several previous investigations.\(^{82-85, 93, 120}\) When particles are far away from channel wall, the drag force reduces rapidly.\(^{96, 99}\) Therefore, in the case of positive lift, the net force increases. Consequently the positive lift coefficient increases.
We also investigated the long dimension and AR effect on positive lift coefficient (Fig. 13c). As channel width increases from 50 to 200 μm (height fixed at $H \sim 27$ μm), the slopes of the linear fitting curves also show slight change accordingly. It can be easily explained in terms of shear rate along the long dimension ($W$) which is parallel to the positive lift. Increasing the long dimension alters the local shear rate near channel width and thus affects the lift force. Expanding dimension leads to low velocity at given $Re$, which also impacts the lift. However, the effect of long dimension is minor compared to that of the short dimension.

Microchannel AR has a non-linear effect on the positive lift coefficient (Fig. 13c). To reduce the possibility of four focusing positions which may introduce potential errors, we tested the AR effect for the value much smaller than 1 according to the condition of rotation effect described in the theory section. Basically, first decreasing AR from $\sim$0.54 to $\sim$0.27, $C_L^+$ increases and then it drops with further diminishing AR, which implies an optimal AR offering the maximum coefficient. Possibly this is due to the modification of shear rate distribution.

Overall, our results show that lateral migration along microchannel wall is primarily dominated by the rotation induced lift (positive lift force $F_l^+ \sim a^3$). The positive lift coefficient has shown the dependence on $Re^{-0.5}$, analogous to the negative lift coefficient. It also appears to scale inversely with particle diameter $a$ and 2 power $H$. These experiments therefore lead us to an expression for the positive lift coefficient as

$$C_L^+ \sim \frac{H^2}{a \sqrt{Re}}, W > H$$

(8)

Since we have already provided the positive coefficients for four particles, it is easy to estimate the coefficients for other particles in different channels using expression (8). Particle behavior in the microchannel is then precisely predicatable in the combination of negative and positive coefficients. Thus a general design rule for inertial microfluidic channel is then permitted.
We have verified this expression by scaling the particle size and channel dimensions correspondingly. Briefly 10 μm particles were injected into 50×27 μm² (Width × Height) channel; 20 μm and 43.2 μm particles were tested in channels with heights of 50 and 100 μm respectively. The focusing performances of the three sets were shown in Fig. 14 which indicates similar focusing length of ~8 mm. Next, we further discuss our experimental work and its implications.

![Fig. 14](image)

**Fig. 14** Evaluation the combination of particle size and channel dimensional effects. Three particle suspensions were tested in different channels with dimensions scaled linearly. The curves of FWTM indicate similar convergence at ~8 mm downstream position for the three cases.

**Discussion**

Based on our experimental work, particle focusing in rectangular microchannels occurs in two steps. Randomly distributed particles flowing through the channel first migrate from the center to the long length of cross-section forming two broad bands near walls. Then, particles further aggregate to the middle of the long wall and get stabilized forming tight streams. Our results conclusively show that the first step occurs rapidly, while the second step is slow.
Different mechanisms are associated with these processes. Our previous works 70, 71 have pointed out that shear induced lift (here we call it negative lift) is responsible for the lateral migration toward the channel wall. As particles migrate closer to walls, the rotation induced lift (positive lift) gains strength while the shear lift is balanced with wall induced lift force. The positive lift then drives particles from the corner up the velocity gradient toward the middle point of the wall, where velocity profile is symmetric. This point is the stable position.

Our experimental results indicate that both negative and positive lift coefficients exhibit an inverse dependence on flow ($C_L \sim Re^{-0.5}$) and a quadratic dependence on the smallest microchannel dimension. However, particle diameter exhibits different influences—negative coefficient is scales with $a^{-2}$ while positive coefficient scales with $a^{-1}$. This difference has led to a distinct particle-size dependent feature between negative ($F_L^- \sim a^2$) and positive ($F_L^+ \sim a^3$) lift forces. Based on our two-step focusing behavior in low aspect ratio rectangular microchannels, the complete focusing length for particle migration to their two stable position centered long lengths can be derived from equation (5) as

$$L = \frac{3\pi\mu D_R^2}{4\rho U_f a^3} \left( \frac{H}{C_L} + \frac{W}{C_L} \right), \quad W > H$$

(9)

Considering that $C_L^+ < C_L^-$ as in Fig. 15 and $W>H$ in a rectangular microchannel, $\frac{W}{C_L^+} > \frac{H}{C_L^-}$. Hence positive lift is dominating the channel length required for complete focusing. One have to note that this expression is for particle migration to the two positions centered $W$ since particles may occupy additional two positions centered $H$ depending on flow condition and particle size as in previous discussion. Equation (9) is applicable to other channel with different cross-section, such as square and circular ones. In as square microchannel, $H=W$ and in round microchannel, $W=0$ and $H$ equals to diameter. Hence in round channel, only the first stage happens that is dominated by shear induced lift force ($C_L^-\,$). As a result, particles form Segré annulus near walls in circular
microchannel. Therefore equation (9) can be the general guidelines for microchannel design which takes the advantages of inertial migration.

Our results further show that the negative lift coefficient is much larger in magnitude (~10×) than the positive lift coefficient for the three particle diameters tested. The results, presented in Fig. 15, also suggest that the negative lift (10⁻⁸ N) is larger than the positive lift (10⁻⁹ N). This is in agreement with the accepted view that rotation induced lift is usually small but it becomes significant when close to wall. And it is also in good agreement with Saffman’s results which show shear induced lift is generally much larger than lift due to rotation.¹¹⁴,¹¹⁵ Nevertheless, positive lift is very important in the case of narrow channels since it dominates and determines length needed for complete focusing. We note, however, that the coefficients obtained in this work are the average along the particle migration path since lift coefficient is as a function of position in the flow.⁹²,⁹⁶,⁹⁹,¹¹²

Ultimately, we confirmed the proposed underlying mechanisms responsible for the dissimilar particle migration behaviors towards and away from microchannel walls. Compared and analyzed with previous numerical results, our outcomes suggest that shear induced lift (negative lift) is the main leading force that drives particles away from channel center and toward the walls. As particles approaching channel wall a counteracting wall induced lift arises primarily due to the vortices generated according to wake. Once these two forces balance each other the rotation induced lift (positive lift) takes control and acts on the particle resulting in a net force along the wall toward the center. Particles then migrate to the stable positions centered the faces where little spinning presents due to minimum shear rate. This spinning induced force is considered to be only significant close to walls.
Fig. 15 Comparison of negative and positive lift coefficients in the same channel. Note the difference between their measuring paths. Negative lift coefficient was based on the experimental results obtained in a 50 μm × 100 μm channel and the positive coefficient was based on a 100 μm × 50 μm channel. Particle sizes were 10, 15, and 20 μm respectively. Although they are distinct in directions, the magnitude of negative lift coefficient is ~10 times larger. It implies fast formation of two bands but slow migration in single stream ordering.
CHAPTER 4

INERTIAL TRAPPING FOR ISOLATION

This chapter introduces a simple device for isolation and enrichment of cells. The effect of vortices generated in microchannel is used for high sensitive and size-selective particle isolation. The high throughput isolation in the simple system is shown both numerically and experimentally. To understand the working principle, series of experiments were carried out to investigate the multiple parameters that influence the performance. The device is capable of isolating particles with concentration as low as 1 per mL. The different vortex shapes of particle vortex were examined and the size-dependence nature has been quantitatively characterized as threshold Reynolds number, which also relies on the length scale of cavity opening within the channel. The results reveal the optimal flow conditions, which are found to rely on channel length prior to cavities. These detailed investigations of the approach provide a practical overview and design rules for its implementation in specific applications.

Introduction

As already discussed, size-based separation and sorting is critical in many biological applications including sample preparation\textsuperscript{122,123}, blood cell sorting\textsuperscript{4,124,125} and cancer cell isolation.\textsuperscript{9,10} Conventional techniques usually utilize membrane to do filtration or concentrate/separate cells using centrifugation based equipment.\textsuperscript{126-128} While widely used, they
suffer from clogging. To overcome the limitation, a number of platforms have been developed to realize membrane-free filtration in microscale.

Recently, several groups reported on a passive approach based on the formation of microvortices to fulfill size-selective separation. Vortex is formed by modulating channel geometry which is essentially takes the advantage of conventionally so-called dead volume. Expansions right after a straight channel combined high flow rate have been utilized to create microvortices. Multiple promising potential applications employing this method have been demonstrated, such as extraction of plasma from blood and isolation of rare cells. Recent work also proposed an automated on-chip cell labeling which is supposed to simplify the process and save time. However, the separation efficiency is found to be only~20% which substantially limits its implementation in real world. Moreover the high flow rate for cell isolation may undermine the yield since the excessive shear rate may break cells. Although these works have successfully demonstrated the cell isolation and sample preparation in the systems, the separation size has to be 40 μm from blood cells in which the size of majority is around 8μm. In terms of proposed isolation of circulating tumor cells (CTCs), this size differentiation could present a problem in practice since the size of CTCs could be much smaller. Again because of few events of CTCs in blood, the selectivity and sensitivity are essential for follow-up analysis such as standard molecular assay. However these critical parameters remain less explored. The compromised performance of this approach is primarily due to the less explored working principle and unclear mechanism.

A series of experiments was conducted to explore in detail on how the size-based trapping occurs and what the factors affect the performance. Multiple variables such as flow condition, size of trapping region and concentration were investigated and the data generated
help better understand the mechanism. Our results have shown that the size dependence property of trapping can be characterized by threshold Reynolds number ($Re_{th}$) which is the triggering value of flow condition and the sample concentration has displayed an interesting effect on $Re_{th}$.

In order to help decipher the trapping mechanism, we also examined the particle behavior in the recirculating chambers. Due to the non-continuous process for sample preparation, we have also carefully looked at the time point for sample releasing and reloading in terms of separation efficiency. In short we believe that our broad and detailed research can be good guideline in the case of device design for specific application. Our results suggest optimization solutions that could overcome the aforementioned limitations and thus enhance device performance, for example improving the efficiency, size selectivity and yield (cell separation).

**Design principle**

Our previous work \(^{70, 71}\) has demonstrated that particles in rectangular straight microchannel migrate away from the center and equilibrate near the long edges because the wall induced lift force balances shear induced lift force. Therefore randomly distributed particles will form two bands along the sidewalls in high aspect ratio microchannel as illustrated in the straight segment of the channel depicted in Fig. 16a. The net lift force acting on a neutrally buoyant particle flowing in Poiseuille flow can be estimated using equation (2) in Chapter 3. According to Stoke’s Law, \(^{70, 71}\) the net lift force shall equal to the drag force ($F_D$) expressed in equation (3). Substituting $F_D$ in equation (2) with equation (3), the lateral migration velocity can then be rewritten as in equation (4). In other words particle migration in the Microchannel is highly size-dependent ($\sim a_p^3$). As a result large particles will migrate into their equilibrium positions faster than small ones in the same channel. Nevertheless, eventually all of them will focus into trains as long as $a_p/H>0.07$. \(^{71}\) In this case, by design proper outlet system, particles might be separated.
Fig. 16 Schematic of the device concept. (a). Lift and shear forces balance and focus cells before the expansion. This balance is disrupted in the expansion region, leading to vortices and size-based cell trapping. Simulation result (CFD-ACE+) indicating vortex formation (b) and the velocity magnitude profile (c) along the dot and dash line in (b), and experimental verification using fluorescent particles (d).
based on size in high aspect ratio straight channel.\textsuperscript{63, 71}

However, the separation efficiency and purity of the product are highly limited by the gap between the different streaks which is usually on several micros. If we add an expansion right after full focusing of particles, the force balance at equilibrium position is disrupted since wall induced lift force substantially reduces due to the long distance to the side wall. Therefore shear induced lift is dominant again and particles automatically regain lateral migration velocity directing them toward the expansion. According to equation (4), the velocity is strongly dependent on the particle size and flow rate as well. For a specific expansion requiring minimum lateral migration velocity ($U_L$), the average flow velocity ($U_f$) required for trapping is approximately proportional to the $-1.5$ power of particle diameter. As a result under the right flow condition we are able to push only large particles into the expansion while let small ones pass through. Then large particles can be isolated from the mixture in a much easier way.

