Fun with Molding

An Introduction to Photolithography and Micromolding

2.674 Module I

(Labs 1 & 2)

Fall 2016

Pappalardo II Micro/Nano
Teaching Laboratory

Safety Notes

This lab involves the use of chemicals (UV-curable epoxy, acetone, and PDMS rubber) and UV light sources. As was explained in the first lecture and safety training, and as always when dealing with chemicals, it is necessary to wear your gloves at all times and to avoid contact with the chemicals. The UV light sources are designed to prevent exposure of the users' eyes or skin to significant amounts of UV light during normal usage. Do not attempt to circumvent the safety precautions, for example by lifting the UV light to point it at your eyes.

General Overview

Advances in micro- and nanotechnologies rely critically on micro- and nanofabrication technologies for creating useful micro/nanostructures and devices. In Module 1, we will take a closer look at some of these techniques, which fall under the broad category of *Microfabrication Technology*.

The techniques of microfabrication have developed rapidly and are now used to make a wide range of structures, from integrated circuits to microelectromechanical systems (MEMS). Integrated circuits are comprised of a series of patterned layers of different materials usually on a silicon wafer that define all of the necessary features for transistors, resistors, etc. For example, a transistor's source and drain are defined by injecting dopants into a silicon wafer; the transistor's gate is separated from the rest of the transistor by a thin layer of silicon dioxide; and the interconnects that wire up the transistors into circuits are made of either polycrystalline silicon or metal lines separated from each other where necessary by more silicon dioxide. These structures and other structures are created by different microfabrication techniques that include various methods to selectively *deposit* and *remove* different materials starting from a substrate, such as a silicon wafer. A typical process to pattern the necessary device layers on top of the substrate would proceed as follows. A uniform (unpatterned) layer of a material is deposited on top of the substrate. Then, a process called *photolithography* is carried out. Photolithography uses light to define features on a substrate. Following the deposition of the material to be patterned, a thin (~1 μm) layer of a *photosensitive* material called photoresist is deposited on top of the first layer. Then, a pattern of light is projected on the photoresist, in a process known as *exposure*. This

pattern of light originates from a photomask, a glass plate with a micro-patterned chrome coating on one face. Ultraviolet light shines through the photomask and onto the photoresist layer. Where the light shines on the photoresist, it modifies the photoresist to make it either harder to remove (negative resist) or easier to remove from the surface (positive resist). For example, the incident light may cross-link the polymers in a negative tone photoresist and make it insoluble in the 'developer' solution, and thereby harder to remove. The tools that are used for these processes are typically large, expensive, and complex. Figure 1 shows a photograph of a stepper, a high-precision photolithography tool that positions a mask over a substrate precisely and shines UV light through it in a very controllable manner, along with a photograph of a mask aligner, a less precise photolithography tool.

Figure 1. A stepper from Nikon (left) and a mask aligner from EV (right).

The photoresist is then selectively removed from the surface in a chemical process called developing; leaving what is essentially a stencil right on the surface of the substrate. Once the resist is developed, the pattern is transferred to the substrate and/or the layers that have been deposited on top of it. For example, the substrate or the deposited material might be etched away in the exposed regions, or additional material might instead be added in those same exposed regions. Finally, all the photoresist is removed using a harsher removal process known as *stripping*, leaving behind the patterned substrate. This process is repeated with subsequent layers, until the integrated circuit is complete, with layer upon patterned layer and no empty spaces between them.

Figure 2 shows an example of a typical photolithography process, with a simplified cross-sectional diagram of an integrated circuit, showing the various layers defined on top of each other using photolithography.

