Separation of magnetic beads in a hybrid continuous flow microfluidic device

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ABSTRACT
Magnetic separation of biological entities in microfluidic environment is a key task for a large number of bio-analytical protocols. In magnetophoretic separation, biochemically functionalized magnetic beads are allowed to bind selectively to target analytes, which are then separated from the background stream using a suitably imposed magnetic field. Here we present a numerical study, characterizing the performance of a magnetophoretic hybrid microfluidic device having two inlets and three outlets for immunomagnetic isolation of three different species from a continuous flow. The hybrid device works on the principle of split-flow thin (SPLITT) fractionation and field flow fractionation (FFF) mechanisms. Transport of the magnetic particles in the microchannel has been predicted following an Eulerian-Lagrangian model and using an in-house numerical code. Influence of the salient geometrical parameters on the performance of the separator is studied by characterizing the particle trajectories and their capture and separation indices. Finally, optimum channel geometry is identified that yields the maximum capture efficiency and separation index.

1. Introduction
Magnetic separation of immunologically linked biological entities on functionalized magnetic beads offers a promising route for miniaturizing clinical diagnostic applications. Nonmagnetic moieties of a wide spectrum of biophysical and biochemical traits can be bound to micron-scale magnetic beads and can be separated by the application of externally applied magnetic field [1]. Immunomagnetic separation technique offers several advantages over other kinds of separation methods envisaged in microfluidic devices: it offers facile, non-contact maneuverability of the magnetic particles (conjugated with biomaterials) with the help of external magnetic field; magnetic bead-analyte conjugates have strong magnetic contrast in most of the biological media, facilitating magnetic transport and magnetic diagnosis; availability of magnetic particles over a wide range of particle size and the diversity of biofunctionalization offers easy choice of particles to suit a specific application. However, selective separation of magnetic microspheres (and the tagged biomaterials) in a microfluidic environment is a challenging task. For example, the simplest design of magnetic trap [2] cannot be used to separate beads of different magnetophoretic mobility. Magnetic Split flow thin fractionation (SPLITT) allows microfluidic separation of magnetic beads of different mobility into co-flowing streams separated by thin splitters at the outlets of the microchannel [3]. This is achieved by imposing a magnetic field gradient along the transverse direction of the polydisperse suspension flow through the microchannel. Field flow fractionation (FFF) is another kind of microfluidic separation method, developed by Giddings [4], which adopts a flow-based chromatography type fractionation technique. Microspheres of different mobility are separated, by using externally applied field in the transverse direction, into streams that branch out from the main microchannel at different axial locations along the flow. FFF offers the advantages of simultaneous separation and measurement, and hence, is useful in bio molecules and cell separation and diagnosis [5] and biosensors [6]. While FFF design is less compact, SPLITT designs are more vulnerable to cross-contamination. It is therefore essential to maintain the separation throughput and minimize the non-specific crossover in SPLITT device by appropriately designing the microchannel and the magnetic field. Hoyos et al. [7] created a localized magnetic field by applying Halbach array which offered improved magnetic selectivity for transverse separation inside the SPLITT channel. System throughput can also be tuned by changing the channel layout. Although, the literature is replete with studies on FFF and SPLITT devices, to our knowledge, there is no report on integrating features of both the designs to develop a hybrid separator.
Operating regimes of magnetophoretic FFF and SPLITT have been characterized earlier by this group, where the influence of salient design and operating parameters on the device performance have been analysed [3,8]. Both the types of designs were found to offer narrow operating windows for which the capture efficiency and separation indices were high. It is intuitive from these prior studies that operating the FFF or SPLITT devices with more than two particles is extremely sensitive to any variation of parameters.

