Continuous particle separation in spiral microchannels using dean flows and differential migration

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Microparticle separation and concentration based on size has become indispensable in many biomedical and environmental applications. In this paper we describe a passive microfluidic device with spiral microchannel geometry for complete separation of particles. The design takes advantage of the inertial lift and viscous drag forces acting on particles of various sizes to achieve differential migration, and hence separation, of microparticles. The dominant inertial forces and the Dean rotation force due to the spiral microchannel geometry cause the larger particles to occupy a single equilibrium position near the inner microchannel wall. The smaller particles migrate to the outer half of the channel under the influence of Dean forces resulting in the formation of two distinct particle streams which are collected in two separate outputs. This is the first demonstration that takes advantage of the dual role of Dean forces for focusing larger particles in a single equilibrium position and transposing the smaller particles from the inner half to the outer half of the microchannel cross-section. The 5-loop spiral microchannel 100 µm wide and 50 μ m high was used to successfully demonstrate a complete separation of 7.32 μ m and 1.9 μ m particles at Dean number De = 0.47. Analytical analysis supporting the experiments and models is also presented. The simple planar structure of the separator offers simple fabrication and makes it ideal for integration with on-chip microfluidic systems, such as micro total analysis systems (µTAS) or lab-on-a-chip (LOC) for continuous filtration and separation applications.

1. Introduction

Separation and filtration of microparticles based on size is essential for many applications such as biochemical and environmental assays,^{1,2} micro/nano-manufacturing,³ and clinical analysis.4 Traditional methods for separation and removal of microparticles from solutions involve the use of a membrane filter, which are typically limited by the membrane pore size, making them inefficient for separating a wide range of particles. Clogging and high costs associated with membrane-based filtration on the microscale have resulted in the development of a number of membrane-less separation techniques. Sedimentation, field-flow fractionation (FFF),^{5,6} hydrodynamic chromatography (HDC),7,8 pinched flow fractionation (PFF),9 electrophoresis,10,11 dielectrophoresis,12 acoustic separation,13 diffusion-based extraction,14,15 deterministic lateral displacement,16-18 centrifugation,19-22 and inertial focusing23,24 are some of the techniques recently demonstrated for separation and concentration of particles and biological molecules.

Microscale membrane-less separation techniques have seen an exponential growth in recent years, and have been shown to work well for micro and nano particles from large cells (>100 μ m) to small proteins (<10 nm).²⁵ Most of these techniques work in

batch mode rather than continuous flow mode, requiring precise injection of small sample volumes in the separation section of the microfluidic network. Yet, as Pamme²⁵ points out in a recent review, most lab-on-a-chip (LOC) applications require continuous on-chip separation of particles for faster analysis and detection. These techniques are thus not an attractive choice for filtering and separating particles in large sample volumes (~mL) due to long analysis times. In addition, the external force fields required for their functionality 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, dependence on particle charge and mobility presents constraints on the type of particles that can be analyzed.

Passive membrane-less microfluidic devices have the potential to overcome these limitations. One example is the "H-filter"^{14,15} that filters and extracts biomolecules and particles by taking advantage of differences in their diffusion coefficients. This purely diffusion-based separation mechanism can be readily used to filter smaller particles (<1 μ m) from a mixture with larger particles, such as extraction of antibiotic cephradine from blood.¹⁵ However, due to their small diffusion coefficients, large particles such as cells cannot be filtered efficiently as diffusion times and lengths become impractically long for most LOC applications. Another example of a passive microfluidic separation is based on size-dependent lateral displacement of particles in microchannels with micropillar arrays.¹⁶⁻¹⁸ In this

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approach, particles are sorted using arrays of obstacles spaced w apart, with particles larger than the critical diameter $d_c = 20\% \times 2w$ following a deterministic path and forming separate streams that can be collected individually at the outlet. The device was successfully used to demonstrate efficient sorting of 0.8 µm, 0.9 µm and 1 µm microspheres.

