MICROFLUIDIC DESIGN:
SOFT LITHOGRAPHY

LAB MANUAL

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INTRODUCTION

Microfluidics is an exciting, and relatively new field that offers researchers the ability to conduct experiments at the microscale offering greater efficiency and control in experimentation. Currently microfluidic technologies have provided novel experimentation in areas such as DNA analysis, polymer formation, cell sorting, pathogen detection, chemical reactions, and much more. While reactions at the macro level can take hours, microfluidic devices reduce the time to fractions of a second as well as reducing the quantity of reagents. Furthermore, integrated microfluidic devices condense all aspects of an experiment onto a single chip giving them the synonym: lab-on-a-chip.

Using this manual, students will learn soft lithography techniques for device construction. It is important for students to learn soft lithography, for it is the primary method for generating microfluidic devices because of its low cost, relatively fast prototyping, great accuracy, and outstanding precision. With this technique micro and even nano scale experiments can be conducted with highly repeatable results. Ultimately, the techniques students learn in this manual will be the basis for all future microfluidic experimentation.

Source: www.gene-quantification.de/lab-on-chip.html
EQUIPMENT

(a) needles  (b) syringes  (c) biopsy punchy  (d) wafer tweezers  (e) flat tweezers  (f) scalpel

Silicon wafer  Teflon tubing
(a) Wash bottles (b) Teflon tubing (c) PDMS (d) PDMS curing agent

UV Power Meter

Hot plate

Glass slides

Syringe pump
TECHNIQUES

Technique 1: Soft Lithograph Device Fabrication

This technique is the basis for all soft lithography based fabrication. It is important to master this technique for it is not only an efficient and accurate method of device production, but also a technique that can be carried on into research and industry. Refer to the illustration below as you read through Technique 1.

Technique 1A: Transparency Creation

The first technique in soft lithography fabrication is designing a UV mask—similar to an overhead transparency—that outlines the blueprint of the microfluidic device. This step is important because it will be used to selectively expose a negative photoresist coating produced in the next two techniques. Refer to part C \( \rightarrow \) D in the illustration (pg 7) and the introduction to Technique 1C for further details.

Procedure

Create a microfluidic design that meets the demands of the experiment at hand.

There are only two simple rules to UV mask design:
1. Features where liquid will flow in the device are clear.
2. All other features such as pillars, filters, etc are to remain black

PDMS Column Filter

Depending on the precision desired, the design can be created in AutoCAD, Adobe Photoshop, VectorWorks or any other software that provides an accuracy no less than 1200 dpi. For a rough UV mask or transparency, a printer may be used at a 1200 dpi setting; however, it is recommended that a professional printing service that specializes in high dpi printing be used for best results.
Technique 1B: Preparation of Silicon Wafer

A silicon wafer is typically used as the substrate for the photoresist pattern, but first the silicon wafer must be cleaned before any photoresist is added.

Safety and Protocol

1. Wear gloves at all times.
2. Safety glasses are to be worn when working with solvents.
3. Hold wafers only with wafer tweezers.
4. Use primer under running fume hood.
5. Dispose of solvents in labeled waste containers located under fume hood.

Procedure

1. Sonicate a silicon wafer for 10 minutes in acetone.
2. Rinse wafer with isopropyl alcohol (IPA).
3. Dry wafer with nitrogen gas.
4. Bake wafer for 5 minutes at 200°C.
5. Allow wafer to cool, before soaking wafer in a thin layer of primer for 5-10 minutes. Seal primer bottle immediately after use.
6. Rinse wafer with IPA and dry with nitrogen.
6. Place the cleaned wafer is a large petri dish for storage and transport.
Technique 1C: Photoresist Patterning

Although there are several negative photoresists, SU-8 is commonly used to produce microfluidic devices with depths ranging from 20 to 200 micrometers. In this technique, SU-8 will be spin coated onto a clean silicon wafer to produce a pattern of a desired thickness. The coated wafer will then be exposed to ultra-violet (UV) light that passes through the UV mask created in Technique 1A. Photoresist exposed to the UV light will be cross-linked in the shape of the UV mask. The unexposed, uncross-linked coating, can then be removed using SU-8 developer leaving a hardened pattern on the silicon wafer. This is called a stamp that will be used to mold an elastomer in the next technique.

