Canto II 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 15 for details)
- ❖ Make sure the FACS Flow container is fully filled (see page 16 for details)

1. Turn on the external pressure (on the opposite wall)

2. Turn on the Canto II

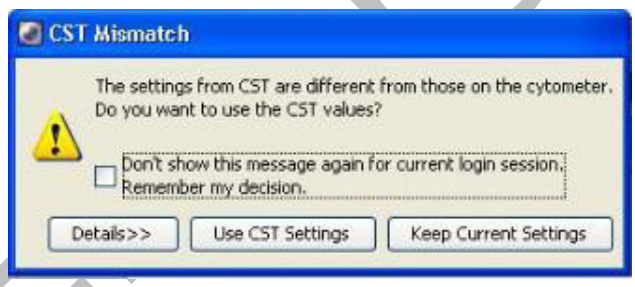
3. Turn on the Computer

Log into Windows using User Name **Admin** and password: **BDIS#1**.

4. Launch the BD FACSDiva Software

- ❖ Log in FACSDiva software with your personal login name and password.

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



5. Start Fluidics Startup

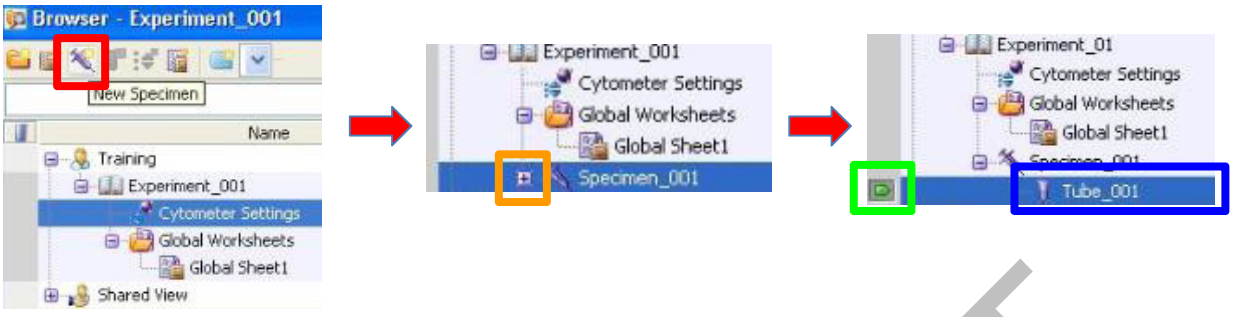
Starting the **Fluidics startup** (7 min)

- Choose Cytometer > Fluidics Startup.

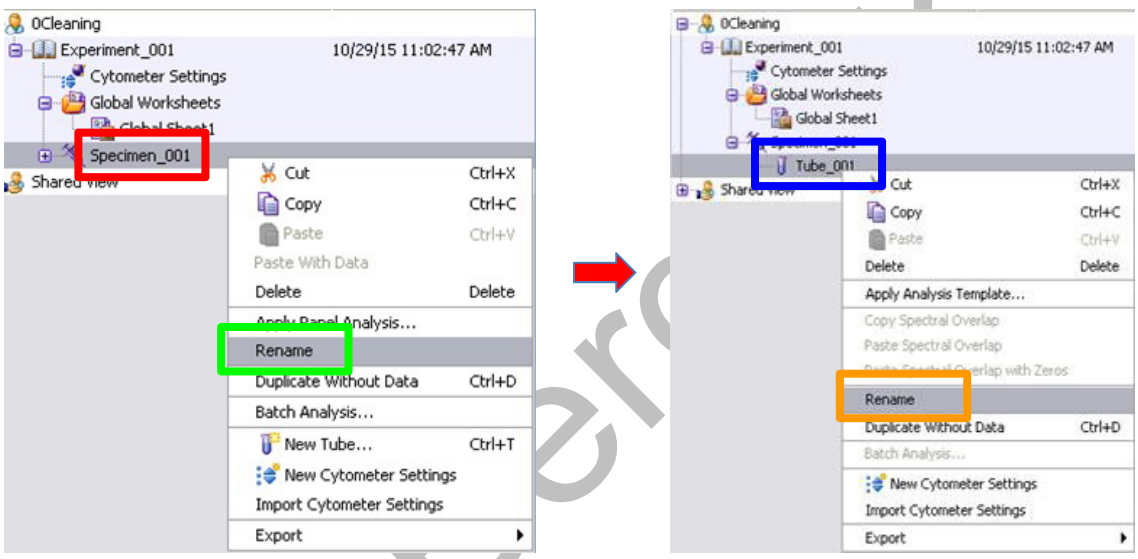
6. Creating and working with experiments in BD FACSDiva Software

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

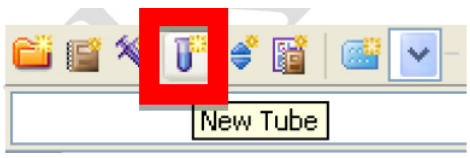
❖ Select **New Specimen** expands the **Specimen** to show **Tube 001**. Highlight the tube with the **Tube Pointer**.



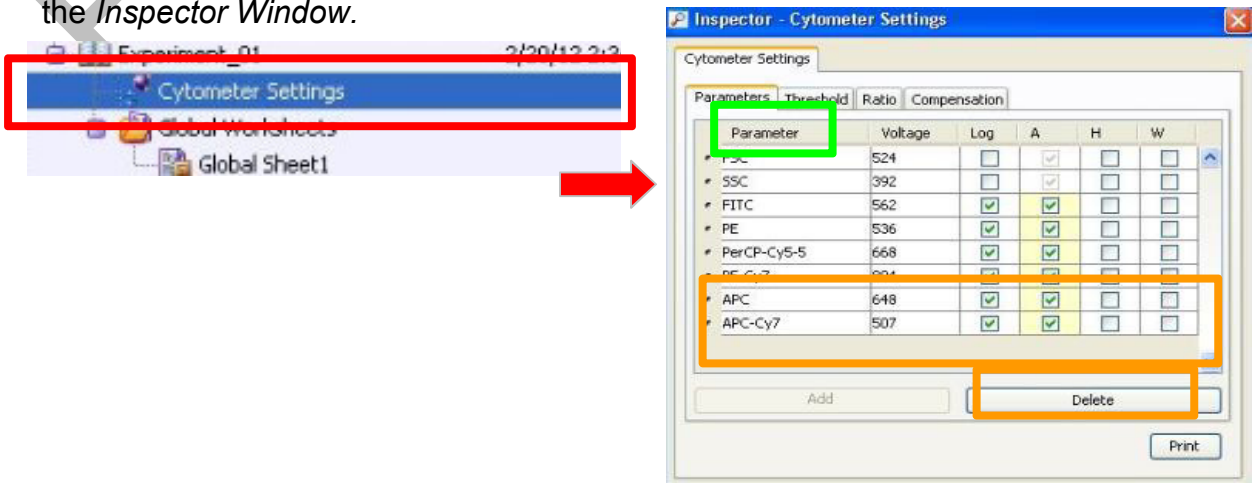
❖ Right click on **Specimen_001** > **Rename** if necessary; Right click on **Tube _001** & **Rename** if necessary.



