

# **GemStone User Guide**

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## **GemStone User Guide**

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This guide is intended for GemStone version 1.0 and minor revisions.

### **Quick Jumps:**

[Quick Start](#)

[Overview Section](#)

[Tutorials](#)

[Reference Section](#)

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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:

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

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## Quick Start

### Install GemStone.

On a Macintosh:

If you received an electronic download, extract the GemStone Client Mac Installer into a temporary folder on the Macintosh computer for installation. Then, launch the installer program and follow directions on the screen.

If you received an installation CD, insert the CD and double click on the installer package. Then follow the directions on the screen.

On a PC:

If you received an electronic download, extract the GemStone Client Windows Installer into a temporary folder on the Windows computer for installation. Then, launch the installer program and follow directions on the screen.

If you received an installation CD, insert the CD. If a browser does not automatically open, double click on the CD and then double click on default.htm. Dismiss any warning message from the browser. Click on the Install GemStone 1.0 link and follow the directions on the screen.

### Redeem the Battery Certificate.<sup>1</sup>

Run GemStone.

Click OK to close the banner dialog.

Choose the Charge Battery command in the Battery menu.

Select the Redeem Battery Certificate via the Wizard option<sup>2</sup>.

Click Next.

Enter the Battery Certificate Code.

Click Next.

### Get Busy!

The complete GemStone User Guide, as well as the latest videos and tutorials can be accessed online at [www.vsh.com/documentation](http://www.vsh.com/documentation).

We recommend starting with the [Tutorials](#).

For assistance, please contact technical support at 207.729.6767 or tech@vsh.com.

<sup>1</sup> If your version included a Battery Certificate, it is included as part of the installation CD packaging or as part of an email received after an online purchase has been made.

<sup>2</sup> This option requires that your computer be connected to the Internet.



## **Latest Versions**

To find the latest versions for GemStone and its documentation, visit the Verity Software House web site at [www.vsh.com](http://www.vsh.com). This site provides a complete version of the latest revision of the online documentation, patches, and technical support information for the program.

You can also email us at [tech@vsh.com](mailto:tech@vsh.com) to determine whether or not you have the latest version. Please include the version that appears on the start-up dialog box, and the file date for the program with your email.



## **New Features in GemStone**

This section contains major changes in GemStone.

### **Build 1.0.35**

#### **Build 1.0.34**

- + Created a region animation system that enables the identification of any population in all GemStone graphic systems. (cbb, 01Jan10).
- + Created a heat map system capable of revealing very small populations that do not belong to a model. (cbb, 01Jan10).
- + Increased the speed of drawing dots by 10%. (cbb, 01Jan10).
- + Fixed occasional problem with 1P scaling. (cbb, 21Dec09).
- + Statistics have been added to the properties of region labels. (cbb, 20Dec09).

#### **Build 1.0.33**

- + The accuracy of fitting multiple cell types has also been improved. (cbb, 08Dec09).
- + The accuracy of fitting has been improved substantially, especially with higher exclusion probabilities. (cbb, 08Dec09).
- + AutoAnalysis did not work correctly if there wasn't at least one Constant parameter profile. Fixed. (cbb, 20Nov09)
- + A bug was fixed with synthesizing data that was created in version .32. (cbb, 19Nov09).

#### **Build 1.0.32**

- + Documents now store the active cell type and return to that cell type after reading in the document. (cbb, 12Nov09).
- + All tabs for parameters and cell types are now at the bottom of the application. This makes selecting the page of interest more efficient. (cbb, 12Nov09).
- + GemStone stored documents are now more compact. (cbb, 12Nov09).
- + GemStone will no longer decrement the battery for files that are exported via the Export Model Data command. This will prevent users from being charged for generated data files they have created, for example when following the GemStone tutorials. (cmb, es,tc,bb 10Nov09)
- + When loading a document, you can now gate it without the file being loaded in. (cbb, 08Nov09).
- + The program now has a Model Heatmap Graph cell type graphical object. This graph allows you to see how every event fits the model. A powerful way to use this new object is to use Enrich to fill the packet with the events you want to investigate. Once the packet is enriched, type in 0 for the Exclusion Probability cell type property. This forces the system to classify just about every event in the packet. You can then use the heatmap to find small populations that don't fit the model well. (cbb, 08Nov09).

- + The global ColorByGateCombination now works on classified and unclassified data. Regions now have a property, Color By Region Combination, that allows you control what regions are creating the color combinations. This enhancement makes it much easier to find outlier populations in your modeling. (cbb, 08Nov09).
- + The vector states now have the ability to trace medians and modes. The medians are so much better than means that they are now the default method for drawing the vectors. (cbb, 07Nov09).
- + When editing properties in a table pressing Enter or Return will move down one row and begin an edit. (cmb, es, 3Nov09)
- + Prevented tool tips from blocking user input when shown on Mac systems. (cmb, tc, 3Nov09)
- + Program now gives you an opportunity to save your document before quitting. (cbb, 31Oct09)
- + <Control>S now saves the document to the last model with no dialog shown. (cbb, 02Nov09).
- + Program now gives you an opportunity to save your document before quitting. (cbb, 31Oct09).
- + Fixed a subtle bug on the mac that caused it to crash if the cursor was on a cell type parameter profile shrink tool when the system was analyzing data (ES). (cbb, 29Oct09).
- + New surface graphics have been added to two-parameter and parameter profile graphs. (cbb, 24Oct09).

### **Build 1.0.31**

- + A new surface graphics option has been added to parameter state graphs. (cbb, 22Oct09).
- + A new program banner file has been created that expires in Feb, 2010. (cmb, 21Oct09).
- + When a GS document is read in, it always updates the matched database parameter's transform so that when the associated data file is read in, it looks exactly the same as on the model's author's system. (cbb, 07Oct09).
- + Added a preference for the fidelity plot in the cell type's frequency plot (Preffer). (cbb, 06Oct09).
- + Improved the ability of old versions of the program to read new versions of GemStone documents. (cbb, cmb 05Oct09).

### **Build 1.0.30**

- + GemStone now optimally smoothes mean and standard deviation values. (cbb, 25Sep09).
- + When a CDP relation was deleted, it wasn't properly deleting the back relation. (cbb, 22Sep09).
- + Fixed a bug that would cause the definition points of a model to be incorrectly positioned when the model was saved and later opened. The problem was setup by changing the name of a database parameter with a model loaded that included that parameter. (cbb, cmb, 18Sep09)

## GemStone User Guide

- + GemStone no longer remembers the pathway to a document if it is executed from an email attachment. (cbb, 18Sep09).
- + Increased the maximum size of the information table from 20 to 200. (cbb, 18Sep09).
- + Add a "Paste from clipboard" option to context menus for parameter overlay. (cbb, 06Sep09).
- + Add a "Set all match status flags" option for the cell type actions. (cbb, 06Sep09)
- + Allow multiple parameter resolutions to be merged (Amnis files). (cbb, 05Sep09)



## **System Requirements**

### **Minimum System Configurations**

PC: Windows XP, Vista or greater with 1GB memory and 50MB free disk space for program files.

Macintosh: OSX 10.3.9 or greater with 1GB memory and 50MB free disk space for program files.



## Installation and Setup of GemStone Client

GemStone Client software is easy to install on a Mac or Windows machine. In order to open and analyze your own data files, it needs a "software battery". The software battery allows GemStone to open and analyze a pre-defined number of files.

If you purchased GemStone Client with a Battery Certificate, you can use the certificate to create a software battery after you install the software. You can also obtain charges for a software battery from the Verity Software House web site or from a Battery Server for GemStone if your institution has purchased and installed one on your network.

### Windows Version

GemStone for Windows requires Windows XP, Vista or greater.

#### To install GemStone Client for Windows

- Log on to your computer with an account that has Administrator privileges. Most operating systems require administrative rights during installation.
- If you have an installation CD, insert the program CD into the CD-ROM drive. If you downloaded the software, extract the installation files from the download into a temporary folder.
- Run the program named **Setup** or **Setup.exe**. Follow the directions on the screen to complete the installation.

#### To run GemStone Client for Windows

- 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 choose All Programs. Then navigate to the Verity folder, and choose GemStone Client.

#### To install a software battery or battery certificate

- See the section [How to Work with Software Batteries](#) .

### Macintosh Version

GemStone for Mac requires OSX 10.3.9 or greater. You will need to have administrative rights to install the software.

#### To install GemStone Client for Macintosh

- Log on to your computer with an account that has Administrator privileges. Most operating systems require administrative rights during installation.

- If you have an installation CD, insert the program CD into the CD-ROM drive. If you downloaded the software, extract the installation files from the download into a temporary folder.
- Run the **GemStone Installer** program. Follow the directions on the screen to complete the installation.

### **To run GemStone Client for Macintosh**

- 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 GemStone Client for Mac application in the Applications/GemStone folder.

### **To install a software battery or battery certificate**

- See the section [How to Work with Software Batteries](#) .

## Installation and Setup of Battery Server for GemStone

You will need to register Battery Server for GemStone with Verity Software House in order to unlock the program on the computer. This can be done using the registration wizard, the online registration at [vsh.com](http://vsh.com), or by contacting technical support. The Serial Number and CD Key provided with your software are required for registration, along with some information that identifies the computer on which the software is installed.

### Windows Version

GemStone for Windows requires Windows XP, Vista or greater.

#### To install Battery Server for GemStone (Win)

- Log on to your computer with an account that has Administrator privileges. Most operating systems require administrative rights during installation.
- If you have an installation CD, insert the program CD into the CD-ROM drive. If you downloaded the software, extract the installation files from the download into a temporary folder.
- Run the program named **Setup** or **Setup.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.

#### To run Battery Server for GemStone (Win)

- See the section [How to Work with Battery Server for GemStone](#) .

#### To install a software battery or battery certificate

- See the section [How to Work with Software Batteries](#) .

### Macintosh Version

GemStone for Mac requires OSX 10.3.9 or greater. You will need to have administrative rights to install the software.

#### To install Battery Server for GemStone (Mac)

- Log on to your computer with an account that has Administrator privileges. Most operating systems require administrative rights during installation.
- If you have an installation CD, insert the program CD into the CD-ROM drive. If you downloaded the software, extract the installation files from the download into a temporary folder.
- Run the **Battery Server Installer** program. 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.

### **To run Battery Server for GemStone (Mac)**

- See the section [How to Work with Battery Server for GemStone](#) .

### **To install a software battery or battery certificate**

- See the section [How to Work with Software Batteries](#) .

## Registering your new software

### Why You Should Register

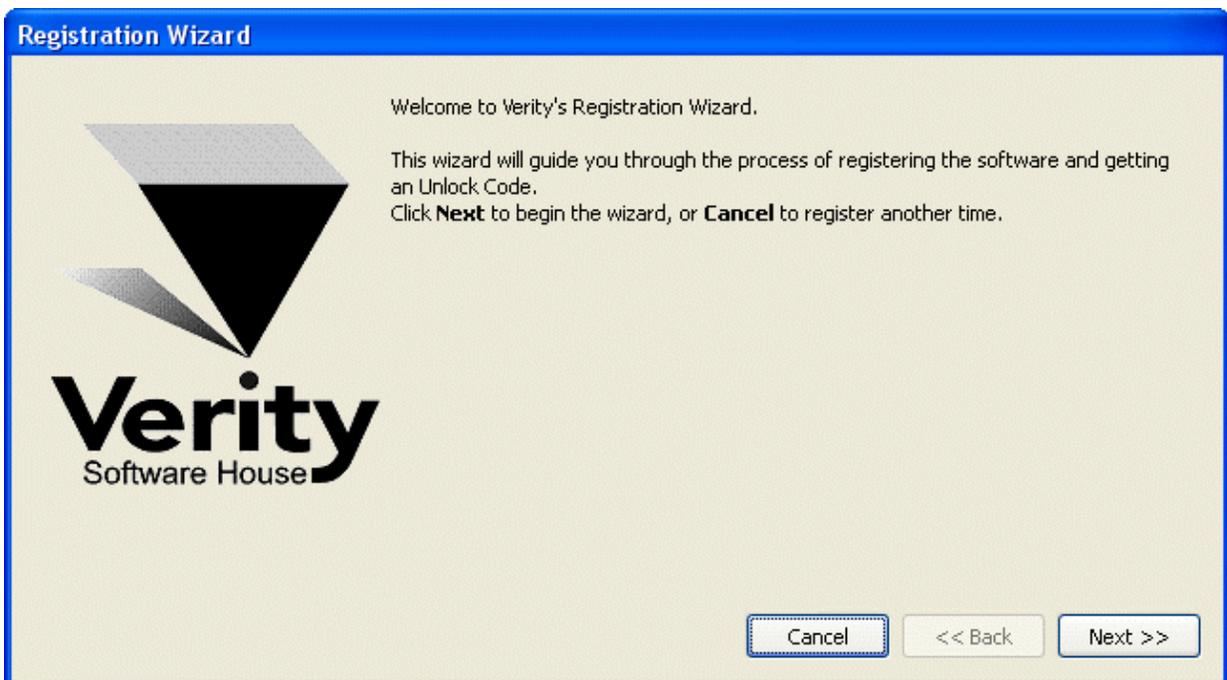
With this version of GemStone, registration is more important than ever before. In fact, *your software will not be fully functional until you register*. Here's why.

This version of the program uses software security that allows it to be used without a hardware lock. Software security requires you to register your software in order to receive an **Unlock Code** to make the program fully functional. You can run the program up to 10 times prior to registering the software.

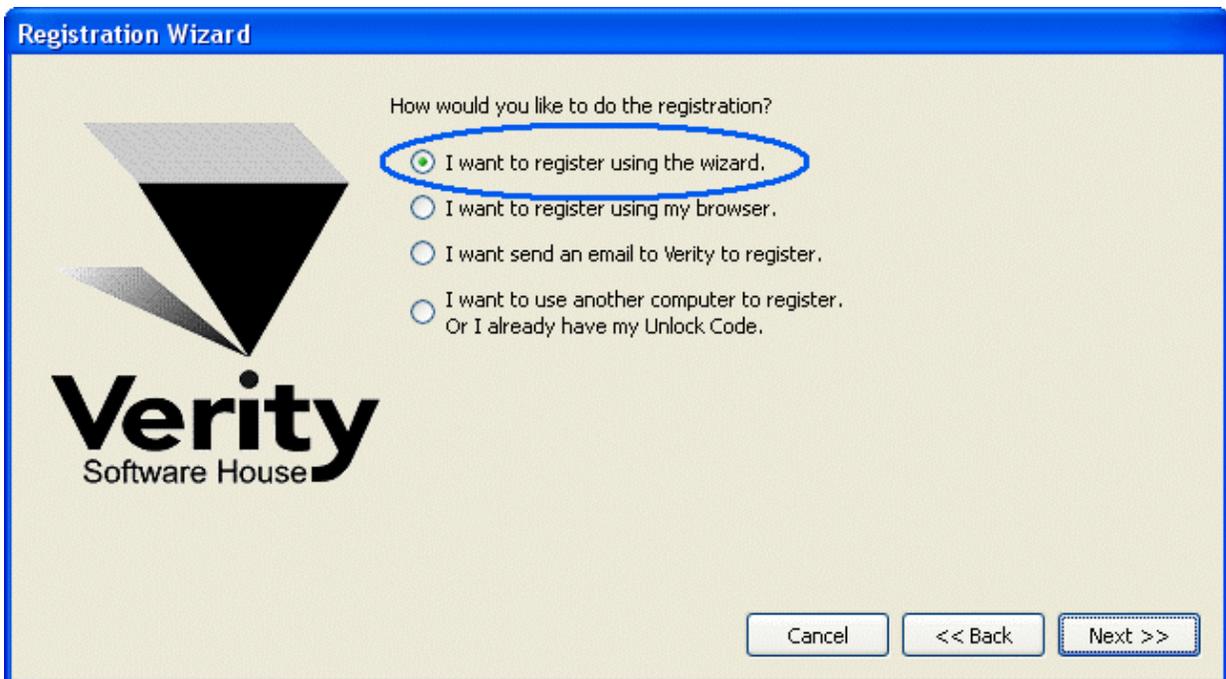
Of course, registration is useful for other reasons, too. We can inform you of bug fixes and updates, and provide you with better technical support.

