

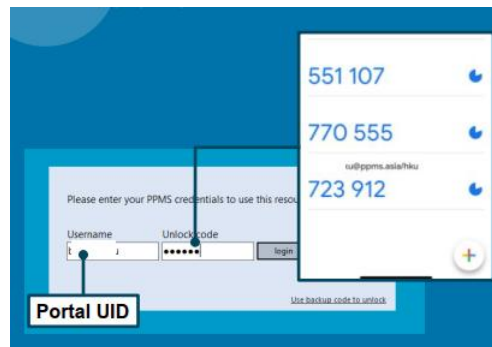


Imaging and Flow Cytometry Core

BD LSRT Fortessa Standard Operation Protocol

A. Log in the Window and Tracker

1. Press Ctrl+Alt+Delete
2. Account: **User**
Password: posted under the bottom of the computer screen
3. Log in Tracker





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B. Log in the BD FACSDiva Software

1. Key in **username** and **password** and click **OK** to log in.
*Please contact the Technical Staff in charge if you do not have an account.
**Please contact the Technical Staff in charge if you forget password.



2. Click **Use CST Settings** when pop-up message as below is shown.



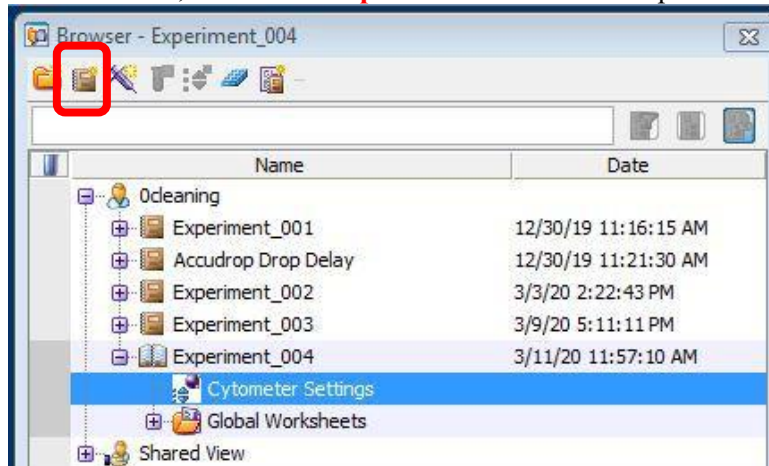


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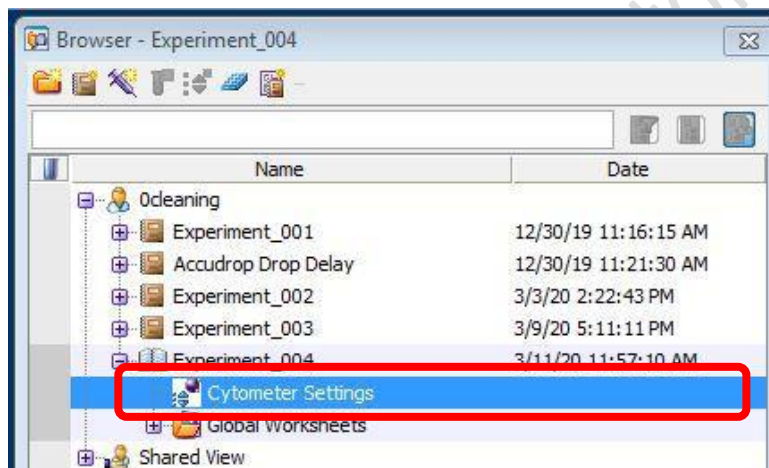
C. Experiment Setup

1. Setup New Experiment

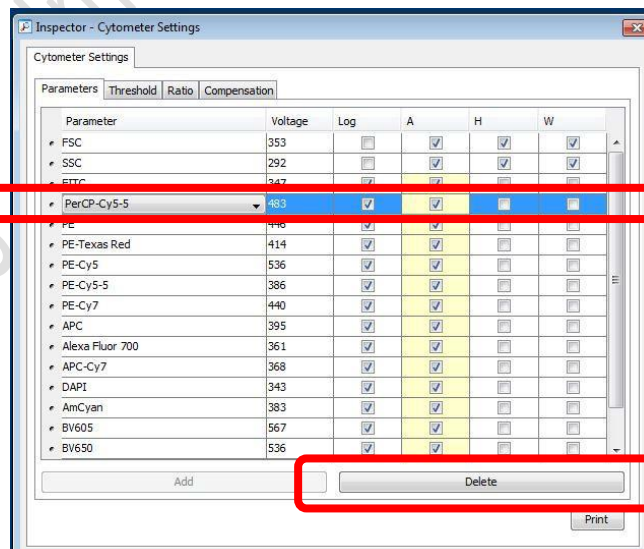
1.1 Go to Browser, Click **New Experiment** icon. A new experiment will be created