Recently centrifugal-based particle separation has been demonstrated using flows in curvilinear microchannels.²⁰⁻²² As the technique is membrane-free it eliminates issues arising from membrane fouling, can be performed in a continuous flow mode allowing the processing of large sample volumes in a short time, does not require an external force field and thus is easy to fabricate and can be applied to any particle type ranging from biological samples such as cells to micrometer-sized colloidal particles. Gregoratto et al.²⁰ demonstrated continuous separation of large volumes of dilute suspensions using spirallyshaped microchannels. To minimize the effects of secondary flows (Dean vortices), high aspect ratio (~10) microchannels were fabricated in silicon using the DRIE process. The design was tested with 1 µm, 8 µm and 10 µm polystyrene particles and a 3.5 fold increase in concentration of 10 µm particles was achieved at the outlet for an input velocity of 0.27 m s⁻¹ (flow rate of 2 mL min⁻¹). The principle of centrifugal separation was also used by Seo et al.,^{21,22} whose design was similar to the spirally wound channels by Gregoratto et al.²⁰ except for an additional "S"-shaped unit at the center for reversing flow direction. At flow velocities of 92 mm s⁻¹, an impressive 200-fold concentration enhancement of the 10 µm particles was demonstrated; for smaller 6 µm and 3 µm polystyrene particles under similar flow condition, 9.3 and 1.4 times concentration enhancement was achieved. In another recent work, Carlo *et al.*^{23,24} separated microparticles using an asymmetric serpentine channel geometry. They observed that in an asymmetric curvilinear channel, particles with $a_p/D_h > 0.07$ (where a_p is the particle diameter and D_h is the microchannel hydraulic diameter) tend to occupy a single equilibrium position. They used this principle to separate 10 µm particles from a mixture of 10 µm and 2 µm particles; the 2 µm particles remained dispersed across the entire microchannel while the 10 µm particles formed a continuous ordered stream and were collected separately.

In this work, we describe a passive spiral microfluidic device for *complete* separation of particles using centrifugal-based differential migration. Fig. 1 schematically illustrates the concept. Our design achieves a complete separation between two particle sizes using Dean drag to transpose the smaller particles and inertial lift forces coupled with Dean drag to equilibrate the larger particles. The combined effect of these forces results in the formation of distinct particle streams based on their size, which in turn are collected at separate outlets by taking advantage of the laminar flow in the outlet microchannels. Although inertial migration coupled with Dean drag has been recently used by others²⁰⁻²² to focus or concentrate large particles (~10 μ m), operation and design of these devices have not been reported in detail. Herein, for the first time we demonstrate both numerically



Fig. 1 (a) Schematic of the proposed spiral microparticle separator. The design consists of two inlets and two outlets with the sample being introduced through the inner inlet. Neutrally buoyant particles experience lift forces (F_L) and Dean drag (F_D), which results in differential particle migration within the microchannel. (b) Microchannel cross-sections illustrating the principle of inertial migration for particles with $a_p/D_h \sim 0.1$. The randomly dispersed particles align in the four equilibrium positions within the microchannel where the lift forces balance each other. Additional forces due to the Dean vortices reduce the four equilibrium positions to just one near the inner microchannel wall.

and experimentally how Dean forces can play a dual-role to achieve a complete separation of particles. Based on the results presented in this work, particle separators can be designed for continuous separation of microscale, and possibly nanoscale, particle mixtures over a wide dynamic range.

2. Design principle

Fluid flowing through a curvilinear channel experiences centrifugal acceleration directed radially outward leading to the formation of two counter-rotating vortices known as Dean vortices in the top and bottom halves of the channel.^{26–28} The magnitude of these secondary flows is quantified by a dimensionless Dean number (*De*) given by:

$$De = \frac{\rho U_{\rm f} D_{\rm h}}{\mu} \sqrt{\frac{D_{\rm h}}{2R}} = Re \sqrt{\frac{D_{\rm h}}{2R}}$$
(1)

where ρ is the density of fluid medium (kg m⁻³), $U_{\rm f}$ is the average fluid velocity (m s⁻¹), μ is the fluid viscosity (kg m⁻¹ s⁻¹), R is the radius of curvature (m) of the path of the channel, and Re is the flow Reynolds number. For a straight microchannel, De =0, indicating absence of Dean flows. In curved channels, Deincreases with higher curvature (smaller R), larger channel size (larger $D_{\rm h}$), and faster flows (higher Re). Dean flows increase in magnitude with increasing De and have recently been used to demonstrate efficient fluid mixing in microchannels.²⁸ Particles flowing in a curvilinear channel experience a drag force due to the transverse Dean flows. Depending on particle size, this drag force ($F_{\rm D}$) causes particles to move along the Dean vortices (*i.e.* circulate), and thus move towards either the inner or outer channel wall.