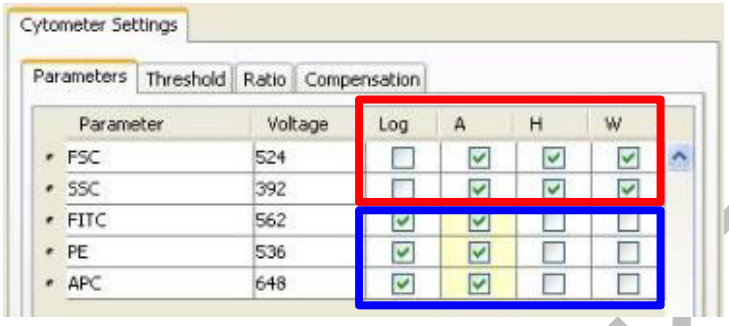
❖ Click the New Tube buttom to create new tubes. Rename them if necessary.



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

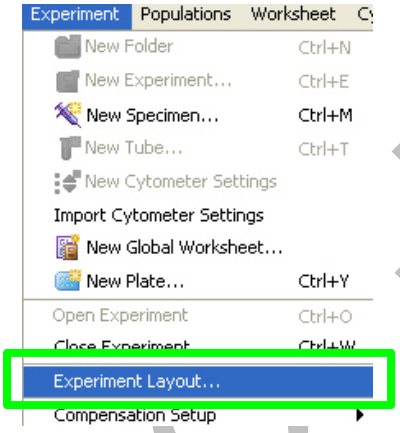


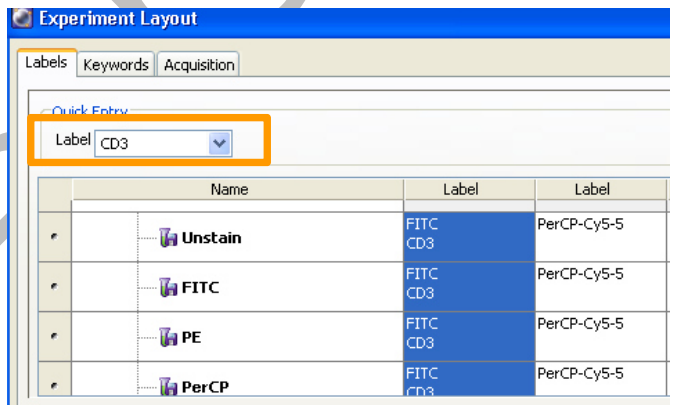
❖ **FSC** (measure *Cell Size*) and **SSC** (measure *Cell Granularity*) are a **MUST** for all kind of analysis and they should be kept in **linear scale.** Please check '**A**'rea, '**H**'eight and the '**W**'idth of **FSC** and **SSC**. **'Log' and 'A'rea boxes should be checked for fluorescence channels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



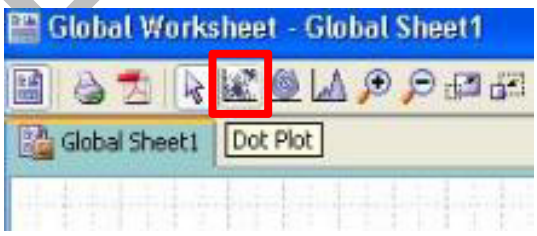
❖ Choose **Experiment** > **Experiment Layout** and define labels for each parameter.

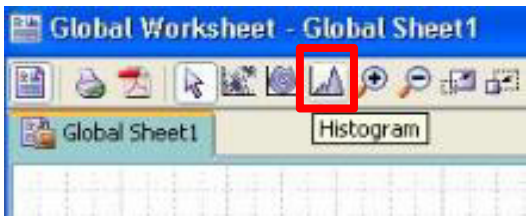
- Select the column of fluorescence channel and enter a label in the **Quick Entry**
- **Label field**

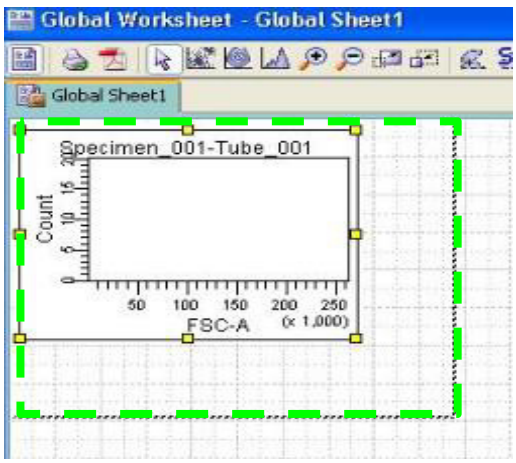




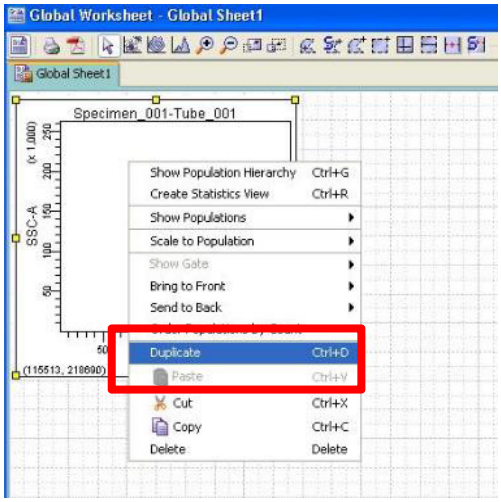
❖ Select **Dot Plot** or **Histogram** & move the cursor onto the **blank worksheet**.



