University of Wollongong

Research Online

Faculty of Engineering and Information
Sciences - Papers: Part B

Faculty of Engineering and Information
Sciences

2017

Flow rate-insensitive microparticle separation and filtration using a microchannel with arc-shaped groove arrays

Qianbin Zhao

University of Wollongong, qz260@uowmail.edu.au

Dan Yuan

University of Wollongong, dy983@uowmail.edu.au

Sheng Yan

University of Wollongong, sy034@uowmail.edu.au

Jun Zhang

Nanjing University of Science and Technology, jz218@uowmail.edu.au

Haiping Du

University of Wollongong, hdu@uow.edu.au

See next page for additional authors

Follow this and additional works at: <https://ro.uow.edu.au/eispapers1>

Part of the [Engineering Commons](#), and the [Science and Technology Studies Commons](#)

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au

Flow rate-insensitive microparticle separation and filtration using a microchannel with arc-shaped groove arrays

Abstract

Inertial microfluidics can separate microparticles in a continuous and high-throughput manner, and is very promising for a wide range of industrial, biomedical and clinical applications. However, most of the proposed inertial microfluidic devices only work effectively at a limited and narrow flow rate range because the performance of inertial particle focusing and separation is normally very sensitive to the flow rate (Reynolds number). In this work, an innovative particle separation method is proposed and developed by taking advantage of the secondary flow and particle inertial lift force in a straight channel ($AR = 0.2$) with arc-shaped groove arrays patterned on the channel top surface. Through the simulation results achieved, it can be found that a secondary flow is induced within the cross section of the microchannel and guides different-size particles to the corresponding equilibrium positions. On the other hand, the effects of the particle size, flow rate and particle concentration on particle focusing and separation quality were experimentally investigated. In the experiments, the performance of particle focusing, however, was found relatively insensitive to the variation of flow rate. According to this, a separation of 4.8 and 13 μm particle suspensions was designed and successfully achieved in the proposed microchannel, and the results show that a qualified particle separation can be achieved at a wide range of flow rate. This flow rate-insensitive microfluidic separation (filtration) method is able to potentially serve as a reliable biosample preparation processing step for downstream bioassays.

Disciplines

Engineering | Science and Technology Studies

Publication Details

Zhao, Q., Yuan, D., Yan, S., Zhang, J., Du, H., Alici, G. & Li, W. (2017). Flow rate-insensitive microparticle separation and filtration using a microchannel with arc-shaped groove arrays. *Microfluidics and Nanofluidics*, 21 (55), 1-11.

Authors

Qianbin Zhao, Dan Yuan, Sheng Yan, Jun Zhang, Haiping Du, Gursel Alici, and Weihua Li

Flow rate-insensitive microparticle separation and filtration using a microchannel with arc-shaped groove arrays

Qianbin Zhao^a, Dan Yuan^a, Sheng Yan^a, Jun Zhang^{*b}, Haiping Du^c, Gursel Alici^a and Weihua Li^{*a}

^a School of Mechanical, Materials and Mechatronic Engineering, University of Wollongong, Wollongong, NSW 2522, Australia, Fax: +61 2 4221 3238; Tel: +61 2 4221 3490; E-mail: weihuali@uow.edu.au

^b School of Mechanical Engineering, Nanjing University of Science and Technology, Nanjing 210094, China; E-mail: junzhang@njust.edu.cn

^c School of Electric, Computer and Telecommunication Engineering, University of Wollongong, Wollongong, NSW 2522, Australia

Abstract

Inertial microfluidics can separate microparticles in a continuous and high-throughput manner, and is very promising for a wide range of industrial, biomedical and clinical applications. However, most of the proposed inertial microfluidic devices only work effectively at a limited and narrow flow rate range because the performance of inertial particle focusing and separation is normally very sensitive to the flow rate (Reynolds number). In this work, an innovative particle separation method is proposed and developed by taking advantage of the secondary flow and particle inertial lift force in a straight channel ($AR=0.2$) with arc-shaped groove arrays patterned on the channel top surface. Through the simulation results achieved, it can be found that a secondary flow is induced within the cross-section of the microchannel and guides different-size particles to the corresponding equilibrium positions. On the other hand, the effects of the particle size, flow rate and particle concentration on particle focusing and separation quality were experimentally investigated. In the experiments, the performance of particle focusing, however, was found relatively insensitive to the variation of flow rate. According to this, a separation of 4.8 μm and 13 μm particle suspensions was designed and successfully achieved in the proposed microchannel, and the results show that a qualified particle separation can be achieved at a wide range of flow rate. This flow rate-insensitive microfluidic separation and filtration method is able to potentially serve as a reliable biosample preparation processing step for downstream bioassays.

