Title:	Classification:
Cytek® Aurora User Training	User Training
Effective Date: 09/16/2020 **Revision Date:** 08/21/2025 **ID:** UT SOP008.6	Page 1 of 22

Table of Contents

1. SpectroFlo Software 2
Getting Started Menu 2
Experiments 3
Instrument Controls 4
Worksheets 6
2. Experiment Set-up 6
Experiment Wizard 6
Unmixing Options 8
3. Between Users and Shutdown Procedures 10
End of Experiment Cleaning 10
4. References 10

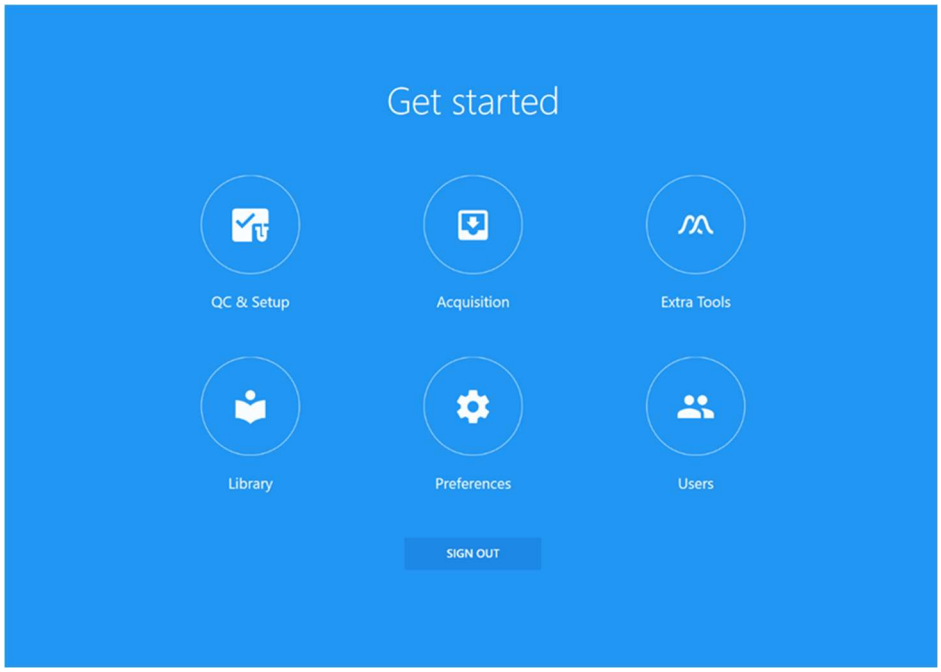
Less Commonly needed Sections.

5. Startup 10
6. Daily QC 11
7. Loader Overview 12
Using the Loader 12
Loader Settings in Experiment 14
Experiments in Plate Mode 16
8. Additional Cleaning Procedures 17
Monthly and As Needed Cleaning 17
9. Troubleshooting 19
10. Revisions 21
11. Training Notes 22

1. SpectroFlo Software

Getting Started Menu

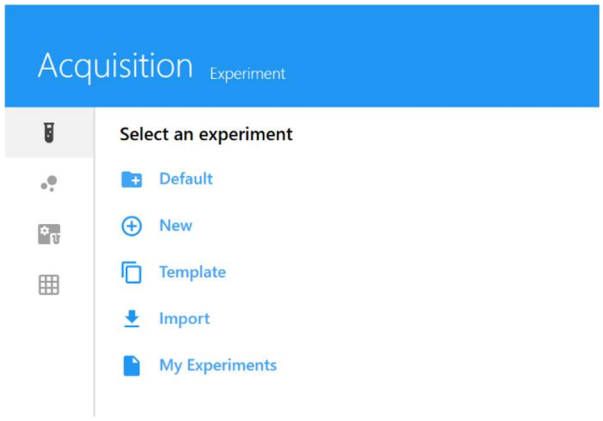
This menu displays six (6) modules to access after logging into SpectroFlo.



Module	Brief Description
QC & Setup	Access Daily QC menu
Acquisition	Create experiments and acquire data. Experiments are made from Templates or from new
Extra Tools	FCS Files can be unmixed or compensated using virtual filters
Library	Stores experiment Templates, worksheet Templates, user settings, fluorescent tags, Loader settings, etc.
Preferences	Software preferences to customize software
Users	Admin tools and user management

Experiments

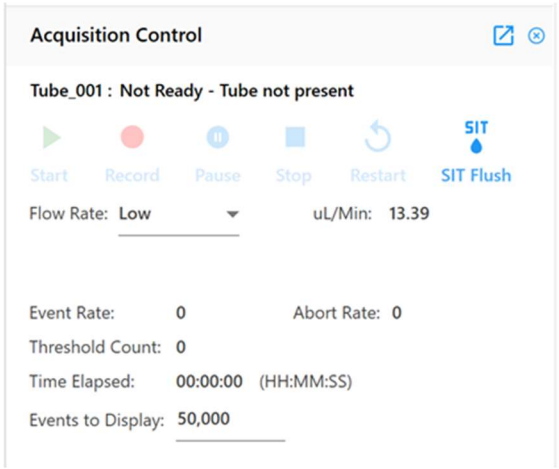
Opening an Experiment



- **Default**: Opens a new experiment with one tube and a set of labels and fluorescent tags in a default experiment worksheet template. This is user configurable.
- **New**: Opens New Experiment Wizard
- **Template**: Open a previously saved Template
- **Import**: Import an experiment ZIP file. Experiments downloaded from Cytek Cloud.
  My Experiments is cleared out by Staff weekly.

Acquisition Controls

The Acquisition Control Panel is used to control experiment level settings when acquiring and recording samples. Start and Record options are only available when a tube is present on the SIP.



- **Start Button**: Select to start acquisition
- **Record Button**: Select to record data. Record will automatically start acquisition
- **Pause Button**: Pause recording. While paused, flow rate may be adjusted.
- **Stop Button**: Stop acquisition and recording
- **Restart Button**: Clear accumulated data and restart acquisition counters.
- **SIT Flush**: Perform SIT Flush
- Flow Rate Options
  - Low: ~15 µL/min
  - Medium: ~30 µL/min
  - High: ~60 µL/min
- Acquisition Counters display real-time counts
  - Event Rate
  - Abort rate
  - Threshold Count
  - Time Elapsed
- Events to Display: Number of events to display during acquisition

Instrument Controls

Instrument Control Panel to adjust experiment level instrument settings.

