

Setting Up an Experiment Using the Plate Loader on the Cytex Aurora Spectral Analyzer

Important things to Remember:

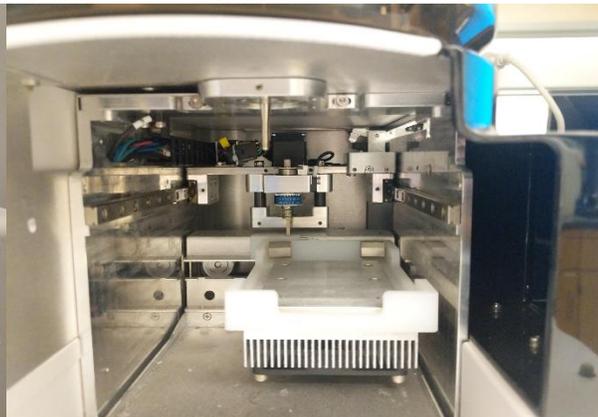
1. You must have single stained controls and unstained controls for your sample
2. The single stain controls MUST match what fluorochrome you have in your sample.
3. Controls can be on cells, beads, or a mixture of both.
4. Normal rules of compensation apply (controls must be as bright as or brighter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

Instructions

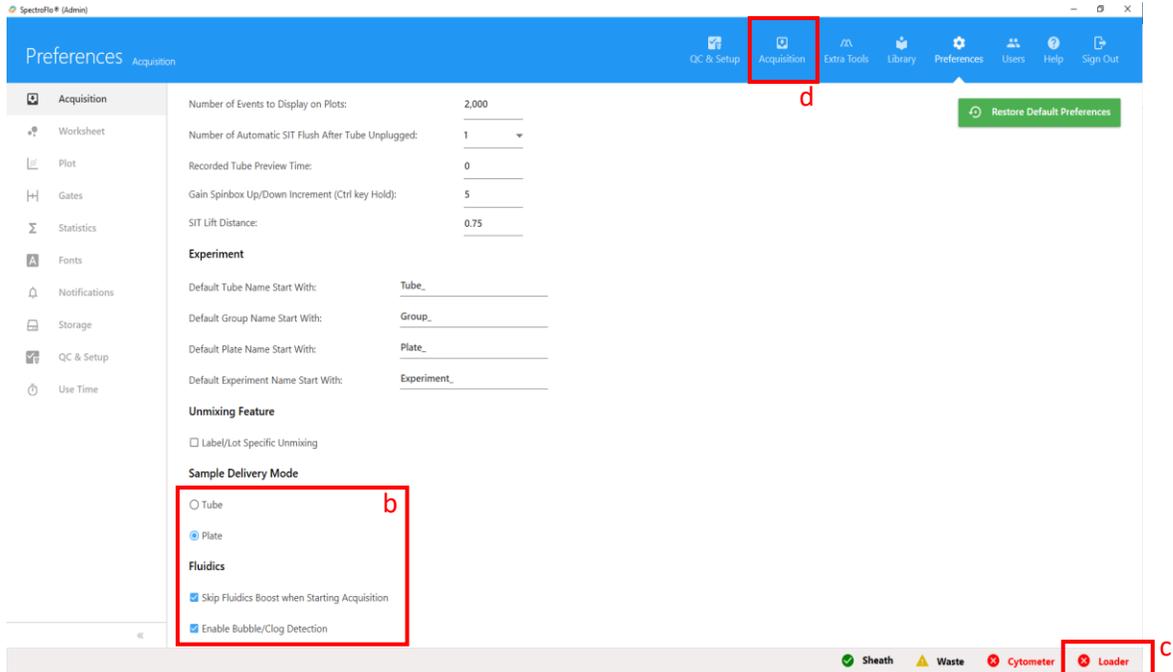
1. If the software isn't open already, double click on the icon for SpectroFlo and Log In to the software



2. Move the loader into position
 - a. Remove the tube of DI water from the SIP
 - b. Lift up on the silver handle (the Loader lever) on the loader
 - c. Slide it towards the front of the machine
 - d. Lower the silver handle down into the slot

