DESIGNING A MICROFLUIDIC SYSTEM: CHANNEL FABRICATION AND FLOW DELIVERY

A Thesis

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Master of Science

by

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ABSTRACT

This thesis presents the process of fabricating a microfluidic mixer and the accompanying fluid delivery system. The device utilizes SUEX epoxy Thick Dry Film Sheets (TDFS) as a photoresist and PMMA as the substrate with features patterned by standard UV lithography. SUEX TDFS is a dry film negative photoresist sheet with a thickness ranging from 100 to 1000 μm. A 200 μm SUEX TDFS is used to seal the microfluidic channels. The sealed device was tested for mixing process under our home made microscope. The air-pressure delivery system was fabricated with acrylic and tested with different types of microfluidic channels.

BIOGRAPHICAL SKETCH

Yunsheng Wang is currently a Master of Science student at Cornell University. She attended Hefei University of Technology from 2009 to 2014. She spend three years at Northern Arizona University from 2010 to 2013, and graduated with a Bachelor of Science degree in Applied & Engineering physics. She came to Cornell University in the summer of 2014 and began Master of Science study in Applied & Engineering Physics. She is pursuing her research on fabricating Microfluidic mixer under the director of Professor Lois Pollack.

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A lot of Thanks to my parents and my parents-in-law as well. Without their love and funding, I could not be at Cornell. Thank you for their generously love and countless time have been spent on me and my son. Finally, to my caring, loving, and supportive husband, Yaohui: Your encouragement and support are very much appreciated and duly noted.

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PDMS : polydimethylsioxane

PMMA : polymethyl methacrylate

COC : Cyclic Olefin Copolymer

PET : Polyethylene terephthalate

CHAPTER 1

INTRODUCTION AND MOTIVATIONS

Microfluidic mixing devices have found application in a large and growing number of studies of fast kinetic biomolecule reactions, offering automation and high-throughput screening while consuming small sample volumes [1]. Rapid mixing of multiple samples is a straightforward method of studying chemical and biological reaction processes, with applications to studies of protein folding kinetics [2] [3], rapid crystallization [4] [5] and cell sorting [6] [7]. Microfluidic mixers were coupled with a variety of optical detection methods such as custom-built microscope to obtain time-resolved data necessary for investigating structural and thermodynamic properties of intermediate states.

Numerous microfluidic mixer designs have been developed to study the mixing process at the micron scale with millisecond time resolution: stopped-flow mixers [8], turbulent continuous-flow mixers [9] [10], and laminar-flow mixers [11]. We implement laminar-flow mixers, since they provide greater uniformity of, and control over, mixing conditions, while offering short dead times and microliter sample volume consumptions [12]. In a laminar flow mixer, the flow rate is very low and the mixing process is driven by diffusion. Therefore, to achieve rapid mixing in a short time, the inner stream in the mixing region has to be as thin as possible [11]. Knight et al., designed a laminar-flow mixer that can achieve microseconds mixing time, nanoliter sample consumption and submicro second time resolution [11]. Park et al., designed a laminar-flow mixer that can achieve a uniform mixing with minimal dead time [13].

Mixers of those designs have successfully been used to study protein folding kinetics coupled with small angle X-ray scattering [14], Forster resonance energy transfer [15], and UV fluorescence spectroscopy [16].

The mixer of Knight et al., and Park et al., were fabricated by replica molding with polydimethylsiloxane (PDMS). With this method, their only need to use a single microfabricated silicon mold to rapidly build prototypes at a low cost. Although successful results have been achieved by this technique, the lower reproducibility and structure limitations of PDMS have restricted the application of the laminar-flow mixer.

To ensure the wide application of laminar-flow mixer, here we present a microfluidic device fabricated with SUEX Thick Dry Film Sheets (TDFS). SUEX is a dry film photoresist with various thickness ranging from 100 to 1000 μm, and can be patterned with standard UV and X-ray lithography on different types of substrates in any shape [17]. Besides, a new delivery system: pressure-drive system is illustrated and tested to provide an alternative to syringe pumps.

Chapter 2 describes the design and fabrication of the microfluidic mixer. The design is duplicated from Wunderlich et al [18]. The microfluidic mixer is fabricated using a new photoresist SUEX.

Chapter 3 introduces the design and fabrication process of a multiple-solution pressure-driven-system for the microfluidic mixer. We designed a pressure-driven system that is easy to setup and refill. The reservoir of the pressure-driven system was fabricated from acrylic using laser and machine cutting. The theory and characterization behind the pressure-driven-system are shown in this chapter. The

pressure driven system was calibrated with a 25 cm long tube. The device repeatability was tested by applying the pressure-driven-system to two sets of microfluidic channels with same dimensions. The impact of material on device performance was also tested by using the pressure-driven-system on two sets of microfluidic channels with same dimensions but built from different materials.

Chapter 4 presents the pressure-driven system as applied to the microfluidic mixer duplicated from Wunderlich et al [18]. The imaging was acquired via a homemade optical system. Here, we describe our initial results with the mixer and offer suggestions for improvement for future work.

Chapter 5 contains a summary of the results and recommendations for future applications of the microfluidic mixer and pressure-driven-design.

CHAPTER 2

FABRICATION OF THE MICROFLUIDIC MIXER

2.1 Microfluidic Mixer Utilizing SUEX

Due to the simplicity and speed of the process, most researchers are interested in fabricating microfluidic mixers using Polydimethylsiloxane (PDMS) and soft lithography [12] [19]. With this technique, researchers need only build a master with structures in a clean room using microlithographic techniques such as photolithography, micromachining, e-beam writing, and relief structures etched in metal or Si [20]. This master can then be used to rapidly prototype the PDMS stamps outside the clean room. The process of fabricating the PDMS stamp is shown in Fig. 1. The liquid PDMS is poured over the silicon master, and peeled off after curing. This technique brings benefits, such as money and time savings, but it also has its own disadvantages when applied to experiments listed below.

First, as shown in Fig. 1**,** the aspect ratio of the microstructure is limited by the softness of PDMS. When the height (h) and width (d) ratio is too high ($h \geq 10d$), the wall will tend to collapse. If the height and width ratio is too low ($d \geq 20h$), the PDMS will sag [20]. Second, the microchannel made in PDMS tends to bend when bonded to a glass cover, as shown in Fig. 2. The microstructure will easily be deformed. Third, the cured PDMS will shrink a little bit ($\sim 1\%$), besides, the cured PDMS can be easily swelled by some nonpolar organic solvents such as toluene and hexane [21]. This will change the channel cross section area.

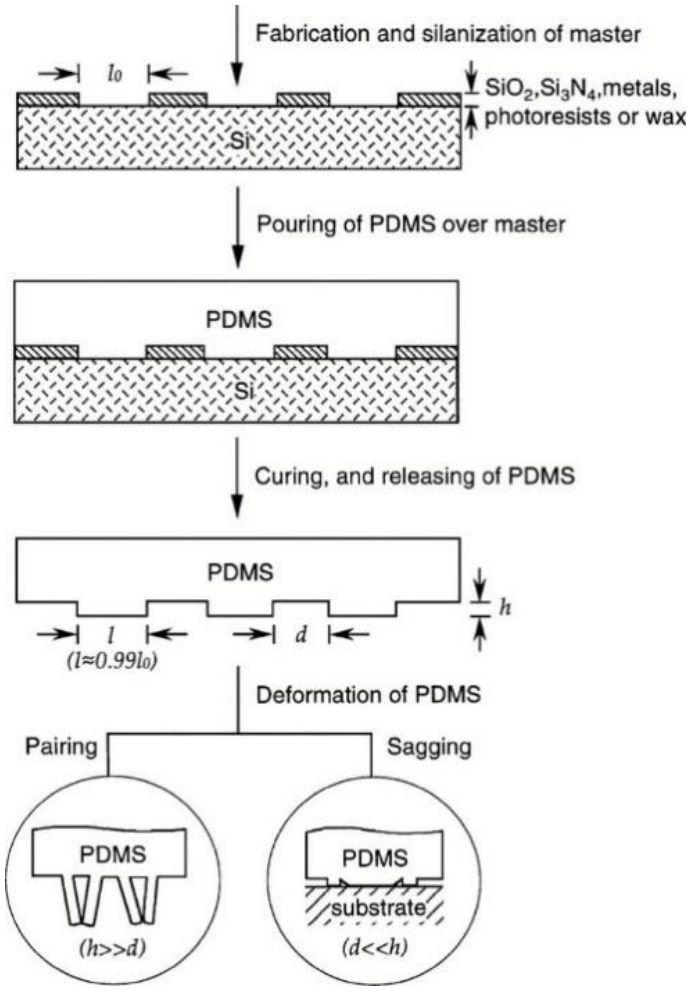


Figure 1 Schematic illustration of the procedure for fabricating PDMS stamps from a master having relief structures on its surface [21].

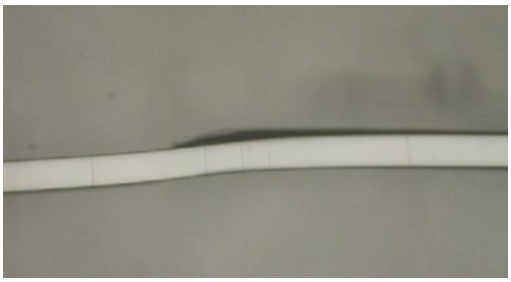


Figure 2. Microscope image of the bend PDMS microchannel when bond with cover glass.

In order to overcome the above deficiencies of PDMS, we use a new photoresist, SUEX, to fabricate the mixer. The performance will be given in chapter 4. SUEX is an epoxy-based negative photoresist and a Thick Dry Film sheet. The heights of SUEX vary from 100μm to 1mm, with the dry film protected by two throw-away layers of protective polyester (PET) film. Standard UV and X-ray lithography are able to pattern features [22]. SUEX has similar advantages to polymer materials used in standard microfabrication, such as being inexpensive, transparent to UV/visible light, having easily modifiable surface properties and improved biocompatibility [23]. The negative photoresist SUEX is chosen to fabricate the channel directly based on several advantages compared with SU-8. Below are the advantages of fabricating microfluidic mixers using SUEX compared with SU-8:

1. Uniform height
2. Clean and no edge bead effects.
3. Reproducible (wafer-to-wafer) and can be layered reproducibly.
4. Can be cast on acrylic, glass, copper in any shape.
5. Less fabrication time (around two hours) compared with SU-8 (up to few days' fabrication)

The fabrication process of SU-8 and SUEX are compared in Fig. 3. Compared with SU-8, the most convenient way of using SUEX is it does not require to adjust the spin speed and bake condition to get a desired height. The soft bake and post-exposure bake time of SUEX is much shorter than SU-8 as shown in Fig 3 c) and d). Besides,

the SUEX surface is more uniform than SU-8, because SU-8 cannot wet low surface energy substrate [24].

