I, Xiao Wang, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Electrical Engineering.

It is entitled:
Inertial microfluidic vortex cell sorter

Student's name:  Xiao Wang

This work and its defense approved by:

Committee chair: Ian Papautsky, Ph.D.
Committee member: Chong Ahn, Ph.D.
Committee member: Chia Chi Ho, Ph.D.
Committee member: Susan Kasper, Ph.D.
Committee member: Andrew Steckl, Ph.D.
INERTIAL MICROFLUIDIC VORTEX CELL SORTER

A thesis submitted to the
Graduate School of the University of Cincinnati
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in the Department of Electrical Engineering and Computing Systems
of the College of Engineering and Applied Science

2016

by

Xiao Wang

B.E., Huazhong University of Science and Technology, China, 2010

Committee Chair: Ian Papautsky, Ph.D.
ABSTRACT

Sorting and purification of target cells from complex cellular samples is critical for downstream cell biology research, biomedical research and clinical diagnostics. In this work, a novel microfluidic platform is developed to achieve size-based sorting of cells with high performance and systematic versatility. The platform uses inertial effect of fluid and microscale vortices in designed microfluidic units to align and sort cells. A microfluidic unit was first developed to enable bimodal sorting of cells with high sorting resolution and tunability. Microparticle mixture with only 2 µm difference was continuously sorted with > 90% efficiency. Two sorting units were then integrated into specific arrangements to enable versatile cell sorting functions. A serial sequencing arrangement enabled new multimodal sorting function. Multi-sized or heterogeneous microparticle mixtures were sorted continuously into three subpopulations of different sizes with > 90% efficiency. Alternative arrangement of the two units enabled new double sorting and purification function. The larger target cells can be extracted from the small non-target cells two times sequentially yielding highly purified target cell product. Spiked human cancer stem-like cells (HuSLCs) were sorted from human blood samples with enriched concentration and significantly enhanced purity. The two distinct arrangements exhibit the systematic versatility of the vortex cell sorting platform for efficient sorting of complex cellular samples which will open new opportunities for size-based cellular sample preparation in cell biology, biomedical research and clinical diagnostics.
ACKNOWLEDGMENTS

First, I would like to express my sincerest gratitude to my advisor, Dr. Ian Papautsky for his guidance and advice during my PhD studies. His expertise, integrity and passion for research have always inspired me to put my heart into scientific research and to discover the beauty within science and nature. He has always valued my opinions and encouraged me to pursue my research interest. He is a gentle, supportive, cheerful and open-minded advisor which made working with him a truly joyful and wonderful experience. I could not be more fortunate to have him as my advisor. Without his support and advice, this work would not have been possible.

I would like to thank Dr. Susan Kasper and Dr. Chia-chi Ho, Dr. Chong Ahn and Dr. Andrew Steckl for being a part of my committee and for their valuable time, support, encouragement and comments on my research. I would like to give special thanks to Jeff Simikins and Ron Flenniken from ERC cleanroom for their patient instructions on cleanroom facilities and their warm help on fabrication processes. I would like to thank Dr. Necati Kaval from Sensor Instrument Facility for his expertise and help on building an important system for my research. I would also like to thank the administrative staff, Janie Runck, Tony Seta and Terasa Hamad at the department office for their willingness to help and the ability to process things efficiently.

Many thanks to my colleagues from the BioMicroSystems Lab Li Shen, Jian Zhou, Mike Ratterman, Yuguang Liu, Xing Pei, Nivedita, Prithviraj Mukherjee and Craig Murdock for their continuous support and friendship over the years. It has been a great pleasure to work with them.
I would like to extend my thanks to students I have once closely worked with Jacob Buenger, Nadja Dindic and Hua Gao for their contributions on previous projects. Special thanks to high school student Xiaodi (David) Yang for his contribution on several important experiments for one of my journal papers. I would also like to thank Dr. Josh Hagen from Air Force Research Laboratory for his funding support on several of my projects.

I would like to thank my friends Zhaoyun Wang, Wenpeng Yan, Yezhou Wang for their friendship. I would like to express my sincere gratitude to Chef Chu from Amerasia restaurant for his friendship, inspiration and delicious dishes and recipes. Although cooking seems not relevant to academic research, his work ethic and passion for inventing phenomenal dishes have truly inspired me that the only way to become successful in my career is to spend time and constantly devote my love into my daily work.

Finally but most importantly, I would like to express my sincerest and deepest gratitude to my parents and my wife. I would like to thank my parents Yu He and Lian Wang for their unconditional love and their continuous support. Since I was a child, they always educated me to be a man with integrity, honesty, empathy and generosity. They led me to appreciate the beauty within art, music and technology. They support and encourage me to pursue what I am passionate about. I could not be more fortunate to have such wonderful parents. I would like to thank my wife (and also my lab mate) Wenjing Kang for her love, care, comfort and support in my everyday life since we met. She has always been the angel in my world giving me strength and courage to move forward. Our journey together has been full of happiness. I am the luckiest man to have her as my wife.
TABLE OF CONTENTS

LIST OF FIGURES ........................................................................................................ viii
LIST OF TABLES .......................................................................................................... xiii
LIST OF SYMBOLS ...................................................................................................... xiv

CHAPTER 1 INTRODUCTION ....................................................................................... 1
  Microfluidic cell sorting techniques ....................................................................... 1
  Inertial microfluidics ............................................................................................... 3
  Motivation ................................................................................................................ 7
  Scope of work .......................................................................................................... 8
  Summary .................................................................................................................. 9

CHAPTER 2 EXPERIMENTAL METHODS .................................................................. 11
  Device fabrication ................................................................................................... 11
  Sample preparation ............................................................................................... 18
  Experimental setup ............................................................................................... 20
  Data analysis ......................................................................................................... 22

CHAPTER 3 INERTIAL MICROFLUIDIC VORTEX-AIDED SORTING ..................... 27
  Device principle .................................................................................................... 28
  Results and discussion ......................................................................................... 32
  Summary .............................................................................................................. 40

CHAPTER 4 INTEGRATED VORTEX SORTER FOR MULTIMODAL SORTING ....... 43
  Device principle .................................................................................................... 44
  Results and discussion ......................................................................................... 46
  Summary .............................................................................................................. 54

CHAPTER 5 INTEGRATED VORTEX SORTER FOR DOUBLE PURIFICATION ....... 55
  Device principle .................................................................................................... 56
  Results and discussion ......................................................................................... 58
Summary ..........................................................................................................................70

CHAPTER 6 CONCLUSIONS ............................................................................................72

REFERENCES ................................................................................................................76
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Schematics of the two-stage model of inertial focusing</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>Schematics of soft lithography. (a) spin coating negative photoresist SU-8. (b) Selective exposure using a photo mask under UV light. (c) Development using SU-8 developer. (d) Casting PDMS on the SU-8 mold and curing on a hotplate. (e) Peeling off the PDMS chip and treating with oxygen plasma. (f) Bonding the PDMS chip on a glass substrate.</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Photograph of a master mold made of MX5050 dry film photoresist on a glass substrate.</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>Photograph of a PDMS device</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>Schematics of epoxy chip fabrication. (a) Thermal aging of the original PDMS chip. (b) Double cast PDMS on aged PDMS chip. (c) New PDMS chip with the reversed structures. (d) Punch inlet and outlet ports and insert Teflon wires. (e) Insert Teflon wires into PEEK tubes. (f) Cast epoxy on the mold. (g) After curing, remove PDMS mold and Teflon wires. (h) Bond the epoxy chip with the epoxy-coated glass substrate.</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>Microscopic bright-field images showing the expansion of microchannels made of PDMS and epoxy after introducing certain flow rate ($Q = 275 \mu$L/min). PDMS channel expands to 45 µm while epoxy channel remains the original dimension.</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>(a) Bright-field image of HuSLCs. (b) Zoom-in bright field and phase-contrast images of HuSLCs. (c) The size distribution of HuSLCs.</td>
<td>21</td>
</tr>
<tr>
<td>8</td>
<td>Processing microscopic high-speed bright-field images for visualization of particle trajectories. a) A single bright-field images. b) Stacking of 200 images in ImageJ unveiling the trajectories of particles.</td>
<td>22</td>
</tr>
<tr>
<td>9</td>
<td>Fluorescent images showing sorting of particles with different sizes. a) Pseudo-colored fluorescent streaks of FITC labeled 18 µm microparticles. b) Pseudo-colored fluorescent streaks of TRITC labeled 15 µm microparticles. c) Stacked image showing size-based sorting of the microparticles.</td>
<td>24</td>
</tr>
<tr>
<td>10</td>
<td>(a) Concentrations of 15 µm and 7 µm diameter microparticles from samples collected from middle and side outlets. The red and blue bars represent 7 and 15 µm diameter microparticles correspondingly. (b) Sorting efficiency of 15 µm and</td>
<td></td>
</tr>
</tbody>
</table>
11. (a) Bright microscopic image showing sorting of microbeads with continuous size distribution from 7 µm to 23 µm. 200 images are stacked to illustrate the particle trajectories. (b) Histogram of the normalized count indicates the sorting cutoff.

12. (a) Schematic of the vortex-aided inertial microfluidic design. Flow resistances $r$ of the side outlet channel and $R$ the main outlet channel can be modulated to adjust the sorting cutoff of the device. (b) Size-selective separation in microchambers with three outlets. Red dash line represents the boundary streamline. The black arrow represents the flow direction. $F_s$ and $F_w$ represent shear-gradient induced lift force and wall-induced lift force.

13. Theoretical investigation of size-based separation. (a) CFD-ACE+ model showing streamline distribution in two symmetric microchambers with three outlets. The red solid line indicates the boundary of main flow and sheath flow, with $R$ and $r$ representing the channel resistance of the main and side outlets. (b) Schematic illustrating the relationship between the input flow rate and the separation cutoff diameter, with $U_l$ representing lateral migration velocity. (c) The close-up images form the numerical model show the entrance and exit regions of the separation unit. The $F_s$ is the shear-gradient induced lift force. The $d_p$, $d_b$ and $d_m$ are the particle focusing position, boundary position and migration distance needed for a particle to enter the sheath flow. The red solid line represents boundary streamlines (d) CFD-ACE+ model illustrating the cross-sectional view of the 3-D boundary streamlines. The red area represents main flow, while the blue area indicates sheath flow. (e) Close-up of the upper left channel quadrant (indicated as black dashed lines in panel (d)). Channel resistance ratio $r/R$ influences the boundary position and migration distance for particles to reach the boundary streamline.

14. Bright-filed images at various downstream positions illustrating the focusing and sorting of 21 µm and 15 µm diameter microparticle mixture.

15. (a) Experiments with tracer-beads (TRITC) demonstrating geometric evolution of the vortex as $Re$ increases. Only one of the two symmetric chambers is shown. The white dashed line indicates the vortex area. (b) Experimental (red circle) and simulation (blue circle) measurements showing the increasing vortex size at $0 < Re < 400$. (c) ESI CFD-ACE+ simulation demonstrates geometric progression of the vortex at different $r/R$, and (d) the corresponding quantitative measurements of vortex dimension from both numerical models (blue circles) and experimental (red circles) at for $1 < r/R < 100$.

16. Experimental investigation of the separation resolution and tunability of the cutoff diameter. (a) Particles with continuous size distribution are induced in a device with $r/R = 5.4$ and $Re = 110$ to find out the separation resolution. Particles with diameter $a > a_c$ migrate inside sheath flow exiting through the side outlets (blue dash lines), while particle with diameter $a < a_c$ elute from the main outlet (red dash lines). A few large particles ($a > 25$ µm) are recirculating in the vortices.
(orange dash lines) due to the large $F_s$ that can push them across the sheath flow into the microvortices. (b) Normalized histogram of main and side outlet samples indicate separation cutoff size (grey arrow) and separation resolution using a device with $r/R = 5.4$ and $Re = 110$. (c) Tuning separation cutoff size by changing $Re$ ($r/R = 5.4$). (d) Tuning separation cutoff size by changing $r/R$ ($Re = 110$).

17. The setup for studying the influence of the flow rate $Q$ and the resistance ratio $\sigma$ ($\sigma=r/R$) of the side and the middle outlets on the sorting cutoff diameter. (a) AutoCAD layout of a single sorting unit with tunable resistance ratio. O1 is the middle outlet. O2 and O3 are the side outlets. The black crosses indicate the outlets that are blocked by the plugs. The red solid line represents the resistance channel of the middle outlet. The two blue solid lines represent the resistance channels of the side outlets. (b) A photograph of the actual device with the tunable setup.

18. High-resolution sorting of 21 µm from 18.5 µm diameter particles in a vortex-aided device. (a) The top bright-field image shows separation at the device chamber. The lower three images show particles at the inlet, side outlet and main outlet. The black dots are 21 µm diameter non-fluorescent particles in bright-field view. The white dots are fluorescent 18.5 µm diameter particles. (b) Histograms of inlet, side outlet and main outlet samples indicate the efficient separation. (c) Concentration of 21 µm and 18.5 µm diameter particles in inlet, side and main outlet samples. Normalized count shows a separation efficiency of ~90% for both particles. Error bars represent standard deviation ($n = 3$).

19. Continuous extraction from blood sample. (a) Separation of 21 µm diameter particles from human blood in a device with $r/R = 10$. The top bright-field image shows separation at the device chamber. The lower three images show the particles at the inlet, side outlet and main outlet. (b) The concentration of 21 µm diameter particles in the side outlet increases 5× as compared to the inlet. (c) Normalized count of 21 µm diameter particles in side and main outlets shows a capture efficiency of 86%, while 99% RBCs exit through main outlet. Error bars represent the standard deviation of three individual experiments.

20. Inertial microfluidic multimodal separation. The device consists of a high-aspect ratio channel for particle focusing followed by sequencing of two sets of microchambers for multimodal separation. The inset schematics indicate particle size distribution before and after separation, with $a$ and $f$ represent particle size and frequency. $a_{hc}$ and $a_{lc}$ represent high-pass cutoff and low-pass cutoff for multimodal separation.

21. (a) Microfluidic resistance network of the cascaded device. (b) The analogous electrical circuit model of the microfluidic resistance network. The two red dots represent the two sorting chambers. The fluidic resistance is analogous to the electric resistance. The flow rate is analogous to the current.

