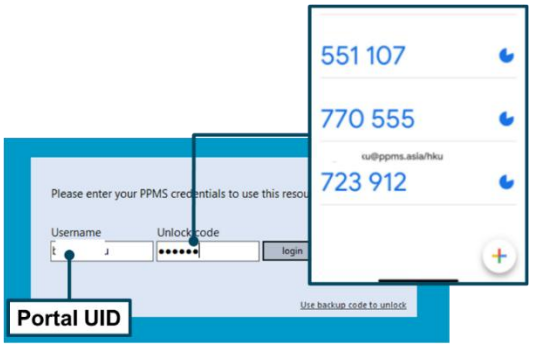
BD FACSymphony A5 SE Standard Operation Protocol

Basic Operation – Tube mode

A. Tracker Login



B. Log in the Window

1. Press Ctrl+Alt+Delete
2. Account: **User**

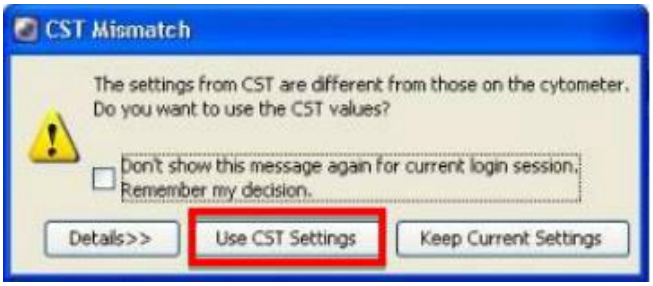
   Password: posted under the bottom of the computer screen

C. 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.

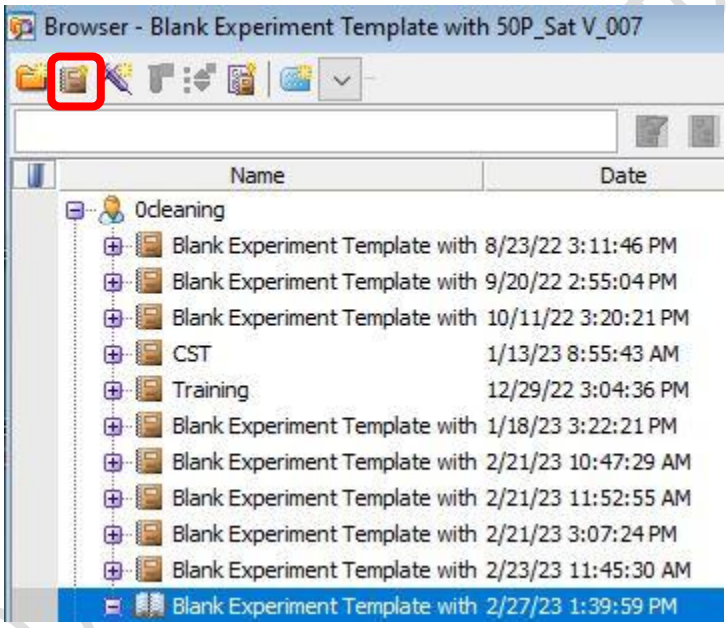
2. Click **Use CST Settings** when pop-up message as below is shown.



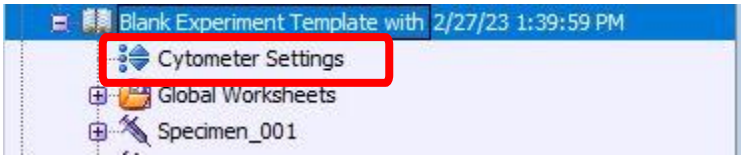
C. Experiment Setup

1. Setup New Experiment

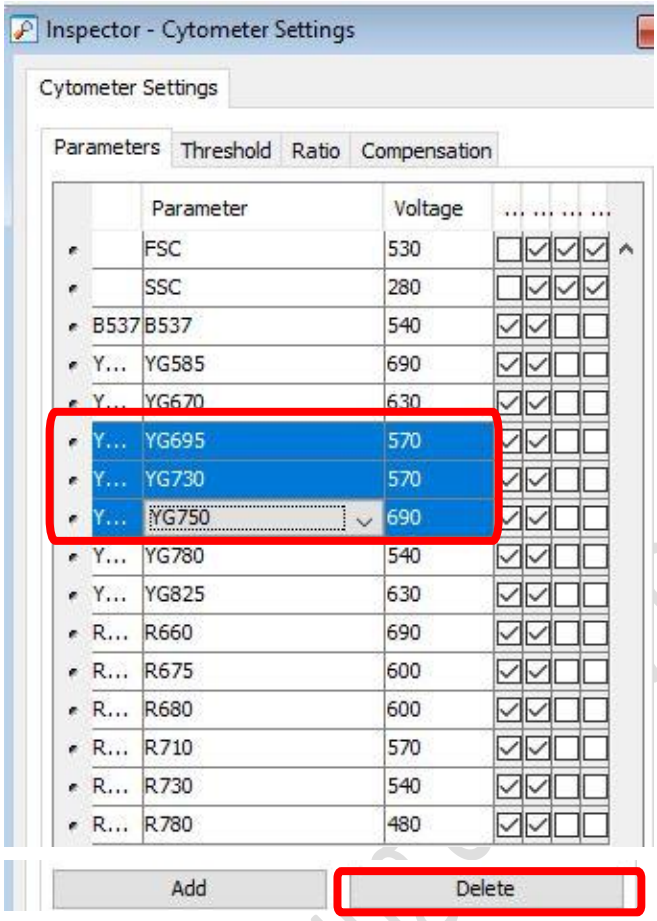
1.1 Go to Browser, Click **New Experiment** icon. A blank experiment template will be created.



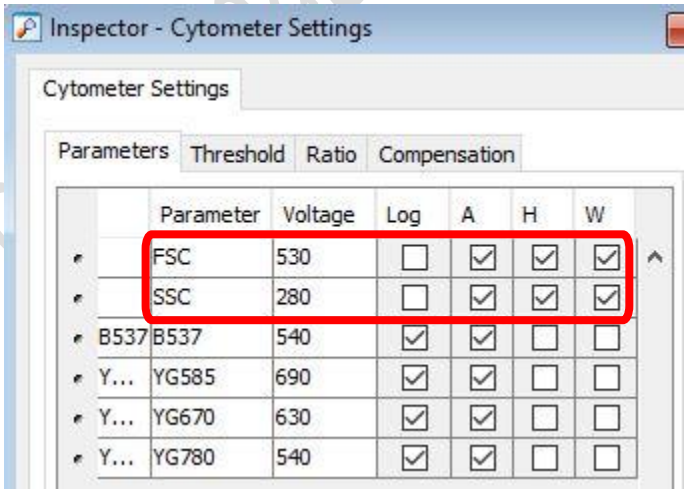
1.2 Click **Cytometer Settings** under the newly created Experiment



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



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



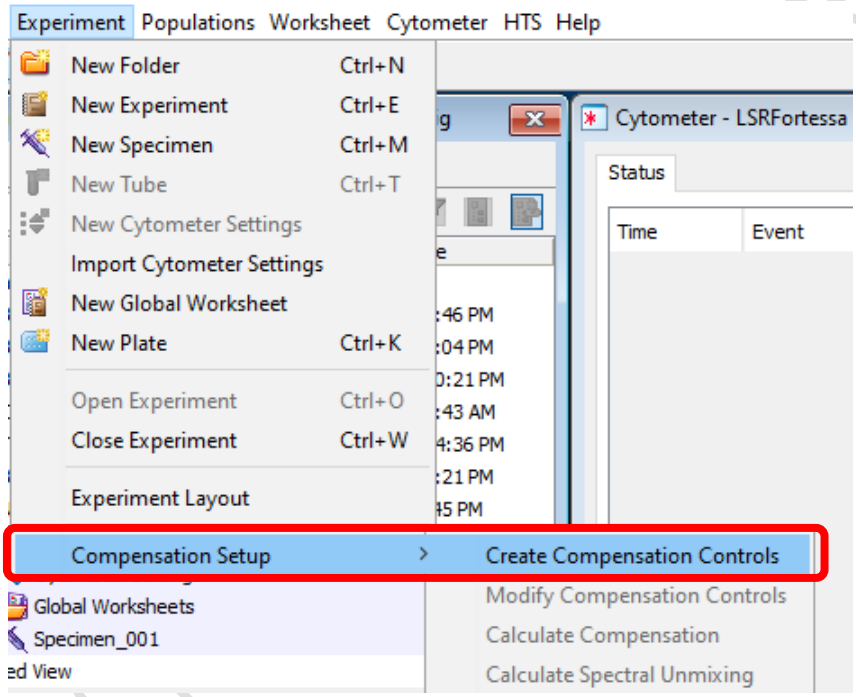
1.5 Keep *Log* boxes of FSC and SSC **unchecked**

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

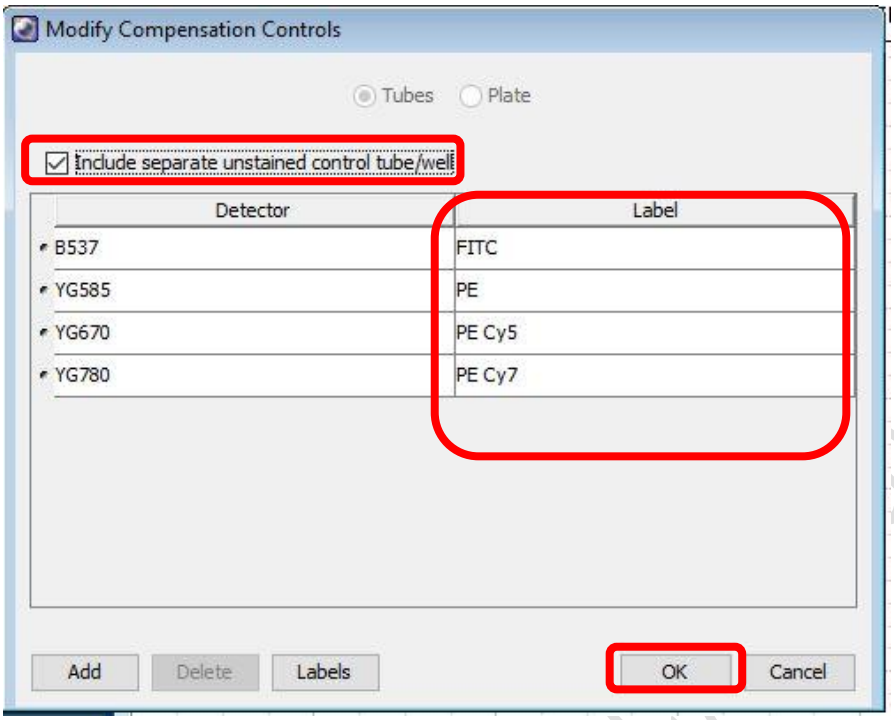
* If you are doing **cell cycle** or **DNA content** analysis, please keep log box of your DNA specific fluorescence channel **unchecked**.

