CANTO II 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

MACHINE SETUP:

* \*Prior to logging in to Diva software- Check liquid tank levels: Top off Shutdown solution tank with DI Water and Sheath tank with FACS Buffer. Before reconnecting tubing to wet cart use syringe to draw out any air bubbles in liquid lines.
* Empty waste tank making sure to not get the filter wet. Refill with bleach to first marked line on outside of tank so that a full tank is > or = 10% solution.
* Log in to Diva software and allow it to connect
* Prime Tanks (Cytometer->Cleaning Modes->Prime Tank After Refill-> Check all boxes)
* Check bubble filters on wet cart for air pockets. If air, find Flow Manager for assistance before proceeding.
  + NOTE: When you see errors of "\_\_\_\_ laser power low" in software, it's almost always due to air in these filters. Contact immediately.
* Perform Fluidics Startup (Cytometer-> Fluidics Startup)
  + NOTE: only necessary for 1st user of the day
* Give the lasers 10-15 minutes of warm-up time.

SAMPLE PREP:

* You MUST filter samples immediately prior to loading on machine using 70uM mesh.
* Add EDTA to prevent clumping (when possible)
* Use a concentration that does not exceed 15k events/second when acquiring at Medium
* Run 10% Bleach solution every 20-30 samples for 5 mins, then dH2O to prevent clogging

RUNNING SAMPLES:

* Using two hands move the support arm to the left applying no vertical pressure to the arm and load tube on to the probe being cautious not to bend the probe. Once secured in place let arm spring back to center.
* Begin acquiring tube using software controls and change speed of acquisition using sample speed drop down menu on acquisition dashboard.
* For control samples, adjust voltages and settings while acquiring.
* For experiment samples, immediately begin recording once tube is loaded to maximize the event numbers.
* If no events, check for cracks in tube or liquid around top or on rubber fitting. Replace tube if cracked or dry and re load.
* If still no events, possible clog has occurred. Load tube of 10% Bleach and mark meniscus with marker. Run a Clean Flow Cell (Cytometer->Cleaning Modes->Clean Flow Cell). If the liquid level does not move, repeat 2-3x. If liquid level still does not move, contact Flow Manager before proceeding. If liquid level moves, follow 2-3 clean flow cells with 2-3 more of DI Water.
* If samples show events but are pressed up against the FSC axis, likely an air bubble. Run a Degas Flow Cell (Cytometer-> Cleaning Modes-> Degas Flow Cell)
* Run samples at a maximum event rate of 15-20k per second.

SHUTTING DOWN:

* After the last sample, run 10% Bleach for 5 mins and dH2O for 5 mins
* If you are the last person on the machine, or there is no user on the machine for hours perform Fluidics Shutdown making sure the Shutdown Solution container is full of DI Water before doing so (Cytometer-> Fluidics Shutdown)
* To create an experiment template for repeated use in Diva, right-click the 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 and D: Drive once transferred to RIA3 server. You should have no experiments with data present in the software or on the computer itself.
* Power down machine or leave on for next user.
* Turn off the computer if you are the last user.

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