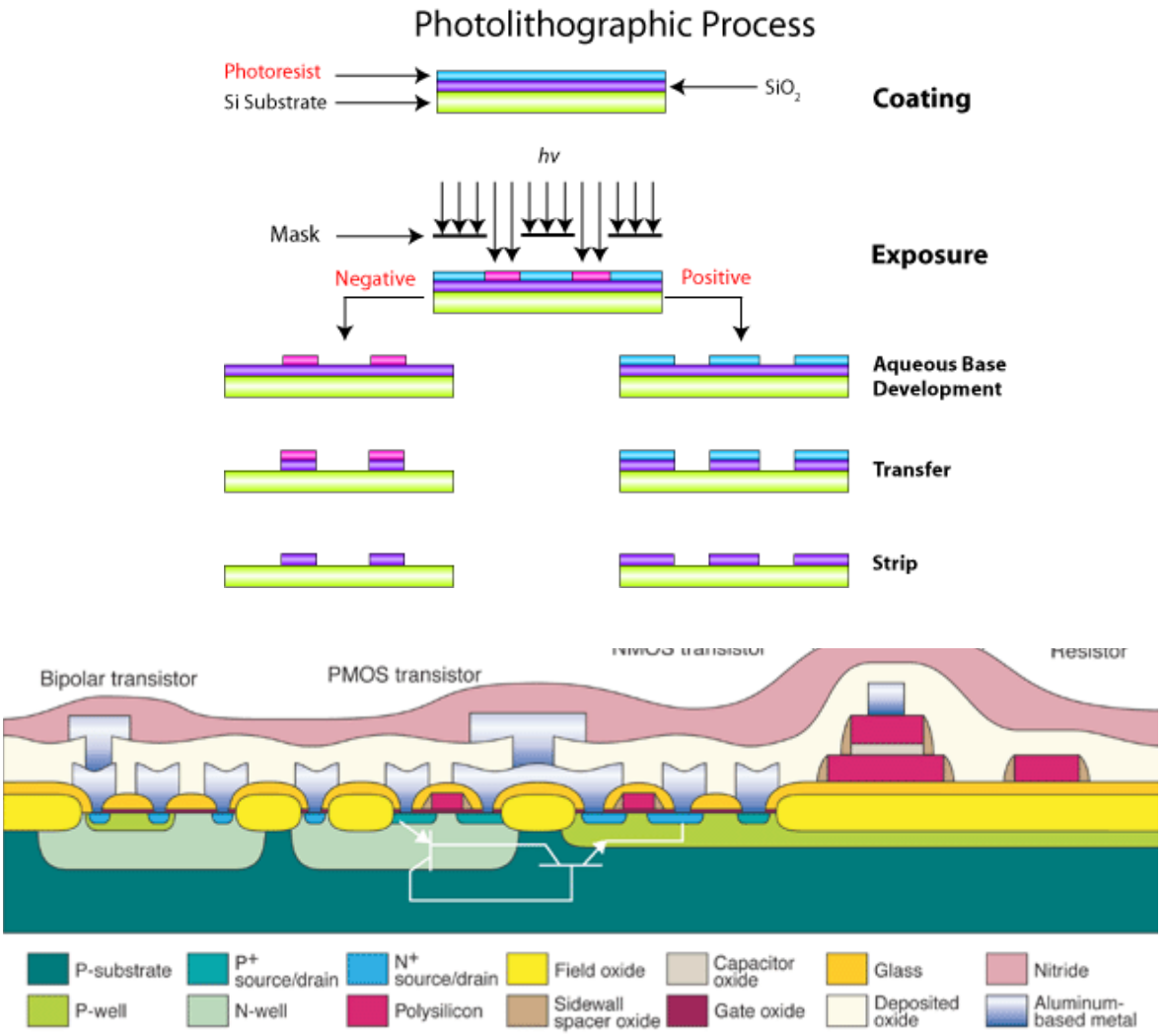

Figure 2. Top: Example of a photolithographic process to pattern silicon oxide on a silicon wafer using positive and negative photoresists. Image courtesy Bowling Green State University Center for Photochemical Sciences. Bottom: Cross-sectional schematic diagram of a CMOS integrated circuit, showing the various layers patterned on top of one another. From http://www.aero.org/publications/crosslink/summer2003/03.html.

The techniques of microfabrication were first developed in the context of integrated circuit manufacturing, but they have been expanded and adapted for the creation of MEMS. Like integrated circuits, MEMS are typically made of successive layers of different materials, patterned to form the necessary structures. Unlike integrated circuits, MEMS often include empty spaces between or within the different layers. These

empty spaces might serve to define moveable mechanical components, like thin diaphragms that deform when a pressure difference is applied across them, or to leave space for the moveable components to move, or to define channels through which fluid can flow. Creating these empty spaces requires a broader range of microfabrication techniques, including etches that etch deep into the substrate and etches that etch sideways to undercut deposited layers and create the necessary spaces, or bonding techniques for joining two substrates.

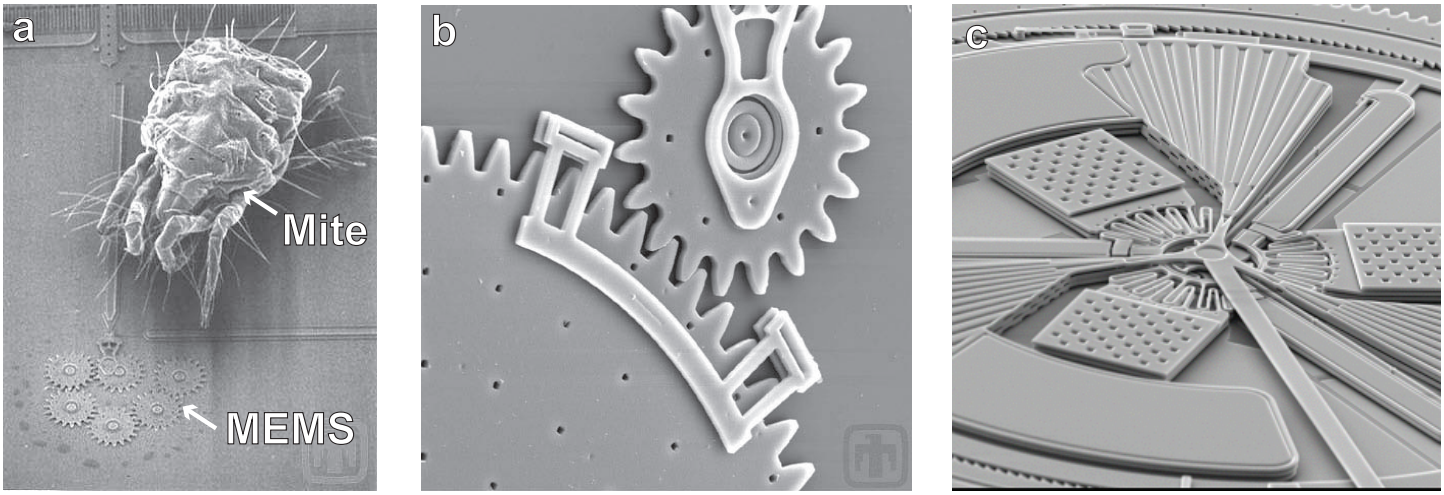

Figure 3. Scanning electron micrographs of MEMS devices. **a)** A MEMS gear chain approached by a dust mite. Note the relative size of gears and mite. **b)** Optical shutter and drive gear. **c)** A MEMS thermal actuator. Pictures and information available at http://www.sandia.gov/mstc/mems_info/movie_gallery.html and http://www.memx.com/image_gallery.htm.

Microfluidics

Within the last decade, the use of microfluidic devices, ranging from single etched channel structures in glass substrates to complex multilayer fluidic networks, for the manipulation of biomolecules has grown exponentially. The popularity of this field can be attributed to its interesting physics & engineering and its versatility for adding value in a wide range of applications. From a biological perspective, recent advances in genomics[1, 2] and proteomics[3] have generated a need for cheaper high-throughput screening[4] platforms. However, the successful design of such platforms requires some knowledge in a variety of disciplines, ranging from engineering (design and manufacture of the microfluidic device) to materials science (selecting the appropriate substrate for device fabrication) to fluid mechanics for the optimization of the flow and/or mixing conditions. With more microfluidic platforms being developed as collaborative projects between laboratories as

well as through industrial partnerships, devices with highly advanced functionalities are being produced for applications such as protein crystallization[5] and tissue engineering that incorporates blood vessels[6].

The development of some of the earliest bio-inspired microfluidic devices in the last decade originated as an extension of MEMS-based technology for analytical chemistry applications. The miniaturized 'total chemical analysis system' (μTAS) proposed by Manz and Widmer in 1990[7], incorporating automated flow injection, chromatography, and electrophoresis, provided the foundation for the chip-based analysis of biomolecules. Using photolithography and established chemical etching techniques, the first microfluidic devices, consisting of a network of micron-scale channels etched in planar glass substrates, created an enabling platform for the manipulation and characterization of small biomolecules at a fraction of the cost of conventional automated systems.

