B49008AA10
August 2014

CytoFLEX Flow Cytometer Quick Start Guide

For detailed instructions, refer to the *CytoFLEX Flow Cytometer Instructions for Use*.

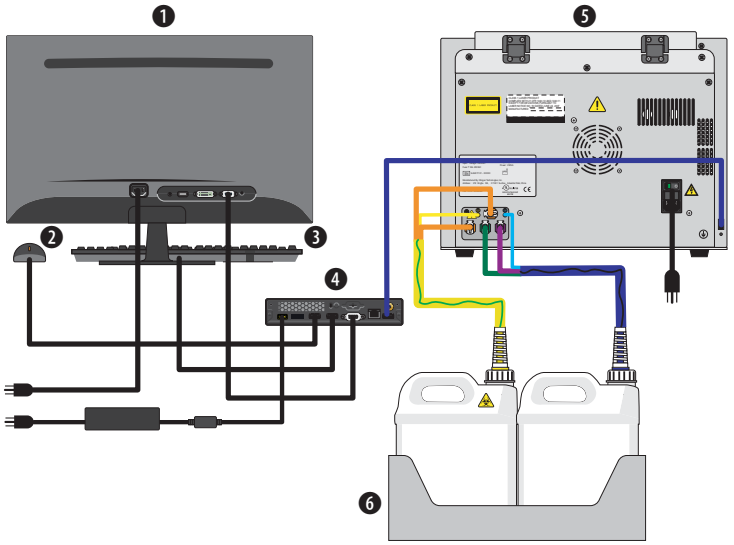
Work Flow

Start up → **QC** → **Create Experiment** → **Adjust Instrument Settings and Compensation** → **Record Data** → **Shut down**

Start Up

Instrument Preparation

1. Ensure all system connections are connected correctly.



1. Monitor
2. Mouse
3. Keyboard
4. Computer
5. Cytometer
6. Fluid Container holder

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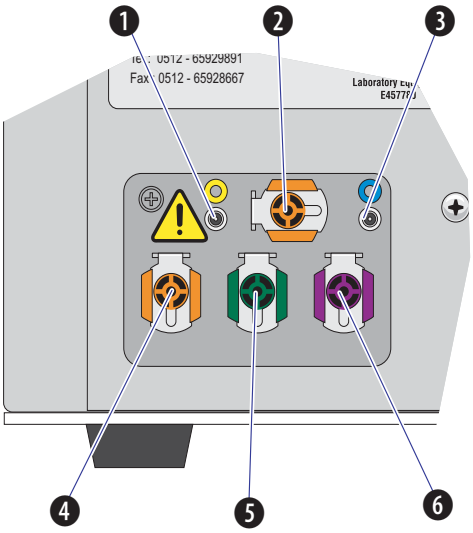
2. Ensure that all of the tubing and the cables are connected to the instrument according to the color code.



1. **Waste level sense.**Connects to the waste liquid sensor cable.
2. **Flow cell waste out.** Connects to the flow cell waste tubing.
3. **Sheath fluid level sense.** Connects to the sheath fluid sensor cable.
4. **Waste out.** Connects to the waste liquid tubing.
5. **Sheath return.** Connects to the sheath fluid tubing.
6. **Sheath fluid in.** Connects to the sheath fluid tubing.

3. Verify that the dongle has been connected to the USB port.

⚠ CAUTION

Risk of instrument damage. Remove the sheath fluid container from the Fluid Container holder and fill away from the instrument to prevent spills that could damage the instrument circuitry.

4. Remove the sheath fluid container from the Fluid Container holder and fill the sheath fluid container with the supplied sheath fluid.
5. If necessary, remove the right side cover and fill the Deep Clean solution with a mixture of 1 part Contrad 70 and 1 part DI water.

⚠ WARNING

Risk of chemical injury from bleach. To avoid contact with the bleach, use barrier protection, including protective eyeware, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

6. Empty the waste container if necessary. Add 400 mL of 5 to 6% bleach to the waste container.

Instrument Start Up

1. Turn on the power switch on the back cover of the instrument, located just above the power cable.

2. Log on to the computer and double-click to start CytExpert.
   a. Ensure that the **Connected** icon on the Status Bar near the bottom-left side of the display is green.

