# The mother machine handbook

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## Videos:

**http://www.youtube.com/user/JunLabHarvard?feature=mhum**

### 1. *E. coli* growing in the mother machine:

http://www.youtube.com/watch?v=5PyoO4S3BU0

### 2. Video guide for ‘Making Mother Machines’:

http://www.youtube.com/watch?v=RGfb9XU5Oow

## Reference:

P. Wang*, L. Robert*, J. Pelletier, W. Dang, F. Taddei, A. Wright, S. Jun.
*Robust growth of Escherichia coli*. Current Biology 20, 1099-1103 (2010).

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# 1. Building the Mother Machine Wafer in Cleanroom

**Brief history**

We used to travel to CNF at Cornell to make a fancy silicon/quartz version of what is now known as ***the mother machine***. For many months, however, there was always something wrong with every silicon/quartz device we made; trial-and-error is inevitable in development. One day, Wei Lien Dang, a very bright former student in the lab suggested to use SU-8 to build his "micropiston." The micropiston was the device we wanted to build and use to isolate, confine and manipulate the chromosomes from *E. coli*. Although not as precise as dry etching, SU-8 was indeed much simpler and cheaper to work with, and it became immediately clear that SU-8 can be used to build the micropiston consisting of dual layers with feature sizes as small as 1 um. The first generation of the micropiston we made at CNF served its purpose very well. We even started to grow cells in the micropiston, aka the "mother machine" (they are the two different names of the same device).

In 2009, we completely moved to CNS at Harvard. This revised cleanroom protocol is optimized for the equipments at LISE at Harvard, but both the new as well as experienced users will have to go through a period of trial-and-error to make a good wafer. The procedure in this protocol is general enough so that it can be used with little change for other cleanrooms.

Former members of the lab should be acknowledged for their significant contributions to building the micropiston/mothermachine. In particular, Wei Lien Dang (grad student) was brave enough to explore the SU-8 version of the device; Wei and I learned a lot about general fabrication process from Peter Galajda (post-doc) when we knew nothing about microfluidics; I did not expect much when Ping Wang (post-doc) followed me to CNS, but Ping soon became a master mother-machine builder.

**Lessons learned**

1. *The simpler the better*. Don't try to make your microfluidic device fancy.
2. *Length scales matter*. Devices with ~1um feature size will take a longer to make than the ones with ~ 5um. Above 5um is fairly straightforward.
3. *Be brave*. Even if you know nothing (e.g., you are a theoretical physicist), you can make similar devices starting from scratch as long as you are determined and resourceful. It can take from weeks to months. Usually you will find nice folks in the cleanroom who are happy to help.
4. *Be honest with yourself*. Congratulations – you have made your first device! But remember that building the device is only the beginning. It will take much, much longer to do the experiments properly using the mother machine or its variations, although you may be tempted to publish something quick and dirty without doing proper controls. If the cells grow healthy in the mother machine, the quality of data should be what we reported in the following paper (reprint can be requested to Suckjoon Jun, or just Google around):

   P. Wang*, L. Robert*, J. Pelletier, W. Dang, F. Taddei, A. Wright, S. Jun. *Robust growth of Escherichia coli*. Current Biology 20, 1099-1103 (2010).

### Summary

The SU-8 mother machine mold is made in the following three main steps: (1) Metal deposition to print the alignment marks on the Si wafer. (2) Building the growth channels. (3) Building the main trench channels. Each step requires a specific mask, and L-EDIT file of the mask design can be obtained from us (see above).

### Preparation

1. Clean Si wafer with Piranha solution (conditionally essential depending on the situation)
2. Bake at 150 C for 10 minutes (this is to dehydrate the substrate)

### Printing the alignment marks

Part I: spincoat the wafer with photoresist and expose to prepare the alignment marks for metal deposition.

Spin-coater setting: (ramp) 100rpm/s → (spin) 500 rpm for 5 seconds → (ramp) 300rpm/s → (spin) 3000 rpm for 30 seconds.

