A mixer, based on the Dean vortex, is fabricated and tested in an on-chip format. When fluid is directed around a curve under pressure driven flow, the high velocity streams in the center of the channel experience a greater centripetal force and so are deflected outward. This creates a pair of counter-rotating vortices moving fluid toward the inner wall at the top and bottom of the channel and toward the outer wall in the center. For the geometries studied, the vortices were first seen at Reynolds numbers between 1 and 10 and became stronger as the flow velocity is increased. Vortex formation was monitored in channels with depth/width ratios of 0.5, 1.0, and 2.0. The lowest aspect ratio strongly suppressed vortex formation. Increasing the aspect ratio above 1 appeared to provide improved mixing. This design has the advantages of easy fabrication and low surface area.

Introduction

Mixing is one of the critical issues for the development of microfluidic systems. In order to perform most chemical reactions on a chip, the reagents must be combined and mixed thoroughly. This includes fluorescent tagging, protein digestion, and de novo synthesis. Unfortunately, the movement of solutions in microfluidic systems is most often characterized by laminar flow. When two solvent streams are combined in one channel, they tend to travel in parallel paths. The only mixing that occurs is diffusional transport across the interface of the two solvent streams. As an example, when two streams of water are combined in a 100 μm wide channel, it will take roughly 50 s for the solutes in one stream to diffuse across the channel. At a typical flow rate of 1 mm s⁻¹, a channel length of 5 cm will be necessary for complete mixing. Both the time required and the length of the channel are not practical for many real world applications.

Numerous approaches have been used to increase the rate of mixing in microfluidic systems. These can be divided into two broad categories: active and passive. Active systems require the application of an external force or field. These may include the incorporation of piezoelectric elements, pulse flow mixers, thermal bubble mixers, acoustic mixers, and micromachined magnetic stirrers. Such systems can be an effective means of mixing, but they are complicated and expensive to fabricate, and are more prone to failure than passive mixers.

In passive mixing, stationary structures aid in the mixing of the fluid passing over or through them. The movement of the fluid provides the only energy put into the system. At present passive mixers show the ruggedness and ease of fabrication necessary for use in real world applications. There are two mechanisms by which passive mixing is implemented. The most common approach is to subdivide the flow of the inlet streams and create multiple, parallel mixing points. The time necessary for two streams to mix through lateral diffusion scales with the square of their widths. By dividing the flow and creating multiple, interlaced streams, the rate of diffusional mixing can be greatly increased.

Besson et al. did this by creating a series of bifurcations within the channel. The two inlet streams each passed through a series of 4 bifurcations to create 16 separate flow streams, which were then combined in 16 separate and parallel mixing chambers. A similar series of bifurcations, run in reverse, recombined the streams into a single channel with 32 individual flow streams, 16 of each liquid.

Other designs with different morphologies have been presented, but all work on similar principles. While this technique is effective, with each bifurcation, the resistance to flow through the channel is increased. The higher pressures require more expensive pumps and more rugged and expensive chips. An added filtration step will also most likely be needed when using real world samples to prevent clogging as the channels become narrower.

A second approach is to perturb the laminar flow after the two solutions have been introduced into the same channel. Any perturbation of the flow that creates lateral movement will stretch the interface and increase the rate of mixing. Stroock et al. have demonstrated one such system in which the bottom of a channel was patterned with a series of diagonal ridges, which deflected the flow immediately over them. A herringbone pattern molded into the floor of the channel created two opposing recirculations in the channel perpendicular to the direction of flow. This deformed and stretched the interface into a spiral. Several sets of herringbones, offset from one another, further subdivided and deformed the interface and greatly facilitated mixing. The depth of the troughs was typically one fifth to one quarter the height of the channel. The effect on the resistance to flow was minimal when compared to systems that subdivide flow, and there were no constrictions which could be prone to clogging. Concurrently with Stroock, Johnson and Locascio developed a topologically equivalent mixing system based on a series of troughs ablated into the bottom of the channels with a KrF excimer laser. The troughs were bigger than the ridges used by Stroock and had a correspondingly greater influence on the flow.12 Both these systems show promise as mixers, but have some drawbacks. The manufacture of small features within the channels increases the complexity of the manufacturing process over one in which the channel is the unit feature. Also, the ridges or troughs greatly increase the surface area of the system and increase the likelihood of fouling. The relatively narrow features may also be difficult to wet under some circumstances, trapping air bubbles.

