

รายงานวิจัยฉบับสมบูรณ์

ไมโครแชนเนลแบบเกลียวร่วมกับเส้นใยแก้วนำแสงสำหรับแยกและนับอนุภาคแบบต่อเนื่อง

Optical Fiber Integrated Spiral Microchannels for Continuous Particle Separation and
Counting

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โครงการวิจัยนี้ได้รับทุนสนับสนุนจาก เงินรายได้มหาวิทยาลัยสงขลานครินทร์

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ชื่อโครงการ (ภาษาไทย) ไมโครแชลแนลแบบเกลียวร่วมกับเส้นใยแก้วนำแสงสำหรับแยกและนับอนุภาคแบบต่อเนื่อง

ชื่อโครงการ (ภาษาอังกฤษ) Optical Fiber Integrated Spiral Microchannels for Continuous Particle Separation and Counting

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กิตติกรรมประกาศ

ผู้วิจัยขอขอบคุณทุนวิจัยจากเงินรายได้มหาวิทยาลัยสงขลานครินทร์ รหัสโครงการ SCI550147S สำหรับเงินทุนสนับสนุนการวิจัยนี้ และขอขอบคุณอาจารย์ที่ปรึกษา รศ.ดร.ปณิต ถาวรังกูร ที่ให้การสนับสนุนตลอดระยะเวลาทำโครงการ

บทคัดย่อภาษาไทยและภาษาอังกฤษ

Separation of microparticles is involved in numerous industrial and research applications especially in the field of biology, environment and medicine. This work shows the fabrication of spiral microfluidic channels integrated with neodymium magnet for real time separation of the synthetic microparticles with and without magnetic properties. Involving the forces inside the spiral microchannel, suspended non-magnetic particles (6 μm) can be focused near the inner wall of the channel. While the magnetic particle (1 μm) induced with magnetic field can be at the area near the outer wall of the channel. The result shows a very high separation efficiency of 77% and 80% for 6 μm and 1 μm sizes of microparticles, respectively. The merit of the design deals with a simple, cheap and sufficient microparticle separation device. It can be used in diverse applications for fast and low-cost real biological sample (e.g. blood or cells) analysis.

การคัดแยกอนุภาคขนาดเล็กระดับไมโครเมตรมีความสำคัญในทางอุตสาหกรรมและทางการวิจัย โดยเฉพาะทางชีววิทยา สิ่งแวดล้อมและการแพทย์ งานวิจัยนี้แสดงให้เห็นถึงการสร้างท่อการไหลของของ

ไหลที่มีรูปร่างเป็นก้นหอยร่วมกับแม่เหล็กนีโอดีเมียมสำหรับการคัดแยกอนุภาคสังเคราะห์ที่มีและไม่มีคุณสมบัติทางแม่เหล็กออกจากกันแบบเรียลไทม์ โดยใช้หลักการของแรงที่เกิดภายในท่อการไหลที่โค้งงอ อนุภาคที่ไม่มีคุณสมบัติแม่เหล็ก (ขนาด 6 ไมโครเมตร) สามารถถูกโฟกัสไปอยู่บริเวณผนังด้านในของท่อ ในขณะที่อนุภาคที่มีสมบัติแม่เหล็ก (ขนาด 1 ไมโครเมตร) จะถูกเหนี่ยวนำโดยแม่เหล็กนีโอดีเมียมไปยังบริเวณผนังด้านนอกของท่อ ผลการศึกษาแสดงให้เห็นถึงประสิทธิภาพในการแยกที่ดีมาก ให้ประสิทธิภาพ 77% สำหรับอนุภาคขนาด 6 ไมโครเมตร และ 80% สำหรับอนุภาคขนาด 1 ไมโครเมตร ข้อดีของโครงสร้างที่ได้ศึกษาคือ เป็นโครงสร้างที่ง่าย ไม่ซับซ้อน ราคาถูก และมีประสิทธิภาพดีสำหรับการแยกอนุภาคที่มีขนาดต่างกัน อุปกรณ์นี้สามารถนำไปประยุกต์ใช้ได้หลากหลายทั้งทางการแพทย์และการวิจัย เช่นการวิเคราะห์ตัวอย่างจริงทางชีววิทยาเช่น เลือด หรือ เซลล์สิ่งมีชีวิต

Introduction

Separation of microparticles (e.g. cells or synthetic particles) by size is an important step in many applications such as medical assays, environmental and biochemical studies [1-3]. Particle separation using microfluidic system has currently received a lot of attention since it can be an alternative to bulky and expensive conventional techniques [4]. The conventional techniques are, for example, membrane-based filtering system and flow cytometry. The filtering technique, however, is limited by the membrane pore size which can only separate a limited range of particle sizes. Membrane clogging and high costs are the other drawback of this technique. Another conventional technique, the flow cytometry, is considered a powerful cell analysis technique which is able to count, sort and analyze biological particles. Although it can separate and isolate particles according to their sizes, the commercial systems available today are expensive, bulky, require high maintenance and highly trained operators [5].

Besides particle separation has gained a high level of interest over the past decade since it is used in various areas such as medicine, chemistry and microbiology in order to purify and determine the suspended particles or cells in solution [6]. Commercial particle separation methods use bulky, complex, and expensive instruments and require a large amount of samples [7].

In the last few decades, the use of microfluidic devices (sometimes called “Lab-on-a-chip” or “Micro Total Analysis Systems (μ TAS)”) to manipulate particles/bioparticles has been on the increase due to their advantages such as speed of analyses, the resolution of separations, small sample and reagent required, and many other advantages [3, 8-12]. Therefore, a microfluidic device is a promising candidate for the separation of different sizes of microparticles. The research works published in recent years involve complicated systems such as multiple channels used to focus the particles [13] and sorting by optical switching technique [14]. This work proposes to develop a simple and efficient microfluidic system cooperating with neodymium magnet for focusing and separating two sizes of microparticles. By using a spiral channel; only single inlet reservoir is needed. The particles with proper size will be focused to a certain area in the channel using the forces inside the spiral channels and separated into one outlet. For the particle size that cannot be focus inside the spiral channel, the magnetic force was used to pull them to another outlet.

