

# Protocol - Mother Machine Experiment

Links - [Ze Master Note](#)

## Log

20150812 - Started to make major updates. Added Steve's multiplex technique.

20151124 - Edit including using oxygen during plasma clean.

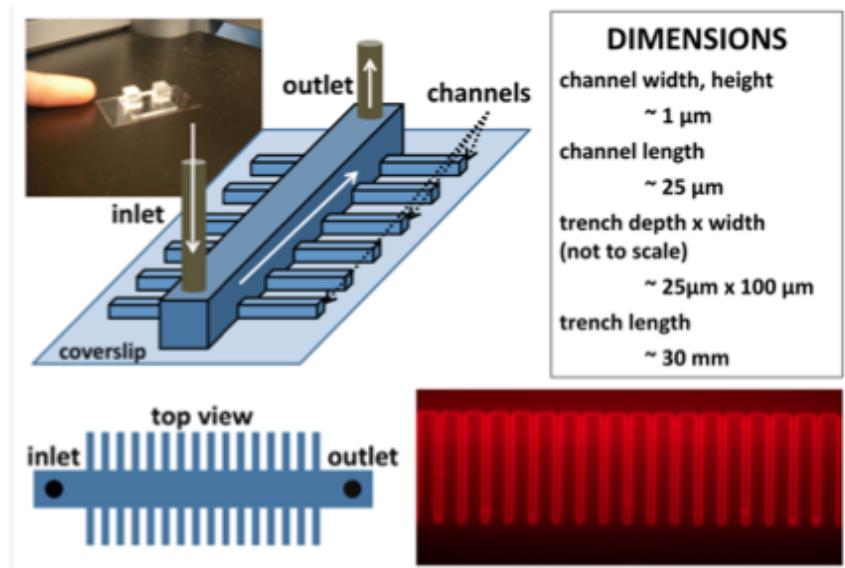
20160809 - Updated for incremental changes. Added pictures. Removed not-helpful analysis section [jt]

20170215 - Added some details about loading, but honestly the whole end of the protocol could use some love.

## Background

Observation of the growth of single cells allows for insight that is precluded by observations on the population level. For example, intrinsic variability in growth rates between otherwise isogenic bacteria (i.e. those with identical genomes) is masked by measuring the growth rate of a batch culture as in a normal growth curve experiment. The mother machine is a microfluidics device that allows for the individual observation of singular bacteria. In the device, thousands of individual bacteria are sequestered in narrow channels that line a long, central trench (see Schematic and Literature below). Cells at the end of the channels (referred to as the ‘mother’ cells) continuously divide, pushing the daughters in a single-file line towards the trench, where they are inevitably washed away by the relatively quick flow in the central trench. Tracking the mother cells over many generations provides unique understanding and perspective of the cell cycle, cell size control, and cell aging.

Schematic of the mother machine: Channels, housing bacteria, are arranged along a central trench. Medium flows down the length of the trench, and bacteria in the channel are supplied with fresh nutrients via diffusion. From Supplemental Experimental Information in Wang, P., Robert, L., Pelletier, J., Dang, W. L., Taddei, F., Wright, A., & Jun, S. (2010). Robust growth of *Escherichia coli*. *Current Biology: CB*, 20(12), 1099–103. doi:10.1016/j.cub.2010.04.045



## Literature

Wang, P., Robert, L., Pelletier, J., Dang, W. L., Taddei, F., Wright, A., & Jun, S. (2010). Robust growth of Escherichia coli. *Current Biology: CB*, 20(12), 1099–103. doi:10.1016/j.cub.2010.04.045

Movie S1: Growth of E. coli in 12 fields of view over 400 minutes.

 NIHMS203820-supplement-02.mp4

3 MB

## Protocol

Here is a video that shows both making the PDMS device and preparing the device on the glass slide. It won't be exactly how we do it, but in general it is the same: [How to make the “mother machine”](#).

