Release Notes

Thermo Fisher Scientific
Attune™ NxT Software v3.1.1

In the following pages you will find instructions describing:

1. New software features
2. Known software/system issues with troubleshooting guidance
3. Software Bugs that have been fixed
4. Software installation instructions

Please note that it is critical for you (the customer) to review the installation instructions completely prior to installing or operating the Attune™ NxT Software.

Installation instructions are included at the bottom of this document for your reference.

New software features implemented in Version 3.1.1

Enhancements to experiment setup and annotation

- Workspace selection now persists when changing samples
- Filmstrip view now persists when changing samples
- Application view now persists when changing samples
- FCS filename is now applied to sample name when a FCS file is imported

Enhancements to Instrument controls:

- Improved instructions during system decontamination
- 1-button export of log files for field service assistance

Enhancements to data analysis and data processing:

- Adjust HyperLog™ transition value directly on plots using transition slider bars

Thermo Fisher Cloud Connected

- Register an Attune NxT Flow Cytometer with the Thermo Fisher Cloud to view performance test data on the cloud
- Upload and download experiments and experiment data directly to Thermo Fisher Cloud for easy of sharing and data storage

Software Bugs Fixed In Version 3.1.1

[EE-16471] – Upgrading to v3.1 from software where the new beadlot (2029773) has been imported fails to upgrade.
[EE-6403] - On failed Performance Test, the laser delays are not reverted to the last known "good value"
[EE-10383] - Wait before record does not consistently send clear command to instrument

[EE-10799] - Get error 1096 when trying to create a tube experiment after launching software without DESkey device, plugging DESkey in, and then selecting to retry launching SW

[EE-10809] - Location value is not validated correctly when importing a sample list CSV
[EE-10863] - Copy and pasting overlay plot with legend, crops the legend and does not use correct decimal places
[EE-11060] - Sample naming inconsistency on sample list import - samples in different groups being compared for uniqueness
[EE-11074] - Remaining time estimator is using well run protocol when estimating tube compensation
[EE-11247] - Duplicate names are not checked correctly when editing gate names using the edit gates dialog
[EE-11252] - Changing range in a bivariate plot change the count in another bivariate plot by 1 or 2 units
[EE-11337] - Database utility fails if the automatic backup is set to a network path
[EE-11470] - Attune won't save the global settings when Attune logout with a new created user account
[EE-11662] - SW crash when undoing multiple changes to the hyperlog transform value
[EE-11830] - Boost mode checkbox is displayed when it shouldn’t be
[EE-11831] - Stain names are not displayed on compensation controls when an experiment is duplicated, created from a template, or imported and upon initial login
[EE-12596] - Update $PnS for a parameter when updating keywords if target of label name has changed
[EE-13898] - When changing samples using the heat map the current view of the heat map is not persisted switches to work space view
[EE-14023] - Clear button no longer clears data during acquisition or on completion of run mode
[EE-14092] - Sample list view is not updated on importing FCS file
[EE-14584] - Automatic Update of Experiment Level Run Protocol has severe lag on 96 and 384 well plates
[EE-15019] - Attune Software not acknowledging scheduled backups
[EE-15020] - Database Utility not backing up to mapped public directories
[EE-15029] - FCS keywords can be missing area, height, width keyword info
[EE-15135] - Attune not clearing on volume Wait To Record with volume Stop Criteria
[EE-15792] – Restoring previously deleted derived gates, did not correctly recalculate the gating statistics

The following sections describe known issues with the Attune™ NxT v3.1 software. Where possible we have outlined steps to work around the known issue.

Guidance for Installing the Attune™ NxT version v3.1 software:

- See the "Installation Troubleshooting Guidance" at the end of this document.

Guidance for Instrument Start Up and Performance Test using the Attune™ NxT v3.1 Software:

- **Power on the auto sampler before the Attune™ NxT cytometer**. If you do not turn the auto sampler on prior to starting the instrument the auto sampler is not recognized.

- If you encounter problems with Startup, close the software, power off the instrument and repeat power on procedure in the correct order.
- **Stopping startup** will not stop the auto sampler initialization. Wait for the auto sampler initialization to complete before running the startup function again.
  Run a SIP Sanitize following Performance Test

Issue ID	Description of Known Issue	Suggested Action
EE-4545	Running startup after running a shutdown doesn't always reset the startup icon in the collection panel to run/record	Repeat Startup by pressing "Startup" from Instrument tab of Ribbon bar or from collection panel.
EE-9831	Occasionally baseline will fail due to "not enough beads found"	Increase bead concentration for baseline test only. Use 10 drops PT beads/4mLs PBS or Attune Focusing Fluid.
EE-15416	Syringe Pump Error – Step Loss Plunger error observed when starting a plate experiment immediately after Performance Test.	After Performance Test perform a SIP sanitize. If error is observed, follow instructions in dialog.

