

A guide to analyze Attune NxT FCS data files in FCS Express 6 software

The acquisition and analysis software package for the Invitrogen™ Attune™ NxT Flow Cytometer enables powerful, user-defined, experimental analysis via an intuitive, easy-to-use interface. Advanced tools are provided for both acquisition and analysis, which are simple enough for users at all experience levels. Invitrogen™ Attune™ NxT Software has been designed to maximize data analysis efficiency. It uses the Microsoft™ Windows™ 7 64-bit system to provide fast refresh rates for large data sets of up to 20 million events created by multilaser, multiparameter instrumentation.



Collecting data on the Attune NxT Flow Cytometer creates FCS 3.0 or 3.1 data files. Importing these data into third-party flow cytometry analysis software requires setting the preferences of that software to properly display data. This instruction guide is intended to help the user open and adjust certain settings in FCS Express™ 6 software (De Novo Software, Glendale, CA) to properly display and facilitate the analysis workflow when using Attune NxT FCS data files. This guide is not meant to be a comprehensive resource for all features and functions available in FCS Express 6 software. We suggest referring to the user guide or visiting the De Novo Software website (denovosoftware.com) for help with the features not covered here. These instructions cover the required steps for version 6 of FCS Express software; other versions may require modifications to these instructions, as menus and placement of settings may have changed.

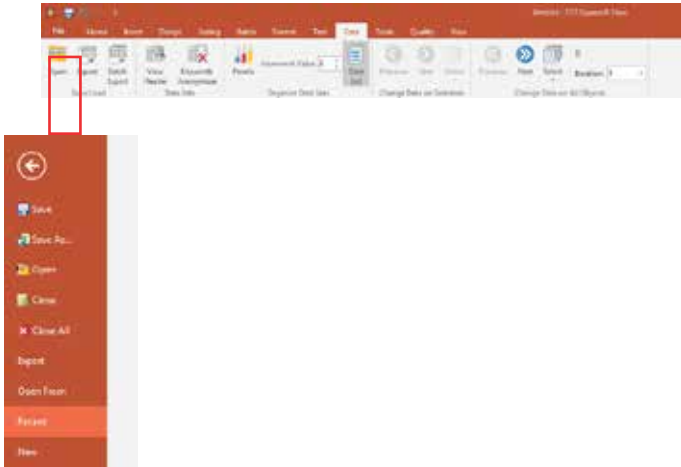
FCS Express software version 3 or higher is required to open Attune NxT FCS 3.0 data files, and FCS Express software version 4 or higher is required for Attune NxT FCS 3.1 data files.

Note: The De Novo Software website (denovosoftware.com) also offers online tutorials and other helpful information.

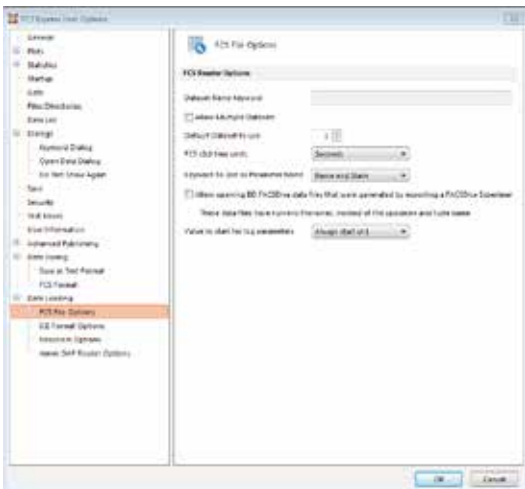


Configuring FCS Express 6 software

1. Before analyzing Attune NxT Software–acquired FCS files using FCS Express 6 software, you can configure FCS Express 6 software to read the parameter names embedded in the Attune NxT FCS file.
2. Press on the **“File”** tab of the ribbon bar and open the **“Options”** menu.



