LSRFortessa Operation Protocol

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

- ❖ Make sure the waste tank is empty and refilled with 500 ml clean (see page 16 for details)
- ❖ Make sure the FACS Flow container is fully filled (see page 16 for details)

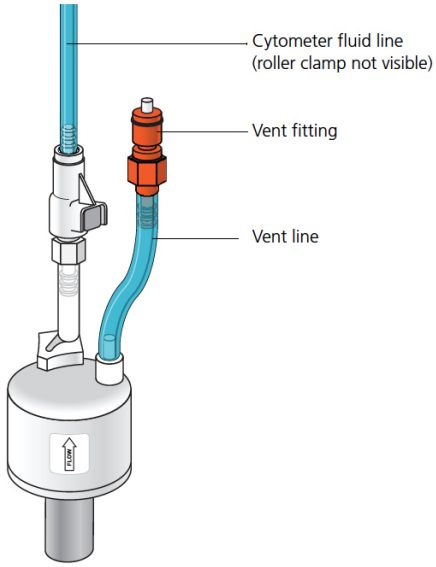
1. **turn on the computer**
   - ❖ Login into Windows using User Name **Admin** and password: **BDIS#1**.
2. **Turn on the FACSflow supply system**
3. **Turn on the main power switch of the cytometer** (Green button right side)

❖ **Please make sure that the green lights on both - instrument BD LSR-Fortessa and FACS Flow system are actually "ON".**

Wait 30 minutes for the lasers to warm them up before run your samples

❖ **Check Fluidics:**

- ➢ **Check if the BD LSR Fortessa fluidic system is functioning properly:**
  - Remove the tube with MQ water from the sip (Sample Injection Port);
  - Push "RUN" and "High" fluidic control buttons;
  - Check if the buffer starts dripping from the sip:
    - If "yes" - please proceed to the step IIb;
    - If "not" - please push the "Standby" button immediately and report the problem to FCF Staff (see the notes on the end of the document).
- ➢ **Check if the trap filter** attached to the pressurized plastic tank **is free of air bubbles:**
  - If bubbles are visible, gently tap the filter body with your fingers to dislodge the bubbles and force them to the top.
  - Note: When removing air bubbles, do not vigorously shake, bend, or rattle the sheath filter - you might damage it.
  - Direct the vent line into a beaker and press the small button at the end of the vent fitting against the side of the beaker until a steady stream of fluid empties from the filter.
  - Tilt the filter and verify that no trapped air remains in the filter.
  - Repeat steps 3 and 4 until no air is observed in the filter.

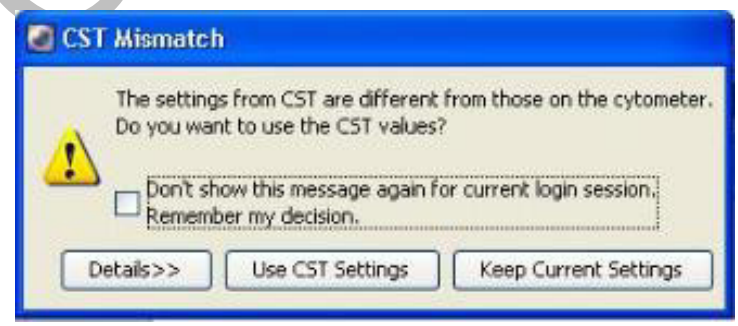


- **Check the sheath line for air bubbles.**
  - Open the roller clamp at the fluidics interconnect (if necessary) to bleed off any air in the line. Collect any excess fluid in a waste container.
  - Close the roller clamp.

4. **Launching the BD FACS DIVA Software**

❖ Log in FACS DIVA software with your personal login name and password.

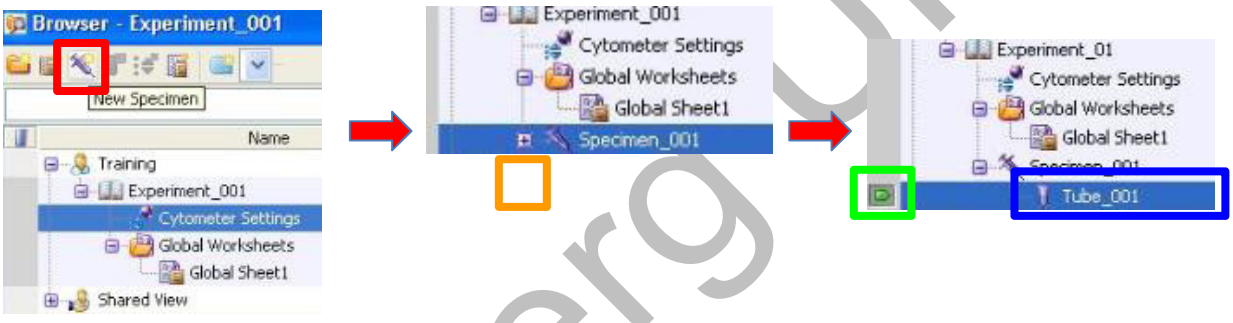
❖ **Always** click **"Use CST Settings"** when pop-up message as below appears.



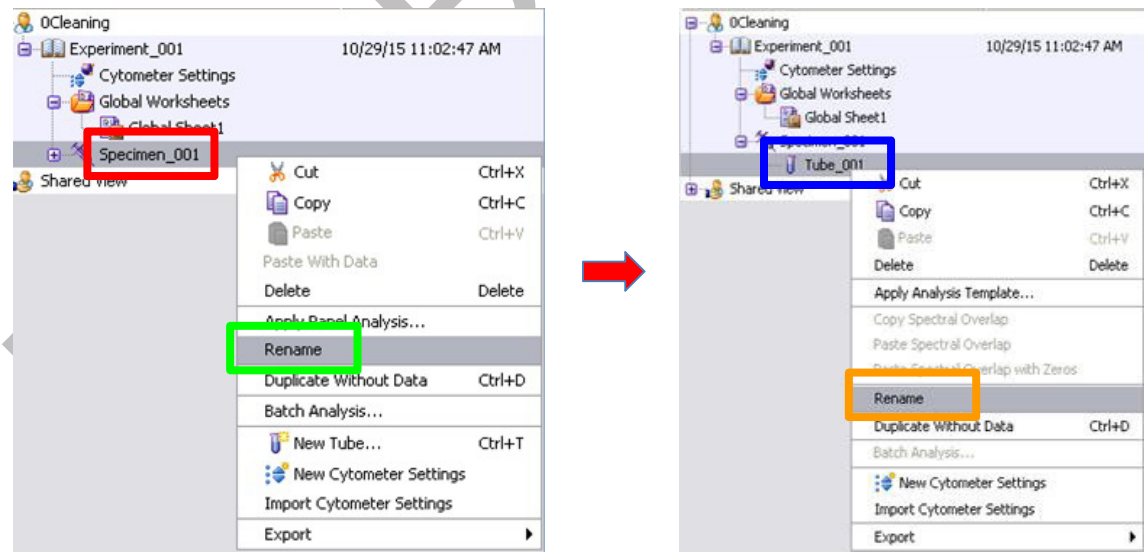
5. Creating and working with experiments in BD FACS DIVA Software