2. Setup Compensation (for Multi-color panel)

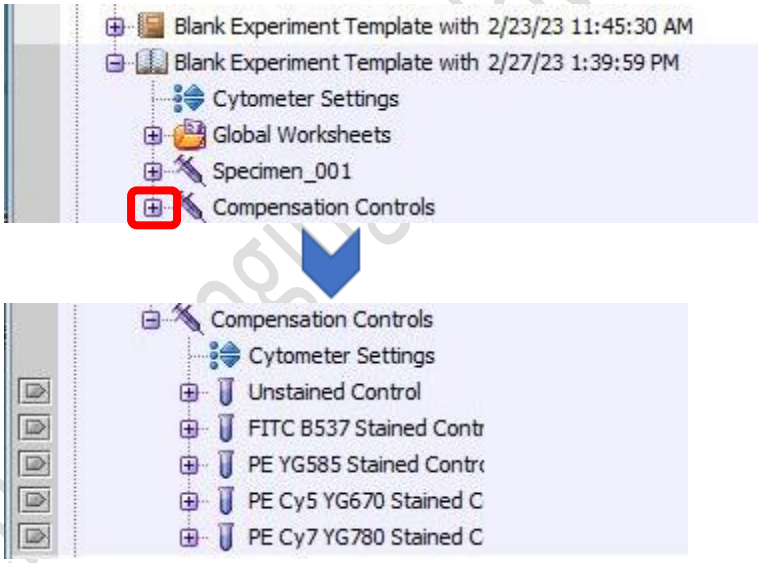
2.1 Click *Experiment* > *Compensation Setup* > *Create Compensation Control*



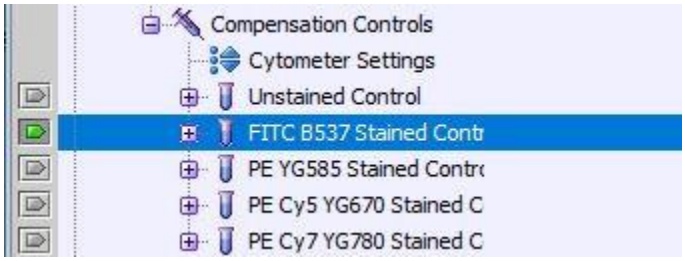
2.2 If any one of your single stain controls is known to be 100% positive, i.e. no negative population, check the box "Include separate unstained control tube/well". Label the detectors and click "OK".



2.4 Expand the Compensation Control Specimen



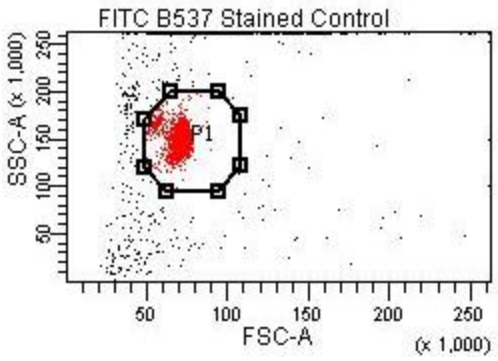
2.5 Click the tube pointer of the first tube



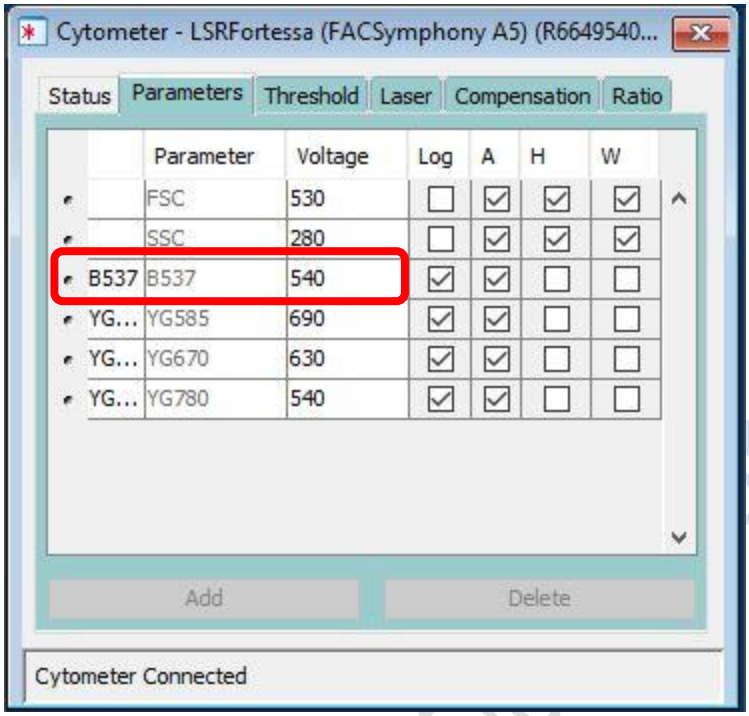
2.6 Load the single stain controls on the sample stage according to the tube label, i.e. run FITC single stain when the tube pointer is pointing at "FITC B537 Stained Control"

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

2.8 Go to **Normal Worksheet**, move the P1 gate to include major cluster. Adjust FSC and SSC PMT Voltage if needed.

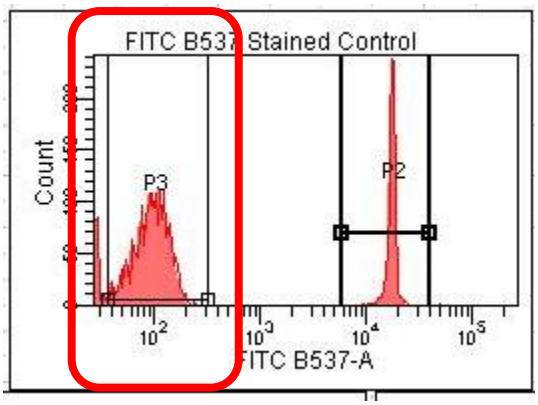


2.9 Go to Cytometer window, fine tune the corresponding fluorescence PMT voltage to have best separation of negative and positive peak



2.10 Move the interval gate (P2) to include the positive peak

2.11 Use interval gate to gate out negative peak (P3)

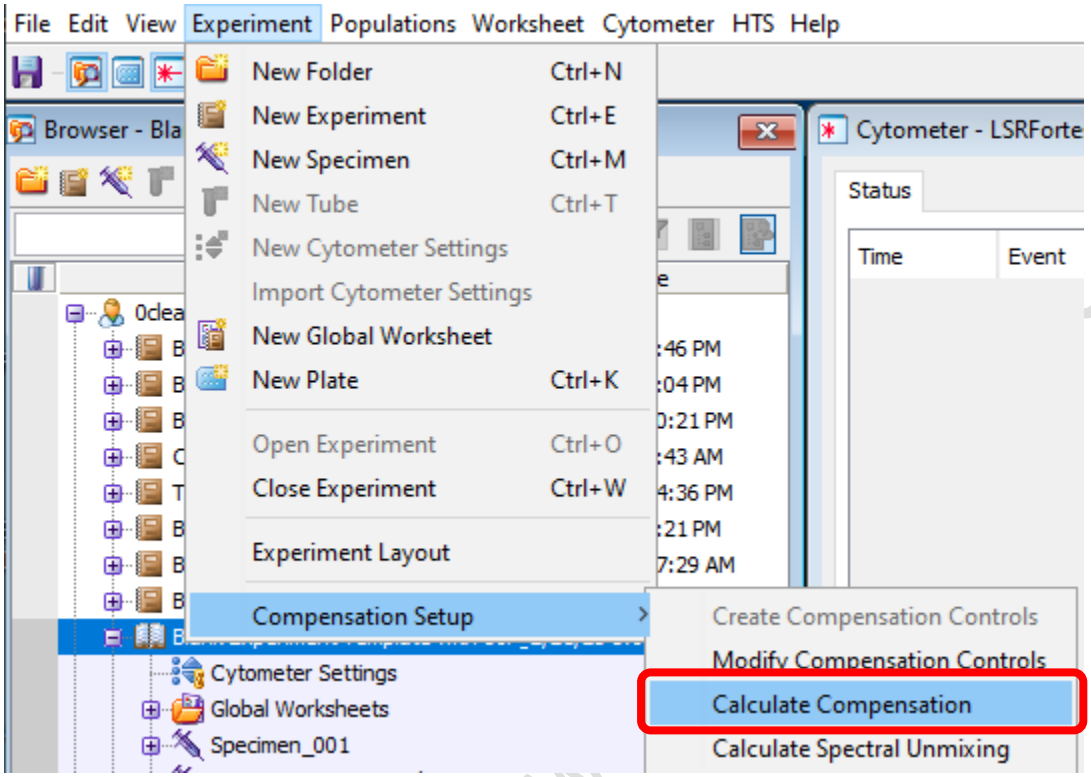


2.12 Go to Acquisition Dashboard, Click "*Stop Acquiring*".
***DO NOT Record Data at this point.**

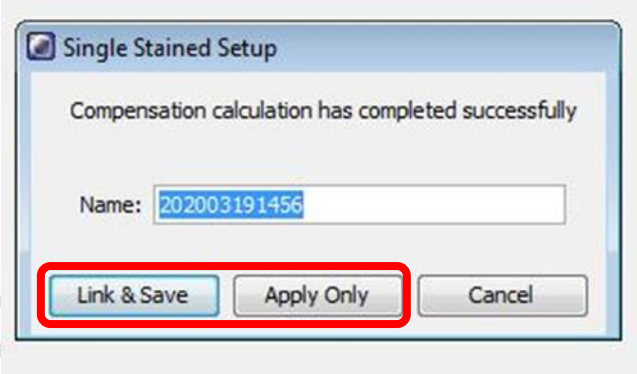
2.13 Repeat step 2.5 – 2.12 with all the single stain controls.

2.14 After optimizing the PMT voltage of ALL the fluorescence channels, load each single stained control and click *Record Data* for ALL single stain controls

2.15 Click *Experiment > Compensation Setup > Calculate Compensation*



2.16 Click *Link and Save* for the most stringent practice, i.e. cannot adjust PMT voltage anymore OR Click *Apply Only* for some flexibility on PMT voltage adjustment of your samples.