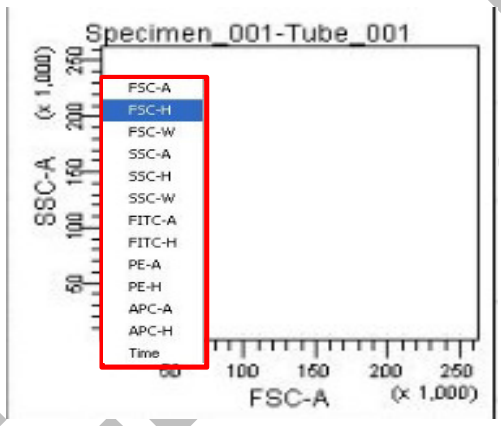




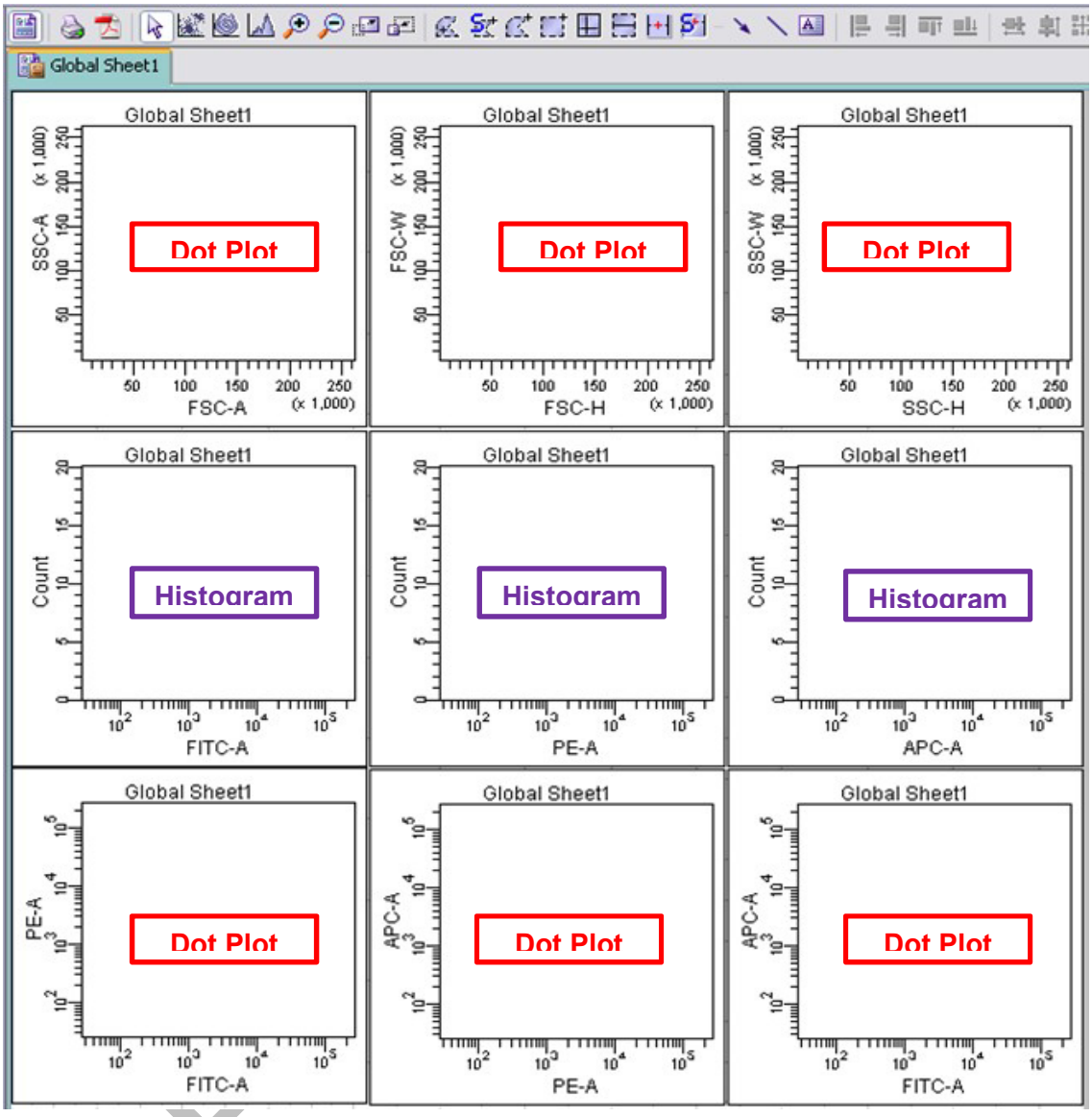
❖ Right click on a plot & **Duplicate** to create another plot of the same type.