Microfluidic devices for biological and chemical applications have been fabricated from a wide range of substrates, including glass, silicon, hard polymers (polystyrene, polyvinylchloride, polymethylmethacrylate (PMMA) and elastomeric[*] materials (silicone rubber, polyurethane). Although some of the earliest microfluidic devices were manufactured from silicon and glass using standard MEMS technology[8-10], there has been a growing trend in the use of less expensive polymeric materials for microfluidic devices[11]. While devices made from silicon and glass are typically chemically etched or micro-machined in a time-consuming process, techniques such as embossing, casting and injection molding can be applied to rapidly prototype devices from hard and elastomeric polymers. Currently, a silicone rubber known as polydimethylsiloxane (PDMS) is the most popular material for rapid prototyping of microfluidic devices in research laboratories due to its ease of use in the laboratory, low cost, and properties that are suitable for a wide range of applications. In contrast, hard polymers are usually preferred for commercial device development due to their robustness and amenability for large scale manufacture by processes such as hot embossing and injection molding.

Broadly, the technique of replica molding of micro- and nanostructures by casting elastomeric materials, including but not limited to silicone rubber, polyurethanes, and isoprenes, against patterned substrates is known

[*] Elastomers are polymers in which the polymer chains are sparsely cross-linked and are relatively free to move. It results in properties such as viscoelasticity, low Young's modulus, and high yield strain.

as *Soft Lithography*. In a broad sense, Soft lithography refers to a set of methods for fabricating or replicating structures using "elastomeric stamps, molds, and conformable photomasks". We will explore the use of soft lithography to engineer surfaces in Module 3. It is called "soft" because it uses soft elastomeric materials. Soft lithography can be used to replicate and construct features measured down to the nanometer scale[12]. The technique was first introduced by the laboratory of Professor George Whitesides[12] and its popularity grew with its adaptation in many laboratories of microfluidics researchers.

Soft lithography has some unique advantages over other forms of lithography, such as photolithography and electron beam lithography[12-14]. Its benefits include being

- substantially less costly than traditional photolithography,
- well-suited for applications in biotechnology,
- well-suited for applications in plastic electronics,
- uniquely suited for applications involving large or non-planar (non-flat) surfaces,
- compatible with a larger choice of pattern-transferring methods than traditional lithography techniques (more "ink" options),
- free of the need of a photo-reactive surface to create a nanostructure, and
- capable of enabling the creation of structures with smaller details than photolithography in laboratory settings (~30 nm vs. ~100 nm).

You will learn a great deal more about microfluidics and soft lithography in the remainder of the course. You will become familiar with two methods commonly used in making microfluidic structures. The first approach, which will be explored in the Lab #1, is to create a pattern from a very thick UV-sensitive material such as photosensitive epoxy, which can be poured or spin-cast onto a support structure like a glass slide, and is selectively hardened to form the final device structure after washing away uncured parts. The second common approach, which we will explore in the Lab #2, is replica molding. The inverse replicas are typically made by casting elastomer such as PDMS (polydimethylsiloxane) or polyurethane over the master pattern, curing it, and

peeling it off of the master pattern. The inverse replicas may then be sealed to glass substrates or to other replica layers in order to form more complex final structures, which has a network of sealed flow paths or multiple layers of flow paths on top of each other. As an introduction to replica molding, we will become familiar with molding with PDMS and will be making structures that will later be visualized in a scanning electron microscope (SEM) and an atomic force microscope (AFM) in Labs #7 to #11.

Lab Report Considerations – Please read this carefully!!!

Prepare your lab report according to the Lab Write-up Questions listed at the end of each lab. Please only answer the questions; it is not necessary to provide background, methods, etc. unless specified in the questions. If you have made interesting observations or insightful comments, you can include them in the lab report. The lab reports should be clear, concise and presentable. This means, that you will present data in informative, well-formatted, easily accessible tables and graphs where appropriate. When there are multiple images or graphs to be shown to convey a specific idea, observation, or result, arrange them in labeled panels (e.g. a, b, c, d) of a single figure (for an example, see Fig. 3 in this document). Write clear captions and include scale bars when showing an image. Points will be based on technical accuracy, clarity, and presentation; critical observations/insights or work of high quality may be given bonus points. Points will not be awarded for guesswork without any justification, or for writing a long report of poor quality.

Lab #1 – Benchtop Photolithography and Millifluidics

I. Laboratory Objectives

In this section of the module, we will:

1) **Fabricate** simple millifluidic channels using UV-curable Loctite (a brand of adhesives) and a benchtop photolithography process. The resulting millifluidic system will laminate two flows together to enable observation of the extent of diffusive mixing.
2) **Assemble** the single-layer millifluidic laminar flow devices by sealing a cap plate to the device, and making the appropriate fluid connections.
3) **Test** the fabricated devices by flowing two different fluids through the device, one through each side of the flow channel, to visualize flow patterns and diffusive mixing at the millifluidic scale.
4) **Explore** different flow channel geometries to see if they affect the mixing results.

II. Background

Soft lithography represents a significant advance in how quickly microfluidic devices may be made, and in the level of resources that is required to make them. However, conventional soft lithography still requires a certain amount of microfabrication equipment to make the master molds or final SU-8 structures in the first place. More recently, it has been demonstrated that millifluidic devices (like microfluidics, but with less precise, often millimeter-scale features) and master patterns may be made by only using equipment that is available on the benchtop[15]. Instead of using conventional photolithography and microfabrication tools to define the patterns, the desired structures may be defined using transparencies as photomasks, a handheld UV light source instead of a conventional photolithographic exposure tool, and a UV-curable Loctite epoxy as the photosensitive material. The fabrication process itself illustrates the basic principle of photolithography that is used to make high-end integrated circuits and MEMS devices. The patterned Loctite may be used to form the final device (as in this lab), or it may be used as a master pattern off of which one can mold the final pattern. Although the devices that you will create today are millifluidic devices rather than microfluidic devices, they

still demonstrate several of the defining characteristics of microfluidic devices. You will examine some of these characteristics in this lab.

