Size-Based Separation of Microparticles in a Multilayered Microfluidic Device

Ki-Hwan Nam and David T. Eddington

Abstract—The current analysis of microbial populations in diverse environments is stymied by the inability to culture the vast majority of microbes in a laboratory setting. Consequently, considerable work is currently underway to identify new microbes based on genetic characterizations, and one limitation is that the majority of sequencing schemes will miss rare populations of microbes as large numbers are pooled for analysis. One approach to overcome this obstacle is to presort the microbes from a heterogeneous field sample based on size, as many microbes live in small clusters, and then analyze the various strains using in situ hybridization. In this paper, a multilayered microfluidic device has been developed for the size-based separation of heterogeneous microbial samples as a first step toward this goal. This device is fabricated using polydimethylsiloxane and soft lithographic techniques. The device includes three successive constrictions with reducing heights and was employed to successfully separate a heterogeneous population of microparticles and field samples obtained from Lake Michigan. The device was found to trap the microparticles and environmental samples with efficiency between 89% and 98% when the particles were larger than the constrictions and 97% for the unknown environmental sample.

Index Terms—Microfluidic, microparticles, multilayered, separation.

I. INTRODUCTION

THE EARTH contains an enormous amount of microbial diversity with microbes inhabiting a wide variety of environments leading to complex ecosystems and communities. Less than 1% of all microbes have been successfully cultured in the laboratory, and fewer than half of the recognized bacterial types contain their cultured representatives [1], [2]. Consequently, the current analysis of microbial populations in diverse environments is based on genetic characterizations such as comparative ribosomal-ribonucleic-acid (rRNA) gene analysis [3]–[9], community deoxyribonucleic-acid (DNA) hybridization efficiency [10], and metagenomic gene inventories [11], [12].

A limitation to these techniques is that most sequencing schemes will miss rare populations of microbes as large numbers of microbes are pooled for analysis. One approach to overcome these obstacles is to presort the microbes from a heterogeneous field sample based on size as microbes cluster...
the second constriction channel are trapped and accumulated, and smaller particles pass through the second constriction channel. The third constriction then traps the majority of the remaining particles. The height, width, and length of each constriction channel can be varied according to the size of particles required to be separated. In this paper, a multilayered microfluidic device containing one, two, or three constriction channels, respectively, was employed according to the sample type.

II. MATERIALS AND METHODS

A. Device Fabrication

Polydimethylsiloxane (PDMS) microfluidic devices with multiple heights were fabricated with one molding step as described previously [27]–[30]. For more details, the schematic illustration to describe the process step by step is shown in Fig. 3. Briefly, the process consists of fabricating an SU-8 master and molding micropatterned PDMS plates. To construct a basic one-layer device using the methods of soft lithography [31], SU-8 negative photoresist (MicroChem Corporation, MA) was spun on a clean silicon wafer (Laurell Technologies Corporation, Model WS-400B-6NPP/NITE, North Wales, PA) with defined speed for specific thickness and was exposed to UV light (OmniCure, S1000, Mississauga, ON, Canada) through a photomask (Fineline Imaging, Colorado Springs, CO) as described in the manufacturer’s guidelines. The unpolymerized resist was washed away, resulting in micropatterned channel networks. The process to make the monolayer structure was repeated four times with four different photomasks representing each layer to achieve a multilayered microfluidic network. PDMS prepolymer was cast on the micropatterned SU-8 master, and a PDMS microfluidic device was released. Next, the microfluidic device was bored to make inlet and outlet ports. The PDMS mold was bonded using O₂ plasma treatment (Plasmatic Systems, Inc. Plasma-Preen II-862, North Brunswick, NJ) with a flat glass slide (Fisher Scientific, 35 × 50-2, Pittsburgh, PA) to form the microchannel structure. Silicone tubes (Cole Parmer, IL) with inner and outer diameters of 1/8 and 1/8 in., respectively, were inserted into the ports to interface the device with syringes. The height of each constriction channel was controlled by the type of SU-8 (2000.5, 2005, 2010, and 2015) and spin speed following standard processing procedures.

B. Height Variation of Constriction Channels

The variation of the heights of the constriction channels by applied flow pressures was measured with fluorescein isothiocyanate (Sigma Aldrich, St. Louis, MO) at 50 μM. The fluorescent solution was injected into the device via standard tubing and pumping (Harvard apparatus compact infusion pump, Model 975, Holliston, MA). Each image acquired was taken every 5 min after the flow rate was changed to allow equilibration. The intensity of fluorescence and height variation were analyzed with Image J and shown in Fig. 4.

