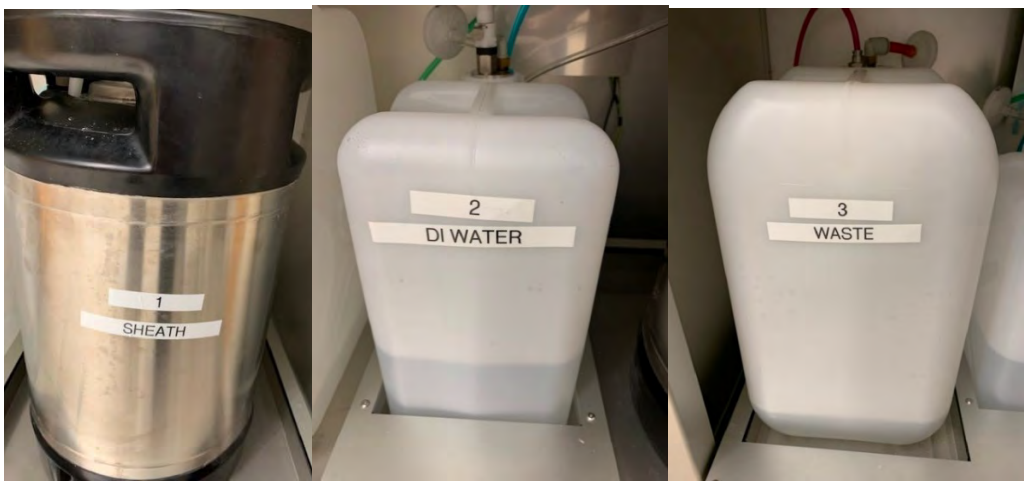
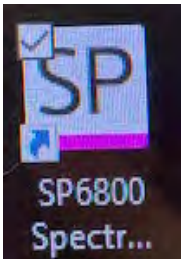
SOP to operate Sony Spectral	Date: 11/14/2019
Responsible for the SOP: Mariza Miranda	
Responsible for the machine: Andria Doty, Mariza Miranda, Anthony Boullosa	Page 1/15

1) Check the level of the sheath tank (1), water (2) and waste (3) before you start.

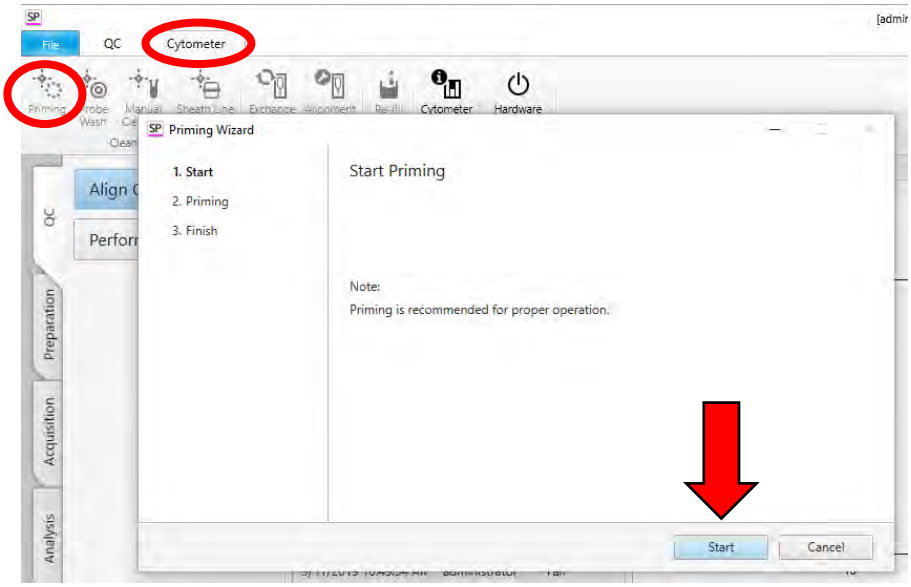


2) Turn on the compressor (4) (button in the back) and the machine (5)

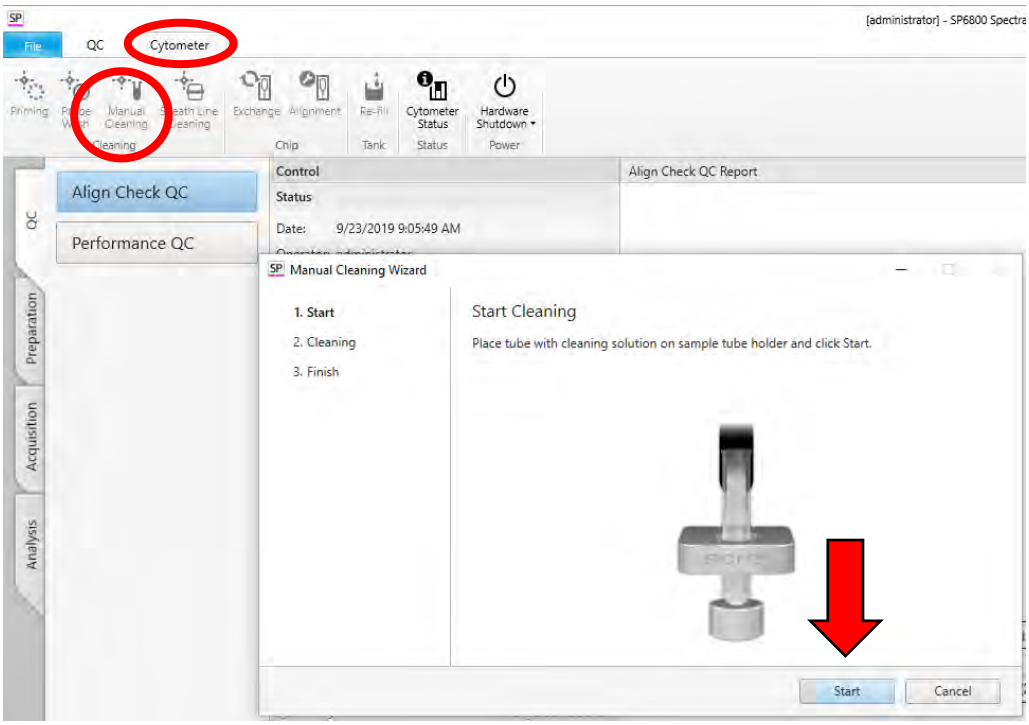
3) Login in the computer, start the TIMER (self service), and open the Sony spectral program (SP6800 Spectral).