22. (a) Stacked bright field images illustrating separation of 21µm, 18.5µm and 15µm diameter particles into O1, O2 and O3. (b) The histograms of the samples from
inlet and outlets indicate after multimodal separation, the purities of 21µm, 18.5µm and 15µm diameter particles are dramatically elevated from 38%, 29.3%, 32.7% (inlet) to 89.4% (O1), 80.7% (O2), 95.9% (O3) respectively. ........................................48

23. (a) The concentration plot shows enrichment of concentration of 21µm, 18.5µm and 15µm diameter particles by 2.4×, 3.8× and 1.7× which is due to separation of input sample volume into different outlets (n = 3). (b) The normalized count shows that the separation efficiencies for 21µm, 18.5µm and 15µm diameter particles are 78%, 87% and 99% respectively indicating efficient multimodal separation of particles with only 3µm difference in size (n = 3). ........................................49

24. (a) By tuning flow rate and resistance network, the separation bandwidth is increased from 3µm to 8µm. (b) As shown in the stacked bright field images, the 21µm diameter particles are extracted from O1, while both 18.5µm and 15µm diameter particles elute from O2. The 11µm diameter particles exit through O3. (c) Bright field images at each outlet channel illustrate the successful separation. ...............49

25. (a) By tuning input flow rate and resistance network, the passband location can shift towards a higher cut-off diameter, while maintaining the separation bandwidth. Bright field images taken at two sequenced microchambers (b), and outlets O1, O2 O3 (c) demonstrate a successful separation of the 23µm, 21µm and 18.5µm diameter particles. In all images, the scale bar is 50 µm. ........................................50

26. (a) The concentration plot shows the concentrations of 21µm, 18.5µm, 15µm and 11µm diameter particles are enriched 1.9×, 2×, 2× and 1.5× correspondingly after multimodal separation (n=3). (b) The normalized count shows that the separation efficiencies for 21µm (from O1), 18.5µm (from O2), 15µm (from O2) and 11µm (from O3) diameter particles are 98%, 87%, 75% and 72% respectively indicating successful separation after tuning bandwidth (n=3). (c) The concentration plot indicates obvious enrichment of 23µm and 21µm diameter particles by 2.6× and 3.6×. (d) The normalized count shows that the separation efficiencies for 23µm (from O1), 21µm (from O2), 18.5µm and 15µm (from O3) diameter particles are 99%, 73%, 98% and 93% indicating successful separation after tuning the passband location. ........................................................................52

27. (a) Heterogeneous mixture of microparticles was sorted into three different size distribution after passing the device. (b) Size distribution of inlet particle mixture. The distribution was normalized to the highest count at a specific particle diameter. (c) Size distributions of samples from O1, O2 and O3. The size distribution in each outlet was normalized to the highest count within that outlet...........53

28. (a) Integration of two vortex sorting units for double sorting and purification. (b) Schematics illustrating details of the device operation. Panel (i) – (iv) matches panel in (a). ........................................................................................................57

29. Experimental proof-of-concept. (a) Inertial focusing of microbeads in the upstream microchannel. Fluorescent image was taken at 20mm downstream. (b) Sorting of the larger beads from the smaller beads in the first sorting unit. (c) Larger beads with the remaining smaller beads flow downstream to the second sorting unit with buffer flow accelerating the flow from inlet 2. (d) Double
30. The contour map showing the sorting cutoff diameters at flow rates from 80 ~ 200 µL/min and resistance ratios from 4.3 to 10.2. 

31. Integration of the vortex sorting system. (a) The fluidic resistance network of the integrated system. The \( Q_s \), \( Q_b \), and \( Q_1 \sim Q_5 \) indicate sample flow rate, buffer flow rate and flow rates at different channels of the device, respectively. (b) The analogous electrical circuit model, with flow rate analogous to current and fluidic resistors analogous to electric resistors. (c) Simplified electrical circuit model from (b), with 1 and 2 indicating the two electric circuit junctions. 

32. Tuning the sorting cutoffs of the integrated system. (a) Stacked bright field images showing sorting of a mixture of 23 µm, 18.5 µm and 7 µm at the indicated flow conditions and fluidic resistance ratios. Both the 23 µm and the 18.5 µm diameter microbeads are sorted to O3. (b) Adjusting the flow rates of inlet and buffer leads to only the 23 µm diameter microbeads being sorted to O3. (c) Modifying the resistance network with the same flow condition as (a). Only the 23 µm diameter beads are sorted to O3. A 30-50 images were stacked to indicate trajectories of the microparticles. 

33. Sorting of large microparticles from diluted human blood. (a) Stacked bright-field microscopic images demonstrating sorting of 23 µm diameter microbeads from 18.5 µm diameter microbeads and 0.5 % diluted blood in the two sorting units. 50 images are stacked to illustrate the complete trajectories of microbeads and cells. The red, blue and green dash lines indicate the trajectories of microbeads and cells. (b) Microscopic bright-field images of inlet and outlets. The inset images are photographs of samples collected from different outlets. (c) Concentration plot showing the 23 µm, the 18.5 µm diameter microbeads and the RBCs concentrations from inlet, O1, O2 and O3. (d) Purity plot showing the purity of the 23 µm microbeads in inlet and O3. (e) Separation efficiency of RBCs, the 18.5 µm and the 23 µm diameter microparticles from O1, O2 and O3 respectively. 

34. Sorting of HuSLCs from blood. (a) Stacked bright-field microscopic images demonstrating the sorting of HuSLCs from 1% diluted blood in the two sorting units and corresponding side outlets. The red, blue and green dash lines indicate the trajectories of cells. The green arrows indicate the HuSLCs being sorted into side outlets. The scale bars are 50 µm. (b) Microscopic bright-field images of inlet and outlets. The scale bars are 50 µm. (c) Zoom-in phase-contrast image taken at O3 illustrating HuSLCs (the green arrows) and RBC (the red arrow). (d) Concentration plot of the HuSLCs and the RBCs in inlet and O3. (e) Purity plot of the HuSLCs in inlet and O3. (f) Separation efficiency of the RBCs and the HuSLCs from O1, O2 and O3.
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Summary of microparticles used in experiments</td>
<td>20</td>
</tr>
<tr>
<td>2.</td>
<td>Summary of parameters for evaluating a microfluidic cell sorting device</td>
<td>23</td>
</tr>
</tbody>
</table>
LIST OF SYMBOLS

\( a \) = Particle diameter
\( a_c \) = Cutoff diameter
\( C_L \) = Lift coefficient
\( C_{L^-} \) = Negative lift coefficient
\( C_{L^+} \) = Positive lift coefficient
\( D_h \) = Hydraulic diameter
\( F_D \) = Drag force
\( F_s \) = Shear-gradient-induced lift force
\( F_w \) = Wall-induced lift force
\( h \) = Channel height
\( I \) = Current (electric)
\( L \) = Channel length
\( Q \) = Flow rate
\( r \) = Fluidic resistance of side outlet
\( R \) = Fluidic resistance of main outlet
\( \text{Re} \) = Reynolds number
\( R_s \) = Separation resolution
\( U_f \) = Flow velocity
\( U_L \) = Lateral migration velocity
\( v \) = Volume of sample

\( w \) = Channel width

\( \mu \) = Viscosity

\( \rho \) = Density
CHAPTER 1

INTRODUCTION

Microfluidic cell sorting techniques

Sorting and purification of target cells from complex cellular samples are critical for downstream cell biology research, biomedical research and clinical diagnostics [1-6]. Conventional approaches such as centrifugation [7] and fluorescent-activated cell sorting (FACS) [3, 8] are the most widely-used approaches in research laboratories and hospitals. Centrifugation sorts cells and other microparticles based on their densities [7]. It has been used for separation of red blood cells (RBCs), white blood cells (WBCs) and blood plasma or enrichment of cellular samples. Centrifugation is convenient and straightforward to perform, however it lacks the precision and sensitivity to sort complex sample matrices or to extract rare cells. FACS sorts cells based on fluorescent labels [3, 8]. Before flowing through a FACS system, fluorescently labeled antibodies are introduced to a cell sample to label the target cells. The sample is then flowed through the FACS system, and cells with fluorescence labels get detected and deflected from the cell stream by electric field. FACS system enables high throughput, high precision analysis and sorting of cell samples, however it requires antibodies specific to surface antigens of the target cells, bulky and expensive instrumentation, and trained personnel to perform successful sorting.
The burgeoning of microfluidics [1, 9-15] has enabled new techniques for sorting and purification of biological microparticles which significantly reduces the size of the system and offers faster sample processing with much higher spatiotemporal precision, lower cost and less human interactions [2, 16]. Microfluidic cell sorting techniques can be divided into two categories: active and passive techniques. Active microfluidic systems rely on external force fields to manipulate cells in microchannels. Optical [17-19], magnetic [20], dielectrophoretic [21-23], electrophoretic [24] or acoustic [25, 26] have been used to sort cells based a variety of sorting markers. In optical sorting [17-19], light is used to deflect cells based on size and refractive index. In magnetic sorting [20], cells of interest are first labeled by antibody-coated magnetic beads. As the sample is flowed in microchannel with designed magnetic field, labeled cells are deflected from the cell streams and sorted downstream. Dielectrophoretic and electrophoretic sorting both rely on the electric field. Dielectrophoretic sorting uses non-uniform electric field to sort cells based on polarizability [21-23], while electrophoretic sorting uses uniform electric field to deflect cells based on their surface charge [24]. Acoustic sorting uses standing acoustic waves generated by external piezoelectric actuators to sort cells based on size, density and compressibility [25, 26]. Active microfluidic sorting techniques provide a wide choice of sorting markers which is beneficial for comprehensive analysis and sorting of complex sample matrices, however they offer limited throughput and require sophisticated external controls.

Passive microfluidic techniques rely on physical geometry of microchannels and related hydrodynamics to separate cells without the assistance of external forces, thus they have significantly simplified design, fabrication and operation of devices. A variety of passive microfluidic techniques has been developed such as deterministic lateral displacement [27, 28],
hydrodynamic filtration [29, 30], pinched flow fractionation [31-33], and inertial microfluidics [34]. Deterministic lateral displacement uses a large array of micropillar structures with designed gap to deflect cells based on size [27, 28]. By designing the arrangement and the distance of the micropillars, this technique can sort particles with size from nm to µm. Hydrodynamic filtration device consists of a central channel with multiple pairs of side channels [29, 30]. As a cell mixture flows in the central channel, they are first entrained near the side walls by constant draining of liquid through side channels. Since smaller cells are placed closer to the side walls, they are first extracted to side channels. Larger cells are extracted further downstream. Pinched flow fractionation achieves sorting by introducing a side flow to pinch cells in a narrow channel [31, 32]. Cells are forced into different lateral positions based on their sizes in the short narrow channel and are spaced out into different streams as they enter the downstream expansion region. Although this passive sorting techniques achieve sorting of cells without external forces, one shared limitation is the low throughput ranging from ~ µL/hr to ~ µL/min [16]. Thus it requires significant amount of time to process a biological sample with large volume such as a standard 7.5mL blood draw from patient. Thus a high-throughput and high-precision microfluidic cell sorting technique will be extremely beneficial for processing biological or environmental microparticle samples.

**Inertial microfluidics**

A recently introduced microfluidic technique, termed inertial microfluidics, has been proved to be a power tool for passive and label-free cellular sample preparation in cell biology and clinical diagnostics [34-38]. The technique uses the inertial effects of fluid around individual particles to position them within the flow [39-41]. These fluid forces cause cells to
migrate across streamlines and ordered in equilibrium positions [37, 42-45]. The inertial migration of particles in microchannels follows the balance of the lift forces and occurs in two stages [42]. Initially, particles are subjected to the shear-gradient-induced lift force $F_s$ that pushes them across streamlines towards channel walls and the wall-induced lift force $F_w$ that acts to balance the shear-gradient induced lift force (Figure 1). Driven by these two dominant lift forces, the particles settle along each sidewalls into bands where $F_w$ balances $F_s$. Once this initial equilibrium is reached, near channel walls particle motion is dominated by the rotation-induced lift force $F_\Omega$ [46]. As a result, particles migrate to the center points of walls in this second stage. This model of inertial focusing is generally applicable to rectangular microchannels of any aspect ratio at finite $Re$, and can be used to aid design of inertial microfluidic systems. The maximum downstream length $L$ necessary for particles of diameter $a$ to focus and fully equilibrate can be described as

$$L= \frac{3\pi \mu D_h^2}{4\rho U_f a^3} \left( \frac{w}{C_L^-} + \frac{h}{C_L^+} \right), \ h>w$$

(1)

where $\mu$ is fluid viscosity, $\rho$ is fluid density, $U_f$ is the average flow velocity, and $D_h$ is the hydraulic diameter ($D_h = 2wh/(w+h)$ for a channel $w$ wide and $h$ high). $C_L^-$ is the negative lift coefficient related to the first stage migration and $C_L^+$ is the positive lift coefficient related to the second stage migration [42]. The equation illustrates a strong dependence of the focusing length on particle diameter ($L \sim a^{-3}$). Thus, larger particles will require much less focusing length than the smaller ones.

By designing the microchannel geometries, the focused cells can be further differentiated into different positions based on their size [35, 36, 47-70], shape [71] or deformability [72], leading to label-free sorting, purification and enrichment of cells. Different from other microfluidic cell sorting techniques in which $Re \ll 1$ [16, 34], the inertial forces start to affect
the cell positions only at $Re > 1$. Therefore, inertial microfluidics, due to its mechanism, has much higher throughput than other microfluidic cell sorting techniques. Based on inertial microfluidics, a wide range of applications such as sheath-less flow cytometry [73-77], cell media exchange [78], label-free cell sorting [35, 36, 47-70, 72, 79], volume reduction [80], cell encapsulation [81, 82] and rare cell isolation [52, 61, 83] have been demonstrated with a throughput orders of magnitude higher than other techniques.

