MICROFLUIDICS AND MICROFABRICATION
in a Chemical Engineering Lab

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Microfluidic systems are widely used in biomedical, chemical, and biological applications. These include molecular separation and sensors, DNA and protein patterning, analysis and sorting of cells, and high throughput screening.[1-4] Microfluidic technology promises to: reduce sample volume and waste; increase speed of analysis; achieve high performance, integration, and versatility; and make miniaturization, automation, modularization, and parallelization easier. One of the great strengths of microfluidics is the ability to integrate separate processes in one chip; this offers new capabilities to control molecules in time and space.

The control of fluid in microdevices is important and presents special challenges. The effects that become dominant in the microscale are laminar flow, diffusion, surface tension, and fluidic resistance.[2] Micromixing is an important process on a microscale. Fluid behavior in a microfluidic device is much different than flow in a macroscopic device. In microfluidic devices of channels less than 1mm, fluids flow in a laminar fashion. In laminar flow, two moving streams in contact with each other do not mix except by diffusion or using non-passive mechanisms such as acoustics or electrokinetics.[5,6]

The selection of material and fabrication methods used in microfluidic application depends on the final use. Traditional silicon and glass micromachining have been the choice of the microelectronics industry, and are well suited for microelectromechanical systems (MEMS). The intrinsic stiffness of these materials poses a challenge to biological applications, however, as well as the fabrication of microfluidic valves and pumps. Soft lithography shows great promise in versatility for microfluidic applications. Soft lithography refers to nano- and microfabrication with elastomeric materials. Soft elastomeric polymers such as poly(dimethylsiloxane) (PDMS) are optically transparent and allow micro features to be replicated with high fidelity. Their fabrication processing is simple (cures at low temperatures, seals easily, and releases from delicate features of a mold). In addition PDMS is non-toxic to cells and can undergo surface chemistry changes if needed.[7] Because of its relative simplicity, it is an ideal model system to introduce undergraduate students to microfabrication. Jablonski, et al.,[8] demonstrated simple device fabrication in PDMS in an undergraduate lab to study the break-up of air bubbles in aqueous flow. Students studied bubbles in channels as a model for intravascular embolism. Students had the opportunity to

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fabricate microdevices in PDMS much like as described in this micromixer paper; however, the design problem, area of fluid mechanics, and analysis are different. This paper presents an approach where each group created its own unique design to mix two fluids and subsequently assessed the effectiveness of the design. PDMS devices are now not only in research but in undergraduate education; they can be used to study a variety of topics in fluid mechanics at the microscale with applications in heat transfer, separations, and biochemical and biomedical analysis.

We have developed a lab to train students in a new technology that allows the manipulation of small volumes and exploits phenomena at the microscale level. Students in the class CHEME 4010 – Molecular Principles of Biomedical Engineering were asked to design, fabricate, and test a microfluidic microdevice made out of PDMS to mix a dye and water. They assessed the degree of mixing at different flow rates and provided recommendations for an improved micromixer design.[9]

This experiment was conducted over three weeks. In Week 1, students in groups of three to four designed the micromixer using AutoCAD.® This was given as an assignment after a 1-hour AutoCAD® tutorial. In Week 2, students fabricated the micromixer using soft lithography (~ 2 hours) and in Week 3 students tested the micromixer with a dye solution and water using a microscope, computer, and image analysis software (~ 2 hours).

LABORATORY DESCRIPTION

Theory

Due to laminar flow in the microfluidic device, passive mixing relies solely on molecular interdiffusion. The diffusive flux (j) of a solute equals $D.\frac{\partial c}{\partial x}$, *i.e.*, diffusion coefficient (D) times the gradient of species concentration $\frac{\partial c}{\partial x}$. In order to characterize convective/diffusive flow the following dimensionless numbers are commonly used—the Reynolds number (Re = Ud/v), the Peclét number (Pe=Ud/D), and the Fourier number ($Fo=T_r/T_m$). Here U, d, and v denote the average velocity, the diameter or the transverse diffusion distance, and the kinematic viscosity, respectively. T_r and T_m denote the average residence time and the diffusive mixing time, respectively, defined as $T_r=L/U$ and $T_m=d^2/D$, where L denotes the longitudinal length. By equating T_r and T_m and knowing the diffusion coefficient (D) of dye in water, students can design a micromixer with appropriate length and width to mix dye and water.