1 Introduction

Microfluidic technology has been popular and attracted a wide attention because of its potential applications in the fields of disease diagnostics and biological assays (Adams et al. 2008; Hoshino et al. 2011; Nagrath et al. 2007). Separation or filtration of target microparticles from a mixture based on the particle physical and biological characteristics is one of the most essential requirements in many biomedical applications, such as blood preparation (Lee et al. 2011a) and circulating tumor cells (CTCs) isolation (Lee et al. 2013). Compared with the conventional separation techniques, such as differential centrifugation and physical filter, the microfluidic technique could be a simpler and more efficient method, which avoids the destruction of cell viability (Xie et al. 2010) due to a high acceleration and intensive labour to clean the filter.

According to the principle utilised, microfluidic separation technologies can be categorized as either the active or passive microfluidic separation method. The active separation methods include the acoustophoretic (AP) (Shi et al. 2008), dielectrophoretic (DEP) (Çetin and Li 2011; Li et al. 2012), magnetophoretic (MP) (Liu et al. 2009) and fluorescent-activated separation method (Wang et al. 2005), which rely on external force fields as the source of manipulation force. In contrast, the passive separation methods depend entirely on the channel geometry and intrinsic hydrodynamic force for functionality, including the deterministic lateral displacement (DLD) (Huang et al. 2004; McGrath et al. 2014), pinched-flow fractionation (PFF) (Yamada et al. 2004), hydrophoresis (Choi et al. 2007; Choi et al. 2008) and inertial microfluidics (Di Carlo 2009; Zhang et al. 2016). The active separation method always has a better ability to control the target particle precisely and high separation efficiency. The intricate fabrication to introduce the external field and requirement of pre-treatment steps, however, can be the obstacle for their integration with downstream analysis and makes the operation of most active separation methods rather complex (Bhagat et al. 2010). Compared with the active separation method, the passive method always only requires a simple fabrication of device but has the high-throughput and easy-integration characteristics, which is able to be adopted in many biological assays (Amini et al. 2014).

Inertial microfluidics relying on the particle inertial migration and secondary flow can provide qualified particle focusing and separation functionality in a high-throughput manner (Di Carlo 2009). Meanwhile, inertial microfluidics is label-free, which can avoid the potential damage on target bioparticles by labelling (Hur et al. 2012). In the regime of inertial microfluidics, the secondary flow is always employed to facilitate the inertial focusing and separation. The performance of inertial microfluidic separation method can benefit from the secondary flow to: (1) eliminate the limitations of channel's cross-section shape; (2) reduce the requirement of long length of channel for particle migration; (3) improve the separation efficiency and (4) enhance the applicability of inertial microfluidics for different applications.

Due to the benefits of employing secondary flow into inertial focusing and separation, a controllable secondary flow has been investigated and exploited extensively and deeply in many inertial microfluidic devices. Spiral (Kuntaegowdanahalli et al. 2009), serpentine (Zhang et al. 2014b) and expansion-contraction structures (Lee et al. 2011a) which can induce counter-rotating secondary flow have been studied to separate microparticles by inertial microfluidics. Bhagat *et al.* employed the spiral microchannel taking advantage of the dean flow and particle inertial migration to achieve effective separation at the Reynolds number ~ 5 (the corresponding flow rate was 10 $\mu\text{l}/\text{min}$) (Bhagat et al. 2008). Meanwhile, Park *et al.* reported a separation of polystyrene beads of 4 μm and 10 μm using a contraction-expansion array (CEA) channel at the flow rate of $\sim 242 \mu\text{l}/\text{min}$. Working together with the inertial lift force, the Dean-like secondary flow induced by the CEA

structure guides the large particle to the CEA side, while the small one shifts towards the opposite side through the Dean flow (Lee et al. 2011b). Our group has also developed a continuous separation method using a serpentine channel. A high efficient separation of both large and small polystyrene beads as well as neuron and glial cells can be achieved at the flow rate between 550 $\mu\text{l}/\text{min}$ to 600 $\mu\text{l}/\text{min}$ (Jin et al. 2016). However, in these works, the separation performance is highly affected by the flow rate applied and the available working range is very limited, which requires a precise control of the input flow rate and significantly hinders the on-chip integration with downstream assays.

This paper presents a flow rate-insensitive particle separation and filtration method using a straight channel (AR=0.2) with arc-shaped groove arrays. The arc-shaped groove structure induces a secondary flow vortex, which guides different-size particles into the differential equilibrium positions in order to achieve an effective particle separation by size. The development of the secondary flow vortex induced by the groove structure is investigated numerically, which is critical for the distribution of the microparticles. Besides, effects of the particle size, flow rate and particle concentration on the focusing and separation performance of microparticles in the proposed microchannel are investigated experimentally. Finally, according to the focusing properties of different-size particles in the microchannel, a separation of 13 μm and 4.8 μm particles is successfully demonstrated using the proposed microchannel within a wide range of flow rates. This innovative separation and filtration method shows qualified performance with a wide range of available working flow rates, which is beneficial for the integration of microfluidic system.