As a passive cell-sorting technique, microchannel geometry determines the mechanism and performance of cell sorting. A wide range of channel geometries has been explored for inertial microfluidic cell focusing and sorting. We [36, 47, 58, 62, 63] and others [70, 72] developed straight channels to order cells into equilibration positions for size-based particle separation, blood filtration, and deformability-based cell separation. Lee et al. [50, 51] used expansion/contraction geometries to induce secondary flow for blood plasma separation (with ~62 % yield). Di Carlo et al. [44, 84] developed wavy channels for cell focusing and filtration

![Figure 1. Schematics of the two-stage model of inertial focusing.](image)
which can separate 3 µm from 4.5 µm diameter particles with efficiency of 50%. We [35, 49, 54] and others [55, 65, 66, 69, 85-90] also used curved or spiral channels to generate cross-sectional secondary vortices. Combining the secondary vortices with inertial forces, the spiral channels exhibit >90 % separation efficiency at high-throughput (~1 million cells /min) [49, 54].

Recently, a novel approach has been reported by several investigators for selective isolation and trapping of cells from a mixture. The approach uses a straight channel to inertially focus cells, followed by microchambers to generate laminar vortices for trapping of larger cells. Sollier et al.[91, 92] reported trapping blood cells for high purity plasma extraction, while Hur et al.[48, 52, 62] introduced devices in which particles and cells were trapped and subsequently released on demand. In a parallel independent work, we reported size-based selection of particles in rectangular channel expansions [62]. Due to its high selectivity and trapping mechanism, these devices offer multiple promising potential applications, including extraction of plasma from blood and isolation and enrichment of rare cells such as circulating tumor cells from blood.

While these devices can capture target cells at ultra-low concentrations (~100 cells/mL), there are several disadvantages: 1) their non-continuous two-step operation is complicated and requires a complex fluidic setup [93]; 2) the release/washing step inevitably decreases the capture efficiency [94]; 3) The sorting cutoff of the device depends on the flow rate and the size of the chamber which cannot be adjusted after fabrication; 4) the number of cells trapped by each vortex is limited, with saturated vortices leading to loss of the target sample. Although the limit of trapping capacity can be subverted by paralleling chambers into an array [52], the overall isolation process still presents a challenge when large sample volumes must be processed (e.g., >10 mL).
Motivation

Sorting of cellular samples and extraction of target cells are critical for subsequent biomedical research and clinical diagnostics [6, 8, 95]. A real-world cellular sample is often a complex matrix containing several types of cells with distinct or continuous physical sizes and different concentrations [96]. Cells of the same type may also have different sizes depending on their growth stages [97]. For example, in a normal human blood sample [98, 99], red blood cells (RBCs) have a diameter of ~6 µm and a concentration of ~10^9/mL. White blood cells (WBCs) have a range of diameter from 10 ~ 20 µm depending on the subpopulations and an overall concentration of ~10^5/mL. In a blood sample from a patient with metastatic cancer, besides these normal blood cells, there are also rare presence of circulating tumor cells or clusters (CTCs) with size from 10~30 µm and a concentration of 1~100 /mL [67, 100-102]. In heart tissue samples [103], cardiac myocytes have a continuous size distribution with an average size of 15 µm. Non-myocytes also have a continuous size distribution with an average size of 10 µm. Due to the size and concentration complexity of cellular samples, the capability to sort target cells from non-target cells with high precision, efficiency, purity and throughput will be extremely helpful. Current inertial microfluidic devices however have several limitations for separating such samples. First, as a size-based technique, they have limited sorting resolution which prevent them from efficient size-based sorting of cells with small size difference (1 ~ 2 µm) [48-51, 54, 62, 65, 69, 70, 104]. Second, they only provide bimodal sorting with a single sorting cutoff diameter which prevent them from processing cellular samples with multiple different sized cells [35, 47, 51, 54, 57, 70, 87, 88]. Third, they lack versatility and easiness of modifying the sorting cutoff sizes according to the sample size distribution which leads to extra burden of redesigning and refabricating the devices. Thus developing an inertial microfluidic
cell sorting devices with high resolution, powerful multimodal sorting ability as well as the
versatility of systematic modification will be beneficial for size-based cellular sample
preparation as well as processing samples containing any type of microscale particulates.

**Scope of work**

In this work, an inertial microfluidic sorting platform based on vortex-aided sorting
scheme was developed to overcome the limitations of previous inertial microfluidic devices. The
development of the inertial microfluidic sorting platform include three steps. First, an inertial
microfluidic cell sorting unit was designed to enable continuous vortex-aided sorting [59]. With
the optimization of the device geometry and flow condition, the sorting unit can successfully sort
particles or cells with high resolution in a continuous fashion. Sorting of 21 µm and 18.5 µm
diameter microparticles was demonstrated with > 90 % efficiency which is the highest sorting
resolution among the existing inertial microfluidic devices. After developing the single sorting
unit, an integrated sorter was developed by sequentially cascading two sorting units and
engineering the surrounding fluidic siphoning channels [60]. Instead of bimodal sorting with a
single cutoff diameter, the integrated device enables high-resolution, multimodal size-based
sorting functionality. Complex particle mixture such as a mixture of three different sized
particles can be sorted continuously with high efficiency. More importantly, the sorting cutoffs
of the device can be adjusted by simply changing the flow rate or modifying the resistance of
fluid siphoning channels without the burden of refabricating the device. Third, an alternative
way of integrating the sorting units was developed to enable new double sorting and purification
function. The function is achieved by connecting the high-pass outlet of the first sorting unit to
the inlet of the second sorting unit. With properly designed surrounding fluidic resistance
channel network, the larger target cells can be sorted from the small background cells two times sequentially. It can generate highly purified target cell product even for samples containing background cells with concentration several orders of magnitude higher than target cells. The two distinct integration topologies indicate the versatility of building the vortex-aided cell sorting unit to enable different sorting functions for samples with different size-distributions and compositions. Using the powerful sorting device, we demonstrated sorting of spiked HuSLCs from human blood samples leading to target cell product with enriched concentration and significantly enhanced purity.

Summary

Following this introduction, Chapter 2 will describe the experimental methods used in this work including the standard soft lithography and the new replication approach using epoxy. Following the fabrication approaches, experimental methods used in inertial microfluidic research will be described including particulate sample preparation, observation and imaging of inertial phenomena and data analysis.

Chapter 3 will introduce the design of the inertial microfluidic cell sorting device based on inertial focusing and continuous microscale vortices. First, the inertial focusing mechanism and vortex-aided sorting mechanism will be described. Then, the sorting resolution and tunability of the unit will be comprehensively investigated. Following the characterization, high-resolution sorting of a mixture of 21 µm and 18.5 µm diameter microparticles will be demonstrated. Lastly, extraction of large particles from blood will be presented.

Chapter 4 will introduce the first arrangement of two sorting units for multimodal sorting of complex particle samples. The device principle and integration approach will be introduced. Sorting of a mixture of three different sized microparticles will be demonstrated as a proof of
concept. To demonstrate the versatility and tunability of the device, modification of the sorting bandwidth and passband location will be demonstrated. Finally, sorting of heterogeneous particle sample with continuous size distribution will be demonstrated.

Chapter 5 will introduce the second arrangement of two sorting units for double sorting and purification of large target cells. Following the introduction of integration principle, the relationship among the flow rate, channel resistance setup and resulting sorting cutoff diameters will be investigated comprehensively. A theoretical equivalent electric circuit model will then be introduced to guide the design of the channel resistance network. To exhibit the function of double sorting and purification, sorting of spiked rare large target particles or HuSLCs from human blood will be demonstrated.

Chapter 6 will summarize this work with future directions.
CHAPTER 2

EXPERIMENTAL METHODS

In this chapter, the experimental methods used in the development of inertial microfluidic devices of this work are described. Generally devices were fabricated in polydimethylsiloxane (PDMS) [105-107] using soft lithography, although rigid epoxy [80] was used in high pressure situations. Fluorescent polymer microparticles were used as surrogates for cells [57, 60, 64]. The samples from different outlets were collected and analysed to evaluate and optimize device performance. Once the operational parameters were determined, real biological samples such as blood or other cellular suspensions were prepared and introduced into the devices for size-based sorting.

Device fabrication

Microfabrication of a master mold

The most widely-used method to fabricate microfluidic devices is soft lithography [105-107] – a fabrication process that includes microfabrication of a master mold, casting and curing of PDMS on the mold and sealing the PDMS chip on a glass substrate. The first step of soft lithography is to fabricate a master mold using SU-8 (SU-8 2050, Microchem Corp.) or dry film photoresist (MX5050, Dupont) (Figure 2). In detail, a silicon wafer was first cleaned using acetone, isopropyl alcohol (IPA) and deionized (DI) water. The silicon wafer was then dipped in
buffered oxide etchant (BOE) for 30 sec to remove oxide layer. After that, the wafer was dehydrated in a 150 °C oven for 15 min and cooled to room temperature. Following the cleaning, SU-8 2050 negative photoresist was spun on the wafer into desired thickness using a spin coater. The spin speed and time can be found in the SU-8 2050 data sheet. The wafer was then soft-baked for 10 min at 65 °C followed by 10 min at 95 °C on a hotplate. After soft baking, the wafer was loaded on a mask aligner and contacted with a photomask with microfluidic channel pattern on it. To achieve vertical side wall, a long pass filter was installed under the UV lamp to eliminate UV radiation < 350 nm. The exposure energy is related to the thickness of the SU-8. Thicker SU-8 film requires longer exposure. The detailed information can be found on the product data sheet. Following the UV exposure, the wafer was baked again on a hotplate for 10 min at 65 °C followed by 15 min at 95 °C. Followed the post exposure bake, 

**Figure 2.** Schematics of soft lithography. (a) spin coating negative photoresist SU-8. (b) Selective exposure using a photo mask under UV light. (c) Development using SU-8 developer. (d) Casting PDMS on the SU-8 mold and curing on a hotplate. (e) Peeling off the PDMS chip and treating with oxygen plasma. (f) Bonding the PDMS chip on a glass substrate.
the wafer was immersed in SU-8 developer to remove the unexposed region. Finally, the SU-8 mold was cleaned using oxygen plasma in a plasma system (CS-1701 RIE, March Plasma System) at 40 cfm of O₂ flow and 200 mW RF power for 1 min and was ready for PDMS casting.

Alternatively, the master mold can be fabricated using dry film photoresist MX 5050. In detail, glass slides were used as substrates instead of silicon wafers due to better adhesion against dry film [60]. A glass slide was first cleaned with IPA and DI water and then dehydrated on a hotplate at 200 °C for 10 min. After dehydration, dry film photoresist was laminated on the substrate using a laminator (XRL-120, Western Magnum Corp.) at 85 °C at a lamination speed of 1.2 m/min. After lamination of the film, the substrate was loaded on a mask aligner with the designed photo mask and exposed under UV for 40 s at 5 mW. After exposure, the substrate was placed on a hotplate at 100 °C for 10 mins followed by development with 0.75% wt potassium carbonate. One should note that the stop point of the development is critical in this process. Overdevelopment could lead to detachment of the structures from the substrate. Figure 3 shows a PDMS master mold made of dry film photoresist.

As the materials for making a PDMS master mold, SU-8 and MX5050 have their own advantages and drawbacks. SU-8 has good adhesion to a silicon wafer substrate thus is robust during repetitive PDMS casting and peeling. If spin-coating properly, SU-8 can be deposited onto the substrate without bubbles which makes it suitable for making large-area of repetitive structures such as micropillar array or microchamber array. Fabrication of structures with small dimension (~10 µm) or high aspect ratio (e.g. w : h = 1 : 3) can also be easily achieved using SU-8. The drawback of the SU-8 is that due to the inconsistency of environmental condition or spin
coating, the heights of the structures may vary by several microns among different batches of molds or different locations on the same mold. Opposite to SU-8, dry film has pre-determined thickness, thus ensures the uniform heights of the structures over the entire substrate. The chemical reagents involved in the dry-film process are much less toxic than SU-8 process which makes it friendlier to human health and environment. Although the MX5050 provides uniform thickness, the available thicknesses of film are limited to 20 µm, 30 µm, 50 µm and 100 µm which prevents it from be used for fabricating devices with more arbitrary heights such as 25 µm. Besides the limitation in height, bubbles with sizes ranging from µm to mm maybe generated during lamination which may further lead to distorted structures. Furthermore, the adhesion of the dry film on the substrate is not as robust as SU-8. One needs to be extremely careful during the development of the mold or peeling PDMS from the mold to avoid the detachment of structures from substrates.

**Figure 3.** Photograph of a master mold made of MX5050 dry film photoresist on a glass substrate.
PDMS casting and bonding

After fabricating the master mold with SU-8 photoresist or dry film photoresist, the mold was placed into a petri-dish. PDMS base and curing agent (Sylard 184, Dow Corning) with mass ratio of 10:1 was dispensed into a mixing cup and vigorously stirred until well mixed. The mixture was then placed into a vacuum chamber and degassed for 30min ~ 1h until gas bubbles completely disappeared. The degassed mixture was poured onto the PDMS master mold slowly to avoid any bubbles. The petri-dish was then placed on a hotplate and cured for 2 h at 80 °C. The outlines of the cured PDMS chips were cut using a scalpel and the chips were peeled off slowly with a tweezer. The inlet and outlet ports were punched with a 14 gauge flat syringe needle or 1.5 mm outer diameter biopsy punch (World Precision Instruments). To bond the device, the PDMS chip and a 1 mm thick glass slide were cleaned using a low-residue tape (3M). After cleaning, the chip and the substrate were treated with oxygen plasma using hand-hold plasma surface treater (BD-20AC, Electro-Technic Products, Inc.). Alternatively surface treatment of PDMS and glass substrate can be conducted in reactive ion etching system (CS-1701 RIE, March Plasma System) at 40cfm O₂ flow, 100mW RF power for 25 s. The chip was then placed on the substrate to complete the bonding. To enhance the strength of the bonding, the device was placed on a hotplate at 60 °C for 15 min immediately after bonding. Figure 4 illustrates a successfully fabricated PDMS device.

Alternative fabrication of devices using epoxy

Cell focusing and sorting in inertial microfluidic devices always require high flow rate ($Re = 1$~$100$). PDMS microchannels, due to the material nature, may get expanded in such high flow and high pressure conditions. Such expansion distorts the channel walls and further affects the performance of inertial focusing and separation. To maintain the shape of the channel at high
pressure, a material with higher rigidity such as epoxy can be used to fabricate the devices instead of PDMS.