### How to Register

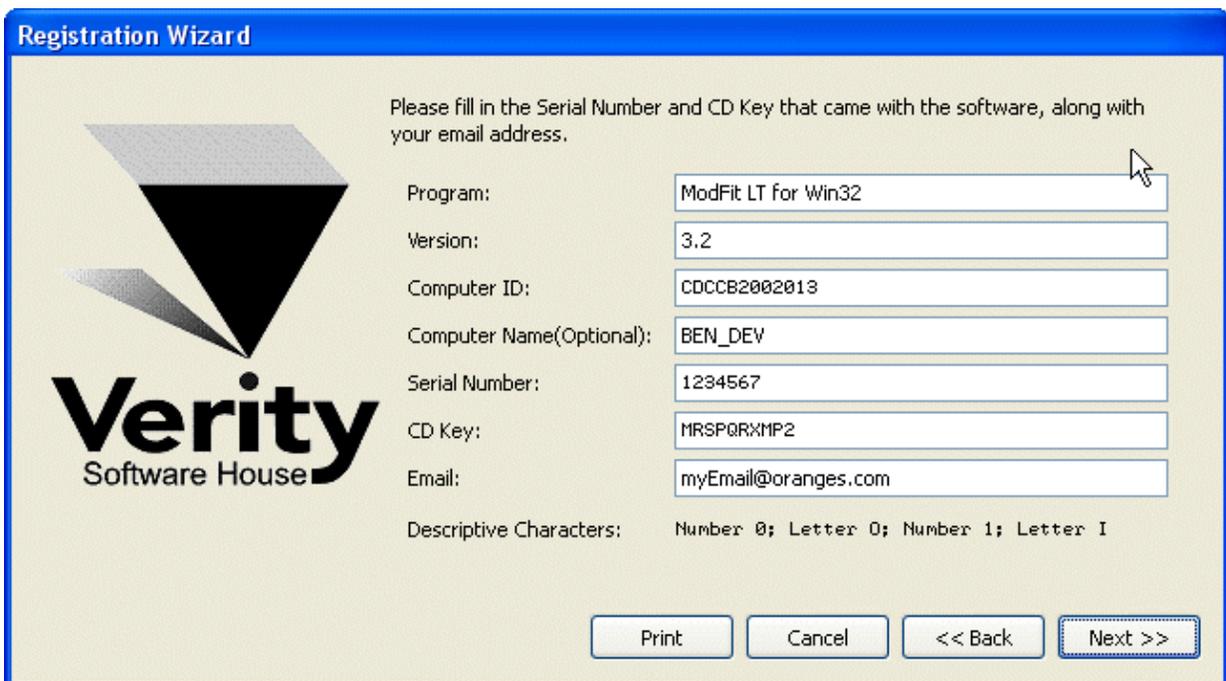
When you start the program for the first time, it will automatically start the Registration Wizard. You can access this dialog at any time by clicking the *Registration* button on the dialog box that is displayed when the program starts up.



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



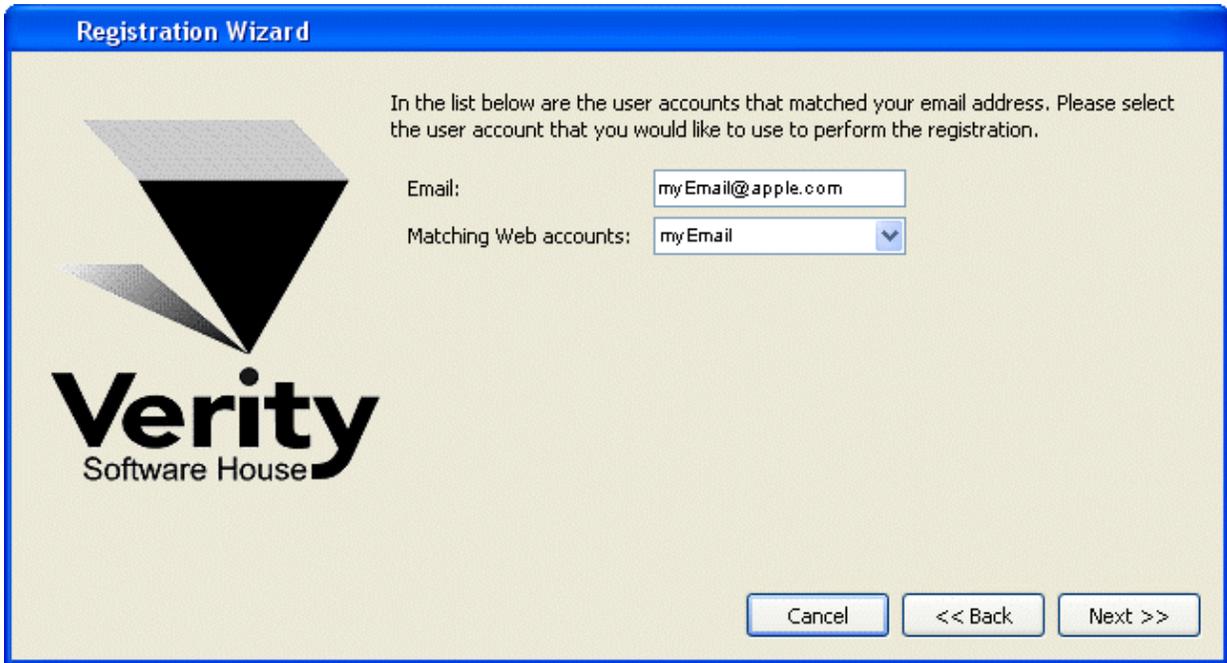
The wizard will ask how you want to register the program. Select the appropriate option and click **Next**.



Then 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* field to identify your computer. 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

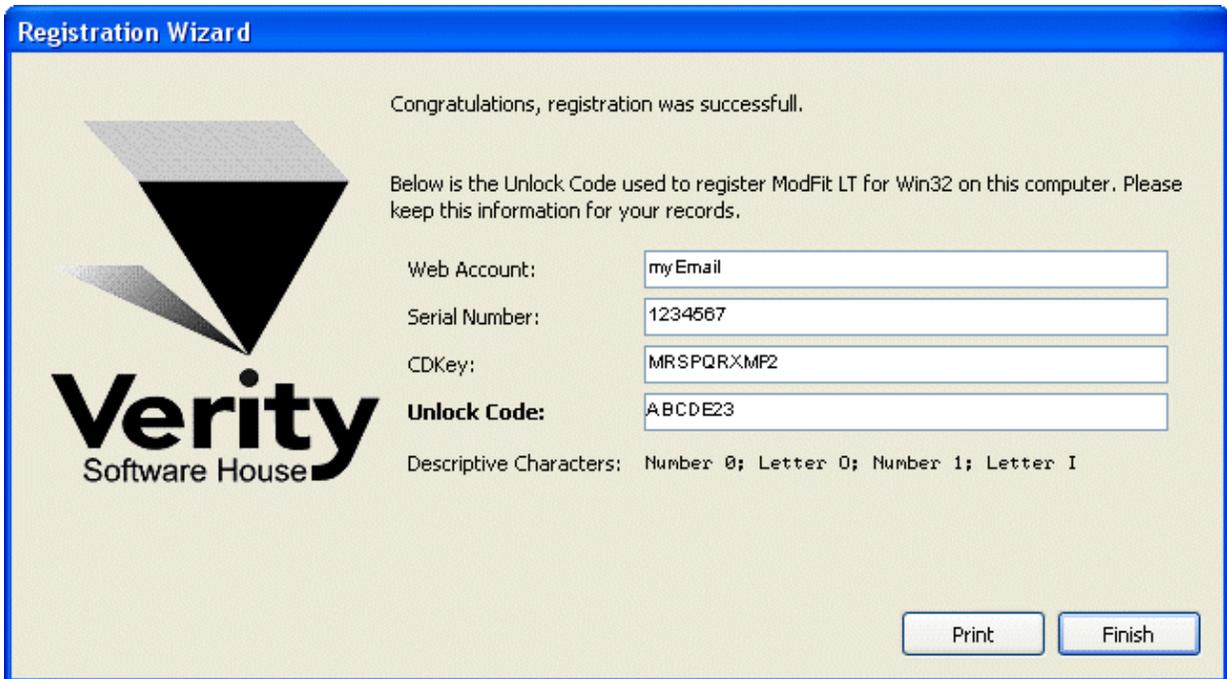
appear; they are case-sensitive. If you have lost or misplaced the Serial number or CD Key, contact Verity technical support at tech@vsh.com or (207) 729 6767. For the *Email* field, make sure to enter a valid email address. Otherwise, you may not receive the Unlock Code and the program will not be fully 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.



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.



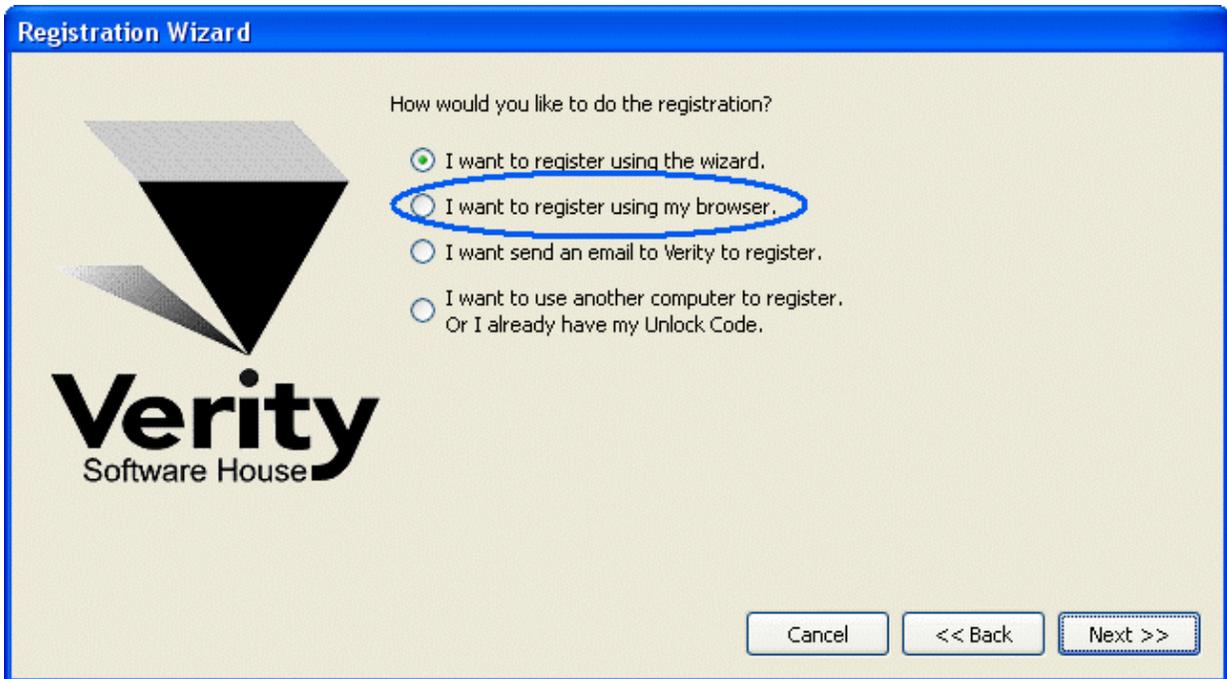
You can print the registration information by clicking the **Print** button. Click **Finish** to close the wizard and continue working with the program

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**

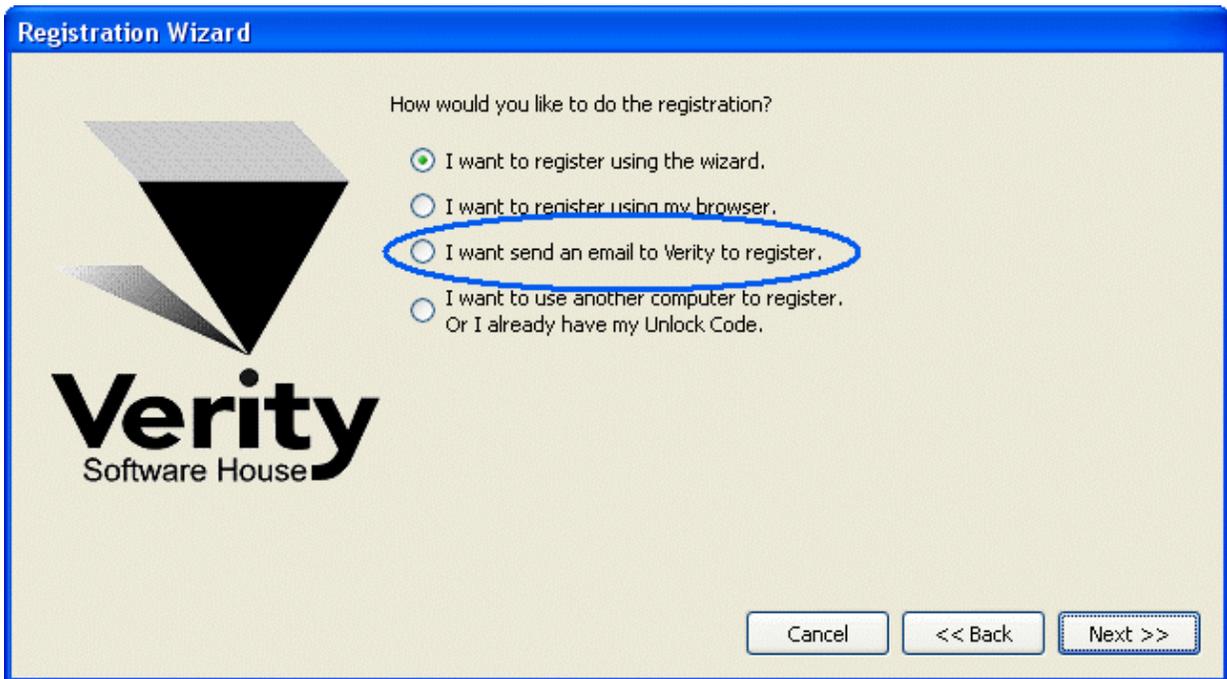
Sometimes firewalls can prevent the registration wizard from connecting to vsh.com. In this case, you can try registering with your browser. Choose the second option in the list and click **Next** to launch your browser with the correct registration information.



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.

### **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**

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USA

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Fax: (207) 729 5443

## **Overviews**

### **Overview Section**

The following sections provide overviews of some of GemStone's key concepts.

[Overview: Workspace Names and User Interface](#)

[Overview: What is a Probability State Model?](#)

[Overview: Building a Model](#)

[Overview: Using a Model](#)

## Overview: Workspace Names and User Interface

The annotated graphic below shows the GemStone workspace, along with the names for the various elements that it is comprised of.

The screenshot displays the GemStone software interface, which is used for probability state modeling. The interface is divided into several panels and toolbars, each annotated with a callout box:

- Main toolbar:** Located at the top left, containing standard file and editing icons.
- Parameters tab:** A tab at the top of the main workspace area.
- Cell Types tab:** Another tab at the top of the main workspace area.
- Global properties panel:** A panel on the left side of the main workspace, displaying global settings.
- Parameter Profiles widget:** A widget on the right side of the main workspace, showing parameter profiles.
- Cell Type canvas:** The central area of the main workspace where data is visualized.
- File Database panel:** A panel on the left side, listing various files and their properties.
- Cell Type Properties panel:** A panel on the left side, showing detailed properties for a selected cell type.
- Batch Levels panel:** A panel on the left side, used for managing batch processing levels.
- Parameter Profiles Properties panel:** A panel on the left side, showing properties for parameter profiles.
- Batch Options panel:** A panel on the left side, used for configuring batch processing options.
- Cell Type selection:** A panel on the left side, used for selecting different cell types.
- Workspace toolbar:** A toolbar located within the main workspace area.
- Parameter Profile plot:** A plot within the main workspace showing parameter profiles.
- Parameter Overlay plot:** A plot within the main workspace showing parameter overlays.
- Parameter Profile toolbar:** A toolbar located within the main workspace area.
- Shrink tool:** A tool located within the main workspace area.
- Zone Statistics table:** A table located in the bottom right corner, displaying statistics for different zones.
- Status bar:** A bar at the bottom of the interface, providing status information.
- Battery meter:** A battery meter icon located in the bottom right corner.
- Batch Controls toolbar:** A toolbar at the bottom of the interface, used for controlling batch processing.

## Overview: What is a Probability State Model?

Flow cytometry data analysis focuses on identification of populations in a sample and providing statistical results about those populations. Typically, regions are drawn on univariate or bivariate plots, and then used to create a hierarchy of subsets by "gating" the events. The resulting subsets are analyzed for frequency, intensity, and other common statistics to characterize the cells that were analyzed.

Cytometrists are trained to identify subsets of interest. We learn the characteristics of the subsets and which parameters are used to select them out of the many cell types that our samples contain. The learning curve can be long, and we often find it difficult to explain our plots and strategies with other scientists unfamiliar with cytometry.

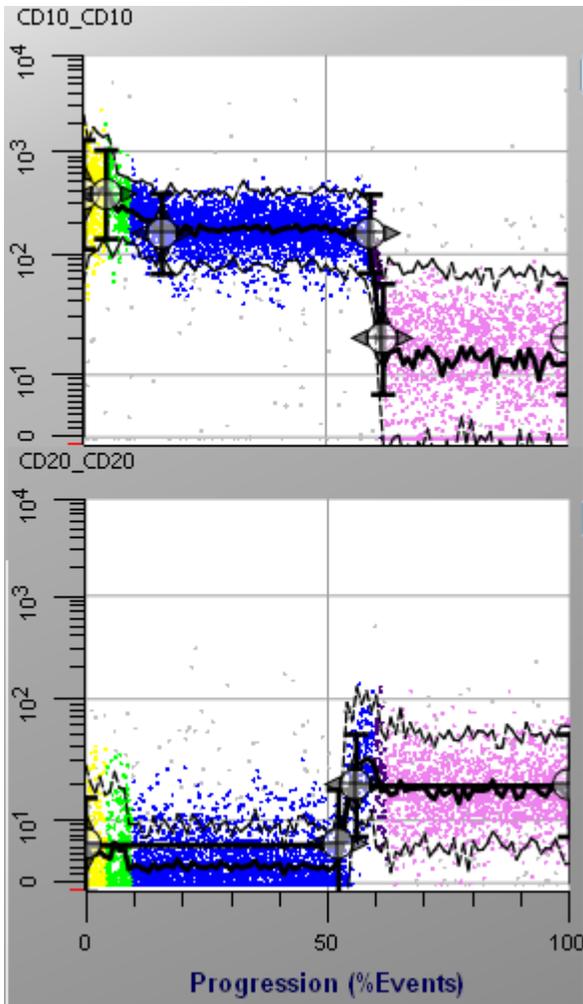
GemStone approaches data analysis quite differently by using a Probability State Model (PSM) to identify and quantify subsets. So what is a Probability State Model, and why is it a better approach?

