LSRFortessa Operation Protocol

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

- ❖ Make sure that the waste tank is empty. If not get a prepared waste container from the closet in the hallway (see page 16 for details)
- ❖ Make sure that the FACS Flow container is fully filled (see page 16 for details)

1. **turn on the computer**
   - ❖ Login into Windows using User Name **BDAdmin** and password: **BDIS#1$$**.
2. **Turn on the FACS Flow 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 up before run your samples

❖ **Check Fluidics:**

➢ **Prime the System**

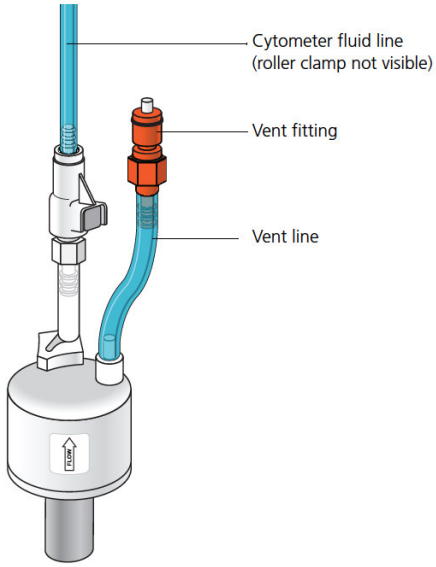
- Remove the water tube from the SIT port
- Press Prime on the Cytometer
- Wait until the red light is gone and repeat the prime function
- Place the water tube back to SIT port

➢ **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. **Log into PPMS** using your User Name and password
5. **Launching the BD FACS DIVA Software**

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

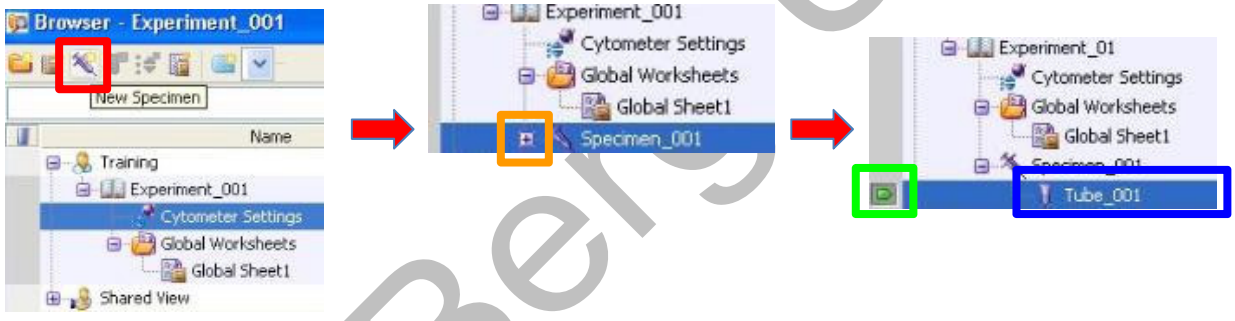
❖ **Always** click **"Use CST Settings"** when pop-up message as below appears.

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

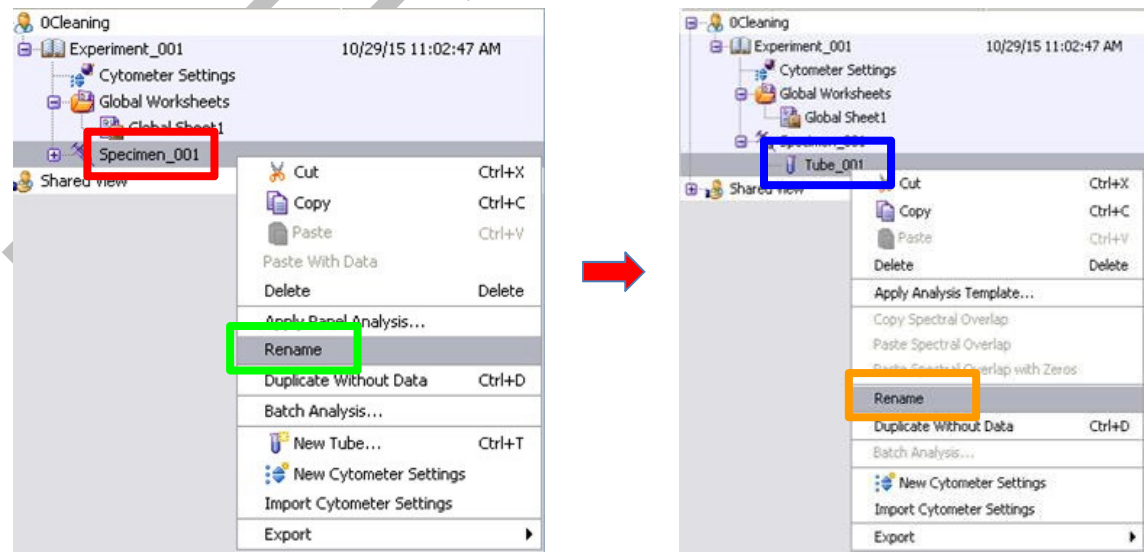
❖ *Browser* toolbar  click **New Experiment** & rename the experiment if necessary or import an old one and duplicate without data.

- **Import an old Experiment:** File or right click --- import --- Experiment --- choose your experiment in your Folder and go to import, open your old experiment --- right click and choose duplicate without data

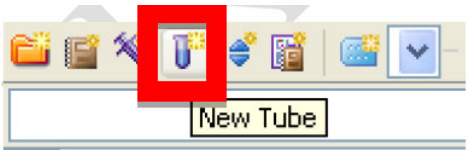
❖ Select **New Specimen** expand the **Specimen** to show **Tube 001**. Highlight the tube with the **Tube Pointer**.