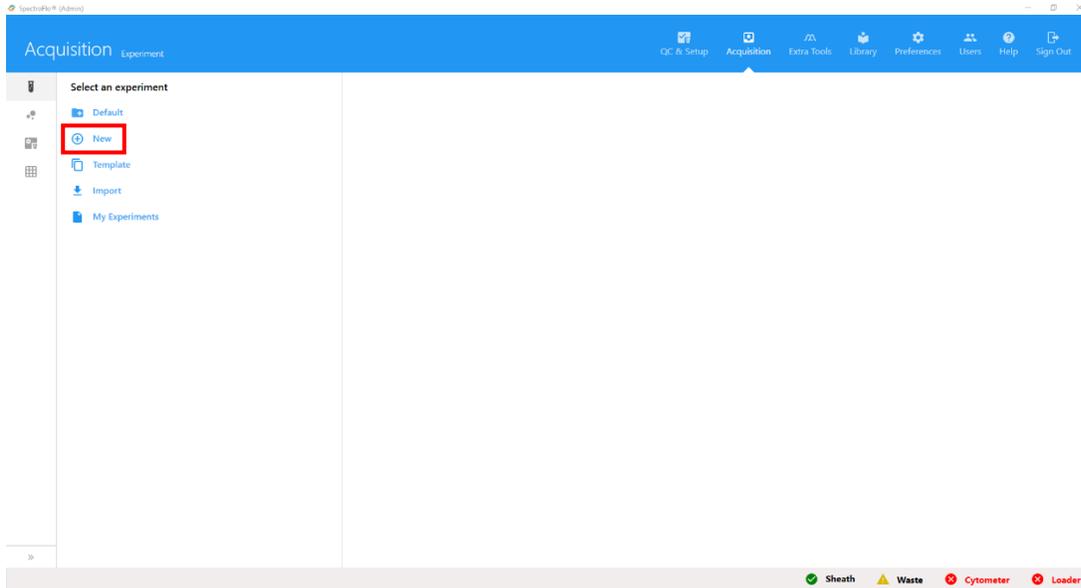


3. When using the plate loader, you need to change the acquisition settings under 'Preferences'
 - a. Click on the 'Preferences' button when you log in. You should now see the following page

