FACS LSRFortessa Standard Operation Protocol
Basic Operation

1. Make sure the following actions have been taken before running your samples.

a. Make sure the waste tank is empty and refill with bleach if the fluid reaches RED line (Please refer to Page 14: Empty Waste Tank Procedure).

b. Make sure the sheath fluid container is fully refilled with sheath fluid (Please refer Page 15: Refill Sheath Container Procedure).

2. Launching the BD FACSDiva Software

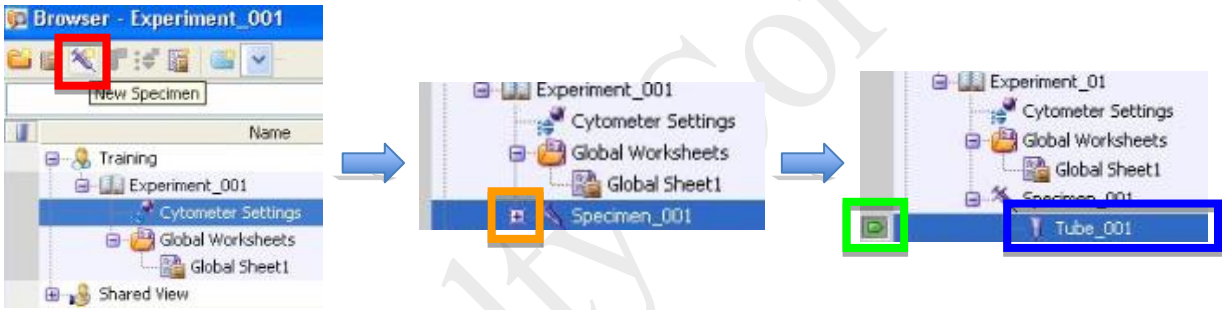
a. Log in FACSDiva software → key in login name and password.
Please contact the administrator to create a new user account.

b. Click **Use CST Settings** when pop-up message as below is observed.

3. Creating and working with experiment in BD FACSDiva Software

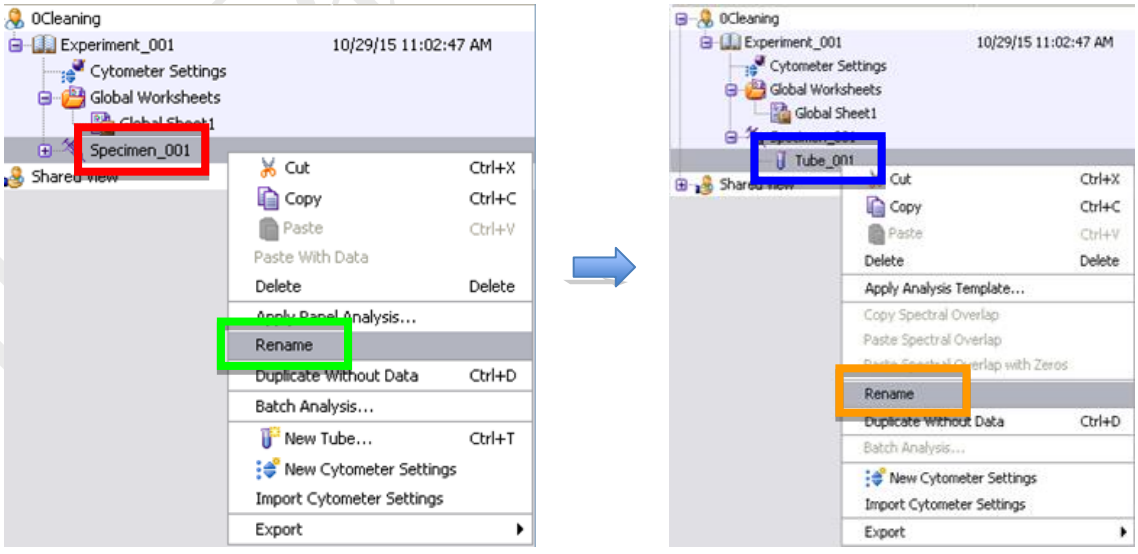
a. *Browser* toolbar → click **New Experiment** → Rename the experiment if necessary.

b. Select **New Specimen** → expands the **Specimen** to show **Tube 001**. Highlight the tube with the **Tube Pointer**.

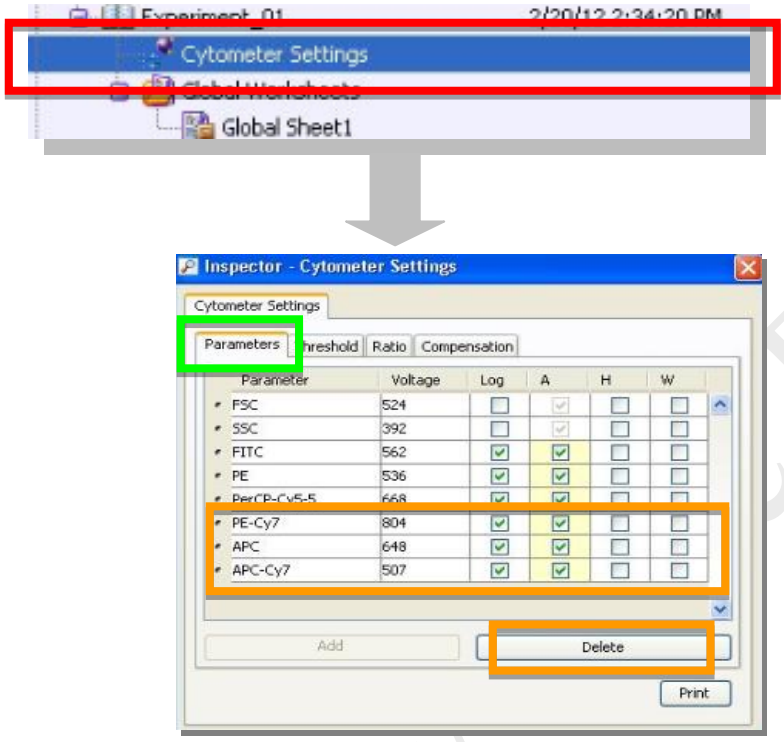


c. Right click on **Specimen_001** →**Rename** if necessary;

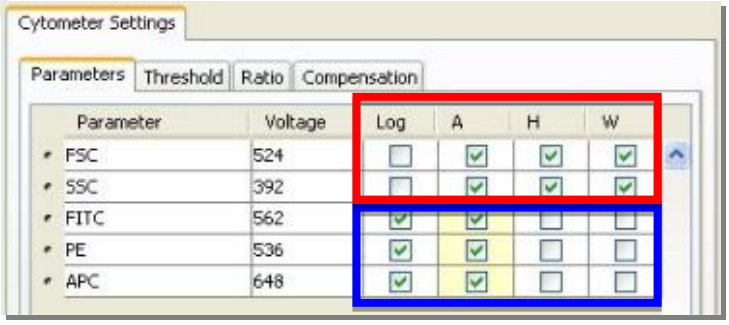
Right click on **Tube _001** → **Rename** if necessary.