Let's examine the words that make up the name to get an idea of what a PSM is.

**Probability:** the statistical likelihood of a particular event occurring. A PSM classifies events using probabilities rather than user-defined gates. The gating approach is subjective, and relatively small errors made in drawing gates are compounded with each subsequent gate in the hierarchy. By using probability, a PSM actually *reduces* the error with each additional parameter that is added to the model. The likelihood that you are actually identifying the cells you are interested in *increases* as you build a PSM.

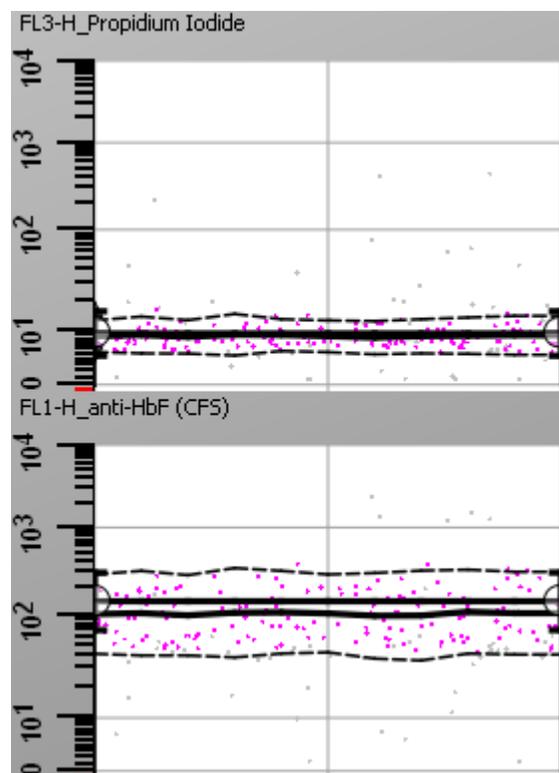
**State:** a set of conditions that discretely characterize something. A PSM probabilistically classifies events into a set of states, based on the characteristics defined for each parameter. The state for each event can be thought of as an additional parameter, one that identifies the particular set of characteristics that define that state. GemStone uses the state as the common, X-axis for its parameter plots. This allows all parameters to be compared on a common axis, making it possible to correlate and compare *all* of the parameters in a single plot.

**Model:** a mathematical representation of a process. A PSM uses fitting routines to classify cells into the most probable states, or to adjust the model to match the data. Using this approach, GemStone can provide objective measurements of how well the model represents the data. There are no hard-edges in a PSM. The modeling process allows populations to overlap based on probabilities.



A PSM allows us to classify events into populations probabilistically based on a model that we define. The model is made up of one or more *Cell Types*. Each Cell Type defines the characteristics of a different subset that we want to analyze. We use what we know about the markers in our experiments to create a set of *Parameter Profiles* for each Cell Type subset. A Parameter Profile uses a set of control points to define how the subset transitions over the state index axis.

The state index axis is typically labeled "Progression", but it does not have to be a progress at all. In fact, simple models can be designed to identify and quantify cell populations where the parameter profiles are simply set for constant intensities.



The PSM approach allows cytometrists to evaluate their analysis more objectively and more scientifically. By using a model, we can measure and report confidence limits. We can evaluate the goodness-of-fit with reduced chi-square (rcs). And we can co-plot our parameters in a single graphic that allows us to examine the coordinated transitions of markers in the cells we analyze.



## Overview: Building a Model

The model-building process is much like the scientific process. It requires careful observation and attention to detail. It requires you to separate what you know from what you do not know. It requires you to move methodically through a process, not moving on to the next step until you fully understand the step you are on.

### Start Simple

There are just a couple basic rules for creating models. The first is to start with the parameters that are the simplest and move toward those that are complex. The second rule is closely related: start with the parameters that are best defined (those that have the most obvious transitions) and move on to those that have more subtle characteristics.

For example, let's say that you are only interested in analyzing a cell subset that shows high expression of some marker, and no other cells have high expression for that marker. This is an easy subset to identify using a constant parameter profile to select the bright cells for the marker. We refer to this as a *selection parameter*. In general, model building starts with the selection parameters.

### Dramatic Transitions

Once the selection parameters are defined, you move on to the markers that show the most obvious, dramatic transitions. For example, if you have one marker that drops 2-decades in intensity in a simple step-down manner and another marker that changes only 1/2 decade in the progression, you would almost certainly choose to model the dramatic, 2-decade shift first. It is easier to position control points on dramatic transitions; control points are used to provide the initial estimates to the modeling system.

### Save As You Go

When designing a model, save the model often. Create a system for naming models so that you have a history of revisions. For example, append a letter or number to the name each time you resave the model. When you need to roll-back to an earlier version, your history of models will be greatly appreciated.

### Test As You Go

As you build a model, test each change that you make. If you add a new parameter and set up a profile for it, try the model out with a few data files to be certain it classifies properly. If you see something you don't expect to see, take the time to decide whether this indicates a problem with the model or something new you didn't know about your data. Research the question to see if there is support in the literature for your observations. Most importantly, don't move on until you have the best explanation or you have modified the model appropriately.

### See also:

[Overview: What is a Probability State Model?](#)

[Model Building 101](#)

## Overview: Using a Model

### Simple Process

Using a GemStone model can be as simple as opening a GemStone document, selecting the data files you want to analyze with it, and reading each file into the model for analysis. In most cases, you will use the [Auto Analysis](#) command after opening a data file into your GemStone model document. This tells GemStone to adjust the model to match the data as best it can and then analyze all events in the file. If you have a model that is designed for the markers in your data files, you can use this simple process.

After the analysis is complete, you may want to save the finished analysis in another GemStone document file so that it can be recalled at a later time. The [Save Document](#) command is used for this purpose. You can also save and print graphics for individual objects or the whole workspace.

### Matching Parameters

If your data files contain markers that have different names in the GemStone document, you have a little more work to do. In this case, you would need to tell GemStone how to match the parameters in your files with the parameters in the model. You only need to do this one time if you save your model to reflect the changes. For details on how to do this, see [How to Remap Parameters in a Model](#) . Once a model is properly set up for the markers in your files, you can use the simple process of opening the model, opening the files, and analyzing them.

### Batch Processing

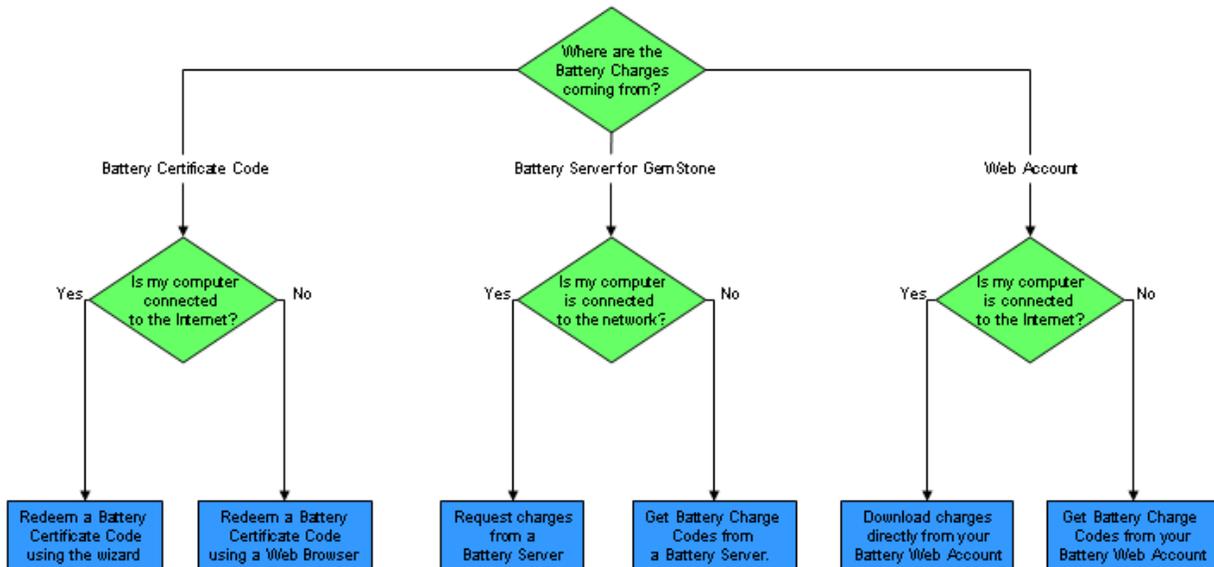
Analysis of sets of data files can be automated by using batch processing options described in the [Batch Processing](#) tutorial.

### Overview: Software Batteries

The primary licensing model for GemStone is a per-use system that uses "software battery" technology. A software battery provides a certain amount of "charge" to GemStone. Each charge can be used to open one data file. The program keeps track of files it has already opened, and only uses a charge the first time it reads a data file. So you can reopen the same file as many times as you like without drawing down the software battery.

There are a number of ways that you can "charge" GemStone's software battery on your computer. This provides flexibility to support single users as well as large institutions with many users. The flow chart below provides an illustration of the different sources for battery charges and how you use each one.

#### How should I charge my GemStone battery?



In order to charge your software battery, determine which of the three sources you will draw from. Then determine whether your computer is able to connect directly to either [www.vsh.com](http://www.vsh.com) or to a Battery Server. Using the flow chart, you can determine the best method for charging you software battery.

You can purchase additional battery charges at anytime by using the online store at the Verity Software House web site, [www.vsh.com](http://www.vsh.com).

**See also:**

[How to Work with Software Batteries](#)

[How to Work with Battery Server for GemStone](#)



## Tutorials

### Tutorials

We are constantly working on new tutorials for GemStone. You'll find them on our web site, [www.vsh.com](http://www.vsh.com), along with many other resources for learning and using GemStone.

[Model Building 101](#)

[Model Building 102](#)

[Model Building 103](#)

[Model Building 104](#)

[Creating a Model for a Multi-Tube Panel](#)

[Model Building 201](#)

[Heat Map and Animated Regions](#)

[How to Remap Parameters in a Model](#)

[Batch Processing](#)

[How to Work with Software Batteries](#)

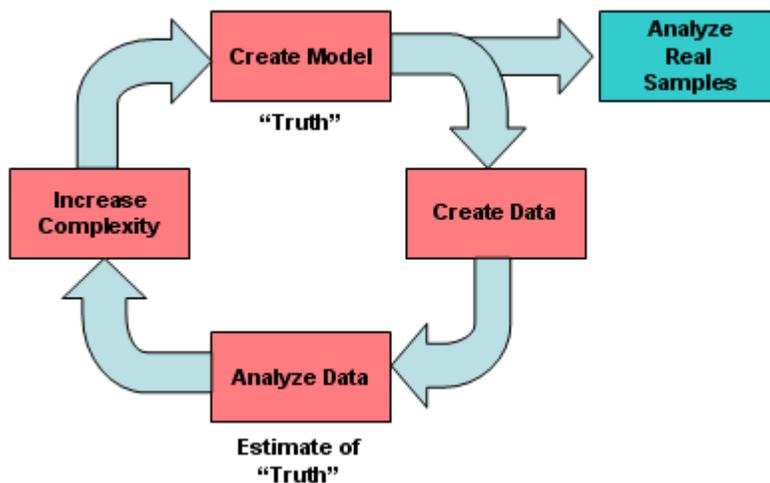
[How to Work with Battery Server for GemStone](#)

## Model Building 101

This tutorial introduces some basic principles behind model building. You will learn about how to build models that represent a single process - a "Cell Type" in GemStone. We will start with the simplest pieces and add a little complexity with each step. A follow-up tutorial, [Model Building 102](#), will continue the model development process where this tutorial leaves off.

### Strategy

Here is a cartoon of how we are going to approach model building.



We start by reading some synthesized listmode data. We create a very simple model for it, and analyze the data with our model. Then, we will add another parameter, enhance the model, and analyze the data. We will repeat the cycle until we have fully developed a model for the data.

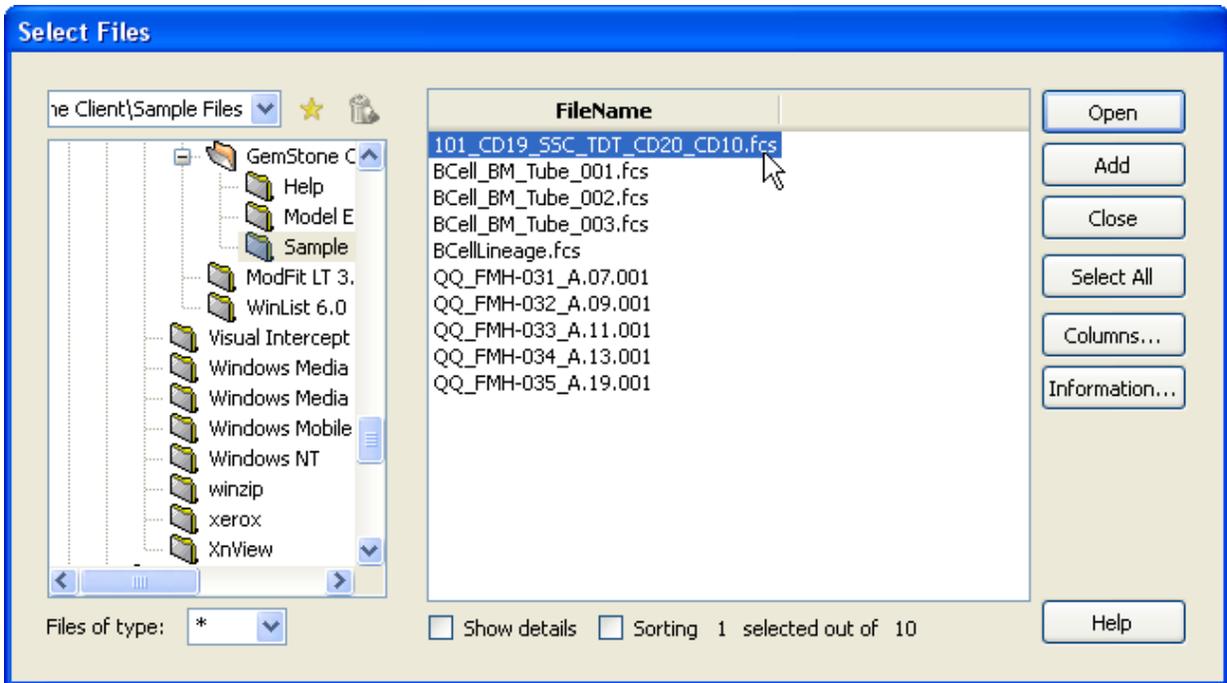
We start with a synthesized data file because we want to define what "truth" is for our data. This file was generated by GemStone, and so we expect an almost-perfect analysis result. "Real" data is easier to understand after we have designed this first model with "toy" data. We will be building the skills and understanding to analyze an actual normal bone marrow file. Our model will help us understand the B-cell lineage and prepare us for "real" data files later on.

### Getting Started

To get started, launch GemStone and pass through the start-up dialogs.

Click the [Select FCS Files](#) button on the main toolbar and navigate to the Sample Files folder. Select the file **101\_CD19\_SSC\_TDT\_CD20\_CD10.fcs**.

Click **Open**. The program will add the file to the File Database panel and read its data.

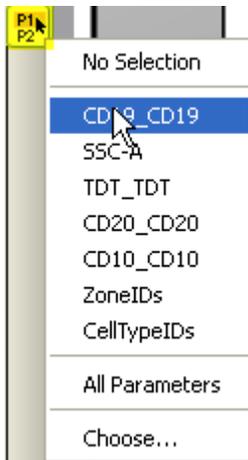


### Choosing CD19 Parameter

Click the Cell Type shrink tool to expose some of the important editors we will be working with. We'll learn a lot more about these editors as we proceed through this tutorial.



Click the last button in the Cell Type toolbar to display a list of parameters in this file. Find **CD19** in the list and select it. (The name might be CD19\_CD19.)



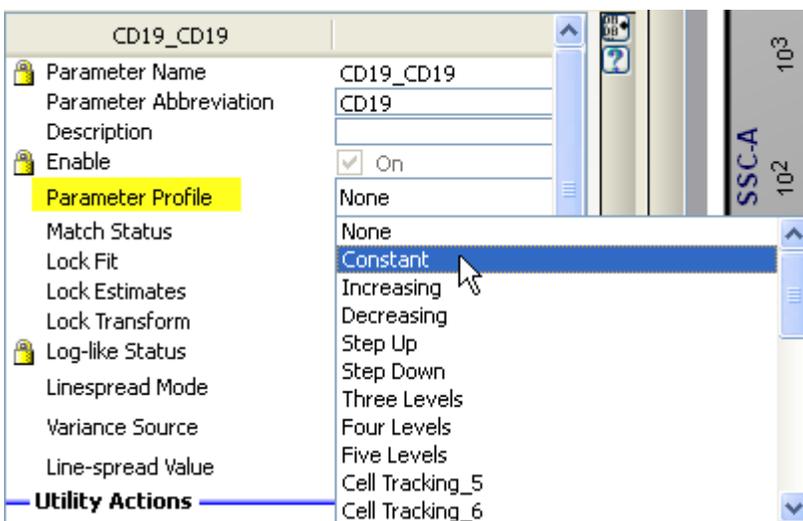
The parameter is added to the Cell Type widget and displayed as a band of dots on a plot.

