BD LSRFortessa Standard Operation Protocol

Basic Operation – Tube mode

A. Log in the Window

1. Press Ctrl+Alt+Delete

2. Key in Password **BDIS#3$$**
   ***No need to switch user**

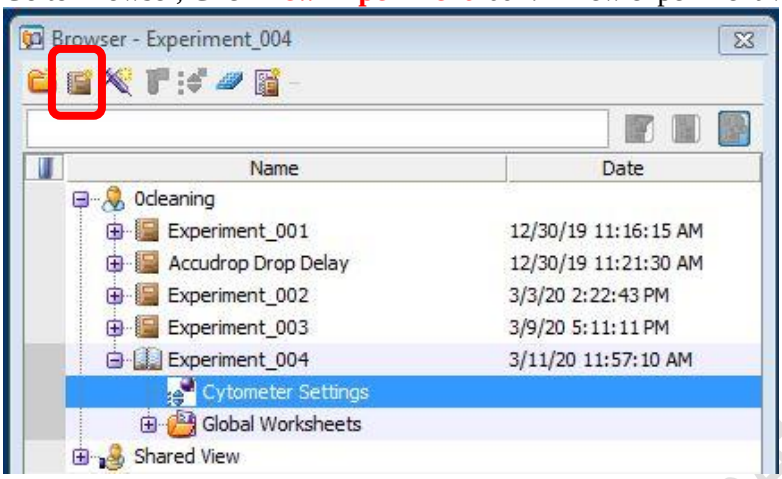
B. Log in the BD FACSDiva Software

1. Key in **user name** 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.

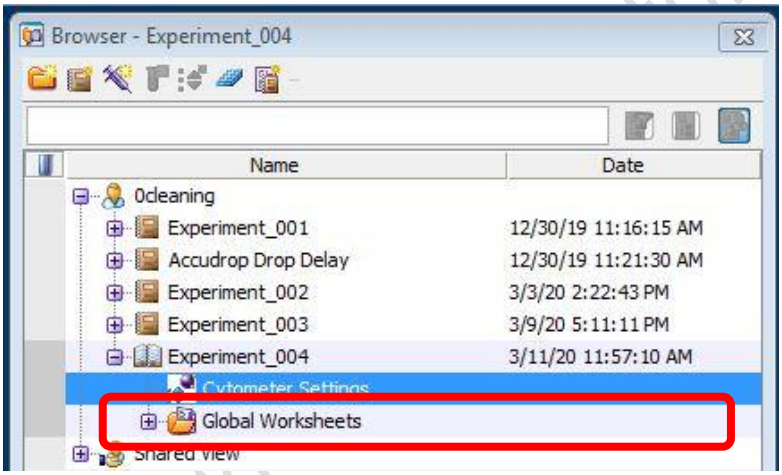
2. Click **Use CST Settings** when pop-up message as below is shown.

C. Experiment Setup

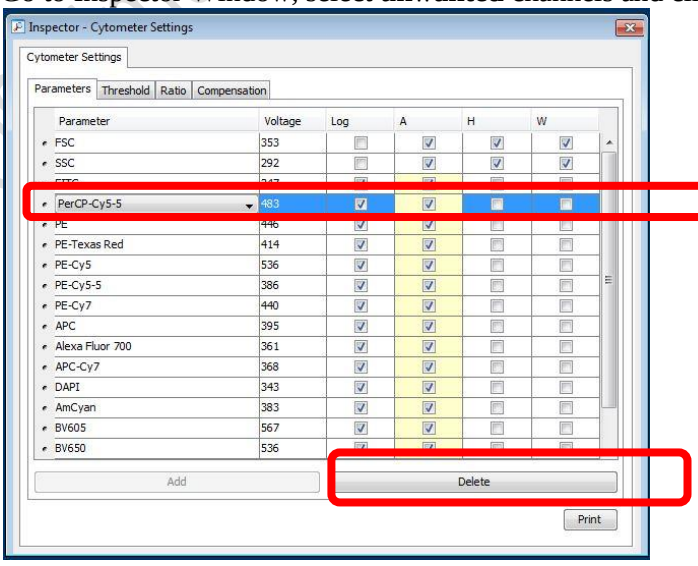
1. Setup New Experiment
   1.1 Go to Browser, Click **New Experiment** *icon*. A new experiment will be created



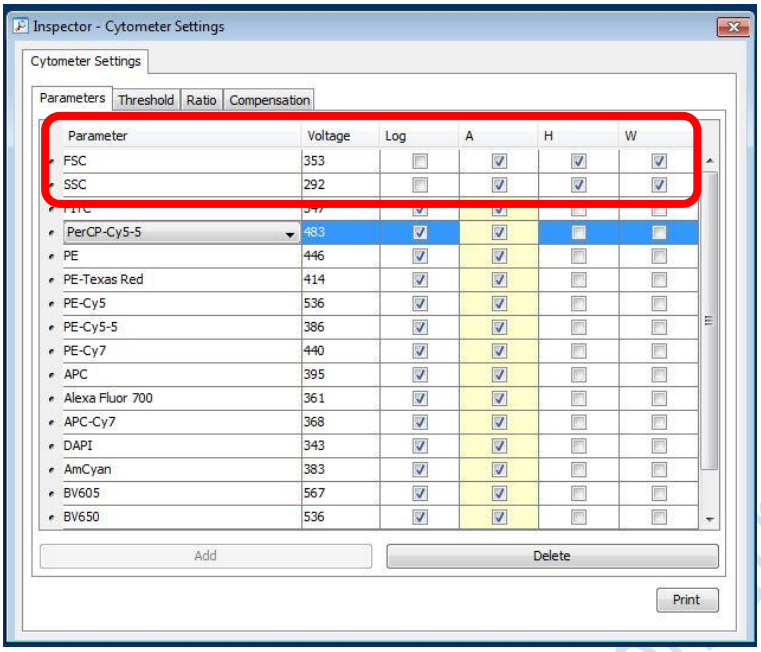
   1.2 Click **Cytometer Settings** under the newly created Experiment



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



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**

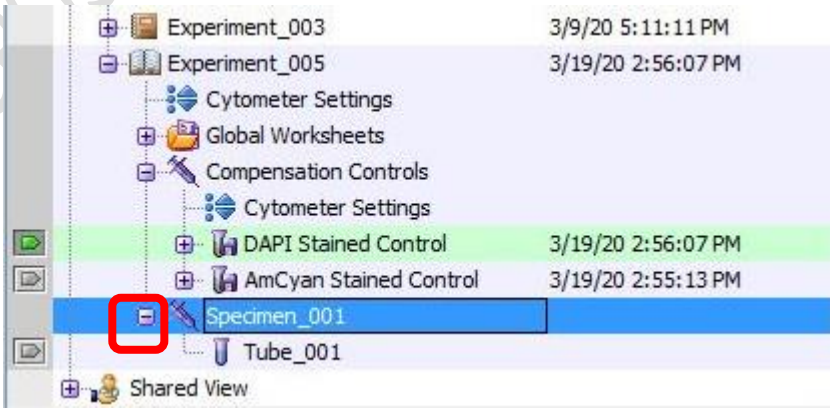
* If you are doing **cell cycle or DNA content** analysis, please keep **log box** of your DNA specific fluorescence channel **unchecked**.