### Media and culture preparation - Do this the day before.

1. Make sure you have reserved your desired microscope! No point of doing any of this if you can't start your experiment.
2. Make desired media. Always sterile filter.
3. Make 4ml 50mg/mL Bovine serum albumin (BSA, [http://en.wikipedia.org/wiki/Bovine\\_serum\\_albumin](http://en.wikipedia.org/wiki/Bovine_serum_albumin)) solution, diluted in the target media. You will use this at the end of the wafer preparation and when preparing the cells to be put in the device, as well as when filling the syringe. Prepare in a falcon tube, sonicate to mix, and then syringe filter into another falcon tube. Avoid bubbles in the final tube. The BSA is later used to quench aldehydes and other functional groups in the PDMS device such that they cannot bind to the cells.
  1. You can store this at room temperature like other sterile media.
4. Make a stock of 1M beta-mercaptoethanol, which will be added to the cell before spinning them down and injecting them in the device to prevent cells from sticking to each other. This can be stored at 4C.
5. Earlier on the day before your experiment inoculate a 1ml falcon tube in the LB with a strain from a glycerol stock cryotube or single colony. Incubate the cell in the shaker at 37C (I like to do it at an angle, but at 1ml or less you can have it straight up and down).
6. Passage the cells into your target media in the evening before you leave.
  1. Unless it conflicts with your experimental conditions, add 1% v/v of your BSA solution to your media (final concentration 0.5mg/mL). This will help the cells not stick to each other while loading and so they don't stick to the PDMS once you have started the experiment.
7. First thing in the morning on the day you want to start your experiment, back dilute your cells again in the target media with 1% BSA solution. These are the cells that you will load into the device.
  1. Generally, it is good to have at least 2 back dilutions in target media is ideal. If you want, you can wash out the LB by spinning them down and resuspending the pellet in desired media before diluting if you want to have super controlled conditions. This is overkill though.
  2. It's good to have multiple dilutions so you can catch the cells at a good turbidity. Back dilute the cells at 1:100, 1:1000, and 1:10,000, for example. You can do 1mL or 3mL. Ideally, you want to harvest the cells when they are mid-log phase (so that there are enough cells but they aren't in stationary

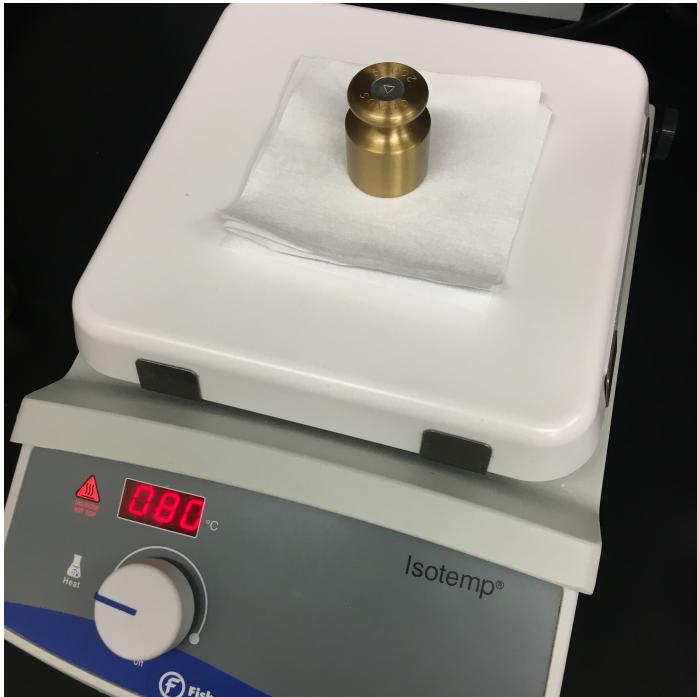
phase), so plan accordingly. You can also try to high dilutions the night before and time it so you can start an experiment earlier in the morning.

8. Do the following steps as you wait for your cells to grow up.

#### **Mother machine device preparation - Morning of experiment.**

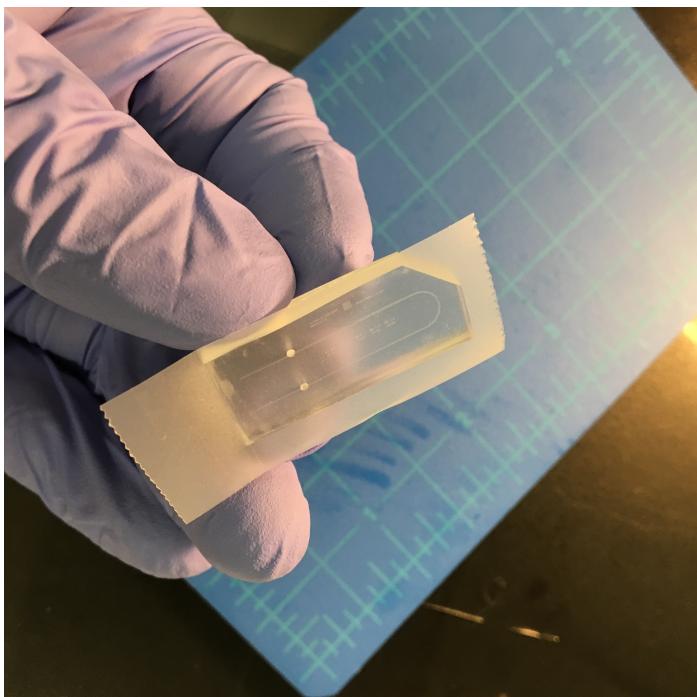
1. Turn on a the plate heater to 65-80C and put the small weights on it. This will be used after plasma cleaning to help bond the device to the Wilco dish.

1.



2. Get your desired mother machine device(s) and take it to the clean room. Thoroughly remove all dust with Scotch tape. Press enough where the tape can remove dirt from the trench and channels, not just the surface of the device. Don't be shy, Scotch tape is cheap.

