

CYTEK® Aurora

Creating an Experiment



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1. Setting up a new experiment
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3. Recording Controls & Unmixing
4. Recording Experimental Samples
5. Data Export
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1. Setting up a new experiment

- 1) Launch SpectroFLO Software.

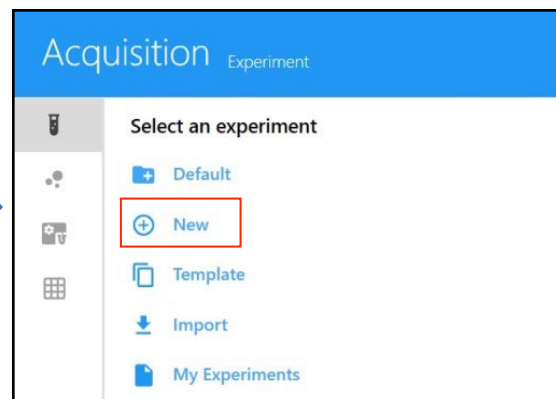
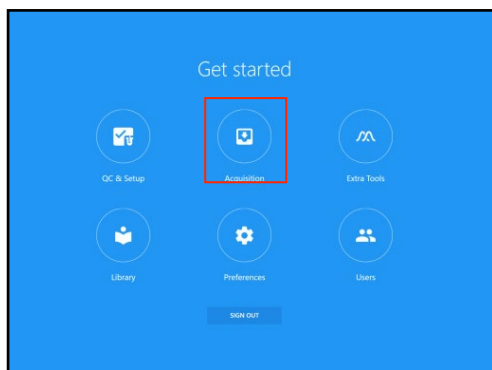
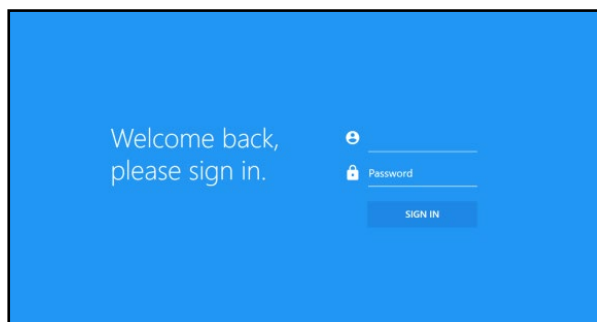
Login name: Lab name

Password: password1

- 2) If you are the first user of the day, follow startup instructions as per **UofU Aurora Startup_Clean**.

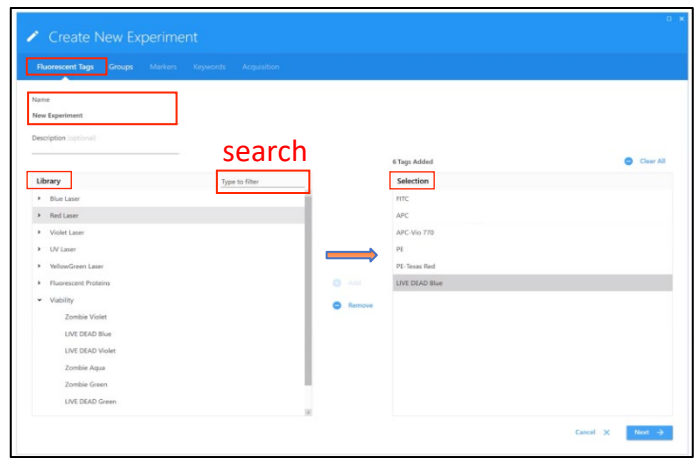
Shutdown guide.

- 3) Select Acquisition from the Get started screen.
- 4) Create a **new** experiment.



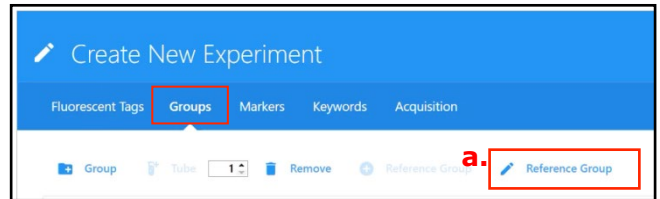
5) Fluorescent Tags Tab

- Rename your experiment
- From the fluorochrome library, search for the fluorochromes in your panel. Double click or highlight and choose **add** to include it in your experiment.
- To confirm the selected list, click **Next**.
- NOTE: DAPI, PI, Zombie or any viability dyes must also be added to your experiment and recorded as single color controls.



6) Create Reference Group Tab (ie single color controls)

- From the Groups select reference group **(a)**.
- SpectroFlo automatically adds an unstained and single color control tubes for each fluorochrome you've added to your experiment.



Note: An unstained sample of the same cell type as your experimental samples must be run as it is a part of the unmixing process **(b)**. You can also Define additional controls for unstained beads **(c)** if desired.