3. Spin MPHP or P20 primer to enhance adhesion; let it sit for 10sec and then spin it off.
4. Spin S1813 (1.3 $\mu$m - 1.5 $\mu$m) photoresist at 3000 rpm for 30 seconds.
5. Soft bake at 115C for 2 minutes (this is to dehydrate the substrate).
6. Use the alignment-mark mask and expose wafer for 4.3 seconds at H-line power = 23.4 mW/cm2. (Note: the UV line power changes over time. Check the up-to-date information posted in the cleanroom and adjust the exposure time to match the dosage. The same advice applies to all UV exposure steps below.)
7. Prepare two containers, one filled with CD-30 developer (300 MIF ok too) and the other with DI water. Develop for 1 minute in CD-30 developer and transfer the wafer to water. Repeat if necessary. Then, wash with DI water and dry it with nitrogen gun.

Part II: actual metal deposition for the alignment marks

8. Deposit copper with target thickness 300 nm.
9. Strip photoresist + copper by sonicating the wafer in acetone for 10-20 minutes, followed by 10 seconds in methanol, followed by another 10 seconds in IPA. Wash with DI water with nitrogen gun. You should see sharp, shiny copper alignment marks on the Si wafer. Visual check to make sure the rest of the wafer surface is copper free.

### Building the growth channels

Spin-coater setting: (ramp) 100rpm/s → (spin) 500 rpm for 5 seconds → (ramp) 300rpm/s → (spin) 3000 rpm for 30 seconds.

10. If necessary, clean the wafer as follows: Sonicate in acetone for 5 minutes → methanol for 10 seconds → IPA for 10 seconds → wash with DI water and dry with nitrogen gun → bake at 200C for 5 minutes to dehydrate.
11. (base layer) Spin SU-8 2000.5 (Note: Baselayer made with SU-8 2002 is more durable but there may be an edge buildup that makes the wafer/mask contact less than ideal upon UV exposure.)
12. (important) Soft bake: 65C for 1 minute → 95C for 1 minute → 65C for 1 minute.
13. Flood expose for 5 seconds at I-line power = 8.8 mW/cm$^2$.

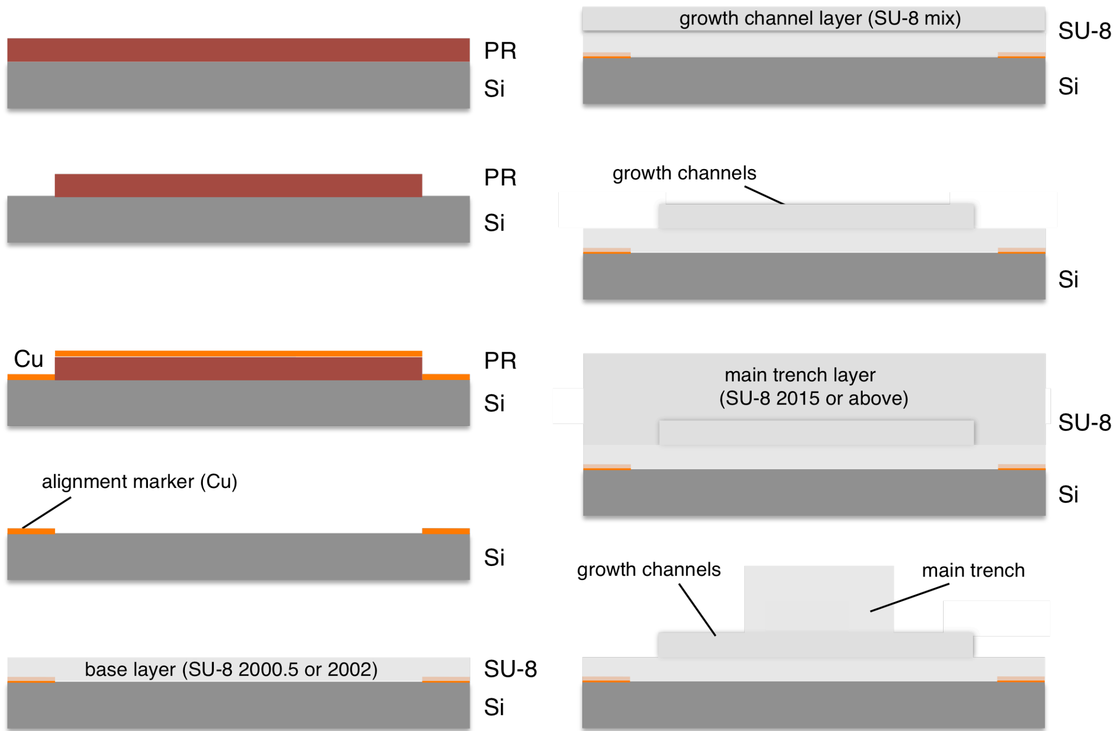
14. Post-exposure bake: 65C for 1 minute → 95C for 1 minute → 65C for 1 minute.
15. Spin SU-8 mixture appropriate for growth channels. For instance, we mix SU-8 2001 and SU-8 2002, where mixing ratio depends on the thickness we want to achieve. It will take some trial-and-error to achieve your ideal thickness.
16. (important) Soft bake: 65C for 1 minute → 95C for 3 minutes → 65C for 1 minute.

