A guide to analyzing Attune NxT FCS data files in FCS Express 7 software

Introduction

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 64-bit Microsoft™ Windows™ 7 or 10 operating system to provide fast refresh rates for large data sets of up to 20 million events created by multilaser, multiparameter instrumentation.

This instruction guide is intended to help the user open and adjust settings in FCS Express™ software (from De Novo Software, Pasadena, CA) to properly display and facilitate the analysis workflow when using the Attune™ NxT FCS data files. These instructions are being provided in part because we understand that users may prefer the workflow or analysis features provided by external software vendors, or may require this software for routine and/or specific analysis tools. This guide is not meant to be a comprehensive resource on all features and functions available in FCS Express software. We suggest that you refer to the user guide or visit the **De Novo Software website** for help with the features not covered here. These instructions will cover the required steps for version 7 of FCS Express software; previous versions may require modifications to these instructions, as menus and placement of settings may have changed.

Methods

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

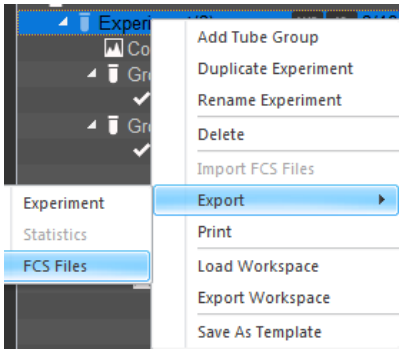
Note: Online tutorials and other helpful information can be accessed on the De Novo Software website, at **https://www.denovosoftware.com/full-access/**, with a free De Novo Software account.

ThermoFisher SCIENTIFIC

Opening Attune NxT FCS data files in FCS Express 7 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.

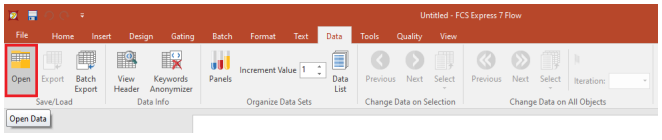


   b. Select **FCS Files/Export** from the dropdown menu.

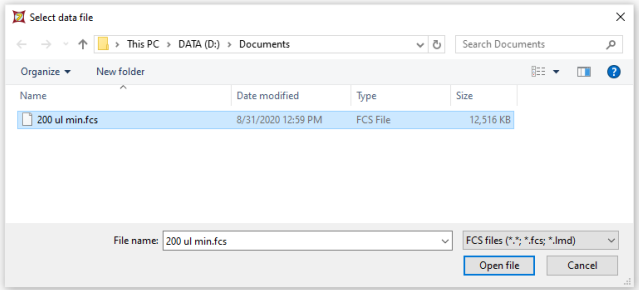
   c. In the file browser dialog box that opens, choose the location to save the exported FCS files to, and select **Save as FCS 3.0 or 3.1** at the bottom of the box.

   d. Click **Select Folder** and the files will be exported.

2. Start FCS Express 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 end of the ribbon bar.

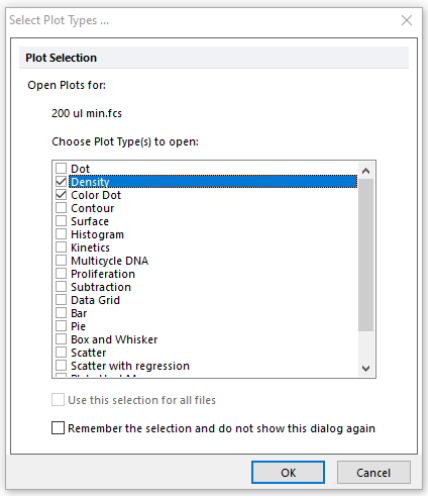


   The following dialog box will appear.



