



Release Notes

Thermo Fisher Scientific
Attune™ Cytometric Software v5.1.1

In the following pages you will find instructions describing:

1. New software features
2. Software bugs fixed in this release
3. Known software/system issues with troubleshooting guidance
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™ Cytometric Software.

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

New software features implemented in Version 5.1.1 (includes 5.0 and 5.1.0)

- Batch import of FCS files
- Improved error recovery in case of missing experiment files (option to reset to default settings)
- Security updates
 - Postgres upgrade from 9.3 to 12.4
 - Service password now SB-327 compliant
 - SAE console updated to use HTTPS by default
 - OpenSSL updated to version 1.1.1
- Expanded plate definition support for both CytKick and CytKick Max
- Support for the Attune CytPix Flow Cytometer
 - User defined image acquisition settings
 - Image Gating to acquire images of specific populations of cells
 - Image frequency and image count settings
 - Image focus and illumination
 - Image size and position
 - Image view and image gallery
 - Image acquisition metrics (image count and image rate)
 - Image measurement tools to measure cell image area
 - Image backgating (on supported plot types)
 - Image customization
 - Filter images from workspace gates

Software Bugs Fixed In Version 5.1.1

- [EE-29248] – Fixed issue where exporting FCS files from experiments collected prior to 5.1 will generate a 1004 error if the experiment is not activated prior to exporting the FCS files in that experiment.
- [EE-28921] – Fixed issue when upgrading the software, the docking pane tabs may be mislabeled.
- [EE-29442] – Fixed issue where drag and drop of workspace badge from old experiments (imported or pre-upgrade) to new experiments w/out activation of old experiment results in crash
- [EE-29349] – Fixed issue where upgrading from 4.2 to 5.X fails due to msdriverinstaller issue
- [EE-29371] – Fixed crash while running a performance test where application was trying to connect to the cloud
- [EE-29374] – Fixed issue where updating FCS Keywords for compensation behaved differently between CytPix and NxT
- [EE-28636] – Fixed issue where there was a silent crash while running a performance test

Software Bugs Fixed In Version 5.1.0

- [EE-15874] - Fix default range for width parameter
- [EE-22840] – Fixed issue where there was a discrepancy between statistics show on plots versus workspace stats boxes for quadrant gates.
- [EE-23386] – Fixed issue causing signal in VL4 or VL5 to dropout while running plates
- [EE-23755] – Fixed issue with Decontamination reminder being displayed each time a new experiment is created and activated.
- [EE-25071] – Fixed issue with SAE audit and action records failing to synchronize with the SAE console.
- [EE-17329] – Fixed issue that when both X and Y axis are set to HyperLog™ and the X axis scale is set to 'manual' scale, any changes to 'manual' or 'automatic' setting of the Y axis will revert the X axis range to default settings (Min: 1, Max: 1048576)
- [EE-22886] – Fixed issue with batch print dialog progress bar hanging after printing multiple samples from the experiment explorer
- [EE-20894] – Fixed issue where having the option to enter reasons for change set for Workspace Text Boxes, and repeatedly clicking on a text box on the workspace resulted in multiple reason for change dialogs being displayed even if no change is made to the text.
- [EE-16644]- Fixed issue where moving a sample from one group to another audits a removal, addition, and update action; instead of just an update record
- [EE-23068] – Removed logging of sensitive information in log files (postgres password)
- [EE-26741]- Fixed issue where acquisition would stop prior to reaching specified stop options
- [EE-26637] – Fixed crash when changing HyperLog™ slider bars during acquisition
- [EE-25882] – Fixed issue where the Others section of the FCS Information panel was not displayed after appending data
- [EE-24203] – Fixed error that occurred when importing a sample list to update experiment annotation
- [EE-23730] – Fixed issue where run protocol settings were incorrectly disabled

- [EE-23175] – Fixed issue where workspace object IDs were duplicated when creating group and sample workspaces or when copying workspace objects from one workspace to another
- [EE-21462] – Fixed issue where modifying the compensation setup parameters was not being reflected correctly on the heatmap
- [EE-24805] – Fixed a data desync that occurred when acquiring samples after working in a service account and logging in as a non-service user
- [EE-22436] – Fixed issue with SAE permission for allowing workspace objects to be copied to the clipboard even when the permission was not given.
- [EE-25907] – Fixed issue with classic autosampler over drawing sample
- [EE-26808] – Fixed issue with CytKick carry over when using boost mode exceeding 1%
- [EE-28387] – Fixed crash when reactivating an experiment after logging out
- [EE-28340] – Fixed issue in image capture settings when toggling between duplicate samples
- [EE-28316] – Fixed issue where software crashes when clicking 'X' on Image Acquisition In Progress dialog displayed when trying to close the application while images are still be saved
- [EE-28313] – Fixed crash in USB communication that caused application to crash
- [EE-28299] – Fixed issue preventing a user from switching between auto and manual scaling of plots
- [EE-28255] – Fixed issue where saving a sample to a new sample doesn't copy Run Protocol settings
- [EE-28250] – Fixed SAE permissions that allowed an Image Container to be cut without permission
- [EE-28101] – Fixed a crash in the database utility that caused backups to fail
- [EE-28074] – Fixed issue with virtual folder management that caused the application to hang up to 10 seconds when opening a File dialog
- [EE-28022] – Fixed crash when plate customize panel settings were changed during acquisition
- [EE-27785] – Fixed issue where derived gate statistics did not update in the heatmap without manually opening each sample
- [EE-27619/ EE-27528] – Fixed issue in CytKick that causes excessive bubbling into sample
- [EE-26953] – Fixed issue where Auto-Refresh is no longer enabled by default
- [EE-26863] – Fixed issue where SAE auditing was not synchronizing with the SAE console
- [EE-26801] – Fixed issue where the common application data used by Attune is created w/ restrictive permissions that was preventing saving of application data
- [EE-26638] – Fixed issue where baseline and performance test fails if a width threshold has been modified in an experiment prior to running the baseline or performance tests

KNOWN ISSUES AND GUIDANCE

The following sections describe known issues with the Attune™ Cytometric v5.1.1 software. Where possible we have outlined steps to work around the known issue.

Guidance for Installing and Upgrading the Attune™ Cytometric v5.1.1 software:

- See the “Installation Troubleshooting Guidance” at the end of this document for more details.



- When upgrading the Attune software, the option to select the Attune only option is only enabled if SAE was not previously installed.

