

INERTIAL FOCUSING IN CURVILINEAR CHANNELS

by

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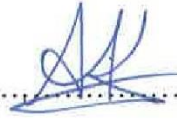
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July 2017

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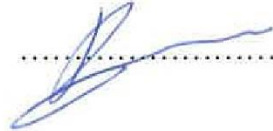
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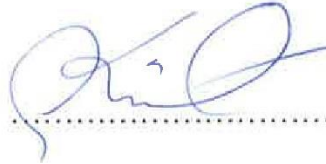
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ABSTRACT

INERTIAL FOCUSING IN CURVILINEAR CHANNELS

ARZU ÖZBEY

PhD Dissertation, July 2017

Supervisor: Prof. Dr. Ali Koşar

Keywords: Microfluidics, Inertial Microfluidics, Particle Focusing, Curvilinear Channel.

Inertial microfluidics has become one of the emerging topics due to potential applications such as particle separation, particle enrichment, rapid detection and diagnosis of circulating tumor cells (CTCs). To realize its integration to such applications, underlying physics should be well understood. This dissertation focuses on particle dynamics in curvilinear channels with different curvature angles (280° , 230° and 180°), where advantages of hydrodynamic forces are exploited as well as cancer cell line focusing in curvilinear channels with curvature angle of 280° . The cruciality of the 3D particle position with respect to inertial forces and Dean drag force is presented by examining the focusing behavior of $20\text{ }\mu\text{m}$ (large), $15\text{ }\mu\text{m}$ (medium) and $10\text{ }\mu\text{m}$ (small) fluorescent polystyrene microparticles, Jurkat, MDA and K562 cell lines for a wide range of flow rates ($400\text{--}2700\text{ }\mu\text{L}/\text{min}$) and corresponding channel Reynolds numbers ($30\text{--}205$). Migration of the particles and cells in lateral and vertical directions and their equilibrium positions are investigated in detail. In the framework of this study's findings, it can be concluded that an increase in curvature angle results in a better separation efficiency. Additionally, two different regions are described: transition region, where the inner wall becomes the outer wall and vice versa, and outlet region. Based on particles' bypassing movement, designing outlets in transition region results in a better separation efficiency. This fundamental approach gives insight into the underlying physics of particle dynamics and offers continuous, high throughput, label-free and parallelizable size-based particle and cell separation.

ÖZET

DÖNEL KANALLARDA ATALET ETKİSİ İLE FOKUSLAMA

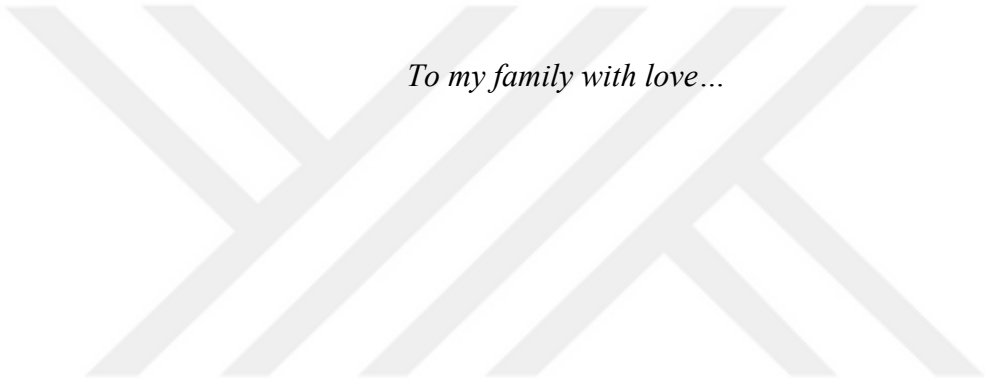
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Anahtar Kelimeler: Mikroakışkan, Atalet Etkili Mikroakışkan, Partikül Fokuslanması, Dönel Kanal.

Atalet etkili mikroakışkanlar, parçacık ayırma, parçacık zenginleştirme, dolaşımdaki tümör hücrelerinin (CTC) hızlı tespiti ve teşhisi gibi potansiyel uygulamalar nedeniyle ortaya çıkan konulardan biri olmuştur. Bu tür uygulamaların entegrasyonunu gerçekleştirmek için temel fizik iyi anlaşılmalıdır. Bu tez, 280° eğrilik açılı eğrisel kanallarda fokuslanan kanserli hücre hattının yanı sıra, hidrodinamik kuvvetlerin avantajlarından yararlanan, farklı eğrilik açılarına (280° , 230° ve 180°) sahip eğrisel kanallarda parçacık dinamiğine odaklanmaktadır. Atalet kuvveti ve Dean sürüklenme kuvvetleri etkisindeki partiküllerin, 3D konumunun önemi, $20\ \mu\text{m}$ (büyük), $15\ \mu\text{m}$ (orta) ve $10\ \mu\text{m}$ (küçük) floresanlı polistiren mikropartiküller ile Jurkat, MDA ve K562 hücre hatlarının fokuslanma davranışı $400\text{-}2700\ \mu\text{L} / \text{dak.}$ debi aralığında ve $30\text{-}205$ Reynolds sayısı aralığında inceleyerek sunulmaktadır. Parçacıkların ve hücrelerin yanal ve düşey doğrultuda hareketi ve denge pozisyonları ayrıntılı olarak incelenmiştir. Bu çalışmanın bulguları çerçevesinde eğrilik açısındaki artışın daha iyi bir ayırma verimi ile sonuçlandığı bulgusuna varılabilir. Buna ek olarak, iki farklı bölge tanımlanmaktadır: iç duvarın dış duvar haline geldiği geçiş bölgesi ve çıkış bölgesi. Parçacıkların baypas hareketine dayanarak, geçiş bölgesini çıkış olarak tasarlanmanın, daha iyi bir ayırma verimi ile sonuçlanacağı düşünülmektedir. Bu temel yaklaşım parçacık dinamiklerinin altında yatan fizik hakkında fikir vermekte ve sürekli, yüksek verimli, paralelleştirilebilir ve boyuta dayalı parçacık ve hücre ayırımı sunmaktadır.



To my family with love...

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LIST OF SYMBOLS

a_p	Particle diameter [m]
C_L	Lift coefficient
Ca	Capillary number
d	Distance [m]
De	Dean number
D_h	Channel hydraulic diameter [m]
F_D	Dean drag force [N]
F_{drag}	Drag force [N]
F_L	Net lift force[N]
$F_{L,deformation}$	Deformability induced lift force [N]
$F_{L,empirical}$	Empirical inertial lift force [N]
F_{LR}	Magnus Lift Force [N]
F_S	Shear gradient lift force [N]
F_{Saf}	Saffman lift force [N]
F_W	Wall induced lift force [N]
h	Height [m]
K	Numerical constant
R	Channel curvature radius [m]
Re_C	Channel Reynolds number
Re_p	Particle Reynolds number

U	Uniform flow velocity [m/s]
U_{De}	Average Dean velocity [m/s]
U_C	Cell migration velocity [m/s]
U_f	Fluid mean velocity [m/s]
U_p	Particle velocity [m/s]
$U_{\max}$	Maximum fluid velocity [m/s]
V	Relative velocity [m/s]
w	width [m]
We	Weber number
γ	Velocity gradient (shear rate) [1/s]
λ	Ratio of particle diameter to channel hydraulic diameter
λ_d	Viscosity ratio
μ	Viscosity [Pa.s]
μ_d	Dynamic viscosity [Pa.s]
ρ	Density [kg/m ³]
σ	Surface tension [N/m]
ν	Kinetic viscosity [Pa.s]
Ω	Angular velocity [m/s]

CHAPTER 1

1. INTRODUCTION

1.1. Microfluidics for Focusing and Separation

Microfluidic and nanofluidic systems are among the fastest growing areas in emerging research and technology. When looking at the number of publications in the literature, it can be recognized that the research and development outputs in these fields have exponentially increased during the last 20 years [1–3]. Apart from the advantages of a compact microfluidic device, cell application offers interesting advantages in terms of providing a lab-on-a-chip platform for diagnosis and treatment [1,4–7].

Microfluidic technologies have many advantages over conventional technologies such as the requirement for a small sample volume, low-cost production, higher sensitivity and improved performance. They take advantage of the fact that the flow characteristics at the microscale may be appealingly different from those at the macroscale. Dominant forces in microfluidics may become negligible at the macroscale. Furthermore, microfluidic technologies facilitate the fabrication of integrative, portable point-of-care (POC) diagnostic devices based on lab-on-a-chip or micro-total-analysis-systems (μ TAS). These devices significantly contribute to biomedical research in the detection, sorting, separation and analysis of cells, especially circulating tumor cells (CTCs) to provide effective diagnosis and therapy.

Microfluidic system originated from the downsizing of gas chromatography system in 1979 upon the development of silicon-based micro-electro-mechanical systems [8]. After 1990, microfluidic applications in subjects such as DNA analysis [9–11], cytometer [12,13], micromixer [14] and microreactors [15] have begun to increase with the capabilities of micro/nano fabrication. On the commercial side, the first microfluidic development began with the appearance of inkjet printers [4].

One of the most important features of microfluidic systems is that they can be integrated into other systems. In particular, the use of Lab-on-a-chip systems has been aimed at enabling several methods, which are used in laboratories in cell applications, to be performed on a single device, and thus, the prominence of microfluidics has become indisputable [6]. The Micro-Total-Analysis System (μ TAS) was designed by combining microfluidic applications such as cytometers, bioreactors and separation in a single system [16]. Very small volumes of liquid are sufficient testing in this integrated system, which only covers a few centimeters square area [1,4–7].

The diagnostic and point-of-care (POC) tests used in the health sector for disease prevention (with the microfluidic system) have not been extensively used for commercial purposes yet, except for a few applications. Especially, particle separation and detection of some cells or biomolecules are one of the most studied topics in this area. With the application of microfluidics based separation systems, mass production of POC test devices will be possible, and low cost and disposable diagnostic devices will be widely available.

Separation methods can be categorized as either active or passive [17,18], where the active methods utilize an external force and passive methods rely on channel topology and internal forces.

1.1.1. Active Particle-Cell Separation Methods and Applications in Microfluidic Systems

Active separation methods require external forces such as magnetic, ultrasonic, optical, thermal and pneumatic forces. Although active techniques provide more accurate results, they suffer from integration of complex components, expensive fabrication and low throughput properties. Additionally, some of active mechanisms such as ultrasonic and thermal methods can damage biological particles.

1.1.1.1. Magnetophoresis

It is possible to separate magnetically sensitive materials in steady flows by applying a magnetic field perpendicular to the flow direction. In cell applications, magnetic particles

are usually coated with the substance necessary to make a specific bond with the cell or protein. Magnetically sensitive objects, such as magnetic microparticles or magnetically labeled cells, are attracted using the magnetic field, which is generated using an electromagnet or permanent magnet (figure 1.1a). The magnetic force depends on the gradient and power of the applied magnetic field and on the magnetization of the particle. Separation of particles with different magnitudes or different magnetizations is possible with this method [19–21].

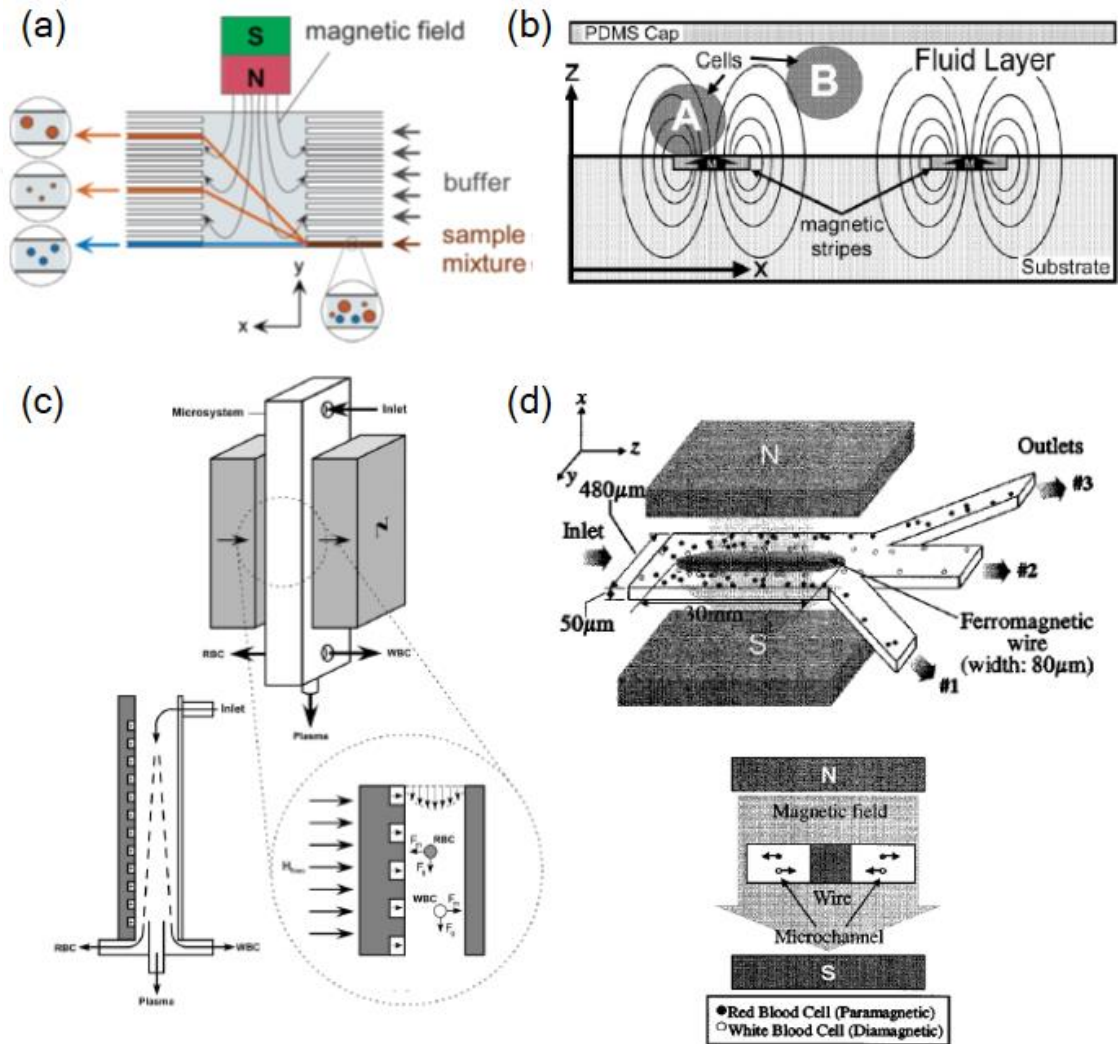


Figure 1.1: (a) Concept of free-flow magnetophoresis [22]. (b) Cross section of the magnetic separation device showing qualitative field lines from magnetized nickel structures embedded into a silicon substrate [23]. (c) Separation of white and red blood cells in the magnetophoretic system [24]. (d) Schematic of magnetophoretic separator consisting of a ferromagnetic wire in the direction of the flow [25].

This technique was first applied by Deng et al. as miniaturized systems [26]. Deng et al. were able to separate almost all of the 4.5 μm big magnetic particles from 6 μm big non-magnetic particles. However, the disadvantage of their system is low output. Pamme et al. separated particles by applying a non-homogeneous magnetic field perpendicular to the flow, using 2.8 μm and 4.5 μm particles with different magnetic susceptibility [22]. Depending on the size and magnetic properties of the particles, their directions in the flow were altered, and particles were focused on different regions. Carr and others have used the same principle, but they added a combined structure, for which they could have 25 different outputs in the microchannel [27]. Blankenstein et al. applied this method to fetal cells [28]. Inglis et al. developed a novel magnetic separation method [23]. In this method, the ferromagnetic bands in the microchannel were produced at a small angle different from the flow direction. Cells were labeled with magnetic nanoparticles and followed the bands (figure 1.1b).

While white blood cells do not exhibit magnetic properties, red blood cells may exhibit magnetic properties depending on whether they are oxygenated or not. Magnetic force can be used not only to separate magnetic particles or magnetically marked particles, but also to separate particles, which do not exhibit magnetic properties. Under the magnetic field, the non-magnetic (diamagnetic) particles can be subjected to a weak pushing force. Furlani used magnetic force to separate white and red blood cells using this feature [24] (figure 1.1c). The separation of red and white blood cells has also been carried out by Han and Frazier [25]. Han and Frazier placed a ferromagnetic wire in the direction of flow into the microchannel and applied magnetic field externally (figure 1.1d). The red and white blood cells were drawn or pushed to the wires depending on their magnetic properties and were collected from different outlets on this count. Zborowski et al. attempted to isolate white blood cells from red blood cells by binding human lymphocytes with ferritin, an iron-rich protein. They utilized the properties of red blood cells as being paramagnetic or diamagnetic and of white blood cells as being diamagnetic [29]. Huang et al. succeeded in separating nucleated red blood cells from the blood of a pregnant woman by designing two modular micro circulating systems [30]. In the first module of this system, the red blood cells without nucleus were collected, and in the second module, 99.99% of the white blood cells were passed through while using the magnetic column to collect the red blood cells of the nucleus.

1.1.1.2. Acoustophoresis

It is possible to separate the particles or biological cells with the help of ultrasonic waves. For this, a continuous wave of sound is sent perpendicular to the flow direction along the microchannel section (figure 1.2). Generally, the sound wave can be controlled with the node located in the center of the microchannel and the wave breakers (anti-node) located at the edges. Particles exposed to sound waves are manipulated with a force directed to the nod or anti-nod. The acoustic force varies according to the acoustic field, the properties of the particles and the surrounded material. The acoustic field is characterized by frequency and amplitude. Accordingly, the lower the applied voltage and the higher the frequency, the higher the acoustic power is. The required frequency is determined by the channel width. For example, if a frequency of 100 kHz is required for a 1 mm width, a 10 MHz wave frequency is required for a 10 μm width. Acoustic force is also proportional to particle volume. It depends on the density and compressibility of the material surrounding the particle as well as on the density and compressibility of the particle. For example, while a solid particle is pushed toward the nod in a liquid such as water, a gas bubble is pushed towards the nod. If the particles in a particular acoustic area have different thicknesses, densities or compressibilities, it is possible to separate the particles with this method [19–21].

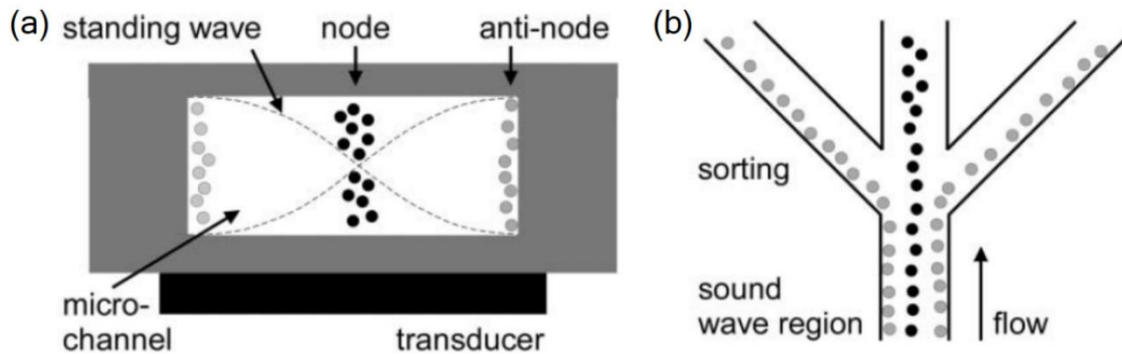


Figure 1.2: Continuous flow separation based on sound pressure. (a) A standing ultrasonic wave is generated over the width of a microchannel by means of an external transducer. (b) Collection of aligned particles from the outlets of the channel [21].

As an example of this method, Petterson et al. investigated the movement of 5 μm particles at a flow rate of 100 $\mu\text{l}/\text{min}$ as well as the effect of acoustophoretic force on blood cells. Sound wave was produced by integrating the silicon chip of the pyroceramic layer [31]. Goddard et al. used ultrasonic acoustic energy to collect the particles at the

central flow streamline. The sound wave was produced by integrating the piezoceramic crystal on the glass capillary tube. 7.8 μm particles and Hamster cells were tested [32,33]. Evander et al. studied the effect of increasing acoustic forces and temperature on cells [34]. Shi et al. provided a pair of transformers on the piezoelectric substrate, bonding the substrate to the PDMS microchannels [35]. It was observed that 1.9 μm polystyrene particles were focused in the microchannel center at 10 $\mu\text{l/min}$. The same group then did different studies using the same method to separate polymer particles and cells [36,37].

1.1.1.3. Optical Separation

The method of separating particles by light has been carried out in such a way that the particles are trapped depending on the polarity of the particles. The optical splitting technique for Particle Focusing has been applied to particles of different sizes or to particles of different light-breaking properties, by forming an interferometric light path [18]. The optical forces are produced using a laser beam, which impacts the particles vertically in the direction of flow. To do this, optical tweezers are used. Optical tweezers were developed by Ashkin as a manipulator [38]. Tightly-focused laser beam was used to capture a single particle.

In optical separation, optical gradient force is created to change the track of particles of different size or refraction characteristics. While particles exposed to sufficiently large magnitude of optical force are caught by optical tweezers kinetically, other particles follow the flow streamline. MacDonald et al. reported that the particle focusing efficiency in this method is about 95% [39]. Chiou et al. developed a novel system for separating particles of different sizes using dielectrophoretic electrodes and a low intensity light source [40]. The same group then separated the HeLa and Jurkat cells using dynamic optoelectronic tweezers [41]. Shah et al. have conducted a separation study using optoelectronic tweezers with an automated system that places HeLa cells in droplets [42].

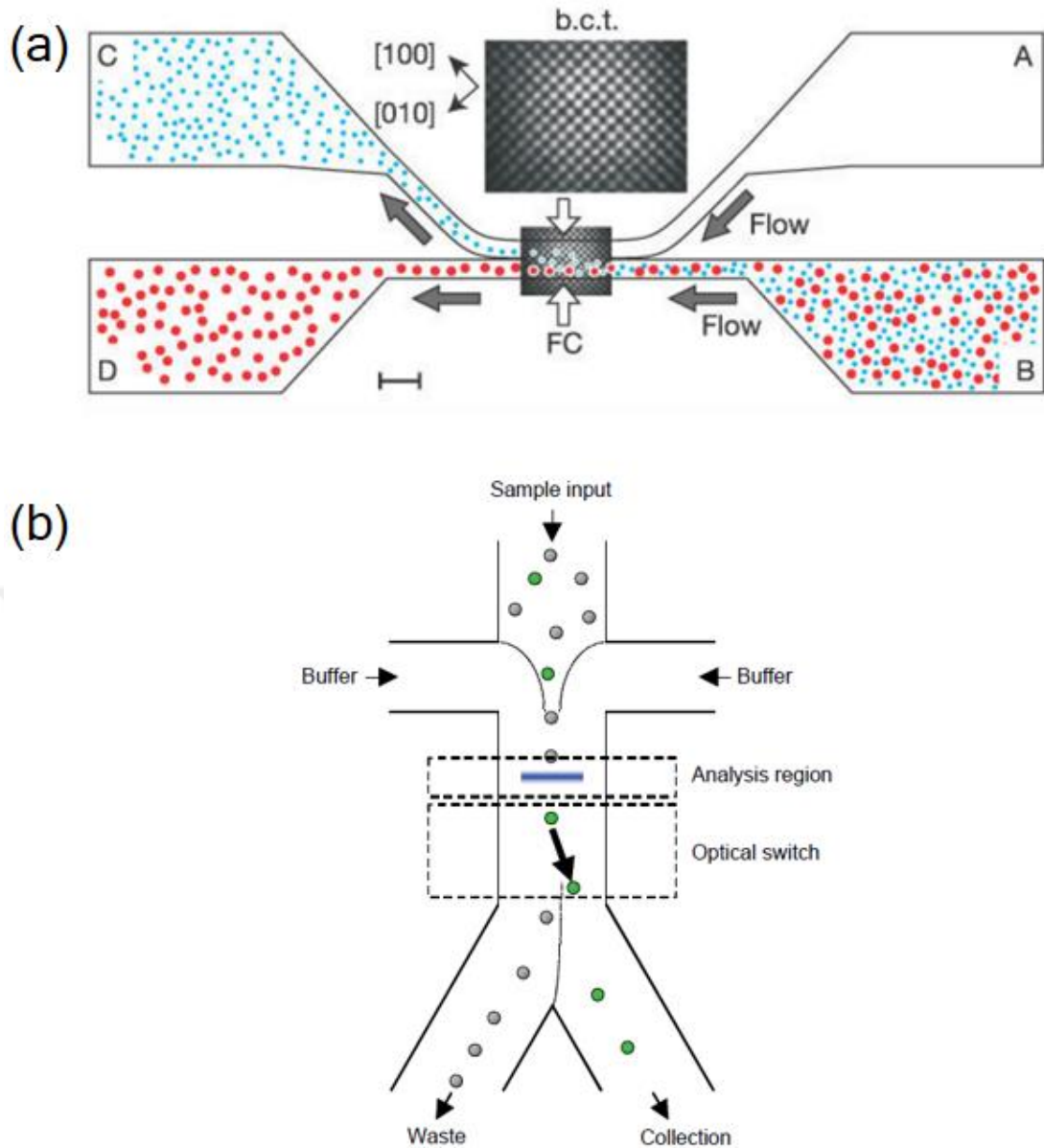


Figure 1.3: The concept of optical fractionation [43]. (b) layout of the microfluidic sorting junction and the optical switch [44]

Optical separation with generating droplets was used by Wang et al. [44] to focus mammalian cells (figure 1.3b), and by Perroud et al. [45] to smear bacterial macrophages. Mac Donald et al. succeeded in separating two types of particles of the same size and having different light diffraction properties [43] (figure 1.3a). 2 μm silica and 2 μm polymer particles go through the microchannel at a flow rate of 1800 $\mu\text{l} / \text{min}$. While the polymer particles passing through the optical path were affected by optical forces and changed the flow direction, the silica particles followed the flow streamline. They reported that a separation efficiency of approximately 100% was obtained. The same

group then carried out the separation of silica particles with four different sizes at a flow rate of 1200 $\mu\text{l} / \text{min}$ [46]. This technique was also used by Ladavac et al. [47].

1.1.1.4. Electrophoresis

Electrophoresis is a powerful technique especially in molecular separation and it can be used in cell separation applications. However, researchers have not been able to draw much attention as a cell sorting method. The most important reason for this is the inability to obtain efficient decomposition due to technical problems related to convection and complicated theoretical structure [18]. The optimal separation technique in this method is a method in which parallel electrophoresis is applied independently from flow. Independent electrophoresis from flow is operated due to flow direction change of the particles in the solute sent to a non-deep reservoir with the electric field, which is applied perpendicular to the flow direction (figure 1.4a). The flow is directed via the hydrodynamic effect in the -x direction, while the electric field is in the y direction. The composition of these two flow vectors determines the observed direction of flow. Depending on whether the molecules are anions or cations, the deviation from the flow direction depends on the ratio of electrical charge to size [21].

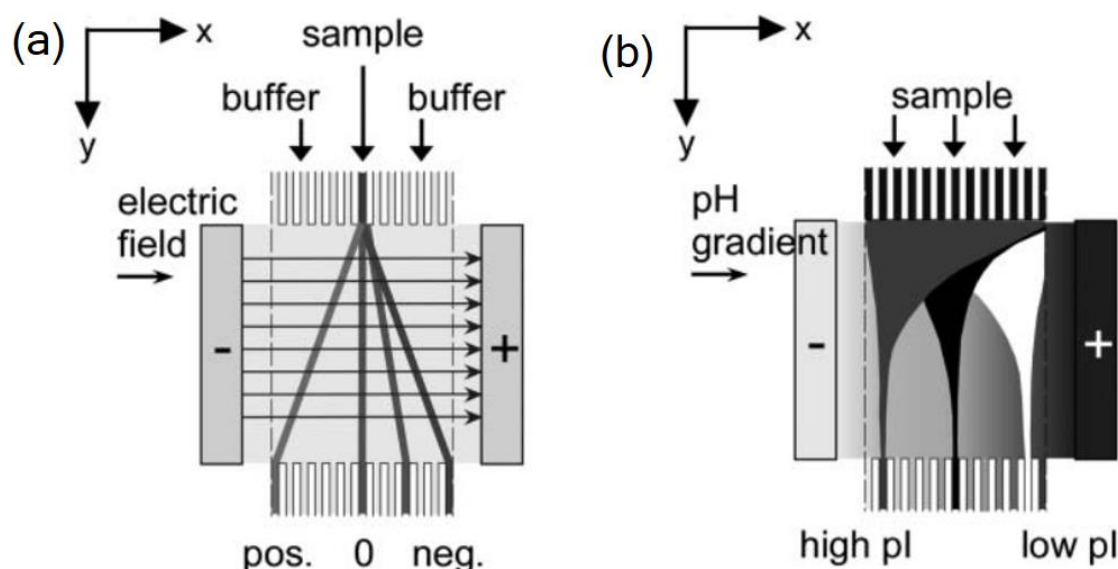


Figure 1.4: Free-flow separation methods usually feature a shallow separation chamber with flow pumped in the y-direction and a field applied in the x-direction. (a) Free-flow electrophoresis, (b) free-flow isoelectric focusing [21].

In a flow-independent isoelectric focusing method, a pH gradient is generated in the x-direction, the flow is given in the y-direction (figure 1.4b). A mixture containing amphoteric molecules such as proteins is sent throughout the width of the separation channel. When the protein enters the area having a different pH from its isoelectric point, the electrophoretic force is applied and the pH gradient moves along the x-direction until it reaches its own isoelectric point. The protein, which is spotted at this point, continues to follow the flow. Proteins with different isoelectric properties can be separated by this method.

1.1.1.5. Dielectrophoresis

Dielectrophoresis (DEP) is formed by an inhomogeneous electric field and can be used as a particle or cell separation method. When a particle is exposed to an electric field, it is polarized along the electric field due to the electric charge. Within the homogeneous electric field, electrostatic forces are counterbalanced in the form of double poles and cause the particle to remain stationary. In the non-homogenous electric field, these forces are different and cause the particle to move. The movement can be in the direction of the electric field or vice versa. If the particle's electrical polarizability is greater than its surrounding material, the particle moves towards the electric field. This is called positive electrophoresis (pDEP) (figure 1.5a). If the electric polarizability of the particle is smaller than the surrounding material, the particle moves away from the electric field and is called negative electrophoresis (nDEP) (figure 1.5b). For this reason, the dielectrophoresis force varies depending on the difference in polarizability of the particle and the surrounding material. The force is also proportional to the particle volume and electric field gradient.

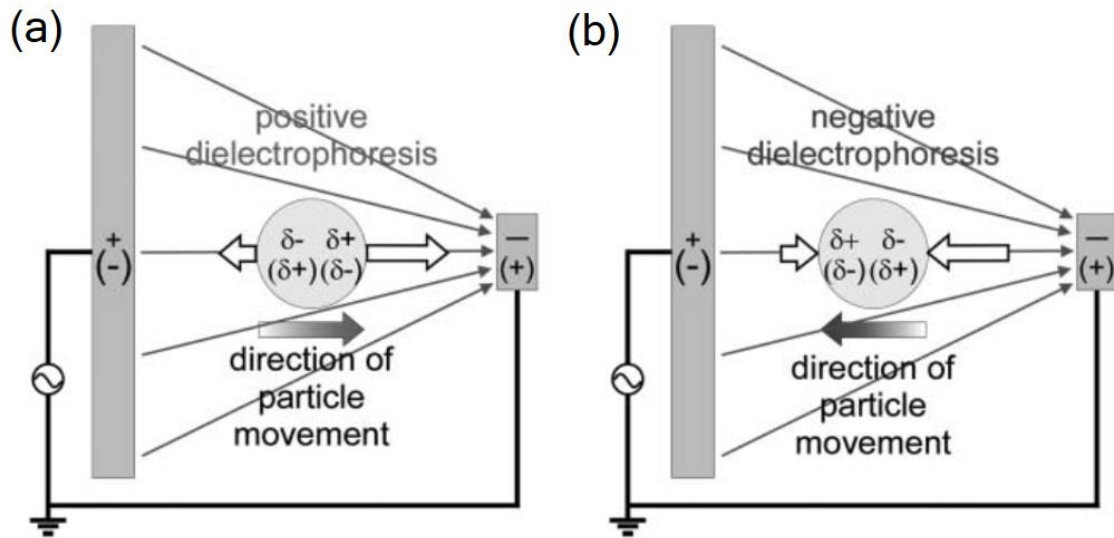


Figure 1.5: The principle of dielectrophoresis (DEP). (a) Positive dielectrophoresis, (b) Negative dielectrophoresis [21].

