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Two simple and rugged designs for creating microfluidic sheath flow

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Abstract

A simple design capable of 2-dimensional hydrodynamic focusing is proposed and successfully demonstrated. In the past, most microfluidic sheath flow systems have often only confined the sample solution on the sides, leaving the top and bottom of the sample stream in contact with the floor and ceiling of the channel. While relatively simple to build, these designs increase the risk of adsorption of sample components to the top and bottom of the channel. A few designs have been successful in completely sheathing the sample stream, but these typically require multiple sheath inputs and several alignment steps. In the designs presented here, full sheathing is accomplished using as few as one sheath input, which eliminates the need to carefully balance the flow of two or more sheath inlets. The design is easily manufactured using current microfabrication techniques. Furthermore, the sample and sheath fluid can be subsequently separated for recapture of the sample fluid or re-use of the sheath fluid. Designs were demonstrated in poly(dimethylsiloxane) (PDMS) using soft lithography and poly(methyl methacrylate) (PMMA) using micromilling and laser ablation.

Introduction

Flow cytometry is a popular technique used to count and evaluate cells and other particles in suspension. In traditional flow cytometers, originally developed by Crosland-Taylor¹ in 1953, the sample solution exits a small tube into the center of a larger tube, carrying clean solution. The larger tube is then constricted so that both streams are reduced in diameter and accelerate. The sample stream is reduced to a diameter roughly the size of the cells or particles to be analyzed, which forces them to travel in single file along a fixed and highly precise trajectory within the flow channel. Because the cells or other particles are positioned so reproducibly, high numerical aperture optics can be precisely aligned to interrogate them. Also, because the cells are following the same path down the channel, they all have the same velocity, which allows the duration and intensity of signals to be correlated with individual cells or particles with low variance.

Because of the success of bench-top cytometers, there have been several attempts to create a miniaturized flow cytometer. Laminar flow makes most microfluidic systems at least theoretically well suited to flow cytometry. In practice, however, emulating the annular design of the traditional cytometers is a difficult fabrication problem. From a purely fluidic perspective, focusing is not entirely necessary. Some researchers have filled the whole channel

with the sample stream.^{2–5} Unfortunately, filling the channel can make optical detection problematic. The cells or particles are evenly distributed across the channel, meaning that they cannot be focused without making the channel small enough to incur a concomitant risk of clogging.⁶ High numerical aperture optics cannot be used, and light scatter off the walls of the channel is unavoidable if the excitation beam has to pass through both an air/solid and a solid/liquid interface before reaching the sample liquid. The fact that particles can take multiple paths through the detection region is often a major contributor to the variance of the data.^{2,4} Additionally, cells and other sample components come into contact with the walls of the channel, which makes fouling a danger.^{7–11} Ladisch and co-workers confined the sample on either side with air.¹² A variety of factors affected the size of the liquid stream, including the hydrostatic pressure and surface tension of the fluid. Unfortunately, light scattering of the walls and the air/water interface is still an issue. In addition, any contamination of the poly (dimethylsiloxane) (PDMS) surface will change or even confound the containment of the sample.

A more robust approach, pioneered by Ramsey's group,^{8,13,14} has been to confine the sample stream on either side with a particle-free aqueous solution. This design is simple and easily fabricated, leading several researchers to imitate it.^{9,10,15–27} Unfortunately, the sample still comes into contact with the top and the bottom of the channel, which necessitates the addition of a dynamic or covalent coating to mitigate the propensity for fouling.^{8–11,25} Also, cells and particles can appear at any depth from the top to the bottom of the channel, so high numerical aperture optics still cannot be used.

A few attempts to sheath the sample stream both horizontally and vertically have been reported.^{26,28–32} Typically, two additional input channels focus the stream vertically as well as horizontally. From the standpoint of cytometry, this is a far better situation, because the sample is now completely isolated from the channel surface, and the position of the particles to be analyzed is fixed. Unfortunately, the addition of another set of sheath inputs brings the total number to four. Their relative flow rates must be carefully controlled or the position of the sample stream will drift. Otherwise, the particles will no longer pass through the laser beam. The best way to ensure even distribution of flow among all the sheath channels is to have a separate pump supplying each stream, but a plethora of pumps substantially increases the expense and complexity of the supporting fluidics.

