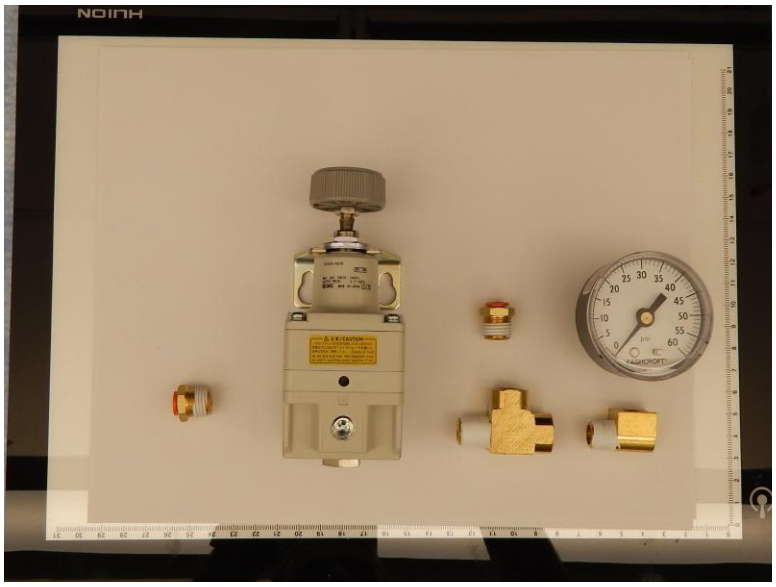
Step-by-Step Setup Guide

Pneumatic Control System Module 1: Flow Control Board and Pressure Manifolds

Part 1: Individual Part Assembly

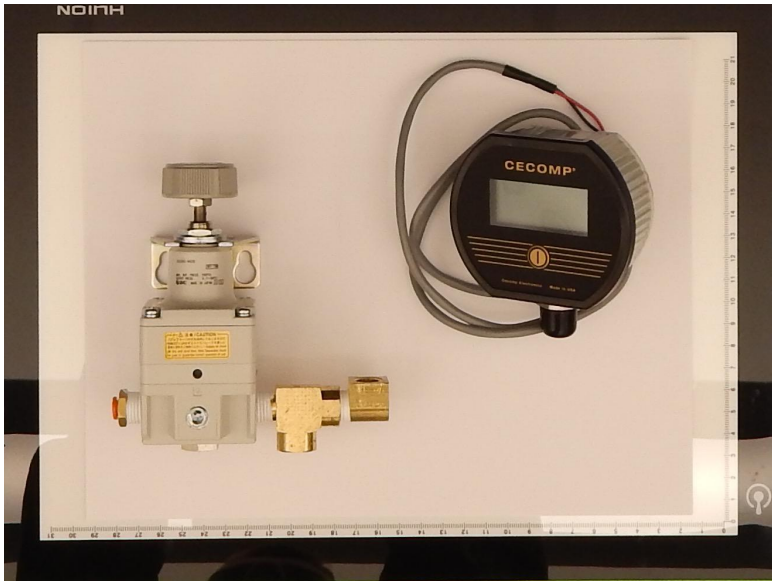
1. Begin by assembling **Parts A - E** for the manifold regulators. These assemblies globally regulate control line reservoir pressures for chip-valve actuation and reservoir loading.

2. Use Teflon tape to tightly wrap the threads of any metal-to-metal male fitting to create a leak-free junction. All metal male fittings must be Teflon-wrapped in all future steps.



3. Assemble the Regulator parts as shown below by carefully threading the male fittings into the corresponding female fittings. Tighten with a wrench to full tightness. This is important to prevent air leaks at junctions.

4. Similarly, assemble **Parts C-E** and **Parts F, G** for the digital manifold regulators. These regulators control flow line pressures when connected to devices via flow control manifolds.

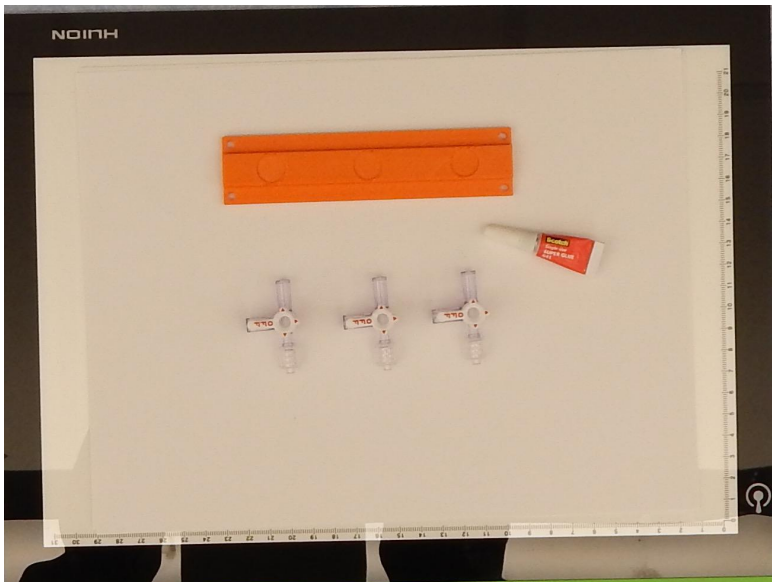


5. Attach the digital regulator display **Part G** last, being careful to properly thread the display to face outward.

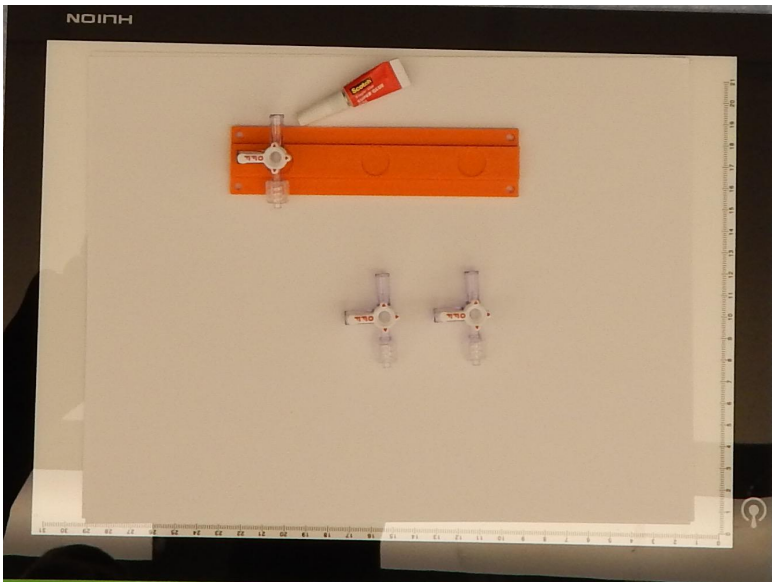
6. Add the push-to-connect tube fitting **Part C** after alignment of the digital display (from the previous step). Tighten fully for a leak free junction.

Before (above) and After (below) addition of **Part C**

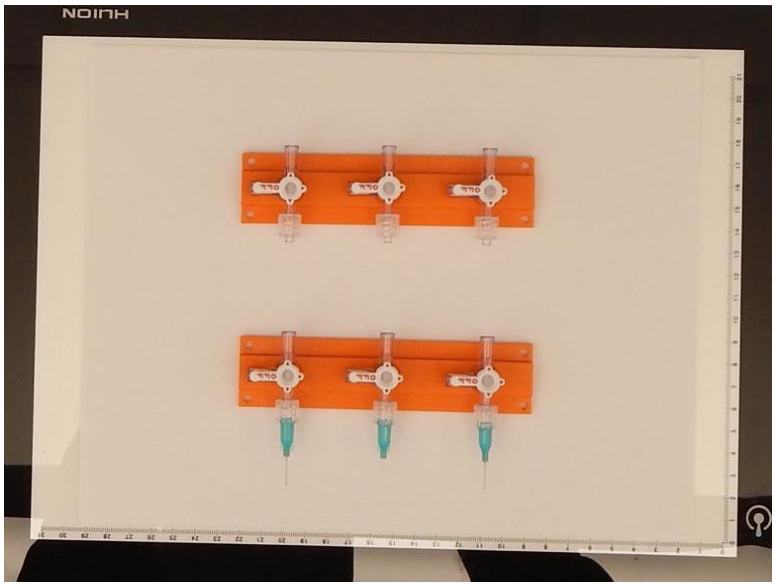
7. Assemble **Parts K - O** and the **3D Printed Part "Flow Control Manifolds *stopcock-mount*"** to make the flow manifolds. These control the pressurization state of individual flow lines by selectively turning on or off the 4-way stopcocks during a device run. Print the 3D printed part with high-density material and let dry at ambient temperature for 1 hour after printing before attempting to glue parts.



8. Using SuperGlue, carefully glue the back of the 4-way stopcock (**Part N**) to the 3D printed flow manifold. When all 3 stopcocks are in place, let dry 1 hour.

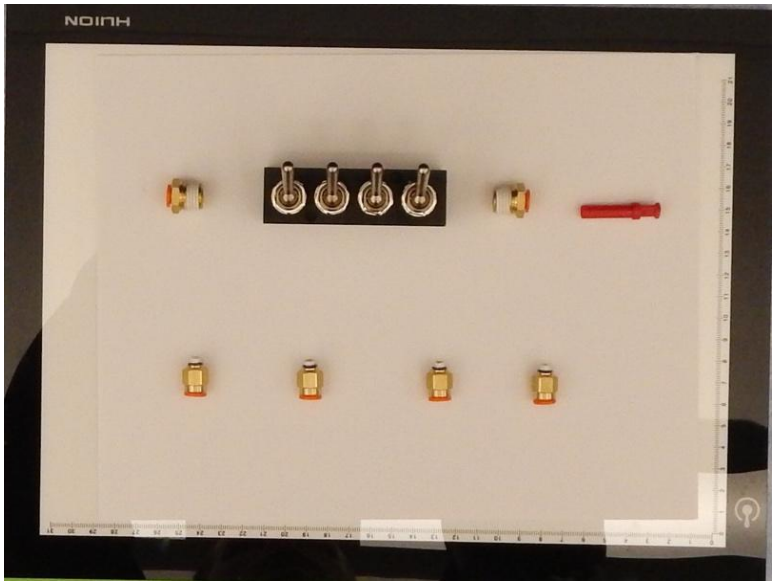


9. When dry, lock on a luer-lock blunt tip needle (**Part O**) to each stopcock. Complete 5 additional manifolds for the full build described in the main text (or the number of manifolds desired for your application).

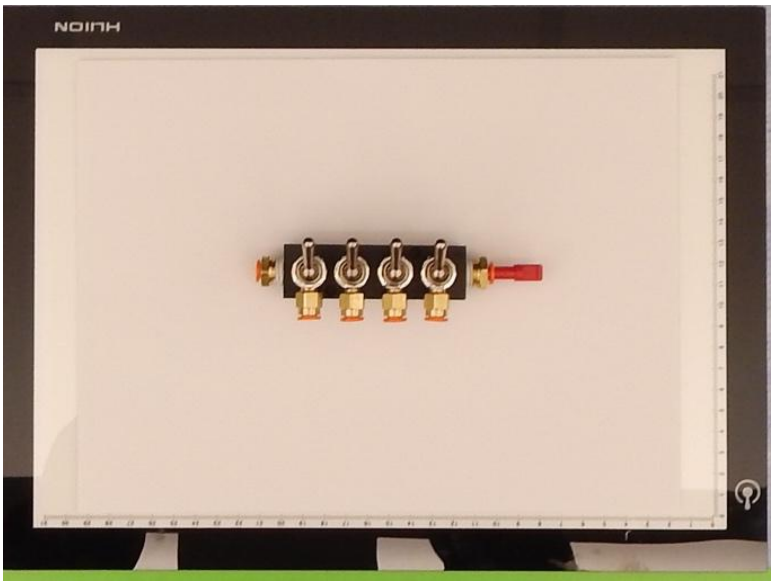


10. Gather **Parts P-S** and **Part C** for global pressure manifold control switch boxes. These switch boxes selectively pressurize control reservoirs connected to the manifold regulators, making it possible to turn control line banks on or off before and after experiments.

11. Thread in the dented toggle switches (**Part Q**) on the outer box (**Part P**).

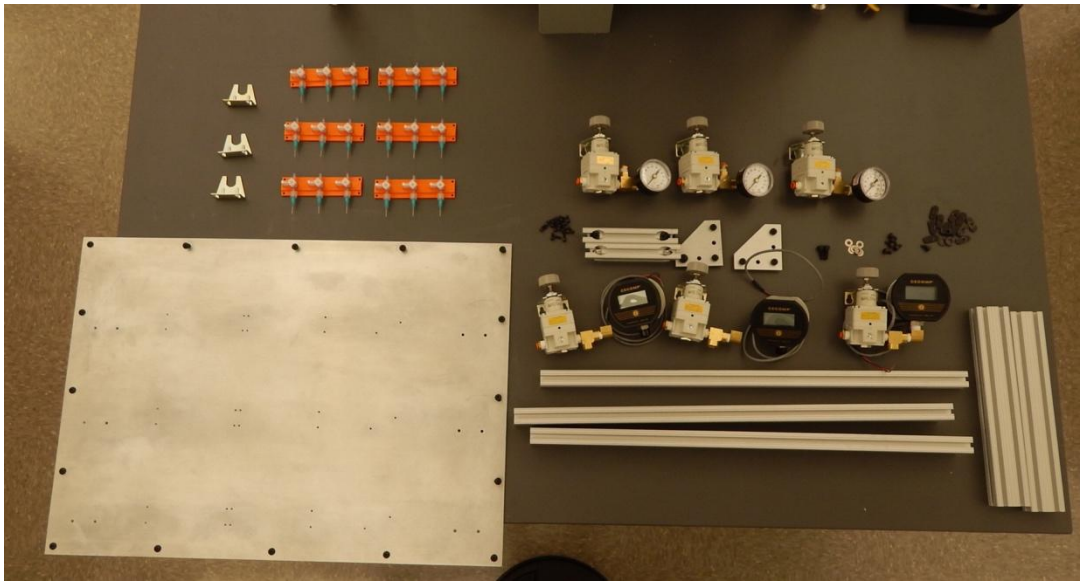


12. Connect the 10-32 UNF connectors (**Part R**) to the 10-32 output of the dented toggle switches and assemble the other connectors (**Parts C, S**) as shown. Set aside.

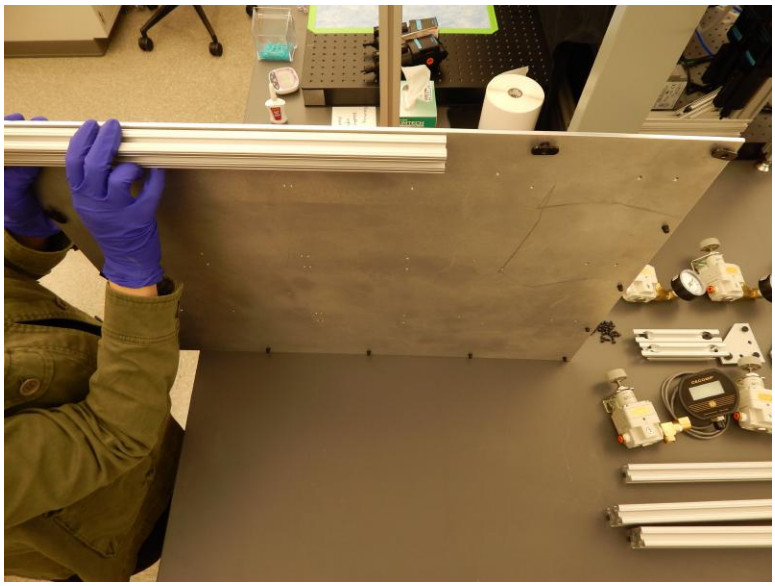


Part 2: Base Board Construction

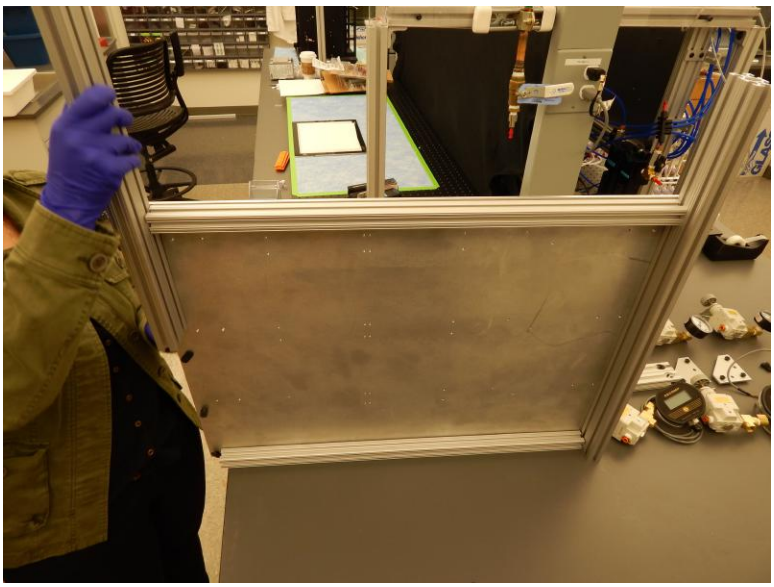
13. Machine the Flow Control Board (**"Base Board *backboard-full*"**) according to the given engineering drawings. Acrylic can be used instead of aluminum, if desired. Holes for regulator attachments must be tapped according to the drawing. Gather **Parts b1-b8** to assemble the Flow Control Board and supporting mounts. Additionally, gather the pressure manifold regulators, digital flow regulators and flow manifolds assembled in **Steps 1-12** of **Part 1**. Place the 80/20 screw fasteners (**Part b5),** without wings, on the Flow Control Board (**"Base Board *backboard-full*"**) as shown.



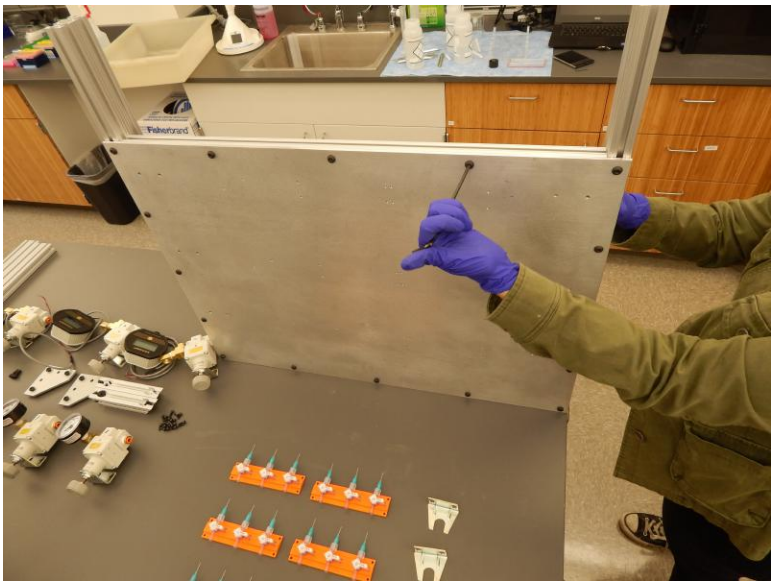
14. Gently attach wings to the end-feed fasteners (**Part b5**) on the top back of the Flow Control Board and gently guide the first piece of 80/20 (**Part b2**) through the wings.



15. Repeat for the 3 other sides of the back Flow Control Board, using the 26" x 2" x 1" 80/20 (**Part b2**) laterally and the double 26" x 2" x 2" 80/20 (**Part b1**) vertically as shown below.

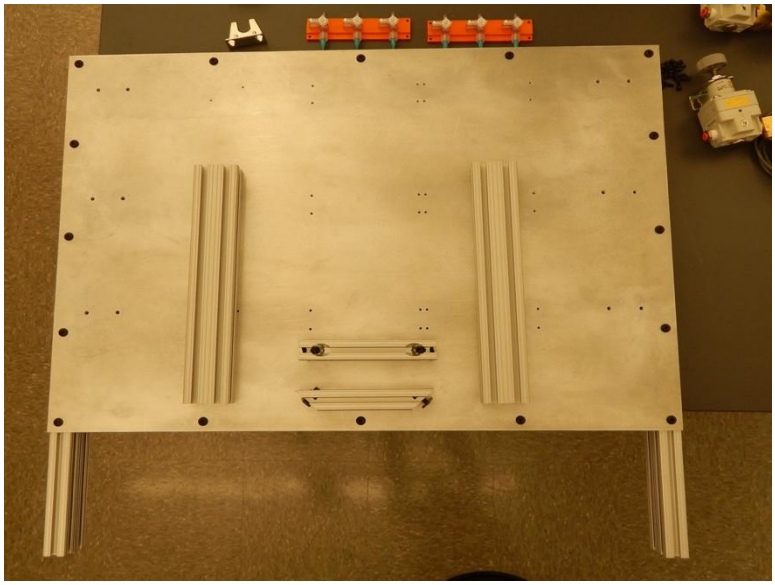


16. Fasten each end-feed fastener (**Part b4**) from the front of the Flow Control Board using an appropriate sized hex key. Check the stability of the rig by moving each 80/20 back and forth, and adjust screws until the fasteners don't move when wiggled.