Guidance for Instrument Start Up and Performance Test using the Attune™ Cytometric v5.1.1 Software:



- **Power on the auto sampler (Attune™ Autosampler, CytKick and CytKick Max) before the Attune™ cytometer (NxT and CytPix).** 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
- If the baseline or performance test fails, restart the application prior to re-running the baseline or 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	Press the “Rinse” command on the instrument ribbon tab to force the application to sync with the instrument state. If the startup icon is not cleared after rinsing, 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.
EE-19783	CytKick autosampler line calibration will fail if the line is not properly primed.	If autosampler line calibration fails (CytKick and CytKick Max only), re-run the startup procedure to prime the lines between the instrument and autosampler.
EE-27811	If application is restarted when waking the instrument from sleep, the autosampler calibration date is lost until the software is restarted	Do not restart the software while the system is waking from sleep; wait until startup completes before restarting the software.
EE-28025	If the application is restarted when waking the instrument from sleep, startup resumes without checking the tube lifter state	Do not restart the software while the system is waking from sleep; wait until startup completes before restarting the software.
EE-28988	Startup does not work after focus and water bottles are reconnected to an autosampler during shutdown.	Power-cycle instrument to commence start up. Do not disconnect the focus and waste bottles during instrument shutdown; ensure waste bottle is empty and focus fluid bottle has sufficient volume prior to shutdown.

Guidance for Setting up an experiment using the Attune™ Cytometric v5.1.1 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 is not automatically active after its creation. The plate experiment is visible in the experiment explorer but is not the experiment viewed in the Attune™ Cytometric Software desktop.	Double click on the new experiment to activate it, create samples or groups of samples using the Experiment ribbon tab, expand the group and double click to activate a sample to setup the workspace, define the run and acquisition criteria, setup instrument settings, and acquire samples.

EE-8864	When creating experiments, the software does not check to see if 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 experiment explorer items, Error -1 is returned, and attempting to open any experiment that doesn't contain XML files, returns an undefined error	Export experiment data from the experiment explorer and then remove experiments to free up disk space
EE-17828	\$LASERCONFIG keyword on model A29004 not updated correctly	Check model number stored in the \$CYT keyword. You can view FCS keywords in the FCS information Panel.
EE-28314	When importing templates, the import button does not become disabled allowing multiple 'Open' file dialogs to be displayed. When this occurs, import of a template file may result in an error 2000.	When importing templates, do not click the import button more than once. Once the Open file dialog is displayed, select the template file you wish to import and click the open button.

Guidance for using the Experiment Explorer and Instrument Settings panel within the Attune™ Cytometric v5.1.1 Software:

Issue ID	Description of Known Issue	Suggested Action
EE-8865 EE-8866	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.
EE-27235	Infrequently, when the instrument model is changed, the instrument settings panel may show the instrument settings of the previous model.	In the event the instrument settings panel does not reflect the active instrument model, restart the software to force an update to the instrument configuration.
EE-27667	Infrequently, the Attune application may fail to completely close which will block a new instance of the Attune Cytometric software from launching.	In the event the Attune Cytometric software fails to launch, check the task manager to ensure that no prior instance of the Attune software is running. If it is, kill the process using the task manager.

EE-16263	When importing an FCS file that was recorded with a user defined window extension, the instrument settings will not update to reflect the user defined window extension	To view the window extension of an imported FCS file, use the FCS Information panel to view the value of the window extension saved in the #WINEXT keyword.
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Guidance for using Compensation within the Attune™ Cytometric v5.1.1 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 whole matching instrument settings in order to be imported into an experiment as compensation controls.
EE-16607	When an experiment is duplicated with read only compensation settings, compensation settings are duplicated even when not selected	If the compensation settings are not required or need to be modified, right click on “Compensation” in the Experiment Explorer and select “Remove Compensation” or select “Compensation Setup” to modify.
EE-27739	Creating compensation setup on an imported experiment that does not match the application’s active instrument model results in “an unknown error”	In order to create or modify experiment compensation ensure that the active model for the system matched the model for the imported experiment.
EE-26562	When the image gallery zoom is set less than 20%, the background of the image gallery is not drawn correctly.	Zoom in until the background of the image gallery is rendered correctly (set the zoom greater than 20%)

Guidance for Acquiring Samples using the Attune™ Cytometric v5.1.1 Software:



- **When the application is run continuously over a period of days, the application's user interface may show artifacts where buttons and dialogs are not rendered correctly. If this is observed, restart the application. It is recommended that the application is restarted on a daily basis to avoid this issue.**
- 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.
- When running plates, it is suggested to limit the number of mixes to 2 or less to prevent bubbles being introduced into the sample.
- 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.
- 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 10²).
- We strongly recommend running the Attune (NxT and CytPix) with a surge protector. Line noise can interfere with the USB connection and cause data desyncs and communication faults in the instrument.

Issue ID	Description of Known Issue	Suggested Action
EE-15379	When collecting additional data and appending an 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 while running a plate, 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	Do not press stop on the last well of a plate run as it will result in loss of any unprocessed sample.

	rinse so there is no chance to recover 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 not 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 reset button (adjacent to the target and label names) 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 be more events than the stop condition.	If a precise number of events is required for the stop condition, ensure that the volume is enough to acquire all events.
EE-9735	Sample recovery may not be available if the application is restarted prior to recovering sample.	Ensure sample recovery is performed prior to closing the software.
EE-27509	Boost Mode (available for CytKick Max) is enabled for all acquisition volumes (at the 500 and 1000 $\mu\text{L}/\text{min}$ flow rates) but is only supported for volumes less than 200 μL .	If boost mode is selected and a volume greater than 200 μL is acquired, the acquisition will not use the boost mode option when acquiring wells with these settings. For boost mode benefits, ensure the well volume is less than 200 μL .
EE-28312	The stop button in the collection panel may fail to stop a run.	If the stop button fails to stop a run, use the instrument ribbon tab’s stop button to stop the run.

EE-29604	When Boost Mode is enabled, the plate time will not reflect changes to flow rate, acquisition time, or stop criteria.	None.
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Guidance for using the Experiment Workspace (Gates, Plots, Stats) within the Attune™ Cytometric v5.1.1 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-28304	When a gate is added to a plot where one of the axes is set to auto scale and the sample does not have data, the gate will be added outside the plot range making it unselectable.	Record a sample or set the axes to manual scale to ensure the gate is drawn within the plot range.
EE-26776	After resizing workspace objects in autolayout mode, the free space that may be created is not automatically filled.	To force the autolayout to complete, move a workspace object to force the filling of the free space that may have been generated by reducing a workspace objects size.
EE-27733	When a plot is selected and instrument settings are modified the plot will become deselected but the customize panel will show the customize settings of the previously selected plot and will not function	Reselect the plot to ensure the customize panel changes apply to the plot selection(s)

EE-27654	Infrequently, when running a sample over existing data, then saving the data and selecting the option to <i>Save to new sample</i> the resulting FCS file may have event IDs starting at greater than 1	In the case that this is observed, the image and events IDs are correctly synchronized but start at an event ID greater than 1
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Guidance for working with Overlays within the Attune™ Cytometric v5.1.1 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.
EE-28315	Samples disappear from sample lists in overlay builder when using arrows to move samples to/from the selected samples list	Close and reopen the overlay builder to reset the sample lists to ensure all samples are populated in the sample lists
EE-28302	When a file is recorded, removed and then re-recorded, a concentration is being displayed during acquisition that may not be correct.	The concentration statistic should not be displayed during acquisition as the volume is unknown while acquiring. When this is experienced, the concentration may be incorrect but will be updated to the correct concentration when the acquisition is complete.