Generally, in dielectrophoresis, particle focusing is achieved using non-uniform AC electric field and electrodes within the microchannel [21,48]. In addition, it is possible to focus the particles by inserting micro insulators in the channel [49–51]. In this method, when the DC field is applied across the channel, the dielectrophoretic force around the micro-insulator causes the particles to be displaced by the electrokinetic flow. Dielectrophoresis with electrodes is typically seen in the negative DEP method [52,53]. The largest electric field occurs on the surfaces of the electrodes and the particles are pushed away from the electrode. The method of separating particles using an insulator was first developed by Cummings and Singh [54]. Cummings and Singh used insulating, cylindrical columns in microchannels and studied the behavior of particles under the DEP effect by providing electric field gradients around these columns. When the dielectrophoretic movement was greater than electrokinetic and Brownian movements, the particles accumulate in the columns [55,56]. This method was then tested on biomolecules [57–61]. Separation of polymer particles and biological cells according to their sizes was also been studied [62–65].

The dielectrophoretic particle separation method was also applied to straight and curved channels. In the experiments conducted by Cummings and Singh using the positive dielectrophoresis method in expanding and constricting channels, focusing of 200 nm latex particles was observed [50]. A similar study was carried out by Xuan et al. using negative dielectrophoresis. In this work, 40 μm particles were observed to be focused at the channel center [66]. Zhu and Xuan worked on focusing of particles in microchannels

using the DC effective AC electric field. 10 μm polystyrene particles were observed to be focused at the channel center [67]. In another similar work, it was aimed to improve the control of the electric field and the particle focusing efficiency using oil [68]. The straight channels with expansion and contraction geometry using oil was later used by other groups to separate particles [69]. Xuan's group investigated particle focusing using DC electrokinetic flow and curved microchannel structure [70–73]. In this method inertial and Dean forces are negligible due to low Reynolds numbers. Since the path length changed, the electric field had a maximum value on the inner wall side and a minimum value on the outer wall side. For this reason, the particles did a lateral motion electrokinetically during the turn. Depending on whether the applied dielectrophoresis was negative or positive, the particles were focused on the inner wall or outer wall [74,75]. Zhu et al. studied focusing of 5 μm particles by dielectrophoresis in spiral channels [71]. Unlike curved channels, it was observed that particles moved toward negative wall at negative DEP, and the rotation did not affect particle motion. For each spiral-turn, particle got closer to the outer wall. Zhu and Xuan examined particle focusing using a double spiral channel geometry [73]. Then, they used double spiral channel geometry and dielectrophoresis method to separate the particles according to their sizes [76].

1.1.2. Passive Particle-Cell Separation Methods and Applications in Microfluidic Systems

Passive separation methods mainly utilize hydrodynamic forces such as surface tension, viscosity, capillarity and inertial forces, which are important in microscale and rely on the channel geometry and surface properties. Fabrication of passive separation devices is relatively simpler and easier to integrate into the lab on a chip system. Moreover, ability of designing parallel microchannels could provide a higher throughput since the external power sources are eliminated. However, most of the passive mechanisms rely on size base separation and it is difficult to separate or detect similar size of particles/cells.

1.1.2.1. Micro Filter

It is possible to filter out microparticles or cells of different sizes, but this technique can provide low separation efficiency. There are four types of micro filtering methods, which can be created with microchannel geometries such as weir, pillar, cross-flow and membrane [77].

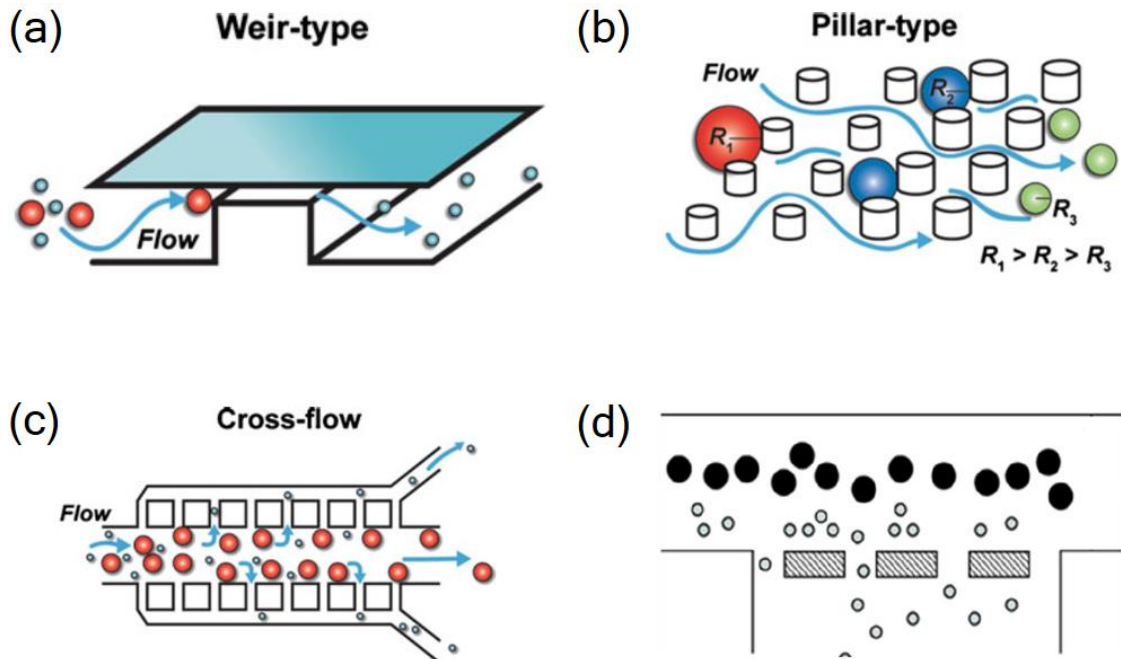


Figure 1.6: (a) Weir-type filtration, (b) pillar-type filtration, (c) cross-flow-type filtration, (d) membrane-type filtration [77].

In weir-type filtration (figure 1.6a), separation of small particles from the dam-shaped structures can be achieved. Crowley and Pizziconi used capillary effect to make blood plasma pass through a planar slit and to make sure that blood cells did not cross because of the narrowness of the capillary, but only limited volumes of plasma could be obtained [78,79]. This method was later developed to allow blood to be separated in higher volumes [80,81]. In the pillar-type filtration (figure 1.6b), the pillars are arranged at a certain distance from each other to direct the flow and the particles, yet, clogging and deterioration of the cells limit this application. Mohamed et al. could isolate fetal nucleated red blood cells (fNRBCs) from maternal cells in cord blood with this design [82]. In the cross-flow-type filtration (figure 1.6c), obstacles are placed at regular intervals, and flow is perpendicular to micropost array or weir filter. As small particles pass through the space of these obstacles, the other particles follow the flow streamline. It is also desirable to reduce the clogging problem. This method was used to separate the plasma from the blood [83], the white blood cells from the bloodstream [84,85], and DNA [86]. In membrane-type filtration (figure 1.6d), holes are formed for passage of certain lengths of cells or particles, but as in other filtration methods, the clogging problem is one of the most important problems in this method. With this method, separation of the cancer cells from the blood by forming holes in the form of eggs or circles was studied [87].

1.1.2.2. Hydrodynamic

The hydrodynamic filtration method is used to separate particles according to channel geometry and flow characteristics. This method presumes that particles follow the streamline at low Reynolds number. In this method, microchannel geometry consists of one or more inlets and outlets, where the flow is separated from the main flow. Smaller size particles can approach the microchannel walls more than larger particles. The ability to remove small particles from the side outlets can vary depending on the velocity of the flow near these outlets. When this flow velocity is low, no particles can be taken from the side outlets, only medium particles can be collected when the speed is medium, and both particle types can pass through the side outlets when the velocity is high.

Yamada and Seki are the first ones to implement this method [88] (figure 1.7). 1 μm and 3 μm particles were introduced at the inlet with a flow rate of 1 $\mu\text{L}/\text{min}$. Separation of 1 μm , 2 μm and 3 μm particles was studied in a different channel design. Accordingly, the

efficiency increased in particle concentrations collected from different outlets [89]. Yamada et al. later used this method to separate the liver cells from the parenchymal tissue at a concentration of 50 $\mu\text{L}/\text{min}$ [90]. In another study, Hela cells were run in the hydrodynamic system to separate at 140 $\mu\text{L}/\text{min}$ [91]. The group then performed hydrodynamic filtration experiments on various types of spherical and non-spherical particles (0.2 - 30 $\mu\text{L}/\text{min}$) [92]. Aoki et al. attempted to focus particles at the channel center by using a flow instead of taking the particles from the side outlets with the hydrodynamic filtration method [93].

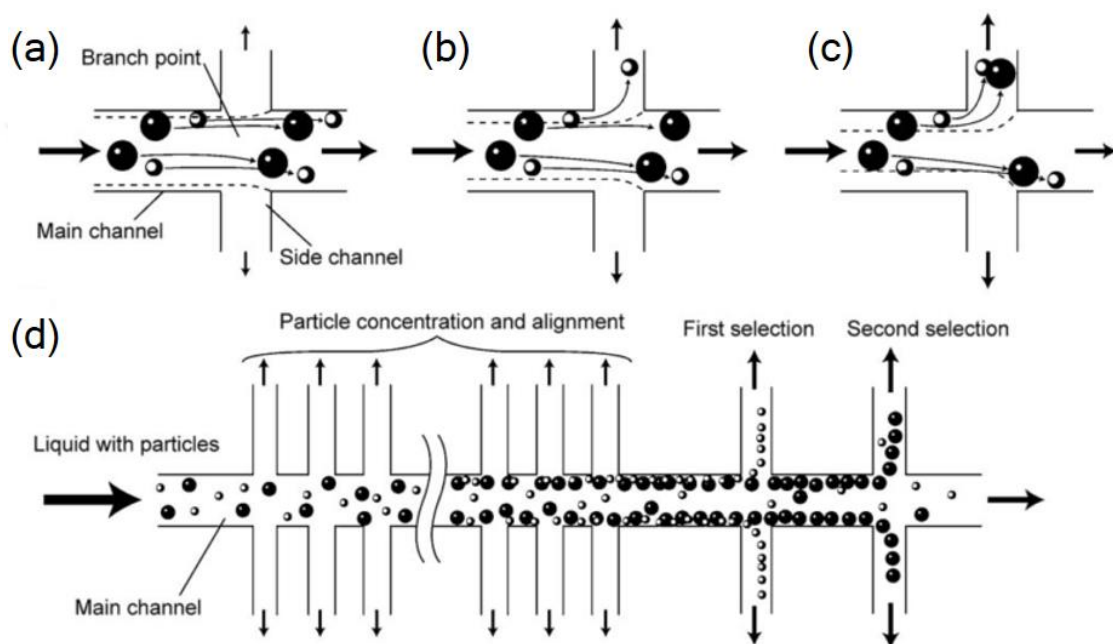


Figure 1.7: Principle of hydrodynamic filtration. (a)–(c) Schematic diagrams showing particle behavior at a branch point; (a) the relative flow rates distributed into side channels are low, (b) medium, and (c) high. (d) Schematic diagram showing particle concentration and classification in a microchannel having multiple branch points and side channels [88].

1.1.2.3. Deterministic Lateral Displacement (DLD)

In this technology, specific arrangement of micropost arrays in the microchannel was utilized to separate particles of larger and smaller than a critical diameter (D_c) [94]. When two different size particles following the same streamline enter the micropost arrays region, the particle which smaller than the critical diameter will remain in the first streamline because its hydrodynamic center is not out with the width of the first

streamline. On the other hand, a particle is larger than the critical diameter is displaced to the next streamline owing to the fact that its hydrodynamic center is out with the boundary of the first streamline. Deterministic lateral displacement (figure 1.8) was first reported by Huang et al. in 2004 to separate 0.8, 0.9 and 1 μm of microspheres with a resolution of 10 nm [95]. After the invention, DLD has been applied to the separation of red blood cells [96], white blood cells [97], platelets [98] and circulating tumor cells [99]. DLD was also coupled with external forces of acoustic [100], electric [101] and gravitational [102] by using shape, deformability and dielectric properties of the particles.

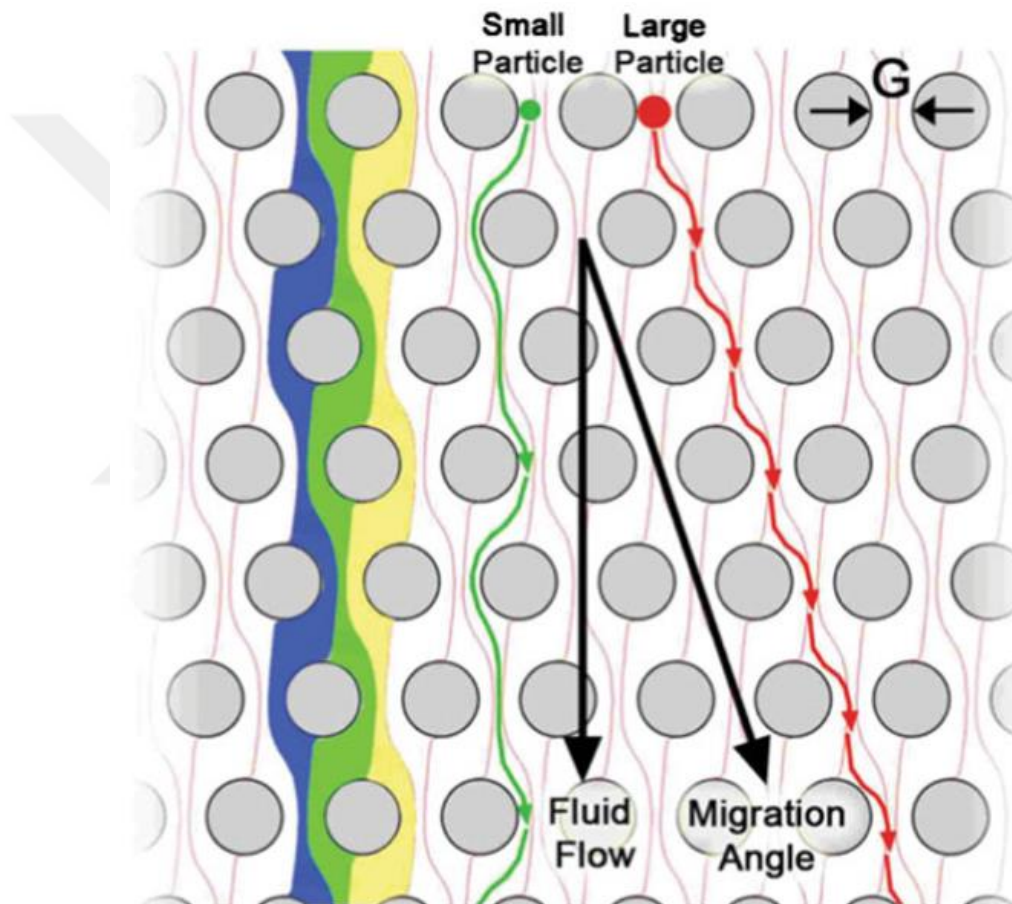


Figure 1.8: Deterministic lateral displacement [103].

1.2. Structure of the Thesis

Focusing particles or cells into a tight stream is of significant interest for separation and detecting purposes. This thesis presents experimental evidence of interesting microparticle and cancer cell line behavior in curvilinear channels. The effect of curvilinearity with different curvature angles (180° , 230° , 280°) on particle focusing behavior was examined in this dissertation by performing inertial focusing of $10\text{ }\mu\text{m}$, $15\text{ }\mu\text{m}$ and $20\text{ }\mu\text{m}$ fluorescent polystyrene microparticles at different channel Reynolds numbers. Furthermore, the decoupling effect of inertial and Dean drag forces on particles and separation potential were revealed. In addition, focusing behavior of Jurkat, MDA and K562 cell lines were investigated. This thesis will provide a valuable contribution to the field of inertial microfluidics by extensively improving understanding of three-dimensional particle dynamics in curvilinear channels.

Chapter 2 presents the literature review of the inertial focusing including the theoretical description of inertial and Dean drag forces and progresses on design parameters.

Chapter 3 presents the forces acting on the particles in inertial microfluidics including major and negligible forces.

Chapter 4 focuses on methodology for fabrication of microchannels, experimental procedures of particle and cell focusing and methods for analysis of images and recorded movies.

Chapter 5 includes the experimental results of curvilinear channels with different curvature angles (180° , 230° , 280°) on particle dynamics behavior. The focusing width and positions were given as well as separation potential of $10\text{ }\mu\text{m}$, $15\text{ }\mu\text{m}$ and $20\text{ }\mu\text{m}$ particles. Moreover, a hypothesis of three-dimensional particle dynamics in curvilinear channels was presented.

Chapter 6 presents the experimental data on particle dynamics of $10\text{ }\mu\text{m}$, $15\text{ }\mu\text{m}$ and $20\text{ }\mu\text{m}$ particles in an asymmetric curvilinear channels with curvature angle of 280° .

Chapter 7 presents the experimental evidence of focusing behavior of Jurkat, MDA and K562 cell lines. Investigation was carried out for separation potential of these cell lines.

CHAPTER 2

2. REVIEW OF INERTIAL FOCUSING

Among the passive separation approaches, inertial microfluidics has been becoming more and more popular due to its high throughput, sensitivity, simplicity and high efficiency. It was first reported by Segre and Silberberg in 1961 [104,105]. Accordingly, it is known that uniformly dispersed particles formed a narrow band at a distance of $0.2D$ away from the channel walls in a circular cross section channel with a diameter D , which is called “tubular pinch effect”. After the discovery of the tubular pinch (TP) effect, many studies can be seen in the literature to explain this behavior. For instance, Matas et al. experimentally confirmed TP effect for a wide range of Reynolds number and found that equilibrium positions shift towards the wall with increasing Re number [106–108]. An increase in the a_p/D_h ratio results in accelerated migration of the particles from the microchannel center towards the circumference as a consequence of the fact that average shear rate experienced by particles augments with increase in the surface area [106–108]. The higher the flow rate becomes, the closer the particles get to the wall [107].

2.1. Inertial Focusing in Straight Channels

The equilibrium position of the particles in straight channels depends on Reynolds number as well as the cross section of the channel (figure 2.1). Considering square microchannel cross section, Kim and Yoo observed that in experiments on square-profile flat channels, particles with low Reynolds numbers ($20 < Re < 30$) are collected in a fine equilibrium position along the channel perimeter [109]. A similar study was done by Chun and Ladd [110]. Broken symmetry results in eight distinct equilibrium positions for low

Reynolds Number ($Re < 100$). While low Reynolds number flows introduce eight equilibrium positions due to a uniform shear gradient on all four sides, high Reynolds number ($Re > 500$) reduces the equilibrium positions to four by making the particles to follow a streamline centering at each channel wall face along the microchannel [109–112]. This was explained by the effect of uniform shear gradient on all four sides of the channel. These particles migrated closer to the microchannel walls as the flow rate increased [108,113–115].

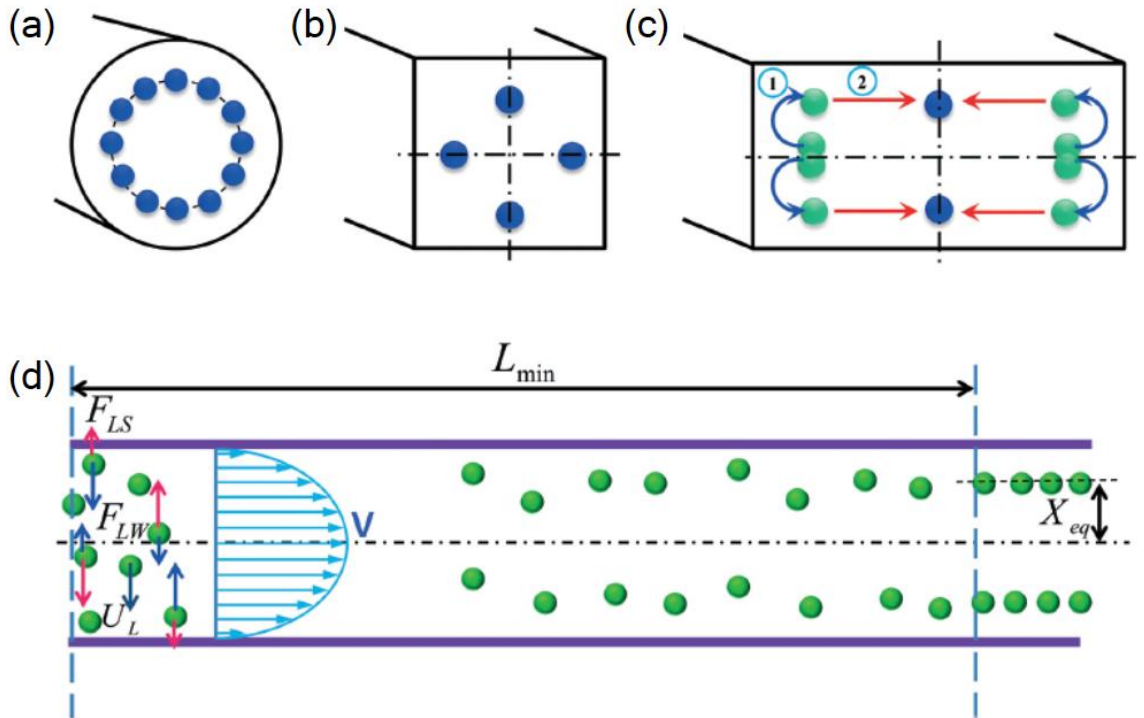


Figure 2.1: Inertial equilibrium positions in a straight channel with different cross sections: (a) circular cross section; (b) square cross section, and (c) rectangular cross section with low aspect ratio (≈ 0.5). Illustration of two-stage migration model proposed by Zhou and Papautsky (d) The lateral migration speed U_L and minimum channel length for particle focusing L_{min} [116].

Analogous to square microchannels, in rectangular microchannels once again eight equilibrium positions were observed. Bhagat et al. experimentally observed that the particles focused along the long side of the channel walls [112,113]. However, since the inertial forces across a wider dimension were weaker, shorter dimensions experienced focusing lines prior to longer dimensions distinguishing the rectangular microchannels from the square ones. Moreover, Zhou and Papautsky experimentally found that two equilibrium positions could be obtained at the center of the channel [117]. They claimed

that not only the shear-induced and wall-induced lift forces acted on particles within flow causing equilibria near microchannel sidewalls, but the rotation-induced lift force also influenced the position of these equilibria. Later, Zhou et al. modified the design of the channels based on their theory [118]. Randomly distributed particles were focused at half of the channels near two channel walls within the high aspect ratio region then when the channel expanded. Since larger particles had a higher migration velocity than smaller particles in low aspect ratio region, separation of larger and smaller particles could be achieved with a high separation efficiency (99%).

The ratio of particle diameter to channel hydraulic diameter a_p/D_h was taken into account to explain how the system parameters affect particle movement [119]. For this purpose, two kinds of Reynolds number were introduced: one is channel Reynolds number and the other is particle Reynolds number, which consists of inertial forces to viscous forces as well as particle to channel hydraulic diameter ratio, one of the most important dimensionless parameter to characterize particle behavior:

$$\text{Re}_p = \frac{\rho U a^2}{\mu D_h} = \text{Re} \frac{a^2}{D_h^2} \quad (2.1)$$

where ρ is fluid density, U is fluid mean velocity, a is particle diameter, μ is fluid viscosity and D_h is hydraulic diameter of the microchannel. If $\text{Re}_p < 1$, dominant viscous drag influences particles to follow fluid streamlines. If $\text{Re}_p > 1$, inertial lift forces become dominant, and lateral migration of the particles can be observed. This study also suggested that wall induced and shear induced lift forces acting on a particle (orthogonal to flow directions) changed depending on the particle position and channel Reynolds number.

According to the literature, successful particle focusing can be achieved by minimum thresholds for ratio of particle diameter to channel hydraulic diameter, $\lambda = \frac{a_p}{D_h}$, and particle Reynolds number, which are $\lambda > 0.07$ and $\text{Re}_p > 1$, respectively [115]. Particle lateral migration velocity (U_L) and the minimum channel length ($L_{\min}$) required for particles to have their equilibrium positions are derived by balancing the Stokes drag with net inertial lift force [112]:

$$F_d = 3\pi\mu a U_L \quad (2.2)$$

$$F_L = \frac{\rho C_L U_f^2 a^4}{D_h^2} \quad (2.3)$$

$$U_L = \frac{F_L}{3\pi\mu a} = \frac{\rho U_f^2 a^3}{6\pi\mu D_h^2} \quad (2.4)$$

$$L_{\min} = \frac{D_h}{2U_L} \times U_f = \frac{3\pi\mu D_h^3}{\rho U_f a^3} \quad (2.5)$$

where C_L is lift coefficient, ρ is fluid density, U_f is fluid mean velocity and $\frac{U_f}{D_h}$ is

shear rate. The lift coefficient is a function of channel Reynolds number and varies between 0.2 and 0.5 in microfluidic applications [119,120]. Recent studies show that particle position plays an important role in the scale of the net lift force so that this force is dominant in particle motion near the centerline [115] and is proportional to [121]:

$$F_L = \frac{f_1 \rho U_f^2 a^3}{D_h} \quad (2.6)$$

and near the wall proportional to [121]:

$$F_L = \frac{f_2 \rho U_f^2 a^6}{D_h^4} \quad (2.7)$$

where f_1 and f_2 are nondimensional lift coefficients that depend on x/h and Reynolds number.

Hur et al. used straight microchannels with a high aspect ratio to demonstrate the purification of adrenal cortical progenitor from digestions of murine adrenal glands [122]. They observed that smaller size cells focused near the channel walls while larger size cells focused near the channel centerline. Mach and Di Carlo used a massively parallelized microfluidic device, which consists of 40 single straight channels located radially and separates pathogenic bacteria from diluted blood [123]. In this study, more than 80% pathogenic bacteria were removed from the system with a high throughput of 400 million cells per minute.

2.2. Inertial Focusing in Curved Channels

Since secondary flows emerged from curvilinear geometry of microchannels especially dominates in regions of such fading lateral force, curvilinearity in rectangular cross sectional microchannels results in enhanced capability to control the number and position of the equilibrium lines along the stream. The equilibrium positions can be further controlled using curved channels [114,124,125]. For this purpose, Dean drag force, deemed to follow Stokes drag relationship, is introduced as a result of the curvilinear channel geometry.

In fluid flows through a curved channel, there is a secondary flow due to difference of velocity between fluid in the centerline and near the walls, and subsequent larger inertia force in the centerline. Therefore, the fluid in the centerline tends to flow outwards and generates pressure gradient. Besides, due to this centrifugal pressure gradient, the fluid near the walls is prone to flow inwards. As a result of tendencies in the fluid, two symmetric vortices form [114,124]. The magnitude of these vortices can be quantified by a non-dimensional number, Dean number (De):

$$De = \frac{\rho U_f D_h}{\mu} \sqrt{\frac{D_h}{2R}} = Re_c \sqrt{\frac{D_h}{2R}} \quad (2.8)$$

where μ is the fluid viscosity and R is the channel curvature radius.

A particle flowing through a microchannel with a rectangular cross section experiences Dean drag force as well as shear lift force. According to Stokes drag law, the Dean drag force acting on a particle can be expressed as [126]:

$$F_D = 3\pi\mu U_{De} a_p \quad (2.9)$$

where U_{De} is the average Dean velocity and is calculated as

$$U_{De} = 1.8 \times 10^{-4} De^{1.63} \quad (2.10)$$

The Secondary flow caused by the Dean vortices exerts a drag force on the particles directed outwards near the channel center, and inwards near the upper and bottom walls [114,124–127]. The combination in magnitude of the net lift force and Dean drag force reduces the number of equilibrium positions into one with a controlled Re_c . In most cases, centrifugal force is negligible because the density of particle is very close to that of fluid.

Early studies estimate the theoretical observations as follows: Particles close to top and bottom walls of the microchannel experience a lateral net lift force F_L and vertical Dean drag force F_D . Due to strong F_D , particles follow the Dean flow. For particles near the outer wall, the direction of F_L and F_D become equal, causing particles to follow Dean flow. Particles near the inner wall, F_L and F_D act in opposite directions [112,128]. Di Carlo et al proposed that the ratio of net inertial lift force to Dean drag force F_L/F_D would be a key parameter for describing behavior in such systems so that when $F_L/F_D \ll 1$ no particle focusing will be observed as the stronger Dean vortices mixes particles and when $F_L/F_D \gg 1$ particle focusing is possible due to inertial lift force [115]. However, preferable condition is when $F_L/F_D = 1$. Computational results presented the magnitude of wall induced and shear induced forces based on the particle location [121]. Di Carlo et al. proposed a new hypothesis based on their observations that the shear induced lift force, not the wall induced lift force, balances Dean drag [129]. A dimensionless parameter (ratio of shear induced lift force to Dean drag) is introduced to better estimate the particle behavior:

$$R_F = \frac{F_L}{F_D} = \frac{2Ra^2}{D_h^3} \quad (2.11)$$

For very low R_F ratio, particles will follow the Dean vortices, while for very high R_F particles will migrate to equilibrium positions. However, for intermediate R_F , equilibrium positions can be comparatively controlled by Dean flow leading to an intriguing new focusing mechanism. The latest findings suggest that inertial equilibrium positions can be controlled if:

$$R_F = \frac{2Ra^2}{H^3} \geq 0.08 \quad (2.12)$$

where H is the narrowest channel dimension. Martel and Toner proposed inertial microfluidics theory with a comprehensive set of studies based on Re number, De number and a_p/D_h ratio [130,131]. Their results showed that as the velocity profile became flattened for wider channels, the shear gradient lift in this wide direction decreased and Dean drag on particle became stronger in wider channels. Moreover, the addition of curvature to microchannels not only added Dean drag to the force balance but also

changed shear gradient lift force via redistribution of the velocity profile. Therefore, a simple ratio of forces was not sufficient enough for scaling of inertial focusing.

Curvilinearity, which generates the Dean drag force, can be divided into two main categories: spirals and serpentine channels. According to the literature, spiral channels achieved better separation efficiency compare to serpentine channels [124]. Gregoratto et al. used a high aspect ratio ($h / w = 10$) spiral microchannels with bifurcation at the exit [132]. Devices of varying geometries were fabricated using conventional silicon micro fabrication processes and were tested by flowing dilute aqueous suspensions of polystyrene particles (diameters of 1 μm , 8 μm and 10 μm) through the devices at various flow rates. A 3.5 fold concentration enhancement of 10 μm particles was achieved in the longer devices at a flow rate of 2 ml/min, whereas the 1 μm particles showed negligible concentration increase at similar flow rates. Similarly, Seo et al. used double spiral channels, where an “S” shaped structure was placed at the center of the channel [133,134]. The experiments were carried out by mixing the polystyrene particles of diameters of 3 μm , 6 μm and 10 μm with DI water. They found that at higher flow rates the exit concentration of 10 μm particles was 660 times greater on the inner wall side than that on the outer wall side.

Bhagat et al. developed a cytometry system based on inertial microfluidics and Dean flow. They reported that this system could provide a throughput of 2100 particles per second [135]. Bhagat et al. conducted experimental studies ot low De numbers to separate 1.9 μm and 7.32 μm particles in five-loop spiral microchannels with two-inputs and two-outputs [136]. At $De = 0.47$, large particles were separated from the outlet at the side of the inner wall, while small particles were collected from the outlet at the side of the outer wall. Later, Bhagat et al. performed a similar study with the subsequent separation of particles of 560 nm, 1.9 μm and 7.32 μm [137]. In another study, Kuntaegowdinahalli et al. achieved 90% efficiency in separating 10 μm , 15 μm and 20 μm polystyrene particles with a throughput of 1×10^6 particles using a spiral microchannel design [128] (figure 2.2). Russom et al. designed a spiral microchannel design with increasing width, and the design incorporated two exit zones with a width of 50 μm and 950 μm [138]. Separation of 3 μm and 10 μm particles was carried out at 3.5 ml / min. 98.5% of the 10 μm particles were taken from the narrow exit on the side of the inner wall of the microchannel, while 92.5% of the 3 μm particles were taken from the other exit zone.

