Fusion Quick Guide v5 *__Download a copy of this instrument Help File__: salk.edu/fccfaccess > Help Files

*13 fluorescence channels (355nm, 405nm, 488nm, 561nm, 640nm) See chart on Fusion or Help Files for filter/detectors

GREEN SECTION: Start up: Preparing the stream and nozzle.

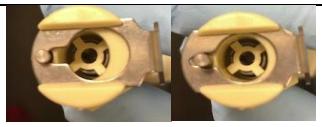
YELLOW SECTION: Running calibration procedures (CS&T and Autodelay)

ORANGE SECTION: Sorting: Designating Sort gates and setting up the collection vessels

BLUE SECTION: Cleaning up and Shutting down.

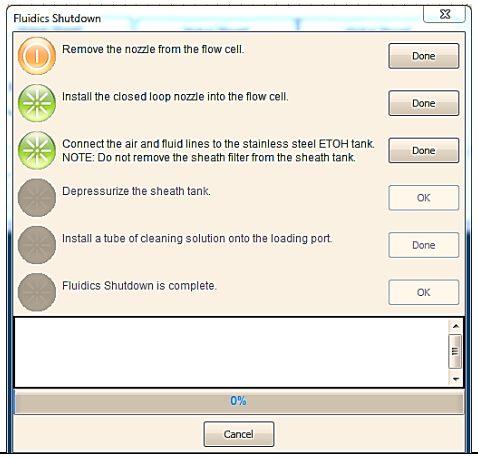
AQUA SECTION: Troubleshooting (broken up into different sections)

Help Files Folder on Fusion Desktop: Contains Troubleshooting Guides, Diva Software Guide and BD Fusion Sorter User Guide

BEFORE start up (1) Check Tanks (2) Complete Tank connections: (i-iii) Sheath Tank (iv & v) Waste Tank	**Sheath Carboy:** Handle the tank aseptically and locate a fresh carboy of buffer before starting (rectangular carboy, NOT ROUND ONES!). IF ETHANOL CHANGEOVER IS NEEDED: Decant ETOH into metal reserve tank, rinse sheath tank with 1L sterile sheath then carefully return open tank to the drawer. **SHEATH TANK:** Fill to upper weld line and secure lid. Complete sheath tank connections (i-iii): *Make sure to press the metal tab on the side of all female couplers before making connections; this is to ensure the correct positioning to receive the male coupler (see photos on right).* (i) Air and (ii) sheath filter (both to top of tank), and (iii) sheath level sensor (to fluidics drawer electronics console at right side of cart). **Waste Tank:** previous user empties and adds 1L bleach before reinstalling - (iv) Check the waste tank has two completed waste connections and (v) one sensor connection: all 3 should connect to ports located at the side of the fluidics drawer.  *Left:* Before pressing side tab (wrong position, non-circular – will damage male coupler). *Right:* After pressing metal side tab of female coupler (forms a circle ready to connect to male coupler). Use small amount of o-ring lube on the black o-ring on the male coupler if difficult to push together to avoid damage.
START: PC, Fusion, Waterbath, Compressor	(1) PC: Use **Admin PW: BDIS#1** to log in. (2) Fusion power: button is on the right side of the unit (Green Button) (3) Waterbath: Requires two steps; ON as well as Enter ↵ button to start the cooling. Check it starts to get cold!! (4) Compressor: Located on the floor next to waterbath; power switch is on back of unit.
START AMS and BSC fan and lights	Both on BSC hood: Aerosol Management System should be set to LO (put in HI for 5min if there is a clog before accessing the sorter). BSC fan must be turned on (blinking = OFF), light button is in the same area on BSC hood.