### Model Building Tip: Start with Selection Parameters

Start building a model with the simplest parameters first, and work toward parameters that have more complicated transitions. Parameters that are constant throughout the progression and that select the populations of interest should be defined first. We refer to these as selection parameters.

### Modeling CD19

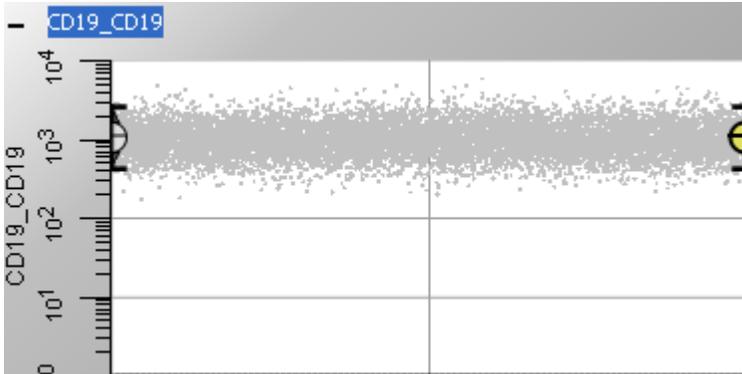
The **CD19** plot is now the "active" plot. Notice that its label is highlighted. We also see that CD19 appears in the Parameter Profile properties panel. In that panel, click the dropdown control next to **Parameter Profile** in the property list to display a list of choices. Choose **Constant** from the list.



### Model Building Tip: Another Way to Select a Profile

You can also select a parameter profile from the context menu of the parameter plot. To do so, right-click the mouse on the parameter plot, select the **Choose Parameter Profile** option, and choose a profile from the list.

You should now see two **Control Points** in the CD19 plot. The vertical placement of the Control Points should be in the center of the band of dots. Click and drag either point to identify center of the CD19+ events at around  $10^3$ .

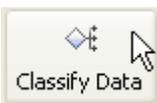


**Model Building Tip: Control Points**

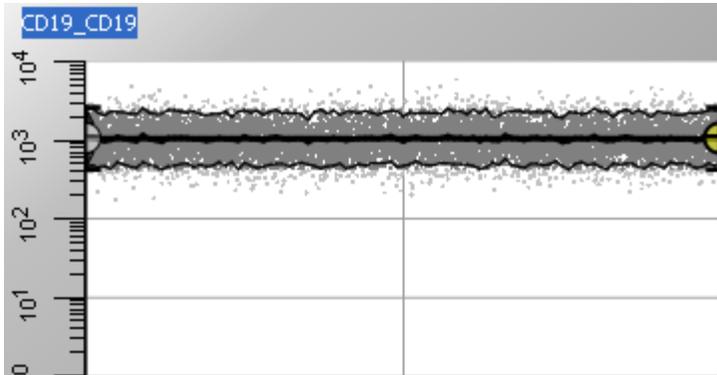
Control Points are the objects that allow you to manipulate a Parameter Profile. They are also referred to as "Control Definition Points" because they define the important transitions in the profile.

At this point, we have set up a very simple GemStone model that will select CD19 positive events. Let's tell GemStone to analyze the data with this model. Our expectation is that we will "classify" most of the events as matching our CellType1 model definition.

Click the **Classify Data** button the main toolbar.



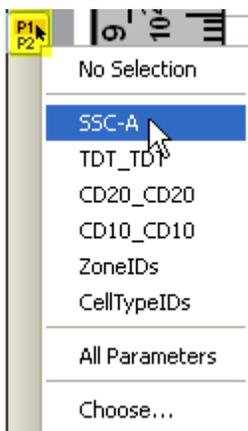
We now see some dots are darker than others, and there are some additional decorations on the plot. The darker dots are those that have been classified by our model as being CD19 positive. Light gray dots are considered "unclassified" – not belonging to a cell type at this point. The graphics show the 95% confidence limits, as well as the mean of the data and the mean of the model.



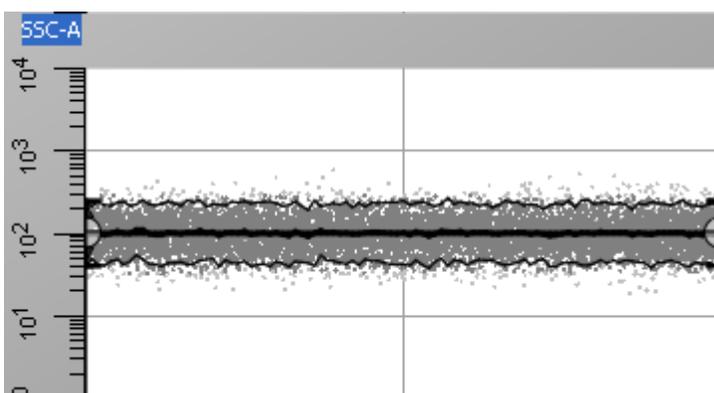
Good so far. We're ready to add SSC to our model.

### Modeling the SSC Parameter

Click the parameter selection tool button and choose **SSC**. A new plot is added to the Cell Type widget, and SSC appears in the **Parameter Profile** properties panel.



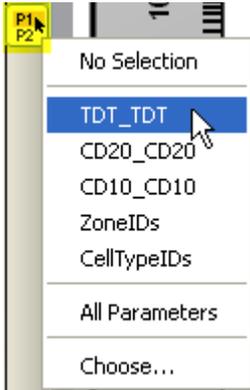
B-cells have a constant, low side scatter measurement, so we will again select a Constant profile. Right-click on the dots in the SSC plot and select the **Choose Parameter Profile**, then choose **Constant** from the list. Position the **Control Points** in the vertical center of the SSC population at around  $10^2$ . Then click the **Classify Data** button on the main toolbar.



**Model Building Tip: Start with Simple, Dramatic Transitions**  
Once the selection parameters are defined in the model, you are ready to move on to parameters that transition during the progression. Start with parameters that have the simplest and most obvious changes. These will help create a backbone for the model. They will provide clues about how the more complex parameters behave.

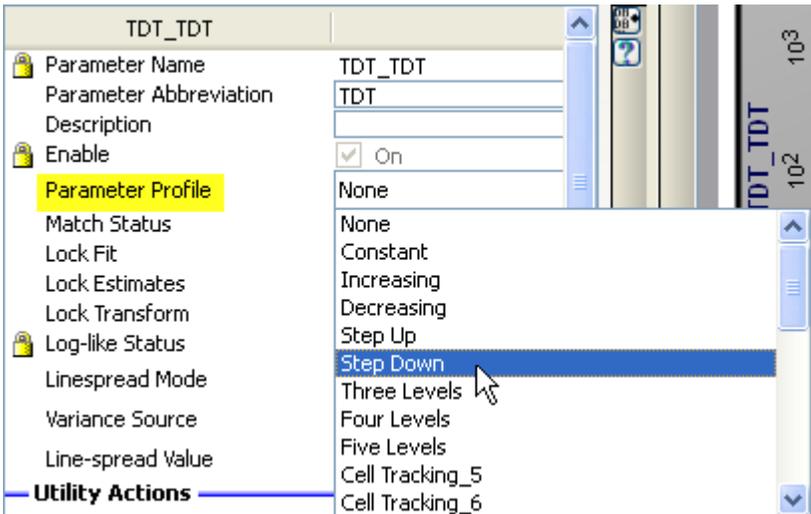
### Modeling the TdT Parameter

Click the parameter selection tool button and choose **TDT**. A new plot is added to the Cell Type widget, and TDT appears in the **Parameter Profile** properties panel.



TdT expression in B-cells is a little more complicated. It starts with elevated expression and then transitions to a low intensity as a function of B-cell progression. We see two bands of dots for TdT, one at about  $10^3$  and another heavier band at about  $10^1$ . We need to use a stair-step profile for this.

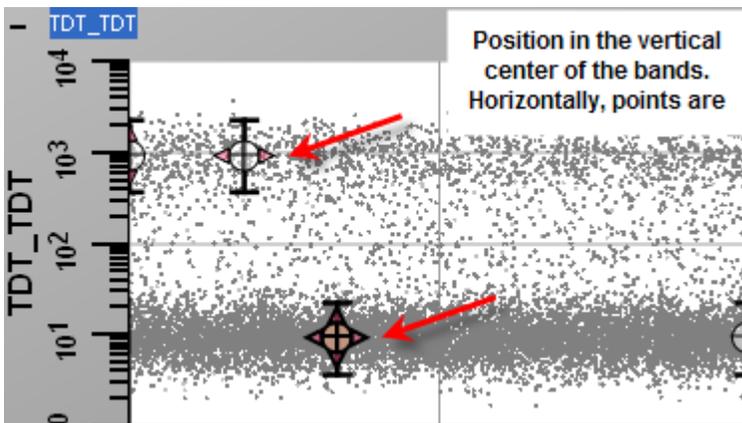
Choose a **Step Down** profile from the dropdown control next to **Parameter Profile**.



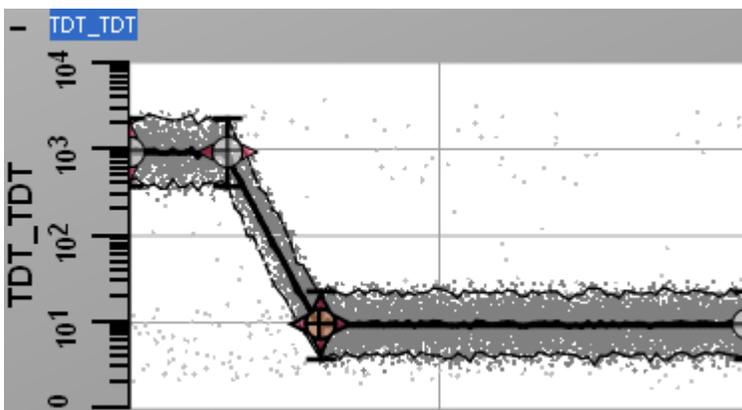
With this profile, the first two points move together in the Y direction, and last two points move together. We need to position the first level on the bright TdT events and the second level on the dims.

Move one of the first two **Control Points** so that it is located at approximately  $10^3$  on the Y-axis – the center of the band of bright events. Since there are fewer events in the upper band, we can position this point near the left end of the X axis.

Now move the third **Control Point** to approximately  $10^1$  on the Y-axis. It should look approximately as shown here.



Click the **Classify Data** button on the toolbar to analyze the data. You can also use Ctrl-A on the PC keyboard, or Apple-A on the Mac keyboard to initiate the Classify Data command. The plot should look similar to this:



GemStone has reordered the events along the progression axis (the X axis), because our model now tells it that the bright TdT events are early in the progression and dim TdT events are later in the progression. This new ordering of events is applied to all of the plots, including CD19 and SSC.

### Using Estimate X Positions

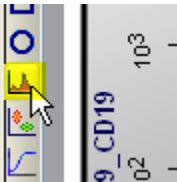
The initial placement of control points for TdT is probably close, but not perfect. GemStone can help us optimize the X positions with the **Estimate X Positions** tool in the toolbar on the right of the TdT profile plot.



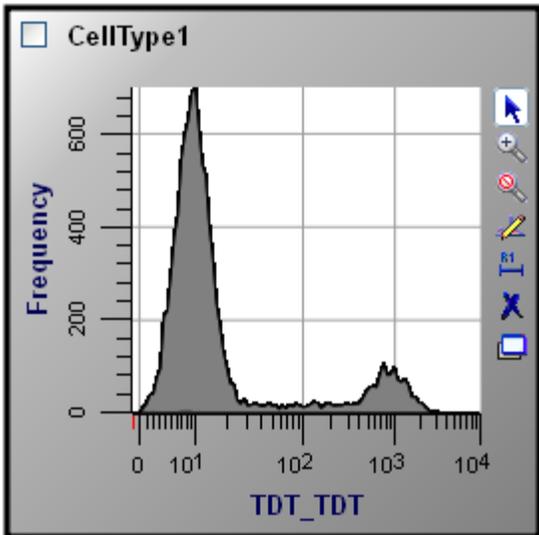
Locate the **Estimate X Positions** button and click it. GemStone will move the definition points on the TdT profile to create a better match for the model. You can click this button more than once and improve the model each time.

### Adding a 1P Plot

We can create some conventional histograms to help us visualize the data. Click the 1P histogram tool in the Workspace toolbar.



In the empty space to the right of the Parameter Plots, click and draw a box for the histogram. You can also click and hold down the Shift key to create a fixed size histogram. When you release the mouse button, the histogram is displayed. Click the X-Axis label on the 1P histogram and choose **TdT**.



### Getting Statistics

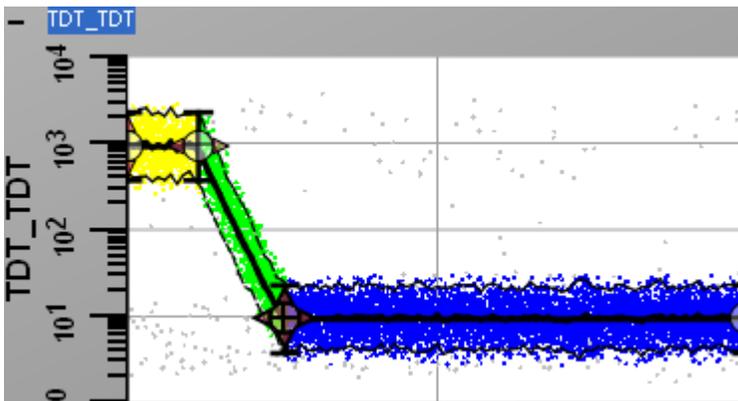
Let's create three "zones" to measure TdT positive, TdT in transition, and TdT negative populations. To do this, we will use the **Control Points** on the TdT profile plot.

Double-click the *second* **Control Point** on the TdT plot. The **Edit BeginDown** dialog appears. "BeginDown" is the name of this point, and this dialog is used to edit properties of it.

Click the <--- **Create Zone** button, and notice that events to the left of the BeginDown point are now colored differently. These events fall into the new **zone** we created for TdT positives. Click **OK** to close the dialog.

Double-click the *third* **Control Point**. The **Edit EndDown** dialog appears. Click <--- **Create Zone** to create the intermediate TdT zone.

Finally, click **Create Zone** ---> to create the TdT negative zone to the right of the definition point. Click **OK** to close the dialog.



The zone coloring is applied to dots in all of the Parameter Profiles. Because of the way [Probability State Models](#) work, you will notice that the system accounts for the overlap between adjacent populations. This is especially evident in the 1P histogram of TdT, where we easily see overlap of the 3 zones.

Now that our statistical zones are created, let's name them. Locate the **Zone Statistics Table** on the canvas and drag it to a convenient location.

To rename a zone, click the zone label and edit the text. Label the zones "TdT+", "TdT trans", and "TdT-", respectively. Notice that the percentages are approximately 10%, 10%, and 80%.

Name	Active	Color	Percent	%Total	E
Total	<input checked="" type="checkbox"/> On		100.00	96.31	9
TdT+	<input checked="" type="checkbox"/> On		11.41	10.99	1
TdT tran:	<input checked="" type="checkbox"/> On		13.83	13.32	1
TdT-	<input checked="" type="checkbox"/> On		74.76	72.00	7

## Saving our Model

As you build models, it is always a good idea to save them as you add complexity. Keep a history of the model's revisions by adding a number or letter to the end of the name each time you save a new version. Let's save our CD19 TdT model now.

Click the **Save Document** button on the main toolbar. The **Save GemStone Document** dialog is displayed. Type "**Model101**" for the file name and click **Save**.

## Conclusions

In this tutorial, we introduced many of the principles of model building with GemStone. Our strategy was to create a simple model, and then to analyze synthesized data with the model. We gradually added complexity and enhanced the model.

This approach is a sort of "self-fulfilling prophecy". We would be very surprised if our model was not able to analyze the generated data properly. However, there are a number of real benefits to this technique. It forces us to describe the characteristics that our real data files will contain. As we add more parameters to our model and synthesize new data, the distributions in our generated data should also become more "realistic". We can take a very complex analysis and break it into incremental steps that can be easily tested.

In our follow-up tutorial, we will add complexity to build a model that can be used with a real B-cell sample.

### See also:

[Model Building 102](#)

[Cell Types](#)

[Cell Type widget](#)

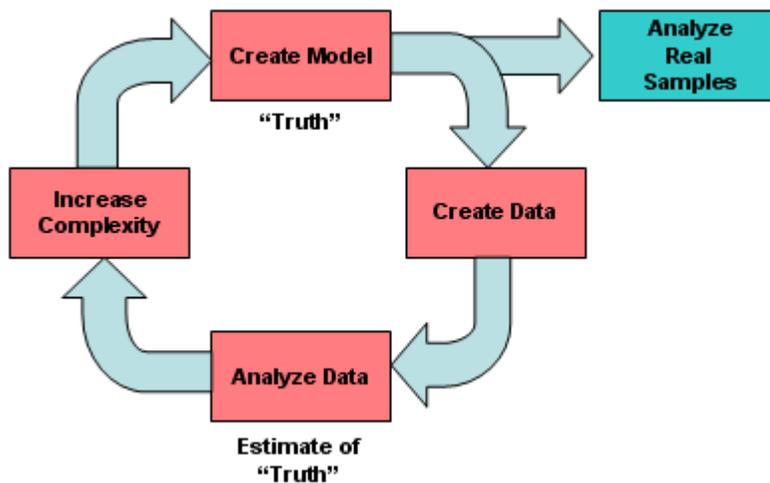
[Parameter Profile Descriptions](#)

## Model Building 102

This tutorial continues where [Model Building 101](#) leaves off. We will add detail to that model to provide a more complete analysis. The Parameter Overlay plot will be used to help visualize the B-cell progression in 5-parameter space.