We present a system that can generate lateral vortices similar to those seen by Stroock, but without the need for ridges or
troughs. The phenomenon on which these chips are based was first explored by Dean for pressure-driven flows in curved circular tubes and more recently has been studied for curved channels of rectangular cross-section, such as the one in Fig. 1A. In these geometries, curvature amplifies a lateral instability that drives a secondary cross-channel flow. The Dean number, defined as

$$D_n = \frac{G w^3}{\mu v^2 R} \frac{2w^{1/2}}{R}$$

(1)

characterizes this secondary flow, where G is the centerline pressure gradient driving the primary flow, R is the channel radius of curvature, w is the channel width, \(\mu\) is the fluid dynamic viscosity, and v is the kinematic viscosity (Fig. 1A). In a straight channel \((D_n = 0)\), pressure-driven flow is unidirectional and develops a parabolic profile with the fluid moving down the center of the channel traveling faster than the fluid traveling along the walls or along the top and bottom. When the flow is directed through a curved channel \((D_n > 0)\), the fluid moving down the center experiences a higher centrifugal force than the surrounding liquid (Fig. 1B). As a result, a pair of counter-rotating vortices forms that ejects fluid from this high-speed core toward the outer wall. At higher values of \(D_n\) and depending on the aspect ratio of the channel cross-section, additional vortices and time-dependent oscillations can form.\(^{13-20}\) We exploit these secondary flows to mix two segregated inflow streams.

**Experimental**

**Fabrication of the low-aspect-ratio chips**

The channels were machined in a PMMA sheet (Lucite CP, ICI Acrylics Inc, Cordova TN) with a Techno-isel CNC router (Techno, Inc., New Hyde Park, NY). Inlet and outlet holes were drilled by the CNC router using a 1/16th inch circuit board bit. The channels were then machined using an appropriately sized end mill. The width of the channel was set by the diameter of the bit. The chip was then cut free of the material using a 1/8th inch end mill. An aqueous cooling liquid (Formula 7, Kool Mist, Santa Fe Springs, CA) was sprayed over the piece throughout the machining process. Fig. 2 shows the channel layout of a typical chip. Four sets of chips were made with radii of 5 mm, 6 mm, 7 mm, and 8 mm. Once the channels had been machined, the chip was cleaned of any particles or debris with a stream of ethanol.

Double-sided tape (ARcare 8890, Adhesives Research, Inc., Glen Rock, PA) was used to fix the chips to glass slides. A portion of tape was cut to roughly match the size of the chip. The backing material was then removed, from one side of the tape, and the tape applied to the glass slide. Another glass slide was then placed over the tape, and the stack was clamped together for one hour to ensure a strong, bubble free, and uniform bond with the glass. The second layer of backing material was then removed and the chip was brought into contact with the tape. The chip was again clamped for one hour to aid bonding. Short lengths of 17 gauge stainless steel tubing were then glued into the inlet and outlet holes using 5 min epoxy (Devcon, Danvers, MA).

**Fabrication of the high-aspect-ratio chips**

Using the end mills to cut the channels directly limits the maximum aspect ratio of the channels to about two. To make a chip with a higher aspect ratio, it was necessary to make the two walls separately. Fig. 3 shows a typical design of a high aspect ratio chip. The inner piece is designed to fit into the outer piece. The interlocking sections make a snug fit and fuse together during the annealing process. The pieces were machined from PMMA using a 0.0500 inch end mill, with all cuts extending completely through the material. The outer
channel was milled into the outer piece, while inner inlet channel and arc were formed by the assembly of the two pieces. The width of the channel at the arc could be controlled by adjusting the radii of the inner and outer pieces.

Once assembled, the two pieces were sandwiched between the top and bottom pieces. The bottom piece contained through-holes for the registry pins. The top piece had these holes as well as holes used for the two inlets and the one outlet. All holes were milled out using the 0.050 inch bit to accommodate 17 gauge tubing snugly. Short lengths of the tubing were inserted into the registry holes to ensure proper alignment, and the stack was clamped between two glass slides using spring clamps. The stack was then immersed in boiling, deionized water for 90 min. The chips were then removed from the water and allowed to cool. The annealing process fused the two central chips to the top and bottom pieces as well as to each other in the interlocking section. The depth of the channels made in this way were fixed by the thickness of the PMMA but the width could be varied from 1 mm down to about 250 μm.

