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Deterministic fractionation of binary suspensions moving past a line of microposts

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Abstract We investigate the motion of suspended particles past a single line of equally spaced cylindrical posts that is slanted with respect to the driving force. We show that such a line of posts can fractionate particles according to their size, with small particles permeating through while the larger particles are deflected by the steric barrier created by the posts, even though the gaps between posts are larger than the particles. We perform characterization experiments driving monodisperse suspensions of particles of different size past the line of posts over the entire range of forcing orientations and present both the permeation probability through the individual gaps between the posts as well as the fraction of permeating particles through the one-dimensional array. In both cases, we observe a sharp transition from deflection to permeation mode that is a function of particle size, thus enabling separation. We then drive binary mixtures at selected orientations of the line of posts and demonstrate high separation purity and efficiency.

Keywords Separations Microfluidics Deterministic · Suspensions - Filtration - Fractionation

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1 Introduction

An important unit operation in a variety of lab-on-a-chip systems is the separation of suspended mixtures of species. As a result, a number of microfluidic devices have been created either to miniaturize conventional separation techniques or to implement novel separation strategies (Stone et al. [2004](#page-7-0); Pamme [2007;](#page-7-0) Li and Drazer [2007](#page-7-0); Kulrattanarak et al. [2008](#page-7-0); Lenshof and Laurell [2010](#page-7-0)). Different aspects of the separation process are emphasized depending on the application, but a common trend is the development of continuous separation processes, which can be readily integrated with downstream operations in a lab-on-a-chip platform (Yamada and Seki [2005](#page-7-0); Huh et al. [2007](#page-6-0); Bernate and Drazer [2011,](#page-6-0) [2012\)](#page-6-0). A popular approach toward continuous separation processes is to create a selective barrier that is permeable to some species but deflects others. Various types of barriers have been explored including dielectrophoretic (Kralj et al. [2006](#page-7-0); Alazzam et al. [2011](#page-6-0)), magnetophoretic (Pamme and Manz [2004](#page-7-0); Zhu et al. [2012;](#page-7-0) Kirby et al. [2012](#page-6-0)), optical (Korda et al. [2002;](#page-7-0) MacDonald et al. [2003](#page-7-0)), and physical barriers (Belfort et al. [1994](#page-6-0); De Jong et al. [2006](#page-6-0); Gossett et al. [2010](#page-6-0); Jaffrin [2012](#page-6-0)). A physical barrier is also at the core of the implementation of cross-flow filtration systems in microfluidic devices. In this method, suspended species are driven by a feed flow that is essentially tangent to a barrier membrane, and only permeable species move through the membrane driven by the pressure-driven cross-flow (Van-Delinder and Groisman [2007;](#page-7-0) Ji et al. [2007](#page-6-0); Aran et al. [2011](#page-6-0)). Let us point out that the main advantage of crossflow filtration, compared to dead-end filtration in which all the flow passes through the membrane, is the reduction in clogging or fouling (VanDelinder and Groisman [2006;](#page-7-0) Ji et al. [2007\)](#page-6-0). Note, however, that in all cases, it is the size of

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the pores that separates between permeating (smaller) and deflected (larger) particles.

Deterministic lateral displacement (DLD) is another particularly promising, two-dimensional (2D) continuous separation method to fractionate a mixture of suspended species (Heller and Bruus [2008](#page-6-0); Huang et al. [2004](#page-6-0)). In this technique, components of different size migrate in different directions as they are driven through a periodic array of cylindrical posts. DLD has been successfully implemented for the fractionation of diverse samples (Holm et al. [2011](#page-6-0); Loutherback et al. [2010](#page-7-0); Green et al. [2009;](#page-6-0) Long et al. [2008;](#page-7-0) Davis et al. [2006](#page-6-0); Inglis et al. [2006](#page-6-0)). In our previous work, we investigated the mechanisms leading to separation in DLD and demonstrated an alternative operation mode, in which suspended species are driven through a 2D array of posts by a constant force, specifically gravity (g-DLD) (Devendra and Drazer [2012](#page-6-0); Risbud and Drazer [2013;](#page-7-0) Bowman et al. [2012](#page-6-0); Luo et al. [2011](#page-7-0); Koplik and Drazer [2010](#page-6-0); Balvin et al. [2009;](#page-6-0) Herrmann et al. [2009](#page-6-0); Frechette and Drazer [2009\)](#page-6-0). The motion of suspended particles in DLD devices exhibits directional locking, in which the migration angle remains constant and equal to a lattice direction over a finite range of forcing orientations (Frechette and Drazer [2009\)](#page-6-0). In particular, for small enough angles between the external force and a line of posts corresponding to a column (or a row) in the square array, the particles move down a lane between two adjacent columns (or rows) without crossing them. Previous experiments also established that as the forcing angle increases (from zero), there is a critical transition angle, above which the particles are able to move across columns in the array (Devendra and Drazer [2012](#page-6-0)). This critical transition angle depends on particle size, thus allowing for separation. Moreover, the highest resolution of separation in a binary mixture was found to occur near the critical transition angle (Devendra and Drazer [2012\)](#page-6-0). These results suggest that a single line or a one-dimensional (1D) array of cylindrical posts in lieu of the entire 2D array, could in principle, be used to fractionate a mixture of particles.