To fabricate devices in epoxy, a reversed PDMS mold needs to be fabricated by double casting PDMS on the original PDMS chip (Figure 5). After peeling from the SU-8 or MX5050 master mold, the original PDMS chip was first thermally aged in an oven at 100 °C for at least 72 h. Thermal aging leads to crosslinking of low molecular weight species and alters the hydrophobicity of the PDMS surface. This surface modification will effectively prevent the double-cast PDMS from sticking to the original PDMS surface. The thermally aged PDMS chip was used as a new mold for double casting of PDMS. After double casting, the new PDMS chip was peeled off from the aged PDMS mold and used as the mold for epoxy casting. To create proper inlet and outlet interface for the epoxy chip, the inlet and outlet ports were punched on the PDMS mold using 0.75 mm OD biopsy punch (World Precision Instruments). Teflon-coated wires with 0.028” diameter (PTFE-coated stainless steel wire, McMaster-Carr) were inserted into PEEK tubes (1/16 OD × 0.030 ID, IDEX Health & Science LLC.). One end of the Teflon wire was then partially inserted into an inlet or outlet port. The epoxy resin (Epox-Acast 690, Smooth-on) was then mixed, degassed and poured on the PDMS mold and cured at room temperature.

Figure 4. Photograph of a PDMS device.
Figure 5. Schematics of epoxy chip fabrication. (a) Thermal aging of the original PDMS chip. (b) Double cast PDMS on aged PDMS chip. (c) New PDMS chip with the reversed structures. (d) Punch inlet and outlet ports and insert Teflon wires. (e) Insert Teflon wires into PEEK tubes. (f) Cast epoxy on the mold. (g) After curing, remove PDMS mold and Teflon wires. (h) Bond the epoxy chip with the epoxy-coated glass substrate.
temperature for ~36 h. After curing, the epoxy replica was peeled from the PDMS mold and the Teflon wires were removed. To bond the epoxy chip to glass, a 1” × 3” glass substrate (Fisher Scientific) was coated with epoxy and cured at room temperature. The epoxy chip was then bonded to the glass substrate irreversibly by placing them on a 60 °C hotplate with gentle pressure by hand. Since epoxy is more rigid than PDMS, the epoxy microchannels can maintain their shapes and dimensions at high flow rate and pressure. As Figure 6 shows, when introducing the same flow rate ($Q = 275 \mu$L/min) to a PDMS device and an epoxy device with the same channel configuration (designed to be 30 µm), the width of the PDMS channel expanded from 30 µm to 45 µm, while the width of the epoxy channel remains the same.

**Sample preparation**

**Preparation of microbead suspensions**

Inertial microfluidics is usually characterized using neutrally-buoyant polymer microbeads in a microchannel [108]. A bead suspension with specific size range is first induced

![Figure 6](image.png)

**Figure 6.** Microscopic bright-field images showing the expansion of microchannels made of (a) PDMS and (b) epoxy after introducing certain flow rate ($Q = 275 \mu$L/min). PDMS channel expands to 45 µm while epoxy channel remains the original dimension.
into the microchannel and images of bead migration in the flow are captured using certain imaging technique. These images are then analysed using corresponding approaches to investigate the inertial focusing or sorting in the device. Once a device is characterized and optimized using microbeads, it can be further tested with cellular samples for real-world applications. The use of microbeads offers a number of advantages, including (i) greater robustness than cells, (ii) ease of preparation of solutions with desired concentrations, (iii) reduced cost, and (iv) availability in a wide range of sizes (from nm to μm), fluorescent labels, materials and density. To prepare neutrally-buoyant microbead suspensions, we prepared a buffer by adding sodium chloride (NaCl) (Sigma Aldrich) to DI water to match the density of microbeads. The amount of NaCl depends on the density of beads. For example, to suspend polystyrene beads with density of 1.05 g/cm³, 50g of NaCl was added to 1L of DI water to achieve the final concentration of 1.05 g/cm³. To prevent particle aggregation, 0.1% v/v Tween 20 (Sigma Aldrich) was added to the buffer. Microbead stock solution or powder was then added to the buffer solution and mixed on a vortex mixer for 1 min until uniformly distributed in the solution. The microbead suspension is ready to use in the following experiments. The information about microbeads used in this work is listed in Table 1.

**Preparation of cellular samples**

One of the potential applications of the presented inertial microfluidic device is sorting of cells based on their physical sizes. To demonstrate the ability of isolation of larger target cells from smaller non-target cells such as blood cells, we spiked HuSCLs into diluted human blood as a model of cellular sample. HuSCLs are a subset of tumor cells that have the ability to self-renew and generate the diverse cells that comprise the tumor [109-111]. First, HuSLCs were cultured for 4 days on a 10 cm primaria plate which is coated with matrigel. HuSLCs were then
trypsinized after they were confluent and diluted to the required concentration. Before the experiment, 8 µL of cell suspension was injected into a hemacytometer to determine the size distribution and concentration. As Figure 7 shows, the HuSLC sample in our experiment has a concentration of $1.3 \times 10^5$/mL and a size distribution from 12 µm to 25 µm with majority from 12 µm to 20 µm. Human whole blood obtained from blood bank was diluted in PBS into 1% v/v suspension. 500 µL of HuSLC sample was spiked into 5mL of diluted blood leading to a final concentration of $1.3 \times 10^3$/mL. The sample was ready to use for sorting on the device.

**Experimental setup**

Before introducing sample, each device was first primed with DI water for microbead experiments or PBS for cellular experiments. To ensure priming without air bubbles, the device was placed in a vacuum chamber and degassed for 15 min. Immediately after degassing,

**Table 1. Summary of microparticles used in this work.**

<table>
<thead>
<tr>
<th>Diameter (µm)</th>
<th>Excitation/Emmision (nm)</th>
<th>Concentration</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.19</td>
<td>365/415</td>
<td>0.1~0.5% v/v</td>
<td>Bangs Labs</td>
</tr>
<tr>
<td>7</td>
<td>480/520</td>
<td>1% v/v</td>
<td>Bangs Labs</td>
</tr>
<tr>
<td>10</td>
<td>480/520</td>
<td>1% v/v</td>
<td>Bangs Labs</td>
</tr>
<tr>
<td>15</td>
<td>540/560</td>
<td>1% v/v</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>15</td>
<td>480/520</td>
<td>1% v/v</td>
<td>Bangs Labs</td>
</tr>
<tr>
<td>18.5</td>
<td>480/520</td>
<td>1% v/v</td>
<td>Polyscience</td>
</tr>
<tr>
<td>21</td>
<td>Bright field</td>
<td>2% v/v</td>
<td>Polyscience</td>
</tr>
<tr>
<td>23</td>
<td>480/520</td>
<td>1% v/v</td>
<td>Polyscience</td>
</tr>
<tr>
<td>5~27</td>
<td>Bright field</td>
<td>0.1% w/v</td>
<td>Cospheric</td>
</tr>
</tbody>
</table>
priming liquid was introduced into the device. Since vacuum temporally forms in the microchannel, priming liquid spreads the entire microchannel automatically without bubbles. This priming method is especially helpful for devices containing large-area and complicated geometries.

To introduce samples into a device with steady flow rate, a sample was first loaded into a 5 mL or 20 mL syringe (Fisher Scientific) and connected to the device using a 1/16” tubing and proper fittings (Upchurch Scientific). The syringe was then loaded on a syringe pump (Legota 180, KD scientific) from which the flow rate can be set to a specific value. Samples passing through the device were collected into different vials for downstream analysis.

![Image](image)

**Figure 7.** (a) Bright-field image of HuSLCs. (b) Zoom-in bright field and phase-contrast images of HuSLCs. (c) The size distribution of HuSLCs.
To visualize trajectories of particles in bright-field, images were taken using an inverted epi-fluorescence microscope (IX71, Olympus Inc.) equipped with a 12-bit high-speed CCD camera (Retiga EXi, QImaging). The exposure time was set to 10 μs and 200 ~ 300 images were sequentially taken with minimum time interval. At high-flow-rate situation, 2 × 2 or 4 × 4 binning can be used to capture particle motion. A complete view of particle motion was established in ImageJ by stacking the images (Figure 8). To take fluorescent images of particle streak velocimetry, we used fluorescently labelled microparticles and used the same imaging setup to sequentially take 20 ~ 50 images with an exposure time of 100 ~ 200 ms. The images were then stacked and pseudo-colored in ImageJ to illustrate fluorescent streaks of microparticles (Figure 9). To visualize size-based sorting, a mixture of different sized microparticles with different fluorescent labels were used with corresponding filter cubes. By switching between different filter cubes, the fluorescent trajectories of different sized particles can be visualized and captured individually. Subsequently, the captured images were pseudo-colored with appropriate colors and merged in ImageJ (Figure 9).

Data analysis

Inertial microfluidic sorting can be quantitatively characterized by analyzing inlet and outlet samples. The parameters often used to evaluate separation performance include

Figure 8. Processing microscopic high-speed bright-field images for visualization of particle trajectories. (a) A single bright-field image. (b) A stack of 200 images in ImageJ unveiling the trajectories of particles.
microparticle separation resolution, purity, and separation efficiency. The definitions are summarized in Table 2. For inertial size-based separation, size distributions of microparticles from and inlet and outlets directly show the separation performance. A simple approach to measure the size distribution is to induce the sample into hemocytometer to form a monolayer of particles and then capture bright field images under a microscope. The images are then analyzed using software such as Image Pro Plus or ImageJ to automatically acquire particle size and count. The data are then plotted into a histogram or a scatter plot. Concentration of microparticles from different outlets indicates sample enrichment or dilution after separation which can be used to evaluate devices for rare cell separation and enrichment. Similarly, the particle concentration of outlet samples can be measured easily with hemocytometer by dividing the count of the particles by the volume. The volume can be calculated as the area of the image multiple by the gap of the hemacytometer (100 µm). While hemacytometers offer a low-cost option, the increased error rate (as high as 10%) will require a larger sample size to maintain the confidence interval. Thus precise measurement of size distribution of low-concentration samples might be challenging.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency (normalized count)</td>
<td>Target particle count from one outlet over total count of target particles from all outlets</td>
</tr>
<tr>
<td>Purity</td>
<td>Target particle count (or concentration) over total particle count (or concentration) in one outlet</td>
</tr>
<tr>
<td>Resolution</td>
<td>Resolution measures the ability of separating two different sized particles and is defined as $a/\Delta a$, where $\Delta a$ is the smallest difference in diameter that can be resolved in a microfluidic device with $&gt;80%$ efficiency at particle average diameter of $a_{ave}$</td>
</tr>
</tbody>
</table>
although centrifugation can be used to increase the concentration. More precise measurement requires facilities such as flow cytometer.

Sorting efficiency and purity are the most critical parameters to characterize the performance of a sorting device. The efficiency can be calculated from the concentration plot following a simple procedure. For example, as shown in Figure 10a, the concentrations of 15 µm diameter particles and 7 µm diameter particles collected from middle and side outlets are first measured using a hemacytometer. The volume of samples from these outlets are also measured. The count of the 15 µm diameter particles from the middle outlet is determined by multiplying the concentration and the sample volume from the middle outlet. The same process can be used to determine count of the 15 µm diameter particles from the side outlet sample. The sorting efficiency for 15 µm diameter particles from the side outlet is then determined as count from the side outlet divided by the total count indicating ~90 % efficiency (Figure 10b). Similar measurement and calculation are carried out for 7 µm diameter particles indicating 99.7 % efficiency from middle outlet. Purity of an inertial sorting device can be characterized from the concentration plot. As Figure 10c shows, the purity of 15 µm diameter particles in the inlet sample is calculated as the ratio of 15 µm diameter particle concentration over the total

![Fluorescent images showing sorting of particles with different sizes.](image)

**Figure 9.** Fluorescent images showing sorting of particles with different sizes. (a) Pseudo-colored fluorescent streaks of FITC labeled 18 µm microparticles. (b) Pseudo-colored fluorescent streaks of TRITC labeled 15 µm microparticles. (c) Stacked image showing size-based sorting of the microparticles.
concentration to be 0.65 %. While after sorting the purity of 15 µm diameter particles from side outlet is calculated to be 65 % a 100× enhancement in purity.

Cellular or microparticle samples often exhibit heterogeneity in terms of size. Thus, precise measurement of the separation cutoff size and the separation resolution of a device helps to determine the proper size range for efficient separation. The two parameters can be measured by inducing commercially available particle mixture with continuous size distribution (Cospheric Inc.) through a device and analyzing the outlet samples. For example, as shown in Figure 11 the particle mixture was pumped through a vortex-aided inertial microfluidic sorter. Microparticles with diameters larger than cutoff exit through side outlets, while microparticles with diameter smaller than cutoff were collected from main outlets. The size distribution of side and main outlets were measured using a hemocytometer. The histogram of normalized particle counts from side and main outlets indicates the cutoff size to be ~18 µm. The separation resolution can also be measured from the histogram. The histogram indicates that the device can separate particles with size difference of \( \Delta a = \sim 1 \mu m \) for \( \eta = >70 \% \), \( \Delta a = \sim 2 \mu m \) for \( \eta = >80 \% \) and \( \Delta a = \)

Figure 10. (a) Concentrations of 15 µm and 7 µm diameter microparticles from samples collected from middle and side outlets. The red and blue bars represent 7 and 15 µm diameter microparticles correspondingly. (b) Sorting efficiency of 15 µm and 7 µm diameter microparticles. (c) Purity of 15 µm diameter microparticles from inlet and side outlets.
~3 µm for η = >90 %. Average diameter $d_{ave}$ of the sortable particles for this device is 18 µm.

Thus the resolution is calculated to be $a/\Delta a = 18/2 = 9$.