- Below the menus for the negative controls, you see your fluorescent tags listed in the left column **(d)**. The next column is Control type **(e)** (beads or cells) must be selected from the dropdown menu to match the kind of control that you will be recording, for each fluorescent tag!
- Select appropriate matching autofluorescent negative **(g)** (eg. Unstained cells, unstained beads, or leave blank for internal negative)
- Hit **Save** to confirm defined controls.

Edit Reference Group

Define Unstained Control Matching Sample Autofluorescence

Name	Control Type	View Stored Unstained Controls
Unstained	Cells	3 Controls

b.

Define Universal Negative Control(s)

Name	Control Type
Unstained Beads	Beads

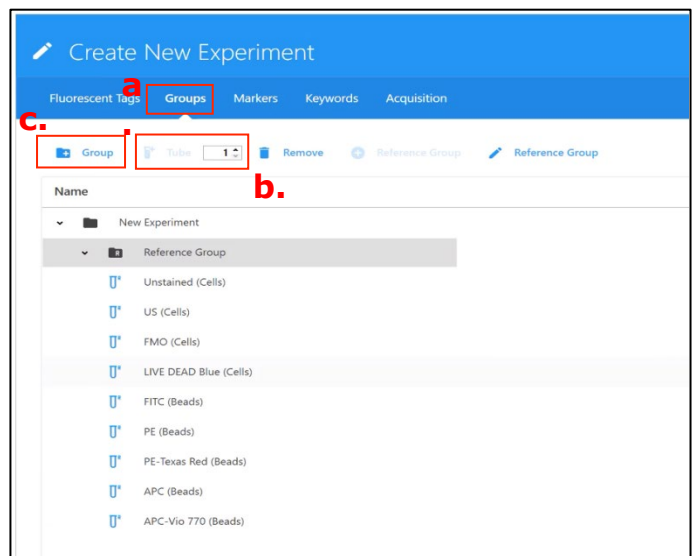
c.

Fluorescent Tag	Control Type	Label	Universal Negative	View Stored Reference Controls
BUV805	Cells		Unstained	1 Control
Super Bright 436	Cells		Unstained	N/A
BV480	Cells		Unstained	1 Control
BV510	Cells		Unstained	1 Control
FITC	Cells		Unstained	1 Control
PerCP-eFluor 710	Cells			N/A
PE-Cy5	Cells			1 Control
APC	Cells		Unstained	1 Control
Alexa Fluor 700	Cells		Unstained	1 Control
APC-Fire 810	Cells		Unstained	N/A
BUV395	Beads		Unstained Beads	1 Control
BV605	Beads		Unstained Beads	1 Control
PE	Beads		Unstained Beads	1 Control

