

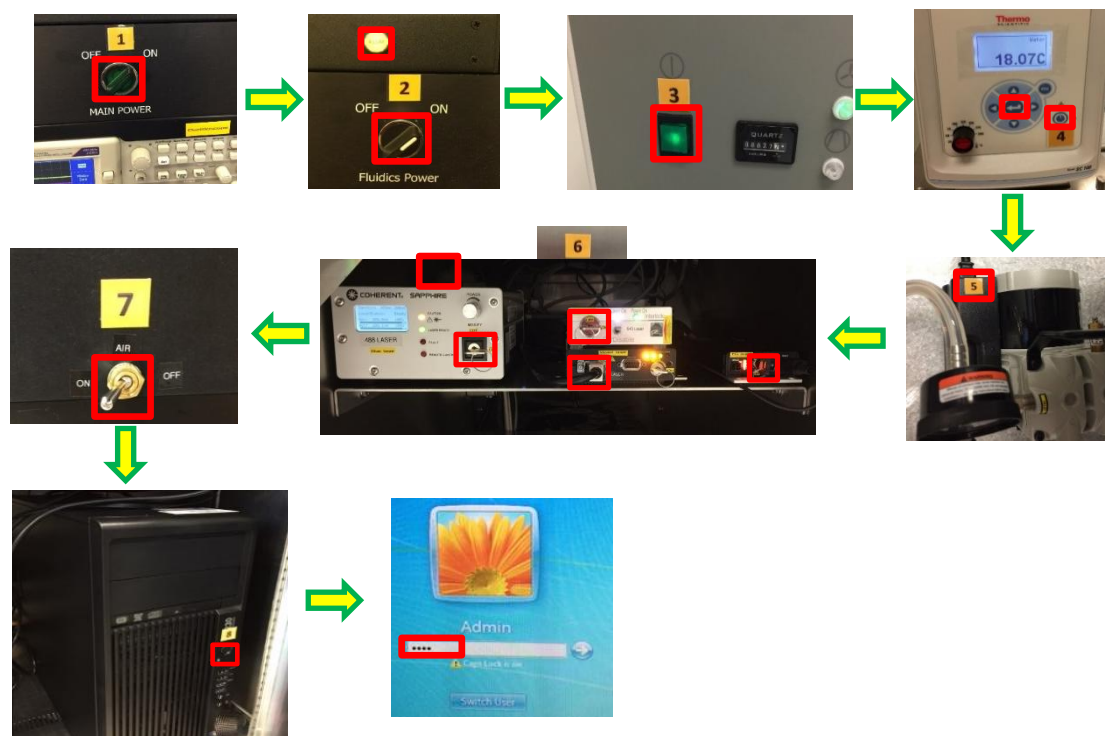
Imaging and Flow Cytometry Core

BD Influx Standard Operation Protocol - System Startup

Before turning on the system, please make sure the sheath tank is full (~6 kg) and waste tank is empty (only containing bottom filled layer of bleach).

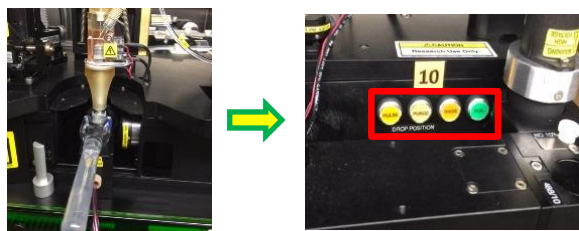
Machine Start Up:

(1) Turn on the “Main Power” → (2) “Fluidics Power” and “ILLUM” → (3) “Air compressor” (gauge must show 20 psi) → (4) Chiller Power and Arrow → (5) Air Pump (gauge must show between 5-10 Hg) → (6) Turn on the power of each laser that is required and warm up for about 20 minutes (blue laser is compulsory to be turned on) → (7) Air Switch at left side of Influx → (8) Computer Power (in the cabinet) → (9) Log in to “Administrator account”, password “BDIS” (twice beep sound will be heard if the server has connected with the instrument successfully).