Guidance for Data Analysis / Data Display using the Attune™ Cytometric v5.1.1 Software:



- Analysis of 3rd party FCS files in the Attune™ Cytometric 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™ Cytometric v5.1.1 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.
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Guidance for running Maintenance Functions:



- The startup and self-test functions can become frozen if the USB is disconnected from the auto sampler or auto sampler is turned off while running the startup or self-test maintenance functions. 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.
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Issue ID	Description of Known Issue	Suggested Action
EE-27057	Infrequently, the instrument may get stuck in a busy state after completing an acquisition	If the instrument appears to be busy after completing an acquisition, click the instrument ribbon’s stop button to return the system to idle.
EE-26965 EE-28348	Infrequently, the instrument may get stuck in a busy state after completing a rinse and may require the instrument to be restarted	If the instrument appears to be busy after completing an acquisition, click the instrument ribbon’s stop button to return the system to idle. If this does not return the system to an idle state, first try restarting the software. If this does not, restart the instrument.
EE-28569	Sanitize SIP duration is longer on Win 10 environment vs Windows 7	When running a SIP sanitize on a plate, the SIP sanitize may take up to 8 minutes.

Guidance for using the External Fluid System (EFS) with the Attune™ Cytometric v5.1.1 Software:



- When both the focusing fluid bottle within the Attune™ Cytometer (NxT or CytPix) and the 10 L cubetainer of focusing fluid on the EFS are empty, **DO NOT disconnect the Attune™ focusing fluid bottle**. Instead, replace the empty cubetainer of focusing fluid on the EFS; once reconnected the Attune™ Cytometer focusing fluid bottle will re-fill as expected.
- If the on-board Attune™ Cytometer focusing fluid is disconnected, the EFS may get in a locked state. Once in a locked state the EFS must be reset by unscrewing the grey EFS electrical cable from the back of the Attune™ Cytometer (NxT or CytPix), waiting 30 seconds, then re-screwing the electrical cable into the Attune™ Cytometer (NxT or CytPix).

Guidance for Setting up user accounts and user options using the Attune™ Cytometric v5.1.1 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 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™ Cytometric v5.1.1 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	If the plate run does not resume using the “resume option” in the automation software, resume the plate run using the driver window.

	not resume, use the driver window to resume.	
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.
EE-12825	Leak detection dialog is not displayed when an AAS leak detection occurs before plate is handed off to AAS in automation mode. Once leak is resolved, system goes into a loop that requires the plate abort.	Abort the process and restart the plate acquisition sequence in the automation software.
EE-28363	Application becomes unstable while running plates with image acquisition using an Orbitor	Do not run plates using automation mode (Orbitor) while acquiring images

Guidance for Connecting to Thermo Fisher Cloud using the Attune™ Cytometric v5.1.1 Software:



- If a timeout occurs while registering the Attune™ Cytometric software to connect to the Thermo Fisher Connect or logging a user into the Thermo Fisher Cloud from the Attune Cytometric 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 Cytometric software and Thermo Fisher Cloud.

Guidance for using the SAE Features in the Attune™ Cytometric v5.1.1 Software:



- Ensure audit settings are configured prior to using the Attune software in SAE mode. Changes to audit settings (selected audit types) will result in audit gaps if experiments are modified after changing the selected audit types used to track changes in experiments.

Issue ID	Description of Known Issue	Suggested Action
EE-20918	'Collection Wells' attribute in plate audit displays wells out of order	There is no mitigation for this issue.
EE-20667	Cancelling the e-Signature report loading progress dialog does not revert back to the previously selected report	Reselect the previous report or select a new report to open the report.
EE-17454	Certain Unicode characters are not supported in the e-Signature report and will not be rendered correctly.	Avoid the use of the following Unicode character sets: Devanagari, Bengali, Gurmukhi, Gujarati, Oriya, Tamil, Telugu, Kannada, Malayalam, Control Pictures, Optical Character Recognition, Miscellaneous Technical, Kanbun, Hangul (Syllables and Compatibility Jamo, and CJK Compatibility Ideographs, and Enclosed CJK Letters and Months
EE-19083	If a user locks their account due to repeatedly attempting to login with invalid credentials, and the SAE service is interrupted while the locked user account is waiting for the end of the timeout period, the account never unlocks after the timeout period has lapsed.	Have and an SAE administrator manually reset the account to active in the SAE console.
EE-19216	The e-Signature report will not be regenerated if the page size or orientation is changed when the workspace is blank (no workspace objects are present).	Make another change to the experiment to obsolete the current unsigned or signed e-Signature report to force a new report to be generated with the expected page size and orientation.
EE-19267	If the DESkey missing dialog is displayed, selecting the no option to retry DESkey authentication will result in a system error.	Logout of the application and ensure the DESkey device is present and correctly functioning. If a network license is used, ensure the remote server is connected and online.

EE-19644	If a user repeatedly enters a correct username with an invalid password and the account lockout policy is setup to lock a user account after a certain number of invalid attempts, the lockout message will not be displayed until the user enters the correct password.	To see the account lockout message in the Attune™ Cytometric software, enter the correct username and password.
EE-20078	When signing an e-Signature report using the pending signatures dialog, the selected meanings are based on the currently logged in users remaining meanings and not the remaining signatures of the signee.	The pending signatures dialog is intended to be used by the actively logged in user and not shared so that other users who also need to sign the report can apply their signatures. Those users should sign in with their own accounts or use the Sign Report dialog workflow.
EE-20482	When data is restored using the Attune Database Utility, the data in the Attune may become out of sync with the data in the SAE Console. The Attune Database Utility will backup the SAE console data but does NOT restore the data automatically. This will result in audit gaps and inconsistencies between the Attune software and SAE console.	Prior to restoring Attune data and the Attune database, backup all SAE data using the SAE console. When restoring the Attune data and database, the SAE data will have to manually copied back to the SAE database folder to restore the SAE data so that it is in sync with the Attune data.
EE-27773	If permissions are set in the SAE console to prevent deletion of cell images this permission will block deletion of other workspace objects if the selection includes both cell image containers and other workspace objects (i.e. plots, stats boxes,etc)	If the permission is set to prevent deletion of cell image containers and the user is needing to delete other workspace object types that they have permissions to deleted, ensure the selection does not include any cell image containers.
EE-27749	If an account is disabled while in active session, its activity is still audited	Changes to account status will not be applied until the user logs out. Certain permissions and restrictions will be applied immediately but auditing of the current session will continue until the user logs out.
EE-17649	If the account lockout duration is changed on the SAE console after an account is locked out due to invalid login attempts, the lockout duration will be based on the original lockout duration settings.	To unlock a locked user account, either wait for the specified lockout time or have an SAE administrator login to the console and reset the account status to active.