We present two designs that can produce fully sheathed flow in easily manufactured devices. The sheath and sample fluids are first introduced into the channel using conventional and easily manufactured geometries. Then a set of grooves wraps the sheath solution around the sample. The two designs require only one or two sheath inlets. They were designed and modeled using the in-house software, Tiny-Toolbox (TT). The diameter of the sheathed sample stream is governed by the relative flow rates of the sample and sheath streams, while the position and shape of the sample stream are controlled by the selection of the grooves.

Experimental

Computational modeling

Fluidic modeling was performed using an in-house Navier–Stokes fluid solver, Tiny-3D, (T3D),³³ coupled with the acceleration algorithm Tiny-Toolbox (TT).^{34–36} TT operates under conditions of Stokes flow and high Peclet number, which makes it broadly applicable to a variety of conditions commonly found in microfluidics. TT provides a roughly 10^5 – 10^6 increase in speed over traditional Navier–Stokes solvers. Briefly, T3D is used to model the field through a length of channel with a pre-defined structure (*e.g.* a pair of downstream-pointing chevrons in the top and bottom of the channel). Stokes flow is assumed, as is a fully developed Poiseuille flow at the start of the grooves, and diffusion is assumed to be negligible.

The reverse Lagrangian paths are then determined to map any given point on the outflow plane with its originating point on the inflow plane, and the information is compacted into a simple 2D advection map.

The initial inflow conditions for the maps are established using standard T or cross-intersections. The design shown in Fig. 1A uses a T-intersection to place the sample stream next to the sheath stream within the channel. The interface between the two streams is vertical, and its horizontal position is a function of the relative volumetric flow rates of the two streams. Because the cytometers are operated in the laminar flow regime, the distribution of the streams is stable and subject only to diffusion unless the streams flow through a perturbing structure.

The distribution of the two streams is taken as the inflow plane of the sheathing structure. The TT algorithm can then apply the map for a given structure to redistribute the two streams. Multiple structures can be modeled by applying the maps for those structures in the same order in which they are placed in the channel. Previously, this technique was used to create highly efficient microfluidic mixers.³⁴ In this paper, it is applied to the somewhat simpler problem of generating sheath flow.

Fabrication of sheath devices

To demonstrate the general utility of the designs, chips were manufactured using fabrication methods commonly available: milling, soft lithography and laser ablation. Many researchers have only one fabrication technique available to them, and it was desirable to demonstrate that the cytometer designs were technique and material independent.

Milling—The milled chips were manufactured according to a previously discussed method.³³ Briefly, the chips were made in poly(methyl methacrylate) (PMMA) (Acrylite FF, CYRO Industries, Parsippany, NJ) using a Haas Minimill (Haas Automation Inc., Oxnard CA). Nominal channel dimensions were 3.2 mm wide by 1.0 mm deep. The grooves were 0.8 mm wide, 0.43 mm deep, and 0.8 mm into the side wall of the channel. The chips were made in two halves with half the channel milled into each. The two halves were aligned, clamped, and bonded together with a low viscosity epoxy (Epotek 301, Epoxy Technology, Billerica, MA).

Soft lithography—Chips were fabricated in PDMS using standard microfabrication procedures.³⁷ Briefly, a master was created on a silicon wafer by sequentially spinning on layers of SU-8 photoresist (Microchem, Newton, MA) and exposing with a photomask containing the desired channel geometries and features. Designs used in this study had three distinct layers. After development, the master was treated with chlorotrimethyl-silane (Sigma-Aldrich, St. Louis, MO) vapor to prevent polymer adhesion. The master was placed into the bottom of a mold and PDMS (Sylgard 184, Dow-Corning, Corning, NY) was poured on top to make layers approximately 1 mm thick. After curing, the PDMS was peeled from the master. Fluidic inlets were cored into the top of the cytometer, and the two halves were then bonded together by treatment in an oxygen plasma. In the microfabricated design only, the top and bottom halves of the cytometer were asymmetric in order to minimize alignment issues during bonding. That is, channels were located only in the bottom half of the device so that only grooves or chevrons would need to be aligned before bonding. Channels were 130 μm high and 390 μm wide. Grooves and chevrons extended 65 μm into the top and bottom of the channels.