Open desktop icons: Tera Term, Coherent Connect (for the Lasers)	(1) Tera Term: Open and leave running > Copy codes from here as text to send the core including any codes if you experience error messages in Diva. *Note: When Cytometer IP address is displayed, Diva can connect to the Fusion* (2) Coherent Connect (CC): START button > fire up all 5 lasers, note that UV laser requires TWO STEPS (slider as well as START button). **Close CC afterwards** (never leave open as it can create issues with lasers and with Diva). Refer to Troubleshooting Guide if Laser is missing from CC and you need it (no signals in QC template with beads).
Open Diva, Choose "Use CST Settings"	Diva: When Tera Term displays the Cytometer IP address, the Fusion is ready to connect to Diva software. Open desktop icon (**Administrator, no PW**). Select Use CST Settings when the window appears. *For Diva Connection issues: restart the Fusion at the Green Power Button (watch Tera Term to see when to open Diva). If fails, restart computer AND Fusion.*
Fluidics Start Up Sonicate nozzle Dry off nozzle and check for o-ring Prepare to install nozzle	On weekdays (M-F), the bottom right corner of the Diva software should say "Fluidics Startup done". If it says "Fluidics Shutdown done", execute Start Up (Cytometer Menu > Fluidics Start Up). *"Fluidics Startup" should always be performed with CLN (closed-loop nozzle) in.* While this is going: Sonicate the nozzle for 5min (in a FACS tube with 2ml H_2O**;** top up the sonicator before starting it if the water level is low - use H_2O from DI faucet). Dry the nozzle gently with a kimwipe AVOIDING the o-ring. Before removing the CLN, prepare the flow cell for the nozzle by clearing it of air/debris: Start the stream for 10s (this evacuates air/debris to waste). Stop the stream, remove the CLN (stow it out of the way) and install the sonicated nozzle (rotate turn key to "up position" to lock nozzle into place). If o-ring detaches from a nozzle: use the spare nozzle and notify FCCF, nozzles are kept in the Fusion storage cubby (left side of the sorter – slide door to open).
Verify **TWO** nozzle settings. Change if needed.	E.g. For 100um nozzle (FCCF default): (1) Verify "**100 micron**" (picture below left: this is the Sort Setup) at the top of the Droplet cam view (FCCF default nozzle), and (2) Verify a matching configuration is displayed in the top left of the main Diva software header (see picture below right, the "100" indicates the configuration for the 100 micron Sort Setup). **Change these two settings if planning to use another nozzle other than what is displayed:** (1) Cytometer Menu > Sort Set up > select nozzle and (2) Cytometer Menu > View Configurations > Select Configuration needed **(must end in 2UV, not FSC-PMT)** > Set Configuration > Exit window. Check that the two settings are now displayed on the appropriate two windows for the new nozzle set up.  
Continue to Install the nozzle Check/clean plates and sort chamber	Check the nozzle area is clean, dry and free from visible saline deposits (clean with kimwipe/q-tip using DI water and dry well). Insert the nozzle only when area around nozzle and sort chamber are free of salt deposits and visibly clean; next, rotate the turn key to secure in place. Sort chamber: Open and check plates are dry and clean without saline deposits (clean with kimwipes: DI water then 70% ethanol, dry thoroughly).
Start the Stream Check waste stream is centered	Turn on the Stream (at the top of the droplet camera window). Waste stream centering: If needed, use the Allen key (in the white tube rack in the BSC); loosen bolt on either side of the chamber just enough to move the chamber to center waste stream within the aspirator. Do not over tighten bolts. **Note: If the Fusion was already calibrated for the same nozzle you plan to use and was left with the stream off (Closed Loop Nozzle in): Install nozzle, start the stream, turn on Sweet Spot and check Drop 1 values. If Drop 1 is nearly the same, it is good to use; re-run Autodelay if needed.*

Purge sheath filter of any air (follow carefully!)	**Open the fluidics drawer and carefully inspect the 0.2um Filter.** DO NOT ROUGHLY HANDLE where the tubing fittings connect to the filter**. Check for bubbles**: the air release cap is located at the top of the filter. Spray cap with 70% ETOH before continuing, dry with kimwipe then hold the wipe to catch any drips: **Point the cap away from you (fluid is under pressure) then with care not to take the cap entirely off or lose it –** open cap just enough to slowly release air. Tighten cap and replace the filter. **Always check for air if drop breakoff is unstable.*
Adjust break off (in the droplet camera view)	Adjust the break off (using Amplitude): Put the first free drop of the stream (aka "Drop 1") in the same position as the sample images for the nozzle in use – the reference images are attached to the computer monitor of the Fusion. Move the break off position higher by increasing the Amplitude value (and vice versa: move down by decreasing the amplitude). Note: when the break off is in the "usual" position for FCCF, the Drop 1 value in the typable box will be close to the actual value (on the grey background). **Unstable breakoff will look like Drop 1 is changing positions constantly ("jumping up and down" as you watch). Check sheath filter for air if this is observed*.