Here, motivated by our DLD results discussed above and the possibility of using steric barriers for separation, we investigate the motion of suspended particles driven past a slanted line of uniformly spaced cylindrical posts by a constant external force. We demonstrate that such a line of posts can, in fact, fractionate a mixture of suspended particles with high purity and efficiency. More specifically, we shall show that depending on the angle between the line of posts and the external force, large particles will be deflected sideways, whereas small particles will permeate through the line of posts. It is important to note that the gap between the posts is larger than the largest particles used here, unlike cross-filtration methods mentioned before. On the other hand, the deflection of particles of a given size is only possible when the projected gap, that is, the gap between posts projected in the direction of the driving force is smaller than the particles. The conceptual idea of the device is shown by the schematic in Fig. 1. This introduces a novel approach to separation based on a steric barrier (in this case, the single line of posts) that is fundamentally different from the traditional approach based on membranes or filters.

2 Experimental section

2.1 Device fabrication and experimental setup

The devices were fabricated using photolithography in a clean room. A negative photoresist (SU8-3025) was spun coated on top of a standard microscope glass slide, exposed to UV light, and developed to obtain a line of cylindrical posts on the glass slide. The thickness of the photoresist and, therefore, the height of the resulting posts, was approximately 40 μ m. The profile of a fabricated line of

Fig. 1 Schematic representing the principle of operation of separation using single line of posts. r_1 and r_2 are representative radii of larger and smaller component in the binary mixture of particles, respectively

posts obtained with a 3D laser scanning microscope VK-X100/X200 (Keyence Corp., Japan) is shown in Fig. 2. The measured diameter of the posts is $2R = 19.5 \text{ }\mu\text{m}$ and the center-to-center separation is $\ell = 40$ µm. Several of these lines of posts were fabricated on a single glass slide. Individual lines of posts were separated by $1,000 \mu m$ from each other to avoid any effect on the particles moving past a given line from a neighboring line. The lines were used singularly in independent experiments. The diameter of the posts and the spacing between them were designed to be comparable to the diameter of the largest particles used in the experiments.

The lines of cylindrical posts were surrounded by double-sided adhesive tape (Grace Bio-Labs, Inc., OR) to create a containing well on the glass slide. We performed experiments using silica particles with a density of 2 $g/cm³$ and average diameter of 4.32 μ m (Bangs Laborarties, Inc., CA), 10, 15, and 20 μ m (Corpuscular Inc., NY). The particles were suspended in 0.1 mM aqueous KOH solution (to prevent sticking). They were then introduced into the well and allowed to settle down at the bottom of the device. Without trapping any air inside, the well was sealed using a glass cover-slip (and double-sided adhesive tape) to eliminate the possibility of convective effects. The device containing the suspended particles was then mounted on a microscope, and the microscope was tilted at an angle, $\phi = 16^{\circ}$, thus exploiting gravity to drive the particles down the slide and past the line of cylindrical posts. The average velocity of the particles in our experiments varied from 0.3 to 5 µm/s and dominates over other modes of transport such as diffusion. In fact, using the Stokes–Einstein equation for the bulk diffusivity of the particles and the distance between posts as the characteristic length, the corresponding Péclet

Fig. 2 Three-dimensional microscopic image of the device using a 3D laser scanning microscope. The color at each point represents the measured height. We indicate the microscope tilt angle ϕ , the forcing angle θ , the diameter of the posts $2R \approx 20 \mu$ m, the spacing between the posts $\ell = 40$ µm, and the height of the posts ≈ 40 µm

numbers are $Pe > 100$, and Brownian motion is negligible. The Reynolds number based on these velocities is $Re < 10^{-3}$, and inertia effects can be ignored (Luo et al. [2011](#page-7-0)). More details on the experimental procedure can be found in Devendra and Drazer [2012.](#page-6-0)