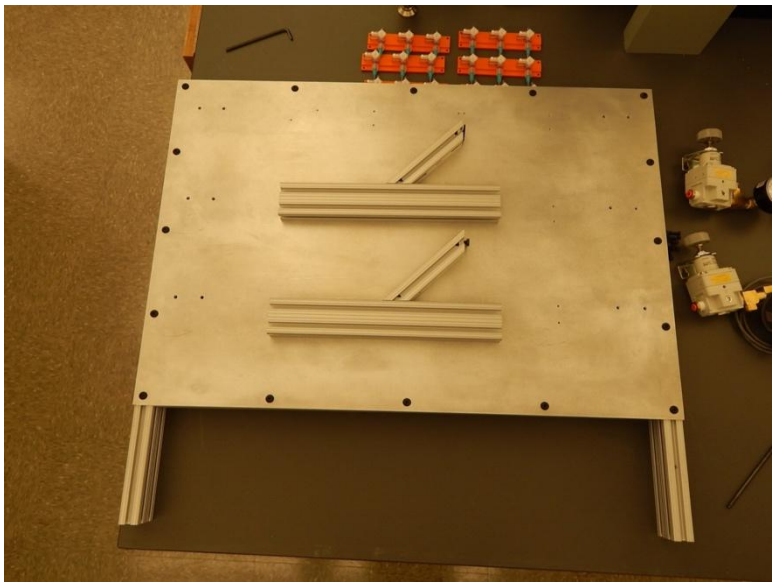


17. When the initial structural support is completed, your setup should look like the following image from the back.

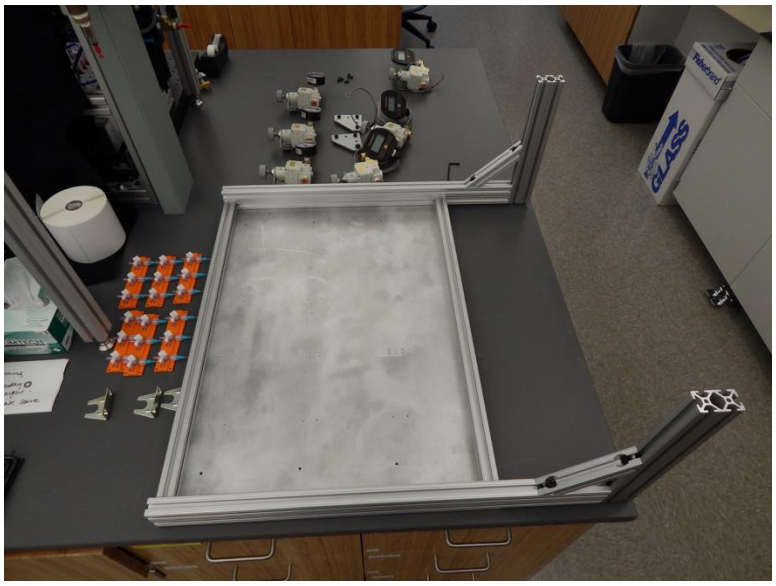
18. Assemble the support legs (**Parts b3, b4**) with accompanying fasteners.



19. Slide the wing nut of the diagonal 80/20 (**Part b3**) into the 12" x 2" x 1" support leg **(Part b2**) and loosely fasten. Allow enough give for the diagonal to easily slide along the leg. This pre-assembly will help during stabilization and alignment.



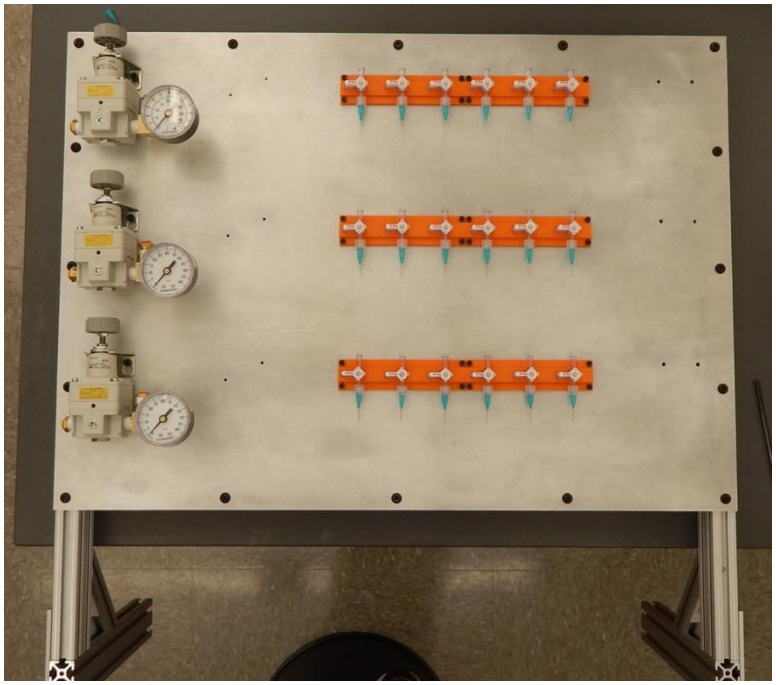
20. Assemble the support legs as shown from the back of the Flow Control Board. Tighten with a hex key and ensure the structure is level upon standing.



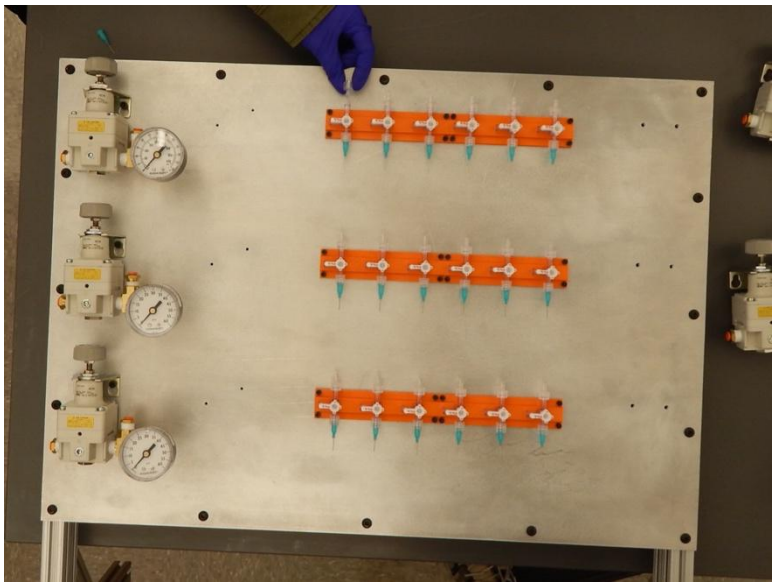
21. Stabilize the support leg structure with a 5-hole corner surface bracket (**Part b8**). Tighten with a hex key.

Part 3: Base Board Attachments

22. Attach the pressure manifold regulators and flow manifold parts with 10-32 screws (**Part s1**) to the shown positions on the Flow Control Board. Tighten with a hex key.

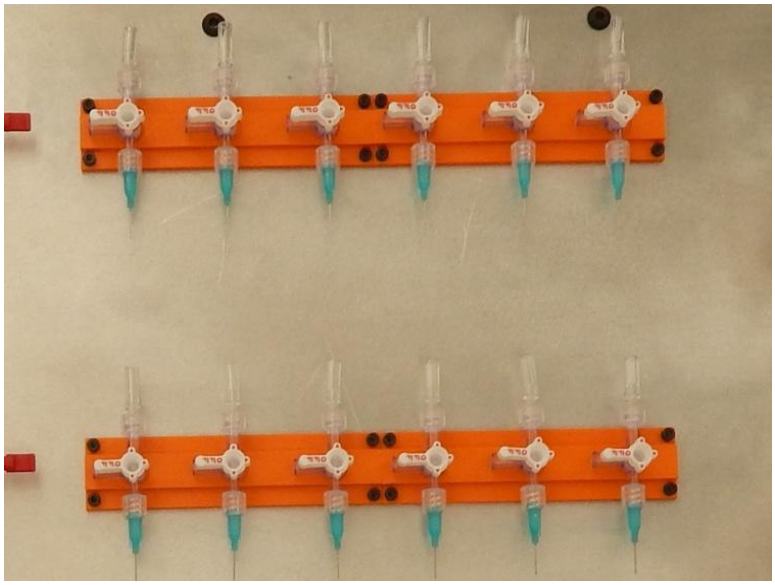


23. Carefully attach the male luer integral ring lock (**Part M)** to the female luer connector on each 4-way stopcock (**Part N**) of the flow manifolds. Optionally, these steps can be completed before affixing the manifolds to the Flow Control Board. In our experience, the board adds additional stability for mounting the manifold components.

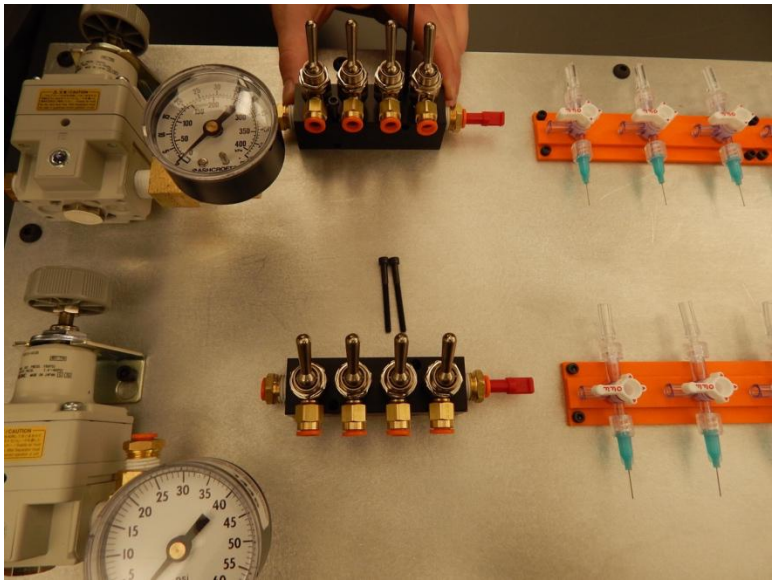


24. Cut approximately 50 - 1/2" segments of **Tubing T2** with scissors. Attach a segment to each integral ring lock (**Part M**) stopcock assembly by firmly pressing the tubing past the barb. Save the remaining segments for the later T-junction top assembly (using **Part K**).

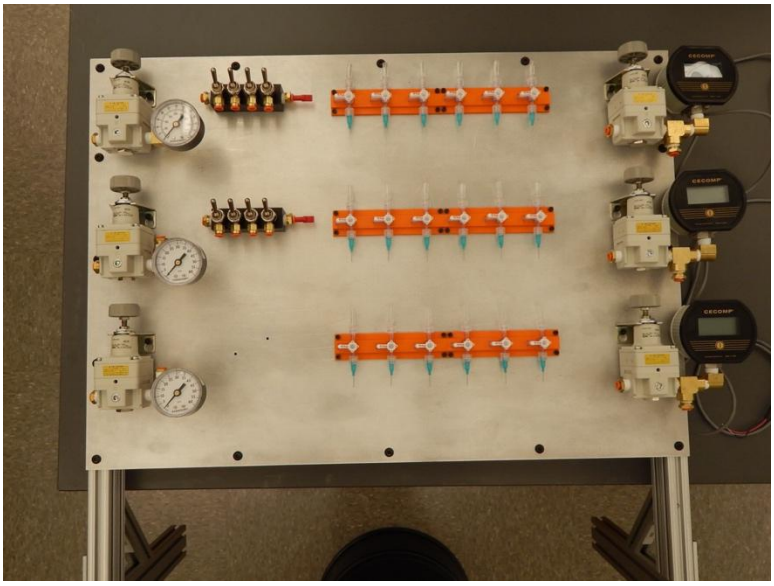
(!) **Tip:** You can use a heat gun to help attach the tubing segment by expanding the tubing.



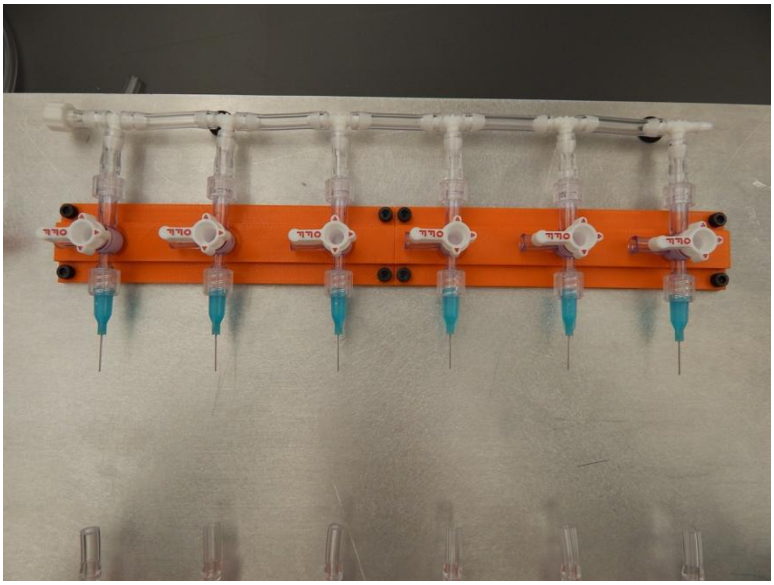
25. Install the manifold control switchboxes on the Flow Control Board with the long mounting screws (**Screw s2)** using a hex key and carefully aligning the control box to the tapped holes as shown.



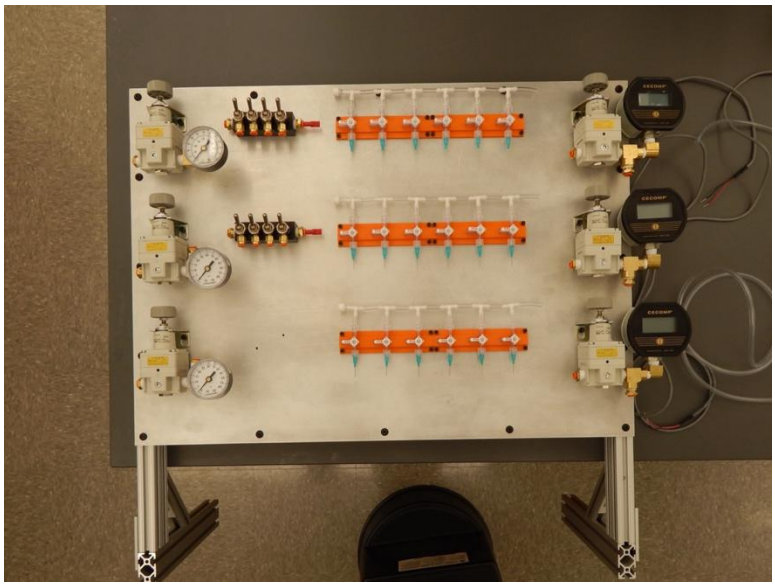
26. Attach the digital flow control regulator assembly from Step 6 with the 10-32 screws (**Screw s1**) at the baseboard positions shown below. At this point, the Flow Control Board should appear like this:



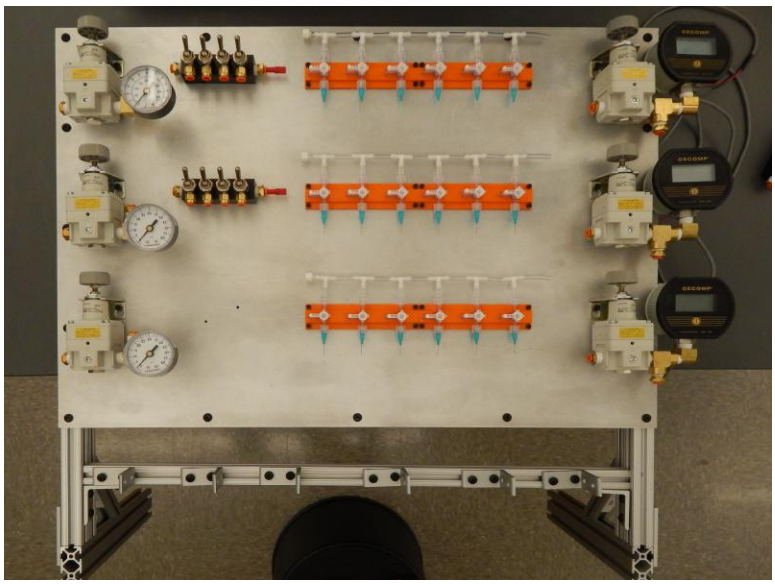
27. Using the 1/2" segments of **Tubing T2** from Step 24, connect the T tube fittings (**Part K**) and luer plug cap (**Part L)** to the 4-way stopcock flow manifold assembly and associated tubing lines as shown below. This step completes the air pressurization system for the flow manifold assembly, which selectively pressurizes flow lines on the microfluidic device.



28. The Flow Control Board assembly should now appear like this:

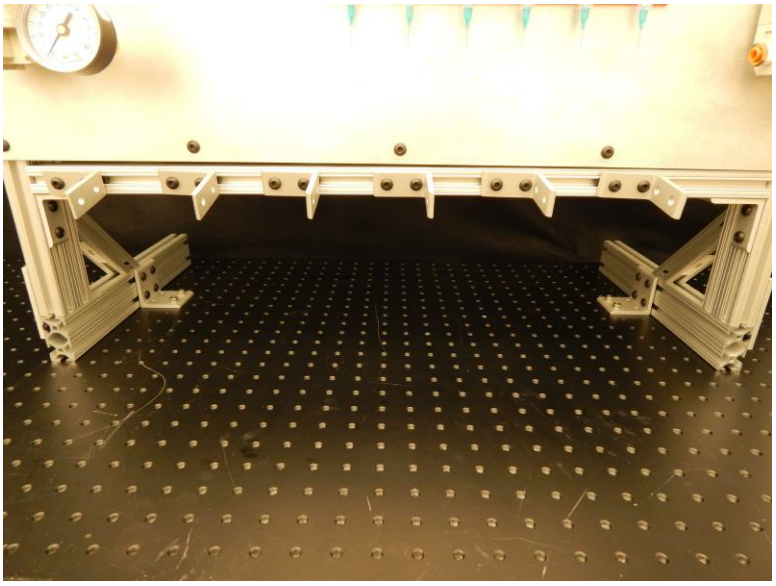


29. After finishing the Flow Control Board regulator and flow manifold components, add on an additional 80/20 26" x 2" x 1" (**Part b2**) support using 2 elbow brackets (**Part b6**) with fasteners to brace the support. The support should be placed 2 inches below the bottom of the board. This support will be used to mount the control water reservoirs and solenoid valve arrays for control line modulation. Additionally, attach 6 more elbow brackets (**Part b6**) at regular intervals along the bottom 80/20 as shown below. These will be used for mounting control valve manifolds in **Module 3.**



Part 4: Tubing Connections

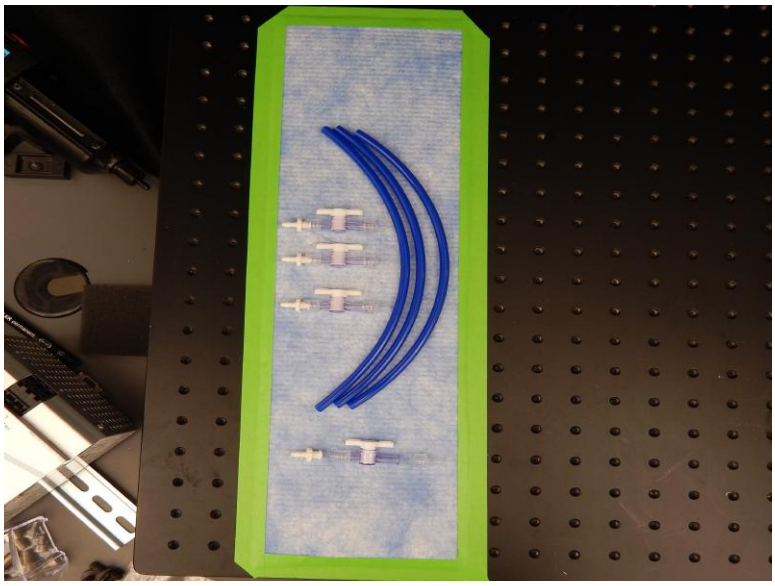
30. After finishing all the baseboard component connections in previous steps, mount the entire assembly to an optical breadboard or bench of your choice. Here we use an extended corner bracket (**Part b7**) on each side of the bottom legs to lock down our setup. Alternatively, c-clamps or other attachment means can be used. Locking down the setup is important before proceeding to future modules if the full build is desired.



