Step-by-Step Operation Guide

Device Operation Guide: Microfluidic Experiment Operation Manual

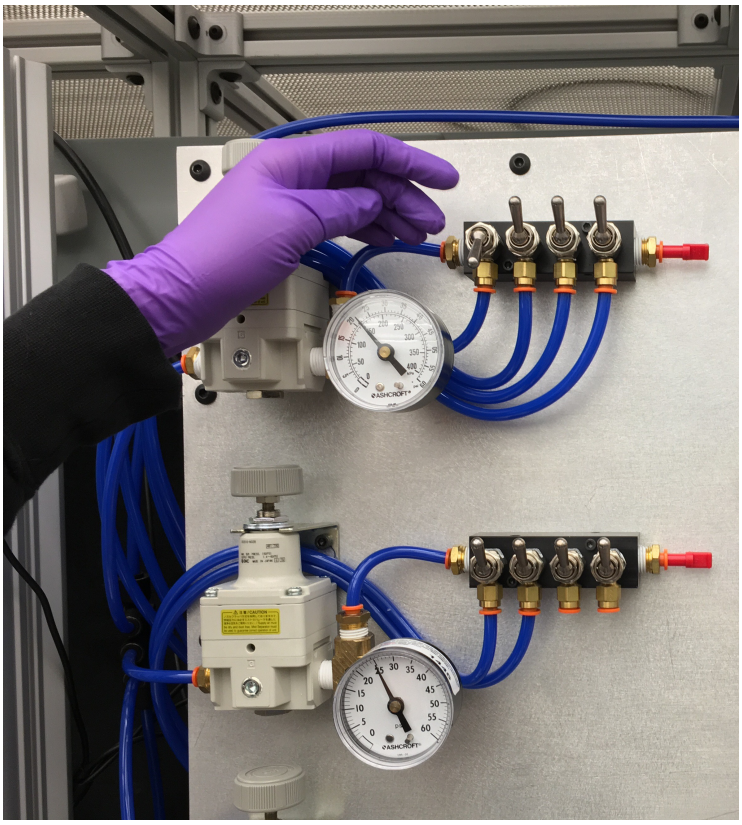
Note: In this guide, we run through our MITOMI experiment described in the Main Text as an example device run.

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.

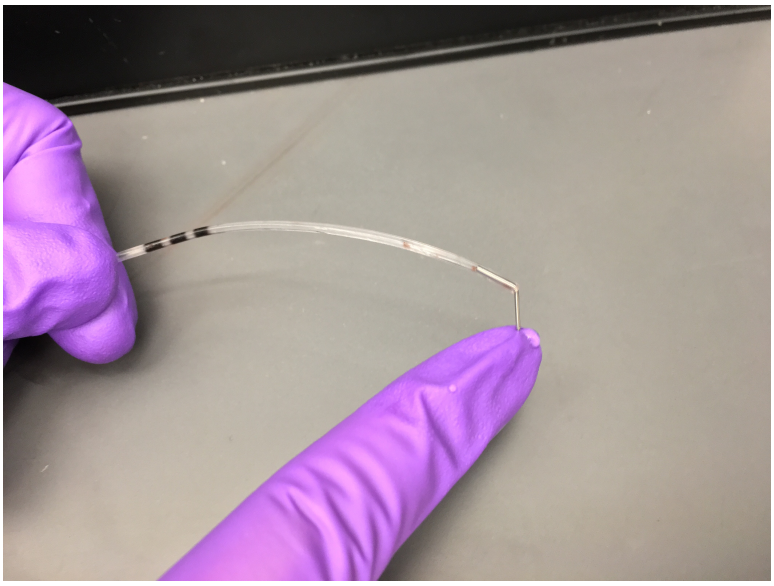
2. Send pressure to the desired **Control Valve Manifolds** by toggling their corresponding switches at the **Control Switchbox** on the **Base Board** of **Module 1**. Set the regulator pressure to the desired valve closure pressure.

   For our MITOMI experiment, we use 15 psi.



