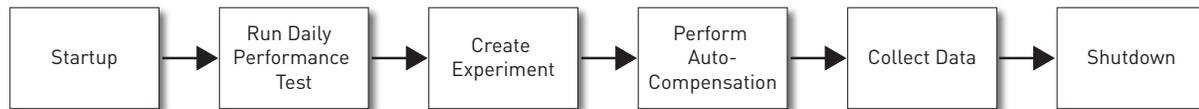


The functions of the Attune® Acoustic Focusing Cytometer are controlled by the Attune® Cytometric Software. Instructions for using the Attune® Acoustic Focusing Cytometer are described below.

For safety and biohazard guidelines, refer to the “Safety” appendix in the *Attune® Acoustic Focusing Cytometer User Guide* (PN 4453328). Wear appropriate protective eyewear, clothing, and gloves.

Workflow



For detailed instructions, refer to the *Attune® Acoustic Focusing Cytometer User Guide* (PN 4453328) supplied with the product or download the user guide from: www.appliedbiosystems.com.

Startup

Before You Begin

1. Check the levels in the fluid tanks located in the fluidics compartment.
2. Turn ON the Attune® Acoustic Focusing Cytometer.
3. Start up the computer and log in to Windows.
4. Launch the Attune® Cytometric Software and login.

Run Startup Function

1. Click **Startup** on the upper-right-corner of the *Login* screen.
2. Follow the instructions provided by the software.
3. Login to the Attune® Cytometric Software.



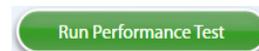
Performance Test

1. Click **Performance Test** on the *Main Menu*.
2. Verify that the lot number of the Attune® Performance Tracking Beads you are using is identical to the bead lot number used in the current baseline.

Current Baseline: 6/9/2010 - 756080
Lot No.: 756080
Expiration Date: 02/11/2013

IMPORTANT! If you are using a new lot of beads, download the appropriate lot information at www.appliedbiosystems.com and perform new baseline calculations.

3. Prepare the Attune® Performance Tracking Bead suspension.
4. Click **Run Performance Test** and follow the instructions provided by the software.

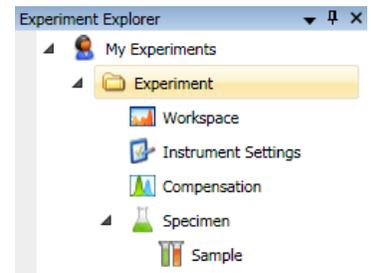


The result of the daily performance test is displayed at the end of the run. You can also review the current and previous reports by clicking **Reports** on the Main Menu.

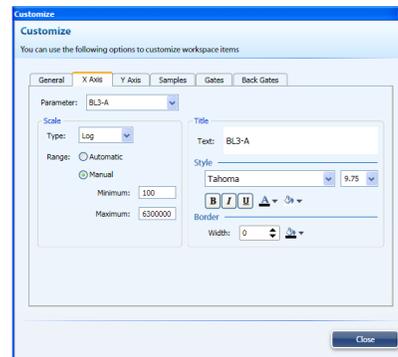
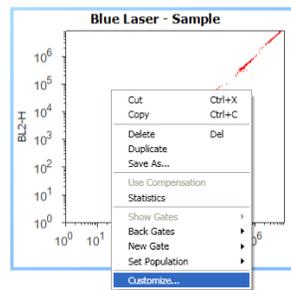


Create Experiment

- From the Main Menu screen, double-click **Blank Template** or a pre-populated **Template** (if available). Alternatively, create a new experiment (right-click on **My Experiments** and select **New Experiment**) or duplicate a saved Experiment (right-click on an existing experiment and select **Duplicate Experiment** using the *Experiment Explorer*).



- To add a *Specimen*, right-click the **Experiment** in Experiment Explorer and choose **Specimen**.
- To add a *Sample* to the Specimen, right-click the **Specimen** and choose **New Sample**.
- To insert a *Plot* to your *Work Area*, select the **Specimen** or the **Sample**, then choose the type of plot from the *Insert* tab.
- To customize the *Plot*, right-click on the plot and select **Customize** to open the *Customize dialog box*.



Auto-Compensation

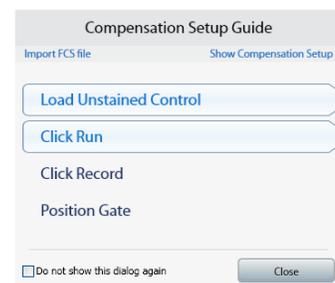
Before You Begin

Prepare *unstained* and *single-stained compensation controls*.

Note: If compensation is required, DO NOT record any samples until all voltages have been set.

