

ModFit LT™ User Guide

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This guide is intended for ModFit LT™ 6.0 and later minor versions.

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License and Warranty

License Agreement

Copyright laws of the United States and international treaty protect this software. Verity Software House provides this software under the following conditions:

Software is intended to be installed and used on one computer. An additional computer license is required for each additional installation.

Network versions of software require one serial number for each simultaneous use. Network versions are intended for installation and use on a local area network; other uses are not supported.

Backups of the software must be used only for archival purposes.

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Verity Software House (VSH) warrants the physical media and the physical documentation, if included, to be free of defects in materials and workmanship for a period of 30 days from date of purchase. If VSH receives notice of defects in materials or workmanship within the warranty period, VSH will replace the defective media or documentation.

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Latest Versions

To find the latest versions for this software and documentation, visit the Verity Software House web site at www.vsh.com. This site provides a complete version of the latest documentation, service packs, and technical support information for the program.

You can also email us at verity@vsh.com to determine whether you have the latest version. Please include the version that appears on the start-up dialog box and the program file date for the program.

The program also has a [Check for Updates](#) command in the Help menu. Use this command periodically to see if there are updates for your software.

Naming Convention

This manual and ModFit LT's analysis results use shortened forms of ploidy terms to improve readability. We use the term "Diploid" when referring to "DNA diploid". Similarly, we use the terms "Aneuploid" and "Tetraploid" when referring to "DNA aneuploid" and "DNA tetraploid" respectively.

When using analysis results in publications or other contexts outside of the software, we suggest that the explicit "DNA" terms be used. See "Convention on Nomenclature for DNA Cytometry", Hiddeman et, al., Cytometry 5:445-446 (1984) for additional details.

New Features

Version 6.0

Projects

Organize your experiments quickly and easily with *Projects* in ModFit LT. Projects collect your analysis setup, output files, and the list of FCS files that have been selected. A project can also contain a copy of the FCS files themselves, making it easy to move and archive your experiments.

Projects also provide improved guidance for the different kinds of analysis ModFit LT can perform. Project types include cell cycle, synchronization cell cycle, cell tracking, and custom.

Projects offer enhanced options for batch processing and collect output into well-defined locations.

User Interface

The user interface has been updated to support high DPI monitors, including updated icons and dynamic sizing.

The MacOS version is now compatible with ARM and Intel processors and MacOS 10.14, 11, and 12

The Windows version is now compatible with Windows 10 and 11.

ModFit LT now supports translation into different languages.

Analysis

Improved logic for internal and external reference populations in Auto Analysis.

Gating keywords update when gates are moved.

A new *normalized* RCS value (*nRCS*) provides better comparison of the goodness of fit for samples with a large number of cells.

The VLog transform is now available for log-like data.

Output

SVG output has been greatly improved.

PDF output now offers user-selectable resolution.

Database wizard now adds all results from the Sync wizard.

Bug fixes

MF-267: Ranges disappear from range view when a report is saved, auto saved, or opened.

MF-275: Additive Factor displays histogram channel values even in ADC mode.

MF-609: Hyp, Dip cycles change order in Choose Model.

MF-725: Sync wizard adjust dialog shows peak at a negative position. Corrected.

MF-608: Changing the G2/M to Disabled in Choose Model turns off S-phase, even if manually checked to enable.

MF-711: Cell tracking model fails for DDE data from WinList.

MF-620: Failure with the cell tracking wizard and generations that are off scale.

MF-411: Shortcut keys for actions only work on the current tab.

MF-753: Save Image context menu output does not use correct background.

MF-602: Wide-open gate is eliminating events on the axis.

MF-295: Canceling during batch processing can lead to unexpected behavior.

MF-312: Result text is clipped at 50% zoom.

MF-306: Update results after switching the axis scale.

MF-735: Crash after editing full model while using the sync wizard.

MF-661: Fixed issue in remote connection with WinList that could occur if the FCS file name contained a Unicode character (e.g. the micro symbol).

MF-385: Crash if the user deleted a gate and then clicked on the axis to change measurements on the gate that should no longer be displayed.

MF-340: Crash if the user opened an FCS file, showed the range view, clicked New, and then loaded an FCS file.

MF-353: Crash if the user deleted the last tissue type in the S-Phase Cutoffs dialog.

MF-351: Crash if the user loaded an FCS file that had fewer measurements and a gate referred to measurements that did not exist in the data.

MF-342: Crash if the user used a Gaussian component shape for S-phase.

MF-332: Crash if the user opened malformed FCS files that contained \$PnR (range) values that were negative.

MF-318: Crash if the user opened an pre version 5 report with gate 2 or 3 active and the lower number gates inactive.

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MF-328: Crash if the user opened certain LT 3.2 reports and clicked cancel in the analysis setup dialog.

And additional bug fixes...

System Requirements

NOTE: Your software will only run on the operating system you select at the time of purchase.

Minimum System Configurations

For PC Version: Windows 10 version 1607 (build 14393) or newer, Windows 11 or greater with 50MB free disk space for program files.

For Mac Version: MacOS 10.14, 11.x, 12.x on ARM or Intel processor, 50MB free disk space for program files.

Optimal Configurations

Faster CPUs with more cores, SSD drives, and greater RAM will improve performance.

Installation and Running ModFit LT

IMPORTANT NOTES:

- **Stand-alone versions of the program are designed to be *installed and registered on a single computer*.**
- You must have administrative rights to the computer during installation and registration of the software. After registration, the program can be run by users with restricted access to the computer.
- You will need to register the software with Verity Software House in order to unlock the program on the computer. This can be done using the online registration process or by contacting technical support.
- If your program is an update version, you will need the serial number for the previous version in order to register and unlock the software.
- If your program is an additional computer license, you will need the serial number for the primary license in order to register and unlock the software.

Windows Stand-alone Version

Stand-alone version is designed to be installed and registered on a single computer.

Installation

- Log on to your computer with an account that has Administrator privileges. You *must* have administrative rights during installation.
- If you downloaded the software, extract the installation files from the download into a temporary folder. If you have an installation CD, insert the program CD into the CD-ROM drive.
- Run the program named **ModFitLTSetup.exe**. Follow the directions on the screen to complete the installation.
- See the section on [registering your software](#) for information on how to register your software.

Using the software

- Log on to your computer with your own user account. This should be the account that you normally use on the computer.
- Open the Start menu and navigate to the Verity folder. Choose ModFit LT 6.0.

Macintosh Stand-alone Version

Stand-alone version is designed to be installed and registered on a single computer.

Installation

- Log on to your computer with an account that has Administrator privileges.
- If you downloaded the software, extract the installation files from the download into a temporary folder. If you have an installation CD, insert the CD into the CD-ROM drive.
- Run the **ModFit LT Installer**. Follow the directions on the screen to complete the installation.

Using the software

- Log on to your computer with your own user account. This should be the account that you normally use on the computer.
- Start the program by double clicking on the **ModFit LT** application in the Applications/ModFit LT folder.

Registering your new software

Why You Should Register

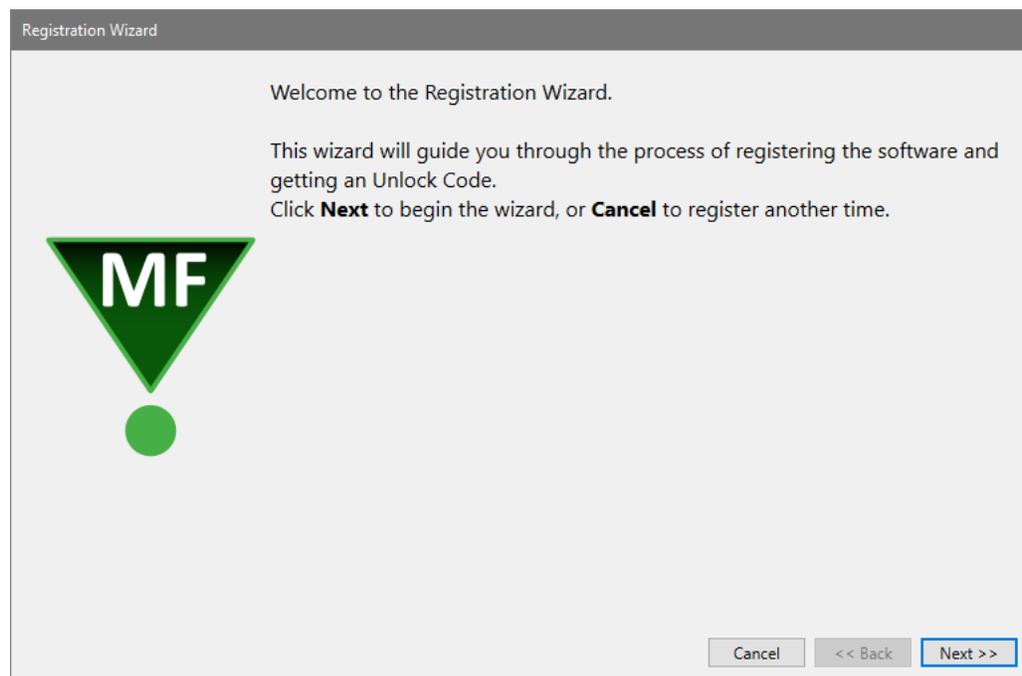
Registration is a key step in setting up the software to run on your computer. In fact, *your software will not be fully functional until you register*. Here's why.

This version of the program uses software security, which requires you to register your software in order to receive an **Unlock Code** to make the program fully functional. An unregistered installation will be fully functional for 30-days, and then become a "trial version" with limited functionality until it is registered.

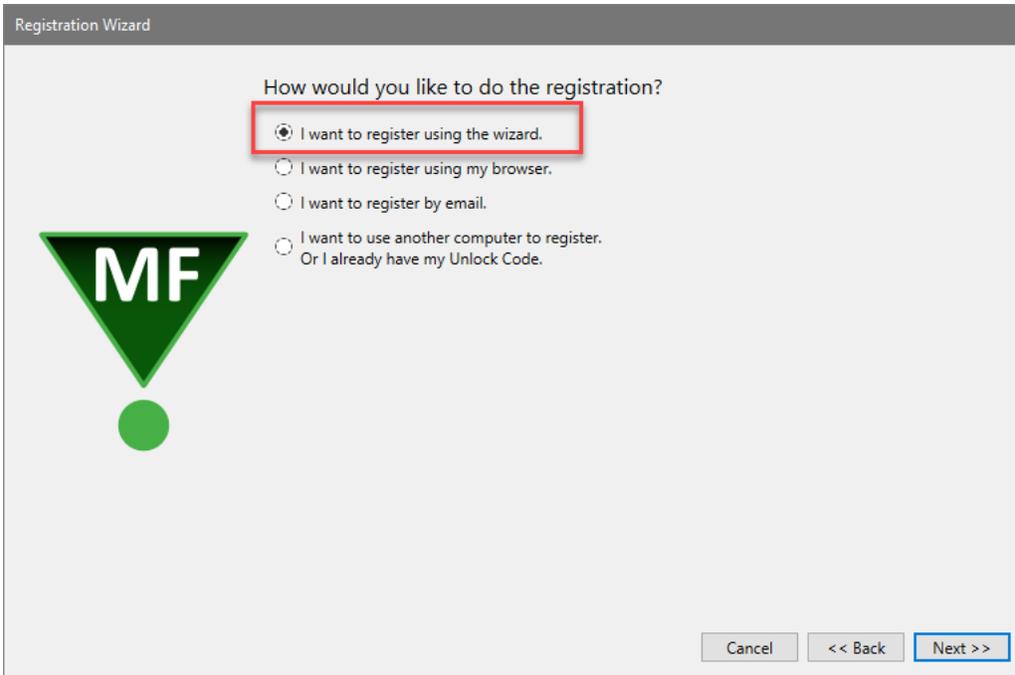
Of course, registration is useful for other reasons, too. We can inform you of bug fixes and updates, and provide you with better technical support.

How to Register

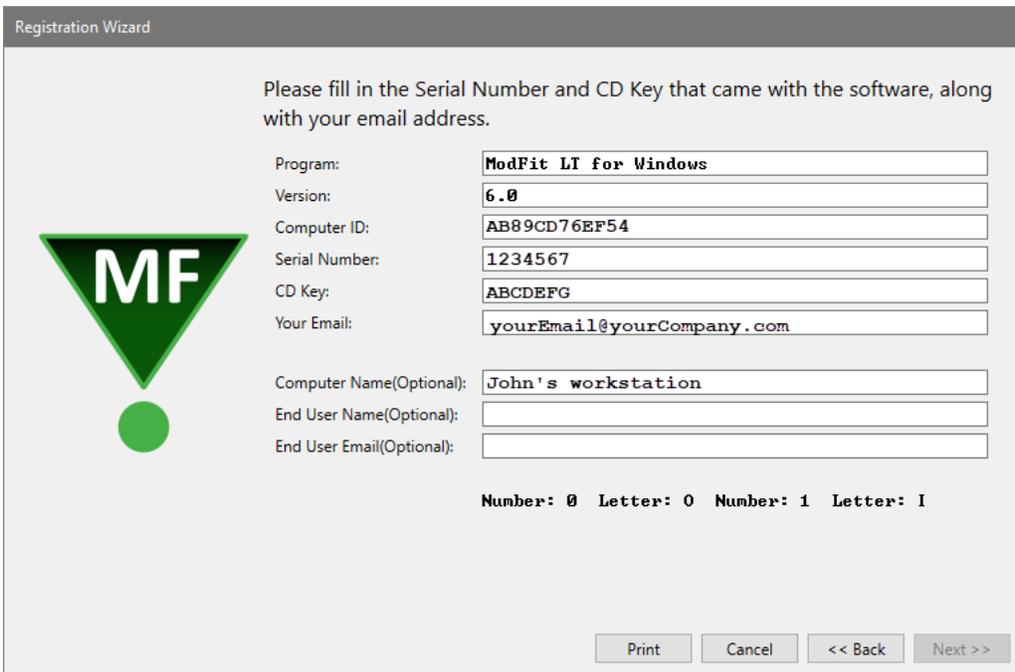
When you start the program for the first time, it will automatically start the Registration Wizard. You can access this dialog at any time by clicking the *Registration* button on the dialog box that is displayed when the program starts up.



Click the **Next** button to begin the registration process.



The wizard will ask how you want to register the program. Select the appropriate option and click **Next**.



The next step in the wizard shows some information about the program. There are 3 fields that you need to fill in for this step: *Serial #*, *CD Key*, and *Email*. You can also enter or edit the *Computer Name*, *End User Name*, and *End User Email* fields.

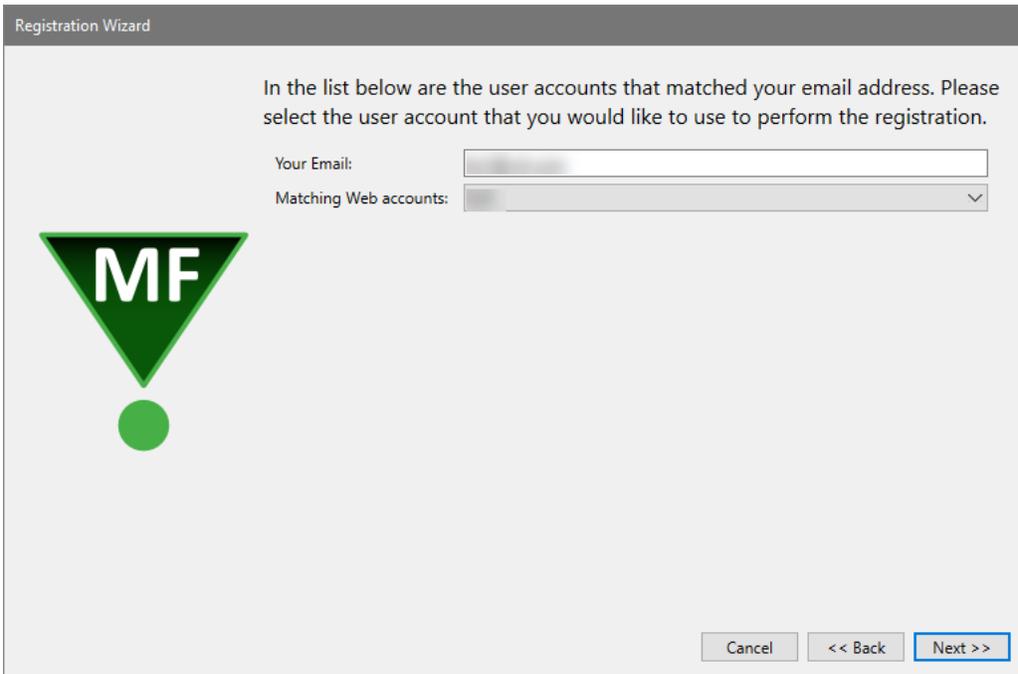
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The *Serial number* and *CD Key* can be found on the outside of the box the program came in, or in email if the program was provided to you by download. Enter these exactly as they appear; they are case-sensitive. If you have lost or misplaced the Serial number or CD Key, contact Verity technical support at tech@vsh.com or (207) 729 6767.

For the *Email* field, make sure to enter a valid email address. Otherwise, you may not receive the Unlock Code and the program will not be fully functional.

Click **Next** to continue the registration process.

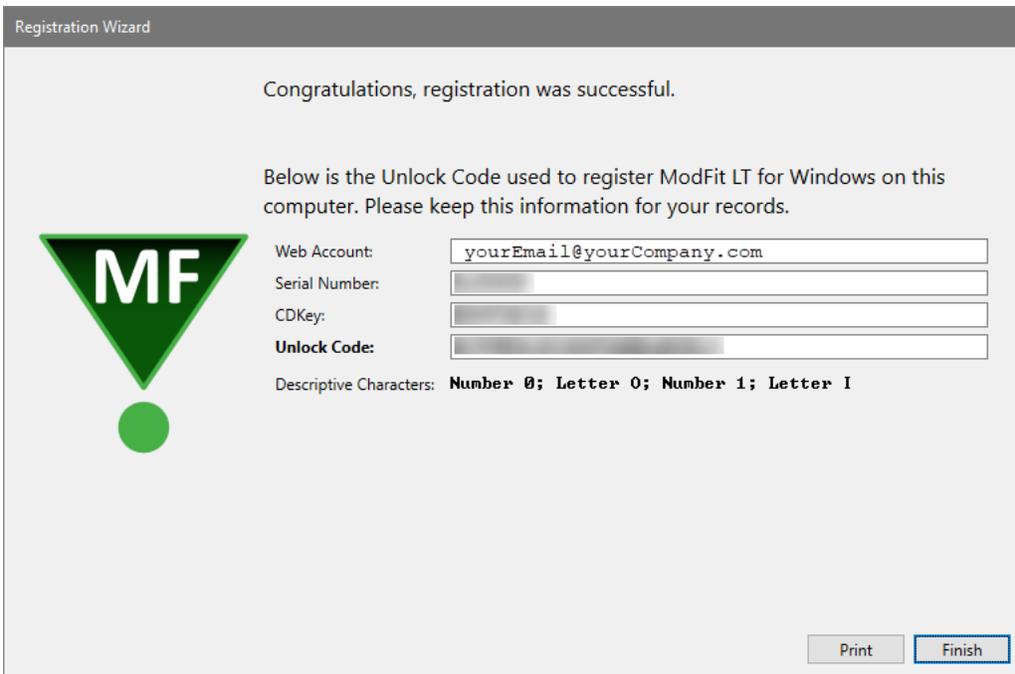
If you have more than one user account at vsh.com, you will be presented with another step. This step *only* appears if you have more than one account.



The image shows a 'Registration Wizard' dialog box. At the top, it says 'Registration Wizard'. Below that, it reads: 'In the list below are the user accounts that matched your email address. Please select the user account that you would like to use to perform the registration.' There are two input fields: 'Your Email:' followed by a text box, and 'Matching Web accounts:' followed by a dropdown menu with a downward arrow. On the left side of the dialog, there is a green inverted triangle containing the white letters 'MF' and a small green circle below it. At the bottom right, there are three buttons: 'Cancel', '<< Back', and 'Next >>'.

Choose the web account that you want to use for registration, and then click **Next**.

At this point, the program will request an unlock code from the registration server at vsh.com. If successful, you will see the confirmation step and the Unlock Code for your computer.



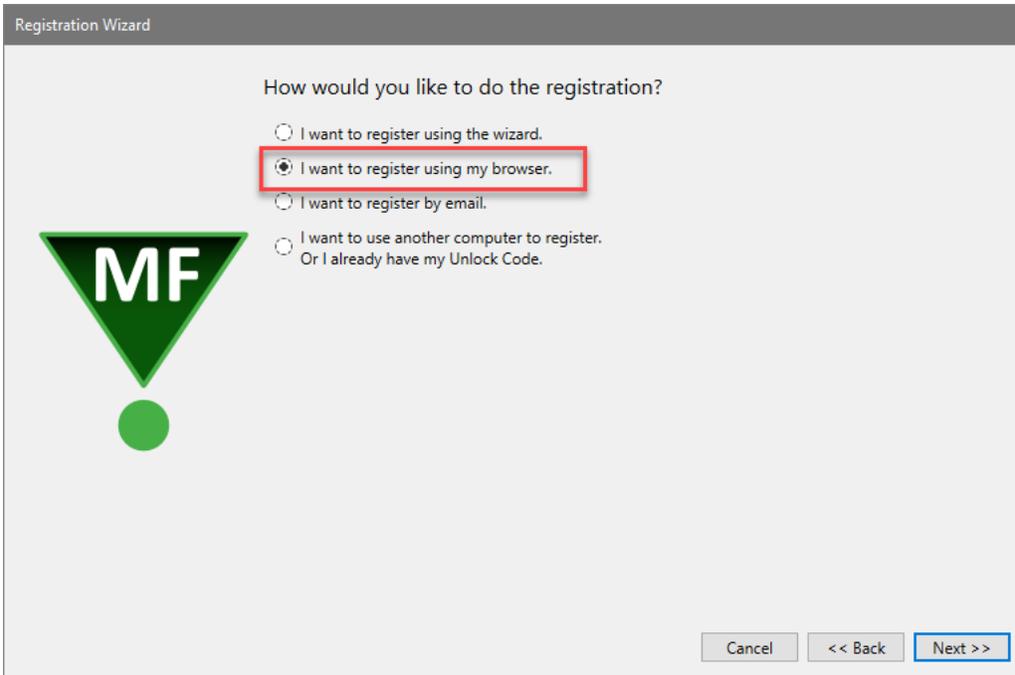
You can print the registration information by clicking the **Print** button. Click **Finish** to close the wizard and continue working with the program

If there was a problem with the registration, an informative message will explain the problem and steps to take to correct it.

Once you have completed the registration successfully, the program will be fully functional.

If the registration wizard cannot connect to vsh.com...

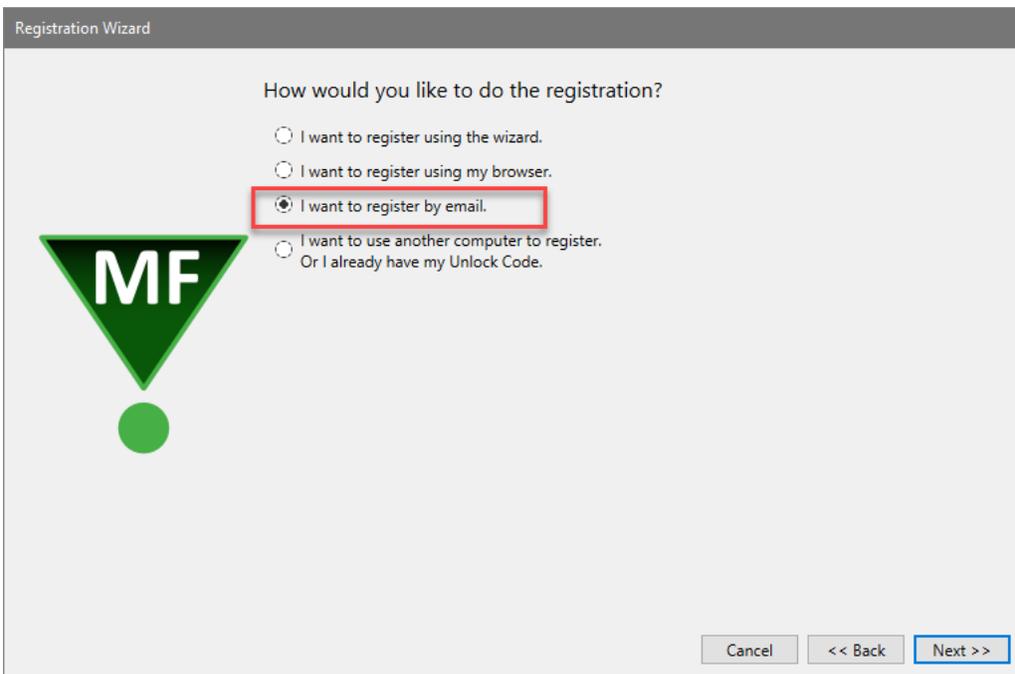
Sometimes firewalls can prevent the registration wizard from connecting to vsh.com. In this case, you can try registering with your browser. Choose the second option in the list and click **Next** to launch your browser with the correct registration information.



Follow instructions on screen to complete the process.

To register your software using email...

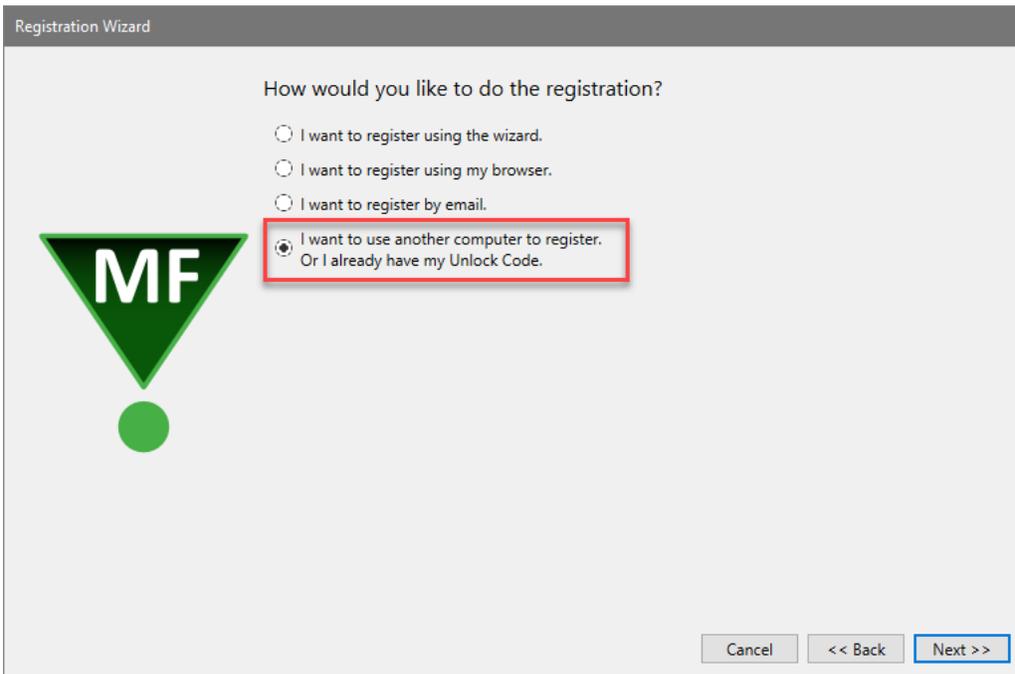
The registration wizard can help you send email to Verity Software House with your registration information. Choose the third option in the list and click **Next** to create an email message.



Follow instructions on screen to complete the process.

To register your software using another computer...

When the installation computer does not have internet access but another computer does, you can use the fourth option in the list and click **Next** to begin the process.



On the installation computer, write down the Computer ID displayed in the Register Software dialog.

Next, go to the computer that has Internet access, launch a browser, and navigate to www.vsh.com.

Click the **Register Software** link.

If you already have a web account, login with your user name and password. If you do not have a web account, create a new account that will be associated with this registration.

After you login, the **Software Registration** page will be displayed on the web site. Fill in the information in the form on this page to register the product. If you are registering an *update version*, you will need the serial number of the previous version to complete the registration. If you are registering an *additional user license*, you will need the serial number of another copy of the program that is registered to your institution.

Getting an Unlock Code is a two-step process. First, you fill in and submit the registration form on the web site. This sends a registration request to Verity. Your Unlock Code is then emailed to you, so make sure you provide a valid email address.

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When you receive your Unlock Code via email, paste or type it into the Unlock Code field in the Registration dialog box on the installation computer. When possible, use Copy and Paste to do this, to ensure that the characters are correct. Then click the OK button.

Your program will become fully functional once the Unlock Code is entered correctly.

If you do not have Internet access...

If you do not have access to the Internet, you can send the registration information below to Verity Software House by fax, mail, or telephone. Our technical support staff can register the software for you with the information you provide, and supply you with the Unlock Code to enter into the Registration Wizard.

Here is the information we need. You can print this page and fill it in.

These fields are found in the Registration dialog box.

Program Name:
Program Version:
Computer ID:
Serial number:
CD Key:

User Information

Name:
Institution:
Address:
City:
State/Province:
Zip/Postal Code:
Country:
Phone:
Fax:
Email:

Our technicians will use the information below to create a web user account for you on our web site.

Name to use on VSH web:
Password to use on VSH web:
A question we can ask to verify who you are:
The answer to the question to verify who you are:

If you are updating from a previous version, you will need to supply the serial number of the previous version.

Serial Number of previous version:

Contact Verity Software House

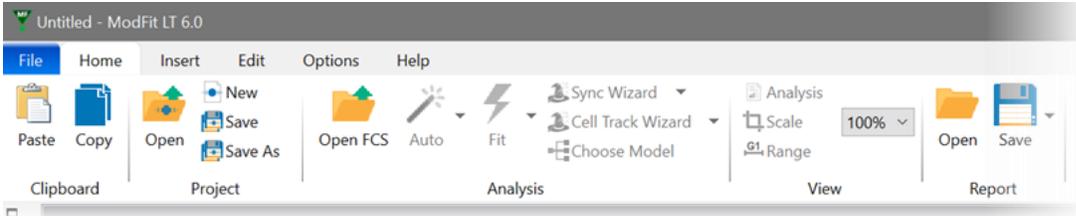
[Click here for contact info.](#)

Tutorials

Auto Analysis with a Cell Cycle Project

This tutorial will introduce you to some of the key features of ModFit LT, including creating a new project, adding files to it, and setting up the processing options for Auto Analysis.

- Start ModFit LT by following instructions in [Installing and Running ModFit LT](#).
- Click the OK button on the opening dialog, if it appears.

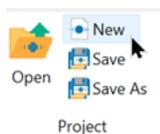


The ModFit LT ribbon bar appears at the top of the application window. Buttons are grouped to give you quick access to the most common commands in ModFit LT.

Create a new project

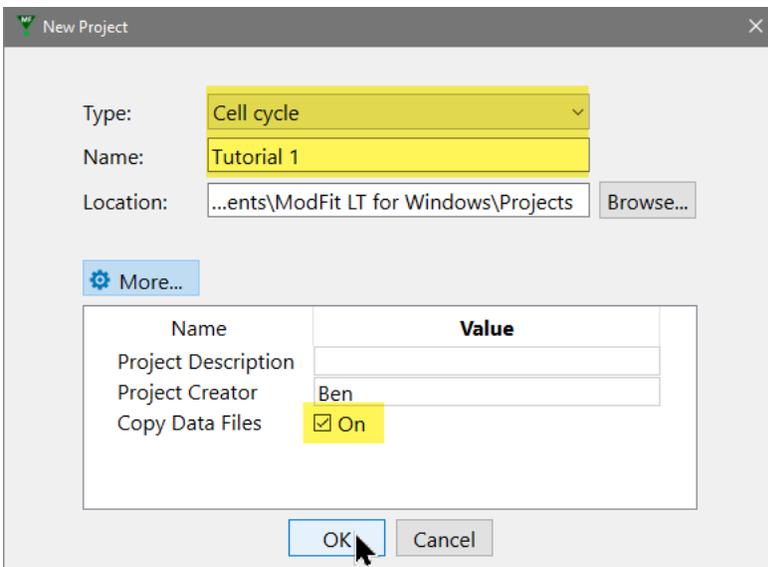
We start by creating a new project.

- Click the **New** button in the **Project** panel on the ribbon.



The New Project dialog is displayed.

- Choose **Cell Cycle** for the Type.
- For the Name, enter **Tutorial 1**.
- Click the **More** button and enable the **Copy Data Files** option.
- Click **OK**



ModFit LT will create a folder in your Documents ModFit LT Projects folder named Tutorial 1. When we select FCS files for this project, ModFit LT will copy the files into the project folders.

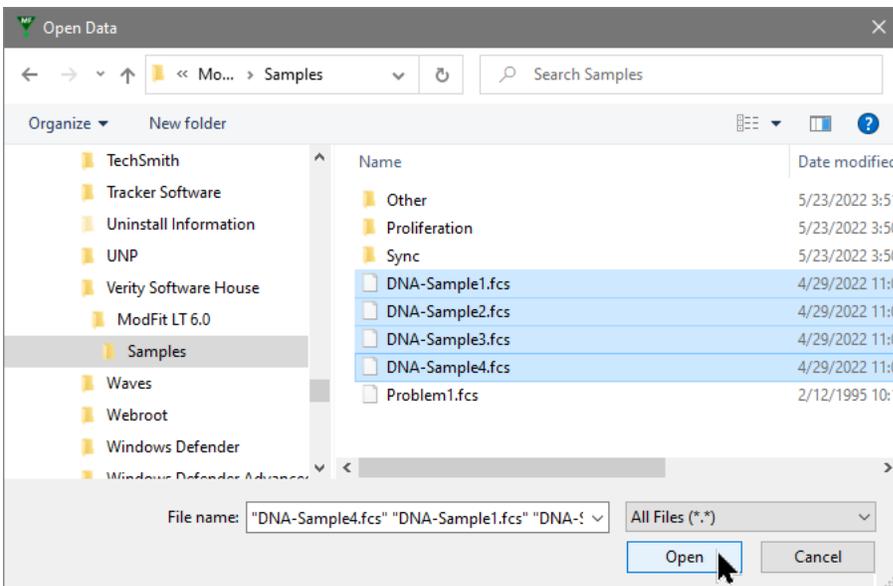
What is a project?

A *project* in ModFit LT is a folder on disk used to organize an analysis or experiment. It keeps track of the kind of analysis you're doing, the files you analyze, the output of the analysis, and even a database of analysis results. For more on projects, see [Projects in ModFit LT](#).

Add files to the project

With our Cell Cycle project created, let's add some FCS files.

- Click the Open FCS button on the ribbon. The Open Data dialog is displayed.
- Navigate to the location where ModFit LT is installed:
 - Windows: Program Files\Verity Software House\ModFit LT 6.0
 - MacOS: Applications\ModFit LT 6.0
- In the **Samples** folder, select the four (4) DNA sample files: DNA-Sample1.fcs, DNA-Sample2.fcs, DNA-Sample3.fcs, and DNA-Sample4.fcs.
- Click **Open**.

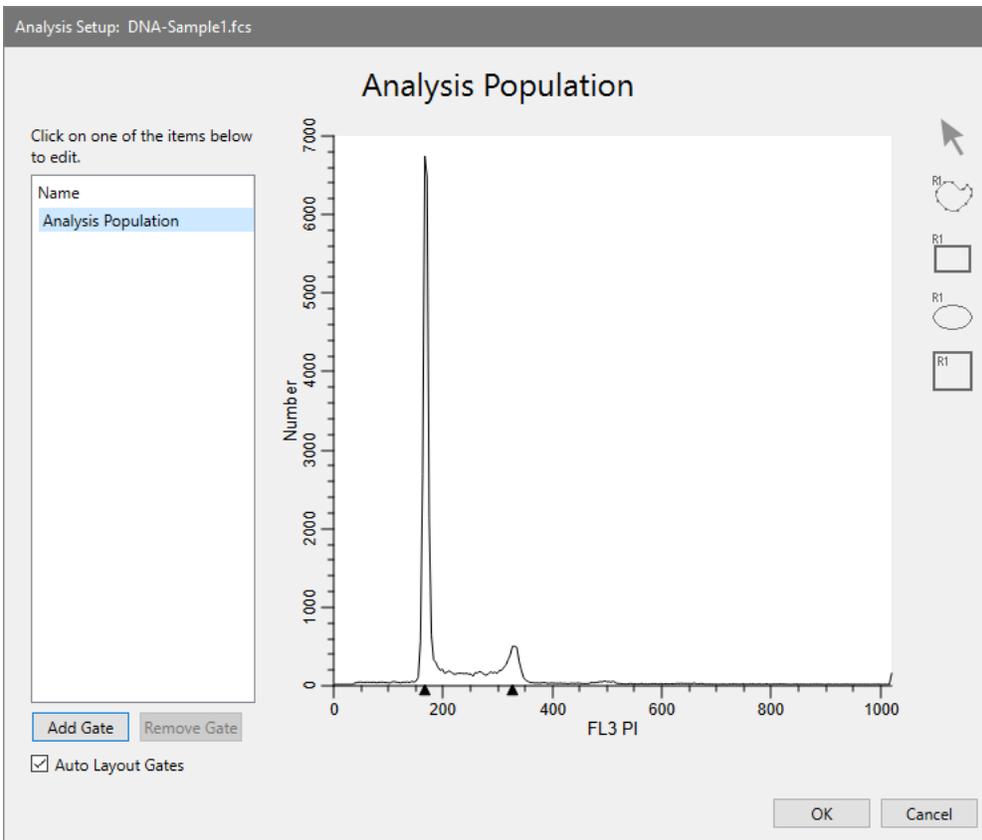


The files will be added to the file list for the project, and the first file will be opened.

Select the measurement to analyze and setup a gate

When the first file is opened, the Analysis Setup dialog is displayed. This dialog allows us to set up one or more gates and to select the measurement that we want to analyze.

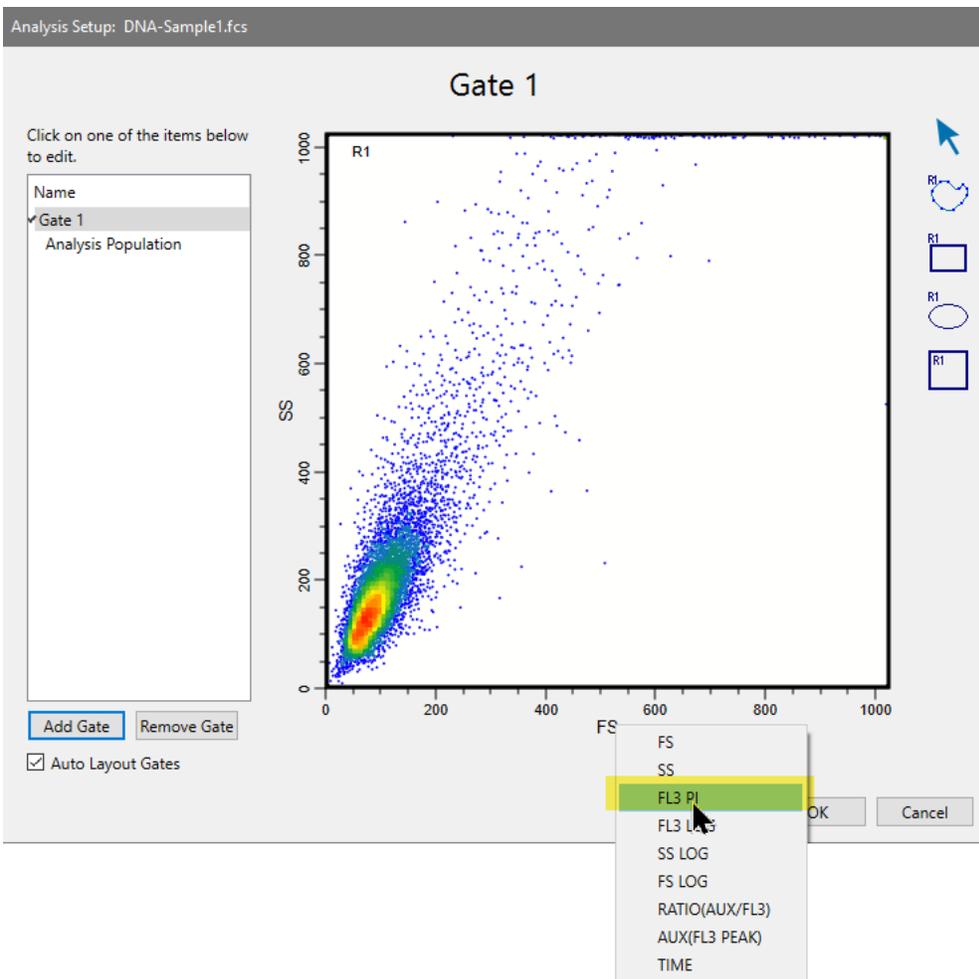
The measurement that we want to analyze is already correctly selected: **FL3 PI**.



Let's add a gate so that we can better visualize the data.

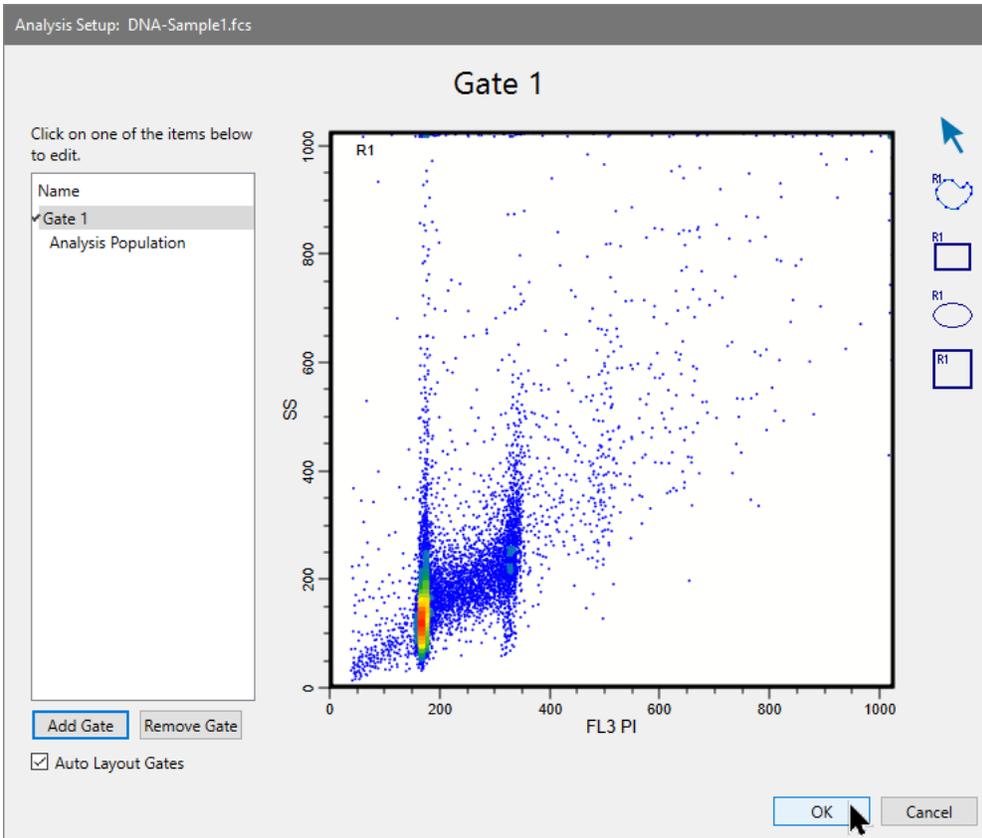
- Click the **Add Gate** button in the dialog. A new gate is added and displayed.
- Click the X-axis label "FS". This opens a list of the measurements in the FCS file.
- Click FL3 PI in the list to display the DNA measurement on the X axis.

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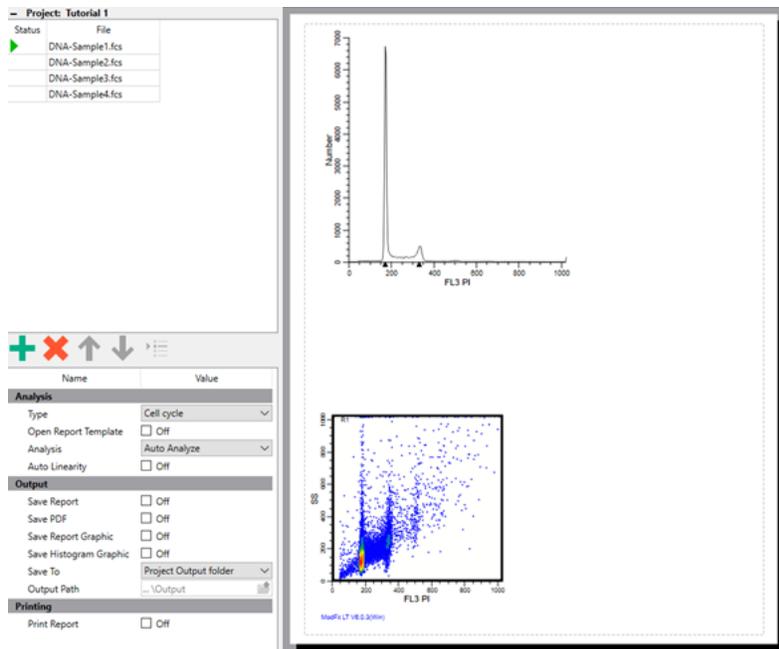
- Click OK.

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The dialog closes and the file is read. The report page shows a 1P histogram of the DNA measurement "FL3 PI", as well as a gate plot of FL3 PI vs SS. On the left of the main window, the FCS files appear in a list. Below the list of files, processing options are shown.

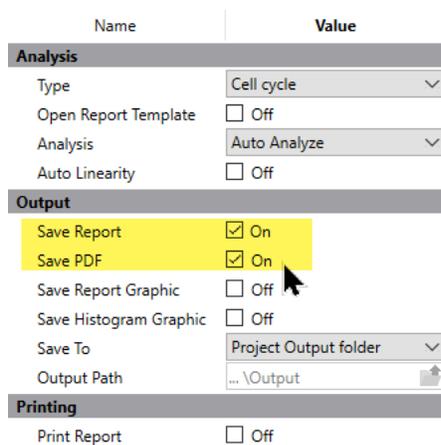
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Choose processing options

The processing options in the lower-left portion of the screen determine what actions ModFit LT will perform when it analyzes each of the FCS files we added to the list. The **Type** is already set to **Cell cycle**; we chose that when we created the project. The Analysis is set to **Auto Analyze**, and that's what we want for this project. Let's enable two output options.

- Enable **Save Report** and **Save PDF** in the options list.



With these settings, each file will be Auto Analyzed, a report will be saved, and a PDF will be created.

Other options

For details about each of the processing options, take a look at [Batch Processing](#).

Processing the files

We are ready to analyze the files in this project. This is done using the controls at the very bottom-left of the main window.

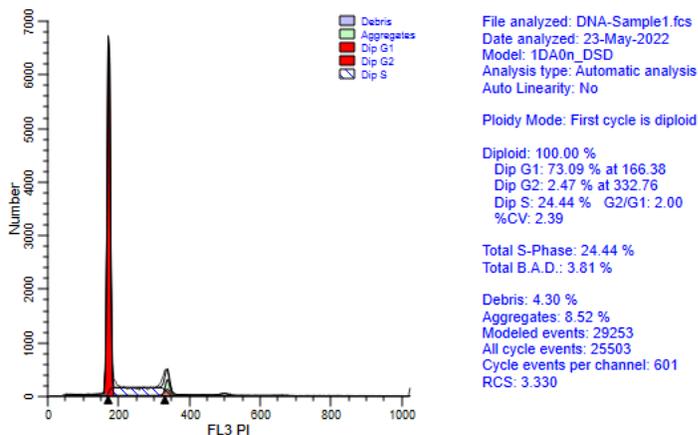
- Click the **Run** button to analyze the first file in the list.



Batch toolbar

The batch toolbar has controls to move through the list of files in your project one at a time, or automatically to the end of the list.

ModFit performs its automatic analysis of the sample. It scans the histogram for peaks, and based on the sizes and positions, ModFit LT determines the number of cell-cycles in the sample. It performs a *non-linear least squares* analysis of the data. The results of the analysis are shown to the right of the DNA histogram.



We can process the rest of the files in the list automatically.

- Click the Auto Advance button on the toolbar.

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Processing the remaining files will not take long. Notice that the file list indicates that a file has been analyzed as each one is processed.

Project: Tutorial 1	
Status	File
✓	DNA-Sample1.fcs
✓	DNA-Sample2.fcs
✓	DNA-Sample3.fcs
▶ ✓	DNA-Sample4.fcs

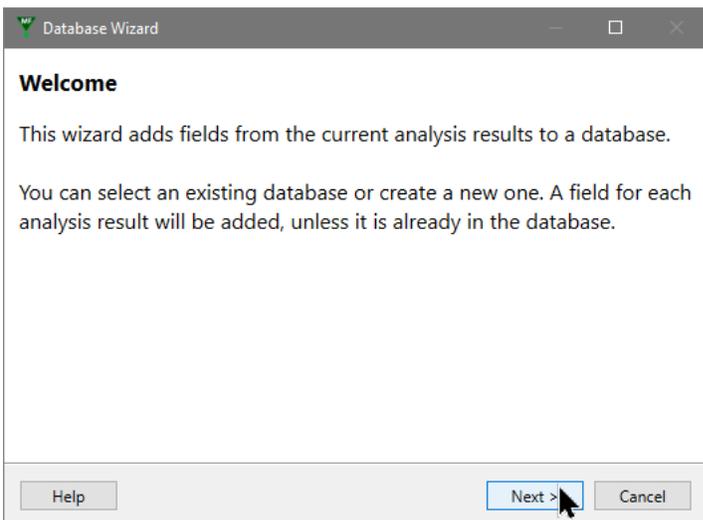
Create a database

Let's use the Database Wizard to set up a database of the analysis results for the project.

- Click the **Edit** tab of the ribbon.
- Click the **Database Wizard** button in the Database panel.



The Database Wizard dialog is displayed.

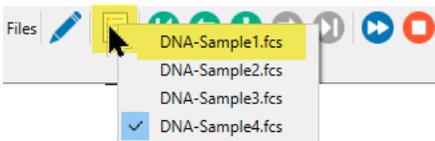


- Click **Next**.
- On the next page of the wizard, select "Tutorial 1" as the database.
- Click **Finish**.

When the wizard closes, a new Database Viewer window appears on the screen. This window will allow us to view records as they are added to the database. The fields in the database were added by the wizard, based on the analysis we performed.

Since it was so quick to analyze the files in our project, let's analyze them again so that the results can be added to the database we just created.

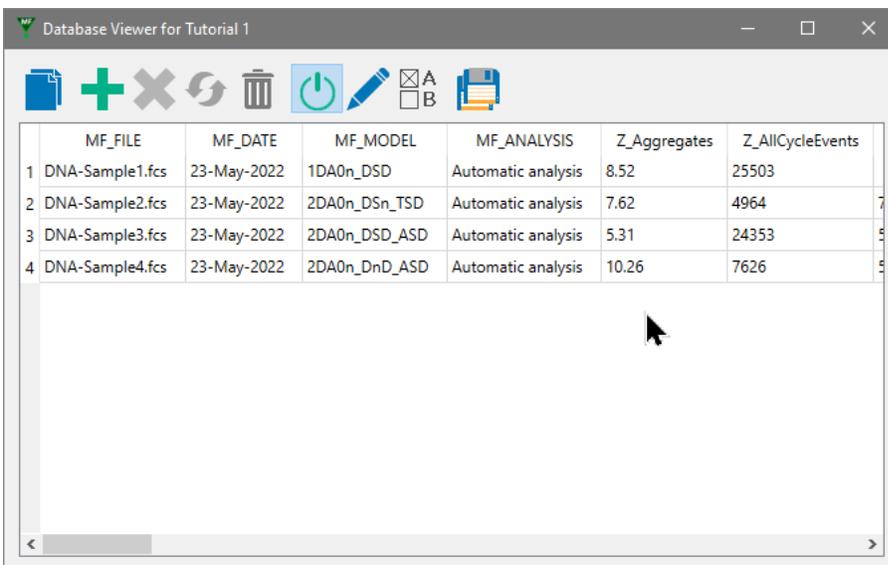
- Click the Choose button on the batch toolbar and select DNA-Sample1.fcs from the menu.



- Click the Auto Advance button to reprocess all four files in the project.



The Database Viewer window fills with four records of analysis from the four files.



	MF_FILE	MF_DATE	MF_MODEL	MF_ANALYSIS	Z_Aggregates	Z_AllCycleEvents
1	DNA-Sample1.fcs	23-May-2022	1DA0n_DSD	Automatic analysis	8.52	25503
2	DNA-Sample2.fcs	23-May-2022	2DA0n_DSn_TSD	Automatic analysis	7.62	4964
3	DNA-Sample3.fcs	23-May-2022	2DA0n_DSD_ASD	Automatic analysis	5.31	24353
4	DNA-Sample4.fcs	23-May-2022	2DA0n_DnD_ASD	Automatic analysis	10.26	7626

Project database

When a new project is created, a database is created for it. Use the Database Wizard to add fields quickly to the project's database. You can show or hide the viewer without affecting the database, and records will be added as files are analyzed as long as the database is "active". A button on the Database Viewer toggles the active state of the database on or off.

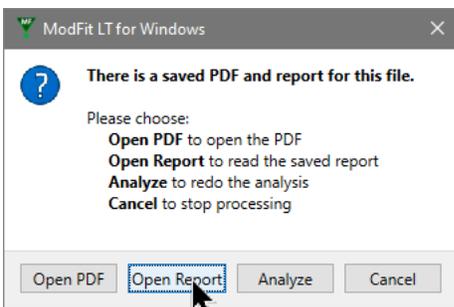
Optional step

Creating a database is an optional step for a project. It is a useful step if you want to export your experimental results to another program, such as Excel, for further analysis.

Review the analysis

At this point, we have a working database and we should also have a PDF and report for each of the FCS files in our project. Let's take a look at a report and PDF.

- Double click the first file in the list. A dialog is displayed with a set of choices.
- Choose **Open Report**



The analysis report for the first file is opened and we can review or modify the analysis.

- Double-click the first file again, and this time choose **Open PDF**.

The PDF for this analysis will open in your computer's default PDF viewer. It will match the report in the ModFit LT window.

Reports can also be opened using the Open Report button on the Home tab of the ribbon.

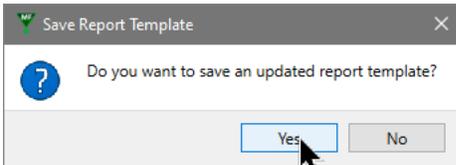
Re-save the project

It's always a good idea to use the Save Project button after making changes to the project. This is done automatically for some changes you make, but not for all.

- Click the **Save** button in the **Project** panel of the Home tab.



- A dialog will appear asking if you want to save an updated report template. Click **Yes**.



Report template

The report template contains information about the measurement selected for analysis, the gate, and any other elements you might add to the report. The template is opened whenever you reopen the project.

Reopen the project

Let's reopen the project to verify that it saved correctly.

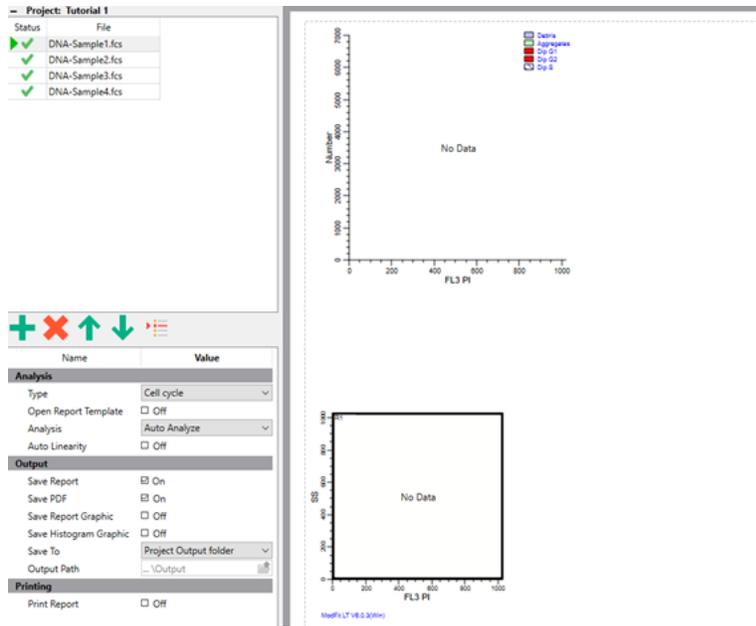
- From the **Project** panel of the **Home** tab, click **Open**.



- The Select a Project dialog is displayed, showing the Projects folder.
- Select **Tutorial 1** in the list and click the **Select Folder** button.

The project will open. The list of files and the processing options should be the same as we left them. The database viewer is shown. The report template is loaded, but it will show **No Data** in the DNA and gate plots.

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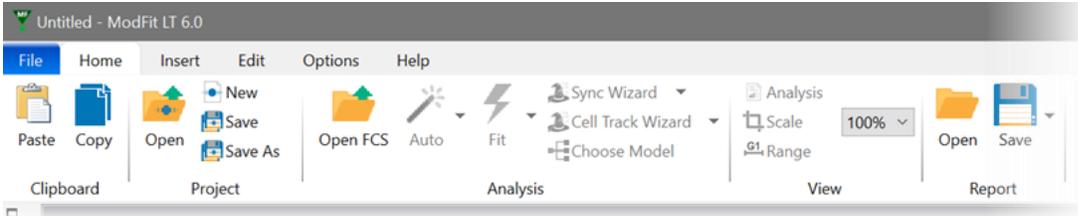
To review one of the reports already analyzed, double-click the file in the list and choose **Open Report**. You can add files to the project to analyze more. You can also save the project under another name and it will be set up just like this project.

Welcome to ModFit LT!

Analysis with a Sync Wizard Project

This tutorial focuses on using the Sync Wizard to analyze samples that were blocked in G0G1 and then released. We'll create a project to help manage the FCS files and output.

- Start ModFit LT by following instructions in [Installing and Running ModFit LT](#).
- Click the OK button on the opening dialog, if it appears.

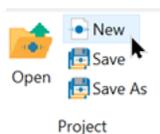


The ModFit LT ribbon bar appears at the top of the application window. Buttons are grouped to give you quick access to the most common commands in ModFit LT.

Create a new project

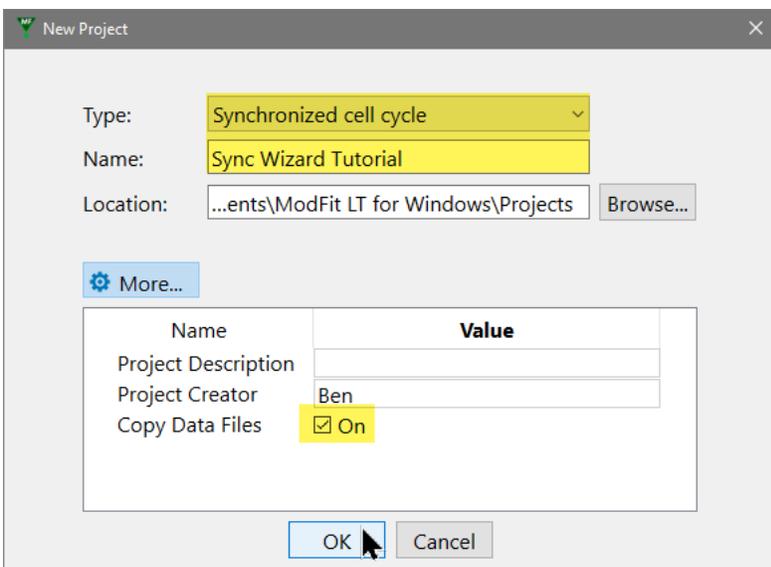
We start by creating a new project.

- Click the **New** button in the **Project** panel on the ribbon.



The New Project dialog is displayed.

- Choose **Synchronized cell cycle** for the Type.
- For the Name, enter **Sync Wizard Tutorial**.
- Click the **More** button and enable the **Copy Data Files** option.
- Click **OK**

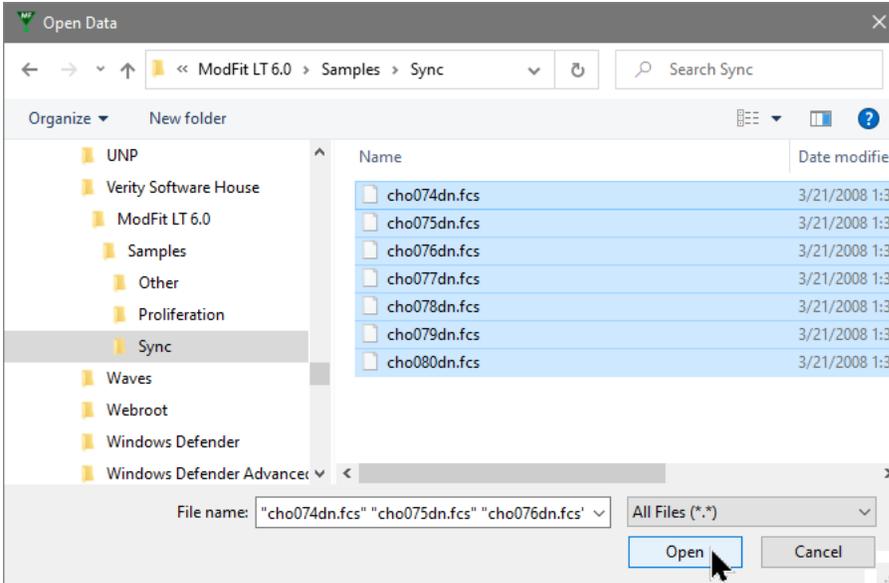


ModFit LT will create a folder in your Documents ModFit LT Projects folder named Sync Wizard Tutorial. When we select FCS files for this project, ModFit LT will copy the files into the project folders. See [Projects in ModFit LT](#) for more info.

Add files to the project

With our project created, it is time to add FCS files.

- Click the Open FCS button on the ribbon. The Open Data dialog is displayed.
- Navigate to the location where ModFit LT is installed:
 - Windows: Program Files\Verity Software House\ModFit LT 6.0
 - MacOS: Applications\ModFit LT 6.0
- In the **Samples\Sync** folder, select the seven (7) sample files: cho074dn.fcs through cho080dn.fcs.
- Click **Open**.



The files will be added to the file list for the project, and the first file will be opened.

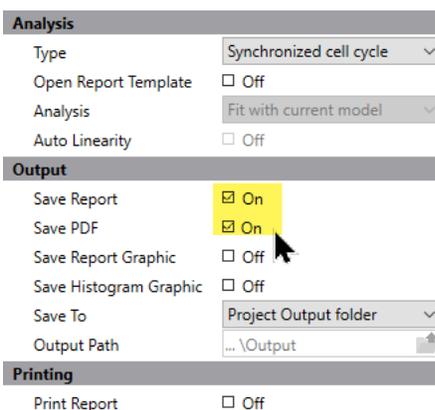
Setting gates

The example files used in this tutorial are *histogram* format FCS files, so there is no gating to set up. In most cases, FCS files are *listmode* format, and ModFit LT would allow you to select the measurement to analyze as well as to set up gates.

Choose processing options

The processing options in the lower-left portion of the screen determine what actions ModFit LT will perform when it analyzes each of the FCS files we added to the list. The **Type** is already set to **Synchronized cell cycle**. The Analysis is set to **Fit with current model**. Let's enable two output options.

- Enable **Save Report** and **Save PDF** in the options list.



With these settings, each file will be analyzed with the Sync Wizard's model, a report will be saved, and a PDF will be created.

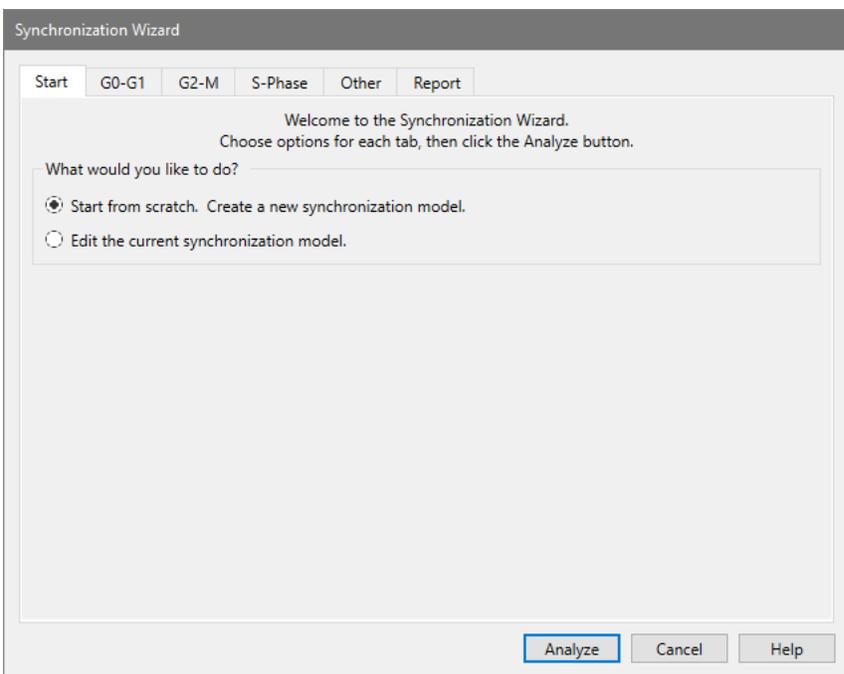
Setting up the Sync Wizard

We are ready to launch the Sync Wizard to set up the analysis.

- Click the **Sync Wizard** button in the Analysis panel of the Home tab.



The **Synchronization Wizard** dialog is displayed, showing the **Start** tab.



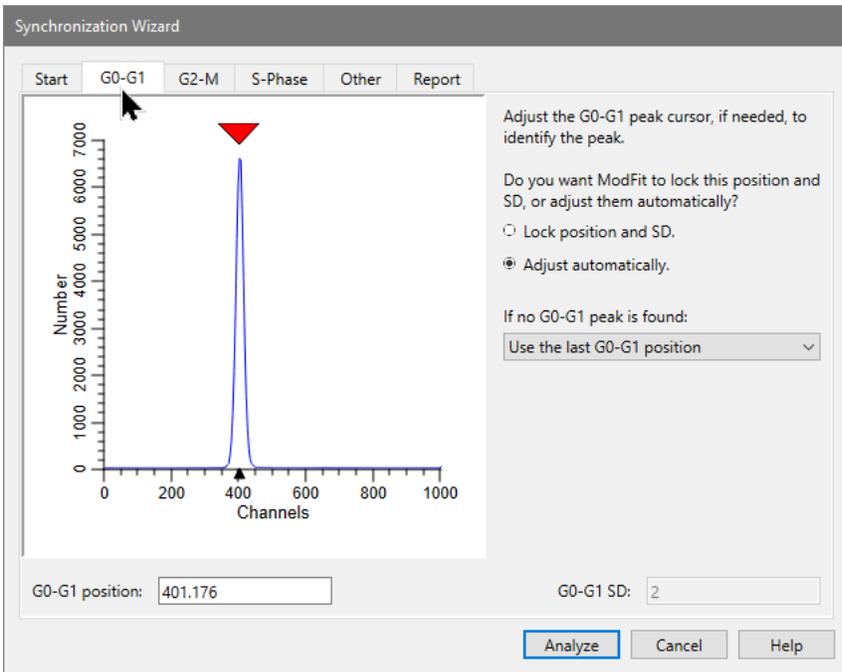
- Click the **G0-G1** tab.

The G0-G1 tab displays the DNA histogram. In this sample, all of the cells are blocked in G0-G1, so we see only one peak.

A red arrow appears above the peak, indicating where ModFit LT has found a peak. The position of this peak is shown in an edit box below the plot. If you move the red arrow with

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your mouse, the value in the edit box updates with the new position. You can also type a position directly into the edit box to set the peak's position. Since the correct peak was found (~401), we will use that value in this tutorial.

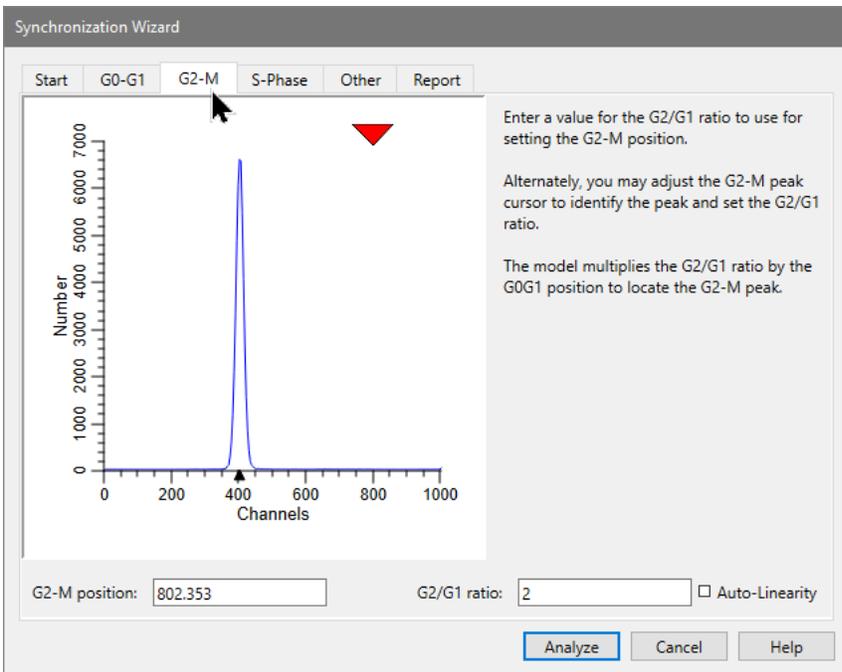


On the right side of the dialog, we can tell the wizard to **Lock position and SD** for the G0-G1 peak or **Adjust automatically**. Since there is a well defined peak, leave this setting on **Adjust automatically**.

Another option tells the wizard what to do if it cannot find a peak in subsequent FCS files. Set this to **Use the last G0-G1 position**.

- Click the **G2-M** tab.

This tab also displays a plot of the DNA histogram. Now the red triangle has moved to ~802 and there is no peak under it.



If there is no peak found on this tab, the wizard will assume that the G2-M position is two times the G0-G1 position. We note this in the **G2-M position** (803) and the **G2/G1 ratio** (2) edit boxes. In the Sync Wizard, the position of the G2-M peak is always dependent on the position of the G0-G1 peak. Similarly, the standard deviation of the G2-M peak is dependent on the standard deviation of the G0-G1 peak.

With the samples for this tutorial, we know that the G2/G1 ratio is closer to 1.92, so we can enter that value in the G2/G1 ratio edit box.

- Enter **1.92** in the G2/G1 ratio edit box.
- Click the **S-Phase** tab

The **S-Phase** tab displays options for the S-Phase shape, number of compartments, and spacing of the compartments.

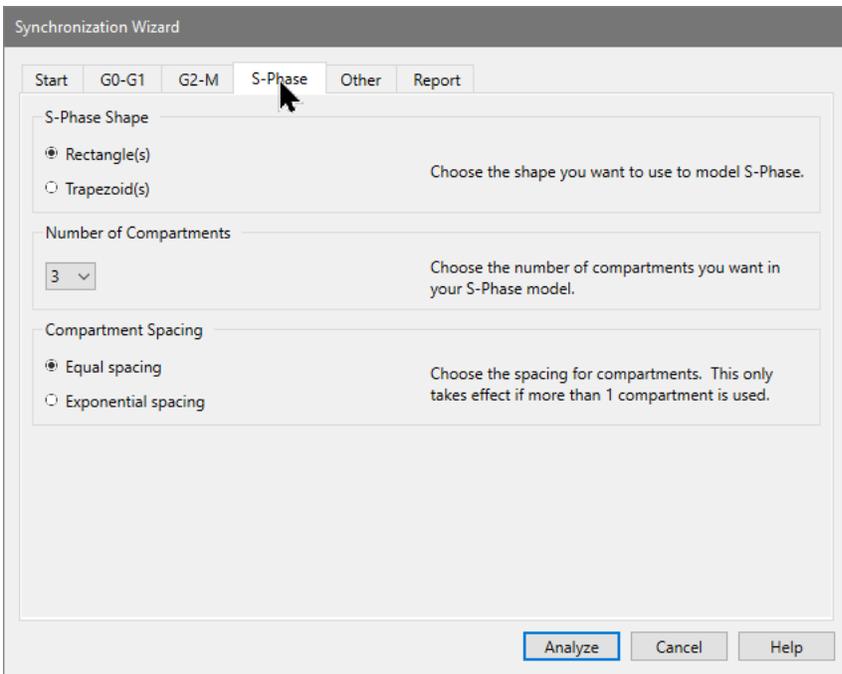
The **S-Phase Shape** can either be Rectangle or Trapezoid. A rectangle is more conservative and robust, and a trapezoid has an additional degree of freedom. Leave the selection on Rectangle for this tutorial.

The **Number of Compartments** determines how many rectangles or trapezoids to use when fitting the S-Phase. More compartments provides more flexibility, but it can also allow the wizard to overfit the data. We'll use 3 compartments in this tutorial.

Leave the **Compartment Spacing** set to Equal spacing.

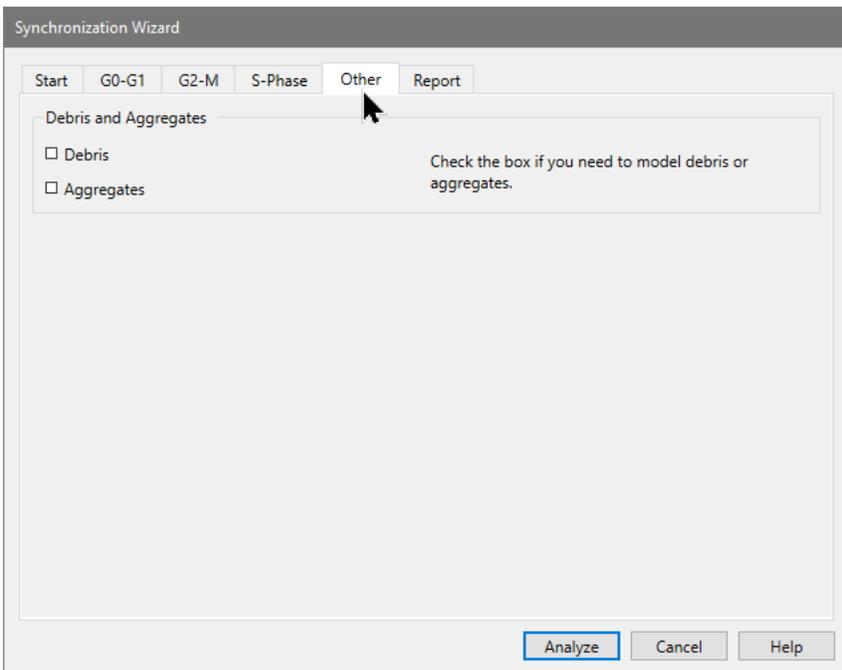
For more on these options, see [Sync Wizard: S-Phase tab](#).