2.2 Image acquisition and data analysis

Images were captured at a frame interval of 2s in bright field, using a Rolera Mgi+ EMCCD camera (Q-imaging, BC) and IPLab software (Biovision Tech, PA). We used manual tracking plugin (by Fabrice Cordelieres) in ImageJ (NIH, MD) to track the particles. The field of view consisted of 21 posts. The stream of particles was not focused from a single starting point, and as a result, the first interacting post for a given particle could be any one of the posts in the field of view. The forcing angle, θ , is defined as the angle between the direction of the driving force (gravity projected onto the plane of the device, i.e. xy -plane in Fig. 2) and the line of posts $(x$ axis in Fig. 2) and was measured by tracking particles in an (open) area free of posts. Experiments were performed independently at forcing angles with no particular increasing or decreasing order to avoid any cumulative error or correlation between the experiments. We only considered particles that travelled at least $100 \mu m$ in the driving direction to reduce fluctuations in the value of the measured forcing angle. The particles moving in the open area and those interacting with the posts were tracked separately. Approximately 20 particles were tracked in each experiment for a given forcing angle and a given particle size. In order to minimize the effect of polydispersity in the supplied particles, especially 10-, 15-, and 20- μ m particles, we used calibration scales during tracking and considered only those particles within 10 % of the specified size. In order to capture the behavior in the dilute limit, the trajectories of particles that interact with another particle were discarded. On occasions, where a large particle blocked a spacing between the posts, the trajectories that interacted with such stuck particles were split into separate trajectories by cropping out the periods during which the interaction with the stuck particles took place. In rare instances, if a post was significantly different in size and shape from the design, the particles that interacted with that specific post were eliminated from the analysis.

3 Results and discussion

The proposed separation method is based on the hypothesis that we can use a single 1D array of cylindrical posts to fractionate a suspension of particles of different size into two streams. The main assumption is that, for a given particle size (and material), there is a critical forcing angle θ_c , which characterizes the motion of particles past the line

of posts. Specifically, particles would be displaced laterally by the line of posts for $\theta < \theta_c$ (deflection mode), but would pass through the line of posts for $\theta > \theta_c$ (permeation mode). The second assumption is that as observed in previous DLD experiments using 2D arrays of posts, the critical angle depends on particle size, thus leading to sizebased separation (Devendra and Drazer [2012\)](#page-6-0). In particular, a polydisperse suspension of particles moving past a line of posts oriented at an angle θ would result in two distinct streams of particles: a permeating stream, composed of particles for which $\theta_c < \theta$, and a deflecting stream moving along the line of posts, composed of all the particles for which $\theta_c > \theta$. Moreover, previous experiments in 2D arrays suggest that the critical forcing angle is an increasing function of particle size, and therefore, there would be a critical size $a = a_c$, where a is the particle radius, such that $\theta_c(a_c) = \theta$ and smaller particles $(a \lt a_c)$ would simply permeate through the line of posts but larger particles $(a > a_c)$ would be deflected.

3.1 Probability analysis

In order to investigate the postulated critical behavior, we analyze the motion and interaction of the particles with individual posts as independent stochastic events in a Bernoulli process. An event here is specific to a particle and a given gap between two consecutive posts. It is defined as a success if the particle passes through the gap and a failure if the particle does not pass through the gap but gets laterally displaced and continues toward the next gap downstream. Then, we define the probability of crossing, p, as the frequency ratio v_c/n , where v_c is the number of successes, and n is the total number of events. We also estimate the uncertainty in the determination of the probability of crossing with the standard deviation $\sigma_{\rm c} =$ $\frac{1}{p(1-p)}$ n $\overline{}$. We characterize the downstream output by defining a permeation fraction, $r_p = v_c/N$, where N is the total number of particles interacting with the line of posts. The uncertainty in the determination of the permeation fraction is estimated with the standard deviation $\sigma_{\rm p} =$ $\frac{1}{p(1-p)}$ N $\overline{}$. In terms of the experimental variables, θ_c is estimated as the average of the lower bound (θ_c^L) and upper bound (θ_c^U) values of the critical transition angle, defined by the largest forcing angle for which $p = 0$ and the smallest measured forcing angle for which $p = 1$, respectively.