d. Fluorescent Tag **e.** Control Type **g.** Universal Negative

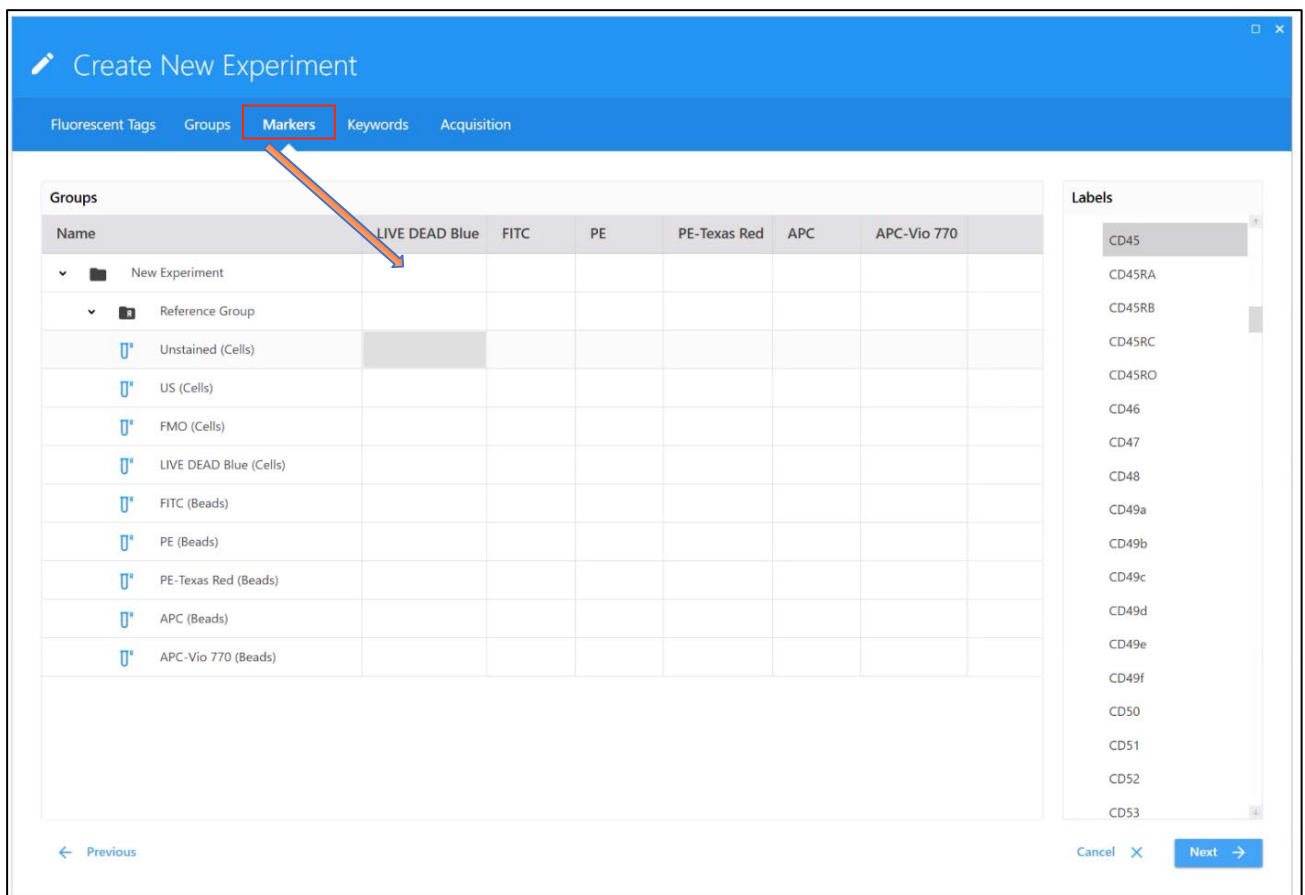
7) Create Experimental Sample Groups

- To add an **experimental sample group**, from the **Groups (a)** tab, enter the number of tubes **(b)** you would like to add in the group, and select + **Group** icon **(c)**. NOTE: You can rename, create or delete tubes and groups later, but creating at least one here will allow you to add labels in the next step.
- If there are different conditions (e.g., different tissue, different cell lines etc.) multiple groups can be created.



8) Markers – labeling

- From the "Markers" tab, set labels for each of your fluorescent tags by double clicking on the text box underneath the fluorochrome name and typing in your desired label. Hit **Enter** on the keyboard to confirm. NOTE: you can have group/tube specific labels; just double click on the text box by the new group name.
- When you are done labeling, click **Next**. NOTE: You can skip the “Keywords” tab and move straight to “Acquisition”.



2. Adjusting User Settings

1) Acquisition Tab:

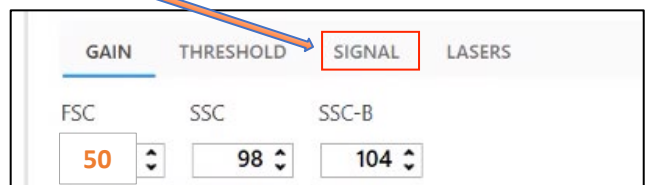
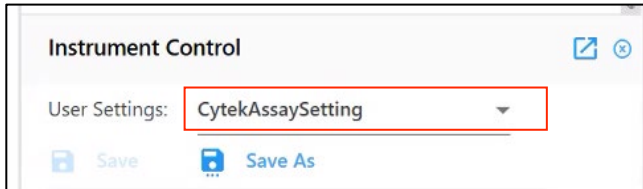
- At this stage you can define and set up the worksheet, stopping gates, events to record for references controls and experimental samples (these options can be modified at any point in the future by clicking in the “Edit” button, which should bring you back to this screen).
- For reference group samples, work from the Default Raw Worksheet. Set **Stopping gate and Events to Record as needed**. For cellular reference controls with rare expression, ensure that at least **1000 positive events** are recorded. Storage gate is not suggested, as it will not record anything that doesn't meet that criteria.

Name	Worksheet	Stopping Gate	Storage Gate	Events To Record	Stopping Time (sec)
▼ New Experiment	Default Raw Worksheet (Raw) ▼	P1 ▼	All Events ▼	10,000	10,000
▼ Reference Group	Default Raw Worksheet (Raw) ▼	P1 ▼	All Events ▼	10,000	10,000
Unstained (Cells)	Default Raw Worksheet (Raw) ▼	P1 ▼	All Events ▼	10,000	10,000
LIVE DEAD Blue (Cells)	Default Raw Worksheet (Raw) ▼	P1 ▼	All Events ▼	10,000	10,000
FITC (Beads)	Default Raw Worksheet (Raw) ▼	P1 ▼	All Events ▼	10,000	10,000
PE (Beads)	Default Raw Worksheet (Raw) ▼	P1 ▼	All Events ▼	10,000	10,000
PE-Texas Red (Beads)	Default Raw Worksheet (Raw) ▼	P1 ▼	All Events ▼	10,000	10,000
APC (Beads)	Default Raw Worksheet (Raw) ▼	P1 ▼	All Events ▼	10,000	10,000
APC-Vio 770 (Beads)	Default Raw Worksheet (Raw) ▼	P1 ▼	All Events ▼	10,000	10,000