EE-27632	Attune is unable to verify SAE user passwords that contains special characters	Do not use passwords containing the following special characters: % & +
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Guidance for using the CytPix Image Acquisition/Analysis Features in the Attune™ Cytometric v5.1.1 Software



- The image acquisition and analysis features are only available when connected to an Attune CytPix Flow Cytometer or when a CytPix experiment is active
- The total images option will allow up to 30,000 images to be collected when this option is checked. When unchecked, the system will determine the maximum number of images based on available RAM (up to 1,048,576 images)
- It is possible to define image gates and storage gates that are mutually exclusive meaning that the acquired images will not have any corresponding FCS file events and will not be displayed in the software

Issue ID	Description of Known Issue	Suggested Action
EE-28248	When acquiring a large number of images (500K+) the software may become slow.	When acquiring a large number of images, wait for the image processing to complete before starting another acquisition.
EE-28228	If other activities are performed that use the status bar's progress bar, the image transfer/saving progress bar will not be displayed until there are new updates to the image transfer/saving progress.	Images are transferred and saved in the background and will update the status bar progress bar while the images are being processed. During acquisition, this background progress is not displayed and it may be interrupted if other functions that show progress are running while the image processing is occurring.
EE-28102 EE-27717	If camera settings are changed very quickly a 'Fatal Error' dialog may be displayed informing the user that the camera could not be stopped or started.	This error will typically resolve itself and no additional action should be required. To avoid this error from being displayed, wait a second between making additional changes to the camera settings (window size and position settings).
EE-27770	When a cell measurement tool is inserted to a cell image container, the measurement tool will not be displayed until the cell image has data to display.	The cell measurement tool will be added in a default location and will be displayed when the cell image container has an image to display.
EE-27053	If the clear button is pressed during an acquisition with less than 20 total	The image counter is updated as new preview images arrive. When there are less than 20 images, no previews will be

	images, the acquired image counter is not reset.	available and the image counter will not refresh correctly.
EE-26813 EE-28223	If the software is closed and immediately reopened it can freeze for up to about 5 seconds as it waits to connect to the camera firmware.	When the application closes, the camera firmware gets reset which means that restarting the Attune software within this period will result in a delay for the application to connect to the camera firmware. After 5 seconds the application should fully open. If the application does not respond after 10 seconds, close and restart the software.
EE-27208	If a user does not have file read/write permissions for the image storage folder before starting acquisition they will not be warned prior to starting the acquisition.	In the case, that a user does not have read/write permissions for the image storage folder, they will only be warned at the point where images are saved where they will be provided an option to choose another location to save the images.
EE-27210	If the storage location where images are saved is filled while saving an image, the image may not be able to be reloaded as the image metadata may be incomplete	Before acquiring samples, the disk space is checked to ensure there is enough space to complete the run. While acquiring, ensure that no other programs are writing files to the same disk location as the disk may be filled up while the Attune application is trying to save image files. In the case that this happens, the image file may be recoverable by using a zip utility (i.e. 7zip) to recover and fix the image file.
EE-27769	Image view and Image gallery images cannot be copied and pasted to external applications during acquisition	Wait for acquisition to complete before copying image view/gallery images to external applications.
EE-26368	When clearing multiple times, preview images from the data acquired prior to clearing may persist until new images arrive	Wait for new preview images to arrive to update any stale images that may persist after clearing the run data.
EE-22599	When multiple measurement tools are added to a cell image, the labels can overlap become illegible	Avoid adding too many measurement tools that could result in the area measurement labels overlapping

Other Known Issues:

Issue ID	Description of Known Issue	Suggested Action
EE-19651	While tooltips (warning balloons) are displayed, the application window background may be rendered with a gray background.	Clear the error resulting a balloon tip message being displayed to allow the main window to be redrawn correctly.
EE-19623	If an experiment name is too long to be display in the heatmap legend, there are no ellipsis symbols to indicate that the name is truncated	Rename the experiment to shorten the name to allow the full experiment name to be displayed in the heatmap legend.
EE-20286	Two characters cut off from experiment title when heatmap is printed with max experiment title length	Rename the experiment to shorten the name to allow the full experiment name to be printed for the heatmap legend.
EE-19184	Certain DPI settings will prevent the Attune Cytometric software from opening when running under Windows 10	If the Attune application does not start, check the DPI settings. The application should work with DPI settings set as follows: 2k Settings – 100%, 125%, 150%, 175% 4k Settings – 100%, 125%, 150%, 175%, 200%, 225%, 250%
EE-18899	Overlay plots don't render correctly when DPI settings are changed	The overlay plots are correctly sized when the DPI settings are set with 2K resolution at 100%. If the resolution is set to 4K and 200% DPI, the plots will be 50% smaller than expected. The zoom settings can be used to scale the images to the appropriate size.
EE-21019	The appearance of the 'File' menu's dropdown may show the dropdown contents spilling outside the dropdown area.	This is cosmetic issue that has no functional impact. Restarting the application may fix the appearance of the file menu.
EE-19651	While balloon tooltips are displayed the background may not correctly paint (background will paint grey)	This is cosmetic issue that has no functional impact. Once the balloon style tooltip is no longer displayed, the background will be correctly painted.
EE-28081 EE-21535	When the Attune is disconnected (powered off or the USB is disconnected) the Attune software may not display the Attune disconnected popup	If the Attune is powered off or the USB is disconnected the status bar alarm icon will be displayed but the popup may not be displayed. If the disconnected icon is displayed in the status bar, ensure the

		Attune is powered on and that the USB is connected. Restart the software if the USB is reconnected while the software is running.
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Installation Instructions - READ ALL INSTRUCTIONS BEFORE PROCEEDING

System Requirements: Quad core processor, 16 GB RAM, 500 GB disk space available. Windows 10 Enterprise LTSC 2019 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.
 - Ensure the monitor is plugged into the display port on the add-on graphics card (PCI slot), and that the drivers for the card have been installed
-



- **It is essential that all data is backed up prior to proceeding to install the Attune™ Cytometric Software v5.1.1. Refer to the “Data Backup” instructions for more information on backing up data.**
 - Resolve any pending signature requests before backing up data. Failure to do so can lead to the inability to sign for the pending request and will require the user to modify the experiment and re-request the signatures.
-

UPGRADE FROM v3.2.1 or earlier to Attune™ Cytometric Software v5.1.1

For computers attached to an instrument please contact your Technical Sales Specialist to obtain a quote for the necessary items to proceed to the upgrade. If you have already purchased Windows 10 upgrade kit, please contact service for installation. Do not attempt to self-install software as it will not work and may damage your system.

