EVALUATION OF A MICROFLUIDIC MIXER UTILIZING STAGGERED HERRINGBONE CHANNELS: A COMPUTATIONAL FLUID DYNAMICS APPROACH

BRIAN HAMA

Bachelors of Science in Chemical Engineering
Cleveland State University
May 2015

Submitted in partial fulfillment of requirements for the degree
MASTER OF SCIENCE IN CHEMICAL ENGINEERING
at
CLEVELAND STATE UNIVERSITY
August 2017
We hereby approve this dissertation
for
Brian A. Hama
Candidate for the Master of Science in Chemical Engineering degree for the
Department of Chemical and Biomedical Engineering
and CLEVELAND STATE UNIVERSITY’s
College of Graduate Studies

_____________________________________
Dissertation Chairperson, Chandra Kothapalli, Ph.D.
Department of Chemical & Biomedical Engineering
Department & Date

_____________________________________
Dissertation Committee Member, Jorge E. Gatica, Ph.D.
Department of Chemical & Biomedical Engineering
Department & Date

_____________________________________
Dissertation Committee Member, Petru S. Fodor, Ph.D.
Department of Physics
Department & Date

_____________________________________
Dissertation Committee Member, Miron Kaufman, Ph.D.
Department of Physics
Department & Date

Student’s Date of Defense: August 17, 2017
Acknowledgements

I would like to thank Dr. Chandra Kothapalli for his guidance as my advisor and mentor as well as encouraging me to achieve above and beyond. Dr. Kothapalli provided copious advice throughout this work from the broader level such as the project’s direction and goals to finer details such as advising on incidental problems and teaching me microfluidic fabrication methods. I would like to acknowledge my committee members Dr. Petru Fodor, Dr. Miron, Kaufman, and Dr. Jorge Gatica for their guidance and support. Specifically, Dr. Fodor was instrumental in teaching me COMSOL Multiphysics and provided extensive assistance with the software. Dr. Kaufman provided the custom Mathcad program used in this work and background on entropic mixing. Dr. Gatica graciously provided the Razel syringe pump and advised on problems with COMSOL.

I would like to recognize my colleagues at Cleveland State University, including Gautam Mahajan for his assistance with the confocal microscope and general help around the laboratory. I thank Edward Jira for his work on SolidWorks to generate the channel design drawings as well as the rest of the lab group: Jyotsna Joshi, Jacqueline Matz, Jennifer Vasu, Mohammadreza “Ryan” Abnavi, and Marissa Sarsfield. I also want to thank the Department of Chemical and Biomedical Engineering staff including Becky Laird and Darlene Montgomery for their tireless work for not only myself, but all the students in the department.

I also acknowledge the California Naonosystems Institute at the University of California for their technical assistance and fabricating the silicon wafer with SU-8 mold.

I would like to acknowledge financial support from the Choose Ohio First Scholarship program and the Department of Chemical and Biomedical Engineering. I also thank the confocal microscopy facility at CSU, which was funded by grant NIH 1 S10 OD010381.
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ABSTRACT

Microfluidic platforms offer a variety of advantages including improved heat transfer, low working volumes, ease of scale-up, and strong user control on parameters. However, flow within microfluidic channels occurs at low Reynolds numbers, which makes mixing difficult to accomplish. Adding V-shaped ridges to channel walls, a pattern called the staggered herringbone design (SHB), might alleviate this problem by introducing transverse flow patterns that enable enhanced mixing. However, certain factors affecting the SHB mixer’s performance remain largely unexplored.

In this work, a microfluidic mixer utilizing the SHB geometry was developed and characterized using computational fluid dynamics based simulations and complimentary experiments. A channel design with SHB ridges was simulated in COMSOL Multiphysics under a variety of operating conditions to evaluate its mixing capabilities. The device was fabricated using soft-lithography to experimentally observe the mixing process. The mixing was visualized by pumping fluorescent dyes through the device and imaging the channels using a confocal microscope.
The device was found to efficiently mix fluids rapidly, based on both simulations and experiments. Varying the Reynolds number or component diffusion coefficients had a weak effect on the mixing profile, due to the laminar flow regime and insufficient residence time, respectively. Mixing effectiveness decreased as the component flow rate ratio increased. Fluid flow patterns visualized in confocal microscope images were highly identical to the simulated results, suggesting that the simulations serve as good predictors of the device’s performance. This SHB mixer design would be a good candidate for further implementation as a reactor.
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CHAPTER I
INTRODUCTION

Microfluidics, the study of the behavior and manipulation of fluids in channels consisting of inner dimensions of less than one millimeter\(^1\), has gained interest over the past two decades because it enables the miniaturization of processes and systems,\(^2\) with applications in clinical diagnostics\(^3\), food security\(^4\), biological processing\(^5\), chemical reaction control\(^6\), and many others\(^7\). Specifically, the small dimensions increase the surface area to volume ratio within microfluidic channels by two to three orders of magnitude\(^1\), which significantly increases control over variables in chemical reactions such as temperature, pressure, volume, and mass transport\(^8\). Conditions in these small volumes are also more uniform, resulting in the suppression of temperature or concentration gradients and dead zones in mixing. This leads to reactions being performed in a more controlled manner without temperature-promoted side reactions. Reactions also become inherently safer\(^9\) because overall volumes are much lower, and runaway exothermic reactions can be managed due to the increased heat transfer. Thus, microfluidic mixers perform significantly better than their macroscale counterparts. This also eliminates the need for the process of scale-up for production at industrial levels.
The rigorous process of scaling a laboratory reactor to full size is entirely circumvented by “numbering up,” which is simply using a large number of the smaller devices to increase production\textsuperscript{10}. Therefore, the more ideal process at the micro-scale is used directly for large-scale production.

Miniaturization has a host of advantages in laboratory research in that it allows experiments to be performed in a single compact device, a technology aptly referred to as lab-on-a-chip (LOC). An LOC’s small size enables the performance of these actions with very small quantities of reactants with low physical space requirements; this leads to the ability to operate many LOCs in parallel for a given amount of space. This enables high-throughput experimentation, which is especially useful for drug discovery and stem cell research, in which large numbers of experiments need to be investigated\textsuperscript{11}. Furthermore, the size of an LOC provides the benefit of portability for use as a diagnostic device\textsuperscript{7} on-site without having to transport materials to a laboratory. This is especially useful where laboratory facilities may not be readily available or in close proximity. Such a system, shown in Fig. 1.1 below, has been developed to diagnose Ebola in a patient via Mechanically Induced Trapping of Molecular Interactions (MITOMI) using only 5 μL of blood sample, which improves the limit of detection by two to three orders of magnitude compared to existing portable MITOMI systems\textsuperscript{12, 13}.
Of the many unit operations that can be performed in microfluidic devices, mixing is of particular interest and is well-studied because it is a necessity to perform chemical reactions, formulate solutions, etc. However, mixing is especially difficult in microfluidics because flow at the micro-scale is highly laminar, with Reynolds numbers (Re) typically less than 100; the orderly flow regime due to relatively strong viscous forces prevents material from being effectively transported across layers of fluid by turbulence\textsuperscript{14}. As a consequence, diffusion is the only mechanism of mass transport, which takes place on much longer time scales than convection\textsuperscript{11}. Mixing at the macro-scale is normally accomplished by imposing turbulence with impellers, mixing pumps, or higher
flow velocity, which increases $Re$ to the turbulent regime$^8$. In microfluidics, these methods are ineffective due to the size of the channels and feasible flow rates in such devices, which restricts the maximum achievable $Re$. However, the distance a material needs to traverse, called the diffusion length, is much smaller in microfluidic devices$^{15}$, and there are many techniques that reduce the diffusion length further to promote mixing.

1.1 Active vs. Passive mixers

The various methods of inducing mixing in microfluidic devices can be classified into two main categories: active and passive. The description of active mixing is kept brief here because this study is focused on passive mixing. In active mixers, some external source$^{16}$ of fluid agitation is employed such as electrophoresis$^{17, 18}$, magneto-hydrodynamic particles$^{17}$, addition of artificial cilia$^{19}$, pressure fields$^{20}$, acoustics$^{21}$, or temperature manipulation$^{22}$. Although active micromixers are usually more efficient than passive mixers$^{23}$, they tend to be difficult to implement in practice due to the need for external equipment and power sources$^{15}$. These devices often feature moving parts that are subject to mechanical failure and may cause clogging within the channels$^{11}$. As a result, passive micromixers tend to be favored in real applications.

1.2 T-junction based passive mixers

Passive mixing utilizes special channel designs to introduce flow patterns that disrupt the orderly laminar flow, increasing contact area between the two fluids and reducing the diffusion length$^{15}$. The simplest design is the T-mixer$^{24}$, which is a juncture where two separate fluids to be mixed come together via separate inlets and mix in a
single main channel. While the design is basic and easy to fabricate, diffusion is the only
driver of mixing. Thus, long channels are required to provide enough residence time for
the fluids to mix properly, as evidenced by the unevenly distributed fluorescence
intensities at the end of the device in the figure below. One way to improve on the T-
mixer is to effectively multiply its effect by parallel lamination\(^{25}\). This is accomplished
by feeding \(n\) channels\(^{26}\) to the device and merging them into one channel. The mixing is
quickened by a factor of \(n^2\) due to the significantly increased contact area between the
two fluids which, in principle, reduces the amount of channel length required. In a similar
vein to the parallel lamination mixer, the split and recombine (SAR) mixer is the parallel
lamination mixer repeated in a series\(^{27}\). While the parallel lamination and SAR mixers
further increase contact area between the two fluids, they are often difficult to fabricate
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mixer, and SAR mixer for comparison.
**Fig. 1.2:** The most basic passive mixers: (Top) the T-mixer and intensity data clearly showing a lack of mixing due to non-uniformity\(^{24}\). Reprinted with permission from Kamholz et al. Copyright 1999 American Chemical Society. (Lower left) Parallel lamination mixer\(^{25}\) with \(n = 2\) splits. Reprinted with permission of Springer. (Lower right) SAR mixer\(^{15}\). Reprinted with permission of Springer.
1.3 Hydrodynamic focusing based passive mixers

Another type of passive mixer uses a method called hydrodynamic focusing. One way this is accomplished is through sheath flow, which involves one fluid being centered in the channel that is surrounded by a “sheath” fluid. The ratio of inner to sheath flow rates affects the diameter of the inner fluid in the channels. As the sheath flow is increased, the inner fluid flow profile shrinks; this leads to improved mixing because of the increased contact area and shortened diffusion length. The results in Fig. 1.3 below were achieved using as little as 5 nL/s of sample fluid, demonstrating that extremely low flow rates can be achieved in this system. Flow focusing is also achieved by inertial and Dean drag forces by designing channels with long curves. The curves in combination with the fluid velocity cause counter-rotating vortices that exert a drag force on the fluids. The drag force is directed toward the outer walls of the bend and inwards to the center from the top and bottom walls of the channel. It follows that the vortices would aid in mixing as the fluids are effectively stirred as they traverse the channel. This type of mixer can be tailored for a variety of biomedical applications. Fig. 1.3 and 1.4 below show the flow focusing micromixers described.
Fig. 1.3: (Top left) Sheath flow is achieved by surrounding a sample (white) with a sheath fluid (dark)\textsuperscript{28}. (Top right) Increasing the flow rate of the sheath fluid reduces the size of the sample flow pattern. (Bottom left) The width of the sample flow is plotted as a function of sheath to sample flow rate ratio. (Bottom right) The time evolution of mixing in the device is plotted. Reprinted with permission from Knight et al., Physical Review Letters, volume 80, page 3863, 1998. Copyright 1998 by the American Physical Society.
Fig. 1.4: (Top left) Fluorescent particles with diameters of 15 μm (green) and 20 μm (red) show the inertial effects experienced during a curve. (Top right) The intensity data as a function of position along the channel width are also shown. (Bottom) Flow profiles of fluorescent particles at different locations and flow rates.

