QUICK REFERENCE GUIDE

Cytek Northern Lights™, Cytek Aurora™, and Cytek Aurora™ Evo Systems

This guide provides basic instructions on startup, shutdown, daily QC, experiment setup, and data analysis using the SpectroFlo® software. For detailed information, refer to the instrument's 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. Ensure the sheath tank is full and the waste tank is empty.
2. Turn on the system and the workstation.
3. Sign in using your unique username and password. Optionally, select whether to sign in to use the system in **Full Spectrum Mode** or **Conventional Mode**.

   3a. In **Full Spectrum Mode**, both raw and unmixed data are saved. Raw data contains all the fluorescence information for all detectors. Unmixed data has been spectrally deconvoluted to separate fluorochromes present on each particle based on its spectral signature from a set of reference controls.

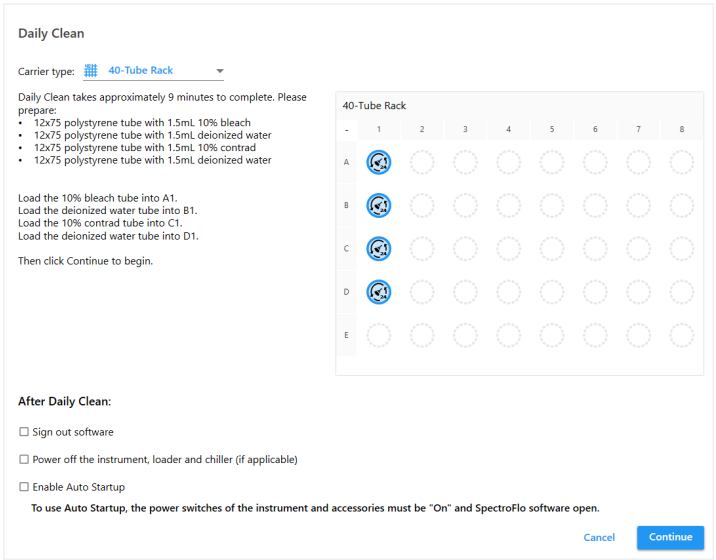
   3b. In **Conventional Mode**, data from one detector is visualized per fluorochrome, based on its peak emission. Data can be compensated automatically, using a set of compensation controls.
4. Allow the cytometer to warm up before running **Daily QC**.

SHUTDOWN

1. In the **Cytometer** menu from either the **QC & Setup** or **Acquisition** module, select **Daily Clean**.
2. Select the carrier type from which you will be running the **Daily Clean**. Follow the prompts on the screen to clean the instrument with 10% bleach, 10% Contrad, and DI water.
3. The sample line should remain submerged in the last tube or well of water. If running from manual tubes, leave the last tube of DI water loaded on the instrument sample port.
4. Turn off the flow cytometer and either **Sign Out** or exit the SpectroFlo software.

DAILY QC

1. Click **QC & Setup** from the **Get Started** menu or select it from the **Top Ribbon** menu.
2. Select the appropriate bead lot number from the **Bead Lot** drop-down menu.
3. Select the appropriate carrier type.
4. Prepare SpectroFlo QC beads (1 drop of beads in 300 µL of sheath solution) into the tube and/or well. Click **Start**.
5. When **Daily QC** is successfully completed, click **View Report** to see the **Daily QC** report.
   NOTE: If the events do not reach less than 1,000 events/second within 30 minutes, repeat the **Long Clean** procedure.



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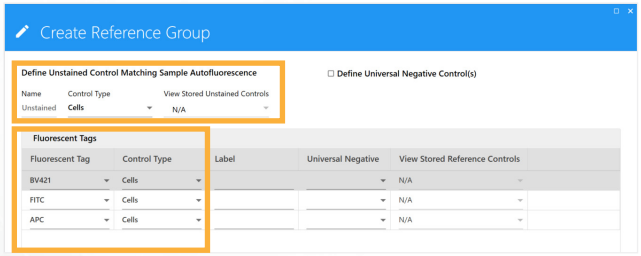
ACQUIRING MULTICOLOR DATA