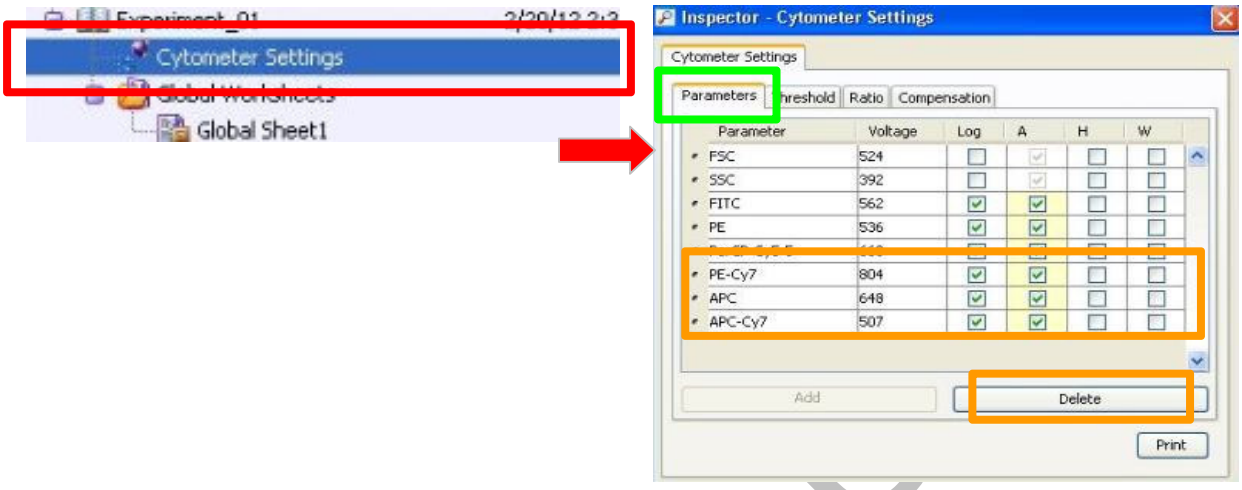
❖ Right click on **Specimen_001** > **Rename** if necessary; Right click on **Tube _001** > **Rename** if necessary.



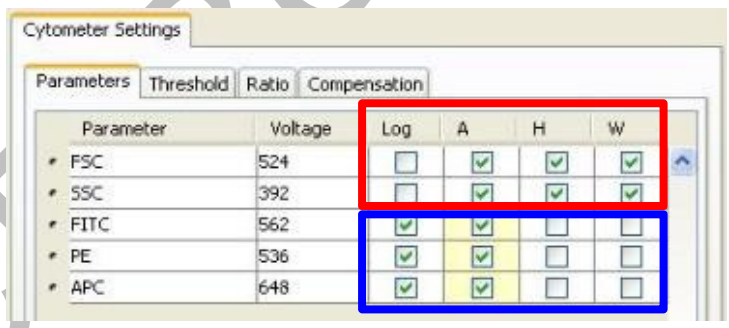
❖ Click the **New Tube** bottom to create new tubes. Rename them if necessary.



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

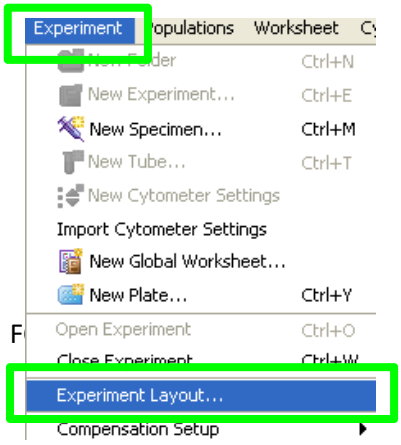


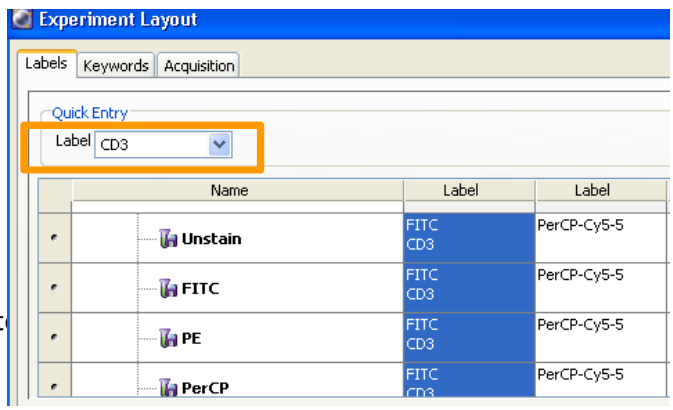
❖ **FSC** (measure *Cell Size*) and **SSC** (measure *Cell Granularity*) are a **MUST** for all kind of analysis and they should be kept in **linear scale.** Please check '**A**'rea, '**H**'eight and the '**W**'idth of **FSC** and **SSC**. **'Log' and 'A'rea boxes should be checked for fluorescence channels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



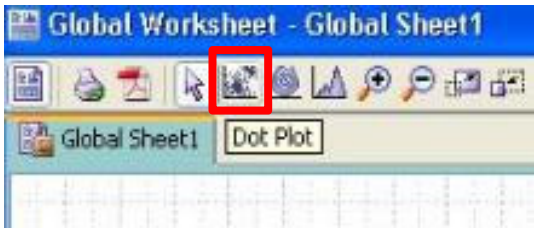
❖ Choose **Experiment** > **Experiment Layout** and define labels for each parameter.

