



**Release Notes**  
Life Technologies®  
Attune™ NxT Software v2.2

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 2.2

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

## **New software features implemented in Version 2.2**

- Concentration statistic
- Export of FCS files to 3.0 format
- Templates-Single User
- Heat Map Analysis
- Import of instrument settings from FCS
- Statistics Default Options
- Export and Print of the Results Table
- Ability to delete plates in batches on the experiment explorer
- Ability to multi-select wells and tubes on the experiment explorer
- Display of the quadrant gates in the edit gates menu
- Ability to set stop options using quadrant and derived gates
- Performance improvements around setting up plates

The following sections describe known issues with the Attune™ NxT v2.2 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 v2.2 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 properly.
- **If the instrument is in sleep mode after shutdown, close the software program completely prior to running start up**
- **Do not stop start up once it has been started.** If stopped you may encounter a blinking amber indicator light. If you accidentally stop start up, shut everything off and restart again.
- **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.
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.

**Guidance for Setting up an experiment using the Attune™ NxT v2.2 Software:**

- **Do not label gate names starting with a number i.e. 7AAD**

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, 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.
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 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.
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

**Guidance for using the Experiment Explorer and Instrument Settings panel within the Attune™ NxT v2.2 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.

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
1206	Experiments are limited to 400 samples; greater than 400 samples can cause software instability	If an experiment requires more than 400 samples, duplicate experiment for additional samples beyond 400.

**Guidance for using Compensation within the Attune™ NxT v2.2 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

- Do not import FCS file as compensation control prior to launching the set up wizard. First, Import compensation file as a sample allowing the instrument settings to update. Second- chose your compensation parameters using the comp set up. Third-import compensation FCS files into the controls of interest. Fourth-apply compensation as necessary.
- Using auto compensation adjustment tools for large event files, response rate for adjustments will be slow. If you need to adjust compensation, make changes after files are acquired.

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.
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 v2.2 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. To improve response rate, turn off auto-scaling or auto refresh functions.
- During Acquisition having any plot axes set to auto-scale can cause excessive CPU usage resulting in system wide performance lag. Recommend all plots set to manual scale during acquisition for optimal performance.
- Acquisition volume in plates is limited to 800 ul acquisition volume
- 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 to respond when making adjustment during acquisition and a blue indicator spinning wheel will be visible. This is expected behavior and 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.

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
688	The complete stop condition option for append does not work if stop condition is based on time or volume	Only use total events or gated events if you require to add data to existing data files
1506	Heat map stats will not update when heat map tab is undocked	Make sure heat map tab is in docked position before using statistical information from heat map; Dock heat map and refresh data.

**Guidance for using the Experiment Workspace (Gates, Plots, Stats) within the Attune™ NxT v2.2 Software:**

- **Do not begin gate label names with a number**
- 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 and disable auto-refresh on home tab.  
In order to be able 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.
- If target and label names are assigned to a parameter prior to recording the corresponding parameter in the plot axis customize panel will be blank. To correct this open another sample prior to continuing analysis

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.
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.
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 v2.2 Software:**

- 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)
- 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. (Note-header row will be copied if you use the send table to function on the statistics ribbon)
- At this time the selection of stats in results view is global to all users and is not persisted with the experiment. (i.e. statistics selected to be displayed in the results table for one experiment will also be selected for other experiments within user profile)

**Guidance for Heat Map Analysis using the Attune™ NxT v2.2 Software:**

- If the heat map displays NA after acquiring a plate, the statistics may be out of date. To correct this change any attribute on any plot on the workspace (i.e. move gate, change name, change scale, add new plot etc.). This will force a refresh of the entire statistics.

Known issue (“Artifact”) ID Number	Description of Known Issue	Suggested Action
1842	The gate list from the heat map set up tab populates incorrectly and allows sample and group level workspace gates to be displayed.	Heat Map view is only available for experiment level workspaces. If you have a group or sample level workspace, the gates that you create will show in the gate dropdown but will not display on the heat map view, do not use these gates.