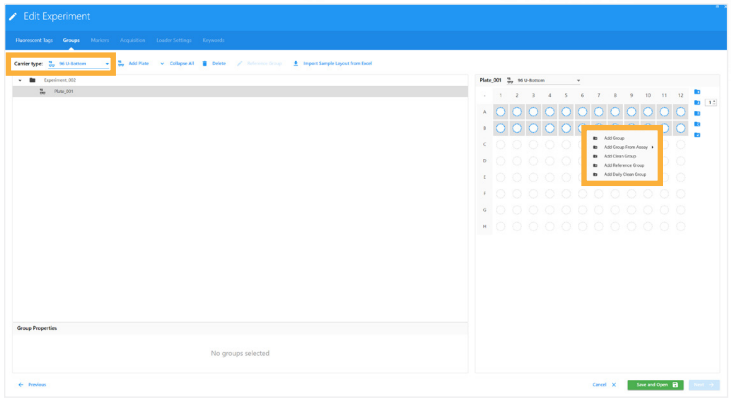
1. Click **Acquisition** from the **Get Started** menu or select it from the **Top Ribbon** menu. The **Experiment** menu allows you to open a new experiment, the default experiment, or an experiment template. Existing experiments that have been previously acquired or imported can be accessed from **My Experiments**.
2. Depending on whether you are in **Full Spectrum** or **Conventional Mode**, data can be either unmixed or compensated once all necessary controls are acquired.

SETTING UP A NEW EXPERIMENT

1. Click **New** in the **Experiments** menu.
2. Enter an experiment name (and description if desired).
3. Select all the fluorescent tags used in the experiment. By default, fluorescent tags are organized by the primary excitation laser.
4. Click **Next** to move to the **Groups** tab. Choose the appropriate carrier type. For tubes or racks, add the needed plate type or rack. Highlight the target tubes or wells and click **Add Reference Group**, or click **+ Reference Group**.
   NOTE: In **Conventional Mode**, single color controls can be added using **Add Compensation Controls** instead of **Add Reference Group**.
5. In the **Create Reference Group** tab, select the control type (**Cells** or **Beads**) for the unstained control. The unstained control needs to be the same cell type and prepared in the same way as the multicolor samples. You may also enter the marker labels associated with each reference control.
   NOTE: In **Conventional Mode**, an unstained control is not needed, as autofluorescence is not being accounted for.



6. Select **Control Type** for each single-stained control (fluorescent tag).
7. Click **Save** to return to the **Groups** tab.
8. For plates or racks, highlight the target tubes from the layout. Click **Add Group** for your samples. For manual tubes, click **+ Group** and add tubes.



9. Double click to edit any of the sample tube or well names. Press **Tab** to cycle through the samples.
10. Click **Next** to move to the **Markers** tab to enter markers for each fluorochrome at the sample, group, or experiment level.
11. Click **Next** to move to the **Acquisition** tab. Edit the available **Acquisition** criteria at the sample, group, or experiment level.

    11a. Use a **Raw** worksheet, such as the **Default Raw Worksheet**, for your reference controls. Use an **Unmixed** worksheet for multicolor data after spectral unmixing.

    11b. Enter the desired stopping criteria (e.g. stopping gate, events to record, volume, time).
12. If a plate or tube rack was entered as a group, edit the loader settings at either the sample, group, or carrier level. Optionally, click **Next** to enter the keywords at the sample, group, carrier, or experiment level.
13. Click **Save and Open** to open the new experiment.

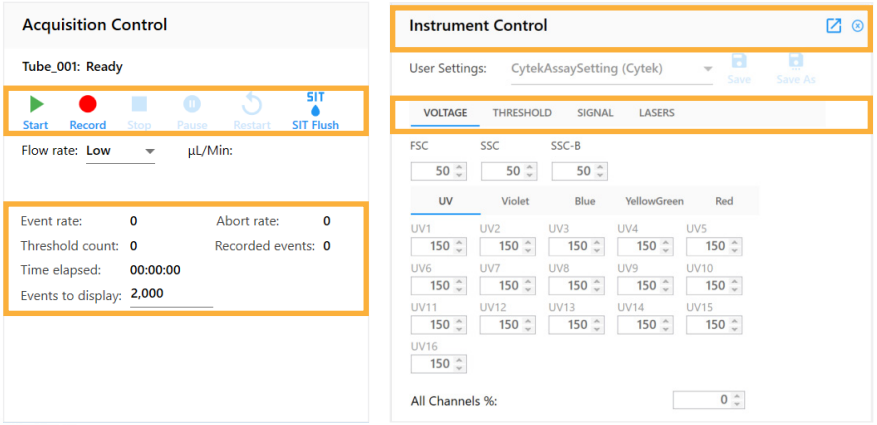
DATA ACQUISITION

1. 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.