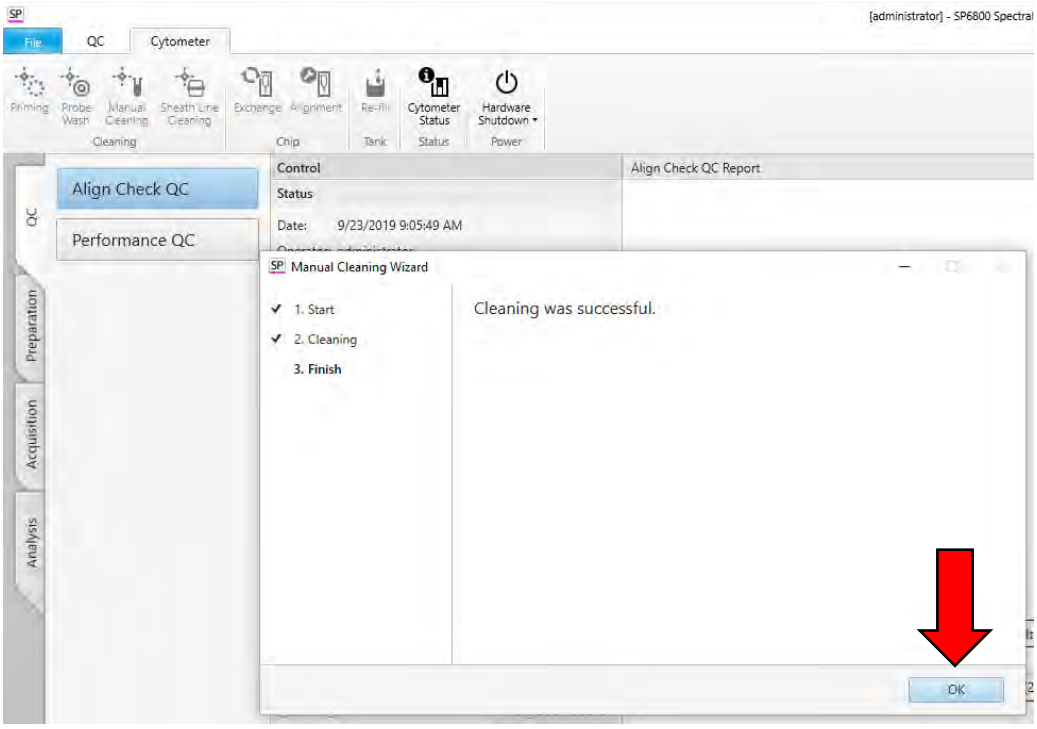


4) When you open the program will pop out a message for Prime the machine >> select **START**. If the message doesn't appear automatic, go to **CYTOMETER** tab and **PRIMING**



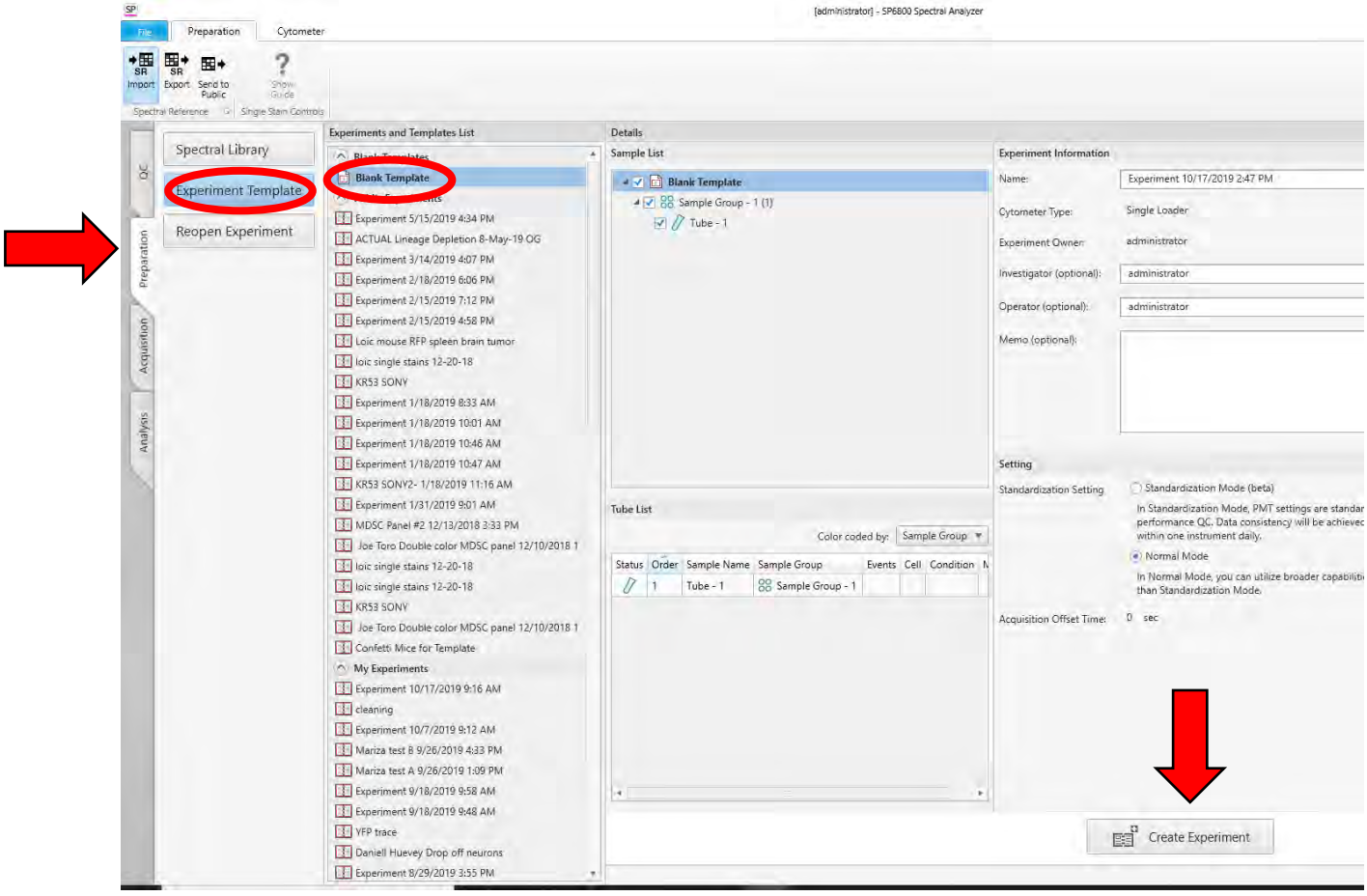
5) After to Prime, go to the tab **CYTOMETER** and run **MANUAL CLEANING** with contrad 5% first, then bleach 30% and water