**Guidance for Exporting/Importing using the Attune™ NxT v2.2 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.
- Import a compensation file as a sample prior to creating compensation set up to ensure that all parameters are correct.
- Prior to importing experiments or FCS files, check the configuration of the instrument that the experiment/fcs files were collected on using the options, configuration tab or the keywords panel. If the configuration doesn’t match the configuration that your software has been set to, prior to importing the experiment, change the configuration to match the experiment. Note- if you receive an error message when trying to export instrument settings (instrument settings can’t be displayed for selected sample, the instrument configuration is not), return to main menu, options, configuration and chose the right configuration and then reopen experiment, group, or sample.

Known issue (“Artifact”) ID Number	Description of Known Issue	Suggested Action
2174	During any batch export of FCS files, if “update keyword” is selected, the \$FIL keyword of all files is set to the sample name of the last file selected (i.e. exporting at the experiment level a plate of wells A1-A10, all would have \$FIL set to A10.fcs)	<ol style="list-style-type: none"> <li>1. Export FCS files individually - \$FIL will update correctly</li> <li>2. If no changes were made to data (target/label fields, compensation), select “ignore” option - \$FIL will remain correct as no updates are made</li> <li>3. If using FCS files in a 3<sup>rd</sup> party analysis</li> </ol>

		package, use Well ID instead of filename to filter samples (this will not work for tube samples as there is no Well ID)
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**Guidance for Shut down or Maintenance features or using the Attune™ NxT v2.2 Software:**

- Self-test 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 auto sampler or remove any cables during operation of instrument or maintenance functions

**Guidance for Setting up user accounts and user options using the Attune™ NxT v2.2 Software:**

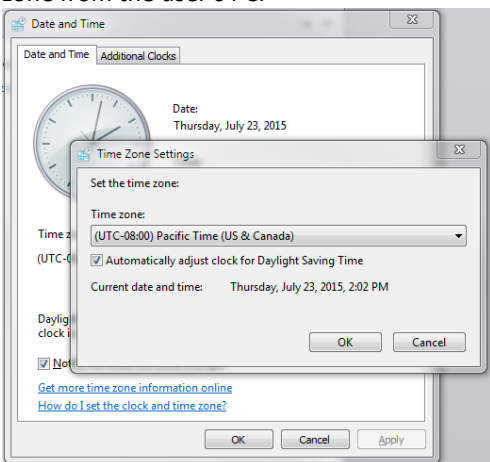
- Email notification is not available in this version of software. Email address can be added but no notification is sent.
- After initial set up of a user account, Forgot password is available. To set up security questions, log into new users account by entering user name and password. From the options 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.

**Guidance for setting up the Attune™ NxT v2.2 Software to ensure correct for time zone settings**

**Adjusting time zone when using Attune NxT software**

When the Attune NxT software is installed, the PostgreSQL database server fixes itself to the time zone where it was installed.

An end user in a time zone different from the installed time zone (usually US/Pacific) can experience problems such as the service user being unable to login because the database server is on a different time zone from the user’s PC.



**Figure 1** Windows Time Zone Settings

To adjust time zone settings

1. Close Attune NxT Software if it is running.
2. Run the Attune NxT Database Utility located C:\program files\lifetechnologies\AttuneNxT\AttuneNxTDatabaseUtility.exeRunning the utility fixes the Postgres Server’s time zone quietly in the background.

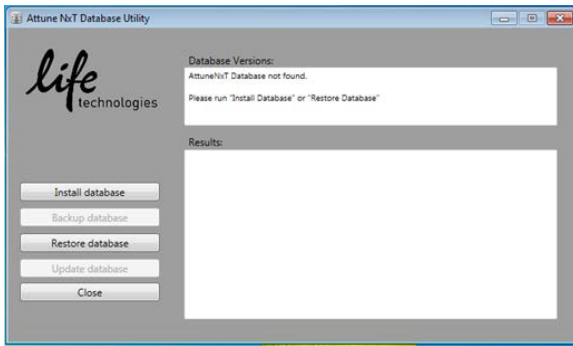


Figure 2 Attune NxT Database Utility



## **Software Bugs Fixed In This Release**

Artf55282- Do not press stop button during baseline as this can cause an intermittent timeout failure of subsequent baseline runs

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.

Artf57858- Software can become unresponsive if start up is run prior to making sure all fluid bottles are plugged in

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.

Artf56058- It is not possible to select multiple compensation controls at once by holding down the control key on the keyboard for performing

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.

