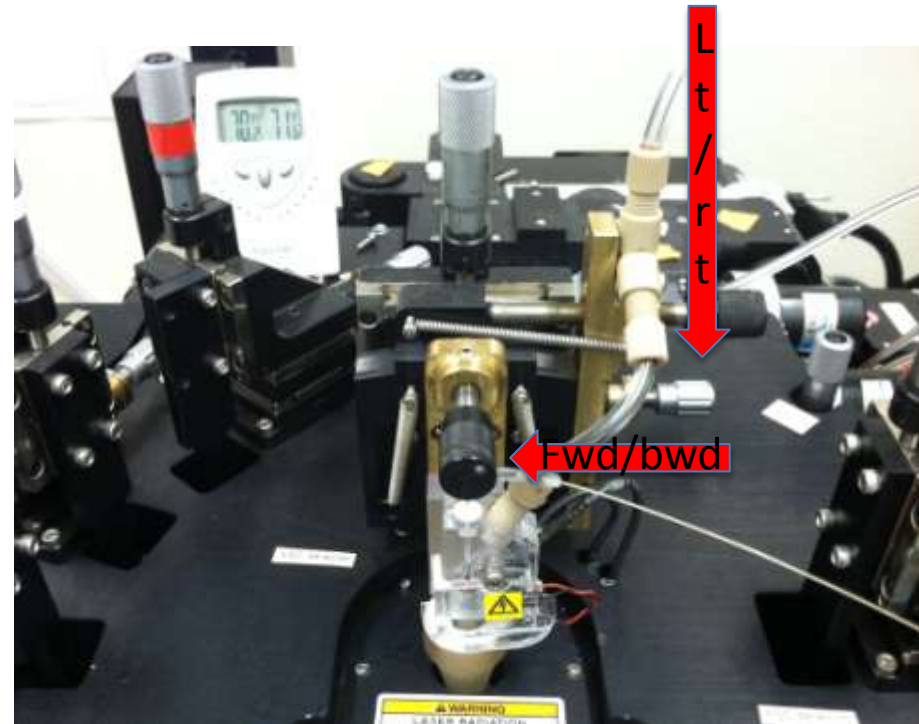


Visual representation of the streams: What to look for!

Video screen 3-1: Sort chamber waste sink



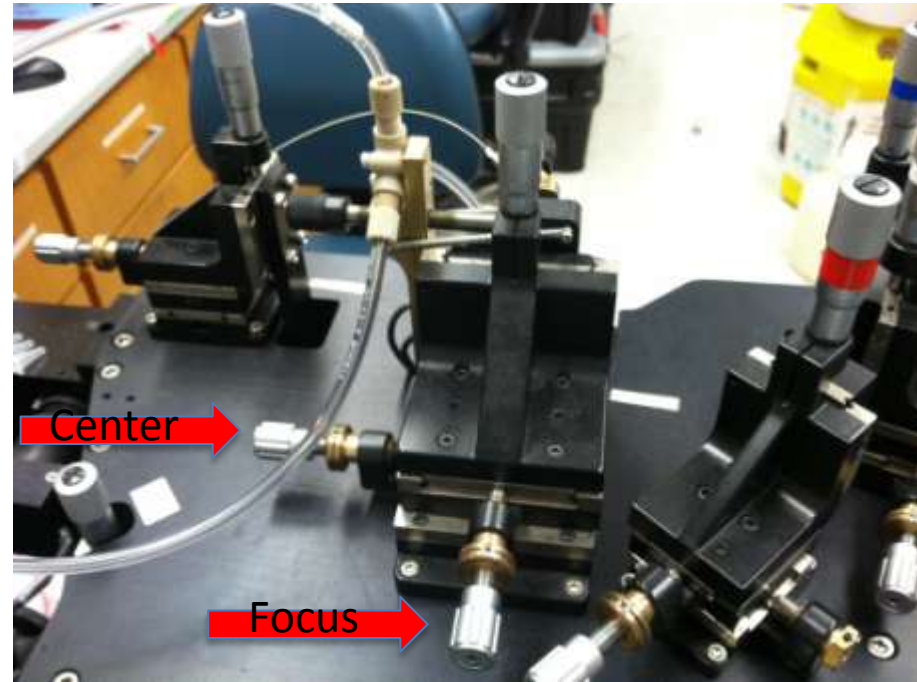
Step One: Want to center the stream directly over the waste sink using the course micrometers

Visual representation of the streams: What to look for!

Video screen 1-1: Laser pinholes



View from the back of Influx



Step Two: Want to center the stream directly over the pin holes and focus the stream to have sharp sides on either end.

Visual representation of the streams: What to look for!