❖ *Browser* toolbar □ click **New Experiment** & rename the experiment if necessary. or import an old one and duplicate without data.

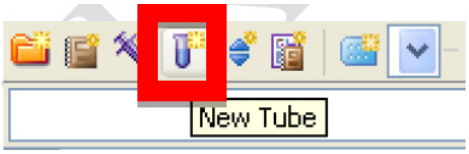
❖ Select **New Specimen** expands the **Specimen** to show **Tube 001**. Highlight the tube with the **Tube Pointer**.



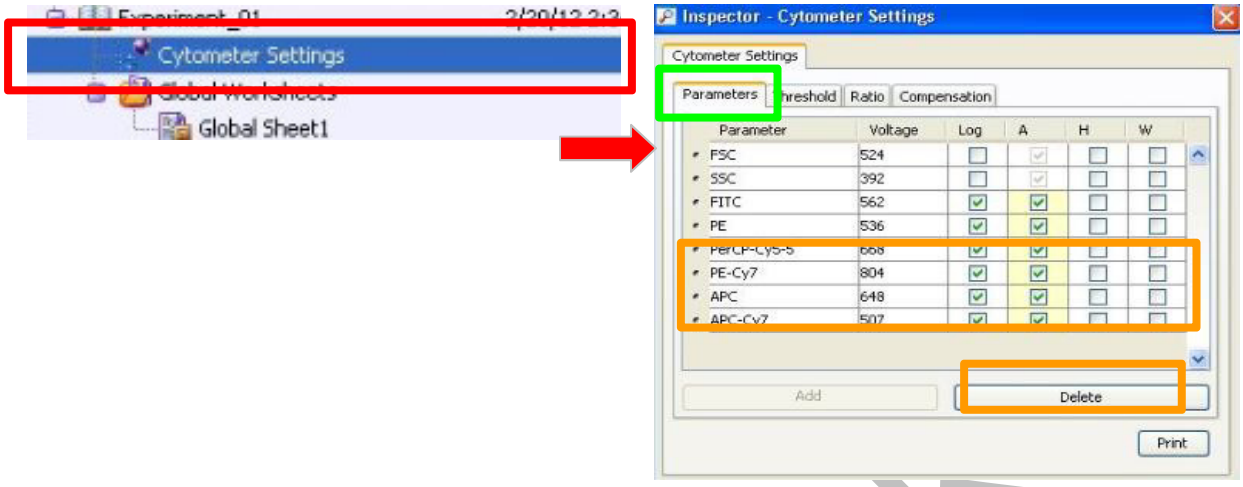
❖ Right click on **Specimen_001** > **Rename** if necessary; Right click on **Tube _001** > **Rename** if necessary.



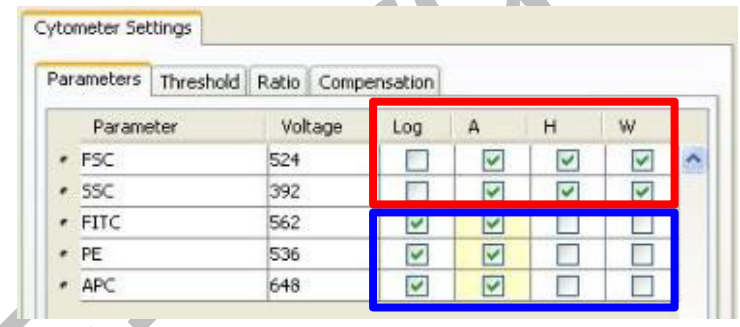
❖ Click the **New Tube** bottom to create new tubes. Rename them if necessary.



❖ Select **Cytometer Settings** > **Parameters** > **Delete** unnecessary parameters on the *Inspector Window.*

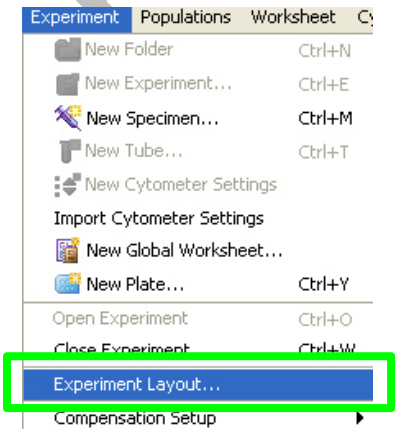


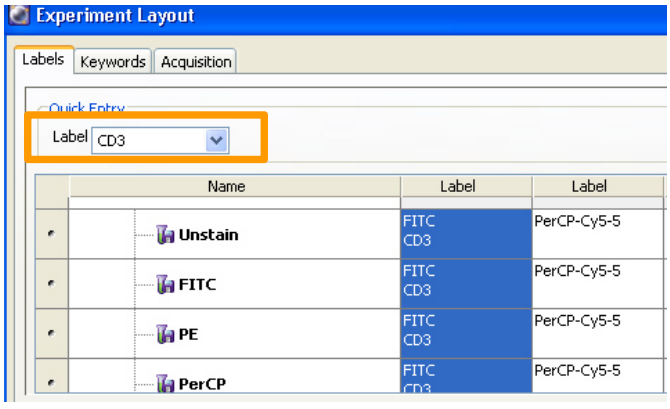
❖ **FSC** (measure *Cell Size*) and **SSC** (measure *Cell Granularity*) are a **MUST** for all kind of analysis and they should be kept in **linear scale.** Please check '**A**'rea, '**H**'eight and the '**W**'idth of **FSC** and **SSC**. **'Log' and 'A'rea boxes should be checked for fluorescence channels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



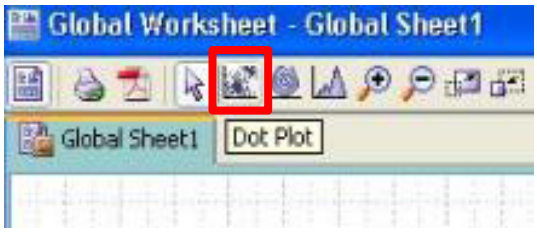
❖ Choose **Experiment** > **Experiment Layout** and define labels for each parameter.