Safety and Protocols

1. Wear gloves at all times while handling SU-8. Double gloving is advised.
2. Safety glasses are to worn at all times.
3. SU-8 and SU-8 developer containers should be sealed immediately after use.
4. Fume hoods must be on when using SU-8 and SU-8 developer. DO NOT turn off hoods until 20-30 minutes after placing SU-8 and SU-8 developer into the appropriate waste containers located inside fume hood. Solid waste (aluminum foil and wipes) go directly into the solid waste container.
5. Replace aluminum foil after every use of the spin coater.
6. Use UV filtered light when working with SU-8.

Figure 1. SU-8 2000 Spin Speed versus Thickness

Spin Speed Chart
Source: http://www.microchem.com/products/su_eight.htm
Spin Coating: SU-8

1. Place wafer onto spin coater.
2. Turn on the pump and select the desired spin coating program. Each program consists of 3 cycles. The first spin cycle runs at 500 revolutions per minute (rpm) for 10 seconds to spread the SU-8 to the edges of the wafer. The coater then ramps up to the user-defined rpm, which controls the thickness of the coating. Refer to the thickness vs. rpm chart for the appropriate spin settings for the desired device thickness. Lastly, a ramp down cycle slowly brings the coated wafer to a stop.
3. Check that the wafer is centered on the spin coater by running a test program at 500 rpm’s. An uneven spin can produce a non-uniform coating.
4. After centering the wafer, pour approximately 1 mL of SU-8 per inch of wafer diameter: a 3 inch wafer uses 3 mL of SU-8.
5. Seal the spin coater and start the program.
6. Once the wafer has stopped, remove the SU-8 meniscus at the edge of the wafer.
7. Perform a pre-bake by placing the wafer on a hot plate at 65°C before increasing to 95°C. The time at each temperature varies depending on the thickness of SU-8. Refer to the pre-bake heating chart for details.

<table>
<thead>
<tr>
<th>THICKNESS</th>
<th>SOFT BAKE TIMES</th>
</tr>
</thead>
<tbody>
<tr>
<td>microns</td>
<td>(65°C) minutes</td>
</tr>
<tr>
<td>25 -40</td>
<td>0 - 3</td>
</tr>
<tr>
<td>45 - 80</td>
<td>0 - 3</td>
</tr>
<tr>
<td>85 - 110</td>
<td>5</td>
</tr>
<tr>
<td>115 - 150</td>
<td>5</td>
</tr>
<tr>
<td>160 - 225</td>
<td>7</td>
</tr>
</tbody>
</table>

Mask Aligner (MA6)

Mask Aligner Safety and Protocols

1. Wear gloves at all times while operating the MA6.
2. UV protective glasses should be worn during wafer exposure.
3. Isopropanol (IPA) is the only chemical used on the mask aligner.
4. Ensure that the nitrogen stream stays on for 20 minutes after turning off equipment. Do not forget to turn off the supply tank.
5. When finished ensure that the mask has been removed from the holder.
6. Replace covers on wafer and mask holders when finished to minimize dust accumulation.
MA6 Procedure

Start Up

1. Turn on the gas and vacuum lines.
   a. N₂ stream – set regulator to 15 psi
   b. Air stream – set regulator to 60 psi
   c. Vacuum – set to 70 cm Hg (13.5 psi) below atmosphere
2. Turn the lamp power box on.
3. Wait 20 seconds before pressing CP. When start is illuminated press START.
4. Power on the Mask Aligner and wait for the computer to boot-up.
5. Press Load, aligner will initialize.