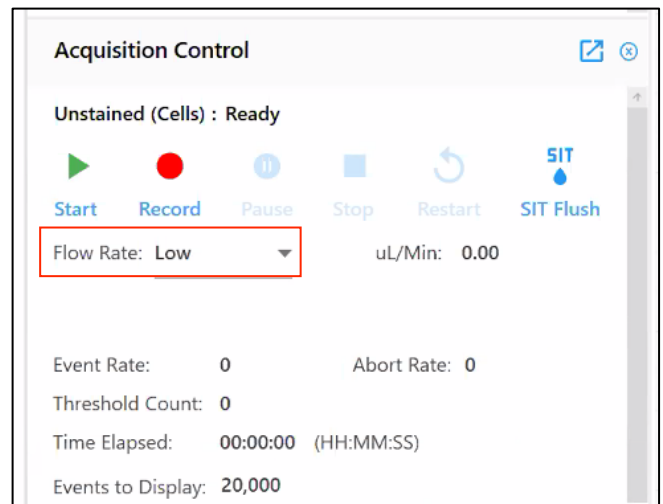
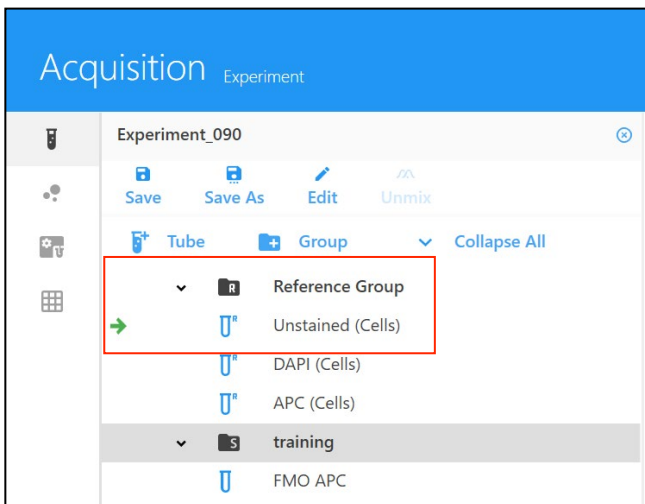
- Select **Save and open**.

3. Recording Controls

- 1) After selecting **save and open**, navigate to the *Instrument Control Tab* (left bottom side) and choose "CytekAssaySetting" for the starting User Settings.
 - Importantly, "CytekAssaySetting" are the optimized starting gains for all detectors on the Aurora. Changes should only be made to bring cells on scale for FSC and SSC. (Consult Flow core staff for further conversations about fluorescent detector gain adjustments.)
 - Chose desired doublet discrimination parameters in Signal tab (A, H, W)



- 2) Load an unstained sample to the loading station and select the appropriate tube from the Reference Group with the **green arrow**.
- 3) In the *Acquisition Control Window*, click on the *Flow Rate* to adjust the acquisition speed and the number of events to display. When you first load the sample, keep the flow rate at **low** and monitor event rate.
 - While Cytek says the instrument can process 30k events per second, we advise to avoid running beyond 10k events per second, and to monitor the abort rate and keep it **under 5%** (for example: if the flow rate is 10,000/sec, the abort rate should not be higher than 500). We always suggest clients to bring extra flow buffer to dilute their samples if needed. Refer to educational seminars about the pros and cons of running low, medium or high.