2 Theory

2.1 Inertial lift force

When particles travel in a straight channel at a moderate Reynolds number, the inertia of particles and fluids will cause a particle lateral migration (Segre 1961; Segre and Silberberg 1962). The reason of particle migration has been recognised as the counteraction of two primary inertial effects: (1) the shear gradient lift force F_{Ls} and (2) the wall lift force F_{Lw} (Zhang et al. 2016).

The net inertial lift force F_L exerting on a rigid microparticle can be expressed as below (Asmolov 1999):

$$F_L = f_L \rho_f \gamma^2 a^4 \quad (1)$$

Meanwhile, this expression can be simplified as:

$$F_L = f_L \rho_f U^2 a^4 / H^2 \quad (2)$$

$$\text{Re} = \rho_f UH / \mu \quad (3)$$

$$H = 2wh / (w + h) \quad (4)$$

where ρ_f , U , γ and μ are the fluid density, maximum velocity, shear rate and dynamic viscosity, respectively. a is the microparticle diameter. H is the hydraulic diameter of the channel (w is the width and h is the height of the channel). The lift coefficient f_L is a function of the lateral position of particles x and the Reynolds number (Re).

Due to the fabrication limitation of the standard photolithography and soft lithography, a majority of channels utilised in microfluidics have rectangular cross-sections. And the equilibrium position and particle migration are closely related with the variation of the channel's cross-section aspect ratio (AR) (the ratio of channel height to channel width). In a straight channel with the square cross-section (AR=height/width=1), particles are going to migrate to four equilibrium positions centred at the faces of the channel at a moderate Reynolds number (Choi et al. 2011). The four equilibrium positions, however, will gradually disappear as the aspect ratio decreases or increases, and it is found that particles tend to concentrate at two equilibrium positions centred at the two longer faces when aspect ratio reduces to 0.5 (Chung et al. 2013). Afterwards, the two equilibrium positions will spread out to two relatively wide bands paralleling with the longer faces when the aspect ratio continuously reduces to 0.2 (Zhang et al. 2014a). Under this situation, particles are still able to migrate to the longer faces due to the sharp velocity profile between two longer faces. The velocity profile between two shorter faces, however, becomes extremely blunted and the induced weak shear gradient lift force is not strong enough to repel particles away from the centre.

2.2 Secondary flow

Besides the inertial migration, secondary flow is another important role of inertial microfluidics and always generated through a curved channel or disturbance obstacle structure due to the mismatch of the fluid velocity between the centre and near-wall regions (Di Carlo 2009). The fluid near the centre of channel has a stronger momentum compared with the fluid near channel's walls. The fluid, as a result, tends to flow outwards and drive the relatively stagnant fluid near the wall inside to the centre, forming a vortex circulation. For the suspending particles in the channel, a drag force of secondary flow (vortex) will exert on them and affect their distribution.

At a low Reynolds number (typically in the microfluidic system), the drag force exerting on a small rigid sphere can be expressed as follows:

$$F_D = 6\pi a\mu(v_f - v_p) \quad (5)$$

where v_f is the velocity of the fluid, and v_p is the velocity of the particle (Gerlach 1998).

2.3 Theory of differential inertial focusing in the microchannel with grooves

Within the channel with arc-shaped groove arrays used in this work, a secondary flow can be induced because the fluid flow tends to be trapped in the expansion groove structure and generates large transverse pressure differences in the cross-sections. The particles, as a result, can be manipulated by the strong secondary flow. In this work, the ratio of the height of the groove structure and straight channel is set as ~ 1 , which can ensure an enough strong secondary flow induced (Chung et al. 2013).

The arc-shaped groove arrays on the top of the straight channel are composed of 50 same single groove structures. In order to simplify the computational fluid dynamics analysis, a simulation of the segment with one single characteristic arc-shaped groove structure was conducted by the finite element software (Figure 1). The simulation results are illustrated by five cross-section plots, which describe the variation process of the flow field at different positions of the microchannel. It can be seen that the velocity arrows (the black arrow in Figure 1) are deflected by the influence of the arc-shaped groove and point to different directions, which means that the former stabilized flow field is disturbed and interfered by the groove structure. Taking five plots together, the arrows depict a complete secondary flow vortex within cross-sections and this secondary flow gradually shifts

following the rotation direction of the vortex. As shown in Figure 1(a-e), it can be found that the horizontal components of velocity arrows in the straight channel part always point to the Y direction, while the arrows in the groove structure part point to the opposite direction (Hoshino et al.) (Hoshino et al.). This is because that the secondary flow vortex exists in both the straight channel and expansion groove structure, and forms a complete circulation, which means that there is only half of the vortex circulation in the straight channel while another half exists in the groove structure.