It was desirable to be able to view the channel from the side. Unfortunately, the milling process leaves a rough surface that appears frosted. A solution of PMMA in toluene was prepared and applied to the chip. This created an optically smooth surface, allowing a clear view through the side of the chip.

### Measurement of dye profiles within the channel

The chips were mounted under a dissecting microscope (Edmund Scientific, Barrington, NJ) and illuminated with a Fiber-Lite High Intensity Illuminator (Dolan-Jenner, St. Lawrence, MA). The microscope was equipped with an Hitachi KP-D50 color digital camera. Images were captured on a PC using a frame grabber (Cyberoptics Semiconductor, Beaverton, OR).

Blue food coloring was used as a stream marker. Dyed water was pumped into one inlet, while pure water was pumped in the other. In all cases, the flow rates of the two inlets were equal. Flow was provided by a peristaltic pump (Ismatec Inc., Glattbrugg, Switzerland), using a home-built pulse dampener. The dyed stream was introduced on the inside of the curve. In these cases, the magnification was reduced to allow the entire arc to be observed in a single image. This process allows the dye profile to be observed at any angle along the curve. For experiments observing the effect of flow velocity on the profile, measurements were taken at a single point (typically 260° from the start of the turn). For these experiments, the magnification of the optics was increased in order to focus on the region of interest. In other experiments, the variation in the dye profile was observed over the length of the channel. In these cases, the magnification was reduced to allow the entire arc to be observed in a single image. This resulted in a corresponding reduction in the spatial resolution of the images, which translated into a reduction of the signal-to-noise ratio of the intensity measurements.

The centroid of the dye was found by the following equation

\[
R_c = \frac{\sum r A(r)}{\sum A(r)}
\]

where \(R_c\) is the radial position of the centroid in pixels, \(A(r)\) is the average absorbance of the response at radial position \(r\). The position is reported in reduced form, so that the inner wall corresponds to 0 and the outer wall corresponds to 1.

### Results and discussion

Fig. 4 shows a typical image of the outlet end of the channel at various flow velocities. The channel has a square 1.27 mm cross-section. The radius of curvature is 5 mm. At low velocities \((Re = 3)\), the flow lines follow the contours of the channel. The dye, which was introduced to the inner half of the channel, only appears to move into the outer half by the process of diffusion. Higher flow velocities allow less time for diffusion to take place, so that one would expect that the amount of dye found in the outer half of the channel would decrease at higher velocities. Instead, the opposite is seen. At \(Re = 10\), the channel appears to be nearly filled with the blue dye. In reality, the clear and blue streams have become vertically stacked with the dyed solution in the middle and the clear on the top and bottom of the channel. This can be seen when the channel is viewed from the side (see below). At still higher velocities \((Re = 16)\), the dyed stream appears to reform.
at the outside of the channel. The longitudinal variation of the radial distribution of the dye is also evident. By $Re = 30$, multiple stripes become visible. This is most likely due to the formation of whorls as the two streams become folded into one another (Fig. 1C). These visual results parallel what is seen when the absorbance profile is plotted (Fig. 5).

The onset and topology (i.e., the number and arrangement of vortices) of the secondary flow has been studied computationally assuming that $w/R$, the ratio of the channel width to the radius of curvature, is small, typically limited to $w/R < 0.1$. This assumption eliminates all but the first order effects of the curvature, and the steady-state velocity field for the channel cross-section can be obtained by solving a simplified two-dimensional problem. The channels in the current study violate this assumption, with $w/R = 0.25$ for the smallest radius tested. Furthermore, our focus is on mixing, so knowledge of the onset and topology of the secondary flow is not sufficient. We must determine the strength of the secondary flow as indicated by the transport of fluid from one side of the channel to the other. The simplest metric for this was to determine the location of the centroid of the dye within the channel. Fig. 6 shows the location of the centroid as a function of Reynolds number for several channels of varying radius and depth.