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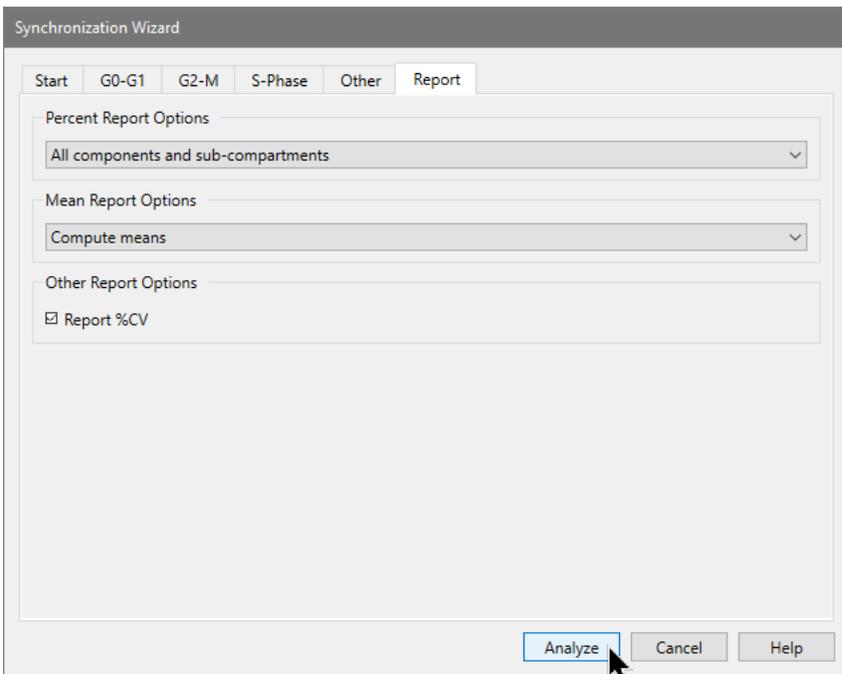
- Click the **Other** tab.

This tab shows a few other analysis options: **Debris** and **Aggregates**. We will leave both of these unchecked for this example.



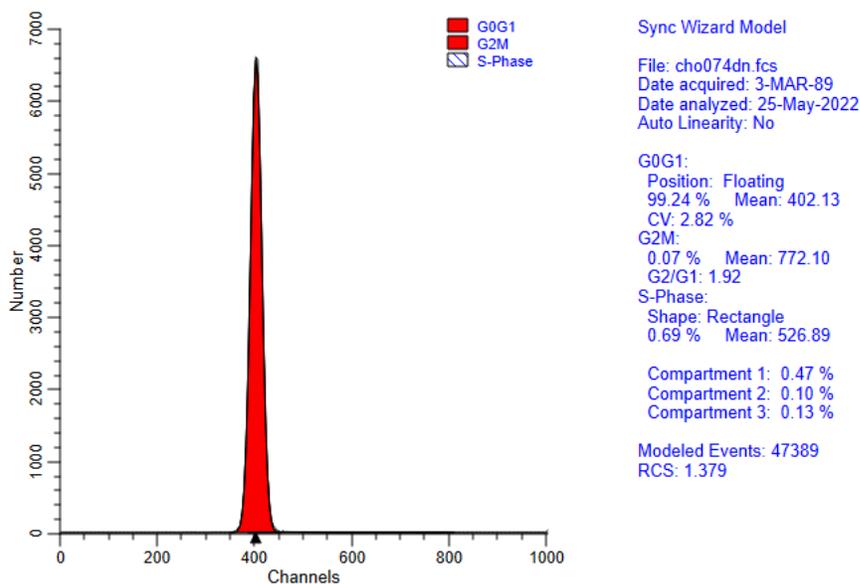
- Click the **Report** tab.

This tab provides some reporting options, which we will not change in this example.



- Click the **Analyze** button to initiate the analysis.

ModFit LT analyzes the data for the first file with the Sync Wizard model and displays the results on the report page. Information about the G0G1, G2M, and S-Phase is reported on the right.



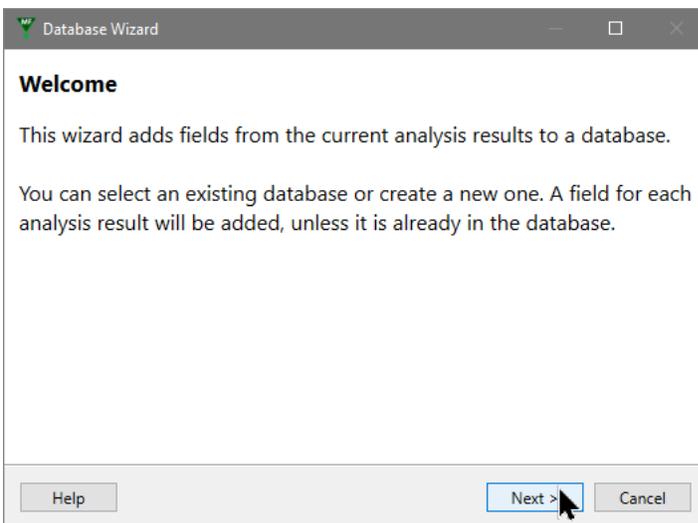
Create a database

Now that we have analyzed a file, we can use the Database Wizard to set up a database of the analysis results for the project.

- Click the **Edit** tab of the ribbon.
- Click the **Database Wizard** button in the Database panel.



The Database Wizard dialog is displayed.



- Click **Next**.
- On the next page of the wizard, select "Sync Wizard Tutorial" as the database.
- Click **Finish**.

When the wizard closes, a new Database Viewer window appears on the screen. This window will allow us to view records as they are added to the database. The fields in the database were added by the wizard, based on the analysis we performed.

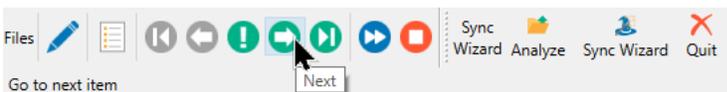
Optional step

Creating a database is an optional step for a project. It is a useful step if you want to export your experimental results to another program, such as Excel, for further analysis.

Processing the files

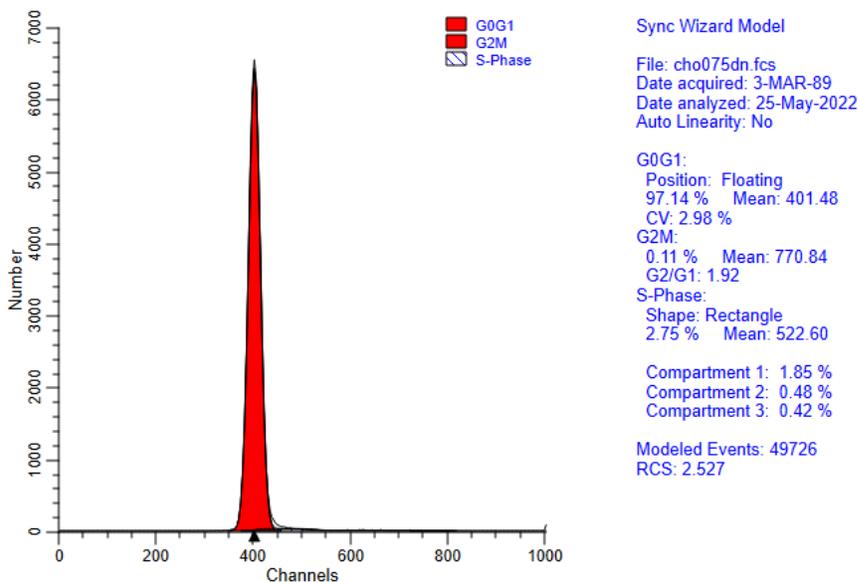
We can process the rest of the files in the list one at a time or automatically. To do this, we'll use the control on the batch toolbar, shown on the bottom-left side of the main window.

- Click the **Next** button on the batch toolbar.



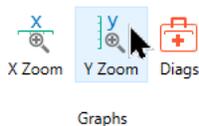
Before ModFit LT reads the next file, it saves the report and PDF for the file we just finished - the first file in the list. It also adds a record to the database for the first file, which appears in the Database Viewer window. Then it reads the next file in the list and analyzes it using the Sync Wizard model.

This second sample is very similar to the first, but more cells have moved into S-Phase.

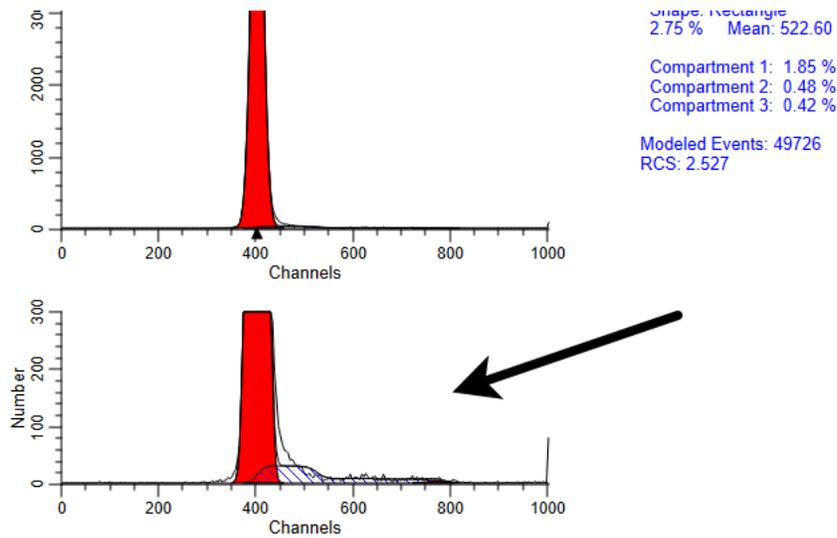


Let's add a zoomed view of the plot to the report so that we can see the S-Phase in more detail.

- In the **Insert** tab of the ribbon, click **Y Zoom** in the Graphs panel.



A zoomed version of the plot is added directly below the full-scale view. The details of the S-Phase are magnified.

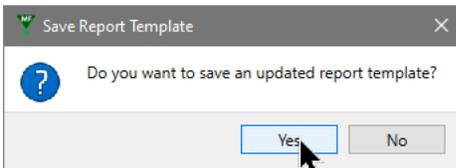


Use the Save Project button after making changes to the project to save your changes.

- Click the **Save** button in the **Project** panel of the Home tab.

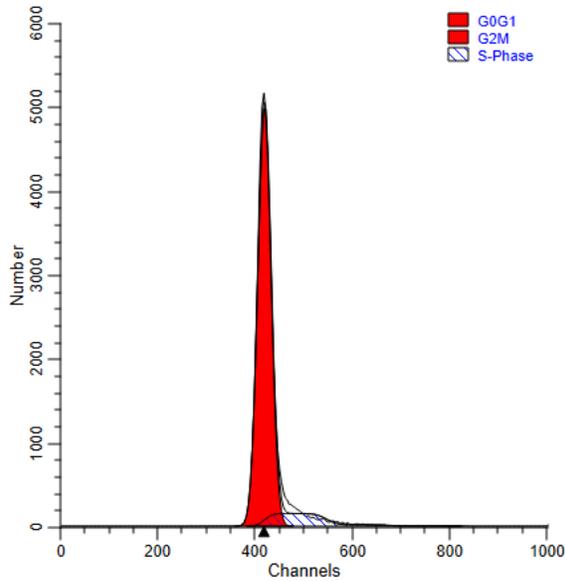


- A dialog will appear asking if you want to save an updated report template. Click **Yes**.



- Click the **Next** button on the batch toolbar, and even more cells have moved into S-Phase.

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Sync Wizard Model
 File: cho076dn.fcs
 Date acquired: 3-MAR-89
 Date analyzed: 25-May-2022
 Auto Linearity: No

G0G1:
 Position: Floating
 88.59 % Mean: 418.24
 CV: 3.24 %

G2M:
 0.02 % Mean: 803.02
 G2/G1: 1.92

S-Phase:
 Shape: Rectangle
 11.38 % Mean: 499.12

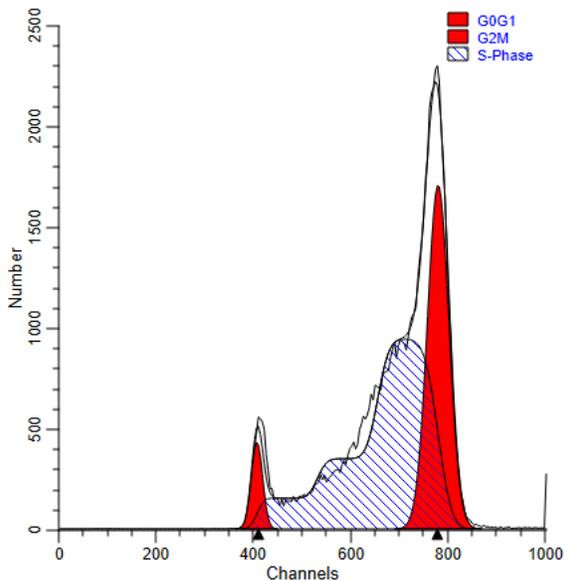
Compartment 1: 10.06 %
 Compartment 2: 1.20 %
 Compartment 3: 0.12 %

Modeled Events: 48076
 RCS: 4.279

- Click the Auto Advance button on the toolbar to process the remaining files in the list.



When it finishes processing, the analysis for the last file remains on the report. The cells in this sample are mostly in S-Phase and G2-M.



Sync Wizard Model
 File: cho080dn.fcs
 Date acquired: 3-MAR-89
 Date analyzed: 25-May-2022
 Auto Linearity: No

G0G1:
 Position: Floating
 4.36 % Mean: 406.20
 CV: 2.83 %

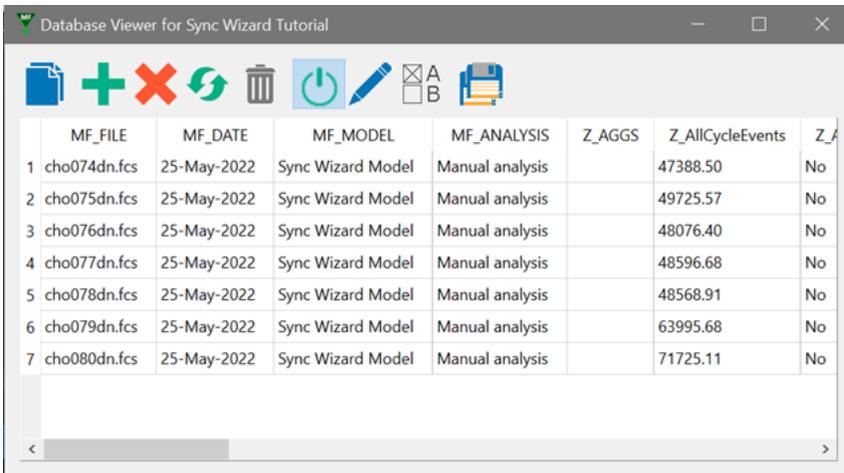
G2M:
 32.98 % Mean: 779.91
 G2/G1: 1.92

S-Phase:
 Shape: Rectangle
 62.66 % Mean: 661.73

Compartment 1: 6.63 %
 Compartment 2: 15.12 %
 Compartment 3: 40.91 %

Modeled Events: 71725
 RCS: 5.260

The database Viewer shows records for all files in the project.

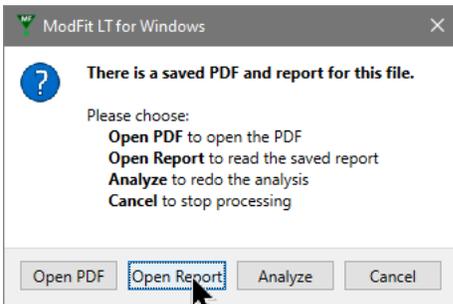


	MF_FILE	MF_DATE	MF_MODEL	MF_ANALYSIS	Z_AGGS	Z_AllCycleEvents	Z_A
1	cho074dn.fcs	25-May-2022	Sync Wizard Model	Manual analysis		47388.50	No
2	cho075dn.fcs	25-May-2022	Sync Wizard Model	Manual analysis		49725.57	No
3	cho076dn.fcs	25-May-2022	Sync Wizard Model	Manual analysis		48076.40	No
4	cho077dn.fcs	25-May-2022	Sync Wizard Model	Manual analysis		48596.68	No
5	cho078dn.fcs	25-May-2022	Sync Wizard Model	Manual analysis		48568.91	No
6	cho079dn.fcs	25-May-2022	Sync Wizard Model	Manual analysis		63995.68	No
7	cho080dn.fcs	25-May-2022	Sync Wizard Model	Manual analysis		71725.11	No

Review the analysis

ModFit LT created a PDF and report for each of the FCS files in our project as we analyzed them. Let's take a look at a report and PDF.

- Double click one of the files in the list. A dialog is displayed with a set of choices.
- Choose **Open Report**



The analysis report for the selected file is opened and we can review or modify the analysis.

- Double-click the same file, and this time choose **Open PDF**.

The PDF for this analysis will open in your computer's default PDF viewer. It will match the report in the ModFit LT window.

Reports can also be opened using the Open Report button on the Home tab of the ribbon.

Reopen the project

Let's see what the project looks like when we open it again.

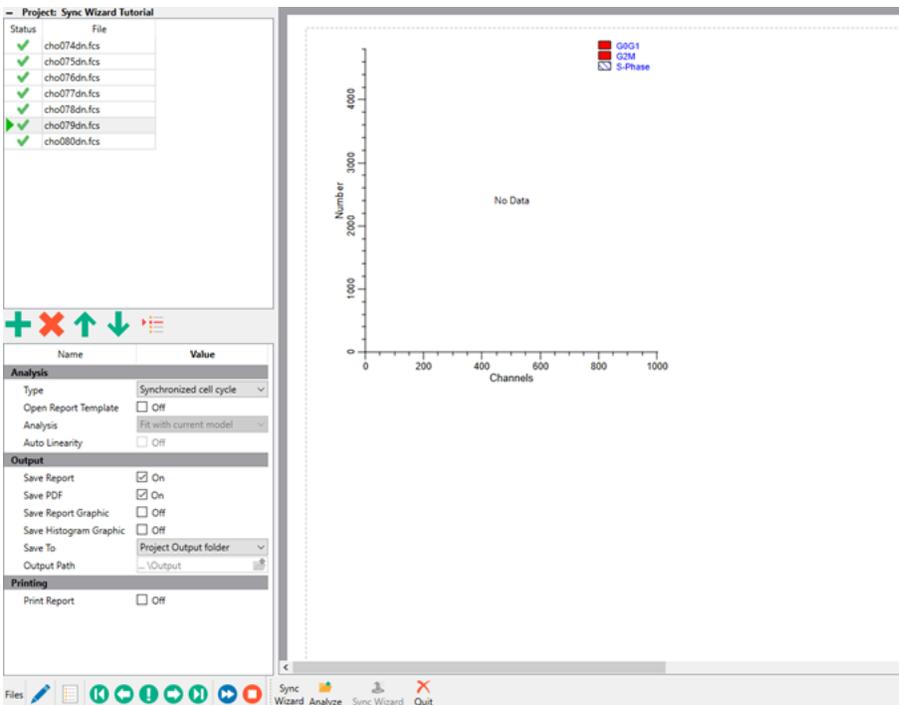
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- From the **Project** panel of the **Home** tab, click **Open**.



- The Select a Project dialog is displayed, showing the Projects folder.
- Select **Sync Wizard Tutorial** in the list and click the **Select Folder** button.

The project will open. The list of files and the processing options should be the same as we left them. The database viewer is shown. The report template is loaded, but it will show **No Data** in the plot.



To review one of the reports already analyzed, double-click the file in the list and choose **Open Report**. You can add files to the project to analyze more. You can also save the project under another name and it will be set up just like this project.

Summary

The Sync Wizard is a tool designed to allow you to analyze samples with perturbed S-Phase and G2-M populations. It is divided into "tabs" that allow you to describe your data and choose options to use in modeling your data.

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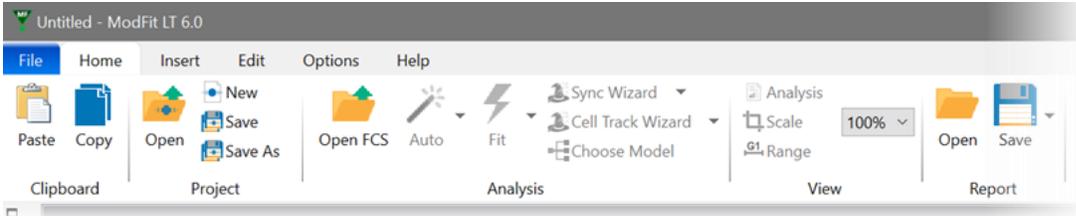
Control the model's flexibility by adjusting the shape used and number of compartments in S-Phase. The default setting, 3 rectangles, is good for most cases. The trapezoid shape more closely approximates the shape of the perturbed S-Phase distribution, but it can provide the model too much flexibility.

Once you have created a model with the Wizard, use the buttons on the batch toolbar to analyze your sample files.

Analyzing with a Cell Tracking Project

The Cell Tracking Wizard is used to analyze samples stained with CFSE or other cell tracking dyes. In this tutorial, we will create a cell tracking project to demonstrate how it works.

- Start ModFit LT by following instructions in [Installing and Running ModFit LT](#).
- Click the OK button on the opening dialog, if it appears.

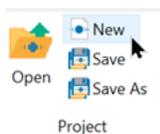


The ModFit LT ribbon bar appears at the top of the application window. Buttons are grouped to give you quick access to the most common commands in ModFit LT.

Create a new project

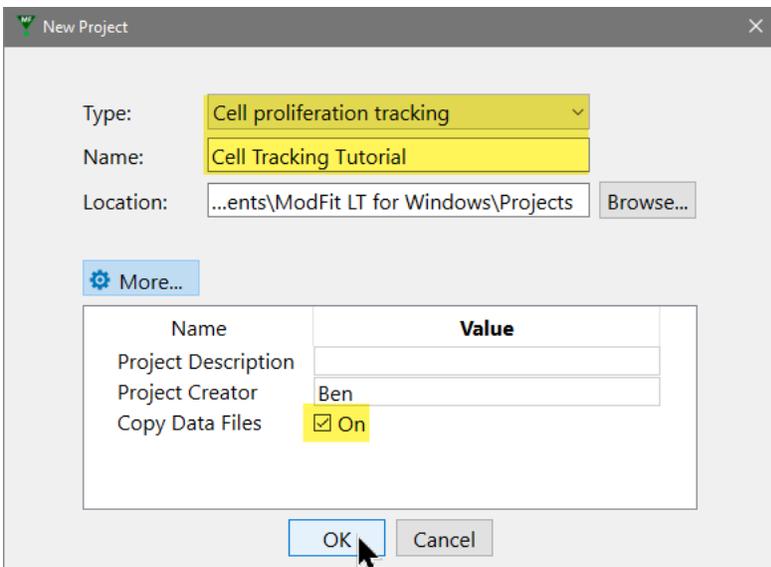
We start by creating a new project.

- Click the **New** button in the **Project** panel on the ribbon.



The New Project dialog is displayed.

- Choose **Cell proliferation tracking** for the Type.
- For the Name, enter **Cell Tracking Tutorial**.
- Click the **More** button and enable the **Copy Data Files** option.
- Click **OK**

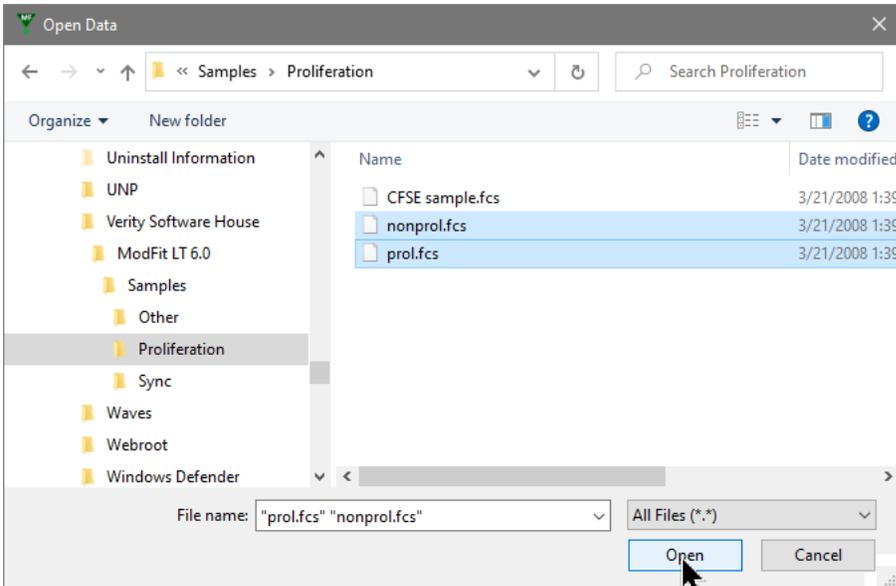


ModFit LT will create a folder in your Documents ModFit LT Projects folder named Cell Tracking Tutorial. When we select FCS files for this project, ModFit LT will copy the files into the project folders. See [Projects in ModFit LT](#) for more info.

Add files to the project

With our project created, it is time to add FCS files.

- Click the Open FCS button on the ribbon. The Open Data dialog is displayed.
- Navigate to the location where ModFit LT is installed:
 - Windows: Program Files\Verity Software House\ModFit LT 6.0
 - MacOS: Applications\ModFit LT 6.0
- In the **Samples\Proliferation** folder, select two files: **nonprol.fcs** and **prol.fcs**.
- Click **Open**.



The files will be added to the file list for the project, and the first file will be opened.

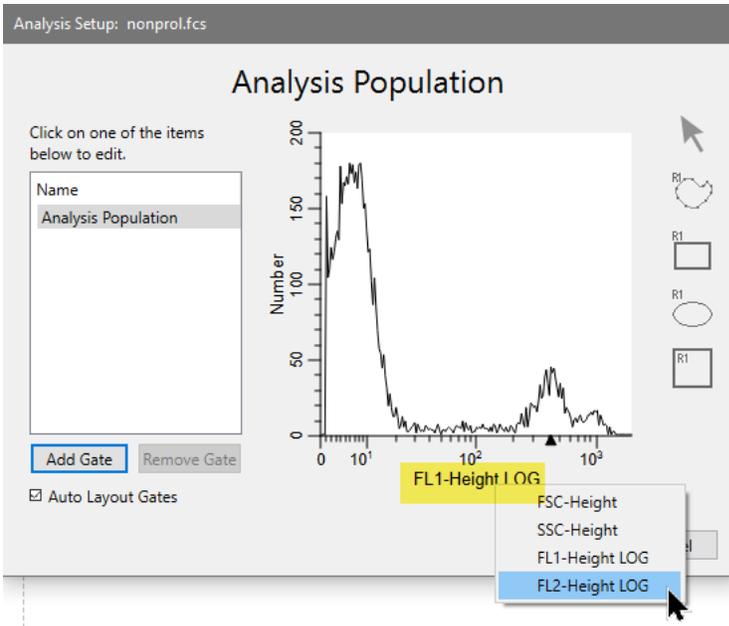
Select the measurement to analyze and setup a gate

When the first file is opened, the Analysis Setup dialog is displayed. This dialog allows us to set up one or more gates and to select the measurement that we want to analyze.

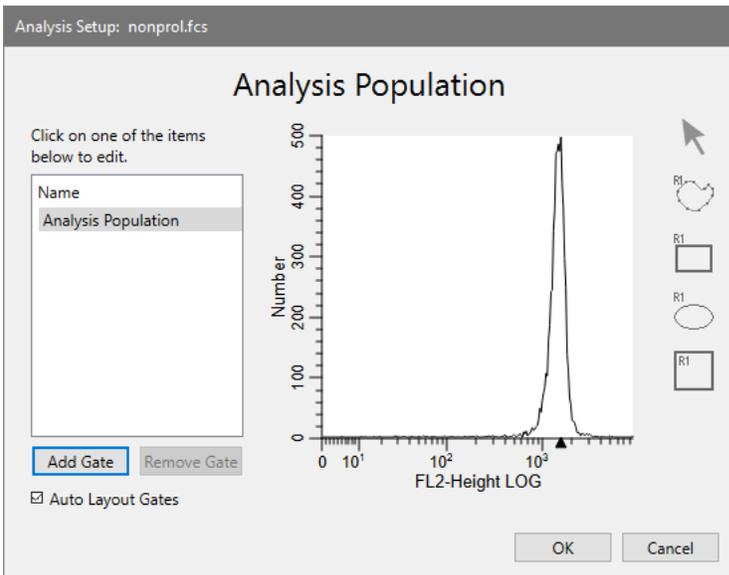
The Analysis Population is showing **FL1-Height LOG**. We want to select a different measurement with the cell tracking dye.

- Click the **FL1-Height LOG** label and choose **FL2-Height LOG** from the menu.

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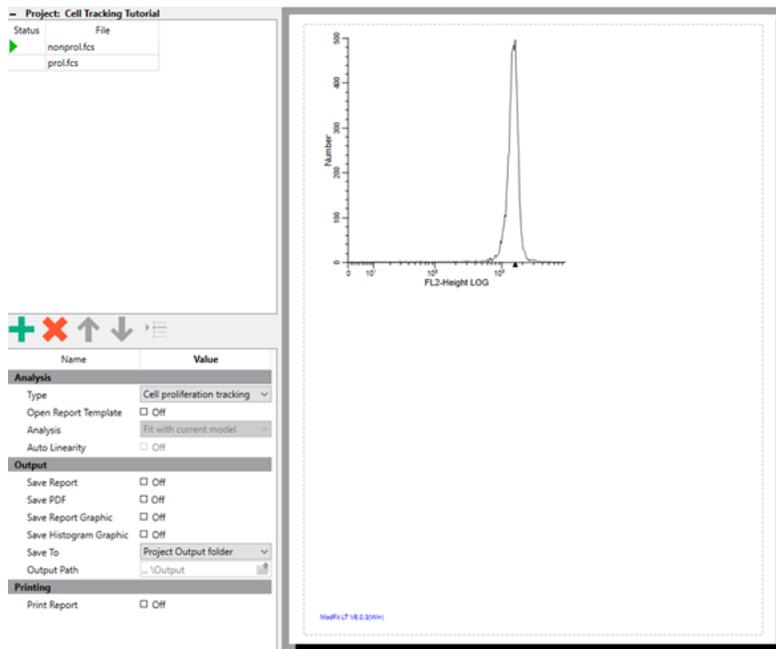
This is the measurement we want to analyze.



If we need to do additional gating, we could add gates now. In this case, we will analyze the data without additional gates.

- Click OK.

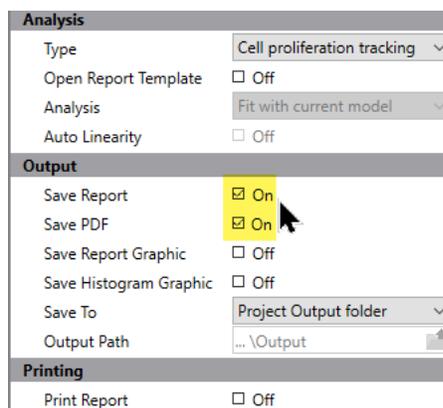
The dialog closes and the file is read. The report page shows a 1P histogram of the cell-tracking measurement "FL2-Height LOG". On the left of the main window, the FCS files appear in a list. Below the list of files, processing options are shown.



Choose processing options

The processing options in the lower-left portion of the screen determine what actions ModFit LT will perform when it analyzes each of the FCS files in the list. The **Type** is set to **Cell proliferation tracking**. The Analysis is set to **Fit with current model**. Let's enable two output options.

- Enable **Save Report** and **Save PDF** in the options list.



With these settings, each file will be analyzed with the Cell Tracking Wizard's model, a report will be saved, and a PDF will be created.

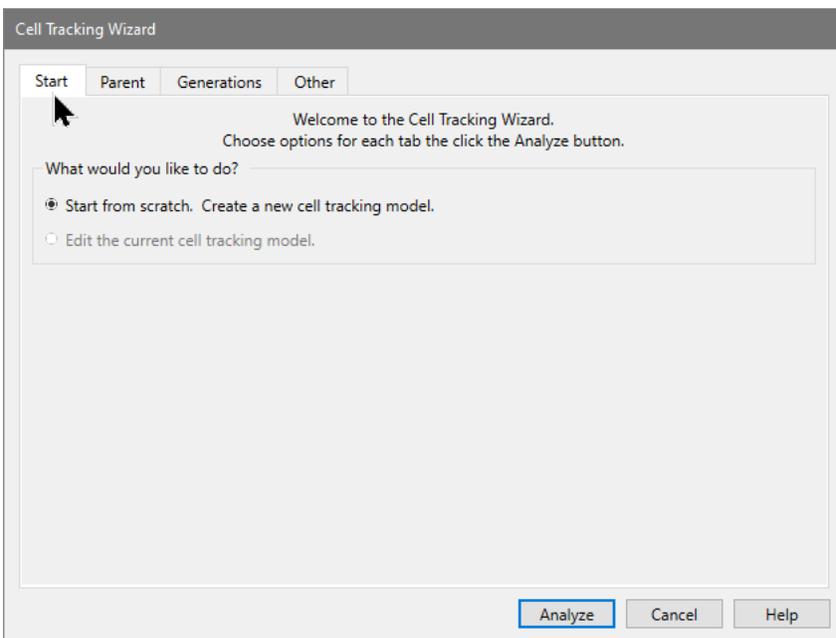
Setting up the Cell Tracking Wizard

We are ready to launch the Cell Tracking Wizard to set up the analysis.

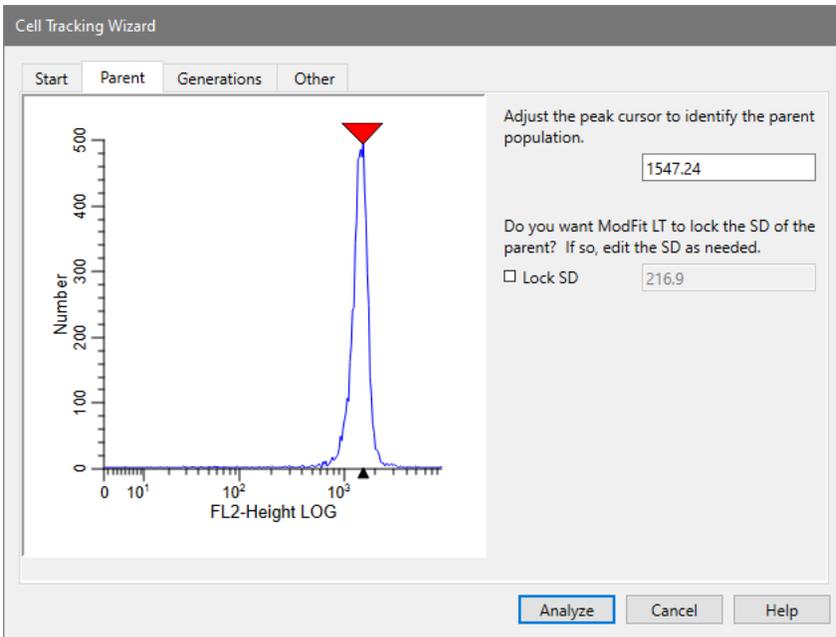
- Click the **Cell Track Wizard** button in the Analysis panel of the Home tab.



The Cell Tracking Wizard dialog is displayed showing the **Start** tab.



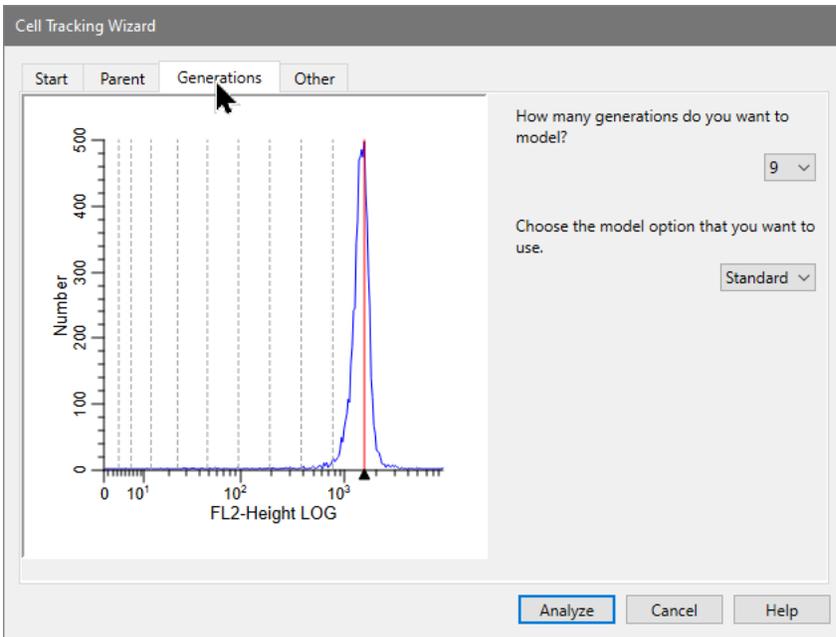
- Click the **Parent** tab.



A plot of the selected measurement is shown on the left. A red triangle identifies the brightest peak that the wizard found in the sample. This is assumed to be the parent population. If the wizard did not find the correct location for the parent population, you can move the red triangle to the correct position or type the position into the edit box on the right. In this sample, the correct position was found so no change is needed.

This tab gives us the ability to **Lock SD** for the parent. This option is useful with noisy data or in cases where the parent population may become very small in subsequent samples. In this tutorial, leave the option unchecked.

- Click the **Generations** tab.

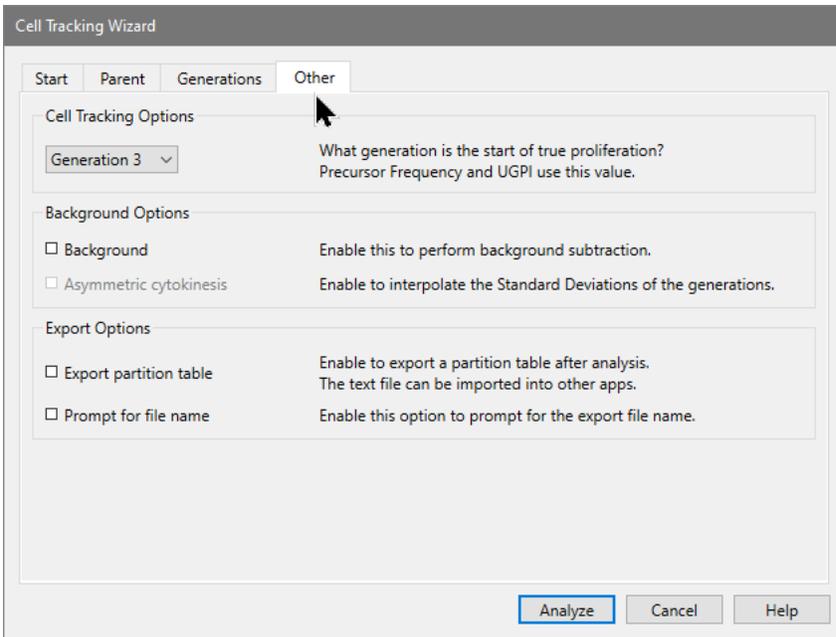


On this tab, the plot indicates the parent position with a red vertical line. Dotted lines are drawn in the computed locations for daughter generations, each having half of the intensity of its predecessor.

The number of generations can be set using the combo box on the right. As we can see, nine (9) is the correct value for this sample so no change is necessary. This value should be set to capture all daughter generations between the parent and 0 on the axis.

We will leave the model option as **Standard** for now.

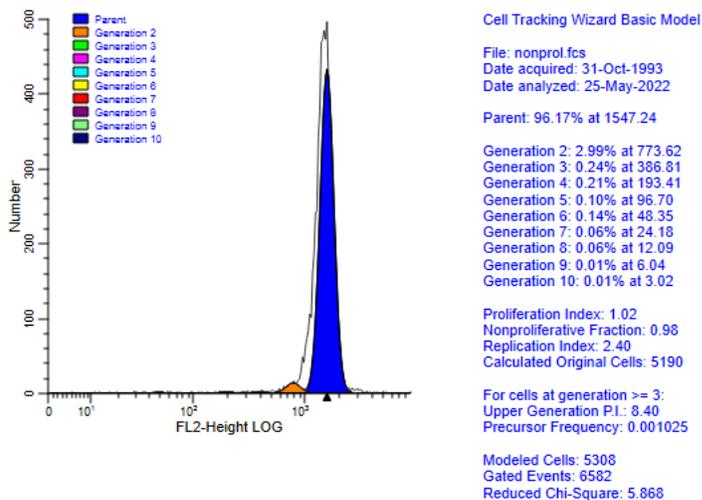
- Click the **Other** tab.



This tab determines the generation that is the start of true proliferation and several other useful settings. These are all in the correct state for this tutorial, so no changes are required.

- Click the **Analyze** button to initiate the analysis.

ModFit LT analyzes the first sample file with the Cell Tracking Wizard model and displays the results on a report page. A plot of the cell tracking measurement and a legend appear on the left. On the right the report shows the position and fraction for the parent and each daughter generation, as well as key results from the proliferation analysis.



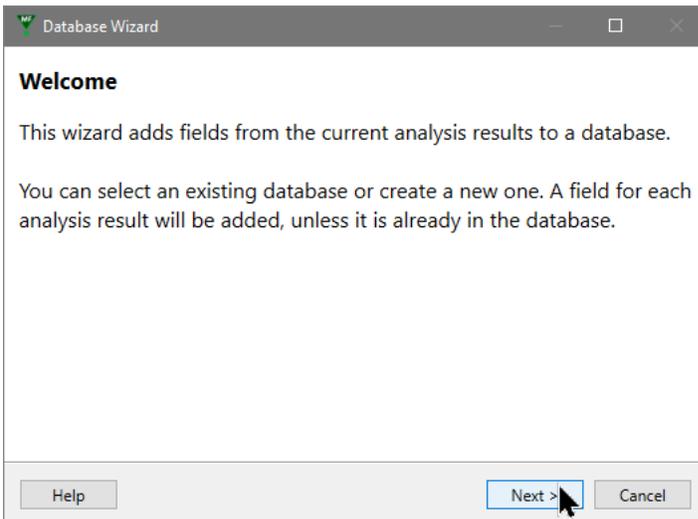
Create a database

Now that we have analyzed a file, we can use the Database Wizard to set up a database of the analysis results for the project.

- Click the **Edit** tab of the ribbon.
- Click the **Database Wizard** button in the Database panel.



The Database Wizard dialog is displayed.



- Click **Next**.
- On the next page of the wizard, select "Cell Tracking Tutorial" as the database.
- Click **Finish**.

When the wizard closes, a new Database Viewer window appears on the screen. This window will allow us to view records as they are added to the database. The fields in the database were added by the wizard, based on the analysis we performed.

Optional step

Creating a database is an optional step for a project. It is a useful step if you want to export your experimental results to another program, such as Excel, for further analysis.

Processing the files

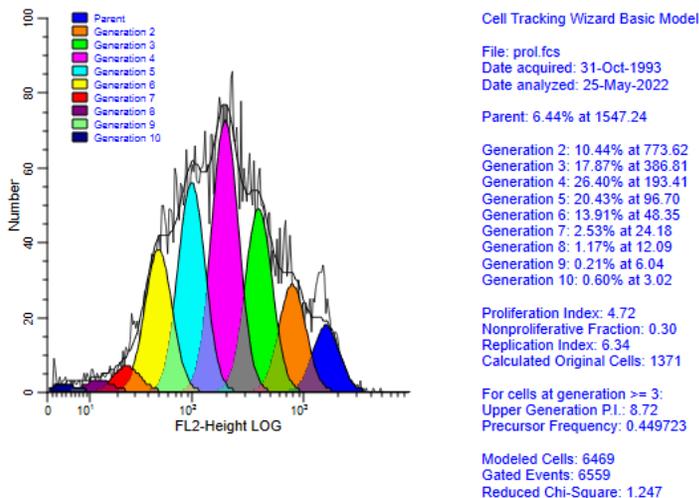
We can process the rest of the files in the list one at a time or automatically. To do this, we'll use the controls on the batch toolbar, shown on the bottom-left side of the main window.

- Click the **Next** button on the batch toolbar.



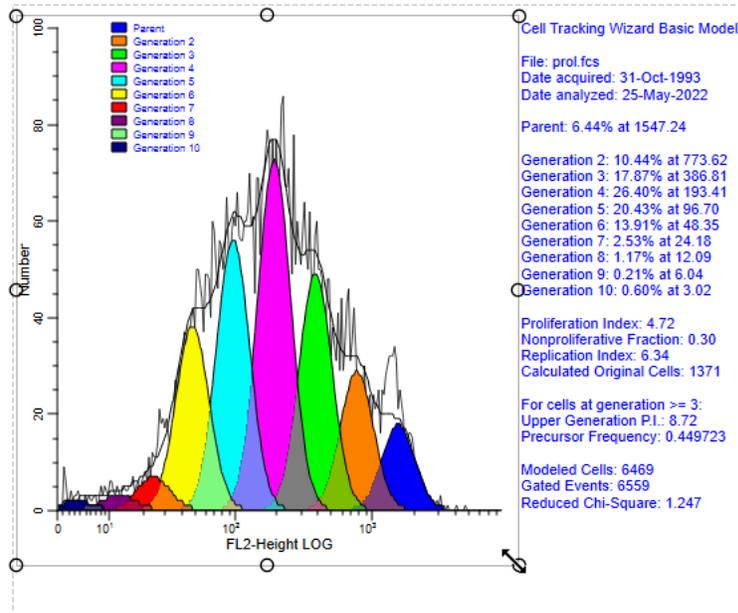
Before ModFit LT reads the next file, it saves the report and PDF for the file we just finished - the first file in the list. It also adds a record to the database for the first file, which appears in the Database Viewer window. Then it reads the next file in the list and analyzes it using the Cell Tracking Wizard model.

In this second sample, most of the cells have divided at least once. The daughter generations have more cells than the parent generation.



Let's make the plot larger so that we can see the populations better.

- Click the plot to select it. Sizing handles will appear on its borders.
- Using the sizing handles, make the plot larger.

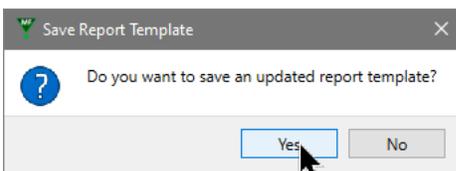


Any time you make revisions to your report layout, use the Save Project button to save your changes.

- Click the **Save** button in the **Project** panel of the Home tab.



- A dialog will appear asking if you want to save an updated report template. Click **Yes**.

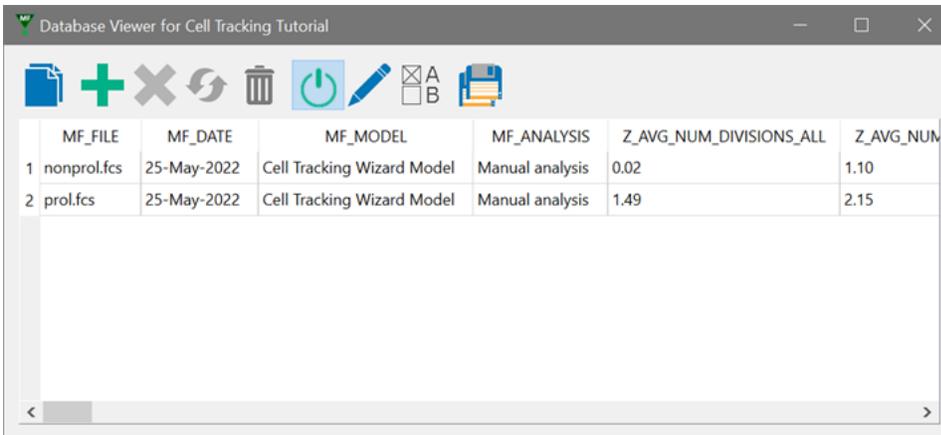


Note

You would need to reanalyze the 2 files in this project to update their reports and PDFs with the modified report template.

If there were additional files in this experiment, we could use the Auto Advance button on the batch toolbar to analyze them. In this case, our two files are analyzed and saved.

The database Viewer shows records for all files in the project.

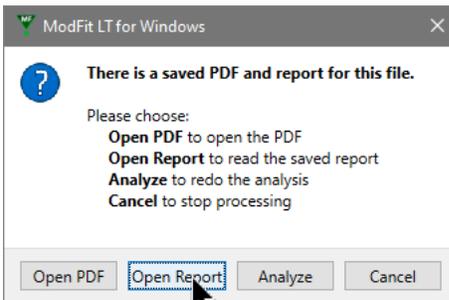


	MF_FILE	MF_DATE	MF_MODEL	MF_ANALYSIS	Z_AVG_NUM_DIVISIONS_ALL	Z_AVG_NUM
1	nonprol.fcs	25-May-2022	Cell Tracking Wizard Model	Manual analysis	0.02	1.10
2	prol.fcs	25-May-2022	Cell Tracking Wizard Model	Manual analysis	1.49	2.15

Review the analysis

ModFit LT created a PDF and report for each of the FCS files in our project as we analyzed them. Here is the quick way to view them.

- Double click the second file in the list. A dialog is displayed with a set of choices.
- Choose **Open Report**



The analysis report for the selected file is opened and we can review or modify the analysis.

- Double-click the second file again, and this time choose **Open PDF**.

The PDF for this analysis will open in your computer's default PDF viewer.

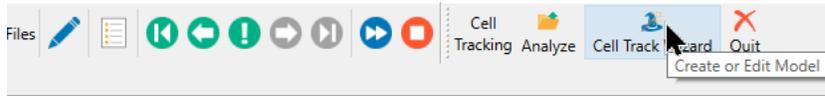
- When you have finished reviewing the PDF, close the PDF viewer.

Adjusting the model

You may be interested in trying several other options in the Cell Tracking Wizard, found in the Other tab. To try these, you need to display the wizard dialog.

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- Click the Cell Track Wizard button on the wizard toolbar at the bottom of the main window. The Cell Tracking Wizard dialog is displayed.



The wizard opens in editing mode so that you do not have to recreate the wizard model from scratch. Visit the tabs in the wizard to make changes you want to try out.

- On the **Other** tab, uncheck **Save project template**. You may not want to save these changes to disk until after you review them.
- Click the **Analyze** button. The sample is analyzed with the modified cell tracking model.

If you decide to keep the changes, use the **Save Project** button in the Project panel of the Home tab to update the project. When prompted to save the report template, click Yes.

Reopen the project

Let's see what the project looks like when we open it again.

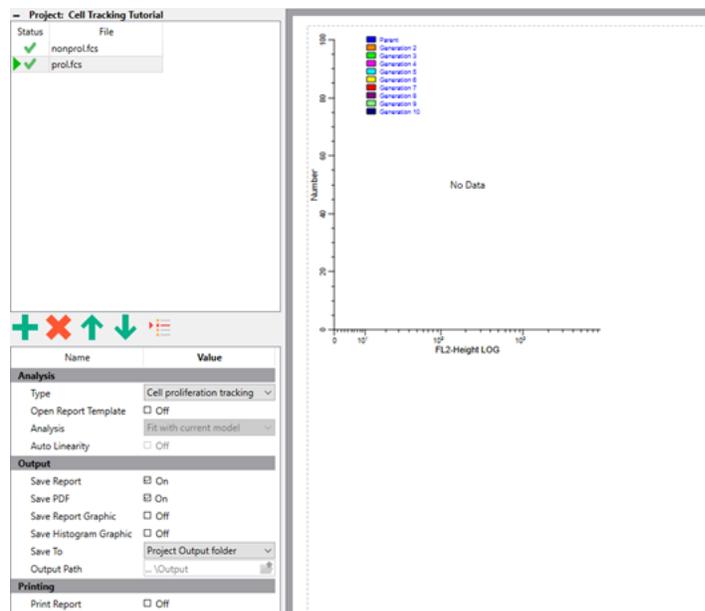
- From the **Project** panel of the **Home** tab, click **Open**.



- The Select a Project dialog is displayed, showing the Projects folder.
- Select **Cell Tracking Tutorial** in the list and click the **Select Folder** button.

The project will open. The list of files and the processing options should be the same as we left them. The database viewer is shown. The report template is loaded, but it will show **No Data** in the plot.

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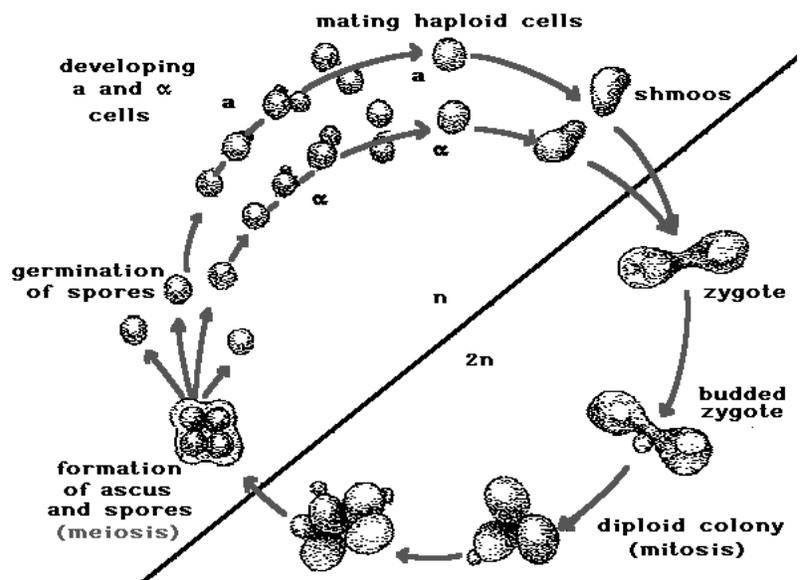
To review one of the reports already analyzed, double-click the file in the list and choose **Open Report**. You can add files to the project to analyze more. You can also save the project under another name and it will be set up just like this project.

Summary

The Cell Tracking Wizard is a tool designed to allow you easily to analyze samples prepared with cell tracking dyes. It is organized by categories into tabs which allow you to describe your data and choose options to use in modeling your data.

Creating a Custom Project

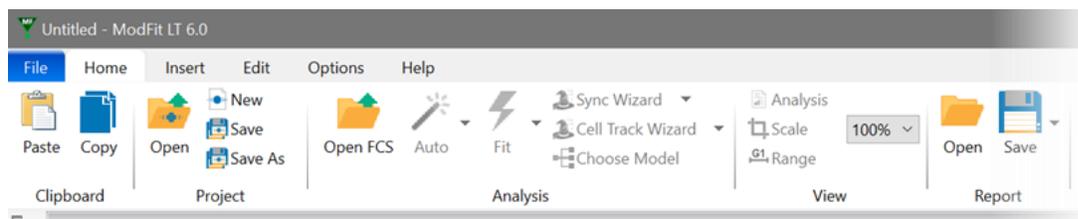
In this tutorial, we will create a custom project to analyze DNA cell cycle in yeast samples. As shown in the diagram below, yeast cell cycle is quite different from mammalian cell cycle. ModFit LT's automatic analysis was not designed to analyze samples from yeast populations. However, ModFit LT allows the user to create models from scratch for novel applications, such as yeast DNA analysis, and we can create a project to organize the model, sample files, and output we generate.



Life Cycle of Yeast, (c) 2005 Genetics Education Network, Kansas State University

We will use a yeast model previously developed and learn how to incorporate the model into a new project. Let's get started.

- Start ModFit LT by following instructions in [Installing and Running ModFit LT](#).
- Click the OK button on the opening dialog, if it appears.

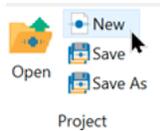


The ModFit LT ribbon bar appears at the top of the application window. Buttons are grouped to give you quick access to the most common commands in ModFit LT.

Create a new project

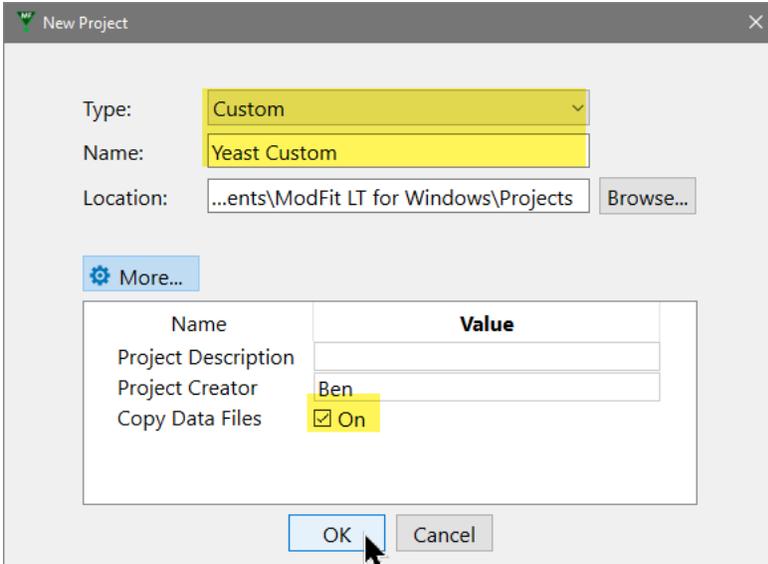
We start by creating a new project.

- Click the **New** button in the **Project** panel on the ribbon.



The New Project dialog is displayed.

- Choose **Custom** for the Type.
- For the Name, enter **Yeast Custom**.
- Click the **More** button and enable the **Copy Data Files** option.
- Click **OK**



ModFit LT will create a folder in your Documents ModFit LT Projects folder named Yeast Custom. When we select FCS files for this project, ModFit LT will copy the files into the project folders.

Open the previously-created report with custom model

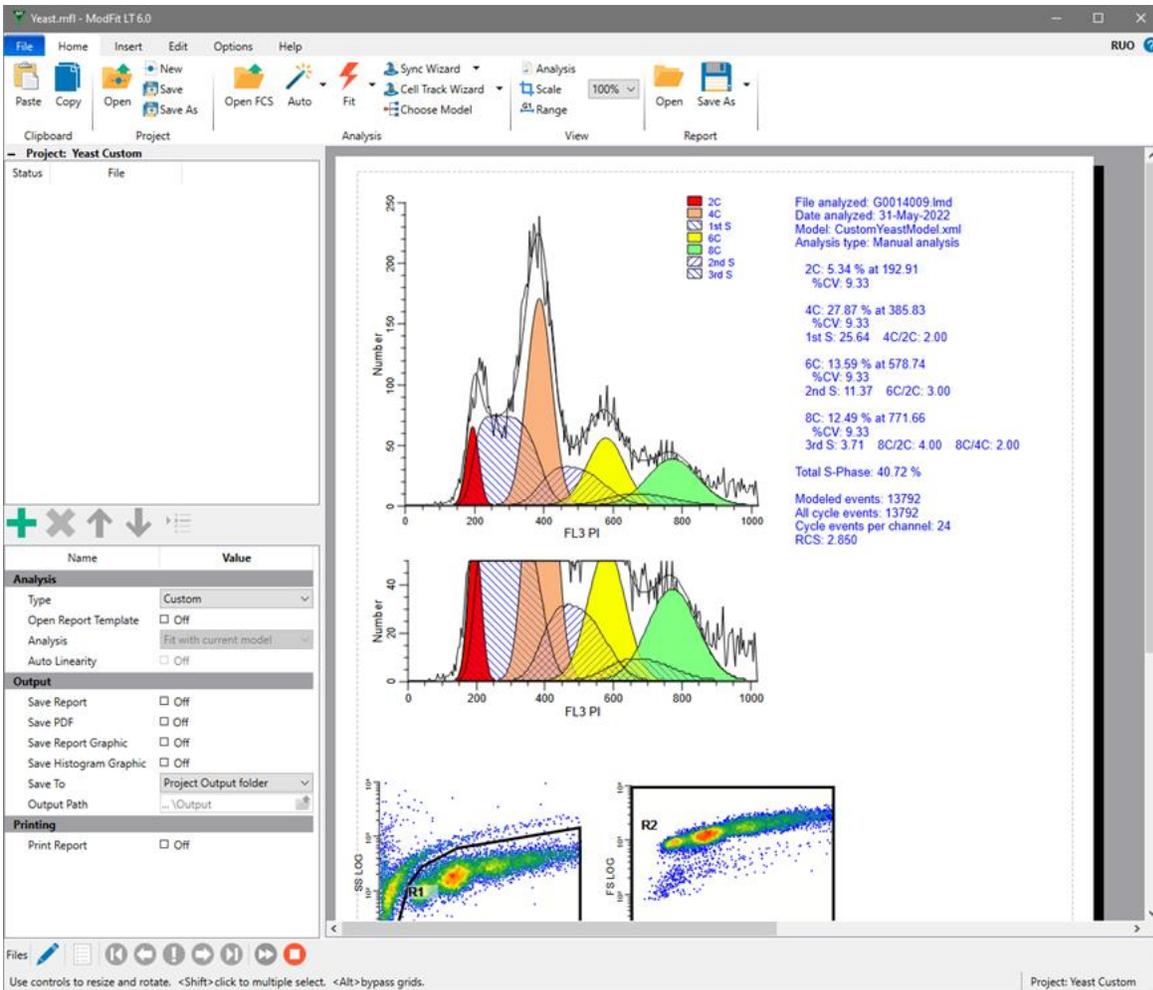
For this example, we have a custom model and report that were created in a previous version of ModFit LT. We will use that as the basis for this new project.

- Click the **Open Report** button in the Report panel of the ribbon. The Open Reports dialog is displayed.



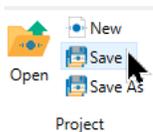
- Navigate to the location where ModFit LT is installed:
 - Windows: Program Files\Verity Software House\ModFit LT 6.0
 - MacOS: Applications\ModFit LT 6.0
- In the **Samples\Yeast** folder, open **Yeast.mfl**.

The report opens in ModFit LT showing the analysis from a previous version of ModFit LT. This report contains a custom model designed to analyze yeast cell cycle.



To make this model part of the new project, all we have to do is save the project again.

- Click the **Save** button in the Project panel on the Home tab of the ribbon.



At this point, our project is all setup to use this custom model as part of the project's report template.

Projects and Report Templates

Each project you create in ModFit LT will contain a *report template* file that is opened each time the project is opened. The report template is automatically given the same name as the project. The report template contains the models, the gates, the report layout, and other information.

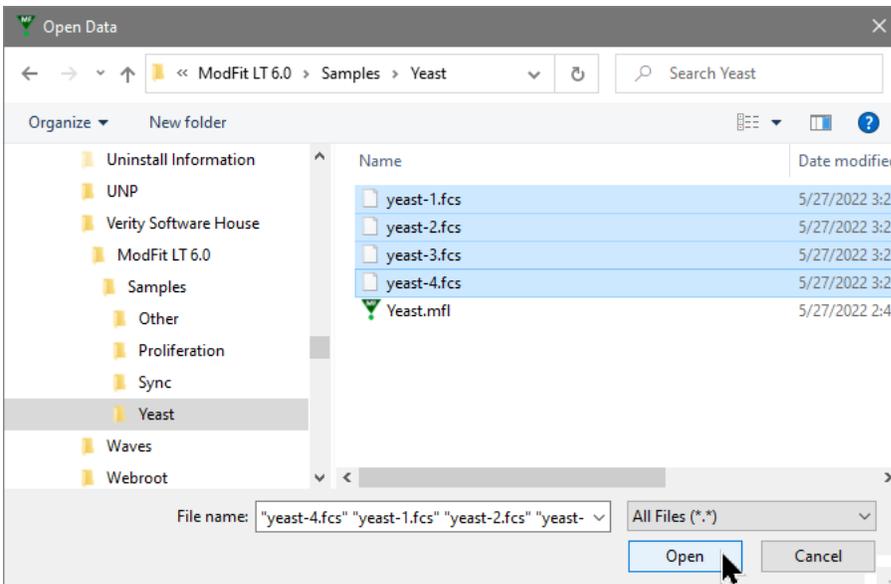
If you open a report or report template manually and want to use it in your

project, simply use the Save Project command and click "Yes" when asked if you want to save the project template.

Add files to the project

With the custom model added to the new project, let's add some FCS files.

- Click the Open FCS button on the ribbon. The Open Data dialog is displayed.
- Navigate to the location where ModFit LT is installed:
 - Windows: Program Files\Verity Software House\ModFit LT 6.0
 - MacOS: Applications\ModFit LT 6.0
- In the **Samples\Yeast** folder, select the four (4) yeast sample files: yeast-1.fcs, yeast-2.fcs, yeast-3.fcs, and yeast-4.fcs.
- Click **Open**.



The files will be added to the file list for the project, and the first file will be opened.

Choose processing options

The processing options in the lower-left portion of the screen determine what actions ModFit LT will perform when it analyzes each of the FCS files we added to the list. The **Type** is already set to **Custom**, which we chose that when we created the project. The Analysis is set to **Fit with current model**, and that's the only option for a custom project. Let's enable two output options.

- Enable **Save Report** and **Save PDF** in the options list.

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Analysis	
Type	Custom
Open Report Template	<input type="checkbox"/> Off
Analysis	Fit with current model
Auto Linearity	<input type="checkbox"/> Off

Output	
Save Report	<input checked="" type="checkbox"/> On
Save PDF	<input checked="" type="checkbox"/> On
Save Report Graphic	<input type="checkbox"/> Off
Save Histogram Graphic	<input type="checkbox"/> Off
Save To	Project Output folder
Output Path	... \Output

Printing	
Print Report	<input type="checkbox"/> Off

With these settings, the custom model will be used to analyze each file after it is read. Then, a report and PDF will be created.

Analyze the first file

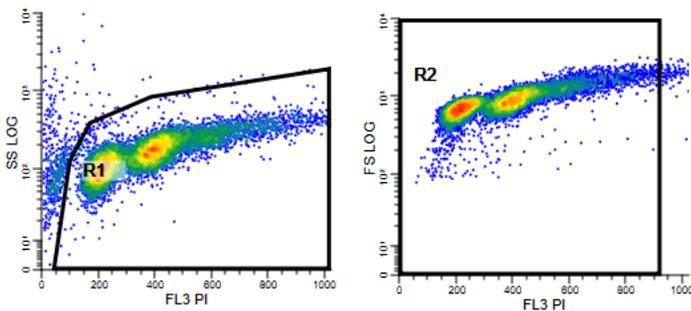
We are ready to analyze the first file. This is done using the controls at the very bottom-left of the main window.

- Click the **Run** button to analyze the first file in the list.



The first file is read and analyzed with the custom yeast model in our project.

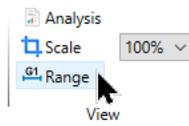
If the gates need to be adjusted, this is the time to do that. The first gate is used to remove some low PI events, and the second gate removes some of the brightest events that may be pegged.



The next step for custom models is to check the positions of the ranges used by the model. Ranges are positioned over the peaks in the sample to provide initial estimates for the model. Here's how to view and adjust the ranges.

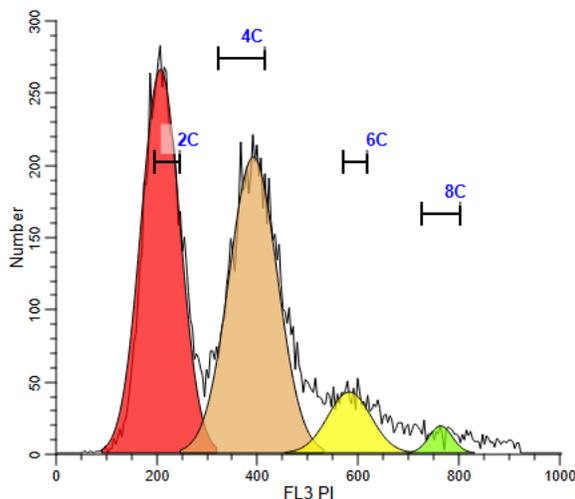
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- Click the **Range** button in the View panel on the Home tab of the ribbon.



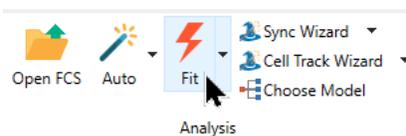
This switches ModFit LT into Range view, where the model's ranges are displayed. The filled area under each range represents the initial estimate for the peak that is under the range. The idea is to position and size the ranges over the peaks to give a reasonable first estimate to the modeling routine.

With this yeast model, the **2C** range should be positioned over the first peak (on the left) at ~ 200 . The **4C** range should be at $\sim 2X$ the position of the 2C range at ~ 400 . The **6C** range should be at $\sim 3X$ the 2C range at ~ 600 , and the **8C** range should be at $\sim 4X$ the 2C range at ~ 800 . If needed adjust the ranges so that they are centered on those positions. Once a range is selected, you can also adjust the width of the range with the handle that appears. The width can be fairly narrow; it is better to provide a narrower width estimate, not an overly broad width estimate.

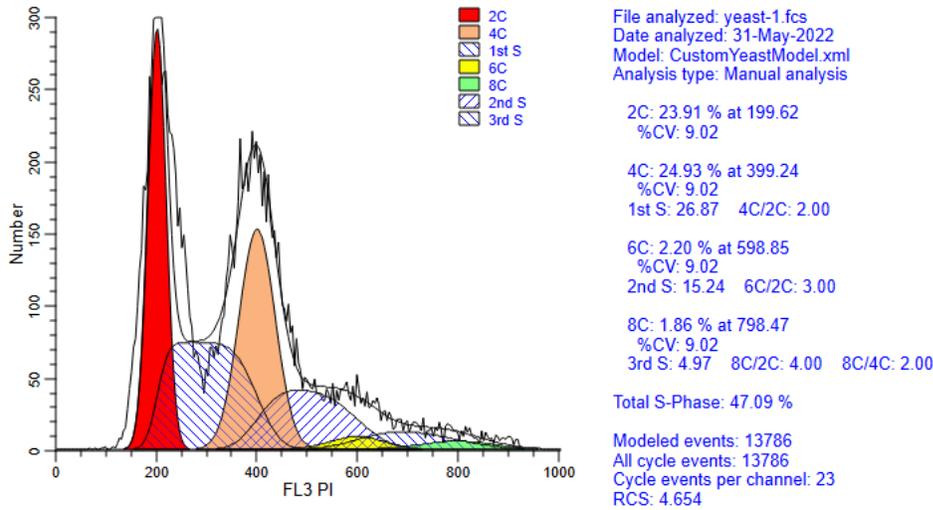


When the ranges are adjusted, you are ready to re-fit the model to the data.

- Click the **Fit** button in the Analysis panel of the Home tab on the ribbon.



ModFit LT switches back to Analysis view and shows the analyzed sample. The analysis will look similar to the one shown below.



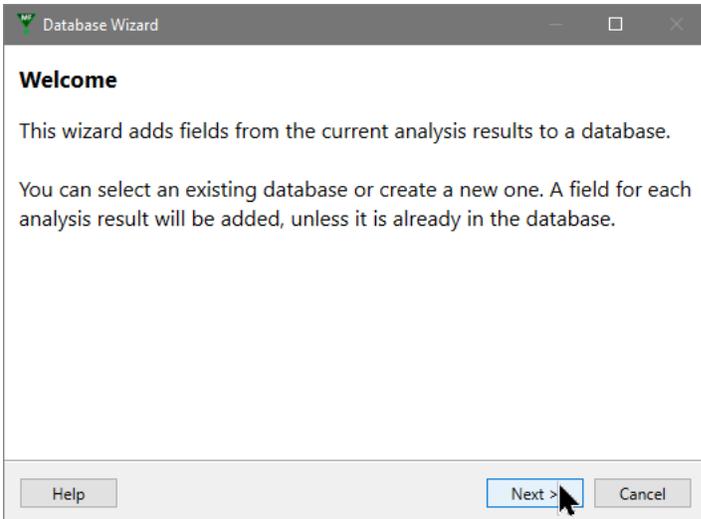
Create a database

Now that we have analyzed the first file, we can use the Database Wizard to set up a database of the analysis results for the project.

- Click the **Edit** tab of the ribbon.
- Click the **Database Wizard** button in the Database panel.



The Database Wizard dialog is displayed.



- Click **Next**.
- On the next page of the wizard, select "Yeast Custom" as the database.
- Click **Finish**.

When the wizard closes, a new Database Viewer window appears on the screen. This window will allow us to view records as they are added to the database. The fields in the database were added by the wizard, based on the analysis we performed.

Processing other files

We can process the rest of the files in the list one at a time or automatically. If the gates or ranges might need to be adjusted, it is best to advance one file at a time through the project.

- Click the **Next** button on the batch toolbar.



Before ModFit LT reads the next file, it saves the report and PDF for the file we just finished - the first file in the list. It also adds a record to the database for the first file, which appears in the Database Viewer window. Then it reads the next file in the list and analyzes it using the project's custom model.

The workflow for each file follows the pattern for the first file:

- Check the gates and make any adjustments needed.
- Click the **Range** button to switch to Range view.

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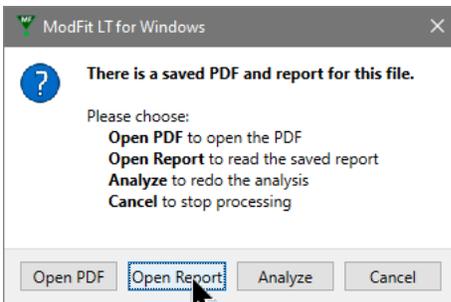
- Adjust the ranges to identify the correct peaks and adjust peak widths if needed.
- Click the **Fit** button to re-analyze the file with any adjustments you made.
- Click **Next** on the batch toolbar to move to the next file.