Laser ablation—The sheath designs were also made in PMMA using a Lamda Physik 220LX excimer laser (Coherent Inc., Santa Clara, CA) operating with ArF (193 nm). As with the milled chips, the chip was made in two symmetrical pieces, each containing one half of the channel. The two ablated halves were then aligned and thermally bonded together at 130 °C for 2 h.

The main channel and grooves were cut with an aperture projecting a 100 μm square on the surface set at 45° to the channel axis. Multiple passes were used to create the channel, which was 450 μm wide by 120 μm deep. Grooves were then cut with the same aperture to a depth of 60 μm .

Experimental cross-sections

The milled chips were used to obtain cross-sectional images of the distribution of the sample and sheath streams within the channel using a previously described method.^{33–35} A 70% fructose solution was used to maintain low Reynolds number flow in the millimeter-sized channels, which ensured that the flow characteristics exactly matched those of the smaller channels. The chips were equipped with a glass window placed at the end of the channel to allow images to be taken looking down the axis of the channel. A laser sheet from an argon ion laser was directed through the chip perpendicular to the axis of the channel. One of the two streams was labeled with rhodamine WT (Bright Dyes, Miamisburg, OH). Images were acquired with an Hitachi KP-D50U (Hitachi Denshi, Ltd.) camera mounted on a dissecting microscope.

Results and discussion

The stripe-based design

The stripe-based sheath flow design requires only a simple T-junction to establish the inflow condition (Fig. 1A), which places the sample and sheath streams side-by-side within the channel. A set of one or more grooves cut into the top and bottom of the channel then transfers a portion of the sheath solution over and under the sample stream and into a common alcove cut into the wall at the downstream end of the grooves. In transit, some fluid leaks out of the groove, isolating the sample stream from the top and bottom of the channel. The alcove becomes filled with the sheath fluid, which then exits along the wall of the main channel. Subsequent pairs of stripes move more sheath fluid to the far side of the sample stream and displace the sample stream further away from the wall. Fig. 1B shows the T3D simulation of a sheath and sample fluid traveling through a sheath device containing four pairs of grooves.

Fig. 2 shows the channel cross-section resulting from 1–5 groove pairs. The inflow condition was a channel with 50% sheath solution on the left (dyed solution) and 50% sample solution on the right. The left column of the figure shows the cross-sections predicted by the Tiny-Toolbox software and the right column shows the experimental cross-sections. As can be seen, only one stripe pair is needed to sheath the flow, but the resulting sample stream is still quite close to the wall. Subsequent stripes transport more sheath solution across the channel to the right, moving the sample stream to the left. Approximately 5 stripes are needed to place the sample stream in the center of the channel.

The size of the sample stream can be controlled by varying the relative flow rates of the sample and sheath streams. Fig. 3A shows the TT model of the sample streams resulting from flow ratios from 1 : 5 to 1 : 100 (sample : sheath). Not surprisingly, increasing the proportional flow rate of the sheath causes the size of the sample stream to shrink, but the center of the stream does not stay in the same place. Instead, one side of the sample stream remains in place while the other grows outward. The small sample stream corresponding to the 1 : 100 flow ratio represents the fluid which was originally flowing in a narrow sheet down the right wall of the channel.

The sample stream can be made quite large while remaining sheathed. Fig. 3B shows a sample stream that constitutes 75% of the flux in the channel (3 : 1, sample : sheath). Much smaller

sample streams have also been demonstrated, such as the one seen in Fig. 3C, which results from a flow ratio of 1 : 1000.

One potentially useful feature of this form of sheath flow is that it is possible to ‘unsheath’ the sample. Because the design operates in the Stokes flow regime, the flow patterns created by the features are reversible. The operations performed on the fluid stream by a given set of features can be reversed by using a second set of features mirroring the first. Fig. 4 shows an example of sheathing and unsheathing performed using the reversed grooves. The ‘wings’ of sheath fluid reaching into the sample side of the channel after the unsheathing are due to errors in the manufacturing process. Even given these rather obvious errors, the sheath and the sample could be separated with roughly 90% efficiency. This ability would be useful in situations where one would want to recycle the sheath fluid, such as continuous monitoring applications.