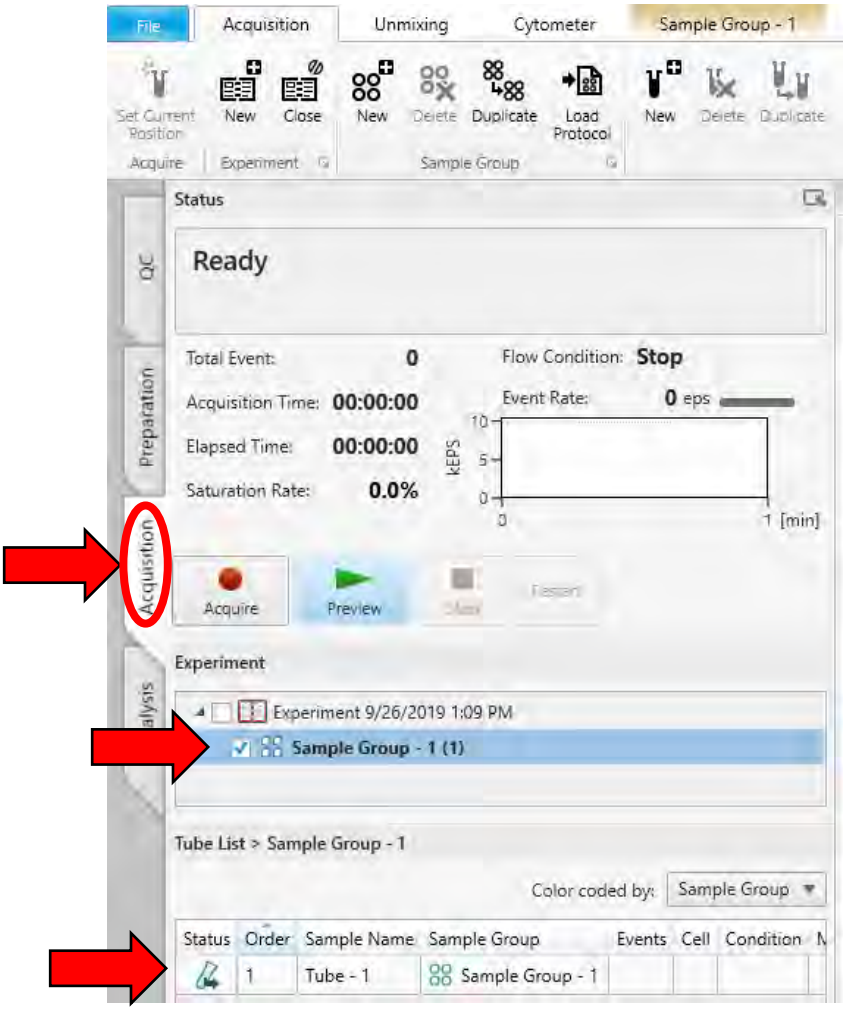


Repeat to clean with bleach and water

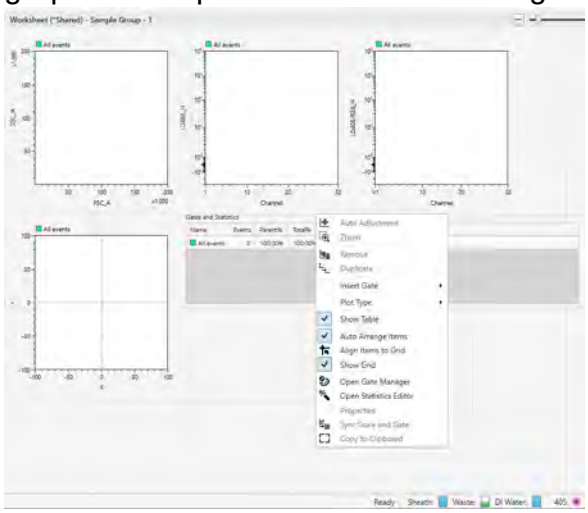
6) Go to the tab **PREPARATION** >> select **PREPARATION** tab on the left side >> Then select **EXPERIMENT TEMPLATE** >> **BLANK TEMPLATE**>> **CREAT EXPERIMENT**

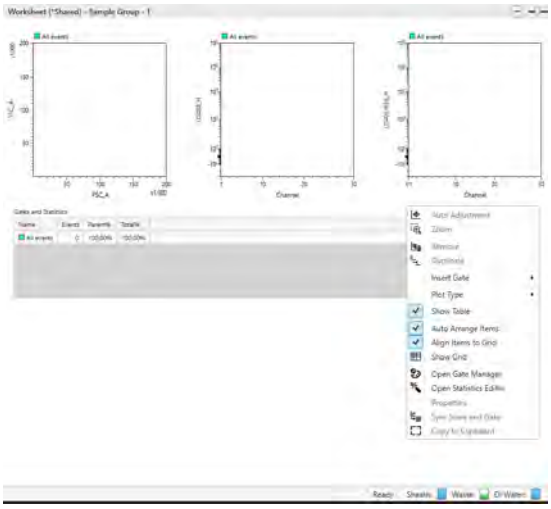


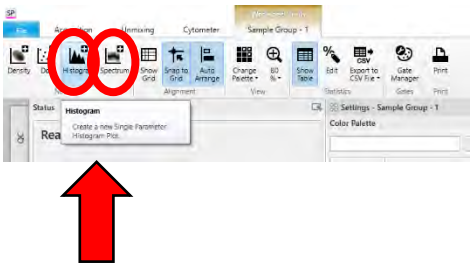
7) It will open a new screen, on the tab **ACQUISITION** select **SAMPLE GROUP** >> It will generate the tube 1

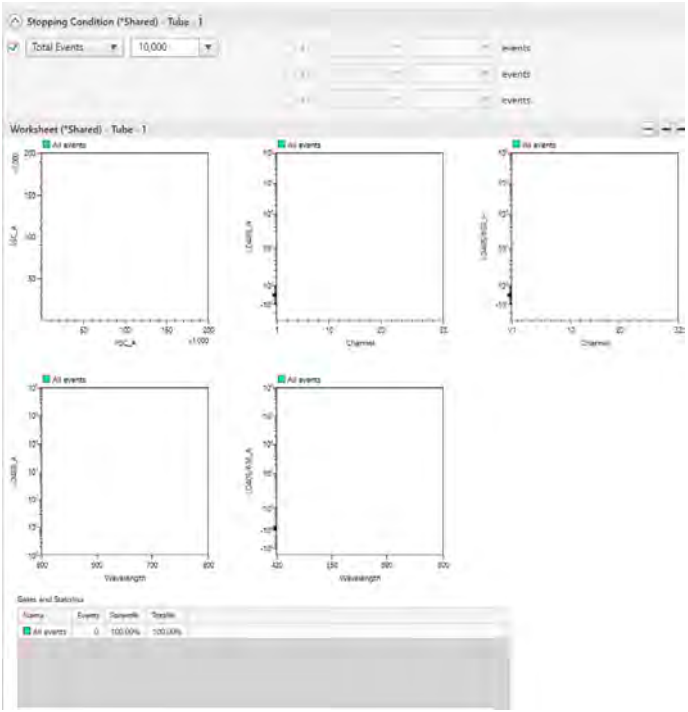


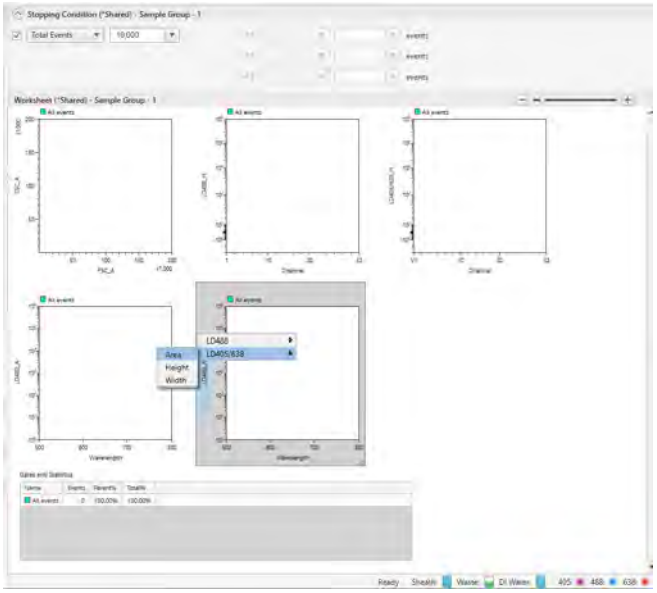
8) Organize your work space: right click and remove lines of grid, add 2 new spectrum graphics and put the axis as wavelength