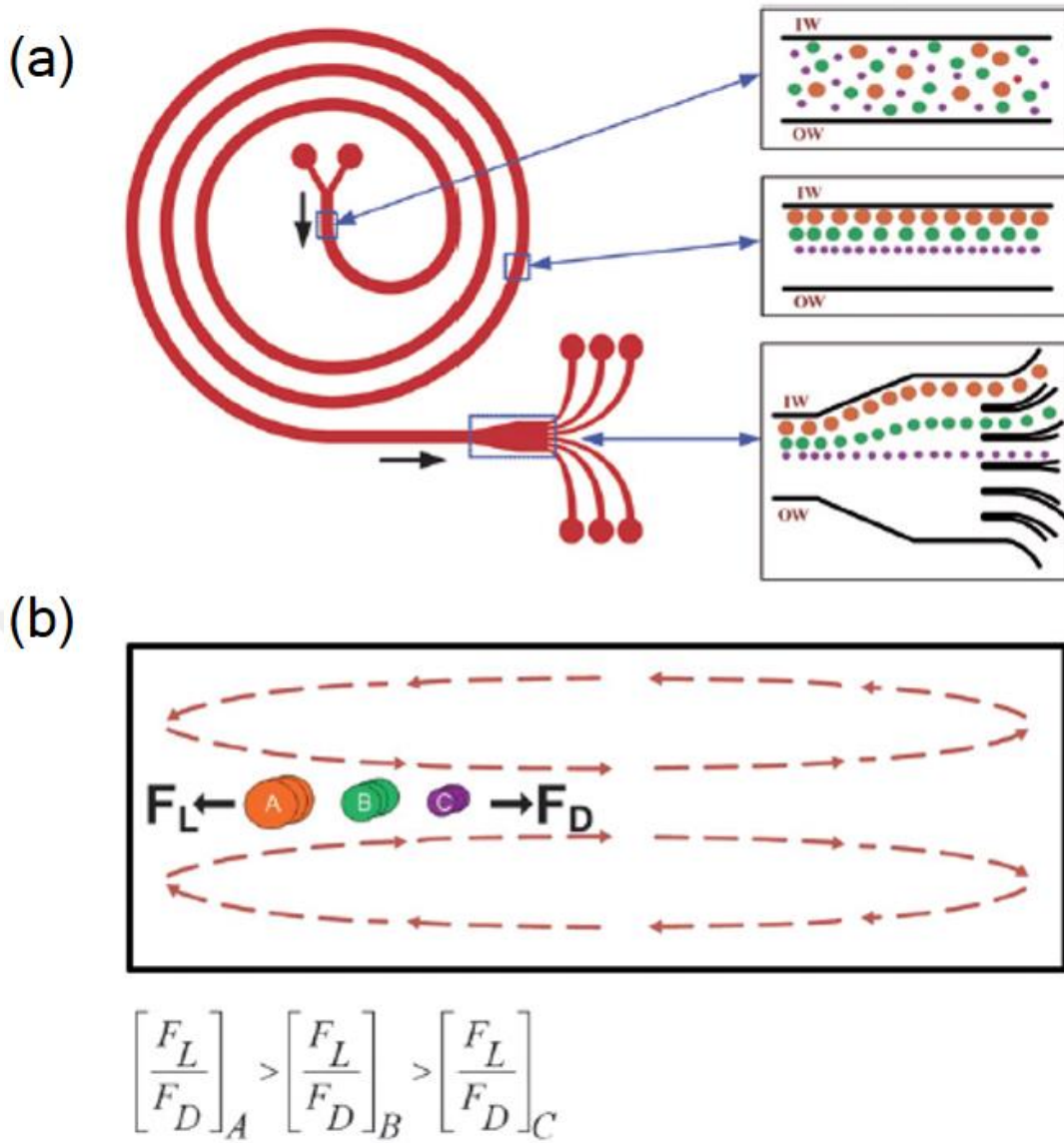


Figure 2.2: (a) Schematic of the spiral microparticle separator. (b) Microchannel cross-section illustrating effects of F_L and F_D on particles [128].

Hou et al. used spiral channels to isolate circulating tumor cells (CTCs) from blood [139]. The device achieved a recovery rate more than 85%. Moreover, a clinical validation with positive enumeration in all samples from patients with metastatic lung cancer were also reported. Sun et al. improved the spiral channels by using double spiral topology, which could enrich MCF-7 and HeLa tumor cells from whole blood (diluted) with a throughput of 3.33×10^7 cells per min. They claimed that double spiral topology provided better focusing behavior of small blood cells. Warkiani et al. reported a multiplexed microfluidics design, where the spiral channels were stacked vertically for ultra-high throughput CTC detection [140]. The clinical use of this chip was demonstrated by

detecting CTCs from 100% of blood samples collected from patients with advanced stage metastatic breast and lung cancers. The CTC recovery rate ranged from 20 to 135 CTCs per mL and obtained under high purity (of 1 CTC out of every 30-100 WBCs). Guan et al. introduced a novel spiral microchannel with a trapezoidal cross section. They reported that a higher separation efficiency compared to rectangular cross section. Later, Wu et al. used spiral channels with trapezoidal cross section to separate leukocytes from blood [141]. Larger blood cells focused near the inner wall, while smaller blood cells were trapped in Dean vortices and focused near the outer wall. Separation efficiency of $>80\%$ was reported. Later, isolating CTCs from blood samples of cancer patients was demonstrated by the same group using slanted spiral microchannels [142]. The device successfully isolated and recovered more than 80% of the tested cancer cell lines spiked in 7.5 ml blood within 8 min with high purity. In the initial clinical investigation, the trapezoid chip successfully isolated CTCs from 10 out of 10 (100%) patients with advanced stage metastatic breast and lung cancer (3–125 CTCs per ml).

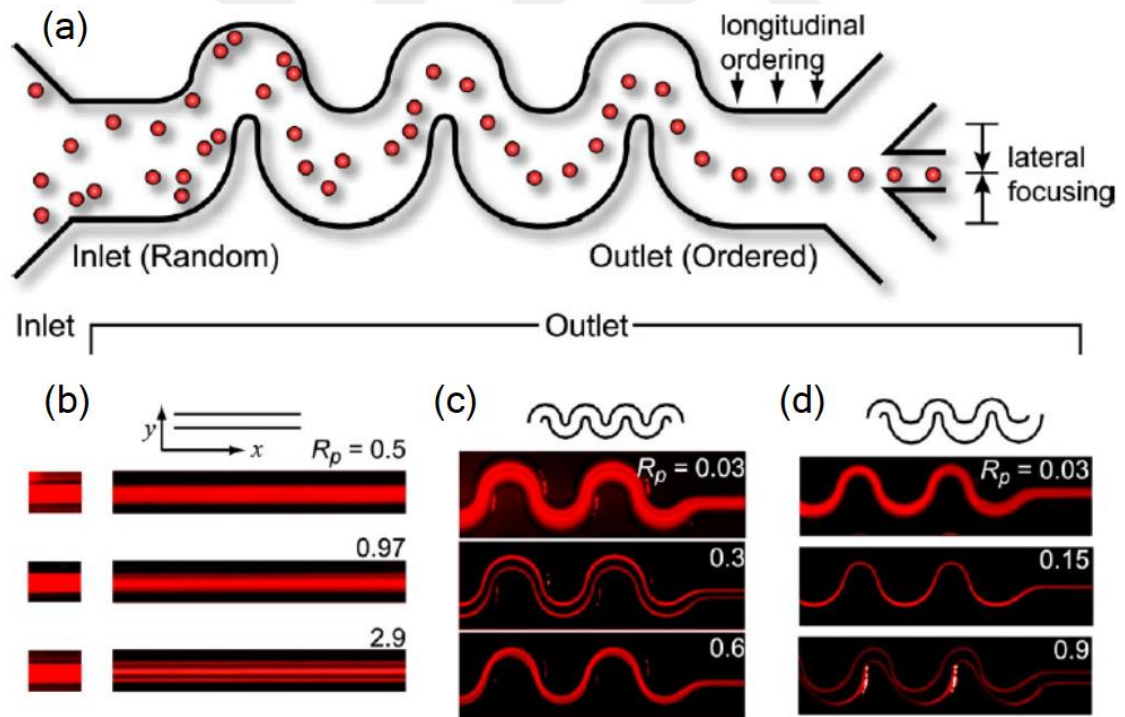


Figure 2.3: Inertial self-ordering. (a) Schematic drawing of the inertial ordering process. (b) Top-down views of fluorescent streak images of flowing $9\ \mu\text{m}$ -diameter particles in a square channel ($50\ \mu\text{m}$) filled with water (c) For a symmetric curving channel the symmetry of the system reduces focusing to two streams. Above a critical Dean number (De) focusing is perturbed. (d) For an asymmetric curving system, focusing down to a single stream is favored. Focusing is again more complex as De increases [115].

Not only spiral channels but also curvilinear serpentine channels were studied as curved channels. Di Carlo et al. carried out extensive particle focusing experiments using straight, symmetrical and asymmetric serpentine curved channels [115]. Experiments on asymmetric curved channels with particle diameters of 2-17 μm and channel hydraulic diameters of 10-87 μm were carried out with a range of Reynolds number of 0.1-225. Double focus of particles in symmetric channels, single focus of particles in asymmetric channels were observed (figure 2.4). This work was later developed with the design of asymmetric microchannels with 5 outlets, varying from 350 μm to 650 μm in width [143]. Separation of 3.1 μm and 9 μm particles was carried out at a flow rate of 0.9 ml / min and 90% of 9 μm particles could be collected on the outlet side of the channel. Gossett and Di Carlo experimentally studied the mechanism of particle focusing in a single turn in U shaped channels for $\text{Re} > 270$ and developed design criteria for separating by examining the particle focusing mechanism of the symmetric and asymmetric geometries [129]. Nguyen's group investigated particle focusing behavior in symmetrical serpentine channels. They observed three focusing patterns as single focusing line in the center, two focusing lines near the walls and transitional focusing [144].

2.3. Inertial Focusing in Straight Channels with Contraction and Expansion

In straight channels with contraction and expansion geometry, Dean-like secondary rotating flows take place within the expansion part. At the channel inlet, randomly distributed particles or cells experience wall induced lift force and shear gradient lift force and migrate to lateral equilibrium positions. When particles or cells reach the expansion region, the wall induced lift force is suddenly reduced, while shear gradient lift force still acts on them. Larger particles or cells are influenced by shear gradient lift force more than smaller particles and pushed away from the channel center to the channel walls in this region. Shear gradient lift force on smaller cells or particles is not sufficient to push away the smaller cells or particles, thus they follow the main flow [145].

Park et al. designed the microchannel series by adding 80 expansion and contraction geometry in the straight channels [146]. They investigated inertial focusing of particles with two sided multiorifice type micro channels in a series of expansion and contraction sections having vortex flows at the side wall corners of wide sections and making another

particle focusing position apart from that in straight channels. They observed that particles focused at two side positions, and particles focused at the centerline for Re_p of 3 to 3.5. Later, this structure was re-arranged, and the outlet zone was expanded [147]. Particles of $2\ \mu\text{m}$, $7\ \mu\text{m}$ and $15\ \mu\text{m}$ were separated at $Re = 70$, $15\ \mu\text{m}$ particles were focused near the channel center, and $7\ \mu\text{m}$ particles focused near the walls of the channel. Besides particle separation, this group also reported biological particle separation studies such as separation of blood plasma from red blood cells [148] and cancer cells from the blood [149]. A similar study was done earlier by Faivre et al. to separate blood plasma [150]. Wu et al. conducted separation experiments of *E. coli* bacteria from blood using kinetic inertia effect [151]. In this study, blood cells were influenced more by inertial forces due to their larger size and were focused on a different region relative to the equilibrium position of *E. coli* bacteria. Wu et al. were able to separate the bacteria with 99% purity. Warkiani et al. developed a detection method for malaria using inertial microfluidics and PCR detection techniques [152]. High aspect ratio channels coupled with pinched flow dynamics were utilized to isolate ring-stage malaria parasite from lysed blood containing white blood cells. In the high aspect ratio region, WBCs migrated and aligned into two streams near the channel walls, while malaria parasites remained unfocused.

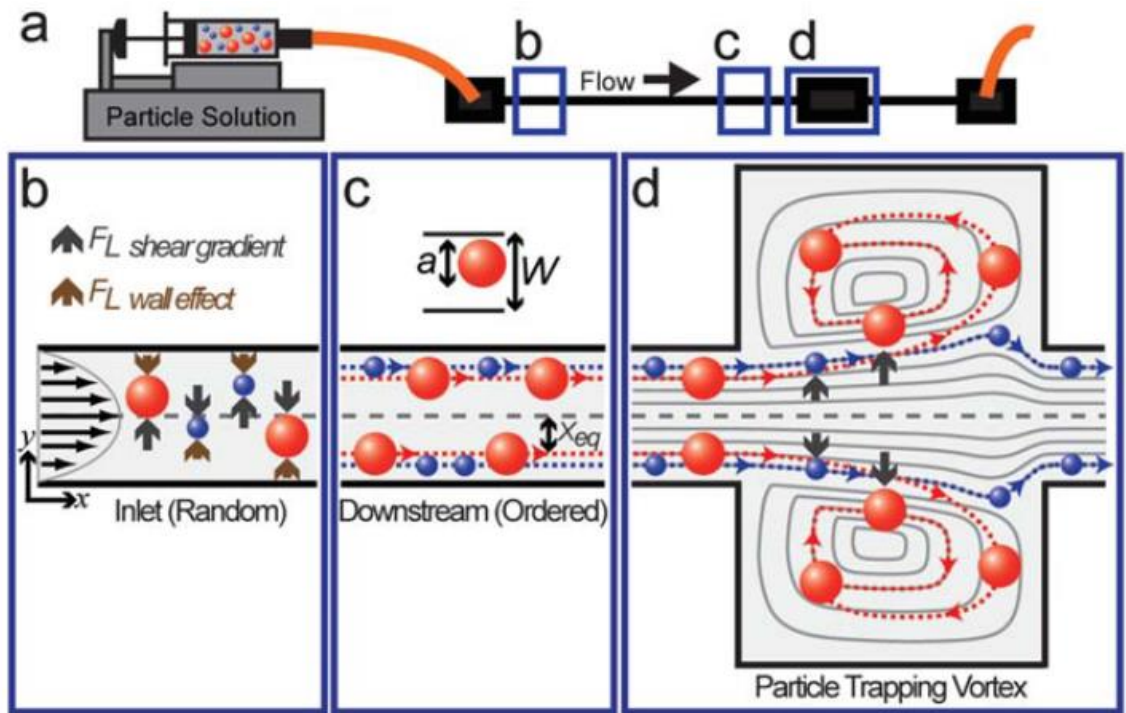


Figure 2.4: Particle trapping mechanism in laminar microvortices [153].

Recently, Di Carlo's group introduced a new approach called "centrifuge-on-a-chip" [153]. The centrifuge chip consisted of a straight channel with contraction and expansion geometry. The approach utilized the irreversible migration of particles into microscale vortices and selectively trapped larger particles in the expansion region (figure 2.4). The group conducted experiments with a variety of bio samples such as cancer cells spiked in blood [153], pleural fluids [154] and blood sample from cancer patients [145]. Wang and Papautsky demonstrated a microfluidics chip consisting of a straight channel and expansion-contraction geometry, where siphoning outlets were placed in the expansion regions [155]. They separated three components of microparticles with small differences in size based on inertial migration.



CHAPTER 3

3. FUNDAMENTALS OF INERTIAL MICROFLUIDICS

3.1. Shear Gradient Inertial Lift Force

The shear gradient inertial lift force occurs owing to the parabolic velocity profile of the resulting Poiseuille flow, which leads to the migration of particles/cells from the microchannel center towards the microchannel walls. A particle in such a flow with flow velocities relative to the particle velocity is demonstrated in figure 3.1. The magnitude of relative velocity of fluid is greater on the upper side of the particle than that on the lower side of the particle, causing a low pressure on the upper side. A shear gradient lift force is generated to push particle from centerline to channel walls until the wall induced lift force balances it [108,156]. In another words, particle moves through the regions where the difference of relative velocities on either side of the particle is minimized. Shear gradient lift force is given as

$$F_s = \frac{\rho U_{\max}^2 C_L a^3}{D_h} \quad (3.1)$$

where ρ is density of fluid, $U_{\max}$ is maximum velocity of fluid, C_L is lift coefficient, a is particle diameter and D_h is hydraulic diameter of the channel. As can be seen in the formula, shear gradient lift force strongly depends on position of particle, fluid velocity and particle diameter and is independent of particle rotation [121,127].

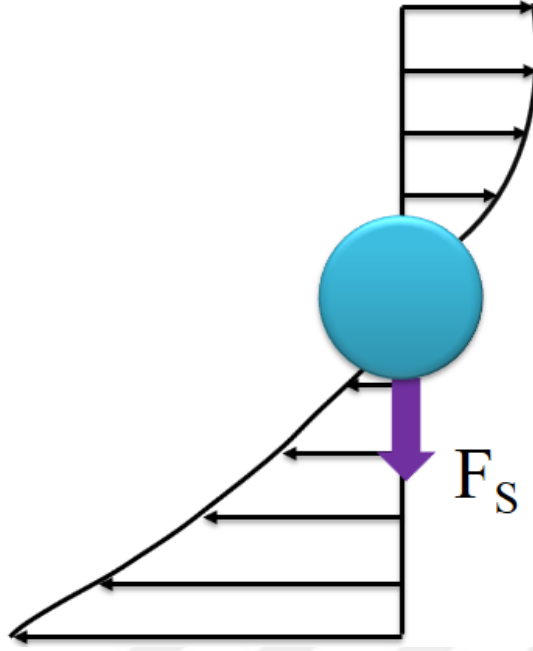


Figure 3.1: Shear gradient lift force.

3.2. Wall Induced Inertial Lift Force

Particles moving through a channel will experience the wall induced inertial lift force due to two major mechanisms [157,158]: a) when particles are close to the walls, the axisymmetry of the wake vorticity distribution, which is generated at the surface of the particle, is broken by the presence of the walls. As a result, an induced velocity emerges and further breaks the symmetry creating a wall induced lift force that pushes particle away from the wall. In addition, the presence of the walls causes the particle to move slightly slower than the fluid [125,127], b) because of the particle-flow interaction, flow streamlines are diverted toward the side of the particle, resulting in an acceleration on the relative fluid velocity on the upper side of the particle (figure 3.2), creating low pressure on this side. Therefore, a wall induced lift force pushes the particle away from the wall. It should be noted that this force decreases with the distance between particle and the wall. Wall induced lift force can be calculated as:

$$F_w = \frac{\rho U_{\max}^2 C_L a^6}{D_h^4} \quad (3.2)$$

where C_w is the lift coefficient that changes with Reynolds number and the position [121,127].

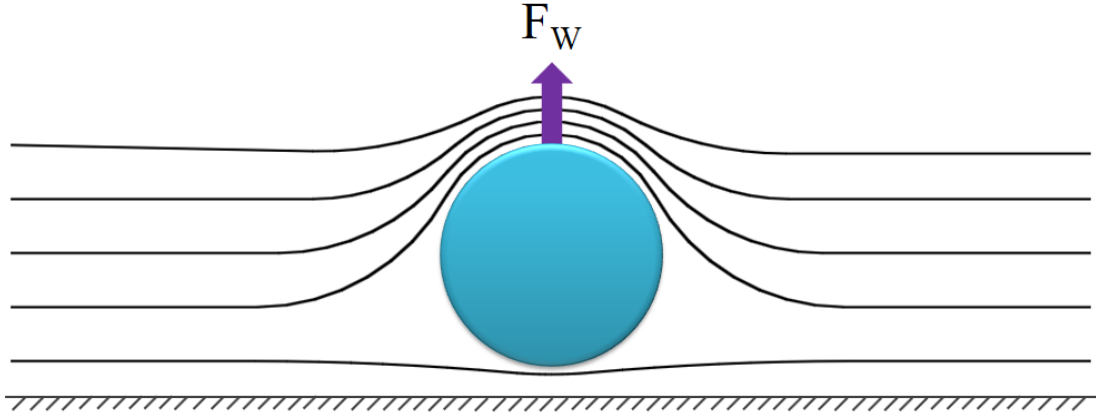


Figure 3.2: Wall induced lift force.

3.3. Net Inertial Lift Force

Inertial microfluidics was first reported by Segre and Silberberg in 1961[104,105]. Accordingly, it is known that uniformly dispersed particles formed a narrow band at a distance of $0.2D$ away from the channel walls in a circular cross section channel with a diameter D . Upon the introduction of the tubular pinch effect (TPE) in the literature [104,105], many studies were performed for better explaining particle focusing [107,119]. In a straight channel, an external force, inertial lift force having two components, acts on the particles. These two components are shear gradient inertial lift force (F_s) and wall induced lift force (F_w). These forces are orthogonal to the flow direction and depend on parameters such as particle position and channel Reynolds number. During migration of a particle through a microchannel, these forces act on the particle in opposite directions. The shear gradient component, which is a result of parabolic velocity profile in Poiseuille flows, pushes the particles from the channel centerline towards the walls. On the other hand, wall-induced component acts in the opposite direction and pushes from the walls towards the centerline. According to the tubular pinch effect, due to balance between these two components of inertial lift force, equilibrium positions for the particles can be attained.

Particle Reynolds number, Re_p , is defined as:

$$Re_p = Re_c \left(\frac{a_p}{D_h} \right)^2 \quad (3.3)$$

where Re_c is channel Reynolds number, a_p is particle diameter and D_h is channel hydraulic diameter. According to the literature, successful particle focusing can be achieved by minimum thresholds for ratio of particle diameter to channel hydraulic diameter, $\lambda = \frac{a_p}{D_h}$, and particle Reynolds number, which are $\lambda > 0.07$ and $Re_p > 1$, respectively [115].

The net lift force, F_L , acts on a particle in Poiseuille flow [119]:

$$F_L = \frac{4C_L \rho U_f^2 a_p^4}{D_h^2} \quad (3.4)$$

where C_L is the lift coefficient, ρ is fluid density, U_f is fluid mean velocity, and $\frac{U_f}{D_h}$ is the shear rate. The lift coefficient is a function of channel Reynolds number and the particle location and varies between 0.2 and 0.5 in microfluidic applications (when $Re_c < 100$, C_L can be approximated to a constant number, 0.5) [119,120]. Moreover, increasing Re_c results in a decrease in net lift force [114]. Further control of focusing positions can be accomplished by using curved channels [114,124].

3.4. Viscous Drag Force

Drag force arises from viscous nature of the fluid when a body moves through a fluid and acts as a resistive force. Stokes drag force is commonly used for microfluidics applications since the relative viscosity of fluid to particle is considerably small:

$$F_{drag} = 3\pi\mu a_p U_r \quad (3.5)$$

In inertial microfluidics, viscous drag force is composed of two components: along 1) the mainstream direction, due to the axial velocity difference between the fluid and the suspended particles (figure 3.3), and 2) the cross section, due to the secondary flow induced by the channel curvature or disturbance structures.

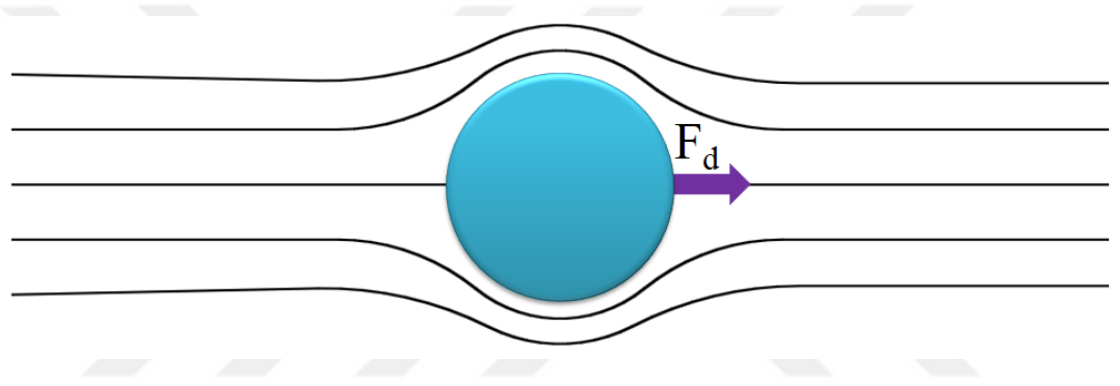


Figure 3.3: Viscous drag force.

3.5. Dean Drag Force

In fluid flows through a curved channel, there is a secondary flow due to difference of velocity between fluid in the centerline and near the walls, and subsequent larger inertia force in the centerline. Therefore, the fluid in the centerline tends to flow outwards and generates pressure gradient. Besides, due to this centrifugal pressure gradient, the fluid near the walls is prone to flow inwards. As a result of tendencies in the fluid, two symmetric vortices form (figure 3.4) [114]. The magnitude of these vortices can be quantified by a non-dimensional number, Dean number (De):

$$De = \frac{\rho U_f D_h}{\mu} \sqrt{\frac{D_h}{2R}} = Re_c \sqrt{\frac{D_h}{2R}} \quad (3.6)$$

where μ is the fluid viscosity and R is the channel curvature radius.

A particle flowing through a microchannel with a rectangular cross section experiences Dean drag force as well as shear lift force. According to Stokes drag law, the Dean drag force acting on a particle can be expressed as [128]:

$$F_D = 3\pi\mu U_{De} a_p \quad (3.7)$$

Where U_{De} is the average Dean velocity and is calculated as

$$U_{De} = 1.8 \times 10^{-4} De^{1.63} \quad (3.8)$$

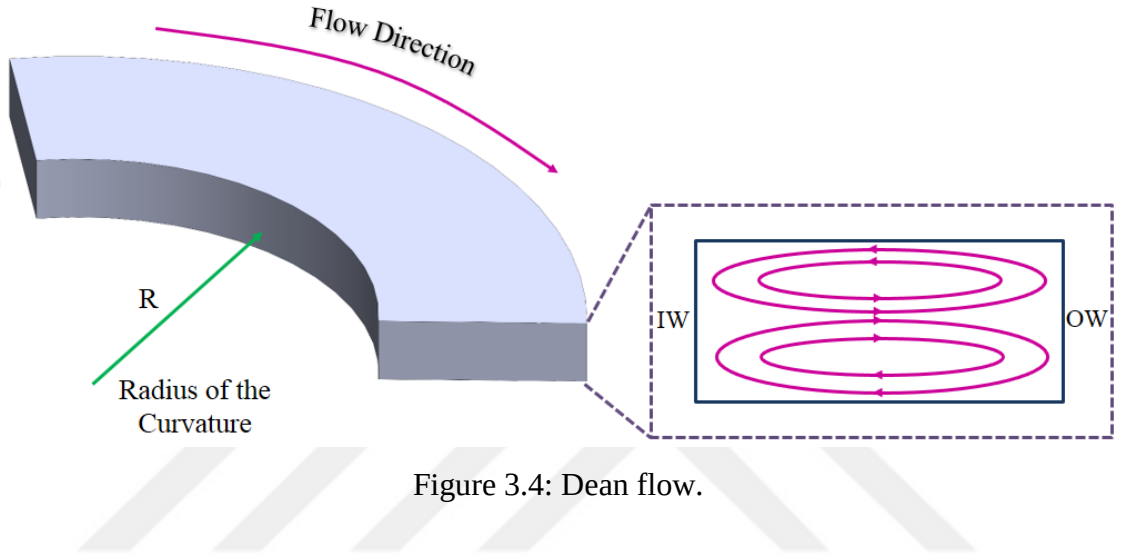


Figure 3.4: Dean flow.

3.6. Magnus Force

Figure 3.5 displays a stationary cylinder rotating with constant angular velocity Ω , in a flow with uniform velocity U . Fluid velocity at the bottom part of the cylinder is lower than the fluid velocity at the upper part, assuming no slip velocity at the surface of the cylinder. Accordingly, the pressure at the bottom part is higher than that at the upper part of the cylinder, creating a lift force to lift the cylinder. This lift force can be calculated for a cylinder as [159]:

$$\vec{F}_{LR} = \pi\rho^2 a \vec{U} \times \vec{\Omega} \quad (3.9)$$

Moreover, considering a rigid sphere rotating in a fluid, instead of a cylinder, this force is called Magnus Lift force and given as [160]:

$$\vec{F}_{LR} = \frac{1}{8} \pi \rho a^3 \vec{U} \times \vec{\Omega} \quad (3.10)$$

In the case of when sphere is not stationary, relative velocity $(\vec{u}_f - \vec{u}_p)$ should be replaced with U :

$$\vec{F}_{LR} = \frac{1}{8} \pi \rho a^3 (\vec{u}_f - \vec{u}_p) \times \vec{\Omega} \quad (3.11)$$

Flow streamline asymmetry due to the rotation of the sphere causes pressure difference which creates Magnus lift force. The direction of this force is perpendicular to the axis of rotation.

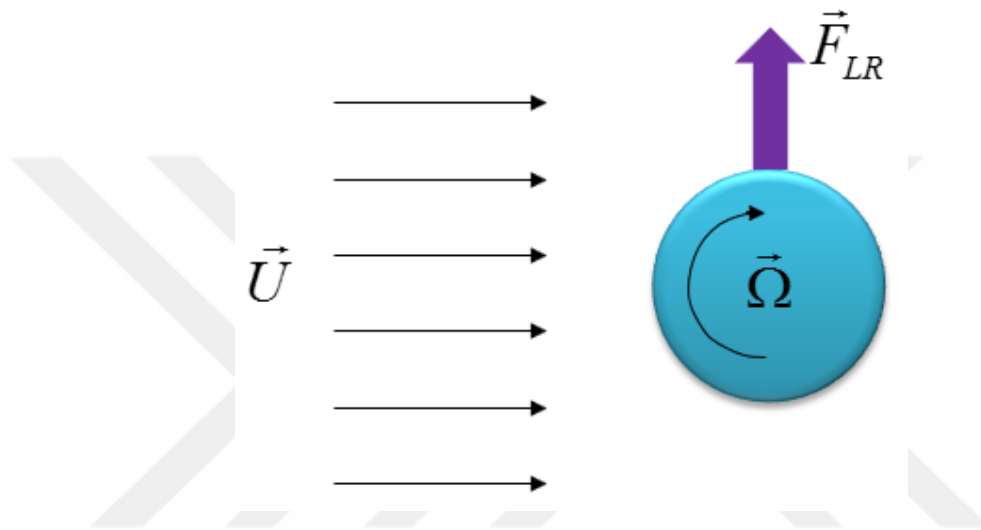


Figure 3.5: Magnus force.

3.7. Saffman Force

Particles flowing through a channel interact with channel walls unless the particle is so small or walls are far away from the particle [127]. The wall influences the particle in two different ways: a) due to the walls, the flow will not be uniform and fluid velocity gradient (shear rate) and accordingly shear-induced particle rotation occur. The extra drag makes the particle lag behind the fluid (figure 3.6a). A lateral force on particles called the Saffman force is generated by this slip-shear motion. b) the flow field around the particle is perturbed by the presence of the walls, when particle is close to the walls (wall-induced lift force). Saffman calculated the lateral lift force on a particle which arises from the interaction of the Stokeslet velocity field of the particle and the velocity gradient of the bulk flow [161]:

$$F_{Saf} = \frac{K}{4} V a^2 (\gamma \nu^{-1})^{1/2} \quad (3.12)$$

where K is a numerical constant (~ 81.2), V is the relative velocity between particle and the fluid, a is particle diameter, γ is the velocity gradient (shear rate), ν is the kinetic viscosity. It is worth mentioning that, this force is independent of the rotation of the particle. However, the particles can rotate by the influence of the shear in reality and a Magnus force can affect the particles [125]. The direction of Saffman force is towards the side where the relative velocity V is maximum (figure 3.6b). The Saffman force direction is through the stagnant walls when particles are lagging the flow, and through the moving walls, when particles are leading the flow.

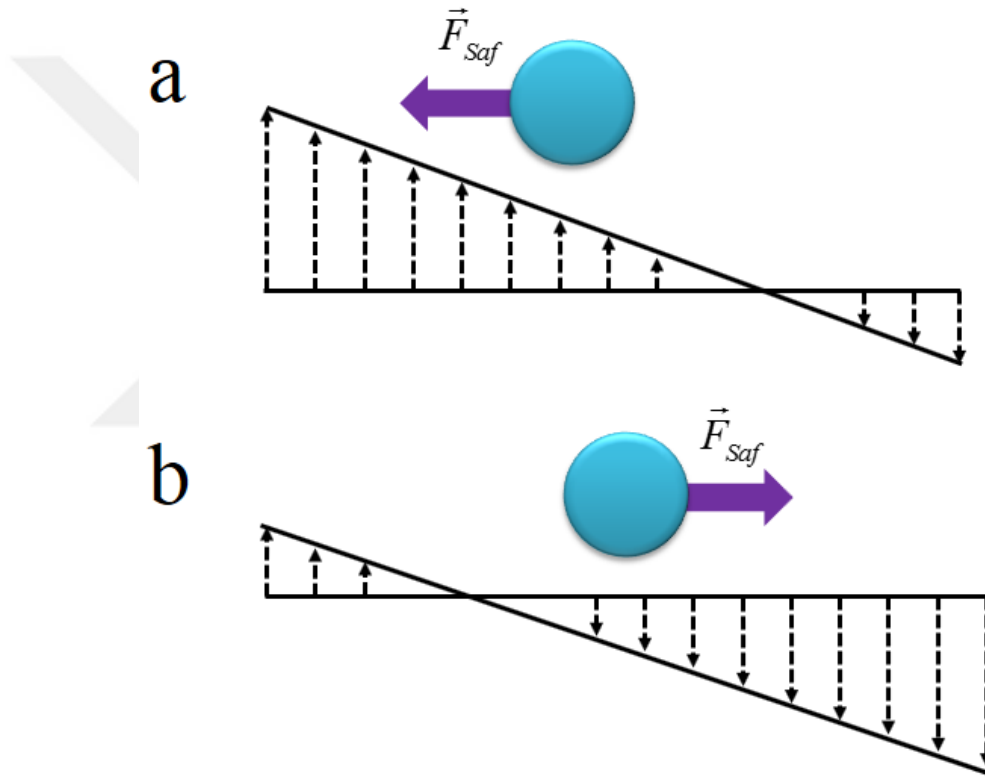


Figure 3.6: Saffman force.