Exposure

6. Check the intensity of the UV light.
   a. Place the UV power meter at the center of the wafer holder.
   b. Press lamp test and record the intensity.
   c. Repeat for positions near the edge of the open mask holder and half way between the middle and edge.
   d. Compute an average intensity.
7. Press edit parameter to change the exposure time.
   a. Use the x directional keys to find the desired setting and change the value or setting type with the y directional keys.
   b. Exposure time is determined by thickness dividing exposure energy by the mean exposure intensity (recorded on UV power meter). This quotient is the seconds required to expose. Ex. If the mold is 25 microns, the mean UV intensity is 22. Then 150 mJ/cm² (exposure energy) divided by 22 (UV intensity) equals 6.81 seconds

Exposure Energy Chart

<table>
<thead>
<tr>
<th>THICKNESS</th>
<th>EXPOSURE ENERGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>microns</td>
<td>mJ/cm²</td>
</tr>
<tr>
<td>25 - 40</td>
<td>150 - 160</td>
</tr>
<tr>
<td>45 - 80</td>
<td>150 - 215</td>
</tr>
<tr>
<td>85 - 110</td>
<td>215 - 240</td>
</tr>
<tr>
<td>115 - 150</td>
<td>240 - 260</td>
</tr>
<tr>
<td>160 - 225</td>
<td>260 - 350</td>
</tr>
</tbody>
</table>

Source: http://www.microchem.com/products/su_eight.htm

8. Press edit programs to change soft contact and continuous exposure setting.
9. Tape the mask to the quartz glass, ensure that the tape does not go over the edges of the glass.

10. Press Change Mask, remove mask holder and flip over to access the clamps that hold the quartz glass in place, place mask (on quartz glass) on the holder and check that the clamps are securely holding the glass, and press enter to start vacuum.
   a. The mask should be facing up so that when the mask holder is placed in the mask aligner the mask will face downwards.
   b. If vacuum does turn on and an error “loss of vacuum” is displayed:
      i. Take the mask off and then place it back onto the holder and press enter again
      ii. If this fails then remove mask and wipe the back of the glass with a wipe and IPA. Use as little IPA as possible. Replace mask and press enter.
      iii. If neither works ask your instructor for assistance.

11. Place the mask holder back into the mask aligner and press change mask. This clamps the mask holder into place.

12. Press Load. A prompt to remove the wafer holder will appear.
   a. Pull out wafer holder
   b. Place wafer on the holder to cover the vacuum holes. Follow the peg positions.
   c. Push wafer holder back into mask aligner

13. Press enter (the microscope will lower for alignment purposes. At this point, ignore alignment. If alignment is necessary, see your instructor).
   a. If the microscope does not move, check the display to see if the wafer holder has moved position. This is due to difference between the alignment distance and the exposure distance. Press exposure again.
   a. b. If the wafer vacuum is lost at any time press enter. The machine should allow a bypass for the wafer vacuum.
   b. If the mask vacuum is lost at any time. Press the unload button, re-position the wafer over the vacuum holes, press load and enter until the wafer holder is clamped down.
   c. If mask vacuum still causes problems, call your instructor.

14. Press exposure. The microscope moves up and the substrate is exposed for the set time.

15. After exposure, press unload and remove the wafer.

16. Press change mask, remove the mask holder, press enter to turn the mask vacuum off, and remove the mask. Replace the mask holder and press change mask. The vacuum should remain off.
**Shutdown**

17. On the computer, click file and shutdown.
18. Power off the mask aligner.
19. Turn off the lamp, vacuum, and air pressure lines.
20. IMPORTANT: The N2 line stays on for 20 minutes to cool the lamp. After twenty minutes turn off the N2 line.
21. Replace any covers that were previously on the mask aligner to prevent dust accumulation

**SU-8 Development**

**Safety and Protocols**

1. Wear gloves at all times. Double gloving is advised.
2. Only use developer under running fume hood.
3. Seal developer bottle immediately after use.
4. Dispose of waste in appropriate containers.

**Procedure**

1. After MA6 exposure, remove the wafer. Place on hot plate at 65°C before increasing to 95°C. See post bake table for time frames.