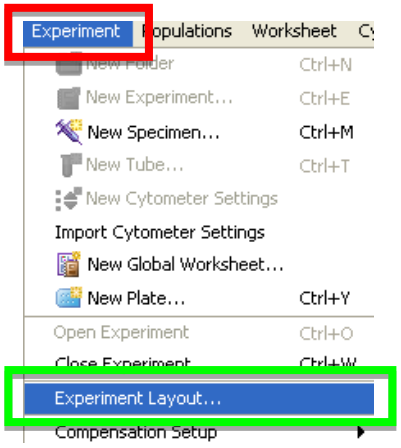
d. Select **Cytometer Settings** →**Parameters** →**Delete** unnecessary parameters on the *Inspector Window*.

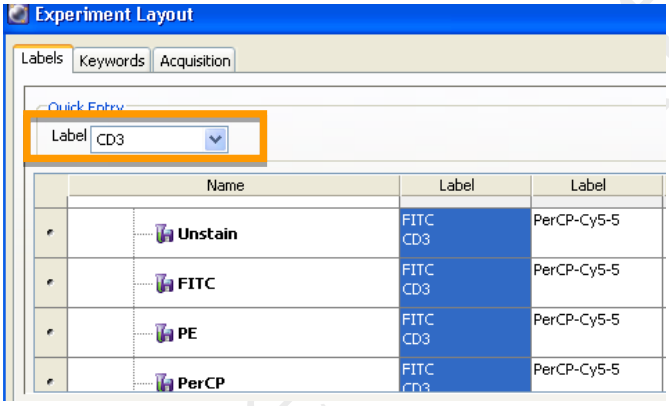


e. **FSC** (measure *Cell Size*) and **SSC** (measure *Cell Granularity*) are **MUST** for all kind of analysis and they should be kept in **linear scale.** Please check '**A**'rea, '**H**'eight and the '**W**'idth of **FSC** and **SSC**. **'Log' and 'A'rea boxes should be checked for fluorescence channels except for cell cycle and/or DNA analysis.**

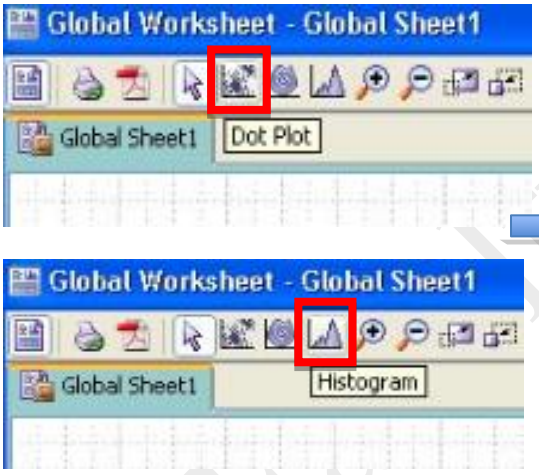


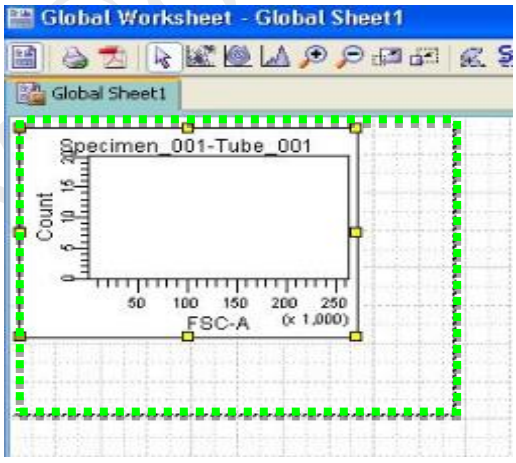
f. Choose **Experiment** → **Experiment Layout** and define labels for each parameter. Select the column of fluorescence channel and enter a label in the **Quick Entry Label field**.



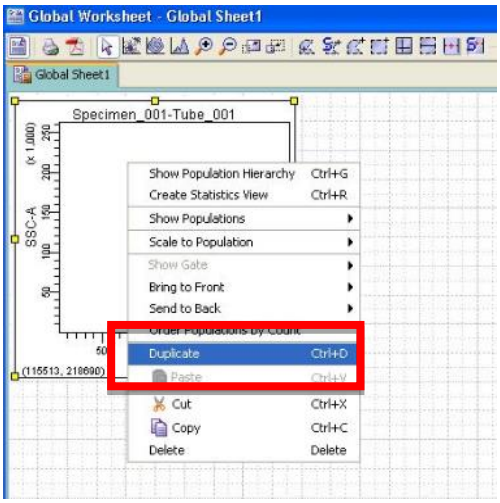


g. Select **Dot Plot** or **Histogram** → move the cursor onto the **blank worksheet**.