In the case of a neutrally buoyant particle in a Poiseuille flow, the particle is force-free and the Stokeslet generated by the lag of the particle relative to the shear flow (slip-shear) is balanced by an opposite Stokeslet originating from the curvature of the base flow so that there is basically no net Stokeslet in the base flow; consequently, no Saffman force acts on the sphere [108]. The Saffman force is more relevant in the case of non-neutrally buoyant particles in a vertical flow, or an additional outer force (electrical or magnetic) [124,162] acting on the particle to lag or lead the fluid flow, creating an obvious net

Stokeslet flow field. In this situation, the Saffman force will direct to the channel centerline when particles lag the flow, or direct toward the channel walls when particles lead the flow.

3.8. Deformability Induced Lift Force

When bio-particles such as cells are used instead of rigid particles, additional lift forces arise on the deformable particles. The velocities and stresses are not the same along the interface of the deformable particle, and particles change their shape to match the velocities and stresses [124]. Hence, the deformability induced lift force, which is perpendicular to the main flow, arises. Characterization of the relative deformation of a droplet can be done by using three dimensionless parameters:

a) Weber Number:

$$We = \frac{\rho_f U^2 a}{\sigma} \quad (3.13)$$

where σ is surface tension,

b) Capillary Number:

$$Ca = \frac{\mu U a}{\sigma h} \quad (3.14)$$

and

c) Viscosity Ratio:

$$\lambda_d = \frac{\mu_d}{\mu} \quad (3.15)$$

where μ_d is the dynamic viscosity of fluid inside the droplet

Chan and Leal derived an analytical expression for deformability-induced lift force, given the condition that the drop or bubble is not too close to the walls [163,164]:

$$F_{L,deformation} = \mu U a \left(\frac{a}{H} \right)^2 \frac{d}{H} f(\lambda_d) \quad (3.16)$$

$$f(\lambda_d) = \frac{16\pi}{(\lambda_d + 1)^3} \left[\frac{11\lambda_d + 10}{140} (3\lambda_d^3 - \lambda_d + 8) + \frac{3}{14} \frac{19\lambda_d + 16}{3\lambda_d + 2} (2\lambda_d^2 - \lambda_d - 1) \right] \quad (3.17)$$

where d is the distance between the drop and the center of the channel.

Stan et al. introduced an empirical formula for both inertial lift forces and drop deformation are small [164]:

$$F_{L,empirical} = C_L \mu U a \left(\frac{a}{H} \right)^3 \left(\frac{d}{H} \right) \quad (3.18)$$

Lift coefficient C_L is determined from experimental measurements.

It should be considered that large portion of cells in real systems are non-spherical. Particle's symmetric or asymmetric shape has an impact on the lifting forces acting on the particle. In recent studies, Hur et al. and Masaeli et al. show that above a critical Reynolds number ($Re \sim 50$) particles predominantly adopted preferred equilibrium positions and “tumbling” rotational axis [165,166]. This rotational axis focusing position was found to be independent of cross-sectional shape and mainly depends on rotational diameter. During the tumbling motion, when the major axis rotates perpendicular to the wall, wall effect lift increases substantially due to the closer distance. As a result, the combined force on these particles is higher than that of spheres of the same volume. Moreover, Hur et al. experimentally demonstrated that in Poiseuille flow the direction of deformability-induced lift force is towards the center [166]. Meaning that, as the deformability induced lift force increases, cells shift towards the channel center. Counterintuitively, as the viscosity ratio decreases, the focusing position shifts back towards to the wall. Therefore, the velocity of migration of the cell stream is also affected by deformability since the overall wall directed lift force magnitude gets smaller.

CHAPTER 4

4. EXPERIMENTAL METHODS

4.1. Fabrication of Microfluidic Devices

PDMS (polydimethylsiloxane) (Sylgard 184, Dow Corning) microchannel devices were fabricated using the standard soft lithography microfabrication technique. Single-side polished 3" silicon wafers were coated with SU-8 3050 photoresist (Microchem Corp.) by means of a spinner (Dorutek) and exposed to UV light via a Mask Aligner UV-Lithography device (Midas System Co., Ltd., MDA-60MS Mask Aligner 4"). The image reversal acetate masks used in the lithography process were produced by a printer with 10,000 DPI (by CAD/Art Services, Inc.). The unexposed area was then developed using SU-8 Developer (Microchem Corp.). PDMS prepolymer and curing agent (Sylgard 184 silicone elastomer kit, Dow Corning) were mixed at a 10:1 (weight:weight) ratio in a plastic weighing dish and cast over the silicon master contained in a glass petri dish. The prepolymer mixture in the master was placed in a vacuum oven (Sheldon Manufacturing, Inc.) and degassed at low pressure (76 mmTorr) for 1 h to remove air bubbles and then cured for 12 h at 75°C. Following curing, solid PDMS was peeled off from the master and cut with a scalpel. Inlet and outlet holes were formed using a 2 mm biopsy puncher. As a pre-process of bonding, microscopic glass slides were cleaned with isopropanol alcohol and deionized (DI) water along with PDMS molds and then dried with N₂ gas. After cleaning, both the PDMS channels and glass slide were placed in an Oxygen Plasma Device (Harrick Plasma Cleaner) and activated for 60 s. Thereafter, the PDMS bond surface was immediately brought into contact with the glass slide to create the enclosed form of the channel and was baked for 15 min at 75°C on a hotplate (Dorutek) to strengthen the bonds.

4.2. Preparation of Particle Suspensions

In our focusing experiments, low particle concentration was preferred (to avoid particle-particle interaction). For this purpose, polystyrene fluorescent microparticles with diameter of 10 μm (Invitrogen), 15 μm (Invitrogen) and 20 μm (Phosphorex) were employed. Dilution process was performed by fixing 1% weight fraction in deionized (DI) water to have the final concentration of less than 0.01 wt.%. Mixtures of two microparticles of different sizes, namely 20 μm and 15 μm , 20 μm and 10 μm , 15 μm and 10 μm , were utilized for separation and enrichment of this study.

4.3. Cell Culture

Human breast adenocarcinoma cell line MDA-MB-231 (20-24 μm), human T lymphocyte peripheral blood acute T cell leukemia cell line Jurkat (11-14 μm) and human bone marrow chronic myelogenous leukemia cell line K562 (16-20 μm) were used to mimic CTCs separation in this thesis. MDA-MB-231 cells were maintained in DMEM high glucose medium (PAN Biotech, P04-03500); Jurkat and K562 cells were maintained in Roswell Park Memorial Institute medium RPMI 1640 (Gibco, 31870-025) in a 5% CO_2 -humidified incubator at 37 $^{\circ}\text{C}$. Culture media were supplemented with 10% heat-inactivated fetal bovine serum (Biowest, S1810-500), antibiotics (50 units/ml penicillin and 50 g/ml streptomycin; Gibco, 15140-122), and L-glutamine (2 mM, Gibco, 25030-024). Additionally, MDA-MB-231 media was supplemented with 1X non-essential amino acids MEM-NEAA solution (Gibco, 11140035).

The cells were splitted every 2 days. Since MDA-MB-231 is an adherent cell line Trypsin EDTA Solution A, 0.25%-0.02% (Biological Industries, BI03-050-1A) was used to detach cells. 1/10 of the detached cells were inoculated in fresh media. Jurkat and K562 are suspension cell lines. Therefore, 1/10 of their total culture were directly inoculated to fresh media without trypsin treatment.

4.4. Operational Procedure

Particle suspensions were introduced to a 50 mL plastic syringe and injected into the microfluidic device at flow rates ranging from 400 to 2700 $\mu\text{L}/\text{min}$ using a syringe pump (Harvard Apparatus PHD 2000) for each experiment. The flow rate was raised by 100 $\mu\text{L}/\text{min}$ every 90 s. The experiments were repeated for three times. Connections between syringe/collection tubes and the microfluidic device were made using TYGON tubing (IDEX Corp., IL) (internal diameter: 250 μm , length: 150 mm) and the corresponding fittings (IDEX Corp., IL). The microfluidics chips were integrated to an inverted phase contrast microscope (Olympus IX72) equipped with a (12-bit) charge coupled with a device camera (Olympus DP 72) and mercury lamp (Olympus U-LH100HG).

The cells were fluorescently stained by using DILC18(3) (Life Technologies, D3911). First, 2.5 mg/ml stock dye solution was prepared in Dimethyl Sulfoxide (DMSO) (Santa Cruz, cs-358801). The cells were re-suspended in PBS (Gibco, 20012-019) containing 3% FBS in 1 000 000 cells/ml concentration. Stock dye was added in ratio of 5 $\mu\text{L}/\text{mL}$ of the cell suspension. MDA-MB-231 and K562 cell lines incubated with the dye for 20 min while Jurkat cell line was incubated for 5 min at 37 $^{\circ}\text{C}$. Then the cells washed with 1 mL PBS at least for 10 times until the supernatant became completely clear. The cells were re-suspended in PBS containing 3% FBS in 150 000 cells/ml concentration. Finally, whole suspension was filtered through 70 μm cell strainer (Falcon, 352350) prior to its injection to the chip system.

4.5. Image Acquisition

Videos were recorded for each flow rate with 600 ms exposure time, for the fluorescent imaging. Post processing of the fluorescent imaging were done using the Olympus (Olympus VS120-S5) and ImageJH software. Composite stacks of discrete frames were obtained with the ImageJH software. The plotting line profile module of the ImageJH software was used across the cross section of channels to measure the migration length and focusing width from A-A' line for transition region and from B-B' line for outlet region. Each line scan was then implemented in the OriginPro-2015 Software to achieve a representative column of pixels in the Re_c maps. A sample of exporting the focusing

position and determination of the focusing width from the intensity graph is given in figure 4.1. Channel Reynolds maps are exported by employing intensity graphs at flow rates varying between 400 $\mu\text{L}/\text{min}$ and 2700 $\mu\text{L}/\text{min}$ frame by frame on A-A' and B-B' cross sections for outlet and transition regions, respectively. These intensity graphs are used to obtain the counter graphs, which give related pixel maps. Phantom V310 high speed camera is was also utilized to record the videos at frame rates of up to 15000 fps and a resolution of 512 $\times$ 384 pixels. The high-speed camera was connected to a PC for storing the videos, post-processing of the images was performed with the Phantom PCC and ImageJ software. The particle velocity was measured using the Phantom PCC 2.7 software.

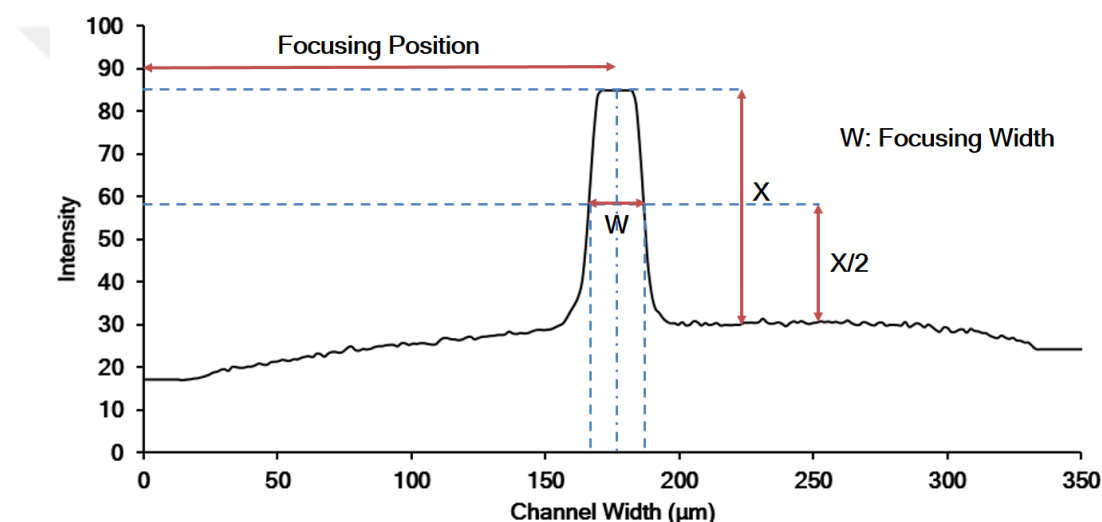


Figure 4.1: Determination of the focusing position and focusing width from fluorescent intensity graph at $Re_c = 144$. (X: maximum intensity, W: Focusing width)

For each flow rate, separate videos from transition and outlet regions were captured and a set of 50 images was overlaid using the ImageJH software to quantify the exact focusing position and width. The plotting line profile module was used across the channel width in the outlet and transition positions to prepare the fluorescent intensity graph. Important parameters and a sample are shown in figure 4.1.

4.6. Simulation

The COMSOL Multiphysics 4.2 software program (COMSOL Inc., Burlington, MA) was utilized to analyze fluid flow at multiple cross sections of the curved microchannels. The dimensions of the 3D model were determined based on the microchannel dimensions used in the experimental study shown in figure 5.1. A 3D Cartesian coordinate system (x, y, z) with the origin located at the cross sectional center of the microchannel structure inlet was used. Full Navier–Stokes equations were solved using single phase and incompressible flow assumptions via the laminar flow model. For an incompressible and steady laminar flow, the governing Navier–Stokes equations can be expressed as

$$\rho(u \cdot \nabla)u = \nabla \cdot [-pI + \mu(\nabla u + (\nabla u)^T)]. \quad (4.1)$$

Various inlet velocities corresponding to Reynolds numbers 1–100 were considered at the inlet of the microchannels. A no-slip velocity condition on the walls and zero pressure at the outlet were chosen as the boundary conditions. Consequently, from the steady-state solution of the laminar flow, maximum Dean flow speeds and axial flow speeds were deduced, and Dean vortices were obtained using the velocity fields at different cross sections. The data obtained from the simulations match with the experimental observations and bolster the experimental results.

CHAPTER 5

5. INERTIAL PARTICLE FOCUSING IN CURVILINEAR CHANNELS WITH DIFFERENT CURVATURE ANGLES

5.1. Introduction

Microfluidic technologies have many advantages over conventional technologies such as the requirement for a small sample volume, low-cost production, higher sensitivity and improved performance [125,167]. They take advantage of the fact that the flow characteristics at the microscale may be appealingly different from those at the macroscale such that the dominant forces in microfluidics may become negligible at the macroscale [168]. Furthermore, microfluidic technologies facilitate the fabrication of integrative, portable point-of-care (POC) diagnostic devices based on lab-on-a-chip or micro-total-analysis-systems (μ TAS) [169]. These devices contribute great benefit to biomedical research in the detection, sorting, separation and analysis of cells, especially circulating tumor cells (CTCs) to provide effective diagnosis and therapy [170–172].

Circulating tumor cells (CTCs) are rare cancer cells which originate from the primary tumors and interfered to bloodstream. Isolation of CTCs from blood is critical owing to the fact that metastatic CTCs may hold different genomic and phenotypic properties which may provide insights for prognosis and effective treatment. Focusing biological particles and cells using microfluidic systems have been implemented as an efficient CTCs enumeration and enrichment method for clinical diagnostics applications [173,174]. Focusing particles and cells into a narrow stream is a requirement for these emerging applications and for understanding the underlying physics of particle/cell focusing in microfluidics [175].

A variety of fundamental focusing and separation approaches have been studied with synthetic microparticles in the framework of microsystems [21,176,177]. From the microfluidics point of view, separation/isolation principles are divided into two categories

depending on the external energy usage: active and passive separation. Whereas active techniques require external forces such as magnetic [178], dielectric [179] and acoustic [180] forces to separate particles/cells, passive techniques mainly utilize hydrodynamic forces [18]. Passive techniques can be further separated into filtration-based, deterministic lateral-displacement-based and inertia-based techniques [171]. Noticeably, active techniques provide more accurate results yet are limited by their low throughput, integration of complex components and expensive production or process requirements. Several recent reviews on microfluidics particle/cell focusing and isolation have enhanced our understanding of separation characteristics and physics [18,175–177,181]. Among these techniques, inertial focusing has gained significant attention because it offers high throughput and effective and precise control for particle and cell manipulation. Despite being an actively studied topic, inertial particle focusing behavior and its underlying mechanisms are not yet fully understood. Different channel types, such as straight [117,118], serpentine [115,129,143,144], spiral [131,136–138] and straight with contraction–expansion arrays [146,147,151], are used in inertial microfluidics, however the separation of particles with a serpentine microchannel has not attracted as much attention as the other types. In serpentine channels, secondary flow directions vary with a sudden change in the channel curvature. As a result, steady state secondary flows cannot be precisely assessed. Recently, the highest efficiency was found as 95% by the Nguyen's group [144]. However, the throughput was not as much as that in spiral channels.

The Dean drag force, is introduced by using a curvilinear channel geometry. The effect of this curvilinear geometry emerges with the formation of two counter-rotating vortices, Dean vortices, which exert a drag force on the particles. This force is directed outwards near the channel center and inwards near the upper and lower walls. Whereas the radial circulation of these Dean vortices is directed towards the outer wall at the midline, while it is directed towards the inner wall at the top and bottom regions of the channel.

In contrast to the studies on inertial microfluidics in the literature, the effect of curvilinearity with a high curvature angle (280°) on particle focusing behaviour is examined in this study by performing inertial focusing of 10 μm , 15 μm and 20 μm fluorescent polystyrene microparticles at different channel Reynolds numbers. Furthermore, the decoupling effect of inertial and Dean drag forces on particles and separation potential are revealed. Because the forces acting on the particles vary depending on their location, the concomitant effect remains unknown. This dissertation

has the potential to provide a valuable contribution to the field of inertial microfluidics by extensively improving our understanding of three-dimensional particle dynamics in curvilinear channels.

5.2. Results and Discussions

The PDMS microfluidics chip used in this study consists of one microchannel design that includes two inlets and three outlets with diameters of 1 mm each. The height and width of the microchannel are 91 μm and 350 μm , respectively. The whole chip comprises 11 curvilinear geometries, each having a curvature inner radius of 800 μm and a curvature angle of 280° . The total chip length is approximately 4.3 cm. A schematic of the chip is shown in figure 5.1.

Our experimental observations demonstrate the need for the analysis to be focused on two specific channel regions: the transition region, figure 5.1c(i), and the outlet region, figure 5.1c(ii). Owing to the sudden rotational change in the transition region, the outer wall becomes the inner wall and vice versa. Thus, to prevent confusion, as illustrated in figure 5.1c, it is best to define the wall between A and B as wall W_1 and the wall between A' and B' as wall W_2 .

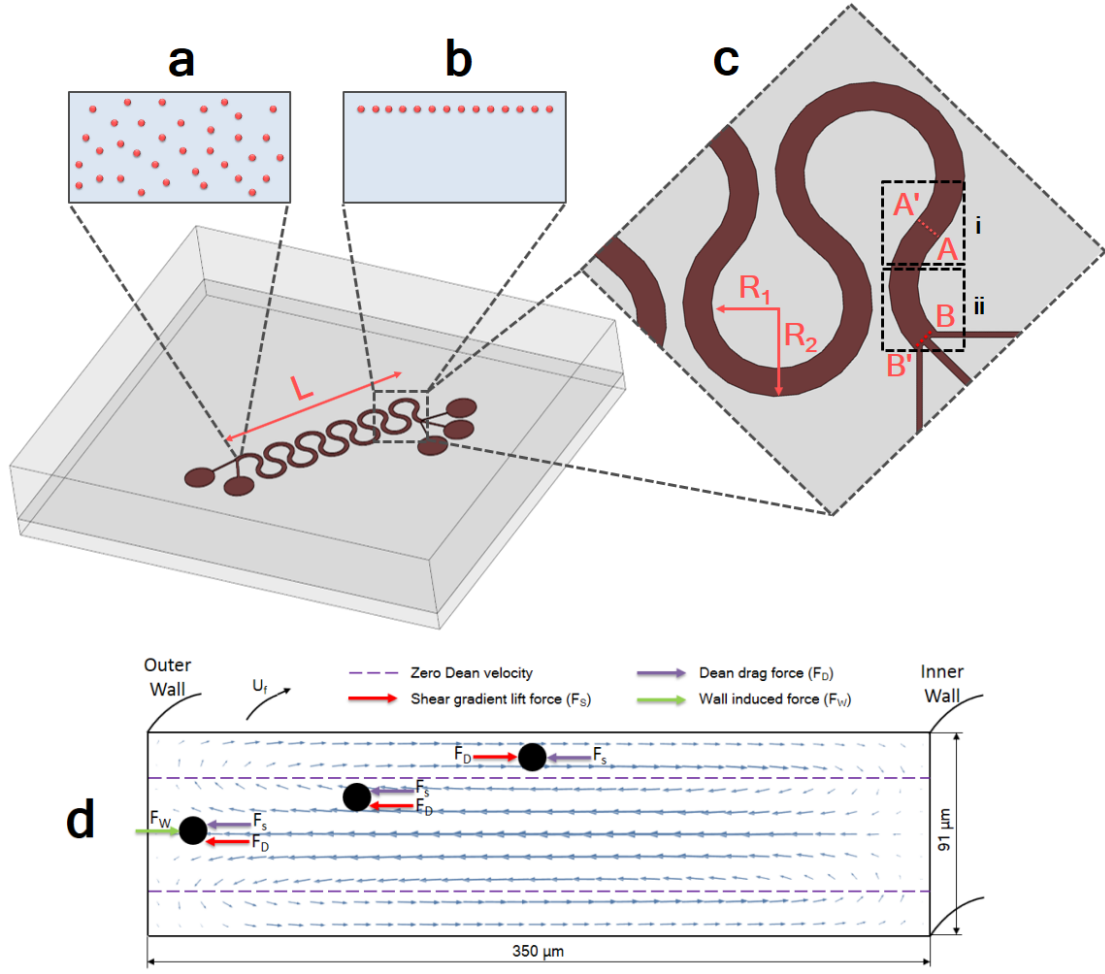


Figure 5.1: Schematic of the particle focusing device. The randomly dispensed particles in the inlet region (a) focus into a single particle stream at the AA' cross section (at the transition region) (b). (c) A close-up view of the channel transition region (i) and the outlet region (ii). ($R_1=800 \mu\text{m}$ and $R_2=1150 \mu\text{m}$ and $L=9.75 \text{ mm}$) d) A force diagram depicting the direction of forces acting on the particle of various locations.

To investigate the effects of curvature on microparticle focusing of microparticles of different sizes, sets of experiments were performed for three different sizes (20 μm (large), 15 μm (medium) and 10 μm (small)) at a wide range of flow rates varying between 400 $\mu\text{L}/\text{min}$ and 2700 $\mu\text{L}/\text{min}$ and corresponding to Reynolds numbers between 30 and 205. Figure 5.2 presents the particle streak positions and focusing width data in the outlet and transition regions. In the figures related to both the transition and outlet regions, the bottom and top of each figure represent walls W_2 and W_1 , respectively.

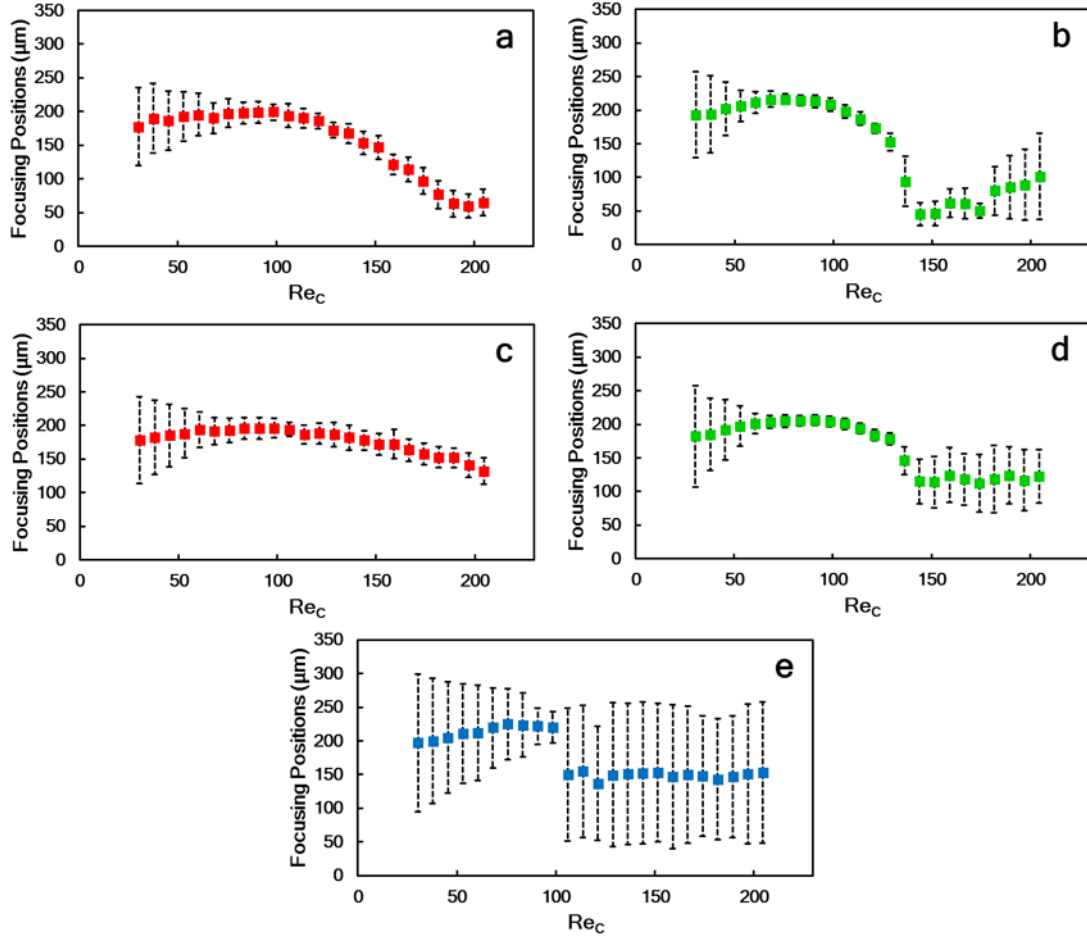


Figure 5.2: Focusing positions of microparticles of three different sizes for the curved microchannel with varying flow rates and corresponding channel Reynolds numbers in the two aforementioned regions: the transition region for particles of sizes a) 20 μm (red), b) 15 μm (green), and the outlet region for c) 20 μm (red) and d) 15 μm (green) e) 10 μm (blue) particles. Plotted error bars indicate the width of the focusing streak.

Initially, at the lowest Re_C ($Re_C = 30$), both large ($a_p = 20 \mu m$; $\lambda = 0.139$) and medium ($a_p = 15 \mu m$; $\lambda = 0.1$) particles pass along the channel by occupying the center of the channel as a wide band, and have a focusing width more than four times the diameter of a single particle. With an increase in Re_C (to 76) for large particles and (to 60) for medium particles, the focusing width decreased to a single streak with a thickness of approximately less than twice the diameter of a single particle. No considerable change is observed in the focusing width of large particles for both transition and outlet regions with a further increase up to the highest value ($Re_C = 205$), (figure 5.2a, figure 5.2c). Medium particles start defocusing in the outlet region, when $Re_C > 136$ (figure 5.2d). Because of the bypassing movement of medium particles, a more sophisticated results

can be seen in figure 5.2b in the transition region by increasing the Re_C numbers. A sudden increase in the focusing width is seen at $Re_C = 136$, while a refocusing trend is observed with a focusing width of almost twice the diameter of a single particle (at $Re_C = 144$) a refocusing behavior with focusing. With a further increased in Re_C ($Re_C > 182$), focusing is disturbed by the dominance of the Dean drag force.

The focusing width of small particles ($a_p = 10 \mu m$; $\lambda = 0.07$) is observed to be in uniform alignment throughout the microchannel at low Reynolds numbers and tends to decrease in size as the Re_C increases to 98. At this Re_C number, the focusing width is almost four times greater than the diameter of a single particle. However, a further increase in the Re_C results in a defocusing trend of the small particles in the outlet region.

Notwithstanding that the focusing position of large particles is very close to the centerline of the microchannel for all Re_C at the outer region, this trend is disturbed, and the particle stream approaches W_2 at the transition region when the Re_C is greater than 98. Care must be taken when interpreting these results because the large particles are observed to be in the equilibrium position near the centerline, owing to the directional magnitude balance between the shear gradient lift and Dean drag forces; however, when the $Re_C > 98$, particles tend to pass through the outer wall upstream and downstream of the transition region. Thus, the particles approach the centerline at the outlet, where the data are taken (B-B' cross section).

The tightly focused stream of the medium particles initially forms close to the centerline, slightly on the W_1 side of the channel in both the transition and outlet regions, when the Re_C is 61. Increasing the Re_C causes the particle equilibrium position to shift through the W_2 side up to $Re_C \sim 136$. However, at this Reynolds number ($Re_C \sim 136$), a sudden increase in the focusing width is observed in both regions between the centerline and the W_2 region. Although this defocusing observation continues up to the highest Re_C in the outlet region, interestingly, a tightly focused streamline is regenerated near the W_2 for a Re_C of 144 in the transition region.

At the lowest Reynolds number, the focusing width of small particles is greater than that of large and medium particles. With an increase in Re_C number to 98, a decreasing trend

can be seen in the focusing width. A sudden defocusing behavior is observed with a further increase in Re_C , (Fig. 5.2e). Prior to this defocusing behavior, the wide particle streak starts to approach wall W_1 with an increasing Re_C . However, this streak is then disrupted by the wall W_2 , which suggests that there is a rotation to the opposite direction of one of the dominant forces, thereby balancing the other dominant forces.

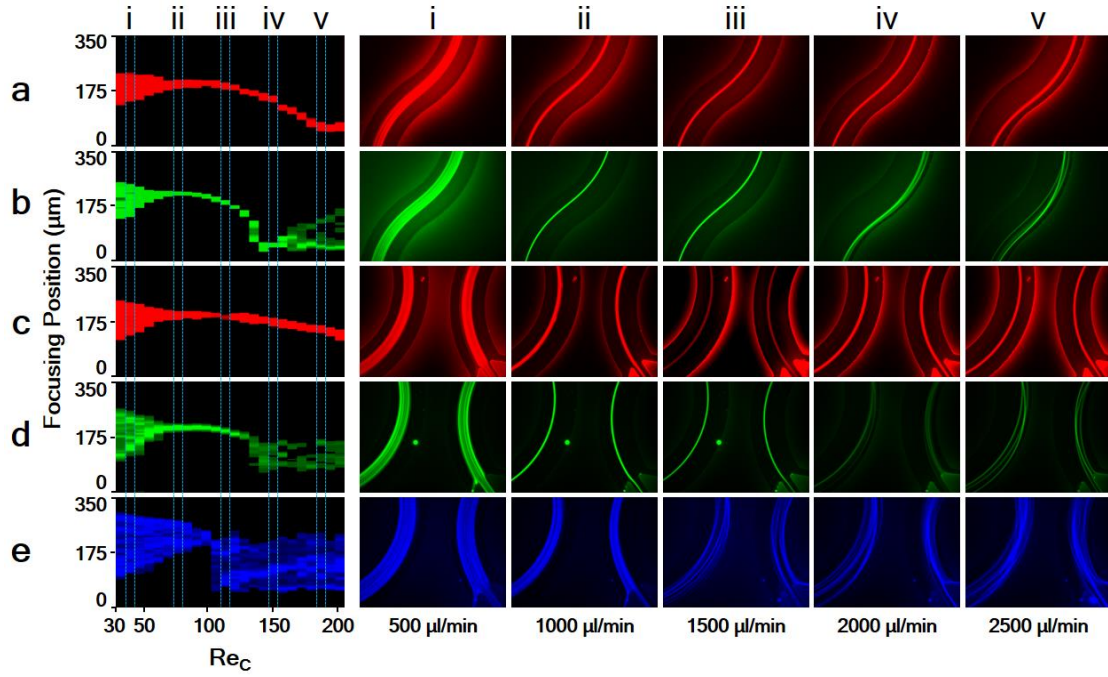


Figure 5.3: Re_C maps: at the transition region for a) 20 μm (red) and b) 15 μm (green) particles and at the outlet region c) 20 μm (red), d) 15 μm (green) and e) 10 μm (blue) particles. Particle streams for different channel Reynolds numbers corresponding to five selected flow rates between 500 and 2500 $\mu\text{L}/\text{min}$ (i–v).