The scalability of this design was demonstrated by creating chips using soft lithography and laser ablation (Fig. 5). In both cases, the flow is from top to bottom, with the sample stream initially on the left side of the channel. The chip made by soft lithography (Fig. 5A) is 390 μm wide by 130 μm deep. The stripes are 30 μm wide by 65 μm deep. A similar device was made using laser ablation (Fig. 5B) and contained a channel 450 μm wide by 150 μm deep. The stripes were cut with a 100 mm square aperture set at 45° relative to the axis of the channel, resulting in squared-off grooves. In both cases, the dye-labeled sample stream is brought to the center of the channel by 5 pairs of stripes. The scale bar represents 200 μm for both images.

Chevron-based design

The stripe design is just one possible configuration that can be used to generate sheath flow. Another design can be seen in Fig. 6. In this design, the sheath solution is split equally between the two side inlets of a cross-intersection to place the sample stream in the center of the channel. A pair of chevrons cut into the top and bottom of the channel then transports sheath fluid from the sides to the middle along the top and bottom, isolating the sample stream from the top and bottom of the channel and compressing it vertically. As more chevron pairs are added, the sample is further compressed. Fig. 6B shows the outline of the sample stream for flow ratios of 4.5 : 1 : 4.5 and 9.5 : 1 : 9.5 as more chevron pairs are added to the channel.

The effect of varying the flow ratio can be seen in Fig. 7, where the number of chevrons is held constant at 4 pairs. Fig. 7A shows the size and shape of the sample stream as predicted by TT, and Fig. 7B shows the experimental cross-sections for those flow ratios when the sample stream is labeled with fluorescent dye. The chip was made by milling using the same technique and general dimensions as the milled groove-based chips.

Unlike the groove-based design, the chevrons place the sample stream in the center of the channel, regardless of the flow ratios. Instead of moving to the side, the sample stream expands or contracts horizontally and symmetrically. Within limits, the height and width of the sample stream can be controlled independently. The number of chevron pairs controls the height of the sample stream while the flow ratio controls the width. The sample stream remains approximately rectangular when the width is relatively small, but begins to develop large lobes at the ends as the proportional flow rate of the sample stream is increased. Unlike the stripe-based design or more traditional annular cytometers, the height of the sample stream at its center is independent of the flow ratio. Fig. 8 shows a plot of the height of the sample stream relative to the height of the channel as a function of the number of chevron pairs as predicted by TT. Ideally, one would want the height of the sample stream to be comparable to the diameter of the particles to be examined. Because height is a function of the number of chevrons and not the flow ratio as in the previous design, a designer must choose an appropriate channel dimension and chevron number. Although the change in height diminishes with subsequent pairs, quite small sample streams can be created. One interesting feature of this design is that

broad, shallow sample streams can be made, which have been demonstrated to have utility in imaging cell analyzers.^{29,38} If a broad-flat ribbon is desired, then the formation of the lobes at either end of the sample stream could probably be reduced or eliminated by altering the shape of the grooves.

Because the sample stream is initially focused horizontally at a cross-intersection, it is possible to change its position laterally by introducing unequal flows through the two sheath inlets. Unfortunately, some distortions of the sample stream result, as can be seen in Fig. 9. The ratio of the sample to the sum of the sheath streams was held constant at 1 : 99 while the ratio between the two sheath streams was, from left to right, 45 : 54, 47 : 52; 49 : 50; 51 : 48, and 53 : 46. As can be seen, deformation of the sample stream sets in quickly as it is moved away from the center of the channel. A thin tendril of fluid runs to the center at the top and bottom of the sample stream. It is likely that alternative groove designs could be found to prevent the formation of the tendrils of fluid.

The chevron-based design has also been demonstrated in chips made using soft lithography, as seen in Fig. 10, where the sample stream has been labeled with an adsorbing dye. Because the sample is not moved laterally when it is surrounded by the sheath fluid, it does not appear to move in the micrographs. There is a slight widening of the stream, consistent with Fig. 6. There is also a slight decrease in color intensity, which is also consistent with the shorter path length after the sample stream has been compressed vertically.

