Protocols for fabrication of microfluidic devices.

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(Based on the procedures done by Sharvari Nadkarni, developed with the help of Loling Song)

Work as much as possible in clean environment, under the flow hood.

MAKING AND CURING PDMS PADS

1. Rinse the master (when reusing) with IPA and rinse off with distilled water. Blow dry with air/argon/nitrogen. If there are pieces of PDMS stuck on it, then try to pick them up with some magic tape (physics stock room). Do not use magic tape on the pattern part of the wafer. If you are using the master for the first time, you can pour PDMS directly.
2. Take a weigh boat (Chemistry stock room) and with a Kim wipe (use texwipes when we get them), dust off any visible particles and plastic pieces and fibers (sometimes from the weigh boat itself).
3. Place the weigh boat on the PDMS balance (Denver Instrument Company XE series, model 300) and zero it. Wear clean gloves. The blue nirtile gloves sometimes have a powder like substance on them. If they get wet, wash them well and dry your hands. Pour the approx 90g PDMS (Polydimethylsiloxane) (Sylgard 184 from Dow Corning – distributor – Wolcott Park Inc.)
Shake the curing agent and pour $1/10^{th}$ of the weight of the PDMS (elastomer/curing agent ratio = 1:10). With a transfer pipette head, thoroughly mix for at least a couple of minutes.
You can also use a plastic Petri dish to pour the PDMS in, however, while removing the excess PDMS after all the stamps are cut, is harder and there are chances of breaking the wafer. The weigh boat is made of soft plastic and can be cut easily.
Do not make the total volume greater than 100 g since then the PDMS is too thick and will not be easy to punch and stick tubing in later on. Also, the inner cavity of the punched part remains hydrophobic and making PDMS too thick makes the chances for air bubbles greater. Do not make it too thin either.

4. Place the dry master in another weigh boat (also dusted to make it clean) and pour the PDMS on top. Cover with another weigh boat and take it to the desiccator.

5. Place in the dessicator (Company and part number) attached to the vacuum pump (Welch, Directorr 8905 Vacuum Pump) without the cover and start the vacuum. The PDMS will start bubbling within minutes. If there is too much PDMS, it spills over, so keep some texwipes under the sample.

6. Let all the air be pulled out. No bubbles should be seen to be coming out of the PDMS. Usually the process takes about 45 minutes. It is okay to release the vacuum to pop

bubbles out if you have been degassing for a long time (e.g an hour and a half). Probably the vacuum is not as good as it can get.

7. Put in the oven (Memmert – Wisconsin Oven) with the cover (another weigh boat) on and bake for 70 minutes at 60 C.

8. Replace the texwipes inside the dessicator if there has been a spill of PDMS. Do not wait till later because the PDMS will cure in 24 hours and it will be harder to get rid of then.

MAKING A CHANNEL FROM THE CURED PDMS

Everything should be handled dry and clean.

1. Cut the PDMS midway between the two patterns using a sharp knife (used from the tool box Precise Accu-Knife Set, Control Company Huston). Wipe the knife with an IPA clean room pre-entry wet wipe (Liberty, Model WW05).

2. Peel off carefully and check that no SU-8 is getting lifted off. Sometimes SU-8 gets lifted off and then the pattern is not good anymore.

3. Protect the entire PDMS surface (channel side) by using strips of "magic tape"(Scotch tape – phys. Stock room). Try to cover the main part with one strip and put two more on either side.

4. Use a 1.5 inches long, 19-gauge, stainless steel needle (75165A755, Dispensing needle, McMaster -Carr) with polished tip (polished by machine shop) to punch holes in the inlets and outlets. Make sure that the needle is clean from the inside (can rinse with ethanol/IPA, not acetone since that can eat up the plastic) and from the outside by wiping with an IPA wipe.

5. Push the needle all the way through from the topside to the channel side. With a precision knife, make an incision in the magic tape at the opposite – channel side so that it is easy to push the needle through. From the top side, push the needle completely out.

6. Remove the PDMS noodle from the core. The noodle should pop out by itself. If it doesn't, use a clean, dry syringe to push some air through and pop the noodle out.

7. Use magic tape to remove any residual PDMS fragments from the needle surface, or carefully scrape them off with the knife before withdrawing the needle from the PDMS. Withdraw from the holder end, slowly and smoothly, holding the stamp down on a hard surface.