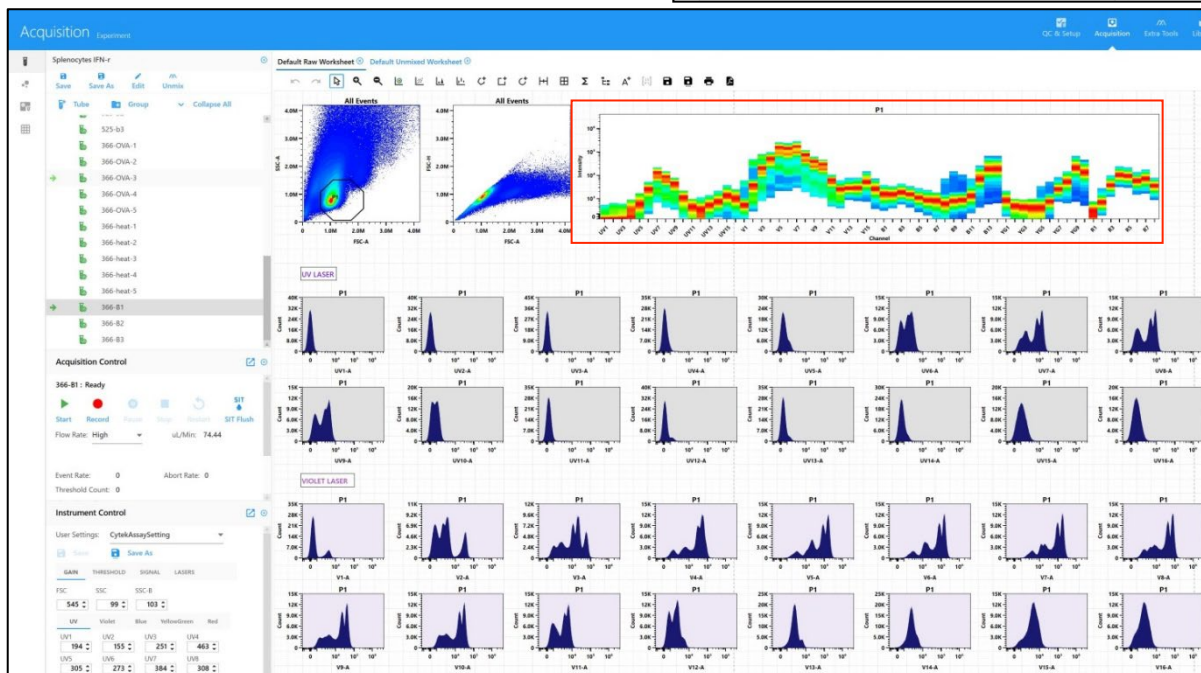
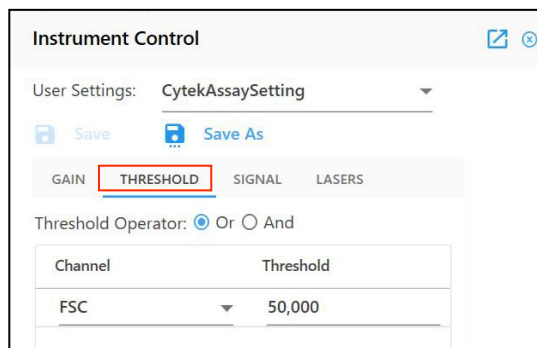


- While running your unstained sample, (Instrument Control Window), adjust gains on the FSC and SSC and the threshold value to put cells in desired place on the plot. **Note:** The FSC and SSC settings from the CytekAssaySettings are optimized for the Spectroflo beads. Thus, reduce the FSC to bring cells on scale - typically, a **good starting point is around 50**.

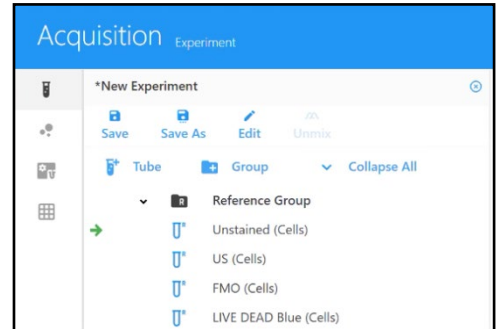
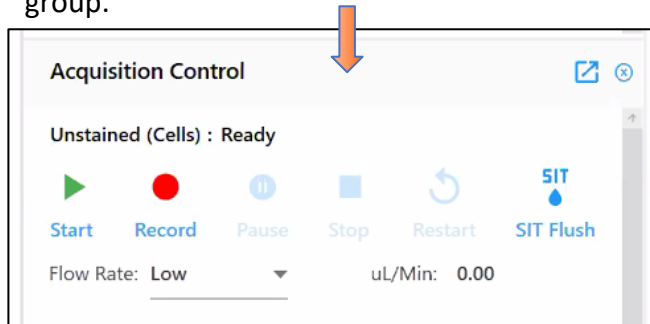
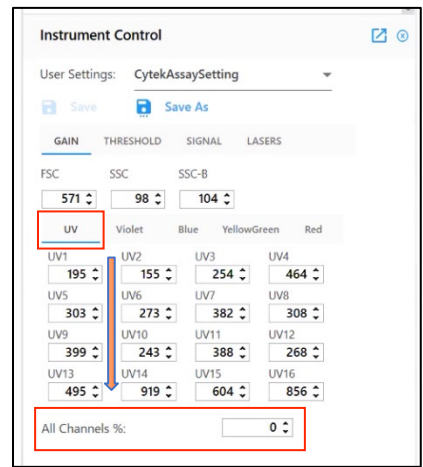


- Please be aware that SSC by default is off the violet laser and that SSC-B is off the blue laser. Either can be used, but the violet SSC is preferred. NOTE: A threshold that is too high may prevent the cytometer from displaying cells of interest (dying/dead or smaller cells in heterogenous populations); a too low one would allow a lot of noise/dead cells to your file (or to your collection tube when sorting). We suggest something around 50,000 when recording bead controls, and around 100k-400k when recording cells, but play around to find something suitable for your samples.

