FORTESSA USER GUIDE

COMPUTER SETUP:

* Turn on computer before turning on the machine
* Windows 10 Username: BDOperator
* Password: BDIS#1
* Open Tera Term application on desktop and leave running in background
* Log in to BD FACSDiva software

MACHINE SETUP:

* Turn on the machine if you are the first user of the day. There is no fluidics startup for the Fortessa.
* Give the lasers 20-30 minutes of warm-up time.
* Check tank levels for FACS buffer and waste
  + To fill FACS buffer unscrew probe and place in holster. Silence alarm by clicking button on the wet cart. Fill cube if above 50% full or replace with new cube and screw in probe.
  + For full waste, unscrew probe and holster on side of cart. Empty waste in the sink and refill with bleach to first mark on outside of tank so that a full tank would create a 10% bleach solution. NOTE: make sure to not get the waste cap filter wet. If so, it needs to be replaced immediately.
* YOU MUST HIT RESTART ON WETCART AFTER REMOVING EITHER PROBE
* Bleed bubble filter (1st) and sheath line just above filter (2nd) into the beaker to remove any air bubbles caught in the lines and filter.
* Move sample probe arm to the side, remove the tube and PRIME 2x
* When red PRIME button goes back to STANDBY, load tube of DI Water, center arm, and RUN for 30 seconds prior to running any samples.
* Be careful as the probes can be easily bent so only move in a vertical motion.

SAMPLE PREP:

* You MUST filter samples immediately prior to loading on machine using 70uM mesh or less.
* Add EDTA to prevent clumping (when possible)
* Use a lower concentration to run at about 15k events/sec on MED speed.
* There may be a sample surge of ~100uL when centering the arm underneath the sample. Use minimum starting volume of 250uL.
* Run 10% Bleach solution every 20-30 samples for 5 mins, then dH2O to prevent clogging

RUNNING SAMPLES:

* Only load sample on to probe when you are ready to start recording immediately, otherwise leave tube of dH2O running.
* Samples are aspirated when tube is loaded if arm is to the side whether in RUN or STANDBY, so once tube is loaded immediately center arm.
* Load sample with machine in RUN and center arm.
* For control samples, adjust voltages and settings while acquiring.
* For experiment samples, immediately begin recording once tube is loaded to maximize the event numbers.
* RUN button turns green when tube is properly pressurized.
* If the Run button is orange instead of green, it indicates there is a pressure issue. Please check either the tube (cracks on tube), liquid around the top, or air in the air bubble filter
* The black disc under the sample should have a gap between the sample tube and never be touching as this can cause the probe to bend. If this is the case immediately screw the disc down.
* Use LO MED HI buttons and SAMPLE FINE ADJ dial to control flow rate.
* Run samples at a maximum event rate of 15-20k per second. LO speed has best sensitivity and cleanest signals.
* If samples show events but are pressed up against the FSC axis, likely an air bubble in the flow cell. PRIME 2x with no tube loaded and run DI Water tube for 30 seconds to stabilize stream.
* If a clog occurs, run 10% Bleach solution until clog is broken down and passed, then dH2O for 5 minutes. If persistent clog, seek help from Flow Core Manager.
* There is no automatic back-flushing of the outer probe, so wipe down probe with EtOH Kimwipe if worried about carry over or alternatively run a fresh tube of water between samples. (This is only for very rare populations, likely not necessary as carry over is very minimal)

SHUTTING DOWN:

* After the last sample, run 10% Bleach for 5 mins and dH2O for 5 mins
* **When you are done, leave DI Water tube loaded on the probe and place machine in STANDBY**
* Power down machine or leave on for next user.
* To create an experiment template for repeated use in Diva, rick-click experiment and DUPLICATE WITHOUT DATA.
* Export the experiment containing data (FCS files and/or experiment file) from Diva database directly to the RIA3 server
* Delete experiments with data from Diva Database. You should have no experiments with data present in the software or on the cytometer computer.
* If you are the last user for the day, turn off the computer.

In the event of a problem, contact Jun Case Rm. 6012C, [jcase88@bwh.harvard.edu](mailto:jcase88@bwh.harvard.edu). THANK YOU!