Materials and Methods

Design

Students used the design template in Figure 1 with constraints to design a micromixer using AutoCAD® (AutoDesk) with two inlets and one outlet that can mix a dye solution with water at a maximum flowrate of 20 μl/min. Food dye was obtained from the local grocery store and had a diffusion coefficient of ~2000 $\mu m^2/s$ (based on similarly sized molecules). The constraints were that the micromixer had to be passive (have no moving parts and rely on shapes and patterns to alter flow) with the width and spacing between channels to be no less than 150 μm. Students also had to submit four copies of their AutoCAD® design arranged so that it fit squarely in the center of a 4-inch silicon wafer and corners were limited to 45 or 90 degrees. The constraints on channel width and spacing and corners were given to ensure high success in soft lithography microfabrication by novice students. Using the maximum flowrate of 20 μl/min, and the diffusive mixing and residence time equations above, students calculate what the minimum theoretical length for mixing should be. This minimum theoretical length guides students in their final design. They also make a plot of theoretical length at which mixing occurs vs. flowrate and compare with experimental results.

The AutoCAD design was made into a photolithography mask by converting it to a pdf file and sending the file to PageWorks, Inc., Cambridge, Mass. The resulting photolithography mask was on emulsion-based transparency paper.

Master and Device Fabrication

The master fabrication occurred in a Class 10,000 clean room. The transparency mask was cut to fit the dimensions (5 in. × 5 in.) of the contact aligner mask holder (HTG, System 3HR). In a fume hood, a 4-inch silicon wafer (Type P, Wafer Works Corp.) was covered with SU-8(50) (MicroChem, Inc.), a negative tone, photosensitive epoxy resist using a spin coater (Speciality Coating Systems Inc., P6700) at a Spread Cycle: 500rpm at 100rpm/sec for 5s and Spin

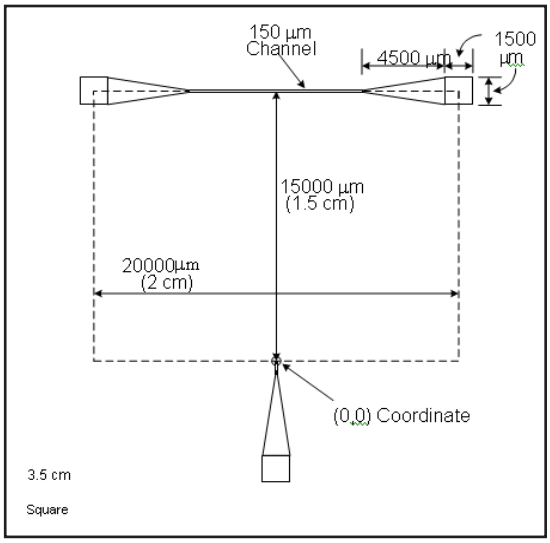

Figure 1. *Design area for the micromixer.*

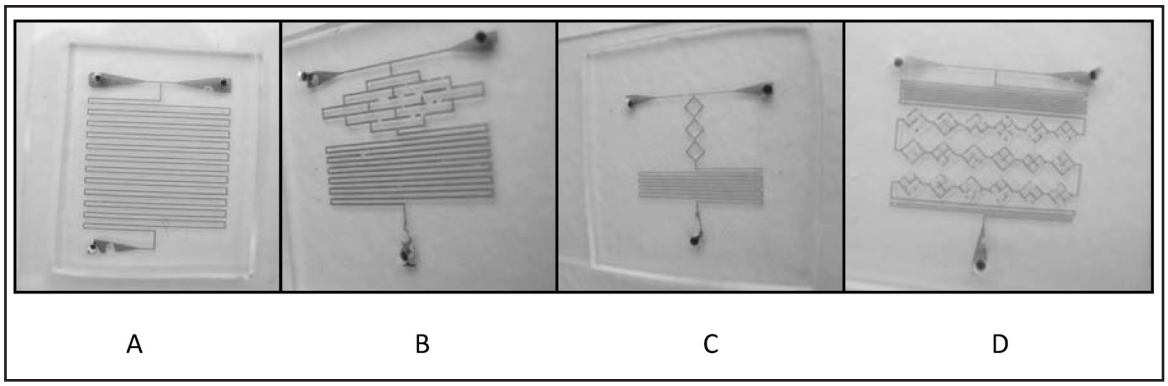

Figure 2. *Examples of four microdevices: A – straight channels, B – bricklike structure, C – diamonds, D – series of diamonds.*

Cycle: 1200rpm at 300rpm/sec for 30 s to produce a SU-8(50) film of 100 μm. The wafer was then baked on a hot plate at 65 ˚C for 10 minutes. After 10 minutes the temperature was increased to 95 ˚C and held at this temperature for 30 minutes.