<table>
<thead>
<tr>
<th>THICKNESS</th>
<th>PEB TIME (65°C)*</th>
<th>PEB TIME (95°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>microns</td>
<td>minutes</td>
<td>minutes</td>
</tr>
<tr>
<td>25 - 40</td>
<td>1</td>
<td>5 - 6</td>
</tr>
<tr>
<td>45 - 80</td>
<td>1 - 2</td>
<td>6 - 7</td>
</tr>
<tr>
<td>85 - 110</td>
<td>2 - 5</td>
<td>8 - 10</td>
</tr>
<tr>
<td>115 - 150</td>
<td>5</td>
<td>10 - 12</td>
</tr>
<tr>
<td>160 - 225</td>
<td>5</td>
<td>12 - 15</td>
</tr>
</tbody>
</table>

Post Bake Heating Chart  
Source: http://www.microchem.com/products/su_eight.htm

2. Place wafer into a developer for time that corresponds with thickness.

<table>
<thead>
<tr>
<th>THICKNESS</th>
<th>DEVELOPMENT TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>microns</td>
<td>minutes</td>
</tr>
<tr>
<td>25 - 40</td>
<td>4 - 5</td>
</tr>
<tr>
<td>45 - 75</td>
<td>5 - 7</td>
</tr>
<tr>
<td>80 - 110</td>
<td>7 - 10</td>
</tr>
<tr>
<td>115 - 150</td>
<td>10 - 15</td>
</tr>
<tr>
<td>160 - 225</td>
<td>15 - 17</td>
</tr>
</tbody>
</table>
3. Remove wafer and use a small amount of IPA on one of the corners of the wafer.
   a. If a white streak occurs, rinse with developer and return to developer bath
   b. If no white streak occurs, rinse entire wafer with IPA.
4. A post develop bake is advised at 120°C for 5-10 minutes.
5. Observe the SU-8 stamp under reflective light microscopy. Observe SU-8 mold
   using reflective light. Record observations. Are the corners smooth? Rigid?
   Uniform? Cracked?
6. If badly cracked, features are indistinct, etc. Technique 1B, C must be repeated

Technique 1D: PDMS Preparation & Curing

This technique uses the SU-8 stamp created in technique 1C to mold PDMS into
the desired pattern of the device.

PDMS Safety and Protocols

1. Use gloves and safety glasses at all times.
2. Avoid spilling PDMS: use plastic wrap or paper towels to prevent PDMS from
   spilling onto machines or tables
3. Clean spills with isopropanol
4. Use chlorotrimethylsilane under running fume hood and immediately after use
5. Dispose of waste in appropriate waste containers

Procedure

Mixing PDMS

1. Mix PDMS and curing agent in a 10:1 ratio in a disposable container: for a 5 inch
   petri dish a total of 90 grams
2. Mix thoroughly for 5 minutes
3. Ensure that there are no clear distinctions between the two fluids

Degas in Desiccator

4. Cover bottom of the desiccator with aluminum foil
5. Punch small holes in aluminum to allow for complete evacuation of the chamber
6. Place PDMS mixture on the aluminum foil
7. Seal desiccator and power on vacuum.
8. Bubbles will emerge as the vacuum removes all gas from the chamber and the
   PDMS. Vent the desiccator to pop the bubbles and to prevent the liquid from
   spilling out into the desiccator. Venting is accomplished by opening the 3-way
valve to the room’s atmosphere.
9. Close the three way valve to return the chamber to vacuum  
10. Repeat steps 8 and 9 until no more bubbles emerge

**Chlorotrimethylsilane Monolayer Formation on Wafer**

11. Clean off wafer with SU-8 stamp using nitrogen gas to remove any dust particles  
12. Place wafer in a 5 inch petri dish  
13. Drop ~0.5 mL of chlorotrimethylsilane around the wafer, do not drop the silane directly on the wafer. Cover with the petri dish top.  
   CHLOROTRIMETHYLSILANE IS VERY TOXIC!  
   DO NOT INHALE OR HAVE SKIN CONTACT!  
14. Cover the petri dish and allow 5 minutes for monolayer formation. After this time all the silane will have evaporated.

**Molding and Curing PDMS to SU-8**

15. Pour degassed PDMS mixture onto chlorotrimethylsilane coated wafer  
16. Repeat degassing process to remove any bubbles formed during the PDMS pour  
17. Bake for 2 hours at 80°C.