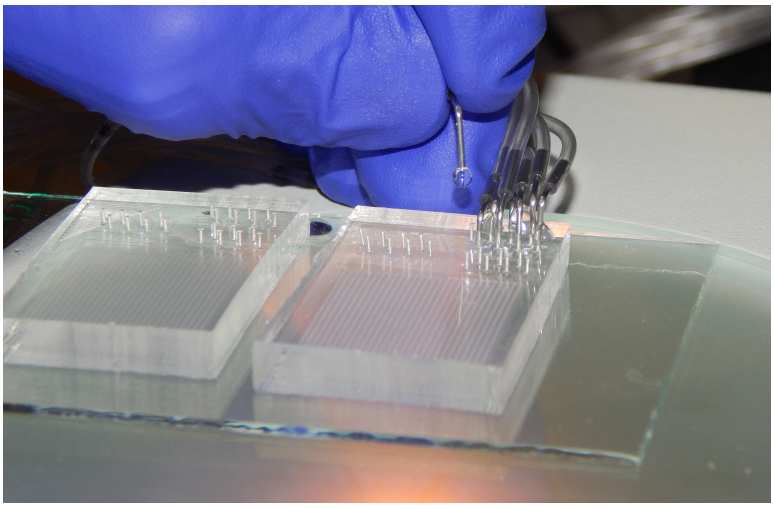
(!) Tip: Make sure valves are OFF (open state) on your Geppetto interface before beginning any run. Water should not be spraying from the control lines. If you find water is spraying when the manifold is pressurized, close the switch to off and change your valve state. You may need to refill the Water 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

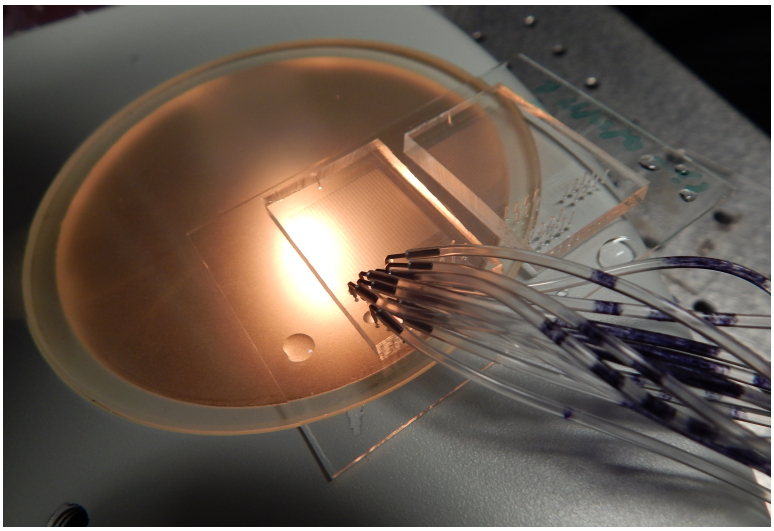
4. Insert pins from the Control Lines according to valve number into the corresponding Control Line Inputs on the device. Again, check that a small bead of water forms on each line before placing the line into the device.



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.

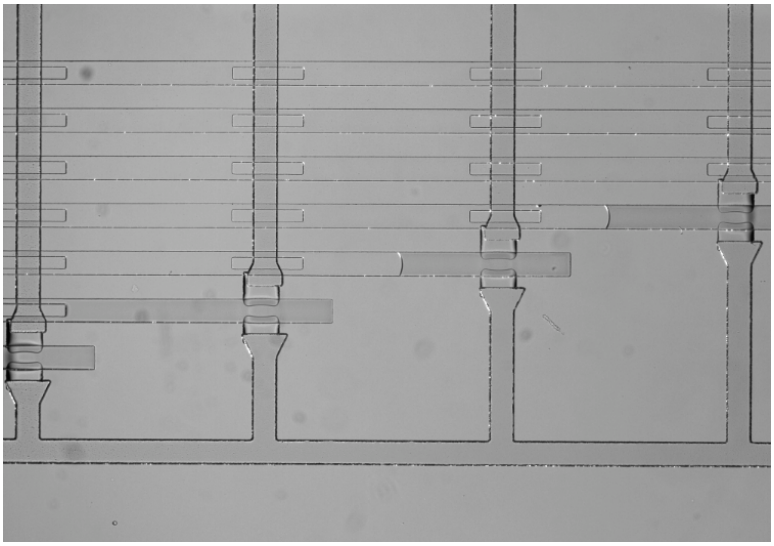
5. When finished, the device with control lines attached should appear as follows:



(!) Tip: While these conditions are typical for most device runs, if long-term cell culture is being or similar applications necessitating sterility are being performed in the microfluidic device, you may want to consider taking additional measures to ensure diffusion from the control channel into the flow layer does not contaminate your experiments. We recommend irradiating or autoclaving control line water or otherwise sterilizing tubes, reservoirs and other pneumatic components. Additionally, to minimize flow layer diffusion, control lines can be loaded with Krytox oil instead of water, which has been successful for our purposes in the past.

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.