Plasma cleaning:
Plasma cleaning is done in the Harrick Plasma cleaner/sterilizer PDC-32G.

1.Clean plasma cleaner cavity with a wipe and give a general wipe to nearby surfaces as well.

2. Leave the previously used filter (0.22 μm pore size- Acrodisc Filters, Pall Corporation, ref. 4192) which should be already on the inlet nozzle) on and close the needle valve. Pre-evacuate the plasma cleaner as follows:
Turn on the pump.
Turn on the power switch of the plasma cleaner.
Leave them on for 1 min – 2 min. The first time you turn on the pump, it takes longer for the necessary level of vacuum to be reached.
Turn on RF to high.
If the vacuum level is right then a bluish violet color will appear. If a pink color appears, or no color appears then it means that the required level has not been reached. For this pre-run it is not so critical, however, when the sample is in it is important that the bluish color appears when the RF is turned on high.

Slowly let air in till through the needle valve till color changes from blue/violet to pink/purple.
As soon as pink/purple color appears, stop letting in the air. Hold this pink/purple plasma for 1 min 30 sec. (You need not wait for this period of 90 seconds when no sample is in – it is optional).
Turn RF off.
Let the air in gradually.
Turn off the Plasma cleaner and the pump off.

Plasma-clean the surfaces of the substrates:
1. Wear clean gloves. Start with fresh gloves if necessary. Also wear gloves that fit your fingers so that when the PDMS and glass slides are picked up by the sides, the material of the gloves does not touch the surface.
2.Give the cavity another clean with IPA wipe.
3. Carefully pick up a prewashed, dried glass cover slip in first into the cavity of the plasma cleaner.
4. Load the PDMS with the channel surface up and remove the tape when the stamp is inside the cavity.
5. Close the nozzle and hold the door (even after the vacuum is reached).
6. Turn on the pump and power supply switch.
Wait for about 45 sec to 1 min.
7. Turn on RF to high. The bluish color should appear right away. Else evacuate more.
8. Release vacuum by letting the air in slowly.
Monitor the color change from blue/violet to pink/purple. There is a small window where the color is a deep pink (almost looks like focused light). This is how I make this process reproducible.
9. Stop letting air in and hold the pink color for 1 min 30 sec.
10. Turn RF off.
11. Let the air in gradually.
12. Turn off the Plasma cleaner and the pump off.

13. When the system comes to 1 atm back again, the lid comes off. Place it on a clean surface. With a long forceps (which should be clean) and fingertips, slowly bring the PDMS slab out taking care not to touch the surface that has been plasma cleaned. Keep it down on a texwipe. With the forceps, bring the glass slide out and pick it up with your fingertips. Slowly bring into contact the surface of the glass (that got exposed to plasma) and the plasma oxidized PDMS surface. They should bond immediately and no air should get trapped in between them. If it does, you can press gently and try to get it out. Do not press too much since that interferes with its natural bonding.

Do not do this process on a clean glass surface because the other side of PDMS can stick to it. This seal is reversible, however, while picking up the PDMS, you can stress the glass – PDMS bonds. Do not assemble it on an Al foil either since it is hard to see if any air has been trapped in between the surface because of the reflection from the foil.

Literature says that PDMS stays hydrophilic for about half an hour after it has been plasma cleaned. (The reference I have is not a formal publication, however, I have seen it in the Whitesides paper – I will have to relocate it).

Pre-Washing the glass slides.

This may seem trivial, but is a very critical process. The bonding of the glass to PDMS is highly dependent on how clean the two surfaces are.

1.In hot water (boil water if the tap water is not hot enough) add soap and in the holder (found in the lab - must have been machined in the machine shop) place glass slides in the slot. The glass slides used are 22 X 40, No.1 (Gold Seal cover glass – chemistry stock room). Soap used is a glassware washing powder – Alconox. (Chem. Stock room).

2.Leave overnight or at least for a couple of hours.
3. Wear new gloves and wash them thoroughly because the blue nitrle gloves have some soap like substance on them.

4. Use the cover slip forceps (Fisher - Chem. Stock room) to pick up the slide. Hold it with the gloves and wash it well under warm tap water for at least a minute till all the soap goes off. Wash the forceps well also.

