



Release Notes
Life Technologies®
Attune® NxT Software v2.1

In the following pages you will find instructions describing:

New software features
Known software/system issues with troubleshooting guidance
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 2.1

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

New software features implemented in Version 2.1

- On plot compensation
- Export of statistics to CSV
- Support of all optional and Attune® custom FCS keywords
- Ability to create and manage multiple user accounts (individual password security)
- System access based on user account privileges
- Levey-Jennings and Performance History reports of baseline and performance test
- Target and Label Fields now carry over to axis labels and can be applied Smart Gate Labeling
- Append and Overwrite of FCS files
- Ability to move gate labels (except quad gates)
- Export and Copy and Paste of plots to external programs (like Power Point)
- Ability to set user options for default settings for gates, plots, fonts, colors, and group/ sample names

Auto Sampler features

- Support of 384 well plates
- Support of Deep Well plates – up to 800 uL draw volumes (no multi-draw)
- Lower Dead Volume – 30 uL from wells (for sample flow rates of 12.5 to 500 uL/min)
- NOTE: it is important to power on the auto sampler before the Attune® NxT cytometer to ensure that the auto sampler is recognized properly.

The following sections describe known issues with the Attune® NxT v2.1 software. Where possible we have outlined steps to work-around the known issue.

Guidance for Instrument Start Up and Performance Test using the Attune® NxT v2.1 Software:

- **Run performance test directly after launching the software application.** If you open an experiment and then run performance test you may experience a failure. Restart the software application and repeat performance test if this occurs.

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf55035	The Area scaling factor (ASF) is not updated automatically after baseline and performance test are completed.	The Area scaling factor may be manually adjusted under the "Advanced settings" section of the Instrument Settings menu prior to acquiring and recording a sample. If the area parameter looks larger than the height parameter, enter a value <1; if area looks smaller than height, enter a value >1.
artf55282	Do not press stop button during baseline as this can cause an intermittent timeout failure of subsequent baseline runs	If a baseline is stopped and the next baseline run results in an error dialog and or a baseline with everything failing, restart the application software and the issue should be resolved
artf56497	The "Run" and "Record" buttons may not replace the "Start Up" button on the Collection panel following instrument startup. This does not happen with 100% frequency.	It is recommended that the user re-run Start Up and the buttons should become active.
artf56687	When an instrument has an auto sampler attached, the software may sometimes become unresponsive if the user stops the Start Up functions after it has been started.	DO NOT stop the Start Up function after it has started.
artf57858	Software can become unresponsive if start up is run prior to making sure all fluid bottles are plugged in	Power cycle the system with the fluid bottles plugged in

Guidance for Setting up an experiment using the Attune® NxT v2.1 Software:

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf53358	When adjusting the size of the polygon region on a plot using the bounding rectangle box, the region may not maintain their 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.
artf53507	If the experiment name, group, sample and parent gate are all displayed in a plot header,	If the names have too many characters, the plot title may not

	the hyperlink to set the parent population and gating hierarchy may not be visible on the plot.	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.
artf53424	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.
artf56476	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 a workspace, run protocol, and acquire samples.
artf56550	When creating experiments, Attune doesn't check to see if it has 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	Archive your data from your experiment explorer to free up disk space
artf57711	After an experiment is created the type of plate (96 or 384-well, as well as well type), cannot be changed using the customize menu.	Ensure that the correct plate type is selected upon experiment set up. If the wrong plate type is selected, delete the experiment and re-start.

Guidance for using the Experiment Explorer and Instrument Settings panel within the Attune® NxT v2.1 Software:

- Target and Label Fields are applied to plot axes but do not show up in statistics tables
- By default, plot axes titles include the \$PnS, or stain name, in addition to the target and label name. To remove the stain name and only display the target and label name, select the plot and then select the customize panel and delete the name in the axis label text boxes.
- When an FCS file is imported to a sample, the instrument settings panel does not update to the sample specific data. It will display the experiment level instrument settings. The target and label names and parameter list are correct in the dropdown list on a workspace plot.