Process the remaining files using these steps.

Review the analysis

ModFit LT created a PDF and report for each of the FCS files in our project as we analyzed them. Let's take a look at a report and PDF.

- Double click one of the files in the list. A dialog is displayed with a set of choices.
- Choose **Open Report**



The analysis report for the selected file is opened and we can review or modify the analysis.

- Double-click the same file, and this time choose **Open PDF**.

The PDF for this analysis will open in your computer's default PDF viewer. It will match the report in the ModFit LT window.

Reports can also be opened using the Open Report button on the Home tab of the ribbon.

Summary

Custom projects are used for samples that don't fit the traditional DNA, synchronization, or cell tracking approaches. In this case, we opened a report file that contained a custom model designed to analyze DNA of yeast samples.

After creating a new, custom project, you can use Open Report or Open Report Template to read a previously created model into the project. You can also create a model from scratch or by using one of the built in tools such as Auto Analysis, the Sync Wizard, or the Cell Tracking wizard. Be sure to use the Save Project command to make your model and report part of the project's report template.

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Use the batch toolbar to process samples in a custom project. You can process files one at a time, adjusting gates and ranges before moving on to the next file.

Manual analysis with ModFit LT

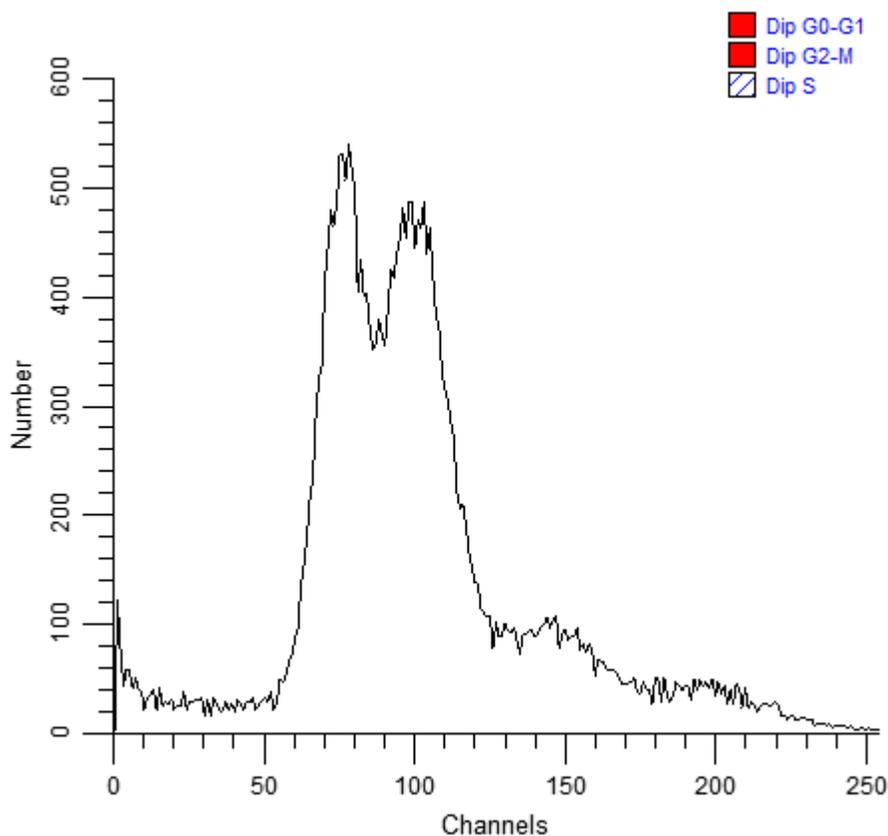
If ModFit LT has difficulty detecting peaks or identifying the ploidy patterns of a sample using automatic analysis, you can use manual analysis to analyze the data.

Manual analysis involves several steps. First, you open the sample you want to analyze. Next, you use the Choose Model dialog box to select a model that matches your assessment of the sample. Once the model is selected, you need to position ranges over important peaks, and finally tell the program to analyze the sample.

When to Use Manual Analysis

Here is an example.

- Click the **Open FCS** button on the **Home** tab of the ribbon.
- Open the file "Problem1.fcs" from the Samples folder.



There are several problems with this histogram, the most important being that its peaks are very broad and indistinct. In order for Auto Analysis to succeed, the important peaks in the histogram must be correctly identified. Peaks are shown by the small black triangles under

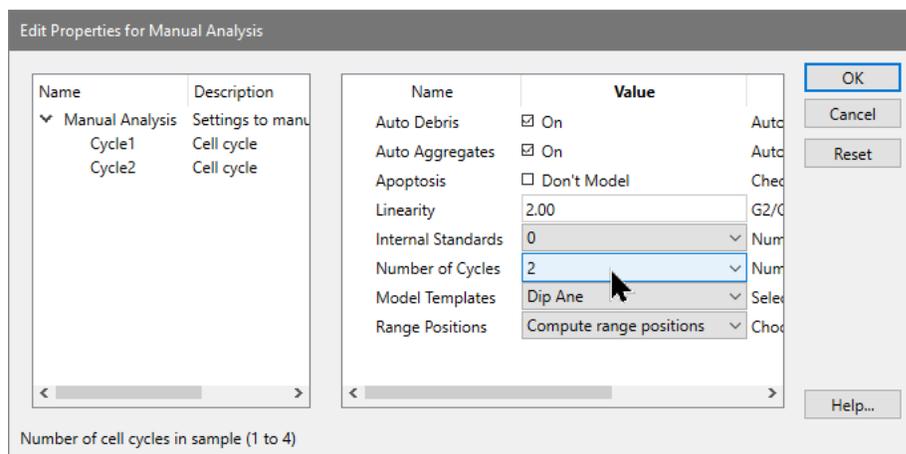
the graphic. The program probably missed one or both of the dominant peaks in the histogram.

This is a circumstance where you must use manual analysis to model the sample. Even if the peaks *are* all detected, you can still continue with this tutorial.

Choosing a Model

- Click the **Choose Model** button on the ribbon bar  **Choose Model** and a dialog is displayed.

This dialog allows you to define the model that you want to use for the histogram. Based on the options you choose, the program will construct a model to match those assumptions. This is a DNA Aneuploid sample, so the selections we make in the dialog need to reflect that.



- Enable **Auto Debris** by checking the Auto Debris checkbox.
- Enable **Auto Aggregates** by checking the Auto Aggregates checkbox.
- Disable Apoptosis* by clearing the check in the Apoptosis checkbox.
- Set **Linearity** to 2.0. This is the default G2/G1 ratio.
- Set **Internal Standards** to 0. There are no internal standards in this file.
- Set **Number of cycles** to 2. We want to model 2 cell cycles in this data file: a Diploid and an Aneuploid cycle.
- For the **Model template**, choose "Dip Ane". This tells the program the ploidy to assign to each cycle.
- For the **Range positions**, choose "Compute range positions". If you use this dialog to modify a model, you might choose "Use current range positions" so that the program did not move existing ranges.
- Click **OK** to confirm your choices and close the dialog.

Adjusting Ranges

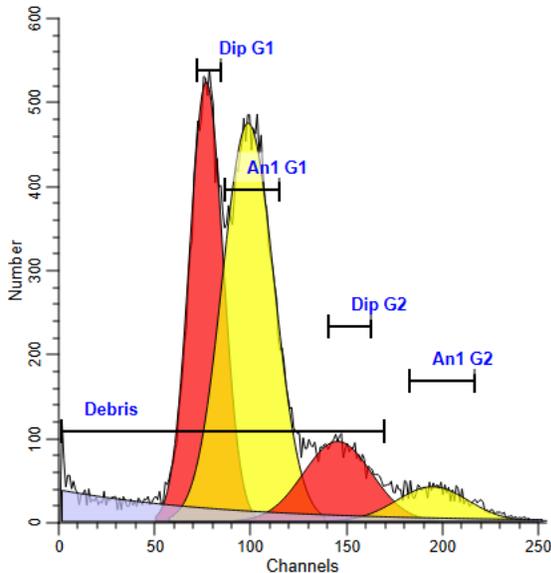
The program will construct the model and try to position the model ranges over detected peaks. It switches to the Range view automatically. You can switch to this view anytime by clicking the Range button on the ribbon.

In the **Range** view, you can position ranges over the peaks in the sample. You move a range by clicking on the range label, and dragging it to a new location with the mouse button down. When finished, you release the mouse button. To adjust the width of a range, click the black handle and drag to choose a new size. If a range is already correctly positioned and sized, do not move or resize it.

- Make sure the **Debris** range is located over the starting point of debris at the left edge of the histogram. This range is always used to identify the start of debris. The right side of the range should be placed at approximately two-thirds of the histogram scale.
- Next, move the **Dip G1** range over the first peak, the DNA Diploid G0-G1. It does not need to be stretched to encompass the peak as long as it is centered on the peak. You should see a red fill in the first peak that indicates the range estimate of the size of the peaks.
- Move the **An1 G1** range over the center of the second peak, the DNA Aneuploid G0-G1. This peak will fill with yellow to indicate the range estimate.

Notice that as you move a range, the left pane on the status bar displays information about the range position. The left and right values are in the units shown on the X-axis; the top is in number of events. When the mouse button is released, the status bar indicates the fitted estimates for the range.

- Move the **Dip G2** range over the portion of the histogram that is about twice the position of the **Dip G1** range. This is the approximate position of the Diploid G2-M.
- Move the **An1 G2** range to about twice the position of the **An1 G1** range. This is the approximate position of the Aneuploid G2-M.

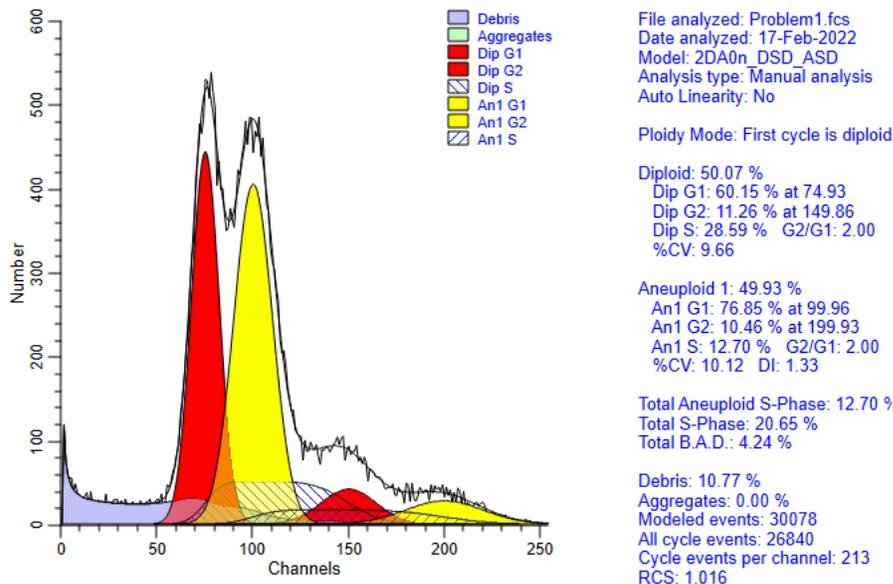


Fitting the Model

With the ranges in position, you are ready to analyze the sample.



- Click the **Fit** button  on the ribbon to perform the non-linear least squares fit. The results will be similar to the one shown here.



- Review the analysis to ensure that the model correctly matches the sample. If it does not, you should make sure you selected the correct model and that its ranges are correctly positioned.

Summary

The manual analysis mode should be used when the peak finder or automatic analysis mode fails, or you need to make your own decisions about how to model a sample. It enables user-intervention at the key points in the analysis: model selection and range positioning.

Models are selected using the **Choose Model** option on the **Home** tab of the ribbon bar.

Ranges used to identify peaks must be positioned so that each range identifies a unique peak; they should not overlap. For example, the Dip G1 range is used to identify the Diploid G0G1 peak, and no other *peak* range should be placed over the same peak. You should not, for example, place the An1 G1 range over the same peak as the Dip G1 range. The Debris

range is the only exception. Since it does not identify a peak, it should be stretched across about two-thirds of the histogram.

See also:

[Model Descriptions](#)

[Choose Model command](#)

[Ranges command](#)

Enhanced Cell Tracking Analysis

Features in ModFit LT and WinList provide unparalleled capabilities to analyze cell proliferation.

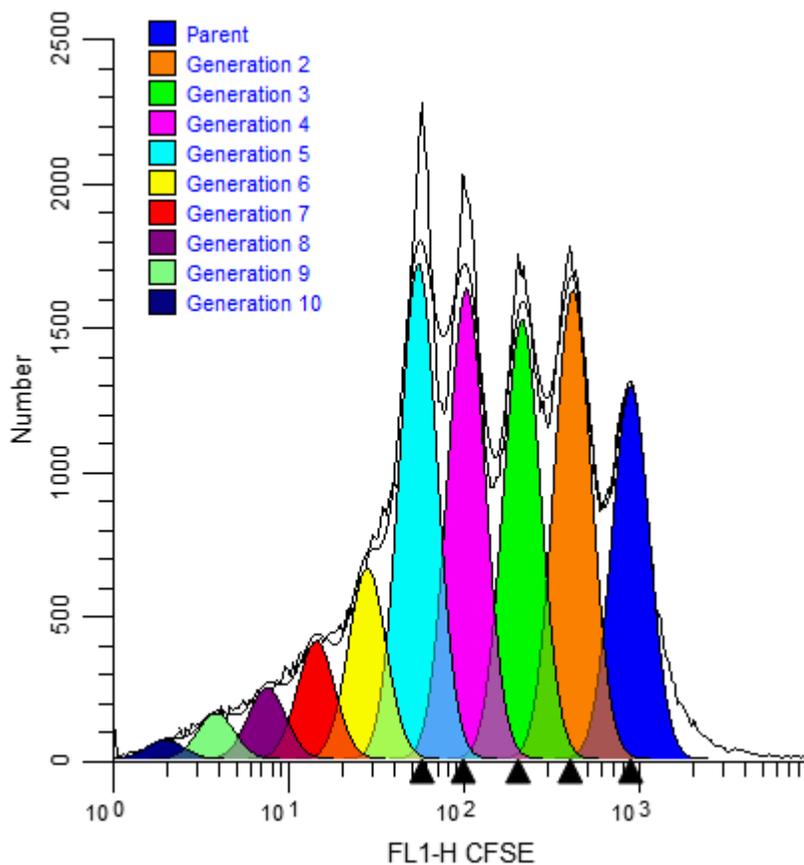
ModFit LT performs sophisticated least squares analysis in just a few clicks of a mouse and reveals the secrets of the proliferation data: proliferation index, non-proliferative fraction, and precursor frequency, and fractions for each proliferating generation. The proliferation results can be exported so that other applications can extend the analysis.

Enter WinList. NStat regions can import the proliferation results from ModFit LT, and then partition the generations according to the proliferation analysis. This enhancement sets the stage for exciting new analysis capabilities.

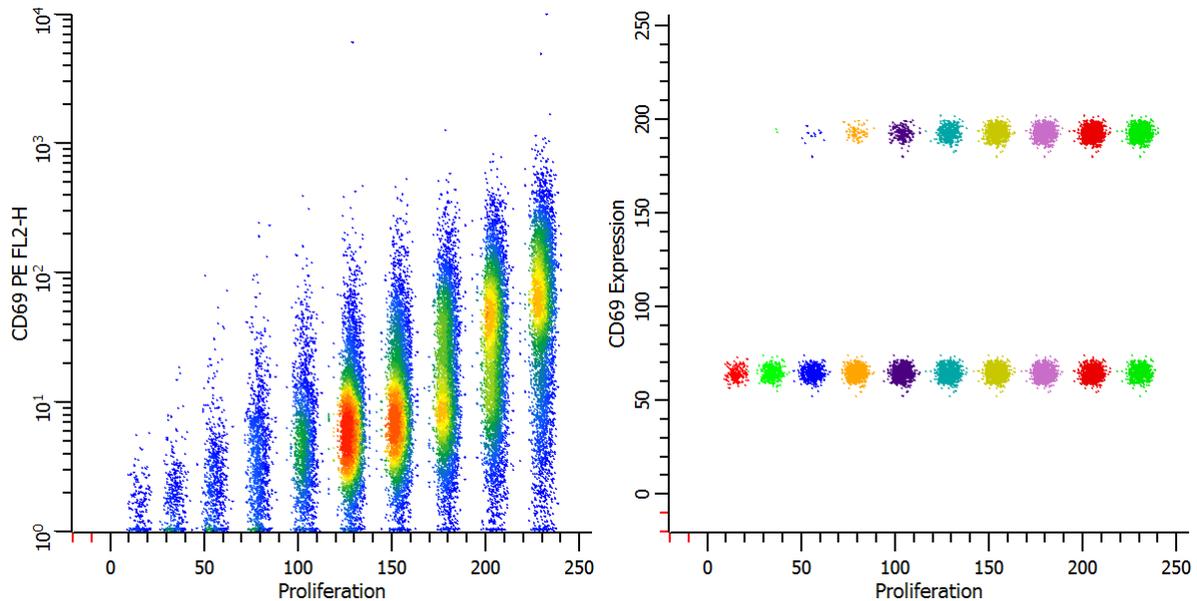
This tutorial will explore these new features to examine a CFSE-stained sample containing several other markers.

The big picture

From the Cell Tracking Wizard in ModFit LT...



... into WinList for exciting, new explorations of proliferation.

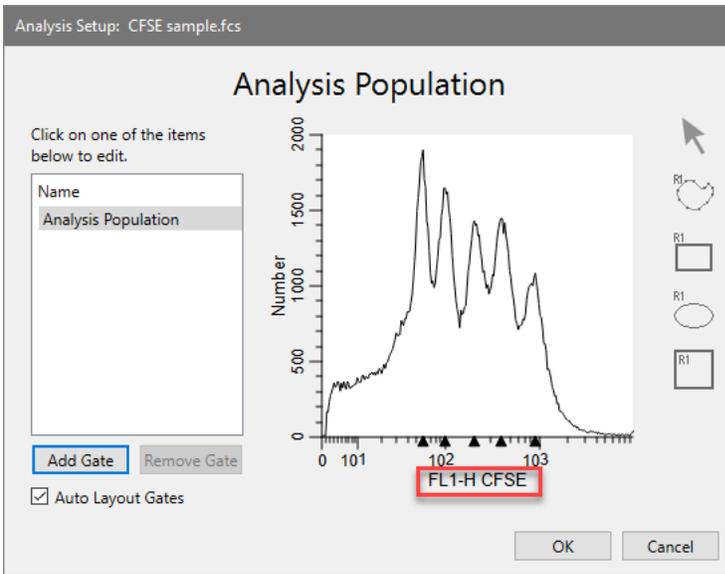


Getting started

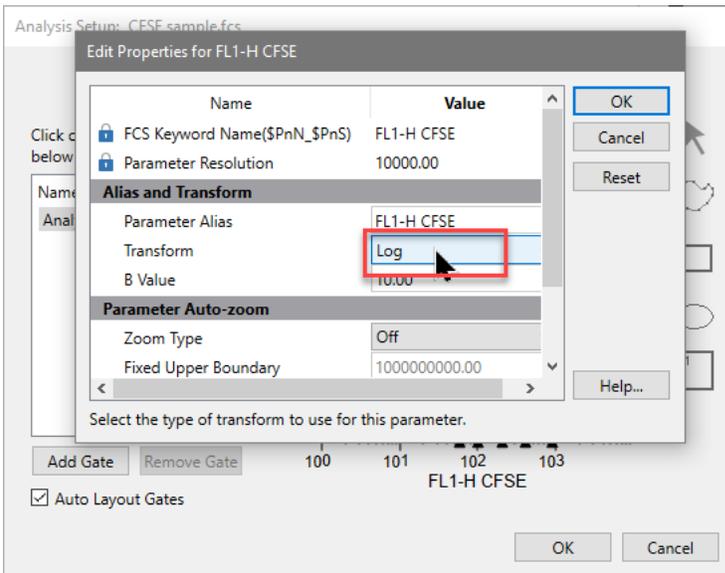
To start, we will use the Cell Tracking Wizard in ModFit LT and export its results. Then, you will set up WinList to import those results into an NStat region.

You will need both ModFit LT and WinList for this tutorial. WinList must be version 9.0 or greater.

- Launch WinList and pass through the opening dialogs.
- Launch ModFit LT and pass through the opening dialogs.
- Using ModFit LT's **Open FCS** command, navigate to the Samples folder located in the *WinList* application folder. Select "CFSE sample.fcs" and click **Open**. Note, the dialog may look different than the one shown below, depending on the version of ModFit LT you are using.



- Choose "FL1-H-CFSE" as the analysis parameter.
- Next, right-click on the histogram and choose **Edit X Parameter**. Choose **Log** for the **Transform** and click **OK**.

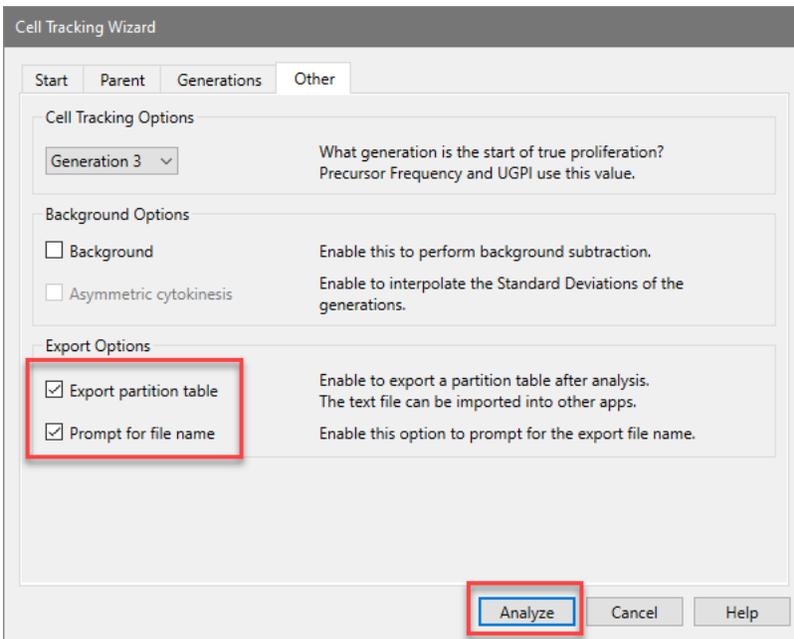


The axis now displays the Log transform. We don't need to create any gates - we'll do that later in WinList.

- Click **OK**.
- On ModFit LT's home tab choose **Cell Track Wizard**.
- In the Cell Tracking Wizard dialog, click the **Generations** tab and choose the "Floating" model option from the drop-down list.

ModFit LT™ User Guide

- Click the **Other** tab, and enable **Export partition table** and **Prompt for file name** checkboxes. These options will cause the program to create the export file after analysis and allow you to enter a name for the export file.



- Finally, click the **Analyze** button to perform the analysis.
- When the analysis is complete, the Choose Location to Save Partitions dialog will be displayed. You can save the file to any location. For this tutorial, save the export file to the desktop so that it's easy to find later.

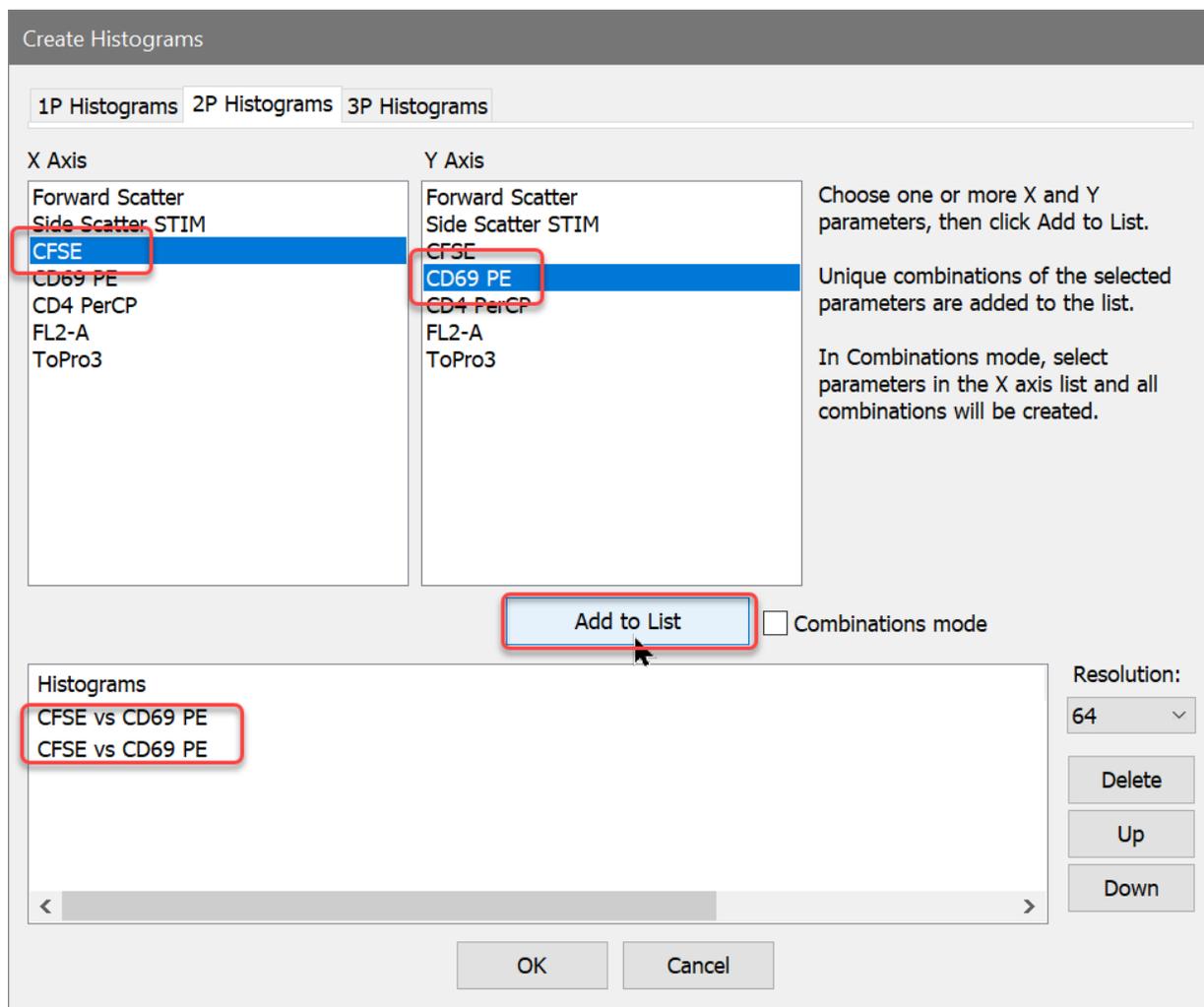
When you choose the Export option for the Cell Tracking Wizard, the program determines the boundaries between adjacent generations and writes that information to the export file. The boundaries are the points of intersection between the Gaussian components in the model.

```
Cell Tracking Wizard Partitions
File    CFSE sample.fcs
Regions 10
Scale   0          256
Gen10   0.00        24.83
Gen9    24.83       44.56
Gen8    44.56       62.77
Gen7    62.77       81.32
Gen6    81.32       98.89
Gen5    98.89       119.50
Gen4    119.50      138.40
Gen3    138.40      157.46
Gen2    157.46      177.94
Parent  177.94      256.00
```

Setting up WinList

- Activate WinList and click the **Open FCS** button on the ribbon bar.

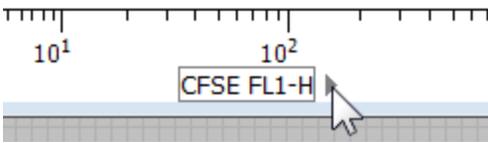
- Navigate to the Samples folder located in the WinList application folder. Select the "CFSE sample.fcs" file and click **Open**.
- In the **Create Histograms** dialog, select **CFSE** in the X Axis list and **CD69 PE** in the Y Axis list. Click **Add to List** *two times*. This will create two copies of CFSE vs. CD69 PE. Click **OK** to close the dialog.



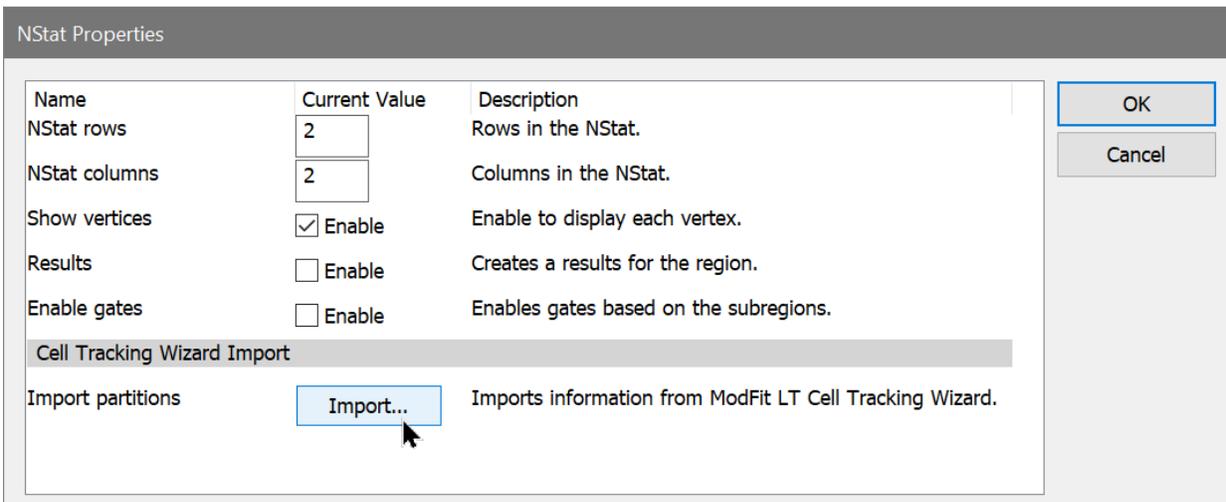
You can change the graphical display for the histograms you've just created. Right click on the histogram and choose Graphics. Try out the different display options to see what you like best..

For this analysis we need to use a standard **Log** transform for the X and Y axis, not HyperLog. The exported information from ModFit LT is in Log units.

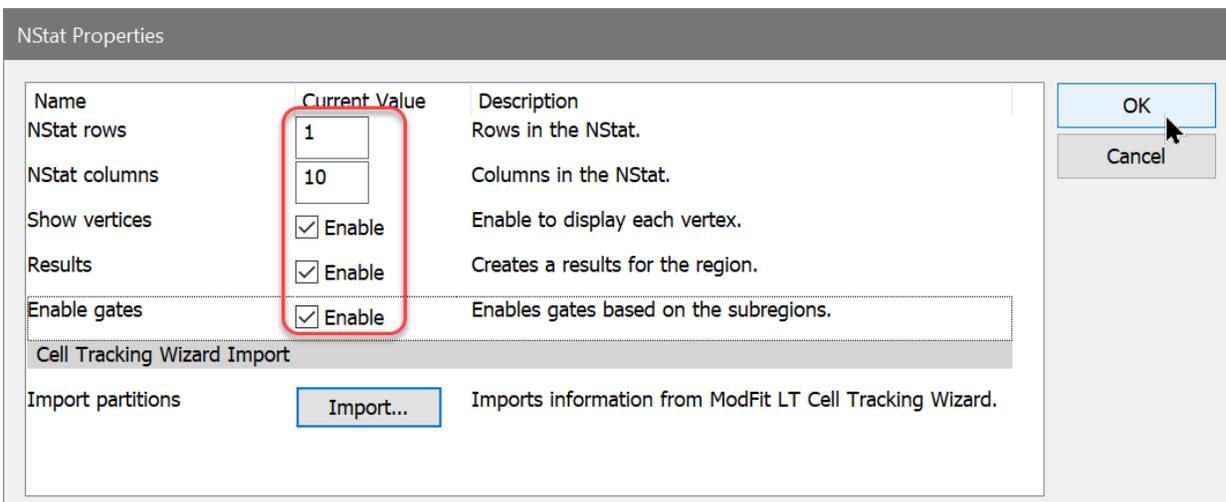
- Move the mouse over the **CFSE** label on the X-axis and click the small triangle that appears. Select **Log** from the list of transformations. Repeat this for the **CD69** Y-axis.



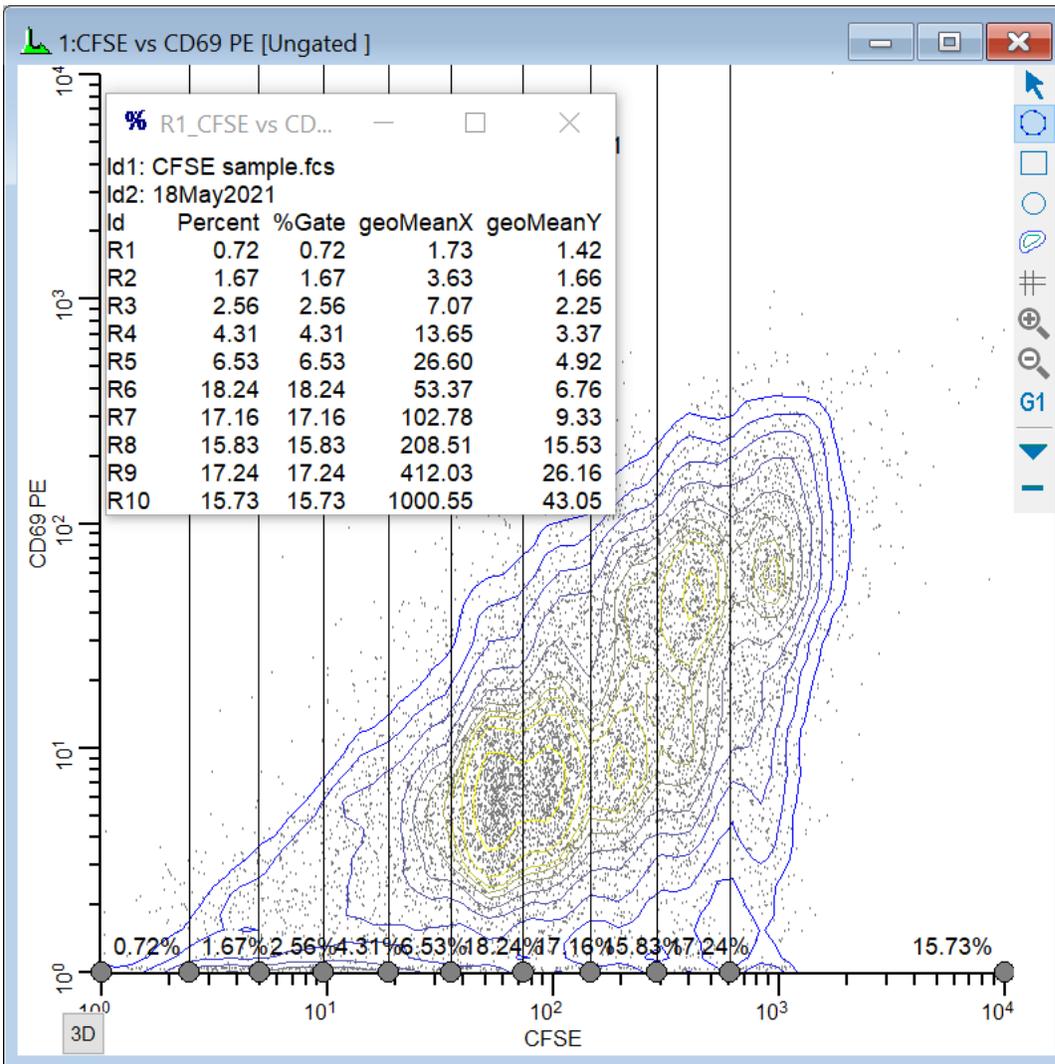
- Click on the title bar of the first dot plot to activate it. Click the NStat region tool in the histogram toolbar.
- In the NStat Properties dialog, click the **Import** button.



- Navigate to the Desktop folder and select CellTrackingWizardPartitions.txt and click **Open**.
- Edit the NStat rows to **1** and check the **Show Vertices**, **Results**, and **Enable gates** checkboxes. The number of columns should already be 10 from the Import. Click **OK**.



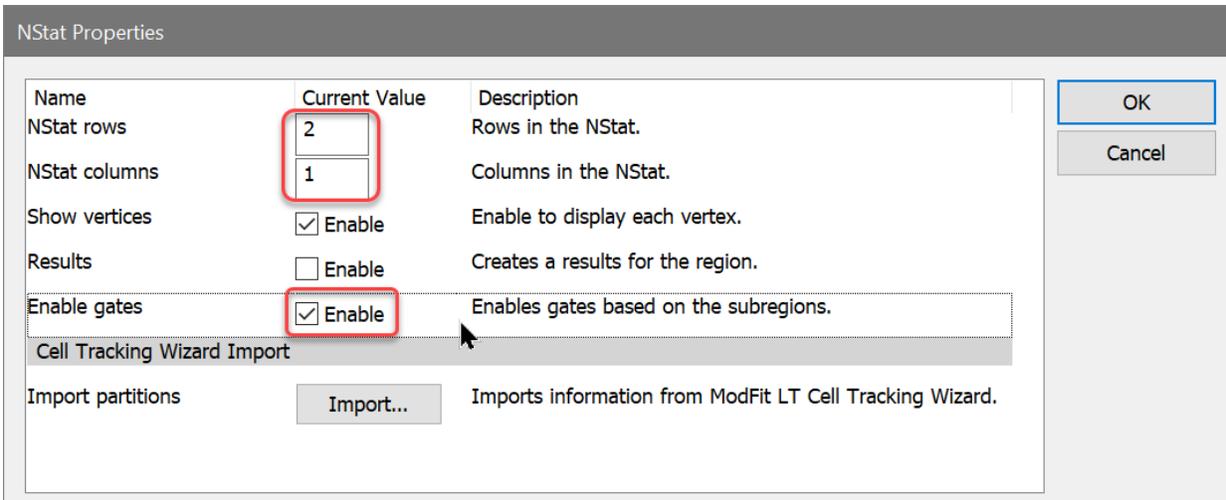
WinList creates the NStat partitions using the imported Cell Tracking information and displays a results window with statistics.



Isolating the generations

At this point, you have completed the basic import process. Let's extend the WinList analysis to explore other markers as a function of the proliferation analysis.

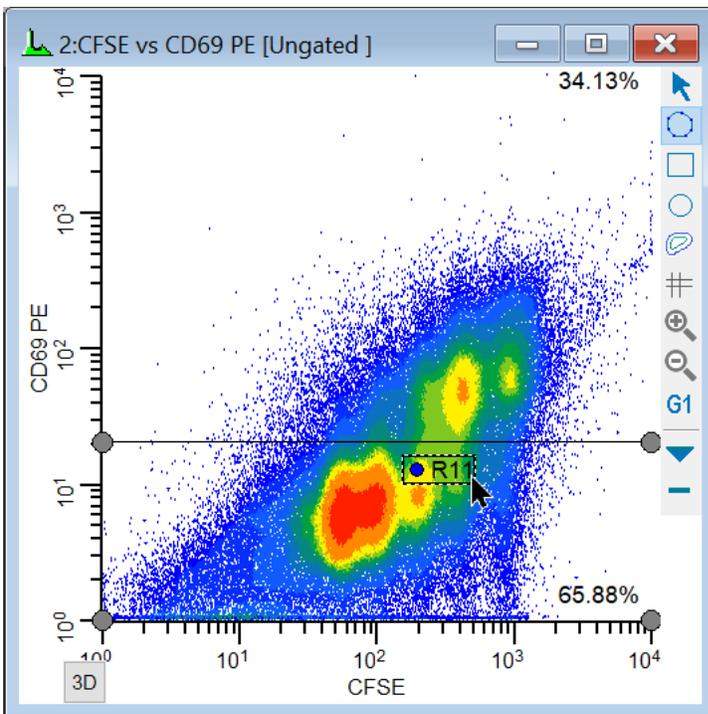
- Click the title bar of the second histogram, and click the NStat region tool button to create another NStat. For this one, set NStat rows to **2** and NStat columns to **1**. Check the **Enable gates** option and uncheck other options. Click **OK**.



Now, one of the histograms contains an NStat that shows the proliferation partitions, and the other histogram has an NStat that will allow us to define the CD69 negatives and positives.

- Drag the region by the label to adjust the second NStat so that it approximately divides the two major populations in the second histogram, as shown.

*You can change the graphics for any histogram by choosing **Graphics** from the right-click context menu for a histogram.*



To analyze and visualize the generations better, we will create a calculated parameter using the *FCOM* function. This function creates a new parameter that will allow us to look at each population independently. It uses gates as input, and we'll use the gates from the NStat regions we just created.

Let's create a few FCOM calculated parameters to help isolate the generations.

- Click the **Add Parameter** button on the main ribbon bar. Alternatively, you can right-click in the data source and choose Add Parameter from the context menu.



- Click the **Add** button in the **Edit Calculated Parameters** dialog.
- Type **Proliferation** in the Name edit box. Type or copy this expression into the Equation edit box, and then click **OK**.

FLOG(FCOM(G1,G2,G3,G4,G5,G6,G7,G8,G9,G10)+0.5)*256/FLOG(1024)+FRND(3)

This expression deserves some explanation. Let's break it apart, starting with the FCOM portion. FCOM is a "gate combination" function. It takes any number of gates as arguments; the result of the equation depends on how many gates are in the expression. With a single gate, there are 2 possible event classifications: an event is "in" or "not in" the gate. With two gates, there are 4 possible event classifications. With the ten gates in our expression, FCOM can produce 1024 different event states.

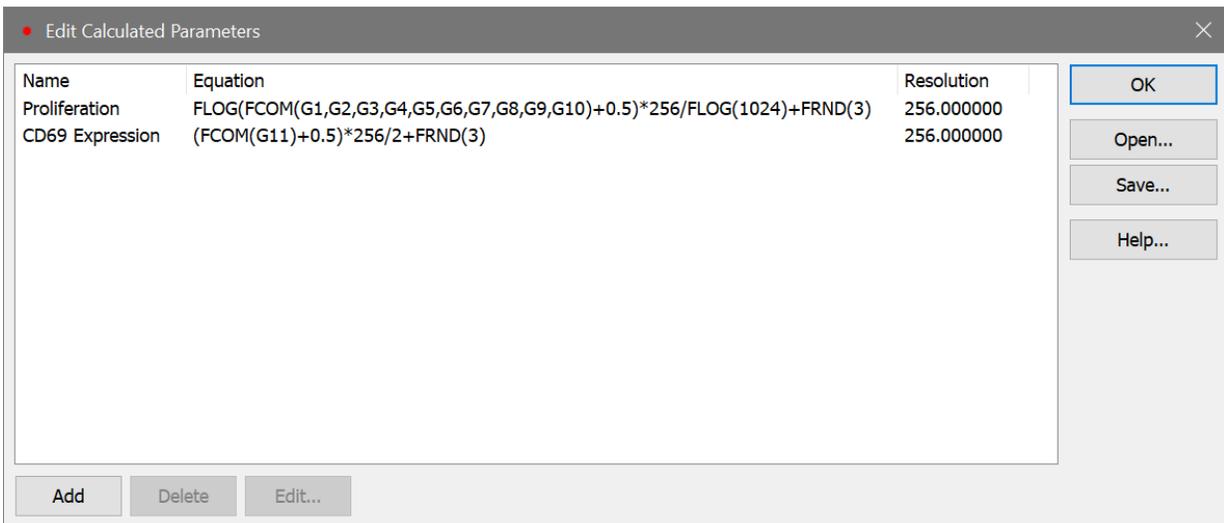
However, since these gates do not overlap at all, most of the 1024 possible states have no events in them. In fact, the only states that contain events are the 10 single-positive event states, resulting in values of 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512. If we plotted the result of the FCOM function as a histogram, we would see 10 spikes of events, and the spikes would be exponentially spaced.

Our goal is to see these 10 spikes *evenly* spaced across the histogram. This is where the FLOG function comes in. By taking the log of the FCOM output and scaling it by 1024, we redistribute the 10 FCOM values evenly across 256 channels. The final portion of the expression, "+FRND(3)", changes the spikes of events into Gaussian distributions so that they can be better visualized.

Take a look at the WinList reference sections on Add Parameters and FCOM for details on the complete set of WinList functions.

- Once again, click the **Add** button in the **Edit Calculated Parameters** dialog.
- Type **CD69 Expression** in the Name edit box. Type or copy this expression, and click **OK**.

$$(FCOM(G11)+0.5)*256/2+FRND(3)$$



- Click **OK**.

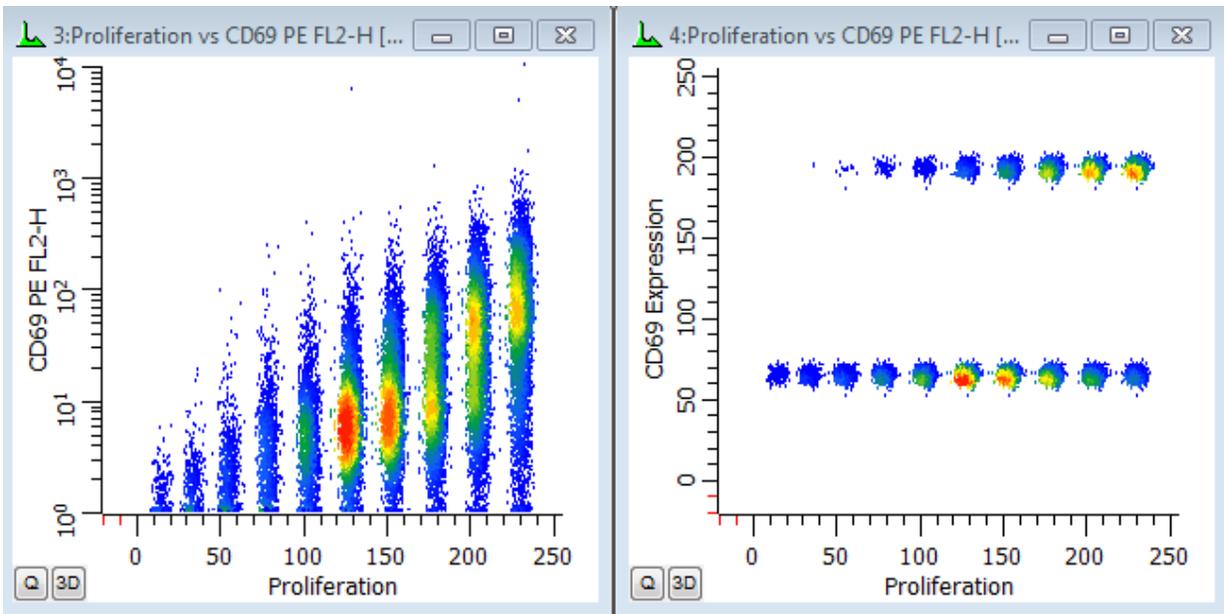
CD69 expression as a function of proliferation

We're now ready to explore this data file in really interesting ways. Wouldn't it be nice to see CD69 as it relates to proliferation, viewing each generation as a discrete cluster? Could we quantify the CD69 expression of CD69-positive cells for each generation? Yes and yes.

- From the **Insert** tab on the ribbon bar, click the **Density** plot. This creates a Density plot in a default location on the data source. Click the X Axis label and choose *Proliferation*. Click the Y Axis label and choose *CD69 PE*.

Let's create another histogram to isolate the CD69 positives and negatives.

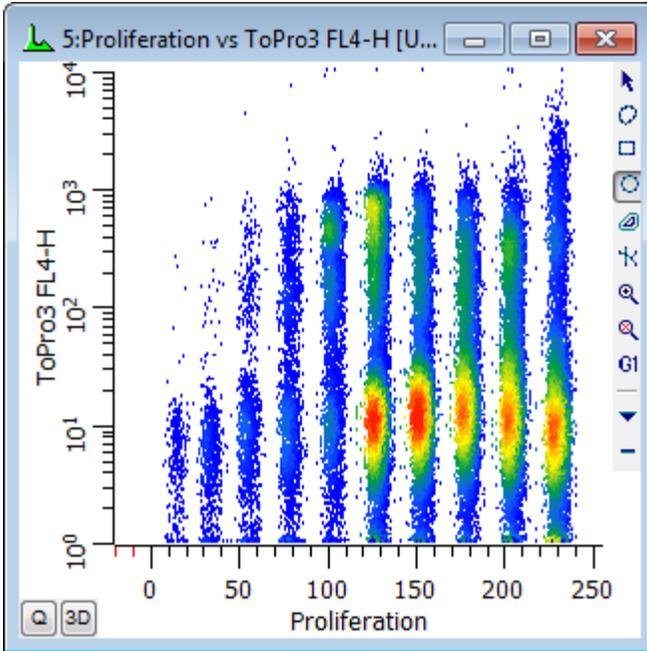
- Right-click on the histogram that we just created and choose **Clone** from the context menu. A duplicate copy of the histogram is created.
- Change the Y Axis to show *CD69 Expression*.



In the graphics we can see the generations separated into discrete populations along the X-axis. That's the first FCOM function we created. The Parent generation has the highest intensity.

The Y-axis shows the continuous CD69 parameter in the histogram on the left. We can see that the parent generation had the greatest expression of CD69, with diminishing expression for each subsequent daughter generation. In the second plot, the CD69 positives and negatives are distinctly separated using the CD69 Expression FCOM parameter that we created. This makes it very easy to get statistics on the "CD69-activated" cells for each generation by simply creating regions on the subsets.

You can, of course, explore how other parameters relate to cell proliferation. Simply create additional histogram to display the parameter combinations you are interested in.



Making life easier

You have done the real work in setting things up with this tutorial. If you save this setup as a WinList protocol or protocol bundle, the calculated FCOM parameters, NStat regions, and selected histogram can be recalled very easily from disk.

When you analyze new data files with ModFit LT's Cell Tracking Wizard, you can export new partition files. Then, open the same data file into your WinList protocol and import the partitions into the NStat region as we have demonstrated in this tutorial. All of the calculated parameters will update based on the new data and partitions.

Creating a report template

One of the best ways to save time with ModFit LT is to design one or more reports to use as templates. You can open a template and use it as the starting point of your analysis, with your customized comments, graphics, and layout.

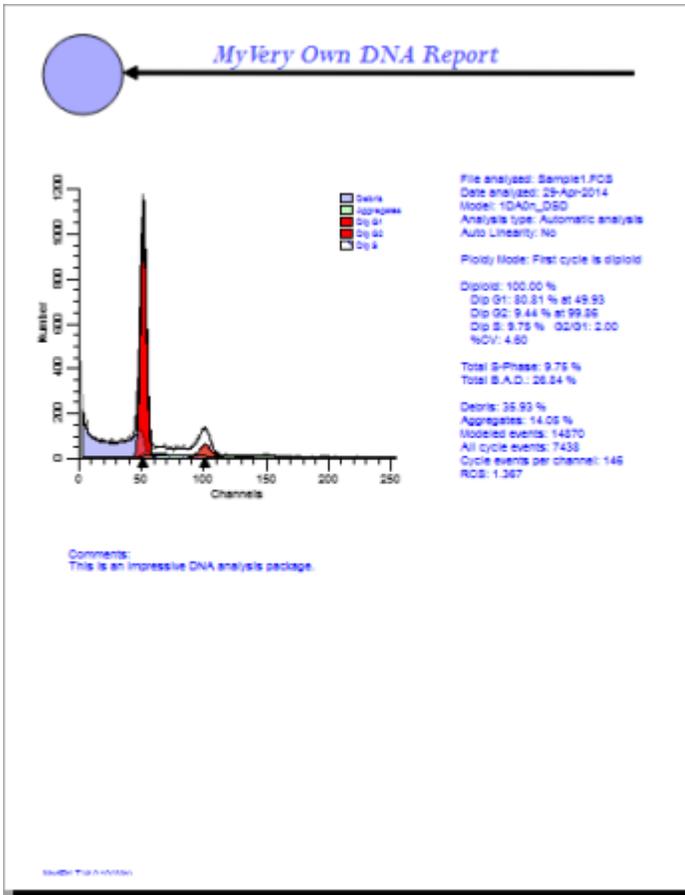
In this tutorial, we will design a simple report using ModFit LT's built-in text and graphics tools.

- Launch ModFit LT.
- Click the **Open FCS** button on the **Home** tab. Choose one of the sample files, and then click **Open** to load it.

You will see a histogram, but no results. In order to display the results, we have to analyze the data.

- Click the **Auto** button to automatically analyze the sample. Once finished, ModFit LT will show us the results in textual format.

Suppose that we want to create a report that looks something like the one pictured below.



We have added four objects to this report and moved things around a bit. The top of the report contains a circle, arrow, and title text, and there is a new free-form text object in the lower half of the report.

We will build a report template like this simple example.

Building the example

- Adjust the **Zoom** so that you can see the whole report page on the screen.
- Click the histogram graphic and drag it down the report page about two inches.
- Move the legend down about two inches.
- Click the results text and drag it down the page, as well. Align the top of the text with the top of the graphic.



- From the **Insert** tab, click the **Ellipse** tool . This will insert an ellipse object onto the report page.
- Move and size the ellipse to resemble the example.
- Double-click the new ellipse object to edit its properties. Change the Fill Color to a color you like, and click OK.

- Click the **Arrow** tool on the **Insert** tab  . An arrow will be inserted into the report.
- Drag the arrow so that its tip is next to the right hand side of the circle. Then, click and drag the other end of the arrow across the page to form a horizontal line.



- Click the **Text** tool on the **Insert** tab  . The **Edit Text** dialog will appear. Click the **Properties** button.
- Click the **Label Font** to display the font dialog. Increase the font size to 26. You can change the font and color as well. Click **OK** when you are done adjusting the font.
- Type "My Very Own DNA Report" (or some other title that suits you) into the text edit box, then click **OK**. The text is inserted into the report.
- Move and resize the text so that it is positioned correctly over the arrow.

OK, we have just one more item to add.

- Click the **Text** tool once again. In the **Edit Text** dialog, type a comment that you might make about the data you analyze. Then click **OK**.
- Move this last text to the lower portion of the report page.

Great. You are done. All that is left is to save the report under some name that we will remember.

- From the **File** menu, click the **Save Report Template** button. In the **Save Template** dialog, enter the name "PRACTICE".
- Click the **Save** button.

Using the template

The report template has been saved. So how does one use a report template? It is really quite simple.

Any time you create a new *project* in ModFit LT, use the **Open Report Template** command in the File menu to open your customized report template. After opening your template, use the **Save Project** button on the Home tab to save your report template as the project's report template.

If you want to use your custom template without creating a project, simply use the **Open Report Template** command in the File menu to open your customized report template. Once the report template is loaded, open a new *data file*, and analyze it. All of the results in the template reflect the new sample.

ModFit LT™ User Guide

If you want to protect your reports from someone accidentally overwriting them, you can make them "read-only". If someone accidentally tries to save a report by the same name, ModFit LT will require the user to choose a new name for their report.

Note: If you have saved reports with a previous version of ModFit LT, you should load them into this version of the program and save them again to upgrade them to the latest version format.

Summary

You can design report templates that include graphics, text, and the layout you want. Templates can be opened in ModFit LT and used as the starting point for the analyses you do with the program. They can save you time and energy, since you design them only once and use them over and over again.

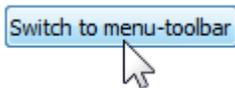
Reference

Main Window

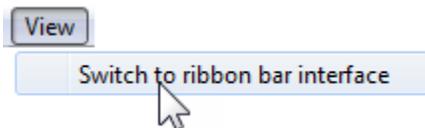
ModFit LT offers two interfaces for the main window. The [Ribbon Bar](#) provides easy access to the most common commands without having to drill down in menus. The [Toolbar](#) and menu interface is a good option for users who want to keep the legacy version of the interface.

The program must be restarted when changing from one to the other.

To switch to the Toolbar and menu interface, select the **Options** tab on the ribbon bar and click the **Switch to menu-toolbar** button.

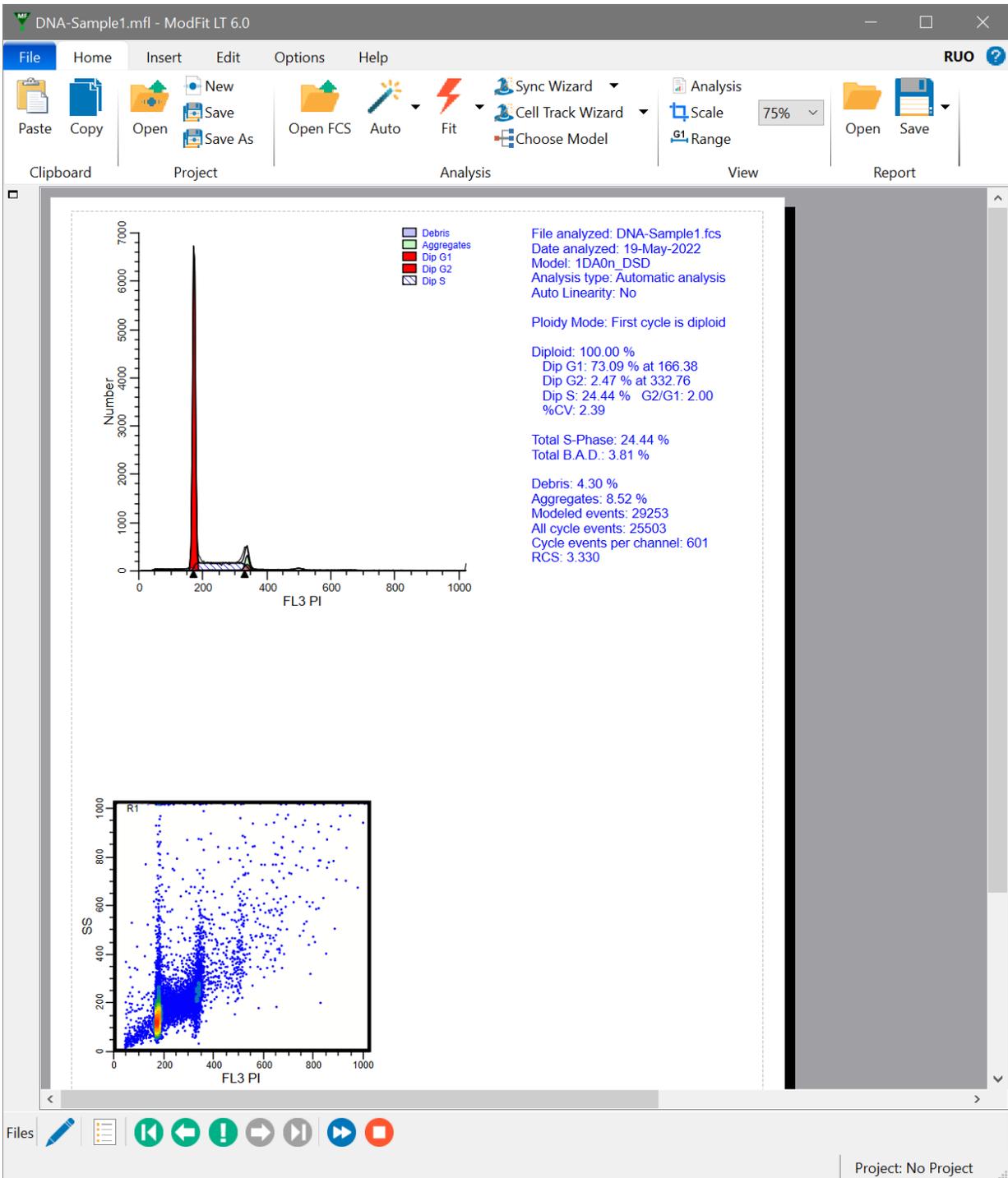


To switch to the Ribbon Bar interface, choose **Switch to ribbon bar interface** in the **View** menu.



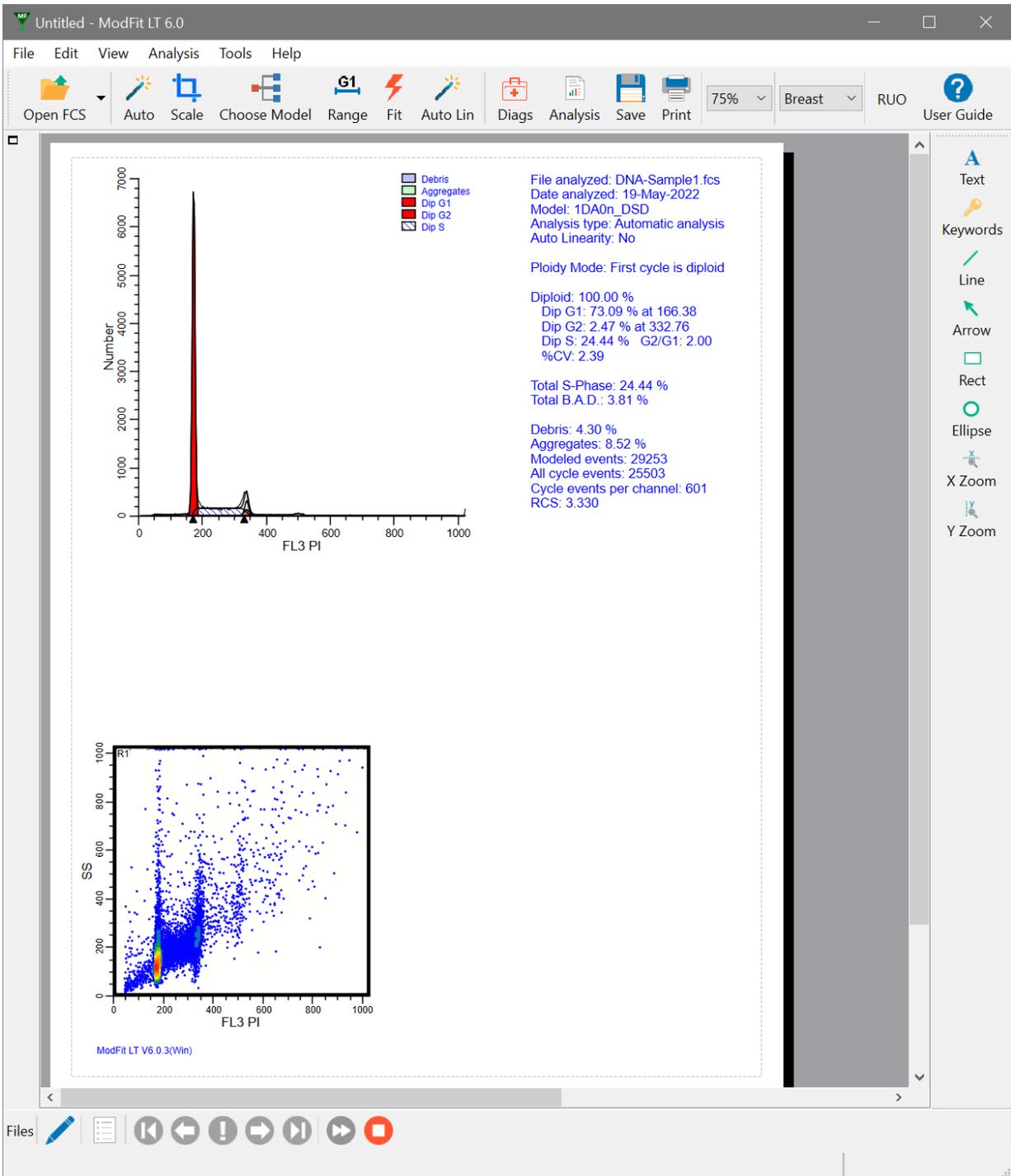
Main window with Ribbon Bar

ModFit LT™ User Guide



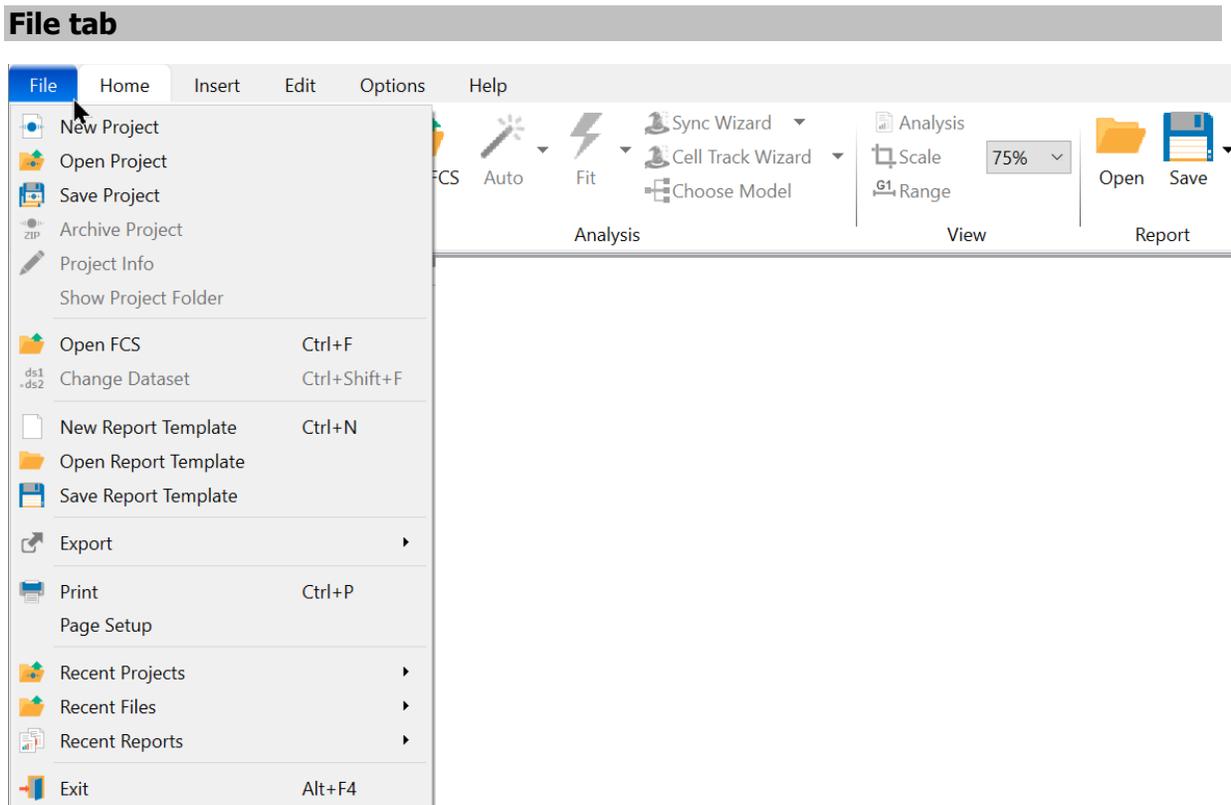
Main window with Toolbar and Menu

ModFit LT™ User Guide



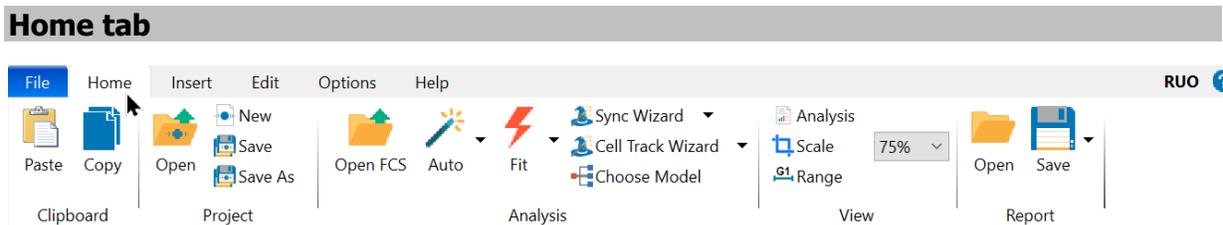
Ribbon Bar

The Ribbon Bar provides easy access to the most common commands without having to drill down in menus. It is divided up into tabs that group commands based on function.



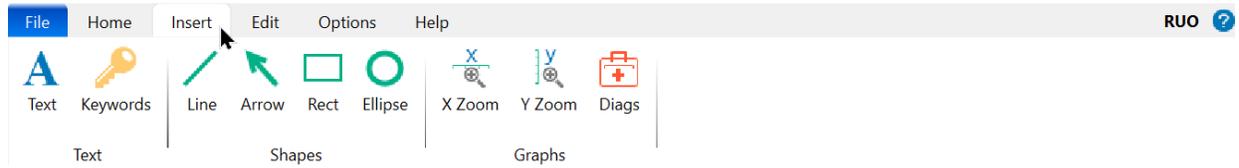
Command	Description
New Project	Create a new project folder
Open Project	Open an existing project
Save Project	Save the project
Archive Project	Archive the project as a ZIP file
Project Info	Display and edit project information
Show Project Folder	Open the project folder in Explorer (Windows) or Finder (MacOS)
Open FCS	Open an FCS data file for analysis
Change Data Set	Select a different FCS dataset

New Report Template command	Create a new, empty report
Open Report Template	Open a report template from disk
Save Report Template	Save a report template
Export PDF , Export Histogram Graphic , Export Report Graphic	Export PDF and graphics
Print	Print the report
Page Setup	Set the page size and margins
Recent Projects	Shows a list of recently-opened projects
Recent Files	Shows a list of recently-opened data files
Recent Reports	Shows a list of recently-opened reports
Exit (Quit)	Closes the program



<u>Clipboard</u>	<u>Project</u>	<u>Analysis</u>	<u>View</u>	<u>Report</u>
Paste	Open Project	Open FCS	Analysis	Open
Copy	New Project	Auto Analyze	Scale	Save
	Save Project	Auto with Auto Linearity	Range	Save As
	Save Project As	Fit with Current Model	Zoom	
		Auto Linearity		
		Sync Wizard		
		Cell Track Wizard		
		Choose Model		

Insert tab



Text

- [Text](#)
- [Keywords](#)

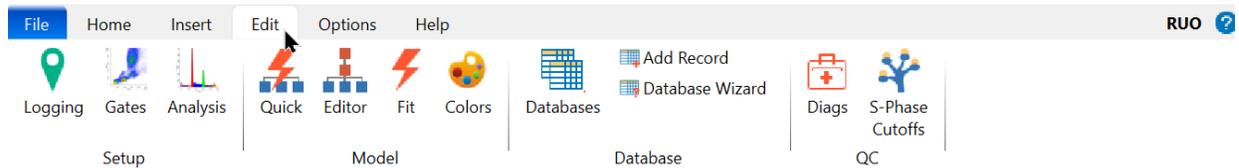
Shapes

- [Line](#)
- [Arrow](#)
- [Rectangle](#)
- [Ellipse](#)

Graphs

- [X Zoom graph](#)
- [Y Zoom graph](#)
- [Fit Diagnostics graph](#)

Edit tab



Setup

- [Logging](#)
- [Gates](#)
- [Analysis](#)

Model

- [Quick Editor](#)
- [Full Editor](#)
- [Fit with Current Model](#)
- [Colors](#)

Database

- [Edit Databases](#)
- [Add Record](#)
- [Database Wizard](#)

QC

- [Fit Diagnostics](#)
- [S-Phase Cutoffs](#)

Options tab



Interface

[Switch to menu-toolbar](#)

Settings

[Report Batch Settings](#)

[Auto Analysis settings](#)

[Peak Finder settings](#)

[Options and Configuration](#)

[User Equations](#)

[Manage settings](#)

[Select Shared Folder](#)
(Only ModFitAdmins in
Compliance mode)

Help tab



Online

[Updates](#)

[Website](#)

[Ask Tech Support](#)

[Make a Suggestion](#)

[Report a Problem](#)

Learn

[Application Info](#)

[User Guide](#)

[About](#)

Toolbar

The toolbar is displayed across the top of the application window, below the menu bar. It can be moved by clicking and holding the mouse down on a blank portion of the toolbar, then dragging to a new location. The toolbar provides quick mouse access to common ModFit LT functions.

The buttons appearing in the Toolbar are shown below along with an explanation of the functions they perform.



Click

To



Open an FCS data file for analysis. This is a split button, which also contains a command to open reports..



Perform an automatic analysis.



Adjust the scaling of the data, and the lower and upper boundaries of the analysis.



Select a model to be used for manual analysis.



Adjust the range positions on the data.



Analyze the data with the current model, scaling, and range positions.



Perform auto-linearity on the current data with the current model.



Evaluate the fit for S-Phase variability and compare with Consensus Conference recommendations.

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Edit and lay out the report.



Automatically name, then save the report.



Print the report.



Select a zoom factor for viewing the report page on the screen.



Select a tissue type for the data sample. By selecting a type, you apply the cutoffs for that type. You must enter your own cutoff values in the S-Phase cutoffs dialog.



This text indicates whether the program is running in ["Compliance" or "RUO" mode](#).



Display the ModFit LT User Guide.

Shortcuts

ModFit LT provides keyboard shortcuts for many of its commands.

Action Name	Windows Shortcut	MacOS Shortcut
New Report Template	Ctrl+N	Command+N
Open FCS	Ctrl+F	Command+F
Open Report	Ctrl+O	Command+O
Save Report	Ctrl+S	Command+S
Print	Ctrl+P	Command+P
Exit (Quit on MacOS)	Alt+F4	Command+Q
Copy command	Ctrl+C	Command+C
Paste command	Ctrl+V	Command+V
Automatic Analysis	Ctrl+A	Command+A
Auto Analyze and Auto Linearity	Ctrl+Shift+A	Command+Shift+A
Fit with Current Model	Ctrl+M	Command+M
Change Data Set	Ctrl+Shift+F	Command+Shift+F

Projects

Projects in ModFit LT

ModFit LT can help organize your work into *Projects*, making it easy to keep files together and to move your work from one place to another.

What is a Project?

A *Project* is a folder structure containing information about your experiment:

- The type of project. For example, projects types can be Cell Cycle, Synchronization Wizard, etc.
- A report template file, which can be customized.
- A list of the FCS files you have added to the project
- The actions that you want to perform when you open an FCS file
- The output that is generated by the analysis, such as reports, PDFs, image files, and database results.
- A copy of the preferences and settings that were in place when the project was saved.
- Optionally, a project folder can also contain copies of the FCS files in your experiment.

What is the Project Type?