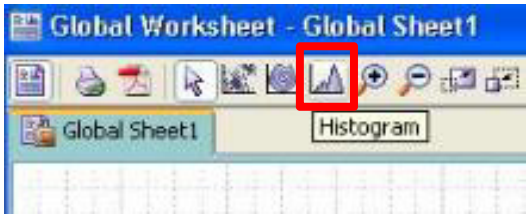
- Select the column of fluorescence channel and enter a label in the **Quick Entry**
- **Label field**

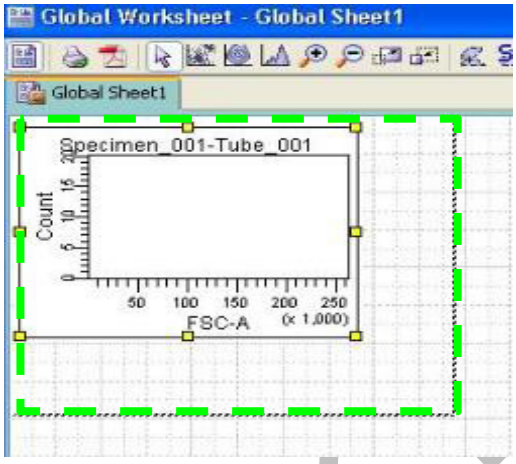




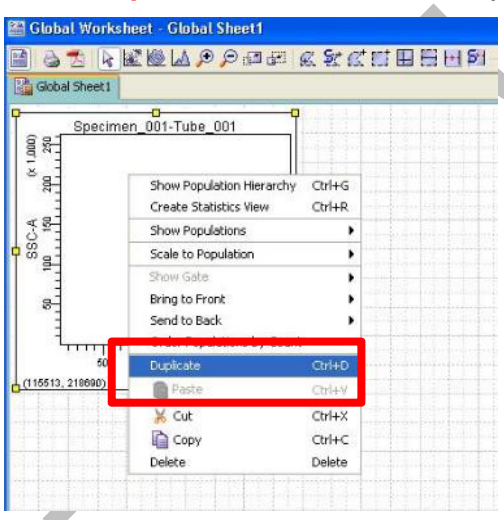
❖ Select **Dot Plot** or **Histogram** move the cursor onto the **blank worksheet**.



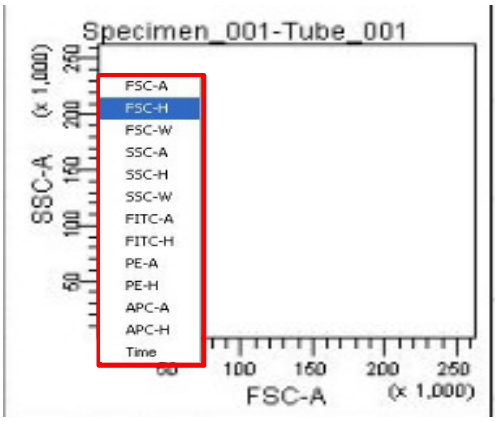




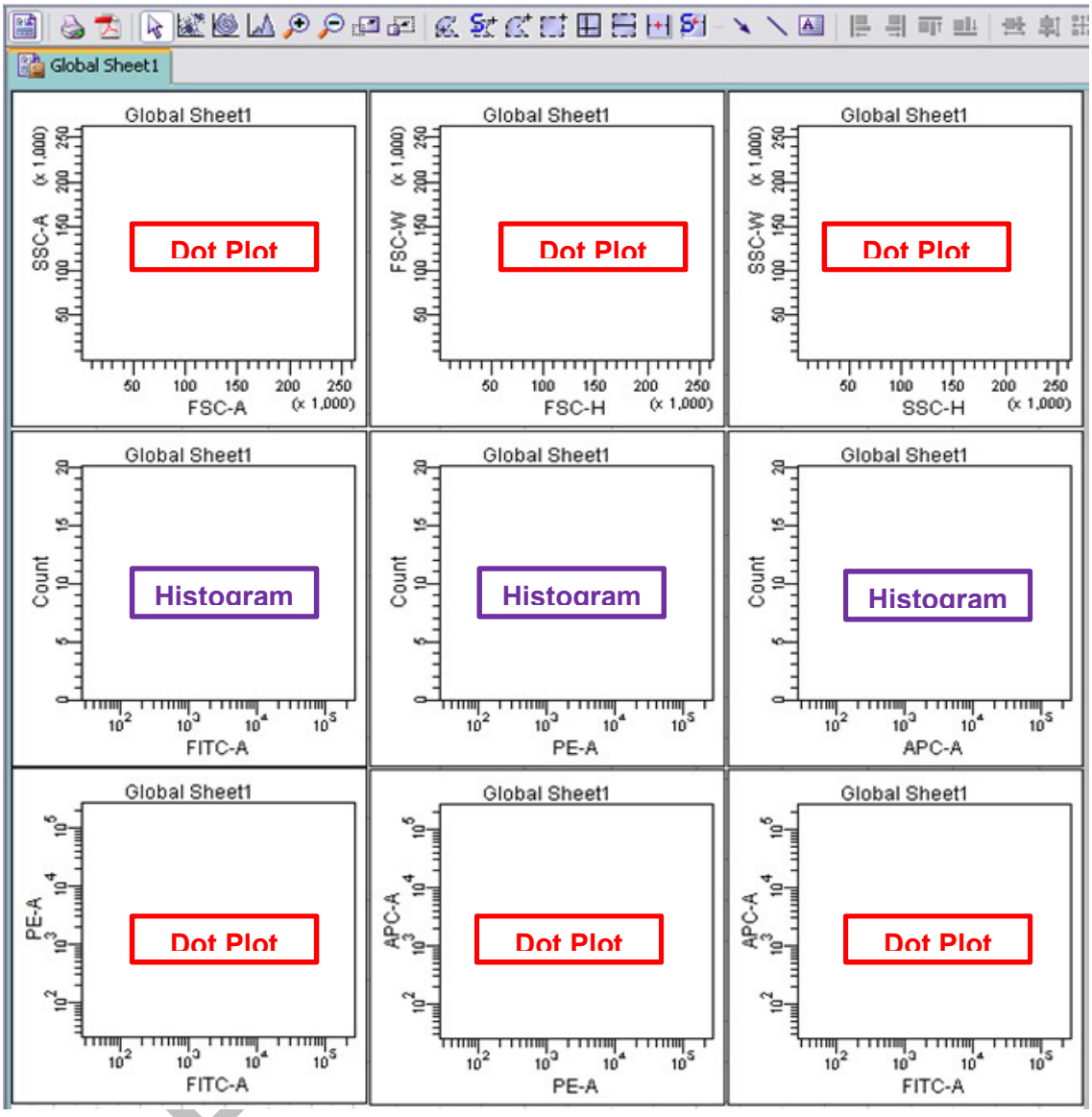
❖ Right click on a plot > **Duplicate** to create another plot of the same type.