The result from this work can be an ideal prototype for the later development of a cheap, real-time, high-throughput, small sample, and portable system for point-of-care or in-the-field real biological sample (e.g. blood) separation and counting.

Objectives

The purpose of this work was to develop a spiral microfluidic device for continuous separation 2 different-size microparticles. One of the particle size was influenced by the forces happened inside the microchannel, and was focused to the same stream line. The other particle was induced by the magnetic according to its magnetic property and can be moved to the different position from the other size.

Literature Review

Microfluidics is miniaturized systems involving a multidisciplinary field such as physics, chemistry, biology, engineering and biotechnology. It uses the advantage of the fluid (liquids and gases) behavior on the microscale for the development. Microfluidics employs

the small size of microchannels and fluid behavior, and thus offers new capabilities in the control and manipulation of small samples in real time. Microfluidics has shown the following significant advantages compared to conventional large-scale systems:

- cost savings owing to low reagent and sample consumptions [14];
- low-cost disposable devices [15];
- high sensitivity [16];
- highly sequential or parallel experimentation leading to the rapid detection performance [14, 17];
- better representative of the natural tissue environment and precise experimental control of the cellular microenvironment [18].

The ability to perform assays with the above advantages has transformed many areas of the applied sciences, such as medicine, pharmaceutical research, and analytical chemistry [19-22].

Separation and counting of microparticles or biological cells based on size are two most important applications of microfluidic systems. Several microscale techniques have been studied and developed in the past two decades for these applications due to the fact that the separation and counting are the essential preparatory and analytical steps in many biological and biomedical assays [15]. Moreover, the steps are also crucial for several environmental and clinical applications ranging from removal of colloidal and supracolloidal residues from wastewater effluents to detection of biologically harmful agents, such as bacteria and protozoa in drinking water to cell separations in cancer therapy [3, 23].

The traditional separation methods of suspended cells/particles in the liquids are membrane-base filtration systems. Drawbacks of the method are clogging of the membrane, high cost, and limit range of membrane pore size. In recent years, there are several studies on the membrane-less separation techniques including the use of electrophoresis [24], dielectrophoresis [25], magnetic field [26]/magnetic particles[27] , optical force [28], acoustic wave [29], Pillar and weir structures [30], pinched flow fractionation (PFF) [31], hydrodynamic

filtration [32], hydrodynamic chromatography (HDC) [33, 34], centrifugation[35], and inertial focusing [36-38]. Some of these techniques have been developed to microscale according to their capability of separating micro and nano particles ranging from larger than 100 μm cells to less than 10 nm proteins [2].

Particle separation in microfluidics can be classified as active and passive techniques [39, 40]. The active technique needs the external force field for the separation, while the passive technique only relies on the channel geometry and inherent hydrodynamic forces. Pamme [2] mentioned that most lab-on-a-chip applications require continuous on-chip separation of particles for faster analysis and detection. Most of the membrane-less separation active techniques in recent years work in batch system considered not an attractive choice for filtering and separating particles in large sample volumes ($\sim\text{mL}$) due to long analysis times, and requiring precise injection of small sample volumes in the separation section of the microfluidic network. The external force field required in active technique can potentially damage biological macromolecules and cells, and the active sources needed to produce these fields for particle manipulation often make the device fabrication complex and difficult to integrate with conventional LOC components. Finally, type of particles can be limited by the particle charge and mobility.

Passive microfluidic devices have potential to overcome these limitations [41]. One example is the separation using weir structures and microarrays based on size-dependent lateral displacement of particles [42, 43]. The weir structures were demonstrated to isolate leukocytes from blood [44]. Although it is sufficient for carrying out further downstream assays, there is limited efficiency in the target cell isolation. For the microarray technique (Figure 1), it makes use of the asymmetric bifurcation of laminar flow around micrometer-scale obstacles. Although it can efficiently separate microspheres of 0.8, 0.9 and 1.0 micrometers, the device is easily clogged due to the processing of large sample volumes.

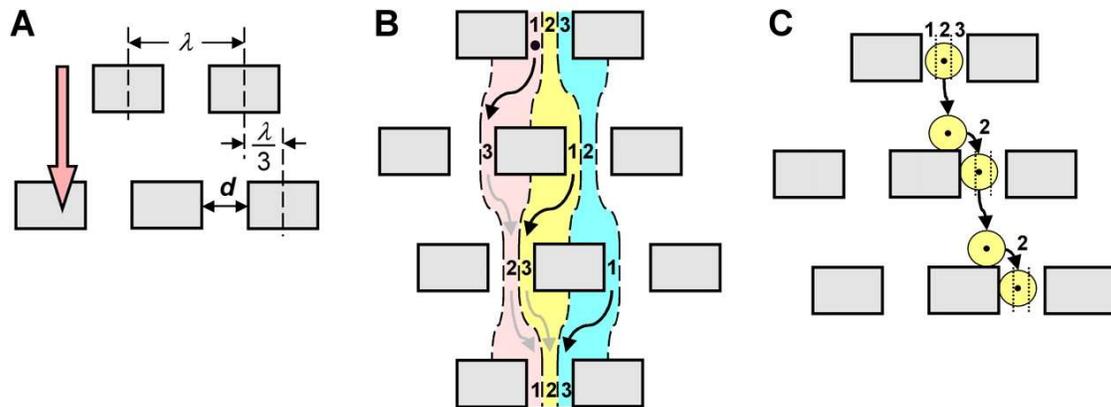


Figure 1. Illustration a separation process using microarrays based on size-dependent lateral displacement of particles. 1(A) Geometric parameters defining the obstacle matrix. A fluid flow is applied in the vertical direction (thick arrow). (B) Three fluid streams (red (left), yellow (middle), and blue (right)) in a gap do not mix as they flow through the matrix. Lane 1 at the first obstacle row becomes lane 3 at the second row, lane 3 becomes lane 2 at the third row, and so on. Small particles following streamlines will thus stay in the same lane. (C) A particle with a radius that is larger than lane 1 follows a streamline passing through the particle's center (black dot), moving toward lane 1. The particle is physically displaced as it enters the next gap. Black dotted lines mark the lanes. (Reprinted from reference [43])

Pinched flow fractionation (PFF) is one of the passive separation micro device technique based on particle size (Figure 2). In two separate inlets, the dilute sample is flowed along with sheath buffer in one of the inlets, and is aligned against a sidewall in the pinched segment. The particles are then separated according their sizes when they flow into the broadened segment because of the expansion of the laminar flow sheet [45]. The advantage of this technique is that it is only based on the laminar flow profile in the segments; however, not only high flow rates ($>4,000$ particles/min) are limited because inertial forces tend to affect particle motion, affecting separation efficiency [15]; but also the solution flow rate in both inlets must be well controlled.

