High Efficiency Hydrodynamic Chromatography in Micro- and Sub-micrometer Deep Channels using an On- Chip Pressure-Generation Unit

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Abstract

In this article, we report the development of a microchip based hydrodynamic chromatographic device that has an integrated pressure-generation unit to drive the mobile phase during the separation process. The pressure-gradient in this system was realized through introduction of a mismatch in electroosmotic flow rate at the junction of two glass channel segments with different depths as has been previously reported by our research group. A fraction of the resulting pressure-driven flow was then guided into an analysis channel to enable the size-based particle separations. The reported device allowed the realization of hydrodynamic chromatography in both micro- and sub-micrometer deep analysis channels as experiments showed the noted approach to yield pressure-driven velocities in our system that were nearly unaffected upon scaling down the depth of the entire fluidic network. Separations with plate numbers in the range of 500-2000 plates/mm were realized in a 3 cm long analysis column for a mixture of 25, 100 and 250 nm diameter polystyrene beads with analysis times as short as 12 s.

The noted separation efficiency exceeds those previously reported in the literature for hydrodynamic chromatographic analysis performed on microchips likely due to a faster mass transfer across the channel cross-section as well as narrower band injections in our assays.

Keywords: hydrodynamic chromatography, microfluidics, micropump, nanofluidics, particle separation, surface conductivity

Introduction

Characterization of colloidal matter, biological macromolecules and polymeric materials is critically important to understanding their properties as well as improving their utility in specific applications [1-3]. In addition, because most natural and synthetic substances of these kinds occur as mixtures with a broad range of size and shape distributions, the separation of their different fractions often becomes necessary for achieving the desired material properties. In the case of colloidal matter, for example, the distribution of particle sizes and shape tends to affect solid state properties such as powder packing, abrasive efficiency, friction and bulk density. Moreover, the rheological properties of granular materials such as drag, mixing and viscosity both in their solid- and melt-states are known to be significantly influenced by the noted size/shape distributions. Hydrodynamic chromatography (HDC) presents a powerful approach to realizing macromolecular and colloidal separations relying on the preferential sampling of the different flow streamlines by the particles based on their size and shape among other properties [4-6]. This separation method can be practiced in open or packed columns and has been applied to analyzing a variety of sample mixtures ranging from those of particulate matter to viruses and DNA. Moreover, the ability to couple HDC with a multitude of detection methods such as light scattering (both multi-angle static and quasi-elastic), viscometric and refractometric techniques has further empowered the material characterization capabilities of this

separation method [7]. In particular, continuous measurements performed by such an array of detectors now makes it possible to establish the mutual interdependence of size, molar mass, shape, structure and compactness across the elution profile of the sample in a single analysis [8].

Over the years there has been a concerted effort to improve the resolving power of HDC methods in order to broaden their applicability to a wider range of samples. In this regard, miniaturization of this technique using narrow capillaries and microchannels has proven to be particularly promising yielding peak efficiencies that were previously unattainable [9-13]. This is because band broadening in HDC methods is known to be dominated by the contribution from slow analyte mass transfer (mixing) across the transverse dimension of the channel particularly at large flow velocities [9,11,13]. Such a limitation leads to concentration gradients for the particles/macromolecules across the channel cross-section yielding band broadening similar to that described by Taylor and Aris for point-like particles [14,15]. While Giddings and others have shown that this contribution to plate height tapers off at large mobile phase velocities in packed columns [16-18], the classical linear increase in its magnitude with flow velocity can be expected to hold true for open channels/capillaries [13]. Blomet *al.* demonstrated HDC separations in 1 μm deep channels yielding plate numbers as high as 1200 plates/mm for 150 nm sized particles transported at the rate of about 0.7 mm/s [10]. Interestingly, the peak efficiency was noted to increase with flow velocity in that work suggesting band broadening to be governed by axial diffusion under the chosen experimental conditions. More recently, Prof. Liu's group demonstrated highly efficient DNA separations using the HDC method reporting plate numbers again over 1000 plates/mm using 2 μm diameter capillaries. In their case however, the plate heights were seen to increase with flow velocity indicating slow analyte mass transfer in the mobile phase to be dictating band dispersion [13]. Both these works suggest that further

improvements in the efficiency of the HDC technique may be possible through realization of faster separations in narrower analysis columns. In addition, the ability to inject shorter sample bands in the system may allow maximize these benefits realized through such high-speed miniaturized assays.