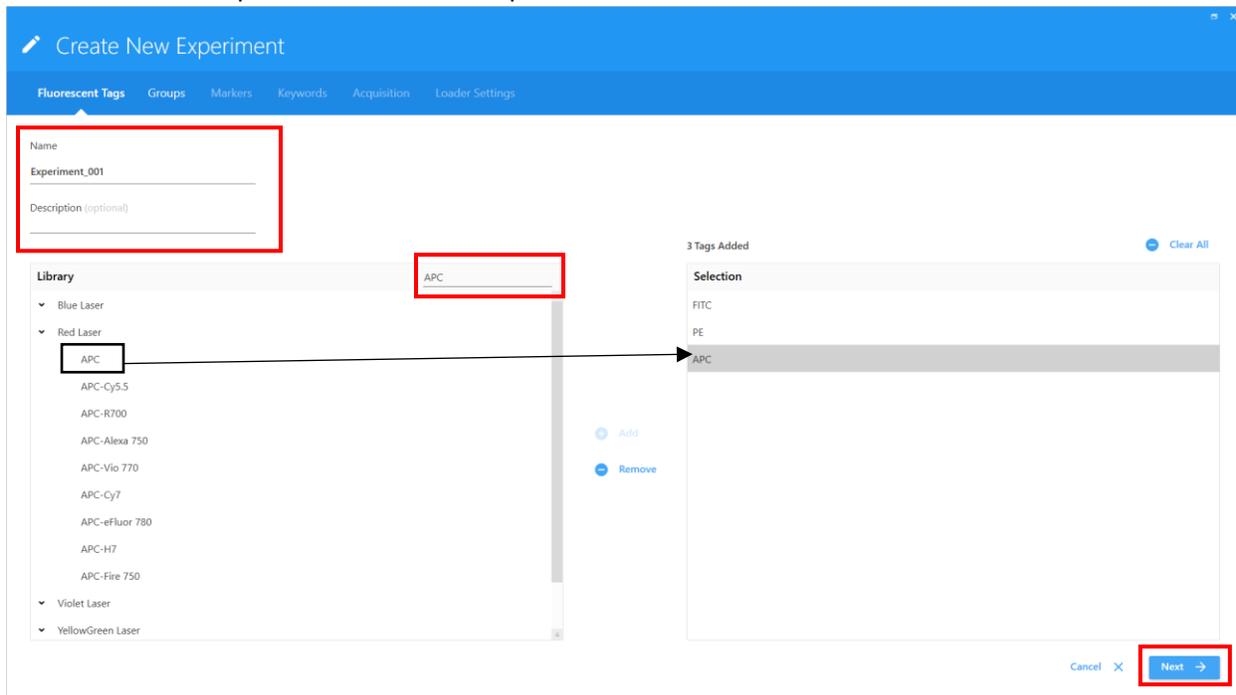


- b. Under 'Acquisition' > 'Sample Delivery Mode'
 - i. Click on the button next to 'Plate' to change the software into plate mode
 - ii. Also click the 'Skip Fluidics Boost' (optional) and 'Enable Bubble/Clog Detection' buttons
 1. Skip Fluidics Boost – Will not boost your sample to the flow cell, so you won't lose sample during the initial stabilization
 2. Enable Bubble/Clog Detection – you can set this to stop recording when it encounters a bubble or a clog, so you don't record an entire plate while there is an issue.
 - c. Check that the loader is turned on (this icon should be a green check mark). If not, there is a switch located on the back of the plate loader unit that you can use to turn it on
 - d. Click on 'Acquisition' in the top bar to begin setting up your experiment

