Simulations and Experimental Analysis of High-Aspect-Ratio Diffusive Micro-Mixers

Amit Maha
Louisiana State University and Agricultural and Mechanical College

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A Thesis

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by

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<tr>
<td>ρ</td>
<td>Fluid density</td>
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<tr>
<td>ν</td>
<td>Kinematic viscosity</td>
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<td>ΔP</td>
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<td>Volume of fluid, m³</td>
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<tr>
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<tr>
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<tr>
<td>w</td>
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Abstract

Passive (diffusional) mixing has been used in designing high-aspect-ratio micro-mixers for the purpose of performing the Liagase Detection Reaction (LDR). A simple model was used to design such mixers optimized for pressure drop or time required to deliver a prescribed volume of mixture. The types of mixers considered are simple, cheap, and durable and can perform over a broad range of volumetric flow rates at reasonably modest pressure drops. The fluids typically have a very low diffusion coefficient of $1.2\times10^{-10}\text{m}^2/\text{s}$, and thus diffusional mixing can only be effective in high-aspect-ratio micro-channels. A realizable aspect ratio of 6 has been considered initially because it is easily releasable using the LIGA technique.

Numerical simulations were performed on various diffusional-based micromixer configurations. Two variants of a Y-type mixer with contraction and several variants of a mixer employing jets in cross-flow have been simulated. The various mixers have been evaluated in terms of volumetric mixing efficiencies and maximum pressure drops. One of the mixers with jets-in-cross-flow was found to perform best. In addition, the effect of jet width and expansion after the mixing were assessed.

Experimental validations for the jets-in-cross-flow mixer were performed. The mixer was manufactured using a micromilled brass mold insert hot embossed into a Polymethylmethacrylate (PMMA) substrate, which was then covered with 0.125mm PMMA coverslip. A chemiluminescence technique was applied for the first time to make qualitative observations of the mixing zones. Quantitative mixing efficiency experiments were performed by using Rhodamine B fluorescent dye solution and de-ionized water. The experimental results show good agreement with numerical simulations.
Chapter 1. Introduction

Microfluidic devices have been the focus of a rapidly increasing amount of research activity in recent years. These microfluidic devices are being used widely in the areas of biology and biotechnology. Applications include DNA assays, cell sorting, high throughput screening, chemical reactors and many more. There have been various mixing designs available in the literature (Nguyen et. al., 2005). Some of the designs include simple T-shape and Y-shape mixers. The requirements for mixing fluids in a small length and time have also led to the development of dynamic mixers. Most of the available mixers have been designed by etching in silicon substrates. The bio-chips of interest here are used in combinations with surface modifications on the microchannels for performing various tests for different applications. Silicon chips are not very easy to be adapted for such applications, and thus current work at LSU has been oriented towards the development and use of polymer bio-chips.

Our research effort has focused on designing and developing an integrated microfabricated microfluidic mixer to carry out the Ligase Detection Reaction (LDR) assay for the detection of low abundant cancer diagnostic markers. In the LDR technique, a solution mixture (consisting of 1 to 5 different chemical reagents of various concentrations, which are used depending on the nature of diagnostics) is mixed with the product of the Polymerase Chain Reaction (PCR). Therefore, an effective micro-mixer is required for preparing various compound solutions. Mixing in microchannels is challenging. Under typical operations, flows in these channels are laminar (Re<1) and the benefits of turbulent mixing are not present. Transport is dominated by diffusion, which is a slow process. Thus many efforts have been directed towards the development of “dynamic” micromixers, which
bring to bear additional complexity and associated difficulties in fabrication, implementation and testing. In an effort to take advantage of High Aspect-Ratio-Microstructure technology, we have focused on passive (diffusional) mixing designs in high aspect-ratio microchannels.

The characteristic Diffusion Length scale is proportional to \((D_t)^{1/2}\) (where \(D\) is the diffusion coefficient and \(t\) is the time needed for diffusion). It is evident that to reduce the time for complete mixing, the diffusion length must be reduced. Hence, small width channels must be considered for mixing. Increasing the contact surface area between the fluids can enhance diffusional mixing. Increasing the depth of the channels in the mixers lead to large contact surface area of the mixing fluids. As the flow rates required for mixing large volumes of fluids are increased, the pressure drop and the mixing lengths required for mixing the fluids will increase. Therefore, reducing mixing lengths and more importantly pressure drop are also additional aspects to be considered in designing diffusion-based micromixers. A simplified analysis has been carried out on diffusion mixers for arriving at optimum designs in terms of time-to-full-mixing for a given mixture volume and set pressure drop. In addition, a variety of mixers were designed and simulated. Some of the mixing strategies involve bringing the different fluids in small aspect ratio (larger width – larger crosssectional area - lower pressure drop) channel and then combining them in a narrow mixing channel of minimum pressure drop for the production of the required volume of mixture. At the end of all the mixing channels, the mixed fluid can be directed via an expansion into a lower aspect ratio channel (and take advantage of the associated pressure recovery).

The devices designed were comprised of micro-channels of widths ranging from 20 mm to 100 mm and aspect ratios ranging from 3-20. The numerical predictions for mixing of fluids were performed using commercial software (Fluent Inc., Lebanon, NH) by solving the multi-dimensional diffusion equation coupled with the equations of motion. The samples and
the reagents that would be mixed have $D=10^{-7}-10^{-5}$ cm$^2$/s at room temperature. An average diffusion coefficient of $10^{-6}$ cm$^2$/s was used for the simulations. The total flow rates used were of the order of tens of nanoliters per second, with equal flow rates of incoming constituents. The mixing efficiencies for various micro-mixer designs were estimated and compared with numerical simulations.

A review of various micromixers from the literature is provided in Chapter 2. In addition, a simple theory for estimating mixing lengths, channel widths and mixing times are also presented. Chapter 3 provides numerical simulation results on various types of micromixer designs. The experimental results are compared with numerical simulations in Chapter 4. Finally future work and conclusions are provided in Chapter 5.
2.1 Literature Review

It is difficult to mix solutions in microchannels because flows in these channels are laminar, and mixing is primarily based on diffusion of species across the channels. The literature on micromixing can be classified into two types of mixers: active mixers and passive mixers.

2.1.1 Active Micromixers

Active mixers use various techniques by applying external forces and active control of the flow field to enhance mixing. In one of the earliest active micromixers pressure field disturbance was used. Deshmukh et al (2001) reported a T-mixer using pressure disturbance. The mixer was fabricated in silicon using Deep Reactive Ion Etching (DRIE). An integrated planar micropump drives and stops the flow in the mixing channel to divide the mixed liquids into serial segments and make the mixing process independent of convection. Another alternative method to pressure disturbance is the generation of a pulsing velocity Niu et. al. (2003). The pressure disturbance was achieved by a source–sink system controlled using a computer. This design is partly similar to that of Evans et al (1997). The performance of the mixing process was related to the pulse frequency and the number of mixing units. Volpert et al (1999) developed an active micromixer for improving the mixing of two fluids in a microchannel. The flow through the main channel of the micromixer was unsteadily perturbed by three sets of secondary flow channels, enhancing the mixing. Lee et al (2001) and Niu et al (2003) designed a micromixer, which employed unsteady pressure perturbations superimposed on a mean stream to enhance the mixing. Oddy et al (2001) developed an electrokinetic process to stir micro- and nanoliter volumes using sinusoidal
oscillation of electroosmotic flow. Solomon et al (1996) performed experiments comparing long-range chaotic mixing of miscible and immiscible impurities in a time-periodic flow by passing current to produce an alternating magnetic field resulting in producing alternating vortex structures. Suzuki et al (2002 and 2003) produced a micromixer based on magnetic force inducing mixing on flow seeded with magnetic beads. Hong et al (2003) performed numerical study of mixing on a herringbone pattern based on a configuration of Stroock et al (2002). Lu et al (2002) developed a moving magnetic bar, which rotated by rotating magnetic field and caused mixing in the channel. The micromixer design was based on numerical predictions. Rapid mixing was found in a large chamber with a 3X3 mixer array rotating at 600 rpm. Erickson et al (2002) performed numerical predictions of microfluidic mixing in a T-shaped channel using electrokinectically driven fluids and influencing the surface heterogeneity on the channel walls. The predictions indicated that by introducing these heterogeneous regions on the wall, the mixing channel length could be reduced by 70%.

### 2.1.2 Passive Micromixers

In passive mixers no external energy source is required as an input for enhancing the mixing mechanism. Although active mixers may effectively provide rapid mixing, the additional mechanical and electronic devices add complexity. These additional devices used in these mixers need extra energy and may be difficult to fabricate or integrated on a Lab-on-A-Chip device and may not be suited for various type of reagents. Additionally, the electrical field and heat generated by active control may damage biological samples (Chung et al, 2004). Different methods and substrates have been used to fabricate each, but it is generally agreed that passive mixers are easier to fabricate and simpler in design than active mixers. Song et al (2003) performed mixing by chaotic advection in droplets by moving them in
microchannels. Stroock et al (2002) presented a passive method for mixing streams of steady pressure-driven flows in microchannels at low Reynolds number by chaotic advection. The length of the channel required for mixing grows only logarithmically with the Peclet number, and hydrodynamic dispersion along the channel is reduced relative to that in a simple, smooth channel. Wong et al (2004) fabricated micro T-mixers on a silicon substrate covered with a Pyrex glass plate to enable observation and characterization of mixing performances. The goal was to test the feasibility of using T-mixers for rapid mixing. It was shown that for a micro T-mixer with a mixing channel having a hydraulic diameter of 67 µm, an applied pressure of 5.5 bar was sufficient to cause complete mixing within less than a millisecond after the two liquids made contact. Chung et al (2004) proposed microfluidic self-circulation in a mixing chamber to improve mixing performance. The mixing chamber was 4 mm in diameter and 500 µm deep, and the two channels, 500µm x 500 µm in cross-section, for a total volume of 20 µL. The self-circulation of a microfluid in the mixing chamber was achieved by pumping of the working fluids from opposite ends in a circular chamber. Bertsch et al (2001) studied two geometries, a series of stationary rigid elements that formed intersecting channels to split, rearrange and combine component streams and a series of short helical elements arranged in pairs; each pair comprised of a righthanded and left-handed element arranged alternately in a pipe. Song et al (2003) described an experimental test to predict scaling of mixing of solutions by chaotic advection inside droplets that move through winding microchannels. Glasgow et al (2003) demonstrated the merits of flow rate time dependency through periodic forcing. Their study used mixing in a simple "T" channel intersection by numerical simulation and experimentally mixing two aqueous reagents. The channels segments were 200µm wide by 120 µm deep. Knight et al (1998) demonstrated mixing on a silicon chip by hydrodynamically focusing of fluorescein. The mixers were
etched on silicon comprising of a rectangular cross section of depth 10µm. The inlet streams are controlled by the ratio of side to inlet pressure ratios. Hibara et al (2001) utilized multiplayer flow of fluids in a 70µm wide and 30µm channel to observe miscible liquids water and acetone interface. Shastri et al (1998) performed experiments by sending fluids through two concentric capillary tubes. Pabit et al (2002) also used coaxial capillaries with ID 20µm and 100µm for the two fluids. The process yields similar result to that of hydrodynamically focusing by Knight et al (1998). Here the flow from the inner capillary is squeezed by the flow from the annular region surrounding the inner capillary tube. Liu et al (2004) developed a two-fluid mixing by creating a three-dimensional serpentine channel and compared the results to that from a square wave channel and the herringbone pattern. Liu et al (2000) also performed passive mixing in three-dimensional serpentine microchannels. The serpentine design enhances chaotic advection and improves mixing. Gobby et al (2001) performed numerical simulations to study the characteristics of T-type micromixers with varying inlet angles. The simulations were performed for mixing gases of different viscosity, operating in laminar flow regime. Wang et al (2003) also performed numerical investigations with patterned grooves. These grooves are similar to the groves produced in the herringbone pattern. Park et al (2004) produced micromixer with a breakup process of splitting the fluid at each stage of the repeating mixer design unit. Veenstra et al (1999) designed a diffusional micromixer in which two-inlet fluids are brought into a small width microchannel to reduce the overall length required for diffusion and the mixture is expanded into a wide channel. The exit is split into two equal channels and the extent of mixing is studied in one of the exit channel from the split. Therrialult et al (2003) described mixing in three-dimensional microvasculator networks by chaotic mixing. The network of channels consists of smooth cylindrical channels (10-300µm). The network is similar to the serpentine model, where the
channels form a complex three-dimensional right-angled bend channels. Wang et al (2003) performed numerical predictions of placing rectangular obstacles at different orientation with the channel to enhance mixing. Stroock et al (2002) developed chaotic mixer for microchannels that had herringbone pattern in a 200µm wide microchannel. This pattern helps in rotating the fluid along the streamwise direction.

The development of micromixers has been progressing rapidly in recent years. From the early devices made of silicon and glass, a number of polymeric micromixers have been fabricated and successfully tested. Due to their simple designs, passive micromixers found the most applications in analytical chemistry. While conventional parallel lamination mixers work well at low Reynolds numbers and low Peclet numbers, micromixers based on chaotic advection can be designed to suit a wide range of Reynolds numbers. Mixing with chaotic advection does not depend on the Peclet number. Appendix F provides a comparative table for various active and passive micromixer designs from the literature.

2.2 **Simple Theory**

Consider a simple micro-scale binary diffusion mixer as a rectangular microchannel with two-equal area inlets shown in Figure 2.1. In addition, define the following variables and parameters:

- \(Q_1\): Flowrate on fluid 1
- \(Q_1\): Flowrate on fluid 2
- \(Q\): Total Flow rate (\(Q_1 + Q_2\))
- \(w\): Width of the microchannel
- \(H\): Height of the microchannel
- \(L_m\): Length of the microchannel required for complete mixing
- \(AR\): Aspect ratio = \(H/w\)
O: Volume of the mixture required or desired

$D_{12}$: Binary mass diffusion coefficient

$\mu$: Fluid viscosity

$\rho$: Fluid density

Furthermore, the assumptions that the two fluids that are mixed are dilute solutions, which have similar viscosities ($\mu_1=\mu_2$), densities ($\rho_1=\rho_2$) and are being pumped into the mixer at the same flow rates ($Q_1=Q_2$).

It is possible to define some scales to convert some to the above defined parameters into non-dimensional form (subscript ‘s’ refers to scales, subscript ‘p’ refers to variables after being scaled and subscript ‘o’ refers to optimal solution).

Length scale, $L_s = O^{1/3}$
Time scale, $t_s = \frac{(L_s)^2}{D_{12}}$

Flow rate scale, $Q_s = L_s \cdot D_{12} = L_s \cdot \frac{\nu}{Sc}$

Pressure scale, $p_s = \rho(\nu/L_s)^2$

The time required for complete mixing is the same as the sum of time for the diffusion to the half width of the channel and the time required to produce the required volume of mixed fluids

$$\text{Time for complete mixing} = \frac{w^2}{4D_{12}} + \frac{O}{Q} \quad (1)$$

Channel length = (average velocity) * (time to diffuse half width of the channel)

$$\text{Channel length} = \frac{Q}{4 \cdot AR \cdot D_{12}} \quad (2)$$

The parameters such as length of mixing channel, time to complete mixing, pressure drop, flow rate, width of the channel and aspect ratio (that influence the performance of a simple micromixer) can be expressed in terms of the scaled variables defined above:

$$\text{Length of Mixing Channel}, \quad L_{dp} = \frac{Q_p}{4 \cdot AR} \quad (3)$$

$$\text{Time to Complete Mixing}, \quad t_{M,p} = \frac{w_{p}^2}{4} + \frac{1}{Q_p} \quad (4)$$

Pressure drop (from laminar flow in rectangular channels),

$$\Delta p_p = -\frac{Q_p^2}{\frac{1}{3} \cdot AR^2 \cdot w_{p}^4 \cdot Sc \cdot \left[ 1 - \frac{1}{AR} \cdot \frac{192}{\pi^5} \cdot \sum_{n=1,3,5}^{\infty} \frac{1}{n^5} \tan \left( n \cdot \frac{\pi}{2} \cdot AR \right) \right]} \quad (5)$$
The pressure drop is a function of flowrate, aspect ratio and width of the channel. This micromixer can be used in two ways:

a) To produce continuous flow of mixed solution (mixing time is a function of flow rate and aspect ratio only).

b) To produce a required volume (O) of mixed solution.

Biomedical applications typically require a fixed volume of mixed solution. For producing a fixed volume of mixture volume the optimal conditions are subjected to variables of channel width, aspect ratio, flow rate, and pressure drop. By limiting any two parameters, it is possible to optimize the remaining parameters. Some limits for these parameters are set due to manufacturing constraints (aspect ratio) and available power requirements (pressure drop to drive the fluid through the channels). Therefore, an optimal solution for channel width, time for mixing and flow rate can be solved by setting limits on the aspect ratio and pressure drop. An optimal time to produce a fixed volume of a mixed solution can be estimated in terms of aspect ratio and pressure drop. The corresponding optimal channel width and volume flow rate can also be calculated.