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**Technique 1E: Glass Bonding**

This is the final technique in producing a PDMS microfluidic device. PDMS is bonded to a glass substrate forming a complete seal for the channels in the previous technique.

**Procedure**

**Cutting Out PDMS Mold**

1. Using a scalpel, cut the PDMS around the device leaving a 0.5 cm border.  
2. Using a biopsy punch, cut holes at the inlets and outlets. Push the punch all the way through the PDMS and then remove the cut PDMS with tweezers  
3. Clean the surface containing features with tape  

**Bonding PDMS Mold to Glass**

4. Place a clean glass slide and PDMS mold (device face up) onto the plasma cleaner tray and insert the tray into the plasma cleaner.  
5. Turn on vacuum pump with the cover plate held over the plasma chamber.  
6. Turn the needle valve such that 0.4 torr reads on the pressure gauge.  
7. Turn the RF power on and set the intensity to high. Check that the plasma coils
are glowing a faint pink color.
8. Allow the plasma cleaner to run for 90 seconds before turning the RF intensity to
off and switching the RF and pump power to off.
9. Open the ball valve to the atmosphere while holding the cover plate until the
pressure gauge reads room pressure.
10. Remove glass and PDMS mold from the chamber.
11. Attach the PDMS mold to glass by placing the device face down and wait for 30
seconds.
12. Insert tubing into the inlet and outlet punched area, refer to Technique 2. Inject
water to ensure that there are no leaks.
13. If leaks exist the Technique 1D must be repeated.
Technique 2: Syringe and Teflon Tubing Preparation

Safety and Protocols

1. Wear gloves and safety glasses at all times.
2. Needles are sharp! Avoid puncturing skin.

Procedure

1. Place head of syringe well below the surface of the fluid in it respective container
2. Withdraw fluid slowly, especially if the liquid is highly viscous.
3. Withdraw past desired volume in order to reduce air bubbles in the next steps
4. Remove syringe from container and wipe the head.
5. Turn the syringe upward and flick to dislodge air bubbles
6. Push the plunger inwards to remove air bubbles (this minimizes the amount of bubbles entering the device).
7. Attach a clean needle to syringe and remove the needle cap
8. Point the syringe head up again and push liquid out of the syringe through the needle until fluid flows out of needle
9. Cut Teflon tubing to the desired length. Insert the needle into one side of the tubing. Avoid puncturing the tubing as well as your skin.
10. Cut the other end of the tubing at an angle to allow for easier insertion into microfluidic device’s inlets and outlets.
Technique 3: Pump and Syringe Operation

Procedure for Chemyx N3000

Consult specific pump manual and instructor for variations between different pumps.

1. Turn pump on
2. Select standard infusion and hit enter.
3. Select the syringe being used under “Find Syringe” or input the syringe dimensions manually.

Select infusion or withdraw:

4. Infusing
   a. Ensure plunger of syringe makes contact with moving plate (plate can be moved manually my turning top knob once to unlock/lock)
   b. Begin infusing by pressing enter

5. Withdrawing
   a. Lock end of plunger to plate with withdrawal holder
   b. Lock finger flange of syringe into the other withdrawal holder

Quick Keys:
F1= Fast forward   F2=Forward
F3=Reverse         F4=Fast Reverse
Technique 4: Profilometer Operation

Protocols and Safety

1. Wear gloves at all times.
2. DO NOT touch the leveling stage. Only the instructor should try to level the device.

If not using a Dektak system, the parameters found in the procedure such as scan rate can still be utilized on other systems.