5. Hold the cover slip with the forceps under running distilled water for a final rinse.

6. Hold under the hood and blow dry with argon or nitrogen (Air gas Company). Once one end becomes clean, hold the cover slip by the edges with gloves. Dry the gloves on a Kim wipe while holding the cover slip with the forceps. This is done because the forceps are not always clean. This way, the residue left near the place where the forceps touch the slide, also goes off with the water.

7. Place in the slide box. This should be dry and clean to begin with.

Prepare this before punching a stamp.

MAKING CONNECTIONS FOR THE LIQUID DELIVERY

If you are connecting a dry stamp, pre-fill all the syringes and tubing even before you cut and punch a fresh stamp. There are advantages in filling dry.

1. You fill directly from the tubing, by capillary action and this solution is usually buffer, so it is clean and filtered.
2. Air bubbles trapped in the system pop out if you flow long enough.

Two types of syringes are used:

1. 500 ml Hamilton Gastight syringe with Teflon Luer Lock (1700 Series,Cat. No. 81220, Hamilton Company).
2. 3 ml BD sterilized plastic syringe with Luer Lock. (BD cat. no-309585, distributor – Fisher Sci.)

24-gauge tubing with hub (Hamilton, Cat. No 90624, Teflon Tubing with Kel-F Hub) is connected at the outlet end of a three-way stopcock (company).
Connect the Hamilton syringe at the horizontal end of the stopcock and the 3ml syringe at the side inlet. First fill the 3ml syringe with DB and connect to the side inlet with the tubing connected at the outlet. Fill the tubing completely with DB. Flow out some excess so that the tubing gets cleaned in the process. Close off the tubing side with the switch. Fill the rest of the stopcock with the 3ml syringe connected so that water overflows from the other side. Fill the Hamilton syringe with DB completely. Ensure that there are no air bubbles trapped. Tap the syringe to make the bubbles rise if any are in the syringe and push them out. While connecting to the stopcock, make some excess liquid come out of both the Hamilton syringe and push the 3ml syringe so that water overflows from the stopcock. In this situation, make the connection between the two water fronts and push the gastight syringe in place. Now, remove the 3ml syringe and refill it so that there are no air bubbles in it. Repeat a similar procedure, now, pushing out excess from the gastight syringe through the stop cock on one side and pushing excess from the plastic syringe on the other. And connect the two water fronts. This process should ensure that there are no air bubbles in the system.

Air bubbles can be a very major problem and cause pulsing making the gradient unstable.
It is very important to have the system bubble free.

For the outlet and cell inlet, connect 1/2 inch long, 23 gauge needle tips (75165A684, McMaster Carr) to 24-gauge tubing (WTFT 24, Physics stock room). Connect a one-way

stopcock to the luer lock tip. With another 3 ml syringe, push some liquid through so that the tubing gets cleaned and then keep the tubing filled and the syringe connected.

After the stamp is assembled after plasma cleaning, as soon as possible transfer it to the location of the microscope. (You can keep it in clean, filtered water for later use, however that increases the chances of air bubbles and dirt getting in the channel).

Put a drop of DB on one of the inlets. If you put it on the other inlets there will be an effective pressure built up and capillary action wont act by itself. The liquid should start flowing immediately and within minutes the channel is completely filled. Insert the tubing through the pre punched hole at the inlet where you put the drop. You should not have to apply too much force. That may lift off the pad if its not stuck very well.

Next connect the cell inlet tubing with stopcock and syringe connected. If you are loading cells, you can draw some cells in before connecting. Make sure you do not have too much DB in the syringe else you will get a huge dilution factor. Once the tubing is in, close the stopcock and take the syringe out. Open the stopcock before connecting the next tubing – the outlet tubing. Do a similar procedure – draw cells, insert tubing, close the stopcock and remove syringe. Open the stopcock.

At this time there will be three tubings connected. Usually, the channels should be completely filled by this time. There may be a small air bubble either at the cell inlet or at the other inlet. Use the infusion syringe pump (Harvard Apparatus, PHD2000, Infusion) to flow from one inlet to the other so that there is DB coming from the hole and then connect the remaining inlet tubing. Make sure that when you do this there is at least one hole that is open. If the cell inlet and outlet both have their stopcocks closed then this can pressurize the system and lift the channels. By now, there should be no air bubbles or some at the cell inlet/outlet. In that case, you can flow for a while till they come out. You will have to re-load cells in that case.

