**Release Notes**
Thermo Fisher Scientific
Attune™ NxT Software v2.4

In the following pages you will find instructions describing:

New software features
Known software/system issues with troubleshooting guidance
Software Bugs that have been fixed
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 Version 2.4

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

# New software features implemented in Version 2.4

- Area Scaling Factors (ASF) are now applied from Performance Test (Advanced/System Settings)
- Import and Export of Workspaces
- Import and Export of Compensation Settings
- Support for deep well plates up to 2 mLs
- User options to set Plot label display name ($PnN vs. $PnS)
- Implemented load instrument settings and workspace on New Experiment dialog
- Support for Blue, Red, Yellow (BRY) instruments
- Sample recovery to return unused sample to a tube or preloaded sample in a plate
- Drag and Drop of Experiment Explorer Attributes (Instrument Settings, Workspace, Compensation)
- Filter Configuration for system wide annotation of fluorophores
- Implemented context menus on the heat map
- Completion of optional FCS file keywords (ie $EXP, $INST, $OP, $CELLS, etc.)

**The following sections describe known issues with the Attune™ NxT v2.4 software. Where possible we have outlined steps to work-around the known issue.**

**Guidance for Installing the Attune™ NxT version 2.4 software:**
- See the "Installation Troubleshooting Guidance" at the end of this document.

**Guidance for Instrument Start Up and Performance Test using the Attune™ NxT v2.4 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.
- **Stopping startup** will not stop the auto sampler initialization. Wait for the auto sampler initialization to complete before running the startup function again.

| Issue ID | Description of Known Issue | Suggested Action |
|---|---|---|
| EE-2274 | Without functional FSC and SSC, Baseline and performance test will sometimes fail to create proper gates on BL1 and will fail the performance test. | Verify that FSC and SSC are working correctly. Follow recommended on screen troubleshooting steps. |
| EE-2336 | During start up, selecting to cancel dialog that says Check fluid bottles causes series of issues that requires power cycling the Attune™. | Do not cancel the check fluid dialogs dialog during startup. Correct the fluidics issue and proceed by clicking OK. |

**Guidance for Setting up an experiment using the Attune™ NxT v2.4 Software:**

| Issue ID | Description of Known Issue | Suggested Action |
|---|---|---|
| EE-445 | When adjusting the size of the polygon gate on a plot using the bounding rectangle box, the gate vertices may not maintain their relative positions. | Polygon gates can be made smaller or larger by moving the individual points on the gate itself or by adjusting the bounding box and then adjusting the individual point that was not maintained. |
| EE-2185 | If the experiment name, group, sample and parent gate are all displayed in a plot header, the hyperlink to set the parent population and gating hierarchy may not be visible on the plot. | If the names have too many characters, the plot title may not show the hyperlink. Decrease the length of the names to view the hyperlink or set the parent population by right-click within the plot to bring up a context menu where parent population may be selected. On customize menu, deselect experiment and or sample name to further shorten the plot title label. |
| 53424 | 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. |
| 56476 | 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. |
| 56550 | When creating experiments, the software | Export experiment data from the experiment |

| | doesn't check to see if it there is enough disk space to create the necessary files. 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 | explorer and then remove experiments to free up disk space |
|---|---|---|
| EE-3496 | If samples are named using any of the following words, the FCS file will not be recorded: CON, PRN, AUX, CLOCK$, NUL, COM1, COM2, COM3, COM4, COM5, COM6, COM7, COM8, COM9,LPT1, LPT2, LPT3, LPT4, LPT5, LPT6, LPT7, LPT8, LPT9 | Do not name any samples using these words. The use of these words is case sensitive and they are reserved by the operating system. |

**Guidance for using the Experiment Explorer and Instrument Settings panel within the Attune™ NxT v2.4 Software:**

- Target and Label Fields (ie CD4-FITC) are applied to plot axes but do not show up in statistics tables
- The default width threshold has been updated to 1.0.  This will only apply to new software installation and newly created user accounts. It is recommended that existing users change their default width threshold to 1.0.