Procedure

1. Turn on profilometer
2. Wait a few seconds, initialize Dektak
   a. If the stage was not raised at the end of last scan, allow it to raise before opening program.
   b. If communication between software and hardware does not occur.
      i. Click retry
      ii. Close program and open again.
      iii. Turn off program and machine before repeating
      iv. Ask for assistance.
3. Open the cover and place sample onto translational stage.
4. Click the tower down button.
5. Once the tip has been lowered onto the surface, the x, y, and theta translational stages can be turned to orient the surface as necessary.
   a. DO NOT try to change the x, y, and theta stages while the tower is lowering. The tip has to touch the surface and then retract. Moving the stages at this time will cause alignment problems.
   b. If moving the sample, raise the tower before moving sample.
4. The scan length and duration can be changed under the Scan Routines Window. Click on scan length to bring up the input window.
   a. The scan length and duration can be altered to change to the scan rate. A good scan rate is <1 um/sample.
   b. Resolution of the feature size can be changed under the Measure Range option. Ex. a feature is 100 um then use the 524 um setting.
   c. All other options should not be altered.
5. Move the y at least 200 um away from the first scan feature. This allows the tip to orient itself along the surface as well as provide a flat analysis region.
6. Click on Run Currently Active Scan Routine. Current setup has the plot displayed in real time with the image behind.
7. Once completed the plot will appear in the data analysis screen.
8. Auto leveling of the base line should immediately occur. If not, select analysis
and step detection. Change the distance to the max distance scanned and click apply.

7. If auto level does not occur change the detection method from first step to every step or vice versa.

9. Export data with the Export Scan Data button.

10. Click Switch to Sample Positioning Window to return to the tower controls.

11. Shut down: Make sure that the tower has been raised. Close the program before turning off the instrument.
EXPERIMENTS

Experiment 1: Adjacent Flow

At the macro level miscible liquids undergo chaotic mixing when brought together. Conversely, at the micro level Reynolds number (Re) can remain below one. A standard equation for Reynolds number is \( Re = \frac{pVD}{\mu} \), where \( \mu \) is the dynamic viscosity. If this condition is met, no mixing occurs and liquids move in what is called laminar flow. This property of microfluidic devices allows for designs that can flow adjacent streams without mixing due to flow conditions. In this experiment, the student will flow a water stream with fluorescent salt and an adjacent stream that will only contain \( \text{H}_2\text{O} \).

Objective

The objective of this experiment is to observe laminar flow and the novel experimental conditions microfluidics offer. As this is the first experiment and an explicit design is not given, the student will learn the basic techniques of both microfluidic design and fabrication.

Safety and Protocols

1. Wear gloves and safety glasses at all times.
2. Avoid skin contact with fluorescent salt, this may cause skin irritation and prolonged exposure can cause skin damage.
3. Fluorescent salt will stain clothing.
4. Dispose of waste in appropriate waste containers.

Procedure

1. Design a simple device that possess:
   a. 2 inlets and 2 outlets
   b. A large channel where both fluids will flow adjacent each other
   c. Conditions for laminar. Note: Several design schemes are possible.
2. Fabricate this device using Technique 1.
3. Fill a 1 ml syringe with water.
4. Fill a 1 ml syringe with fluorescent water.
5. Place the syringes in the syringe pump and connect Teflon tubing to both the syringe and channel. Refer to Technique 2 and 3 for tubing and syringe pump operation.
6. Connect the outlets with Teflon tubing to a small disposable container. Secure the tubing to the container with tape.
7. Run both pumps at the lowest speed possible for consistent flow
8. Place the device under a fluorescent microscope and observe the channel where both fluids meet, record observations.
   a. With time both fluids should occupy an equal volume of the channel
   b. If this does not occur check for obstructions or other factors that could hinder flow.
9. Develop a method to check that laminar flow is occurring. How does your method compare to the definition of laminar flow?
10. Increase flow rate of one fluid to the highest operable setting, record observations. How does flow rate effect laminar flow?
11. Increase flow rate of both fluids to the highest operable setting, record observations.
12. Alter flow rates to different ratios: 1:1, 2:1, 5:1, 10:1. Record the effects on laminar flow any changes in the two fluid streams.

Questions

1. Was laminar flow observed or did mixing occur? Why?
2. When the speeds increased was laminar flow observed or was turbulent mixing observed? Why?
3. Compute the Reynolds number for the different channels in the microfluidic device. Does the compute Re number fall in the laminar flow regime? Are your observations comparable to your experimental results?
4. Using the effective diameter of your channel, compute the flow rate necessary to produce turbulent flow. Use water’s viscosity for all your calculations.
5. Discuss the results of your calculations as compared to what you observed in this experiment.