17. Use the growth-channel mask and expose the growth channel features; use 3 cycles for 4.7 seconds for each cycle at I-line power = 8.8 mW /cm$^2$ with long-pass UV filter. This step may require considerable trial-and-error.
18. (important) Post-exposure bake: 65C for 1 minute → 95C for 3 minutes → 65C for 1 minute.
19. Develop in SU-8 developer for 1 minute, followed by IPA for 10 seconds. Wash with DI water and dry with nitrogen.
20. Measure the dimension of the growth channels, especially their thickness.

**Building the main trench**

Spin-coater setting: (ramp) 100 rpm/s → (spin) 500 rpm for 5 seconds → (ramp) 200 rpm/s → (spin) 1500 rpm for 60 seconds.

21. Spin SU-8 2015 resist.
22. Soft bake: increase the temperature very slowly (10-30 minutes) from 65C to 95C; and then, decrease slowly to room temperature.
23. (edge removal)
    (a) Prepare a 5ml syringe filled with SU-8 developer (PGMEA) with a thin needle (eg, gauge 27). Set the spin-coater as follows: ramp at 100 rpm/s → 500 rpm for 20 seconds → ramp up 1000 rpm/s → 3000 rpm for 30 seconds.
    (b) Spin the wafer. As soon as the speed reaches 500 rpm, bring the tip of the needle close to the edge of the spinning wafer, and eject PGMEA so that SU-8 is stripped exposing about < 5mm Si surface at the edge of the wafer. Continue for the entire 20 seconds at 500 rpm.
    (c) As the speed ramps up, PGMEA on the Si wafer will dry fast because of centrifugal force.
24. Use the main-trench mask and expose the deep channel features; use 7 cycles for 6 seconds for each cycle at I-line power = 8.8 mW /cm$^2$ with long-pass UV filter. Again, this step may require considerable trial-and-error.
25. Post-exposure bake: 65C for 1 minute → 95C for 5 minutes → 65C for 1 minute
26. Develop in SU-8 developer for 5-10 minutes, followed by IPA for 10 seconds. Wash with DI water and dry with nitrogen.
27. Hard bake: 65C for 1 minute → 95C for 1 minute → 150C for 5 minutes → 95C for 1 minute → 65C for 1 minute.