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

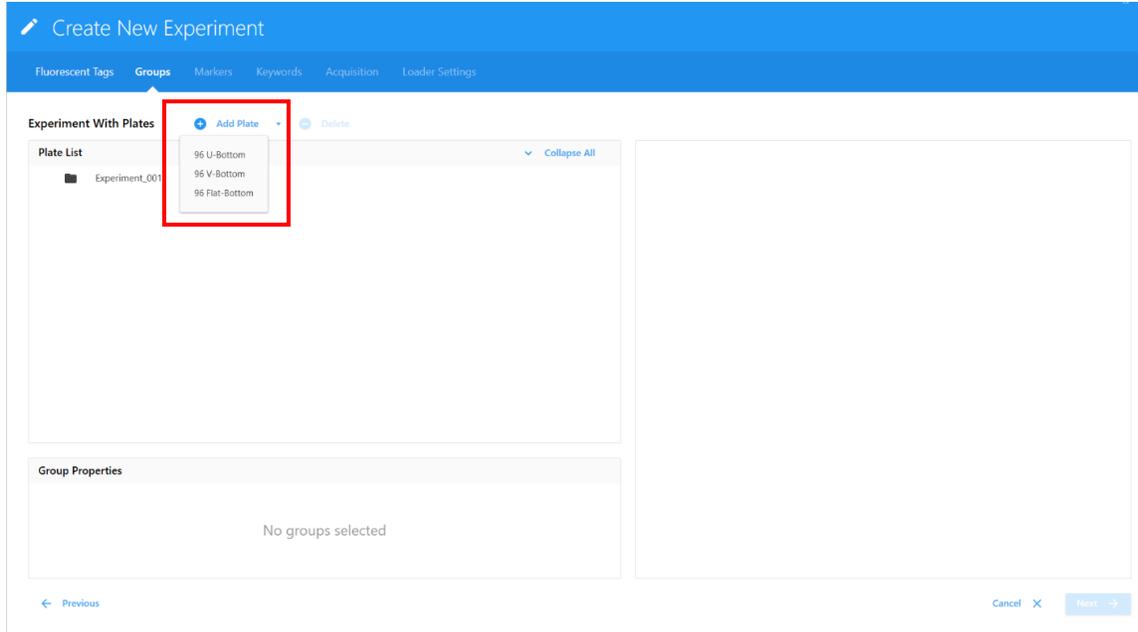


5. The 'Create New Experiment' wizard will open



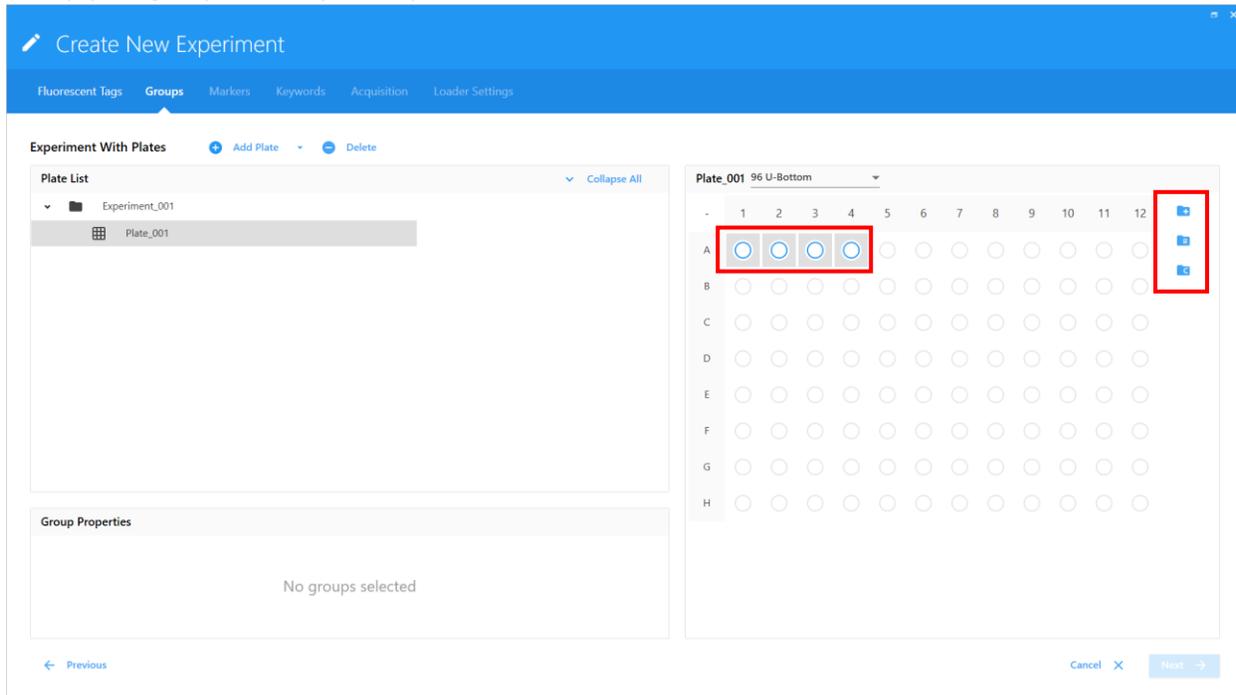
- Name your experiment – the more unique the name is the better. This helps us if we need to retrieve your data from the backup servers.
- Add fluorochromes to your experiment
 - Type the fluorochromes in the 'Type to filter' box
 - Double click on desired fluorochrome to add it to your experiment
 - **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****
- Click **Next**

6. You will now be on the page to set up your plates and groups, it should look like the following image



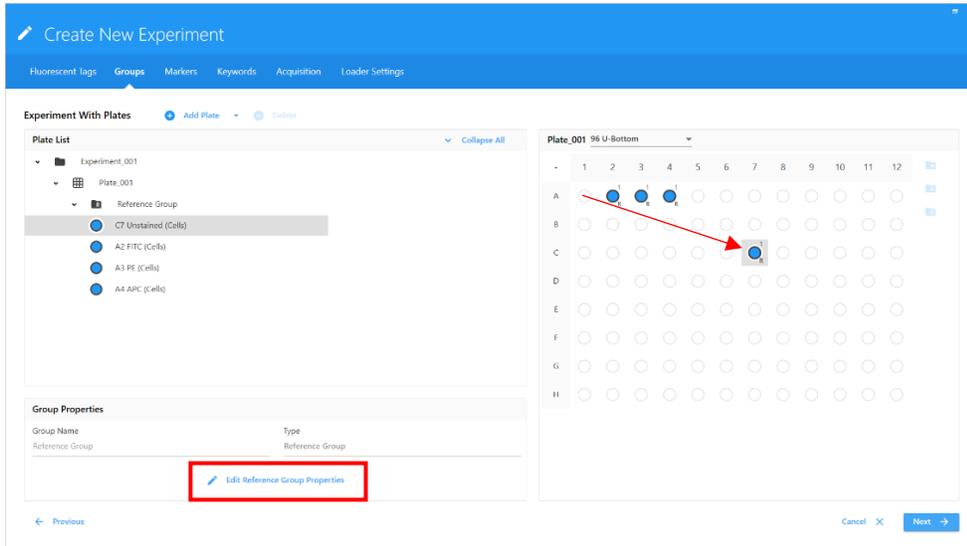
- a. Click on the ' + Add Plate ' button to get a drop down of plates you can select. This will add a plate to the 'Plate List'.
- b. Currently, the software only supports 96-well V, U, and Flat Bottom plates.