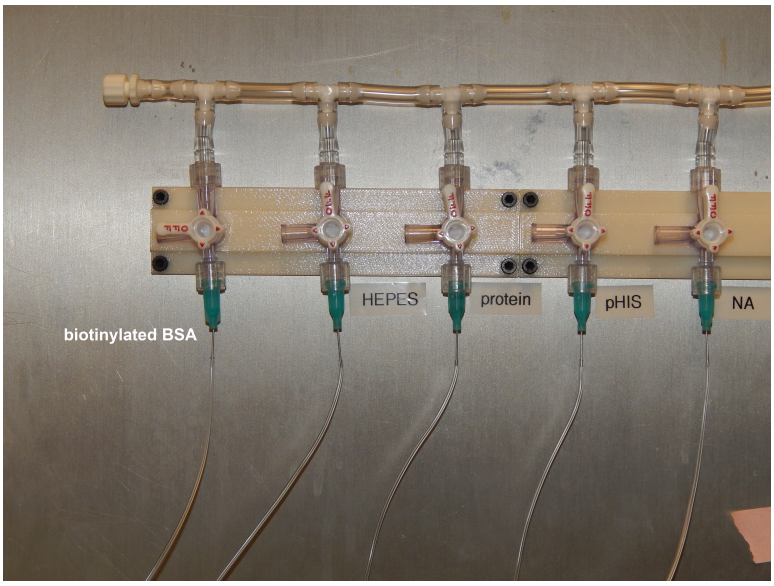
7. Now you are ready to insert your flow lines to load samples of interest into the device. To do so, load Tygon lines (**Tubing T5** with a pin **P1** at the end) of the appropriate length to reach the device with reagents of interest. Attach these lines on the **Flow Manifold** of interest and connect 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.

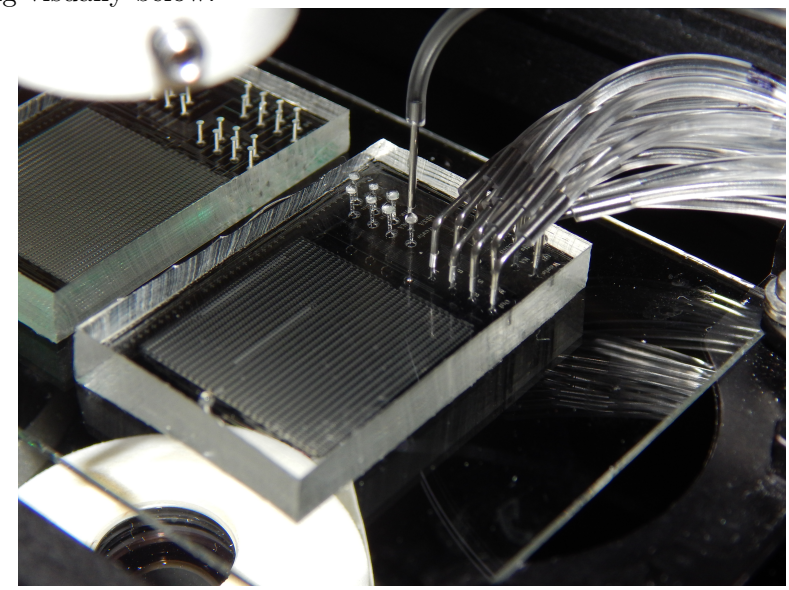
   In our MITOMI example run, we have connected a flow line containing 100uL of 2mg/mL biotinylated-BSA in water to our device for the beginning script. We then opened the biotinylated-BSA line on the manifold.



(!) 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.

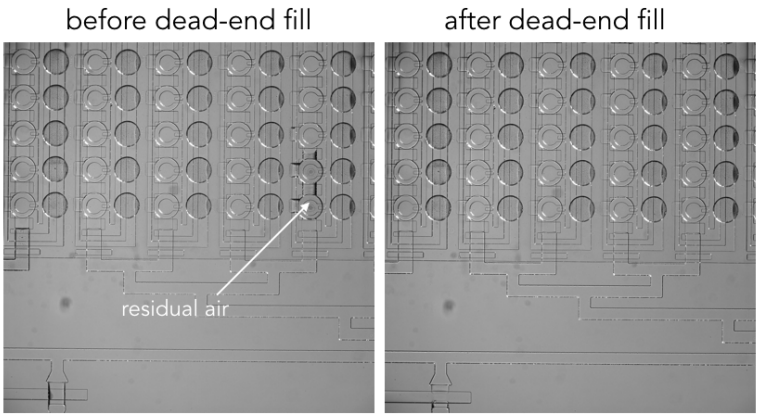
9. Using Geppetto, flow in the reagent used for de-bubbling by releasing any valves blocking flow.

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



(!) Tip: If you are operating your device in different temperature conditions (***e.g.*** in an incubator), be aware water evaporates more quickly. We recommend refilling the control lines often or operating the device under high humidity (~90%) in these settings.

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).