In addition to the Dean drag $F_{\rm D}$, particles in a curvilinear channel experience pressure forces²⁹ and inertial lift forces.^{30,31} The net lift force $(F_{\rm L})$ acting on the particles is a combination of the shear-induced inertial lift force F_{IL} and the wall-induced inertial lift force F_{WL} . In Poiseuille flow, the parabolic nature of the velocity profile results in a fluidic shear-induced inertial lift force F_{IL} that acts on particles and is directed away from the microchannel center. As the particles move towards microchannel walls, an asymmetric wake induced around particles generates a wall-induced inertial lift force F_{WL} away from the wall.²³ The magnitude of these opposing lift forces varies across microchannel cross-section, with the wall-induced lift forces dominating near the microchannel walls and the shear-induced lift forces dominating near the microchannel center. The particles thus tend to occupy equilibrium positions where the oppositely directed lift forces are equal and form narrow bands.³²⁻³⁶ Chun et al.32 showed that preferential focusing of particles is dominant for particles with a_p/D_h ratio ~0.1. Fig. 1b schematically illustrates this principle of inertial migration. For a rectangular microchannel the number of equilibrium positions where the shear-induced lift force (F_{IL}) and the wall-induced lift forces $(F_{\rm WL})$ balance each other reduces to four at low Re.²³ Adding a component of Dean drag $(F_{\rm D})$ further reduces the four equilibrium positions to just one near the inner microchannel wall.18

Particles dispersed in a spiral microchannel get entrained in one of the two Dean vortices that are formed at the top and bottom half of the microchannel (Fig. 1). The Dean drag force and the inertial lift forces tend to dominate the migration of neutrally buoyant particles flowing in microchannels at $Re \sim 1$. Particles flowing near the top and bottom microchannel walls experience strong lateral flows due to Dean drag F_D and are pushed towards the inner and outer microchannel walls. Near the outer microchannel wall, the net lift force (F_L) acts along the direction of F_D and the particles continue to follow the Dean vortices independent of their size. However, near the inner microchannel wall, F_L and F_D act in opposite directions and depending on the magnitude of these forces, particles will either equilibrate and form a focused stream or continue to re-circulate in the Dean vortex.

In this work, we take advantage of the size dependence of the forces that act on particles flowing in curvilinear channels, namely the Dean drag and the inertial lift forces. The spiral geometry in this work will cause the bigger 7.32 μ m particles $(a_p/D_h \sim 0.1)$ to occupy a single equilibrium position near the inner microchannel wall. On the other hand, the smaller 1.9 μ m particles $(a_p/D_h \sim 0.3)$ experience higher viscous drag due to the Dean flows and will continue to re-circulate along the Dean vortices and can be transposed to the outer half of the microchannel. Thus, the device uses inertial migration of larger particles and the influence of Dean drag on smaller particles to achieve a complete separation of 1.9 μ m and 7.32 μ m polystyrene particles.

3. Methods

3.1. Microchannel design and fabrication

Fig. 2 illustrates the spiral microchannel fabricated in polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning). The design consisted of a 5-loop spiral geometry with two inlets and two bifurcating outlets. The microchannels were 100 μ m wide and 50 μ m high with 250 μ m spacing between two successive loops. The initial radius of curvature of the spiral was 3 mm and the total length of the microchannels was ~13 cm.



Fig. 2 Photograph of the 5-loop spiral microchannel fabricated in PDMS (the microchannel is filled with a blue dye for visualization). The inset SEM image illustrates a section of the spiral microchannel.