Particles are initially located at two long band regions paralleling with long faces of the channel due to the inertial migration when travelling in the straight channel ($AR=0.2$). Then, when particles pass through the segment combined with the groove structure, the induced transverse secondary flow will guide particles in a horizontal and vertical displacement (depicted as the grey arrows in Figure 1) within cross-sections. Because the horizontal component of the flow field direction in the straight channel points to Y direction all the time, particles are going to be pushed to the Y direction as well (shown as the particle distribution in Figure 1 (a-e)). For different-size particles, the particle migration can be different because of the effect of the particle size. Large particles are guided by the secondary flow towards the Y direction and balanced with the inertial lift force near the channel sidewall. After travelling through the enough repetitive grooved structures, all large particles could be focused into a narrow area (nearly a line) near the sidewall. However, the inertial lift force on the small particle is much smaller and is hard to balance the secondary flow drag force. As a result, small particles are prone to follow the rotating streamline of secondary flow. And the only possible focusing position for small particles may be the core of vortex near the channel centre where the magnitude of secondary flow velocity is minimum. So it is expected that a large portion of small particles will be concentrated and focused near the channel centre when they travel through this area, which has been validated by the experimental results. However, it should be noted that a few of small particles may still be retained within the rotating streamlines and not able to be focused.

At the same time, it can be seen that the whole secondary flow vortex is not symmetric and the flow field near the left wall region is more stable than other regions, because the size of the arrow which depicts the magnitude of the secondary flow is smaller compared with other regions. And the horizontal component of arrows is also much smaller near the interface between the straight channel and groove structure, which means that particles there almost receive a vertical drag force merely and this drag force can be balanced by the inertial lift force. As a result, both large and small size particles located at above regions can be more stabilized and the results of experiments even demonstrate that particles can be focused at these equilibrium positions at a wide range of flow rates. Due to differential focusing positions of large and small particles, separation or filtration of different-size particles can be achieved utilising this proposed microchannel with arc-shaped groove arrays.

3 Materials and methods

3.1 Design and fabrication of the microchannel

The microchannel used in this work mainly consists of two parts, a straight channel ($AR=0.2$) with arc-shaped groove arrays, which guides the particle distribution, and an expansion channel with 5 outlets which is used for collection of different-size microparticles (Figure 2a). The front part of the channel is a stacked structure composed of a straight channel ($AR=0.2$) and 50 arc-shaped grooves. For the straight channel, the cross-section is a $200 \times 40 \mu\text{m}$ (width (W) $\times$ height (h)) rectangular. 50 grooves are arranged with $50 \mu\text{m}$ spacing (H_s) on the

top of the straight channel. The groove (shown as Figure 2b) is arc-shaped with a small curvature of 600 μm (R_1) and a large curvature of 650 μm (R_2). And the height of the groove structure (H_g) is 38 μm . Figure 2c is the optical microscopic image of a segment of the channel with groove structures captured by the CCD camera. The details of the fabrication of this microchannel can be found elsewhere (Yan et al. 2014).

3.2 Preparation of particles

The commercial fluorescent polystyrene microparticles were purchased from Thermo Fisher Scientific. The 13 μm (CAT. NO. 36-4, 16%CV) and 3.2 μm particles (CAT. NO. R0300, 5%CV) are red fluorescent, while the 9.9 μm (CAT. NO. G1000, 5%CV) and 4.8 μm particles (CAT. NO. G0500, 5%CV) are green fluorescent. Microparticles were suspended in deionized (DI) water and 0.1% w/v Tween 20 (Sigma-Aldrich, Product NO. P9416) was also added and stirred well to prevent the particle aggregation and adhesion to the channel walls.

3.3 Experiment setup

The microfluidic device was placed on an inverted microscope (CKX41, Olympus, Japan). Particle suspensions were injected into the microchannel by a syringe pump (Legato 100, Kd Scientific) at different flow rates. And a mercury arc lamp was used to illuminate the particle trajectories within the channel for the observation and recording. A CCD camera (Optimos, Q-imaging, Australia) was used to capture the fluorescent images at a 20 ms exposure time. All images were then processed using the image processing software (Q-Capture Pro 7, Q-imaging, Australia). And these images were obtained by stacking 50 consecutive frames in order to analyse the particle distribution.

3.4 Numerical simulation

In order to analyse and explain the mechanism of this filtration method, a numerical simulation of flow field was conducted by the finite element software (COMSOL Multi-physics 5.1, Burlington, MA). A 3D model of the straight channel (AR=0.2) with a single characteristic arc-shaped groove structure was built in COMSOL. The steady laminar flow physics was selected due to the low Reynolds number of the microfluidic system. The boundary condition was set as no slip on the channel walls and the physical property of fluid was chosen as incompressible flow. The order of finite element was set to P1+P1, the default setting of COMSOL Multiphysics. And the component was meshed by the free tetrahedral mesh at the default finer level (1488007 elements in total) to ensure the accuracy of numerical results. At the inlet, the velocity of flow was set as 2.083 m/s (the corresponding flow rate and Reynolds number were 1000 $\mu\text{l/min}$ and 145.83 respectively), and the relative pressure was set as 0 Pa at the outlet. The equations to govern steady incompressible flow are as below:

N-S equation:

$$\rho_f \vec{v}_f \cdot \nabla \vec{v}_f = -\nabla P + \mu \nabla^2 \vec{v}_f \quad (6)$$