- **CytekAssaySettings** are recommended as a starting point (optimized with CD4/CD8 lymphocytes for resolution).
  - Note you may need to adjust Gains depending on your experimental design and question
- **Gain:** Adjust gains for detectors. Use All Channels % to increase/decrease all gains in selected laser by percentage. FSC gain range: 1-1,000. SSC gain range: 10-10,000.

***Note**: Gain optimization may improve the overall resolution of your experiment. To optimize gains first assure all reference controls are 1. titrated, 2. have the expected peak channel, and 3. match reference spectral signatures. Next collect an all-color sample and confirm that the fluorescent signal is always on-scale (<4x10^6). If any detector is off scale decrease all lasers uniformly (e.g. All Channels -10%) until all signals are on-scale. If all the signals are well below 4x10^6 increase all lasers uniformly (e.g. All Channels 10%) until the brightest detector approaches 4x10^6). *For further optimization of gains consult flow core staff to design a plan for your specific experiment*. If gains are altered make sure to save the gain settings!

- **Threshold:** Set threshold parameter and minimum channel value. This is helpful to remove electronic noise, debris, or small cells (e.g. RBCs) from the recorded data.
- **Signal:** Select measurements for each parameter. Select area, height, and/or width. Width can only be selected for one channel per laser.
  Lasers: - *ask Core staff before you proceed with this.* In some cases, experiments will require optimizing the area scaling factors or the laser delays for scatter or fluorescent channels. For example, if you are unable to view your cells (very large) on the lowest FSC gain setting, lowering FSC area scaling factor (e.g. 0.5) would be appropriate*. When area scaling is optimized detector Area and Height will be approximately equal*.

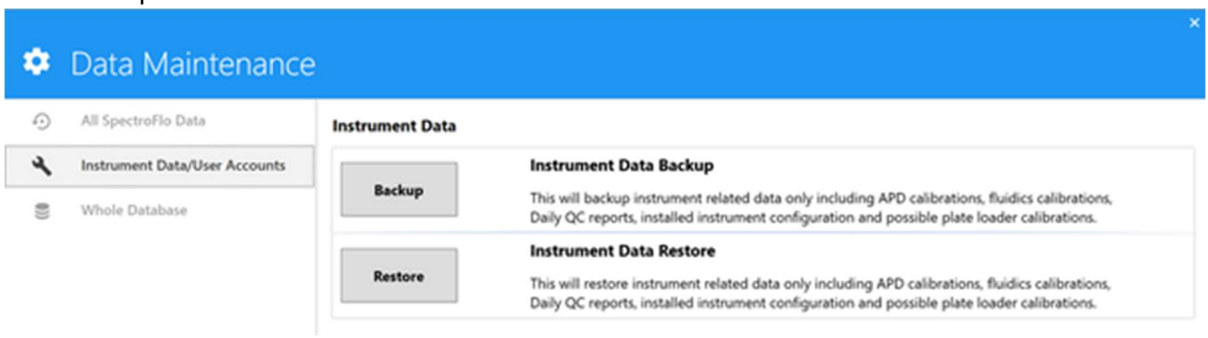
FCS Files

FCS files generated from an experiment are stored in the CytekExport folder by default. The type of FCS file that exports depends on the experiment. If no unmixing was performed*, raw data files only* will export. If live unmixing was performed while acquiring data, *raw data files AND unmixed data files* will export. If samples were unmixed post-acquisition, *unmixed data files only are exported. Note data files are deleted from the software every week by staff.*

To export data files:

1. *ZIP of Experiment Contains: FCS files [raw and unmixed], worksheet(s), CytekAssaySettings, Loader Settings, etc. From Acquisition > Experiment; close your current experiment.: Navigate to My Experiments> Select experiment> Export (ONYEN folder)*

2. *FCS files only: Navigate to File Explorer> OS (C:) > CytekbioExport> FcsFiles> Experiments> Find your username (ONYEN)*
3. *The daily QC files are used for unmixing and are found in File Explorer> OS (C:) > CytekbioExport>Setup (QC files are date and timestamped)*
    a. *If you are performing unmixing off the cytometer you will need to have the daily QC files associated with the .fcs files to be unmixed. Individual files can be copied as described above; batched files are available via* the Data Maintenance utility when SpectroFlo is closed (***Ask core staff for help with this process***). Click the Instrument Data/User Accounts menu, and click Backup next to Instrument Data Backup.

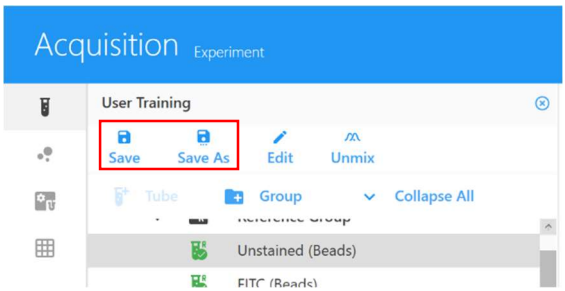


    b. Transfer the exported ZIP file to the analysis workstation. Open the Data Maintenance Utility (again, make sure SpectroFlo is closed as only SpectroFlo or the Data Maintenance utility can be open at once), click the Instrument Data/User Accounts menu, and click Restore next to Instrument Data Restore. Close the Data Maintenance utility. Open SpectroFlo, log in, and verify that the QC reports are visible in the QC & Setup module.

Experiment Templates

Experiment Templates contain:

- *Fluorescent tags used*
- *Reference controls*
- *Groups/tubes*
- *Labels*
- *Worksheets*
- *Stopping criteria for acquisition*
- *It DOES NOT include CytekAssaySettings, those need to be saved and input separately.*