C. Separation of Fluorescent Microspheres

The trapping efficiencies for various sized particles were measured in two-layered microfluidic devices composed of a single constriction channel with a specified height. Three types of devices were prepared to measure trapping efficiencies according to a variation of the size of particles (Dₚ) for the height of constriction channels. The heights of constriction channels (h) constructed in each device were 7 μm (7.0 ± 0.6 μm), 3 μm (3.2 ± 0.6 μm), or 0.8 μm (0.8 ± 0.4 μm), respectively. Moreover, six types of particles were tested within each device with concentrations of 6.05 × 10⁴ particles/mL for 10-μm particles (D = 9.922 ± 0.330 μm), 2.30 × 10⁵ particles/mL for 6-μm particles (D = 6.058 ± 0.146 μm), 3.00 × 10⁵ particles/mL for 4.5-μm particles (D = 4.869 ± 0.246 μm), 4.20 × 10⁹ particles/mL for 3-μm particles (D = 2.986 ± 0.083 μm), 8.50 × 10⁵ particles/mL for 2-μm particles (D = 2.156 ± 0.114 μm), and 2.27 × 10⁶ particles/mL for 1-μm particles (D = 1.022 ± 0.010 μm) (Fluoresbrite Plain YG, Polysciences, Inc., Warrington, PA). Different concentrations of each particle were used to ensure that the volume of each particle injected was similar with each other for these early studies. Moreover, before the injection of a single
were driven into the device at 0.2 μm/min for 40 min. After the removal of unloaded particles from the inlet, successively, water was injected into the device for 1 h to distribute the particles to the constriction channels.

In the second set of experiments, a four-layered microfluidic device composed of three successive constriction channels of differing heights was used to measure trapping efficiencies according to the concentration of a heterogeneous mixture of fluorescent microspheres. The heights of each constriction channel were 7, 3, and 0.8 μm, respectively. For a heterogeneous mixture of fluorescent microspheres, 10-, 4.5-, and 2-μm-diameter particles of the same concentration were mixed, and three dilutions of the mixed particles were prepared to quantify the concentration-of-particles effect on separation. The three concentrations of each particle were 5.0 × 10^6 particles/mL (C_1), 1.5 × 10^5 particles/mL (C_2), and 4.5 × 10^4 particles/mL (C_3). The particle injection time and method were the same with the previous experimentation.

D. Separation of Environmental Samples

Environmental samples collected from Lake Michigan were injected into the separation device. Prior to separation, the sample was filtered using a 10-μm isopore membrane filter (Millipore, Billerica, MA) to remove large particles and aggregates and then injected into a water-filled multilayered microfluidic device composed of three successive constriction channels of differing heights for 3 h at room temperature. Next, continuous diamidino-2-phenylindole (DAPI) staining of the DNA of the microbes was accomplished in the device to facilitate visualization of the microbes and distinguish from non-living debris. The original concentration obtained from Lake Michigan was 1.37 × 10^6 cells/mL (±5.75 × 10^4 cells/mL). For the separation, the environmental sample was injected into the device at 0.2 μL/min for 30 min, and successively, water was injected into the device for 1 h to distribute the particles to the constriction channels.

E. DAPI Staining of Environmental Samples

Two percent of paraformaldehyde (Sigma Aldrich, St. Louis, MO) stock solution adjusted at pH 7.2–7.4 to fix the microbes was injected into the device where the microbes were already trapped at 4 °C for 6 h. The microbes required fixation to ensure that their morphology is preserved during the experiment. After fixation of the microbes, 1× phosphate buffered saline (PBS) solution (Sigma Aldrich, St. Louis, MO) was injected into the device at room temperature for 1 h. Then, a 1-μg/mL DAPI (Dilactate, Sigma, St. Louis, MO) suspended in 1× PBS solution was injected to stain the DNA of the microbes. DAPI facilitates the identification of the microbes and distinguishes microbes from debris in the sample. Finally, 1× PBS solution was injected into the device for 1 h to remove excess DAPI solution.

F. Microscopy and Particle Counting

Once all particles were trapped at constriction channels, each constriction channel was imaged on a fluorescent microscope (Olympus IX71). Images were acquired at 10×, 20×, and 32× magnification using a digital camera (QImaging, RETIGA-SRV, Surrey, BC, Canada), and the number of trapped particles of differing sizes at each constriction channel was counted and analyzed with ImageJ to measure trapping efficiencies. For each data point, averages of three images were compiled to ensure adequate sample size.

