Contents

Page

1 Introduction

Lab-on-a-chip essentially refers to integrated microfluidics devices, which scale down fluid treatment to the micro-meter range. At such dimensions, the surface area to volume ratio of the fluid is greater than, say, at the centi-meter range, making surface forces dominant in the fluid mechanics. Flow patterns are therefore interesting to study by subjecting different channel designs to testing and photographic techniques. Applications of microfluidic devices are already present in the biochemical field, such as gene analysis and studying polymerase chain reactions.

Question

What is the definition of the Reynold's number? Estimate the Reynold's number for water in a channel of 100µm width (double the thickness of hair) and flow at 1mm/s.

Reynold's number is a dimensionless quantity defined as the ratio of the inertial force to the viscous force experienced by the fluid flowing in a particular channel. Its expression is

$$Re = \frac{ud}{v}$$

where u = fluid velocity; d = hydraulic diameter; v = kinematic viscosity.

The kinematic viscosity v of water at 20ºC is about **$1x10^{-6}$ m^2/s**. Substituting this value and also that of d = 100µm and u = 1mm/s, the Reynold's number for water at 20ºC is about Re = 0.1.

2 Objective

The aim of this experiment is to familiarise with the process of fabricating a microfluidic device. Additionally, a simple channel design will be used to explore how controllable variables, such as channel widths and fluid pressures, affect bubble creation and flow patterns.

3 Preliminary Questions

With what type of material does the liquid come into contact?

Upon fabrication, the microfluidic device is simply a piece of patterned PDMS (poly-dimethyl siloxane) bonded to a blank substrate. This means that the liquid comes into contact with the PDMS and glass microscope slide.

What does bonding mean and how is it achieved?

Bonding refers to chemical adhesion of the patterned PDMS onto the microscope slide. Both the PDMS and the glass slide have silanol groups, i.e. –Si-OH. When treated by plasma, water molecules are removed from the surfaces, leaving –Si-O or –Si. Shortly thereafter, when the surfaces are pressed together, new bonds are formed between them, namely –Si-O-Si- bonds. This provides the adhesion needed to prevent fluid leakage when the microfluidic device is in use.

What exactly is SU-8? Is it a positive or negative photoresist? Who invented it?

SU-8 is a high contrast, epoxy based negative photoresist invented by International Business Machines (IBM).

How expensive is 1 litre of SU-8? Why is yellow light important?

According to the research members, a 500ml bottle of SU-8 costs between US$500-600. That amounts to S$1,250-1,500 per litre, which is pretty expensive.

Yellow light is the result of filtering all UV radiation and blue light from the fluorescent tubes. UV and blue light tend to have shorter wavelengths compared to other colours in the visible spectrum. It is important to have yellow light because the photoresist undergoes chemical reactions when exposed to UV light, evident by the fact that UV light is used at the flood exposure stage. Thus, yellow light prevents premature exposure which can disrupt the photolithography process.

What fixes the glass plate on the axis of the spin coater?

The centre of the spin coater is connected to a vacuum pump. When the glass slide is placed there and the pump is turned on, the vacuum generated fixes the slide on the axis of the spin coater by suction.

When do you punch holes for the PDMS supply lines (before/after bonding)?

Once the PDMS is cured and peeled from the patterned substrate, the supply lines are created by punching holes. This is done before bonding the PDMS onto the blank substrate.

4 Device Fabrication Procedure

Overview

- Designing of Device
- Printing of Photomask
- Substrate Cleaning
- Spin Coating of Photoresist
- Soft Bake
- UV Exposure
- Post-exposure Bake
- Development
- PDMS Preparation
- PDMS Curing
- PDMS Bonding

4.1 Designing of Device

There were several ideas as to how bubble channels of different shapes and densities were to be created. Designing of the fluid channels on the microfluidic device took into account the relative positions of the gas and liquid inlets/outlets and also the widths of the channels at different locations. Some of the preliminary designs, created using the software "Inkscape", are showed below.

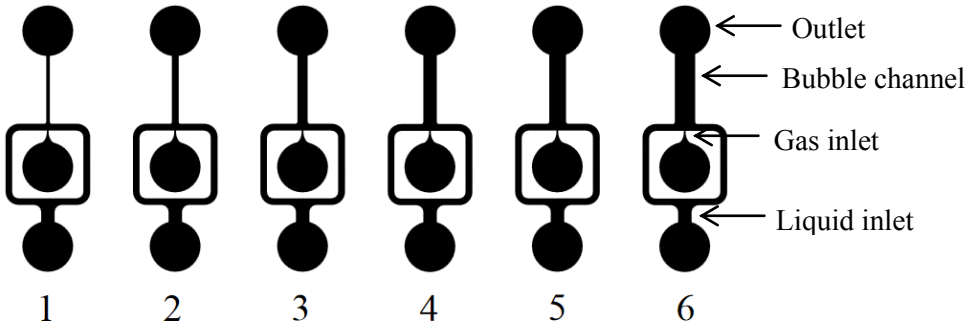


Figure 1: T-junction design with 6 different widths of the bubble channel

Figure 2: Y-junction design (blue – liquid; green – gas; black – bubbles)