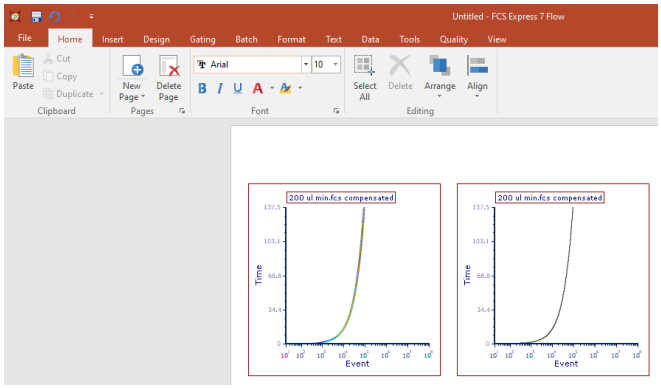
3. Navigate to the folder where the exported Attune NxT FCS data files are located, select the file to be opened, and click **Open file**.

4. Select the plot types you would like the FCS Express software to generate automatically after opening the file, by checking the appropriate boxes in the **Select Plot Types …** option box. Click **OK**.



Tip: You can select one or more plot types to generate in the workspace after opening. You can also add plots by using the buttons for **1D Plots** and **2D Plots** on the **Insert** tab on the ribbon bar. Alternatively, you can use the **Data List** button on the **Data** tab, which will allow you to drag and drop other files from your directory folder to the layout.

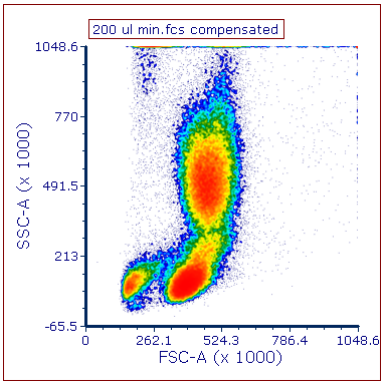
If, in the previous step, you selected plots to be generated, you will see a workspace that looks something like the image below:



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

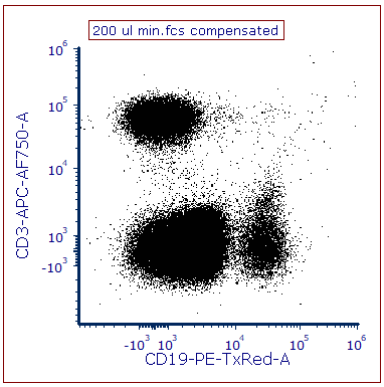
In most cases, no additional adjustments to plots or scaling are needed to visualize the Attune NxT FCS data in FCS Express software. The steps below outline how to change parameters and make adjustments to axis scaling if desired.

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



2. Add any additional plots to the layout that will display compensated fluorescence parameters, using the preferred plot type (density, color dot, etc.)

   If data were compensated at the time of acquisition, the compensation will be automatically applied to the plots in the FCS Express software.

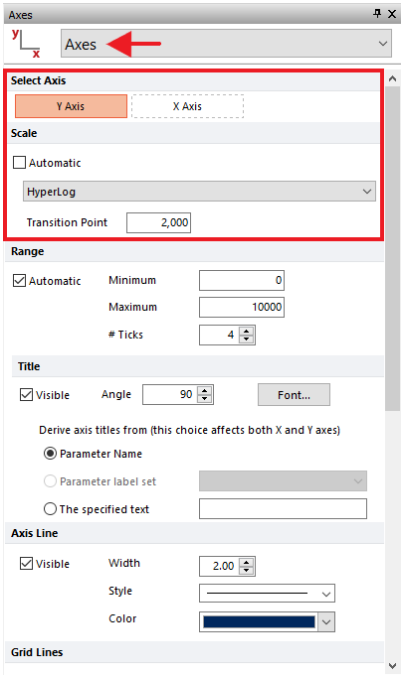


Note: With the advent of multiple fluorescence cytometry, compensation of these signals has been necessary to properly interpret the data. Unfortunately, since compensation is a subtractive process, it can produce negative and zero-valued data. The log transform is undefined for these values and, as a result, forces computer algorithms to truncate these values, creating a few problems for cytometrists.