III. RESULTS

A. Multilayered Microfluidic Devices

The ability of the device to separate fluorescent microparticles and environmental samples was analyzed. The dimensions of this multilayered microfluidic device for size-based bacterial separation, composed of a distribution channel that is 15 × 18.8 μm with 18-μm height including three successive constriction channels of differing sizes, are shown in Fig. 2. The dimensions of each constriction channel are defined as follows: w is the smallest distance between the posts inside the constriction channels, h is the height of constriction channels, and l is the length of constriction channels. The dimensions of w, h, and l of each constriction channel starting from the inlet were 36 × 32 × 7 μm, 22 × 26 × 3 μm, and 15 × 18 × 0.8 μm, respectively, and the total volume of a microfluidic
channel was 4.5 μL. The size and shape of the distribution channel was chosen to ensure that the flow was well distributed and that entrapped air completely purged from the channel.

To determine the best ratio \( R_1 \) of \( h \) to \( w \), while preventing constriction channels from collapsing onto the glass substrate, several dimensions were explored: 0.8, 2, 3, 4.5, and 8 μm tall and 15, 24, and 34 μm wide. The range of \( R_1 \) used earlier was between 0.50 and 0.02. When the \( R_1 \) was larger than 0.1, the constriction channel was open, and solutions freely passed through the channels, but when it was lower than 0.05, the constriction channel was closed because the ceiling collapsed onto the substrate. The range between 0.1 and 0.05 had some open channels and some collapsed channels. The smallest dimension that was able to be generated with transparent photomasks was 15 μm. Chrome photomasks could be used to generate features smaller than 15 μm; however, the 15-μm dimension was chosen as a cutoff point to reduce fabrication costs. The \( R_1 \) of each 7-, 3-, and 0.8-μm-tall constriction channel included in the multilayered microfluidic devices used for bacterial separation was 0.19, 0.14, and 0.05, respectively. The 0.8-μm channels were partially collapsed; however, the device was still able to separate the samples.

The multilayered microfluidic device was made of PDMS which is an elastomeric material and thus can deform under the pressure used to drive the flow [35]. Consequently, the relationship between heights of constriction channels and applied flow rates was examined, as shown in Fig. 4. As the flow rates were increased, the heights of constriction channels were increased in proportion to the applied flow rates, and the heights of constriction channels were extended up to 1.7 times of the original height by the applied flow pressure. Faster flow rates were not applied due to water leakage at these rates at tubing connectors. In this paper, flow rates for the injection of particles into the device is one of the functions of trapping efficiency, and the height variation by applied flow rates gives rise to a variation of the trapping efficiency of the device. After due consideration of experimental time and trapping efficiency, a constant flow rate of 0.2 μL/min was determined for trapping particles and microbes to minimize this phenomena.

### B. Single-Constriction Devices With Microparticles

For the quantitative comparison of the trapping efficiency of the device composed of individual constriction channels for several different-sized fluorescent microspheres, trapping efficiency is defined as follows:

\[
E_{\text{Particle, Single}}[\%] = \frac{N_{\text{Trapped}}}{N_{\text{Trapped}} + N_{\text{Passed}}} \times 100. \tag{1}
\]

The trapping efficiency \( E_{\text{Particle, Single}} \) of the microfluidic device composed of a single-height constriction channel was determined by the number of trapped particles at the constriction channel \( (N_{\text{Trapped}}) \) and the number of particles that passed through the constriction channel and located at the outlet port \((N_{\text{Passed}})\). After the injection of particles into the device, there were few particles in the distribution channel, sticking to the PDMS or glass surfaces, but the number was negligible, compared with the number of trapped particles; therefore, it was not included in these calculations. Single-constriction devices were fabricated to first quantify the trapping efficiency of individual constriction heights without the compounding variable of trapped particles blocking smaller particles at the constrictions. The trapping efficiencies for the heights of each constriction channel with respect to particle size were first calculated in three types of multilayered microfluidic devices. Each device composed of a single type of constriction channel with 7-, 3-, and 0.8-μm height, respectively, was assessed with six types of particles of differing sizes, as shown in Fig. 5. The separation efficiency is governed by the ratio \( R_2 \) of the particle’s diameter \( (D_p) \) to the height of the constriction channel \( (h) \). When \( R_2 > 1 \), approximately 98% of these particles were trapped at the constriction channel; when \( R_2 = 1 \), approximately 80% of the particles passed through the channel; and when \( R_2 < 1 \), approximately 94% passed through the constriction channel. In conclusion, the constriction channels should be designed to trap microparticles that are slightly larger than the height of the constriction to compensate for the compliant nature of the microchannel walls and the deformation of the microfluidic channels by an applied flow pressure.