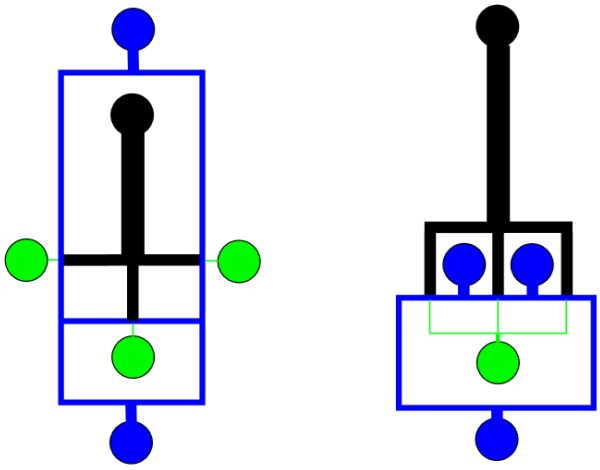


Figure 3: Designs with multiple inlets (blue – liquid; green – gas; black – bubbles)

Multiple inlets can potentially complicate the fluid flow, especially at the junctions, and introduce difficulties in controlling the variables. As such, it is decided that only 1 liquid and 1 gas inlet would be used. The T-junction design in Figure 1 was adopted, using 2 different widths for the gas input channel, namely 50µm and 75µm. For the 50µm gas channel, it is combined with a 100µm, 200µm or 300µm bubble channel. For the 75µm gas channel, it is combined with a 150µm, 300µm or 400µm bubble channel. These 6 designs are summarised in Figure 4.

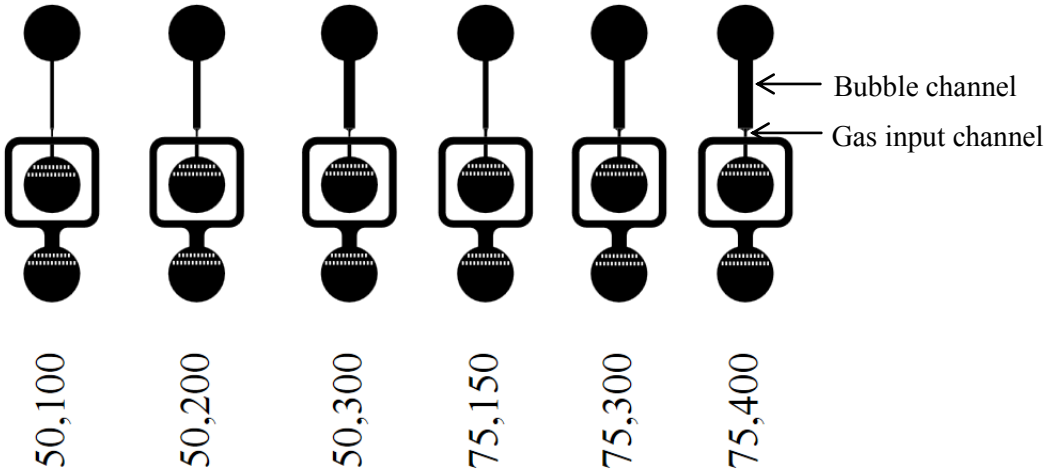


Figure 4: Final design with varying gas input width and bubble channel width

Using these different widths allows us to determine the conditions needed for the formation of bubbles with various patterns in the bubble channel.

4.2 Printing of Photomask

Once the designs were finalised, they are sent for high resolution printing. It is similar to conventional printing except that the high resolution patterns are printed on transparent plastic instead of paper.

4.3 Substrate Cleaning

The substrate used is a glass microscope slide. In a cleaning procedure, several slides are cleaned together. Each slide is firstly rinsed with acetone and ethanol, along with gentle wiping using ethanol-soaked wipes. The slides are then placed vertically in a container filled with ethanol. This container, in turn, is transferred to a sonicator so that the slide surfaces can be cleaned using ultrasound mechanism. After about 30 minutes of ultrasound treatment, the slides are individually rinsed with ethanol again, followed by distilled water. Lastly, each of them is blown dry using an air gun and then stored for future use.

