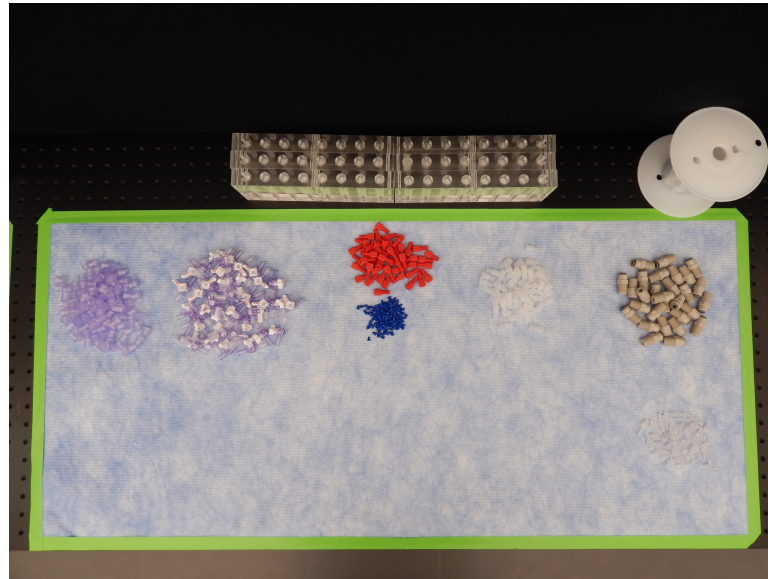


## Step-by-Step Build Setup Guide # 3

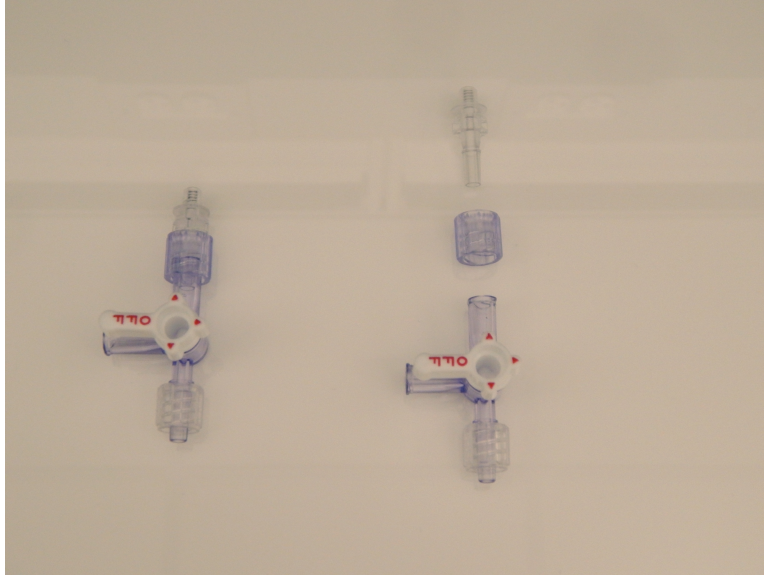
### Pneumatic Control System Module 3: Control 8-Valve Solenoid Manifolds

#### 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.



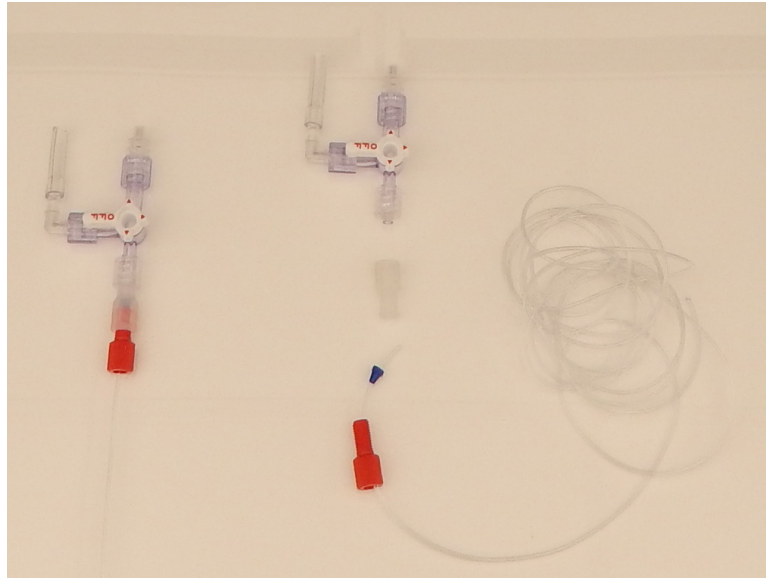
3. Continue the assembly by connecting the barb elbow (**Part c**) using one snap luer lock ring (**Part b**). Cut 1" of **T2 Tubing** and attach to the smaller edge of the barb elbow. Tubing lengths must be precise, as these will be connected across the manifold using spanning T joints (**Part K**)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