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf56058	It is not possible to select multiple compensation controls at once by holding down the control key on the keyboard for performing	The FCS file from each compensation control must be manually exported using the right-

	functions like delete, remove, or export FCS files.	click context menu.
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Guidance for using Compensation within the Attune® NxT v2.1 Software:

- If you make an error in the compensation set up wizard and need to make changes, you must delete 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
- If compensation for the experiment has never been set up and you import an FCS file with embedded compensation values, the compensation values will not be compensated and you will need to push the "Apply Compensation" button.

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf54564	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.
artf54815	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.
artf55173	If a sample has an FCS file with an embedded spillover matrix the matrix from the FCS file will not be able to be used after the file is imported into a new experiment.	If an FCS file is imported into an experiment, enter spillover values into the spillover matrix manually by selecting the "View Matrix" button of the Compensation tab of the Ribbon bar. Ensure that "OK" or "enter" are pressed following data entry.
artf56527	The "Compensation Setup" button may be available from the compensation ribbon during acquisition	Do not launch compensation setup during acquisition. It is best practice to set up compensation prior to starting your experiment to ensure correct instrument set-up.
artf57907	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 v2.1 Software:

- The default setting for FSC voltage is set very low; increase to at least 400 to put most common cell types on scale using a FSC vs. SSC scatter plot.
- 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 is expected behavior and does not have any impact on the completion of the acquisition.
- Keep the tube lifter in the DOWN position when using the auto sampler.
- Derived gates cannot be used as a stop option.

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf56764	"Load plate into auto sampler" dialogue box will be shown if the Attune® Auto Sampler is unplugged while the software is open	Do not unplug the auto sampler while the software is open. If the "load plate into auto sampler" dialogue is observed after unplugging the system, click the "cancel" button to remove the dialogue box.
artf57941	When adding more sample or trying to acquire more events, the complete stop condition option is only available for the first addition to time, volume, and events stop criteria. After that, you will only be able to append data not complete the stop criteria therefore amount left of stop criteria will not apply anymore.	Only use the complete stop conditions option once.

Guidance for using the Experiment Workspace (Gates, Plots, Stats) within the Attune® NxT v2.1 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.

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf52417	Plots will appear blurred at low resolutions.	Keep plot resolution greater than 256x256 for best visualization. Resolution can be adjusted under the Customize panel when a plot is selected.
artf54881	The names/labels for quadrants may not display properly.	Names and labels can be truncated depending upon the font size used and or length of name. Reduce the font size/spacing and if possible shorten name length.
artf55134	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.

artf55596	The software will be unresponsive if accidentally add an incompatible gate type to a plot in the film strip view such as: <ul style="list-style-type: none"> • Adding a rectangle, oval, or quadrant gate to a histogram plot • Adding a histogram gate to a dot plot, density, or precedence density plot. 	To correct this press the Esc button on the keyboard.
artf55603	Changing the coordinates or rotation of an oval gate using the customize menu does not work.	Resize and reshape an oval gate through manipulation of the object within the plot.
artf57459	The icons 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
artf57610	Plot titles in auto layout mode are limited to 35 characters and the title including Experiment, Group, Sample, and Parent Gate will not be visible when >35 characters are used in auto layout.	Keep plot titles to less than 35 characters by limiting title name or do not include hierarchical elements (such as Experiment, Group and Sample) in title name. Alternatively view the plot in freeform mode and resize the plot until all elements are visible.

Guidance for Data Analysis / Data Display using the Attune® NxT v2.1 Software:

- On the “Results” view, right clicking on a column header will display a list of statistics, some of which are not currently available.
- 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 manually.

Known issue (“Artifact”) ID Number	Description of Known Issue	Suggested Action
artf55357	The results table will revert to default settings and not maintain user-selected statistics once the software is closed.	The statistical measure of interest can be recalculated by the software by selection of the measure from the “Statistics” ribbon. Re-select the statistics of interest when the software is re-launched.
artf56533	A statistics box will report a statistical value for a disabled parameter if the experiment workspace is made that includes a parameter that is disabled AFTER the workspace is finalized. In this instance the statistics box inserted into the workspace will show a value of “0” for every measure associated with the disabled parameter instead of ignoring the disabled parameter.	Do not include plots in a workspace for disabled parameters. If a plot is inserted in a workspace for a disabled parameter, a brick symbol will appear on the plot. Delete this plot before inserting the statistics box.

