Release Notes

Life Technologies®
Attune® NxT Cytometric Software v 2.0.0

In the following pages you will find instructions describing:

1. Software installation
2. New software features and fixes
3. Known software/system issues with troubleshooting guidance
4. System Decontamination 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 2.0 ("Attune Software v2.0"). Two different instructions are provided: one for installation of software on a new system and one for software upgrade from a previous software version. Please ensure that you are following the correct instructions before beginning

Installation Instructions:

IMPORTANT: All instruction must be exactly followed as described and if they are done in a different order, then the correct installation will not result (either a new install or an upgrade from v1.1 to v2.0 of the Attune Software

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

Attune Software v2.0 Installation (New Install only, if upgrading from v1.1 see section below)

Software Installation
Note: Do not launch the Attune NxT 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)
3. It is recommended that no other applications are running while completing these steps.
4. Unzip the AttuneNxT.zip and run "setup.exe". Follow the instructions and accept all defaults.
5. After install is complete, open a Windows Explorer window and navigate to the Attune NxT install folder, default <C:\Program Files\LifeTechnologies\AttuneNxT\>
6. From this folder, install the drivers and database as described below.

Database and Driver Installation

1. Attune®NxT Third Party Dependencies

1.1. Navigate to the Attune installation directory. The default is <C:\Program Files\LifeTechnologies\AttuneNxT\>

1.2. Run the file "AttuneNxtThirdPartyDependencies.exe" and accept all defaults in any windows that are displayed during the installation.

**Important: During installation pop-up dialogue boxes associated with the installer may be minimized. Pay close attention to extra windows that may require your attention.

2. Attune®NxT Datastore Installation

2.1. At the completion of the 3rd party installation, select the option to "Setup Database" at the bottom of the dialog.
NOTE: If the dialog was closed prior to selecting this option, the database installer can be found in < C:\Program Files\LifeTechnologies\AttuneNxT\dbinstall>. From this directory, run <AttuneNxtDatabaseSetup.exe> to continue.

2.2. Select the option to "Install New Database" and select "OK". Click "Yes" in the warning dialog to continue the installation.

2.3. If prompted to enter a password, enter "life".

2.4. Once the database has been installed, close the dialog.

3. Attune®NxT Driver installation

3.1. Navigate to the Attune installation directory, USBDriver folder. The default is < C:\Program Files\LifeTechnologies\AttuneNxT\UsbDriver>

3.2. Run < AttuneNxTDriverInstaller_x64.exe> and follow the instructions to install the Attune®NxT Cytometer USB driver.

Cytometer Firmware Installation

1. Update the Attune Cytometer Firmware

1.1. Navigate to the Attune installation directory.

1.2. Turn on the Attune Cytometer and ensure that is connected to the PC.

1.3. Wait at least 1 full minute before running the FW installer after the instrument has been powered on.

1.4. Run <FwUpdater.exe>

1.5. Power cycle the instrument after the installation is complete.

Attune Software v2.0 Upgrade from v1.1 Installation

Software Installation

Note: Do not launch the Attune NxT until the completion of all the installation steps. This upgrade will only upgrade the software from v1.1

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).
3. It is recommended that no other applications are running while completing these steps.
4. Uninstall the existing Attune NxT 1.1 application using "Programs and Features" found in the control panel. It should show up as "Attune NxT Cytometric Software". This will not affect previously collected data.
5. Uninstall the existing "AttuneNxtThirdPartyDependencies" if it is listed in the list of installed programs.
6. Unzip AttuneNxT.zip and run "setup.exe". Follow the instructions and accept all defaults.
7. After install is complete, open a Windows Explorer window and navigate to the AttuneNxT install folder, default <C:\Program Files\LifeTechnologies\AttuneNxT\>

8. From this folder, perform the steps as described below.

Database and Driver Installation

1. Attune®NxT Driver installation

1.1. It is necessary to update the Attune USB driver to version to version 2.0.0.5625.

1.2. Navigate to the Attune installation directory, USBDriver folder. The default is < C:\Program Files\LifeTechnologies\AttuneNxT\UsbDriver>