In this article, we report the development of a microchip based HDC device that relies on an on-chip pumping unit for generating the pressure gradient in the separation channel. The noted pressure-drive was realized by producing a mismatch in the electroosmotic flow rate at the junction of two channel segments with different depths [19-21]. In our previous works, we have shown that this approach to pressure-generation is particularly conducive for miniaturization of pressure-driven separations yielding flow velocities in the separation channel that are nearly unaffected upon scaling down the depth of the entire fluidic network [22,23]. Moreover, the small dead volume in the system allows for high dynamic control over this pressure gradient, which otherwise can be challenging to accomplish during the sample injection process using external pumps. Taking advantage of these aspects of our system, high efficiency HDC separation of fluorescent nanoparticles were performed in micro- and sub-micrometer deep channels yielding plate numbers over 2000 plates/mm. In addition, we were able to resolve individual peaks from a mixture of 25, 100 and 250 nm diameter beads in a 3 cm long channel in less than 12 s of separation time.

Experimental Procedure

Device fabrication: For fabricating the micro-/nanofluidic devices employed in this work, bottom and cover plates made from borosilicate glass were purchased from Telic Company (Valencia, CA). While the purchased cover plates had both their faces unprotected, the bottom ones came with a thin layer of chromium and photoresist laid down on one of their surfaces.

90 Custom designed photomasks created through Fineline Imaging Inc. (Colorado Springs, CO)
91 were used to pattern the desired channel layout onto the bottom plate using standard
92 photolithographic methods [24,25]. The channel network shown in figure 1 was used in our
93 experiments with all of its segments being 200 μm wide and uniformly deep except for the
94 shallow region marked in segment C. The length of the separation channel in our device
95 (segment F) was chosen to be 3.5 cm. After completion of the photo-patterning process, the
96 photoresist layer was cured in microposit developer MF-319 (Rohm and Haas) and the
97 chromium layer removed along the channel network with a chromium etchant (Transene Inc.).
98 The fluidic ducts were then etched to a depth between 0.24 and 2.2 μm using a solution of
99 buffered oxide etchant purchased from Transene Inc. To realize the shallow region in segment C,
100 a small fraction of this channel (about 2mm long) was manually covered with a layer of
101 photoresist followed by etching the remaining fluidic network to its final depth. The protective
102 photoresist and chromium layers on the bottom plate were subsequently removed using acetone
103 and chromium etchant solutions, respectively. Access holes were drilled at the channel terminals
104 using a microabrasive powder blasting system (Vaniman Inc.) to allow the introduction of liquid
105 reagents/sample into the micro-/nanochannels. Finally, the micro-/nanofluidic network was
106 sealed off by bringing a glass cover plate in contact with the bottom plate in deionized water and
107 allowing the two plates to bond at 550°C for 12 hrs [26]. Cylindrical glass reservoirs with internal
108 volumes of about 150 μL were affixed over the access holes using a ultraviolet light curable glue
109 (Norland Products Inc.) to serve as a source or sink for the liquid flow within the micro-
110 /nanochannels.