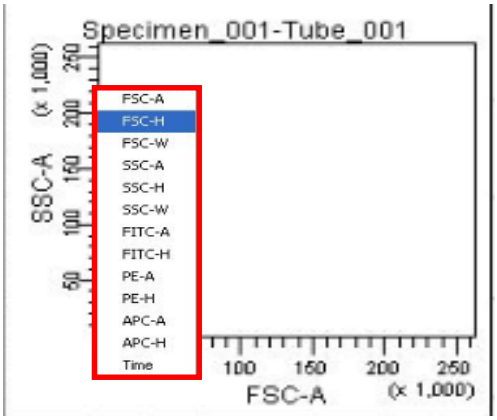




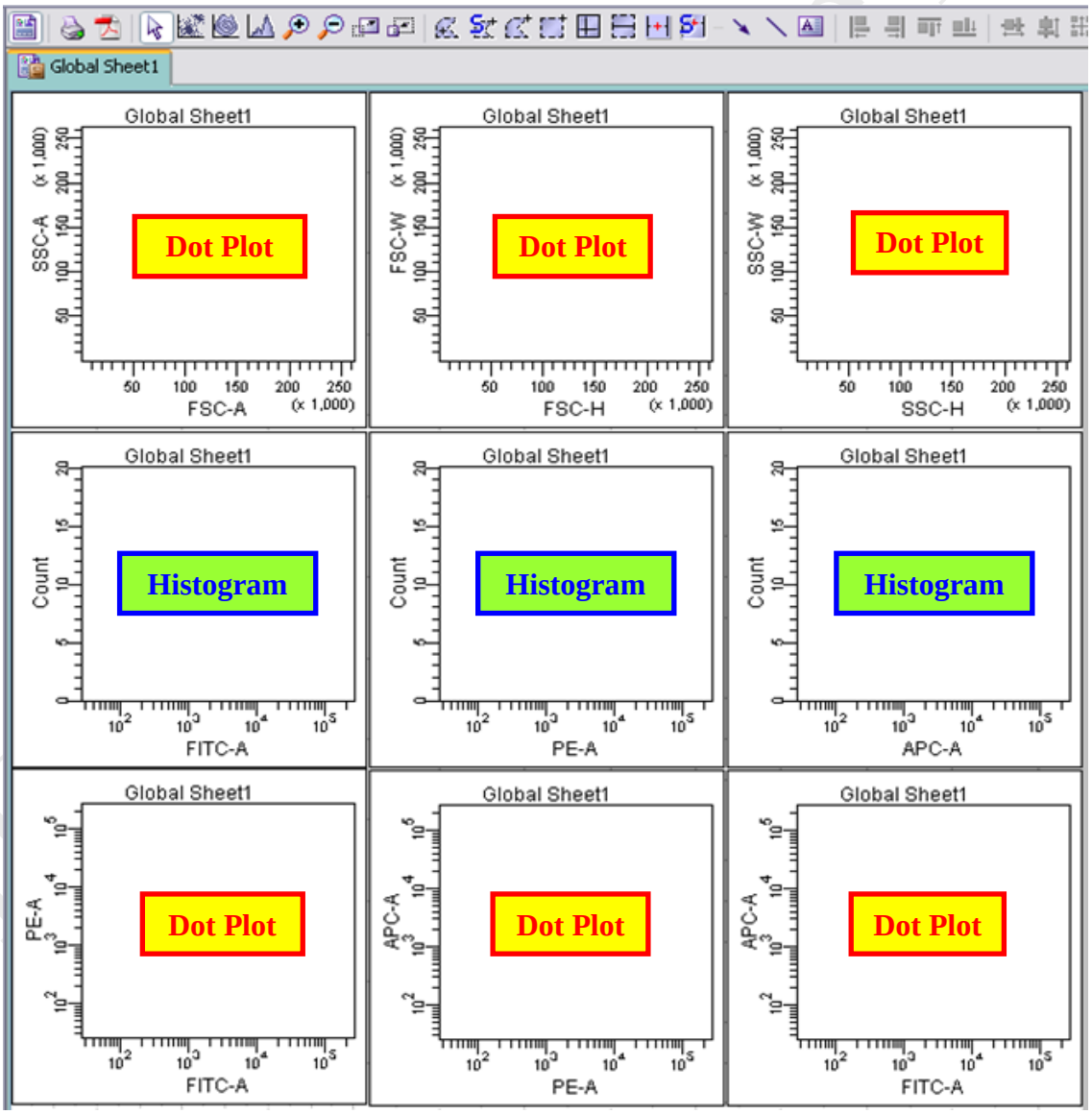
h. Right click on a plot → **Duplicate** to create another plot of the same type.



i. Select each individual **axis**, and opt from a list the preferred parameter.

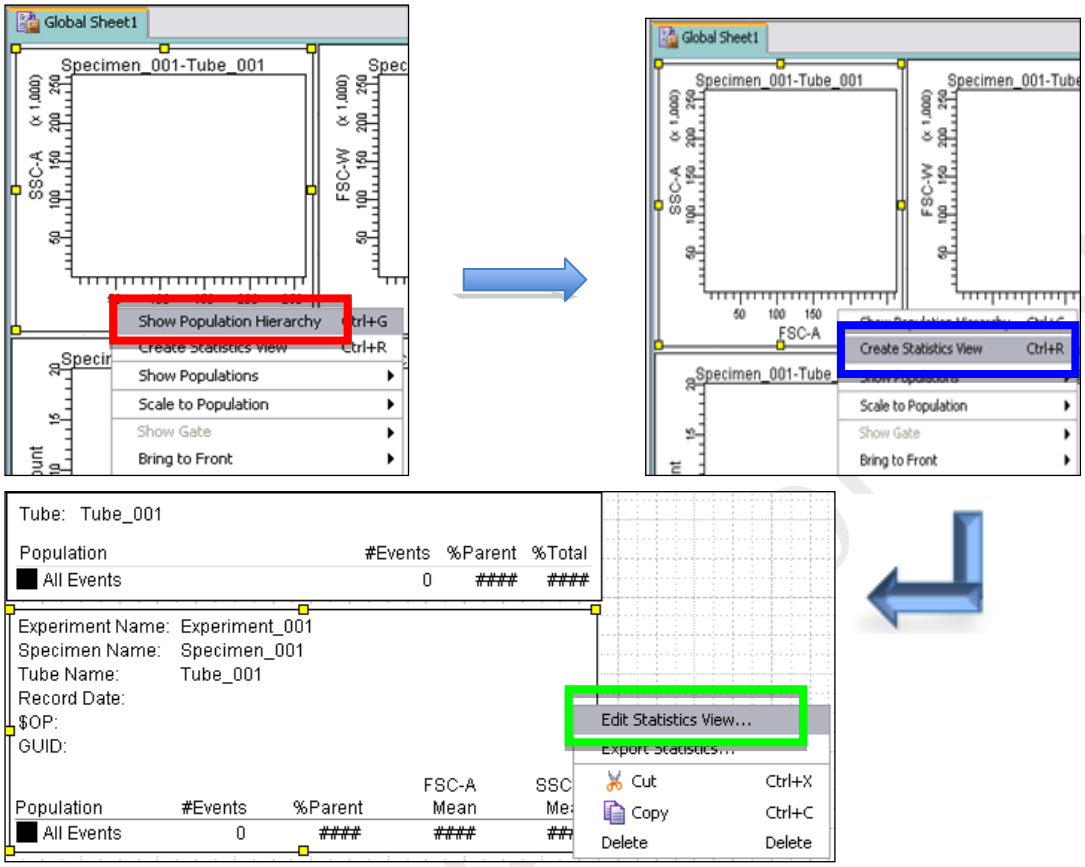


j. Below shows a template of plots used in routine analysis.

