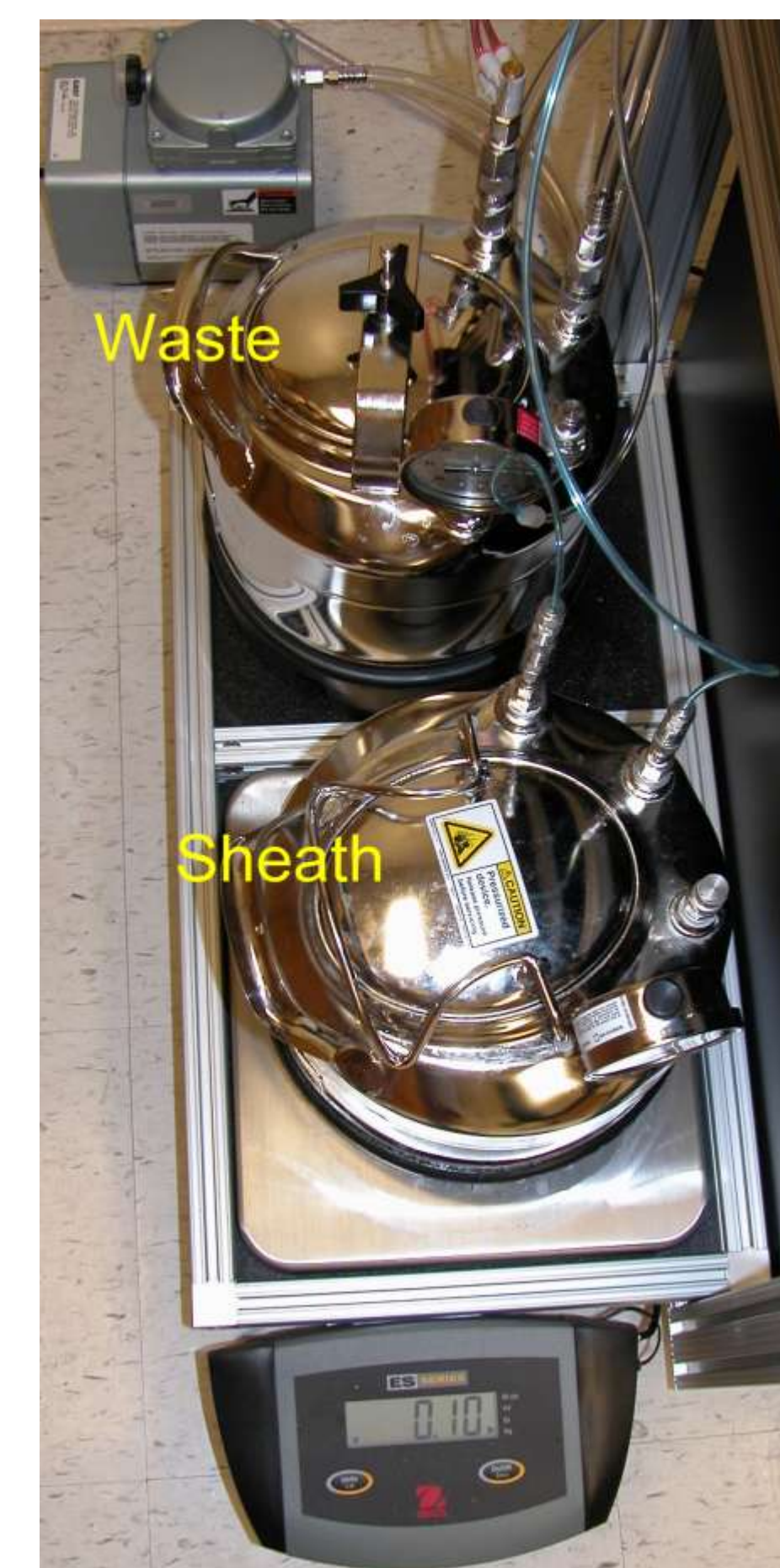


Start up

1. Add up to 6L of 0.2 μm filtered sheath fluid into sheath fluid reservoir
 - Attach both the waste and sheath fluid reservoirs to cytometer
2. Sonicate nozzle for 5 minutes in ddH₂O (as necessary)
3. Apply vacuum and air supply
 - Sheath pressure will reach its max at $\sim 18.5''$ Hg
4. Start Lasers (requires a 15-30 minute warm up)
 - Turn on main switch and wait 15 seconds
 - Activate laser using key switch, note power level on blue laser should be approximately 100%



