

A guide to analyze Attune NxT FCS files in ModFit LT software

Background

Analyses of cell cycle and cell proliferation, or cell tracing, often require sophisticated modeling software that are not provided by acquisition software. In this instructional guide, we have provided step-by-step directions to simplify the process of importing Invitrogen™ Attune™ NxT Software-generated flow cytometry standard (FCS) files into ModFit LT™ software (Verity Software House, Topsham, Maine).

Please refer to the ModFit LT™ documentation and the official website of Verity Software House as needed for complete instructions on using the features provided by the ModFit LT software package. The steps provided here are applicable for both cell cycle and cell proliferation data files generated on the Invitrogen™ Attune™ NxT Flow Cytometer.

Software requirements

The Attune NxT Flow Cytometer writes FCS files using FCS as default and is also capable of generating FSC 3.0 data files. For native support of FCS 3.1 data files, ModFit LT software version 4.0 or higher is required.

The Attune NxT data files can be exported as FCS 3.0 data files that can be opened and analyzed using ModFit LT software version 3.2 or higher.

For more information, consult the Verity Software House at vsh.com



Cell cycle analysis using ModFit LT software 4.0

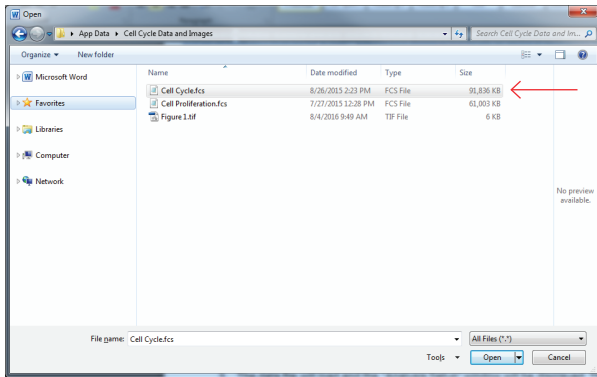
Export Attune NxT data files as described in the Attune NxT Software Users Guide (Pub. No.100024236) https://tools.thermofisher.com/content/sfs/manuals/100024236_AttuneNxT_SW_UG.pdf

To open standard cell cycle data files generated by the Attune NxT software in ModFit LT platform:

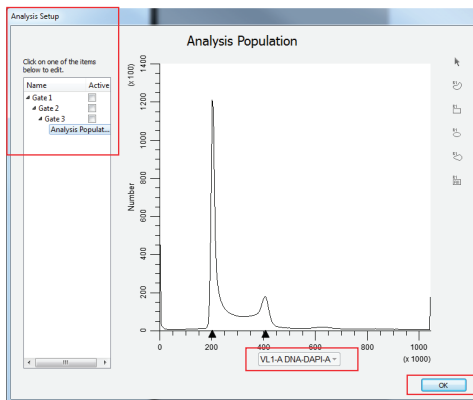
1. Launch ModFit LT software and click the **Open File** icon in the upper left. Alternatively, click **File** → **Open Data** from the command bar dropdown menu.



2. Navigate to the folder where the Attune NxT FCS 3.1 data files are located. Select the appropriate file that you want to analyze and click **Open**.

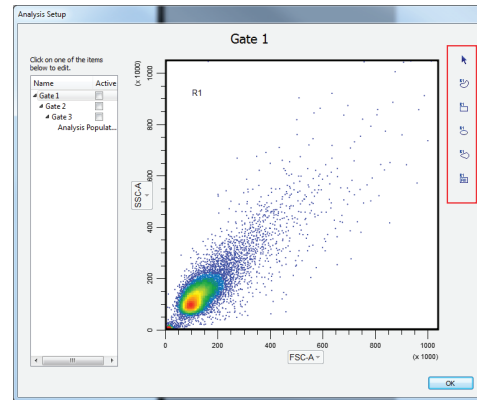
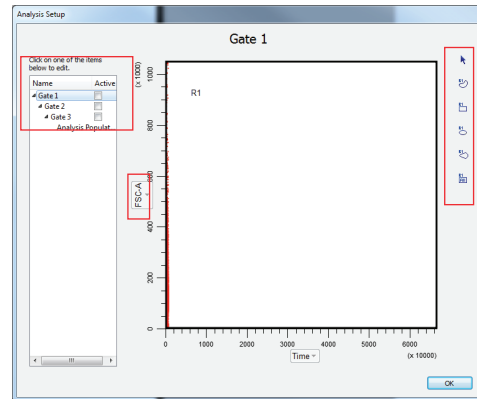