The spiral microchannel was fabricated using standard soft lithography methods.³⁷ Briefly, a 50 μ m thick layer of SU-8 photoresist (2075, Microchem Corp.) was patterned on a single-side polished 3" silicon wafer using conventional photolithography techniques. PDMS prepolymer mixed in a 10 : 1 ratio with the curing agent was then cast on the SU-8 master to replicate microchannel features. After curing, the PDMS mold was peeled from the SU-8 master and O₂ plasma bonded to a 1 mm thick glass slide to complete the microchannel. Input and output ports were cored using a 14 gauge syringe needle prior to bonding.

3.2. Numerical simulations

The spiral geometry was modeled using a commercial computational fluid dynamics software CFD-ACE+ (ESI-CFD Inc., Huntsville, AL). The simulation environment was verified for transient incompressible flows. The physical properties of water were applied to the fluids participating in the simulation (density $\rho = 1000 \text{ kg m}^{-3}$ and dynamic viscosity $\mu = 10^{-3} \text{ kg m}^{-1} \text{ s}^{-1}$). A diffusion coefficient $D = 10^{-10} \text{ m}^2 \text{ s}^{-1}$ was used for the fluids in the simulations. Fluid flow rates of 5 μ L min⁻¹, 10 μ L min⁻¹, and 20 µL min⁻¹ was specified at the input, while the outlet was set to a fixed-pressure boundary condition. The FLOW and CHEM modules in CFD-ACE+ were used to solve for fluid flow mixing in the spiral microchannels. The SPRAY module was used to solve for motion of the particles dispersed in the fluid. Polystyrene particles 7.32 μ m and 1.9 μ m in diameter (ρ = 1050 kg m⁻³) were dispersed into the inner flow stream at the inlet (inner inlet). By assigning a zero input velocity, the particles were forced to flow at the same velocity as the fluid at the inlet. The Algebraic MultiGrid (AMG), the Conjugates Gradient Squared (CGS), and the Preconditioning (Pre) solvers were used for pressure, velocity, and species corrections. A second order limiting scheme was used for solving the species diffusion and a first order upwinding scheme was used to solve for particle motion. The convergence limit for mass fraction was set to 10^{-6} and the simulations were run for ~2000 time steps until flow and particles reached the outlet.

3.3. Characterization

To experimentally evaluate particle separation, one syringe was filled with fluorescently labeled polystyrene particles (Bangs Laboratories) in DI water (0.05% volume fraction) while the other with DI water only. Using syringe pumps (NE-1000X, New Era Pump Systems) two 5 cc syringes were driven at 5 μ L min⁻¹ ($U_{\rm f}$ = 33 mm s⁻¹, De = 0.23), 10 μ L min⁻¹ ($U_{\rm f}$ = 66 mm s⁻¹, De = 0.47) and 20 μ L min⁻¹ ($U_f = 130$ mm s⁻¹, De =0.94). During testing, high speed images of the channel were captured at various locations downstream using an inverted epifluorescence microscope (Olympus IX71) equipped with a 12-bit CCD camera (Retiga EXi, QImaging). A stack of 300 images (Z-stacked) from each location was then overlaid to create composite images. Grayscale line scans across the microchannel width of the composite images recorded using ImageJ[®] helped analyze the particle migration. Polystyrene particles 1.9 µm and 7.32 µm labeled with Dragon Green (FITC) or Plum Purple (DAPI) fluorophores were used to characterize and demonstrate separations. The images were then overlaid to create a composite image indicating the particle position within the microchannel.

