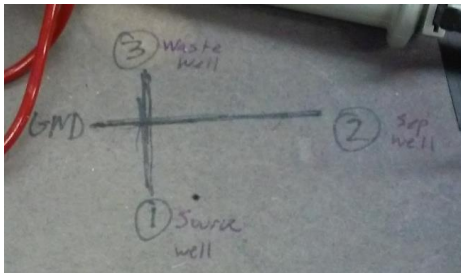
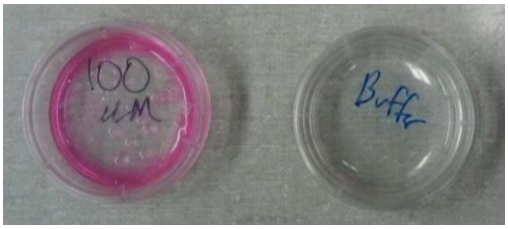
Running the system

1. Locate a microfluidic device.



2. Verify that the microfluidic device (MFD) is functional
    a. Use the microscope to make sure that the MFD channels are clear
    b. Put de-ionized (DI) water into the source (or insertion) well.
    c. Watch the channels under the microscope to ensure osmotic flow in all channels
    d. If liquid is not flowing down one or more channels, use the vacuum to assist the flow
    e. Recheck the MFD under the microscope. Make sure there are no bubbles or leaks in the channels. If there are bubbles, use the vacuum to suck them out.
    f. Once you are confident the MFD is functioning correctly, use the vacuum to suck out all of the DI water. Verify with the microscope

3. Insert buffer solution into the MFD
    a. Start with the source well
    b. Use the microscope to verify proper flow.
    c. If needed, use the vacuum to assist flow or remove bubbles
    d. Once the channels are all full of buffer solution, insert buffer solution into the other wells
    e. Use the microscope to make sure you don't have any bubbles



4. Insert fluorophore into the insertion well
    a. First, remove most but not all of the buffer solution from the insertion well. By leaving some of the buffer solution in the well, you reduce the chances of introducing bubbles.
    b. Then put the fluorophore solution into the insertion well
    c. Use the pipet to mix the buffer and fluorophore
    d. Use the microscope to make sure you don't have any bubbles

5. Secure MFD on optical bench
    a. Place MFD on sample holder
    b. Use tape to make sure the MFD won't slide around

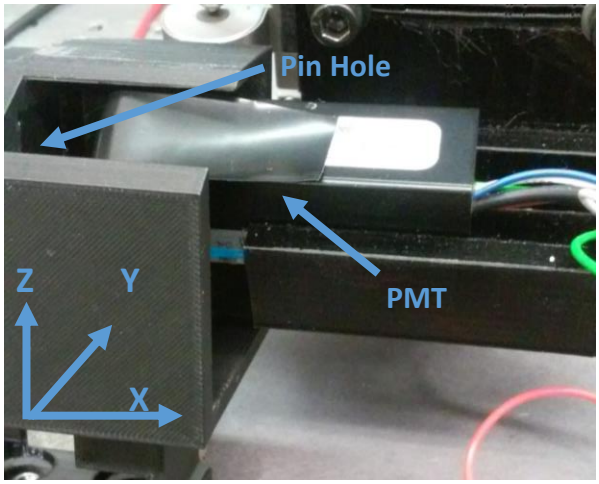
6. Turn on optical bench systems
    a. Turn on power to XYZ stage
    b. Start XYZ control python script.

    c. Turn on laser
        i. The laser should cast a scatter pattern onto the white sheet of paper that is taped to the top of the XYZ stage

    d. Turn on PMT (BUT DO NOT UNCOVER UNLESS THE LIGHTS ARE OFF)

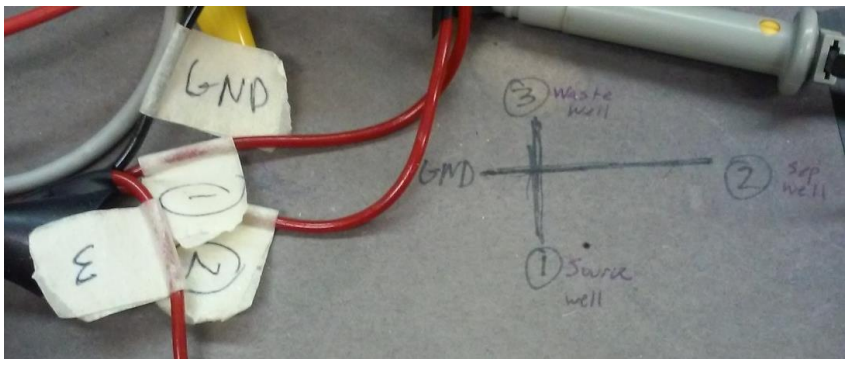
    e. Make sure the oscilloscope is on and that at least one of the cables is attached to the PMT output. The PMT output should never be allowed to exceed 4 V