Artf56527- The "Compensation Setup" button may be available from the compensation ribbon during acquisition

Artf56764- "Load plate into auto sampler" dialogue box will be shown if the Attune® Auto Sampler is unplugged while the software is open

Artf54881- The names/labels for quadrants may not display properly.

Artf55134- The time it takes to open the plot "Preview" panel is dependent upon the number of parameters enabled

Artf55596- The software will be unresponsive if accidentally add an incompatible gate type to a plot in the film strip view such as:

- Adding a rectangle, oval, or quadrant gate to a histogram plot
- Adding a histogram gate to a dot plot, density, or precedence density plot.

Artf55603- Changing the coordinates or rotation of an oval gate using the customize menu does not work.

Artf55357- The results table will revert to default settings and not maintain user-selected statistics once the software is closed.

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.

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.

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

689 - Crash on append

639 - Time zone issue with data base

691/724/940 – Improved application performance

814 – Fixed application start up issue with blinking amber light in non-4 laser systems

992 - Levey-Jennings data now displays correctly prior to previous upgrades upgrade issues

867 –Improved support for import/export of large experiment files

935 - Changing histogram plot to bivariate plot in customize menu cause application crash

## **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 NxT Software v2.2 Installation - UPGRADE FROM v2.1 (If installing new for the first time, see section below)**

#### **Software Installation**

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

**Step 1-** Restart the computer and cytometer before beginning these instructions.

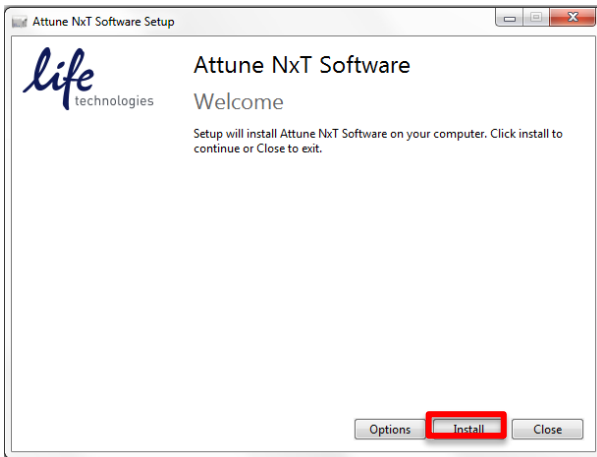
**Step 2-** Log into Windows as an INSTR-ADMIN account and password: INSTR-ADMIN. This is the default administrator account. (Note- if your instrument is networked, please make sure that the administrator privileges have not been removed by your local IT department.) **Do not run any other application while completing these steps.**

**Step 3-** Unzip (select “extract all files”) the AttuneNxTv2.2.zip

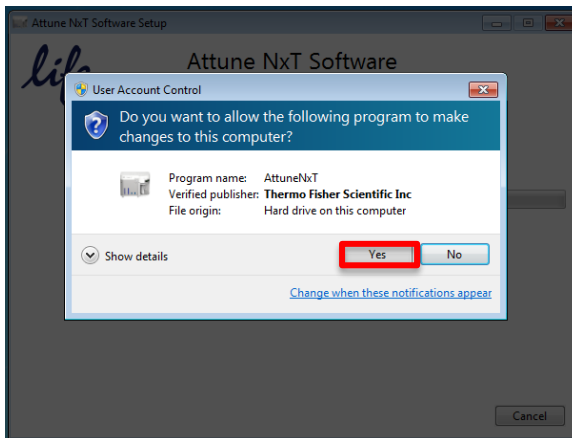
3A- Double-click “SetupAttuneNxt.exe”.

**Step 4-** Install the software (Note-Follow the instructions and accept all defaults (see screenshots and details below))

4A. Select “Install”

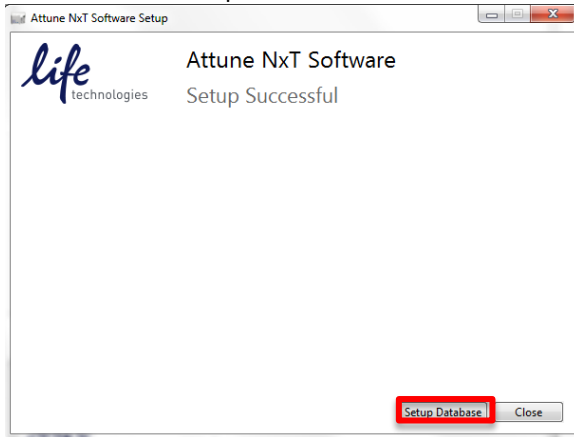


4B. Select “Yes”