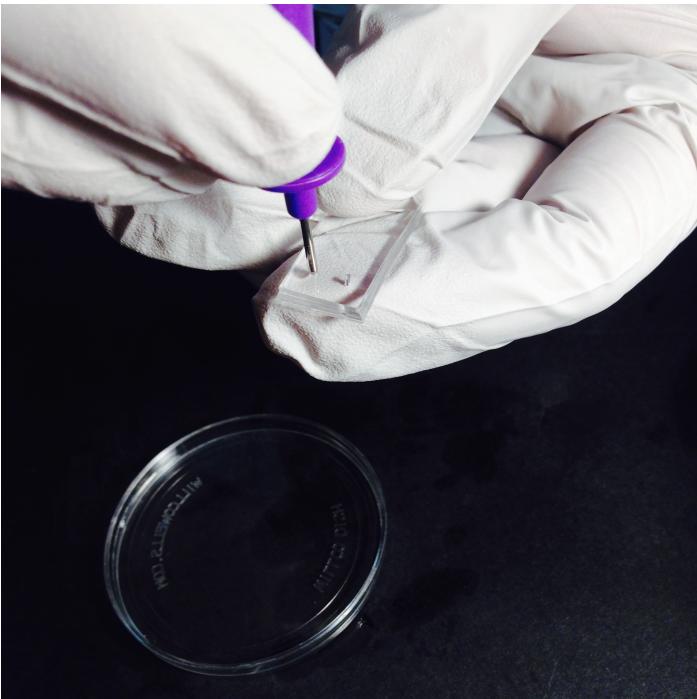
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2. There is a top and a bottom; the bottom side has the features on it which you can identify in the light.
3. Also, use the stereoscope to inspect the device for any damage or scratches.

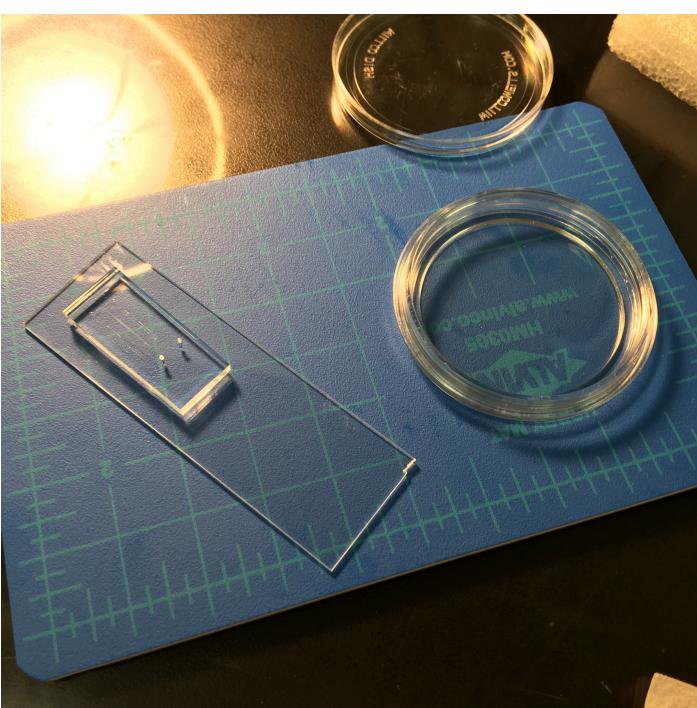
- ... use the stereoscope to inspect the device for any damage or scratches.
3. Use a small punch to poke 2 holes in the wafer at either end of the central trench. Poke them from the bottom side. Place the device between your thumb and middle finger, with your index finger on the back (top) of the device. Enter the punch perpendicular to the bottom (feature) side of the device. Press the end of the punch to remove the core, then twist out the punch from the direction in which it entered.

1.



2. Alternatively (and better), you can punch holes in your devices before you clean them (see the making PDMS devices protocol). This way, tiny pieces of PDMS created by the boring process are removed and won't clog the device during the run.
3. You can use the stereoscope to help make sure the hole is in the right place.
4. Place the wafer on a clean glass slide with the bottom (feature) side up. Clean it a bit more with tape just in case. You can also clean it with ethanol and blow dry it off with the filtered air spout in the chemical hood.

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5. Get a Wilco slide and put it--just the glass part--and the glass slide with the wafer on it into the plasma cleaner.

