

Aurora (Analyzer) Training Guide

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Startup

Check fluidics tanks

- Empty waste tank, add ~1 cup bleach.
- Fill Sheath tank if necessary.

Turn on Computer

- Choose the Operator account (no password).

Turn on Aurora

- The button can be found on the left side panel.
- Place a tube of DI water on the SIP.
- Log into SpectroFlo software.



Setting Up an Experiment in Tubes

Create New Experiment

- Choose Acquisition Module.
- A wizard will guide you through the steps of creating a new experiment.
 - Click **New** in the Acquisition Experiments window.
 - Enter an experiment name.
 - Select all the fluorescent tags used in the experiment.
 - Click Next at bottom of the window or “Groups” at the top of the window
 - Select **+ Reference Group**. (Must always have an unstained cell control for autofluorescence subtraction.)
 - Type Labels for each of the Fluorochromes (optional).
 - Save Reference Controls.
 - Select the Default Raw Worksheet (Raw) for the reference controls.
 - Select the stopping gate (P1), events to record, stopping time, and stopping volume. It will stop at whichever one it reaches first.
 - Click **Save and Open** to open the new experiment.
- Place the Unstained Control on the SIP.
- Set the Flow Rate to Low (10ul/minute).
- Click **Start** to run sample. (The start arrow will appear when the tube is correctly placed on the cytometer.)
- Make FSC and SSC gain changes to get your sample on scale. (CytekAssaySetting should be chosen in the Instrument Control window.)
- Run and Record controls.

Unmixing

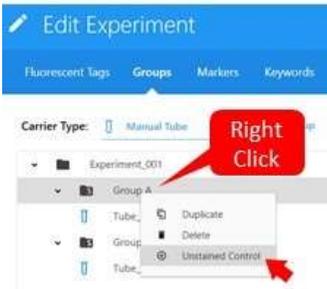
- Click on the Unmix button once all Reference Controls are recorded.
- The first window that appears allows you to choose controls from the library.
- If you want to subtract autofluorescence check the box on the bottom left of the window.
- Adjust the positive and negative gates for each Fluorochrome.
- Click “**Live Unmixing**” in the bottom right corner.

Adding Groups

- In the experiment window, click **+Group**. A Group with one tube will be added. Name the Group and Tube.
- Click Edit at the top of the experiment window.
 - More tubes can be added.

- Click on the group name and add “Unstained Control” if this group will have a different unstained control than the one collected in the previous Reference Controls.
- Type Marker Names.
- Choose an Unmixed Worksheet and Stopping Rules.
- Click Save and Open.

*** How to add group-specific unstained controls (when different cell types or stimulation conditions are analyzed within the same experiment):



Setting Up an Experiment in Plates

Create New Experiment

- Turn the plate loader switch to the on position.



- **Remove tube from the SIP if present.**
- Move the loader arm to the forward position.
- Open the **Preferences tab**, click on **Cytometer** and choose the type of plate you are using under **Default Carrier Type**.
- Choose the Acquisition Module.
- A wizard will guide you through the steps of creating a new experiment.
 - Click **New** in the Acquisition Experiments window.
 - Enter an experiment name.
 - Select all the fluorescent tags used in the experiment.
 - Click Next at bottom of the window or “Groups” at the top of the window.
 - Click Add Plate.
 - Highlight the wells with single color controls and select **R for Reference**. (**It is highly recommended to perform Unmixing of Reference Controls in tubes.) (Must always have an unstained cell control for autofluorescence subtraction.)
 - Type Labels for each of the Fluorochromes (optional).
 - Click Next 2 times or “Acquisition” in the top toolbar.
 - Under Acquisition Settings Select the stopping gate (P1), events to record, stopping time, and stopping volume. It will stop at whichever one it reaches first.

- Click Next or “Loader Settings” and choose Mix Time, Mix Speed, SIT Flush Times, Sample Recovery, Stage Temperature, Record Data Delay Time.
- Click **Save and Open** to open the new experiment.
- Set the Flow Rate to Slow (10ul/minute)
- Click Start to Run the Sample.
- The SIP will calibrate the A1 well, Clean the SIP, and return to collect sample.
- Make FSC and SSC gain changes to get your sample on scale. (CytexAssaySetting should be chosen in the Instrument Control window.)
- Run and Record controls.

Unmixing

- Performed the same as in Tubes.