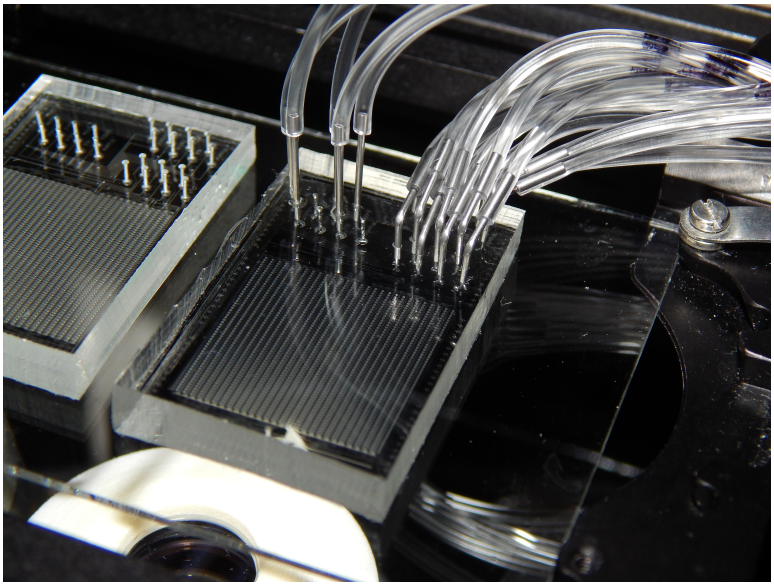
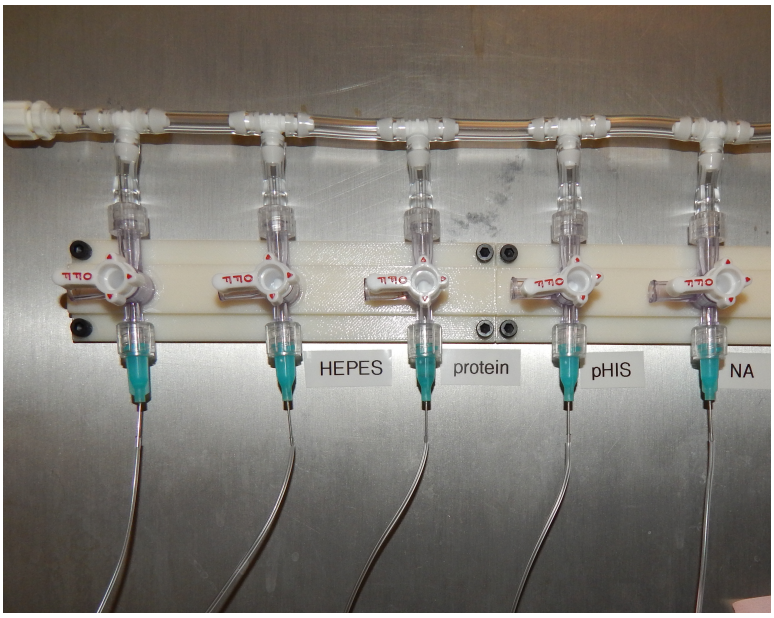


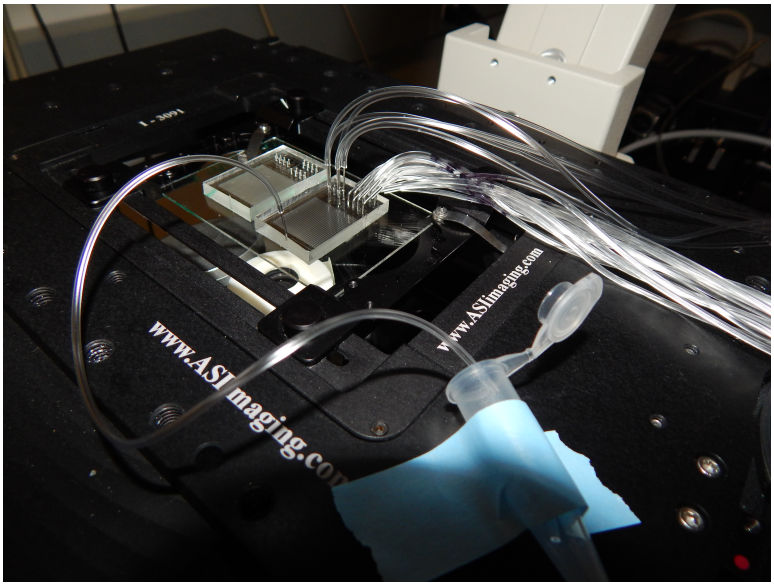
Table 1. Surface chemistry components necessary for surface immobilization of a GPF-tagged protein.

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!