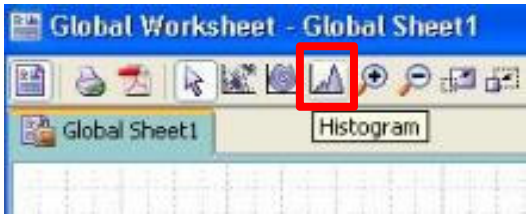
- Select the column of fluorescence channel and enter a label in the **Quick Entry**
- **Label field**

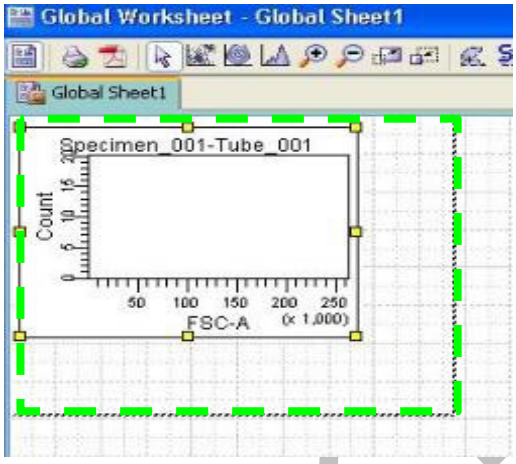




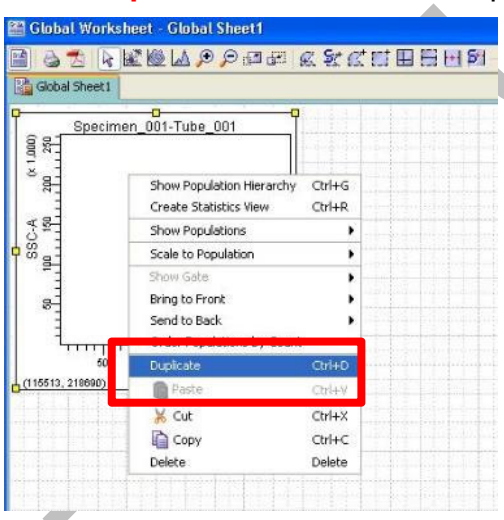
❖ Select **Dot Plot** or **Histogram** move the cursor onto the **blank worksheet**.



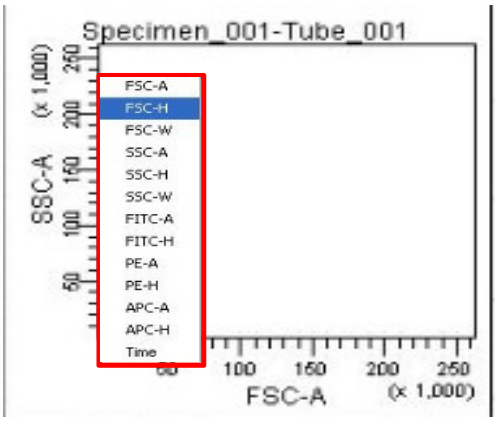




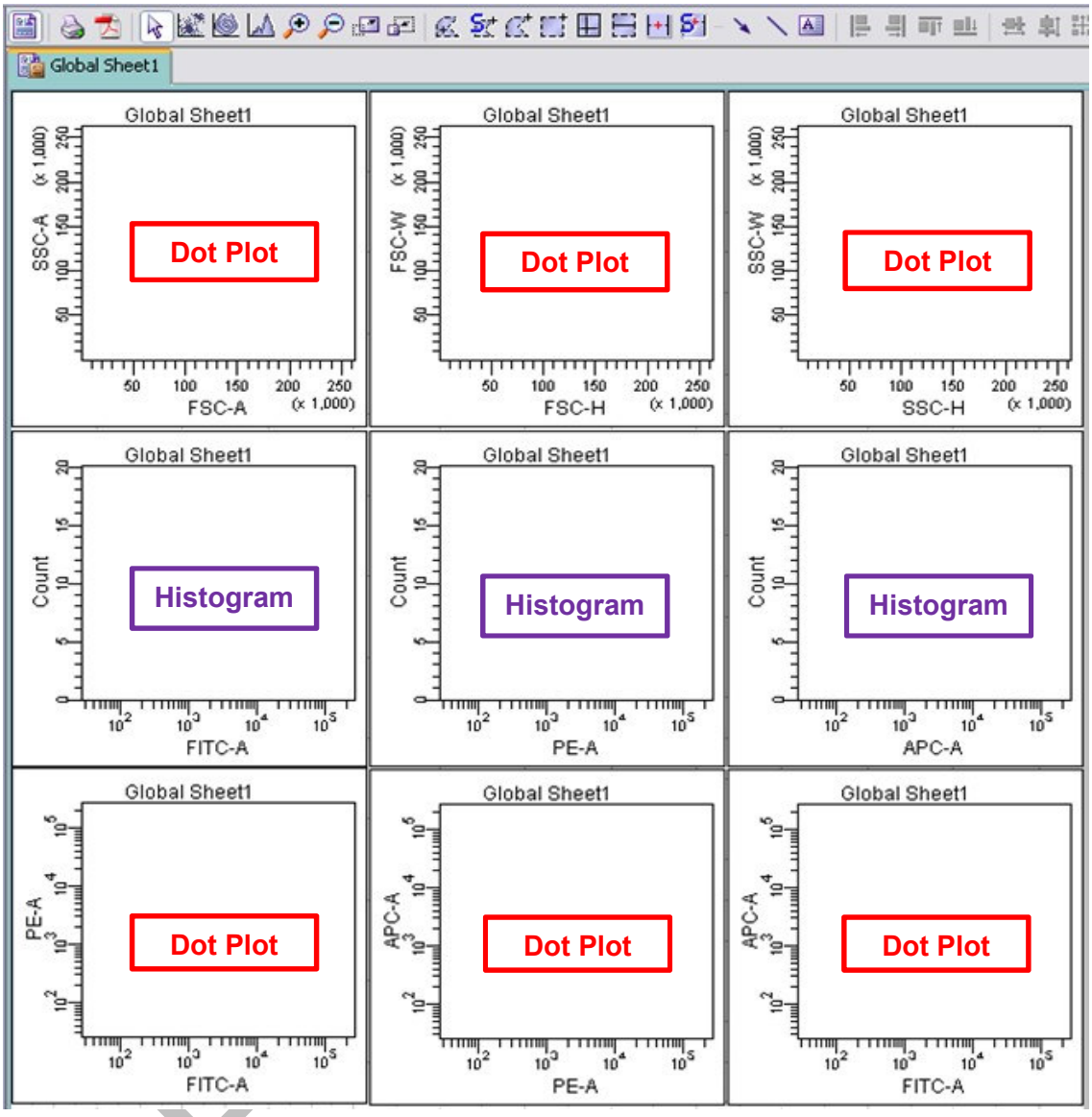
❖ Right click on a plot > **Duplicate** to create another plot of the same type.