Characteristics of fluid flows on the small scale

One key characteristic of flow through small-scale systems is the relative importance of inertial and viscous effects in the fluid that flows through the device. Viscosity is a material property of fluids, and it is a measure of a fluid's resistance to being deformed by a stress. For example, water has a lower viscosity (about 10^{-3} Pa-s) than cooking oils (of order 10^{-1} Pa-s); water is "thinner" and flows more readily. Inertia, of course, describes the extent to which a fluid tends to continue going in the direction in which it was previously flowing; faster flows have behavior that is more strongly affected by their inertia. In microscale (and to a lesser extent, in milliscale) systems, fluid mechanics is strongly influenced by scaling laws. Viscous effects become relatively more significant, and inertial forces become less significant. The relative importance of inertia and viscosity is captured in a dimensionless number called the Reynolds number. The Reynolds number, *Re,* is defined as $Re = d\rho u/\mu$, where d is the hydraulic diameter (essentially the characteristic dimension of the channel through which the fluid flows), ρ is the density of the fluid, u is the flow velocity, and μ is the viscosity of the fluid. The hydraulic diameter, d, represents the actual diameter for a pipe with circular cross-section, and may be approximated as $d = 4A/p$ for noncircular cross-sections, where A is the cross sectional area of the pipe and p is its wetted perimeter. Note that the velocity is in the numerator and the viscosity is in the denominator; higher values of the Reynolds number indicate that inertial effects are becoming more important, while lower values indicate that viscous effects are becoming more important. For flow in pipes, values of *Re* above about 2300 correspond to turbulent flow, while values below that level indicate laminar flow. In microfluidics, the flow is almost always laminar because the hydraulic diameter is so small. In turbulent flow, material randomly moves from one part of the pipe or channel to another. This random advection (*Recall*: advection is transport of thermal energy, solutes, etc. *via* motion of fluid) rapidly mixes the fluid. In the absence of turbulence, fluid moves only along the pipe or channel and there is little or no motion across the pipe cross-section.

In the absence of turbulence, the dominant mechanism by which fluids and solutes mix at the microscale is typically diffusion (with a few, very carefully engineered exceptions). Diffusion is the process by which a chemical species moves from a region of higher concentration to a region of lower concentration due to the underlying random motion of the molecules or particles of the chemical species. If you start with a slab of liquid that contains a given species, the characteristic length scale over which that species will diffuse in a period of time t is given by $\sqrt{Dt}$, where D is the diffusivity of the species. The diffusivity of molecules/ions in water ranges from 10^{-9} m^2/s for ions to 10^{-11} m^2/s for macromolecules such as proteins. For systems in which two liquids (or two different solutes in the liquid) have the opportunity to mix while flowing through the system, the diffusion length (and hence the extent of mixing) may be determined by the transit time of the liquids through the system.

The devices that we will microfabricate today laminate two flows together to observe the extent of mixing. The mixers have two inlet ports at one end and two outlet ports at the other end, as shown in Figure 3. Between the inlet and the outlet ends of the device is a flow channel through which two flows injected at one end can flow together to the other end, at which point they are once again separated into separate exits. We will have the opportunity to examine diffusion and advection in the context of these devices during this lab.

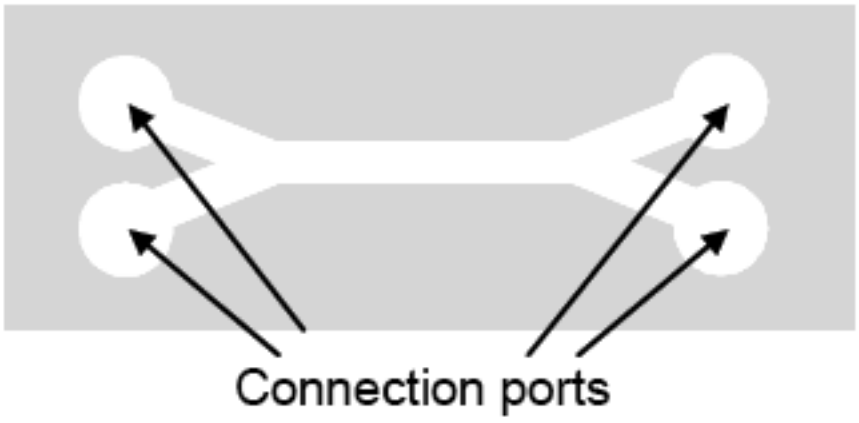

Figure 4. Schematic diagram of a mask pattern, showing the locations of the four connection ports.

III. Experimental

Preparation: Each station should have the following materials:

- One tube of UV-curable Loctite (Loctite 3108)
- Glass slides
- Transparency masks
- Scotch tape
- Blank transparencies
- Plastic slides to turn into spacers
- Plastic slides with holes drilled
- Paper plate work surface
- Nitrile gloves (choose the appropriate size from the available boxes)
- Tygon tubing
- Luer stubs with Luer lock adapters
- Syringes
- Plastic cups
- Blue and yellow food coloring

A) *Fabrication of the Flow Channels by Benchtop Photolithography*

Loctite 3108 is UV-curable; it hardens when exposed to UV light. The ambient UV light is enough that the Loctite must be stored in an opaque package, but is little enough that we don't have to worry about any unintentional curing while we work with it in the lab. Because Loctite 3108 hardens (cross-links) when exposed to UV light, we can use it as a *negative resist*. The exposed parts will remain permanently on the substrate surface, while the unexposed parts do not harden and are readily removed (the opposite happens in *positive resist*). We will use the UV-hardened Loctite to form the walls of our flow channels.

Step 1: Prepare the Loctite resist on the substrate

In conventional microfabrication, photoresist and photocurable epoxies such as SU-8 are dispensed in a layer with even thickness using a spinner. The spinner rotates the substrate after the photoresist is dispensed (and sometimes even while it is dispensed). The spin speed and the viscosity of the resist determine the ultimate layer thickness. Instead of using a spinner, our benchtop process uses mechanical stops to ensure that the resist layer (and hence the flow channels) have (about) the desired height.

Put on your nitrile gloves. You will keep them on for the rest of the chemical processing in the lab. Place a glass slide on the paper plate, and cut out a small plastic spacer to put over each end of the slide (**Careful!!!** Make sure that no body part is in the way of the cutting blade in case you slip while cutting). Using the calipers or micrometer provided, measure and **record** the thickness of the plastic spacer. A good measurement consists of measuring the thickness multiple times to get a good idea of the average thickness and its standard deviation. Then place the spacers over the far ends of the glass slide. Dispense a line of Loctite down the center of the glass slide. Cut out and place a piece of blank transparency on top of the line of Loctite. Place a glass slide on top of the blank transparency, and press down on the glass slide over the spacers in order to ensure uniform spacing between the transparency and the underlying glass slide. (If you press in the middle, the glass slide will bow and ruin your layer thickness.) Once the glass slide has been pushed down as far as it goes, remove the upper glass slide, flip the whole assembly (Wei's flip) and place the transparency mask in place. Now your resist is prepared.