1.4 Chaotic advection based passive mixers

Finally, a third type of passive mixer accomplishes mixing through chaotic advection with special channel designs. In general, advection is the transport of material due to a fluid’s bulk motion. This occurs naturally in any microfluidic device, but the laminar flow regime restricts advection to only occur in-line with the fluid’s streamlines.
While turbulent flow naturally produces chaos in a fluid, chaotic advection mixers use special channel designs to introduce disturbances. These disturbances differ from true chaos seen in turbulent flow in that they do not change with respect to time or experiment performed (i.e., the behavior does not change if the flow is restarted in a new “experiment” in the device). Therefore, the term “chaotic advection” is used only due to the naming convention in the field of microfluidics. The previously described passive mixers do not manipulate the advection of fluid; rather they rely on diffusion or the inertia of the fluid. In chaotic advection mixers, the channels are shaped such that they forcibly alter the unidirectional paths that the fluid would normally take. Ultimately, these micromixers are designed to introduce transverse flows that cause fluid to move in many directions. The disorderly flow then functions similarly to natural turbulence in that the interfaces between the two fluids are stretched and folded repeatedly, which dramatically increases contact area between them. The large contact areas allow diffusion to complete the mixing in a very short amount of time, resulting in a uniform concentration profile. There are a variety of ways of achieving this including F-shaped channel arrangements, two-layer crossing channel mixers (TLCCM), and 3D serpentine designs. As shown in Fig. 1.5 below it is obvious that the TLCCM outperformed the 3D serpentine mixer based on the concentration profiles.
Another method of introducing disturbances in flow is the addition of grooves to the channel walls. One of the simpler designs, the slanted groove mixer (SGM) features slanted grooves that span the width of the channel. Confocal microscopy results showed that the fluid undergoes a helical flow pattern as it passes over the grooves (Fig. 1.6)\textsuperscript{35}. A more complicated variant of the SGM, the connected-groove micromixer (CGM) features slanted grooves spanning the width of the channel and on the side walls. The ridges can be staggered - which means that a set of ridges in one direction is followed by a set of

---

\textbf{Fig. 1.5:} Channel designs and concentration profiles\textsuperscript{34} for a,b) two varieties of TLCCM and c) 3D serpentine. Concentration profiles were taken via confocal microscopy at various mixing cycles $N$ at locations $A_N$. Reproduced from \textsuperscript{34} with permission of The Royal Society of Chemistry. Below, a schematic for d) F-shaped mixing configuration is shown\textsuperscript{33}.
ridges in the opposite direction, or non-staggered- in which the same ridge is simply repeated along the entire channel. Both variants of the CGM were investigated and shown to produce a spiraling flow pattern (Fig. 1.7)\textsuperscript{36}. Computational fluid dynamics (CFD) simulations were performed and compared with the experimental results via confocal microscopy. Both versions of the CGM were found to perform better than the SGM, with the best results coming from the staggered CGM because it produced two co-rotating flows instead of the singular rotation in the SGM and non-staggered CGM. Overall, the staggered CGM achieved mixing 50% faster than the SGM\textsuperscript{36}.

![Fig. 1.6: Schematic of SGM showing the helical flow pattern along with experimental results from confocal microscopy\textsuperscript{35}. From A.D. Stroock, S.K.W. Dertinger, A. Ajdari, I. Mezic, H.A. Stone, G.M. Whitesides, 2002. Chaotic Mixer for Microchannels. Science Magazine 295, 647-651. Reprinted with permission from AAAS.](image-url)
**Fig. 1.7:** (Left) Schematics of two variants of the CGM. (Right) The spiral-shaped flow patterns that these mixers generate are illustrated. (Below) Comparison of CFD simulations with experimental results from confocal microscopy. Reprinted from Chemical Engineering Science, volume 63, Yang et al., Fluids mixing in devices with connected-groove channels, 1871-1881, Copyright 2008, with permission from Elsevier.

Finally, chaotic advection mixers could utilize the staggered herringbone (SHB) geometry, which is similar to the SGM and CGM and consists of asymmetrical V-shaped grooves. A set of grooves with the shorter end on the left-hand side is then followed by another set of grooves with the short end on the right-hand side. This completes one mixing cycle, which is repeated many times throughout a device. The SHB arrangement normally features the ridges with their vertices pointing in the opposite direction of the flow; the opposite orientation (with the vertices pointing in the direction
of flow) is referred to as the reverse staggered herringbone mixer. The geometry could be further modified by employing ridges that come up out of the walls into the main channel (positive) instead of going into the walls (negative). The traditional SHB configuration is forward and negative. The standard SHB geometry has been shown in both CFD simulations and experiments to be effective at inducing mixing in microfluidic devices by adding rotational and extensional flows, which fosters more intimate contact between the mixing fluids. Research efforts have concluded that specifics on the design and operation of SHB microfluidic mixers vary depending on the desired application since all variants of the SHB are effective, as seen in Fig. 1.8 below. The fluid flow rate may be increased to reduce mixing time, but reducing the flow rate may be considered to reduce the channel length needed or to address pressure limitations in a device.
Fig. 1.8: (Above) Visual representations of the different SHB ridge configurations. (Below) Fluorescence imaging of mixing in each configuration after three mixing cycles\textsuperscript{41}.

The SHB mixer has been well-characterized in many aspects, but there are some characteristics that have not been fully addressed. While the forward-negative SHB has been thoroughly investigated, the literature contains very few studies on other configurations. One study that comes close to achieving this by Kwak et al.\textsuperscript{41} provides a
comprehensive comparison between the different configurations with simulations and fluorescence microscopy, but lacks confocal microscopy results and a detailed quantification of mixing beyond normalized intensity profiles. Further, the simulation analysis was mostly qualitative for the sake of comparison between the different configurations and did not include any mixing quality computations. Another difficulty in the literature on the SHB mixer is that the design of the ridges, i.e., the actual shape, inter-ridge spacing, and overall dimensions, varies greatly across different studies. While the design by Kwak et al. features ridges of 50 μm width and depth, studies such as Williams et al. use ridges of 85 μm width and 50 μm depth and Du et al. use ridges 12.5 μm wide and 7 μm deep. Additionally, the CFD simulations in the literature largely treat $Re$ as the primary variable for mixing evaluations. However, there are plenty of other factors that could affect the mixing performance of the SHB mixer such as diffusivities of species or the ratio of the inlet component flow rates which remain unexplored.

The objective of this study is to design, fabricate, characterize, and implement a microfluidic micromixer with potential applications to continuously synthesize nanoparticles. The list of applications of nanoparticles and nanostructured materials is seemingly endless, with applications in chemical synthesis, polymer-based consumer products, fuel cells and batteries, medicine, and many more. The mixer was first designed using computer-aided design (CAD) software and simulated with a CFD software package; the simulation results were used as a benchmark to be compared to the experimental performance of a fabricated version of the device to evaluate its feasibility in applications that demand rapid and thorough mixing. Obviously, this is not limited to
nanoparticles; other potential uses for such a device lie in biological research such as protein folding\textsuperscript{42}, focused drug delivery to study cell behavior\textsuperscript{43}, or maintaining concentration gradients within cells\textsuperscript{43}.
CHAPTER II
THEORY

At its core, microfluidics is an application of fluid mechanics at sub-millimeter dimensions, with the addition of mass transport in this work. It is therefore necessary to visit the theoretical basis for microfluidic flow behavior by delving into the fundamentals of fluid mechanics. Then, to understand the mechanisms behind mixing in microfluidics, discussion will shift to mass transport. Before the fluid flow problem can be solved, it is necessary to understand the assumptions and boundary conditions that apply to this study.

2.1 Assumptions

1. Two dilute species are to be mixed, referred to as A and B in this document. They both enter the device at 10 μM concentration each (for instance), but may have different diffusivities (driven by molecular weight of species). They may both enter the device at different flow rates.

2. Here, we assume that the device is operating at steady state, meaning that conditions are not changing over time. One set of time-dependent studies were performed to observe the transient conditions prior to steady state.
3. Operating conditions are at 25 °C (room temperature) and atmospheric pressure.

4. The fluid is incompressible and Newtonian. Incompressibility implies that the specific volume of the fluid is constant within the flow, and a Newtonian fluid’s viscosity does not change with respect to shear rate.

5. Physical properties of the fluid are similar to water because the desired application of the device involves aqueous ionic solutions. The density and viscosity of water were taken to be 1000 kg/m$^3$ and 0.001 Pa·s, respectively.

6. The species diffusivity is assumed to be that of FITC – Dextran at a concentration of 10 μM and 25 °C. In simulations, four molecular weights (1 kDa, 10 kDa, 100 kDa, and 1000 kDa) with corresponding diffusivities$^{44}$ ($D_l = 2.91 \times 10^{-10}$ m$^2$/s, $9.25 \times 10^{-11}$ m$^2$/s, $3.16 \times 10^{-11}$ m$^2$/s, and $1.1 \times 10^{-11}$ m$^2$/s, respectively) were considered. For brevity, we refer to each diffusion coefficient by its molecular weight from here onward (i.e., D1, D10, D100, and D1000).

7. The cartesian coordinate system is defined for a single straight channel with the origin at the bottom left corner of the main channel inlet; $x$ is in the direction of the flow through the channel, $y$ is the vertical direction starting at the interface between the main channel and the ridges and pointing into the main channel, $z$ is the horizontal direction starting at the left-hand side of the channels and pointing to the right-hand side as shown in Fig. 2.1.
8. The device is oriented such that all channels are at the same elevation. Therefore, hydrostatic effects are neglected.