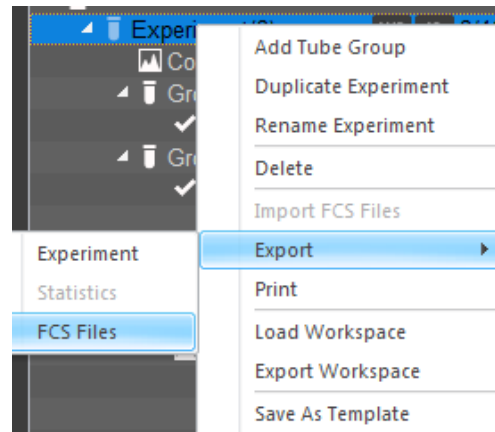
3. Within the **“Options”** menu, select **“Data Loading”** and click on FCS file options. Under the **“Keyword to Use as Parameter Name”**, select **“Name and Stain”**. This will ensure that the target and label fields entered in the Attune NxT Software settings are automatically transferred to the axis labels within FCS Express 6 software.



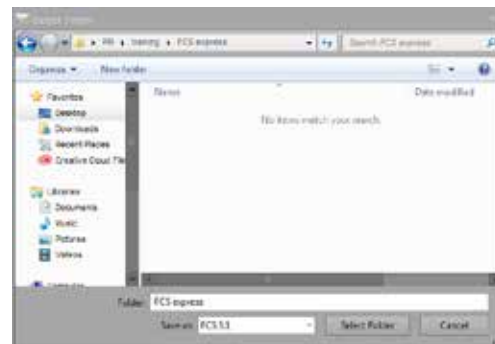
Opening Attune NxT FCS data files in FCS Express 6 software

1. The **“File Save (Export)”** dialog box uses the standard Microsoft™ Windows™ browser, which opens to the last-visited directory where files were saved, and allows you to locate and select files for exporting. Experiment files have the default extension (.fcs). FCS files can be easily exported from Attune NxT Software using these easy steps:

- a. Right-click on the name of the experiment in the Experiment Explorer.



- b. Select **“FCS Files/Export”** from the dropdown menu.
- c. In the file browser dialogue box that opens, choose the location to save the exported FCS files to and select **“Save as FCS 3.0 or 3.1”** on the bottom of the box.
- d. Click **“Select Folder”** and the files will be exported.

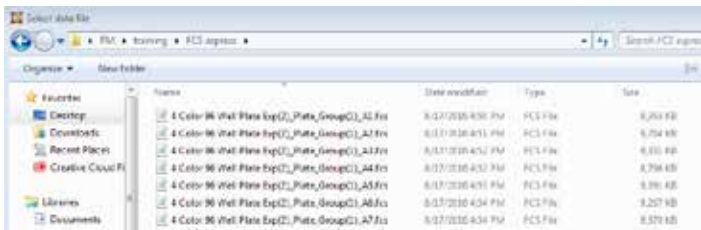


2. Launch FCS Express 6 software, and once the application has opened, click the **“Data”** tab on the ribbon bar and then click the **“Open”** icon in the **“Save/Load”** icon group on the left-hand side of the ribbon bar.
3. From the FCS Express 6 Welcome page, start a new layout by pressing **“New Layout”**.

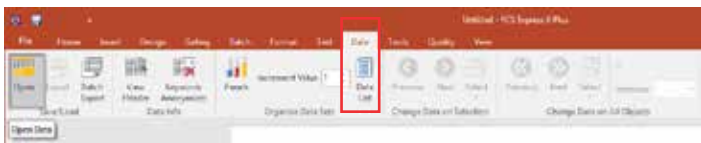
- Once the new layout has opened to a blank page, click the **“Insert Tab”** and select a plot type to insert into the blank layout.



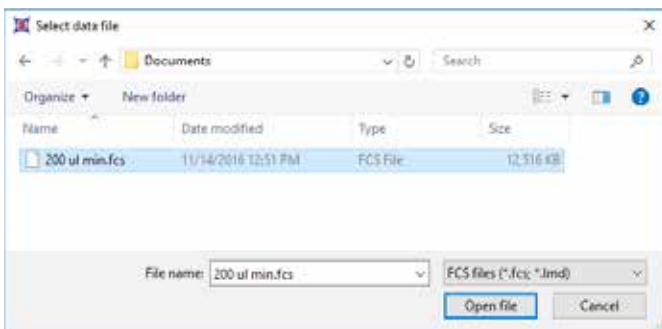
- Select a plot type to automatically open a file browser dialog box. From the file browser dialog box, select the appropriate file to open.



- Once a file is selected, the plot type selected in step 5 above will be created using the designated file. You can add other files associated with the experiment to the layout by clicking on **“Data List”** from the Data tab and pressing the plus **“(+)”** button.