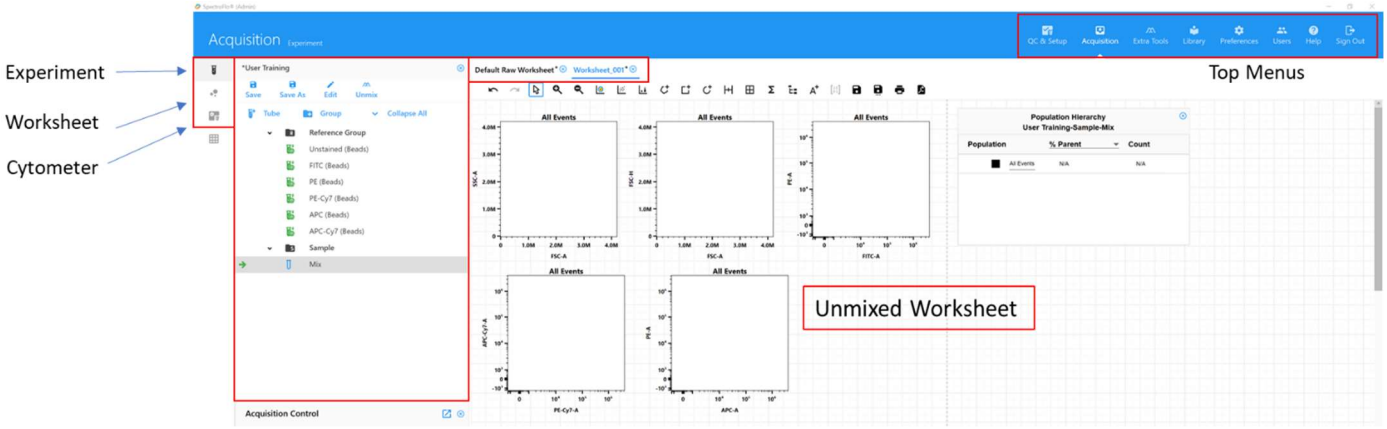


Use the Save As selection to save the Experimental Template.

Worksheets

Worksheets are used to visualize the data in the experiment. Experiments require at least ONE worksheet. Worksheets contain plots, gates, annotations, statistics, and population hierarchy. Templates can be loaded or saved via the Worksheet menu on the left side of the software.

Type	Brief Description
Raw	*View all parameters for unmixed data. The plots reflect the channel names, e.g. B1-A or V4-A*
Unmixed	View parameters selected while setting up experiment, e.g. FITC-A. Only available after data is unmixed.
Template	View data from a previously created and saved worksheet

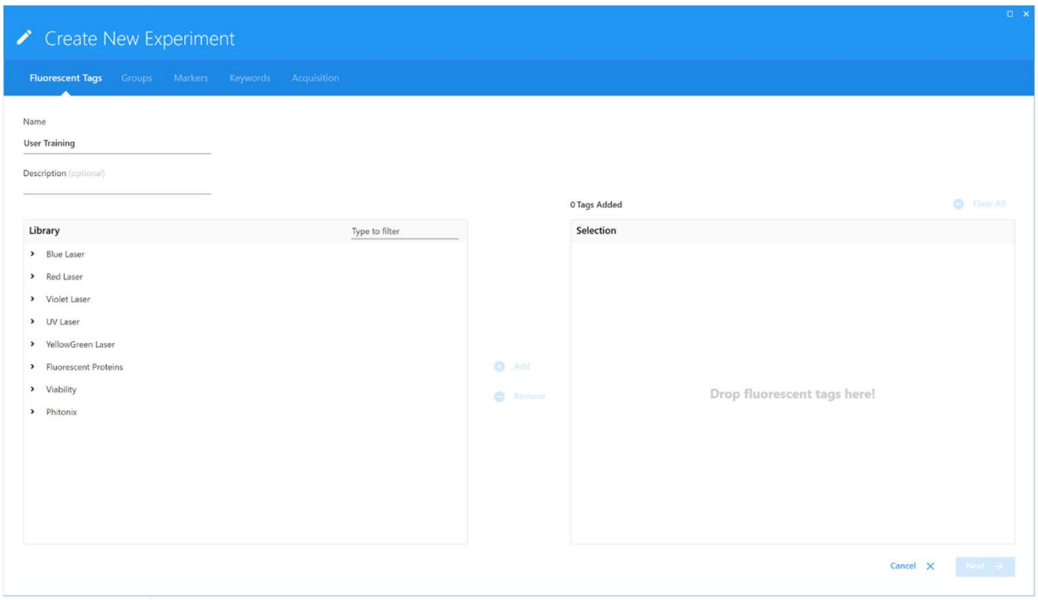


2. Experiment Set-up

Experiment Wizard

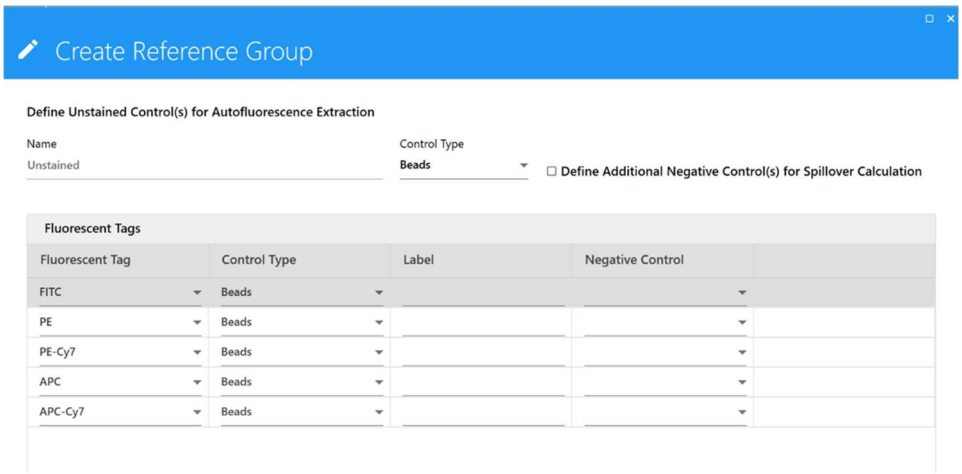
*<u>*Note*</u>: The Cytek Cloud Experiment Builder utility has off cytometer functionality essentially identical to the Experiment Wizard; consider setting up your experiment in advance using this tool.

- Click Acquisition from the Top Menu or Get Started Menu.
- Click **New** in the Experiment menu to create a New Experiment or select from a previously saved Template.
- Follow the New Experiment Wizard to name the experiment, select fluorescent tags, define reference group controls, etc.



  - **IMPORTANT**: Define an unstained control for autofluorescence by selecting its control type (beads or cells). The control **must** be prepared in the same manner as the samples to ensure accurate unmixing.

- You may select **Define Extra Unstained Control(s) for Spillover Calculation** to use different unstained control(s) for reference controls. For example, if you run beads as your controls and they only have positive peaks, you will need an extra control of unstained beads.



- Acquisition Settings
  - Select Default Raw Worksheet (Raw) for the Reference Group and Sample groups if unmixing will be done post-acquisition.
  - Select Default Unmixed Worksheet (Unmixed) or any other saved unmixed worksheet for your sample groups if you are performing live unmixing.

- Click **Save and Open** when completed with all steps.
- Cytek recommends running a tube of 3 mL of DI water at high flow rate for 5 minutes prior to running samples.
- Click **Start** to view the data to adjust Gain Settings; click **Record** to when ready to record the data.