To explore the separation based on trapping mechanism, we constructed a 3D model (ESI ACE+) which comprised of 1mm high aspect ratio straight channel and a pair of expansions (Fig. 16b). Flow module was used to trace the streamlines of water. To increase the accuracy, we took the advantage of unstructured mesh and used triangle mesh type, creating 3,137,926 cells with everyone sized less than 2 µm. The model was constructed to feature as 45 µm width, 90 µm height and total length of 2.3 cm, including the 300 µm ×300µm chambers in the middle. Multiple $Re$ were tested to ensure that vortices can be formed in the expansions. Combination of Z-cut and X-cut were implemented to find out the streamlines and velocity magnitude crossing the two vortex centers. The vortices were formed in the chambers as illustrated by the traces of streamlines.
We obtained the distribution of velocity magnitude (Fig. 16c) along the dash line delineated in Fig. 16b. Compared to the magnitude in the center, the velocity in the chambers is very low which means once particles enter the expansion they are dominated by drag force. Drag force keeps particles following the streams implying the visualization of vortices by introducing fluorescent micro beads. The form of vortices may vary depending on the flow rate since it might be primarily controlled by interaction between centrifugal force and drag force. The model guided us to design series of microchannels featured as different dimensions to further exploit and characterize the isolation of particles taking advantage of the temporary absence of walls and the low flow velocity in chambers.

**Experimental**

To investigate the mechanism of trapping particles by microvortex based on size, we designed a series of microchannels featured as different chamber sizes and various channel height as well as distinct focusing lengths. In order to validate the numerical results, we first fabricated a microchannel with 1 mm straight segment and 300 $\mu$m $\times$ 300 $\mu$m chambers symmetrically located at both sides of the channel. The height was 100 $\mu$m and width was 45 $\mu$m. Then another series of microchannels with chamber sizes varying from 300 $\mu$m $\times$ 400 $\mu$m, 400 $\mu$m $\times$ 400 $\mu$m to 500 $\mu$m $\times$ 400 $\mu$m were implemented to explore the dimensional effect on trapping particles. The focusing lengths of the three channels were 25 mm and the widths were fixed to 45 $\mu$m. Since the channel height affects the focusing performance, we also varied the channel height from 100 $\mu$m to 150 $\mu$m. All devices were fabricated in polydimethylsiloxane (PDMS) by soft photolithography described in Chapter 2. Preparation of particle suspension

Six fluorescent labeled particles were used to explore the size dependent trapping of the devices. Their diameters were 6 $\mu$m (Polyscience Inc.), 7.32 $\mu$m (Bangs Laboratories Inc.), 9.94
μm (Bangs Laboratories Inc.), 10 μm (Invitrogen®, Life Technologies), 15 μm (Invitrogen®, Life Technologies) and 20 μm (Polyscience Inc.). First, the particle suspensions were diluted in deionized water to volume fraction (VF) of 0.025% (56,800 particles/mL) to avoid particle-particle interaction. Then we also used particle concentrations of 568,000 /mL (VF=0.25%), 5,680/mL, 568/mL, 56/mL, ~6/mL and ~1/mL to further examine the exhibited concentration dependence and to find out the capacity of each device. A small drop of Tween-20 was added to each suspension to avoid potential clogging issue.

The general method was to image the device using high speed microscope in both bright field and fluorescent filter cubes. The sharp bright field images were analyzed and the number of particles trapped in the chambers were counted using Image J®. The pseudo-colored images present in this work were also completed by Image J®. Fluorescent images were obtained using DAPI, FITC and TRITC filter cubes. Images in Fig. 18 were obtained by overlying of 100 frames for better visualization. The approximate channel walls in this work are indicated by dash lines. The bright field images for collecting data with respect to trapping capacity (maximum number of particles that can be trapped in the chambers) were captured every 30s after stabilizing flow in the channels for 90s at each Re.

**Demonstration and characterization**

To demonstrate the concept of particle trapping in the device, 20 μm particles were introduced into a high aspect ratio (AR) channel with symmetrical cavities. Fluorescent image in Fig. 16d shows that particles regained lateral velocity and migrated into the cavities. The particle traces in the chambers clearly indicates the formation of vortices as predicted in the numerical model in Fig. 16b. Particle recirculation other than random trajectories imply low fluid velocity which is suggested by the model in Fig. 16c, leading to drag dominance of particle motion in the
cavities. In combination of particle trajectories and the streamlines present in the numerical model, particle recirculation is across multiple vertical planes in this case, suggesting the non-planar nature of the vortices.

Since the lateral migration velocity is strongly depending on flow velocity and particle size as in equation (4), we expected that particle trapping would require minimum velocity and would show size-selective property. 20 μm particles were introduced into the channel at different $Re$ to explore the effect of flow velocity. Fluorescent image in Fig. 17a indicates no particles were trapped in the cavities at $Re=54$. Increase of $Re$ has led to the observation of particle recirculating in the expansions as shown in the blue trajectories in Fig. 17b. Repeated experiments have helped us identify the threshold Reynold’s number ($Re_{th}$) which is corresponding to the minimum velocity required for particle trapping. For 20 μm particles, $Re_{th}$ was determined to be ~69. Particle size effect on trapping is also present in Fig. 17b. At $Re=69$, 20 μm particles were observed in the cavities while 15 μm particles were freely flowing through the expansion at the same time as indicated by blue and yellow trajectories, respectively. This size-based trapping permits the isolation of large particles out of mixture containing small species. Some other groups recently reported on sample preparation taking advantage of this property. In order to characterize the size-dependent trapping, five particles were tested in the same channel featured as 300 μm × 300 μm cavities to identify their $Re_{th}$. The results were plotted with respect to particle size in Fig. 17c, which shows exponentially decay of $Re_{th}$ as particle size increase.

The curve seems to be in agreement with the inference of equation (10), which implies the minimum flow rate (corresponding to $Re_{th}$) may scale with -1.5 power of particle size. However our outcomes has suggested the dependence on -1.2 power of size as expressed in
Fig. 17 Size dependence of particle trapping. (a) Pseudo-colored fluorescent image illustrating no trapping observed at $Re=54$ for 20 μm particle. (b) Superimposed fluorescent images indicating trapping of 20 μm particles (blue rings, DAPI) while 15 μm particles pass through (yellow streams, TRITC) at $Re=69$. (c) The dependence of threshold Reynolds number on particle size for channels with size differentiated chambers. The curves represent the calculated threshold $Re$ for different particles and the error bars for 300 μm × 300 μm chambers were the standard deviation of at least three data points. The concentrations of suspensions were 56,800 particles/mL.
following equation,

\[ Re_{th} = \frac{940000}{L_o \times a^{1/2}} \]  

(10)

where \( L_o \) is the length of expansion opening in \( \mu m \) and \( a \) is the particle diameter in \( \mu m \). Using this equation we have obtained the prediction curves for the five particles in other channels with larger cavities. It turns out that these predictions are highly consistent with experimental data points as in Fig. 17c. For the same particle, larger expansion moves the curve downward which means smaller \( Re_{th} \). For example, the \( Re_{th} \) for 10 \( \mu m \) particle is \(~170\) when \( L_o=300 \ \mu m \); however, it tremendously reduces to \(~110\) at \( L_o=500 \ \mu m \). This is possibly due to the longer trapping window length that offers more time for lateral migration. This equation actually provides a simple practical way to manage the flow condition for handling biological sample, such as isolation of cells which may be sensitive to shear rate and easy to damage.

While length of opening shows significant effect in terms of threshold \( Re \), we found less effects of other dimensions of chambers, such as chamber width \( (L_c) \) and channel height. Careful comparison of \( Re_{th} \) obtained for different channel heights, we found that higher channel involves slightly larger \( Re_{th} \), for instance, \( Re_{th}\approx85 \) for 100 \( \mu m \) height and \( Re_{th}\approx94 \) for 150 \( \mu m \) height channels with both featured as 300 \( \mu m \times 400 \mu m \) chambers for 20 \( \mu m \) particles. This effect may be due to higher channel requires larger flow rate to sustain the same flow velocity as in lower channel. In addition, high aspect ratio of chamber \( (L_o/L_c) \) also tends to increase the \( Re_{th} \). For 20\( \mu m \) particle, \( Re_{th}\approx69 \) for 300 \( \mu m \times 300 \mu m \) chambers but \( Re_{th}\approx85 \) for 300 \( \mu m \times 400 \mu m \) with all other dimensions identical. Possibly the deeper chambers possess more fluid which requires more energy to trigger the recirculation. As a result it requires higher the flow rate for deeper cavities and thus larger \( Re_{th} \) is necessary for trapping to occur.
During the determination of threshold $Re$, we noticed that the particle representation of vortex varies with particle diameter, chamber size and flow rate ($Re$). Fluorescent images in Fig. 18a-c show dissimilar particle vortices of three particles in the same channel at their $Re_{th}$. While 20 $\mu$m and 15 $\mu$m particles occupied single orbit in one chamber, two orbits were observed for small particles (7.32 $\mu$m) as in Fig. 18c. According to numerical model in Fig. 16b, these orbits shall not be in the same z-plane since parts of them were in superposition. The absence of multiple orbits for small particles is possibly as a result of limited space. This implication was further evidenced in comparison of Fig. 18a and d. Careful examination of these two figures, we found that the single orbit in Fig. 18a was possibly a complex of two orbits since the thickness of fluorescent trajectory is heavier than that in Fig. 18d. Both were results of 20 $\mu$m particles, but two orbits were observed in larger chamber. Although 15 $\mu$m particles were operated at $Re_{th}$ which is larger, the size of vortex is smaller than that of 20 $\mu$m particles (Fig. 18a-b). This observation might be resulted from the interaction of centrifugal and drag forces. Large particles experience relatively stronger centrifugal force that keeps particles further away from center toward the chamber walls.

Besides of particle size and chamber space, the flow rate is also an important fact affecting the particle recirculation as in Fig. 18d-f. Increasing $Re$ has led to asymmetric expansion of recirculation orbits which were distorted at high $Re$. As discussed previously, higher $Re$ will increase the recirculation velocity in the chamber as in Fig. 16c. The larger velocity generates larger centrifugal force that overcomes drag force and thus pushes particles outward forming extended orbits. In addition, the spacing between orbits in Fig. 18d were then filled with particles at larger $Re$ (Fig. 18e), indicating enhanced trapping capacity. Further
Fig. 18 Shapes and evolution of vortices of three particles in 300 μm × 300 μm (a-c) and 400 μm × 400 μm (e-f) at different flow conditions. 20 μm (a), 15 μm (b) and 7.32 μm particles trapped in the chambers at their threshold Re, respectively. All volume fraction used were 0.025%. (d) Multiple orbits of 20 μm particles at Re=154. (e) Increasing sizes and particle densities of vortices at Re=205. (f) Expansion of vortices and loss of particles at high Re. The volume fractions of (e-f) were 0.0025%. All images were pseudo-colored for better visualization.
increased Re seems to lose particles in the chamber as the intensity reduced (Fig. 18f). These results imply there is an optimal Re in terms of trapping capacity.

To determine the trapping capacity, we have imaged the chambers in bright field using high speed camera. Time lapsed images in Fig. 19a-d reveal the progressive feeding of particles into the cavities. While it was not clear how many orbits in at 10s, two orbits were evident after 60s. As we discussed previously, these two orbits may not be in the same z-plane but they were close to each other. Particle number after 60s were observed to be approximately the same (Fig. 19e), which indicating the trapping capacity of chambers. This number could be primarily due to the confinement of walls as well as the orbits that can be fit, as shown in the fluorescent images in Fig. 18a and d.

Since the centrifugal force is one of the determinative factors for orbit occupation, flow velocity shall also affect the trapping capacity. Higher velocity generates larger centrifugal force and thus expands the orbits outward. Hence, more particles can be accommodated in the recirculating tracks. As a consequence, capacity increases. To explore effect of flow rate in detail, multiple Re were tested using the same particle suspension in the same device. The results were present in Fig. 19e. The flat regions of these curves clearly indicate the trapping capacity. There are small peaks prior to the flat regions, implying over feeding of particles. Interestingly, increasing Re first elevates the capacity and then it serves in the opposite way as the flat region at $Re=325$ tremendously lowered that is consistent with the decreased intensity in Fig. 18f. For example, ~40 particles were counted at $Re=222$ but less than 10 were observed at $Re=325$. Based on these curves, the optimal flow condition might be around $Re=239$ as shown in Fig. 20.