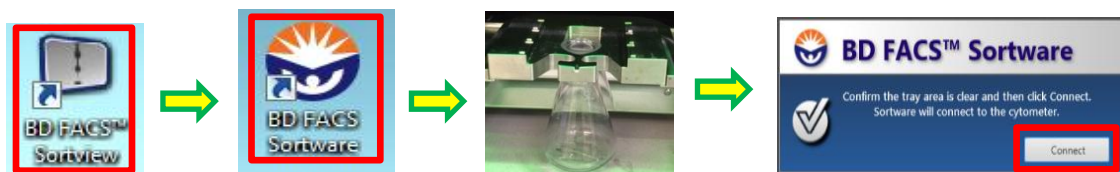


Imaging and Flow Cytometry Core

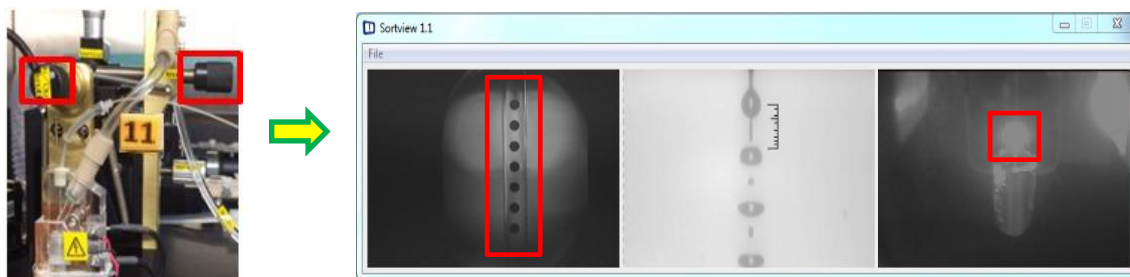
(10) Place the flush bucket and debubble reservoir under the nozzle assembly. Fill in MQ water and start the debubbling process with Purge/Rinse/Pulse/Long peak modes until all the bubbles are removed followed by turning on the stream with “RUN” button. Remove the debubble reservoir and flush bucket.



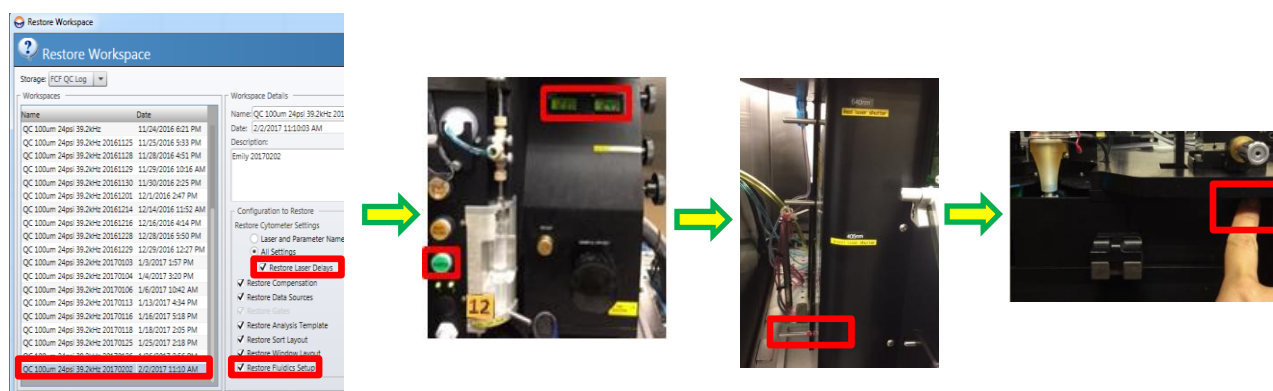
Double click **BD FACS Sortview** → **BD FACS Software** → Make sure the flask is in place → Click **Connect**.



(11) Start aligning the stream by adjusting the Horizontal, In/Out, Left/Right and the focus knobs until the brightest spot of the fluorescent signals and a sharp image of the stream and are obtained.

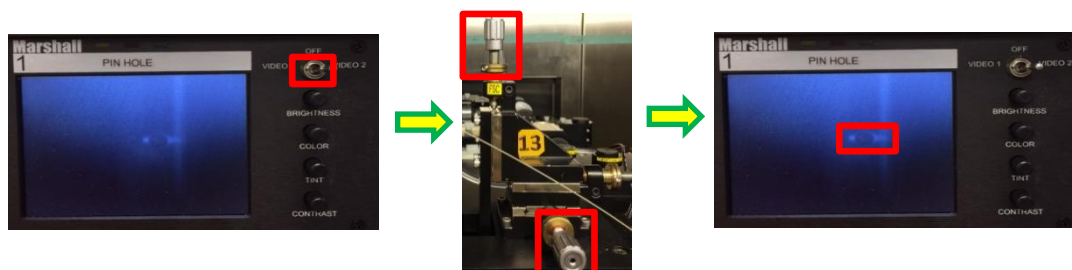


(12) Restore the latest QC workspace for 100um nozzle in “FCF QC Log” folder → restore Laser Delay and Fluidics Setup. Load Rainbow beads and start running the sample → Adjust the sample flow rate, sample pressure > sheath pressure 1psi → Open Blue Laser shutter → close the door in front of the nozzle and activate laser by presenting your finger onto the sensor.