Guidance for Export Import of Experiments, FCS files using the Attune® NxT v2.1 Software:

- The software supports FCS3.1 formats

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf56354	The first recorded FCS file in an experiment is recorded as DefaultSampleName.fcs, even if the name is changed. All subsequent fcs files will be recorded with the correct file name.	
artf57789	Target and label name edits made after recording a sample are not updated in FCS file.	If using a 3 rd party analysis software package, make all changes to target and label fields prior to recording. If using the NxT software exclusively, this should not be an issue because the experiment files are updated.

Installation Instructions:

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

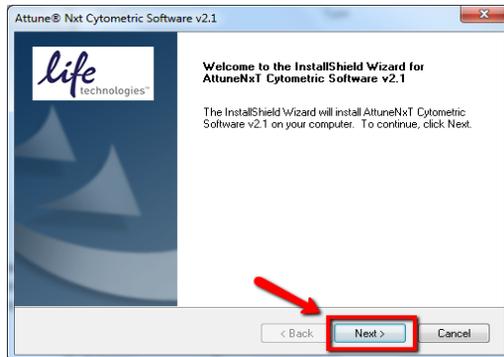
Attune Software v2.1 Installation – NEW INSTALL (If upgrading from a previous version, see sections below)

Software Installation

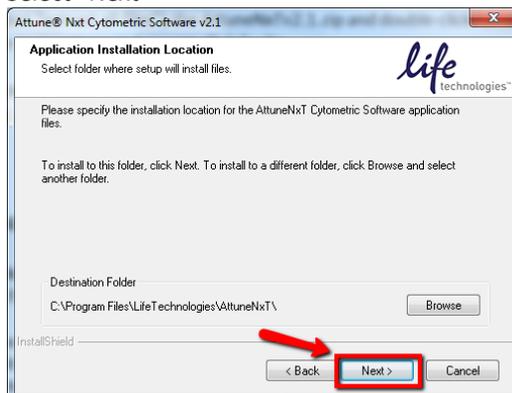
Note: Do not launch the Attune NxT application until the completion of all the installation steps.

1. Restart the computer before beginning these instructions.
2. Log into Windows as an administrator. (Unless changed the default administrator user account is: INSTR-ADMIN and password: INSTR-ADMIN). Note: It is recommended that no other applications are running while completing these steps.
3. Unzip (select “extract all files”) the AttuneNxTv2.1.zip
4. Open the USB3_FOR NEW INSTALL, INSTALL ME FIRST folder and double-click the “RENESAS-USB3-Host-Driver-30230-setup.exe” file.
5. Follow the instructions and accept the terms of agreement. Once complete, click “Finish”.
6. Plug in the Deskey device and power on the Attune® NxT cytometer
7. Double-click “setup.exe” in the “Attune NxT_Build 2.1.0.2009” folder.
8. During the installation process described below, you may have a windows notification popup from the toolbar stating that a driver installation was not completed. These messages can be ignored. They do not affect the installation process.
9. Follow the instructions and accept all defaults (see screenshots and details below)