3. Once the data file loads, the **Analysis Setup** window will be displayed after the data file has opened in ModFit LT software. The ModFit LT software automatically detects and sets the parameter to the cell cycle parameter.

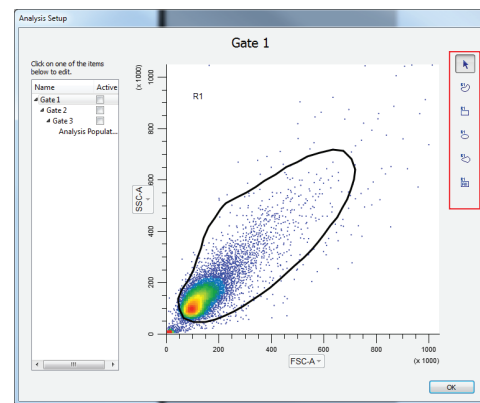


4. If the parameter chosen automatically by ModFit LT software is incorrect, select the parameter that was used for the cell cycle staining by clicking on the x-axis parameter name. A dropdown list of all available parameters is displayed.
5. If desired, defined gates can be created to isolate a specific population of cells. If the analysis does not require any gating, click **OK** to complete the analysis setup. To create a gate, click on one of the predefined gates under **Analysis Setup** to define a gating population:

- b. Define the x and y parameters for the plot by clicking on the x-axis and y-axis parameter names and selecting the desired parameter.

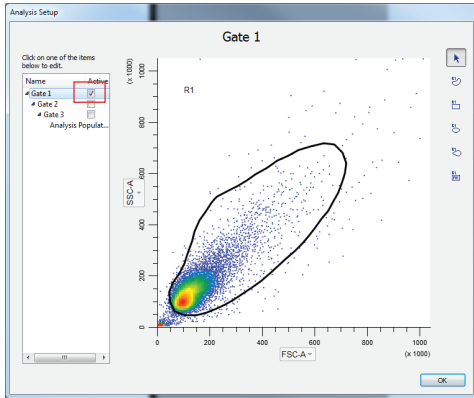


- c. Use the gating tools on the right side of the **Analysis Setup** to draw a gate around the population of interest.

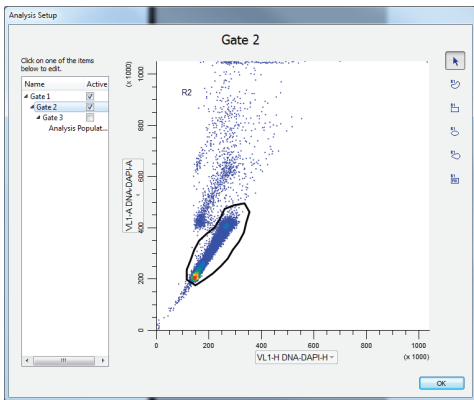


- a. Select **Gate 1**. The plot will update based on the selected gate, and by default, will use the first two parameters within the FCS file.