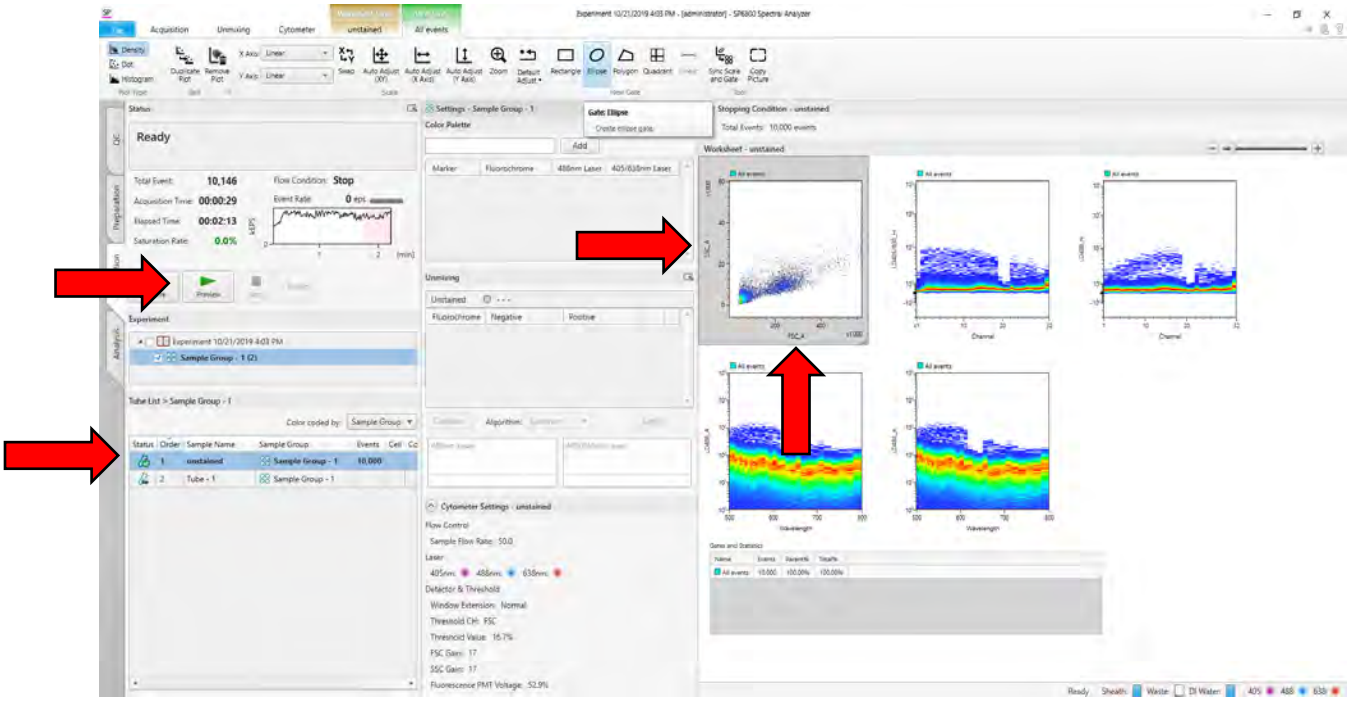




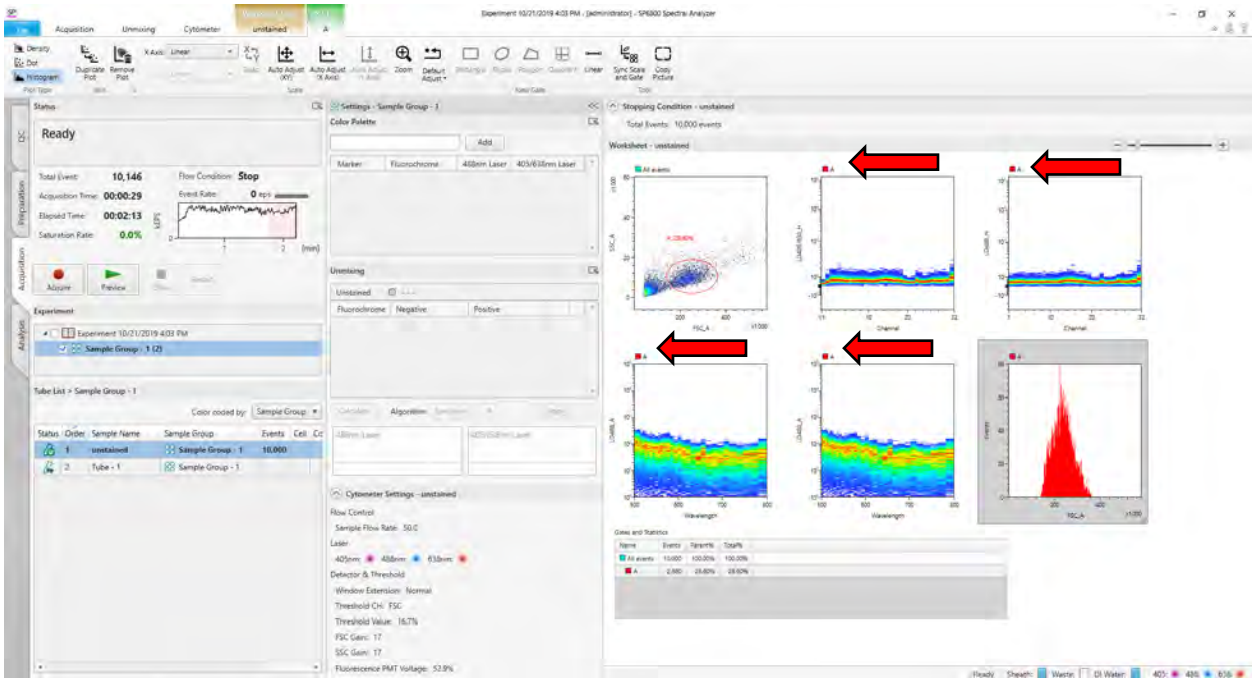


Change the FSC-A for the color that you are working

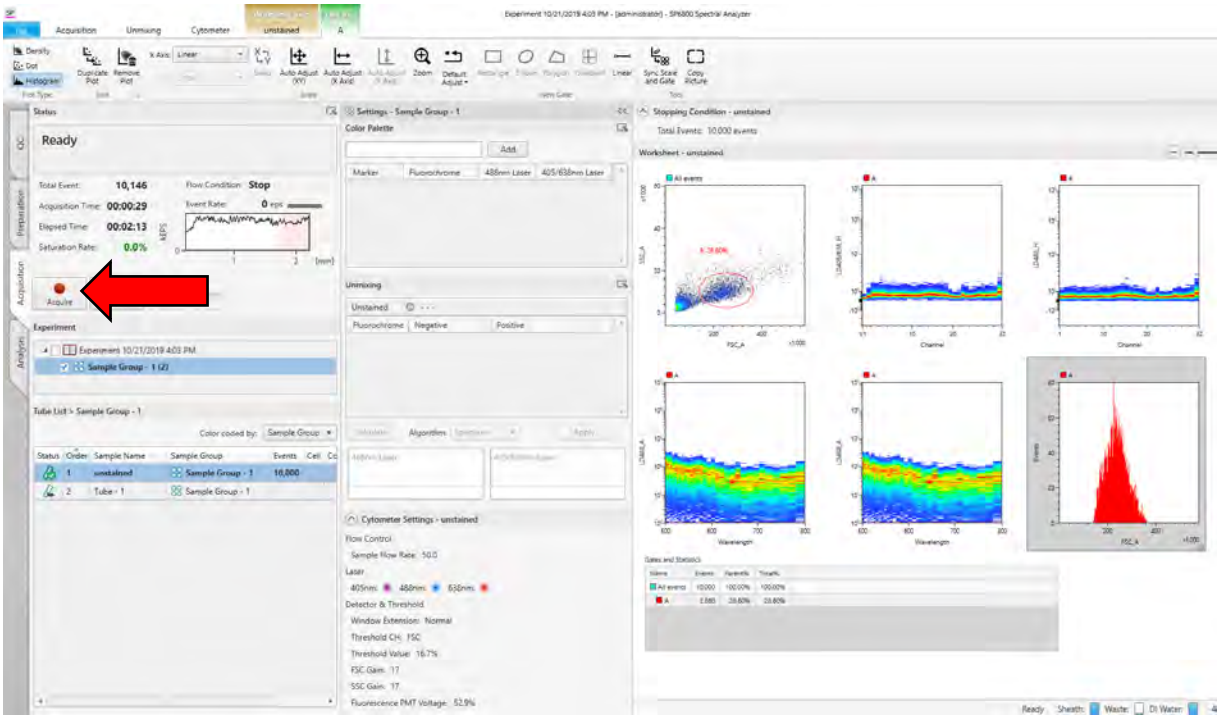
9) Select the tube 1, rename it as UNSTAINED and insert the tube in the holder on the machine. Click in **PREVIEW** to find your cells/beads adjusting the SSC-A and FSC-A:



