Start up

1. Add up to 7L 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 ~18.5” Hg

4. Start Lasers (requires ~15 minute warm up)

 -Turn on main switch and wait 15 seconds

 -Activate laser using key switch, note power level on blue laser should approximately 100%

5. Power up Electronics console (Take opportunity to press ILLUM)

6. Start computers (fresh boot daily) and initiate Sortware software package

Flush Plumbing

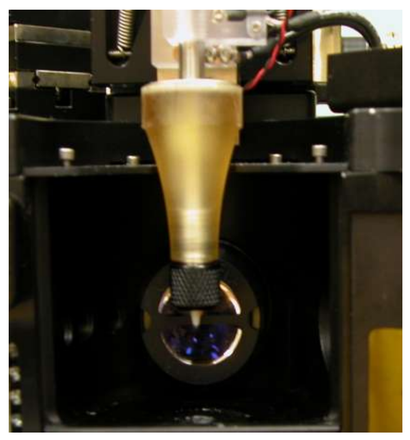
1. Place the Flush bucket under the nozzle apparatus and press RINSE and allow to run for about 10 sec; Press RINSE to stop. (Fills plumbing

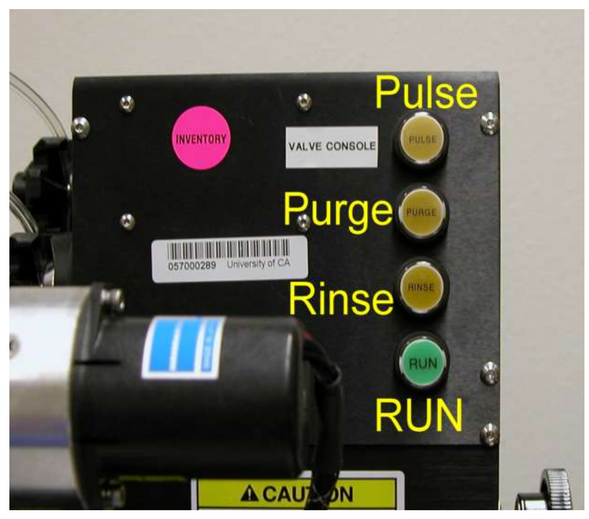
2. Clean Nozzle

 -Flush both sides of nozzle with 0.2 μm filtered ddH_2O

 -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

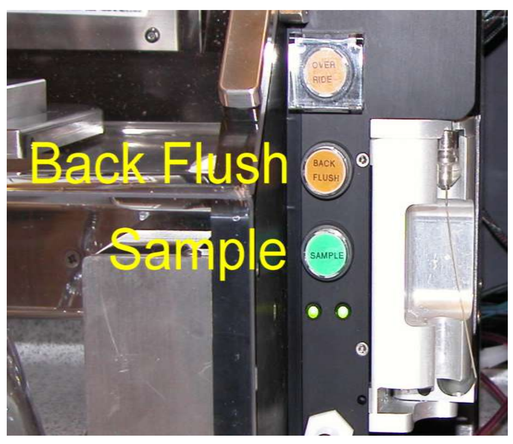
6. Press PURGE to remove bubbles from the line, allow fluid to reach Y.