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

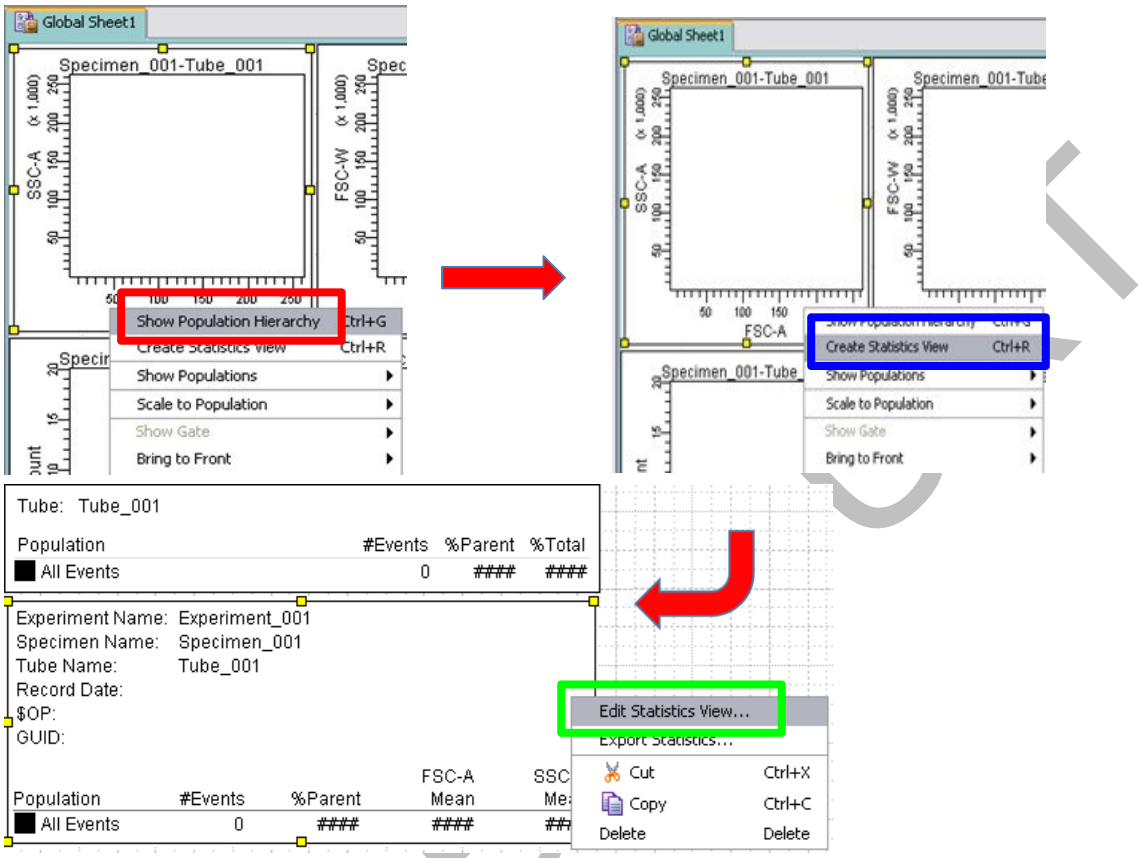


❖ Below shows a template of plots used in routine analysis.

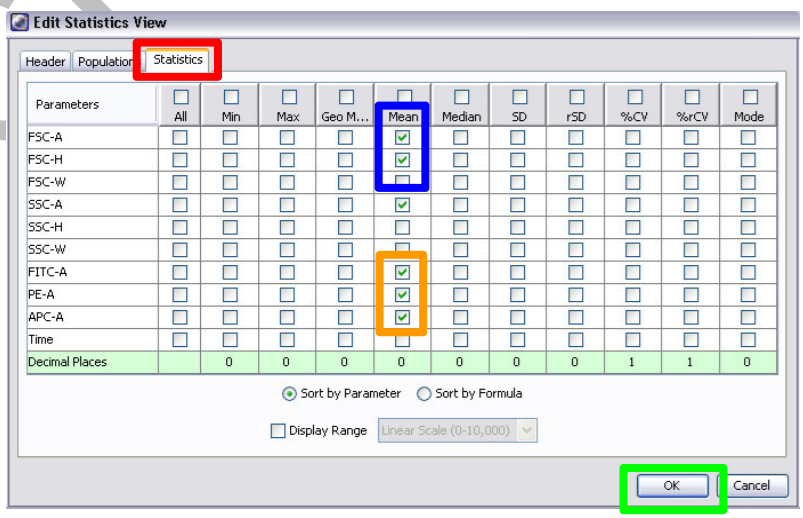


❖ Right click on the plot > **Show Population Hierarchy**

- Right click on the plot > **Create Statistics View** & right click on the statistics view
- > **Edit Statistics View**

