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Hydrophoretic Sorting of Micrometer and Submicrometer Particles Using Anisotropic Microfluidic Obstacles

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We describe a hydrophoretic device that uses rotational flows induced by regularly patterned obstacles only on the top wall for preparing samples of biological particles, including micrometer and submicrometer particles, and DNA molecules. Many of the current continuous separation devices based on physical fields are limited to the separation of cells and micrometer-sized particles due to their dependence on a particle volume, and the purely hydrodynamic separation of macromolecules such as DNA or protein complexes remains a challenge. Hydrophoresis is entirely based on hydrodynamics using rotational flows induced by anisotropic obstacles. Different sizes of micrometer and submicrometer beads, as well as DNA molecules, were separated into distinct trajectories using two kinds of hindrance mechanisms. Continuous separation of these particles was achieved using the obstacles, demonstrating the potential of hydrophoresis for biological sample preparation on the micro- and nanoscales, with the advantages of continuous flow and sheathless passive operation.

The rapid evolution of analytical processes implemented on microchips has been accompanied by a great demand for high-resolution separation techniques for a wide range of applications related to complex biological samples. Cells and macromolecules are typically found in a heterogeneous mixture that requires precise identification of target cells or molecules. Many physical fields have been used for the separation of microparticles such as cells and bacteria, including dielectrophoretic, magnetic, optical, and acoustic fields. In these field-based methods, the force acting on particles is proportional to their volume. The level of the force acting on macromolecules is several femto-Newton's or less, which is insufficient to manipulate nucleic acids or proteins in flows. Dielectrophoretic trapping of DNA toward the high-field region has been proposed using dielectric constrictions and nanotubes, but these methods are limited by difficult sample recovery and low sample throughput. One common approach for purifying macromolecules is to use electric fields in gels or microfabricated sieve structures. Molecular separation in the nanoscopic structures is achieved by repeated interaction between charged molecules and nanopores in Ogston, entropic, and electrostatic sieving modes. In other approaches, DNA molecules are continuously separated by diffusion in asymmetric obstacle arrays.

Flow-based separation methods such as field-flow fractionation and hydrodynamic chromatography have been used to separate biological samples in a broad range of sizes from micrometer-sized particles to macromolecules. Both techniques use a parabolic flow profile in which analytes are distributed differentially according to their size, mass, and other physical properties. In this way, particles have different retention times along different velocity paths, which leads to limitations on sample throughput and the integration of multistep microsystems. Recently, microfabricated devices have been designed to separate biological samples continuously by asymmetric bifurcation of laminar flow and size-dependent alignment of particles toward a channel wall. In the former, particles interact with an obstacle matrix in asymmetric ways, which enables high-resolution separation of macromolecules. However, this method requires precise sheath

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control of a sample flow to prevent particles from flowing on multiple paths, which limits its application in integrated microsystems. More recently, a hydrophoretic separation system was proposed that uses slanted or anisotropic obstacles to induce hydrodynamic interaction between the obstacles and the particles subjected to rotational flows induced by the obstacles.\(^ {24,25} \)

Although this hydrophoretic sorting system has the advantage of sheathless sorting of microparticles, it is limited to the separation of microparticles such as blood cells and microbeads.

In this paper, we describe a new hydrophoretic separation method that uses rotational flows induced by regularly patterned obstacles only on the top wall. Previous hydrophoretic separation devices required the alignment and bonding of two poly(dimethylsiloxane) (PDMS) layers,\(^ {24,25} \) which made it difficult to fabricate submicrometer channels due to channel collapse and to integrate with other substrates such as glass. To overcome this limitation, we designed a microchannel with anisotropic obstacles patterned only on the top wall, as shown in Figure 1. We characterize its performance experimentally for hydrophoretic ordering using 6–15 \( \mu \)m polystyrene beads to measure the effects of the ratio of the particle size to the channel size, the number of the obstacles, the slant angle of the obstacles, and the flow speed. We also demonstrate that this separation method is effective on the submicrometer scale by using 0.5–1.1 \( \mu \)m polystyrene beads, \( \lambda \)-phage, and micrococcus DNA molecules. The hydrophoretic device requires only a single cast of PDMS, making it easy to fabricate micrometer and submicrometer channels. Our results show the potential of using hydrophoresis for biological sample preparation on micro- and nanoscales with the advantages of continuous flow and sheathless passive operation.