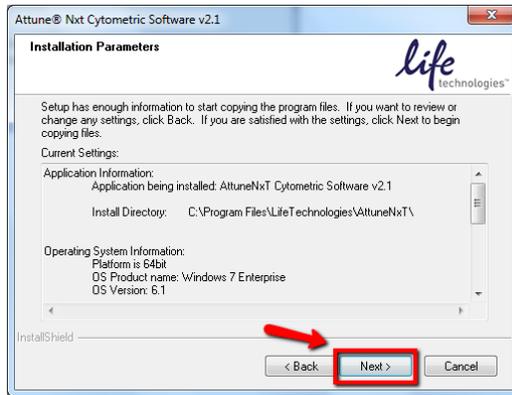
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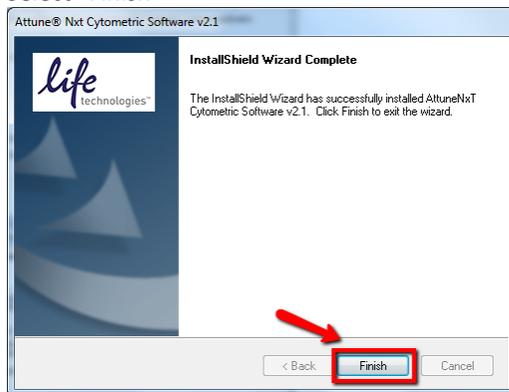
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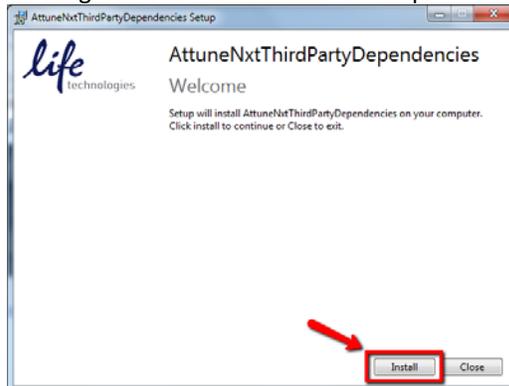
- Select “Next”



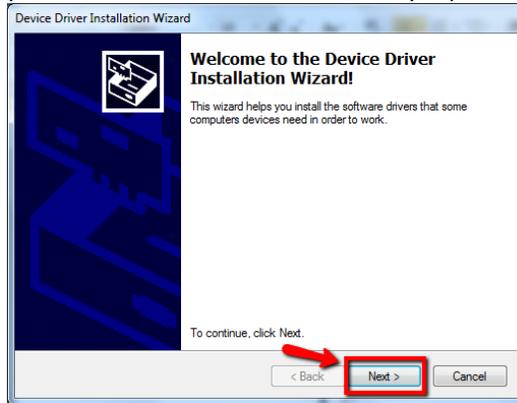
- Select “Finish”



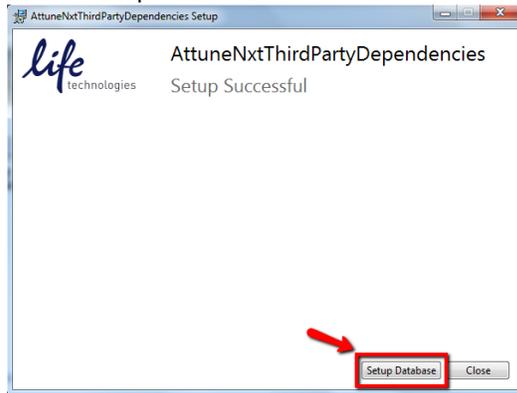
- The next window, shown below, may be minimized by default. If so, look in your toolbar to bring the window into view to complete the installation. Select “Install”



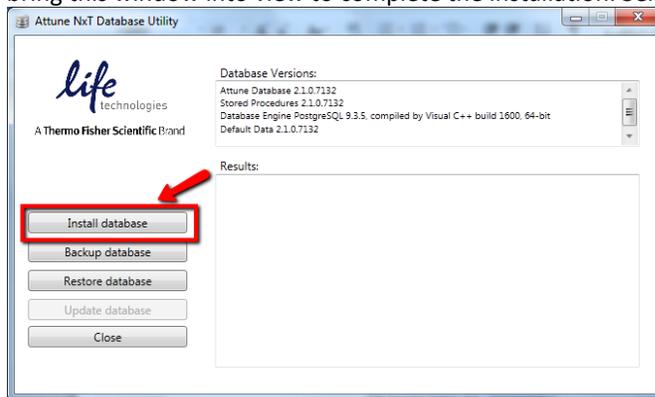
- An additional window will open after clicking “Install”. On this window, select “Next”. Once complete, select “Finish”. It is perfectly normal to get a message saying that the driver installation could not complete, still select “Finish”. This window will close, taking you back to the “AttuneNxTThirdPartyDependencies Setup” window.