The Re_C maps and representative row images provided in figure 5.3 present a better understanding of particle focusing behavior over a wide range of channel Reynolds numbers. These maps are obtained by taking the intensity line graphs, where the line is on the A-A' cross-section for the transition region and on the B-B' cross-section for the outlet region at different flow rates. Following that, the counter graph, which gives pixel maps corresponding to the intensity line graph data, is obtained. Re_C maps for both the transition and outlet regions are constructed frame by frame with the bottom and top edges of each graph representing walls W_2 and W_1 , respectively.

Large particles initially form a wide particle streak band near the centerline of the channel with a low Re_C ($Re_C \sim 38$), figure 5.3a(i), figure 5.3c(i). At this stage, owing to low flow velocity, the particle motion behavior is slightly influenced by the Dean drag and inertial lift forces. Increasing the Re_C ($Re_C \sim 75$) results in the formation of a single stable particle stream near the centerline (falling on the W_1 side) along the microchannels. Owing to an increase in the shear gradient lift force, this stream migrates to wall W_2 in the transition region. After a further increase in the Re_C , a transverse motion of focusing particles is observed. Particles moving into the transition region do not migrate along the essential flow stream but instead migrate perpendicular to this stream as they move closer to the outer wall rather than remaining at the channel centerline. Although this position is preserved at the transition region, and the particle stream remains close to W_2 , the Dean vortices directions change with the sudden switch of the channel curvature, and, thus, the particle location is disturbed as they move to the centerline from W_2 in the outlet region. The higher the flow rate becomes, the closer the particles move to W_2 in the transition region.

Similar to large particles, medium particles are initially focused near the centerline at a low Re_C and form an equilibrium line that is closer to W_1 than that of large particles. However, this streak does not migrate as it does for large particles. Instead, sudden defocusing behavior is observed at $Re_C \sim 136$; see Fig. 3b. Following this transition behavior, particles are refocused near W_2 at $Re_C \sim 144$. When the Re_C is further increased, a second defocusing effect can be observed in figure 5.3b(v) and figure 5.3d(v) owing to the dominant Dean drag force.

During all experiments, no effective focusing line was observed for small particles. However, the bypassing movement was more dominant. This expected result agrees with the previous studies mentioned above, which report a minimum threshold of $\lambda > 0.07$ for inertial focusing behavior to occur [115]. Even so, achieving a focusing width of almost four times the diameter of a single particle using small particles suggests the possibility of focusing small particles with precise geometries.

One of the main implications of this study is to establish the cruciality of the velocity profile shift with each turn and the effect of this change on the 3D position of the particle and the direction and magnitude of dominant forces acting on the particles. Previous simulation studies have shown that Dean vortices preserve a reasonably constant pattern

and direction throughout the applied flow rates in spiral channels [182]. However, because of sudden turns in this case, the direction changes to the opposite direction rather than following a constant pattern. From a cross-sectional perspective, these two counter-rotating vortices follow a pathway that is parallel to the z-axis from the inner wall to the outer wall at the mid-section of the channel, whereas this pathway is in the opposite direction near the top and bottom walls as observed from planes 1 and 5 in figure 5.5b. The vertical position of the particles is predominantly influenced by the vertical component of the inertial lift force.

For the majority of the spiral geometry studies, the stable equilibrium line is observed close to inner wall [128]. Accordingly, the Dean drag force acts in the same direction as the net lift force near the outer wall, thereby causing particles to continue migrating along the circulation route of the two symmetric counter-rotating vortices. However, near the inner wall, the Dean drag force acts in the opposite direction, resulting in one stable equilibrium position of particles inside the microchannel. It should be noted that for this case, the particle's vertical position must be close to the channel's vertical center because the direction of the Dean vortices is towards the channel outer wall near the channel's vertical center. In addition, the findings of Martin et al. on the effect of curvature ratios suggest a decrease in the shear gradient near the centerline as the velocity profile shifts due to an increase in curvature [130]. Because of this decrease, the particle's vertical position moves closer to the center on the inner half of the microchannel, resulting in the formation of an equilibrium position near the inner wall in spiral channels. Nevertheless, the case becomes more sophisticated for alternating curvilinear geometry because secondary flow directions vary with the sudden change in the channel curvature. Therefore, emerging steady state secondary flow conditions are not predictable precisely [124,125]. In straight channels, owing to the parabolic velocity profile, velocity maxima are formed at the centerline so that the magnitude of the lift coefficient and hence the lift force are zero at that point. However, the introduction of curvature to microchannels not only generates Dean drag to the force balance but also alters the velocity maxima. A single particle path is demonstrated in figure 5.4 and figure 5.5. When the particle is at position 1, velocity maxima are formed near the inner half of the channel. Towards the transition region (position 3), velocity maxima shift through the centerline of the channel. In the transition region, the inner wall becomes the outer wall. Beyond this point, velocity maxima shift from the centreline through the inner wall as particle approaches to the outlet

region (position 5). A continuous velocity maxima shift along the cross-section, results in a differential change not only in the horizontal component but also in the vertical component of the shear gradient lift force, which continuously alters the 3D position of the particle. This effect is amplifying with a decrease in the particle size. It is well known that shear gradient lift force direction is from the velocity maxima through the channel walls.

The formation of single stable particle streams of both large and medium particles is observed near the centerline at different Re_C . An increase in Re_C , results in a transverse motion of large and medium particles. However, the force balance of large particles is different than that of medium particles during transverse motion. Although, large particles remain focused until the maximum Re_C , a defocusing trend is observed for medium particles when $Re_C > 136$. According to our results, there are three different cases which need to be discussed: 1) When both large and medium particle streams are near the centerline at lower Re_C (figure 5.4a), 2) transverse motion of medium particles at higher Re_C (figure 5.4c) and 3) transverse motion of large particles at higher Re_C (figure 5.5). For analyzing the particle dynamics of these situations from a different perspective, force diagrams of all cases are prepared by using our simulation results. Force diagrams of cases 1 and 2 are preferred to be given in figure 5.4 while the force diagram of case 3 is presented in figure 5.5 because of its similarity to case 1.

In case 1 (Figure 5.4a), when the particles are in position 1, where the velocity maxima are in the inner half of the channel, the horizontal component of shear gradient lift force pushes particles from velocity maxima to the outer wall. At this position, the particles vertical position is supposed to be above the zero Dean velocity line in the upper half of the channel and below the zero Dean velocity line in the lower half of the channel, where the Dean drag force direction is through the inner wall. Thus, the particles' lateral position is observed near the channel centerline, where the shear gradient lift force and Dean drag force balance each other.

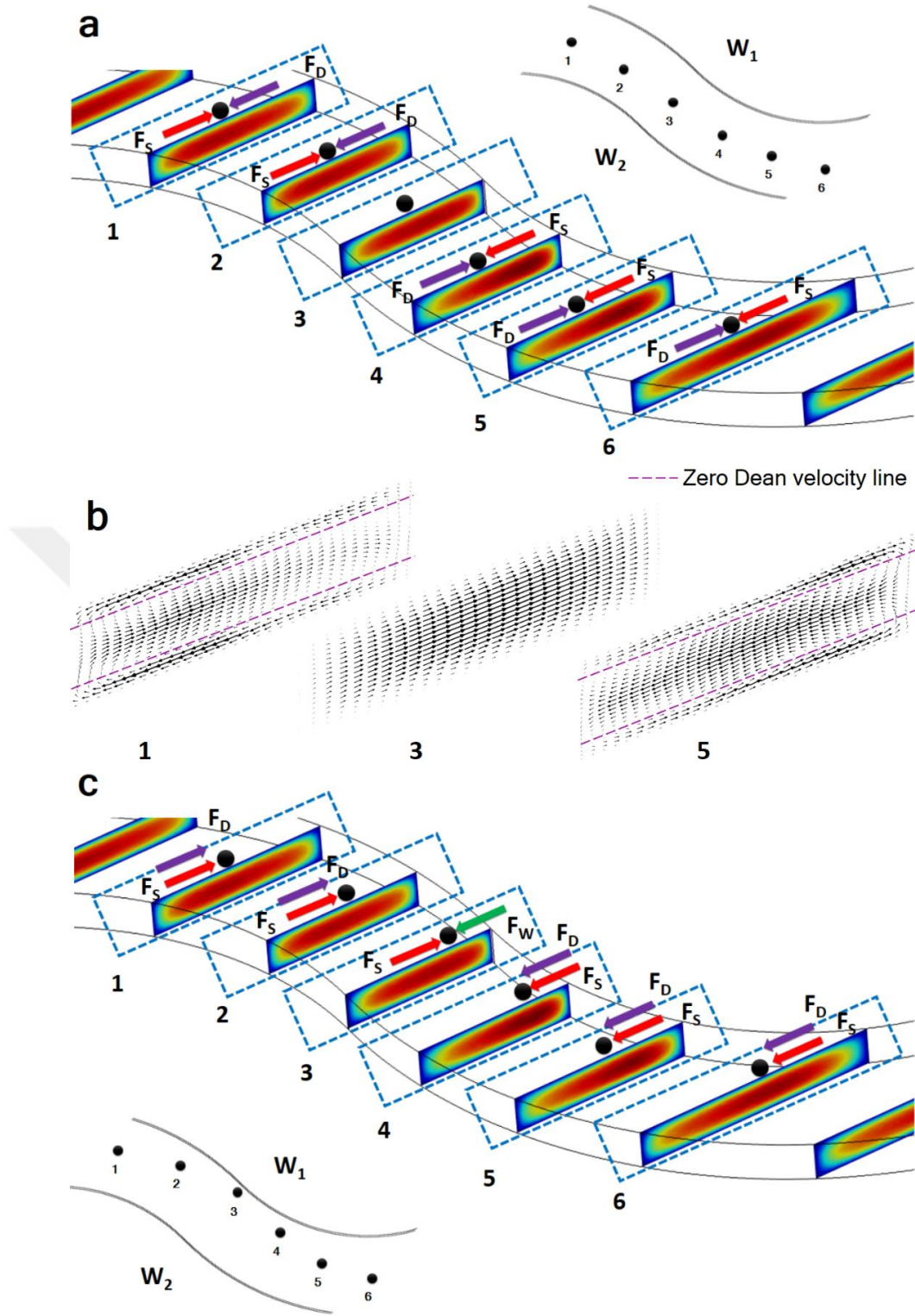


Figure 5.4: (a) Simulated velocity profile in the transition region and the forces acting on the particles where focusing occurs near the centerline. (b) Proportional Dean flow vectors with zero Dean velocity lines for particle positions 1, 3 and 5. (c) Simulated velocity profile in the transition region and the forces acting on the medium particles where transverse motion is observed. Dominant forces acting on the particles are F_s (shear gradient lift force), F_D (Dean drag force) and F_w (wall-induced lift force).

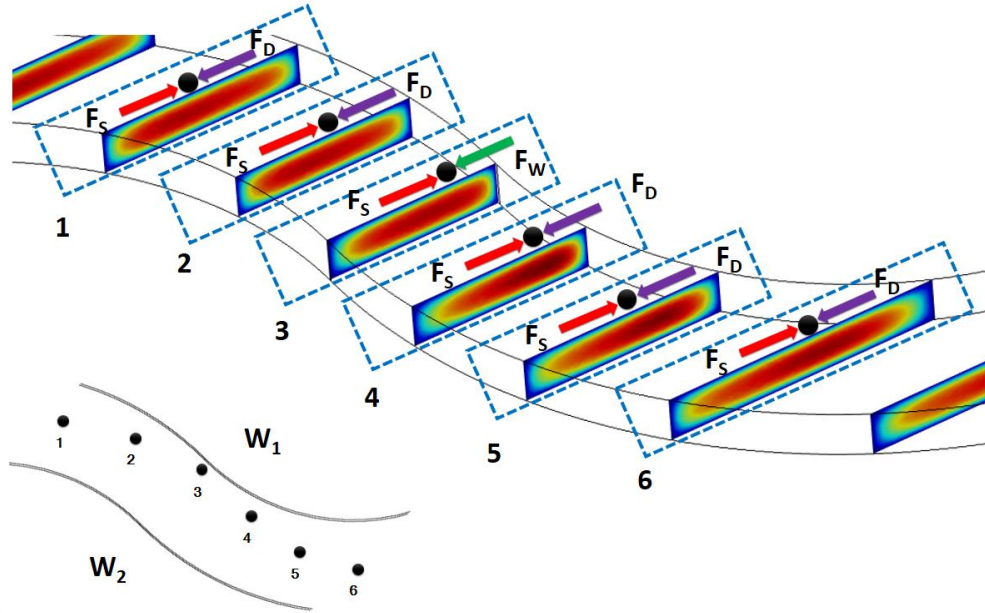


Figure 5.5: Simulated velocity profile in the transition region and the forces acting on the particles where transverse motion of large particles is observed. Dominant forces acting on the particles are F_s (shear gradient lift force), F_D (Dean drag force) and F_w (wall-induced lift force).

When particles move to position 2, the velocity maxima approach the channel center, leading to a slight increase in the shear gradient lift force on the particles not only in the horizontal direction but also in the vertical direction. When the vertical component of the shear gradient lift force increases, the particles are pushed closer to the top and bottom of the channel walls, leading to a slightly stronger Dean drag force through the inner wall. Thus, particles preserve their previous lateral position. When the particles move further towards position 3 (the transition region), the velocity maxima are formed at the center of the channel, resulting in a lift coefficient magnitude of zero and hence an absence of the lift force. However, because of the sudden directional curvature change (as the inner wall becomes the outer wall and vice versa), the curvature effect becomes negligible. Thus, the Dean drag force becomes insignificant. At that point, the particles follow the streamlines and preserve the previous lateral position until they reach the redistributed velocity profile region. Beyond position 3 (transition region), the direction of the curvature changes and it should be noted that whenever the curvature changes, the direction of the forces changes to the opposite direction. In position 4, because of the change in the curvature, the velocity maxima that are concentrated in the centre of the channel now shift towards W_2 . Again, the horizontal component of shear gradient lift

force is through the outer wall, and the Dean drag force is the counterbalancing force at the centerline. In position 5, the velocity maxima draw closer to the inner wall, causing the shear gradient lift force on the particles to slightly decrease in both vertical and horizontal directions. This decrease leads to a shift in the particles' vertical direction towards the zero Dean velocity line, and so the Dean drag force becomes weaker.

In case 2 (the transverse motion of medium particles) (figure 5.4c), while Dean drag force is in the opposite direction compared to case 1, horizontal component of shear gradient lift force direction is the same as that in situation 1. It is well known that an increase in flow velocity causes a decrease in the lift coefficient [183]. Hence, not only horizontal, but also vertical components of the lift force decrease with increasing flow velocity compare to case 1. As a result, the particles approach the vertical center of the channel or beyond the zero Dean velocity line. Upon crossing this vertical position, the particles experience a change in the direction of the Dean drag force, which is now directed towards the outer wall.

In this case (figure 5.4c), when the medium particles are in position 1, where the velocity maxima are in the inner half of the channel, a combined effect of the Dean drag force and horizontal component of the shear gradient lift force (both of which are in the same direction) causes the particles to migrate through the outer wall. In this position, a slight defocusing trend can be seen in figure 5.3b(iv). The reason for this defocusing trend is the absence of the counter force near the centerline of the channel for medium particles. When the particles move through position 2, the velocity maxima approach the channel center, causing a slight increase in the shear gradient lift force on the particles. The particles move closer to W_2 under the influence of the shear gradient and Dean drag forces. When the particles move further towards position 3 (the transition region), the velocity maxima form at the centerline. Again, because of the sudden directional curvature change, the curvature effect become negligible so that the Dean drag force becomes insignificant. Now, the shear gradient lift force is the major force and pushes particles through the wall, whereas the wall-induced lift force is the balancing force. Beyond this position, the sudden change in outer and inner wall causes the Dean vortices to change direction, and the particles move through the higher shear area. In position 4, because of the curvature changes, the velocity maxima form in the inner half of the channel. The Dean drag force is through the outer wall. Therefore, the particles are pushed to the centreline from the wall under the influence of the shear gradient lift force and Dean

drag force. Again, there is no balancing force, so that a slight defocusing trend can be observed near the outlet region, figure 5.3d(iv). In position 5, the velocity maxima draw closer to the centerline, causing the shear gradient lift force to push particles towards the outer wall, whereas the Dean drag force is through outer wall. An increase in defocusing behavior can be noticed as particles approach to this position, figure 5.3d(iv).

This defocusing behavior is not observed for large particles in case 3 (figure 5.5) due to scaling of the shear gradient lift force with a_p^3 . Thus, the magnitude of the vertical component of this force is still sufficient to push large particles above the zero Dean velocity line as in case 1, while it is not sufficient for medium particles.

An increase in Re_C results in a greater lift force compared to Dean drag force ($F_L \propto u_f^2$, $F_D \propto u_f^{1.63}$). As a result, large particles migrate to W_2 . Force balance in this case is similar to case 1, except the transition region. Although velocity maxima are near the centerline in transition region for all cases, the shear gradient lift force becomes negligible in case 1 (particles are near the centerline) because of the difference in particle lateral positions, while particles experience a shear gradient lift force through the W_2 in case 3 (particles are near the W_2) in the transition region. As the particles approach the outer wall, the wall-induced lift force becomes more dominant and balances the other two forces (the Dean drag force and shear gradient lift force). The only countering force here is the wall-induced lift force near the channel wall. A slight lateral position difference between 15 and 20 μm particles can be observed when the Reynolds number is 38 such that the medium particles are slightly closer to the outer wall compared with the larger particles. Without a full assessment of all three-dimensional effects, this result could at first glance be unexpected because the shear gradient and Dean drag forces change with a_p^3 and a , respectively, so that the expected lateral position would be closer to the centerline for medium particles compared with large particles because the order of magnitude of the shear gradient results in a significant effect on large particles. However, the shear gradient also scales with a_p^3 in the vertical direction and pushes larger particles to the top and bottom channel walls, where the Dean drag force is more dominant. Medium particles should be closer to the zero Dean velocity region. Therefore, the Dean drag force on large particles is greater than the Dean drag force on medium particles, so that the stable equilibrium position of large particles is observed to be closer to the centerline.

The main focus of this study is to investigate the effect of velocity maxima shift on the net inertial lift force (F_L) and direction change of the Dean Drag force (F_D) according to particles 3D position in the microchannel qualitatively. The change of main flow velocity profile can directly affect F_L and F_D , hence the force balancing and particle dynamics. Our simulation results do not consider the interaction between the particle and the flow. In fact, recent simulation studies show that flow field could be changed by the presence of the particles [124,184]. However, our simplified hypothesis can give a qualitative insight of the inertial microfluidics physics in curvilinear channels. On the other hand, running the simulations with particles for getting quantitative data is a challenging problem due to certain reasons (code complexity, computational resources etc.), and it is our long-term vision to develop simulation methods that are capable of performing this simulation.

Our findings suggest that carefully tailoring geometrical parameters could result in a curvilinear design, which could easily meet the requirement for a high throughput size based on separations for a vast variety of biomedical and other applications.

By looking at the streak positions of particles in the transition region (figures 5.2d and 5.2e), it can be clearly observed that the behavior of particles is different from that in the outlet region. As illustrated in figure 5.2d, the focusing width of large (20 μm) particles decreases with increasing flow rate, down to approximately double the particle diameter. At later stages, the equilibrium line starts to change its position from the center towards W_1 . The migration of medium (15 μm) particles to W_1 is faster than that of the 20 μm particles with increasing flow rate in the transition region, so there is a considerable distance between the equilibrium lines of the large and medium particles at an optimal Reynolds number. However, this distance decreases at that optimum Reynolds number in the outlet region.

One of the main reasons of this distance in the transition region is vertical position difference of medium and large particles at a given Re_C . When Re_C is low, the vertical positions of both large and medium particles are supposed to be above the zero velocity line, where the Dean drag force direction is from the outer wall to the inner wall and the shear gradient lift force balances the Dean drag force near the centerline. However, an increase in Re_C results in crossing medium particles the zero Dean velocity line. Beyond this vertical position, medium particles experience a switch in the direction of the Dean

drag force, which is now directed towards the outer wall. Therefore, as particles approach to the transition region, the shear gradient force and Dean drag force act on medium particles in the same direction and cause a lateral migration through the W_2 , while they act on large particles in such a way that they balance each other near the centerline. In the outlet region, while the Dean drag force acts on large particles from the outer wall to the inner wall and balances the shear gradient lift force near the centerline, it acts on medium particles from the inner wall to the outer wall. The Dean drag force and the shear gradient lift force act on medium particles in the same way and cause a defocusing trend near the outlet region. A more detailed analysis can be found in the Discussion section.

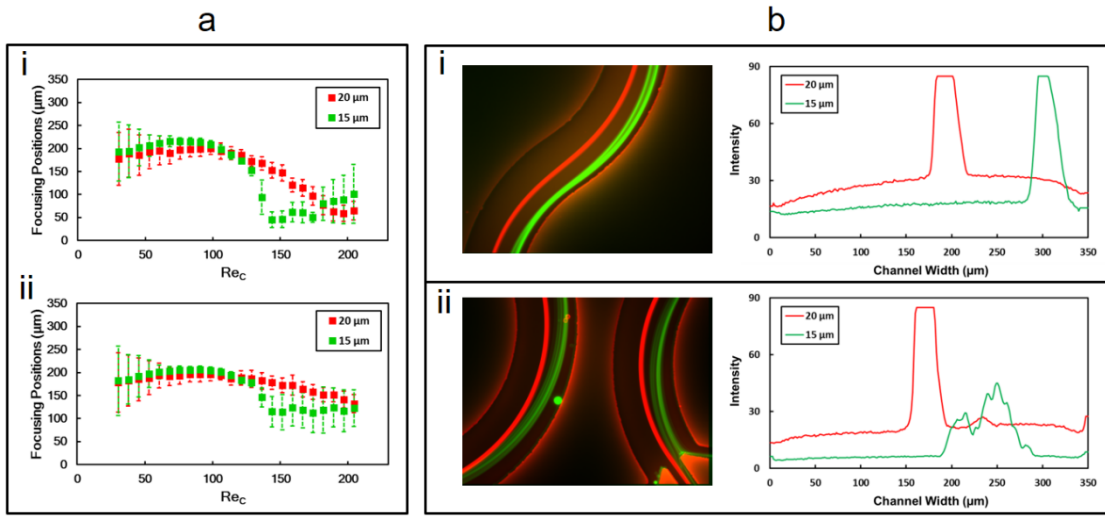


Figure 5.6: (a) Particle lateral position data from both 15 μm and 20 μm particles in the transition region (i) and in the outlet region (ii). (b) particle streams of 20 μm (red) and 15 μm (green) particles in the channel transition region (i) and in the outlet region (ii) when Re_c is 144; the corresponding intensity line graphs are presented to the right of each panel.

This study is a fundamental particle focusing study for better assessing the underlying physics of inertial microfluidics, which may lead to design novel microfluidic devices with a high separation efficiency. In such a curvilinear geometry, our findings suggest that designing the outlets of the curvilinear channel at the transition region could offer a better separation efficiency of particles with sizes of 20 μm and 15 μm. The optimal channel Reynolds number for such a separation of 20 μm and 15 μm particles is approximately 144 (figure 5.6) in the transition region. Furthermore, for this Reynolds number, the corresponding distance between the particle focusing streams of the large

and medium particles is nearly $108\ \mu\text{m}$, which is three times larger than the diameters of the particles combined together.

To reveal the curvature angle effect, channels having curvature angles of 180° and 230° were tested. The curvilinear channels with a width of $350\ \mu\text{m}$ and a height of $90\ \mu\text{m}$ used to demonstrate focusing behavior of the $20\ \mu\text{m}$, $15\ \mu\text{m}$ and $10\ \mu\text{m}$ polystyrene particles. The fabricated devices consisted of 10 curvilinear geometries with one inlet and three outlets, each having the inner radius of $800\ \mu\text{m}$. The total chip length of channels with curvature angles of 230° and 180° channels are approximately $33\ \text{mm}$ and $26\ \text{mm}$, respectively. A schematic view of the chips displayed in figure 5.7.

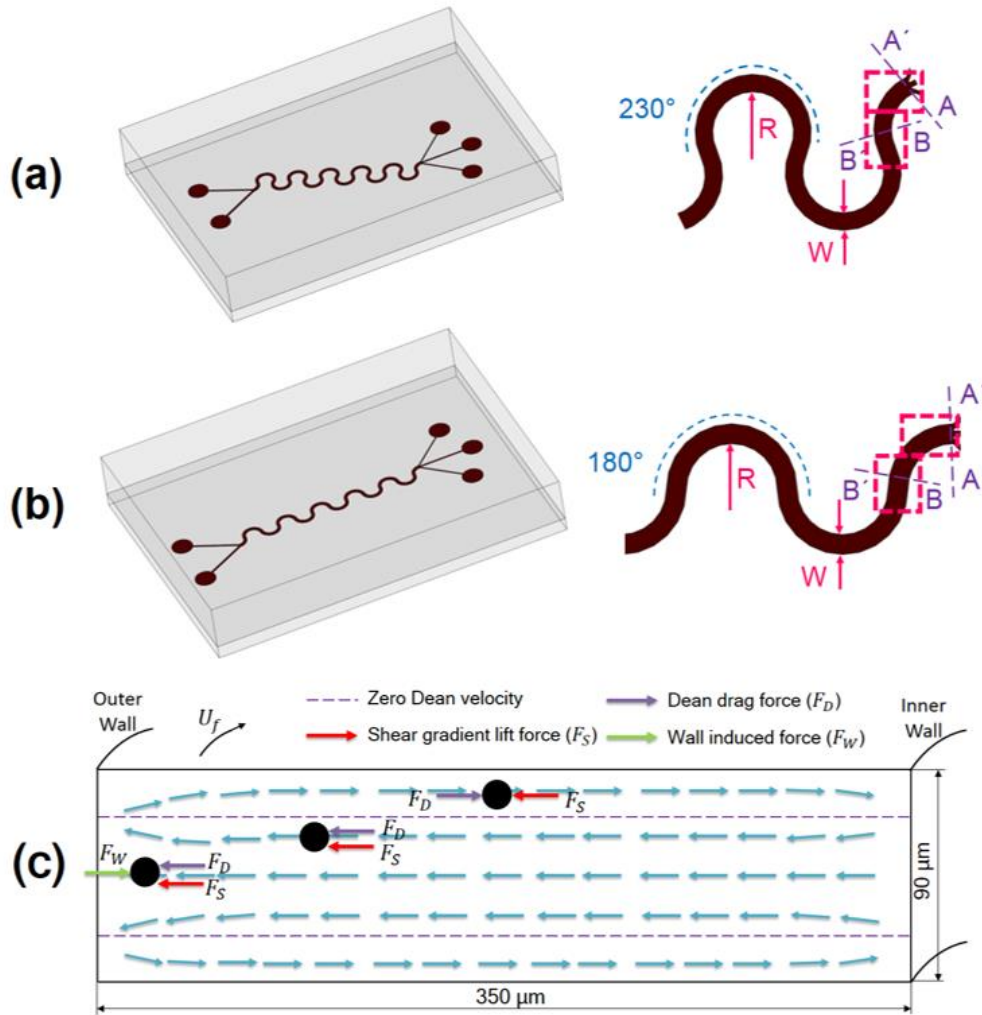


Figure 5.7: Schematic of the particle focusing chips with curvature degree of : (a) 230° and (b) 180° . In both channels A-A' and B-B' cross sections are related to outlet and transition regions, respectively. ($R = 800\ \mu\text{m}$ and $W = 350\ \mu\text{m}$) (c) forces acting on particles at different locations.

According to particle focusing position results, it can be claimed that particles tend to make transverse motion by means of Dean flow. Therefore, it is appropriate to focus on two specific channel regions: the transition region and the outlet region (figure 5.7). Due to the sudden rotational change, the inner wall becomes the outer wall and vice versa. Thus, at the transition region, the walls cannot be defined as outer and inner walls. The walls labelled at the transition region as W_1 for wall between A and B and as W_2 for wall between A' and B'.

The effects of curvature angle on microparticle focusing with different sizes were investigated by performing sets of experiments using particles with three different sizes (20 μm (large), 15 μm (medium) and 10 μm (small)) at a wide range of flow rates varying between 400 $\mu\text{L}/\text{min}$ and 2700 $\mu\text{L}/\text{min}$, which correspond to Reynolds numbers between 30 and 205.

Particle streak position and focusing width of 20 μm (red), 15 (green) μm and 10 μm (magenta) particles for different flow rates are presented in figure 5.8 at the outlet and transition regions of the curvilinear channel with the curvature angle of 230° . To clearly display the particles focusing positions and focusing widths over a wide range of channel Reynolds numbers, the Re_c maps and representative row images are given in figure 5.9. The bottom and top positions of each figure in the transition region represent walls of W_2 and W_1 , and those in the outlet region represent inner and outer walls, respectively.

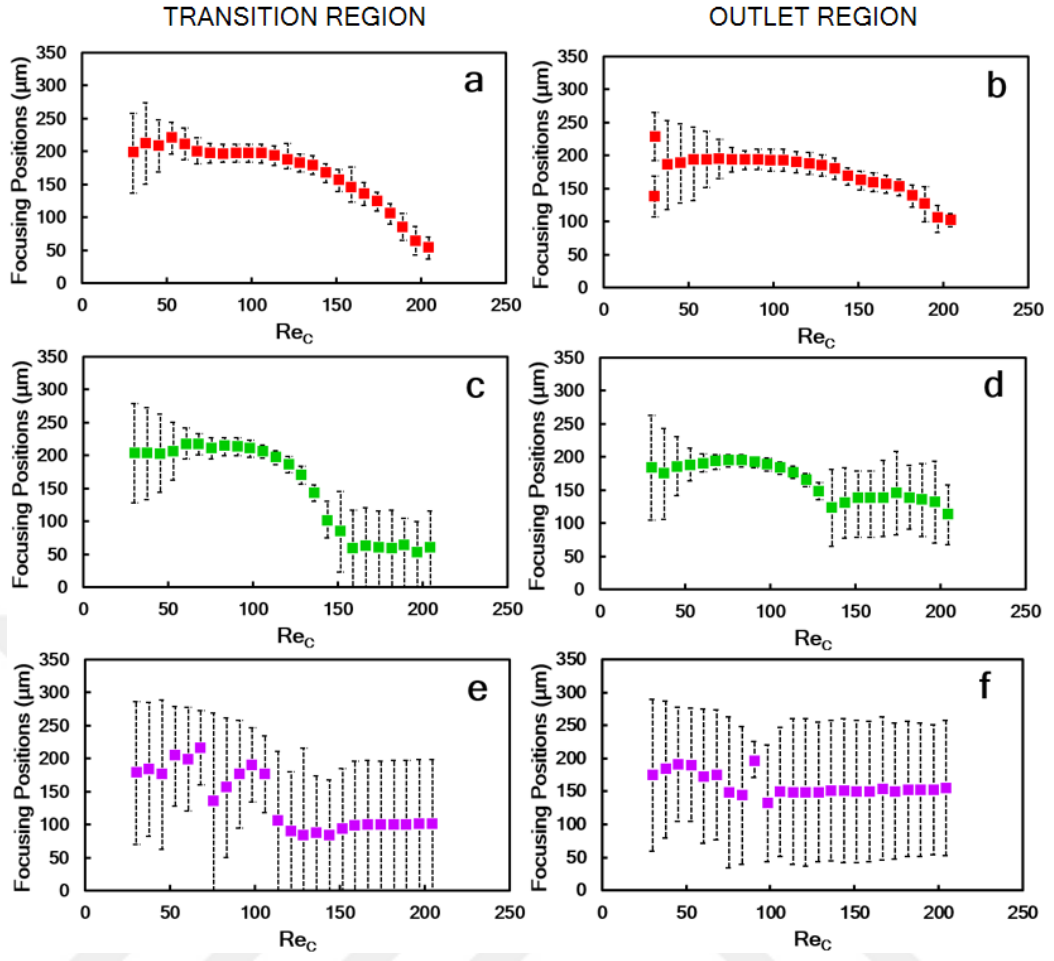


Figure 5.8: Focusing positions of microparticles in a curvilinear channel with a curvature angle of 230° varying flow rates and corresponding channel Reynolds numbers at: transition region; a) $20\ \mu\text{m}$, c) $15\ \mu\text{m}$, e) $10\ \mu\text{m}$ and outlet region; b) $20\ \mu\text{m}$, d) $15\ \mu\text{m}$, f) $10\ \mu\text{m}$. Error bars show the width of the focusing streak.