PR
Si
PR
Si
Cu
PR
Si
alignment marker (Cu)
Si
base layer (SU-8 2000.5 or 2002)
SU-8
Si
growth channel layer (SU-8 mix)
SU-8
Si
growth channels
Si
main trench layer
(SU-8 2015 or above)
SU-8
Si
growth channels
main trench

Joe Levine, a very bright and hardworking graduate student from Elowitz Lab at Caltech, visited us in 2010 to learn how to make/use the mother machine. Joe wrote a detailed and thorough protocol when he was with us, and here we include it with his permission. The protocol will be very helpful especially for those who have no prior experience in PDMS-based microfluidics.

Thanks to James Pelletier (undergrad in Jun lab), we also have a video companion to the protocol. See:

http://www.youtube.com/watch?v=RGfb9XU5Oow

and

http://www.youtube.com/user/JunLabHarvard?feature=mhum

# Making Mother Machines: From Molds to Microscopy

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These notes describe how to create PDMS 'mother machine' devices from wafer molds and prepare them for use in microscopy. The overall work flow requires two days plus an overnight to make and treat the devices. Preparing the finished devices for use in a microscopy assay takes ½ - ¾ of a day and ends with movie set up.

Workflow Summary

Day 1: Pour uncured PDMS onto a wafer mold and cure overnight. (~1-2 hrs + O/N).

Day 2: Remove PDMS device layer from wafer, cut out individual devices, and chemically treat them to remove residual uncured PDMS (~ 8 hrs + O/N).

Day 3: Plasma clean devices and bond them to a glass coverslip for microscopy, passivate the internal surfaces to protect the cells, load cells into channels (~ ½ day total). Finally, set up device for microscopy, and start movie.

You can separate these days if necessary. For example, cured devices can be removed from molds and stored covered at room temperature for many days. Chemically treated devices can be left covered at room temperature probably for about a month.

A few general precautions are essential. First, many protocol steps involve manual manipulations that should be done carefully to minimize damaging the devices or the mold. Damage to a device or improper device assembly, especially at late stages, is difficult to recover from. The silicon/SU-8 wafer molds also need to be handled with extreme care, since the patterned molds can be easily damaged and the wafers are extremely difficult and time consuming to replace.

# Abbreviated Directions

**Day 1**: **Preparing PDMS devices**

1. Prepare one petri dish for each wafer to be used, and line with smooth, flat heavy duty aluminum foil. Place wafer in dish, blow off any dust, and cover.
2. Mix 10g total volume of PDMS + linker (10:1 ratio) for each device (9.0g PDMS, 0.9g linker) for 10 minutes, and degas.
3. Pour degassed PDMS + linker onto device in wafer, being careful to both cover the entire wafer and to not spill.
4. Cover petri dish containing wafer and place at 65C to cure overnight.

**Day 2**: **Removing Devices from Wafer, and Chemical Treatment.**

1. Remove petri dish with wafer and cured PDMS from 65C.
2. Carefully peel cured PDMS device layer from the wafer, treating the mask surface gently. Keep cured device layers in original petris dishes w/o foil.
3. Cut out individual devices punch small hole in corner for drying.
4. Wash and stir devices for 2 hrs once in pentane.
5. Wash and stir devices for 2 hrs in acetone, 3X. Use fresh acetone each time.
6. String up device and hang-dry in fume hood overnight.

**Day 3**: **Bonding Devices to Slides, Passivation, Loading Cells.**

1. Prepare boiled salmon sperm & BSA mixture (1:4), 10ul total for each device.
2. For each device that will be prepared, cut out a thick PDMS mounting block.
3. Plasma clean top side of PDMS devices and PDMS mounting blocks for 4 seconds. Join together and allow them to bond at 65C for 10 minutes.
4. Plasma clean bottom/feature side of PDMS devices for 4 seconds. Plasma clean glass imaging slide for 10 seconds. Join together and allow them to bond at 65C for 10 minutes.
5. Inject 10ul salmon-sperm/BSA passivating mixture into device inlet until it flushes through and emerges from outlet.
6. Place uncovered devices at 37C for at least 1 hr to passivate.
7. Spin down the bacterial culture you want to image and concentrate it heavily (e.g. concentrate E. coli OD 0.2 culture 1:50). Inject concentrated cells into passivated device inlet.
8. Place devices + cells at 37C for at least 1 hr to allow cells to load into growth channels.
9. Prepare growth media for experiment in microfluidic pump, with BSA.
10. Connect pumped media to device inlet and waste tube to device outlet. Flush cells out of main trench.
11. Set desired pumping speed for experiment, prepare microscopy setup, and start time-lapse experiment.