Optimal channel width, \( w_{po} = \frac{2}{\left( Q_{po} \right)^{\frac{1}{2}}} \) \( \text{(6)} \)

Optimal time to complete diffusion, \( t_{Mpo} = \frac{2}{Q_{po}} \) \( \text{(7)} \)

Optimal volume flow rate,

\[
Q_{po} = \left[ -\Delta p_{po} \cdot \frac{16}{3} AR^2 Sc \left[ 1 - \frac{192}{\pi^{\frac{5}{2}}} \sum_{n=1,3,5} \frac{1}{n^5} \tanh \left( \frac{n \pi}{2} AR \right) \right] \right]^{\frac{1}{4}}
\] \( \text{(8)} \)
When the optimal time for complete diffusion was scaled with the optimal time for complete mixing for an aspect ratio of 1, the scaled optimal time shows a single curve for any pressure drop (Refer to Figure 2.3). The figure shows that the optimal time is reduced by 90% when the aspect ratio is increased from 1 to 100. This reduction in mixing time happens due to the reduction in length (high AR) in the diffusion direction, since the fluid needs only half the width of the channel to diffuse in order to fully mix.

The optimal channel length required for complete mixing was scaled by the optimal length for an AR of 1 is shown in Figure 2.4. For lower aspect ratios the solution asymptotically converges to an increased length ratio of 24 with respect to the optimal channel length at AR=1. For higher aspect ratios, the length in the diffusion direction is reduced and therefore a shorter length of the channel is required to move the fluid through
The optimized flow rate was proportional to the volume of the mixture to be generated and inversely proportional to the time. As the aspect ratio increased, the optimal time for complete mixing was reduced. This increased the flow rate as aspect ratio increases. Figure 2.5 shows the optimized flow rate scaled with respect to the optimal flow rate at AR=1 plotted as a function of aspect ratio. For an AR of 5, the optimized flow rate increases by 200% compared to that at an AR of 1. Another parameter to consider is the width of the channel. Width is important with respect to manufacturing limits in terms of aspect ratio and height or depth of the microchannel (depending on the process of manufacturing). It is intuitive from discussion so far that ultimately reducing the width only (changes AR) and
affects the distance in the diffusion direction (cross-stream). This reduction in width increases pressure drop. Therefore, to reduce the overall pressure drop, overall length of the channel must be minimized. Bear in mind that the pressure drop is inversely proportional to the square of the width of the channel, but only proportional to the length of the channel.

In contrast for the production of fixed mixture volume in a fixed production time, the optimal pressure drop (in microchannels with larger widths and larger cross section areas - low aspect ratios) decreases as the aspect ratio is increased. Figure 2.7 shows the optimized pressure drop scaled with respect to optimal pressure drop at AR=1 plotted as a function of aspect ratio. For an AR=2, the optimized pressure drop decreases by 80% compared to that at AR=1.
Figure 2.6: Optimized width scaled with respect to optimized width at an aspect ratio of 1

Figure 2.7: Optimized pressure drop scaled with respect to optimized pressure drop at an aspect ratio of 1 for production of finite mixture volume in a finite production time
This theory provides some basic understanding by setting constraints and limitations on some of the influencing parameters for designing diffusional micromixers. The limitation of this theory is that it does not include mixing generated due to cross-stream gradients (multi-component diffusion) and convective mixing. Numerical simulations would be an excellent tool for providing a better understanding and designing better micromixers. Numerical simulations will account for multi-component diffusion and convective mixing along with diffusional mixing. Chapter 3 deals with the concepts and designs for various types of micromixers. The performance of these designs will be evaluated using numerical simulations.
Chapter 3. Design and Numerical Simulations

3.1 Objectives for the Micromixer

An integrated micromixer on a Bio-Chip is required for obtaining mixtures of small quantities of volumes (nano-liters) of reagents for various applications in the area of Biotechnology (DNA assays, cell sorting, chemical reactors etc.). In addition, these micromixers need to be simple, cheap, durable perform over a relatively broad range of flows and be of small volume. Reagents with low diffusion (Diffusion Coefficient = $10^{-5}$-$10^{-7}$ cm$^2$/s) typically are used in various applications. Furthermore, they should be able to produce mixtures relatively fast (Order of seconds-milliseconds) with good mixing efficiency (over 80%) and sustain very low pressure drop (less than 0.5psi). These specifications must be met under the condition that reagents with low diffusion in aqueous solutions (Diffusion Coefficient = $10^{-5}$-$10^{-7}$ cm$^2$/s) are typically used in the various applications, and that turbulence or hydrodynamic instabilities are untenable on the micro-scale. Invariably, almost all of the mixing enhancing schemes, passive or active, aim at reducing the diffusion length associated with the mixing device predominantly by folding contact surfaces multiple times. Reduction of diffusion length can be realized if the mixture constituents are brought into contact in high-aspect-ratio micro-channels of small width.

3.2 Design Idea

As discussed in Chapter 2, the designs available in the literature have been classified into active and passive micromixers. The active mixers use complex driving mechanisms and electronics to mix the reagents very effectively. This increases the overall complexity of and cost of the final Biotechnology product, which may be prohibitive especially if the product is to be disposable. On the other hand, passive mixers use geometrical variation to enhance
diffusional micromixing. Passive mixers are relatively easy to manufacture and do not carry any additional complexity compared to active mixers and can be easily incorporated within Lab-On-A-Chip device.

Deep and Narrow channels (High Aspect-Ratio) channels can be manufactured using the LIGA (LIGA is the German acronym for X-ray lithography (X-ray Lithographie), Electroforming (Galvanoformung), and Molding (Abformung)) technique. They provide a large contact area between the mixture constituents and reduce the length scale in the
diffusion direction, which in-turn reduces the overall time for mixing. Furthermore, the mixing channel length is reduced as is the pressure drop.

One method of reducing the overall pressure drop, albeit at the expense of increased device volume, is to start with low aspect ratio inlets (low pressure drop) and bring the reagents into a short high aspect ratio micromixer while exiting into a low aspect ratio microchannel for necessary process or analysis.

3.3 Mixer Designs

Six mixer design configurations were evaluated considered as shown in Figures 3.1 – 3.6. The parameters shown on the figures were chosen in accordance with theoretical estimates and manufacturing capabilities.

3.4 Numerical Simulation

Numerical simulations were used to examine the effectiveness of the above micromixer designs. The Navier-Stokes Equations including species transport equations were solved using the FLUENT 5.4 and 6.1.2 solvers (FLUENT Inc., Lebanon, NH). The mixing channels were meshed using hexahedral elements with grid refinement where appropriate to capture high gradient and curvature zones. The reagents that will be used in the actual LDR devices have diffusion coefficient in the order of $10^{-9}$-$10^{-11}$ m²/s. The simulations were performed for a median $D_{12} = 1.2 \times 10^{10}$ m²/s. A nominal depth of the mixer channels was 150µm and the width of the channels varied from 12.5µm to 50µm. All of the geometries used in simulations had a plane of symmetry with respect to the half depth of the channels, so only the half depth of the channels was used for carrying out the laminar flow simulations by applying a symmetry boundary conditions at the channel half depth. This allowed further refinement of the grid thus enhancing the spatial resolution of the simulations. The
coordinate system in regards to width, half-depth and the length of the mixing channels is along the x, y, and z coordinates respectively.

The total number of nodes for the simulations has varied from 1,000,000 – 1,500,000 based on the complexity of the designs for the mixers. Grid independence study was performed for each design. The numerical results presented in this thesis show the mixing results for various designs. These simulations also include pressure recovery results from channel expansions after the mixing chamber.

For all fluid flows, FLUENT solves conservation equations for mass and momentum. For flows involving heat transfer or compressibility, an additional equation for energy conservation is solved. For flows involving species mixing or reactions, a species conservation equation is solved. The equation for conservation of mass, or continuity equation, can be written as follows:

\[
\frac{\partial \rho}{\partial t} + \sum \left( \rho u_i \right) = 0 \quad (9)
\]

Conservation of momentum in the i direction in an inertial (non-accelerating) reference frame is described by:

\[
\frac{\partial}{\partial t} \left( \rho u_i \right) + \frac{\partial}{\partial x_i} \left( \rho u_i u_j \right) = - \frac{\partial p}{\partial x_i} + \frac{\partial \tau_{ij}}{\partial x_j} + \rho g_i + F_i \quad (10)
\]

where p is the static pressure, \( \tau_{ij} \) is the stress tensor (described below), and \( \rho g_i \) and \( F_i \) are the gravitational body force and external body forces in the i direction, respectively. In this study these body forces will be set equal to zero.

The stress tensor \( \tau_{ij} \) is given by:
\[\tau_{ij} = \left[ \mu \left( \frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i} \right) \right] - \frac{2}{3} \mu \frac{\partial u_i}{\partial x_i} \delta_{ij}\]  

(11)

where \( \mu \) is the molecular viscosity and the second term on the right hand side is the effect of volume dilation. FLUENT solves the multi-component diffusion energy equation in the following form:

\[\frac{\partial}{\partial t} (\rho Y_i) + \nabla \cdot (\rho \vec{v} Y_i) = -\nabla \cdot \vec{J}_i + R_i + S_i\]  

(12)

where \( Y_i \) is the local mass fraction for the \( i \)th species, \( R_i \) is the net rate of production of species \( i \) by chemical reaction and \( S_i \) is the rate of creation by addition from a dispersed phase plus any user defined sources. The above equation is solved for \( N-1 \) species where \( N \) is the total number of fluid phase chemical species present in the system. Since the mass fraction of the species must sum to unity, the \( N \)th mass fraction is determined as one minus the sum of \( N-1 \) solved mass fractions. \( D_{ij} \) is the diffusion flux of species \( I \), which arises due to concentration gradients. Using the dilute approximation, for laminar flows with \( D_{i,m} \) as the diffusion coefficient for species \( i \) in the mixture:

\[\vec{J}_i = -\rho D_{i,m} \nabla Y_i\]  

(13)

For multicomponent systems it is not possible, in general, to derive relations for diffusion fluxes containing a gradient of only one component. Maxwell-Stefan equations are used to obtain diffusive mass flux.

\[\sum_{j=1}^{N} \frac{X_i X_j}{D_{ij}} (\vec{V}_j - \vec{V}_i) = \vec{d}_i - \frac{\nabla T}{T} \sum_{j=1}^{N} \frac{X_i X_j}{D_{ij}} \left( \frac{D_{T,j}}{\rho_j} - \frac{D_{T,i}}{\rho_i} \right)\]
where \( X \) is the mole fraction, \( \bar{V} \) is the diffusion velocity, \( D_{ij} \) is the binary diffusion coefficient and \( D_t \) is the thermal diffusion coefficient. If the external force is assumed to be the same on all species and the pressure diffusion is negligible, then \( \bar{d}_i = \nabla X \).

### 3.5 Estimates Based on Theory

The theory for flow in rectangular ducts has been well established. Since, the fluids that will be used for our application are liquids, the estimated Knudsen numbers, \( \text{Kn} = \lambda_L / l \) computed for various mixer design dimensions were less than 0.001. So, the continuum hypothesis was used for estimating the flow behavior. When two fluids are brought into contact along the height of the channel cross-section, the diffusional time to full mixing can be estimated from the previous chapter as 

\[
\tau = \frac{1}{AR^2} \frac{H^2}{4D_{12}},
\]

while the channel length necessary is

\[
L = \frac{1}{4AR} \frac{Q}{D_{12}},
\]

in terms of the binary diffusion coefficient, \( D_{12} \), the total volumetric flow rate, \( Q \), the aspect ratio, \( AR \), and the channel height, \( H \). Using laminar flow theory the equation relating the pressure drop over the length of channel necessary for full mixing can be estimated as (rearranging equation (8)):

\[
-\Delta P = 3 \rho Sc_{12} \left( \frac{Q \cdot AR}{H^2} \right)^2 \left[ 1 - \frac{192}{\pi^5} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^5} \tanh \left( \frac{(2n+1)\pi AR}{2} \right) \right]^{-1},
\]

where, \( \rho \) is the density and \( Sc_{12} \) is the Schmidt number. The time required to obtain a total volume of mixed fluid, \( O \), from two streams of equal flow rates was estimated as \( t_{\text{tot}} = \tau + O/Q \). Figure 3.7 shows the relationships between the diffusion time, length, channel width and pressure drop as functions of the channel aspect ratio. In both cases the diffusion time was
1.3sec. For a modest aspect ratio (AR=6) and flow rate the pressure drop was kept at acceptably low levels (Figure 3.7 (a)). To obtain one order of magnitude higher flow rate than Figure 3.7(a), the pressure drop increased by 100-fold; which was unacceptable. Keeping the channel width fixed and increasing the channel aspect ratio by 4-fold the pressure drop was reduced to within acceptable levels (Figure 3.7 (b)). In this case the total time required to obtain 7.5μL of mixture was 11.3 seconds, while in the first case the total time for the same mixture volume was 101.3 seconds. The advantages of using high-aspect-ratio channels for diffusional mixing are clear. Consequently such channels were used to devise effective mixers for applications such as the LDR, PCR and others, especially when multiple stages of mixing are required and the pressure drop per stage needs to be minimal and the mixture production time relatively small.

In order to minimize the overall pressure drop, the diffusional mixer designs only incorporated high aspect ratio channels in the mixing region. The inlet fluid channels had relatively low aspect ratio (low pressure drop). The fluid from these inlet channels were forced into a high aspect ratio mixing chamber (higher pressure drop in the mixing region). After mixing the combined fluids were released into a low aspect ratio chamber reducing the overall pressure drop. If the aspect ratios are kept constant throughout the entire mixer, then an increased pressure drop was incurred with no benefit. The expansion of the exit stream also helps in terms of pressure recovery by converting some of the dynamic pressure into static pressure.

3.6 Numerical Simulation Results

3.6.1 Mixing Efficiency

It is important to distinguish various mixing geometries by evaluating their performance. The most important metrics of performance are an appropriately defined
\[ D_{12} = 1.2 \times 10^{-10} \text{ m}^2/\text{s} \]

Volume Flow = 0.075 \( \mu \text{L/s} \)
Height = 150 \( \mu \text{m} \), \( \tau = 1.3 \text{s} \)

\[ \text{Width (} \mu\text{m}) = 25, \quad \text{Length (mm)} = 26.04 \]
\[ \Delta P (\text{kPa}) = 10.82, \quad U (\text{mm/s}) = 20 \]

(a): Estimates based on AR=6

\[ D_{12} = 1.2 \times 10^{-10} \text{ m}^2/\text{s} \]

Volume Flow = 0.75 \( \mu \text{L/s} \)
Height = 600 \( \mu \text{m} \), \( \tau = 1.3 \text{s} \)

\[ \text{Width (} \mu\text{m}) = 25, \quad \text{Length (mm)} = 65.1 \]
\[ \Delta P (\text{kPa}) = 62.14, \quad U (\text{mm/s}) = 50 \]

(b): Estimates based on AR=24

Figure 3.7: Mixing length variation on aspect ratio
mixing efficiency and the overall pressure drop. The mixing efficiency defined by Equation 15 was used by Wang et. al. (2003) in evaluating their mixers:

\[
\varepsilon = 1 - \frac{\int_{0}^{l} |c_e - c_\infty| dx}{\int_{0}^{l} |c_i - c_\infty| dx} \times 100\%,
\]

(15)

where, \(\varepsilon\) is the efficiency of the mixer, \(c_e\) is the mass concentration distribution across the transverse direction at the exit of the mixer, \(c_\infty\) is the concentration of a completely mixed fluids, \(c_i\) is the initial concentration before mixing and \(l\) is the width of the channel. This definition ignores the fact that the concentration distribution depends on both cross-sectional coordinates. Because the concentration varies over the cross-sectional area of the exit, a modified version of Equation (15) was introduced by D. Erickson and D. Li (2002) and is given in Equation (16):

\[
\varepsilon = 1 - \frac{\frac{1}{A_e} \int_{A_e} c_e - c_\infty dA}{\sum_i \frac{1}{A_i} \int_{A_i} c_i - c_\infty dA} \times 100\%\)
\]

(16)

where, \(A_e\) is the area of the exit, \(A_i\) is the area of the inlet.

This definition of efficiency is adequate under “static” conditions. In practice, the mixing is done under flowing conditions, and what really matters is the rate at which the mixed product is produced at the end of the mixer channel exit. It is important to evaluate the mixing efficiencies in terms of flow rates. For example it is possible to have two different mixers with same the inlet area concentrations and exit concentrations having different
velocity profiles through the exit. These mixers will produce mixed products at different rates because various concentrations have different mass flow rates. The efficiency must be evaluated on how well the mixer is able to mix mass flow rates of different species. A further modification of the mixing efficiency of Equation (16) to include the flow effect is shown in Equation 17.

\[
\eta = 1 - \frac{\int_{A_e} \rho_e V_e |c_e - c_\infty| dA}{\sum_{i} \int_{A_i} \rho_i V_i |c_i - c_\infty| dA} \times 100\%
\]

(17)

where, \(c_e\) is the exit concentration distribution, \(V_e\), \(\rho_e\) are the exit velocities and densities of the mixture, \(V_i\) and \(\rho_i\) are the velocities and densities of fluids at the inlets. The ratio of flow rates given in equation (17) above can be described as the ratio of deviation of flow rate from the ideally mixed situation to the flow rate deviation from the ideal mixed flow rate at the inlets. Most of the reagents for our case have properties comparable to that of water, and thus the ratio of densities in the efficiency calculation would equal to unity. The efficiency equation (17) describes the ratio of deviation of unmixed flow rate to the deviation of unmixed flow rate at the inlet of the mixers. And one minus this ratio will provide the efficiency of mixing fluids for steady flow rates (for unsteady flows a time averaged scheme may be used).