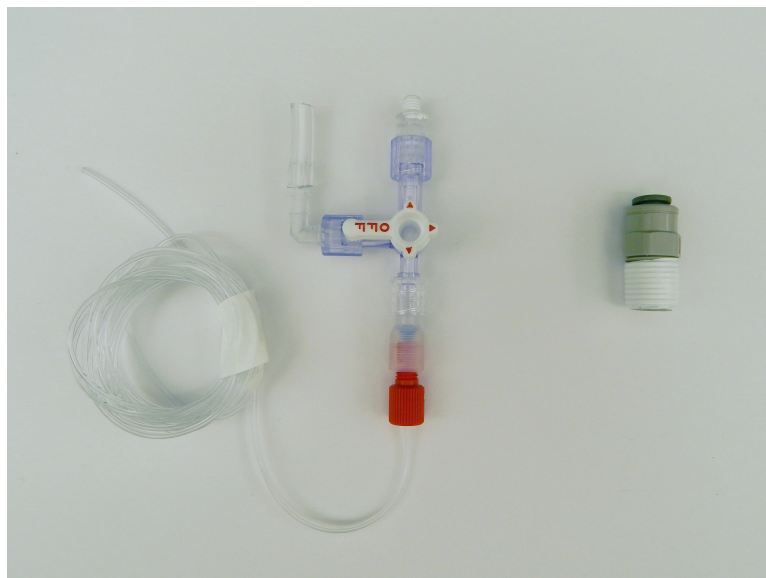
4. Assemble the flangeless ferrule assembly (**Parts d, e, and f**) as shown below with 14" of Tygon tubing (**T5**). This tubing will become the control lines that are ultimately connected to the microfluidic device.

(!) **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.

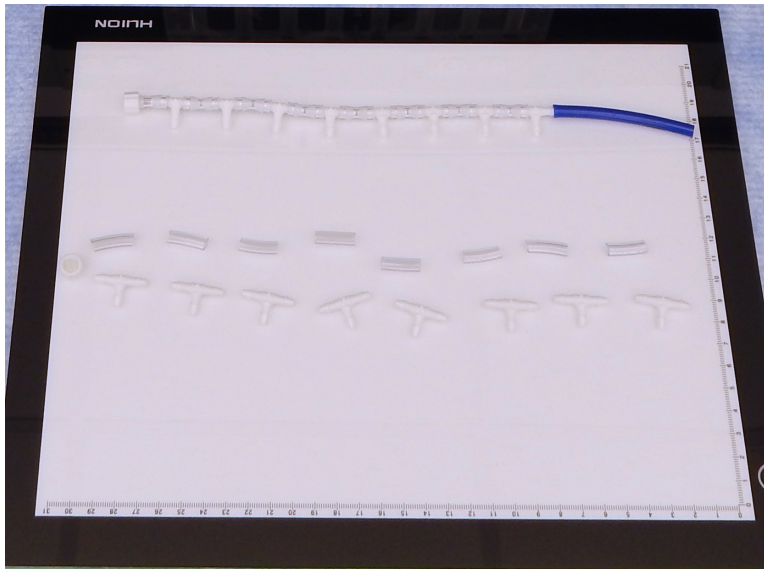


8. Align the stopcocks parallel to one another. Remove the barbed elbow assembly (**Part c**, **Tubing T2**) from the 4-way stopcock (**Part N**) and superglue the stopcock connectors to prevent leaks. It is important to perform this operation now, despite the disassembly,

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**.