2.17 Switch **Normal worksheet** to **Global worksheet**

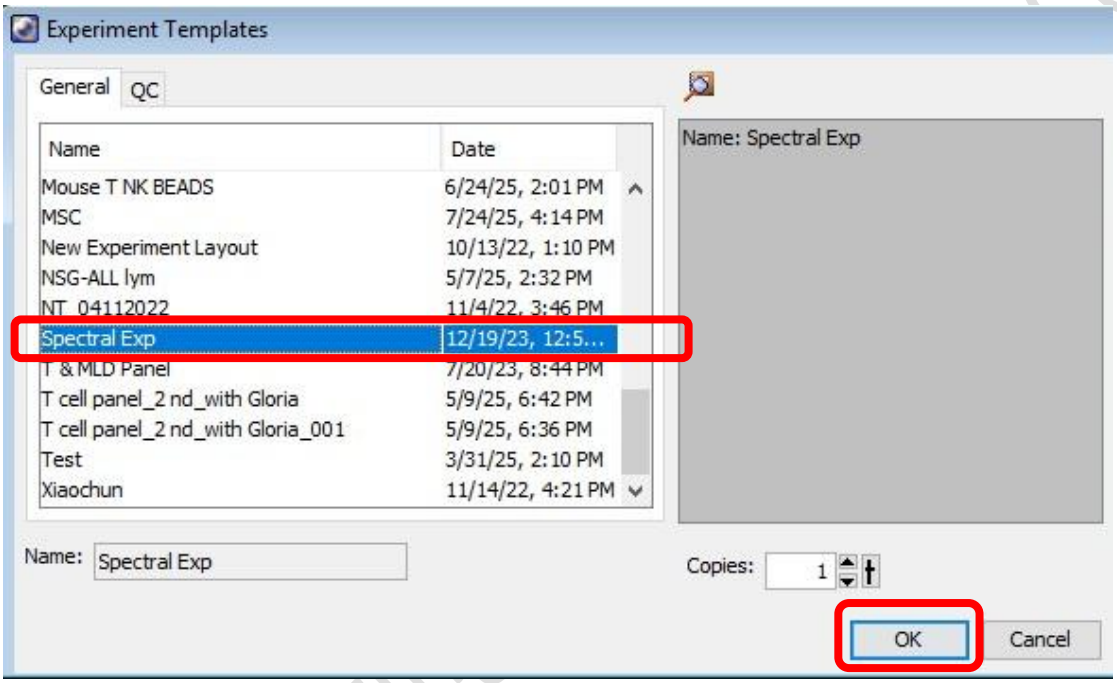
3. Spectral functions

*** Do not delete any fluorescent parameters. Doing so will remove the ability to perform spectral unmixing.**

3.1 Setting up a new spectral experiment

3.1.1 Click *Experiment* > *New Experiment*

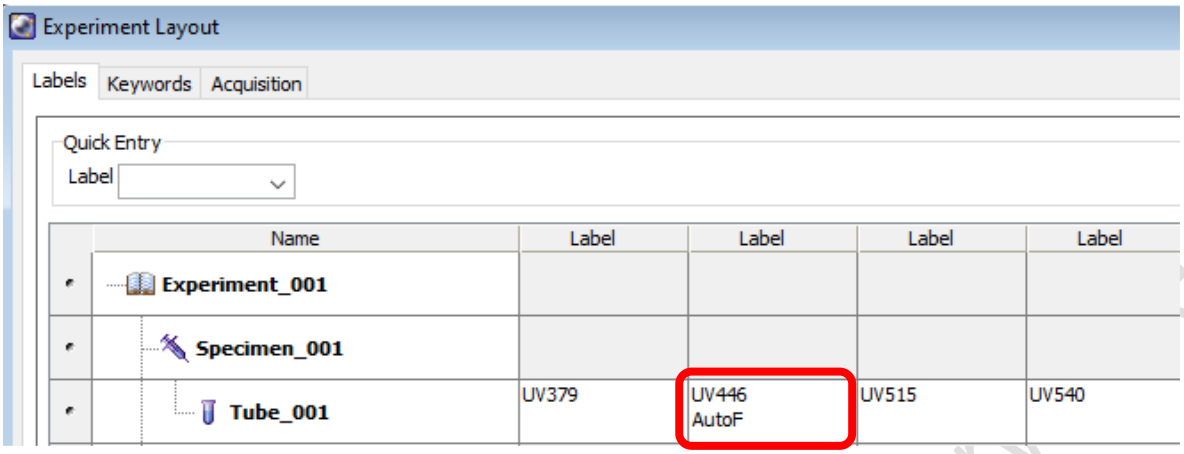
The Experiment Templates dialog is displayed. select the *Spectral Exp* and click OK



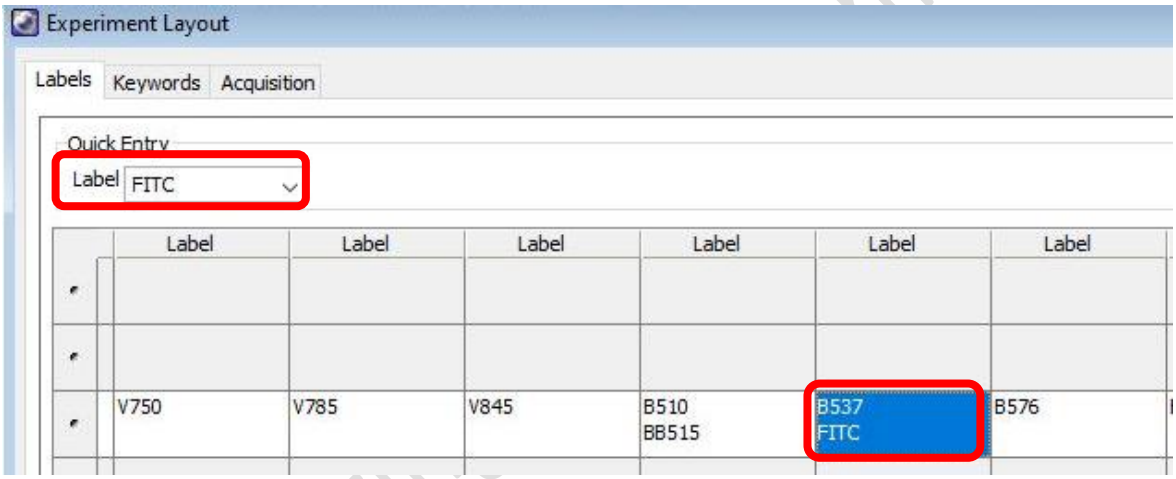
3.1.2 Click *Experiment* > *Experiment Layout* to assign parameter labels for each reagent to an individual primary detector.

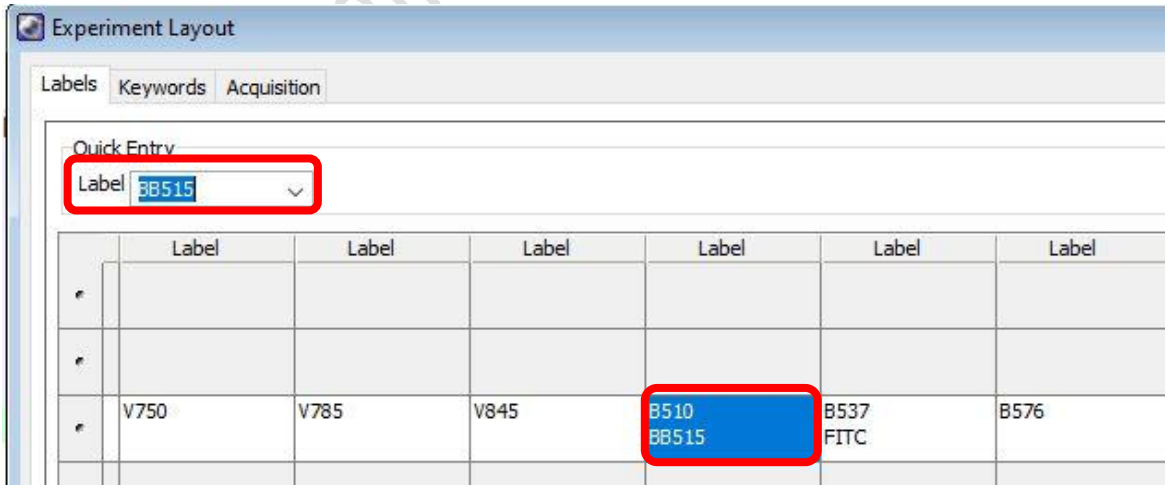
*Typically, both the dye and the antibody names are added to the label, as shown in the following example for FITC and BB515 staining.

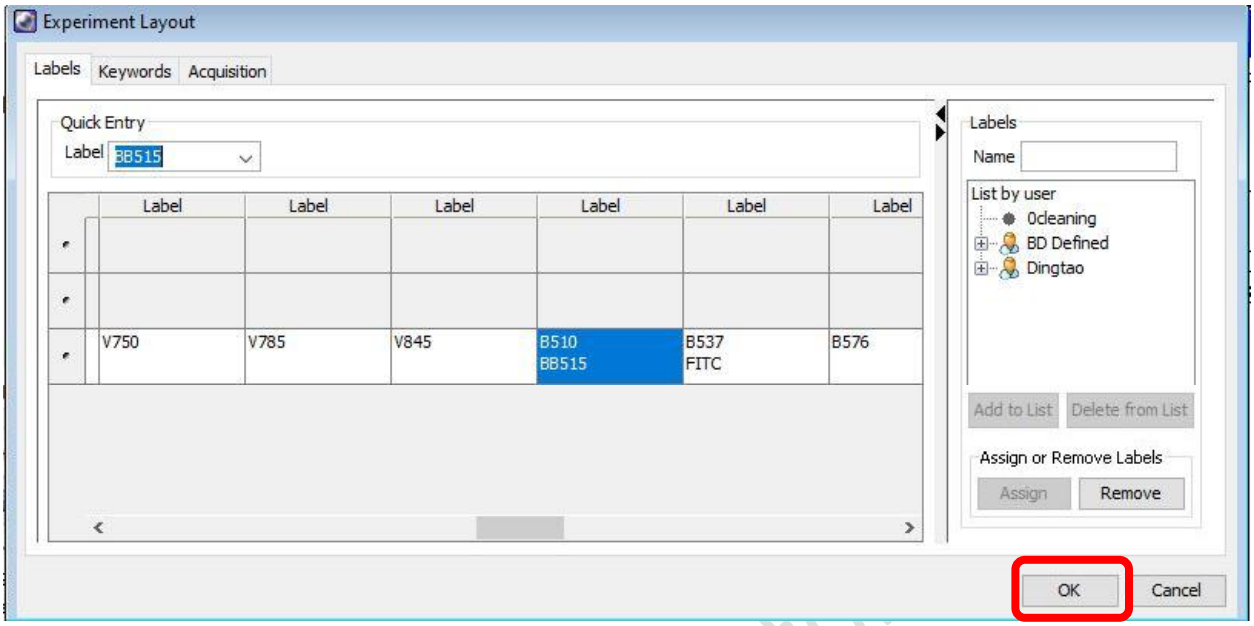
*To add an **autofluorescence** control to your experiment, assign the label "**AutoF**" to parameter **UV446**. If that parameter is already assigned to another fluorochrome in your experiment, select any of parameters **UV379–UV540 or V427–V540 for AutoF**.



Note: The autofluorescence control is only supported in spectral workflows, not compensation workflows.