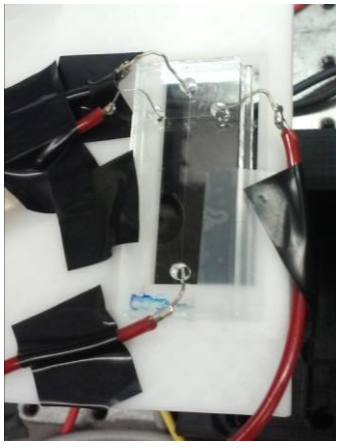
7. Turn off room lights
    a. Monitor lights are okay, as are phone screens and other small light sources as long as they aren't directed at the PMT
    b. Uncover the PMT
    c. Monitor PMT output on the O-scope to make sure it never goes above 4 V.
8. Position the laser so it goes through the source well
9. Align the PMT – this is iterative
    a. Use the X positioning screws on the PMT stage to pull it away from the pin hole.
    b. The O-scope should pick up a significant signal level
    c. Adjust the Z and Y position to maximize the PMT output signal level
    d. Move the X position closer to the pin hole
    e. Repeat steps c and d until moving the PMT closer to the pin hole doesn't increase signal level. At this point you should have found the focal point



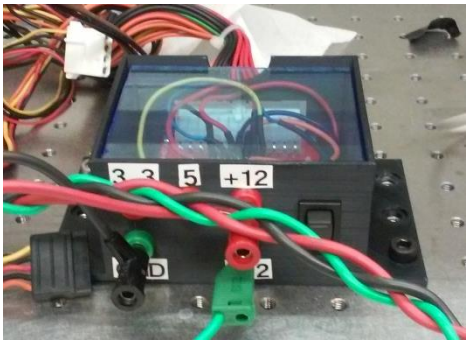
10. Move laser to source channel just left of where the channels cross

11. Put high voltage leads into the wells.
    a. The cables are labeled GND and numbers 1 through 3. Put the labeled leads into the corresponding wells

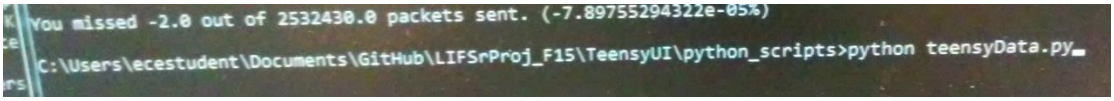




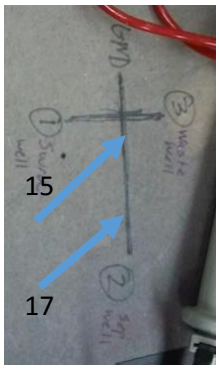
12. Turn on high voltage system



13. Turn on data collection program



    a. Click start to begin data collection
    b. Click HV on
    c. Click Calibrate
14. Run Insertion
    a. Click the insertion button on the data collection program. This will automatically set the high voltage system to generate flow from the source well to the waste well.
    b. You should see a rise in the o-scope signal level as the fluorescence flows into the channel
15. Realign laser to the separation channel just below the crosshair



16. Run separation
    a. Click the separation button on the data collection program
    b. You should see a brief spike of signal as the fluorescence in the crosshair passes down the channel. This close to the crosshair it shouldn't take more than a few seconds to see the spike.
17. If you are able to see the spike close to the crosshair, repeat steps 14 and 15 but align the laser lower on the separation channel
    a. If you can see a spike lower on the channel then the experiment worked as intended.
18. Once you have finished your test, whether successful or unsuccessful, shut down the system.
    a. Properly close the data collection program as detailed elsewhere
    b. Close the XYZ program
    c. Turn off the high voltage, laser, XYZ power, and PMT
    d. Cover the PMT (at this stage you may turn the lights on)
    e. Remove the HV leads from the MFD

19. Remove the MFD from the system and clean it out
    a. Use the vacuum to remove all of the buffer and fluorophore from the channels
    b. Fill the channels with DI water to clean out any residue left by the buffer and fluorophore
    c. Use the vacuum to remove ALL of the DI water. Use the microscope to make sure
20. Put everything away
    a. Put the buffer and fluorophore back in the refrigerator
    b. Put working MFD's back in the case and the non-working ones into their container
    c. Throw away the used tips from the mechanical pipettes
    d. Tidy up the optical bench and surrounding area. Shorts can damage the system.

COMMON ROADBLOCKS:

If you don't see a rise in signal level during step 14, you may not be getting flow. This can be caused by bubbles in the channel. This holds true for steps 16 and 17. Bubbles are your worst enemy.

Lack of flow can also happen if the high voltage leads are placed incorrectly or incompletely. Make sure all leads are in the correct well and are all the way in the well.

It is unlikely that you will see the data spike in the data collection program even if you see it on the O-scope. Post-processing will show you whether or not you got good data.

The data collection program can run very slowly and create prohibitively large files if left to run for long periods of time. Only turn on the data collection program right before you plan to run Insertion or Separation and make sure you turn it off as soon as you have finished with it. Even if you plan on running Insertion and Separation more than once, it can be advantageous to reset the data collection between runs.