For maximizing the efficiency of the microfluidic separation device, here we propose a hybrid device bearing the features of both an FFF and a SPLITT and analyze the separation performance. A homogeneous suspension (in a buffer liquid) of particles of different magnetophoretic mobility is introduced into the channel through one inlet, while another inlet carries the buffer solution. The particles are separated through three different outlets. For separation of the particles, an appropriately designed magnetic field is imposed. The objective of this study is to prescribe the geometrical parameters such that the three types of particles get collected selectively at their designated outlet streams with minimum cross-contamination.

### 2. Theoretical formulations

Fig. 1 illustrates the schematic diagram of the hybrid separator that has a length $L$ and width $H$ through which a steady pressure-driven flow is analysed. The device comprises of two inlets (Inlet$_1$ and Inlet$_2$) and three outlets viz., Outlet$_1$, Outlet$_2$ and Outlet$_3$.

A homogeneous aqueous buffer suspension carrying three different particle types of equal number density is introduced through Inlet$_1$ whereas Inlet$_2$ allows only the aqueous buffer solution. For generating a magnetic field gradient in the channel a magnetic line dipole is positioned at a location ($X_{m dip}, Y_{m dip}$) (see Fig. 1) in such a manner that the magnetic particles experience a magnetophoretic movement in the transverse direction, eventually leading them through the outlets Outlet$_1$, Outlet$_2$ and Outlet$_3$.

With the proper geometrical orientation of the outlets, the particles with larger and smaller magnetophoretic mobility should escape through the outlet streams Outlet$_1$ and Outlet$_2$, respectively, while the nonmagnetic particle is expected to be carried out through Outlet$_3$ (as can be seen in Fig. 1). Particles moving with the carrier fluid inside the channel will experience a magnetic body force ($F_{mb}$), viscous drag force ($F_d$) by the carrier fluid, the gravitational force ($F_g = 4/3 \rho \pi r^3 g$) and the thermal Brownian force, ($F_B = R_d \sqrt{2 m k_b T/d t}$, where $R_d$ is a random normal number vector whose value lies between 0 and 1, $k_b$ is the Boltzmann constant, $T$ is the absolute temperature and $d t$ is the time interval over which the Brownian force is resolved) [9].

The Lagrangian motion of a single particle, influenced by these forces can be expressed as

\[
\frac{d^2 \mathbf{r}_p}{d t^2} = \mathbf{F}_{mb} + \mathbf{F}_d + \mathbf{F}_g + \mathbf{F}_B
\]

Brownian force becomes negligible for particles exceeding 40 nm [10]; on the contrary, their size (~ 1 µm) and mass (~7.5×10$^{-15}$ kg) renders the inertial and gravitational forces negligibly small. Therefore, the forces which can play major role in the present study are the magnetic and drag forces, which can be respectively expressed as [11]

\[
\mathbf{F}_d = -6\pi \eta K \mathbf{r} \times \mathbf{B}
\]

The wall drag coefficients $K_{m dip}$ and $K_{m dip}^2$ (for the drag forces in, respectively, the parallel and perpendicular directions to the wall) components can be expressed as $K_{m dip}^1 = [1−9/16]^{-1}$, and $K_{m dip}^2 = [1−9/8]^{-1}$ where, $\xi$ is the ratio of the particle diameter to its distance from the wall [12] and the effective magnetic susceptibility $\chi_{eff}$ [13] of the particle is

\[
\chi_{eff} = \frac{B}{1 + (\xi/3)}
\]

Thus, reckoning the significant forces on a particle, Eq. (1) can be written as

\[
\mathbf{v}_p = \frac{1}{6\pi \eta K_{m dip}} \mathbf{F}_d
\]

The instantaneous position of any particle can be calculated by integration of Eq. (4), once the initial position of the particle is specified.