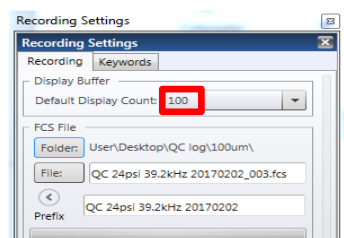


Imaging and Flow Cytometry Core

(13) Switch the video channel to Video 2 on the Oscilloscope. Start aligning the horizontal and vertical knobs corresponding to Forward Scatter to the pinhole and stream by referring to the fluorescent signals exciting by blue laser.



Set the “Display event rate” to 100.



(14) Open the shutters of the other lasers, preferably with this sequence: “Red” → “Violet” → “Yellow-green”. Open the shutter of each laser and highlight each dot plot in the Software by referring to its corresponding channel. Start adjusting the vertical knob of each laser to fit its designated pinhole followed by optimizing the “Laser Delay” to make sure the signal of each peak is located at the center of its corresponding bucket which shown on the oscilloscope (applies to all lasers except Blue laser).

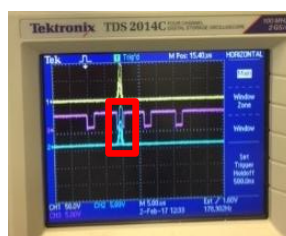
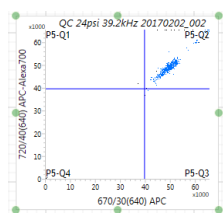
- Blue Laser: vertical knob → 1st pinhole
- Red Laser: vertical knob → 7th pinhole
- Violet Laser: vertical knob → 5th pinhole
- YG Laser: vertical knob → 3rd pinhole



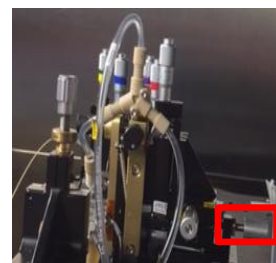
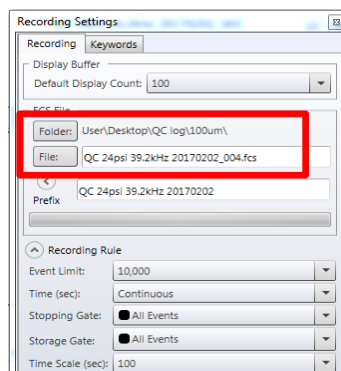
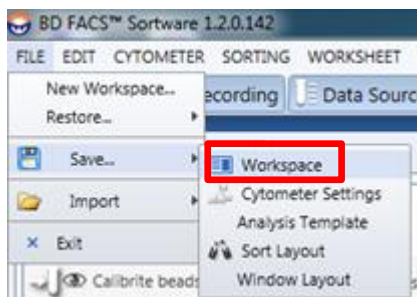


Imaging and Flow Cytometry Core

(15) Slightly adjust the horizontal knob to achieve highest signal strength and tightest CV. When all the alignments have been done, select QC Log folder to record FCS file → save workspace in FCF QC Log folder.