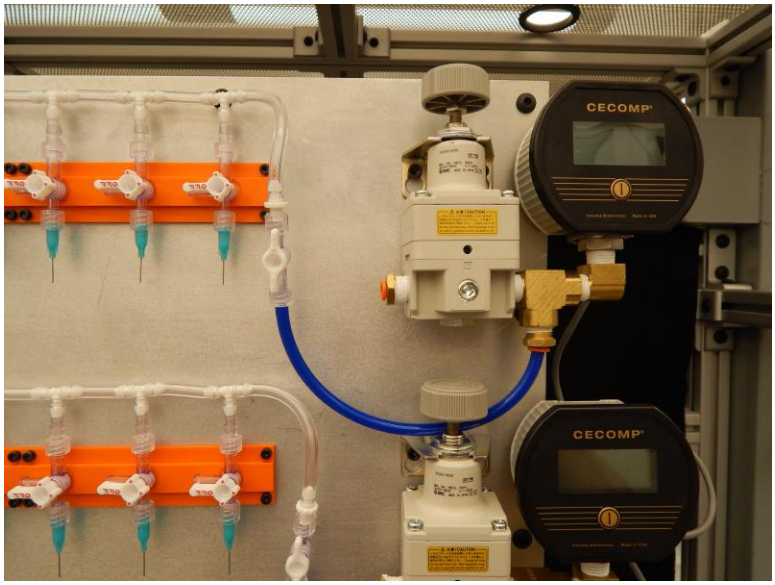
31. To begin tubing connections, assemble generous 7" cuts of **Tubing T1** and **Parts H-J**. These parts are used to connect the flow regulator assemblies to the flow manifold and selectively pressurize the manifolds to prevent back pressure. We use these assemblies as additional level of control. **Parts H-J** can be optionally excluded from the build if global manifold control is not needed.

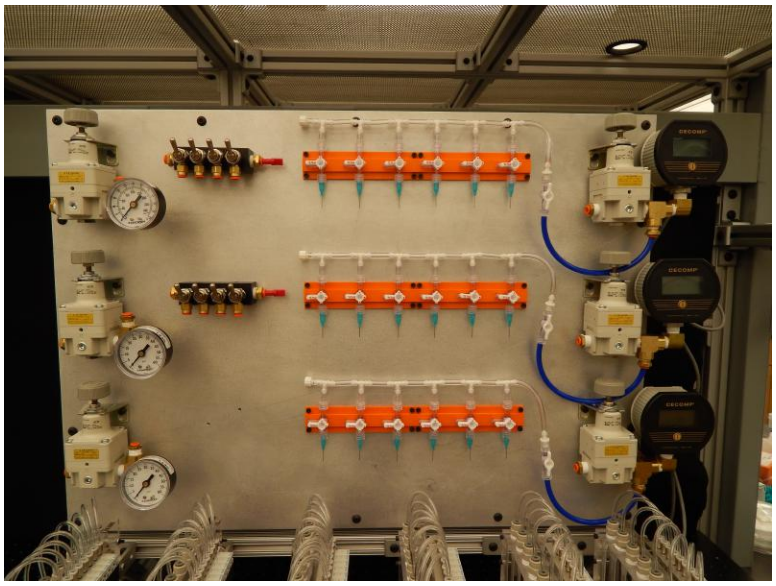
Pause Point!

(!) **Tip:** It is arbitrary when you decide to complete your tubing connections during your build. We often proceed to **Modules 2, 3** and **4** before completing tubing connections so that we can complete all final connections at one time. If you decide to do so, return here after completion.

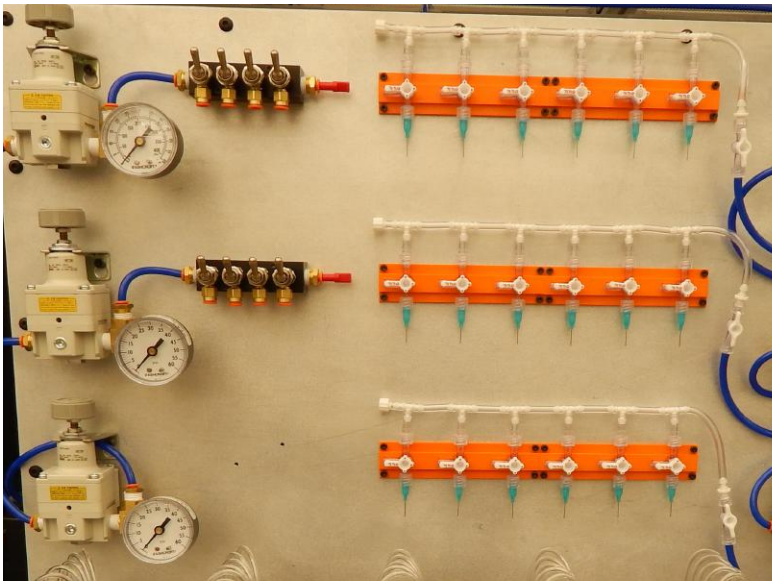


32. Using the push-to-connect fittings (**Part C**) attach **Parts H-J** and **Tubing T1** as shown in the Module schematic. Use **Tube T2** to complete the connection to the flow manifolds (**Part K – T2 – J**).





33. Subsequently, use small 3" cuts of **Tubing T1** to connect the control regulators to the control manifolds on the left side of the setup as shown below.



You're finished with Module 1!
Proceed to subsequent modules, if you haven't already.

Step-by-Step Build Setup Guide # 2

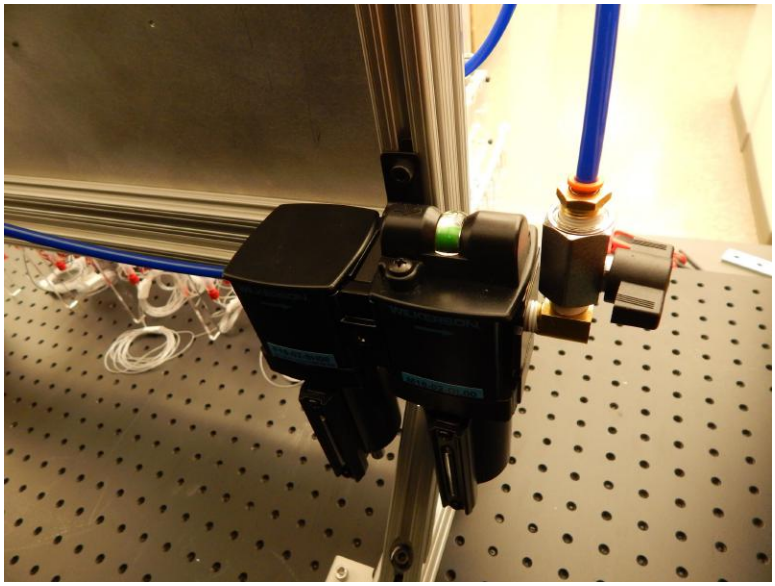
Pneumatic Control System Module 2: Filter Assembly to House Air

(!) Tip: You can replace the filters in this assembly with disposable syringe filters to conserve costs, if the filters are switched frequently. For long-term use, we don't recommend this modification as dust and other air contaminants can decrease the lifetime of regulators if disposable filters are not replaced regularly.

1. Begin by assembling **Parts T – Y** and **Parts C, E,** and **S** for the dual filter assembly. Use **Tubing T1** for the connections between components.

2. Join the two filters using the Filter Joiner Clamp, **Part X,** with **Filter V** facing closer to the house air connection (follow filter arrows). Attach **Part C** to **Filter V**.

3. Assemble the other filter parts to **Filter Y** as given on the module overview schematic, leaving approximately 3-4" of **T1** between each connection from the filter assembly. Attach to the flow control board. Connect longer segments of **T1** to span to each regulator on the Flow Control Board in the following step.

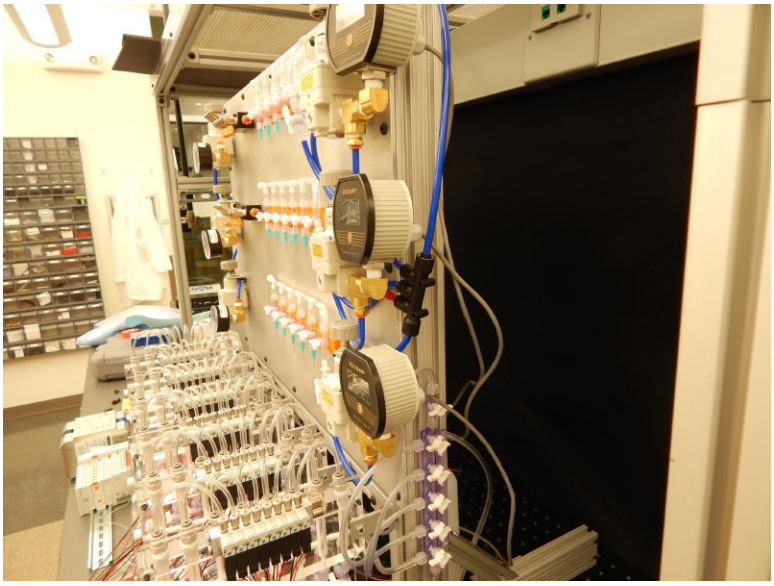


4. Create long segments of **T1 Tubing** to deliver compressed air to each regulator on both sides of the board using the 3-way push-to-connect manifolds (**Part T**) split. Connect the regulators to the 3-way push-to-connect manifolds on the left side as shown below.

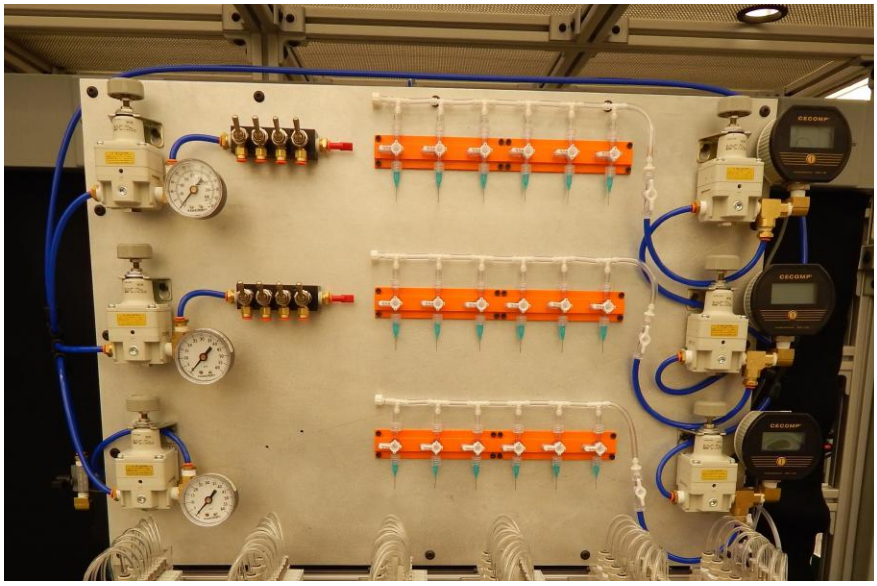
(!) Tip: We typically run tubing to air connections at the very end of all assemblies (shown by the completed control valve manifolds in the above image). This is an arbitrary decision; you can connect tubing at any time. We just find it helpful to do it all at once.

5. Using the end of the **Part T** run tubing to the right side. Complete the same connections for the right side using another **Part T**. Use the red end-stop plug (**Part S**)

to cap the end connection of **Part** T on the right side. These connections pressurize the regulators installed in **Module 1**.



6. The completed setup for air delivery from the filter assembly should appear as follows:



7. Finally, connect the whole filter assembly to a source of high pressure (>40 psi) air. House air or compressed air sources can be used. We typically mount to compressed air using a high pressure regulator connected to **Parts C** and **V** and then run **T1** from the filter assembly to the air source. Connections will vary based on your lab's air access.

(!) Tip: We've found compressors to be difficult to use as an air source because the regulators we've chosen use open pressure feedback, which means they leak a bit to maintain tight pressure control. This leakage causes compressors to cycle on frequently, making the lab environment excessively noisy.

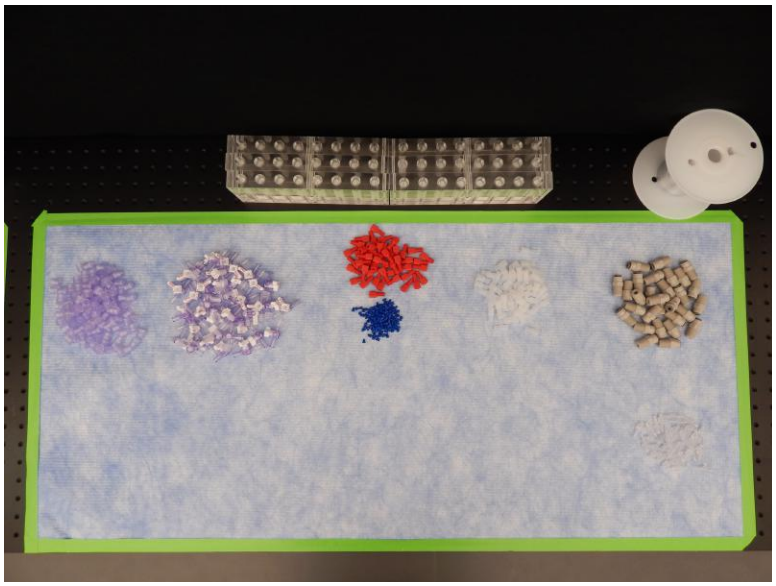
You're finished with Module 2!

Step-by-Step Build Setup Guide # 3

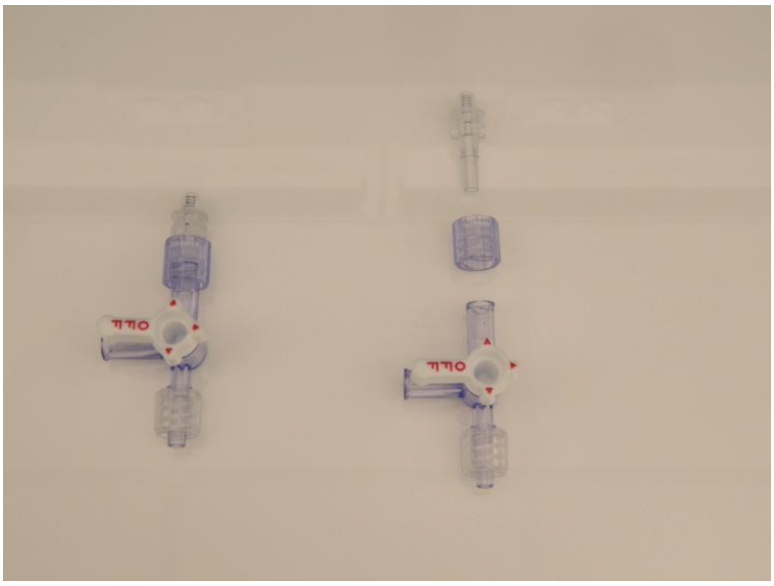
Pneumatic Control System Module 3: Control 8-Valve Solenoid Manifolds

Part 1: Small Parts Assembly of the Control Valve Manifolds

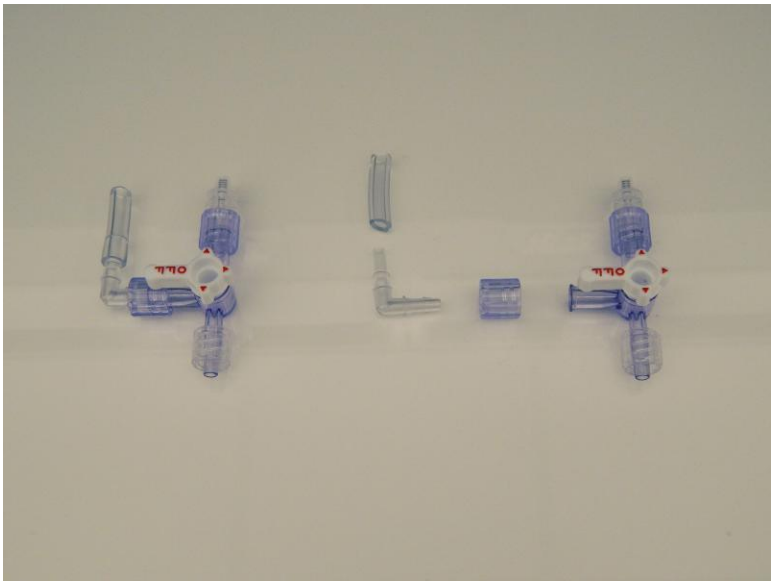
1. Begin by assembling the **Machined Parts "Water Reservoir Stand *reservoir-stand*"** (not shown) and **"Control Water Reservoirs *reservoir*"** (shown below) as well as **Parts a – h** and **Parts N, L, K** for the control 8-valve solenoid manifold build. These manifolds will hold water to push into the control lines when pressurized. They connect with the solenoid valve arrays (**Part h**), which toggle between pressurized air and atmosphere.



2. Assemble one 4-way stopcock (**Part N**) to one snap luer lock ring (**Part b**) and one tapered thread to luer connector (**Part a**) as shown below. For our setup, we built 6 8-valve reservoirs for 48 control line inputs, which requires 48 sets of connector assemblies. Adjust according to your own needs.



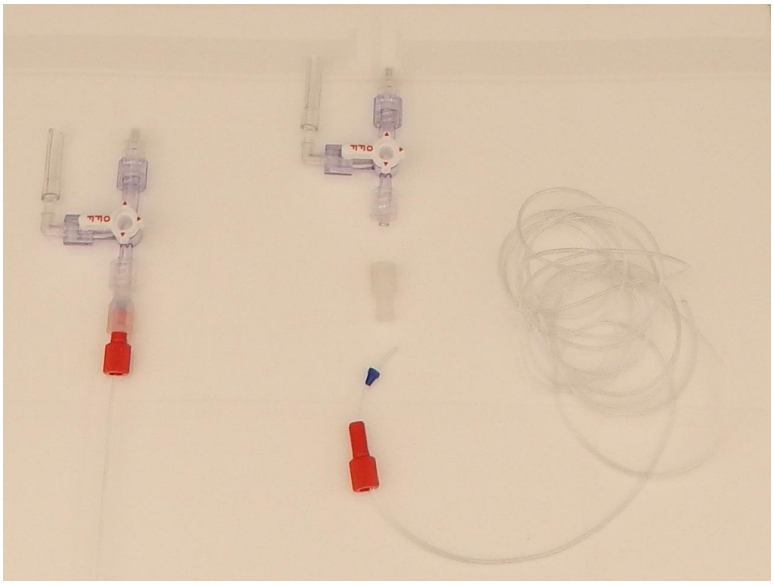
3. Continue the assembly by connecting the barb elbow (**Part c**) using one snap luer lock ring (**Part b**). Cut 1" of **T2 Tubing** and attach to the smaller edge of the barb elbow. Tubing lengths must be precise, as these will be connected across the manifold using spanning T joints (**Part K**) in a later step.



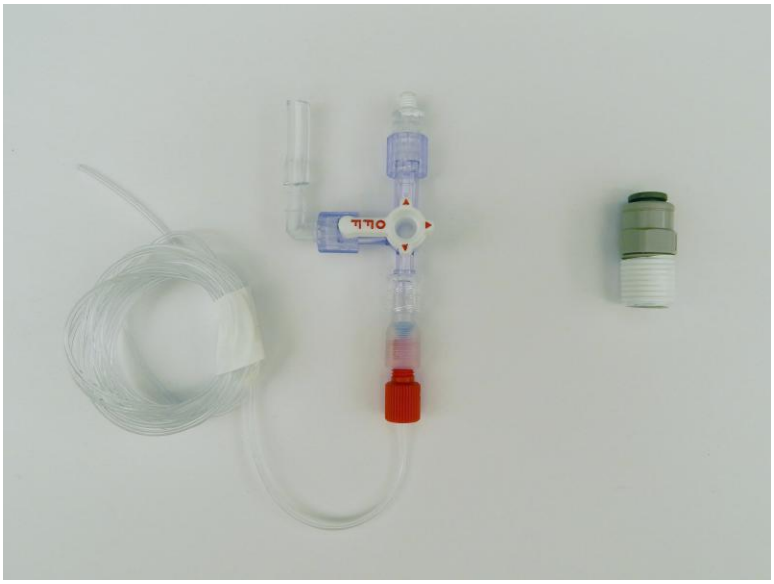
4. Assemble the flangeless ferrule assembly (**Parts d, e,** and **f**) as shown below with 14" of Tygon tubing (**T5**). This tubing will become the control lines that are ultimately connected to the microfluidic device.

(!) Tip: We prefer long tubing here as this allows us the flexibility to move the chip around from the reservoirs to different imaging stations or other equipment. However, long tubing can increase valve response times so we have found 12-14" to be optimal. Tygon lengths can be

adjusted to your own needs but **make sure the tubing length is sufficient** to span the entire distance from the pneumatic control station to the ultimate device location during experiments.



5. To keep the assembly orderly, wrap the long Tygon tubing for each assembly into a manageable coil and secure with tape. Build the number of control line assemblies required; here, we show a setup with 48 control lines. Apply Teflon tape on the tapered thread of **Part a** and **Part g** as shown, in preparation for assembly of the control manifold.