The project Type determines how the project will be used:

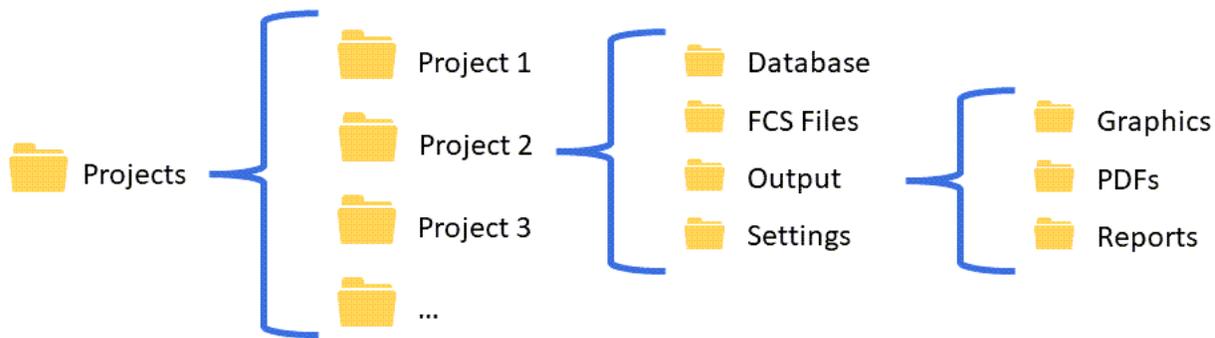
Project Type	Usage
Cell cycle	Cell cycle analysis using Auto-Analysis or a model created with the Choose Model dialog. Supports multiple cell cycles, debris and aggregate subtraction
Synchronized cell cycle	Analyze samples that have been synchronized (e.g. blocked in G0-G1 and then released). Supports one cell cycle, debris and aggregate subtraction
Cell proliferation tracking	For cells stained with cell-tracking dyes such as CFSE. Computes all cell proliferation statistics (e.g. Proliferation Index, Replication Index, Precursor Frequency, etc.)
Custom	For an unrestricted analysis with a custom model

ModFit LT will enable the tools that are appropriate for the type you select.

Where are Projects stored?

Projects are stored in a folder named Projects within the ModFit LT folder in the user's Documents, by default. Each project is contained in its own folder within the Projects folder. It is recommended that Projects are stored in the default Projects folder to make it quick and efficient to open projects. However, users can save a Project to any writable location, and Projects can be opened from any readable location accessible to the user.

The diagram below illustrates the structure a Project folder.



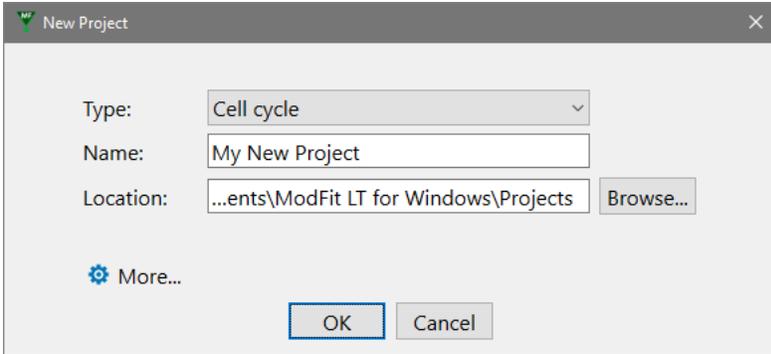
Folder	Contents
Database	Contains the tab-delimited database file and related files.
FCS Files	If the project is set up to Copy Files, the FCS files added are copied into this folder.
Output	Graphics, PDFs, and Reports are stored in folder under the Output folder.
Settings	The program settings that were in place when the project was last saved are contained in this folder.

Do I have to use Projects?

Projects are intended to make your work easier to organize and structure, but there is no requirement to use Projects.

New Project

This command creates a new project. When selected, the New Project dialog is displayed.



Type

The project Type determines how the project will be used:

Project Type	Usage
Cell cycle	Cell cycle analysis using Auto-Analysis or a model created with the Choose Model dialog. Supports multiple cell cycles, debris and aggregate subtraction
Synchronized cell cycle	Analyze samples that have been synchronized (e.g. blocked in G0-G1 and then released). Supports one cell cycle, debris and aggregate subtraction
Cell proliferation tracking	For cells stained with cell-tracking dyes such as CFSE. Computes all cell proliferation statistics (e.g. Proliferation Index)
Custom	For an unrestricted analysis with a custom model

ModFit LT will enable the tools that are appropriate for the type you select.

Name

Enter a name for the project. The name will be used to create a project folder on disk, so the characters in the name have to be compatible with file names on your operating system.

Location

By default, projects are saved in the ModFit LT subfolder of the user's Documents folder and in most cases you do not need to edit the location. However, you can select any location accessible to your computer using the Browse button.

More

Click the More button to show additional properties of the new project.

Project Description

Use this field to describe your project. It can also be left blank.

Project Creator

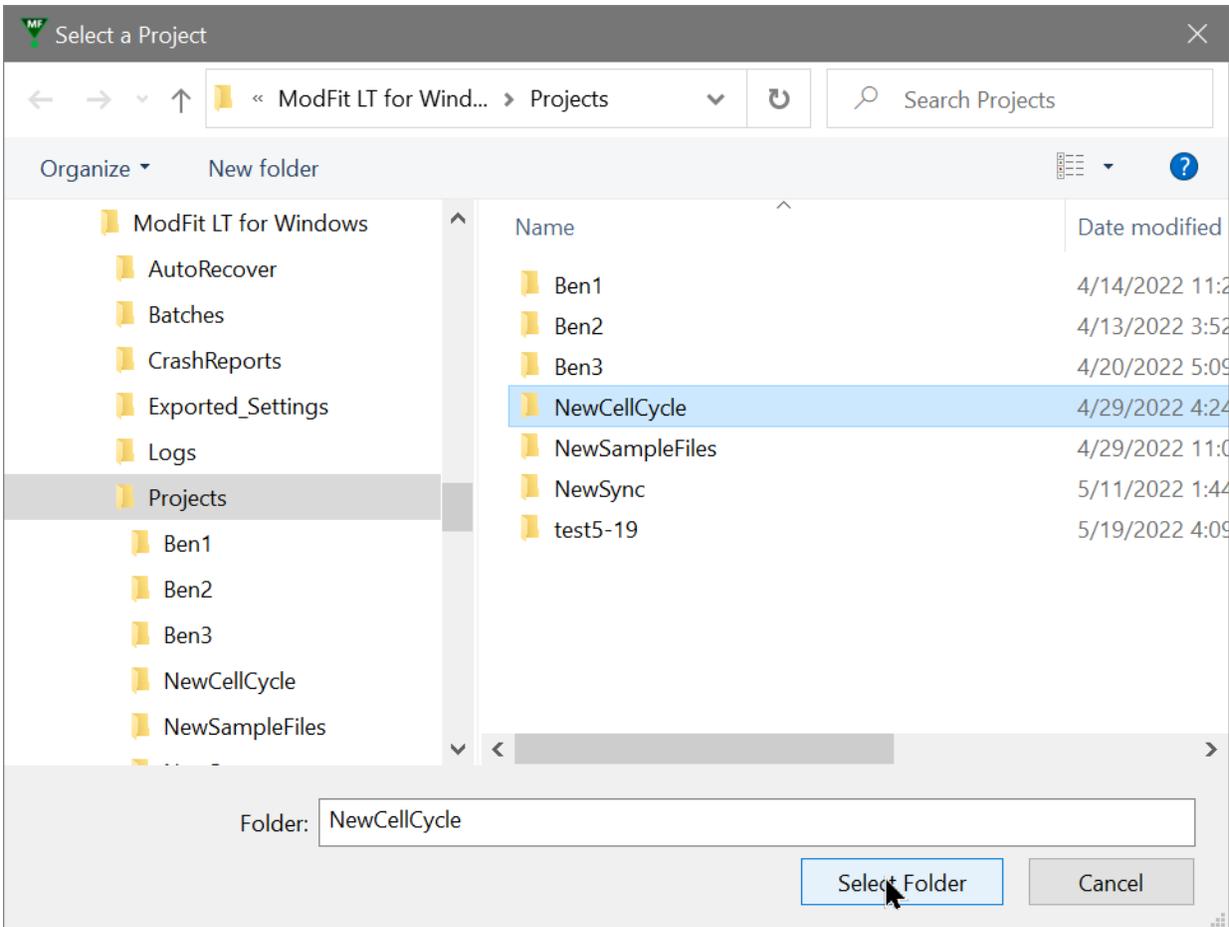
By default, the creator is the user logged onto the computer. This field can be edited.

Copy Data Files

When this option is enabled (checked), FCS files that are added to the project are copied into the project folder. This makes it easier to move projects to different locations.

Open Project

This command displays the Select a Project dialog:



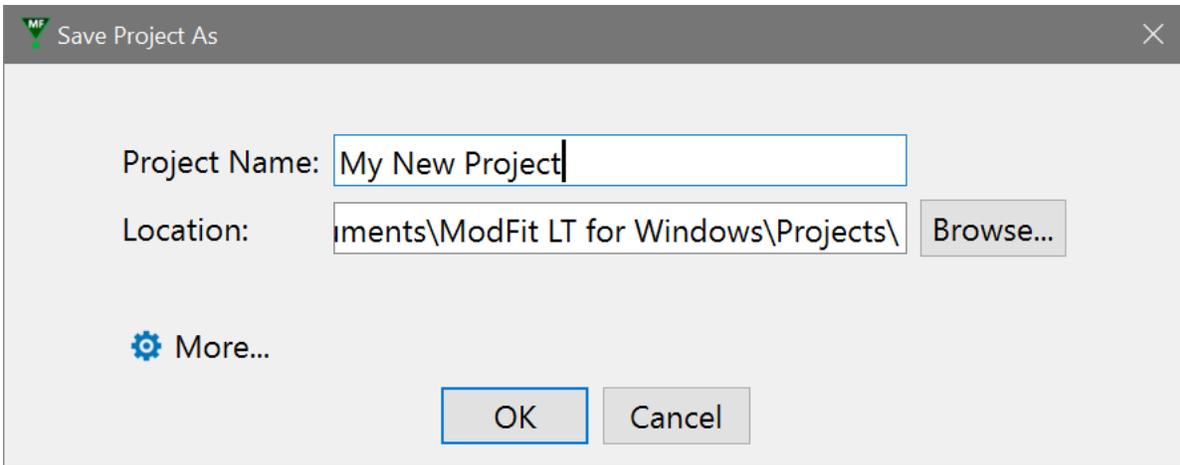
A project is a folder on disk, usually located in the ModFit LT\Projects folder under the user's Documents. Find the project folder you want to open, select it in the list, then click the **Select Folder** button to open the project.

Save Project

This command will save the current state of the project, including the files added, the layout of the report template, and the batch processing options.

Save Project As

Use this command to save the current project with a new name. This command displays the Save Project As dialog.



The current project's file list, batch settings, and report template are copied to the new project folder. If the current project has the Copy Data Files option enabled, the FCS files that have been added to the project will be copied to the new project.

Archive Project

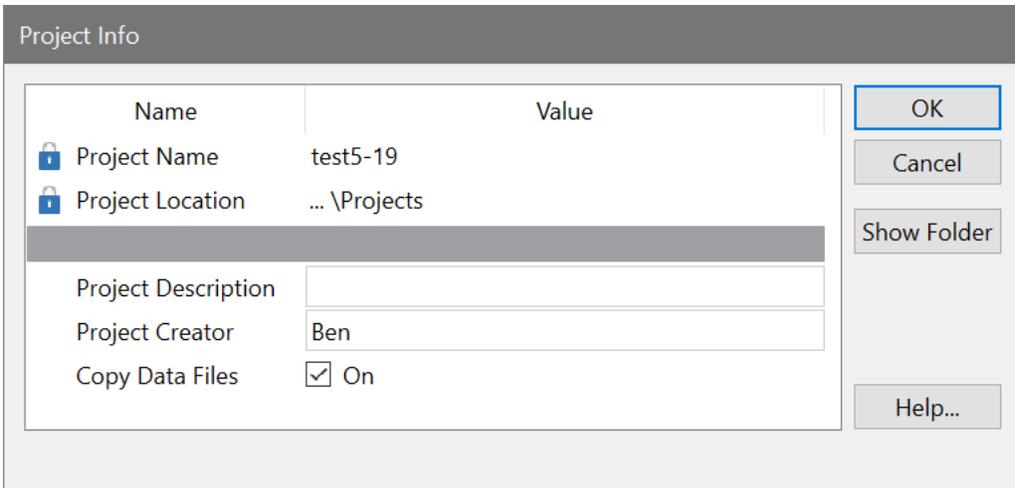
This command creates a Zip file of the current project. The file is written to the ModFit LT\Projects folder in the user's Documents folder. A message is displayed when the archive operation is completed.

The Zip file compresses the project files and makes it easy to move the project to another location.

Files in the original project folder are not changed by the archive operation. They remain on the disk in the project folder.

Project Info

This command displays the Project Info dialog to show the properties of the project:



Several properties can be edited in this dialog. In addition, the **Show Folder** button provides a convenient way to open the project's location in Explorer (Windows) or Finder (MacOS).

Show Project Folder

Use this command to open the project folder in Windows Explorer or MacOS Finder. It provides a quick way to get to the actual folder on disk where the project files are stored.

Data Files

Open FCS



Use this command to open a data file. Choosing this option will display the [File Open dialog](#) to select a file to open.

A data file is an FCS (Flow Cytometry Standard) format histogram or listmode data file. It can also open some formats of ASCII-encoded histogram files. You can load one data file at a time into a report document.

If a listmode file is selected, the [Analysis Setup](#) dialog is displayed to allow selection of the analysis parameter and to set up gates.

Batch Processing

When more than one file is selected in the Open FCS dialog, they are added to a batch list that appears on the left side of the main window. See [Batch Processing](#) for details.

To select a batch of files, click the mouse on the first item you want to select and drag the mouse over sequential files in the list. Alternately, you can click the first item, hold down the Shift key and click the last item to select a group of files. Hold down the Ctrl key to select non-adjacent files in the list.

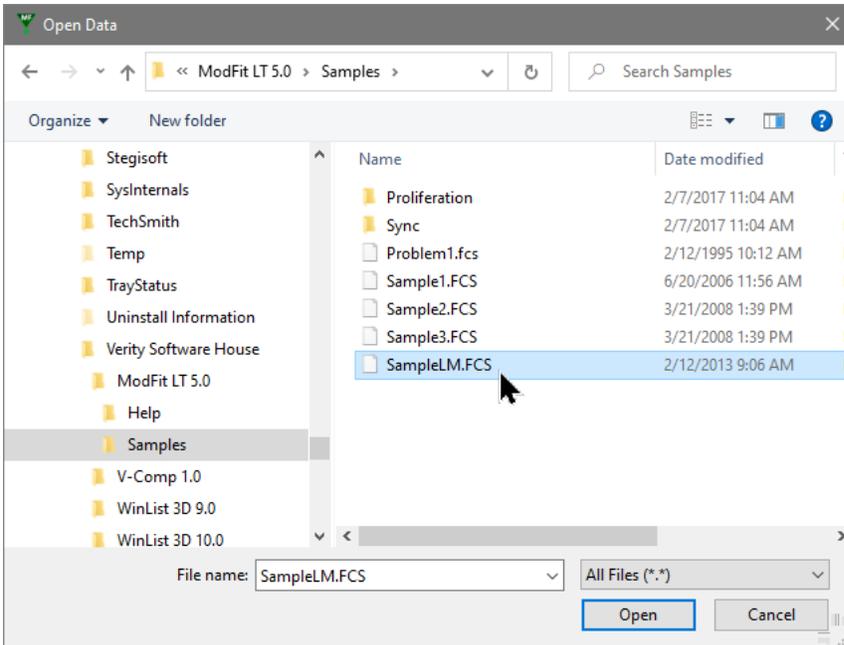
Shortcuts

Keys: CTRL + F on PC; Command + F on Mac

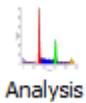
File Open dialog box

This dialog is used to open data files or reports in ModFit LT. This is the standard File Open dialog box for the operating system, so its appearance and features are different on Windows and Mac versions.

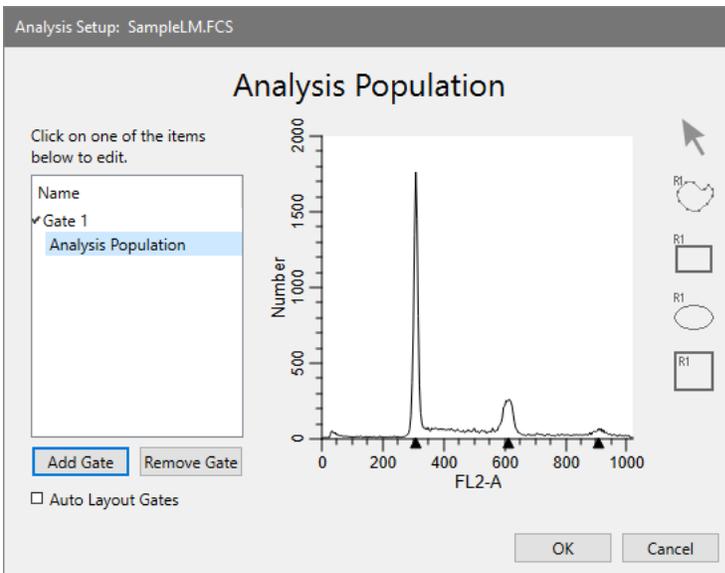
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Analysis Setup



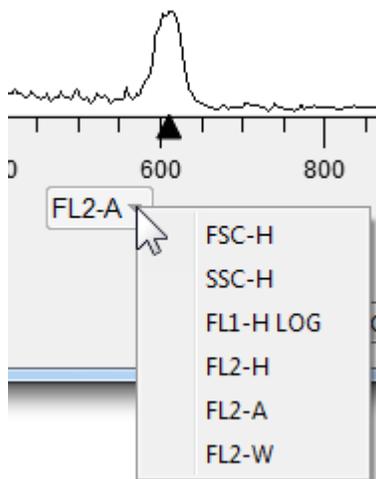
When you open an FCS **listmode** file, ModFit LT will present a dialog that allows you to choose which parameter you want to analyze and parameters to use for gating.



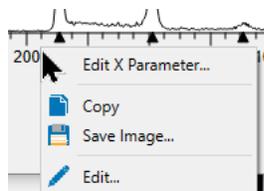
The dialog contains a tree on the left side showing available gates and the analysis population. A graphical view of the selected item is shown on the right side of the dialog.

Analysis Population

Choose the parameter you want to analyze by clicking the label on the X axis and selecting a parameter from the list.



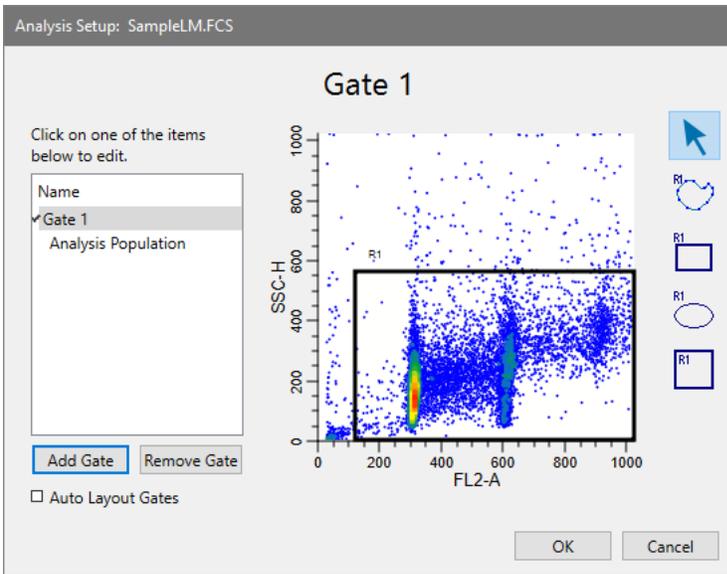
You can right-click the X-axis and choose Edit X Parameter to change the transformation used for the parameter. See [Edit Properties for Parameter](#) for additional info.



Gates

After you choose the Analysis parameter, you can create gates. Use the **Add Gate** button to create a new gate, and the **Remove Gate** button to remove an unneeded gate. Gates are logical "AND" gates, which means that events must satisfy all active gates to display in the analysis histogram.

Select a gate item in the tree to display the gate in the right-hand panel of the dialog. With the item selected, click the X- and Y-axis labels to pick the gating parameters for the plot. Use the region tools on the right to define a gating region on the plot.



Graphics for the gating plot can be edited by right-clicking the plot area and selecting [Edit Properties](#). See [Edit 1P Graphics](#) and [Edit 2P Graphics](#) for additional details.

Define each gate for your analysis. The active gates will appear on the report page and can be modified there once created.

The **Auto Layout Gates** option can be enabled to automatically organize gates on the report. Uncheck this option if you create your own arrangement for gates.

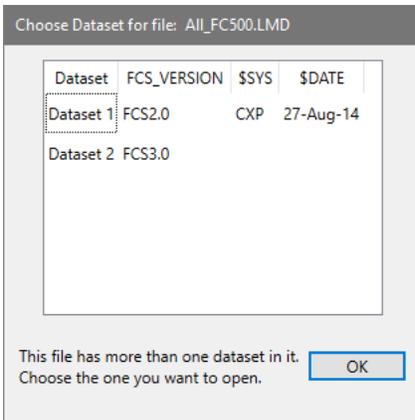
Click OK to finish the gate and analysis population selection.

Change Data Set

This dialog is used to choose a "data set" for some FCS files. For example, Beckman Coulter files from FC500, Navios, and Gallios instruments commonly create 2 data sets in each FCS file: one in FCS2 format and the other in FCS3 format.

If the file you select contains more than one data set, the Change Data Set dialog will be displayed.

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Select the data set from the list of choices, then click OK. The program will re-read the FCS file, extracting the data from the selected dataset for gating and analysis.

Recent Files list

This menu is a convenient list that shows the most recent data files that have been opened in ModFit LT. Select a file from the list to open the file for analysis.

Analysis

Auto Analysis



Use this command to automatically analyze the current data file.

The program uses the settings from the Auto Analysis Settings dialog, along with Peak Finder Settings, to analyze the data file.

What Does Auto Analysis Do?

When you load a data file, ModFit LT identifies peaks it finds in the sample. Automatic analysis looks at the peak positions, standard deviations, and other information about the sample and attempts to match the data to a ploidy-specific model. The model is constructed, ranges are positioned around the peaks, peak estimates are computed, and a non-linear least squares analysis is performed on the data. In addition, ModFit LT tests the S-Phase measurements for statistical confidence if the S-Phase assessment system is activated.

When automatic analysis finishes, ModFit LT displays the analysis results in graphical and textual form on the report.

See also:

[Strengths and Limitations of Automatic Analysis](#)

Shortcuts

Keys: CTRL + A on PC; Command + A on Mac

Auto Analyze and Auto Linearity



Use this command to automatically analyze the current data file and then to perform Auto Linearity on the file.

See [Auto Analysis](#) and [Auto Linearity](#) for additional details.

Shortcuts

Keys: CTRL + Shift + A on PC; Command + Shift + A on Mac

Fit with Current Model



Use this command to apply a model to the analysis data with current scaling and range positions. This performs a non-linear least squares analysis of the data.

If you use Automatic Analysis, you will not need to use this command. It is done for you.

Use this command after you have opened a data file, chosen a model, and positioned the model's ranges. If you change the model using the Quick Model Editor, the Full Model Editor, or by positioning ranges, you need to use this command to re-fit the data with the model.

See also:

[Choose Model](#)

[Ranges](#)

Auto Linearity

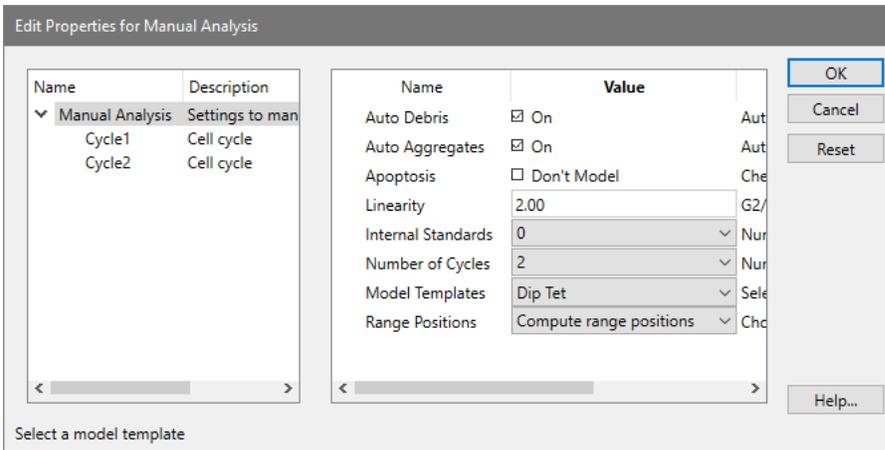


This command initiates the auto-linearity process with the current model and data. Auto linearity adjusts the G2M and aggregate components based on iterative fitting process, and sets the linearity to the one that achieves the best fit.

Choose Model



Use this command to select a model to use in the manual analysis of the data. A model consists of a set of components, which are linked together based on the choices you make in this dialog.



Selecting a model does not actually perform an analysis. After choosing a model, you need to



position its ranges and click the **Fit** button  to analyze the data with the model.

The left portion of the dialog contains a tree diagram of the model. The top of the tree is the Manual Analysis branch, and the choices you need to make when selecting a model are described below. Also shown in the tree is a branch for each cell cycle you want to model. The number of cycles you see will depend on what you choose for "Number of cycles" in the dialog.

After making your initial model selections with the Manual Analysis branch selected, you can fine-tune the model by selected each cycle branch and adjusting its properties. See the [Edit Properties for Cycle](#) dialog box for details on cycle properties.

AutoDebris

To enable debris modeling, check this option. The AutoDebris component can model debris from fresh, frozen, pulverized, and paraffin samples. If the sample shows little or no debris, you may choose to disable this option.

AutoAggregates

Check this option to enable a model component that automatically fits most forms of aggregation. Aggregates can form in the sample as cells and cell fragments stick together. Because all sizes and shapes of cells can form aggregates, the AutoAggregate component is a continuous distribution. Its shape is unique for each data file, and is based on probabilities of cells in each channel sticking together. To disable the AutoAggregates component, uncheck this option.

Apoptosis

Note: this is **not** the recommended approach to use for Apoptosis. Secondary markers are much more robust and accurate.

Check this option to model an apoptosis distribution in the sample. A Gaussian will be used to model a peak, and a range to identify the peak will be displayed.

Linearity

Enter a linearity value for the sample. This value can be determined by dividing the G2M position for a cycle by the G1 position for the same cycle. The program will use the linearity value for computing positions for G2M populations that are not clearly defined peaks, and for the AutoAggregate model component.

Standards

Use this option to select the number of internal standards that appear as distinct peaks in the sample.

Number of cycles

Choose the number of cell cycles that appear in the sample. Each cell cycle contains model components for G1, S-Phase, and G2M populations.

Model templates

After choosing the number of cycles above, select a model template that best describes the cycles. The templates are named with abbreviated forms of the common cell cycle names: "Dip" is Diploid, "Ane" is Aneuploid, "Tet" is Tetraploid, "Hyp" is Hyper-Diploid, and "NearDip" is Near-Diploid.

Notice that the model template name determines the relative positions of the cycles in the model. For example, with 3 cycles, there is a template for "Dip Ane Tet" and another for "Dip Tet Ane". The "Dip Ane Tet" template is designed for the Aneuploid appearing between the Diploid and the Tetraploid populations; the "Dip Tet Ane" is designed for the Aneuploid appearing above (to the right of) the Tetraploid population.

The available model templates will change depending on the number of cell cycles you have selected to model.

Range positions

This option allows you to determine whether or not the program will re-position ranges upon choosing OK. If you have already selected a model and are making some adjustments in this dialog, choose "Use current range positions". If you are selecting a new model, choose "Compute range positions" to determine the initial locations for the ranges.

OK

Click this button to create a model matching your selections and display the ranges for the model. After clicking OK, position the ranges and click the Fit button on the toolbar.

The selected model will be used until 1) another model is selected using Choose Model, 2) an automatic analysis is performed, 3) a new report document is loaded, or 4) you close the program.

Cancel

Click this button to close the dialog without creating a model.

Reset

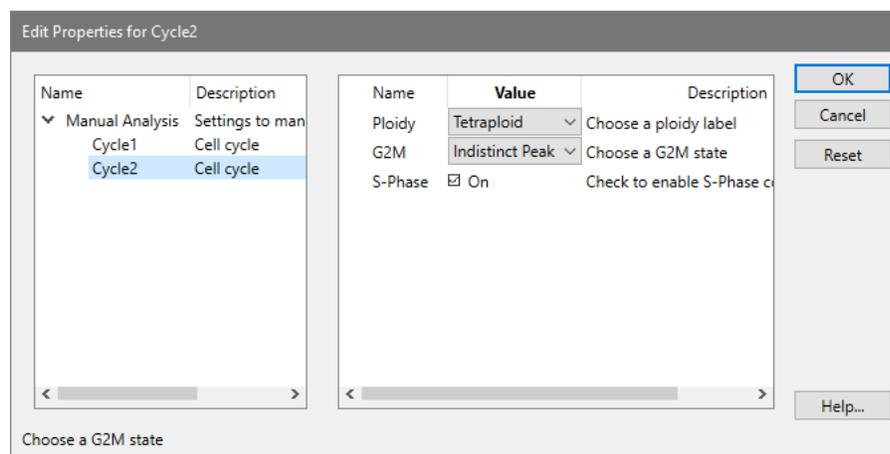
Click this button to restore the dialog to a default model.

See Also

[Edit Properties for Cycle](#)

Edit Properties for Cycle

These options appear when you chose a Cycle in the tree on the left side of the [Edit Properties for Manual Analysis](#) dialog box. The options allow you to fine-tune the properties for a set of components used to model one cell cycle in the model.



Ploidy

This option allows you to select a different ploidy label for the cell cycle. The model template you select in the Edit Properties for Manual analysis dialog determines the default value for this option.

Range labels and results reported on the report are based on the ploidy names assigned to cell cycles.

G2M

There are three options available for the way G2M is modeled: *Disabled*, *Visible peak*, and *Indistinct peak*. When Disabled is selected, no G2M will be modeled for the cycle. Choosing Visible peak allows the program to adjust the mean position of the G2M component for the best fit, the lowest reduced chi-square (RCS). If the peak for G2M is not clearly defined, choose Indistinct peak. With this setting, the position of the G2M is based on the G1 position multiplied by the linearity factor.

S-Phase

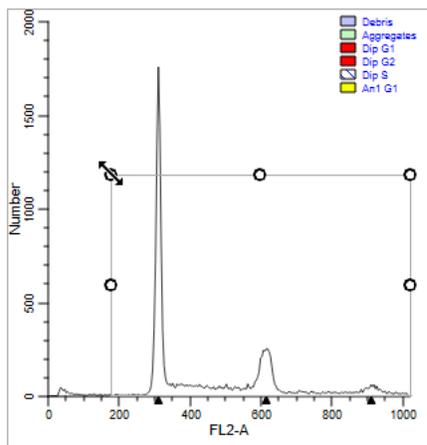
This checkbox allows you to enable or disable the S-Phase component for the cycle. Normally, you should disable an S-Phase if another more important cycle is significantly overlapping this cycle. For example, in the near-Diploid model, the Diploid S-Phase is disabled because the DNA Index (DI) for the Aneuploid cycle is less than 1.3 and therefore the S-Phases are virtually superimposed on one another.

Scale command



Use this command to manually adjust the scaling of the loaded histogram data.

A rectangle encompassing the data is displayed. Sizing handles appear on the edges of the rectangle. Position the mouse over a sizing handle, click and hold the button down, and drag to position a new boundary.



Data below the left boundary and above the right boundary will be excluded from the analysis. Adjusting the top of the scaling rectangle re-scales the data on the Y-axis graphically, but has no effect on the analysis.

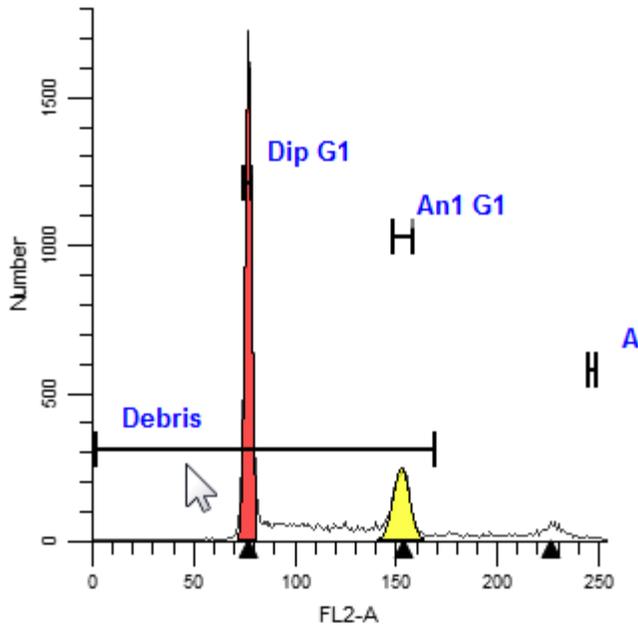
Normally when each new data file is opened, the program will automatically scale the data. See the section on the [Options and Configuration](#) dialog box to learn how to disable the auto-scaling feature.

Range command



Use this command to manually adjust the ranges for the model.

ModFit LT uses specific ranges to identify important parts of a histogram, usually peaks. Automatic Analysis positions ranges for you. However, if you need to correct a range position or if you manually select a model for analysis, you will need to position ranges yourself.



The rule of thumb for positioning ranges is to make sure each range identifies one, unique peak. Ranges should be positioned so that they do not overlap one another. When a range is positioned correctly, you will see the associated portion of the histogram filled in with a color indicating the range estimate.

The exception to the rule for placing ranges is shown in near-Diploid histogram. In these histograms, you may need to place ranges so that they are not centered on the peaks. Instead, move the ranges slightly apart to help differentiate the two, overlapping peaks.

Only move or resize ranges that need to be adjusted. By leaving correctly positioned ranges alone, you help improve user-to-user consistency.

The vertical position of ranges has no impact on the range estimate. You can arrange ranges vertically so that they are easy to read and access.

Ranges and their uses

Debris This range provides an estimate of debris. The left side should be placed at the highest portion of the beginning of debris. The right side should extend approximately two-thirds of the histogram scale. The vertical position has no impact on the estimate.

Std1 This range is used to identify the first internal standard, if there is one. If you have named the internal standard, the name of the range is changed to the name of the standard. Center the range over the first standard peak.

Std2 This range is used to identify the second internal standard, if there is one. If you have named the internal standard, the name of the range is

changed to the name of the standard. Center the range over the second standard peak.

- Apop** This range identifies the apoptosis peak, if you have enabled the apoptosis component. The range should be centered over the peak.
- Dip G1** This range identifies Diploid G0-G1 peak. The range should be centered over the G1peak.
- Dip G2** This range identifies Diploid G2-M peak. The range should be centered over the G2 peak.
- An 1 G1** This range identifies an Aneuploid G0-G1 peak. The first Aneuploid cycle will use An1, the second An2, and so forth. The range should be centered over the G1 peak.
- An 1 G2** This range identifies an Aneuploid G2-M peak. The first Aneuploid cycle will use An1, the second An2, and so forth. The range should be centered over the G2 peak.
- G1** This range is used by the Sync Wizard to identify the Diploid G0-G1 peak. The range should be centered over the peak.
- Parent** This range is used by the Cell Tracking Wizard to identify the Parent population.
- Gen 1** This range is used by the Cell Tracking Wizard to identify a daughter generation population, if the Floating option is selected on the Generations tab of the wizard dialog. Gen1 is the generation closest to the parent, Gen2 is to the left of Gen1, etc.

Fit Diagnostics command



Diags

Use this command to add a report object that displays a summary of the Fit Diagnostics dialog box on the report. The object shows a legend of the elements found in the Fit Diagnostics dialog box. The object can be moved to any convenient location on the report.

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2.96	% CV
23174	Cell Number
309	Avg. Cells Per Channel
n/a	Aneuploid Fraction
4.22	% B.A.D.
3.55	RCS

To edit the properties of the object, double click on the object after it has been inserted into the report.

Fit Diagnostics dialog

This dialog displays information about the current analysis, comparing key aspects with DNA Consensus Conference recommendations and user-defined cutoffs.

If the S-Phase Assessment system is active, the S-Phase Error Analysis for diploid, aneuploid, and total (average) S-Phase computations are shown in the middle portion of the dialog. In DNA Diploid models, the standard deviation for diploid S-Phase is computed. In DNA Aneuploid models, the standard deviations for aneuploid and total (average) S-Phases are computed.

Probability of the S-Phase value falling into a user-defined cutoff category is shown in the listbox at the bottom of the dialog.

Quality of Histogram and Fit			
%CV	2.20	%Aneuploid	1.16
Cell #	11265	%B.A.D.	10.38
Avg Cell #	146	RCS	3.24

Key: Good Fair Poor

S-Phase Error Analysis			
% Diploid S	0.00	+/-	0.00
% Aneuploid S	0.00	+/-	0.00
% Total S	0.00	+/-	0.00

S-Phase Assessment not active.

OK Cancel

When pressed, **%CV**, **Cell #**, **Avg Cell #**, **% Aneuploid**, **%B.A.D.**, and **RCS** buttons display reference text explaining the generally accepted guidelines for each criterion. The

status boxes display green for results in the Good range, yellow for results in the Fair range, and red for Poor results.

In the S-Phase Error Analysis section, you can view error distribution plots by clicking the **% Diploid S**, **% Aneuploid S**, or **% Total S** buttons. Next to the buttons, the computed S-Phase percentage appears with its standard deviation. Values will only be computed if the S-Phase assessment system is active. The [S-Phase cutoffs dialog](#) controls whether the system is active or inactive.

See also:

[Histogram and S-Phase Assessment](#)

[S-Phase cutoffs command \(Edit menu\)](#)

S-Phase Cutoffs



Research and Compliance Mode note:
 In RUO mode, all users can perform these tasks.
 In Compliance mode, only **ModFitAdmins** have permission to perform them.

Use this command to review, edit, and create cutoff ranges for different tissue types. The cutoff ranges you enter are used by the Fit Diagnostics routine to assign S-Phase percentages into low, intermediate, or high classifications.

Refer to the DNA Consensus Conference report (Cytometry 14, p471-500, 1993 (c) Wiley-Liss, Inc.) for information on establishing cutoffs in your laboratory.

S-Phase Cutoffs

Activate Assessment System

Current Tissue: Double click on item to set current tissue.

Tissue	Diploid		Aneuploid		Total	
	Lo->Int	Int->Hi	Lo->Int	Int->Hi	Lo->Int	Int->Hi
Breast	<input type="text" value="5.0"/>	<input type="text" value="10.0"/>	<input type="text" value="10.0"/>	<input type="text" value="20.0"/>	<input type="text" value="10.0"/>	<input type="text" value="20.0"/>
All	5.0	10.0	10.0	10.0	20.0	20.0
Breast	5.0	10.0	10.0	10.0	20.0	20.0
Colon	5.0	10.0	10.0	10.0	20.0	20.0
Prostate	5.0	10.0	10.0	10.0	20.0	20.0

< _____ >

To enable the S-Phase assessment system, check the **Activate Assessment System** checkbox.

To disable the S-Phase assessment system, clear the check from the **Activate Assessment System** checkbox.

To add a tissue cutoff, enter a tissue type in the Tissue edit box. Then enter the thresholds for low-to-intermediate and intermediate-to-high for each ploidy type. Click the **Add** button to add the new type to the list of choices.

To set a tissue type as the **Current Tissue**, double click the entry in the listbox. The current tissue is displayed at the top of the dialog and on the Toolbar in the Tissue type drop-down listbox.

To edit a tissue cutoff, click the listbox entry for the tissue you want to edit. Make changes to the ranges in the edit boxes, and then click **Replace** to update the entry.

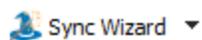
To delete a tissue cutoff, click the listbox entry you want to delete. Then click the **Delete** button.

To accept changes to the dialog, click **OK**.

To discard changes to the dialog, click **Cancel**.

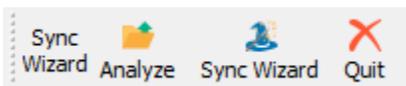
Sync Wizard

Sync Wizard->Create or edit model



The Synchronization Wizard guides you through the process of creating or editing a model to analyze synchronized cell lines. You can initiate the wizard by selecting **Sync Wizard->Create or edit model** from **Home** tab of the ribbon bar. A dialog will appear containing several tabs, each presenting you with choices relative to a particular aspect of the model. The wizard provides full functionality for creating and editing a synchronization model while maintaining a simple interface.

When starting a Sync Wizard session, you should select each tab to adjust any settings to your data. When you press the **Analyze** button, the Wizard dialog will close, the model will be constructed, and the data file analyzed. In addition, the Wizard toolbar will appear at the bottom of the main window, allowing you to open files, return to the Wizard, or exit the Sync Wizard.



If you need to adjust a setting in the model, re-enter the Sync Wizard dialog by clicking the Wizard button on the Wizard toolbar, or selecting **Sync Wizard->Create or edit model**. When editing a model, you only need to review the tabs containing options you want to change. For example, you might view the G0-G1 tab to change the model from "Adjust automatically" to "Lock the position", then choose **Analyze** to close the dialog.

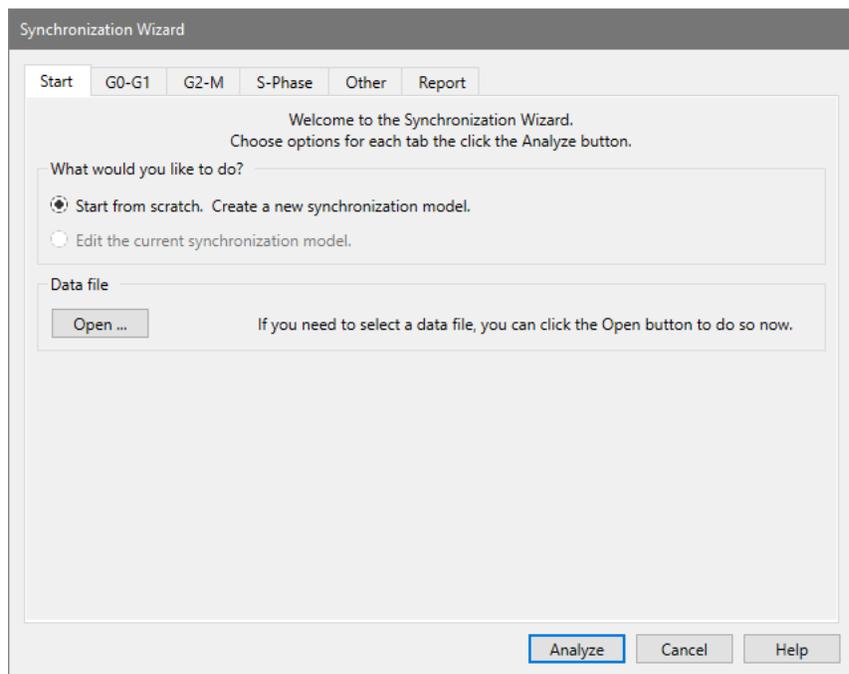
ModFit LT remains in Sync Wizard mode until you quit the Wizard, either by clicking the X on the Wizard toolbar, by selecting **Sync Wizard->Quit**.

You can save an active Sync Wizard session in a report to preserve your settings for the synchronization model you create.

Sync Wizard: Start tab

You decide whether to create a model, or edit the one you already created on this tab. The option to edit is disabled if the Sync Wizard is not already running. Once a model has been created, you can re-enter the Wizard to edit the model's settings.

Use the **Open** button to select a data file, if you haven't already selected the file you want to start with.



Sync Wizard: G0G1 tab

The purpose of this tab is to allow you to identify the G0-G1 peak. A graphic of the currently loaded data file is displayed with a cursor used to identify the position of the G0-G1.

The program will position the cursor automatically over the first peak in the sample. If this is not the correct peak for your analysis, position the G0-G1 peak cursor by clicking on the red triangle at the top of the graphic and dragging it over the peak. Alternately, you may type a channel value into the **G0-G1 position** edit box.

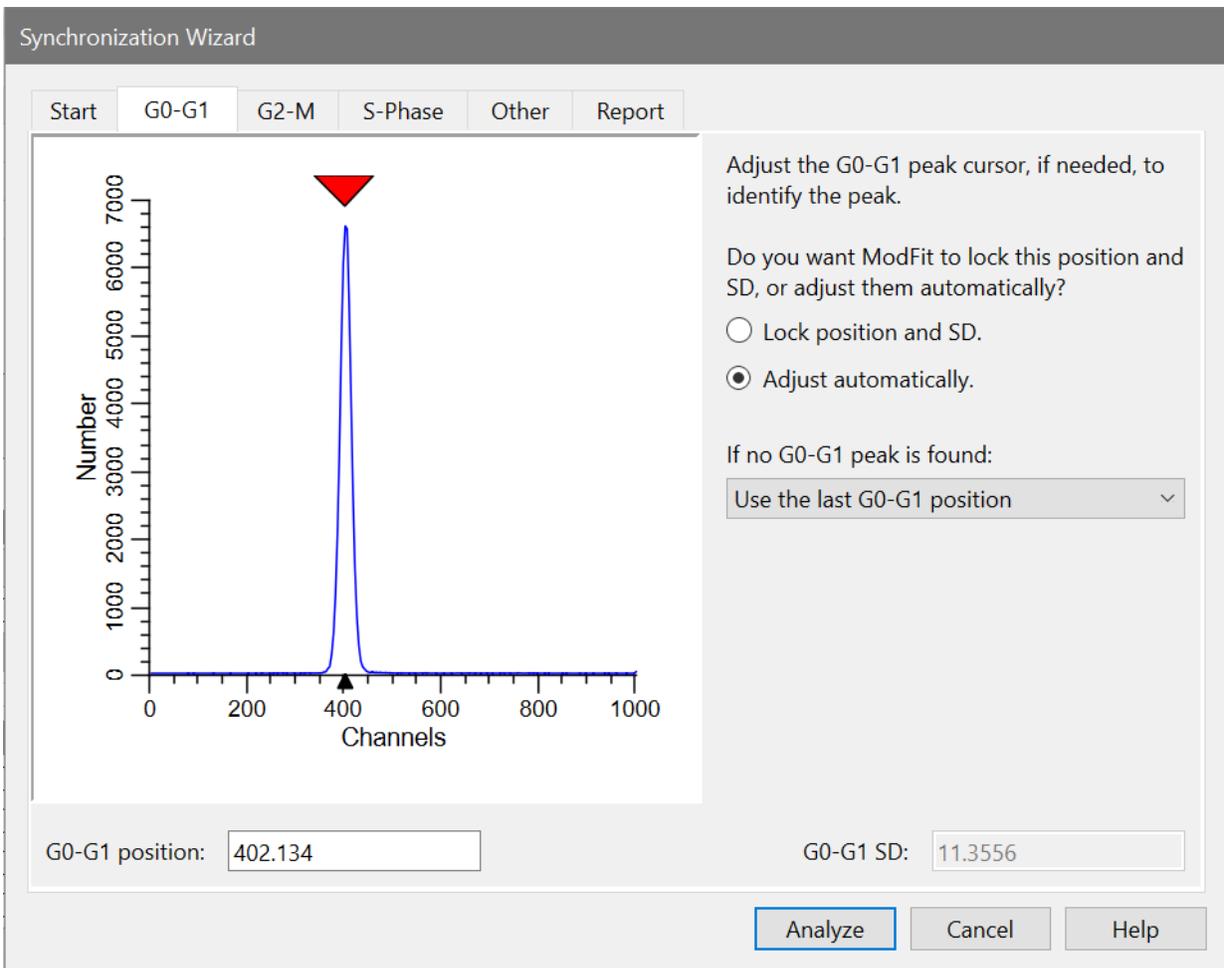
When you close the Wizard dialog by choosing **Analyze** and you are creating a model from scratch, ModFit LT will position the G0G1 range around the channel position you indicate. The range is placed so that it encompasses several channels to the left and right of the peak position.

When editing a model, the Sync Wizard does not adjust the range position on the data. You can manually adjust the range by clicking the Range button on the toolbar and moving or resizing the G0G1 range.

The Sync Wizard allows you to "lock" the channel position, or to let the program adjust the position within the G0G1 range. When you choose **Lock the position**, ModFit LT will not try to find a better peak position. This setting is useful in case where the G0-G1 peak is indistinct or not present. You must also specify a standard deviation for the peak with this option.

Choosing **Adjust automatically** allows the program to look for a peak within the range of channels encompassed by the G0G1 range, initially plus or minus 8 channels from the identified peak position. If the program cannot locate a peak within the G0G1 range, the [Sync Wizard Adjustment dialog](#) is displayed with options on how to adjust the model.

Two options are provided for the case where no G0-G1 peak is found in a sample. **Use the last G0-G1 position** causes the program to use the last known G0-G1 location if it can't find a peak. **Prompt for action** displays the [Sync Wizard Adjustment dialog](#) so that the user can be alerted to the issue and decide what to do when it happens.



Sync Wizard: G2M tab

The purpose of this tab is to allow you to identify the G2/G1 ratio. This ratio is used to determine the position of the G2-M peak relative to the G0-G1 peak. A graphic of the currently loaded data file is displayed with a cursor used to identify the position of the G2M peak.

You may adjust the G2/G1 ratio three ways: by entering a new ratio in the **G2/G1 ratio** edit box, by entering a channel position in the **G2-M position** edit box, or by dragging the red triangle cursor over the G2-M position on the graphic.

The model created by the Sync Wizard will multiply the G0-G1 peak position and standard deviation by the G2/G1 ratio appearing on this tab to compute the G2-M mean position and standard deviation. The position of the G2-M peak is always, therefore, dependent on the position of the G0G1 peak. Similarly, the standard deviation of the G2-M peak is dependent on the standard deviation of the G0G1 peak.

An option is provided to perform **Auto-Linearity** on the sample. When enabled, the program will attempt to determine the best G2/G1 ratio for each sample.

The screenshot shows the 'Synchronization Wizard' window with the 'G2-M' tab active. The main plot area displays a histogram with 'Number' on the y-axis (0 to 7000) and 'Channels' on the x-axis (0 to 1000). A single sharp peak is visible at approximately 400 channels. Below the plot, the 'G2-M position' is set to 804.269 and the 'G2/G1 ratio' is set to 2. The 'Auto-Linearity' checkbox is unchecked. The 'Analyze' button is highlighted in blue.

Sync Wizard: S-Phase tab

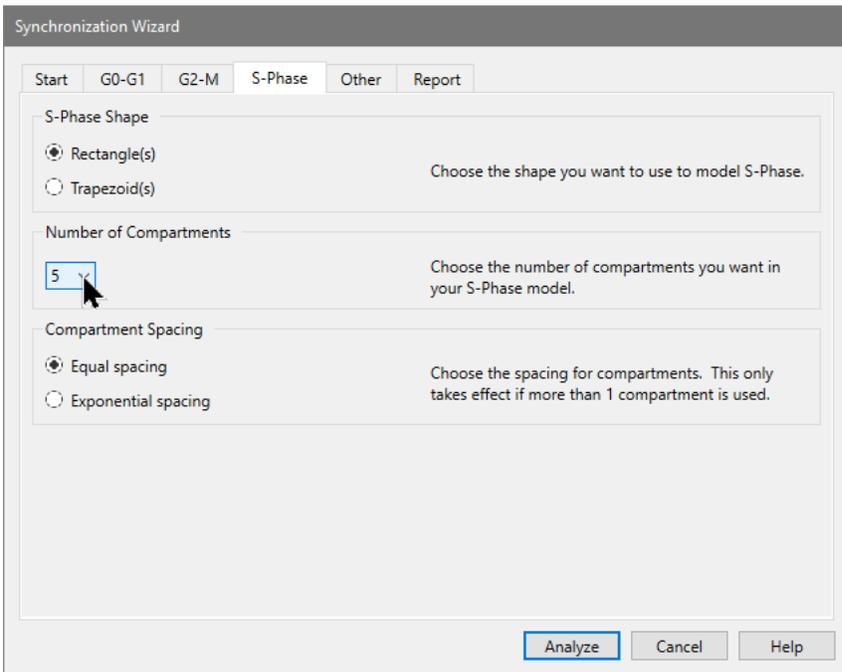
The purpose of this tab is to allow you to control the aspects of modeling S-Phase.

You may adjust the shape used for S-Phase by selecting either **Rectangle** or **Trapezoid**.

The **Number of Compartments** section allows you to pick how many trapezoids or rectangles you want to use. By choosing more compartments you allow the S-Phase portion of the model more freedom to conform to your data. However, giving too much freedom may allow the S-Phase portion of the model to take over data that truly belong to G0-G1 or G2-M.

If you use more than one compartment in S-Phase, you can choose the spacing of compartments. Choosing **Equal spacing** distributes the compartments evenly between G0-

G1 and G2-M populations. Choosing **Exponential spacing** causes compartments to increase exponentially in size.

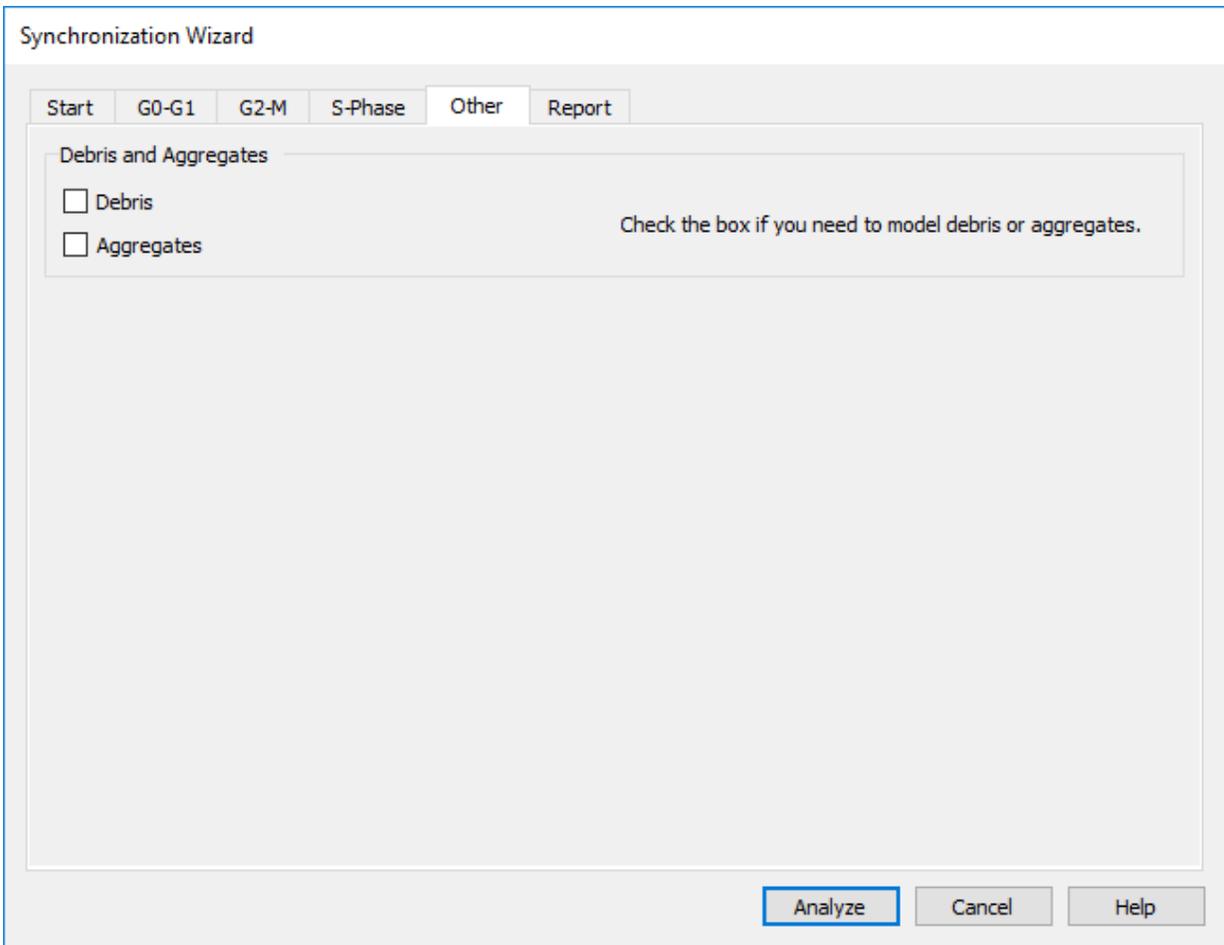


Sync Wizard: Other tab

The purpose of this tab is to allow you select miscellaneous options related to the synchronization model you are creating.

If you need to perform debris subtraction, enable the **Debris** checkbox. The Sync Wizard will add a debris component appropriate for fresh and frozen samples.

If your samples contain aggregates that you want to model, enable the **Aggregates** checkbox. The Sync Wizard will add an aggregate compensation component to the model.



Sync Wizard: Report tab

The purpose of this tab is to allow you to control what information is reported by the Sync Wizard model.

For Percent report options, you may choose to report percentages for all components and sub-compartments, or only the primary components.

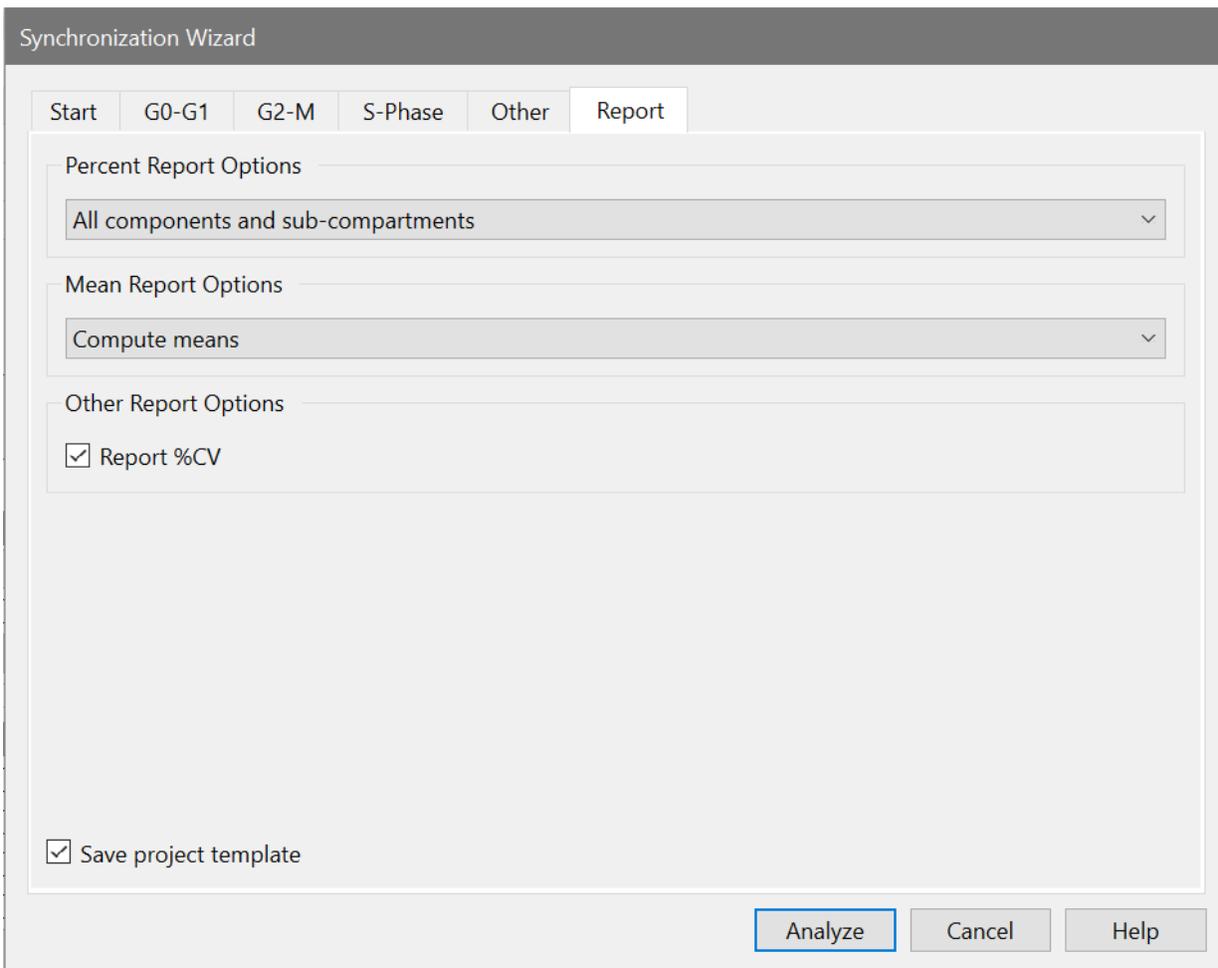
For Mean report options, you may choose whether or not to report means.

You can include information derived from the data file keywords and other demographic information by selecting "Show demographics".

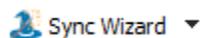
If you would like to display the %CV for the G0-G1 peak, enable the **Report %CV** check box.

The first time the wizard is run for a project, it will automatically save a report template. The template is opened automatically when the project is reopened. If the wizard is run again after the initial setup, the **Save project template** checkbox will be displayed on the Report

tab of the wizard. Check the option to update the saved report template, or uncheck it if you do not want to save the changes to the wizard.



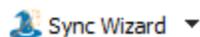
Sync Wizard->Open Data and Analyze



Use this command to open a data file into ModFit LT and analyze the file with the model created by the Synchronization Wizard. The standard File Open dialog is displayed for selection of a file or files. After selection, the program loads and analyzes the first file.

The batch toolbar can be used to advance through and process a set of files in the batch. This toolbar appears at the bottom left of the main window. See [Batch Processing](#) for additional details.

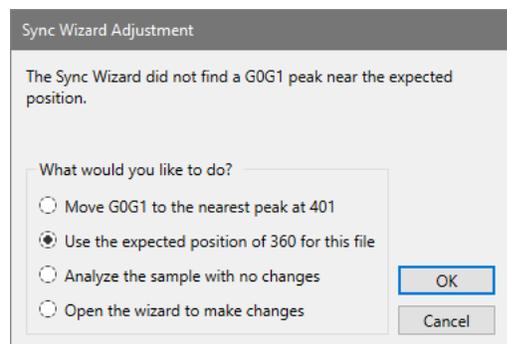
Sync Wizard->Quit



Use this command to exit the Synchronization Wizard. The Wizard toolbar will be hidden.

Sync Wizard Adjustment dialog

This dialog is displayed when the Sync Wizard model is set to adjust the position of the G0-G1 peak automatically but there is no peak found under the G0-G1 range.



The Wizard offers several options:

Move G0G1 to the nearest peak: This choice tells the wizard to move to the nearest peak.

Use the expected position: This choice tells the wizard to use the last-known position of the G0G1 peak.

Analyze the sample with no changes: This choice tells the wizard to continue, even though it has not found a peak.

Open the wizard to make changes: This choice opens the Sync Wizard so that you can make changes.

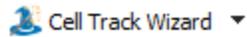
If the sample has a visible G0-G1 peak (identified by the peak finder with a black triangle) on the graphic, you can choose to let the Sync Wizard move the range. It is likely that the position has shifted from one sample to the next, and it is safe to keep the model in "Adjust automatically" mode.

If the peak suggested by the dialog is *not* the G0-G1 population, you should choose to lock the G0-G1 position in its last location. In synchronization experiments, the G0-G1 population can disappear in the course of the treatment. Locking the position to a previously identified value is the best way to handle these samples.

You can choose to analyze with no changes if you are confident that the model is still appropriate for the data. Alternatively, you can open the wizard to make adjustments yourself.

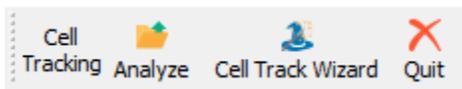
Cell Tracking Wizard

Cell Tracking Wizard->Create or Edit Model



The Cell Tracking Wizard guides you through the process of creating or editing a model to analyze cell tracking dye samples. You can initiate the wizard by selecting **Cell Tracking Wizard->Create or edit model** from the **Home** tab of the ribbon bar. A dialog will appear containing tabs, each presenting you with choices relative to a particular aspect of the model. The wizard provides full functionality for creating and editing a Cell Tracking model while maintaining a simple interface.

When starting a Cell Tracking Wizard session, you should select each tab to adjust any settings to your data. When you press the **Analyze** button, the Wizard dialog will close, the model will be constructed, and the data file analyzed. In addition, the Wizard toolbar will appear at the bottom of the main window, allowing you to open files, return to the Wizard, or exit the Cell Tracking Wizard.



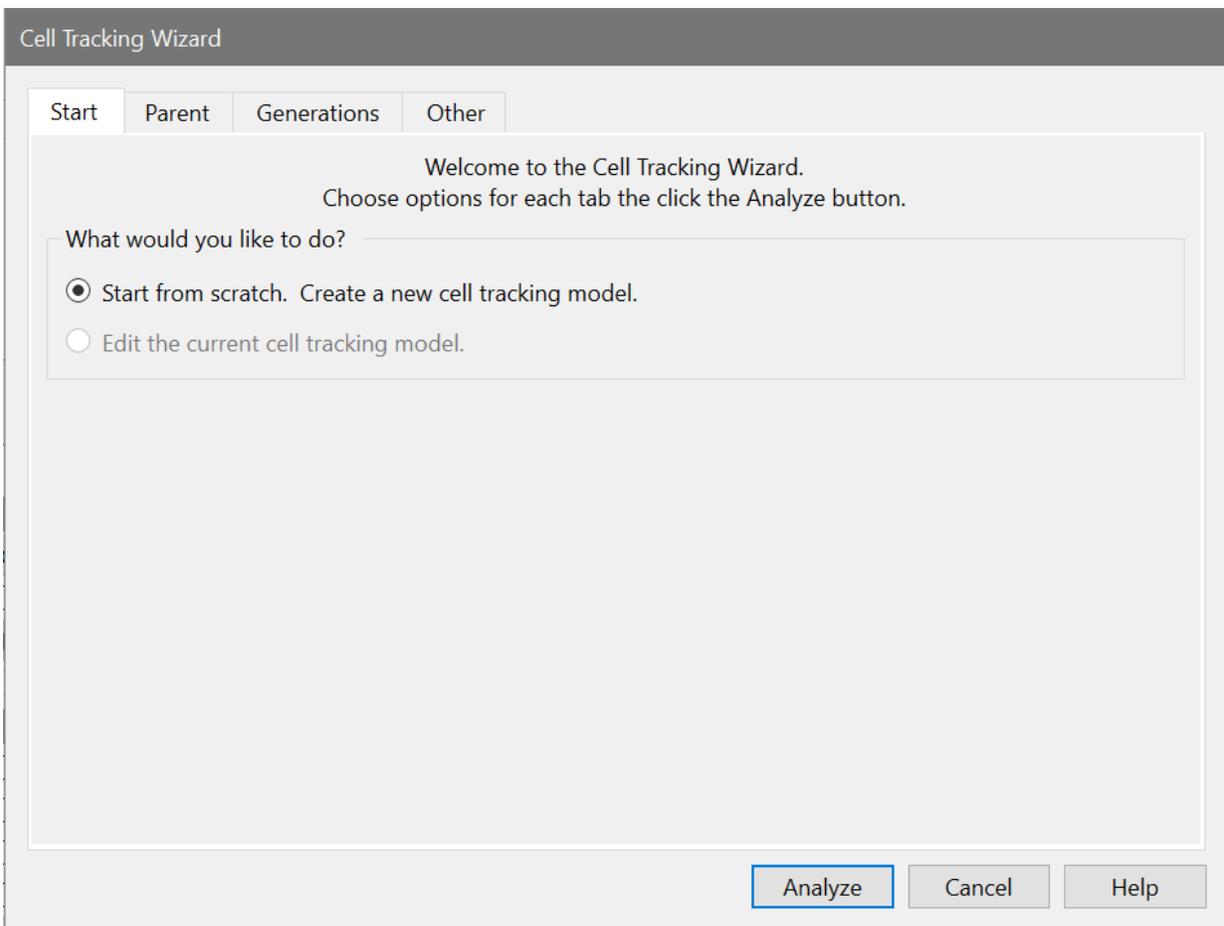
If you need to adjust a setting in the model, re-enter the Cell Tracking Wizard dialog by clicking the Wizard button on the Wizard toolbar. When editing a model, you only need to review the tabs containing options you want to change.

ModFit LT remains in Cell Tracking Wizard mode until you quit the Wizard, either by clicking the X on the Wizard toolbar.

You can save an active Cell Tracking Wizard session in a report to preserve your settings for the Cell Tracking model you create.

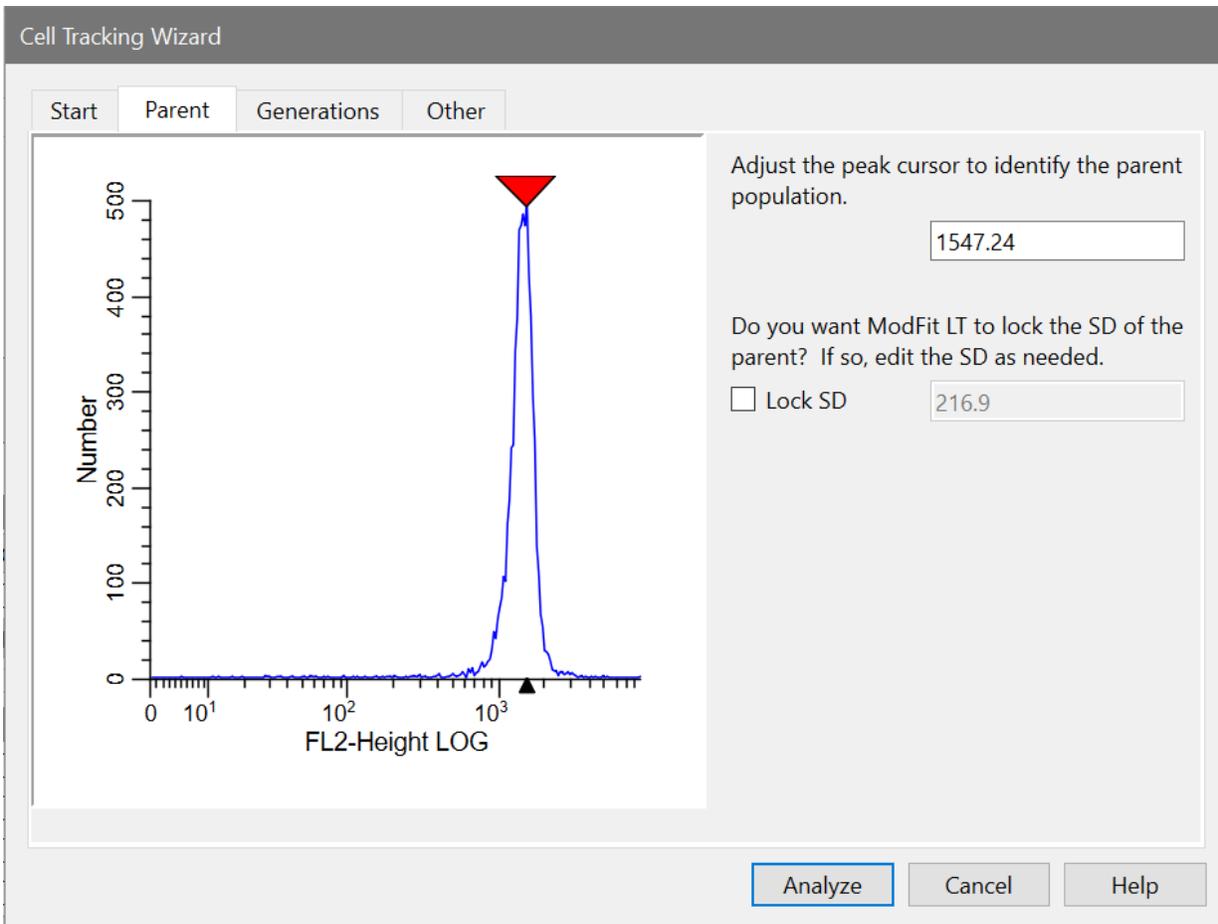
Cell Tracking Wizard: Start tab

You decide whether to create a model, or edit the one you already created on this tab. The option to edit is disabled if the Cell Tracking Wizard is not already running. Once a model has been created, you can re-enter the Wizard to edit the model's settings.



Cell Tracking Wizard: Parent tab

This tab allows you to locate the peak that represents the parent population in the sample. The program automatically positions the cursor over the right-most peak in the histogram. If this is not the correct peak for your analysis, position the parent peak cursor by clicking on the red triangle at the top of the graphic and dragging it over the peak.

