

## 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

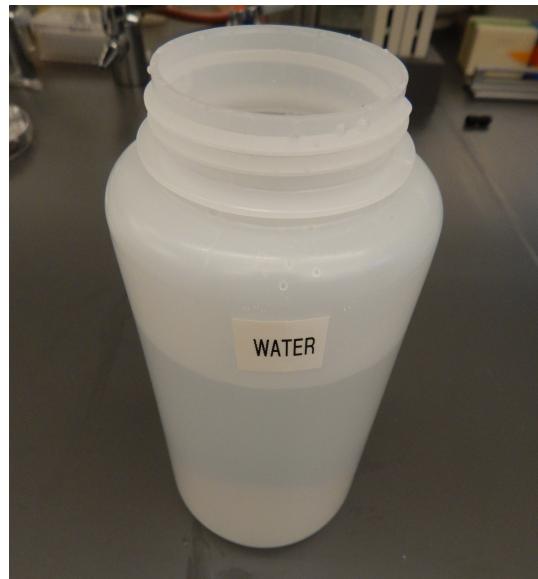
1. Make sure you have completed all Modules and that your completed Setup has all air and water connections completed, similar to the following image.



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.



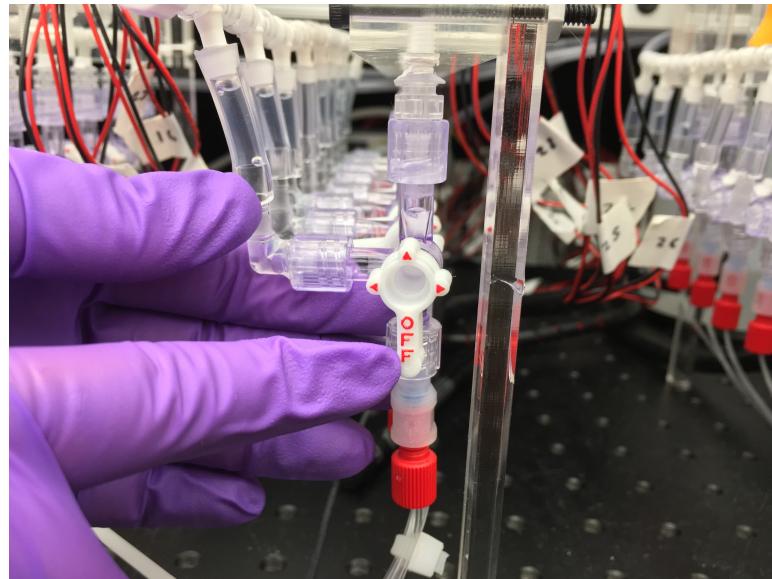
5. Reassemble the Control Loading assembly when finished.



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**).

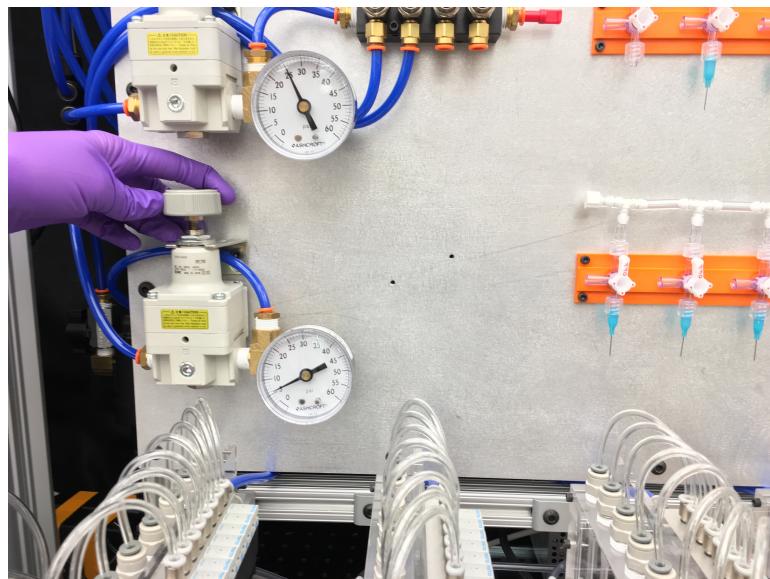
When loading water, place the stopcock OFF down (to activate the water loading path). This will allow water to flow from the master reservoir into the individual reservoir once pressure is applied to the master reservoir



**(!) 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.

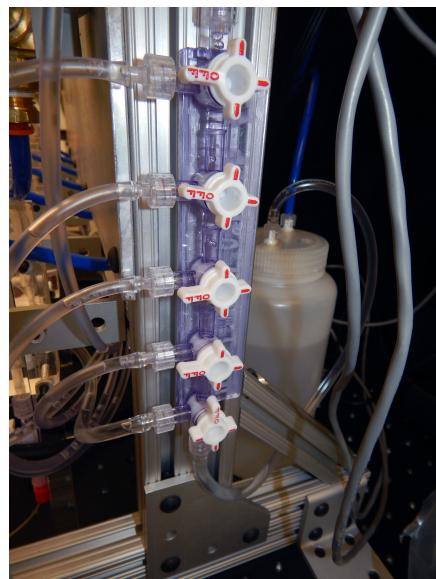
7. Pressurize the Control Loading Regulator on the **Base Board** to <5 psi.



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.

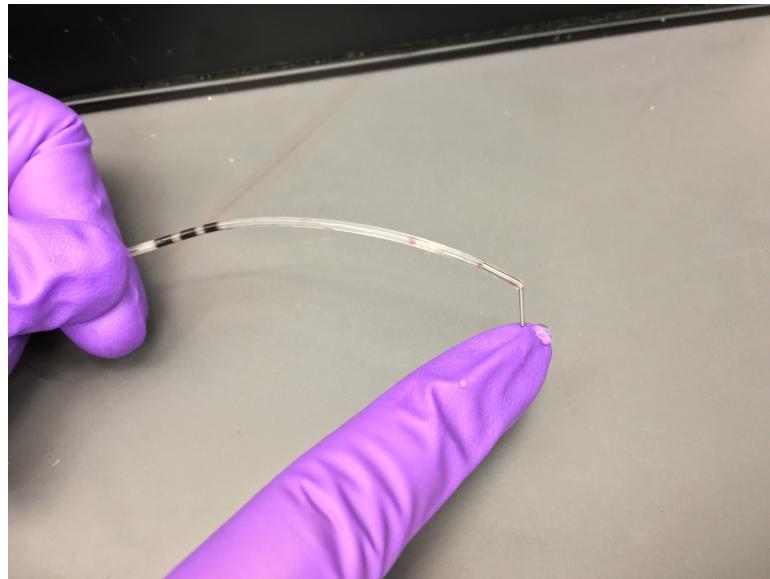
#### Part 2: Priming Control Lines

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!