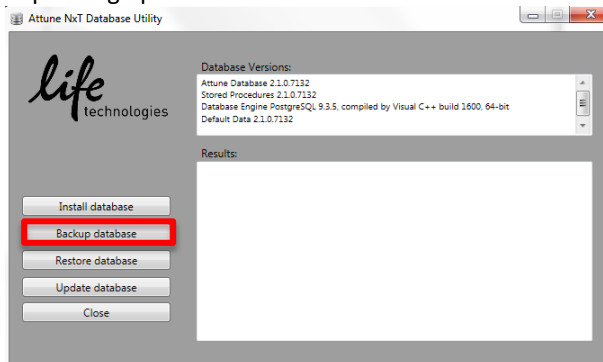
## Step 5- Setup Database

### 5A. Select "Setup Database"

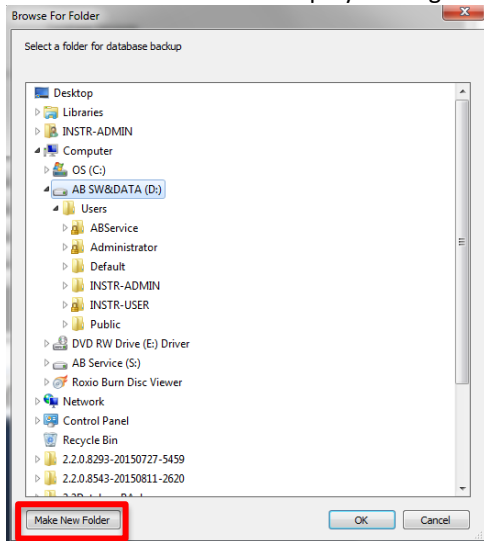


Note-The next window, shown below, may be minimized by default so look in your toolbar to bring this window into view to complete the installation.

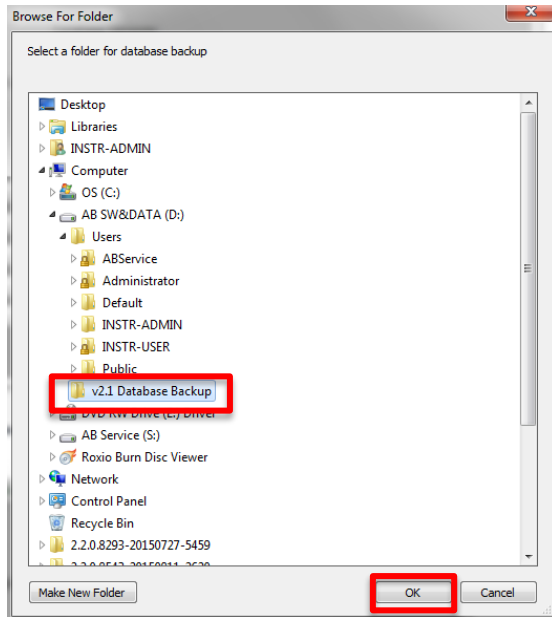
5B. Database Backup to update all existing experiments from the current version of the software, select "Backup database" as shown below. Note- backing up the database can take some time depending upon the amount of data stored in the database.



### 5C. Select location of back up by clicking Make New Folder



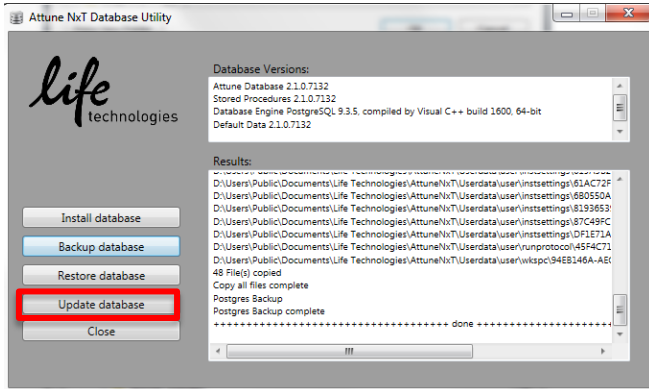
5D. Create New Folder on D: Drive and name v2.1 Database Backup