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

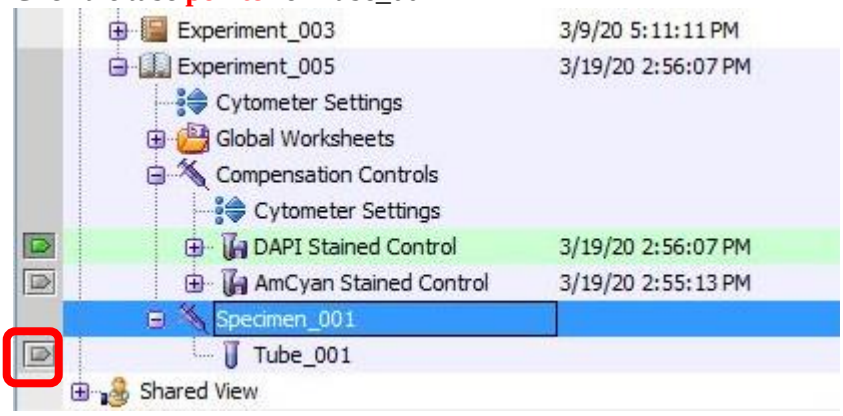
3. Setup Plots and Tables

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

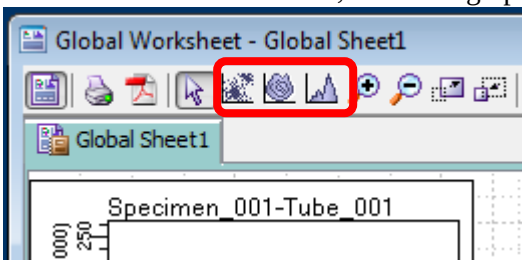
3.2 Expand Specimen_001



3.3 Click the tube **pointer** of Tube_001



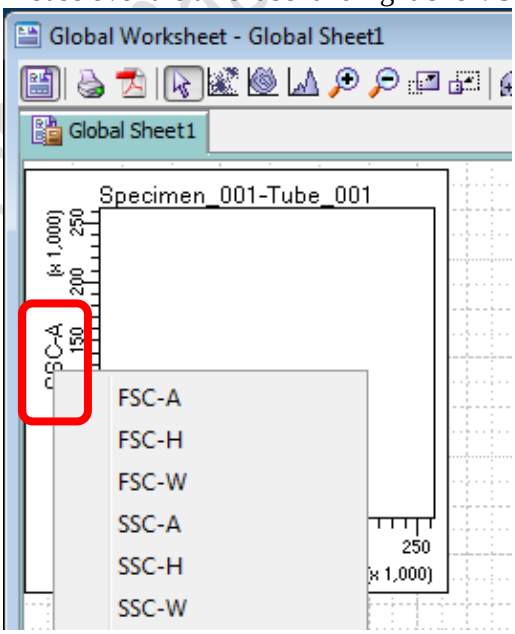
3.4 Go to Global Sheet Window, Click the graph type icon



Icon	Type
	Dot Plot
	Contour Plot
	Histogram

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

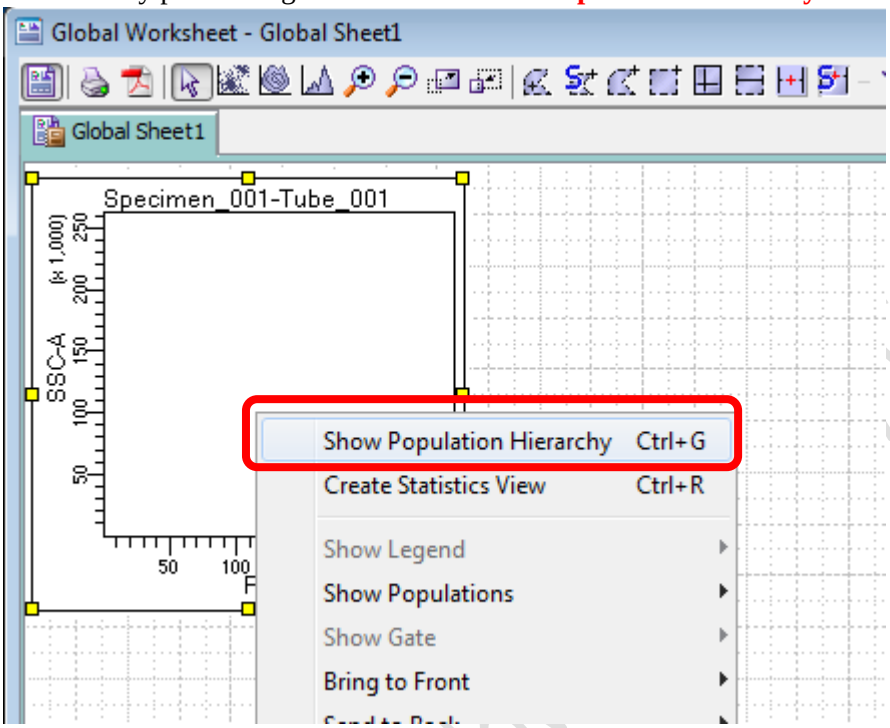
3.6 Mouse over the axis label and right click. Select the parameters of interest from the list.



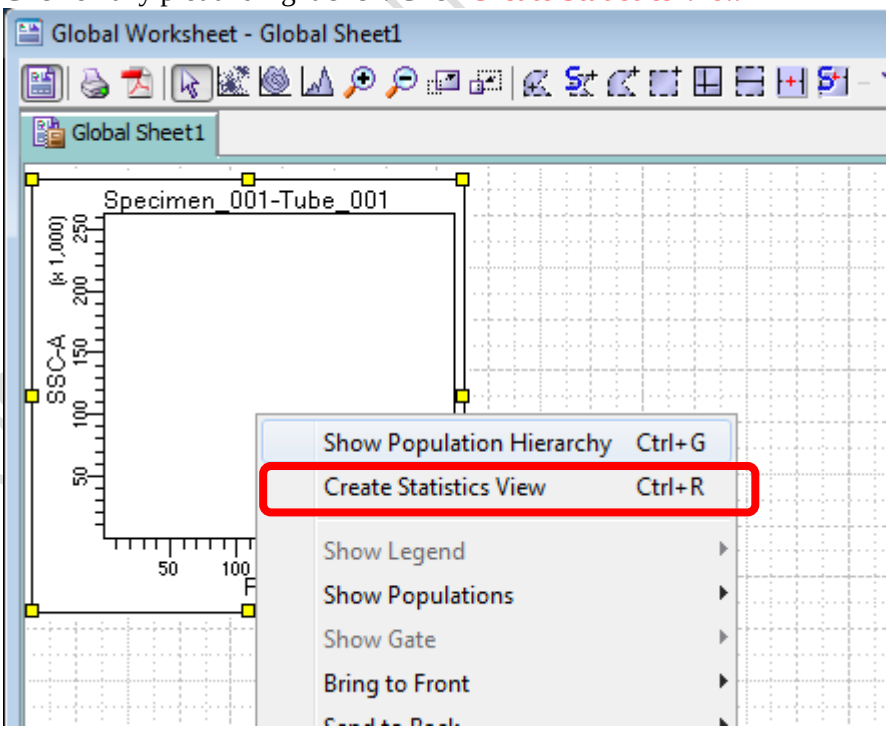
3.7 Repeat step 3.4 – 3.6 until all plots needed is created.