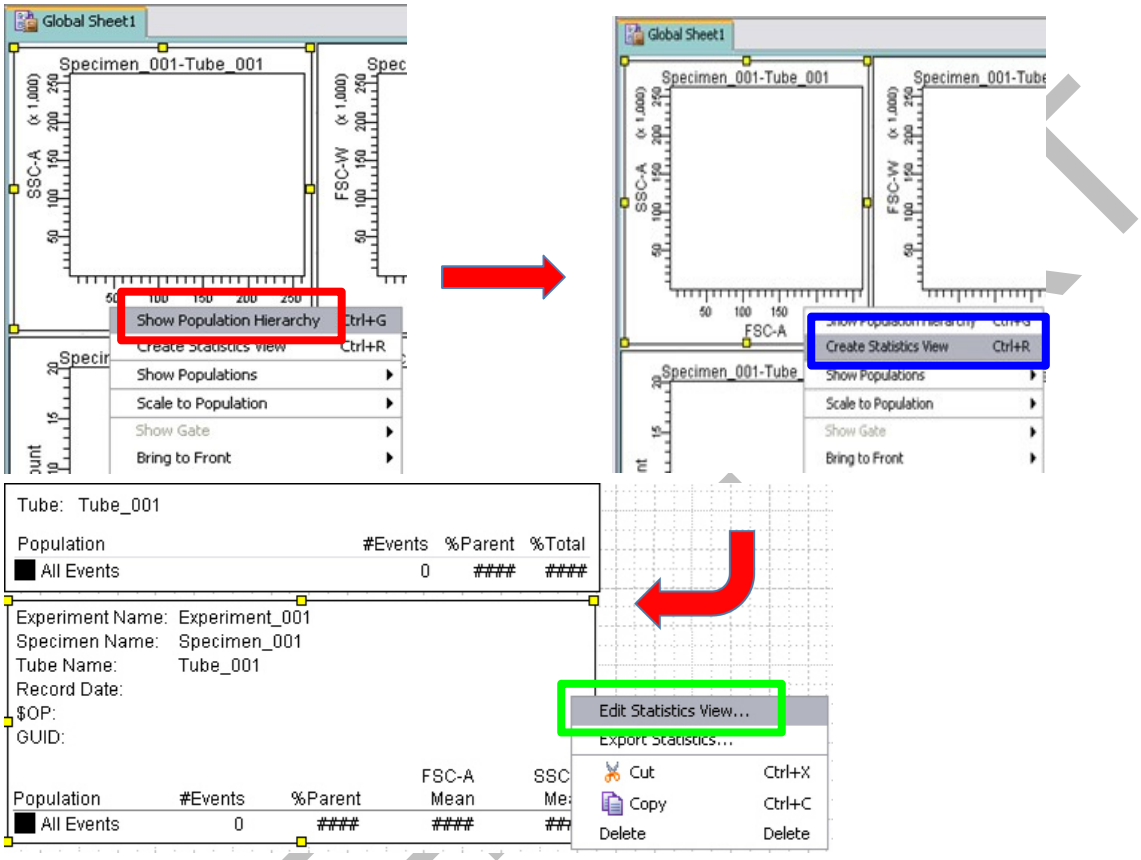
❖ Select each individual **axis**, and choose from a list of offered parameters the preferred one.



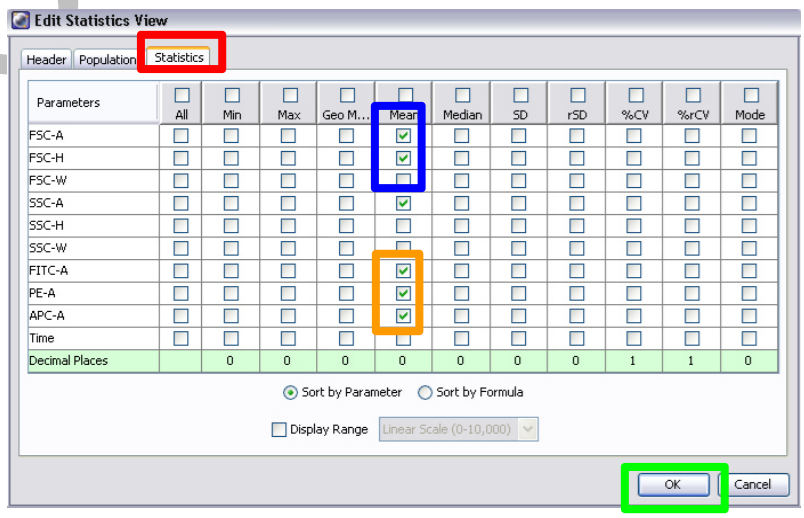
❖ Below shows a template of plots used in routine analysis.



- 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**

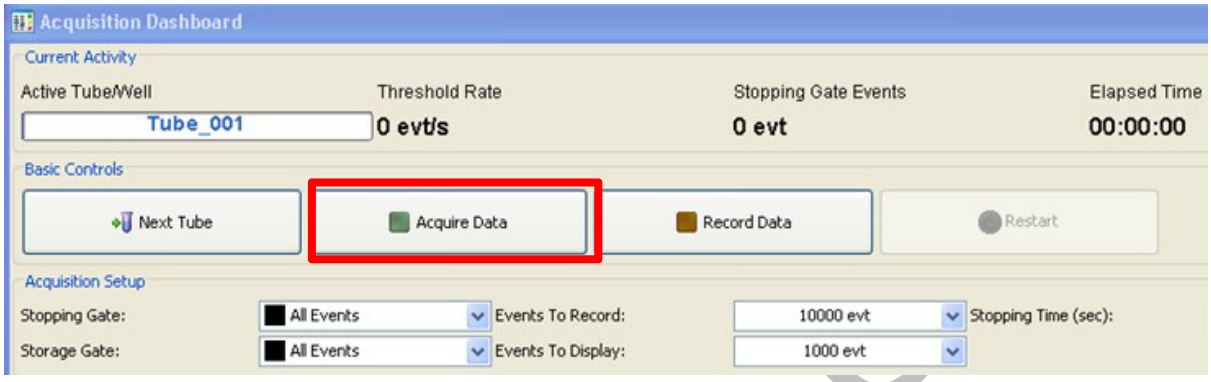


- Select ***Statistics*** tab & tick **mean** of **FSC-A** and **FSC-H** & tick the **mean** of the **'A'rea of the parameters** & click **OK**