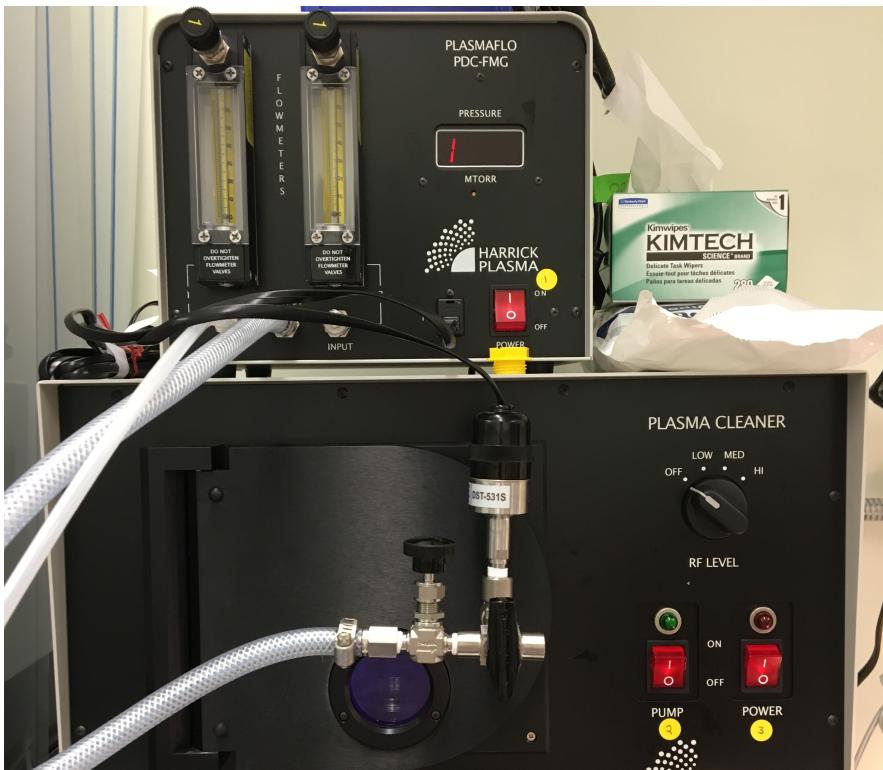
1.



2. If you are worried that your Wilco dish is not clean you can also put a drop of ethanol on that and blow dry it off.
6. Plasma clean. There are many strategies here but this is the gist.

1. Turn pump on and ensure flow valve is pointed down such that flow is blocked. Allow the pressure to drop to 1000mTor. Switch flow valve to left so air can be siphoned in. The pressure should jump, allow it to fall to 1000mTor again. Turn power on for 20 seconds (less time sensitive). Turn on high power for 15 seconds (time sensitive). Shut everything (pump, plasma level, plasma cleaner power) off. Return chamber to atmospheric pressure by opening flow valve to the right, but do it slowly as to not blow the devices around.

2.





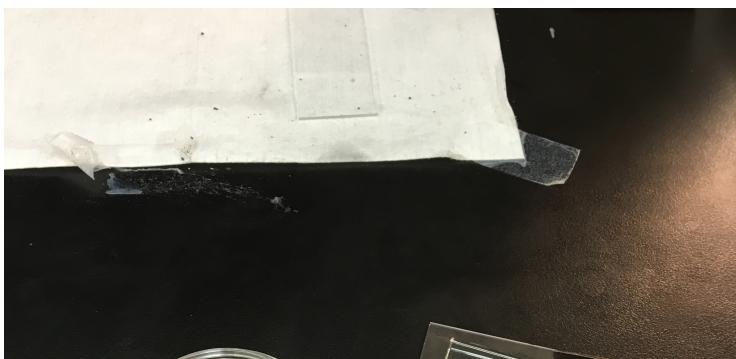
3. If the vacuum is slow, make sure all the seals around the vacuum hose going from the plasma cleaner chamber (on back of machine) to the pump are tight.
4. You can also use pure oxygen during the plasma cleaning process. Turn on the tank (you need less than 10psi) next to the freezer and continue as normal. Be sure to close it after you are done so it doesn't get wasted.

5.



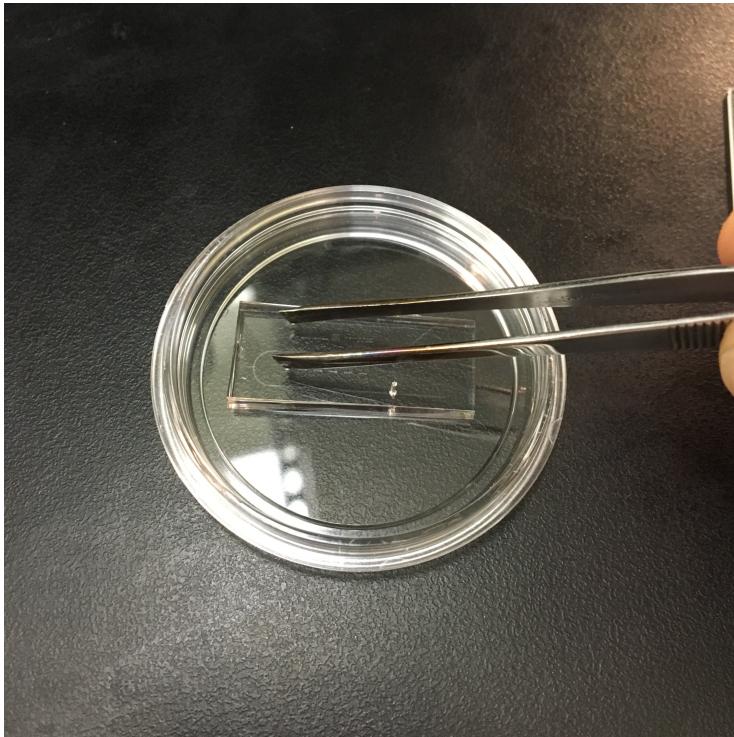
6. You can also backfill the tank with oxygen and then evacuate it a few times before you start the treatment. This will ensure that very little of the original air is in there, and that there is very little water vapor.
  7. If you use oxygen, you likely won't be able to see the plasma. If you use air, it will be purple the aurora borealis.
7. Anneal the device to the Wilco dish. This is the most time sensitive part. Remove wafer from the glass slide with tweezers and place on Wilco slide with the bottom (feature) side facing down. Press down gently with tweezers and then with fingers to ensure there are no bubbles. You can press down hard around the sides, but be careful not to damage the channel features. Because the PDMS is reactive at this point, if any of the channel features touch the device then they will bind the glass and occlude the channels.