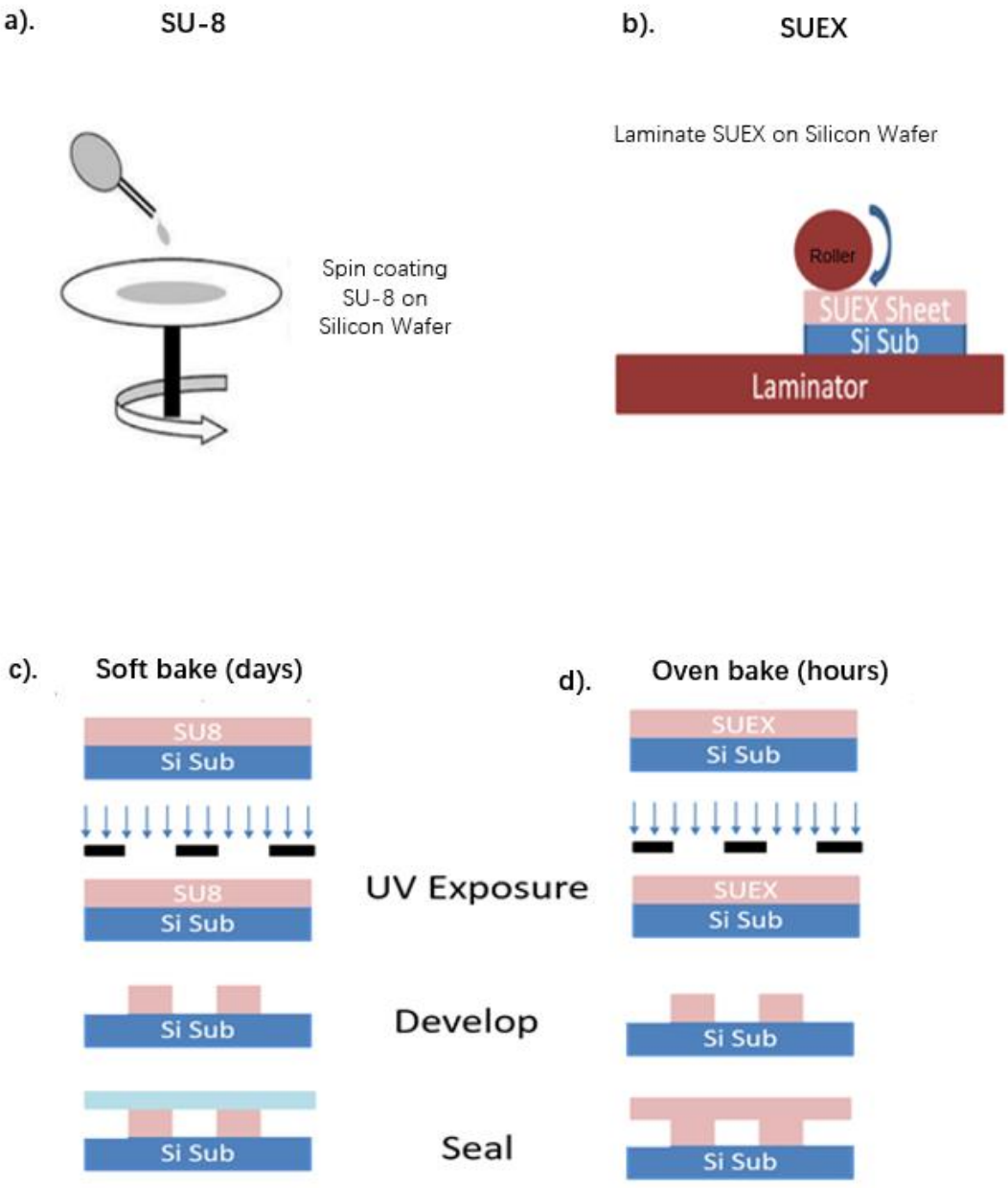


Figure 3. Fabrication process comparison between SU-8 and SUEX. a. Spin coat SU-8 on silicon wafer. (b) Laminate SUEX on silicon wafer. (c) Pattern process for SU-8. (d) Pattern process for SUEX

2.2 Selection of The Substrate of the Microfluidic mixer

There are several standards required for the substrate of a microfluidic mixer.

1. Transparent to UV light
2. Easy to machine for tubing connections
3. Compatible with SUEX

Several types of material satisfy all of these standards: silica, glass and some polymer materials. Silicon and glass however are too brittle to machine. Therefore, polymer materials are advantageous as substrates based on their good mechanical and machining properties. As shown in Table 1 [25], compared with other polymer materials, COC, PET and PMMA have better UV transparency. Besides PMMA are compatible with cross linked SU-8 [26]. Since SUEX has the same properties as SU-8, it is compatible with PMMA. Based on all of these standard requirements, we chose to use PMMA as the microfluidic mixer's substrate.

Table 1. Summary of Physical properties of common microfluidic polymer materials [25]

Polymer	Acronym	T_g (°C)	T_m (°C)	CTE (10^{-6}°C^{-1})	Water absorption (%)	Solvent resistance	Acid/base resistance	Optical transmissivity	
								Visible	UV[a]
Cyclic olefin (co)polymer	COC/COP	70–155	190–320	60–80	0.01	Excellent	Good	Excellent	Excellent
Polymethylmethacrylate	PMMA	100–122	250–260	70–150	0.3–0.6	Good	Good	Excellent	Good
Polycarbonate	PC	145–148	260–270	60–70	0.12–0.34	Good	Good	Excellent	Poor
Polystyrene	PS	92–100	240–260	10–150	0.02–0.15	Poor	Good	Excellent	Poor
Polypropylene	PP	−20	160	18–185	0.10	Good	Good	Good	Fair
Polyetheretherketone	PEEK	147–158	340–350	47–54	0.1–0.5	Excellent	Good	Poor	Poor
Polyethylene terephthalate	PET	69–78	248–260	48–78	0.1–0.3	Excellent	Excellent	Good	Good
Polyethylene	PE	−30	120–130	180–230	0.01	Excellent	Excellent	Fair	Fair
Polyvinylidene chloride	PVDC	0	76	190	0.10	Good	Good	Good	Poor
Polyvinyl chloride	PVC	80	180–210	50	0.04–0.4	Good	Excellent	Good	Poor
Polysulfone	PSU	170–187	180–190	55–60	0.3–0.4	Fair	Good	Fair	Poor

T_m melting temperature. CTE coefficient of thermal expansion

2.3 Selection of Sealing Material of the Microfluidic Mixer

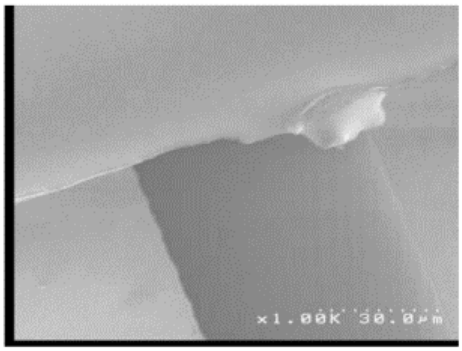
Compared to other alternatives, like glass and polymers, SUEX is more convenient to seal the channel, and bonds is very well [27]. The process of using SUEX to seal the channel is very simple: laminate a 200 μm thickness SUEX sheet and expose it with a certain UV dosage, then bake at 65 degrees for 5 minutes.

It is very important to decide a proper lamination temperature. If the lamination temperature is too high, the SUEX will flow into the channel and affect the channel size. If the lamination temperature is too low, then the adhesion between the SUEX will not be sufficient to provide adequate bonding. As shown in Fig. 4**,** the sagging across a 50μm channel at different lamination temperatures. The sealing process is showing in Appendix A**.**

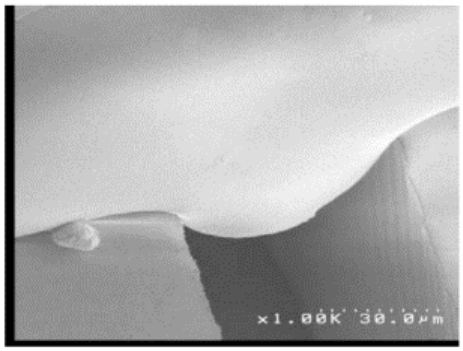
2.4 Device Design [18]

The device design and key elements are shown in Fig. 5. The center and two side inlets allow the sample solution to mix in the mixing region. The width of the channel is 50μm, then the channel narrow to 8 μm to ensure a rapid mixing. The observation channel then widens to 50 μm to allow a sufficient signal to be detected by the microscope. Filters (schematic shown in Fig. 5 b) are designed in the center and side

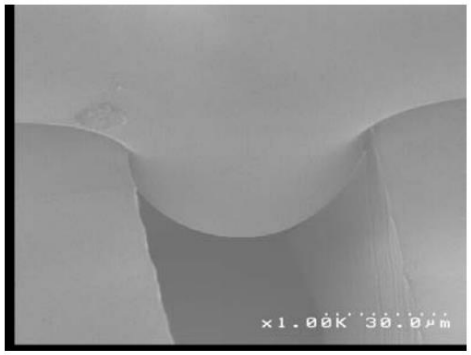
channels to prevent dust and other particles entering the device and clogging the narrow mixing region.



Lamination at 47˚C



Lamination at 55˚C



Lamination at 62˚C

Figure 4. The sagging of the SUEX coversheet across the 50um channel at different lamination temperature [27].

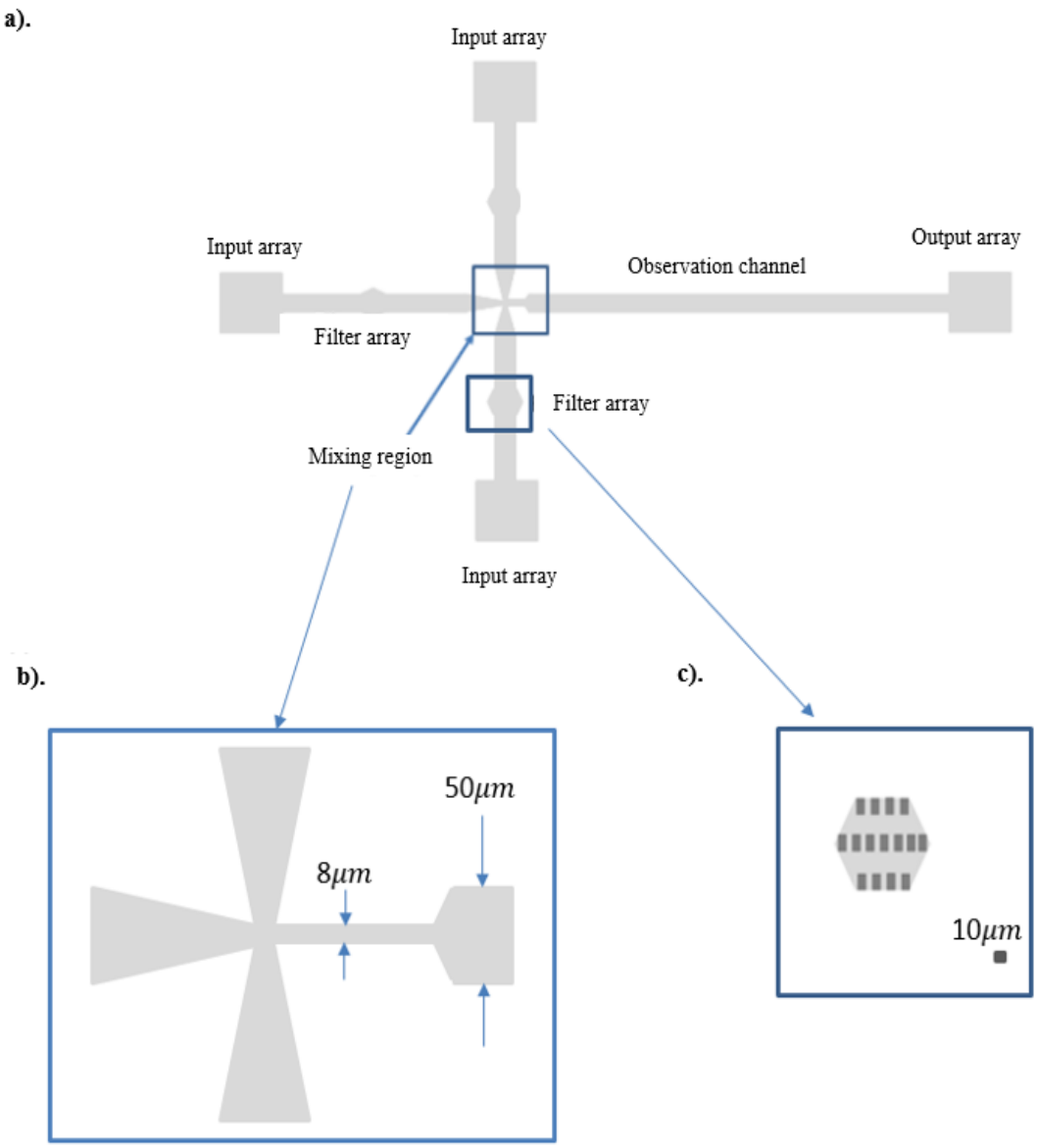


Figure 5. Design of the microfluidic mixer. a). Layout of the microfluidic mixer. Liquid is driven through the three inlets towards the mixing region. The center and two side inlets allow the sample solution to mix in the mixing region. The filter array is used to prevent clogging of the device. b). The width of the channel is 50 μm, then the channel narrow to 8μm to ensure a rapid mixing. c). Filter array with three rows of post separations (10 μm) connected to the inlet post array to prevent small particle from entering the channels and blocking the mixing region. The depth of all features is 100 μm.