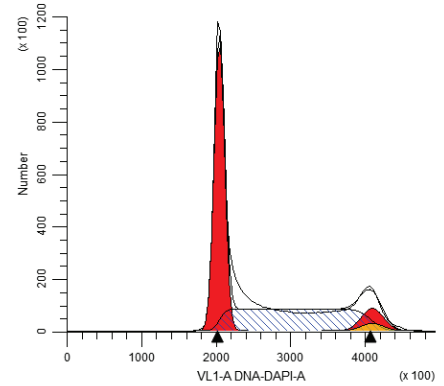
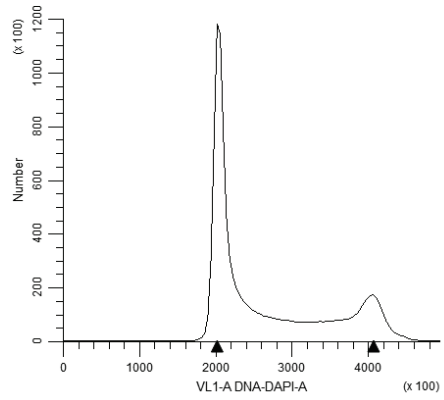
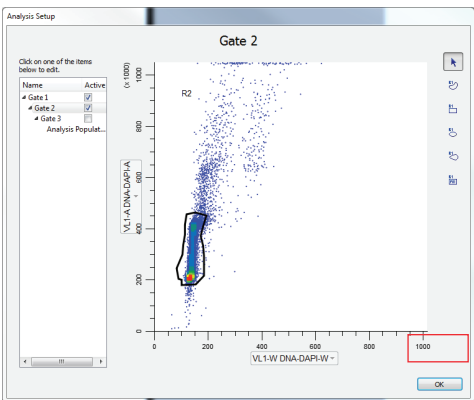
d. Enable the gate by checking the **Active** checkbox to the right of the gate name.



e. To add a second gate, repeat steps a–d above, selecting for the parameters of interest (i.e., doublet discriminator using area vs. width or area vs. height).



6. Click the **OK** button in the **Analysis Setup** dialog when all parameters and gates have been defined. The gating hierarchy is listed in the panel on the left.



7. To complete and optimize the modeling of the data, follow the ModFit LT™ instructions.

Cell tracing and proliferation analysis using ModFit LT software v4.0

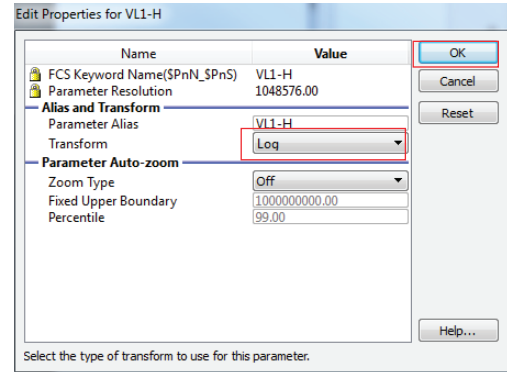
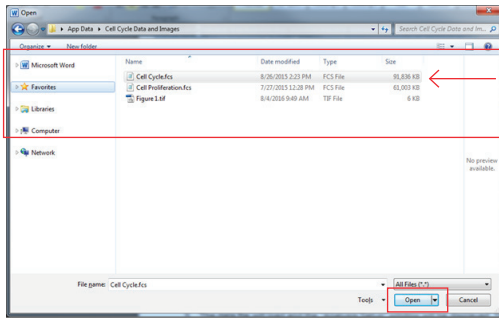
Export Attune NxT data files from the Attune NxT software. Update any keywords as required.

To open standard cell cycle data files generated by the Attune NxT software in ModFit LT software:

1. Launch ModFit LT software and click the **Open File** icon in the upper left. Alternatively, click **File** → **Open Data** from the dropdown menu on the command bar.