1.2 Click **Cytometer Settings** under the newly created Experiment



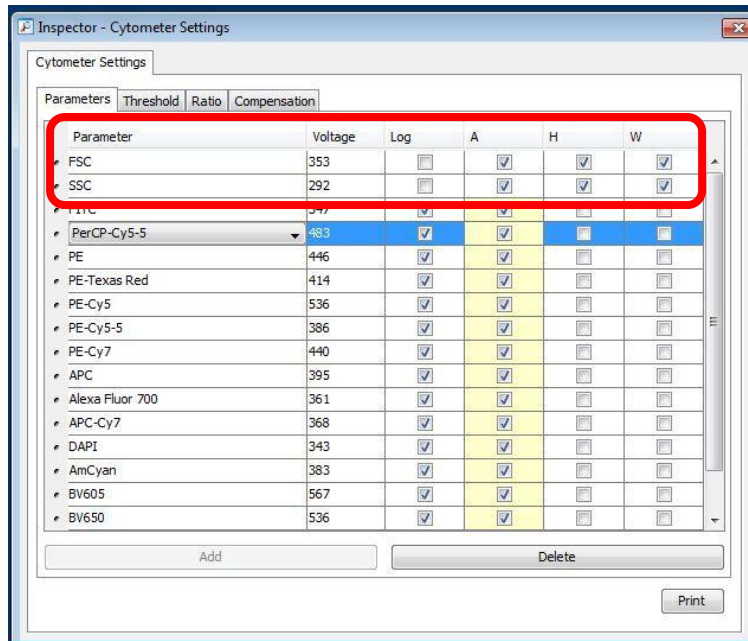
1.3 Go to Inspector Window, select **unwanted** channels and click **Delete**





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1.4 Check **H** and **W** boxes of FSC and SSC



1.5 Keep **Log** boxes of FSC and SSC **unchecked**

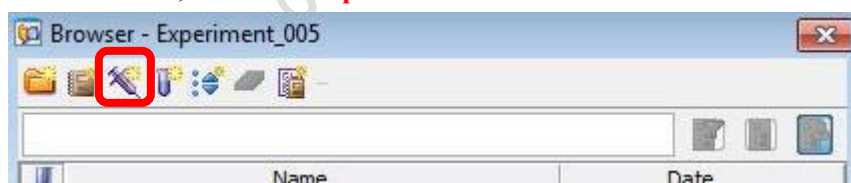
1.6 Keep **Log** boxes of all fluorescence channels **checked**

* If you are doing **cell cycle or DNA content** analysis, please keep **log box** of your DNA specific fluorescence channel **unchecked**.

2. Setup Compensation – Please refer to **FACSDiva compensation automated protocol**

3. Setup Plots and Tables

3.1 Go to Browser, Click **New Specimen icon**



3.2 Expand Specimen_001

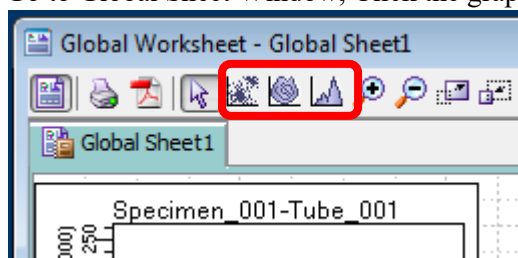





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3.3 Click the tube **pointer** of Tube_001



3.4 Go to Global Sheet Window, Click the graph type icon

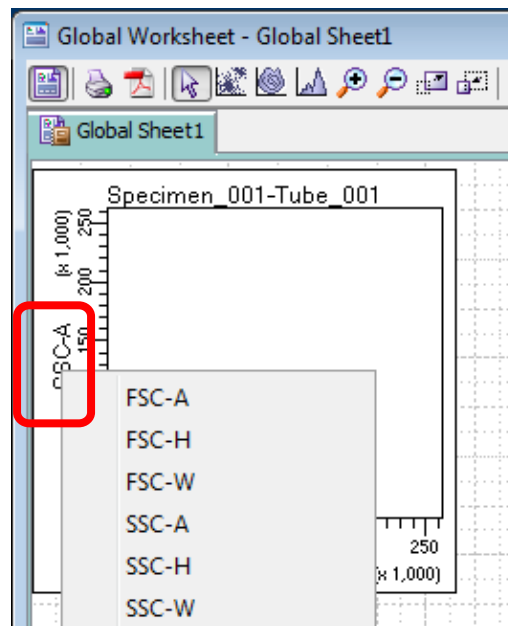


Icon	Type
	Dot Plot
	Contour Plot
	Histogram

3.5 Click on the blank area of Global Worksheet window to create a new plot.

3.6 Mouse over the axis label and right click. Select the parameters of interest from the list.

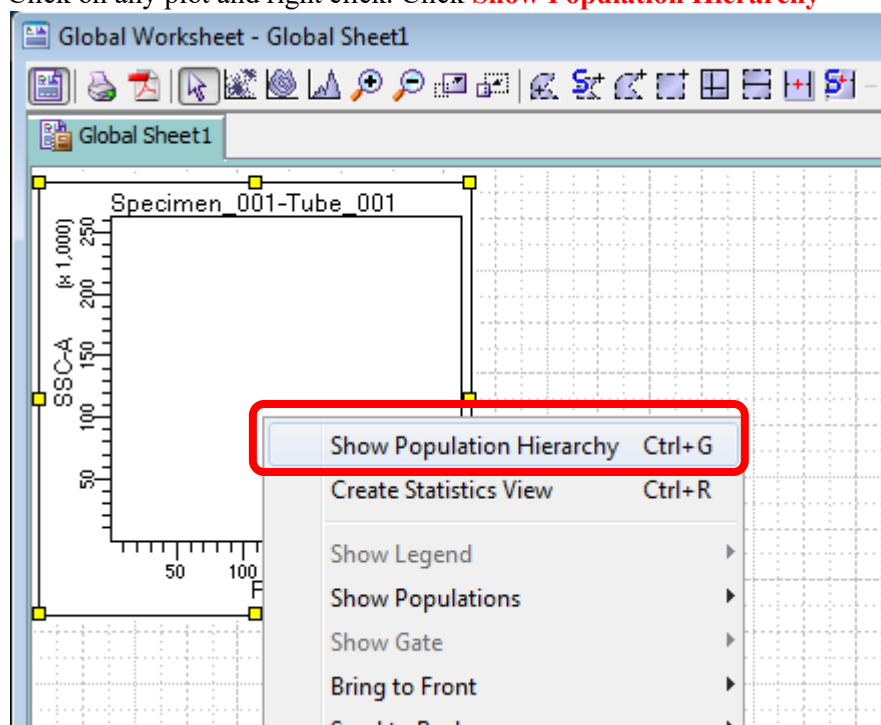
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3.7 Repeat step 3.4 – 3.6 until all plots needed is created.