3.6.2 Two Inlet Mixers with Contractions

This design consisted of two parallel inlets separated by a 5µm wall. The areas of the inlets were in the ratio 1:2. The fluids leaving the inlets 25µm wide for blue and 12.5µm wide for red were released into a 1mm long straight channel after which these streams are
focused into a 25µm-wide channel (3mm length) via a contraction (Y-type). The simulation results for this design are shown in Figures 3.8(a) – 3.8(e).
Fluid 1 (blue, top inlet) had an entrance width of 25 µm at a flow rate of 37.5 nL/s. Fluid 2 (red, bottom inlet) had an entrance width of 12.5 µm with the same flow of 37.5 nL/s. The maximum pressure drop obtained from the inlets to the exit of the mixer was 1552 Pa for a total flow rate of 75 nL/s. The time for the fluid to go through the mixer is approximately 0.25 s.

A second method of contraction was performed on the same inlet geometries by bending and contracting the initial channel into a single 2 mm long 25 µm wide channel with a total mixer length of 4 mm. This was done to explore possible benefits due to the flow in the bend. Additionally, this geometry was of interest to make the mixer more compact and in multiplexing configurations (Figures 3.9(a)-3.9(f)).

![Figure 3.9: Fluid mixing in a U-type high-aspect-ratio channel with parallel inlets](image)

- (a) top view of center-plane concentration contours
- (b) bend concentration details
- (c) inlet velocity and concentration distribution
- (d) stream-wise velocity contours exit region (negative numbers indicate flow in –z direction)
- (e) velocity distribution (before and after the bend)
- (f) exit-plane concentration contours (half channel depth)
The simulations carried out for the U-bend type mixer had the same flow rates and initial conditions with those used in the Y-type mixer. The maximum pressure drop obtained for this design was 1410 Pa for the same total flow rate of 75nL/s and the through-time of approximately 0.25s. Comparison of the mixing efficiencies for the Y-type and U-bend type mixers are given in Figure 3.10.

The objectives for these simulations were to evaluate mixing performance and pressure drop with a contraction and the effect of the bend on mixing. The U-bend type mixer provided better performance both in terms of mixing efficiency and pressure drop. The bend allowed the transition of the fluid into a high aspect ratio channel in a smoother fashion than an abrupt contraction leading to a higher pressure drop. The efficiency calculations based on Equation (17) showed higher values than the calculations based on Equation (16)
because most of the completely mixed regions were in the center of the channel
where the velocities were maximum (Figures 3.8(b) and 3.9(d)), that produce well mixed
volumes of the fluids.

3.6.3 Jets in a Cross Flow

This design consisted of a central straight channel with a modestly high aspect ratio
(AR=6 as before) and up to two channels with twice the aspect ratio feeding into it at 90
degrees. High-aspect-ratio jets in cross-flow introduced the second fluid into the first fluid,
which flowed in the main channel. The configuration was similar to hydrodynamic focusing
on silicon chips performed by Knight et. al. (1998) to study fast reaction kinetics. The
objectives were to evaluate mixing performance and pressure drop for one and two jets in
cross-flow, at different jet offsets and with or without contraction. The main idea behind this
design was to enhance convective mixing in the cross-stream direction as the velocity scales
in the cross-stream direction do not contribute towards mixing for laminar flows.
Figure 3.11: Fluid mixing in Jets with cross-flow (X2J) channel
(a) top view of center-plane concentration contours
(b): Stream-wise velocity contours near exit
(c): Fluid mixing from lower inlet
(d) Fluid mixing from upper inlet
(e) exit-plane concentration contours (half channel depth)
3.6.3.1 1 mm Offset Jets

The jets were set to offset by 1mm so that the pressure drop is reduced while achieving good mixing efficiency. The two fluid inlets (red) separated by a 1mm injected jets of the same fluid through a width of 12.5μm with a combined flow rate of 37.5nL/s into a 25μm channel that is carrying the second fluid (blue) also with 37.5nL/s flow rate. The area ratio of each jet inlet to that of the main mixing channel was 1:2. The simulation results for this case are shown in Figures 3.11 (a)-(e). A maximum pressure drop of 1620 Pa was
obtained for this mixer design while the time it takes the fluid to go through the mixer is approximately 0.25s.

3.6.3.2 One Jet with Contraction

The first fluid (red) was injected through a single jet of width 12.5µm with a flow rate of 37.5nL/s into a 50µm channel that was carrying the second fluid (blue), also with 37.5nL/s flow rate. Following the jet the main channel is contracted into one (25µm width) so that the aspect ratio was 6. The simulation results for this case are shown in Figures 3.12(a) – 3.12(c). A maximum pressure drop of 970 Pa was obtained for this mixer design while the time it

![Figure 3.13: Fluidic mixing for 2 jets in a cross flow with a contraction (X2JC) (a): top view of center-plane concentration contours (b): concentration variation in the contraction (c): exit-plane concentration contours (half channel depth)
takes the fluid to go through the mixer is approximately 0.25s.

3.6.3.3 Two Jets with Contraction

The efficiency calculations for the Y-type mixer showed a sudden increase in mixing in the contraction region. A case was designed with the combination of the Jets in Cross-Flow (X2J) type mixer with the Y-type mixer. The results showed that the two jets in cross-flow separated by 1mm distance prior to the contraction with individual jet channel widths of 12.5µm with equal flow rates of fluid 1 (red) totaling 37.5nL/s. These jets are injected into a
main channel 50\(\mu\)m wide carrying fluid 2 (blue) with a flow rate of 37.5nL/s. The simulation results for this design are shown in Figures 3.13(a) - 3.13(c). A maximum pressure drop of 1448 Pa was obtained for this mixer design while the time it takes the fluid to go through the mixer is approximately 0.25s.

3.6.3.4 Jets with Opposite Inlets

An alternative case on jets in cross flow was simulated where the jet inlets were opposite to each other. This simulated design similar to the hydrodynamic focusing devices of Knight et. al. (1998). The inlet conditions and flow rates were identical to the all the cases described above. The simulation results are shown in Figure 3.14(a)- 3.14(c). The maximum pressure drop for a length of 1mm mixer is 550 Pa. The efficiencies based on Equation (17) can be evaluated and are shown in Figure 3.15. The distance between the inlet jets were varied from being opposite to an offset of 1mm. The 1mm offset jets performed very similar in terms of mixing efficiency but had the least pressure drop.
Figure 3.16: Effect of addition of 6mm mixing channel length to Y-type mixer
(a): top view of center-plane concentration contours
(b): exit-plane concentration contours (half channel depth)
(c): Pressure drop along the length of the micromixer
(d): Efficiency with the addition of 6mm Extension.
Figure 3.17: Effect of addition length of 12mm mixing channel length to the original Y-type mixer
(a): top view of center-plane concentration contours
(b): exit-plane concentration contours (half channel depth)
(c): Pressure drop along the length of the micromixer
(d): Efficiency with the addition of first and second 6mm Extensions.
Figure 3.18: Effect of addition of 6mm mixing channel length to X2J (1mm offset) mixer
(a): top view of center-plane concentration contours
(b): exit-plane concentration contours (half channel depth)
(c): Pressure drop along the length of the micromixer
(d): Efficiency with the addition of 6mm Extension.
The advantage of shifting the second jet by 1 mm offset is shown in the pressure drop evaluation as well as the efficiency estimates (Figure 3.15). There have been additional studies performed to evaluate the offset distance, with offsets of 25 µm, 50 µm, 200 µm and 1 mm modeled. The 1 mm offset jets provide the lowest pressure drop without compromising on the mixing efficiency. The reason for the least pressure drop when the jets were placed at an offset of 1 mm is that the total flow rate for each case remains same but the total flow rate does not flow through the same channel length.

3.7 Increasing Mixing Channel Length

The intent in this study was to determine the length over which most of the mixing had taken place and the point beyond which increasing the length would have diminishing returns. Then put it in perspective relative to simple theory predictions. By increasing the

![Figure 3.19: Fluidic mixing for 2 jets in a cross flow with a jet width of 25 µm (X2J)]

(a): top view of center-plane concentration contours
(b): concentration variation at the first inlet
(c): concentration variation at the second inlet
length of the mixing channel, the mixing efficiency is increased at the expense of pressure drop. Because of limitation on grid size, simulating the mixer with this additional length is not feasible. But, the numerical simulation software Fluent provides the ability to store boundary profiles (storing all variables on a given boundary). A rectangular microchannel with the same height, width, mesh setting and boundary conditions (using the boundary profile data) at the end of the original mixing channel was constructed and simulated to produce the effect of having additional channel length to the original micromixer design. But, the length of this new mixing channel was limited due to the grid size. Therefore, a round off length of 6mm was chosen to accommodate computation limit by the Fluent solver. This

Figure 3.20: Fluidic mixing for 2 jets in a cross flow with a jet width of 50µm (X2J) (a): top view of center-plane concentration contours (b): concentration variation at the first inlet (c): concentration variation at the second inlet
study was performed on the best (X2J with offset) and the worst (YP) mixers in terms of the mixing efficiency.

It is evident from Figures 3.16 that increasing the length of the mixing channel is still not enough to provide comparable results with that from the simulation results obtained from jets in a cross flow mixer. Furthermore, the pressure drop has increased by more than a factor of two. An additional length of 6mm was added to improve mixing in the Y-type channel. The results from this simulation are shown in Figure 3.17.

The result of adding an extra mixing length channel bumped up the efficiency to 87.2%. But, in achieving comparable mixing to the X2J configuration, the pressure drop rose by a factor of four.

![Figure 3.21: Fluidic mixing for 2 jets in a cross flow with a jet width of 125µm (X2J)](image)

(a): top view of center-plane concentration contours
(b): concentration variation at the first inlet
(c): concentration variation at the second inlet
Adding the extra length to the jets in cross flow mixer (1mm offset inlets), showed relatively less improvement in terms of increasing efficiency. This was evident since the diffusion was proportional to concentration gradients. There were relatively higher concentration gradients, both stream-wise and cross-stream direction, for the Y-mixer.

3.8 Effect of Changing Jet Width

A parametric study was performed by see the effect of changing the width of the jets for the X2J (1mm offset inlets) mixer, but maintaining the same flow rates in each case. The initial jet width (12.5mm) for the simulation used for simulations in previous cases was half the width of the mixing channel providing an aspect ratio of 12 (Assuming AR=12 being able to manufacture). In this study, the width was changed by factors of 2, 4 and 10 resulting in jet widths of 25, 50 and 125 microns. The results are shown in Figure 3.19. – 3.21.

As the jet width increased, the penetration of the jets into the main stream decreased.
as a result of the decrease in average velocity. The average velocity dropped from 10mm/s in the 12.5µm to 0.1mm/s in the 125µm jet inlet. This significantly changed the velocity profiles in the fluid region emerging from the jets into the mixing channel. There were very low velocity regions near the wall of the microchannel, where the diffusion dominated the convective mixing. This affected the contact line between the mainstream fluid and the fluid from jet. The diffusion and the difference in the velocities affected the mainstream fluid to enter the channel where the jets were present.

The mainstream fluid (blue) was dragged into the jet channels ranging from a few micrometers for the jet width of 25µm to 60µm for the jet width of 125µm. The velocities

![Figure 3.23: Effect of addition of expander to the X2J mixer (1mm offset)](image)

(a): Concentration distribution along the length of the channel
(b) pressure drop along the length of the channel
decreased with increasing jet channel width because the flow rate is maintained for all the simulated cases. The mixed regions are spread over a large area due to diffusion domination. This can be clearly seen by comparing Figure 3.21 and Figure 3.11. The high concentration of the fluid (red) from the jet inlet prevails for substantial length into the channel. Figures 3.11, 3.19, and 3.20 shows that the high concentration of the fluid from the first jet inlet located near the 100µm axial location (Z) prevails until the inlet from the second jet (~1mm axial distance). For the case where the jet width was 125µm the fluids were well mixed. The average velocity in the jet channel is 1mm/s. Because of these low velocities and the movement of main channel fluid (blue) into the jet channels, better mixing is obtained.

Figure 3.24: Effect of addition of expander to the Y-mixer
(a): Concentration distribution along the length of the channel
(b) pressure drop along the length of the channel
Figure 3.21 showed the high concentration was reduced along the axial length of the channel. In addition, the area of mixing region between the jet inlets was increased as compared to Figures 3.11, 3.19 and 3.20. These observations reflected the mixing efficiency curve shown in Figure 3.22.

3.9 Effect of Expander at the End of the Mixing Channel

Once the required reagents or fluids are mixed in a high aspect ratio microchannel, in order to reduce the burden of pressure drop, the flow is expanded into a low aspect ratio microchannel. The expansion after the mixing channel was performed by using a 7° angle. This resulted in expanding the width of the microchannel from 25µm to 86.16µm. The length of the expansion chamber was set to 500µm. The overall pressure drop for the X2J mixer (1mm offset) was ~100 Pa greater than that for the Y-mixer. But, from the efficiency calculations the additional pressure drop was used in mixing the fluids better. The process of expanding the mixing channel restricted the heavy penalty in pressure drop only to the mixing channel. Once the mixing is complete there is no need for spending additional pressure drop to move the fluid through additional high aspect ratio microchannels. Bear in mind that one of these type of micromixers would be incorporated into the final Lab-On-A-Chip device. As the complete device would have many complex networks, it was imperative that each subsystem in the design must be optimized to reduce overall pressure drop.

3.10 Design Comparisons

It is evident from the simulation results that the concentrations at the exit from the jets in a cross flow (X2J with 1mm inlet offsets) show nearly a uniform mixing. From the mixing efficiency calculations (η), it was found that the jets in a cross flow provide the best mixing by 86%, while the Y-mixer performed worst only mixing 49%. The efficiency Figure 3.10 also shows a sudden increase in the mixer efficiency of Y-mixer at the location of the
contraction. By changing the jet width to 125 µm the efficiency has increased to 90% due to the main channel fluid entering the jet channels where the local velocities are low increasing diffusional mixing. The laminar flows show parabolic profiles (Figures 3.8(c) and 3.9(c)) for the flows in designed micromixer channels. The well-mixed regions are in the center of the mixing channels where velocities are highest (Figures 3.8(b), 3.9(d) and 3.11(b)) resulting in high mass flow rate of well-mixed products. Therefore, the mixing efficiency based on flow rate (equation 17) is higher than the efficiencies area based average concentration (equation 16)). Overall the Jets in Cross-Flow type mixer configuration performed best for the design requirements.

### 3.11 Accuracy of Numerical Simulations

Bejat (2001) had demonstrated the velocity distribution comparison between simulation and analytical solution at the symmetry lines on a rectangular cross section geometry using various mesh schemes. The results were based on various mesh schemes to capture the laminar profile. Boundary layer type mesh scheme was used for all the

<table>
<thead>
<tr>
<th>Mixer type</th>
<th>η (after 1mm mixing) (%)</th>
<th>η (after 4mm mixing) (%)</th>
<th>Max. pressure drop (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>21</td>
<td>49</td>
<td>1552</td>
</tr>
<tr>
<td>U-bend</td>
<td>25</td>
<td>55</td>
<td>1410</td>
</tr>
<tr>
<td>X2J (1mm offset inlets)</td>
<td>52</td>
<td>86</td>
<td>1620</td>
</tr>
<tr>
<td>X1JC</td>
<td>38</td>
<td>68</td>
<td>970</td>
</tr>
<tr>
<td>X2JC</td>
<td>32</td>
<td>72</td>
<td>1448</td>
</tr>
<tr>
<td>X2J (opposite inlets)</td>
<td>53.3</td>
<td>85.8</td>
<td>1730</td>
</tr>
<tr>
<td>X2J (25µm jet inlets, 1mm offset)</td>
<td>42</td>
<td>84.6</td>
<td>1680</td>
</tr>
<tr>
<td>X2J (50µm jet inlets, 1mm offset)</td>
<td>42.3</td>
<td>84.3</td>
<td>1660</td>
</tr>
<tr>
<td>X2J (125µm jet inlets, 1mm offset)</td>
<td>78</td>
<td>90.5</td>
<td>1640</td>
</tr>
</tbody>
</table>
simulations above. Along the streamwise direction, the grid was placed at uniform intervals but the grid density was varied depending on the gradients observed in the flow field. The difference between the important physical quantities obtained from the simulation data and analytical solution are compared for accuracy. The important physical quantities are:

- Velocity distribution
- Pressure drop
- Concentration distribution

The concentration distribution at the exit was also very important. Any numerical error due to grid density or lack of modeling will result in error in estimating the efficiency curve for the mixer. The numerical diffusion error upstream of the exit gets amplified progressively throughout the length of the channel. Using unstructured mesh in the contraction and bend regions provided errors larger than 5% when compared with structured mesh. Therefore, all the simulations were performed using structured mesh. For every
simulation, the error was estimated with respect to analytical solution. After grid refining, the physical quantities were compared with the coarse grid in order to adequately resolve the numerical solution. The error for the various physical quantities mentioned above were calculated as follows:

\[
\text{Error}(\%) = \left( \frac{\phi_{\text{theory},i,j} - \phi_{\text{sim},i,j}}{\phi_{\text{theory},i,j}} \right) \times 100
\]  

where \( \phi \) is the physical quantity (pressure, velocity and concentration) compared with simulation results and theoretical predictions at the every node locations of the grid.