9. There is no accumulation or depletion of material in the device.

2.2 Boundary conditions

The channel geometry is shown below in Fig. 2.2 for the first three mixing cycles of the device. The rectangular channels are 200 μm wide with 50 μm height and the ridges are 35 μm wide and recessed into the lower wall 50 μm deep. One section is defined as a grouping of five consecutive identical ridges, with one complete mixing cycle consisting of two sections: one with the vertices left of center and one with the vertices right of center. The first mixing cycle is shown in more detail in Fig. 2.3. Complete dimensions of the ridges are shown in Fig. 2.4. The defined geometry and corresponding labeling of cycles and sections in Fig. 2.2 are referenced through the
remainder of this document. All boundary conditions are described in the following list and summarized in Table 2.1 and Fig. 2.5.

![Diagram of mixing cycles](image)

**Fig. 2.2**: Three complete mixing cycles taken from different orientations in COMSOL. The direction of flow is along the x-direction. A) Angled view that indicates the defined cycles and sections along the channel length. B) Side view from xy-plane. C) Top-down view from xz-plane D) Cross-sectional view from yz-plane.
Fig. 2.3: A) Close diagonal view of the first mixing cycle. The channels are also shown in the B) xz-plane, C) xy-plane, and D) yz-plane with dimensions indicated.

Fig. 2.4: Enlarged view of herringbone ridges with dimensions indicated.
1. Inlet flow is for $Re = 0.01, 0.1, 1, 10,$ and $100$ (all in laminar range) which corresponds to velocities in the range of $u = 0.0125 \text{ cm/s}, 0.125 \text{ cm/s}, 1.25 \text{ cm/s},$ $12.5 \text{ cm/s},$ and $125 \text{ cm/s}.$ The corresponding flow rates were divided between components A and B.

2. $R_{AB}$, defined as the ratio of flow rate of component A to that of component B, was varied over $1, 2,$ and $3.$

3. The inlet concentration profile is a step change along the $z$-direction of the channel to simulate two different fluids being introduced to the device side-by-side. The step function is defined with respect to an initial concentration $c_{0A} = c_{0B} = 10 \mu M$:

   - $c_A = c_{0A}$ for $z \leq 100 \mu m$
   - $c_A = 0$ for $z > 100 \mu m$
   - $c_B = 0$ for $z \leq 100 \mu m$
   - $c_A = c_{0B}$ for $z > 100 \mu m$

4. The no-slip condition applies to all walls in the device. Local fluid velocity at all walls is zero.

5. Flow is pressure driven and exits to atmospheric pressure, which is expressed by setting the outlet gauge pressure to zero.
Table 2.1: Summary of boundary conditions used to solve the fluid flow problem in COMSOL.

<table>
<thead>
<tr>
<th>Boundary Condition Type</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inlet flow</td>
<td>$Re = 0.01, 0.1, 1, 10, 100; u = 0.125 cm/s, 1.25 cm/s, 12.5 cm/s, 0.125 cm/s</td>
</tr>
<tr>
<td>Component flow rate ratio</td>
<td>$R_{AB} = 1, 2, 3$</td>
</tr>
<tr>
<td>Inlet concentration</td>
<td>$c_{0A} = c_{0B} = 10 \mu M$</td>
</tr>
<tr>
<td>Wall effects</td>
<td>No-slip; $u = 0$ at walls</td>
</tr>
<tr>
<td>Pressure</td>
<td>$P_{outlet} = 0$</td>
</tr>
</tbody>
</table>

![Diagram of fluid flow](image)

**Laminar Flow (SPF)**

Equations

\[
\begin{align*}
\frac{\partial \mathbf{u}}{\partial t} + (\mathbf{u} \cdot \nabla) \mathbf{u} &= -\left(\frac{1}{\rho}\right) \nabla p + \nu \nabla^2 \mathbf{u} + \mathbf{g} \\
\frac{\partial \rho}{\partial t} + (\nabla \cdot \mathbf{u}) &= 0
\end{align*}
\]

Boundary Conditions

1. $t = 0$: $\mathbf{u} = 0$
2. $t > 0$: $u_x = u_0$ and $u_y = u_z = 0$
3. $z = 0, z = w$: $\mathbf{u} = 0$
   $y = 0, y = h$: $\mathbf{u} = 0$
4. $t \to \infty$: $\frac{\partial \mathbf{u}}{\partial t} = 0$

**Transport of Diluted Species (TDS)**

Equations

\[
\frac{\partial c_i}{\partial t} - \nabla(D \nabla c_i) + \nabla(\mathbf{u} c_i) = 0
\]

Boundary Conditions

1. $t = 0$: $c_A = c_B = 0$
2. $t > 0$ and $x = 0$:
3. $z \leq \frac{w}{2}$: $c_A = c_{A0}$ and $c_B = 0$
   $z > \frac{w}{2}$: $c_A = 0$ and $c_B = c_{B0}$

Fig. 2.5: Illustration of boundary conditions in the fluid flow problem.
### 2.3 Relevant equations for momentum and mass transport

One of the most distinctive features in microfluidics is a highly laminar flow regime, which is characterized by the Reynolds number\(^{(45)}\):

\[
Re = \frac{\rho u L}{\mu}
\]  

(1)

where \(\rho\) is the fluid density, \(u\) is the magnitude of velocity, \(\mu\) is the fluid’s viscosity, and \(L\) is the characteristic length of the geometry being examined. In this study, the channels are rectangular, so the characteristic length is evaluated according to definition\(^{(45)}\):

\[
L = 4 \left( \frac{A_c}{P_w} \right)
\]  

(2)

where \(A_c\) and \(P_w\) are the cross-sectional area and wetted perimeter of the channel, respectively. For a rectangular duct with width \(a\) and height \(b\), the characteristic length can be computed as:

\[
L = 2 \left( \frac{ab}{a+b} \right)
\]  

(3)

By definition, \(Re\) is a dimensionless number that denotes the ratio of inertial to viscous forces experienced by the fluid. When \(Re\) is small, the flow is laminar, meaning that all streamlines in the fluid are parallel with no cross flow. When \(Re\) becomes sufficiently large (i.e., critical Reynolds number), the flow becomes chaotic; streamlines cross paths with each other and eddies form in the fluid. In a typical macroscopic flow scenario such as pipe flow, this turbulent flow occurs at \(Re = 4000\), with a transition between laminar and turbulent flow occurring at Reynolds numbers between 2100 and 4000\(^{(46)}\). In microfluidics, the values of \(L\) and \(u\) are very small compared to those seen in macroscopic settings. Consequentially, microfluidic flow is always laminar; typically, \(Re < 100\), with many devices operating at \(Re < 1\).
To solve the fluid velocity profile in a given geometry, the Navier-Stokes equation of motion and continuity equation for momentum transport are employed for an incompressible fluid with no accumulation\textsuperscript{46}:

\[
\frac{\partial \mathbf{u}}{\partial t} + (\mathbf{u} \cdot \nabla) \mathbf{u} = -\frac{1}{\rho} \nabla p + \nu \nabla^2 \mathbf{u} + \mathbf{g} \quad (4)
\]

\[
\frac{\partial \rho}{\partial t} + (\nabla \cdot \rho \mathbf{u}) = 0 \quad (5)
\]

where \( \mathbf{u} \) is the velocity as a vector, \( p \) is pressure, \( \nu \) is the kinematic viscosity of the fluid, and \( \mathbf{g} \) is gravity as a vector. In the steady state studies, we neglected the time derivatives due to Assumption 2 and the gravitational term due to Assumption 8. By Assumption 4, the fluid is incompressible, so \( \nabla \cdot \mathbf{u} = 0 \). Equations 4 and 5 are in their most general forms, so they can be evaluated for one, two, or three dimensions in Cartesian, cylindrical, or spherical coordinates. These equations are used to determine the velocity profile of the fluid for all locations in the device, which is necessary to solve the mass transport equations.

In the device studied in this work, mass transport occurs due to both diffusion and convection. Diffusion is the movement of matter within a fluid due to a concentration gradient. This is expressed by Fick’s First Law of Diffusion\textsuperscript{47}:

\[
J_{\text{diffusion},i} = -D_i \nabla c_i \quad (6)
\]

where \( J_{\text{diffusion},i} \) is the diffusive flux vector of species \( i \), and \( D_i \) and \( c_i \) are the diffusion coefficient and concentration of species \( i \), respectively. A species will diffuse through a fluid from areas of high concentration to low concentration, as indicated by the negative sign in Equation 6.

Though the microfluidic device in this work operates in the laminar flow regime, it does introduce transverse flow patterns due to its geometry, which directs the transport
of a species $i$ through convective action. This is accounted for by the convective flux\textsuperscript{47}, $J_{\text{convection},i}$, expressed as:

$$J_{\text{convection},i} = uc_i$$

(7)

The two fluxes obtained from Equations 6 and 7 are additive, yielding the total flux of species $i$ due to both diffusion and convection.

$$J_{\text{total},i} = J_{\text{diffusion},i} + J_{\text{convection},i} = -D\nabla c_i + uc_i$$

(8)

Finally, the flux expression is substituted into the continuity equation for mass transport in an incompressible fluid with no accumulation, given as:

$$\frac{\partial c_i}{\partial t} + \nabla \cdot J_{\text{total},i} = 0$$

(9)

Substitution of equation 8 into equation 9 yields the Convection-Diffusion Equation\textsuperscript{47}:

$$\frac{\partial c_i}{\partial t} - \nabla(D\nabla c_i) + \nabla(uc_i) = 0$$

(10)

As with the momentum transport equations, we neglected the time derivative in steady state studies due to Assumption 2. This equation is used to calculate the concentration profile at all locations in the device using the velocities from Equations 4 and 5 for $u$.

Further, this equation is also in its most general form and may be used to solve more than one particular problem. A useful way to quantify the relative importance of the convective and diffusive terms is through the Péclet number\textsuperscript{46}:

$$Pe = \frac{ul}{D}$$

(11)

Defined as the ratio of convective transport to diffusive transport, $Pe$ is an indicator for which mechanism is dominant in the flow. When $Pe > 1$, convection is more significant than diffusion, and the opposite applies for $Pe < 1$. 

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CHAPTER III
MATERIALS & METHODS

3.1 Channel design and computational fluid dynamics simulations using COMSOL Multiphysics® modeling software

The micromixer was first designed in SolidWorks® Premium 2016-17 3D CAD software. The design consisted of two layers, to be combined in fabrication. As mentioned previously, the top layer contained the main channel, which was rectangular with a width of 200 \( \mu m \) and height of 50 \( \mu m \). The main channel contained 27 straight sections 17300 \( \mu m \) in length, each connected by two 90 degree bends to form a serpentine path. This shape was mirrored and repeated on the other half of the device. The channels also contained designated port locations for two inlets, one outlet, and up to three ports with many different uses such as measuring temperature and pH or serving as an outlet depending on application and need. The bottom layer of the channel design was comprised of the SHB ridges, which were 50 \( \mu m \) deep. The ridges were asymmetrical and spanned the width of the main channel. One complete cycle was 1886 \( \mu m \) in length and consisted of a set of five ridges with the shorter segment on the left followed by a set of five ridges that were of a mirrored configuration. Each straight pass in the main channel
contained nine complete cycles, with a total of 486 cycles in the device. **Fig. 3.1** shows a two-dimensional drawing of the complete mixer layout.