* **Essential Plots:** FSC-A VS SSC-A; FSC-H VS FSC-W; SSC-H VS SSC-W

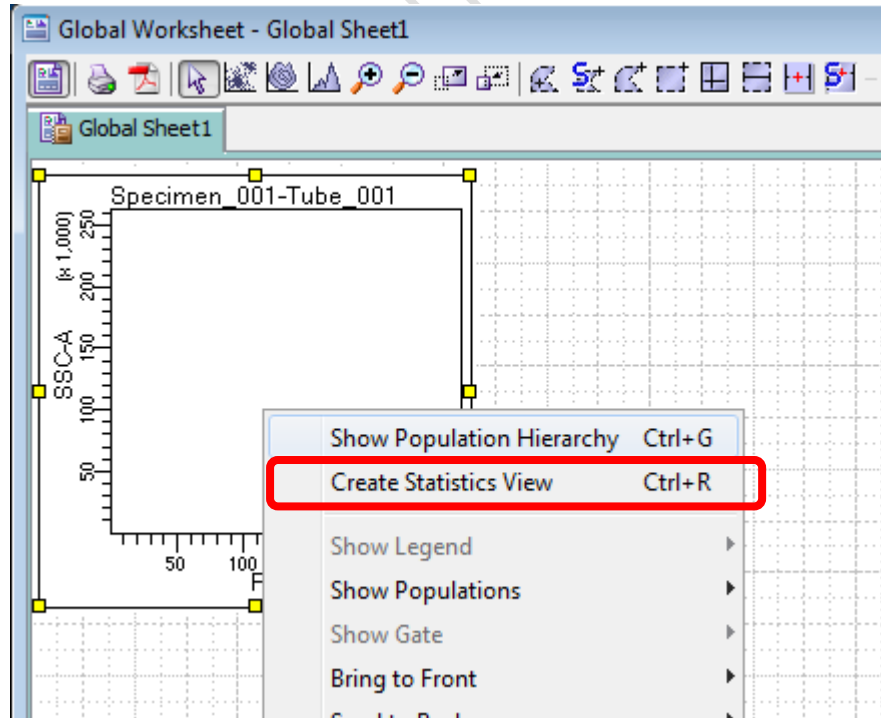
3.8 Click on any plot and right click. Click **Show Population Hierarchy**





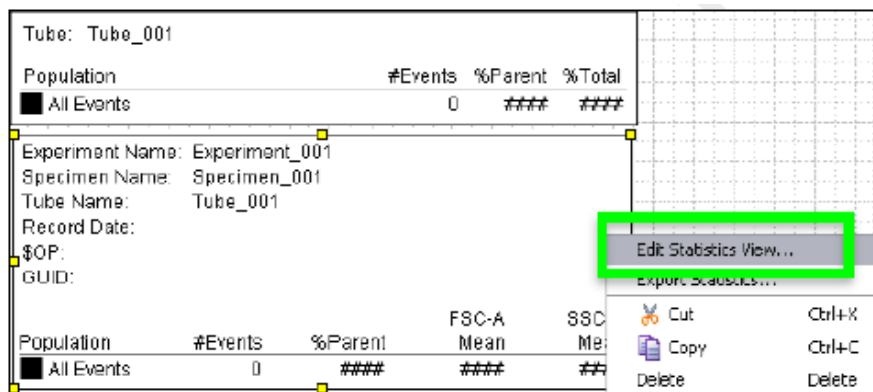
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3.9 Click on any plot and right click. Click **Create Statistics View**