## Day 1: Preparing PDMS for forming devices.

1. Glove up.
2. Prepare one large petri dish (150x15mm, VWR #25384-326) for each individual wafer used. Cover the bottom and sides of the petri dish with heavy duty aluminum foil and thoroughly smooth it with a fiber free cloth (e.g. VWR Spec-Wipe #21913-211) The foil catches any uncured PDMS that spills later during pouring and curing, and must be flat and smooth so as to not tilt the wafer and spill the PDMS, or allow any spilled PDMS to flow under the wafer.
3. Carefully remove the wafers you want to use, one at a time, and place them each patterned side up in an individual petri dish.
4. Blow air or N2 over the wafer, petri dish, and aluminum foil to remove dust. Be careful to prevent air from blowing under the dish and making it fly away. Cover the dish when finished.
5. Mix the PDMS and and linker together (Sylgard 184 silicone elastomer kit, 3097358-1004) in a small aluminum dish (e.g. VWR 57mm aluminum dish #25433-010). Use ~10g total per wafer, 9.0g of PDMS and 0.9g of linker. ~10% error is tolerable. This amount gives a good device thickness on a 4'' wafer. Mix PDMS and linker together for ~ 10 minutes until it looks milky and bubbly.
6. Degas the PDMS/linker mixture in a vacuum dessicator.
    a. Place the PDMS/linker mixture, still in the Al dish, into a vacuum dessicator and securely close the lid. (**See Illustration**)
    b. Pump down the dessicator with a vacuum source.
    c. As gas escapes the PDMS bulk it will form bubbles on the surface. Periodically disturbing the vacuum with a brief influx of air will break these bubbles, accelerating degassing and reducing spillage.
    d. Degassing is complete when the PDMS/linker mixture is completely clear and bubble free. This can take ~ 20 minutes.
    e. Remove the degassed mixture from the dessicator.
7. Back at the bench, center the wafer in the petri-dish. This will help later in case of PDMS/linker overflow.
8. Pour PDMS/linker mixture onto wafer.
    a. Pour the mixture onto the wafer center and spiral it outwards to eventually cover maybe ½ the surface area.
    b. Pour at a medium speed close to the wafer. Pouring too fast risks uneven converage, while drizzling adds unwanted bubbles. An ~1 cm width stream is about right.
9. Tip/tilt the petri dish to spread the entire PDMS/linker mixture and coat the wafer evenly and completely. This should be done **carefully** to avoid spilling.
    a. The goal is to coax the mixture edge right up to the edges of the wafer.
    b. PDMS is quite viscous and can build up enough momentum to spill.

    c. When the mixture edge is close to the wafer edge, tip/tilt in little pulses to controllably move the mixture edge front.
    d. If the mixture does not cover the wafer, it will be difficult to separate from the wafer after curing without damaging the wafer surface.
    e. If the mixture runs over the wafer edge, the surface tension holding the mixture on the wafer will be broken and it will run off during curing, leading to excessively thin devices.

10. A few small bubbles may remain in the PDMS/linker top layer after coating. If so place the covered petri dish with PDMS/wafer @ 4C for 10-20 minutes. This should degas the remaining few bubbles. The very last few can be puctured with clean Aluminum foil.
11. Place PDMS/wafer in the covered petri dish in a 65C oven/incubator to cure overnight. Make sure the wafer rests on a flat level surface to prevent the PDMS mixture from spilling, since it loses its viscosity at 65C.