2.5 Fabrication Process

The microfluidic mixer is completely fabricated by polymer materials. The PMMA was predrilled with 1 mm holes and laser cut into 100 mm square and used as the substrate. Then a 96 mm diameter and 100 μm SUEX sheet was laminated onto the PMMA substrate (Fig. 5 b). The actual height of the SUEX sheet was measured to be 98 μm. The mask for lithography was made by Heidelberg Mask Writer DWL 2000. The AB-M contact aligner was used for photolithographic process to pattern the channels on the SUEX: the mask was placed in contact with the top of the SUEX sheet and the SUEX is exposed to UV light (1200) for 93 seconds with two intervals. Then bake it on hot plate and developed in PGMEA (EBR-10A). After clean with Isopropanol a 96 mm diameter 200 μm high SUEX was laminated on top of the structure to seal the channel. Fig. 6 show the complete microfabrication process. See Appendix A for fabrication protocol.

2.6 Final Device and Results

The final device is shown in Fig. 7. The pillar inside the filter has collapsed due to the small contact area between SUEX and PMMA substrate. This results in a low bonding stress which is not enough to prevent collapse during development. Due to this, we applied an outer 2 μm filter between the sample reservoir and the tubing as an alternative to the filter inside the channel. This will be discussed in detail in Chapter 4.

In order to remove the pillar inside the device, slow stirring during the developing process is required. See Appendix A for a detailed fabrication process.

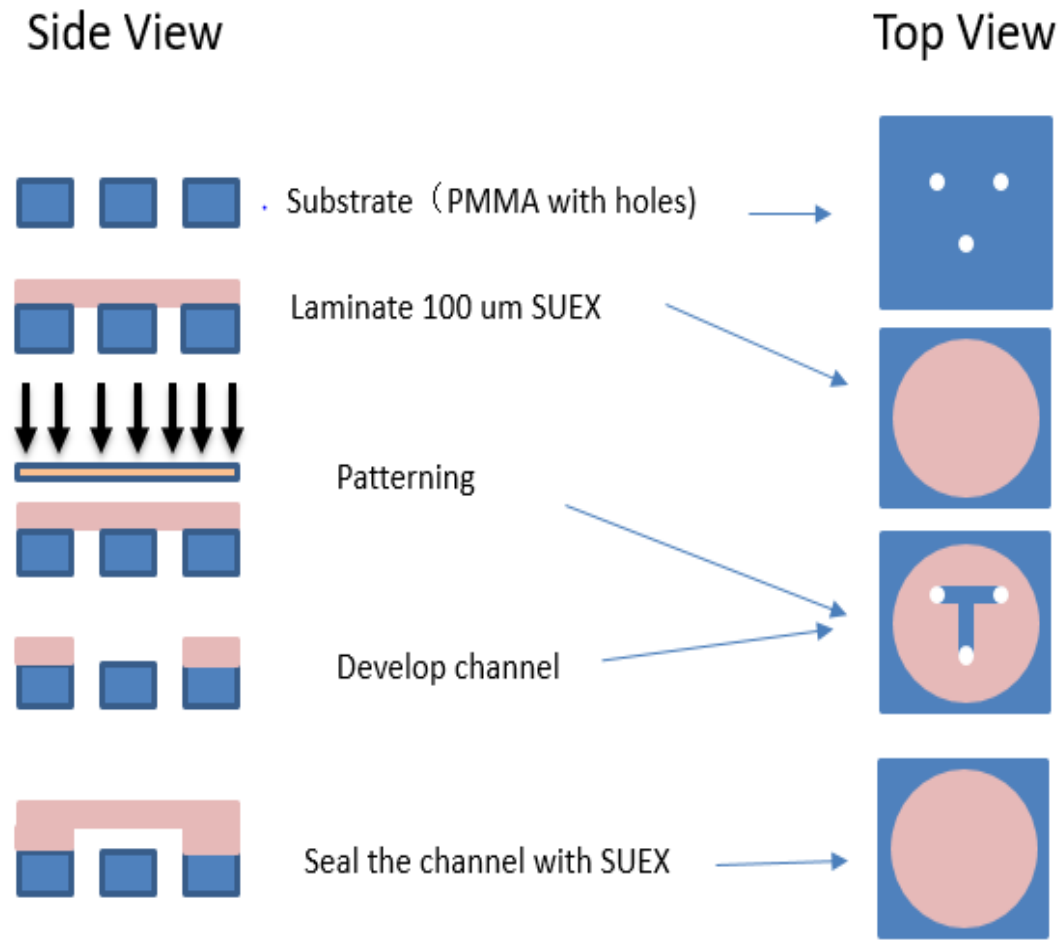
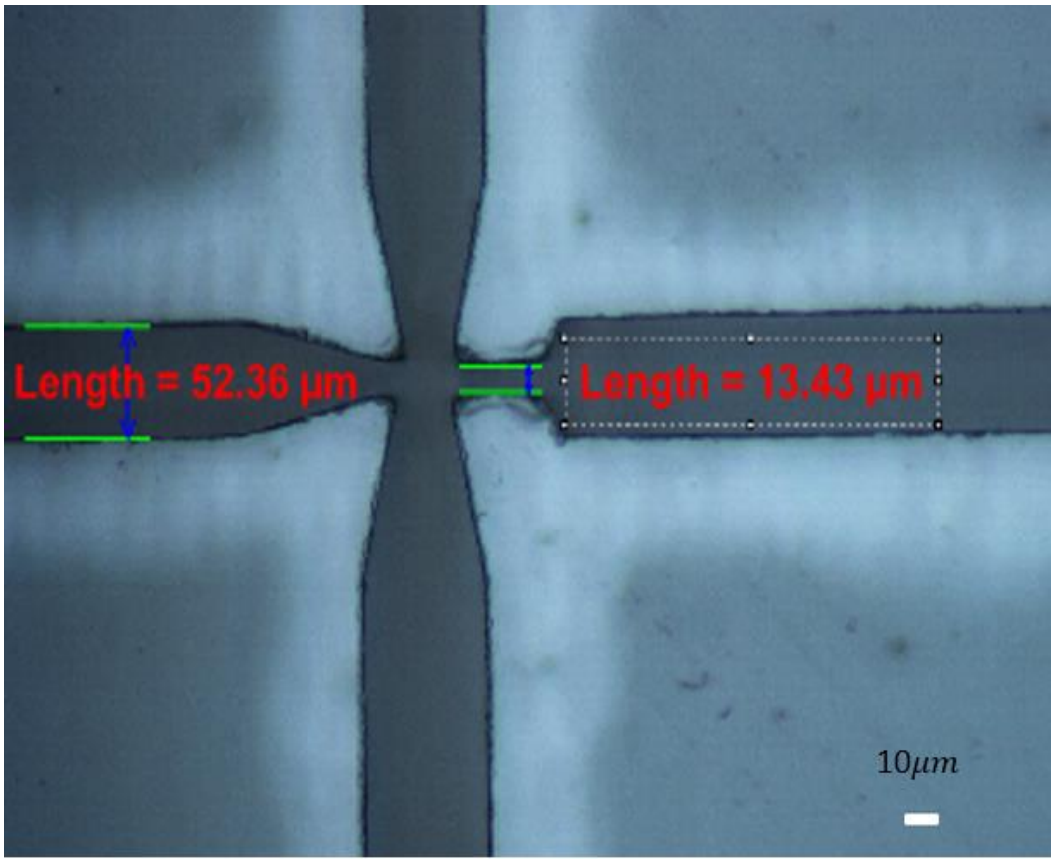
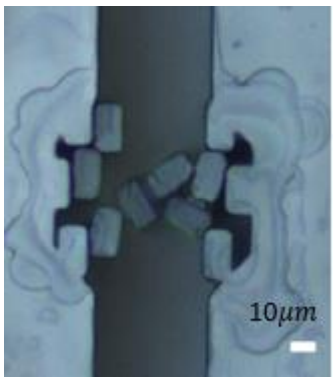


Figure 6. The complete fabrication process of the mixer utilizing SUEX

a).



b).



c).

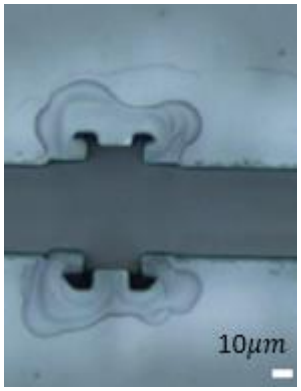


Figure 7. The microfluidic mixer fabricated by SUEX. The scale bar is 10µm. (a) Micrograph of the mixing region. (b) Micrograph of the filter region in the side channel (c) Micrograph of the filter region in the center channel.

CHAPTER 3

MUTIPLE USE PRESSURE-DRIVEN SYSTEM

3.1 Design and Fabrication Process

Syringe pumps are the most commonly used delivery system to provide flow to microfluidic systems [13] [28] due to their ease of set up and control. However, vibrations of the syringe pump generate periodic variations of the flow rate [29]. Moreover, it is hard to determine the actual flow rate during the transient period due the response time of the syringe pump (minutes to hours) [30]. We built a stable and a fast response time air-pressure-driven system to overcome the limitations of the syringe pump system.

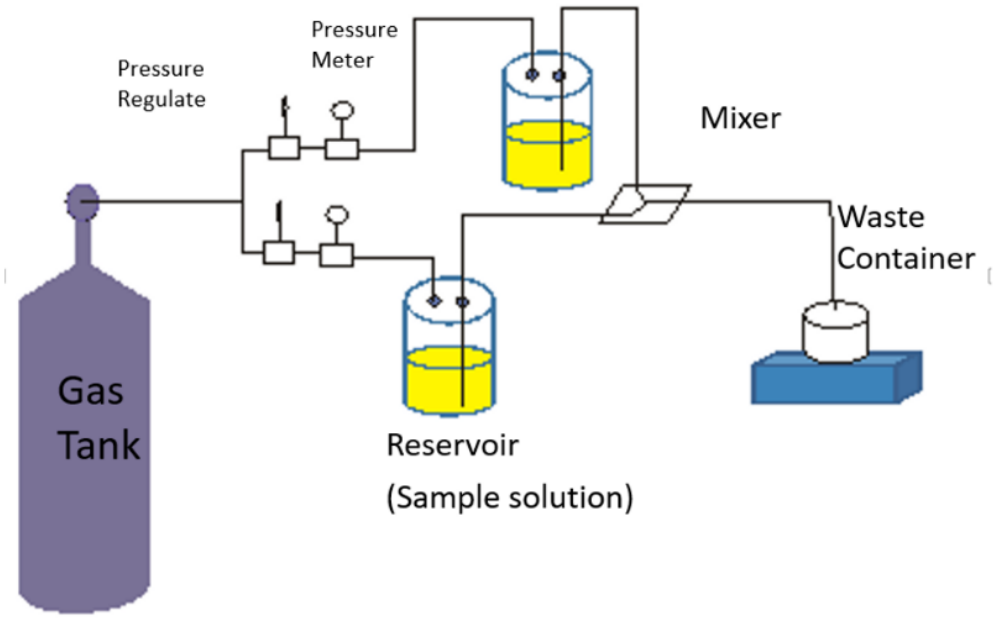


Figure 8. Schematic drawing of the pressure-driven system

As shown in Fig. 8, the air-pressure-driven system contains a gas tank which was used to provide pressure (the gas tank was replaced by the wall pressure for safety reasons), the pressure was controlled by a 100 psi pressure regulator and recorded by pressure meter. A 3/8'' tubing was used to connect the pressure valve to the reservoir which was used to load the sample solution. When pressure is applied to the reservoir, the sample solution is pushed into the tube and injected into the mixer channel. The number of reservoirs needed depends on the amount of inputs of the mixer. As shown in Fig. 2, the mixer has two inputs, so two reservoirs were used.