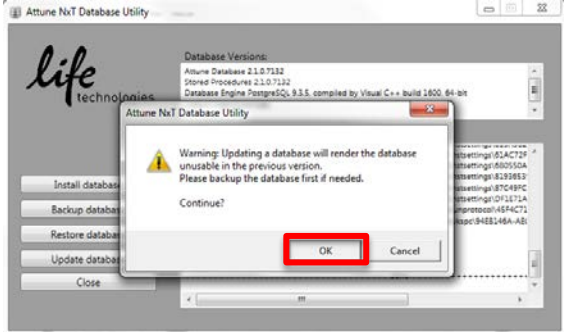
5E. Click OK

Step 6: Update Database

6A. After the database backup is complete as indicated in the results section, select "Update Database"

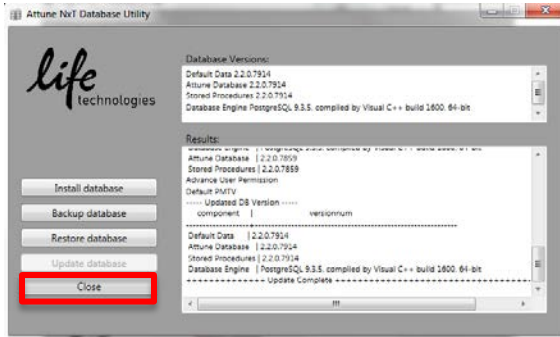


Note: A warning notification will pop up in a separate window.



6B. Select "OK", as the database backup is already complete. The pop-up will close and the update will proceed.

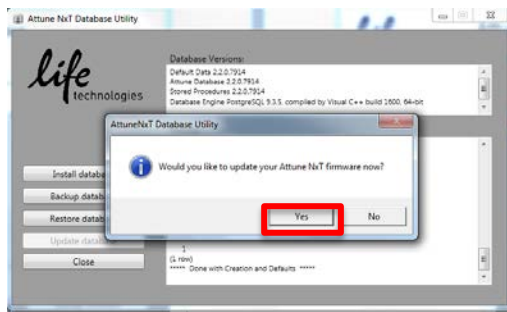
6C. Close the Database window after database update is complete.



Note- the window will close and a pop up window asking to update the Attune NxT firmware will appear.

**Step 7: update firmware**

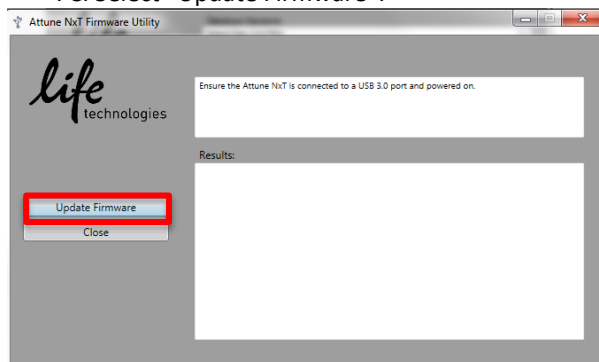
7A. Select Yes to update firmware



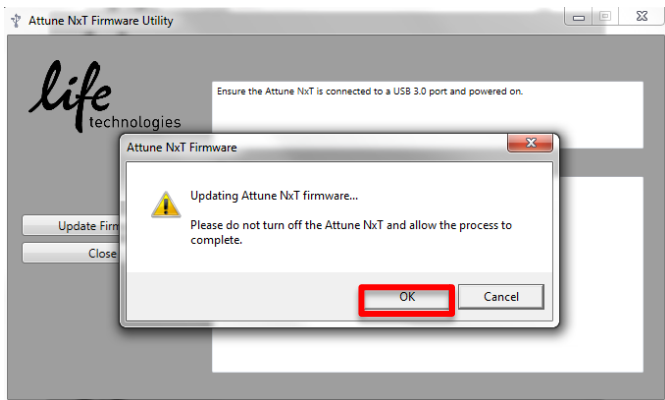
Note- the pop-up window will close automatically upon selection.

7B. Ensure Attune NxT instrument is connected and powered on and all programs are closed including Attune NxT software. Note if software has been opened before running firmware updater turn off instrument and turn it back on before proceeding to next step.