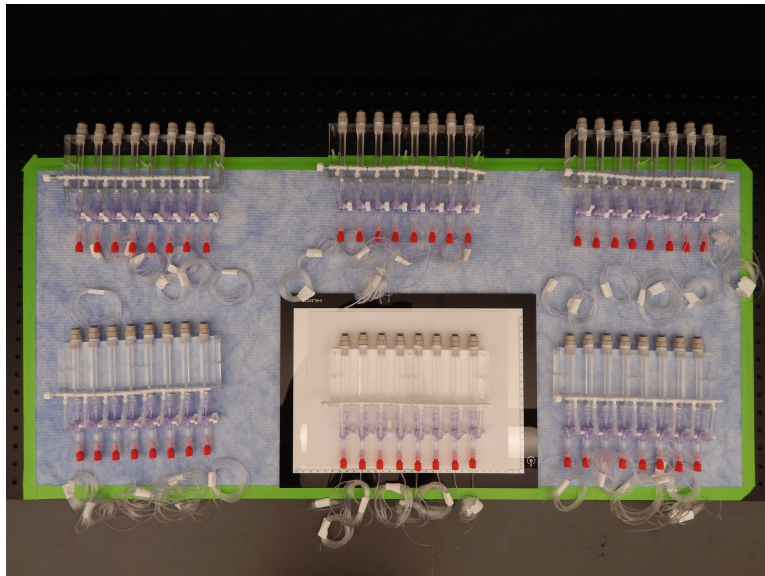


10. Attach the horizontal tubing segment to the elbow joint assemblies with **Tubing T2** connectors carefully.

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

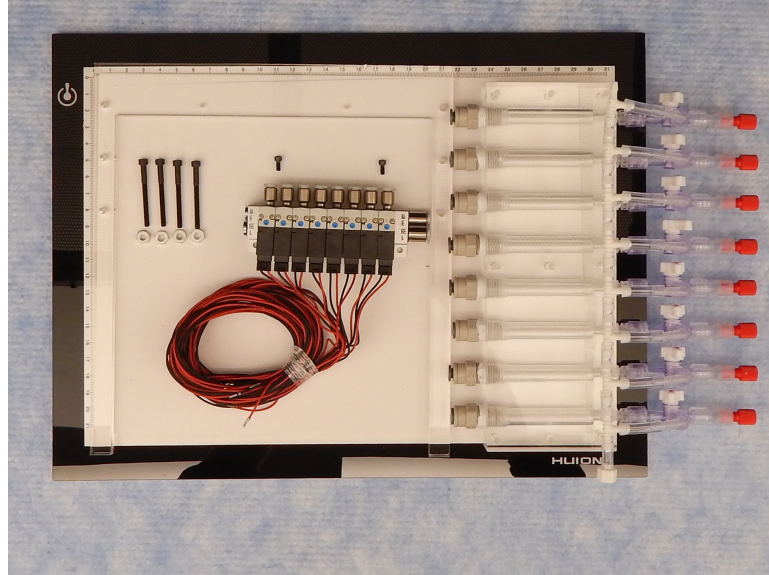


11. Complete 6 control manifold assemblies, or as many as required for your needs.

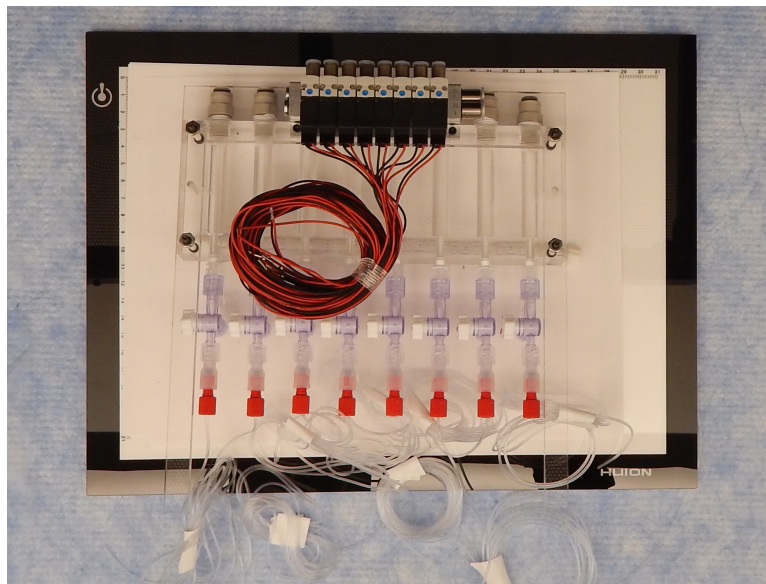


## Part 2: Valve Integration and Full Manifold Mounting

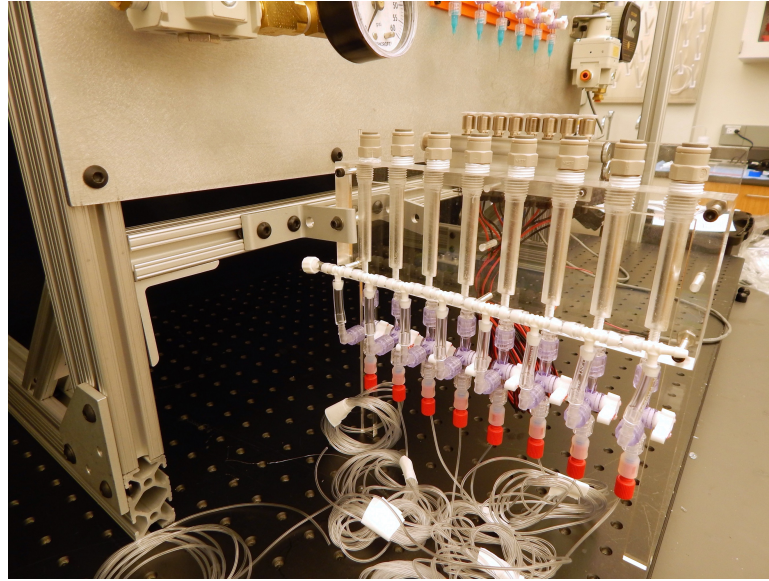