Video screen 1-1: Laser pinholes

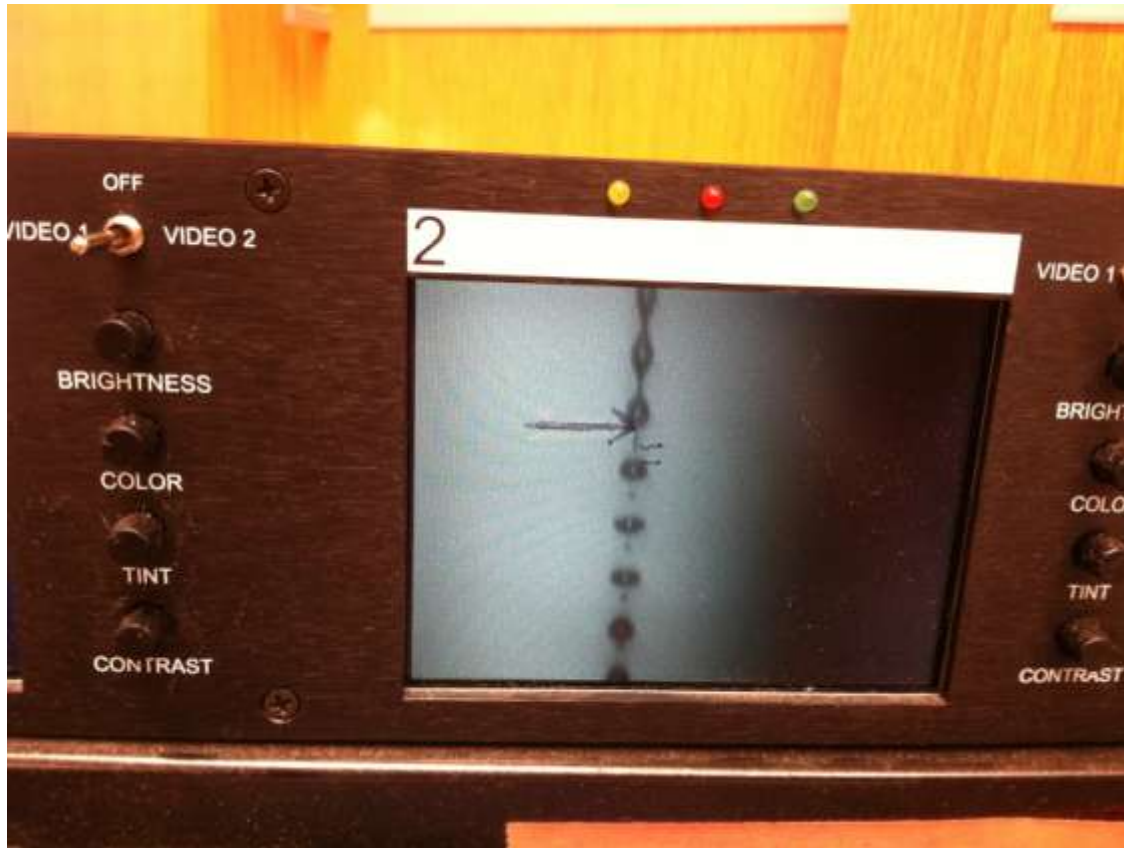


Step Three: Move nozzle tip to a position just above the view visible on video screen 1.