The existence of optimal flow condition could be resulted from several essential factors. On one hand, at high Re the excessive centrifugal force is unable to create even larger orbit due
Fig. 19 Demonstration of 20 μm particle trapping and enrichment in a 500 μm × 400 μm channel. (a-d) Time-lapse images illustrating trapping of progressively larger number of particles. (e) Number of trapped particles as a function of time at various Re. The flat regions of the curves imply the capacity of the different Re. The small peaks of Re=256 and Re=325 indicate the optimal flow condition is Re<256. The concentration used was 568 particles /mL.
to wall confinement. On the other hand, particle-particle interaction takes place due to the high velocity in such a compact chamber and the particle begins to jump from one z-plane to another as implying by the blurry boundary of fluorescent vortices in Fig. 17f. These factors lead particles in the chamber to escape. In addition, the high Re offers particles very large travelling velocity, which could tremendously limit the time for lateral migration. This time-limited case stops feeding particle into the chambers. Unlike losing particles, before optimal Re, the increasing flow velocity can elevated the centrifugal force which not only create large focusing orbits but also serves to help particle occupy multiple z-planes.

Regardless of the capacity, the small volume of the chambers offers very high concentration as long as particles get trapped. As shown in the right vertical axis in Fig. 19c, the concentration of particles in the cavities was calculated on the order of $10^5$. The enhancement factor was $1000\times$ when initial concentration was 568 particles /mL. It is very promising if only 1 particle /mL was injected and then the concentration gain would be $10^5\times$ or more. Actually, we conducted additional set of experiments to capture particles in extremely low concentration suspension. Table 2 shows the result which implies the potential application in isolation of rare cells such as circulating tumor cells in blood.

As recent work 65 proposed that channel height would affect particle focusing in straight channel, it may also modify the trapping outcome. We fabricated two channels with 100 μm and 150 μm in height respectively to investigate the aforementioned effect on trapping capacity and time efficiency. The total number of particles trapped in the two devices are plotted as a function of Re in Fig. 20a. It seems that higher channel captured more particles in the chambers.

However, close examining at Fig. 20b, the trapping capacities ($2\times$ maximum number of two chambers in the same channel) only exhibit slight difference. As a result, the channel height
Fig. 20 Dimensional effects with respect to $Re$. (a) Height effect on total number of particle trapped. (b) Doubled maximum number of particles trapped in the two chambers which shows clearly the height doesn’t affect the trapping capacity much. (c) Time-lapsed curves indicating the saturation and time efficiency of the two heights. In (a-c), blue items represent the data obtained from channel with 100 μm height and red dots were those acquired from 150 μm height channel. The chamber sizes are 500 μm × 400 μm. The error bars are the standard deviation of 5 data points running at $Re=239$ after 90 second each time. (d) Comparison of total number of particle trapped vs. $Re$ in 4 types of chambers. The inset is the normalized curves which show where are peaks more explicitly. Channel heights were 100 μm. In all the four figs, particle size were 20 μm and concentration were 5680/mL, $VF=0.0025\%$. 
doesn’t affect capacity very much. Nevertheless, we would expect much smaller channel height (e.g. 50 μm) may serve to reduce the capability of trapping since there could be space-limited for particle recirculation. Although less effect on capacity, the larger height performed better in terms of time efficiency illustrating in Fig. 20c. The higher can be saturated faster than lower channel. Since the devices were tested at identical Re, the flow velocity in higher channel was slower than that in lower channel. Thus particles in former channel had more time to migrate into the chambers. Hence, higher channel could reach capacity faster.

As discussed previously, the chamber size can affect the trapping capacity. To further explore its effect, we conducted additional experiments in different devices. The results are present in Fig. 20d. Apparently, larger chamber provides more room for particle recirculation and as a result more particles were observed in the chambers. For example, 500 μm × 400 μm chambers captured as high as 80 particles while the number was less than 10 in the smallest chambers. In addition increasing any length of the two chamber dimensions (LO and LC) enhanced the capacity significantly. Unexpectedly all curves share similar optimal Re at which

**Table 2.** Tests of extremely low concentration in channel with 500 μm ×400 μm chambers at Re=256. Control was input only DI water.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>#1</th>
<th>#2</th>
<th>#3</th>
<th>Control</th>
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</thead>
<tbody>
<tr>
<td>Particle in 10mL</td>
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<td>9</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Volume used (mL)</td>
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<td>9</td>
<td>4.5</td>
<td>10</td>
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<td>4</td>
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<td>0</td>
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<tr>
<td>Particle lost</td>
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<td>0</td>
<td>1–2</td>
<td>0</td>
</tr>
</tbody>
</table>
maximum number of particles were captured. This should not be coincidence if comparing channel dimensions. More specifically, the focusing length of the channels may determine the optimal $Re$ as all these devices shared the same channel length before the chambers (focusing length).

To confirm the hypothesis, two devices with distinct focusing lengths (1mm and 25mm) but identical chambers were tested using the same particle suspension. Particles trapped in the cavities were counted and plotted as a function of $Re$ in Fig. 21a. Interestingly the peak shifts to left side by approximately 90 units. Specifically, the peak is at $Re\approx240$ for longer channel while $Re\approx150$ for shorter one. These results suggest the threshold $Re$ ($Re_{th}$) reduces as focusing length decreases. This experimental outcome may be explained as following: not all the focusing positions along the long length of the channel contribute to particle trapping. Previous work (Bhagat et al. 2008b, Bhagat et al. 2008c) disclosed that particles would focus into two bands (multiple positions) along the side walls in high aspect ratio channel while other researchers also found particle focusing in the center of channel walls (Di Carlo et al. 2009). Our speculation would be that particles focus into multiple positions along side walls in short channel; for long channel, particles could focus into their stable positions which locate in the middle of the walls. In this case, short channel provides more chance for particle to migrate into the channel. As in Fig. 16d, the particles seemed to travel into the chambers from the top and bottom and then screwed into the middle plane. But this pattern was never observed in long channel (Fig. 17).

These evidences imply there are preferred positions for particle to migrate into cavities which essentially determine the $Re_{th}$. These positions could be at quarters of channel height. These results provide another practical way to reduce the $Re_{th}$ for handling biological sample.
Fig. 21 Peak shift due to different factors. (a) Focusing length effect on trapping 20 μm particles. The red and blue curves represent data collected from channel with 25 mm and 1 mm focusing length, respectively. (b) The trapping capacity of 20 μm (red) and 15 μm (blue) particles. The channels featured as 500 μm × 400 μm chambers, 150 μm height. All particle concentrations were 5680 /mL.
In addition to the left-shift of peak, decrease of focusing length also substantially compromises the trapping capacity. Maximum of ~20 particles were captured in shorter channel compared to ~90 particles in longer channel. The underestimated capacity might be due to the effect of particle concentration. Higher concentration tends to squeeze more particles into the cavities as implied in comparison of Fig. 18e and Fig. 19d. Short focusing length is not sufficient for particles to migrate to the channel height. As a result the local concentration near sidewalls is smaller than that in longer channel. Therefore, fewer particles were observed. We will come back to this effect again later in this work.

Unlike that the short focusing length shift peak to left side, smaller particle moves the curve to the opposite side. Although the curve for 15 μm particle in Fig. 21b is incomplete due to limitation of PDMS which can’t sustain even higher Re, the trend is manifest. Moreover, this finding is in highly consistent with the size dependent feature that we discussed early in this work: Smaller particles require larger flow rate to get sufficient lateral migration velocity as in equation (10). According to these curves, we are able to only trap large particles while let 15 μm particles freely flow through (Fig. 18b) when below specific Re. In an appropriate range, we may get significantly high species ratio in the device even at high Re. For instance, at Re=239, more than 100 large particles were in the chamber while only a few (<10) 15 μm beads. Either of these two cases serves promising for particle isolation.

As mentioned previously, particle concentration also affects the trapping performance, which guided us to systematically examine the effect. We used 20 μm particles diluted in the factor ranging from 10 to 10^6 × with the lowest concentration of ~6 particles /mL. Re_{th} for different concentrations were determined experimentally. The results were plotted in Fig. 22a. At low concentration, the Re_{th} tends to converge at high value; in contrast, increasing concentration
renders to a lower $Re$. The error bars for high concentration are narrower than those of less particle suspensions. This might be due to the susceptibility of low concentration to particle loss, e.g. single particle stuck in the channel could result in remarkable change of the concentration for 6 particles/mL suspension. It could also be explained as few particles in the solution employ less chance to occupy the trapping preferred focusing position we proposed antecedently. This hypothesis comes to be more reasonable when looking at the unchanged peaks in Fig. 22c. If all particles were focused into preferred positions, there should be no concentration effect on $Re_{th}$, since particles at certain $Re$ in the same location experience identical forces which control their behaviors.

We further explored the concentration effects on trapping efficiency and capacity. Fig. 22b shows the trapping curves of the tested three concentrations as functions of time at $Re=239$. Very consistently of the three tests, the particles were captured continuously in a quite linear way until the chambers were saturated (Fig. 22b). And the low concentration requires more time to reach the capacity. The total numbers of particles that trapped corresponding to each $Re$ were present in Fig. 22c. The curve shifts down as concentration decreases. Considering the smaller change of $Re_{th}$ of the low concentration suspensions, we would expect their capacity curves to be similar as indicated by the first two data points of the lowest concentration. Hence, the peak of lowest curve was underestimated (limited by experimental condition). In terms of the capture efficiency, which is the number of particles trapped divided by total particles injected, the trapping is low-concentration preferred as lower concentration exhibits higher capture efficiency. This feature is actually appealing if taking the device in application of rare cell isolation. The efficiency eventually goes down because either the chamber gets saturated (high concentration suspension) or the injection rate is higher than trapping rate (slope before saturation). These
**Fig. 22** Concentration (# of particles /mL) effect on (a) threshold $Re$, (b) time-efficiency (the slope before saturation), (c) capacity at various $Re$ and (d) capture efficiency. The concentration is plotted in logarithmic scale to enhance visualization. The devices were featured as 500 $\mu$m ×400 $\mu$m chambers and 150 $\mu$m height. Both (b) and (d) were at $Re=239$. 
curves also provide a practical guideline in the case of particle/cell isolation as it tells when to release the captured sample.

We proposed to use this approach to extract and enrich rare cells. In this case, the concentration ratio between the noise (e.g. blood cells) and the target (e.g. rare cell) can be as high as $10^6$, which remains challenging till now. In Fig. 23 we have successfully demonstrated the isolation of cells. Particle spiked blood as first utilized to explore the practical potential of cell extraction. Both large cells (measured as $\sim 12 \, \mu m$) and $20 \, \mu m$ particles were observed in the trapping regions. Again two orbits for blood cells further confirm that space control the trajectories of the particles/cells in the expansion. The bright fluorescent rings in Fig. 23b imply that HPET cells were intact when recirculating in the restriction area. According to Fig. 17c, $12 \, \mu m$ cells should not be trapped; however the concentration effect took place as implied in Fig. 22a, which compromised the performance. Cell concentration was at least an order larger than that of particles. We conducted additional set of experiments to evaluate its effect in detail using the heterogeneously populated particle mixture. Mixtures of either $20 \, \mu m$ and $15 \, \mu m$ particles or

![Fig. 23](image)

**Fig. 23** Demonstration of cell isolation. (a) Representative image of running blood spiked with particles; both $20 \, \mu m$ particles and $12 \, \mu m$ cells are trapped. (b) Trapping of fluorescently-labeled human prostate epithelial tumor (HPET) cells ($15\sim22 \, \mu m$). The concentration was $\sim 2500$ cells/mL.
20 μm and 7.32 μm particles featured as various concentration ratio (Table 3) were introduced into the channel at Re=239. Our tests have demonstrated the successful extraction of one 20μm particle out of the mixture with 100,000 small particles.