Continuity equation:

$$\nabla \cdot \vec{v}_f = 0 \quad (7)$$

Non-slip boundary condition:

$$\vec{v}_w = \vec{0} \quad (8)$$

where $\vec{v}_f$ is the velocity vector and P is the pressure of flow field, respectively; $\vec{v}_w$ is the fluid velocity vector at the channel walls; $\nabla = \vec{i} \frac{\partial}{\partial x} + \vec{j} \frac{\partial}{\partial y} + \vec{k} \frac{\partial}{\partial z}$, and ∇^2 is the Laplace operator: $\nabla^2 = \nabla \cdot \nabla = \vec{i} \frac{\partial^2}{\partial x^2} + \vec{j} \frac{\partial^2}{\partial y^2} + \vec{k} \frac{\partial^2}{\partial z^2}$.

4 Results and discussion

4.1 Effects of the flow rate and particle size

Figure 3a shows the microscopic images of different-size particle trajectory patterns at different flow rates within the microchannel. In this series of experiments, four different-size particles with the diameter of 3.2 μm , 4.8 μm , 9.9 μm and 13 μm were employed to investigate the effects of particle size on focusing performance in the microchannel. And the result is critical for the following particle separation and filtration performance. The particle suspensions were injected into the microchannel through the syringe pump, and the concentration of 3.2 μm , 4.8 μm , 9.9 μm and 13 μm particle suspensions were 5×10^5 counts/ml, 5×10^5 counts/ml, 10^5 counts/ml and 10^5 counts/ml respectively. The input flow rate of this series of experiments was increased from 400 $\mu\text{l/min}$ to 1400 $\mu\text{l/min}$ (the corresponding Reynolds number is from 58.332 to 204.162). First, it was observed that the particle focusing results were different between large particles (9.9 μm and 13 μm in diameter) and small particles (3.2 μm and 4.8 μm in diameter), which agreed well with the proposed focusing theory. The small particles were prone to be concentrated near the middle of the channel, while the large particles tended to be focused to one side of the channel. From the normalized intensity profiles of large particles (9.9 μm and 13 μm) at different flow rates (Figure 3b), it was found that particles were pushed to one side of the channel and concentrated at one equilibrium position. Meanwhile, the focusing performance was improved when the flow rate increased from 400 $\mu\text{l/min}$ to 1200 $\mu\text{l/min}$. The reason is that a higher flow rate could induce a stronger secondary flow within the channel so that the particles guided by the secondary flow drag force were able to be concentrated more quickly and efficiently. The focusing performance, however, was gradually worse as the flow rate exceeded 1200 $\mu\text{l/min}$. This may be explained by the fact that the violent interaction between particles destroyed the stabilized focusing patterns when a stronger drag force exerting on particles in a small region. On the other hand, from the normalized intensity profiles of small particles (3.2 μm and 4.8 μm) at different flow rates (Figure 3b), the small particle focusing performance was insensitive with the increase of the flow rate compared with the large particles. The small particles could be concentrated well at a wide range of the flow rate from 600 $\mu\text{l/min}$ to 1400 $\mu\text{l/min}$. However, the small particles were also found to be trapped into the groove structure due to the small size, and a small proportion of them were still not able to be focused at the end of the channel.

In order to analyse and evaluate the particle trajectory pattern and focusing performance quantitatively, the particle focusing band was measured as the length between two points where the intensity crossed 50% threshold in the normalized intensity profile. And if the measured focusing band width is less than 2 times the diameter of the particle, it could be recognized as a successful particle focusing (Martel and Toner 2012). The different-size particle focusing patterns at the flow rate of 1000 $\mu\text{l/min}$ were chosen to be assessed by this method, and we found that all the tested particles had a well-qualified focusing performance. As shown in the Figure 3b, the normalized intensity profile of the particle trajectory patterns demonstrated that different-size particles could be focused well at the flow rate of 1000 $\mu\text{l/min}$, as the measured focusing bands were all smaller than 2 times the diameters of particles. In the normalized intensity profile of 3.2 μm particles, however, it should

be noted that the fluorescent intensities measured were not all concentrated near the equilibrium position within the channel, which should not be ignored compared with other particles. The drag force and inertial lift force exerting on 3.2 μm particles could be not strong enough to reach a stabilized condition and particles with small size might have a high possibility to be trapped into the groove structure even at the equilibrium position. Therefore, a number of 3.2 μm particles still dispersed randomly within the channel and cannot be concentrated. In summary, the experimental results agreed well with the proposed theory, but the particle size was found to be a very important factor for the focusing efficiency. Large particles (13 μm and 9.9 μm) were focused well in this channel, while the focusing performance of small particles was affected severely by the particle size.