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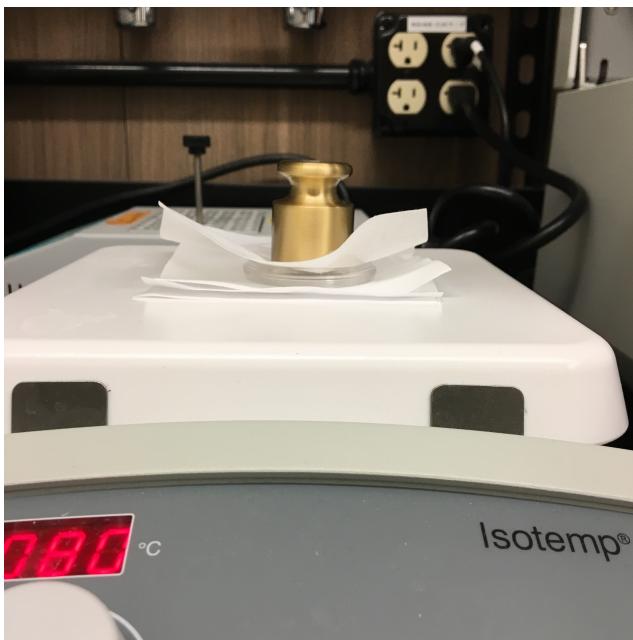


2.



8. Put the device on the hotplate at for 5-20min in order to ensure a good bond. You can put a non-woven cloth over the device and then put a weight on it.

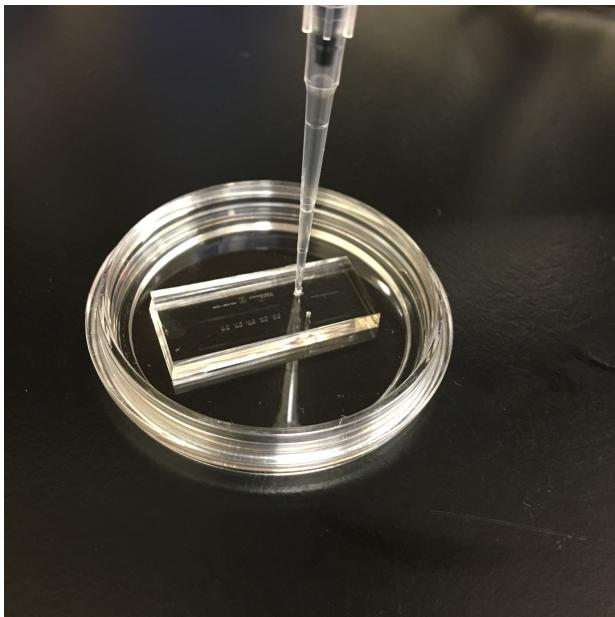
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2. You can always use the weight as a little iron to press the device to the Wilco dish
9. Load the devices with BSA. Use a 10ul pipet with small tips to put 10ul 50mg/ml BSA solution into the

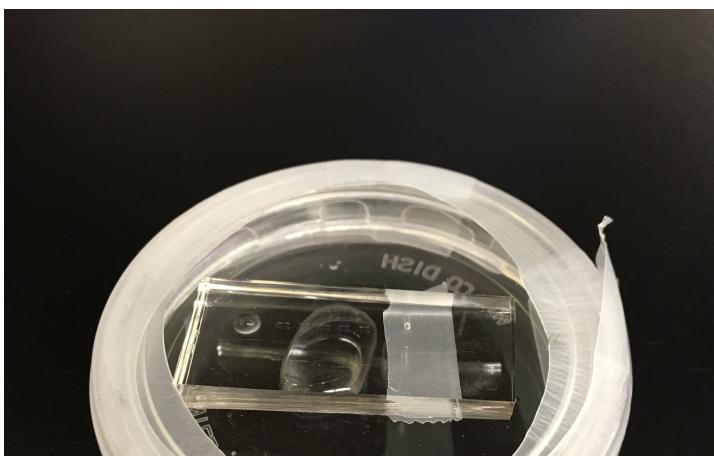
device. Fully depress the plunger and hold it so the fluid goes all the way through. Do not release the plunger! Hold you the pipet tip at the top where it connects to the pipet. With the plunger fully depressed, pull up on the pipet to remove the it from the tip. This is to ensure you do not suck any air back into the device.