6. Assemble the control line assemblies to the **Machined Part "Control Water Reservoir *reservoir*."** These will eventually be loaded with water by the stopcock to barbed elbow

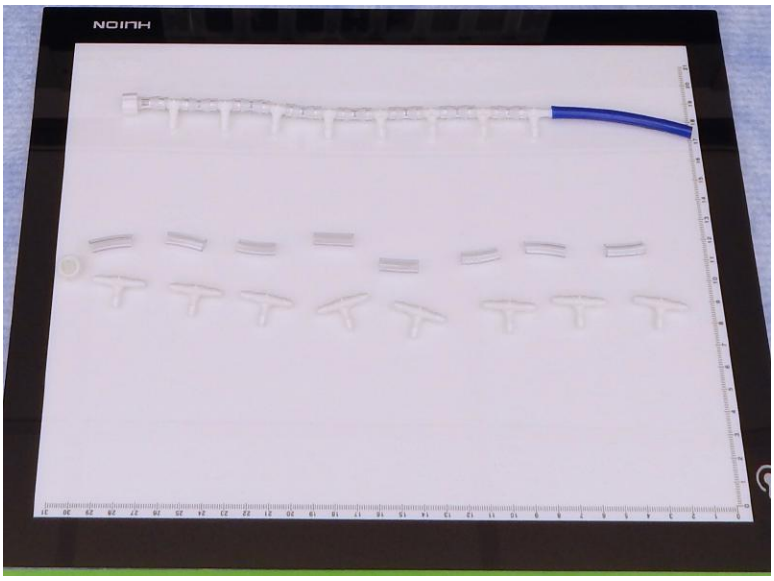
(**Part c**) connections built in **Module 4**. The stopcock will toggle between a (1) water loading mode (vertical, in line with reservoir) to refresh control lines and (2) an active state (horizontal, outward from page) where pressurized water is sent from the reservoir to downstream control lines to actuate on-chip valves. Apply superglue at the junction after threading the tapered end of **Part a** into the reservoir to prevent leaks.

7. Attach **Part g** to the top of the assembly for each of the 8 channels.

8. Align the stopcocks parallel to one another. Remove the barbed elbow assembly (**Part c, Tubing T2**) from the 4-way stopcock (**Part N**) and superglue the stopcock connectors to prevent leaks. It is important to perform this operation now, despite the disassembly,

because it will ensure each stopcock is precisely aligned horizontally in space with the rest of the control loading assembly. Re-attach the elbow joint assemblies.

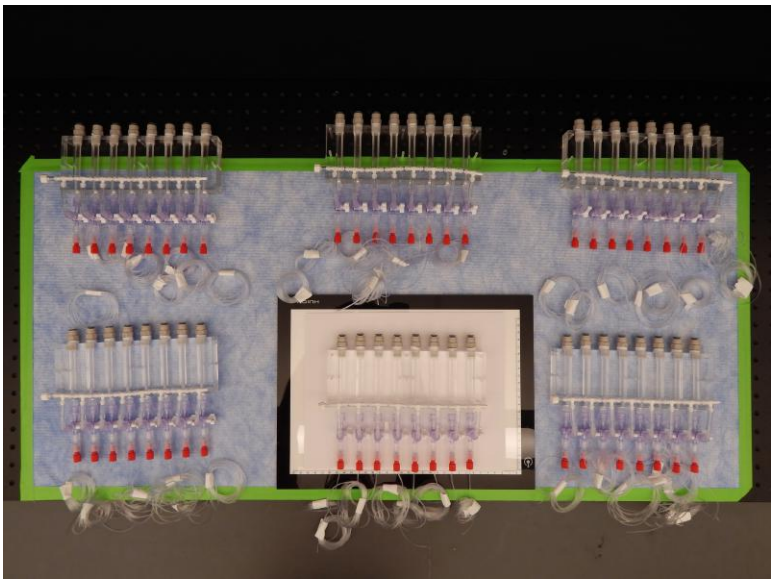
9. Assemble the horizontal tubing assembly according to the Module schematic as shown below. Use ~1" regular segments of **Tubing T2** to connect the T tube fittings (**Part K**). This is similar to the flow manifolds you assembled in **Module 1**. Attach an end cap (**Part L**). This assembly (loading port) will be attached to **Tubing T1** segments from the Control Loading module in **Module 4**.



10. Attach the horizontal tubing segment to the elbow joint assemblies with **Tubing T2** connectors carefully.

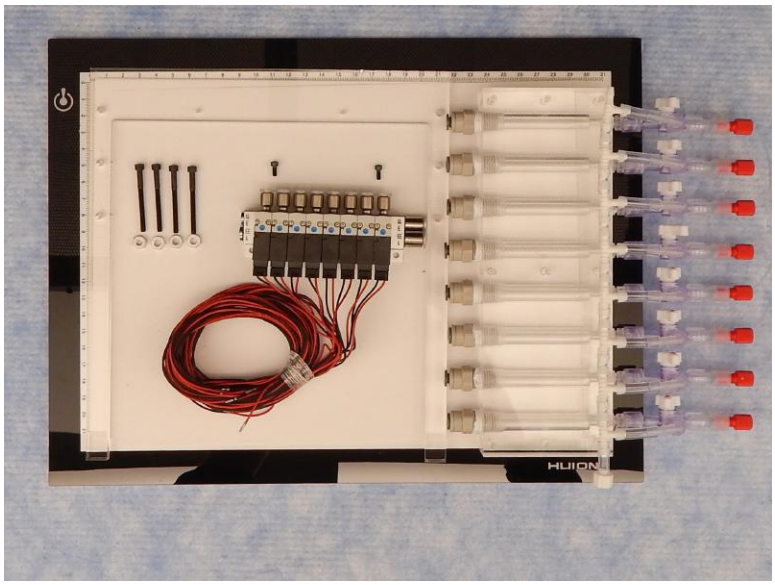
(!) Tip: You can use a heat gun to help attach the tubing segment by loosening the tubing.

11. Complete 6 control manifold assemblies, or as many as required for your needs.

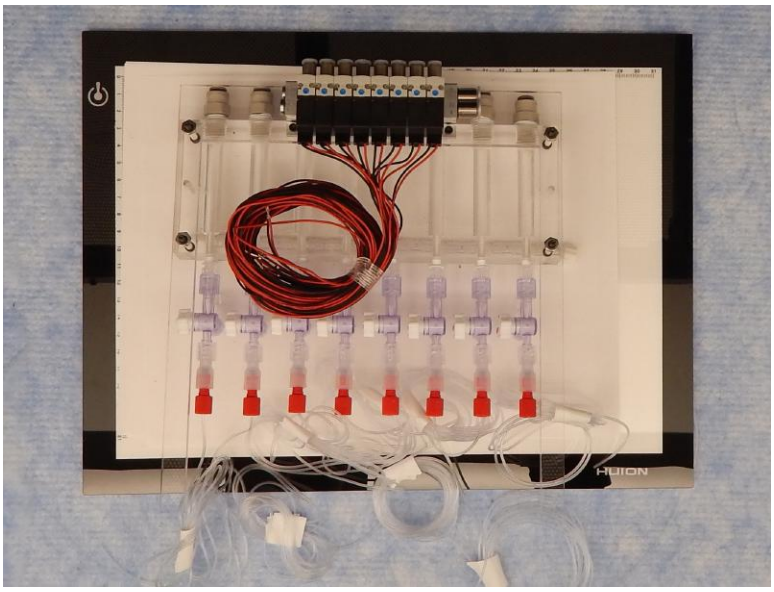


Part 2: Valve Integration and Full Manifold Mounting

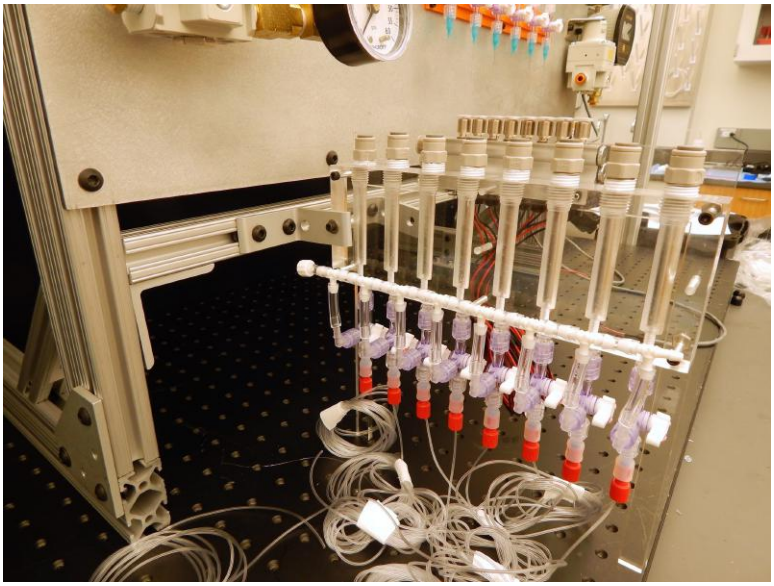
12. Gather the **Machined Parts "Water Reservoir Stand *reservoir stand*,"** 8-Valve Solenoid Array (**Part h**), and **Screws s2**, **s3** as shown.



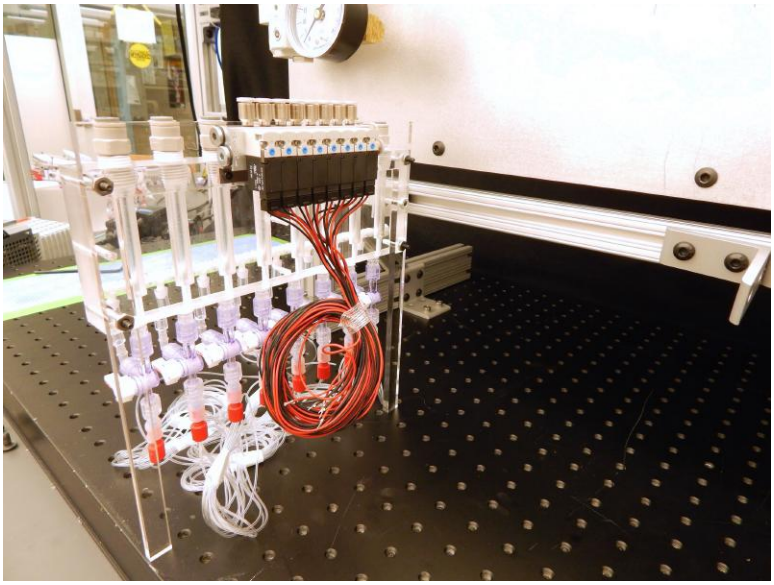
13. Mount the completed Control Manifold assembly built in Steps 1-11 to the Water Reservoir Stand using **Screw s2** in the tapped holes as shown. Mount 8-Valve Solenoid Array (**Part h**) to the back of the assembly with **Screw s3** as shown.



14. Attach the mounted elbow bracket **Part b6** (mounted in **Module 1**) to the back of the Water Reservoir Stand as shown.

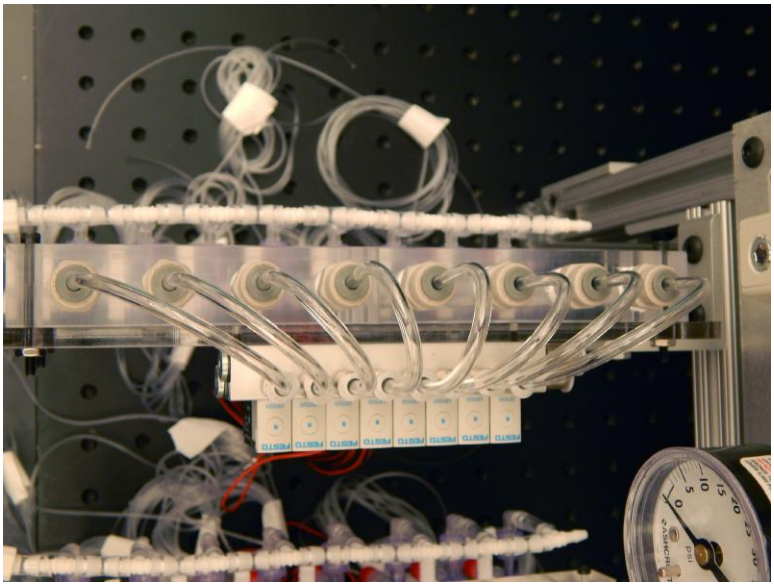


15. Here's the assembly visualized from another angle.



16. Complete the mounting for all 6 Control Valve Manifolds.

17. Cut 2" segments of **T3 Tubing** to connect the solenoids outlets with the water reservoir assemblies. This will allow the solenoid to pressurize the water input.



18. Here is a final view of all 6 assembled control manifolds for a total of 48 independent solenoid driven control lines.

Part 3: Control Line Indexing

19. In this Part of **Module 3**, you will unravel, index, and label your control lines ***e.g.*** the Tygon **T5** tubing assembly associated with each valve, that will ultimately connect to your microfluidic device.

    In **Module 5**, you will index and label the electrical connections associated with each valve (red and black wires per each solenoid). It is arbitrary whether you choose to index

the valves wiring or fluidic control lines first (or choose to index and label them at the same time). If you would like to proceed with labelled both the valve lines and wires at the same time, complete this Part in tandem with **Module 5** Part 1.

20. Starting with the leftmost manifolds, label each Control Manifold from 1 – 6 (or your completed number of manifolds) and each solenoid valve (and associated control line) with the valve number, starting at 0 (***e.g.*** Manifold 1 = Valves 0-7, indexed for each valve starting from closest to you on the solenoid array). These global Manifold labels will help you keep track of the individual control line and solenoid wire labels as you proceed.

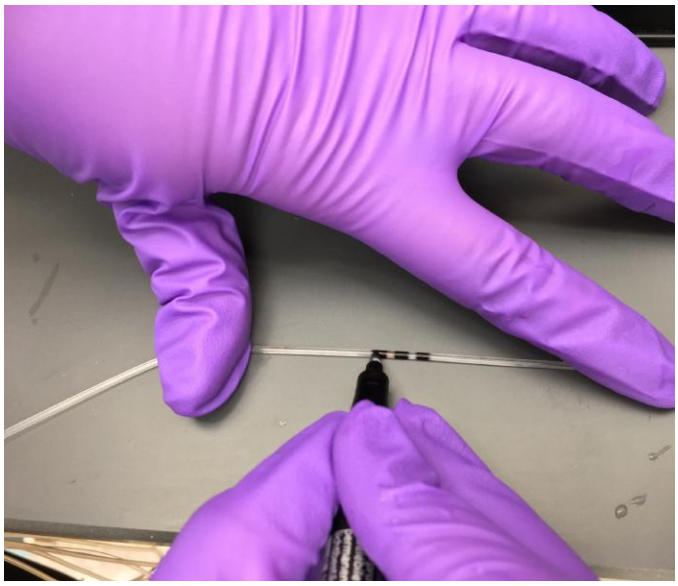
    Labels for Manifold 1 (left) to Manifold 6 (right) on a completed setup are shown below.

21. Gently unravel the control lines associated with each valve one at a time. Cut to to the desired length. We recommend completing this step and Steps 22-23 per each valve individually before proceeding to the next valve.

(!) Tip: Make sure your control line length is enough to reach comfortably across all the manifolds to the device mount on your imaging system, bench, or any location in which you will be operating your microfluidic device and performing experiments. We typically make control lines of ~22-24".

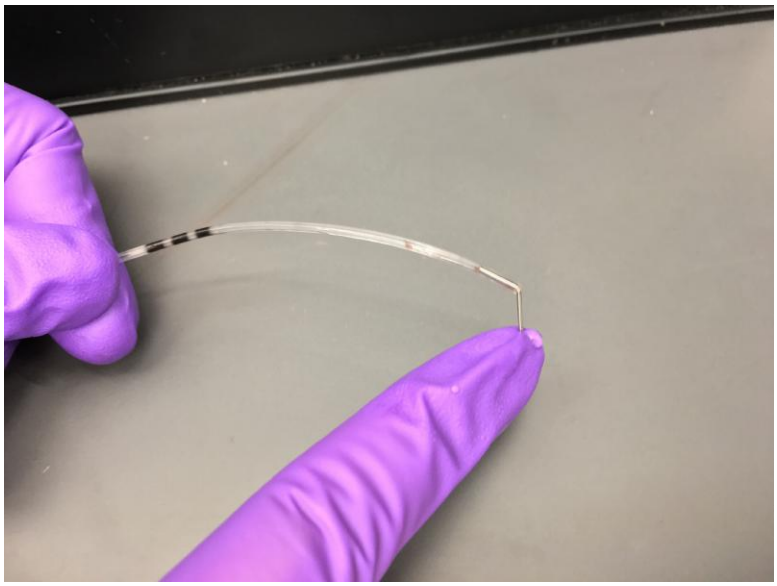
22. Label the bottom of each control line with the Valve number. You can use a label maker, if desired. We recommend using a block encoding using Sharpie on each line. To do this, we mark a set of Sharpie rectangles for each Valve number starting at 0. (0= 1 small rectangle, 1 = 2 small rectangles, 3= 3 small rectangles, 4= 4 small rectangles, 5= Long rectangle, 6= Long rectangle + 1 small rectangle, and so on for each Manifold).

    Below is shown the labelling for Valve 2 on Manifold 6.



23. Next, insert a steel blunt pin (**P1**) at the end of each control line. We bend the pins to easily interface with the microfluidic device.

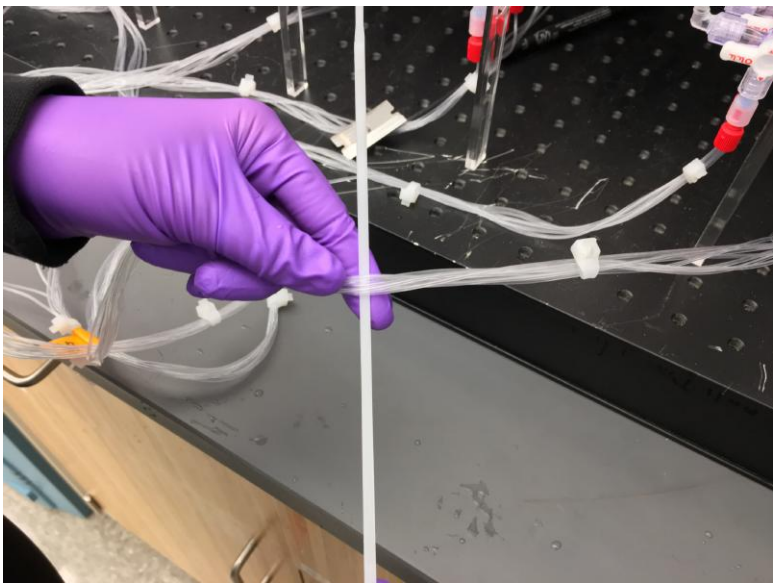
Note: Microfluidic inlet holes must be punched to the same size as the pins used in this step otherwise the connection to the microfluidic device will not be leak-free.



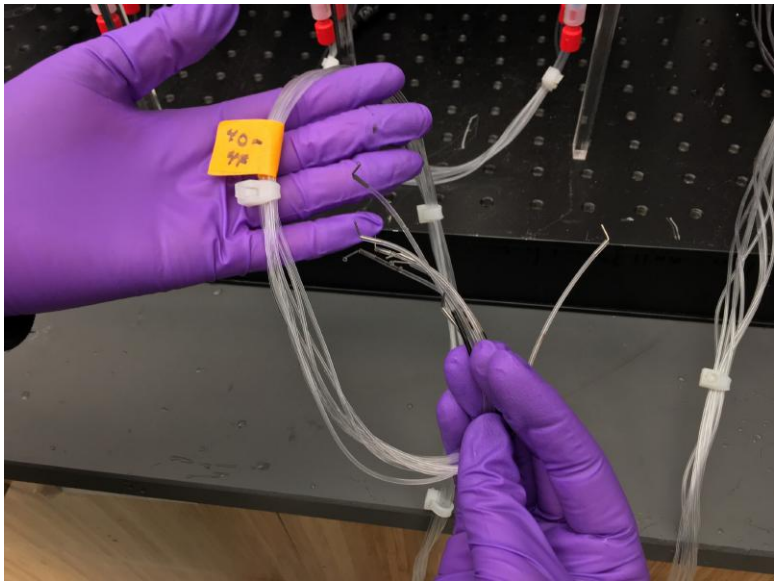
24. Repeat these steps for all control lines in a Manifold set.

Note: In this picture, water is seen coming out of some of the lines but at this point, your setup should not yet have had water run through the lines so don't worry about that just yet.

25. After labelling and inserting pins for all the control lines in a Manifold, use cable ties or tape to collect and organize the control lines. We recommend leaving ~6" at the pin-end of each control line set so that connecting lines to the device can still be flexible.



26. Label your Manifold control line set at the last cable tie. This helps you keep track of the valve numbers (as related to Manifold origin) in high complexity devices with many valves from multiple Manifolds.