| Issue ID | Description of Known Issue | Suggested Action |
|---|---|---|
| 1206 | Adding more than 400 samples to an experiment can cause software instability. | If an experiment requires more than 400 samples, duplicate the experiment for additional samples beyond 400. |
| EE-2343 | If a filter is applied to the experiment explorer that returns no results and a new experiment is created, the software will hang. | Remove any filters that are applied to the experiment explorer before creating a new experiment. Creating a new experiment when the experiment explorer is completely empty (ie User folder is also missing) will cause the software to hang requiring that the application is restarted. |
| EE-2329 | If a filter is applied to the experiment explorer before any experiments are created, clearing the filter will not fully restore the experiment explorer. | Do not apply a keyword or date filter to the experiment explorer if the explorer is already empty of any experiments.  If this is done, log out and log in to reset the experiment explorer. |
| EE-2753 | Groups containing wells can be duplicated using the Experiment Explorer's duplicate group command. | Do not duplicate groups containing wells as they will be mismapped on the plate which can cause problems during plate acquisition. |
| EE-2675 | All parameters are available from the threshold dropdown list even if that parameter is disabled so threshold can be set off deselected parameter. | Only select parameters that are enabled as only enabled parameters will be considered in thresholding logic. |

**Guidance for using Compensation within the Attune™ NxT v2.4 Software:**

| ⚠ | • | If you make an error in the compensation set up wizard and need to make changes, you must remove the compensation and then re-start the compensation set up process. |
| | • | 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 |
|---|---|---|
| 54564 | 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. |
| 54815 | The HyperLog™ scale is not available for compensation control samples using the right click menu. | To set the axis of compensation controls using a HyperLog™ axis scale, adjust the axis scale using the customize panel. |
| 57907 | Undoing the movement of a gate on a compensation control will not update the compensation matrix | Do not use the undo function when making adjustments to the gates or markers on the compensation workspace. Adjust the gates back to the previous position if necessary. |

**Guidance for Acquiring Samples using the Attune™ NxT v2.4 Software:**

| ⚠ | • | At very high event rates 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**. |
| | • | 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. |
| | • | Mixing in the auto sampler is limited to 2 or less mixes |
| | • | Keep the tube lifter in the DOWN position when using the auto sampler. |
| | • | 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. |
| | • | If the sample syringe pump stalls during a rinse or other fluid function, the software will not display an error message. If the rinse function appears to stall or no events are displayed during acquisition, check that the sample syringe pump (located on the left side of the instrument) is moving. If the pump is not moving, run the unclog function to re-initialize the pump. If the error state continues, power cycle the instrument. |

| Issue ID | Description of Known Issue | Suggested Action |
|---|---|---|
| EE-688 | The complete stop condition option for append does not work if stop condition is | Only use total events or gated events if you require to add data to existing data files |

| Issue ID | Description of Known Issue | Suggested Action |
|---|---|---|
| | based on time or volume | |
| EE-2714 | Overwriting existing sample causes the collection panel to stop updating when the workspace contains lots of plots | Be patient when overwriting existing samples when the workspace contains lots of plots. |
| 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-2536 | When a rinse or other fluid function is performed that removed the remaining sample from the sample line, the sample remaining progress bar is not cleared. | The sample remaining progress bar in the collection panel does not clear itself if a fluid function is run that purges the sample from the system. |
| EE-2934 | Canceling an acquisition by clicking the cancel button on the dialog that is shown if the tube lifter is dropped during a multidraw acquisition, will stop the acquisition but leave the software in a state where it things acquisition is still in progress. | If an acquisition is canceled using the dialog presented when the tube lifter has been dropped during a multidraw, the software will need to be restarted. |

**Guidance for using the Experiment Workspace (Gates, Plots, Stats) within the Attune™ NxT v2.4 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.
- In order to move gate names, make sure that gate names are small enough to display entire name on plot; Quadrant gate names can't be moved.

| Issue ID | Description of Known Issue | Suggested Action |
|---|---|---|
| 55134 | 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. |
| 57459 | The buttons on the workspace toolbar are not active when a new plate node or experiment is activated | Define a group/sample and activate a well sample for the workspace tool bar to become active |
| EE-677 | If a quadrant color is changed and the quad gate name is changed, the color of the gate label changes to black. | Restart application to get quad gate labels to show with the color selected for that gate. |

**Guidance for Data Analysis / Data Display using the Attune™ NxT v2.4 Software:**

⚠
- Analysis of 3rd party files in the Attune NxT software is not currently supported. Background calculation of statistics may not complete if 3rd party FCS files are included in an experiment.
- On the "Results" view, right clicking on a column header will display a list of statistics, some of which are not currently available (i.e. cell cycle statistics).
- 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 v2.4 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.