1.3. Run < AttuneNxTDriverInstaller_x64.exe> and follow the instructions to install the Attune®NxT Cytometer USB driver.

2. Attune®NxT Third Party Dependencies

2.1. Navigate to the Attune installation directory. The default is <C:\Program Files\LifeTechnologies\AttuneNxT\>

2.2. Run the file "AttuneNxtThirdPartyDependencies.exe" and accept all defaults in any windows that are displayed during the installation.

**If Third Party Dependencies are already installed and were NOT uninstalled in the previous section, the software will prompt you to uninstall the dependencies first before proceeding. Select OK.

3. Attune®NxT Datastore Upgrade

This involves two different steps to upgrade the database. First a "backup" step, then an "update" step. Follow the directions precisely as outlined below.

3.1. At the completion of the 3rd party installation, select the option to "Setup Database" at the bottom of the dialog.
NOTE: If the dialog was closed prior to selecting this option, the database installer can be found in < C:\Program Files\LifeTechnologies\AttuneNxT\dbinstall>. From this directory, run <AttuneNxtDatabaseSetup.exe> to continue.

3.2. Run <AttuneNxtDatabaseSetup.exe> and select "Backup Database" (Recommended before update). NOTE: This may take considerable time depending on the amount of data previously collected.

3.3. **IMPORTANT: Once the backup is complete, select "Update Existing Database".**

3.4. If prompted to enter a password, enter "life".

3.5. Click "Close" when prompted.

Firmware Installation

1. Update the Attune Cytometer Firmware

1.1. Navigate to the Attune installation directory.

1.2. Ensure that the Attune Cytometer is powered on and connected to the PC.

1.3. Wait at least 1 full minute before running the FW installer after the instrument has been powered on.

1.4. Run <FwUpdater.exe>

1.5. Power cycle the instrument after the installation is complete.

Software Features

The Attune® NxT v2.0 software includes the following features:

Automated Instrument Functions:

- Automated Performance Testing: Baseline and Performance Test, including report
- Automated Maintenance: Start Up, Shutdown, Rinse, Sanitize Attune SIP, Deep Clean, Sanitize, System Decontamination, Autosampler Calibration

Sample Acquisition:

- Sample acquisition from flow cytometry tubes, microfuge tubes, or standard 96 well plates using 12.5 µL/min, 25 µL/min, 100 µL/min, 200 µL/min, 500 µL/min, or 1000 µL/min flow rates.
- Use of round bottom plates is recommended for optimal mixing. Instrument Control functions: Run, Record, Pause, Stop, Clear
- Creation of plate experiments using the plate set up ("Heat Map"view):
    - Create multiple groups and/or samples within a plate *(single experiment per plate only)*
    - Create as many individual run protocols as desired per plate
    - There is a maximum number of 3 mixes recommended at this time
    - Collect samples from the entire plate or only a subset of the plate

Data Visualization Tools:

- Workspace flexibility: create different workspaces for samples, groups, or an entire experiment
- Experiment Workspace for easy setup including:
    - Histogram Plot, Dot Plot, Density Plot, Precedence Density Plot; Histogram marker, Rectangle Gate, Polygon Gate, Quadrant Gate and Derived Gates (customized logic gates)
    - "Plot Preview" function for easy incorporation of ready-made plots
    - Data display using Log, Linear and HyperLog™ transform
- Experiment Workspace Layouts: Auto layout or Freeform Workspace options
    - Plot resize tools: use to make plots identical sizes in Freeform mode
    - Plot organization tools: align left or right in Freeform mode
    - Copy and paste gates between plots; change gate type after creation
    - Unfilled or opaque gates

Statistical Measurements Available:

- Count, % Total, % Gated; Mean, Median, Mode; Standard Deviation, Robust Standard Deviation; % CV, Robust % CV;
- *concentration measurement is not available at this time*