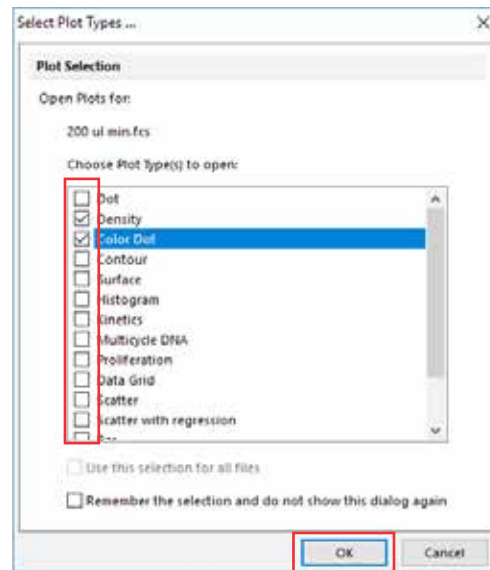


The following **“Select data file”** dialog box will appear after clicking on the plus **“(+)”** button from the Data List, allowing you to add additional data files.



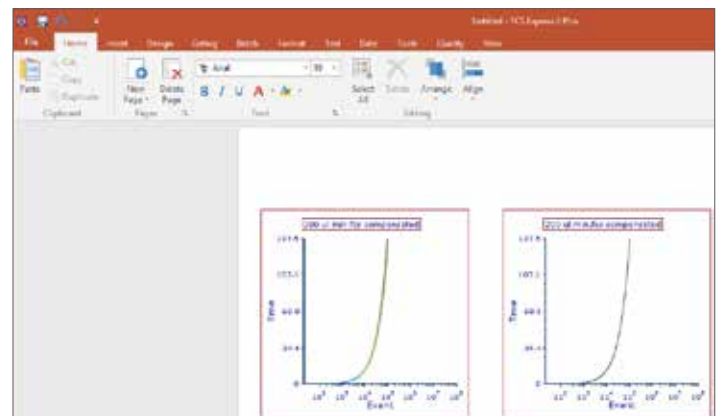
- Data files in the data list can then be dragged and dropped into the layout to insert additional data files and plots.

- You can select the plot types you would like FCS Express 6 software to generate automatically after dragging and dropping the data file from the data list by checking the appropriate boxes in the **“Choose Plot Type(s) to open”** box. Click **“OK”**.



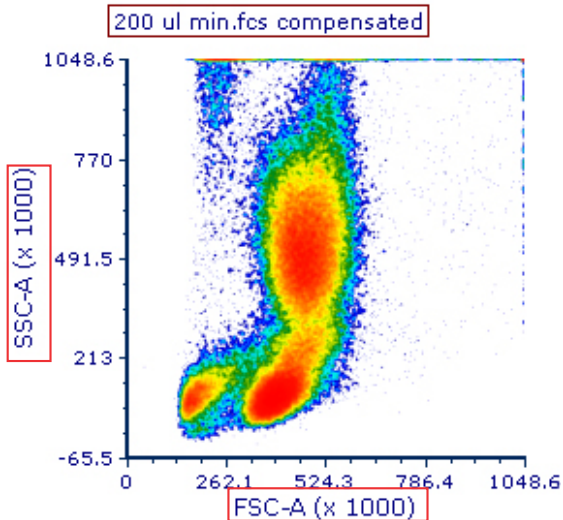
Tip: You can also select one or more plot types to generate in the layout after dragging and dropping. Instead of dragging and dropping, you can also add plots by using the buttons for 1D/2D plots on the **“Insert”** tab on the ribbon bar. Alternatively, you can use the **“Data List”** button on the **“Data”** tab to open the Data List. Simply drag and drop other files from your directory folder into the Data List.