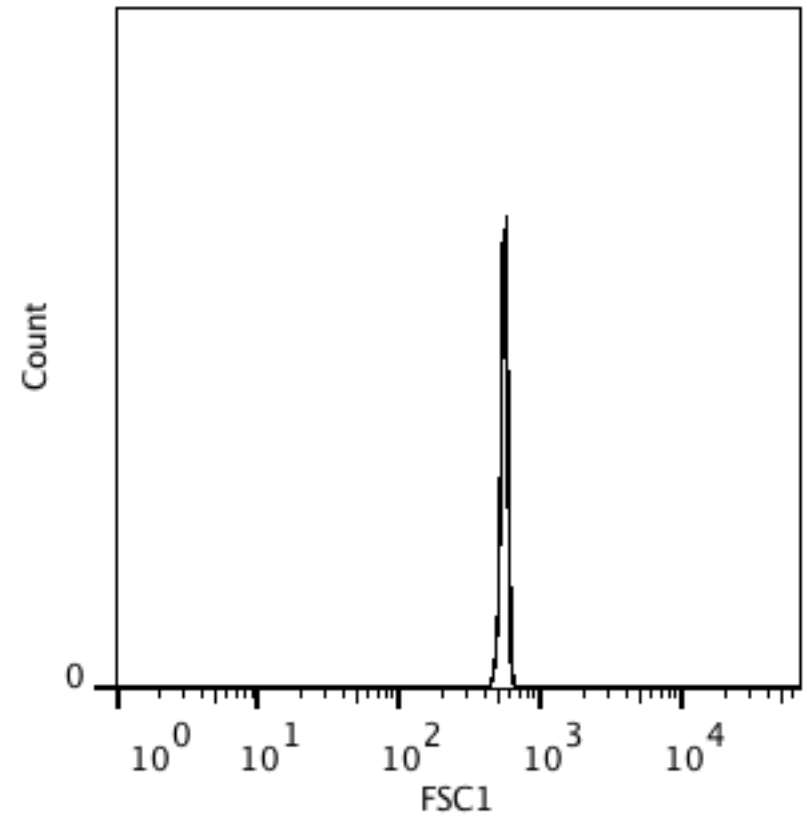
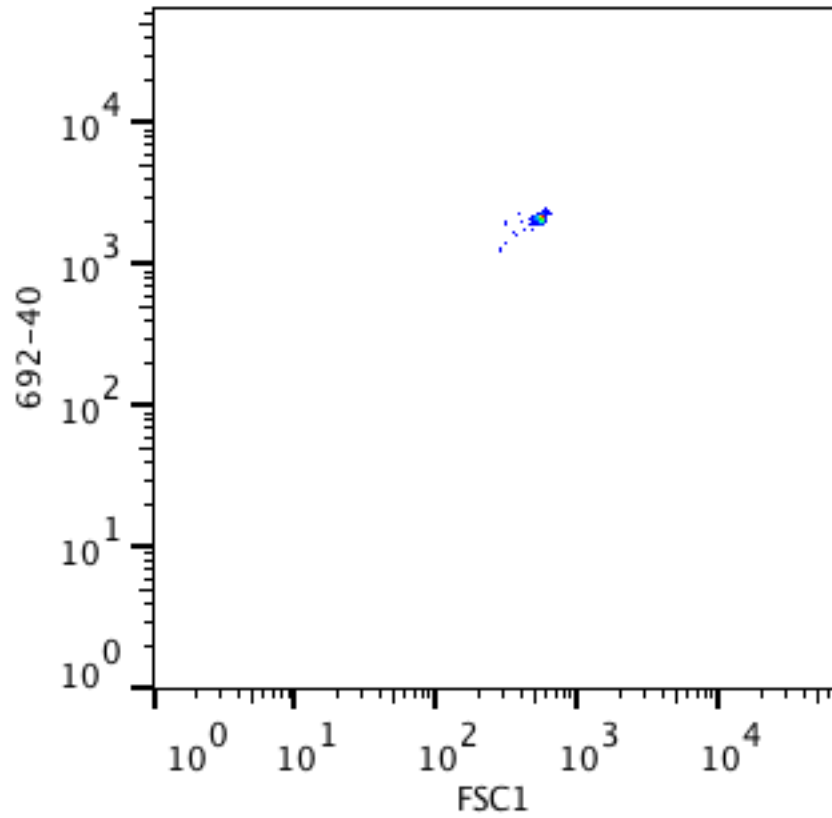
Visual representation of the streams: What to look for!

Video screen 2- Drop formations



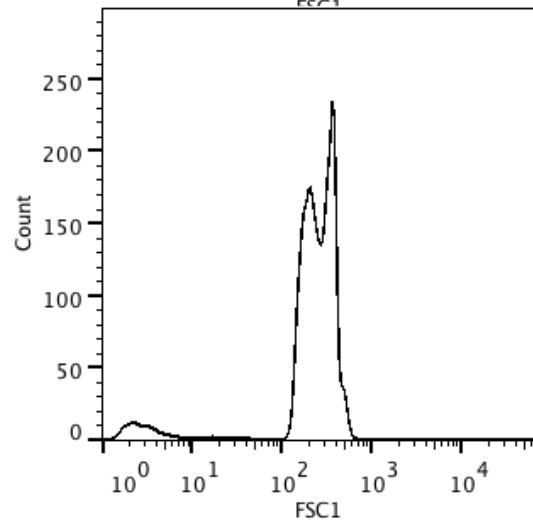
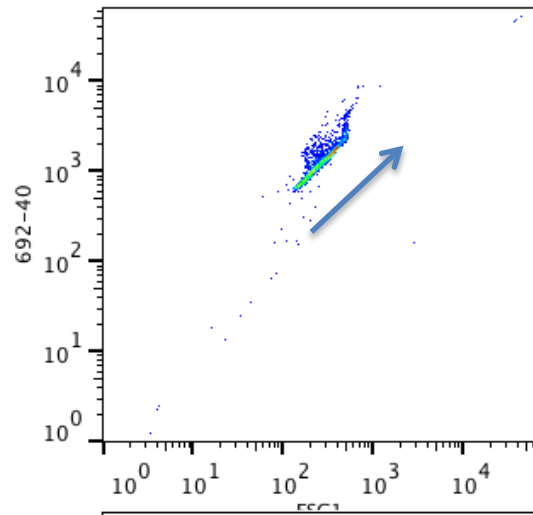
Step Four: If the previous three steps work well then the stream should situate itself to the right of the hand written arrow.