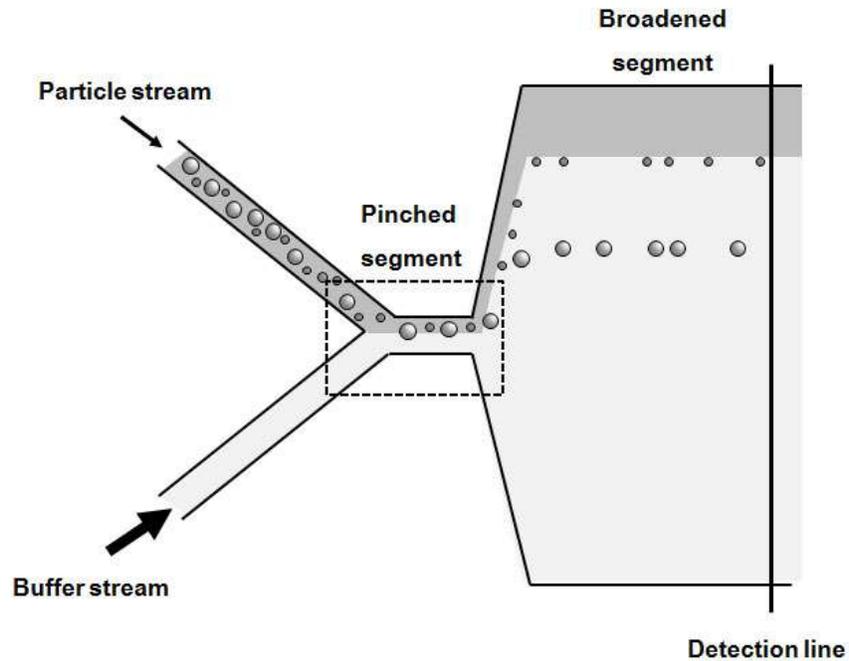


Figure 2 Principle of pinched flow fractionation. In the pinched segment, particles are aligned to one sidewall regardless of their sizes by controlling the flow rates from two inlets.[45]

Another technique for passive separation of microparticles is hydrodynamic filtration (Figure 3). The technique is based on channel design and flow control. The solution with suspended particles is pumped to an inlet to the main channel, and the particles will be separated by their sizes to several branches of small outlets located at the side wall along the way of the main channel. As the smaller particles can occupy the stream closer to the main channel wall than the bigger particles, they will enter the earlier located side channels while the bigger ones will enter channels located quite a distance away [32, 46].

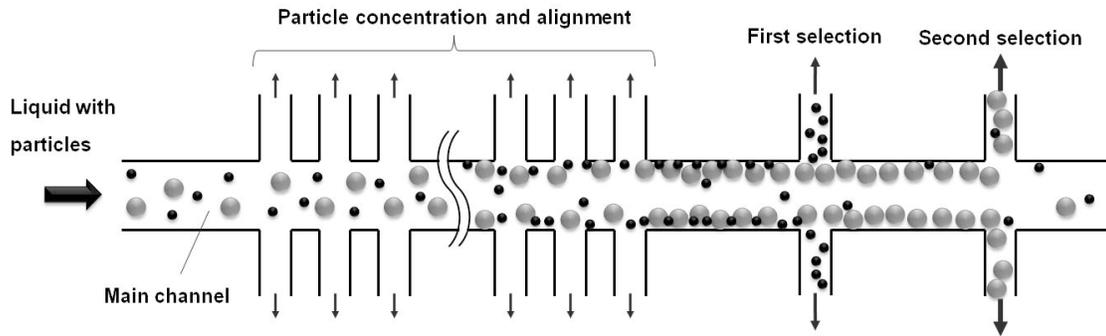


Figure 3 Schematic diagram showing particle concentration and classification in a microchannel having multiple branch points and side channels. [32]

Inertial lift forces have also been applied as one of the passive technique to manipulate and separate the microparticles in microfluidics. Forces acting on the particles in the flow stream precisely manipulate the particle position inside the flow. With the additional curved designed microchannels (as shown in Figure 4, for example) , the separation is achieved from these forces equilibrating the particles at different positions within the microchannel cross-section based on their size relative to the microchannel dimensions [35, 38, 41]. Centrifugal-based particle separation applies the inertial lift forces by using spiral designed microchannels. This technique has been getting attention since it has several advantages which are [4]:

- membrane-less;
- capable of analyzing large sample volume in continuous flow mode;
- no external force field required;
- easily-fabricated;
- applied to wide range of particle types ranging from biological samples to micron-sized microparticles.

