

7. Remove the wafer from the H<sub>2</sub>O, and rinse with acetone, isopropanol, and blow dry with filtered N<sub>2</sub>.

Note: After all wafers have been processed, remove the Piranha mixture from heat, and allow the mixture to cool to room temperature. Once the mixture is cooled, it can be discarded in acid waste. However, do not tightly close the acid waste container for 24-48 hours after adding Piranha. Residual reactions can still occur, which would lead to production of dangerous pressure in a sealed container.

## Soft Lithography

Microfluidic devices are constructed from a translucent, gas-permeable polymer and bound to a glass coverslip, which allows for observation using fluorescence microscopy. Polydimethylsiloxane (PDMS) is gas permeable, and has the unique property of having the same optical properties as water, which simplifies imaging of aqueous systems(Ferry et al., 2011).

### Mixing PDMS

1. Check wafer. If dirty, rinse with isopropanol (NO ACETONE) and blow dry with filtered N<sub>2</sub>.
2. Wrap wafer in a single layer of aluminum foil. Ensure a vertical rim of ~1" around the entire wafer. Wrap wafer so that when you pour PDMS, none of it leaks and collects between the wafer and the foil.
3. Add 3.5g of catalyst to 35g of PDMS base (1:10 ratio) in a weigh boat and mix thoroughly for 5 minutes. The mixture should go from clear to cloudy (due to the incorporation of air bubbles into the mixture).
4. Place the weigh boat in the desiccator and apply vacuum. The bubbles in the PDMS mixture should enlarge. Remove the vacuum to pop the bubbles. Repeat application and removal of vacuum until all bubbles are gone (it usually takes 20-25 mins to remove all the bubbles; if the bubbles are removed quickly then it may be a sign of the base being too old).
5. Pour mixed PDMS onto the foil-wrapped wafer. Place foil-wrapped wafer in desiccator.
6. Repeat cycles of vacuum application and removal to eliminate any bubbles in the PDMS.
7. Place wafer at 80°C for 2-12 hours.

Note: Overnight baking is beneficial during the spring, summer and fall, as the increased humidity negatively affects binding. The baking time can be decreased to 2 hours during the drier winter months.

### Preparing Monolith

1. Remove wafer from 80°C oven. Let cool to room temperature.
2. Remove aluminum foil from bottom of the wafer and then slowly lift monolith from wafer. The best way to remove the monolith is to lift to the middle, then move around the wafer and lift. Repeat until the monolith is freed from the wafer. Return wafer to storage.
3. Trim circular edges from the monolith using a sharp blade; do not drag the blade along the monolith surface, instead make single deep cuts into the monolith.
4. Cover both large sides of the monolith with parafilm.
5. Cut monolith into quarters.
6. Punch ports in each quarter with biopsy punch - either using the microscope after removing parafilm or you can look at the ports through the parafilm and punch without the microscope. Punch with the FEATURE SIDE up (punch through the feature side, not the other side).

7. Clean punched quarters with tape and then wrap punched quarters in parafilm.
8. Dice individual chips.
9. Completely submerge chips in methanol in a crystallization dish.
10. Sonicate in methanol for 480 seconds (8 minutes)
11. Discard methanol and add new methanol
12. Sonicate again in methanol for 480 seconds (8 minutes)
13. Place chips at 80°C for 30 minutes

## **Binding**

1. Turn on O<sub>2</sub> to 0.4-0.5 scfm
2. Turn on UVO cleaner. Run UVO cleaner for 5 minutes to warm up.
3. Clean 24x40mm #1.5 coverslips by rinsing with isopropanol and blowing dry with N<sub>2</sub>.
4. Clean chips with scotch tape. Briefly: place tape on feature side of chip. Using finger nail, apply gentle pressure to features. Remove tape. Repeat twice more (3x total). Clean the other side with tape also
5. Place 2-4 chips (feature side up) and cover slips in UVO cleaner; place chips and cover slips in the middle section of the UVO cleaner in between the screws. Run for 3 minutes
6. Once done in UVO cleaner, quickly invert chips onto cover slips. Place them in the 80°C oven top shelf overnight.