4.4 Spin Coating of Photoresist

The photoresist is the basis of creating the intended patterns onto the substrate. In this experiment, a negative photoresist is used, SU-8, which is a viscous and colourless organic liquid. Using a dropper, the SU-8 is transferred onto a clean substrate and allowed to spread out on the surface. The substrate is then placed at the centre of the spin coater and the vacuum pump turned on.

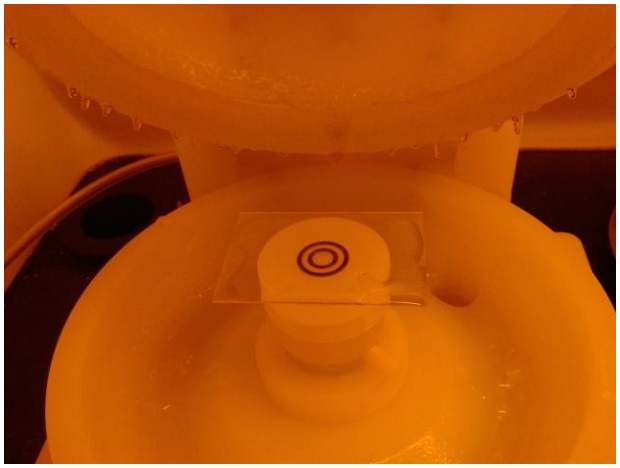


Figure 5: Substrate with Photoresist on Spin Coater

The following program is keyed into the spin coater, after which the substrate is removed from the spin coater.

Step	Time	RPM
1	10s	500
2	50s	2000
3	10s	500

Table 6: Spin Coating Steps

A total of 4 substrates are prepared in order to make room for any mistakes that are anticipated in the fabrication process.

4.5 Soft Bake

After spin coating, each substrate is placed on the hot plate and baked at 95ºC for 4 minutes.

Figure 7: Soft Bake on Hot Plate

4.6 UV Exposure

The substrates are then ready for UV exposure using the mask. Since the photoresist used is negative, the corresponding mask used is dark field. As illustrated in Figure 4, the mask is loaded onto the mask holder whereas the substrate is loaded onto the sliding holder below it. Alignment of the substrate to the mask in the x, y and z directions are done manually. Care is exercised in placing the substrate near but not in contact with the mask. Such proximity will ensure that diffraction effects during exposure are kept to a minimum, resulting in better resolution of the patterns.

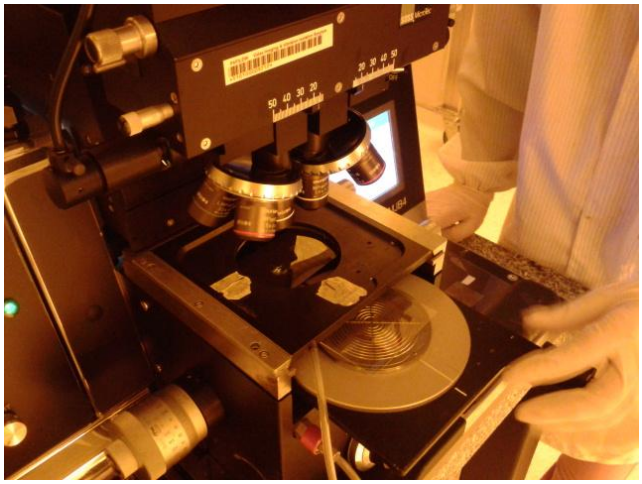


Figure 8: Alignment of Mask and Substrate in UV Irradiator

After alignment is done, the set-up undergoes a flood exposure of UV light for 5s. Exposed portions of the photoresist on the substrate experience a chemical reaction, rendering them insoluble in the developer, which will be added at a later step.

4.7 Post-exposure Bake

Immediately after exposure, the sample is subjected to a heat cycle again. It is placed onto a hot plate for 4 minutes with a temperature setting of 95ºC.

4.8 Development

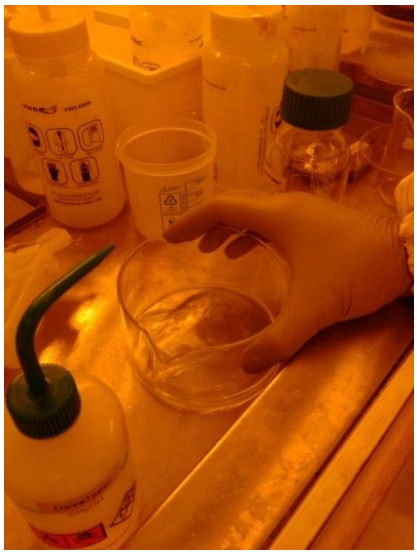


Figure 9: Developing of Sample

After the sample is left to cool, it is placed into a dish of developer for about 2 minutes. Constant swirling is done to ensure good mixture. After the sample is removed, it is rinsed with developer fluid and then distilled water. Finally, it is blown dry using compressed air. The result is a master mold with the intended patterns to be used in fabricating the PDMS.