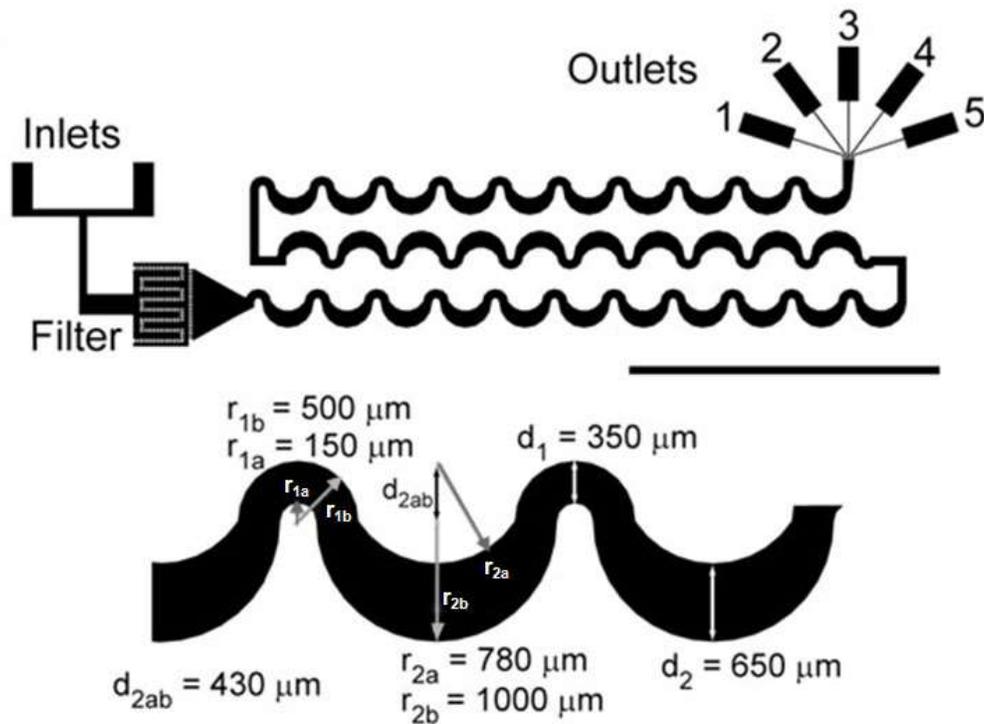


Figure 4 Example of a particle separation device design using inertial lift force. A top down drawing of the separation system is shown to scale (scale bar is 1 cm). The device consists of inlets, a coarse filter (50- μm cutoff), 62 asymmetric separation turns, and 5 outlets to collect the filtrate. A closeup of the separation turns shows the detailed geometric values used for successful separation. The small and large turns have widths of 350 and 650 μm , respectively. For the large turn, the center of the two curves defining the channel is offset by 430 μm , creating a widening arc. (Reprinted from reference [38])

The technique has been used in many applications, for example, separating cells and particles by deformability [47], and continuous blood separation [48].

To focus the particles into a single stream, the symmetry of rectangular channels has to be introduced with repetitive curvatures. The addition of curvature introduces a secondary cross sectional flow field perpendicular to the flow direction (called Dean flow). For a low-aspect-ratio (width \gg height) rectangular geometry, the lift force along the dimension defined by the channel height is dominant and it is expected that particles will tend to focus into two laterally broad focusing positions (top and bottom of the rectangular

channel) (Figure 5A) [49, 50]. In the curved channel, a secondary rotational flow-field perpendicular to the flow direction, located above and below the plane of symmetry of the channel [37, 51] (Figure 5B). The transverse Dean flow introduces a drag force (F_D) that moves particles at the top and bottom laterally toward the inner wall; however, if they are closer to the mid-plane in the z-direction, the particles are pushed toward the outer wall and recirculated following the top or bottom stream lines allowing them to quickly find their lateral equilibrium positions.

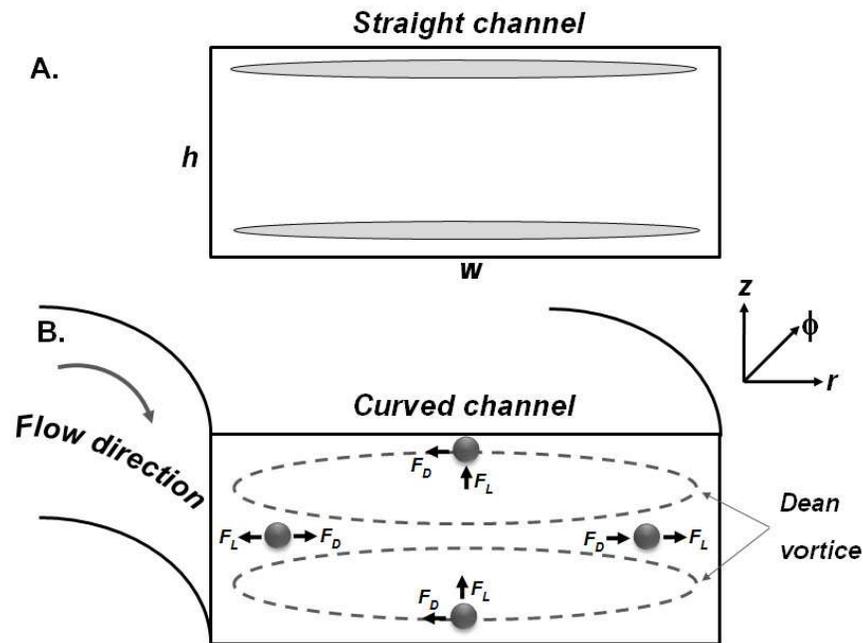


Figure 5 Schematic illustration of particle focusing points along the height of the channel for straight rectangular (A) and curved (B) channels. Neutrally buoyant particles flowing in curved channels experience a combination of inertial lift (F_L) and Dean drag (F_D) forces.

The work from Carlo et al. [37] demonstrated that particles with $a_p/D_h > 0.07$ in an asymmetric curvilinear channel (where a_p is the particle diameter and D_h is the microchannel hydraulic diameter defined as $D_h = 2wh/(w+h)$ where w and h are the width and height of the channel; for typically channel $D_h = 4A/P$ for a microchannel with cross-sectional area A and perimeter P) tend to occupy a single equilibrium position. With this

ratio, they were able to separate 10- μm particles from the mixture with 2- μm particles as the 10 microns formed an ordered stream and were collected separately. Bhagat et al. [41] achieved a complete separation between two particles using Dean drag to transpose the smaller particles and inertial lift forces coupled with Dean drag to equilibrate the larger particles. By combining these forces, the formation of distinct particle streams based on their size can be achieved and collected at the outlets using the advantage of laminar flow. Figure 6 demonstrates how the particles arrange in the inlet and outlet before and after passing through the spiral microchannel.