27. Repeat for all the Manifolds until indexing and labelling of the control lines is complete.

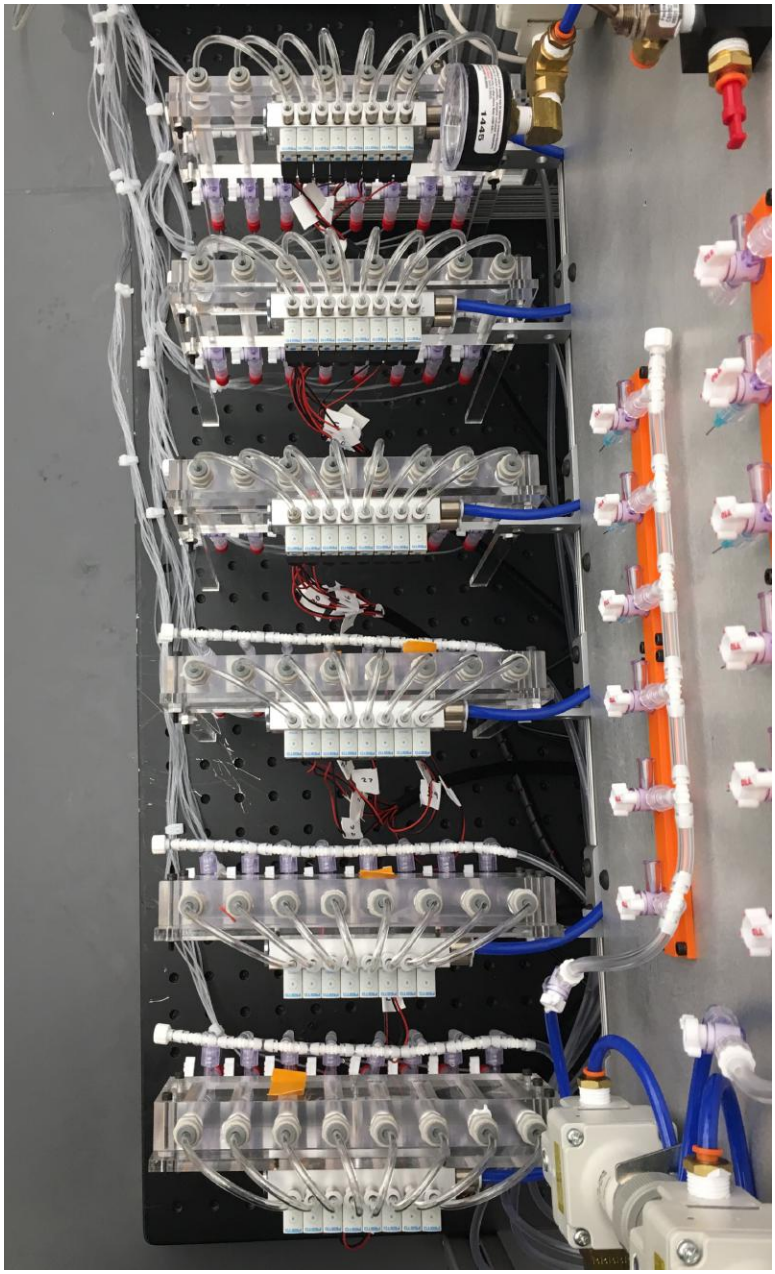
Part 4: Global Connections

28. Before proceeding to Global Connections, complete the **T1 Tubing** connections for the Base Board regulators explained in **Module 1**.

29. On the side of the solenoid valve array, **Part h**, you will find two ports. The top port will be connected to the control manifolds which can be tuned to the desired pressure from the regulators in **Module 1**. The bottom port is open to atmospheric pressure. When addressed, the solenoid valve toggles between atmosphere and pressurized air. Connect a long piece (~5-7") of **T1 Tubing** from the Control Switch Box outlet corresponding to the Manifold # (in order of valve number) to the top port of the Solenoid Valve array as shown in the following pictures.

(!) Tip: These two ports can be used to control different two-state valve toggling between air sources of your choice, for instance, vacuum and atmospheric pressure. For Quake-style valves, we recommend the use of compressed air (regulated via the regulators in **Module 1**) connected to Port 1 (top). Port 2 (bottom) should be open to atmospheric pressure.

Connections to each Solenoid Array Top Port (below).



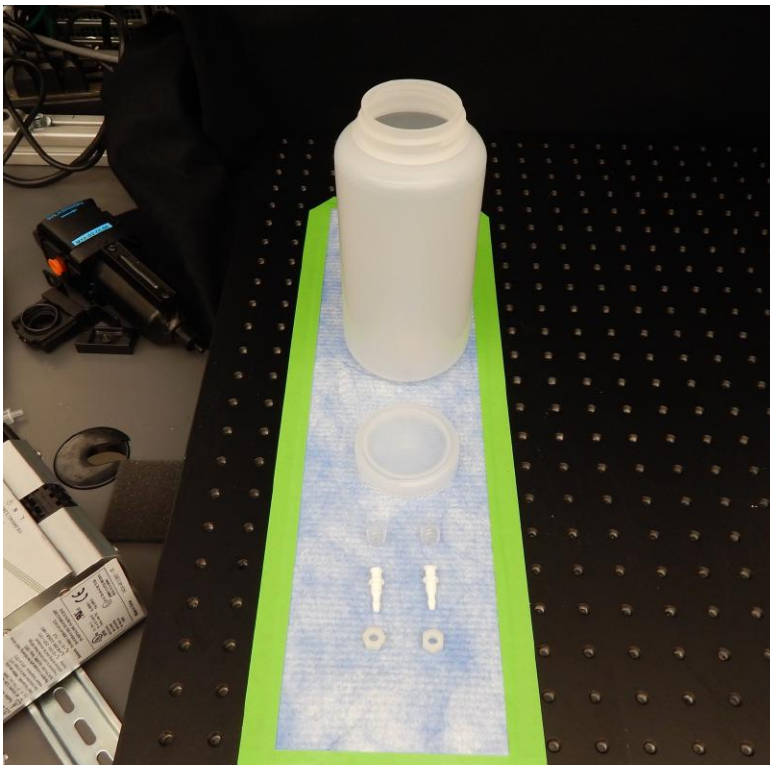
30. The completed setup for **Modules 1, 2,** and **3** should appear as the following image. Note the manifold connections for the 6 manifolds from the Control Switchbox from **Module 1**.

You're finished with Module 3!

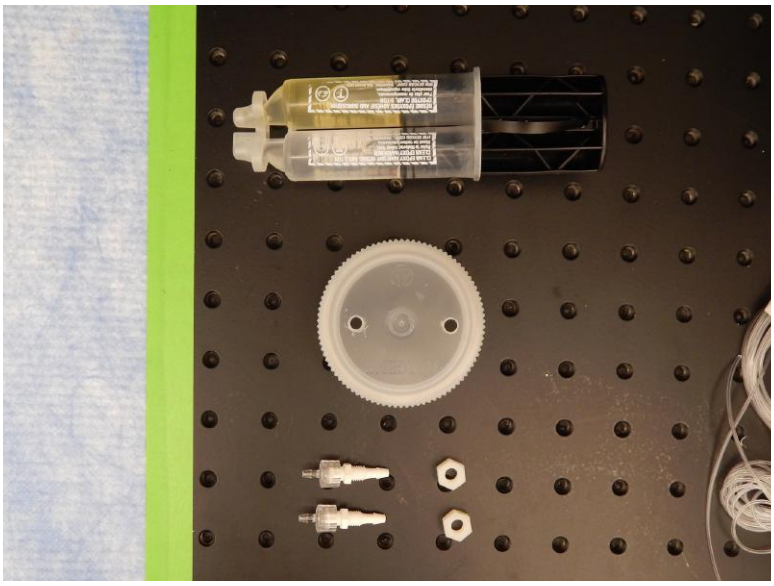
Step-by-Step Build Setup Guide # 4

Pneumatic Control System Module 4: Control Reservoir Loading Assembly

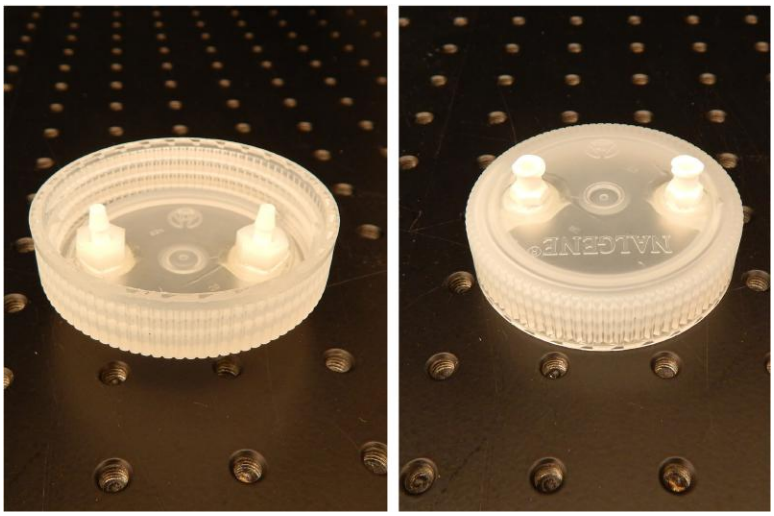
1. Begin by assembling **Parts i-k** and **Parts M, H, J, I** for the water reservoir assembly. This assembly is used to load the water reservoirs of the control manifolds built in **Module 3**. The reservoirs (**Module 3**) are then pressurized when the manifolds are in the active state. The water is pushed into the control lines and, eventually, control channels in the chip to actuate on-chip valves. This assembly replenishes lost water during device operation.



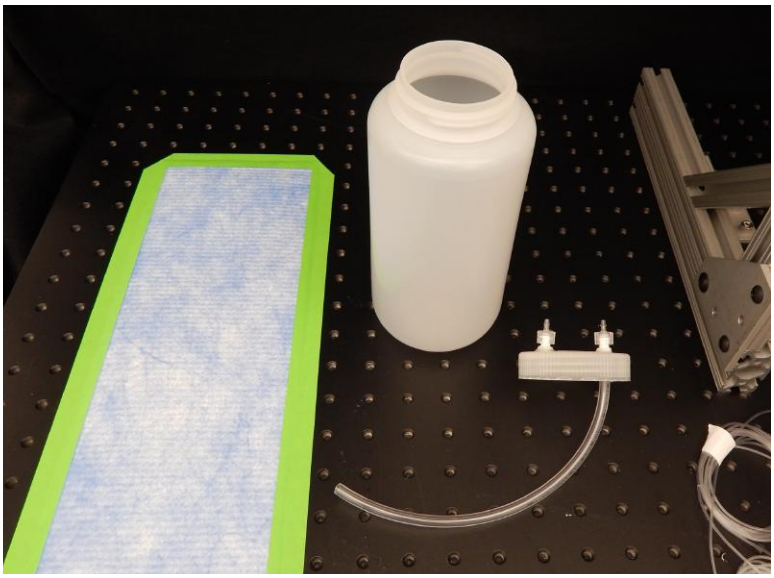
2. Using a drill cut out two 1/4" holes through the cap of the Nalgene bottle, **Part j**. Assemble one set of male integral ring (**Part M)** and female luer to barb (**Part J**) connectors and one set of male integral ring, Series 400, (**Part H**) and female luer to barb (**Part J**) connectors, according to the schematic.



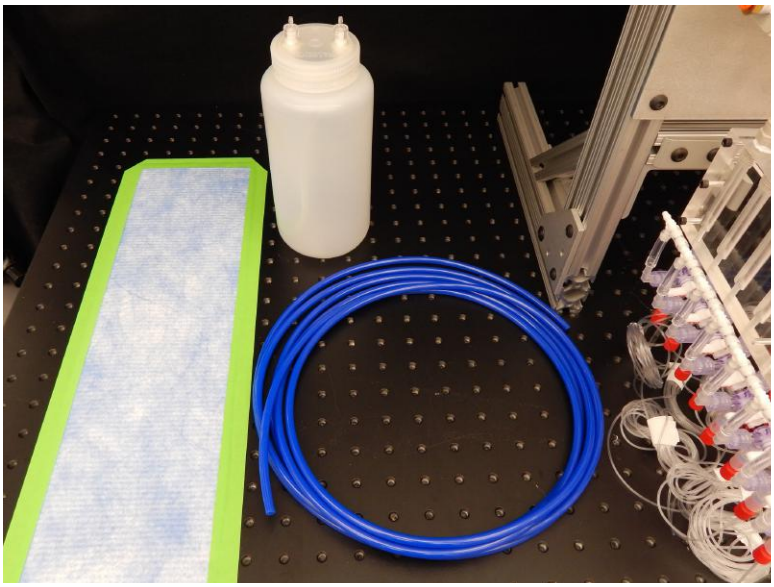
3. Feed the **Part M** and **J** assembly through the cap on both sides and lock with the Nylon hex nut (**Part i**) as shown below, such that the female luer ¼" connector is facing outward from the cap (note: **Part M** was removed for purposes of demonstration). Apply epoxy or Super Glue to seal the holes on both sides of the cap. This helps prevent leaks. Let dry 1 hour.



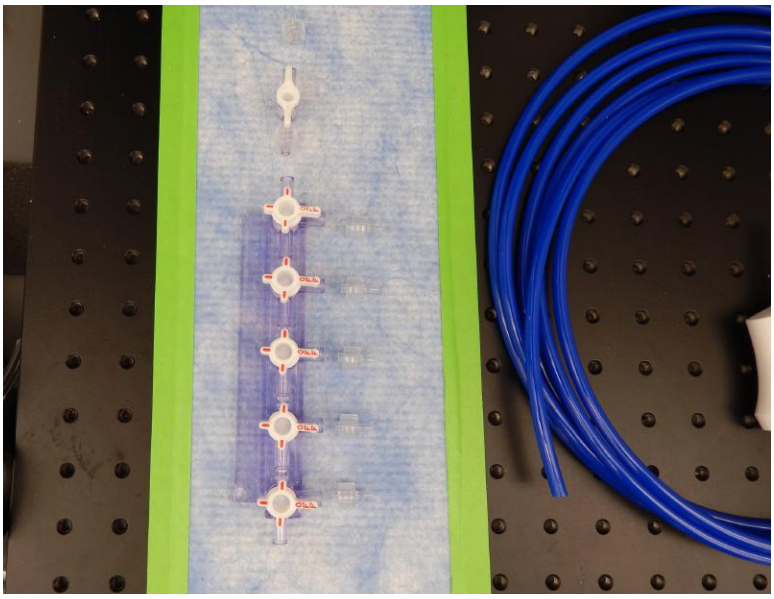
4. Attach ~4" of **T2 Tubing** to one side of the bottle on the inside of the cap as shown. Note the side you choose.



5. Assemble the Nalgene bottle (**Part j**) and cap assembly, noting the side with the tubing. This side will be used as a straw for moving water to load the control reservoirs while the other side will be used as a pressurization port to pressurize the bottle for pressure-driven loading when connected to a regulator.



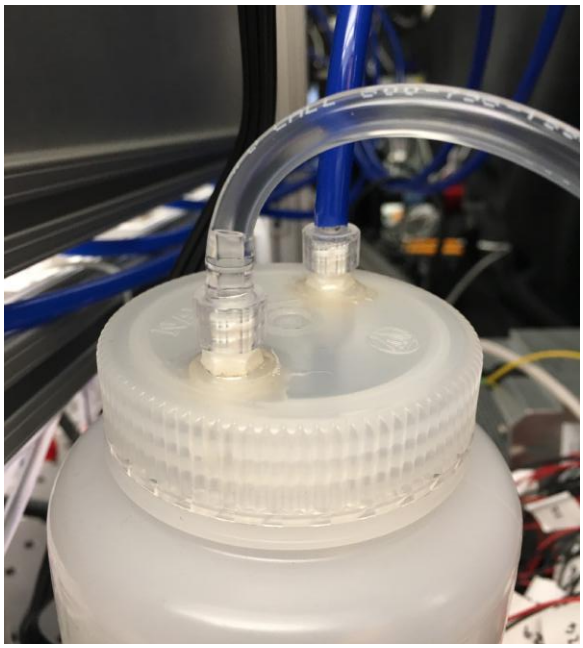
6. Assemble a 5-way manifold (**Part k**) and 1-way stopcock assembly (**Parts I, H**). Connect male integral rings (**Parts M**) to each outlet as shown below. Assemble according to the Module schematic. Scale accordingly to the number of control manifolds you chose to build in **Module 3** (each stopcock controls 1 manifold).

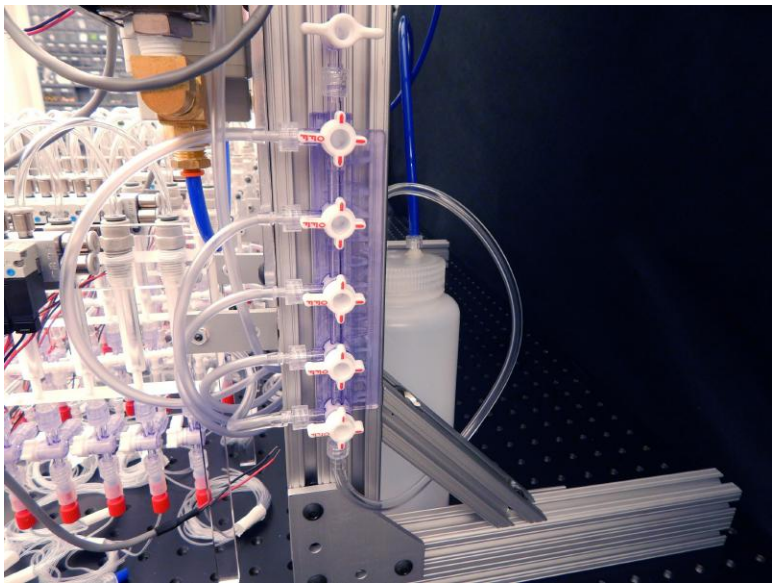


7. Using epoxy or Super Glue mount the assembly to the right 80/20 rail closest to the digital gauges.

(!) Tip: We use Scotch Super Glue or High Performance repair glue for mounting this assembly directly to the 80/20. We've also had success with 2-Ton epoxy, but not 30-minute fast-dry epoxies.

8. Connect **T2 Tubing** to from the bottle connector that contains the 'straw' to the bottom of the assembly as shown. Connect **T1** tubing from the bottle to left Regulator 3 (Control Loading Regulator) of **Module 1** as shown in the schematic. This regulator will be used to pressurize the control reservoir loading. Connect **T4** tubing from each stopcock of the 5-way manifold and the end of the 1-way stopcock to the loading port of the Control Manifolds built in Module 3. Write down the manifold order of connections to use when loading the reservoirs. Each stopcock loads water to 1 manifold. You will load the water into the reservoirs in the **Operation Guide**.





You're finished with Module 4!

Step-by-Step Build Setup Guide # 5

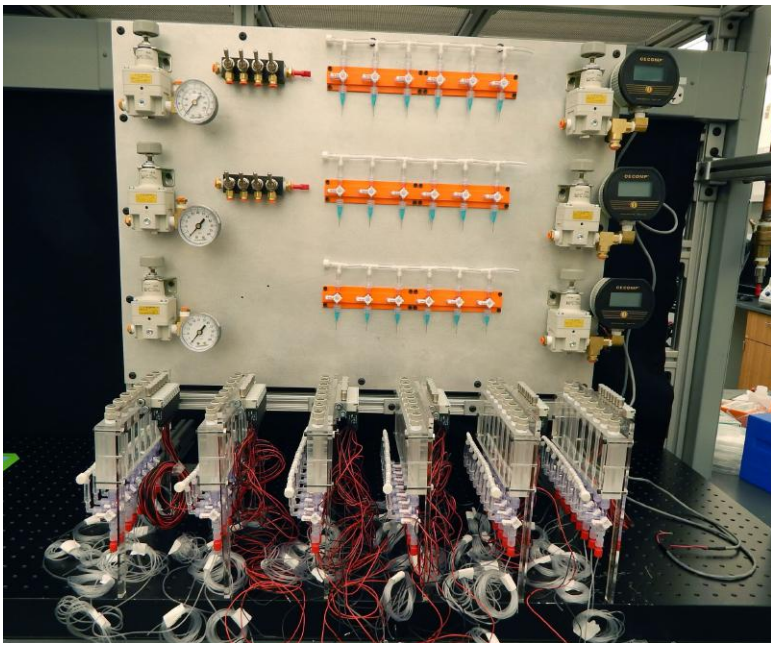
Pneumatic Control System Module 5: WAGO Digital Control

Part 1: Solenoid Valve Indexing

1. Gently unravel the red and black wires connected to the solenoid valve arrays (**Part h**) from the completed Control Line Manifolds from **Module 3**.

Note: Here we show unindexed control line coils, but you should have previously completed control line indexing in **Module 3**. It is arbitrary whether valves or control lines are indexed and labelled first as long as the indexing is consistent.

(!) Tip: In this Module, the individual solenoid connections will be established to a Modbus controller that essentially acts as a programmable logic circuit (PLC) for fast, remote control of the valve states. It is therefore important to keep track of the individual solenoid electrical connections by clearly indexing them by valve number. We label at the **bottom** (near the end of the lines) and **top** (near the solenoid valve array) of each wire set.