Viewing Options:

- "Zoom In" tool that uses a "filmstrip" for easy navigation between plots;
- Display all events or only a subset of events
- Customizable Application Layout: move and dock the Collection, Customize, Instrument Settings and Experiment Explorer panels where you would like
- FCS information viewer: quick access to information on individual FCS file
- Compatible with multiple monitor displays *(note that a single monitor is included with a base package of the Attune® NxT Acoustic Cytometer).*

Compensation:

- Auto-compensation set up and auto-set up of compensation workspaces
- Acquisition of compensation controls from tubes
- Background fluorescence set using a negative gate or unstained control
- Auto-calculation of compensation

- *Manual adjustment of compensation through direct changes to spillover matrix*

File Management:

- Import/ Export of files and experiments
- Data files are stored in FCS 3.1 format
- Compatible with import of FCS files in 3.0 and 3.1 format

User Management & Security:

- 3 user accounts are available—"admin","user" and "service" accounts

The Attune® NxT v2.0 software includes the following improvements to system features:

- Event rates up to 35,000 events per second
- Acquisition of large event files (>1 Million)
- Progress bar that indicates instrument progress during maintenance functions
- Foreground calculations to update all data for all samples
- Updated LED indicator lights:

LED Color	Function/step
Blue (fade)	Warm up
Blue (solid)	Warm up complete
Blue (flashing)	Startup and instrument functions (except rinse)
Green (solid)	Startup complete or instrument idle
Green (flash)	Data/sample acquisition
Multicolor (fade)	Shutdown complete/sleep mode
Amber (blink)	Instrument Errors

Important Software Fixes included in Attune® NxT v2.0 software:

Sample Acquisition:

- Threshold settings are now saved upon duplication of experiments.
- Multi-draw of samples is now supported for tube samples.

Instrument Settings Panel

- Issues associated with selection and de-selection of parameters during acquisition has been addressed.

Maintenance Functions

- Improved stability of fluidics functions-shutdown and decontamination.

The following sections describe known issues with the Attune® NxT v2.0 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 v2.0 Software:

- Power on the instrument **and wait at least 2 minutes** prior to launching the software to allow the system ample time for system self-test. If the software is launched before self-test is completed, the "Start-Up" button may not be enabled and/or the Performance Test module may not complete.
    - If the instrument and Autosampler are in "sleep" mode following an instrument shutdown, turn both the instrument and Autosampler off using the power switches located at the back of the instrument prior to launching the Attune® NxT software. (Note: the Autosampler must be turned on first, then the instrument.)
- Prepare performance tracking beads using **3 drops of beads per 2mLs of Phosphate Buffered Saline (PBS)**. If the bead solution is too dilute, the performance test algorithm may time out and fail.
- **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.
artf56687	When an instrument has an Autosampler 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.
artf56690	The performance test will sometimes stop prematurely if the user starts the performance test while the tube lifter is in the lower or down position. This does not occur with 100% frequency.	Only initiate performance test with the tube lifter in the up position. If the performance test finishes after a few seconds, close the software, restart the application, rerun Start Up and re-run the performance test.

Guidance for Setting up an experiment using the Attune® NxT v2.0 Software:

- We recommend that the user set up the experimental workspace and gating strategy prior to starting acquisition.
- Use the "Heat Map View" to define wells used in a plate experiment and define run protocol(s) for the experiment

- Do not move or “undock” the heat map view otherwise it can become inactivated when another panel is activated.
- DO NOT USE a quadrant gate as a parent gate.

Known issue (“Artifact”) ID Number	Description of Known Issue	Suggested Action
artf52800	When trying to change the parameter used on a plot axis after right-click on an axis label, the yellow and violet laser parameters are listed in the “plot context menu” in longest to shortest bandpass filter order. This differs from the order of the parameter listed in the "Instrument Settings” panel where they are listed from shortest to longest bandpass filter.	Ensure that the correct parameter is selected for the plot when you are using the plot context menu to make changes to axis parameters.
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.
artf54546	When gates are selected in a plot, new plots cannot be added without first deselecting the gate.	Deselect the gate prior to adding a plot.