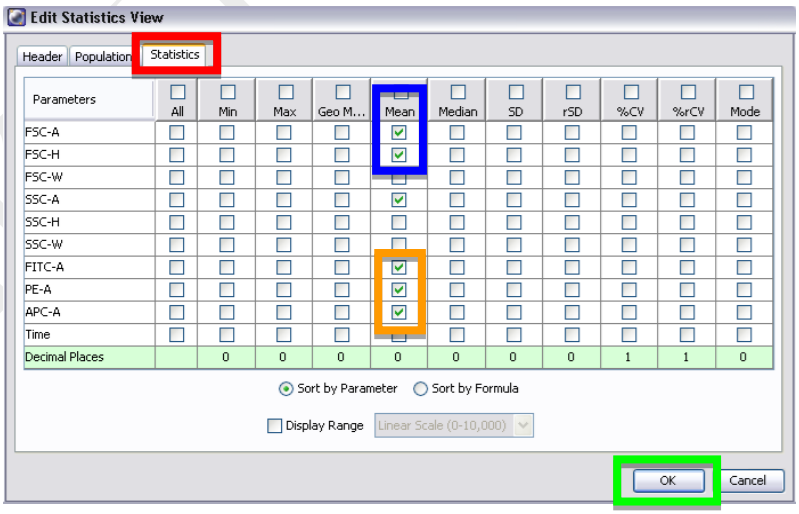


k. Right click on the plot → **Show Population Hierarchy**

Right click on the plot → **Create Statistics View** → right click on the statistics view → **Edit Statistics View**



l. Select ***Statistics*** tab → tick **mean** of **FSC-A** and **FSC-H** → tick the **mean** of the **'A'rea of the parameters** → click **OK**

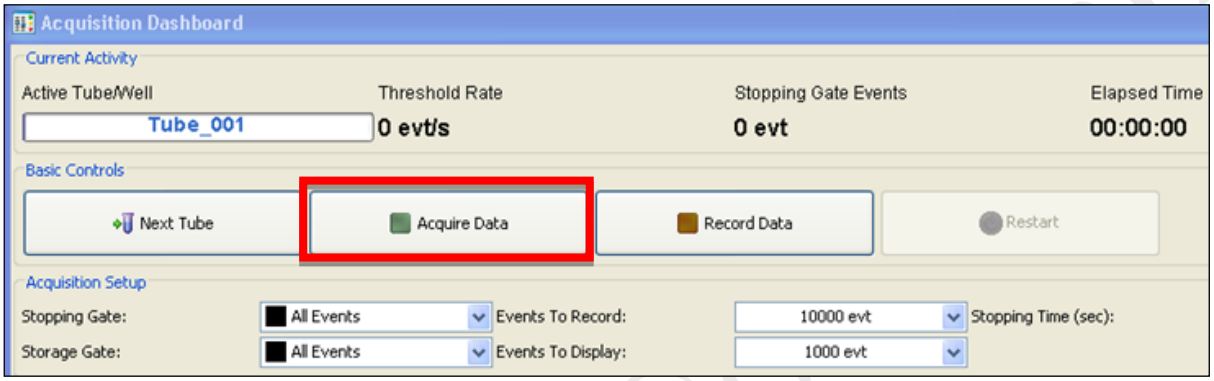


4. Procedures for sample acquisition

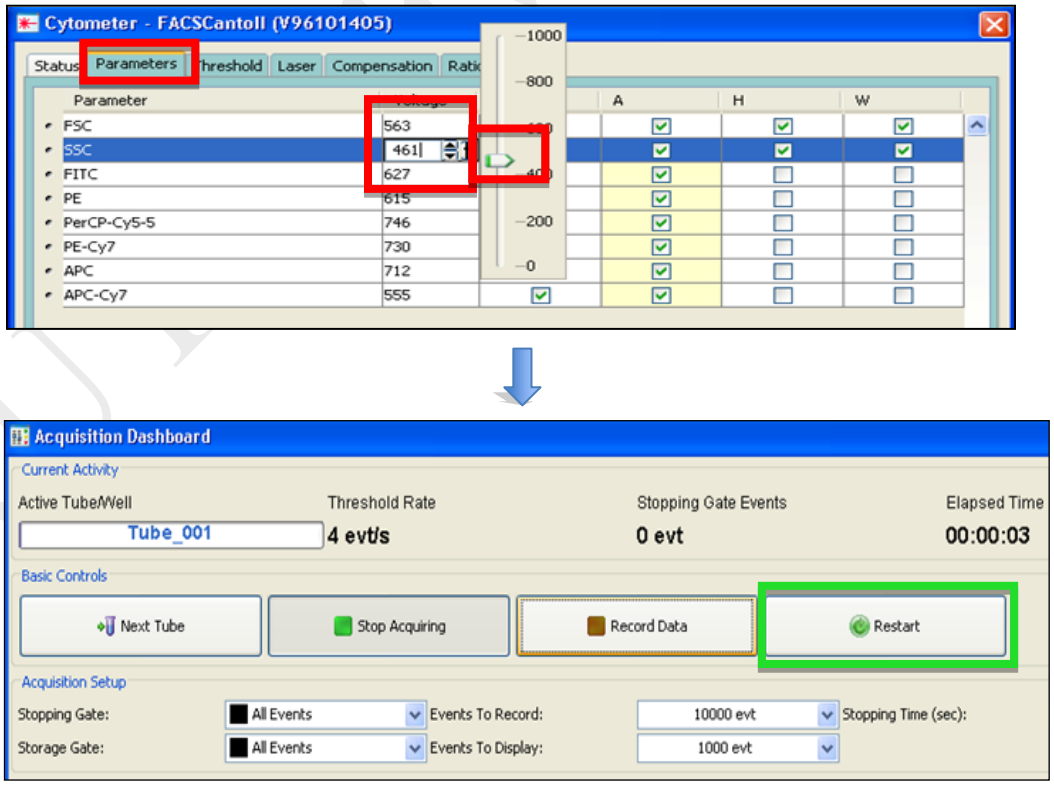
a. Press "**RUN**" and "**LO**" on fluid control panel.

b. **Gently tab the tube to mix your sample** → put your sample tube on SIP → Run the unstained/negative control sample before other sample tubes.