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

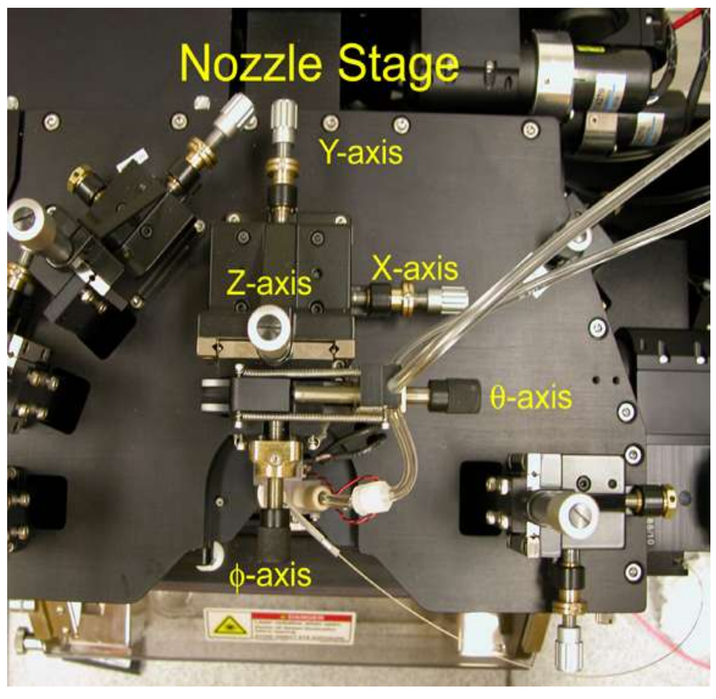
Align Stream

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 sortware workspace file (Lookup under Training Folder; Startup17Oct12).

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.

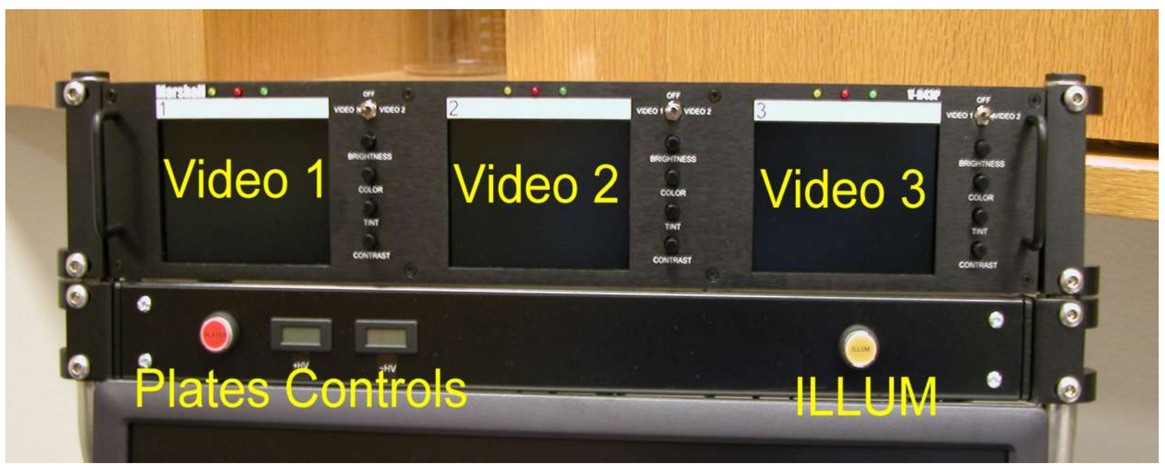
4. Steer laser to a position between the 1st and 2nd pinhole on video screen 1