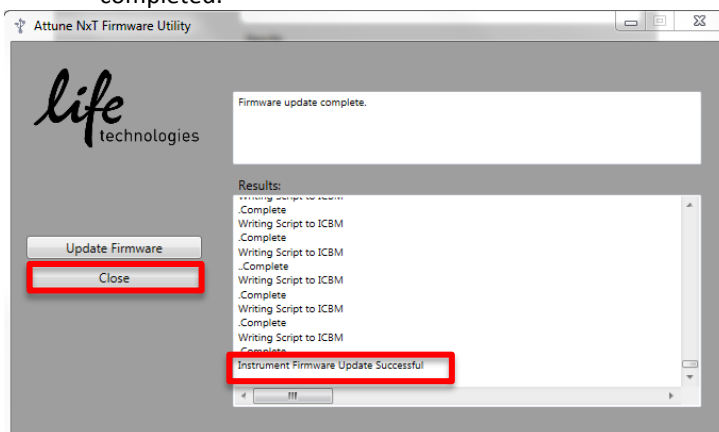
7C. Select "Update Firmware".



7D. Select OK. Note-Do not disturb instrument computer during firmware update. Note- this process can take several minutes.



7E. Verify Firmware update has been successful and Close Firmware window if successfully completed.



7F. Turn off and Turn on the computer and cytometer. Once the Attune® NxT instrument and the computer have been powered on again, double -click the shortcut icon “Attune® NxT Software” that has been created on the desktop to launch the application software.

**Step 8:** Run startup by following the on-screen instructions

**Step 9:** Run a new baseline before proceeding

## Attune™ NxT Software v2.2 Installation - UPGRADE FROM v2.0.1 or earlier

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

## Attune NxT Software v2.2 Installation – NEW INSTALL

### Software Installation

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

**Leave the Attune™ cytometer powered off until installation complete** (Note- you will be instructed to power on the Attune™ cytometer in instructions below)

**Step 1-** Turn on the computer.

**Step 2-** Log into Windows as an INSTR-ADMIN account and password: INSTR-ADMIN. 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.) **Do not run any other applications while completing these steps.**

**Step 3-** Unzip (select “extract all files”) the AttuneNxTv2.2.zip

3A. Double-click “SetupAttuneNxt.exe”.

**Step 4-** Open the USB\_FOR NEW INSTALL, INSTALL ME FIRST folder

4A. Double-click the “RENESAS-USB3-Host-Driver-30230-setup.exe” file.

4B. Follow the instructions and accept the terms of agreement.

4C. Once complete, click “Finish”.

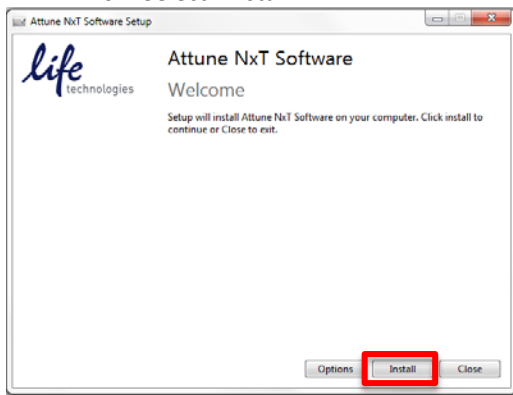
**Step 5-** Plug in the Deskey device and power on the Attune® NxT cytometer

**Step 6-** Install the software (Note-Follow the instructions and accept all defaults (see screenshots and details below))

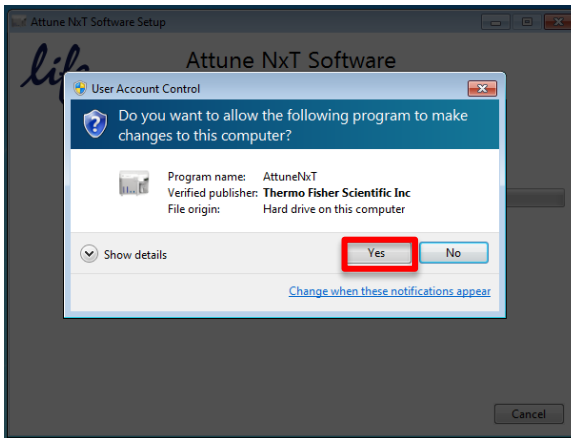
6A/ Double-click “SetupAttuneNxt.exe” in the “Attune NxT\_Build 2.2.0.8543 folder.