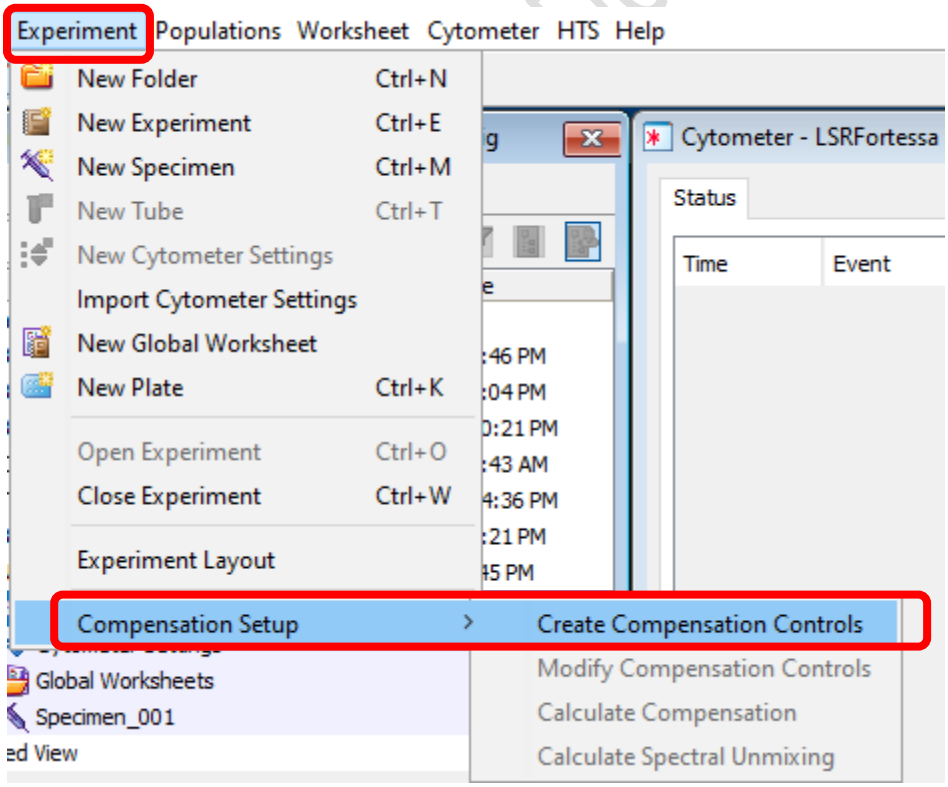




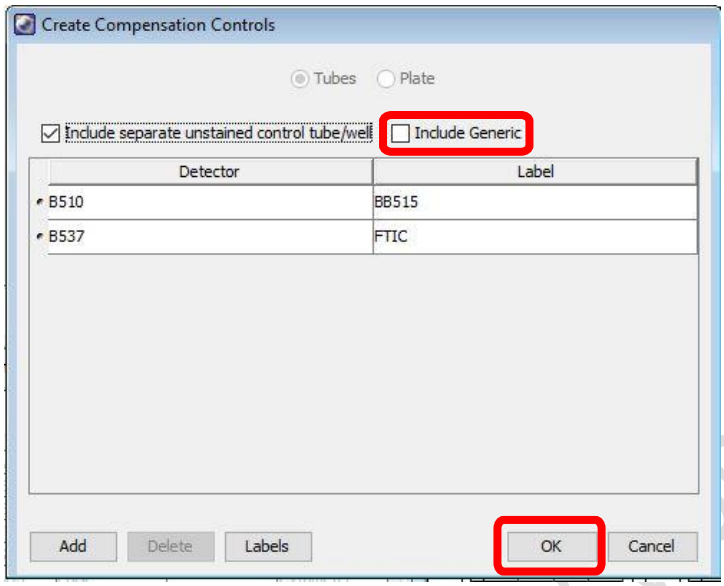


3.2 Setting up the Spectral Unmixing algorithm

3.2.1 select *Experiment* > *Compensation Setup* > *Create Compensation Controls* from the main menu.



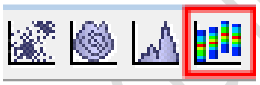
3.2.2 Leave the checkbox Include Generic in its default (deselected) state.

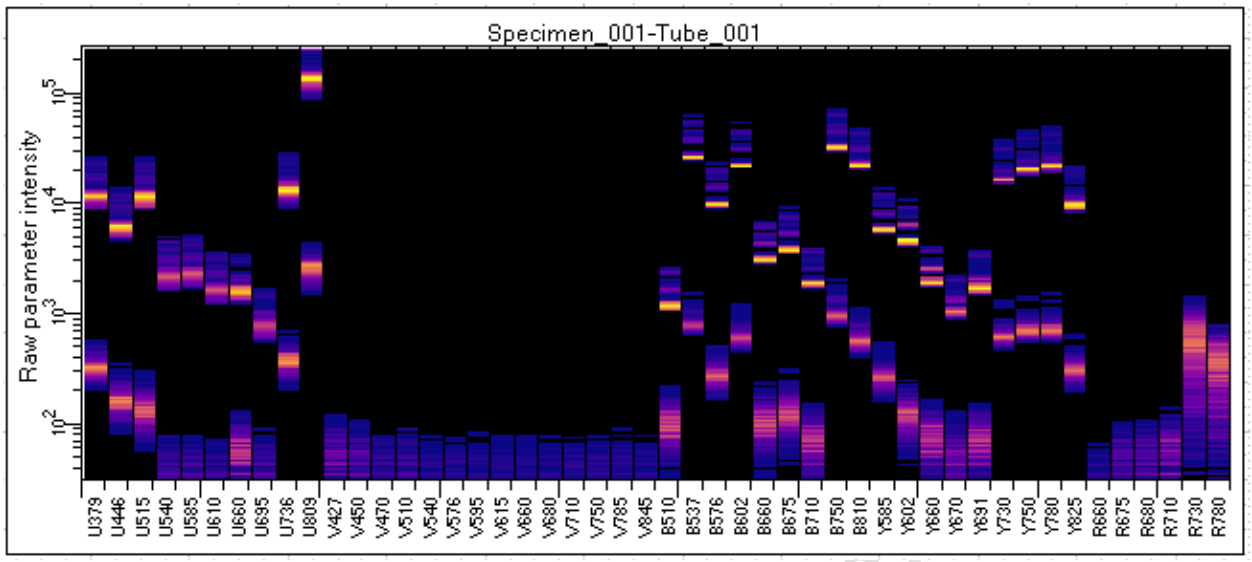


3.2.3 Click OK to create the controls. If you assigned AutoF to a detector in the experiment layout, an AutoF control tube will be displayed from the Compensation Controls specimen from the experiment Browser window.

3.2.4 Adjust the flow cytometer settings as needed for your experiment, including PMT voltage and area scaling.

Note: For spectral experiments, create a spectral plot to assist with adjusting PMT voltage settings to ensure that the signal across all detectors is on-scale, not just the signal for the primary detector for each fluorochrome.

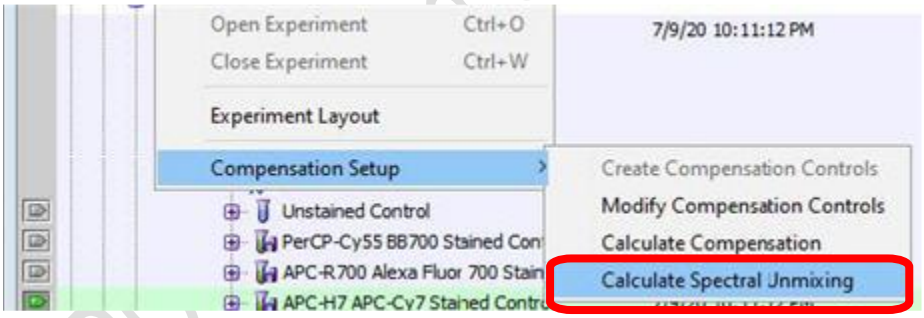


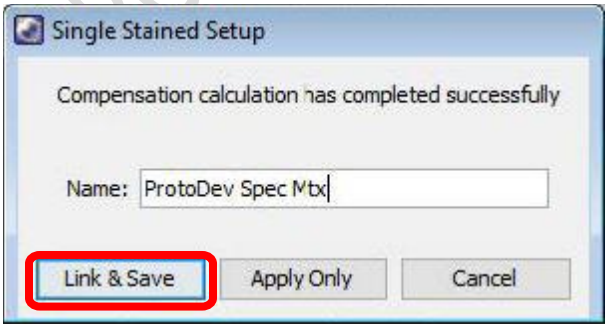


Note: The plot supports the display of specific populations or combinations of populations and displays live data during acquisition. The spectral plot does not support zoom, biexponential scaling, or gate drawing.

3.2.5 Record each of the single-color controls after voltage adjustments by ± 30 according to the selected template after voltration.

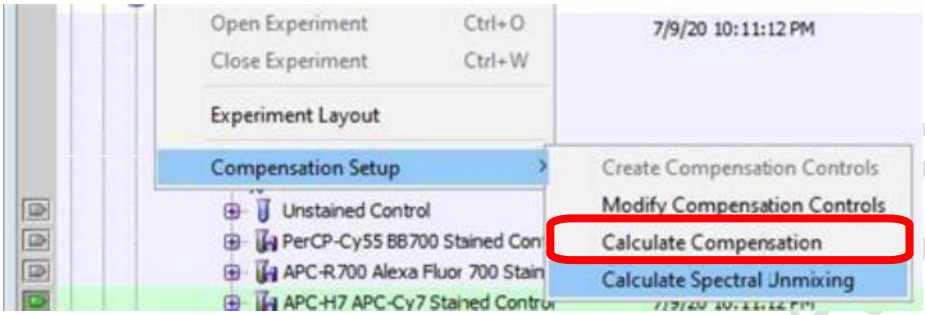
3.2.6 Select *Experiment* > *Compensation Setup* > *Calculate Spectral Unmixing*.



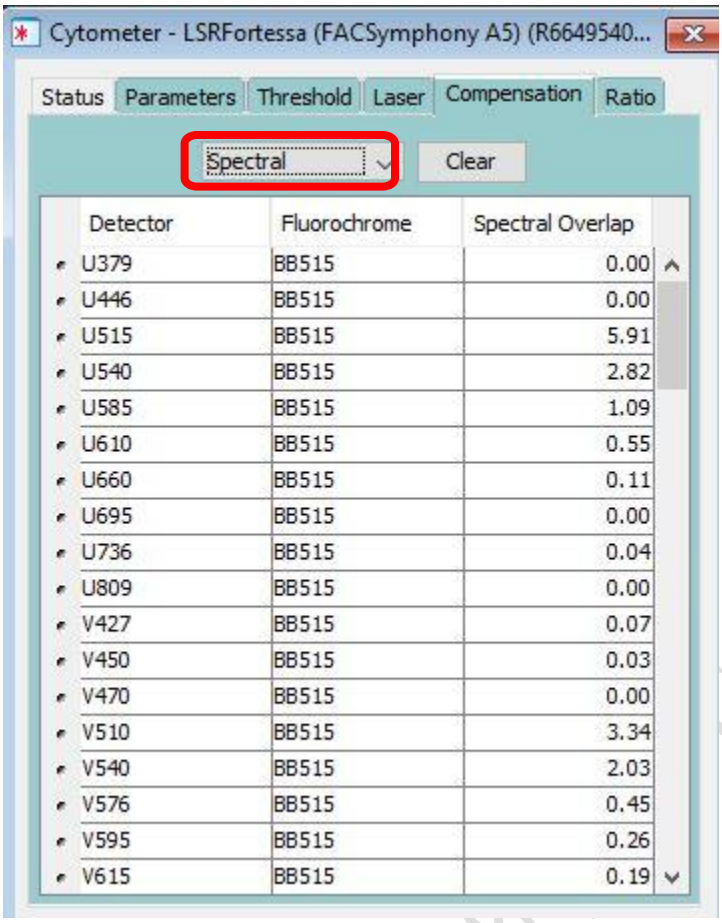


Click *Link and Save* for the spectral application, you cannot adjust PMT voltage anymore.