Alignment Trouble shooting: Ideal Bead profile



Alignment Trouble shooting: What knobs to adjust under each circumstance

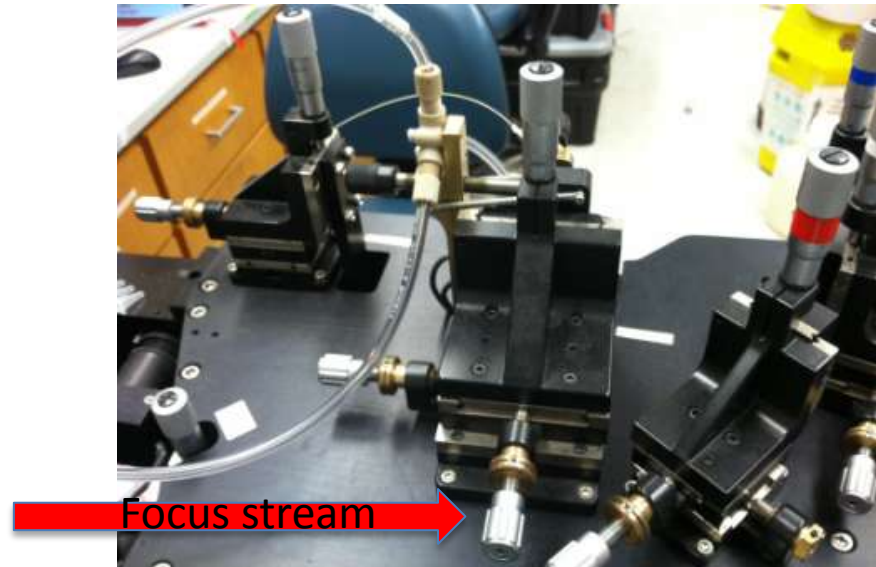
1. Diagonal bead profile



488nm laser alignment

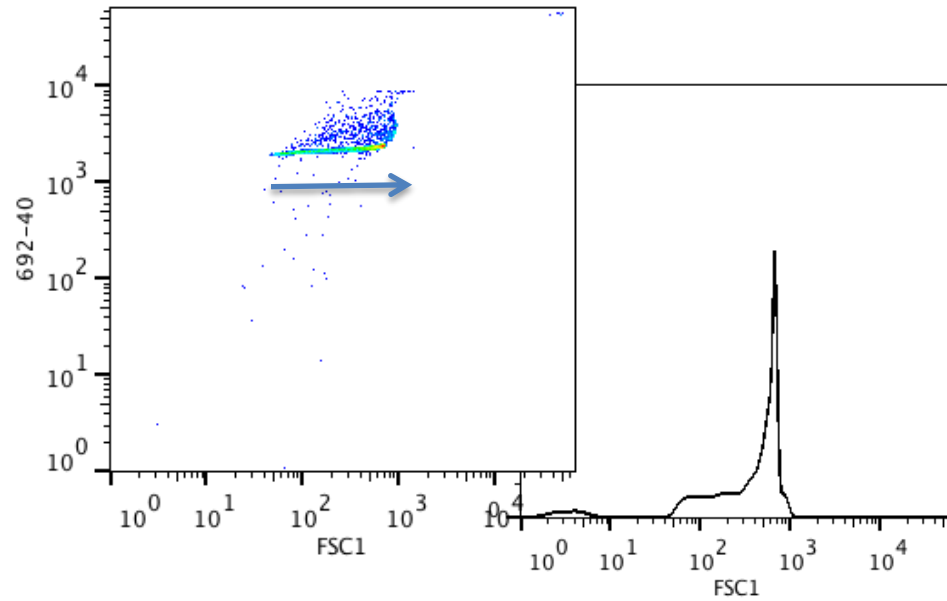


Stream Focus alignment

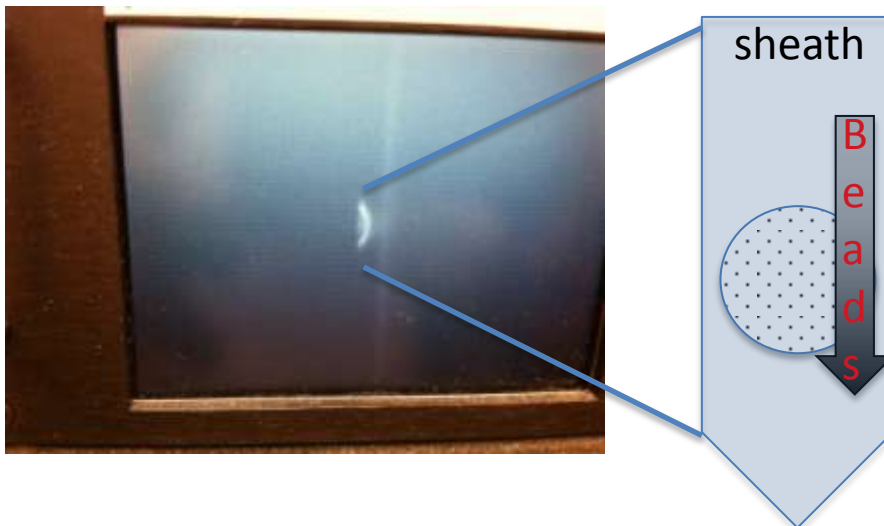