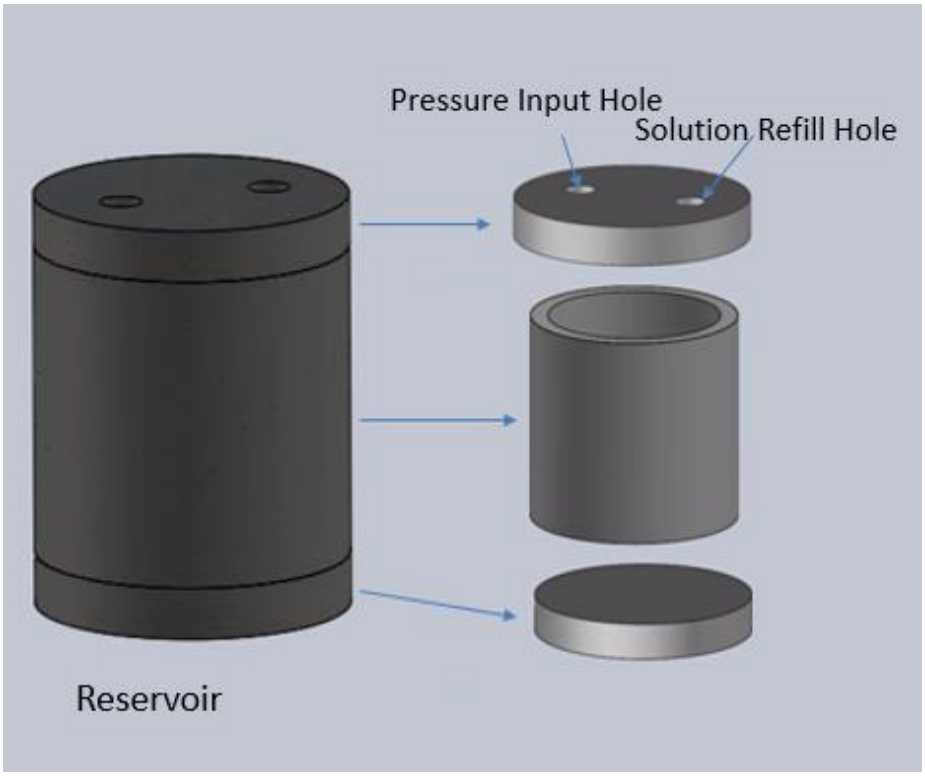


Figure 9. Schematic of the reservoir fabrication process

The reservoir fabrication process is shown in Fig. 9. The reservoir was made of acrylic. The cylindrical tank was machined by a one foot long acrylic tube, the top and

bottom covers were laser cut from acrylic sheets. 10 minutes epoxy was used to seal the cylindrical tank with the top and bottom cover. Two holes were drilled and taped on the top cover, used to apply pressure, load the sample solution and output the sample solution to the mixer. Two pipe fittings were screwed into the reservoir by hand. The detailed fabrication process is shown in Appendix B

3.2 Theory of The Multiple Use Pressure-driven System

According to Bernoulli's principle, for incompressible flow at any arbitrary point along a streamline the total work done by weight and pressure plus the kinetic energy are constant as shown in equation 1:

$$\frac{1}{2}\rho v^2 + \rho g z + p = C \quad \textbf{(1)}$$

However, when applying Bernoulli equation to a piping system between two points, the head loss due to pipe friction, entries, exits, fittings should be taken into consideration. The Bernoulli Equation becomes:

$$\frac{P_1}{\rho g} + \frac{1}{2g} {v_1}^2 + z_1 = \frac{P_2}{\rho g} + \frac{1}{2g} {v_2}^2 + z_2 + h_{loss} \quad \textbf{(2)}$$

h_{Loss} is the total losses in the pipe and duct system. There are two types of losses derived in the piping system: major losses due to friction and minor losses due to change of velocity in bends, valves, and fittings etc. The major loss in pipes can be expressed as:

$$h_f = f\frac{L}{D}\frac{V^2}{2g} \quad (3)$$

Where f is the friction coefficient derived by Weisbach [31], based on the roughness of the pipe and relate to the Reynolds Number of the flow. This is only valid for fully developed, steady, and incompressible flow [31]. L is the length of pipe, D is the diameter of the pipe, and D is the flow velocity.

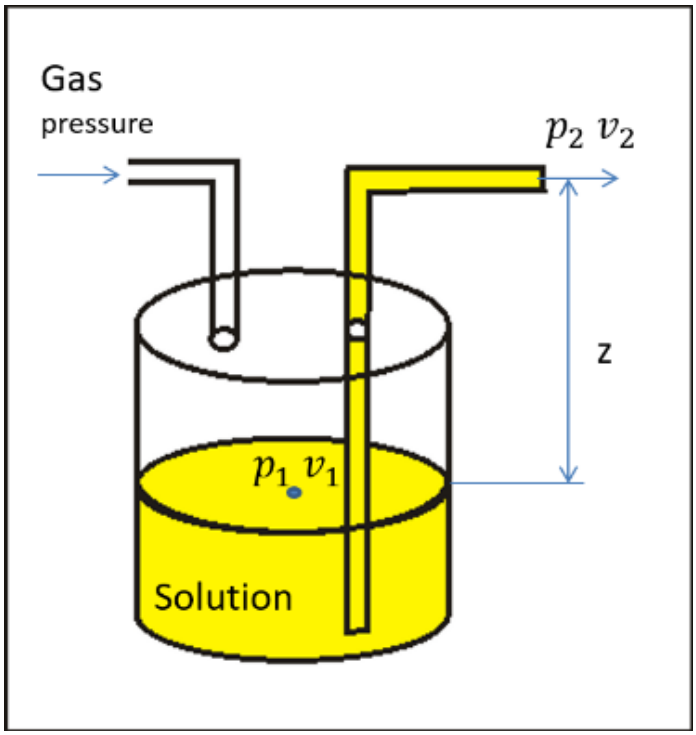


Figure 10. Schematic of two points connected by a streamline in a piping system.

Reynold's number is used to estimate the flow is laminar or turbulent, and it can be expressed as

$$\mathrm{Re} = \frac{VD}{\nu} \qquad \textbf{(4)}$$

ν is the viscosity of the flow. When Re≤ 2000, the flow is laminar, Turbulent flow occurs when Re≥ 10000 [31]. In between this region, we have transition flow. In our experiment, the highest Reynold's number is 58.2 ± 2. This means the flow is laminar.

The friction coefficient is related to Reynolds Number. For a laminar flow, the surface roughness will not affect the friction coefficient [32] and can be expressed as

$$f = \frac{K}{Re} \qquad \textbf{(5)}$$

Where K is the geometry factor depends on the channel geometry. For a circular tube the geometry factor is 64 [33].

The flow is not fully developed when it enters tube or channel, the distance for the flow to fully developed (the transient distance) [34] is expressed as:

$$I_d \approx (0.5 + 0.65Re)d \qquad \textbf{(6)}$$

The minor losses in pipes are expressed as:

$$\Delta h_m = \sum K \frac{V^2}{2g} \qquad \textbf{(7)}$$

K is the sum of the loss coefficients in the pipe. For different types of pipe entrance, exit, and transition, it have an associated loss coefficient [33].

3.3 Experiment Setup Calibration

A 25cm long tube was used to calibrate the pressure-driven system as shown in Fig. 11.

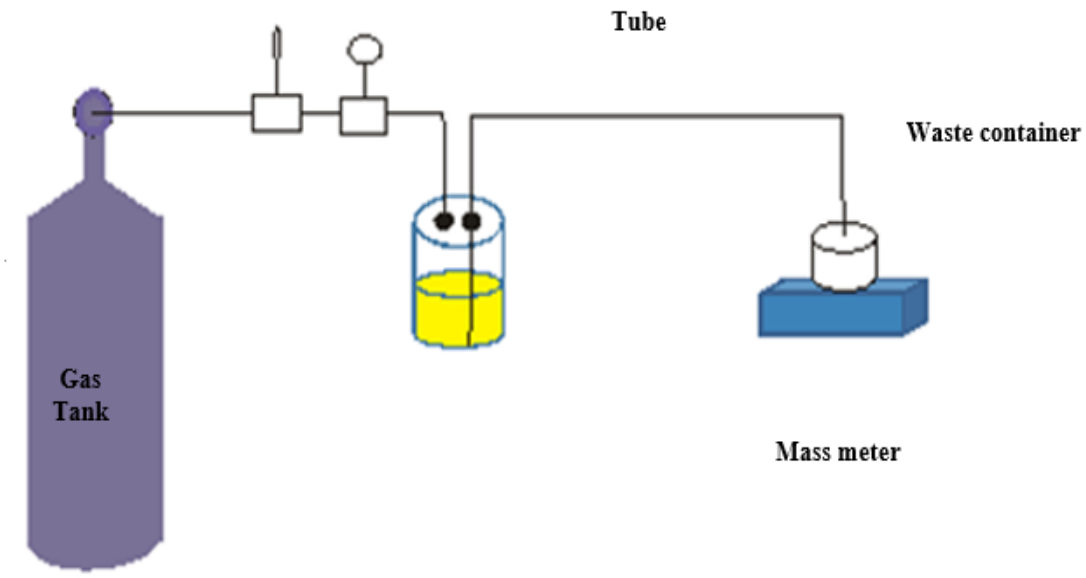


Figure 11. Schematic of the experiment setup used to calibrate pressure-driven-system.

A mass meter was used to record the output mass accumulation throughout the experiment, with the pressure change recorded by a pressure meter. The output flow rate of the tubing was derived from the measured mass flow rate. The calibration measurements were taken for 0.1, 0.2, 0.3, 0.4, and 0.5 kg/cm^2(1 kg/cm^2 = 14.223 psi). For each pressure, the mass rate was computed by measuring the liquid mass accumulated from a container during 60 seconds. The relationship between the flow

rate of the tube and the input pressure is shown in Fig. 12. The theory curve was derived via Eq. 2. As shown in Fig 12, the experimental results and theory is within the error bar, indicating there is no leaking issues and the design is ready to be applied to the mixer. The experiment data is shown in Appendix C.