7. Set up your groups on the plate layout



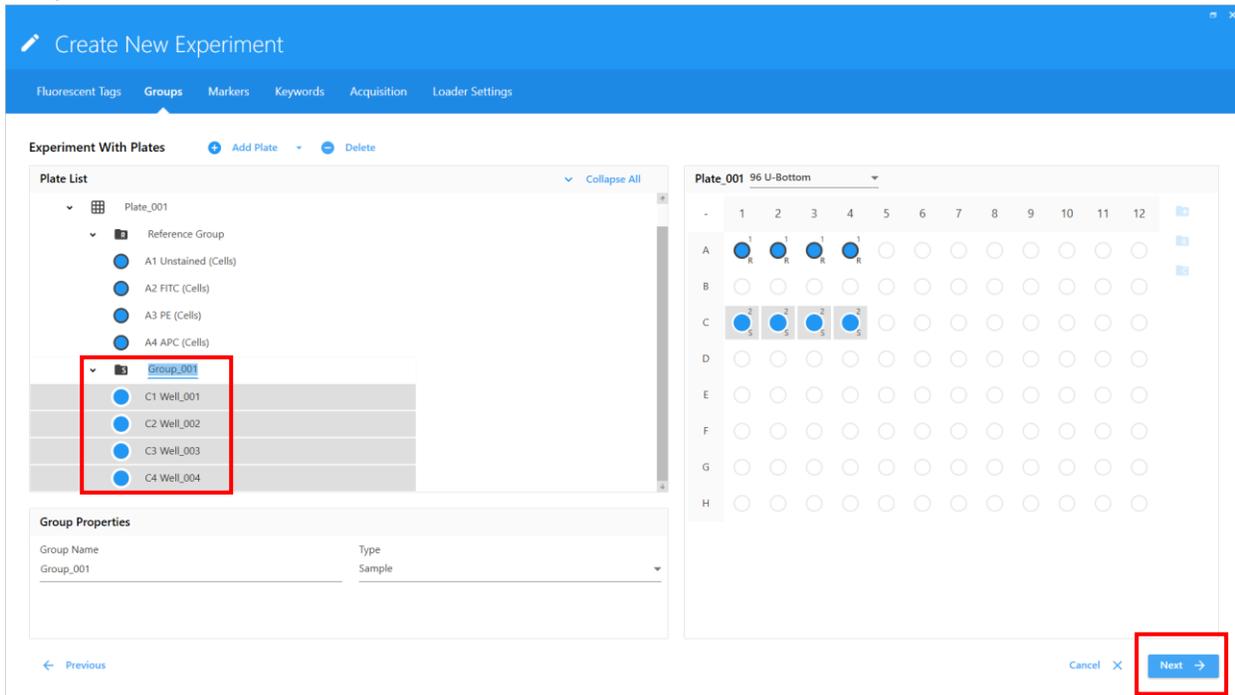
- a. You have three options on the right hand side of the plate diagram
 - i. ' + ' is for experimental groups
 - ii. ' R ' is for the reference group (single stain controls)
 - iii. ' C ' is for a cleaning group
- b. Create your reference group first
 - i. Highlight the wells containing your single stain controls and click ' R '

- ii. Confirm all your single stain controls (fluorochrome, what they are stained on, if they need a universal negative, etc.)
 - iii. Hit Save
8. ****THE SOFTWARE WILL REORDER YOUR CONTROLS BY LASER AND DETECTOR, FROM V1 to R8****