Guidance for Setting up an experiment using the Attune™ NxT v3.1 Software:

Issue ID	Description of Known Issue	Suggested Action
EE-8862	When the plot type is changed using the right-click customize menu, the newly created plot will have the default scale of the previous plot.	If you change plot types using the customize menu, ensure that x-and y-axis scale ranges are set appropriately.
EE-8863	A plate experiment isn't automatically active after it is created. The plate experiment is visible in the experiment explorer but is not the experiment viewed in the Attune™ desktop.	Double click on the new experiment to activate it, create samples or groups of samples on heat map tab, expand the group and double click to activate a sample to create a workspace, define the run and acquisition criteria, and create a workspace, run protocol, and acquire samples.
EE-8864	When creating experiments, the software doesn't check to see if it there is enough disk space to create the necessary files.	Export experiment data from the experiment explorer and then remove experiments to free up disk space

	This results in missing files and XML load failures. If there isn't enough room to create the EE nodes, Error -1 is returned, and attempting to open any experiment that doesn't contain XML files, returns an undefined error	

Guidance for using the Experiment Explorer and Instrument Settings panel within the Attune™ NxT v3.1 Software:

Issue ID	Description of Known Issue	Suggested Action
EE-8865 EE-8866 EE-11292	Adding more than 400 samples to an experiment can cause software instability. This can be done using the experiment ribbon tab's new sample button which does not correctly check the sample count within the experiment.	If an experiment requires more than 400 samples, duplicate the experiment for additional samples beyond 400.

Guidance for using Compensation within the Attune™ NxT v3.1 Software:

- The gate names on histogram plots within compensation samples are shown in large text and cannot be customized
- When using "on-plot" adjustment tools for large (>1 million) event files, response rate for adjustments will be slow.

Issue ID	Description of Known Issue	Suggested Action
EE-8867	Parameters that are deselected by the user will become re-enabled if the user switches between area and height measurements AFTER the parameters were deselected.	Ensure that the desired area or height parameter is selected prior to selecting parameters for compensation controls. If area or height measurement is changed, ensure that the correct parameters are selected prior to sample acquisition.
EE-11352	A compensation control cannot be imported as a compensation control if the scatter (FSC or SSC) voltages differ from other controls.	All compensation controls must have matching instrument settings in order to be imported into an experiment as compensation controls.

Guidance for Acquiring Samples using the Attune™ NxT v3.1 Software:

- At high event rates (i.e. 20k/sec) on very large data collections, you may observe a slowing of the data update on the screen (~5 sec between updates). This does not have any impact on the completion of the acquisition. To improve response rate, **turn off auto-scaling** or **auto refresh functions** (located within the Home ribbon tab).
- If any plot axes are set to auto-scale during acquisition, excessive CPU usage may occur resulting in a system wide performance lag. It is recommended **that all plots are set to manual scale during acquisition** for optimal system performance.
- For large data files, especially on workspaces that have statistics boxes, software response may be slow if the workspace is modified during acquisition. When this occurs, a blue indicator spinning wheel will be visible. This does not have any impact on the

completion of acquisition. It is recommended that if you are acquiring large files, wait to make adjustments on the workspace until after the file has completed.

- It is suggested to limit the number of mixes to 2 or less to prevent bubbles being introduced into the sample.
- Keep the tube lifter in the DOWN position when using the auto sampler.
- By default the option to **exclude coincident events is turned OFF**. To exclude coincident events, a user must select the "Exclude coincident events" option in the Threshold section of the Instrument Settings panel.
- By default the first two decades are displayed on dual parameter and histogram plots set to *log* scale. Ensure that voltage settings are optimized so that all populations are set above the noise (greater than 10^2).