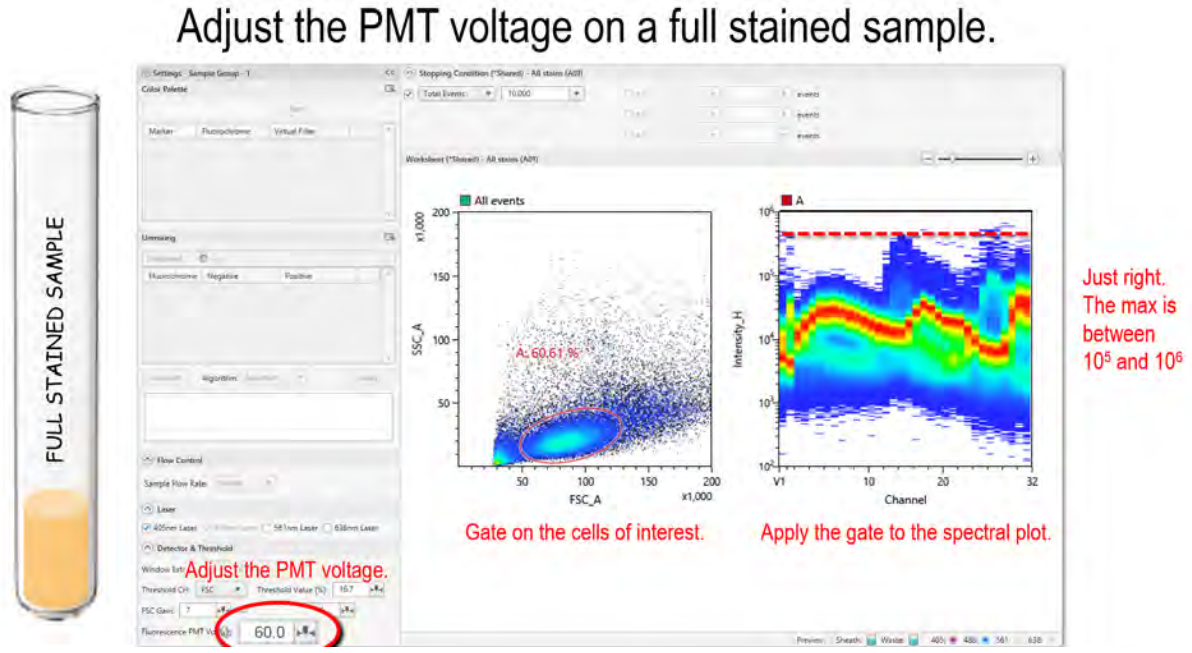
10) Put the gate in your cells/beads while you are seeing in Preview, then show the gate A on all the graphics

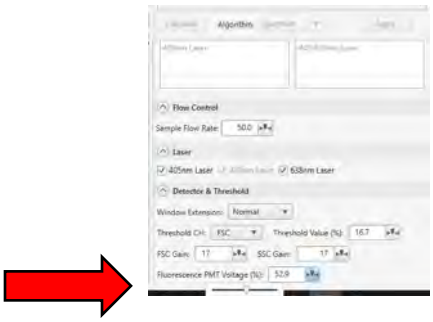


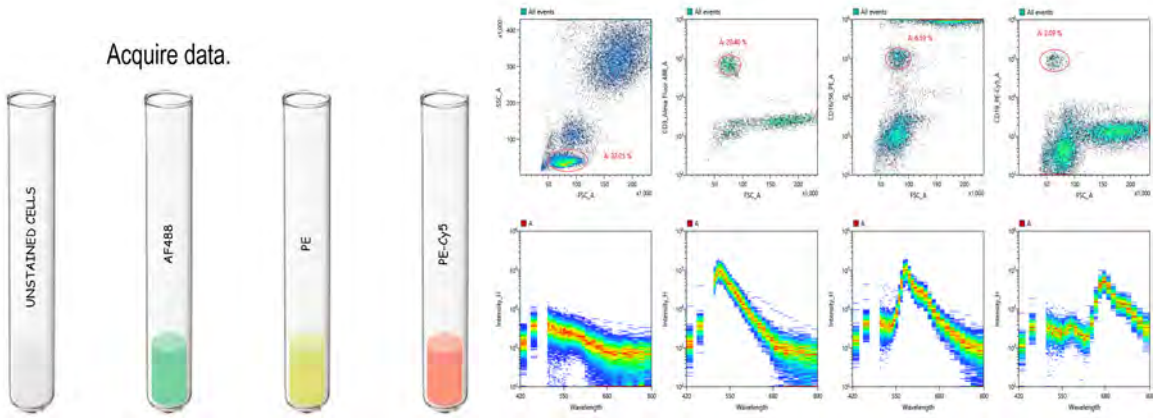
11) Once that the gate is set for your cells/beads click on **ACQUIRE** to record



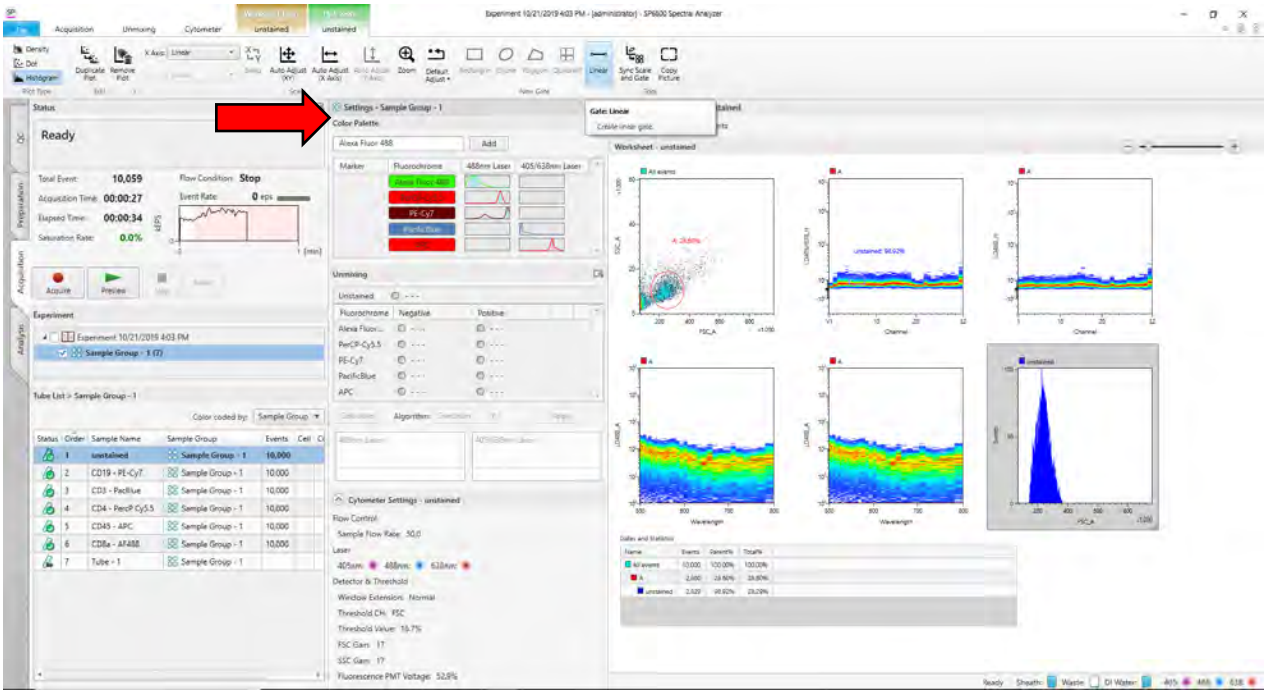
12) After record the first tube (unstained), the next tube will appear automatic: rename (fisrt color, ex.PE), adjust the voltage (arrow) when you are in **PREVIEW**, and then, **ACQUIRE.**