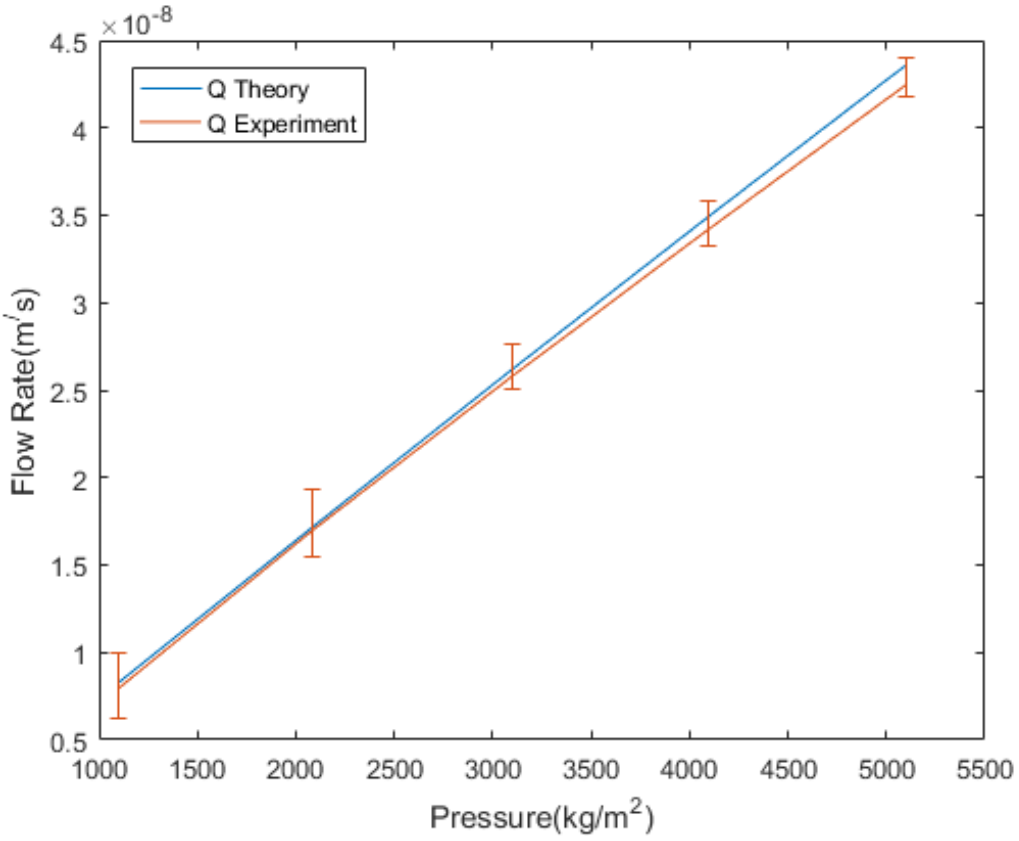


Figure 12. Relationship between the flow rate of the tube and the input pressure

3.4 Design Characterization

Three sets of different single channel sizes were designed (200μm, 100μm and 50μm) as shown in Fig. 13.

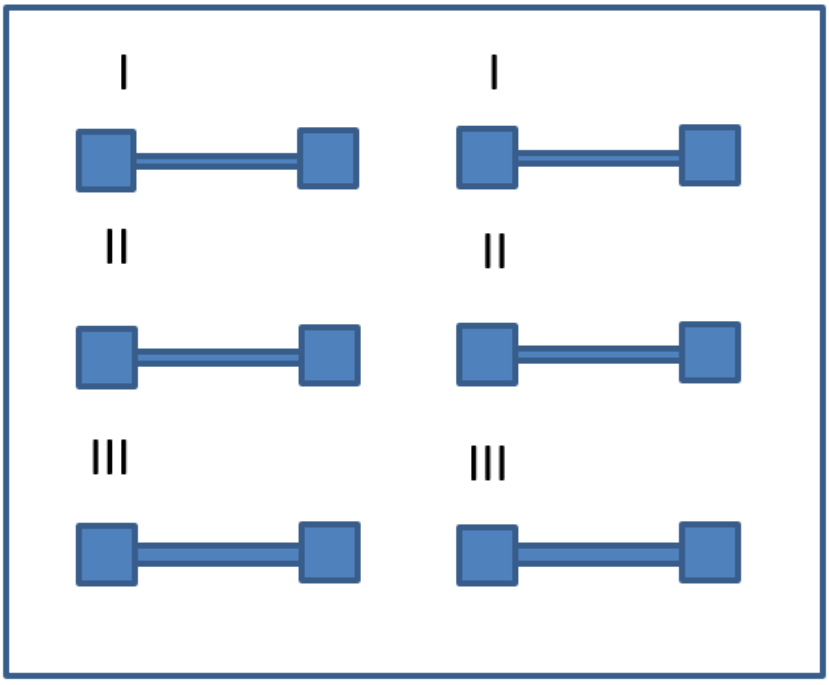


Figure 13. The design of three sets of different size single channel. The channel size are I: 50μm, II: 100μm and I: 200μm.

Device repeatability was tested by applying the pressure-driven system to same sized but independently fabricated microfluidic mixers. The impact of material on device performance was tested by applying the pressure-driven system to devices with the same channel sizes but made of PDMS and SUEX. All the measurements were

taken for 0.1, 0.2, 0.3, 0.4, and 0.5 kg/cm^2(1 kg/cm^2 = 14.223 psi). For each

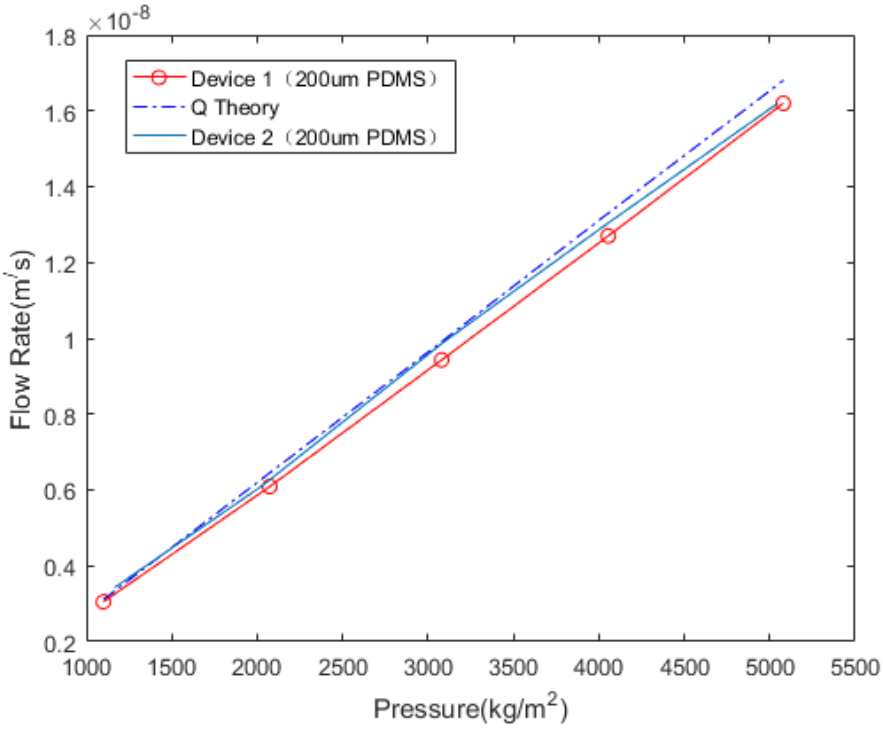
designated pressure, the mass rate was computed by measuring the liquid mass accumulated from a container during 60 seconds. The experimental data are shown in Appendix C.

3.5 Analysis

The results of device repeatability and the impact of material on device performance are shown in Fig. 14. The experimental data is shown in Appendix C.

From Fig. 14 a and b, there are significant differences between devices, especially between the different materials. Compared with SUEX, the device made with PDMS is closer to theory. According to Eq. 3, 4 and 5 the wall roughness can be ignored if the flow is laminar. However, Hetsroni, Pogrebnyak and Yarin [35] studied the fluid flow in micro channels, and found that even for laminar flow the friction coefficient was influenced by the channel roughness. Besides, the channel roughness will also decrease the value of Reynolds number. Therefore, we conclude that the major cause for the variation between the devices made with different materials (PDMS and SUEX) is the channel roughness. As shown in Fig. 15 the channel made with SUEX is rougher than channel made with PDMS. Therefore, the friction losses for SUEX is much higher than PDMS which is consistent with Fig. 14 b. Besides, it was obviously to notice that the influence of friction coefficient will increase as the channel size decrease.

a).


×10^-8
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
Flow Rate(m^3/s)
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
Pressure(kg/m^2)
Device 1（200um PDMS）
Q Theory
Device 2（200um PDMS）

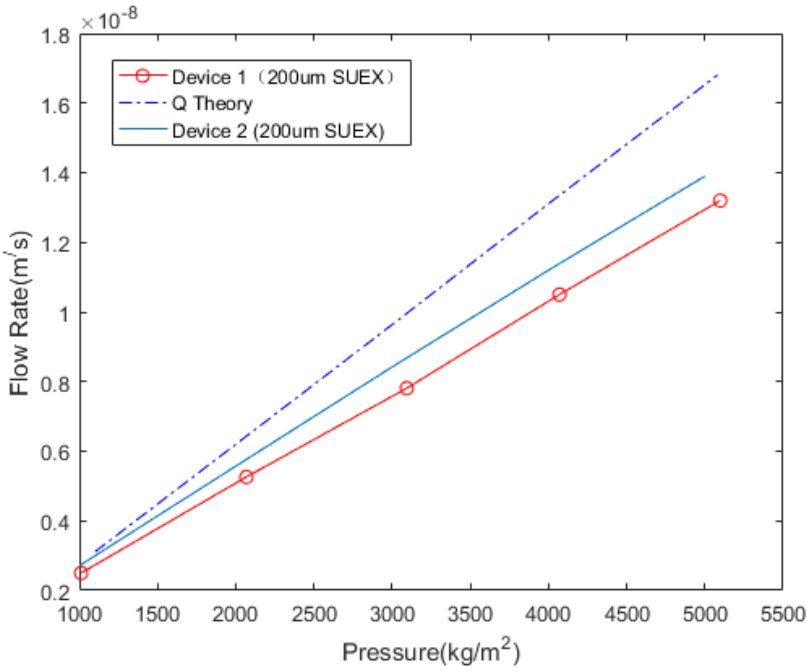
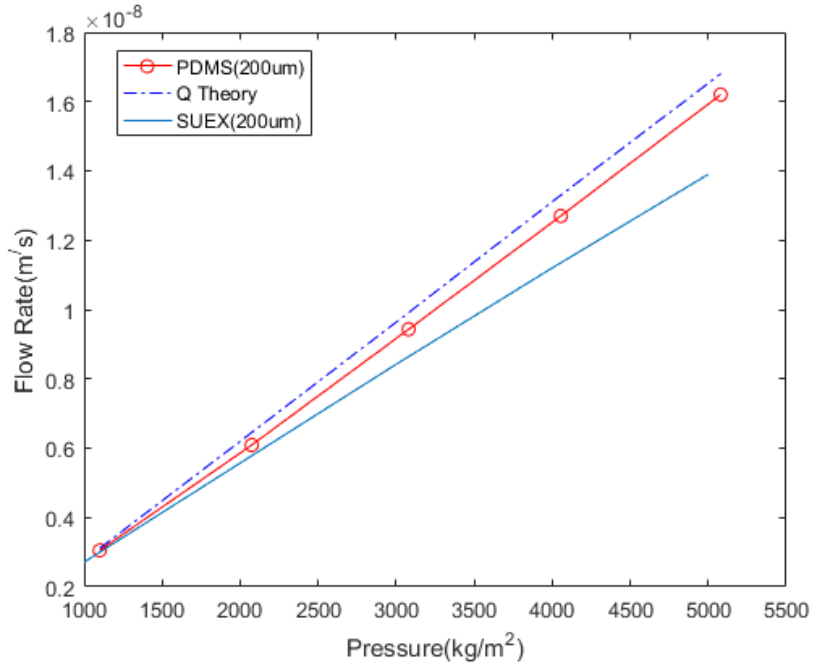

×10^-8
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
Flow Rate(m^3/s)
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
Pressure(kg/m^2)
Device 1（200um SUEX）
Q Theory
Device 2 (200um SUEX)

Figure 14 (continued)

b).