Prior to upgrading you must backup your data to migrate the data from the pre-Windows 10 release. See the “Data Backup” section for instructions on how to backup your data.

INSTALL OR UPGRADE FROM v4.0, v4.2, v4.2.1 to Attune™ Cytometric Software v5.1.1

Overview:



*These steps are only relevant for installation or upgrade on an Attune instrument

Software Installation



If upgrading, ensure **All Data** is backed up to an external storage device before performing the upgrade process.

Step 1 Restart or power on the computer.

Step 2 Ensure that the DESkey USB key that is used to run the software is plugged into the computer.

Step 3 Log into Windows as an administrator.

For the Instrument PC, the default username and password are:

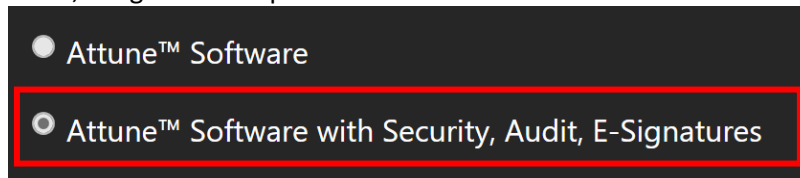
User: INSTR-ADMIN

Password: INSTR-ADMIN

Step 4 Unzip (select “extract all files”) the Attune_5.1.1.zip file to the desktop.

Step 5 Install the software:

- a. Double-click “SetupAttune.exe” in the “Attune 5.1.1” folder.
- b. To install the 21 CFR Part 11 option, select the “Attune™ Cytometric Software with Security, Audit, E-Signatures” option.

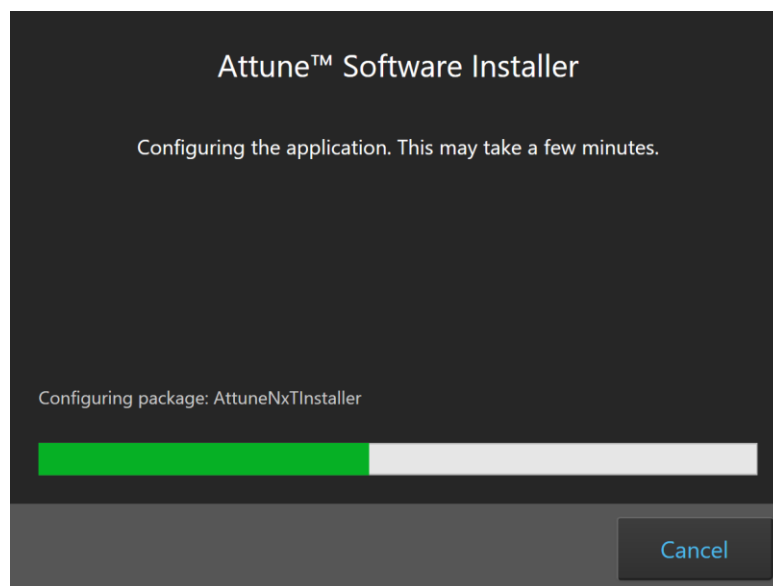


NOTE: If you have not purchased the 21 CFR Part 11 option, the SAE features will not be available in the software. When upgrading from an installation where SAE was previously installed, this option will automatically be selected.

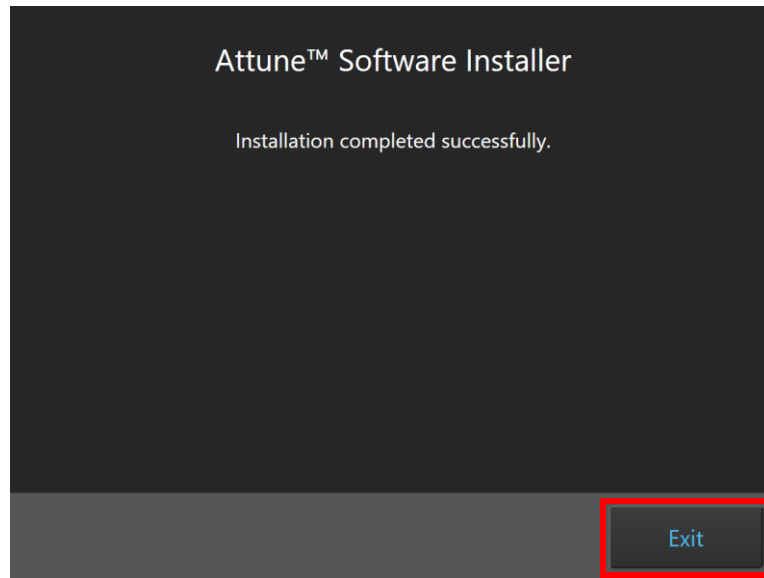
- c. Select “Install” to start the software installation.



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 “SetupAttune.exe”



Step 6 When the installation has completed, the “Installation completed successfully” message is displayed. Close the installer by clicking the “Exit” button (shown below).



Step 7 Once the software installation is complete, the instrument firmware will be checked (if the instrument is connected and powered on) and, if an update is required, the firmware update utility will be launched.