The Hyperlog scale is a log-like transform that admits negative, zero, and positive values [1]. Attune NxT Software offers users the choice of selecting hyperlog scaling. If you would like to change the axis scaling, there are several options to choose from—linear, log, hyperlog, and biexponential. To change the axis scaling to hyperlog, follow the optional steps below:

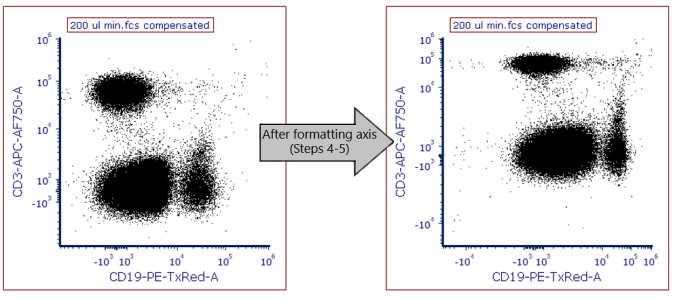
3. Select the plot, right-click, and select **Format** to bring up the formatting window.

4. Click on the drop-down menu at the top of the formatting window and select **Axes**.



5. Select **Y Axis**, and under **Scale**, select **HyperLog** from the drop-down menu. Enter a value into the **Transition Point** field. The plot will automatically update to display the new settings.

6. Select **X Axis** and repeat step 5.



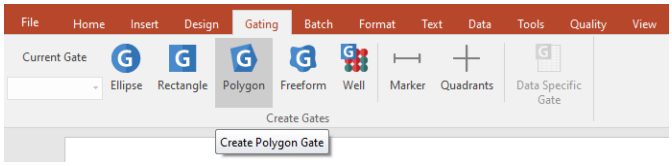
Tip: We have found that values between 1,000 and 3,000 often provide the best hyperlog scaling and display of compensated data files. However, values above or below this range are also acceptable.

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

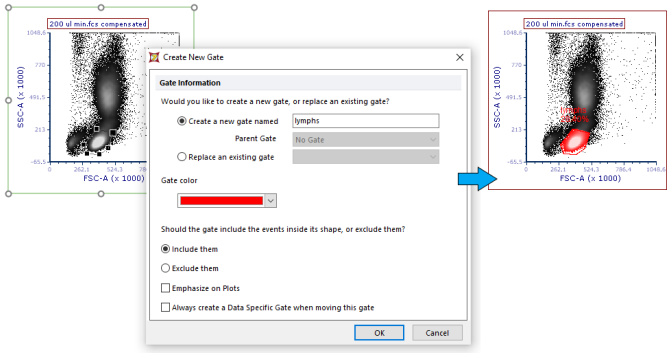
The Attune NxT Flow Cytometer allows users to easily generate concentration-based statistics for absolute counts in FCS Express software by inclusion of the $VOL keyword at the time of acquisition. The steps below show how to utilize the $VOL keyword in FCS Express software to determine absolute counts for data and gated populations.

A. Draw a gate around the region of interest.

1. Click on the desired gate type from the **Gating** tab on the ribbon bar. In this example, we've chosen a **Polygon** gate.

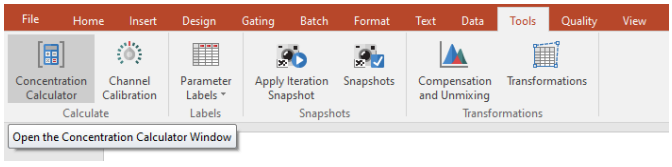


2. Draw the gate around the region of interest on the plot, and if desired, name and change the color of the gate, and click **OK**.