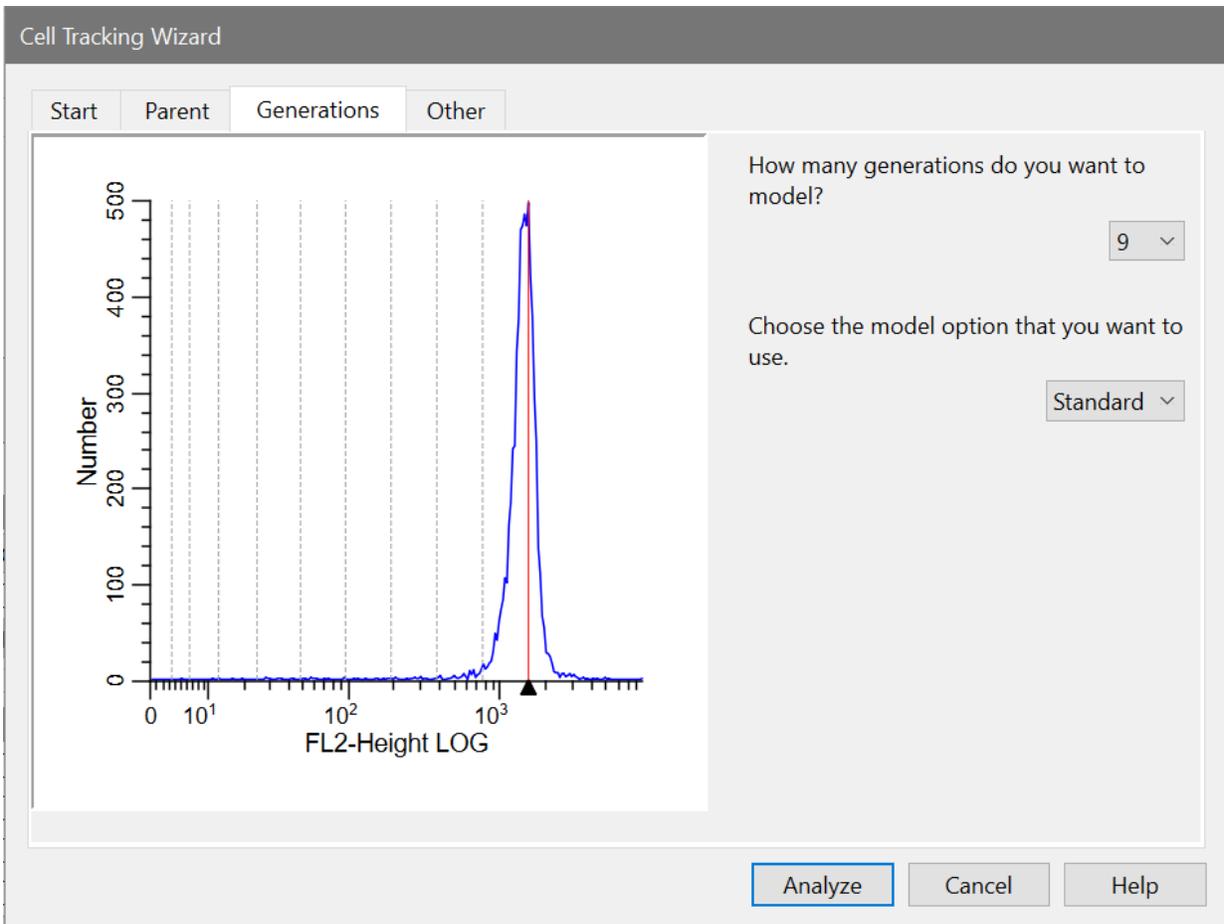


Alternately, you may type a channel value into the **Parent** channel edit box.

This tab also allows you to "lock" the standard deviation (SD) of the Gaussian component used to model the parent population. The **Lock SD** option is most useful when analyzing samples in which there is a great deal of proliferation. In these cases, the parent population is often difficult for the model to resolve, and the modeling process may broaden the SD of the parent too much. When this option is enabled, the SD value can be edited.

Cell Tracking Wizard: Generations tab

This tab displays the information about how the generations will be modeled.



There is an editor for the number of **generations** to model. The number of generations should be based on the range between the parent population and the background of the measurement. For example, if the parent has a mean of 1000 and an unstained population has a mean of 10, the maximum number of discernible generations is five, with means at 500, 250, 125, 62.5, 31.25, and 15.625. The variability of the populations also has an impact on when the last generation will no longer be discernible from background.

A dropdown box is provided to select between the **Standard** and **Floating** option for modeling generations.

In the Standard mode, the mean position of each generation is dependent on the position of the parent generation. Each generation is positioned at half the intensity of the previous generation.

The floating option allows the mean of each generation to be computed independently of the other populations. This option provides much greater flexibility to the model and works well for samples with visually distinct generations. The floating option is *not* recommended for samples where generational boundaries are not obvious to the eye.

The graphic displays a red line where the parent generation is positioned and dotted lines for each generation.

Cell Tracking Wizard: Other tab

The purpose of this tab is to allow you select miscellaneous options related to the cell tracking model you are creating.

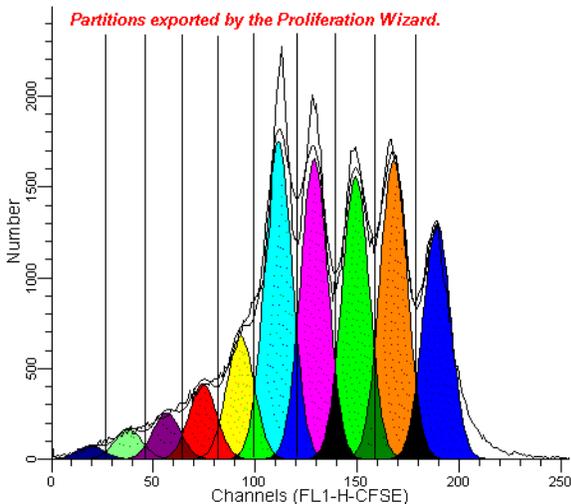
The **Cell Tracking Options** group allows you to identify the generation that is considered the start of true proliferation. This value is used in computing the Precursor Frequency and the Upper Generation Proliferation Index (UGPI). For a description of the use of Precursor Frequency, see "A flow cytometric method to estimate the precursor frequencies of cells proliferating in response to specific antigens", Givan, A., et al, Journal Of Immunological Methods Vol. 230, 1999 Elsevier Science B.V.

If you need to perform background subtraction, enable the **Background** checkbox. The Cell Tracking Wizard will add a rectangle to model background.

When you enable Background subtraction, another option, **Asymmetric cytokinesis**, becomes available. With this option checked, the standard deviations of the generations are broadened by interpolation along the background rectangle. If this option is not enabled, all generations have the same standard deviation as the parent population.

With the Asymmetric cytokinesis option enabled, the program sets the name of the model to "Cell Tracking Wizard Cytokinesis Model". If the option is not checked, the model name is "Cell Tracking Wizard Basic Model".

Two export options can be enabled for the Cell Tracking Wizard. When **Export partition table** is enabled, the program will save a text file containing the "boundaries" of the generations. These are the points of intersection of the adjacent generations, illustrated below.

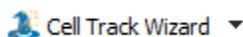


The export file is created each time the wizard analyzes a data file. By default, the export file name is *ProlifWizardPartitions.txt*. If you enable the **Prompt for file name** option, the wizard will display a File Save dialog before storing the partition file, allowing you to choose a file name and location.

The partition file can be imported into other applications, like WinList, for further analysis. It is a simple, text format file that is readable by spreadsheets, databases, and word processors.

The first time the wizard is run for a project, it will automatically save a report template. The template is opened automatically when the project is reopened. If the wizard is run again after the initial setup, the **Save project template** checkbox will be displayed on the Other tab of the wizard. Check the option to update the saved report template, or uncheck it if you do not want to save the changes to the wizard.

Cell Tracking Wizard->Open Data and Analyze

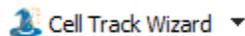


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Use this command to open a data file into ModFit LT and analyze the file with the model created by the Cell Tracking Wizard. The standard File Open dialog is displayed for selection of a file or files. After selection, the program loads and analyzes the first file.

The batch toolbar can be used to advance through and process a set of files in the batch. This toolbar appears at the bottom left of the main window. See [Batch Processing](#) for additional details.

Cell Tracking Wizard->Quit



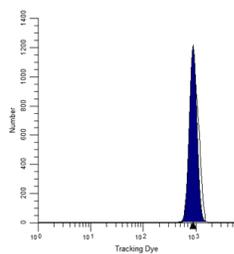
Use this command to exit the Cell Tracking Wizard. The Wizard toolbar will be hidden.

Cell Tracking Example

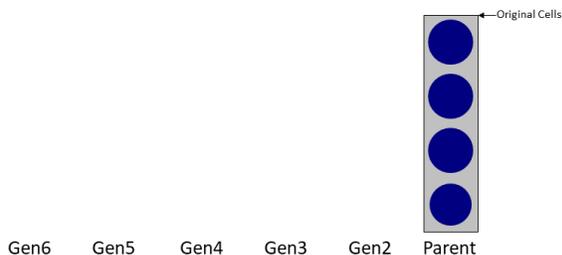
Here is an example of a cell-tracking study that helps to explain the results that the Cell Tracking Wizard displays.

Suppose that we take samples at three time points: T=0, T=3h, and T=6h. For this simple cell tracking example, we will start at time T=0 with four cells stained for cell tracking.

For the population of cells at T=0, here's what we might see, and some example calculations for cell tracking equations.



Initial Cell Culture, Time T=0

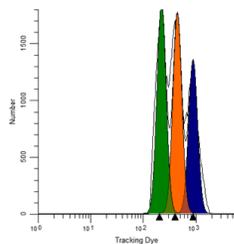


Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=1}^n N_i$	4+0+0+0+0	4
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^n N_i$	0+0+0+0	0
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	0/2+0/4+0/8+0/16+0/32	0
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=1}^n \frac{N_i}{2^{i-1}}$	4/1+0/2+0/4+0/8+0/16+0/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=1}^n N_i} * 100$	(0+0+0+0+0)/(4+0+0+0+0)*100	0
Precursor Frequency; g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	(0/4+0/8+0/16+0/32)/(4/1+0/2+0/4+0/8+0/16+0/32)	0
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=1}^n N_i}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	(4+0+0+0+0)/(4/1+0/2+0/4+0/8+0/16+0/32)	1
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	(0+0+0+0+0)/(0/2+0/4+0/8+0/16+0/32)	N/A
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=1}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	(1*0/2+2*0/4+3*0/8+4*0/16+5*0/32)/(0/2+0/4+0/8+0/16+0/32)	N/A
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=1}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	(0*4/1+1*0/2+2*0/4+3*0/8+4*0/16+5*0/32)/(4/1+0/2+0/4+0/8+0/16+0/32)	0
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=1}^n \sum_{j=1}^{i-1} \frac{N_i}{2^{i-1}}$	((0/2)+(0/2+0/4)+(0/2+0/4+0/8)+(0/2+0/4+0/8+0/16)+(0/2+0/4+0/8+0/16+0/32))/(0+0+0+0+0)	N/A
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	4/(4/1+0/2+0/4+0/8+0/16+0/32)	1
Upper Generation PI; g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	(0+0+0+0)/(0/4+0/8+0/16+0/32)	N/A

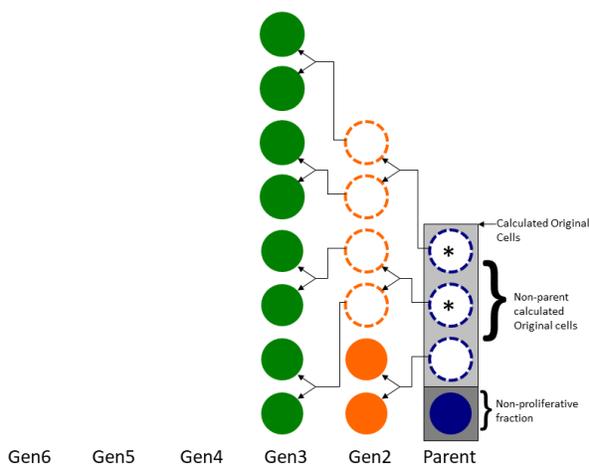
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We can now look at the sample at time T=3 and compute the same equations on the current population of cells. Note that the filled in circles above represent cells that currently exist and the unfilled, dashed circles are cells that have divided into two daughter cells.

* Note, the circles containing an asterisk are those that went on to “true” proliferation. True proliferation is defined by g, the index of the user defined generation where true proliferation began. In this example, “true” proliferation begins at generation 3. Cells that remain in the parent or generation 2 did not truly proliferate.



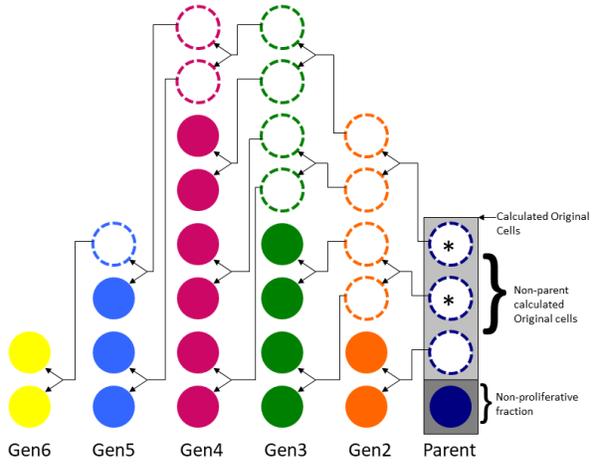
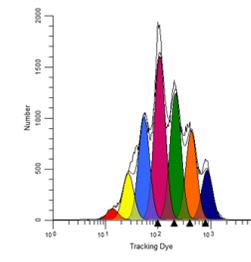
Time Point 3, T=3



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=1}^g N_i$	1+2+8+0+0	11
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^g N_i$	2+8+0+0	10
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^g \frac{N_i}{2^{i-1}}$	2/2+8/4+0/8+0/16+0/32	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=1}^g \frac{N_i}{2^{i-1}}$	1/1+2/2+8/4+0/8+0/16+0/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^g N_i}{\sum_{i=1}^g N_i} * 100$	(2+8+0+0)/(1+2+8+0+0) * 100	90.9
Precursor Frequency; g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=3}^g \frac{N_i}{2^{i-1}}}{\sum_{i=1}^g \frac{N_i}{2^{i-1}}}$	(8/4+0/8+0/16+0/32)/(1/1+2/2+8/4+0/8+0/16+0/32)	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=1}^g N_i}{\sum_{i=1}^g \frac{N_i}{2^{i-1}}}$	(1+2+8+0+0)/(1/1+2/2+8/4+0/8+0/16+0/32)	2.75
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^g N_i}{\sum_{i=2}^g \frac{N_i}{2^{i-1}}}$	(2+8+0+0)/(2/2+8/4+0/8+0/16+0/32)	3.33
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=1}^g (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=1}^g \frac{N_i}{2^{i-1}}}$	(1*2+2*8+4*3+0/8+4*0/16+5*0/32)/(2/2+8/4+0/8+0/16+0/32)	1.67
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=1}^g (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=1}^g \frac{N_i}{2^{i-1}}}$	(0*1+1*2+2*8+4*3+0/8+4*0/16+5*0/32)/(1/1+2/2+8/4+0/8+0/16+0/32)	1.25
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=2}^g \sum_{j=1}^{i-1} \frac{N_j}{2^{j-1}}$	((2/2)+(8/8+4/4)+(0/2+0/4+0/8)+(0/2+0/4+0/8+0/16)+(0/2+0/4+0/8+0/16+0/32))	7
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=1}^g \frac{N_i}{2^{i-1}}}$	1/(1/1+2/2+8/4+0/8+0/16+0/32)	0.25
Upper Generation PI; g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^g N_i}{\sum_{i=g}^g \frac{N_i}{2^{i-1}}}$	(8+0+0)/(8/4+0/8+0/16+0/32)	4

Here is our final time point T=6. Note that the filled in circles above represent cells that currently exist and the unfilled, dashed circles are cells that have divided into two daughter cells.

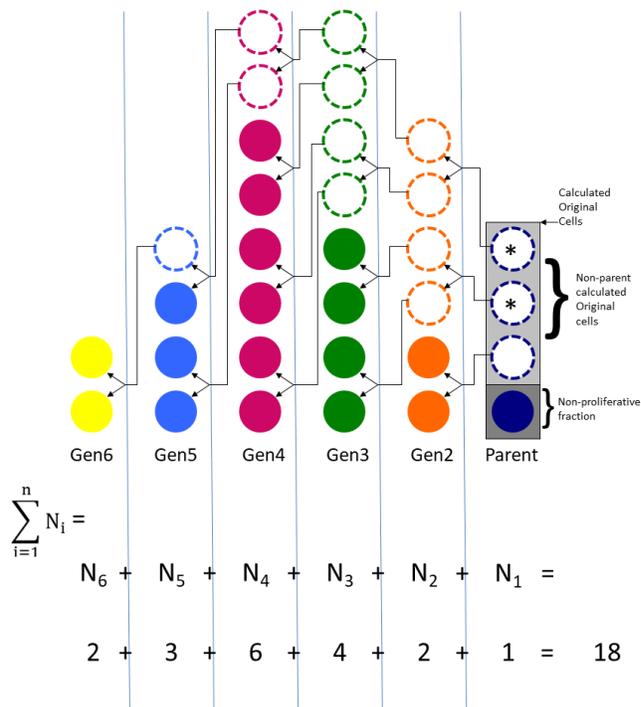
Time Point 6, Time T=6



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=1}^g N_i$	$1+2+4+6+3+2$	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^g N_i$	$2+4+6+3+2$	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^g \frac{N_i}{2^{i-1}}$	$2/2+4/4+6/8+3/16+2/32$	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=1}^g \frac{N_i}{2^{i-1}}$	$1/1+2/2+4/4+6/8+3/16+2/32$	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^g N_i}{\sum_{i=1}^g N_i} * 100$	$(2+4+6+3+2)/(1+2+4+6+3+2) * 100$	94.4
Precursor Frequency; g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=2}^g \frac{N_i}{2^{i-1}}}{\sum_{i=1}^g \frac{N_i}{2^{i-1}}}$	$(4/4+6/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=1}^g N_i}{\sum_{i=1}^g \frac{N_i}{2^{i-1}}}$	$(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)$	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^g N_i}{\sum_{i=2}^g \frac{N_i}{2^{i-1}}}$	$(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=2}^g (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=2}^g \frac{N_i}{2^{i-1}}}$	$(1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(2/2+4/4+6/8+3/16+2/32)$	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=1}^g (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=1}^g \frac{N_i}{2^{i-1}}}$	$(0*1/1+1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=1}^g \frac{N_i}{2^{i-1}}$	$((2/2)+(4/2+4/4)+(6/2+6/4+6/8)+(3/2+3/4+3/8+3/16)+(2/2+2/4+2/8+2/16+2/32))$	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=1}^g \frac{N_i}{2^{i-1}}}$	$1/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.25
Upper Generation PI; g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^g N_i}{\sum_{i=g}^g \frac{N_i}{2^{i-1}}}$	$(4+6+3+2)/(4/4+6/8+3/16+2/32)$	7.5

The number of Modeled Cells is dependent on the number of cells in the original culture and the amount of cell division. The number of modeled cells will increase with proliferation. This value is used mostly for computing other cell tracking statistics.

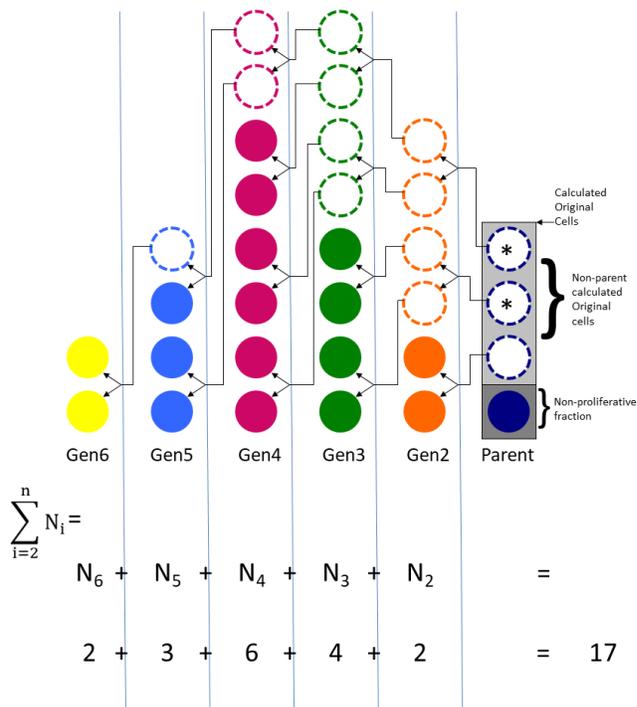
Modeled Cells



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=1}^n N_i$	1+2+4+6+3+2	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^n N_i$	2+4+6+3+2	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	2/2+4/4+6/8+3/16+2/32	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=1}^n \frac{N_i}{2^{i-1}}$	1/1+2/2+4/4+6/8+3/16+2/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=1}^n N_i} * 100$	$(2+4+6+3+2)/(1+2+4+6+3+2) * 100$	94.4
Precursor Frequency; g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(4/4+5/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=1}^n N_i}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)$	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=2}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(2/2+4/4+6/8+3/16+2/32)$	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=1}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(0*1/1+1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(1/1+2/2+8/4+0/8+0/16+0/32)$	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=1}^n \sum_{j=1}^{i-1} \frac{N_i}{2^{i-1}}$	$((2/2)+16/2+4/4)+16/2+6/4+6/8+(3/2+3/4+3/8+3/16)+12/2+2/4+2/8+2/16+2/32)$	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	1/(1/1+2/2+4/4+6/8+3/16+2/32)	0.25
Upper Generation PI; g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(4+6+3+2)/(4/4+6/8+3/16+2/32)$	7.5

Non-parent Cells are modeled cells not in the parent generation. The number of Non-parent cells will be zero in the initial culture and will increase with proliferation. This value will always be less than Modeled Cells. This value is used mostly for computing other cell tracking statistics.

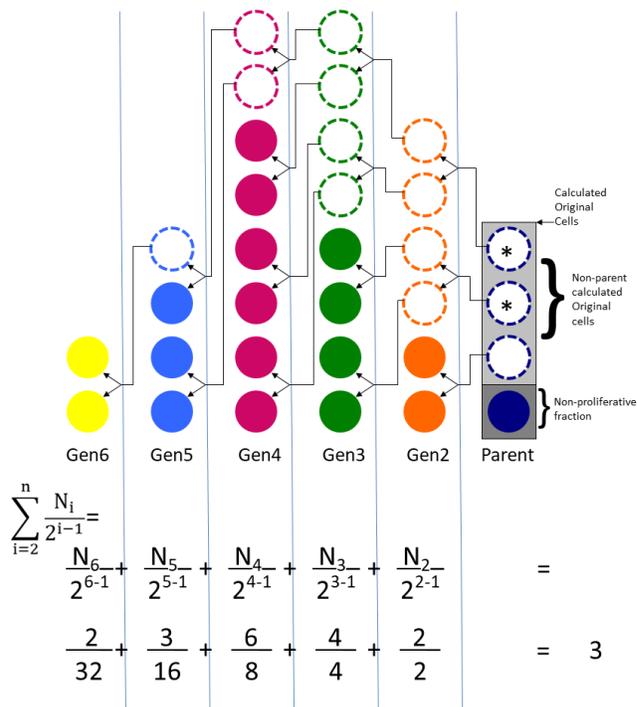
Non-parent Cells



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=2}^n N_i$	1+2+4+6+3+2	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^n N_i$	2+4+6+3+2	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	2/2+4/4+6/8+3/16+2/32	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	1/1+2/2+4/4+6/8+3/16+2/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n N_i} \cdot 100$	$(2+4+6+3+2)/(1+2+4+6+3+2) \cdot 100$	94.4
Precursor Frequency; g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(4/4+6/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)$	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=2}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1 \cdot 2/2+2 \cdot 4/4+3 \cdot 6/8+4 \cdot 3/16+5 \cdot 2/32)/(2/2+4/4+6/8+3/16+2/32)$	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=2}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(0 \cdot 1/1+1 \cdot 2/2+2 \cdot 4/4+3 \cdot 6/8+4 \cdot 3/16+5 \cdot 2/32)/(1/1+2/2+8/8+0/16+0/32)$	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	$((2/2)+1+6/2+4/4)+1+6/2+6/4+6/8+(3/2+3/4+3/8+3/16)+1+2/2+2/4+2/8+2/16+2/32)$	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	1/(1/1+2/2+4/4+6/8+3/16+2/32)	0.25
Upper Generation PI; g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(4+6+3+2)/(4/4+6/8+3/16+2/32)$	7.5

Non-parent Calculated Original Cells are the cells in the original culture that divided and gave rise to the modeled Non-parent Cells. The number of Non-parent Calculated Original Cells will be zero in the initial culture and will always be less than the Calculated Original Cells. This value is used mostly for computing other cell tracking statistics.

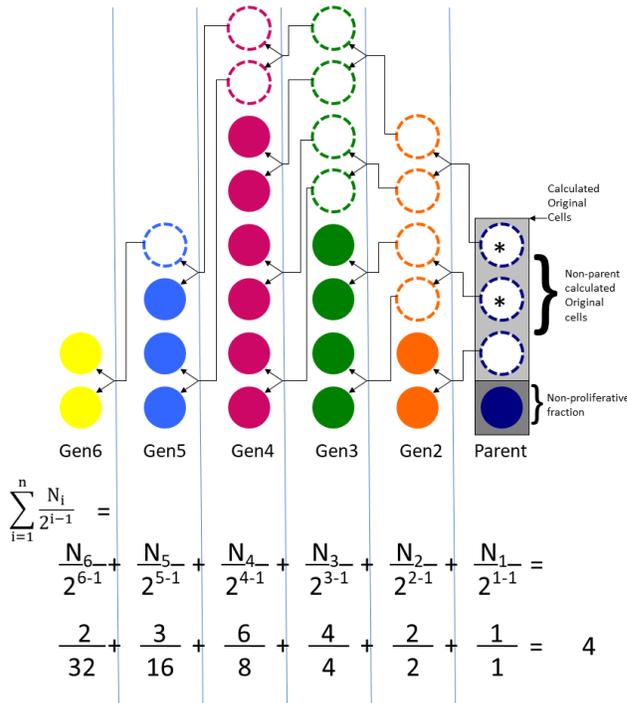
Non-parent Calculated Original Cells



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=2}^n N_i$	$1+2+4+6+3+2$	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^{g-1} N_i$	$2+4+6+3+2$	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	$2/2+4/4+6/8+3/16+2/32$	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	$1/1+2/2+4/4+6/8+3/16+2/32$	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n N_i} * 100$	$(2+4+6+3+2)/(1+2+4+6+3+2) * 100$	94.4
Precursor Frequency: g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(4/4+5/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)$	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^{g-1} N_i}{\sum_{i=2}^{g-1} \frac{N_i}{2^{i-1}}}$	$(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=2}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(2/2+4/4+6/8+3/16+2/32)$	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=2}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(0*1/1+1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(1/1+2/2+8/8+4/4+0/16+0/32)$	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	$((2/2)+16/2+4/4)+16/2+6/4+6/8+(3/2+3/4+3/8+3/16)+12/2+2/4+2/8+2/16+2/32)$	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$1/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.25
Upper Generation PI: g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(4+6+3+2)/(4/4+6/8+3/16+2/32)$	7.5

Calculated Original Cells are the cells in the original culture, both those that divided and those that did not. The number of Calculated Original Cells will be the same as Modeled Cells in the initial culture. This value is used mostly for computing other cell tracking statistics.

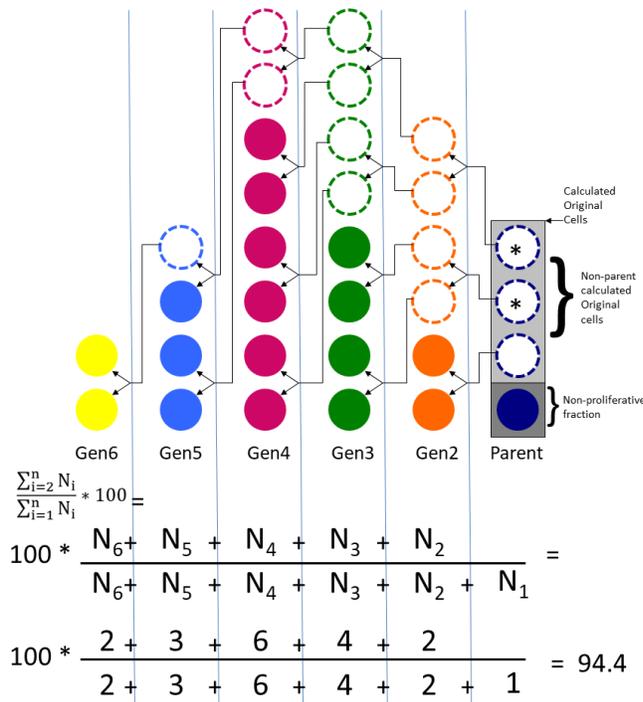
Calculated Original Cells



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=1}^n N_i$	1+2+4+6+3+2	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^n N_i$	2+4+6+3+2	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	2/2+4/4+6/8+3/16+2/32	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	1/1+2/2+4/4+6/8+3/16+2/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=1}^n N_i} * 100$	(2+4+6+3+2)/(1+2+4+6+3+2)*100	94.4
Precursor Frequency: g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	(4/4+6/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=1}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=2}^n (i-1) * \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	(1*2+2*2+4*4+3*6+6*8+3*16+5*2/32)/(2/2+4/4+6/8+3/16+2/32)	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=2}^n (i-1) * \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	(0*1+1*1+2*2+2*4+3*6+6*8+3*16+5*2/32)/(1/1+2/2+8/4+0/8+0/16+0/32)	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	((2/2)+4/4+6/8+3/16+2/2+2/4+2/8+2/16+2/32)	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	1/(1/1+2/2+4/4+6/8+3/16+2/32)	0.25
Upper Generation PI: g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	(4+6+3+2)/(4/4+6/8+3/16+2/32)	7.5

Percent Divided is the percent of cells in the final culture that divided at least once. The Percent Divided will be zero in the initial culture and will increase to at most 100%.

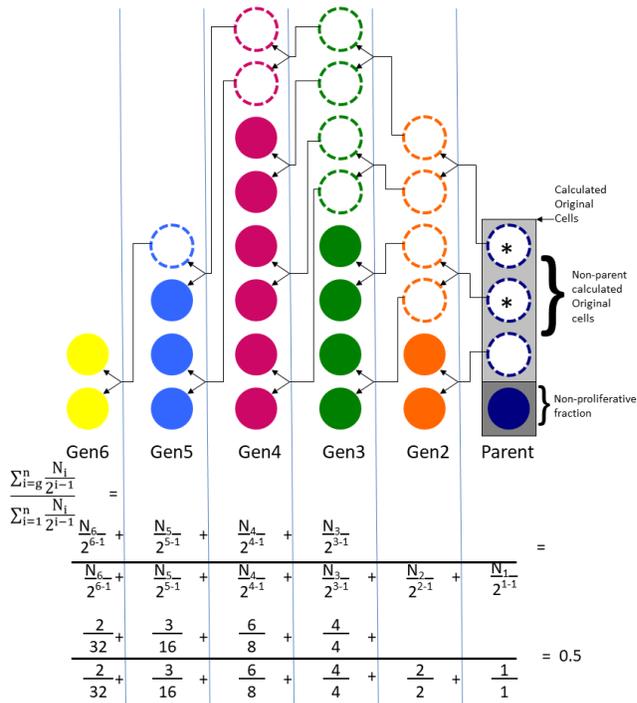
Percent Divided



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=1}^n N_i$	1+2+4+6+3+2	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^n N_i$	2+4+6+3+2	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	2/2+4/4+6/8+3/16+2/32	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	1/1+2/2+4/4+6/8+3/16+2/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=1}^n N_i} * 100$	(2+4+6+3+2)/(1+2+4+6+3+2)*100	94.4
Precursor Frequency: g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	(4/4+6/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=1}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=2}^n (i-1) * \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	(1*2+2*2+4*4+3*6+6*8+3*16+5*2/32)/(2/2+4/4+6/8+3/16+2/32)	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=2}^n (i-1) * \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	(0*1+1*1+2*2+2*4+3*6+6*8+3*16+5*2/32)/(1/1+2/2+8/4+0/8+0/16+0/32)	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	((2/2)+4/4+6/8+3/16+2/2+2/4+2/8+2/16+2/32)	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	1/(1/1+2/2+4/4+6/8+3/16+2/32)	0.25
Upper Generation PI: g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	(4+6+3+2)/(4/4+6/8+3/16+2/32)	7.5

Precursor Frequency is the number of cells in the original culture that divided at least enough to get to the specified generation of “true” proliferation. In this example the specified generation is three. The Precursor Frequency will be zero in the initial culture and will increase with “true” proliferation to a maximum of 1.0.

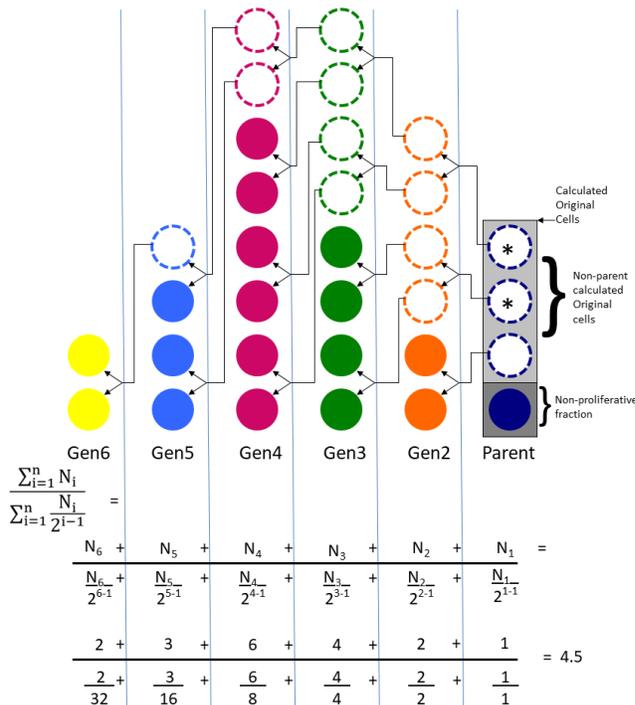
Precursor Frequency



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=1}^n N_i$	$1+2+4+6+3+2$	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^n N_i$	$2+4+6+3+2$	17
Non-Parent Calculated Original cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	$2/2+4/4+6/8+3/16+2/32$	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=1}^n \frac{N_i}{2^{i-1}}$	$1/1+2/2+4/4+6/8+3/16+2/32$	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=1}^n N_i} * 100$	$(2+4+6+3+2)/(1+2+4+6+3+2) * 100$	94.4
Precursor Frequency; g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(4/4+6/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=1}^n N_i}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)$	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=1}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(2/2+4/4+6/8+3/16+2/32)$	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=1}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(0*1/1+1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=1}^n \sum_{j=1}^{i-1} \frac{N_i}{2^{i-1}}$	$((2/2)+(4/2+4/4)+(6/2+6/4+6/8)+(3/2+3/4+3/8+3/16)+(2/2+2/4+2/8+2/16+2/32))$	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$1/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.25
Upper Generation P; g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index; excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(4+6+3+2)/(4/4+6/8+3/16+2/32)$	7.5

Proliferation Index is the number of modeled cells divided by the cells in the original culture. This is the average number of cells that one original cell became. The Proliferation Index will be one in the initial culture and will increase with proliferation to a maximum of $2^{(\maxGen-1)}$, where maxGen is the highest generation modeled.

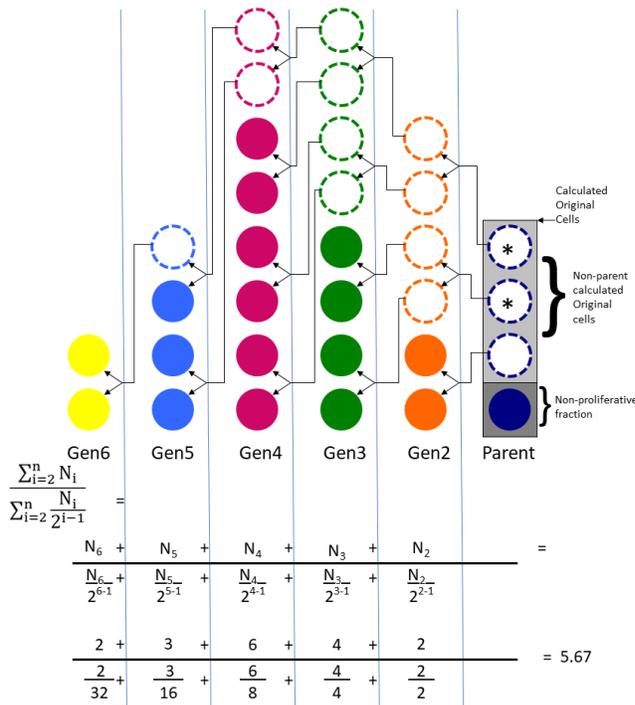
Proliferation Index



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=1}^n N_i$	1+2+4+6+3+2	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^n N_i$	2+4+6+3+2	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	2/2+4/4+6/8+3/16+2/32	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	1/1+2/2+4/4+6/8+3/16+2/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=1}^n N_i} * 100$	$(2+4+6+3+2)/(1+2+4+6+3+2) * 100$	94.4
Precursor Frequency: g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(4/4+5/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=1}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1+2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=2}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(2/2+4/4+6/8+3/16+2/32)$	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=2}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(0*1/1+1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	$((2/2)+1/1+2/2+4/4+6/8+3/16+2/32)+((3/2+3/4+3/8+3/16)+1/2+2/2+4/2+8/2+16+2/32)$	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	1/(1/1+2/2+4/4+6/8+3/16+2/32)	0.25
Upper Generation PI: g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(4+6+3+2)/(4/4+6/8+3/16+2/32)$	7.5

Replication Index is the number of daughter cells divided by the number of cells in the original culture that divided. The Replication Index will be undefined (0/0) in the initial culture and will increase with proliferation to a maximum of 2^(maxGen-1), where maxGen is the highest generation modeled.

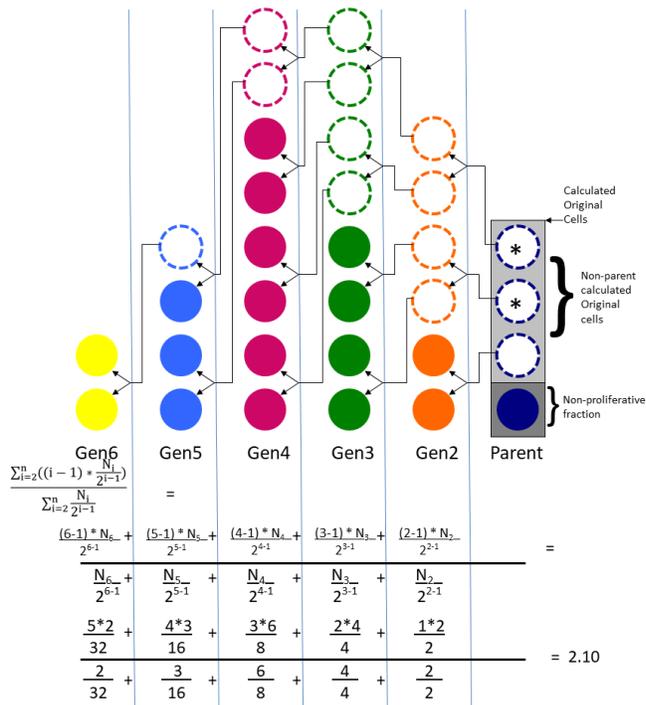
Replication Index



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=2}^n N_i$	1+2+4+6+3+2	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^{g-1} N_i$	2+4+6+3+2	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	2/2+4/4+6/8+3/16+2/32	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	1/1+2/2+4/4+6/8+3/16+2/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^{g-1} N_i}{\sum_{i=2}^n N_i} * 100$	$(2+4+6+3+2)/(1+2+4+6+3+2) * 100$	94.4
Precursor Frequency; g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(4/4+6/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)$	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^{g-1} N_i}{\sum_{i=2}^{g-1} \frac{N_i}{2^{i-1}}}$	$(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=2}^n (i-1) * \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(2/2+4/4+6/8+3/16+2/32)$	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=2}^n (i-1) * \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(0*1/1+1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	$((2/2)+4/4+6/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$1/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.25
Upper Generation PI; g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(4+6+3+2)/(4/4+6/8+3/16+2/32)$	7.5

Proliferating Average Number of Divisions is the sum of the number of divisions that resulted in the cells in each generation divided by the Non-parent Calculated Original Cells. The Proliferating Average Number of Divisions will be undefined in the initial culture and will increase with proliferation to a maximum of maxGen-1 where maxGen is the highest generation modeled.

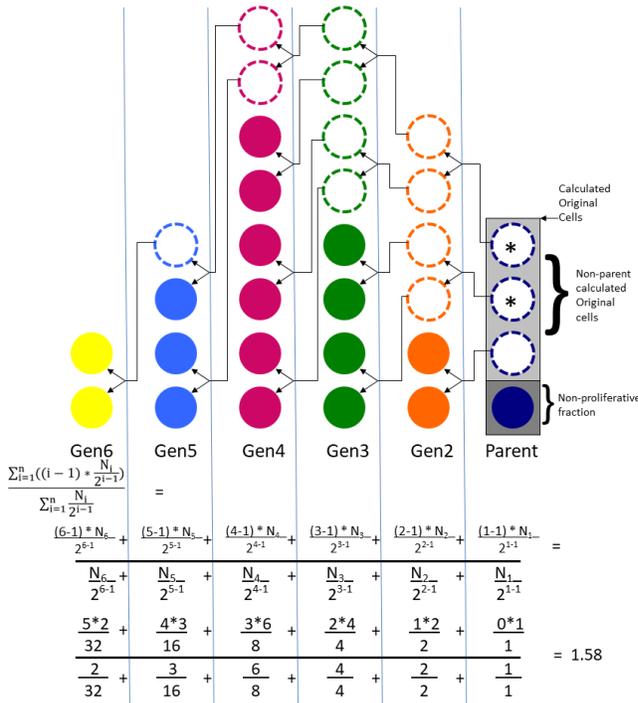
Proliferating Average Number of Divisions



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=2}^n N_i$	1+2+4+6+3+2	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^n N_i$	2+4+6+3+2	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	2/2+4/4+6/8+3/16+2/32	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	1/1+2/2+4/4+6/8+3/16+2/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n N_i} * 100$	$(2+4+6+3+2)/(1+2+4+6+3+2) * 100$	94.4
Precursor Frequency; g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(4/4+5/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)$	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=2}^n ((i-1) * \frac{N_i}{2^{i-1}})}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(2/2+4/4+6/8+3/16+2/32)$	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=2}^n ((i-1) * \frac{N_i}{2^{i-1}})}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(0*1/1+1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	$((2/2)+4/4+3/8+2/16)+(1/1+2/2+4/4+6/8+3/16+2/32)$	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$1/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.25
Upper Generation PI; g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(4+6+3+2)/(4/4+6/8+3/16+2/32)$	7.5

Average Number of Divisions is the sum of the number of divisions that resulted in the cells in each generation divided by the Calculated Original Cells. The Average Number of Divisions will be zero in the initial culture and will increase with proliferation to a maximum of maxGen-1 where maxGen is the highest generation modeled.

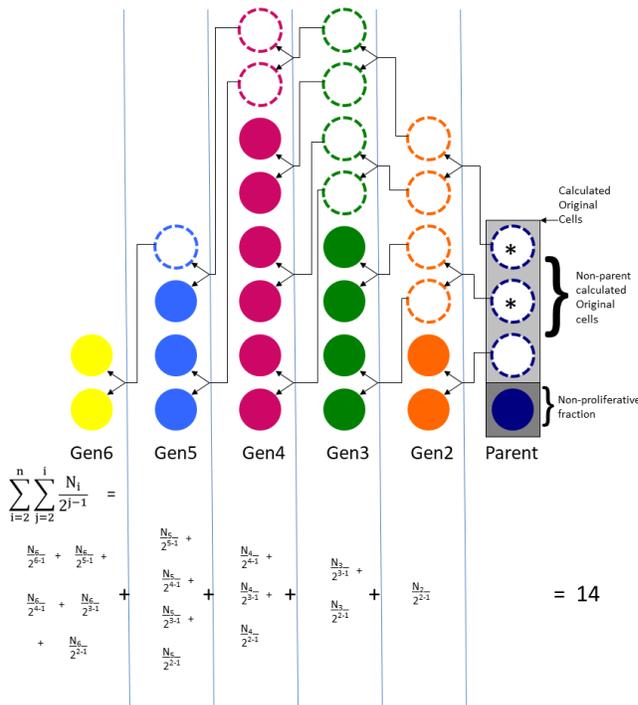
Average Number of Divisions



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=1}^n N_i$	1+2+4+6+3+2	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=1}^{g-1} N_i$	2+4+6+3+2	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=1}^n \frac{N_i}{2^{i-1}}$	2/2+4/4+6/8+3/16+2/32	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=1}^n \frac{N_i}{2^{i-1}}$	1/1+2/2+4/4+6/8+3/16+2/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=1}^{g-1} N_i}{\sum_{i=1}^n N_i} \cdot 100$	$(2+4+6+3+2)/(1+2+4+6+3+2) \cdot 100$	94.4
Precursor Frequency; g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(4/4+5/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=1}^n N_i}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)$	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=1}^{g-1} N_i}{\sum_{i=1}^{g-1} \frac{N_i}{2^{i-1}}}$	$(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=1}^n ((i-1) \cdot \frac{N_i}{2^{i-1}})}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(1 \cdot 2/2+2 \cdot 4/4+3 \cdot 6/8+4 \cdot 3/16+5 \cdot 2/32)/(2/2+4/4+6/8+3/16+2/32)$	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=1}^n ((i-1) \cdot \frac{N_i}{2^{i-1}})}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(0 \cdot 1/1+1 \cdot 2/2+2 \cdot 4/4+3 \cdot 6/8+4 \cdot 3/16+5 \cdot 2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=1}^n \frac{N_i}{2^{i-1}}$	$((2/2)+(4/4)+(6/8)+(3/16)+(2/32))/(1/1+2/2+4/4+6/8+3/16+2/32)$	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$1/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.25
Upper Generation PI; g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(4+6+3+2)/(4/4+6/8+3/16+2/32)$	7.5

Number of Divisions is the total number of divisions that resulted in the cells in each generation. The Number of Divisions will be zero in the initial culture and will increase with proliferation.

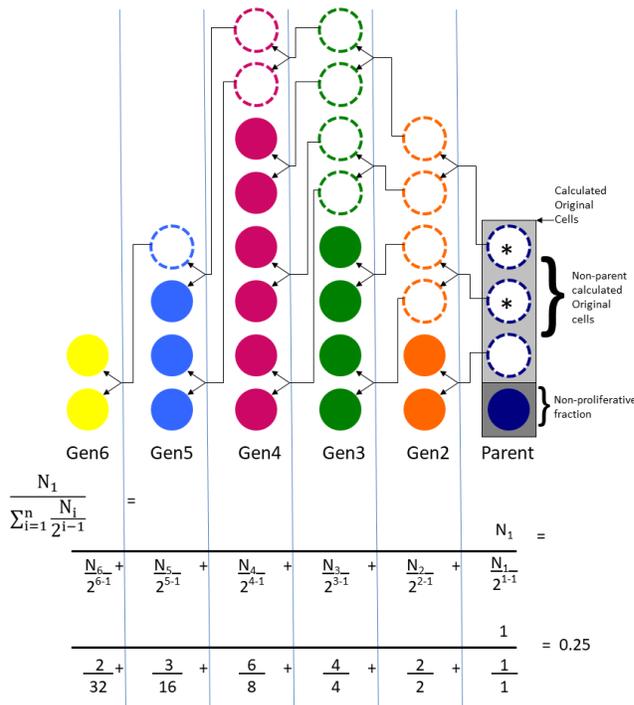
Number of Divisions



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=2}^n N_i$	1+2+4+6+3+2	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=2}^n N_i$	2+4+6+3+2	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	2/2+4/4+6/8+3/16+2/32	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	1/1+2/2+4/4+6/8+3/16+2/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n N_i} \cdot 100$	$(2+4+6+3+2)/(1+2+4+6+3+2) \cdot 100$	94.4
Precursor Frequency; g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(4/4+5/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)$	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=2}^n N_i}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=2}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(1^*2/2+2^*4/4+3^*6/8+4^*3/16+5^*2/32)/(2/2+4/4+6/8+3/16+2/32)$	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=2}^n (i-1) \cdot \frac{N_i}{2^{i-1}}}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$(0^*1/1+1^*2/2+2^*4/4+3^*6/8+4^*3/16+5^*2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=2}^n \frac{N_i}{2^{i-1}}$	$((2/2)+(4/2+4/4)+(6/2+6/4+6/8)+(3/2+3/4+3/8+3/16)+(2/2+2/4+2/8+2/16+2/32))$	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=2}^n \frac{N_i}{2^{i-1}}}$	$1/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.25
Upper Generation PI; g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(4+6+3+2)/(4/4+6/8+3/16+2/32)$	7.5

Non-proliferative Fraction is the number of modeled cells divided by the cells in the original culture. The Non-proliferative Fraction will be one in the initial culture and will decrease with proliferation to a minimum of zero.

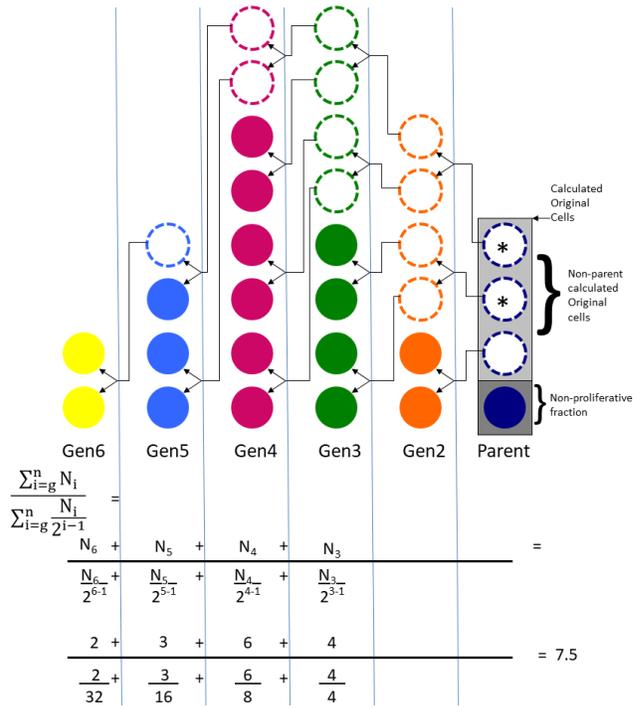
Non-proliferative Fraction



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=1}^n N_i$	1+2+4+6+3+2	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=1}^{n-1} N_i$	2+4+6+3+2	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=1}^{n-1} \frac{N_i}{2^{i-1}}$	2/2+4/4+6/8+3/16+2/32	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=1}^n \frac{N_i}{2^{i-1}}$	1/1+2/2+4/4+6/8+3/16+2/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=1}^{n-1} N_i}{\sum_{i=1}^n N_i} * 100$	$(2+4+6+3+2)/(1+2+4+6+3+2) * 100$	94.4
Precursor Frequency; g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(4/4+6/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=1}^n N_i}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)$	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=1}^{n-1} N_i}{\sum_{i=1}^{n-1} \frac{N_i}{2^{i-1}}}$	$(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=1}^n (i-1) * \frac{N_i}{2^{i-1}}}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(2/2+4/4+6/8+3/16+2/32)$	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=1}^n (i-1) * \frac{N_i}{2^{i-1}}}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$(0*1/1+1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=1}^n \frac{N_i}{2^{i-1}}$	$((2/2)+(4/4)+(6/8)+(3/16)+(2/32))/(1/1+2/2+4/4+6/8+3/16+2/32)$	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_1}{\sum_{i=1}^n \frac{N_i}{2^{i-1}}}$	$1/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.25
Upper Generation Proliferation Index; g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(4+6+3+2)/(4/4+6/8+3/16+2/32)$	7.5

Upper Generation Proliferation Index is the number of modeled cells divided by the cells in the original culture for generations greater than g, which is 3 in this example. This is the average number of cells that one original cell became, excluding those in generations less than g. The Upper Generation Proliferation Index will be undefined in the initial culture and will increase with proliferation to a maximum of 2^(maxGen-1), where maxGen is the highest generation modeled.

Upper Generation Proliferation Index



Value	Description	Equation	Calculation	Result
Modeled Cells [Z_MODELED_CELLS]	All filled, solid circles	$\sum_{i=g}^n N_i$	1+2+4+6+3+2	18
Non-Parent Cells [Z_NON_PARENT_CELLS]	All filled, solid circles not in the parent generation	$\sum_{i=g}^n N_i$	2+4+6+3+2	17
Non-Parent Calculated Original Cells [Z_NON_PARENT_ORIGINAL_CELLS]	Circles with a light gray background	$\sum_{i=g}^n \frac{N_i}{2^{i-1}}$	2/2+4/4+6/8+3/16+2/32	3
Calculated Original Cells [Z_ORIGINAL_CELLS]	Circles with a light or dark gray background	$\sum_{i=g}^n \frac{N_i}{2^{i-1}}$	1/1+2/2+4/4+6/8+3/16+2/32	4
Percent Divided [Z_PERCENT_DIVIDED]	100 times Non-parent Cells divided by Modeled Cells	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n N_i} * 100$	$(2+4+6+3+2)/(1+2+4+6+3+2) * 100$	94.4
Precursor Frequency: g=3 [Z_PRECURSOR_FREQUENCY]	Circles containing * divided by Calculated Original Cells	$\frac{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(4/4+5/8+3/16+2/32)/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.5
Proliferation Index [Z_PROLIF_INDEX]	Modeled Cells divided by Calculated Original Cells	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(1+2+4+6+3+2)/(1/1+2/2+4/4+6/8+3/16+2/32)$	4.5
Replication Index [Z_REPLICATION_INDEX]	Non-parent Cells divided by Non-parent Calculated Original Cells	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(2+4+6+3+2)/(2/2+4/4+6/8+3/16+2/32)$	5.67
Proliferating Average Number of Divisions [Z_AVG_NUM_DIVISIONS_PROLIF]		$\frac{\sum_{i=g}^n (i-1) * \frac{N_i}{2^{i-1}}}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(2/2+4/4+6/8+3/16+2/32)$	2.10
Average Number of Divisions [Z_AVG_NUM_DIVISIONS_ALL]		$\frac{\sum_{i=g}^n (i-1) * \frac{N_i}{2^{i-1}}}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(0*1/1+1*2/2+2*4/4+3*6/8+4*3/16+5*2/32)/(1/1+2/2+8/8+6/16+0/32)$	1.58
Number of Divisions [Z_TOTAL_DIVISIONS]	All unfilled, dashed circles	$\sum_{i=g}^n \frac{N_i}{2^{i-1}}$	$((2/2)+16/2+4/4)+16/2+6/4+6/8+(3/2+3/4+3/8+3/16)+2/2+2/4+2/8+2/16+2/32)$	14
Non-proliferative Fraction [Z_NONPROLIF_FRACTION]	Number for filled parent circles divided by Calculated Original Cells	$\frac{N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$1/(1/1+2/2+4/4+6/8+3/16+2/32)$	0.25
Upper Generation PI: g=3 [Z_UPPER_GEN_PROLIF_INDEX]	Similar to Proliferation Index, excludes cells with a generation index of less than 'g'.	$\frac{\sum_{i=g}^n N_i}{\sum_{i=g}^n \frac{N_i}{2^{i-1}}}$	$(4+6+3+2)/(4/4+6/8+3/16+2/32)$	7.5

Reports

Reports

The documents created by ModFit LT are called reports. Each reports contains the analysis information for one data sample, and can be saved to disk and re-opened by the program.

When you start ModFit LT, a simple report is created for you automatically. It contains several objects which are common to all reports: a graphical element which displays the histogram data, a textual element which displays analysis results, and the gates that have been created. The histogram is not displayed until there is a data file loaded. The text objects remain hidden until you have analyzed the data.

To see these elements, load a data file into ModFit LT. The histogram will be displayed. In addition, if you load listmode data, you can create gates on the data. The gate graphics will appear on the report, allowing you to manipulate the gates without reloading the sample.

Report Tools

ModFit LT reports can include additional text and graphics that you add to the report with report tools. These tools are found on the **Insert** tab of the ribbon bar. In toolbar mode, the Report toolbar is displayed.

The table below shows the report tools and their functional descriptions. Click a tool for additional information.

Tool	Description
 Text	Adds free-form text objects.
 Keywords	Adds keyword information extracted from the data file you analyze.
 Line	Adds a solid line.
 Arrow	Adds an arrow.
 Rect	Adds a filled rectangle.



Adds a filled ellipse.



Adds a graph zoomed on the X-axis to magnify the Diploid G1 component.



Adds a graph object zoomed on Y-axis to magnify S-Phase and aggregates.



Adds the fit diagnostics graph to the report.

Moving and resizing report objects

All of the objects on the report can be moved, and most objects can be resized. This allows you to place objects where you want them on the report page, and adjust the size of graphics elements.

- To move an object, click and hold the mouse button down on the object, then move the object. Release the mouse to set the new position.
- To resize an object, click the object to select it. Handles will appear on the object. Click and hold the mouse button down on one of the handles. As you move the handle, the object will resize. Release the mouse to set the new size.

New Report Template



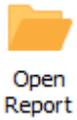
Use this command to create a new report template.

Any existing objects on a report, including analysis results, are discarded, and new objects are created in their default locations.

Shortcuts

Keys: CTRL + N on PC; Command + N on Mac

Open Report Template



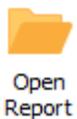
Use this command to open a report template from disk. The report template can define the kind of analysis, gating, and objects that appear on the report page.

Save Report Template



Use this command to save a report template. The report template can define the kind of analysis, gating, and objects that appear on the report page.

Open Reports command



Use this command to open one or more existing ModFit LT report documents. Choosing this option will display the [File Open dialog](#) to select reports to open.

A Report document contains an analysis of data and additional report objects that you add to the document.

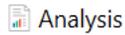
A batch of reports may be selected at one time. When more than one report is selected, the [Report Batch Settings](#) dialog is displayed to allow you to set options for batch processing. Then, the [Report batch toolbar](#) will be displayed at the bottom of the application window. This toolbar will allow you to navigate through the selected reports, and provides additional batch processing options.

To select a batch of reports, click the mouse on the first item you want to select and drag the mouse over sequential files in the list. Alternately, you can click the first item, hold down the Shift key and click the last item to select a group of files. Hold down the CTRL key to select non-adjacent files in the list.

Shortcuts

Keys: CTRL + O on PC; Command + O on Mac

Analysis command



Use this command to display the report view.

Save command



Use this command to save the document with its current name and folder.

When you save a document for the first time, ModFit LT displays the [Save As dialog](#) so you can name your document. The name for the report is based on the data file currently loaded into it. ModFit LT takes the name of the data file and adds ".mfl" to create the report name.

Once a report document has been saved, this command will not display the Save As dialog; it will simply resave the report.

If you want to change the name and directory of an existing document before you save it, choose the [Save As command](#).

The document contains the model, data, gates, and any additional elements that you may have added to the page.

Shortcuts

Keys: CTRL + S on PC; Command + S on Mac

Save Report As

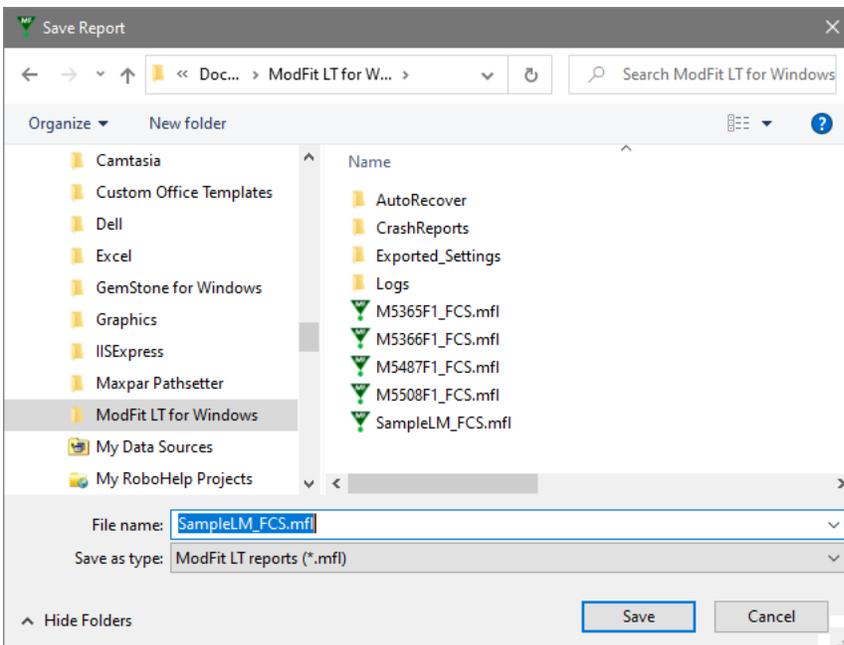


Use this command to save and name the ModFit LT report document. ModFit LT displays the [Save As dialog](#) so you can name your document.

To save a document with its existing name and folder, use the [Save command](#).

File Save As dialog box

This dialog is used to save reports in ModFit LT. This is the standard File Save dialog box for the operating system, so its appearance and features are different on Windows and Mac versions.



Print command



Use this command to print a ModFit LT report to the selected printer. The Print dialog box is displayed allowing you to select printing options.

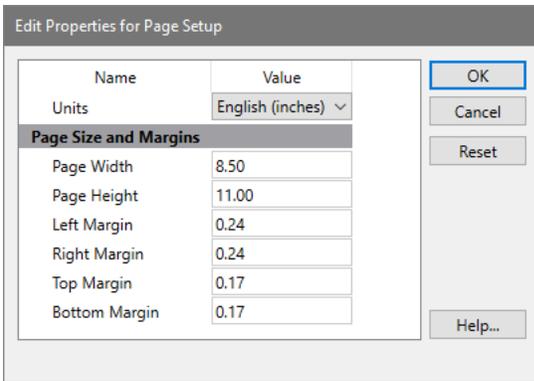
Shortcuts

Keys: CTRL + P on PC; Command + P on Mac

Page Setup command

Use this command to set up characteristics of printed pages, including paper size and margins.

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Zoom command



Use this command to change the magnification of the report document as viewed on the screen. Increase the zoom to magnify the image, or decrease it to reduce the image size.

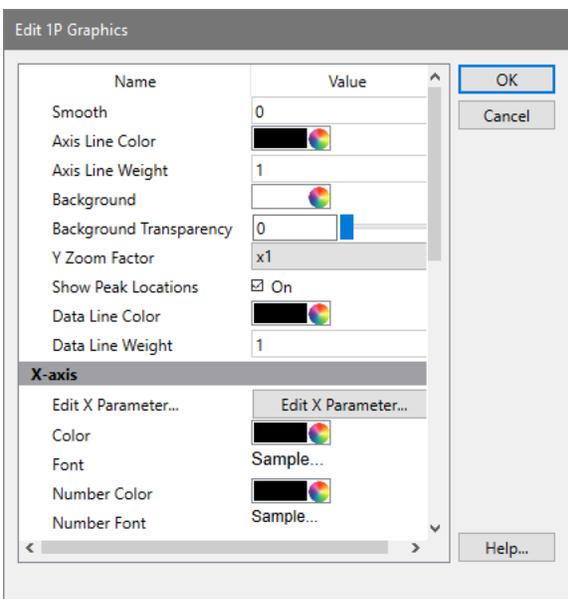
This has no effect on printed output.

Recent Reports list

This menu is a convenient list that shows the most recent report files that have been opened in ModFit LT. Select a file from the list to open the report.

Edit 1P Graphics

This dialog is used to change the graphic properties for 1P histograms.



Smooth

This value determines the amount of smoothing for the histogram. Set to 0 for no smoothing.

Axis Line Color

Choose the color of the axis line.

Axis Line Weight

Choose the weight of the axis line.

Background

Choose the color of the background page.

Background Transparency

Sets the transparency of the background.

Y Zoom Factor

Select a zoom factor for the Y direction.

Show Peak Locations

Enable this option to see where ModFit found peaks in the file. The peaks are shown as triangles on the axis.

Data Line Color

Choose the color of the data line.

Data Line Weight

Choose the weight of the data line.

X-axis and Y-axis properties

Edit X (or Y) Parameter

Displays a dialog to [Edit Properties for Parameter](#), to edit transformations, the name, etc.

Color

Choose a color of the axis label.

Font

Select the font of the axis label.

Number Color

Choose the color of the axis tic numbers.

Number Font

Select the font of the axis tic numbers.

Auto Minimum

Enable this option to automatically set the minimum of the axis.

Minimum Value

When Auto Minimum is *not* enabled, this value sets the minimum value displayed by the axis.

Auto Maximum

Enable this option to automatically set the maximum of the axis.

Maximum Value

When Auto Maximum is *not* enabled, this value sets the maximum value displayed by the axis.

Auto Calculate Major Tics

Enable this option to automatically determine the major tic unit for the axis

Number of Major Tics

When Auto Calculate is *not* enabled, this value sets the major tic unit of the axis.

Auto Calculate Minor Tics

Enable this option to automatically determine the minor tic unit for the axis.

Number of Minor Tics

When Auto Calculate is *not* enabled, this value sets the number of minor tics per major tic on the axis.

Actions

OK

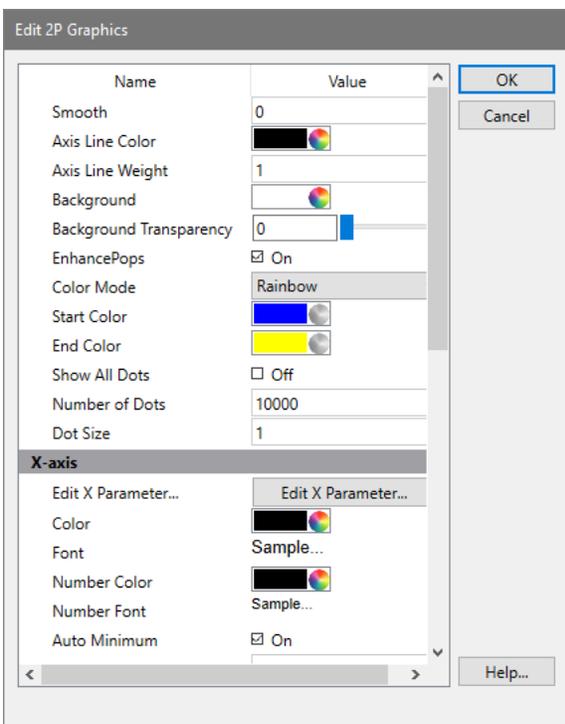
Click this button to accept the changes and close the dialog box.

Cancel

Click this button to discard any changes and close the dialog box.

Edit 2P Graphics

This dialog is used to change the graphic properties for 2P plots.



Smooth

This value determines the amount of smoothing for the histogram. Set to 0 for no smoothing.

Axis Line Color

Choose the color of the axis line.

Axis Line Weight

Choose the weight of the axis line.

Background

Choose the color of the background page.

Background Transparency

Sets the transparency of the background.

Enhance Pops

Enable this option if there are small populations you need to visualize in the graph.

Color Mode

Choose between Rainbow and Gradient color density graphics.

Start Color

For Gradient color mode, this is the color of low frequency events on the axis.

End Color

For Gradient color mode, this is the color for the highest frequency events..

Show All Dots

When enabled, all events in the plot are displayed. If unchecked, the number of dots is controlled by the Number of Dots edit field.

Number of Dots

When Show All Dots is unchecked, this field determines the number of dots to display on the plot. Fewer dots may appear if there are fewer events in the plot.

Dot Size

Enter a size for dots. A value of 0 draws a dot 1 pixel in size.

X-axis and Y-axis properties

Edit X (or Y) Parameter

Displays a dialog to [Edit Properties for Parameter](#), to edit transformations, the name, etc.

Color

Choose a color of the axis label.

Font

Select the font of the axis label.

Number Color

Choose the color of the axis tic numbers.

Number Font

Select the font of the axis tic numbers.

Auto Minimum

Enable this option to automatically set the minimum of the axis.

Minimum Value

When Auto Minimum is *not* enabled, this value sets the minimum value displayed by the axis.

Auto Maximum

Enable this option to automatically set the maximum of the axis.

Maximum Value

When Auto Maximum is *not* enabled, this value sets the maximum value displayed by the axis.

Auto Calculate Major Tics

Enable this option to automatically determine the major tic unit for the axis

Number of Major Tics

When Auto Calculate is *not* enabled, this value sets the major tic unit of the axis.

Auto Calculate Minor Tics

Enable this option to automatically determine the minor tic unit for the axis.

Number of Minor Tics

When Auto Calculate is *not* enabled, this value sets the number of minor tics per major tic on the axis.

Actions

OK

Click this button to accept the changes and close the dialog box.

Cancel

Click this button to discard any changes and close the dialog box.

Report tools

Text tool



Text

Use this command to add editable text to the report document.

A dialog will appear allowing you to enter text. After typing the text, click OK on the dialog. The text will appear on the document. It can be dragged anywhere on the page. Its bounding rectangle can also be resized.

To edit the text, double click anywhere on the text block.

To drag text, click the text and hold down the mouse button. With the button depressed, move the mouse. Release the button to set the new position.

To add text to the Flash Comment list, choose the Save button in the Edit Text dialog. The standard File Save dialog is displayed. Type in a name for the comment file and click OK. Once the comment has been stored on disk, you will be prompted to type a description for the comment. Enter the description and click OK. The Edit Text dialog will now display the description in the Flash Comment list.

To add a stored comment to the report, choose the Text report tool. The Edit Text dialog appears. From the Flash Comment list, click the comment you want to add. The text of the comment will appear in the Edit Text dialog. Click OK to add the comment.

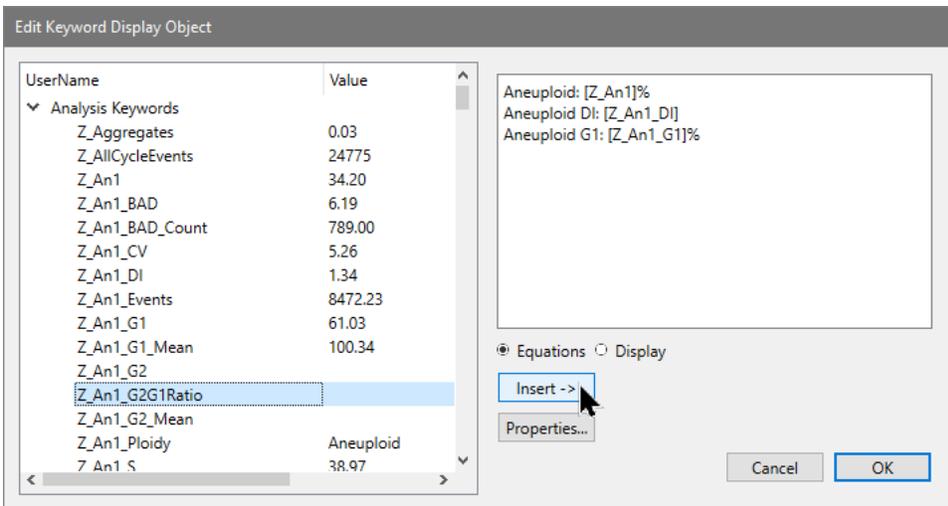
Keywords tool



Keywords

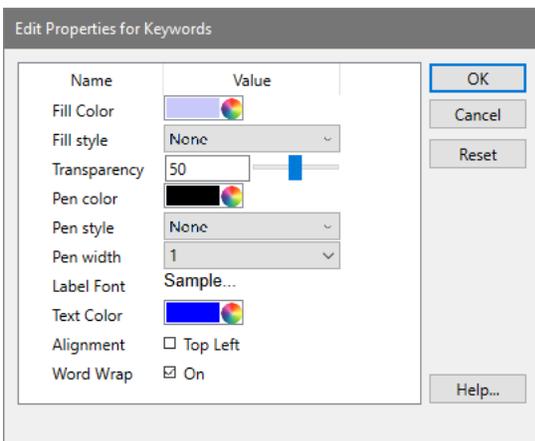
Use this command to add keywords from your data file and analysis results to the report document. Some keywords are stored in the file at the time it is created by the acquisition software. ModFit LT allows you to access these keywords and place them in the report. In addition, you can access the analysis results as keywords to place on the report. A list of the keywords appears in the [Keywords](#) section.

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To move the object after it is inserted, click on it and hold down the mouse button. With the button depressed, move the mouse. Release the button to set the new position.

Click the Properties button to set the font, alignment, and coloring for the object.



Line tool



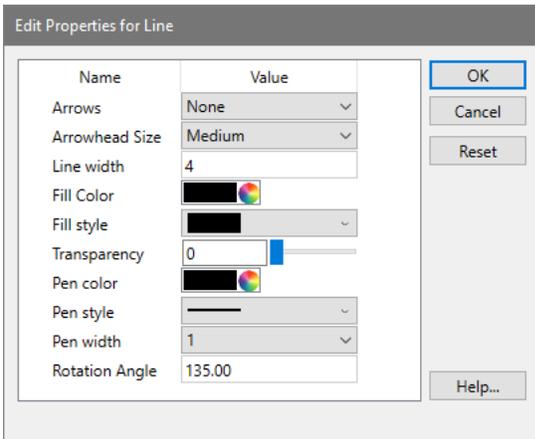
Line

Use this command to add lines to the report document. Lines can be dragged into position anywhere on the report.

To drag a line, click on the object and hold down the mouse button. With the button depressed, move the mouse. Release the button to set the new position.

To change the size or angle of a line, click on the line to make it the active object. Handles will appear on each end of the line. Click and hold one of the handles to reposition. The line will reorient as you move the handle.

To change the properties of a line, double click on the line.



Arrow tool



Use this command to add lines with arrowheads to the report document. Arrows can be dragged into position anywhere on the report.

To drag an arrow, click on the object and hold down the mouse button. With the button depressed, move the mouse. Release the button to set the new position.

To change the size or angle of an arrow, click on the line to make it the active object. Handles will appear on each end of the arrow. Click and hold one of the handles to reposition. The arrow will reorient as you move the handle.

To change the properties of an arrow, double click on the arrow.

Rectangle tool

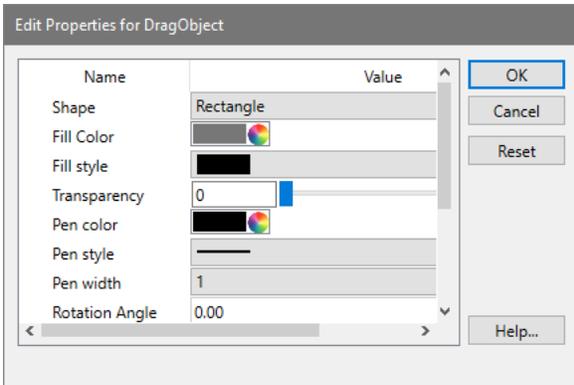


Use this command to add rectangles to the report document. Rectangles can be dragged into position anywhere on the report, and have selectable colors.

To drag a rectangle, click on the object and hold down the mouse button. With the button depressed, move the mouse. Release the button to set the new position.

To change the size of a rectangle, first click on it to make it the active object. Click and hold one of the handles to reposition that corner of the rectangle. Each of the handles can be moved in the same manner.

To change the properties of a rectangle, double click on the rectangle.



Ellipse tool



Use this command to add ellipses and circles to the report document. Ellipses can be dragged into position anywhere on the report.