## Day 1: Preparing the PDMS blocks to hold microfluidic tubes.

These blocks are prepared identically to the PDMS/linker for the devices, except the mixture is prepared in a larger volume and cured for two nights.

1. Mix 42g of PDMS and 4.2g of linker in a standard sized Petri dish (e.g. for normal bacterial work).
2. Stir for **30 minutes**.
3. Degas the mixture, taking care to prevent overflow.
4. Place the covered petri dish in the 65C oven to cure. These thick PDMS blocks should be cured for at least 2 days to eliminate residual uncured PDMS.

## Day 2: Removing Cured PDMS devices from the wafer.

Once the PDMS device layer has cured overnight on the wafer it is removed and kept in a large covered petri dish. The wafer is then gently wiped of uncured PDMS and returned to storage. While this procedure only takes a few minutes, the detailed instructions are to help ensure no damage is done to the wafer or the cured PDMS devices.

1. Glove up!
2. Remove petri dishes with wafer / cured PDMS devices from the 65C oven.
3. Remove the cured PDMS layer from the wafer.
    a. Use the tip of a sharp scalpel to gently separate the PDMS from the wafer at the wafer's edge.
    b. Grab the separated PDMS with some tweezers, and slowly and gently peel the cured PDMS layer off of the wafer.
    c. The peeling motion should be done along the axes of the growth channels.
4. Wipe any uncured PDMS from the back of the wafer off with a fiber-free wipe. Do **not** wipe the patterned side.

## Day 2: Chemical Treatment of PDMS Devices.

Cured PDMS devices need to be chemically treated before use, to remove any residual uncured PDMS. The devices are first individually cut from the cured PDMS sheet. They are then stirred in a pentane bath, which flows into the polymer matrix and expands it, allowing uncured PDMS to dilute out. The devices then washed three times in acetone baths to remove the pentane. Finally, the acetone is air-dried off overnight. All work is done in a fume hood.

1. Cut individual devices out of the PDMS layers with a scalpel. As a rule of thumb, leave about 1 cm along each side of the large channels, and maybe 5 mm or so around any fine features.
2. Punch a 2 mm hole in the corner of each device using a hole punch (Harris Uni-core). Keep the hole away from any device features. This hole will be used to hang devices for drying after chemical treatment.
3. Prepare a beaker of Pentane in a fume hood. 200 ml of pentane in a 500 ml beaker is sufficient to treat over 20 devices. Add a magnetic stir bar and stir at 1-5 rotations per second. Grab individual devices with tweezers and drop them quickly into the liquid. Try not to partially submerge them for prolonged periods, to prevent deformation. Cover the beaker tightly with foil to keep the pentane from evaporating. Let the devices stir and wash for at least 2 hours. Make sure all devices mix and none stick to the beaker wall.
4. Once pentane wash is done, prepare a fresh beaker of acetone (same size beaker and same volume acetone as pentane). Use tweezers to gently pick individual devices out of the pentane and transfer them quickly to the acetone. Also transfer the stir bar. Once all transfers are complete, cover the beaker tightly with foil to prevent acetone evaporation, and set stirring for ~1-5 rotations per second, and let the devices stir and wash for 2 hours.
5. Repeat the acetone wash two more times in fresh acetone.
6. After the 3rd and final acetone wash, prepare a thread drying line in the fume hood. The devices will hang dry on this overnight.
7. Remove the devices from the acetone, and string them up on the drying line using the punched holes. Make sure the devices do not touch each other.

## Day 3: Plasma cleaning and device assembly.

In this step, we assemble the full device onto a glass surface for imaging. The final assembly consists of the device, a thick PDMS block in which to mount fluid tubes, and a glass slide. **See illustration**. The assembly procedure requires plasma cleaning the various device surfaces before bonding them. At the end, the device is passivated with a mixture of BSA and Salmon Sperm to coat the PDMS surfaces and prevent them from killing the cells.

Note on device orientation: If small letters and numbers are readable when looking at the device, you at facing the non-featured side, and the side away from you contains the features.