Guidance for using the Experiment Explorer and Instrument Settings panel within the Attune® NxT v2.0 Software:

- The “Target” and “Label” fields associated with each parameter (e.g., FSC, SSC, BL1, etc.) **should not be used at this time** for axis labeling. Instead, plot axis may be labeled individually using the Customize menu. Select a plot, navigate to the Customize panel and change the axis label under the “Text” field associated with each axis.
- Dragging and Dropping items in between experiments is not recommended as it may cause a crash.

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf56057	The Experiment Explorer tree may become cluttered.	To simplify the Experiment Explorer tree, minimize experiments or export experiments to another location.
artf56058	It is not possible to select multiple compensation controls at once by holding down the control key on the keyboard for performing functions like delete, remove, or export FCS files.	The FCS file from each compensation control must be manually exported using the right-click context menu. If a compensation control is not needed, we recommend deleting all compensation controls and restarting compensation setup from the beginning BEFORE any samples are acquired and data recorded.

Guidance for using Compensation within the Attune® NxT v2.0 Software:

We recommend that users prepare, acquire, and record compensation controls with each experiment.

- It is not possible to delete or add individual compensation control samples without deleting the entire compensation module. If a control must be deleted or added, right click on "Compensation" within the Experiment Explorer, select "Remove Compensation", and then re-start the compensation set up process.
- It is possible to remove the FCS file associated with a compensation control. Note that once a single compensation control is recorded in the auto-compensation module, fluorescence voltage settings are fixed.
- Prior to setting up compensation ensure that parameters are selected on the instrument settings panel prior to using the compensation set up wizard.
- We recommend that gates are not adjusted while recording compensation controls. Wait until the sample is finished recording to adjust the gate and marker region.

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf53408	The "Reset" button on the Spillover matrix is not functional.	Prior to making adjustments on the Spillover matrix, make note of the calculated values so that you can retype the entries into the matrix if necessary.
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	To set the axis of compensation

	compensation control samples using the right click menu.	controls using a HyperLog™ axis scale, adjust the axis scale using the customize panel.
artf55007	If the user sees that compensation matrix doesn't make sense (for example a row in the matrix is 0 across the entire row) or if the matrix doesn't seem to update when typing values into the matrix box; the matrix may not have updated.	Adjust the gates on all of the controls, open another experiment, re-open the original experiment and this should force the compensation matrix to refresh.

Guidance for Acquiring Samples using the Attune® NxT v2.0 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.
- DO NOT deselect all available parameters while recording a sample or the software will become unresponsive.
- Keep the tube lifter in the DOWN position when using the Autosampler.
- Derived gates cannot be used as a stop option.
- Derived gates should not be used as a parent gate to set additional derived gate.

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf55921	The "Exclude coincidence events" function located in the Instrument Settings panel in the software does not exclude coincidence when selected.	When the "exclude coincidence" tick box is checked, coincident events are not being excluded. To exclude coincident events, UNCHECK the box.
artf55949	Changing plots during acquisition on large event files can cause a time delay before the plot type is updated or system crash.	If collecting large event files >100,000 events, do not adjust any of the plot parameters during acquisition. After acquisition, parameters can be adjusted. Note that for large files sizes there may be some delay in refresh of the data.
artf55968	When the tube lifter is lowered during sample recording, an error message dialogue box with a "tube lifter lowered during acquisition" warning will not be able to be closed by clicking "OK" unless the tube lifter is left in the lowered position.	DO NOT lower the tube lifter during acquisition and record; allow the sample to record to completion prior to lowering the tube lifter. If the tube lifter is lowered during acquisition and record an error dialogue box will alert the instrument operator that the tube lifter was lowered. KEEP THE TUBE LIFTER LOWERED before clicking "OK" on the dialogue box; the remaining sample will be analyzed to completion.
artf56073	The software may display an incorrect	Final gate adjustment should be