Figure 10: Close-up of Patterns on Master Mold

4.9 PDMS Preparation

Liquid PDMS is prepared by mixing the base and curing agent in the weight ratio of 10:1. Here, using an electronic balance, 32.6g of base agent is mixed with 3.23g of curing agent. The mixture is stirred in a plastic cup for a few minutes.

Because air bubbles are introduced during the stirring process, it is subsequently placed in a vacuum chamber to be de-gased. When the vacuum is switched on, foam rises from the PDMS mixture. Once the foam reaches the brim of the cup, air is let in to allow the foam to subside. This cycle of decreasing and increasing the chamber pressure is repeated several times.

Figure 10: Vacuum Chamber

4.10 PDMS Curing

The edges of the master mold are taped up to form a box-like structure to hold the liquid PDMS. Once the PDMS is de-gased, it is poured onto the master mold at a low height. The samples are then placed in a convention oven to be de-gased again and cured. The curing process is done at a temperature range of 60-80ºC for about an hour.

Once the PDMS is hardened due to the curing, the patterned portions are cut and peeled from the substrate. Holes are then punctured by a needle at the inlets and outlets of the designs.

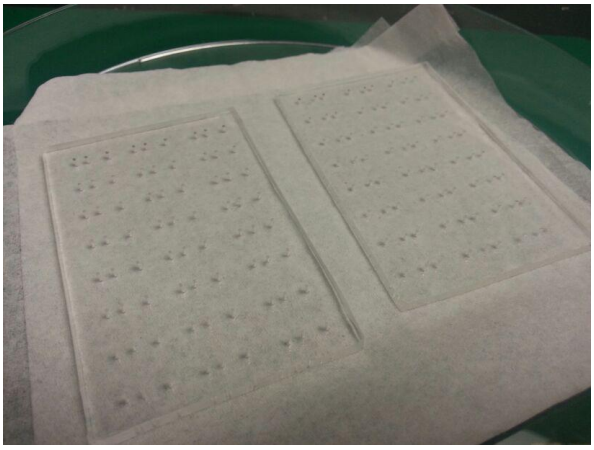


Figure 11: Patterned PDMS after Peeling

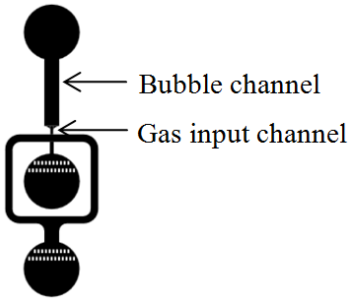
4.11 PDMS Bonding

Figure 12: Plasma Cleaner

The peeled PDMS is now ready to be bonded to a clean substrate. Each PDMS and a new substrate are placed into the plasma cleaner with their bonding surfaces facing upward. Under vacuum conditions, the plasma is turned on for about 10s. Upon removal from the plasma cleaner, the treated surfaces of the PDMS and substrate are pressed manually together. At this stage, the microfluidic device is successfully fabricated.

5 Testing of Device

As illustrated in Figure 4, the basic design of the microfluidic device has 1 gas inlet, 1 liquid inlet, an outlet and a bubble channel to observe bubble patterns. The 6 sets of dimensions fabricated are summarised as follows.



Design	Gas input channel width	Bubble channel width
1	50µm	100µm
2	50µm	200µm
3	50µm	300µm
4	75µm	150µm
5	75µm	300µm
6	75µm	400µm

Table 13: Channel dimensions

The microfluidic device is secured onto a microscope using sticky tape as shown in Figure 14. Before a selected design is used, it is first flushed with ethanol to wet the channels and remove any dirt. Tubes with needles at the end are connected to the selected design at the 2 inlets and the outlet. The liquid input is a 10mL syringe filled with distilled water and a surfactant additive mounted on a syringe pump. The gas input is a gas controller supplying air. Because the knob of the gas controller is too coarse to properly control the gas input pressure, an oscilloscope capable of fine adjustments is connected to the gas controller and used to control the gas pressure instead. As such, the magnitude of gas pressure is calibrated to a voltage reading on the oscilloscope.

A high speed camera is mounted onto the microscope to capture and transmit images into the software “Photron Fastcam Viewer”. Trial runs are then conducted using randomly selected designs to familiarise with the equipment.