4. Results and discussion

Presence of Dean flows and their extent in our spiral microchannel was confirmed numerically by modeling dye flows at various flow conditions (Fig. 3). At lower Dean number (De = 0.23), the dye gradually moves from the outer inlet towards the inner outlet following the Dean vortices. The magnitude of these Dean vortices increases with increasing fluid velocity due to the higher centrifugal force acting on the fluid. At De = 0.47, the two dyes have switched positions by the time the flow



Fig. 3 Modeling results indicating dye movement within the spiral microchannels for De = 0.23, 0.47 and 0.94. The cross-section images illustrate the extent of Dean flows for increasing Dean numbers.

reaches the outlet. Further increasing the flow rate to De = 0.94, results in a complete recirculation of the two dyes by the time the flow reaches the outlet. These results suggest that for our microchannel geometry, $De \sim 1$ is high and results in complete flow recirculation. Since the focus of this work is on particle separation, complete recirculation is counter-productive and should be avoided. Therefore, we will limit the flows to De < 1, which corresponds to maximum input flow of 20 µL min⁻¹ or $Re \sim 10$.

Ookawara *et al.*^{38,39} formulated the expression for the average Dean velocity for a given De as

$$\bar{U}_{\text{Dean}} = 1.8 \times 10^{-4} De^{1.63} (\text{m s}^{-1})$$
 (2)

Depending on particle size, these transverse secondary Dean flows tend to entrain particles in one of the two vortices formed and force them to follow fluid movement within the vortices. The drag force exerted on a particle due to these flows can then be obtained by assuming the Stokes drag:

$$F_{\rm D} = 3\pi\mu \bar{U}_{\rm Dean} a_{\rm p} = 5.4 \times 10^{-4} \ \pi\mu \ De^{1.63} \ a_{\rm p} \ (\rm N) \tag{3}$$

In addition to the Dean drag, the neutrally buoyant particles also experience shear induced inertial lift forces and wall induced lift forces. The viscous drag force is responsible for the particle flowing in the channel and has no consequence in differential migration of particles. To account for the lift forces (the inertial and wall-induced), Asomolov³¹ derived an expression describing the magnitude of the lift forces as a function of the particle position across the channel cross-section.

$$F_{\rm L} = \rho G^2 C_{\rm L} a_{\rm p}^4 \,(\mathrm{N}) \tag{4}$$

where G is the shear rate of the fluid (s⁻¹) and $C_{\rm L}$ is the lift coefficient which is a function of the particle position across the channel cross-section assuming an average value of 0.5.²³ The average value of G for a Poiseuille flow is given by $G = U_{\rm max}/D_{\rm h}$, where, $U_{\rm max}$ is the maximum fluid velocity (m s⁻¹) and can be approximated as $2 \times U_{\rm f}$. From these expressions, it is clear that

Table 1 Summary of the Dean drag force (F_D) and the net lift force (F_L) acting on 1.9 μ m and 7.32 μ m particles for varying Dean numbers

Particle diameter/µm	Dean number (De)	$F_{\rm D}/{ m N}$	$F_{\rm L}/{ m N}$
1.9	0.23	3×10^{-13}	6.5×10^{-15}
	0.47	9×10^{-13}	2.6×10^{-14}
	0.94	3×10^{-12}	1×10^{-13}
7.32	0.23	1.1×10^{-12}	1.4×10^{-12}
	0.47	3.6×10^{-12}	5.7×10^{-12}
	0.94	1.1×10^{-11}	2.3×10^{-11}

these two forces $(F_{\rm D} \text{ and } F_{\rm L})$ depend on the particle size $(a_{\rm p})$, with the lift force $(F_{\rm L})$ increasing rapidly for increasing particle size $(F_{\rm L} \propto a_{\rm p}^4 \text{ while } F_{\rm D} \propto a_{\rm p})$. By varying the ratio of these two forces, differential migration of particles based on size can be used to achieve a complete separation of a mixture of 1.9 µm and 7.32 µm polystyrene particles.

The Dean drag force ($F_{\rm D}$) and the net lift force ($F_{\rm L}$) acting on 1.9 µm and 7.32 µm particles flowing in a 100 × 50 µm (W × H) microchannel at the three Dean numbers tested in this work are summarized in Table 1. The Dean forces dominate over the lift forces and influence the migration of 1.9 µm particles for all three flows. The 1.9 µm particles are then expected to get entrained in the Dean vortices and re-circulate along with the fluid flow. For 7.32 µm particles, the Dean drag forces acting on the particles at the three flows are less than the corresponding net lift forces. Thus, for 7.32 µm particles the lift forces dominate and the particles align along the four equilibrium positions illustrated in Fig. 1(b).