111 **Device operation:** The reported microchip was prepared for an experiment by rinsing the entire
112 fluidic network with 0.1M sodium hydroxide followed by deionized water, methanol and

113 deionized water again in that order for 20 min.each. Analyte injection into the separation channel
114 (segment F) was accomplished by placing the colloidsamplein reservoir 1 while filling the
115 remaining reservoirs with deionized water. The application of high voltages to reservoirs 1 and 2
116 (0.5 kV and 3 kV, respectively) under these conditions while electrically grounding reservoirs 3
117 and 4 then allowed the realization of a sample flow profile that was suitable for band injections
118 (see figure 1, stage A). Reservoir 5 was electrically floated in our experiments by not placing an
119 electrode in it to realize an electric field-free condition in the separation channel (segment F). In
120 this situation, liquid/sample flow in this segment was entirely pressure-driven irrespective of the
121 voltages applied at the different channel terminals. In order to introduce the analytebands into
122 segment F, a gated injection scheme was used [27]. This scheme was implemented by
123 electrically grounding reservoir 2 for 0.1 s during which the samplewas allowed to fill up the
124 entire injection cross (see figure 1, stage B). Following this step, the electric potentials at
125 reservoirs 1 and 2 were switched to the operation voltages (φ and 6φ , respectively) to be used in
126 the experiment, which also forced a fraction of the sample that had filled up the injection cross to
127 be swept into segment F (see figure 1, stage C). Notice that the voltage applied at terminal 2 was
128 always chosen to be 6 times the voltage applied at terminal 1 in all stages of our microchip
129 operation, as it yielded sample injections into the analysis channel with relatively minor tailing
130 of the analyte band. Also, the voltages applied in stages A and B of our microchip operation
131 were consistently held at the same value irrespective of the magnitude of φ to minimize
132 variations in the volume of the injected sample plug. The analyte bands thus injected into
133 segment F were detected 3 cm downstream of the injection cross via the laser induced
134 fluorescence technique. For these measurements, a laser beam (125 mW Argon ion tunable laser,
135 Melles Griot) of wavelength 467 nm was used to excite the fluorescent colloidal sample and the

resulting signal collected using a photomultiplier tube (Hamamatsu Photonics) after passing it through a 514 nm longpass optical filter (Semrock Inc.). The sequence of voltages applied to the different channel terminals in the noted assay were executed using a commercial electrical power supply (LabSmith high voltage sequencer) while the process of data acquisition by the photomultiplier tube was controlled with an in-house written LABVIEW program. The peak shapes were analyzed in our work with numerical data analysis tools available in MATLAB. The visualization of the analyte bands was realized in this study using an epifluorescence microscope system (Nikon) with bandpass excitation (465-495 nm) and emission (515-555 nm) optical filters. The fluorescence images of the sample injection process were recorded in our experiments using a CCD camera (Roper Scientific).

Results and Discussion

Retention time: Our initial experiments in this project focused on measuring the effect of different operating parameters on the retention time of the fluid and the fluorescent polystyrene beads in the separation channel, i.e. segment F. Note that their transport through the separation channel was entirely pressure-driven due to the absence of an electric field in this particular channel segment [25,28]. For the fluid flow measurements, 10 μ M samples of a neutral fluorescent tracer dye (Rhodamine B base) prepared in deionized water were injected into the separation channel using the operating procedure described in the previous section. In figure 2(a), we have presented data obtained from one such set of measurements made using a microchip that had a channel depth of 0.52 μ m in its shallow region and 1.3 μ m in the remaining fluidic network. The figure shows a linear increase in fluid velocity with the applied voltage at reservoir 1 (φ) attaining values as large as 3 mm/s for $\varphi = 0.5$ kV. This linear trend was expected as the pressure-gradient in segment F was produced using electrokinetic forces which