	statistical value if gates are moved rapidly and/or repeatedly during acquisition. This error does not occur in all instances where a gate is moved during acquisition.	made after a file is recorded.
artf56078	After recording a sample, there is a brief period of time during which the run protocol of the previously recorded sample can be altered, leading to incorrect run protocol settings recorded to the experiment file.	To ensure that the run protocol is recorded correctly in an experiment, do not adjust the run protocol until moving to a new sample tube in which no data has been recorded.
artf56650	The "High throughput" option check box on Autosampler collection criteria is not available.	Manually enter high throughput run protocols for each well (50 µl of sample, 500 µl/min, 1 rinse, 1 mix) to minimize run time per plate.
artf56654	Blank stop options boxes can lead multiple warning boxes	Do not delete default stop option criteria and leave blank (for example if collecting 30 seconds, put 0 in minutes box).
artf56709	After running a tube sample, any portion of the sample remaining inside the sample loop within the instrument can potentially contaminate the first well of a plate experiment if a rinse cycle is not manually performed following tube acquisition.	The tube lifter MUST BE LOWERED following tube acquisition prior to running a plate. DO NOT ACQUIRE a plate sample with the tube lifter in the raised position. After running a tube sample, make sure that the tube lifter is lowered and a rinse cycle is initiated.

Guidance for using the Experiment Workspace (Gates, Plots, Stats) within the Attune® NxT v2.0 Software:

- Do not use quad gates as parent gates.
- Use less than 50 characters when naming gates.
- It is not possible to copy and paste plots from the Attune® NxT v2.0 software at this time. We recommend that plot images be captured from the software using a screen capture until the copy/paste/save as functions are enabled in the 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.
- When setting up derived gates with two words for the gate name, use parentheses (", ") to enclose both words.
- Do not set up workspace with more than 399 individual plots.

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf50038	Plot alignment tools will align plots to the object to the furthest side of the page (Left aligns to the most left object, right to the most right, top to the most top)	Auto layout mode may also be used to automatically align plots in grid fashion.
artf50040	The plot resizing tools ("make same height" and "make same width) may not resize plots based on the reference plot selected.	To use the plot resize tools, select the reference plot by selecting a plot, then hold down the control button on the

		keyboard before selecting additional plots. The reference plot is the first plot selected and the width and height of other plots will be resized to this plot using the plot resize tools.
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.
artf53509	The names/labels of gates may be written in a different color font than the gate to which they correspond if the end user changes the gate color using the gate customization menu.	If you change the color of a gate using the gate customization menu, check the color of the gate name/label to avoid future confusion.
artf54881	The names/labels of different quadrants within a quad gate are truncated after 31 characters	Do not use gate names/labels that include more than 31 characters with a quadrant gate.
artf55024	The parent gate hyperlink may disappear when the workspace is shrunk below 71% or above 162% using the slider bar size adjustment scale.	The parent gate hyperlink will reappear when the size of the workspace is set between 71% and 162%using the size adjustment slider bar.
artf55025	If greater than 9 gates are inserted in a workspace, the colors of the gates will be repeated unless the gate color is changed manually by the user.	If different gate colors are required, manually change the color of the gate by selecting the gate and changing the line color option using the Customize panel.
artf55134	The plot “Preview” panel can take up to 30 seconds to open.	If all or most parameters are active, wait at least 30 seconds after clicking on the Preview panel to allow the panel to open. Do not attempt to do any other function during this time. Deselecting parameters will decrease the time it takes to open the panel.
artf55203	The software will not automatically update a group name in the header of a statistics box immediately following name revision.	To update the group name in the header of the statistics box, either re-activate the current sample by double-clicking it OR open another sample in the experiment and check that the name in the header has been updated.
artf55224	Derived gates can only be created using regions.	If creating derived gates, be aware that they can only be created using regions.
artf55595	The customize panel does not update properly when the “Zoom In” film strip view is open.	If customization in the “Zoom In” view is necessary, use the navigation arrows on the page to scroll through the plots to force the customize panel to update with the desired changes. Alternatively, customize plots while not in the film strip zoom view.
artf55596	The software will be unresponsive if an inappropriate gate is added to a plot such as: • Adding a rectangle, oval, or	When the software is unresponsive, press the Esc button on the keyboard. Alternatively, open the window of another application or minimize the