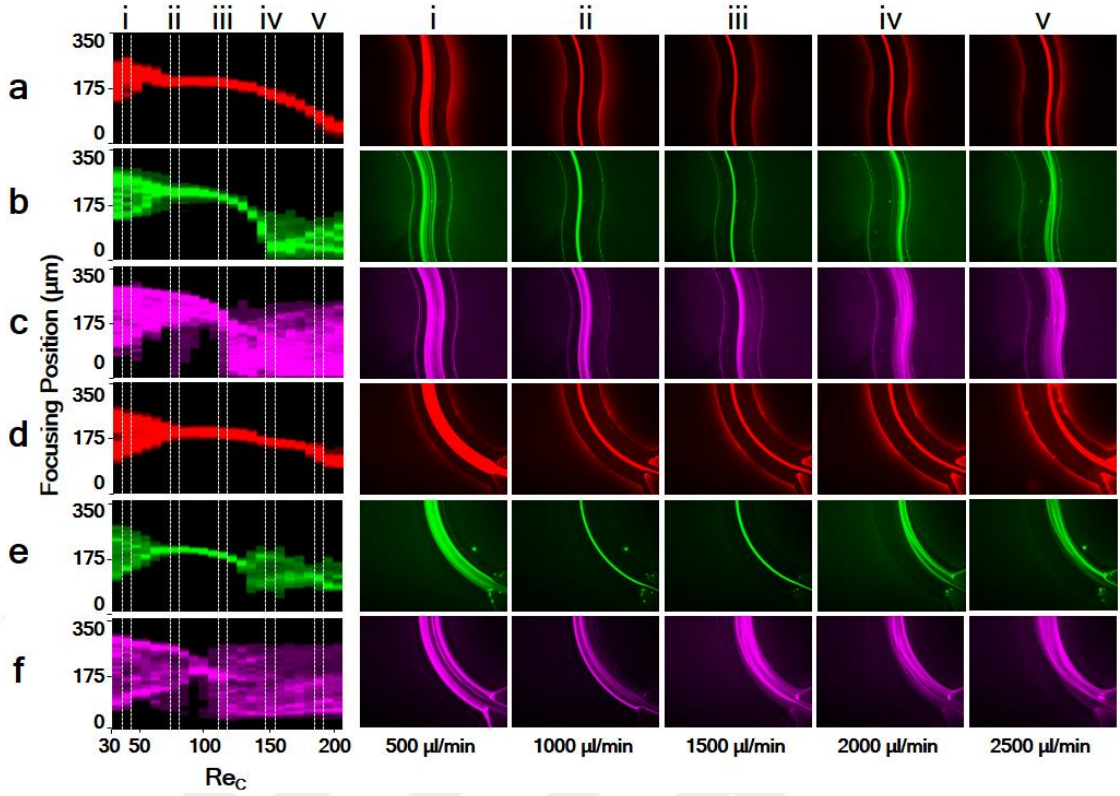


Figure 5.9: Re_C maps of the curvilinear channel with a curvature angle of 230° at: transition region; a) $20\ \mu\text{m}$, b) $15\ \mu\text{m}$, c) $10\ \mu\text{m}$ and outlet region; d) $20\ \mu\text{m}$, e) $15\ \mu\text{m}$, f) $10\ \mu\text{m}$. Particle streams for different channel Reynolds numbers corresponding to five selected flow rates between 500 and $2500\ \mu\text{L}/\text{min}$ (i–v).

Large particles ($a_p = 20\ \mu\text{m}$; $\lambda = 0.139$) initially pass along the channel near the centerline, slightly closer to W_1 as a wide band, which was the size of almost four times the diameter of the particle at transition region at low Re_C ($Re_C \sim 30$) (figures 5.8a and 5.9a) due to relatively small inertial lift and Dean drag forces combined with low flow velocity. Meanwhile, interestingly, two focusing lines can be observed at the outlet region (figures 5.8b and 5.9d). With the increase in the Re_C ($Re_C \sim 75$), a single particle streak with a width of less than twice the diameter of a single particle near the centerline is seen in both transition and outlet regions, where the streak is still slightly closer to W_1 in transition region and outer wall in outlet region. This result agrees with our previous explanation given above. Considering a large particle passing through the transition region to the outlet at low flow rates, it can be stated that Dean drag force acts in the direction from the outer wall to the inner wall since particle is assumed to be above the zero Dean velocity line in the upper half of the channel and below the zero Dean velocity line in the lower half of the channel, while shear lift force acts from the inner wall to the

outer wall. Indeed, the horizontal component of shear gradient lift force direction is always from velocity maxima through the channel walls and velocity maxima shifts from inner wall to the centerline when the particle approaches to transition region, and from centerline to inner wall when particle passes through the outlet region. When a particle is at the transition region, it follows the streamline and preserve the previous lateral position until it reaches the redistributed velocity profile region, because there is neither shear gradient lift force (velocity maxima are formed at the center) nor Dean drag force (curvature effect becomes negligible due to the sudden directional curvature change). It should be noted that the magnitude of Dean drag and shear lift forces depends on the flow velocity and the particle 3D position.

An increase in Re_C ($Re_C \geq 144$) leads to a larger lift force compared to Dean drag force ($F_L \propto U_f^2$, $F_D \propto U_f^{1.63}$). However, with a further increase in the flow velocity, the lift coefficient decreases and the lift force gradient becomes less than Dean drag force gradient. As a result, this stream migrates to wall W_2 in the transition region and to inner wall in the outlet region. Force directions are more or less the same, except the transition region owing to the fact that particle is closer to W_2 at higher Re_C . Hence, a wall induced lift force becomes dominant, which pushes particles through the centerline, while a shear gradient lift force repels particles from velocity maxima to W_2 . It should be taken into consideration that, this particle trajectory demonstrates a transverse motion of the particles along the channel, where the particles do not follow the essential flow stream but instead migrate perpendicular to this stream as they move closer to the outer wall or W_2 rather than remaining at the channel centerline. The higher the Re_C , the closer the particles to the W_2 in the transition region. Regarding the focusing width, our observations indicate that between $83 \leq Re_C \leq 205$, the focusing width is approximately the same (figures 5.8a, 5.8b, 5.9a, 5.9d).

Medium particles ($a_p = 15 \mu m$; $\lambda = 0.1$) are initially focused near the centerline, closer to W_1 in transition region and outer wall in outlet region. as a wide band at low Re_C , similar to the large particles owing to the reason that particles are slightly influenced by Dean drag and shear lift forces at low flow velocity. Increasing Re_C to $Re_C \sim 68$, a tightly focused particle streak with a width of less than twice the diameter of a single particle is obtained near the centerline, slightly closer to W_1 in transition region and closer to the

outer wall in outlet region is observed (figures 5.8c, 5.8d, 5.9b, 5.9e). The streak position of medium particles is slightly closer to W_1 than large particles at transition region as a result of the fact that shear gradient and Dean drag forces change with a_p^3 and a , respectively. In another words, the ratio of shear gradient lift force to Dean drag force is greater for the large particles so that large particles are pushed to the centerline more than medium particles. However, it should be noted that not only the horizontal component, but also the vertical component of shear gradient lift force scales with a_p^3 . Thus, large particles should be pushed through the top and bottom channel walls more than medium particles and medium particles should be closer to the zero Dean velocity line. Unlike large particles, the single streak of medium particles does not migrate to W_2 . Instead, a defocusing trend for particles is observed at $Re_C \sim 144$ for transition region and at $Re_C \sim 136$ for outlet region.

It is well known that the lift coefficient sorely depends on the flow velocity and an increase in the flow velocity results in a decrease in the lift coefficient [119]. Therefore, not only the horizontal, but also the vertical component of the lift force decreases with an increase in flow velocity. Consequently, medium particles approach the vertical center of the channel, beyond the zero Dean velocity line, where Dean flow direction changes and is now directed towards the outer wall. Considering a medium particle going through the transition region along the channel, a combined effect of shear gradient lift force and Dean drag force exists on the particle in the same direction, from inner wall to outer wall. Hence, a defocusing trend is observed in both transition (figures 5.8c, 5.9b) and outlet (figures 5.8d, 5.9e) regions up to the highest Re_C ($Re_C \sim 205$) due to the absence of the counter acting force near the centerline.

An effective focusing line cannot be observed for small particles ($a_p = 10 \mu m$; $\lambda = 0.069$), except at $Re_C \sim 68$ for the transition region (figures 5.8e, 5.9c) and $Re_C \sim 91$ for the outlet region (figures 2f, 3f). In general, the bypassing movement is dominant. This result is presumable and consistent with the previous studies, which reported with a minimum threshold of $\lambda > 0.07$ for inertial focusing [185]. However, even with a $\lambda = 0.069$, the tendency of focusing in a wide band at $Re_C \sim 68$ in the transition region and $Re_C \sim 91$ in the outlet region should be taken into consideration for the enrichment purposes. Thus,

the possibility of focusing small particles with a width of approximately four times the diameter of a single particle can be achieved with suitable geometries.

This section of the thesis is a fundamental study to understand the underlying physics of microparticle focusing and inertial microfluidics, and can contribute to the effective novel microfluidic separation devices. Our experimental observations highlight that there is a shift in particle focusing behavior between transition and outlet regions. Our findings suggest that carefully designing the outlets at the transition region in curvilinear channel, could easily offer a better separation or enrichment efficiency of particles. In addition, increasing the curvature angle leads to a better separation of 20 μm and 15 μm diameter particles (figures 5.10 and 5.13). Fluorescent images of particle separation and enrichment potential of the curvilinear channel with curvature angles 180° and 230° are illustrated in figures 6 and 7. In addition, high speed image samples for each particle size and their trajectories can also be seen. The optimum separation of 20 μm and 15 μm particles is achieved at $\text{Re}_C \sim 144$ at the transition region in the curvilinear channel with curvature angles 230° with the corresponding distance between the particle focusing streams of the large and medium particles is approximately twice as the diameter of large particles (40 μm) (Figure 5.10a). At this Re_C , the average speeds of particles are measured as; $u_{p,20\mu\text{m}} = 1.78 \text{ m/s}$ and $u_{p,15\mu\text{m}} = 1.97 \text{ m/s}$ (figure 5.10a). As illustrated in figure 5.10b, the enrichment (not the pure separation) of 20 μm and 10 μm particles can be achieved at $\text{Re}_C \sim 136$ in the transition region since the large particles pass through near the centerline, while most of the small particles travel between centerline and W_2 ($u_{p,20\mu\text{m}} = 1.71 \text{ m/s}$ and $u_{p,10\mu\text{m}} = 1.83 \text{ m/s}$). Similarly, enrichment of 15 μm and 10 μm particles can be acquired at $\text{Re}_C \sim 121$ in the transition region. According to figure 5.10c, the particle stream of medium particles is seen near the centerline, while most of the small particles travel between centerline and W_2 ($u_{p,15\mu\text{m}} = 1.54 \text{ m/s}$ and $u_{p,10\mu\text{m}} = 1.65 \text{ m/s}$).

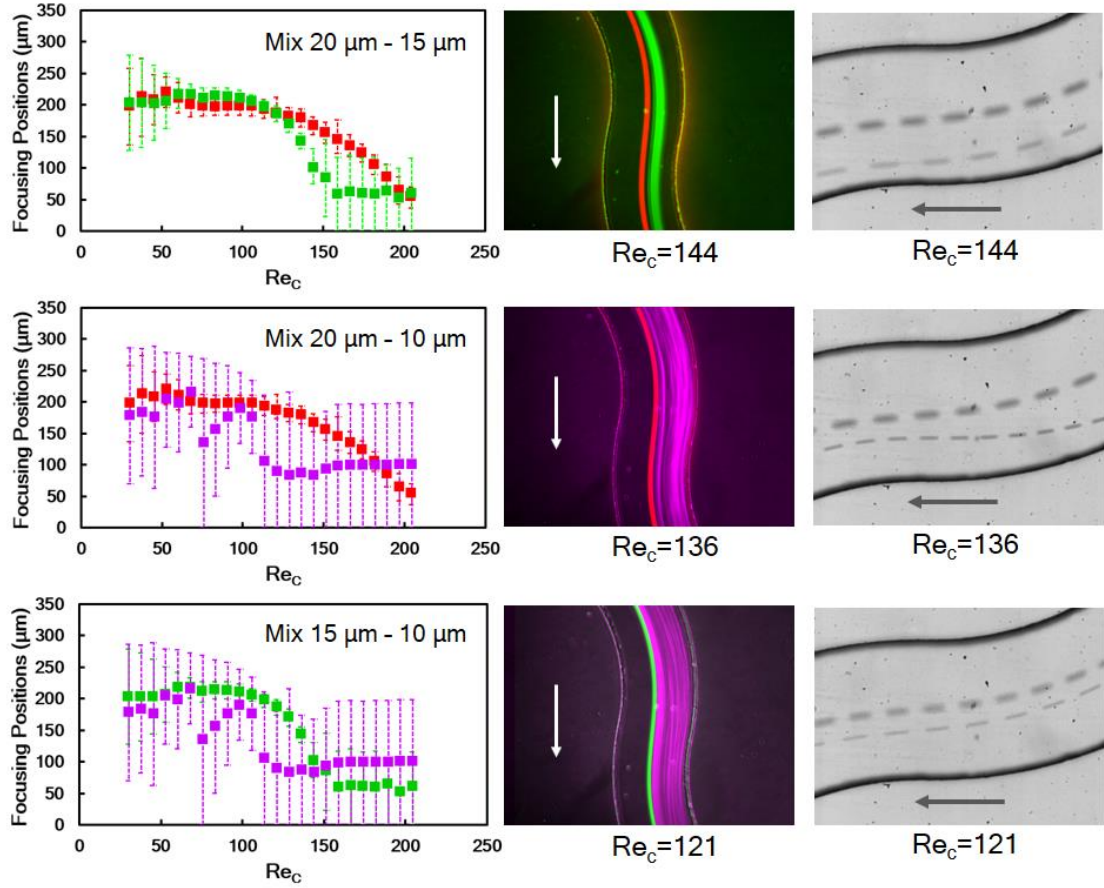


Figure 5.10: Focusing positions of microparticles at transition region in the curvilinear channel with curvature angle of 230° for different flow rates (left column), particle streams for optimum separation or enrichment potential channel Reynolds number (middle column), and high speed images of an example for each particle size and their trajectories (right column) for mixed sets of microparticles, 20 μm and 15 μm, 20 μm and 10 μm, 15 μm and 10 μm. The arrows represent flow direction in the channel.

The particle streak positions and focusing widths corresponding to 20 μm (red), 15 (green) μm and 10 μm (magenta) particles are presented for different channel Reynolds numbers in figure 5.11 at the outlet and transition regions of the curvilinear channel with the curvature angle of 180° . The Re_C maps and representative images are given in figure 5.12. The bottom and top of each figure in the transition region represent walls of W_2 and W_1 , and inner and outer walls in the outlet region, respectively.

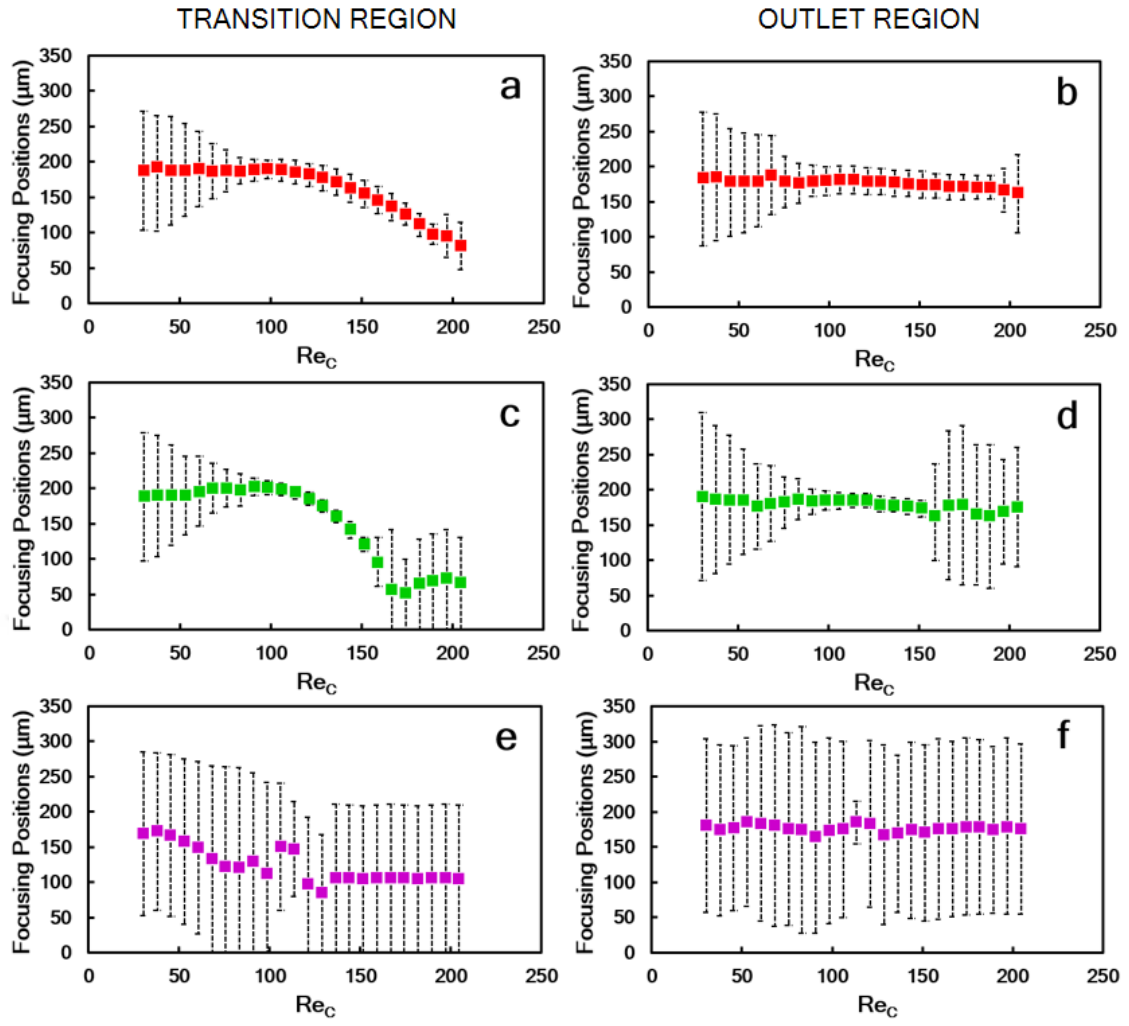


Figure 5.11: Focusing positions of microparticles in the curvilinear channel of curvature angle of 180° with varying flow rates and corresponding channel Reynolds numbers at: transition region; a) 20 μm , c) 15 μm , e) 10 μm and outlet region; b) 20 μm , d) 15 μm , f) 10 μm . Error bars show the width of the focusing streak.

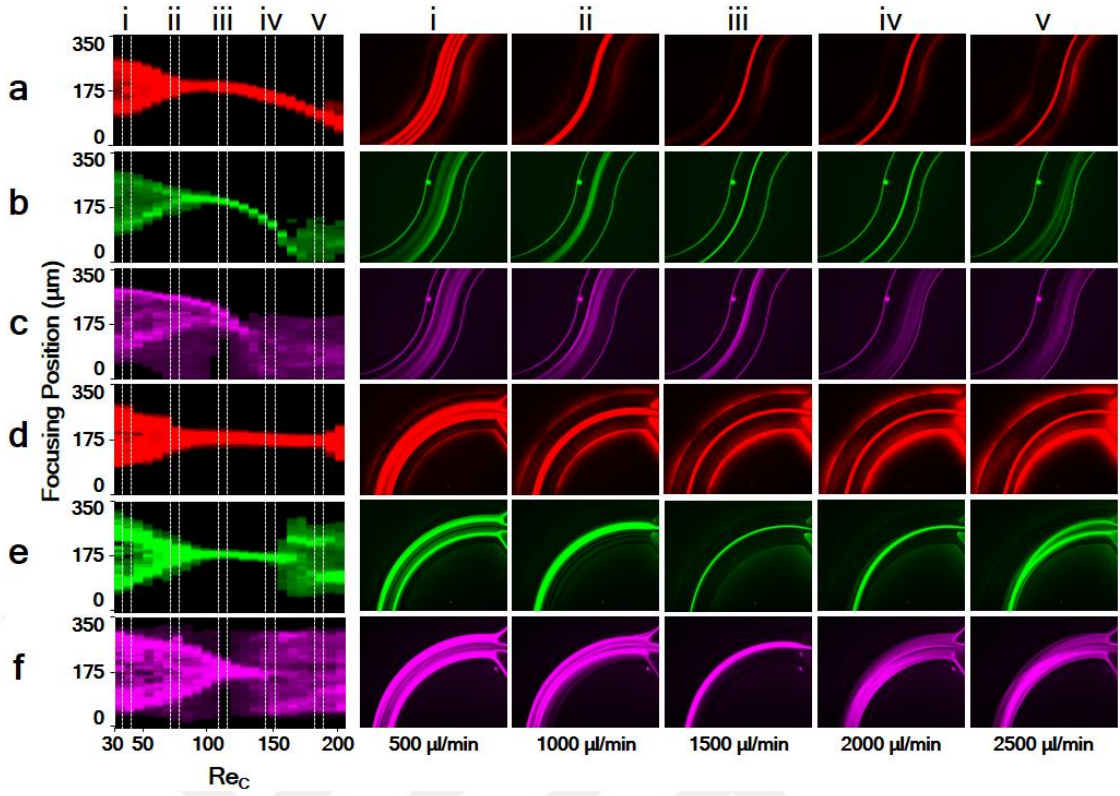


Figure 5.12: Re_C maps of curvilinear channel with curvature angle of 180° at: transition region; a) $20\ \mu\text{m}$, b) $15\ \mu\text{m}$, c) $10\ \mu\text{m}$ and outlet region; d) $20\ \mu\text{m}$, e) $15\ \mu\text{m}$, f) $10\ \mu\text{m}$. Particle streams for different channel Reynolds numbers corresponding to five selected flow rates between 500 and $2500\ \mu\text{L}/\text{min}$ (i–v).

A very widely focused stream of large particles ($a_p = 20\ \mu\text{m}$; $\lambda = 0.139$) forms near the centerline, occupying almost half of the channel width in transition (figure 5.11a, 5.12a) and outlet regions (figures 5.11b, 5.12d) at low Re_C ($Re_C \sim 30$). The focusing width is greater in transition region of this channel in comparison the channels with the larger curvature angle (figure 5.13a). With an increase in the Re_C up to 91, the width of the stream gradually decreases to a single particle streak in the transition region, while a slightly wider stream can be observed in the outlet region. This result is also consistent with our previous explanation. Here, considering force directions, the horizontal component of shear gradient lift force acts from velocity maxima to outer wall while Dean drag force acts from outer wall to inner wall, since the particles are assumed to be above the zero Dean velocity line in the outlet region. When the particles are travel through the centerline in the transition region, particles neither experience shear gradient lift force nor Dean drag force. Hence, they follow the streamline until reaching the region of velocity profile. A further increase in Re_C ($Re_C \geq 144$), does not change the force direction but

the force magnitude due to flow velocity increase ($F_L \propto U_f^2$, $F_D \propto U_f^{1.63}$) in outlet region. Because of this, the stream of medium particles experiences lateral migration through the wall W_2 in transition region. However, it has more or less the same position in the outlet region as a result of transverse motion. Because particles are now closer to W_2 , not the centerline, shear induced force acts on the particles through W_2 and wall induced lift force acts as a counterbalancing force in transition region. With a further increase in Re_C ($Re_C \geq 144$) a defocusing trend can be seen in both transition (figures 5.11a and 5.12a) and outlet regions (figures 5.11b and 5.12d).

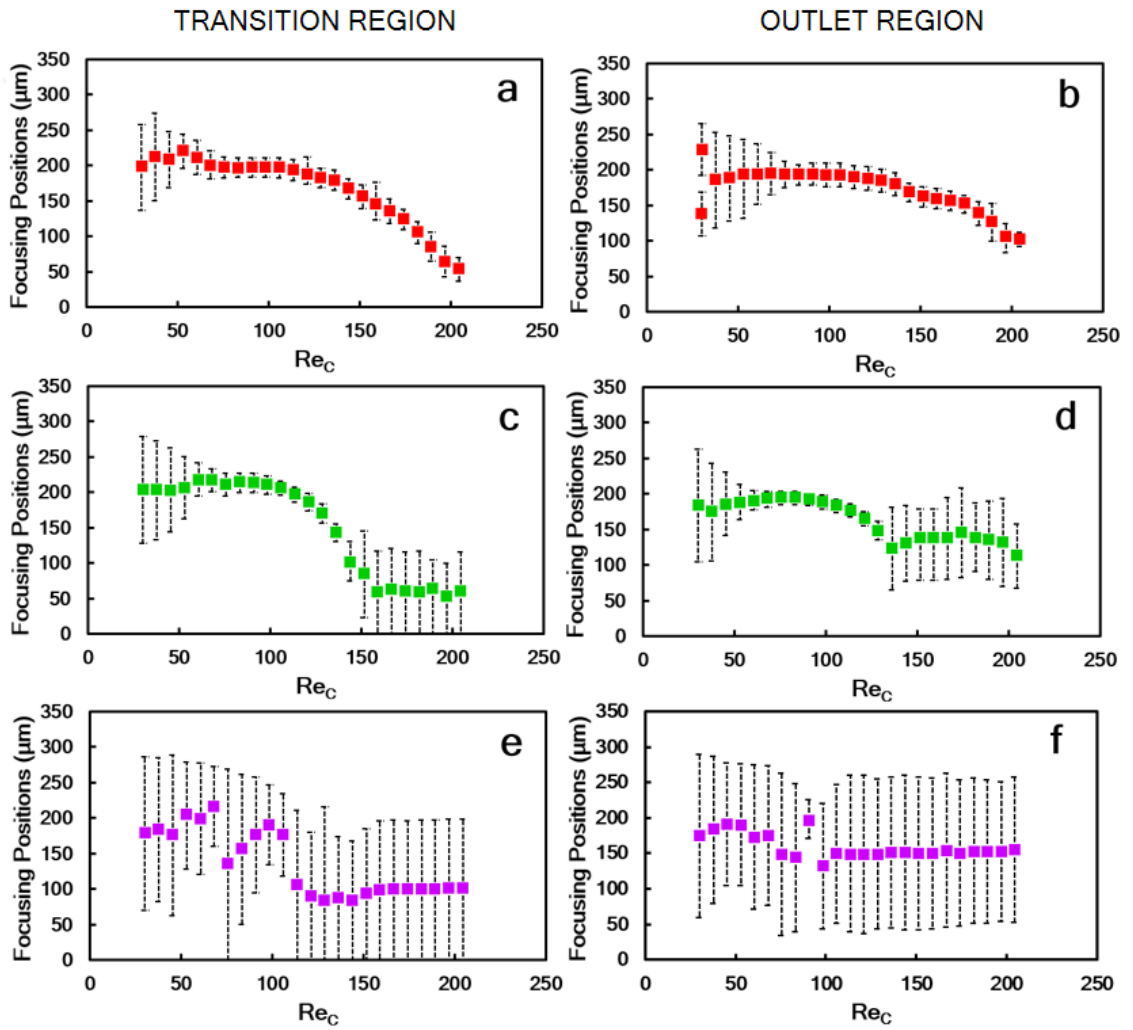


Figure 5.13: Focusing positions of microparticles in a curvilinear channel with a curvature angle of 230° for varying flow rates and corresponding channel Reynolds numbers at: transition region; a) $20\ \mu\text{m}$, c) $15\ \mu\text{m}$, e) $10\ \mu\text{m}$ and outlet region; b) $20\ \mu\text{m}$, d) $15\ \mu\text{m}$, f) $10\ \mu\text{m}$. Error bars show the width of the focusing streak.

The focused stream of medium particles ($a_p = 15 \mu\text{m}$; $\lambda = 0.1$) initially forms within a very wide band, with a width of more than the channel half width, near the centerline in both transition and outlet regions of the channel (focusing width is wider at the outlet region) at low Re_C ($Re_C \sim 30$) due to small effect of Dean drag and shear gradient lift forces. The focusing width is larger in the transition region of this channel in comparison to the channels with a curvature angle of 230° (figure 5.13b). Similar to large particles, an increase in the Re_C up to 98, the focusing width of medium particles gradually decreases to a single particle streak width in both transition (figures 5.11c, 5.12b) and outlet regions (figures 5.11d, 5.12e). However, this tight stream is relatively closer to the centerline in the outlet region than in the transition region. Additionally, comparing the streak positions of large and medium particles, the focusing position of medium particles is slightly closer to W_1 than large particles in transition region, because shear gradient and Dean drag forces alter with a_p^3 and a , respectively, and large particles are repelled to the centerline more than medium particles. The particles then start to migrate through the W_2 upon a further increase in Re_C ($Re_C \sim 114$). An increase in flow velocity leads a decrease in the lift coefficient which is the reason for the reduction in shear gradient lift force. Since the shear gradient lift force direction is from velocity maxima to outer wall and Dean drag force is from outer to inner wall under this condition, this reduction makes particles to approach to inner wall just before the transition region and consequently to W_2 in transition region. Instead of Dean drag force, wall induced lift force is now introduced to the system as particles approach to the W_2 . When $Re_C \sim 144$, medium particles start with transverse motion. The reason for the transverse motion is reduced lift coefficient. Not only the horizontal component, but also the vertical component of shear gradient lift force decreases with the increase in flow velocity. Therefore, the lateral position of the particles should be below the zero velocity line on the top and above the zero velocity line on the bottom, where Dean drag force direction is shifted to the opposite direction. At $Re_C \sim 144$, the stream of medium particles in channels with curvature angle of 180° closer to the centerline, while large particle stream approaches to W_2 in channels with curvature angle of 230° in transition region (figure 5.13b). A combined effect of shear gradient and Dean drag force repels particles through outer wall in outlet region. However, a focusing like trend can be recognized in the transition region at $Re_C \sim 152$

due to the balance of shear gradient and Dean drag forces on one side and wall induced lift force on the other side. Besides, defocusing behavior of particles increases with the channel Reynolds number.

Small particles threshold of particle diameter to channel hydraulic diameter ratio is 0.069 ($\lambda = 0.069$), and its claimed that when this ratio is below 0.07, inertial focusing is not occurred. Our results on the curvilinear channels with curvature angle of 180° agree with this finding. However, a focusing-like trend is observed when $Re_C \sim 91$ for the outlet region, possibly because shear gradient lift force acting on small particles is not as much as that on medium and large particles due to the fact that this force scales with a_p^3 . On the other hand, Dean drag force scales with a_p . Therefore, bypassing movement of particles becomes more dominant. In general, focusing width of small particles in the channels with curvature angle of 230° is less than that of in the channels with curvature angle of 180° (figure 5.13c).

In general, our observations indicate that the curvilinear channel with curvature angle of 230° could offers a better separation or enrichment efficiency. However, according to figure 5.14a, corresponding to the channel with curvature angle of 180° , the distance between the particle focusing streams of the large and medium particles is approximately $10 \mu m$ at $Re_C \sim 174$. Thus, separation of $20 \mu m$ and $15 \mu m$ size particles could possibly be achieved. The average velocities of a large and a medium particle is $u_{p,20\mu m} = 2.02 \text{ m/s}$ and $u_{p,15\mu m} = 2.20 \text{ m/s}$. Neither successful separation nor enrichment is possible for $20 \mu m - 10 \mu m$ and $15 \mu m - 10 \mu m$ particle mixtures, because small particles travel through the channel in defocused at almost all the Re_C . However, optimum enrichment for $20 \mu m - 10 \mu m$ particle mixture ($u_{p,20\mu m} = 1.67 \text{ m/s}$ and $u_{p,15\mu m} = 1.80 \text{ m/s}$) at $Re_C \sim 129$ and of $15 \mu m - 10 \mu m$ particle mixture ($u_{p,20\mu m} = 1.57 \text{ m/s}$ and $u_{p,15\mu m} = 1.76 \text{ m/s}$) at $Re_C \sim 129$ could be achieved (figure 5.14).

