Cytek® Aurora Quick Reference Guide

This quick reference guide provides the basic instructions on startup, shutdown, daily QC, experiment setup, and unmixing using SpectroFlo® software. For detailed information, refer to the *Aurora User's Guide*. Use this quick reference guide only after you have become familiar with the procedures outlined in the user's guide.

Startup

1. Check the sheath and waste tanks; fill and/or empty, if necessary.
2. Turn on the workstation, then turn on the cytometer.
3. Ensure that a tube of DI water is loaded on the SIP, then start SpectroFlo software and log in.
4. Wait 30 minutes to allow the cytometer to warm up before running Daily QC.

Shutdown

1. In the Cytometer menu from either the QC & Setup or Acquisition module, select **Fluidics Shutdown**.
2. Follow the prompts to load a tube with 3 mL of 10% bleach, followed by a tube with 3 mL of DI water, followed by 3 mL of 30% contrad, followed by 3 mL of DI water.
3. Leave the tube of DI water on the SIP. Make sure the SIT is submerged in the water.
4. Exit SpectroFlo software.
5. Turn off the cytometer.

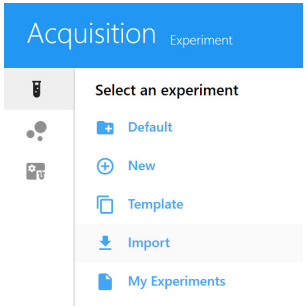
Daily QC

1. Load a tube of SpectroFlo beads (1 drop of beads in 300 µL of sheath or PBS).
2. Click **QC & Setup** from the Get started menu.
3. Select the current bead lot number from the Bead Lot menu.
4. Click **Start**.
5. When Daily QC successfully completes, click **View Report** to see the Daily QC report.

If QC fails, follow the guidelines in the Daily QC Failed dialog that appears.

Data Acquisition

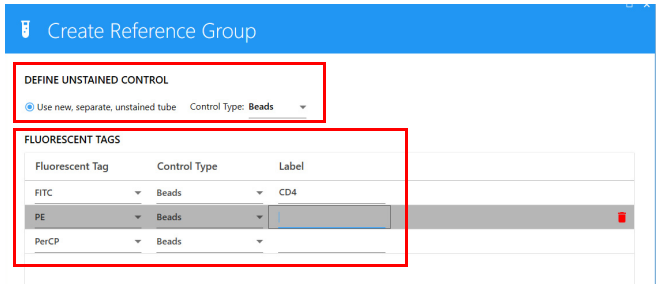
Click Acquisition from the Get started menu. The Acquisition Experiment menu allows you to open a new experiment, the default experiment, or a template. You can also import experiments, as well as access all your experiments, original and unmixed, from My Experiments.



Setting Up a New Experiment

A wizard guides you through the steps of creating a new experiment.

1. Click **New** in the Acquisition Experiments menu.
2. Enter an experiment name.
3. Select all the fluorescent tags used in the experiment. Tags are organized in default groups by laser.
4. Select ⊕ **Reference Group** to create a group for tubes that will be used as reference controls.
5. Select the control type (Cells or Beads) for the unstained control. The unstained control needs to be of the same type and prepared in the same way as the samples.

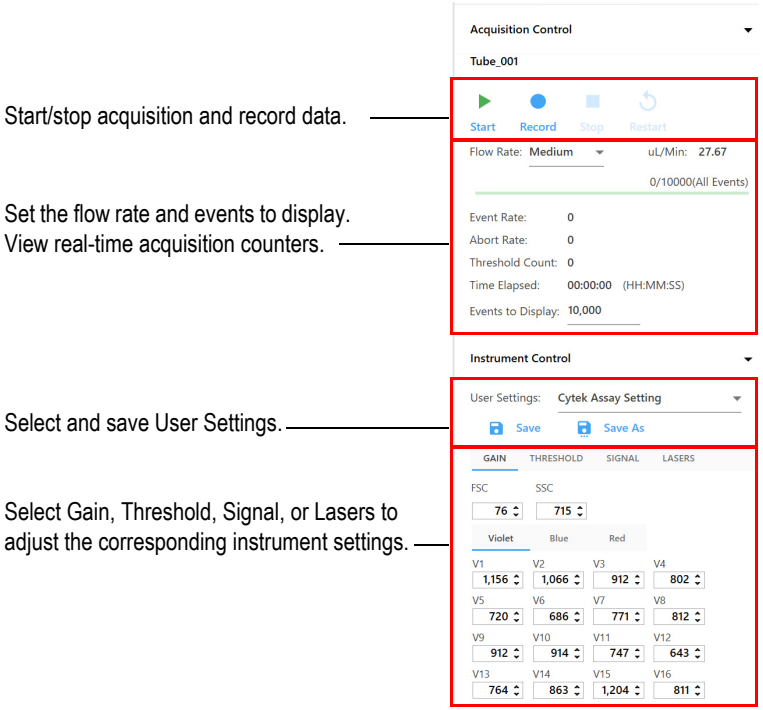


Then select the control type for each single-stained control (fluorescent tag).