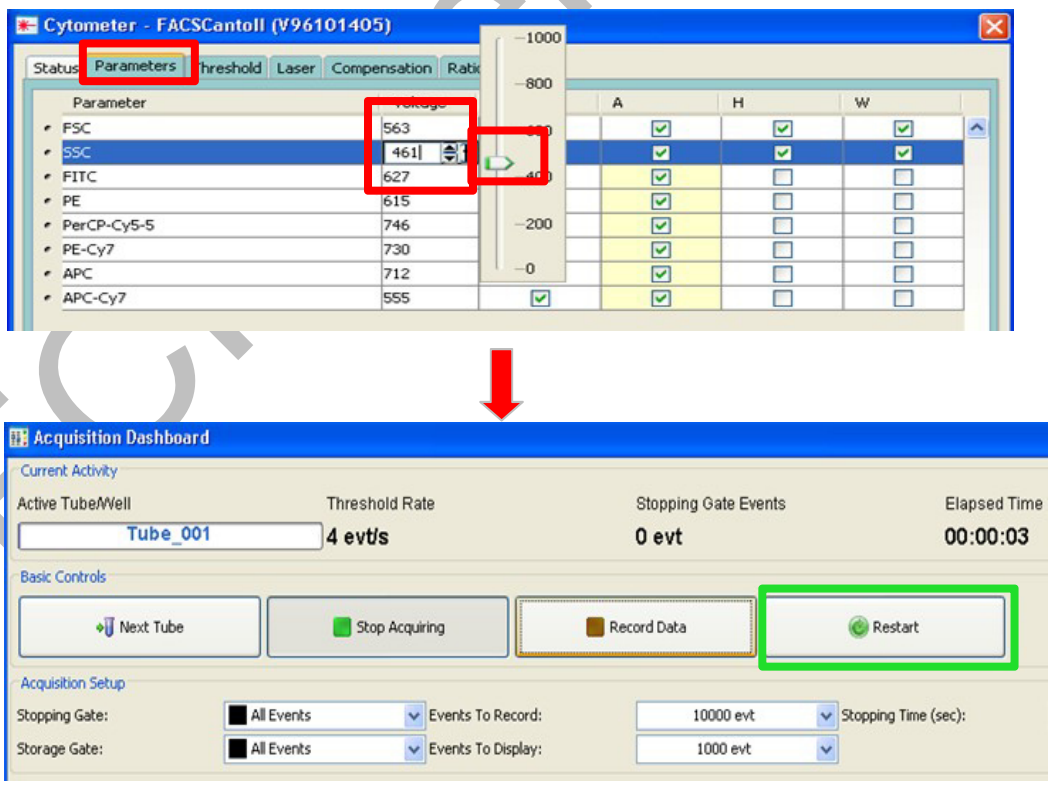


7. Procedures for sample acquisition

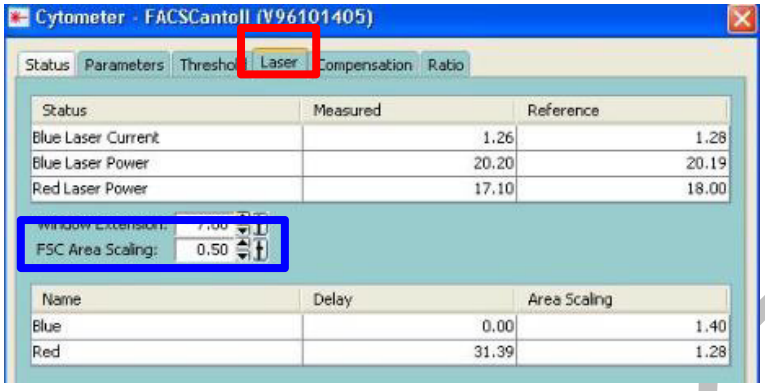
- **Gently tab the tube to mix your sample** & put your sample tube on SIP (sample injection port) & Run the unstained/negative control sample before other sample tubes.
- Acquisition Dashboard & **Acquire Data**
- Choose **Flow Rate low, medium** or **high** to acquire your samples



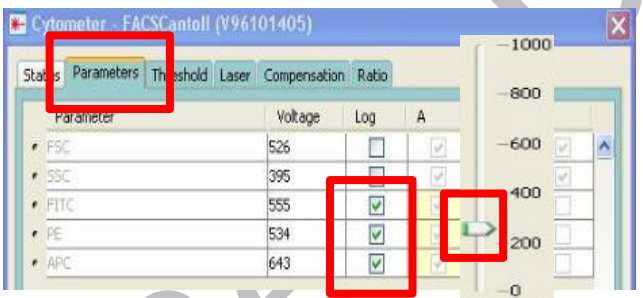
- Identify the population of interest by adjusting the voltage of **FSC** and **SSC** on **Parameters**
- Press **Restart** to accelerate the changes.



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



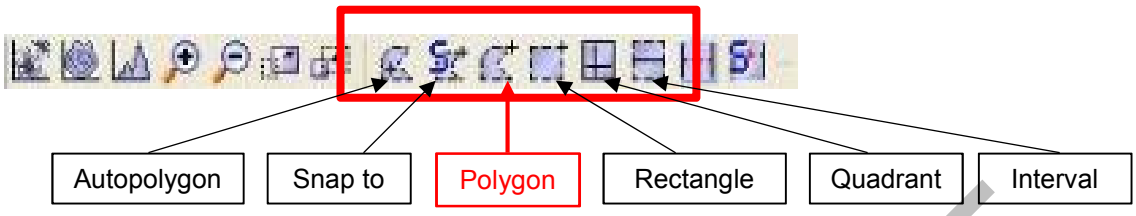
- Adjust voltage of each of the fluorescent channels; preferably the peak of the "negative" population (mean in statistic view) of interest is greater than **ZERO** but less than **10^2**.



- Click Stop Acquiring on Acquisition Dashboard.
- Repeat the steps above with the positive control sample tubes. Adjust the voltage of corresponding channels if their signal peaks are **outside** the limit of the histograms.

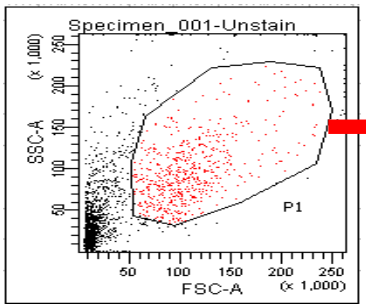
8. Creating Gates

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

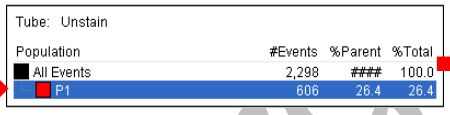


- Gate the cells of interest according to the following sequence by using:

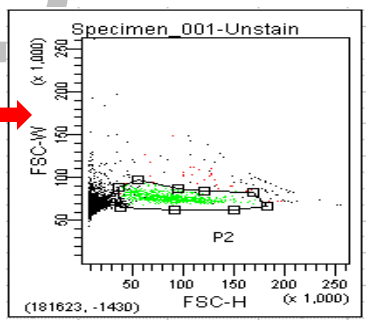
Create a polygon gate (P1) to gate the main population on the FSC-A vs SSC-A dot plot

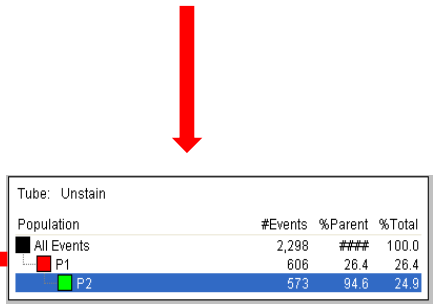


Highlight P1 population in "Population Hierarchy'