c. Acquisition Dashboard → **Acquire Data**

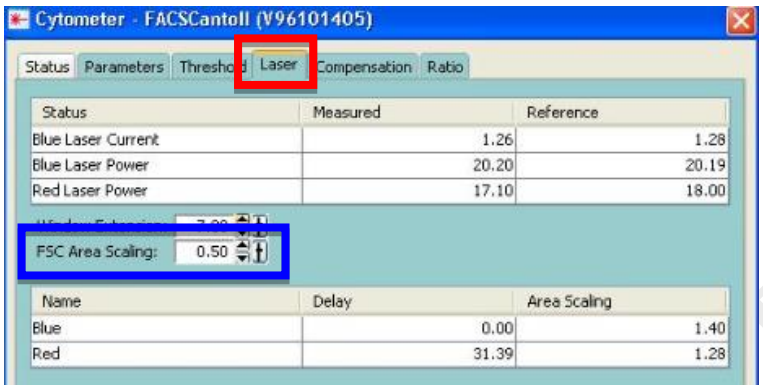


d. Identify the population of interest by adjusting the voltage of **FSC** and **SSC** on **Parameters** → Press **Restart** to accelerate the changes

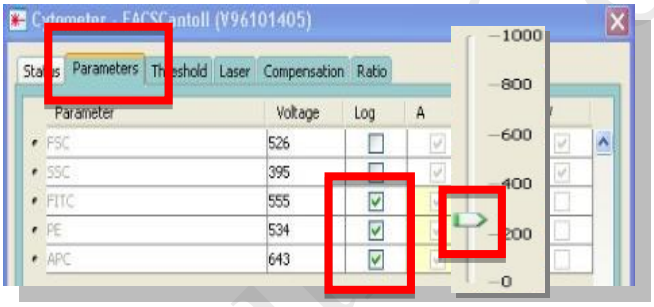


e. Cytometer → **Laser**→Adjust the **FSC Area Scaling** until the **mean** of **FSC-A** and **FSC-H** are APPROXIMATELY SAME.

Return to *Parameters* tab and finely re-adjust voltage of **FSC** and **SSC**.



f. Adjust voltage of each of the fluorescent channels; preferably the peak of population of interest is greater than **ZERO** but less than **10^2**.

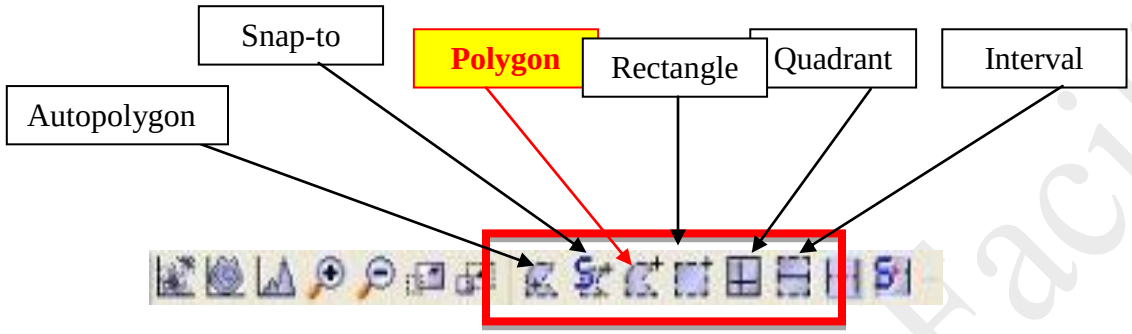


g. Click Stop Acquiring on Acquisition Dashboard and replace your sample with DI H2O.

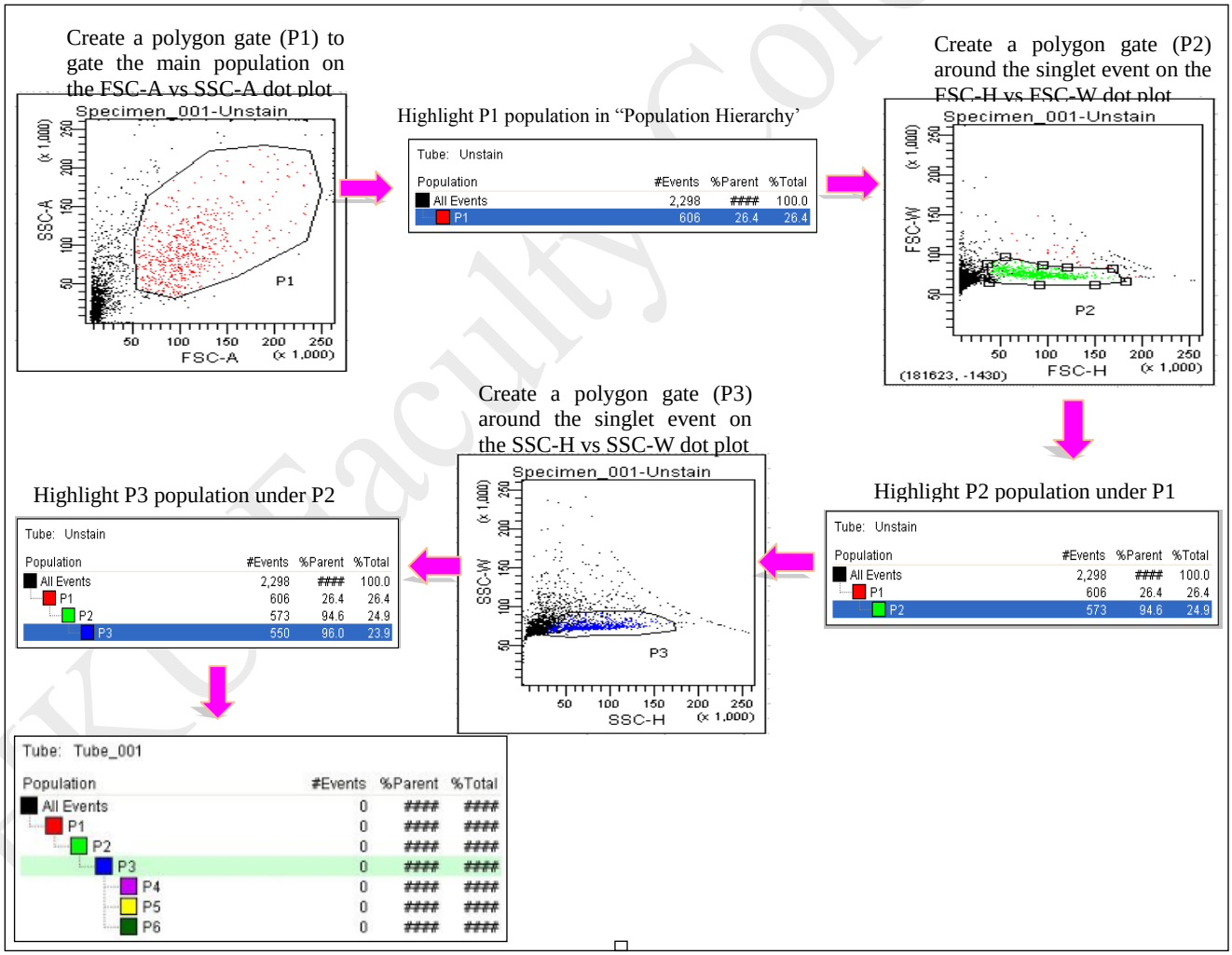
h. Repeat above steps with the positive control sample tubes. Adjust the voltage of corresponding channels if their signal peaks are outside the limit of the histograms.