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

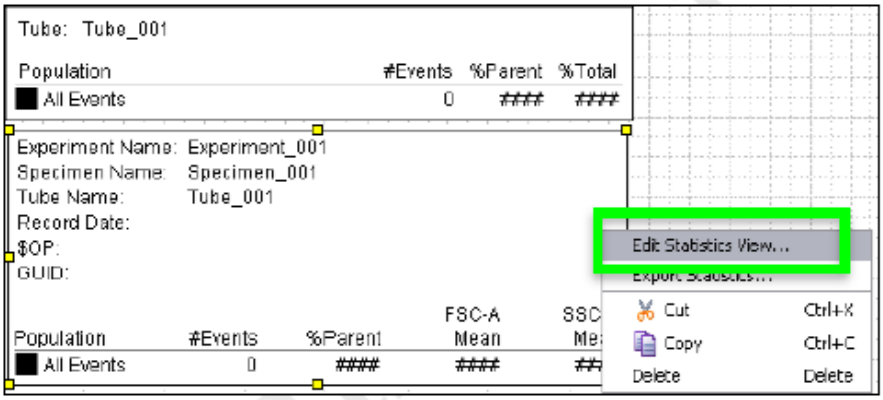
3.8 Click on any plot and right click. Click **Show Population Hierarchy**



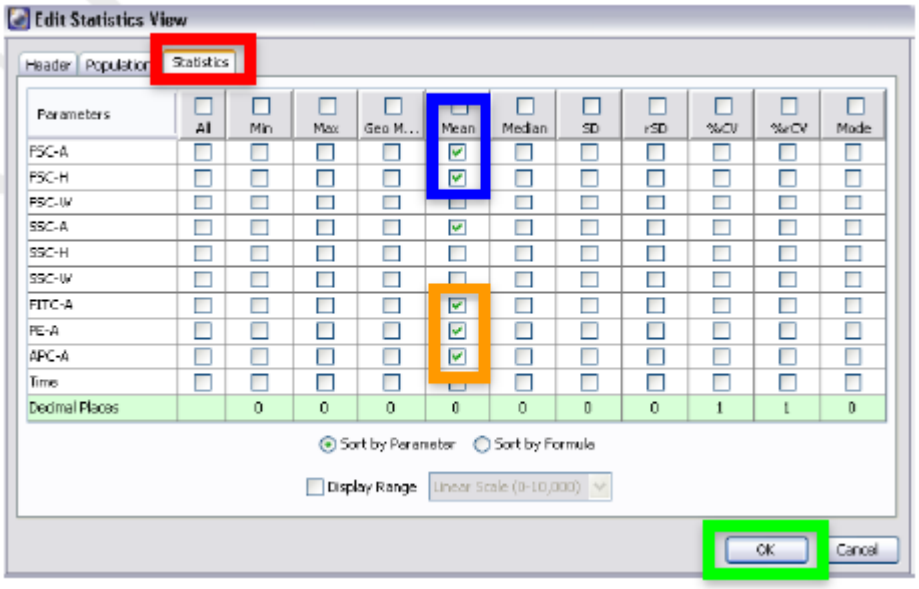
3.9 Click on any plot and right click. Click **Create Statistics View**



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**

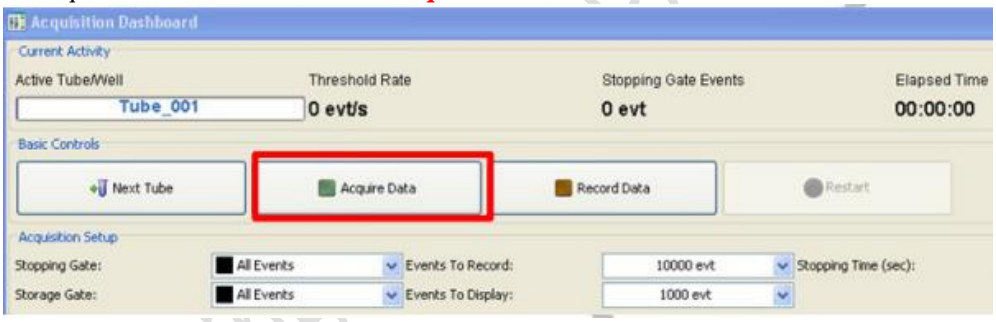


D. Sample Acquisition

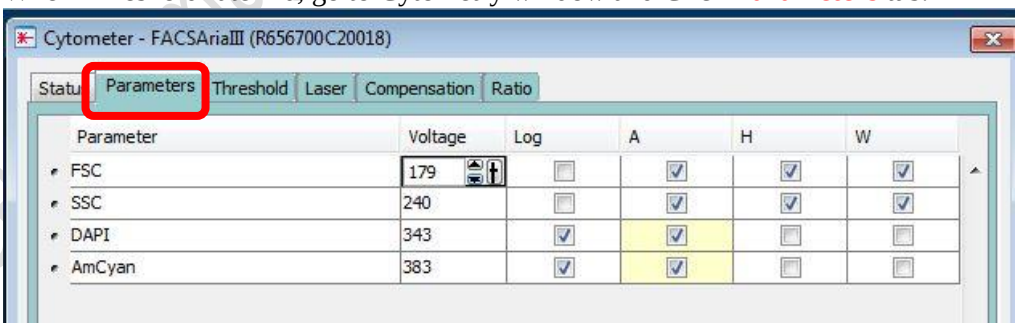
1. Gently tab or pulse vortex your sample and put your sample tube on SIP (Sample injection port).
   *Run the unstained/negative control sample before stained samples.
   !! DO NOT return the support arm to the centre without putting a tube filled with liquid on SIP!!

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

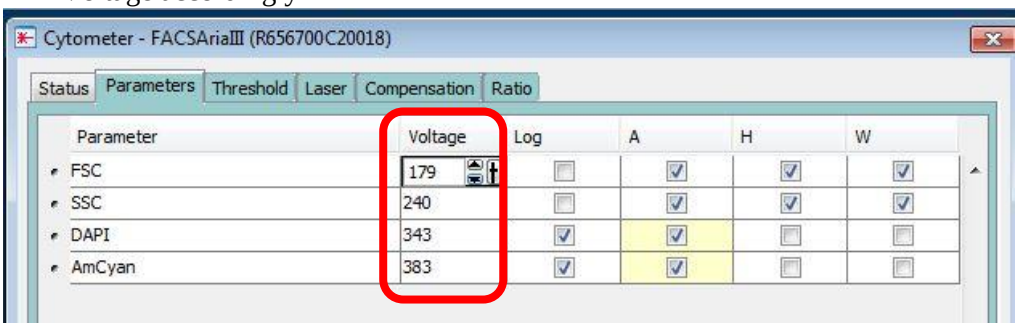
3. Go to Acquisition Dashboard, Click **Acquire Data**