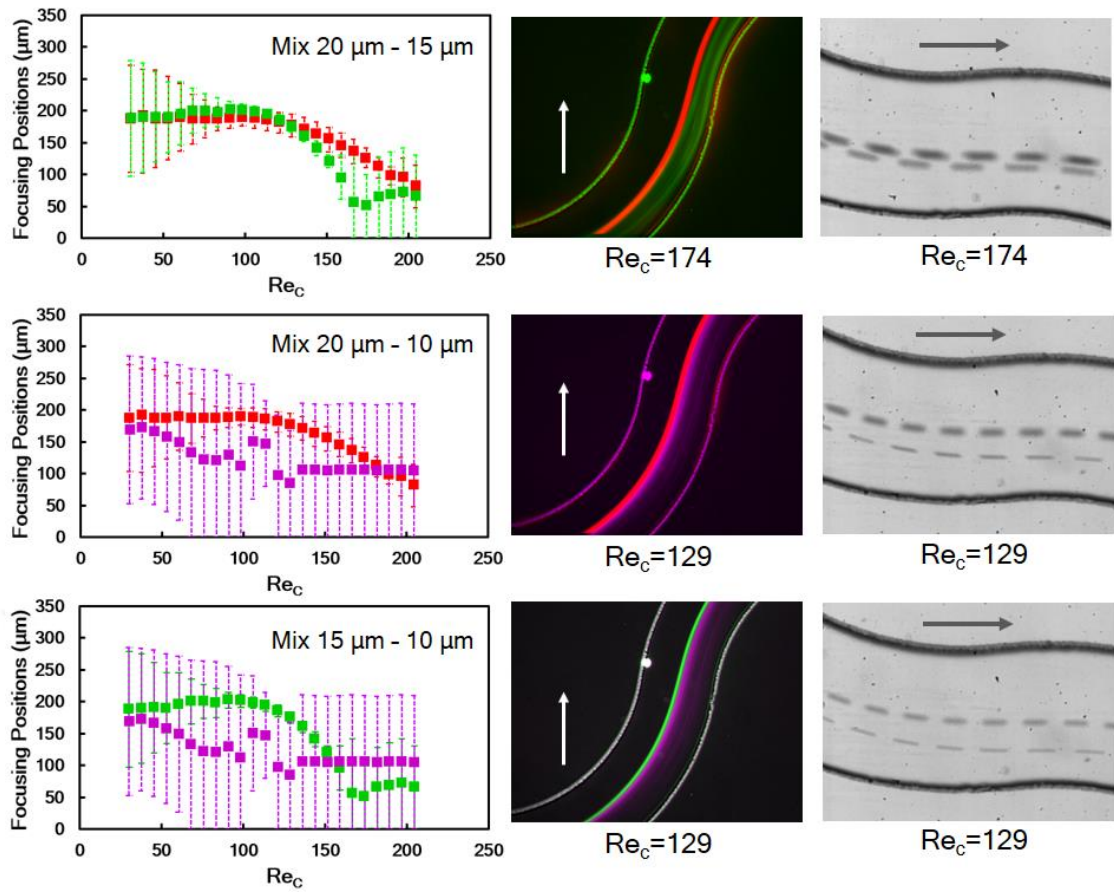


Figure 5.14: Focusing positions of microparticles at transition region in the curvilinear channel with curvature angle of 180° for different flow rates (left column), particle streams for optimum separation or enrichment potential channel Reynolds number (middle column), and high speed images of an example for each particle size and their trajectories (right column) for mixed sets of microparticles, 20 μm and 15 μm, 20 μm and 10 μm, 15 μm and 10 μm. The arrows represent flow direction in the channel.

CHAPTER 6

6. INERTIAL FOCUSING OF PARTICLES IN ASYMMETRIC CURVILINEAR CHANNELS WITH CURVATURE ANGLE OF 280°

6.1. Introduction

While the first term of “inertial microfluidics” essentially originates from fluid inertia, the second term stands for small volumes. Although it is difficult to combine these two aspects, designing a proper device which consists of the larger geometries than those of conventional microfluidic device yet subject to remaining in the limits of microscale dimensions, offers relatively higher fluid inertia, leading to Reynolds numbers higher than 1. Since the straight channels are not as efficient as curved channels or straight channels with constriction and expansion geometry, from the particle/cell separation perspective, inertial microfluidics is often associated with Dean flows, which can be obtained in channels with both curved geometry and constriction and expansion geometry.

Inertial microfluidics has attracted much attention by virtue of its potential to be used in portable point of care (POC) diagnostic devices, lab on a chip systems and micro total analysis systems (μ TAS), especially for separation or enrichment purposes. Among the focusing and separation methods, inertial microfluidics is distinguished by its high throughput, simple topology, relatively easy fabrication and basic sample preparation requirement features. Despite being one of the emerging topic in microfluidics, the underlying physics of combined inertial microfluidics and Dean flow is not yet fully understood due to its complex nature and lack of 3D visualization techniques. In addition to the low cost and portability of microfluidic systems to be used for biomedical and chemical purposes, new mechanisms and new forces in microfluidics play an important role [173,174]. Furthermore, fabrication of portable point of care (POC) diagnostic devices as lab on a chip or micro total analysis systems (μ TAS) could be simplified and accelerated with new technologies [168,186]. The fabricated devices partake in

tremendous convenience for biomedical applications in detection, sorting, separation and analysis of cells for effectual diagnosis and therapy [170,171,181].

Focusing of particles into a tight stream is vital for the counting (cytometer) and separation requirements [173–175]. Fundamental particle focusing principles can be classified as active and passive separation [176]. Active separation approaches take the advantage of external forces such as magnetic [178], dielectric [179], acoustic [187] and optic forces [188], while passive separation approaches rely mainly on the microchannel topology and hydrodynamic forces [177] and can be further categorized to hydrodynamic filtration, pinch flow fractionation, deterministic lateral displacement and inertial microfluidics [171]. Remarkably, active techniques offer more accurate outcomes [21]. Yet, these techniques have some restrictions, such as low throughput, complex component integrations and expensive requirements for process or production [21]. Recently, some review studies on sorting and isolation of particles and/or cells assisted in understanding of characteristics and mechanisms of separation [17,18,21,175,181].

Among the passive separation approaches, inertial microfluidics has been becoming more and more popular due to its high throughput, sensitivity, simplicity and high efficiency. It was first reported by Segre and Silberberg in 1961 [104,105]. Accordingly, it is known that uniformly dispersed particles formed a narrow band at a distance of $0.2D$ away from the channel walls in a circular cross section channel with a diameter D . Since then, theoretical explanations of particles behavior have been provided [107,189,190]. Recently, the scope was extended to applications in microfluidics and different topologies such as straight [112,113,118], spiral [120,128,130,136,138], serpentine [115,129,143,144] and straight with constriction-expansion arrays [145–148]. Some comprehensive studies [129–131,191] and reviews [114,117,124,127] on inertial focusing can be found in the literature.

The aim of this chapter is to reveal the asymmetry effect in a microfluidic channel with curvature angle of 280° on inertial particle focusing of $20\text{ }\mu\text{m}$, $15\text{ }\mu\text{m}$ and $10\text{ }\mu\text{m}$ fluorescent polystyrene microparticles at a channel Reynolds Numbers range of 30-205. The combined effect of Dean drag and inertial forces were qualitatively investigated in detail to provide insight to particle behavior and to better understand 3D particle dynamics in curvilinear channels. Accordingly, asymmetry dramatically alters focusing characteristics of the particles in such a way that increasing asymmetry provides a better

separation and enrichment and forces acting on particles vary depending on the 3D position of particles in curvilinear channels.

6.2. Results and Discussion

A schematic view of the asymmetric chip is displayed in figure 6.1. Particle focusing positions are not the same along the microchannel, because particles tend to make transverse motion by the effect of Dean flow. To investigate the particle motion appropriately, it is preferred to focus on two specific channel regions: the transition region and the outlet region (Figure 6.1). Due to the sudden rotational change, the inner wall becomes the outer wall and vice versa. Hence, at the transition region, the walls cannot be defined as outer and inner walls. The walls labelled at the transition region as W_1 for wall between A and B and as W_2 for wall between A' and B'.

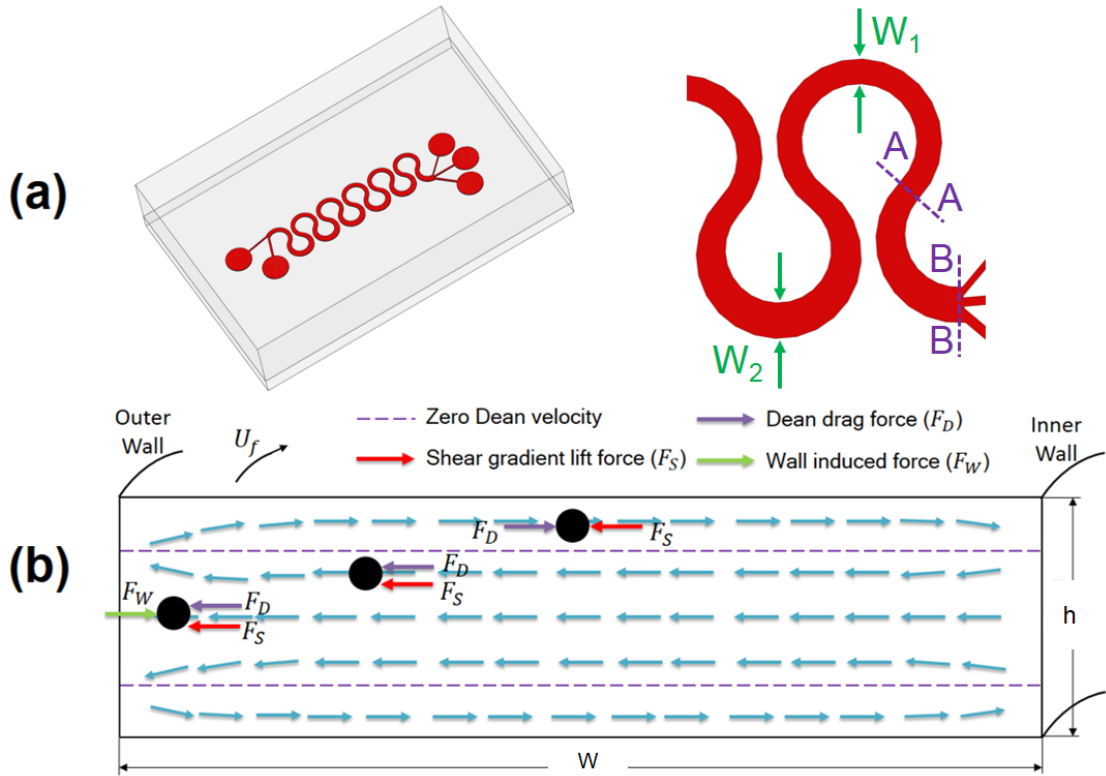


Figure 6.1: (a) Schematic of the particle focusing device. A close-up view of the channel transition region (A-A) and the outlet region (B-B). ($W_1=350 \mu\text{m}$ and $W_2= 500 \mu\text{m}$) (b) A force diagram depicting the direction of forces acting on the particle of various locations.

Mean velocity profile has an extremely significant effect on particle behavior. In straight channels, parabolic velocity profile has a maximum at the centerline and zero velocity at the walls. Shear gradient force acts on the particle from velocity maximum (centerline) to walls in both vertical and horizontal directions. However, at the centerline, it becomes zero owing to the fact that the magnitude of lift force becomes zero. Along with the introduction of curvature to the channel topology, the secondary flow (Dean flow) forms due to velocity mismatch of fluid elements between the centerline and near the wall [114], which alters the maxima of the mean velocity profile. Dean flow consists of two counter-rotating vortices parallel to the z -axis. The flow direction is from the inner wall to the outer wall at the mid-section of the channel, while it is from the outer wall to the inner wall near the top and bottom walls, above the zero Dean velocity line in the upper half of the channel and below the zero Dean velocity line in the lower half of the channel (figure 6.1b). In spiral channels, Dean vortices preserve their pattern up to a certain Dean number, which is within the limits of microfluidics separation studies [120]. The simulation studies showed that main flow velocity maxima are close to the inner wall [130,192]. A particle near the inner wall is subject to a Dean drag force, where the direction is from the inner wall the outer wall. On the other hand, the net lift force (a combination of shear gradient lift force and wall induced lift force) acts on the particle from the outer wall to the inner wall in spiral channels. These two forces act in the same direction, when particles are near the outer wall. Hence, particles continue to move along the circulation route of the counter-rotating vortices. Moreover, shear gradient lift force decreases near the centerline, as velocity profile shifts due to an increase in curvature. Thus, the vertical position of particle moves closer to the center on the inner half of the channel, resulting in an equilibrium position near the inner wall in spiral channels. It should be taken into consideration that particle position in y direction is also significant to define Dean drag force direction and the abovementioned phenomena in spiral channels can only occur when particle vertical position is below the zero Dean velocity line in the upper half of the channel and above the zero Dean velocity line in the lower half of the channel, where the direction of Dean flow is from the inner wall to the outer wall. The directions of secondary flow vary with a sudden change in the curvature of the microchannel, and the case becomes more sophisticated for alternating curvilinear geometry. Thus, it is very difficult to precisely predict the emerging steady state secondary flow conditions.

Furthermore, we demonstrated in Chapter 5 that towards to the transition region, velocity maxima shifted to the centerline of the channel. Thereafter, the inner wall becomes the outer wall beyond the transition region. Beyond this location, velocity maxima shift from the centerline to the inner wall in curvilinear channels with a curvature angle of 280° . The change in the shear gradient lift force happens not only in the horizontal direction but also in the vertical direction, due to a continuous velocity maxima shift along the cross section. Besides, this effect is amplified with a decrease in the particle size.

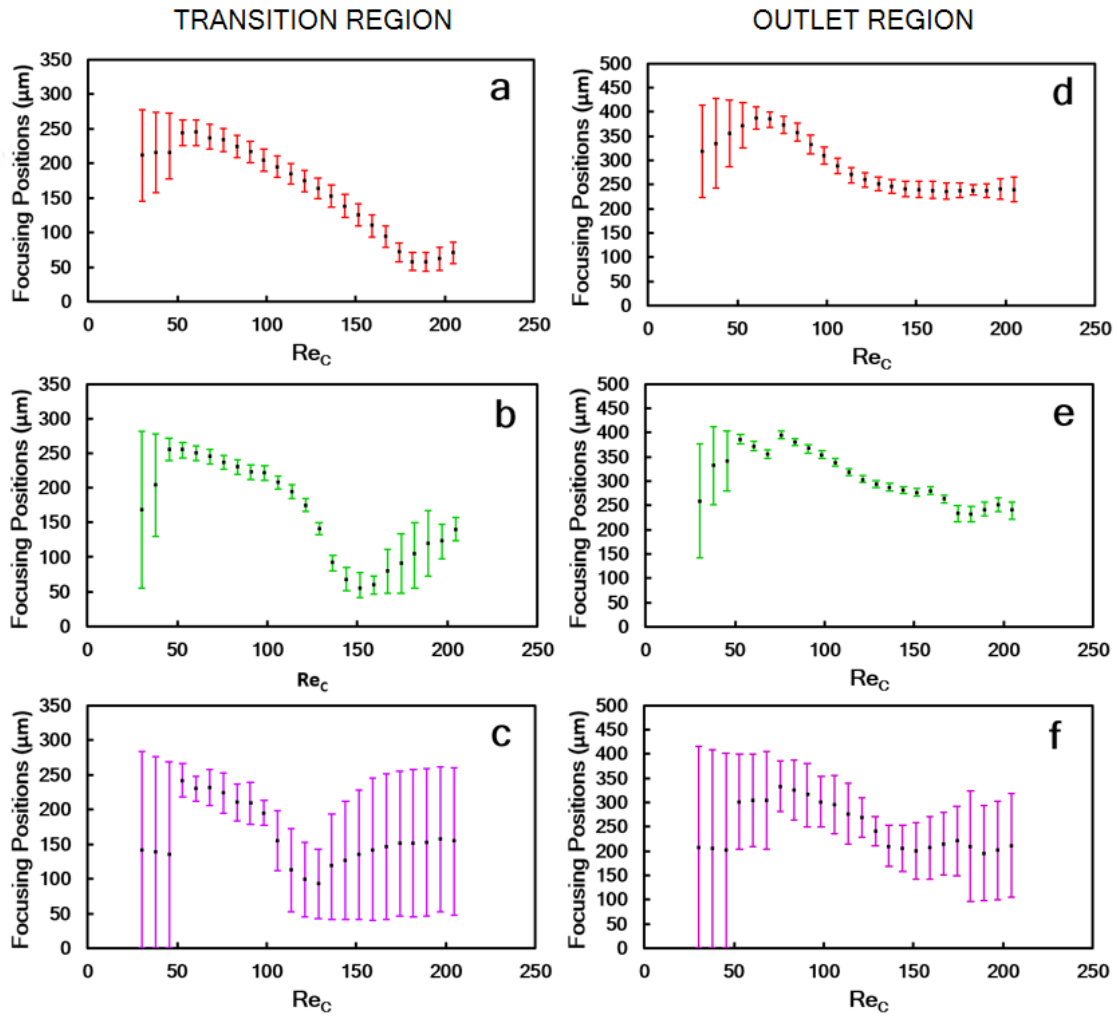


Figure 6.2: Focusing positions of microparticles in a curvilinear channel with a curvature angle of 230° for varying flow rates and corresponding channel Reynolds numbers at: transition region; a) $20\ \mu\text{m}$, c) $15\ \mu\text{m}$, e) $10\ \mu\text{m}$ and outlet region; b) $20\ \mu\text{m}$, d) $15\ \mu\text{m}$, f) $10\ \mu\text{m}$. Error bars show the width of the focusing streak.

The Re_C maps and representative row images are given in Figure 6.2 to clearly display the particles focusing positions and focusing widths over a wide range of channel

Reynolds numbers. The bottom and top positions of each figure in the transition region represent walls of W_2 and W_1 , and those in the outlet region represent inner and outer walls, respectively.

Large particles ($a_p = 20 \mu\text{m}$; $\lambda_1 = 0.139$, $\lambda_2 = 0.129$) initially pass along the channel near the centerline, in the transition region, slightly closer to W_1 as a wide band, which has the size of almost five times the diameter of the particle at transition region at low Re_C ($Re_C \sim 30$) (figures 6.2a and 6.3a) due to relatively small inertial lift and Dean drag forces combined with low flow velocity. Meanwhile, as the particles approach to the outlet region, this wide band in the transition region becomes larger (the focusing width widens almost twice in size) in the outlet region (figures 6.2d and 6.3d) as a result of increased Dean drag force and decreased shear induced lift force. With an increase in the Re_C ($Re_C \sim 53$), a single particle streak with a width of almost twice the diameter of a single particle near the centerline is seen at transition region, where the streak is still slightly closer to W_1 at transition region. At this Re_C , the particle focusing behavior is not the same in the outlet region. Although initial wide band decreases its width along with an increment on the flow velocity, at this Re_C it couldn't reach a single particle stream width. and this wide stream is still closer to the outer wall at the outlet region. It can be claimed that the ratio of net lift force to Dean drag force F_L/F_D is higher in the transition region because channel hydraulic diameter D_h is smaller in this region. Similar to symmetric channels with curvature angle of 280° (Chapter 5), shear lift force acts from the velocity maxima to the outer wall while Dean drag force acts from the outer wall to the inner wall in the outlet region because particles are assumed to be above the zero Dean velocity line in the upper half of the channel and below the zero Dean velocity line in the lower half of the channel. When a particle approaches to the transition region, F_s increases and F_d decreases, leading to a tighter particle stream. On the other hand, when a particle approaches to the outlet region, F_s decreases and F_d increases. As a result of mixing effect of Dean vortices, single particle stream in the transition region widens in the outlet region. However, increasing the flow rate, both F_s and F_d increases ($F_L \propto U_f^2$, $F_D \propto U_f^{1.63}$). Therefore, at $Re_C \sim 53$, not only in the transition region, but also in the outlet region large particles focus in a tight stream. With a further increase in the flow

velocity, the lift coefficient decreases and the increase in the shear lift force becomes less than that of Dean drag force. As a result, this stream migrates to wall W_2 at the transition region and to inner wall at the outlet region. Particle stream approaches to W_2 at higher Re_C ($Re_C > 172$), in the transition region, hence a wall induced lift force comes into play, which pushes particles through the centerline. On the other hand, shear gradient lift force is still present and pushes particles from velocity maxima to W_2 . It is worth mentioning that, this particle trajectory indicates a transverse motion of the particles along the channel, where the particles do not follow the essential flow stream but instead migrate perpendicular to this stream as they move closer to the outer wall or W_2 . Regarding the focusing width, our observations indicate that between $53 \leq Re_C \leq 205$, in the transition region (figures 6.1a, 6.1b, 6.2a and 6.2d) and $68 \leq Re_C \leq 189$ at the outlet region (figures 6.1a, 6.1b, 6.2a and 6.2d) the focusing width remains approximately the same.

Medium particles ($a_p = 15 \mu m$; $\lambda_1 = 0.1$, $\lambda_2 = 0.09$) are focused in a wide band, which covered half of the channel width, near the centerline in the outlet region, while no focusing behavior observed in the transition region at $Re_C \sim 30$ because particles are slightly influenced by Dean drag and shear lift forces at low flow velocity. Increasing Re_C to $Re_C \sim 45$, a tightly focused particle streak with a width of less than twice the diameter of a single particle is obtained near the centerline, slightly closer to W_1 at transition region however, this tight stream was not observed in the outlet region (figures 6.1 and 6.2). Similar to large particles, as the particles approach to the outlet region, Dean drag force increases and shear gradient lift force decreases due to increasing D_h . Single particle stream migrates to W_2 gradually increasing the Re_C . Shear gradient and Dean drag forces scales with a_p^3 and a , respectively. Not only the horizontal component, but also the vertical component of shear gradient lift force scales with a_p^3 . Thus, large particles should be pushed through the top and bottom channel walls more than medium particles at the same flow rate, and medium particles should be closer to the zero Dean velocity line. The vertical position of medium particles is supposed to be below the zero Dean velocity line in the upper half of the channel and above the zero Dean velocity line in the lower half of the channel when they start transverse motion. In this case, although the direction of shear gradient lift force is the same, from velocity maxima to W_2 , Dean drag

force direction changes as from W_2 to centerline in the transition region. It is well known that the lift coefficient sorely depends on the flow velocity and an increase in the flow velocity results in a decrease in the lift coefficient [119]. Therefore, not only the horizontal, but also the vertical component of the lift force decreases with an increase in flow velocity. Consequently, medium particles approach to the vertical center of the channel, beyond the zero Dean velocity line, where Dean flow direction changes and is now directed towards the outer wall. Considering a medium particle travelling through the transition region along the channel, a combined effect of shear gradient lift force and Dean drag force exists on the particle in the same direction, from inner wall to outer wall. A defocusing trend for particles is observed at $Re_C \sim 144$ for transition region.

Small particles ($a_p = 10 \mu m$; $\lambda_1 = 0.069$, $\lambda_2 = 0.064$) did not focus at $Re_C \sim 30$ in both the transition and the outlet region. Increasing Re_C to $Re_C \sim 53$, particles tend to focus in a wide band, which has the size of almost five times the diameter of the particle at the transition region and the width of this focusing band was almost the same until the channel Reynolds number is increased to $Re_C \sim 106$. However, the focusing band migrates through the centerline because, the direction of F_s is from velocity maxima to the inner wall, F_d direction is from the outer wall to the inner wall and F_s increases more than F_d ($F_s \propto U_f^2$, $F_d \propto U_f^{1.63}$). A sudden defocusing behavior is observed when increasing Re_C to $Re_C \sim 106$ probably because vertical position of small particles approaches to center of the channel, pass the zero Dean velocity line and as a result, Dean drag force direction is reversed. Dean drag force and shear induced force directions become the same, through the W_2 at the transition region and through the outer wall at the outlet region. Therefore, particles approach to W_2 until Re_C is increased to $Re_C \sim 129$ at the transition region. Further increment of Re_C result in a mixing effect of the particles because Dean drag force overcomes the shear gradient force. As mentioned before, lift coefficient decreased increasing the flow velocity and at some point Dean drag force becomes larger than shear gradient force. One interesting note about small particles is that although the ratio of particle diameter to channel hydraulic diameter ($\lambda = a/D_h$) is smaller than 0.07, particles tend to focus. However, the width of this focusing band is at least in the size of four times the diameter of the particle. Previous studies claimed a minimum threshold of $\lambda > 0.07$ for inertial focusing, but the maximum focusing width was considered as in the size of four times the diameter of the particle.

However, it should be taken into account that particles can form a wide band even with a $\lambda = 0.064$.

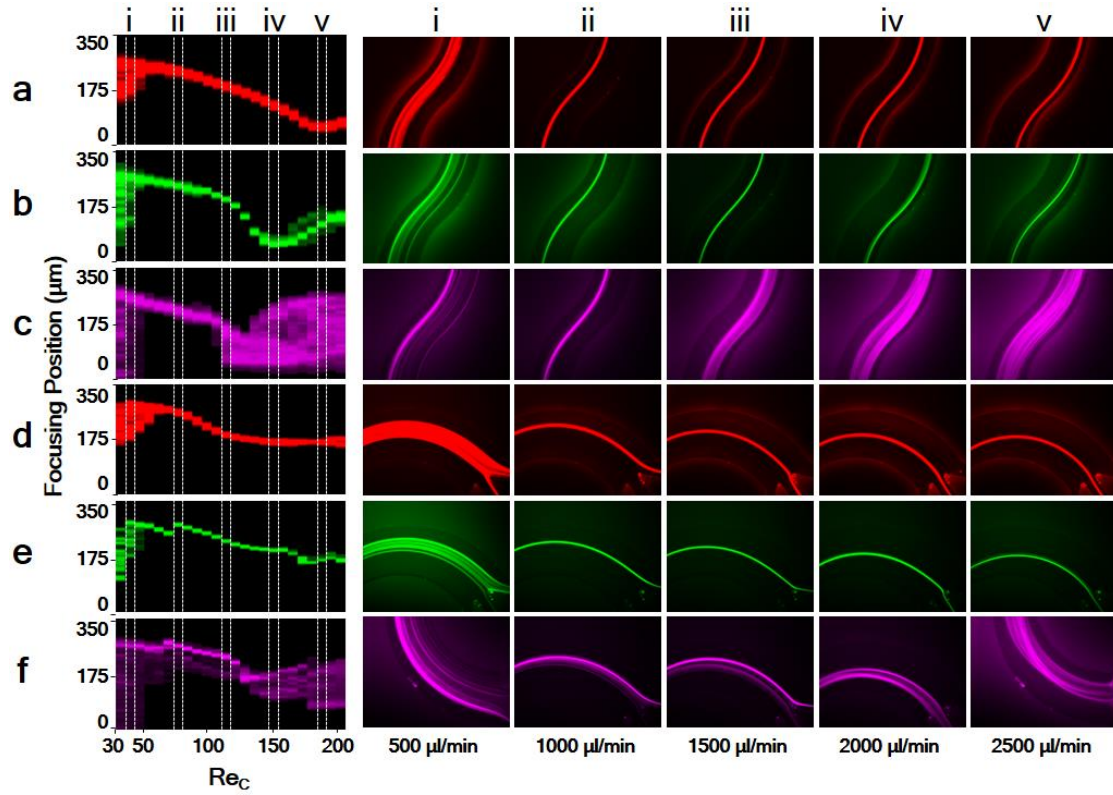


Figure 6.3: Re_C maps of the asymmetric curvilinear channel with a curvature angle of 280° at: transition region; a) $20\ \mu\text{m}$, b) $15\ \mu\text{m}$, c) $10\ \mu\text{m}$ and outlet region; d) $20\ \mu\text{m}$, e) $15\ \mu\text{m}$, f) $10\ \mu\text{m}$. Particle streams for different channel Reynolds numbers corresponding to five selected flow rates between 500 and $2500\ \mu\text{L}/\text{min}$ (i–v).

Our experimental observations highlight that there is a shift in particle focusing behavior between transition and outlet regions. Our findings suggest that carefully designing outlets at the transition region in curvilinear channels, could easily offer a better separation or enrichment capability. Fluorescent images of particle separation and enrichment potential of the asymmetric curvilinear channel with curvature angle of 280° are illustrated in figure 6.4. The optimum separation of $20\ \mu\text{m}$ and $15\ \mu\text{m}$ particles is achieved at $Re_C \sim 144$ at transition region in the asymmetric curvilinear channel with a curvature angle of 230° . The corresponding distance between the particle focusing streams of large and medium particles is approximately twice as the diameter of large particles ($40\ \mu\text{m}$) (figure 6.4a). As illustrated in figure 6.4b, the enrichment of $20\ \mu\text{m}$ and

10 μm particles can be achieved at $Re_C \sim 121$ at transition region since large particles pass through near the centerline, while most of small particles travel between the centerline and W_2 . Similarly, enrichment of 15 μm and 10 μm particles can be obtained at $Re_C \sim 121$ at the transition region. According to figure 6.4c, the particle stream of medium particles is seen near the centerline, while most of small particles travel between the centerline and W_2 .

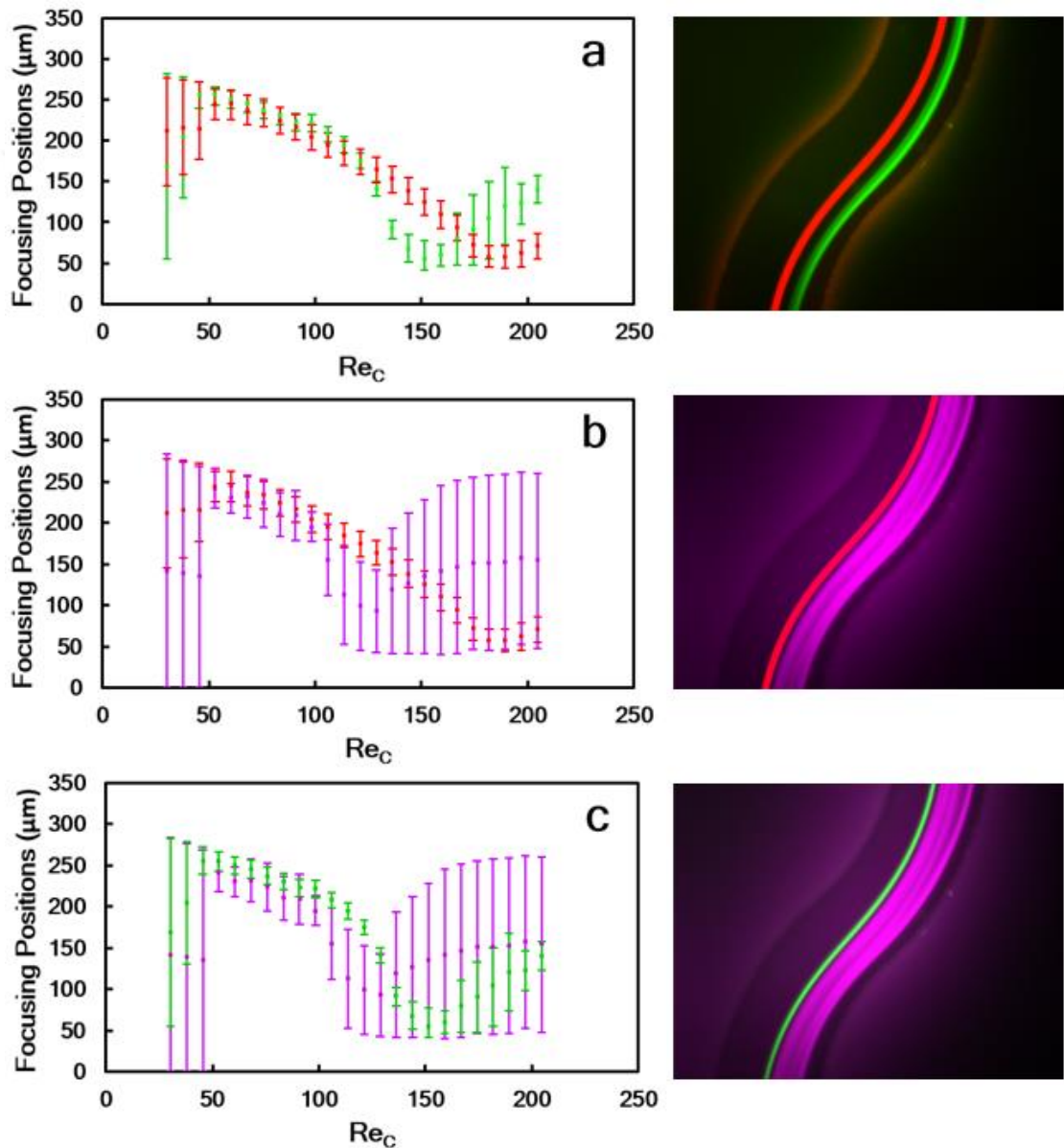


Figure 6.4: Focusing positions of microparticles at transition region in the curvilinear channel with a curvature angle of 230° for different flow rates (left column), particle streams for optimum separation or enrichment potential channel Reynolds number (right column) a) 20 μm and 15 μm , b) 20 μm and 10 μm , c) 15 μm and 10 μm .