Unmixing Options

Live Unmixing

Live Unmixing will allow to view unmixed data live while acquiring:

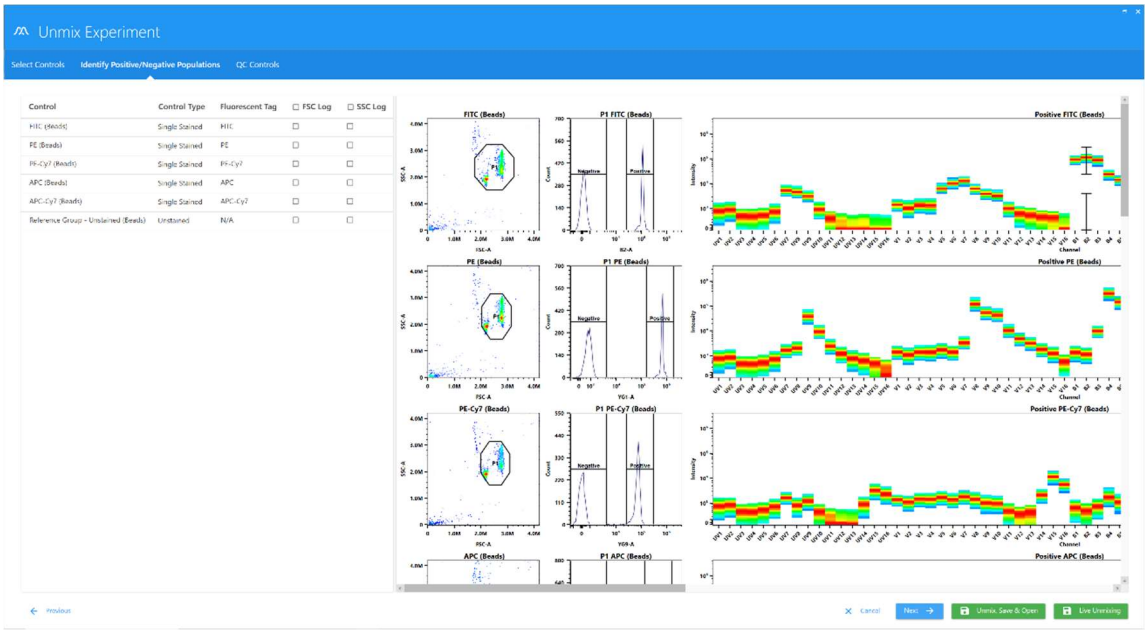
- Record all reference controls
  - For reference controls you will need single stained samples for each fluorescent marker and an internal or universal unstained sample with matching background autofluorescence. In some cases, autofluorescence signatures on beads will not match those on cells if using bead standards, you need to confirm that single stained beads accurately unmix the corresponding single color cell samples.

- The number of events to record depends on the target population. You need approximately 1000-2000 events in both the negative and positive populations to accurately unmix. It is recommended to save at least 50,000 total events.
- If you have a heterogenous population and want to utilize AF extraction, it's recommended to record as many cells as possible.

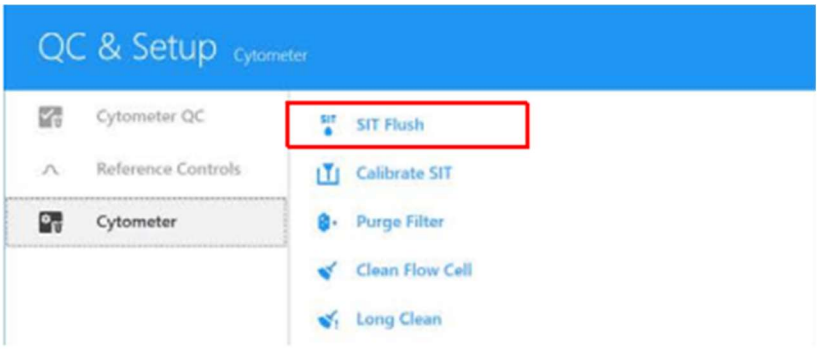
- Click **Unmix** in the Experiment Controls.
  - Check Autofluorescence Extraction, if desired (this is helpful if your sample has a single strong autofluorescence background); adjust gates to reflect negative and positive populations and peak emission channels.
- Click **Live Unmixing** when complete.
- View samples in an unmixed worksheet. Both raw and unmixed FCS files are saved.

Post-Acquisition Unmixing

- Acquire all reference controls and samples
- Click **Unmix** in the Experiment Controls
  - Check Autofluorescence Extraction, if desired; adjust gates to reflect negative and positive populations and peak emission channels.
- Click **Unmix, Save & Open**
- A new analysis experiment is created automatically. A new set of unmixed FCS files are saved.



3. Between Users and Shutdown Procedures



End of Experiment Cleaning

- If operating in plate mode, you must switch to tube mode prior to performing Fluidics Shutdown
- If there is a user following you in the day, perform **Fluidics Shutdown** function in the Cytometer menu, DO NOT shut the instrument off. Simply, log off.
- If last user of the day, navigate to the Cytometer menu and select **Fluidics Shutdown** and follow the wizard steps
  - **Turn instrument off by pressing the button on the left side.**
- Click Done and ensure the SIT is submerged in DI water.
- Logout of the software, then computer.

4. References

- Aurora Quick Reference Guide
- Aurora User Guide
- Cytek Resources- Instrument Cleaning Recommendations