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

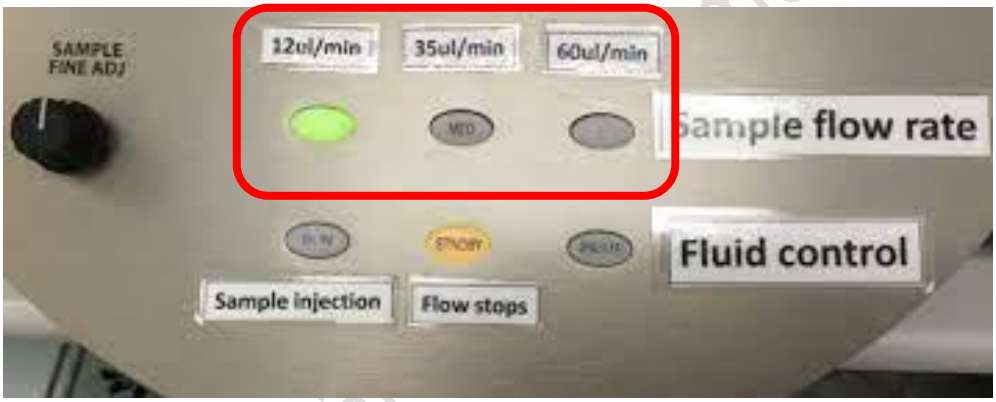


5. Adjust PMT Voltage accordingly



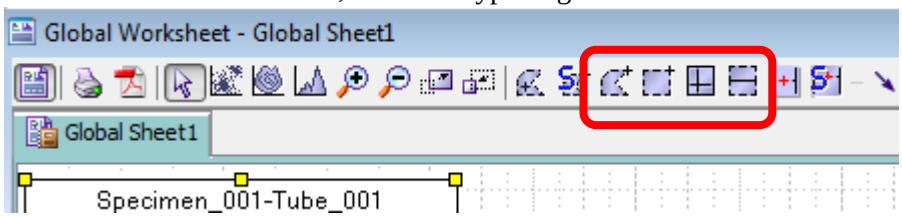
Channel	Suggested Voltage range for mammalian cells
FSC	450-600
SSC	250-310
Fluorescence	300-850

6. Adjust **Sample flow rate** on the fluidics control panel if needed (optimum Threshold rate 2000 – 5000 evt/s)



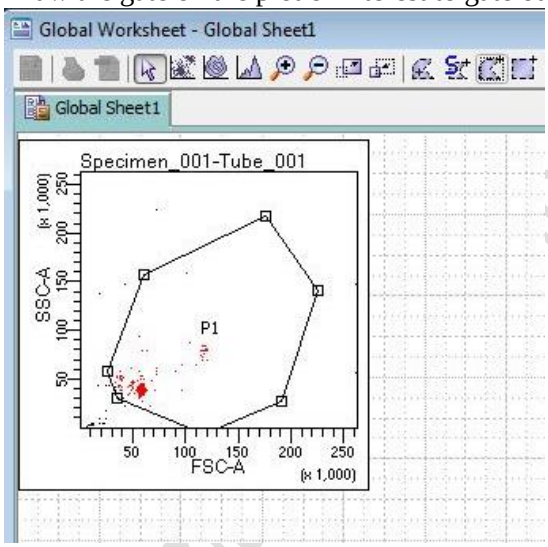
E. Create Gates

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

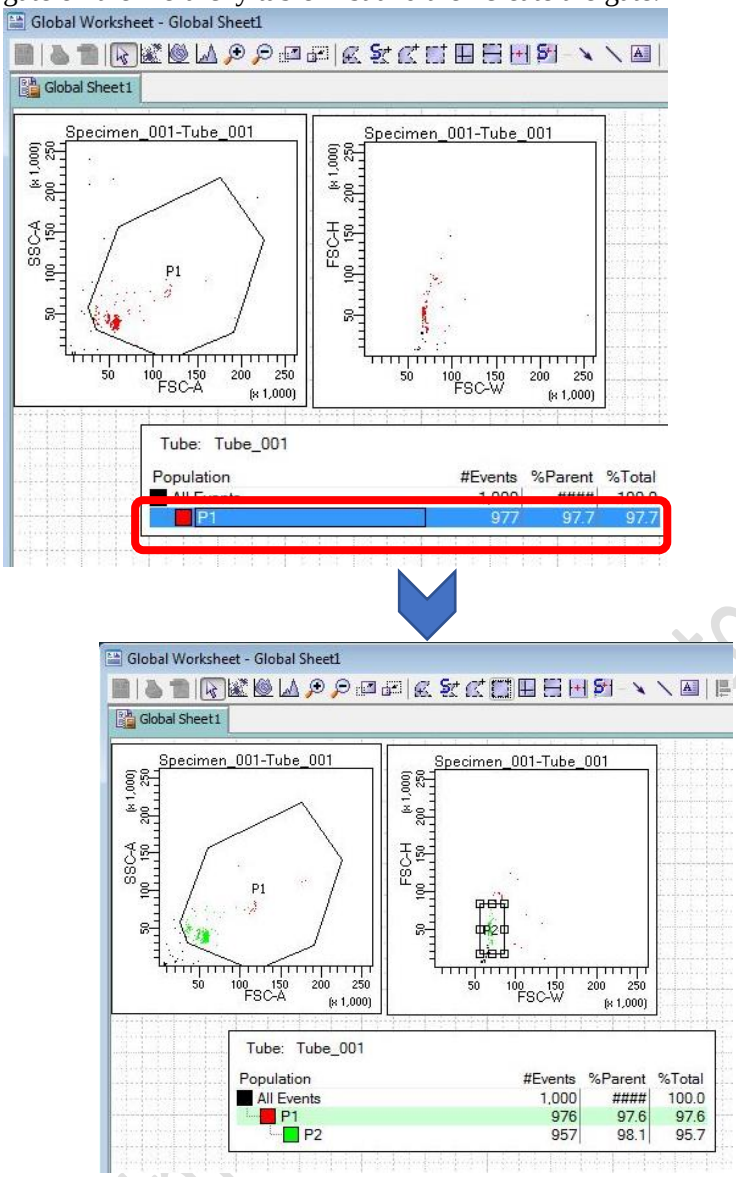


Icon	Type
	Polygon Area Gate
	Rectangle Area Gate
	Quantrad Gate
	Interval Gate

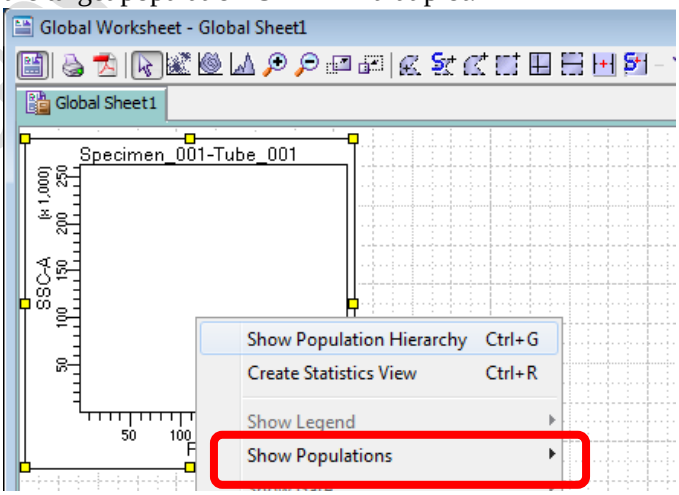
2. Draw the gate on the plot of interest to gate out target cluster /peak