Bead QC ("before") Automated CS&T Sweet Spot ON	(1) Check CST bead profile histograms (~1 drop in 300ul) in QC template, if CVs look OK then (2) execute CS&T if first user. (3) Turn on Sweet Spot. Make PDFs of CST report and bead profile to send to the core. **Wide bead CVs:** perform flow cell cleaning, OR can likely use with caution but check with FCCF about post-use flow cell cleaning. **Changing to another size nozzle:** CS&T must be run for the new nozzle in order to calibrate laser delays to the new settings (check waste is centered also)
Run Auto-Delay (Drop delay timing)	**Before starting: (i) Observe the droplet camera** view and make sure the breakoff (Drop 1 position) appears stable when Sweet Spot is engaged. **(ii) Observe the stream camera** view with/without using Test Sort and verify that sort streams are stable (do not collapse intermittently) and are well defined (i.e. no spraying). *For these issues: check the sheath filter for air, re-sonicate the nozzle. For Intermittent "fuzziness" to Test Streams or "extra streams", the nozzle may need to be re-sonicated multiple times (try up to 2x more before moving to the spare nozzle and informing the core). If waste stream is problematically "wide" - adjust for residual charges on 4th, 3rd, 2nd drop (see later section "Set up Sort Streams").* **Prepare for Drop Delay calibration (Autodelay):** Open the nozzle specific Drop Delay experiment. Use Test Sort button then optimize (1) Accudrop laser for maximal brightness (turn silver knob) and (2) adjust left stream position using its deflection slider (engage the Optical Filter button > position the left stream within the left square). Load Accudrop beads (1 drop in 2ml) and start sorting all the beads (use the NOT P1 gate already set up) to the left. Press AutoDelay and follow the prompts: should give an inverted U shape afterwards. Try making new beads first, then see Troubleshooting if there are any issues.
Drop 1 and Gap	**Monitoring changes:** After calibration (CS&T and Autodelay) and turning on Sweet Spot, type in the actual Drop 1 and Gap values. Run Autodelay IF Drop 1 changes by more than 10 units -> update Drop1/Gap values again.
Sterilize sorter and work area	**Run 10% bleach** for 5min (Tip: start setting up plots, open your template/a new experiment while running bleach). Backflush sample line for 2min to wash out bleach (Cytometer Menu > Cleaning Modes). **Sterilize work area and collection tube holders** with 70%ETOH.
Select Sort Device Set up Sort Streams	Select Sort Device (eg 4-way for eppis or FACS tubes and 2-way for 15ml) then Use Test Sort and adjust streams to be used (up to 4). Should be tight, not spraying (otherwise sonicate nozzle again). See troubleshooting if needed (or try the spare nozzle). **Waste stream should be tightly focused; adjust residual charges if waste seems "fuzzy"** or wide: Adjust in this order, optimizing each for "thinnest" waste stream before moving on: 4th, 3rd and 2nd drop.