12. Gather the **Machined Parts “Water Reservoir Stand *reservoir stand*,”** 8-Valve Solenoid Array (**Part h**), and **Screws s2, s3** as shown.



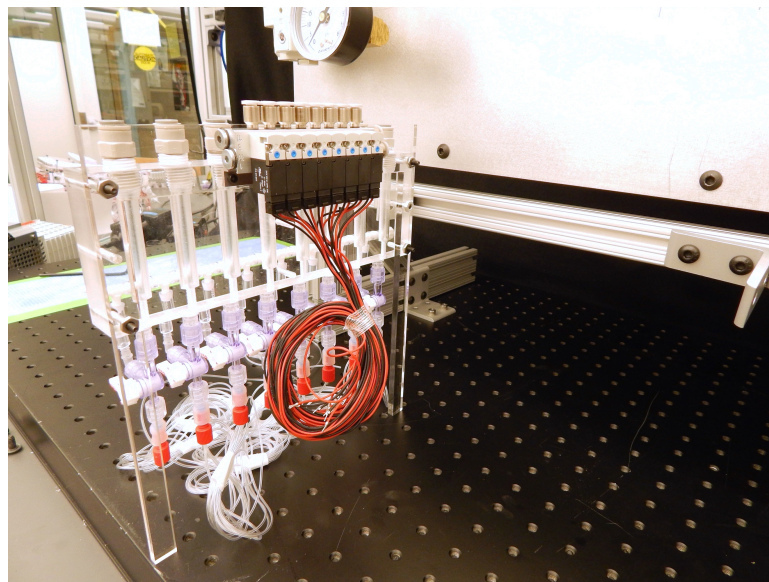
13. Mount the completed Control Manifold assembly built in Steps 1-11 to the Water Reservoir Stand using **Screw s2** in the tapped holes as shown. Mount 8-Valve Solenoid Array (**Part h**) to the back of the assembly with **Screw s3** as shown.



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



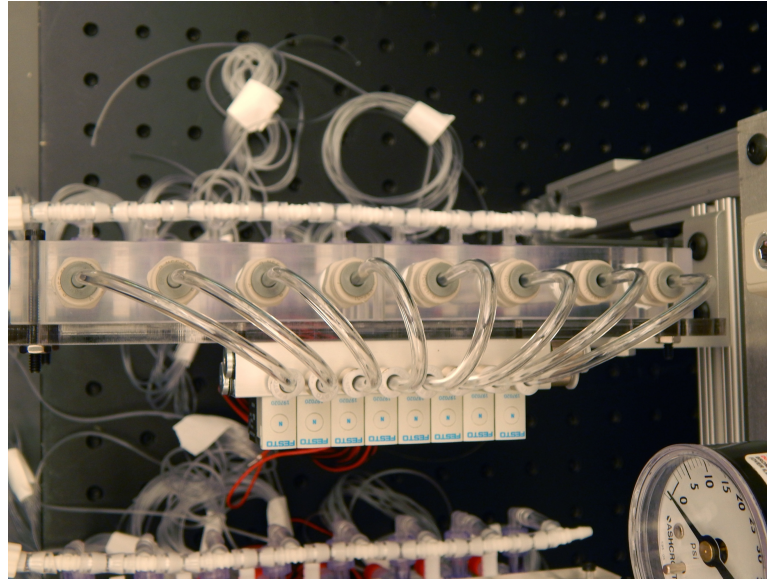
15. Here's the assembly visualized from another angle.