4.2 Effects of the particle concentration

The concentration of particle suspensions was one of the influence factors to the separation and filtration throughput. Under the same condition, increasing the concentration of particle is an effective method to achieve a high-throughput yield. However, the higher particle concentration may influence the particle focusing performance as well. Therefore, we conducted a series of experiments on 9.9 μm particle suspensions with three different concentrations (10^5 counts/ml, 5×10^5 counts/ml and 10^6 counts/ml respectively). The flow rate was increased from 200 $\mu\text{l/min}$ to 1400 $\mu\text{l/min}$. The microscopic fluorescent images (Figure 4a) were the particle trajectory patterns of suspensions with different concentrations captured at the same segment of the channel. Through the comparison of corresponding normalized intensity profile (Figure 4b), it can be found that as the particle suspensions concentration increased, the particle focusing band gradually became wider. The focusing performance and position, however, were nearly the same on the same flow condition. The reason of the wider particle focusing band can be explained that larger quantities of particles were travelling within the channel and concentrated at the equilibrium position in order that more intensive interaction between particles was induced. To evaluate the focusing performance quantitatively, the focusing band width of three different concentration particle suspensions at the flow rate of 1000 $\mu\text{l/min}$ was measured by the assessment method mentioned above. Shown as the Figure 4b, except the measured focusing band width of 10^5 counts/ml particle suspensions, another two focusing band width were both larger than 2 times the diameter of the particle (20 μm), which indicates the deterioration of the focusing quality of 9.9 μm particles with higher concentration. This is not beneficial for single stream particle focusing. However, for particle separation and filtration, the particle focusing performance and distribution of 5×10^5 counts/ml and 10^6 counts/ml particle suspensions are still qualified because there is significant distance between the different-size particle focusing bands. Therefore, the focusing position and band width are the key factors for the success of particle separation and the increase of particle concentration could be an effective approach to improve the throughput yield.

4.3 Particle separation by size within a wide flow rate

To evaluate the separation performance of this method, the particle suspensions containing 13 μm particles (8×10^4 counts/ml) and 4.8 μm particles (10^6 counts/ml) were injected into the channel with arc-shaped groove arrays. The input flow rates were set as 400 $\mu\text{l/min}$, 600 $\mu\text{l/min}$, 800 $\mu\text{l/min}$, 1000 $\mu\text{l/min}$ and 1200 $\mu\text{l/min}$ to investigate the optimal flow rate range for particle separation. As shown in Figure 5, the microscopic images at the segment with arc-shaped groove arrays show that 13 μm (red) and 4.8 μm particles (green) was successfully separated at the flow rates of 600 $\mu\text{l/min}$, 800 $\mu\text{l/min}$, 1000 $\mu\text{l/min}$ and 1200 $\mu\text{l/min}$. And the images at expansion part with 5 outlets show that a majority of 13 μm particles could be collected from the outlet i and

very few 13 μm particles entered the outlet ii within this flow rate range. Meanwhile, 4.8 μm particles trajectory patterns at the outlets did not change much from the flow rate 400 $\mu\text{l/min}$ to 1200 $\mu\text{l/min}$. Most of them flowed into the outlet iv, while few 4.8 μm particles were dispersed randomly within the channel and flowed into other outlets. Through the statistical calculation of the data collected, we calculated the purity and recovery rate of 13 and 4.8 μm particles (Jin et al. 2014; Loutharback et al. 2012; Zhou et al. 2013). The purity of 4.8 μm particle was >99.9% and recovery rate was ~80%. On the other hand, the recovery rate of 13 μm particle was >99.9% and the purity was ~35%. The data were collected at different flow rates within the flow rate range to confirm the critical value.

5 Conclusions

A flow rate-insensitive size-based microparticle separation and filtration method has been studied in this work by using a straight channel with arc-shaped groove arrays. In this method, different-size particles can be focused differentially at the distinct equilibrium positions, and the focusing performance is insensitive with the change of flow rate. Through the simulation analysis of flow field within the channel cross-sections, it can be found that a secondary flow is induced by the arc-shaped groove structure and guides the randomly distributed particles to new equilibrium positions, which large particles are pushed to one side of the channel and small particles are concentrated near the middle of the channel. Meanwhile, the focusing quality is improved with the increase of particle size, and particle focusing position and quality is not very sensitive to the flow rate within the tested flow rate range. According to these, particle suspensions containing 13 μm and 4.8 μm particles were successfully separated using this channel, and a qualified efficiency of separation could be achieved at a wide range of flow rates (600 $\mu\text{l/min}$ to 1200 $\mu\text{l/min}$). In conclusion, this research provides an innovative particle separation and filtration method with a wide working flow rate range and could work as a reliable biosample pre-treatment for downstream analysis.

Acknowledgement

This work is partially supported by UOW-CSC Scholarship.