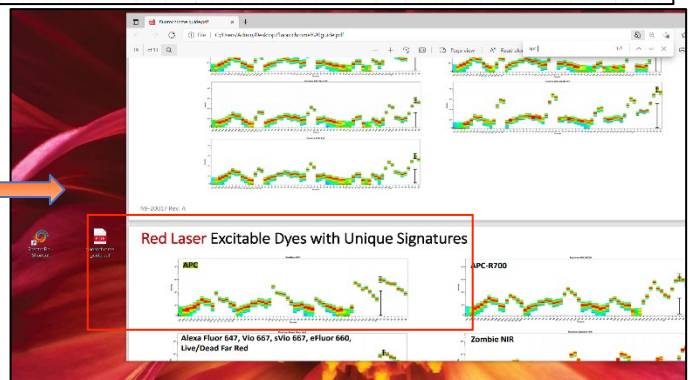
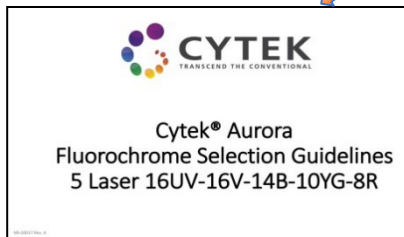
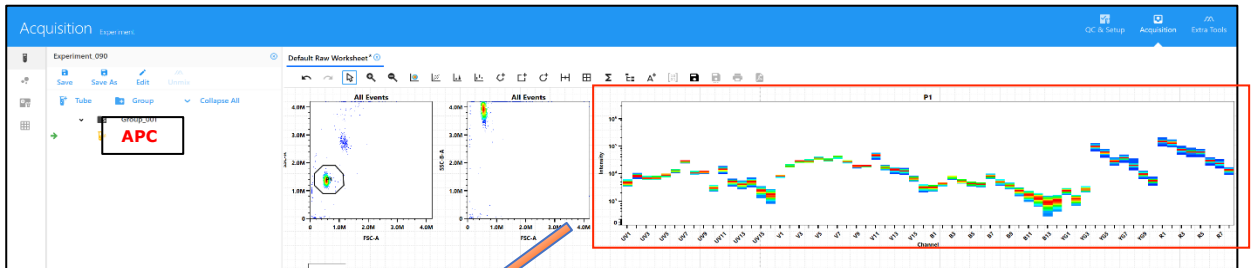
- After the unstained control, run a small aliquot of an experimental sample, which is expected to have the brightest signal. Check the full spectrum plot to ensure that all signal is on scale.



- **IMPORTANT:** If any data is off scale for fluorescence, **never adjust that individual detector, but instead reduce the gain for "all channels%" of that laser.** Gain reduction is % based and should only be reduced for all laser lines until the signal is on scale. (Consult Flow core staff for further conversations about fluorescent detector gain adjustments.)
- When all parameters are set properly, Reference Controls are ready for acquisition. Therefore, click **Start** and **Record** for each sample from the reference group.



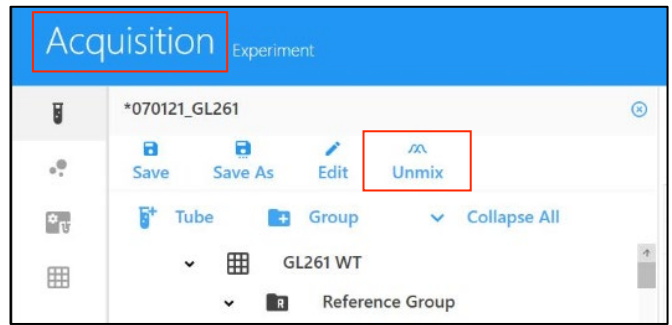
- 5) Check each reference control to ensure that the full spectrum signature matches what is expected. Compare the signature from the full spectrum plot in SpectroFlo with an image from the [Cytek's fluorochrome guide](#):
- If a fluorochrome is not listed on Cytek's fluorochrome guide, please search for the spectra from spectrum.cytekbio.com and remember to select 5L configuration.



- 6) After all reference group samples are recorded, the "Unmix" button, that was previously greyed out, should now be clickable.

4. Unmixing

- 1) From the Acquisition Control Window click on **unmix**.
- 2) After that, you will have to select which controls (beads or cells) that are going to be used for the unmixing.