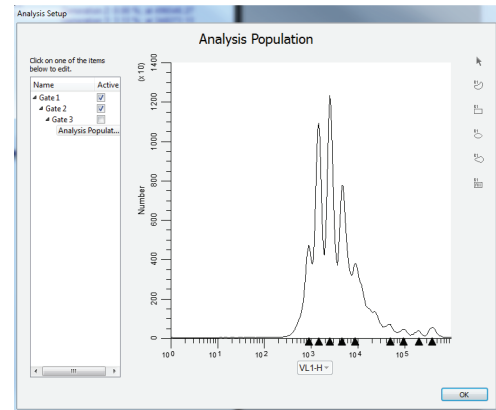
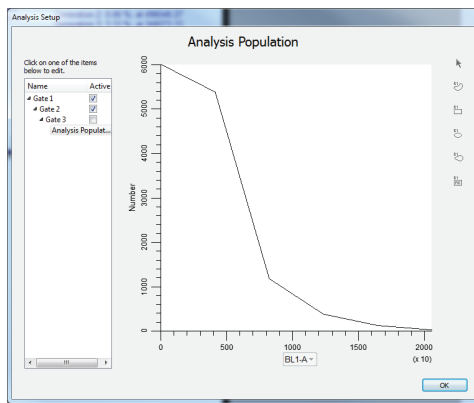


2. Navigate to the folder where the Attune NxT FCS 3.1 data files are located. Select the appropriate file that you want to analyze and click **Open**.



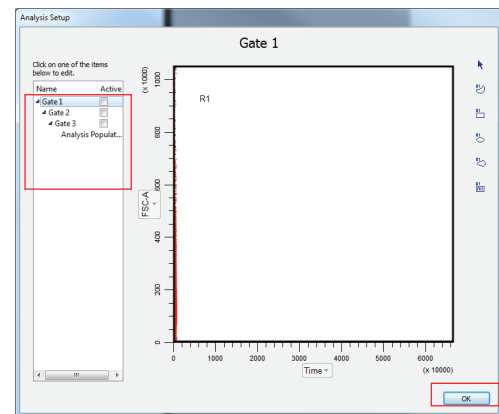
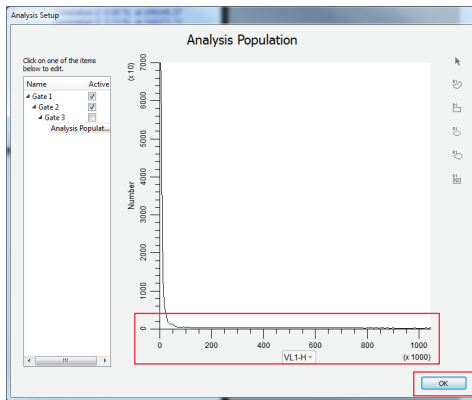
- a. Image will be displayed in log scale.

3. The data file will load and the **Analysis Setup** window will be displayed.



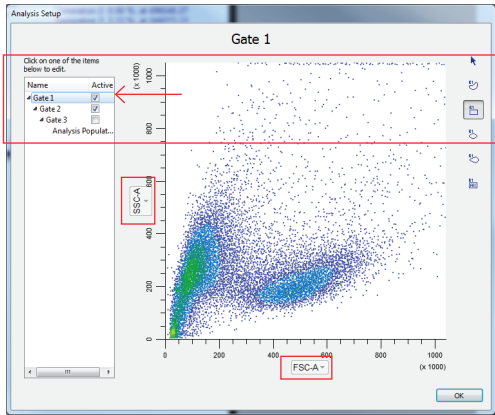
4. Select the parameter appropriate for the stain used by clicking on the x-axis parameter name. A dropdown list of all available parameters is displayed.