References

- Adams AA et al. (2008) Highly efficient circulating tumor cell isolation from whole blood and label-free enumeration using polymer-based microfluidics with an integrated conductivity sensor *Journal of the American Chemical Society* 130:8633-8641
- Amini H, Lee W, Di Carlo D (2014) Inertial microfluidic physics *Lab on a Chip* 14:2739-2761
- Asmolov ES (1999) The inertial lift on a spherical particle in a plane Poiseuille flow at large channel Reynolds number *Journal of Fluid Mechanics* 381:63-87
- Bhagat AAS, Bow H, Hou HW, Tan SJ, Han J, Lim CT (2010) Microfluidics for cell separation *Medical & Biological Engineering & Computing* 48:999-1014
- Bhagat AAS, Kuntaegowdanahalli SS, Papautsky I (2008) Continuous particle separation in spiral microchannels using dean flows and differential migration *Lab on a Chip* 8:1906-1914
- Çetin B, Li D (2011) Dielectrophoresis in microfluidics technology *Electrophoresis* 32:2410-2427
- Choi S, Song S, Choi C, Park J-K (2007) Continuous blood cell separation by hydrophoretic filtration *Lab on a Chip* 7:1532-1538
- Choi S, Song S, Choi C, Park JK (2008) Sheathless focusing of microbeads and blood cells based on hydrophoresis *Small* 4:634-641
- Choi YS, Seo KW, Lee SJ (2011) Lateral and cross-lateral focusing of spherical particles in a square microchannel *Lab on a Chip* 11:460-465

- Chung AJ, Gossett DR, Di Carlo D (2013) Three dimensional, sheathless, and high - throughput microparticle inertial focusing through geometry - induced secondary flows *Small* 9:685-690
- Di Carlo D (2009) Inertial microfluidics *Lab on a Chip* 9:3038-3046
- Gerlach T (1998) Microdiffusers as dynamic passive valves for micropump applications *Sensors and Actuators A: Physical* 69:181-191
- Hoshino K, Huang Y-Y, Lane N, Huebschman M, Uhr JW, Frenkel EP, Zhang X (2011) Microchip-based immunomagnetic detection of circulating tumor cells *Lab on a Chip* 11:3449-3457
- Huang LR, Cox EC, Austin RH, Sturm JC (2004) Continuous Particle Separation Through Deterministic Lateral Displacement *Science* 304:987-990
- Hur SC, Brinckerhoff TZ, Walthers CM, Dunn JC, Di Carlo D (2012) Label-free enrichment of adrenal cortical progenitor cells using inertial microfluidics *PLoS One* 7:e46550
- Jin C, McFaul SM, Duffy SP, Deng X, Tavassoli P, Black PC, Ma H (2014) Technologies for label-free separation of circulating tumor cells: from historical foundations to recent developments *Lab on a Chip* 14:32-44
- Jin T, Yan S, Zhang J, Yuan D, Huang X-F, Li W (2016) A label-free and high-throughput separation of neuron and glial cells using an inertial microfluidic platform *Biomicrofluid* 10:034104
- Kuntaegowdanahalli SS, Bhagat AAS, Kumar G, Papautsky I (2009) Inertial microfluidics for continuous particle separation in spiral microchannels *Lab on a Chip* 9:2973-2980
- Lee MG, Choi S, Kim H-J, Lim HK, Kim J-H, Huh N, Park J-K (2011a) Inertial blood plasma separation in a contraction–expansion array microchannel *Applied Physics Letter* 98:253702
- Lee MG, Choi S, Park J-K (2011b) Inertial separation in a contraction–expansion array microchannel *Journal of Chromatography A* 1218:4138-4143
- Lee MG, Shin JH, Bae CY, Choi S, Park J-K (2013) Label-free cancer cell separation from human whole blood using inertial microfluidics at low shear stress *Analytical chemistry* 85:6213-6218
- Li M, Li S, Cao W, Li W, Wen W, Alici G (2012) Continuous particle focusing in a waved microchannel using negative dc dielectrophoresis *Journal of micromechanics and microengineering* 22:095001
- Liu C, Stakenborg T, Peeters S, Lagae L (2009) Cell manipulation with magnetic particles toward microfluidic cytometry *Journal of Applied Physics* 105:102014
- Loutherback K, D'Silva J, Liu L, Wu A, Austin RH, Sturm JC (2012) Deterministic separation of cancer cells from blood at 10 mL/min *AIP advances* 2:042107
- Martel JM, Toner M (2012) Inertial focusing dynamics in spiral microchannels *Physics of Fluids* (1994-present) 24:032001
- McGrath J, Jimenez M, Bridle H (2014) Deterministic lateral displacement for particle separation: a review *Lab on a Chip* 14:4139-4158
- Nagrath S et al. (2007) Isolation of rare circulating tumour cells in cancer patients by microchip technology *Nature* 450:1235-1239
- Segre G (1961) Radial particle displacements in Poiseuille flow of suspensions *Nature* 189:209-210
- Segre G, Silberberg A (1962) Behaviour of macroscopic rigid spheres in Poiseuille flow Part 2. Experimental results and interpretation *Journal of Fluid Mechanics* 14:136-157
- Shi J, Mao X, Ahmed D, Colletti A, Huang TJ (2008) Focusing microparticles in a microfluidic channel with standing surface acoustic waves (SSAW) *Lab on a Chip* 8:221-223
- Wang MM et al. (2005) Microfluidic sorting of mammalian cells by optical force switching *Nature biotechnology* 23:83-87
- Xie Y, Zheng D, Li Q, Chen Y, Lei H, Pu LL (2010) The effect of centrifugation on viability of fat grafts: an evaluation with the glucose transport test *Journal of Plastic, Reconstructive & Aesthet Surgery* 63:482-487
- Yamada M, Nakashima M, Seki M (2004) Pinched flow fractionation: continuous size separation of particles utilizing a laminar flow profile in a pinched microchannel *Analytical chemistry* 76:5465-5471
- Yan S, Zhang J, Alici G, Du H, Zhu Y, Li W (2014) Isolating plasma from blood using a dielectrophoresis-active hydrophoretic device *Lab on a Chip* 14:2993-3003
- Zhang J, Yan S, Li W, Alici G, Nguyen N-T (2014a) High throughput extraction of plasma using a secondary flow-aided inertial microfluidic device *RSC Advances* 4:33149-33159
- Zhang J, Yan S, Sluyter R, Li W, Alici G, Nguyen N-T (2014b) Inertial particle separation by differential equilibrium positions in a symmetrical serpentine micro-channel *Sci Rep* 4:4527
- Zhang J, Yan S, Yuan D, Alici G, Nguyen N-T, Warkiani ME, Li W (2016) Fundamentals and applications of inertial microfluidics: a review *Lab on a Chip* 16:10-34
- Zhou J, Giridhar PV, Kasper S, Papautsky I (2013) Modulation of aspect ratio for complete separation in an inertial microfluidic channel *Lab on a Chip* 13:1919-1929