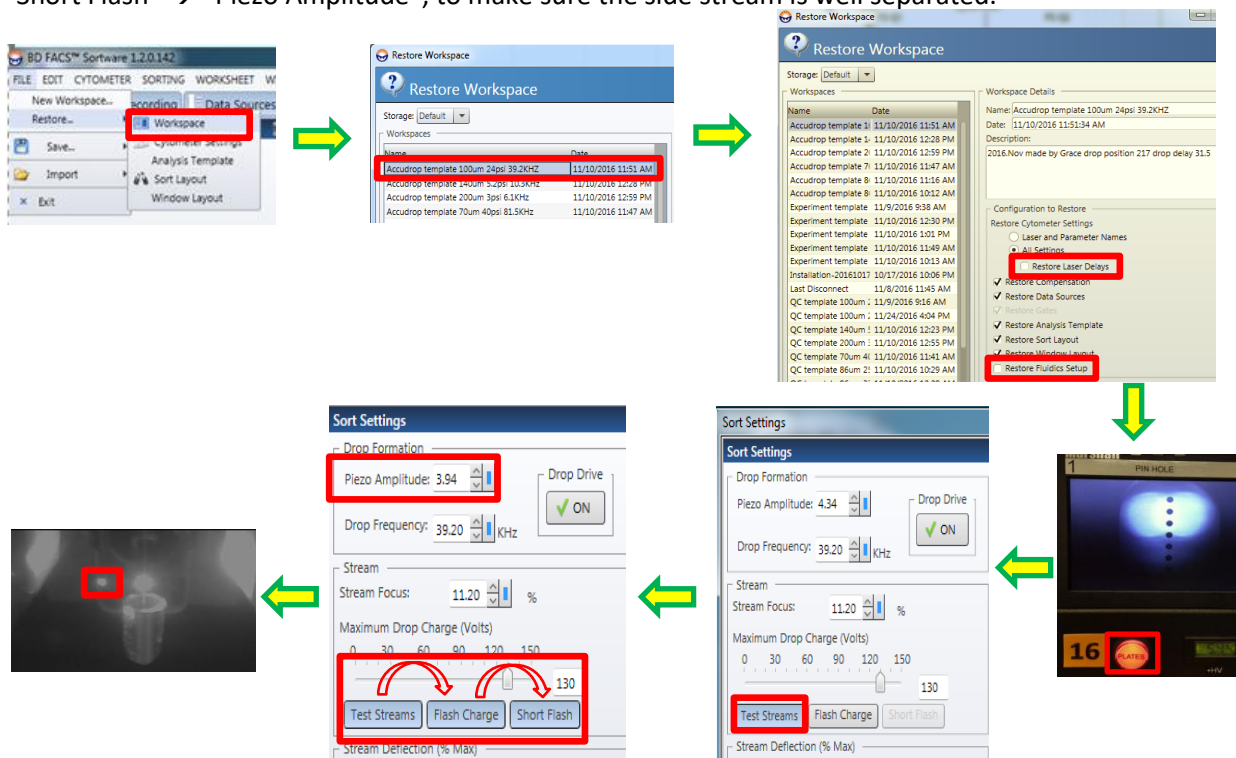
Detector	Wavelength	Log	Thru	Label
✓ PMT 1	15.50	✓	✓	PM1
✓ PMT 2	29.50	✓	✓	PM2
✓ PMT 3	39.50	✓	✓	PM3
✓ PMT 4	42.50	✓	✓	PM4
✓ PMT 5	42.50	✓	✓	PM5
✓ PMT 6	43.50	✓	✓	PM6
✓ PMT 7	51.49	✓	✓	PM7
✓ PMT 8	51.11	✓	✓	PM8
✓ PMT 9	48.82	✓	✓	PM9
✓ PMT 10	51.88	✓	✓	PM10
✓ PMT 11	38.50	✓	✓	PM11
✓ PMT 12	41.85	✓	✓	PM12
✓ PMT 13	35.50	✓	✓	PM13
✓ PMT 14	54.57	✓	✓	PM14
✓ PMT 15	48.48	✓	✓	PM15