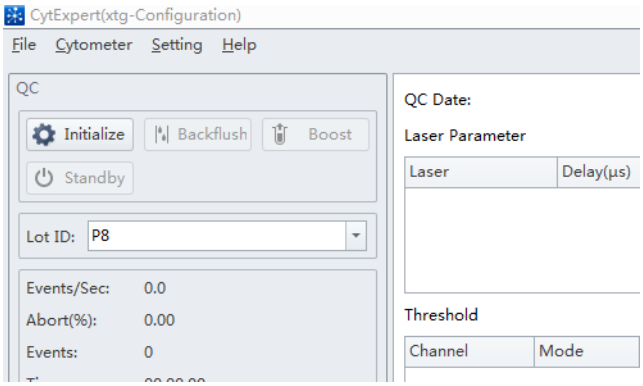
   b. If the icon is not green, ensure that the instrument USB is securely connected to the computer and restart the computer.

3. Select **System Startup Procedure** in the Cytometer menu and follow the software prompts.

Instrument QC

IMPORTANT Verify the detector configuration. Ensure that the instrument settings are properly configured for the QC experiment. The QC experiment may not complete or may end in erroneous results if incorrect settings are chosen. Beckman Coulter recommends using the factory configuration and ensuring that the proper optical filters are in place.

1. Select **Start QC** in the QC menu. The Acquisition screen is now replaced by the QC screen.



⚠ CAUTION

Risk of erroneous QC results. Different lot numbers correspond to different target value information. Selecting the wrong lot number will lead to erroneous QC results.

2. Ensure that the QC fluorosphere lot number is selectable in the Lot No. drop down menu. If the lot number is not selectable, import the lot-specific target value file. Refer to Importing Lot-Specific Target Values in Chapter 4, Instrument Quality Control of the CytoFLEX Flow Cytometer Instructions for Use.
3. Prepare a QC sample by adding 3 drops of Beckman Coulter supplied QC fluorospheres and 1 mL of DI water into a 75-mm test tube.

IMPORTANT For each new Lot number, download the corresponding target value file and refer to Step 2.

4. Using the Lot No. drop-down list

on the left side of the screen, select the Lot No. corresponding to the QC fluorospheres.

5. Select

, insert the prepared QC sample tube into the sample tube holder, then select

to start QC.

   NOTE QC may take several minutes. The minimum flow rate for the QC fluorospheres is 100 events/second. The results are displayed and saved automatically once it is completed.

   - Green icons in the Result section indicate *Pass*.
   - Red icons in the Result section indicate *Fail*.

6. If QC fails, prime the instrument, initialize, and repeat QC.

   NOTE If QC fails two times in a row on the same day, contact your Beckman Coulter Representative.

Data Acquisition

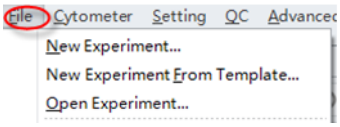
Create Experiment

1. Select the CytExpert desktop icon

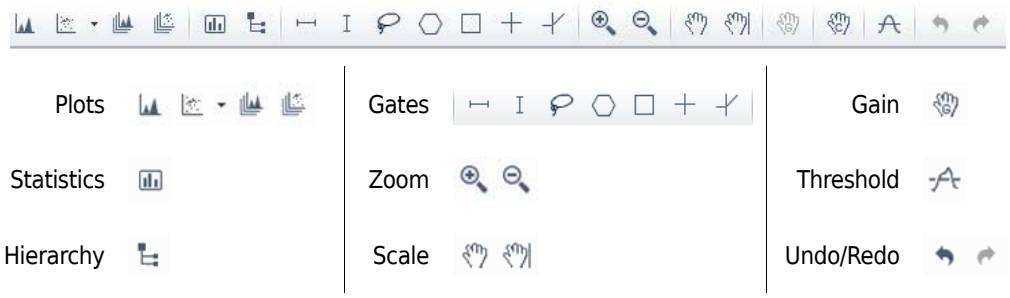
to open the software.
2. From the Start page, select **New Experiment** or **New Experiment from Template** to start a new experiment, or **Open Experiment** to continue an existing experiment.

   NOTE Templates contain information on hardware and software settings including channels used, gain settings, flow rate, and stop criteria.