Set up collection holder/ACDU	Use Test Sort (open Waste Drawer) to check position of sort streams for depositing into tubes for your sort. Adjust deflection slider for each stream needed. **Ask us about high precision targeting for low number sorts – eg for 10x*
Cooling: Sample and collection	Sample Cooling is usually at 4C (turn on/off under Cytometer Menu > Sample temperature). Collection is cooled by the waterbath (separate to sample): If collection cooling is needed, **check the waterbath has cooled down, connect the collection holder** (make sure you press in the metal side tab in before connecting couplers).
Load Template/ Create Experiment Set up FSC/SSC Set up aggregate discrimination	Load a template: Experiment Menu > New Experiment > Salk Users > Choose your template. Make a new experiment: icons are located at the top of the Browser window (Experiment icon > Specimen icon > Expand the specimen and click on the green arrow next to the first Tube). For most applications: Type 200V into FSC and SSC as a starting place (leave in linear display mode). Check Width (W) for FSC and SSC and remove unused fluorescence channels using the Delete button at the bottom of the Parameters Tab (ask staff for help if needed) Browser window: Icons highlighted in red: (left to right) Experiment, Specimen and Tube. The Sort Layout is highlighted in green. 
Run samples (use Flow Rate 1 at first, and pause sample when drawing gates!)	Filter each sample using a filter cap tube or cell strainer basket: samples MUST be free from clumps or viscous material to prevent nozzle or sample line clogs. When sorting cells, please treat with DNAse I (see salk.edu/fccfaccess > Help files > Sample Prep Protocols). Can use both BD polystyrene OR polypropylene FACS tubes. Acquisition Dashboard: Set the Flow Rate to 1, then use the Load button to raise the sample onto the port. Use Acquire button to start or pause the sample (eg while drawing gates).
Adjust voltages, do compensation	Do not record anything until everything has been checked and all voltages adjusted. (1) Make sure bright events are not off scale – it is a good idea to briefly look at a fully stained sample or two (bright dots must be brought back onto the plots!), check any negative controls. Check single color comps one at a time and adjust as needed (each one must be brightest in own channel). (2) Perform compensation if needed (multi-color assays).

Activate bi-exponential display Check/draw gates Set up Sort Layout Enter sort # limit Sort Precision	(1) Bi-exponential display should be enabled for fluorescence plots (highlight the plot then go to the Inspector window and check the box) to ensure populations are properly displayed. (2) Draw/adjust gates: View gating relationships using the Population Hierarchy window (right click any plot on the white area > Choose it from the top of the list). Tip: To link gates, click on a gate in the Hierarchy before drawing the next gate. (3) Open a Sort Layout (icon located in the Browser- **see photo in section above**). For sort purity, put the smallest % populations in the middle positions for 3-4 way sorts: add the gates to each position by right clicking the position > selecting gate. (4) Designate sort limits if desired: Enter the limit under the Target Events box of the Sort Layout. (5) Select Precision (desired sorting mode): Most popular modes are "4-way Purity" (1 drop is sorted for each sort decision, while sorting for purity) and "Single Cell" (1 drop is sorted but in this case, besides purity, precise counting is critical). In Single Cell mode, drops are only sorted if target is in the center of the drop (i.e. more sample is wasted in this mode, but the sort count is very accurate as it is less likely to get any empty drops sorted; ask us for more info if needed). The other "Purity" mode can also be used; this mode will sometimes sort 2-drops (based on cell position within the drop) to increase final recovery, and thus produces a higher sort volume overall
Sorting *Install your collection tubes!	**Install collection tubes then start the sample (Acquire) > click on the Sort button in the Sort Layout. Increase flow rate to maximize speed and sort efficiency.** Generally, should be 75%+ (or even as high as 95%+) depending on sample type: dilute sample if efficiency is low, or talk to staff for suggestions. Sample stickiness will affect efficiency (typically helpful to treat with DNAse I) and cause sort streams to be splattery/fuzzy. "Stickiness" or viscosity will disrupt efficient droplet formation. If sort streams are splattery, it may be necessary to stop frequently and dry the plates (if it is even possible to sort at all) so sample quality needs to be addressed. If you need to unload the sample to filter or resuspend, you should be able to use the Resume button to keep the sort count going. **Backflush for 2min in between your sort samples** (can also run bleach if samples are difficult).