**Figure 11.** (a) Bright microscopic image showing sorting of microbeads with continuous size distribution from 7µm to 23µm. 200 images are stacked to illustrate the particle trajectories. (b) Histogram of the normalized count indicates the sorting cutoff.
CHAPTER 3

INERTIAL MICROFLUIDIC VORTEX-AIDED SORTING

This chapter introduces an inertial microfluidic cell sorting unit based on continuous microscale vortices for size-based sorting of large particles with high size resolution, high efficiency and high purity [59]. The design uses a high-aspect-ratio channel upstream to inertially focus cells into equilibrium positions near microchannel sidewalls. Follows the focusing channel, a single pair of chambers with siphoning channels is designed to generate vortices for continuous size-based sorting. During operation, the larger cells are selectively-entrained by inertial lift force into sheath flow and are released through the siphoning side channels, while the smaller cells remain in main flow and exit through the main outlet. The sorting principle, resolution and tunability of the microfluidic geometry were investigated with numerical models and experiments. High-resolution sorting of polystyrene particles with size differences of less than 2 µm was demonstrated with high throughput (2.5× 10⁴ /min), high efficiency (~90 %), and high purity (>90 %). To verify the feasibility of biological sample separation for potential clinical applications, continuous separation using blood spiked with particles was also demonstrated, illustrating 86 % separation efficiency and 5× enrichment. As a cell sorting device, the design has several advantages, including simple geometry, small footprint, ease of paralleling and cascading, continuous one-step operation, as well as ultra-high
sorting resolution. The described approach is applied to sort cells and particles quickly and effectively with broad applications in cellular sample preparation.

**Device principle**

The vortex-aided design consists of four major components: a high-aspect-ratio channel for inertial particle focusing; two symmetric chambers for vortex formation; two side outlets at the corners of the chambers for sheath flow formation and extraction of large particles; and a main outlet for exit of small particles (Figure 12). The focusing channel is 10 mm long with a 50 \( \mu \text{m} \times 100 \mu \text{m} \) \((w \times h)\) cross-section; each capture chamber is \(500 \times 500 \mu \text{m}^2\). The length of outlet channels can vary to modulate fluidic resistance ratio \((r/R)\) of the side \((r)\) and main \((R)\) outlets for optimizing the device performance.

The first critical component of the system is the high-aspect-ratio channel aimed at focusing particles or cells into first stage as two bands along channel sidewalls. Using equation (1) and lift coefficients we presented in our recent work [42], \(L_1 = 1.6 \text{ mm}\) and \(L_2 = 12.8 \text{ mm}\) for the first and second stage focusing of 20 \(\mu\text{m}\) diameter particles in 100 \(\mu\text{m} \times 50 \mu\text{m} \) \((h \times w)\) channel \((C_L^- = 0.03 \text{ and } C_L^+ = 0.007 \text{ at } Re = 110)\). Accordingly, a 10mm long channel was designed to ensure large particles (~20 \(\mu\text{m}\) diameter) focus in the first stage which was the preferable focusing condition for extraction of larger particle in this vortex-aided separation. This preferred focusing condition will be discussed in more detail in the following section.

Next, we discuss the basis of size-selective hydrodynamic-vortex separation. As illustrated in schematics (Figure 12b) and numerical simulation (Figure 13a), flow in a microchamber is separated into the main and sheath components, exiting through the main and side outlets, respectively. Sudden expansion of the microchannel modulates lateral flow velocity
Figure 12. (a) Schematic of the vortex-aided inertial microfluidic design. Flow resistances $r$ of the side outlet channel and $R$ the main outlet channel can be modulated to adjust the sorting cutoff of the device. (b) Size-selective separation in microchambers with three outlets. Red dash line represents the boundary streamline. The black arrow represents the flow direction. $F_s$ and $F_w$ represent shear-gradient induced lift force and wall-induced lift force.
Figure 13. Theoretical investigation of size-based separation. (a) CFD-ACE+ model showing streamline distribution in two symmetric microchambers with three outlets. The red solid line indicates the boundary of main flow and sheath flow, with $R$ and $r$ representing the channel resistance of the main and side outlets. (b) Schematic illustrating the relationship between the input flow rate and the separation cutoff diameter, with $U_l$ representing lateral migration velocity. (c) The close-up images form the numerical model show the entrance and exit regions of the separation unit. The $F_s$ is the shear-gradient induced lift force. The $d_p$, $d_b$ and $d_m$ are the particle focusing position, boundary position and migration distance needed for a particle to enter the sheath flow. The red solid line represents boundary streamlines (d) CFD-ACE+ model illustrating the cross-sectional view of the 3-D boundary streamlines. The red area represents main flow, while the blue area indicates sheath flow. (e) Close-up of the upper left channel quadrant (indicated as black dashed lines in panel (d)). Channel resistance ratio $r/R$ influences the boundary position and migration distance for particles to reach the boundary streamline.
and leads to formation of microscale vortices. Presence of these vortices has been confirmed experimentally and numerically [52, 61]. Neutrally-buoyant particles entering the expansion region experience a sudden absence of the microchannel wall and the significantly reduced wall-induced lift force $F_w$. Thus, the dominating shear-gradient induced lift force $F_s$ drives particles to migrate towards the sheath flow (Figure 12b). The magnitude of $F_s$ scales as $F_s \propto U_r^2 a^2$, where $U_r$ is flow velocity and $a$ is particle diameter [59, 61, 112, 113]. Assuming that $F_s$ is balanced by the Stokes drag ($F_D = 3\pi \mu a U_L$), the lateral migration velocity of these particles scales with particle size as $U_L \propto U_r^2 a$. Consequently, the larger particles migrate across the streamlines faster than the smaller particles ($U_L \propto a$). Once the larger particles cross the boundary streamline between the main and the sheath flows, they become entrained and exit through side outlets. Meanwhile, the smaller particles remain in the main flow due to insufficient lateral migration (Figure 12b). It is this vortex-initiated lateral migration that enables continuous separation of different-sized particles at high resolution.

To demonstrate continuous particle separation in the device, a mixture of 21 µm and 15 µm diameter polystyrene particles was injected into the device at input $Re = 110$ (Figure 14). The device operation only requires a single syringe pump and includes only a single step of sample injection, thus reducing the complexity and costs of operation. The stacked images in Figure 14 illustrate the expected behavior – extraction of the 21 µm diameter particles from the mixture. Particles first order at equilibrium positions near microchannel sidewalls. As they travel to the vortex chamber, the 15 µm diameter particles exit through the main outlet while the 21 µm diameter particles enter the chambers following the sheath flow and exit through the side outlets. In the following sections, we investigate the flow conditions and the outlet resistance network as the two factors critical to device performance.
Results and discussion

Morphology of microscale vortices

Microscale vortex is the critical component for size-based sorting in the presented device. The morphology of microscale vortices depends on the input flow rate and the resistance ratio \( r/R \). First, we studied the vortex geometric progression at increasing \( Re \) both numerically and experimentally using a device with fixed a \( r/R = 10 \) (Figure 15a-b). At \( Re = 11 \), the vortex is underdeveloped due to the low flow velocity. As the \( Re \) increases to 88, the area experiences an increase of two orders of magnitude (growing stage) occupying 50% of the chamber. The remaining 50 % of the chamber area is occupied by the sheath flow. As \( Re \) increases from 133 to 333, the flow velocity increases causing the vortex area to increase from 60 % to 70 % (stable stage) with 30 % of the chamber area as the sheath region. Further increase of \( Re \) has very limited impact on the area of the vortex because the side outlet is continuously siphoning flow.

The resistance network influences vortex geometry by tuning the siphoning rate of flow

![Image](image.png)

Figure 14. Bright-filed images at various downstream positions illustrating the focusing and sorting of 21 µm and 15 µm diameter microparticle mixture.
in the side outlet. The CFD-ACE+ simulations at $Re = 88$ show the increase of vortex dimension as $r/R$ increases (Figure 15c). The vortex area at various $r/R$ was measured from both simulation and experimental results (Figure 15d). At $r/R = 1$, considering the current distribution in a parallel circuit, flow is distributed evenly in the three outlets ($Re_{Q1} = Re_{Q2} = Re_{Q3} = 29.3$). Accordingly, the vortex occupies only $\sim 36\%$ of the chamber area due to the fast release of flow from side outlet. At $r/R = 10$, the vortex area increases to $\sim 50\%$ because the flow gets siphoned much slower through the side outlet ($Re_{Q1} = Re_{Q2} = 7.3$ and $Re_{Q2} = 74$). As the ratio increases further to $r/R = 50$, the vortex further enlarges and occupies $73\%$ of the chamber area.
Particle dynamics in the microscale vortices

For a cell sorting device, the ability to adjust sorting cutoff diameter is essential for efficiently processing cellular samples with different size distributions. From the discussion of inertial migration above, the lateral migration velocity $U_L$ of a particle is strongly related to the flow velocity in the microchannel $U_f$ ($U_L \propto U_f^2$). Faster input flows lead to faster lateral migration and longer lateral migration distances for all particles (Fig. 12b), resulting in a smaller cutoff diameter $a_c$. This offers the possibility of tuning cutoff diameter through simply adjusting input flow rate.

Another parameter that influences the separation cutoff diameter is the position of the boundary streamline $d_b$, which determines the lateral migration distance $d_m$ needed for a particle to reach the boundary (Figure 12c). Our numerical results illustrate the 3-dimensional boundary at the entrance of the chamber (Figure 12d) and show that position of $d_b$ can be precisely tuned by engineering the channel fluidic resistance ratio $r/R$, where $r$ is the resistance of a side outlet and $R$ is the resistance of the main outlet. As Figure 10e shows, at $r/R < 2$ initial equilibrium position of all particles is within the sheath flow boundary, leading to non-selective extraction of all particles through side outlets. As the boundary streamline shifts towards the side wall at higher $r/R$, the initial equilibrium position of particles shifts into the main flow, leading to a possible separation. Since larger $r/R$ leads to longer migration distance $d_m$, the cutoff diameter $a_c$ increases at larger $r/R$. This offers the possibility of tuning cutoff diameter through resistance modification of outlet channels. In summary, our theoretical analysis indicates that the microfluidic geometry provides size-based, continuous separation functionality with the capability of tuning cutoff diameter.
Resolution and tunability of the sorting unit

Having discussed the basic principles of the hydrodynamic-vortex sorting, we experimentally demonstrate resolution and tunability of separation. As described in the introduction section, the resolution of sorting plays a critical role on the performance of size-based sorting. In the first set of experiments, solution of polymer microspheres ($4 \times 10^4$/mL) with continuous size distribution from 10 µm to 27 µm was pumped into a single microchamber device with $r/R = 5.4$ at $Re = 110$ ($Q = 500$ µL/min). In Figure 16a, we show that microparticles with diameter larger than the set cut-off size ($a > a_c$) migrate into the sheath flow as expected, exiting separation channel through side outlets. Microparticles with $a < a_c$ elute from the main outlet. This is the typical operation of a bi-modal separator. Histograms in Fig. 16b confirm the cutoff size of $a_c = 14$ µm, given the experimental conditions. More importantly, the results show that particles with size difference of $\sim 1$ µm can be separated with $>70\%$ efficiency (green arrows). The data further show that differences of $\sim 2$ µm can be separated with higher, $>80\%$ efficiency (blue arrows), while differences of $\sim 3$ µm can be separated with even higher, $>90\%$ efficiency (orange arrows). The separation resolution $R_s$ (see Methods for definition) of this hydrodynamic-vortex platform is $R_s > 10$, which is at least $2 \times$ higher than in previous inertial microfluidic designs [35, 36, 49, 51, 52, 63-65, 67-69]. The high resolution stems from the size-dependent inertial migration at the vortex region, which permits the differentiation of lateral positions of different sized microparticles across the boundary. This high resolution feature is extremely beneficial for separating particulate mixture with small size difference.

Next, tuning of the separation cutoff diameter was explored in order to demonstrate flexibility of the microfluidic platform. As was suggested in the theoretical discussion above, $a_c$
Figure 16. Experimental investigation of the separation resolution and tunability of the cutoff diameter. (a) Particles with continuous size distribution are induced in a device with \( r/R = 5.4 \) and \( Re = 110 \) to find out the separation resolution. Particles with diameter \( a > a_c \) migrate inside sheath flow exiting through the side outlets (blue dash lines), while particle with diameter \( a < a_c \) elute from the main outlet (red dash lines). A few large particles \( (a > 25 \, \mu m) \) are recirculating in the vortices (orange dash lines) due to the large \( F_s \) that can push them across the sheath flow into the microvortices. (b) Normalized histogram of main and side outlet samples indicate separation cutoff size (grey arrow) and separation resolution using a device with \( r/R = 5.4 \) and \( Re = 110 \). (c) Tuning separation cutoff size by changing \( Re \) \( (r/R = 5.4) \). (d) Tuning separation cutoff size by changing \( r/R \) \( (Re = 110) \).
can be precisely tuned by modulating the input flow and channel resistance ratio $r/R$. Two sets of experiments were conducted to measure the change in $a_c$ as a function of these two parameters. First, as shown in Figure 16c, increasing $Re$ from 44 to 110 at $r/R = 5.4$ leads to a linear decrease in the cutoff diameter from 25 μm to 14 μm. This suggests that $a_c$ scales with the first order of input flow velocity as $a_c \propto U_f$. Second, as shown in Figure 16d, increasing $r/R$ from 5.4 to 10 at $Re = 110$ leads to a parabolic increase in the cutoff diameter from 14 μm to 19 μm. This suggests that $a_c$ scales with the resistance ratio as $a_c \propto (r/R)^{\frac{1}{2}}$. The experimental results confirm that a combination of these two system parameters permit control of the cutoff diameter with broad tunable range.

The tuning of the sorting cutoff is convenient to perform without extensive labor of redesigning and refabricating the devices. The flow can be easily tuned by setting new values on a syringe pump. The resistance ratio can be modified without the need of refabricating the device using the following approach. We use our customized PDMS/tubing plugs to selectively block the outlets to achieve different resistance ratios. The plugs are fabricated by injecting PDMS into the tubing and cure at 80 °C for 2 hr. After curing, the tubings were cut into short pieces and used as plugs. We can switch the positions of the O2 and O3 to change the length of side outlets providing $r/R$ from 2 ~ 22. For example, in Figure 17, we block all the outlets except the 4th outlets. This setup provides a resistance ratio $\sigma$ of ~ 8. If we move further to 5th outlets, the resistance ratio $\sigma$ increases to 10.