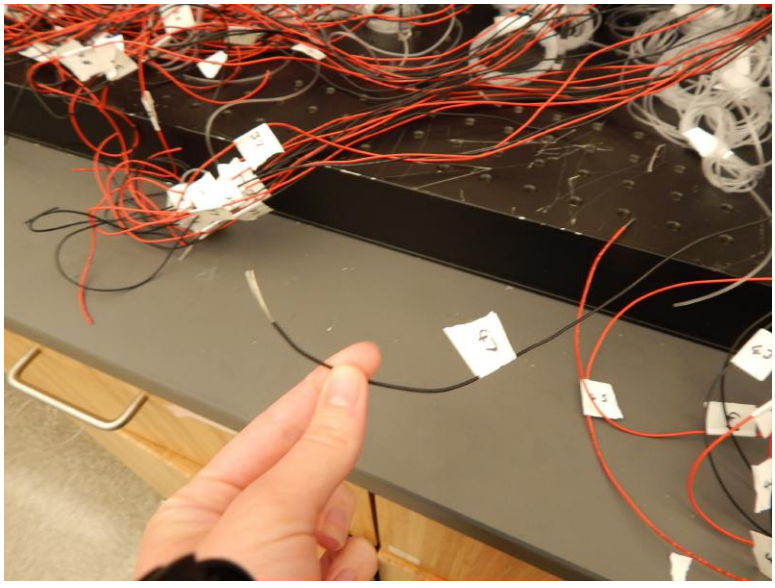


1. Each red and black wire set controls the positive voltage and ground connections, respectively, for each solenoid. Starting with the leftmost manifolds, label the top and bottom of the red and black wires associated with each solenoid valve with the valve number, starting at 0 (***e.g.*** Manifold 1 = Valves 0-7, indexed per each red and black wire). Place the bottom label ~7" from the end of the wire (to make room for stripping

and cutting later). These valve and manifold numbers should be consistent with those made in **Module 3**. Our labels are shown below.

(!) **Tip:** It is important to label ***both*** the top and bottom wires with the valve number as we later will place black hosing around all the wires from one manifold to organize and secure them. Without the top labels, it becomes difficult to isolate individual valves connections if there arises a problem or if the setup must be dissembled (for moving around, for instance). Use a label maker or solvent resistant markers on lab tape when labelling the solenoids.

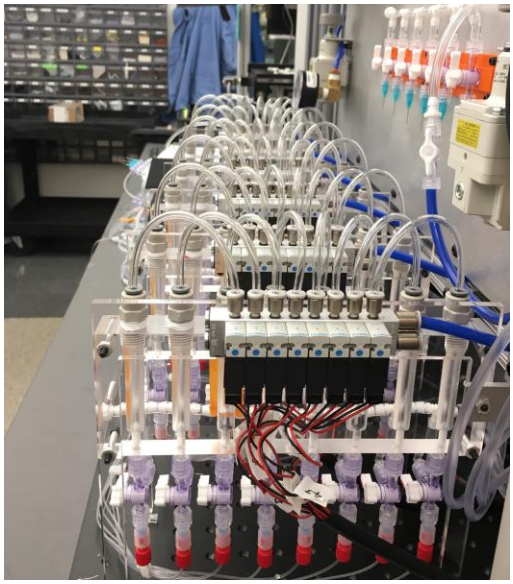
2. With a wire cutter, cut ~4" from the end of each red and black wire set and strip the wire back ~1/2". The exposed wire will be used to connect +V (red) and ground (black) for each valve into the WAGO Fieldbus Controller to enable digital valve control.



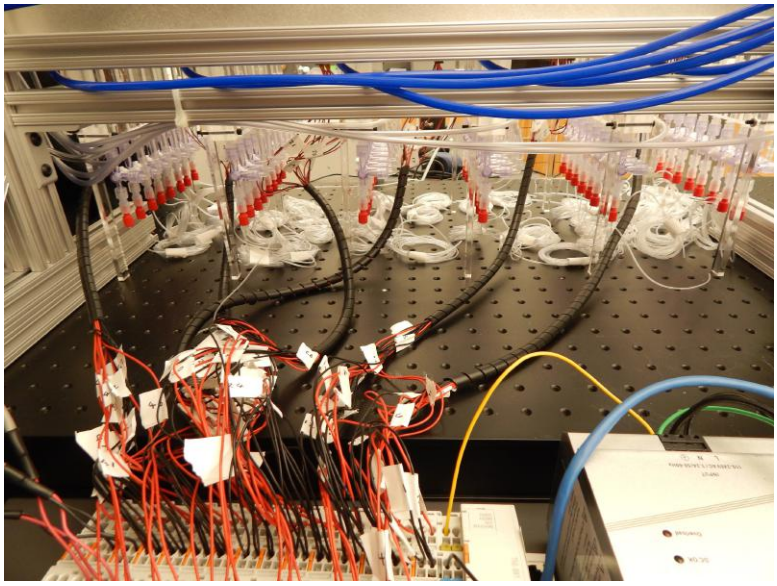
3. Cut ~14" pieces of **Tubing T5**. Collect all the solenoid wires from one manifold and gently snake the **T5 Tubing** up the collection of wires to organize and secure them This step tidies up the wires so that wiring the WAGO Modbus controller is easy and less messy.

(!) **Tip:** Start from the bottom of the collection of wires, wrap the first end around the whole group, and thread the tubing upward by coiling to the right. Stop coiling when the tubing reaches the labels near the solenoid array. **Be sure to keep labels at both the top and bottom of each wire visible**. **T5** should terminate tubing ~2" from valve solenoid arrays, as shown above.

4. The completed manifolds should appear as following:



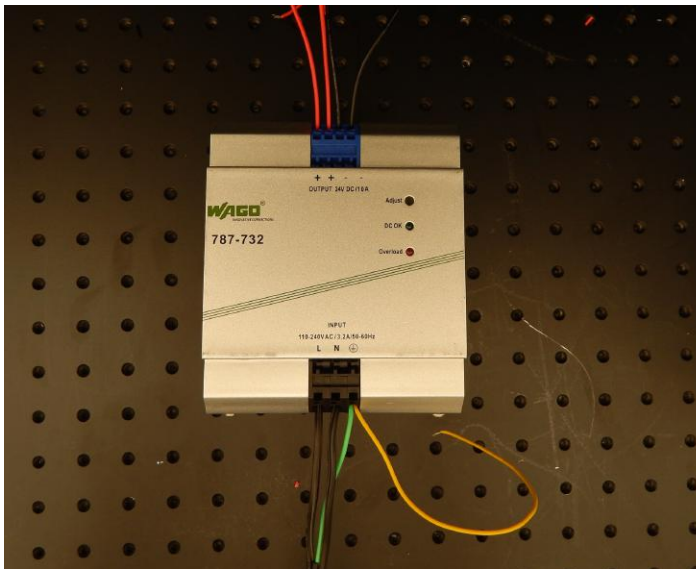
5. After **Module 5**, the valve wiring collections wired into the WAGO will appear like this image.



Part 2: WAGO Modbus Setup

(!) Tip: The following steps are the **coolest** but also most complicated of the whole setup build. In these steps, you will wire the connections from each individual solenoid valve (in the order of the valve numbers) to a MODBUS controller, the WAGO. This will allow for fast programmable control of each individual solenoid valve corresponding to each individual control line as set up in **Module 3**. We will assemble the WAGO **Parts W1-W4** according to the manufacturer's instructions. You can follow along here or skip to the solenoid wiring if you chose to use the manufacturer's tutorial.

6. Wire the Power Supply Module of the WAGO controller (**Part W2**). First, cut 3 sets of ~6" of 18G red and black wire (**Part o**). Strip each wire approximately ½" on the top and bottom. Insert the wires to the 24V DC output lanes at the top of the brick (red: +, black: -). These wires are used for powering the rest of the WAGO. Similarly, wire the input lanes at the bottom of the brick by stripping wires within the 24V power supply (**Part n**) and inserting them into the L, N and ground lanes as shown. These connections are used to externally power the Power module brick. Add an additional wire (shown in yellow here, but black **Part o** wire is just fine) to the final lane of the input lanes. The completed wiring should look like this:

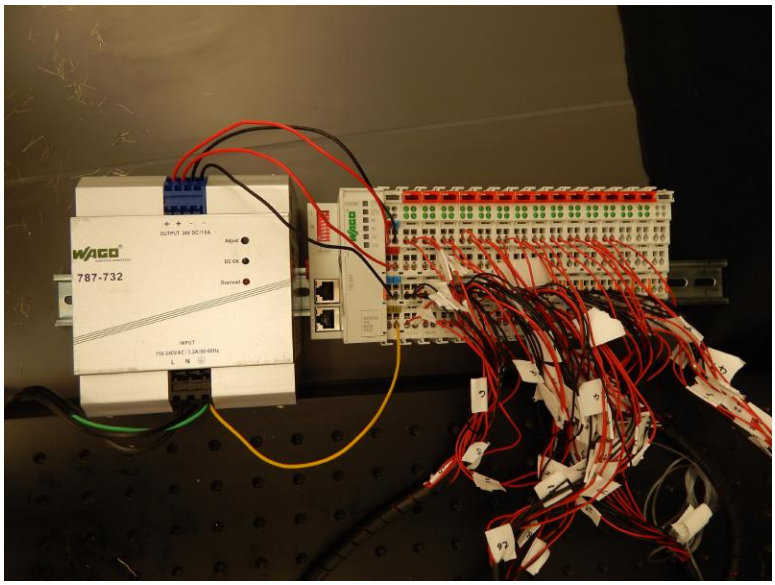


7. Mount the wired Power Supply Module **Part W2** to the DIN rail, **Part W5**. This will help stabilize later components.

8. Assemble **Parts W1, W3,** and **W4** by first mounting the I/O controller (**Part W1**) onto the DIN rail and then clicking in subsequent digital output (DO) modules (**Part W3**) using the orange tabs on the end of each module, ending with the end module (**Part W4**). You will need the following number of digital output (DO) modules (**Part W3**) for your build since each DO Module controls 4 valve states and (separately) powers up to 2 regulators.

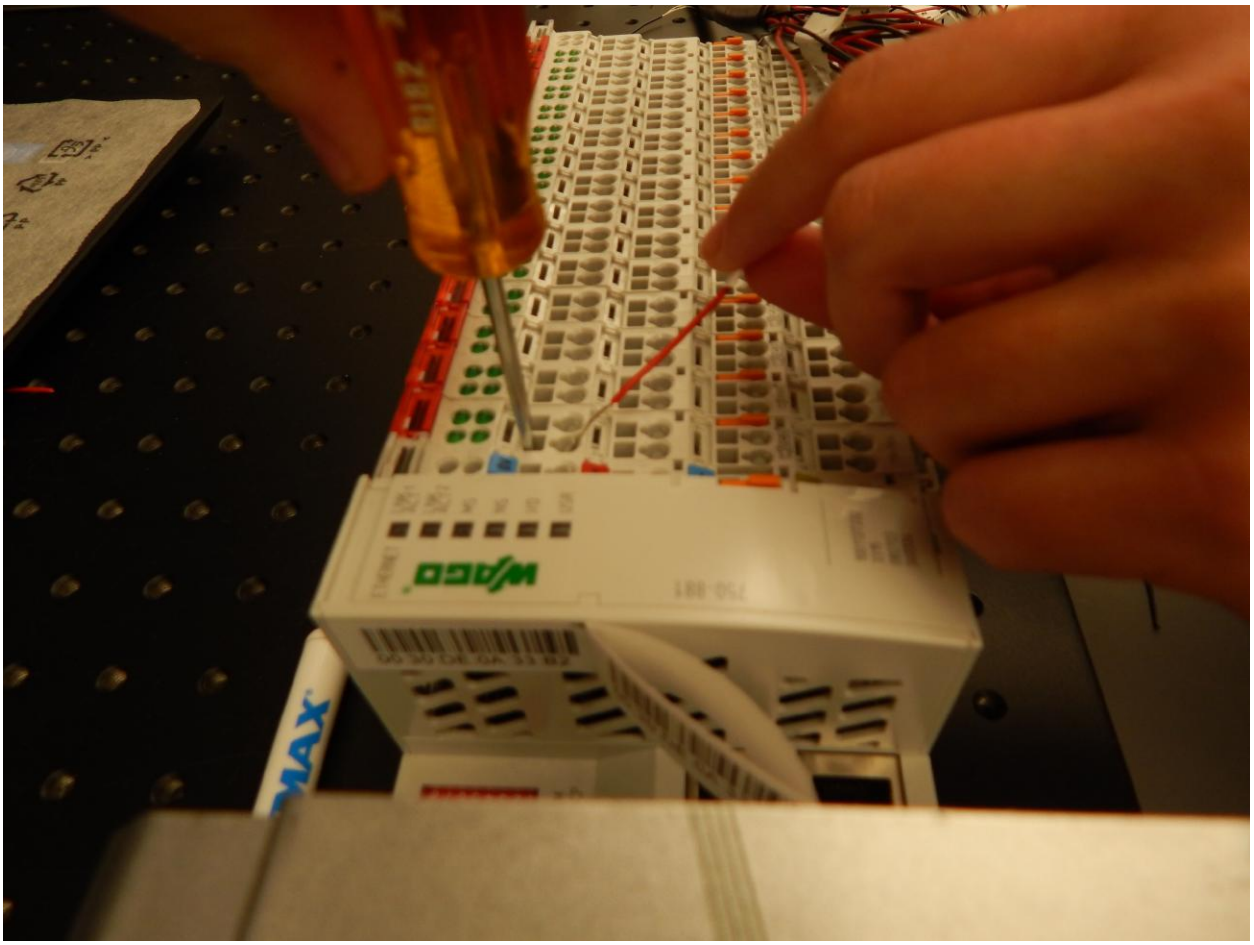
$$\#\ of\ needed\ DO\ modules = \frac{\#\ of\ Control\ Lines}{4} + \frac{\#\ of\ Digital\ Flow\ Regulators}{2}$$

The WAGO assembly should now look like this (minus all the wires):



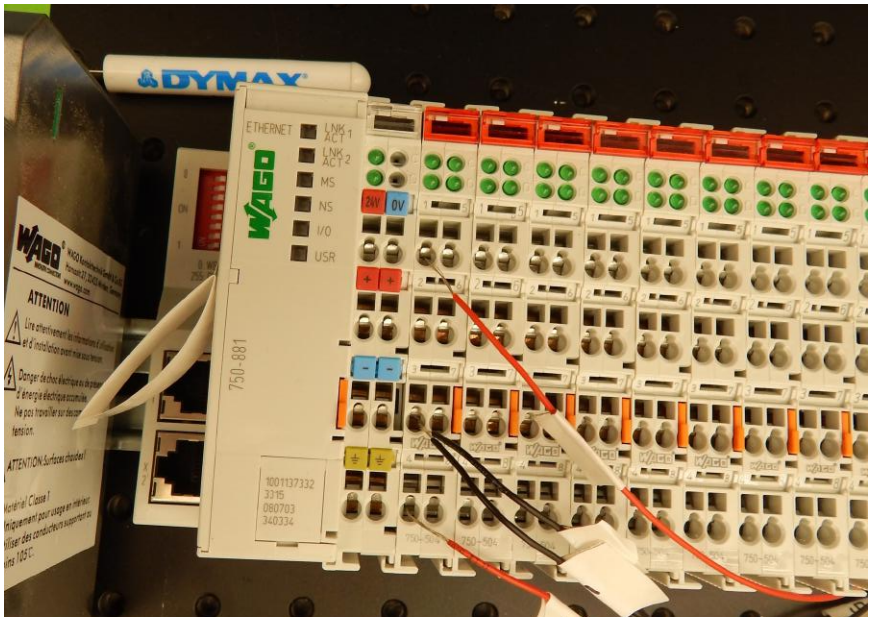
(*) Modifier: Assemble only the amount of digital output (DO) modules (**Part W3**) that are needed for your build. Alternative modules, such as combined digital input (DI) and digital output (DO) modules are available from WAGO Inc. for more specialized purposes, such as addition of other PLC-controllable instrumentation (for instance, we also control UV curing lamps with our WAGO) to customize your build. The WAGO system is highly modular; components can easily be exchanged or added ***after*** the build if later functionality is desired.

9. Begin wiring connections from the digital output (DO) modules to the individual solenoids. You will wire in the order of the valve numbers. Each DO module contains 4 outputs to control 4 solenoids. The 4 green lights at the top of each module from top left to bottom right register the valve state. Each column of a single DO module controls 2 outputs so they must be wired together. To wire, use a small flat head screw driver to pull back the metal tongue in the rectangle above the circular breakout connector and insert the wires into the circular breakout as shown below. Practice once before proceeding.

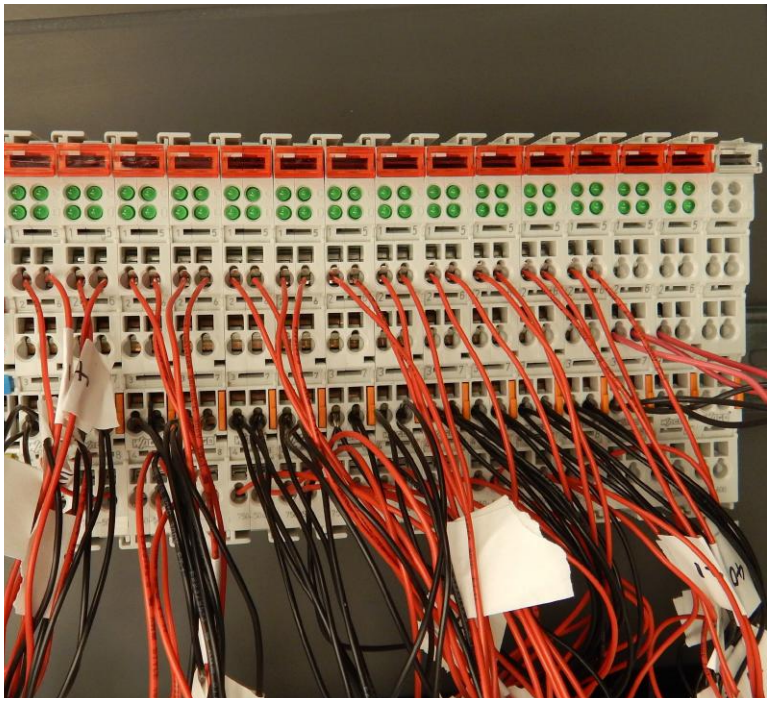


10. Starting with the first DO module (**Part W3**) closest to the I/O controller module (**Part W1**), put the red wire for Valve 0 in the left breakout of the 1st row (labeled position 1 on the Module). Put the red wire for Valve 1 on the left breakout of the 4th row (labeled postion 4 on the Module). Put the two black wires for both Valve 0 and Valve 1 in the left breakout of the 3rd row (position 3 on the Module).

(!) **Tip:** Here you are attaching the red leads to the 24V pulse outputs and the black leads to the Module ground, which interfaces with the global ground. If you are interested in further information about the WAGO wiring, check out the WAGO guide for this module 750-504. There are some excellent diagrams on the manufacturer's site that explain the internal WAGO wiring in greater detail.



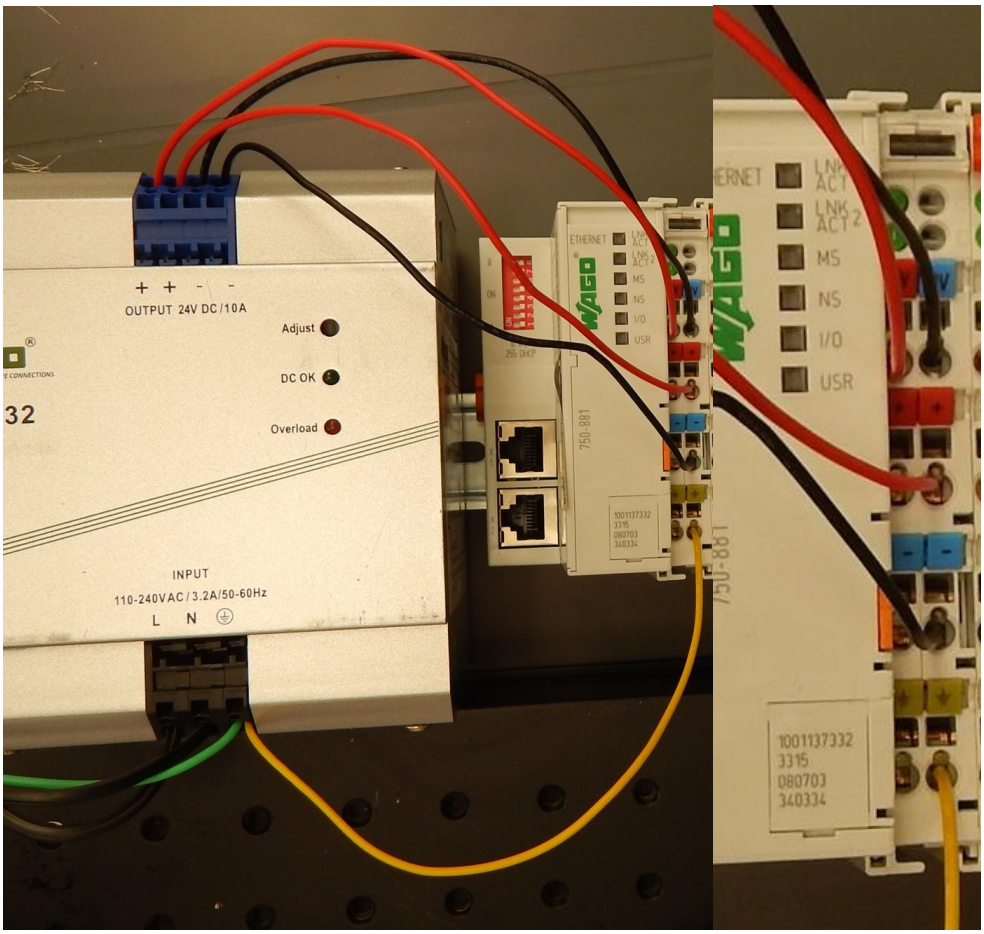
11. Repeat for Valves 2, 3 using the right breakouts for the first module then continue wiring all the rest of the valves. Your WAGO build should now look like this.