Anydesk setup	Set up the remote monitoring if you are planning to leave the sort unattended (information is on the BSC hood). Do not leave LCMV or primary patient sorts unattended.
Mid sort cleaning	Depending on sample type and quality, sort duration, and if there was observed quality degradation (data looks weird, scatter looks unusual, event rate fluctuating) periodic cleaning is recommended. Running bleach for 5min periodically should pre-emptively prevent problems in most cases. Filter samples regularly if they are problematic (a blocked sample line will require staff attention to replace the tubing) or retreat with DNAse I.
Nozzle clog	Sweet Spot will shut off the stream if the clog is bad enough; but partial clogs may not trigger this (i.e. will need to manually stop the Stream). In the Droplet camera view this will look like a shift to the side (compared to earlier), or oddly shaped droplets. Stop the stream, and switch the AMS to HI for 5min. After 5min, remove the nozzle and sonicate (same as for Set Up). While this is proceeding, clean and dry the sort chamber, plates and nozzle area (exercise necessary biosafety precautions cleaning these areas). After reinstalling the nozzle and turning on the stream, follow Set Up steps to adjust the breakoff. Turn on Sweet Spot, use Test Sort to check if stable side streams are produced (re-sonicate if not). Compare Drop 1 actual value to starting Drop 1 typed in the box (will need to re-calibrate if more than 10 units difference). Run bleach for 5min, backflush and resume sorting.
Clean and Leave it *For regular hours -Open laser door -Delete old data -Sign Clipboard -Send Files to FCCF	Sample line cleaning (this does not clean Flow Cell well!): Backflush to remove residual sample, **run bleach for 5min**, backflush 2min. After cleaning, record a CST bead file (on Flow Rate 1) in the QC template. Make PDF of "after" bead file. **Before leaving:** Based on how bead CVs look, find out from us if you should set up Flow Cell soak (in upcoming section) or just install a tube of bleach and leave it to run. e.g. at Flow Rate 5 is safe (less chance of clog). The Fusion has an Autostop feature so a tube can be run unattended. **Clean work areas, delete old data from any previous session. Sign off clipboard. Open the laser interlock door before leaving.** Send PDFs/Files to core.
Clean & Leaving with stream off *Alternate option	**For long breaks between users*:** After cleaning/bead QC, stop the Stream, **install Closed Loop Nozzle.** Put a tube of 1.5% Citranox on the sample port. Cytometer > Cleaning Modes > Clean Flow Cell (do 2x). **Clean work areas/delete old data/sign off clipboard. Open laser interlock door.** Send PDFs/Files to core. **When ready to use, re-check Drop 1 etc.*
Clean *If last user	Same as above, and if needed, do a Flow Cell soak (see below) e.g. if bead CVs are wide or scatter is "weird".
Flow Cell Soak *Use any time as needed –skip if bead CVs ok	Stop the Stream, **install Closed Loop Nozzle.** Put a tube of 30% contrad on the sample port. Cytometer > Cleaning Modes > Clean Flow Cell (do 2x). Leave to sit up to 2h, then start the stream for at least 30s (CLN in). Stop the stream, change nozzle and re-check bead CVs. Record CST bead file in Flow Rate 1 to check CVs in the QC template. Make PDF of "after" bead file to send to core. If you're having trouble, proceed to shut down and email us what you have done and how it looks so we can follow up in case we need to look for problems that are not related to cleanliness.