Images and experimental data illustrating distribution of $1.9 \,\mu\text{m}$ particles across the microchannel are shown in Fig. 4(a). At De = 0.23, the 1.9 µm particles were uniformly distributed across the width of the channel at the outlet due to the weak Dean forces that are not sufficient to move all particles towards the outer half of the channel. When the flow was increased to De = 0.47, the particles experience greater $F_{\rm D}$, and nearly 95% of the particles migrated from the inner half at the inlet to the outer half by the time the flow reached the outlet. Increasing flow further to De = 0.94 resulted in a complete transfer of particles to the outer half in the second loop due to stronger Dean flows, and a complete re-circulation by the time the particles reach the outlet. These experimental results were confirmed by numerical models; modeling images of the microchannel indicating migration of 1.9 μ m particles at De = 0.47 are shown in Fig. 4(b).

To verify the inertial migration and equilibration near the inner microchannel wall, the 7.32 μ m particles were introduced into the spiral geometry through the inner and outer outlet. Fig. 5(a) shows the averaged experimental images confirming particle distribution across the microchannel for 7.32 μ m particles introduced through the inner and outer inlet at De = 0.47. Also shown in the figure are the normalized distribution of 7.32 μ m particles at the microchannel inlet and outlet. Irrespective of where the particles were introduced into the microchannel, at the outlet the particles tend to form a narrow band of particles focused towards the inner wall for all three flow velocities, unlike the 1.9 μ m particles. This behavior is primarily attributed to the inertial migration and equilibration of the particles due to stronger lift forces. For our 100 × 50 μ m

channel dimensions, the hydraulic diameter is $D_{\rm h} = 67 \,\mu{\rm m}$ and thus for 7.32 $\mu{\rm m}$ particles $a_{\rm p}/D_{\rm h} = 0.11$ (>0.1) which yields particle equilibration. The lift forces then align the particles in the four equilibrium positions as was schematically shown in Fig. 1(b).^{23,32-34} Due to the curvilinear nature of the spiral geometry in this work and strong lateral Dean flows at the top and bottom of the microchannel, the equilibrium positions were further reduced to one near the inner wall, as Fig. 5(a) indicates.²³ Corresponding modeling images of the microchannel indicating the equilibration of 7.32 $\mu{\rm m}$ particles near the inner wall are shown in Fig. 5(b). These results agree with the experimental data confirming particle focusing.

Inertial migration and equilibration of particles is highly size dependent.^{23,24,30–32} To investigate the threshold a_p/D_h ratio above which particles begin focusing in the designed spiral microchannel geometry, polystyrene particles 1.9 µm, 4 µm, 5 μ m, and 7.32 μ m in the diameter were modeled. Fig. 6 shows the particle migration across the microchannel outlet at De =0.47; all particles were introduced through the inner inlet of the microchannel. The results show that the 1.9 µm particles $(a_p/D_h = 0.03)$ and the 4 µm particles $(a_p/D_h = 0.06)$ are more influenced by the Dean forces rather than the inertial lift force, and thus follow the fluid flow migrating away from the inner wall of the microchannel. However, the 5 μ m ($a_p/D_h = 0.07$) and the 7.32 μ m ($a_p/D_h = 0.11$) particles migrate and equilibrate near the inner microchannel wall. These results are in agreement with the work by Carlo *et al.*,²³ validating that particles with $a_p/D_h \ge$ 0.07 tend to focus and occupy a single equilibrium position. These results also suggest that a complete separation between particles smaller and larger than the 5 µm threshold should be possible in our microchannel geometry.