3.2.7 When the software calculates spectral unmixing, it also calculates **compensation** values (as if you had selected *Experiment* > *Compensation Setup* > *Calculate Compensation*).



Compensation setup does not take autofluorescence into account. By default, when you select spectral unmixing, Spectral is selected in the dropdown menu on the Compensation panel of the Cytometer window as shown in the following:



Detector	Fluorochrome	Spectral Overlap
U379	BB515	0.00
U446	BB515	0.00
U515	BB515	5.91
U540	BB515	2.82
U585	BB515	1.09
U610	BB515	0.55
U660	BB515	0.11
U695	BB515	0.00
U736	BB515	0.04
U809	BB515	0.00
V427	BB515	0.07
V450	BB515	0.03
V470	BB515	0.00
V510	BB515	3.34
V540	BB515	2.03
V576	BB515	0.45
V595	BB515	0.26
V615	BB515	0.19

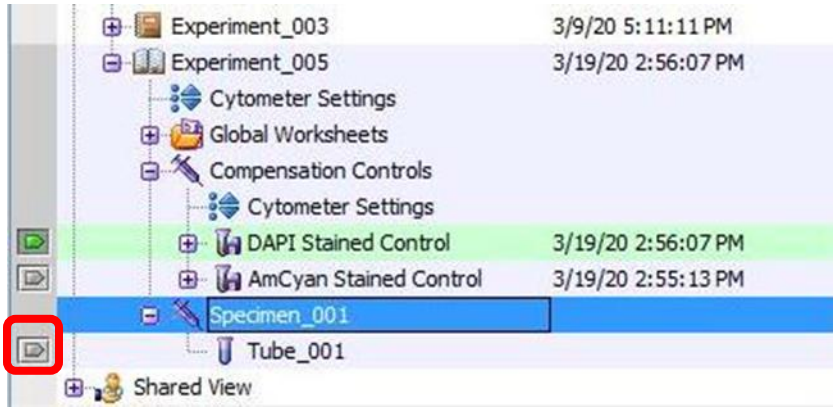
You can switch between **spectral unmixed** and traditional **compensation** values, by toggling between *Spectral* and *Compensated* in the dropdown menu, to compare the plot data in each case.

4. Setup Plots and Tables

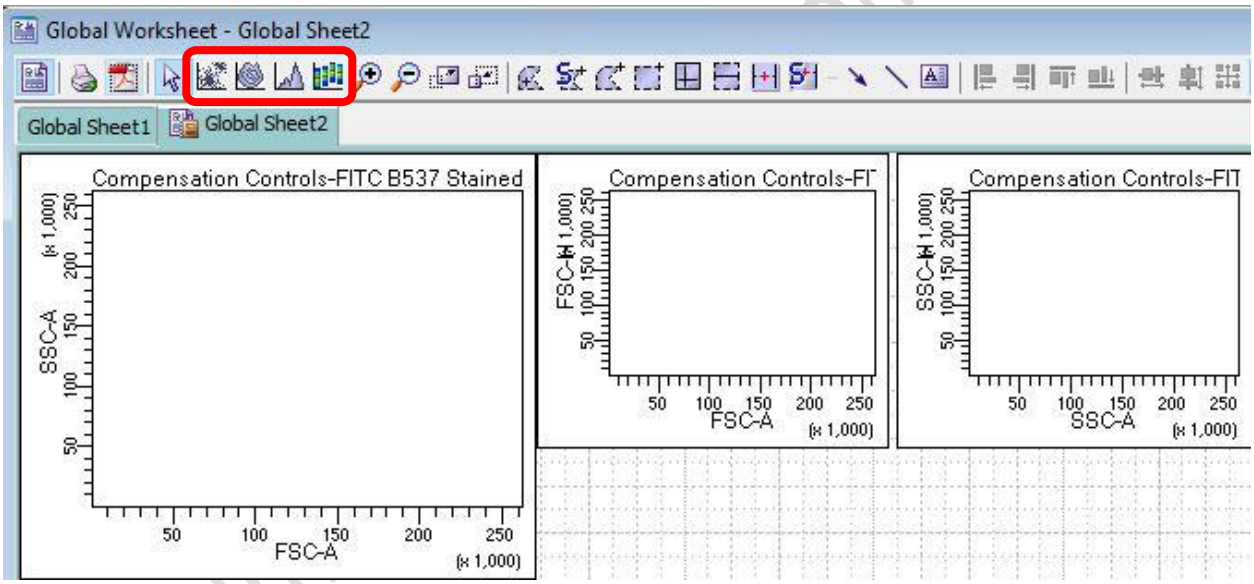
4.1 Go to Browser, Click New Specimen icon

4.2 Expand Specimen_001

4.3 Click the tube **pointer** of Tube_001



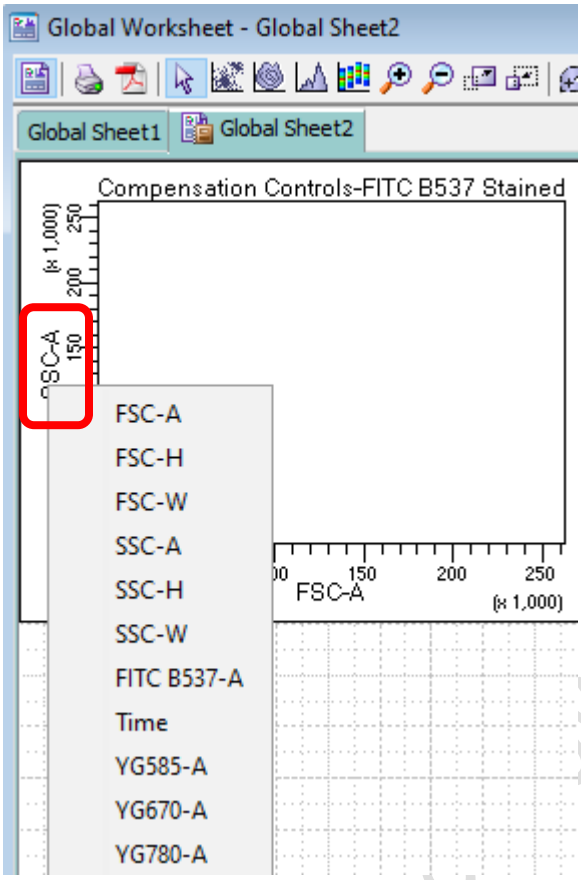
4.4 Go to Global Sheet Window, Click the graph type icon



Icon	Type
	Dot Plot
	Contour Plot
	Histogram
	Spectral plot

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

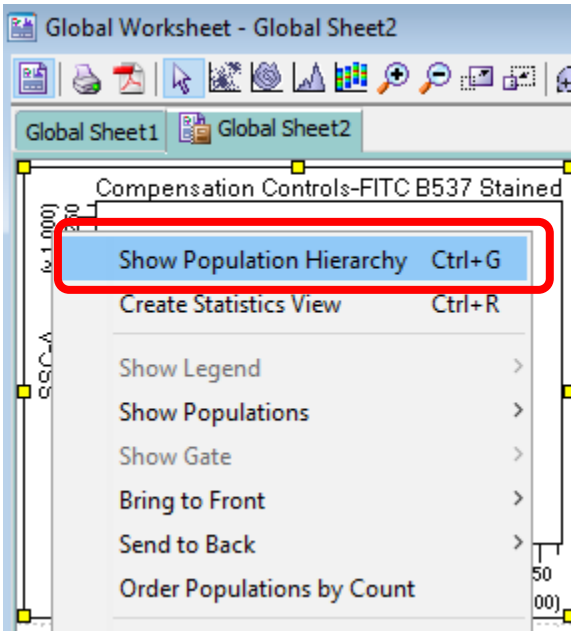
4.6 Mouse over the axis label and right click. Select the parameters of interest from the list.



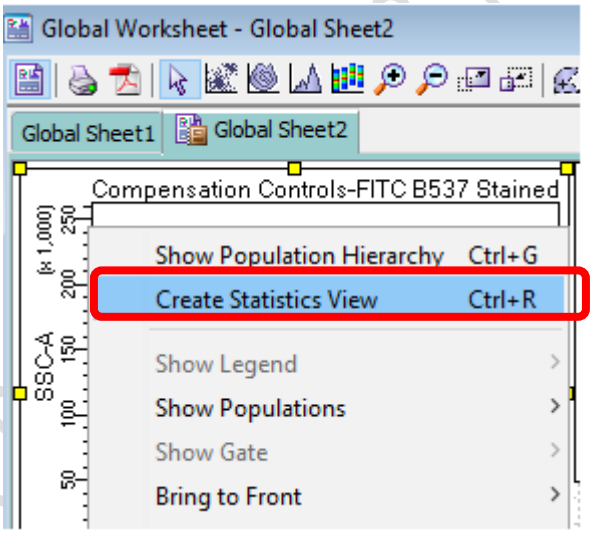
4.7 Repeat step 4.4 – 4.6 until all plots needed is created.