Alignment Trouble shooting: What knobs to adjust under each circumstance

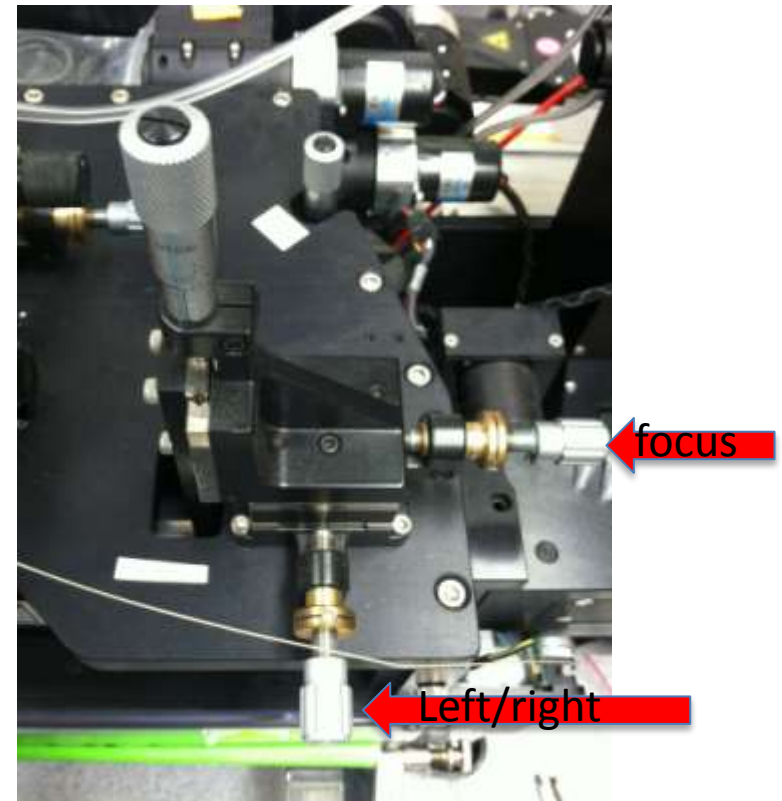
1. Horizontal bead profile



Video screen 1-2

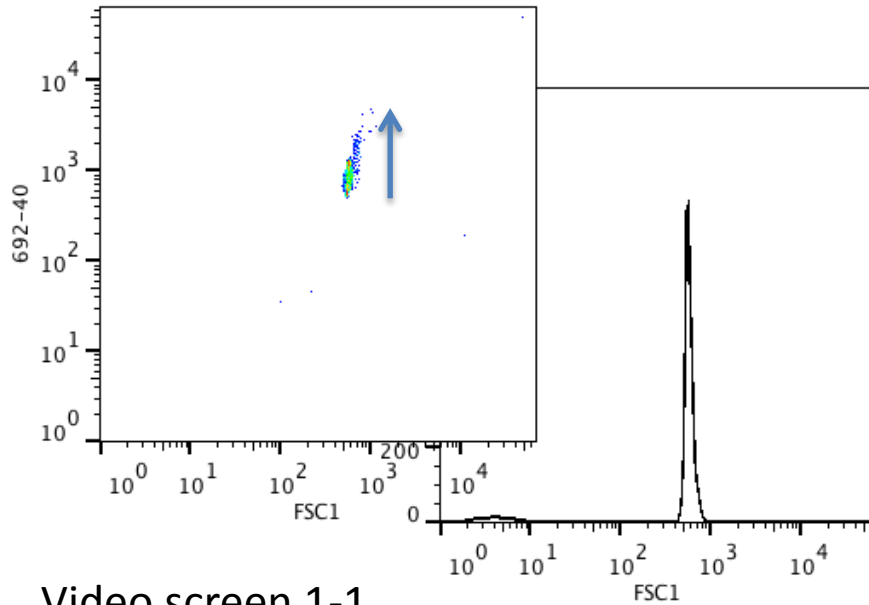


FSC detector alignment



Alignment Trouble shooting: What knobs to adjust under each circumstance

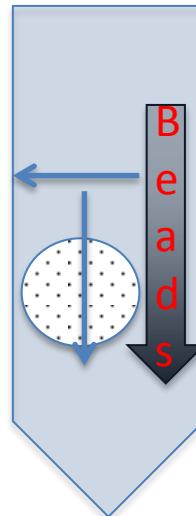