If, in the previous step, you selected the plots to be generated, you will see a workspace that looks something like this:

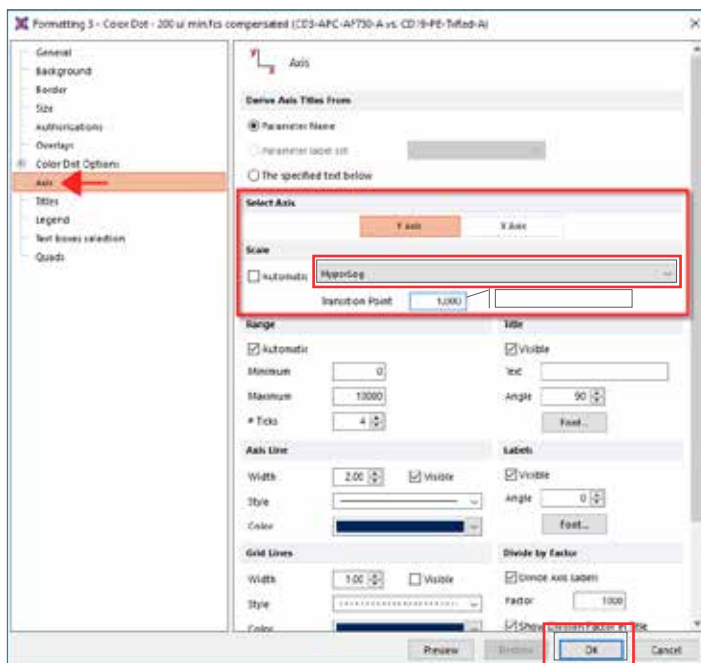


Configuring FCS Express 6 software to display Attune NxT FCS data files correctly

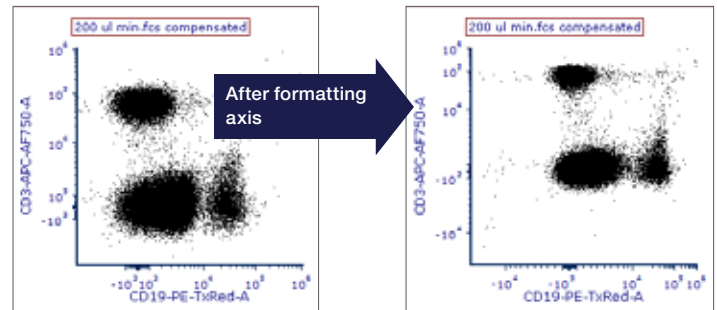
1. Click on each axis title and select the parameter you would like to display. In this example, forward scatter (FSC) is displayed on the x-axis and side scatter (SSC) on the y-axis.



Add to the workspace any plots that will display compensated fluorescence parameters using the preferred plot type (density, color dot, etc.). If modification of the data scaling for the x- or y-axis is needed, highlight the plot that contains the fluorescence parameter, right-click, and select “Format” to bring up the following option settings.



2. Select “Axis” from the panel of options on the left side of the window.
3. Select “Y Axis”, and under “Scale”, select “HyperLog” from the dropdown menu. Enter 1,000 in the “Transition Point” field (or any transition point value you would like to use).
4. Select “X Axis” and repeat step 3.
5. Choose “OK”, and data should be scaled correctly, as shown.



Note: For “Scale”, you may only need to select “HyperLog” if the data have compensation applied to them. You can select “Log” or “Linear” if one of these methods is preferred for displaying your data. The “Transition Point” value can also be optimized for displaying compensated data files. We have found that a value between 1,000 and 3,000 provides the best scaling and display of compensated data files. However, values above or below these are acceptable, depending on the data being displayed.

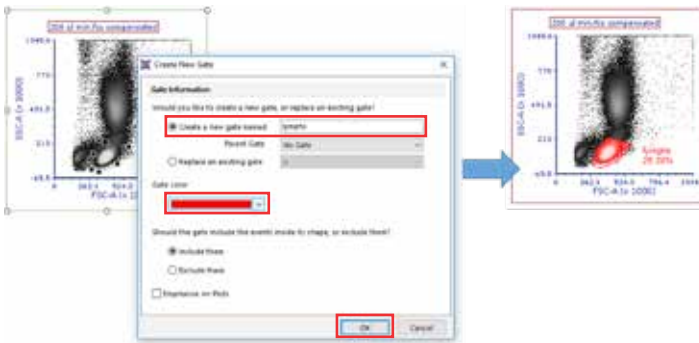
Note on compensation: Samples acquired on the Attune NxT Flow Cytometer using fluorescence compensation will have a compensation matrix that is exported with the FCS file. Within FCS Express 6 software, you can use the acquisition-associated compensation matrix or you can re-create a compensation matrix within the software using the FCS Express compensation tools. To learn more about the FCS Express compensation tools, please visit denovosoftware.com.