Figure 3.25 shows that maximum error in numerical estimation for all the simulations after the grid refinement was 2% that obtained from analytical solution. The pressure drop estimates show less than 1% error when compared with analytical solution. The concentration distributions are compared at the exit plane. The results show that the numerical results from the refined grid used in the simulations are acceptable.
3.12 Conclusions

The numerical simulations were performed based on theoretical analysis based on manufacturing limitations and acceptable pressure loss. The numerical results show good agreements with analytical solutions. The efficiency for the mixers was estimated based on both the flow rate and area. Both the efficiencies converge for complete mixing in the channel (leads to long channel length). The dramatic increase in the width of the jet inlet channel led to better mixing in the channel and thus provided better efficiency. Overall, the jets in a cross flow type micromixer perform better than other designs simulated.
Chapter 4. Experimental Results

4.1 Device Fabrication

Most of the fabrication of micromixers was based on technologies of micro electromechanical systems (MEMS). The basic substrate materials were silicon and glass. Recently, arising from the need for low cost and biocompatibility, polymers have been extensively used for making micromixers. A number of polymeric fabrication techniques are readily available. Polymeric bulk micromachining such as hot embossing, injection molding, casting and laser ablation, realized structures in a polymer substrate, while polymeric surface micromachining creates movable polymeric microstructures using a sacrificial layer.

Different microfabrication methods were employed to manufacture micromixers for the performing experiments. The four methods used were:

- SU-8 lithography (direct);
- Laser Ablation (direct);
- Micromilling (indirect);
- LIGA (indirect).

Barrett (2004) has worked extensively on each process and provides detailed information in his thesis. Brass (353 brass alloy) was micromilled (using a Kern MMP – Microtechnic, Murnau-Westried, Germany) to produce a mold insert consisting of the jets in

![Figure 4.1: X2J mixer sketch](image)
cross flow mixer was manufactured based on simulation results (Barrett 2004). Hot embossing of the micromixer pattern from the brass mold insert was performed using a HEX 02 embossing machine (JENOPTIK Mikrotechnik, Jena, Germany) at the LSU Center for Advanced Microstructures and Devices (CAMD). Refer to Appendix E for a detailed drawing of the mold insert design.

The brass mold insert manufactured contained three similar X2J (1mm offset) mixers (Figure 4.3) based on performance measured on the basis of simulation results obtained in chapter 3. The first two designs had the same dimensions as that simulated in chapter 3. In the past some of the embossed chips with an aspect ratio of 12 were difficult to manufacture. Therefore, in the third design, the width on all the channels was doubled (Addition of similar mixers increases additional mixers to test with minimal embossing process). The micromixers produced using micromilling had rounded corners due to the tool radius used in manufacturing the brass mold insert. The SEM images in Figures 4.4 - 4.9 further show the details of the mixer on the mold insert. From the previous chapter it was evident that the mixing efficiency was directly proportional to the geometry of the mixing device. Therefore additional simulation was performed based on the manufactured dimensions of the brass mold insert. The result from these new numerical simulation results were compared with the

Figure 4.2: X2J mixers micromilled on brass alloy.

Figure 4.3: X2J mixers micromilled on brass alloy (top view).
experimental observations on the PMMA embossed product.

Figure 4.4: Mold insert SEM image showing inlet port that supplies jets to the mixing channel.

Figure 4.5: Mold insert SEM image showing inlet port that supplies fluid to the main channel of the mixer.

Figure 4.6: Mold insert SEM image showing jets and main channel.

Figure 4.7: Mold insert SEM image showing exit port.

Figure 4.8: Mold insert SEM image showing close up view of jets and the main channel.

Figure 4.9: SEM image of the micromixer manufactured by hot embossing mold insert into PMMA.
4.2 Experimental Setup

An inverted fluorescence microscope (IX70 Olympus, Melville, NY) was used for performing analysis of the jets in cross flow with inlets offset (X2J, Figure 4.11) micromixer. The details of various components used are described in the thesis of Bejat (2001). The original stage of the microscope was replaced by H107 stage (resolution ±1µm) from PRIOR Scientific (Rockland, MA). The stage could be controlled by a manual joystick for coarse adjustment and also by PC using a RS232 connection from the serial port. The light source used in the experiments was a mercury lamp, which provided a broadband spectrum (Appendix G) of radiation ranging from UV to IR.

Figure 4.10: Schematic of the microscope and imaging setup

Figure 4.11: Picture of the microscope and imaging setup
4.2.1 Filter Cube

Two sets of filter cubes that were used for performing the experiments. The first filter set was a brightfield filter (U-MF2, Olympus, Melville, NY), which allowed visualizing the sample using white light while blocking the harmful UV radiation. This filter set was used in performing the mixing experiments using chemiluminescence. The second filter set was designed for samples with green light excitation (U-MWG2 from Olympus). The spectrum of the filter set is provided in Appendix G. This filter set was used in performing mixing experiments using Rhodamine B dye (excitation = 546nm, emission = 590nm) with deionized water.

4.2.2 Schematic of the Experimental Setup

The hot embossed PMMA micromixer channels were mounted on the Olympus IX 70 inverted microscope (Figure 4.11) for experimental evaluation. The inlet and exit ports were drilled using a 1 mm diameter drill bit from the opposite side of the embossed surface. These holes provide access to the channels. The plastic chip was cleaned to remove any debris from drilling. The cleaned PMMA chip was covered using a 0.125mm cover sheet of PMMA to seal the channels. The sealing was performed by thermal bonding of the cover sheet with the embossed chip under uniform pressure.

In order to connect the flow supply from the syringe to the microchannel, a plastic adapter was attached to the drilled holes. Using plastic tubing (1533, 1/16” X 0.03”) equipped with Ferrule connector (P-259X, 1/16”) and a flangeless nut (P-251X, 1/16”) from Upchurch Scientific, the tubing was attached to the plastic adapter. The other end of the tubing was attached to the syringe to establish flow into the microchannels. The micromixer chip was mounted on the microscope stage using a plastic sheet and four spring-loaded screws to sandwich the chip between the sheet and the stage. This restricted any relative
movement between the chip and the stage. The 40X objective was used in capturing images on to the CCD (charged coupled device) camera located below the microscope (Figure 4.10 and 4.11).

4.3 Experimental Results

There were two different experiments performed in order to assess the quality of micromixers. The first experiment was performed to qualitatively assess mixing by using Chemiluminescence. The second experiment was performed to quantitatively evaluate mixing by using Rhodamine B dye (ACROS, CAS#81-88-9, laser grade) fluorescent solution. Quantitative data was extracted by recording the fluorescence intensity on to a CCD camera.
4.3.1 Chemiluminescence Experiment

Chemiluminescence is the generation of electromagnetic radiation as light by the release of energy from a chemical reaction. While the light can, in principle, be emitted in the ultraviolet, visible or infrared region, those emitting visible light are the most common. They are also the most interesting and useful. Chemiluminescent reactions can be grouped into three types:

1. Chemical reactions using synthetic compounds and usually involving a highly oxidized species such as a peroxide are commonly termed chemiluminescent reactions.

2. Light-emitting reactions arising from a living organism, such as the firefly or jellyfish, are commonly termed bioluminescent reactions.

3. Light-emitting reactions, which take place by the use of electrical current, are designated electrochemiluminescent reactions.

The qualitative mixing experiment was performed using the SuperSignal ELISA Femto Maximum Sensitivity Substrate kit from PIERCE Biotechnology (Prod # 37075, Rockford, IL). The kit consisted of a Luminol/Enhancer and a stable Peroxide solution. A mixture of equal portions of the above solutions was used in detecting the presence of HRP (Horseradish Peroxide). A solution of HRP when used with the ELISA kit produces chemiluminescence (photons emitted ~ 425nm, blue). The mixing experiment was performed by pumping the equal part mixture of the ELISA kit into the main channel and the HRP solution was pumped through the side jets. The diffusion started at the interfaces of these fluids, the light generated due to chemiluminescence is captured on the CCD camera. The images were captured using a 40X oil immersion objective using 4X4 binning on the pixels to increase signal to noise ratio. A field of view of 332 X 256 pixels corresponded to 220 X
170 µm physical dimension. The motorized stage (PRIOR) was moved by 200µm and 100 images were taken at various locations along the length of the micro mixer (20µm overlap was maintained between the each set of 100 image frame sets). This provided a 20µm overlap between the images. The images were combined using Matlab. This provides a complete picture of diffusion mixing (due to chemiluminescence).

Figure 4.13: Micromixer image under room lights

Figure 4.14: Chemiluminescence mixing experiment with total flow rate of 10µL/min

Figure 4.15: Mixing front observed at the first jet inlet.

Figure 4.16: Mixing front growth between the jet inlets.

Figure 4.17: Second mixing front observed at the second jet inlet.

Figure 4.18: Complete mixed chemiluminescence signature at the exit of the micromixer channel (in the expander region)
Additional experiments were performed at lower flow rates of 5µL/min and 2µL/min. The results for these experiments are shown in Figure 4.19 and 4.20. The chemiluminescence technique provides a neat way of identifying mixing fronts. Figure 4.15 shows the mixing front at the first jet inlet.

This mixing front continued to grow in the channel due to diffusional mixing (Figure 4.16). When the second jet (containing HRP) solution interacted with the fluid in the main channel, a second mixing front was produced (Figure 4.17). The mixing continued to progress through the high aspect-ratio channel and a uniformly mixed product was obtained at the exit of the mixing chamber (in the expansion region, Figure 4.18). This experiment qualitatively validated the design. But, the quality of mixing is strongly dependent on the rate of diffusion and the flow rate used. The rates of diffusion were unknown for the reagents used in this experiment. In addition, the simulations performed were based on two fluids. In this case, we have a combination of three reagents. Furthermore, this is a reaction process where the reagents are converted into products emitting light as a by-product. The concentration continues to change along the length due to diffusional mixing and chemical reaction. Hence, it was essential to perform some quantitative experiments to compare numerical simulations and assess the design and manufacturing quality.

At lower flow rates (Figures 4.19 and 4.20), the mixing was completed before
reaching the exit. The designed flow rate simulated in Chapter 3 was 2.25 µL/min. The images obtained at these flow rates do not show a sharp boundary for the mixing front. In addition, the damköhler number (ratio of reaction time to diffusion time) was small. On the other hand when the flow rate was increased by an order of magnitude, the light emission was detected near the exit region indicating that the species did not have enough time in the high aspect ratio channel to mix.

4.3.2 Calibration Results

In order to perform quantitative experiments, calibration experiments were performed in order to maintain a standard throughout the experimental procedure. First set of calibration experiments were performed to obtain the physical resolution of the microscope objectives (10X and 40X magnification). The experiment consisted of using an Olympus micrometer scale with 0.01mm line spacing grating on a glass slide. The images of the micrometer scale were captured on the CCD camera at various binnings using the 10X and the 40X magnification objectives. The scale was placed in the horizontal and vertical directions on the plane of the stage. For each set of magnification and binning, pixels were counted for the actual physical distance on the scale. Statistical analysis was performed on the observed images. Some sample images captured using 10X and 40X magnifications using 4X4 binnings are shown in Figures 4.21 – 4.24.

The second set of calibration experiments were performed using the Rhodamine B fluorescent dye solution. The goal of these calibration experiments was to estimate the intensity variation as a function of various molar concentrations of the Rhodamine B solution. The calibration experiment was performed by preparing ten different concentration solutions ranging from 1.44X10^{-5}M to 1.44X10^{-6}M with the variation in molar concentration between each solution changing by 10% (1.44X10^{-6}M). These solutions were pumped into
the micromixer channels and images were obtained. The intensity was not only a function of concentration but also a function of the height of the channel. Therefore, the calibration experiments were performed in the molded X2J mixer channel. The final experiment to get quantitative data was also performed using the same mixer. The camera settings such as the brightness, contrast, exposure time and the binning were kept constant for each solution. The camera settings were obtained by repeated experiments using the highest (1.44x10^{-5}M) and the lowest (1.44x10^{-6}M) concentration solutions. The camera parameters were adjusted to obtain a good image resolution in terms of intensity distribution at the highest and lowest concentrations of Rhodamine B fluorescent solutions.

The calibration curve in Figure 4.25 shows a non linear relationship between the intensity distribution and the concentration. This non linear behavior was due to inner filter effects (an apparent decrease in emission quantum yield and/or distortion of bandshape as a result of reabsorption of emitted radiation). In order to reduce these effects, the calibration experiment was performed again at lower concentrations. At lower concentrations there was a possibility of noise affecting the data acquired. The lowest concentration solution in the previous calibration experiment was set as the maximum concentration. Again ten solutions

<table>
<thead>
<tr>
<th>OBJECTIVE Magnification</th>
<th>Binning</th>
<th>Avg. Pixels/µm X</th>
<th>µm/Pixel X</th>
<th>Avg. Pixels/µm Y</th>
<th>µm/Pixel Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X</td>
<td>1X1</td>
<td>1.54</td>
<td>0.65</td>
<td>1.52</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>2X2</td>
<td>0.78</td>
<td>1.28</td>
<td>0.78</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>3X3</td>
<td>0.51</td>
<td>1.98</td>
<td>0.51</td>
<td>1.98</td>
</tr>
<tr>
<td></td>
<td>4X4</td>
<td>0.37</td>
<td>2.71</td>
<td>0.39</td>
<td>2.60</td>
</tr>
<tr>
<td>40X</td>
<td>1X1</td>
<td>6.10</td>
<td>0.16</td>
<td>6.07</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>2X2</td>
<td>3.05</td>
<td>0.33</td>
<td>3.02</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>3X3</td>
<td>2.04</td>
<td>0.49</td>
<td>2.02</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>4X4</td>
<td>1.52</td>
<td>0.66</td>
<td>1.51</td>
<td>0.66</td>
</tr>
</tbody>
</table>
were prepared with a variation of concentration by 10% between the solutions. The new range for the concentration variation was from 1.44X10^{-6}M to 1.44X10^{-7}M.

The new calibration curve showed a linear trend between the intensity measured by the CCD camera and the change in the concentration of Rhodamine B solution. Bindhu et. al. (2001) had measured the quantum yield of Rhodamine B lase dye. They have reported fluorescence quantum yield decreasing from 90% to 20% steadily as the concentrations of the Rhodamine dye increases from 10^{-6}M to 10^{-4}M. This also confirms the non linear
behavior of the calibration curve in the previous experiment performed with higher concentrations of Rhodamine B laser dye.
Table 4.3: Camera conditions for Rhodamine B fluorescent dye intensity calibration using 1.44X10^{-6} M solution

<table>
<thead>
<tr>
<th>Objective</th>
<th>Binning</th>
<th>Concentration (M)</th>
<th>%</th>
<th>Contrast</th>
<th>Brightness</th>
<th>Exp time (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40X 4X4</td>
<td></td>
<td>1.44E-06</td>
<td>100</td>
<td>30</td>
<td>-50</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.30E-06</td>
<td>90</td>
<td>30</td>
<td>-50</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.15E-06</td>
<td>80</td>
<td>30</td>
<td>-50</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.01E-06</td>
<td>70</td>
<td>30</td>
<td>-50</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.64E-07</td>
<td>60</td>
<td>30</td>
<td>-50</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.20E-07</td>
<td>50</td>
<td>30</td>
<td>-50</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.76E-07</td>
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<td>30</td>
<td>-50</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.32E-07</td>
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<td>30</td>
<td>-50</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.88E-07</td>
<td>20</td>
<td>30</td>
<td>-50</td>
<td>0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.44E-07</td>
<td>10</td>
<td>30</td>
<td>-50</td>
<td>0.7</td>
</tr>
</tbody>
</table>

CURVE FIT DATA: \( R^2 = 0.994 \)

\[
Y = A + B \times \text{(Conc)}
\]

\[
A = 8.087 \times 10^{-2}
\]

\[
B = 8.324 \times 10^{-3}
\]

Figure 4.26: Intensity calibration curve for Rhodamine B fluorescent dye using 1.44X10^{-6} - 1.44X10^{-7} M solution
4.3.3 Rhodamine B Dilution Experiment

The mixing experiments were performed using Rhodamine B solution based on the calibration experiments. The results from the second calibration experiment were chosen as a basis since the intensity trend shows linearity with concentration. The experiment consisted of pumping Rhodamine B solution \((1.44 \times 10^{-6} \text{M})\) through the side jets at the simulated flow rate of 37.5nL/s. The main channel was pumped with deionized water at the simulated flow rate of 37.5nL/s. The KD scientific syringe pump (Figure 4.15) was used in driving the fluids through the mixer. This fluorescence experiment did not provide clear boundary for the mixing front as that shown in the chemiluminescence experiment. This was because the image obtained from the chemiluminescence depends on light generated due to mixing whereas the fluorescence image shows intensity wherever there is a presence of the rhodamine in the channel. The fluorescence signature in the mixer was captured on the CCD camera using the U-MWIG2 filter cube (Olympus) and the mercury lamp as the source. The images were again captured using a 40X oil immersion objective using 4X4 binning on the pixels (Brightness, contrast, and exposure times were set based on Table 4.3).