Overview:



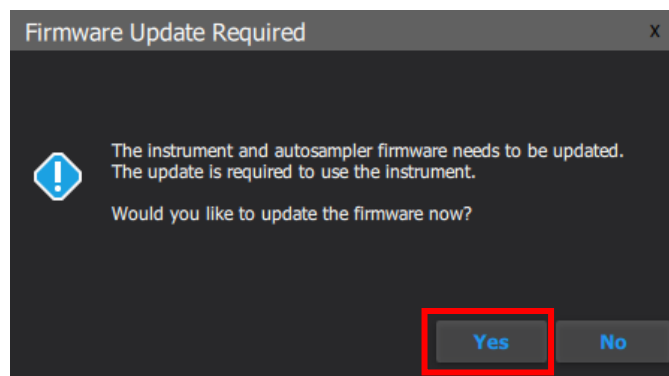
*These steps are only relevant for installation or upgrade on an Attune instrument

Firmware Installation - Launched from 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.
- The firmware update process should take less than 15 minutes.
- **NOTE:** The firmware installation will appear differently depending on whether the firmware utility is launched as a standalone utility or directly from the installer or upon startup of the Attune application.
- **Power Cycle** (turn off, then on) both the autosampler and instrument to complete the firmware update. The instrument and autosampler must be powered on in a specific order. Power on the autosampler first, then power on the instrument.

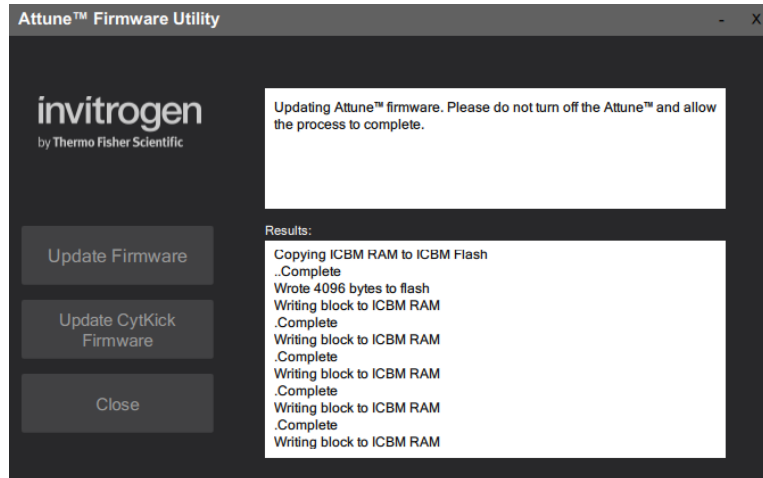
-
- Step 1** Prior to installing the software, connect the Attune to a USB 3.0 CPU port on the BACK of the PC.
- Step 2** Ensure the Attune is powered on and POST (Power-on self-test) has completed (the front panel LEDs are solid blue or green, NOT fading in and fading out).
- Step 3** Install or update the Attune software. Wait for the installer to complete.
- Step 4** After the software installation has completed, the firmware may need to be updated. **The firmware updater utility will automatically launch if the instrument is powered on and connected.**



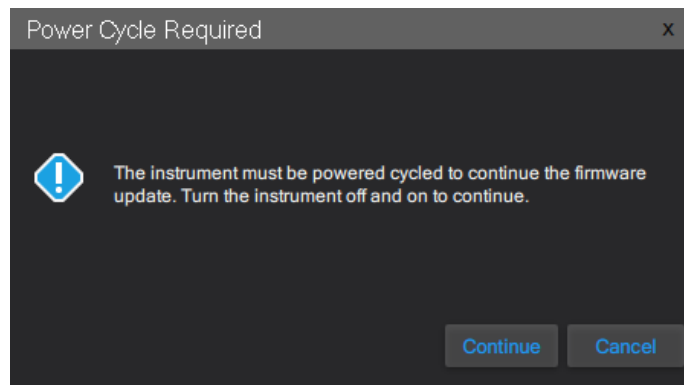
- Step 5** To start the firmware update, click the “Yes” button.



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



Step 8 If prompted, power-cycle the Attune to continue the update. Wait for the instrument to complete POST before selecting “Continue” (the front panel LEDs are solid blue, NOT fading in and out).



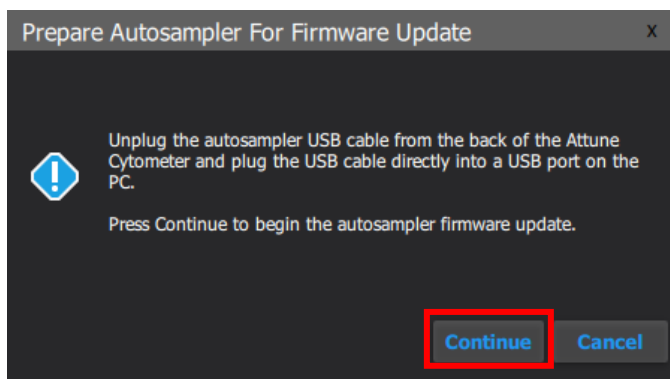
Step 6 Wait for the firmware update process to complete.



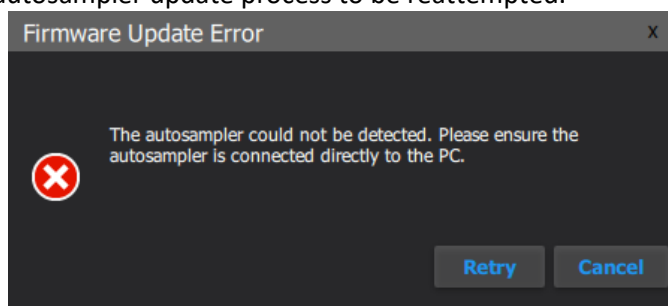
- If an error occurs during the update, power-cycle the instrument. The Firmware Utility can be kept open. Wait for the instrument to finish POST after the power-cycle and try the update again.
- If an error occurs again, power-cycle the instrument, restart the Firmware Utility (FWUtility.exe located at C:\Program Files\LifeTechnologies\AttuneNxT), and move the Attune connection to the USB 3.1 expansion card. Try the update again. If an error persists, contact technical support.

Step 7 Once the instrument firmware has completed, the autosampler firmware may need to be updated.

Step 8 To update the autosampler firmware, unplug the autosampler USB cable from the back of the Attune Cytometer and plug the USB cable directly into a USB port on the PC. When ready, click the “Continue” button to begin the autosampler firmware update.

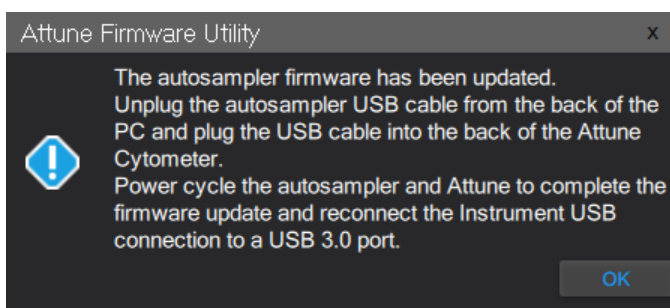


NOTE: If the autosampler is not detected within 1 minute, a dialog will be presented allowing for the autosampler update process to be reattempted.