5. Creating Gates

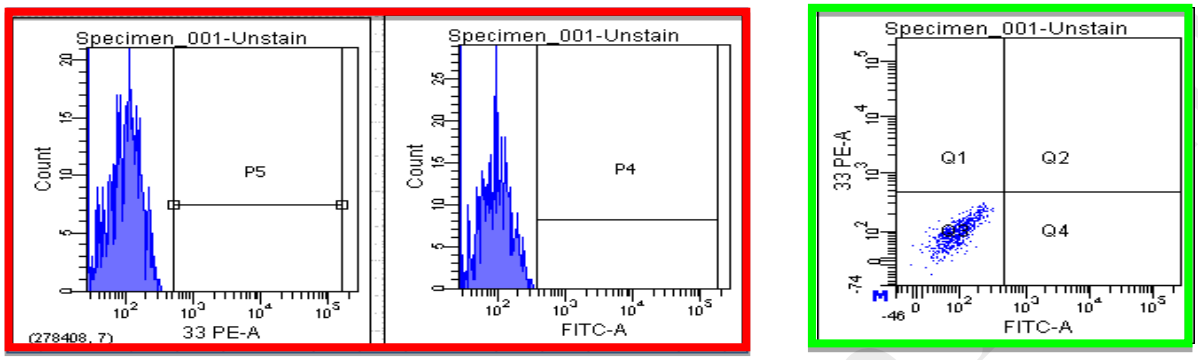
a. Set the current tube pointer to the following tube and use **Polygon Gate** to gate the targeted cells.



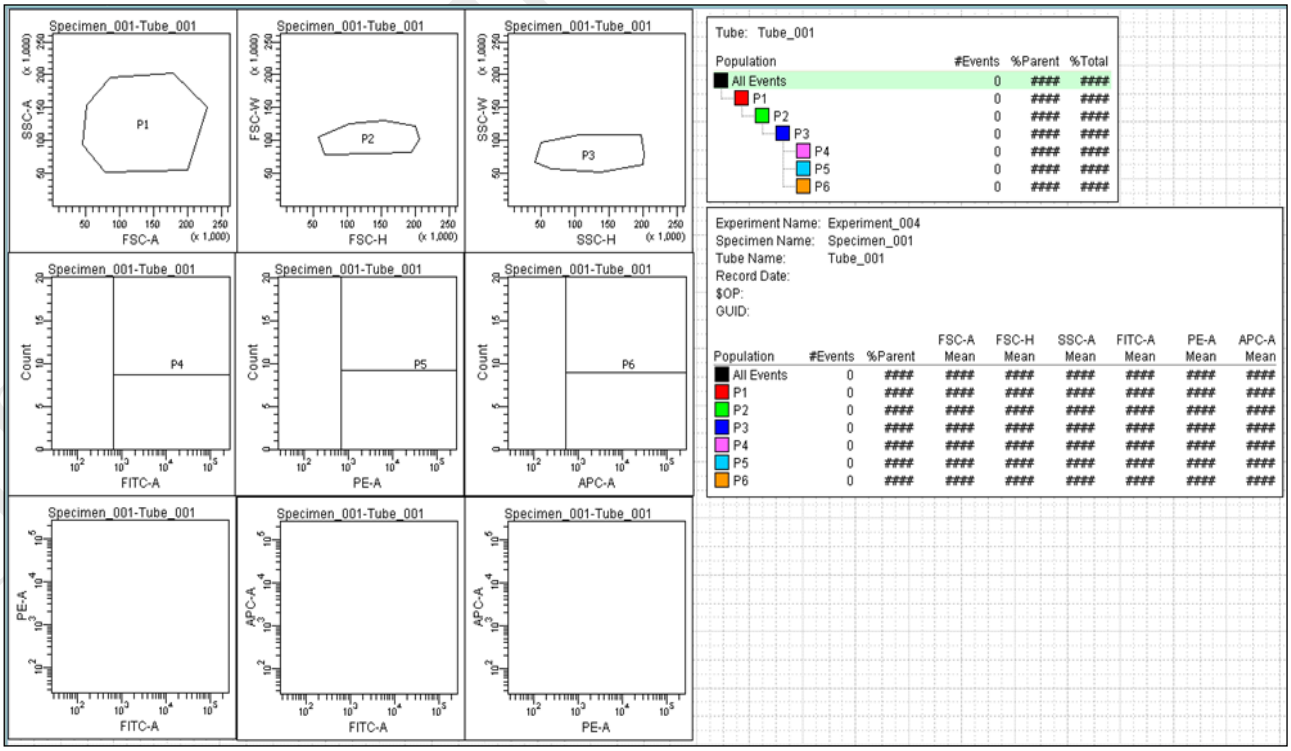
b. Gate the cells of interest according to the following sequence by using:



c. To define fluorescence positive signal, **interval gate (P4, P5,…)** beyond negative peak of fluorescent channels in histogram plot of unstained samples can be created. For over multiple fluorescence channels, **quadrant gate** could be created to define single/double positive signals **(Q1; Q2; Q3; Q4….)**.



* Note that *P1* is the children of *All Events* and the parent of *P2* population; *P2* population is the children of *P1* population and the parent of *P3* population and the grandparent of *P4*, *P5*, and *P6* populations. Thus, on the hierarchy table, users should highlight the *P1* population when a gate for *P2* population is drawn, the *P2* population when a gate for *P3* population is drawn, and the *P3* population when gates for *P4*, *P5*, and *P6* populations are drawn.



6. Recording data for all samples

a. **Gently tab the tube to mix your sample**, then put your sample tube on SIP (sample injection position); Run the unstained sample before other sample tubes.

b. Press "**RUN**" and "**LO**" on fluid control panel.

c. Acquisition Dashboard →**Acquire data** → **Record data**

d. Please pay attention to the sample tube to make sure it will not run dry!

e. Click "Stop Acquiring" to stop acquire the data if necessary then unload your sample.

f. Repeat above steps for each sample.