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.

6B. Select “Install”

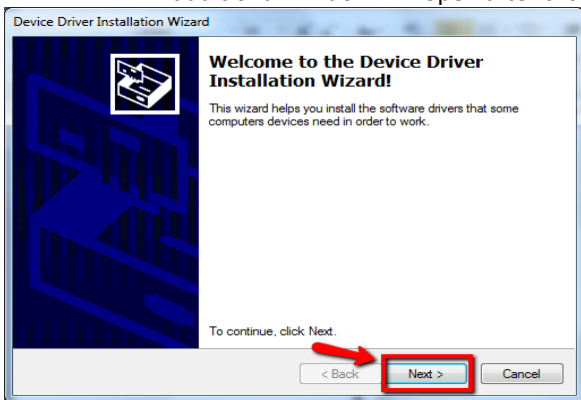


6C-Select "YES"



**Step 7- Install Device Driver**

7A-An additional window will open after clicking "Install". On this window, select "Next".

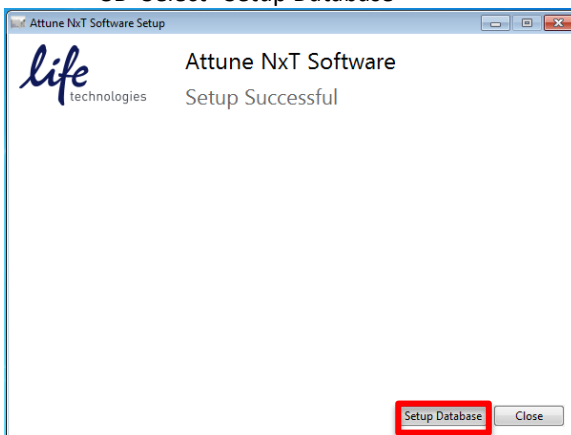


7B- Once complete, select "Finish".

**Step 8- Setup Database**

8A-Now Turn on Attune™ NxT cytometer

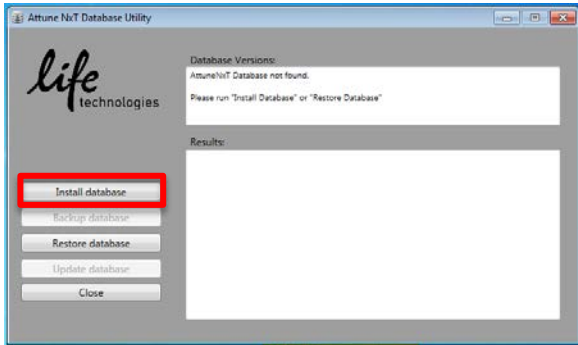
8B- Select "Setup Database"



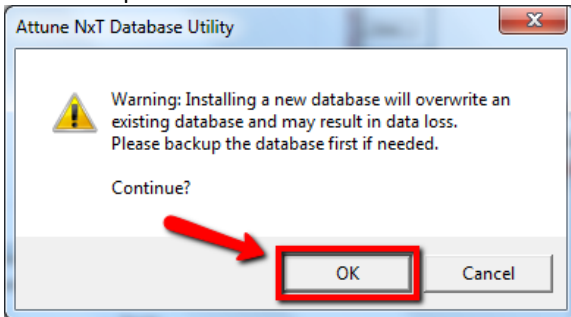


**Step 9-** Install Database (Note-The next window, shown below, may be minimized by default so look in your toolbar to bring this window into view to complete the installation.

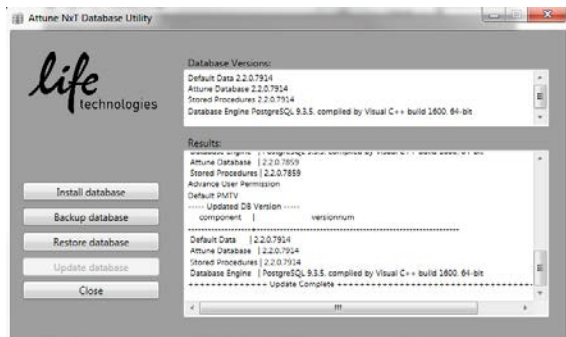
9A- Select "Install Database"



9B. Approve overwriting the existing database (Note-A warning notification will pop up in a separate window. Select "OK".