Additional Resources can be found within the Aurora User Community Forum

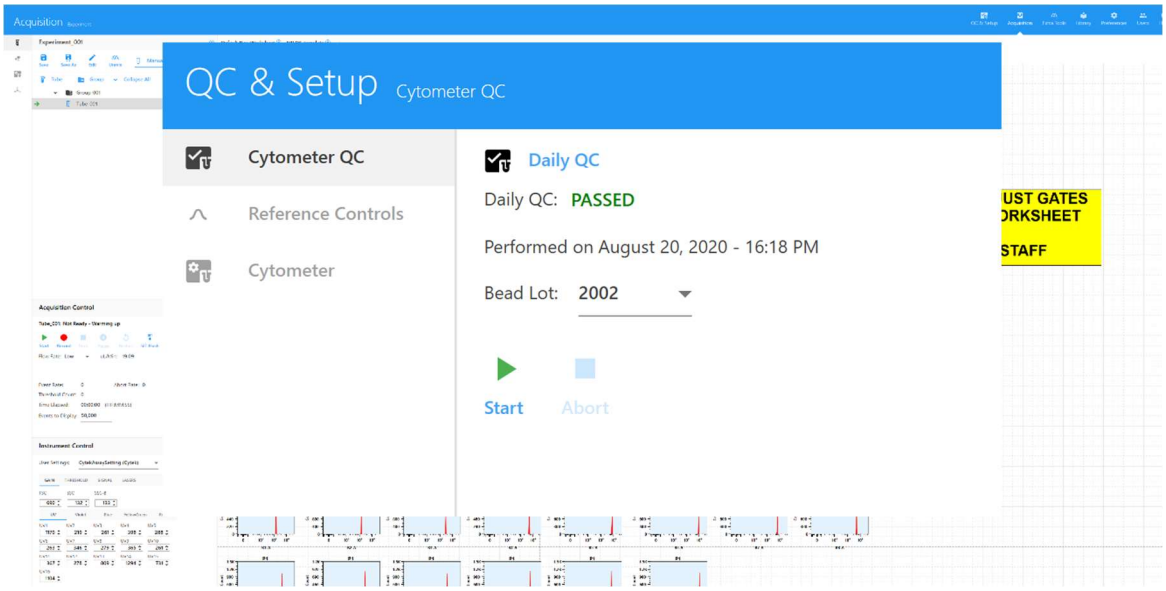
5. Startup

- Check Supply and Waste containers:
  - Empty Waste container in the sink – Add bleach (~10% final concentration).
  - Fill Sheath container with Filtered Deionized Water

- Turn on the workstation, login with ONYEN and password.
- Turn on Cytometer- Left side of instrument.
- Ensure a tube of DI water is on the SIP, then open SpectroFlo software and log in.
- **Wait 30 minutes to allow cytometer to warm up before running Daily QC**. The Software will start a 'Warm-up' timer in the lower right corner of the software once logged in.
  - Note that it is not recommended to run samples during the 30 min warm up, but you are still able to if desired. This only applies for the first time the instrument is turned on in the morning.
- Load a tube of 3 mL DI water and run at a high flow rate for 5-30 minutes or while the system is warming

6. Daily QC

- Load a tube of SpectroFlo beads (1 drop beads into 300 µL filtered DI water) onto the SIP.
- Found in the Fridge of Rm B020
- Open a Default experiment and open "AW QC Template" Worksheet Template
  - Run beads on Low and observe statistics. Make sure all values are below 7% (see current rCVs on worksheet template). The lower the better. If needed, Clean Flow Cell followed by running DI water on High for 5 minutes.
- Click **QC & Setup** Tab.



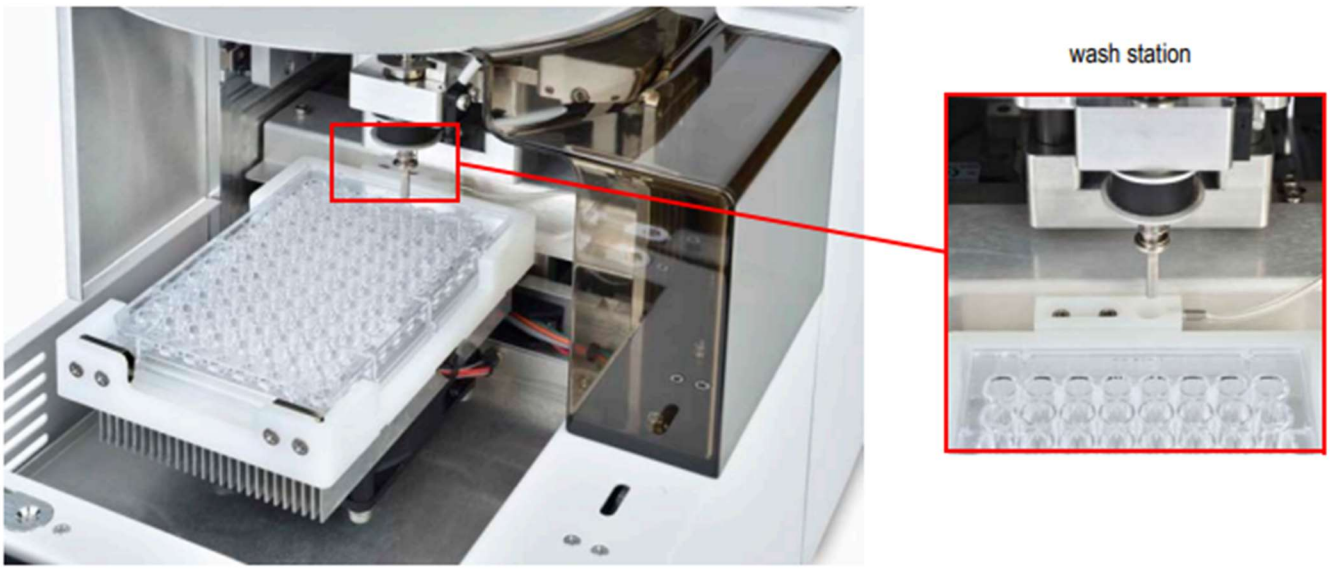
- Select the current bead lot number from the drop-down menu then Click **Start**.
- Event Rate should be greater than 150 evts/s
- It will automatically collect 10,000 events

- When Daily QC successfully completes, click **View Report** to see Daily QC report.

*Note: If QC fails, follow guidelines in the Daily QC Failed Dialog that appears.

7. Loader Overview

The Loader resuspends samples via a mixing probe. This probe is not effective for cells that tend to fall to the bottom of the plate. Be prepared to bring a multi-channel pipette to resuspend cells. The Loader plate stage houses a metal element that can cool/heat from 4°-30℃. The wash station at the back of the plate stage is used to clean the probe after each well.

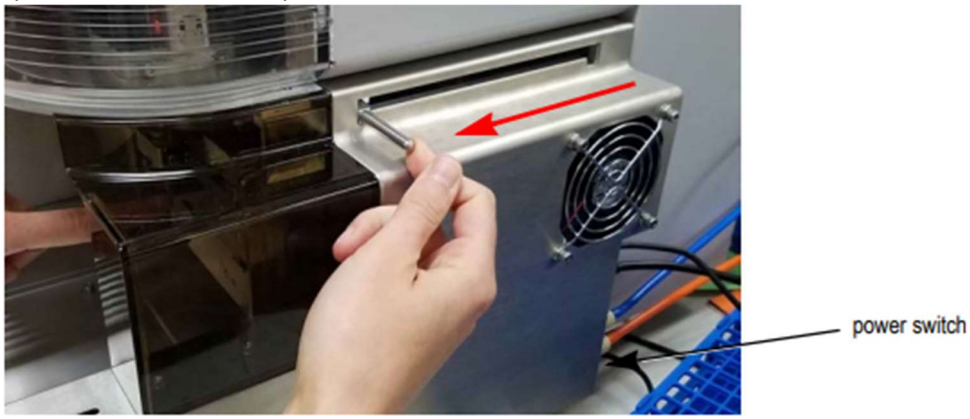


Loader settings are user-adjustable, including mixing speed, mixing duration, the number of SIT flushes between samples, sample recovery, and data record delay time. Pre-defined Loader settings include the default settings for high throughput mode, standard mode, and low carryover mode.