You can also enter the labels associated with each fluorescent tag.

6. Click **Save**.

7. Create groups for your samples and add tubes by selecting ⊕ Group and/or Tube.
8. Select labels for the sample tubes.
9. Click **Next** when all tubes have been created and labeled.
10. Add keywords to sample tubes.
11. Select the worksheets for acquisition. Worksheets can apply to all tubes in the experiment, group, or individual tubes.
    - Select the Default Raw Worksheet (Raw) for the reference controls.
    - Select the desired (or Default) Unmixed Worksheet for the samples.
12. Select the stopping gate, events to record, and stopping time.
13. Click **Save and Open** to open the new experiment.
    The panes in the left column contain the controls used for acquisition.



13. Click **Start** to view the data. Start is enabled when a tube is loaded. View all the controls and make any instrument adjustments necessary to ensure populations are on scale before you begin recording.
14. Click **Record** when you are ready to begin acquisition.

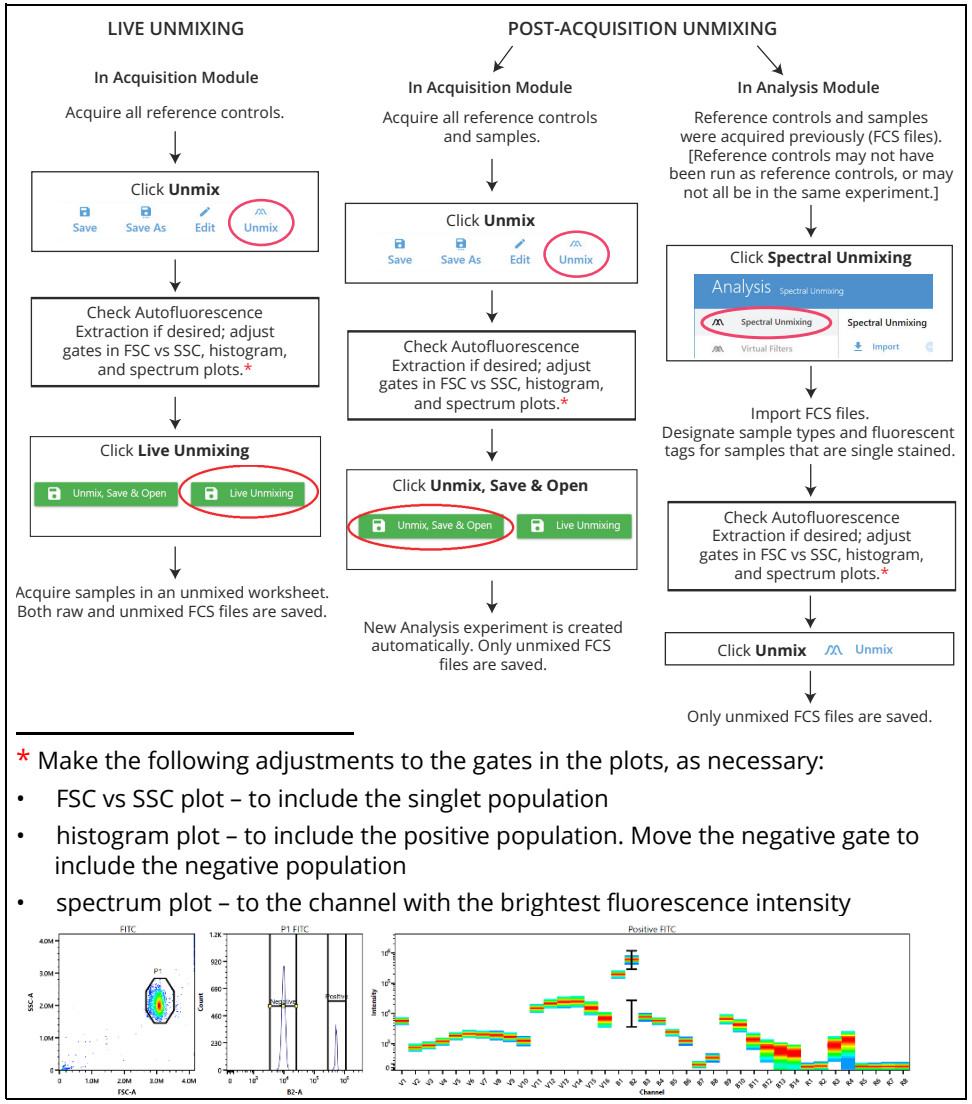
To unmix, follow the appropriate workflow in the following section.

■ Unmixing

Raw vs Unmixed Data

Both *raw* and *unmixed* data are saved. Raw data, where no unmixing is performed, contains all fluorescence information for each detector. Unmixed data has been spectrally deconvolved to separate fluorochromes present on each particle based on a set of reference fluorochrome spectra. Data can be unmixed during or after acquisition, once all needed controls are acquired.

Unmixing Workflows



* Make the following adjustments to the gates in the plots, as necessary:

- FSC vs SSC plot – to include the singlet population
- histogram plot – to include the positive population. Move the negative gate to include the negative population
- spectrum plot – to the channel with the brightest fluorescence intensity