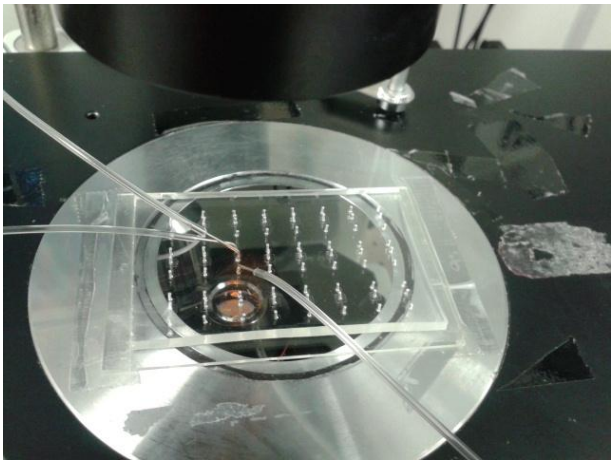


Figure 14: Microfluidic device on the Microscope

Figure 15 & 16: Syringe mounted on syringe pump (left); Gas Controller (right)

6 Hypothesis and Results

There were 4 controllable variables in this experiment, namely the gas input channel width, the bubble channel width, the gas pressure and the liquid flow rate. Before any testing was done, it is hypothesized that the bubble channel width would primarily determine the overall pattern of bubbles, such as whether they will be orderly or scattered and whether they will be closely packed or not.

Additionally, it is hypothesized that the gas pressure would determine the size of the bubbles formed. As for the liquid flow rate, it is thought that it would also have a bearing on the bubble patterns. More importantly, it determines whether bubbles would form at all or not, since a liquid flow that is too slow would likely not be strong enough to pinch the gas into bubbles.

The effects on bubble formation by the abovementioned 4 variables are explored one at a time. The results are as follows.

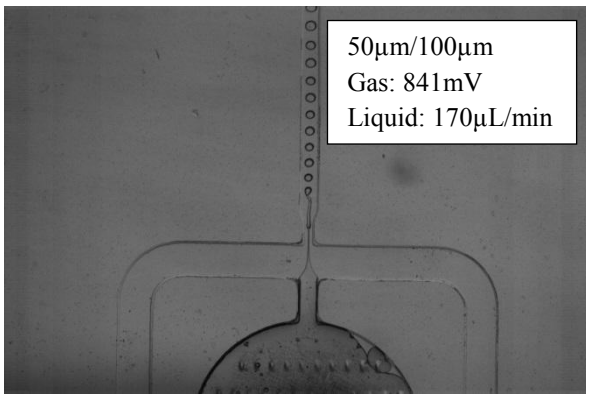
6.1 Gas Input Channel Width

By means of the trial runs, it is found that bubbles are more easily formed using the narrower 50µm gas input channel width. The 75µm gas input channel had a higher tendency to produce a gaseous stream in the bubble channel instead of bubbles. A likely reason is that a narrower gas input channel allows the liquid to exert more pressure on the gas coming in so as to pinch it into bubbles.

6.2 Gas Pressure

The gas pressure as displayed by the gas controller has the units of pounds per square inch (psi). The oscilloscope which was used to tune the gas pressure made use of calibrated voltage signals. Throughout the experiment, the range of voltage used is from about 200mV to 1000mV. This corresponds to a gas pressure of 3psi to 15psi.

The hypothesis that gas pressure determines the size of the bubbles is true. This can be illustrated using the example of the 50µm/100µm design, as shown in the figures below. In this example, the liquid flow rate is kept constant at 170µL/min.



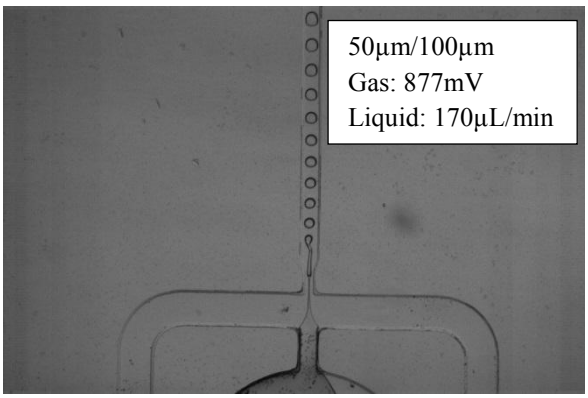
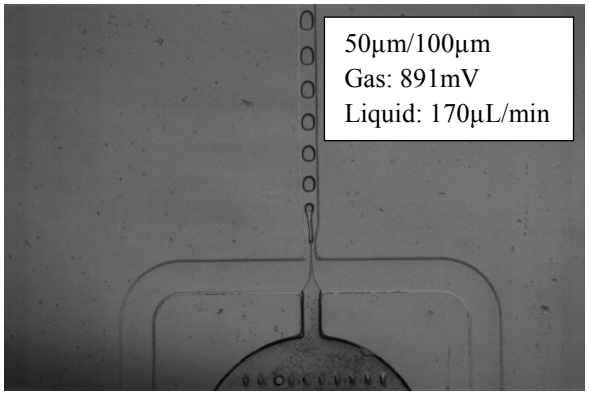