1. Glove Up!
2. Prepare BSA/Salmon-Sperm passivation buffer to be used later.
    a. Defrost BSA (10mg/ml stock solution) and Salmon-Sperm (10mg/ml stock solution). Passivation buffer is 3 parts BSA to 1 part Salmon-Sperm. You need 10ul of buffer per device: 7.5ul of BSA and 2.5ul of Salmon-Sperm. You will also need to add buffer to 1:100 to the media you use during the experiment, e.g. 10ul of passivation buffer for each 1ml of media.
    b. Boil Salmon-Sperm for 10 minutes to denature it. Once it cools to room temperature mix it with the BSA at the appropriate ratio, and leave it at room temperature.
3. Select the devices for your experiment, and place them feature side down in a petri dish. The petri dish will hold the devices during plasma cleaning, and should be sized appropriately for your plasma cleaner.
4. Cut out PDMS mounting blocks for each device, and place them smoothest side up in the petri dish along with the devices. The blocks should be:
    a. Large enough to not rupture when tubes are inserted
    b. Large enough to cover all fluid inlets and outlets of the device.
5. Use scotch tape to clean off PDMS surfaces, removing dust and dirt.
6. Plasma clean the PDMS devices and mounting blocks according to your machine's protocol.
7. Place the mounting blocks onto the appropriate devices to cover the inlet and outlets. The smooth face of the mounting block which faced upwards during plasma cleaning should contact the top face of the device. Press the surfaces together firmly, making sure to expel any bubbles and ensuring complete contact.
8. Incubate the devices at 65C for 10 minutes to bond the two devices to the mounting blocks, then remove to room temperature.
9. Punch holes into the device and mounting blocks to create an inlet for fluidic tubing.
    a. Turn the device upside down and place the top/mounting-block side onto a folded fiber free cloth on the bench top.

   b. Punch holes through each end of the device's main trench, down all the way through the bonded PDMS mounting block, using a Harris Uni-core 0.75mm punch. Lining the punch up by eye with the main trench can be a bit tricky at first. Expel the punched PDMS core using the Uni-core push button.

10. Plasma clean the device bottom surface and also a glass slide, to bond them.
    a. The glass slide may have to be cleaned slightly longer than the PDMS device. This will need to be optimized for your particular set up.
    b. Carefully lay the plasma cleaned device bottom surface onto the plasma cleaned glass surface. Laying the device down on the glass like one lays carpet onto a rug helps minimize bubbles.
    c. Gently tap away any bubbles with blunt tweezers.
11. Incubate the joined device/glass at 65C for 10 minutes to bond them. Then remove the devices from the incubator/oven.
12. Inject passivation buffer into the device to passivate interior surfaces.
    a. Take up 10ul of passivation buffer into a standard 10ul pipette tip.
    b. Insert the pipette tip + buffer into one of the holes in the PDMS mounting block.
    c. Plunge the pipette down to the first stop and hold it.
    d. Passivation buffer should slowly flow through the mounting block hole into the device, eventually emerging from the other holes in the mounting block. This process may take up to a few minutes.
    e. **No leaks should occur**. If a device springs a leak at this point it should be discarded.
    f. Once fluid starts to emerge from all other mounting block holes, gently withdraw the pipette tip from the mounting block (with pipette still plunged). No air bubbles should have crept into the mounting block channels.
    g. Seal the mounting block inlets and outlets with masking tape
    h. Place the device at 37C, uncovered, for at least one hour, to allow the passivation buffer to be drawn into the growth channels via evaporation.
13. Load cells into the device.
    a. Cells should be concentrated to an extremely high density before loading. For example, E. coli grown in rich media are taken at OD600 = 0.2 and concentrated 20-50 fold for loading. You will have to optimize the exact density for your particular experiments.
    b. Take up 10ul of concentrated cells into a 10ul pipette tip.
    c. Insert the pipette tip + buffer into one of the holes in the PDMS mounting block.
    d. Plunge the pipette down to the first stop and hold it.
    e. You should see the cloudy cell suspension slowly flow down the mounting block channel, into and along the device main trench, and finally up and out of the other mounting block channels. This process may take a few minutes.

f. Carefully remove the pipette tip as before, and seal the inlets and outlet with masking tape.
g. Place the device at 37C, uncovered, for at least one hour, to allow the cells to be drawn into the growth channels. You will have to optimize the time according to your experimental conditions. Periodically check the device with your microscope to monitor loading.