While some traditional methods of generating sheath flow are easily clogged due to very small flow openings, the groove-based designs presented here generate very small-diameter core streams without reducing the channel size and thus mitigate clogging. Clearly, the stripe configuration for producing sheath flow has an advantage in that it requires only one inlet for sheath flow, greatly reducing the number of pumps compared to other microfluidic systems that completely ensheath a core fluid.^{26,28–32} Once the number of stripes is established for a given channel aspect ratio, the sheathing will remain essentially in the center of the channel unless the flow rate is significantly changed. Although the chevron design requires one more pump than the stripe design, it does offer a degree of flexibility. By adjusting the sheath flow rates, the core lateral position and width can be changed to suit current needs.

We showed that these microfluidic groove-based sheath flow designs can be manufactured using common micromachining methods. Either soft lithography or laser ablation systems will suffice for creating the sheath flow channels. Although the macrofluidic flow channels emulating the behavior of microfluidic channels were manufactured on a standard CNC milling machine for the purpose of visualizing the results and verifying the TT predictions, these channels could be used in applications requiring sheath flow of viscous fluids.

Conclusions

We have demonstrated a pair of microfluidic cytometer designs that create complete sheath flow on a microfluidic chip. In the stripe-based design, the sheath and sample streams are placed side-by-side within the channel by a T-intersection. The grooves then wrap the single sheath stream around the sample stream. The chevron-based design requires a cross-intersection to place the sheath on either side of the sample in a manner similar to many previous microfluidic cytometers. Unlike the previous systems, however, the chevron grooves then isolate the sample from the top and bottom surfaces.

Both designs have inherent strengths and weaknesses. In the stripe-based cytometer, the number of stripes determines the position of the sample stream. Once the chip has been manufactured, this cannot be changed and the position is very stable. For many applications, this stability is a strength, but the lack of flexibility makes it unsuitable if the experimenter

wishes to be able to adjust the position or width of the sample stream. In those cases, the chevron-based design can provide the needed flexibility, at the expense of adding a second sheath inlet that must be controlled. With the current design, minor deformations occur when the sample stream is moved away from the center, but these can most likely be eliminated with some minor adjustments of the shape of the chevron.

We have demonstrated that these sheath flow designs can be easily manufactured *via* a variety of fabrication paths. Any researcher capable of making two-level microfluidic components has the ability to make these designs. It should also be noted that although the aspect ratio of all the channels presented here was approximately 1 : 3; this was a design convenience resulting from the features used to populate the TT. Variations of these designs will be equally effective at other aspect ratios.

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Fig. 1. (A) Isometric schematic of the stripe-based sheath flow design. (B) Computational model of the sample solution (blue) being sheathed in the sheath solution (red) using Tiny-3D.^{32,34}

Fig. 2. Channel cross-sections resulting from a 50 : 50 flow ratio and varying numbers of stripes: (A) 1 stripe through (E) 5 stripes. Models developed using TT are on the left, while experimental cross-sections are shown on the right.

Fig. 3.
 (A) Size and shape of the sample stream as a function of flow ratio for a design with 6 stripes.
 (B) Cross-sections form a sheath flow chip with 4 pairs of stripes and a flow ratio of 3 : 1 (sample : sheath). (C) The same chip with a flow ratio of 1 : 1000.

Fig. 4. Demonstration of unsheathing: (A) channel cross-section before sheathing; (B) channel cross-section after sheathing by 4 stripe pairs; (C) channel cross-section after unsheathing by a reversed set of 4 stripe pairs.

Fig. 5. Optical images of stripe-based sheath flow devices made *via* soft lithography (A) and laser ablation (B). The sample stream has been labeled with a rhodamine dye. In (A) it is seen by its absorbance, in (B) it is seen by its fluorescence. The scale bar represents 200 μm for both images.

Fig. 6.

(A) Isometric schematic of the chevron-based sheath flow design. The arrows indicate introduction of the respective sheath and sample solutions. (B) Shape of the sample stream modeled as a function of the number of chevron pairs. The outlines depict the shape of the sample stream resulting from flow ratios of 4.5 : 1 : 4.5 (top) and 9.5 : 1 : 9.5 (bottom) with 1–6 chevron pairs.

Fig. 7. Size and shape of the sample as a function of the flow ratio for a design with 4 pairs of chevrons: (A) predicted cross-sections; (B) experimental cross-sections.

Fig. 8.
Stream height as a function of the number of chevron pairs.

Fig. 9.
Shape and position of the sample stream as it is moved from the center of the channel.

Fig. 10.
Image of a dye-labeled stream passing through a chevron-based sheath design.