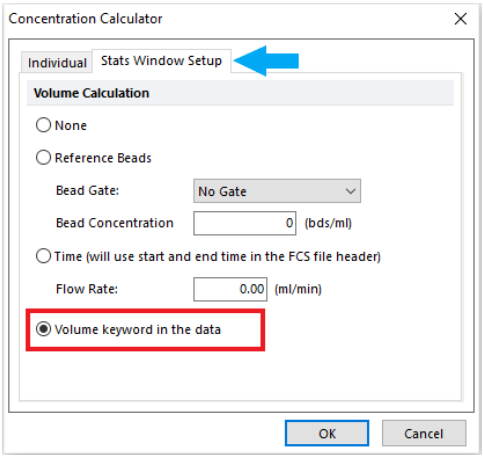


B. Calculate the concentration of the gated events based on the $VOL keyword.

1. Click on **Concentration Calculator** from the **Tools** tab on the ribbon bar.



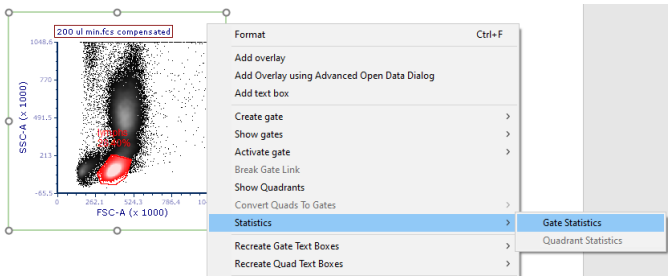
2. Click the **Stats Window Setup** tab.



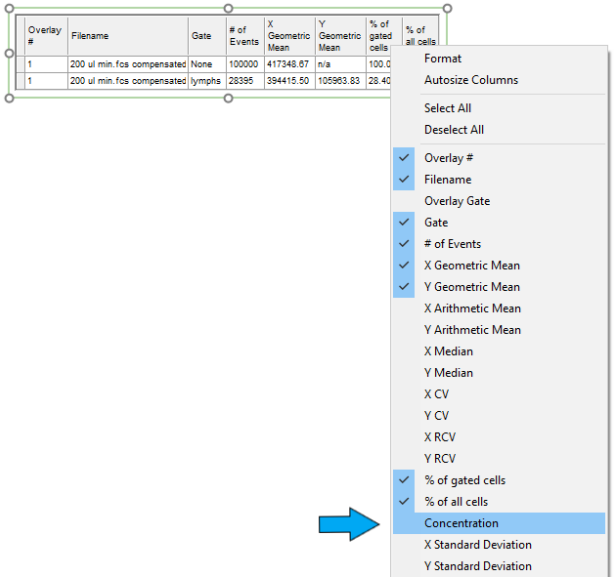
3. Select the **Volume keyword in the data** radio button in the **Volume Calculation** options. Click **OK**.

C. Display the concentration of the gated events based on the $VOL keyword.

1. Select the desired plot, right-click, and select **Statistics**, then **Gate Statistics**.



2. Right-click on the newly created statistics table and select **Concentration**.

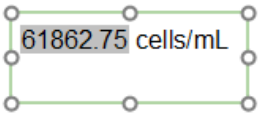


invitrogen

3. The concentration of events for each gate will be displayed in a new column on the statistics table, based on the $VOL keyword of the FCS file.

Overlay #	Filename	Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of gated cells	% of all cells	Concentration
1	200 ul min.fcs compensated	None	100000	417348.67	n/a	100.00	100.00	217864.92
1	200 ul min.fcs compensated	lymphs	28395	394415.50	105963.83	28.40	28.40	61862.75

4. (Optional) Drag and drop the **Concentration** column to your layout page. The concentration will now appear as dynamic text that changes as you adjust your gates or change your data files. You may type free text, e.g., “cells/mL”, after or before the inserted concentration statistic in the text box.



Reference

1. Bagwell CB (2005) Hyperlog—a flexible log-like transform for negative, zero, and positive valued data. *Cytometry A* 64(1):34-42.

Find out more at **thermofisher.com/attune**

ThermoFisher SCIENTIFIC