3.2 Experimental results

In Fig. 3, we present the probability of crossing, p , as a function of the forcing angle, θ , for all particle sizes and the entire range of forcing directions. Each probability value is obtained from an independent experiment at the corresponding forcing angle. In all cases, we observe that the value of the probability is initially zero for a range of forcing directions and sharply transitions to $p = 1$ over a small range of forcing angles. This behavior is consistent with the directional locking observed in the 2D arrays (Devendra and Drazer [2012\)](#page-6-0) and with the existence of a critical angle at which the motion of a given size of particles transitions from deflection to permeation, as discussed above. It is also clear in Fig. 3 that the critical angle is different for particles of different size, thus indicating the potential for using a single line of posts for separation. Both θ_c^L and θ_c^U increase with the size of the particles. The measured values are shown in Table [1](#page-4-0), where we also estimate θ_c as the average between θ_c^{L} and θ_c^{U} . The estimated critical angle also shows the same trend as in 2D arrays (Devendra and Drazer [2012\)](#page-6-0).

The actual separation, however, is better characterized by the fraction of particles that permeates through the line of posts, that is, the permeation fraction, r_p . In Fig. [4,](#page-4-0) we present r_p as a function of the forcing angle, θ , for all particle sizes. The transition from deflection to permeation is steeper, by definition of r_p , with the permeation angles at which $r_p = 1$, θ_p^U , typically reduced by $\approx 2^\circ$ compared to θ_c^U . Note that by definition, $\theta_p^U \leq \theta_c^U$ and $\theta_{\rm p}^{\rm L} = \theta_{\rm c}^{\rm L}$. The measured values are shown in Table [1,](#page-4-0) where we also estimate θ_p as the average between θ_p^L and $\theta_{\rm p}^{\rm U}$.

Fig. 3 Probability of crossing, p , for 4.32-, 10-, 15-, and 20-µm particles as a function of forcing angle

Table 1 Critical forcing angles for all particles in single species experiments

Particle diameter (μm)	$\theta_{\rm c}^{\rm L}$	$\theta_{\rm c}^{\rm U}$	θ_c	θ_{p}^{L}	θ_{p}^{U}	$\sigma_{\bf n}$
4.32	$14^{\circ} + 3^{\circ}$	$20^{\circ} + 2^{\circ}$	$17^{\circ} + 4^{\circ}$	$14^{\circ} + 3^{\circ}$	$18^{\circ} + 1^{\circ}$	$16^\circ + 4^\circ$
10	$14^{\circ} \pm 2^{\circ}$	$23^{\circ} + 3^{\circ}$	$19^\circ + 4^\circ$	$14^{\circ} + 2^{\circ}$	$23^{\circ} + 3$	$19^\circ + 4^\circ$
15	$20^{\circ} \pm 1^{\circ}$	$27^{\circ} \pm 1^{\circ}$	$23^{\circ} + 2^{\circ}$	$20^{\circ} + 1^{\circ}$	$25^{\circ} \pm 1^{\circ}$	$22^{\circ} \pm 3^{\circ}$
20	$25^{\circ} \pm 2^{\circ}$	$32^{\circ} \pm 2^{\circ}$	$29^\circ + 4^\circ$	$25^{\circ} + 2^{\circ}$	$30^{\circ} + 2^{\circ}$	$27^{\circ} \pm 4^{\circ}$

Fig. 4 Permeation fraction, r_p , for 4.32-, 10-, 15-, and 20-µm particles as a function of forcing angle

3.3 Binary experiments and separability

Based on the results discussed above, obtained for different species separately, we selected specific forcing directions to investigate the separation of binary mixtures of 4.32 and 15- μ m particles and of 10- and 20- μ m particles. The purpose is to identify specific forcing directions where smaller particles permeate through the line of posts, while the larger particles deflect and to measure the separation efficiency and purity. In Fig. 5, we present particle trajectories from two representative examples of separation in binary mixtures.