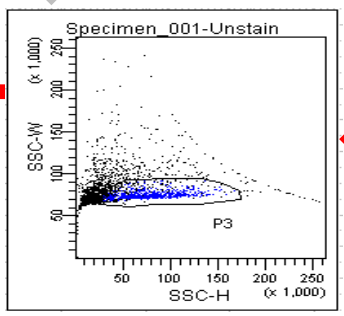
Create a polygon gate (P2) around the singlet event on the FSC-H vs FSC-W dot plot



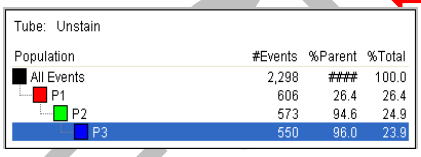


Highlight P2 population under P1

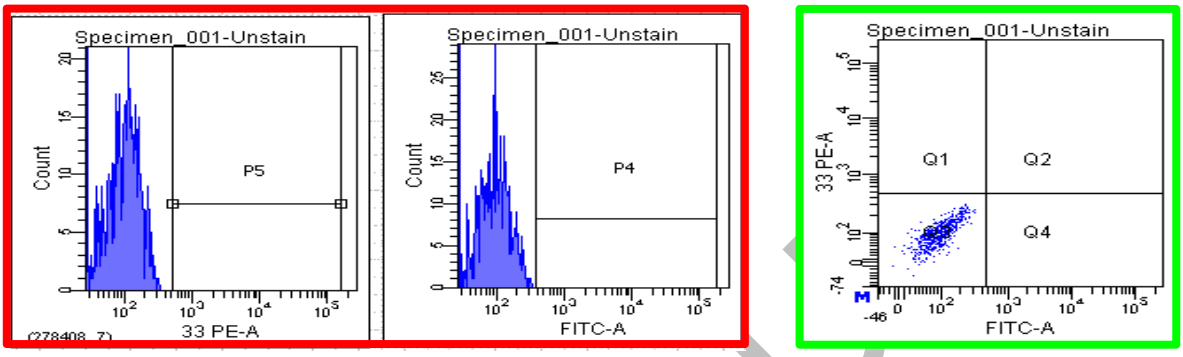
Create a polygon gate (P2) around the singlet event on the FSC-H vs FSC-W dot plot



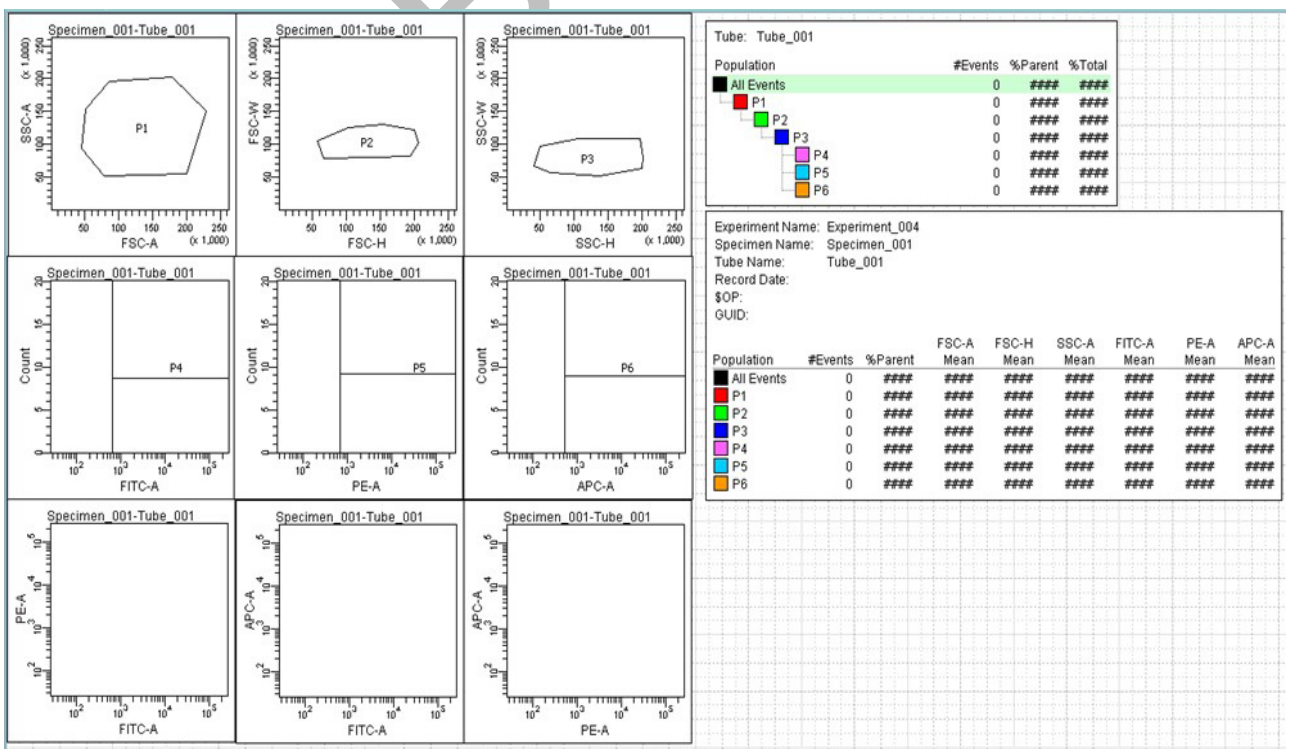
Highlight P3 population under P2



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



* Note that *P1* is the child of *All Events* and the parent of the *P2* population; *P2* population is the child of the *P1* population and the parent of the *P3* population and the grandparent of the *P4*, *P5*, and *P6* populations. Thus, on the hierarchy table, users should highlight the *P1* population when a gate for the *P2* population is drawn, the *P2* population when a gate for the *P3* population is drawn, and the *P3* population when gates for the *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 (sample injection port); Run the unstained sample before other sample tubes.
- 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 the steps above for each sample.