**EXPERIMENTAL SECTION**

**Device Design and Fabrication.** We fabricated the hydrophoretic device incorporating the anisotropic microfluidic obstacles in PDMS using soft lithographic methods. The mold for the obstacles and the microchannels was defined on a Si wafer using two-step photolithography. For microparticle applications, the channels were 50 \( \mu \)m wide \((W_{ch})\), and the obstacles had a gap height of 20 \( \mu \)m \((H_{ob})\), a thickness of 12 \( \mu \)m \((L_{ob})\), and a pitch distance of 21 \( \mu \)m \((D_{ob})\). For applications involving submicrometer particles and DNA, the channels were 50 \( \mu \)m wide and 5.1 \( \mu \)m deep, with \( L_{ob} = 21 \mu m, D_{ob} = 21 \mu m, \) and the obstacles at a fixed angle of 30°. The other dimensions such as the gap height of the obstacle ranged varied depending on the application. The deviations of all dimensions were less than 7.5%.

**Sample Preparation.** Fluorescent polystyrene beads with nominal diameters of 0.52, 0.75, 1.1, 6 (actually 6.1), and 15 (15.4) \( \mu \)m were purchased from Polysciences (Warrington, PA) and Molecular Probes (Eugene, OR). The beads were prepared in 2% pluronic F68 solution (Sigma-Aldrich, St. Louis, MO) in concentrations of approximately 7.8 \( \times \) 10\(^4\), 4.1 \( \times \) 10\(^4\), 3.0 \( \times \) 10\(^4\), 5.4 \( \times \) 10\(^2\), and 1.7 \( \times \) 10\(^2\)/\( \mu L\), respectively. Nondyed polystyrene beads with diameters of 8, 10 (10.1), 12 (11.9), and 15 (14.8) \( \mu \)m were obtained from Sigma-Aldrich. These beads were prepared in 2% pluronic solution in concentrations of approximately 3.7 \( \times \) 10\(^3\), 6.5 \( \times \) 10\(^2\), 2.8 \( \times \) 10\(^3\), and 2.5 \( \times \) 10\(^3\)/\( \mu L\), respectively, and had a coefficient of variation (CV) of less than 5% for particle size. For DNA separation, \( \lambda \)-phage (Takara Bio Inc., Tokyo, Japan) and micrococcus (Sigma-Aldrich) DNA were labeled.
with fluorescence dye (YOYO-1; Molecular Probes) in TBE 0.5× buffer. The dye to DNA base pair ratio was about 1:10 in the final concentration of approximately 20 ng/µL.

**Position Measurement.** The beads and DNA molecules were introduced into the hydrophoretic device using a syringe pump (Pump 11 Pico Plus; Harvard Apparatus, Inc., Holliston, MA). The mean flow speed at the obstacle gap was calculated from the applied volumetric flow rate. The trajectories of the fluorescence beads and DNA were imaged using fluorescence microscopy (TS100; Nikon, Tokyo, Japan) with a long exposure time of 1 s or less. For the accurate measurement of nondyed beads, their positions were recorded at the 1 mm-wide expanded outlet of the device. The measured positions were then converted to corresponding positions in the 50 µm-wide anisotropic region under the assumption of linear amplification of fluid streamlines.

**Computational Fluid Dynamics Simulation.** Simulations of the pressure fields and gradients induced by the anisotropic obstacles were performed with commercial software (CFD-ACE++; ESI, Huntsville, AL).