**Size-based sorting of particles with high resolution**

Due to the high resolution of the vortex-aided sorting, the device is able to separating particles or cells with small size difference while remaining ~90 % efficiency and >90 % purity.
A mixture of 21 µm and 18.5 µm diameter particle (~2.5 × 10^4 /mL each) was injected in the device at $Re = 110$ and $r/R = 5$. The experimental observations at the chamber illustrate syphoning of the 21 µm diameter particles from the side outlets, while the 18.5 µm particles travel to the main-outlet. The images of the inlet and outlet samples in Figure 18a demonstrate the population of each type of particles indicating the successful separation. The quantitative analysis of downstream particle samples indicate highly purified 21 µm particles from side outlet and 18.5 µm from main outlet (Figure 18b). The purity of 21 µm diameter particles from the side outlet is 93% while the purity of 18.5 µm from main outlet is 91%. In addition, the concentration of 21 µm particles from the side outlet is enriched 3× than the inlet. The enrichment is caused by the sample volume distribution from side and main outlet (Figure 18c). Since $r/R = 5$, the sample volume of the two side outlets $V_{side}$ and the total sample volume $V_{total}$ has the relationship of $V_{side}/V_{total} = 2/7$. Normalized counts show a ~90% separation efficiency of 21 µm diameter particles from side outlet and 18.5 µm from main outlet (Figure 18c).

Figure 17. The setup for studying the influence of the flow rate $Q$ and the resistance ratio $\sigma (\sigma = r/R)$ of the side and the middle outlets on the sorting cutoff diameter. (a) AutoCAD layout of a single sorting unit with tunable resistance ratio. O1 is the middle outlet. O2 and O3 are the side outlets. The black crosses indicate the outlets that are blocked by the plugs. The red solid line represents the resistance channel of the middle outlet. The two blue solid lines represent the resistance channels of the side outlets. (b) A photograph of the actual device with the tunable setup.
Figure 18. High-resolution sorting of 21 μm from 18.5 μm diameter particles in a vortex-aided device. (a) The top bright-field image shows separation at the device chamber. The lower three images show particles at the inlet, side outlet and main outlet. The black dots are 21 μm diameter non-fluorescent particles in bright-field view. The white dots are fluorescent 18.5 μm diameter particles. (b) Histograms of inlet, side outlet and main outlet samples indicate the efficient separation. (c) Concentration of 21 μm and 18.5 μm diameter particles in inlet, side and main outlet samples. Normalized count shows a separation efficiency of ~90% for both particles. Error bars represent standard deviation ($n = 3$).
Continuous extraction of large particles from blood sample

To demonstrate the potential to extract larger cells such as CTCs from blood, we spiked 21 µm diameter non-fluorescence particles to mimic larger cells into diluted human-blood (particle concentration \( \sim 1 \times 10^4 \) /mL, RBC concentration \( \sim 1 \times 10^6 \) /mL) and pumped the sample through device for separation. The separation phenomenon and the resulted outlet sample are presented in bright-field image Figure 1a. Particles follow the sheath streamlines to the side-outlets, while RBCs exit from the main-outlet. Compared to inlet sample, particles are concentrated 5× in the side-outlet (Figure 1b). Normalized count shows \( \sim 86 \% \) particle sorting efficiency, while nearly all \( \sim 99 \% \) RBCs exit from the main outlet (Figure 1c). These results indicate that the device is very promising in efficient extraction of large cells from mixture continuously. Furthermore, the operation of device only requires one syringe pump to deliver sample thus lowering the complexity and cost of instruments while improving the convenience of using.

Summary

This chapter presents an inertial microfluidic sorting unit using vortices and accompanied sheath flow for continuous size-based cell sorting with high resolution and high tunability. The device consists of a high-aspect-ratio channel for inertial focusing of cells followed by two symmetric chambers with three outlets for continuous sorting of larger cells from small ones. The device exhibits higher resolution than previous bimodal inertial microfluidic separators, which permits more precise separation of cell samples with small difference in size or continuous size distribution. Moreover, the cutoff diameter of separation can be easily tuned by input flow rate and fluidic resistance network. The device successfully separated 21 µm from 18.5 µm diameter particles with >90 % purity and \( \sim 90 \% \) efficiency indicating the high resolution, high
Figure 19. Continuous extraction from blood sample. (a) Separation of 21 µm diameter particles from human blood in a device with $r/R = 10$. The top bright-field image shows separation at the device chamber. The lower three images show the particles at the inlet, side outlet and main outlet. (b) The concentration of 21 µm diameter particles in the side outlet increases $5 \times$ as compared to the inlet. (c) Normalized count of 21 µm diameter particles in side and main outlets shows a capture efficiency of 86%, while 99% RBCs exit through main outlet. Error bars represent the standard deviation of three individual experiments.
efficiency and purity. Besides, we presented separation of large particles from human-blood sample with an efficiency of 86 % and 5× enrichment by concentration.

Simple geometry of this unit suggests that it is possible to cascade several units into an integrated inertial microfluidic system with multiple sorting cutoffs and capable of separating particle or cell mixtures of three or more sizes in a single run. It can potentially be used for one-step separation of cellular or particle samples with more complex components. The vortex-aided inertial microfluidic unit provides a simple cellular sorting platform with high resolution, efficiency and ease of cascading and paralleling. We envision numerous applications in preparation of cell or particle sample in biological, biomedical, environmental research areas and clinical diagnostics.
CHAPTER 4

INTEGRATED VORTEX SORTER FOR MULTIMODAL SORTING

Most inertial microfluidic devices are only capable of bimodal separation with a single cutoff diameter and a well-defined size difference [35, 36, 51, 52, 63-66, 68, 69, 114]. These limitations inhibit efficient separation of complex real-world samples, which often include heterogeneous mixtures of multiple microparticle components. In this chapter, we demonstrate an inertial microfluidic device that achieves efficient multimodal separation of mixture of three components of microparticles with small differences in size [60]. Our approach uses the inertial microfluidic vortex-aided sorting unit presented in Chapter 3 as a building block. By sequencing two sorting units and designing proper surrounding fluidic resistance network, we demonstrate continuous multimodal separation of complex microparticle mixtures into three components with high resolution. Further, due to the tunability of the cutoff diameter in each stage, separation bandwidth or pass-band location can be modified in a wide range exhibiting the versatility of the system for sorting of samples with different size range.

Our approach offers a number of benefits, in addition to the unique multimodal separation functionality, which significantly broaden applications of inertial microfluidics in sorting of complex microparticle samples. First, the straightforward system design permits the cutoff diameters to be easily and precisely tuned by adjusting input flow or fluidic resistance of outlet
channels. Second, the device offers the highest reported separation resolution which leads to more precise separation of samples with smaller size differences. Third, the continuous nature of the device operation lends itself to high throughput and high volume applications, especially if the microchannels are paralleled. And finally, the device has a single inlet and requires only one syringe pump for sample delivery, which largely reduces complexity of external instrumentation often associated with electrical, optical or acoustic separators (leading to the “chip in a lab” modality). Ultimately, this straight planar geometry allows easy integration with downstream detection or measurement techniques permitting automated on-chip sample preparation and processing for a broad range of applications.

Device principle

The multimodal separation chip uses microfluidic hydrodynamic-vortices to continuously separate microparticles based on size. These vortices form by the sudden expansion of channel cross-section and act to selectively siphon microparticles from the main channel into side outlets with high resolution. With addition of an upstream microchannel that focuses particles into equilibrium positions near sidewalls, the system can efficiently sort microparticles from the highly-ordered streams on the basis of their size. The mechanism of inertial focusing and vortex-aided bimodal sorting in a single sorting unit have been described in detail in Chapter 3, and thus it will not be repeated here.

The multimodal functionality is achieved by sequencing multiple focusing channels and vortex units (Figure 20). At first glance, one might expect the cascading of vortex units to be straightforward, a simple matter of fabricating multiple units in sequence. However, due to the siphoning of flow from the side outlets, the input flow rate for the second sorting microchamber is lower than the first sorting chamber. Consequently, the microchannel resistance network has
**Figure 20.** Inertial microfluidic multimodal separation. The device consists of a high-aspect-ratio channel for particle focusing followed by sequencing of two sets of microchambers for multimodal separation. The *inset* schematics indicate particle size distribution before and after separation, with $a$ and $f$ represent particle size and frequency. $a_{hc}$ and $a_{lc}$ represent high-pass cutoff and low-pass cutoff for multimodal separation.
to be carefully designed to match the input flow rate of each microchamber to provide proper cutoff size. In Figure 21, an analogue electrical circuit model [115] is presented to aid design of fluidic resistances of the cascaded system. In the model, the red dots represent the two microchambers where the current (flow) is siphoned into three branches. The input flow rate $Q_c$ ($I_c$) of the second chamber is determined by the resistance ratio of $R_1$ and $R_c$ which further consists of paralleling of $R_2$, $R_3$ and $R_4$. To achieve multimodal separation, $Q_{in}$ has to match $R_1/R_c$ to provide the higher cut-off diameter for high-pass separation in the upstream chamber. Meanwhile, $Q_c$ has to coordinate with $R_3/R_4$ to offer the lower cut-off diameter for low-pass separation in the downstream chamber.

**Results and discussion**

**Multimodal separation of complex microparticle mixture**

The multimodal separation was validated through separation of a mixture of 21 µm, 18.5 µm and 15 µm diameter polymer microspheres using the device shown in Figure 20. At flow rate $Q_{in} = 525\mu$L/min ($Re = 116$), the upstream microchamber provides high-pass separation with

---

**Figure 21.** (a) Microfluidic resistance network of the cascaded device. (b) The analogous electrical circuit model of the microfluidic resistance network. The two red dots represent the two sorting chambers. The fluidic resistance is analogous to the electric resistance. The flow rate is analogous to the current.
$a_{hc} \sim 20 \mu m$ with resistance ratio $R_i/R_c = 4$. As the flow is separated at the upstream microchamber, the downstream flow rate $Q_c$ is decreased to $\sim 350 \mu L/min$ ($Re = 77$). Thus, resistance ratio $R_3/R_4 = 5.6$ is designed for the downstream microchamber enabling low-pass separation with $a_{lc} \sim 17 \mu m$. Using these system parameters, the cascaded device successfully separates the particle mixture into its components into O1, O2 and O3 correspondingly (Figure 22a). The histograms and sample images before and after separation indicate dramatic enhancement of purity of each sized particles (Figure 22b). Furthermore, the 21 $\mu$m, 18.5 $\mu$m and 15 $\mu$m diameter microparticles are enriched by 2.4×, 3.8× and 1.7× after separation, as Figure 23a shows. The separation efficiencies were measured to be 78 %, 87 % and 99 % respectively, which confirms the device can perform high-resolution multimodal separation of complex particle mixtures without compromising efficiency (Figure 23b).

Size distributions of microparticle or cellular mixtures vary from sample to sample. Size range of interest may also vary. Thus, the tunability of separation at individual stage becomes significant in sample preparations. Here, we demonstrate the versatility tuning of the separation bandwidth (Figure 24) and passband location (Figure 25). Such capabilities lead to successful separation of mixtures into components with different size range. First, we are able to precisely tune separation bandwidth by using proper combination of input flow and resistance network (Figure 24a). To widen the bandwidth, we fixed the resistance ratio of the upstream chamber $R_i/R_c$ to maintain the high-pass separation with $a_{hc} \sim 20 \mu m$, while use a smaller ratio $R_3/R_4 = 4.9$ to enable low-pass separation with a smaller $a_{lc} \sim 12 \mu m$. Under this modification, the 21$\mu$m diameter particles still exit through O1, while both 18.5 $\mu$m and 15 $\mu$m diameter particles are extracted from O2. The 11 $\mu$m diameter particles instead elute through O3 as shown in experimental images of microchambers (Figure 24b) and outlets (Figure 24c). Such
Figure 22. (a) Stacked bright field images illustrating separation of 21µm, 18.5µm and 15µm diameter particles into O1, O2 and O3. (b) The histograms of the samples from inlet and outlets indicate after multimodal separation, the purities of 21µm, 18.5µm and 15µm diameter particles are dramatically elevated from 38%, 29.3%, 32.7% (inlet) to 89.4% (O1), 80.7% (O2), 95.9% (O3) respectively.
Figure 24. (a) By tuning flow rate and resistance network, the separation bandwidth is increased from 3µm to 8µm. (b) As shown in the stacked bright field images, the 21µm diameter particles are extracted from O1, while both 18.5µm and 15µm diameter particles elute from O2. The 11µm diameter particles exit through O3. (c) Bright field images at each outlet channel illustrate the successful separation.

Figure 23. (a) The concentration plot shows enrichment of concentration of 21µm, 18.5µm and 15µm diameter particles by 2.4×, 3.8× and 1.7× which is due to separation of input sample volume into different outlets (n = 3). (b) The normalized count shows that the separation efficiencies for 21µm, 18.5µm and 15µm diameter particles are 78%, 87% and 99% respectively indicating efficient multimodal separation of particles with only 3µm difference in size (n = 3).
Figure 25. (a) By tuning input flow rate and resistance network, the passband location can shift towards a higher cut-off diameter, while maintaining the separation bandwidth. Bright field images taken at two sequenced microchambers (b), and outlets O1, O2 O3 (c) demonstrate a successful separation of the 23µm, 21µm and 18.5µm diameter particles. In all images, the scale bar is 50 µm.
changes in separation indicate clear broadening of separation bandwidth from 3 µm to 8µm. Quantitative measurements of concentration and efficiency are demonstrated in Figure 26a-b. One should also note that narrowing of the bandwidth is also possible by using larger ratio $R_3/R_4$.

Second, it is able to adjust the passband location while maintaining the bandwidth (Figure 25). We used larger resistance ratio $R_1/R_c \sim 5.5$ to provide high-pass separation with larger $a_{hc} \sim 22$ µm in the upstream chamber. Similarly, we applied a larger resistance ratio $R_3/R_4 \sim 11$ to upshift the $a_{lc}$ of downstream microchamber. Consequently, 23 µm diameter particles instead of 21 µm diameter particles are found in O1, while 21 µm and 18.5 µm diameter particles are separated in the downstream microchambers as demonstrated in experimental images of both microchamers (Figure 25b) and outlet channels (Figure 25c). This result indicates successful offsetting of passband location of the device by 3 µm. Quantitative measurements of concentration and efficiency are demonstrated in Figure 26c-d. In summary, we have demonstrated two important abilities for tuning multimodal separations in the device including the modulation of bandwidth and passband location. Such versatility can greatly benefit sample preparations of different complex mixtures according to size distribution or help precisely selecting cells or particles with size of interest.