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

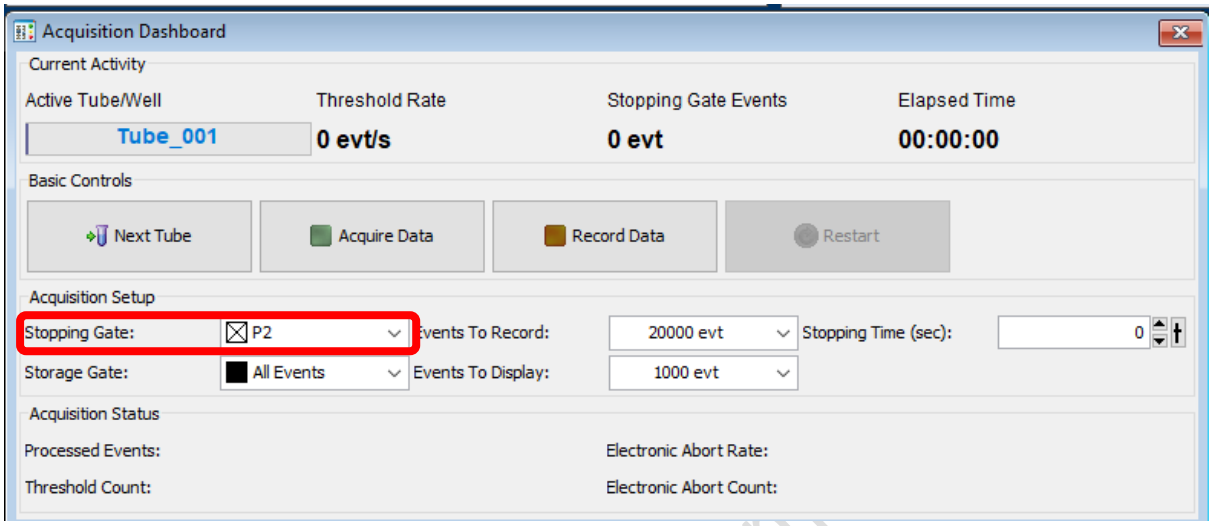


4. Click on target plot and right click, Click *Show Population* > *Target population* to visualize the target population ONLY in that plot.

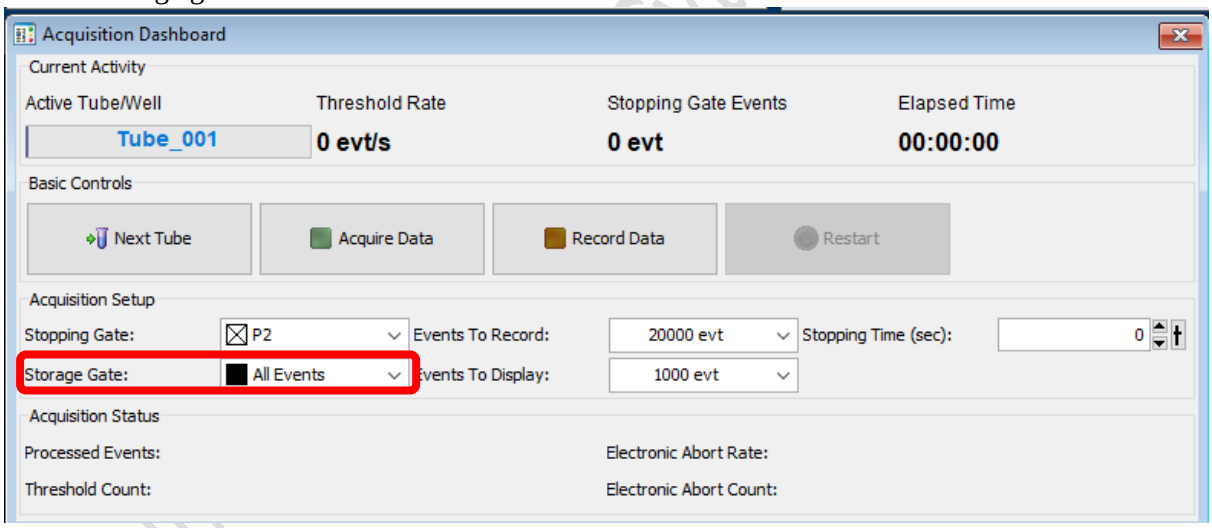


F. Data Recording

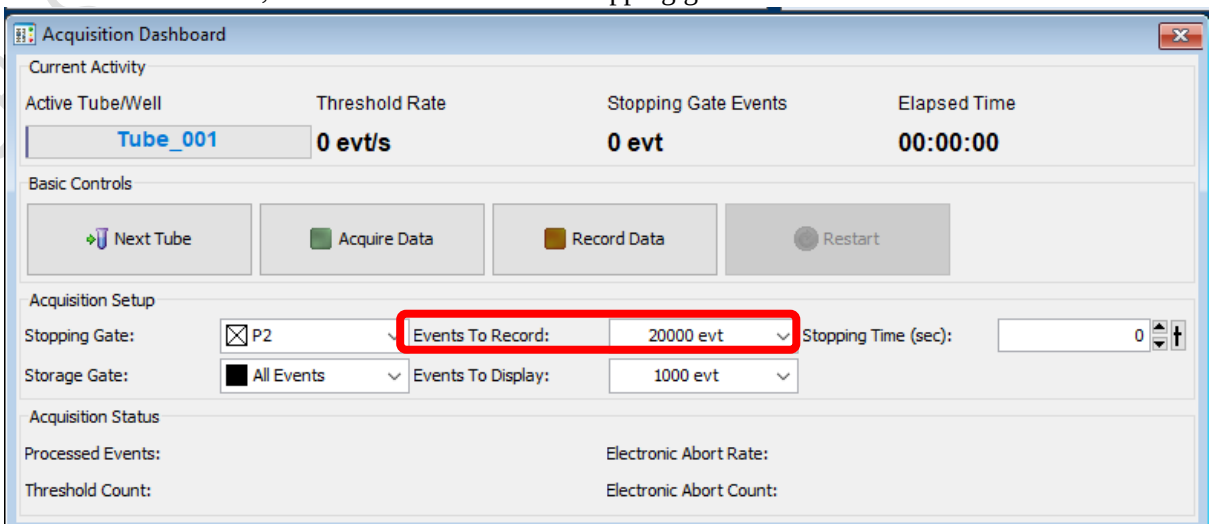
1. Go to Acquisition Dashboard, set **Stopping gate** to singlet gate or live cell gate