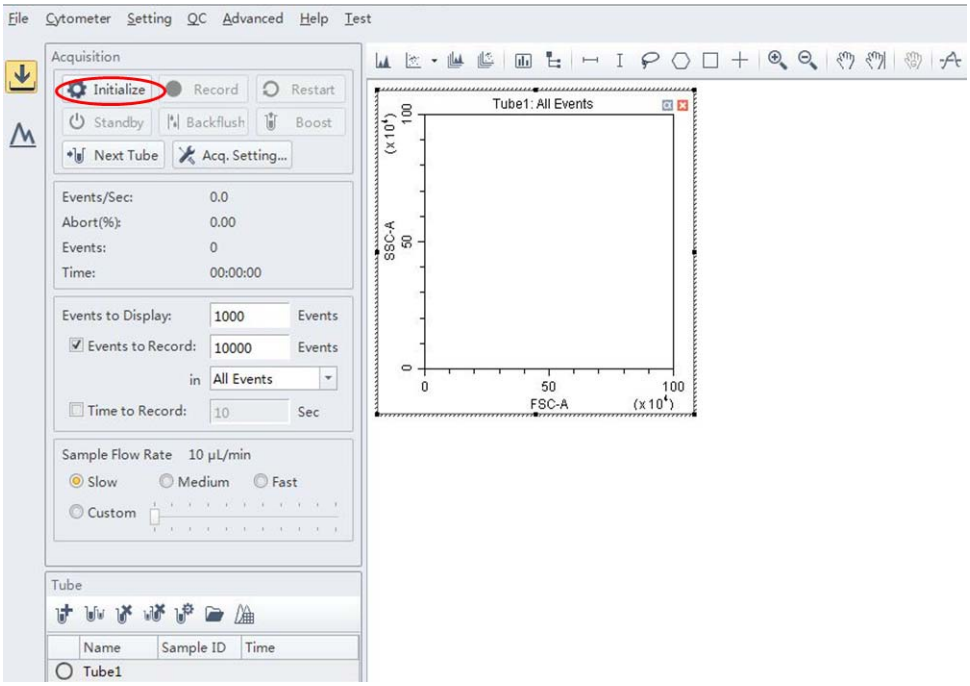
Alternatively, select **New Experiment, New Experiment from Template**, or **Open Experiment** from the File menu.



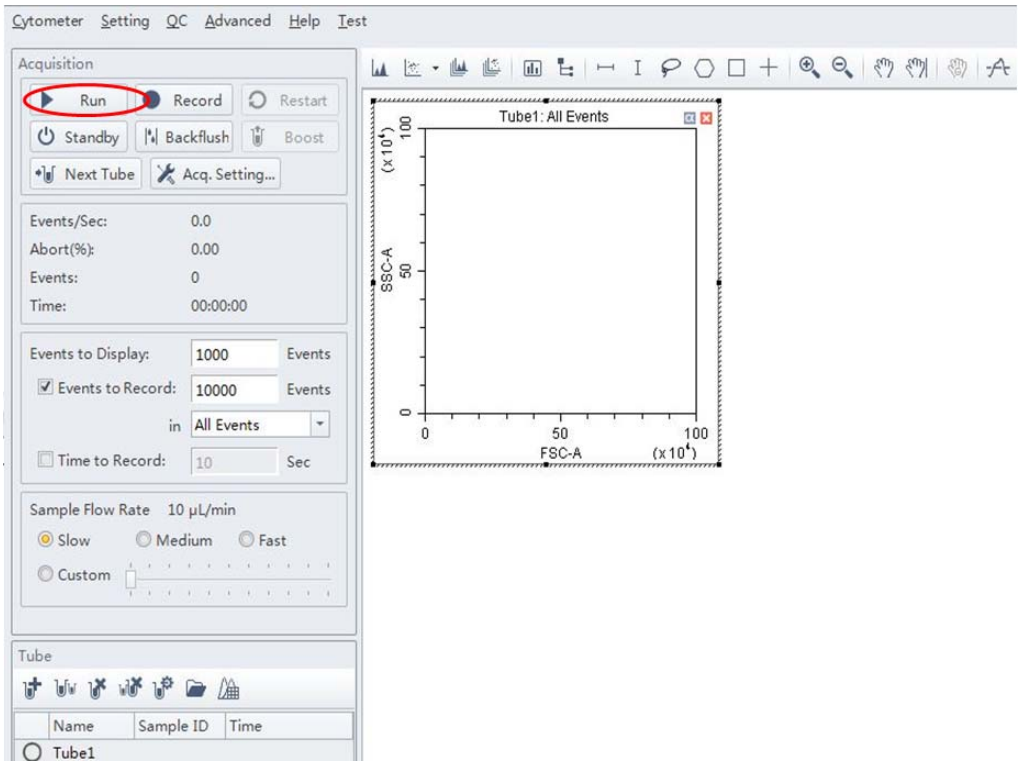
3. If a tube has not been created, use the Tube toolbar buttons to create a new tube. To modify a tube's property, highlight the tube name then select located on the bottom, left section of the Acquisition screen.
4. Familiarize yourself with the toolbar.