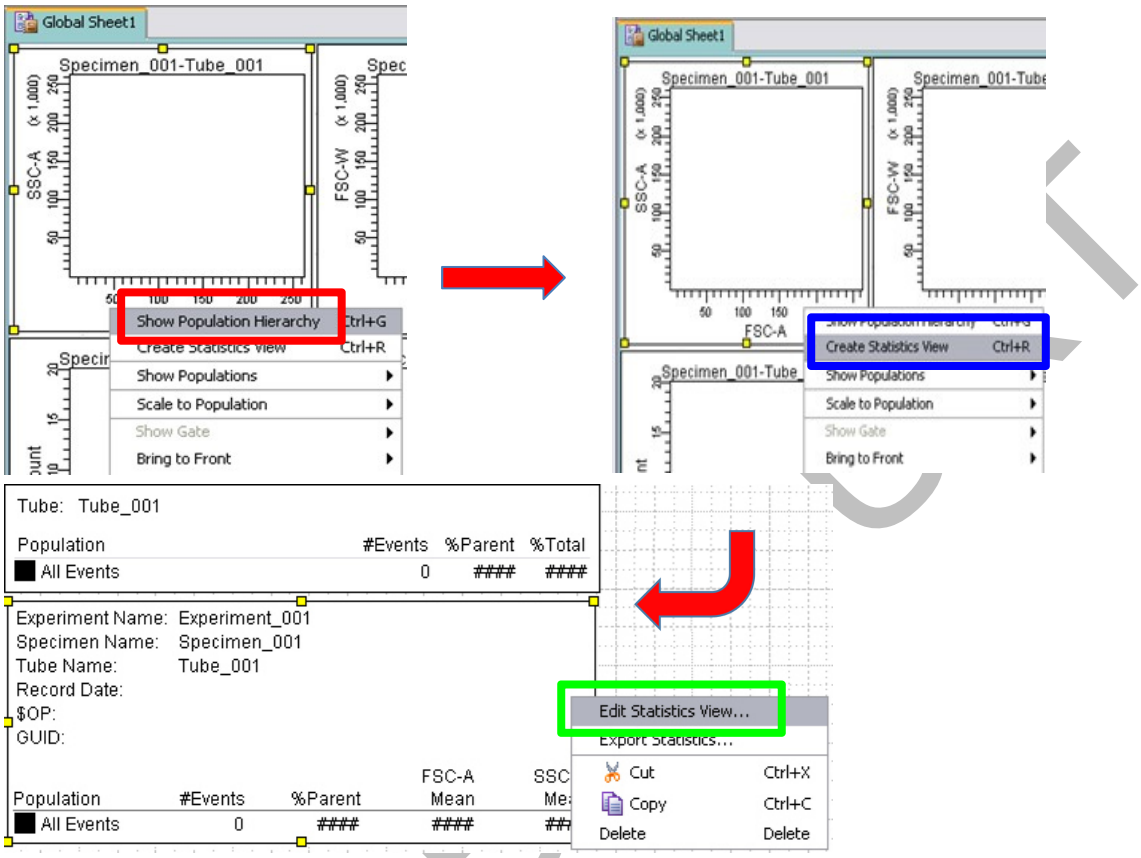
❖ Select each individual **axis**, and choose from a list of offered parameters the preferred one.



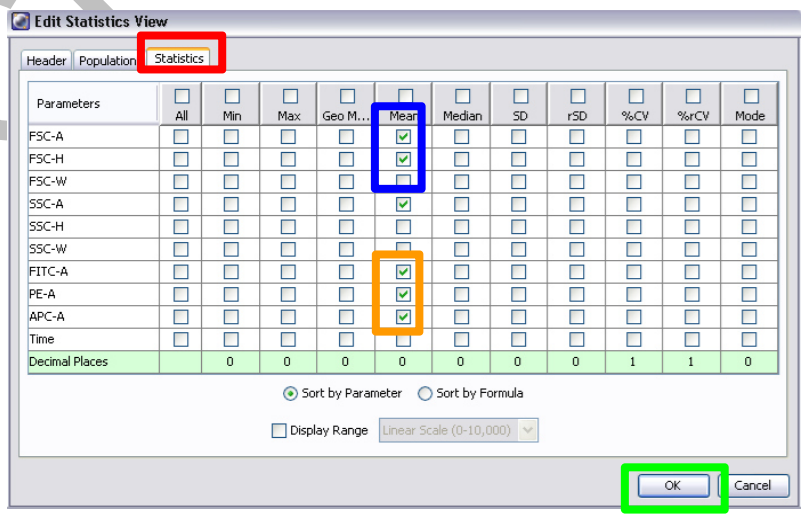
❖ Below shows a template of plots used in routine analysis.