5. Power up Electronics console (Take opportunity to press ILLUM)
6. Start computer (fresh boot daily) and initiate Spigot software package



Flush Plumbing

1. Place the Flush bucket under the nozzle apparatus and press RINSE and allow to run for about 20 sec; Press RINSE to stop.

2. Clean Nozzle

- Flush both sides of nozzle with 0.2 μm filtered ddH₂O
- Install nozzle (Remove the Flush Bucket first)

3. Press RUN to restart stream

4. Press BACK FLUSH, let run for 30 seconds then press RUN

5. Place the Flush bucket under the nozzle and place nozzle purge reservoir under the nozzle submerging the nozzle in 0.2 μm filtered sheath fluid

6. Press PURGE to remove bubbles from the line, allow fluid to reach Y.

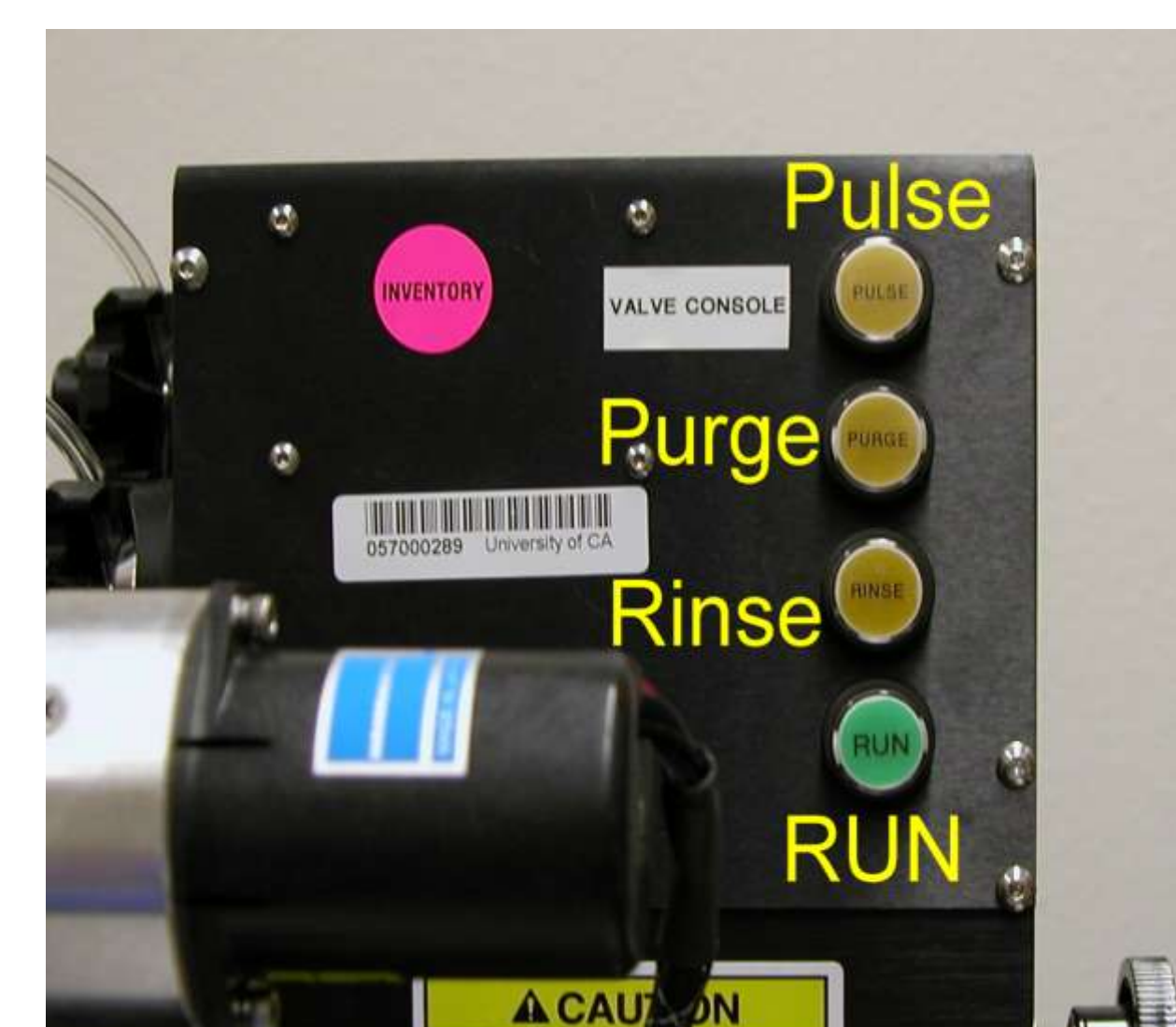
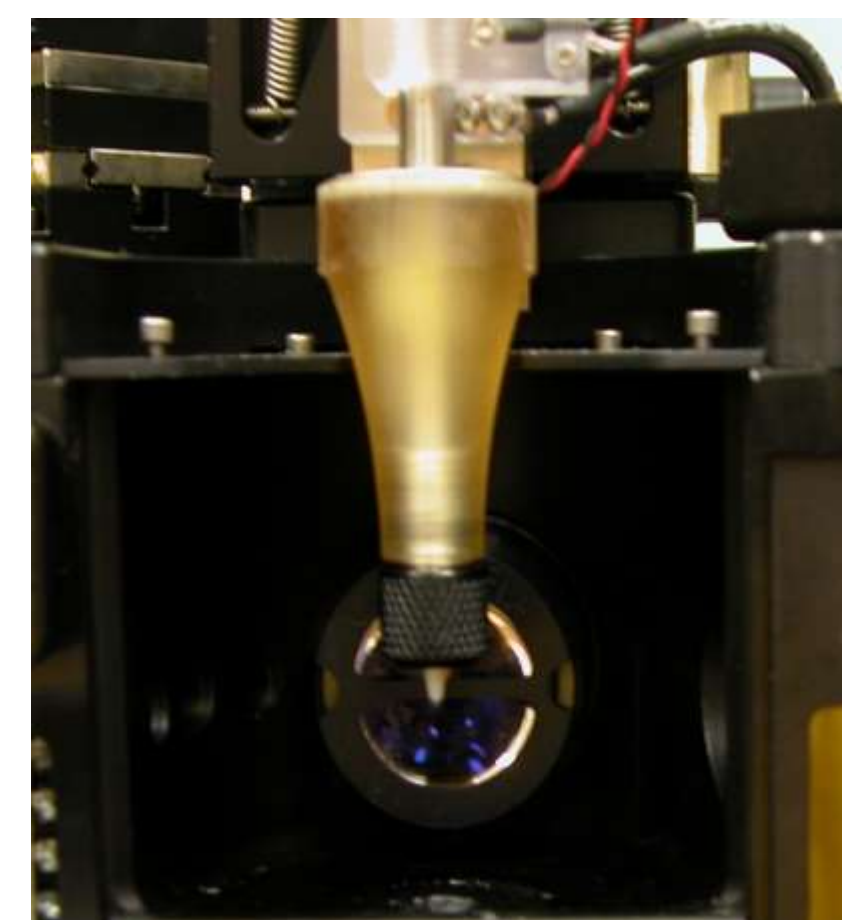
7. Press PULSE several times to coax remaining bubble from plumbing