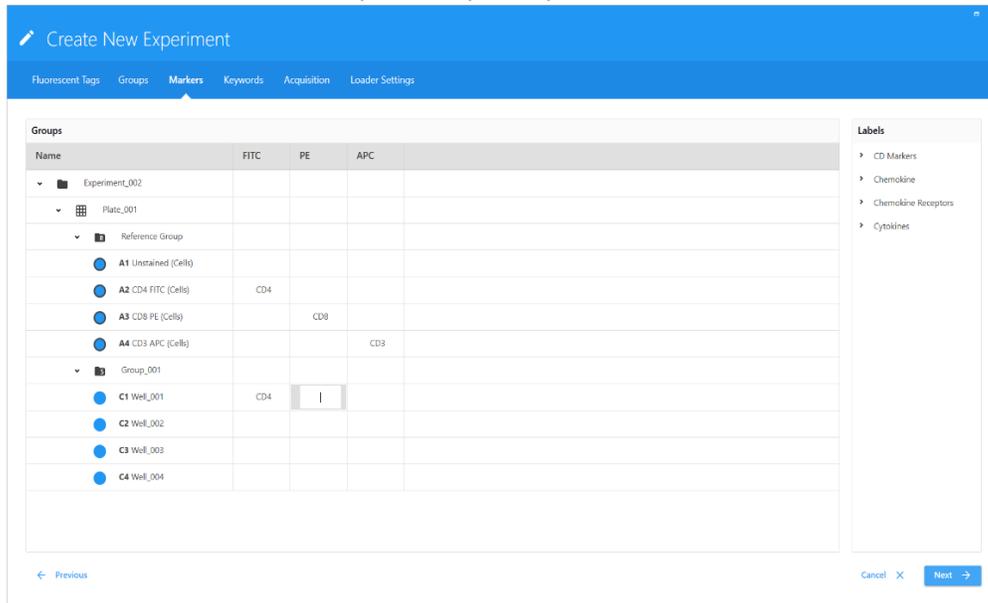
- a. You can re-order your controls by clicking and dragging to move them into the correct well.
- b. Click on 'Edit Reference Group Properties'
 - i. This opens a window to allow you to assign labels to your fluorochromes, and assign negative controls (if needed)

9. Create your experimental (sample) groups the same way, clicking and dragging to highlight the wells that your samples are in



- a. You can rename the group and well names here.

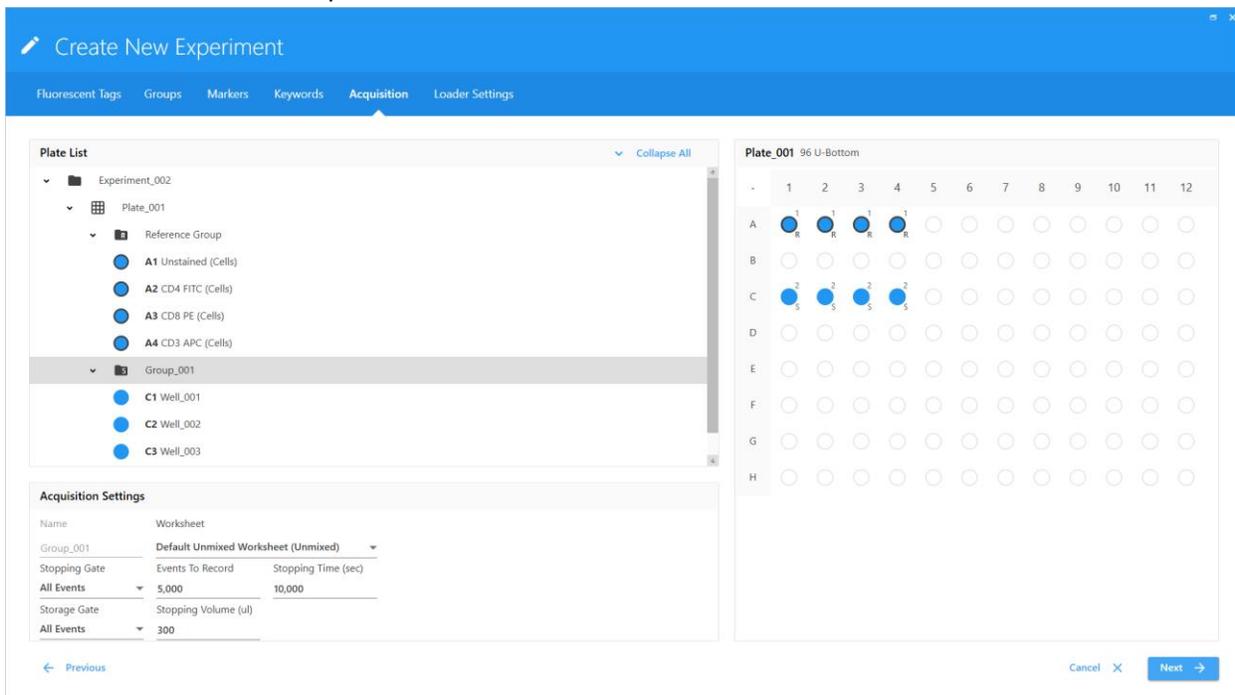
- b. The well location can only be changed by clicking and dragging, similar to moving single stained controls around
 - c. When you are done setting up groups hit 'Next'
10. You will now be on the 'Marker's tab
- a. If you labeled your controls they will show up here
 - b. You can label the markers on your samples if you want