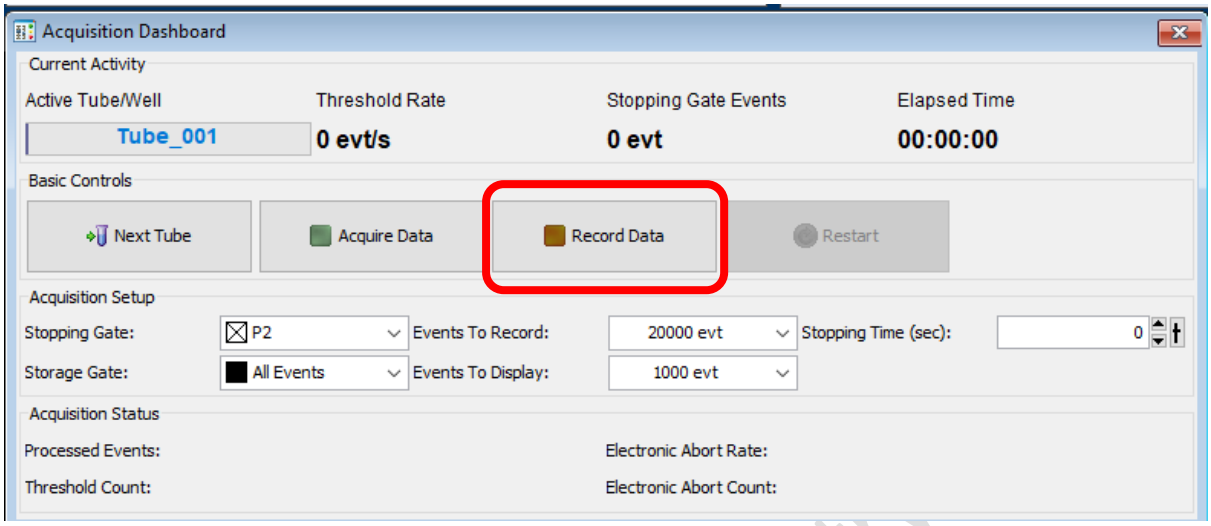
2. Set the Storage gate to All Events



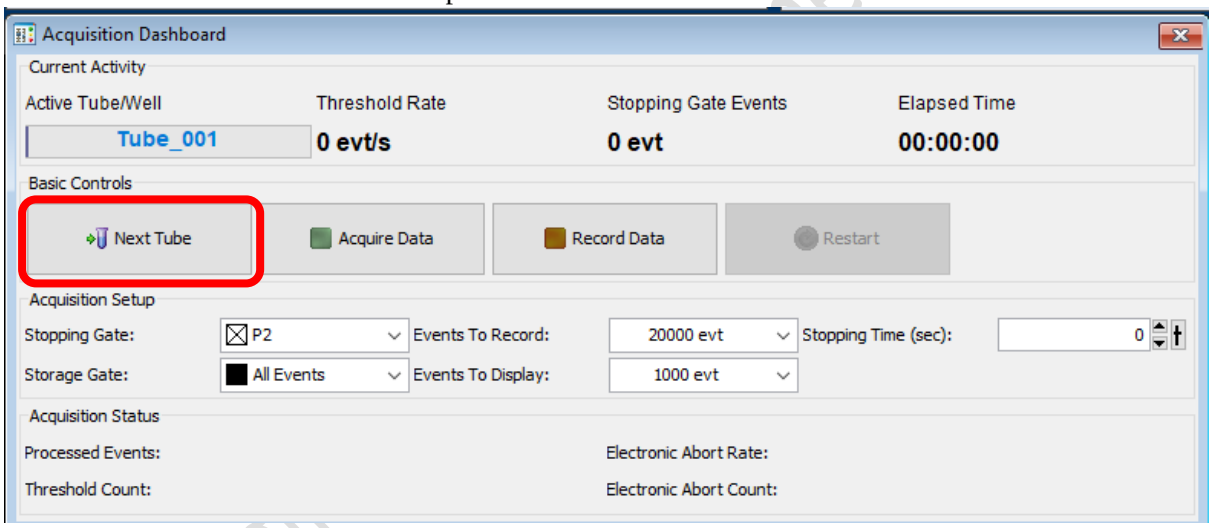
3. Set Events To Record, i.e. events number out of stopping gate to be recorded