10. Machine Cleaning

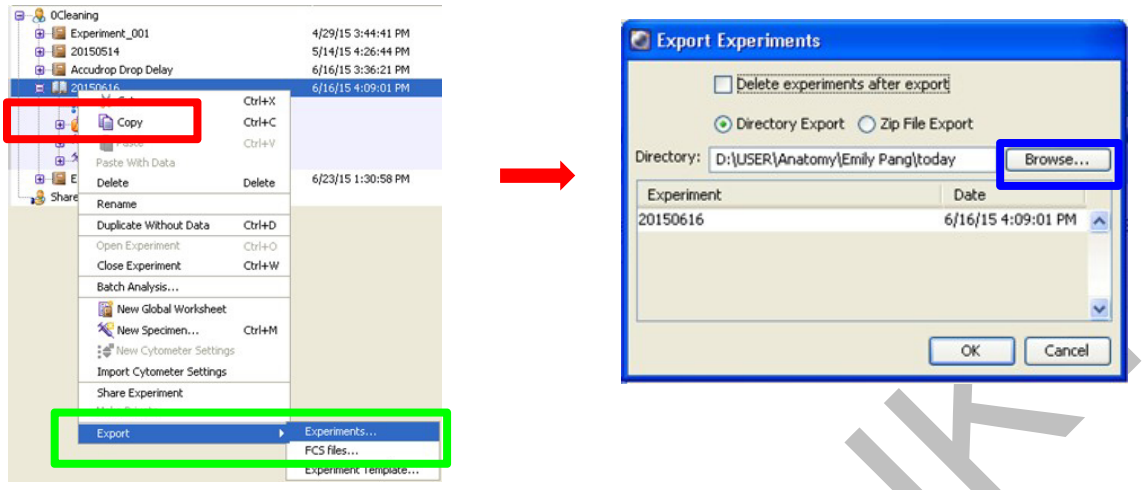
Cleaning procedure between each user is required.

- Prepare **3** mL of each cleaning solution (FACS Clean, FACS Rinse, Milli-Q H_2O).
- Install a tube of FACS Clean solution on the SIP and let it run for **5** minutes with the flow rate set to high.
- Repeat this step with BD™ FACSRinse solution & Milli-Q H_2O.
- Remove the tube from the SIP.

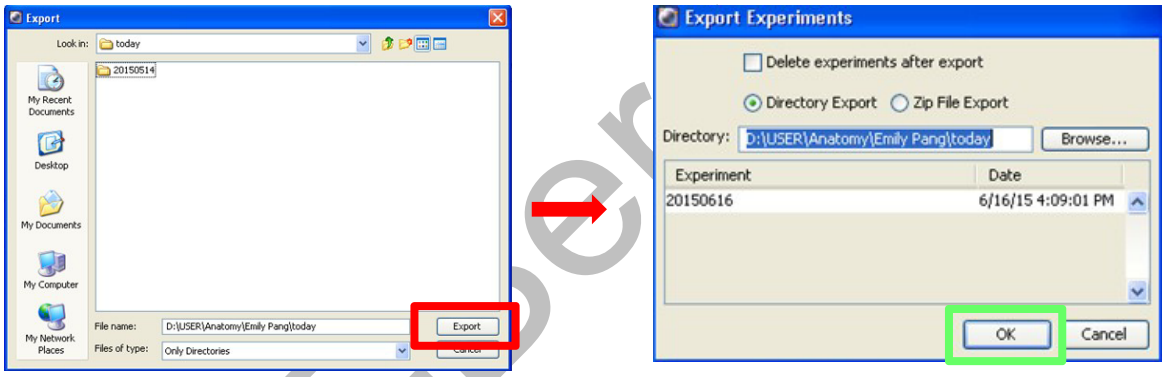
Note: Please be sure in all the cases not to exceed the maximum allowed volume of 2 ml in the tube.

11. Export FCS Data / Experiment

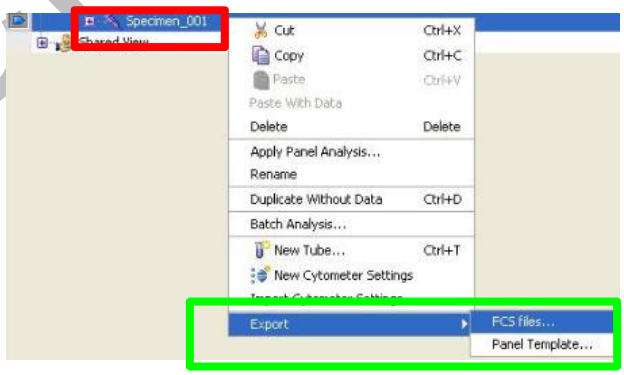
❖ To save Experiment, right click on the **Experiment Export** > **Experiment** > **Browse** to choose the destination folder (**D/year/AG/name)**.



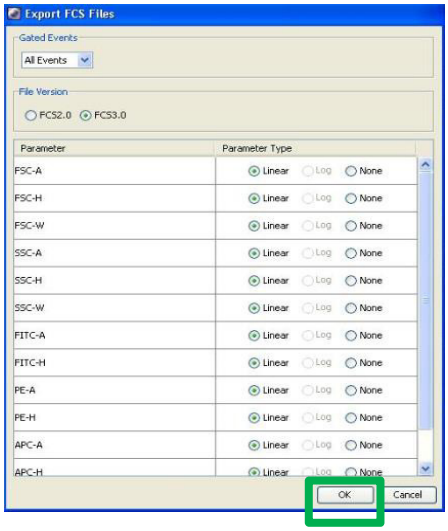
❖ Create a new folder and rename, then click **Export** > **OK**

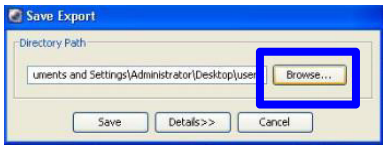


❖ To save FCS files, right click on **Experiment** > **Export** > **FCS Files**

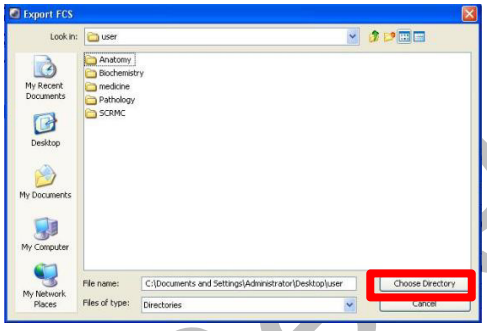


❖ Select **FCS 3.0** > **OK** > **Browse** to choose destination folder (**D/year/AG/name)**.