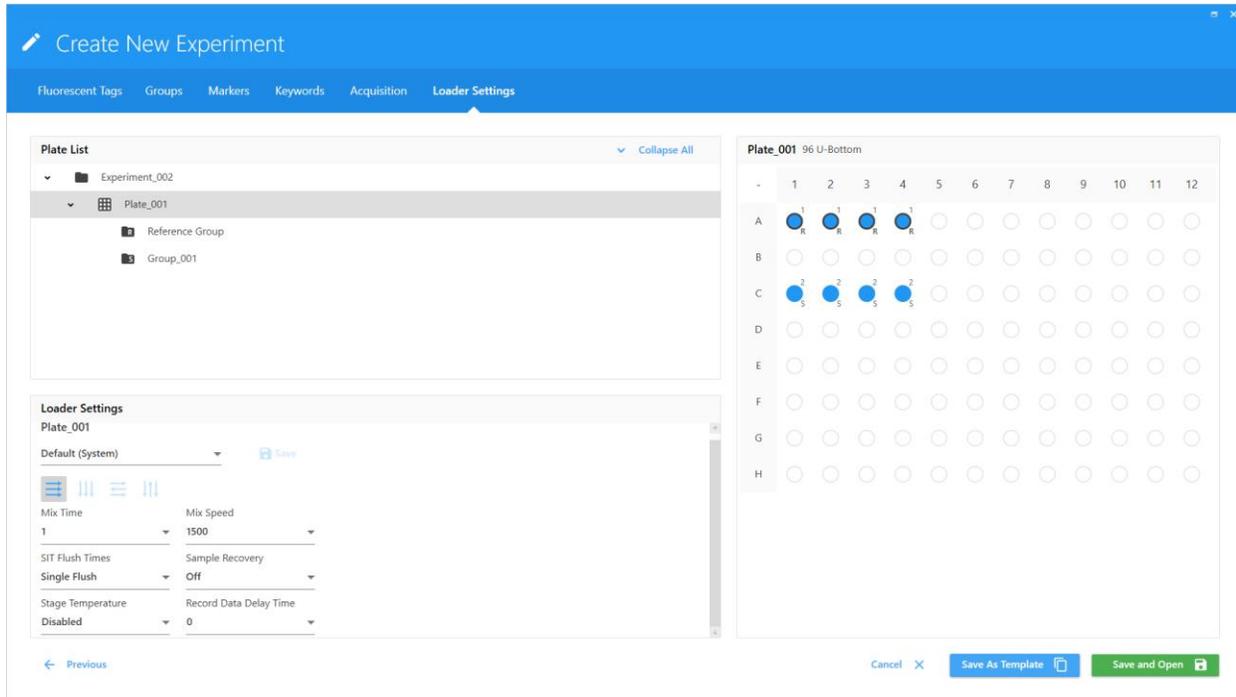
- c. Double click in the box next to your sample, in the fluorochrome column, and fill in your marker.
 - i. Hitting 'Tab' will just move the cursor to the next space and you can type in the marker. This allows you to move through marker labeling quickly
- d. Once you are done labeling markers hit 'Next'