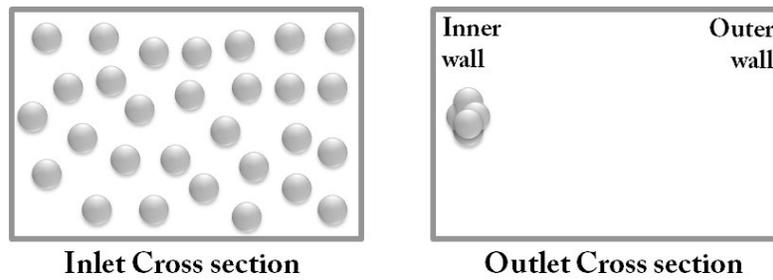


Figure 6 Microchannel cross-sections illustrating the particle position: particles are randomly dispersed before effected by the forces inside the spiral channel (left), particles are aligned in a single stream at the mid of the inner wall of the channel after passing through the spiral channel.

Due to the curvilinear channel geometry, the centrifugal acceleration directed radially outward leads to the formation of two counter-rotating vortices known as Dean vortices in the top and bottom halves of the channel [52] (Figure 5B). The value of these flows is quantified by a dimensionless Dean number (De) given by:

$$De = \frac{\rho U_f D_h}{\mu} \sqrt{\frac{D_h}{2R}} = Re \sqrt{\frac{D_h}{2R}} \quad (1)$$

Where ρ is the density of fluid medium (kg m^{-3}), U_f is the average fluid velocity (m s^{-1}), μ is the fluid viscosity ($\text{kg m}^{-1} \text{s}^{-1}$), R is the radius of curvature (m) of the path of the channel, and

$Re [= \rho U_m D_h / \mu$ where U_m is the maximum channel velocity] is the flow Reynolds number. [41]

The average Dean velocity for a given De is given by

$$\bar{U}_{Dean} = 1.8 \times 10^{-4} De^{1.63} \quad (2)$$

and the Dean drag force (F_D) exerted on particles can then be obtained using Stokes' Law

$$F_D = 3\pi\mu\bar{U}_{Dean}a_p = 5.4 \times 10^{-4} \pi\mu De^{1.63} a_p \quad (3)$$

where a_p is particle diameter.

In a straight microchannel there is no Dean flows; therefore $De = 0$. In curved channels, De increases with higher curvature (smaller R), larger channel size (larger D_h), and faster flows (higher Re). With increasing De , Dean flow increases in magnitude. Particles flowing in a curvilinear channel experience a drag force due to the transverse Dean flows.

The low-aspect-ratio microfluidic spiral device had been demonstrated by Russom et al. [4] how the two dominant forces, F_D and F_L (illustrated in Figure 5), interplay to trap particles flowing through a curved channel at an equilibrium position. The relative magnitude of the F_D and F_L acting on the particle can cause particle focusing (dominant lift) or mixing (dominant Dean flow).

The net lift force (F_L) acting on a particle is given by

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

where U_f is the average flow velocity and L_C is the characteristic length. Therefore, the lift force is a function of microchannel cross-section, flow velocity, and particle size.

Particles dispersed in a spiral microchannel flow along one of the two Dean vortices are forced to follow fluid movement (Figure 5B). The Dean drag force and the inertial lift

forces tend to dominate the migration of neutrally buoyant particles flowing in microchannels at $Re \sim 1$. Chun et al. [53] showed that preferential focusing of a particle is dominant for particles with a_p/D_h ratio ~ 0.1 . Bhagat et al. [41] reported the spiral microchannels which can separate 7.32- μm particles ($a_p/D_h \sim 0.1$) and 1.9- μm particles ($a_p/D_h \sim 0.03$). The bigger particles formed a single stream after passing through the channel while the smaller ones experienced higher viscous drag due to the Dean flows still continued to re-circulate along the Dean vortices and were transposed to the outer half of the microchannel.

Methodology

a. Design and experimental setup

The optimal dimensions and fabrication of spiral microfluidics (Figure 1) was studied. The optimal parameters will be considered by calculation and from the literature review. These involve hydraulic diameter (D_h), radius of curvature (R), Dean number (De), number of spiral loops of the microchannel etc. To achieve these parameters, several minor parameters will also be considered, for example, height (h) and width (w) of channel, particle size (a_p), density of buffer medium (ρ), fluid velocity, and fluid viscosity (μ). The ratio of a_p and D_h should fall in the range of 0.07 to 0.1 to get the focused particle stream in the microchannel. The successful fabricated chips were show in figure 7(a).

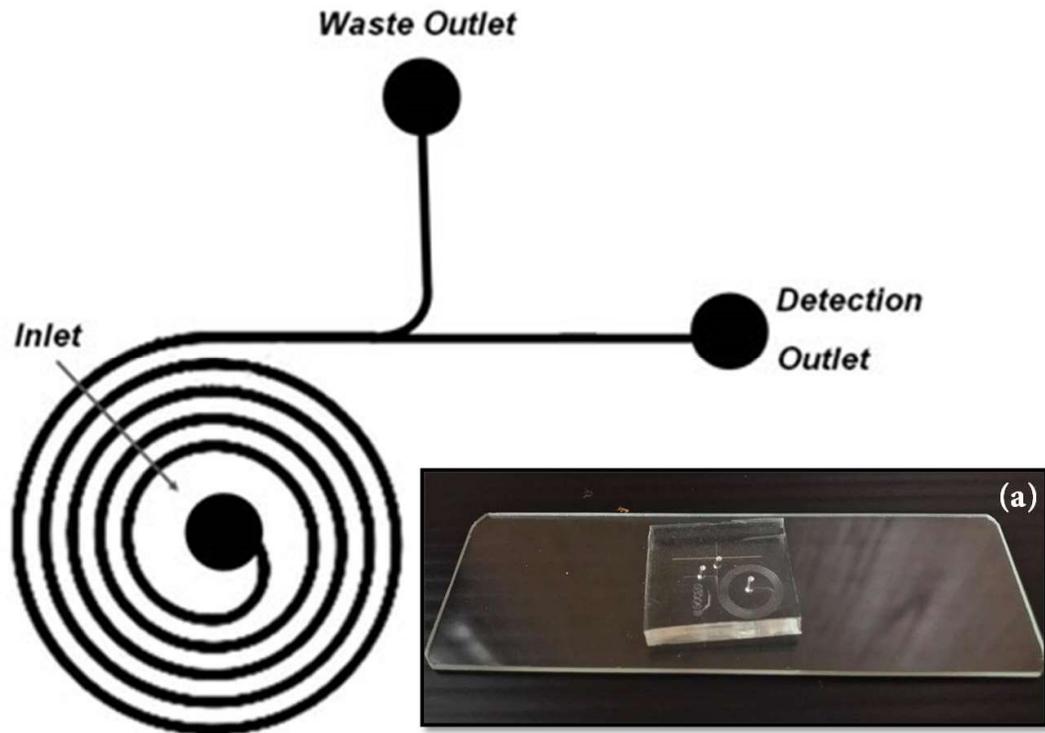


Figure 7. A proposed design of the spiral microfluidics for particle separation and counting (a) a successfully fabricated chip.