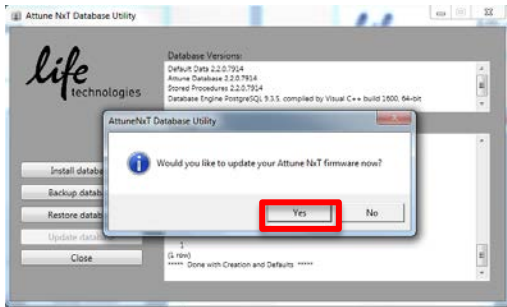


9C. Close the Database window after database update is complete



**Step 10-** Update firmware

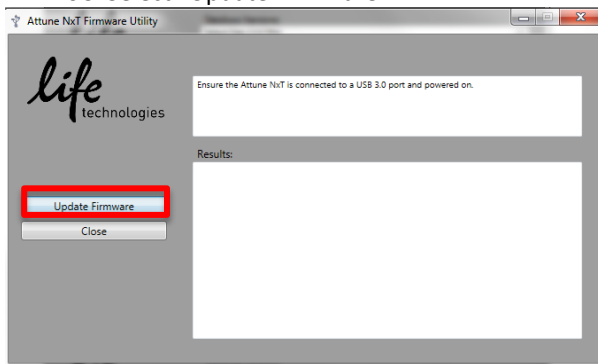
10A. Select Yes to update firmware



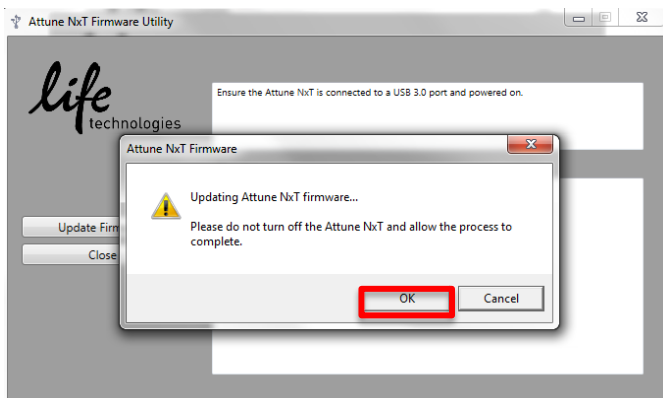
Note- the pop-up window will close automatically upon selection.

10B. Ensure Attune NxT instrument is connected and powered on and all programs are closed including Attune NxT software. Note if software has been opened before running firmware updater turn off instrument and turn it back on before proceeding to next step.

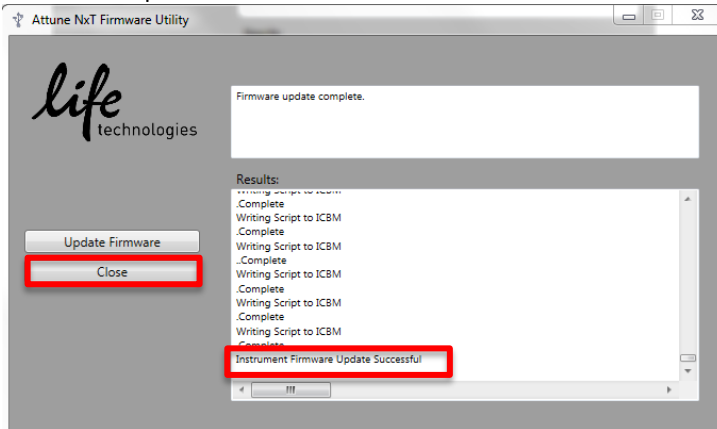
10C. Select "Update Firmware".



10D. Select OK. (Note-Do not disturb instrument computer during firmware update. Note- this process can take several minutes.)



10E. Verify Firmware update has been successful and Close Firmware window if successfully completed.



10F. Turn off and Turn on the computer and cytometer. Once the Attune® NxT instrument and the computer have been powered on again, double -click the shortcut icon “Attune® NxT Software” that has been created on the desktop to launch the application software.

**If you encounter this message, please double-click the “SetupAttuneNxT.exe” and re-run the installer**