1. Vertical bead profile



Stream Focus alignment

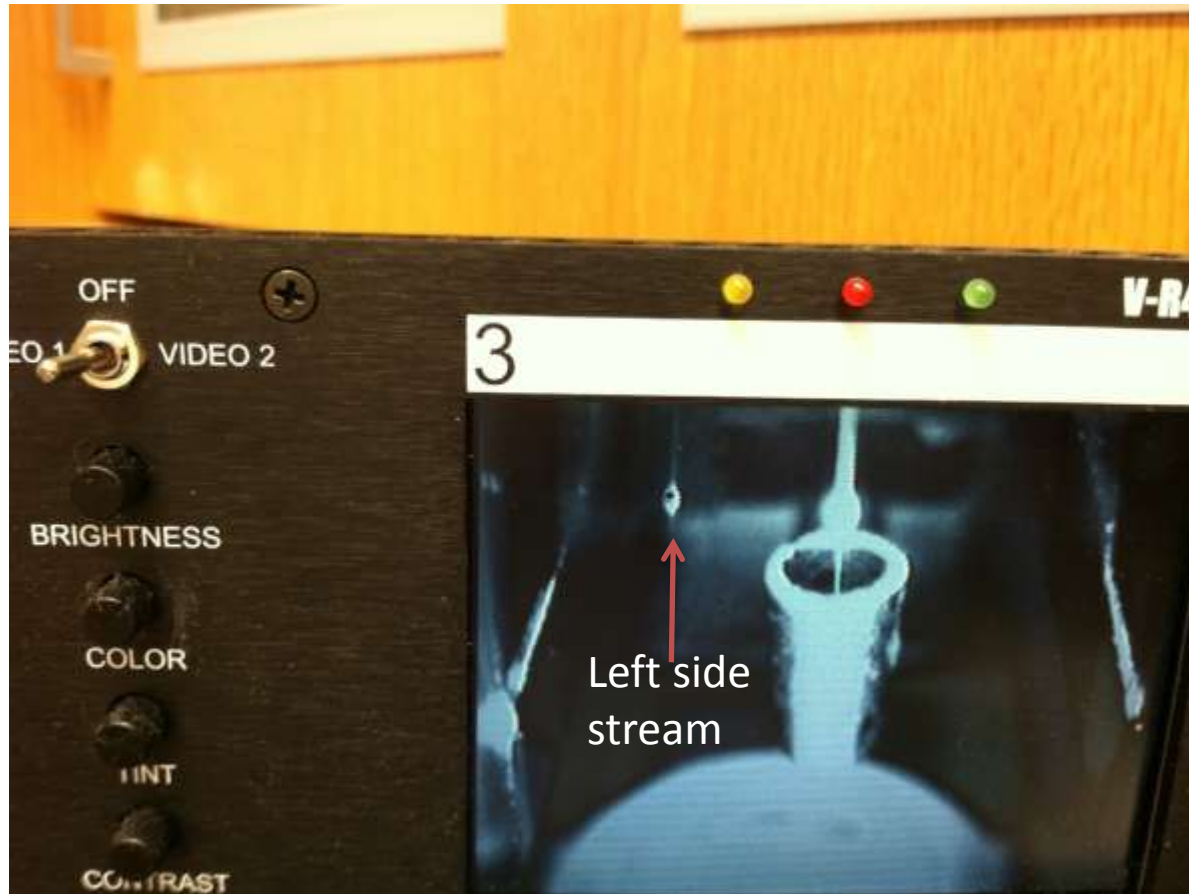


Video screen 1-1



Sorting: Stream, Visuals and Strategies

Video screen 3- Sort Stream (position dictated by the software)



Place side stream over the hand drawn dot. This will roughly correspond to the center of a tube on a two tube sort...