5. Create the desired histograms or dot plots using the Plots tool .
6. Set the desired sample flow rate, then select Initialize on the Acquisition panel to start the lasers and fluidics.



7. Load a sample tube into the sample port, then select Run to start acquisition and display data.



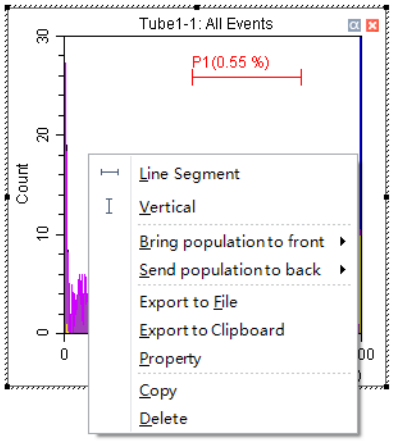
8. Use the Threshold tool or select Acq. Setting... to set discrimination to eliminate undesired populations on the plot.

9. Use the Scale and Gain tools or select Acq. Setting... to move the population displayed on the plot to the desired location.

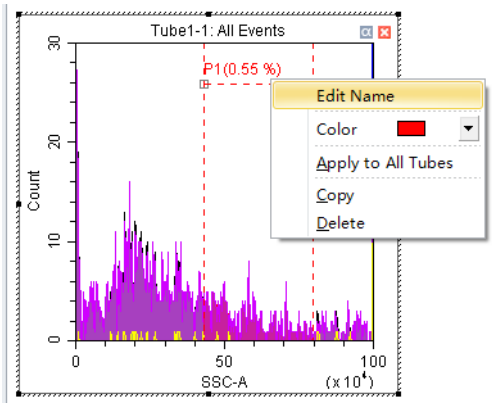
Adjusting Settings and Compensation

Plots, Gates, and Statistics Management

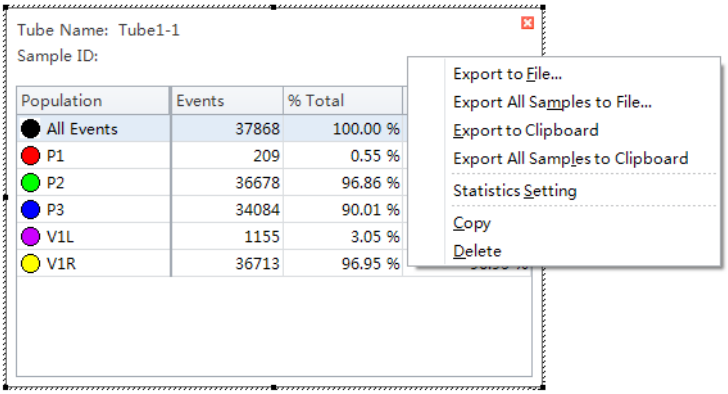
1. To modify the attributes of a plot, gate, statistics table, or population hierarchy, select the plot and right click to select the attribute options. See examples below.
   - Plot properties (right click on the gate)