12. Wire the global ground wire (yellow wire from the ending input lane) to the first module of the I/O controller base (**Part W1**) as shown below. Insert the striped wire in the bottom right input of the module. This connects the input ground to the shared module

ground. Next wire the power supply to the positive and negative leads from the output of the Power Supply Module (**Part W2**) as shown.

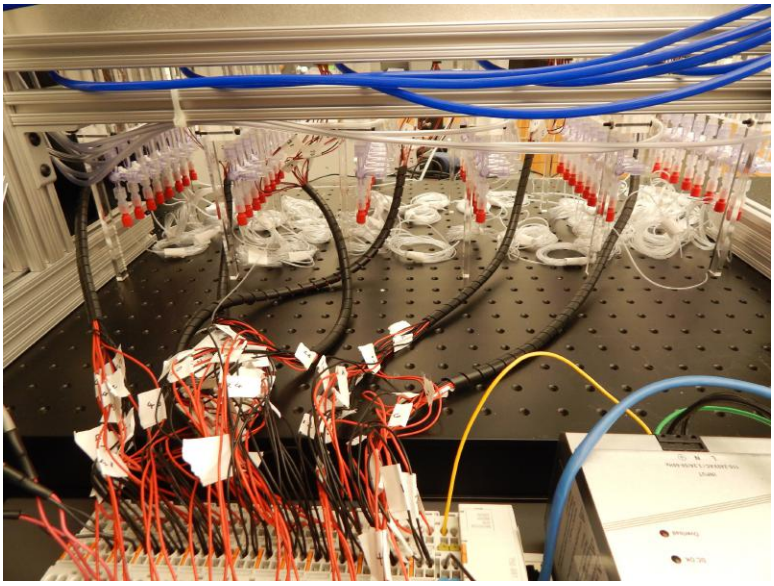
(!) Tip: The I/O module powers the rest of the DO module blocks and establishes block-wide ground ("global ground"). It can be identified by lack of an orange top.

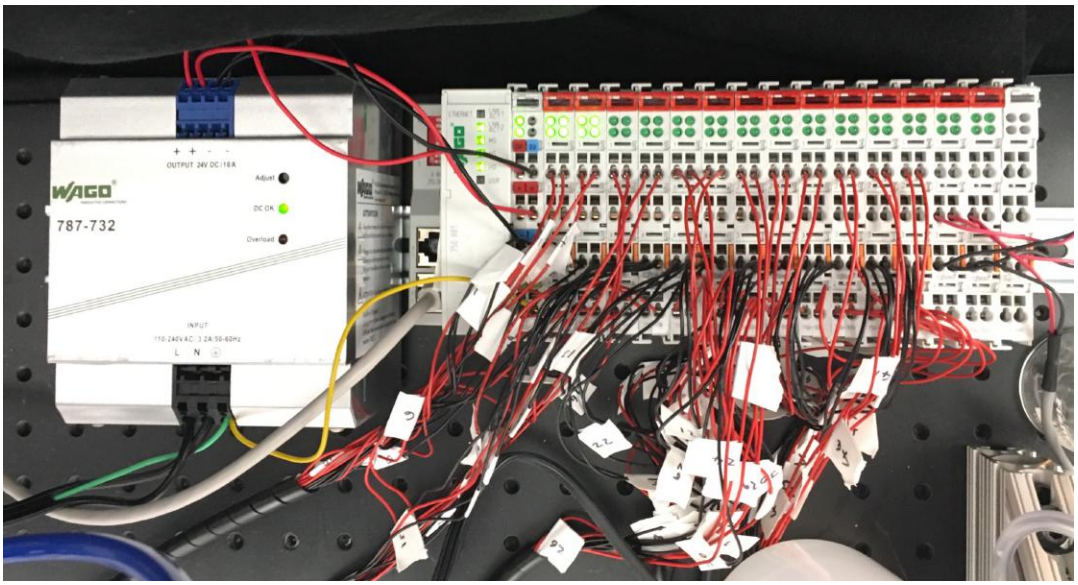


13. Next, connect the Ethernet cable, **Part m**, to the top Ethernet port (shown above) of the WAGO I/O (**Part W1**) and to your computer. Change the WAGO dipswitch (top red box on **Part W1**) to the desired WAGO Ethernet address. We use 192.168.1.3 but this address may depend on your institutional requirements. Use the manufacturer instructions to change the dipswitch as desired.

14. Plug in the WAGO power (**Part n**) to an outlet.

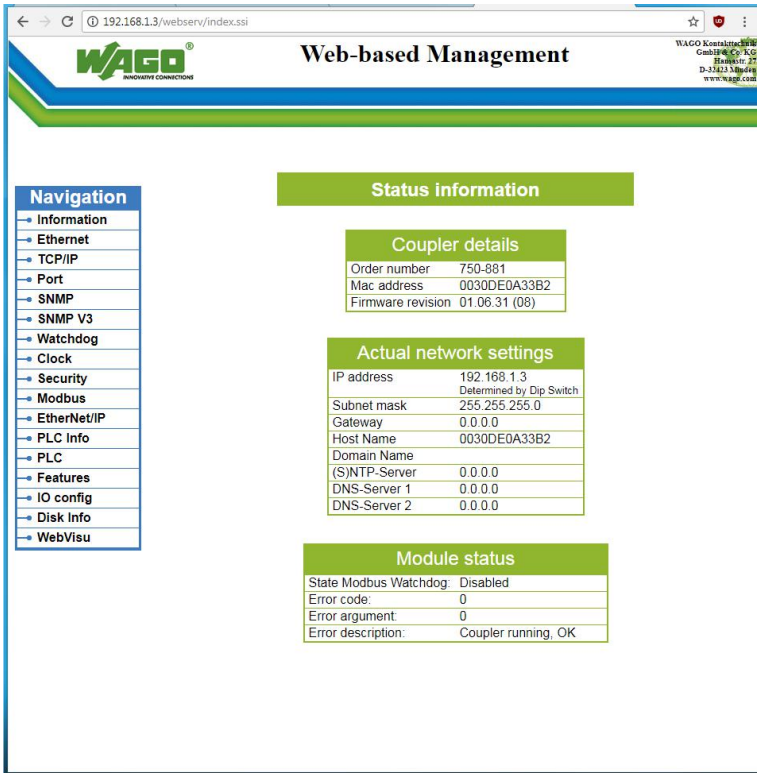
15. The WAGO assembly, with all the wiring connected, should now appear as the following images.





You should see the power lights and connectivity lights (**Parts W1, W2**) on in your setup upon powering the WAGO but valve state lights (top of **Parts W3**) may or may not be active yet depending on the initialization parameters.

16. In a browser, type in the WAGO Ethernet address you set in Step 13. You should see the following display.



If you do not, change your WAGO Ethernet settings under your Network Settings to have the following subnet. At startup, the Watchdog timer (under the Watchdog tab at the WAGO Ethernet website shown below) should be set to 0. If it is not, set it as such on this site.

17. Now proceed to set up your WAGO device interface using our control software according to the Geppetto Read Me as described in the main text of the article.

Congratulations!
You have finished all the Modules for the Build!

Proceed to Device Operation and Setup

Step-by-Step Operation Guide

Device Operation Guide: Microfluidic Experiment Operation Manual

Note: In this guide, we run through our MITOMI experiment described in the Main Text as an example device run.

Part 1: Pre-Run Priming

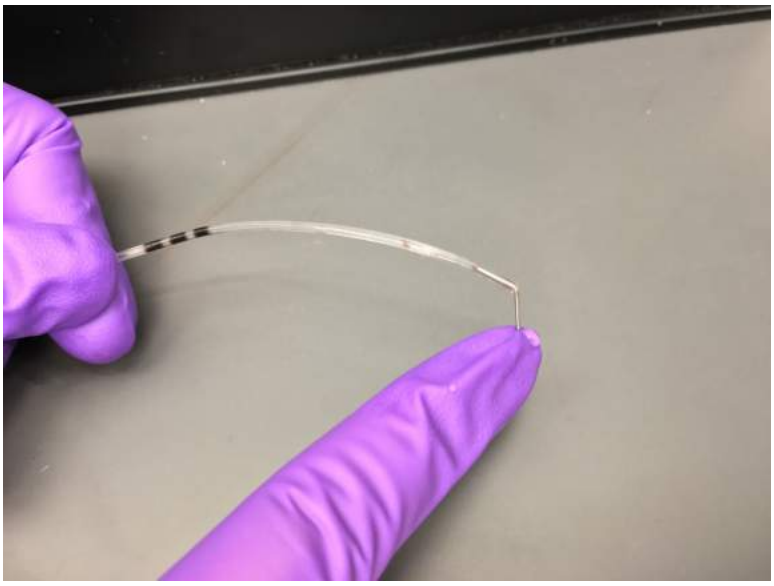
1. Before any device run, make sure **Control Manifold** stopcocks for the valves of interest are horizontal (valve pressurization function is active), control lines are organized and available, and the **Geppetto** GUI or script for your run is loaded.

2. Send pressure to the desired **Control Valve Manifolds** by toggling their corresponding switches at the **Control Switchbox** on the **Base Board** of **Module 1**. Set the regulator pressure to the desired valve closure pressure.

   For our MITOMI experiment, we use 15 psi.

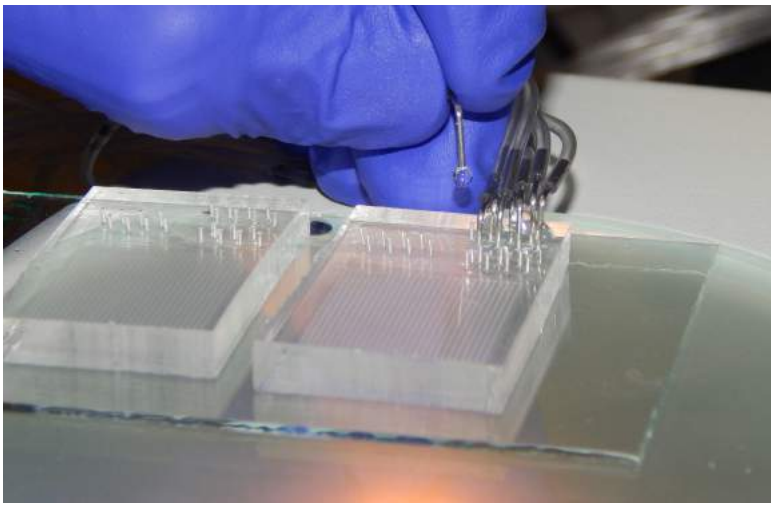
(!) **Tip:** Make sure valves are OFF (open state) on your Geppetto interface before beginning any run. Water should not be spraying from the control lines. If you find water is spraying when the manifold is pressurized, close the switch to off and change your valve state. You may need to refill the Water Reservoirs. This switchbox is used as a safety feature to guard against depleting all your control lines.

3. Prime the device control lines by quickly pulsing the valve state (on-off) for each valve until a bead of water forms at the end of each line. Follow the steps from the **Operation Start Guide** Part 3 to do so before each run.



Part 2: Device Setup

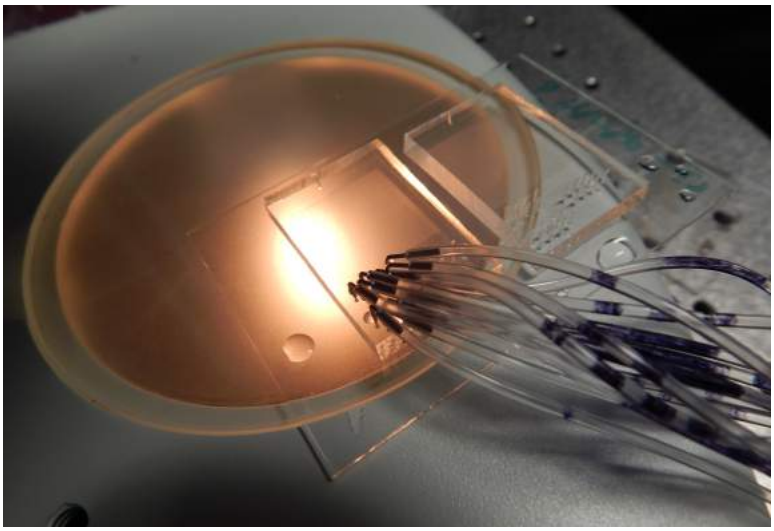
4. Insert pins from the Control Lines according to valve number into the corresponding Control Line Inputs on the device. Again, check that a small bead of water forms on each line before placing the line into the device.



Control Lines inserted in the MITOMI device for an experimental run.

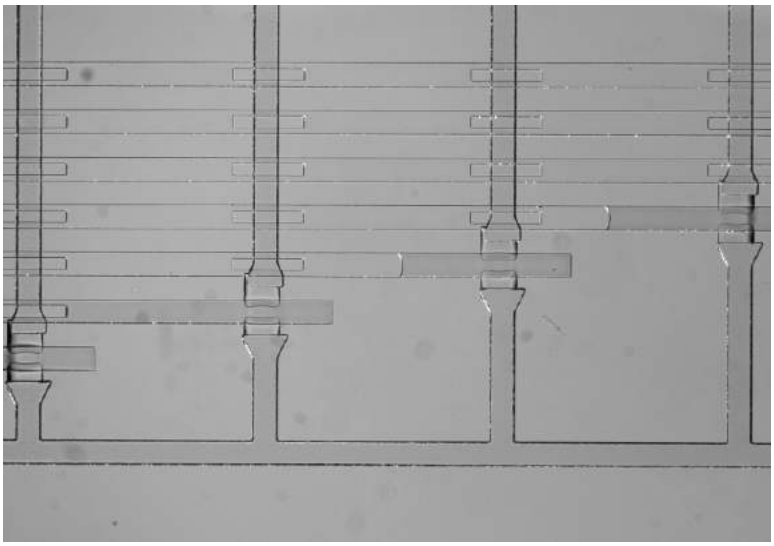
(!) **Tip:** Don't force the pins all the way to the bottom of a microfluidic device. This may delaminate your device when valve pressure is applied. Instead insert them firmly into the device but only about halfway down the full thickness of the PDMS.

5. When finished, the device with control lines attached should appear as follows:



6. De-bubbling the device is an important step to remove air bubbles from the control lines for consistent valve closure. We do so using a process called "dead end fill."

   Using Gepetto, fill control lines one at a time with water at your desired valve closure pressure (in this case, 15 psi).



Air in control lines being pushed out by water during the initial actuation step.

(!) **Tip:** We suggest actuating each control line one-at-a-time and watching under a microscope to ensure that the pins have been inserted in the correct order and that there are no defects in the device.

7. Now you are ready to insert your flow lines to load samples of interest into the device. To do so, load Tygon lines (**Tubing T5** with a pin **P1** at the end) of the appropriate

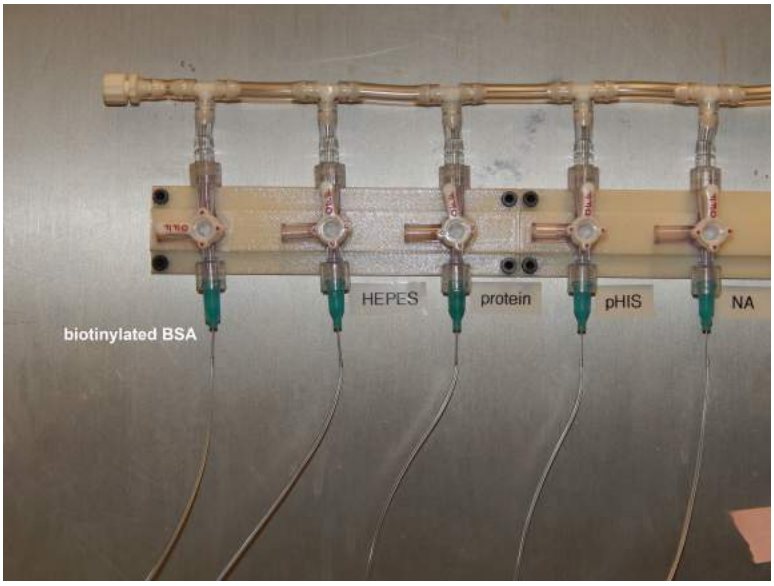
length to reach the device with reagents of interest. Attach these lines on the **Flow Manifold** of interest and connect the pin to the device.

When ready, pressurize the corresponding **Flow Regulator** on the **Base Board** to the desired inlet pressure. Open the global **Flow Manifold** stopcock (**Part i**). Leave the individual **Flow Manifold** stopcock (**Part N**) corresponding to the flow line closed until the experimental run has begun and valves are appropriately actuated.

(!) Tip: As part of device priming, we also de-bubble the flow channels of the device for better flow performance. We recommend picking a flow reagent (like water) to first de-bubble your device before loading precious samples. Below we use a BSA mix for de-bubbling and surface treatment. Whatever reagent you choose, the de-dubbling process of "dead end fill" takes approximately 5 minutes with a flow through of reagent then dead end pressurization.

8. Actuate your valve state for beginning the device run using Geppetto. Now open the individual **Flow Manifold** stopcocks (**Part N**) for each line of interest for your experimental run.

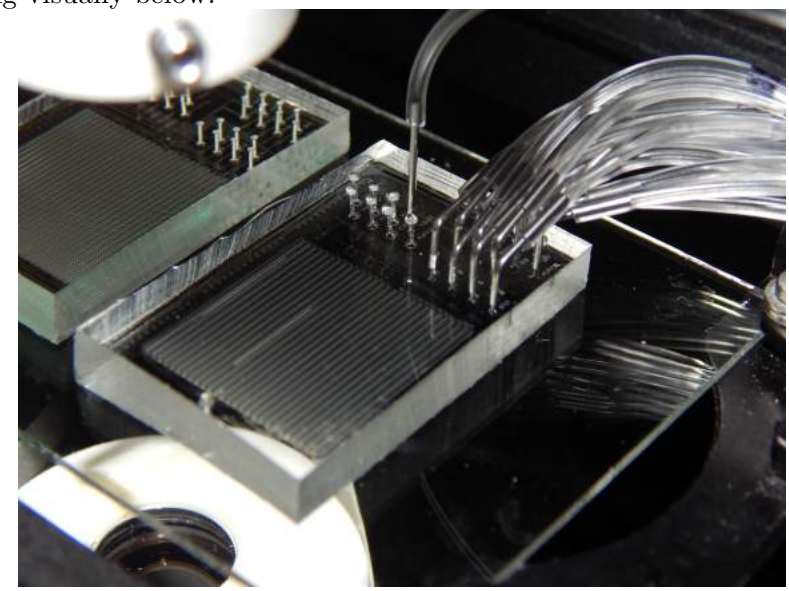
   In our MITOMI example run, we have connected a flow line containing 100uL of 2mg/mL biotinylated-BSA in water to our device for the beginning script. We then opened the biotinylated-BSA line on the manifold.



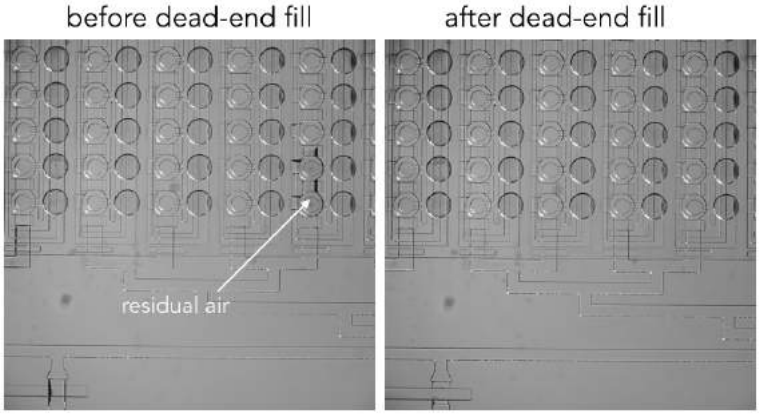
(!) Tip: You can leave lines closed at the Flow Manifold stopcocks until needed in your experimental sequence. It's your preference based on how you have configured your valve states.

9. Using Geppetto, flow in the reagent used for de-bubbling by releasing any valves blocking flow.

In our MITOMI experiment, we flow in ~7 pL of the BSA reagent. You can see the chamber filling visually below.



10. After ~5 min of reagent flow, close an outlet valve on your device to "dead end fill" the flow reagents. and dead-end fill the remainder of the device until no air remains (the remaining air will be forced out through the PDMS) (~5 min).



This completes device priming. Now you can proceed to your experimental sequence. When finished, turn off all the valves and depressurize the manifolds by reversing the earlier steps of this manual.

In the following steps, we will demonstrate an experimental protocol for a MITOMI device run as described in the Main Text.