- 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**

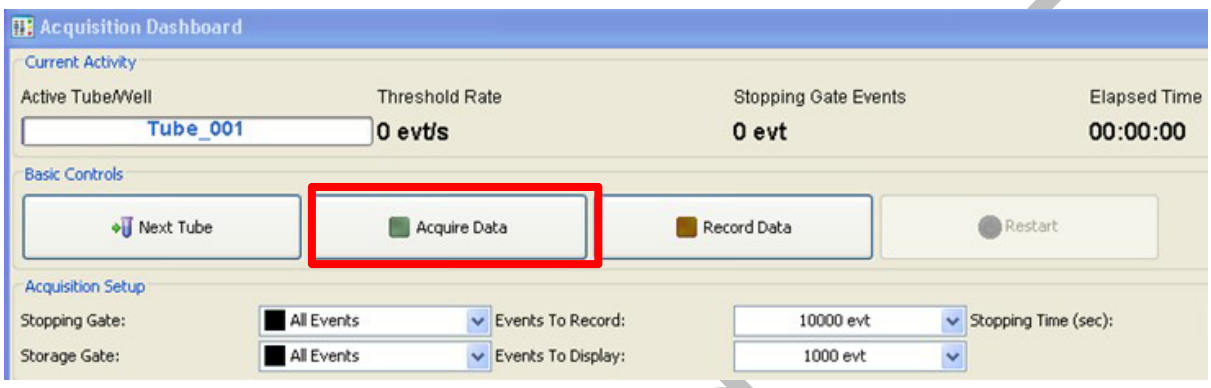


- Select ***Statistics*** tab > tick **mean** of **FSC-A** and **FSC-H** > tick the **mean** of the **'A'rea of the parameters** > click **OK**

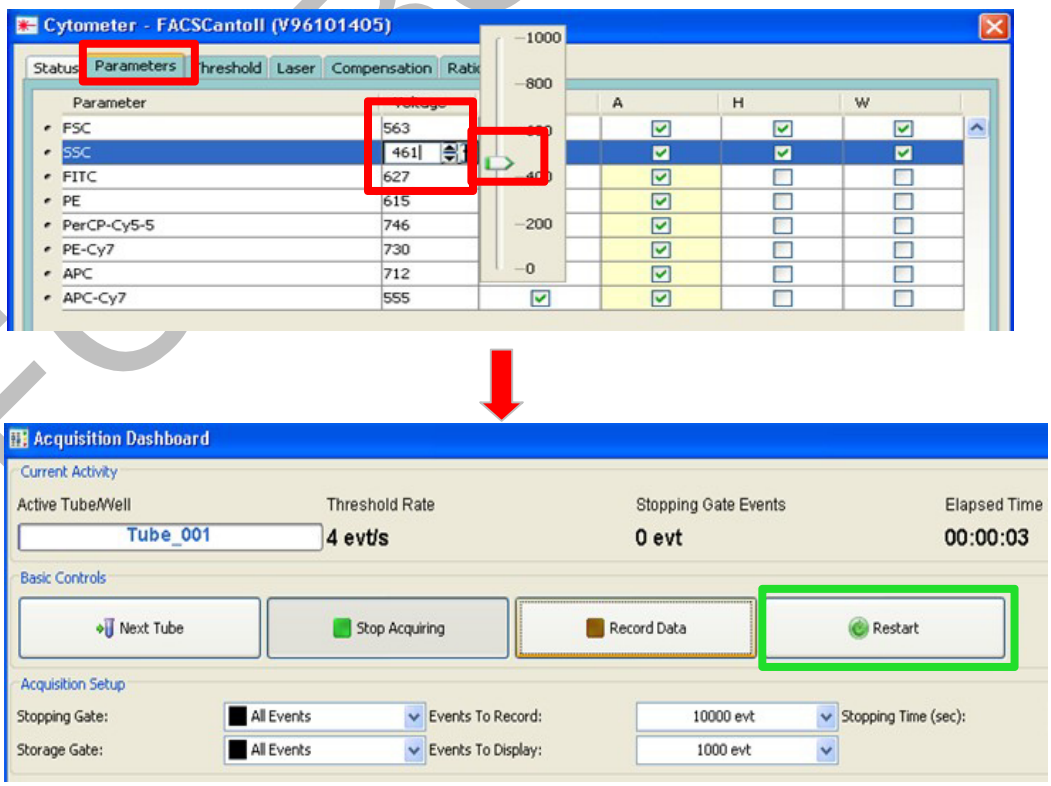


6. Procedures for sample acquisition

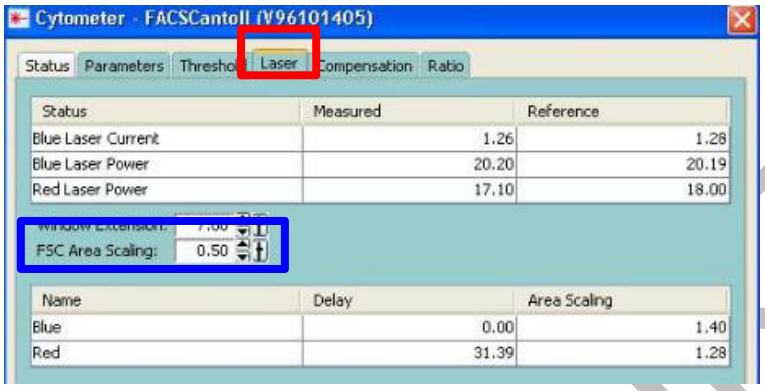
- Press "**RUN**" and "**LO**" on fluid control panel.
- **Gently tab the tube to mix your sample** & put your sample tube on SIP (Sample injection port) & Run the unstained/negative control sample before other sample tubes.
- Do not run the machine without putting a tube filled with fluidics on SIP
- Acquisition Dashboard> **Acquire Data**
- **NOTE: The sample is acquired the whole time being on the SIP!**



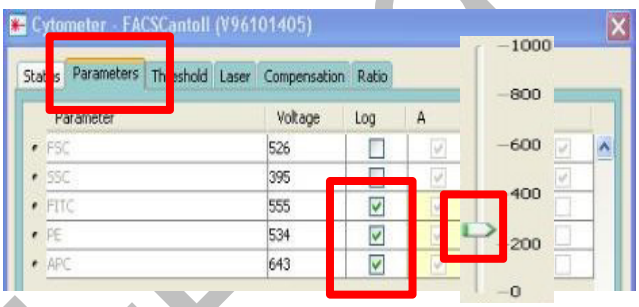
- Identify the population of interest by adjusting the voltage of **FSC** and **SSC** on **Parameters**
- Press **Restart** to accelerate the changes.