Table 3. Extraction of particles from mixture with various concentration ratios. Binary mixture of 20 μm and 15 μm particles and binary mixture of 20 μm and 7.32 μm particles were tested in channel with 500 μm × 400 μm chambers. “Yes” represents trapping of 20 μm particles from mixture was observed. The ratio was initial concentration of large particle over that of small particles.

<table>
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<th>1:10²</th>
<th>1:10³</th>
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<td>Yes</td>
<td>Yes</td>
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<tr>
<td>20:7.32</td>
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<td>N/A</td>
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</table>

Summary

We have successfully demonstrated the simple planar microfluidic structure for isolation and enrich of particles based on size by taking advantage of the geometrically generated micro vortices. The formation of the vortices was predicted by the numerical model and validated by the particle experiment. Since the particle migration strongly depending on flow condition, we identified the threshold Re (Re_{th}) to characterize the particle behavior in the device. Furthermore, we proposed an empirical equation to predict Re_{th}, which help others to design channel for their specific application. We also found the capacity of each channel with different dimensions corresponding to Re, which reveals the optimal flow condition for trapping as well as for separation. Many effort were paid on the effect of concentration, we discovered the concentration dependence of Re_{th}, trapping capacity and capture efficiency as well as time efficiency. By comparison of focusing length effect, Re_{th} trend and the unchanged peak of
capacity-Re curves at distinct concentrations, we suggest there are preferred positions for trapping along the long length of the straight channel. In consideration of separation applications, we experimentally verify the feasibility of isolation and enrichment of both extremely low concentration (~1 particle /mL) and very high selectivity (species ratio $10^5:1$). More encouraging cell isolation has been demonstrated in the device using HPET cells.
CHAPTER 5

INERTIAL FILTRATION AND RECONCENTRATION

Purification and concentration are essential in many biomedical and environmental applications, yet conventional techniques based on centrifugation and flow cytometry are insufficient due to the multitude of time-consuming and laborious steps and the need to process considerable amount of sample and. Approaches using microfluidics to address these shortcoming have not been completely successful, leading to either loss of target or slow removal of waste fraction (only 1/3 per stage). In this work, for the first time we present an approach based on inertial microfluidics that offers superior performance of purification and concentration (>3×) in a low aspect ratio channel with nearly 100% efficiency. Following filtration, viability and proliferation assays show that cancer cells preserve their functionality which is critical for follow-up processing and analysis in life science research.

Introduction

Concentration and purity of bioparticles can be very critical in the process of detection, monitoring and analysis in both environmental and biomedical applications, such as monitoring pathogen levels in water and detection of rare cells in blood stream. In particular, isolation of viable and functional cells from complex sample matrices remains challenging in both research and clinical settings. Large amount of sample is generally necessary to obtain reproducible analysis and thus continuous processing could be helpful. Conventionally centrifuge is one of the most common instruments in biological, medical and clinical laboratories
for sample preparation which typically requires multiple steps of centrifugation and resuspension. This process is usually time-consuming and laborious for diagnostic and research. Another standard approach for cell sorting and separation in clinical application and biomedical research is the flow cytometry which provides extraordinarily rich information regarding the input sample. However it again suffers from time-consuming and costly issues due to the typical requirement of large amount of sample to process.

Microscale devices potentially help address these challenges since they are typically high throughput and require small reagent and thus reduce cost. Localization of particles and cells in a physical region is the most common way in terms of filtration and concentration. While membrane-based method is widely used in filtration, the physical and chemical properties of membrane such as pore size and charge limit the outcomes. Furthermore these systems are normally unable to concentrate sample because of the little reduction of volume of output. Another mostly used approach of localization involves mechanical trapping other than membrane filtration. While these microfluidic devices may help purify and are able to re-concentrate sample, they generally requires multiple steps including trapping and washing which lead to complicated process control and considerable amount of time. Most of these prototypes were demonstrated using particles, which have not yet demonstrated for cell filtration. In addition working condition of low flow rate further downgrades their performance.

Similar approach but based on microvortex was reported for automated sample preparation. Unfortunately the system is subjected to low efficiency and purity.

Recent development of inertial microfluidics offered a promising alternative to continuous filtration and enrichment. A number of systems based on inertial focusing of particles/cells have been reported in literature. Although these
investigators demonstrated particle ordering in preferable positions within the channels, little has been done in terms of characterizing devices for separation and purification, such as description of separation efficiency, enrichment factor and the feasibility of applying to biological sample. While the devices are based on cell deformability and have shown good efficiency, they are not generally applicable since not all cells are highly deformable. Furthermore, these approaches display moderate separation efficiency which means loss of sample during operation.

Continuous filtration in a simple straight high aspect ratio (AR) microchannel has been proposed by several groups and it has successfully demonstrated the fractionation of red blood cells (RBCs) from blood based on size only. This approach is quite suitable for processing large amount of sample since it can be easily paralleled to be extremely high throughput. Yet its performance in terms of purification and concentration is moderate due to the only one waste outlet out of three. Moreover, little of cell biological properties such as viability and proliferation have been reported. Yet, they are very critical in the research and diagnostics since follow-up analysis such as molecular characterization is usually required.

In this work, we describe a straight microchannel design capable of highly efficient cell filtration along with preservation of viability and proliferation. The system offers a much higher improvement in purity and concentration, while sustaining the high throughput. We first demonstrate that particles can be focused into two positions in the center of long length within cross-section, unlike the broad sheaths as shown in previous work. Following the demonstration, we characterize the focusing length for multiple particles. The capability of ~100% efficiency of the system, combined with the flexible outlet configuration, offers precise control of sample concentration after processing. Moreover, we characterize performance of our system and show its capability of cell separation and concentration using blood and cancer cells.
Design principle

Particles flowing in a circular pipe were found to form a narrow band (Segré annulus) near walls,\textsuperscript{80,81} which moves closer to walls as $Re$ increases.\textsuperscript{93,94} Recent work on the microscale reported similar results.\textsuperscript{84} These findings show that the equilibrium position of particles is $\sim 0.2D$ ($D$ is tube diameter) away from wall at low $Re$, shifting to $\sim 0.05D$ at higher $Re$.\textsuperscript{84} The formation of the Segré annulus has generally been interpreted as a result of interaction of shear and wall induced lift forces.\textsuperscript{70,71,100} When neutrally buoyant particles flow through the tube, shear-induced lift force, which is as a result of parabolic nature of velocity profile in Poiseuille flow,\textsuperscript{91} drives particles away from channel center toward the wall. As particles approach channel wall, another force induced by the presence of the wall acts on particles in the opposite direction. This wall-induced lift force increases and eventually balances the shear-induced lift, resulting in a force-free position within tube cross-section adjacent to the wall. This force balance stops particle migration, leading to formation of the Segré annulus.\textsuperscript{80,84,93}

While the counteracting forces successfully explain the Segré annulus near wall, particle migration in microchannels of non-circular cross-section, such as square, is still difficult to explain. While the aforementioned theory suggests that particles in a square channel exhibit similar migration behavior and create a Segré-annulus-like band, a number of recent studies\textsuperscript{85,100} using confocal and holographic microscopy have reported that particles actually occupy four positions centered each face rather. Simulation results by Chun and Ladd\textsuperscript{95} also suggest four and eight positions depending on $Re$. Both numerical and experimental results imply that particles may laterally migrate “parallel to” in addition to “toward” the wall.

To improve the understanding of particle migration and resolve the discrepancy between the theory and the experimental results, we carefully re-examined the literature regarding the
forces exerting on particles flowing in sheared flow. We now believe that particle behavior is primarily dictated by the lift force, although the mechanism is generally complicated. Nevertheless, two primary principles have been identified as vorticity in the continuous-phase and rotation of the particle. Saffman lift is the main type of vorticity induced lift subjected to linear shear gradient. Feng et al. have shown that particle flowing in Poiseuille flow experiences shear induced lift force analog to Saffman lift. This shear induced lift as described in current theory for annulus formation, is responsible for particle migration toward channel walls. This lift, however, is generally neglected since the rotation induced lift is believed to be an order of magnitude smaller compared to shear induced lift in unbounded shear flow.

In the presence of nearby wall, rotation-induced lift becomes important when the shear is large. Particles flowing in Poiseuille flow rotate as a result of parabolic velocity profile which leads to non-zero shear rate and the drastically increased shear rate close to wall. A number of numerical models of the near-wall case have shown that particles translating near wall experience shear, wall and rotation induced lift force. While the first two should cancel at annulus positions, the rotation lift may dominate particle motion. Rotation lift is found to be directed upward the velocity gradient, and thus in the case of Poiseuille flow near wall it drives particles toward the middle of wall where shear rate is zero. If apply rotation effect to particle migration in square microchannel, particles in the corners would further migrate to the center of each edge undergoing rotation induced lift due to parabolic. Indeed, this prediction coincides with experimental results we mentioned before. In summary, our hypothesis for particle migration in microchannel is subjected to two mechanisms: 1) particles away from channel walls primarily undergo shear induced lift force that drives them away from channel
center towards channel walls; 2) once the shear induced lift is balanced with the wall induced force and particle is sufficiently close to walls, particle rotation induced lift begins to dominate and directs particle motion parallel to walls toward their middle points where are the four equilibrium positions.

This model of two-stage particle migration is also applicable to round ducts, which exhibit a radially symmetrical velocity profile and resulting in zero shear rate for concentric contours. When applied to rectangular microchannel, the model predicts both four and two positions depending on $AR$ and $Re$. Unlike the square channel which offers identical shear rate distribution along all edges, rectangular channel produces distinct shear rate patterns for long and short edges. $^71$ Short edge generates larger shear rate and thus particles migrate mainly parallel to short walls and toward the long edges. Due to the large shear rate, rotation induced lift take the control of particle motion which is migration to the center of long edges. On the other hand, due to considerable small shear rate, particles close to short walls do not translate themselves to middle points which are the other potential equilibrium positions. They tend to migrate to the long edges instead because of the dominant shear induced lift force and preferential migration behavior can be observed, as illustrated in Fig. 24a. However, increasing $Re$ (larger shear rate in the direction of long edges) or $AR$ close to unity (square channel) disrupts the preferential migration and activates the other two potential equilibrium positions. In addition, increasing particle size also serves to increase the preferential property of lateral migration since particle size determines local shear rate. $^92, 99$

In this Chapter, we take the advantage of the preferential migration in rectangular microchannel for particle and cell filtration. Based on the new model of particle focusing, particles migrate and occupy two positions centered the long edges under proper $Re$. Previous
Fig. 24 Schematic and concept of purification design. (a) Topview and cross-section view of particle migration in a rectangular straight channel. (b) Concept of purification.
work \cite{70, 71, 100} has pointed out the strong size-dependence of migration velocity as expressed in equation (4) in Chapter 3. Then the channel length required for particle focusing can be derived in equation (5). Hence, small particles require much longer channel for full focus. By cutting the channel length to that of fully focusing large particles, we can extract large particles from the central outlet in low AR channel (Fig. 24b). From equation (6), as long as the focusing length can be measured we are able to obtain lift coefficient which is an average along migration path since it is as a function of particle position.\textsuperscript{92, 100} Then, using the coefficient we can precisely predict particle focusing length.

**Results and discussion**

Since our device concept builds on the basis of two-position focusing hypothesis, it is essential for us to validate the assumption before any further steps. We fabricated numerous devices by standard soft lithography described in Chapter 2. We introduced uniformly dispersed particles of 20 \( \mu \text{m} \) in diameter into rectangular microchannels and imaged every 1 mm downstream length to observe the migration behavior as described in Chapter 2. Bright field and fluorescent images in Fig. 25 have clearly revealed the two locations of particle entrainment at \( \sim 12 \) mm downstream. Very interestingly we found that particles actually migrated toward the long length (width in the figures) much faster than they translated themselves to the final equilibrium positions. As indicated in Fig. 25c, two broad bands were observed in less than 5 mm downstream length while it took 12 mm for them to reach the two positions centered the channel widths (Fig. 25a-b). This result has shown the broad-band focusing in rectangular microchannel is the transient state before particles stabilized into final equilibrium positions.