To drag an ellipse, click on the object and hold down the mouse button. With the button depressed, move the mouse. Release the button to set the new position.

To change the size of an ellipse, first click on it to make it the active object. Click and hold one of the handles to redefine the size and shape of the ellipse. Each of the handles can be moved in the same manner.

To change the properties of an ellipse, double click on the ellipse.

X Zoom Graph



Use this command to add a graph object to the report that shows a zoomed view of the histogram. The inserted graph can be moved and resized on the report.

The X Zoom graph object is especially useful for visually identifying near Diploid populations. It is designed to crop the X-axis so that only the Diploid G1 peak is on scale.

To edit the object zoom settings, double click on the object after it has been inserted into the report. Then, adjust the X-Axis minimum and maximum values to include the portion of the histogram you want to view.

You can set the default values for this object using the [Options and Configuration](#) dialog box.

Y Zoom Graph



Use this command to add a graph object to the report that shows a zoomed view of the histogram. The inserted graph can be moved and resized on the report.

The Y Zoom graph object is designed to enhance low-level histogram data. It provides a detailed view of S-Phase and small G2M and aggregate forms. Use the graph to verify the fit of the model and ensure the correct model is selected.

To edit the object zoom settings, double click on the object after it has been inserted into the report. Select a new Y Zoom factor to increase or decrease the magnification of the histogram.

You can set the default values for this object using the [Options and Configuration](#) dialog box.

Fit Diagnostics command



Diags

Use this command to add a report object that displays a summary of the Fit Diagnostics dialog box on the report. The object shows a legend of the elements found in the Fit Diagnostics dialog box. The object can be moved to any convenient location on the report.

2.96	% CV
23174	Cell Number
309	Avg. Cells Per Channel
n/a	Aneuploid Fraction
4.22	% B.A.D.
3.55	RCS

To edit the properties of the object, double click on the object after it has been inserted into the report.

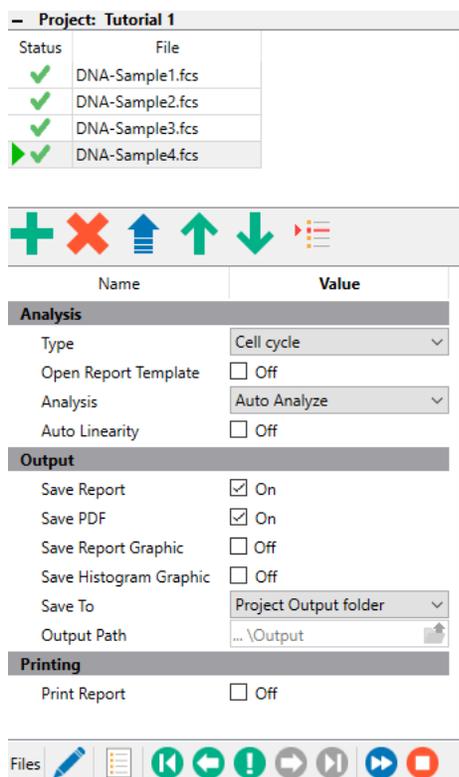
Batch Processing

Batch Processing

ModFit LT provides powerful batch processing of data files and reports. Batch processing of data files supports automatic analysis, saving and printing of reports, and databasing of results, in addition to other features. When processing batches of reports, you can re-analyze data, perform AutoLinearity adjustments, re-save and print reports, and store results in databases.

Batch Processing FCS Files

On the left side of the main window, ModFit LT displays a list of FCS files in the batch. Tools are provided to add, remove, read, and move files in the list, as well as to set the file that is currently active. The actions that will be performed on the files can be selected in the lower portion, and controls to move through the batch appear at the bottom.



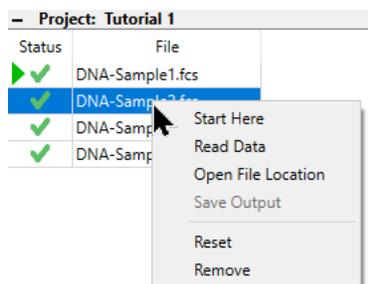
Batch Processing Reports

The **Options** tab contains entries for [Report Batch Settings](#). To process of batch of reports, use the [Open Report](#) button on the Home tab to select the set of reports you want to process. Upon opening the set of reports, the [Report Batch Settings](#) will be displayed, and the [Report Batch Toolbar](#) is used to process the report files.

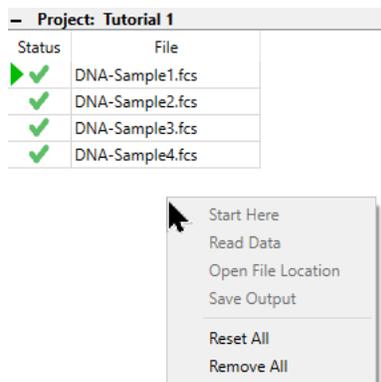
Batch Context Menu

The batch context menu is displayed when you right-click in the file list.

When the mouse is right-clicked on an item in the batch, the following menu is displayed:



When the right-click is on white space in the batch list, a different menu is shown:



Start Here

Select this command to set the current item in the list. The item will be processed by the next batch processing command.

Read Data

Select this command to set the current item in the list and read the FCS data, without performing additional batch processing.

Open File Location

Select this command to open the folder on disk where the file is located.

Save Output

This command is enabled for the current batch item if it has been analyzed. Selecting the command causes the batch processing options to be performed on the file, such as saving and printing a report.

Reset Status/Reset All

This command resets (clears) the status flag for the selected file in the list, or if no file is selected, for all files in the list.

Remove/Remove All

When this command is selected for one file, ModFit LT will prompt for confirmation that you want to remove the file from the batch. If confirmed, the file will be removed from the batch list. If the file is contained in a project, it will also be removed from the project folder.

When the command is selected not for a specific file, it can remove *all* files from the batch list. ModFit LT will prompt for confirmation that you want to remove the file from the batch.

File Batch Settings

These properties determine what actions the program will perform when processing FCS files in the batch list. They appear at the bottom of the batch list panel on the left side of the main window.

Analysis	
Type	Cell cycle 
Open Report Template	<input type="checkbox"/> Off
Analysis	Auto Analyze 
Auto Linearity	<input type="checkbox"/> Off

Output	
Save Report	<input checked="" type="checkbox"/> On
Save PDF	<input checked="" type="checkbox"/> On
Save Report Graphic	<input type="checkbox"/> Off
Save Histogram Graphic	<input type="checkbox"/> Off
Save To	Project Output folder 
Output Path	... \Output 

Printing	
Print Report	<input type="checkbox"/> Off

Analysis

Type

The Type identifies the kind of analysis you want to perform:

Type	Usage
Cell cycle	Cell cycle analysis using Auto-Analysis or a model created with the Choose Model dialog. Supports multiple cell cycles, debris and aggregate subtraction
Synchronized cell cycle	Analyze samples that have been synchronized (e.g. blocked in G0-G1 and then released). Supports one cell cycle, debris and aggregate subtraction
Cell proliferation tracking	For cells stained with cell-tracking dyes such as CFSE. Computes all cell proliferation statistics (e.g. Proliferation Index)
Custom	For an unrestricted analysis with a custom model

Open Report Template

Enable this option to open the project's report template before each file in the batch is analyzed. This ensures that each file is analyzed with the same starting conditions.

When using a project, ModFit LT will open the project's report template when the project is opened. For most types of analysis, the report template does not need to be re-opened for each file analyzed and this option can be left unchecked. This would be true for most Cell Cycle, Synchronization, and Cell Tracking projects.

If you have a custom model for your project, you may want to enable this option.

Analysis

Choose what analysis option you want to occur when a new data file is opened. The options available are listed below:

No analysis: No analysis is performed.

[Fit with current model](#): The Fit command is performed with the current model.

[Auto Analyze](#): Automatic analysis is performed.

Auto Linearity

Enable this option to perform [automatic linearity adjustment](#) for each file.

Output

Save Report

Enable this option to save a report for each data file. The report is automatically named based on the name of the FCS file analyzed. Saving occurs after any analysis has been performed when moving to another batch item. Reports are saved in the Reports folder in the Output Path.

Save PDF

Enable this option to generate a PDF version of the report. PDFs are saved in the PDFs folder in the Output Path.

Save Report Graphic

This option saves a report graphic for each file analyzed. Graphics are automatically named based on the data file that is loaded, as described in [Export Report Graphic](#). Graphics are saved in the Graphics folder in the Output Path.

Save Histogram Graphic

This option saves a histogram graphic for each file analyzed. Graphics are automatically named based on the data file that is loaded, as described in [Export Histogram Graphic](#). Graphics are saved in the Graphics folder in the Output Path.

Save To

Output can be saved to the Project Output Folder or to a Specified Output Path. When Specified Output Path is selected, the Output Path field is used to select the output location.

Output Path

If the Save To option is set to Specified Output Path, reports will be saved to the path selected here. Click the Folder button to select a new location for output.

Printing

Print Report

Enable this option to automatically print a report to the current printer. Printing occurs when moving to another batch item.

File Batch Toolbar

This toolbar is displayed at the bottom of the ModFit LT application window when you select more than one file in the Open FCS dialog.



Most of the buttons on the toolbar are used to navigate through the batch. The left-most button is used to edit the properties of the batch processor. See [File Batch Settings](#) for more on file batch properties.

As you navigate through the batch, the actions in the File Batch Settings are performed on each file analyzed. This allows you to perform a series of operations just by moving to another batch item, such as automatic analysis, saving and printing reports. The function of each button is described below.



Show or hide the batch panel



Select from a list of batch items.



Go to the first file in the batch.



Go to the previous file in the batch.



Redo the current file in the batch.



Go to the next file in the batch.



Go to the last file in the batch.



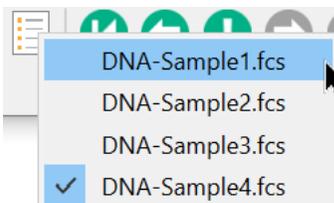
Auto-advance through the batch.



Stop the auto-advance process.

Choose Batch

This menu is displayed when the Choose button on the File Batch or Report Batch toolbar is clicked. It allows you to choose a batch item to open.



The menu lists all items in the batch. To select an item, click on it in the list. The item will be opened.

Report Batch Settings



This option displays the Edit Properties for Report processor dialog box, allowing you to choose what happens during batch processing of *reports*. The dialog is displayed automatically when you select more than one report for analysis.

Name	Value	Description
Analysis	No analysis	Analysis option when a report is processed.
Add Zoom Objects	<input type="checkbox"/> Off	Automatically add X and Y zoom objects to report.
Save Report	Don't re-save report	Select a save option.
Print Report	<input type="checkbox"/> Off	Print the report.
Save PDF	<input type="checkbox"/> Off	Save a PDF of the report.
Save Graphics	None	Save graphics of the report and analysis histogram.
Overwrite Existing Files	<input checked="" type="checkbox"/> On	Overwrite existing output files.
Prompts		
Database Prompt	<input type="checkbox"/> Off	Prompt to confirm save record?

Buttons: OK, Cancel, Reset, Help...

Batch processing options apply when you select more than one report in the Open Reports dialog box. When more than one report is selected, the [Report Batch toolbar](#) is displayed at the bottom of the application window. You can navigate through the batch one report at a time, or auto-advance through the batch of reports.

Analysis

Choose what analysis option you want to occur when a new data file is opened. The options available are listed below:

No analysis: No analysis is performed.

Auto-analyze: Automatic Analysis is run the data in the report.

Auto-analyze and Auto Linearity: Automatic Analysis and Auto Linearity are performed on the report data.

Fit with current model: The Execute Model command is executed on each data file.

Fit and AutoLinearity: An AutoLinearity adjustment is performed for each report, and the model is finally fit with the best linearity setting. See [Using AutoLinearity](#) for more on this option.

Add Zoom Objects

Enable this option to automatically add an X-Zoom and Y-Zoom graph to each report that is processed. These objects allow close examination of the low level events such as debris, aggregates, and S-Phase, and provide a magnified view of the area around the Diploid G1 peak.

Save Report

This drop-down provides three report saving options described below. Saving occurs after any analysis has been performed, and again when moving to another batch item.

Don't re-save report: The report is not resaved.

Re-save report: The report is resaved, using the save report name.

Auto-name and save report: The report is resaved using the Auto Save Report command. The name of the report may change from the original name with this option.

Print Report

Enable this option to automatically print the report to the current printer. Printing occurs when moving to another batch item.

Save PDF

Enable this option to automatically generate a PDF version of the report. The PDF is created in the same location as the Save Report Path.

Save Graphics

This option allows you to save graphics for the histogram or report. Graphics are automatically named based on the data file that is loaded, as described in [Export Histogram Graphic](#) and [Export Report Graphic](#). The save operation occurs when moving to another batch item. The options available are listed below:

No: No graphics are saved to disk.

Histogram: Histogram graphics are saved.

Report: Report graphics are saved.

Histogram and Report: Both Histogram and Report graphics are saved.

Database prompt

Enable this option if you want to be prompted to store the analysis in any active databases. If you clear this checkbox and databases are active, records will be stored without prompting. If databases are inactive, this option is ignored.

Report Batch Toolbar

This toolbar is displayed at the lower right of the ModFit LT application window when you select more than one file in the Open Reports dialog.



Most of the buttons on the toolbar are used to navigate through the batch. The left-most button is used to edit the properties of the batch processor. See [Report Batch Settings](#) for more on report batch properties.

As you navigate through the batch, the actions and options in the Report Batch Settings dialog are performed on each file that is opened. This allows you to perform a series of automatic operations just by moving to another batch item, such as rerunning the analysis and printing reports. The function of each button is described below.



Edit the properties of the batch.



Display the [Choose Batch](#) dialog to select a batch item to open.



Go to the first file in the batch.



Go to the previous file in the batch.



Redo the current file in the batch.



Go to the next file in the batch.



Go to the last file in the batch.



Auto-advance through the batch.



Stop the auto-advance process.



Exit out of batch processing reports

Clipboard

Copy command



Use this command to copy the report or a single object to the clipboard.

Copying to the clipboard replaces the contents previously stored there.

To copy a single object, click the object to select it, and then choose the Copy command. The object will be placed in the clipboard.

To copy the entire report, make sure no object is selected in the report, and then choose the Copy command. The report will be copied as a graphic image to the clipboard.

Shortcuts

Keys: Ctrl + C on PC; Command + C on Mac

Paste command



Use this command to insert a copy of the clipboard contents onto the report. This command is unavailable if the clipboard is empty or if ModFit LT does not support the format of the data.

When the clipboard contains more than one format at a time, ModFit LT prompts you to select the format you want. Select the format you want from the list and click OK to paste the object.

After it is pasted, the object may be selected and dragged to a new location on the screen.

Supported formats include the following:

PC version: Text, Bitmap, PNG

Mac version: Text, Bitmap, PNG

Shortcuts

Keys: Ctrl + V on PC; Command + V on Mac

Databases

Databases



Databases

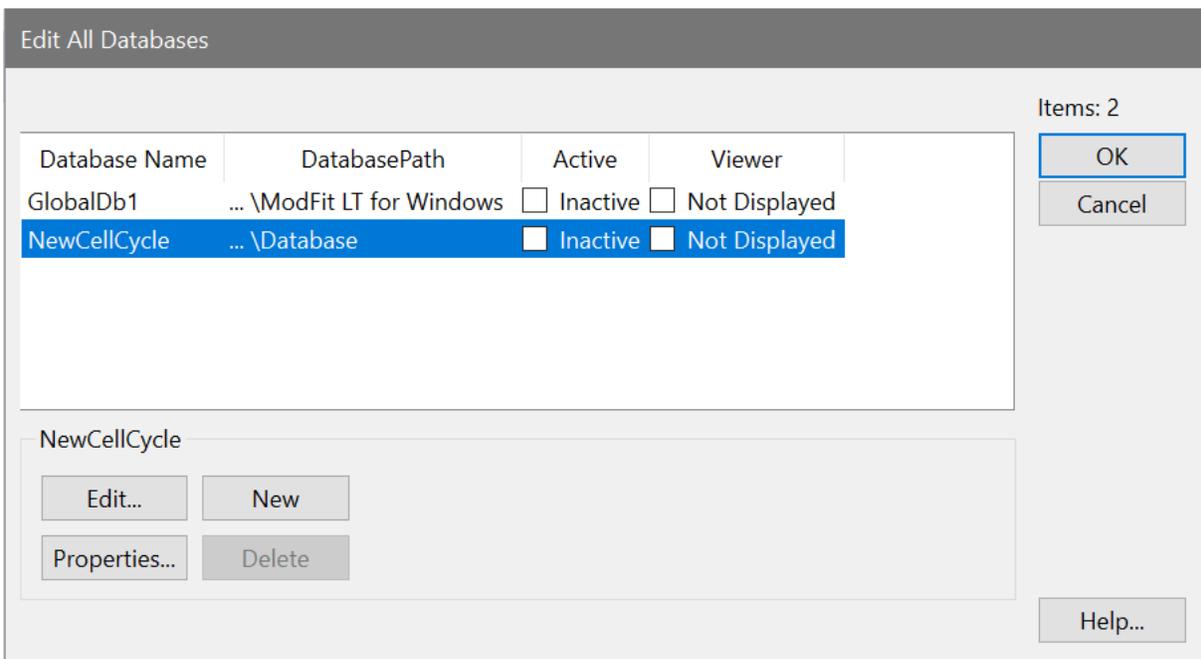
[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*

Use this dialog to add, delete or edit one or more databases. See [Databasing with ModFit LT](#) for more on databases.

The Edit All Databases dialog can be displayed by choosing **Edit Databases** from the **Edit** tab.



Overview

ModFit LT can have one or more databases defined at any time. The database format is ASCII-formatted, tab-delimited database. Any FCS keyword or analysis result can be mapped to a database field. A complete description of the functionality of the database system can be found in the [Databasing with ModFit LT](#) discussion.

Edit

If a database is selected, this option shows the [Edit Keyword Map](#) dialog box. You can also double-click the database item to activate this option.

Properties for the selected item

This button appears in the group of buttons below the listbox. It allows you to edit properties for the selected item in the list.

If a database is selected, the [Edit Properties for Database](#) dialog will be displayed.

New

This command creates a new, empty database.

Delete

This option will delete the selected database from the list.

OK

Put away the Edit All Databases dialog box.

Help

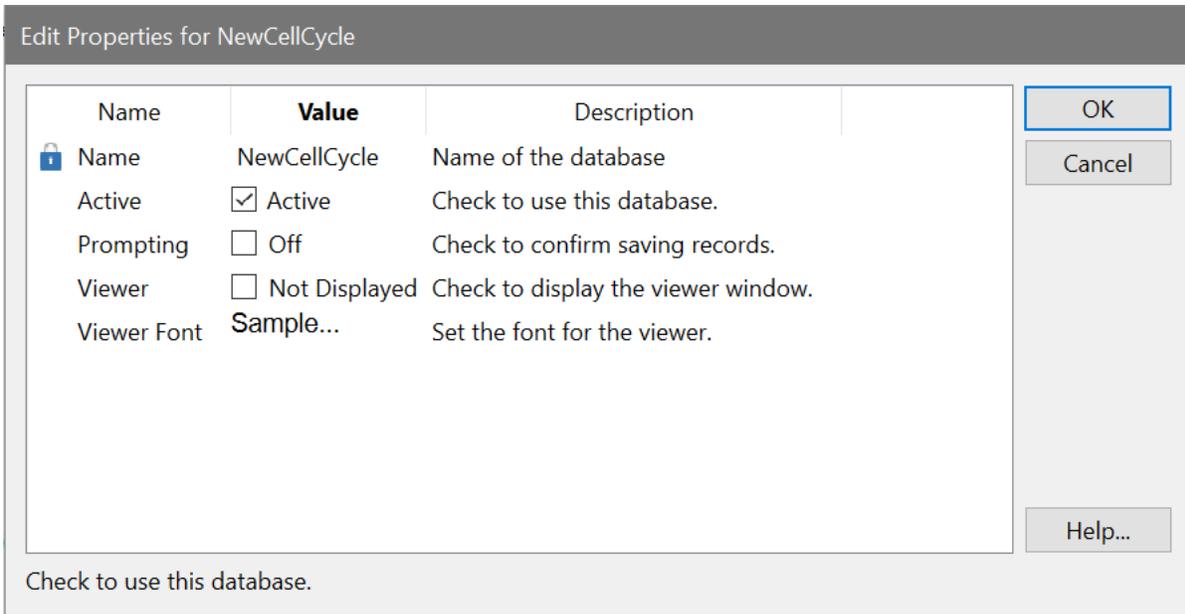
Show this help topic.

Edit Properties for Database

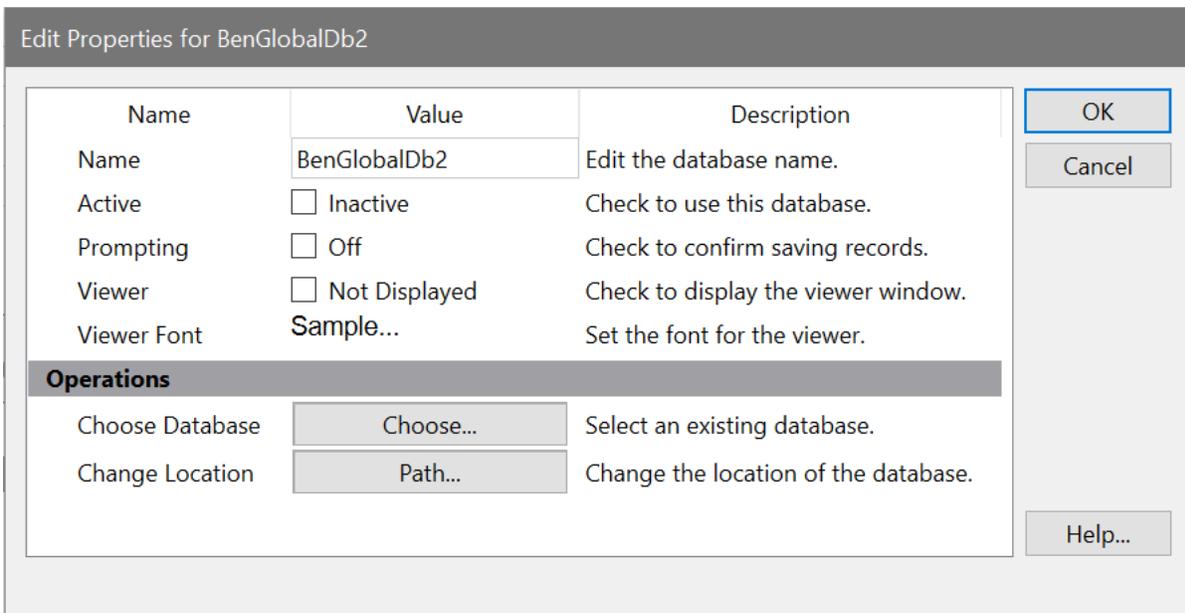
Purpose: To activate, display, and set other attributes of a database. See [Databasing with ModFit LT](#) for more on databases.

The Edit Properties for Database dialog can be displayed by choosing **Edit Databases** from the **Edit** tab, selecting the database you want, then clicking the **Properties** button. If the [Database Viewer](#) is displayed, clicking a button on the viewer's toolbar can also access this dialog.

When a *project* is created in ModFit LT, a database is automatically created for the new project in the Database folder of the project. The properties for a project database are shown here:



If you create a database using the New button in the Databases dialog, the database is considered to be a *global* database and is available for use by any project or non-project analysis. Additional properties are shown for global databases:



Overview

Each database can be activated independently. Additionally, the database can be set for prompting or automatic mode, and a viewer window can be displayed. This dialog also allows you to set the path and other properties for the database.

Active

Check this option to activate the database. When the database is active, it will attempt to store a record each time an analysis is performed. Uncheck this option to disable a database; records will not be saved in databases that are not active.

Prompting

Check this option if you want ModFit LT to prompt you each time it is ready to save a record. The prompt will allow you to confirm or cancel the saving of the record into each active database. Uncheck this option for automatic operation where ModFit LT stores the information without prompting.

Viewer

Check this option to display a [Database Viewer](#) for the database. The viewer displays the fields in columns and each row represents a record in the database. Additional options are available on the viewer window.

Viewer Font

Select a font to use for the database viewer.

Choose database (for global databases only)

This option allows you to choose an existing database on disk. Click the "Link to" button. A file selection dialog box will appear, allowing you to navigate to the existing database and select it. Linking to an existing database will rename the database in memory to match the one selected on disk, and the associated database map on disk will be loaded as well.

Change location (for global databases only)

This option allows you to change the path for the defined database. Click the "Path" button to display a Choose database location dialog. Navigate to the location in which you want to store the database.

If a database of the same name already exists in that folder, you will be offered three choices. First, you can choose to overwrite the database with the one you have defined. Secondly, you can choose to use the database that exists in the new location, replacing the structure you have defined in memory. This is convenient if you have a database already defined that you want to use. Or you can choose to cancel the action.

OK

Save property settings and put away the Edit Properties for Database dialog box.

Cancel

Close the dialog and discard any changes you have made.

Help

Show this help topic.

Edit Keyword Map

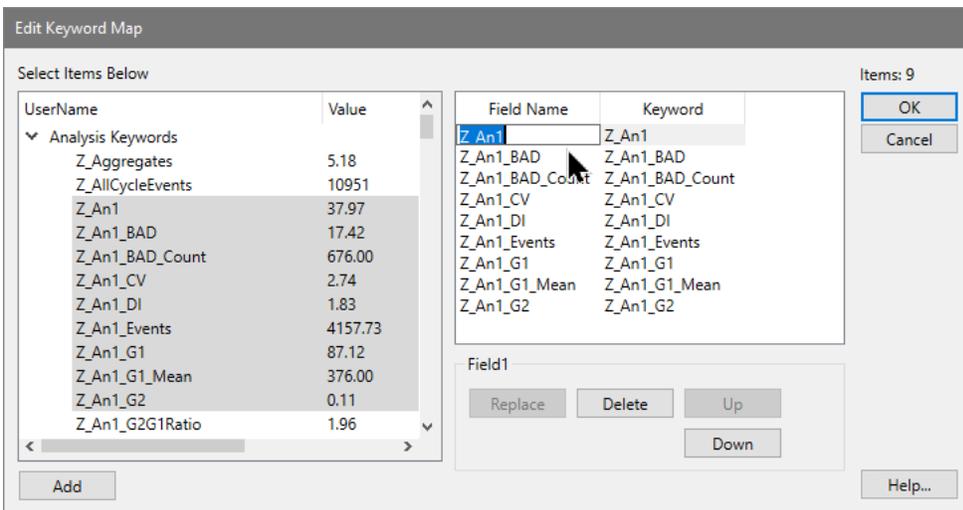
Research and Compliance Mode note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*

Purpose: Select the information you want to save in a database. See [Databasing with ModFit LT](#) for more on databases.

The Edit Keyword Map dialog can be displayed by choosing **Edit Databases** from the **Edit** tab, selecting the database you want to edit, then clicking the **Edit** button. If the [Database Viewer](#) is displayed, clicking a button on the viewer's toolbar can also access this dialog.



Overview

Each database in ModFit LT has its own structure, made up of a set of keywords to database and a matching set of fields in a database. The Edit Keyword Map dialog determines how the keywords are "mapped" to fields in the database.

Results Tree

This tree expands to display all of the available groups of keywords and the keywords in each group. A branch of the tree can be expanded by double clicking the mouse on it. Select a branch of the tree by clicking once on it.

This tree is used to select the keywords you want to store in the database. It allows multiple selection. See the discussion in [Databasing with ModFit LT](#) for more on proper selection of information to database.

Map Definition

This list displays the field names and the keywords they relate to. The first column showing the field name can be edited; other columns are for display only. To edit a field name, click once on the field to select it, then click again after a moment to enter edit mode.

Add

Click this button to add the keywords selected in the Results Tree to the database definition shown in the Map Definition list. Fields are named automatically to show the source of the information. You can also double-click on a keyword to add it to the definition.

Replace

The option is available when you select an item in the tree and an item in the Map Definition list. It allows you to map a new keyword to an existing field in the database.

Delete

Click this button to delete the fields selected in the Map Definition list from the database.

Up/Down

These buttons allow you to change the order of fields in the Map Definition list. Select one or more fields in the list, and then use the Up and Down buttons to move the item(s).

OK

Save changes to the database. If records already exist in the database, the structure of the records will be revised to match the new structure you have defined.

Cancel

Close the dialog and discard any changes you have made.

Help

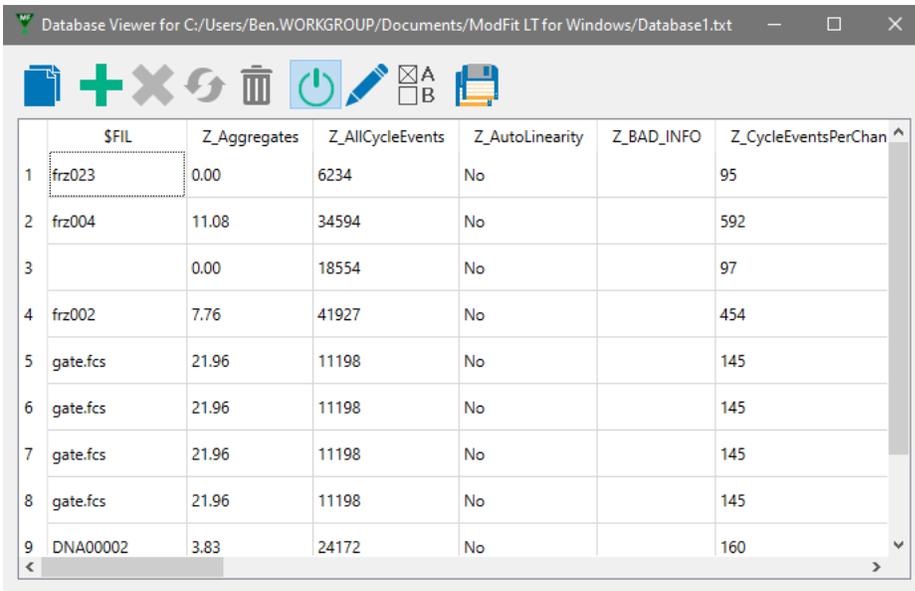
Show this help topic.

Database Viewer

Purpose: Display the contents of a database.

The Database Viewer can be displayed by choosing to show the viewer in the database properties. To do so, choose **Edit Databases** from the **Edit** tab, select the database you want, and then click the **Properties** button. In the [Edit Properties for Database](#) dialog, enable the viewer and click OK.

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	SFIL	Z_Aggregates	Z_AllCycleEvents	Z_AutoLinearity	Z_BAD_INFO	Z_CycleEventsPerChan
1	frz023	0.00	6234	No		95
2	frz004	11.08	34594	No		592
3		0.00	18554	No		97
4	frz002	7.76	41927	No		454
5	gate.fcs	21.96	11198	No		145
6	gate.fcs	21.96	11198	No		145
7	gate.fcs	21.96	11198	No		145
8	gate.fcs	21.96	11198	No		145
9	DNA00002	3.83	24172	No		160

Overview

This window can be displayed for any database you have created. The viewer displays the fields defined in the database as column headings. Each record in the database is shown as a row of information. The window can be scrolled horizontally and vertically if there is information out of view. Each column can be resized by dragging the partition between the column headings with the mouse.



At the top, a toolbar provides buttons to manually add, delete, and replace records.

Click the copy button to copy the database as text to the clipboard.

Click the "+" (add) button to add the current analysis information to the database.

Click the "-" (delete) button to delete the selected records from the database; if the button is grayed, there are no records selected.

Click the replace button to store the current analysis into an existing record in the database.

Additional buttons activate and deactivate the database, display the Edit Keyword Map dialog, display the Edit Properties for Database dialog, and backup the database.

See [Databasing with ModFit LT](#) for more on databases.

Add Record

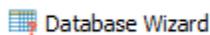


This button appears on the **Edit** tab of the ribbon bar. It can be used to manually trigger the addition of a record to active databases.

See also:

[Databases](#)

Database Wizard



Research and Compliance Mode note:

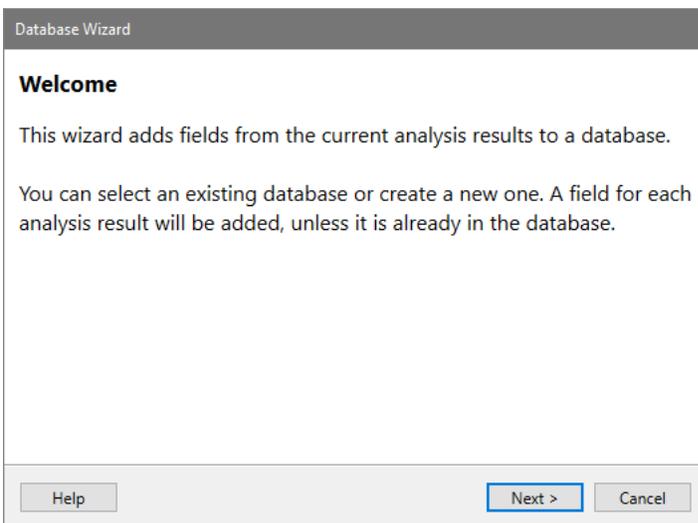
In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*

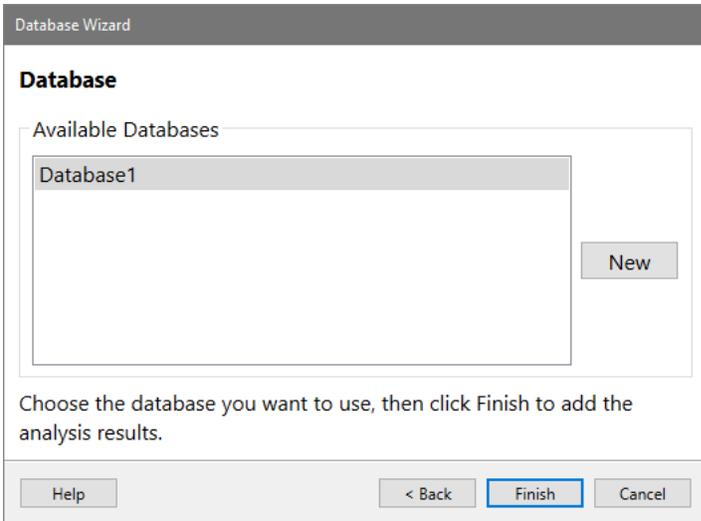
The Database Wizard is a tool that makes it easy to set up a database for the ModFit analysis results.

To use the wizard, start by [opening a data file](#) and analyzing it so that you have a set of analysis results on the report. Then, start the database wizard by clicking this button, found on the **Edit** tab of the ribbon bar.

The wizard's greeting page will display.



Click **Next** to proceed. The Database page of the wizard will display.



Select an existing database from the list or use the **New** button to create a new one. Once you have made your selection, click the **Finish** button to complete the wizard.

The wizard will add fields to the selected database for all of the analysis results that are shown on the report. If a field is already in the database, it will not be added again.

Exporting

Export PDF

Use this command to export the report to PDF format on disk.

When you select this option, the File Save dialog is displayed. Navigate to the location where you want to store the file, and then enter a name for the file. Click the Save button to store the file on disk.

Exporting is also available in batch processing. See [File Batch Settings](#) and [Report Batch Settings](#) for more.

Export Histogram Graphic

Use this command to export the graphics for the histogram to a file on disk. The default format of the graphic can be selected using the Options and Configuration dialog. Supported formats currently include Jpeg (jpg), Tiff (tif), Portable Network Graphics (png), and Scalable Vector Graphics (svg).

When you select this option, the File Save dialog is displayed. Navigate to the location where you want to store the graphic file, and then enter a name for the file. Click the Save button to store the file on disk.

Exporting is also available in batch processing. See [File Batch Settings](#) and [Report Batch Settings](#) for more.

See also: [Options and Configuration](#)

Export Report Graphic

Use this command to export the graphics for the entire report to a file on disk. The format of the graphic can be selected using the Options and Configuration dialog. Supported formats currently include Jpeg (jpg), Tiff (tif), Portable Network Graphics (png), and Scalable Vector Graphics (svg).

When you select this option, the File Save dialog is displayed. Navigate to the location where you want to store the graphic file, and then enter a name for the file. Click the Save button to store the file on disk.

Exporting is also available in batch processing. See [File Batch Settings](#) and [Report Batch Settings](#) for more.

See also: [Options and Configuration](#)

Export Fit Data

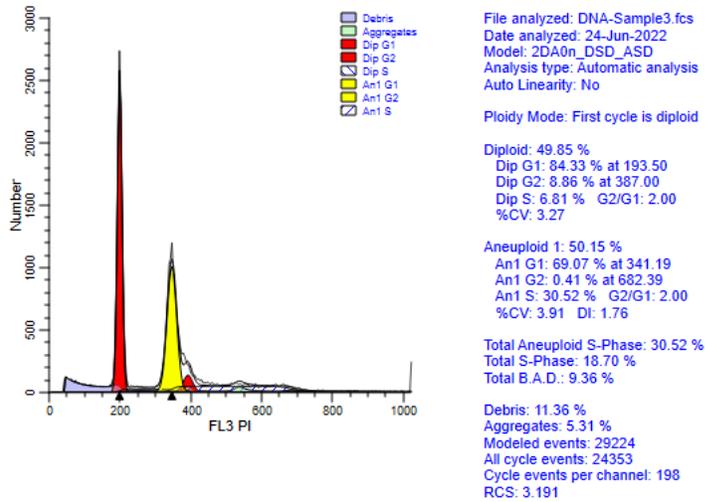
This command appears in the Export sub-menu of the File menu. After analyzing an FCS file, the command can be used to export the model components and raw data to a comma-

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separated value (CSV) file. The CSV file can be read by spreadsheet apps, such as Microsoft Excel, which can create additional charts and perform additional calculations on the data.

The output will depend on the model that was used to analyze the data. The first column in the output will be the raw data values for each channel in the 1P histogram. The second column is the "total" of all model components, or the overall fit. The next columns will represent each active component in the model. Finally, a column for "residuals" is included.

Here is an example. Suppose ModFit LT has performed the analysis shown below for a DNA Aneuploid sample.

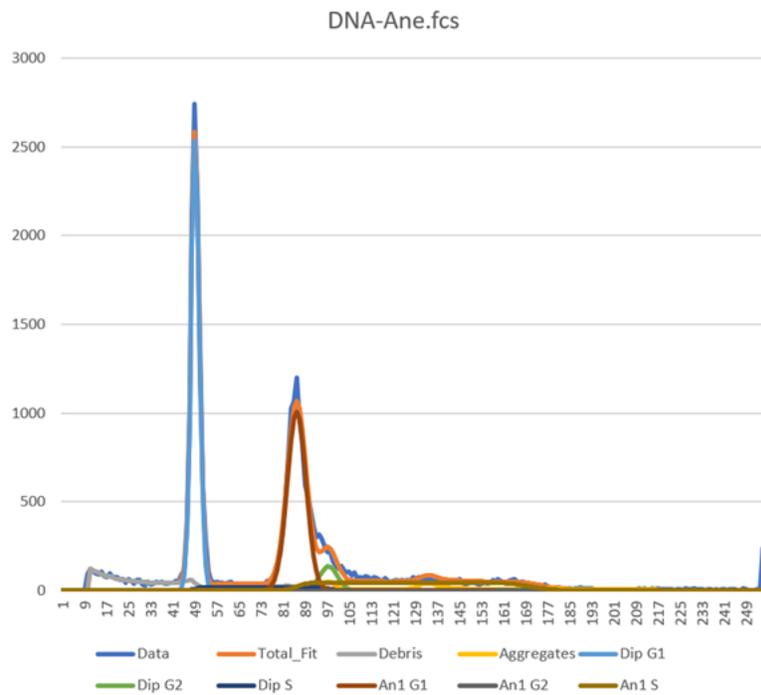


The Export Fit Data command would generate the following output:

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FL3 PI_Intensity	Data	Total_Fit	Debris	Aggregate	Dip G1	Dip G2	Dip S	An1 G1	An1 G2	An1 S	Residuals
0	0	0	0	0	0	0	0	0	0	0	0
4.01569	0	0	0	0	0	0	0	0	0	0	0
8.03137	0	0	0	0	0	0	0	0	0	0	0
12.0471	0	0	0	0	0	0	0	0	0	0	0
16.0627	0	0	0	0	0	0	0	0	0	0	0
20.0784	0	0	0	0	0	0	0	0	0	0	0
24.0941	0	0	0	0	0	0	0	0	0	0	0
28.1098	0	0	0	0	0	0	0	0	0	0	0
32.1255	0	0	0	0	0	0	0	0	0	0	0
36.1412	88	0	0	0	0	0	0	0	0	0	88
40.1569	124	119	119	0	0	0	0	0	0	0	5
44.1725	108	112	112	0	0	0	0	0	0	0	-4
48.1882	96	104	104	0	0	0	0	0	0	0	-8
52.2039	88	98	98	0	0	0	0	0	0	0	-10
56.2196	105	92	92	0	0	0	0	0	0	0	13
60.2353	81	86	86	0	0	0	0	0	0	0	-5
64.251	76	81	81	0	0	0	0	0	0	0	-5
68.2667	94	77	77	0	0	0	0	0	0	0	17
72.2824	76	73	73	0	0	0	0	0	0	0	3
76.298	72	69	69	0	0	0	0	0	0	0	3
80.3137	72	66	65	0	0	0	0	0	0	0	6
84.3294	59	63	62	0	0	0	0	0	0	0	-4
88.3451	63	60	60	0	0	0	0	0	0	0	3
92.3608	45	58	57	0	0	0	0	0	0	0	-13
96.3765	64	55	55	0	0	0	0	0	0	0	9
100.392	51	53	53	0	0	0	0	0	0	0	-2
104.408	40	52	51	0	0	0	0	0	0	0	-12
108.424	58	50	50	0	0	0	0	0	0	0	8

A chart can be easily created in Microsoft Excel based on the exported data:



Logging

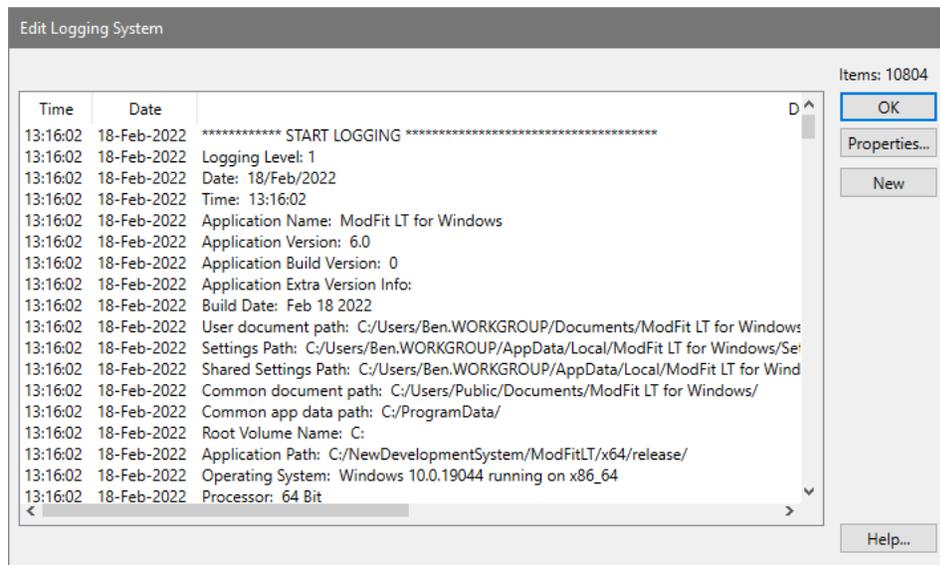
Edit Log



Research and Compliance Mode note:
In RUO mode, users can turn logging on and off, and set properties.
In Compliance mode, logging is always enabled.

ModFit LT allows you to create a log of operations that you perform. This is useful if you have any difficulties with the program and need to interact with the Technical Support team. Logging is always enabled when the program is running in Compliance mode.

To view the current log, click the Logging button on the **Edit** tab of the ribbon bar.

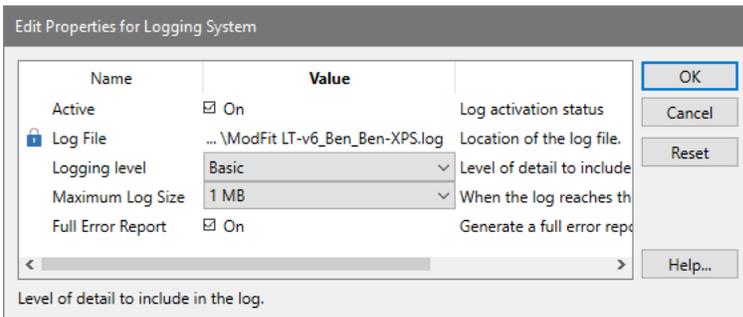


To adjust properties of the log, click the **Properties** button. See [Edit Properties for Log](#) for details on the log settings.

Edit Properties for Log

Research and Compliance Mode note:
In RUO mode, users can turn logging on and off, and set properties.
In Compliance mode, logging is always enabled.

This dialog allows you to activate and adjust the program's logging features, useful in isolating problems that you may have in working with the program.



Active

To enable logging of program operations, check this option. This file is a plain text file, with fields separated by tabs. It can be opened in most text editors, or attached to email messages.

To disable logging, clear the check from this checkbox.

Log File

This read-only field shows the name of the log file on disk.

Logging Level

This field selects the level of detail stored in the log. Choose from "Basic" and "Comprehensive". Comprehensive logging is typically only used when working with a support technician at Verity Software House to determine the cause of a problem.

Maximum Log Size

Select the maximum size for the log file. If this is set for Unlimited, the log will grow without limitation (not recommended).

Full Error Report

Generally, this option should not be enabled. However, Technical Support may ask you to enable this option to determine the cause of a problem.

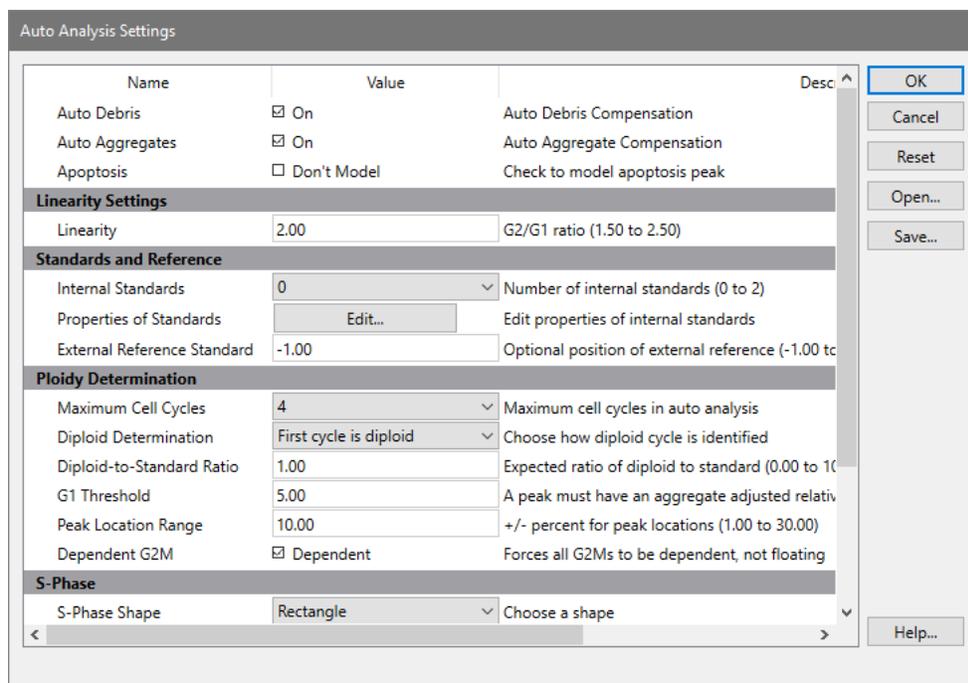
Preferences

Auto Analysis Settings



[Research and Compliance Mode](#) note:
 In RUO mode, all users can perform these tasks.
 In Compliance mode, only **ModFitAdmins** have permission to perform them.

This dialog is used to configure several properties of the Auto Analysis command.



AutoDebris

When this option is checked, the program will enable the AutoDebris component to model debris in the data file during Auto Analysis. Debris is often created in the sample preparation process. If the option is not checked, the AutoDebris component will be disabled.

AutoAggregates

When this option is checked, the program will enable the AutoAggregate component to model aggregation in the data file during Auto Analysis. Aggregation in the sample can produce a continuum of particles that are stuck together, significantly affecting S-Phase estimates. If the option is not checked, the AutoAggregate component will be disabled.

Apoptosis

NOTE: We strongly recommend that this option should be disabled in your configuration. To get more robust and accurate results, use additional markers for apoptosis detection.

If this option is checked, the first peak detected after any internal standards will be considered an apoptotic population and modeled with a Gaussian.

Linearity Settings

Linearity

This value is used in the computation of some G2M positions, and in the AutoAggregate model component. The default value is 2.0, as G2M peaks are expected to appear at two times the associated G1 peak. Adjust this value to match the known linearity setting for your instrument.

When Auto Analysis does not detect a distinct G2M peak for a cycle, it assumes the location of the G2M peak to be at the G1 position times this linearity setting. This is called a *dependent* G2M, because its position depends on the G1.

The linearity value is used by the AutoAggregate model component to determine the expected locations of peaks created by aggregation.

Standards and References

Internal Standards

Select the number of internal standards that your data files contain.

Most DNA internal standards are designed to appear to the left of the first cycle's G1 population. By default, the program will assume that the first peaks it finds should be assigned to the number of standards you select here. Use the Properties of Standards button below to give the program additional information about the internal standards in your samples.

Properties of standards

Click this button to display the [Standards](#) dialog. This dialog allows you to edit the names and expected locations of internal standards in your samples. By entering expected locations, the program can more accurately determine which peaks are standards, and where the Diploid population occurs. See the description of *Diploid determination* below for more on using this value to assign ploidy.

External reference standard

If you enter a value of an external standard location, you can make use of this value in assigning ploidy labels to the cycles that AutoAnalysis finds. For example, if you run a normal Diploid control at a particular channel, you can enter that channel position in this edit box.

See the description of *Diploid determination* below for more on using this value to assign ploidy.

Ploidy Determination

Maximum cell cycles

Select the maximum number of cell cycles that Auto Analysis can model. By default, this is set to "Unlimited". If the program detects numerous false peaks in your data files, you may want to specify a maximum of 3 or 4 cycles. See the Peak Finder Settings for more information about finding peaks.

Diploid determination

This option determines the method that Auto Analysis will use to identify the Diploid cycle in your data files.

First cycle is Diploid

This is the default selection. Use this option if you want the first (leftmost) cell cycle always to be classified as the Diploid cycle.

Based on external reference

Use this option if you have entered an expected location for an external reference above, and you want to use that position as the basis for identifying the Diploid cycle. The expected Diploid location will be computed as the *External reference standard* location multiplied by the *Diploid-to-Standard ratio*.

Based on Standard1 and Based on Standard2

Use this option if you have entered expected location(s) for internal standards above in *Properties of standards*, and you want to use one of the standards to compute the expected location of the Diploid cycle. The expected Diploid location will be computed as the *Expected position* of the standard multiplied by the *Diploid-to-Standard ratio*.

Examples:

1. Suppose you have one internal standard in your samples that is normally found at channel 38 in the DNA histogram, and that the normal Diploid G1 location is 1.3 times the standard's position. You would enter the expected location of the standard using *Properties of standards*, select *Based on Standard1* for the *Diploid determination* option, and enter 1.3 in the *Diploid-to-standard* edit box.

2. Suppose you run a normal Diploid control that is positioned at channel 55 everyday, and that you expect this to be the location of Diploid cycles in your test samples as well. You would enter 55 in the *External reference standard* field, select *Based on external reference* for the *Diploid determination*, and enter 1.0 for the *Diploid-to-Standard ratio*.

Diploid-to-Standard ratio

This value is used if you have the *Diploid determination* option selected for Based on external reference, Based on Standard1, or Based on Standard2. The expected location for the Diploid G1 is computed by multiplying this value times the expected location of the reference or standard.

When *Diploid determination* is set for First cycle is Diploid, this value is not used.

G1 threshold

This value sets the minimum relative height for a peak to be considered a G1, after adjusting the height for aggregates. The relative height is a value relative to the largest peak, where the largest peak is 100%. The default setting for G1 threshold is 5%. To allow smaller peaks to be considered G1 peaks, lower the value. To make the setting more restrictive, increase the setting.

Peak location range

This value controls how much variation the program allows in looking for a peak at an expected location. It is a percentage of the expected location. If, for example, the expected location of the Diploid G1 is channel 50 and this value is set for 10%, Auto Analysis will look for a peak between channel 50 plus and minus 5 channels, or 45 to 55.

Dependent G2M

With this option enabled, Means and Standard Deviations for all G2M components will be dependent on the associated G1 components in models created by Auto Analysis. This setting creates more consistent analysis results from one operator to the next, and it is recommended. With the option disabled, any G2M component that has a well-defined Peak Finder peak will have a floating Mean and Standard Deviation. Those without well-defined peaks will be dependent on associated G1 components.

S-Phase

S-Phase shape

This option controls the mathematical function that is used to model S-Phase.

Rectangle

This is the default selection. It provides the most conservative estimate of S-Phase and is the recommended option.

Trapezoid

This option provides one additional degree of freedom over the Rectangle.

Polynomial

This option adds two degrees of freedom over a rectangle.

Number of compartments

Select the number of compartments to use in modeling S-Phase. By default, this option is 1, the recommended value.

DI for S-Phase

Enter a DNA index value (DI) below which the program will disable S-Phase modeling for the lower of two overlapping cycles. The default and recommended value is 1.3.

When two cell cycles are significantly overlapped, the S-Phases can be arbitrarily inflated or deflated in the modeling process. In this situation, it is better to disable the S-Phase for the lower-positioned cycle. The DI for S-Phase setting controls the threshold for this decision.

Tetraploid

G2M Threshold

This value determines when a peak at a G2M location is considered to be too large for a G2M peak, and is classified as a G1 peak for another cycle. The value used in making this decision is a simple statistical fit of the peak, *not* the modeled result for the peak.

The value is a percentage of the estimated area of the associated G1 peak. By default, the G2M can be up to 15% of its associated G1 before it is reclassified as a G1 of another cycle.

Tetraploid location range

This value controls how much variation the program allows in looking for a potential Tetraploid peak. It is a percentage of the expected location.

Open

If you have previously saved Auto Analysis settings to disk, you can use the Open button to load those settings into the program.

Save

Use this option if you want to store the current Auto Analysis settings to a file on disk. The standard file save dialog box will be displayed, allowing you to navigate to a location and name the file to save.

Reset

Click this button to restore the Auto Analysis values to the factory default settings.

OK

Click this button to accept the changes you have made and close the dialog.

Cancel

This option closes the dialog and discards any changes you might have made.

Help

This option displays the on-line help for this dialog.

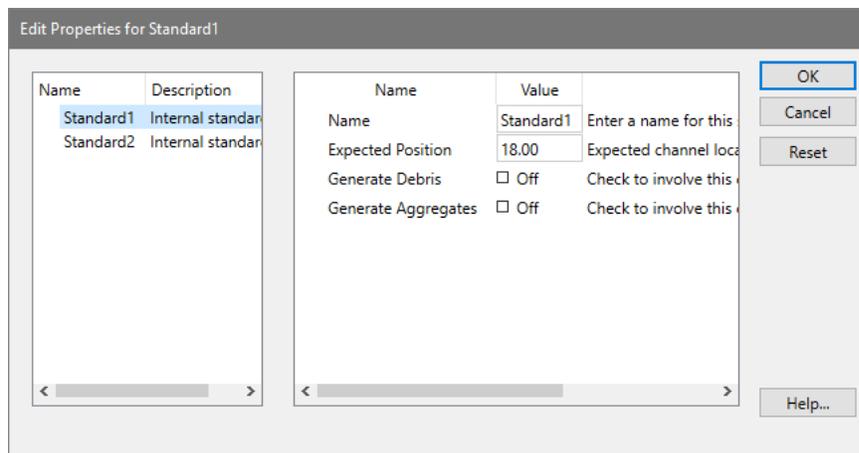
Standards

Research and Compliance Mode note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** have permission to perform them.*

This dialog allows you to edit how ModFit LT interprets the internal standards in your data files.



If your files contain internal standards, you should fill in the expected location for each standard in this dialog. Doing so will assist the program in identifying standard peaks, as well as to correctly classify cell cycles. After you enter location values in this dialog, you can choose a ploidy determination method in the Auto Analysis Settings dialog that makes use of the location.

Select the standard that you want to edit in the list on the left and the right hand list will show the properties that you can adjust for the standard.

See also: [Properties for Standards](#)

Properties for Standards

Name

Enter a name for the standard. This name will be assigned to a range for the standard peak. The range can be viewed and adjusted by clicking the Range button on the toolbar.

Expected position

Enter the channel location on the histogram where the standards peak is typically found. You should ensure that your protocols for running samples on your flow cytometer consistently maintain this position for the standard.

Generate debris

When checked, internal standard components are considered to be involved in the generation of debris in the histogram.

Note: Models that are created by AutoAnalysis ignore internal standards from debris generation.

Generate aggregates:

When checked, internal standard components are considered to be involved in the generation of aggregates in the histogram.

Note: Models that are created by AutoAnalysis ignore internal standards from aggregate generation.

See also: [Standards](#)

Peak Finder Settings



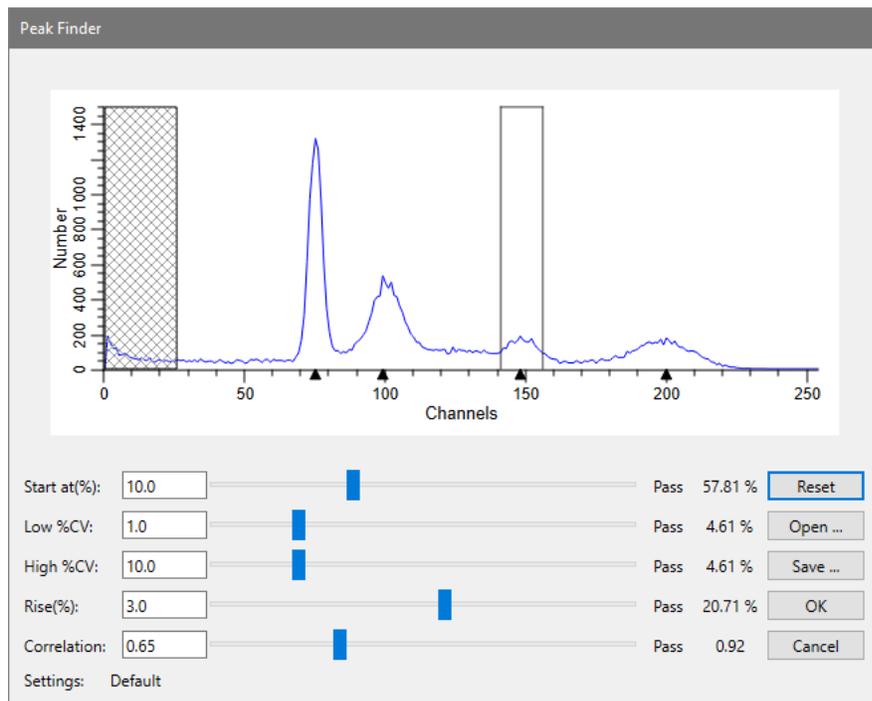
Research and Compliance Mode note:
In RUO mode, all users can perform these tasks.
*In Compliance mode, only **ModFitAdmins** have permission to perform them.*

The Peak Finder dialog adjusts the settings used by the system to determine how peaks will be identified in your samples. The peak finder statistically determines what populations are peaks in the histogram data, and then applies filters to remove unwanted peaks from consideration.

The peak finder is of fundamental importance in Automatic Analysis. Therefore, it is very important to approach changes to the peak finder with care and thoughtfulness.

Changes to these settings will affect how the program defines a peak, which in turn will alter ploidy detection, model selection, and analysis results in Automatic Analysis. Test any changes you make on a reasonable sampling of your data files. Make changes to improve the

overall effectiveness of the peak finder, not to handle an exceptional sample. Exceptional samples should be handled using [manual analysis](#).



The dialog displays the analysis histogram. The graphic indicates the currently identified peaks with black triangles at the base of the histogram. In addition, there are controls that allow you to adjust the peak finder settings and see the effect immediately.

As you move your mouse pointer over the histogram, boundaries of peaks are drawn over the data, illustrated in the graphic above. The status of the peak for each criterion is shown next to the control for the filter. Using this information, you can determine why the peak finder excludes peaks, and make adjustments to the filters accordingly.

Start at

The value controls the point at which ModFit LT starts looking for peaks. It allows you to skip over channels where your data has debris that may be misinterpreted as a peak. The value is a percentage of the histogram scale, and higher values move the starting point to higher channel location.

Low %CV

This value controls the low %CV cutoff for data to be considered a peak. %CV reflects the broadness of the peak; those peaks which have %CV lower than this filter are excluded. To remove more narrow peaks, increase the value. To allow more peaks, lower the value.

High %CV

This value controls the high %CV cutoff for data to be considered a peak. %CV reflects the broadness of the peak; those peaks which have %CV higher than this filter are excluded. To

allow more peaks to pass the filter, increase the value. To reduce the number of peaks that pass the filter, reduce the value.

Rise

This value controls how far a peak must rise above adjacent data to be considered a real peak. Peaks that extend above adjacent data less than this value are excluded from the peak finder. To increase the minimum rise for a peak and reduce the number of passing peaks, increase this value. To reduce the filter and allow more peaks to pass, reduce the value.

Correlation

This value controls the minimum correlation coefficient allowed for a peak to pass this "shape" filter. Each potential peak is fit with a Gaussian curve; the resulting correlation coefficient is compared to this filter. To increase the minimum allowable correlation coefficient and reduce the number of passing peaks, increase the value. To increase the number of peaks that pass the filter, reduce the value.

Open

If you have previously saved peak finder settings to disk, you can use the Open button to load those settings into the program. This is useful if you analyze sets of data that have different peak characteristics.

Save

Use this option if you want to store the current peak finder settings to a file on disk. The standard file save dialog box will be displayed, allowing you to navigate to a location and name the file to save.

Reset

Click this button to restore the peak finder values to the factory default settings.

OK

Click this button to accept the changes you have made and close the dialog.

Cancel

This option closes the dialog and discards any changes you might have made.

Help

This option displays the on-line help for this dialog.

Options and Configuration

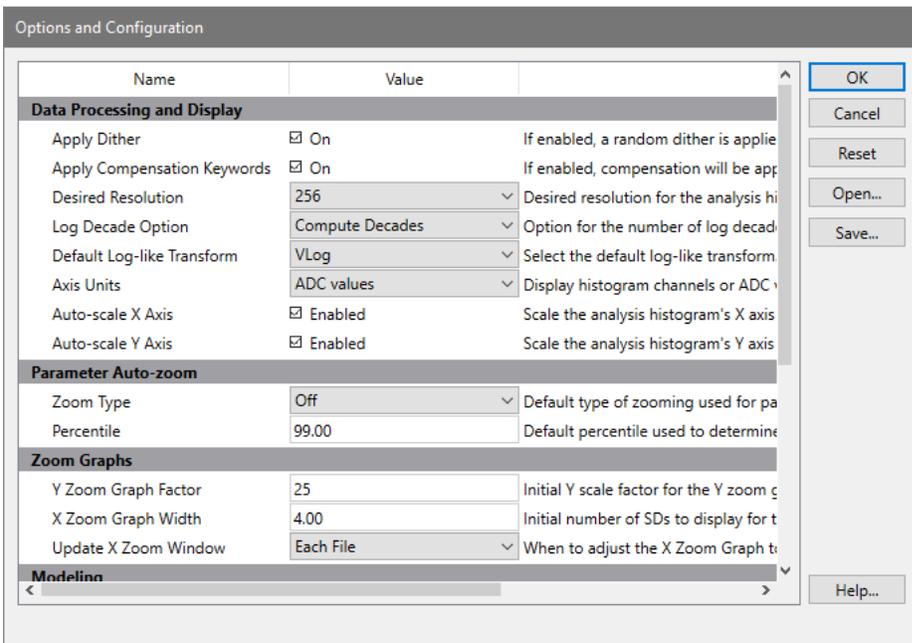


Research and Compliance Mode note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** have permission to perform them.*

This dialog is used to set program configuration options.



Data Processing and Display

Apply Dither

When enabled, a small random value is added to listmode events to make the distributions continuous. This is enabled by default.

Apply Compensation Keywords

When enabled, the program will read compensation keywords from the FCS file and apply compensation defined in those keywords.

Desired resolution

Select the desired resolution for the analysis histogram. It is strongly recommended that you use the default setting of 256 channels to improve performance and consistency. The histogram will be displayed and analyzed at the desired resolution, provided that the parameter's resolution is greater than or equal to the desired resolution.

Log Decade Option

This option determines how many decades will be displayed for listmode data. Choose between "Computed Decades" and "Force Four Decades". Computed decades are based on the ADC resolutions stored in the FCS file keywords.

Default Log-like Transform

Choose the transform to use for log distributed data, by default. Options include Log, HyperLog, Hyperbolic Sine, and VLog.

Axis Units

Choose between "Histogram channels" and "Data (ADC) values". Historically and by default, ModFit displayed the axis with histogram channel tic marks, and results were reported on that scale. To display the axis with the true resolution of the FCS data, choose "Data (ADC) values"; this will also change results to be on the ADC scale.

Auto-scale X axis

Auto-scale Y axis

These options control the auto-scaling of the main histogram graphic. When checked, each data file is automatically scaled on the X-axis and/or Y-axis as it is opened. The Y scaling is set to the height of the highest peak. The low X scale is set to 0. The high X scale is set to the highest channel that contains data, to a maximum of resolution minus 1.

Uncheck one or both of these options to leave the scaling unchanged as new data files are opened. This is convenient for studies where it is useful to see all histograms on a fixed scale.

Parameter Auto-zoom

Zoom Type

Choose how this parameter should be zoomed when ModFit reads it.

Off: no zooming is performed.

To highest used channel: zooms to the highest channel that has events in it.

To fixed upper boundary: zooms to the channel defined in the Fixed Upper Boundary edit box below.

To Percentile: zooms to a percentile location in the data.

Percentile

This field is used when To Percentile is selected for Zoom Type. It defines the percentile of data to include in the zoomed area.

Zoom Graphs

These settings determine the default values for X and Y Zoom graph objects. These objects can be placed on the report to show the histogram zoomed in the X or Y scale, respectively.

Y Zoom Graph Factor

Enter the initial value for Y Zoom graph objects. The value is the number of times to magnify the scale, e.g. 25 means multiply data points on the Y scale by 25.

X Zoom Graph Width

Enter the initial width as a number of standard deviations to display in the X Zoom graph object.

Update X Zoom Window

The X Zoom window object on the report can adjust to the position of peaks in each file, or only when created. Select the appropriate option.

Modeling

Dip G2M Fraction

This value is used to compute the relative area of the Diploid G2M in Tetraploid models. Because the Diploid G2M in a Tetraploid model is in the same location as the Tetraploid G1 peak, the program disables modeling of the Diploid G2M. After the model fit is completed, the program adjusts the Tetraploid G1 by subtracting a portion of its area and assigning it to the Diploid G2M. The adjustment is computed by multiplying the Diploid G1 area by the Dip G2M Fraction. The default value is 0.08, or 8%, which means that the Diploid G2M area will be 8% of the Diploid G1, and the Tetraploid G1 will be reduced by the same area.

Display nRCS

This option enables the display of the "Normalized Reduced Chi Square", or nRCS, on the report. The nRCS is an RCS value computed on files with > **Target Area for nRCS events**. When RCS is computed on files with a large number of events, it increases simply due to the additional events. This makes it difficult to determine whether the fit is good or bad. The nRCS addresses this by scaling the data to the **Target Area for nRCS events** and then computing RCS on that. The result is a value which behaves well even with large event counts.

Target Area for nRCS

This value determines the number of events to use for the nRCS calculation. See the discussion of nRCS above.

AutoLinearity

These settings control the AutoLinearity function of the program. This feature is available when batch processing reports, and in the Quick Model Editor. See [Using AutoLinearity](#) for more on this feature.

Low linearity

This field sets the lowest value used by the AutoLinearity operation. Values for this field must be between 1.85 and 1.94, inclusive.

High linearity

This field sets the highest value used by the AutoLinearity operation. Values for this field must be between 1.95 and 2.2, inclusive.

Miscellaneous

Copy/Save DPI

This option controls the quality of the graphics copied to the clipboard and saved to files by the program. DPI stands for dots per inch, with larger values providing higher quality images and larger file sizes.

Default Graphic output

Choose the desired graphic format to create when exporting histogram and report graphics. Export commands are found in the File menu and in the File and Report Batch Settings dialog.

Supported formats currently include bitmap (bmp), JPeg (jpg), Tiff (tif), Portable Network Graphics (png), and Scalable Vector Graphics (svg).

PDF Output Quality

This option controls the quality of the PDF files created by the program. Generally, higher quality PDF files are larger than lower quality files.

Parameter Database

ModFit LT keeps track of which parameters from the FCS file you have used for gating and analysis. This command allows you to view and edit settings for these parameters, including whether they are linear or log. See [Edit Parameter Database](#) for more information.

Save Recovery Backup

ModFit can save your work periodically so that if there is a problem you can recover. Choose the time interval for automatic backups.

Status Bar Text

The field determines what will be displayed on the status bar. By default, it is set to "Project: [MF_PROJECT_NAME]", which displays the word Project: followed by the name of the current project. Any of ModFit's keywords can be used in this field, surrounded by square brackets, and the resulting expression will appear on the status bar.

Startup Action

This option determines what happens when ModFit LT is opened. By default, it will **Load the last project**. Other options include **Don't open or create a project**, **Open a project**, and **Create a new project**.

Copy Data into Project

This preference determines the default state for the "Copy Data Files" option in the [New Project](#) dialog. Enable this option if files should typically be copied into the project folder. Disable the option if files should typically *not* be copied into a project folder.

Open

If you have previously saved configuration settings to disk, you can use the Open button to load those settings into the program.

Save

Use this option if you want to store the current configuration settings to a file on disk. The standard file save dialog box will be displayed, allowing you to navigate to a location and name the file to save.

Reset

Click this button to restore the configuration values to the factory default settings.

OK

Click this button to accept the changes you have made and close the dialog.

Cancel

This option closes the dialog and discards any changes you might have made.

Help

This option displays the on-line help for this dialog.

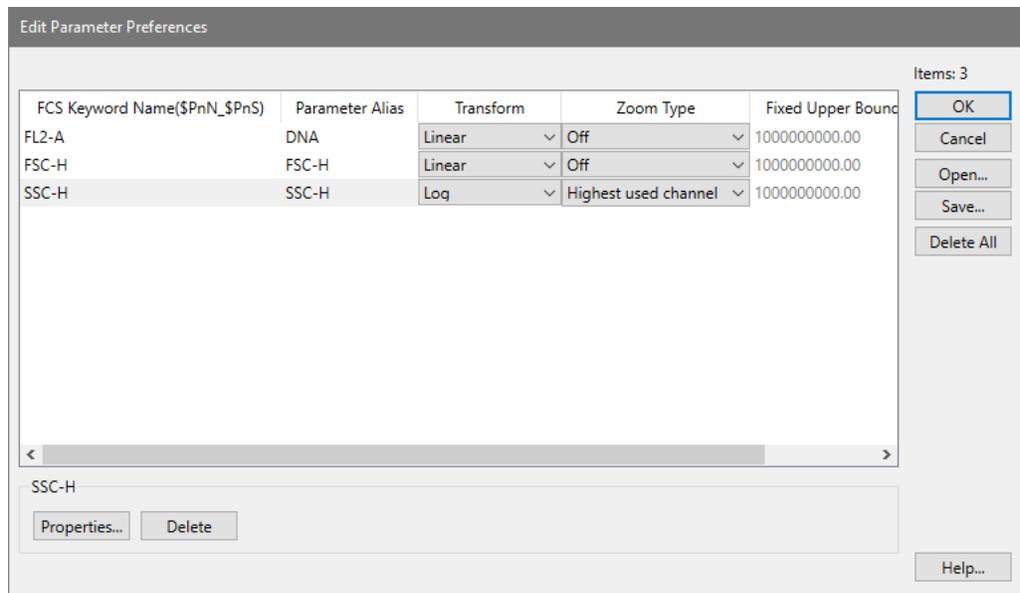
Edit Parameter Database

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** have permission to perform them.*

This dialog displays the parameters that have been customized by the user.



FCS Keyword Name(\$PnN_\$PnS)

This field identifies the entry and cannot be edited. The label is composed of two keywords for a specific parameter in the FCS file: the \$PnN and \$PnS keywords. These keywords allow ModFit to distinguish your preferences for each uniquely-identified parameter.

Parameter Alias

This field can be edited. It is displayed on graphs that display this parameter.

Transform

Select the transformation that you want to use for this parameter. Choices include:

- Linear
- Log
- HyperLog
- Hyperbolic Sine
- VLog

Zoom Type

This field allows you to set up automatic parameter "zoom". It is useful for parameters where only a small portion of the dynamic range is used. For example, with DNA samples acquired on an Accuri instrument, only a small portion of the channels are used to store data.

The default setting is for zooming to be "Off". Other options include:

"Highest used channel": zooms the data to exclude any empty channels on the high end of the parameter's range.

"Fixed upper boundary": zooms the data to exclude channels above a specified channel.

"Percentile": zooms the data to exclude channels above the specified percentile of the data area.

Fixed Upper Boundary

This field is used if Zoom Type is set for "Fixed upper boundary". It defines the channel to which you want to zoom. Channels above this channel will be excluded.

Percentile

This field is used if Zoom Type is set for "Percentile". Enter the percentile of data you want to include.

OK

Click this button to accept the changes and close the dialog box.

Cancel

Click this button to discard any changes and close the dialog box.

Open

If you have previously saved parameter preferences to disk, you can use the Open button to load those preferences into the program.

Save

Use this option if you want to store the current parameter preferences to a file on disk. The standard file save dialog box will be displayed, allowing you to navigate to a location and name the file to save.