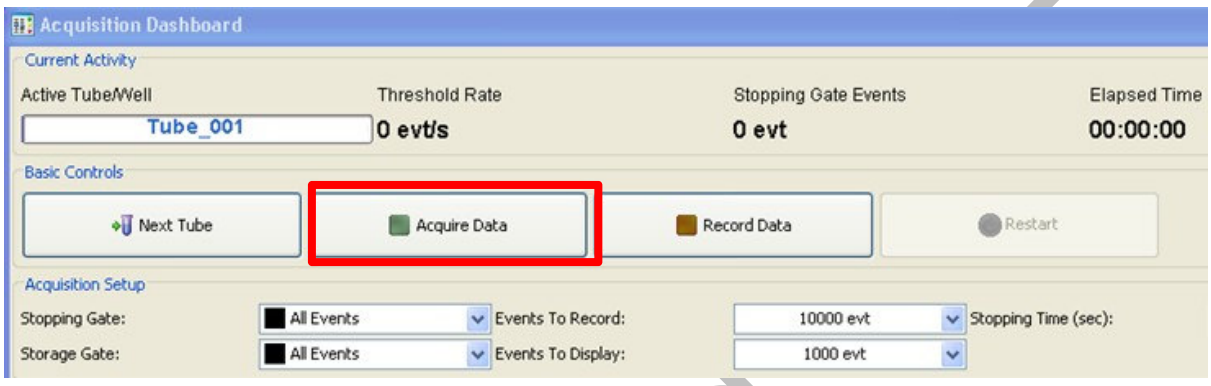


❖ Select ***Statistics*** tab > tick **mean** of **FSC-A** and **FSC-H** > tick the **mean** of the **'A'rea of the parameters** > click **OK**

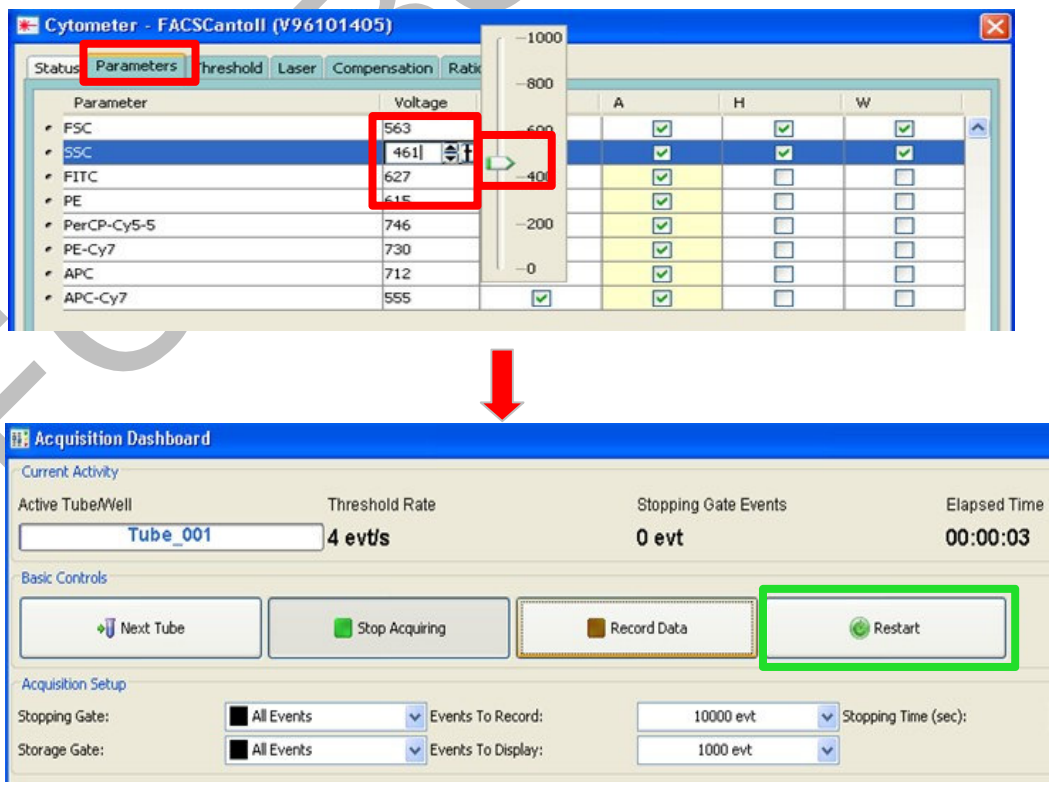


7. Procedures for sample acquisition

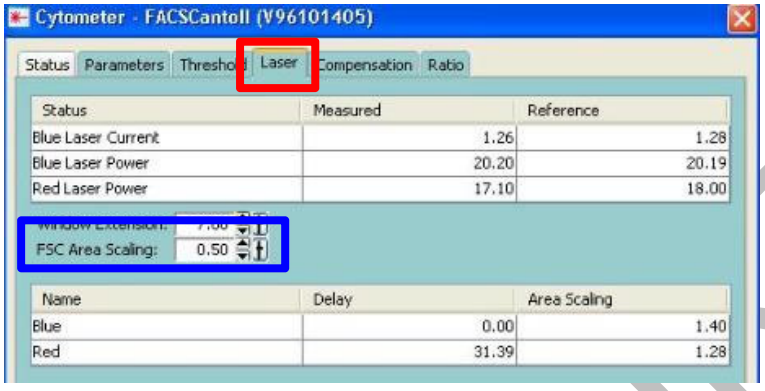
- Press "**RUN**" and "**LO**" on fluid control panel.
- **Gently tab the tube to mix your sample** & put your sample tube on SIP (Sample injection port) & Run the **unstained/negative** control sample before other sample tubes.
- Do not run the machine without putting a tube filled with fluidics on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