Unlike the “steady” images of sideview, which display two bands after few millimeters, particle migration in topview shows diverse behaviors as they flow down the channel. Three
Fig. 25 Demonstration of particle focusing into two positions centered the long lengths of channel cross-section. Topview of bright field (a) and corresponding fluorescent (b) images shows particle lateral migration and equilibration in rectangular microchannel. Three streams were observed before complete focusing into one stream. The bright field images indicate particle migration from corners to middle points leading the two side streaks to merge into the primary particle train in center. (c) Images of sideview at progressive downstream positions in accordance to part a and b. Compared with those topview images we have identified particle positions within the channel as indicated in the illustration of cross-section near right bottom corner of each image. Particles in all the images were 20 μm in diameter and the randomly distributed suspensions were introduced into the channel at $Re=120$. Dimensions of cross-section are 50 μm wide and 100 μm in length.
streams including two weak ones in corners were observed (e.g. 5 mm in Fig. 25b) before they merged into single streak. The bright field image at 5 mm and 10 mm (Fig. 25a) indicates particles in the corners migrated and joined the central train leading to absence of particles everywhere except the middle position. The temporary formation of minor streaks can be the result of two facts. According to Zeng et al. 96 particles close to wall experiences extra drag force due to the added-viscous effect. Particles in the corners are surrounded by two nearby walls which could double the inertial drag. Hence, it compromises the net force acting on particles and reduces the lateral migration velocity. On the other hand, once they leave the control of drag, they get accelerated immediately because of the high shear rate and merge into the central stream almost in a sudden way. This is why the intensity of side streams kept reducing but we never observed particles in the gap between streams in fluorescent images (Fig. 25b). However, comparing the single particle near wall in bright-field image at 5 mm and 10 mm we are able to tell particle was migrating away from sidewalls.

We further characterized the focusing length for precise prediction of particle ordering using intensity data obtained by line scan of each image captured every ~1 mm downstream (Fig. 26a). Full width at half maximum (FWHM) of intensity peak has been used by us and others to determine how tight the stream is focused. However, in this case due to the temporary formation of side peaks a different approach is needed. It is possible that particles in corners are still feeding into the center, but middle stream may have already been tight. Thus, we utilized full width at tenth of maximum (FWTM) of peak instead to find out the focusing position. In this approach, various $Re$ were tested to reveal the migration behavior both qualitatively and quantitatively. The progressive aggregation of particles was shown in Fig. 26b. For example curve at $Re=120$ which is corresponding to Fig. 25b, the flat region before sharp slope indicates
Fig. 26 Characterization of focusing length and lift coefficient. (a) Normalized fluorescent intensity as a function of channel width at consecutive channel positions as in Fig. 2(a-b). Gradually vanishing side-peaks and sharpening primary peak indicates the conjoining of particle trains. (b) FWTM as a function of downstream length at various Re revealing particle migration across channel width. Increasing Re tends to shift the curve to right indicating longer focusing length and to slightly elevate the flat region on right side. (c) Positive lift coefficient plotted with respect to $Re^{-0.5}$ for different particle size. Small particles employ large lift coefficient which shows inverse dependence on particle size. (d) Focusing length as a function of Re. Both low and high Re lead to a longer channel length for particle entrainment. Small particles require substantially longer channel. The prediction curves are obtained based on positive lift coefficient. Solid symbols indicate experimental results. All figures here are obtained from 20 μm particles in a 100 μm × 50 μm channel.
existence of side streams and in contrast the even curve after represents focusing into the stable two positions. Similarly, the sharp drop implies particles leaves drag control and accelerate toward their destination. We took the turn point from sharp slope to flat curve as the focusing length ($L$) for particles to complete the inward migration. According to the curves, increasing $Re$ extends the first flat region and thus requires much longer channel for them to be fully focused. In other words, higher $Re$ requires longer focusing length. As a result, particles in corners may never merge into the center in the case of excessive $Re$. In addition high $Re$ tends to reduce the tightness of focused streams as the other flat region levels up. Again this outcome may be due to the enhanced drag force at large $Re$.

The average lift coefficient along the migration path ($W/2$) can be obtained using equation (6). Since the particles were moving upward the velocity gradient undergoing rotation induced lift force, we denoted the coefficient here as positive lift coefficient. The data attained for three particles were plotted as a function of $Re^{-0.5}$ in Fig. 26c. Intriguingly, all the three curves show high linearity with respect to $Re^{-0.5}$, which is implied in Saffman’s expression.\textsuperscript{112, 114} Enhanced $Re$ diminishes the coefficient which indicates its low $Re$ preferred feature. Moreover small particles employ large coefficient which exhibits an inversely dependence on particle size at the same $Re$ based on our data. This suggests the hyperbolic relation between focusing length and particle size ($L\sim a^{-2}$).

Based on the lift coefficient obtained experimentally, prediction curves for different particles can be depicted using equation (5). The curves plotted as a function of $Re$ are shown in Fig. 26d. The solid symbols represent experimental data points. Notice that the curve for 7.32 $\mu$m particle was drawn according to relation of $L\sim a^{-2}$. These curves have shown that either high or low $Re$ increases the focusing length, which suggests the optimal $Re$ for each particle in terms
of shortest length for equilibration. There is a pretty wide region which shows approximately the same focusing length. The larger the particle is, the wider the “flat” region goes.

With the guidance of prediction curves, we designed a low $AR$ straight channel with equally divided three outlets as in Fig. 27. In order to characterize the device for filtration we introduced particle mixture into the channel and collected the outcomes from each outlet. The normalized particle distribution is present in Fig. 27a. As expected, nearly all 20 $\mu$m particles were recovered at central outlet and small particles were evenly distributed among the three outlets. According to the prediction curve of 7.32 $\mu$m particle in Fig. 26d, it requires 60 mm channel length which is much longer than our device (10 mm). Therefore small particles remains uniformly distributed across channel width as indicated by the green pattern in the small inset. Since the 20 $\mu$m particles collected in outlet #2, the filtration efficiency can be $\sim$100%. And for running one stage, we are able to remove 2/3 of waste which means 3× of purification factor. If one redesigns the outlet system to have larger side outlets, the purification factor could be much higher than 3. In addition multiple stages can be easily cascaded to further enhance the purity without loss of sample. This can be very useful in many applications, such as re-concentrate cell sample.

One potential application could be filtration of large cells (e.g. WBCs and CTCs) from blood. We have demonstrated the feasibility of this suggestion using particle spiked blood as presented images in Fig. 27b-c. Initially randomly dispersed blood cells and particles were introduced into the channel (Fig. 27b) and after $\sim$10 mm flowing through the channel, we observed well-focused particle train in the middle and it directly entered the central outlet (Fig. 27c). RBCs which behave like 7.32 $\mu$m particles were present everywherein the channel. We did not observed WBCs due to their relative rare population and difficulties imaging in the bright
Fig. 27 Purification results of particle mixture and particle spiked blood. (a) Filtration of 20 μm particles from mixture of 20 and 7.32 μm particles. Large particles (yellow stream in inset) were collected at central outlet and small particles were approximately distributed evenly in all three outlets. The column diagram implies clearly the ~100% efficiency which means almost no particle lost through two waste outlets. The particle concentration was on the order of $10^5$ per mL. (b) Bright field image of inlet indicating randomly suspended particle (20 μm) and blood cells. (c) Particles exiting from central outlet and blood cells (RBCs) from every outlet. $Re = 50$ for all results showing in this figure and channel length was ~10 mm.
field. However, WBCs were most likely in the same train as 20 \( \mu \text{m} \) because similar size would replicate the behavior as in comparison of RBCs and 7.32 \( \mu \text{m} \) particles.

To further explore the capability of biological sample purification, we performed experiments with LNCaP cells and successfully showed re-concentration of LNCaPs by a factor of 3. Images in Fig. 28a clearly demonstrate cell focusing in the channel and that cells behave similar to particles. Again uniformly suspended cells became ordered progressively and were then recovered from central outlet. Cells collected at each outlet were then plotted in Fig. 28b. At \( Re=50 \) we only observed cells at central outlet. As a result, the concentration of collected cells was enhanced three times accordingly. When \( Re \) increased few cells were also collected at side outlets. When \( Re=100 \) cells were found to distribute evenly at all three outlets which means no purification achieved. The corresponding efficiency is given in Fig. 28c. The efficiency reduces from 100\% to 33.3\% as \( Re \) increases to 100. According to Fig. 26d, this device shall offer good performance for cells in diameter of 20 \( \mu \text{m} \) when \( Re<100 \). The compromised efficiency for \( Re=70 \) is primarily due to the size distribution of cells. Although the mean cell size is comparable to 20 \( \mu \text{m} \), there were many small cells which required longer channel for full focus.

The cell viability and yield followed the same trend as efficiency, decreasing rapidly with faster flow. At \( Re=50 \) we found \( \sim83.3\% \) viable cells and the same amount of yield since all cells were collected from outlet #2. At \( Re > 70 \) we recovered \( \sim64\% \) viable cells. Dissimilar to viability, yield displayed a continuous drop as \( Re \) increased. The reducing cell viability is mainly related to high shear rate generated on the cell surface at large \( Re \). High shear rate tends to break the cells. The other fact we observed during experiment may also associate with the lowering viability. We noticed that at high flow rate cells would collide with the sharp corners between
Fig. 28 Validation of single-stream cell focusing in the channel. (a) Bright field images showing development of LNCaP-cell train at different downstream positions at $Re=50$. These results have demonstrated that LNCaP cells mimic particle behavior flowing in microchannel. (b) Cell collected at each outlet at three $Re$. (c) Efficiency, yield and viability. Increasing $Re$ renders to a decrease for all of the three outcomes. At $Re=100$, cells were collected evenly from each outlet. In contrast viability remains approximately the same as $Re=70$. Approximate 83% of cells were found to be viable at $Re=50$. The channel was 10 mm in length.
outlets. The lower yield at high $Re$ it is as a consequence of the combination of low efficiency and viability.

To clarify the biocompatibility of the device we performed an assay to test the proliferation of cells collected from the device. Cell number was counted every day after running through the device. We plot the results for both control and experiment as a function of time in Fig. 29. The two curves are approximately overlapped which indicates cells collected from device basically functionalize the same as those never touched the device. In case of rare cell isolation, one of the great challenges is preservation of cell viability and cell functionality for follow-up analysis, such as molecular characterization.\textsuperscript{10} Cell proliferation is especially important for monitoring response to drug and treatment as well as for drug development. Although not 100% yield was achieved, ~83% exhibits its potential application in purification of rare cells in blood. Even the only commercialized system of CTC isolation was reported that significant number of CTCs captured by CellSearch\textsuperscript{®} were apoptotic and that the portion of

![Fig. 29 Proliferation result showing cells were functional as control.](image)

91
apoptotic cells increased with counting.\textsuperscript{10,150,151} The cell loss in our system was probably caused by the shear stress which was calculated to be maximum \( \sim 600 \text{ dyn/} \text{cm}^2 \) at long walls. However it can be reduced by lowering flow rate and enlarging the channel. Nevertheless the cell proliferation has shown the preservation of cell function in our system. In addition these results have shown the biocompatibility of our system for other more robust cell (e.g. WBC & RBC) filtration.

Filtration of rare cells such as CTCs has been demonstrated successfully using HPET cell spiked blood as in Fig. 30. To distinguish HPET cells from blood, they were fluorescently stained. While blood cells were evenly distributed, HPET cells possibly as well as white blood cells formed single stream and exited the channel from central port in fluorescent image (Fig. 30b). Comparing these two images, we know that HPET cells behaved in a similar way as 20 \( \mu \text{m} \) particles.