Delete All

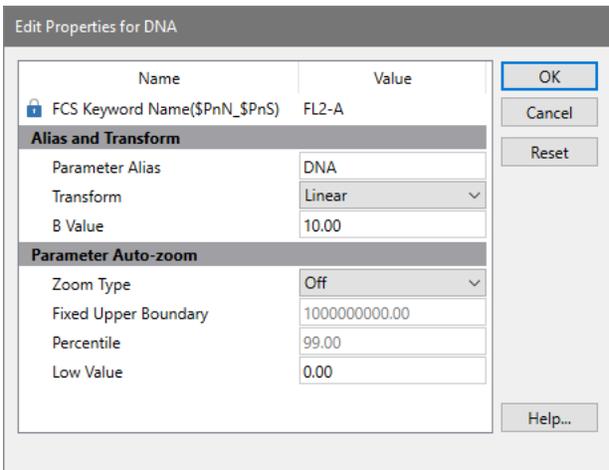
Click this button to clear out all entries from the parameter preferences. It is recommended that you Save your parameter preference to a file prior to deleting all, in case you want to restore settings to a previous state.

Edit Properties for Parameter

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.



FCS Keyword Name

This is the name from the FCS file for the parameter.

Alias

This field contains the name to display when this parameter is used in ModFit. It can be edited, and it appears on the axis of the graphs that display the parameter.

Transform

Choose either a Log or Linear transform to use for this parameter.

Parameter auto-zoom

Zoom Type

Choose how this parameter should be zoomed when ModFit reads it.

Off: no zooming is performed.

To highest used channel: zooms to the highest channel that has events in it.

To fixed upper boundary: zooms to the channel defined in the Fixed Upper Boundary edit box below.

To Percentile: zooms to a percentile location in the data.

Fixed Upper Boundary

This field is used when To fixed upper boundary is selected for Zoom Type. It defines the channel location to zoom to.

Percentile

This field is used when To Percentile is selected for Zoom Type. It defines the percentile of data to include in the zoomed area.

OK

Click this button to accept the changes and close the dialog box.

Cancel

Click this button to discard any changes and close the dialog box.

Edit User Equations



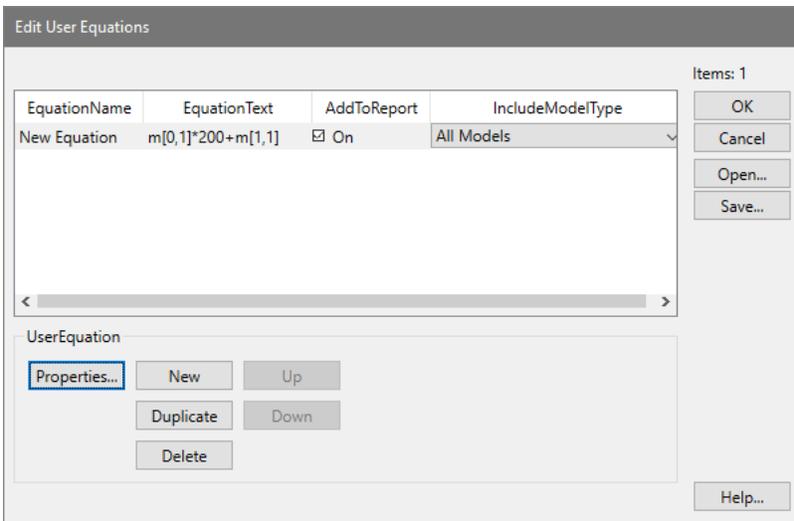
[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*

ModFit LT allows you to create additional equations to compute when the model is executed, either with Auto Analysis or Execute Model. Equations that you enter here are remembered when the program closes. They can be added to databases and displayed on the ModFit LT report page as keywords.

These equations must follow the syntax described in the section on [Equation syntax](#).



To add a new equation, click **New** and enter the equation (see [Equation syntax](#) for details).

To change an equation, select the equation in the list and click the **Properties** button, or edit the equation directly. See [Edit Properties for Equation](#) for details.

To display an equation on the report, check the **Add to Report Results** checkbox.

Use the **Model Type** drop-down list to determine which model types will compute and display your equation.

To save the current equations to disk so that they can be opened on another computer, click the **Save** button.

To open a previously saved set of equations from disk, click the **Open** button.

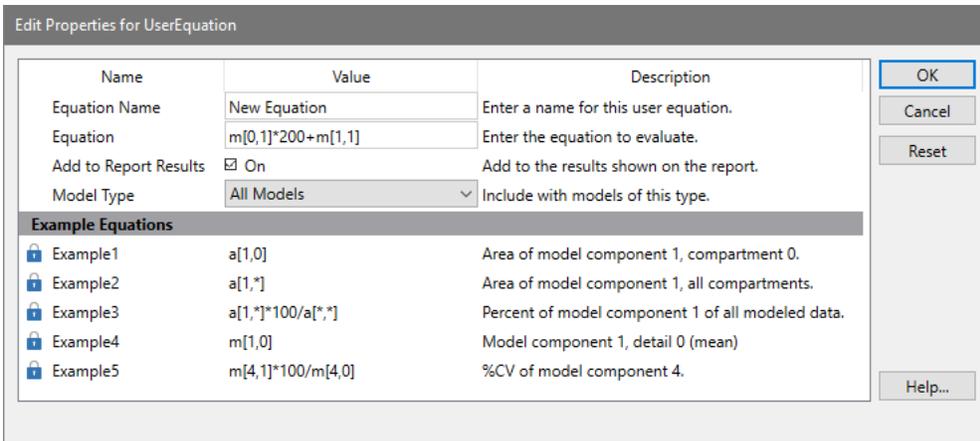
Edit Properties for Equation

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*

ModFit LT allows you to create additional equations to compute when the model is executed, either with Auto Analysis or Execute Model. These equations must follow the syntax described in the section on [Equation syntax](#).



You can enter a name for each equation and the equation expression. The name will be used as a keyword name, prefixed with "USER_", and can be selected for databasing and display on the report.

For example, if you name your equation, "SumOfAneuploidAreas", you can select the keyword "USER_SumOfAneuploidAreas" to display on the report using the Tools menu, Keywords command. You can also select this keyword to be databased using the Edit menu, Databases command.

Settings Manager

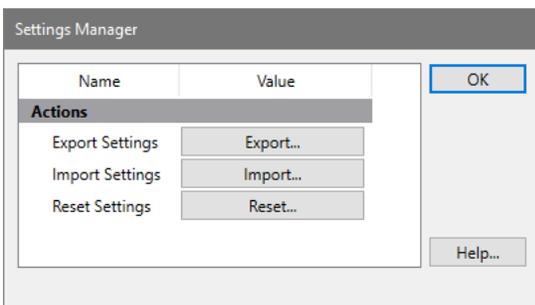


Research and Compliance Mode note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** have permission to perform them.*

This dialog allows you to manage the program settings. You can back-up settings so that they can be transferred to other computers or for archival purposes. Similarly, you can select settings from previous back-ups and load them as current settings.



Export Settings

Click this button to export the current settings. The standard file save dialog will be displayed. Navigate to the location where you want to save the settings, type a file name, and click **Save**.

Import Settings

Click this button to import the settings. The standard file open dialog will be displayed. Navigate to the settings file that you want to open, select it, and click **Open**.

Reset Settings

Click to clear the current settings and reset to defaults. The program will confirm that you want to perform this operation. If you proceed, it will offer to restart the program for you.

OK

Click this button to close the dialog box when finished importing or exporting settings.

Research and Compliance Modes

Research and Compliance Modes

ModFit LT can be set up to run in either a Research-Use-Only mode, or in Compliance mode. There are some differences in how the program operates in each mode.

- Compliance mode depends on operating system user groups to control the level of access to program features. Research mode allows any user to perform any operation.
- Compliance mode displays a login dialog when the ModFit LT is starting up to verify the user and determine the group to which the user belongs.
- Compliance mode enables logging so that there is a record of the activities of each user. Research mode can be run with logging enabled or disabled.
- Compliance mode is designed to support clinical labs and can be part of a compliance plan for the US-FDA's 21 CFR Part 11 regulations. Research mode does not provide compliance with 21 CFR Part 11.
- Both modes use the same intuitive interface and ModFit LT engine.

The program displays which mode it is operating in. In the Ribbon bar interface, the mode is displayed in the upper right corner of the ribbon:



In the Toolbar interface, the mode is displayed on the main toolbar next to the Help button.

Setting Up Research Use Only Mode

After initial installation, ModFit LT will automatically be in Research mode. There is no further configuration required.

To revert to Research mode after setting up Compliance mode, the user groups that were created for Compliance mode must be removed. Once the user groups are removed, the program will revert to Research mode.

Setting Up Compliance Mode

Setting up Compliance mode is something that should be performed by IT personnel.

While it is not a complicated task, it requires administrative access rights and a clear understanding of user groups and permissions. It is therefore something that we strongly recommend be performed by the IT team at your institution. The task involves creating several *required* ModFit LT user groups for your computer or domain, and then adding users to those groups. Once the groups are defined, the program automatically operates in Compliance mode. [Click here for details.](#)

21 CFR Part 11 Compliance Matrix

This matrix provides a simple summary of how ModFit LT in Compliance mode helps you with 21 CFR Part 11 compliance. [Click here for details.](#)

Setting Up Compliance Mode

Setting up Compliance mode should be performed by your IT team. It requires administrative rights and a clear understanding of your network operating system.

The task involves creating several *required* ModFit LT user groups for your computer or domain, and then adding users to those groups. Once the groups are defined, the program automatically operates in Compliance mode.

ModFit LT User Groups

There are three (3) user groups for Compliance mode: **ModFitAdmins**, **ModFitEditors**, and **ModFitUsers**. Optionally, you can define an additional user group, **ModFitResearchers**, for research users within the Compliance setting who are not restricted by Compliance requirements.

The table below shows the kinds of features that group members can access.

Feature	ModFitAdmins	ModFitEditors	ModFitUsers	ModFitResearchers	RUO Mode Any user
Set shared folder location	Yes	No	No	No	No
Edit Preferences	Yes	No	No	Yes	Yes
Check for Updates	Yes	No	No	Yes	Yes
Edit Logging	Yes	No	No	Yes	Yes
Create and Edit Models	Yes	Yes	No	Yes	Yes
Edit Databases	Yes	Yes	No	Yes	Yes
Select Models	Yes	Yes	Yes	Yes	Yes
Add files to batch	Yes	Yes	Yes	Yes	Yes
Run a batch of files	Yes	Yes	Yes	Yes	Yes
Log and Preference folder	Shared	Shared	Shared	Per user	Per user

Setting Shared Folder location

When Compliance mode is enabled, there is an additional command that appears on the [Options tab](#) of the ribbon bar for **ModFitAdmins**. In Toolbar mode, the command appears in the Edit menu.

The **Select Shared Folder** command is used to select a **settings** folder that is shared by all users in Compliance mode. The common settings folder ensures that all Compliance-mode users work with the same configuration.

ModFitAdmins can use this command at any time to set or change the location for shared settings. Other users will not see this command.

Creating User Groups

The program requires at least one of the ModFit LT user groups to be defined in order to run in Compliance mode. ModFit LT user groups can be defined for your computer or for your entire domain using tools provided by the operating system. This is typically done by information technology personnel because it requires administrative rights.

The advantage of defining the user groups at the domain (network) level is that the groups are defined *for all computers* on the domain at the same time. If you define the user groups on an individual computer, those groups are available *only on that computer*.

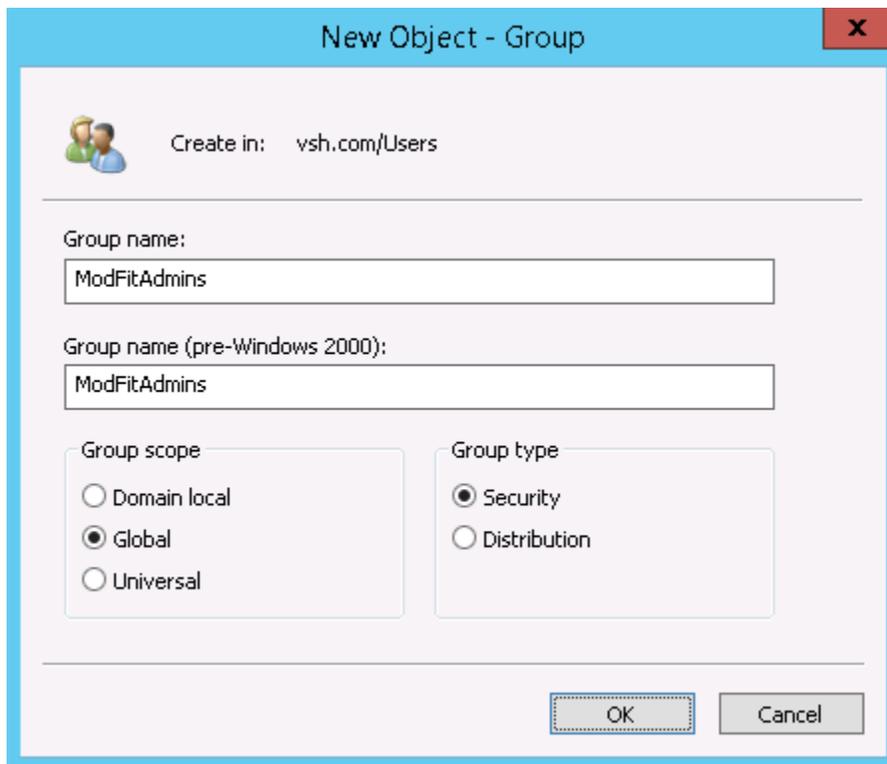
Below we describe how to create user groups on a Windows server or on a Windows compute as examples. The specific methods you need to use may differ depending on the version of the operating system you are working with.

After you have defined the user groups and added users to them, ModFit LT will detect the groups when it starts up and operate in Compliance mode automatically.

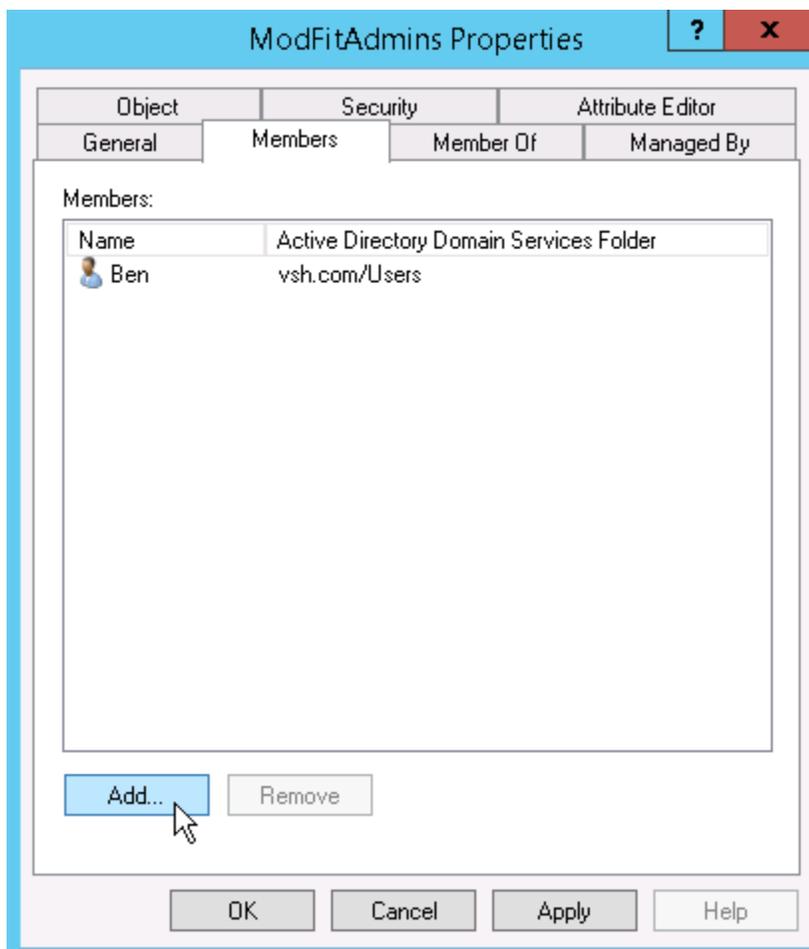
Windows Server User Groups

Follow these steps to define user groups for an entire network or domain. **Note: if you use this technique, you do not need to define user groups on each individual computer.**

- On a domain server, launch the "Active Directory Users and Computers" administrative tool.
- Right click the "Users" folder in the left panel and choose New->Group. The New Group dialog is displayed.



- Type **ModFitAdmins** for the group name and click OK.
- Once the group is created, double-click the group in the list to display the Properties for the group.
- Click the **Members** tab and click **Add** to select members for the ModFitAdmins group. You should have at least one user in the ModFitAdmins group. Members can be added by typing user names, or by using the dialog's search features.

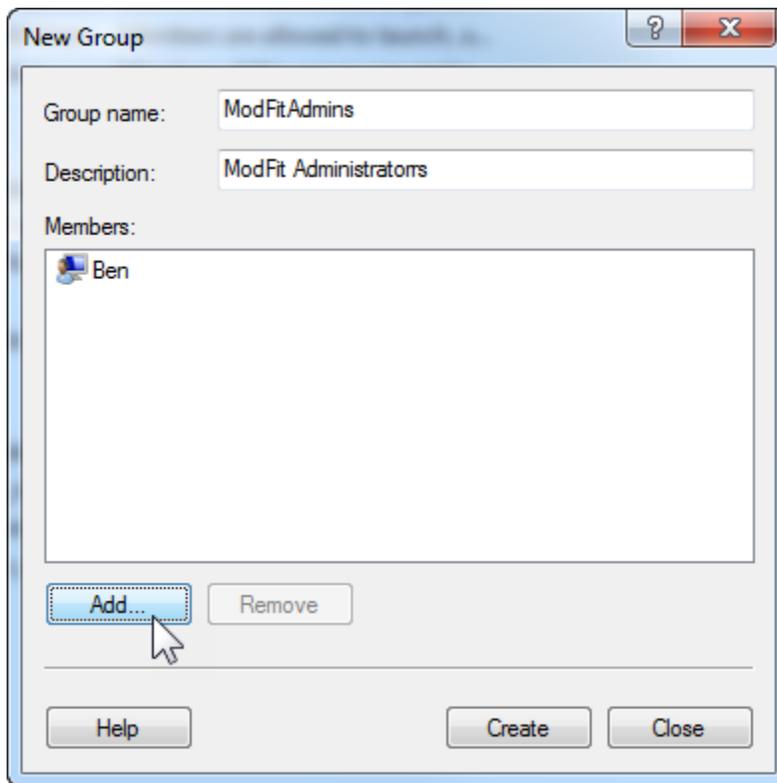


- Repeat this process for the **ModFitEditors**, **ModFitUsers**, and optionally **ModFitResearchers** groups, adding appropriate users to each group.
- Once the groups are created and users are added to them, the program will automatically use Compliance mode when it starts up and is connected to the domain.

Windows Computer User Groups

Follow these steps to define user groups for an individual Windows computer. **Note, if you already defined user groups for your domain, you do not need to define the groups again for each computer.**

- On a Windows 7 computer, right click Computer and choose "Manage". For Windows 10 and newer, right click the Windows button (lower left) and choose "Computer Management". The Computer Management app will display.
- Expand "System Tools" and "Local Users and Groups" in the left panel.
- Right click the "Groups" folder and choose "New Group". The New Group dialog is displayed.



- Type **ModFitAdmins** for the group name and enter a description for the group.
- Click **Add** to select members for the ModFitAdmins group. You should have at least one user in the ModFitAdmins group. Members can be added by typing user names, or by using the dialog's search features.
- Click **Create** to create the new local user group.
- Repeat this process for the **ModFitEditors**, **ModFitUsers**, and optionally **ModFitResearchers** groups, adding appropriate users to each group.
- Once the groups are created and users are added to them, the program will automatically use Compliance mode when it starts up on that computer.

Mac OS X User Groups

Follow these steps to define user groups for an individual Mac OS computer.

- On a Mac OS computer, launch System Preferences. Choose "Accounts" or "Users and Groups", depending on the version of the OS.
- Click the lock icon to authenticate to make changes. This will require administrative rights.
- Click the "+" button under the list of users and groups. The "New Account" dialog is displayed.
- Change the New Account type to "Group".
- Type **ModFitAdmins** for the group name and click "Create Group".
- Select the users you want to be members of the **ModFitAdmins** group. You should have at least one user in the **ModFitAdmins** group.

- Repeat this process for the **ModFitEditors**, **ModFitUsers**, and **ModFitResearchers** groups, adding appropriate users to each group.
- ModFit LT will run in Compliance mode the next time it starts.

See also:

[21 CFR Part 11 Compliance](#)

21 CFR Part 11 Compliance

ModFit LT can be part of your lab's compliance strategy for 21 CFR Part 11 when it is setup and operated in [Compliance mode](#). It has been developed under a quality management system and validated with a suite of quality assurance tests. The table below summarizes how the key elements of Part 11 relate to ModFit LT and its use in Compliance mode settings.

Section	Summary of Rule	Compliance Strategy
11.10	Controls for closed systems	ModFit LT can be accessed only through the user interface; it is a closed system.
11.10 (a)	Validation and accuracy.	ModFit LT uses externally-generated data to validate its results and employs proprietary binary format, static PDF, and/or CRC methods to ensure valid files.
11.10 (b)	Record generation and readability	ModFit LT generates Xml-format model files, CRC-checked log files, and static PDF that are human-readable and machine-readable.
11.10 (c)	Record protection and retrieval	Record retention and retrieval plan is managed by the laboratory's file management practices.
11.10 (d)	Authorized access	ModFit LT uses the operating system's user login validation, and user group membership determines permissions within the software.
11.10 (e)	Audit trails	ModFit LT employs a CRC-checked logging system with time stamps, and log files are preserved. Log files cannot be deleted by ordinary means.
11.10 (f)	Operational system checks	ModFit LT enables and disables portions of the UI to enforce permitted sequencing of steps and events.
11.10 (g)	Authority checks	ModFit LT uses the operating system user groups and login validation to assure authorized access.
11.10 (h)	Device checks and validity	ModFit LT performs validation checks on the FCS data files.
11.10 (i)	Education, training and experience	ModFit LT provides a User Guide, technical support, and tutorial videos to assist with the proper, intended use of ModFit. Specific on-site training may be arranged.
11.10 (j)	Electronic signatures	This is an individual laboratory requirement beyond the scope of use of ModFit LT.

11.10 (k)	Systems documentation	ModFit LT version releases are accompanied by an updated User Guide. Verity employs document control procedures.
11.30	Controls for open systems	ModFit LT is designed as a closed system.
11.50	Signature manifestations	ModFit does not require or offer electronic signatures.
11.70	Signature linking	ModFit LT does not require or offer electronic signatures.
11.100 (a)	Unique electronic signatures	Your laboratory must ensure that electronic signatures are unique to each user on the network.
11.100 (b)	Identity verification	Each laboratory must verify its own employment records and IDs.
11.100 (c)	Signature verification	Each laboratory must verify electronic signature eligibility with the FDA.
11.200 (a)(1)	Two-component signing	ModFit LT does not require or offer electronic signatures.
11.200 (a)(2,3)	Authenticity and falsification	ModFit LT does not require or offer electronic signatures.
11.200 (b)	Biometric signatures	ModFit LT does not utilize biometric IDs.
11.300 (a)	Controls for identification	ModFit LT uses the operating system user groups and login validation to assure unique user identities.
11.300 (b)	Identification aging	The individual laboratory IT structure must control password checks and aging requirements.
11.300 (c)	External ID validation	Not applicable. ModFit LT does not utilize external tokens, cards, RFID, or other physical identity-confirming devices.
11.300 (d)	Unauthorized use	The individual laboratory IT structure must control login authenticity and manage safeguards to prevent unauthorized use.
11.300 (e)	External device testing	Not applicable. ModFit LT does not utilize external tokens, cards, RFID, or other physical identity-confirming devices.

Customizing Models

Model Descriptions

This section contains descriptions of the models used by ModFit LT.

Model naming

When Auto-analysis or Choose model commands are used to create models, the program uses the following logic to create a name for the model.

<i>Position in name</i>	<i>Meaning</i>
1	Number of cycles
2	'D' if AutoDebris is enabled; 'n' if not
3	'A' if AutoAggregates is enabled; 'n' if not
4	Number of standards
5	'A' if Apoptosis is enabled; 'n' if not

For each cycle in the model, the name includes the following:

An underscore (_) character;

The first character of the cycle's ploidy, i.e. D=Diploid, A=Aneuploid, T=Tetraploid, H=Hypodiploid;

'S' if S-Phase is active, or 'n' if not; and

'F' if G2M is active and floating, 'D' if active and dependent, 'n' if G2 inactive. Floating means that the mean position and standard deviation are adjusted during the modeling process. This gives more freedom to the model, but the peak must be unambiguous. Dependent means that the position and standard deviation of the G2M are computed by multiplying the G1 position and standard deviation by a factor of 2.0 or your chosen linearity setting. Inactive means that the G2M is not modeled.

For example, the model name "3DA0n_DSD_AnD_ASF" can be interpreted to mean that there are 3 cycles, AutoDebris is enabled, AutoAggregates is enabled, zero standards, and apoptosis is not being modeled. The first cell cycle is Diploid, with S-Phase active and a Dependent G2M. The second cycle is Aneuploid, S-Phase is turned off and the G2M is dependent. The third cycle is also Aneuploid, S-Phase is enabled, and the G2M position is allowed to float.

Ranges and their uses

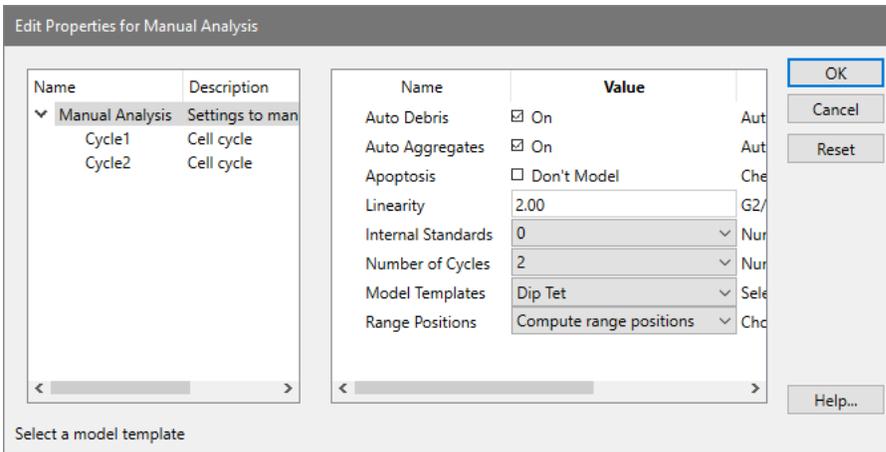
ModFit LT™ User Guide

The table below shows the ranges used by ModFit LT and the populations they are used to identify.

<i>Range</i>	<i>Usage</i>
Debris	Left side placed at start of downward sloping debris, right side extended through roughly 2/3 of the histogram. Provides estimates for AutoDebris component.
Stnd 1	Identifies the peak of the first standard. The range should be positioned to encompass the standard peak. If you have named your standards, the range will show the name you assign. See Auto Analysis Settings for details on naming standards.
Stnd 2	Identifies the peak of the second standard. The range should be positioned to encompass the standard peak. If you have named your standards, the range will show the name you assign.
Dip G1	Identifies the G1 of the Diploid cycle. The range should be positioned to encompass the peak.
Dip G2	Identifies the G2 of the Diploid cycle. The range should be positioned to encompass the peak.
<i>Cycle G1 and G2</i>	Identify the G1 and G2 for each additional cycle. The range should be positioned to encompass the associated peak.

When you use automatic analysis, ModFit LT constructs a model for you based on the peaks it can identify in the data sample. It looks at how many peaks there are, their standard deviations, areas, and relative locations, then takes its best shot at deducing the ploidy, debris, and aggregation aspects of the sample. It builds a model that matches what it finds. The black triangles at the base of the histogram show the peaks the program has identified.

When you select a model yourself using the [Choose Model](#) option, you are presented with the dialog pictured below. With this dialog, you make the decisions about the sample.



Colors

ModFit LT allows you to edit the colors used by the model using the [Colors command](#). The default color assignments are listed in the table below.

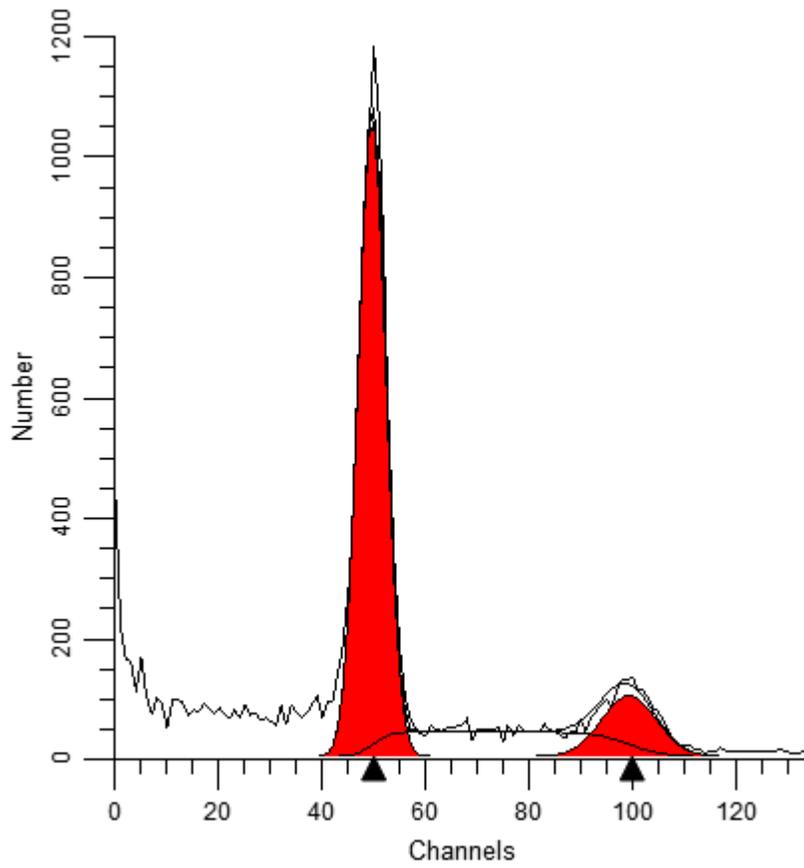
<i>Component</i>	<i>Color</i>
Diploid G0-G1	red
Diploid G2-M	red
Diploid S-Phase	reverse-hatched blue
Aneuploid G0-G1	1 st is bright yellow; others become darker shades of yellow
Aneuploid G2-M	1 st is bright yellow; others become darker shades of yellow
Aneuploid S-Phase	forward-hatched blue
AutoAggregates	light green
AutoDebris	medium blue
Apoptosis	sky blue
1 st Standard	dark gray
2 nd Standard	light gray

Component Shapes

The parts of ModFit LT's models are comprised of several component shapes. The table below shows the shapes used by the models, and where they are used.

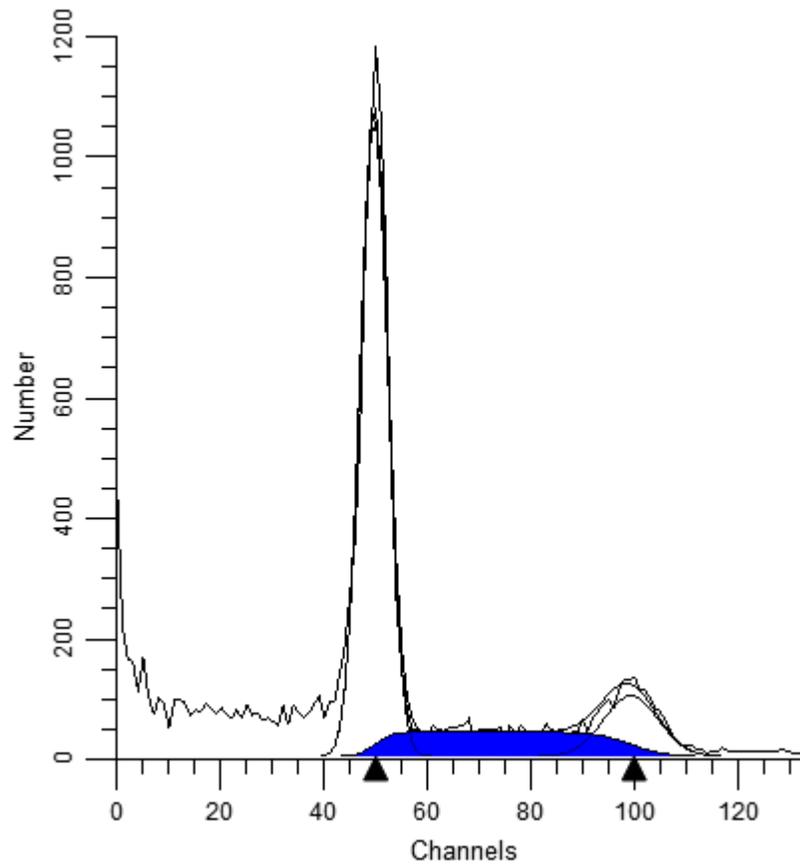
<i>Component</i>	<i>Shape</i>	<i>Uses</i>
------------------	--------------	-------------

Gaussian



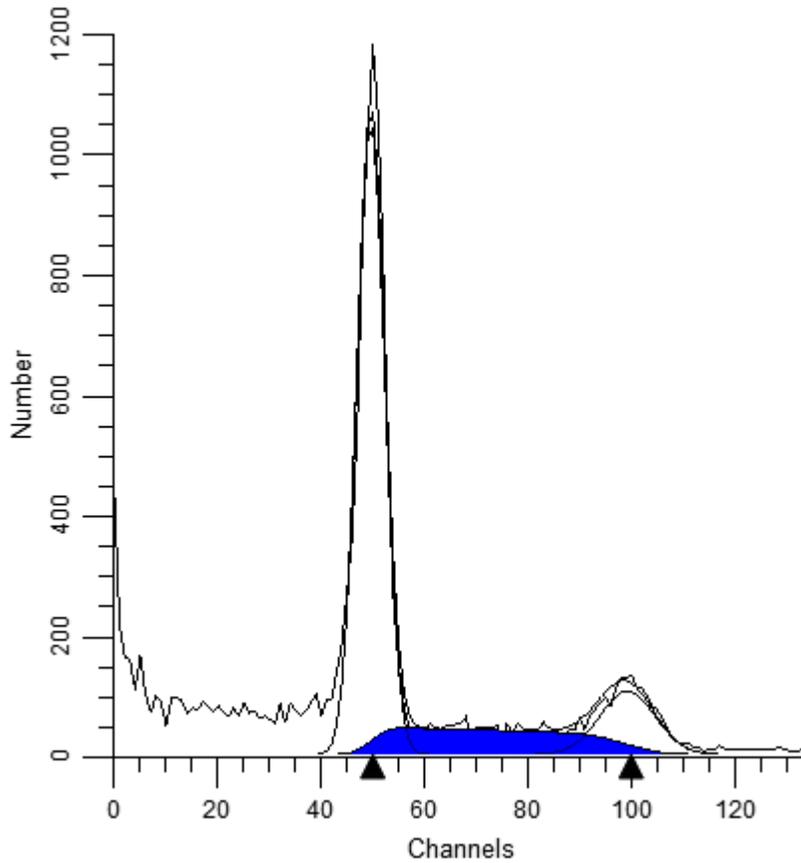
Gaussian components are used to model peaks. They are often linked to ranges.

Rectangle



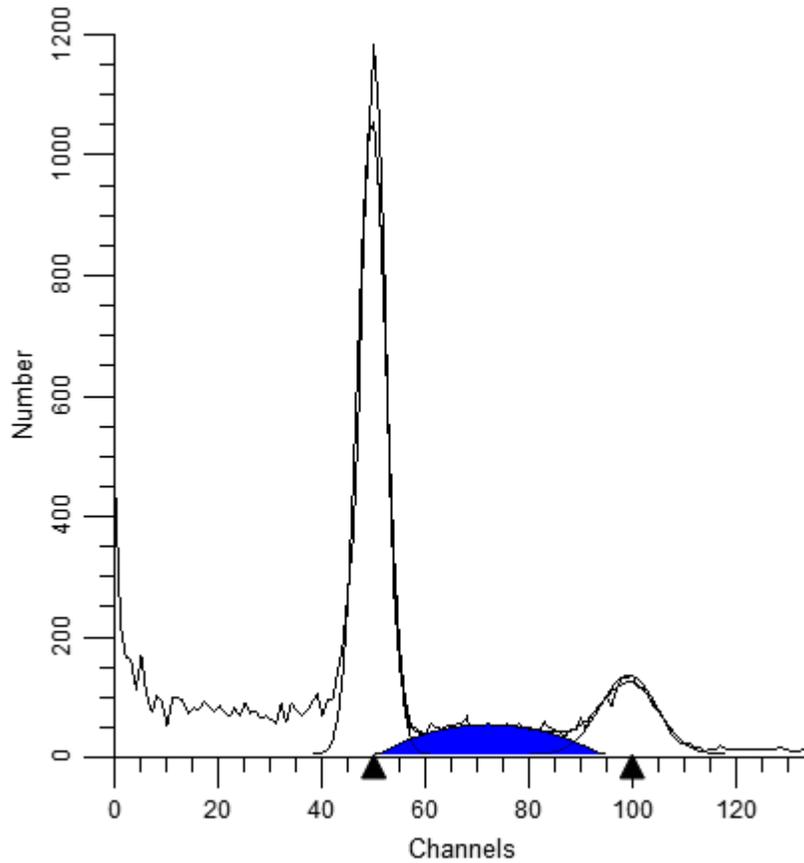
This component is typically used to model S-Phase. The peaks on either side of it determine boundaries and standard deviations.

Trapezoid



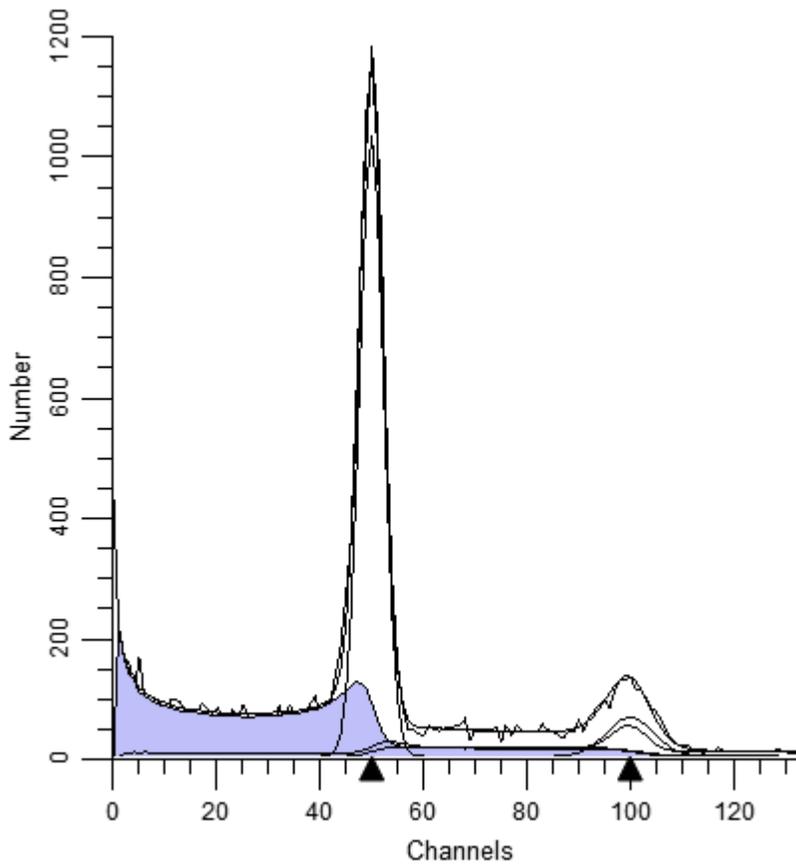
This component can be used to model S-Phase. It has an additional degree of freedom over the rectangle and generally yields higher S-Phase values. The peaks on either side of it determine boundaries and standard deviations.

Polynomial



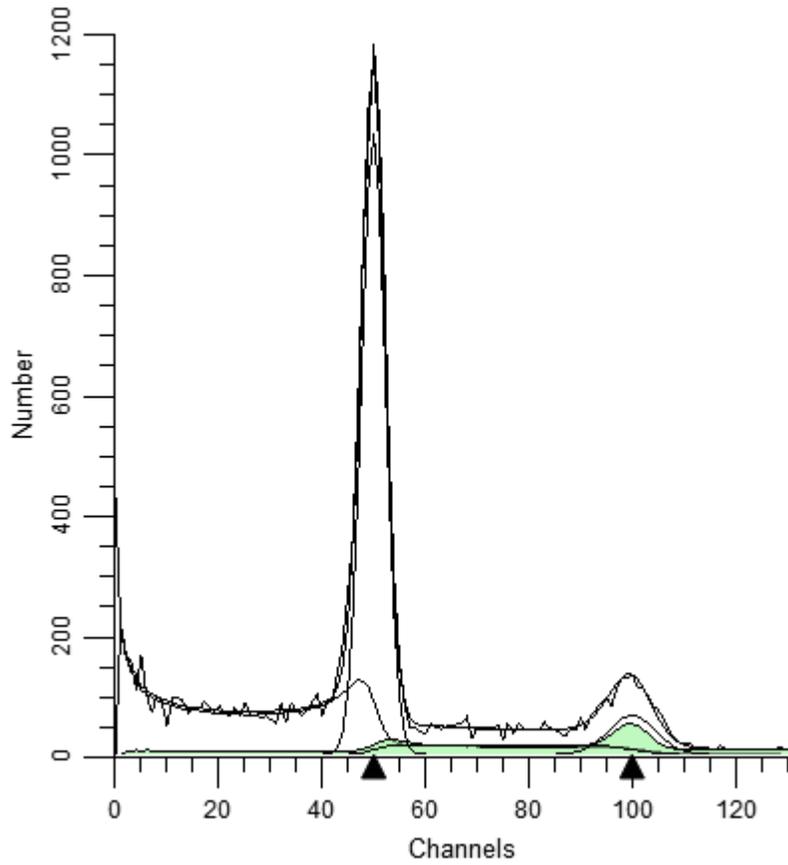
This component can be used to model S-Phase. It has an additional degree of freedom over the trapezoid and should be used with caution. The peaks on either side of it determine boundaries and standard deviations.

AutoDebris



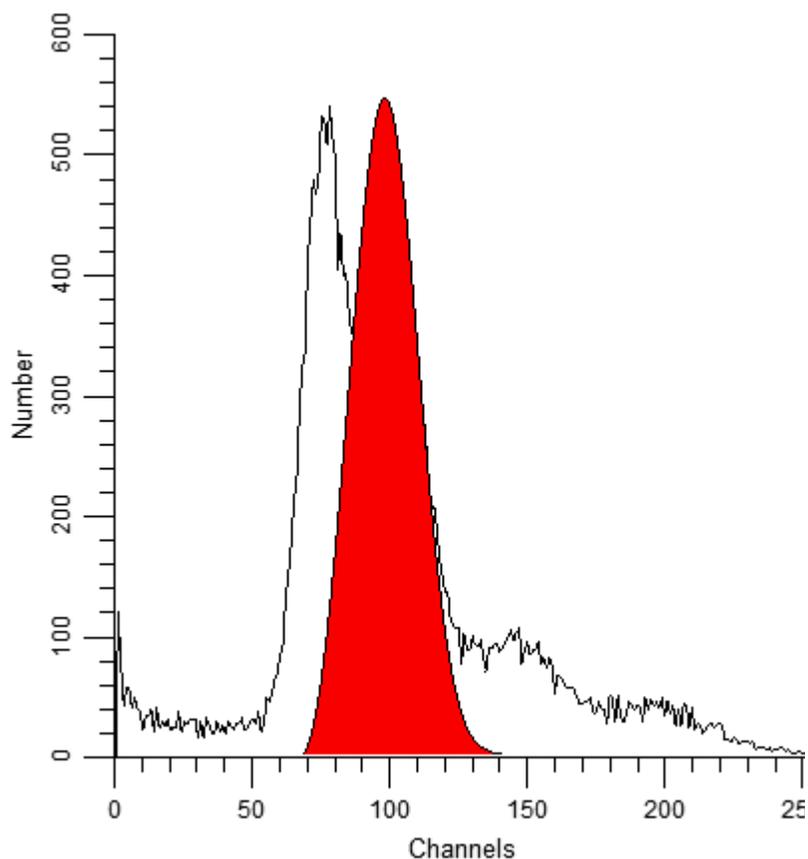
The AutoDebris component is used to model debris. Its starting point is based on the position of the Debris range.

AutoAggregate



This component is used for aggregate compensation. Its shape is unique to each histogram. It does not use a range.

Weibull



This component can be used to model skewed peaks in custom models. Contact Verity technical support for assistance with this component.

See also:

[Choose Model command \(Analysis menu\)](#)

[Manual analysis with ModFit LT](#)

[Analysis Results](#)

Colors command



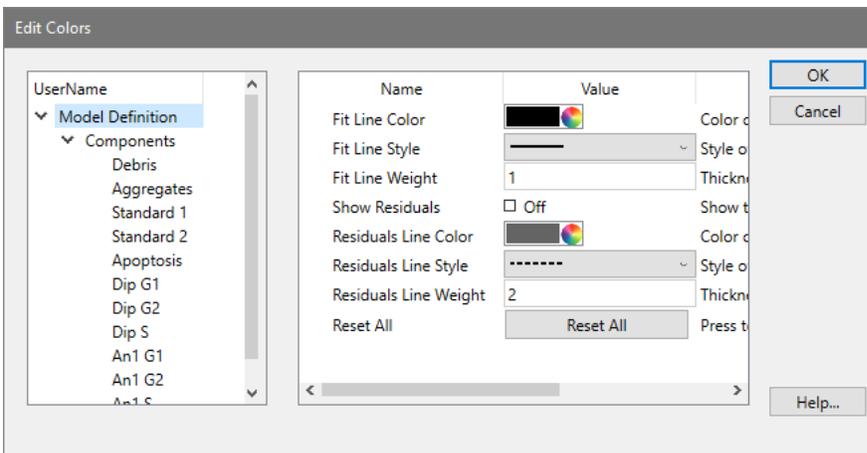
Colors

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*

Use this command to adjust colors and other graphical aspects of the model. You can change color, fill style, and transparency for colors in the current model. The changes you make can also be remembered and applied to other models.



Model Definition

With the Model Definition selected in the tree on the left, you can edit characteristics of the fit line displayed on the modeled histogram. The color, style, and weight of the fit line can be adjusted.

Components

To choose a new color for a model component, click the color swatch next to the component. The standard dialog for choosing colors will appear. Select a new color and click OK.

To choose a new fill style for a model component, click the sample fill for the component. A drop-down list of choices will appear. Select a new fill style from the list.

Each component can either be transparent or opaque. This determines how overlapping components are drawn. To make a component transparent, enable the checkbox in the Transparent column. Uncheck the option to make a component opaque.

OK

When you click OK, you will be asked whether you want to apply the changes to all models, or just the current model. If you choose to apply to all models, the changes are stored for future sessions of ModFit LT. If you choose to apply the changes only to the current model, the changes will be lost when you load a new model, perform an Auto Analysis, or close the program.

Reset All

Click this button to clear all color selection overrides.

Cancel

Click this button to discard changes to colors.

Quick Model Editor



Overview

The Quick Model Editor allows you to make changes to the current model. It is available when the model you are using is created by AutoAnalysis or by using the Choose Model option.

Changes you make with this dialog are applied to the model when you click OK. In order to see the changes to the model, you need to re-analyze the data by clicking the Fit button or choosing Execute Model from the Analysis menu.

Name	Value	Description
AutoDebris	<input checked="" type="checkbox"/> On	Enables AutoDebris compensation
AutoAggregates	<input checked="" type="checkbox"/> On	Enables AutoAggregate compensation
Linearity	2.00	Edit the G2/G1 ratio to use (1.50 to 2.50)
Internal Standards	0	Number of internal standards (... to ...)
G2M		
Cycle1 G2M	<input type="checkbox"/> Not Visible	Allows modeler to float G2M position when checked
Cycle2 G2M	<input type="checkbox"/> Not Visible	Allows modeler to float G2M position when checked
S-Phase		
Cycle1 S-Phase	<input checked="" type="checkbox"/> On	Enables S-Phase for the cycle
Cycle2 S-Phase	<input checked="" type="checkbox"/> On	Enables S-Phase for the cycle

AutoDebris

This checkbox determines whether the AutoDebris model component is active or not. When checked, AutoDebris is activated. The AutoDebris component is used to model debris in data. It can be left active even when samples display little or no debris, because its shape and amplitude are based on probability distributions unique to each sample.

AutoAggregates

When this option is checked, the AutoAggregates model component is activated. This component is used to model the continuum of aggregate forms found in many samples. It can be left active even when there is little or no aggregation in the sample, because its shape and amplitude are based on probability distributions unique to each sample.

Linearity

This field modifies the appropriate G2M dependency settings and modifies the aggregate model component's linearity setting. When the user indicates that a G2M is not visible, the program makes the position and standard deviation of the G2M model component dependent on the G1 by this linearity setting. It updates the linearity setting for the aggregate model component as well.

Internal standards

This option determines the number of peaks attributed to standards. Standard peaks typically occur below the first G1 peak. However, you can configure the expected locations for standards in your samples using the [Auto Analysis Settings](#) dialog box.

G2M Section

The options in this section of the dialog allow you to control how the G2M for each cycle is modeled. If the option is checked (*Visible*), the program will use the associated G2M range to estimate the position and it will allow that position to be adjusted by the modeling process. If the option is *not* checked, the position of the G2M will be computed by multiplying the G1 position by the linearity value.

Generally, if a G2M is a well-defined peak that does not overlap another peak, you can enable the option and the model will provide a more accurate result. However, if the G2M peak is not clearly defined or if it substantially overlaps another peak, uncheck the option. If the option is checked when a peak is *not* well defined, the model will produce less accurate results.

S-Phase Section

The options in this section control the state of the S-Phase model component for each cell cycle in the model. If a check appears in the checkbox, the S-Phase component is active and the program will attempt to model it. Clear the check to disable the S-Phase component for the model.

In samples such as near Diploid histograms, the S-Phases of two or more cycles can be significantly overlapped. This can create problems for the modeling process. In these cases, it is better to disable one of the S-Phase components, normally the Diploid S-Phase.

OK

Apply the settings to the current model and put away the dialog box.

Cancel

Close the dialog and discard any changes you have made.

Reset

This option resets the the properties in this dialog to their default states.

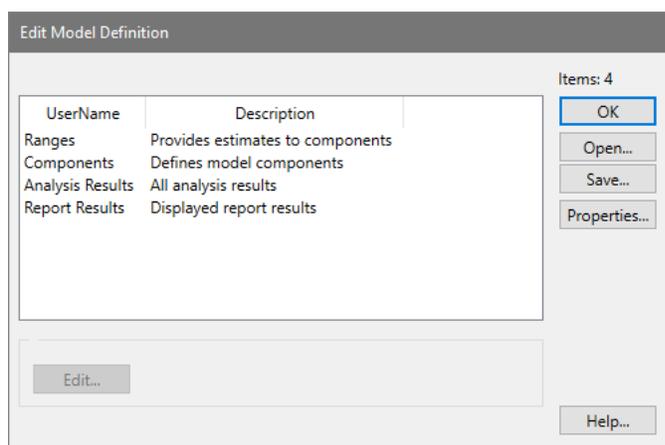
Full Model Editor



[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*



This dialog allows editing of existing models and creation of new models. It can be used to change any and all aspects of a model, and therefore should be used with great care.

The listbox displays the aspects of the model that can be edited: ranges, components, and results.

Ranges:

Ranges are used to provide estimates for the model components. They are displayed when you click the Range button on the ribbon bar. Most ranges are used to identify peaks in the model, and associated model components use the range position, size, and statistical data as a starting point in the modeling of the histogram. See [Edit Ranges](#) for more on this topic.

Components:

Components of the model define the mathematical pieces and their interdependencies. There are components to model debris, aggregates, peaks, and broad distributions. Each component, in turn, has a set of details that further describe the properties of the component. See [Edit Properties for Component](#) for more on this topic.

Analysis Results:

Analysis results are defined using equations. They can show simple information, such as keywords stored in the data file, or sophisticated mathematical expressions based on details of model components. See [Edit Analysis Results](#) for more on this topic.

Report Results:

Report results define what analysis information will be displayed on the report. See [Edit Report Results](#) for more on this topic.

OK:

Click this button to close the dialog and apply the changes to the current model.

Open:

This option allows you to open a previously saved model from disk. The File Open dialog box is displayed, allowing you to navigate to and select the model. Note: Models from ModFit LT 2.0 and earlier cannot be opened with this option.

Save:

This option allows you to save the current model to disk for later use. The File Save dialog is displayed, allowing you to navigate to the location where you wish to store the model.

Help:

Click this button to display online help for this topic.

Edit:

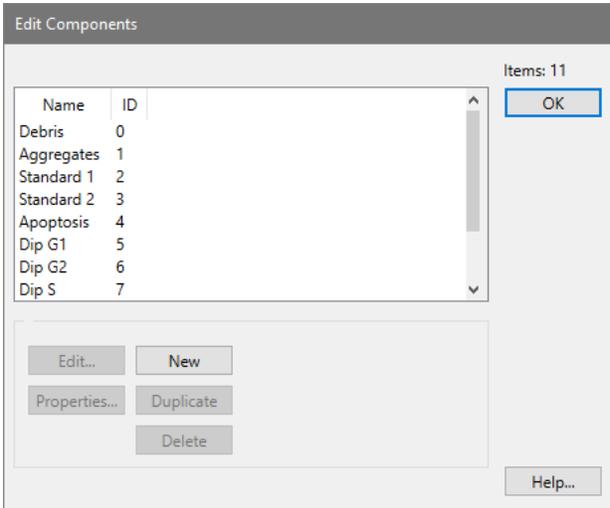
This button is enabled when one of the list entries is selected. Clicking the button will display the edit dialog for the selected item.

Edit Components

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*



This dialog allows you to add, delete, and edit components of the model. Each model component mathematically describes a piece of the model. The collection of components attempts to fully define the histogram as a mathematical model.

Component names can be edited by clicking slowly once on the name, then clicking again. The list item will enter edit mode; press the Tab key or click another item to accept the revision.

Select a component in the list, and then click one of the action buttons to modify it.

OK:

Click this button to close the dialog and accept the changes.

Help:

Click this button to display online help for this topic.

Properties:

This button is enabled when one of the list entries is selected. Clicking the button will display the edit properties dialog for the selected item.

Edit:

This button is enabled when one of the list entries is selected. Clicking the button will display the edit dialog for the selected item.

Copy:

Click this button to copy the selected component. The new copy will be added to the end of the component list, and can be selected for editing.

Delete:

Click this button to delete the selected component. If other components are dependent on the deleted component, they will become inactive. Use great care when deleting components to ensure the remaining model components are not adversely affected.

See also:

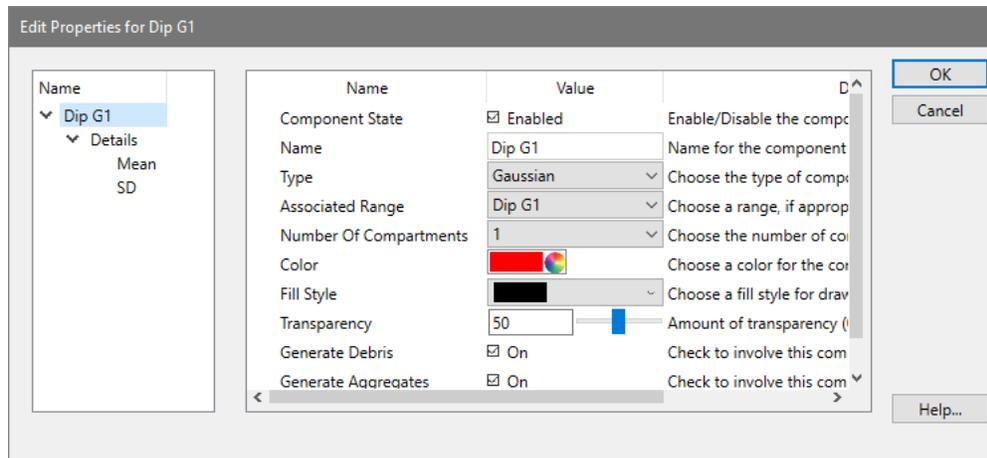
[Edit Properties for Component](#)

Edit Properties for Component

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*



This dialog allows you to edit properties for a single model component. In addition, you can select and edit the properties for each detail of the component. Component details vary depending on the type of component.

A tree showing the component and its details is displayed on the left side of the dialog. Initially, the component itself is selected, and the list on the right shows the properties of that component.

When you click one of the details in the tree on the left, the properties for that detail will appear. See [Edit Properties for Detail](#) for more on this topic.

Component State:

This checkbox shows the current state of the component.

Name:

This edit box displays the name of the component. It can be edited by clicking inside the edit box and typing new text.

Type:

This drop-down list displays and selects the type of mathematical expression used for this component. Changing the type may also change the details, shown in the tree on the left of the dialog. See [Model Descriptions](#) for details on each model component type.

Associated range:

This drop-down list displays and selects the model range, if any, used to provide estimates to this model component. In most cases, ranges identify peak locations. They appear on the histogram when you click the Range button on the toolbar, or select Position ranges from the Analysis menu. If a component is not associated with a range, the selection will display "No range".

Number of compartments:

This list selects the number of sub compartments for the component. Some components, like the Polynomial, do not support more than one compartment. Setting this to 0 will disable the component.

Color:

Click this button to display the standard color selection dialog and choose a new color for the component.

Fill Style:

This list contains graphical fill options to use when drawing the component. Using a fill pattern allows an additional degree of transparency to overlapping components, in some cases, making it easier to review the model.

Transparency:

This option determines whether the component coloring will be transparent or not.

Generate Debris:

Enable this option if the population associated with the component contributes to debris in the sample. The debris modeling uses this to determine the expected shape of debris.

Generate Aggregates:

Enable this option if the population associated with the component contributes to aggregates in the sample. The aggregate modeling uses this to determine the expected shape of aggregates.

OK:

Click this button to accept the changes and close the dialog.

Cancel:

Click this button to cancel changes and close the dialog. This button may be disabled if changes are made that cannot be cancelled.

Help

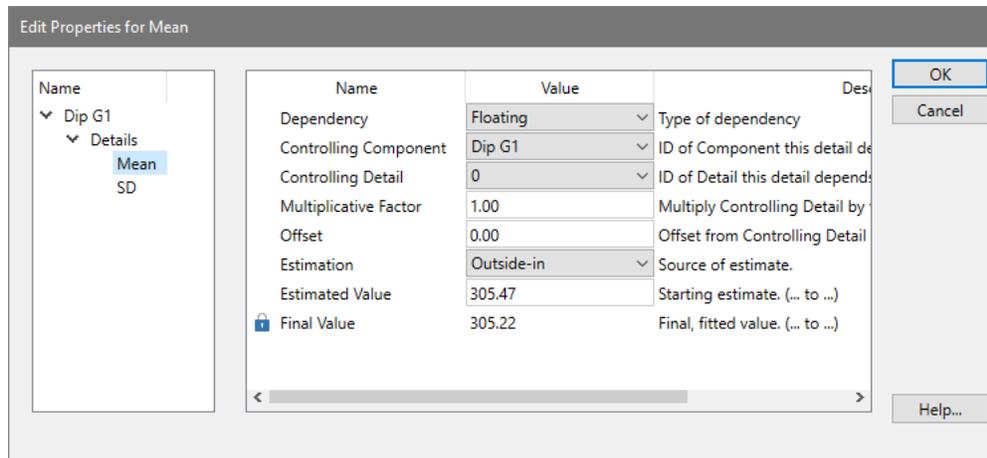
Click this button to display online help for this topic.

Edit Properties for Detail

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.



This dialog allows you to edit the properties of a model component detail. The details of a component will depend on the particular component type. The properties of each detail define how the detail is related to other component details, how it is computed, and the source of its estimates. See [Edit Properties for Component](#) for additional information.

Dependency:

This option determines how the detail is computed. *Floating* means that the modeling process will adjust the detail to find the best value. Floating details are commonly used for means and standard deviations of well-defined peaks modeled with Gaussian component. *Dependent* details are computed directly by applying a multiplicative and/or additive factor to the value of the controlling detail. *Fixed* details are not adjusted during the modeling process; their values are determined either by a static value or from a range estimate. *Interpolate* can be selected to compute the value of the detail by interpolation. It is only applicable to standard deviation details. An example best describes the use of this option. Suppose you have a model that describes a diploid population. Now suppose there is an extra peak in S-Phase that you believe should be broadened in the same way as the S-Phase is broadened. You would choose Interpolate for the standard deviation of the extra peak, and make its controlling component and detail the S-Phase lower standard deviation. The upper boundary of the interpolation is automatically the upper standard deviation.

Controlling component:

If *Dependent* or *Interpolate* is selected as the Dependency option, the controlling component determines what model component this detail is dependent upon. You must also select a

Controlling detail (below) to complete the dependency. If the Dependency option is *Floating* or *Fixed*, the controlling component is ignored.

Controlling detail:

If *Dependent* or *Interpolate* is selected as the Dependency option, the controlling detail determines the detail of the Controlling Component on which this detail depends. If the Dependency option is *Floating* or *Fixed*, the controlling component is ignored.

Multiplicative factor:

If *Dependent* is selected as the Dependency option, the final value of the controlling component's detail is multiplied by the multiplicative factor. As an example, the G2M component's mean position is often made dependent on the G1 component's mean by a factor of 2.

Offset:

If *Dependent* is selected as the Dependency option, this value is added to the final value of the controlling component's detail, after it has been multiplied by the multiplicative factor.

Estimation:

This drop down provides choices for the source of the estimate for the detail. *Outside-in* and *Inside-out* draw the estimate from simple statistics performed before the modeling process. *Range* draws the estimate from a range associated with this component. *User-entered estimate* uses the value entered in the Estimated value field as the source of the estimate.

Estimated value:

This field displays the estimate that was used for the detail when the model was last run. If Estimation is set to *User-entered estimate*, the value will not be adjusted. In all other cases, the value may change each time you choose Fit, depending on how the details dependencies are set.

Final value:

This read-only field displays the result of the last modeling process for this model.

OK:

Click this button to accept the changes and close the dialog.

Cancel:

Click this button to cancel changes and close the dialog. This button may be disabled if changes are made that cannot be cancelled.

Help

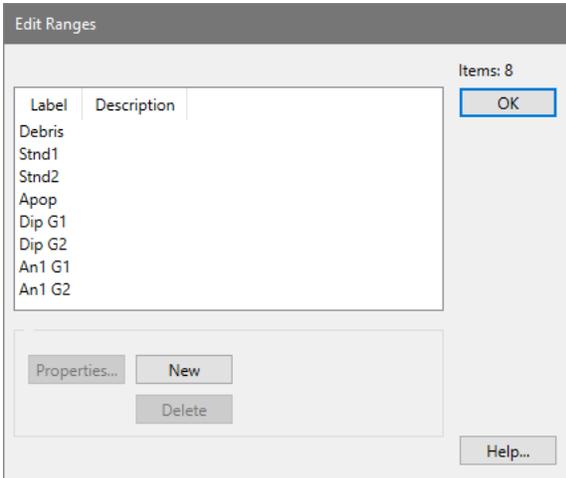
Click this button to display online help for this topic.

Edit Ranges

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*



This dialog allows you to add, delete, and edit ranges of the model. Ranges are graphical elements that you can position on the histogram. They are used to supply estimates to model components.

Range names can be edited by clicking slowly once on the name, then clicking again. The list item will enter edit mode; press the Tab key or click another item to accept the revision. See [Edit Properties of Range](#) and [Edit Properties for Component](#) more on this topic.

OK:

Click this button to close the dialog and accept the changes.

Help:

Click this button to display online help for this topic.

Properties:

This button is enabled when one of the list entries is selected. Clicking the button will display the edit properties dialog for the selected item.

Delete:

Click this button to delete the selected range. If components are dependent on the deleted range, they will become inactive. Use great care when deleting range to ensure model components are not adversely affected.

Properties:

This button is enabled when one of the list entries is selected. Clicking the button will display the edit dialog for the selected item.

Edit Properties of Range

[Research and Compliance Mode](#) note:

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*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*

Name	Value	Description
Label	Dip G1	Name Of The Range
Active	<input checked="" type="checkbox"/> Active	Changes activation state
Left	297.16	Channel of left edge of range. (-1.00 to ...)
Width	16.06	Width of the range (-1.00 to ...)
Y Position	70.00	Height of the range (0.00 to 100.00)
Color		Color for simple analysis
Type Of Estimate	Gaussian	Estimate associated with this range.
Auto-position to	No peak	Choose an auto position option

This dialog allows you to edit the properties of a model range. Ranges are graphical elements that you can position on the histogram, used to supply estimates to model components.

Label:

This edit box displays the name of the range. It can be edited by click inside the edit box and typing new text.

Active:

When checked, the range is displayed on the histogram (in Range view) and it can be used as an estimation source for model details. When unchecked, the range will not be displayed and any components that use the range will be deactivated.

Left:

The edit box shows the location of the left side of the range. You can edit the field manually, or position the range graphically to change the value. To display ranges, click the Range button on the toolbar, or choose Position Ranges from the Analysis menu.

Width:

The edit box shows the width of the range. The right side of the range is positioned at Left plus this value.

Y Position:

This value determines the vertical location of the range label. The value is a percentage of the Y scale.

Color:

Click this button to display the standard color selection dialog and choose a new color for the range.

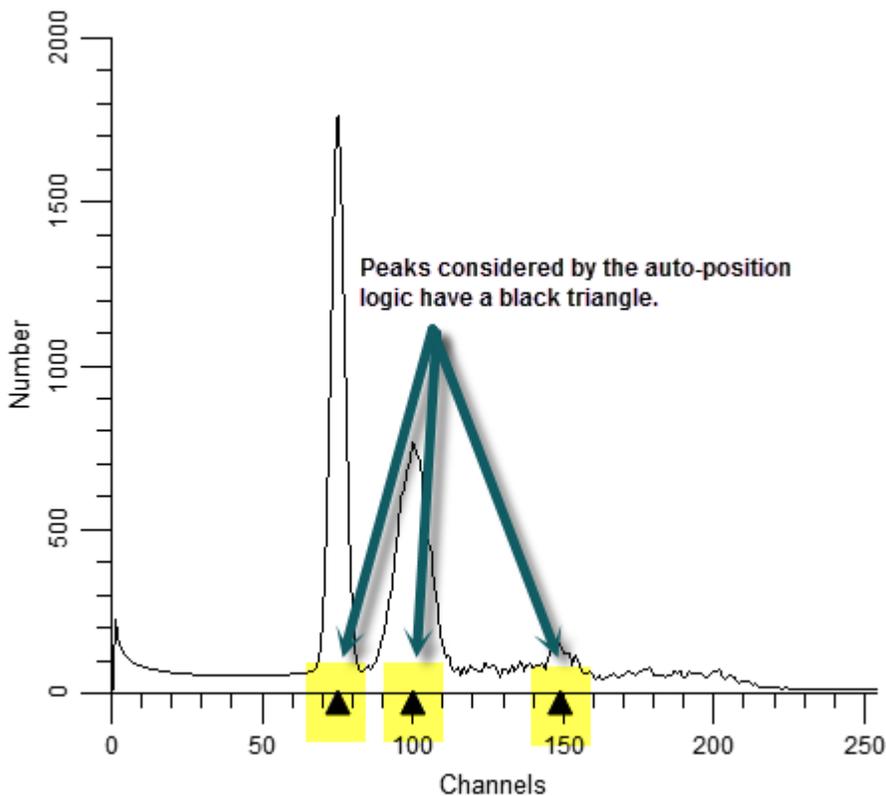
Type of estimate:

There are three types of range estimates: *Gaussian*, *Debris* and *Aggregate*. Most ranges are *Gaussian* type, used to identify peaks in the histogram. There is typically only one *Debris* estimate range in a model, used by the AutoDebris component for estimation of the debris. The *Aggregate* estimate can be associated with the AutoAggregate component, but is typically not used.

Auto-position to:

In most models, the ranges are positioned and sized by the user for manual analysis. Automatic analysis positions ranges based on the peaks it finds in the data. However, you can create your own custom models, too, and for those models you can tell ranges how to position themselves.

The peaks that are considered by the auto-positioning logic are those that show a black triangle on the main histogram.



No peak: This turns off auto-positioning and leaves the range where it is.

- Closest peak: Moves the ranges to the closest peak.
- Dimmest peak: Moves the ranges to the dimmest peak.
- Brightest peak: Moves the ranges to the brightest peak.
- Largest peak: Moves the ranges to the largest peak (by area).
- Smallest peak: Moves the ranges to the smallest peak (by area).
- Tallest peak: Moves the ranges to the tallest peak.
- Shortest peak: Moves the ranges to the shortest peak.

OK:

Click this button to accept the changes and close the dialog.

Cancel:

Click this button to cancel changes and close the dialog. This button may be disabled if changes are made that cannot be cancelled.

Help

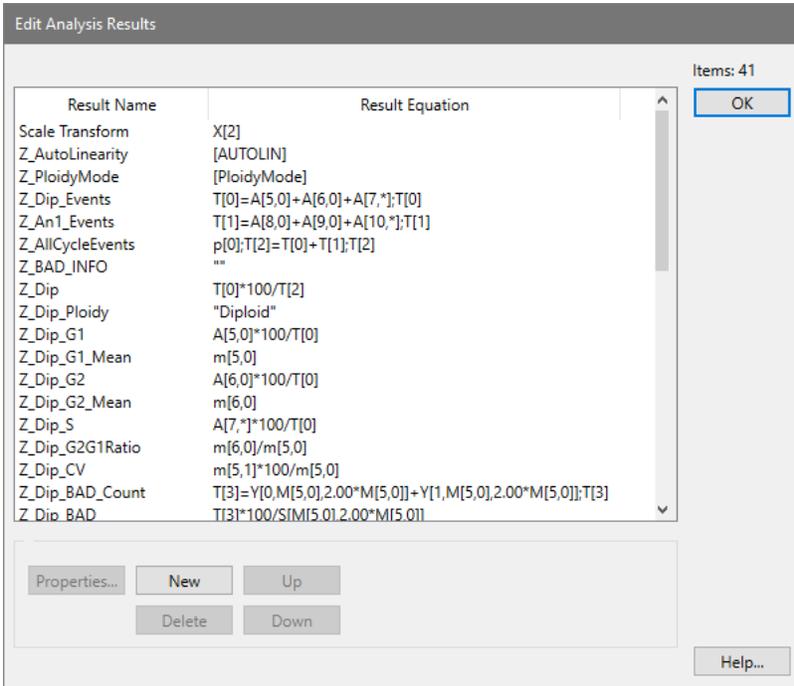
Click this button to display online help for this topic.

Edit Analysis Results

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*



This dialog displays the list of analysis results that are computed for the current model. Each item in the list has a **Result Name** and a **Result Equation**. The equation uses ModFit LT's [equation syntax](#) to compute the results.

For models that are created by ModFit LT's Auto Analysis, Choose Model, Sync Wizard, and Cell Tracking Wizard, there is little need to edit the Analysis Results. The main use for this dialog is for models that you design yourself from scratch, or models you customize from a ModFit LT model.

The items in this list are available for databasing as well as being added to the report.

Edit Properties for Analysis Result

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

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Name	Value	Description
Result Name	Z_Dip_Events	Name of the result.
Result Equation	T[0]=A[5,0]+A[6,0]+A[7,*];T[0]	The evaluation of this equation will be the result value.

This dialog is used to edit an Analysis Result. It allows editing of the **Result Name** and the **Result Equation**.

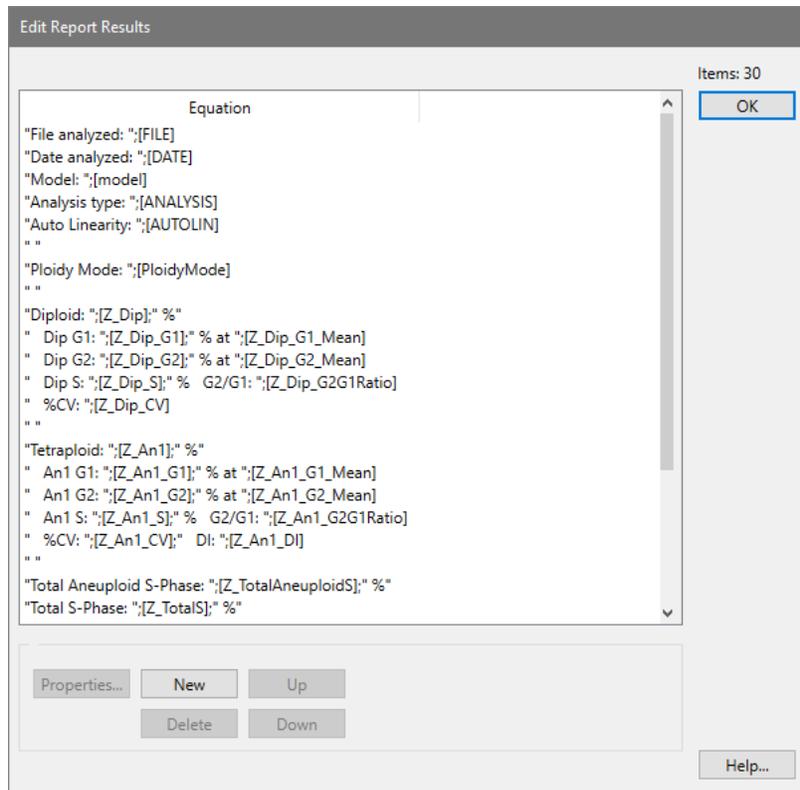
See [Equation Syntax](#) for details on writing equations.

Edit Report Results

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*



This dialog allows you to edit the equations used to display results on the report.

You can edit equations by selecting an item in the list and clicking Properties. Alternately, you can edit an equation directly by clicking once slowly to select the equation, then clicking again to enter edit mode. To leave edit mode, press the Tab key or click another item in the list.