For the square channels, the dye begins to move outward almost immediately in all the chips. Only at the lowest $Re$ does diffusion become important as a form of lateral mass transfer. All four plots appear to peak at a Reynolds number of approximately 20. This corresponds to the point where the bulk of the dye has been transferred to the outer half of the channel.
Higher flow rates cause the dye stream to travel from the inner half to the outer half and then begin to return to the inner half by the time it reaches the detection region at 260°.

It is interesting to note that the position of the maximum of the four plots does not significantly change, even though w/R changes by 60%. This implies that the magnitude of the secondary flow is inversely proportional to the radius. When the radius is increased by some factor q, the secondary flow velocity decreases by 1/q, but the distance the flow stream must travel to reach the measurement point (at a fixed number of degrees from the start) has increased by the same factor q. This linear dependence implies that no threshold for rolling exists, contrary to published theoretical models.14 It is likely that the difference lies in the assumptions made in the models. In particular, high aspect ratio channels were modeled by imposing a periodic boundary condition, thus neglecting the effects of the top and bottom of the channel. It is possible that the models will prove more accurate for channels with extremely high aspect ratios.

Effect of aspect ratio

A set of channels was made having a constant width of 1.27 mm, but varying depth. The low aspect ratio channels had a depth of 0.64 mm, while the high aspect ratio channels were 2.54 mm deep. This provided channels with aspect ratios of 0.5, 1.0, and 2.0. Fig. 6 shows the plots of the centroid position for the channels as a function of Reynolds number. The profiles were taken 260° into the curve. The maximum corresponds to the value of Re necessary to transfer the bulk of the dye solution to the outer half of the channel. As can be seen, this occurs at a higher flow rate for the shallow channels, while two deeper channels appear to roll at roughly the same rate.

The aspect ratio 1.0 and 2.0 channels appear to behave similarly in terms of the Re necessary to establish rolling, but the deeper channels appear to be more effective mixers. This can be seen when the profiles are observed (Fig. 5). For the square channel, the bulk of the dye shifts from the inside to the outside, then returns to the inside as the flow is increased. In the deeper channel, on the other hand, the bulk of the dye shifts to the outside, but then appears to split into two separate streams. This is most likely due to the formation of a whirl along the axis of the channel, as depicted schematically in Fig. 1C. Such a whirl will have a higher interfacial area and more rapid mixing. This implies that a deeper channel may be preferable for a mixer.

The deeper channel has a lower velocity for a given Reynolds number. As a result, it is not surprising that at the lowest flow rates, diffusion becomes an important source of mass transfer. It should be noted that the movement of the dye centroid is only useful in identifying the onset of rolling as dye is initially transferred outward from the inner half of the channel. The complex profiles that develop at higher velocities (Fig. 4) indicate that the dye is not merely moving back and forth, but is most likely developing whorls parallel to the axis of the channel.

Angular measurements

Using the aforementioned method, it was possible to establish the dye profile at several positions along the turn. Fig. 7 plots the reduced position of the centroid as a function of angular distance from the start of the curve and under varying Reynolds numbers. The 5 mm radius chip with an aspect ratio of 2 was used for this experiment. At lower velocities, the rolling is almost nonexistent, but quickly becomes evident as the velocity is increased. In general, two effects have been observed when the Reynolds number is increased. Firstly, a shorter distance is required for the centroid to reach its maximum value. Secondly, the magnitude of the maximum value of the centroid decreases. The easiest explanation for both these phenomena is that increasing the flow rate leads to increased heterogeneity in the lateral flow field. At moderate flow rates, the bulk of the dye stream moves to the outer half of the channel before it then begins to move inward again. At higher flow rates, portions of the dye stream reach their outermost extent and begin to travel inward before other portions have reached their respective maxima.

This can most clearly be seen in Fig. 8, where the profiles at several points along the channel are plotted for a flow with Re = 60. The bulk of the dye can be seen to be moving outward at 90° and 120°, but its outer edge has not yet reached the wall. At 150°, the outer edge continues to move outward, but some portion of the dye is now moving inward, causing the band to spread. By 210°, the dye distribution has become bimodal as the two substreams have moved apart. Near the exit, at 263°, three bands can be seen, as represented by three local maxima in the plot.