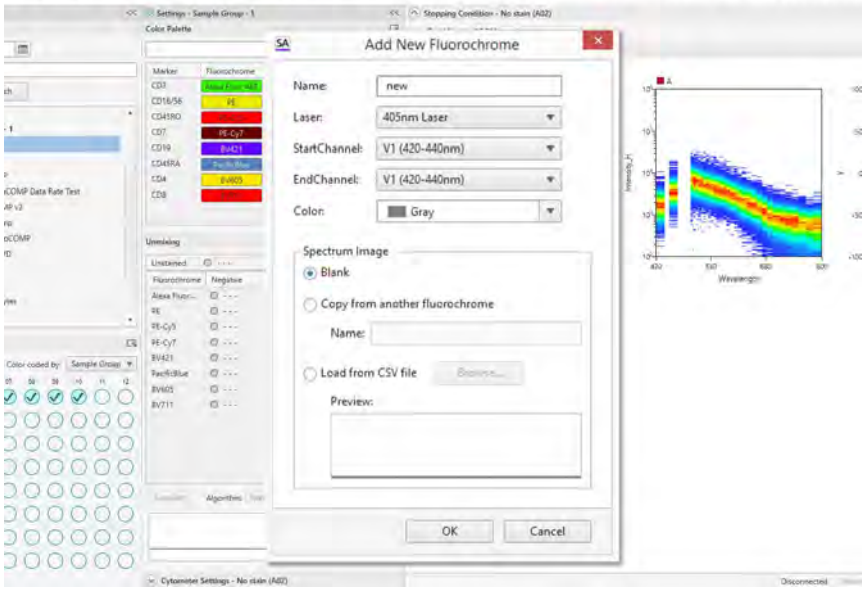




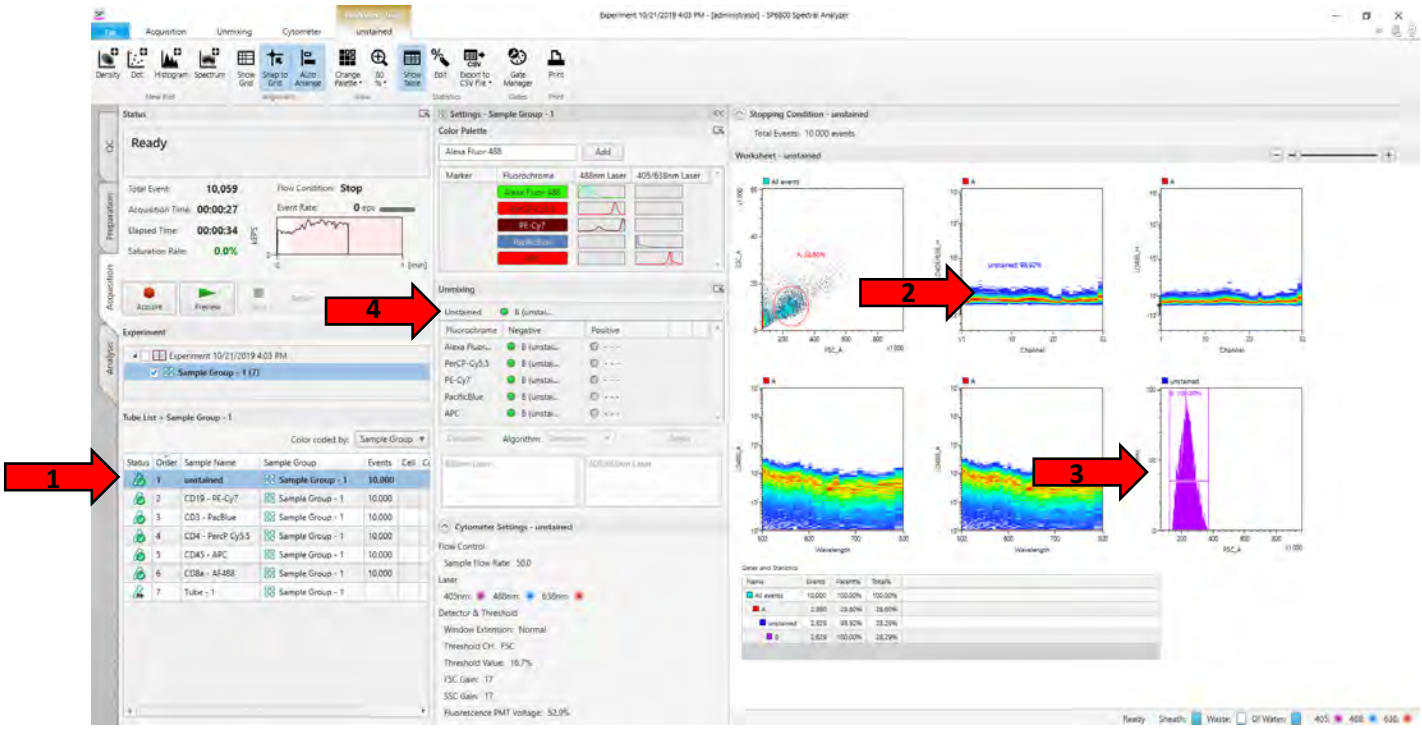
13) After record all the fluorochromes/antibodies, add them to the **COLOR PALETTE** one by one.