scale linearly with the electric field in the system. We would like to point out that in all our devices, the depth in the shallow region of the pumping unit was chosen to be 40% of that in the remaining microchannel network. Such a choice was observed to nearly maximize the pressure-driven velocity in segment F for a given set of voltages applied at the channel terminals as has been noted in our previous publications [19,23]. For the particle velocity measurements, we introduced samples containing 0.05 wt% of polystyrene beads prepared in deionized water and estimated their flow velocity in segment F. The 25, 100 and 250 nm diameter particles used in our experiments were again seen to migrate at a rate that depended linearly on the parameter ϕ with the larger beads traveling faster than the smaller ones due to their inability to access the slower moving streamlines close to the channel walls and tendency to sample smaller interstitial volumes in the conduit (see figure 2(a)). The relative retention time for each of the particles in this situation was estimated as the ratio of the fluid velocity to the particle velocity in the separation channel. Figure 2(b) shows this ratio to decrease with the particle size yielding values as small as 0.74 for the 250 nm diameter beads when migrated through a 1.3 μm deep channel. In figure 3, the dependence of the fluid and particle velocities on the channel depth was explored in order to arrive at the experimental conditions that were most conducive for separating the chosen particle mixture. It can be seen from the figure that although the fluid velocity decreased monotonically for shallower conduits, the particle flow velocity exhibited a weak maximum. The decrease in the fluid velocity however was only about 17% going from a channel depth of 2.2 to 0.6 μm similar to that seen in our previous studies [23]. Such reduction likely occurred due to a lower electroosmotic flow in the shallower fluidic networks resulting from partial overlap of the electrical Debye layers around their top and bottom channel walls [29,30]. On the other hand, the origin for the weak maximum in the particle flow velocity can be understood based on two

competing effects. While the advection of these beads slowed down with a decrease in the channel depth, their inability to access the slower moving streamlines in the shallower ducts countered this effect by speeding up their migration rate relative to the average fluid velocity. The latter effect is better demonstrated in figure 3(b) which shows a clear decrease in the relative retention times for the particles as their diameter becomes comparable to the channel depth. Overall, the differential in the flow velocity for the beads was seen to maximize for the shallowest channel used in our experiments indicating this to be a prudent choice for our HDC analysis.

Peak efficiency: Following the flow velocity measurements discussed above, we evaluated the peak efficiency for the particle bands in our device which can significantly influence the resolving power of our HDC assays. To this end, we estimated the plate height for the fluorescent bead samples as a function of the applied voltage and the channel depth in the system. In figure 4(a), we have included data obtained from this study which show the plate height measured in a 0.6 μm deep channel to increase with ϕ for the two larger particles having diameters of 100 and 250 nm. This is not surprising keeping in mind that the diffusion coefficients for such particles tend to be fairly small causing their band dispersion to be limited by mass transport across the channel depth. In this situation, the plate height for the 100 and 250 nm diameter beads can be expected to increase with flow velocity which in turn scales linearly with the applied voltage in the system. For the 25 nm diameter particles however, a minimum in the plate height was observed with increasing values of ϕ painting a more complete picture predicted by the van Deemter equation [31]. Importantly, we were able to attain plate heights below 0.5 μm for the smallest fluorescent beads which also never exceeded 3.5 μm for the larger ones in a 0.6 μm deep channel. In figure 4(b), the effect of the depth in segment F was investigated on the peak

205 efficiency of the particle bands to further establish the cause for band broadening in our system.
206 The figure shows a nearly monotonic increase in the plate height with this depth for all 3 particle
207 sizes again highlighting the dominating contribution from flow dispersion on the width of their
208 corresponding peaks. Overall, the plate height was seen to increase by about 10-fold for the
209 largest particlesize and nearly 4-fold for the 25 nm diameter bead going from a 0.6 to 2.2 μm
210 deep channel. These measurements corresponded to plate numbers as large as 2000 and 500
211 plates/mm for the 25 and 250 nm diameter particles respectively, which significantly exceed
212 those previously reported in the literature for microchip based HDC analysis. The variation in the
213 recorded plate height with channel depth further established that shallow ducts were a better
214 choice for our HDC separations and will likely yield the highest separation resolutions attainable
215 with our design.