### C. Triple-Constriction Devices With Microparticles

In addition to the trapping efficiencies of three individual constrictions, particle distribution in a multilayered microfluidic device composed of three successive 7-, 3-, and 0.8-μm-tall constriction channels was calculated with a heterogeneous mixture of particles to simulate an environmental sample with a distribution of particles. Moreover, 10-, 4.5-, and 2-μm-diameter microspheres were chosen for a heterogeneous mixture of particles at three concentrations as described previously. Successively trapped particles based on size at each constriction channel were shown in Fig. 6. The resulting particle distribution \( (D_{\text{Constriction}}) \) for each concentration of heterogeneous population of particles at each constriction channel is shown in Fig. 7(a). It indicates a relative number of each type of trapped particles at each constriction channel. The number was directly counted from images taken by a fluorescent microscope. A heterogeneous population of particles of varying sizes was separated at each desired constriction channel by size in serial order. At concentration \( C_3 \), 89% of particles trapped at the 7-μm-tall constriction channel were 10-μm-diameter particles and 94% of particles trapped at the 3-μm-tall constriction channel was 4.5 μL.
The particle distribution \( D_{\text{Particle}} \) of the triple-constriction microfluidic device is determined by the number of each type of particles trapped at each constriction channel \( N_{\text{Trapped, Each}} \) divided by the total number of each type of particles trapped at all constriction channels \( N_{\text{Trapped, Total}} \). Even though there were few particles in the distribution channel sticking to the PDMS or glass surfaces and few 2.0-\( \mu \)m particles were in an outlet, the number was negligible, compared with the number of trapped particles; hence, they were not reflected in the trapping efficiencies. As shown in Fig. 7(b), at a concentration of \( 1.35 \times 10^5 \) particles/mL of a heterogeneous population of particles composed of the same amount of three types of particles, 96% of 10-\( \mu \)m-diameter particles injected into the device were trapped at the 7-\( \mu \)m-tall constriction channel, 89% of 4.5-\( \mu \)m-diameter particles were trapped at the 3-\( \mu \)m-tall constriction channel, and 93% of 2.0-\( \mu \)m-diameter particles were trapped at the 0.8-\( \mu \)m-tall constriction channel. In this case, unexpected small particles trapped at larger constriction channels were not mostly blocked by previously trapped particles, but stuck to larger particles.

In addition to trapping microparticles in the microfluidic device, for the collection of trapped microparticles, an extraction channel was directly linked to the microfluidic devices composed of a single constriction channel with defined heights, as shown in Fig. 8. The inlet channel of the extraction channel was linked at the downside of one end of the constriction channel, and an outlet was linked at the topside of another end of the constriction channel. This device was designed to simultaneously make a parallel stream with the constriction channel and a backward stream from the outlet to the inlet of a distribution channel, which is opposite to the direction of particle injection, to collect the trapped microparticles effectively. The flow was analyzed through tracking fluorescent particles. Consequently, at least approximately 94% of trapped particles were successfully extracted from the device.

### D. Triple-Constriction Devices With Environmental Samples

A field sample of water was collected from the shoreline of Lake Michigan and stored at 4 °C until processed by the device. Lake Michigan has extremely diverse kinds of microbes composed of various bacteria including not only *Escherichia coli*, *Bacillus*, *Bacteroides*, *Enterococci*, *Psychrotrophs*, and *Mesopeltes* (cold- and warm-loving bacteria, respectively) but also many other small unknown bacteria and microbes such as phytoplankton, zooplankton, and small algae [32]–[34]. Most bacteria living at Lake Michigan are typically 0.2–5.0 \( \mu \)m in length even though the size is dependent on the shape such as cocci, rods, or threadlike even in the same species. *E. coli*, is typically rod-shaped and about 2 \( \mu \)m long. The device used with the environmental sample was the same triple constriction as described previously. Images of environmental microbes trapped at each constriction channel are shown in Fig. 9. For the quantitative comparison of the trapping efficiency of the device with a heterogeneous population of bacteria obtained from Lake Michigan, it is defined as follows:

\[
E_{\text{Bacteria}}[\%] = \frac{C_{\text{Inlet}} - C_{\text{Outlet}}}{C_{\text{Inlet}}} \times 100.
\]
The trapping efficiency ($E_{\text{bacteria}}$) of the multilayered microfluidic devices for bacterial separation is the concentration of bacteria at the outlet ($C_{\text{Outlet}}$) subtracted from the concentration of injected bacteria ($C_{\text{Inlet}}$) divided by the initial concentration of bacteria. The initial concentration of the environmental sample obtained from Lake Michigan was $1.37 \times 10^6$ cells/mL ($\pm 5.75 \times 10^4$ cells/mL), and the concentration at the output was $3.90 \times 10^4$ cells/mL ($\pm 3.30 \times 10^3$ cells/mL).

The majority of microbes injected into the device were trapped at the constriction channels with a trapping efficiency of 97%.

IV. DISCUSSION

The multilayered microfluidic device described in this paper provides a simple, economical, and effective procedure for simultaneous separation and concentration of a heterogeneous population of particles based on size. The use of constrictions of differing heights in the device simplifies this approach. The distribution channel had a height of 18 $\mu$m, and the $l$ and $w$ of each constriction channel were minimized to minimize the fluidic resistance and pressure required to drive the fluid through the channel. Most particles were trapped at the entrance of each constriction channel, but few particles were trapped under the constriction channel when the size of the particles matched with the size of the constriction channel because of the variation of the heights of constriction channels, as shown in Fig. 4. In addition, the volumes of fractionated material at each constriction channel could be quantified through image analysis. Circular microposts of 400 $\mu$m were created in the distribution channel, and rectangular microposts were also constructed in each constriction channel to support the thin and wide microchannels from collapsing. The distance between two rectangular posts ($w$) was determined to be higher than 0.05 of $R_1$, the ratio of $h$ to $w$, and the minimum dimension of $R_1$ at each constriction channel was used to ensure that the microchannel ceiling did not collapse.

One of the major differences of this multilayered microfluidic device compared with other devices designed for particle separation [13]–[26] is that the device is able to passively trap the particles without external controls. Once the height of constriction channels is defined, particles introduced into the device will separate based on size. Moreover, this device is specified for the separation of a heterogeneous population of particles where many different diameters or groupings are expected to be encountered. A heterogeneous population of particles is separated into several subpopulations, and the number of subpopulations can be controlled by the number of constriction channels. In addition, the size of particles to be separated is also easily determined by the height of the constriction channel and can be a first step toward identifying an unknown microbial sample.

The separation efficiency of the device with various sized particles was assessed based on the heights of individual constriction channels. When the diameter of the particles was
larger than the height of constriction channels, more than 98% of particles were trapped at the constriction channel. However, when the height of the constriction channel was lower than 1.0 \( \mu \text{m} \), as shown in Fig. 5(c), approximately 60% of 1-\( \mu \text{m} \) particles passed through the constriction channel even though these particles were 20% larger than the height of the constriction. This is most likely a result of channel deformation under the pressure-driven flow, as shown in Fig. 4. When a flow rate of 0.2 \( \mu \text{L/min} \) was used for particle injection, the 0.8-\( \mu \text{m} \)-tall constriction channel was extended to 0.9 \( \mu \text{m} \) (±0.2 \( \mu \text{m} \)). Even though the height of the constriction channel was higher than the particle diameter, when 1-\( \mu \text{m} \)-diameter particles were injected into the device with this flow rate, the reason 60% of the particles passed the constriction channel was this deformation of the 0.8-\( \mu \text{m} \)-tall constriction channel by an applied flow pressure. Consequently, controlling the flow rate is also important in the operation of the device. As the flow rates were increased, some of the trapped particles at constriction channels passed through the constriction channels as a result of the higher pressure associated with the higher flow rates leading to decreased trapping efficiency. Therefore, flow rates lower than 0.20 \( \mu \text{L/min} \) were used to drive particle flow in the microfluidic channel to minimize particles being forced through the constrictions.