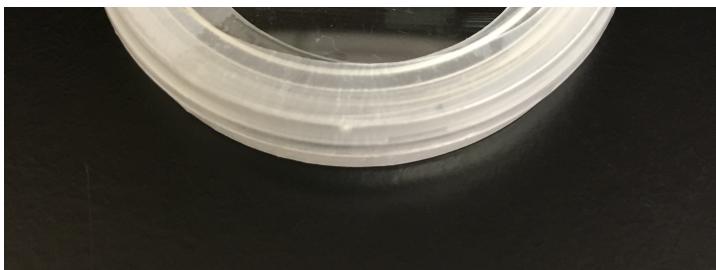
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2. Wait until you can see liquid coming out the outlet hole. It's good to have enough pressure on the fluid to make sure any air inside the growth channels are forced out through the PDMS and the entire device is wetted.
3. You can remove the pipet and leave the tip standing vertical in the device, and then use a 3mL syringe to force more liquid in, but be careful not to break the bond with the Wilco slide.
4. Be careful when removing the pipet tip as not to delaminate the device. Just wiggle it out slowly.
5. The internal volume of the device is much smaller than 10uL, so just push enough through so it comes out the other side and you introduce no bubbles into the device.
10. If not using immediately, store the device in a way that prevents evaporation.
  1. Dry the device top and put scotch tape over the holes. Cover the Wilco slide with the wafer inside. Put few drops of water on the underside of the cover to further reduce evaporation, and place upside down. You can further put parafilm around the edge. It will be good for a couple of hours, or instead use water in place of BSA in step 9, then put it in the fridge and it will be good for a week.

2.





3. Alternatively, you can cut the pipet tip off near the tip, leaving that part protruding from the device. Putting another pipet tip on the outlet, even before loading BSA, can help you see the liquid going through the device. It will also prevent evaporation of the liquid in the device.
11. Check out the Devices in the scope to see that the aren't funky or anything. Make sure there aren't any air bubbles and that the device is not delaminated already.

#### **Prepare Darwin chamber for inoculation and data acquisition.**

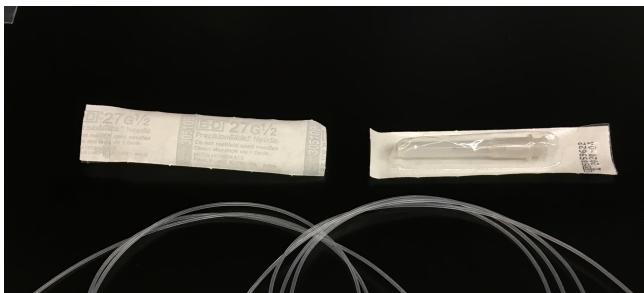
1. Get a small beaker or 50mL Falcon tube for the effluent. Put a few mL of delicious Citrus II in there so the waste cells don't grow up and smell bad.
2. If it doesn't have one already, put a 500mL Erlenmeyer flask with water at the correct temperature in the Darwin chamber incubator. This is to further stabilize the temperature of the media on its way to the device.

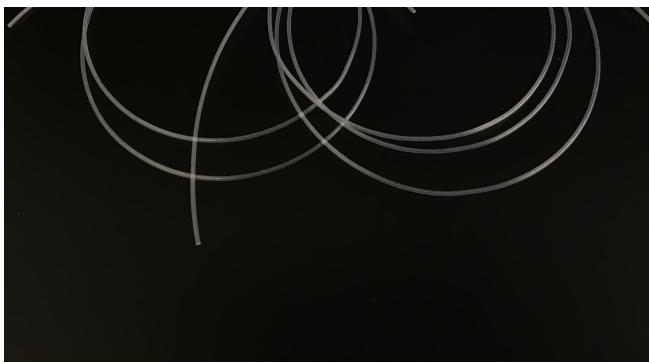
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3. Get the (previously cleaned—see clean up below) inlet and outlet tubing. The inter tubing needs a 27gauge needle. You can cut off the end of this needle with wire cutters as to minimize the chance of stabbing yourself, but make sure the flow is unobstructed. You can cut off the ends of the tubing itself to make sure the ends are flat and to get rid of any potential contamination. Make sure the tube doesn't have any obstructing kinks.