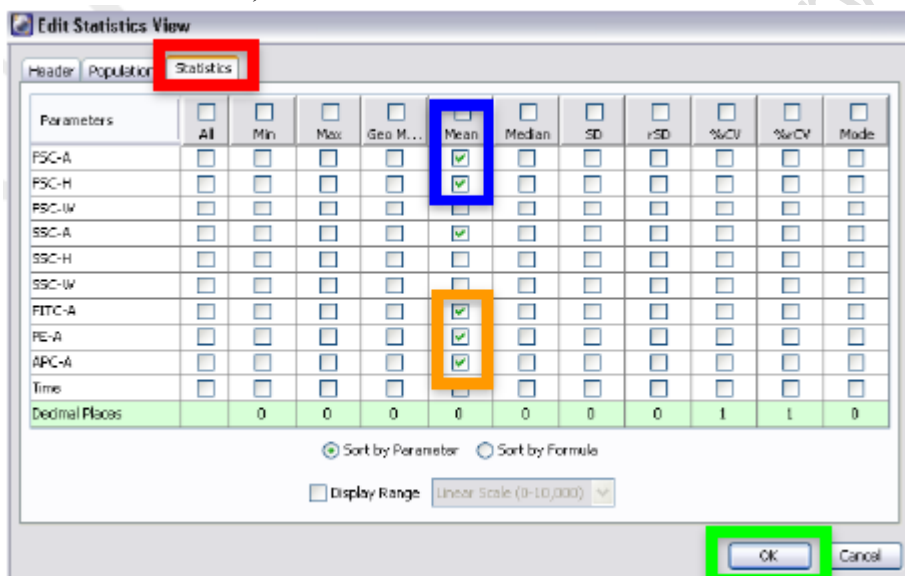


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3.10 Right Click on Statistics View table, select **Edit Statistics View** to select statistics of interest to be shown in the table.



3.11 Click **Statistics** Tab, check the boxes of interested statistics and then click **OK**



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D. Sample Acquisition

1. Gently tab or pulse vortex your sample and put your sample tube on SIP (Sample injection port).

*Run the unstained/negative control sample before stained samples.

!! DO NOT return the support arm to the center without putting a tube filled with liquid on SIP!!

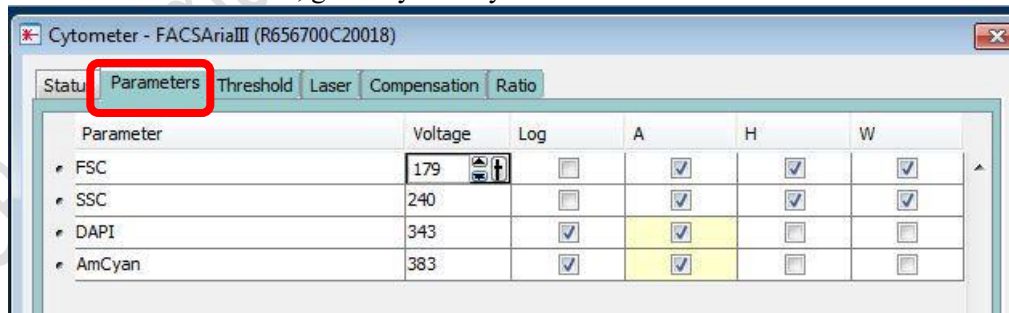
2. Press **RUN** and **LOW** on the fluidics control panel.



3. Go to Acquisition Dashboard, Click **Acquire Data**

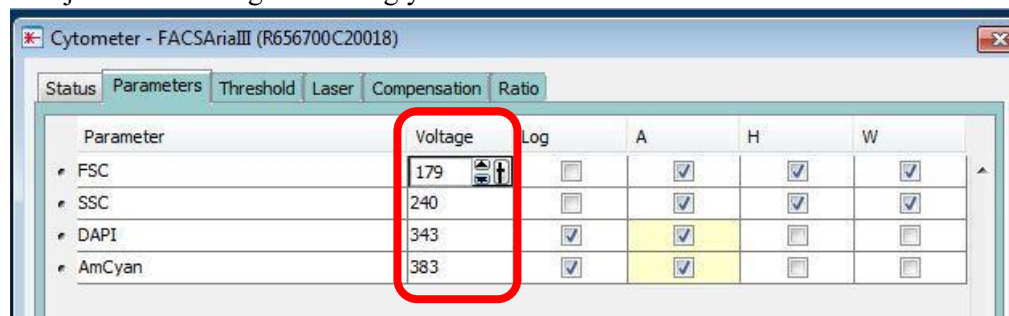


4. When Threshold rate > 0, go to Cytometry window and Click **Parameters** tab.

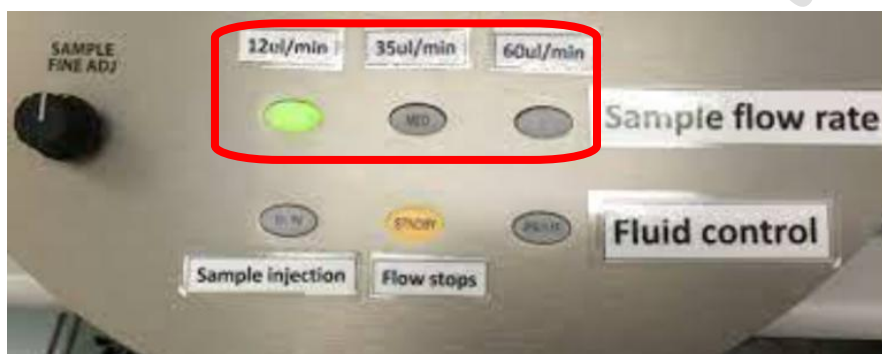


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5. Adjust PMT Voltage accordingly



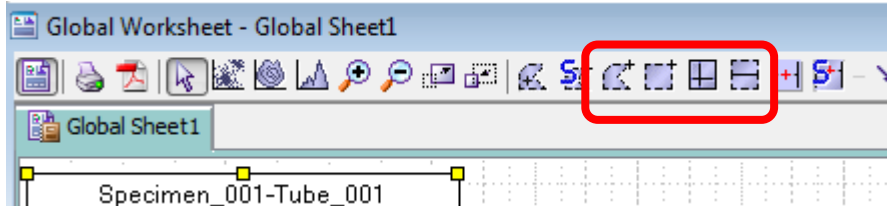
6. Adjust **Sample flow rate** on the fluidics control panel if needed (Maximum Threshold rate 8000 evt/s)