**Resolution Calculation.** The CV for particle position as a measure of resolution was calculated by \( CV = \sigma_x (dD/dx) \times 100\% \), where \( D \) is the particle diameter, \( x \) is the measured position, and \( \sigma_x \) is the standard deviation of \( x \). The size selectivity \( \alpha \) of the hydrophoretic device is defined as \( \alpha = CV(D/100) \). The separation resolution \( R_s \) between two DNA streams is defined as \( R_s = 0.5(\Delta x / (\sigma_1 + \sigma_2)) \), where \( \Delta x \) is the distance between the peaks of two streams and \( \sigma \) is the standard deviation of each stream width. We used a Gaussian function fit to determine the means and widths of DNA streams in their fluorescence profiles.

**RESULTS AND DISCUSSION**

**Hydrophoretic Device Principle.** The design of the hydrophoretic device consists of regularly patterned obstacles with an anisotropic resistance with respect to the fluid flow as shown in Figures 1 and 2. The hydrophoretic device allows two kinds of separation mechanism based on size and deformability to enable the separation of biological samples. Upon application of a fluid flow along the y-axis, the anisotropic fluidic resistance of the obstacles generates rotational fluid streams as shown in Figure 2.

**Hydrophoretic Ordering of Microparticles.** To demonstrate the steric hindrance mechanism of microparticles, we injected 15- and 6-µm beads into the hydrophoretic device, which was 38 µm deep (\( H_{ob} \)) with obstacles 18 µm high (\( H_{ob} \)) (see Experimental Section). Figure 4a shows the fluorescence trajectories of the beads upon application of a fluid flow along the y-axis (approximately 6.7 and 3.3 mm/s for the 15 and 6 µm beads, respectively). The corresponding volumetric flow rates were 0.4 and 0.2 µL/min, respectively. The rotational flows induced by the anisotropic obstacles deflected both types of beads along the x-axis. When the beads reached the right sidewall, two different patterns of particle flow were observed: the 15 µm bead in hydrophoretic ordering and the 6 µm bead in free flow. The 15 µm bead in hydrophoretic ordering reached and stayed at the obstacle gap serves as a molecular sieve. A large molecule with a radius of gyration that is greater than the obstacle gap does not immediately pass through the gap, but moves toward the left sidewall. The smaller molecule, however, follows the hydrophoretic ordering, separated from the large molecule.

![Figure 2](image2.png)

*Figure 2. Microfluidic device incorporating anisotropic microfluidic obstacles. The device consists of a prefilter to remove particles with a diameter greater than 20 µm, the obstacles, and an expanded outlet region. The width \( W_{ob} \) of the anisotropic region is 50 µm; the other dimensions varied according to the application. See the Experimental Section for the exact dimensions (scale bar = 100 µm).*

![Figure 3](image3.png)

*Figure 3. Hydrophoretic separation of macromolecules. The obstacle gap serves as a molecular sieve. A large molecule with a radius of gyration that is greater than the obstacle gap does not immediately pass through the gap, but moves toward the left sidewall. The smaller molecule, however, follows the hydrophoretic ordering, separated from the large molecule. 1b,c,24-26 Particles suspended in the fluid flow migrate toward the right sidewall following the rotational streams. At that time, the particle motion is determined by a kind of steric hindrance mechanism.27 Steric hindrance occurs when the obstacles prevent rotational flows of large particles that are observed in relatively smaller particles as illustrated in Figure 1d. In short, the particle–obstacle interaction deflects the large particles from their streamline and leads to equivalent flow paths for different size particles; this is called hydrophoretic ordering. A particle with a diameter that is similar to the obstacle gap (\( H_g = H_{ob} - H_{ob} \)) will steer its position toward the center of the z-axis due to the particle–wall interaction. The particle thus follows stream 1 and stays near the right sidewall without deviation. In contrast, small particles following streamline 2, separate from the large particles as shown in Figure 1d.*
right sidewall without deviation. The 6 \( \mu \text{m} \) bead moved across the channel from the right to left sidewall, following the rotational flows. Figure 4a shows a small part of the separation channel, thereby showing only one transition of the 6 \( \mu \text{m} \) bead. However, as flowing through the whole channel, 6 \( \mu \text{m} \) beads followed the streamlines traveled back and forth between one sidewall and the other, following the rotational flows.

