

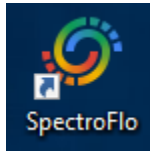
Setting Up an Experiment on the Cytex Aurora Spectral Analyzer

Important things to Remember:

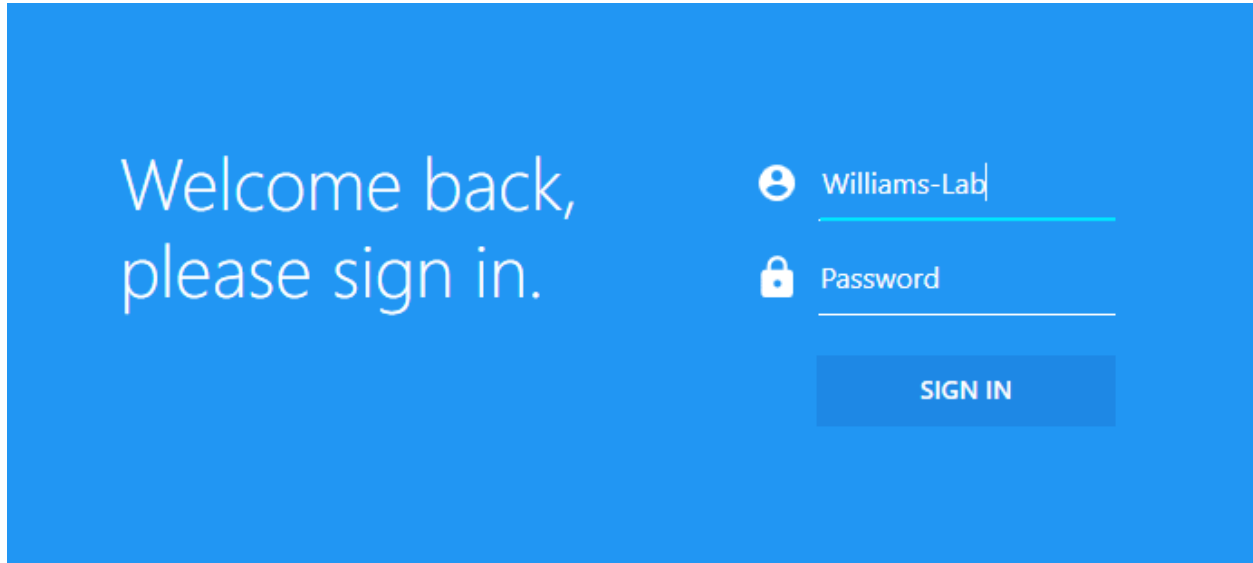
1. You must have single stained controls and unstained controls for your sample
2. The single stain controls MUST be exactly what you have in your sample
3. Controls can be on cells, beads, or a mixture of both
4. Normal rules of compensation apply (controls must be as bright as or brighter than your sample, etc.)
5. 50-character limit for experiment/template/worksheet names and 30-character limit for User Settings Names

Instructions:

1. If the software isn't open already, double click on the icon for SpectroFlo

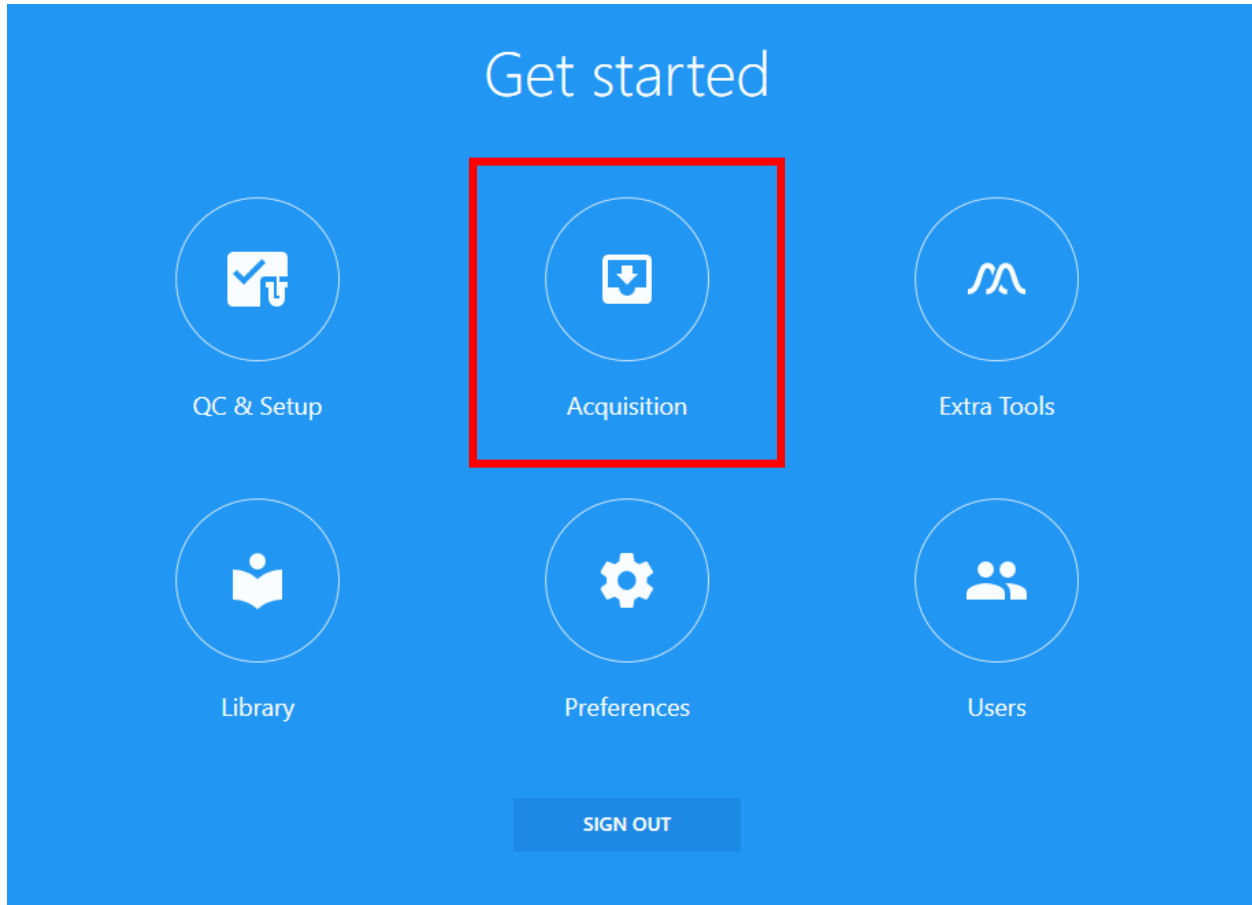


2. Log in

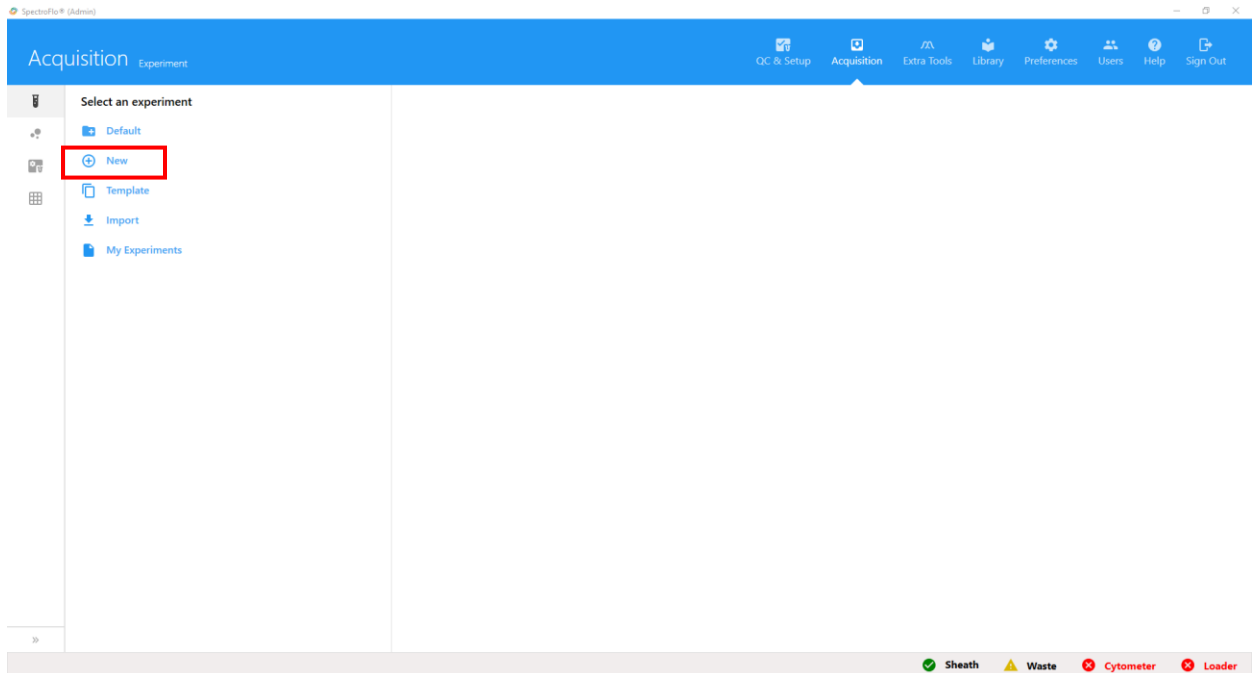


- a. Username has a drop-down menu. Begin typing your username in and click on your lab name from the drop-down menu that appears.
- b. Your password will be the one given to you during training