In Fig. 5a, the forcing direction is $\theta \approx 15^{\circ}$ and the 4.32lm particles exhibit a finite permeation fraction $(0 < r_{\rm p} < 1)$, whereas the 15-µm particles are completely deflected along the direction of the line of posts ($r_p = 0$). In Fig. 5b, the forcing direction is $\theta \approx 24^{\circ}$, and all the 10-µm particles permeate through the posts ($r_p = 1$), while all the 20-µm particles are completely deflected ($r_p = 0$). These figures graphically illustrate the probability analysis

Fig. 5 Representative trajectories during the fractionation of two different binary mixtures. In both cases, we see permeation of the smaller particles in the mixture and deflection of the larger ones. a A mixture of 4.32- and 15-µm particles, $\theta = 15^{\circ}$. **b** A mixture of 10- and 20-µm particles, $\theta = 24^{\circ}$

discussed above, as well as the proposed mechanism for separation.

In Fig. [6,](#page-5-0) we present the probability of crossing at the selected forcing directions, both in a binary mixture of 4.32- and 15-µm particles (Fig. [6a](#page-5-0)) and of 10- and 20-µm particles (Fig. [6c](#page-5-0)). We also present the permeation fraction, r_p , of the smaller particle and the deflection fraction, $r_d = 1 - r_p$, of the larger particle in both binary mixtures (Fig. [6b](#page-5-0), d). High purity and efficiency of separation are achieved when both r_p and r_d are close to 100 % for smaller and larger particles, respectively. In the case of 10 and 20-µm particles, one of the force orientations ($\theta \approx 23^{\circ}$) results in ideal separation. On the other hand, for the mixture of 4.32 - and 15 -µm particles, we approximate the optimum separation angle as the average between the estimated critical angles from the experiments. The data are summarized in Table [2](#page-5-0).

Fig. 6 Probability of crossing for a binary mixture of a 4.32- and 15-um particles and of c 10- and 20-um particles. Permeation fraction for 4.32- and 10-um particles and deflection fraction for 15- and

Table 2 Estimated optimum forcing angles for maximum separability in binary experiments

Particle diameter (μm)	θ_{p}	$r_{\rm p}$ (%)	$r_{\rm d}$ (%)
4.32	$18^\circ \pm 3^\circ$	83 ± 6	17 ± 6
15	$18^\circ + 3^\circ$	4 ± 4	96 ± 4
10	$23^{\circ} \pm 1^{\circ}$	100	
20	$23^{\circ} + 1^{\circ}$		100

4 Conclusions

We presented a simple and novel method for continuously separating components in a binary mixture using a single line of cylindrical posts. We initially performed characterization experiments, by driving monodisperse

20- μ m particles in a binary mixture of **b** 4.32- and 15- μ m particles and of d 10- and 20-µm particles

suspensions through a line of posts over the entire range of forcing orientations and analyzed the results in terms of the probability of a particle to permeate through it. In each case, we observed a sharp increase in the permeation probability over a small range of forcing angles, indicating a transition from deflection to permeation mode as the forcing angle increases from zero. We also showed that the critical transition angle increases with particle size. We finally used these characterization experiments to fractionate binary mixtures at selected force orientations, showing excellent purity and efficiency of separation at angles close to the critical transition angle of the larger particles.

Let us finally note that, the probability of a particle to permeate through the line of posts depends on the number of posts. It is expected that a longer line of posts increases the probability of crossing and thus, the permeation fraction. Hence, increasing the number of posts results in a shift toward lower values of the forcing angle at which all particles permeate, i.e., smaller θ_{p}^{U} . On the other hand, small variations in the size and shape of posts, as well as other effects such as Brownian motion of the suspended particles, could induce early transitions and lower the purity (or efficiency) of the separation. In this sense, a single line of posts is more sensitive to defects than a matrix of posts. Therefore, in a specific application, one should treat the number of posts as a variable used to optimize separation quality or resolution.

The separation approach proposed here has similarities with cross-flow fractionation, but it is important to note that in this case the size of the pores (openings between cylindrical posts) is larger than the size of the suspended particles. Therefore, the system is in principle less prone to clogging compared with traditional membrane-based filtration systems relying on the retention of particles larger that the membrane pores. Moreover, using post-post separations larger than the particles could also prevent clogging at larger volume fractions (and throughput) of the suspended mixture (VanDelinder and Groisman [2006](#page-7-0); Bacchin et al. 2011; Henry et al. 2012) The study of clogging in this and similar microdevices is an important aspect that requires further attention (Wyss et al. [2006](#page-7-0); Bacchin et al. 2011; Agbangla et al. 2012). Finally, let us mention that this method could be enhanced into multicomponent separation by positioning consecutive lines of posts at increasing angles with respect to the force (or other arrangements).

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