To demonstrate the capability of separating heterogeneous sample, microparticle mixture with continuous diameter distribution from 10 µm to 27 µm (Figure 27b) was introduced into a cascading device at $Q_{in}=0.5$ mL/min ($Re=110$) (Figure 27a). With resistance ratio $R_1/R_c = 5.5$, the upstream microchamber provides high-pass separation with $a_{hc} \sim 24$ µm. As the flow is separated at the upstream microchamber, the downstream flow rate $Q_c$ decreased to ~370 µL/min ($Re=81$). With resistance ratio $R_3/R_4=11$, the downstream microchamber enables low-pass
Figure 26. (a) The concentration plot shows the concentrations of 21µm, 18.5µm, 15µm and 11µm diameter particles are enriched 1.9×, 2×, 2× and 1.5× correspondingly after multimodal separation (n=3). (b) The normalized count shows that the separation efficiencies for 21µm (from O1), 18.5µm (from O2), 15µm (from O2) and 11µm (from O3) diameter particles are 98%, 87%, 75% and 72% respectively indicating successful separation after tuning bandwidth (n=3). (c) The concentration plot indicates obvious enrichment of 23µm and 21µm diameter particles by 2.6× and 3.6×. (d) The normalized count shows that the separation efficiencies for 23µm (from O1), 21µm (from O2), 18.5µm and 15µm (from O3) diameter particles are 99%, 73%, 98% and 93% indicating successful separation after tuning the passband location.
Figure 27. (a) Heterogeneous mixture of microparticles was sorted into three different size distribution after passing the device. (b) Size distribution of inlet particle mixture. The distribution was normalized to the highest count at a specific particle diameter. (c) Size distributions of samples from O1, O2 and O3. The size distribution in each outlet was normalized to the highest count within that outlet.
separation with $d_{lc} \sim 21 \, \mu m$. With these design parameters, the cascading device successfully sort heterogenous sample into three different distribution (Figure 27c). Majority of the particles with diameter $> 24 \, \mu m$ was separated into high pass outlet O1. Particles with diameter of $21 \sim 24 \, \mu m$ was extracted from band pass outlet O2 while particles with diameters $< 21 \, \mu m$ was mainly separated into low pass outlet O3.

**Summary**

In summary, this chapter presents a microfluidic device capable of versatile multimodal separation of microparticle samples based on sizes, with high resolution and high tunability of separation cutoff diameter. Unlike the conventional bimodal microfluidic separators, the presented device provides a novel multimodal separation functionality. This feature significantly expands the capability of the microfluidic separator for sample preparation of complex mixtures of microscale particulate components such as cell mixtures. We envision this versatile microfluidic multimodal separator will open new opportunities in the microfluidic separation field for size-based separation of complex particulate materials from biological, environmental to synthetic microparticles for a wide range of applications including industrial microparticle purification, cellular sample preparation, biomedical research and clinical diagnostics.
CHAPTER 5

INTEGRATED VORTEX SORTER FOR DOUBLE PURIFICATION OF CELLULAR SAMPLES

Sorting of target cells with high purity from complex cell samples is challenging yet essential for downstream research. It becomes even more challenging in applications involving samples with a large number of background cells and only a small fraction of target cells (e.g. isolation of CTCs from human blood) [66, 83, 100, 116], since it requires the device to not only extract the target cells with high yield, but also to remove background cells with extremely high efficiency. Although some inertial microfluidic devices demonstrate sorting of microbeads or cells with efficiency >95 % [49, 63, 114], it is often insufficient to obtain highly purified target cells due to multiple orders of magnitude difference in target/non-target cell numbers. Furthermore, cell samples have different size distributions, thus the flexibility of tuning the sorting cutoffs is critical for maintaining the performance of the sorting. Nevertheless, for most of the inertial microfluidic devices, the sorting cutoff can only be modified by re-designing and re-fabricating of the device, which leads to extra development time, cost, and possibly delays in processing of time-sensitive biological samples.
In this chapter, an integrated vortex-based inertial microfluidic chip with new topology is introduced for continuous double sorting and purification of biomicroparticles with high efficiency and purity. The continuous vortex-aided sorting chamber [59] presented in Chapter 3 is used as a unit and integrated by connecting the high pass outlets of the first unit into the inlet of the second unit. With properly designed flow conditions and fluidic resistance network, the larger target cells are first sorted in the first unit and then pass into the second unit to be double-sorted, leading to the product with significantly increased purity. Tuning of the sorting cutoffs of the integrated device is possible by simply modifying the input flow rate or the fluidic resistance network, which allows the flexibility and versatility of sorting cellular samples with different size distributions. To demonstrate the feasibility of sorting of relatively rare cells as well as efficient removal of a large number of background cells, human blood spiked with HuSLCs was successfully sorted with ~90 % efficiency as well as removing RBCs with ~99.97 % efficiency. With the double sorting functionality and the flexibility of cutoff tuning, we envision this inertial microfluidic vortex sorter can potentially serve as a feasible tool for sorting and purification of rare larger cells from cellular mixtures for applications ranging from cell biology research to clinical diagnostics.

Device principle

The device presented in this chapter uses the vortex-aided sorting unit as a building block for high resolution size-based sorting of cells. To enable sorting and purification of the larger target cells from the large number of the smaller background cells, two vortex sorting units are integrated with the side outlets of the first sorting unit connected to the inlet of the second unit (Figure 28a). Cellular sample is introduced into the device from inlet 1 and focused along the sidewalls driven by the inertial lift forces (Figure 28b). Downstream, the first sorting unit
recovers larger cells from the smaller background cells. To remove the remaining background cells and further purify the large targets, the sorting product from the side outlets of the first unit flows downstream into the second unit. To create proper flow condition for the sorting in the second unit, buffer is introduced from inlet 2 to accelerate the flow. As a result, large target cells are sorted to outlet 3 (O3) leading to double-purified product, while the remaining small background cells are sorted into outlet 2 (O2).

As a proof of concept, we introduced a mixture of 18.5 µm and 7 µm diameter microbeads into the device to mimic cellular samples with physical sizes similar to CTCs and red

Figure 28. (a) Integration of two vortex sorting units for double sorting and purification. (b) Schematics illustrating details of the device operation. Panel (i) – (iv) matches panel in (a).
blood cells (RBCs) (Figure 29). The mixture was introduced into the device at a flow rate $Q_s = 190 \, \mu L/min (Re = 80)$ and uniformly distributed in the $30 \, \mu m \times 50 \, \mu m (w \times h)$ microchannel near inlet 1 (Figure 29a). Downstream, the 18.5 $\mu m$ diameter beads focused into two streams near the side walls, while the 7 $\mu m$ diameter microbeads focused into four streams at the center of each wall. This transformation of focusing positions from two to four in a rectangular microchannel has been reported previously [43, 117] and occurs as $Re$ exceeds the threshold values which depend on the size of the microparticle and is lower for smaller microparticles. This phenomenon benefits sorting in our device because a portion of the smaller background particles will focus near top and bottom walls which ensures they exit through the middle outlet. In the first sorting unit, the 18.5 $\mu m$ and the 7 $\mu m$ diameter microbeads were sorted into side outlets and middle outlet O1 respectively (Figure 29b). The 18.5 $\mu m$ and the remaining 7 $\mu m$ diameter beads flew downstream and merged with the buffer inlet 2 at $Q_b = 90 \, \mu L/min (Re = 38)$ (Figure 29c). The microbeads were then sorted again in the second sorting unit leading to pure 18.5 $\mu m$ diameter beads in O3 (Figure 29d).

**Results and discussion**

**Size-based sorting in a single vortex unit**

The devices presented in Chapters 3 and 4 were designed for sorting larger beads or cells with diameter $>10 \, \mu m$, and thus offered limited performance for sorting of smaller cells such as RBCs. In this chapter, to enable sorting of human blood samples, the dimensions of the focusing microchannel was reduced to $30 \, \mu m \times 50 \, \mu m (w \times h)$ with a microchamber with fixed width $Wc$ and length $Lc$ of $500 \, \mu m \times 500 \, \mu m$. To the design of the unit with smaller dimensions, a comprehensive investigation of the sorting cutoff diameter is necessary.
Figure 29. Experimental proof-of-concept. (a) Inertial focusing of microbeads in the upstream microchannel. Fluorescent image was taken at 20 mm downstream. (b) Sorting of the larger beads from the smaller beads in the first sorting unit. (c) Larger beads with the remaining smaller beads flow downstream to the second sorting unit with buffer flow accelerating the flow from inlet 2. (d) Double sorting in the second chamber. 30–50 images were stacked to indicate trajectories of the microparticles.
Using the method of Chapter 3, the sorting cutoff diameter was quantitatively characterized over a wide range of inlet flow rate \( Q \) from 80 µL/min to 200 µL/min and channel resistance ratio \( r/R \) from 3.3 to 10 (Figure 30). To determine the cutoff, we introduced microbead mixtures with continuous size distribution from 7 µm to 23 µm into the device and measured the cutoff diameter at each combination of \( Q \) and \( \sigma \) to form a contour map of cutoff diameter. As the contour map in Figure 29 shows, the cutoff diameter of a single sorting unit can be tuned from 12.5 µm to 23 µm at the given range for \( Q \) and \( \sigma \). For example, at a fixed flow rate of \( Q = 100 \) µL/min, the cutoff diameter increases from 18 µm to 23 µm as \( \sigma \) increases from 4.6 to >8.5. At \( r/R = 4.6 \), the cutoff diameter decreases from 21 µm to 12.5 µm as \( Q \) increases from 80 µL/min to 200 µL/min. Note that at \( r/R = 3.3 \), the boundary of the sheath flow and the middle flow is closer to the channel center. Thus the majority of the microbeads are initially focused very close to or inside the boundary leading to insufficient sorting.[59] These results

![Figure 30](image.png)

**Figure 30.** The contour map showing the sorting cutoff diameters at flow rates from 80 ~ 200 µL/min and resistance ratios from 4.3 to 10.2.
suggest that the cutoff of a sorting unit can be easily tuned over a wide range by modifying the flow rate or resistance ratio of the outlet channels.

Integration of two vortex units using electric circuit model

In the integrated system, the input flow rate for each sorting unit needs to match its resistance ratio $r/R$ to provide proper cutoff. We used an electrical circuit model analogous to the fluidic resistance network [115] to aid the analysis and the design of the device. As Figure 31 shows, the fluidic resistance network of the integrated vortex sorter consists of two inlets for sample and buffer with the flow rates of $Q_s$ and $Q_b$, fluidic resistors $R_1 \sim R_5$ of outlet channels and connection channel between the two units, as well as outlet ports O1 ~ O4. In the analogous electrical circuit model (Figure 31b), the flow rate and the fluidic resistor are analogous to the current and the electric resistor respectively. The resistance induced by the microchambers is much smaller than the surrounding microchannels, thus is neglected in the electric circuit model. As Figure 31c shows, the model can be rearranged and simplified to contain two current sources $I_s$ and $I_b$ and three resistors $R_1$, $R_2/2$ and $R_6$.

In this integrated system, the combination of the flow rate $Q_s$ and $\sigma_1$ (defined as the resistance ratio of the first chamber) determines the cut off of the first chamber. Similarly the combination of the flow rate $Q_3$ and $\sigma_2$ (defined as the resistance ratio of the second chamber) determines the cut off of the second chamber. Different from the single unit, the resistance ratio $\sigma_1$ is not only related to the channel resistance $R_1$ of the middle and $R_2$ of the side outlets, but also the downstream components. To calculate $\sigma_1$, we use Kirchhoff’s first law at junctions 1 and 2 as:

$$I_1 + 2I_2 = I_s \quad (2)$$

$$2I_2 + I_b = I_3 \quad (3)$$
Combining these two equations with resistors $R_1, R_2, R_6$, the $\sigma_1$ is given as:

$$\sigma_1 = \frac{I_1}{I_2} = \frac{R_2 + \frac{2R_6(1+I_b)}{I_s}}{R_1 + \frac{2R_6Q_s + 2R_1Q_b}{Q_s R_1 R_6}} - \frac{Q_s R_1 > Q_b R_6}{(4)}$$

The resistance ratio of the second unit $\sigma_2$ is given as:

$$\sigma_2 = \frac{I_4}{I_5} = \frac{R_5}{R_4}$$

which is similar to a single unit because of the shared electric ground of all three outlets O2, O3 and O4. Using equations 4 and 5, one can calculate the resistance ratios $\sigma_1$ and $\sigma_2$ based on the dimensions of each channel and the input flow rates $Q_s$ and $Q_b$, and then use the contour map (Figure 30) to estimate the cutoff of each sorting unit.

**Tuning the sorting cutoffs in an integrated system**

The flexibility of tuning the sorting cutoffs according to size distribution and target of interest in a given sample is critical for effective size-based sorting of different samples. In the integrated system, the sorting cutoff can be conveniently adjusted by the flow rate or the resistance network according to equations 4 and 5. Figure 32a and b demonstrate tuning of the cutoff using the flow rate. We introduced a mixture of 23 $\mu$m, 18.5 $\mu$m and 7 $\mu$m diameter beads into the device at $Q_s = 190$ $\mu$L/min with buffer flow $Q_b = 90$ $\mu$L/min at $\sigma_1$ and $\sigma_2 = 7$. The $Q_3$ is calculated to $\sim 130$ $\mu$L/min. Under this conditions, the cutoffs of the first and second sorting units are $\sim 16$ $\mu$m and $\sim 18$ $\mu$m respectively. As Figure 32a shows, both the 23 $\mu$m and the 18.5 $\mu$m diameter beads were sorted into the side outlets of the first stage. Downstream, both beads were sorted into O3. As we reduced the flow rates $Q_s = 170$ $\mu$L/min and $Q_b = 60$ $\mu$L/min, the resistance ratios $\sigma_1$ and $\sigma_2$ remain the similar values. The $Q_3$ is then calculated to $\sim 100$ $\mu$L/min.
Figure 31. Integration of the vortex sorting system. (a) The fluidic resistance network of the integrated system. The $Q_s, Q_b$, and $Q_1$-$Q_5$ indicate sample flow rate, buffer flow rate and flow rates at different channels of the device, respectively. (b) The analogous electrical circuit model, with flow rate analogous to current and fluidic resistors analogous to electric resistors. (c) Simplified electrical circuit model from (b), with 1 and 2 indicating the two electric circuit junctions.
This upshifts the cutoffs of the first and the second unit to ~17 µm and ~21 µm respectively. Thus, instead of extracting both the 23 µm and the 18.5 µm diameter microbeads, only the 23 µm diameter microbeads are extracted from O3, illustrating successful modification of the cutoff. We can also fix the flow rate and tune the cutoffs by only adjusting the resistance ratios. As Figure 32c shows, we fix the flow rates $Q_a = 190 \mu$L/min and $Q_b = 90 \mu$L/min and increase the resistance ratio $\sigma_2$ from 7 to 10. The cutoff of the second unit increases from ~18 µm to ~21 µm. As a result, the 23 µm diameter beads were successfully extracted through O3 while the 18.5 µm diameter beads exited through O2 with the remaining 7 µm diameter microbeads. These results demonstrate the flexibility and convenience of tuning the sorting cutoffs indicating the versatility of the system for processing samples with different size distributions.