**Guidance for Shut down or Maintenance features or using the Attune™ NxT v2.4 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 (ie deep clean, shutdown…)

**Guidance for Setting up user accounts and user options using the Attune™ NxT v2.4 Software:**

⚠
- Email notification is not available in this version of software. Email address can be added but no notification is sent.
- After initial set up of a user account, the "Forgot password" option is available if security questions have been created. To set up security questions, log into new users account by entering user name and password. From the options window, select user management, select edit user, and select security questions. The user will be prompted to input three security questions and answers (note- they are case sensitive) If the user forgets their password, they can then click forgot password and it will ask them to answer two of the three questions and after answering correctly the software will prompt them to reset their password.

| Issue ID | Description of Known Issue | Suggested Action |
|----------|---------------------------|------------------|
| EE-21118 | Creating a username without letters or numbers (e.g., of "**...**" ), will cause the software to crash | Don't create a username without characters or numbers |

## Software Bugs Fixed In This Release

* [EE-68] - Invalid gate equation seen when a gate name includes any number and any of the following symbols: "+-/*|!&"
* [EE-69] - Target and label edits notification icon is lost if user changes sample and reactivates sample
* [EE-72] – Prevent Attune NxT Database Utility from running if Attune NxT is running
* [EE-74] - $BTIM (beginning time) and $ETIM (ending time) are written backwards in the FCS file
* [EE-80] - When batch exporting FCS file if any group other than a single sample is chosen and Update header is selected the header for all Files will use $FIL from the last sample in the grouping
* [EE-93] - Bug: Sometimes $ETIM not recorded or is 00:00:00
* [EE-101] - Importing FCS file with embedded matrix into new experiment does not allow user to apply matrix to experiment
* [EE-299] - When importing/exporting compensation XML, the instrument settings should also be included so that when importing compensation, the instrument settings are updated to match the comp settings for which they were created
* [EE-534] - When choosing to update FCS keywords on export, the data keywords (ie BEGINDATA) are duplicated
* [EE-75] - Application always initializes as a 4 laser system, so a user's settings for laser configuration do not persist across sessions
* [EE-303] - admin options - service and sysadmin needs cannot reset offline instrument configuration
* [EE-321] - Update Statistics on status bar freezes sometimes
* [EE-352] - On Heatmap the default display setting is gradient and not threshold
* [EE-353] - In new experiment from template dialog notes field is one lone line of text up to 500 characters. It should be 5 rows of 100 characters
* [EE-355] - Instrument rinse function stalls after baseline
* [EE-357] - Startup with software is non-functional after shutdown
* [EE-358] - Pressing 'Enter' while in the Compensation Setup View causes the child window to close.
* [EE-363] - Ensure system log can display all available logs (currently limited to 10000 entries)
* [EE-364] - Polygon Gate Coordinates shown in status bar are incorrect
* [EE-369] - Sample name with decimal in name (ie 12.5) is truncated (ie 12) in the $FIL recorded in the FCS file.
* [EE-386] - When multi-selecting experiments to delete in the experiment explorer, plate experiments are also selected for deletion
* [EE-402] - Implement Import Beadlot functionality in the Baseline Setup View
* [EE-417] – Fixed issue with duplicated naming adding a 1
* [EE-443] – Fixed issue with area scaling factor precision (increased to 2 decimal places)
* [EE-466] - When auto plot range is turned on CPU usage maxes out to 99%
* [EE-467] - Time and Event parameters are not serialized when Appending Data
* [EE-482] - Statistics on gates not available in zoomed in view
* [EE-550] - [Instrument Settings] Populate filter configuration combobox in instrument settings parameters group with available filter configurations for logged on user
* [EE-595] - Nested derived gates cannot be used without typing out the full equation of the corresponding derived gate
* [EE-619] - Workspace, instrument settings, run protocols were not saved prior to exporting or duplicating experiments
* [EE-638] - Crash running performance test at 12% after viewing the workspace