The motorized stage (PRIOR) was moved by 200µm and images sets of 100 frames were taken at each location along the length of the micromixer. This also resulted in an overlap of 20µm between image sets. Using the linear calibration curve from Figure 4.26, the mixing efficiency was determined by performing averages over the 100 frames for each location.
image location along the length of the mixing channel. The averages were calculated at three different locations on each image frame:

1. Average over the beginning of frames of the image set (100 frames)
2. Average over the end of the frames of the image set (100 frames)
3. Average over the entire set of frames (100) of the image

Johnson et. al. (2002) performed an experimental investigation of T-microchannel mixers in polycarbonate. They reported diffusion coefficients of $2 \times 10^{-6} \text{cm}^2/\text{s}$ for their experiments using an average velocity of 8 mm/s in the mixing channel running under electroosmotic flow. In a simple T-microchannel, a mixing efficiency over 80% requires a length of 2.3cm. On the other hand, Imanaga et. al. have studied cell-to-cell diffusion of fluorescent dyes in paired ventricular cells using Rhodamine B dye. They have reported a diffusion coefficient of $8 \times 10^{-7} \text{cm}^2/\text{s}$. There is almost an order of magnitude difference for the
diffusion coefficient. This has led us to perform additional simulations by changing the simulation diffusion coefficient by a factor of 2 to cover this range in variation of diffusion coefficient for the Rhodamine B.

The additional simulations used the diffusion coefficients of \(2 \times 10^{-6} \text{ cm}^2/\text{s}\) and \(0.5 \times 10^{-6} \text{ cm}^2/\text{s}\) (providing similar order of magnitude difference in diffusion coefficients). Mixing efficiencies obtained from these simulations are shown in Figure 4.33 and Figure 4.34. The efficiencies calculated from the experimental images are reported in Figure 4.33 and Figure 4.34.

The set of 100 image frames corresponding to every 200\(\mu\text{m}\) axial displacement were concatenated using MATLAB code provided in Appendix B. The program evaluates the
efficiency based on the three methods discussed above. Figure 4.33 shows the experimental efficiency results obtained (evaluating efficiencies at the beginning, end, and the average over the entire frame sets). Figure 4.34 shows a clear picture of one of the methods (based on average over the frame) including the standard deviation estimated from the experimental data. The mixing efficiency curve obtained (including the standard deviation) is well within the bounds of mixing efficiency obtained from numerical simulations (variation of diffusion coefficient). Experimental estimate shows that the micromixer manufactured by direct micromilling (with 50µm-curved radii instead of sharp bends) provides mixing efficiency of 86% ± 1.8%.

The numerical simulations used for various micromixer designs used a concentration of 100% (1M) for fluid flowing through the jets (red). Figure 4.36 shows the simulation
results based on the experimental Rhodamine B concentrations used. The results show identical efficiency curves. As the channel length increases, the efficiencies calculated based on flow rates approached the efficiencies calculated based on cross section area.

![Diagram showing efficiency curves for various Rhodamine B concentrations.](image)

**Figure 4.35:** Compare efficiency for various inlet concentrations based on numerical simulations for X2J (1mm offset) micromixer

Using the simple mixer theory, to produce a 10nL volume of mixture (reagents having a diffusion coefficient of $1.2 \times 10^{-10} \text{m}^2/\text{s}$ in an aspect ratio=6 mixing channel), with the manufactured channel width of 27µm would require an optimal total flow rate of 6.568nL/s. The experimental efficiency calculations based on this optimized flow rate is shown in Figure 4.36. Simulations were also performed based on the flow rate obtained from simple
theoretical calculations. Furthermore, 15% deviations to the optimal flow rates corresponding to 7.55nL/s and 5.58nL/s were also adjusted to the experiments to observe sensitivity of the optimal flow rate on mixing efficiency (Figure 4.36). The flow rates are very small to significantly affect the operation of the micromixer. The pressure drop obtained from simulation corresponds to 80 Pa.

But for a diffusion coefficient of $10^{-10} \text{m}^2/\text{s}$, the optimal flow rate from simple theory calculations corresponds to 5.487nL/s to produce a 10nL of mixed fluid. Again simulation and experimental results for these conditions were performed. The mixing efficiency for this case is shown in Figure 4.35. The pressure drop obtained from simulation corresponds to 70 Pa.

The mixing efficiency based on optimal flow rates show greater than 90% within 2mm length of the mixing channel. The optimal flow rate would produce a 10nL of mixed product mixing efficiency 97% for a length of 4mm in less than 5s. Using the simulated
conditions, the volume of 10nL of mixed product will be generated in less than 0.4s (keeping within the limits of pressure drop).

4.4 Conclusions

Hot embossing mixer structures from a brass mold insert into a PMMA substrate produced experimental chips. These micromixer designs were micromilled leading to rounded corners. Additional simulations were performed in order to compare the experimental observations due to variation in mixer design (rounded corners from micromilling). The chemicaluminescence experiment was done to observe the mixing region qualitatively. Rhodamine B fluorescence solution was used in experimentally evaluating the mixing efficiency of jets in cross flow micromixer. The experimental results show that the jets in cross flow mixer perform a 1:1 mixing with a pressure drop of less than 0.5psi and a mixing efficiency of 86%.

Figure 4.37: Optimal mixer efficiency to produce a 10nL product
Chapter 5. Conclusions and Recommendations

This work has been focused on designing and developing an integrated microfabricated passive microfluidic diffusional micromixer to carry out the Ligase Detection Reaction (LDR) assay for the detection of low abundant cancer diagnostic markers. Various micromixers were designed and simulated. The designs were evaluated based on mixing efficiency and pressure drop criteria.

The design objective was to incorporate large contact areas between the mixing reagents; since narrow channels reduce the length scale in the diffusion direction. This reduced the overall time for mixing. Furthermore, the mixing chamber length must be minimized to reduce the pressure drop to carry out the mixing process. The mixer designs were evaluated based on pressure drop and mixing efficiency. The mixing efficiency was evaluated based on the basis of both flow rate and area at the exit and the inlets. The presence of well-mixed fluids at the high velocity regions (center of the channel), the efficiency based on flow rate shows higher than that estimated based on area. But, when the length of the mixing channel was increased, the two efficiencies converged. The addition of an expander at the exit of the mixing channel helps in recovering some of the pressure loss.

The jets in cross flow micromixer showed better performance. The numerical simulations were validated using two experimental techniques. The qualitative results for mixing were obtained by the method of chemiluminescence. The mixing efficiency was determined quantitatively by using Rhodamine B fluorescent dye solution and de-ionized water. The experimental observations show close agreement with the numerical simulation estimates. It is almost impossible to achieve 100% mixing in a practical situation as the length and the width requirements affect the pressure drop.
Future study may need to consider experimentally estimating efficiency based on flow rate. Manufacturing techniques must be refined to produce even higher aspect ratio (~20) to reduce channel length required for mixing. Embossing patterns on two different substrates and bonding them together to yield high aspect ratio microstructures can also be achieved. Alignment along with the quality of bonding will be a key issue for this process. If the bonding process does not successfully seal the channels, there may be leakage at the seam. Use of glass cover slip will provide better experimental results not only for the effectiveness of micromixer but also for various other processes involved on the diagnostic chip device. In addition, a few prototype LDR devices incorporating the mixer would provide better understanding and limitations for the micromixer as well as the complete Lab-On-A-Chip device.
References

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Appendix A: Fortran Files to Calculate Mixing Efficiency

A.1 Calculate the Efficiency Based on Cross-Section Area of the Channel

! Calculating mixer efficiency on a slice
PROGRAM Calculate_Mixer_Efficiency
IMPLICIT NONE
EXTERNAL RINDEX
! CALCULATIONS BASED ON HALF CHANNEL DEPTH
! MAY NEED TO MAKE NECESSARY ADJUSTMENTS FOR COMPLETE DEPTH
!---------------------End of Variable Declaration-------------------------

!-------------------------Main Program -----------------------------------
!--------------------------- READING THE DATA FILE-----------------------------------------

READ(5,8) scanLine    ! TITLE = 'Fluent 6.0.12
READ(5,8) scanLine    ! VARIABLES = "X"
num_var = 1     ! number of variables
equal_sign = "="    ! set character = to equal_sign
double_quote_sign = ""
! Loop to find number of variables

READ(5,8) scanLine    ! TITLE = 'Fluent 6.0.12
READ(5,8) scanLine    ! VARIABLES = "X"
num_var = 1     ! number of variables
equal_sign = "="    ! set character = to equal_sign
double_quote_sign = ""
! Loop to find number of variables
num_var = num_var + 1
READ(5,9) flag_char
ENDDO
BACKSPACE(5) ! Go to the starting of previous line since

! while loop reading for number of variables
READ(5,9) flag_char
! While loop to to discard remaining lines between
DO WHILE( flag_char .ne. "Z") ! the variables and stating of Zone T=
  READ(5,9) flag_char
ENDDO
BACKSPACE(5)
8 FORMAT(A50) ! Format to read/write 50 characters
9 FORMAT(A1) ! Format to read/write 1 character

!-----------------> HEADER for output file in TECPLOT format<-----------------------------------
WRITE(6,*) 'TITLE    = "******PUT THE TITLE HERE***********"'
WRITE(6,*) 'VARIABLES= "Z"
WRITE(6,*) "e"
WRITE(6,*) "dp"
WRITE(6,*) "C_max"
WRITE(6,*) "x_C_max"
WRITE(6,*) "y_C_max"
WRITE(6,*) "C_min"
WRITE(6,*) "x_C_min"
WRITE(6,*) "y_C_min"
WRITE(6,*) 'ZONE T="h (ZONE LABEL)"
WRITE(6,*) 'I=, F=POINT'

!----------------->End of Header for the output file.<---------------------------------------------------
!---------------------------------------------------------------------------------------------------------------

!------> Insert the correct areas and velocities corresponding to the simulation conditions
below<<-------
Ai_water = 75 * 50 ! Area of Water Inlet
Ai_DNA = 75 * 12.5 * 2 ! Area of DNA inlet
A_e = 25 * 75 ! Exit Area
A_i = Ai_water + Ai_DNA ! Total Inlet Area
V_i_water = 10 ! Inlet Velocity of water in mm/s
V_i_DNA = 5 ! Inlet Velocity of DNA in mm/s
C_{i\_dna} = 1.0
C_{i\_water} = 1.0
C_{inf} = (A_{i\_water}*\text{DABS}(C_{i\_water}-C_{i\_dna}) + 
A_{i\_DNA}*\text{DABS}(C_{i\_dna})/(A_{i})  \quad \text{! } C_{inf} = 0.5 \text{ for eq flow rates & eq areas}

\text{Inlet conc. distribution} = ((C_{inf}-(C_{i\_water}-C_{i\_dna}))*A_{i\_water} + 
(C_{i\_dna} - C_{inf})*A_{i\_DNA})/(A_{i})  \quad \text{! Inlet conc. distribution}

flag_{p} = 1  \quad \text{! For evaluating reference pressure}

DO WHILE (.NOT. EOF(5))
\text{! Loop for reading all the zones}

READ(5,8) scanLine  \quad \text{! ZONE } = "Slc: Z = ....."
\text{eq\_sign\_loc} = \text{RINDEX}(scanLine,equal\_sign)
\text{db\_quote\_loc} = \text{RINDEX}(scanLine,double\_quote\_sign)
\text{loc\_string} = scanLine(eq\_sign\_loc+1:db\_quote\_loc-1)
READ(loc\_string,*) location
\quad \text{! location = real number from Slc: Z = .....}

READ(5,8) scanLine
\text{eq\_sign\_loc} = \text{RINDEX}(scanLine,"N=") + 2
\quad \text{! Find the index of N= character +2}
\text{db\_quote\_loc} = \text{RINDEX}(scanLine," E=")
\quad \text{! Find the index of E= character}
\text{integer\_value} = scanLine(eq\_sign\_loc : db\_quote\_loc-2)
\quad \text{! Integer value lies between the two indexes as Number of Nodes}
READ(integer\_value,*) N
\quad \text{! Read number of nodes}

\text{eq\_sign\_loc} = \text{db\_quote\_loc} + 3
\quad \text{! Move the index to the character after E=}
\text{db\_quote\_loc} = \text{RINDEX}(scanLine,"ZONE")
\quad \text{! Find the index of ZONE}
\text{integer\_value} = scanLine(eq\_sign\_loc : db\_quote\_loc-3)
\quad \text{! Scan the integer value between the two indices}
READ(integer\_value,*) E
\quad \text{! Read Number of Edges}

READ(5,8) scanLine
\quad \text{! Ignore line with DT=(DOUBLE DOUBLE....)}
READ(5,8) scanLine  \quad \text{! Ignore line with DATAPACKING=POINT}

DO i=1,N
\quad \text{! Read the nodes into an array}
READ(5,*) (node(i,k),k=1,num\_var)
ENDDO

DO j=1,E
\quad \text{! Read the edges into an array}
READ(5,*) DL(j,1), DL(j,2), DL(j,3), DL(j,4)
ENDDO
WRITE(*,*) 'Reading File'

! Finding Volumetric flowrate Concentration for each element
C_tot = 0.0 ! Initialization for C_tot
C_max = 0.0 ! Initialization for C_max
C_min = 1.0 ! Initialization for C_min
P_tot = 0.0 ! Initialization for P_tot
A_tot = 0.0 ! Initialization for A_tot

DO j = 1, E
  delta_x = find_delta(node(DL(j,1),1), node(DL(j,2),1), node(DL(j,3),1), node(DL(j,4),1))
  delta_y = find_delta(node(DL(j,1),2), node(DL(j,2),2), node(DL(j,3),2), node(DL(j,4),2))
  C = get_avg(node(DL(j,1),8), node(DL(j,2),8), node(DL(j,3),8), node(DL(j,4),8)) ! Averaging
  P = get_avg(node(DL(j,1),4), node(DL(j,2),4), node(DL(j,3),4), node(DL(j,4),4)) ! Averaging
  IF (C_max .le. C) THEN
    C_max = C
    x_C_max = get_avg(node(DL(j,1),1), node(DL(j,2),1), node(DL(j,3),1), node(DL(j,4),1)) ! Averaging
    y_C_max = get_avg(node(DL(j,1),2), node(DL(j,2),2), node(DL(j,3),2), node(DL(j,4),2)) ! Averaging
  ENDIF
  IF (C_min .ge. C) THEN
    C_min = C
    x_C_min = get_avg(node(DL(j,1),1), node(DL(j,2),1), node(DL(j,3),1), node(DL(j,4),1)) ! Averaging
    y_C_min = get_avg(node(DL(j,1),2), node(DL(j,2),2), node(DL(j,3),2), node(DL(j,4),2)) ! Averaging
  ENDIF
  V_e = get_avg(node(DL(j,1),7), node(DL(j,2),7), node(DL(j,3),7), node(DL(j,4),7)) ! Avg. velocity for each element
  C_element = delta_x * delta_y * DABS(C-C_inf) !Vol Concentration deviation for each element
  P_element = delta_x * delta_y * P !Applied Force on each element
  P_tot = P_tot + P_element !Adding forces from each element
  C_tot = C_tot + C_element !Adding Concentration deviation from all elements
$A_{\text{tot}} = A_{\text{tot}} + \Delta x \times \Delta y$

!Volume flow rate on each element

ENDDO

WRITE(*,10) C_{\text{tot}}, P_{\text{tot}}
10 FORMAT('C =', E11.4, 'P=', E11.4)

!------------------- Finding domain/slice extension/boundary for calculating $M_{\text{eff}}$

xmin = node(1,1)
xmax = node(1,1)
ymin = node(1,2)
ymax = node(1,2)

DO i=2, N
  IF(xmin .ge. node(i,1)) xmin = node(i,1)
  IF(ymin .ge. node(i,2)) ymin = node(i,2)
  IF(xmax .le. node(i,1)) xmax = node(i,1)
  IF(ymax .le. node(i,2)) ymax = node(i,2)
ENDDO

!---------------------------------------------------------------------------------

Area = (xmax-xmin)*(ymax-ymin)

! Total Fluid Area per slice (could be different if the cross section area is changing)

WRITE(*,*) 'Area = ', Area

slice_conc_distribution = C_{\text{tot}}/A_{\text{tot}}

! slice concentration Distribution

$M_{\text{eff}} = (1 - \text{slice}\_\text{conc}\_\text{distribution}/\text{Inlet}\_\text{conc}\_\text{distribution})\times 100$

! Efficiency based on flow-rates

WRITE(*,20) M_{\text{eff}}
20 FORMAT(' M_{\text{eff}} = ', F6.2, '%')

$P = P_{\text{tot}}/\text{Area}$

IF (flag_p .eq. 1) THEN
  $P_{\text{ref}} = P_{\text{tot}}/\text{Area}$
ENDIF

!----- if $P_{\text{ref}}$ is known, remove the if statement and move $P_{\text{ref}}$ before the DO loop

!----- and set the correct value for $P_{\text{ref}}$

dp = P_{\text{ref}} - P

! Calculate pressure drop

!-------------------Write the calculated quantities/variables to the output file---------------------