![Fig. 30](image.png) Demonstration of filtration of HPET cells from blood. (a) Bright field image showing blood cell distribution in the three outlets, and (b) the corresponding fluorescent image indicating cell focusing and filtration in central outlet. Blood spiked with HPET cells was introduced into the channel at \( Re=50 \). HPET cell concentration was 2500 cells/mL and blood was diluted for 100x. Note the fluorescent material in part b implies broken cells which released fluorescent dye attaching to channel walls.
Summary

In this work, for the first time we have successfully demonstrated sample purification and re-concentration in a simple low AR straight channel. The experiment of filtration of 20 μm particles from mixture has revealed not only the ~100% efficiency but also the precisely 3× enhancement of concentration for a single run which is outperformed than its high AR counterparts.63, 71 These high AR channels employ only one waste outlet for small species but two for target collection. Although the waste outlet was designed to be ~2.5× in width as those of target outlets, the enrichment factor was reported only ~2× for single-stage run. Although high factor (~4×) was achieved by two-stage running, it was still much smaller than the single-stage operation (6×) of our approach given the same outlet configuration. While ~60% removal of small species which is comparable to our result, our device is capable of ~90% removal for second stage which is higher than 80% in previous work. In addition the ~100% efficiency which indicates no particle loss combined with single target outlet provides a practical way for precise re-concentration of sample without involvement of conventional centrifuge and re-suspension steps, which potentially enables an automated sample preparation substantially reducing the input of labor and time especially in life science laboratories and clinical diagnostics.

Our cell filtration experiments have shown similar efficiency as particles (~100% at Re=50) and indicated the preservation of cell functionality after processing through the device. Although it is unable for complete cell separation, these findings further envision the potential applications such as aforementioned sample preparation. While the capture efficiency and yield are comparable to other immunoselection based microfluidic platforms,68, 109 our throughput is supreme that is >10× faster for a single channel. If massively parallel the channels which has been demonstrated to be quite easily,63 our approach is capable of extremely fast operation. In
addition the filtration we described is purely relied on inertial forces in a planar geometrically
confined channel based on size. Therefore it doesn’t require the complicated fabrication process
and the time-consuming incubation of coating antibody as in the herringbone-chip.\textsuperscript{109}
Considering the high cell viability as well as the throughput, our system serves a good alternative
for cell purification and re-concentration.
CHAPTER 6

MODULATING INERTIAL FORCE FOR COMPLETE SEPARATION

In this chapter, we report for the first time a new simple microfluidic device that offers ~100% separation efficiency and >90% purity. More importantly, it is very easy to design the outlet system due to the considerable gap between focused-particle streams. Our approach is based on manipulation of inertial forces acting on particles in a modulated AR for complete separation device. Our previous work \textsuperscript{70, 71} have demonstrated filtration of particles in straight channel as particles flowing in straight channel migrate toward walls forming particle-free region in the channel center.\textsuperscript{70, 71, 100, 106} However, in this work our detailed exploration has revealed that particles in rectangular channel can be further focused into two positions centered channel long lengths under the control of dominant inertial force. Our results have shown that different inertial force dominates particle migration away and near to wall. As a result we manipulate the dominated inertial force by modulating channel aspect ratio to relocate the equilibrium positions.

Design principle

We constructed a simple concept of modulation of aspect ratio for complete separation (MARCS) which takes advantage of the two-position focusing in rectangular channel. In the MARCS system, particles first flow through a sufficiently long high AR (height/width>1) channel (region #1 in Fig. 31) and migrate to the two equilibrium positions at centers of channel sidewalls. Then, expansion of the channel into a low AR (region #2 in Fig. 31) alters the shear rate distribution along walls and causes the equilibrium positions to shift to the centers of
channel top and bottom. Hence, particles refocus in the center if sufficient channel length is available, as indicated by the trajectory of green particles in Fig. 31.

Now, let us consider the case of introducing particle mixture into the aforementioned channel (Fig. 31). At first, the long channel of region #1 ensures all particles (both green and red in Fig. 31) migrate to their equilibrium positions in the center of sidewalls. After the expansion, the shift of equilibrium positions causes particles to regain migration velocity which is highly size-dependent as described in equation (4), toward their destinations in the middle of channel width. Since large particles (green) migrate much faster than small spheres (red), they complete the refocusing first. If the channel length is limited to only refocus green particle, we are able to separate the large from the small by designing the three outlet system in the MARCS device.

Balancing the Stoke’s drag with the net lift force acting on a particle, we can derive the lateral migration velocity $U_L$ expressed in equation (4) and the channel length $L$ required for particle focusing from equation (5). Since the particle migration distance $L_m$ is approximately half the channel width in both regions, the channel length is dependent on particle size. As a result, focusing lengths in region #1 and #2 are dominated by the small particle and the large particle, respectively.

**Experimental**

To maximize the separation efficiency and purity, it is essential to precisely determine the downstream lengths necessary for each focusing region. Although equation (5) can be a good guidance at low $Re$, the unknown lift coefficient $C_L$ compromises the accuracy of the predictive equation. Thus, we first designed series of microchannels to experimentally determine the focusing length for the separation species. Since the particles were expected to focus into two positions centered the sidewalls, we used low $AR$ microchannels measure the focusing length
Fig. 31 Schematic of the design concept of MARCS. Mixture of particles first flows through a high aspect ratio (AR) channel (region #1) where they migrate to equilibrium positions near the channel height. In this step, small particle requires longer channel and is the limiting case. Once all particles are fully focused into stable positions, channel expands into a low AR (region #2), which modifies the inertial lift. This switch of AR shifts equilibrium positions to the middle of channel width. Thus, particles regain migration velocity and move toward the new stable positions. Due to the high dependence of migration velocity on particle size, large particles complete the refocusing much faster than small spheres. Therefore to separate the mixture, the length of region #2 is determined by large particle.
(essentially rotating the high AR channel on its side). After obtaining accurate focusing lengths for each region, we designed the new channel consisting of high AR and low AR segments for realizing a complete separation.

Microchannels were fabricated via soft lithography as discussed in Chapter 2 with height of either ~27 μm or ~50 μm. For improved visualization and imaging, fluorescent microparticles were used to perform the experiments and measurements. To minimize the particle-particle interaction we diluted particle suspensions (Bangs Laboratory Inc., Polyscience Inc., and Lift Technology Inc.) in deionized water to reach the volume fraction (VF) of 0.025%. For characterization of efficiency and purity, we initially mixed 9.94 μm with 20 μm diameter particles in the ratio of 1:1. A small drop of Tween-20 (Fisher Scientific.) was added to the particle suspensions to minimize clogging of microchannels.

Human whole blood (Hoxworth Blood Center, Cincinnati, OH) was diluted in saline solution to achieve different VF. We prepared 50%, 2.5%, 1%, 0.5%, 0.01% and 0.005% hematocrit samples, corresponding to the dilution factors of 1×, 20×, 50×, 100×, 500× and 1,000×. For particle spiked blood, we directly added 20 μm particles into the diluted blood sample.

To determine focusing length, high-speed images at successive downstream positions were captured using an inverted epi-fluorescence microscope. At least 100 frames were obtained at each position at the same Re; multiple Re were tested to draw the prediction curve in Fig. 33d. To achieve better signal processing and stream visualization, 100 frames at the same position were stacked using ImageJ®. Fluorescent intensity data were obtained from line scan for quantitative assessment of focusing. When the mixture of particles was tested, we took images at each channel position alternatively using different filter cubes (FITC and TRITC). Then the grey
scale images from different filter cubes but identical location were merged forming RGB composite picture which explicitly shows the separation process (Fig. 34). Dashed lines in the fluorescent images represent the approximate locations of channel walls.

To characterize the efficiency and purity of the design, a particle suspension (9.94 μm and 20 μm) was prepared in the initial ratio of 1:1 and injected into the channel; samples were collected at each outlet. In control condition, we ran a mixture through syringe and the connecting tubing without passing through the channel. The output samples were counted using a hemocytometry to determine particle population. For convenient comparison with control, samples from outlet #2 and from outlets #1&3 were diluted to the same volume as the control. Each sample was counted at least 3 times to reduce potential errors; mean values with standard deviation were reported.

**Design validation**

As discussed in Chapter 3, particles in rectangular channel occupy only two equilibrium positions near the center of the long sides in channel cross-section under proper conditions. Although previous work has shown this focusing behavior, the number of stable positions in the channel remains controversial. Thus, we first conducted a number of experiments with particle suspensions to confirm focusing behavior. Both low AR and high AR channels were investigated under controlled conditions.

The resulting fluorescent images are shown in Fig. 32. To clearly demonstrate focusing, it is necessary to obtain both topview (e.g. looking from channel width) and sideview (e.g. looking from channel height) images. Since it is not possible to directly acquire the sideview images using an inverted microscope, two microchannels were used instead, with a high AR channel providing imaging of sidewalls of the low AR channel (see Fig. 32). For example, 50 × 27 μm²
Fig. 32 Demonstration of two-position focusing in rectangular channel. (a-c) The 7.32 μm particles flowing through 50 μm × 27 μm channel at Re=40 (20×). Randomly distributed particles were injected into the channel. Fluorescent image (looking from channel width) indicates particles were everywhere across the channel width. After ~16 mm downstream length, particles were found aggregating in the middle point of channel width. The corresponding image of sideview (looking from channel height) shows two streams near side walls. (d-f) Similarly 20 μm particles were observed to achieve complete focusing into two positions at ~8 mm downstream length in 100 μm × 50 μm channel at Re = 20. (10×). All particle suspensions were diluted to be 0.025% volume fraction to minimize particle-particle interaction. Fluorescent images were pseudo-colored and each image was stacked of 100 frames to enhance both visualization and accuracy.
(width × height) channel (Fig. 32a-b) was paired with 27 × 50 μm² (width × height) channel (Fig. 32c) to show the two specific focusing locations within the rectangular channel cross-section for 7.32 μm particle. We consider images captured from low AR channel as topview (or bottomview, e.g. Fig. 32a-b and Fig. 32d-e) and those from high AR channel as sideview (e.g. Fig. 32c and Fig. 32f) as indicated in the right upper corner of each image.

Our results, for the first time, experimentally demonstrate two focusing positions in rectangular microchannel. Fig. 32b shows that after 16 mm downstream length, a randomly distributed suspension of 7.32 μm diameter particles (Fig. 32a) is entrained into single stream in the middle of channel, with two streams observed from the sideview at identical downstream position (Fig. 32c). According to the evolution of inertial forces corresponding to particle position within the cross-section, particles near the central channel axis first migrate toward the walls, which could happen fast due to the large shear force. Indeed, we have observed the two streams in the sideview within 2 mm downstream length for 7.32 μm from sideview. However, at least 9 mm channel length is required for observation of single stream from topview. Thus the particle translation along channel walls is a slow process compared to that toward the walls. This result is actually suggested by the net forces acting particle near wall where the small rotation induced lift ⁹⁶, ¹¹⁴, ¹¹⁵ is dominant. Similar results have been obtained for 20 μm diameter particles in the other channel. These results also confirm that a longer channel is required for smaller particles to complete entrainment, as implied in equation (4).

We next characterized the channel length required for full focusing, which is essential in terms of device performance. We measured the intensity distribution across the channel width from fluorescent image to evaluate the focusing progress. Consecutive downstream positions were measured and the results are reported in Fig. 33a. Small side peaks were observed at Re=70.
Fig. 33 Determination of focusing length in rectangular microchannel. (a) Normalized intensity across channel width at multiple channel positions at $Re=70$. Three streams were first observed before fully focused as indicated by the small side peaks. Particle size was 7.32 μm in 100 μm × 50 μm channel. (b) FWTM as a function of downstream length at three $Re$ for 7.32 μm particle. After certain length, the curves become flat. The length at turn point is considered as focusing length. Higher $Re$ tends to require longer channel for full focus. (c) Focusing length obtained from b as a function of $a^2$. (d) Focusing length with respect to flow condition ($Re$). Solid points indicate the experimental results and the curves are the predictions for 7.32 μm ($L_1$) in 50 μm × 27 μm and 20 μm ($L_2$) in 100 μm × 50 μm respectively. The anticipated total channel length for designing concept is the blue curve which is as result of the sum of the other two curves in accordance to relation of $L(Re) = L_1(Re) + L_2(0.5Re)$. 
However these two peaks eventually disappeared as downstream length increased. The formation of three streams was also observed in square microchannel. The temporarily streams can be explained as the added viscous effect which imposes extra drag force due to two-wall boundary in the corners. This added viscous effect decreases rapid as particles move away from wall. Due to the additional side streams, the common approach of using full width at half maximum (FWHM) of peak is no longer applicable. Therefore, full width at tenth of maximum (FWTM) of peak was used and plotted as a function of downstream length (Fig. 33b). The curves gradually decrease before a sharp drop, which indicates a sudden acceleration of migration velocity meaning that particles are out of corner influence. Particles at higher Re were found to stay within corner region longer and the curve for low Re steps down in a more mild way. This is because that small Re limits shear rate while large Re push particles even closer to wall where drag force increases due to wall-added viscous effect. Nevertheless, all curves converge after specific channel length.