* [EE-639] – Instrument Settings panel grayed out after opening main menu and creating a new experiment
* [EE-655] – Updated support URL to thermofisher.com
* [EE-758] – Fixed issue causing a data desync when running an instrument at high events rates when connected to a USB 2.0 port
* [EE-759] – Fixed issue when running at high event rates which resulted lost data
* [EE-763] – Fixed issue where control points on a quad gate were not always displayed
* [EE-790] – Fixed issue where stopping a plate from the instrument ribbon and then running a rinse caused rinse  to stall at 95%
* [EE-943] - Value of 0.1 ASF is not persisted in the system settings and is not transferred to advanced settings
* [EE-958] – Fixed issue when viewing/editing a sysadmin service account in the user management (via edit user) where both accounts showed that all permissions are available
* [EE-961] - "Automatically update Experiment level IS" checkbox on IS tab - checked state is not necessarily reflective of functionality being applied
* [EE-971] - Use Comp from file and or experiment Button is flashing so fast it looks unchecked
* [EE-987] - A gate on each control needs to be altered to get compensation updated and the view matrix button to become available
* [EE-992] - If an experiment hasn't been expanded during an instance of the software, export FCS files at the experiment level is disabled in the right-click context menu
* [EE-1017] – Fixed issue where entering label names in the instrument character caused an invalid character warning
* [EE-1060] – Fixed issue where the Heatmap would display NA for certain samples after acquisition requiring a gate or plot to be modified to force a refresh of the heatmap statistics
* [EE-1075] - Fixed the button to check configuration to display the current instrument configuration
* [EE-1178] – Fixed issue with heatmap not updating if the first sample activated didn't contain an FCS file
* [EE-1182] - If instrument loses power user is no longer informed of Attune connection status other than no icons on status bar
* [EE-1365] – Fixed application crash when loading workspace having multiple plots
* [EE-1366] - Plot Context menus are not available when zoomed in (filmstrip view)
* [EE-1399] – Fixed issues where performance test fails with "Internal System Error"
* [EE-1464] – Fixed issues with cut and paste from IS panel Voltages
* [EE-1545] – Fixed issue where starting a plate run on a plate that already has samples recorded and clicking cancel did not cancel acquisition and would start acquisition from a well that was already acquired
* [EE-1592] – Fixed default hyperlog value and set to 1000
* [EE-1597] - Importing FCS files with missing parameters results in the instrument settings XML being limited to those parameters
* [EE-1615] - In customize panel for plots copy and paste via keyboard shortcut does not work, context menu does work
* [EE-1616] – Fixed issue where the scroll position of the workspace was not reset when viewing compensation controls
* [EE-1620] – Fixed issue where the use experiment comp radio button was not available even though comp controls collected and sample collected.
* [EE-1628] – Fixed issue where importing sample as comp control results in XML containing channels instead of parameters
* [EE-2190] - When both the x and y axis are set to hyperlog and the scale setting is changed, the other axis loses its hyperlog setting