	quadrant gate to a histogram plot • Adding a histogram gate to a dot plot, density, or precedence density plot. This issue has been observed in both filmstrip zoom view and in regular workspace view. Often the mouse cursor will be replaced by a circle with a line through it when not posed over a plot.	Attune® NxT v2.0 software.
artf55967	The software may not allow manual adjustment to axis scales.	If the axis scaling range does not change upon manual entry, temporarily switch the range view from "manual" to "automatic", then back to "manual" range again and enter the desired values.
artf56132	Plots cannot be inserted when a gate is selected or activated.	Deselect/deactivate the gate before inserting a plot into the workspace.
artf56519	Creating a statistic box on blank workspace causes the software to crash.	Prior to adding a statistics box, make sure the workspace has at least one plot.

Guidance for Data Analysis / Data Display using the Attune® NxT v2.0 Software:

- On the "Results" view, right clicking on a column header will display a list of statistics, some of which are not currently available.
- When viewing files within experiments a parameter mismatch error or file error may occur. If this is observed, open a different sample and then reselect the sample; the file should display correctly without errors.
- *Note that statistics are not reported for derived gates.*

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf52579	A statistics box inserted in the workspace for a specific single plot will NOT display the parental hierarchy in the statistics box.	Use a "global statistics box" to indicate gating hierarchy and statistics associated with each region or gate. To insert this type of statistics box, press the "statistics" button on the "workspace" ribbon without selection of an individual plot. If the gating hierarchy is not required to be shown, a "plot specific" statistics box may be created by selecting "statistics" from the workspace ribbon while a specific plot is selected.
artf54100	System locale settings not applied to numerical values displayed in workspace statistics tables.	Note that results will be displayed using a US-based number format utilizing decimal places.
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 v2.0 Software:

- Individual FCS files may be exported by right clicking on a sample and selecting "Export FCS File" from the context menu; holding down the control key (Ctrl) on the keyboard while selecting multiple samples will allow bulk export of FCS files from sample tubes but not compensation controls.
- Experiment files may be exported by right click of the selected experiment. Files are exported as an ".atx" file. The ".atx" file extension may be changed to ".zip" to later manually extract FCS files from the experiment (if required).
- FCS files cannot be exported from a gate.
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Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf56328	The software does not permit import of an experiment file (".atx") if the experiment explorer has no previous experiments in it.	Create a new "blank" experiment. Once the blank experiment is created the end user will be able to import previously generated experiment files.
artf56438	Run protocols are not persisted after export of experiment.	Check run protocols after duplication of an exported experiment prior to running samples
artf56445	The software does not display FCS 2.0 files correctly.	The Attune® NxT v2.0 software is currently unable to support analysis of FCS 2.0 files. Do not import FCS 2.0 files at this time.

Guidance for Shutdown and Instrument Maintenance using the Attune® NxT v2.0 Software:

DO NOT shut off the computer while the Attune® NxT application is performing shutdown.

Known issue ("Artifact") ID Number	Description of Known Issue	Suggested Action
artf54707	The instrument doesn't wake up after it is put through an instrument Shutdown cycle.	Once Shutdown is complete, turn the instrument completely off using the on/off switch located on the back of the instrument. Turn the instrument

		on and wait two minutes before restarting the software the next time the instrument is used.
artf56638	The Autosampler may go into an error state if a plate is in Autosampler during automatic calibration.	Remove the plate, restart the software, and re-run automatic calibration.