Step 2: Expose and develop the Loctite resist

To expose your Loctite, you will need a photolithographic mask. For this lab, we will use transparency masks. Transparency masks are usually printed on special printers with very dark, space filling ink; this ensures that no light can leak through the black part. For our rather crude purposes, we will use several layers of regular transparency. Cut out two units of the mask pattern from the provided transparencies, carefully align one pattern over the other, and affix them in place with tape, carefully ensuring that the resulting transparency mask does not curl up. Look through the resulting sandwich at the light, and note how much darker it gets. If you

really, really want to prevent light leakage through the dark regions, you can repeat this exercise and add a third layer of transparency.

Carefully transfer the glass slide-Loctite-transparency sandwich and your transparency mask to the UV exposure station in the lab (Caution! UV light is hazardous). (Hint: it helps to stick something like a piece of transparency or a slide under the entire sandwich so you don't make a mess of your resist.) The station consists of a UV light and an acrylic sheet. The UV light exposes the Loctite to cure it, and the transparency mask ensures that only the desired regions of the Loctite are exposed. The acrylic sheet goes underneath the sample; because acrylic absorbs UV, it ensures that the light doesn't bounce off the table and expose the sample a second time from below. Place the sample on the acrylic sheet, position the transparency mask on top of the Loctite, and position the UV light over the sample. Turn on the UV light, and expose the sample for about 35 s (ask your instructors regarding the exposure time to use). Switch off the UV light, remove the sample, and use a paper towel to wipe off any Loctite that may have leaked onto the acrylic sheet.

Back at your workstation, peel (slowly and carefully) the upper transparency off the glass slide. (The transparency with Loctite gunk on it goes into the solid hazardous waste bin.) Can you see the exposed pattern? Still wearing your gloves, take your exposed glass slide to the fume hood, where there is a squeeze bottle of acetone, a funnel, and a waste bottle to collect the acetone waste. Open the waste bottle, place the funnel in the opening, and, working directly over the funnel, squirt acetone from the squeeze bottle onto your exposed pattern in order to develop away the unexposed Loctite (**Careful!!!** Don't squeeze the bottle too hard to ensure the acetone does not splash. Make sure that your goggles are properly protecting your eyes). When the pattern is cleared, close up the waste bottle and proceed to the sink to rinse off your sample. It should still be tacky. Dry it off with the air gun and inspect it. How did it come out? If it didn't come out right, you can try defining the pattern again with different experimental parameters. What changes do you think might help? Finally, using the plastic rulers, measure and **record** the dimensions of your mask and Loctite patterns.

The following details need to be recorded in your lab notes for answering the lab report questions.

- Record your exposure time, the thickness of the plastic spacers, and the dimensions of the mask and as-fabricated patterns.

- Sketch the pattern as defined on the transparency and as it appears in the hardened Loctite, including key dimensions (i.e. feature widths). **Measure and record the width of the channel on the mask and in the Loctite at three different locations.**

B) *Assembling the Millifluidic Device*

In this part of the lab, you will first create an upper cap plate with flow connections and then cap your device with it.

Your device will be capped with a plastic slide with four holes pre-drilled in it such that they align with the ports of the device. First, you must connect the fluid connectors (Luer adapters) to the plastic slide with Loctite epoxy and cure them in place. You can find your own best way to do this, but here is a suggestion: First, draw a circle around each hole in the plastic plate with a lab marker; this will help you align your Luer adapters to the holes. Place two Luer adapters over the holes on one end of the plastic slide, and run a bead of Loctite around each adapter. (This can be a little tricky; you can try using a piece of blank transparency as a spreading tool in order to get a nice bead of Loctite around each adapter.) Repeat the process with the other end of the plastic slide, so that you have connectors for all ports. Once you have the adapters glued in place, harden the Loctite by exposing it to the UV light for several minutes. (Optionally, you can add more Locktite around the Luer adapters and cure additionally 5-10 minutes to reinforce the contact.)

Now you are ready to assemble your cap plate onto the device. If you didn't expose your Loctite for too long, it will still be rather tacky. Aligning carefully, press the cap plate onto the flow channels, pressing firmly around the edges to ensure that it sticks well. Finally, place the entire device back under the UV light and cure it for about 5 minutes to harden the Loctite (it requires 15-20 min to fully cure, but we found 5 min is sufficient for our experiments). Now you have a millifluidic channel!

C. Testing

In this part of the lab, you will connect your device to the test apparatus and use the apparatus to flow two different fluids through the two different halves of the channel to observe what happens.

Step 1: Set up the test apparatus

First, mix up yellow and red colored water in plastic cups, using water and the food coloring provided. Load one syringe with each liquid; try to get them filled to the same level, as this will ultimately make it easier to dispense the two liquids at the same rate. Attach Luer adapters onto the connection ports of the two syringes. Connect the Luer stubs in the inlet ports to the syringes with a length of Tygon tubing. Connect two more lengths of Tygon tubing to the outlet ports, and route those tubes into a plastic cup to catch the effluent.

Step 2: Flow liquids and observe output

With one person timing the flow's travel through the main channel, *gently* depress the two syringes in order to obtain simultaneous flows of both yellow and blue liquids. **Record** the time required for the flows to traverse the length of the channel. Observe and sketch/photograph the patterns of yellow, red, and/or orange.

D. Exploration (with your own channel geometry)

At this point, you probably have some theories on what might affect the results of this experiment. Different mask patterns are available for you to try different flow geometries; you can also draw your own suggested mask pattern on a blank piece of transparency with a black magic marker and try that. (If you're extra careful, you can usually get the black regions black enough to keep enough light out.) You can even think of making more complex channels by patterning both the top and bottom slides. Make sure that your entry and exit ports align with the holes on the plastic slides. Try the experiment with the new pattern. What do you see in terms of mixing behavior? Can you get the two streams to mix in your device?

Lab #1 write up questions:

1. Pattern transfer (*3 points*):

   a) We suggest you flip the glass/Loctite/transparency assembly before the UV exposure through the mask (this is called Wei's flip). Flipping the whole assembly (mask-glass-Locktite-transparency) helps to better separate the transparency from the hardened Loctite while maintaining adhesion to the glass slide. Can you explain why this flipping helps? (Hint: Look up Beer-Lambert law, and see how the UV exposure at the Loctite/transparency interface compares to that at the Loctite/glass interface)

b) What is the measured width of the millifluidic channel on the mask and the actual width that was obtained in the Loctitie pattern (mean ± std. dev.)? Briefly explain one reason that you think could have caused the actual width of your channel to be different from that on the mask.