The equivalent position of 15 \( \mu \text{m} \) beads is about 9 \( \mu \text{m} \) from the right sidewall after passing through more than 40 obstacles (Figure 1 in the Supporting Information). To ensure the complete ordering of microparticles, we used the hydrophoretic device with 80 anisotropic obstacles at a flow speed of 33.3 mm/s (2 \( \mu \text{L/min} \)). On a microscale level, the diameter of particles assuming hydrophoretic ordering affects their equivalent flow paths. Particles in the diameter range 10–15 \( \mu \text{m} \) were clearly resolved into their own equivalent positions as shown in Figure 4b. The distribution of particle streams shifted upward as the obstacle angle increased from 10° to 50°. The maximum transverse flow in the staggered herringbone mixer was achieved for \( \theta = 45° \). In a similar manner, the transverse flow in a hydrophoretic device with \( \theta = 50° \) can be greater than in a device with \( \theta = 10° \), which results in the upward shift of the position distribution of differently sized particles as the obstacle angle increases. The respective CVs for 10–15 \( \mu \text{m} \) beads at 10° are 5.6%, 2.6%, and 0.9% (see Experimental Section). The respective CVs for 10–15 \( \mu \text{m} \) beads at 50° are 3.6%, 2.0%, and 2.2%. The size selectivity values for 10–15 \( \mu \text{m} \) at 10° are 0.6, 0.3, and 0.1 \( \mu \text{m} \), respectively. The size selectivity values for 10–15 \( \mu \text{m} \) at 50° are 0.4, 0.2, and 0.3 \( \mu \text{m} \), respectively.

The flow speed also affects the equivalent flow path of each particle in hydrophoretic ordering. The particle Reynolds number \( Re_p \) is defined as the ratio of the particle inertia to the viscous force; \( Re_p = \frac{D^2U}{\mu D_b} \), where \( \rho \) is the fluid density, \( D \) is the particle diameter, \( U \) is the maximum fluid velocity, and \( \mu \) is the dynamic fluid viscosity. \( D_b \) is the hydraulic diameter, defined as \( D_b = \frac{2wh}{w + h} \), where \( w \) and \( h \) are the width and height of the channel, respectively.28,29 For \( Re_p \gg 1 \), the particle inertia is dominant. At such a high value of \( Re_p \), inertial lift forces can affect the equilibrium position of particles under hydrophoretic ordering. As \( Re_p \) increased from 0.1 to more than 0.7, the inertial lift away from the anisotropic obstacle resulted in the shift of the equivalent position of the 15 \( \mu \text{m} \) beads toward the left sidewall, i.e., inertial shifting as shown in Figure 5. With the use of the repeated symmetric patterns of the anisotropic obstacles, this hydrophoretic device can easily augment the focusing throughput for high-throughput analysis without inertial shifting (Figure 2 in the Supporting Information). In the parallel-ized channel, the focusing throughput of 15 \( \mu \text{m} \) beads (\( Re_p = 0.07 \)) was 57 particles/s (20 \( \mu \text{L/min} \)).

The degree of the particle deflection along the x-axis is determined by \( H_{ob} \) and thereby by the induced pressure gradients. As \( H_{ob} \) was changed from 18 to 2 \( \mu \text{m} \) for a fixed obstacle gap (\( H_g = 20 \mu \text{m} \)) and at a fixed obstacle angle (\( \theta = 10° \)), the 15 \( \mu \text{m} \) beads followed the applied flow along the y-axis without crossing as shown in Figure 6a. In the simulation, the magnitude of the pressure gradient at \( H_{ob} = 18 \mu \text{m} \) was 3 times higher than at \( H_{ob} = 2 \mu \text{m} \) (Figure 3 in the Supporting Information). The rotation of the fluid at \( H_{ob} = 2 \mu \text{m} \) was not sufficient to drive particle crossing or hydrophoretic ordering. At \( H_{ob} \geq H_g \), the magnitude of the pressure gradient is saturated and assures hydrophoretic ordering. The equivalent positions of particles at \( H_{ob} = 43 \mu \text{m} \) were similar to ones at \( H_{ob} = 18 \mu \text{m} \) (Figure 6b). The respective CVs for 10–15 \( \mu \text{m} \) at \( H_{ob} = 43 \mu \text{m} \) are 1.0%, 4.3%, and 1.3%. The size