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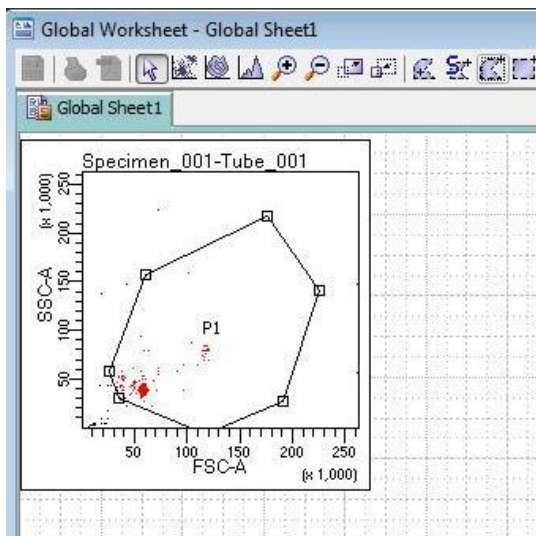
E. Create Gates

1. Go to Global Sheet Window, Click the type of gate needed



Icon	Type
	Polygon Area Gate
	Rectangle Area Gate
	Quantrad Gate
	Interval Gate

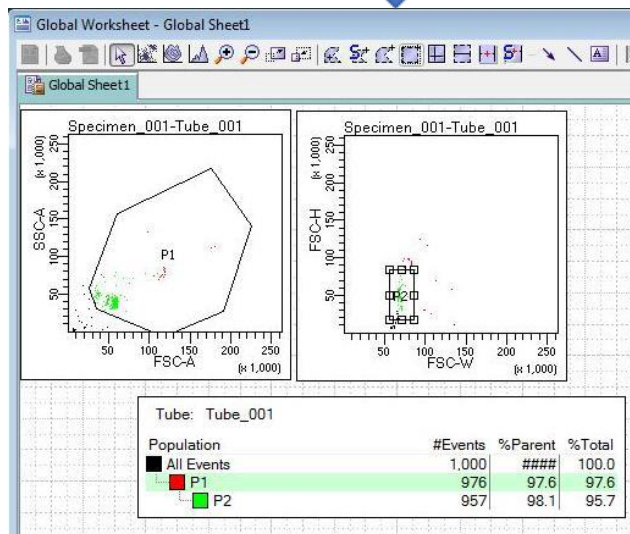
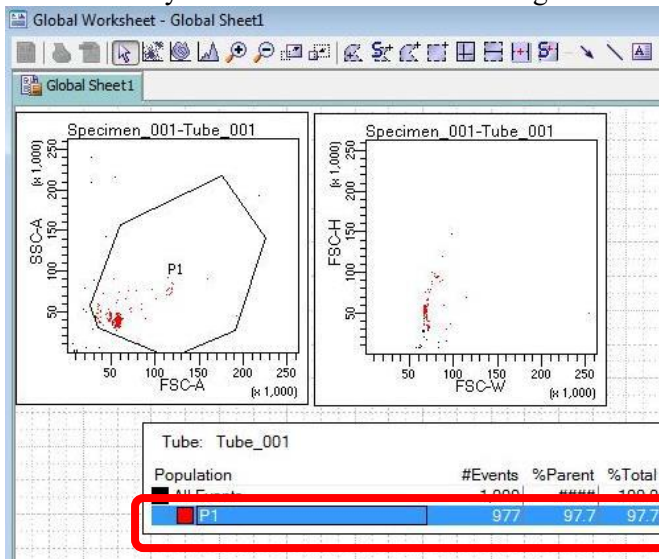
2. Draw the gate on the plot of interest to gate out target cluster /peak





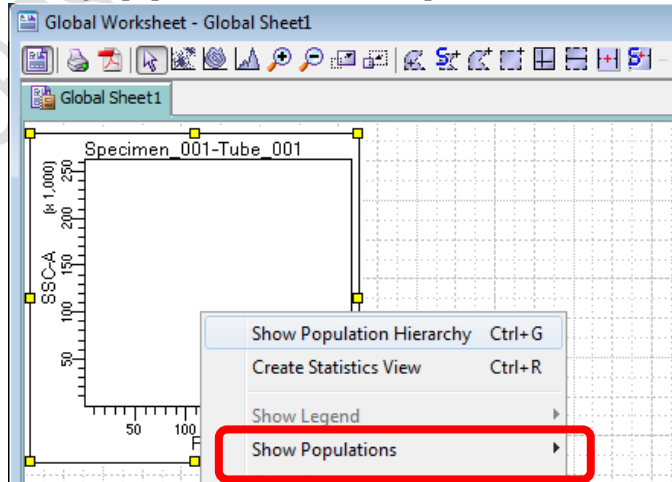
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3. If you want to create a new population out of parent population, highlight the parent gate on the hierarchy table first and then create the gate.



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4. Click on target plot and right click, Click *Show Population > Target population* to visualize the target population ONLY in that plot.