2. Flow behavior (*2 points*):

   a) What is the (approximate) Reynolds number of the flow in the channel of your device? List the values of all parameters and measurements that you used and show your calculations.

   b) Estimate the flow rate required to obtain turbulent flow in the millifluidic device. By how many orders of magnitude is this flow rate higher than the actual flow rate in your experiment?

3. Mixing behavior (*3 points*):

   Since we don't get turbulence at the feasible speed of flow, we can assume diffusion is the only mechanism to mix two color streams in this device. To get a handle on mixing, we can write:

$$\frac{\textit{timescale for diffusion across channel width}}{\textit{timescale for advection along channel length}} \sim \frac{W^2/D}{L/U} = \frac{LU}{D}\left(\frac{W}{L}\right)^2 = Pe\left(\frac{W}{L}\right)^2$$

   Here, W is channel width, L is channel length, U is flow velocity, D is diffusivity of the species under consideration, and Pe is a dimensionless number called the Péclet number, which compares diffusion to advection (when $W \approx L$). For mixing to occur, the diffusive and advective timescales should be comparable, *i.e.* the above ratio should be on the order of 1. For short channels, the advective timescale, L/U, is smaller than diffusive timescale, W^2/D, so the fluid exits the channel before diffusion has a chance to mix the species. If the millifluidic channel could be made longer, estimate the required length of the channel for the two color streams to diffuse (mix) completely across the channel before exiting, while keeping U and W the same as in your experiment.

4. Design for mixing (*2 points*):

   The design objective for the second geometry (your own design of the mask pattern) is to mix the two color streams before they exit the device.

   a) Explain the design rationale of your second geometry.

   b) If you have achieved mixing, present a photo of your test device. If not, suggest what technique could be used to improve mixing.

Lab #2 – Introduction to PDMS Rubber Molding

I. Laboratory Objectives

In this laboratory, we will become familiar with the processing of (poly)dimethylsiloxane (PDMS, a kind of silicone rubber) for its application to the replica molding of nano and microstructures. We will:

1) **Develop** protocols for the preparation of the two-part PDMS rubber (Dow Corning Sylgard 184)
2) **Explore** the processing parameters and materials properties integral to PDMS molding
3) **Fabricate** structures from substrates patterned in nature with nanoscale features (leaves) and in everyday objects (a CD), as well as micron scale patterns from silicon molds. We will save these replica molds for making microfluidic devices, microcontact printing, and characterization by Scanning Electron Microscopy and Atomic Force Microscopy in later Labs.

II. Background

Technologies such as injection molding and embossing are useful for large-scale fabrication of a variety of plastic components. In the 1990s, when microfluidics was still an emerging field, silicon microfabrication techniques were adapted to make microfluidic components and devices. However, the expensive equipment required and long processing times were not very compatible with microfluidics: Devices failed easily due to clogging or breaking, and there was a need to make disposable devices to prevent contamination of biological samples. Plus, it was difficult to make valves and pumps in silicon, and it was impermeable to oxygen, which was required for keeping cells and tissues alive. Polydimethylsiloxane (PDMS), introduced by the Whitesides group[16], revolutionized the field of microfluidics by enabling rapid prototyping of the devices, ease of making interconnects, valves, and pumps, transparency, and permeability to oxygen. Today, majority of microfluidics researchers use PDMS for fabrication.

PDMS commonly used for microfluidics comprises two parts, a polymer and a cross-linker (Figure 4) with a small amount of catalyst. The cross-linking can occur at room temperature (takes 1-2 days), and can be accelerated by heating it to a higher temperature. The ratio of the polymer to cross-linker and the curing time/temperature determine the number of cross-links and hence the mechanical properties of PDMS. Due to its

elastomeric nature, making interconnects in PDMS is very easy by simply punching a hole and inserting tubing. It also makes it easy to peel off cast PDMS from a mold. In addition, the polymer is permeable to air, so cells are not starved of oxygen in PDMS devices. In addition to its bulk properties, surface properties of PDMS are very interesting and useful. They enable facile bonding of PDMS to glass, allow for tunable wettability, and can also generate interesting nanoscale features that can be used to make nanofluidic channels.

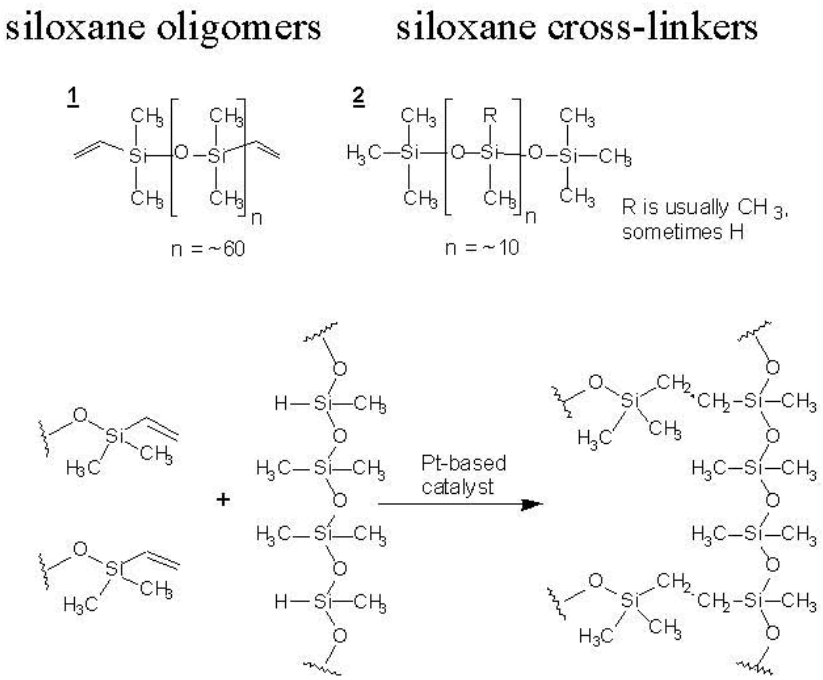

Figure 4. PDMS polymer and cross-linker.