Calculating concentration in FCS Express 6 software using the Attune NxT \$VOL keyword

1. Draw a gate around the region of interest.
 - a. Click on the desired gate type from the “Gating” tab on the ribbon bar. In this example, we’ve chosen a “Polygon” gate.



- b. Draw the gate around the region of interest on the plot, and if desired, name and change the color of the gate, then click “OK”.

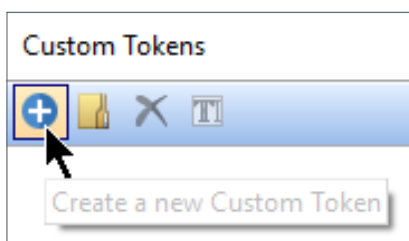


2. Create a “Custom Token” that uses the \$VOL keyword to automatically calculate the concentration of the gated events.

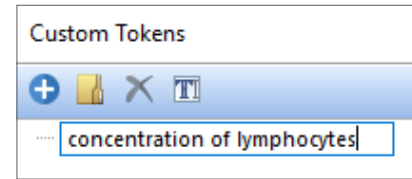
- a. Click on “Custom Tokens” from the “View” tab on the ribbon.



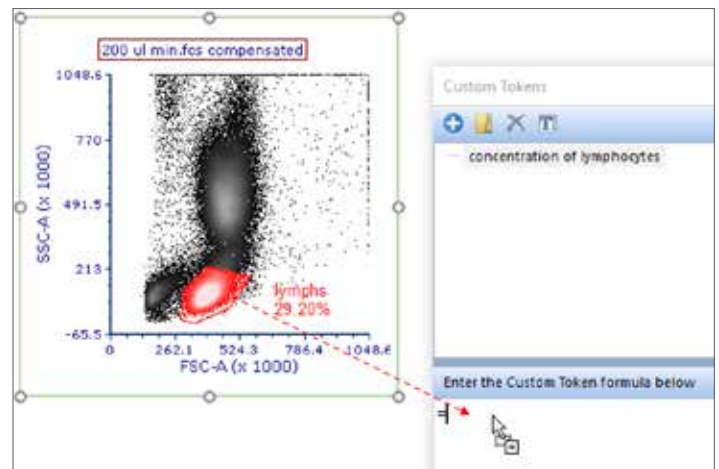
- b. Click the blue plus button (“+”) to add a new “Custom Token”.



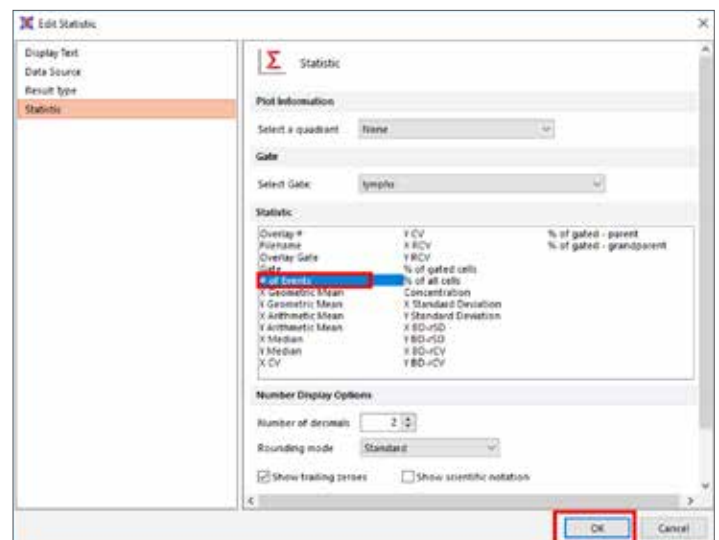
3. Optional: Type a descriptive name for the “Custom Token”.



4. Type an equals sign (“=”) in the lower formula pane of the “Custom Tokens” window.
 5. Drag the gate of interest to the formula pane to the right of the equals sign from step 4.