For reloading, preferably use the outlet because even if there is an air bubble that gets in, it will go out eventually and wont affect the flow.
While reloading, keep flowing while disconnecting the outlet tubing. Once you see some Db come out from the hole, stop the flow. Open the cell inlet stopcock if it is close. Draw some cells into the syringe and connect the tubing. Cells will immediately start flowing from the outlet to the cell inlet. Close the stopcock; disconnect the syringe from the outlet. Close the cell inlet stopcock. This should hold some cells in the main channel. Give them about an hour to settle.

Usually if cells are loaded this way, they should just remain in the area between the outlet and the cell inlet and they do not flow back into the network. If you use gravity, you may have a larger proportion of cells flowing backward because of the difference in pressures. Even if that happens it is not a real problem.

Give the cells a good amount of time (1- 1.5 hours) to settle and then start flowing at 0.5 μl/min. The cells usually tolerate this speed.

If they are round or old they may not attach.

Concentrating cells :-

Take 10 ml of cell culture and with a balance, centrifuge for 900 rpm for 20 seconds. Remove supernatant 9 ml gently. Shake remaining 1 ml up and down gently and transfer into a vial using a pipette. Depending on the concentration desired and how old the cells are this centrifuging process can be modified.

If you want very low density, you can use the cell culture without any centrifugation.

MAKING REUSABLE MASTERS

(NBTC facility – enter with certified I card)

Spinning wafers:
(The wafers are obtained from CNF staff – 4-inch wafers, n-type or p-type doesn't matter.

A) Preliminaries:

1. Logon at the terminal. This switches on the spinner. (If you are planning to use the hot plate on the spinner, logon two hours prior so that the plate reaches equilibrium.)

2. Before entering the clean room ensure you have everything you need. Get the PDMS balance from the chemistry room (currently it is the balance to your left as soon as you enter the room). (Company – Navigator, least count =0.1g)

3. Wipe down everything and gown as per rules.

4. Switch on the vacuum system in the side room.

5. When you are starting a new spinning session, change the Aluminum foil, which was used by the earlier user. This is because SU 8 is given time to dry before the foil is removed so that it doesn't spill everywhere while cleaning up. You do not have to change the Al foil when you are done, but leave the SU 8 to dry and the next user will replace it. Give the lid of the spinner a wipe with acetone and the general working area should be given a wipe with acetone. SU 8 readily dissolves in acetone.

6. There are 3 hot plates needed for the spinning process - at 200 C, 65 C and 95 C.

For the 65 and 95 C, use the digital hotplates (VWR 555) in the clean room. (These belong to the BMEP lab but you can use them). For the 200 C you can use a similar kind of hot plate or the analog hotplate belonging to the NBTC (Themodyne, Type 1900).

Set the digital Hotplates –

1. Plug in, Swtich on. Press the on/off to on.
2. Temperature will start rising to set point and the timer will start counting down.
3. To change the set point, press the 'up' or 'down' key till you hit the temperature you want to reach.
4. When you leave the button, for a while the system will sense that no change has taken place in the set temperature. The set point will then blink and the actual temperature will start increasing or decreasing to your set point.
5. Similarly adjust the time setting. You can reset the time in the middle of the count down as well.

Level the hotplates:
It is important that after SU8 is spun, it is kept on a leveled surface. As best as possible, using paper and the adjustments on the plate itself, level the two hotplates at 65 and 95 with a leveler. The plate at 200 C need not be leveled.

Program the spinner:

Spin parameters depend on which resist (SU-
8 50, 25,10, 5) you are using and what thickness you want to spin. Choose the resist in such a way that for the desired thickness, the corresponding parameters lie in the middle of the spin speed curve (MicroChem catalog).
The SU-8 is spread in one cycle and spun in another. So first ramp up to 500 rpm at 100 rpm/sec and hold. Total time =10s (5secs to ramp and 5 secs to hold). Next ramp to 2000 a 400 rpm/sec. Total time =35 sec

Program instructions –

Press – Prog
Enter -1
Vel. 0 = 500
Rmp =100
Time=10
Vel 1=2000
Rmp=400
Time=35
Vel 2=clear and enter (ends the program).