The surface of a material is considered to be different from the bulk material due to interactions between it and the environment. An obvious example is oxide coatings on metals, however, polymers and other materials can undergo surface rearrangements of molecules or atoms as well to accommodate changes in its surrounding environment. The surface region is informally defined by the zone where the structure and composition, influenced by the interface, differs from the bulk composition and structure[17]. The surface region has its own unique reactivity and properties – catalysis (for example, as used in petrochemical processing) capitalizes on special surface reactivity and microfluidics devices must always consider how the substrate surface interacts with solvents or solutes. When a hydrophobic material is exposed to a hydrophilic environment, as is the case with PDMS and water, the interfacial energy is high. In order to reduce the

interfacial energy that builds up when an environment of polar substances (i.e. water or a hydrophilic polymer like acrylamide) come in contact with nonpolar substances (i.e. air or PDMS), components from the material may move toward or away from the surface depending on their polarity and the polarity at the interface. Many materials can undergo a reversal of surface structure when transferred from air into a water environment as occurs with the hydroxylated polymer pHEMA (used in contact lenses), whose surface changes from methyl groups in air to hydroxyl groups in water[18].

The specific characteristics of the surface of a material can be explored through surface analysis techniques such as contact angle measurement, electron, X-ray, and other spectroscopies, atomic force microscopy, scanning tunneling microscopy, ellipsometry, and other techniques. In regards to contact angle measurements, the behavior of a drop of a liquid on a surface gives information about the chemical properties of the surface. For example, waterproofing sprays that cause water droplets to form beads on treated clothing are typically containing hydrophobic fluoropolymers (one example is Teflon). The degree to which a liquid beads on a surface is described by the contact angle. Contact angle is a quantitative way to describe the attraction that a drop of liquid has for the substrate to which it was added and it may be affected by the heterogeneity or roughness of the material surface. In general, the higher the affinity the liquid has for the substrate, the more the liquid is likely to spread, thus reducing the angle formed by the drop at the solid-liquid interface. This spreading phenomenon is known as wettability. Energetically, the contact angle describes the balance between the attractive force that holds the molecules together in the droplet (cohesive force) and the attraction of the liquid molecules for the molecules that make up the substrate (adhesive force). At equilibrium, the energy corresponding to the cohesive and the adhesive forces is at a minimum[17].

The experiments that you will perform today are highly relevant characterization techniques to be used in many areas including processing and manufacturing to bioengineering and drug delivery. It is always necessary to consider the chemistry and texture of an interface as we will see in the following experiments how interactive the surface of a material can be.

III. Experimental

Preparation: Each laboratory group should have the following materials at their bench for this experiment:

- One box two-part cure PDMS elastomer (Dow Corning Sylgard 184)
- One stack of small Petri dishes (60 mm diameter)
- One stack of large Petri dishes (100 mm diameter)
- One 1.5 mL tube of water with food coloring
- One 1.5 mL tube of light mineral oil (aliphatic, linear chain hydrocarbon with ~20 carbon atoms)
- One 1.5 mL tube of tetradecane (aliphatic, linear chain hydrocarbon with 14 carbon atoms)
- Three 1 cc syringes with blue luer stub attachments
- Dry leaves
- Pieces of a CD (compact disc) with the plastic layer peeled off
- Silicon wafers with photoresist patterns

A) Molding with PDMS Rubber

Step 1: Preparation of the PDMS elastomer

Sylgard 184 is a two-part rubber RTV elastomer (A, the base and B, the curing agent). RTV stands for "Room temperature vulcanization", meaning that the rubberization of the elastomer, by monomer cross-linking, starts to occur at room temperature as soon as the two parts (containing defined ratios of monomer and catalyst) are combined in the proper ratio. PDMS is a hydrophobic silicone rubber material much like the formulations that you would use for waterproofing projects such as sealing the cracks around your bathtub. For this experiment, we will use the optimal ratio of part A:B as specified by the manufacturer, 10:1 A:B. We are going to study the importance of mixing/degassing characteristics of PDMS. You will be initially preparing **two** 40 gram samples of PDMS. Take the elastomer kit over to the weighing scale on the benchtop. Next to the weighing scale, there is a stack of disposable cups. Follow the steps below:

a) Mix the two parts of PDMS: 1) Put a cup on the scale; 2) tare the scale to read 0.0 g; 3) Pour ~40 g of part A (large container), followed by ~4 g of part B (small bottle) into the cup. **Record** the actual weights of each part of PDMS for your lab write-up.

b) Put the cup with elastomer into the beige adapter cup for the polymer mixing apparatus. **Record** the mass of the cup/adapter with PDMS.

c) Put the cup/adapter in the Thinky Supermixer. The function of the Supermixer is to mix and degas the two parts of Sylgard 184, leaving you with a mostly gas-free, homogenized mixture for casting on the silicon molds.

d) Adjust the *mass balance* within the apparatus to match the combined mass of the polymer-filled cup/beige adapter (**This step is important; a mass imbalance can damage the mixer**).

e) Prepare the sample using a mix/degas protocol (program 1). To start the mixer, insert the container and adapter, close the door, and select either program 1 using the soft keypad on top of the mixer. **Record** the times for mix and degas for the run. Push start. (If the Thinky mixer is not available, PDMS can be mixed by hand, but a longer vacuum step is needed to remove bubbles).

Step 2: Molding Test Patterns

Next, we will make PDMS casts of several commonly found objects with patterns to examine the fidelity of the molding process. Pour ~1-2 mm deep layers of PDMS over the following objects provided on your bench (marked Step 2):

- Leaves (back side of oak, front side of Lotus) in bottom of Petri dish*
- Section of a CD (compact disc) with tracks exposed

*To get the leaves to adhere to the bottom of the Petri dish, apply a bit of the prepared PDMS to the top side of a leaf to stick it down.

Place samples in the 80° C oven and bake. **Record** the time for which the samples were baked.

Step 3: Molding Microfluidic Devices and Stamps

Now we will cast PDMS into the shapes and geometries needed to make microfluidic channels stamps for microcontact printing. Again, use the same Sylgard PDMS sample from Step 1. Pour ~5 mm deep layers of PDMS over the following molds provided on your bench (marked Step 3):

- Silicon wafer mold 1: Microfluidic devices and stamps
- Silicon wafer mold 2: Microfluidic mixing and electrokinetic separation devices

Place these samples in the vacuum chamber and turn on the vacuum pump to fully de-gas. It is helpful to occasionally release the vacuum to burst the bubbles. While waiting for samples to de-gas and bake, let us examine PDMS castings that have been previously prepared by the instructors and teaching assistants.

B) Surface chemistry properties of PDMS

The PDMS elastomer is intrinsically hydrophobic, so we would expect it to repel water. In this section of the laboratory, we will look at the interaction of PDMS with different solvents, as the wettability of PDMS has important consequences in its ultimate use as a substrate for microfluidic devices. To begin, cut the PDMS sample provided to you into two pieces and place each part in a separate large Petri dish. Label one dish as *untreated* and set it aside. Take the second part to the air plasma machine. Place the sample on a provided glass slide, close the plasma bonder door, start the pump to evacuate the chamber down to a pressure of 600-700 mTorr, and expose to plasma for 45 s.