### Strategy

Remember the cartoon from the first tutorial? We analyzed synthesized data, analyzing the simplest parameters first. We then add to our model and cycle through the testing process again, until we have developed it with enough detail to handle data that has been acquired on a flow cytometer.



### Open Model101.gs

To get started, let's open the model we created in Model Building 101.

Click the **Open Document** button on the main toolbar. In the Open Document dialog, navigate to and open "**Model101.GS**". Click the **Open** button.

The program will open the model and the simulated data that we generated with it. At this point, the model defines CD19, SSC, and TdT for our B-cell lineage.

### Read the File

When GemStone opens a document, it also *closes* the current FCS file. This is because the document itself contains some of the data for the file it last analyzed. So, we need to tell GemStone to read our test file again so that we can add more parameters to the model.

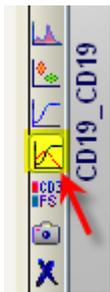
The quickest way to read a file again is to double-click the file in the File Database panel. Do that now.

Status	FileName
▶ ✓ L1	101_CD19_SSC_TDT_CD20_CD10.fcs

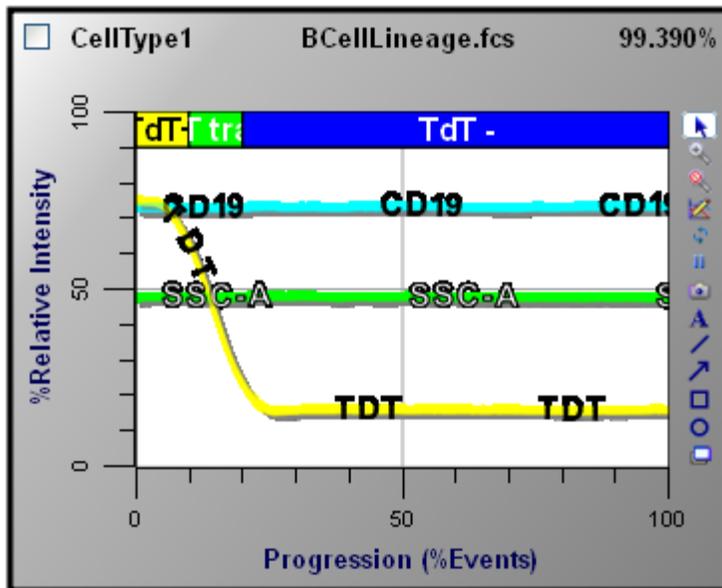
### Adding a Parameter Overlay Plot

The Parameter Overlay plot is one of the most useful graphical tools in GemStone. It presents all of the parameters in our model in one, correlated plot. Each parameter appears as a ribbon of color on the plot. Let's create one now.

Click the Overlay button on the Workspace toolbar to select the overlay drawing tool.



In an empty portion of the Cell Type canvas, click and drag to define a rectangle for the overlay plot. When you release the mouse, the plot will be displayed. The progression for CD19, SSC, and TdT is shown.

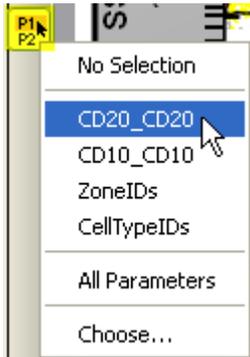


We will refer to this plot as we develop the model with additional parameters.

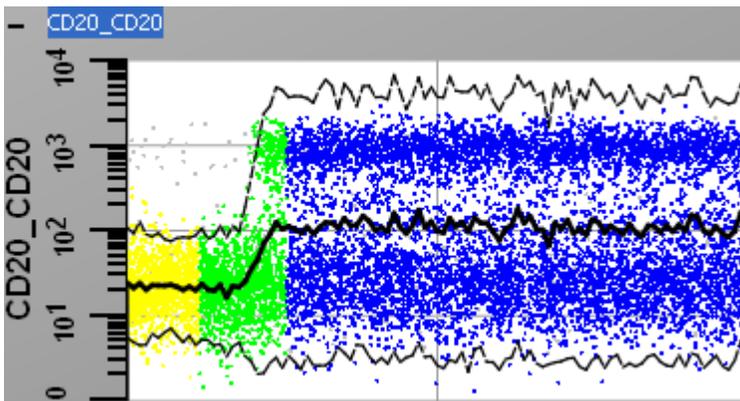
### Modeling CD20

Let's add CD20 to the model.

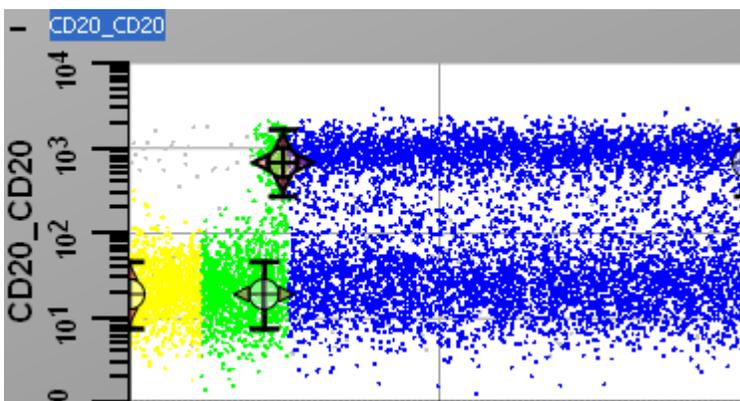
Click the parameter selection tool button and choose **CD20** from the list. A new plot is added to the Cell Type widget, and CD20 appears in the **Parameter Profile** properties panel.



CD20 is not expressed until part way through the progression, and we can see that without even modeling the parameter.



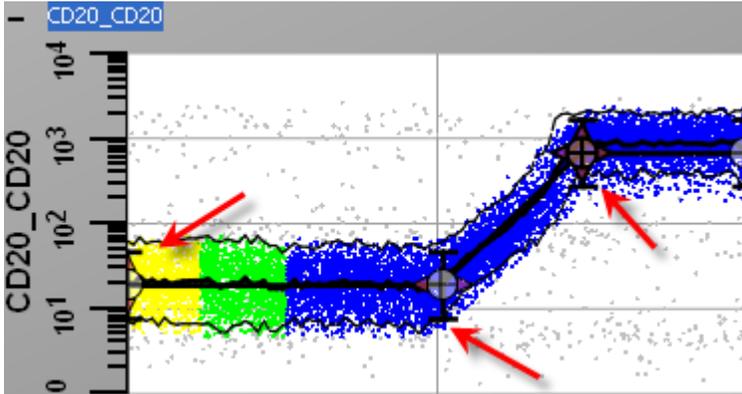
We will use a step-up profile for CD20. Right-click the **CD20** plot and select **Choose Parameter Profile** in the context menu. From the list, choose **Step Up**. Position the Control Points approximately as shown in the illustration below:



Now we can tell GemStone to **Estimate X Positions** and it will automatically adjust the positions and analyze the data. Click the **Estimate X Positions** button.



Now if we look closely at the result, we can see that the CD20 fit needs improvement.

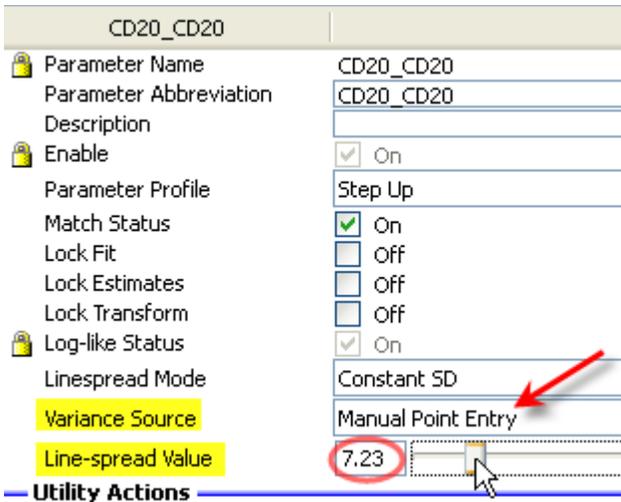


Notice that the little "whiskers" on the Control Points do not match the 95% confidence lines on the data. This is because the data has a different "line spread" than our parameter profile. Think of line spread as a measurement of broadness, or standard deviation. The parameter profile uses the whiskers on the Control Points as an estimate of line spread. In the low-intensity region, our model's line spread is not as wide as it should be for this data. If we look at the bright CD20 events, we see that the Control Point whiskers are actually a little wider than the 95% confidence lines. The model's line spread is wider than it should be. So we need to learn how to adjust the line spread for Control Points. We start by selecting one of the Control Points that we want to adjust.

Click the **second** Control Point to select it.

Next, in the Parameter Profile panel change the **Variance Source** to **Manual Point Entry**.

Now, move the **Line-spread Value** slider to adjust the line spread to about 7.



Notice now that the whiskers for the first two Control Points are wider than they were, and that they more closely match the 95% confidence limits of the data. In most cases, it is good to set the line spread to be a little wider than the observed data; the automatic adjustment routines in GemStone give better results with slightly wider settings.

Click the **third** Control Point to select it.

Move the **Line-spread Value** slider to adjust the line spread to about 3.5. You may also need to adjust the vertical position of the definition point.

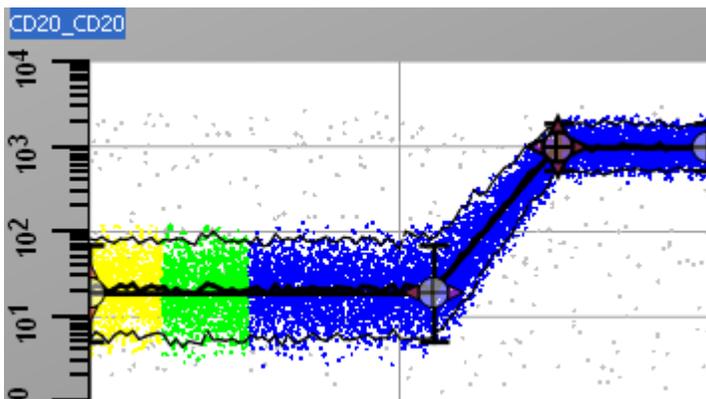
#### Model Building Tip: Use the Scroll Wheel for Line Spread Adjustments

If your mouse has a scroll wheel, there's a quick and easy way to adjust the line spread for a Control Point. Position the mouse over the center of the Control Point you want to adjust, and then roll the scroll wheel. Scrolling up increases the line spread; scrolling down decrease the line spread.

Click the **Estimate X Positions** button to allow GemStone to adjust the model and reanalyze the data.

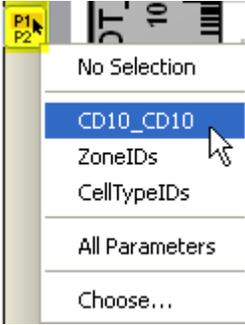


Our CD20 profile now matches the data more accurately. The low-intensity events are modeled with a broader line-spread, and the high-intensity events have a narrower spread.

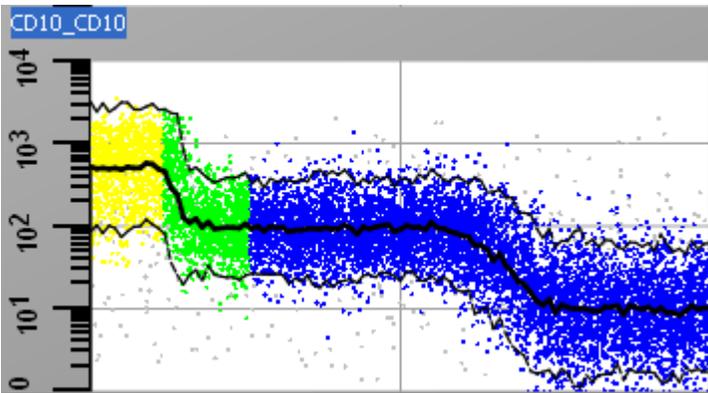


#### Modeling CD10

Click the parameter selection tool button and choose **CD10** from the list.



Now we see one of the most interesting aspects of a GemStone model. We have not yet attempted to model CD10, and yet the structure of the CD10 progression is plainly visible. Three distinct levels of expression are shown, and the levels match our expectations for CD10.

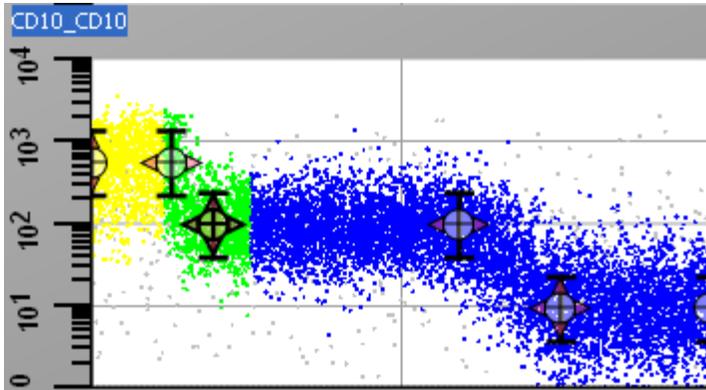


For CD10, we know that it is highly-expressed early, followed by a moderate expression and finally a low expression. How did GemStone figure this out? In each of the previous parameters, we had to select a parameter profile and position Control Points before the events were properly distributed along the progression axis.

What has happened is that our model now has enough structure to reveal structure in parameters that we have not modeled. Our TdT profile defined a transition early in the progression. CD20 defined a transition later in progression. These pieces of the model create enough of a scaffold to order the progression of events. We can use this to explore markers that are completely unknown.

Let's go ahead and model CD10 so that our model has an even stronger scaffold.

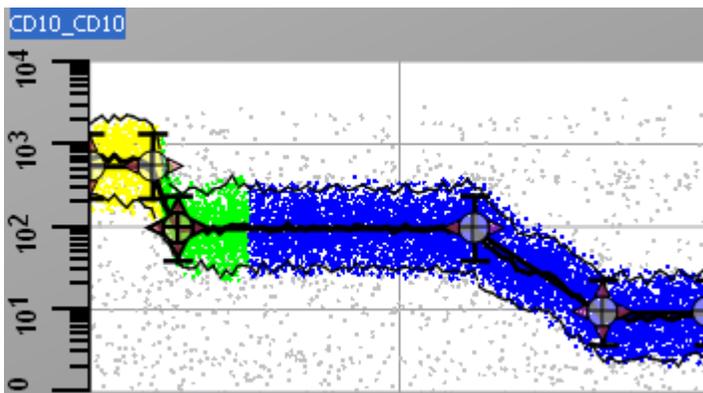
Right-click the **CD10** plot and select **Choose Parameter Profile** in the context menu. From the list, choose **Three Levels**. Position the Control Points so that they match the transitions in the data as shown in the illustration below:



Now click the **Estimate X Positions** tool to allow GemStone to fine-tune the Control Point locations and analyze the data.

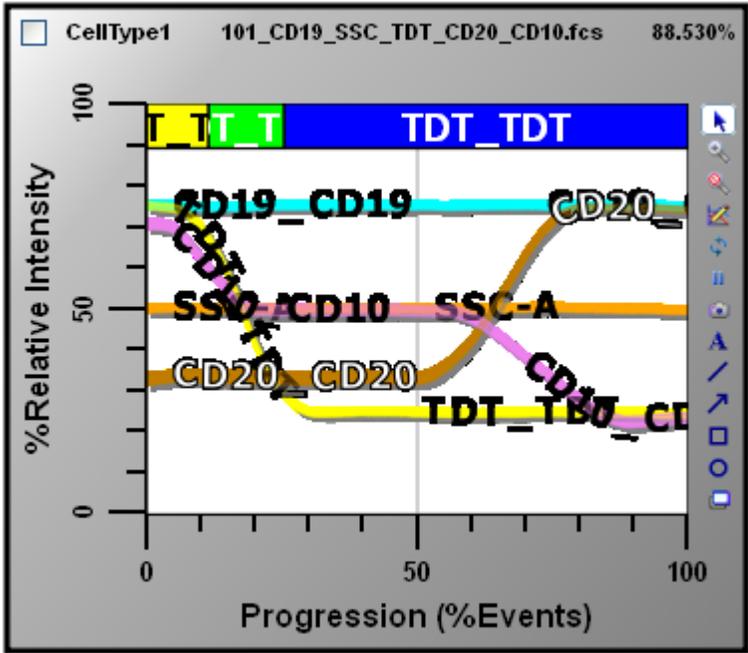


Our fit now looks like this:



Just as we saw with CD20, the line spread for the CD10 profile could use a little fine-tuning too. The whiskers on the control points are a little narrow. If you would like to adjust these, go ahead and do so using the same process we used for CD20.

If we look at our Overlay Plot, we now see the coordinated expression of CD19, TdT, CD20, and CD10. The light scatter parameter is also displayed.