- Cytometer > **Laser** & Adjust the **FSC Area Scaling** until the **mean** of **FSC-A** and **FSC-H** are APPROXIMATELY THE SAME.
- Return to *Parameters* tab and finely re-adjust voltage of **FSC** and **SSC**.



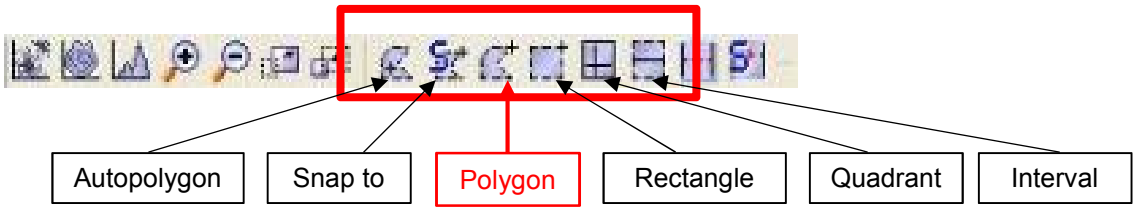
- Adjust voltage of each of the fluorescent channels; preferably the peak of the "negative" population (mean in statistic view) of interest is greater than **ZERO** but less than **10^2**.



- Click Stop Acquiring on Acquisition Dashboard and replace your sample with DI H_2O.
- 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.

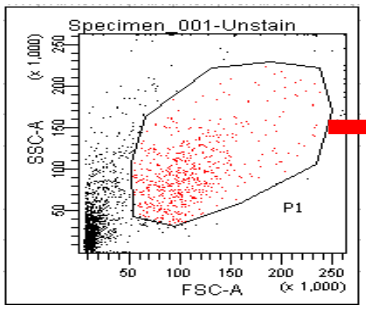
7. Creating Gates

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



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

Create a polygon gate (P1) to gate the main population on the FSC-A vs SSC-A dot plot

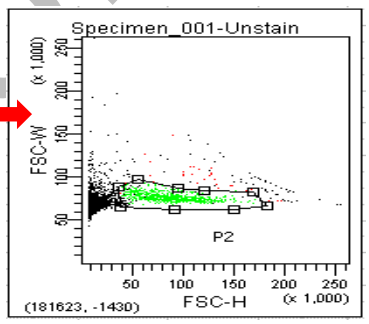


Highlight P1 population in "Population Hierarchy'

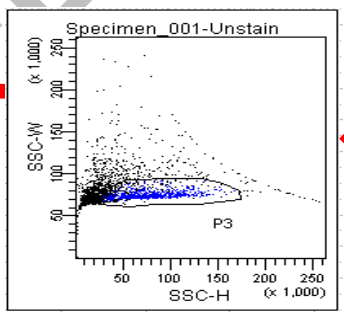
Tube: Unstain

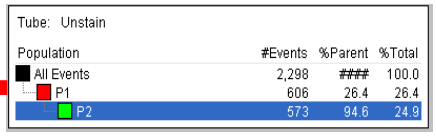
Population	#Events	%Parent	%Total
All Events	2,298	####	100.0
P1	606	26.4	26.4

Create a polygon gate (P2) around the singlet event on the FSC-H vs FSC-W dot plot



Create a polygon gate (P2) around the singlet event on the FSC-H vs FSC-W dot plot



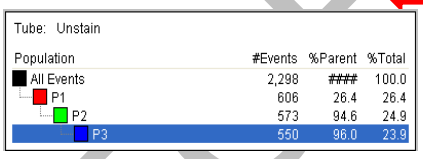


Tube: Unstain

Population	#Events	%Parent	%Total
All Events	2,298	####	100.0
P1	606	26.4	26.4
P2	573	94.6	24.9

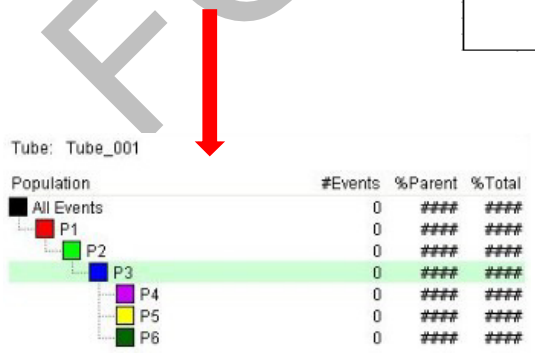
Highlight P2 population under P1

Highlight P3 population under P2



Tube: Unstain

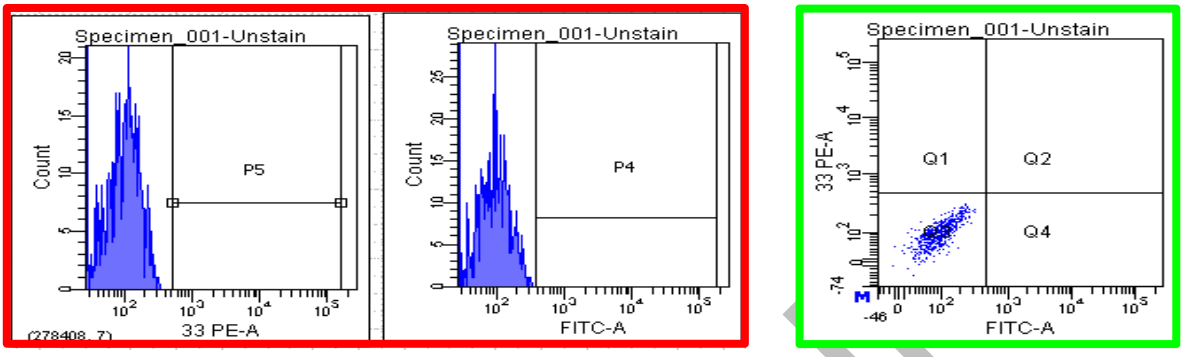
Population	#Events	%Parent	%Total
All Events	2,298	####	100.0
P1	606	26.4	26.4
P2	573	94.6	24.9
P3	550	96.0	23.9