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F. Data Recording

1. Go to Acquisition Dashboard, set **Stopping gate** to singlet gate or live cell gate

The screenshot shows the 'Acquisition Dashboard' window. Under 'Current Activity', 'Active Tube/Well' is 'Tube_001', 'Threshold Rate' is '0 evt/s', 'Stopping Gate Events' is '0 evt', and 'Elapsed Time' is '00:00:00'. In the 'Acquisition Setup' section, 'Stopping Gate' is set to 'P2' (highlighted with a red box), 'Events To Record' is '20000 evt', and 'Stopping Time (sec)' is '0'. 'Storage Gate' is set to 'All Events' and 'Events To Display' is '1000 evt'. The 'Basic Controls' section has buttons for 'Next Tube', 'Acquire Data', 'Record Data', and 'Restart'. The 'Acquisition Status' section shows 'Processed Events' and 'Threshold Count' on the left, and 'Electronic Abort Rate' and 'Electronic Abort Count' on the right.

2. Set the Storage gate to All Events

This screenshot is identical to the previous one, but the 'Storage Gate' is now set to 'All Events' (highlighted with a red box). The 'Stopping Gate' remains 'P2'.

3. Set Events To Record, i.e. events number out of stopping gate to be recorded

This screenshot is identical to the previous ones, but the 'Events To Record' value is now '20000 evt' (highlighted with a red box). The 'Stopping Gate' remains 'P2' and 'Storage Gate' remains 'All Events'.



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4. Click **Record Data**

The screenshot shows the 'Acquisition Dashboard' window. The 'Current Activity' section displays 'Active Tube/Well' as 'Tube_001', 'Threshold Rate' as '0 evt/s', 'Stopping Gate Events' as '0 evt', and 'Elapsed Time' as '00:00:00'. The 'Basic Controls' section contains four buttons: 'Next Tube', 'Acquire Data', 'Record Data' (highlighted with a red rectangle), and 'Restart'. The 'Acquisition Setup' section includes 'Stopping Gate' (set to 'P2'), 'Events To Record' (20000 evt), 'Stopping Time (sec)' (0), 'Storage Gate' (set to 'All Events'), and 'Events To Display' (1000 evt). The 'Acquisition Status' section shows 'Processed Events' and 'Threshold Count' on the left, and 'Electronic Abort Rate' and 'Electronic Abort Count' on the right.

5. Click **Next Tube** to create a new sample

The screenshot shows the 'Acquisition Dashboard' window after clicking 'Next Tube'. The 'Current Activity' section remains the same. The 'Basic Controls' section shows the 'Next Tube' button highlighted with a red rectangle. The 'Acquisition Setup' and 'Acquisition Status' sections are identical to the previous screenshot.

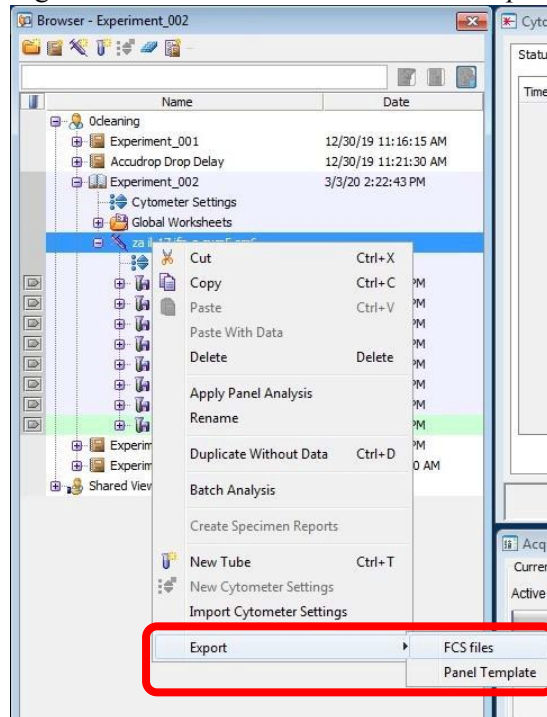
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G. Data Export