**Fig. 3.1**: Complete schematic of channel layout of the microfluidic device. One complete mixing cycle of 1886 μm was repeated nine times along each straightaway of 17300 μm. There were 27 of these straight segments on the first half of the device, which was repeated on the other half. Three sensor ports were positioned as optional outlets or for probing sensible conditions in the flow.
The expected performance of the device was evaluated using COMSOL Multiphysics 5.2 simulation software with a Chemical Engineering Module. The studies conducted on the device utilized the Laminar Flow (SPF) and Transport of Diluted Species (TDS) physics interfaces. The TDS interface was configured to handle the two species to be mixed. The first simulations we built in COMSOL used a Stationary study, which corresponds to a steady state operation. The geometry was constructed within the software by first drawing 2D shapes and “extruding” them out of the work plane to make 3D objects, shown in Fig. 3.2. The main channel and SHB ridges were drawn in separate but coinciding work planes and extruded in opposite directions to form the two-layered geometry. To avoid excessive processor load and computing times, only three mixing cycles were included in our simulations, and the two separate inlet channels that lead up to the first mixing cycle were omitted except for the cases where \( Re \) and \( R_{AB} \) were manipulated. Instead, the inlet was constructed as a single stream of fluid incorporating a stepwise change in concentration across the channel to imitate two separate fluids entering side by side. Preliminary testing showed that both geometrical configurations (with and without inlet channels) produced equivalent results (< 1% deviation), so results were not compromised by using one over the other. One case involving a geometry twice this length (six mixing cycles) was studied to confirm that simulation beyond the third mixing cycle yielded no additional benefit regarding mixing efficiency. It is important to note that the simulated length was only a small portion of the overall design shown in Fig. 3.1; this was to increase the residence time of fluid within the channels. For the proposed application of nanoparticle synthesis, the particles should be “aged” to reach a desired size.
To facilitate viewing and displaying of simulation results for post-processing, the geometry was then partitioned into smaller subsections. More specifically, the partitions were placed between the end of one half mixing cycle and the beginning of the next half cycle. These partitions were prepared by unifying the subsections into one object. This allowed for greater control over which portions of the geometry were displayed at a time within COMSOL’s interface while also retaining the original geometry.

**Fig. 3.2:** The geometry was formed by formulating 2D drawings that were “extruded” out of the work plane. (Top) First, the main channel was drawn with a simple rectangle and extruded in the positive y-direction by 50 μm. (Bottom) The ridges were formed by drawing them along the location of the main channel rectangle, then extruding in the opposite direction by 50 μm. The geometry was completed by merging the two sets of shapes together. Units of 2D drawings are mm.
Next, various aspects of the simulation were entered into COMSOL such as material properties and boundary conditions. The initial concentrations $c_{0A}$ and $c_{0B}$ of 10 $\mu$M as well as diffusion coefficients $D_A$ and $D_B$, depending on the case investigated, were specified. Next, the identity of the fluid was selected to be liquid water because the desired application of the device involves aqueous ionic solutions. Upon selecting this, COMSOL supplied its stored physical properties on water automatically.

The physics interfaces were then configured according to the boundary conditions of our fluid flow problem. In the SPF interface, the inlet and outlet locations were specified by selecting the appropriate faces in the geometry through which fluid would enter and leave. This interface was also where the inlet velocity (and $Re$ or $Q$ by extension) was entered. For the cases where $Re$ and $R_{AB}$ were manipulated, the inlet velocity of each fluid was entered separately. The two streams were set such that the desired $R_{AB}$ was achieved and the two velocities added together to match the velocity corresponding to the desired $Re$ in the device. In the case of the outlet, the pressure was set to zero in order to incorporate pressure-driven flow that exits to atmospheric pressure. With this setting, COMSOL automatically calculated the required pressure at the inlet to achieve the velocity at the inlet.

In the TDS interface, the inlets and outlet for the diluted species were specified at the same locations as in the SPF settings. The diffusivities of the two species were also entered for this physics interface. When $Re$ and $R_{AB}$ were manipulated, the two inlet channels contained pure component A or component B. Otherwise, a concentration gradient was used such that the left-hand side of the channel was pure component A with no component B, and the right-hand side was pure component B with no component A.
The inlet concentration profile of component A was created by defining a step function within COMSOL that was centered at \( z = 100 \) \( \mu \text{m} \) and took on a value of 1 when \( z < 100 \) \( \mu \text{m} \) and a value of 0 when \( z > 100 \) \( \mu \text{m} \). In the concentration input, the expression took the form \( c_{0A} \times \text{step}(z) \). This was repeated for component B, except a second step function was defined that took a value of 0 when \( z < 100 \) \( \mu \text{m} \) and a value of 1 when \( z > 100 \) \( \mu \text{m} \). A representative image of this inlet concentration profile is shown below in Fig. 3.3.

![Inlet concentration profile](image)

**Fig. 3.3:** Inlet concentration profile created by the described step change across the channel. The resulting profile was the same when two separate inlets of pure fluid are merged at this location.

The geometry was divided into a mesh of tetrahedral elements containing 524,000 domains, 64,000 boundaries, and 6000 edges, which was in the typical range of number of elements for CFD simulations\(^{37}\). The mesh was a crucial step in setting up simulations because it had a large impact on both the computational time and quality of results. The size of the elements used in the mesh affected the total number of elements needed to fill the geometry (i.e., smaller elements led to needing more elements in the mesh). Having
smaller elements made the mesh finer, which yielded higher resolution in the simulation results. However, this also increased the computational demand and, by extension, solution time. It became obvious that the mesh size had to be optimized to yield results of sufficient quality without requiring excessive computing time. The aforementioned mesh characteristics were the result of such optimization by trial and error. Typical solution time was 2-4 hours, depending on the specific case being simulated.

To set up a computation, a study was configured to give COMSOL instructions on how to solve the fluid dynamics problem. First, a stationary (or steady state) study was created, which was composed of two steps. The first step utilized the SPF physics interface and its settings, and then the second step used SPF results in combination with the TDS interface to compute the concentration profile. Various settings could be manipulated to alter the method by which COMSOL performed calculations, from the type of numerical solver to the tolerance in solution. During a computation, the fluid flow was simulated within each mesh element, with the results assembled together to generate the final solution. All computations in this work employed the Generalized Minimum Residual (GMRES) method of solving the system. In the SPF step, Equations 3 and 4 were solved for each mesh element to compute the velocity profile in the device. Drawing from the resultant velocity data in the SPF step, the concentration profile was solved during the TDS step using Equation 10.

The system was also simulated under non-steady state conditions to gain an understanding of how mixing was established upon startup of the device. The same simulation setup for the Re sweep was used as a basis for creating a time-dependent study in COMSOL. The only difference from the stationary study was that a range of times had
to be entered. The result was snapshots of the conditions in the device at different points in time, which were assembled into video files consisting of 40 frames played at 5 frames per second. The main goal was to observe the development of the steady state concentration profile and to determine approximately how much time was required to achieve this.

Table 3.1 lists the studies examined in all CFD simulations including variables manipulated and held constant. For the four diffusion coefficient values considered in Study 3, there were ten non-repeating pairs of $D_i$ between the two components A and B. These combinations are found in Table 3.2.

**Table 3.1**: All studies performed in COMSOL with manipulated and constant quantities.

<table>
<thead>
<tr>
<th>Study #</th>
<th>Description</th>
<th>Variables Manipulated</th>
<th>Variables Held Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Re Sweep</td>
<td>$Re = 0.01, 0.1, 1, 10, 100$</td>
<td>$D_A = D_B = D1$; $R_{AB} = 1$</td>
</tr>
<tr>
<td>2</td>
<td>Extended Channel</td>
<td>Number of mixing cycles</td>
<td>$Re = 1$; $D_A = D_B = D1$; $R_{AB} = 1$</td>
</tr>
<tr>
<td>3</td>
<td>Diffusivity Sweep</td>
<td>$D_i = D1, D10, D100, D1000$</td>
<td>$Re = 1$; $R_{AB} = 1$</td>
</tr>
<tr>
<td>4</td>
<td>$R_{AB}$ Sweep</td>
<td>$R_{AB} = 1, 2, 3$; $Re = 0.1, 1, 10, 100$</td>
<td>$D_A = D_B = D10$</td>
</tr>
<tr>
<td>5</td>
<td>Transient Study</td>
<td>$Re = 0.1, 1, 10, 100$</td>
<td>$D_A = D_B = D1$; $R_{AB} = 1$</td>
</tr>
</tbody>
</table>

**Table 3.2**: All combinations of diffusion coefficients examined within Study 3 (diffusivity sweep).

<table>
<thead>
<tr>
<th>Pairing notation</th>
<th>Component A</th>
<th>Component B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D1</td>
<td>D1</td>
</tr>
<tr>
<td>2</td>
<td>D1</td>
<td>D10</td>
</tr>
<tr>
<td>3</td>
<td>D10</td>
<td>D10</td>
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<td>5</td>
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<td>D100</td>
<td>D100</td>
</tr>
<tr>
<td>10</td>
<td>D1000</td>
<td>D1000</td>
</tr>
</tbody>
</table>
3.2 Mixing quality evaluation

When computations were complete, the velocity and concentration profiles could be plotted and displayed according to user preference. Cross sectional plots of the concentration profile were captured as images after each half mixing cycle in the device using $yz$ planes at different values of $x$. There were six sections and two components (each with their own concentration profile), resulting in 12 images per simulation, with the exception being Study 2 in which there were 24 images. This was not done on the time-dependent studies in Study 5.

The concentration profile images generated in COMSOL were then used to evaluate the mixing quality through a quantity known as the mixing index, $M$. We used a statistical approach to determine $M$ by evaluating the Shannon Entropy\(^4\). This method depends on only the probability distribution of intensities from a grayscale fluorescence image:\(^5\):

$$S_j(species) = - \sum_{c=1}^{2} p_{c/j} \ln(p_{c/j})$$

(11)

$$S_{locations}(species) = \sum_{j=1}^{N} p_j S_j(species)$$

(12)

where $S_j(species)$ is the entropy of mixing of all species at the location of bin $j$, $p_{c/j}$ is the probability of finding a particle of species $c$ conditional on the bin $j$, $S_{locations}(species)$ is a spatial average of the entropy of mixing of species conditional on location, and $p_j$ is the probability of finding a particle irrespective of species in bin $j$. To find the mixing index, $M$, we normalized the entropy of mixing with $\ln(c)$, yielding values between 0 (perfect segregation) and 1 (perfect mixing).

$$M = S_{locations}(species)/ \ln(c)$$

(13)
In the studied device, $M$ was evaluated with a custom-written Mathcad (version 15) program (courtesy of Prof. Miron Kaufman) incorporating equations 11-13. The concentration profile images were converted to grayscale and served as input to the program. Because there were separate concentration profiles for components A and B, there were also two sets of $M$ calculated, referred to as $M_A$ and $M_B$, respectively.