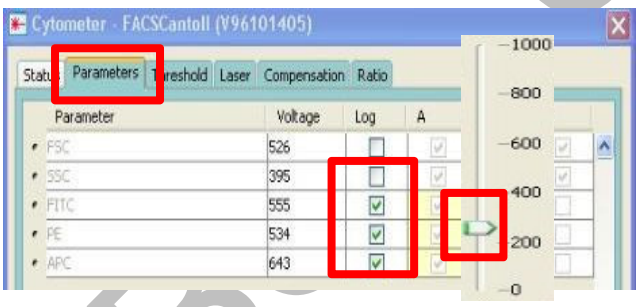
- Identify the population of interest by adjusting the voltage of **FSC** and **SSC** on **Parameters**
- Press **Restart** to accelerate the changes.



- 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**.



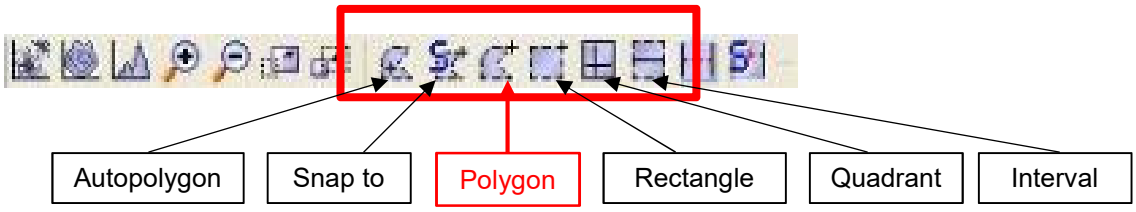
- Adjust voltage of each of the fluorescent channels if necessary



- 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 (Off-Scale).

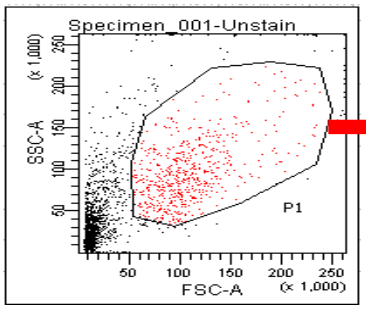
8. Creating Gates

❖ Set the current tube pointer to the following tube and use **Polygon Gate** to gate the target 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

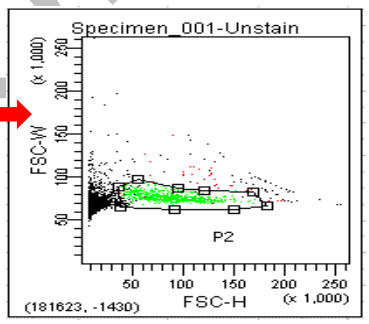


Highlight P1 population in "Population Hierarchy'