A master mold of the spiral microchannel was fabricated by collaborating with a research team at the TMEC (Thai Microelectronics Center), Bangkok, Thailand, using standard soft lithography methods. The 100- μm thick SU-8 photoresist was patterned with the reversed design on the silicon wafer using conventional UV lithography techniques. To fabricate the microfluidic chip, a mixture of PDMS pre polymer and its curing agent (10:1 ratio) (Sylgard 184; Dow Corning, MI) was poured on the master mold and cured for 4 hrs in a conventional oven at 60 °C. A pre-cleaned PDMS replica was then bonded on a cleaned glass slide by treating their surfaces with an oxygen plasma (PDC-32G, Harrick plasma, USA) at 200 mTorr, 200W. The schematic diagram of the chip fabrication is shown in Figure 8.

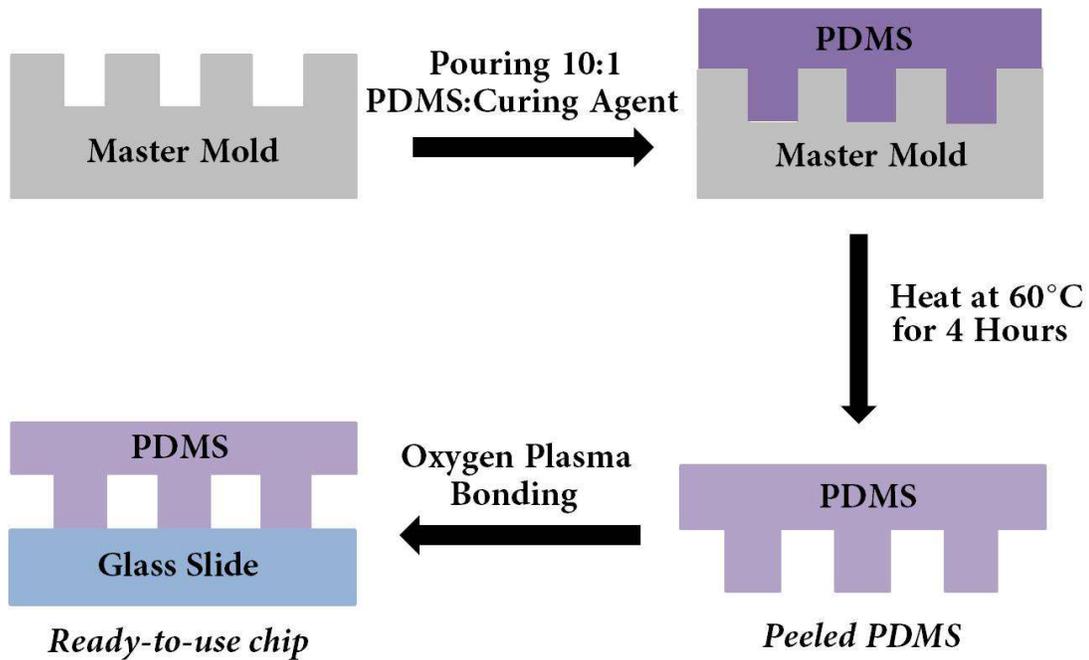


Figure 8. A schematic diagram of the spiral microchannel fabrication

b. Data Analysis

A behavior of the flow was monitored at the straight part of the channel near the outlet in order to ensure that all the particles have already reached their equilibrium positions. The position of each particle was analyzed using a program named Tracker Video Analysis and Modeling Tool.

c. Materials and methods

Polystyrene (6 μm) and SiMAG-Silanol microsphere (1 μm) were employed as the analyzed samples because not only it is convenient to control their size, but there are also a wide range of sizes available in the market making them the best candidate for the cell representative. The stock of 6- μm sphere solution were prepared by dispersing 1 drop of microparticle standard (size 6 μm standard based on Polystyrene monodisperse) (Fluka Analytical, Switzerland) in 2 mL DI water. The 6- μm size sample was prepared by mixing 400

μL of stock with 1500 μL of DI water and 50 μL of 1% of TWEEN20 to prevent particles to stick together and to the channel wall.

The stock of 1- μm magnetic sphere solution were prepared by dispersing 20 μL of SiMAG-Silanol size 1 μm (Chemicell, Berlin, Germany) in 1 mL of DI water. The 1- μm size sample was prepared by mixing 40 μL of stock with 1000 μL of DI water and 50 μL of 1% of TWEEN20.

The mixed particle size sample was prepared by mixing 20 μL of 1- μm magnetic sphere stock solution, 1 drop of 6- μm sphere, 150 μL of 1% of TWEEN20, and 1500 μL of 1% of DI water.

d. Experimental Setup

The system consisted of an inverted microscope (IX70-S8F2, Olympus Optical Co.,Ltd.,Japan), a CCD camera (8050/4070/340 Series Scientific-Grade Digital Camera, USA) , a computer, a microsyringe pump (781101,KD Scientific Inc.,USA) and a syringe kit.

The flow rates of the particle suspended solution were optimized. The results were analyzed using video recorded files of flowing particles from a CCD camera which is connected to an inverted optical microscope. Non-modified (without fluorescence or modified surface) two-size microspheres; 1 and 6 μm) were studied; 1- μm particles have magnetic property. The aim is to focus the 6- μm particles to the inner wall of the spiral channel and separated into the inner outlet while the 1- μm particles were induced by external magnetic field (figure 9) and come out at the outer outlet.