6. If desired, defined gates can be created to isolate a specific population of cells. If the analysis does not require any gating, click **OK** to complete the analysis setup. Otherwise, click on one of the predefined gates under the **Analysis Setup** to define a gating population:

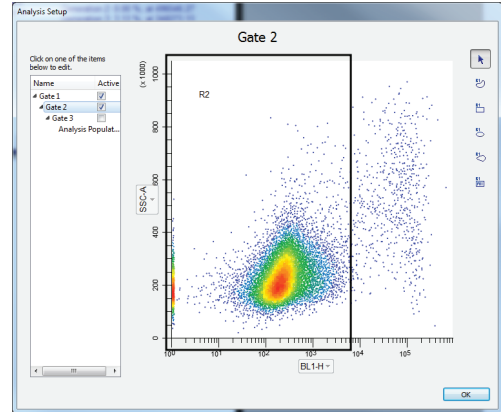


5. Ensure the parameter is set to log scale. To change axis scaling, right-click on the x-axis and select **Edit X Parameter**. Select **OK**. Ensure that the image is displayed correctly. In future analyses, ModFit LT software will remember the scaling of the selected parameter.

a. Select **Gate 1**. The plot will update based on the selected gate, and by default, will use the first two parameters within the FCS file.

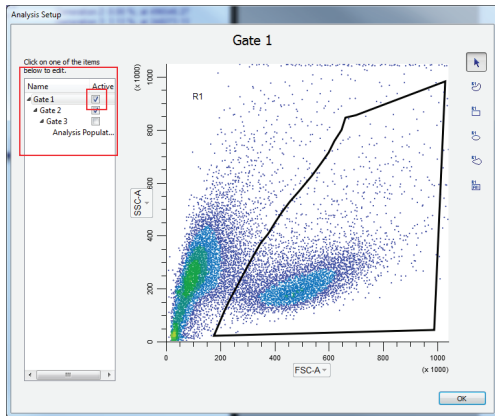


e. To add a second gate, repeat steps a–d, selecting the parameters of interest (i.e., doublet discriminator using area vs. width or area vs. height).

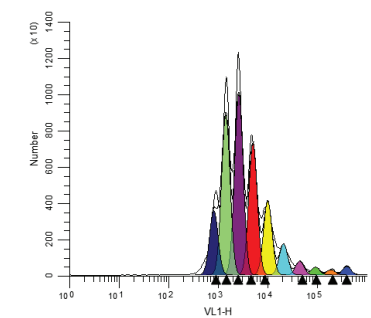
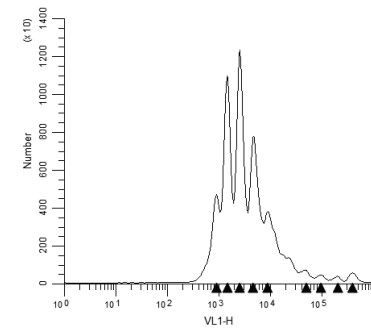
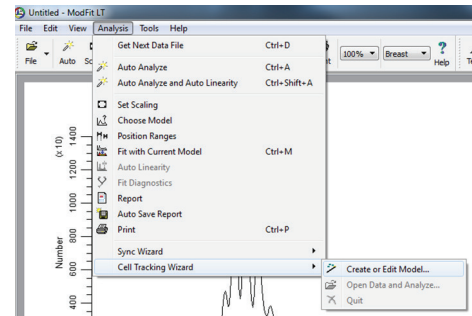


- b. Select the x and y parameters for the plot by clicking on the x-axis and y-axis parameter names and selecting the desired parameter.
- c. Draw a gate around the population of interest using the gating tools on the right side of the **Analysis Setup**.

- 7. Click the **OK** button on the **Analysis Setup** dialog when all parameters and gates have been defined.
- 8. To complete and optimize the modeling of the data, navigate to the **Analysis** menu, click the **Cell Tracking Wizard** options and choose **Create or Edit Model...** The **Proliferation Wizard** dialog will appear.



d. Enable the gate by checking the **Active** checkbox to the right of the gate name.