6. Choose “# of Events” and click “OK”.

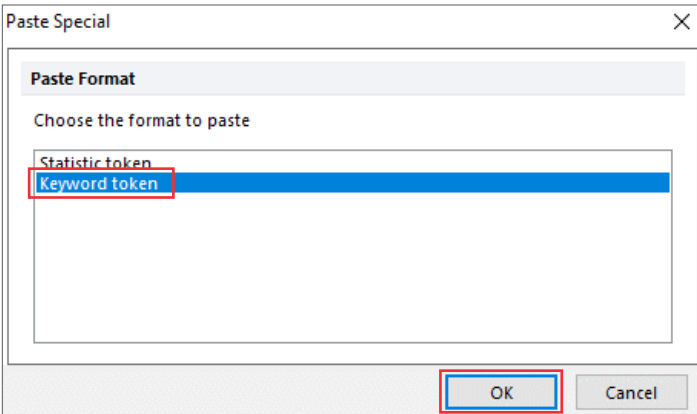


7. Type a divided by sign (“/”) after the inserted statistic from step 6.

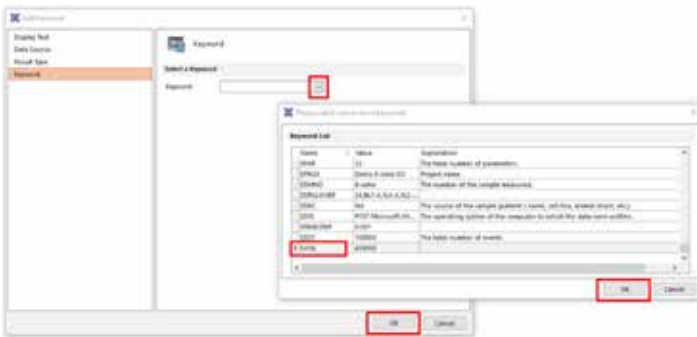
- Drag the plot by its border to the “**Custom Tokens**” window to the right of the divided by sign from step 7.



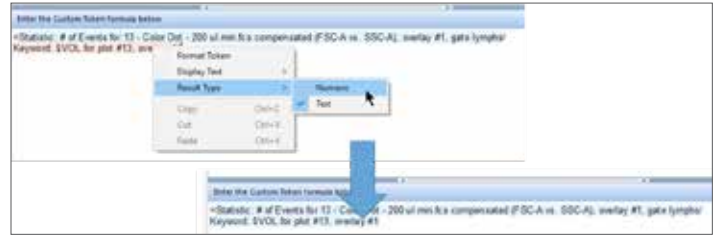
- Choose “**Keyword token**” and click “**OK**”.



- Click on the ellipsis (“...”), scroll to and select “**\$VOL**”, and click “**OK**” twice.



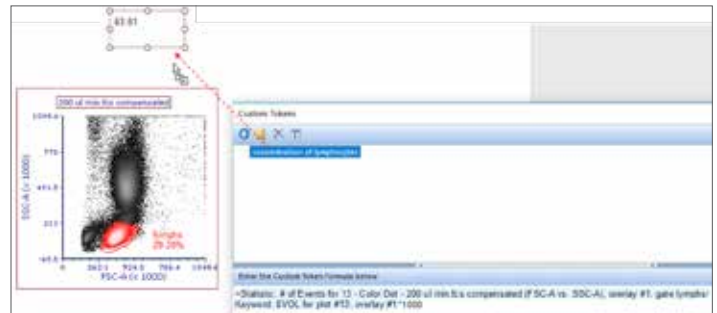
- Right-click on the inserted “**\$VOL**” keyword to change the “**Result Type**” to “**Numeric**” (*upper pane*). The keyword token will now appear highlighted in blue (*lower pane*).



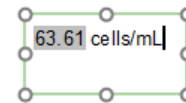
- Type “***1000**” after the inserted “**\$VOL**” keyword from step 11 so that the concentration will appear in cells/mL instead of cells/μL.



- Drag and drop the “**Custom Token**” to your layout page. The concentration will now appear as dynamic text that changes as you adjust your gates or change your data files.



- Optional:** You can type free text (e.g., “**cells/mL**”) after (or before) the inserted concentration statistic in the text box.



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Note that once the “**Custom Token**” has been defined, any new data files loaded into the layout will automatically display the concentration, eliminating the need to re-create the “**Custom Token**”. Additionally, a new “**Custom Token**” can be easily created for other gates by copying and pasting the original “**Custom Token**”, changing the name of the new “**Custom Token**”, and changing the gate that defines the statistic.

These instructions are based on our understanding of the default display of the data in the respective software.

Find out more at thermofisher.com/attune

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