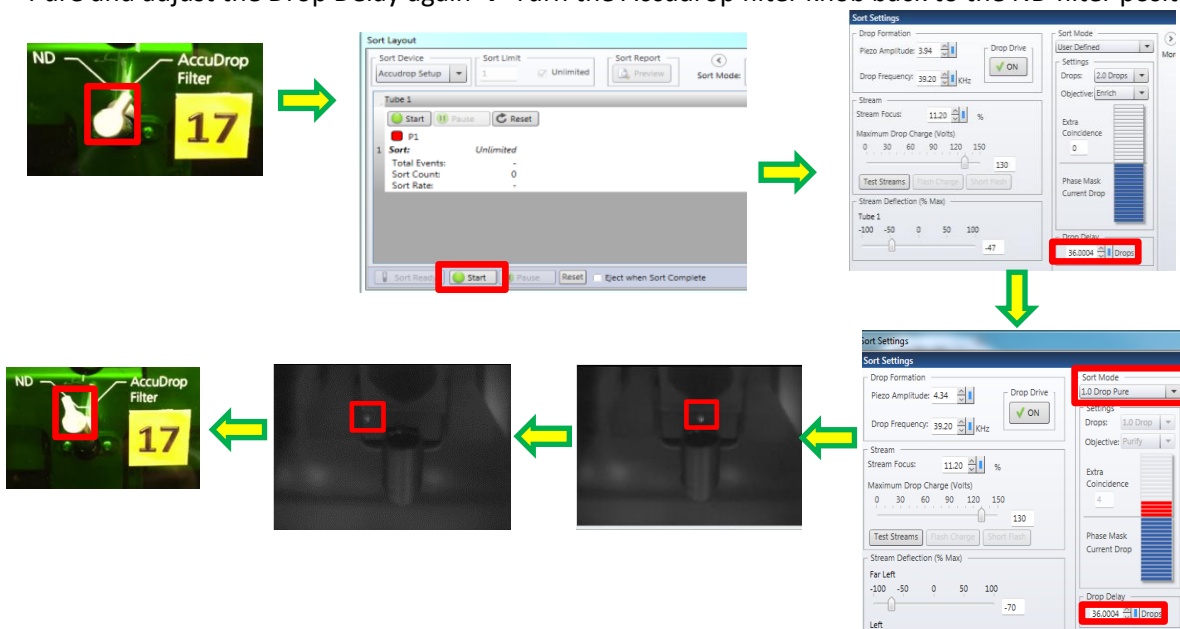
Imaging and Flow Cytometry Core

Accudrop Test:

- File → restores Accudrop workspace → uncheck “Laser Delay” and “Fluidics Set up” → (16) turn on the voltage of deflection plates → turn on “Test Stream” → “Piezo Amplitude” → “Flash Charge” → “Short Flash” → “Piezo Amplitude”, to make sure the side stream is well separated.

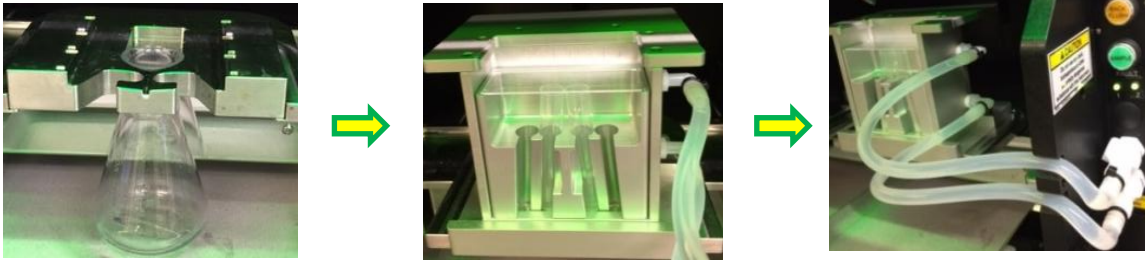


- Load Accudrop beads → (17) turn the filter knob to Accudrop filter → run accudrop beads → click “Start” in the sort layout → adjust the “drop delay” until the left stream is as bright as possible while nearly no fluorescent signals can be detected in the mainstream. Change the Sort Mode to 1.0 Drop Pure and adjust the Drop Delay again → Turn the Accudrop filter knob back to the ND filter position.