Cell cycle analysis using ModFit LT software v3.2

FCS files may be easily exported from the Attune NxT Software using these easy steps:

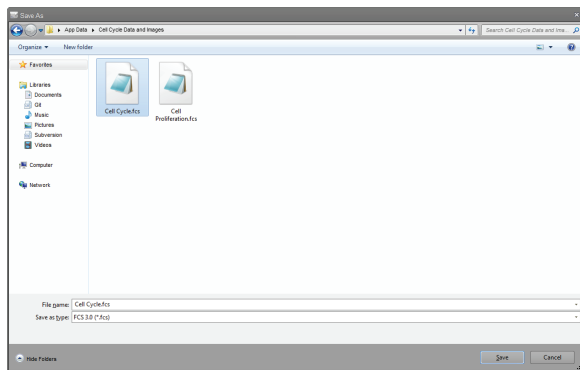
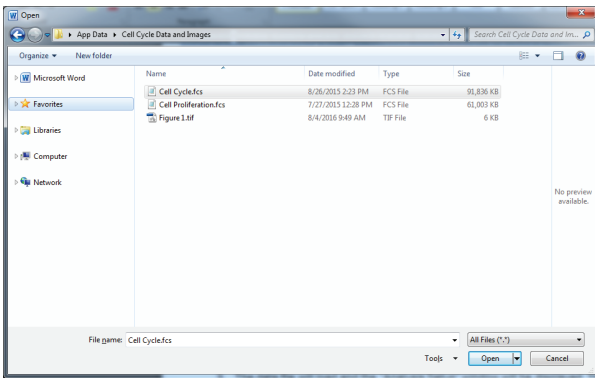
- Right-click on the name of the experiment in the experiment explorer.



- Select **Export /FCS files** from the dropdown menu.

- 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** on the bottom of the box.

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

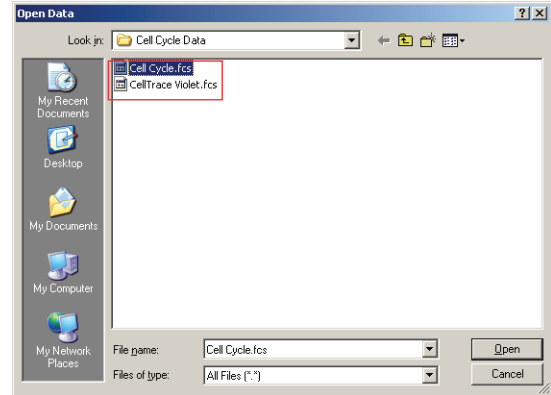


To open standard cell cycle data files generated by the Attune NxT software:

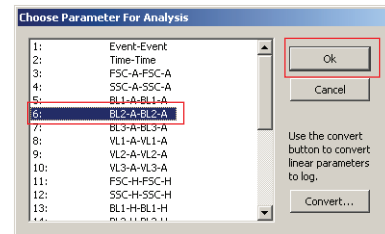
- Launch ModFit LT software and click the **Open File** icon in the upper left. Alternatively, click **File** → **Open Data** from the dropdown menu on the command bar.



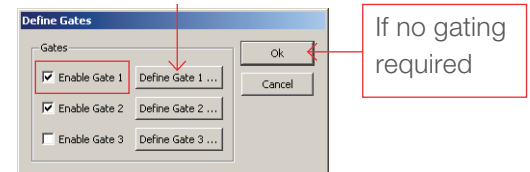
- Navigate to the folder where the Attune NxT FCS 3.0 data files are located, select the file, and click **Open**.



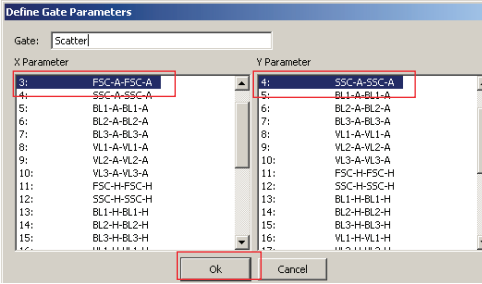
- The option box, as shown below, will open. After the option dialog box has opened, select the parameter that was used for the cell cycle stain (e.g., BL2-A for propidium iodide, VL1-A for Invitrogen™ FxCycle™ Violet stain). Select **OK**.