B) Spinning:

1. Wear fitting gloves. Pick up a wafer by the sides. If you want, you can pre-clean the wafer by spinning acetone/IPA before you spin SU-8. Or pre-clean and blow dry under

the hood. (The wafers out of the box are clean and sometimes, this procedure may make them dirtier than they started with).

2. Place it on the 200 C hotplate for two minutes. Timers are available in the NBTC.

3. With a wafer tweezers (available with CNF staff), pick up the wafer and place it on the spinner chuck.

4.Ensure that the vacuum is on; else the chuck will fly off.

5. Press 'Run' on control panel.
Program? Is displayed. Press '1'.
Hit start. The display will say "testing centering" . If the wafer wobbles while spinning, the centre of the wafer is not at the center of the chuck. Observe which direction the throw seems to be when the chuck rotates. Move the wafer in the other direction. Press 0 to re-test. When the wafer is centered, no wobbling will be seen. Blow off any dust particles seen by eye, with the Nitrogen gun.

6. Get the SU-8 50 (MicroChem – Product no. Y131269, negative, radiation sensitive resist) bottle (group bottle is a smaller bottle labeled) from the chemicals cabinet. SU-8 50 is highly viscous and so pouring directly from the big bottle is not advisable since it is hard to control. *Pour SU-8 from the bigger bottle to a smaller, preferably amber, bottle (since the resist is light sensitive) well in advance. (at least a day or two). If you pour it just before you spin, then in the transfer, some air bubbles get in and they remain when you pour SU-8 onto the wafer. They can make the surface of the SU-8 non-uniform after spinning.*

7. Weigh the SU-8 bottle before pouring (keep its lid on). With a steady hand, pour about 4 g of SU-8 onto the centered wafer. Pour in the center of the wafer, with the hand very close to the wafer. When you want to stop, slowly change the angle of the bottle, while rotating it. There will be a point when the stream will cut off itself from the rest of the poured SU-8. Take care not to have any streaks of SU-8 on the master before you spin.

8. Close the lid and press start. Wipe with acetone any SU8 that may be sticking to the sides of the bottle and on its rim. SU-8 is soluble in acetone and hardens quickly. So wiping it off right away prevents the bottle cap from getting stuck to the bottle in due course of time. With the bottle cap on, weigh the bottle again and record amount of SU-8 used for the wafer. (Ideally it shouldn't matter if SU 8 is in little excess since it would just get spun off.)

9. Pre bake:
When the spread and spin cycles are over, press reset. Open the lid and pick up the wafer by the sides. If there have been no air bubbles or dust particles in the SU-8, the

spinning should give a smooth looking surface. Sometimes, it seems like air bubbles have burst in the process and there are "crater" like depressions.
And if there is a slight non-uniformity, then there will be interference fringes. If overall, the coat looks smooth, you can use it.
(Once a wafer is spun, SU8 is hard to get rid off. It dissolves in acetone, but it is too viscous to get it off and reuse the wafer is you make a mistake. It is better to start with a fresh wafer.)
Pick up the wafer by the sides and place it on the hotplate at 65 C for 6 minutes. (Abe Stroock's suggestion is to leave it here longer for non-uniformities to relax. It will not affect other procedures and the final master. However, I have not tried this. May be worth waiting for 10 minutes instead). Plate should be leveled.
Place a Pyrex cover or a Pyrex container as a cover on the wafer, leaving a gap for the SU-8 solvent vapor to escape. (Do all these things under the hood). This is done to prevent dust particles from falling in during this.

10. After 6 (or 10) minutes, pick up the wafer and place on the hotplate at 95 for 20 minutes. Again, keep a cover on it with a support under it so that vapor can escape.

11. Place in the wafer tray (obtained in physics stock room) with face (SU-8 side) up and store on a level surface so that the SU8 level stays uniform.

12. Once the SU8 dries, the wafers are ready to expose. I have exposed wafers sometimes within an hour after spinning, sometimes a month after spinning. I have not seen a preference for one over the other. It is conceivable that keeping them too long is not a good idea because they may be getting exposed to some ambient light (although the light in the clean room is yellow light). Also store with an Al foil wrapped around. Do not take the wafers out of the clean room boundaries since they will get exposed to white light.

Exposing:

1.Logon outside the clean room. Once inside, switch on the power switch and the lamp. A screeching noise is heard when the lamp ignites. Let the lamp warm up and equilibrate for at least 15 minutes after switching the lamp on.