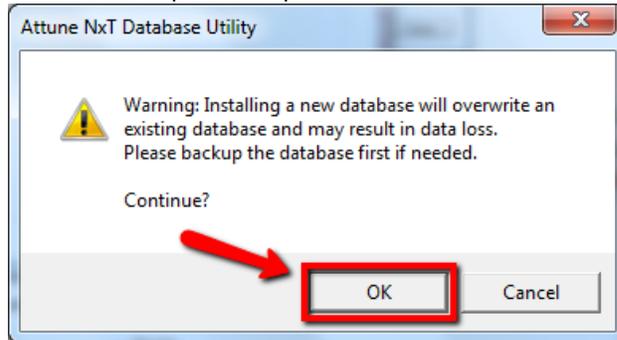
- Select “Setup Database”



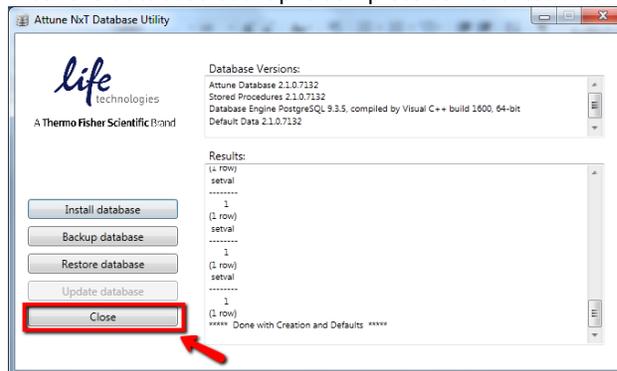
- The next window, shown below, may be minimized by default so look in your toolbar to bring this window into view to complete the installation. Select “Install Database”



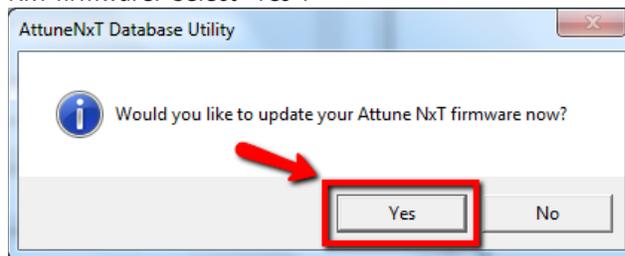
- A warning notification will pop up in a separate window. Select “OK”. The pop-up will close and the update will proceed



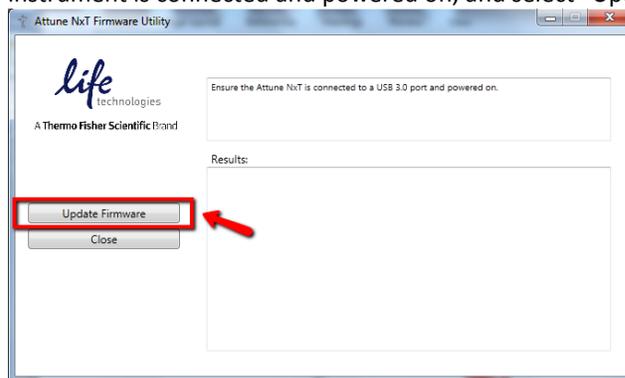
- After the database backup is complete as indicated in the results section, select “Close”



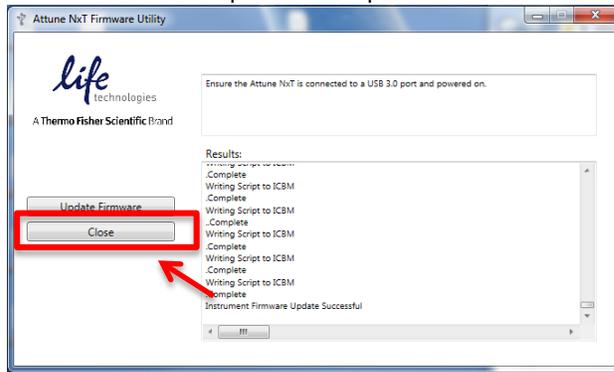
- The previous window will close and a pop up will display asking to update the Attune NxT firmware. Select “Yes”.



- The pop-up will close and the window below will be displayed. Ensure the Attune NxT instrument is connected and powered on, and select “Update Firmware”.