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

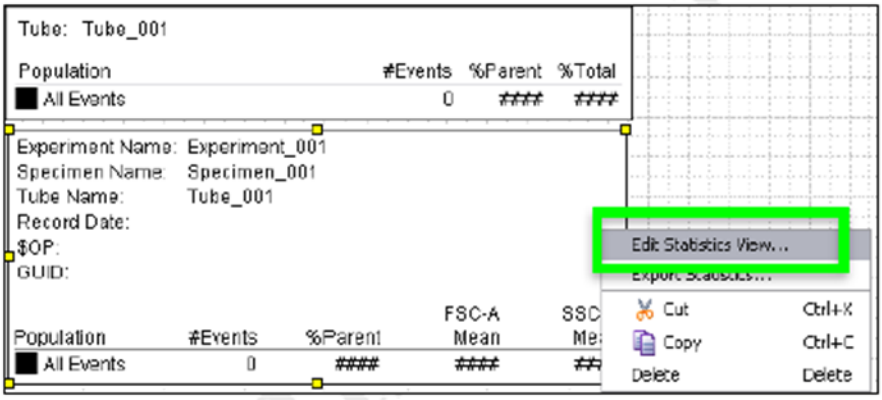
4.8 Click on any plot and right click. Click **Show Population Hierarchy**



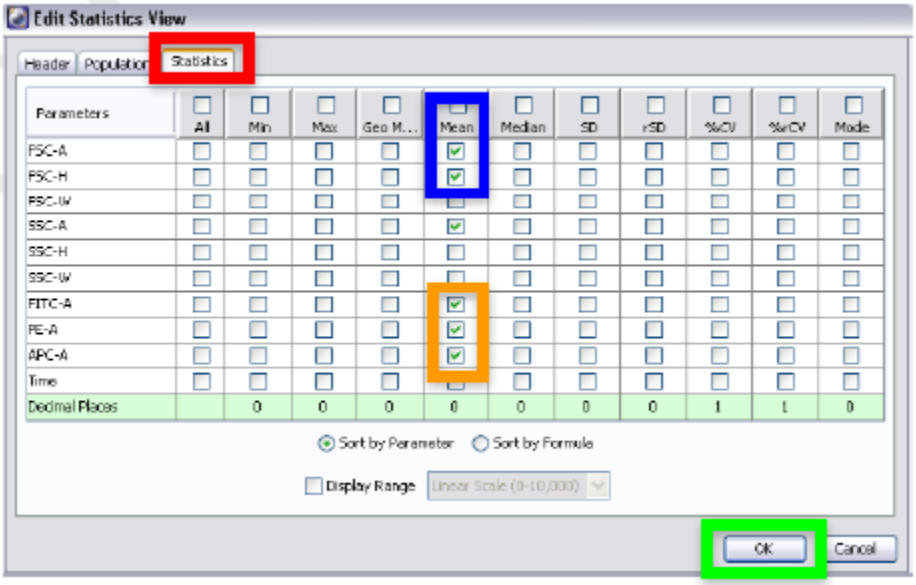
4.9 Click on any plot and right click. Click **Create Statistics View**



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



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



D. Sample Acquisition

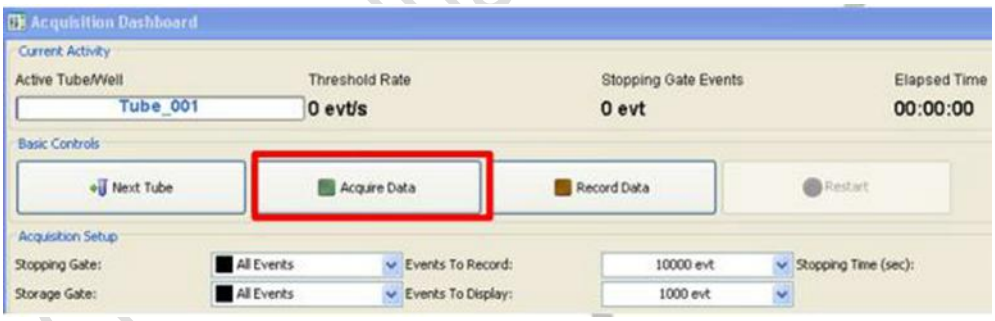
1. Gently tab or pulse vortex your sample and put your sample tube on SIP (Sample injection port).

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

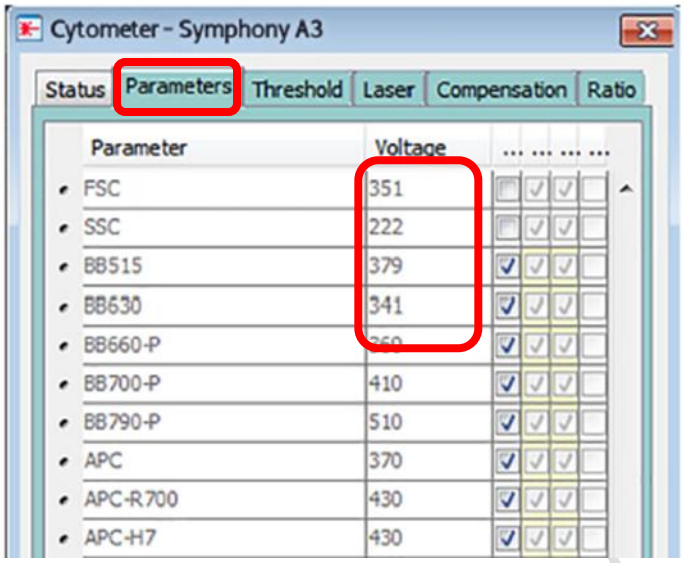
!! DO NOT return the support arm to the center without putting a tube filled with liquid on SIP!!

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. Adjust PMT Voltage accordingly.

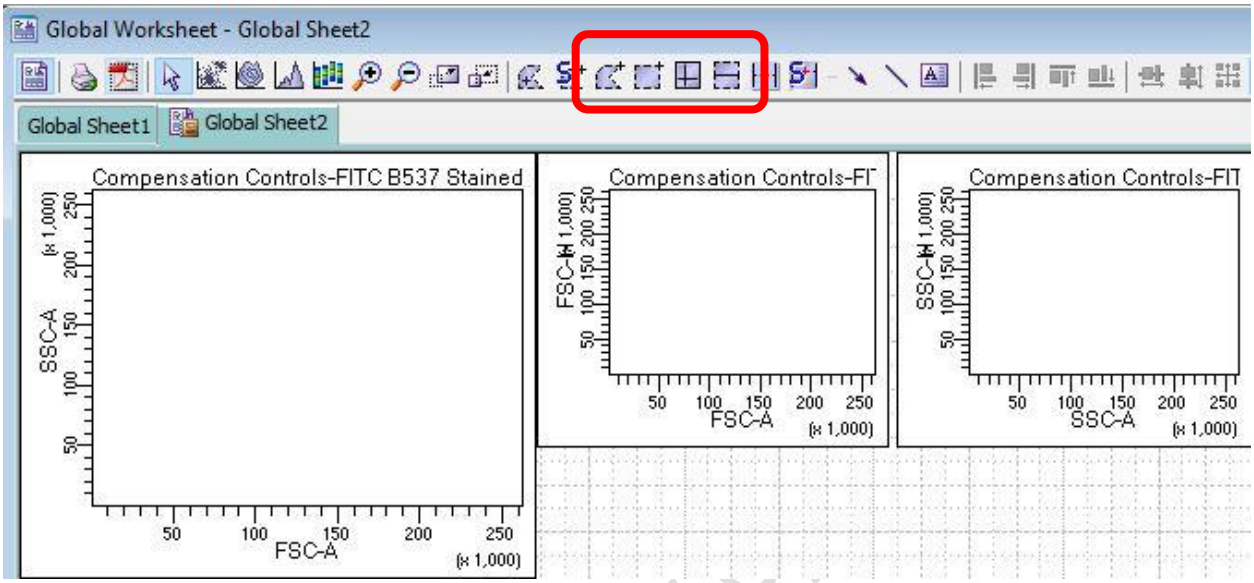


Channel	Suggested Voltage range for mammalian cells
FSC	600-800
SSC	250-310
Fluorescence	300-850

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

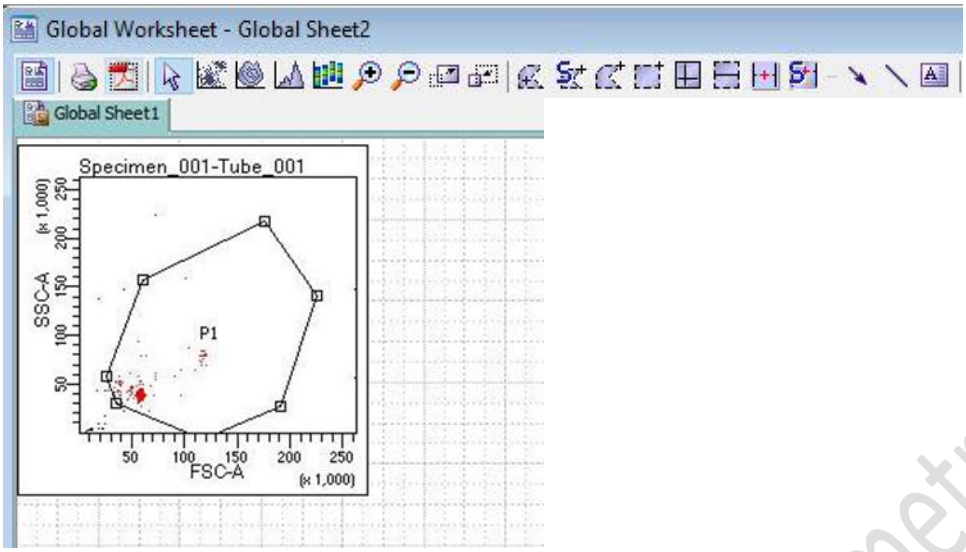
E. Create Gates

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

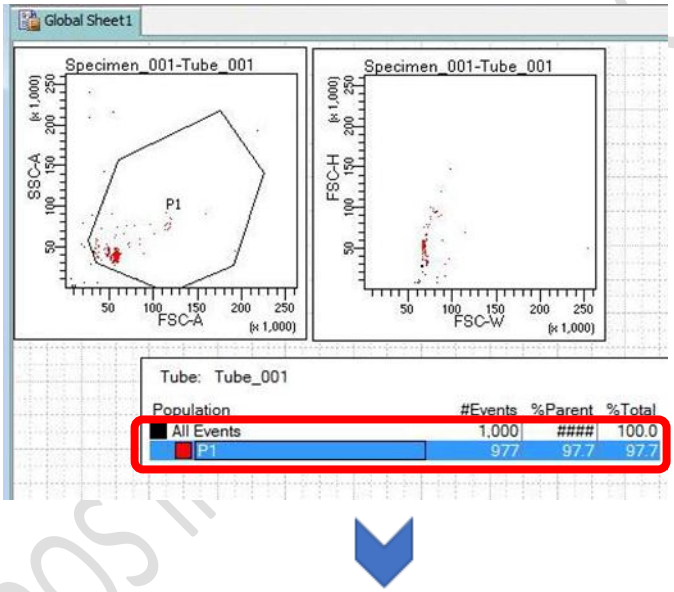


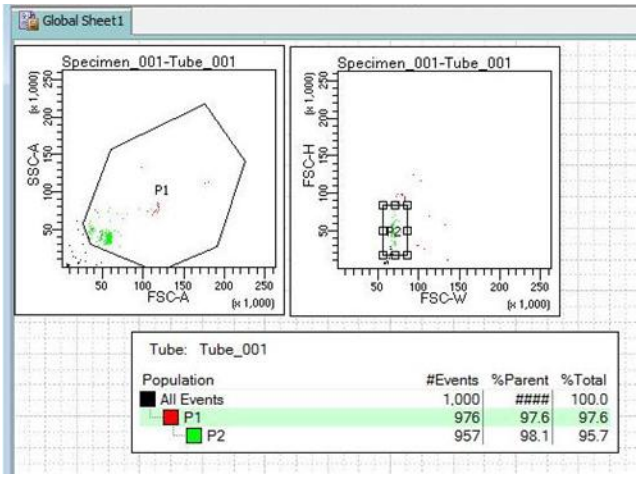
Icon	Type
	Polygon Area Gate
	Rectangle Area Gate
	Quantrad Gate
	Interval Gate

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

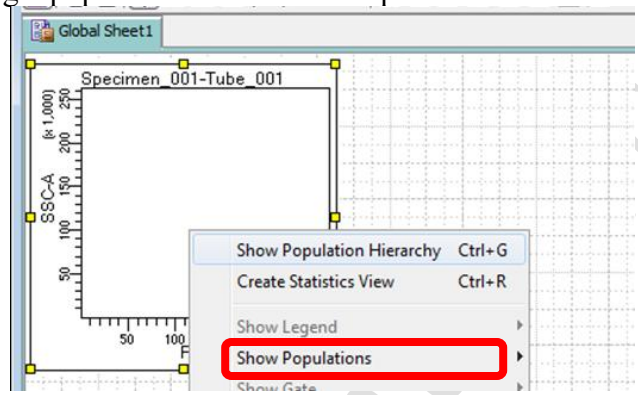


3. If you want to create a new population out of particular parent population, highlight the parent gate on the hierarchy table first and then create the gate.