Fig. 1 Finite element analysis of flow field at modulated Reynolds number calculated by COMSOL Multi-physics 5.1. The 3D model of a segment with one characteristic arc-shaped groove structure of the microchannel was divided by five cross-sections to investigate the variation of flow field. **(a-e)** These five simulation results are depicted by the combination of the velocity arrow plot and pressure contour plot. The velocity arrow plot shows the velocity direction within cross-sections and the size of arrow is proportional to the magnitude of the flow velocity. The pressure contour plot, meanwhile, shows the variation of pressure induced by the groove structure, which generates and drives a rotational secondary flow within cross-sections. With the simulated flow filed, different-size particle migrations are expected. **(i-v)** are the corresponding colour bars of **(a-e)**, and the units is Pa.

Fig. 2 (a) A schematic drawing of the microchannel (not to scale) used in this work. (b) Zoom in the characteristic arc-shaped groove structure and the characteristic dimensions of it. (c) A optical image of the arc-shaped groove structure captured by the CCD camera.

Fig. 3 (a) Optical microscopic images captured by the CCD camera of different-size particle trajectory patterns. The particle sizes were 3.2 μm, 4.8 μm, 9.9 μm and 13 μm respectively. The flow rates applied were 400 μl/min, 600 μl/min, 800 μl/min, 1000 μl/min, 1200 μl/min and 1400 μl/min (the corresponding Reynolds numbers were 58.332, 87.488, 116.664, 145.83, 174.996 and 204.162). (b) The normalized intensity profile of different-size particle trajectory patterns at 1000 μl/min and the comparisons from 400 μl/min to 1400 μl/min. (i) The 3.2 μm particle's; (ii) The 4.8 μm particle's; (iii) The 9.9 μm particle's; (iv) The 13 μm particle's.

Fig. 4 (a) Optical microscopic images captured by the CCD camera of the 9.9 μm particle trajectory patterns of different concentration suspensions. The concentrations of 9.9 μm particle are (i) 10⁵ counts/ml, (ii) 5×10⁵ counts/ml and (iii) 10⁶ counts/ml respectively, and the flow rates applied were 200 μl/min, 400 μl/min, 600 μl/min, 800 μl/min, 1000 μl/min, 1200 μl/min and 1400 μl/min (the corresponding Reynolds numbers were 29.166, 58.332, 87.488, 116.664, 145.83, 174.996 and 204.162). (b) The normalized intensity profile of 9.9 μm particle trajectory patterns of different concentration particle suspensions at 1000 μl/min and the comparison from 200 μl/min to 1400 μl/min. (i) 10⁵ counts/ml particle suspensions'; (ii) 5×10⁵ counts/ml particle suspensions'; (iii) 10⁶ counts/ml particle suspensions'.

Fig. 5 Separation (filtration) of 13 and 4.8 μm particles. The applied flow rates were 400 $\mu\text{l/min}$, 600 $\mu\text{l/min}$, 800 $\mu\text{l/min}$, 1000 $\mu\text{l/min}$ and 1200 $\mu\text{l/min}$ (the corresponding Reynolds numbers were 58.332, 87.488, 116.664, 145.83 and 174.996). And the optical microscopic fluorescent images of particle trajectory patterns were captured at the segment with arc-shaped groove arrays and expansion channel with 5 outlets. 13 μm particles were red fluorescent and 4.8 μm particles were green fluorescent.