WRITE(6,35) location, M_{\text{eff}}, dp, C_{\text{max}}, x_{\text{C}_{\text{max}}}, y_{\text{C}_{\text{max}}}, C_{\text{min}}, x_{\text{C}_{\text{min}}}, y_{\text{C}_{\text{min}}}
35 FORMAT(E11.4, E11.4, E11.4, E11.4, E11.4, E11.4, E11.4, E11.4, E11.4)

flag_p = flag_p + 1

END DO

40 FORMAT(E17.9)

CLOSE(5) ! Close input file
CLOSE(6) ! Close output file

END

!-----------------------------------------------------------------------------------^End of Main Program^----------------------------------
REAL*8 FUNCTION find_delta(x1,x2,x3,x4)
    ! Calculate delta_x or delta_y for each node
    REAL*8 temp, dx1, dx2, dx3, dx4, x1, x2, x3, x4
    dx1 = DABS((x1-x2))
    dx2 = DABS(x2-x3)
    dx3 = DABS(x3-x4)
    dx4 = DABS(x4-x1)
    temp = dx1
    IF(temp.le.dx2) temp = dx2
    IF(temp.le.dx3) temp = dx3
    IF(temp.le.dx4) temp = dx4
    find_delta=temp
RETURN
END

REAL*8 FUNCTION get_avg(nu m1, num2, num3, num4)
    ! Calculates the average of 4 numbers
    REAL*8 num1, num2, num3, num4
    get_avg = (num1 + num2 + num3 + num4)/4.0
    RETURN
END

A.2 Calculate the Efficiency Based on Flow Rate

! Calculating mixer efficiency on a slice
PROGRAM Calculate_Mixer_Efficiency
IMPLICIT NONE
EXTERNAL RINDEX
! CALCULATIONS BASED ON HALF CHANNEL DEPTH
! MAY NEED TO MAKE NECESSARY ADJUSTMENTS FOR COMPLETE DEPTH
! -------------------Variable Declaration-----------------------------
INTEGER i, k, N, E, j, DL(5000,4), num_var, eq_sign_loc, db_quote_loc
INTEGER flag_p, RINDEX
REAL*8 node(5000,15), delta_x, delta_y, C_element, C_tot, find_delta, M_eff
REAL*8 xmin, xmax, ymin, ymax, C, C_i_water, C_inf, A_e, Ai_water, Ai_DNA,
A_i
REAL*8 Vi_water, Vi_DNA, Q_tot, C_i_dna
REAL*8 Inlet_conc_distribution, slice_conc_distribution
REAL*8 C_max, C_min, x_C_max, y_C_max, x_C_min, y_C_min, location, dp, P
REAL*8 P_tot, P_element, P_ref, Area, get_avg, V_e
CHARACTER scanLine*50, flag_char, equal_sign, double_quote_sign,
loc_string*20
CHARACTER integer_value*6

!---------------------End of Variable Declaration-------------------------
!-------------------------Main Program -----------------------------------
!--------------------------- READING THE DATA FILE-----------------------------------------
!--This program reads all the slices from the input file created in TECPLT ver. 10
!--------------------------------------------------------------------------------------------
OPEN(5,STATUS='UNKNOWN',FILE='***** Insert the Input File here*****')
! Open the input file
OPEN(6,STATUS='UNKNOWN',FILE='*****Insert the Input File here*****')
! Open the output file
READ(5,8) scanLine
! TITLE = 'Fluent 6.0.12
READ(5,8) scanLine
!  VARIABLES = "X"
READ(5,9) flag_char
! Check for "" character
num_var = 1
! number of variables
equal_sign = "=
! set character = to equal_sign
double_quote_sign = \\
! set character " to double_quote_sign
DO WHILE( flag_char .eq. double_quote_sign)
! Loop to find number of variables
    num_var = num_var + 1
    READ(5,9)flag_char
ENDDO
BACKSPACE(5)

8   FORMAT(A50) ! Format to read/write 50 characters
9   FORMAT(A1) ! Format to read/write 1 character
!-----------------> HEADER for output file in TECPLT format<-------------------------------
WRITE(6,*) 'TITLE    = "*******PUT TITLE HERE******"'
WRITE(6,*) 'VARIABLES= "Z"'
WRITE(6,*) "h"
WRITE(6,*) "dp"
WRITE(6,*) "C_max"
WRITE(6,*) "x_C_max"
WRITE(6,*) "y_C_max"
WRITE(6,*) "C_min"
WRITE(6,*) "x_C_min"
WRITE(6,*) "y_C_min"
WRITE(6,*) 'ZONE T="h (*****PUT ZONE LABEL********)"'
WRITE(6,*) 'I=, F=POINT'

!----------------------------> IMPORTANT! BEFORE USING TECPLOT<----------------------
!---------------> Manually count and add the number of lines generated by this program--------
!---------------> and inlcude in the output data file after I= XX where XX= # of lines/slices----
!-------------------------------------------------------------------------------------------------------------
WRITE(6,*) 'DT=(', ('DOUBLE ', k=1,num_var), ')'

!----------------->End of Header for the output file.<---------------------------------------------------
!--------------------------------------------------------------------------------------------------------------

!------> Insert the correct areas and velocities corresponding to the simulation conditions below<-------

Ai_water = 75 * 50
! Area of Water Inlet

Ai_DNA = 75 * 12.5 * 2
! Area of DNA inlet

A_e = 25 * 75
! Exit Area

A_i = Ai_water + Ai_DNA
! Total Inlet Area

Vi_water = 10
! Inlet Velocity of water in mm/s

Vi_DNA = 5
! Inlet Velocity of DNA in mm/s

C_i_dna = 1.0
C_i_water = 1.0
C_inf = (Ai_water*DABS(C_i_water-C_i_dna)*Vi_water +
Ai_DNA*DABS(C_i_dna)*Vi_DNA)/(Ai_water*Vi_water + Ai_DNA*Vi_DNA)
! C_inf = 0.5 for equal flow rates

Inlet_conc_distribution = ((C_inf-(C_i_water-C_i_dna))*Ai_water*Vi_water +
(C_i_dna - C_inf)*Ai_DNA*Vi_DNA)/(Ai_water*Vi_water + Ai_DNA*Vi_DNA)
! Inlet conc. distribution

flag_p = 1
! For evaluating reference pressure

DO WHILE (.NOT. EOF(5))
! Loop for reading all the zones

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READ(5,8) scanLine
   ! ZONE = "Slc: Z = ......"
eq_sign_loc = RINDEX(scanLine,equal_sign)
db_quote_loc = RINDEX(scanLine,double_quote_sign)
loc_string = scanLine(eq_sign_loc+1:db_quote_loc-1)
READ(loc_string,*) location
   ! location = real number from Slc: Z = ......

READ(5,8) scanLine
   eq_sign_loc = RINDEX(scanLine,"N=") + 2
      ! Find the index of N= character +2
db_quote_loc = RINDEX(scanLine," E=")
      ! Find the index of E= character
integer_value = scanLine(eq_sign_loc : db_quote_loc-2)
      ! Integer value lies between the two indexes as Number of Nodes
READ(integer_value,*) N
      ! Read number of nodes
eq_sign_loc = db_quote_loc + 3
      ! Move the index to the character after E=
db_quote_loc = RINDEX(scanLine,"ZONE")
      ! Find the index of ZONE
integer_value = scanLine(eq_sign_loc : db_quote_loc-3)
      ! Scan the integer value between the two indices
READ(integer_value,*) E
      ! Read Number of Edges
READ(5,8) scanLine
   ! ignore line with DT=(DOUBLE DOUBLE....)
READ(5,8) scanLine
   ! ignore line with DATAPACKING=POINT

DO i=1,N
   ! Read the nodes into an array
   READ(5,*) (node(i,k),k=1,num_var)
ENDDO

DO j=1,E
   ! Read the edges into an array
   READ(5,*) DL(j,1), DL(j,2), DL(j,3), DL(j,4)
ENDDO

WRITE(*,*) 'Reading File'
! Finding Volumetric flowrate Concentration for each element
C_tot = 0.0
   ! Initialization for C_tot
C_max = 0.0
   ! Initialization for C_max
C_min = 1.0
   ! Initialization for C_min
P_tot = 0.0
   ! Initialization for P_tot
Q_tot = 0.0

DO j = 1, E
    delta_x = find_delta(node(DL(j,1),1), node(DL(j,2),1), node(DL(j,3),1), node(DL(j,4),1))
    delta_y = find_delta(node(DL(j,1),2), node(DL(j,2),2), node(DL(j,3),2), node(DL(j,4),2))
    C = get_avg(node(DL(j,1),8), node(DL(j,2),8), node(DL(j,3),8), node(DL(j,4),8)) ! Averaging
    P = get_avg(node(DL(j,1),4), node(DL(j,2),4), node(DL(j,3),4), node(DL(j,4),4)) ! Averaging
    IF (C_max .le. C) THEN
        C_max = C
        x_C_max = get_avg(node(DL(j,1),1), node(DL(j,2),1), node(DL(j,3),1), node(DL(j,4),1)) ! Averaging
        y_C_max = get_avg(node(DL(j,1),2), node(DL(j,2),2), node(DL(j,3),2), node(DL(j,4),2)) ! Averaging
    ENDIF
    IF (C_min .ge. C) THEN
        C_min = C
        x_C_min = get_avg(node(DL(j,1),1), node(DL(j,2),1), node(DL(j,3),1), node(DL(j,4),1)) ! Averaging
        y_C_min = get_avg(node(DL(j,1),2), node(DL(j,2),2), node(DL(j,3),2), node(DL(j,4),2)) ! Averaging
    ENDIF
    V_e = get_avg(node(DL(j,1),7), node(DL(j,2),7), node(DL(j,3),7), node(DL(j,4),7)) ! Avg. velocity for each element
    C_element = V_e * delta_x * delta_y * DABS(C-C_inf) ! Vol Concentration deviation for each element
    P_element = delta_x * delta_y * P ! Applied Force on each element
    P_tot = P_tot + P_element ! Adding forces from each element
    C_tot = C_tot + C_element ! Adding Concentration deviation from all elements
ENDDO
WRITE(*,10) C_tot, P_tot
10 FORMAT('C =', E11.4, 'P=', E11.4)

!--------------  Finding domain/slice extension/boundary for calculating M_eff
xmin = node(1,1)
xmax = node(1,1)
ymin = node(1,2)
ymax = node(1,2)

DO i=2, N
    IF(xmin .ge. node(i,1)) xmin = node(i,1)
    IF(ymin .ge. node(i,2)) ymin = node(i,2)
    IF(xmax .le. node(i,1)) xmax = node(i,1)
    IF(ymax .le. node(i,2)) ymax = node(i,2)
ENDDO

Area = (xmax-xmin)*(ymax-ymin)
! Total Fluid Area per slice (could be different
! if the cross section area is changing)
WRITE(*,*) 'Area = ', Area
slice_conc_distribution = C_tot/Q_tot
! slice concentration Distribution
M_eff = (1 - slice_conc_distribution/Inlet_conc_distribution)*100
! Efficiency based on flow-rates
WRITE(*,20) M_eff
20 FORMAT(' M_eff = ', F6.2, '%')
P = P_tot/Area
IF (flag_p .eq. 1) THEN
    P_ref = P_tot/Area
ENDIF
!
.dp = P_ref - P
! Calculate pressure drop

!-------Write the calculated quantities/variables to the output file-------------------------
WRITE(6,35) location, M_eff, dp, C_max, x_C_max, y_C_max,
C_min, x_C_min, y_C_min
35 FORMAT(E11.4, E11.4, E11.4, E11.4, E11.4, E11.4, E11.4, E11.4, E11.4)
END DO
40 FORMAT(E17.9)
CLOSE(5)
!
CLOSE(6)
!
END

!------------------------------------------------------------------------^End of Main Program^-------------------
!---------------------------------------------------------------------------------------------------------------
!---------------------------------------------------------------------------------------------------------------
! Procedures and Functions
!-------------------------------------------------------------------------------
REAL*8 FUNCTION find_delta(x1,x2,x3,x4)
    ! Calculate delta_x or delta_y for each node

REAL*8 temp, dx1, dx2, dx3, dx4, x1, x2, x3, x4
dx1 = DABS((x1-x2))
dx2 = DABS(x2-x3)
dx3 = DABS(x3-x4)
dx4 = DABS(x4-x1)
temp = dx1
IF(temp.le.dx2) temp = dx2
IF(temp.le.dx3) temp = dx3
IF(temp.le.dx4) temp = dx4
find_delta=temp
RETURN
END

!-----------------------------------------------------------------------------------------------
REAL*8 FUNCTION get_avg(num1, num2, num3, num4)
! Calculates the average of 4 numbers
REAL*8 num1, num2, num3, num4
get_avg = (num1 + num2 + num3 + num4)/4.0
RETURN
END
Appendix B: Matlab Files

B.1 Matlab File Used in Constructing Calibration Curve

```matlab
clc; clear all; % Clear screen
% Clear all variables from the memory
frame_index = 1; % Index number for the frame number to read from the multi-image TIFF file
image_file_ext = '.tif'; % Extension of the image file
num_image_sets = 10; % Number of image sets
num_frames = 80; % Number of frames
tecplot_datafile = strcat('I:\amaha\Image_processing\Calibration_Rhb_case4\','Rhb_Calib_case4_Curve', '_tec.dat') % Create tecplot filename based on image filename
fileptr = fopen(tecplot_datafile, 'w'); % open the tecplot data file for appending
fprintf(fileptr, 'TITLE = "Rhb Calibration Curve"
VARIABLES =
"Dilution"
"Average Intensity"
"Standard Deviation"
"Concentration"
');
fprintf(fileptr, 'ZONE T="Rhb Calibration Curve"
I=10, F=POINT, DT=(DOUBLE, DOUBLE, DOUBLE, DOUBLE)
')

for n_images = 1 : num_image_sets % Name of the multi-image TIFF file assigned to image_filename variable
    image_filename = 'Rhb_Calib_case4_';
    file_nmbr = n_images*10; % File number generated based on loop counter
    image_filename = strcat(image_filename,num2str(file_nmbr), image_file_ext) % Filename to read images based on the set or part
    min_row_ind = 100; % Min index for row to calculate average
    max_row_ind = 120; % Max index for row to calculate average
    min_col_ind = 100; % Min index for column to calculate average
    max_col_ind = 120; % Max index for column to calculate average
    Int_array = zeros(1); % Initialize the Intensity array
    for frame_index = 1 : num_frames % Loop to go over all the frames
        frame_index % Display frame index number
```
B.2 Matlab File to Combine Images

% Amit Maha
% Program to read images from a multi-image TIFF file
% Program writes the calculated efficiencies into a single tecplot format file
% corresponding to each input file
clc; % Clear screen
clear all; % Clear all variables from the memory
image_file_ext = '.tif'; % Extension of the image file
num_image_sets = 22; % Number of image sets
m_pixel = 0.66; % microns per pixel
delta_pixel = floor(200/m_pixel);
for n_images = 1 : num_image_sets
**B.3 Matlab File to Calculate Efficiency from Experimental Images in TIFF Format**

```matlab
% Program to read images from a multi-image TIFF file
% Program writes the calculated efficiencies into a single tecplot format file
% corresponding to each input file

clc; % Clear screen
clear all; % Clear all variables from the memory

frame_index = 1; % Index number for the frame number to read from the multi-image TIFF file

image_file_ext = '.tif'; % Extension of the image file
file_path = 'I:\amaha\Image_processing\X2J_R50_Rhb_case4_exp1\Efficiency\';
num_image_sets = 16; % Number of image sets
m_pixel = 0.66; % microns per pixel
num_frames = 100; % Number of frames
z_p = 15; % Number of pixels per z location to estimate the intensity
```

```matlab
% Name of the multi-image TIFF file assigned to image_filename variable
image_filename = strcat('Part_', num2str(frame_index), image_file_ext) % Filename to read images based on the set or part

imgfile_info = imfinfo(image_filename,'tif') % Obtaining information about the image_file variable

[Img_X, map] = imread(image_filename, 1); % read the first frame to estimate the size of the image
frame_size = size(Img_X); % Size of each frame
no_of_columns = frame_size(2); % Total number of Columns per frame
no_of_rows = frame_size(1);
for i = 1 : delta_pixel
  for j = 1 : no_of_rows
    Img_Y(j, floor((n_images-1)*delta_pixel) + i) = Img_X(j,i);
  end
end
imwrite(Img_Y, map,
'I:\amaha\Image_processing\X2J_R50_Rhb_case4_exp1\Combined_Images\X2J_R50_Rhb_exp_combined_image.tif', 'Compression', 'none', 'Description', 'Combined Image', 'Resolution', [33 26], 'WriteMode', 'overwrite');
```
dist_increment_img_sets = 200; % Distance increments between the image sets = 200 microns