To achieve a complete separation between 1.9 μ m and 7.32 μ m particles, it is essential to determine the length of the spiral microchannel needed for the 1.9 μ m particles to migrate to the outer half of the microchannel and for the 7.32 μ m particles to focus near the inner wall. The two particles can then be collected separately at the two outlet arms. Using Asomolov's³¹ lift force equation and assuming Stokes drag, expression for the particle lateral migration velocity (U_L) can be written as

$$U_{\rm L} = \frac{\rho U_{\rm max}^2 a_{\rm p}^3 C_{\rm L}}{3\pi\mu D_{\rm h}^2} \quad ({\rm m \ s^{-1}})$$
 (5)

The channel length $(L_{\rm I})$ necessary for the particle to completely focus at one of the equilibrium positions is then given by

$$L_{\rm I} = \frac{U_{\rm f}}{U_{\rm L}} \times L_{\rm M} \quad (m) \tag{6}$$

where $L_{\rm M}$ is the migration length (m). Similarly, the channel length required for Dean migration ($L_{\rm D}$) is given by

$$L_{\rm D} = \frac{U_{\rm f}}{\overline{U}_{\rm Dean}} \times L_{\rm M} \quad (m) \tag{7}$$

For 1.9 μ m particles to migrate over a distance of 100 μ m (or ~2 × channel half width) to ensure that all particles from the inner half of the microchannel migrate into the outer half of the microchannel, the required channel lengths are calculated



Fig. 4 (a) Fluorescent images illustrating distribution of 1.9 μ m particles across the microchannel inlet and outlet (bright white lines indicate the approximate position of the microchannel walls) at De = 0.23 (top row), De = 0.47 (middle row), and De = 0.94 (bottom row). The normalized particle dispersion across the microchannel width are also shown. The particle migration is similar to the dye migration due to stronger Dean forces entraining the particles within. (b) Panels illustrating the modeled 1.9 μ m particle distribution across the microchannel width at inlet and outlet at De = 0.47. The results show excellent agreement with experimental findings. Particles were introduced through the inner inlet.

to be 20 cm, 12.5 cm and 8 cm for De = 0.23, De = 0.47 and De = 0.94 flows respectively. Thus, De = 0.47 flow is appropriate for the 13 cm long microchannel in this work. For 7.32 µm particles at De = 0.47 to migrate over a length scale of 50 µm to ensure that all particles occupy equilibrium positions, 16 cm of microchannel length is required. However, as Dean flows in our spiral channel will actually aid lateral migration of particles toward their equilibrium positions, we expect the channel length needed for particle focusing to be less than the value calculated from eqn. (6). Therefore, from these results the 13 cm long spiral microchannel of this work with De = 0.47 should yield a complete separation of the 1.9 µm and 7.32 µm particle mixture.

To experimentally demonstrate separations, a mixture of 1.9 μ m and 7.32 μ m particles (0.05% volume fraction) was introduced through the inner inlet of the microchannel. For visualization, the two particle types were labeled with different fluorophores: the 1.9 μ m particles were labeled with Plum Purple

and the 7.32 µm particles with Dragon Green. Images at the inlet and outlet of the spiral microchannel were captured using DAPI and FITC filter cubes. Fig. 7(a) illustrates the overlaid images of the microchannel at the inlet and outlet positions. Although the particle mixture was introduced through the inner inlet, by the time the flow reached the outlet the 1.9 µm particles have all migrated into the outer half of the channel and were collected at the outer outlet. On the other hand, the 7.32 µm particles occupied the equilibrium position near the inner microchannel wall and were collected at inner outlet. Corresponding model images of the microchannel cross-sections supporting these experimental results are shown in Fig. 7(b). A line scan through the microchannel output (immediately prior to bifurcation) indicating the normalized particle dispersion across the microchannel is shown in Fig. 7(c). These results show that the two particles were successfully separated from one another using the spiral design.



Fig. 5 (a) Fluorescent experimental images illustrating distribution of 7.32 μ m particles across the microchannel inlet and outlet at De = 0.47 (white lines indicate the approximate position of the microchannel walls). These results show that particles occupy an equilibrium position near the inner microchannel wall when particles are introduced through either the inner or outer inlets. Corresponding line-scans indicating the normalized distribution of 7.32 μ m particles are also shown in the figure. (b) Modeling results corroborating the equilibration of 7.32 μ m particles introduced through the inner inlet near the inner microchannel walls at De = 0.47.