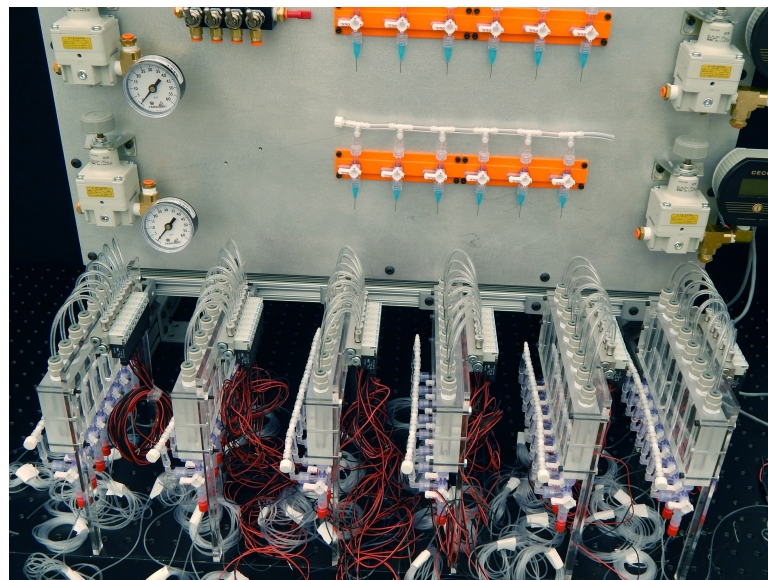
16. Complete the mounting for all 6 Control Valve Manifolds.

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.

- 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.

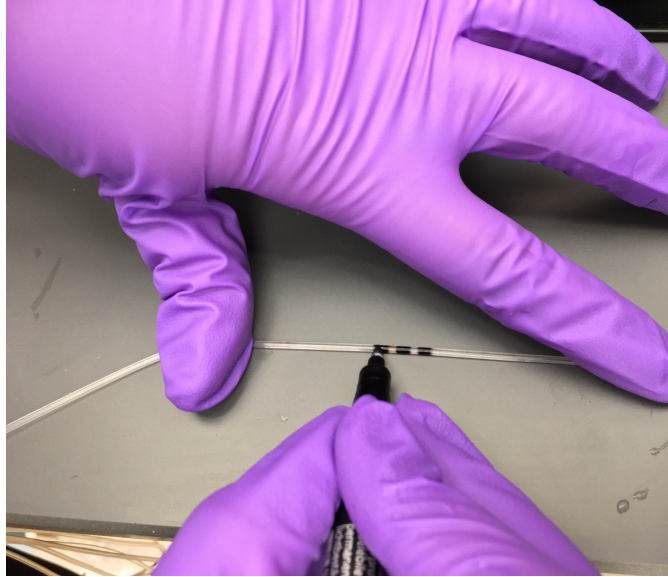


- Gently unravel the control lines associated with each valve one at a time. Cut to to the desired length. We recommend completing this step and Steps 22-23 per each valve individually before proceeding to the next valve.

**(!) 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”.

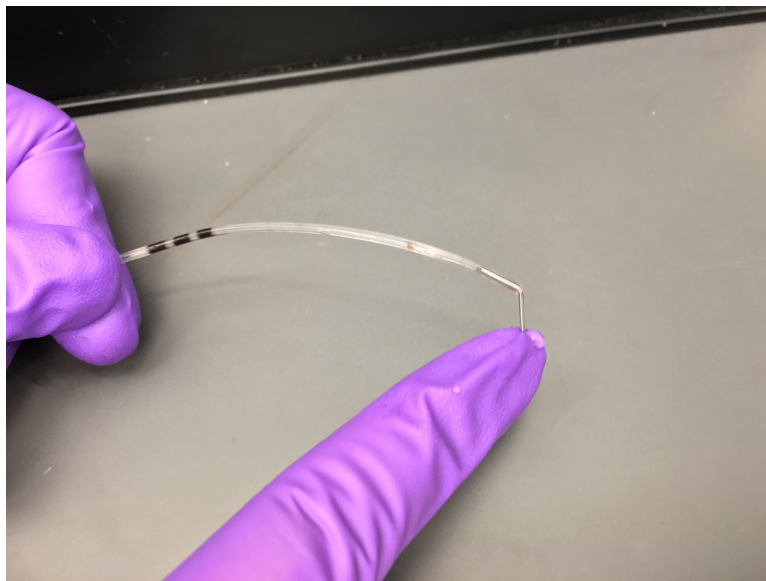
- Label the bottom of each control line with the Valve number. You can use a label maker, if desired. We recommend using a block encoding using Sharpie on each line. To do this, we mark a set of Sharpie rectangles for each Valve number starting at 0. (0= 1 small rectangle, 1 = 2 small rectangles, 3=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