Figure 17 & 18: 50µm/100µm design with oscilloscope reading of **841mV** (left) and **877mV** (right)



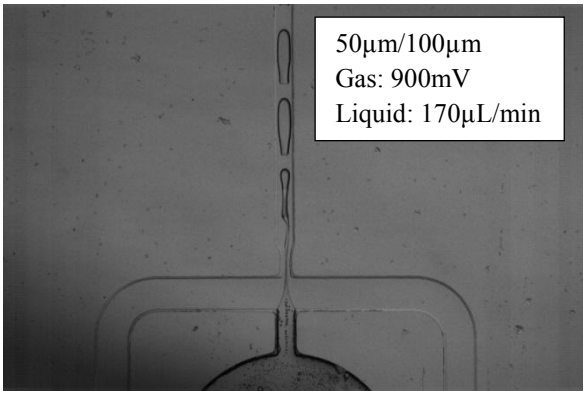


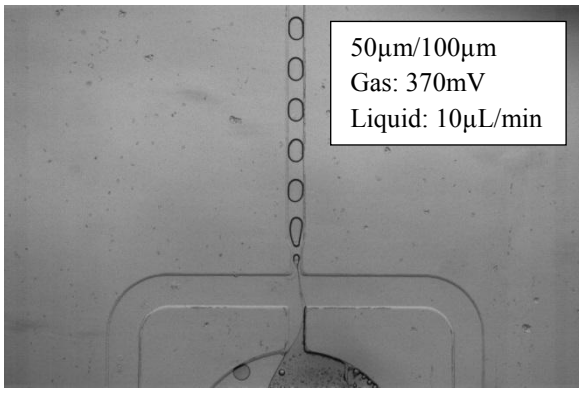
Figure 19 & 20: 50µm/100µm design with oscilloscope reading of **891mV** (left) and **900mV** (right)

Figures 17-20 indicate that a progressive increase in gas pressure results in a noticeable increase in bubble size. The bubbles are generally circular in shape but due to the narrow bubble channel of just 100µm, from 891mV onwards, the bubbles are compressed by the channel walls. At 900mV, the bubbles become substantially elongated. At even higher gas pressure, only a gaseous stream is observed instead of bubbles.

The phenomenon of increasing bubble size with increasing gas pressure and the appearance of a gaseous stream at high pressures are observed in all of the 6 designs.

6.3 Liquid Flow Rate

The effect of liquid flow rate on bubble formation is rather interesting to observe. Again, the example of the 50µm/100µm design is used to illustrate the effect. In this example, the oscilloscope reading, which controls the gas pressure, is kept constant at 370mV.



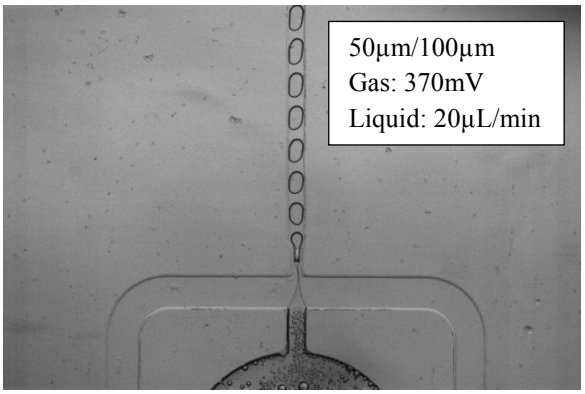
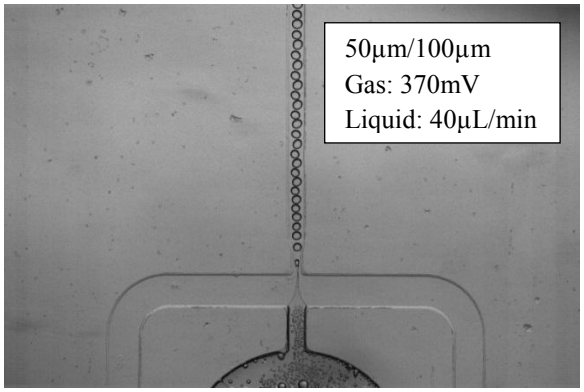


Figure 21 & 22: 50µm/100µm design with liquid flow rate of **10*µ*L/min** (left) and **20*µ*L/min** (right)