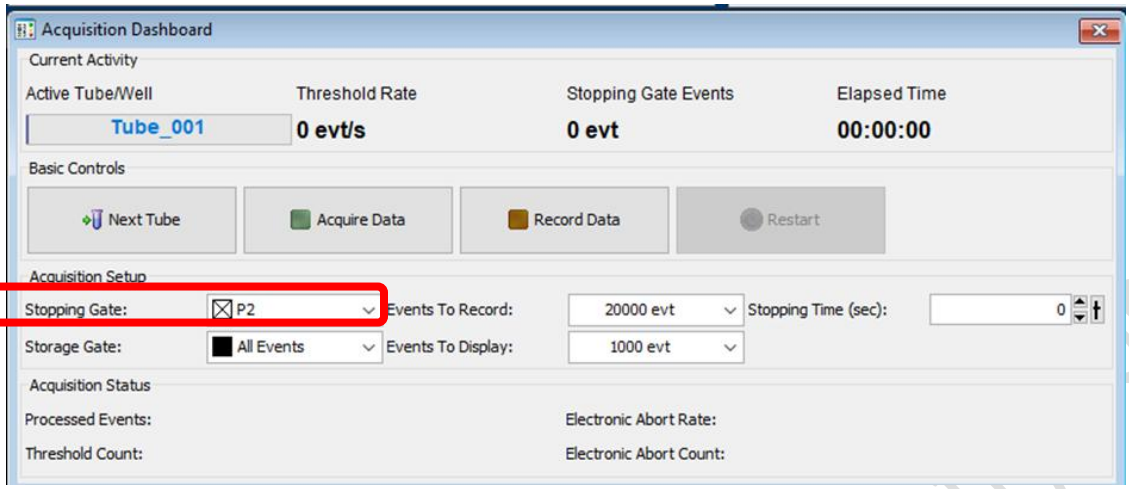


4. Click on target plot and right click, Click *Show Population* > *Target population* to visualize the target population ONLY in that plot.

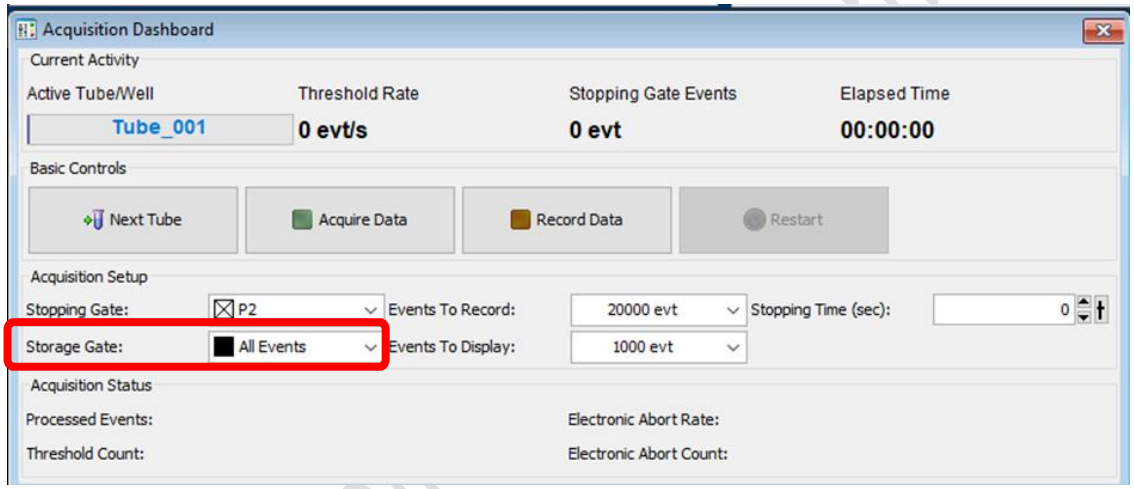


F. Data Recording

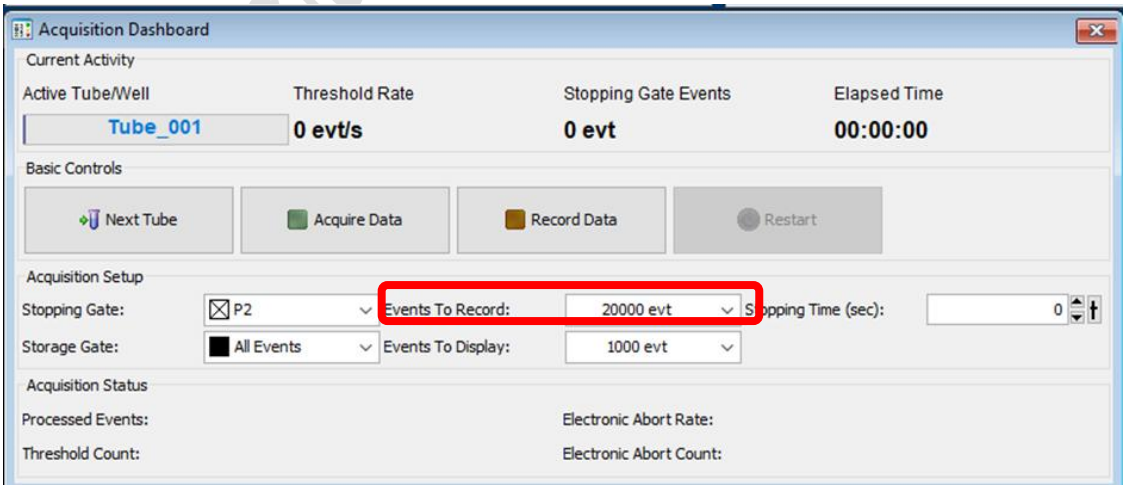
1. Go to Acquisition Dashboard, set Stopping gate to singlet gate or live cell gate



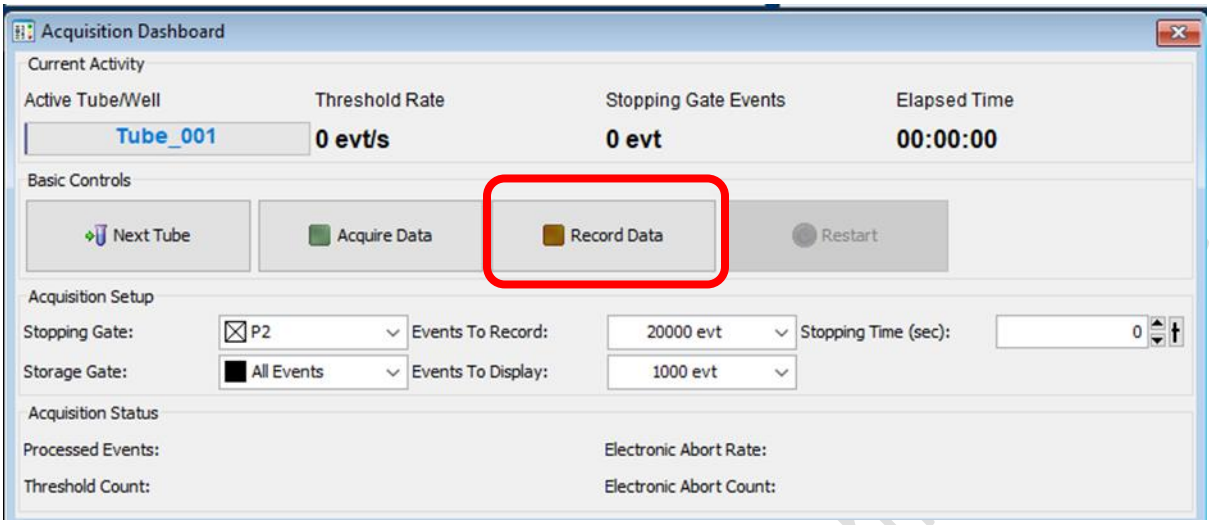
2. Set the Storage gate to All Events



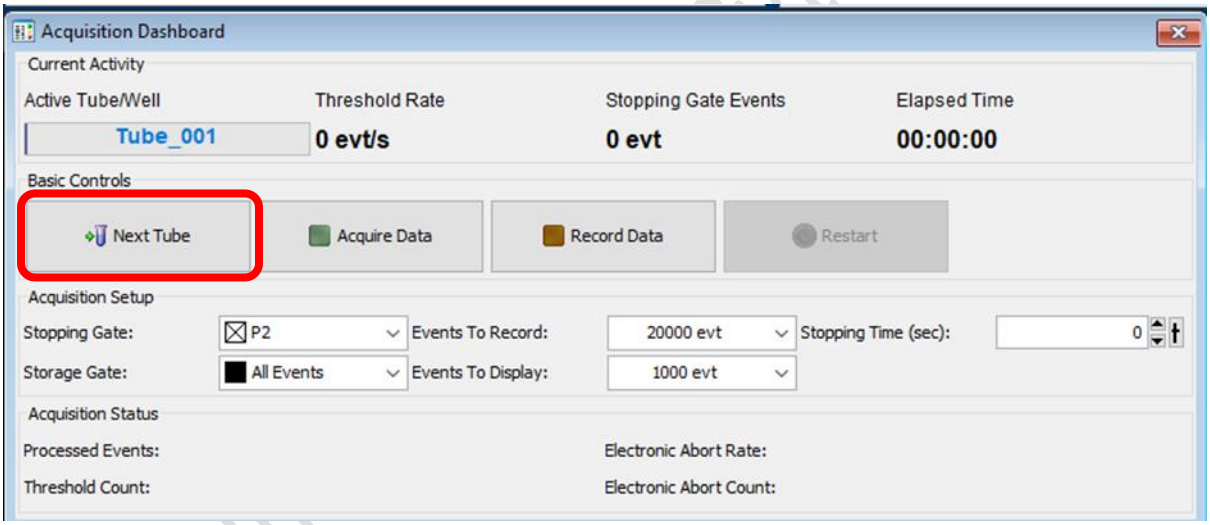
3. Set Events To Record, i.e. events number out of stopping gate to be recorded