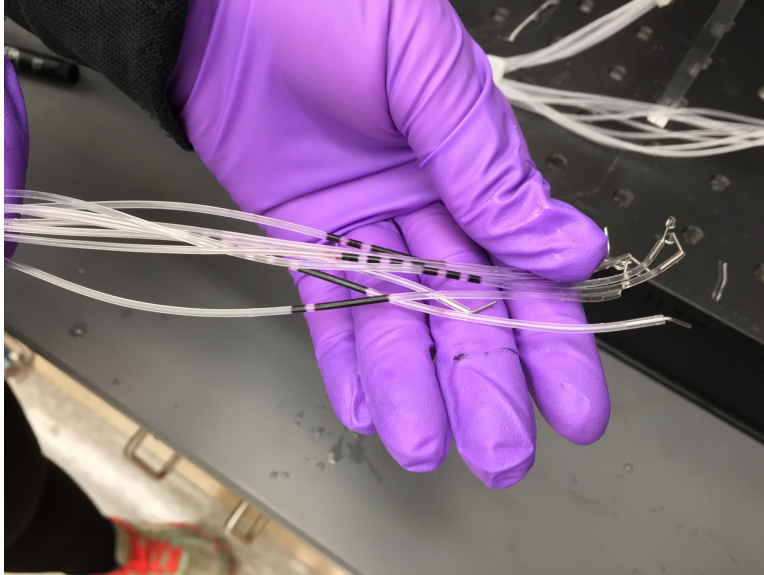


23. Next, insert a steel blunt pin (**P1**) at the end of each control line. We bend the pins to easily interface with the microfluidic device.

*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.