2.Blow off the mask (kept in the clean room – has the group name on it) with a nitrogen gun. Keep a hotplate at 65C ready for placing the exposed wafers.

When in the HTG room, keep the wafers on the table, beyond the curtains, to avoid any unwanted exposure by the light.

The following procedure is for flood exposure.

3.Put the exposure time to 0000 if it is not already. Clear the flood exposure area and give the area a wipe with did. water. Place a clean uncrumpled texwipe and place the mask on the wipe. Bring the head of the HTG above the mask. Press test to shine the light and

align it so that the mask is symmetrically below the light. This should be done quickly to avoid exposure to the light. With scotch tape from the other clean room, hold the texwipe down on the base. Mark the position of the mask with a pen and draw its outline.

4.Bring the head to the position above the wafer chuck by pressing cycle. It will momentarily expose light because although the setting is on 0, there is still some time for which it shines the light. This is done just so that the HTG head is out of the way when you are adjusting the wafer position.

5.Place the wafer as accurately as you can in the middle of the marked area so that it is symmetric w.r.t the mask. Place the mask on top and gently give a tap to ensure that there is no gap between the wafer and the mask.

6. Bring the head of the HTG over the mask by pressing "cycle" twice and set the exposure time (in seconds).

7. Press expose and wait till exposure is over. Look away from the lamp.

8. Pick up the mask and the wafer. Place it face down on the tray. (Do not move the HTG head before this. Pressing cycle when the HTG head is over the flood expose area, will make the HTG head go above the chuck and exposure will start. Even if you have reduced the time back down to zero, this could cause an unnecessary exposure of the mask if the light is leaking or you are not careful. If you have your original time setting then this exposure will last that long.)

9. Take the wafers to the other clean room with the hot plates. Place the wafer on the hotplate at 65. (At this stage it is not so critical to put a cover on, but it may still help keep dust away). After a minute, increase the setting to 75. When it reaches roughly 75, leave it there for a minute and increase to 85 C. Increase thus in steps of 10 C till you reach 95. Keep at 95 for about 3-4 minutes. The temperature sometimes rises beyond the setting. (For ex. Instead of 75 C it may hover around 75.8.) As long as it is close to the setting and in equilibrium, it should be okay.
Ramp down: After the wafer has been at 95 for about 4 minutes, slowly ramp down, decreasing in steps of 10 like in the ramp –up case.
When the wafer is near 65, you can take it out from the hot plate and hold it in your palm till it cools off. Then place in developer.

Ramp up and Ramp down each take about 20 minutes. So total process is 40 minutes.

Note: *This is very different from the Microchem protocols, which requires a bake at 65 C for 1 min and at 95 C for 5 minutes. However, this makes the SU-8 crack since it has a small coeff of thermal expansion. So a slow ramp up and a slow ramp down are worth the wait.*

10. While the wafer is sitting on the hotplate, pour 200ml of the SU-8 developer (PM acetate – Microchip Product no. Y020100) into a clean Pyrex container. Wear the thick green nitrile gloves. The SU-8 developer and IPA are in the chemicals cabinet. Pour approx. same amount of Isopropyl Alcohol in another container. When the wafer is in the developer, shake gently so as to have a rinsing effect and take away the unexposed SU-8. Do this for 6 minutes and then pour out the SU-8 into the SU-8 waste bottle. Refill with approx. same or little less quantity of developer and redo the process for 4 minutes. Pick up with the tweezers and put in IPA for about two minutes and shake gently. If there is a whitish film observed, it means that the development is not over. Put back in fresh developer and rinse with IPA again.

11. Give another spray the IPA from the squeeze bottle and blow dry with the nitrogen gun.

12. Keep face down in the tray and use the Olympus microscope (Olympus B01) in the clean room to make sure that the master looks good and the features are well defined.

Sometimes, the SU-8 may not get completely cross-linked usually if exposure is not enough. When this happens, the SU-8 starts getting washed off from the pattern and fringes can be seen because of varying thickness of SU-8. If exposure is too much, then the features do not get very well resolved. Especially near the narrower parts of the pattern (e.g. junction of the 10 channels in our case). One needs to figure out an optimum set of parameters that work for the requirements of the master.