Part 3: MITOMI Experimental Example

1. After priming the device as demonstrated in the previous steps, insert lines containing the remaining solutions necessary for surface chemistry and protein pull-down (Table 1).

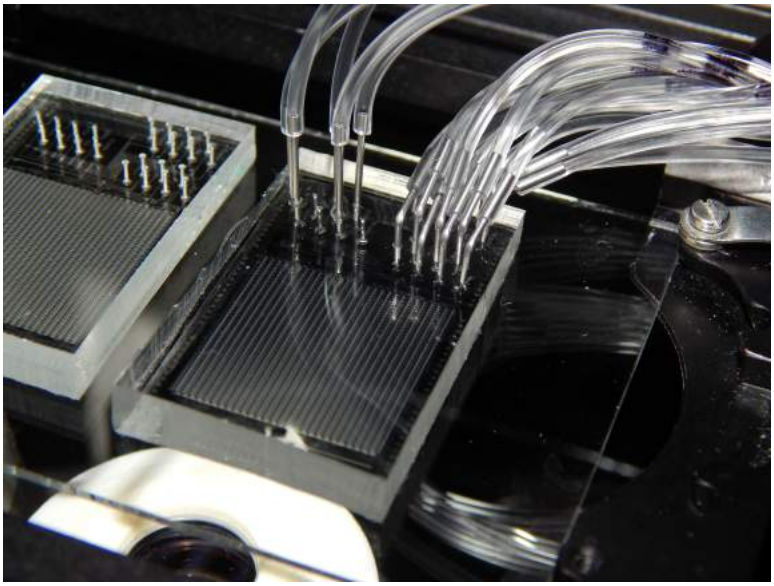
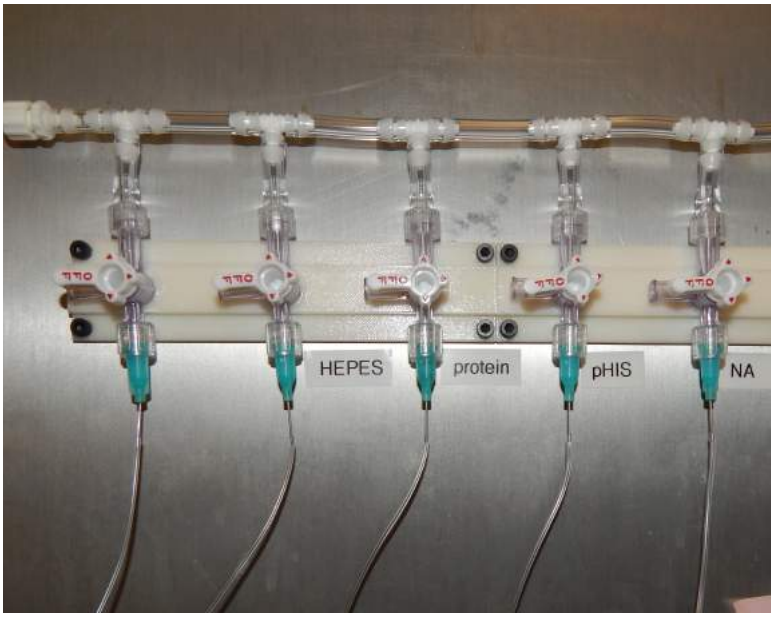


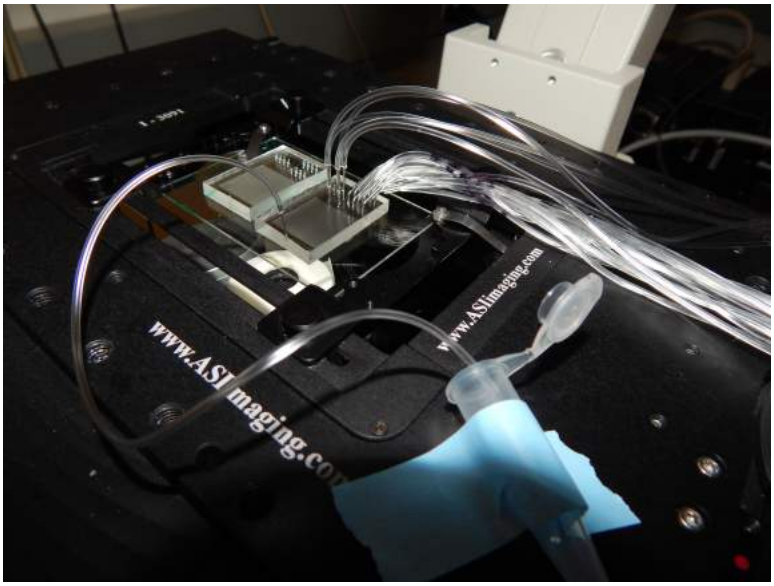
Table 1. Surface chemistry components necessary for surface immobilization of a GPF-tagged protein.

Component
Biotinylated BSA (diluted to 2 mg/ml in water) (Pierce; ThermoFisher cat. 29130)
PBS
Neutravidin (diluted to 1 mg/ml in PBS) (Pierce, ThermoFisher cat. 31000B)
PBS
Biotinylated BSA
PBS
Biotinylated anti-GFP antibody solution, diluted to 0.1 mg/ml in PBS (Abcam, ab6658)
PBS
eGFP (BioVision, cat. 4999) (310nM eGFP, 10 mg/ml BSA in PBS)

2. Open the **Flow Manifold** stopcock corresponding to each solution flow line. Set pressure to 3-4 psi. Note that at this point the valves inside the MITOMI device should be closed to prevent flow through the chip.



3. Insert an empty Tygon line (**Tubing T5**) into the outlet of the device, to collect the flow-through. This facilitates automated setup by preventing a pool of liquid forming at the outlet of the device.



4. Confirm that all the valves controlling pressure to the flow lines are open, and that the device valves are in the desired state before beginning an automated setup script.

5. Run the Geppetto script for your experiment.

Congratulations you've finished your first device experiment!

Step-by-Step Operation Guide

Operation Start Guide: Initialization for Priming the Microfluidic Setup

Part 1: Loading the Water Reservoirs using the Control Loading Module

1. Make sure you have completed all Modules and that your completed Setup has all air and water connections completed, similar to the following image.

2. Confirm your flow (digital) gauges are powered.

3. Check control and flow regulators for air flow when the compressed air is connected by turning the pressure of each regulator to ~10 psi and ensuring that air is flowing through. If you notice a leak (strong hissing noise), you can isolate the leak by spraying soapy water along the connections until you find where bubbles form. Replace or tighten any leaky components.

Part 2: Loading the Water Reservoirs using the Control Loading Module

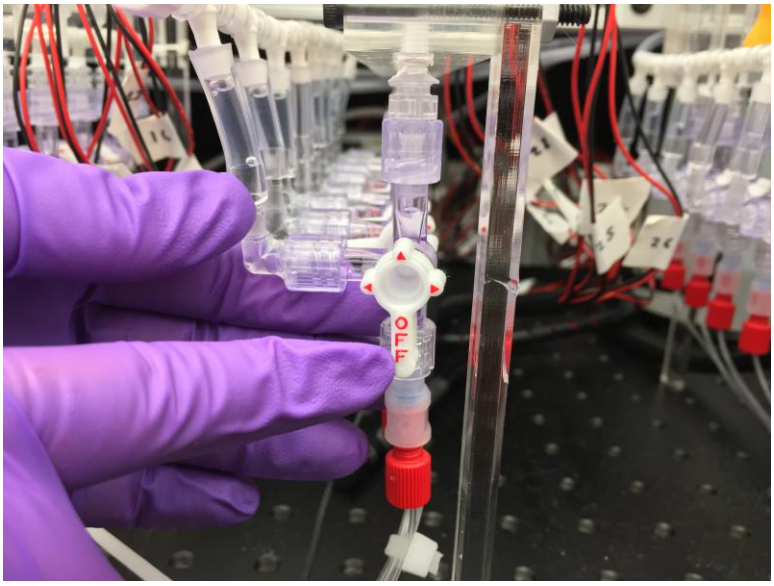
4. To operate control lines on a multilayer microfluidic device, we use pressurized water driven into the microfluidic control channels to close valves. Water must be loaded into the **Control Manifold Water Reservoirs** to be able to drive water through the control lines that connect to the microfluidic device. To do so, load the Control Loading Module Nalgene bottle (**Part j**) with filtered water.

5. Reassemble the Control Loading assembly when finished.

6. On the Control Manifolds, locate the control line tubing assembly 4-way stopcocks (**Part N**).

   As mentioned, these stopcocks toggle between the water loading function to load the water reservoir with water (OFF faces down toward control line assemblies) and the control line pressurization function for driving water into control lines (OFF faces horizontal toward **Part c**).

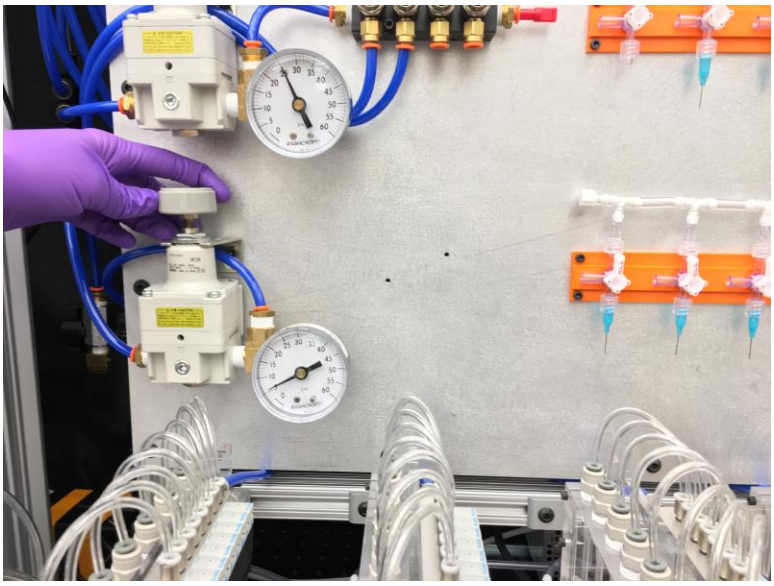
When loading water, place the stopcock OFF down (to activate the water loading path). This will allow water to flow from the master reservoir into the individual reservoir once pressure is applied to the master reservoir



(!) Tip: We typically load water individually for each valve-associated reservoir. That means that all other stopcocks MUST NOT be in the water loading function (that is, they should all have OFF facing horizontal). You can load multiple valves at once if desired but be sure to return each stopcock to its control line pressurization function (OFF facing horizontal) before proceeding.

DANGER: Forgetting and leaving stopcocks facing downward during attempted device operation will reflux water outward into your solenoid array components when the solenoid is actuated. This is messy and requires cleanup so never forget to have stopcocks in the right state for your desired function.

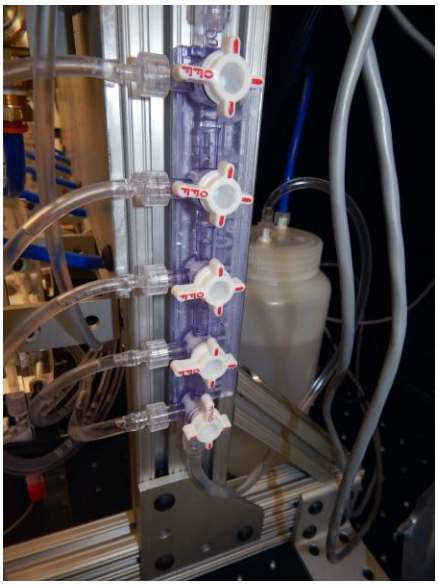
7. Pressurize the Control Loading Regulator on the **Base Board** to <5 psi.



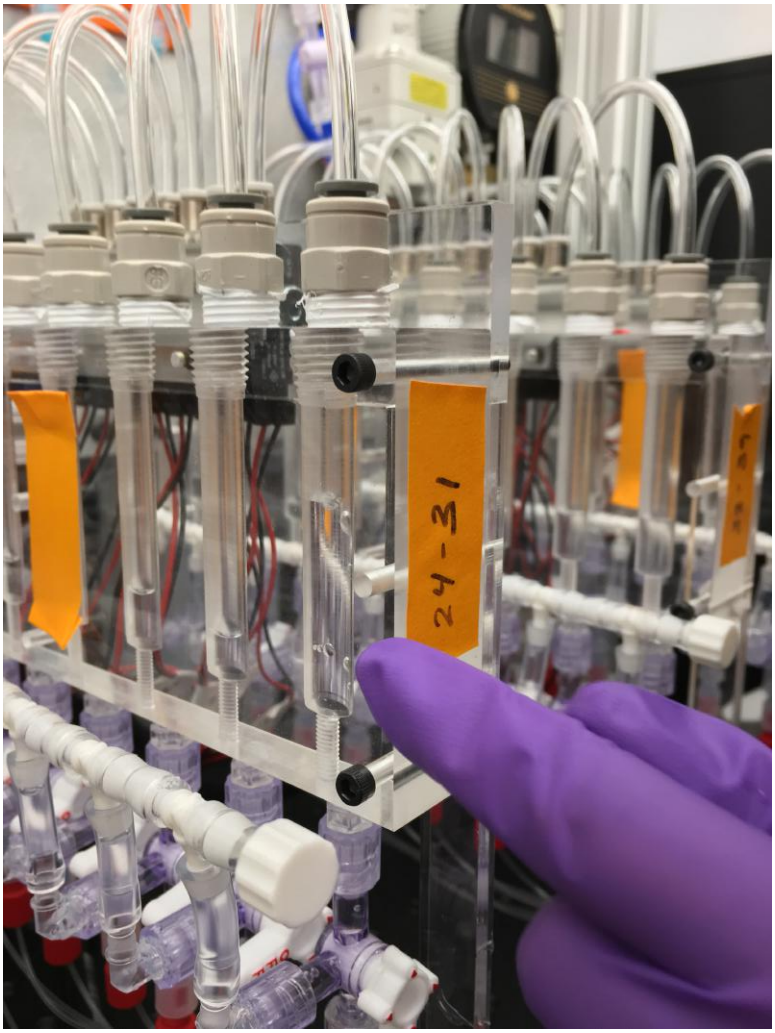
8. Open the appropriate stopcock valve on the Control Loading Manifold (**Part k**) from **Module 4** for the Control Manifold you are filling. This allows water to flow from the Control Loading water vessel to the valve bank you hope to fill. Remember, in **Module 4** each stopcock was associated with an individual Manifold.

   Close the stopcock when the reservoir desired is filled.

DANGER: Only load 1 Manifold at a time. You must watch the water reservoirs as they fill. They fill quickly (~5 s for all 8 channels). We have overfilled Water Reservoirs by attempting to fill too many at the same time, which is messy and takes time to clean up.

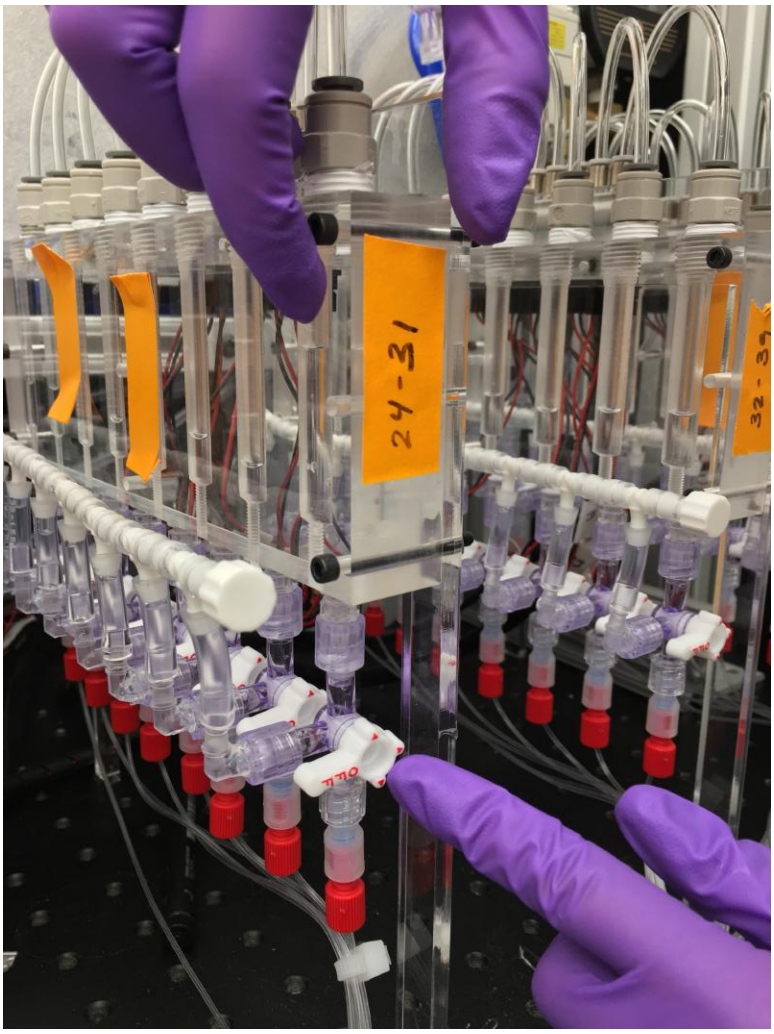


Stopcock open to fill Manifold 1 (bottom stopcock).



Water filling the reservoir for Valve 24.

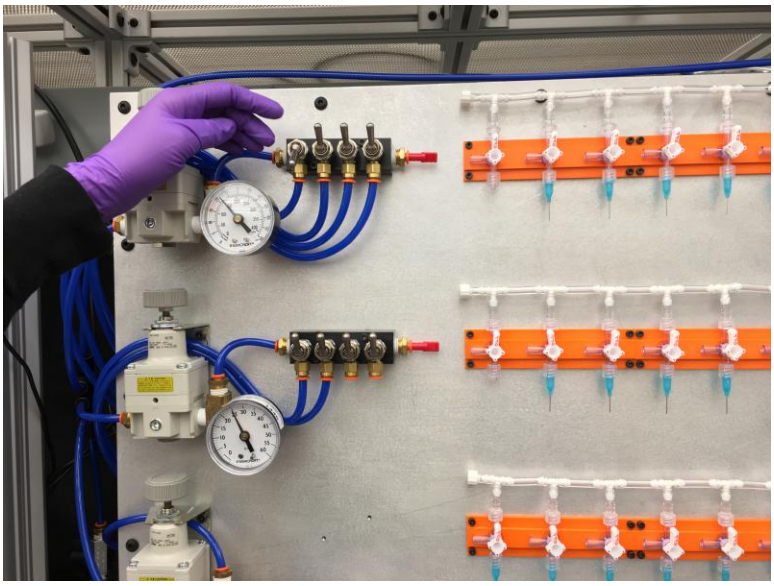
9. Close the associated stopcock (OFF to horizontal) on the Control Manifold after the reservoir is filled.



10. Repeat for all Manifolds and associated Valves. Remember to return the Control Manifold stopcocks to the horizontal position each time when finished.

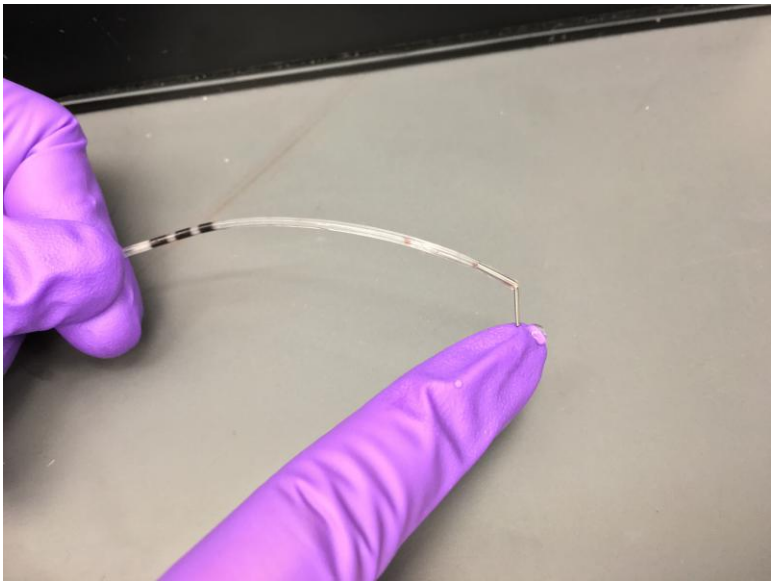
Part 2: Priming Control Lines

11. Now that the **Control Manifold Water Reservoirs** are loaded with water, air must be pushed out of the new control lines before connecting them to a microfluidic device. In order to do so, first send pressurized air to the **Control Manifolds** by activating the Manifold toggle for each Manifold on the **Base Board Manifold Switchbox** as shown.



12. Using the Geppetto GUI or the CLI scripting interface, gently turn on each valve for ~1s to send water all the way down control line until you see a water bead form at the end. Turn off the valve. Repeat for all valves.

(!) **Tip:** This is called priming the line and is important to eliminate air bubbles in the control line. We typically complete this valve pulsing **before each experiment.**



You're now ready to proceed to Device Operation!

Congratulations your Setup Build and Initialization is Finished!