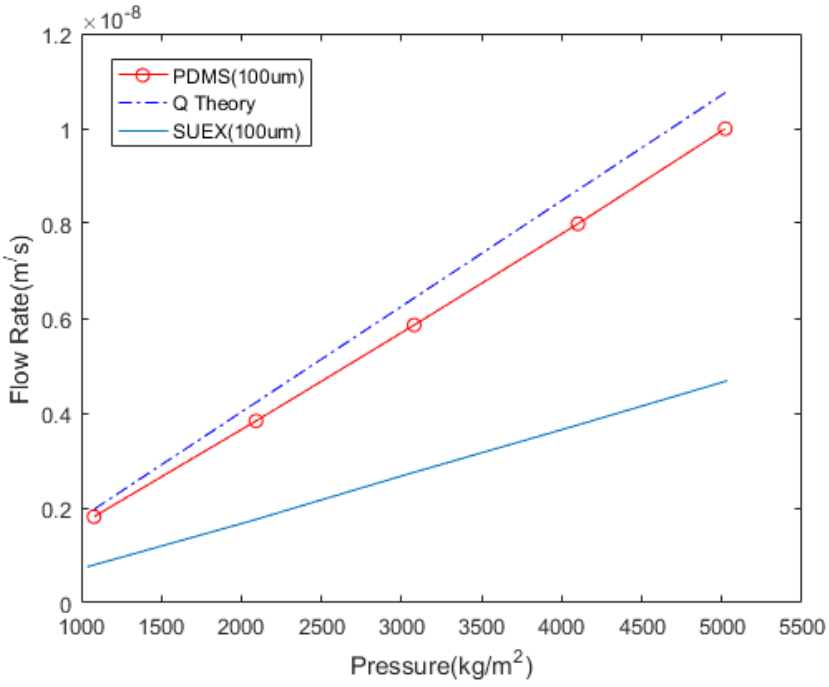


Figure 14 (continued)

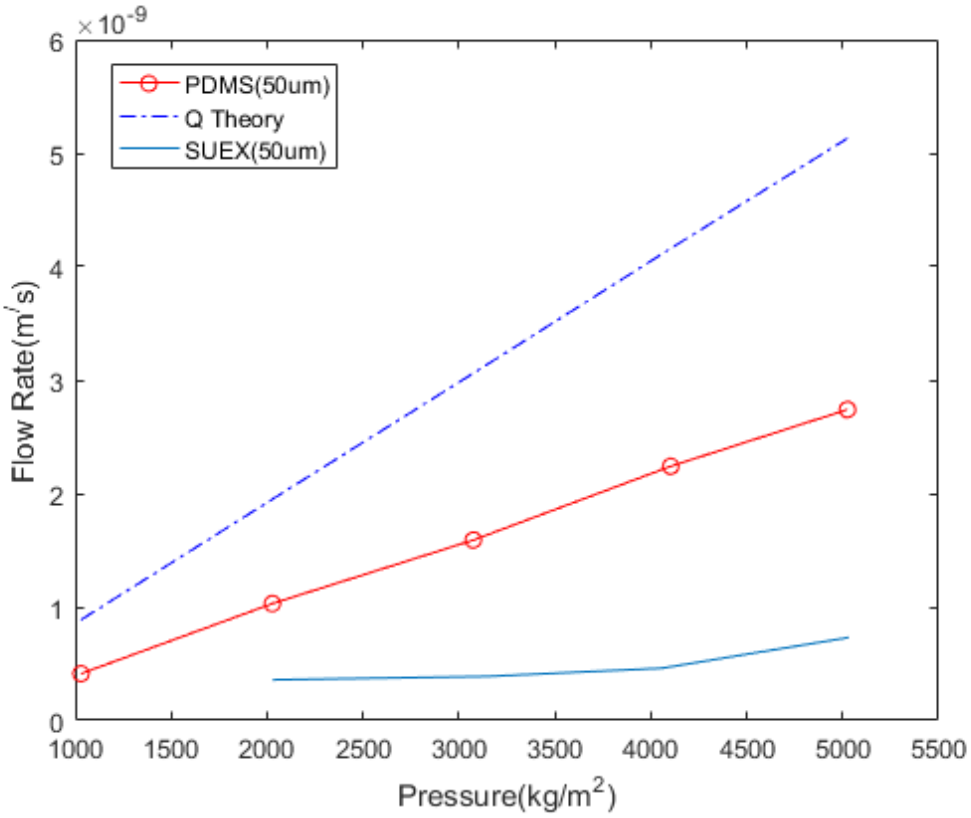


Figure 14. The relationship between the flow rate of single channel microfluidic mixer and the input pressure. (a) 200μm single channel microfluidic mixers made with PDMS and SUEX. (b) 200μm, 100μm, and 50μm single channel microfluidic mixers made with PDMS and SUEX.

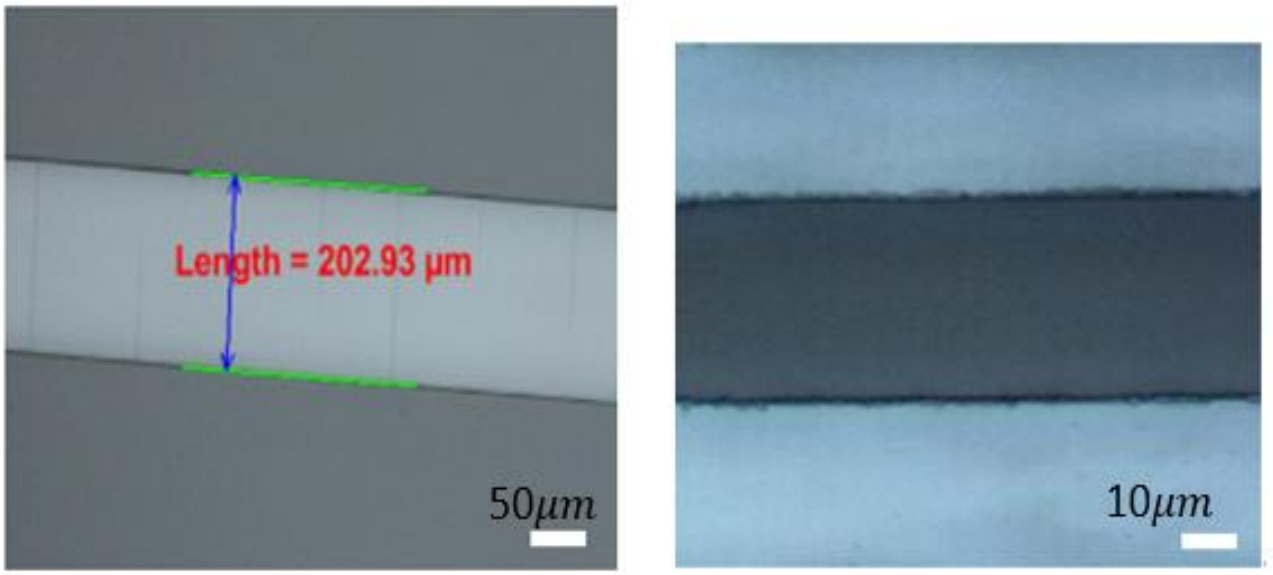


Figure 15. Micrographs of single channel made with different materials. (a). Channel made with PDMS. (b). Channel made with SUEX. The scale bar is 50 µm.

Due to the impact of material and poor device repeatability, it is hard to use theory to estimate the flow rate inside the channel. The best way to determine the channel flow rate is by utilizing a flow meter. Even though, from Fig. 12, the linear relationship between pressure and flow rate mean the pressure-driven system is stable. Compare with syringe pump, pressure-driven system is easy to refill sample solution and can handle fluids volume to liters which promised a long time running experiment.

CHAPTER 4

TESTING

4.1 Experiment Setup

In this part, the microfluidic mixer made with SUEX was connected to the designed pressure driven system and tested under our home made microscope system as shown in Fig. 16.

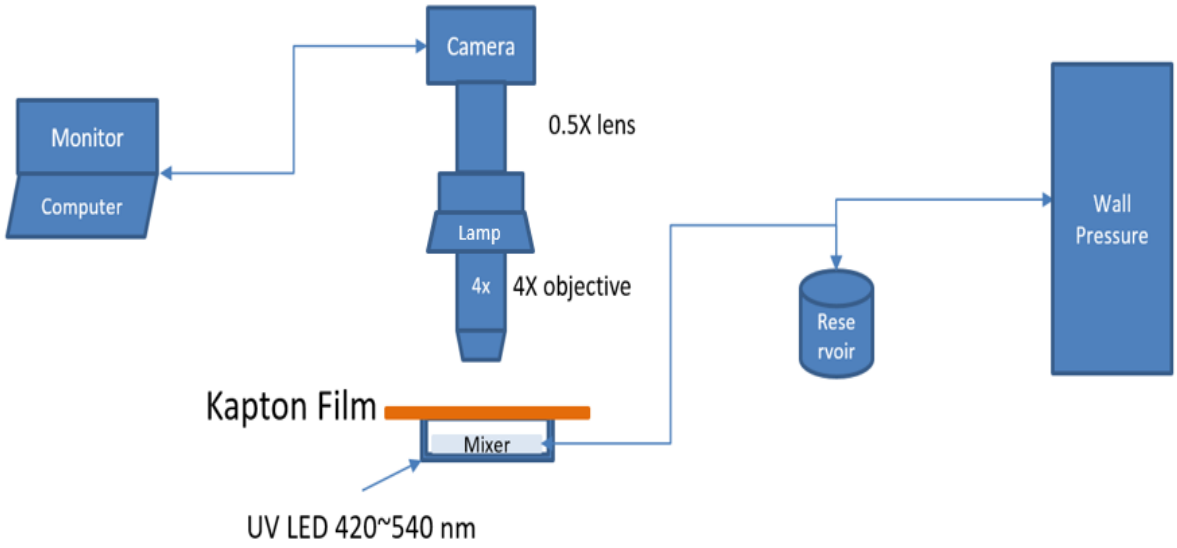


Figure 16. Schematic of Scienscope Microscope system and the experiment setup

The homemade microscope consisted of a Scienscope MZ7-CP-05, with a Scienscope stage. Adequate lighting was achieved by setting the fiber optic annular ring light to maximum brightness. Besides, a 420~540 nm UV-LED light was applied under the mixer to excite the fluorescein flowing in the mixer. A 50 um Kapton film

was applied above the UV-LED light and the mixer to filter wavelengths below 470nm. To improve the spatial resolution, we used a 4X objective with 0.1 NA and 22mm working distance. A 0.5X coupler lens was used to concentrate light onto the camera sensor.

Since the UV-LED light source was not focused, and the Kapton film can't filter all the background noise, it is hard to collect sufficient fluorescence signal. In order to collect the data, high concentration fluorescein (500 μM) was made from powder and filtered through a 0.2 μm filter. A 200 μm single channel was used to calibrate the set up. DI water was hand filtered through 0.2 filter. Filtered DI water was used as buffer and was introduced first to collect buffer to buffer mixing image (background). Then 500 μm filtered fluorescein was infused into the center channel and filtered DI water was used in the other two channels to collect the mixing image. A background subtracted image was obtained by subtracting background image from the mixing image.

4.2 Results and Discussion

Fig. 17 and Fig. 18 illustrate the calibration and quenching result obtained from the home made microscope. It is clear that the mixing image (Fig. 18) is inferior to the single channel image (Fig. 17). This is because our homemade microscope was not sufficiently optimized to observe quenching. Due to time and resources limitations, the

image was taken for analysis. The profile of the fluorescence intensity along the streamline is shown in Fig.19. Since the fluorescence intensity in the input center channel is very low, it is hard to tell if the fluorescence intensity is decreasing after mixing. Even though the signal is low, we are still able to see fluorescence in the observation channel is less than the input center channel (Fig. 19). This shows that the mixing process was taking place in the mixing region.