Adding Groups

- In the experiment window, click Edit at the top of the experiment window.
- Choose **Groups**.
- Highlight the wells that contain samples and choose **+** to add a Group.
- Add Group Name (Optional).
- Click Next and add Marker Names (Optional).
- Click Next 2 times or click on Acquisition in toolbar.
 - Under Acquisition Settings Select an Unmixed Worksheet, the events to record, stopping time, and stopping volume. It will stop at whichever one it reaches first.
 - Click Next or “Loader Settings” and choose Mix Time, Mix Speed, SIT Flush Times, Sample Recovery, Stage Temperature, Record Data Delay Time.
 - Click **Save and Open** to open the new experiment.
- Set the Flow Rate to Slow (10ul/minute)
- Click Start to Run the Sample.
- The SIP will calibrate the A1 well, Clean the SIP, and return to collect sample.
- Make FSC and SSC gain changes to get your sample on scale. (CytexAssaySetting should be chosen in the Instrument Control window.)
- Run and Record controls. (Samples can be recorded by Well, by Group or by Plate.)

Exporting

- Save the Unmixed Worksheet, Assay Settings, and the Experiment.
- Close the Experiment.
- Open “My Experiments”. Right click the experiment and choose Export. This will export your experiment as Raw and Unmixed FCS files.
- Transfer experiment to 3rd party drop box (e.g. OneDrive)

Repeating an Experiment

- Duplicate an experiment with Reference Data (by right clicking on the experiment in the My Experiments window in Acquisition and clicking Duplicate with Reference Data).
- Click “Unmix” and “Live Unmixing” which will update all the reference controls to that day’s QC.
- Click Edit in the Experiment window, Add additional Group(s), Additional Group Specific Unstained Cell controls.
- Acquire new group specific unstained controls.
- Click Unmix, Set FSC/SSC gate on population of interest in the new unstained cell control.
- Click Live Unmixing.

Clean (performed after each user, takes about 3 minutes)

- Under Cytometer icon (left toolbar) choose **Clean Flow Cell**
- Place a tube with 3ml of FACSClean (10% Bleach) on the SIP and click Continue.
- Place a tube with 3ml of DI water on the SIP and click Continue.
- Log out of SpectroFlo Software.

Shutdown (performed by last user of the day, takes about 6 minutes)

- Under Cytometer icon (left toolbar) choose **Fluidics Shutdown**.
- Fill a FACS tube with 3ml 10% bleach (FACSClean). Click Continue.
- Fill a FACS tube with 3ml DI water. Click Continue.
- Fill a FACS tube with 3ml Contrad. Click Continue.
- Fill a FACS tube with 3ml DI water. Click Continue.
- Click Done.
- Sign out of SpectroFlo Software.
- Turn off the Cytometer.

Additional Tips

Reference Controls from the Library

- Reference Controls can be saved in the Reference Control Library if experiments will be repeated often.
 - Open the QC & Setup Module and click on Reference Controls, New Reference Controls.
 - Choose Fluorochromes.
 - Define the Unstained Control.
 - Define control type – beads or cells.
 - Type in labels and/or lot numbers (optional)
 - Click Next.
 - Load the Reference Control and preview the sample. Adjust FSC and SSC if necessary.
 - Once settings are adjusted click Next.
 - Record all the Reference Controls and adjust gates. (Gates are permanent after saving.)
 - Select Save to save Reference Controls.
- To use controls from the library:
 - Choose Acquisition Module, New Experiment.
 - A wizard will guide you through the steps of creating a new experiment.
 - Click **New** in the Acquisition Experiments window.
 - Enter an experiment name.
 - Select all the fluorescent tags used in the experiment.
 - Select **+ Reference Group** and delete the Reference Controls. (Must always have an unstained cell control for autofluorescence subtraction.)
 - Save Reference Controls.
 - Select the Default Raw Worksheet (Raw) for the reference controls.
 - Select the stopping gate, events to record, and stopping time. It will stop at which ever one it reaches first.
 - Click **Save and Open** to open the new experiment.
 - Run the Unstained Reference Control.
 - Click Unmix, Next, and gate unstained Reference Control. Choose Live Unmixing.
- Proceed with the rest of the experiment as usual.

Preferences Tab

- To set the SIT Lift Distance

- Flow Rate speeds should meet the below criteria, if not set SIT Lift Distance to 1.5.
 - Low = 10ul/min
 - Med = 30ul/min
 - High = 60ul/min

Troubleshooting

Instrument will not perform SIT Flush

- Check that the bar at the top of the SIP has dropped.
 - Ask staff to demonstrate.
 - Push down with pen tip and or squirt some DI water to loosen salts.

Flow Rate is lower than expected

- Check the SIT Lift Distance under the Preferences tab and raise if necessary.
- Filter the samples.
- Dilute the samples.

Cytometer will not Connect

- Place a tube on the SIP if not present.
- If tube is present shut down cytometer and computer. Reboot computer first then turn on cytometer.