- Once the firmware update is complete as indicated in the results section, select “Close”



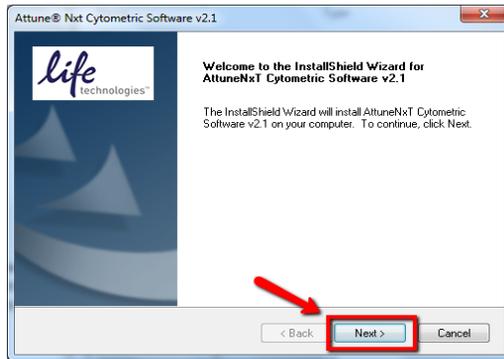
- The install is now complete. Power cycle the Attune® NxT instrument.
- Once the Attune® NxT instrument has been powered on again, double-click the shortcut icon “Attune® NxT Software” that has been created on the desktop to launch the application software.

Attune Software v2.1 Installation - UPGRADE FROM v2.0.0/v2.0.1

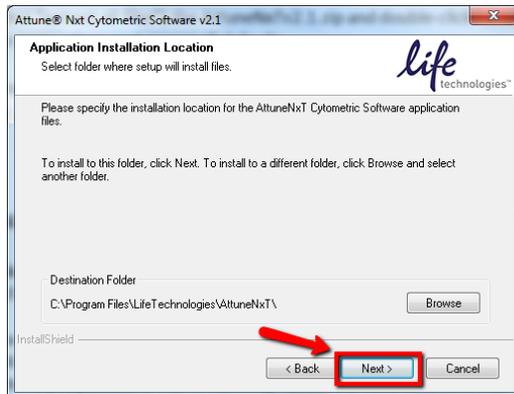
Software Installation

Note: Do not launch the Attune NxT application until the completion of all the installation steps.

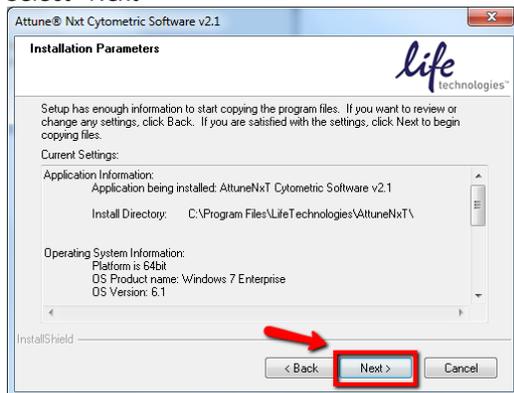
1. Restart the computer and cytometer before beginning these instructions.
2. Log into Windows as an administrator. (Unless changed the default administrator user account is: INSTR-ADMIN and password: INSTR-ADMIN). Note: It is recommended that no other applications are running while completing these steps.
3. Unzip (select “extract all files”) the AttuneNxTv2.1.zip and double-click “setup.exe”.
4. Follow the instructions and accept all defaults (see screenshots and details below)
 - o Select “Next”



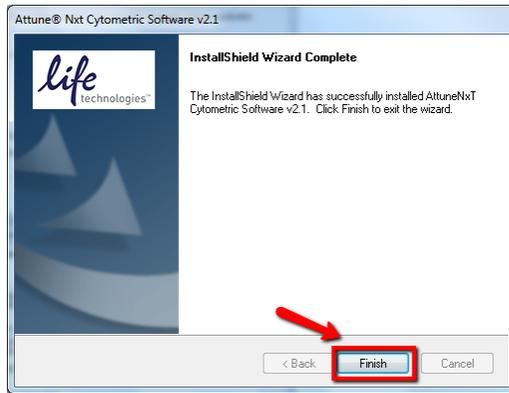
- o Select “Next”



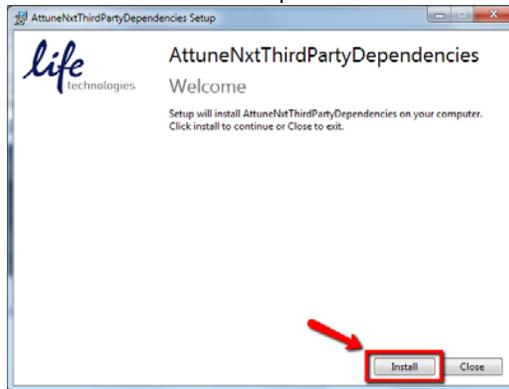
- o Select “Next”