Issue ID	Description of Known Issue	Suggested Action
EE-15379	When collecting additional data and appending a FCS file, the "complete stop condition" option does not work if the stop condition is based on time or volume	Select "total events" or "gated events" when completing the stop option during append.
EE-2967	If a fluid bottle is disconnected when a sample has been preloaded, sample recovery will return the sample to the wrong well.	If a fluid bottle error occurs while processing a plate, do not recover the sample back to the sample plate. Load a clean plate to recover the sample and transfer the sample back to the correct well in the original sample plate.
EE-5572	Pressing stop during acquisition on the last well of a plate run automatically runs a rinse so there is no chance to recover sample.	Do not press stop on the last well of a plate run as it will result in loss of any unprocessed sample.
EE-9195	When acquiring samples, the software pre allocates enough memory to complete the acquisition based on the run protocol settings and the display events value. If the software cannot allocate enough memory a warning is displayed to the user stating that they will only be able to collect a certain number of events.	If this warning is displayed, select the "Do not show me this message again" option on the dialog. The message will not be displayed for the life of the current user session. This message will be displayed again for a new user or when the current user logs in again and this condition arises.
EE-11067	The remaining time will not be calculated and displayed when transition from run to record.	The remaining time will only be displayed while processing a plate or while processing a tube sample using the one click record.
EE-11045	Tabbing through the area scaling factor edit controls in the advanced system settings panel is registered as a change to the instrument settings and will result in a sample showing a numbered IS badge if the sample has sample level instrument settings.	If the area scaling factors do not need to be changed, do no click on or tab through the ASF controls in the advanced system settings panel.
EE-10941	If a target or label name is modified post recording and then manually edited back to the original value, the change will not persist.	Use the notification button to revert the target and label names to their recorded values.
EE-8284	If the stop on event occurs at or near the same time as the volume is exhausted the stop on event condition will not be used and there will	If a precise number of events is required for the stop condition, ensure that the volume is sufficient to acquire all events.

	be more events than the stop condition.	

Guidance for using the Experiment Workspace (Gates, Plots, Stats) within the Attune™ NxT v3.1 Software:

- With large event files, there can be a slow response in between commands. Faster response rates can be achieved with fewer parameters selected and using a decreased number of plots and gates on workspace. Disabling the auto-refresh on home tab will also improve system performance.
- Quadrant gate names can't be moved.
- To export statistics directly from a statistics box, double click the statistics to enable the "Export stats" option in the right click context menu.
- Printer preferences may not be correctly displayed for certain printers.
- The width parameter scale range will default to 1,048,576. Set the maximum scale range to 1024 for ease of viewing.

Issue ID	Description of Known Issue	Suggested Action
EE-8868	The time it takes to open the plot "Preview" panel is dependent upon the number of parameters enabled	Deselect unneeded parameters prior to opening "Preview" panel.
EE-5793	When orientation is changed and then grid size of workspace is changed, the next workspace of differing grid size viewed may cause plots to occupy more grid spaces than originally set.	This can be fixed by forcing the workspace objects to snap back to the default grid locations by toggling between freeform and auto layout.
EE-10849	Default plot image format is blank for new users in export tab of options dialog.	Go to the export options and update the default export option or select the image format when saving plots.
EE-9849	Gate fonts may show up bigger than expected in subsequent plate experiments.	To fix the font sizes force the screen to refresh by changing the view.

Guidance for working with Overlays within the Attune™ NxT v3.1 Software:

- Gallery plots cannot be printed from the software. In order to print gallery plots, copy and paste them to an external application that supports printing.
- When printing overlay plots, the size of the printed plot can be adjusted using the zoom setting. Ensure the Overlay view's zoom setting is less than 400% otherwise the plots may be too big to print on a page.
- No warning is given if attempting to overlay FCS files that were acquired using different instrument settings. Ensure that samples were acquired using the same settings prior to using this feature.
- Do not create overlays from plots with axes set to auto scale as the data may not render correctly.

Issue ID	Description of Known Issue	Suggested Action
EE-4539	If an overlay plot is created from a sample that does not contain data, the X axis label will not be blank.	When data is recorded, the overlay axis label will correctly reflect the selected parameter.

Guidance for Data Analysis / Data Display using the Attune™ NxT v3.1 Software:

- Analysis of 3rd party FCS files in the Attune NxT software is not supported. Background calculation of statistics may not complete if 3rd party FCS files are included in an experiment.
- Data in the Results Table can be copied but the header row is NOT copied and must be entered into a secondary data analysis program such as Microsoft® Office Excel. (Note- header row will be copied if you use the send table to function on the statistics ribbon)

Guidance for Exporting/Importing using the Attune™ NxT v3.1 Software

- When exporting FCS files from experiments that have experiment level compensation, you may be asked to update the compensation values in the exported file even though the compensation values have not changed. Select ignore or update.
- When exporting FCS files and updating keywords is required, ensure the experiment that the samples belong to is active.