Sorting: Stream, Visuals and Strategies

Video screen 3- Sort Stream with Flash Charge and Stream Focus (software)

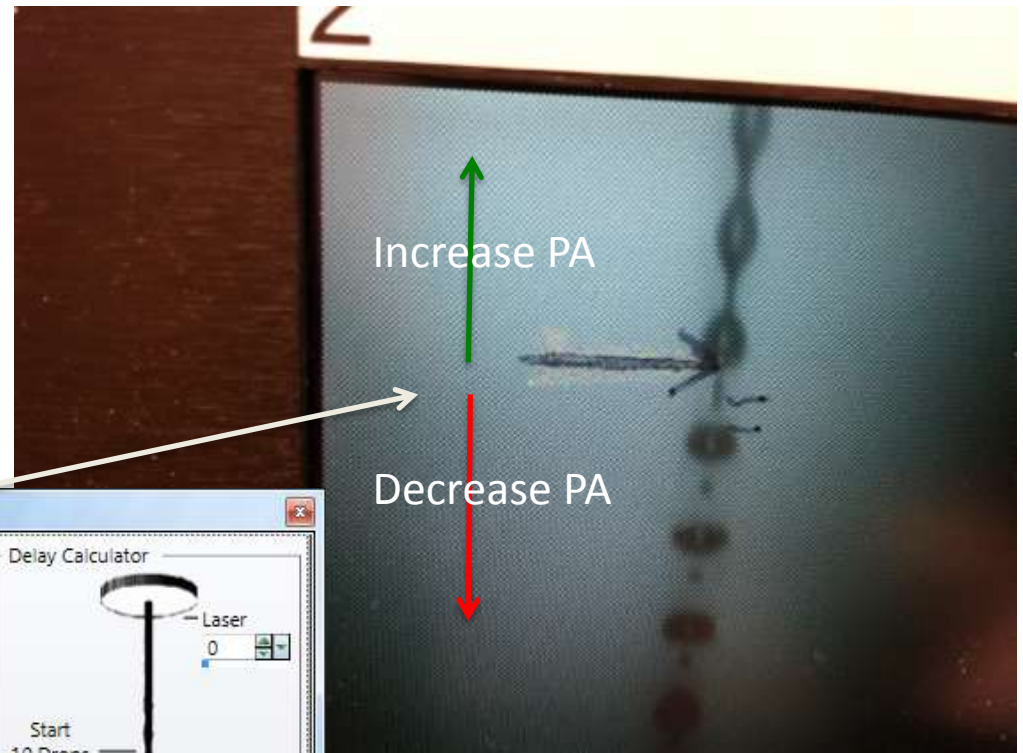


Set Piezo Amplitude so as to place side stream on the hand drawn black dot and to resolve a smaller side stream opposite the left side stream. Use Stream Focus to draw the right “flash” stream as close to the main stream as possible.

Sorting: Stream, Visuals and Strategies

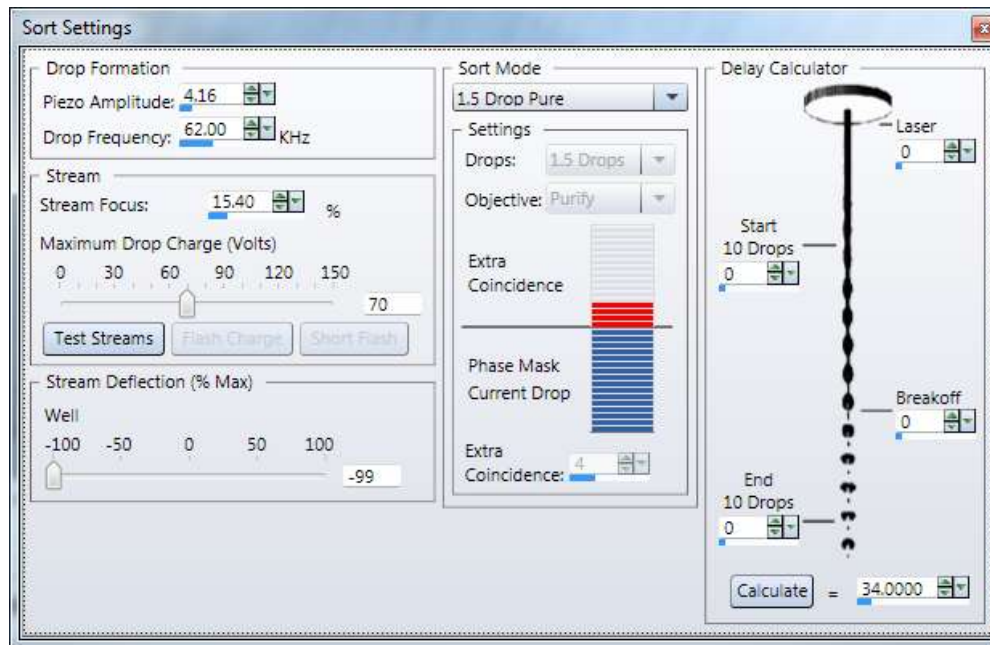
Video screen 2- Breakoff point

Want breakoff point to correspond to hand drawn arrow. Breakoff point will go up with an increase in the PA. Breakoff point will go down with a decrease in the PA.