Regular Shutdown: Turn off stream Remove nozzle Install CLN Final Flush of FC Laser door open Empty waste Clean up work areas	**For Su-Th (non holiday) unless directed otherwise,** you will do a "regular" (non ethanol) shutdown leaving 1x PBS in the system: Turn off stream and remove nozzle. Stow nozzle in labeled FACs tube (should be in the white tube rack already). Submerge nozzle with filtered MQ water for storage. **Install Closed Loop Nozzle (LEAVE IN)** *remembering to lock CLN in place with turn-key* and repeat these 3 steps with bleach, then water, then 1.5% citranox. (1) Perform a Clean Flow Cell cycle -> wait 3min (2) Start the stream for 10s (3) Load the tube of cleaning agent and run on Flow Rate 11 for 2min; unload. Turn off the Stream. Leave the laser interlock door open. Empty Waste (add 1L bleach after!) Clean/tidy work areas.

Ethanol shutdown **Fridays/holidays* Laser door open Empty waste Clean up work areas	 **Fri/weekend/holidays do 70% ETOH shutdown for storage unless booked (e.g. then the 70% ETOH shutdown may move a day or so to be done ONCE over the break)**. Check ahead with staff/ check calendar /note board on the BSC for instructions before "ethanol" shutdown: 1. Disconnect air, sheath and waste lines, and sheath sensor (use quick release to disconnect air, sheath and waste lines, then gently disconnect the black sheath level sensor from the electronics console panel: pull straight outwards to unplug it). 2. Remove tank, empty sheath in the sink, then fill sheath tank with 70% Ethanol from the metal reserve tank. Add 70% Ethanol until it reaches halfway from bottom of tank to the LOWER weld line inside the tank (indicated in picture to the left). Close lid and swish ethanol so it covers the entire inside of the tank. 3. Reconnect tank. 4. Place a tube of 70% Ethanol on the SIT. 5. Perform automated Fluidics Shutdown. Go to Cytometer -> Fluidics shutdown. Just click Done within the Fluidics Shutdown window (see picture to the right for reference). Leave the laser interlock door open. Empty Waste tank (add 1L bleach after!). Clean/tidy work areas. 
Export Data	Right click on the experiment in the browser→ recommend to (1) "**Export Experiment**" first (browse, locate to Desktop) then repeat a second time choosing (2) "**Export FCS Files**" (choose FCS3) browse, locate to Desktop)→ "replace all" FCS files. This gives you a folder with your template as well as correctly names data files.
Connect to Filer	Go to "Computer". If need to map to Lab share: Right click Computer and select map network drive. Type in path and lab, e.g. \\filer.ad.salk.edu\I**MPL-K (*your lab)** "Cut and Paste" data to server (DO NOT LEAVE ON DESKTOP)
Delete old data	In the browser: Right Click → Delete (Important: Prevents Diva getting buggy and slow!)
Close software/PC	Exit Diva and Tera Term (no need to turn off lasers in CC as you're about to turn the system off anyway).
Power off/take pics	(i) BSC fan, (ii) BSC light, (iii) AMS, (iv) Green power button OFF on Fusion, (v) waterbath, (vi) compressor
Email PDFs/photos Restart PC. Red bin.	Send relevant photos/files: (see above 6 photos), photo of (legible) clipboard signed off, before/after bead profiles, any CS&T report. Restart PC. Empty red bin if full or you were running LCMV samples.
TROUBLESHOOTING	
General computer issues.	**Anything that can be computer related**, email IT@salk.edu and provide the Anydesk info on the front of the BSC. This includes Filer connection, Diva or Coherent Connect not opening, computer freezing/crashing/blue screen etc.
Sheath tank doesn't pressurize	(i) Check air supply: Air supply tubing line should be connected to the top of the tank and compressor must be ON (ii) Make sure o-ring hasn't fallen in to the tank: If so, retrieve, dump sheath, rinse tank VERY well with ethanol and fill with fresh sheath. Recommended to perform an ethanol shutdown at the end of the day for sterility. (iii) A quick release coupler could be damaged (e.g. nicked o-ring on male coupler). Check sheath and air coupler at the top of the tank, looking for a ripped or nicked black o-ring. Ask core staff for help replacing and adding o-ring lube.