This behavior would seem to imply that the shape, and not just the magnitude of the secondary flow profile is strongly affected by the Reynolds number. To test this, three profiles were plotted, in which the product of the angle of travel and the Reynolds number were held constant (Fig. 9). The profiles are very different, proving that the lateral flow field is a nonlinear function of Re.
Side view

It is clear from the images that the flow streams are stratifying vertically, but the top view alone is insufficient to show the nature of that stratification. Previous computational studies hypothesized that at higher aspect ratios, additional vortex pairs are formed, but substantial elongation of the initial vortex pair is seen before any additional bifurcation occurs. This result is in contrast to Taylor vortices that form with roughly unit aspect ratio between rotating concentric cylinders or Dean vortices in curved channels of infinite extent in the z direction. It is unknown what aspect ratio would be required to create additional vortex pairs for the channel width, curvatures, and flow rates in the current study, and whether the additional vortex pairs would affect significantly the amount of mixing produced by the secondary flow.

The fabrication and testing of channels with aspect ratios greater than three provided insight into the formation of additional vortex pairs within the parameter range of the current study. Unfortunately, the direct excavation of such a channel could not be realized due to the aspect ratios of the end mills available. Instead, channels were manufactured by bringing two machined pieces of PMMA into close proximity to form the opposing walls of the channel (Fig. 3). The depth of the channel was set by the thickness of the PMMA stock. The width could be controlled to within 10 μm by the placement of registry pins. This method was used to manufacture a channel 5.3 mm high and 500 μm wide. The radius of the turn was 4 mm. Fig. 10 shows a view of the channel though the side of the chip, with dye introduced along the inner half of the channel. At Re < 1 (Fig. 10, top), the channel appears uniformly blue. At higher flow rates, the movement of the outer clear solution inward along the top and bottom edges can be seen (bottom). This is consistent with the presence of only two rotation zones, so under the current values of curvature, channel width, and flow rate a single pair of vortices is produced even though the aspect ratio of the cross-section exceeds 10. Had additional vortex pairs been present, more clear stripes would have appeared wherever the outer solution was being moved inward. It is interesting to note that evidence of rolling is seen at quite low flow rates when viewed from the side. This can be attributed to the location of the center of rotation of the vortex (seen as the interface between dyed and clear solution), which is roughly 500 μm from the top and bottom of the channel. The dyed solution moves outward in the region between the two centers, while the clear solution is moving inward above and below them. This means that the inward flowing streams must transfer an equal volume of fluid through a much smaller area, and consequently travel much faster.

Conclusions

We have shown that it is possible to generate lateral mixing in fluidics systems by simply directing flow around a bend. The nature of the mixing is very similar to that generated by Stroock’s herringbone ridges, but does not require an increase in the surface area of the channel, which increases the likelihood of fouling or trapped bubbles.

One issue that needs to be addressed is the degree to which this technique can be miniaturized. Achieving the Reynolds numbers necessary to generate the vortices will become increasingly difficult as the channels are made smaller due to the pressures necessary to achieve the appropriate flow rates. It may be possible, however, to increase the driving force through changes in channel geometry. It has already been shown that decreasing the radius of curvature reduces the distance necessary to generate rolling. While increasing the aspect ratio slightly decreased the degree of curvature reduces the distance necessary to generate rolling, it appears that mixing may have been enhanced. Further research is needed to establish the limits of this system.

The Reynolds numbers used here are easily attainable in larger microfluidic channels. This not only means that this technique can be used with relatively little modification of current techniques, but that the phenomena discussed here must be considered when designing microfluidic systems. This will become particularly important as attempts are made to increase the throughput. A chip designed for purely viscous flow may become particularly important as attempts are made to increase the pressures necessary to achieve the appropriate flow rates. It may be possible, however, to increase the driving force through changes in channel geometry. It has already been shown that decreasing the radius of curvature reduces the distance necessary to generate rolling. While increasing the aspect ratio slightly decreased the degree of rolling, it appears that mixing may have been enhanced. Further research is needed to establish the limits of this system.

Acknowledgements

The authors thank Stephanie Fertig, Carolyn Kaplan, and Elaine Oran for their indispensable advice on the theory, design...
and implementation of the experiments presented in this paper. The work was supported by NRL PE62236.

References

12 T. J. Johnson and L. E. Locascio, Lab Chip, 2002, 2, 135–140. This paper demonstrated that grooves as well as ridges will work, and that these systems also function under electroosmotic flow.