Placed near Outlet$_1$ (as shown in Fig. 1), the line dipole has a strength $P$. In a practical MEMS device, such a line dipole may be produced by a pair of parallel conductors, carrying currents in opposite directions, and a soft magnetic core to buttress the field. The resulting magnetic field $H$ at any location ($r, \phi$) from the virtual origin of the line dipole, can be expressed as [14]

\[
H = \frac{P}{r^2} (\hat{e}_r \sin \phi - \hat{e}_\phi \cos \phi)
\]

The drag force on the particles is influenced by the continuum phase (the host buffer liquid) velocity. The continuum phase follows the conservation of mass and momentum as specified by

\[
\frac{d\rho}{dt} + \nabla \cdot (\rho \mathbf{V}) = 0,
\]

\[
\frac{d\mathbf{V}}{dt} = -\nabla P + \nabla \cdot \tau - \mathbf{J} \mathbf{E}_r.
\]

where $\tau$ denotes the viscous stress, $\lambda$ the local particle density [15] and the last term in Eq. (7) signifies the reaction of $\mathbf{F}_d$ (i.e., the force applied on the particle by the liquid). On the walls of the channel and the guide block, no slip boundary condition is considered. At the two inlets Inlet$_1$ and Inlet$_2$, identical plug flow velocity profiles ($U_{in}$) are considered, while zero gauge pressure is specified at all the outlets.

### 3. Numerical simulations

An Eulerian-Lagrangian approach was considered for this work for the particle-laden flow through the microchannel. The coupled mass and momentum equations for the liquid phase were solved using SOLA – an explicit finite difference technique [16]. Under a steady flow, the fluid phase was first solved by the Eulerian approach. Particle tracking was then completed in a ‘frozen’ flow-field. The drag force by the liquid on the particle and its reaction i.e., force exerted by particle on liquid was calculated, and then again the fluid phase was solved by considering the revised body force in the momentum equation. Particle trajectories were then re-calculated in the revised flow-field, and these sequences were repeated until the largest deviation of the momentum source term within the domain between two consecutive steps of iteration fell below a pre-set convergence criterion. Details about the numerical scheme may be found elsewhere [11]. Following a grid
independence study, a 150×90 mesh configuration was chosen for the present simulations. The numerical code was validated [11] by comparing the particle trajectories with those obtained through analytical solution by Nandy et al. [17]. Also, the simulations are validated with experimental results of particle capture efficiency in a simple “in-line trap” configuration of Modak et al. [18].

4. Results and discussions

4.1. Particle transport for the base case

Simulations are conducted for a given set of particle and flow parameters (see Table 1), while the salient device geometry is chosen as described in Table 2 (see also Fig. 1). Fig. 2 shows trajectories of 100 large (2 μm radius, denoted by red lines) magnetic particle clusters, 100 small (1 μm radius, denoted by cyan lines) magnetic particle clusters and 100 nonmagnetic (0.5 μm radius, denoted by black lines) particle clusters released from Inlet, i.e. 0.0015×0.002 (m). Initially, at the entry region of the channel all three types of particle clusters primarily experience the fluid drag force, as the magnetic force is relatively weak there due to large distance from the dipole. As the particles are advected downstream nearer to the line dipole, magnetic particles experience stronger magnetic force in the transverse direction, and the particles begin to show deviation towards the dipole.

Larger magnetic particles exhibit higher magnetophoretic mobility than the smaller ones. On the contrary, the nonmagnetic particles experience only the drag force and therefore, they follow the streams. Because of the combined drag and magnetic force fields, the particles are fractionated at their designated outlets. It is evident from Fig. 2 that 4 large and 49 small clusters of magnetic particles are captured in the Outlet1. Outlet2 receives 42 small magnetic particle clusters along with 9 clusters of small nonmagnetic particle clusters released from Inlet1 (i.e. 0.0015×0.002). Magnetic particle clusters that has entered into the channel. Thus intermingling; larger magnetic particle clusters should be collected at their designated outlets with very little net magnetic particles are designated to Outlet3. Therefore, the device performance is characterized here by capture efficiency (CE) i.e., the ratio of number of particle clusters (large magnetic, small magnetic and nonmagnetic) collected at their designated outlets (i.e., Outlet1, Outlet2 and Outlet3 respectively) to the number of the corresponding particle clusters that has entered into the channel. Thus

\[
CE = \frac{\text{Number of large magnetic particle clusters captured at the Outlet1}}{\text{Total number of the large magnetic particle clusters entered into the channel}}
\]