Issue ID	Description of Known Issue	Suggested Action
EE-11060	When importing from the sample list, sample name uniqueness is not being checked between groups resulting in samples being appended with a number even though they are unique within their imported group.	Either ensure all names are unique within the sample list or rename the sample after importing the sample list to remove the appended number.

Guidance for Shut down or Maintenance features or using the Attune™ NxT v3.1 Software:

- The self-test function can become frozen if the USB is disconnected from the auto sampler or auto sampler is turned off during self-test. Do not turn off the auto sampler or remove any cables during operation of the instrument or while running maintenance functions (i.e. deep clean, shutdown…)
- While running the decontamination function a "Check Fluid Bottle" warning dialog will be displayed at each step the bottles are removed. **DO NOT** press "cancel" from these dialog messages as this will cancel the entire script.

Guidance for Setting up user accounts and user options using the Attune™ NxT v3.1 Software:

- After initial set up of a user account, the "Forgot password" option is available if security questions have been created. See the User Guide for instructions on how to setup password reset questions.

Guidance for Database Backup using the Attune NxT Database Utility Program:

- If the automated backup is currently set to "OFF", and it is then turned "ON", the Status Page will continue to state that the backup is "OFF" until the Database Utility is closed and re-opened.

Guidance for Automation Mode using the Attune™ NxT v3.1 Software:

- Do not reuse barcodes when running in automation mode.

Issue ID	Description of Known Issue	Suggested Action
EE-8654	If a clog occurs while in automation mode, two dialogs may be displayed.	Close both dialogs and manually select "unclog" from the instrument tab of the ribbon bar. Follow onscreen instructions to unclog the system.
EE-8667	If a bubble error occurs while in automation mode, the plate will pause allowing the SIP sanitize function to be run. Once the SIP sanitize procedure has completed, the run can be resumed in the automation software. If the plate run does not resume, use the driver window to resume.	If the plate run does not resume using the "resume option" in the automation software, resume the plate run using the driver window.
EE-7612	If a user closes the software during an automated run, then restarts the software and re-enables automation mode, restarting the automation run will cause the automation software to hang.	Do not select the restart plate operation in the automation software. Abort the run in the automation software and setup a new run in the dashboard to continue.

Guidance for Connecting to Thermo Fisher Cloud using the Attune™ NxT v3.1 Software:

- If a timeout occurs while registering the Attune NxT software to connect to the Thermo Fisher Cloud or logging a user into the Thermo Fisher Cloud from the Attune NxT software, ensure that the AttuneCloudService is running.
- Slow internet speeds may result in time outs when connecting to the Thermo Fisher Cloud. If the software is registered to connect to the Thermo Fisher Cloud, the cloud connection error indicator may be displayed in the status bar.
- NOTE: The AttuneCloudService uses python to manage the connection between the Attune NxT software and Thermo Fisher Cloud.

Installation Instructions: PLEASE READ ALL INSTRUCTIONS BEFORE PROCEEDING

System Requirements: Quad core processor, 16 GB RAM, 500 GB disk space available. Windows 7 64 bit Professional with Service Pack 1 set to **US English**.

- The instrument must be **powered on and connected** to the computer for the firmware updater to run at the end of the installer.
- **DO NOT** update the firmware if the instrument is in a sleep state. The indicator lights on the front of the instrument will fade in/fade out in multiple colors during the sleep state. **Power cycle** (turn on and off) the instrument prior to running the firmware updater.
- **DO NOT** launch the software application until all installation steps have been completed.
- **DO NOT** run any other applications while completing these steps.
- No change to existing login credentials will occur during the software upgrade.

INSTALL AND UPGRADE (v2.1 – v2.6.1 to Attune™ NxT Software v3.1.1)

Overview:

Software Installation

- Ensure ***All Data*** is backed up to an external storage device before performing the upgrade process.

Step 1 Download Attune NxT v3.1.1 software from https://www.thermofisher.com/global/en/home/global/forms/attune-nxt-software-download-registration.html. Save the folder to the Attune NxT instrument computer.

Step 2 Restart or power on the computer and cytometer.

Step 3 Ensure that the DESkey USB key that is used to run the software is plugged into the computer.