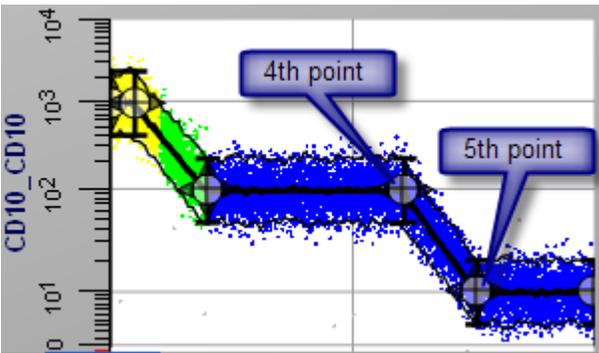
**Redefine Zones**

The zones we initially created are not showing us all that we know at this point. Let's redefine the zones to cover the progression in more detail.

In the **Cell Type Properties** panel, find the **Edit Zones** entry and click the **Edit** button. A dialog appears showing the zones we created.

Select the last zone in the list, **TdT-**, and then click the **Delete** button. Click **OK** to close the dialog.

In the **CD10** plot, double-click the 4th control point.



Click the **<--Create Zone** button and then click **OK**.

Double-click the 5th control point.

Click the **<--Create Zone** button. Next, click the **Create Zone-->** button. Finally, click **OK**.

We now see dot coloring that reflects 5 zones. Let's edit the names of these zones.

To rename a zone, click the zone label in the **Zone Statistics** table and edit its label. Label the zones **Total**, **I**, **II**, **III**, **IV**, and **V** respectively

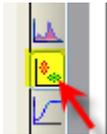
CellType1

Name	Active	Color	Percent	%Total	Events
Total	<input checked="" type="checkbox"/> On		100.00	88.53	8853
I	<input checked="" type="checkbox"/> On		11.50	10.18	1018
II	<input checked="" type="checkbox"/> On		13.52	11.97	1197
III	<input checked="" type="checkbox"/> On		37.50	33.20	3320
IV	<input checked="" type="checkbox"/> On		20.50	18.15	1815
V	<input checked="" type="checkbox"/> On		16.98	15.03	1503

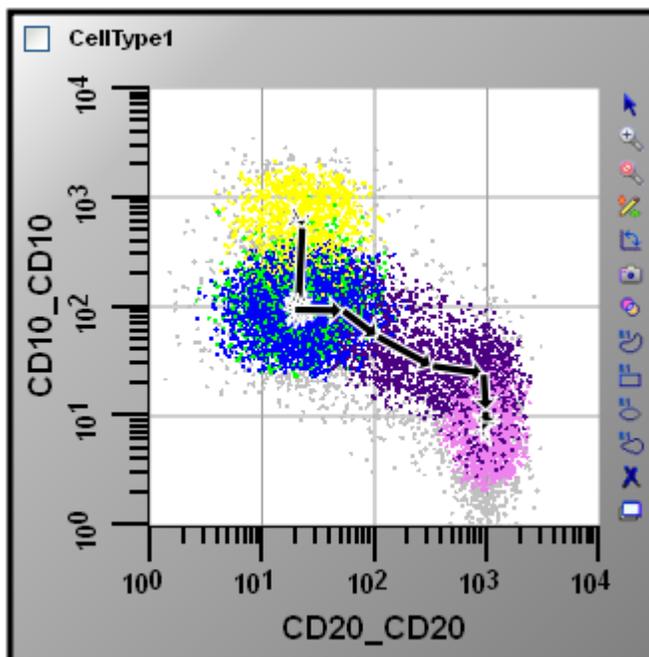
### Create a 2P Plot

We can create a standard dot plot to see whether our model is producing data that looks like what we expect.

From the Workspace toolbar, select the **2P histogram tool** and draw a rectangle for the histogram in an empty space on the Cell Type canvas.



Select **CD20** for the X-axis and **CD10** for the Y-axis by clicking on the axis label and choosing the parameter from the list.



Our model has created events that form a familiar pattern of CD10 vs. CD20 expression for B-cells. Because we have defined these in terms of a *progression*, GemStone can overlay arrows that show us how B-cells develop, even in the dot plot.

Let's save our new, improved model.

From the main toolbar, click the **Save Document** button. The **Save GemStone Document** dialog is displayed. Type "**Model102**" for the file name and click **Save**.

We are ready to try this model out on a real B-cell sample, which is the subject for [Model Building 103](#).

**See also:**

[Model Building 103](#)

[Cell Types](#)

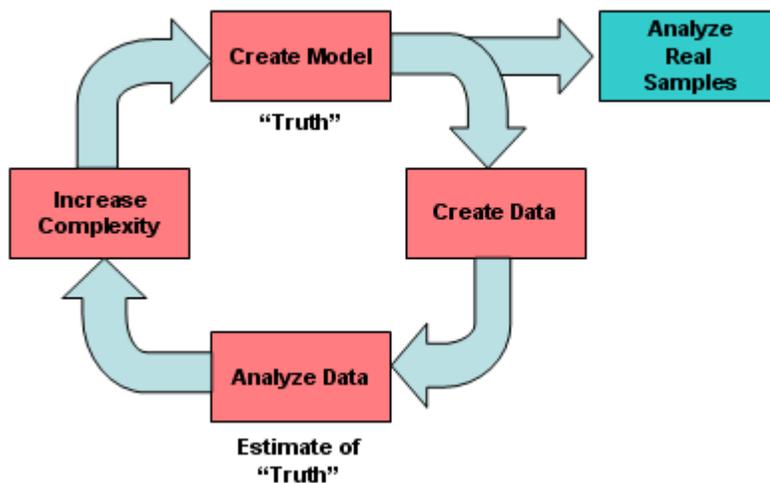
[Cell Type widget](#)

[Parameter Profile Descriptions](#)

## Model Building 103

In this tutorial, we will put our B-cell model to the test and use it to analyze a real, B-cell sample file. We will start with the model we created in [Model Building 101](#) and [102](#), and we will work through the process of adjusting the model to a real sample. At that point, we should be able to select the additional parameters that are in our sample file and see how they behave in the B-cell progression – without having to model them ourselves.

At this stage, we are jumping out of our model development cycle and into the real world of analyzing real samples.



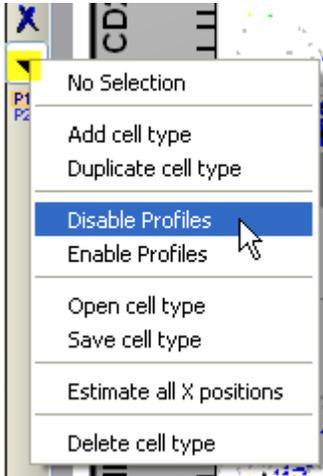
### Open the Model

On the main toolbar, click the **Open Document** button. Select "**Model102.gs**" and click the **Open** button to load our working model.

### Disable the Profiles

Normally, when GemStone opens an FCS data file, it automatically analyzes the file with the model it currently has open. In making the transition from synthesized data to real data, we do not want this to happen because we want to see the unanalyzed data from our real sample. So we are going to want to turn off the pieces of the model. We can do this with one action.

Click the **Utility Action** button on the **Workspace** toolbar, and select **Disable Profiles** from the menu.



All of our plots will redraw without color, showing all events in an unanalyzed state.

### Model Building Tip: Use Disable Profiles

Disable Profiles is used to turn off the profiles for all parameters in the cell type. This can be useful if you want to restart the model building process but don't want to reconstruct the model from scratch.

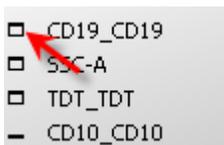
### Open the Data File

On the main toolbar, click the **Select FCS Files** button. Navigate to GemStone's **Sample Files** folder, select **BCellLineage.fcs**, and click **Open**. The file is added to the File Database and read into our model.

Now we will enable the pieces of our model, one at a time, starting with the CD19 and SSC **selection parameters**. Selection parameters, as we learned in Model Building 101, are those that have **constant** parameter profiles and are used to select populations of interest. In this case, CD19 and SSC will be used to select B-cells from our sample file.

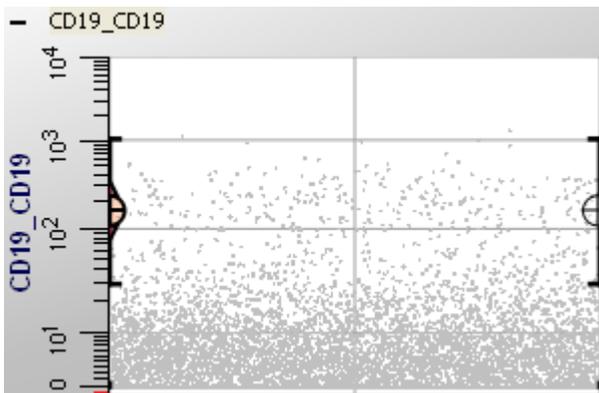
### Adjust and Enable CD19

Click the shrink tool for the **CD19** Parameter Profile plot to expand the plot.



The B-cells are those with high expression of CD19. We need to move the CD19 **Control Points** down to identify the correct cells. Click and drag one of the control points to identify the band of CD19 positive cells.

We also need to adjust the line spread to encompass the bright CD19 events. Use the **Line-spread** slider or mouse scroll wheel to adjust the slider to select a broad band of bright CD19 events.



You might worry that we are being too generous in selecting with CD19 here. That is intentional at this point: we don't want to exclude dim CD19 events yet. As we adjust the model, those dim events will start to drop out.

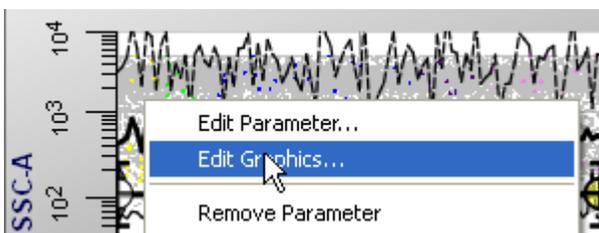
Click the **Enable** tool on the CD19 parameter profile to turn it on , and then click the **Classify Data** button on the main toolbar .

### Turn Off Means and Confidence Limits for SSC

We're ready to move on to SSC. Click the shrink tool for the **SSC** Parameter Profile plot to expand the plot.

GemStone is trying to be helpful by showing 95% confidence limits and the mean of the analyzed events. In this case, however, the extra graphics are making it difficult to see what we need to see. Let's turn off the extra graphics.

Right-click in the **SSC** plot area and choose **Edit Graphics** from the context menu.



In the **Edit Graphic Options** dialog, select the **Means** tab. Uncheck the **Enable Means** option.

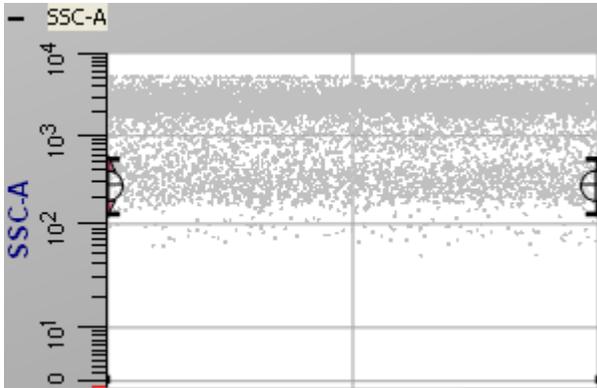
Select the **Conf Limits** tab and change **Line Spread Mode** to **Model SD Bars**.

Select the **Model** tab and uncheck **Enable Model**.

Click **OK**. The SSC plot should now display only the dots and the control points.

### Adjust and Enable SSC

Slide the **Control Points** for SSC up, centering the points on the low-intensity band of events. Adjust the line-spread for the profile. The result should look something like this:

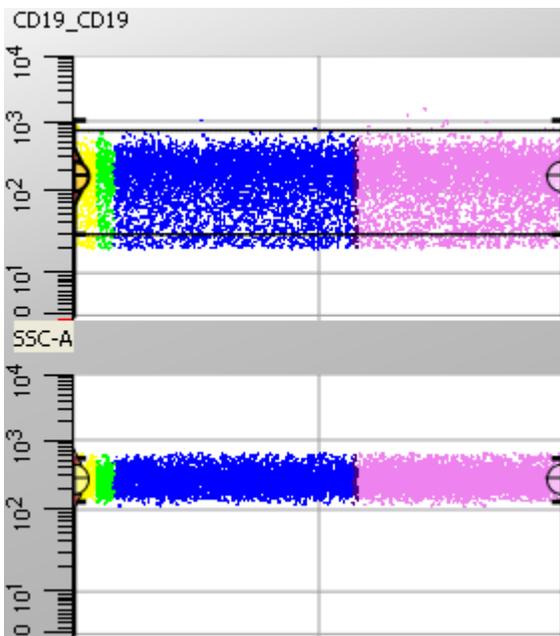


Click the **Enable** tool on the SSC parameter profile to turn it on  , and then click the **Classify Data** button on the main toolbar  .

### Enrich

B-cells are a relatively small percentage of the cells in this sample, less than 3%. That can make things difficult to visualize. So we'll use a feature called **Enrich Data** to search out all of the B-cells (those that have high CD19 and low SSC) and select them exclusively. We do this after adjusting our **selection parameters** and before we move on to the parameters that transition during the progression.

On the main toolbar, click the **Enrich Data** button. Now we've got plenty of B-cells to analyze.



**Model Building Tip: Use Enrich Data to See More of the Cells of Interest**

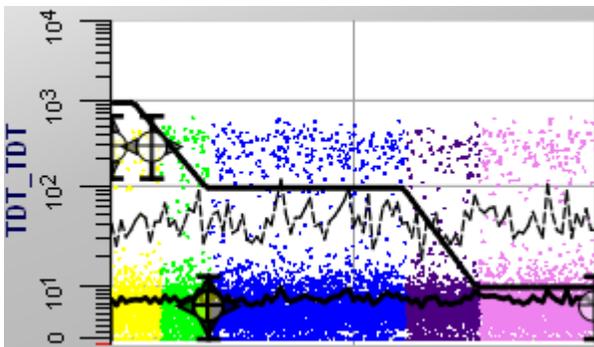
Using Enrich Data allows you to include for consideration only those events that match the current model. This allows concentration of events that represent a small fraction of the entire sample.

### Adjust and Enable TdT

Click the shrink tool for the **TdT** Parameter Profile plot to expand the plot.

Here we can see two bands of events: a sparse band of high-intensity and a dense band of low-intensity TdT. Let's move our **Control Points** down to the approximate centers of the bands.

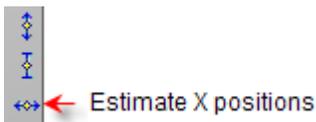
Adjust the control points down as shown:



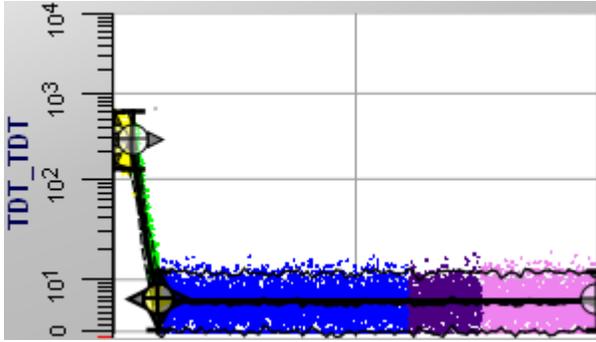
### Model Building Tip: Place Definition Points at the Right Intensity

When determining the initial position for the definition points, it is most important to get close to the right intensity level. The definition point should be placed near the center of the band of data it represents. The line spread should be set to approximate the variance of the data. The state index position of the definition points can be estimated based on the density of the dots at the given intensity.

Use the **Estimate X Positions** adjuster tool for TdT to fine-tune the control point positions.



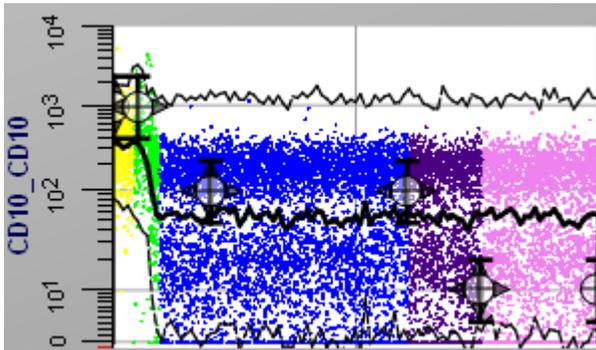
The profile now shows the step-down progression we designed into our model.



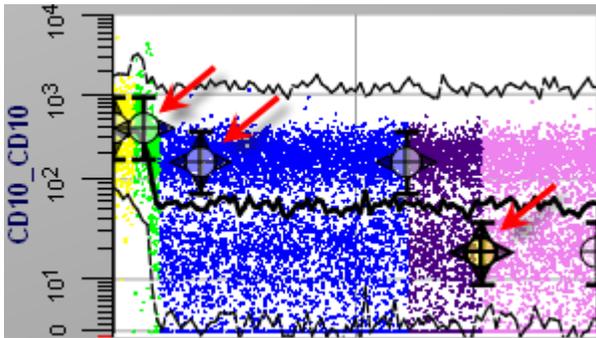
At this point, we have selected B-cells and created the first structure in the progression backbone using the TdT profile. GemStone has re-ordered the events so that the high-intensity TdT events are at the start of the progression, the low-intensity TdT events are at the end of the progression, and intermediate TdT events are in the transition between high and low. This order is reflected in *all* of the parameter profile plots, and we start to see the hidden structure appearing in the other parameters.