CHAPTER 7

7. INERTIAL FOCUSING OF CANCER CELL LINES IN CURVILINEAR CHANNELS

7.1. Introduction

Metastatic tumors originate from primary tumors and are spread through the lymphatic system or bloodstream to the other parts of the body. Due to some genetic mutations, millions of cells shelter in primary tumors and accomplish maintaining cellular functions such as proliferation. Owing to uncontrolled continuous cell proliferation, primary tumor forms, and some rare cells from this tumor become invasive and are released from the primary tumor and start to circulate through to the bloodstream. Most of these cells so-called “circulating tumor cells (CTCs)” die while circulating in the bloodstream, while only 0.01% of them will likely lead to metastasis [193]. CTCs might circulate as a single cell or they might clump and form CTC clusters of 2 to 50 individual tumor cells. Although CTCs are extremely rare in blood [194], CTC clusters increase metastatic risk, compared to single CTCs [195]. Primary tumors barely cause cancer deaths, however metastatic cancers ultimately account for 90% of mortality [196]. Thus, development of isolation or detection methods for CTCs will play an important role for assisting oncologists to accurately interpret cancer characteristics while enhancing effective treatment strategies.

Conventional diagnostic strategies for primary tumors include evaluation of clinical symptoms, which is based on cancer type and cancer location, relies on imaging techniques such as Magnetic Resonance Imaging (MRI), Computed Tomography (CT), Magnetic Resonance Spectroscopy (MRS), Positron Emission Tomography (PET), Radiography (X-ray), ultrasonography and tissue biopsy examination under the microscope [197]. However, these methodologies are deficient owing to the fact that examination is subjective and depends on the evaluation of oncologists and pathologists,

moreover taking the tissue specimens leads to an inconvenience to patients and may not even be considered as an option. In addition, imaging techniques are not convenient and not frequently usable because of radiation and cost concerns. Moreover, primary tumor sampling cannot give sufficient information about prognosis and treatment [198]. These problems might be solved by a new diagnostic concept : liquid biopsy requiring isolation of CTCs or cell-free circulating tumor DNA (ctDNA) from blood [199].

Besides the fact that CTCs may shed light on the evaluation of the cancer stage and determination of therapeutic techniques, CTCs could possibly provide real time monitoring of tumor progression as representative biomarkers. Metastatic CTCs may hold different genomic and phenotypic information in comparison to primary tumor cells or other CTCs as a result of secondary mutation so-called tumor heterogeneity, which constitutes a significant debate in the literature [200–202]. Molecular and cellular analysis of CTCs might contribute to developing novel personalized cancer treatments [199,203]. However, concentration of CTCs has been estimated to be 1 to 10 CTCs per 10^7 - 10^9 blood cells [204]. Hence, achieving pure CTCs from the blood is still challenging.

Currently, many CTC enrichment techniques require specific antibodies against epithelial cell surface protein, epithelial cell adhesion molecule (EpCAM). One of the most common commercially available macroscale CTC enrichment methods approved by US Food and Drug Administration (FDA) is the CellSearch® system (Veridex, LLC, Raritan, NJ, USA), which is used to determine prognosis of metastatic colon, breast and prostate cancers [205,206]. CellSearch applied magnetic field to enrich the labeled CTCs with EpCAM-conjugated magnetic particles in bulk purification. One of the limitations for this method is the long process time. In addition, many cells are damaged during sample preparation. Although the results for CTCs enumeration and enrichment are satisfactory in CellSearch, this method could not provide a suitable platform for antitumor-response prediction or determination of personalized therapy. Therefore, novel approaches become essential for cancer diagnostics in order to overcome the limitations of conventional CTCs-separation systems while providing better solutions for high cost, insufficiency, long processing time, low-throughput and complexity.

Microfluidic technologies come into play with many advantages over conventional technologies such as requirement of small sample volume, low-cost production and processing higher sensitivity and improved performance [207,208]. Furthermore, microfluidic technologies also facilitate manufacturing of integrative, portable point of

care diagnostics devices based on lab on a chip or micro-total-analysis-systems (μ TAS). From the microfluidics point of view, separation/isolation principles can be divided into two categories depending on the external energy usage; active and passive separation [176]. While active techniques require external forces such as magnetic [209], dielectric [179] and acoustic [180] to separate particles/cells, passive techniques utilize mainly hydrodynamic forces [18]. Passive techniques can be further separated into filtration based, deterministic lateral displacement based and inertia-based ones [171]. Noticeably, active techniques provide more accurate results yet are limited by the low throughput, integration of complex components and expensive production or process requirements. A number of CTCs enrichment techniques are studied in microfluidics platforms. Affinity-based isolation is the most common approach to separate circulating tumor cells. EpCAM coated cell surfaces are attached to the antibody (anti-EpCAM) coated surfaces. Target cells should withstand against the shear force, which makes flow speed and throughputs low. For the purpose of increasing the surface to volume ratio, many different channel structures were used such as silicon microposts [210], herringbone chip [211], nanopillars [212]. Instead of the antibodies, aptamers [213] and hydrogels [214] are also being used to capture CTCs. Magnetic micro/nano particles conjugated with specific antibodies are attached to the corresponding cells [215]. With magnetic gradients, labeled cells are captured from the blood. This technique is called as immunomagnetic cell isolation [216]. A label-free technique is the dielectrophoretic (DEP) cell isolation, in which a non-uniform electric field is used to polarize cells, and with DEP forces, cells can be manipulated. In this case, DEP forces depend on the dielectrical properties of each cell types and their surrounding medium [217]. In an ultrasonic field, acoustic radiation force originating from the differences in acoustic contrast factors, compressibility and density of the particles/cells and the medium accumulates suspended particles/cells in a carrier fluid through nodes or anti nodes of the standing acoustic wave [218]. Hence, particles/cells with distinct acoustic contrast factors can be directed to the desired position and be collected from different outlets.

Hydrodynamic filtration has been an appealing technique and isolates cells with respect to size and deformability [219]. Not only larger cells but also lower deformable cells can be retained on the one side of the microfilter. Higher deformable cells can pass through the microfilter as well as smaller cells and can be collected from the outlet. Various types of micro filters have been studied for cell separation such as microweirs, micropillars,

cross-flow and porous membrane [220]. Likewise, in the deterministic lateral displacement method, size and deformability of cells are significant parameters. While larger cells/particles (larger than critical hydraulic diameter) follow a lateral deterministic streamline around the micro-pillars placed in a microchannel, smaller cells/particles maintain original down flow streamlines through pillar gaps [94]. Several recent microfluidics cell sorting/isolation reviews can be found in the literature to better understand separation characteristics and physics [221–226].

A variety of fundamental focusing and separation approaches has been studied with synthetic microparticles in the framework of microsystems [21,177]. However, there are some limitations, when live cells come into play such as the need for labeling the cells with an agent depending on the force and the possibility of external forces to damage the cells. In this work continuous, high throughput, label-free and parallelizable size based cell sorting and separation was performed in symmetrically curved channels taking the advantage of inertial microfluidics and Dean flow physics while avoiding cell death or damage. Cell focusing behavior for different cell types with different flow rates was reported by taking the cell viability and deformability into consideration. The purpose is to understand the curvature effect on inertial fluidics physics as well as to achieve an efficient cell separation. Owing to the fact that the forces acting on the cells alter depending on their location, and the concomitant effect is still unknown, this study has the potential to provide a valuable contribution to the field of inertial microfluidics.

After the determination of the optimum parameters to enrich the circulating tumor cell lines, focusing behavior of MDA-MB-21 (Human breast adenocarcinoma cell line), K562 (human bone marrow chronic myelogenous leukemia cell line), Jurkat (human T lymphocyte peripheral blood acute T cell leukemia cell line) was examined at various Reynolds numbers and Dean numbers. Assessment of cancer cell migration and equilibrium positions of these cells will lead to design futuristic microfluidic innovations for precise detection and diagnosis of cancer in point of care devices with lower costs.

7.2. Results and Discussion

A typical PDMS microfluidics chip consists of one microchannel design with one inlet, and three outlets. The height of the microchannel is $91\text{ }\mu\text{m}$, while the width and radius of curvature are $350\text{ }\mu\text{m}$ and $800\text{ }\mu\text{m}$, respectively. The whole chip comprised of 11 curvilinear geometries, each having the radius of curvature of $800\text{ }\mu\text{m}$ with an angle of curvature of 280 degree . The total chip length was 9.75 mm . An illustration of the device is provided in the Figure 7.1. Our goal is to apprehend the effect of curvature angle on cell focusing and separation and to provide new insight into inertial microfluidics considering curved channels. Since the angle of the curvature has momentous effects on focusing; the geometry of the micro device is mainly based on this feature. As the angle of the curvature increases, the Dean flow gets stronger hence the strength of the secondary flow proportionally depends on the angle of the curvature. According to this, sudden rotational change (between the inner and outer wall) leads to a better separation efficiency rather than serpentine channels where the angle of curvature is 180° . Additionally, this design is compatible with high throughput designs since many parallel channels could be placed in a rather small footprint area.

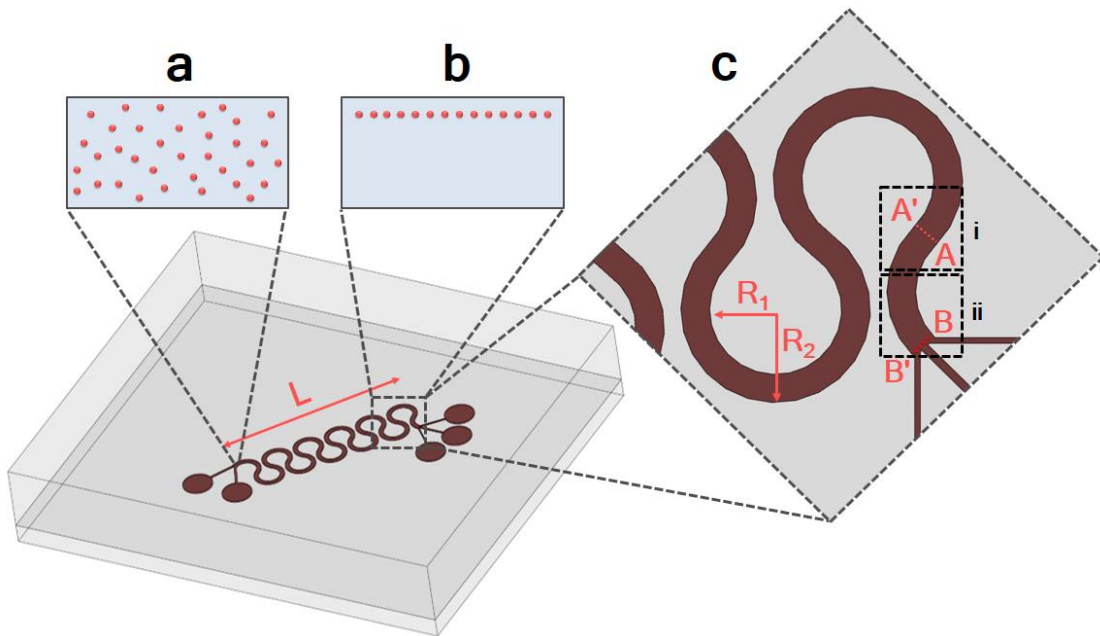


Figure 7.1: The randomly dispensed cells in the inlet region (a) focus into a single particle stream at the AA' cross section (at the transition region) (b). (c) A close-up view of the channel transition region (i) and the outlet region (ii). ($R_1 = 800\text{ }\mu\text{m}$ and $R_2 = 1150\text{ }\mu\text{m}$ and $L = 9.75\text{ mm}$)

To better comprehend how inertial lift and Dean drag forces affect the cell motion in curvilinear microchannels, we examined cell distribution in the transition region at various flow rates (400-2500 $\mu\text{l}/\text{min}$). As explained in Chapters 5 and 6, separation potential was observed only in the transition region. Based on this data, focusing experiments of cancer cell lines of Jurkat (11-14 μm), MDA (20-24 μm) and K562 (16-20 μm) were conducted only in the transition region. Due to the sudden rotational change, the inner wall becomes the outer wall and vice versa. Hence, at the transition region, the walls cannot be defined as outer and inner walls. The walls labelled at the transition region as W_1 for wall between A and B and as W_2 for wall between A' and B'. For each cell line type, channel Reynolds number (Re_c) was increased by increasing the flow rate until focusing was disturbed by dominant Dean drag force. the Re_c maps and representative row images are given in Figure 7.2 to clearly display the particles focusing positions and focusing widths over a wide range of channel Reynolds numbers. The bottom and top positions of each figure in the transition region represent walls of W_2 and W_1 , respectively.

To better understand the relation between the forces in a flow, a cross sectional perspective is crucial. In circular microchannels, particles form a thin equilibrium band along the channel periphery [104,105]. An increase in the a_p/D_h ratio results in accelerated migration of the particles/cells from the microchannel center towards the circumference in consequence of the average shear rate experienced by particles/cells augmenting with increase in the surface area. As particles/cells approach to the channel walls, an asymmetric wake induced around particle introduced a force, named wall induced lift force, on the particle/cell directed through the channel centerline because of increasing pressure. It should be noted that particle/cell will move slightly slower than fluid on this area.

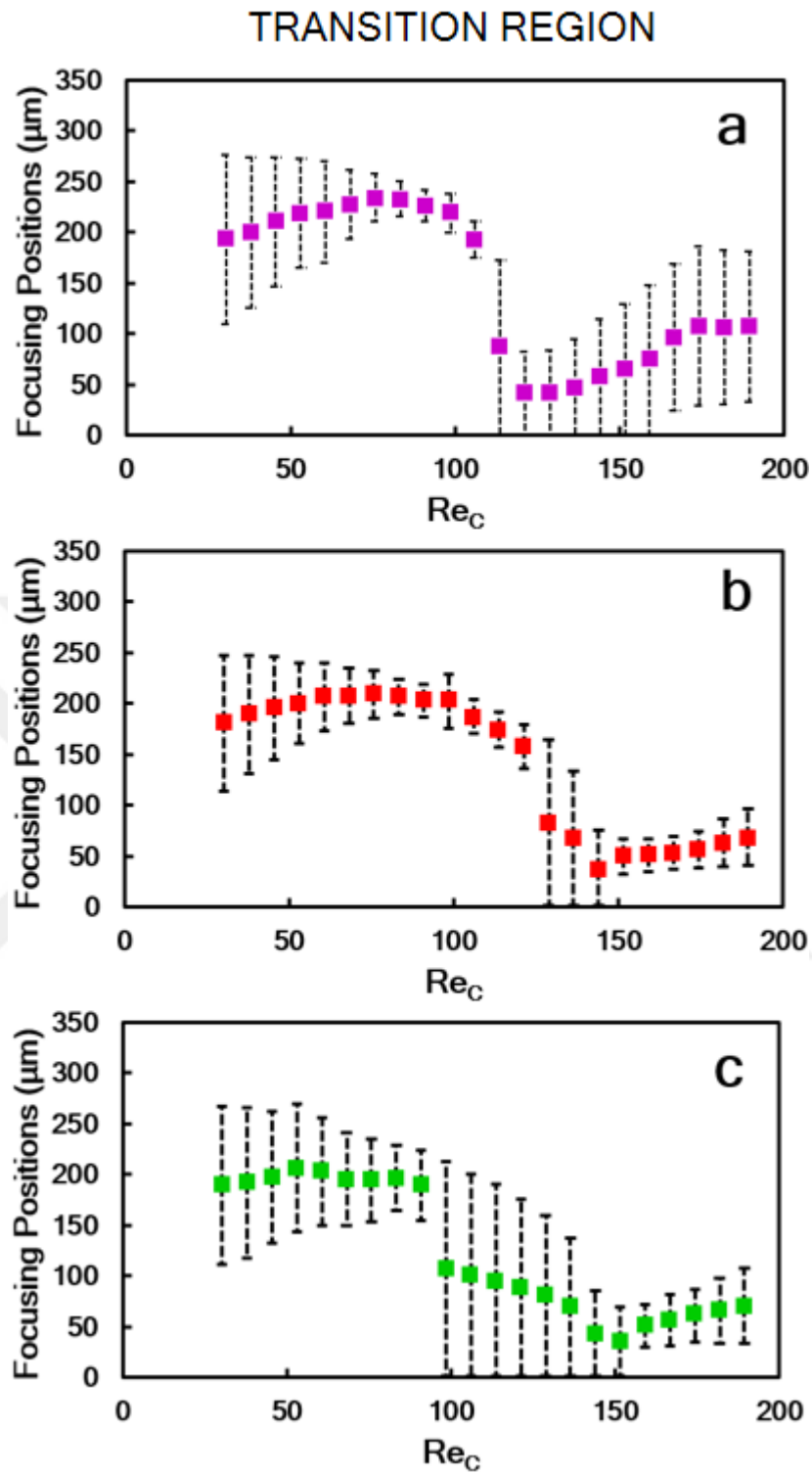


Figure 7.2: Focusing positions of cell lines for the curvilinear microchannel with varying flow rates and corresponding channel Reynolds numbers in the the transition region for cell lines of (a) Jurkat (magenta) (b) MDA (red) (c) K562 (green). Plotted error bars indicate the width of the focusing streak.

At low Re_C , no focusing line can be observed in Jurkat cell lines, instead, cells form a very wide band near the centerline due to relatively small inertial lift and Dean drag forces (figures 7.2 and 7.3). Increasing the Re_C ($Re_C \sim 83$) results in formation of thinner focusing band (focusing width of $35 \mu m$) near the centerline, slightly closer to the W_1 . However, the focusing band migrates through the centerline when flow rate is increased because the direction of F_s is from velocity maxima to the inner wall, F_d direction is from the outer wall to the inner wall and F_s increases more than F_d ($F_s \propto U_f^2$, $F_d \propto U_f^{1.63}$). A sudden defocusing behavior is observed when increasing Re_C to $Re_C \sim 114$ because vertical position of small particles approaches to center of the channel, passes the zero Dean velocity line and as a result, Dean drag force direction is reversed. At this flow rate, Dean drag force and shear gradient lift force directions are the same through the W_2 . With a further increase in Re_C , a transverse motion of focusing cell line is observed. As a result of high curvature ratio, transverse motion of cell streams can be observed around a turn as a function of increasing channel Reynolds number. Cells do not migrate along the essential flow stream but perpendicular to this stream as they move closer to the W_2 at the transition region instead of remaining at channel center. This implies that cell migration velocity U_C is perpendicular to the average fluid velocity U_f at the transition region. A further increase in Re_C to $Re_C \sim 144$ results in a mixing effect of the particles because Dean drag force overcomes to shear gradient force. As mentioned before, lift coefficient decreased increasing the flow velocity and at some point Dean drag force becomes larger than shear gradient lift force.

Likewise, MDA cell lines initially focus in a wide band (around $130 \mu m$) near the centerline at low Re_C ($Re_C \sim 30$) (figures 7.2 and 7.3) due to relatively small inertial lift and Dean drag forces combined with low flow velocity. With an increase in the Re_C ($Re_C \sim 83$), a single cell streak with a width of almost twice the diameter of a single cell near the centerline is seen at transition region, where the streak is slightly closer to W_1 at transition region. With increasing flow rate, both F_s and F_d increases ($F_L \propto U_f^2$, $F_D \propto U_f^{1.63}$). With a further increase in the flow velocity, the lift coefficient decreases and the increase in the shear lift force becomes less than that of Dean drag force. As a result,

this stream migrates to wall W_2 at the transition region. Particle stream approaches to W_2 at higher Re_C ($Re_C > 172$), in the transition region, hence a wall induced lift force comes into play, which pushes particles through the centerline. On the other hand, shear gradient lift force is still present and pushes particles from velocity maxima to W_2 . MDA cell lines also approach to the W_2 at high Re_C , indicating a transverse motion of the cells along the channel, where the particles do not follow the essential flow stream but instead migrate perpendicular to this stream.

According to our observations, focusing behavior of K562 cell lines are very similar to MDA cell lines possibly due to the small difference in size between them (approximately 1-2 μ m). At the lowest channel Reynolds number ($Re_C \sim 30$), a wide focusing stream with approximately 160 μ m width is seen for K562 cell lines, which is wider than that for MDA ones. With an increase in the channel Reynolds number, both cell lines focusing stream becomes tighter. This behavior continues up to the channel Reynolds number of 129 for MDA cell lines, while at lower Reynolds number ($Re_C \sim 98$) K562 cell lines start to defocus and fill the nearly half of the channel near the W_2 . The decrease in focusing line width near the W_2 continues up to channel Reynolds number of 152, beyond which the focusing line moves away from the wall.

The geometry of the current device results in different sorting locations for each cell line. Gossett et al. observed similar behavior with particles passing through the curved channels [129]. The results also agree with Martel and Toner's results stating that considering the simple force ratio is not enough for the new designs [130,131]. Taking into account that focusing capability of the micro device cannot be determined just by the forces, there are additional parameters for determining the location of focusing line such as a cell dimension, cell location across the channel, shape of cells, deformability.

Qualitatively, cells are chosen according to their diameter dimensions; Jurkat (11-15 μ m) (this cell line was chosen for mimicking the white blood cells), MDA (15-20 μ m) and K562 (15-20 μ m). In this thesis, we were able to confirm that effects of inertial forces depend on sizes of the cells. For instance, inertial forces have more effects on bigger cells ($F_L/F_D \sim a^3$). The smaller cells exhibit focusing in two regions; near the centerline for $83 \leq Re_C \leq 106$ and near the W_2 for $121 \leq Re_C \leq 144$. Based on the quantification of the largest cells, it is observed that they focus near the centerline for $68 \leq Re_C \leq 114$ and near

the W_2 for $144 \leq Re_C \leq 189$. Thus, it can be deduced that every cell has distinct focusing location across the channel due to size difference. However, intriguingly, the transition between the different regimes of focusing appears the same for various sizes of the cells.

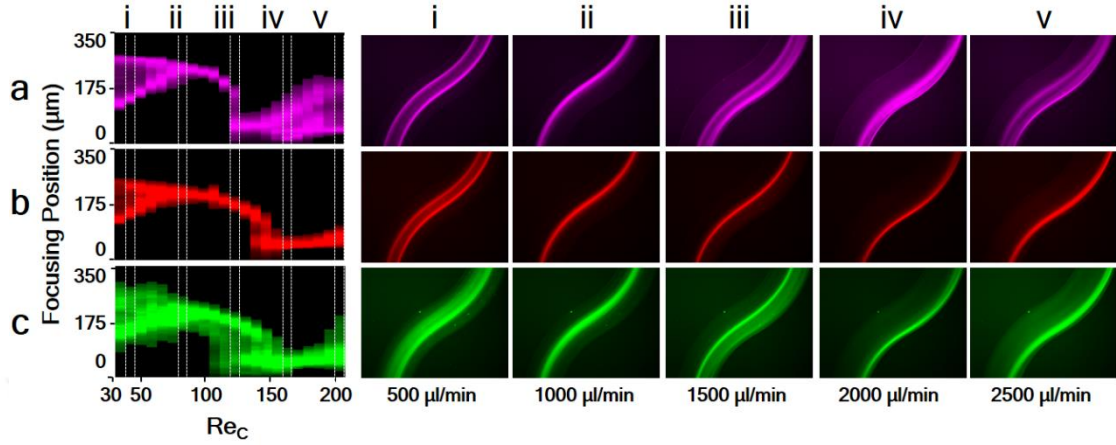


Figure 7.3: Re_C maps: at the transition region (a) Jurkat cell line (magenta), (b) MDA cell line (red), (c) K562 cell line (green). Cell line streams for different channel Reynolds numbers corresponding to five selected flow rates between 500 and 2500 μ L/min (i–v).

Moreover, the effects of cell shape and deformability must be also underlined. It should be considered that many cells in real systems are non-spherical. Paxricle's/Cell's shape (symmetric or asymmetric shape) has an impact on lifting forces acting on the particle/cell. In recent studies, Hur et al. and Masaeli et al. showed that above a critical Reynolds number ($Re \sim 50$) particles predominantly adopted preferred equilibrium positions and “tumbling” rotational axis [165,166]. This rotational axis focusing position was found to be independent of cross-sectional shape and mainly depended on rotational diameter. During the tumbling motion, when the major axis rotates perpendicular to the wall, wall effect lift increases substantially due to the closer distance. As a result, the combined force on the particle/cell is higher than that of a sphere of the same volume. Our approximation in this study is generally based on the cells having an ellipsoidal shape. This estimation can be justified with the fact that cells are elastic and as they move in a fluid at a high velocity they will become more longitudinal. Thus, deformability of bioparticles/cells can also lead additional lift forces. As the cell flows along the channel, it maintains a shape. In contrast with a symmetric particle, deformable particle can have a net lift leading to its lateral migration. Hur et al. experimentally demonstrated that in

Poiseuille flow, the direction of deformability-induced lift force is towards the center. As the deformability induced lift force increases, cells shift towards the channel center. Counterintuitively, as the viscosity ratio decreases, the focusing position shifts back towards to the wall. Thus, the velocity of migration of the cell stream becomes affected by deformability as a result of a smaller the overall wall directed lift force magnitude. Accordingly, for achieving the equilibrium position, the length of the channel should be enough. Therefore, we designed our channel with more number of curvatures to have a better design regarding this issue.

As previously mentioned, spiral type microchannels have been extensively used in the literature for focusing or separating particles/cells [227]. Our design allows occupying the same footprint as straight channels, which makes parallelization possible with sufficiently parallel optical detection integration as well as achieving high efficiency similar to spiral channels. This feature is useful for ultra-high throughput applications such as flow cytometers with the advantages of reducing cost and size.

Our experimental observations highlight that there is a shift in particle/cell focusing behavior between transition and outlet regions. Our findings suggest that carefully designing outlets at the transition region in curvilinear channels, could easily offer a better separation or enrichment capability. Fluorescent images of cell separation and enrichment potential of the curvilinear channel with curvature angle of 280° are illustrated in figure 7.4. The optimum separation of Jurkat and MDA cell lines is achieved at $Re_C \sim 121$ at transition region in the asymmetric curvilinear channel with a curvature angle of 230° . The corresponding distance between the particle/cell focusing streams of Jurkat and MDA cell lines is approximately $95 \mu m$ (figure 7.4a). As illustrated in figure 7.4b, the enrichment of Jurkat and K562 cell lines can be achieved at $Re_C \sim 121$ at transition region since large particles/cells pass through near the centerline, while most of small particles/cells travel between the centerline and W_2 . It should be noted that white color represents combination of Jurkat and K562 cell lines.

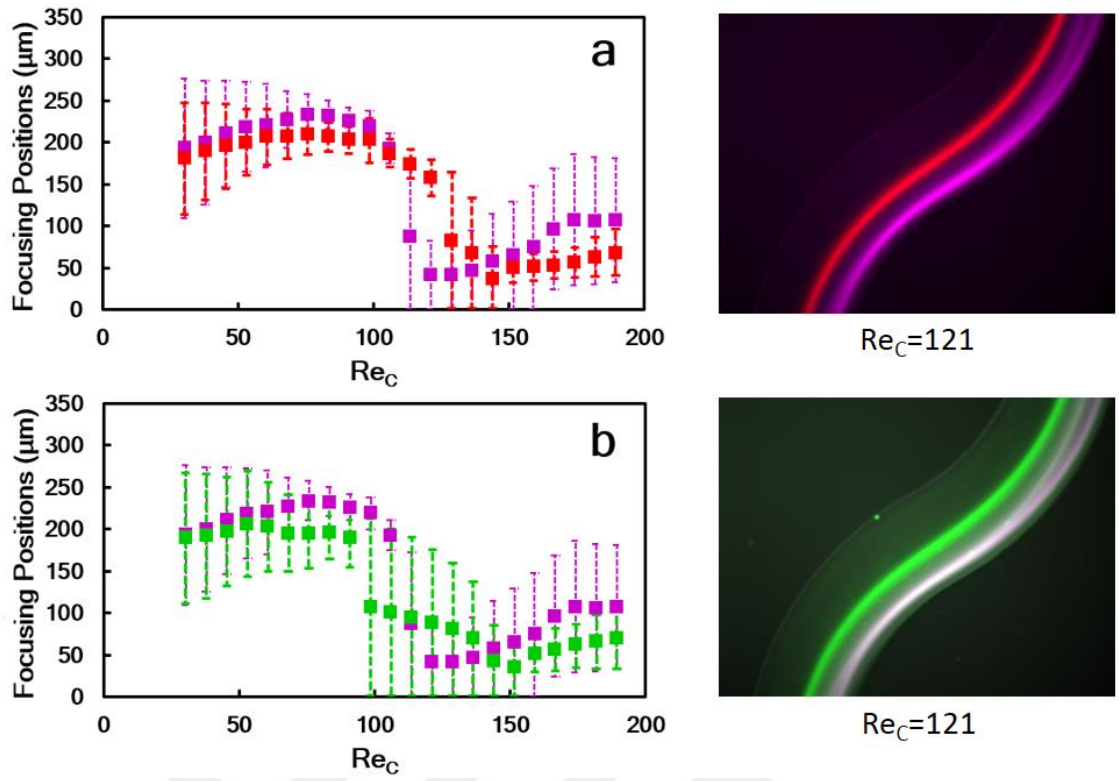


Figure 7.4: Focusing positions of cancer cell lines at transition region in the curvilinear channel with a curvature angle of 230° for different flow rates (left column), cell streams for optimum separation or enrichment potential channel Reynolds number (right column) a) Jurkat and MDA b) Jurkat and K562 cell lines.

CHAPTER 8

8. SUMMARY

A variety of fundamental focusing and separation approaches has been considered with synthetic microparticles in the framework of microsystems. Among these techniques, inertial focusing has gained significant attention because it offers high throughput and effective and precise control for particle and cell manipulation. Despite being an actively studied topic, inertial particle focusing behavior and its underlying mechanisms have not been yet fully understood. Different channel types such as straight, serpentine, spiral and straight with contraction–expansion arrays were used in inertial microfluidics, while the separation of particles with serpentine channels has not attracted as much attention as the other types. Motivated by the promising results, this thesis focuses on inertial focusing in curvilinear channels. This thesis makes several important contributions to the literature of inertial microfluidics along with some major conclusions:

- A) The effect of curvilinearity with curvature angle of 280° , 230° and 180° on particle focusing behavior was examined in this thesis by performing inertial focusing of $10\text{ }\mu\text{m}$, $15\text{ }\mu\text{m}$ and $20\text{ }\mu\text{m}$ fluorescent polystyrene microparticles at different channel Reynolds numbers (30-205). Furthermore, the decoupling effect of inertial and Dean drag forces on particles and their separation potential were revealed. Because the forces acting on the particles vary depending on their location, the concomitant effect remains unknown. The results showed that a larger curvature degree offered better separation or enrichment efficiency.
- B) The effect of channel asymmetry was studied. Breaking symmetry resulted in better separation potential especially for the $10\text{ }\mu\text{m}$ particles.
- C) In addition, it was revealed that not only lateral positions but also vertical positions of particles depend on the flow velocity, which dramatically alters the force balance on the particles. Moreover, it was reported that smartly designing

outlets at transition region leads to a better separation or enrichment potential in curvilinear channels. As a result, this study has the potential of providing a valuable contribution to the field of inertial microfluidics by significantly improving our understanding about curvature angle effect and three-dimensional particle dynamics in curvilinear channels.

- D) Furthermore, inertial focusing of cancer cell lines (MDA, K562 and Jurkat) in curvilinear channel with a curvature angle of 280° was presented for a wide range of channel Reynolds numbers. The results indicated that separation of MDA and Jurkat cell lines (Jurkat cell lines were used to mimic white blood cells) with high efficiency is possible at the transition region of the symmetric curvilinear channels. However, not the pure separation but enrichment of K562 and Jurkat cell lines may be achieved.
- E) A continuous, high-throughput and parallelizable size-based particle focusing technique with a high separation or enrichment potential was developed in a specific symmetrical curved channel by taking advantage of inertial microfluidics and Dean flow physics. The design allows almost the same footprint to be occupied as straight channels, which enables parallelization with parallel optical detection integration and achieves high efficiency relative to spiral channels.
- F) The optimum Reynolds number for separation potential of 20 and 15 μm particles for symmetric curvilinear channels with curvature angles of 280° and 230° and asymmetric curvilinear channels with curvature angle of 280° is 144. However, we found that separation of these particles in symmetric curvilinear channels with curvature angle of 180° is almost impossible. Furthermore, Jurkat and MDA cells can be separated using curvilinear channels with curvature angle of 280° when Reynolds number is 144.

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