Using the Loader

The loader must be calibrated for the plate type being used. *This option is only available to Admins, ask Staff for help*.

1. Turn the power to the Loader on. The power switch is located on the back of the loader near where the fluidic lines connect.
2. Remove the tube on the SIP
3. Carefully pull the lever towards you.

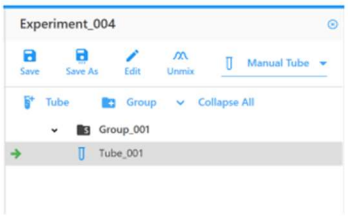


4. Perform a SIT calibration to your plate.
5. If necessary, click **Eject** from the Acquisition module to eject the stage
6. Load a plate on the stage so the A1 is located in the front-left corner. Gently push the plate against the back of the stage, then press down on the front edge of the plate to secure it with the clips in the holder.
7. Click **Load** from the Acquisition module to load plate, then click **Start** to begin acquisition.
8. To run tubes, select Manual Tube as the carrier type option in the experiment. This is helpful if Reference Controls are in Tubes while experimental samples are in a plate.

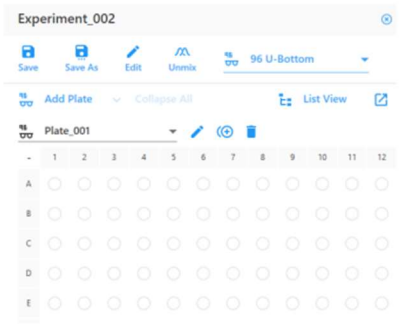
***Note**: There is a dead volume of **35 uL** sample per well. Recommended minimum volume of **50 uL** sample per well. Max volume is 200 uL.

Loader Settings in Experiment

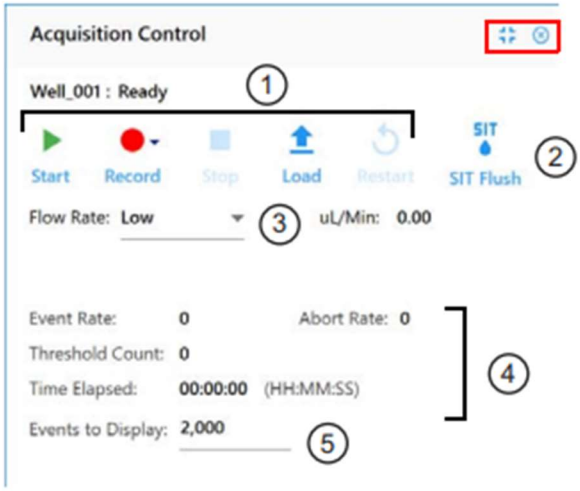
Default Experiment List View



Default Experiment Plate View Once Plate is Added



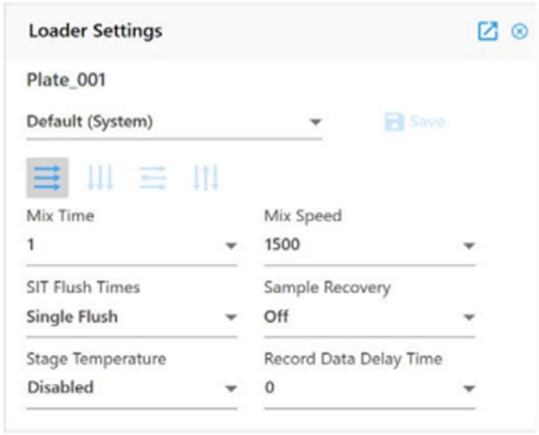
Acquisition Controls



1. Start/Record/Stop/Load/Eject/Restart
2. SIT Flush
3. Flow Rate: Low (~15 uL/min), Medium (~30 uL/min), High (~100 uL/min)
4. Event Rate/Abort Rate/Threshold Count/Time Elapsed
5. Events to Display

Loader Settings

Several default Loader settings are available. You can select the Default, High Throughput, or Low Carryover Loader Setting, depending on your application. You can also create custom settings. Loader settings are enabled once a plate is added. Adjust the settings before acquisition.