In contrast to this entropic method of evaluating mixing, other research groups have used alternate approaches. One of the more common methods is by examining fluorescence intensity images and either evaluating the standard deviation\textsuperscript{35} of the intensity distribution or normalizing the intensities to generate a distribution plot\textsuperscript{41}. Another method is by calculating the coefficient of variation (CV)\textsuperscript{38}, which is widely used for evaluating distributions in many other fields besides microfluidics. Its general definition is $CV = \sigma / \mu$, where $\sigma$ is the standard deviation and $\mu$ is the numerical average of the quantity being measured.

\textbf{3.3 Device Fabrication}

The physical micromixer device was fabricated using well-established soft-lithography techniques. In principle, polydimethylsiloxane (PDMS) is molded to the channel design and is bonded to a glass slide to form complete channels. PDMS is a polymeric organosilicon compound composed of silicon and organic groups. It is a material of choice in microfluidics because it is mechanically tough, transparent, non-toxic, chemically inert, offers flexibility in crosslinking, and non-flammable\textsuperscript{50,51}. PDMS is also an economical alternative to other fabrication methods such as photolithography on glass cover slides or silicon wafers\textsuperscript{9,52}. Its mechanical properties as an elastomer are
customizable depending on the ratio of the base elastomer to crosslinking agent, and it easily molds to surfaces during the curing process. Plasma oxidation treatment alters the hydrophobic surface chemistry of PDMS to make it become hydrophilic by adding hydroxide groups. Plasma treated PDMS and glass can be irreversibly bonded to each other due to the rearrangement of chemical bonds, forming a permanent seal. In this work, all fluids were aqueous and thus had an affinity for the hydrophilic walls. It is likely that using nonpolar fluids would affect the fluid flow in the channels due to surface tension effects. A mold of our micromixer device was made on a silicon wafer by rendering a negative of the channel design with SU-8 photoresist techniques. SU-8 is a polymer containing eight epoxy groups and is a photoresist, which means that its material properties change when it is exposed to light\(^{53}\). Fig. 3.4 below shows representations of the molecular structures of PDMS and SU-8.

![Molecular structures of PDMS and SU-8](image)

**Fig. 3.4:** Molecular structures of PDMS\(^{51}\) and SU-8\(^{53}\) used in this work.

The SolidWorks design files containing the channel design were sent to California NanoSystems Institute (University of California, Santa Barbara), where the silicon wafer mold was developed. Briefly, for the fabrication of the mold, SU-8 (MicroChem) was
poured onto a silicon wafer (10 cm diameter) and cured. Then, the desired pattern from SolidWorks is formed on the surface by applying a mask that covers only the locations where the pattern is to be made. Then, the rest of the wafer is exposed to UV light such that it becomes soluble in a development solvent, which is used to remove the SU-8. Because of the pattern masking, the SU-8 not exposed to light remains unaffected by the solvent and remains in place. This creates a negative of the design on the silicon wafer that serves as the mold for the channels. The wafer accommodates the channel design in duplicate, allowing for two devices to be made per batch. The micromixer was fabricated using the following procedure, with the steps also represented in Fig 3.5.

1. Before the mold was first used, it was first silanized by depositing a layer of Tridifluoro – (Tridecafluoro – 1, 1, 2, 2 – Tetrahydrooctyl) – 1 – Trichlorosilane (SigmaAldrich) on the surface. An open petri dish containing 75 μL of the silanizing agent and the exposed mold in its own dish were placed under vacuum in a desiccator for 15 minutes. The mold was removed from the desiccator and the remaining silanizing agent was discarded. The deposited layer acts as a protective barrier for the mold and also facilitates delamination of cured PDMS from the surface. This step was repeated every several uses of the mold.

2. PDMS (Dow Corning, Sylgard 184 Silicone Elastomer Kit) solution was formulated by measuring 28 g of elastomer base and 2.8 g of curing agent to achieve a crosslinking ratio of 10:1 and mixed well. For the first use of the mold, a larger amount of PDMS was used to fill the entire dish. Subsequent devices were cut out from the same area around the pattern in the mold.
3. PDMS mixture was placed under vacuum for up to one hour to remove air bubbles introduced during the mixing process.

4. PDMS was then poured onto the mold and vacuumed again to remove any air bubbles introduced during this step.

5. The mold was placed into an oven at 70 °C to accelerate the curing of the PDMS. The PDMS cures to form a crosslinked polymer within two hours at this temperature.

6. After removal from the oven, the molded devices were carefully cut out with a sharp knife, taking care not to scratch the surface of the wafer. The two devices were then separated from each other by cutting them apart. Beyond this step, the feature side with the channels was always kept facing upward.

7. From this point onward, attention is focused on a single device, although many devices could be prepared simultaneously during these steps. The inlets, outlet, and probe ports were cored using a biopsy punch at the designated sites in the channel design. The punches were made to go completely through the PDMS layer from the feature side to the opposite face.

8. The device was initially cleaned of dust and other large debris with adhesive tape.

9. A bath of deionized (DI) water was set on a hot plate and brought to a boil. The cut out microfluidic devices were boiled for 15 minutes, stirring occasionally. This step leached any uncured PDMS residues from the device to ensure a clean surface during bonding.

10. The device was removed from the water with tongs and carefully dried with a low-lint tissue. The dry device was then placed onto a clean petri dish and labeled.
11. A microscope cover glass (Fisherbrand, 45 × 50 mm) was cleaned of any residues using a 70% ethanol solution and dried with a low-lint tissue.

12. The PDMS piece was bonded to the cover glass using a plasma treatment. A high frequency generator (Electro-Technic Products, model BD-10A) was brought into close contact (within 5 mm) with the feature side of the PDMS and the face of the glass to be bonded, covering all surface area with the generated plasma arc in duplicate.

13. The PDMS piece was carefully lifted and flipped over so that the feature side was facing down and placed onto the glass surface. Gentle, non-point pressure was applied to facilitate complete surface contact and to smooth out any air bubbles at the interface.
Fig. 3.5: Images of each step in the micromixer fabrication process. A) The SU-8 mold is clean and ready for use. B) The SU-8 mold (left) is prepared for its first use with a silanizing agent (right). C) PDMS solution is prepared and poured into the mold. D) The loaded mold is vacuumed to remove air bubbles. E) After baking for two hours, the cured
PDMS is cut out from the mold. F) A biopsy punch is used to form the inlet and outlet ports. G) Adhesive tape is used to remove dust and other particulates. H) The device is boiled to leech PDMS residues from the surfaces. I) The device is bonded to a glass slide by treating the surface with plasma. J) Closer view of plasma treatment. K) The treated PDMS is placed onto the treated glass and allowed to laminate. L) After lightly pressing the PDMS to the glass, all air pockets are removed.

**Fig. 3.6**: Complete devices are kept in petri dishes to keep them clean. They are mounted to the dish with tape for use under a microscope.

### 3.4 Experimental setup and operation

A fluid delivery system was developed with the intention that tubing could be easily connected to and disconnected from devices (**Fig. 3.7**). First, a 10 mL syringe (Henke Sass Wolf, 10 mL Norm-Ject) was snugly fitted to PVC tubing (Clearflex 60, 1/8 inch inner diameter). This tube was fitted to smaller tubing (Clearflex 60, 1/16 inch inner diameter). The end of this tube was then connected to a 90 degree elbow joint (McMaster-Carr), which was then connected to a pipette tip that was trimmed to size. Connections between the syringe and tubes were made using tubing ties and the connection to the pipette tip was sealed with epoxy (Loctite). This tubing system was duplicated so that there was one for each of the two inlet ports. To collect fluid leaving
the device at the outlet, a similar tubing setup was created. A pipette tip was connected to the 1/16 inch tubing, which was connected to the 1/8 inch tubing. This tube emptied into a petri dish for disposal.

![Tubing setup](image)

**Fig. 3.7**: Images of the tubing system for the microfluidic device. (Lower Left) Overall image of a tubing-syringe set. (Upper Left) Close up of the syringe. (Upper Right) Connection between the larger and smaller tubing sizes. (Lower Right) Pipette tip connected to the elbow joint and smaller tubing size.

Each syringe was loaded into a syringe pump (either Harvard Instruments PicoPlus, cat no. 70-2213; or Razel, model no. A-99 FM) to pump fluid to the device. The pumps were both calibrated for operation at flow rates in the range of 5-500 μL/min by a bucket and stopwatch method: Briefly,

1. A plastic test tube was weighed empty.
2. The syringe to one of the pumps was loaded with water, and the tip was purged of air before testing.
3. A pump speed setting was entered into the pump, and the tubing outlet was inserted into the test tube.

4. The pump was started and the stopwatch was started. Water exiting the tubing was collected in the test tube. The amount of time spent collecting fluid varied greatly depending on the pump speed. In the case of the PicoPlus pump, the setting was flow rate (μL/min) whereas the Razell pump settings were simply a percentage (%) of the maximum speed. For each run, time was allotted such that a measureable amount of fluid was collected each time. Run times varied from 30 seconds for higher flow rates to 60 minutes at low flow rates.

5. When enough fluid accumulated, the pumps were stopped and the time was recorded.

6. The test tube was then weighed with the collected fluid and recorded.

7. Steps 2 through 6 were repeated twice, resulting in three runs per pump setting.

8. Steps 2 through 7 were repeated for various flow rates ranging from 5 to 500 μL/min.

The flow rate for each run was calculated from the collected mass and time data. First, the mass of the water collected was determined from the difference between the wet and dry masses of the tube.

\[ m_{\text{water}} = m_{\text{wet}} - m_{\text{dry}} \]  

(14)

The volume of water collected was then determined using the density of water at room temperature.

\[ V_{\text{water}} = \frac{m_{\text{water}}}{\rho_{\text{water}}} \]  

(15)
The volumetric flow rate of water for the run was calculated from the time required to collect the volume of water.

\[ Q = \frac{V_{\text{water}}}{t} \]  \hspace{1cm} (16)

For each pump speed setting, the average of the three runs was calculated, yielding pump setting versus \( Q \) data. The pump setting varied depending on the pump examined. Linear fits were performed on the data at different ranges of flow rates (5-20 \( \mu \text{L/min} \), 20-100 \( \mu \text{L/min} \), and 100-500 \( \mu \text{L/min} \)) because the slope of the data was slightly different at especially high and low flow rates. The resulting calibration curves are as follows.