References:

Figure 4. Size-dependent ordering of microparticles. (a) Trajectories of microbeads passing through the slanted obstacles. The channels are 50 \( \mu \text{m} \) wide and 38 \( \mu \text{m} \) deep with obstacles 18 \( \mu \text{m} \) high at an angle of 10°. A fluid flow was applied along the y-axis at flow speeds of 6.7 and 3.3 mm/s for the 15 and 6 \( \mu \text{m} \) beads, respectively. The corresponding volumetric flow rates were 0.4 and 0.2 \( \mu \text{L/min} \), respectively. The 15 \( \mu \text{m} \) bead entering from the left sidewall traverses the channel (top). The 15 \( \mu \text{m} \) bead entering from the right sidewall flows along the y-axis without deviation. The 15 \( \mu \text{m} \) beads in hydrophoretic ordering reach and stay at the right sidewall without deviation. In contrast, the 6 \( \mu \text{m} \) bead travels back and forth between one sidewall and the other, following the rotational flows without hydrophoretic ordering (bottom). Colored fluorescent trajectories were superimposed on the bright field images (scale bar = 100 \( \mu \text{m} \)). (b) Measured position distributions for bead diameters ranging from 8–15 \( \mu \text{m} \) at two different obstacle angles for a flow speed of 33.3 mm/s (2 \( \mu \text{L/min} \)).

Figure 5. Measured position distributions of 15 \( \mu \text{m} \) beads as a function of flow speed. The channel was 50 \( \mu \text{m} \) wide (\( W_{ob} \)) and 38 \( \mu \text{m} \) deep (\( H_{ob} \)), with obstacles 18 \( \mu \text{m} \) high (\( H_g \)) and an angle of 10° (\( \theta \)). For \( Re_p \gg 1 \), the particle inertia is dominant. At such high particle Reynolds numbers, inertial lift forces can affect the equilibrium position of particles under hydrophoretic ordering. Because of the effect of inertia, the ordering position shifts toward the left sidewall along the anisotropic obstacle. The width of the fluorescent peaks corresponds closely to the diameter of the bead.
The ratio of the volume efficiency by collecting the separated beads into two reservoirs with 120 anisotropic obstacles. The beads were injected into the channel with \( H \) and 0.2 \( \mu m \) of 1.1 and 0.52 \( \mu m \) can separate large particles in hydrophoretic ordering from low size selectivity that is insufficient to resolve the 5.1 \( \mu m \) beads. The streams of these beads were not resolved, and the right sidewall due to the steric hindrance mechanism as shown in Figure 7d, where the flow speed was 5.1 \( \mu m / s \). For \( D/H_g \geq 0.5 \), the obstacle gap \( H_g \) begins to hinder the rotational flow of particles induced by the anisotropic obstacles and leads to hydrophoretic ordering. As the obstacle gap is reduced in nanoscale, this device could perhaps be used even to separate biomolecules such as protein complexes.

**Figure 6.** Effect of the height of the anisotropic obstacle on hydrophoretic ordering. (a) Trajectories of 15 \( \mu m \) beads for two different obstacle heights (\( H_{ob} \)): 43 \( \mu m \) (top) and 2 \( \mu m \) (bottom). The height of the obstacle gap (\( H_g \)) is 20 \( \mu m \) for both channels (scale bar = 100 \( \mu m \)). The 15 \( \mu m \) bead entering from the left sidewall traverses the channel with \( H_{ob} = 43 \mu m \) (top). The 15 \( \mu m \) bead entering from the right sidewall flows along the \( y \)-axis without deviation. The 15 \( \mu m \) beads in hydrophoretic ordering reach and stay at the right sidewall without deviation. In contrast, the 15 \( \mu m \) bead injected into the channel with \( H_{ob} = 2 \mu m \) flows along the \( y \)-axis without deflection since transverse flow is insufficient to force particles to cross (bottom). (b) Measured position distributions for bead diameters ranging from 8 to 15 \( \mu m \) in the device with \( H_{ob} = 43 \mu m \). The flow speed was 33.3 \( mm / s \) (2 \( \mu L / min \)). The equivalent positions of particles at \( H_{ob} = 43 \mu m \) were similar to ones at \( H_{ob} = 18 \mu m \) in Figure 4b.