A = -6.860846560412470000E+000;
B =  1.152120850446460000E+002;

for n_images = 1 : num_image_sets
    image_filename = 'Section_'; % Name of the multi-image TIFF file assigned to image_filename variable
    image_filename = strcat(image_filename,num2str(n_images), image_file_ext) % Filename to read images based on the set or part
    imgfile_info = imfinfo(image_filename,'tif') % Obtaining information about the image_file variable
    [Img_X, map] = imread(image_filename, 1); % read the first frame to estimate the size of the image
    frame_size = size(Img_X); % Size of each frame
    no_of_columns = frame_size(2); % Total number of Columns per frame
    min_row_ind = 96; % Min index for row to calculate average
    max_row_ind = 166; % Max index for row to calculate average
    min_col_ind = 1; % Min index for column to calculate average
    max_col_ind = no_of_columns; % Max index for column to calculate average
    Int_array_begin = zeros(1); % Initialize the Intensity array
    Int_array_end = zeros(1); temp_Int_array_frame = zeros(1);
    count_int_array_begin = 1; count_int_array_end = 1;
    for frame_index = 1 : num_frames
        % Loop to go over all the frames
        [Img_X, map] = imread(image_filename, frame_index);
        % read the frame # based on index used
        Int_array_frame = zeros(1);
        count_int_array_frame = 1;
        for j = min_col_ind : max_col_ind
            % Loop to go over the columns
            temp_sub = double(Img_X(i,j)) + 1;
% 1 index offset as matlab index starts from 1
if j<=15
    Int_array_begin(count_int_array_begin,1) = map(temp_sub, 1);
    % Intensity Array to calculate mean, std etc
    count_int_array_begin = count_int_array_begin + 1;
else
    if j>(max_col_ind - 15)
        Int_array_end(count_int_array_end,1) = map(temp_sub, 1);
        % Intensity Array to calculate mean, std etc
        count_int_array_end = count_int_array_end + 1;
    end
end

Int_array_frame(count_int_array_frame,1) = map(temp_sub,1);
count_int_array_frame = count_int_array_frame + 1;

end % End for the column loop
end % End for the row loop

temp_Int_array_frame(n_images,1) = mean(Int_array_frame);
temp_std_array_frame(n_images,1) = std(Int_array_frame);

end % End for the frame loop

z_location = j-2;
% Calculate the z location based on pixel number relative to frame
% Calculate the z location by addition of relative distance = z_location*m_pixel
% and linear motion of the frames by 200 microns based on image sets
begin_location(n_images,1) = 1100 + (7-30)*m_pixel + (n_images-1)*dist_increment_img_sets;
end_location(n_images,1) = 1100 + (max_col_ind-7-30)*m_pixel + (n_images-1)*dist_increment_img_sets;
frame_location(n_images,1) = 1100 + ((min_col_ind+max_col_ind)/2-30)*m_pixel + (n_images-1)*dist_increment_img_sets;

mean_intensity_begin(n_images,1) = mean(Int_array_begin)
% Calculate the mean intensity from the array
mean_intensity_end(n_images,1) = mean(Int_array_end)
% Calculate the mean intensity from the array
mean_intensity_frame(n_images,1) = mean(temp_Int_array_frame)
% Calculate the mean intensity from the array

std_intensity_begin(n_images,1) = std(Int_array_begin)
% Calculate the standard deviation from the array
std_intensity_end(n_images,1) = std(Int_array_end)
% Calculate the standard deviation from the array
std_intensity_frame(n_images,1) = max(temp_std_array_frame)
% Calculate the standard deviation from the array

% Using the curve fit equation from calibration experiment for
% converting intensity to dilution
% Given equation Y = A + B*X, where X = Intensity and Y = Dilution
\[
\text{dilution\_begin}(n\_\text{images}, 1) = \frac{(A + B \times \text{mean\_intensity\_begin}(n\_\text{images}, 1))}{100};
\]
\[
\text{dilution\_end}(n\_\text{images}, 1) = \frac{(A + B \times \text{mean\_intensity\_end}(n\_\text{images}, 1))}{100};
\]
\[
\text{dilution\_frame}(n\_\text{images}, 1) = \frac{(A + B \times \text{mean\_intensity\_frame}(n\_\text{images}, 1))}{100};
\]

% Perform mixing efficiency based on dilution
\[
C_{\text{inf}} = 0.5;
\]
\[
C_{i\_H2O} = 1;
\]
\[
C_{i\_Rhb} = 1;
\]
\[
\text{efficiency\_begin}(n\_\text{images}, 1) = \frac{(1 - |\text{dilution\_begin}(n\_\text{images}, 1) - C_{\text{inf}}|/0.5)\times100}{100};
\]
\[
\text{efficiency\_end}(n\_\text{images}, 1) = \frac{(1 - |\text{dilution\_end}(n\_\text{images}, 1) - C_{\text{inf}}|/0.5)\times100}{100};
\]
\[
\text{efficiency\_frame}(n\_\text{images}, 1) = \frac{(1 - |\text{dilution\_frame}(n\_\text{images}, 1) - C_{\text{inf}}|/0.5)\times100}{100};
\]
end

\[
\text{tecplot\_datafile} = \text{strcat(file\_path,'X2J\_R50\_Rhb\_case4\_expl\_tec\_efficiency.dat')}
\]
% Create tecplot filename based on image filename

\[
\text{fileptr} = \text{fopen(tecplot\_datafile, 'a+');}
\]
% open the tecplot data file for appending
\[
\text{fprintf(fileptr, 'TITLE = "\%s"\nVARIABLES = \"Z\"\n"e"\n"Average Intensity"\n"Standard Deviation"\n', tecplot\_datafile);
\]
\[
\text{fprintf(fileptr, 'ZONE T="beginning of the frame" \nI=16, F=POINT, DT=(DOUBLE, DOUBLE, DOUBLE, DOUBLE)\n');}
\]
for \(n\_\text{images} = 1 : \text{num\_image\_sets}\)

\[
\text{fprintf(fileptr, '\%15.9f \%15.9f \%15.9f \%15.9f \n', begin\_location(n\_\text{images}, 1), efficiency\_begin(n\_\text{images}, 1), mean\_intensity\_begin(n\_\text{images}, 1), std\_intensity\_begin(n\_\text{images}, 1));}
\]
end

\[
\text{fprintf(fileptr, 'ZONE T="end of the frame" \nI=16, F=POINT, DT=(DOUBLE, DOUBLE, DOUBLE, DOUBLE)\n');}
\]
for \(n\_\text{images} = 1 : \text{num\_image\_sets}\)

\[
\text{fprintf(fileptr, '\%15.9f \%15.9f \%15.9f \%15.9f \n', end\_location(n\_\text{images}, 1), efficiency\_end(n\_\text{images}, 1), mean\_intensity\_end(n\_\text{images}, 1), std\_intensity\_end(n\_\text{images}, 1));}
\]
end

\[
\text{fprintf(fileptr, 'ZONE T="average over the entire frame" \nI=16, F=POINT, DT=(DOUBLE, DOUBLE, DOUBLE, DOUBLE)\n');}
\]
for \(n\_\text{images} = 1 : \text{num\_image\_sets}\)

\[
\text{fprintf(fileptr, '\%15.9f \%15.9f \%15.9f \%15.9f \n', frame\_location(n\_\text{images}, 1), efficiency\_frame(n\_\text{images}, 1), mean\_intensity\_frame(n\_\text{images}, 1), std\_intensity\_frame(n\_\text{images}, 1));}
\]
end
\[
\text{status} = \text{fclose(fileptr);}
\]
% Close the tecplot data file
Appendix C: LabView GUI for Instruments

Figure C.1: LabView GUI created to interact with PRIOR microscope stage and syringe pump
Appendix D: Numerical Simulation Results of Various Mixer Designs

<table>
<thead>
<tr>
<th>Offset Distance (µm)</th>
<th>Jet Velocity (each jet, Red) (mm/s)</th>
<th>Cross Stream Velocity (Blue) (mm/s)</th>
<th>Height of the channel (µm)</th>
<th>Length of the mixer (mm)</th>
<th>Total Flowrate (nL/s)</th>
<th>Pressure Drop (Pa)</th>
<th>Efficiency (η%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>10</td>
<td>150</td>
<td>1</td>
<td>56.25</td>
<td>360</td>
<td>24</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>10</td>
<td>150</td>
<td>1</td>
<td>75</td>
<td>510</td>
<td>53</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>10</td>
<td>150</td>
<td>4</td>
<td>75</td>
<td>1800</td>
<td>86</td>
</tr>
<tr>
<td>0</td>
<td>20</td>
<td>10</td>
<td>150</td>
<td>4</td>
<td>93.75</td>
<td>2790</td>
<td>88</td>
</tr>
<tr>
<td>0</td>
<td>50</td>
<td>50</td>
<td>150</td>
<td>4</td>
<td>375</td>
<td>9100</td>
<td>65</td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>10</td>
<td>150</td>
<td>1</td>
<td>56.25</td>
<td>390</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>10</td>
<td>150</td>
<td>1</td>
<td>56.25</td>
<td>385</td>
<td>27</td>
</tr>
<tr>
<td>200</td>
<td>5</td>
<td>10</td>
<td>150</td>
<td>1</td>
<td>56.25</td>
<td>420</td>
<td>26</td>
</tr>
<tr>
<td>1000</td>
<td>5</td>
<td>10</td>
<td>150</td>
<td>2</td>
<td>56.25</td>
<td>625</td>
<td>38</td>
</tr>
<tr>
<td>1000</td>
<td>10</td>
<td>10</td>
<td>150</td>
<td>2</td>
<td>75</td>
<td>825</td>
<td>71</td>
</tr>
<tr>
<td>1000 (only one inlet, the other inlet is provided with zero velocity)</td>
<td>20</td>
<td>10</td>
<td>150</td>
<td>4</td>
<td>75</td>
<td>1860</td>
<td>53</td>
</tr>
<tr>
<td>1000 (double the cross-section area for all the inlets)</td>
<td>10</td>
<td>10</td>
<td>150</td>
<td>4</td>
<td>75</td>
<td>1620</td>
<td>87</td>
</tr>
<tr>
<td>1000</td>
<td>10</td>
<td>10</td>
<td>150</td>
<td>4</td>
<td>150</td>
<td>430</td>
<td>63</td>
</tr>
</tbody>
</table>

Table D1: Comparing various X2J mixer configurations

Details on the parameters used in simulations of various micromixer designs:

**D.1 Simple Two-Inlet Mixer (5µm Separation Wall Between the Inlets)**

Simulation details:

**Inlet Channel Details:**
- Red inlet width = 22.5µm
- Blue inlet width = 22.5µm
- Height = 75µm
- Length = 0.5mm

**Mixing Chamber Details:**
- Width = 42.5µm
- Height = 75µm
Average velocity = 2mm/s (Red) Length = 7mm
Average velocity = 10mm/s (Blue)
Diffusion coefficient = $1.2 \times 10^{-10} \text{m}^2/\text{s}$

**D.2 U-Bend Mixer**
There were many simulations performed on this type of mixer:
Using unstructured mesh in the bend region. This simulation result showed considerable numerical diffusion compared to hexahedral mesh. Therefore, the results from this case were discarded.
Using hexahedral mesh in the bend region
Enhanced the simulation in 2 by making the mesh fine using hexahedral mesh
Fine mesh simulation comparable to X2J mixer

_U-Bend Mixer (short length, fine mesh results)_
Simulation details:
Inlet Channel Details:
Red inlet width = 25 µm
Blue inlet width = 12.5 µm
Height = 75 µm
Length = 0.5 mm
Average velocity = 5 mm/s (Red)
Average velocity = 10 mm/s (Blue)
Diffusion coefficient = $1.2 \times 10^{-10} \text{m}^2/\text{s}$

**D.3 U-Bend Mixer (Fine Mesh Results)**
Simulation details:
Inlet Channel Details:
Red inlet width = 25 µm
Blue inlet width = 25 µm
Height = 75 µm
Length = 0.5 mm
Average velocity = 5 mm/s (Red)
Average velocity = 10 mm/s (Blue)
Diffusion coefficient = $1.2 \times 10^{-10} \text{m}^2/\text{s}$

**D.4 U-bend Mixer (Comparing X2J Mixer)**
Simulation details:
Inlet Channel Details:
Blue inlet width = 25 µm
Red inlet width = 12.5 µm
Height = 75 µm
Length = 0.5 mm
Average velocity = 10 mm/s (Red)
Average velocity = 10 mm/s (Blue)
Diffusion coefficient = $1.2 \times 10^{-10} \text{m}^2/\text{s}$
D.5 Y-Mixer
There were many simulations performed on this type of mixer:
Using unstructured mesh in the contraction region. This simulation result showed considerable numerical diffusion compared to hexahedral mesh. Therefore, the results from this case were discarded.
Using hexahedral mesh in the bend region
Enhanced the simulation in 2 by making the mesh fine using hexahedral mesh
Fine mesh simulation comparable to X2J mixer

D.5.1 Y-Mixer (fine mesh)
Simulation details:
Inlet Channel Details: Mixing Chamber Details:
Red inlet width = 25$\mu$m Initial width = 42.5$\mu$m
Blue inlet width = 12.5$\mu$m Width after contraction = 20$\mu$m
Height = 75$\mu$m Height = 75$\mu$m
Length = 0.5mm Length = 1.5mm
Average velocity = 5mm/s (Red) Average velocity = 10mm/s (Blue)
Average velocity = 10mm/s (Blue)
Diffusion coefficient = 1.2x10$^{-10}$m$^2$/s

D.5.2 YP Mixer (comparing with X2J mixer)
Simulation details:
Inlet Channel Details: Mixing Chamber Details:
Red inlet width = 25$\mu$m Initial width = 42.5$\mu$m
Blue inlet width = 12.5$\mu$m Width after contraction = 20$\mu$m
Height = 75$\mu$m Height = 75$\mu$m
Length = 0.1mm Length = 1.5mm
Average velocity = 10mm/s (Red) Average velocity = 10mm/s (Blue)
Average velocity = 10mm/s (Blue)
Diffusion coefficient = 1.2x10$^{-10}$m$^2$/s

D.6 X2J Mixer
There were many simulations performed on this type of mixer:
Common Simulation details for this case study:
Inlet Channel Details: Mixing Chamber Details:
Blue inlet width = 25$\mu$m width = 25$\mu$m
Red inlet width = 12.5$\mu$m
Height = 75$\mu$m
Length = 0.1mm Height = 75$\mu$m
Diffusion coefficient = 1.2x10$^{-10}$m$^2$/s

D.6.1 X2J Mixer (no offset, fine mesh)
Simulation details:
Inlet Channel Details: Mixing Chamber Details:
Blue inlet width = 25$\mu$m width = 25$\mu$m
Red inlet width = 12.5$\mu$m
Height = 75 µm   Height = 75 µm
Length = 0.1 mm  Length = 4 mm
Average velocity = 10 mm/s (Red)  Length = 0.1 mm
Average velocity = 10 mm/s (Blue)  Average velocity = 5 mm/s (Red)
Diffusion coefficient = 1.2x10^{-10} m^2/s  Average velocity = 10 mm/s (Blue)

**D.6.2 X2J Mixer (25 µm center to center offset)**
Simulation details:
Inlet Channel Details: Mixing Chamber Details:
Blue inlet width = 25 µm  width = 25 µm
Red inlet width = 12.5 µm  Height = 75 µm
Height = 75 µm   Length = 0.1 mm
Average velocity = 5 mm/s (Red)  Length = 1 mm
Average velocity = 10 mm/s (Blue)  Diffusion coefficient = 1.2x10^{-10} m^2/s

**D.6.3 X2J Mixer (50 µm offset)**
Simulation details:
Inlet Channel Details: Mixing Chamber Details:
Blue inlet width = 25 µm  width = 25 µm
Red inlet width = 12.5 µm  Height = 75 µm
Height = 75 µm   Length = 0.1 mm
Average velocity = 5 mm/s (Red)  Length = 1 mm
Average velocity = 10 mm/s (Blue)  Diffusion coefficient = 1.2x10^{-10} m^2/s

**D.6.4 X2J Mixer (200 µm offset)**
Simulation details:
Inlet Channel Details: Mixing Chamber Details:
Blue inlet width = 25 µm  width = 25 µm
Red inlet width = 12.5 µm  Height = 75 µm
Height = 75 µm   Length = 0.1 mm
Average velocity = 5 mm/s (Red)  Length = 1.5 mm
Average velocity = 10 mm/s (Blue)  Diffusion coefficient = 1.2x10^{-10} m^2/s

**D.6.5 X2J Mixer (1 mm offset)**
Simulation details:
Inlet Channel Details: Mixing Chamber Details:
Blue inlet width = 25 µm  width = 25 µm
Red inlet width = 12.5 µm  Height = 75 µm
Height = 75 µm   Length = 0.1 mm

Average velocity = 5mm/s (Red)
Average velocity = 10mm/s (Blue)
Diffusion coefficient = $1.2 \times 10^{-10}$ m$^2$/s

**D.6.6 X2J Mixer (1mm offset)**
Simulation details:
Inlet Channel Details:
Blue inlet width = 25 µm
Red inlet width = 12.5 µm
Height = 75 µm
Length = 0.1 mm
Average velocity = 10 mm/s (Red)
Average velocity = 10 mm/s (Blue)
Diffusion coefficient = $1.2 \times 10^{-10}$ m$^2$/s

Mixing Chamber Details:
width = 25 µm
Height = 75 µm
Length = 2 mm

**D.6.7 X2J Mixer (1mm offset, coarse mesh)**
Simulation details:
Inlet Channel Details:
Blue inlet width = 25 µm
Red inlet width = 12.5 µm
Height = 75 µm
Length = 0.1 mm
Average velocity = 5 mm/s (Red)
Average velocity = 10 mm/s (Blue)
Diffusion coefficient = $1.2 \times 10^{-10}$ m$^2$/s