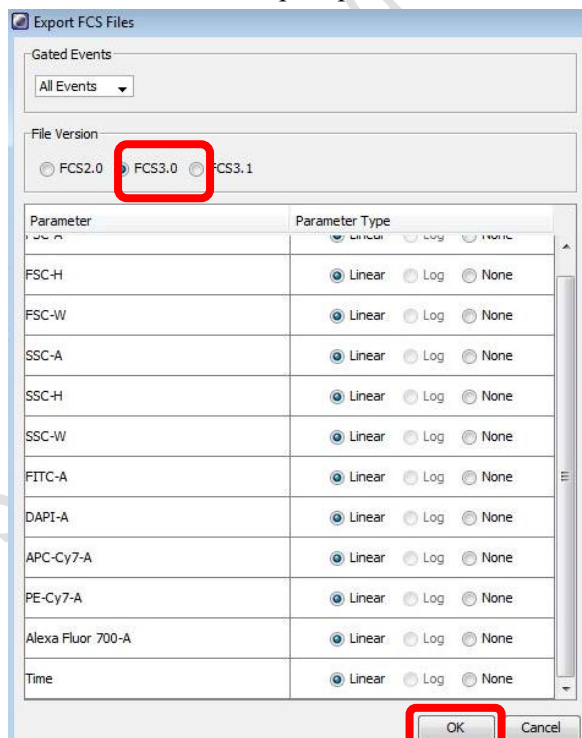
1. FCS file

1.1 Go to Browser window, Select the Tubes / Specimen of interest.

1.2 Right Click over the selection and click *Export > FCS file*



1.3 Select *FCS 3.0* and keep all parameters Linear. Click *OK*

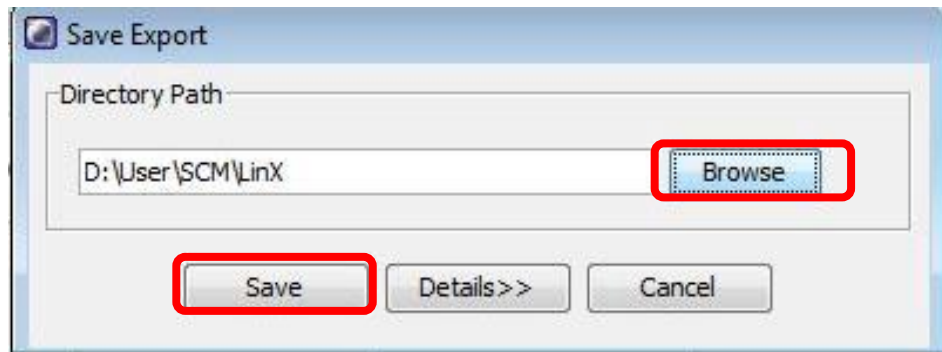




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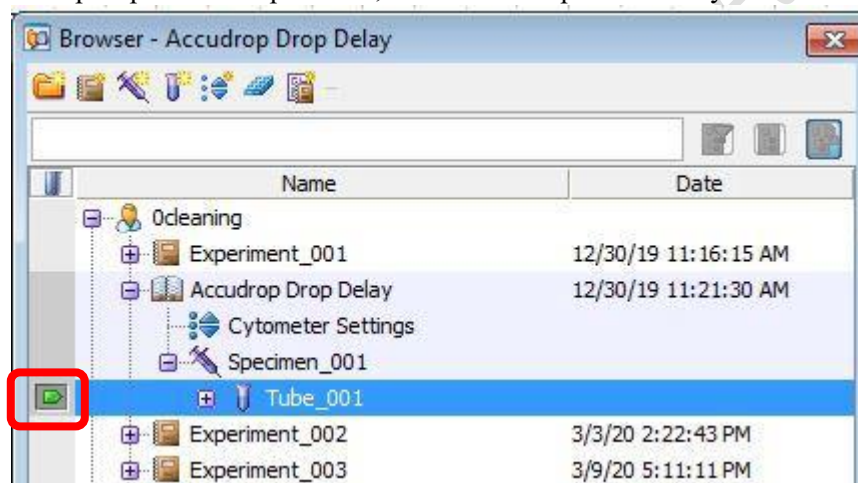
1.4 Click **Browse** to choose the destination (D:/User/Department/Personal Folder)

1.5 Click **Save**



2. PDF file

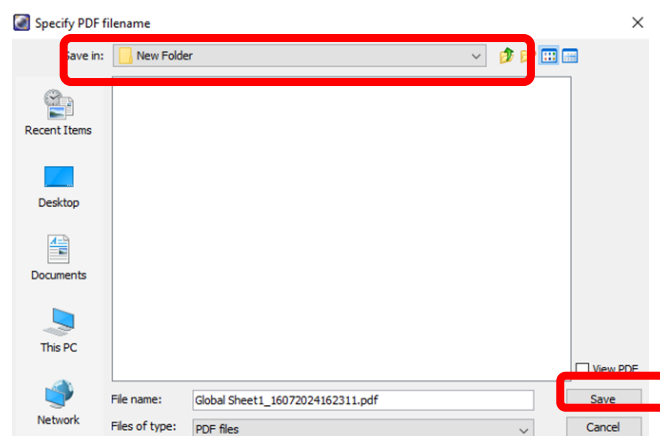
2.1 To export pdf of multiple tubes, Click the tube pointer of any tubes



2.2 Click PDF icon



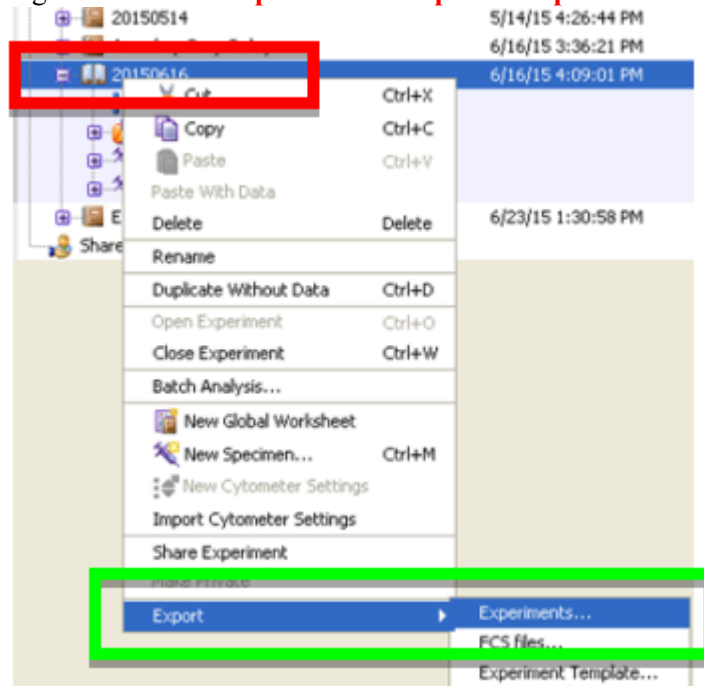
2.3 Choose the destination (D:/User/Department/PersonalFolder) and click *Save*