4. Click **Record Data**



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

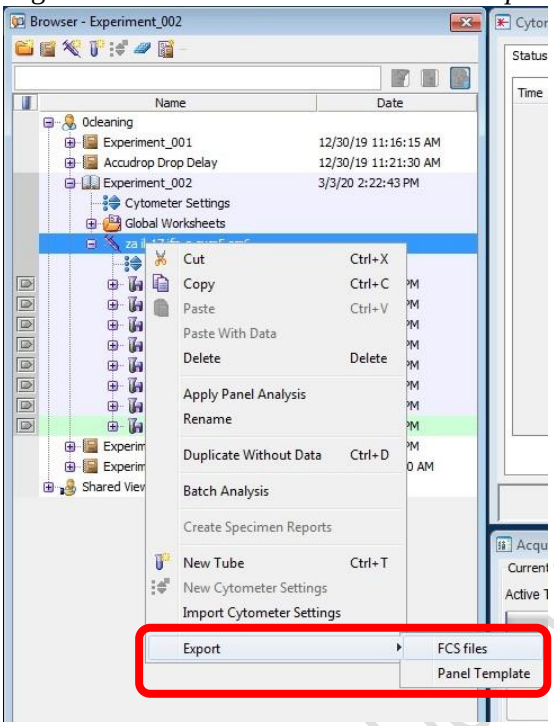


G. Data Export

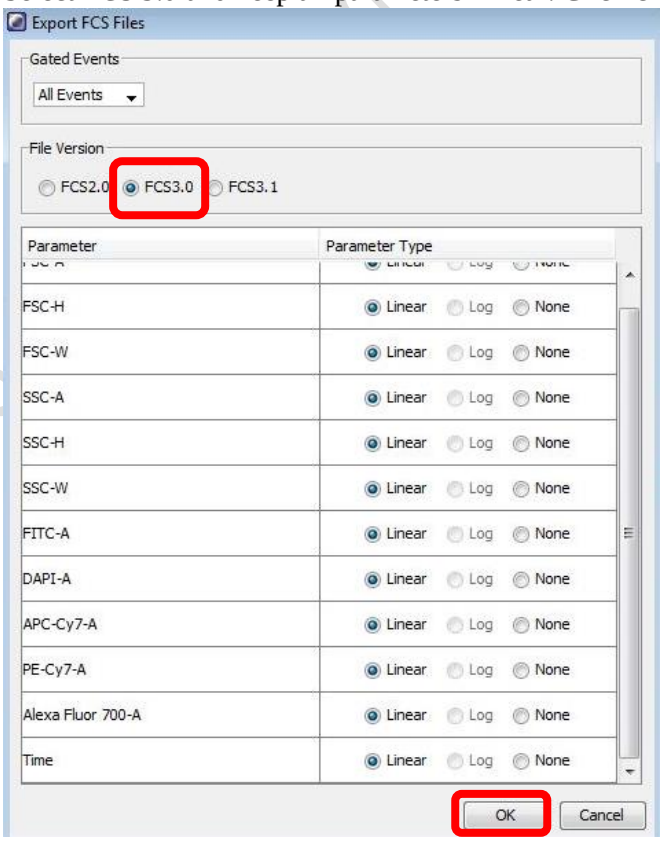
1. FCS file

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

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

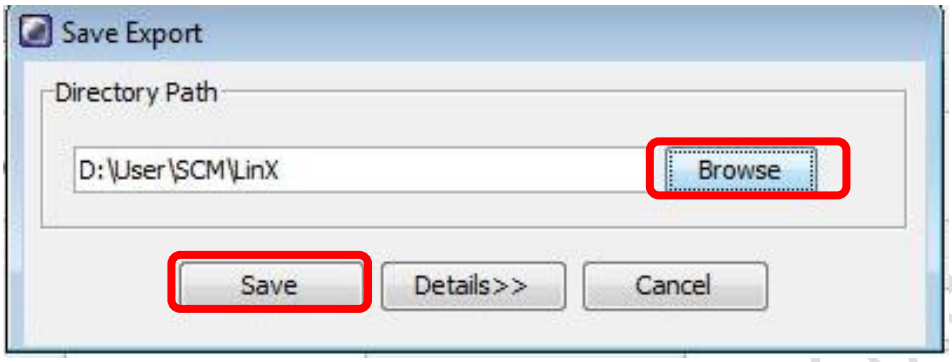


1.3 Select *FCS 3.0* and keep all parameters Linear. Click *OK*



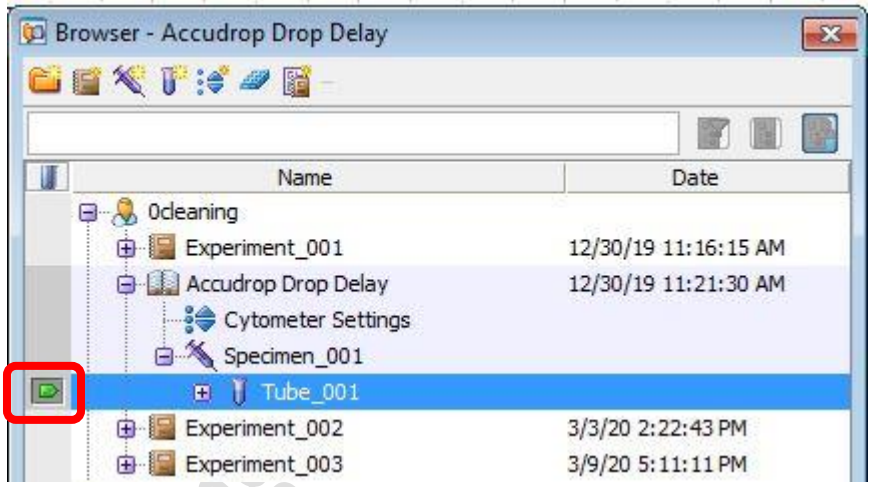
1.4 Click **Browse** to choose the destination (D:/User/Department/PersonalFolder)

1.5 Click **Save**



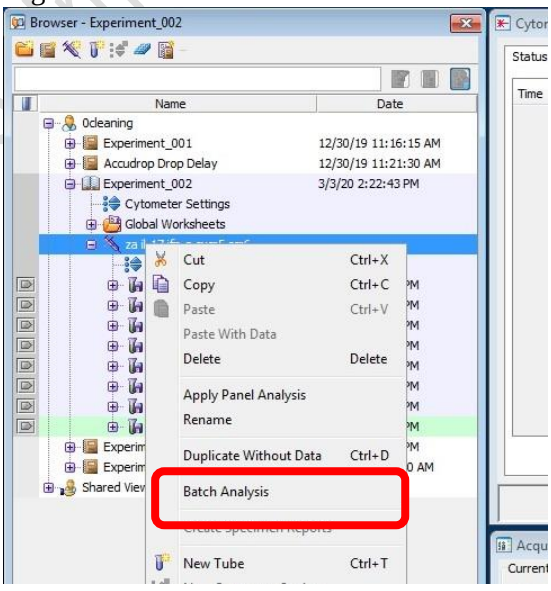
2. PDF file

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

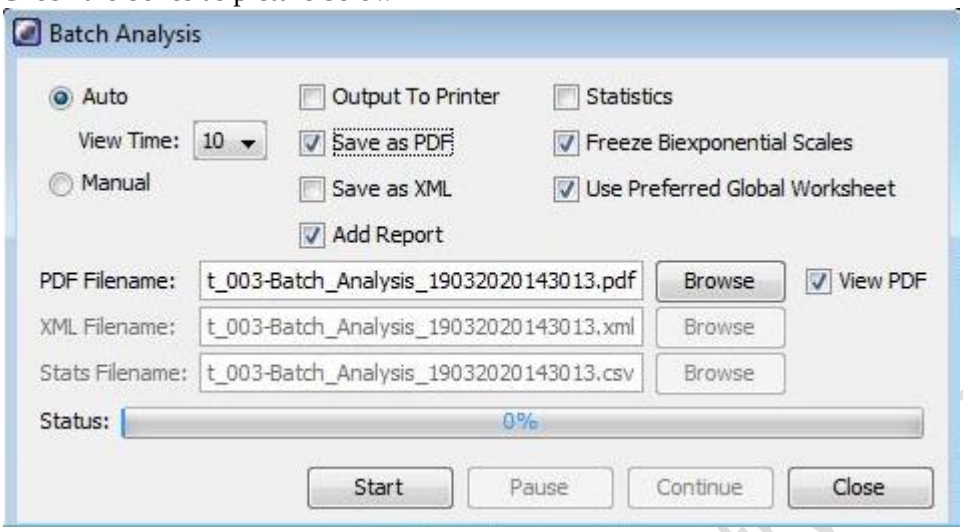


2.2 Select the wanted tubes/ Specimen.

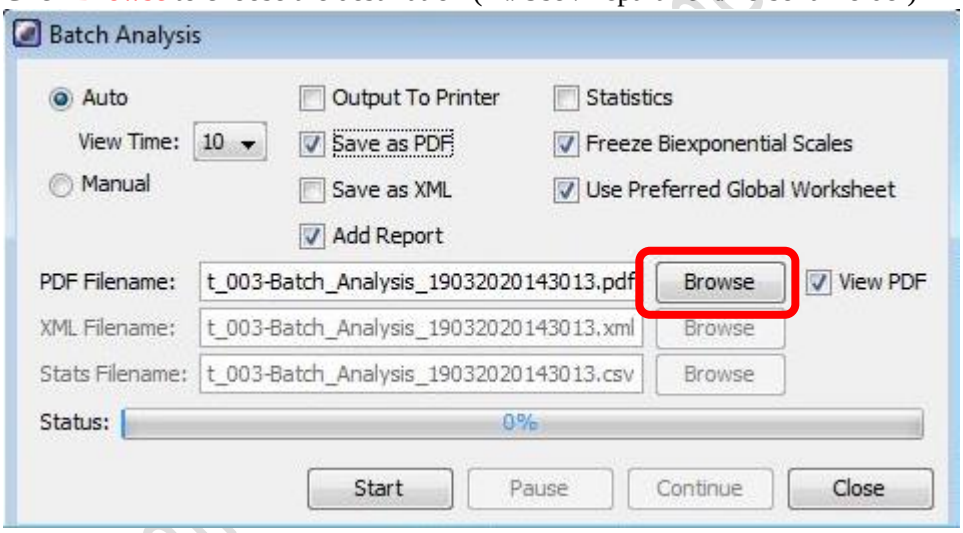
2.3 Right Click over the selection and click **Batch Analysis**