The baked SU-8(50) wafer was exposed to UV light (405 nm, 65 secs) through the mask containing the student group's design using a contact aligner (HTG, System 3HR). The unexposed resist was removed with SU-8(50) developer (MicroChem, Inc.) to produce 100 μm tall design structures on the wafer. The silicon wafer with SU-8(50) structures (Master) was then used as a mold to form the student's design in PDMS. PDMS (Sylgard 184 kit, DowCorning) was mixed for 8 minutes in a plastic cup with a plastic fork at a 10:1 ratio of base to hardener. The aerated mixture was degassed in the cup using a vacuum dessicator by pressuring and de-pressuring the chamber for approximately 10 minutes until all the bubbles were removed. Once fully degassed it was poured over the SU-8 master taped in 150 × 15mm Petri dish and baked in a 60 ˚C oven overnight.

The device was made by cutting a rectangular piece of PDMS around the design and punching holes for the inlet and outlets using a 16G flat needle (Becton Dickson). The PDMS device was sealed to a glass slide using a plasma sterilizer (Harrick Plasma) after plasma exposure for 1 minute.

Testing

After sealing the microdevice, tubing (0.02in ID, 0.02in wall) was inserted in the inlet and outlet holes and the inlet tubing was connected to 5 ml syringes filled with water and food dye, respectively. Water and dye were run through the microdevice using a syringe pump (Harvard Instruments, PHD2000). Each group's device was tested at three flowrates pre-determined by the group (range 5-20 μl/min). The objective was to mix the dye and water. Mixing was visualized using a microscope (Nikon SMZ1000), CCD camera (Sony DXC-390), and video capture software (StreamPix, Norpix). Images of specific features of the microdevice were taken at various flowrates and were analyzed for the degree of mixing using ImageJ (public domain Java based program from NIH).

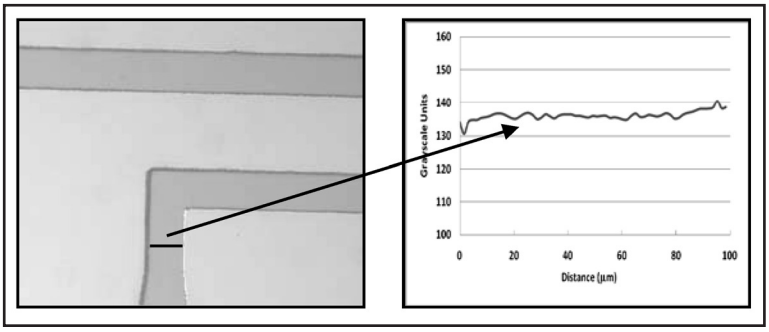

Figure 3. *Successful mixer showing a fully mixed exit stream.*

Given a gray-scale image, ImageJ can calculate the average gray value along a line or of an area as well as generate histograms and surface plots of gray values. Using these functions, the amount of mixing on the chip can be accurately judged. A well-mixed region would contain a uniform amount of gray value. The amount of gray value was measured across each channel and converted to a data table by ImageJ and then graphed and the slope attained linearly using Excel™ . The plots represent the average color gradient of the original images. Less mixing was represented by a steeper slope in the mixing regions and a relatively flat slope represented better mixing by diffusion; complete mixing was represented by a uniformly zero slope.

$$\text{Slope of Mixing Profile} = \left| \frac{\Delta(\text{GrayValue})}{\Delta(\text{Distance})} \right| \qquad (1)$$

TYPICAL RESULTS

Students proposed and tested a variety of designs from a series of straight channels to a combination of channels with mixing features such as diamonds, bricklike structures, or straight channels. Some examples are shown in Figure 2. Most students were able to mix the dye and water with constraints given. Figure 3 shows a successful micro-mixer. The dye is completely mixed at a flowrate of 20 μl/min. The device consisted of a series of straight channels where the length was long enough to allow the diffusion time to be less than or equal to the residence time. Some features that were

not as successful were the split and recombine technique using brick and diamond structures as shown in Figures 4 and 5. The objective of the splitting was to break up the flow of each individual stream into two streams, effectively halving the velocity and increasing residence time, and to decrease the diffusive length d to a number less than the channel diameter. Dividing the two fluids into progressively more layers by splitting and recombining the flow can decrease the thickness of the fluid layers. As shown by Figures 4 and 5, however, it can also separate the two streams, thus preventing contact and hence diffusion.