7. Machine Cleaning

a. **Cleaning procedure between each user is required.**

a. Prepare **3** mL of each cleaning solution (FACS Clean, FACS Rinse, Milli-Q H2O).

b. Press "**RUN**" and "**HIGH**" on fluid control panel.

c. Install a tube of FACS Clean solution on the SIP with the support arm to the side (vacuum on) and let it run for **1** minute.

d. Move the tube support arm under the tube (vacuum off) and allow the cleaning solution to run for **5** minutes with the sample flow rate set to HI.

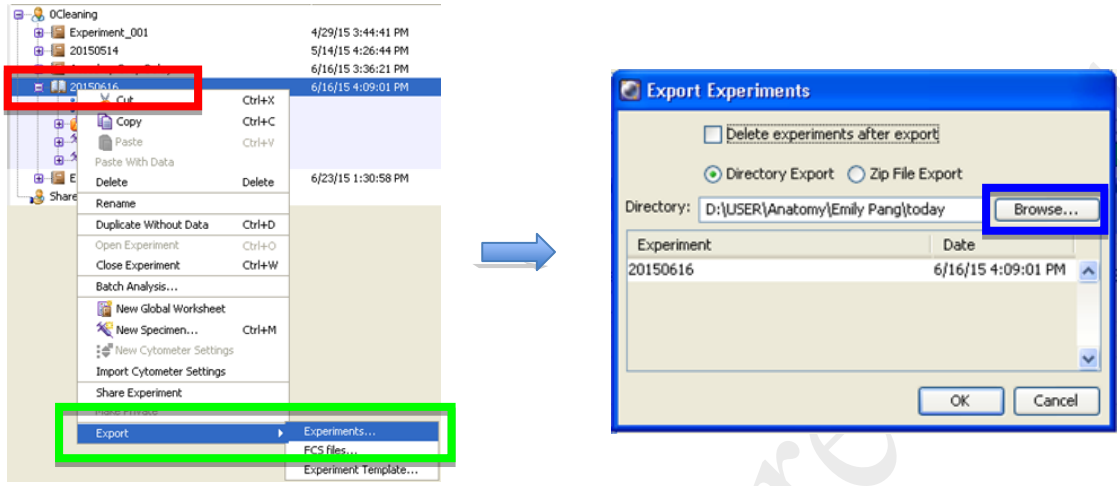
e. Repeat steps 2 and 3 with BD™ FACSRinse solution.

f. Repeat steps 2 and 3 with Milli-Q water.

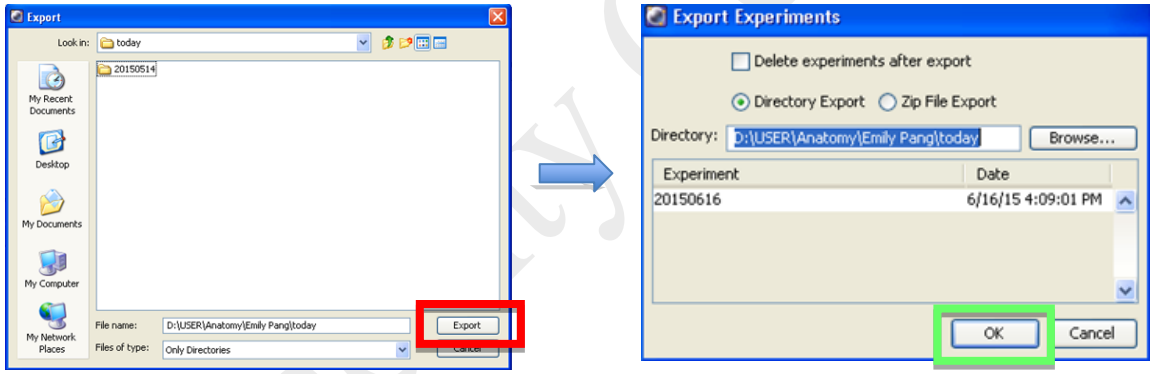
g. Make sure the fluidics control panel shows **LO** and **STANBY** when the entire process of cleaning is complete.

8. Export FCS Data / Experiment

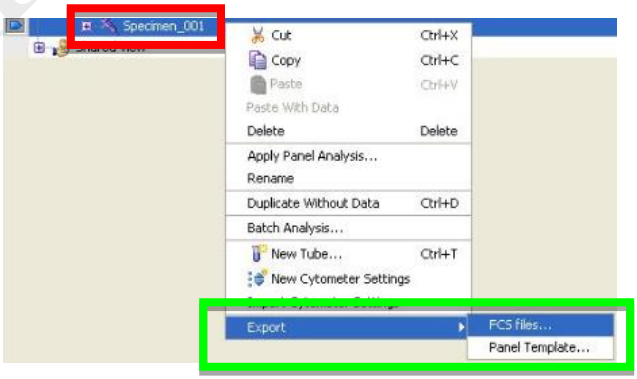
1. To save Experiment, right click on the **Experiment** → **Export** → **Experiment** → **Browse** to choose the destination folder.



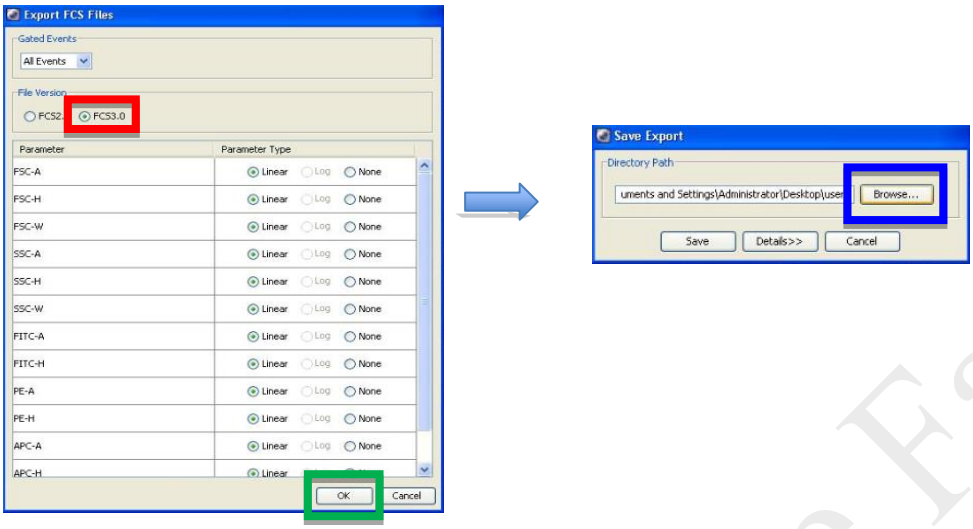
2. Create a new folder and rename, then click **Export** → **OK**