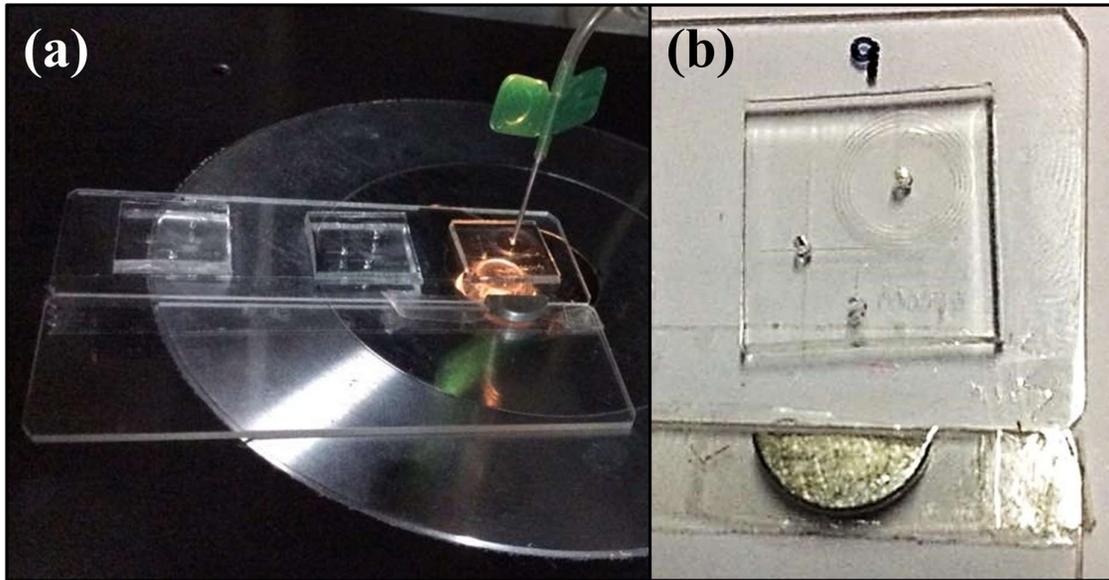


Figure 9 The spiral microfluidic chip incorporated with neodymium magnet for particle separation (a) The flowing particles are monitored under an inverted microscope (b) The position of neodymium magnet

Result and Discussion

a. Flow rate optimization for polystyrene microsphere

The flow behavior of 6- μm polystyrene microsphere were study inside the designed spiral microchannel using the flow rate between 1.5-2.3 $\mu\text{L}/\text{min}$. The particles flow at random position with the flow rate lower than 1.5 and more than 2.3 $\mu\text{L}/\text{min}$. The result shows in Figure 10. The flow rate of 1.9 $\mu\text{L}/\text{min}$ gave the highest separation efficiency which can separate $77 \pm 1\%$ of the particles into the inner wall of the channel.

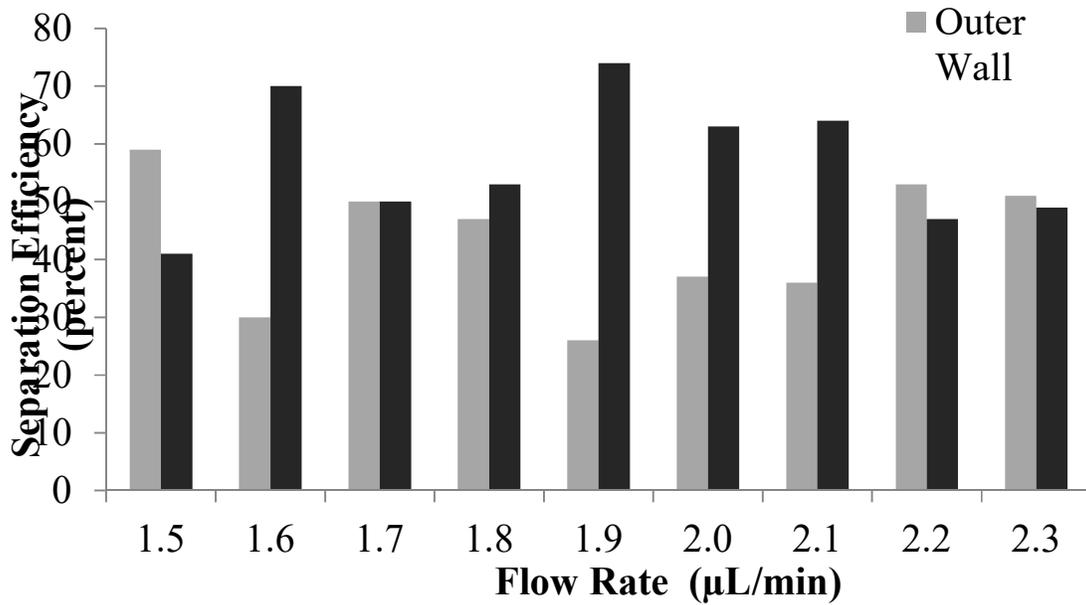


Figure 10 The separation efficiency of 6- μm polystyrene microsphere at different flow rates in the spiral microchannels.

b. Magnetic force optimization for magnetic microsphere separation

According to the design of the spiral microchannel which cannot use to focus a very small particle size (less than 2 micrometers). The 1- μm magnetic microspheres were separated by the utilization of the magnetic force from the neodymium magnet. The position of placing the magnet was studied at 6, 8, 10 and 12 mm from the outlet channel using the optimized flow rate of 6- μm polystyrene microspheres, 1.9 $\mu\text{L}/\text{min}$. The result was found that 8-mm distance gave the highest separation efficiency; $80 \pm 0\%$ of all the flowing particles were pulled to the outer wall of the channel and come out from the outer outlet.

The interference of the magnetic field on the 6- μm polystyrene microspheres were also study. By placing the neodymium magnet at 8 mm from the channel, the flow behavior at the optimized flow rate was monitor. The result (Fig. 11) shows that there is no interference on the flow of 6- μm spheres.