To validate the separation functionality of the multilayered microfluidic device composed of three successive constriction channels of differing heights for size-based separation, a heterogeneous mixture of fluorescent microspheres composed of 10-, 4.5-, and 2-\( \mu \text{m} \)-diameter particles was introduced into the devices with constrictions of 7-, 3-, and 0.8-\( \mu \text{m} \)-tall constrictions. More than 90% of the particles were trapped at the appropriate constrictions. It is important to note that this trapping efficiency is highly dependent on particle concentrations as higher concentrations will rapidly clog the constrictions and reduce trapping efficiency. In order to operate the device at optimal efficiency, lower concentrations should be used. This is clearly shown in Fig. 7, where \( C_1 \) and \( C_2 \) have significantly lower trapping efficiencies than \( C_3 \); hence, the initial concentration is important to avoid the problem in this device. This device will yield the best trapping efficiency at this concentration level. The major obstacle for successful operation of this device is the accumulation of trapped particles creating a channel obstruction and consequent trapping of smaller particles than expected. Consequently, in this paper, we found that this obstacle can be overcome when the initial concentration of particle solution was lower than 1.0 \( \times \) \( 10^6 \) particles/mL and the particles were injected into the device for shorter than 30 min; hence, the problem can be avoided as an initial concentration of samples lowers or particle injection time is reduced. Additionally, when the length of a constriction channel is extended, the problem will be also decreased. As a result, this device is useful for the separation of a heterogeneous particle population based on size in a small volume with low concentration. Moreover, the accumulation of particles in a downstream constriction channel might affect trapping efficiencies in upstream constriction channels due to the expected increase in the absolute pressure upstream over time due to the accumulation of particles and increasing of the resistance. In this paper, however, as the particles were continuously trapped and accumulated at constriction channels, this did not alter the trapping ability of the device. As the particles are spherical and the layer of trapped particles at each constriction channel was less than two, the flow of smaller particles was able to pass among the trapped particles.

In this paper, size-based microbial separation from environmental samples was achieved in this multilayered microfluidic device. A heterogeneous population of environmental microbes was separated into three subpopulations classified by size relevant to the number of constriction channels, and they were successively concentrated without significant loss of the population along the constriction channels. In addition, the environmental sample was injected into the device for 30 min, and successively, water was injected for an additional 60 min to make all introduced microbes trapped at constriction channels. During the process, deforming bacteria were not observed. Even though size-based separation was achieved in this device
with a heterogeneous mixture of particles, the quantification could not be done in the same manner as with the microparticles due to microbial overlapping at the constrictions. All large microbes were trapped at 7-μm-tall constriction channels. This technique would prove useful for when even small amounts of environmental pathogens would prove disastrous such as monitoring drinking water.

The device can also be constructed as an inexpensive diagnostic that can rapidly process field samples without the intermediate culturing step that is currently required for detection of environmental pathogens. Moreover, it achieves a reduction in the required sample volume, highly effective simple process, and high portability. In addition, for the preparation of the sample, additional treatments such as filtration, centrifugation, and storage are not required in this system, innovatively reducing process time. If used in conjunction with in situ hybridization assays, the device could be used to process, trap, and identify microbes of interest for environmental monitoring.

Finally, for more detailed separation, the heights of constriction need to be more minutely divided such as 1, 2, 3, 4, and 5 μm because the size range of the bacteria living in the lake is limited to 0.2–5.0 μm in length. The detailed separation of bacteria and further characterization of the trapped bacteria in a multilayered microfluidic device is in progress for further studies.

V. CONCLUSION

In this paper, a multilayered microfluidic device has been developed for the size-based separation of heterogeneous particle samples. This microfluidic device overcomes a number of limitations on the current analysis of microbial populations in diverse environments by working as a presorter. The central principle of this microfluidic separation is based on the size-dependent relationship between the size of particles and the constriction-channel heights in the device. Each constriction channel of differing height was designed to trap larger particles than the height of each constriction channel. To validate the function of the device for size-based separation, a heterogeneous mixture of fluorescent microspheres composed of 10-, 4.5-, and 2-μm-diameter particles was injected into the multilayered microfluidic devices, and more than 90% of each particle was successfully trapped at the 7-, 3-, and 0.8-μm-tall constriction channels, respectively. Trapping efficiency was dependent on the concentration of the populations so that, as the concentration was decreased, the trapping efficiency of this microdevice for each sized particle was increased at each matched constriction channel. More importantly, environmental samples obtained from Lake Michigan were injected into the microfluidic device, and they were homogeneously distributed and separated into three parts by size at each constriction channel. Moreover, bacteria were simultaneously concentrated according to size without loss of the population along the constriction channels.

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REFERENCES

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