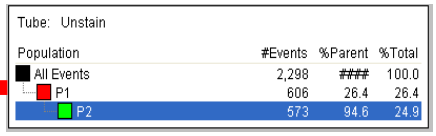
Tube: Unstain

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



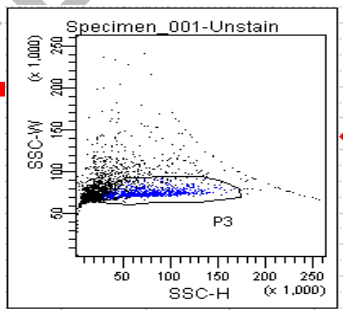
Highlight P2 population under P1



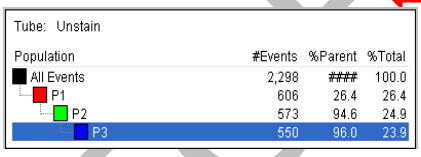
Tube: Unstain

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

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



Highlight P3 population under P2



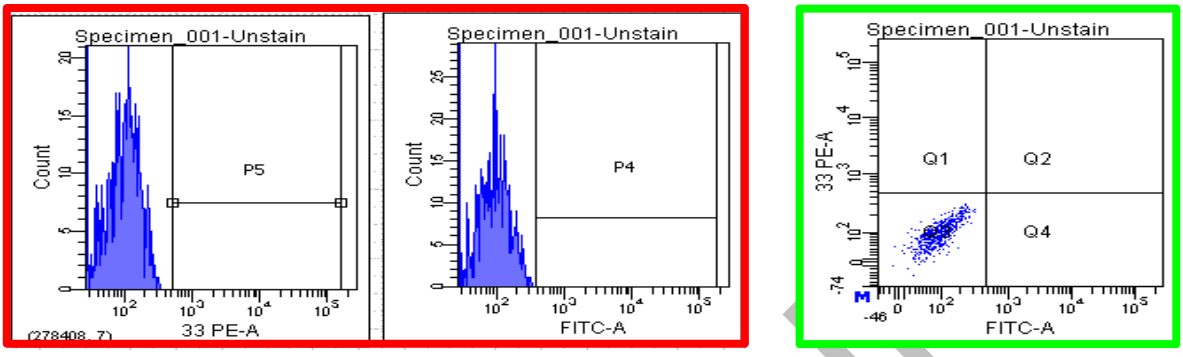
Tube: Unstain

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

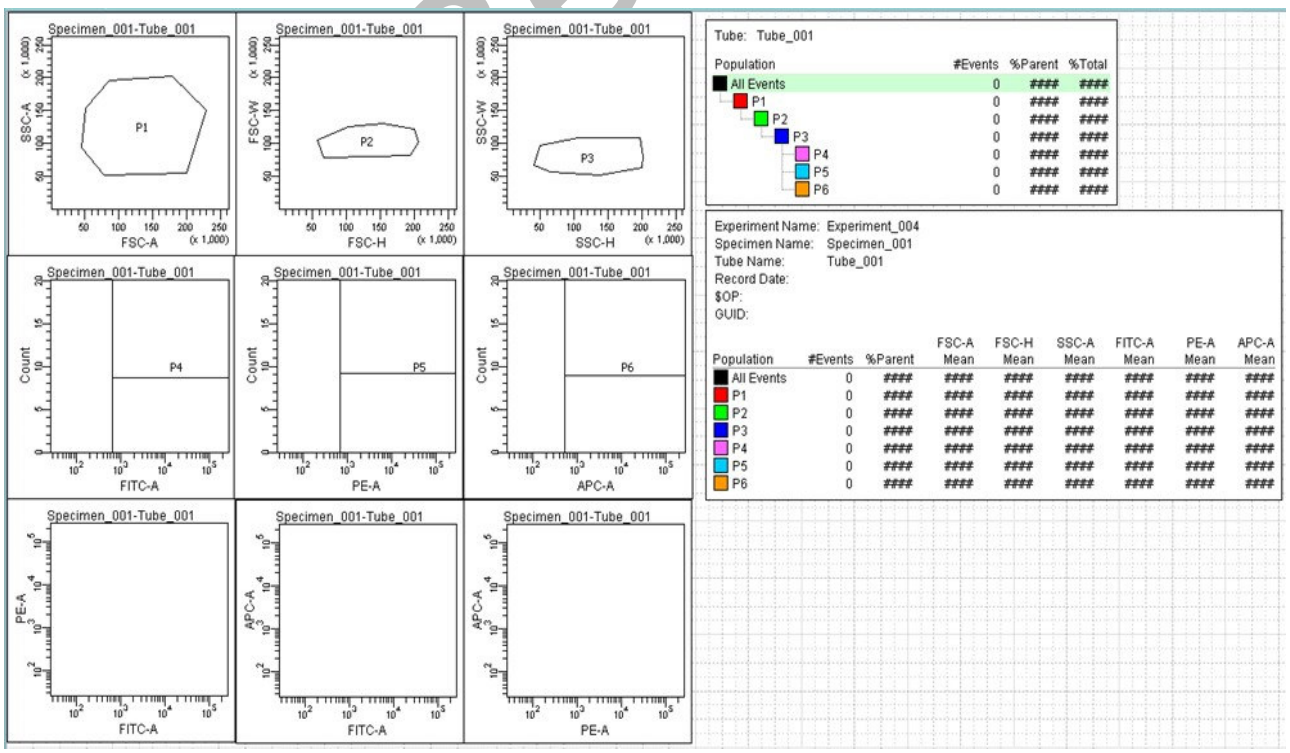
Tube: Tube_001

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.



9. 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.
- Before you start recording, wait a short time until the number of cells per second (Threshold/Rate) is stable

- 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.

10. Machine Cleaning

Cleaning procedure between each user is required.

- Prepare **2.5** 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 HIGH.
- 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 2.5 ml in the tube.

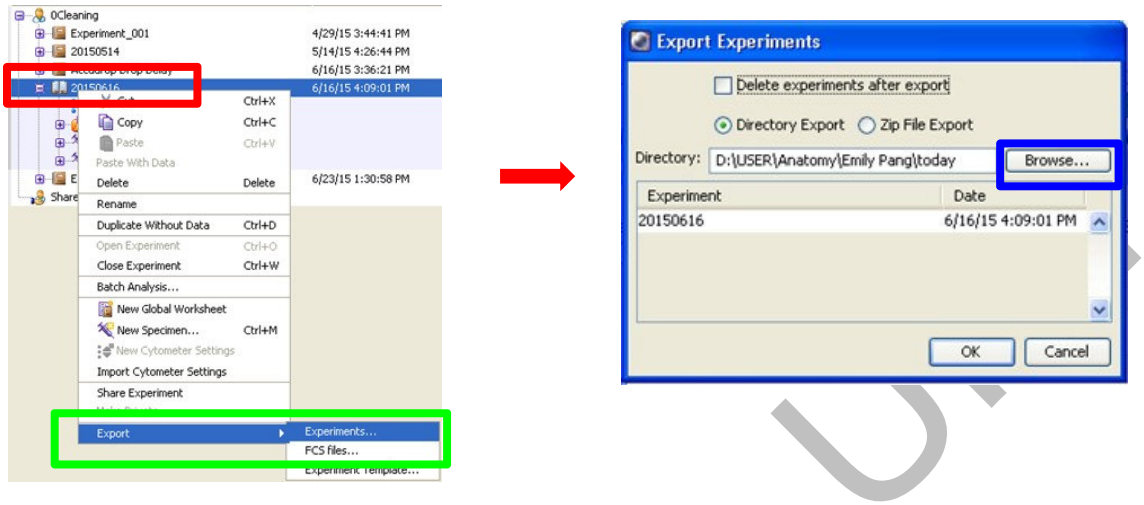
11. Export FCS Data / Experiment

❖ To save Experiment, right click on your created/measured **Experiment** > **Export** > **Experiment** >