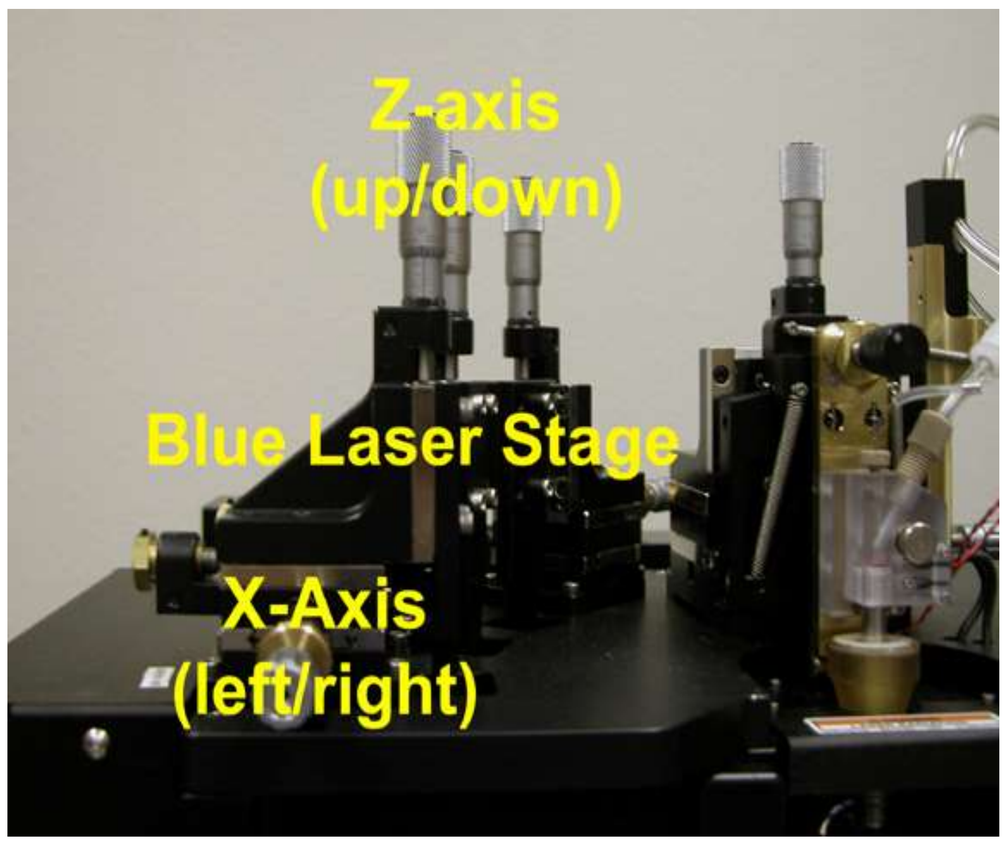
5. Fill sample tube (BD polypropylene falcon tube- Cat #35-2063) with 1 mL of sheath fluid or nanopure water and 1-2 drops of alignment beads (UR 3μm beads)

6. Load tube on to the sample line 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, SSC and the blue laser detector 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 from these lasers are not as tight.





Sample Sort

1.On a new work “page” generate a dot plot with the axis of Pulse Width vs FSC1.

2.Select an appropriate “gate” shape icon to isolate an “alignment bead” profile. Generate a gate by holding down a mouse click and surrounding the bead profile. The bead data points will highlighted by a gate selective color scheme. This gate will be referred to as a “mother” gate.

3.Create “daughter” bead gates in dot plots for FSC1 vs SSC and FSC1 vs 692.

4. Separate 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. Highlight the Sort Settings Window pane. Set the drop frequency to 62 kHz corresponding to a sheath pressure of 27.5 psi and a sample pressure of 28.5 psi. 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 ~221 on the drop position meter.

2. With the TEST STREAM function activated, set Deflection Charge to a value that generates a 30% angle away from center and a stream focus that reduced fanning to a minimum from the center stream. 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 Drop Delay to 33.5. A break-off point at drop position of ~221 should correspond to a delay of ~33.5. Still will be corrected in the calibration

4. In the Sort Layout window pane, select a Calibration sort. Press Eject. Place a clean microscope slide on the front position of the slide/two tube adapter tray. Press SORT READY.

5. Press Start. A slide will be generated with a matrix of 25 drops. The center drop formation will correspond to a delay setting of 33.5. Droplets above this position and below this position will be offset by +/- 0.1 units added to each drop. Under fluorescence microscopy find the drop containing 20 beads.

7. Set the drop delay setting to reflect this drop position (Likely very near 33.5- if not see me!).

Shut Down

1. Run 5% Bleach solution through sample line for 5 minutes (Critical after you've run live culture or when working with an especially concentrated sample); run nanopure ddH_2O 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. Release the pressure via the pressure valve and empty Sheath Fluids Reservoir. Rinse the interior of the reservoir with Nanopure water and dispose of this volume.

6. Add ~1L of Nanopure fresh de-ionized water to the reservoir and reattach; Restart AIR to rebuild the pressure.

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 ddH_2O 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 5-10 minutes; Turn AIR off.

10. Remove Sheath and Waste reservoirs emptying out any remaining fluids; air dry the reservoirs upside down at their appropriate stations.

11. Shut down the remaining controls: Lasers, Electronics Panel, both Computers.

12. Clean off the Sort Chamber Area.