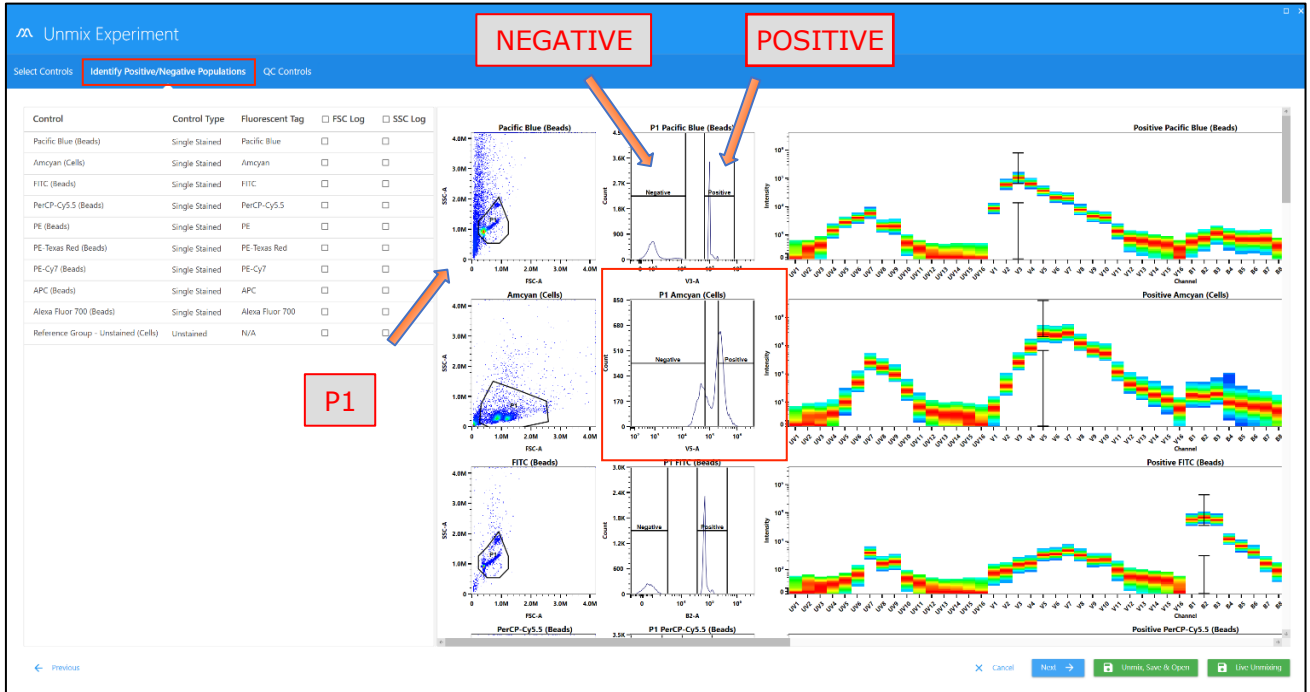
The screenshot shows the 'Unmix Experiment' interface. The 'STAINED CONTROLS' table is highlighted with a red box. The table has columns for 'From Library', 'Fluorescent Tag', 'Control', 'Universal Negative', and 'Generic'. The 'Unmixing Model' dropdown menu is also highlighted with a red box, showing 'Spectral Unmixing With AF Extraction' selected. The 'Next' button is visible at the bottom right.

From Library	Fluorescent Tag	Control	Universal Negative	Generic
<input type="checkbox"/>	BUV805	BUV805 (Cells)	Reference Group - Unstained (Cells)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	Super Bright 436	Super Bright 436 (Cells)	Reference Group - Unstained (Cells)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	BV480	BV480 (Cells)	Reference Group - Unstained (Cells)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	BV510	BV510 (Cells)	Reference Group - Unstained (Cells)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	FITC	FITC (Cells)	Reference Group - Unstained (Cells)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	PerCP-eFluor 710	PerCP-eFluor 710 (Cells)	Reference Group - Unstained (Cells)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	PE-Cy5	PE-Cy5 (Cells)	Reference Group - Unstained (Cells)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	APC	APC (Cells)	Reference Group - Unstained (Cells)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	Alexa Fluor 700	Alexa Fluor 700 (Cells)	Reference Group - Unstained (Cells)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	APC-Fire 810	APC-Fire 810 (Cells)	Reference Group - Unstained (Cells)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	BUV395	BUV395 (Beads)	Reference Group - Unstained Beads (Beads)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	BV605	BV605 (Beads)	Reference Group - Unstained Beads (Beads)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	PE	PE (Beads)	Reference Group - Unstained Beads (Beads)	<input checked="" type="checkbox"/>
<input type="checkbox"/>	PE-Cy7	PE-Cy7 (Beads)	Reference Group - Unstained Beads (Beads)	<input checked="" type="checkbox"/>

- Cytek Aurora allows to extract a sample's auto fluorescence and treat it as a separate parameter, which can improve the resolution for specific, well defined cell types. Therefore, you can click the box "Auto Fluorescence as a Fluorescent Tag" and click on Next. NOTE: this can get tricky if your cells have a complex autofluorescence profile. (Consult Flow core staff for further conversations about AF extraction.)