We considered particles fully focused into two positions once the FWMTM stabalalized, with downstream position at this point defined as focusing length. Thus, although we observed roughly single stream for 7.32 μm particles at 9 mm downstream length, the focusing length was quantitatively determined to be ~16 mm according to the curves in Fig. 33b. Since the rotation lift ($F_\Omega$) is dominates in the near wall region, the net lift force ($F_L$) is approximately the same as $F_\Omega$. According to Rubinov & Keller’s analytic solution, $F_\Omega \sim a^3$. Substituting this into equation (4) we then obtain $U_L \sim a^2$. Thus, the focusing length ($L$) is proportional to $a^{-2}$. from equation (5). Hence, we plotted the focusing lengths with respect to $a^{-2}$ as shown in Fig. 33c. Intriguingly the curves display highly linear feature ($R^2>0.99$). Based on this relation ($L \sim a^{-2}$) a
small change of particle size would result in a considerable adjustment of focusing length which actually implies a high separation sensitivity.

To provide a guideline for designing our new separation concept (MARCS), the focusing lengths were plotted as a function of $Re$. The solid symbols represent the experimental measurements for the two channels corresponding to region #1 and #2 in Fig. 31. The projected curves were attained based on the experimental data and equation (5). As we discussed previously, the length of region #1 ($L_1$) is limited by small particle (e.g. 7.32 μm) and the focusing length of large particle (e.g. 20 μm) determine length of region #2 ($L_2$). To find the optimal flow condition which requires shortest total channel length ($L=L_1+L_2$), we also depicted it in the same chart following the relation of $L(Re) = L_1(Re) + L_2(0.5Re)$. Because we expanded the channel width (region #2) for two times which doubled hydraulic diameter compared to region #1 but the flow rate remains unchanged, the $Re$ in region #2 is a half of that in region #1. From the curve we decided the input optimal $Re \sim 40$ and the total length is ~26 mm ($L_1 \approx 18$ mm and $L_2 \approx 8$ mm). To ensure complete focusing of 7.32 μm particles, we used a more conservative value $L_1\approx 20$ mm. Since previous work ⁶³ has reported that the angle of expansion may affect particle migration, we added an extra 10 mm to region #2.

**Results and discussion**

Through multiple tests using particle mixture, we have successfully demonstrated our concept of complete separation in MARCS system (Fig. 34). The fluorescent images captured progressively downstream have exactly shown the evolution of particle migration which is schemed in Fig. 31. These results have successfully shown the manipulation of equilibrium positions by modulating channel $AR$. Due to the limited options of fluorescent particle, we show
the complete separation of 20 \(\mu m\) (green, FITC) from 9.94 \(\mu m\) (red, TRITC) particles rather than from 7.32 \(\mu m\) (green, FITC) which is not distinguishable.

We introduced the particle mixture of 20 \(\mu m\) and 9.94 \(\mu m\) suspensions into the channel and observed the development of particle streams. Randomly distributed particles (Fig. 34a) first formed streaks near the channel height (Fig. 34b) mainly undergoing shear and rotation induced lift forces successively. Although only two streams were shown in Fig. 34b, there were actually four. The fact that FITC is much brighter than TRITC filter cube leads to the overlapped streams mainly exhibiting green rather than yellow (composite of green and red). Nevertheless once they separated from each other four streaks turned up as shown in Fig. 34c at ~28 mm down channel. Because of the tremendous difference of migration velocities between the two species according to equation (4), 20 \(\mu m\) particles tended to relocate themselves in a much rapid manner. Hence when we observed merging of the two green streams, small particles seemed to stay still. Green streaks were found to almost complete the joining at ~30 mm (Fig. 34d) and single green stream were observed after ~31 mm (Fig. 34e). Finally, the mixture was separated by the equally widened outlets (Fig. 34f) — 20 \(\mu m\) spheres exited central outlet (#2) and 9.94 \(\mu m\) particles were collected at two side outlets (#1 & #3). According to the relation of \(L \sim a^{-2}\), it requires at least 32 mm (current 16mm) length of expanded channel to reposition the 9.94 \(\mu m\) particles to the central outlet. Therefore the separation was fulfilled quite easily. Note that at input \(Re=40\) large particles were found to accomplish the relocation at ~31 mm (Fig. 34e) which is ~3 mm longer than prediction. This extra 3 mm is resulted from channel expansion where the steady flow is disrupted and keeps changing. We actually tested even larger expansion which required even longer channel for particle refocusing.
Fig. 34 Demonstration of complete separation. (a) A mixture of 9.94 μm (TRITC, red) and 20 μm (FITC, green) particles were injected into the channel whose layout is in Fig. 31. (b) Particle streams in the transition region. Since the small particles were less bright, it is hard to observe when red and green stream overlapped. However, a light yellow can be observed due to the composition of red and green. (c) Large particles migration to the center of channel width forming two merging streaks (green) and leaving two red streams close to side walls. (d) Conjoining green streaks while red streams remained in wall region. (e) Completed refocusing of large particles forming single streams in the middle at downstream length ~31mm. (f) Particles differentiated from distinct outlets. All images are stacked of 100 frames and pseudo-colored.
Particles collected at the three outlets were then counted using hemocytomery and the results were displayed in Fig. 35. Since the symmetrical outlet systems and the behavior of particle migration, we mixed samples from outlet #1 & #3 to give better comparison. As discussed previously, the control is considered as the actual or effective input to eliminate the effect of sedimentation. Fig. 35a is the separation result at input \( Re = 40 \). Notice that despite the initial ratio of 20 \( \mu m \) and 9.94 \( \mu m \) particles were 1:1 the ratio of control was approximately 1:3. This change of species ratio is due to the effect of sedimentation. In fact particles were not exactly neutrally buoyant and their density is slightly larger than water. Large particles settle down to the bottom of syringe faster than that of small spheres. However the total numbers of particles collected (either 20 \( \mu m \) or 9.94 \( \mu m \)) at outlets is found to be comparable to those of control. Hence, it is reasonable to consider the control as input and thus no particle loss during operation.

Our results are very promising in terms of separation efficiency and purity. Almost every 20 \( \mu m \) particle was collected at outlet #2 which offers ~100% efficiency. Efficiency is defined as number of large particles at outlet #2 over total number of large particles injected. Moreover only ~2 small particles out of ~32 large particles were found at outlet #2 which suggests the purity > 90% for large particles. And in case of small particles at two side outlets the purity \( \approx 100% \) as almost no 20 \( \mu m \) particles were found as shown in Fig. 35a in column of outlet #1 & #3. Here purity is defined conventionally as the percentage of target in the sample (e.g. # of 20 \( \mu m \) particles over the sum of 20 \( \mu m \) and 9.94 \( \mu m \) particles at outlet #2 and # of 9.94 \( \mu m \) particles over the sum of 20 \( \mu m \) and 9.94 \( \mu m \) particles at outlet #1 & #3). The efficiency and purity we reported here are even higher than those obtained in spiral channel of which outlet system is tricky to design\(^6\). Theoretically our design is able to provide both ~100% efficiency and purity.
Fig. 35 Characterization of efficiency and purity. (a) Particle concentrations of samples collected from control and three outlets at $Re=40$. The control which was collected right after connection tubing without running through device for the same time of performing though device. To convert the effect of sedimentation which gradually lowered the input concentration during operation, control is considered as input here. The volumes of samples from outlets were reduced to ~1/3 due to flow fractionation. Thus in order to compared with control, sample collected from outlets (#2, #1&3) were diluted to the volume of input. Error bars represent the stand deviation of three counts. The mixture injected comprised 9.94 μm and 20 μm particles. (b) Efficiency and purity for various $Re$. Again error bars here represent the stand deviation of three measurements.
The underestimated purity is mainly due to the $AR$ defect. As described in theory section in order to realize 100% separation it is necessary to completely focus all particles into two positions in region #1 (Fig. 31). We found that even 7.32 $\mu$m particles were fully focused into two positions in $27 \times 50 \, \mu m^2$ ($AR\approx1.85$) at 16 mm downstream length (Fig. 32). However, the actual dimensions of region #1 we fabricated are $\sim32 \times 50 \, \mu m^2$ ($AR\approx1.56$). We know that channel $AR$ is essentially responsible for particle preferential equilibration: the far away from 1 the better for two position focusing. Thus the compromised $AR$ of our actual device causes a small amount of 9.94 $\mu$m particles remaining in the middle of channel width. These particles don’t move after expansion and are eventually collected at outlet #2. On the other hand the sizes of particles are not uniform. In addition the resistance of each outlet also plays an important role in the separation process. We will come back these points again later in this paper. Basically redesign the channel $AR$ will improve the purity. Nevertheless our device has been demonstrated to achieve complete separation in terms of both efficiency and purity.

The separation performance was further characterized as in Fig. 35b. The red curve represents the separation efficiency. It turns out that except at $Re=30$ the flow condition shows very little influence on efficiency which maintains nearly 100%. Actually the efficiency of our device is expected to be 100% as long as sufficient long channel. In other words 13 mm (16 mm subtracted transition length of 3 mm) channel length after expansion is always able to entrain 20 $\mu$m particles in the channel center and exit from outlet #2 if actual $Re$ is within the range of 10 and 120 according to Fig. 33d. However at low $Re$ (e.g. 15) which means small flow rate, the flow distribution becomes very susceptible to channel resistance of outlets. For example, at input $Re=30$ which is equivalent to $Re=15$ in region #2 we observed that adjusting connecting tubing at outlets would result in the displacement of particle position and affect their preferred outlet.
Even for high $Re$ adjusting the resistance can affect the collection. But for $Re > 30$, it is relatively easy to balance the resistance between outlet #1 and #3 and thus to ensure large particles to exit from outlet #2. In contrast, for small $Re$ the flow is too slow to overcome the tiny difference of resistances between two side outlets. The unbalanced flow bifurcation at outlet compromises the efficiency at small $Re$ (e.g. 30 in Fig. 35b). Excessive $Re$ can also reduce the efficiency caused by insufficient channel length.

Similarly, much higher purity was found at $Re > 30$. But a maximum purity near $Re = 40$ was observed. The peak here can be related to the optimal focusing length in Fig. 33d. While the compromised purity at $Re = 30$ is due to the same reason of low efficiency, the slight drop of purity after $Re = 40$ is primarily caused by the combination of the defect in channel $AR$ and the fact that small particles are more sensitive to flow rate. On one hand the actual $AR = 1.56$ which is smaller than the supposed value of 1.85 render small amount of 9.94 $\mu$m particles to occupy the other two potential positions located at center of top and bottom. On the other hand increasing $Re$ enhances drag force that is responsible to keep particles in still at top and bottom of channel. In other words high $Re$ compromises preferential equilibration in rectangular channel. As a result the purity decreases slowly with increasing $Re$. The purity can be further improved by optimizing the channel dimensions based on our analysis. Nevertheless our current results have shown high-profile efficiency and purity.

One important application we proposed for our system is separation of CTCs from blood. CTCs are normally much larger than blood cells. To demonstrate the feasibility we used particle spiked blood. Since whole blood is very viscous and the volume fraction of blood cells is approximately 50%, it is necessary to dilute it to achieve Newtonian fluid and minimize cell-cell interaction. But we have not yet seen the proper dilution factor. Therefore before validation of
Fig. 36 Effect of blood concentration on focusing in the channel. (a) Single bright field image showing 50× dilution of blood focusing at downstream length ~35 mm at $Re=50$. (b) Result of binary and stacking of 100 frame process of image in part a. (c) Measurements of half peak width corresponding to blood dilution. The inset shows the average intensity obtained from 5 positions of each processed image such as the one in part b.
separating particles from blood, we conducted another set of experiments to determine the good
dilution factor in the way of measuring half peak width of intensity distribution. Because blood
cells are not fluorescent, we used bright field images (Fig. 36a) at ~35 mm channel length for
blood sample diluted in different factors. To obtain the intensity distribution, we then reversed
the color (Fig. 36b) and stacked 100 frames for each dilution factor. Line scans were used to
obtain intensity data across channel width. Curves of normalized intensity distribution are plotted
in the inset of Fig. 36c. Each curve is the average of 5 measurements. The FWHM attained from
the major peak of the two is plotted as a function of dilution factor in Fig. 36c. Little change of
half peak width is found after 50× dilution. The blood cells could be well focused closer to side
walls.