Compensation Setup

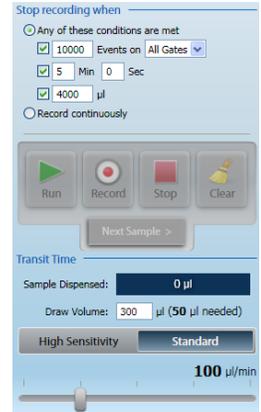
- Click **Compensation Setup** in the Home tab.
- Select the type (Area vs Height) of compensation you want to perform and the desired channels, then click **OK**.
- The *Compensation Workspace* will open to the **Unstained** control.
- Proceed to Optimize the controls.



Optimize the Controls

Optimize Instrument Settings for Unstained Control

1. Ensure the *Compensation Workspace* is set to the Unstained control and install the unstained control on the sample injection port..
2. Define the **Collection Criteria** using the *Collection Panel* and click **Run**.
3. Resize the R1 gate over the population of interest. This gate shape and location can be applied to all compensation controls by right-clicking and selecting **Apply gate shape to all controls**.
4. Adjust the **FSC and SSC voltages, Threshold, and Fluorescence Channels** using the *Instrument Configuration* tab to place the population on scale and to remove unwanted events and background.
5. Remove the unstained control from the sample injection port.



Optimize Instrument Settings for Single-Stained Controls

1. Continue with the single-stained controls using the Unstained control workspace to optimize each control.
2. Install the first single-stained control on the sample injection port.
3. On the *Collection Panel*, click **Next Sample**.
4. Using the same collection criteria and optimization procedures, adjust the instrument settings and gate for the population of interest



Record the Controls

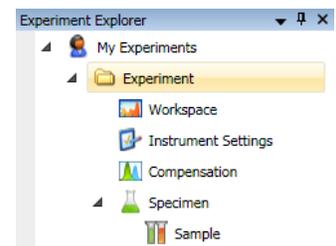
1. Set the collection criteria to the desired settings (stop criteria, acquisition volume, collection mode and rate).
2. Follow the instructions provided by the **Compensation Setup** guide. For each control, record using the desired collection criteria on the appropriate *Compensation Workspace*.
3. Repeat the process for each of the single-stained controls, ensuring the R1 (scatter) and R2 (histogram) gates are set on the appropriate populations.
4. Once all controls are recorded, proceed to running and collecting sample data.
 - If compensation is available, compensation is turned on by default. To turn compensation off for all plots on the workspace, click the **Globally Use Compensation** button.
 - When compensation is available, all fluorescent bivariate plots will have sliders bar enabled allowing for adjustment of compensation in real-time.



Collect Data

Create Experiment

1. Create an **Experiment** that contains the appropriate plots, gates, and statistics needed for data collection.
2. Double-click the desired **Sample** in *Experiment Explorer* to activate it.
3. Define the **Collection Criteria** in the *Collection Panel*.



Run Sample

1. Install the tube containing your sample on the sample injection port and lift up the tube loader to the active position.
 2. Click **Run**. Wait a short time for the sample to equilibrate.
 3. While still in the Run mode, adjust the **PMT voltages and Threshold** values for the appropriate channels using the Instrument Configuration tab. Make sure the events are on scale.
- Note:** If compensation controls have been recorded, only the threshold and voltages for FSC and SSC can be changed.



Gate Population of Interest

1. Select the desired **Plot** in the Space.
2. Select the appropriate **Gate** from *Gating Tools* in the *Insert* tab.
3. Set the **Gate** on the population of interest.



Record Data

1. Click **Record** to start data collection. Data is recorded until any of the conditions set in Collection Criteria is satisfied.
Note: You can click **Resume** and **Record** instead of **Run** to resume the data collection from that point on.
2. Click **Stop** to stop the data collection. The Attune® Cytometric Software automatically saves the data in a unique FCS file.



Sanitize the System Between Users

It is recommended that the following procedure is used to sanitize the system between users.

1. At standard 1000 $\mu\text{L}/\text{min}$, run 1 mL of Milli-Q® water.
2. Repeat with freshly prepared 10% Bleach (1 part of 5.25% sodium hypochlorite to 9 parts of water).
3. Repeat with Milli-Q® water.

Shutdown

IMPORTANT! Perform the following shutdown procedures at least once a day, even if the instrument is intended for continuous use.

1. Check the levels in the fluid tanks. Ensure that the wash solution tank is at least half-full.
2. Return to the *Main Menu* screen, log off and select **Shut Down** from the upper-right corner of the *Login* screen.
3. Select **Standard** or **Custom** wash cycle on **Shut Down** prompt screen.
4. Click **Next** and follow the instructions provided by the software.



5. The software automatically performs the shutdown operation and powers down the Attune® Acoustic Focusing Cytometer.

IMPORTANT! If you intend to leave the Attune® Acoustic Focusing Cytometer in the shutdown state for longer than two weeks, perform system flush and leave the instrument in deionized water to prevent salt crystals from clogging the fluidics system.

For research use only. Not intended for any animal or human therapeutic or diagnostic procedures.

NOTICE TO PURCHASER: REFER TO THE ATTUNE® ACOUSTIC FOCUSING CYTOMETER USER GUIDE (PN 4453328) FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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