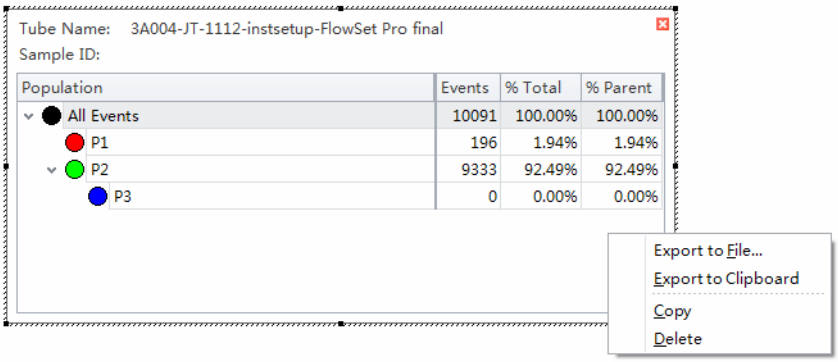
   - Gate properties (right click on gate)



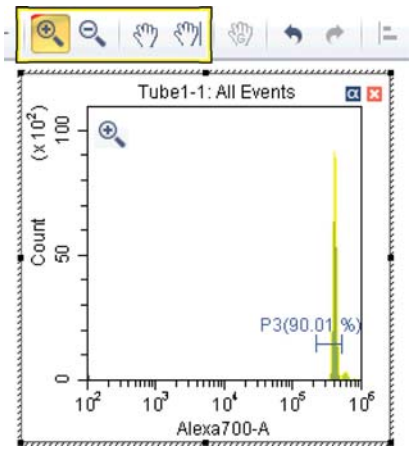
- Statistics Table (right click on statistics table)



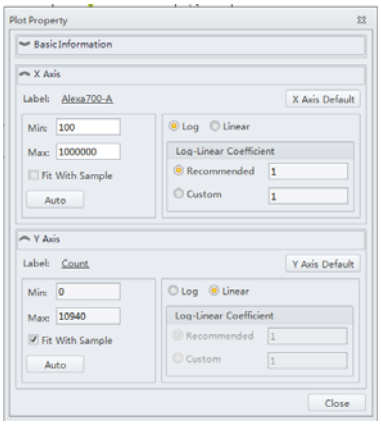
- Population Hierarchy



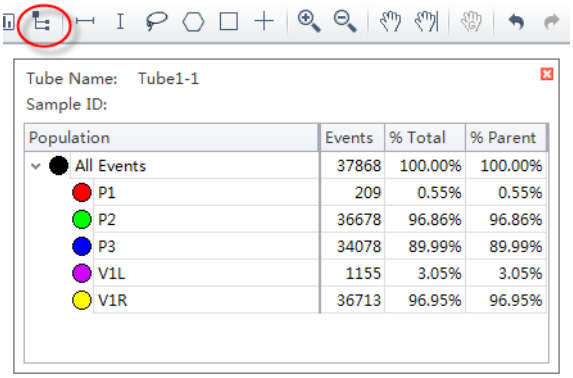
2. Modify the scale range on a plot by either using the Zoom In/Out tools or the pan tools to define the display area around the population on the plot, or by double-clicking near the axes on the plot to view the Plot Property dialog.