Mixing Chamber Details:
width = 25 µm
Height = 75 µm
Length = 4 mm

**D.6.8 X2J Mixer (1mm offset, coarse mesh)**
Simulation details:
Inlet Channel Details:
Blue inlet width = 25 µm
Red inlet width = 12.5 µm
Height = 75 µm
Length = 0.1 mm
Average velocity = 10 mm/s (Red)
Average velocity = 10 mm/s (Blue)
Diffusion coefficient = $1.2 \times 10^{-10}$ m$^2$/s

Mixing Chamber Details:
width = 25 µm
Height = 75 µm
Length = 4 mm

**D.6.9 X2J Mixer (1mm offset, fine mesh)**
Simulation details:
Inlet Channel Details:
Blue inlet width = 25 µm
Red inlet width = 12.5 µm
Height = 75 µm
Length = 0.1 mm
Average velocity = 10 mm/s (Red)
Average velocity = 10 mm/s (Blue)

Mixing Chamber Details:
width = 25 µm
Height = 75 µm
Length = 4 mm
Diffusion coefficient = 1.2x10^{-10} m^2/s

D.6.10 X2J Mixer (1mm offset, fine mesh 2)
Simulation details:
Inlet Channel Details:  Mixing Chamber Details:
Blue inlet width = 25µm  width = 25µm
Red inlet width = 12.5µm
Height = 75µm
Length = 0.1mm
Average velocity = 10mm/s (Red)
Average velocity = 10mm/s (Blue)
Diffusion coefficient = 1.2x10^{-10} m^2/s

D.6.11 X2J Mixer (1mm offset, double cross-section area)
Simulation details:
Inlet Channel Details:  Mixing Chamber Details:
Blue inlet width = 25µm  width = 25µm
Red inlet width = 12.5µm
Height = 75µm
Length = 0.1mm
Average velocity = 10mm/s (Red)
Average velocity = 10mm/s (Blue)
Diffusion coefficient = 1.2x10^{-10} m^2/s

D.6.12 X2J Mixer (1 inlet)
Simulation details:
Inlet Channel Details:  Mixing Chamber Details:
Blue inlet width = 25µm  width = 25µm
Red inlet width = 12.5µm
Height = 75µm
Length = 0.1mm
Average velocity = 20mm/s (Red)
Average velocity = 10mm/s (Blue)
Diffusion coefficient = 1.2x10^{-10} m^2/s

D.7 XIJC Mixer
XIJC Mixer (1 inlet before contraction)
Simulation details:
Inlet Channel Details:  Mixing Chamber Details:
Blue inlet width = 25µm  width = 25µm
Red inlet width = 12.5µm
Height = 75µm
Length = 0.1mm
Average velocity = 20mm/s (Red)
Average velocity = 10mm/s (Blue)
Diffusion coefficient = 1.2x10^{-10} m^2/s
D.8 X2JC Mixer

X2JC Mixer (1mm offset)
Simulation details:
Inlet Channel Details:  Mixing Chamber Details:
Blue inlet width = 50 µm  width = 25 µm
Red inlet width = 12.5 µm
Height = 75 µm
Length = 0.1 mm
Average velocity = 10 mm/s (Red)  Length = 4 mm
Average velocity = 10 mm/s (Blue)
Diffusion coefficient = 1.2x10^{-10} m^2/s

D.9 X2J Inlets R50 Mixer

X2J Mixer (1mm offset, jet inlets having 50 µm radius of curvature)
Simulation details:
Inlet Channel Details:  Mixing Chamber Details:
Blue inlet width = 25 µm  width = 25 µm
Red inlet width = 12.5 µm (Initial)
Red inlet width = 12.5 µm + 100 µm = 112.5 µm (final)
Height = 75 µm
Length = 0.1 mm
Height = 75 µm
Average velocity = 10 mm/s (Red)  Length = 4 mm
Average velocity = 10 mm/s (Blue)
Diffusion coefficient = 1.2x10^{-10} m^2/s
Appendix E: AutoCAD Micromixer Drawings

Figure E.1: AutoCAD drawing layout for X2J mixers manufactured by micromilling

Figure E.2: X2J micromixer drawing layout details
<table>
<thead>
<tr>
<th>Variables</th>
<th>X2J_1</th>
<th>X2J_2</th>
<th>X2J_3</th>
</tr>
</thead>
<tbody>
<tr>
<td>width of the mixing chamber Wm</td>
<td>µm</td>
<td>25.00</td>
<td>25.00</td>
</tr>
<tr>
<td>width of the jet into the mixer W2</td>
<td>µm</td>
<td>12.50</td>
<td>12.50</td>
</tr>
<tr>
<td>width of the channels that carry bulk jet fluid to the mixing chamber 4*W2</td>
<td>µm</td>
<td>50.00</td>
<td>50.00</td>
</tr>
<tr>
<td>width after 7deg expansion W_o</td>
<td>µm</td>
<td>86.16</td>
<td>86.16</td>
</tr>
<tr>
<td>Length of the channel of jet fluid at top L_F1</td>
<td>µm</td>
<td>29250.00</td>
<td>29250.00</td>
</tr>
<tr>
<td>Length of the channel of jet fluid at bottom L_F2</td>
<td>µm</td>
<td>28250.00</td>
<td>28250.00</td>
</tr>
<tr>
<td>Length of the channel of jet fluid to L_F1 L_R1</td>
<td>µm</td>
<td>225.00</td>
<td>225.00</td>
</tr>
<tr>
<td>Length of the channel of jet fluid to L_F2 L_R2</td>
<td>µm</td>
<td>725.00</td>
<td>725.00</td>
</tr>
<tr>
<td>Length of the main stream fluid from the feed port to the first jet L_c</td>
<td>µm</td>
<td>7500.00</td>
<td>7500.00</td>
</tr>
<tr>
<td>distance between the jets in the mixing channel delta_L</td>
<td>µm</td>
<td>1000.00</td>
<td>1000.00</td>
</tr>
<tr>
<td>Length of the mixer L_m</td>
<td>µm</td>
<td>3000.00</td>
<td>3000.00</td>
</tr>
<tr>
<td>Length from L_F1 to to L_jc L1</td>
<td>µm</td>
<td>462.50</td>
<td>462.50</td>
</tr>
<tr>
<td>Length from L_F2 to L_jc L2</td>
<td>µm</td>
<td>962.50</td>
<td>962.50</td>
</tr>
<tr>
<td>Length for channel expansion L_d</td>
<td>µm</td>
<td>500.00</td>
<td>500.00</td>
</tr>
<tr>
<td>Length after expansion to the exit port L_o</td>
<td>µm</td>
<td>1000.00</td>
<td>1000.00</td>
</tr>
<tr>
<td>Radius of the ports R</td>
<td>µm</td>
<td>750.00</td>
<td>750.00</td>
</tr>
<tr>
<td>Length between the input feed ports L_fp</td>
<td>µm</td>
<td>20000.00</td>
<td>20000.00</td>
</tr>
<tr>
<td>Length of the inlet jet channel with width W2 L_ji</td>
<td>µm</td>
<td>250.00</td>
<td>250.00</td>
</tr>
<tr>
<td>Length of the contraction channel of jet fluid from 4*W2 to W2 L_jc</td>
<td>µm</td>
<td>250.00</td>
<td>250.00</td>
</tr>
<tr>
<td>Depth/Height of the channel depth, H</td>
<td>µm</td>
<td>150.00</td>
<td>150.00</td>
</tr>
</tbody>
</table>

Table E.1: Dimension details for the X2J micromilled mold insert layout drawing
# Appendix F: Compare Mixing Times From Literature Review

Table F.1: Comparing various designs based on active Micromixers

<table>
<thead>
<tr>
<th>Paper</th>
<th>Type</th>
<th>Channel height (µm)</th>
<th>Channel width (µm)</th>
<th>Channel Length (mm)</th>
<th>Flow rate (µl/min)</th>
<th>Avg. Velocity (mm/s)</th>
<th>Re</th>
<th>Time to produce 10nL (s)</th>
<th>Materials / Manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deshmukh et. al. (2001)</td>
<td>Pressure</td>
<td>78.0</td>
<td>400.0</td>
<td>1.5</td>
<td>1.6850</td>
<td>0.9000</td>
<td>0.0100</td>
<td>2.0230</td>
<td>Silicon-glass, DRIE</td>
</tr>
<tr>
<td>Suzuki et. al. (2002)</td>
<td>Pressure</td>
<td>35.0</td>
<td>160.0</td>
<td>N/R</td>
<td>0.1010</td>
<td>0.3000</td>
<td>0.2300</td>
<td>5.9410</td>
<td>KOH etching</td>
</tr>
<tr>
<td>Oddy et. al. (2001)</td>
<td>Electrokinetic</td>
<td>100.0</td>
<td>1000.0</td>
<td>1.0</td>
<td>1.0000</td>
<td>0.1670</td>
<td>1.5000</td>
<td>6.5880</td>
<td>PDMS / wet-etched Borofloat glass</td>
</tr>
<tr>
<td>Lee et. al. (2001)</td>
<td>Electrokinetic</td>
<td>25.0</td>
<td>200.0</td>
<td>N/R</td>
<td>0.1500</td>
<td>0.5000</td>
<td>0.0100</td>
<td>4.0000</td>
<td>N/A</td>
</tr>
<tr>
<td>Shin et. al. (2005)</td>
<td>Electric Field</td>
<td>20.0</td>
<td>60.0</td>
<td>20.0</td>
<td>5.0400</td>
<td>70.0000</td>
<td>10.0000</td>
<td>0.4050</td>
<td>Pyrex / wet etching</td>
</tr>
<tr>
<td>Lu et. al. (2002)</td>
<td>Magnetic</td>
<td>70.0</td>
<td>750.0</td>
<td>4.0</td>
<td>0.1700</td>
<td>0.0540</td>
<td>0.4800</td>
<td>77.6030</td>
<td>PDMS / KOH wet etching</td>
</tr>
<tr>
<td>Suzuki et. al. (2003)</td>
<td>Magnetic Beads</td>
<td>80.0</td>
<td>100.0</td>
<td>N/R</td>
<td>0.0500</td>
<td>0.1040</td>
<td>0.0220</td>
<td>12.0000</td>
<td>KOH etching</td>
</tr>
<tr>
<td>He et. al. (2001)</td>
<td>Electroosmotic</td>
<td>10.0</td>
<td>5.0-27</td>
<td>0.2</td>
<td>9e-4 - 4.86e-3</td>
<td>0.3000</td>
<td>0.001-0.004</td>
<td>667 - 125</td>
<td>N/A</td>
</tr>
<tr>
<td>Evans et. al. (1997)</td>
<td>Source-sink</td>
<td>100.0</td>
<td>600.0</td>
<td>N/R</td>
<td>N/R</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>KOH etching</td>
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</table>
Table F.2: Comparing various designs based on passive Micromixers

<table>
<thead>
<tr>
<th>Paper</th>
<th>Type</th>
<th>Channel height (µm)</th>
<th>Channel width (µm)</th>
<th>Channel Length (mm)</th>
<th>Flow rate (µl/min)</th>
<th>Avg. Velocity (mm/s)</th>
<th>Re</th>
<th>Time to produce 10nL (s)</th>
<th>Pressure drop (Pa)</th>
<th>Materials / Manufacture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gobby et. al. (2001)</td>
<td>T-mixer (Simulation)</td>
<td>300</td>
<td>500</td>
<td>1.9-2.25</td>
<td>2.7</td>
<td>0.3</td>
<td>0.1</td>
<td>7.72</td>
<td>9-28.9</td>
<td>N/A</td>
</tr>
<tr>
<td>Knight et. al. (1998)</td>
<td>Focusing</td>
<td>10</td>
<td>10</td>
<td>N/R</td>
<td>0.3</td>
<td>50</td>
<td>0.5</td>
<td>2</td>
<td>10 (psi, inlet pressure)</td>
<td>PDMS</td>
</tr>
<tr>
<td>Veenstra et. al. (1999)</td>
<td>T-mixer</td>
<td>200</td>
<td>100</td>
<td>1.65</td>
<td>2.00</td>
<td>1.667</td>
<td>N/R</td>
<td>1.29</td>
<td>N/R</td>
<td>Silicon / glass</td>
</tr>
<tr>
<td>Wong et. al. (2003)</td>
<td>Cross</td>
<td>40</td>
<td>30</td>
<td>N/R</td>
<td>360-720</td>
<td>5000-10000</td>
<td>170-340</td>
<td>0.16-0.08</td>
<td>N/R</td>
<td>Ceramic</td>
</tr>
<tr>
<td>Wong et. al. (2004)</td>
<td>T-mixer</td>
<td>50</td>
<td>100</td>
<td>N/R</td>
<td>2100</td>
<td>7000</td>
<td>500</td>
<td>0.00285714</td>
<td>N/R</td>
<td>Silicon / glass</td>
</tr>
<tr>
<td>Wu et. al. (2004)</td>
<td>Y-mixer</td>
<td>50</td>
<td>900</td>
<td>N/R</td>
<td>0.729</td>
<td>0.27</td>
<td>0.02</td>
<td>8.23045267</td>
<td>N/R</td>
<td>PMMA</td>
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<tr>
<td>Seidel et. al. (1999)</td>
<td>Injection</td>
<td>20-30</td>
<td>280-600</td>
<td>N/R</td>
<td>N/R</td>
<td>N/R</td>
<td>N/R</td>
<td>N/A</td>
<td>N/R</td>
<td>Silicon / glass</td>
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<tr>
<td>Voldman et. al. (2000)</td>
<td>Injection</td>
<td>70</td>
<td>820</td>
<td>1</td>
<td>5.0-30</td>
<td>N/R</td>
<td>N/R</td>
<td>2.1-4.1</td>
<td>N/R</td>
<td>Silicon / glass</td>
</tr>
<tr>
<td>Wang et. al. (2002)</td>
<td>Cylindrical obstacles</td>
<td>100</td>
<td>300</td>
<td>N/R</td>
<td>0.306</td>
<td>0.17</td>
<td>0.25</td>
<td>1.961</td>
<td>N/R</td>
<td>Quartz</td>
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<tr>
<td>Lin et. al. (2005)</td>
<td>3d Vortex micromixer</td>
<td>1000</td>
<td>830 (dia)</td>
<td>N/A</td>
<td>N/R</td>
<td>N/R</td>
<td>1.0-6.0</td>
<td>N/A</td>
<td>N/A</td>
<td>Silicon / glass</td>
</tr>
<tr>
<td>Mengeaud et al. (2002)</td>
<td>Zig-zag shaped channels</td>
<td>48</td>
<td>100</td>
<td>2</td>
<td>0.0012-0.072</td>
<td>1.3 - 40</td>
<td>0.25 - 270</td>
<td>8.4 - 20</td>
<td>N/A</td>
<td>Mylar</td>
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<td>------------------------</td>
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<tr>
<td>Liu et al. (2000)</td>
<td>3D serpentine</td>
<td>150</td>
<td>300</td>
<td>N/R</td>
<td>10-1200</td>
<td>30 - 350</td>
<td>6.0 - 70</td>
<td>0.06-0.007</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Park et al. (2004)</td>
<td>3D serpentine</td>
<td>50</td>
<td>100</td>
<td>4</td>
<td>N/R</td>
<td>N/R</td>
<td>1.0 - 50</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Stroock et al. (2004)</td>
<td>Patterned wall</td>
<td>70</td>
<td></td>
<td>N/R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Madou et al. (2001)</td>
<td>CD-like platform</td>
<td>63.5-254</td>
<td>127-508</td>
<td>N/R</td>
<td></td>
<td></td>
<td>N/R</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<tr>
<td>Shastry et al. (1998)</td>
<td>Concentric Capillary with bead</td>
<td>N/A</td>
<td>250 (dia)</td>
<td>5</td>
<td>150</td>
<td>1000</td>
<td>N/R</td>
<td>4.00E-05</td>
<td>N/R</td>
<td>GLASS</td>
</tr>
<tr>
<td>Pabit et al. (2002)</td>
<td>Concentric Capillary</td>
<td>100</td>
<td>100</td>
<td>N/R</td>
<td>300</td>
<td>200</td>
<td>14</td>
<td>N/R</td>
<td>N/R</td>
<td>Fused Silica</td>
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<tr>
<td>Losey et al. (2002)</td>
<td>Multiple parallel inlet channels with integrated filter structures</td>
<td>300</td>
<td>625</td>
<td>20</td>
<td>2000</td>
<td>N/R</td>
<td>N/R</td>
<td>N/A</td>
<td>0.54 (atm)</td>
<td>Silicon microfab. Technology</td>
</tr>
<tr>
<td>Yang et al. (2004)</td>
<td>array of impinging micro jets</td>
<td>1000</td>
<td>N/A</td>
<td>5</td>
<td>20</td>
<td>1.67</td>
<td>0.145</td>
<td>204.3</td>
<td>SU8</td>
<td></td>
</tr>
</tbody>
</table>
Appendix G: Experimental Instruments Data Sheets

Figure G.1: Mercury source lamp emission spectrum

Figure G.2: Filter cube spectrum
Vita

Amit Maha was born in Hyderabad, Andhra Pradesh, India, in 1979. He completed his high school studies in 1996 and joined the undergraduate studies program in mechanical engineering at the Louisiana State University, Baton Rouge, in 1996. He finished his undergraduate studies in 2000. He joined Louisiana State University, Baton Rouge, in 2001 to pursue his master’s degree. He expects to receive his master’s degree in December 2005.