Waste: Diva says full but tank is empty	Float sensors stuck by salt crystals. Try "banging" the tank against the floor firmly, to break/jiggle the crystals free
Diva connection issues	**Be sure to use Tera Term and open Diva at the right time**, when the Cytometer IP Address is displayed. (i) If cannot connect, try manually connecting from the Cytometer Menu. (ii) If that fails, leave Tera Term open, close Diva, and turn off Fusion. Start Fusion after 2min and watch Tera Term, open Diva when appropriate. (iii) If that fails, try turning both the PC and the Fusion off and following the start up procedure again.
Laser not showing in Coherent Connect	**Two possibilities, either laser is not actually firing, OR it is simply not talking to CC. Proceed as follows** (i) **Restart CC:** Try closing CC, waiting a minute and reopening. (ii) **USB hub cable reseat:** Try unplugging, waiting 1min then plugging in the laser USB hub ethernet cable: This is located (look for the label "All Lasers: Coherent USB Hub") on the side near the Power Button. To release: squeeze and hold in the tab on the underside of the connector (be sure to hold the tab), then pull the Ethernet connector straight outwards. To reconnect: Push straight in to the port (will "click" in). (iii) **Restart the lasers:** The power button of the Laser power strip is located on the lower front left door of the Fusion (marked off "NO" with tape). Turn off, wait 2min, turn on (don't bother restarting the entire Fusion, this is sufficient for restarting the lasers). Repeat if needed (iv) **Check if laser is producing signals using beads** (only if UV laser is not as UV absolutely needs CC to start): 4 laser are configured to auto-fire upon start up of the Fusion (no CC needed to turn these 4 lasers on). Checking for peaks while running beads in the QC template. **Do not run CS&T if any lasers are missing.**

Unstable breakoff or unstable streams (keep collapsing)	**Check for air**, purge the filter as described previously. Adjust the amplitude to manually put Drop 1 in a similar position to pictures on side of monitor before turning on Sweet Spot. Try resonicating the nozzle, or try a spare nozzle (kept in the side cubby).	
Break off/droplet image looks weird	**(1) Partial clog/dirty nozzle**: re-sonicate up to 2 more times then try the spare one from the storage cubby); **(2) wet camera lens or (3) Sort settings are incorrect:** Try reloading the Sort Setting, verify values (frequency, Drop 1, amplitude) are in the ball park compared to the examples stuck to the side of the Fusion computer monitor. Try clicking the the Attenuation icon if the image suggests the break off is below the viewing area (i.e. drops not showing on the camera). Example pics and instructions are shown in "Troubleshooting: Break off issues and Nozzle problems" located in the Fusion Help Guide folder on the desktop.	 **Attenuation icon** (in Sort Stream window)
No events showing	**First check that laser interlock door closed (if laser interlock door is open, the lasers will be shuddered and not hit the flow cell). If laser interlock door is closed, then blue laser may be off (blue laser is needed for FSC which is the trigger parameter). R**estart laser power button if blue laser is not on.	
No events due to sample line clog	If there are no events, but the lasers are on and the interlock door is shut. First shut off the stream, insert CLN, then preform a clean flow cell with a full 5mL facstube of DiH20 while watching the fluid level. If the fluid level in the tube decreases, you know that the sample line is NOT blocked. If the fluid level does not decrease, the sample line is clogged. You may unclog by turning on the stream with CLN in and running a tube of 30% contrad solution for 5-10 minutes. Then turn off the stream again, and perform another clean flow with diH20 to check if clog is still there. Clear out contrad by running 5-10 minutes of diH20.	
No events due to DIVA error	Restart Diva and check to see events.	