216 As has been noted before, the increased band broadening for the larger particles in our
217 experiments was believed to be a result of slow mass transfer of these entities across the lateral
218 dimensions of the channel which is consistent with the data reported by Op de Beeck *et al.* [11]
219 and Zhu *et al.* [13]. In the paper by Zhu *et al.* for example, the plate heights for a 20 kbp DNA
220 sample was reported to be 5-6 times greater than that for a 75 bp DNA specimen for eluent
221 velocities in the range of 3-4 mm/s through a 2 μm diameter capillary. In our present work, the
222 band broadening for the 250 nm beads similarly exceeded that for the 25 nm ones by a factor of
223 10 in a 2.2 μm deep conduit for comparable particle velocities which is reasonable in view of the
224 fact that large aspect ratio rectangular channels tend to yield greater hydrodynamic dispersion
225 compared to circular capillaries. Likewise, 500 nm particles were seen to yield 5-10 fold higher
226 plate heights compared to the 100 nm ones in the HDC separations reported by Op de Beeck *et al.*
227 which were performed in a micro-pillar array column with inter-pillar distance of about 2.5 μm

but employing lower flow velocities (~ 0.1 mm/s). Interestingly, in a different work by Blomet *al.* [9], the authors actually noted a decrease in plate height going from a 26 nm to a 155 nm diameter particle sample upon transporting them through a 1 μ m deep and 0.5 mm wide microfluidic channel using pressure-drive. The flow velocities employed in that particular work however was over 5-times slower compared to those in our current experiments suggesting the peak efficiency in Blomet *al.*'s assays to be limited by longitudinal diffusion. We would also like to point out that the plate heights realized for the Rhodamine B base peaks in our present work were somewhat larger than those observed for the 25 nm particles (data not shown) which was counterintuitive. However, from our past experience we know that Rhodamine B base has a tendency to stick to our negatively charged glass channels especially when deionized water was used as the mobile phase [23] whereas the polystyrene beads owing to their slightly anionic nature (confirmed by the vendor, Thermo Fischer Scientific) did not exhibit any such noticeable interactions with the channel walls. The adhesion of the Rhodamine B base molecules to the glass channel walls was inferred in our work from the strong tailing noted for its peak in the chromatogram.

Particle separations: The separation performance of our device was assessed by injecting bands of the 3-particle mixture into segment F and quantitating the resolution for the peaks recorded on the chromatogram. For these experiments, the microchip with a 0.6 μ m deep separation channel was chosen as it yielded the highest differential in the particle velocity and the smallest plate heights for the recorded fluorescent peaks. In figure 5(a), we have presented a chromatogram that shows a complete separation between the particle peaks in a 3 cm long channel at $\varphi = 0.5$ kV. An optical image included in the top left corner of this figure shows the same process but was recorded about 0.5 cm downstream of the injection cross in order to visualize all of the 3

separated particle bands as they migrated through the analysis channel. This image however suggests noticeable tailing of the particle zones at this location which primarily was a consequence of the injection process. These analyte bands nevertheless were observed to become increasingly symmetric as they traveled through the separation channel evident from the optical image included below it for the 25 nm particle zone recorded 3 cm downstream of the injection cross. Unfortunately, it was not possible to image all 3 particle zones at this region at the same time due to the limited field-of-view of our imaging microscope. The resolving power of our separations in this situation was assessed using a PMT detector focused at this location yielding the chromatogram shown in figure 5(a). In figure 5(b), we have plotted the resolution for these peaks estimated from such chromatograms as a function of the operating voltage in the device which shows a decrease in the noted quantity for larger values of φ . This trend suggests that the increased broadening of particle bands at higher operating voltages completely offsets the benefits derived from a faster separation in our system consistent with the data included in figures 3 and 4. In general, the separation resolution was seen to drop by a factor of 1.5-2.5 upon increasing φ from 0.1 to 0.5 kV. We also noticed some significant tailing of the 250 nm particle band in our experiments whereas the other two peaks in the chromatograms appeared relatively symmetric. Finally, the resolving power of our HDC device was observed to moderately diminish upon increasing the channel depth in the fluidic network from 0.6 to 1 μm . This observation was again consistent with the dominating effect of flow dispersion in our system particularly for the 100 and 250 nm diameter fluorescent beads.