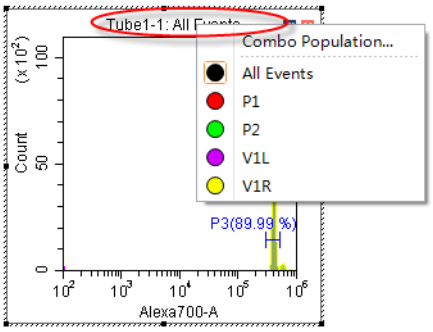
Or

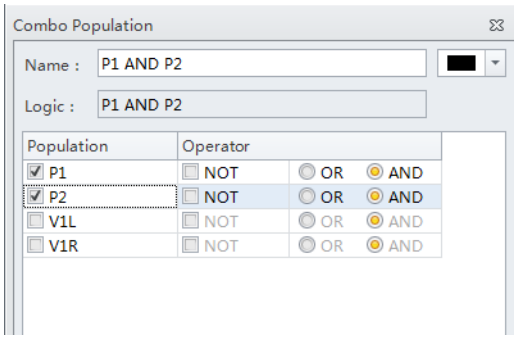


3. Use the Gates tools to gate populations, and the Hierarchy tool to inspect and manage gated populations.

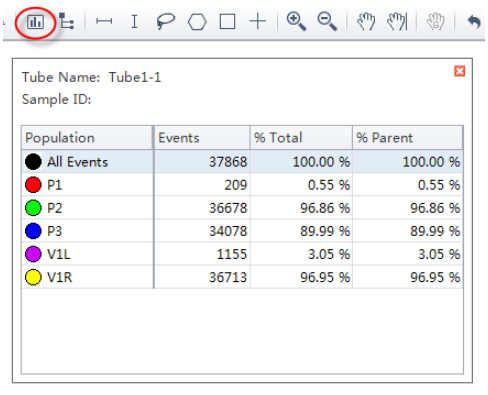


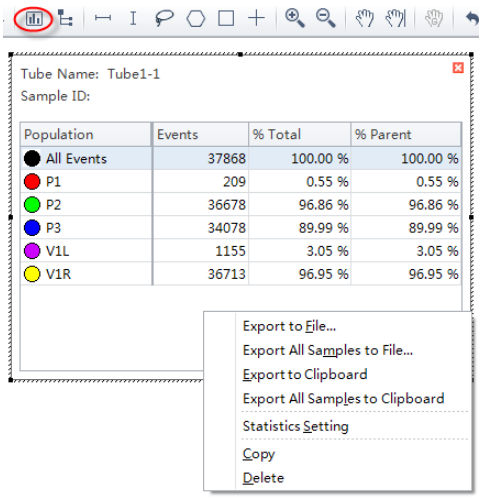
4. Display populations within a gate or Boolean logic of gates (Combo Population) on a plot by left-clicking the title of the plot.





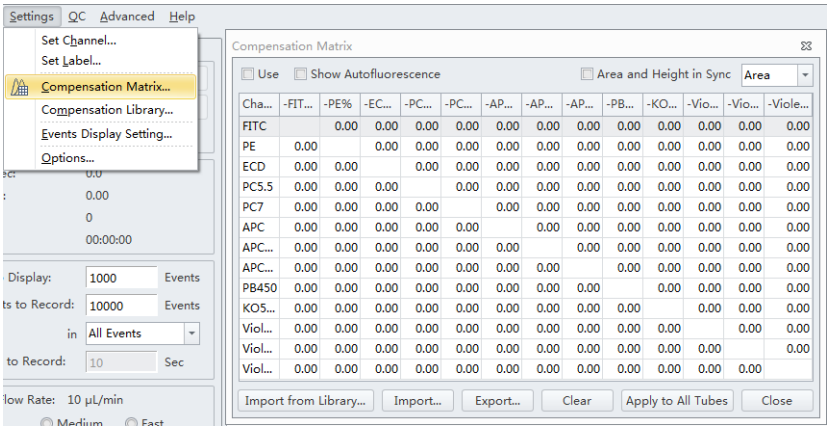
5. Create a Statistics Table by selecting the Statistics tool . The desired statistics can be displayed by right-clicking the panel and then selecting the Statistics Setting in the pop-up window.





Compensation

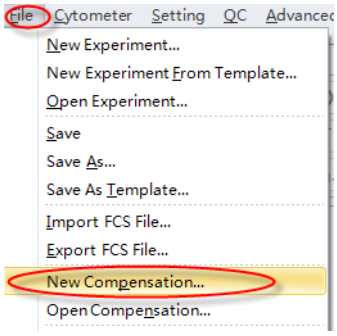
1. Select **Compensation Matrix** in the Setting menu to open the Compensation Matrix window to compensate for the fluorescence spillover.



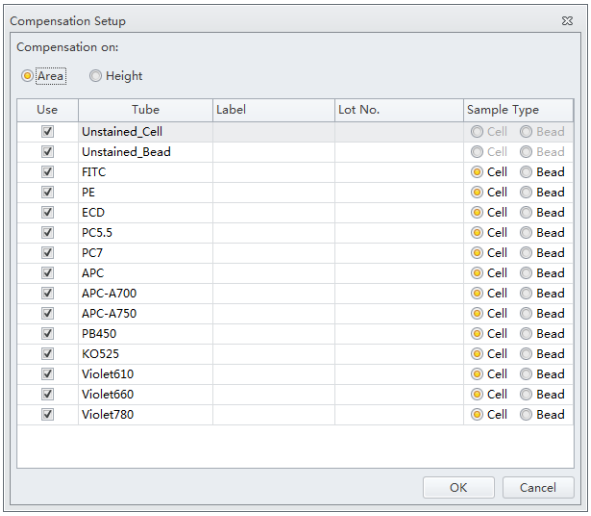
A compensation matrix can be modified manually, or imported from a prior compensation matrix file, or exported for use in other experiments.