Take a 1 cc syringe with a blue luer stub adapter on it (on your benchtop), draw up ~0.2 cc of the water dye solution and eject a small drop (~1 mm diameter) on the top surface of your air plasma-treated and untreated PDMS parts. Repeat the process with the mineral oil solution, placing the water and oil droplets side-by-side. **Sketch/photograph** the droplet shapes to estimate the contact angles. Next, place two more droplets of each type on untreated PDMS, and two more on plasma-treated PDMS and **sketch/photograph** the droplets.

The air plasma treatment alters the surface chemistry of the PDMS by oxidizing the methyl groups and replacing them with silanol (Si-OH) groups on the areas exposed to plasma. The –OH groups are polar and can

form hydrogen bonds with water, making the surface hydrophilic. While this effect is temporary (lasting only a few hours), it is anticipated that it will affect the wettability of the PDMS.

Now, let's think for a minute about what makes a material elastic, like the PDMS rubber that we are playing with in this lab. From a molecular perspective, we can think of cured PDMS as a mass of thousands of crosslinked strings with a repeating backbone of silicon and oxygen (Figure 4), with lots of air spaces in between the strings. The degree of elasticity of PDMS is based on its ability to return back to its original shape when stretched. So, as we deform a material like PDMS elastically, the individual strands stretch out, compressing the material. When we remove the deformation load, the material snaps back and the nanoscale air spaces return, just like decompressing a sponge. Now, there are several ways we can alter the material properties an elastomer like PDMS. One obvious way is stretching it too far, past its ultimate tensile strength, which would cause it to snap. A second way, which may not seem obvious, is altering the "sponginess" of the bulk elastomer using solvents. Solvents that soak into elastomers like PDMS displace the nanoscale air pockets, altering the stretchiness of the material.

We have established that PDMS is intrinsically hydrophobic. Would you expect PDMS to soak up water? What about oil? To investigate, let's put a few more droplets on top of another piece of PDMS (not treated with plasma). Using the provided syringes, place ~1 mm droplets of water, mineral oil, and tetradecane on top of a device. Let it sit for 3 minutes. Wipe off droplets and look at the surface of the PDMS under reflected light.

A) Molding with PDMS Rubber

Step 4: Remove Molds from Oven and Vacuum Chamber

At this time, you should be able to remove the leaf and CD molds from the oven. Place the de-gassed microfluidic devices and stamps into the oven at 80° C and bake till cured. **Record** the time for which the molds were baked.

Step 5: Examination of The Molded PDMS

You will now examine the leaf and CD molds under the microscope. **Acquire** images of the leaf and CD topography that you molded. Next, peel of pieces of the PDMS from the leaf and CD to examine the leaf and CD molded PDMS. Note the features that you can see. **Acquire** images of the leaf and CD molded PDMS at a low/moderate and high magnification.

Step 6: Removal Of The Cured PDMS From The Silicon Molds:

- To separate the cured elastomer from the master mold, use a clean razor blade from the box on the benchtop. Using the razor blade (**Caution!**) first cut the cured PDMS elastomer around the devices. Don't worry about being precise. The important thing is to **avoid cutting or even touching the molded pattern structures with the razor blade**. You may find it useful to hold the razor blade at an angle and observe its reflection in the silicon wafer to make sure it touches the wafer. Then, **pull** the razor blade, making sure not to push down on the silicon. **Be careful not to apply excessive pressure since the silicon mold is rather brittle and breaks easily.** (And it takes a few hours for your TA to make it, so the result will be a not-so-happy TA!)
- Once you make the cut around the devices, gently peel the cured elastomer from the mold. You can use the tweezers provided to lift up one corner, and then use your hands to peel the device. **Make sure you do not touch the device features on the mold, since it will cause damage to the mold. It is also very important that you avoid touching the device area of the peeled elastomer, and not place it in contact with any surface, since a dirty device will inevitably leak. Always place the device side up.**
- Store the PDMS in a petri dish and label it with your names for later retrieval.

C) Examination of Cured PDMS samples

The instructors have cured PDMS in petri dishes under identical conditions, except that a cover was placed on the PDMS in one of the dishes before curing. In addition, some parts of the petri dishes were scoured with a

razor blade prior to pouring PDMS. Examine the cured PDMS samples made by the instructors and teaching assistants. Hold them up to the light. Note the presence/absence of gas bubbles in the four samples, writing down your observations. Answer the questions in the lab report.

Lab #2 Write-up Questions:

1. Effect of oxygen plasma treatment (*3 points*):

   a) Estimate the contact angles for water and oil droplets on untreated PDMS, and on PDMS after oxygen plasma treatment. You can also present representative photographs/sketches of the droplets.

   b) Based on your experiments, can you deduce whether plasma treatment makes the PDMS more hydrophilic or hydrophobic?

   c) Polymers can display complex behaviors. In case of PDMS, the polymer chains can 'move around'. Parts of polymer chains that are at the surface can get buried while chains that were buried can get exposed to the surface. If you leave your plasma treated PDMS untested for a day, will the contact angle for water remain the same as right after treatment? If not, will it increase or decrease? Explain why.

2. Swelling of PDMS (*2 points*):

   a) Do you see any swelling on the PDMS surface? If so, for which liquids?

   b) From your observations, rank the liquids from highest to lowest in terms of (favorable/attractive) interaction with PDMS.

   (FYI: There are extensive theories dealing with polymer-solvent interactions. A classic theory is the Flory-Huggins theory that describes the free energy change associated with mixing of a polymer into a solvent in terms of the enthalpy of interaction between solvent and polymer described in terms of a χ (chi) parameter, and the entropy change accompanying dissolution. The situation in PDMS is more complex because the polymer chains are cross-linked in a few places and are not completely free to move).

3. Molding of microstructures (*3 points*):
   a) Present photographs of the leaf and the CD, and of the PDMS molded using leaf and CD under low and high magnifications that you obtained during class. Please include a scale bar on each photograph.

4. Examining cured PDMS samples (*2 points*):

   a) Do you notice anything different at places where the plastic surface was scoured? Form a hypothesis to explain your observation.

   b) Do you notice a difference in occurrence of bubbles between the samples that were covered and those that were not? Form a hypothesis to explain this behavior.

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