DISCUSSION

All groups came up with thoughtful ideas and designs for mixing dye and water. They tested various flowrates and determined that the slower flowrates more effectively mixed the dye and water, which for some students was counterintuitive to their way of thinking since they are more familiar with convective mixing. Slower flowrates increased the residence time and allowed enough time for diffusion. Generally, to design the appropriate overall length (L) of channels within the microdevice, the residence time T_r was equated with the mixing time T_m for various flowrates (U).

$$T_r = \frac{L}{U}, T_m = \frac{d^2}{D} \qquad (2)$$

As part of their lab report students were asked to propose other methods of mixing that did not rely on diffusion alone. Some examples were to include baffles that cause local turbulence[10] as well as to induce counter-current circular flows with rotation axes aligned with the axis of the channel. Strook, et al.,[11] uses herringbone-shaped grooves or chevrons on the floor of the channel. Thin mixed fluid layers form by the chaotic swirling when the two streams pass over the chevrons. Another method that does not rely solely on molecular diffusion for mixing solutions at this scale is bubble-induced acoustic actuation. Air bubbles in a liquid medium can act as an actuator and vibrate when a sound wave is applied. As the bubble vibrates with the applied acoustic field a cavitation effect is caused, or a bulk fluid flow around the air bubble. This bulk fluid flow greatly increases convectional mixing and drastically reduces the length of mixing.[12]

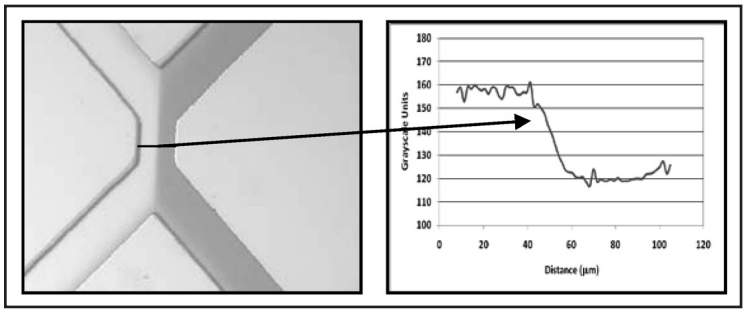

Figure 4. *Diamond structure causes streams to split and not mix.*

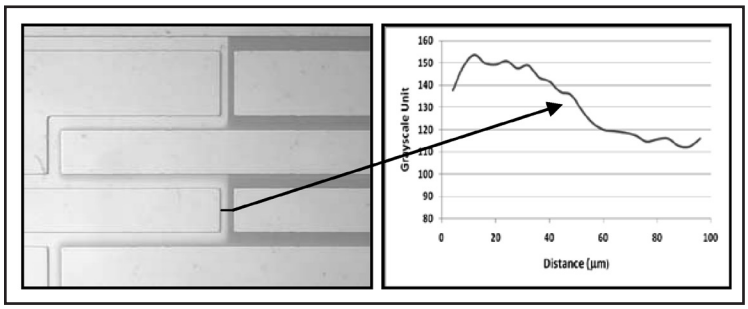

Figure 5. *Bricklike structure can cause inefficient mixing.*

SUMMARY OF EXPERIENCES

This experiment allowed students to get first-hand experience with design, microfabrication, and soft lithography. They were given a design goal and using basic fluid mechanics and their knowledge of diffusion, came up with a design that they tested. This gave students ownership of their work and many students took their devices home. Students were required to write a lab report in the format of a scientific paper with a section for Recommendations for Improvement. This section allowed students to analyze their design ideas after having tested them and many came up with new ideas based on their results as well as other groups results. For example, the idea of splitting and offsetting proposed by some groups did not work as well as conceived and resulted in separate fluid streams as well as allowing air bubbles to become entrapped that were hard to dislodge. Many students refined their ideas and proposed straight channels or angled splitting and recombining channels to ensure both streams were split, not separated.

A survey of this lab was given for three years, assessing level of interest, difficulty in performing the lab, and improved knowledge of microfluidics. The results of this survey are summarized in Figure 6. Results show 90% of the students found the lab extremely interesting. Most students found that the lab had a medium amount of difficulty and 90% felt after taking this lab that they knew significantly more about microfluidics and could design a micromixer to mix an enzyme and substrate (a relevant biochemical micromixing scenario). Some comments were "lab was unique and interesting," "my favorite lab," "enjoyed designing and analyzing microchip from beginning to end," and "learned CAD, image processing, and photolithography!".