NOTE The Compensation Library holds compensation matrices that were previously generated from specific gain settings. However, compensation calculations are Gain-independent. CytExpert generates a new, adjusted compensation matrix from the Compensation Library based on the new gain settings. This can be achieved by selecting **Import from the Compensation Library**.

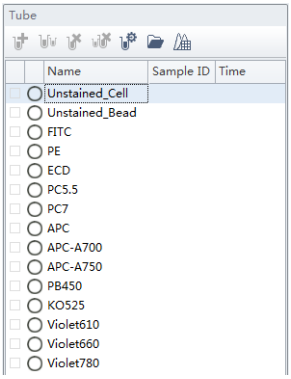
2. If a new, automated compensation is needed, generate as follows:
   - **a.** Prepare all necessary unstained and single color fluorospheres and cells.
   - **b.** Select **New Compensation** in the File menu.

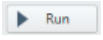


c. Select the corresponding tubes in the pop-up Compensation Setup window, then select **OK**.



CytExpert automatically creates a list of matching empty tubes in the Tube panel as well as all the plots needed to gate the positive and negative populations.



d. Load the corresponding unstained or single color sample fluorospheres or cells and select 

in the Acquisition panel.

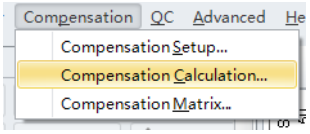
e. If necessary, adjust the scatter gate and the positive and negative population gates in the corresponding histogram.

f. Once the gates are in satisfactory locations, select

.

g. Repeat steps d-f for all tubes.

h. After collecting data for all necessary compensation samples, generate the compensation matrix by selecting **Compensation Calculation** in the **Compensation** menu.



Recording Data

1. Select Run to start acquisition and display data. This data is saved but can be overwritten by changing the instrument setting during the run, or by repeating the run.

   NOTE Data saved during the run has a blue label next to the tube. This blue label indicates that data was saved during the run, but not recorded.

2. Select Record to display and save data. Using Record prevents the user from modifying data at a later time.

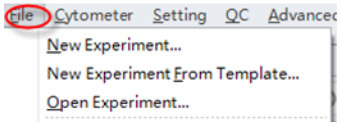
   NOTE Recorded data has a green label next to the tube. This green label indicates that data was recorded during the run.

3. The recording ends when one of the stop conditions is met. Alternatively, select Stop in the Acquisition panel at any time to stop data collection and unload the sample tube.

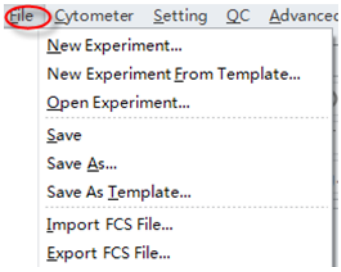
4. Add a new tube to the tube list of the current experiment by using the Tube tool or select Next Tube. New tubes assume all current tube settings unless otherwise modified.

Data Analysis

1. If necessary, turn on the Workstation.
2. Open the CytExpert software.
3. To open the saved experiment(.xit file), select **Open** from the start page or the File menu.