**PicoPlus pump**

\[ \text{Setting } \left( \frac{\mu \text{L}}{\text{min}} \right) = 1.0236Q + 7.7485 \]  
\[ Q > 100 \frac{\mu \text{L}}{\text{min}} \]  \hspace{1cm} (17)

\[ \text{Setting } \left( \frac{\mu \text{L}}{\text{min}} \right) = 1.1064Q - 2.3886 \]  
\[ 20 \frac{\mu \text{L}}{\text{min}} \leq Q \leq 100 \frac{\mu \text{L}}{\text{min}} \]  \hspace{1cm} (18)

\[ \text{Setting } \left( \frac{\mu \text{L}}{\text{min}} \right) = 0.9958Q - 0.2462 \]  
\[ Q < 20 \frac{\mu \text{L}}{\text{min}} \]  \hspace{1cm} (19)

**Razel pump**

\[ \text{Setting } (\%) = 0.1336Q - 0.7801 \]  
\[ Q > 100 \text{ \%} \]  \hspace{1cm} (20)

\[ \text{Setting } (\%) = 0.1389Q - 0.3687 \]  
\[ 20 \text{ \%} \leq Q \leq 100 \text{ \%} \]  \hspace{1cm} (21)

\[ \text{Setting } (\%) = 0.1254Q + 0.0461 \]  
\[ Q < 20 \text{ \%} \]  \hspace{1cm} (22)

These curves yield the pump setting as a function of desired flow rate from the pump. Therefore, the flow rate is substituted for \( Q \) to calculate either the PicoPlus’ flow rate input or the Razel’s speed percentage.

The flow within the channels was imaged using an inverted fluorescence microscopy to evaluate experimental mixing quality. Solutions of 10 kDa FITC – dextran and 10 kDa rhodamine B – dextran fluorescent dyes at 10 \( \mu \text{m} \) were prepared beforehand.
and stored until needed. The dextran solutions were always kept in opaque containers away from light.

1. The syringes were each filled with one of the dextran solutions, being sure that there was no air present. While filling, the air in the length of tubing would inevitably be drawn into the syringe, so it was necessary to purge this air before proceeding. This was accomplished by drawing in about half of the syringe’s capacity, then purging the air before filling the rest of the way.

2. The two syringes were loaded into the syringe pumps and pumped briefly to purge fluid through the two connecting tips.

3. The pumps were set to the appropriate settings to produce the desired flow rate in the device using the appropriate calibration curves. The device was operated for flow conditions at \( Re = 10, R_{AB} = 1, \) and \( D_A = D_B = D_{10} \).

4. The device was positioned under the Zeiss Axio Vert.A1 fluorescence microscope (shown in Fig. 3.8) or Nikon A1 Rsi confocal microscope at this time. To better stabilize it while under the microscope, the device was taped down to its petri dish. The inlet tubes were then connected to the device via the inlet ports, and the outlet effluent tube was connected to the outlet port with the end of this tube emptying into a collection container such as a petri dish or beaker.

5. Both pumps were started simultaneously, and fluid flow within the device was monitored as it approached steady state. Images for both color (red and green) channels were taken at various locations in the device including the inlets, sections 1-6, the outlet, and arbitrary intermediate positions between section 6 and the outlet. Fluorescence imaging was performed at magnifications of 5×, 10×, and
Images were later post-processed using ImageJ (version 1.51j8) by merging each pair of images into a composite of two color channels. The confocal microscope is comprised of diode lasers and an argon multi laser. Using a 10× objective lens, the mixing of the two streams was visualized in the xz-plane in y-stacks (according to the coordinate system established in Fig. 2.1). The laser excitation of FITC and Rhodamine occurred at $\lambda = 488$ nm and $\lambda = 543$ nm, respectively. The emitted fluorescent was detected between $\lambda = 500$-$550$ nm and $\lambda = 590$-$620$ nm for FITC and Rhodamine, respectively. Images were taken using a Galvano scanner with an image size of 512 × 512 pixels with each pixel being 0.49 μm in size. At each location, a stack of 40 images was taken to cover the height of the channel. The stacks were post-processed with ImageJ in a similar manner.

6. To shut down the device, the pumps were both stopped, and the inlet tubes were disconnected from the ports and pumps. The syringe plungers were pulled back slightly to draw a small amount of air into the tips on the tubing; this prevented fluid leakage while handling the tubing.
3.5 Problems and safety

During operation of the system, it was important to be aware of potential safety concerns. First, the pressure drop required to pump fluid from the inlet to the outlet of the device was substantial, meaning that the device required time to pressurize before both fluids began moving to the outlet. It was common to see fluid flowing back and forth from one inlet to the other during startup as the two pumps “fought” against each other. This occurred until the pumps built up enough pressure to force fluid through the device. When the device was fully pressurized, there was a risk of fluid leakage at the inlet ports,
so the device was always operated in a container that could hold the liquids being fed to it.

Ultimately, the large pressure drop in the device placed a limitation on the length of channel that the pumps could feasibly push fluid through. When operating along the full length of the channels, the device encountered many problems such as leaks and mechanical failure; the PDMS could potentially be separated from the glass by the stress. As a result, the second sensor port was used as the outlet instead of the originally intended port. Therefore, only half of the original device was utilized during experiments.

At the expense of residence time in the channels, the operational reliability of the device increased dramatically as a result. Within this portion of the device, some preliminary stress testing was performed to determine its maximum flow rate tolerance. The device was able to run up to \( Re = 25 \) with no issues. At higher \( Re \) ranging from 25 to 100, small leaks were frequently observed in the connection between the tubing and device at the inlet ports, but the device was still operable. When \( Re > 100 \), the leaks at the inlet ports became considerable, which most likely would impact performance due to larger volumes of fluid failing to enter the device. The leaking at the inlets was most likely due to the way that the tubing connected to the device; the snug fitting of the pipette tip relied on static friction between the tip and surrounding PDMS to maintain a seal. The pressure at higher \( Re \) was probably too large for the tips to stay in place.

The pressure was also a concern when detaching tubes from the device after use; fluid could forcibly spray out as the pressure was released. Therefore, when operating the device, especially with hazardous chemicals, protective clothing, eyewear, and gloves should be used. To mitigate the spraying of fluid, the device was allowed to rest for
several minutes so that it could depressurize; fluid would continue to exit the outlet until the pressure dissipated.

Furthermore, it was also imperative that the device itself was handled carefully. Each device was bound to a very thin glass slide, which could break easily and cause injury to the operator. When handling the device, care was taken to avoid bending it or subjecting it to excessive force. The corners of the glass were more likely to be damaged, especially if the PDMS layer did not cover the entirety of the slide.
CHAPTER IV
RESULTS & DISCUSSION

4.1 Computational fluid dynamics simulations

Within each study, many CFD simulations were performed for each set of operating conditions, yielding spatial concentration data and plots. For all except Study 5, cross sections of the concentration profile in the channels were used to compute the mixing index $M$ at different locations and for each species. In this section, we use $M$ to refer to $M_A$ and $M_B$ collectively due to the nature of the results. Each case within a study has a set of concentration profile plots and a plot of $M$ versus section number in the device. For brevity, we present representative concentration profile plots (all in mol/L) in this section and include the rest in the Appendix.

The channel design achieved rapid mixing due to the shape of the herringbone ridges. Simulation results for the velocity profile in the channels (Fig. 4.1) indicated that the flow was warped and rotated about itself due to transverse velocity components being added. The ridges acted as troughs which redirected fluid along their lengths. Because the ridges themselves were diagonal to the direction of the channel, fluid within the ridges was transported horizontally along the $z$-axis until it reached the ridge’s vertex. At this
point, the fluid must exit the ridge, so it moved upward back into the main channel. This displaced the local fluid above the vertex, which caused rotation in the main channel reminiscent of natural convective flow. Specifically, there were two counter-rotating vortices formed due to the existence of the vertex in each ridge. If there was no vertex (i.e., a slanted groove that covered the entire width of the channel), there would be one vortex about the geometric center of the channel as seen with the SGM and CGM in Fig. 1.6 and 1.7. As a result, appreciable \( y \) and \( z \) velocity components were visible at locations just above the ridges. Fig. 4.1 and 4.2 below show the velocity plots at the seventh ridge in the channel (second ridge of section 2), which was arbitrarily selected for this analysis. These images are representative of all ridges at all locations in the studied geometry; obviously, the ridges that are in the mirrored configuration of this ridge yield a mirrored velocity field. The neighboring ridges were hidden from view to better show the velocity field in the single ridge.
Fig. 4.1: A) The velocity profile as seen in the cross section of the channel (yz-plane shows circulation of fluid from the outside walls towards the vertex of the ridge. B) The side view (xy-plane) of the ridge shows fluid dropping downward into the ridge. The fluid moves along the ridge until it is forced upward at the vertex. C) The top-down view (xz-plane) of the channel shows some horizontal motion being imparted on the fluid as it passes over the ridge. These arrows were plotted very close to the bottom of the main channel (y = 5 μm). D) This is the same view as in C) except arrows were plotted at many values of y as in A) and B).
Fig. 4.2: A) The velocity field in the ridge shows fluid entering the ridge from the outside and travels to the vertex in a curved path. B) The downward and upward velocity components due to the ridge can be seen more clearly by showing arrows only at $y = 5 \mu m$. C) The path of the fluid moving along the ridge is evident when only the velocity field in the ridge is shown.

A similar analysis was performed by Itomlenskis et al.\textsuperscript{37} for the standard SHB channel design. Their geometry was similar to our design, making it highly comparable; the ridges and width of the main channel were of identical dimensions, but the main channel’s height was 150 \( \mu m \) instead of 50 \( \mu m \). As can be seen in Fig. 4.3, similar circular flow patterns were seen in the channel cross section. The only major difference is that the direction of rotation is opposite of our results. This is obviously due to the fact that our design uses the reverse SHB configuration.
Fig. 4.3: Velocity field of standard forward SHB geometry\textsuperscript{37}. The circular flow occurs in the opposite direction of what was observed in the current study due to the reversed ridge configuration.

4.1.1 Study 1: Effect of Reynolds number

In Study 1, \( Re \) was varied over five orders of magnitude to determine the effect on mixing. It can be seen in Figs. 4.4 and 4.5 that mixing occurs rapidly because \( M \) always reaches values greater than 0.990 by the end of the sixth section in this study. Appendix A contains the other concentration profile images. The concentration profile shows the segregated colors at the inlet becoming progressively indistinct along the simulated length. The multidirectional flow is clearly seen as the two species are rotated around each other as they pass over the herringbone ridges. The velocity field from Figs. 4.1 and 4.2 is most clearly seen in the cross sectional plots within the first mixing cycle; the green color moves towards the left side of the channel above the vertex of the ridges and the red is displaced to the right side in the first section of the channel. As expected, the concentration profiles of the two species are identical and \( M_A = M_B \) because \( D_A = D_B \). The results show a very weak response to changing \( Re \); the curves in the plots of \( M \) vs section number are nearly the same. The ultimate value of \( M \) at section 6 is 0.999 for \( Re = 0.01, 0.1, 1, \) and 10, with virtually no differences in the curves except in the case of \( Re = 0.01, \)
in which $M$ takes somewhat higher values at earlier sections of the device. This is most likely due to increased residence time in the channel so that diffusion has a stronger effect. For $Re = 100$, the final value of $M$ is 0.992, suggesting slightly worse mixing quality. The effect is more evident in the overall shape of the $M$ vs section number plot compared to the other four $Re$ tested; the curve increases less rapidly, approaching the asymptotic value of 1 at a slower rate than the others. The most likely reason for this is that the overall residence time of the fluid in the simulated length is short enough at high $Re$ such that diffusion has no time to contribute to the mixing over substantial distances. The device’s design is meant to shorten the diffusion length, but it does not drop to zero. This means that no matter how finely-divided the two species become, there is some finite length that must be traversed by diffusing molecules. It just so happens that at $Re = 100$, the residence time is so short that the diffusion is not completing the mixing quite as effectively as it does in the other cases. In macroscopic applications, increasing $Re$ would normally facilitate mixing because flow becomes turbulent. However, even the highest $Re$ investigated in this work is well within the laminar regime.