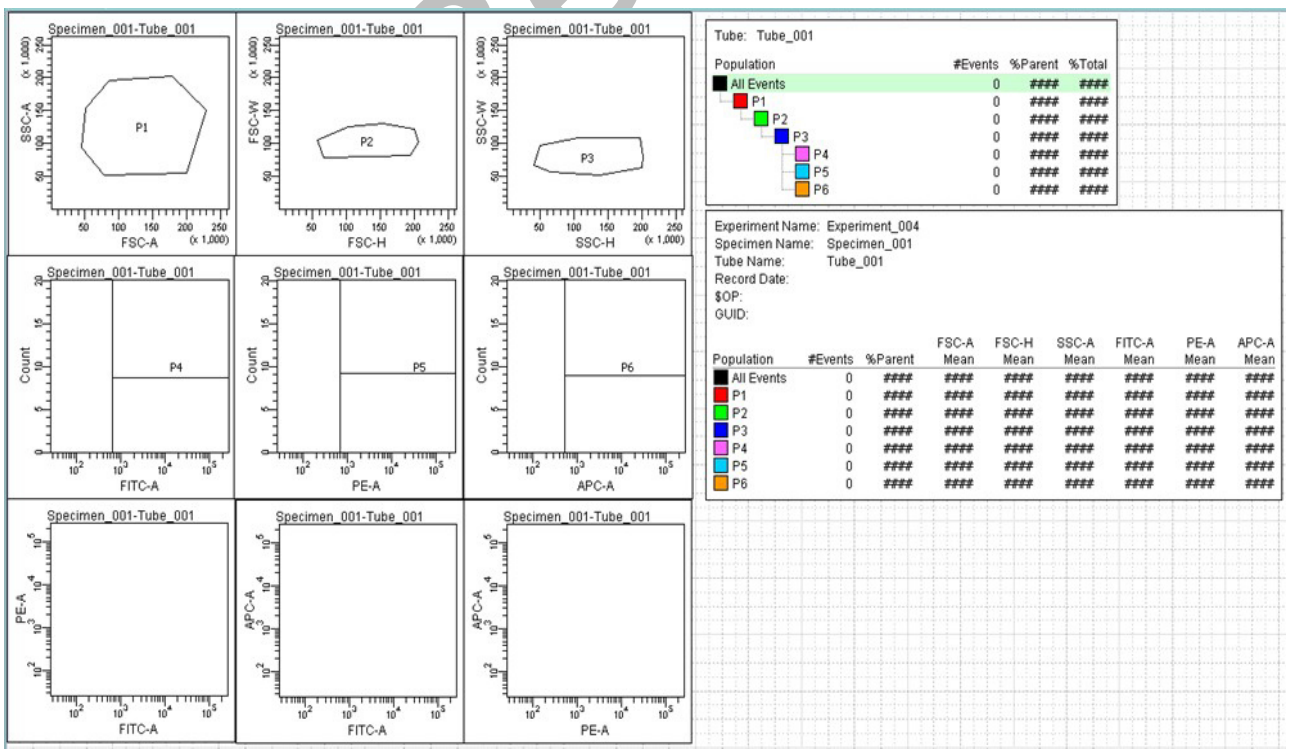
Tube: Tube_001

Population	#Events	%Parent	%Total
All Events	0	####	####
P1	0	####	####
P2	0	####	####
P3	0	####	####
P4	0	####	####
P5	0	####	####
P6	0	####	####

- 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.



8. Recording data for all samples

- **Gently tab the tube to mix your sample** then put your sample tube on SIP. Run the unstained sample before other sample tubes.
- Press "**RUN**" and "**LOW**" on fluid control panel.
- Acquisition Dashboard > **Acquire data** > **Record data**

- **Please pay attention to the sample tube to make sure it will not run dry!**
- Click "Stop Acquiring" to stop acquire the data if necessary then unload your sample.
- Repeat above steps for each sample.

9. Machine Cleaning

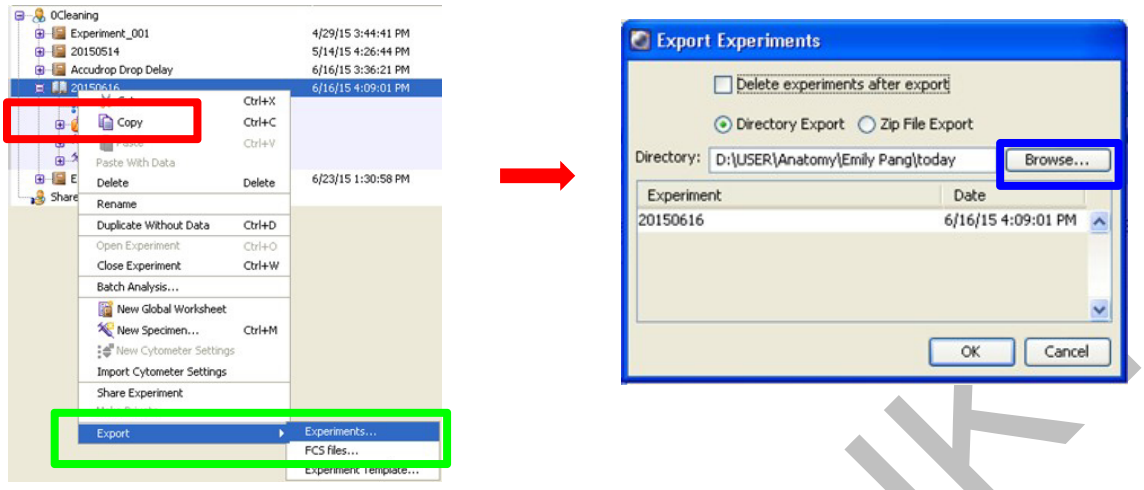
Cleaning procedure between each user is required.

- Prepare **3** mL of each cleaning solution (FACS Clean, FACS Rinse, Milli-Q H_2O).
- Press "**RUN**" and "**HIGH**" on fluid control panel.
- Install a tube of FACS Clean solution on the SIP and allow the cleaning solution to run for **10** minutes with the sample flow rate set to HI.
- Repeat step with BD™ FACSRinse solution and with Milli-Q H_2O.
- Push "Standby" button and leave tube with 1 ml of MQ Water on the sip.