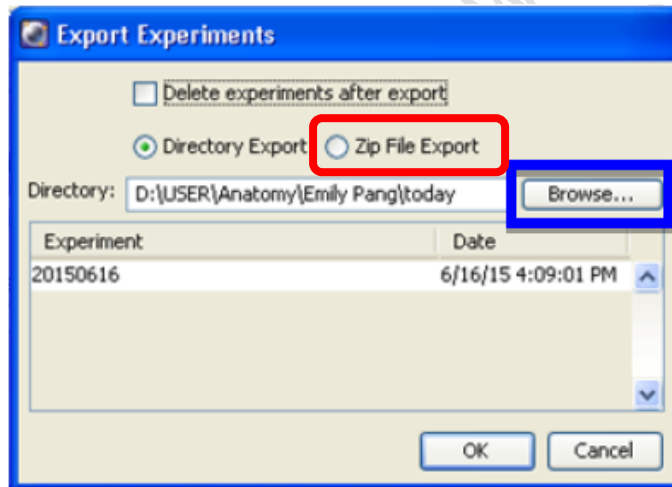
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3. Experiment

3.1 Right click on the **Experiment > Export > Experiments**

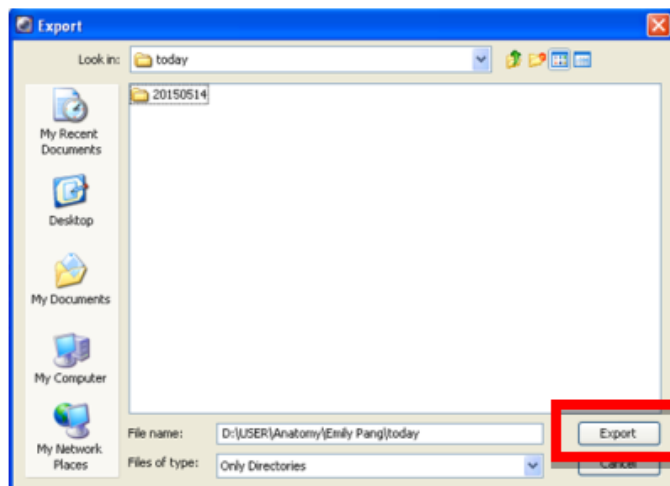


3.2 Choose **Zip File Export** and click **Browse** to select the file destination

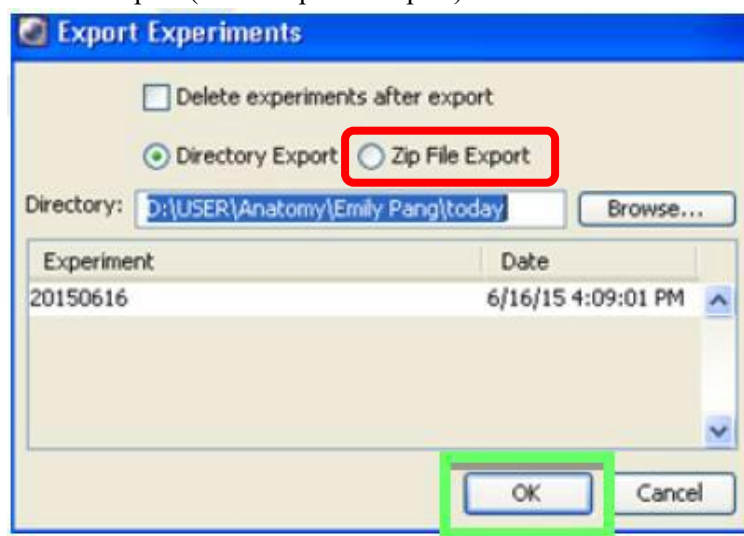


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Select the file destination and click **Export**.

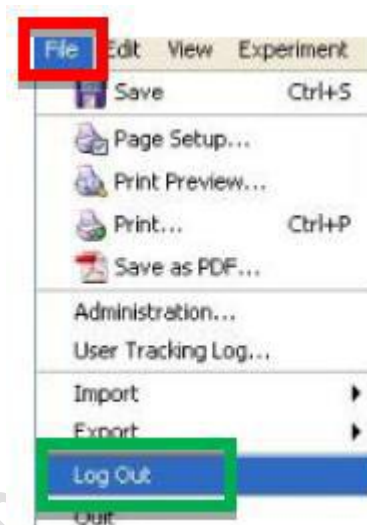


3.4 Click **OK** to export (select Zip File Export)



H. Software Log out

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





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I. System Cleaning

***Cleaning procedure will not interfere with the data export and analysis.**

1. Prepare 3 mL of each cleaning solution (Solution 1: FACSClean; Solution 2: FACSRinse; Solution 3: MilliQ water)
2. Press **HIGH (60 uL/min)** and **RUN (Sample Injection)** on fluidics control panel
3. Load the tube with Solution 1 on the SIP with the support arm on the side for 1 minute
4. Move the support arm under the tube and run for another 4 minutes
5. Repeat step 3 and 4 with Solution 2 and Solution 3
*If PI stain is used, please clean the system 9 minutes instead of 4 for Solution 2
6. After cleaning with Solution 3, press **LOW (12 uL/min)** and **STANDBY (Flow Stop)**