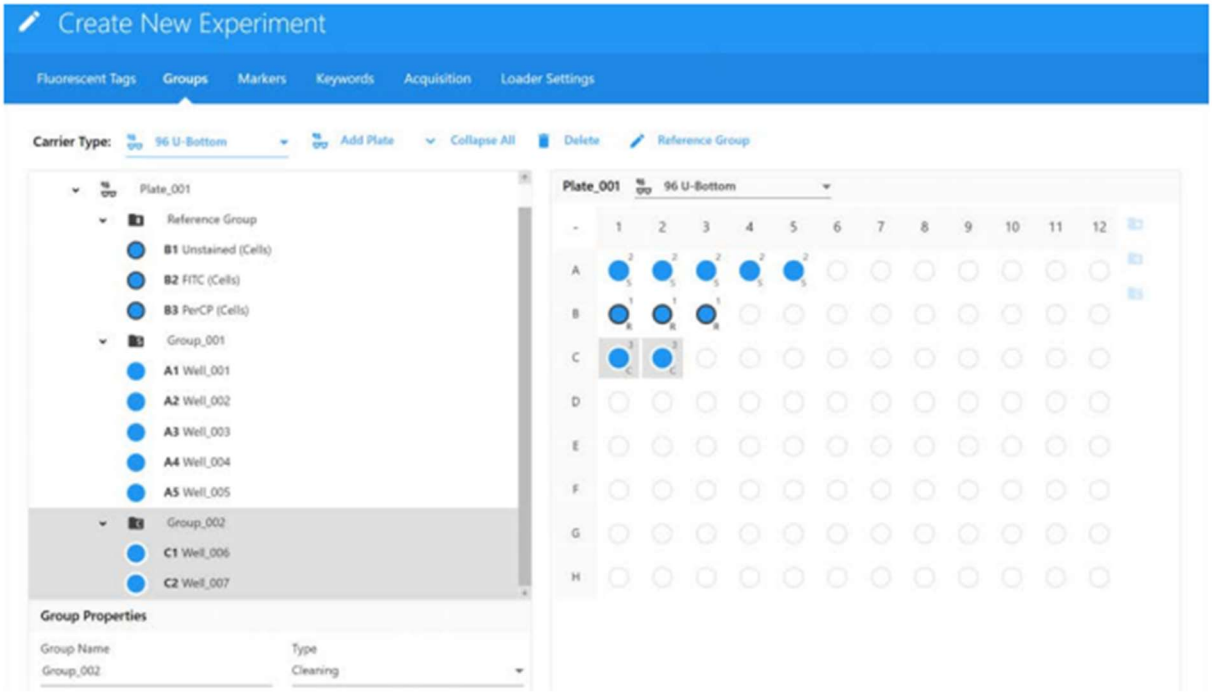


Settings	Description
Acquisition order	Select the order that you want the plate to run. Wells are acquired by: • row from left to right (A1-A12, B1-B12, etc) • column from top to bottom (1A-1H, 2A-2H, etc) • row from left to right, then right to left (A1-A12, B12-B1, C1-C12, etc) • column from top to bottom, then bottom to top (1A-1H, 2H-2A, etc)
Mix time	Select time (in seconds) that each well is mixed. You can also disable the mix time.
Mix Speed	Select the speed at which the mixer spins (in RPM).
SIT Flush Times	A SIT flush is performed over the wash station after each acquisition. Choose single flush, double flush, or disabled if you do not wish to perform a SIT flush
Sample Recovery	Allows any remaining sample that is left in the SIT after acquisition is complete to be deposited back into the well.
Stage Temperature	Select the plate stage temperature (4°–30°C).
Record Data Delay Time	Select the time in seconds you wish to preview data from a well before recording begins once you click Record.

Experiments in Plate Mode

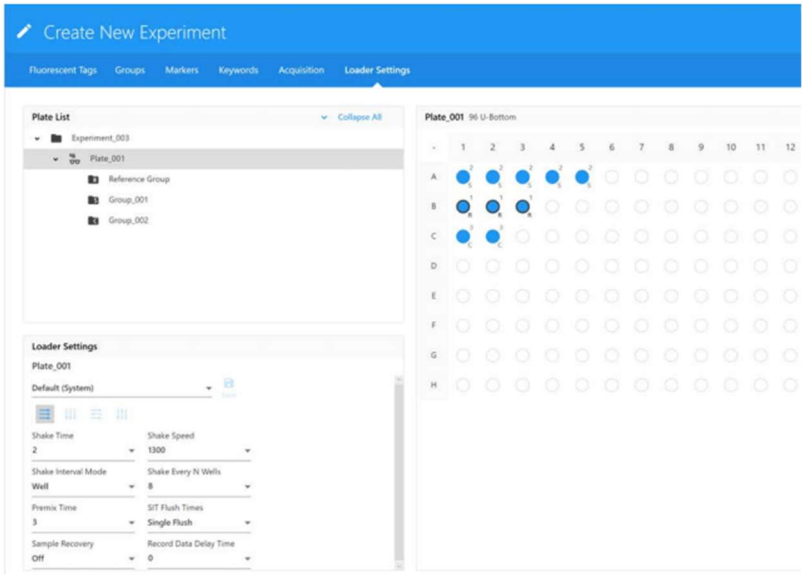
**When running sticky samples, we recommend adding cleaning wells between samples to thoroughly clean the mixing probe. For example, add two wells, one with 10% bleach and the other with DI water. At the end of a plate, consider adding a group of four wells, two with 300 µL of 10% bleach and two with 300 µL of DI water. Program a long mix (15 seconds at 1500 rpm) to thoroughly clean the mixing probe.

1. Ensure correct Carrier Type is selected, then Click **Add Plate**. A plate image will appear on the right and allow Groups to be added.



2. Click in the plate image to select a well, or click and drag to select multiple wells corresponding to the wells in the group you wish to add, then click the appropriate icon to the right of the plate to define the sample types in the group:
    a. To add a group for samples, click **+**. Samples are labelled as **S.**
    b. To add a group for reference controls, click **R**.
    c. To add a group for cleaning well(s), click **C**. For example, you can add DI water to wells to rinse the SIT and clean the mixing probe to prevent carryover.
3. When all groups have been defined and samples are added, Click **Next**.

4. Continue with creating the New Experiment
5. Define Loader Settings, then click **Save and Open**



8. Additional Cleaning Procedures

Clean Flow Cell (Sticky Samples/Clog)

Clean the flow cell after completing an experiment with a sticky dye such as propidium iodide, acridine orange, or thiazole orange. Cleaning the flow cell is also recommended after acquiring large quantities of highly concentrated bead solutions or if you suspect a clog. If running sticky samples, perform this procedure using Contrad instead of 10% bleach between experiments.

- In the Cytometer Menu, select **Clean Flow Cell**
- Follow instructions. If a clog is suspected, using Contrad instead of Bleach for the first step.

Monthly and As Needed Cleaning

Perform Long Clean function monthly (Staff only)

- Prepare at least 1 L of 10% Bleach and two tubes, one containing 3 mL 10% bleach and one containing 3 mL DI water.

- Locate Long Clean sheath bypass assembly and a new sheath filter, if needed.
  - Note: Cytek recommends replacing the sheath filter about once per year; the field service engineer will replace this for you during preventative maintenance visits.
- In the "QC & Setup" or "Acquisition module", under the Cytometer tab, click on Long Clean and follow the instructions. For additional details, watch the video tutorial Performing a Long Clean.

Contrad Soak as Needed (Staff Only)

Perform Contrad Soak as needed. The frequency depends on how often the system is used, and what types of samples are going through the system. If you notice increasing amounts of debris in your FSC vs SSC plot, and/or increasing FSC %rCV in the system's QC data, running this procedure will help clean out the flow cell.

- Reserve one hour for the next morning following the Contrad soak to wash out the Contrad from the system.
- Perform a Fluidics Shutdown using four tubes, each containing 3 mL 1% Contrad 70.
- After completing Fluidics Shutdown, unload the 1% Contrad tube and replace it with a fresh tube containing 3 ml of DI water.
- Power off the system and close SpectroFlo as instructed in the pop-up window and leave the system alone so the flow cell can soak overnight.
- The next morning, power on the system, open SpectroFlo, log in, wait for the software to connect to the cytometer, and perform a Clean Flow Cell using two tubes each containing 3mL DI water.
- Load a new tube containing approximately 3mL of DI water onto the SIP and run it at high flow rate for 30 minutes.
- Perform QC and make sure it passes before running experiments.