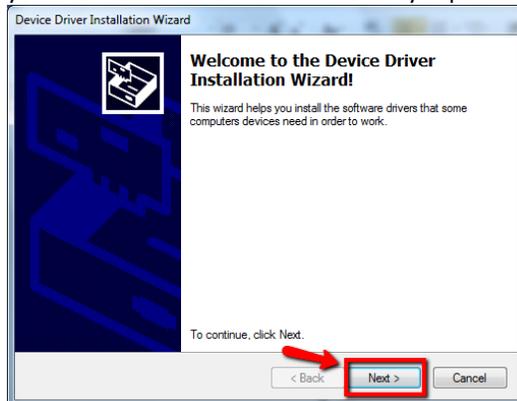
- o Select “Finish”



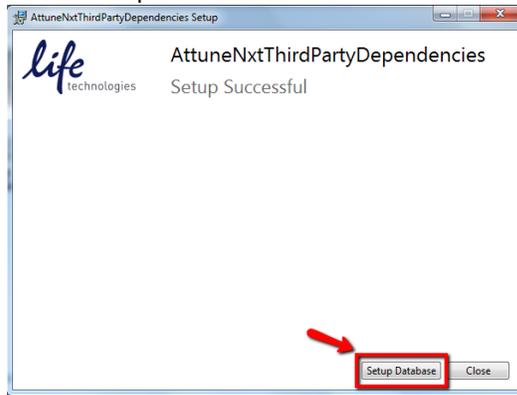
- o The next window may be minimized by default. If so, look in your toolbar to bring the window into view to complete the installation. Select “Install”.



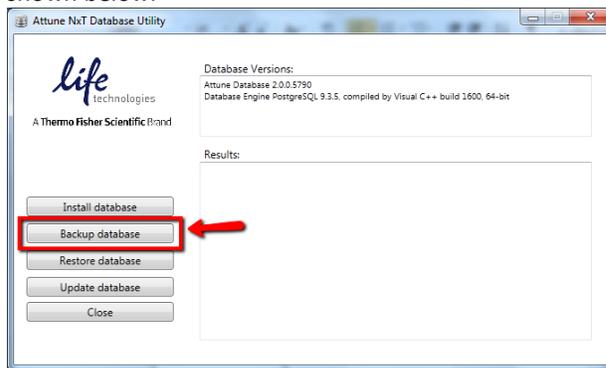
- o An additional window will open after clicking “Install”, on this window, select “Next”. Once complete, select “Finish”. It is perfectly normal to get a message saying that the driver installation could not complete, still select “Finish”. This window will close, taking you back to the “AttuneNxTThirdPartyDependencies Setup” window.



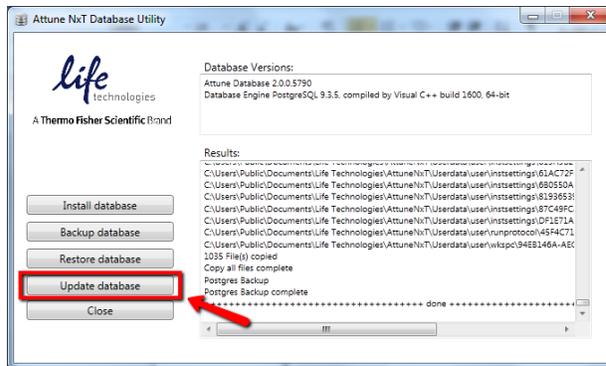
- Select “Setup Database”



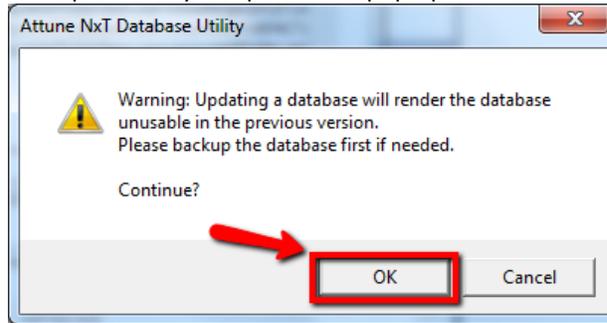
- The next window, shown below, may be minimized by default so look in your toolbar to bring this window into view to complete the installation. To update all existing experiments from the current version of the software, select “Backup database” as shown below.