- Do not use the USB 3.1 Expansion Card to upgrade the autosampler firmware. The autosampler may not be detected if the USB 3.1 Expansion Card is used. Connect the autosampler to either a USB 2.0 or USB 3.0 port built in to the PC.

Step 9 Once the autosampler firmware has completed, unplug the autosampler USB cable from the back of the PC and plug the USB cable into the back of the Attune Cytometer.



Step 10 Reconnect the Attune connection on the PC to the USB 3.1 expansion card.

Step 11 Power Cycle (turn off, then on) both the autosampler and instrument to complete the firmware update. The instrument and autosampler must be powered on in a specific order. Power on the autosampler first, then power on the instrument.

Step 12 Click the 'Close' button to exit the firmware update utility.

Step 13 The software application and instrument are now ready to use.

Step 14 Launch the software using login credentials used in earlier software versions.



- If the software reports the instrument is not connected, wait for the instrument to complete POST, then restart the software.
- If the software is still reporting that the firmware is out of date, repeat the firmware update process.

Firmware Installation - Standalone Installation



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. In this case, follow the same process described above to update the firmware.

Step 1 Connect the Attune to a USB 3.0 CPU port on the BACK of the PC.

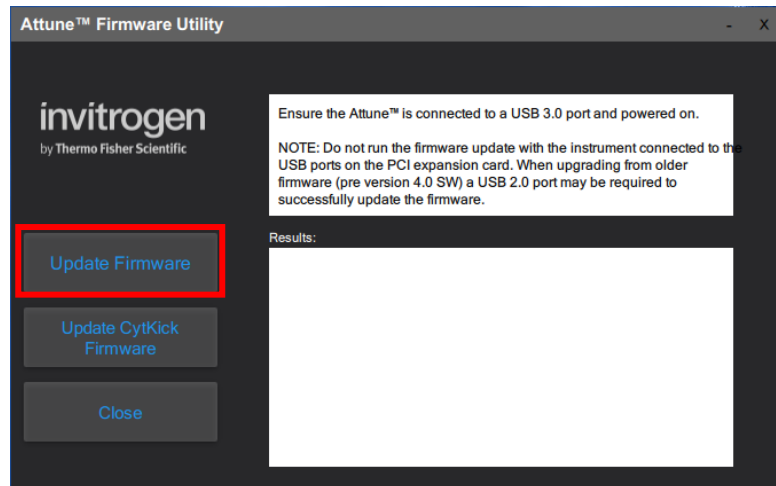
Step 2 Ensure the Attune is powered on and POST (Power-on self-test) has completed (the front panel LEDs are solid blue or solid green, NOT fading in and fading out).

Step 3 Launch the firmware utility “FWUtility.exe” located in “C:\Program Files\LifeTechnologies\AttuneNxT”

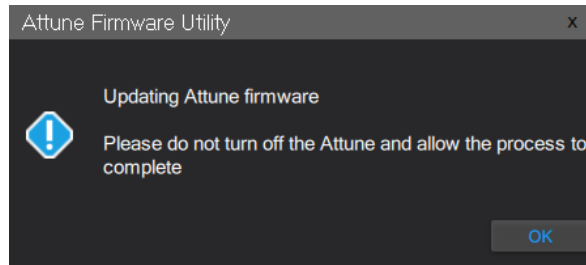


When updating instrument firmware using the standalone utility, the firmware can be updated selectively for either the instrument or autosampler.

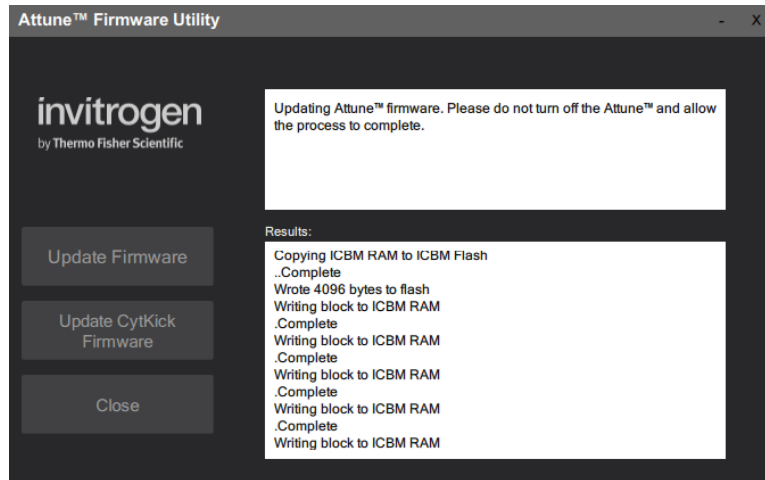
Step 4 To update the Attune Cytometer firmware select the ‘**Update Firmware**’ button.



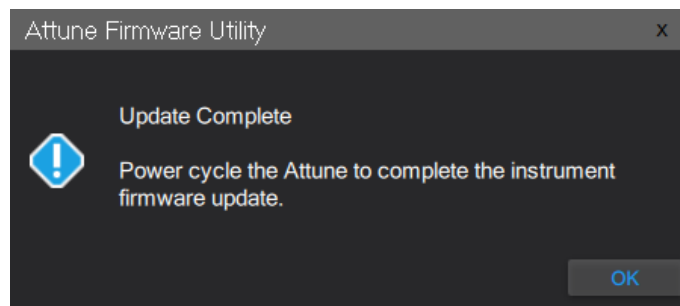
Step 5 Click “OK” to confirm the request to update instrument firmware (shown below).



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



Step 6 Once the firmware has completed, the “Update Complete” dialog is displayed indicating the firmware update was successful:



Step 7 Reconnect the Attune connection to the USB 3.1 expansion card.

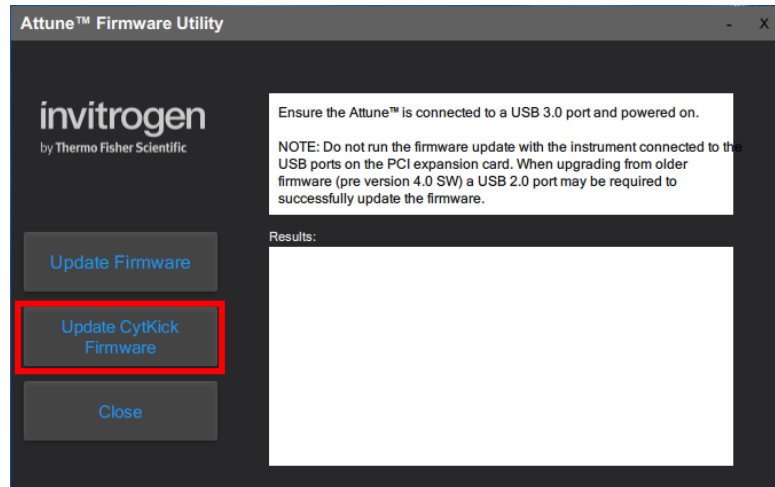
Step 8 **Power Cycle** (turn off, then on) both the autosampler and instrument to complete the firmware update. The instrument and autosampler must be powered on in a specific order. Power on the autosampler first, then power on the instrument.