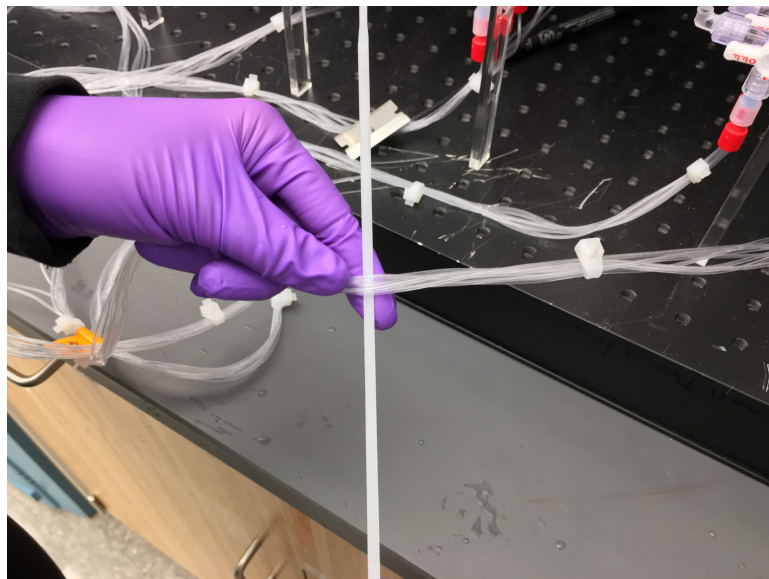


24. Repeat these steps for all control lines in a Manifold set.

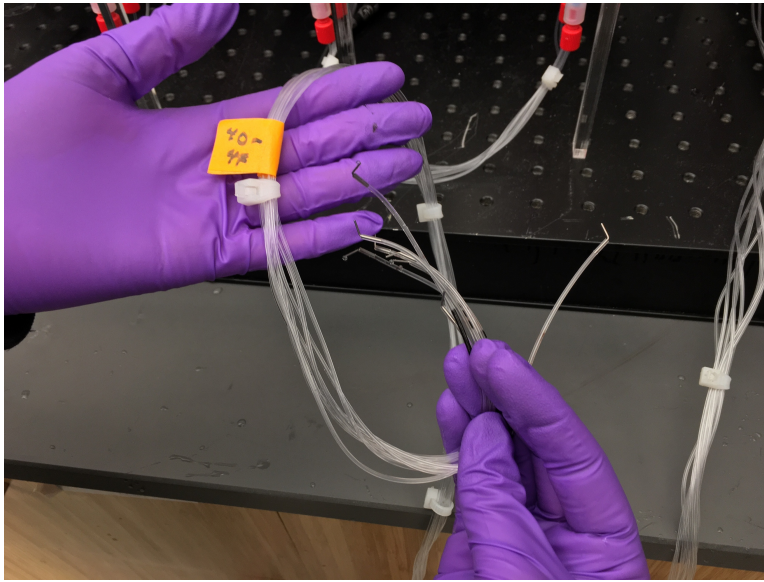


**Note:** In this picture, water is seen coming out of some of the lines but at this point, your setup should not yet have had water run through the lines so don't worry about that just yet.

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.

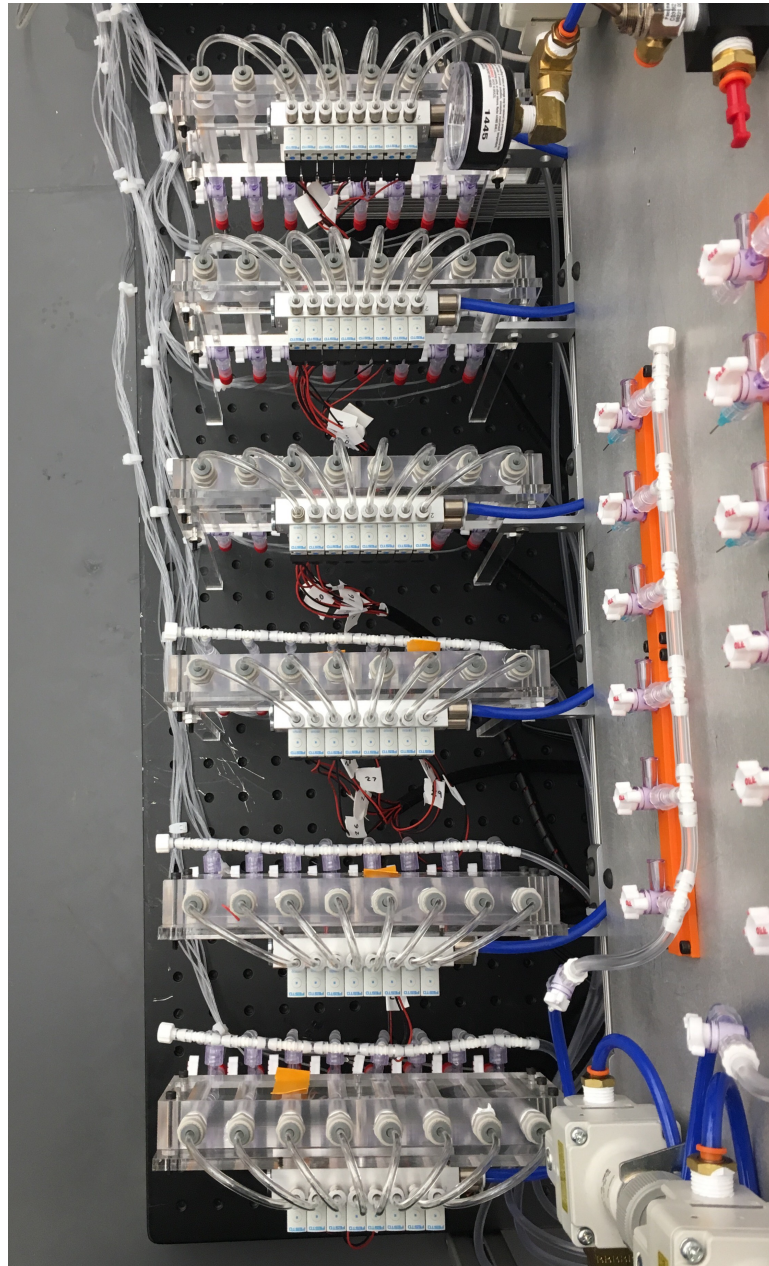
#### Part 4: Global Connections

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!