❖ 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 **duplicate without data**. This retains the mask, the labeled samples and the stored cytometer settings
- It is **not allowed to store more than 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).

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 PPMS schedule:**

- **During the daytime**, we keep the analyzers **"ON" except** there is a gap between users longer than 3 hours;
- If you finish your BD Canto II 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 Canto II** by Starting the **Fluidics shutdown** (7 min)

❖ Choose Cytometer > Fluidics Shutdown.

❖ Turn-off the instrument

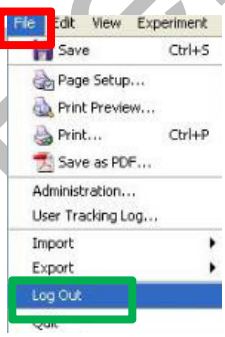
❖ Turn-off the pressure of the external air supply drain (valve on the opposite wall)

❖ Empty the waste,

❖ check and fill up Clean or shut-down solution if necessary

13. Log Out

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



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

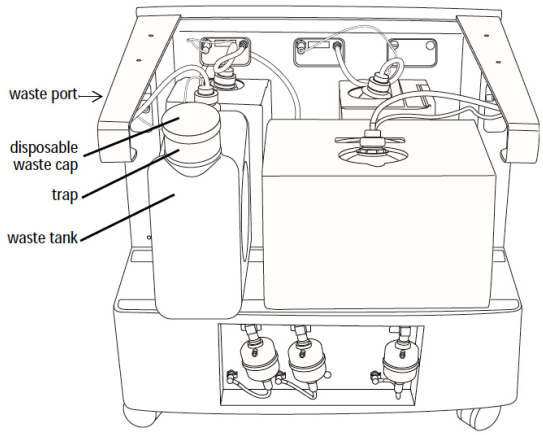
14. Sign in the labbook (next to the computer) for documentation

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

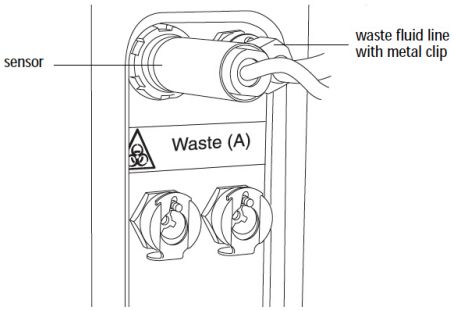
During business hours *— get to Room 581/527 and tell the FCF Berg staff member.*

After hours *— e-mail to fcf-berg@med.uni-tuebingen.de; leave the note about the problem on the instrument's keyboard;*

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



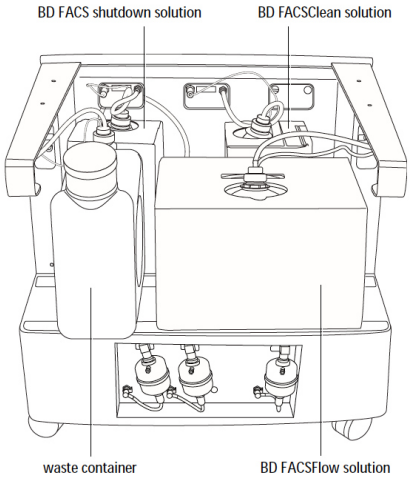
- Ensure the cytometer is not acquiring events.
- Detach the waste container's sensor and fluid line from the fluidics cart waste port.
- Pull the sensor straight out.
- Press the metal clip on the fluid line.



The waste tank can become pressurized when the cytometer is running. Always disconnect the tank from the fluidics cart before you empty it. Wait at least 30 seconds for pressure to dissipate before you remove the waste cap or level sensor cap.

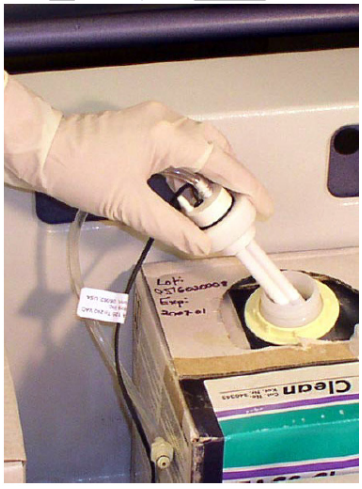
- Remove the disposable waste cap and attached trap from the container; place the assembly on the bench label-side up.
  Do not wet the cap. If you see liquid inside the trap, remove the drain plug and fully drain the liquid before you replace the plug.
- Empty the waste.
- Add approximately 500 ml of clean to the empty waste container (10 L container).
- Screw the cap assembly onto the tank and hand-tighten until it is fully closed.
- Re-attach the sensor and fluid lines.

Refill Flow/Clean or shut down solution Containers (before and/or during measurement)

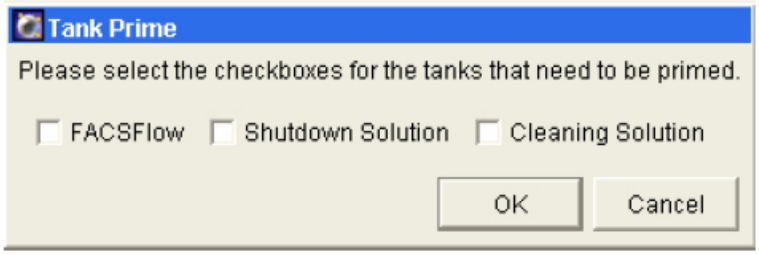


- Ensure the cytometer is not acquiring events.
- Detach the sensor and fluid line from the cart.
- Pull the sensor straight out.

- Press the metal clip on the fluid line.
- Unscrew the cap on the container.
- Remove the cap and sensor assembly and set it aside.



- Put a new container onto the fluidics cart.
- Replace the cap assembly and hand-tighten it until it is fully closed.
- Reattach the sensor line and fluid line to the cart.
    - To attach the sensor line, gently rotate until the connection aligns, and then push.
    - To attach the fluid line, push the coupling into the port until it clicks.
- **Prime the fluidics** when you refill a tank during measurement.
    - Use the “Prime After Tank Refill” command to remove air from the fluidics lines after you changed a container.
        - Choose Cytometer (Instrument) > Cleaning Modes > Prime After Tank Refill.
        - Select the checkboxes for the containers you changed; click OK.



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