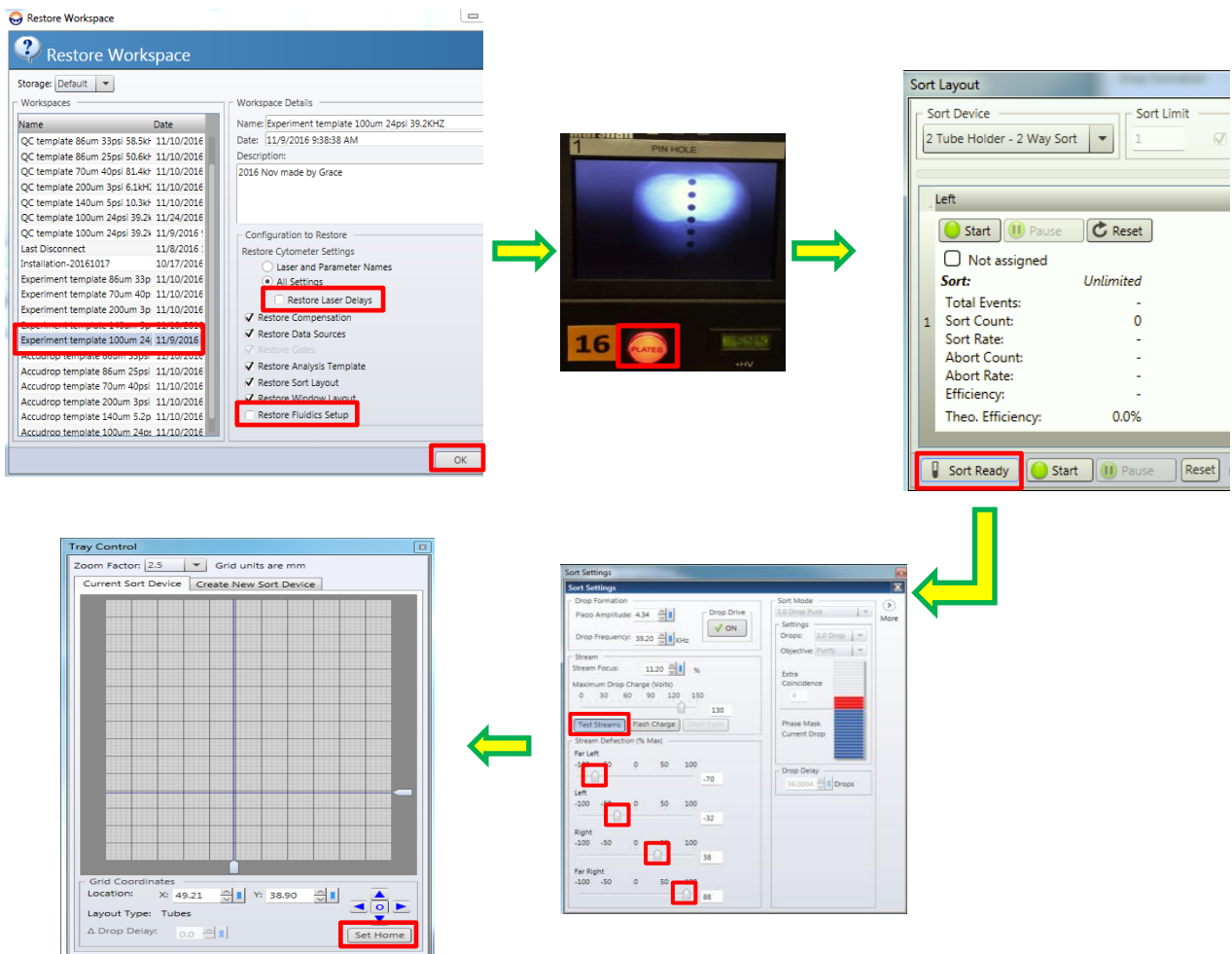


Imaging and Flow Cytometry Core

- Turn off the voltage of deflection plate → remove the cone-shaped flask → place the desired collection device and dummy tubes into the sort chamber → place a slide on top of the dummy tubes → connect the tubes to the ports if cold condition is needed



- Restore a workspace for “Experiment” in Default folder → charge deflection plates → double click “Test Stream” to adjust the position of the side streams → “Set Home”.



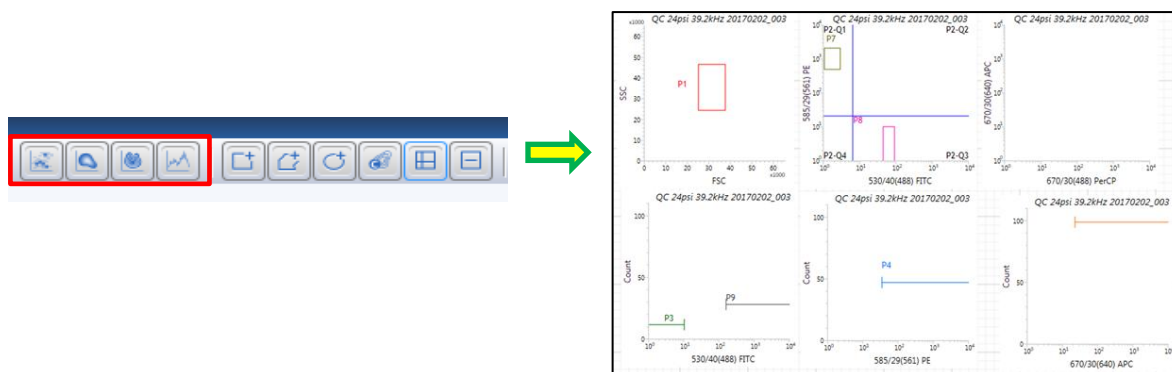


Imaging and Flow Cytometry Core

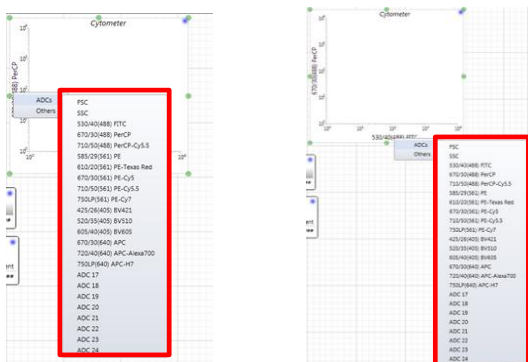
- Turn off the voltage of deflection plates → replace the dummy tubes with the collection tubes that contain complete medium. The cytometer is now ready for your experiment.