A screenshot of the "Sort Settings" software interface. The interface is divided into several sections:

- Drop Formation:** Piezo Amplitude: 4.16, Drop Frequency: 62.00 KHz.
- Stream:** Stream Focus: 15.40 %, Maximum Drop Charge (Volts) slider at 70. Buttons: Test Streams, Flash Charge, Short Flash.
- Stream Deflection (% Max):** Well slider at -99.
- Sort Mode:** 1.5 Drop Pure.
- Settings:** Drops: 1.5 Drops, Objective: Purify.
- Phase Mask Current Drop:** A bar chart showing a red bar at the top and blue bars below.
- Extra Coincidence:** 4.
- Delay Calculator:** A diagram of a stream with a laser at the top. Start: 10 Drops, End: 10 Drops, Breakoff: 0. Calculate = 34.0000.

Sorting: Stream, Visuals and Strategies



Sort mode:

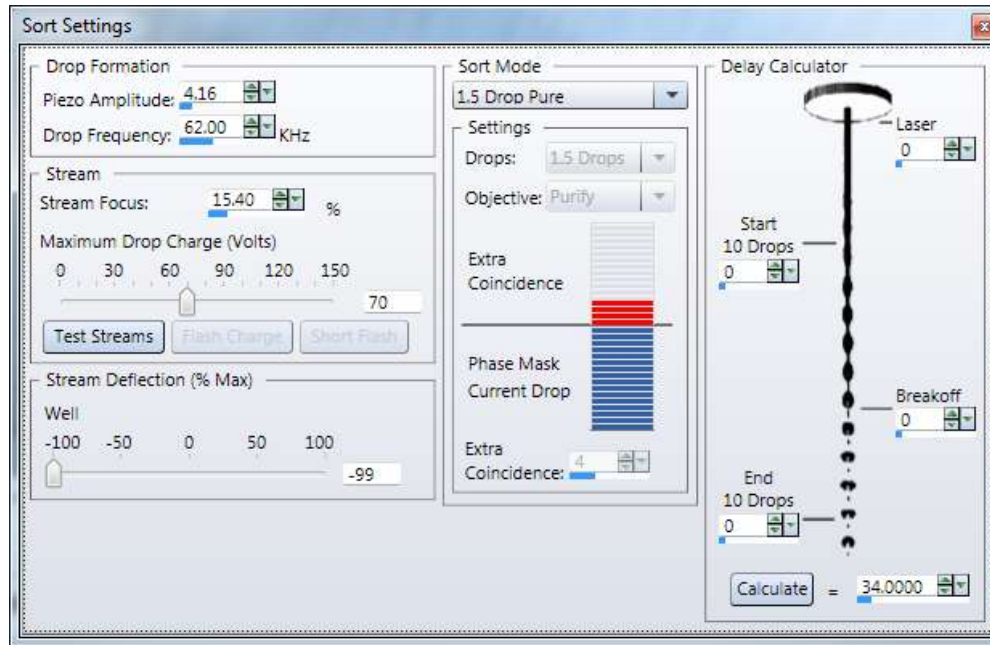
Drops: Number of drops eligible to be sorted

1 drop = sort only one drop at a time

1.5 drops = potential sort 1 and $\frac{1}{2}$ drops - useful when cell of interest is at the edge of a drop

2.0 drops = potential to sort 2 whole drops

Sorting: Stream, Visuals and Strategies



Sort mode:

Objective: How to sort, Stringency

Pure Yield = highest discrimination, will only sort if in middle of drop, no coincidence

Pure recovery = high discrimination, will sort within the boundary of 1.5 drops

Enrich = lowest discrimination, will sort any drop containing your cell, even in the event of coincidence

Sorting: Stream, Visuals and Strategies

Typical settings:

70um nozzle:

Sheath Pressure	18.5	27.5	33.0
Sample Pressure	19.0	28.5	33.9
Frequency	49.10	62.00	74.00
Drop Position	213	223	201
Drop Delay	30.1	33.6	43.1

140um nozzle:

Sheath Pressure	4.0
Sample Pressure	4.5
Frequency	9.40
Drop Position	226
Drop Delay	18.1