### Adjust and Enable CD10

The CD10 plot shows 3 levels of intensity, just what our model is designed for. The first level of high-intensity events is clustered at the beginning of the progression. Then we see a slightly lower intensity band of dense dots, and a diffuse band of low intensity events.

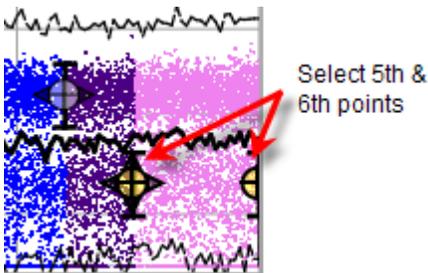


Once again, we'll adjust the positions of the **Control Points** to get a good starting estimate. Move the controls approximately as shown, paying attention primarily to the Y-axis positioning to place the points in the middle of the bands.



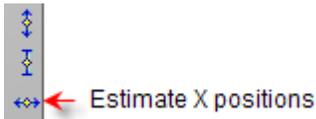
Now here is a new trick. Notice that the width of the CD10 negative band of dots is significantly wider than the intermediate and high CD10 bands. We can help GemStone out here by adjusting the line spread for that low-intensity band to be wider than the other bands. Here is how we do that.

Click the **5th** Control Definition point in the plot. This will select the point.

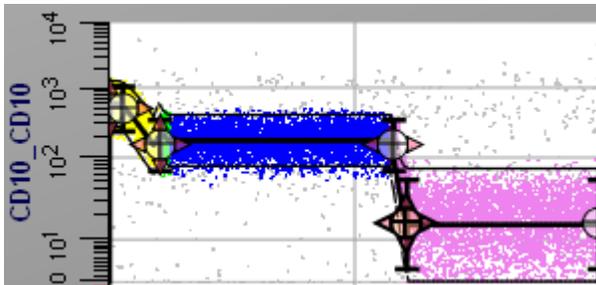


Now position the mouse over the 5th point and use the mouse scroll wheel to adjust the line-spread wider. If you do not have a scroll wheel on your mouse, use the Line-spread slider in the Parameter Profile panel.

This adjustment will make it easier for the auto-adjusting functions. Use the **Estimate X Positions** adjuster tool for TdT to fine-tune the control point positions.



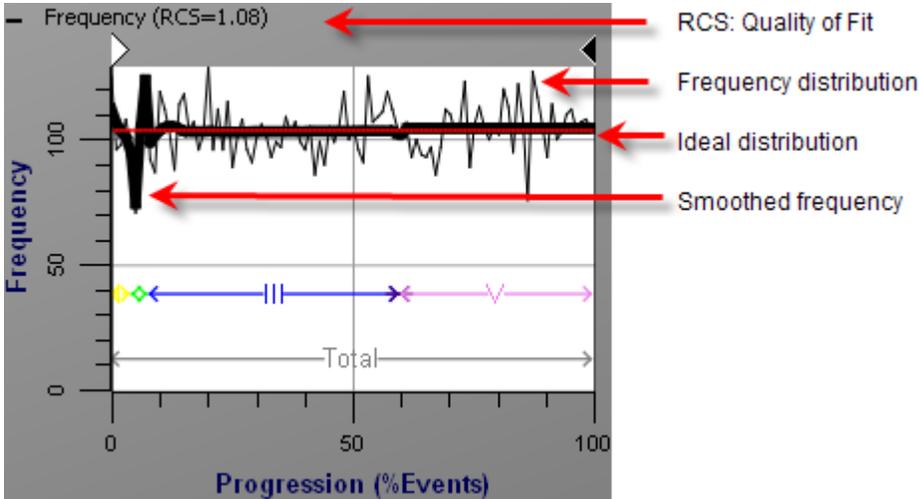
The finished result has matched our data well.



### Using the Frequency Plot

How do we know that the model is really matching the data well? We use the [Frequency Plot](#) to help make that assessment. This is the bottom element in the Cell Type widget. There are 3 things we examine at on this plot to determine how well the model is working.

At the top, **RCS** shows us the reduced chi square value, which is a statistical measure of how well the model matches the data. A value approaching 1.0 is just about perfect. Typical values can be between 3 and 7 with more complex models.



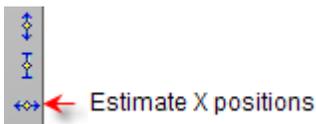
Across the plot is a jagged black line showing us the **frequency distribution** for each state in our model. There is also a horizontal red line showing the **ideal distribution** of events. The ideal distribution simply takes the number of events we are working with and divides that by the number of states in the progression. A Probability State Model attempts to distribute events in this manner, so this is ideally what our model would do. The frequency distribution line shows how our model is actually distributing events, and it should bounce above and below the ideal line as we see in our model so far. The **smoothed frequency** shows a weighted average frequency distribution. See the discussion on [what is a Probability State Model](#) for more on this.

So, our model is looking pretty good at this point.

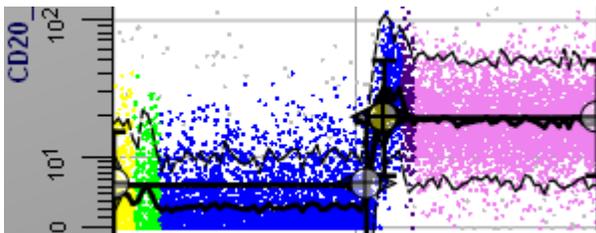
### Adjust and Enable CD20

The CD20 plot shows two levels of intensity.

Move the **Control Points** to the vertical center of the dense bands of dots. Adjust the line-spread for the control points. Then click the adjuster button.



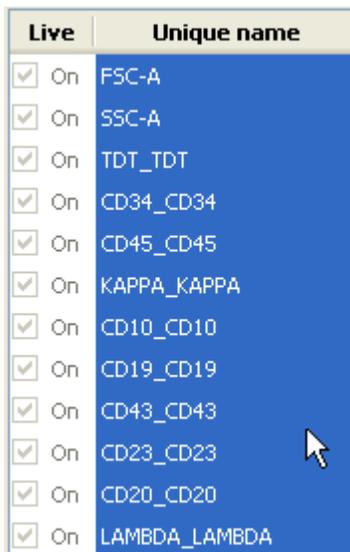
This gives us a pretty good fit with the data. We can see an up regulation that then drops off again, which is what our Three Levels profile is designed for. However, this Step Up profile is also providing a very good fit, so we'll keep it. We can always come back and change it to a Three Level profile later.



### Add the Remaining Parameters

This data file has many more parameters than we are currently modeling. What do you suppose will happen if we display the remaining parameters without defining any expectations in our model? Will there be any structure in them, or will they be evenly distributed bands of dots on the profile plots? Let's find out.

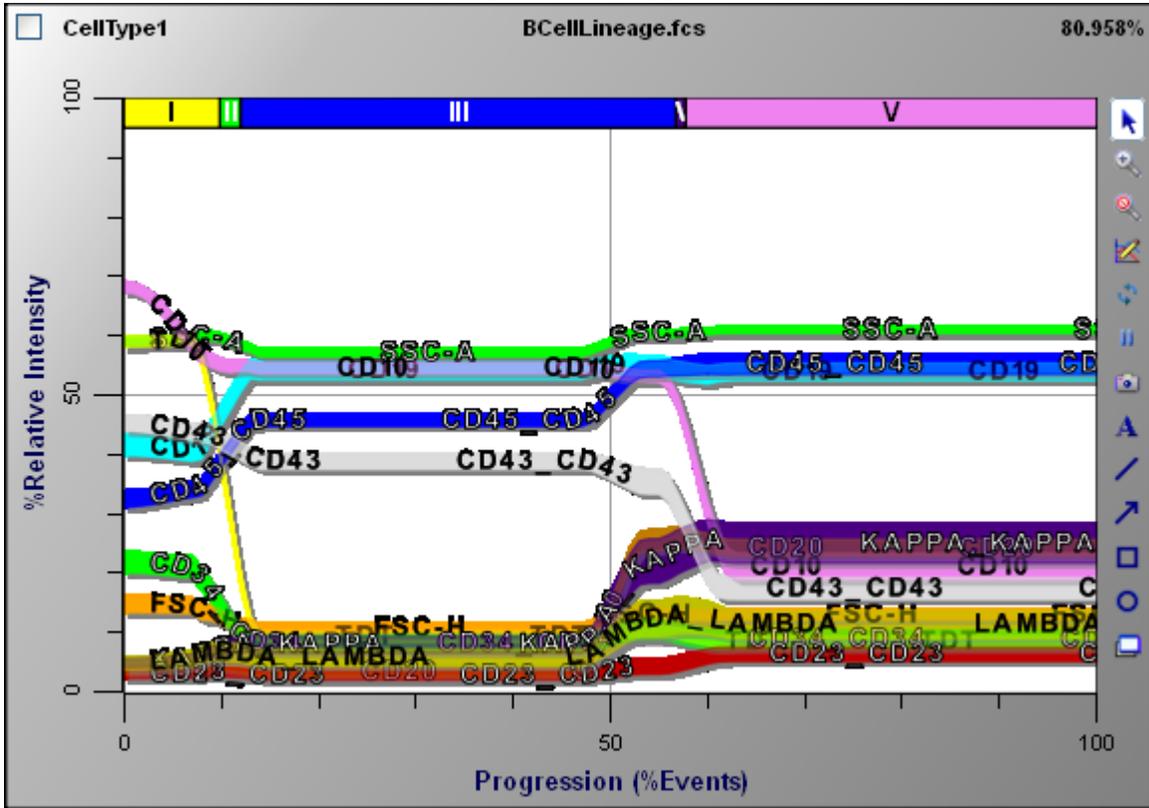
In the **Cell Type Properties** panel, click the **Edit** button for **Select Parameters**. Select all of the "Live" parameters in the list on the left side of the dialog (even the ones we have already added), and then click the **Add** button.



Live	Unique name
<input checked="" type="checkbox"/> On	F5C-A
<input checked="" type="checkbox"/> On	S5C-A
<input checked="" type="checkbox"/> On	TDT_TDT
<input checked="" type="checkbox"/> On	CD34_CD34
<input checked="" type="checkbox"/> On	CD45_CD45
<input checked="" type="checkbox"/> On	KAPPA_KAPPA
<input checked="" type="checkbox"/> On	CD10_CD10
<input checked="" type="checkbox"/> On	CD19_CD19
<input checked="" type="checkbox"/> On	CD43_CD43
<input checked="" type="checkbox"/> On	CD23_CD23
<input checked="" type="checkbox"/> On	CD20_CD20
<input checked="" type="checkbox"/> On	LAMBDA_LAMBDA

Click **OK** to close the dialog.

Now with 11 parameters loaded, the best way to see the other parameters is to look at the **Parameter Overlay** plot.



Even without creating a model for CD45, CD43, CD34, CD23, and others, we can see how these parameters behave with B-cells in our progression. Our model defined the progression well enough that the other parameters reveal information without any effort on our part. For example, we can see that CD45 is lowest at the start of the progression, it increases in zones I and II, and then levels off in zone III. It elevates again at the end of III and levels off in IV and V.

**Saving our Model**

We have made several enhancements to our B-cell model. This model is now capable of analyzing real data, so we should save a new copy of it.

From the main toolbar, click **Save Document**. Type "**Model103.gs**" for the file name and click **Save**.

**Other Enhancements**

If you are familiar with B-cell maturation, you know that at some point along the way a B-cell will either express Kappa or Lambda, not both. When you have a branch like this, you generally need more than one **Cell Type** to properly characterize the process. This will be the focus of [Model Building 104](#), the last in our series.

**See also:**

[Model Building 104](#)

[Cell Types](#)

[Cell Type widget](#)

[Parameter Profile Descriptions](#)

## Model Building 104

This tutorial introduces the use of more than one Cell Type in a model. It continues the model-building process from [Model Building 101](#), [102](#), and [103](#).

When we finished Model Building 103, we had developed a fairly detailed model of B-cell maturation. We used a 12-parameter FCS file to test the model, and we were able to observe correlated transitions in marker expression even for parameters that we did not model.

Our model does not yet account for a branch that occurs in B-cell maturation. As the cells mature, they will either express Kappa or Lambda, but not both. We want to capture this with our model to quantify the relative fractions of Kappa and Lambda cells, and to see if other markers have different characteristics for the two subsets.

To do this, we will create a second Cell Type. In general, whenever you need to analyze more than one population or subset, you will need to create a separate Cell Type for each. We will handle the mechanics of doing that here.

### Open the Model

We will start by opening the model that we have developed.

Click **Open Document** on the main toolbar. Select "**Model103.gs**" and click **Open**.

The model will be read and displayed in its last state.

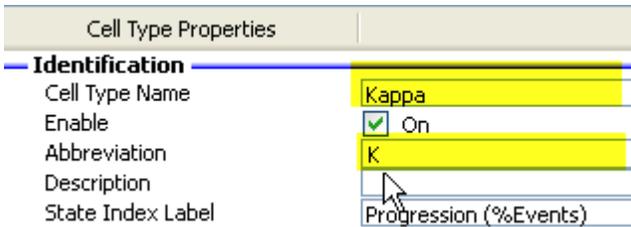
### Cell Type 1: The Kappa Cell Type

We are going to make the first Cell Type for Kappa B-cells. We will select Kappa cells using a Constant profile on the *Lambda* parameter to identify Lambda-negative cells. The advantage to this approach is that our model will be able to show clonal excess for Kappa cells. Saying that another way, the model will not filter out events that are *very* positive for Kappa.

Click the Cell Type shrink tool to expose the editors we will be working with.

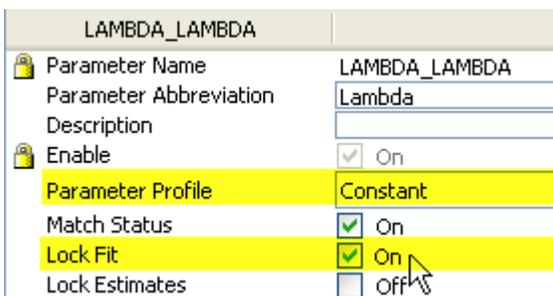


In the Cell Type Properties panel, change the **Cell Type Name** to "**Kappa**", and edit the Abbreviation to "**K**".

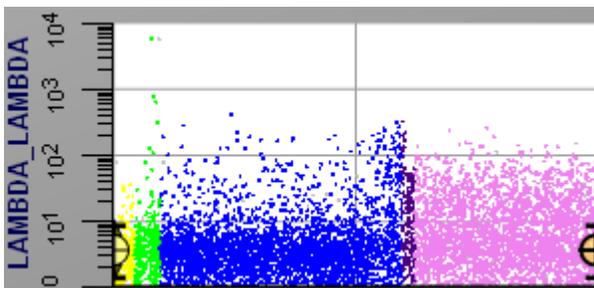


Locate the **Lambda** parameter in the Cell Type widget. It should be the last parameter. Click the **Lambda** label to activate the parameter.

In the Parameter Profile property panel, choose **Constant** for the **Parameter Profile**. Also, enable the **Lock Fit** checkbox. This tells GemStone not to move this profile, which is very important when identifying "negatives" in this manner.



Position the **Control Points** for the profile to identify the low-intensity Lambda events as shown.



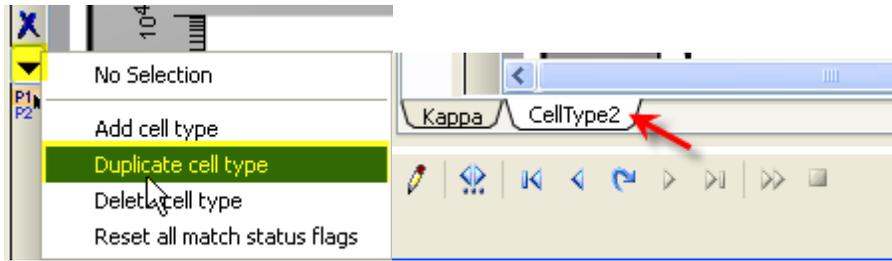
We will not analyze the data yet. First, let's create the Lambda Cell Type.

### Duplicate Cell Type

The only difference between the Kappa and Lambda Cell Types will be in the use of the Kappa and Lambda parameters. All of the other parts of the model for these two Cell Types will be the same. That means that we can duplicate the work we have done already and make a few changes in the second Cell Type to model the Lambda cells.

Click the **Utility Action** button on the **Workspace** toolbar, and select **Duplicate Cell Type** from the menu.

GemStone will make a copy of all of the elements in our Kappa Cell Type, and name the copy "CellType2". It also switches to the tab for CellType2.



**Model Building Tip: Duplicate Cell Type**

Use the Duplicate Cell Type command to make a copy of an existing Cell Type. The command will copy the model definition, as well as the layout and graphics for the Cell Type.

**Cell Type 2: The Lambda Cell Type**

Click the Cell Type shrink tool to expose the editors for CellType2.



In the Cell Type Properties panel, change the **Cell Type Name** to "Lambda", and edit the Abbreviation to "L".



Now we need to make two more changes. We have to turn **off** the parameter profile for Lambda in this Cell Type. Then, we need to model the Kappa parameter to select Kappa negative events.