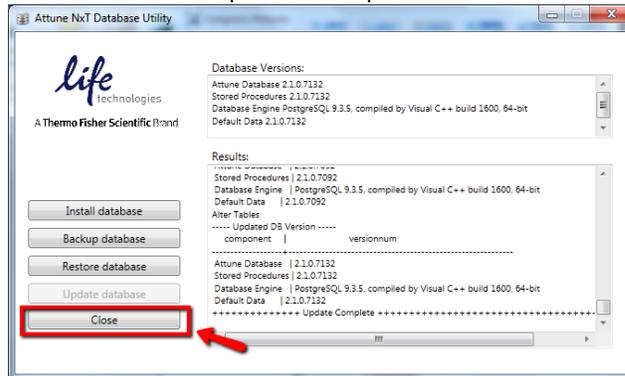
- After the database backup is complete as indicated in the results section, select “Update Database”



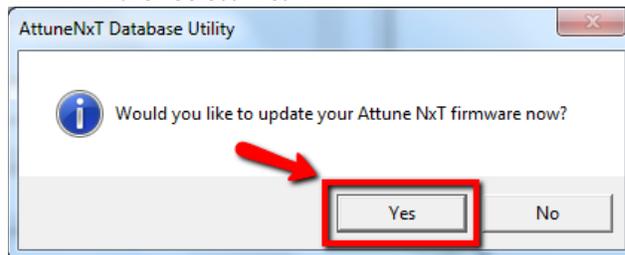
- A warning notification will pop up in a separate window. Select “OK”, as the database backup is already complete. The pop-up will close and the update will proceed.



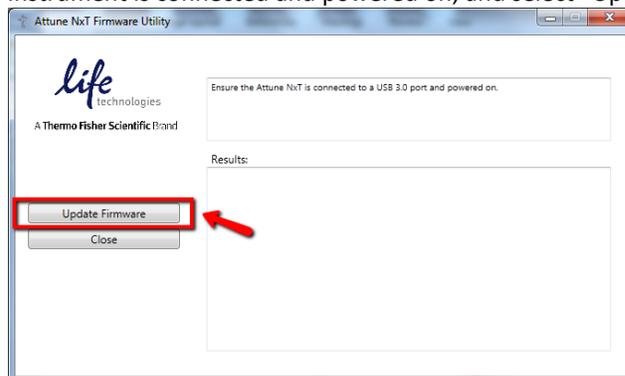
- After the database update is complete as indicated in the results section, select “Close”



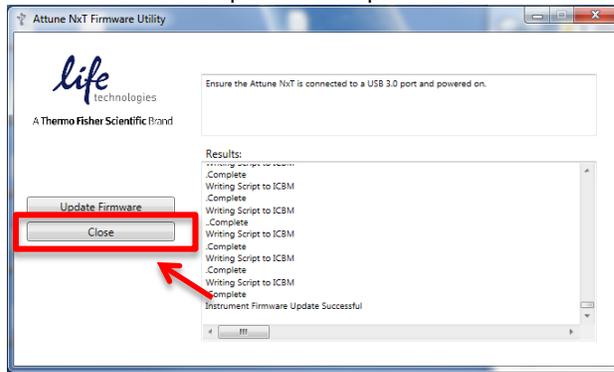
- The previous window will close and a pop up will display asking to update the Attune NxT firmware. Select “Yes”.



- The pop-up will close and the window below will be displayed. Ensure the Attune NxT instrument is connected and powered on, and select “Update Firmware”.



- Once the firmware update is complete as indicated in the results section, select “Close”



- The upgrade is now complete. Power cycle the Attune® NxT instrument.
- Once the Attune® NxT instrument has been powered on again, double-click the shortcut icon “Attune® NxT Software” that has been created on the desktop to launch the application software.

Attune Software v2.1 Installation - UPGRADE FROM v1.1 or earlier

For customers that are currently using Attune® NxT Software v1.1 or earlier, please contact technical support or your local field service engineer for the upgrade to v2.1.