Intermingling of different particles is practically unavoidable, leading to the possibility of collection of a few clusters of particles other than the designated ones at a particular outlet. Therefore, the performance of the device cannot be justified with CE alone. To quantify how good the purity of the separated streams is, separation index (SI) of the device is also evaluated as follows:

\[
SL = \frac{\text{Number of large magnetic particle clusters captured at outlet1}}{\text{Total number of particle clusters captured at the outlet1}}
\]

As an extension to our previous work [3], here we intend to realise the effect of channel geometry on the capture efficiency (CE) and separation index (SI). In our previous study we have done wide parametric variations in terms of a group variable \(\Pi = (a^2 P^2/\eta U_a)\). Here we focus on the effect of variation of the key channel dimensions on the device performance, while all the parameters are kept at their base value (as listed in Table 1).

4.3. Effect of channel geometry

Fig. 1 shows the schematic diagram of the hybrid device with the channel dimensions. Layout of the flow passage can be altered by adjusting the relative locations and widths of the inlets and outlets. Our objective is to identify how these salient design parameters can influence CE and SI of the device. In our simulation, this variation is achieved by changing the transverse dimensions of the blocks B1, B2, B3, B4 and B5 and the longitudinal dimensions of the blocks B2 and B3 (see Fig. 1). The total length \(L\) and width \(H\) of the separator chip and the dipole position \((X_{mag}, Y_{mag})\) are, however, kept fixed at their base values throughout the simulation. Fig. 3 describes the effect of flow passage area on the device performance. Starting from the base configuration (Table 2 and Fig. 2) the heights of blocks \(B_2\) and \(B_3\) (\(H_2\)) are simultaneously decreased in steps of 500 μm and those of \(B_1\) and \(B_4\) (\(H_1\)) are equally increased, keeping the widths of the inlet2 \((L_2)\) and outlet2 \((O_2)\) unchanged. This way, the flow passage is progressively dilated; at the same time the separation between the

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**Table 1**

<table>
<thead>
<tr>
<th>Fluid and Particle Parameters</th>
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<td>(\eta) (Pa-s)</td>
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**Table 2**

<table>
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<th>Range (mm)</th>
<th>Parameters</th>
<th>Base values (mm)</th>
<th>Range (mm)</th>
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<td>Constant</td>
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* \(H_i\) and \(L_i\) denote the height and length of different sections of the channel; \(L_0\) and \(O_0\) denote the inlet and outlet dimensions as indicated in Fig. 1. Overall device dimension: \(L=6\) mm and \(H=2\) mm; the line dipole \(P\) (Fig. 1) is placed at \(X_{mag}=5\) mm and \(Y_{mag}=-0.7\) mm.
dipole and the flow passage decreases. As can be seen from Figs. 2 and 3(a) and (b), nonmagnetic particle clusters, which experience only drag force (and therefore follow the streamlines emanating from Inlet 1), are directed through Outlet 3. With increased $H_1$ and $H_4$ more number of streamlines from Inlet 1 passes through Outlet 3. As a result $CE_3$ increases slightly with the passage area (Fig. 3(c)). At the same time, due to decrease of $H_2$ and $H_3$ the lower bound of the flow passage is pushed down. This leads more number of small magnetic particles, which previously passed out through Outlet 2, to now escape through Outlet 1 (see Fig. 3(a) and (b)). This results in a reduction of $CE_2$ and $SI_2$ (Fig. 3(c) and (d)). Also, due to increased $H_4$, and reduction in $H_3$, trajectories of the large magnetic particles deviate downward, thereby trapping most of those particles on the wall of B2. The base value of $O_1$ is so chosen that 4 clusters of large magnetic particles escape through