System Decontamination:

System decontamination should be performed on once every three months. **The steps below must be followed precisely in the order described for the decontamination process to successfully complete. If steps are missed, please restart the decontamination process.**

1. Place the tube lifter in the lower (down) position.
2. From the instrument ribbon, press the "Decontaminate System" button.
3. Follow the steps for bottle prep as described in the pop-up dialogue instruction box.
4.

**Special Note – the tube lifter needs to remain lowered until fluids in the focusing fluid and shutdown bottles have been exchanged as described in the pop-up dialogue box. If the bottles are disconnected while the tube lifter is in the raised or "up" position, it is likely that the decontamination function will not complete. If this happens, cycle power on the instrument and restart the decontamination function.

   a. During the bottle preparation period, a 'Check Fluid Bottles' pop-up box will appear when the black fluid sensor (radio) cable is un-plugged. DO NOT CLICK THE OK button until all bottle steps are completed.
   b. Once fluids have been exchanged in the focusing fluid and shutdown bottles, confirm that both the black fluid sensor (radio) cable and fluid lines have been plugged into the instrument. Click "OK" on the "Check Fluid bottles" dialogue box once the cables and fluid lines have been reconnected.

5. After clicking the "OK" button on the "Check Fluid Bottle" dialogue box, raise the tube lifter with the clean empty tube.
6. Click the 'Next' button to initiate the first phase of the decontamination process.

When complete with Phase 1, a second instruction window will pop up.

7. Lower the tube lifter and remove the tube that is now filled with bleach.

**Special Note – the tube lifter needs to remain lowered until fluids have been exchanged as described in the pop up dialogue box. If the bottles are disconnected while the tube lifter is in the raised or "up" position, it is likely that the decontamination function will not complete. If this happens, cycle power on the instrument and restart the decontamination function.

   a. Follow the steps for bottle prep in instruction window #2. During the bottle preparation period, a 'Check Fluid Bottles' pop-up box will appear when the fluid sensor (radio) cable is un-plugged. Note the "OK" button should not be clicked until all bottle steps are completed.
   b. Once fluids have been exchanged, confirm that both the black fluid sensor (radio) cable and fluid lines have been plugged into the instrument. Click "OK" on the "Check Fluid bottles" dialogue box once the cables and fluid lines have been reconnected.

8. After clicking the "OK" button on the "Check Fluid Bottle" dialogue box, raise the tube lifter with the clean empty tube.
9. Click the 'Next' button to initiate the second phase of the decontamination process.

When complete with phase 2, a third pop up window may or may not show up.

10. Lower the tube lifter and remove the tube filled with deionized water.

**Special Note – the tube lifter needs to remain lowered until fluids have been exchanged as described in the pop up dialogue box. If the bottles are disconnected while the tube lifter is in the raised or "up" position, it is likely that the decontamination function will not complete. If this happens, cycle power on the instrument and restart the decontamination function.

11. Follow the steps for bottle prep in instruction window #3.
    a. During the bottle preparation period a 'Check Fluid Bottles' pop-up box will appear when the black fluid sensor (radio) cables are un-plugged and remains even after re-plugging in the cables.
    b. Ensure all of the bottles have the proper volume of Focusing, Wash and Shutdown fluids.
12. Once fluids have been replaced in all bottles, confirm that both the black fluid sensor (radio) cables and fluid lines are plugged into the instrument. Click "OK" on the "Check Fluid Bottles" pop-up dialogue box.
13. After all bottles have been plugged in, click the OK on the pop-up box.
14. Turn the instrument "off" using the ON/OFF switch on the back of the instrument.
15. Replace focusing fluid filter; please refer to the user manual for detailed instructions.
16. Turn the instrument "on" using the ON/OFF switch on the back of the instrument.
17. Run Startup three times using the "Start Up" button located on the Instrument ribbon.
18. The instrument is now fully decontaminated and ready to use.