It is worth noting, however, that the differences between the final value of $M$ for $Re = 100$ and those at $Re = 0.01, 0.1, 1,$ and 10 are extremely small (less than 1%). It is reasonable to conclude from this that $Re$ has no impact on the effectiveness of the device. This is also the case in the forward configuration of the SHB, as demonstrated by Strook et al. They found that the flow pattern was independent of Reynolds number for $1 < Re < 100$ as well as in Stokes flow at $Re < 1$ when the ratio of the ridge height to channel height, $\alpha$, was less than 0.3. Our results mimic this trend, although $\alpha = 1$ for our design.
Fig. 4.4: Representative concentration profile in the channels at $Re = 0.1$, $D_A = D_B = D1$, and $R_{AB} = 1$. The rotational flow generated by the ridges is most evident in the first section; green is migrating leftward toward the vertex of the ridge and red is being forced down and to the right.
4.1.2 Study 2: Effect of higher mixing cycles on mixing efficiency

Study 2 was performed using a geometry that contained twice as many mixing cycles (six) to observe the continued mixing beyond the third stage cutoff in the other studies, and the results can be seen in Fig. 4.6 and 4.7. This was a single case simulation at $Re = 1$ with equal diffusivities in components A and B. The same mixing trends in cycles 1, 2, and 3 seen in Study 1 are replicated in these results. In cycles 4, 5, and 6, the mixing simply continues towards completion. Considering that $M$ is approximately 0.990 or higher by the time the fluid enters the fourth cycle, changes in the mixing
completeness are extremely small in the asymptotic approach to 1. The successive cycles further increase $M$ until values of 1.000 are yielded by the calculations in the custom-written Mathcad program, which likely means that any deviations from perfect mixing are smaller than what can be expressed by the significant figures reported by the software. While this may appear to be uninteresting or trivial, it shows that the mixing is continued to completion without reverting to a segregated state. Laminar flow, by nature, is highly orderly to the point where fluid deformations in laminar flow can be undone by reversing the deformation sequence. The result of this simulation shows that the mixing taking place in the channels is complex enough the fluid does not form pockets of segregated species or return to a more orderly state.
Fig. 4.6: Representative concentration profile in the channels at Re = 1, $D_A = D_B = D_1$, and $R_{AB} = 1$. The continued progression of mixing is evident as the concentration becomes more uniformly distributed across the channel.
Fig. 4.7: Mixing profile for the doubled geometry. The value of $M$ continues to approach 1 in the succeeding mixing cycles.

4.1.3 Study 3: Effect of diffusivity

The effect of diffusivity and various combinations of diffusivities on $M$ was evaluated in Study 3 and is shown in Figs. 4.8 to 4.12. The concentration profiles for the other pairings investigated in this study can be found in Appendix B. It is apparent that the diffusion coefficient has a very weak effect on the mixing occurring in the device; all cases investigated yield $M \geq 0.989$ by the time the fluid reaches section 6 (nearly identical to the results of Study 1), and the same asymptotic trend is observed throughout all diffusivity pairings. As mentioned previously, this is most likely due to the short residence time of fluid in the studied channel length as well as the fact that diffusion is naturally a slow process. Flow at $Re = 1$ is already quite slow ($u = 0.0125$ cm/s), so the only way for diffusivity to have a significant effect on mixing is to establish an extreme situation such as $Re \ll 0.1$. However, the syringe pumps used in this study had a lower limit of 5 μL/min, corresponding to $Re = 0.67$, which means that a different experimental setup would be required.
The mixing of components of considerably different diffusivities, such as in pair 4 which utilizes D1 and D1000, does cause $M$ to approach 1.000 at a slightly lower rate than the other cases as well as create a small difference between $M_A$ and $M_B$. As molecular weight increases, diffusivity decreases, so the transport of material is hindered. This is likely responsible for the slightly slower evolution of mixing along the length of the channel. This result is in agreement with work conducted by other groups. Liu et al.\textsuperscript{54} observed in the standard SHB that diffusive mixing components were enhanced at lower $Re$ and higher diffusivity. This group used a geometry with different dimensions, but the general observation is in agreement with our results. However, it is important to note that whether the diffusivities are larger, smaller, or very different from each other, the overall result is largely the same each time. This was also noted by Itomlenskis et al.,\textsuperscript{37} who determined that diffusion’s contribution to mixing at $Re \approx 1$ is no more than 10\% for the SHB mixer.
Fig. 4.8: Representative concentration profile in the channels at $Re = 1$, $D_A = D_1$, $D_B = D_{1000}$, and $R_{AB} = 1$. Changing the diffusivities yielded similar results across all combinations, which were also very similar to the results in Study 1.
Fig. 4.9: Mixing index results with respect to channel position and \( D_B \), with \( Re = 1 \) and \( R_{AB} = 1 \). In these plots, \( D_A = D1 \).

Fig. 4.10: Mixing index results with respect to channel position and \( D_B \), with \( Re = 1 \) and \( R_{AB} = 1 \). In these plots, \( D_A = D10 \).
Fig. 4.11: Mixing index results with respect to channel position and $D_B$, with $Re = 1$ and $R_{AB} = 1$. In these plots, $D_A = D_{100}$.

Fig. 4.12: Mixing index results with respect to channel position and $D_B$, with $Re = 1$ and $R_{AB} = 1$. In these plots, $D_A = D_{1000}$.

These results are also in agreement with the values of $Pe$ that occur in the system. Table 4.1 shows $Pe$ calculated using Equation 11 for each $Re$ simulated. The value of $D$ was taken to be $D_1$, since it is the largest. It can be seen that $Pe >> 1$, implying that convective transport is vastly more significant than diffusive transport, even when $D$ is largest for the lowest molecular weight. This is within expectations because while the
laminar fluid flow is devoid of convective turbulence, mixing is driven by transverse flows generated by the herringbone ridges. The calculated \( Pe \) are also consistent with diffusion being too slow of a process to be significant in the evolution of mixing. Strook et al.\(^{35} \) also investigated mixing performance of their SHB mixer as a function of \( Pe \). Over the range of \( 2 \times 10^3 < Pe < 9 \times 10^5 \), the channel length required for 90% mixing changed by a factor of 2.4. According to the authors, this is considered to be a small change. Our mixer falls within this range of \( Pe \), but the mixing length did not change appreciably across all cases discussed thus far. This may be due to differences in the channel designs, or in the evaluation of mixing. Strook’s group evaluated mixing by the standard deviation of the intensity distribution from confocal images.

**Table 4.1**: Calculated \( Pe \) for each \( Re \) in this work at \( D = D1 \), which is the largest of the four diffusion coefficients examined.

<table>
<thead>
<tr>
<th>( Re )</th>
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<tr>
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<tr>
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### 4.1.4 Study 4: Effect of \( R_{AB} \) on mixing efficiency

The effect of \( R_{AB} \) was investigated in Study 3 with the results exhibited in Fig 4.13 to 4.17, and the results are more dramatic than the previous studies. Appendix C contains the concentration profiles of the other cases in this study. It is apparent that the mixing becomes less effective as \( R_{AB} \) is increased. \( M \) still approaches an asymptotic maximum over the length of the channel, but the value of this maximum is smaller at larger \( R_{AB} \). This is illustrated by the section 6 value of \( M \) for both components being
0.990 at $R_{AB} = 1$, whereas $M = 0.920$ and $0.810$ at $R_{AB} = 2$ and $3$, respectively. This is most likely due to less contact between the two fluids, since a larger volume of the channels is occupied by component A when $R_{AB} > 1$. There is less contact area between components A and B, resulting in more pockets of component A taking longer to encounter component B. This trend occurs consistently when repeated at various values of $Re$. 
Fig. 4.13: Representative concentration profile in the channels at Re = 10, $D_A = D_B = D_{10}$, $R_{AB} = 3$
Fig 4.14: Effect of $R_{AB}$ on $M$ with $D_A = D_B = D1$. Reynolds number was varied in this study. Here, $Re = 0.1$.

Fig 4.15: Effect of $R_{AB}$ on $M$ with $D_A = D_B = D1$. Reynolds number was varied in this study. Here, $Re = 1$. 
Fig 4.16: Effect of $R_{AB}$ on $M$ with $D_A = D_B = D1$. Reynolds number was varied in this study. Here, $Re = 10$.

Fig 4.17: Effect of $R_{AB}$ on $M$ with $D_A = D_B = D1$. Reynolds number was varied in this study. Here, $Re = 100$.

Studies showing the effect of the flow rate ratio of the fed components seem to be very uncommon, so drawing literature comparisons was difficult. One study by Kastner et al.\textsuperscript{55} involved formulating liposomes from 1,2-dioleoyl-3-trimethylammoniumpropane (DOTAP) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) with a SHB
mixer consisting of a channel with 200μm width and 78 μm height and ridges that were 50μm wide and 31 μm deep. The flow rate ratio (FRR) of DOTAP and DOPE was varied from 1 to 5 and the total flow rate (TFR) was varied from 0.5 mL/min to 2 mL/min. The results show that as FRR increased, liposome size decreased, polydispersity decreased, and transfection efficacy decreased initially but then increased marginally. This was attributed to a lower amount of solvent present in the mixture, so only smaller liposomes could be produced. These results agreed with our results in that the minority fluid was spread more thinly across the channels.

4.1.5 Study 5: Transient study

In the time-dependent studies, the concentration profile was observed to develop in the same manner for all Re investigated (Fig. 4.18). The other cases in this study are found in Appendix D. A “front” of fluid containing the two components side by side passes through the channel, leaving behind the steady state concentration profile seen in the previous studies. As soon as the initial front of fluid moves through a location, the steady state profile is immediately established. This behavior is within expectations since the laminar flow produces the same flow pattern nearly instantaneously. In other words, the flow pattern that has been shown thus far is not only effective at mixing, but it is also consistent and develops the same way each time. It is for this reason that the flow is not truly “chaotic,” as described previously in Chapter I. The only effect that Re had on the approach to steady state was the amount of time required. Table 4.2 shows the approximate times required to reach steady state for each Re investigated. This was determined by analyzing the resulting video file for each simulation and noting the time
at which the “front” of fluid passed through the outlet of the third cycle. Not surprisingly, the times were different by one order of magnitude for each increase in $Re$ by one order of magnitude.