11. Skip the 'Keywords' tab

12. You will now be on the 'Acquisition' tab



- a. Here you will set up the acquisition settings for all of your samples (controls and sample groups)
 - i. This can be done at the experiment, plate, group, or well level. It is recommended to set this by group level
 - ii. Click on the Group you would like to edit
 1. Set the worksheet; Reference Group will ALWAYS be set on RAW, Sample Groups can be set on Unmixed
 2. Set the Stopping Gate; You initially won't have any gates to choose from until you create them on the corresponding worksheet
 3. Set the following; Events to Record, Stopping Time, and Stopping Volume
 - a. Events to Record – Set at target # of events
 - b. Stopping Time – Can leave at max (10000 sec), unless you have calculated how much time each well needs to run so you can finish in your scheduled time
 - c. Stopping Volume – Set to 15ul less than the total volume in your wells
 - b. When you are done click 'Next'
13. You will now be on the 'Loader Settings' Tab



- a. The drop-down menu gives you 3 options to choose from
 - i. Default is usually adequate
 - ii. High Throughput is OK if you do not care about carryover between wells, it disables SIT Flush and Mixing
 - iii. Low Carryover takes the longest time, it washes the probe and mixer twice between each well
- b. You can also set you own settings for each variable in the list and save it for later use
 - i. Mix Time – amount of time in seconds the mixer spins in the well
 - ii. Mix Speed – RPM of mixer
 - iii. SIT Flush Times – number of times the mixer flushes/washes in the washing well
 - iv. Sample Recovery – If you hit a stopping condition it can return the unused sample back into the well
 - v. Stage Temp – Can keep cells warm or cool depending

- vi. Record Delay Time – number of seconds between sample starting and when the software begins recording
 - c. Once you have set all of these click 'Save and Open'
14. On the left-hand side of the screen, press 'Eject' in the Acquisition module to bring the plate stage forward
15. Load the plate so that position A1 is located in the front-left corner, and press down on all edges of plate to secure it in the holder
 - a. Press the 'Load' button to load your plate
 - b. This allows the plate loader stage to home correctly on the A1 position
16. Perform a SIT Calibration
 - a. Fill an empty well with DI water
 - b. Add the well as a separate group on your plate map
 - c. Run Acquisition on this well
 - d. Check that the flow rate matches what is expected for the flow rate setting
 - i. Low – about 20ul per minute
 - ii. Medium – about 30ul per minute
 - iii. High – about 60ul per minute
 - e. If the flow rate is very low (0-2ul per minute) raise the height for the lift distance and re-run the well
 - i. Go to the Cytometer Tab
 - ii. Select Calibrate SIT
 - iii. Change SIT lift distance
 1. This number is the amount of millimeters that the SIT tube raises off the bottom of the well
 - iv. Go back to your experiment and re-run the acquisition on the well
 - f. Repeat step E until the flow rate matches the expected flow rate for the setting you are on
17. Run your Reference Controls as normal and perform the unmixing process
18. After that, you can choose to record either a single well, an entire group, or the entire plate automatically.
 - a. If you choose to the 'Record plate' option and some wells already have recorded data, you will be given the option to either skip the recorded wells, overwrite the recorded wells, or append to the files.
19. To clean the instrument, switch back to tube mode:
 - a. Eject plate using the button in Acquisition module and remove plate
 - b. Retract the empty plate stage by pressing the 'Load' button
 - c. Set back to tube mode by going to 'Preferences' and setting 'Sample Delivery Mode' back to 'Tube'
 - d. Push the Loader lever to the back (see Step 4, but move lever back)
 - e. Place a tube of DI water on the SIP
 - f. Perform a SIT calibration to set it back at the proper level for tubes
20. Perform cleaning as described on the Aurora SOP
 - a. Bleach and Water tubes on High flow rate for 3 minutes each
21. Backup your data to OneDrive and delete any older experiments from within Spectroflo.
22. Follow Flow Core Data Management SOP