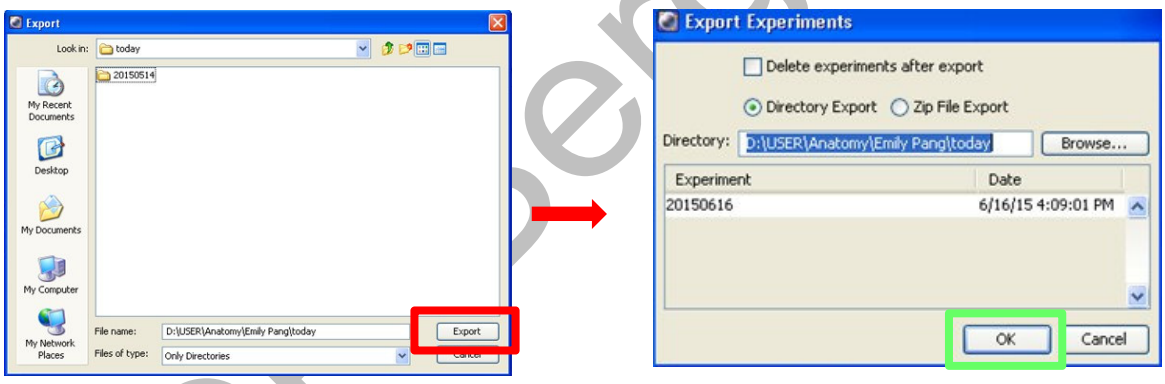
Browse to choose the destination folder.

For external saving: D; Export Userprfile; Groupe and your Folder

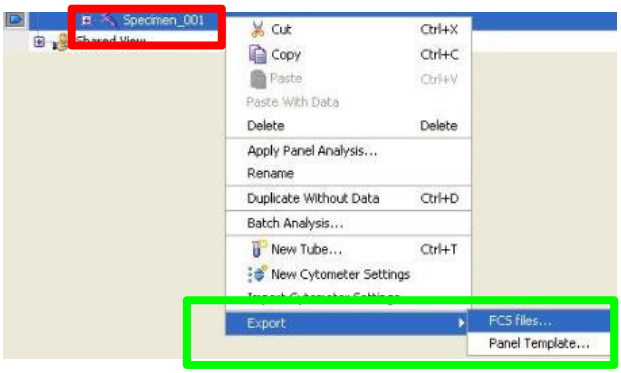
For internal saving: Q; IM; FCF_Berg; FACS; Arbeitsgruppen;…



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



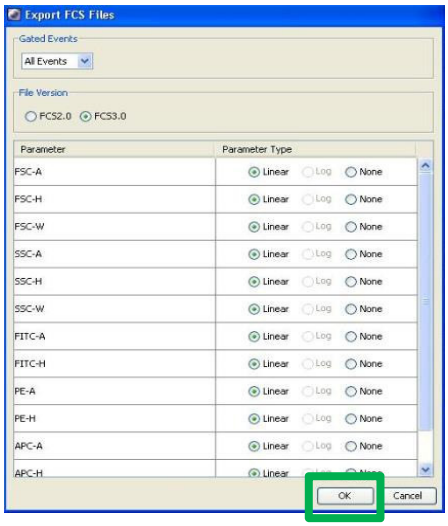
❖ To save FCS files, right click on **Experiment** > **Export** > **FCS Files**

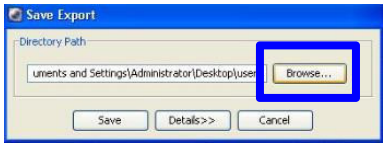


❖ Select **FCS 3.0** > **OK** > **Browse** to choose destination folder

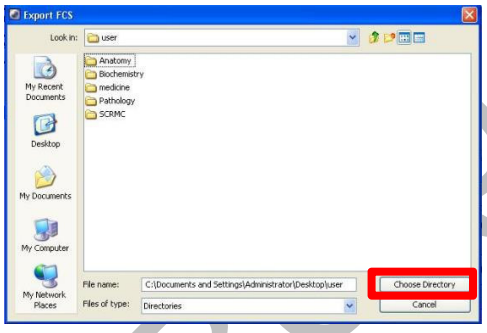
❖ **For external saving: D; Userprofile; Group and your Folder**

❖ **For internal saving: Q; IM; FCF_Berg; FACS; Arbeitsgruppen;…**





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



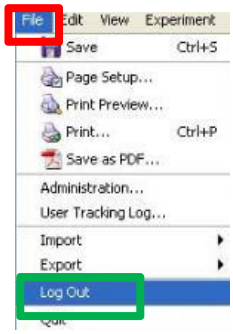
❖ **Duplicate the experiment** if needed again:
- Select the experiment; right click and select **dublicate without data**. This retains the mask, the labeled samples and the stored cytometer settings
- It is not allowed to store more that 3 experiments without data

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

❖ **Mandatory: remove your data (within 7 days) from the hardcopy drive D**

12. Log Out

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



- Log Out PPMS or shut down the Computer

13. 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.

14. 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 and tell the FCF Berg staff member.*

After hours *— e-mail to fcf-berg@med.uni-tuebingen.de; leave a 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.

- Remove the lid from the waste tank
- Get a prepared waste container from the closet in the hallway
- 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)

- If the Alarm sound can be heard on the cart, the alarm must be switched off. To do this, press the button at the bottom of the alarm display on the cart car.
- 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