selectivity values for 10–15 \( \mu m \) at \( H_{ob} = 43 \mu m \) are 0.1, 0.5, and 0.2 \( \mu m \), respectively.

**Differential Ordering and Sorting of Submicrometer Particles.** Hydrophoretic ordering of submicrometer particles was demonstrated with micrometer and submicrometer beads with diameters of 1.1, 0.75, and 0.52 \( \mu m \). To ensure complete ordering of the submicrometer particles, we used a hydrophoretic device with 120 anisotropic obstacles. The beads were injected into the hydrophoretic device with a flow speed of 2.4 \( mm / s \) (0.01 \( \mu L / min \)); the device was 5.1 \( \mu m \) deep with \( H_g = 1.4 \mu m \) (see Experimental Section). The 1.1 and 0.75 \( \mu m \) beads remained near the right sidewall due to the steric hindrance mechanism as shown in Figure 7a. Their equivalent positions were 8.2 ± 2.0 and 12.2 ± 5.1 \( \mu m \) from the right sidewall for the 1.1 and 0.75 \( \mu m \) beads, respectively. The streams of these beads were not resolved, and they overlapped. We can presume that this phenomenon results from low size selectivity that is insufficient to resolve the micrometer and submicrometer beads. The hydrophoretic device can separate large particles in hydrophoretic ordering from relatively smaller particles in free flow. We introduced a mixture of 1.1 and 0.52 \( \mu m \) beads into the hydrophoretic device at a flow speed of 2.4 \( mm / s \) (0.01 \( \mu L / min \)). Figure 7b shows the separation of the 1.1 \( \mu m \) beads in hydrophoretic ordering and 0.52 \( \mu m \) beads in free flow. The equivalent position of the 1.1 \( \mu m \) beads was 10.1 ± 2.3 \( \mu m \) from the right sidewall. We quantified the sorting efficiency by collecting the separated beads into two reservoirs (Figure 4 in the Supporting Information). The ratio of the volume collected was 2:1 for the outlets of the 1.1 and 0.52 \( \mu m \) beads, respectively. We used the hydrophoretic device with 300 anisotropic obstacles at a flow speed of 23.8 \( mm / s \) (0.1 \( \mu L / min \)) for sorting submicrometer particles. The concentration ratio of the beads at the inlet was 2.6:1 for 0.52 to 1.1 \( \mu m \) beads. After separation, the concentration ratio collected at the outlet for the 0.52 \( \mu m \) beads was 261:1 for 0.52 to 1.1 \( \mu m \) beads. The purity of the 0.52 \( \mu m \) beads after separation was increased to 99.6% from the initial value of 72.2%. After hydrophoretic separation, the concentration ratio collected at the outlet for the 1.1 \( \mu m \) beads was 1.4:1 for 0.52 to 1.1 \( \mu m \) beads. The purity of the 1.1 \( \mu m \) beads was enhanced from 27.8 to 41.7%. The sorting throughput was approximately 90 particles/s.

The height of the obstacle gap (\( H_g \)) determines whether particles assume hydrophoretic ordering as shown in Figure 7c. As \( H_g \) was reduced from 1.4 to 0.65 \( \mu m \) keeping \( H_{ob} \) constant at 5.1 \( \mu m \), the beads with a diameter (\( D \)) of 0.52 \( \mu m \) became focused in their equivalent position of 12.9 ± 6.9 \( \mu m \) from the right sidewall as shown in Figure 7d, where the flow speed was 5.1 \( \mu m / s \). For \( D/H_g \geq 0.5 \), the obstacle gap \( H_g \) begins to hinder the rotational flow of particles induced by the anisotropic obstacles and leads to hydrophoretic ordering. As the obstacle gap is reduced in nanoscale, this device could perhaps be used even to separate biomolecules such as protein complexes.