Step 9 Click the ‘Close’ button to exit the firmware update utility.

Step 10 The software application and instrument are now ready to use.

Step 11 Launch the software using login credentials used in earlier software versions.

Step 12 To update the autosampler firmware select the '**Update CyKick Firmware**' button.



Step 13 Follow the instructions to update the autosampler firmware described above.

DATA BACKUP

Software Installation

Step 1: Log into Windows using your credentials.

Step 2 Ensure the Attune™ Cytometric software is not running.

Step 3 Launch the Attune™ Cytometric Database Utility from the startup menu
(**Start > All Programs > ThermoFisher > Attune™ Database Utility**)

Step 4 Log into the Attune™ Database Utility using your Attune™ Cytometric Software login credentials.

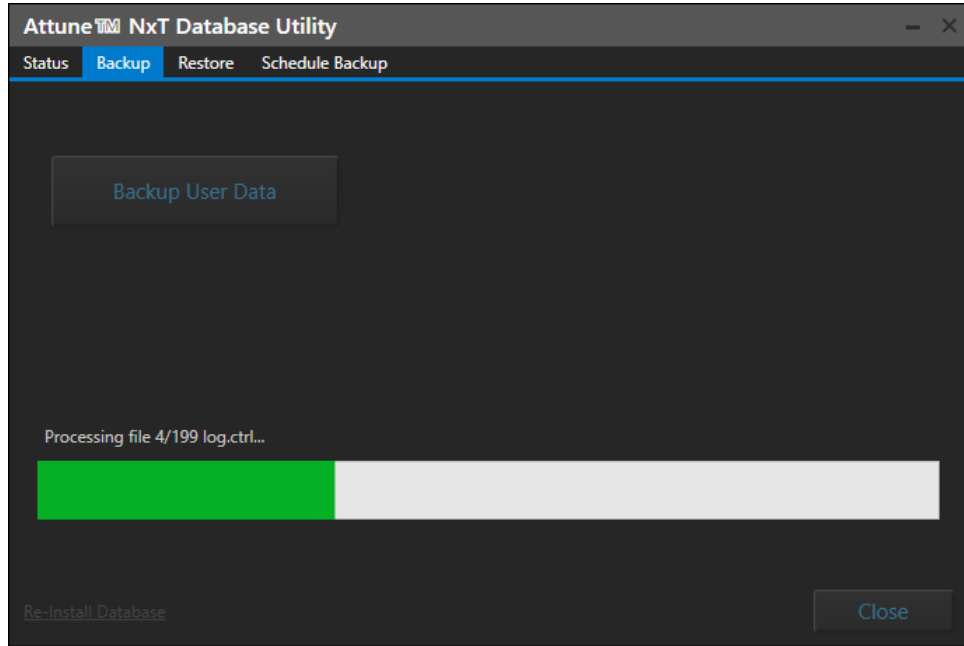
Step 5 Navigate to the **Backup** tab

Step 6 Click the *Backup User Data* button and select a location (network or external hard drive/thumb drive) to backup the data and database.

Step 7 Once a location is confirmed, the backup proceeds and the progress is displayed.



- The backup process takes a snapshot of the database and copies all Attune user data to the specified location. As user data can be quite sizeable, the backup process can take a significant amount of time. Plan accordingly prior to upgrading to account for the backup time.
- If the Security, Auditing, and e-Signature (SAE) module is installed, the backup will also include the SAE database which contains all security configurations, auditing, and e-Signature records. For restoring SAE data, refer to the section 'Restoring Data From Backup'



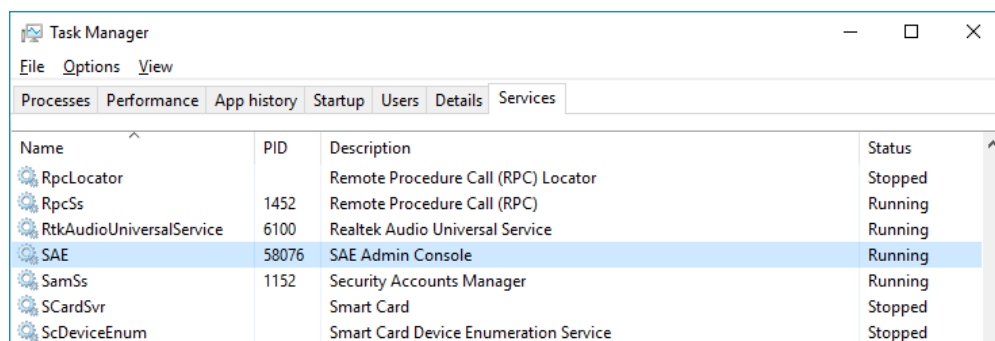
Restoring Data From Backup



- Restoring from a backup will cause current system data to be lost
- Restoring the database from attunenxt.backup without replacing the SAEDB folder with the files located in the SAE_DB.zip will lead to audit issues, and require the user to allow non-SAE files from the SAE Administrator Console.

Step 1 Ensure that the Attune™ Cytometric software is not running.

Step 2 Stop the SAE service via the Services tab of the Windows Task Manager or by entering “net stop SAE” into a command prompt (run as an administrator).



Step 3 Launch the Attune™ Database Utility from the startup menu (**Start > All Programs > ThermoFisher > Attune > Attune™ Database Utility**)

Step 4 Login to the database utility using your Attune credentials and navigate to the **Restore** tab.

Step 5 Click the *Restore User Data* button, then navigate to the location of a SAE backup (network or external hard drive/thumb drive) and select the attunenxt.backup file.

Step 6 Once the attunenxt.backup file is confirmed, press Open.
Restore proceeds and the progress is displayed.

Step 7 Delete the contents of SAEDB directory, C:\Program Files\ThermoFisher\SAEAdmin\SAEDB

Step 8 Unzip the contents of SAE_DB.zip located in the backup directory.

Step 9 Place contents of SAEDB folder extracted from zip in to the SAEDB directory, C:\Program Files\ThermoFisher\SAEAdmin\SAEDB

Step 10 Restart SAE service via Windows Task Manager or “net start SAE” in a command line