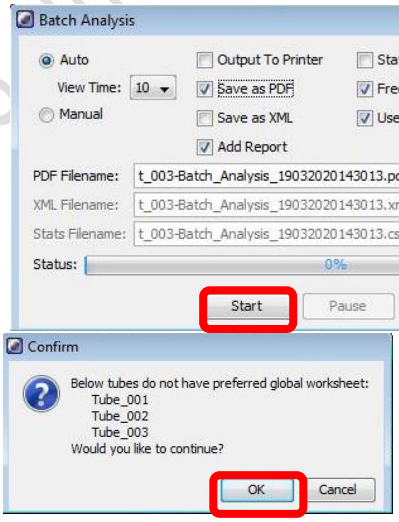
2.4 Check the boxes as picture below



2.5 Click **Browse** to choose the destination (D:/User/Department/PersonalFolder)

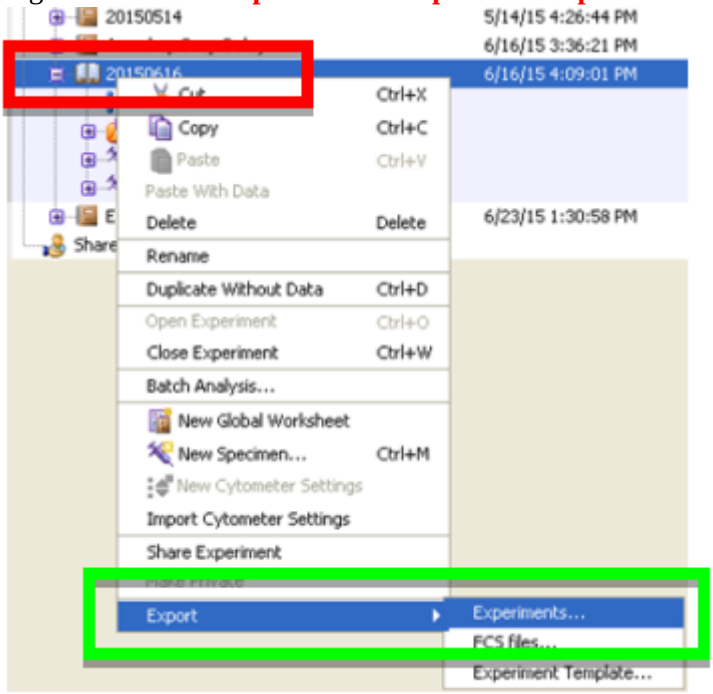


2.6 Click **Start** and then **OK**.

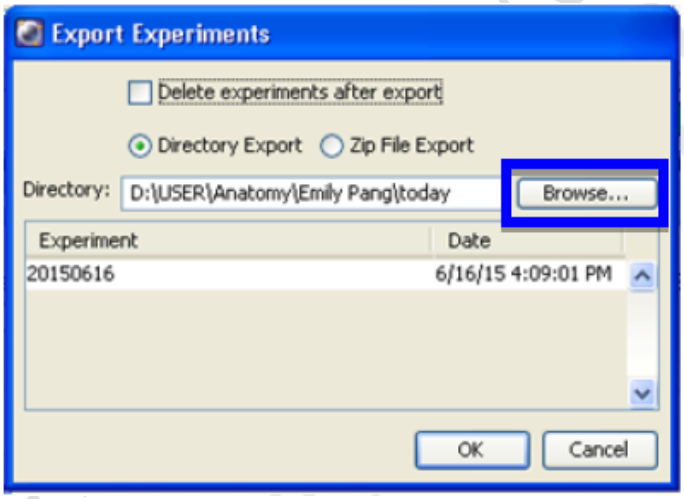


3. Experiment

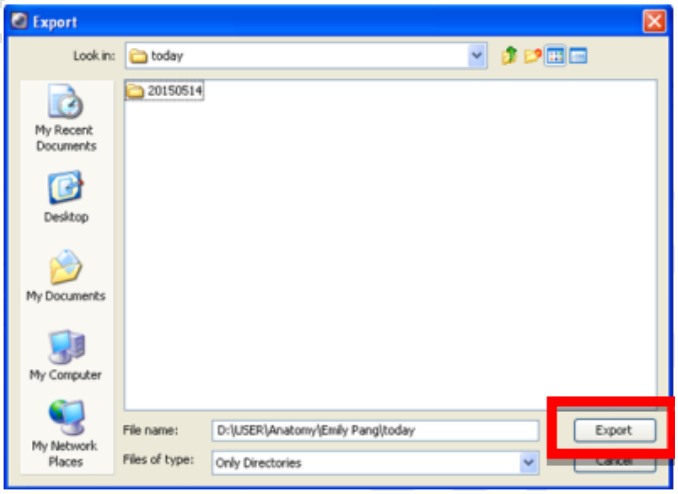
3.1 Right click on the **Experiment > Export > Exerpiments**



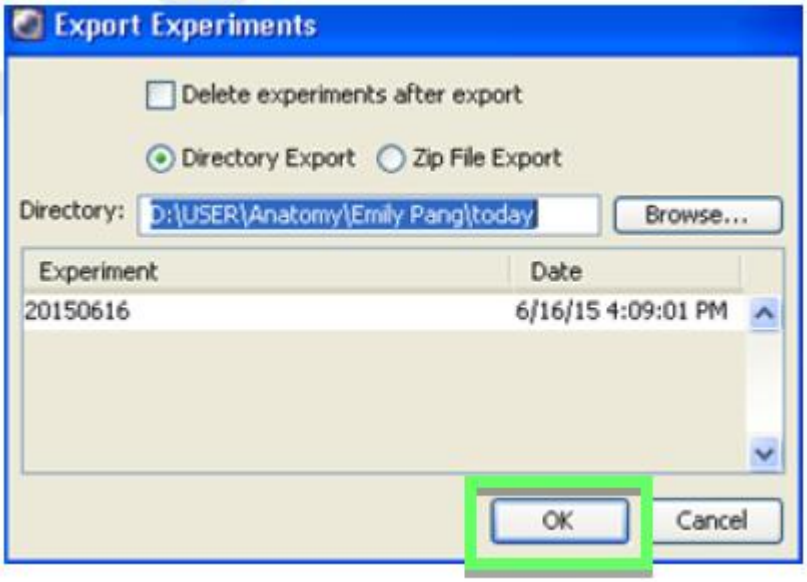
3.2 Click **Browse** to select the file destination



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

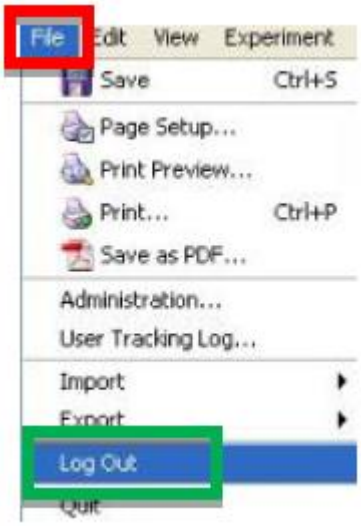


3.4 Click **OK** to export



H. Software Log out

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



I. System Cleaning

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

1. Prepare 3ml of each cleaning solution (Solution1: FACSClean; Solution 2: FACSRinse; Solution 3: MilliQ water)
2. Press **HI (60ul/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 **LO (12ul/min)** and **Standby (Flow Stop)**