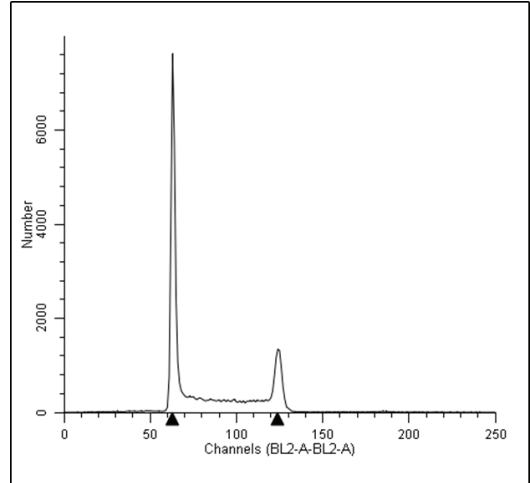
- A second dialog box will open to prompt the user to define gates if a specific population of cells is of interest. To skip this step, click **OK** and proceed to step 7. To isolate a specific population of cells, create gates as defined below. In this example, we will remove aggregates from further analysis by creating two gates. Thus, two gates will be defined.



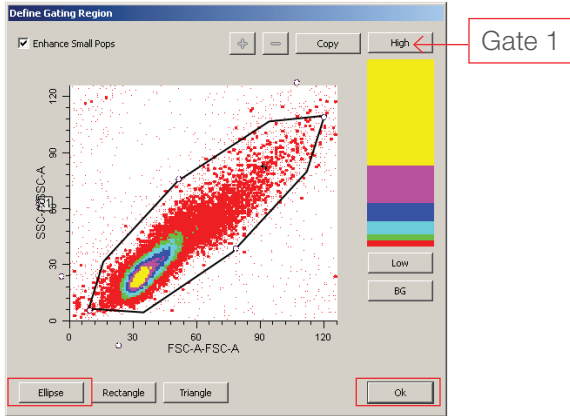
- To create the first gate, click **Enable Gate 1** and select the **Define Gate 1...** button. Selected gates will be listed as shown below.



- Click **OK** on the **Define Gates** dialog box, and a plot will open with the desired histogram displayed.



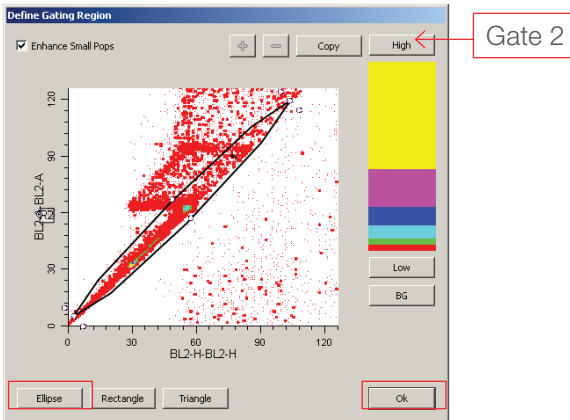
- Select FSC-A for the x-parameter and SSC-A for the y-parameter and select **OK**. This will open up the **Define Gating Region** dialog box as shown below.



- Follow the ModFit LT instructions to complete and optimize modeling of the data.

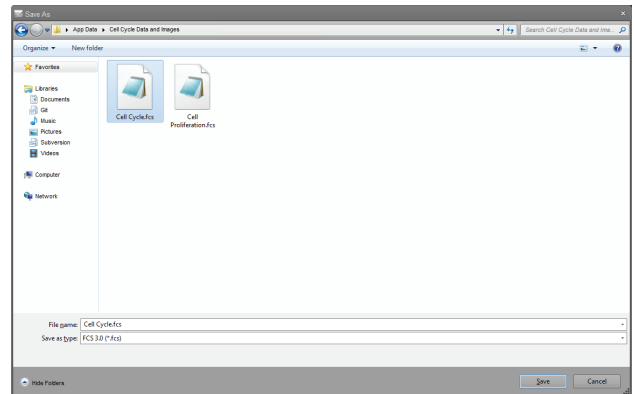
Cell tracing and proliferation analysis using ModFit LT software v3.2

Export Attune NxT data files from the Attune NxT software. Update any keywords as required and select the FCS 3.0 (*.fcs) option as the **Save as type**.