4. Click **Record Data**



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

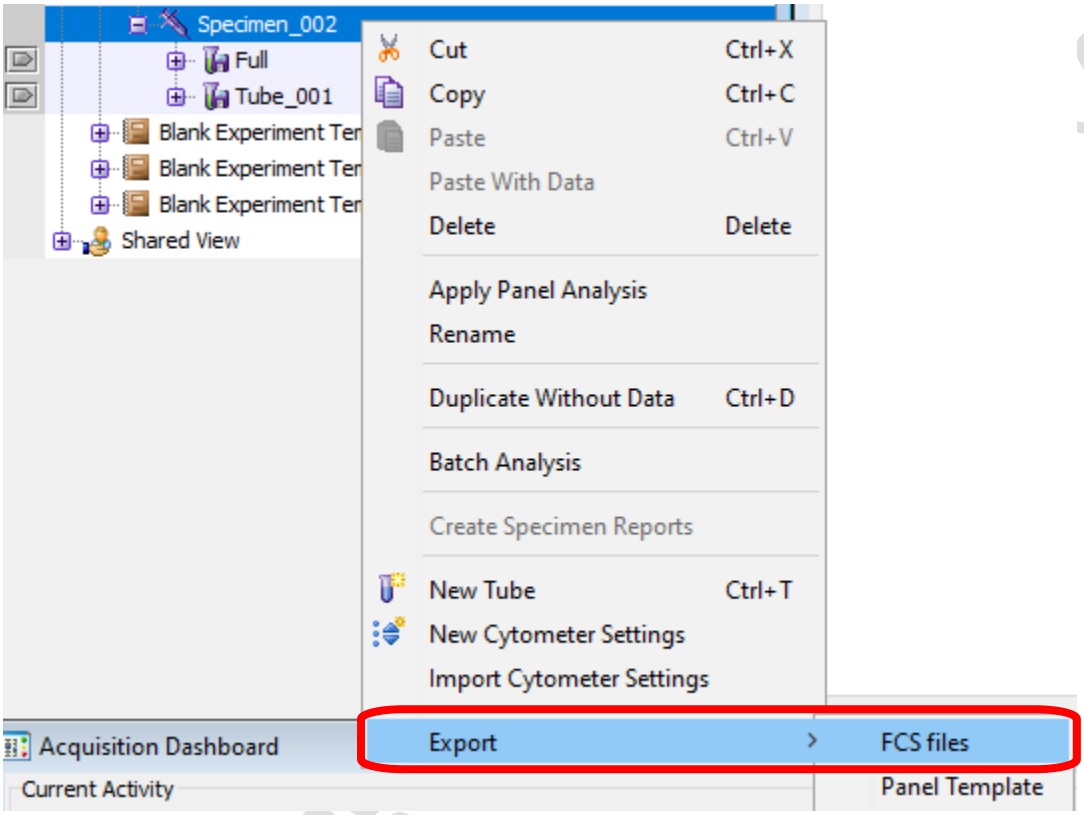


H. Data Export

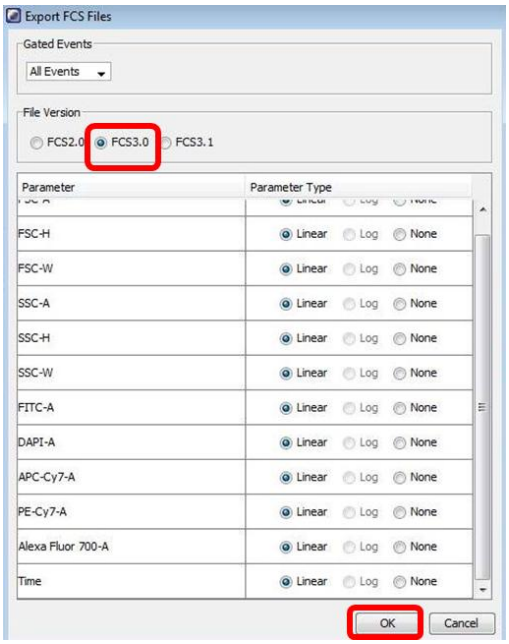
1. FCS file

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

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

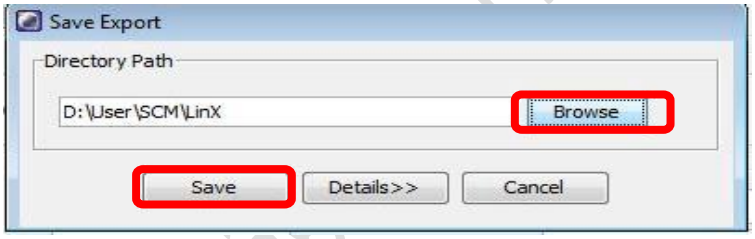


1.3 Select *FCS 3.0* and keep all parameters Linear. Click *OK*



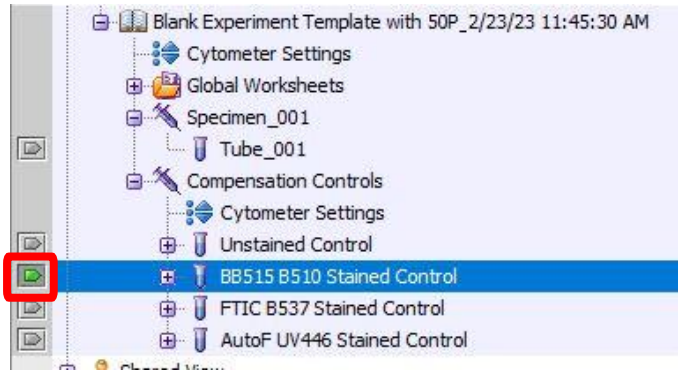
1.4 Click **Browse** to choose the destination (D:/User/Department/PersonalFolder)

1.5 Click **Save**



2. PDF file

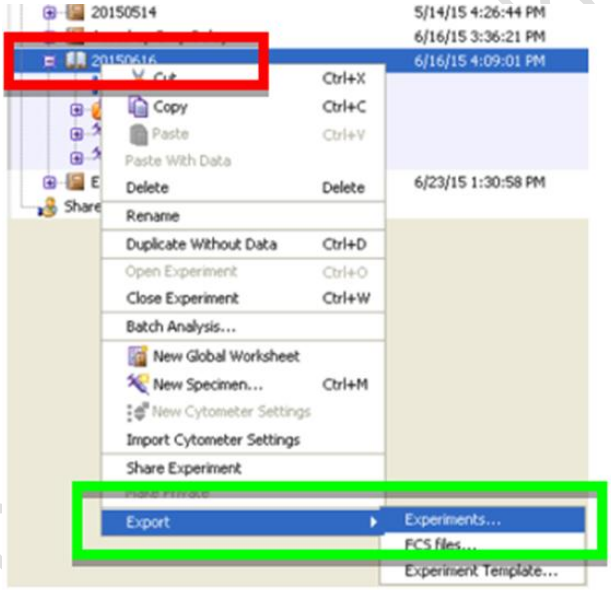
2.1 To export pdf of multiple tubes, Click the tube pointer of any tubes or select the wanted Specimen



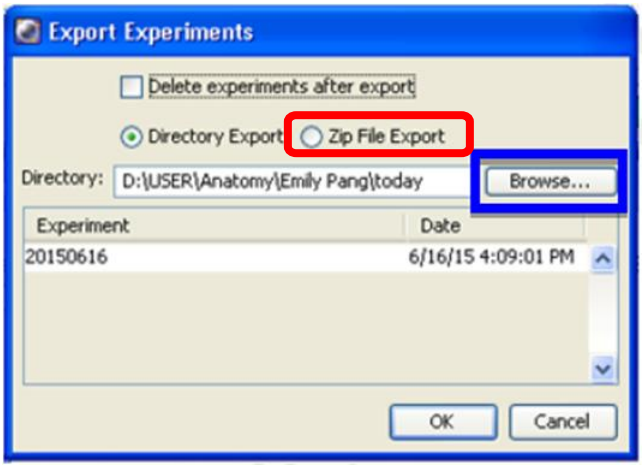
2.2 Click the PDF icon and save to your folder.

3. Experiment

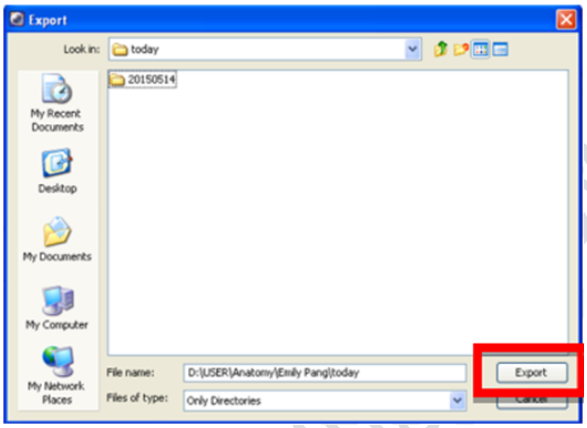
3.1 Right click on the **Experiment > Export > Experiments**



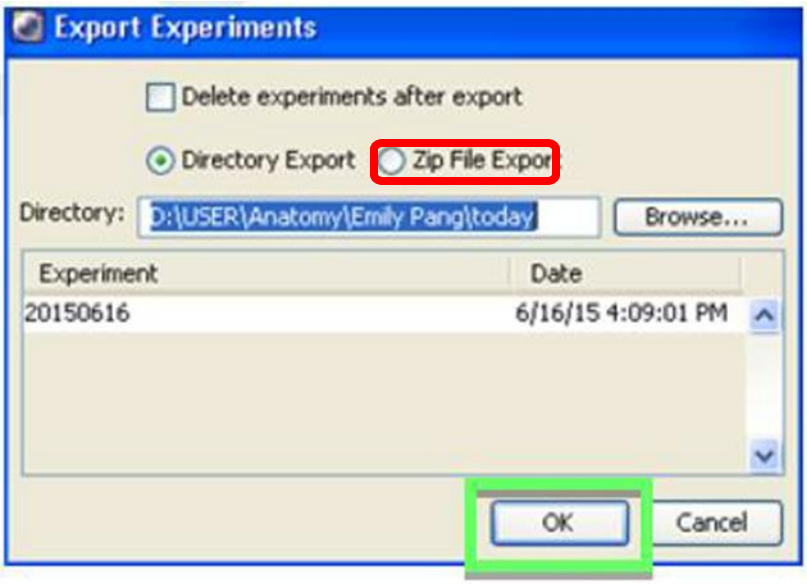
3.2 Choose **Zip File Export** and click **Browse** to select the file destination



3.3 Select the file destination and click Export.

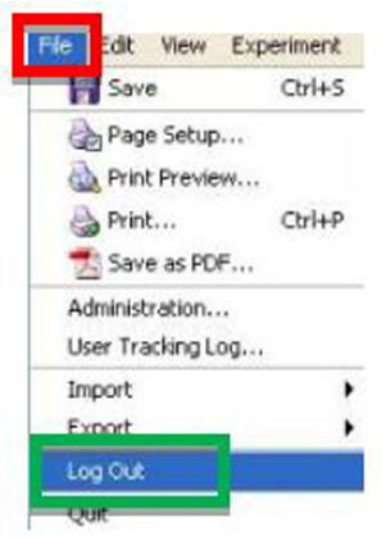


3.4 Click **OK** to export



I. Software Log out

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



J. 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** and **RUN** 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** and **Standby**

H. Log out Tracker before leave