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**Fig. 3.** Particle trajectories for (a) $H_1=H_4=0.0005$ m and $H_2=H_3=0.0005$ m, and (b) $H_1=H_4=0.0004$ m and $H_2=H_3=0.0006$ m. Variation of $CE$ (c) and $SI$ (d) with the passage area of the channel. Area of the passage is increased by simultaneously reducing the heights of blocks B2 ($H_2$) and B3 ($H_3$) in steps of 500 µm and increasing those of B1 ($H_1$) and B4 ($H_4$) equally. Vertical dotted lines denote the base case (blue), case-a (red) and case-b (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Fig. 4.** Particle trajectories for (a) $L_2=0.0041$ m and (b) $L_2=0.0035$ m. Variation of $CE$ (c) and $SI$ (d) with the position of the Outlet 1 (shown in terms of $L_2$). Vertical dotted lines denote the base case.
Outlet1. Lowering the values of $H_2$ and $H_3$ further aggravates the situation. The values of $CE_1$ and $SI_1$ decrease to zero beyond a flow passage area of $6.83 \times 10^{-6}$ m$^2$.

Fig. 4 shows the variation of $CE$ and $SI$ due to the change of the position of Outlet1. This is achieved by simultaneously increasing $L_2$ and decreasing $L_3$, or vice versa, by equal magnitude, so that $O_1$ remains constant. Fig. 4(a) and (b) shows the particle trajectories for $L_2 = 0.0041$ m and 0.0035 m, respectively. When $L_2$ is decreased from its base value of 0.0045–0.0041 m (accordingly, $L_3$ is increased from 0.001 to 0.0014 m), the Outlet1 is shifted upstream by 400 µm. Under this condition, most of the large magnetic particles are found (Fig. 4(a)) to collect at the Outlet1, yielding a large $CE_1$ (~84%) and $SI_1$ (90%). At the same time, with decreased $L_2$ more of the smaller magnetic particles, which were transported to the Outlet1 in Fig. 2, now collect at the Outlet2. This leads to an increased $CE_2$ over the base case. When $L_2$ is decreased further to 0.0035 m (see Fig. 4(b)), some of the large

Table 3
Optimum values of the geometrical parameters.

<table>
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<tr>
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<th>Values (mm)</th>
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Fig. 5. Particle trajectories for (a) $O_2=0.00035$ m, $O_2=0.00065$ m and (b) $O_2=0.0007$ m, $O_2=0.0003$ m, $L_2 = 0.004$ m and $L_3 = 0.0015$ m are chosen from the optimum values observed in Fig. 4. Variation of $CE$ (c) and $SI$ (d) with the relative widths of Outlet2 and Outlet1 (shown in terms of $O_2$).

Fig. 6. Variation of $CE$ (a) and $SI$ (b) with $H_5$. Vertical dotted lines denote the best configuration for the range of study described in Table 2.

Fig. 7. Particle trajectory at optimized channel geometry.
particles skip the Outlet1 and collect at Outlet2. This reduces both CE1 and SI1 as compared to the case of Fig. 4(a). The trajectories of nonmagnetic particles in Fig. 4(a) and (b), and their corresponding CE and SI values do not alter much from the base case. Fig. 4(c) and (d) graphically show the influence of L2 on CE and SI, where a peak of CE1 at L2=0.004 m is observed yielding CE1=97% and SI1=100%. On either sides of L2=0.004 m CE1 decreases; while SI1 remains close to 100% for smaller value of L2 and decreases sharply for L2 > 0.004 m. Fig. 4(b) also shows that CE2 remains high (~100%) for L2 < 0.004 m and decreases monotonically at higher L2. CE3 and SI3 are found almost constant – with increase or decrease of position of Outlet1, there is no effect on CE3 and SI3 because nonmagnetic particles only follow streamlines from I1. In the subsequent section of the paper, we choose L2=0.004 m, since it simultaneously offers high values of CE and SI for all the three types of particles.