**Size Separation of DNA Molecules.** The hydrophoretic device can separate DNA molecules based on another hindrance mechanism (Figure 3). We prepared long DNA molecules of 49 and 115 kb (\( \lambda \)-phage and micrococcus DNA, respectively) in TBE 0.5× buffer. These supercoiled molecules were condensed in the presence of YOYO-1 dye and can be considered to be spherical particles. The corresponding radii of gyration \( R_g \) are 0.863 and 1.45 \( \mu m \) for the \( \lambda \)-phage and micrococcus DNA molecules, respectively.30 These \( R_g \) values are useful measures for defining the critical separation diameter or the gap height (\( H_g \)) of the anisotropic obstacle. Therefore, we fabricated a microchannel with \( H_g = 1.2 \mu m \) between the \( R_g \) values of \( \lambda \)-phage and micrococcus DNA molecules.

Figure 8a shows differential molecular ordering of the \( \lambda \)-phage and the micrococcus DNA for a fluid flow of 111 \( mm / s \) (0.4 \( \mu L / min \)). When driven into the microchannel with 120 anisotropic obstacles, the \( \lambda \)-phage DNA molecules passed freely through the obstacles and followed the hydrophoretic ordering. In contrast, the micrococcus DNA molecules were blocked since their \( R_g \) was greater than \( H_g \) and they moved along the anisotropic obstacle. When the micrococcus DNA molecules enter the obstacle gap that is smaller than their radii of gyration (\( R_g \)), the obstacles presumably work as an energy barrier. The DNA molecules should pay some energy cost for their conformation change to overcome the energy barrier. In the context of this energy cost, the micrococcus DNA molecules can move toward the left sidewall rather than immediately passing through the gap. Therefore, the DNA molecules can travel through alternating thick and thin regions near the left sidewall, repeatedly changing their conformation. Figure 8b shows a separation experiment for the mixture of the two DNA molecules in which the concentration of each molecule was 10 ng/\( \mu L \). The DNA mixture was separated into two streams for the separation time of 0.12 s over the channel length of 5 mm. The separation

resolution for the two DNA streams was 0.44 (see Experimental Section). The separation throughput was $1.7 \times 10^6$ molecules/s (0.4 $\mu$L/min). This rate is over 100 times faster than that of the DNA prism, one of the high-throughput DNA separators. The sample throughput of the hydrophoretic device can be further enhanced by using parallel channels with repeated patterns of anisotropic obstacles.

CONCLUSION

We have proposed a new hydrophoretic device for the separation of micrometer and submicrometer particles based entirely on hydrodynamics. The use of rotational flows induced by the anisotropic obstacles eliminates the precise sheath-control of sample flow, allowing the self-sorting of micrometer and submicrometer particles. Through two different hindrance mechanisms, different sizes of micrometer and submicrometer beads as well as DNA molecules were tracked into their distinct trajectories. We successfully demonstrated differential ordering and sorting of micrometer and submicrometer beads of 0.5–15 $\mu$m in diameter and DNA molecules of 49 and 115 kb. The current implementation may prove to be useful for the separation of binary samples under hydrophoretic ordering and free flow. However, sequential separation in a hydrophoretic device with multiple obstacle–gap heights will enable the extension to multiple-size fractionation. Currently, the sorting throughput of the hydrophoretic device is limited to approximately 90 particles/s and $1.7 \times 10^6$ molecules/s per microchannel. The sorting capacity can be increased by integrating multiple channels. The patterns of the anisotropic obstacles were formed only on the top wall, which enables easy fabrication of submicro- and nanochannels. We believe that this device can be extended to highly integrated microchips performing the separation of biological particles such as cells and bacteria and the sample preparation of biomolecules including DNA and protein complexes.

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