Step 4 Log into Windows as:

User: INSTR-ADMIN and
Password: INSTR-ADMIN

NOTE: This is the default administrator account. (Note: If your instrument is networked, please make sure that the administrator privileges have not been removed by your local IT department.)

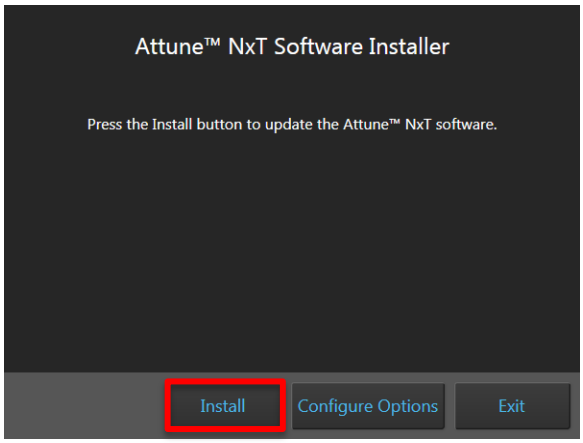
Step 5 Unzip (select "extract all files") the AttuneNxT_3.1.zip file to the desktop.

Step 6 **Complete these steps for NEW INSTALLATIONS ONLY**

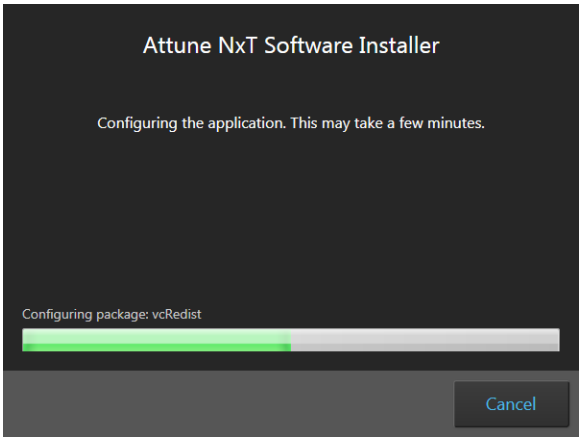
1. Open the USB3_FOR NEW INSTALL, INSTALL ME FIRST folder
2. Double-click the “RENESAS-USB3-Host-Driver-30230-setup.exe” file.
3. Follow the instructions and accept the terms of agreement.
4. Once complete, click “Finish”.

Step 7 Install the software:

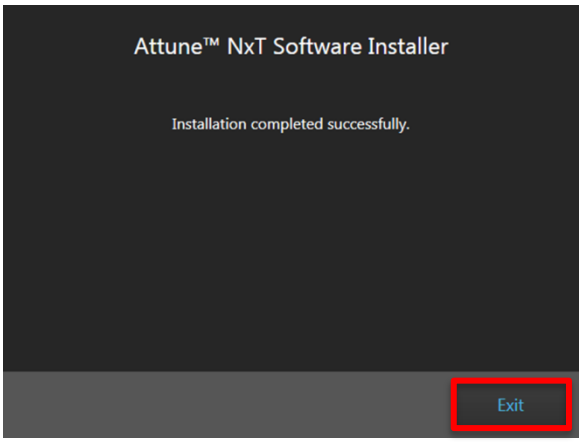
a. Double-click “SetupAttuneNxT.exe” in the “Attune NxT 3.1” folder.
b. Select “Install”



A new window will open as shown below indicating the progress of the software installation. If “Cancel” is pressed during this stage, return to step 4 and restart the process beginning with re-running the “SetupAttuneNxT.exe”



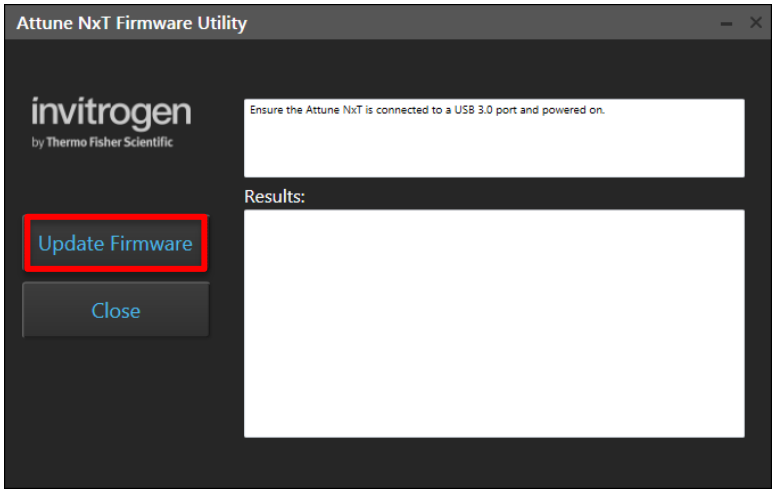
Step 8 When the installer has completed, the “Installation completed successfully” message is displayed. Close the installer by clicking the “Exit” button (shown below).