Fig. 6 Simulation results illustrating migration and equilibration of (a) $1.9 \,\mu$ m, (b) $4 \,\mu$ m, (c) $5 \,\mu$ m, and (d) $7.32 \,\mu$ m particles across the microchannel outlet at De = 0.47. Particles were introduced through the inner inlet. The inner channel wall is at the top of each panel.

Although the use of spiral microchannels has been recently reported for particle concentration,²⁰⁻²² the emphasis in these studies has been on equilibration of larger (~10 μ m) particles and the Dean flows were used to reduce the four equilibrium positions to a single focused particle stream. In our device, however, the Dean forces play a dual critical role to achieving separation. In addition to focusing the larger particles in a single stream, the Dean flows are also used to transpose the smaller particles from the inner half to the outer half of the microchannel cross-section. Consequently, the microchannel length is dependent on the Dean forces that affect smaller particles while the microchannel cross-section (or D_h) is dependent on the larger particles. These design consideration are important in the design of spiral microchannels for separation and extraction of smaller particles. In the future, the presented concept may be extended to separation and extraction of nanoparticles.

Overall, the analytical model presented in this work may be used to design spiral microchannels for separation of particles below and above the particle size thresholds. The threshold particle size in this work was $a_p|_{\text{th}} \sim 5 \,\mu\text{m}$. The same device can be used to separate any particle sizes above and below this threshold value, as long as the threshold particle size remains constant. For separations where a change in the threshold size is needed, new devices must be designed and fabricated. This is an inherent limitation of this passive microfluidic device design. For separations where the threshold changes continuously, active systems such as the ones discussed in the Introduction may offer greater flexibility. Another limitation of this passive device design is the relationship between the particle



Fig. 7 (a) Superimposed fluorescent images illustrating complete separation of 1.9 μ m and 7.32 μ m particles based on size-dependent differential migration at De = 0.47. The Dean drag carries the 1.9 μ m particles to the outer half of the channel (top), which are then collected at the outer outlet (pseudo-colored purple). The larger 7.32 μ m particles $(a_p/D_h > 0.1)$ focus near the inner microchannel wall (bottom) and are collected at the inner outlet (pseudo-colored green). (b) Corresponding modeling results illustrating the complete separation of 1.9 μ m (purple) and 7.32 μ m (green) particles. The particles were introduced through the inner inlet. (c) Line-scan indicating the normalized distribution of 1.9 μ m and 7.32 μ m particles at the outlet for De = 0.47.

size and the microchannel cross-section. Thus, to extend the approach to separation of smaller particles (*i.e.*, nanoparticles), the microchannel cross-sectional dimensions will have to scale down appropriately leading to increased pressure drop (~MPa). However, for the microchannel design investigated in this work at De = 0.47 ($Re \sim 5$) the pressure drop along the 13 cm long channel was ~80 kPa (from the numerical model). Such flows can be easily achieved using functional on-chip pressure generators.⁴⁰ Despite the aforementioned limitations, the spiral microchannel device offers a number of advantages critical to integration in microfluidic LOCs, including the passive (no active circuitry or control) separation principle, the ability to perform a complete separation as opposed to concentration, relatively small device footprint (~1 cm²), and the ability to continuously process large sample volumes.

5. Conclusions

In this work, design of a planar passive spiral microchannel capable of completely separating particle mixture based on differential migration was introduced. The developed design is planar and thus is simple to fabricate and integrate with other LOC components. Particle-based flow modeling with CFD-ACE+ software was used to validate and understand the forces influencing particle flows in a suspension. Particle migration was studied experimentally using fluorescence microscopy and a complete separation of 1.9 µm and 7.32 µm particles was successfully demonstrated by varying the ratio of the lift forces to the Dean forces acting on the particles. Using CFD modeling, we demonstrated that complete separation can be achieved between particles smaller and larger than the \sim 5 µm threshold using our microchannel geometry. As the separation process is entirely based on the particle size, the proposed design is versatile and is capable of separating particulate mixtures over a wide dynamic range. We expect it will enable a variety of environmental, medical, or manufacturing applications that involve rapid, low-cost, continuous separation of microparticles in real-world samples with a wide range of particle components.

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