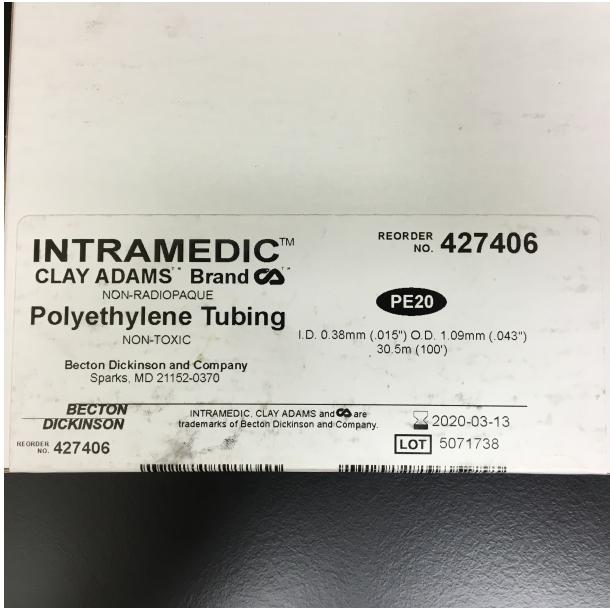
1.





2. The inlet tubing is about a 1m long, the outlet tubing should be just long enough to go from the device to the waste beaker when it is set up on the microscope. The longer inlet tubing is to give you some slack to run through the Erlenmeyer flask. The shorter outlet tubing is to reduce the fluid resistance on that side of the device.

3.



4. Fill a syringe with media. You can use any size from 10-60mL. Get a sterile filter, open it, and put the plastic shell on a culture tube rack with the Luer connection facing up. Remove your sterile syringe from the wrapper, and remove the plunger. Put the plunger back into the wrapper and attach the syringe body to the filter. Transfer media to the syringe by pouring or using a pipet. Put the plunger in the syringe, some liquid will come out of the end. Flip the syringe so as to not lose any more media, and push out all the bubble, keen to evacuate any bubbles. Wipe off any spilled media (with ethanol), and label the syringe.

1.



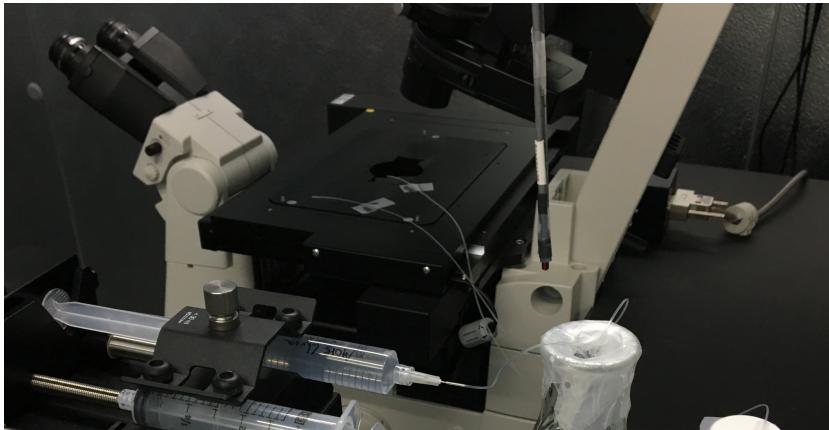


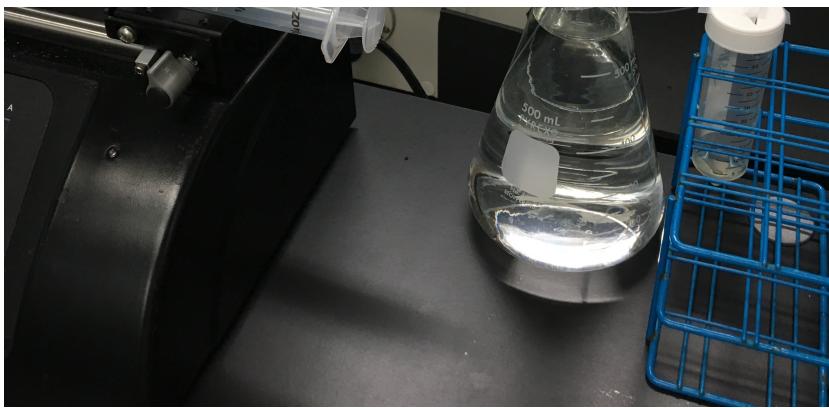
2. Alternatively, you can put the media in a sterile beaker. Using a syringe with a sterile filter, draw in as much media as possible. Push out any excess air, tapping the syringe to free the bubbles. Change to a clean syringe filter.
5. Go to the microscope if you are not there already. Place the syringe in the pump. Securely lock the syringe handles into the pump. Use a second syringe to balance the clamp, but it can be installed backwards as to not interfere with the moving press. Clamp down both syringes.

1.



6. Set up the syringe pump. Be sure to choose the correct brand and size of syringe. Put in your desired flow rate and make sure the units are right (mL/hour)!
7. Hook the syringe up to the inlet tubing via the Luer needle. Allow this to run for some time into the effluent beaker to make sure that flow is continuous and there are no air bubbles. You can use a relatively fast flow for this (5ml/hr) as well as the >> button on the syringe pump to jolt liquid through quickly.
  1. The inlet tubing should be run through a 500ml Erlenmeyer flask filled with water. Put as much slack tubing in there as possible.
  2. Tape the inlet and outlet tubing in the 'ready position.'
  - 3.