While the lab described does use some fairly expensive equipment (contact aligner) and a clean room, a very similar lab with high school students and less-strict mixing criteria (wider channels, slower flowrates) was run for a summer outreach program. In addition, Jablonksi, et al.,[8] used inexpensive materials and simple procedures to produce masters and make a PDMS microfluidic device. A high-resolution printer was used to make a mask and a fixed-distance UV light

source was used to cure SU8(50) in a fume hood, so a clean room is not necessary.

CONCLUSIONS

This paper describes laboratory experiments and a design challenge that can be performed in three weeks with junior/senior chemical engineers. Students are given a problem to design a microfluidic mixer that can be used on a microchip to mix a dye and water at a specific flowrate. The students use computer aided design (AutoCAD) software to design a microscale mixer. They then fabricate their device using polydimethyl siloxane (PDMS). Their devices are tested with microscopes and image analysis software to assess the success of their mixing device in terms of the resulting colorimetric change. Students will come out of this experience with first-hand experience of challenges of mixing and diffusion at a small scale and they will gain skills in microfabrication and image analysis as well as the ability to troubleshoot a design after testing and come up with recommendations.

SUPPORTING MATERIAL

All class protocols for design, fabrication, and testing are available from the author by request at e-mail: sda4@cornell.edu.

ACKNOWLEDGMENTS

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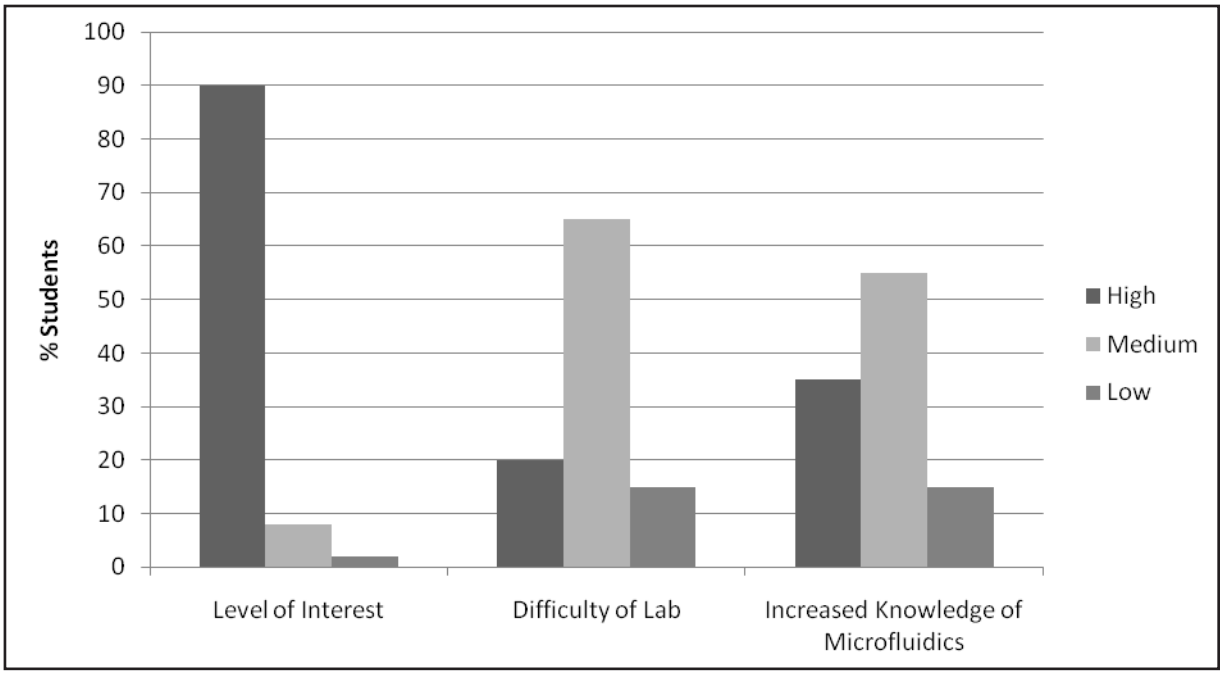

Figure 6. *Chart of student responses to the lab.*