Experiment Set Up:

- You may add or remove the **plots** as you wish according to your experimental design.



- Right click X and Y axes and use **ADCs** to label the parameters accordingly.

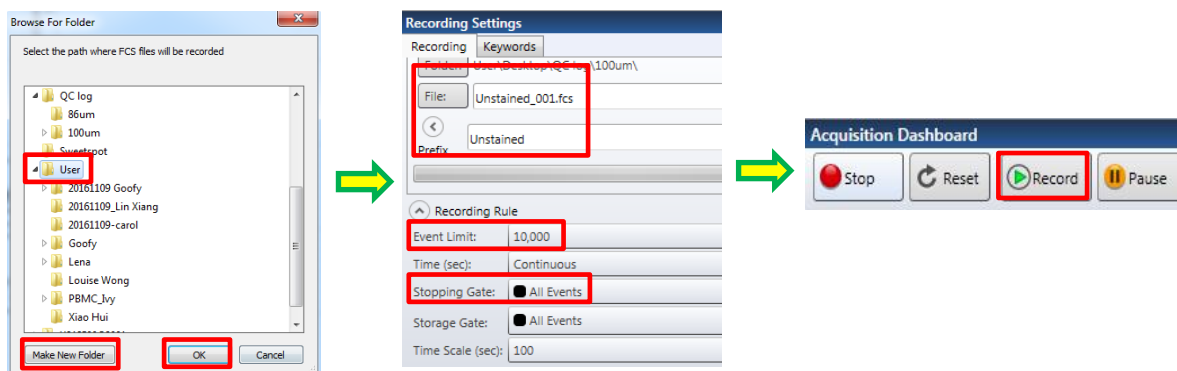


- Load sample, press “Sample” button beside the sample loading port and click “Acquire” in Software.



Imaging and Flow Cytometry Core

- To record FCS file, select “user” folder and create yourself a new folder if you do not have a folder yet. Label the tube name → set event limit and select stopping gate → click “Record”.

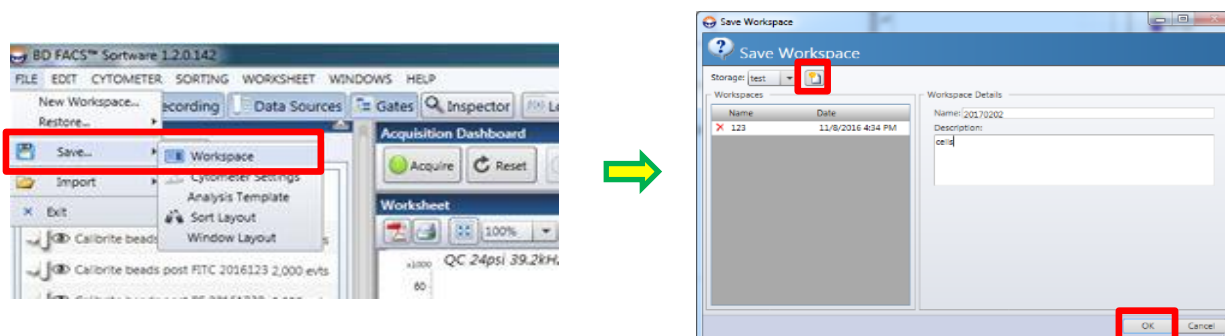


Cleaning and Data Saving:

- Run FACS Clean followed by FACS Rinse and DI water at sample pressure 27.5 psi for 5 minutes each. Leave the tube of water on the sample port.



- Save experiment workspace: File → Save → Workspace → Build a Folder for yourself → Fill in Workspace Details → OK.



- You may save PDF file by clicking “PDF symbol” for each sample and save it in the “user” folder on.