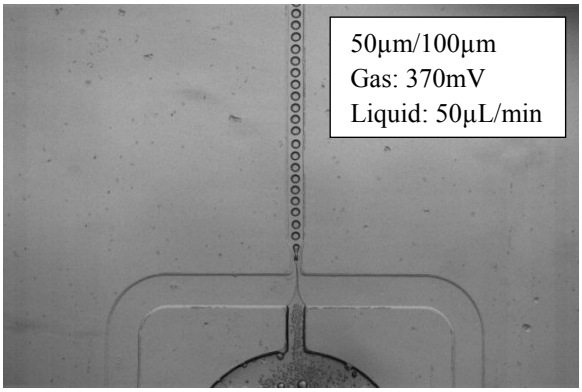


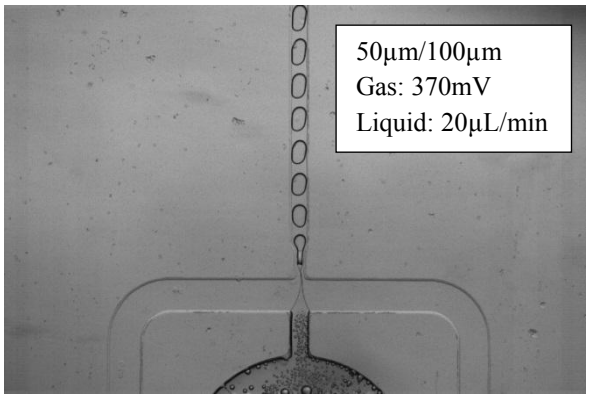
Figure 23 & 24: 50µm/100µm design with liquid flow rate of **40*µ*L/min** (left) and **50*µ*L/min** (right)

Two observations can be made from Figures 21-24. Firstly, as liquid flow rate is progressively increased, the bubbles formed become smaller. A point to note is that the syringe pump controlling the liquid input pushes the syringe using pulses instead of in a continuous manner. An increase in liquid flow rate means these pulses are delivered at a higher frequency. As such, the gas entering the bubble channel is pinched more frequently, resulting in smaller bubbles.

Comparing Figure 23 and 24, it seems that the size of the bubbles did not change. When the liquid flow rate is increased to from 40µL/min to 50µL/min, instead of decreasing in size, the bubbles begin to be spaced out from one another. This implies that although they are no longer pinched by the liquid at a higher rate, they are being carried along in the bubble channel at a higher rate.

6.4 Bubble Channel Width

It is found that the bubble channel width, on its own, does not determine the overall pattern of bubbles formed. However, it does impose an upper limit on the number of bubble columns formed. For instance, in the 50µm/100µm design, bubble size can be made small by adjusting the gas pressure and liquid flow rate. When the bubbles are small enough, 2 columns can be formed, as shown in Figure 26. Since the bubbles cannot be made any smaller, the 100µm bubble channel restricts the number of bubble columns to 2.



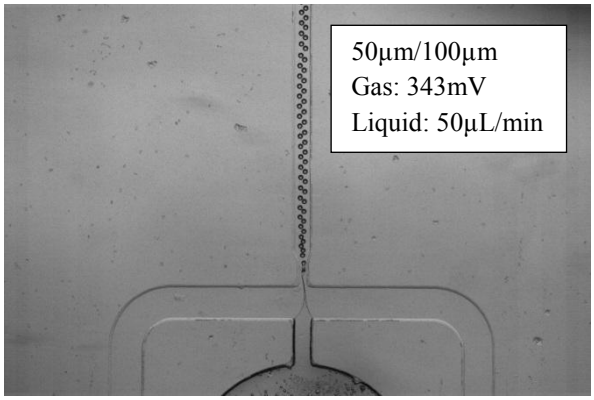
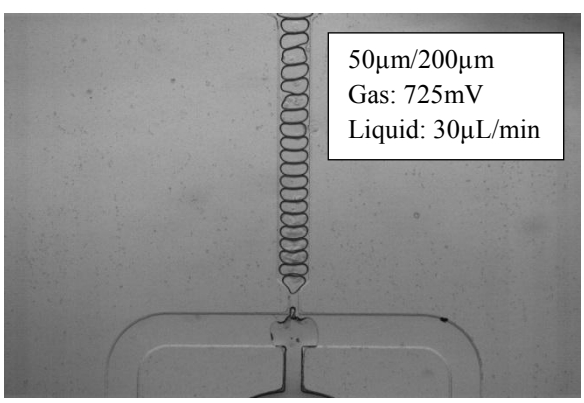


Figure 25 & 26: **50*µ*m/100*µ*m** design with 1 bubble column (left) and 2 bubble columns (right)

With a wider bubble channel of 200µm, there is more room for different bubble patterns. Figure 27 shows how the gas is pinched into bean-shaped bubbles instead of circular ones. Decreasing the gas pressure reduces the bubble size but the relatively wider bubble channel allows for a 1-2-1-2 formation of bubbles.