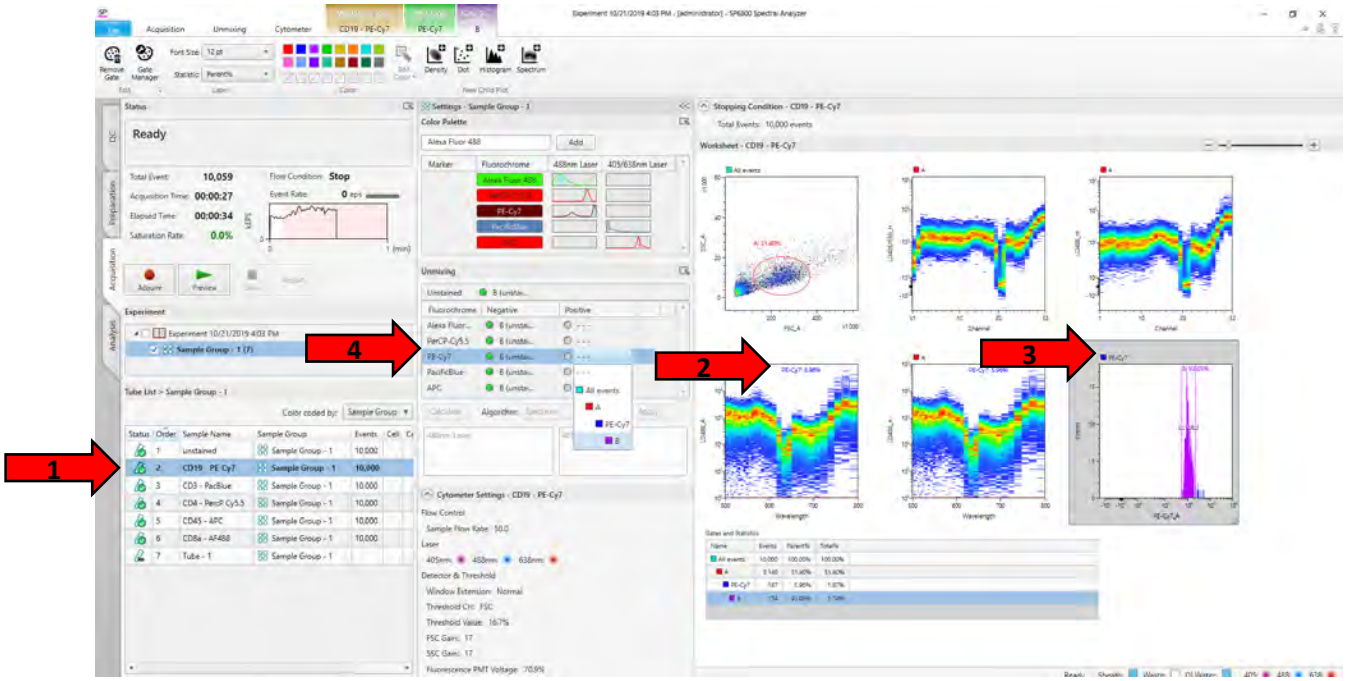
New fluorochromes can be added to the database



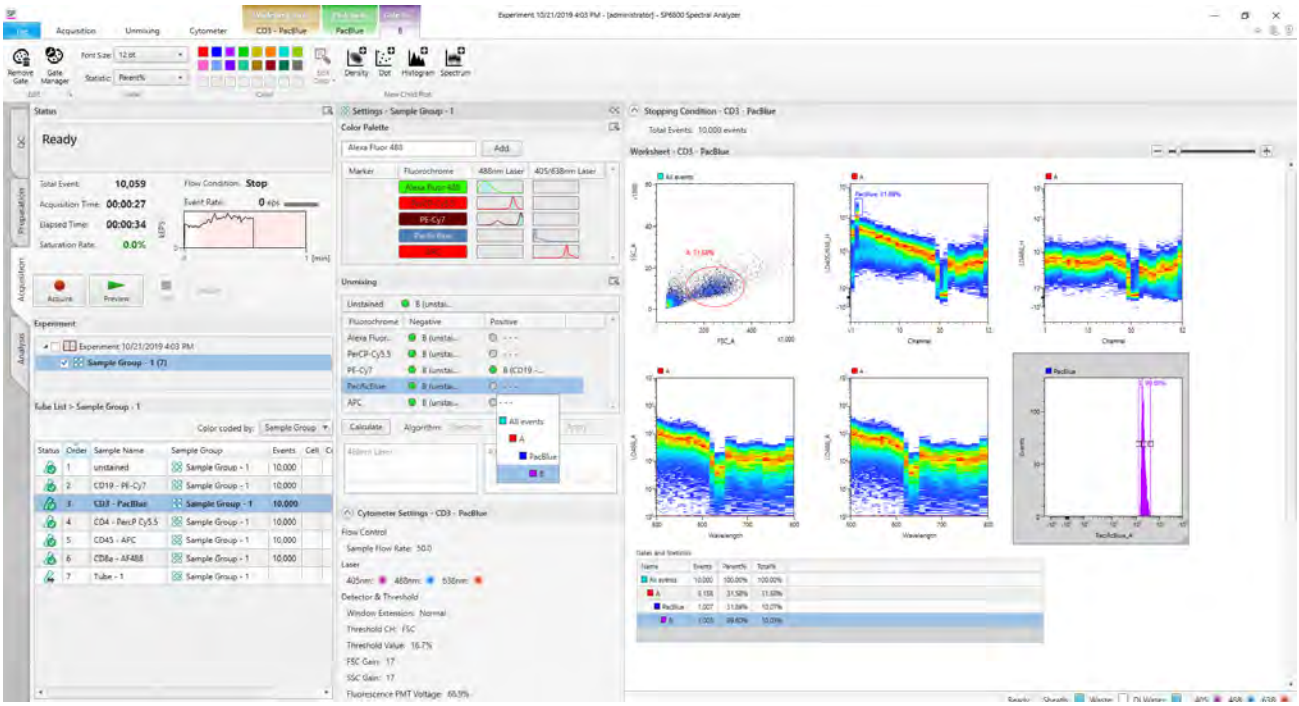
14) After add all the fluorochromes, select the **unstained Tube** (arrow 1). Make a square gate in any of the channel (arrow 2), then ask to show this gate (unstained) on the histogram.
Select the linear gate on the histogram for the negative cells (arrow 3). On **UNMIXING**, double click and assign Negative Spectrum for the unstained and for each fluorochrome (arrow 4).



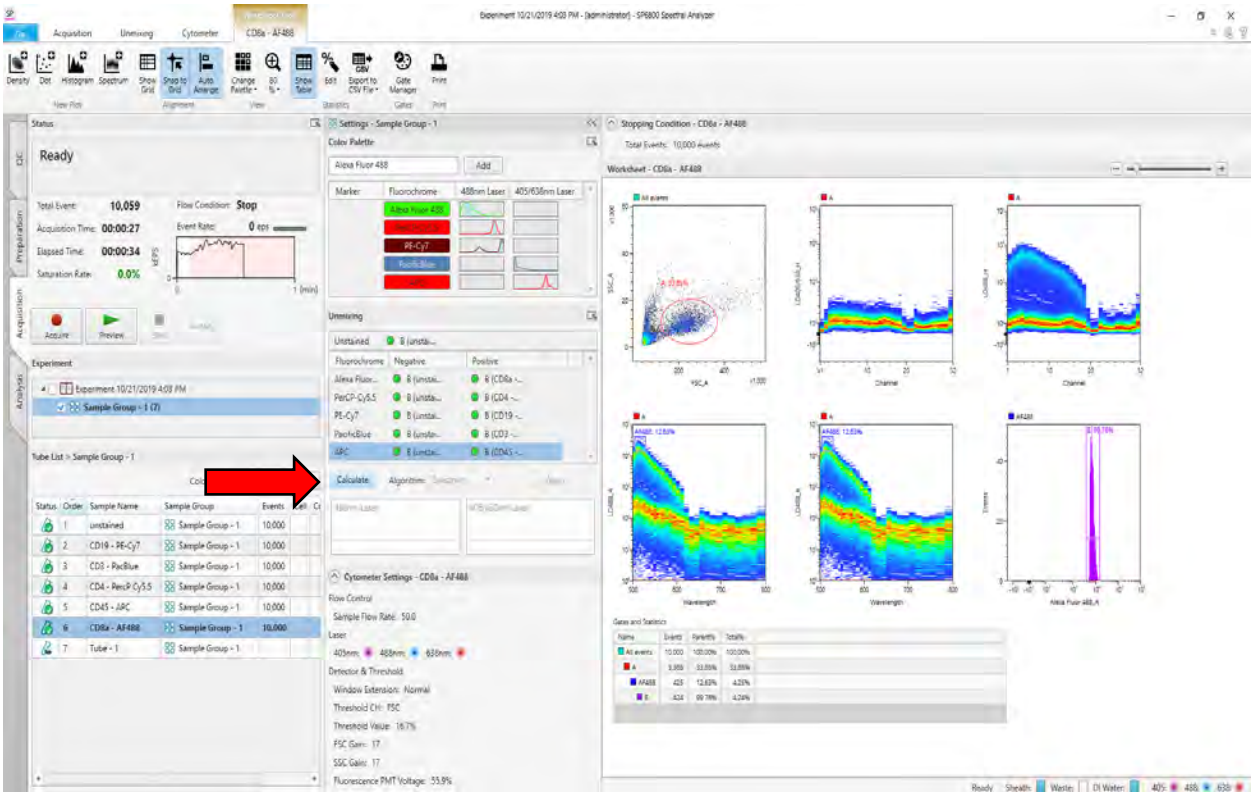
15) Select the next tube with fluorochrome and make a square gate in the highest pick on channel or wavelength graphic corresponding to the channel/wavelength used for the specific fluorochrome. Then, show this gate (i.e. PE-Cy7) on the histogram. Select the linear gate on the histogram for the positive cells. On **UNMIXING**, double click in the fluorochrome and assign the positive gate for the specific color (i.e.B)