8. Press RUN to restart fluids stream

9. Remove nozzle purge reservoir.

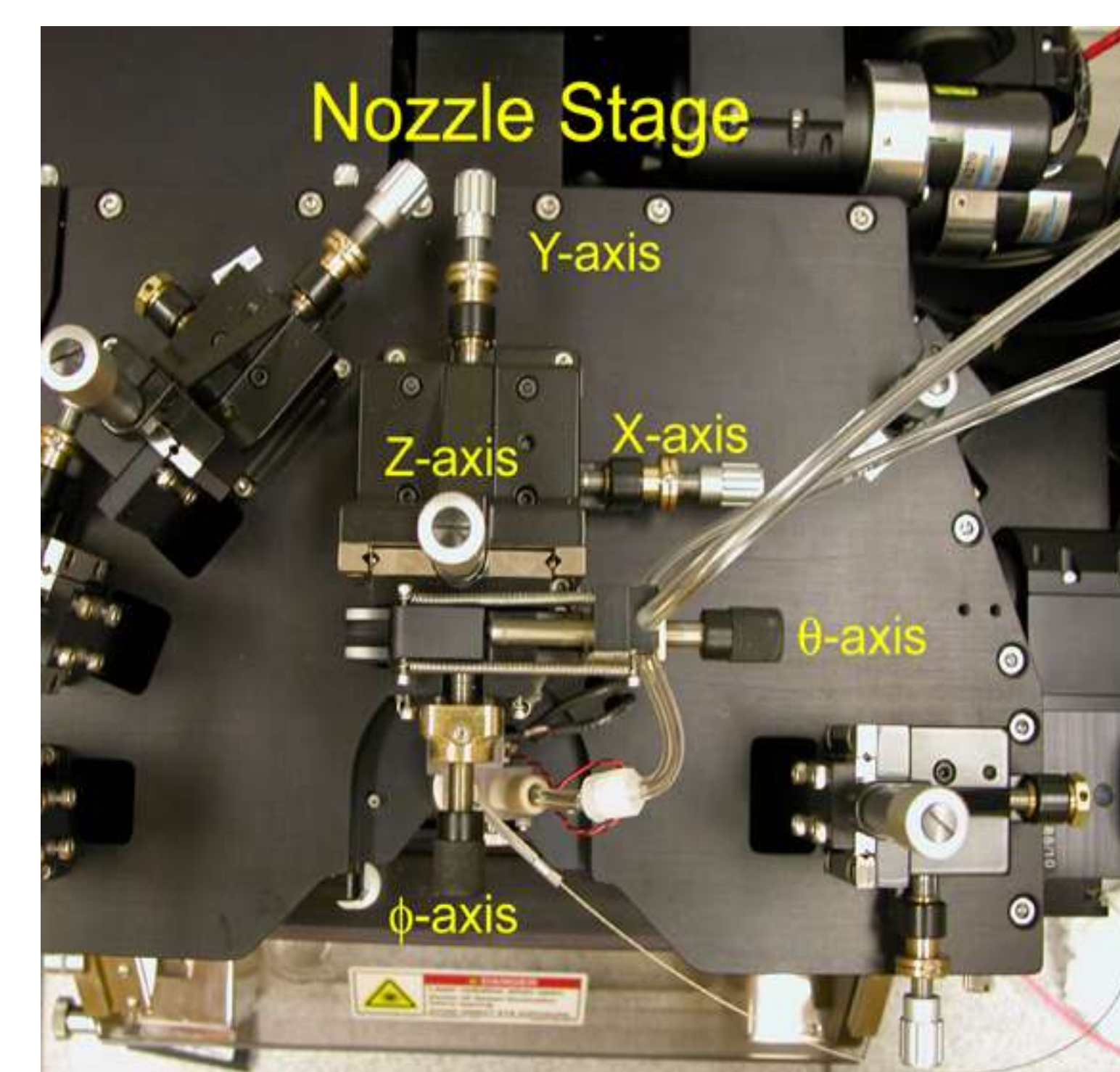
- Dab away any excess fluids from nozzle and the chamber