- ∗ [EE-2206] - Duplicating a name in the experiment explorer can create a name greater than 50 characters; trying to edit this name then results in a Unknown system error
- ∗ [EE-2227] – Fixed issue where the sample remaining indicator is not cleared when a rinse or instrument function that clears the remaining sample in the syringe
- ∗ [EE-2233] - Baseline fails but appears to work after running data in experiment
- ∗ [EE-2239] - Changing active samples leaves previous active sample with black text and removes background color
- ∗ [EE-2281] - Importing FCS file on any control but the first (BL1-H) causes compensation control mismatch
- ∗ [EE-2319] - Crash while updating compensation by changing a compensation gate and then opening a sample
- ∗ [EE-2342] - Immediately pressing stop on the baseline screen doesn't stop baseline - application goes back to the baseline setup screen, but instrument and progress bar keep running
- ∗ [EE-2346] - Software crash when setting plot to quad gate that is set to a derived gate
- ∗ [EE-2430] - Requiring numeric characters in user passwords: user can't create new password or change password at all
- ∗ [EE-2546] - Compensation Calculation Issues: does not handle controls with data where the gates do not include events
- ∗ [EE-2574] - Selecting System log during baseline causes Internal Software errors that require attune shutdown/restart
- ∗ [EE-2597] - Activating another sample while a sample is saving can cause an issue with the saving of the FCS file
- ∗ [EE-2617] - For a sample without data recorded but comp has been recorded for the experiment, parameter checkboxes are available when they shouldn't be
- ∗ [EE-2637] - When saving a tube sample from run mode, a parameter can be unchecked after the file is saved and this change is processed as an IS update
- ∗ [EE-2667] - Auto-hiding collection panel and then right clicking on side bar tabs crashes software
- ∗ [EE-2689] - Repeatable SW crash when sample is renamed to include "&" through the customize menu from the right-click context menu on the heat map
- ∗ [EE-2716] - [AC] Crash if polygon or oval gate is moved during acquisition
- ∗ [EE-2729] - Deleting active plate experiment can cause a crash
- ∗ [EE-2738] - Application infinite busy when switching compensation samples
- ∗ [EE-2767] - Application crashes when logging out if Plate, Experiment, Group or sample notes is in focus
- ∗ [EE-2790] - Attune crash after deleting contents of expression, then hitting ENTER or OK when editing derived gate
- ∗ [EE-2935] - spillover values can't be entered with a decimal in the comp ribbon under adjustment but shift key and mouse click on arrows will move value by 0.1 hundredths can't be entered at all
- ∗ [EE-2976] - Freeze while changing plots to auto range and scale during plate acquisition with heat map detached
- ∗ [EE-3460] - Changing time to FSC-A on daughter plot causes Attune to stop responding

## Installation Instructions:

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

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| ⚠ | • The instrument must be powered on and connected for the firmware updater to run at the end of the installer.<br>• **DO NOT** update the firmware if the instrument is in a sleep state. **Power cycle** (turn on and off) the instrument prior to running the firmware updater.<br>• **DO NOT** launch the software application until completion of all the installation steps.<br>• **DO NOT** run any other applications while completing these steps. |
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## INSTALL AND UPGRADE (v2.1, v2.2, or v2.3 to Attune™ NxT Software v2.4)

**Software Installation**

**Step 1** Turn on the computer and cytometer (restart both if they were previously on). Ensure that the Deskey device that is used to run the software is plugged into the computer.

**Step 2** 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 3** Unzip (select "extract all files") the AttuneNxTv2.4.zip file to the desktop.
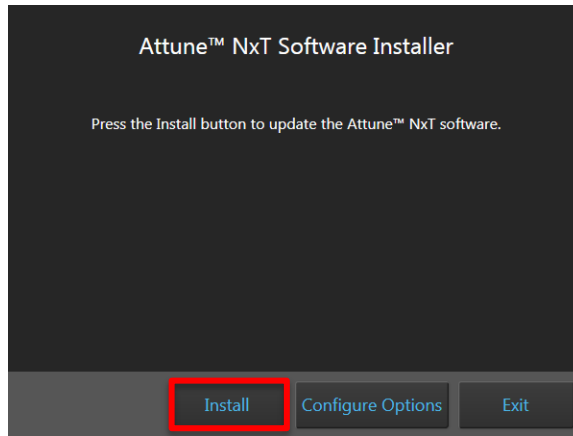
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**Step 4    NEW INSTALL ONLY**

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".
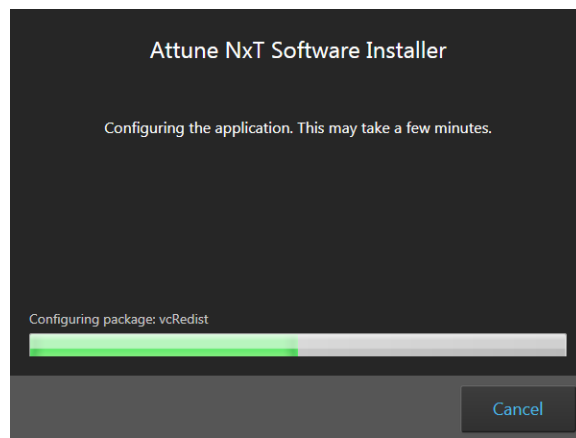5. Plug in the Deskey device and power on the Attune™ NxT cytometer
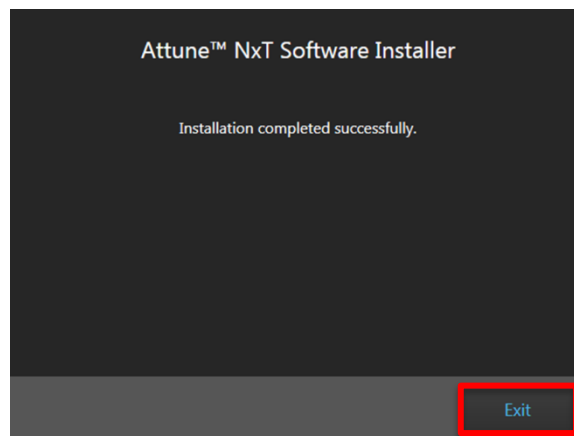
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**Step 5** Install the software:

    a. Double-click "SetupAttuneNxT.exe" in the "Attune NxT_Build 2.4.0.11614 folder.
    b. Select "Install"

You will see a new window open as shown below showing the progress of the software installation.  If for any reason you select "Cancel", start back at step 4 and re-run the "SetupAttuneNxT.exe"
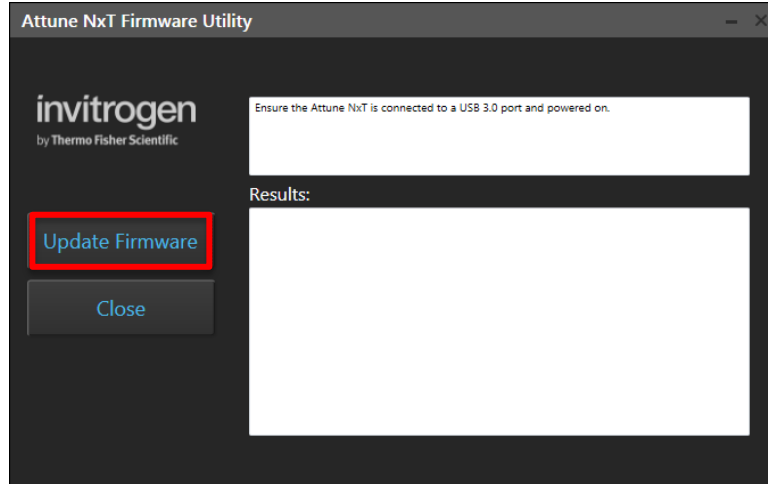


**Step 6**    When the installer has completed, the "Installation completed successfully" message is displayed. Close the installer by clicking the "Exit" button
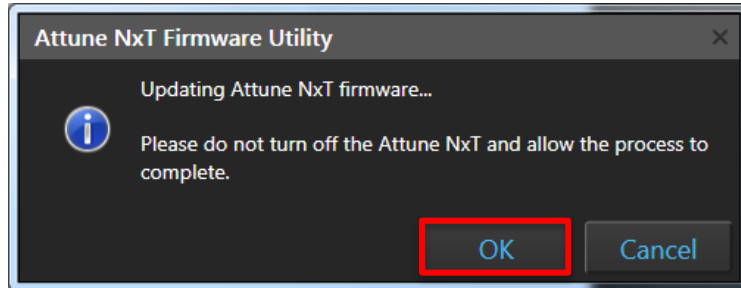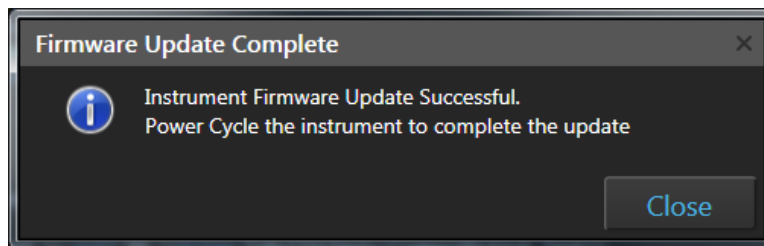
**Firmware Installation**

**Step 1** At the end of the installer, the firmware updater utility will automatically launch if the instrument is powered on and connected.



**Step 2** Select the '**Update Firmware'** button to update the firmware.
**Step 3** Confirm that you want to update the firmware when the firmware update confirmation dialog is displayed by clicking the '**OK**' button:



**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 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.

⚠ 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

Attune™ NxT Software rev 2.4

and connected.

## UPGRADE FROM v2.0.1 or earlier - Attune™ NxT Software v2.4 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.