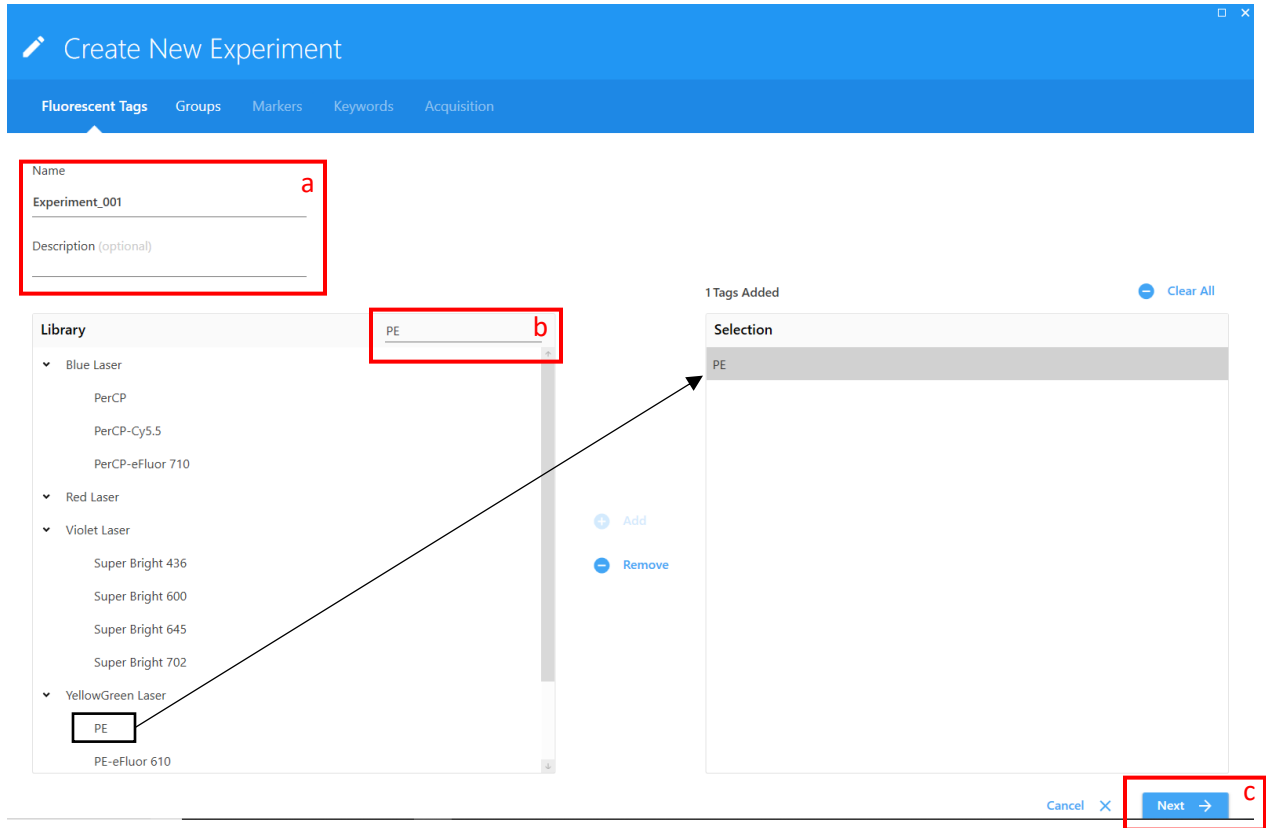
3. Click on **Acquisition**



4. You will now be at the following screen. Click on **+ New**

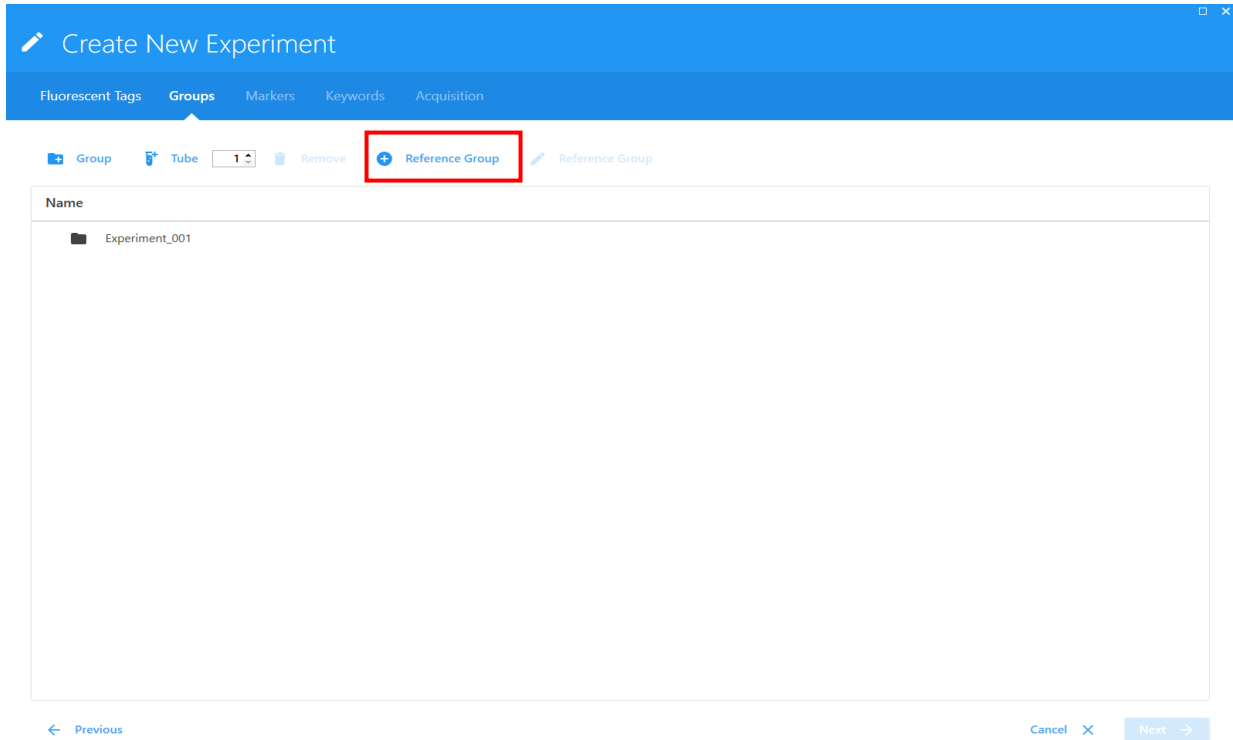


5. The 'Create New Experiment Wizard' will open.



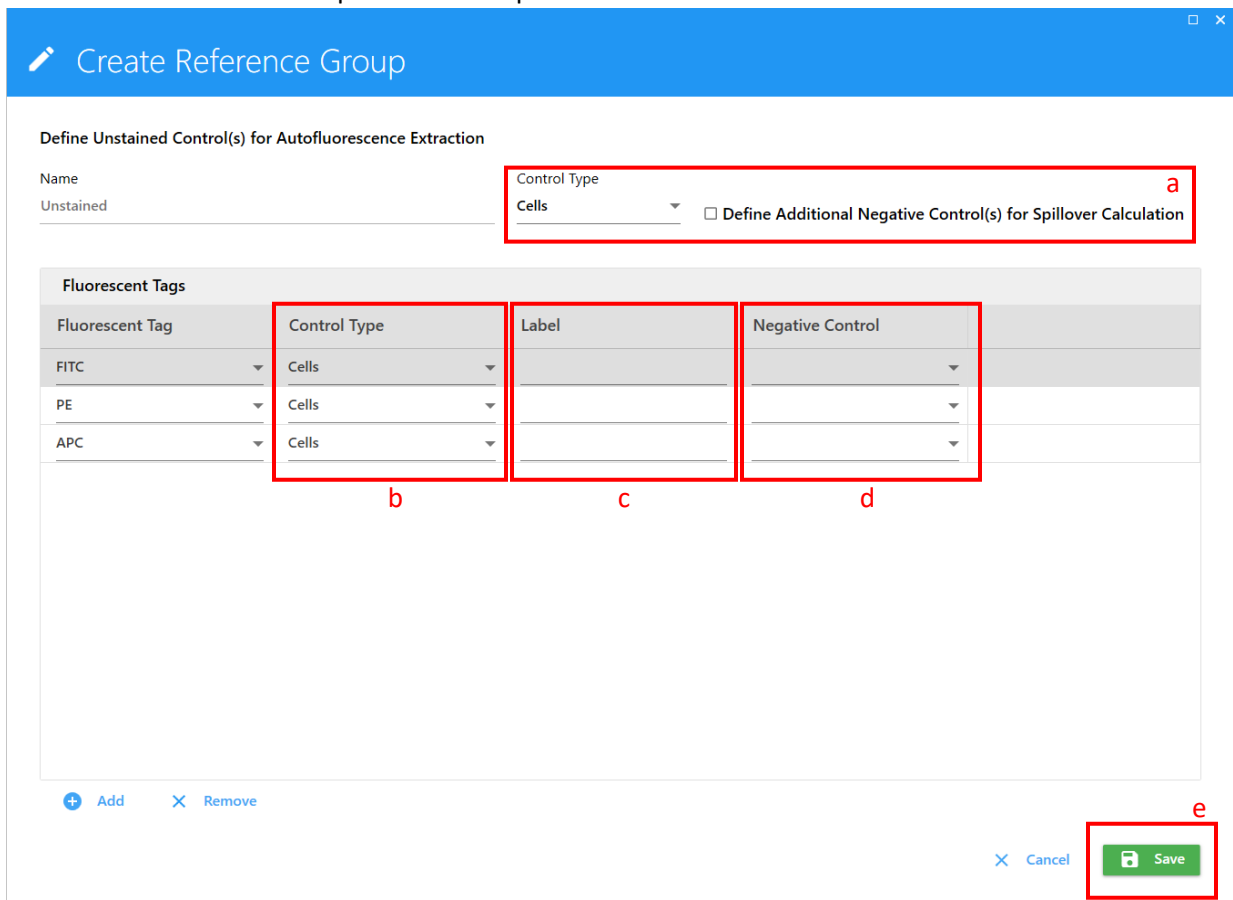
- a. Name your experiment – the more unique the name is the better. This helps us if we need to retrieve your data from the backup servers.
- b. Add fluorochromes to your experiment
 - i. Type the fluorochromes in the 'Type to filter' box
 - ii. Double click on desired fluorochrome to add it to your experiment
 - iii. ****If you are using a fluorochrome that is not in the library, please let one of the flow core staff know. They are easy to add, all we need is the excitation and emission max wavelengths****
- c. Click **Next**