Properties:

This button is enabled when one of the list entries is selected. Clicking the button will display the edit properties dialog for the selected item.

New:

Click this button to add a new equation. The new equation will be added to the end of the list, and can be selected for editing.

Delete:

Click this button to delete the selected equation. Be careful when deleting equations that contain " $T[n]$ " expressions, where n is an integer. These equations compute temporary variables used by other equations; the other equations may fail if they make use of a deleted expression.

OK:

Click this button to close the dialog and accept the changes.

Help:

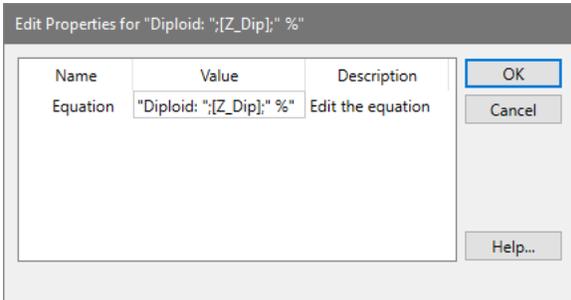
Click this button to display online help for this topic.

Edit Properties of Report Result

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** and **ModFitEditors** have permission to perform them.*



This dialog allows you to edit an equation for a line of results that appears on the report.

Equation:

Type or edit the equation expression. See [Equation syntax](#) for details.

Equation syntax

ModFit equations are expressions that are compiled by the program and evaluated each time the model is analyzed. Equations can contain literal text, keywords from the FCS file being analyzed, and symbols that provide access to the computed results of the analysis. Mathematical operations include addition, subtraction, division, and multiplication, grouping of operations using parentheses, and use of build-in functions.

Strings

String literals are enclosed in quotation marks.

Example equation: "This is text."

Report output: This is text.

Keywords:

Keywords can be drawn from the FCS file and from internally computed values. Keywords are enclosed in square brackets.

FCS keywords vary from file to file, and are generally created by the software that was used to acquire the data file.

Internal keywords include the following:

[DATE]	displays date of analysis.
[TIME]	displays time of analysis.
[FILE]	displays name of data file analyzed.
[REPORT]	displays name of report.
[AUTONAMEREPORT]	displays name generated by AutoNameReport.
[MODEL]	displays the name of the model.
[ANALYSIS]	displays the type of analysis performed, e.g. Automatic, Manual.
[RCS]	displays the reduced chi-square value from the last fit of the model.
[GATE1]	displays name of gate one, or "Off" if no gate
[GATE2]	displays name of gate two, or "Off" if no gate
[GATE3]	displays name of gate three, or "Off" if no gate
[FILEPATH]	displays full path to data file.
[MODELPATH]	no longer supported
[REPORTPATH]	displays full path to the report
[VERSION]	displays the internal version of the program
[LINEARITYFACTOR]	displays the configuration setting for linearity
[EXTREFMEAN]	displays the value for the External Reference Mean position from the Auto Analysis Settings dialog.
[DIPTOSTDRATIO]	displays the value for the Diploid-to-Standard Ratio from the Auto Analysis Settings dialog.
[RANGE n NAME]	displays the name of range with ID of n
[RANGE n LEFT]	displays the left channel position of range with ID of n
[RANGE n RIGHT]	displays the right channel position of range with ID of n

Example equation: "Today is ";[DATE]

Report output shows date of analysis (for example): Today is 28-Feb-2022

Example equation: [RANGE4NAME]

Report output shows name of range with ID of 4: "Dip G1"

Internal functions

Internal functions are used to gain access to component details, areas, and other computed values. Below is a table describing each function. Note that component and compartment indexing start at zero (0).

Function	Description
$A[m, n]$	Returns the area of component m , compartment n . To return the area of all compartments for a component, use an asterisk (*) for n . To return the area of all components, use an asterisk for m .
$M[m, d]$	Returns the value of detail d for model component m .
$T[n]=equation$	Sets the value of temporary variable n to the expression in <i>equation</i> . After setting a temporary variable, you can use the variable without the " <i>=equation</i> " portion to return the value of the equation.
$T[n]$	Returns the value stored in temporary variable n .
$P[n]$	Set the floating-point precision for the rest of the equation to n decimal places.
$Y[m, start, end]$	Returns the portion of the area of component m that falls between channels <i>start</i> and <i>end</i> .
$S[start, end]$	Returns the sum of events between channels <i>start</i> and <i>end</i> .
$U[start, end]$	Returns the mean between channels <i>start</i> and <i>end</i> .
$H[]$	Returns the mean of the highest active cycle component. NOTE: There is no space between the open and close square brackets.
$F[expression]$	Returns the value of a keyword converted into a floating-point number. This allows the keyword to be used in calculations. For example, the \$P1B keyword stores the number of <i>bits</i> used to store a value for parameter 1 in an FCS file. To compute the number of <i>bytes</i> used by parameter 1, the following equation could be used: "Parameter 1 bytes = ";F[[\$P1B]]/8

Mathematical operations

The following operations are supported.

Operation	Usage
Addition	Use the plus symbol (+) between numeric values.
Multiplication	Use the asterisk (*) between numeric values.
Subtraction	Use the minus symbol (-) between numeric values.
Division	Use the slash (/) between numeric values.
Grouping	Use parentheses to group mathematical operations.

Concatenation

Equations can contain multiple elements separated by a semi-colon (;).

Help

Updates



Check for
Updates

Research and Compliance Mode note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** have permission to perform them.*

This command is found on the **Help** tab of the ribbon bar. In the menu & toolbar interface, the command is in the **Help** menu.

Use this command to check the Verity Software House web site for program updates. It will launch your default browser and display a list of available updates for the version you are running.

Ask Tech Support



Contact

This command interacts with your email application to create a new message to Verity Software House Tech Support. A new message is created to the Tech email address and some information about your application in the body of the email.

Fill in the email message with your contact information and the questions you want to ask. A member of the Tech Support team will respond to your question as quickly as possible.

Make a Suggestion



Contact

This command interacts with your email application to create a new message to Verity Software House Tech Support. A new message is created to the Tech email address and some information about your application in the body of the email.

We value your suggestion very highly, and make every attempt to incorporate changes into the software that address suggestions from our clients.

Fill in the email message with your contact information and your suggestion for the program. A member of the Tech Support team will respond to your email as quickly as possible.

Report a Problem



Contact

This command interacts with your email application to create a new message to Verity Software House Tech Support. A new message is created to the Tech email address and some information about your application in the body of the email.

When reporting a problem, please try to include as much detail as possible about the conditions that caused the problem. If at all possible, please try to reproduce the problem and list the steps that will cause it to occur. While it is sometimes possible to correct problems that are not reproducible, it is much more likely that we can fix a problem that we can reproduce.

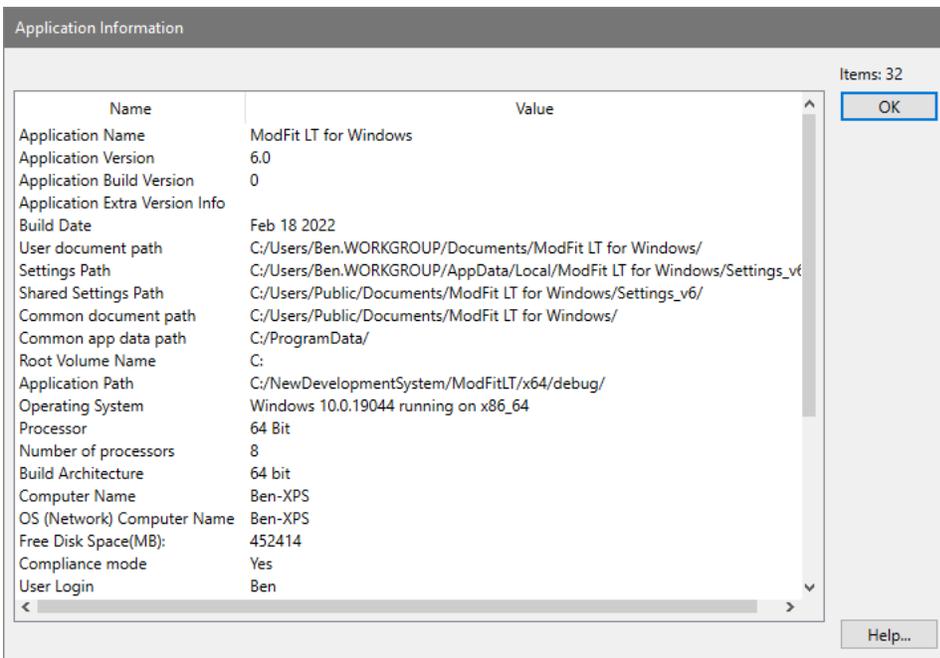
A member of the Tech Support team will respond to your email as quickly as possible.

Application Info



App Info

This command displays a dialog that contains information about the program and its installation. It is useful when interacting with tech support.



User Guide



User Guide

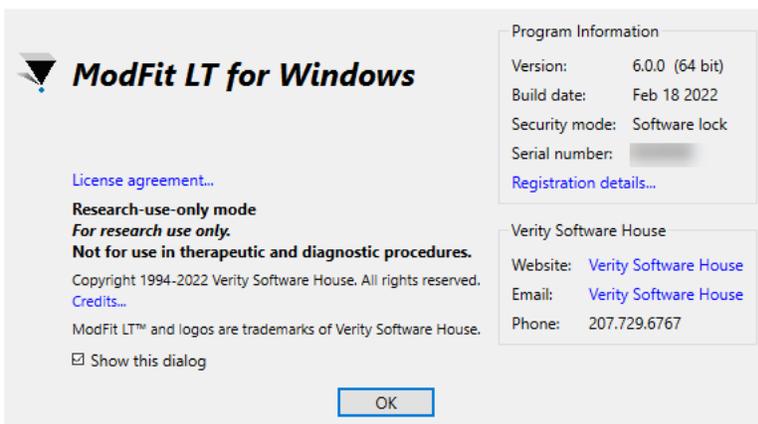
Use this command to display the User Guide in your browser.

About command



About

Use this command to display the About dialog box.



Appendices

Analysis Results

This section describes the results that are displayed by ModFit LT after an analysis has been performed. The results are usually displayed on the upper right side of the report, although you can move the results to anywhere on the report.

There are several types of models available in the program, each with its own set of results. *Standard* models are created by Automatic Analysis and by choosing a model for manual analysis. These models include components and results for debris, standards, aggregates, and apoptosis, as well as for one or more cycling populations. For each of the cell cycles, a G0G1, G2M, and S-Phase component is included.

Sync Wizard models are created when using the Sync Wizard. These models are designed for the special demands of perturbed cell cycle analysis.

The Cell Tracking Wizard creates *Cell Tracking Wizard models*. These models are used to study cell proliferation in samples using cell-tracking dyes and proliferation markers.

The program calculates percentages based on the number of events that are actually modeled. The number of events modeled can be less than the total number of events in a sample if you apply a gate, scale the data to eliminate events, or if the model itself does not fit the data exactly. Suppose, for example, that a listmode file contains 10,000 events. If you gate out 10% of the events, the analysis will be performed on 9,000 events, or 90% of the total events.

Standard Models

<i>Label</i>	<i>Description</i>
Model	This shows the name of the model used for the analysis. For information on how to decode model names, see Model Descriptions .
Analysis type:	This will show either "Automatic analysis" or "Manual analysis", depending on how the model was selected and whether any user-edits were made to the model set up.
Auto Linearity	This will show "Yes" if Auto Linearity was performed, or "No" if it was not.
Ploidy Mode	Displays the Auto Analysis Diploid Determination setting.
Diploid Reference	If the Diploid Determination setting is External Reference, Based on Standard 1, or Based on Standard 2, this result will display the location of the ploidy reference. If First Cycle is Diploid mode, this result is meaningless and is not displayed.
% Cycle	Percent of all events that are associated with cycling populations
%G0G1	G0G1 as a percent of all cells in that cycle
G0G1 mean	G0G1 mean channel
%G2M	G2M as a percent of all cells in that cycle
G2M mean	G2M mean channel
% S-Phase	S-Phase as a percent of all cells in that cycle
G2/G1 ratio	Ratio of G2 mean channel to G1 mean channel

ModFit LT™ User Guide

%CV	Coefficient of variation for G0G1 cells
DI	DNA Index, defined as the mean channel of G1 cells of an aneuploid population compared to Diploid G1 mean channel.
DI based on reference	If the Diploid Determination setting is External Reference, Based on Standard 1, or Based on Standard 2, this result will display the DNA index based on the computed reference position for the Diploid G1.
Total Aneuploid S-Phase	Sum of all Aneuploid S-Phase areas as a percentage of the total (G1G1 + S + G2M) area for all Aneuploid populations. This result is not displayed for Diploid models. However it is computed as a keyword even for Diploid models. For Diploid models, the "tumor" S-Phase is considered to be the Diploid S-Phase, and so the keyword reports the S-Phase of the Diploid.
Total S-Phase	Also called "Average S-Phase". Sum of all S-Phase areas as a percentage of the total area for cycling cells of all populations.
Total B.A.D	Amount of debris and aggregates found within the region between the first G1 and the last G2 peak.
Standards	
Std %	Area of standard as a % of all modeled events minus debris
Std mean	Mean channel position of standard
DI	DNA Index of standard as standard mean channel relative to the Diploid G1 mean channel.
Dip G1 Ratio	Diploid G1 mean channel divided by mean channel of the standard
%CV	Coefficient of variation for standard
%Debris	Debris area as a % of all modeled events
%Aggregates	Aggregate area as a % of all modeled events
%Apoptosis	Apoptosis area as a percent of all modeled events minus debris
Modeled events	Number of events accounted for by all components in the model
All cycle events	Number of modeled events in the G0G1, G2M, and S-Phase components of all cycling populations
Cycle events per channel	All cycle events divided by the number of channels between the first G0G1 and the last G2M of the model.
nRCS	<i>Normalized</i> reduced chi-square, a measure of goodness of fit. This produces an RCS equivalent results as if the sample file contained 10,000 events. See Options and Configuration for more information on this statistic.
RCS	Reduced chi-square, a measure of goodness of fit. Values from 1.0 to 3.0 are good, 3.0 to 5.0 are fair, and greater than 5.0 are poor. Note, RCS will increase as the number of events in the sample file increase. The nRCS can be used in cases where samples routinely have 50,000 or more events.

Tetraploid Model

ModFit LT handles Tetraploid histograms slightly differently than most other ploidy types. Because the Diploid G2M and the Tetraploid G1 peaks are superimposed, the program disables the Diploid G2M during the modeling process. After the model has been applied to the histogram using the Fit with Current Model command or Auto Analysis, the program will add back a specified amount to the Diploid G2M and reduce the Tetraploid G1 by the same amount. This amount is computed as a percentage of the Diploid G1 peak, and the percentage is specified in the Options and Configuration dialog box. The default percentage is 8% (0.08).

Sync Wizard Model

In addition to many of the results described for standard models, the Sync Wizard also computes the percentage for sub-compartments of S-Phase

<i>Result</i>	<i>Computation</i>
S-Phase	Cells in S-Phase as a percent of all the cells in that cycling population
Compartment <i>n</i>	Area of S-Phase sub compartment as a percent of all the cells in that cycling population

Cell Tracking Wizard Model

See the [Cell Tracking Example](#) for an explanation of Cell Tracking results.

Keywords

This section lists and describes the keywords that are created by ModFit LT. These keywords can be selected for display on reports using the Keywords command in the Tools menu, and can be stored in a database as well.

Some keywords are available only when the conditions they relate to exist. For example, keywords related to a second DNA Aneuploid population will only be available if the model that is used contains components for a second Aneuploid cycle.

NOTE: The keywords for second, third, and fourth aneuploid cycles are identical to those for the first, except that they are prefixed with Z_An2, Z_An3, and Z_An4 instead of Z_An1.

Standard Model Keywords	Comment
Z_Aggregates	% aggregates
Z_AllCycleEvents	Sum of events in cycles
Z_An1	% events in first aneuploid cycle
Z_An1_BAD	% BAD in first aneuploid cycle
Z_An1_CV	% CV for first aneuploid cycle G1
Z_An1_DI	DNA index of G1 in first aneuploid cycle
Z_An1_G1	% of first aneuploid cycle in G1
Z_An1_G1_Mean	Mean of G1 in first aneuploid cycle
Z_An1_G2	% of first aneuploid cycle in G2
Z_An1_G2_Mean	Mean of G2 in first aneuploid cycle
Z_An1_G2G1Ratio	G2/G1 ratio for first aneuploid cycle
Z_An1_Ploidy	Ploidy of first aneuploid cycle
Z_An1_S	% of first aneuploid cycle in S-Phase
Z_AneuploidS_Bnd	S-Phase assessment cut-off value
Z_AneuploidS_Class	S-Phase assessment classification
Z_AneuploidS_PValue	S-Phase P-value
Z_AneuploidS_Std	S-Phase assessment standard deviation
Z_CycleEventsPerChannel	Average number of events per channel in cycles.
Z_Debris	% debris
Z_Dip	% events in diploid cycle
Z_Dip_BAD	% BAD in diploid cycle
Z_Dip_CV	% CV for diploid cycle G1
Z_Dip_G1	% of diploid cycle in G1
Z_Dip_G1_Mean	Mean of G1 in diploid cycle
Z_Dip_G2	% of diploid cycle in G2
Z_Dip_G2_Mean	Mean of G2 in diploid cycle
Z_Dip_G2G1Ratio	G2/G1 ratio for diploid cycle
Z_Dip_Ploidy	Ploidy of diploid cycle
Z_Dip_S	% of diploid cycle in S-Phase
Z_DiploidS_Bnd	S-Phase assessment cut-off value

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Z_DiploidS_Class	S-Phase assessment classification
Z_DiploidS_PValue	S-Phase P-value
Z_DiploidS_Std	S-Phase assessment standard deviation
Z_LinearityFactor	Linearity value used in the model
Z_MAE	Mean Absolute Error For details, see https://en.wikipedia.org/wiki/Mean_absolute_error
Z_ModeledEvents	Number of events accounted for by the model
Z_nRCS	Normalized RCS value, which is the RCS computed on the area normalized to 10,000 events.
Z_PloidyStatus	S-Phase assessment ploidy
Z_RCS	Reduced chi-square
Z_RMSD	Root Mean Square Deviation. For details, see https://en.wikipedia.org/wiki/Root-mean-square_deviation
Z_Std1	% of events in first internal standard
Z_Std1_DI	DNA Index of first standard
Z_Std1_DipG1Ratio	DipG1 mean/First Std mean
Z_Std1_Mean	Mean of first standard
Z_Std2	% of events in second internal standard
Z_Std2_DI	DNA Index of second standard
Z_Std2_DipG1Ratio	DipG1 mean/Second Std mean
Z_Std2_Mean	Mean of second standard
Z_TotalAneuploidFraction	Percentage of cells in aneuploid cycles
Z_TotalAneuploidS	(Sum all aneuploid S-Phase areas)*100/(Sum all aneuploid areas). For simple Diploid models, the keyword reports the value of Diploid S-Phase. You may think of this keyword as "tumor S-Phase."
Z_TotalBAD	% BAD for histogram
Z_TotalS	Average or total S-Phase
Z_TotalS_Bnd	S-Phase assessment cut-off value
Z_TotalS_Class	S-Phase assessment classification
Z_TotalS_PValue	S-Phase P-value
Z_TotalS_Std	S-Phase assessment standard deviation

The following keywords store configuration values used by the program. The Auto Analysis Settings, Peak Finder Settings, and Options and Configuration dialog boxes set these values.

Configuration Keywords	Comment
CF_APOPTOSIS	Auto Analysis state for apoptosis
CF_AUTOAGGREGATE	Auto Analysis state for AutoAggregates
CF_AUTODEBRIS	Auto Analysis state for AutoDebris

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CF_MOD_S_COMPARTMENTS	Number of S-Phase compartments in this model
CF_MOD_S_SHAPE	Shape of S-Phase in this model
CF_G2G1RATIO	Auto Analysis state for linearity or G2/G1 ratio
CF_PLOIDY_DIP_STD_RATIO	Auto Analysis state for diploid-to-standard ratio
CF_PLOIDY_MODE	Auto Analysis state for ploidy detection mode
CF_SPHASE_COMPARTMENTS	Auto Analysis state for number of S-Phase compartments
CF_SPHASE_SHAPE	Auto Analysis state for shape of S-Phase
CF_SPHASE_DI_THRESHOLD	Auto Analysis state for DI for S-Phase
CF_TET_RANGE	Auto Analysis state for Tetraploid range
CF_PF_STATE	State for Peak Finder
CF_PF_RISE	Peak Finder setting for rise
CF_PF_LOWCV	Peak Finder setting for low CV
CF_PF_HIGHCV	Peak Finder setting for high CV
CF_PF_CC	Peak Finder setting for correlation coefficient
CF_PF_PCNTSTART	Peak Finder setting for starting threshold

MF keywords

Internal keywords are prefixed with "MF_" and store data computed by the program. They are listed in the table below.

Internal Keywords	Comment
MF_ANALYSIS	Type of analysis performed
MF_AUTODEBRIS_FIRSTCUT	Setting for AutoDebris first cut
MF_AUTODEBRIS_RESOLUTION	Setting for AutoDebris resolution
MF_AUTODEBRIS_SECONDCUT	Setting for AutoDebris second cut
MF_AUTODEBRIS_THIRDCUT	Setting for AutoDebris third cut
MF_DATE	Date of analysis
MF_FILE	Full path name of the data file
MF_FILEPATH	Pathway to the data file
MF_GATE1	Parameters for gate
MF_GATE1_PERCENT	Percent of events in gate
MF_GATE2	Parameters for gate
MF_GATE2_PERCENT	Percent of events in gate
MF_GATE3	Parameters for gate
MF_GATE3_PERCENT	Percent of events in gate
MF_MODEL	Name of the model used in analysis
MF_PLOIDYINDEX	0=Diploid, 1=Tetraploid, 2= One Aneuploid, 3=Two Aneuploid, etc.

MF_RCS	Reduced chi-square
MF_REPORT	Name of the report
MF_REPORTPATH	Pathway to the report
MF_TIME	Time of the analysis
MF_VERSION	Version of the program

USER keywords

These keywords show the results of user-entered equations found in Options and Configuration dialog. The name for the keyword is prefixed with "USER_". This allows you to display and database results of user-entered equations.

C keywords

Keywords that begin with "C_" display the final value for a detail of a model component. The naming convention starts with "C_", followed by the name of the component, an underscore, and the name of the detail. For example, "C_An1 G1_Area" displays the area of the An1 G1 component.

Sync Wizard keywords

When using the Sync Wizard, several additional keywords are made available. These allow you to extract the percentage of each compartment used in modeling S-Phase. If, for example, you use 3 rectangles to model S-Phase, you will find three additional keywords: Z_DIPS_C1, Z_DIPS_C2, Z_DIPS_C3.

Proliferation Wizard keywords

When using the Proliferation Wizard, additional keywords are available to allow you to display and database all of the information generated by the wizard. These keywords are prefixed with "Z_PROLIF", and are named to indicate the value that it stores.

Database

[Research and Compliance Mode](#) note:

In RUO mode, all users can create and edit databases.

*In Compliance mode, databases can only be created and edited by **ModFitAdmins** and **ModFitEditors**.*

This section introduces the concepts and functions of ModFit LT's database features. It is more of a discussion than a tutorial, however there are examples to clarify some aspects of the system.

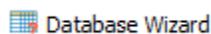
ModFit LT has a simple, powerful database engine that allows you to store large amounts of data easily in one or more database files. You can view databases from within the program, manually add, delete, and replace records, and select the fields you want to store in each database. The databases are created in a simple, text format known as tab-delimited text, which can be read by common database and spreadsheet programs. These features give you a great deal of flexibility in choosing what information to store, where to store it, and how to integrate the information into other database and information systems.

When you create a new *project* in ModFit LT, the program automatically creates a database file for the project.

How do you create a database?

It is best to run an analysis of a data file before you create or edit a database. Open one of your data files and perform an Auto-Analysis. This will fill the program with the information you can choose to store in your database. You can set up a separate database for each kind of analysis you do, one database for everything you do with the program, or anything in between.

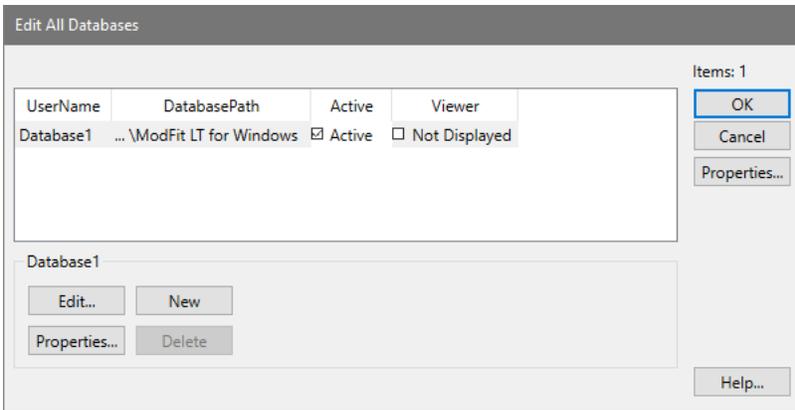
The fastest way to create a database from your results is to use the [Database Wizard](#), found on the **Edit** tab of the ribbon.



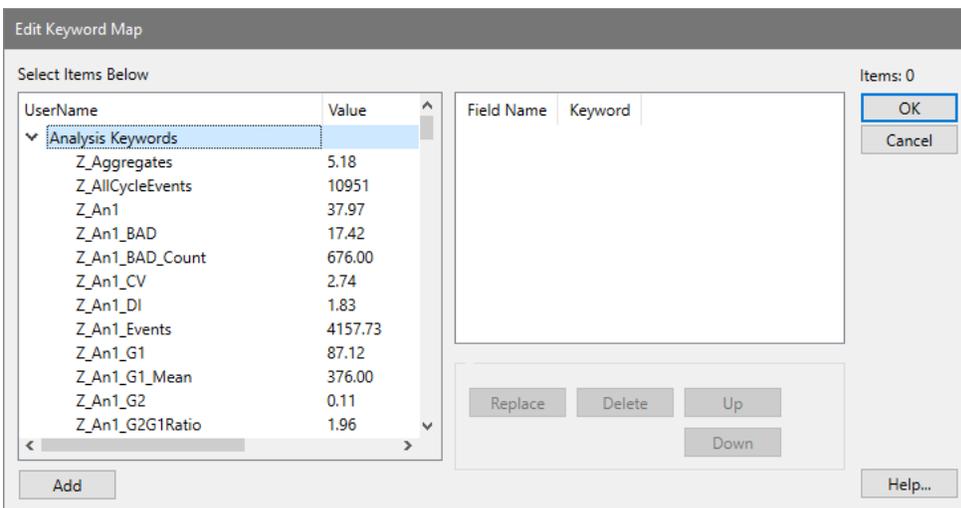
The wizard helps you to select a database (or create a new one) and it adds fields for all of the results that are shown on the current report. This is by far the fastest and easiest way to set up a database.

If you want to select additional fields or have greater control over the database structure, you can click the **Databases** on the **Edit** tab of the ribbon. The Edit All Databases dialog will display.

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There is one database created by the program automatically. This database will not store anything until you tell the program what information you want to save in the database. To do so, you need to select the database in the list and click the **Edit** button. You can also double-click the item in the list to do this.

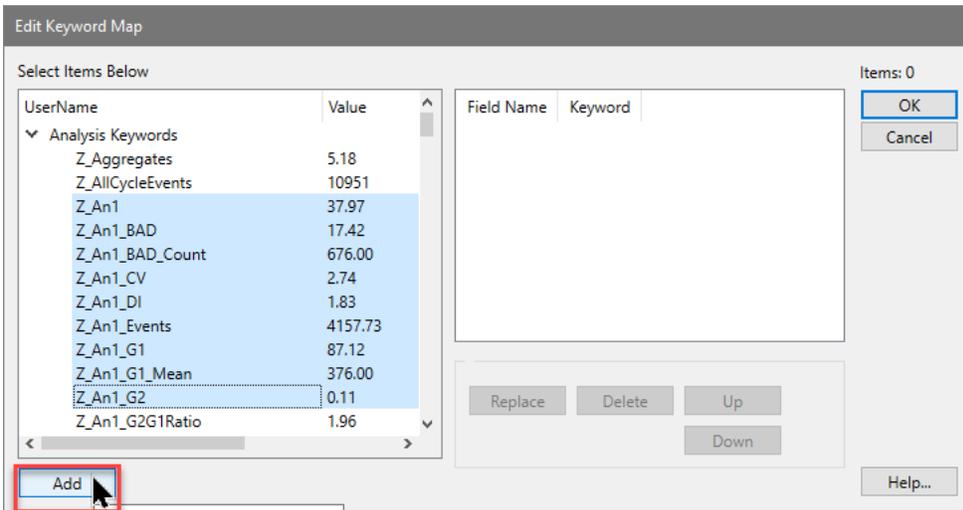


The Edit Keyword Map dialog is displayed. The left side of this dialog presents the information available to store, and the database fields are shown on the right. Initially, the right-hand list is empty.

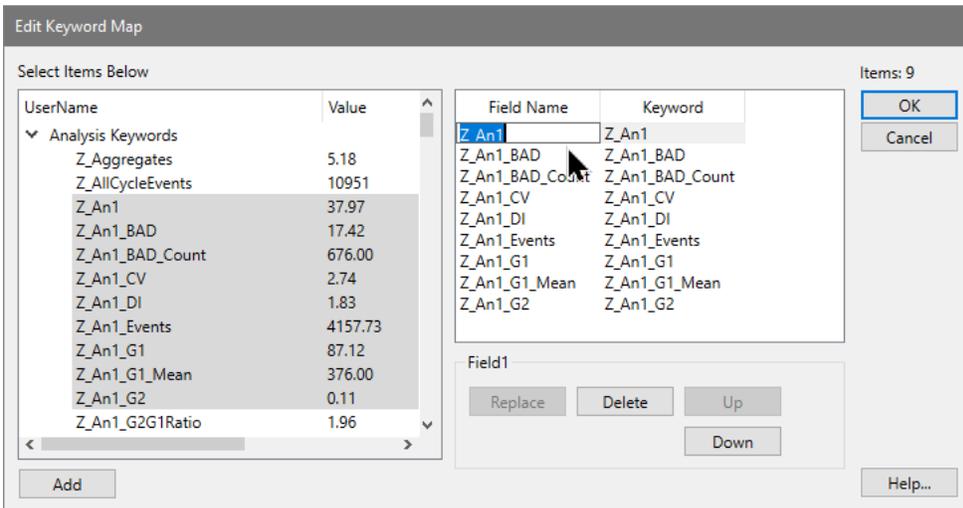
What can you store in the database?

ModFit LT allows you to database virtually any statistic it generates, as well as keyword information from the files that you analyze. The tree on the left of the dialog can be expanded to display branches of keywords. To open or close a branch of the tree, double click the label or click the arrow next to the label. Each branch of the tree contains a set of related keywords.

To add an item to your database definition, select it in the tree on the left and click the **Add** button. A field will be appended to the database. You can also select more than one Keyword at a time and click Add to create multiple fields at once.



The field names initially assigned when you click the Add button are based on the keyword added.



You can edit the names for the fields after they are added to the list, if you wish. To do so, click once on the field name to select it, then after a brief pause, click slowly again. An edit control will appear around the field name, allowing you to type a new name for the field. You can also use the Tab key to move from one field to the next if you need to edit the names of several fields.

To remove one or more fields from the database, select the fields in the list and click the **Delete** button.

To replace an existing field with a new keyword, select the new field in the Keywords list and the one to replace in the list. Then, click the **Replace** button. The name of the field will not change, only the keyword used to evaluate the field.

To change the order of fields in the database, select one or more fields in the list and then use the **Up** and **Down** buttons to move the fields.

When you have finished selecting information to store in the database, click the **OK** button to close the Edit Keyword Map dialog.

What files are created?

When you create a *project* in ModFit LT, there is a database created automatically for the project. It is located in the Database folder of the project, and the project it typically created in the user's Documents\ModFit LT\Projects folder.

You can also create databases outside of a particular project. These are stored in the location shown in the table below. You can choose another location, as long as you have read/write permissions to that folder.

Operating System	Default Location
Windows	C:\Users\[user name]\Documents\ModFit LT for Windows
MacOS	[user name]/Documents/ModFit LT for Mac

A file is created to store a "map" of keywords to database fields. The map file name uses the name of the database object and appends ".**map**" to it. For "Database1", the map file is named "Database1.map".

Next, the program creates the actual database file. This is the tab-delimited, ASCII text file that you may open in other database or spreadsheet programs. The database file name uses the database object name and appends ".**txt**" to it. This file is readable in virtually any program that can open unformatted text. The first line of the file contains the names of the fields separated by tabs; it is known as the database header or definition. Each subsequent line contains a record of information.

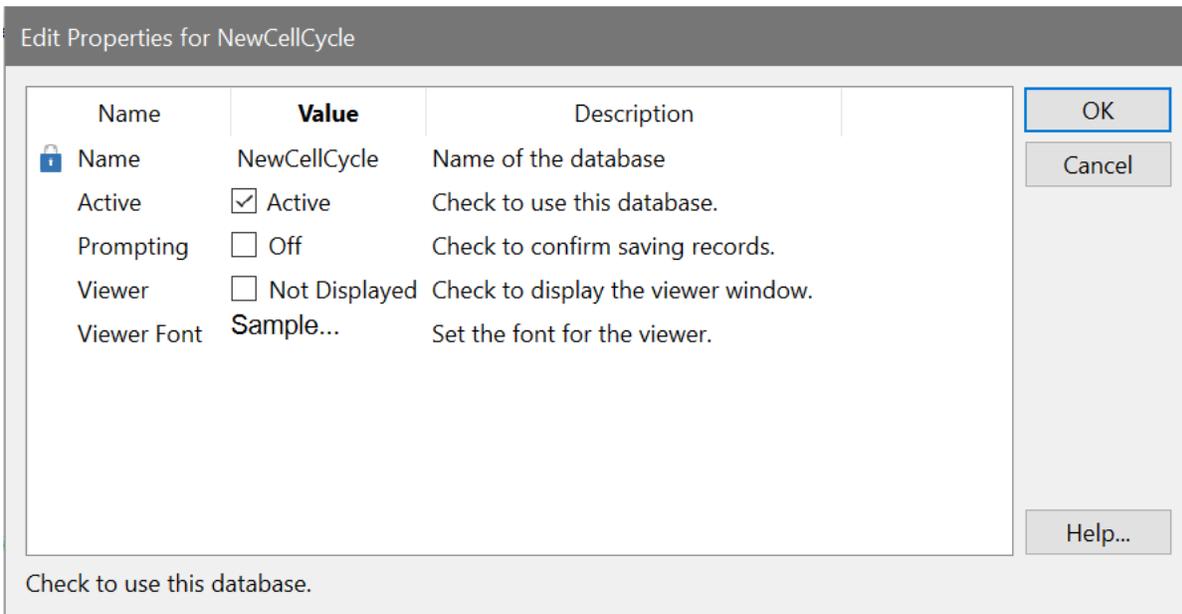
If the database had been created previously, ModFit LT will automatically create a backup of the database before overwriting the existing one. The backup file will have the same name as the database, with "backup" appended to it.

Finally, the program creates a text file called a "**key**" file. This is a text file that explains the database and mapping files. You can open this file in a text editor to review the structure of the database you have created. Its name is based on the database object name, with "key.txt" appended to it.

How do you activate the database?

Once a database has been defined, there are several additional properties you can specify. These properties determine whether the database is active, whether it will prompt you before saving a record, whether or not you want to see the records in a database viewer, and where you want to store the database.

To edit these properties for a database, select the database in the **Edit All Databases** dialog box and click the **Properties** button at the bottom of the dialog. The Edit Properties for Database dialog will be displayed.



The properties are enabled or disabled with simple check boxes. If you create a database outside of a project, there are several additional actions in this dialog. You can use the **Choose** button to use an existing database that you have defined previously. You can change the location of the database using the **Path** button.

In order to store records of information in a database, it must be "active". To make a database active, check the **Active** checkbox in the Edit Properties dialog. Database records are saved after Automatic Analysis or Execute Model commands are performed. Additionally, you can save a record at any time by clicking the "+" button on the database viewer.

If the **Prompting** checkbox is checked, ModFit LT will display a message each time it is about to save a record to the database. You can choose at that time whether or not you want to save the record. If **Prompting** is disabled, the database will save a record without prompting.

To view the database while you work with ModFit LT, check the **Viewer** checkbox. A database viewer window will be created and displayed. The database viewer can be moved, resized, and scrolled horizontally or vertically. It has a toolbar that provides some additional manual database functions. See the topic [Database Viewer](#) for more information.

The size of the font used for the database viewer can be set with the **Viewer font** property.

To select an existing database on disk, click the **Choose** button. A file selection dialog box will appear, allowing you to navigate to the existing database and select it. Linking to an existing database will rename the database in memory to match the one selected on disk, and the associated database map on disk will be loaded as well.

To change the location of a database, click the **Path** button. A path navigation dialog will appear. Navigate to the new location.

How do you rename or create additional databases?

To rename a database, display the **Edit All Database** dialog box by selecting Edit Databases from the Edit menu. Select the database you want to rename and click **Properties**. You can then edit the name for the database. Note, you cannot edit the name of a *project* database.

If a file with the new name already exists, you will be given a chance to overwrite the existing file, use the existing file, or cancel the operation.

When you want to create an additional database, click the **New** button. This will create an empty database with a new name.

Each database has its own map of keywords to fields, and its properties determine whether it is active, prompting, and showing a viewer.

Errors with databases

Since the database system involves creating and manipulating files on disk, there are conditions where errors can occur. If, for example, one of the critical database files is moved or deleted, the program will not be able to use the database. When the program runs into a problem like this, it displays a dialog box of choices.

When you see this dialog, it means that the database "txt" file and the "map" file are out-of-sync with one another. They may have a different number of fields, a different order, different fields defined, or one of the files may be missing.

If you choose the first option, **Use the database and manually create a new map**, the map file is emptied and partially recreated with the fields defined in the database header. In this case, you need to recreate the keyword-to-field "map" using the Edit Keyword Map dialog.

If you choose the second option, **Use the map file and DESTROY the database**, all records in the database txt file are emptied and a new "header" is written into it to match the database map file.

The third option, **Cancel and de-activate the database**, leaves the map and the txt files out-of-sync and deactivates the database, allowing you to investigate the problem before any action is taken.

See also:

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[Edit Databases](#)

[Edit Keyword Map](#)

[Edit Properties for Database](#)

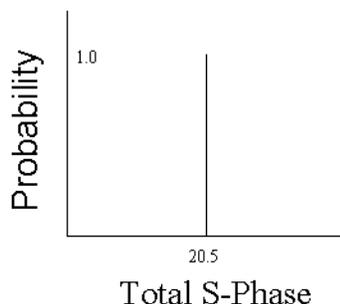
[Database Viewer](#)

Histogram and S-Phase Assessment

ModFit LT contains two systems to evaluate confidence in the S-Phase estimates it computes. The first method evaluates several histogram features that have been published as affecting S-Phase accuracy (Cytometry. Vol. 14. Number 5, 1993.): %CV, Cell Number and %B.A.D. The Prout's Neck DNA Consensus Conference published a set of guidelines for these histogram attributes and ModFit LT uses these published guidelines to provide you with a graphical and quantitative picture of how the histogram satisfies the requirements. By pushing the button next to each of these features you can examine the actual published quote.

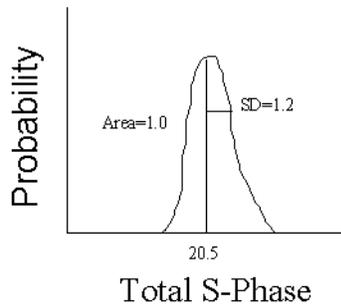
ModFit LT evaluates an additional feature not covered by the Consensus Conference: RCS. Reduced Chi-square is a measure of how well the model fitted the data. Based on our experience with RCS values, we added our own guidelines for this feature.

The second method of S-Phase assessment is unique to ModFit LT and is based on a statistical approach to the problem rather than a consensus of experiences. By using established statistical methods, ModFit LT calculates the error probability distribution about each of its S-Phase estimates (e.g. Total or Average S, Aneuploid S, and Diploid S). The standard deviation of this probability distribution provides a direct quantifiable way of evaluating the confidence of a given S-Phase estimate. If you are getting glassy-eyed on this statistical stuff, let us show you four graphs that will make it crystal clear how this system works. The first graph demonstrates how cell cycle programs used to work.

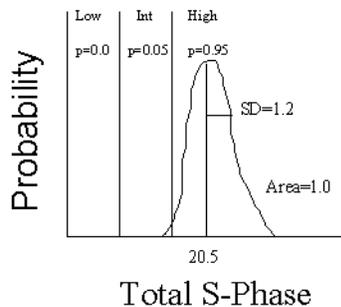


Let's say that your old cell cycle program reported a Total (average) S-Phase estimate, also known as "Average S-Phase", of 20.5%. Since the program did not give you any information on the variability associated with this estimate, you had to assume that it was correct. Another way of saying the same thing is that the probability of the Total S-Phase being 20.5% was assumed to be unity or one. Largely because of this limitation, a number of S-Phase criteria were developed that normally would be conducive to obtaining accurate S-Phase estimates (cell number > 10,000, %CV < 8.0, etc).

ModFit LT has an exclusive statistical method of estimating the probability distribution about each of its S-Phase estimates. Let's take a look at how that changes our figure.



Since ModFit LT calculates the probability distribution about its estimate, you now have a way of quantifying the S-Phase estimate's variability with a standard deviation. Rather than 20.5% in this example, you now know that it is really 20.5% +/- 1.2%. However, ModFit LT takes this S-Phase assessment one step further. By evaluating S-Phase cutoffs against this probability distribution, the program can calculate the probability of the estimate being in a particular category (e.g. Low, Intermediate, or High). Let's look at the next graph to see how this works.

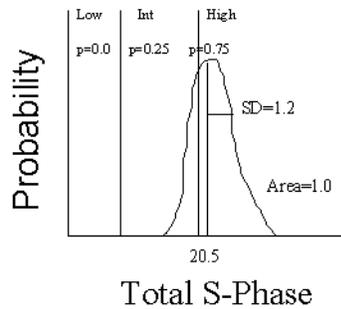


The areas of the probability distribution between each of the S-Phase cutoffs indicate how likely the S-Phase estimate is within that category. For example, the area of the High category is 0.95; thus, there is a 95% chance that the specimen has a high S-Phase with your laboratory's specific S-Phase cutoffs. Normally in statistics, you express the probability of the hypothesis not being correct (p-value). In this case, the p-value is simply 1.0 - 0.95, or 0.05, and it is the p-value which ModFit LT displays.

That's just about all there is to it. Not only does ModFit LT indicate the variability of its estimate, but it also merges your S-Phase cutoffs with the derived probability distribution to provide you with just the information you need to convey to the physician about the proliferative activity of the specimen. Is the S-Phase high? How sure are you of this assessment? You can now answer both questions quantitatively.

We promised you four graphs and have only shown three so far. Suppose the derived S-Phase estimate was one of those borderline calls. It overlaps one of your S-Phase cutoffs. Let's modify the above graph to show that situation.

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How do you classify this specimen? If you choose High, then you have a 25% chance of being wrong. When confronted with this situation it's best to combine two categories. By calling the specimen: Intermediate to High, the probability of being wrong is back to a reasonable level. What you are really saying is that the S-Phase is probably not Low.

If ModFit LT determines that the certainty (probability) of a particular S-Phase level is less than 0.9, it will automatically combine the two most probable categories. If the certainty is still less than 0.9 with the combined categories, then the program will display a question mark (?) next to the corresponding S-Phase assessment.

ModFit LT enables the inspection of the error distributions about each of its S-Phase estimates. If the sample is DNA diploid, only the diploid S-Phase error distribution can be evaluated. If the sample is DNA aneuploid or tetraploid, only the aneuploid and total S-Phase error distributions can be evaluated.

Using Auto Linearity

Purpose:

The purpose of this tutorial is to demonstrate the appropriate use of ModFit LT linearity controls. The goals of the tutorial are listed below.

1. Define linearity and its potential effects on cell cycle analysis.
2. Show an example of a fit with an inappropriate linearity setting.
3. Demonstrate the manual method of adjusting linearity.
4. Discuss the linearity configuration settings.
5. Discuss a recommended approach to modeling with an optimized linearity setting.

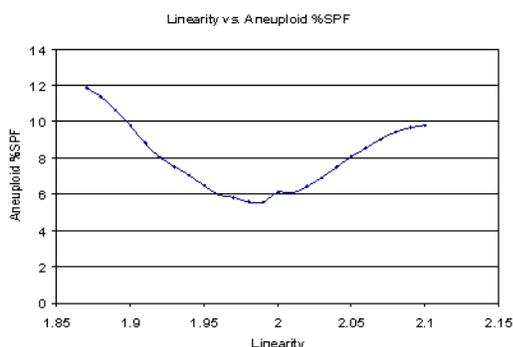
Definition and Potential Effects

Linearity, in this discussion, is defined as the observed ratio between the DNA Diploid G2M and G0G1 positions. Theoretically, this value should be 2.0, but it can vary from this ideal value.

Linearity affects cell cycle analysis in several ways. The first effect is that for models that have indistinct G2M peaks, the program uses the linearity setting to position the G2M in the fitting process. For example, if a G1 peak is found at channel 100, the G2M position would be at 200 with the linearity setting of 2.0. If the linearity setting were 1.9, the G2M position would be 190.

The second major effect is on the aggregation model component. This model component assumes, for example, that doublet positions are twice singlet positions and triplet positions are three times the singlet positions. ModFit LT uses the linearity setting to transform the data so that it is perfectly linear prior to creating the theoretical shape of the aggregate distribution. Thus, linearity can also affect the positions of computed aggregates and affect the model fit and S-Phase estimate.

How important is the linearity setting in estimating the S-Phase fraction? It can change S-Phase estimates significantly, as shown in the next graph.

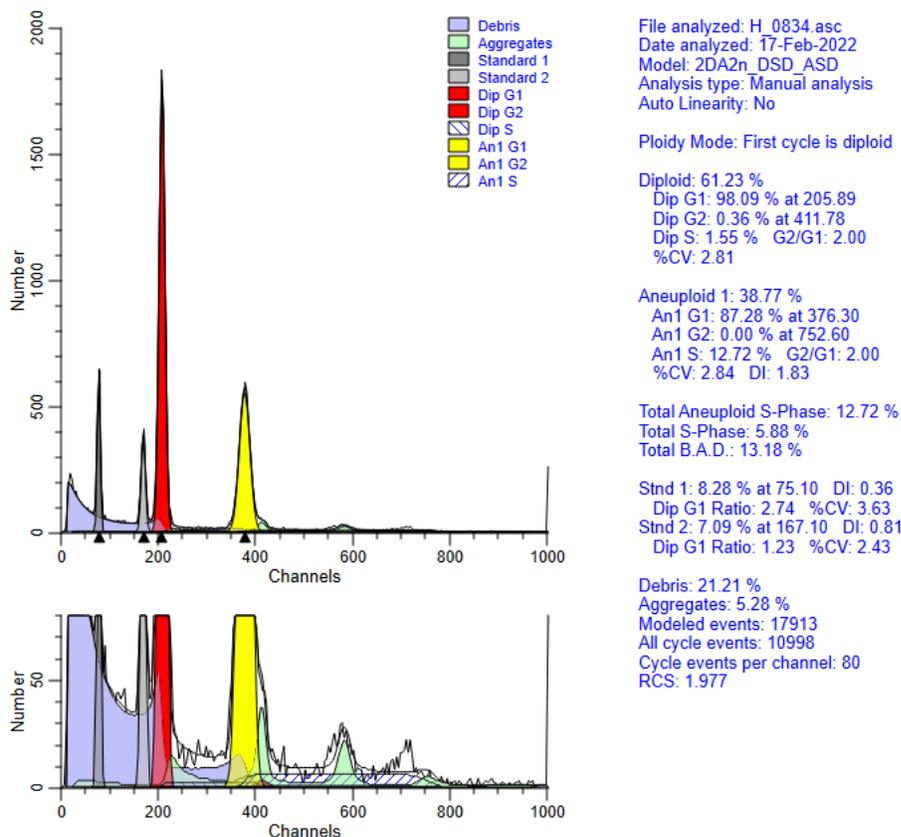


In this particular example, a linearity setting of either 1.90 or 2.1 results in approximately twice the aneuploid S-Phase estimate as a value of 1.99.

Therefore, it is important to optimize this setting for a particular histogram and model in order to obtain accurate and consistent S-Phase estimates.

Example of inappropriate linearity setting

Here is a report demonstrating a model that used an inappropriate linearity setting.



Notice that the Aneuploid S-Phase is quite high and the reduced chi-square is 1.97 for this analysis.

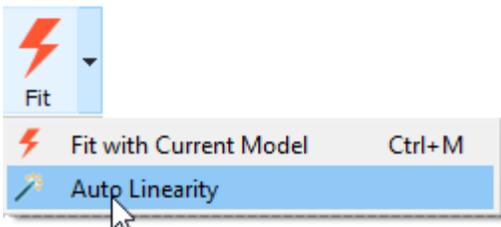
In order to better appreciate the linearity effects, we also show a zoomed view of the graph. This allows us to easily see the Diploid G2M peak and aggregates.

Notice that the diploid G2M model component has disappeared - overwhelmed by the aggregate component.

Automatic linearity detection from Quick Model Editor

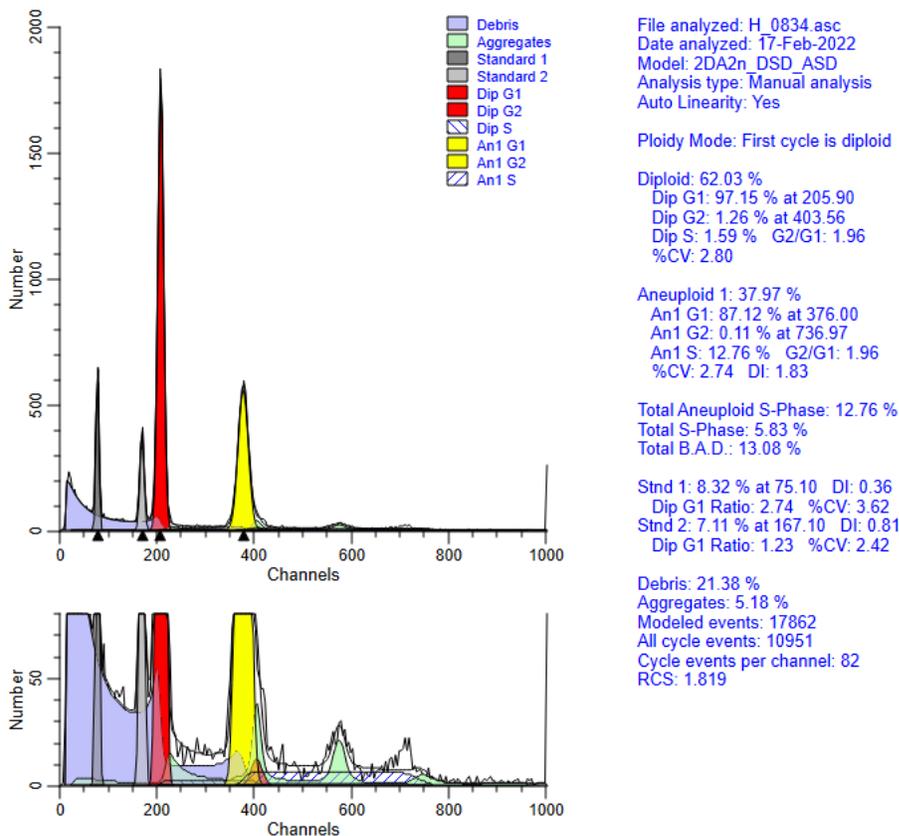
Let's use a feature in ModFit LT that is designed to automatically find this optimal linearity setting.

Click the down-arrow on the right of the **Fit** button on the ribbon bar and choose **Auto Linearity** to trigger the command.



With this method, the program models the data with a set of linearity settings, searching for the one that results in the lowest reduced chi-square. The message status bar in the lower-left corner indicates its progress.

After it has completed this analysis, it re-fits the histogram with the optimized linearity setting. For this example the optimized linear setting is 1.96.

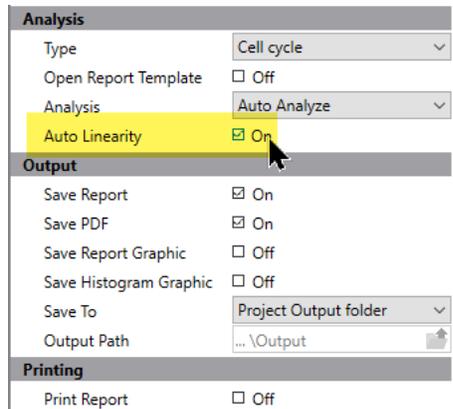


The advantage of using the automatic linearity detection is that it potentially eliminates an important factor in S-Phase estimate variability.

We can also automate the entire process with batch processing.

Automatic linearity adjustment

When you are processing a batch of files in a project, you can enable an option to perform auto-linearity on each sample file. This option is available for Cell Cycle projects.



This setting optimizes linearity automatically for each file.

Linearity configuration settings

How does the program determine the starting and ending linearity value to test in the auto linearity detection system? These settings are stored in the Options and Configuration section of the program. This dialog can be displayed if you have administrative access to the program by choosing the **Configuration** command from the **Options** tab of the ribbon bar.

AutoLinearity	
Low Linearity	1.90
High Linearity	2.05

ModFit LT contains reasonable default values for these settings. If your laboratory has a smaller range of possible linearity values, you can save considerable time by making the Low linearity and High linearity settings more restrictive.

A recommended approach to modeling with an optimized linearity setting

ModFit LT™ User Guide

Our recommended approach to the routine analysis of DNA files is as follows. First, find a good average linearity setting for your laboratory and enter it into the Auto Analysis Settings option in the program. You may also need to tailor other aspects of the auto analysis system as well.

Second, enable Auto Linearity in your Cell Cycle projects to optimize the linearity value for each sample.

Optimizing Auto Analysis

[Research and Compliance Mode](#) note:

In RUO mode, all users can perform these tasks.

*In Compliance mode, only **ModFitAdmins** have permission to perform them.*

This section describes how to configure Auto Analysis to work with your data files. Three configuration dialogs are used to set up the program for Auto Analysis: Peak Finder Settings, Auto Analysis Settings, and Options and Configuration dialogs. By modifying the content of these three dialogs, you can optimize Auto Analysis to handle your data sets.

There are two major benefits to configuring the Auto Analysis system correctly. First, it will reduce the amount of time you have to spend analyzing each file. Of equal importance, you will increase user-to-user consistency of analysis results. The time you spend configuring the system can save analysis time in very few analyses.

Prerequisites

In order to complete this process, you need a data set of twenty or more data files of the same cell type that have been acquired according to the same protocol. Your protocol for sample preparation, staining, and acquisition should be well defined and strictly followed. Since different types of samples may have different characteristics and preparation procedures, you may need to configure the program differently for each type of sample you analyze.

Setting Options and Configuration

From the **Options** tab, choose **Configuration**. This will display a dialog containing a number of configuration settings. Most of these settings do not need to be changed from the program default settings.

One set of values you may need to adjust is found under the **AutoLinearity** section. The Low and High linearity values define the range over which the program can adjust the linearity during AutoLinearity adjustments when the AutoLinearity option is enabled. You should enter the acceptable range for G2/G1 ratios for your data set.

Review other settings in this dialog. For details on each setting, see [Options and Configuration](#).

Make any adjustments required to the values. You may want to save these settings to a file that can be manually loaded back into the program. To do this, use the Save button at the bottom of the list in the dialog. When you need to re-open the saved settings, use the Open button. This also allows you to transfer settings from one computer to another.

Click OK to close the dialog and accept the changes you have made.

Reviewing a Data Set for Important Peaks

Next, the peak finder needs to be tested for your data set.

Click the **Open FCS** button on the **Home** tab. Navigate to the location of your data set and select twenty or more of the files to be analyzed. Click the Open button.

The program will open the first file in the set. If the file is a listmode file, you will need to select the parameter to analyze.

There are two things to notice at this point. At the bottom of the application window, the program now displays a batch navigation toolbar. We will use this to review the data set. Secondly, the first histogram has one or more peaks identified with black triangles along the x-axis. These are the peaks that the program's Peak Finder has identified. When the program is correctly configured for your data set, it will identify the vast majority of the internal standards and G1 peaks for cell cycles in your data set. It will also identify many of the well-defined G2M peaks, but these are not as critical as the G1 peaks. It should not identify many false peaks, that is, portions of the histogram that you do not consider to be real peaks.

To review the data set, we need to tell the program what to do as we navigate through the batch of files. Click the left-most button on the batch control toolbar to display the properties for processing files.

Analysis	
Type	Cell cycle
Open Report Template	<input type="checkbox"/> Off
Analysis	No analysis
Auto Linearity	<input type="checkbox"/> Off
Output	
Save Report	<input type="checkbox"/> Off
Save PDF	<input type="checkbox"/> Off
Save Report Graphic	<input type="checkbox"/> Off
Save Histogram Graphic	<input type="checkbox"/> Off
Save To	Project Output folder
Output Path	... \Output
Printing	
Print Report	<input type="checkbox"/> Off

Set the options as follows: No analysis and do not save or print reports, do not prompt for database or files. This tells the program that all we want to do is open the data files as we navigate through the batch.

Now, here is the process for peak finder review. For each file in the batch, record whether the program has identified all of the internal standards and the G1 peaks for each cell cycle. If the program misses an important peak, note the locations of peaks omitted. The major reasons peaks are missed are that they are too small, too wide, or they do not represent a normal, Gaussian distribution. All of these parameters can be adjusted in the program's peak finder. Also note the locations for any false peaks. For missing peaks and false peaks, try to note the characteristics of the peak, e.g. short, wide, shoulder.

Make notations for the files, and then click the "Next" button on the batch toolbar.



Repeat this process for each file in the data set.

Configuring Peak Finder Settings

Review the notations you made for the data set. If the program correctly identified 95% or more of the peaks in your test data set, you probably do not need to make any adjustments. If the Peak Finder did not perform as well as it should, here is how to make adjustments to the settings.

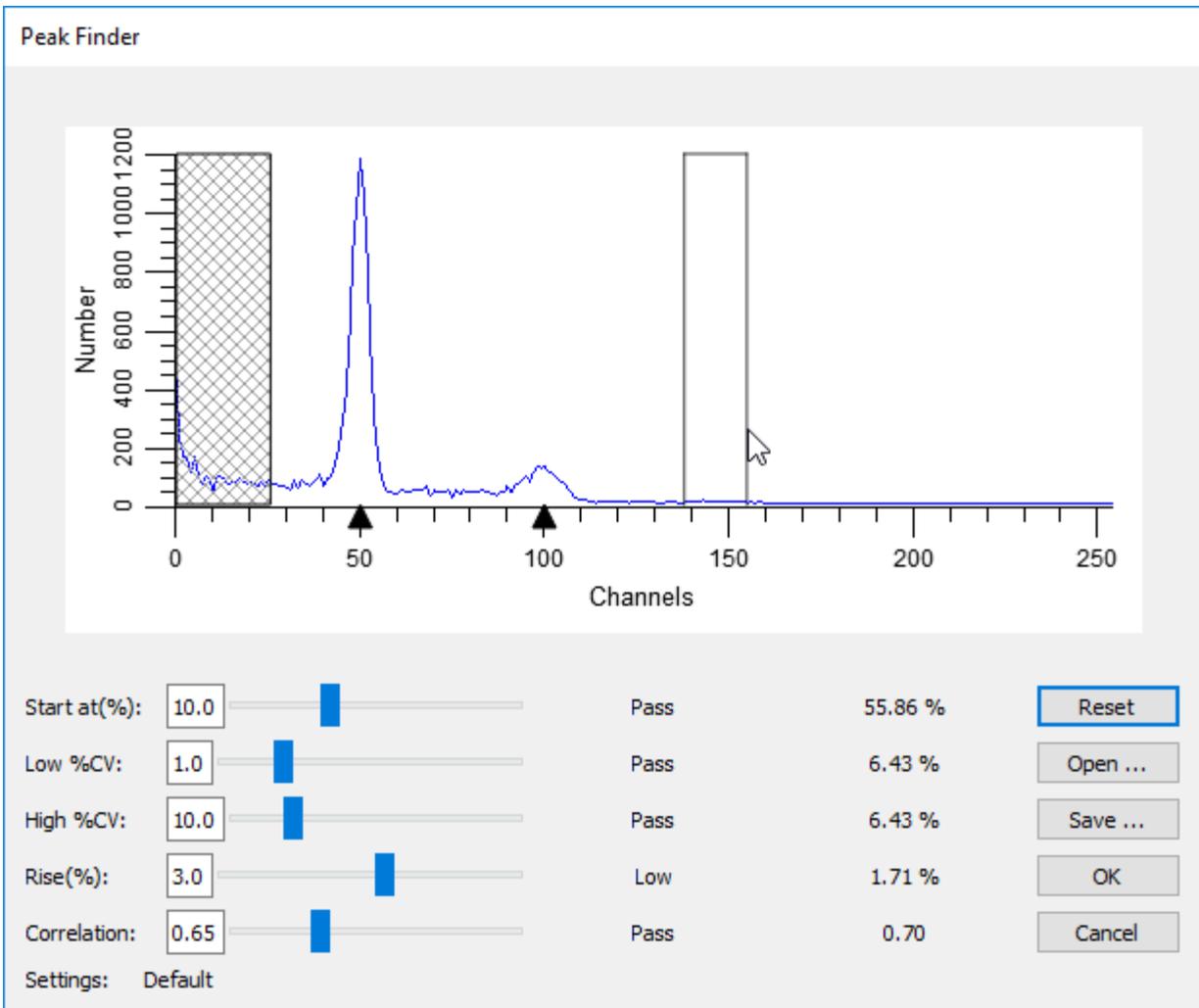
Hopefully, there are some common characteristics to the files that caused problems for the Peak Finder.

Use the batch navigation buttons on the batch toolbar to re-open a histogram that is representative of the mistakes the Peak Finder made.

Next, choose **Peak Finder** from the **Options** tab. The Edit Properties for Peak Finder settings dialog is displayed.

Click the **Show** button at the top of the list of options. This will display a dialog that allows you to see the current histogram, and to understand the characteristics of each peak.

As you move your mouse over the histogram, vertical lines will be displayed showing the boundaries for a peak that the program found. The lower portion of the dialog shows a number of peak filters, as well as the statistics for the peak your mouse is over.



Example of Peak Finder with a "missing peak"

Move the mouse over a peak that the program did not identify. Look at the lower portion of the dialog for one or more of the settings that do not show the word "Pass". Adjust the sliders for the failing settings until all of the settings show "Pass" for the peak.

If no vertical lines appear for a peak, the peak does not qualify as a statistically valid peak. This kind of error cannot be corrected in the Peak Finder.

As you make adjustments, sweep your mouse across the histogram to review the status for all of the peaks. If you find that false peaks now "pass", you may need to compromise on your adjustments. If, however, the false peaks are very small, the program may exclude them from Auto Analysis for not passing other thresholds, so you should not worry about them at this point.

Once you have made adjustments that satisfy the histogram, click OK to close the Peak Finder dialog and return to the Edit Properties for Peak Finder settings dialog. You may at this point choose to save these settings to a file using the Save button at the bottom of the list. Alternately, you may want to write down the new settings. Since this process is

experimental and may require several iterations, it is good to keep track of each set of adjustments.

Click OK to close the dialog.

Now you need to try out the new settings on the data set again. Move to the first file in the batch. Make notations for missing peaks and false peaks, and then move to the next file.

Repeat this process of reviewing and adjusting until the Peak Finder is tuned for your data set.

Adjusting Auto Analysis Settings

The final phase in optimizing Auto Analysis for your data makes use of the Auto Analysis Settings dialog. This dialog controls how the program interprets the peaks in a histogram.

From the **Options** tab, click **Auto Analysis**. The Auto Analysis Settings dialog will be displayed.

The first three options are straightforward. We recommend that you enable AutoDebris and AutoAggregates, as these components are robust and automatic in the way they handle debris and aggregation in samples. The Apoptosis option should be disabled for Auto Analysis unless you have a series of files in which you want to model apoptosis as a Gaussian distribution below the first cycle's G1 peak.

For Linearity, either leave the value at 2.0 or enter the average G2/G1 ratio of a data set. The G2/G1 ratio is easily computed by dividing the mean channel of a G2 peak by the mean channel of the G1 peak of the same cycle. Typical ranges are 1.95 to 2.0.

In the Standards and Reference section, enter the number of internal standards your samples contain. If you have internal standards, you should click the Edit button to set the properties of the standards. The most important property to set for each standard is its expected channel position. By entering an expected position, the program can make better decisions about when peaks shift and where the first cycle begins.

If you want to make use of an external reference standard for determining the location of the Diploid population, you should enter its channel location. This is only required if you want to base the Diploid position on the external reference.

The Ploidy Determination section provides a number of interesting possibilities for assigning ploidy labels to peaks. We will examine these in detail.

First, the Maximum cell cycles entry can in most cases be set to Unlimited. However, if you want to restrict Auto Analysis to creating models with a limited number of cell cycles, select the number of cycles in this drop down listbox.

The Diploid determination list presents 4 options for determining which cycle is labeled "Diploid". The first option, "First cycle is Diploid", simply assumes that the first peak, after any internal standards and apoptosis, is the Diploid G1 peak. This option should be used for paraffin samples. When you use this option, you do not need to enter a value in the related field, Diploid-to-Standard ratio.

You can also choose to base the Diploid cycle decision on the position of one of the internal standards or an external reference channel position. In these cases, you need to enter the expected Diploid-to-Standard ratio. For example, if your 2nd internal standard appears at channel 40 and you expect the Diploid G1 to be at channel 50, you can choose "Based on Standard2" for Diploid determination and enter 1.25 for the Diploid-to-Standard ratio. (Note: The value 1.25 in this example is for PI-stained trout red blood cells in relation to PI-stained human Diploid cells. For DAPI staining or other internal standard cells, the ratio will be other values.)

The G1 threshold field determines how big a peak has to be in order for it to be called a G1 peak. This is a relative height after adjustment for aggregates. In essence, it acts like another filter in the Peak Finder specifically for G1 peaks. If you have a number of small peaks that Auto Analysis is calling G1 peaks and you do not want them classified that way, increase the G1 threshold about 2 or 3 units at a time until they are not longer considered G1 peaks.

The Peak Location Range field determines how much of a shift from the expected locations you will allow. For example, if the value is set for 10 (percent) and the Diploid is expected to occur at channel 50, the program will look for a peak between channel 45 and 55. Generally, values between 5 and 10 are best for this field.

The S-Phase group does not require adjustments for most users. The settings are based on industry-standard recommendations and practices, so if you change these settings, it will be difficult or impossible to compare results in your lab with results of other labs.

The Tetraploid group allows you to set the threshold at which the program will call a peak a Tetraploid G1 instead of a Diploid G2M. Recommended values range from 15 to 25, and you may need to experiment with these values on your data set.

When you finish making your first adjustments to the settings, use the Save button to save the settings to a file on disk. As with other configuration dialogs, you may need to go through several iterations before you find the best settings for your data set.

Choose OK to close the dialog.

Testing the Auto Analysis settings

At this point, you are ready to test the full system.

Change the file processing settings to Auto-analyze each data file that you open.

Click the "go to first" button on the batch control toolbar. The first file will be opened and an Auto Analysis will be performed. Make a notation to indicate whether the program correctly analyzed the data. Pay particular attention to the ploidy classifications for each cycle and the graphical representation of the fit.

Advance to the next file. After the program auto-analyzes the file, make notations about the success or failure of the program. Repeat this process for the entire data set.

After all files have been processed with Auto Analysis, review your notations. The goal is to have the program work automatically for greater than 95% of the files. If you need to make

additional adjustments, repeat the process of reviewing and adjusting until you achieve the best results.

When you have the settings that work best, display the Auto Analysis Settings dialog once again. Save the settings to a file using the Save button. Click OK to close the dialog.

Summary

The process of tuning Auto Analysis for your own data sets involves three dialog boxes: Options and Configuration, Peak Finder Settings, and Auto Analysis Settings. After setting up configuration options, an iterative process is used to find the best Peak Finder Settings for a data set. As the final step, a similar iterative process is used to tune the Auto Analysis Settings to a data set.

Use a data set of samples from the same cell or tissue type. As you perform this process for a data set, save your settings for each dialog box so that they can be reloaded at any time.

You should perform this process for different sample types if the preparation process or the data files themselves have difference characteristics from the data set initially used to optimize Auto Analysis.

You will always find some files in a data set that are unusual when compared with the rest. Do not try to optimize Auto Analysis for *unusual* data, or you will find that it fails more often than it works. Instead, base your decisions on the *typical* data in a data set, and you will find the system has a much higher success rate.

See also:

[Options and Configuration](#)

[Peak Finder Settings](#)

[Auto Analysis Settings](#)

DDE Support

NOTE: This feature is only available on Windows versions of ModFit LT. DDE is *not* supported on the Macintosh.

Dynamic Data Exchange (DDE) in ModFit LT allows the program to be driven by and to provide information to other applications. WinList 3D can use DDE to connect with ModFit LT and to share data with it. In most cases, the DDE scripts provided with WinList 3D are sufficient for this interaction. If you have another application you want to connect with ModFit LT using DDE, please contact Verity Customer Support.

Strengths and Limitations of Automatic Analysis

ModFit LT uses some very sophisticated logic when it attempts to automatically analyze your data. The system has been designed to work with a great variety of data samples, samples that vary in almost an unlimited number of ways. When your data samples fit into the assumptions built into the automatic analysis functions, the program works well in automatically modeling them. Conversely, as you might expect, the automatic systems do not work as well when your samples differ from what it has been designed to analyze.

To better understand the strengths and limitations of automatic analysis, it may be helpful to examine some of the assumptions built into the system.

Peak Finder

When you open a data file with the program, one of the first things the software attempts to do is locate "peaks" in the sample. This step is the foundation of automatic analysis: if it finds the important peaks in the sample, the remaining steps in auto-analysis have a good chance at succeeding. If it cannot detect the important peaks, it will more than likely have difficulty or may fail to automatically analyze the sample.

While it is relatively easy for our eyes to see peaks in a histogram, the computer algorithms that perform this function are anything but simple. The process ModFit LT uses works well with the large library of data files we use to test the system. Here is how it works.

The program performs a statistical analysis of the histogram to identify potential peaks. Then the peak finder begins to filter out peaks that do not meet certain criteria. There are filters for high and low %CV, relative peak height, and how well the peak can be fitted with a Gaussian in a quick analysis. These filters can be viewed in the Peak Finder Settings dialog.

If one or more of the filtering criterion filters out an important peak, the system will likely fail in a later step. For this reason, you can adjust some of the filters used by the peak finder so that the system better matches the data samples analyzed in your laboratory.

A very important note on the peak finder settings: adjusting one or more settings to work with a single sample is a bad idea. The reason is simple: without looking at numerous samples to test your new setting, you may find you have fixed the peak finder for one exceptional sample, and broken it for the typical sample. Weigh your adjustments carefully. Do not make adjustments to accommodate a single sample; use manual analysis to handle the exception.

Ploidy Detection

The peak finder identifies the peaks it finds by displaying a small, black triangle under the peaks on the histogram display. The next step in automatic analysis is to look at the pattern presented by those peaks and attempt to match the pattern to a type of histogram the program knows about. This occurs when you click the Auto button on the toolbar, or choose Automatic Analysis from the Analysis menu.

So just what is an "important" peak?

The most important peaks the program needs to find are peaks for internal standards, apoptosis, and G0G1 peaks for each cycling population. If the program finds these peaks, it generally succeeds in auto-analysis.

You need to tell the program how many standard peaks your sample contains. This is done in the Auto Analysis Settings dialog box. The program assumes that standard peaks, if any, are the first peaks, followed by an apoptosis peak, if selected, and the remaining peaks are cycling population peaks.

If you have correctly informed the program as to how many standard peaks the sample contains and whether or not it has an apoptosis peak, *and* the peaks found by the peak finder are the important peaks, the ploidy detection step is off to a good start.

The remaining step is a pattern-matching step, which ignores standard and apoptosis peaks. Here, the program analyzes relative sizes and positions of the peaks remaining, looking for cell cycles. ModFit LT is designed to handle any number of cell cycles in a histogram, though it is rare to find samples with more than 4 cell cycles.

In general, the more complicated the histogram, the more difficult it is to classify. You may need to manually analyze very complex samples.

Once the sample is classified, a model is created to match the pattern. Then ranges are assigned to important peaks, and the non-linear least-squares analysis of the sample is performed.

Debris and Sample Quality

As you might expect, the success of automatic analysis is closely linked to the quality of the data you want to analyze. Samples with lots of debris or aggregation detract from the program's ability to find peaks or determine the right model to use. Efforts to gate out debris and aggregation sometimes work, but they are usually going to work against the program's built-in functions designed to compensate for debris and aggregates. The better solution is to perfect the sample preparation and acquisition techniques you use. Starting off with a cleaner sample greatly simplifies the peak and ploidy detection operations, and helps ensure that the peaks seen by you and the program are real peaks.

Follow the suggestions of experts to prepare and analyze your samples. Establish standards that must be met before a sample can be considered for analysis. The DNA Consensus Conference (Cytometry. Vol. 14. Number 5, 1993) made many specific recommendations on DNA analysis, many of which relate to sample quality. Your standards should be at least as restrictive as those presented in that report.

Be aware of obvious things, as well. If you accidentally try to analyze forward angle light scatter instead of the DNA parameter, you will end up with nonsensical information when you perform automatic analysis.

Your Responsibilities

Based on this discussion, it is probably obvious that there are numerous ways automatic analysis of your samples can fail. The problem is that the program itself doesn't know when it has failed; only you can make that determination.

You should view automatic analysis with scientific skepticism. You must review the information on the report to ensure all the model matches your understanding of the histogram. You should always review the results of an automatic analysis to ensure that the program has identified the standard peaks, apoptosis peaks, and G0G1 peaks of the cycling populations. In addition, you should verify that the ploidy classification assigned by the program concurs with your knowledge of the sample.

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