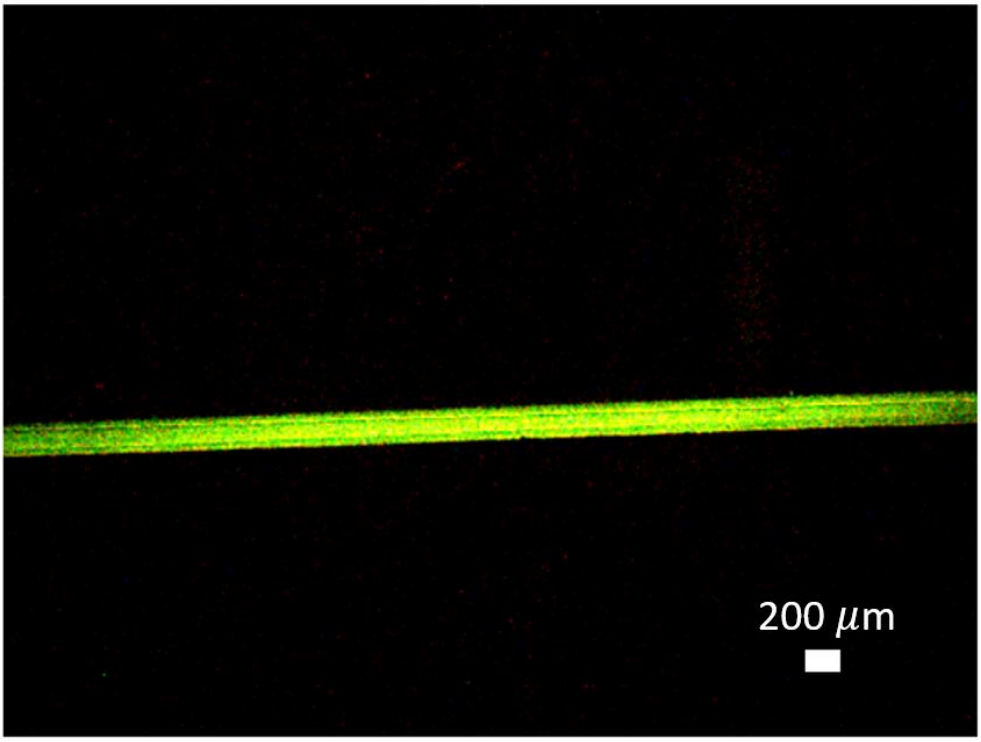


Figure 17. Background subtracted Single Channel image

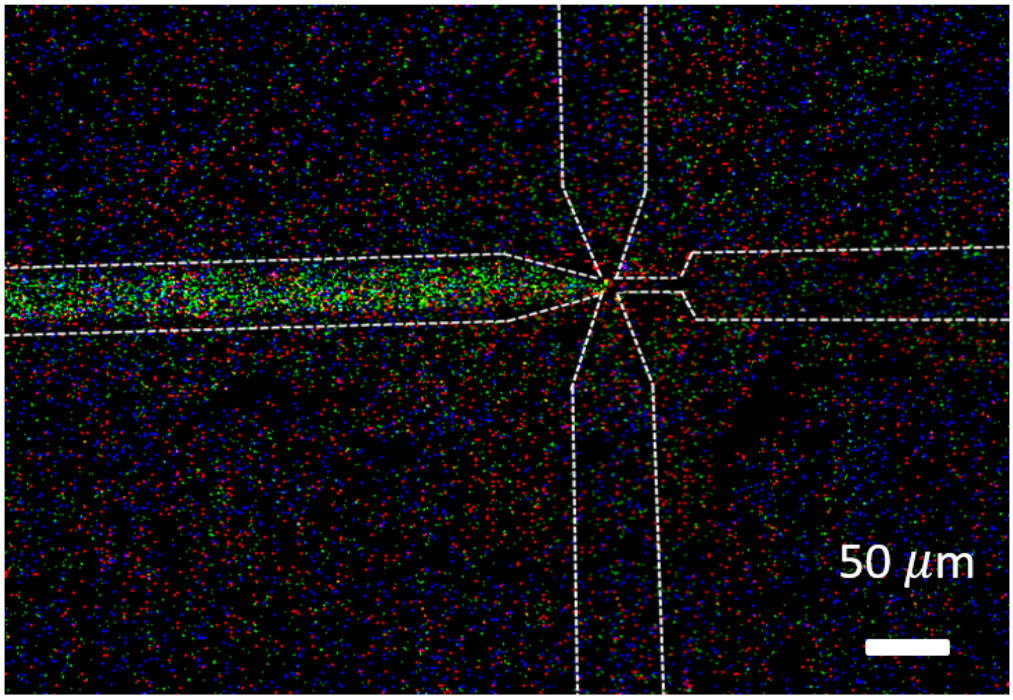


Figure 18. Image of the background subtracted mixing process.

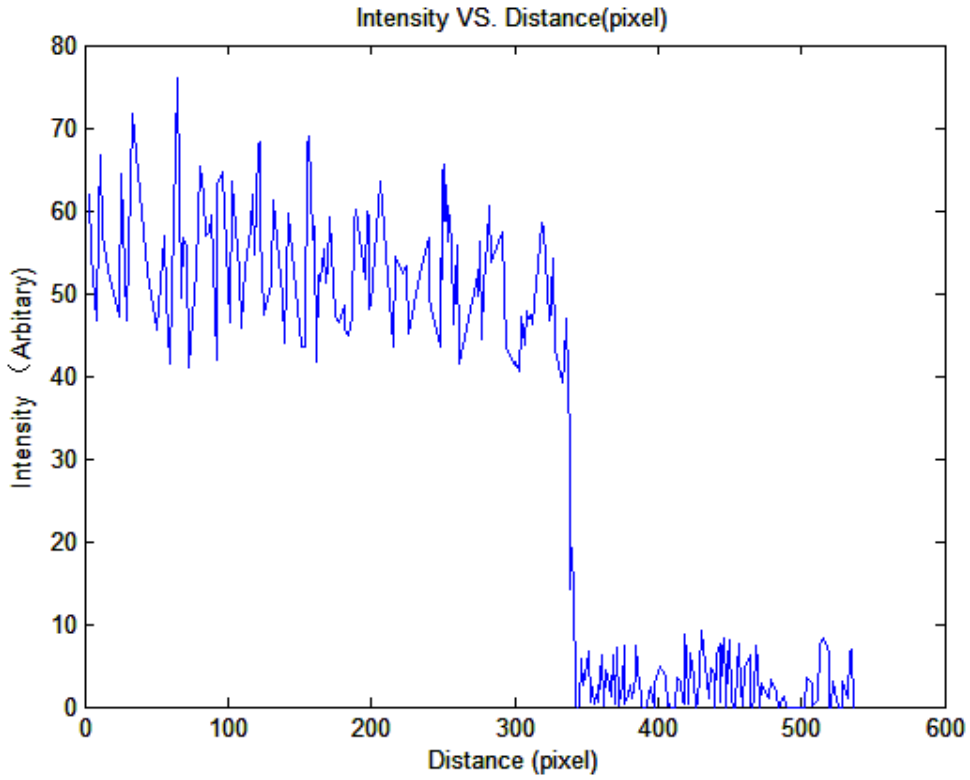


Figure 19. Fluorescence intensity along the streamline

4.3 Devices Performance and Limitations

The microfluidic mixer performance was evaluated from three aspects: SUEX as the cover sealing, bonding stress between SUEX and PMMA, and clogging inside the channel. The SUEX sealing was evaluated by applying pressure via the pressure control system, the highest pressure we applied to the mixer is 100 psi without leaking. In comparison, a well bonded PDMS-glass device can stand operation pressures up to 70 psi [36]. Therefore, SUEX sealing is a successful method compared with PDMS-glass.

The bonding stress between the SUEX and PMMA is not as good as Wunderlich et al.,'s PDMS-glass bonding stress [18], since the pillar inside the filter array were washed away during developing (detail design shown in Chapter 3, section 2.4).

The microfluidic mixer suffers from clogging, even with a 2 um outer filter applied (Chapter 3). As shown in Fig. 20, the image was taken by our home made microscope, the dust is clogged in the mixing region. This is caused by the narrow mixing channel, with 8μm too narrow for small particles to pass through. We failed to fabricate the filter inside the center channel and the side channel, because of the high aspect ratio (10:1 high to width). However, we thought it wouldn't help on solving the clogging issue, since if the dust can pass through the outside 2 um filter, it will also pass the 10 um filter inside the channel (detail design shown in Chapter 3, section 2.4) and clog the narrow mixing region. To solve the clogging issue in the future, the best approach would be to increase the width of the narrow channel. However, this will affect the mixing time.

The SUEX channel were rougher than PDMS, and the overall performance of SUEX were inferior to PDMS (Fig. 14). However, the productivity of SUEX (80%) is better than PDMS (~40%) during our fabrication process. Therefore, with better fabrication protocol, the SUEX will be a better choice to fabricate mixer compare with PDMS.

a) b).

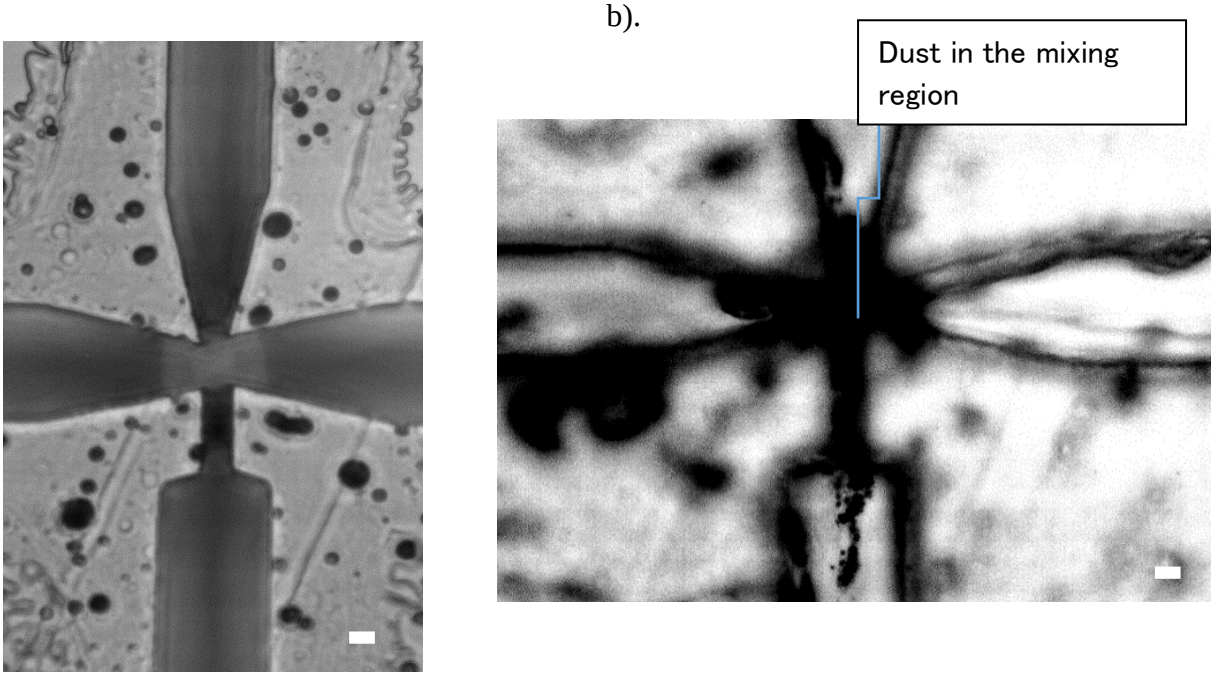


Figure 20. Micrographs of the mixing region of the microfluidic mixer. a). The mixing region before flow fluids. b).The mixing region clogged with dust (black cross area) after flowing filtered DI water. The scale bar is 10 μm.