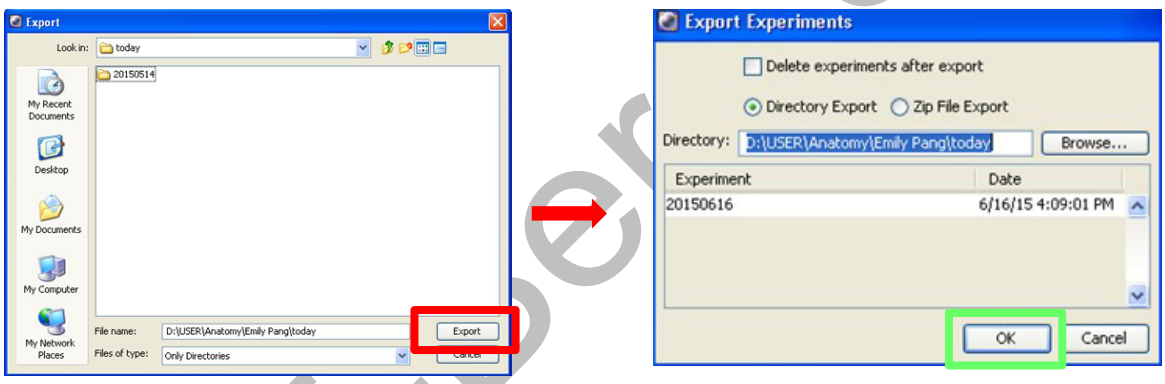
Note: Please be sure in all the cases not to exceed the maximum allowed volume of 2 ml in the tube.

10. Export FCS Data / Experiment

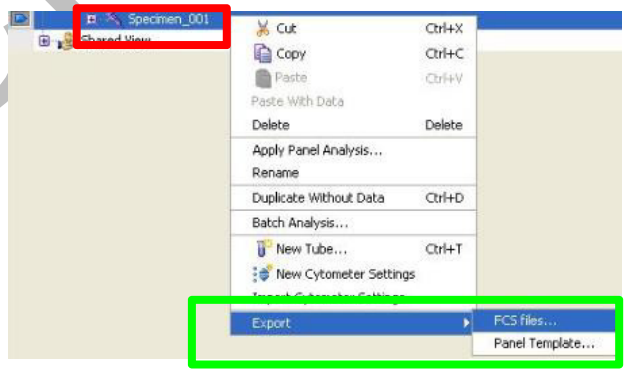
❖ To save Experiment, right click on the **Experiment** **Export** > **Experiment** > **Browse** to choose the destination folder (**D/year/AG/name**).



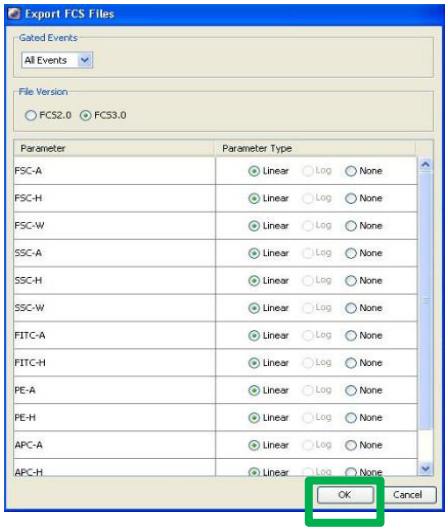
❖ Create a new folder and rename, then click **Export** > **OK**

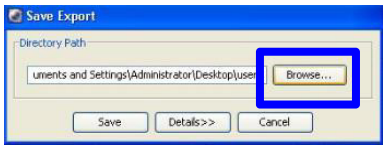


❖ To save FCS files, right click on **Experiment** > **Export** > **FCS Files**

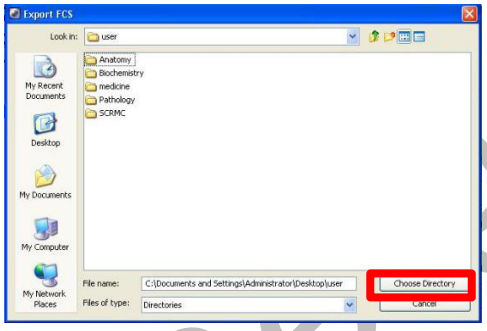


❖ Select **FCS 3.0** > **OK** > **Browse** to choose destination folder (**D/year/AG/name)**.





❖ Create a new folder and rename > **Choose Directory** and save the file.



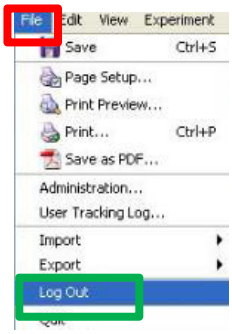
❖ **Duplicate the experiment** if needed again:
- Select the experiment; right click and select **dublicate without data**. This retains the mask, the labeled samples and the stored cytometer settings
- It is not allowed to store more that 3 experiments without data

❖ **Mandatory: Delete the measured experiment** after having FCS data / experiment exported.

❖ **Mandatory: remove your data (within 7 days) from the hardcopy drive D** e.g. by transfer to (S/year/AG/name).

11. Log Out

To log out of FACS DIVA software, go to **File** > **Log Out**



12. Shut down

❖Before you leave please be sure to switch the instrument off if required. **Policy of keeping instruments "ON" or "OFF" depends on the time of the day:**

- **During the daytime**, we keep the analyzers **"ON" except** there is a gap between users longer than 3 hours;
- If you finish your BD LSR-Fortessa experiment **after 6 pm on the weekday or any time on the weekend** and if the next person is not physically present at the site we request to switch "OFF" the **BD LSR Fortessa** and **FACS Flow** system.

❖Please **be sure to logoff Windows** and shutdown the computer before you leave FCF Berg to avoid unnecessary charges.

13. Sign in the labbook (next to the computer) for documentation

Note: *Please report all the problems/concerns to FCF Berg Staff:*

During business hours *— get to Room 581/527 and tell the FCF Berg staff member.*

After hours *— e-mail to fcf-berg@med.uni-tuebingen.de; leave the note about the problem on the instrument's keyboard; proceed on step 9b from the above list.*

❖ **Empty Waste Tank Procedures** (during measurement)

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

- Empty the waste tank.
- Add about 0.5L of clean into the waste tank
- Place the lid back to the waste tank.
- press restart on the FACS Flow supply system
- press PRIME on the cytometer

❖ **Refill Flow Container Procedures** (during measurement)

- Make sure the flow cytometer is in Standby mode (see above).
- Open the lid of the flow container
- Replace by a new flow container
- Close the lid
- press restart on the FACS Flow supply system
- permanently press PRIME (Pump 1 Fill) on the FACS Flow supply system until the light goes out
- press PRIME on the cytometer