4. To import FCS data, select **Import FCS File** from the File menu.

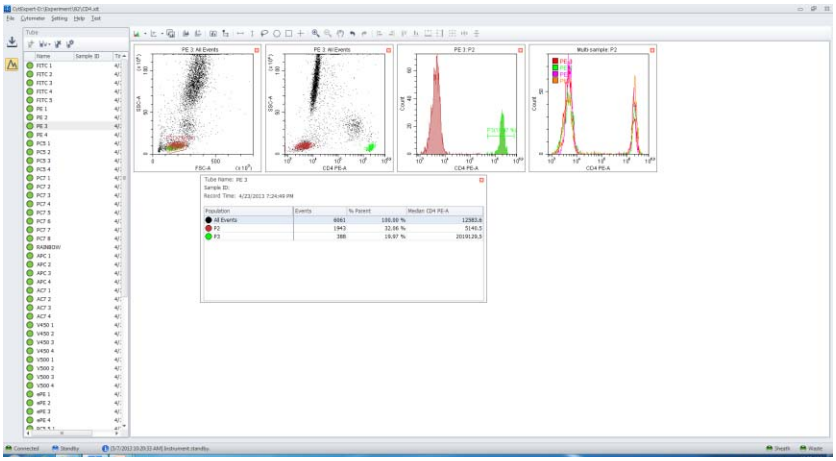


5. To analyze data:

   a. Select the Data Analysis icon for multiple sample analysis or select the Acquisition icon for single sample analysis.

   b. Select the data to be analyzed in the tube panel.

   c. Create plots, gates, Statistics Table, and Population Hierarchy as needed.



6. To export individual plots, right-click the individual plot and select **Export to File** or **Export to Clipboard**. To copy individual plots, select the desired plot, then right-click and select **Copy command or Export to Clipboard**.

   To export the Statistics table, right-click the Statistics table and select **Export to File** or **Export All Samples to File**.

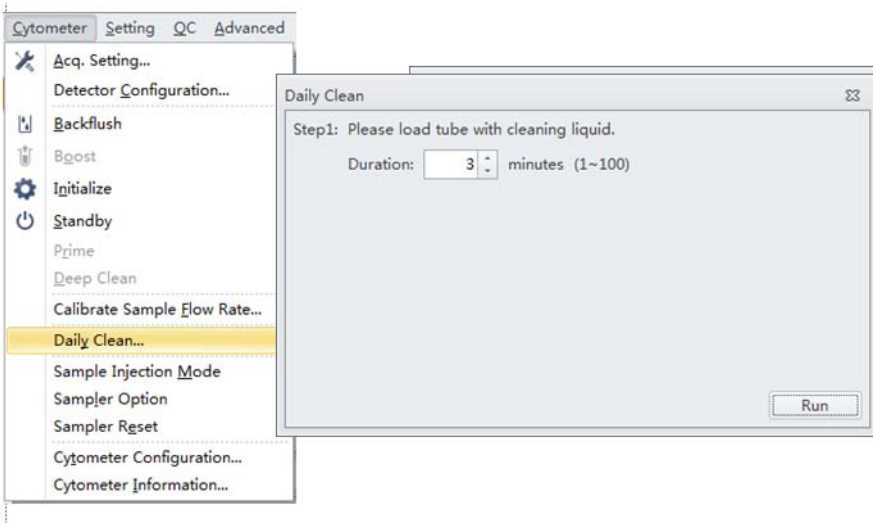
   NOTE **Export to File** exports the individual tube statistics as a single CSV file. **Export All Samples to File** exports all tube statistics as a file in CSV format.

7. Once analysis is completed, close the software, shut down the Workstation, and turn off the main power.

Shut Down

1. Open any experiment from the File menu, and initialize the instrument.

2. Select **Daily Clean** from the Cytometer menu.



3. Follow the Daily Clean procedures, which consist of:
   a. Run FlowClean cleaning fluid for 3 minutes.
   b. Run DI water for 5 minutes.
4. If necessary, empty the waste container.
5. Remove the sample tube and store according to your laboratory procedures.
6. Close the CytExpert software.
7. Shut down the Workstation.
8. Turn off the main power on the Cytometer.

Scheduled Maintenance

1. Clean the flow cell once a month.
   a. When the instrument is in standby, select **Deep Clean** from the Cytometer menu, then wait until the process is finished.

⚠ CAUTION

Risk of instrument damage. The cleaning solution should NOT be left in the flow cell for over 24 hours.

   b. Wait about half an hour, then select **Prime** to remove the remaining cleaning solution.
   c. Wait for the Prime cycle to finish, then run Daily Clean.
2. Replace the peristaltic pump tubing once every 6 months and reset the Maintenance Reminder.
3. Replace the sheath filter once every 6 months and reset the Maintenance Reminder. If necessary, refill the Deep Clean solution with a mixture of 1 part Contrad 70 and 1 part DI water and reset the Maintenance Reminder.

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