**Sorting of cancer cells from blood using the integrated system**

The device enables double sorting of the larger target cells and double removal of the smaller background cells, which can yield highly-purified target-cell product even when cellular samples contain only a small target fraction. To demonstrate the feasibility, we first demonstrated sorting of large microbeads from the diluted human blood (0.5%). We spiked blood with 23 µm and 18.5 µm diameter beads (to mimic relatively rare target cells). The resulting concentrations were ~700 /mL for the 23 µm diameter beads and 18,000 /mL for the 18.5 µm diameter beads in ~1.5×10⁷ /mL RBCs. As Figure 33a shows, majority of the RBCs were sorted into O1 by the first unit, while 23 µm and 18.5 µm beads were sorted to side outlets together with the remaining RBCs. The product from the side outlets was sorted again in the second sorting unit, in which the 18.5 µm beads and the RBCs are separated into O2. The rare 23 µm beads are extracted from O3. The bright-field images (Figure 33b) illustrate successful sorting of the rare 23 µm beads with significantly reduced amount of RBCs compared to the inlet
Figure 32. Tuning the sorting cutoffs of the integrated system. (a) Stacked bright field images showing sorting of a mixture of 23 µm, 18.5 µm and 7 µm at the indicated flow conditions and fluidic resistance ratios. Both the 23 µm and the 18.5 µm diameter microbeads are sorted to O3. (b) Adjusting the flow rates of inlet and buffer leads to only the 23 µm diameter microbeads being sorted to O3. (c) Modifying the resistance network with the same flow condition as (a). Only the 23 µm diameter beads are sorted to O3. A 30-50 images were stacked to indicate trajectories of the microparticles.
sample. Quantitative measurements of concentration (Figure 33c) show a 15,800× decrease of RBC concentration. The purity (Figure 33d) of 23 µm bead concentration increases by ~15,000× from 0.005 % to 71 % in O3. The separation efficiencies for the 23 µm and the 18.5 µm beads are ~ 99 % and ~ 99 % respectively, indicating efficient sorting of larger microparticles from smaller cells using this device (Figure 33e). The overall removal efficiency for the RBCs was ~99.998 % indicating extremely efficient removal of smaller background cells after passing the two sorting units. We note that the removal efficiency of RBCs from the first unit is 94 %, which many may consider as high efficiency; however, some applications such as isolation of CTCs from blood may require more efficient removal of background cells to obtain highly-pure target cell products.

We further demonstrated the sorting of HuSLCs from human blood. Cancer stem-like cells are a subset of tumor cells that has the ability to self-renew and generate the diverse cells that comprise the tumor [109-111, 118]. The cells we used in these experiments were 12 µm to 22 µm in diameter, with the majority measured in the 15 µm to 18 µm range. To sort HuSLCs from blood efficiently, we set the cutoff of each sorting unit to ~15 µm. The diluted human blood (1 %) was spiked with HuSLCs with a concentration of ~1,000 /mL and introduced into the device at $Q_s = 200 \mu$L/min and $Q_b = 90 \mu$L/min. As shown in the stacked bright-field images (Figure 34a), majority of the RBCs flowed to O1 and the HuSLCs was sorted into the side outlet of the first unit, together with the remaining RBCs. Further downstream, the HuSLCs and the RBCs are sorted again in the second unit, leading to more purified and concentrated HuSLCs product from O3. The bright-field microscopic images of samples from the inlet and outlets indicate that HuSLCs are successfully sorted and purified after flowing through the device (Figure 34b-c).
Figure 33. Sorting of large microparticles from diluted human blood. (a) Stacked bright-field microscopic images demonstrating sorting of 23 µm diameter microbeads from 18.5 µm diameter microbeads and 0.5 % diluted blood in the two sorting units. 50 images are stacked to illustrate the complete trajectories of microbeads and cells. The red, blue and green dash lines indicate the trajectories of microbeads and cells. (b) Microscopic bright-field images of inlet and outlets. The inset images are photographs of samples collected from different outlets. (c) Concentration plot showing the 23 µm, the 18.5 µm diameter microbeads and the RBCs concentrations from inlet, O1, O2 and O3. (d) Purity plot showing the purity of the 23 µm microbeads in inlet and O3. (e) Separation efficiency of RBCs, the 18.5 µm and the 23 µm diameter microparticles from O1, O2 and O3 respectively.
We quantitatively measured the concentration, purity and separation efficiency of RBCs and HuSLCs as shown in Figure 34d-f. After sorting, the concentration of HuSLCs is enriched ~10 × while the purity increased ~1,500 ×. The separation efficiency of the HuSLCs from O3 is ~90%. For RBCs, the concentration decreased 265 × in O3 and the removal efficiency is 98% and 99.97% after sorting in the first and second units, respectively. These results suggest our device can efficiently sort and purify relatively rare large cells from a large number of background cells. The 10 × enrichment of the HuSLCs concentration from O3 is because the majority of the fluid flowed to the middle outlet at each of the sorting unit, thus target cells exiting through the side outlets get sorted and enriched simultaneously. This enrichment effect is beneficial especially if the target cells are rare in the samples. The loss of ~10 % of HuSLCs is most likely caused by the cutoff settings of the device. Since the cutoffs are set to ~15 µm, the HuSLCs with size <15 µm as well as the majority of the WBCs with the size of 10~15 µm will exit through O1 or O2. The overall RBC removal (~ 99.97 %) is not as efficient as in previous demonstration (~ 99.998 %) which leads to a smaller reduction of RBC concentration (265× compared to 15,800×) and lower enhancement of HuSLC purity (~1,500× compared to ~15,000×) in O3. There may be two reasons for this: 1) the RBC concentration in the HuSLC sorting experiments is 2× higher than in microbead experiments, which may cause greater interparticle interaction and thus lowering the sorting efficiency; 2) the device for HuSLC sorting used a smaller resistance ratio (σ2 = 5) for the second unit which resulted in the less efficient removal of RBCs. With further optimization on resistance network, the sorting performance can be further improved.
Figure 34. Sorting of HuSLCs from blood. (a) Stacked bright-field microscopic images demonstrating the sorting of HuSLCs from 1% diluted blood in the two sorting units and corresponding side outlets. The red, blue and green dash lines indicate the trajectories of cells. The green arrows indicate the HuSCLs being sorted into side outlets. The scale bars are 50 µm. (b) Microscopic bright-field images of inlet and outlets. The scale bars are 50 µm. (c) Zoom-in phase-contrast image taken at O3 illustrating HuSLCs (the green arrows) and RBC (the red arrow). (d) Concentration plot of the HuSLCs and the RBCs in inlet and O3. (e) Purity plot of the HuSLCs in inlet and O3. (f) Separation efficiency of the RBCs and the HuSLCs from O1, O2 and O3.
Summary

This chapter described an integrated inertial microfluidic sorter for double sorting and purification of larger cells from smaller background cells. Continuous sorting of relatively rare cells (~1,000 /mL) from diluted human blood (~5.2 × 10⁷ /mL) with ~90 % efficiency and ~1,500× increased purity as well as removal of RBCs with ~99.97 % efficiency was successfully demonstrated. The significantly increased purity of target cells after sorting stems from the unique function of the device which extracts the target cells and removes the background cells two times. This function is especially useful for processing cellular samples containing only a small fraction of target larger cells in large amount of small background cells. Although one could achieve the double sorting by flowing the sample two times through a single-stage device, the repetitive sample transfer is less automatic and may lead to damage or loss of the target cells. With the integrated design, the device can continuously generate highly-purified product with much less human interaction.

Moreover, the sorting cutoffs of the integrated device can be tuned by modifying the flow rate or adjusting the fluidic resistance. This flexibility enables convenient modification for sorting samples of different cell sizes. After characterizing the size distribution of a new cellular or bead sample, one could easily modify the cutoffs according to the size distribution of the sample or the size of the target of interest to maximize the sorting performance without the time-consuming redevelopment of the device.

The vortex-aided sorting platform offers several unique advantages, including the efficient removal of high-concentration background cells and sorting of rare target cells, flexible tuning of the cutoffs according to the samples as well as automated continuous sorting with minimal human interactions. We envision this platform has the potential to become a suitable
candidate for sorting and purification of rare cellular components from complex sample matrices in cellular sample preparation applications.
CHAPTER 6

CONCLUSIONS

Cell sorting and purification is often an indispensable sample preparation step in cell biology, biomedical research and clinical diagnostics [6]. Inertial microfluidics has proved to be a powerful technique for label-free, passive cellular sample preparation in cell biology and clinical diagnostics. Although high throughput size-based focusing and sorting of cellular samples have been achieved using the technique, the current inertial microfluidic devices have several key limitations which prevents them from being widely used to process complex real-world cellular samples. As a size-based technique, they have limited sorting resolution and provide only bimodal sorting with a single sorting cutoff diameter which provide limited performance for sorting of complex samples. Furthermore, they lack of flexibility of modifying the sorting cutoff sizes according to sample size distributions. Tuning the cutoff sizes always requires extra burden of redesigning and refabricating the devices.

To achieve high-efficient sorting of complex cellular samples, the first step was to develop a microfluidic geometry as a building block to provide high-resolution cell sorting with tunable and integratable features. This microfluidic geometry consists of a straight high-aspect-ratio microchannel for inertial focusing of cells followed by a pair of expanded microchamber to generate microvortices for size-based cell sorting [59]. Both theoretical and experimental investigations showed that this microfluidic sorting unit can sort microparticles with size
difference of only 1~2µm which is the highest resolution among the existing inertial microfluidic devices. Moreover, the results also suggest that the sorting cutoff diameter can be easily tuned by flow rate and the resistance ratio of the outlets. This tunability made the microfluidic unit versatile for sorting of particle samples with different size distributions without the burden of redesigning and refabrication. Sorting of 21 µm and 18.5 µm diameter microparticles was demonstrated with > 90 % efficiency further confirms capability of sorting particles with very small difference in size. Extraction of large particles (mimic large cells) from dilute human blood was conducted with 86 % separation efficiency and 5× enrichment. All the experimental results suggest that the microfluidic sorting unit developed in this work can provide high-performance size-based cell sorting and tunability of the sorting cutoff.

Similar as integrating transistors into integrated circuits, the microfluidic sorting unit can be integrated to provide versatile sorting functions. First type of integrated cell sorter was developed by cascading two sorting units and engineering the surrounding fluidic siphoning channels [60]. Instead of bimodal sorting function provided by majority of the inertial microfluidic devices, the integrated device has the new multimodal sorting function. Since cellular samples or other real world microparticle samples are always heterogeneous and may contains multiple different-sized particles, such function made it possible to continuously process these complex samples. More importantly, the sorting cutoffs of the integrated device can also be adjusted by changing the flow rate or modifying the resistance network. We demonstrated continuous sorting of 21 µm, 18.5 µm and 15 µm diameter microparticles with > 80% efficiency indicating the successful multimodal sorting with high resolution. To show the systematic tunability, tuning of the sorting bandwidth and passband location were achieved by modifying the flow rate and resistances of the channels. With such tunability, a complex mixture of 23 µm,
21 \, \mu m, 18.5 \, \mu m, 15 \, \mu m and 10 \, \mu m can be successfully sorted into different subpopulations. To demonstrate the capability of sorting heterogeneous cell samples, a microparticle sample with continuous size distribution from 10 to 27 \, \mu m was introduced into the device and sorted into three subpopulations with different size distributions.

An alternative way of integrating the sorting units enables a new double sorting and purification function. The function is achieved by connecting the high-pass outlet of the first sorting unit to the inlet of the second sorting unit. With properly designed surrounding fluidic resistance channel network, the larger target cells can be sorted from the small background cells two times sequentially. Simultaneously, the small non-target cells are also removed two times. This unique function can generate highly purified target cell product even for samples containing non-target cells with concentration several orders of magnitude higher than target cells. Using the second type of integrated device, we demonstrated continuous sorting of relatively rare cells (HuSLCs with \( \sim 1,000 \, \text{/mL} \)) from diluted human blood (\( \sim 5.2 \times 10^7 \, \text{/mL} \)) with \( \sim 90 \% \) efficiency and \( \sim 1,500 \times \) increased purity as well as removal of RBCs with \( \sim 99.97 \% \) efficiency. With further optimization and parallelization of the device, it is possible to extract and purify rare cells such as CTCs from peripheral blood.

Similar as building transistors into an integrated circuit, the vortex building blocks can be potentially integrated into more sophisticated sorters with more versatile functions. For example, Chapter 4 showed cascading of two units for multimodal sorting of large, medium and small cells.[60] The multimodal sorter can be integrated to the second stage of the device discussed herein to enable double filtration of small cells and multimodal sorting of large and medium cells simultaneously. One should note that as more vortex units are integrated into sophisticated network, pressure drop in the device will increase which may cause expansion of PDMS
channels or leaking of the devices. Fabricating the device with more rigid material such as epoxy [119] will be helpful for high pressure condition.

Overall, this work presented a versatile inertial microfluidic platform based on inertial focusing and vortex-aided sorting for size-based sorting of cellular samples with high performance. The central design idea is to first develop a powerful, tunable and integratable microfluidic cell sorting unit and further integrate several units in different ways to enable novel cell sorting functions. With the high-performance and versatility, this platform will open new opportunities in the microfluidic sorting area for preparation of a wide range of cellular samples in cell biology, biomedical research and clinical diagnostics.
REFERENCES