![Figure 4.18](image)

**Fig. 4.18**: Transient development of flow in the channels at $Re = 0.1$, $R_{AB} = 1$, $D_A = D_B = D_{10}$. Snapshots were taken at A) $t = 0.867$ s, B) $t = 5.47$ s, and C) $t = 11.6$ s.

**Table 4.2**: Amount of time required to develop steady state concentration profile with respect to $Re$.

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4.2 Tracing fluid flow using fluorescence and confocal microscopy

Representative fluorescence images taken along the simulated channel length, obtained using Zeiss Axiovert A1 inverted fluorescence microscope, are shown in **Fig. 4.19**. The two different fluids were clearly shown to meet and travel along the inlet channel which leads to the first mixing cycle. The two fluids remained segregated as they flowed side by side in this portion, which featured no ridges. In each successive mixing cycle, the fluids underwent mixing as evidenced by the red and green colors becoming continuously less distinctive. The fluids visibly migrated across the entire width of the channel and became more evenly distributed. At the outlet, the mixed fluids left the
device together with no distinction between red and green. The limitation of this imaging method was that there was no definitive evidence that the mixing was occurring exactly the same way as shown in the CFD results. These images could only show the collapsed 2.5D view of the channels, which provided very little information on what was taking place inside the channels at every \(xz\) or \(xy\) plane. To make up for these limitations, the experiment was repeated using a confocal microscope.

**Fig. 4.19:** Fluorescent images of fluid passing through the microfluidic device at \(Re = 10\), \(R_{AB} = 1\), and \(D_A = D_B = D_{10}\). A) Inlet juncture, B) entrance to the first mixing cycle, C) midway through first mixing cycle, D) entrance to second mixing cycle, E) midway through second mixing cycle, F) entrance to third mixing cycle, G) midway through third mixing cycle, H) end of third mixing cycle, I) Outlet of device. The fluids clearly started off as separate species and became progressively less distinct along the length of the device.
The device was imaged under similar conditions using a confocal microscope. The advantage that the confocal microscope offered was the ability to visualize the flow in multiple layers, each 1-2 μm thick. This allowed us to visualize the flow patterns occurring inside of the channels, instead of a simple top-down view that can only show the channels from the outside. By scanning the channels at many planes, we can more closely compare to the CFD results. This technique appears to be the ideal way to image microfluidic flow, as shown in numerous studies in literature. Confocal images at each of the six studied sections are shown in Figs. 4.20 to 4.25 alongside their respective CFD concentration profiles for select positions along the y-axis. The complete stacks of images are shown in Fig. 4.26 as 3D volume renders using ImageJ. The resemblances between the experimental and simulated flow visualizations are striking. Nearly every detail in the concentration profile computed in COMSOL was replicated in the experimental results, which makes for a strong case that the simulations accurately predicted the real behavior. One limitation of these results is that they were taken along the xz-plane of the system defined for COMSOL simulations. To evaluate the mixing index, the channel cross section in yz-planes is required. The confocal microscope we used did not have capabilities to take slices in any other directions, and reorienting the device was not feasible. Studies in the literature that use confocal imaging commonly show channel cross sections to illustrate mixing mechanisms or to quantify the mixing.

To truly compare to the simulations, quantification of the image stacks is required in future work. This would involve converting each stack of images into a 3D matrix that would effectively be used to reconstruct the cross section of the channel. The result would be intensity data along the cross section that could be used to calculate $M$ as in the
simulation data. Generally, confocal data has been shown to closely match simulation data when comparing cross sections. The work of Amini et al.\textsuperscript{56} and Williams et al.\textsuperscript{38} among others has demonstrated that CFD can accurately predict and compliment confocal microscope images.

\textbf{Fig. 4.20:} Confocal images of the device (Left) with the COMSOL simulation counterpart (Right) for section 1 at $Re = 10$, $R_{AB} = 1$, and $D_A = D_B = D_{10}$.

\textbf{Fig. 4.21:} Confocal images of the device (Left) with the COMSOL simulation counterpart (Right) for section 2 at $Re = 10$, $R_{AB} = 1$, and $D_A = D_B = D_{10}$. 

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Fig. 4.22: Confocal images of the device (Left) with the COMSOL simulation counterpart (Right) for section 3 at $Re = 10$, $R_{AB} = 1$, and $D_A = D_B = D_{10}$.

Fig. 4.23: Confocal images of the device (Left) with the COMSOL simulation counterpart (Right) for section 4 at $Re = 10$, $R_{AB} = 1$, and $D_A = D_B = D_{10}$. 
**Fig. 4.24**: Confocal images of the device (Left) with the COMSOL simulation counterpart (Right) for section 5 at Re = 10, R_{AB} = 1, and D_A = D_B = D10.

**Fig. 4.25**: Confocal images of the device (Left) with the COMSOL simulation counterpart (Right) for section 6 at Re = 10, R_{AB} = 1, and D_A = D_B = D10.
Fig. 4.26: 3D rendering of confocal image stacks for all locations examined.
CHAPTER V
CONCLUSIONS & FUTURE WORK

A microfluidic reverse staggered herringbone mixer was designed, fabricated, characterized, and implemented in both CFD simulations and experiments. The following list summarizes the main observations and conclusions drawn from this work.

- CFD simulations predicted that mixing in the device would be nearly perfect by the third complete mixing cycle, with mixing index values very close to 1 at this stage in the device under most operating conditions. Similar behavior has been seen in other variants of the SHB mixer in the literature.

- Of the variables manipulated in CFD simulations, the value of $R_{AB}$ had the strongest effect on the mixing completeness in the simulated channel length. $Re$ had a very weak effect on the mixer’s performance while the values of $D_A$ and $D_B$ were virtually inconsequential to the results.

- The extended simulated channel length indicated that mixing continued to completion without any reversal of mixing progress.
• Time-dependent CFD simulations showed that steady state flow was established immediately after introducing fluid to the channels. The time to reach steady state decreased with increasing $Re$.

• The device was physically constructed using standard soft lithography techniques and was operated to verify the simulation results. While the fluorescence microscope showed that the flow patterns in the device were at least approximately close to the CFD prediction, the confocal imaging confirmed it by providing a look inside the channels rather than just from the outside.

• It was evident that the simulations in COMSOL accurately predicted the real behavior of the device due to the close resemblance to the confocal imaging. However, the image stacks produced from confocal microscopy need to be quantified (i.e., calculating $M$ in a similar manner to the CFD results) to verify this.

• The staggered herringbone channel design proved to be effective at mixing two fluids together despite the limitations imposed by laminar flow.

A proposed application for this mixer in future work is to synthesize nanoparticles, which is a process that benefits greatly from rapid and uniform mixing. Future work will build on this study by testing the device’s ability to synthesize nanoparticles in experiments. The design of the device may also undergo optimization as some unforeseen practical issues developed in the device fabricated for this study. Chiefly, the operational problems associated with the device’s pressure tolerances are the biggest obstacles, rendering half of the fabricated device unusable. Changes in design parameters such as channel dimensions, spatial arrangement of channels in the overall
device, and stronger connectors to the inlet ports are options to consider when addressing this issue. A more robust fluid delivery system may also benefit the device’s setup. In its current state, the device undergoes a transient startup period as described in Section 3.5. As the pumps pressurize the device, they tend to “fight” each other until fluid flow is fully established, which can cause fluid to flow backwards in one of the inlets. This may be a significant problem especially in reaction applications because this would expose the reactants to each other in the inlet tubing. Possible consequences of this include deposition of solids such as nanoparticles in the tubing which could clog the connecting pipette tips, or an out of control reaction scenario due to the larger volumes of reactants being stored in the tubing compared to the device. Therefore, future work should investigate the development of a tubing system that incorporates a check valve or something similar.
REFERENCES

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APPENDIX A: STUDY 1 CONCENTRATION PROFILES

Fig. A.1: $Re = 0.01$, $R_{AB} = 1$, $D_A = D_B = D1$

Fig. A.2: $Re = 1$, $R_{AB} = 1$, $D_A = D_B = D1$
Fig. A.3: $Re = 10$, $R_{AB} = 1$, $D_A = D_B = D1$

Fig. A.4: $Re = 100$, $R_{AB} = 1$, $D_A = D_B = D1$
APPENDIX B: STUDY 3 CONCENTRATION PROFILES

Fig. B.1: $D_A = D1, D_B = D1, Re = 1, R_{AB} = 1$

Fig. B.2: $D_A = D1, D_B = D10, Re = 1, R_{AB} = 1$
Fig. B.3: $D_A = D1$, $D_B = D100$, $Re = 1$, $R_{AB} = 1$

Fig. B.4: $D_A = D10$, $D_B = D10$, $Re = 1$, $R_{AB} = 1$
Fig. B.5: $D_A = D10, D_B = D100, Re = 1, R_{AB} = 1$

Fig. B.6: $D_A = D10, D_B = D1000, Re = 1, R_{AB} = 1$
Fig. B.7: $D_A = D_{100}, D_B = D_{100}$, $Re = 1$, $R_{AB} = 1$

Fig. B.8: $D_A = D_{100}, D_B = D_{1000}$, $Re = 1$, $R_{AB} = 1$
Fig. B.9: \( D_A = D1000, D_B = D1000, Re = 1, R_{AB} = 1 \)
APPENDIX C: STUDY 4 CONCENTRATION PROFILES

Fig. C.1: $Re = 0.1$, $R_{AB} = 2$, $D_A = D_B = D10$

Fig. C.2: $Re = 0.1$, $R_{AB} = 3$, $D_A = D_B = D10$
Fig. C.3: \( \text{Re} = 1, R_{AB} = 2, D_A = D_B = D10 \)

Fig. C.4: \( \text{Re} = 1, R_{AB} = 3, D_A = D_B = D10 \)
Fig. C.5: $Re = 10$, $R_{AB} = 2$, $D_A = D_B = D10$

Fig. C.6: $Re = 100$, $R_{AB} = 2$, $D_A = D_B = D10$
Fig. C.7: $Re = 100$, $R_{AB} = 3$, $D_A = D_B = D_{10}$
APPENDIX D: STUDY 5 CONCENTRATION PROFILES

Fig. D.1: Top row: Time evolution of mixing for Re = 1, R_{AB} = 1, D_A = D_B = D10. Snapshots were taken at A) t = 0.112 s, B) t = 0.521 s, and C) t = 1.24 s. Middle row: Time evolution of mixing for Re = 10, R_{AB} = 1, D_A = D_B = D10. Snapshots were taken at D) t = 0.006 s, E) t = 0.041 s, and F) t = 0.118 s. Bottom row: Time evolution of mixing for Re = 100, R_{AB} = 1, D_A = D_B = D10. Snapshots were taken at G) t = 0.001 s, H) t = 0.009 s, and I) t = 0.039 s.
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