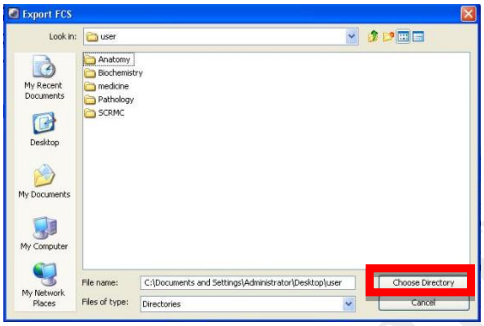
3. To save FCS files, right click → **Specimen** → **Export** → **FCS Files**



4. Select **FCS 3.0** → **OK** → **Browse** to choose folder destination.



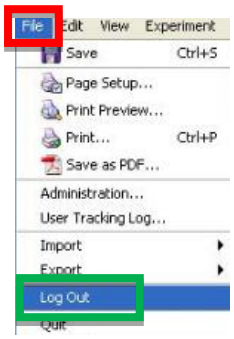
5. Create a new folder and rename → **Choose Directory** and save the file.



6. Delete experiment after having FCS data / experiment exported.

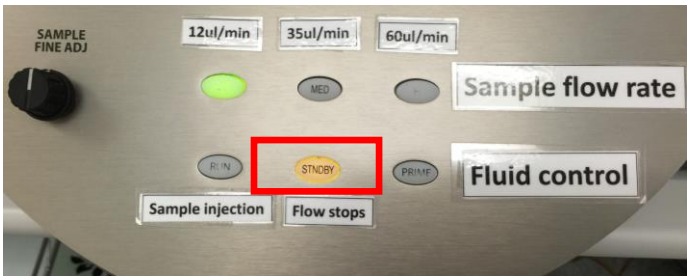
9. Log Out

1. To log out of FACSDiva software, go to **File** → **Log Out**

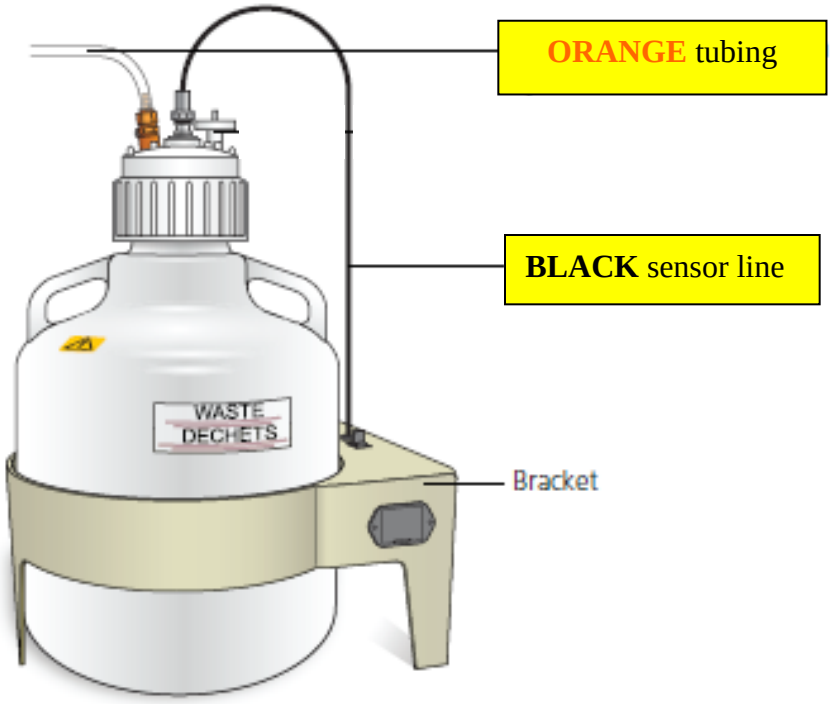


Empty Waste Tank Procedures

1. Make sure the flow cytometer is in **Standby** mode.



2. Diva Software → "Cytometer" → "Standby".
3. Disconnect the **ORANGE** tubing and **BLACK** sensor line from the waste container.

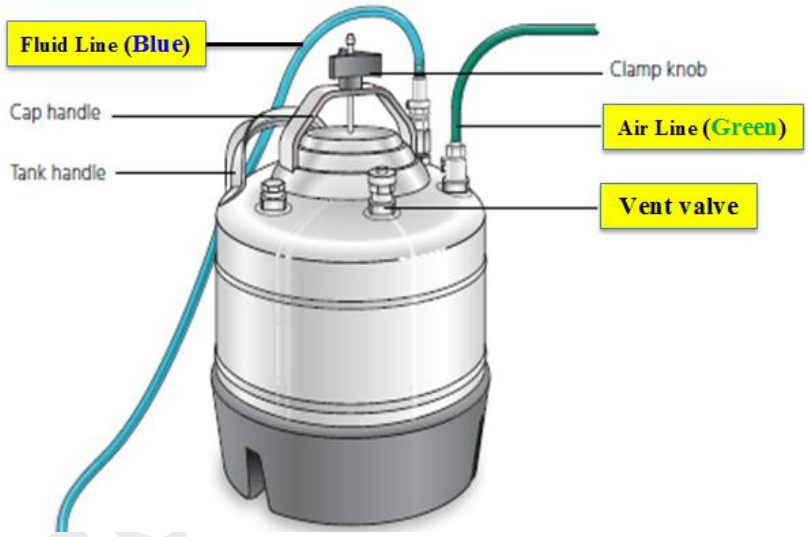


4. Take waste tank to the sink → Remove the lid → Empty the waste tank.
5. Add about 0.5L of bleach into the waste tank → Place the lid back to the waste tank.
6. Reconnect the **ORANGE** tubing and **BLACK** sensor line to the waste tank.

Refill Sheath Container Procedures

1. Make sure the flow cytometer is in **Standby** mode.

2. Disconnect Diva Software: "Cytometer" → "Standby".
3. Disconnect the Air Line **(Green)** and Fluid Line **(Blue)** from the sheath tank.



4. Depressurize the sheath tank by pulling up the **vent valve**.
5. Unscrew the clamp knob and sheath tank → push down to loosen the lid → Tilt the lid to the side to remove it from the tank.
6. Refill the sheath fluid using an air pump until the maximum level.
7. Tilt the lid and close the sheath tank by tightening the clamp knob to finger-tight.
8. Reconnect the Air Line **(Green)** and Fluid Line **(Blue)**.
9. Release the bubbles that trap in the filter if necessary.
10. Reconnect Diva Software: "Cytometer" → "Connect".