Conclusions

To summarize, we have developed a microchip based hydrodynamic chromatographic system in which the pressure-gradient necessary to drive the size-based separations was

274 generated on-chip via electrokinetic means. A mismatch in the electroosmotic flow rate
275 introduced at the junction of two channel segments with different depths was exploited to realize
276 this pressure-drive. Such an approach proved conducive for performing HDC analysis in micro-
277 as well as sub-micrometer deep channels yielding plate numbers as large as 2000 plates/mm for
278 a 25 nm diameter polystyrene bead sample. A detailed study of band broadening in our HDC
279 assays suggested slow analyte mass transfer across the channel depth to be the determining factor
280 for the observed separation efficiency. Nevertheless, the individual peaks for a mixture of 25,
281 100 and 250 nm polystyrene beads could be separated in our device with a resolution greater
282 than 3.5 in just under 12 s. It must be noted that in order to prevent the colloidal particles from
283 entering the pumping unit, we had moved this unit away from the injection/separation region of
284 our device unlike in some of our previous works, e.g. reference 19. This was important as these
285 particles had a tendency to clog the shallow region in segment C significantly cutting down the
286 life time of our HDC system. The noted design however limited the fraction of pressure-driven
287 flow generated in the pumping unit that could be directed into the separation channel to carry out
288 the HDC analysis. In fact, theoretical calculations show that this fraction is less than 10% for our
289 current design but may be improved through use of a fluidic network having different depths in
290 the injection and separation segments [23]. In particular, this fraction may be significantly
291 increased by employing injection arms (segments A and E) that are somewhat shallower than
292 segment F. Such an approach would minimize loss of the pressure-driven flow into reservoirs 1
293 and 4 in the reported device increasing the pressure-gradient across its separation channel. We
294 are currently exploring this strategy for realizing faster separations in sub-micrometer deep
295 analysis channels with plate numbers even larger than that realized in the present work.

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References

- [1] G. Patterson, Physical chemistry of macromolecules, CRC Press, Boca Raton, 2007.
- [2] A. Seidel, Characterization and analysis of polymers, John Wiley & Sons, Hoboken, 2008.
- [3] G.M. Kontogeorgis, S. Kiil, Chapters 8 and 9, in: Introduction to applied colloid and surface chemistry, John Wiley & Sons, Hoboken, 2016.
- [4] K.O. Pedersen, Arch. Biochem. Biophys. Suppl.1 (1962) 157-168.
- [5] E.A. DiMarzio, C.M. Guttman, Macromolecules 3 (1970) 131-146.
- [6] H. Small, J. Colloid Interface Sci. 48 (1974) 147-161.
- [7] A.M. Striegel, A.K. Brewer, Annu. Rev. Anal. Chem. 5 (2012) 15-34.
- [8] A.M. Striegel, Anal. Bioanal. Chem. 402 (2012) 77-81.
- [9] E. Chmela, R. Tijssen, M.T. Blom, J.G.E. Gardeniers, A. van den Berg, Anal. Chem. 74 (2002) 3470-3475.
- [10] M.T. Blom, E. Chmela, R.E. Oosterbroek, R. Tijssen, A. van den Berg, Anal. Chem. 75 (2003) 6761-6768.
- [11] J. Op de Beeck, W. De Malsche, J. Vangeloooven, H. Gardeniers, G. Desmet, J. Chromatogr. A 1217 (2010) 6077-6084.