Firmware Installation

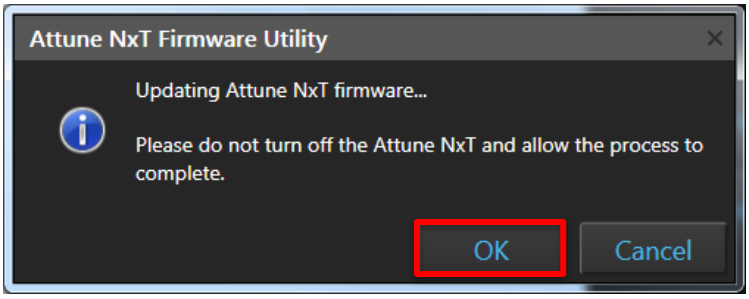
- **DO NOT** update the firmware if the instrument is in a sleep state. The indicator lights on the front of the instrument will fade in/fade out in multiple colors during the sleep state. **Power cycle** (turn on and off) the instrument prior to running the firmware updater.
- The firmware update process should take less than 15 minutes.

Step 1 After software installation has completed, firmware must be updated. **The firmware updater utility will automatically launch if the instrument is powered on and connected**.

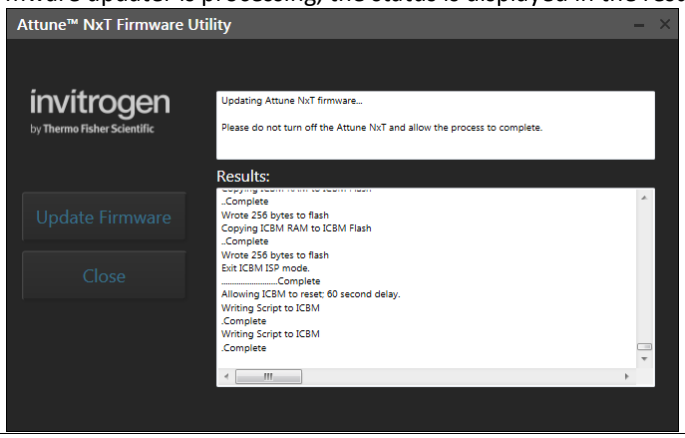


Step 2 From the "Attune NxT Firmware Utility" dialog, select the '**Update Firmware'** button to update the firmware.

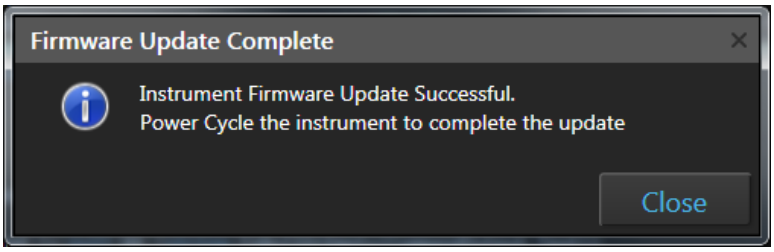
Step 3 Click "OK" to confirm the request to update instrument firmware (shown below).



NOTE: While the firmware updater is processing, the status is displayed in the results window:



Step 4 Once the firmware has completed, the "Firmware Update Complete" dialog is displayed indicating the firmware update was successful:



Step 5 **Power Cycle** the instrument (turn off, then on) to complete the firmware update.
Step 6 Click the 'Close' button to exit the firmware update utility.
Step 7 The software application and instrument are now ready to use.
Step 8 Launch the software using login credentials used in earlier software versions.

If the firmware updater is not completed as part of the installation, you will be prompted to update the firmware the first time the application is launched when the instrument is powered up and connected.

UPGRADE FROM v2.0.1 or earlier - Attune™ NxT Software v3.1.1 Installation

For customers that are currently using Attune™ NxT Software v2.0.1 or earlier, please contact technical support or your local field service engineer.