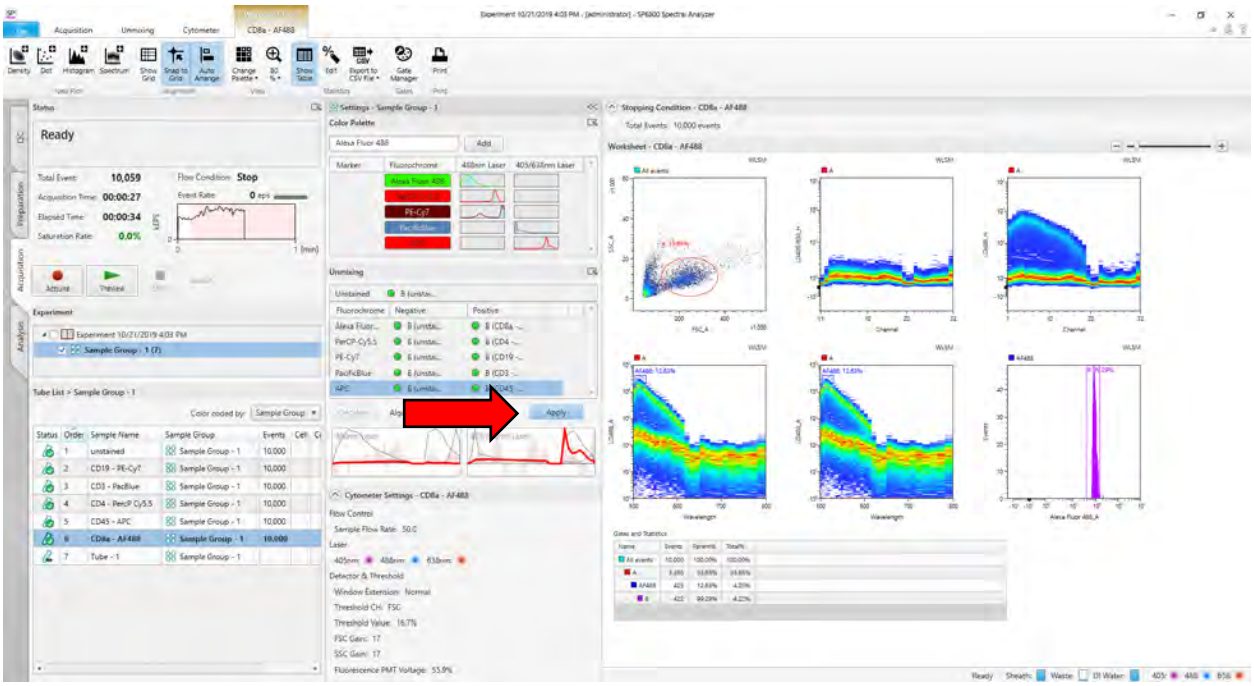


16) Repeat the steps on the item 15 for each individual color (i.e. PacBlue)

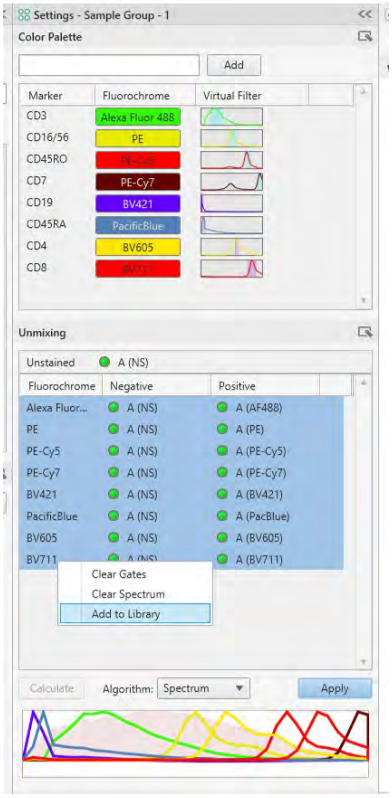


17) After assign the Positive Spectrum for each fluorochrome go to >>> **CALCULATE** >>> **APPLY**



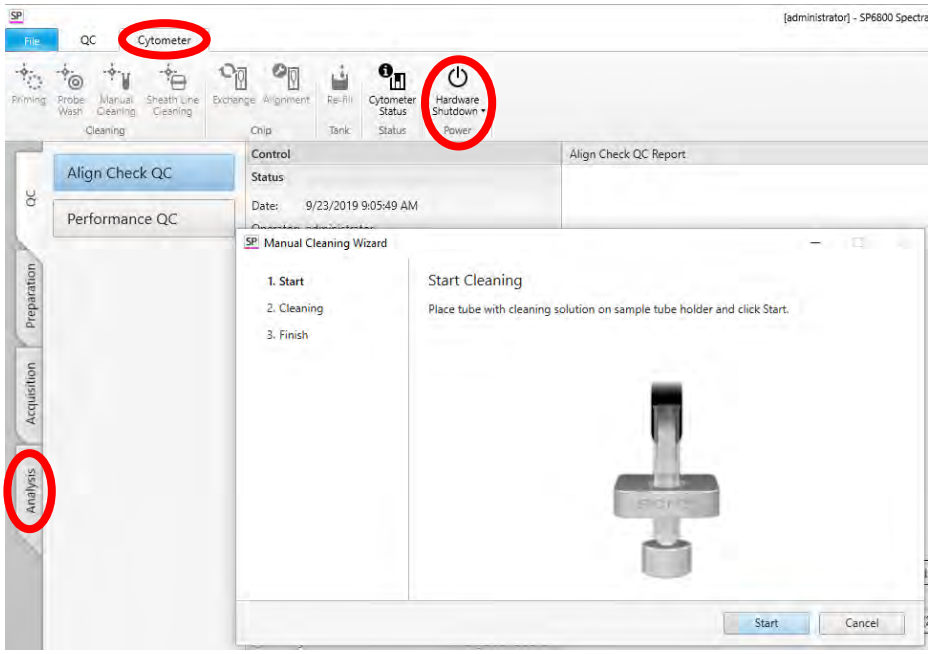


18) Save the Normalized Reference Spectra to the Spectrum Library



19) To proceed the Analysis you need to close your experiment ???

20) To shutdown the machine , go to **CYTOMETER** tab and select **HARDWARE SHUTDOWN >> DAILY CLEAN** and proceed the cleaning with bleach and water.



21) gkdngngk