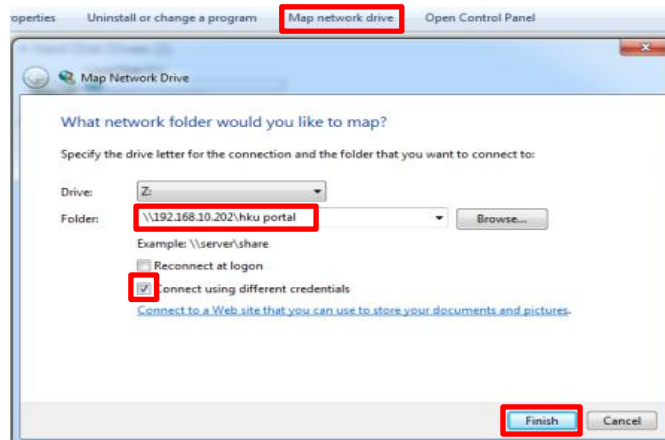


Imaging and Flow Cytometry Core

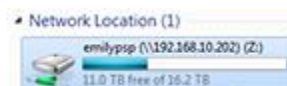


Data Exportation:

1. Double click "Computer" → "Map Network Drive" → Key in your portal ID → check "Connecting using different credentials" → "Finish"



2. a. Save both FCS and PDF files from the "user" folder on the desktop to your network drive.

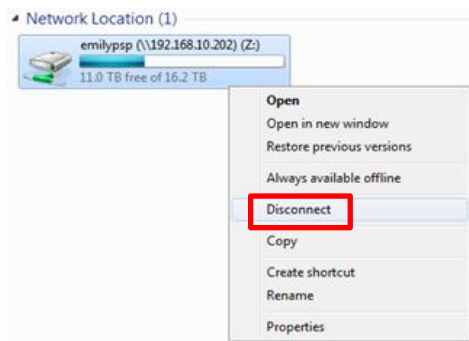


Imaging and Flow Cytometry Core

b. Double click the “Workspace- Shortcut” folder → save your experiment workspace to your network drive.

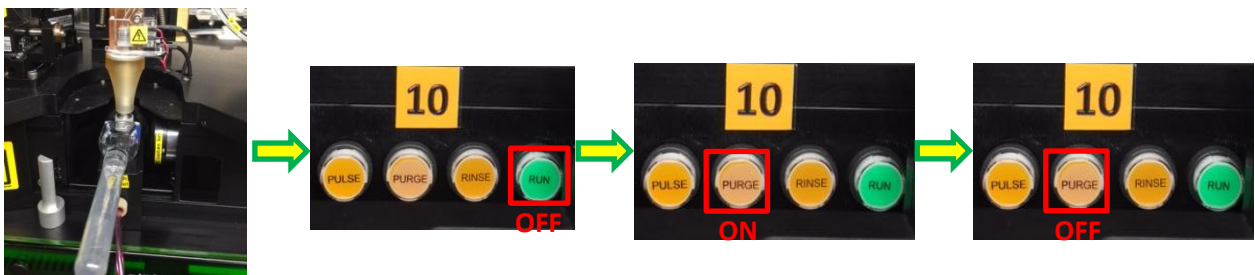


3. Disconnect the Network Drive from the computer.

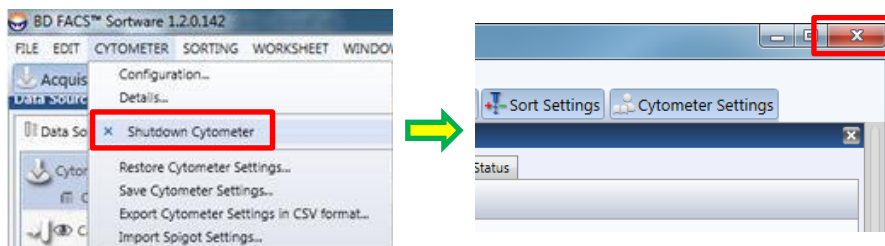


Machine Shutdown:

1. (10) Place the flush bucket and debubble reservoir under the nozzle → fill up debubble reservoir with DI water → press “Run” button to stop the stream → press “Purge” to fill up the flow cell with DI water → turn off “Purge”.



2. (9 & 8) Go to “Cytometer” → “cytometer shutdown” → Exit Software → Shutdown the computer.



Imaging and Flow Cytometry Core

3. Turn off the following items:

Air switch (7) → Lasers (6) → Air pump (5) → Chiller (4) → Air compressor (3) → Fluidics Power and ILLUM (2) → Main Power (1)