The performance of pressure driven system, the highest pressure we applied is around 100 psi without leaking. The average flow velocity achieved is around 50 μm/ms (5 psi) by changing the pressures compare with 2 μm/ms (3 psi) achieved by Wunderlich et al. [18]. The pressure was limited by the wall pressure and the pressure regulator (0~5 psi). The reservoir was glued together, the pressure cannot go very high.

CHAPTER 5

Conclusion and Future Recommendations

In this work we presented a new epoxy material, SUEX, applied to fabricating a microfluidic mixer. The mixer performance was carried out using our homemade microscope and pressure-driven system. The easy fabrication process and successful sealing method (100 psi pressure without leaking) ensures future application.
We also described the advantages of using a pressure-driven system compared to a syringe pump delivery system. The pressure-driven system we designed was characterized by single channel mixers with different channel widths and fabricated in different materials. It demonstrated that the pressure-driven system is flexible, stable and well-suited to variety types of microfluidic mixer.

In summary, for future application the following recommendations should be considered:

(1) A new substrate should be used to get a better bonding stress between SUEX

(2) To develop a better fabrication protocol of SUEX in order to get a smooth channel wall

(3) The narrowed channel (8um in Fig. 5) should be broadened to solve the clogging issue.

(4) A flow meter should be applied to the Pressure-driven system to get an accurate flow rate. With a flow meter, the response time of Pressure-driven system could be measured and used to compare with syringe pump system.

(5) For high pressures, the reservoir (pressure-driven system) should be fabricated in a high pressure standard, such as in metal or bonded with a high pressure glue.

(6) A custom built microscope with high resolution should be used. This will allow better imaging.

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APPENDIX

Protocol for Fabrication of the microfluidic mixer made of SUEX

I. Fabrication of the Mask (Timing 3 hours not include the design time)

- **Mask Design**

a. Use the L-edit software in computer to design the pattern and then save as GDSII file and save in Cornell Nanoscale Science & Technology (CNF) Heidelberg Mask Writer DWL2000 file (This step can be done outside of CNF Clean Room). ! CAUTION For this step, it is very important to notice the mask photoresist is negative or positive, and the pattern you want show up on the mask after develop. For positive photoresist, the exposure area will stay after develop, and for negative photoresist, the unexposed area will stay.

- **Mask Exposure (2hour and 20 minutes)**

a. Bring a new mask which is kept in an orange box, open the file designed before and send it to Heidelberg Mask Writer DWL2000 computer using the computer in CNF Mask Writer room. ! Caution In order to protect the Mask's photoresist, keep the box closed until you have reached an area with only yellow light in CNF.

b. Follow the operation direction to exposure the mask using Heidelberg Mask Writer DWL2000

- **Mask Develop (10 minutes)**

  a. Using mask developer to develop the exposed mask. ! CAUTION The mask should be developed immediately after exposure to ensure the exposure features.

  b. After develop, examine the developed mask features under the microscope. If the features are not correctly exposed, rinse all the photoresist on the mask and exposure again.

  c. If all the features are correct, using the mask developer to chrome etch the developed mask.

- **Strip off Resist (30 minutes)**

  a. Put the developed and etched mask in boat and put it into the first hot bath for ten minutes, then second hot bath for ten minutes, and the third one for five minutes in photolithography room

  b. Rinse the mask in the mask washer and spin dry

  c. Store the mask in the orange case.

II. Fabrication of the Microfluidic Mixer

- **Prepare the PMMA substrate** (30 minutes)

  a. Design the cut pattern using corel draw in lab computer. ! Caution For this step, be aware of the laser cutting mode will be used. If the pattern

is cut via vector mode, the design line width should be in hairline, otherwise choose the right line width for raster mode.

b. Save the design file in Flash Drive and bring it to CNF Versalaser room.

c. Keep the PMMA sheet protect cover while cutting, this is intent to keep the PMMA clean.

d. Open the design file in Versalaser computer and follow the Versalaser operation guide to cut the PMMA sheet to Microfluidic mixer substrate.

e. .Store the cut PMMA microfluidic mixer substrate in a clean box.

- **Laminate** (10 minutes)

a. Bring the PMMA substrate to the CNF SU-8 room. Turn the laminator ON with rollers rolling at least 10 min beforehand. Wipe the rollers with an acetone - wetted ALPHA wipe; not a beta wipe.

b. Remove the PMMA substrate protect cover and Place it on the aluminum square and cover with a PET separator sheet.

c. Remove the shiny PET from the 100um SUEX. Place SUEX side - down on separator sheet.

d. Align SUEX chip and press gently at 12:00 on the wafer for 15 sec to adhere.

e. Laminate SUEX 100 at 65˚C at 1 ft/min roll rate.

- **Post-laminate bake.** (10 minutes)

a. To relax the SUEX & remove trapped air and defects.

b. Keep the hazy PET coversheet on.

c. Place the PMMA substrate on a piece of aluminum foil in the PDMS casting OVEN

d. Bake at 65˚C for 10 min.

e. Store in box for later use.

- **Expose.** (5 minutes) !Caution Remove the hazy PET form the SUEX before exposing.

a. Use 365LP filter. The dose on the ABM aligner is about 8 mJ/cm2 .

b. Expose in 30 - sec intervals with 15 to 30 - sec rests. The expose dose is about 1200-1400 mJ/cm^2

- **Post-expose bke.** (1 hour and 15 minutes) Immediately following exposure.

a. Bake the exposed device on Hot plate at 65˚C for 5 min.

b. Move to 2^{nd} hot plate bake at 85˚C for 10 min

c. Ramp down to room temperature.

- **Develop.** (35 minutes)

a. Face down in PGMEA (EBR - 10A). Slow stirring may detach features.

b. Immerse for 20 - 30 min. Fresh bath (OPTIONAL) 5 - 10 min.

c. Rinse in isopropanol for 5 min and dry thoroughly.

- **Hard-bake**. (10 minutes)

  a. To remove residual developer and improve adhesion. Put the developed device on hot plate and set it to 85 ˚C for 10 minutes then ramp to room temperature.

  b. Store it in a clean box for later use.

- **Measure the height of the device using the P10 Profile**

  a. Put the device on P10 profile plate, center the wafer and vacuum.

  b. Press man/load.

  c. Click focus and find the right place to measure the height of the device.

  d. Press start and adjust to decide the height of the device.

  e. Select man/load to unload

  f. Turn off the Vacuum.

III. Sealing

- **Laminate** (10 minutes)

  a. Bring the device fabricated at II part. Turn the laminator ON with rollers rolling at least 10 min beforehand. Wipe the rollers with an acetone - wetted ALPHA wipe; not a beta wipe.

  b. Remove the shiny PET from the 200um SUEX. Place SUEX side - down on separator sheet.

c. Align SUEX chip and press gently at 12:00 on the wafer for 15 sec to adhere.

d. Laminate 100um SUEX at 47˚C at 1 ft/min roll rate.

- **Expose.** ! CAUTION Remove the hazy PET from the SUEX before exposing.

  a. Use 365LP filter. The dose on the ABM aligner is about 8 mJ/cm^2 .

  b. Expose in 30 - sec intervals with 15 to 30 - sec rests. The expose dose is 1600-1800 mJ/cm^2

- **Post-expose bake**. Immediately following exposure. 2 ramping steps on hot plates.

  a. Bake the exposed device on Hot plate at 65˚C for 5 min.

  b. Move to 2nd hot plate bake at 85˚C for 10 min

  c. Ramp down to room temperature.

  d. Place in a clean case ready for use.

Appendix B

Protocol for Fabrication of the microfluidic mixer made of SUEX

IV. Laser cut the top and bottom reservoir cover

- Prepare a 0.337'' thick Acrylic sheet (about 5''*5'' square large size)
- Design the cut pattern using corel draw in lab computer. ! Caution For this step, be aware of the laser cutting mode will be used. If the pattern is cut via vector mode, the design line width should be in hairline, otherwise choose the right line width for raster mode.
- Save the design file in Flash Drive and bring it to CNF Versalaser room.
- Open the design file in Versalaser computer and follow the Versalaser operation guide to cut the Acrylic sheet to top and bottom reservoir cover.
- Store the cut species in a clean box for later use.
- Tap the three holes on top cover.

V. Machine Cut the Cylinder Reservoir

- Prepare a 1 feet long 1'' OD and 7/8'' ID rod.
- Machine cut it to 2.5 cm long hollow rod to make the cylinder tank.

Glue (pressure stand glue) the bottom and top cover with the cylinder tank.

Appendix C

25cm long tube

P (kg/m^2)	Qtube (m^3/s)	Qtheory(m^3/s)	Up Error bar(m^3/s)	Bottom (m^3/s)
1098	7.93352E-09	8.26277E-09	2.05655E-09	1.75213E-09
2086	1.69927E-08	1.71798E-08	2.27659E-09	1.57727E-09
3098	2.5785E-08	2.61769E-08	1.8947E-09	7.09797E-10
4092	3.41585E-08	3.48854E-08	1.62741E-09	9.13461E-10
5104	4.24938E-08	4.36263E-08	1.45374E-09	6.73942E-10

200 um single channel microfluidic mixer made with PDMS

P (kg/m^2)	Device 1(m^3/s)	Q(Theory (m^3/s))	Device 2(m^3/s)	P for device 2 (kg/m^2)
1100	3.04514E-09	3.10584E-09	3.42576E-09	1167
2074	6.09269E-09	6.45123E-09	6.11575E-09	2032
3080	9.42619E-09	9.90629E-09	9.91347E-09	3095
4055	1.2719E-08	1.32546E-08	1.29554E-08	4042
5080	1.6157E-08	1.67745E-08	1.62212E-08	5048

200 um single channel microfluidic mixer made with SUEX

P (kg/m^2)	Device 1 (m^3/s)	P(2) (kg/m^2)	Device2 (m^3/s)
1011	2.49883E-09	1000	2.708E-09
2068	5.26105E-09	2000	5.567E-09
3094	7.81407E-09	3000	8.42E-09
4070	1.04604E-08	4000	1.12E-08
5098	1.32045E-08	5000	1.392E-08

200 um single channel microfluidic mixer made with PDMS AND SUEX

P(kg/m^2)	Device 1 (m^3/s)	Q(Theory) (m^3/s)	P(kg/m^2)	Device 1 (m^3/s)
1100	3.04514E-09	3.10584E-09	1011	2.49883E-09
2074	6.09269E-09	6.45123E-09	2068	5.26105E-09
3080	9.42619E-09	9.90629E-09	3094	7.81407E-09
4055	1.2719E-08	1.32546E-08	4070	1.04604E-08
5080	1.6157E-08	1.67745E-08	5098	1.32045E-08

100 um single channel Microfluidic mixer made with SUEX and PDMS

P (kg/m^2)	Q (PDMS) (m^3/s)	q(THEORY) (m^3/s)	P(kg/m^2)	Q(SUEX) (m^3/s)
1080	1.81522E-09	1.97068E-09	1038	7.5806E-10
2091	3.83263E-09	4.22394E-09	2057	1.72284E-09
3079	5.85556E-09	6.42588E-09	3020	2.69356E-09
4101	7.99046E-09	8.70352E-09	4070	3.71774E-09
5021	9.99406E-09	1.07538E-08	5032	4.67876E-09

50um single channel Microfluidic mixer made with SUEX and PDMS

P (kg/m^2)	Q (50 um PDMS) (m^3/s)	Q THEORY (m^3/s)	P(kg/m^2)	Q(50 um SUEX) (m^3/s)
1030	4.11766E-10	8.86983E-10	1038	
2028	1.03436E-09	1.94813E-09	2029	3.57859E-10
3075	1.58688E-09	3.06138E-09	3148	3.88374E-10
4104	2.23833E-09	4.15547E-09	4056	4.59635E-10
5025	2.74178E-09	5.13473E-09	5032	7.3139E-10