- 317 [12] X. Wang, V. Veerappan, C. Cheng, X. Jiang, R.D. Allen, P.K. Dasgupta, S. Liu, J. Am.
318 Chem. Soc. 132 (2010) 40-41.
- 319 [13] Z. Zhu, L. Liu, W. Wang, J.J. Lu, X. Wang, S. Liu, Chem. Commun. 49 (2013) 2897-2899.
- 320 [14] G.I. Taylor, Proc. R. Soc. A 219 (1953) 186-203.
- 321 [15] R. Aris, Proc. R. Soc. A 235 (1956) 67-77.
- 322 [16] J.C. Giddings, Dynamics of chromatography: Principles and theory, Marcel Dekker, New
323 York, 1965.
- 324 [17] G. Stegeman, J.C. Kraak, H. Poppe, J. Chromatogr. 634 (1993) 149-159.
- 325 [18] A.M. Striegel, W.W. Yau, J.J. Kirkland, D.D. Bly, Modern size-exclusion liquid
326 chromatography, second ed., John Wiley & Sons, New York, 2009.
- 327 [19] N. Yanagisawa, D. Dutta, Electrophoresis 31 (2010) 2080-2088.
- 328 [20] L. Xia, D. Dutta, Anal. Chem. 84 (2012) 10058-10063.
- 329 [21] L. Xia, D. Dutta, Analyst 138 (2013) 2126-2133.
- 330 [22] G.M. Toh, R.C. Corcoran, D. Dutta, J. Chromatogr. A 1217 (2010) 5004-5011.
- 331 [23] L. Xia, C. Choi, S.C. Kothekar, D. Dutta, Anal. Chem. 88 (2016) 781-788.
- 332 [24] D.C.S. Bien, P.V. Rainey, S.J.N. Mitchell, H.S. Gamble, J. Micromech. Microeng. 13
333 (2003) S34-S40.
- 334 [25] G.M. Toh, N. Yanagisawa, R.C. Corcoran, D. Dutta, Microfluid. Nanofluid. 9 (2010) 1135-
335 1141.
- 336 [26] S.C. Jacobson, A.W. Moore, J.M. Ramsey, Anal. Chem. 67 (1995) 2059-2063.

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Figure 2: (a) Effect of operating voltage on the flow velocity of the fluid and fluorescent particles in the separation channel. (b) Observed variation in the relative retention time for the polystyrene particles as a function of their size. An operating voltage of $\phi = 0.5 \text{ kV}$ was employed for these measurements. The microchips used for generating the experimental data included in both sub-figures had a depth of $0.52 \mu\text{m}$ in their shallow region and $1.3 \mu\text{m}$ in the remaining fluidic network. The error bars included in the sub-figure (b) are based on 5 measurements.

Figure 3:(a) Effect of channel depth on the flow velocity of the fluid and the fluorescent particles in segment F of our device. (b) Observed variation in the relative retention time for the polystyrene particles as a function of the depth in the separation channel. An operating voltage of $\varphi = 0.5 \text{ kV}$ was employed for these measurements. The microchips used for generating the experimental data included in the sub-figures had shallow regions whose depth was 40% of that in the remaining fluidic network. The error bars included in the sub-figures are based on 5 measurements.

Figure 4:(a) Effect of operating voltage on the plate height of the fluorescent particles as recorded on the chromatogram. The depth of the separation channel was $0.6 \mu\text{m}$ in the microchip employed for the experiments. (b) Observed variation in the plate height of the particle peaks as a function of the depth in the analysis channel. An operating voltage of $\phi = 0.3 \text{ kV}$ was employed for these measurements. The microchips used for generating the experimental data included in the sub-figures had shallow regions whose depth was 40% of that in the remaining fluidic network. The error bars included in the sub-figures are based on 5 measurements.

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Figure 5:(a) Hydrodynamic chromatography of a mixture of 25, 100 and 250 nm diameter polystyrene beads in a 0.6 μm deep analysis channel driven by an operating voltage of $\phi = 0.5 \text{ kV}$. While the noted chromatogram was recorded at a separation distance of 3 cm, the optical image included in the top left corner of the sub-figure was obtained about 0.5 cm downstream of the injection cross. The other optical image below it depicting the 25 nm particle band was again recorded 3 cm downstream of the injection cross to highlight the increased symmetry of the sample zones as they migrated through the separation channel. (b) Effect of operating voltage on the separation resolution of the fluorescent particle mixture considered here. The curve indicated by the solid and dotted lines represent data obtained in a 0.6 and 1 μm deep separation channel, respectively. The error bars included in sub-figure (b) are based on 5 measurements.