- 3) Click Next

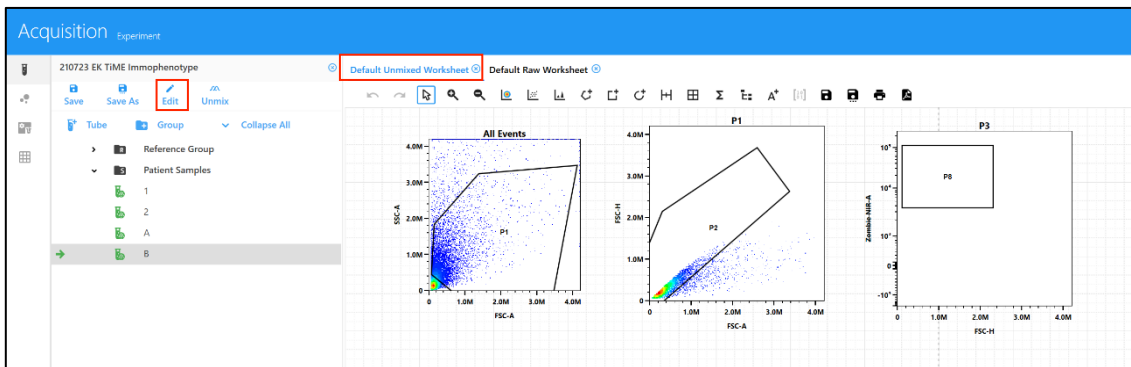
- 3) After selecting all controls, identify an appropriate **P1 gate and positive and negative regions for each single color control**. If you used “unstained/universal negative” as the negative in setup window there wont be a negative gate to adjust. If the positive peaks are not well defined (wide or double peaks merging) or you have a dimly expressed marker we suggest you to draw your bars around the brightest end of them, as depicted in the second line below:



- Once finished, you can hit **Live Unmixing**

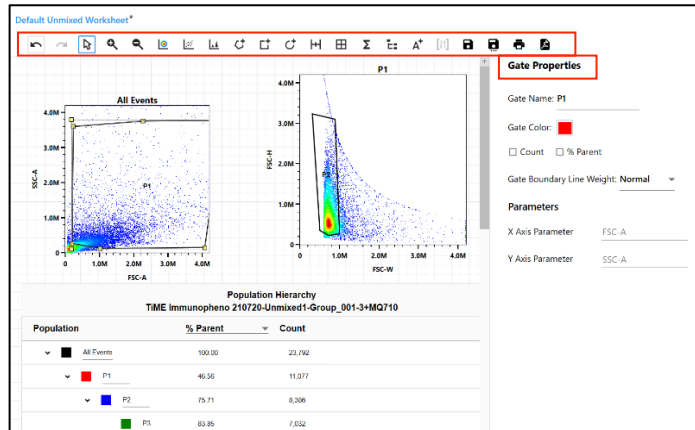
5. Recording Experimental Samples

- 1) After unmixing, a new “**Default Unmixed Worksheet**” tab is created in the acquisition tab. Please never make adjustments to the Default unmixed worksheet and then save/overwrite the worksheet. Instead *Save as* under a different name. After worksheet is saved it will need to be applied to the appropriate groups by “editing” the experiment.



The screenshot shows the 'Save Worksheet Template' dialog box. It has a 'Name' field with the value 'Worksheet 82882'. There is a 'Description (optional)' field. Below the fields, there is a table with columns for Name, Created By, and Type. The table contains one row with the value 'Cytokine Panel', 'Admin', and 'Unmixed'. At the bottom of the dialog, there are 'Cancel' and 'Save' buttons.

- 2) At this point you can start recording your experimental samples, edit the experimental setup, create plots, edit gates properties, and analyze your data.



6. Data export

You have a few options while exporting your experimental data.

1) Exporting FCS files:

- FCS files (both RAW and Unmixed) are automatically saved once samples are recorded. FCS files can be found in this path C:\CytekbioExport\FcsFiles\Experiments\lab folder

7. Cleaning

- If you are not the last user for the day:** Clean the flow cell after your experiment is finished. Go to the **Acquisition module** and select **Clean Flow Cell**. Prepare fresh new tubes of 10% bleach and DI water and follow on-screen prompts.
- If you are the last user for the day:** Clean the analyzer after your experiment. Go to the **Acquisition module** and select **Fluidics Shutdown**.

- Prepare fresh new tubes containing:
- 3 mL of 10% bleach
- 3 mL of DI water
- 3 mL of 30% Contrad 70
- 3 mL of DI water

Fluidics Shutdown: Step 1 of 4

Shutdown takes approximately 6 minutes to complete. You will need:

- 12x75 polystyrene tube with 3mL 10% bleach
- 12x75 polystyrene tube with 3mL deionized water
- 12x75 polystyrene tube with 3mL 30% contrad
- 12x75 polystyrene tube with 3mL deionized water

Load the 10% bleach tube onto the manual port.
Then begin a 6-minute cleaning of the sample line.

Cancel

Continue

Follow the on-screen prompts and **turn off the cytometer at the end of cleaning**. A pop-up window should tell you when to do so.