10. Return the fluids bucket to its home and check for a straight nozzle stream



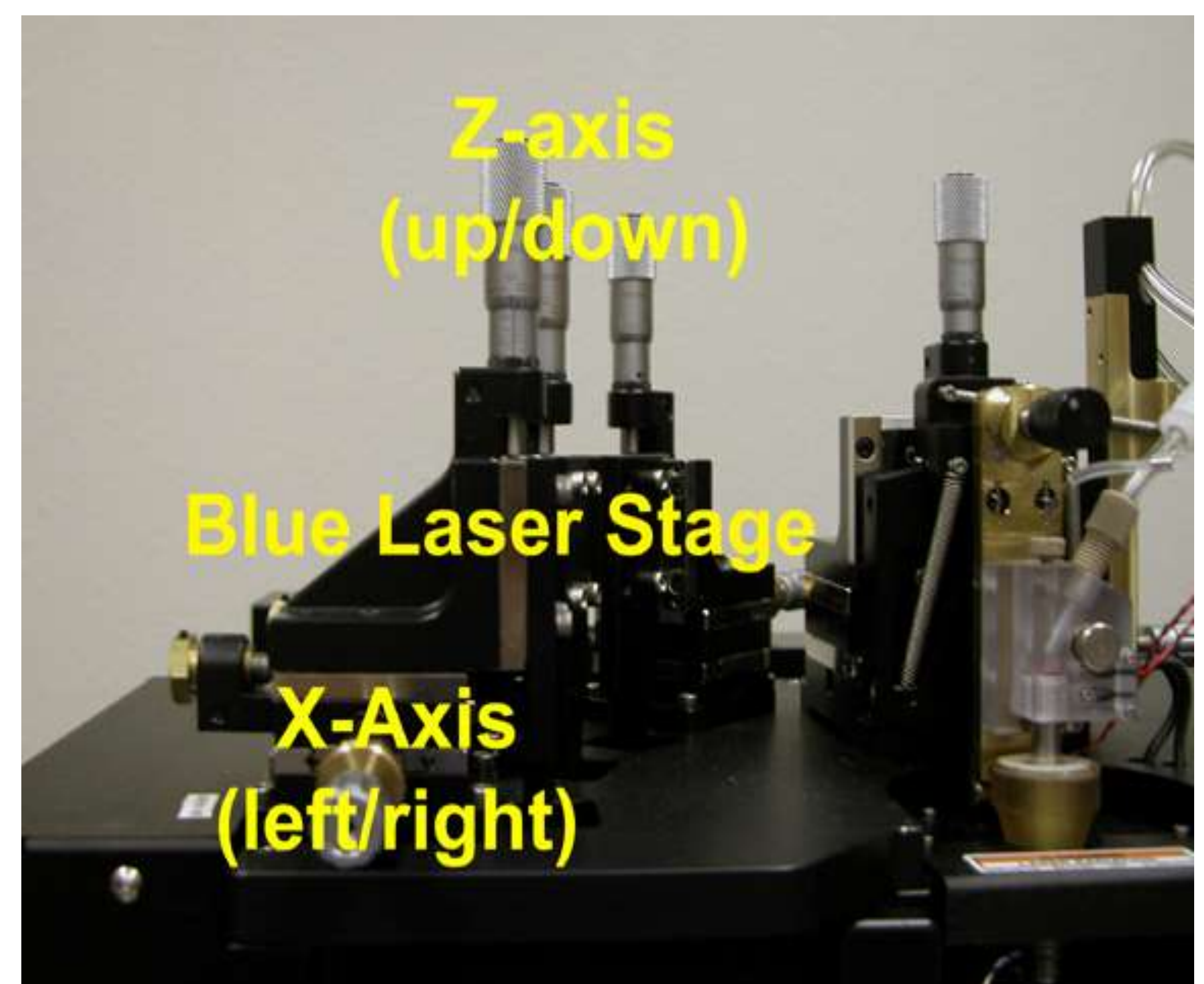
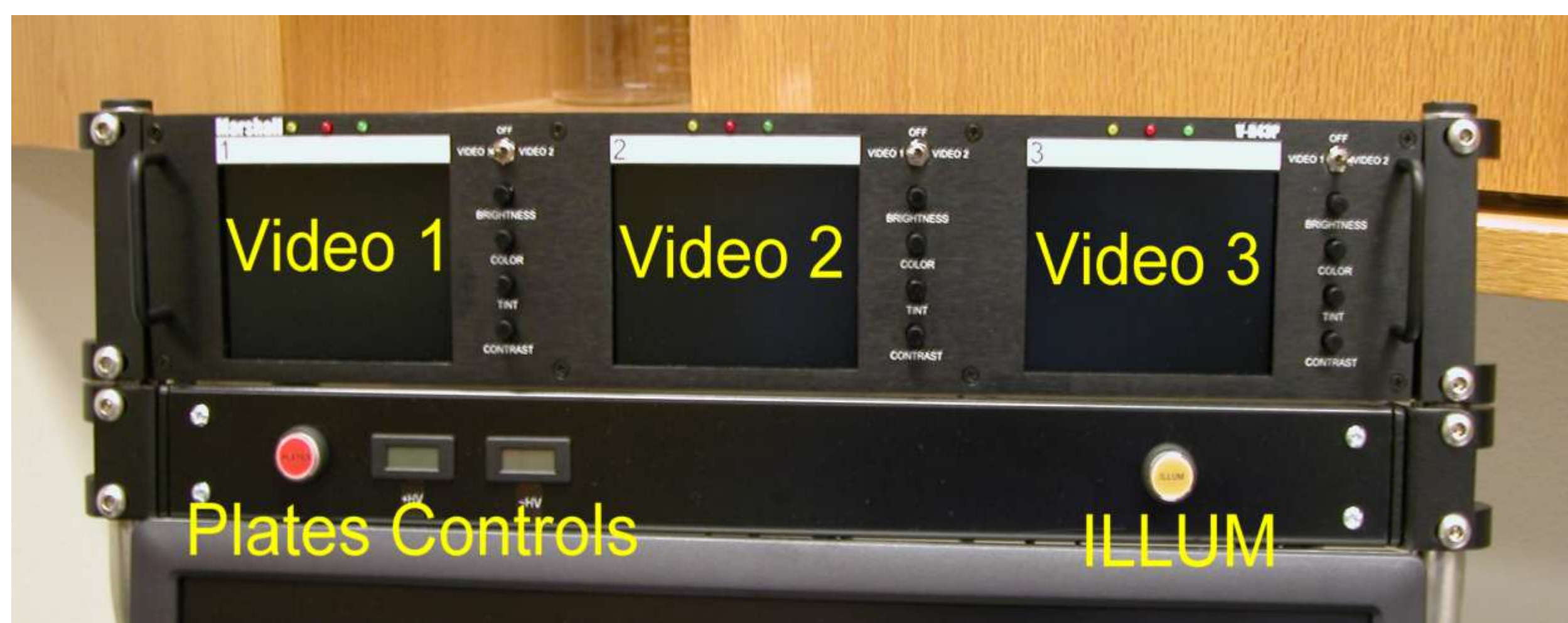
Align Stream