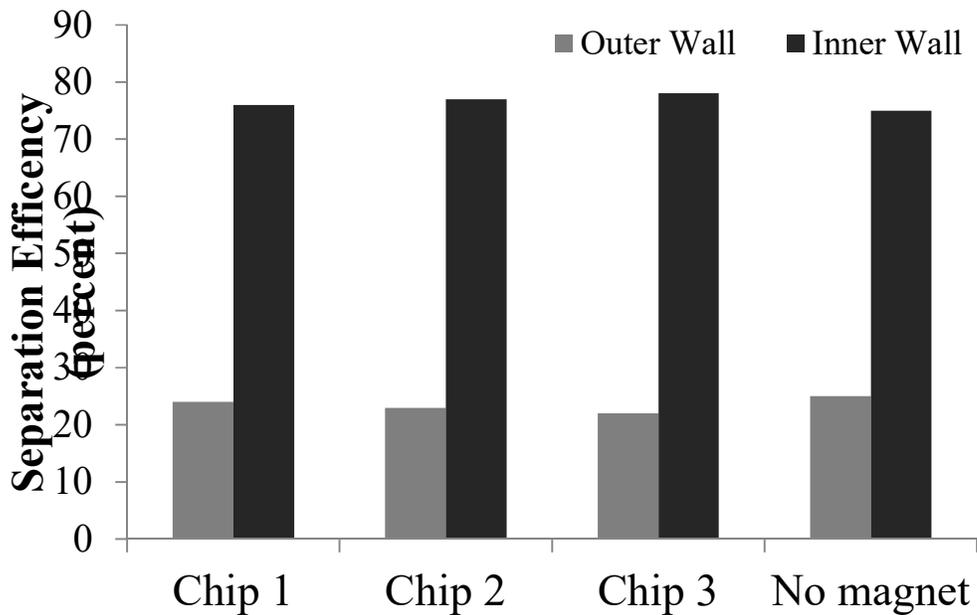


Figure 11 Effect of the magnetic force on the non-magnetic particles in the suspended mixed-size particles solution flowing in the spiral microchannel.

c. Mixed particle separation

The mixture of 1- μm magnetic microspheres and 6- μm polystyrene microspheres were introduced to the spiral channel using the optimized parameters, 1.9- $\mu\text{L}/\text{min}$ flow rate and 8-mm magnet position. The result shows in Figure 12. An $80 \pm 0\%$ of the 1- μm magnetic microspheres were separated to the outer outlet and a $77 \pm 1\%$ of the 6- μm polystyrene microspheres were separated to the inner outlet. The designed microfluidics can give a high separation efficiency for both sizes of particles, and this can be a prototype of several separation applications in field of biological science and medicine.

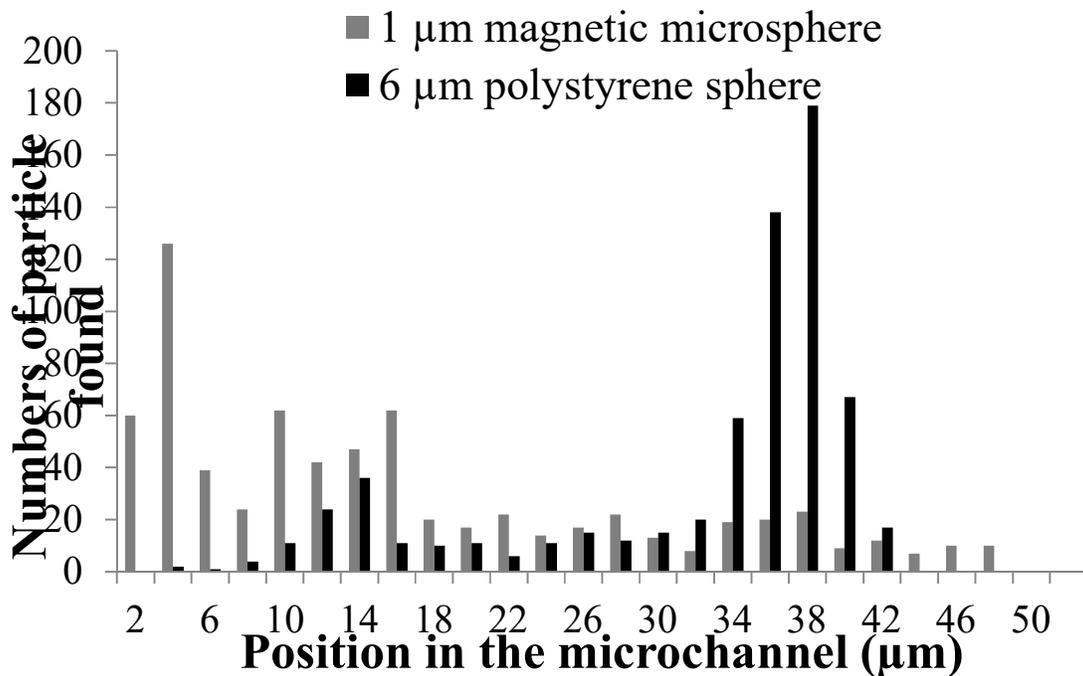


Figure 12 The numbers of 1- μm and 6- μm particles at different positions inside the spiral microchannel with the magnetic force induction monitored at the straight part near the outlets of the channel.

Conclusion

Our spiral microfluidics was successfully separate non-magnetic 6- μm particles from magnetic 1- μm particles with very high separation rate. The optimum flow rate giving highest separation efficiency was 1.9 $\mu\text{L}/\text{min}$ which $77 \pm 1\%$ of 6- μm particles were focused and separated to the inner outlet. The 1- μm particles were manipulated by the magnetic field from hemisphere neodymium magnet placed at 8.00 mm from the channel, and $80 \pm 0\%$ of the particles were separated to the outer outlet. This cheap, easy to fabricate, small amount of sample needed, and small spiral microchannel with cooperated with neodymium magnet can be used to separate real samples with different sizes and magnetic properties such as mixed synthetic particles or biological cells.

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Appendix

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