   1a. Select a **User Setting** from the drop down.
   NOTE: CytekAssaySetting is the recommended starting **User Setting** for the majority of applications.

   1b. Optionally, click through the **Voltage**, **Threshold**, **Signal**, or **Lasers** tabs to adjust the corresponding instrument settings.

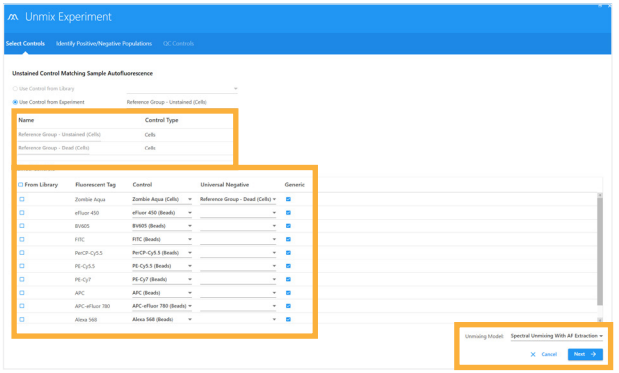
   1c. As needed, save your new **User Setting**.



2. Click **Record** when you are ready to begin acquisition.
NOTE: For spectral unmixing or conventional compensation, follow the appropriate workflow on page 4.

UNMIXING & COMPENSATION

1. Click **Unmix** (or **Compensate**) at the top left of the **Experiment** screen.
2. In the **Unmix/Compensate Experiment** window, select the appropriate controls to use. Click **Next**.
NOTE: In **Full Spectrum Mode**, choose the desired unmixing model. In **Conventional Mode**, an unstained controls is not needed (as autofluorescence is not being accounted for).

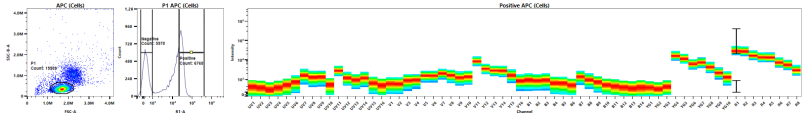


3. Make the following adjustments to the gates in the plots, as necessary:

   3a. FSC vs SSC dot plot - adjust to include the target population (e.g. bead singlets).

   3b. Histogram plot - adjust interval gates around the positive and negative populations.

   3c. Spectral plot (only visible in **Full Spectrum Mode**) - drag the gates to different channels (i.e. peak emission channel) to change the channel displayed in the corresponding histogram plot.



4. Click **Live Unmix**, **Create New Unmixed Experiment**, or **Compensate** based on the current mode and desired workflow (see page 4).

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SPECTRAL UNMIXING

Acquire all reference controls in a raw worksheet.

Acquire all reference controls and samples in a raw worksheet.

Click **Unmix**

Choose which reference controls (e.g. from the experiment or saved in **QC & Setup**), unstained controls, and universal negative to use.

Choose your unmixing model (**Spectral Unmixing** or **Spectral Unmixing with AF Extraction**).

Identify the positive and negative populations for each reference control, adjusting FSC vs SSC, histograms, and spectrum plots.

Click **Create New Unmixed Experiment**

A new experiment will be opened with the spectral unmixing matrix applied to it. Only the unmixed FCS files are saved in this new experiment (the raw FCS files remained linked to the original experiment).

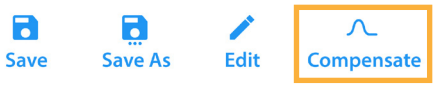
Click **Live Unmix**

The original experiment now has a spectral unmixing matrix applied to it. Both raw and unmixed FCS files are saved.

Continue to acquire any additional samples in an unmixed worksheet.

CONVENTIONAL COMPENSATION

Acquire all compensation controls.



Click **Compensate**

Choose which compensation controls (e.g. **Bead** or **Cell**), and universal unstained to use.

Identify the positive and negative populations for each reference control, adjusting FSC vs SSC and histograms.

Click **Calculate Compensation**

The original experiment now has a compensation matrix applied to it. Continue to acquire any additional samples.

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