Once the optimum position of the Outlet1 is found, we next evaluate the influence of the relative widths of the other two outlets on the performance of the separator. Fig. 5 shows how CE and SI vary with the outlet widths O2 and O3 while their combined width remains constant – thus an increase in O2 (with respect to the base case) is accompanied by an equal decrease in O3, L2 and L3 are chosen at their optimized values of 0.004 m, and 0.0015 m, while all other dimensions remain as per Table 2. Fig. 5(a) and (b) show the particle trajectories for O3=350 and 700 µm, respectively (base value of O3 is 500 µm). The corresponding trends of CE and SI are plotted in Fig. 5(c) and (d). Comparing the trajectories of Fig. 5(a) and (b) with Fig. 2, it is apparent that the particle separation between the Outlet2 and Outlet3 is largely influenced by the flow fractionation. With increased O3, more of the nonmagnetic particles try to escape through Outlet3; CE3 gradually increases and eventually saturates at 100% (Fig. 5(c)). However, with increased O3 (and accompanying reduction of O2) more clusters of the smaller magnetic particles also tend to flow out through it. This leads to a reduction in CE2 and SI2. Similarly, for low value of O2, more clusters of nonmagnetic particles are collected through Outlet2, resulting in a reduction in CE2 and SI2. These relative widths of Outlet2 and Outlet3 do not seem to affect trajectories of the large magnetic particles. As a result CE3 and SI3 remain constant, close to their optimized values observed from Fig. 4. From the plots of Fig. 5(c) and (d), we choose O2 =520 µm and O3 =480 µm as the best performance point (marked by the blue vertical dotted lines in the figures).

Finally, the influence of the width of the block B2 is evaluated with all the geometries corresponding to the optimum condition observed in Fig. 5(c) and (d) marked by blue dotted lines. Fig. 6 shows the variation of CE and SI with variation of H2. Figure 6(a) shows that both CE1 and CE2 increase with H2. As the height of the block B2 increases, both types of magnetic particles (large and small) are diverted closer to the dipole. This increases the average magnetic force on them, enhancing the particle capture. The optimized channel geometry in Fig. 5 is obtained for H2=450 µm, for which the largest values of CE and SI are also observed in Fig. 6. The plots also show a nearly invariant CE2 in Fig. 6 - nonmagnetic particles are not affected by magnetic field, therefore, bringing them closer to the magnetic field (by increasing H2) does not eventually alter their capture efficiency. The SI1 remains saturated at ~100% throughout the range of H2, indicating no trace of the smaller particles in Outlet1. For the conditions investigated in Fig. 6, the optimum value of H2 is found to be 450 µm.

Table 3 summarizes the optimized geometry for the hybrid separator while the particle trajectory for the optimized channel geometry is shown in Fig. 7. The optimum configuration yields CE1=97%, SI1=100%, CE2=88%, SI2=90%, CE3=90%, SI3=88%. These are significantly higher than the previously reported capture efficiencies in FFF [8] and SPLITT [3] configurations. It is important to note that this optimized device performance is achieved with a lower dipole strength (P=1.7 A m) as compared to the previously used dipole strength (P=4 A m) [3]. Thus, the hybrid separator clearly offers a better collection and separation performance, and at the same time offers separation of different three types of particles.

5. Conclusions

The numerical study identifies the optimum channel geometry for optimized performance of magnetophoretic hybrid device to separate biological entities on a microfluidic platform practically for BioMEMS applications. Both CE and SI of the device are found to be strongly affected by the channel geometry. The effect of each salient geometrical parameters of the separator on CE and SI offers the design bases for the best device performance [3]. For the optimized channel geometry reported here, higher CE and SI values are obtained even with relatively lower dipole strength than those observed in our previously reported studies on FFF and SPLITT configurations. The proposed hybrid magnetic separator, therefore, offers an improved design for immunomagnetic separation for biomedical applications.

References