8. Set-up and adjust the microscope. See the microscope Evernote notebook.

**Inoculation - The goal here is to minimize the time between removing cells from the culture and hooking the feed inlet to the device. Ideally this time should be less than 20 minutes.**

1. When ready to inoculate the device, spin down 1ml of a semi-turbid (log phase) sample in a micro centrifuge tube A pellet 0.5-1 mm in diameter is a good size. If the pellet is not big enough, you can put in another milliliter after removing the supernatant and spinning down again.
  1. If the cells are clumping after concentration, add 1mM final concentration beta-mercaptoethanol before spinning down. This reducing agent will decrease cell-cell adhesion.
2. Remove ~980ul of supernatant (maybe less if the pellet is really big). Resuspend the pellet in 20ul BSA solution. Tip: Pipet slowly 10 times, breaking up the pellet by indirectly pushing liquid beneath it.
3. Put ~10ul of cells in the device in the same manner that the BSA solution was added before. Mix well to reduce the clumping of the cells, but be careful not to introduce air bubbles.
4. You will have removed the tape to add the cells, but put it back on to stop flow.
5. Load cells. Centrifuge the device in the fan to force cells into the channels. Depending on the size of the cells and the channels, you may need to centrifuge from 2-20 minutes.
  1. Make sure the channels face outward, so the angular momentum of the fan puts the cells in the channels, not down the trench.
  2. Be sure when you put the device in the fan, that the channels are on the farthest vertex of the main trench out. This means the glass surface of the Wilco dish should be down.
  3. If you do not have an appropriate centrifuge you can let your cells grow into the channels (though they will not be near steady-state when you start the experiment).
6. Place device/Wilco dish on microscope. Be careful not to have any bubbles in the oil.
7. Use the microscope to determine if enough cells have filled into the channel. Check for anything out of the ordinary. This means: clean looking device, good channel loading, no contamination.
8. Now you can use the small microscope instead for checking cell loading.
9. Connect the inlet tubing to the device. Let some effluent come out the outlet port freely.
10. Hook up the outlet tube and tape the end to drain into the effluent flask. Both the inlet and outlet tubing can be taped to the stage with scotch tape to relieve stress where they enter the device.

#### **Setting up the microscope and time course in Elements.**

1. Lightly clamp down device with the red stage screws.
2. Use blue filter - increases the contrast of the image

2. Use blue filter--increases the contrast of the image.
3. Adjust the perfect focus. Navigate to one end of the trench and have the objective look at the middle of the trench, reset the z measure on the microscope, and turn on perfect focus. Navigate down the trench towards the other end. The microscope reads off the the z offset. Adjust the stage as necessary. Note: the small, black hex screws raise and lower the stage. Tightening the screws pushes the stage up. The while clear screws should be disengaged during this process, and *lightly* tightened after adjustment if finished. After adjustment, repeat this step until the left/right offset is less than 5um.
4. Switch to camera mode and open up Elements on the computer if not so already. Press the play button to start the live camera feed.
5. Select your ROI size for the FOVs. First show the entire area, and have the objective centered on the middle of the trench (this is better for the perfect focus). Then select an ROI the shape a long rectangle containing as many channels as possible. Have a buffer zone below and above the channels, though the area the dead-end of the channels is most important.
6. Use the cross tool to make sure that the trench is aligned horizontally, and the channels are vertical. After you have adjusted the Wilco dish, you can tighten the red stage screws. Check if the perfect focus is still within acceptable limits.
7. Choose your FOVs. You can use the space bar to select them. Ideally somewhere between 30 and 40.
8. Adjust the order of your FOVs such cycling through the FOVs leads you circularly back to the start. You could do this by going down one side of the trench and then back up the other side, or 'leap-frogging' down and up one side of the trench.
9. Make sure other parameters are set correctly: picture interval (1-1:30), exposure time (50ms), etc. Ensure that the image is not over exposed by looking at the levels histogram. Make sure perfect focus is on and that Z direction is not checked in the XY table, or else the microscope will have to move in this dimension as well.
10. You can do a 1 time loop to make sure everything is good.
11. Give it a good, descriptive file name and press go. You can set the time to continuous. Save to the SSD.

## **Monitoring and maintenance**

1. Change the syringe in time so there is no time out of flow. Be aware that towards the end of the syringe there is a chance that bubbles in the syringe will be pushed forward and through the tube. Fill the syringe same as above. After you (quickly) switch the Luer lock syringe needle and put the syringe in the pump, make sure the actuating hammer is butt-up against the syringe plunger. You can press the >> button to make sure there will be no lapse in flow.
2. Here are some failure mechanisms (and if you're lucky possible solutions).
  1. The microscope oil can all evaporate. If your dextrous you can put on another 25ul with the pipet from the underside of the microscope stage.
  2. Bubbles in the device will most likely fuck it up. You can try pressing the >> button to force them through but no