1. Place spill flask in the slide/two tube sort tray adapter
2. Focus stream on the waste drain using course axis controls on the Nozzle Stage (Video 3)- looking for centered position over drain
3. Focus stream over the pin holes using the fine axis controls on the Nozzle Stage (Video1)- looking for crisp sides of stream
4. Very often this will place the stream in Video 2 directly adjacent to the arrow etched into the Video 2 screen



Illuminate

1. Open a spigot configuration file (these are often named with the date last used). Highlight the FSC1 vs 692 cytogram plot on the main (left) screen.
2. Close Chamber sliding door, run finger over laser light trigger window.
3. Open BLUE laser SHUTTER; note that this cytometer triggers off the BLUE laser.
4. Steer laser to a position between the 1st and 2nd pinhole on video screen 1
5. Fill sample tube (BD polypropylene falcon tube- Cat #35-2063) with 1 mL of sheath fluid or nanopure water and 1-2 drops of alignment beads
6. Load tube on the sampler and press SAMPLE- may take 30-40 sec for sample to appear on screen (you will see beads flowing in Video Window 1)
7. Steer laser into 1st pinhole using the Z-axis micrometer. Use the FSC and the blue laser axis micrometers to optimize the bead alignment using the cytogram as a reference. Aim for FSC and 692 histogram bead intensity peak CVs of less than 0.010- optimal CV is normally around 0.006.
8. Repeat process for UV and Red laser. Note that Histogram peak CVS off of these lasers are not as tight.



Sample Sort

1. In the Sort Menu chose the Pulse Width vs FSC1 graphical representation of the sample. In middle upper left of the screen, chose to gate the left window and select DRAW WINDOW.
2. Using the right button on your mouse generate a polygonal figure around the bead signal on this cytogram. The bead data points will highlight in GREEN. You will need this later.
3. Repeat this "gating" of beads in cytograms for FSC1 vs SSC and FSC1 vs 692.
4. Separate the uncharged sort plates. Using a clean chem-wipe wash off the interior portion of the deflection plates. Reset the plates and press PLATES button.

Droplet Formation

1. In the SORT CONFIG menu turn on Piezo Drive. Set the drop frequency to 49.1 kHz. Adjust the voltage applied to the Piezo drive until the breakoff point corresponds to the arrow etched on to video screen 2. This break off point should correspond to ~213 on the drop position meter.
2. Set Deflection Charge Amplitude to ~28 V and a stream focus of ~12-16%. Press LEFT and RIGHT TEST DEFLECT. Adjust the deflection angle such that the highlighted sort stream nicks the black ink marks etched into video screen 3 to the right and the left.
3. Set values into Delay Calculator values starting with break off point, up to the nozzle and finishing with the 10 drop count. Press CALCULATE. A break-off point at drop position of ~213 should correspond to a delay of ~29.5.
4. In the SORT TRAY Menu, Press PRESENT TRAY. Place a clean microscope slide on the center position of the slide/two tube adapter tray. Press SORT READY.
5. In the Sorted Sample Container menu chose CALIBRATION SLIDE.
6. From the SORT menu chose the LEFT sort window and press SORT. A slide will be generated with a matrix of 25 drops. Under fluorescence microscopy find the drop containing 20 beads (take 1 or 2).
7. Set the drop delay setting to reflect this drop position (Likely very near 29.5- if not see me!).

Shut Down

1. Run 10% Bleach through sample line for 5 minutes (Critical after you've run live culture or when working with an especially concentrated sample); run nanopure ddH₂O for 5 minutes.
2. Press BACKFLUSH; allow to run for 1 min.
3. Press RUN to turn off the fluidics.
4. Turn AIR control to OFF.
5. Empty Sheath Fluids Reservoir and rinse the interior of the reservoir with Nanopure de-ionized water and dispose of this volume.
6. Add ~1L of Nanopure fresh de-ionized water to the reservoir and reattach; Restart AIR
7. Press RINSE and BACKFLUSH to start the fluidics and let the clean water flow through the system until both the sheath line and the sample line run dry.
8. Press RINSE to turn off the fluidics and remove the nozzle tip; place nozzle assembly in ddH₂O for overnight storage. Detach the sheath fluid plumbing from the Sheath reservoir and attach the two tube fixtures together.
9. Press RINSE and BACKFLUSH allow the system to blow air for 10-15 minutes; Turn AIR off.
10. Remove Sheath and Waste reservoirs emptying out any remaining fluids; air dry the reservoirs on the back bookshelf nearest the HOOD.
11. Shut down the remaining controls: Lasers, Electronics Panel, Computer.
12. Clean off the Sort Chamber Area.