Cameras greyed out (e.g. sort stream camera not "black")	**Rare, connection issue to video board.** See Troubleshooting guide for missing camera stuck to side of Fusion near the Green power button. (1) Leave Diva open > un-plug each camera **labeled T = Top camera, B = Bottom camera port** (twist to the left to release, then pull outwards) > wait 2min > plug back in and twist to the right to secure > Close then reopen Diva. (2) If after reopening Diva cameras are not "fixed", repeat reseating the cables, but before reopening Diva, reboot the Fusion (Green power button, wait 2min before starting up again).	
CS&T warned	**Read message and check the report to determine why it warned.** If it warned because of wide CVs, then most likely the flow cell is dirty, and it may just require some extra cleaning. If the bead profile histograms look good, you may proceed.	
CS&T failed	**Read message and check the report to determine why it failed. First ensure all lasers were on when CS&T was run. Re-check bead profile histograms and that you see ALL lasers on. CS&T will fail if one of the lasers is off.** If failed because of CVs on a laser you were not using anyway, re-check the bead profile histograms, if these look reasonable you can likely proceed to use with caution and notify the core (may need cleaning, or laser alignment by the engineer).	
Accudrop failed	Check side stream positioning, and that accudrop laser is optimized for brightness. Make new beads and re-run.	
Auto Delay Cannot Determine Drop 1	If auto delay program cannot determine drop delay, check that previous Drop1 (located in stream view window) is similar to current Drop1. Note, that if Drop1 is very different from a previous day, that the drop delay will be different (If drop1 increased from previous day, meaning the 1st detached drop is further from the nozzle, Drop delay will need to increase. If drop1 decreased from the previous day, Drop delay will need to decrease).	
Gap value changing	Break off is unstable. Check for air, re-sonicate nozzle. Try spare nozzle.	
Waste stream "fat"	Wide or Fuzzy waste stream: Adjust for residual drop charge on 4th, 3rd, 2nd drop as described previously.	
Test Sort streams not focused or "extra side stream" appearing	Turn off Test Sort and Sweet Spot: re-check Drop 1 and adjust position if needed (using Amplitude) then turn on Sweet Spot. Make sure Drop 1 and Gap is stable (if not, check sheath filter for air) before using Test Sort to check streams. If streams are still fuzzy or extra side stream keeps appearing next to the waste stream, re-sonicate nozzle (repeat a couple of times before trying a spare nozzle).	
Collection holder connectors leaking	O-ring on the make coupler is ripped or nicked. Replace and add a small amount of Parker's o-ring lube just to the o-ring (make sure goes all around the o-ring).	
Streams spraying while sorting	Take off sample, stop stream and check voltage that voltage plates and inside sort block is dry. Stream spraying could also be caused by poor sample quality, try treating with DNAse I (see salk.edu/fccfaccess > Help Files) before sorting. Ensure protein in the sort media is less than 5%	
Sort efficiency low	Sample sticky (quality issues) or too concentrated. Treat with DNAse, dilute, filter. Check sort gate logic also to make biology and sort logic makes sense (no unintended redundancies in the gating strategy causing conflicts).	
Voltage plate arcing	Sample is spraying (sample quality issues), monitor in Stream window – streams should be tightly focused. Address sample prep. Will need to stop and clean plates/sort chamber regularly during the sort.	
Purity issues	Sample may have been spraying during the sort, contaminating the collection. Ensure also that the smallest percentage populations are on the outside positions for 3 or 4-way sorting. Try to improve sample quality.	
Sort volume is high	Smallest nozzle is 70um, ask core staff if it can be used for your sample type.	
Viability issues	Check that tubes are coated with media and streams are well targeted. Talk to staff about options for sorter settings.	
Event rate drops	Sample line may be partially clogged or needs cleaning, run bleach (make sure liquid level goes down, or sample line may be clogged and needing staff attention). Improve sample quality and filter regularly during the sort.	
DIVA software will not close	Hold down Ctrl Alt Delete buttons and force quit the software (be aware that if your data has not been exported, but is in the process of being exported, you want to wait for it to finish BEFORE closing diva)	