6. You will now be at the following screen



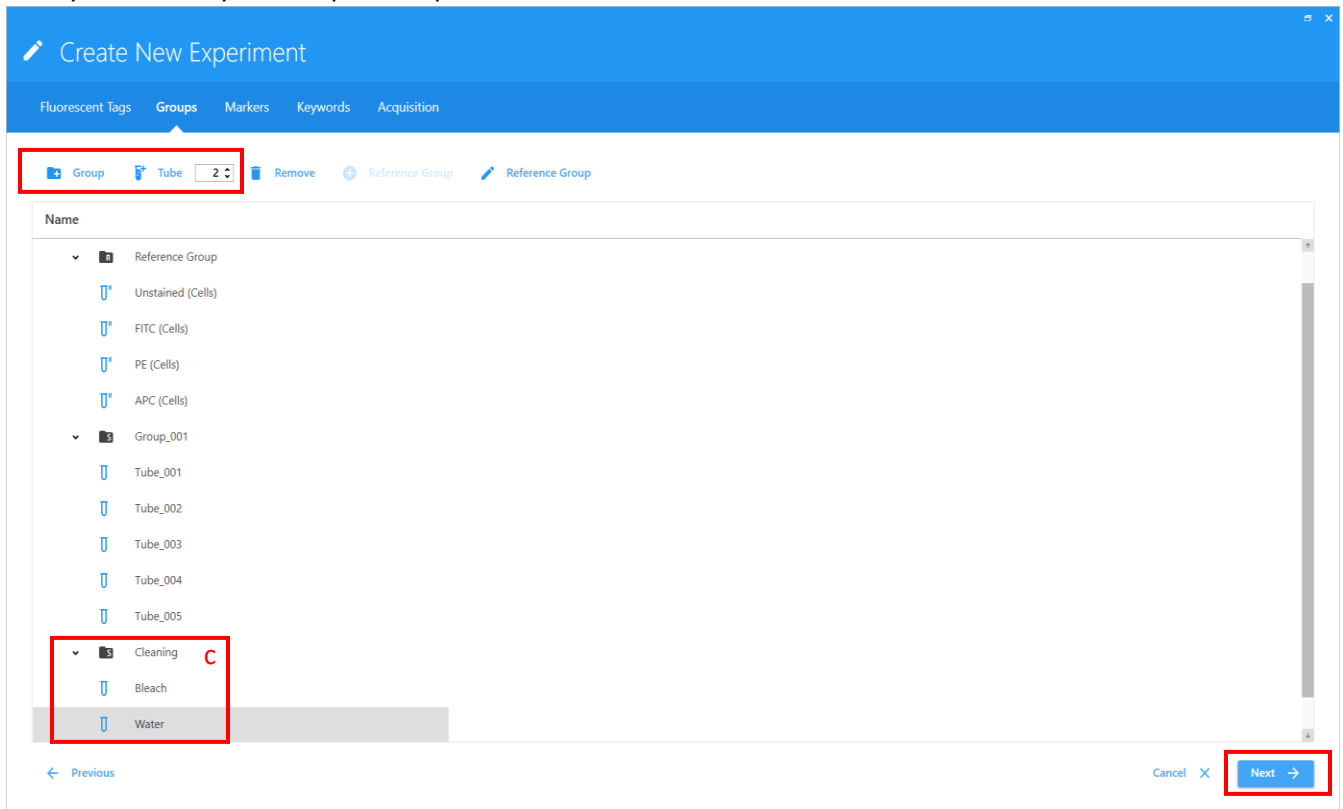
a. Click on **+ Reference Group**

7. The 'Create Reference Group' wizard will open



a. Choose your control type (cells or beads)

- i. If you will be running some single stained controls on beads and others on cells, check the 'Define Additional Negative Controls' box and add an additional negative control and choose its type.
 - ii. If you are running all of your controls on beads, it is still recommended to run an unstained cell control by adding it as an additional negative control.
 - b. Choose the control type (cells or beads) for each of your single stain controls
 - i. If you change the 'Control Type' for the first one in the list, it will change all the controls to match that type. You will have to then individually change all the controls under it to what you want.
 - c. Label your controls, this is the only place you can add labels to the reference controls (i.e. CD4, CD8, etc.)
 - i. You can come back to this wizard at any time
 - d. Select which control you would like the software to use as a negative control for your single stained control
 - i. ONLY do this for single stained controls which do not have a negative population in them
 - e. Click Save when you are done with the above steps
8. Now you can add your Sample Groups



- a. Click on Group and it will create a Sample Group with a single tube in it
 - i. You can create a group with as many tubes as you need. First type the desired number of tubes into the box next to the 'Tube' button, and then hit group. (ex. Typing in 10, and clicking the 'Group' button will create a group with 10 tubes in it)
 - ii. You can also add tubes to your Sample Group in the same manner. Highlight the group you want to add tubes to, type in the number of tubes you want to add, and then click the 'Tube' button
- b. To rename either the Group or Tube just double click on the text (Group_00X or Tube_00X in the above photo) and type in what you want.

- c. Always add a group to your experiment, with two tubes.
 - i. Rename this group to 'Cleaning'
 1. Rename 'Tube_001' to 'Bleach'
 2. Rename 'Tube_002' to 'Water'
 - ii. Change the 'Events to Record' to 1 million or more
 - iii. Set the stopping time for 180 seconds
 - d. When you are done setting up your Groups and Tubes, click 'Next'
9. In the 'Markers' tab you can add fluorochrome marker information to your groups/tubes

The screenshot shows the 'Create New Experiment' window with the 'Markers' tab selected. The 'Groups' table is as follows:

Name	FITC	PE	APC
Experiment_001			
Reference Group			
Unstained (Cells)			
CD4 FITC (Cells)	CD4		
CD8 PE (Cells)		CD8	
CD3 APC (Cells)			CD3
Group_001	CD4		
Tube_001	CD4		
Tube_002	CD4		
Tube_003	CD4		
Tube_004	CD4		
Tube_005	CD4		

The 'Labels' panel on the right shows a list of marker categories: CD Markers, Chemokine, Chemokine Receptors, and Cytokines. The 'Next' button is highlighted with a red box.