Result of particle separation from blood sample is shown in Fig. 37. Diluted blood was
first introduced into the device to examine the behavior. It turns out that red blood cells (RBCs)
were most likely acting the same way as 9.94 μm particles (Fig. 37a) which implies that CTCs
may also mimic the behavior of large particle. Then we injected particle spiked sample at Re=40.

![Fig. 37 Separation of particles from blood. (a) Extraction of red blood cells from outlet #1 and outlet #3 for 1000× dilution at Re=50. (b) Separation of particle spiked blood Re=50. The image was stacked of 15 frames.](image)
It turns that most of blood cells (RBC) travelled out the channel from side outlets and particles were collected at central outlet (Fig. 37b). Thus it is feasible and promising for separation CTCs. A few RBCs were also observed in the central outlet due to the compromised $AR$ which we discussed before and can be redesigned to improve the outcome. The disk-like shape of RBCs may also affect the performance.

To demonstrate a complete separation of cells, blood spiked with HPETs was injected into the channel and the outcome from each outlet was collected as in Fig. 38. The green streak in the middle channel represents HPET cells which were strained fluorescently. Blood cells were not visible in the figure since they were not fluorescent. The autofluorescence of carrier medium outlines the channel geometry and outlet systems. But the medium also caused big issue due to their sticky and fluorescent properties. Cell debris in the medium was observed to attach the channel walls especially in the bifurcation region and the autofluorescent made it different to image the weak HPET cells. Nevertheless most of red blood cells were recovered from side outlets as in Fig. 38b. The clear sample collected in central outlet combined with fluorescent image in Fig. 38a clearly shows complete separation of HPET cells from blood.
Fig. 38 Demonstration of complete separation of HPET cells from blood. (a) Fluorescent image showing entrainment of HPET cells in channel center. Autofluorescence of medium outlines the channel geometry. The bright material was cell debris and other substance released from broken cells. (b) Collected sample from three outlets. Sample #1 and #3 contain red blood cells and sample #2 is clear indicating free RBC content.
Summary

In this work, for the first time, we have successfully demonstrated complete separation of particle sand cells in a simple straight channel—the MARCS system. Our device has shown ~100% efficiency and > 90% purity. Experimental outcomes have also suggested a wide range of $Re$ which possesses promising separation capability. Moreover, we have provided the approaches to further improve the purity and suggestions for maintaining ~100% efficiency. Although the flow rate is on the order of 100 $\mu$L /min ($Re \approx 40$), the throughput can be easily remarkably enhanced simply by massively paralleling the channels based on the straight nature. The successful demonstration of separation of HPET cells from blood suggests very wide application of our approach considering its outstanding performance. We propose to use our design for CTC isolation from blood based on our experimental demonstration of particle separation from blood. The moderate $Re$ is suitable for manipulating biological sample which is not that robust. For those work with diluted blood we provide the guideline in terms of dilution factor. In addition we clearly demonstrated two-position focusing in rectangular channel and characterized the corresponding focusing length with respect to particle size ($L \sim a^2$) and $Re$ which help improve our understanding of particle behavior in microchannel.
CHAPTER 7
CONCLUSIONS

The increasing demand of cell separation has triggered avalanche of developments microfluidic techniques for cell sorting. For example, due to the clinical significance of rare cells such as CTCs, integration of their analysis in clinical routine could benefit millions of patients. However, it is greatly impeded by the absence of satisfactory isolation methods. The ideal platform for capturing CTCs will require a number of characteristics such as high efficiency, sensitivity, specificity, purity and the capability of processing large volume and the preservation of biological properties. In addition, the system should be automated to reduce laborious effort and to achieve easy use. Thus, isolation of CTCs has been identified as a grand challenge facing the microfluidic community. A number of microfluidic platforms, including both active and passive techniques, have been reported for CTC isolation. Some of them show high sensitivity and other may exhibit specificity by integration of immunoselection components. Inertial microfluidics offers a unique opportunity for effective cell sorting including rare cell separation based on size. Nevertheless these systems suffer from a number of critical drawbacks such as moderate separation efficiency and very little throughput. Moreover very little work on purity and cell viability has been done. As a result, developing suitable solution to fulfill the satisfactory isolation of CTCs remains challenging yet urgent in life science research and clinical diagnostics.

Recent exploits of cell sorting systems relying on inertial cell positioning in microchannels, for example the demonstrations of label-free filtration of bacteria from blood and
of MCF cell extraction from blood, suggest a promising potential and active role of inertial microfluidics in development of rare cell isolation techniques. Although powerful capability can be expected, the performance of the existing inertial microfluidic devices for separation is mainly limited to the less explored working principles which post remarkable difficulties to generate design rules. Hence, before moving onto development of platforms for effective cell isolation, mechanisms of inertial cell manipulation using polystyrene particles were explored. Neutral buoyant particles flowing in microchannels show preferential focusing near walls due to the interaction of shear and wall induced lift forces. As a result, particles in a round channel form an annulus near walls and are expected to focus close to perimeter of square channel. In rectangular microchannel, particles focusing near long edge can be expected since the shear rate is asymmetrically distributed along different walls. However actual experiments have revealed distinct focusing behavior of particles. The discrepancy between the predictions and experimental results indicates the limitation of existing hypothesis of two-force dominance. In this work, another force has been proposed to adapt the particle migration and equilibration behavior within microchannel. The experimental results obtained using rectangular microchannel, have suggested the two-stage migration model. In the first stage, particles laterally migrate toward the channel walls primarily undergoing shear induced lift force. As particles approach the wall, it counteracting with the wall induced lift force which eventually cancels the shear force. Then, a much smaller rotation-induced lift force becomes dominant on particle motion and leads to the particle migration along channel walls. This is the second stage. Since the rotational force directs to the smaller shear rate, particles translate themselves to the centers of walls in this stage. Hence particles in different channels can have dissimilar behaviors and distinct equilibrium positions depending on aspect ratio of cross-section because it modulates the distribution of
shear rate. For example, particles in square microchannel can eventually focus into four positions centered the each faces and those in rectangular microchannels can migrate to two stable locations within cross-section. In addition, drag force is found to modify the migration and focusing at high $Re$, which could result in four stable positions in rectangular channel.

In order to generate guidelines for developing inertial microchannels, it is necessary to characterize the primary forces acting on particles. Series of experiments were performed, and for the first time, the lift forces have been successfully characterized experimentally in terms of lift coefficients as a conventional way. The effects of diverse factors such as particle size and channel dimensions have been exploited in detail. Lift coefficients for shear and rotation induced lift forces show similar dependence on flow condition ($\sim Re^{-0.5}$) and short dimension ($H^2$) of cross-section. Nonetheless the lift coefficient for shear-induced lift force is inversely proportional to particle size ($a$) while the coefficient for particle rotation is scaled with $a^2$. Further analysis has revealed that shear force is linearly dependent on $a^2$ which is analogous to Saffman’ lift generated due to vorticity. The rotation force shows a higher order dependence on particle size ($a^3$) which is consistent to Rubinow & Keller’s analysis. Based on the hypothesis of two-stage migration and the characterization of lift coefficients, a general equation was developed for prediction of focusing length in straight microchannel. This part of work can serve as general design guideline for development of inertial microfluidic systems.

With guidelines of the new model, three devices were designed to demonstrate cell separation in this work. The first high-throughput device has been developed for sensitive and size-selective isolation of rare cells such as CTCs. Since the rare cells are normally larger than blood cells, 20 $\mu$m particles have been used to demonstrate and characterize this method. By temporarily removal of channel wall and thus wall-induced lift force, this approach has shown
the capability of extraction of extremely low concentration, as low as one target (20 μm particles) per mL sample which is comparable to CTC concentration in blood and isolation of one target out from $10^5 \times$ other species (7.32 μm particles). Moreover, it has also demonstrated the extreme high factor of enrichment ($10^5 \times$). An empirical expression and the results of dimensional effects have been provided to be guidelines of similar design. Isolation of CTCs has been successfully demonstrated using human prostate epithelial tumor (HPET) cells. While variety of intriguing advantages of this approach is present, there are tradeoffs. Since the cells are trapped into the expansions region which is closed structure, flow will need to stop to release the samples. As a result the isolation can’t be continuous. In addition, while the high throughput (~1.5 mL/min) is suitable for processing large amount of sample, it produces considerable shear rate which could damage cells. Nevertheless it is still a promising alternative for rare cell such as CTC separation.

The second design, which operates continuously, overcomes the limitations of the previous approach. As suggested by the model, particles in low AR rectangular microchannel migrate to their equilibrium positions centered the long length of cross-section and form single stream in the center. Considering the size dependent nature, this is a good opportunity for filtration of large cells from mixture at high efficiency. Indeed by proper design of the outlet systems, this approach has been successfully demonstrated for filtration of 20 μm from 7.32 μm particles at extremely high efficiency (~100%). More encouraging, the experiments using LNCaP cells have shown efficiency as high as 100%. Furthermore preservation of cell properties has been demonstrated by characterization of viability and proliferation. Although increased $Re$ tends to destroy cells due to shear rate, at proper flow rate (225 μL/min) the yield of ~83% has been accomplished. Considering the 100% efficiency which means no sample loss in waste outlets, this design is potentially capable of sample precise re-concentration. Currently a 3×
enrichment ratio for single stage have been documented. It is very easy to achieve different ratio by reconfiguration of outlet system. Although the filtration of cancer cells from blood has been demonstrated, the purity of this system is limited since small species exiting every outlet including the one interested. But it is a great alternative for sample preparation such as purification and concentration without centrifuge.

The third design, based on manipulation of equilibrium positions within the same straight channel, has shown outstanding performance in terms of efficiency and purity. Modification of focusing positions is realized by alteration of channel $AR$ ratio which modulates inertial forces. Strongly size-dependent refocusing after small channel segment leads to a complete separation of particles/cells. The potential efficiency and purity of this approach could both be 100%. Actually the characterization using particles has shown ~100% efficiency and >90% purity for 20 μm particles. For small sample (e.g. 9.94 μm particles), these two outcomes have documented to be ~100%. The size sensitivity of this approach could be very high since the focusing length is scaled with $a^2$. Complete cell separation has been successfully demonstrated using HPET cells spiked blood. Most of blood cells were collected at side outlets while HPET cells were at central output. The results in this work have envisioned its remarkable performance for isolation of CTCs. The high purity and little loss of sample could lead this approach to be an excitingly auspicious alternative of CTC isolation enabling follow-up applications such as molecular characterization and the implementation of its significance in clinical routine.

In short, all the three approaches described in this work highlighted a number of essential advantages for rare cell separation. While they emphasize different advantages, all of them are based on a straight rectangular microchannel. The planar structure of the devices suggests their shared advantages such as simple fabrication and low-cost and thus ease of integration into Lab-
on-a-Chip (LOC) systems to realize sample-to-answer cell sorting and on-chip characterization suitable for CTC detection. In addition, all these design are capable of massively-parallel operation, which could drastically enhance the throughput and reduce operation time of analysis. Ultimately inertial microfluidic platforms demonstrated in this work could facilitate the development of CTC detection in clinical setting.
REFERENCES


16 W. J. Allard et al., "Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases," Clinical Cancer Research 10 (20), 6897-6904 (2004).


55 P. R. Gascoyne et al., "Isolation of rare cells from cell mixtures by dielectrophoresis," Electrophoresis 30 (8), 1388-1398 (2009).


59 M. S. Pommer et al., "Dielectrophoretic separation of platelets from diluted whole blood in microfluidic channels," Electrophoresis 29 (6), 1213-1218 (2008).


133 K. Khoshmanesh et al., "On-chip separation of Lactobacillus bacteria from yeasts using dielectrophoresis," Microfluidics and Nanofluidics 1-10 (2011).