9. Troubleshooting

Common troubleshooting actions. Contact staff if the suggestions do not help.

Observation	Possible Causes	Recommended Solutions
Daily QC does not complete	Wrong QC sample	Ensure you are running SpectroFlo QC beads of the correct lot
	Bead sample not properly mixed	Vortex QC tube for at least 5 seconds
	Bead sample too dilute	Concentrate sample or prepare fresh sample. 1 drop of beads into 300 uL filtered DI water
	Air Bubble in sample line	Run **SIT Flush** or **Purge Filter**
	Degraded Beads	Prepare fresh beads
	Warm-up not done	Let instrument warm up for at least 30 minutes. Run water on High for at least 5 minutes prior to QC
Daily QC failed	Air Bubble in fluidics	Run a **Purge Filter**
	Dirty Flow Cell	Run a **Clean Flow Cell** If problem persists, run **Clean Flow Cell** using 25%-50% Contrad 70 (1%-5% Contrad 100), followed by DI water
	Questionable sample prep	Make fresh beads
	Sample not diluted in same fluid as sheath	Dilute sample in filtered DI water
Air in Sheath filter	Cytometer was not in use for a prolonged period	Run a **Purge Filter**. Check all sheath connections are securely attached. Check for leaks or cracks in the sheath plenum.
	Empty Sheath tank	Refill sheath tank. Run a **Purge Filter**
No events displayed (flow rate lower than expected)	Sample ran dry in tube	Replace tube. Run **SIT Flush**
	Sample not properly mixed	Mix the sample
	Clogged SIT	1. Run a **SIT Flush**. 2. Run **Clean Flow Cell** with 10% bleach followed by DI water If clog persists, replace sample line
	SIT distance is inappropriate (touching bottom of tube or too high)	Run **Calibrate SIT**
No events displayed (flow rate normal)	Insufficient gain for threshold parameter	Adjust the gain for the threshold parameter (FSC by default)
	Threshold too high	Lower threshold
	Laser Delay not correct	Ensure laser delay values match those from daily QC run. Rerun Daily QC if values do not match.
	Threshold set to incorrect parameter	Set threshold to appropriate parameter for application (typically FSC)
	Gated plot with no data in gate	Delete or move the gate

Low sample event rate	Threshold too high	Lower threshold
	Insufficient gain for threshold	Adjust gain for threshold parameter
	Sample not properly mixed	Mix sample to suspend cells homogenously
	Sample too dilute	Concentrate sample. Set Flow Rate to Medium or high
	Clogged SIT	1. Run a **SIT Flush**. 2. Run **Clean Flow Cell** with 10% bleach followed by DI water If clog persists, replace sample line
Erratic Event rate	Partially blocked SIT	Run **SIT Flush** Run **Clean Flow Cell**
	Sticky/Clumpy sample	Vortex, filter, or disaggregate the sample
Data in scatter parameters appear distorted	Air bubble in flow cell	Run **SIT Flush**
	Air in sheath filter	Run a **Purge Filter**
	Dirty flow cell	Run a **Clean Flow Cell**
	Poor sample health	Check the viability of the cells
	Hypertonic buffers	Check the pH of the buffers and fixative
	Incorrect instrument settings	Optimize the instrument settings
High CVs	Air bubble in fluidics	Run a **SIT Flush** and a **Purge Filter**
	Sampe flow rate set to High	Set sample rate to Low or Medium
	Dirty Flow cell	Run a **Clean Flow Cell**. If problem persists, run a Clean Flow Cell using 25%-50% Contrad 70 (1%-5% Contrad 100), followed by DI water
	Questionable sample prep	Verify the sample prep technique
	Air in sheath filter	Run a **Purge Filter**
SIT hitting bottom of well/tube	SIT Lift Distance set too low	Run **Calibrate SIT**

10. Revisions

SOP version Number	Date	**Tracked Changes** (clearly list changes made and why)	Employee
UT SOP 008.2	1/5/2021	Updates Cleaning Protocols	Ayrianna Woody
UT SOP 008.3	4/8/2022	General Updates throughout; formatting	Ayrianna Woody
UT SOP 008.4	11/29/2023	Added Loader and Troubleshooting section	Ayrianna Woody
UT SOP 008.5	5/22/2024	Updated StartUp Section. Bold/red text for importance notes throughout	Ayrianna (Woody) Hedgecock
UT SOP 008.6	08/21/2025	Minor Updates; Gains; Training Notes; Moved less commonly needed items to the end of the training guide.	Bob Immormino

11. Training Notes

Start-up: Check Supply and Waste levels, Login to workstation with onyen, Open instrument software; SpectroFlo
CS&T performance / Quality-control test: Performed weekdays by staff
Create an Experiment: Import a previously run experiment or an experiment template from Cytek Cloud (Acquisition > Import), use the New Experiment Wizard (Acquisition > New), or open a template (Acquisition > Template). **Rename!**
Optimize Gains: If any detector is off scale (>4x10^6) decrease all lasers uniformly (e.g. All Channels -10%) until all signals are on-scale. If all the signals are well below 4x10^6 increase all lasers uniformly (e.g. All Channels 10%) until the brightest detector approaches 4x10^6). Assure reference controls are 1. titrated, 2. have the expected peak channel, and 3. match reference spectral signatures. If gains are altered make sure to **save the gain settings!**

Collect Reference Controls and Unmix: You will need single stained samples for each fluorescent marker and an internal or 'universal' unstained sample with matching background autofluorescence. Once reference controls have been collected use the Unmix wizard. Be sure to compare observed fluorescent signatures with the references available on-line in the Cytek Spectrum viewer.
Generate a gating scheme and collect data: In the Unmixed worksheet add plots to set-up a gating scheme for your experiment. Save your experiment and any updated Worksheets (unsaved items are indicated with an asterisk '*').
Export your Experiment: Close your experiment. Navigate to Acquisition > My Experiments and Right-click on your experiment to export. Temporarily store files on the J:drive (\\ad.unc.edu\med\microbiology\Groups\Flow Facility). The J:drive will be cleared automatically of data 3 months old.
Clean-up: Run Fluidics Shutdown: FACSClean, diH_2O, Contrad, diH_2O. SIT should be down and in water at the end of the process. If you are the last user of the day power off the cytometer.