- a. The 'Reference Group' markers cannot be changed at this stage
 - i. To change the 'Reference Group' you go back to the 'Groups' tab, and click on 'Edit Reference Group'
- b. The marker labels from 'Reference Group' do not automatically drop down and auto-fill into the sample groups
 - i. This is because you can run more controls than you have in a single sample group, and can run multiple panels as separate groups instead of having a separate experiment for each one
 - ii. Markers can be added at the group level or individually at the tube level. Group level is the quickest and easiest to do
- c. Double click in the space at the group level to add a marker for your first fluorochromes
 - i. Hit 'TAB' on the keyboard, which will automatically move you to the next space horizontally and you can type in your next marker.
 - ii. Continue filling in your remaining markers
- d. When you are done click 'Next'

10. IGNORE the 'Keywords' tab

11. You will now be on the 'Acquisition' Tab

Name	Worksheet	Stopping Gate	Storage Gate	Events To Record	Stopping Time (sec)	Stopping Volume (ul)
Experiment_001				1 - 10,000,000	10,000	1 - 3000
Reference Group	Default Raw Worksheet (Raw)	All Events	All Events	10,000	10,000	3,000
Unstained (Cells)	Default Raw Worksheet (Raw)	All Events	All Events	10,000	10,000	3,000
FITC (Cells)	Default Raw Worksheet (Raw)	All Events	All Events	10,000	10,000	3,000
PE (Cells)	Default Raw Worksheet (Raw)	All Events	All Events	10,000	10,000	3,000
APC (Cells)	Default Raw Worksheet (Raw)	All Events	All Events	10,000	10,000	3,000
Group_001	Default Unmixed Worksheet (Unmixed)	All Events	All Events	100,000	10,000	180
Tube_001	Default Unmixed Worksheet (Unmixed)	All Events	All Events	100,000	10,000	180
Tube_002	Default Unmixed Worksheet (Unmixed)	All Events	All Events	100,000	10,000	180
Tube_003	Default Unmixed Worksheet (Unmixed)	All Events	All Events	100,000	10,000	180
Tube_004	Default Unmixed Worksheet (Unmixed)	All Events	All Events	100,000	10,000	180
Tube_005	Default Unmixed Worksheet (Unmixed)	All Events	All Events	100,000	10,000	180

- a. For ease of use, change the worksheet display for your sample group(s) to and unmixed worksheet.
 - i. Always remember, Raw worksheets are to view spectral data, Unmixed worksheets are for unmixed data
- b. Stopping gates and storage gates can be changed once you have built those graphs/gates on your Unmixed worksheet
 - i. You can return to the Experiment Setup Wizard at any point
- c. Set your 'Events to Record' for each group
 - i. For unstained and single stained controls, the number of events to record depends on what your single stain controls are on (cells or beads)
 1. Beads: 5000-10000 events
 2. Cells: 10000+, for lowly expressed markers you will need to record more events.
 - ii. For your sample groups record the number you need, usually based on the lowest expressing population of interest.
- d. Set your 'Stopping Time' for each group
 - i. Unless you need to run a specific amount of time, this setting can be left at default
- e. Set your 'Stopping Volume' for each group
 - i. This can be set 10-15ul LESS than the total volume in your tube. For example, if you have 200ul of sample in your tubes then set this to 185-190ul.
- f. You now have two options; 'Save As Template' and 'Save and Open'
 - i. If you are setting this up on a computer that is not attached to the cytometer, or would like to save this as a template for future use, click 'Save As Template'
 - ii. If you are preparing to immediately run samples on the cytometer click 'Save and Open'
- g. Before you run your first sample perform a SIT Calibration
 - i. Click on the 'Cytometer' icon on the very left hand side of the screen (third one down, looks like a small cytometer)
 - ii. Click on the 'Calibrate SIT' button and then on 'Calibrate SIT' in the box that opens up
 - iii. This makes sure the cytometer correctly measures the distance to the bottom of the tube

12. Backup your data to OneDrive and delete any older experiments from within SpectroFlo.
13. Follow Flow Core Data Management SOP