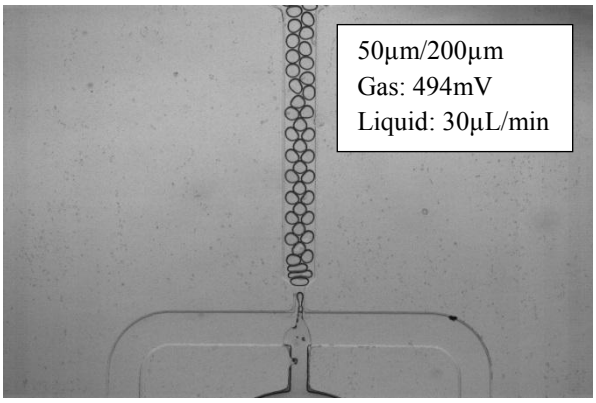
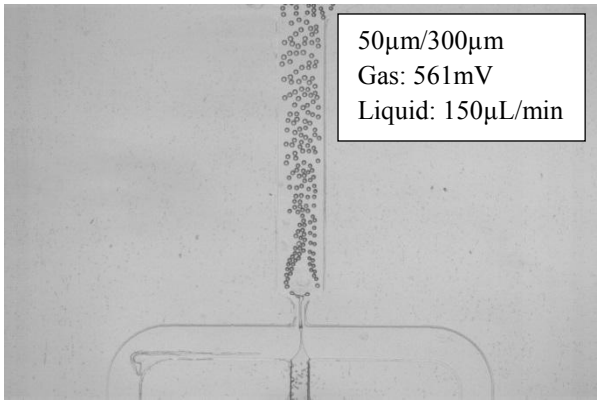


Figure 27 & 28: **50*µ*m/200*µ*m** design with 1 bubble column (left) and 3 bubble columns (right)

Using an even wider bubble channel of 300µm, a disordered bubble flow can be obtained, as shown in Figure 29. This occurred at a relatively low gas pressure which tends to give small bubbles. It is likely because of the wide channel width that causes the bubbles to spread out after being pinched, resulting in a disordered pattern. At a relatively higher gas pressure, the bubbles formed are larger, as shown in Figure 30. As such, they flow through the bubble channel in 2 orderly columns, much like what is observed for the narrower bubble channels.



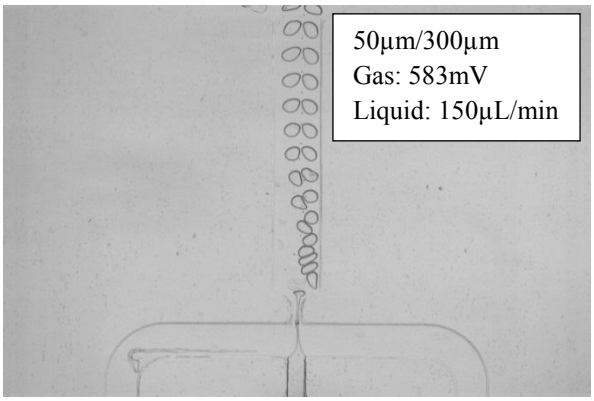


Figure 29 & 30: **50*µ*m/300*µ*m** design with disordered bubble flow (left) and 2 bubble columns (right)

7 Conclusion

Fabricating a microfluidic device involves a tedious sequence of steps. A meticulous approach during fabrication ensures that contamination and damage are kept to a minimum. Fluid interaction between air and water is also explored qualitatively. It is found that bubbles are formed only within certain ranges of gas pressure and liquid flow rate; otherwise a gaseous stream is formed instead. These two variables are also the primary determinants of bubble sizes. In addition, it is found that a wider bubble channel width provides more room for a variety of bubble patterns.

8 Acknowledgements

I would like to acknowledge **Asst. Prof. Claus-Dieter Ohl** for providing the idea and resources to allow me to gain exposure in microfluidic fabrication and testing. I would also like to thank **Mr Chan Chon-U**, **Ms Li Fenfang** and **Mr Leon** for their time and patience in providing guidance throughout this experiment.

9 References

1. faculty.washington.edu
2. *Fluid flow in micro-channels*, by G. Hetsroni et al, International Journal of Heat and Mass Transfer 48 (2005) p. 1984
3. http://www.essom.com/backend/data-file/engineer/engin11_1.pdf