## Day 3: Connecting loaded device to microfluidic pumps.

In this step, we connect the device to microfluidic pumps and mount it on the microscope for imaging. First we prepare tubing that will be used to transfer media into our device. Then, we mount the tubing into the device and begin media infusion.

1. Prepare tubing.
    a. We typically use 0.023'' inner diameter polyethylene tubing (Becton Dickinson Intramedic, Clay Adams 427411). Cut whatever length is necessary to reach from your media pump to your microscope stage, allowing some slack.
    b. To interface the tubing to the PDMS device, insert a small 22 gauge blunt ended needle with luer lock interface (SmallParts, # NE-223PL-C) into the one end of the tubing. This will require some twisting/threading. Once this is inserted, bend the needle to a right angle just above the luer-lock hub, and twist off the hub. Remove any residual plastic. The small ~1/2'' length of metal after the bend will be inserted later into the device's PDMS mounting block.
    c. Insert another blunt ended needle into the other end of the tubing. Leave this one intact. The luer lock hub will be used to connect the tubing to the media syringe.
    d. Prepare as many tubes as you have inlets and outlets on your device. For example, a device with one inlet and one outlet needs one tube connecting the media source to the device inlet, and one tube connecting the device outlet to a waste bottle.
    e. The tubing can be reused many times. After each experiment, flush the tubing out generously with isopropanol and then sterile water.
2. Prepare growth media for experiment.
    a. During a typical experiment we flow media through our device at a rate of 1ml/hour, although your conditions may vary. Therefore for a 24 hour experiment we would prepare perhaps 30ml of media. Make sure to add passivation buffer to your media (1:100).
    b. We dispense our media using 60ml syringes with a Luer-Lok tip (Becton Dickinson, #309653) pumped with a Harvard Apparatus pump.
3. Pump media through tubing and insert tubing into device.
    a. Connect tubing to media filled syringe, and pump media through the tubing to displace all the air, until media is dripping from the other end of the tubing. This can be done at a pump rate of 10-20ml/hr. Displacing all the air prevents any air bubbles from entering the device, which may harm the cells.
    b. Remove masking tape from the mounting block to expose inlets and outlets. Insert the bent metal needle end of the tubing into the inlet hole. Make sure a drop of media is suspended from the end of the

tubing as you insert into the inlet hole, to prevent any bubbles from entering. Dab any excess liquid away with a kim-wipe.

4. Once tubing is inserted into the device, begin to pump media through the device to flush all cells out of the main trench, leaving only cells that have loaded into the growth channels.
   a. We typically pump at rates of 5ml/hr up to (even) 20ml/hr. Excessive rates may cause excessive pressures, which could cause leaks. The higher rates are necessary if you suspect there is a cell-clump clogging the device's main trench.
   b. Pump until **clear** (cell free) liquid begins to emerge from the outlet holes in the mounting block. Dab any excess liquid away with a kim-wipe.
   c. Once clear liquid is emerging from the outlet, turn the pumping rate down to 1ml/hr (or whatever it will be during your experiment).
   d. Insert a tube into the waste outlet, and thread the output of that tube into a waste collection bottle of appropriate size.
5. Mount the device on your microscope stage, and begin your microscopy set up.
6. Continually (and obsessively!) check for leaks the first hour or so of device operation. If no leaks have sprung during this time, the device is probably good for time lapse, although it should be checked every few hours or so.