- Select the type of gate using the buttons at the bottom and draw a region around the population of interest. Select **OK**.
- Additional gates can be defined by selecting the **Enable Gate 2** followed by selecting **Define Gate 2...** This will open the **Define Gate Parameters** for the second gate.

Tip: The width parameter on the Attune NxT software is collected directly from the instrument and will have values that will not scale correctly in ModFit LT software. We suggest that you use the area and height parameters to gate singlet cells if needed.

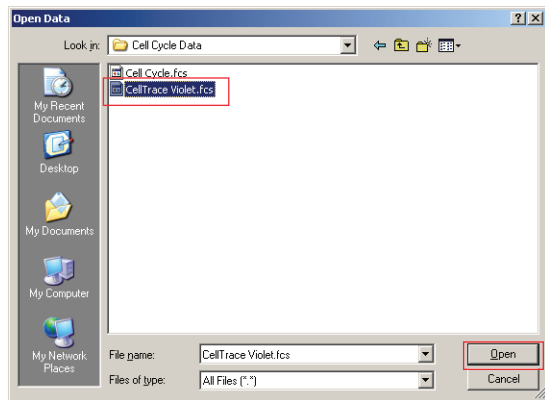


To open standard cell cycle data files generated by the Attune NxT software:

- Launch ModFit LT software and click the **Open File** icon in the upper left. Alternatively, click **File** → **Open Data** from the dropdown menu on the command bar.

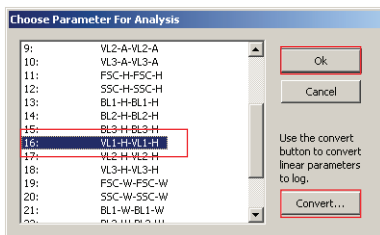


- Navigate to the folder where the Attune NxT FCS 3.0 data files are located, select the file and click **Open**.



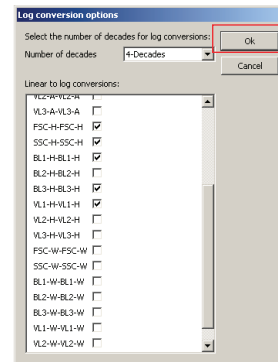
3. The option box, as shown below, will open. Select the parameter appropriate for the stain used in cell proliferation and tracing (e.g., VL1-H for Invitrogen™ CellTrace™ Violet stain).

a. Once the appropriate parameter has been selected, click the **Convert...** button in the lower right corner of the dialog box as shown below. Click **OK**.



b. Ensure that all fluorescence parameters measured during acquisition using the Attune NxT Software are selected in the **Log conversion option** dialog box.

c. Select **4-Decades** from the dropdown menu at the top of the dialog box denoted **Number of decades**, and click **OK**. This will convert the linear fluorescence data collected by digital cytometers like the Attune NxT system to log scale for analysis.



4. Select **OK** on the **Choose Parameter For Analysis** option box from step 3. This will open up the **Define Gates** options. Set any relevant gates to target the population of interest.

Note: See steps 4–6 in “Cell cycle analysis using ModFit LT software 3.2” above for descriptions of defining gates in ModFit LT software.

5. After creating gates, click **OK** to generate a histogram plot specific for the parameter used in the cell proliferation or tracing experiment.

a. From the dropdown menus at the top, select **Analysis** → **Proliferation Wizard** → **Create or Edit Mode I...**

6. For further optimization and modeling of data using ModFit LT software, please refer to instructions available for the ModFit LT software.