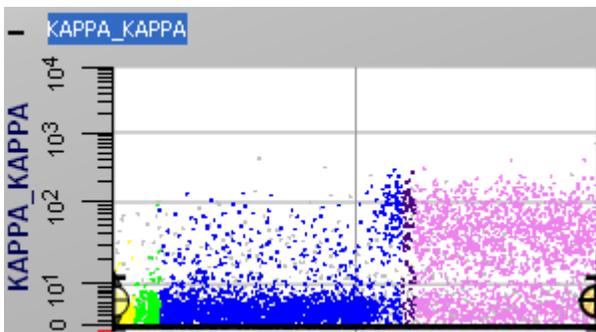
Locate the **Lambda** parameter in the Lambda Cell Type widget. Click the **Lambda** label to activate the parameter.

In the Parameter Profile property panel, choose **None** for the **Parameter Profile**.

LAMBDA_LAMBDA	
Parameter Name	LAMBDA_LAMBDA
Parameter Abbreviation	Lambda
Description	
Enable	<input checked="" type="checkbox"/> On
Parameter Profile	None
Match Status	<input checked="" type="checkbox"/> On

Next, locate the **Kappa** parameter in the Lambda Cell Type widget and click the **Kappa** label to activate the parameter.

In the Parameter Profile property panel, choose **Constant** for the **Parameter Profile**. Enable the **Lock Fit** checkbox as we did for the Lambda profile in the Kappa Cell Type. Position the **Control Points** for the profile to identify the low-intensity Kappa events as shown.



### Saving the B-Cell Kappa Lambda Model

This is a very useful B-cell model at this point. We should save a new copy of it.

From the main toolbar, click **Save Document**. Type "**Model104.gs**" for the file name and click **Save**.

### Auto Analyze the Data

We are ready to analyze our full Kappa-Lambda B-cell model. Let's use **Auto Analysis** to do this. Auto Analysis does many of the steps we have been doing manually: it adjusts the constant selection parameters, it enriches, and it then adjusts the parameter profiles for each of the remaining parameters (except those with the Lock Fit option enabled).

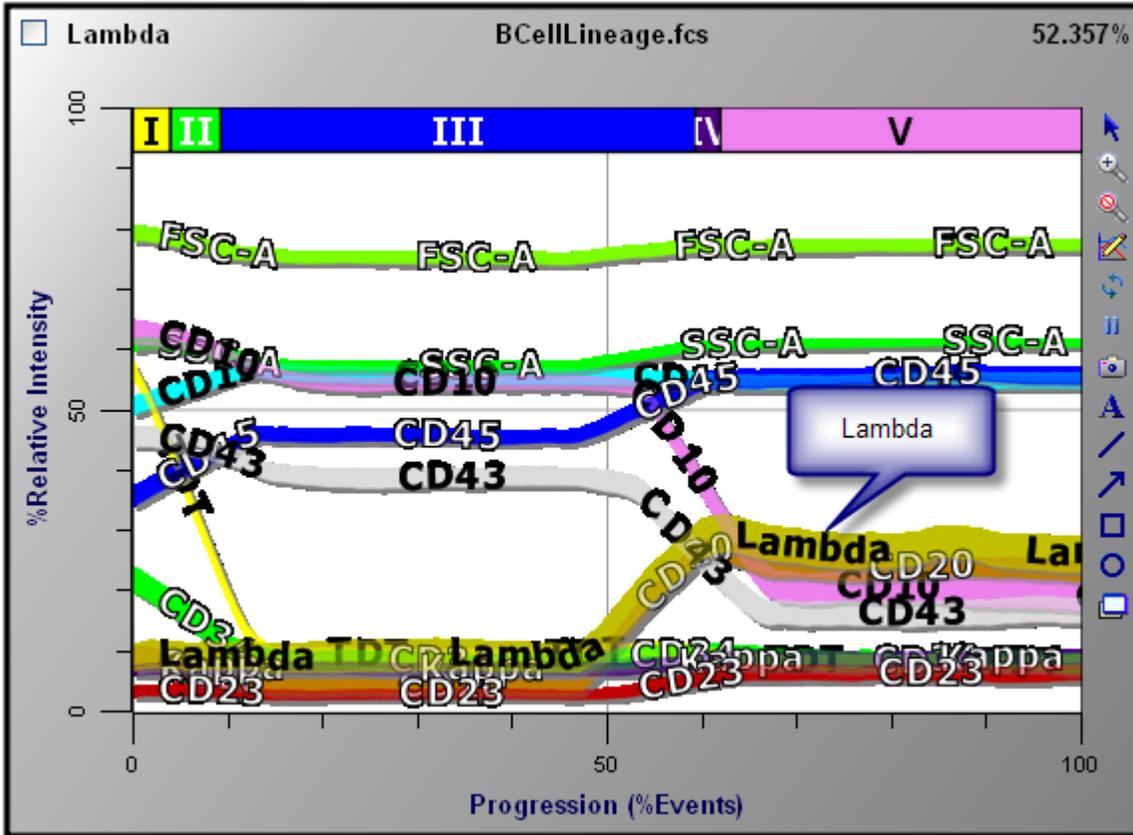
Click the **Auto Analysis** button on the main toolbar.

Like the **Classify Data** command, **Auto Analysis** acts on *all* of the Cell Types that are defined in the model document. An event can be classified as belonging to one of the cell types or it can be considered unclassified. An event can never be classified into more than one of the cell types at the same time. In the model we have created, the Kappa and Lambda Cell Types are assigned events probabilistically.

**Model Building Tip: Stopping Auto Analysis**

There may be a point at which the RCS and frequency distribution are "good enough" and the optimization is no longer making real improvement to the fit of the model. You can stop the current estimation or the auto analysis process by clicking the appropriate check box in the status bar.

Let's take a look at the Parameter Overlay plot for each of our two Cell Types. In the Lambda Cell Type overlay, we can see the elevated expression of the Lambda marker starting late in zone III. The Kappa expression for Lambda cells remains low.



Conversely, in the Kappa Cell Type, the Kappa expression increases in zone IV, and Lambda expression remains low throughout.



Use these principals to develop models for the cells you want to analyze, and you should produce robust models that can be used with GemStone for a great variety of applications.

**See also:**

[Tutorials](#)

[Cell Types](#)

[Cell Type widget](#)

[Parameter Profile Descriptions](#)

## Creating a Model for a Multi-Tube Panel

In the previous [Model Building](#) tutorials, we learned how to build a model for a single-tube B-cell assay. Those tutorials are pre-requisites for this one.

Much of the time we use panels with several tubes - and therefore several listmode files. That presents a different set of problems for analysis. This tutorial will focus on the differences in how you approach model building for panels with more than one tube.

### Concept and Strategy

The model that we build needs to do everything that the single-tube model does. It needs to help us understand the coordinated expression and transitions of the markers in the panel. However, GemStone is going to read the files in our panel one at a time. So, how can we build a model that correlates all markers if they are spread across several tubes?

GemStone helps us with this task by retaining the results for the markers it has analyzed. Here is a simple example. Let's say that tube 1 has CD10 and some other markers, and tube 2 has CD34 among its markers. GemStone will store the means and standard deviations for every state of the CD10 in tube 1 and allow us to correlate it with CD34 in tube 2. After GemStone has analyzed all of the tubes in the panel, the results for the entire panel are available to us. We can display Parameter Overlay plots and view statistics for any of the markers in the panel. We can even look at dot plots for markers that are actually in separate tubes.

Our model-building strategy is similar to the one we used to build our single-tube model. The difference is that we are more dependent on a good set of common parameters. The term "*common parameters*" refers to the set of markers that are common to all tubes in the panel. Using these parameters, we will build the skeleton of a model that will span the several tubes in our panel. The common parameters provide the backbone that allows us to make correlations among all of the markers.

#### **Model Building Tip: Common Parameters**

Common parameters are the parameters that are included in all tubes of a panel. Good common parameters make it possible to create a framework for comparing markers that are not in the same tube.

### Choosing Good Common Parameters

A good set of common parameters is the key to success. For most cytometers, the light scatter parameters are available in every file. Unfortunately, they do not help us correlate markers in most cases. Ideally, the markers we choose to be common will have simple, well-defined transitions in the progression.

For our tutorial, we will set up a model for a 3-tube bone marrow panel, each tube having 7 colors, for a total of 11 fluorescent markers. The tubes have 5 common parameters: CD19 is required in all tubes so that we can identify the B-cells; CD34 will help us identify early B-cells and is a simple, step-down parameter; CD20 is up-regulated later in B-cell maturation;

CD10 starts high and is lost in two later stages; and CD45 will also help identify transitions in the B-cell progression.

Along with these common parameters, tube 1 has CD58 and CD38. Tube 2 contains CD33 and CD13. And finally, tube 3 includes CD9 and CD22, rounding out this panel.

Let's get started.

## Tube 1

### Select the Data Files

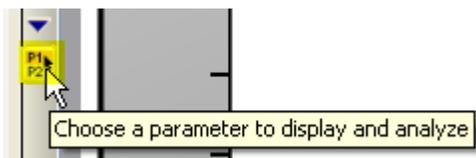
Click the **Select FCS Files** button on the main toolbar. Navigate to the GemStone **Sample Files** folder, and select 3 files in the list: **BCell\_BM\_Tube\_001.fcs**, **BCell\_BM\_Tube\_002.fcs**, and **BCell\_BM\_Tube\_003.fcs**. Click **Open**. The files are added to the File Database and the first file is read into GemStone.

#### Model Building Tip: Renaming Parameters

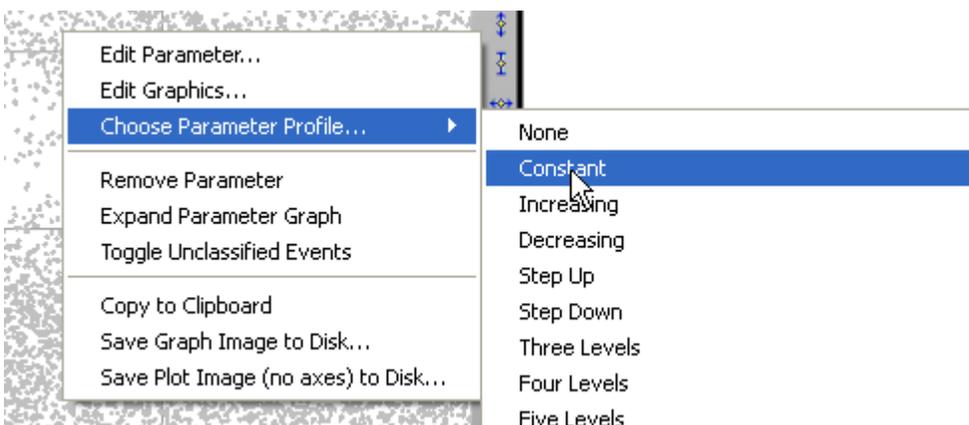
The parameter names for the files in this tutorial may not be especially helpful the first time you read them into GemStone. This can be easily corrected by selecting the Parameter Database command in the Edit menu. In this dialog, you can rename parameters (and do lots of other things, too). For additional details, see [Edit Parameter Database](#).

### Setting up Selection Parameters

We will build the model by first setting up the selection parameters: CD19 and SSC. Click the **Choose Parameter** button in the Workspace toolbar, and select **CD19** in the list.

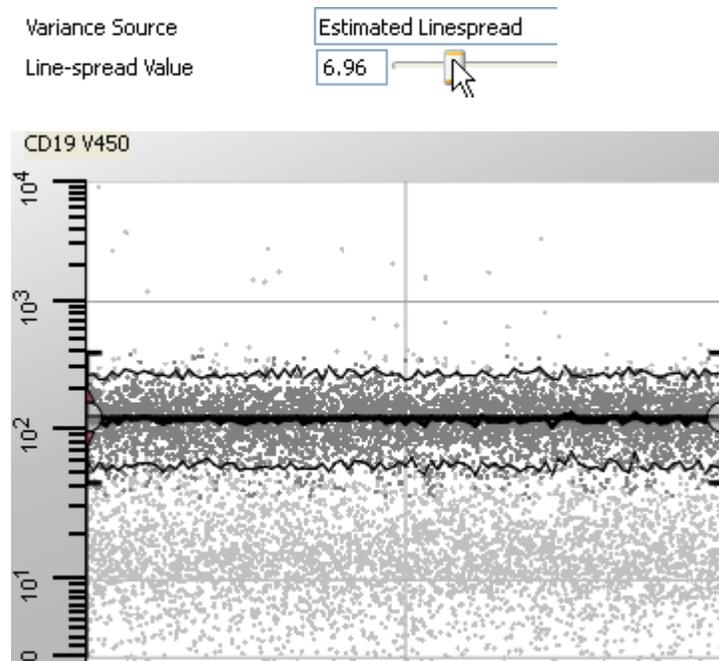


Let's use the context menu in the CD19 parameter plot to select a Constant profile. Right-click inside the **CD19** plot and from the menu, select **Choose Parameter Profile**. In the popup menu, select **Constant**.



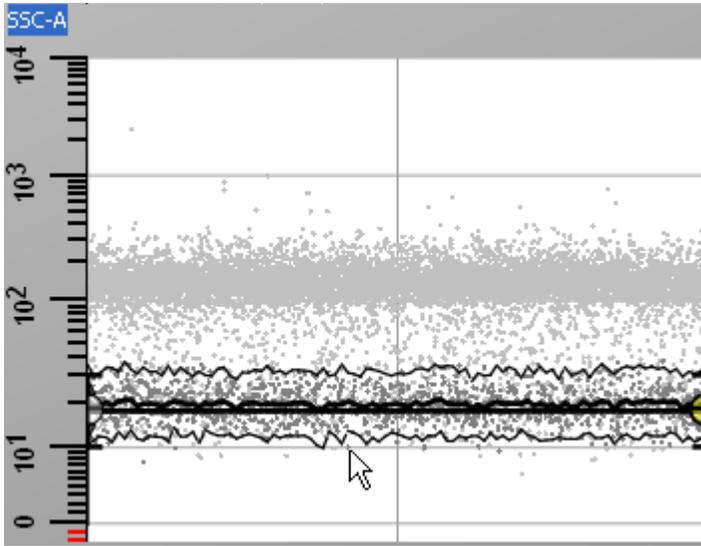
With the markers in this panel, we will *not* be using the three auto-adjustment buttons as we have in other tutorials. Those routines are helpful in many cases, but they are not smart enough to handle the data in these files. We will size and position the definition points manually, and then use the Estimate X-Position button to fine-tune the placement.

Position the profile on the band of high-intensity **CD19** cells. Use the Line Spread slider in the Parameter Profile panel to increase the line spread to about 7.0, and then click the **Classify Data** button on the main toolbar to analyze the events.



Next, we will repeat this process for **SSC** and identify cells with low SSC values.

Click the **Choose Parameter** button in the Workspace toolbar, and select **SSC** in the list. Right-click inside the **SSC** plot and from the menu, select **Choose Parameter Profile**. In the popup menu, select **Constant**. Position the profile on the band of low-intensity **SSC** cells. Click the **Classify Data** button to analyze the events.

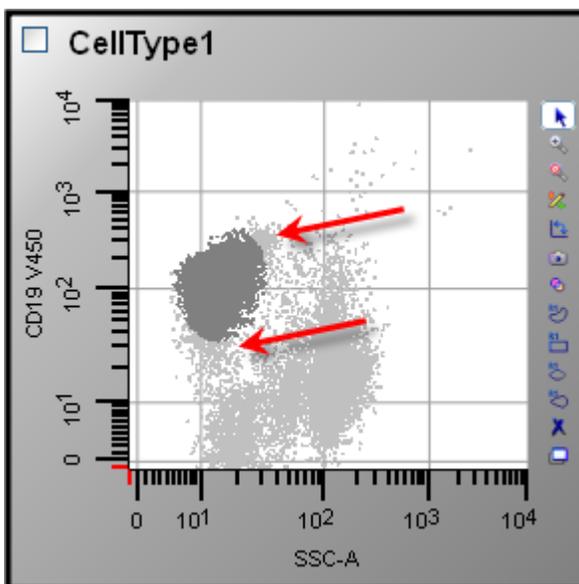


### Verifying with a 2P Plot

Now before we go on, let's see if we have really captured the cells of interest by using a conventional 2P dot plot display of SSC vs CD19. Click the **Create 2P** button in the Workspace toolbar.



Find an empty space on the Cell Type Canvas, click and drag to define a 2P plot. Select SSC on the X-axis and CD19 on the Y-axis.



If we look carefully at the plot, we can see that we have cut off some of the B-cells with low CD19 and some with higher SSC. At this stage in the model-building process, it is very important **not** to eliminate cells that may be of interest. Since we are about to enrich for the cells we have selected, it is better to be a little generous on the edges at this step. So, we need to increase the line-spread settings for both CD19 and SSC before going on.

Click the **CD19** parameter profile plot to activate it, and then increase the line-spread setting to approximately 8.40. Increase the line-spread for **SSC** slightly. Click the **Classify Data** button (or use the short-cut, Ctrl-A on the PC or Command-A on the Mac). You should now see more dark gray events in the 2P plot.

Let's enrich for B-cells. Click the **Enrich** button on the main toolbar.



### Defining the Progression

Now we are ready to really define the progression. We will analyze the common parameters from the simplest to the hardest: **CD34**, **CD20**, **CD10**, and **CD45**.

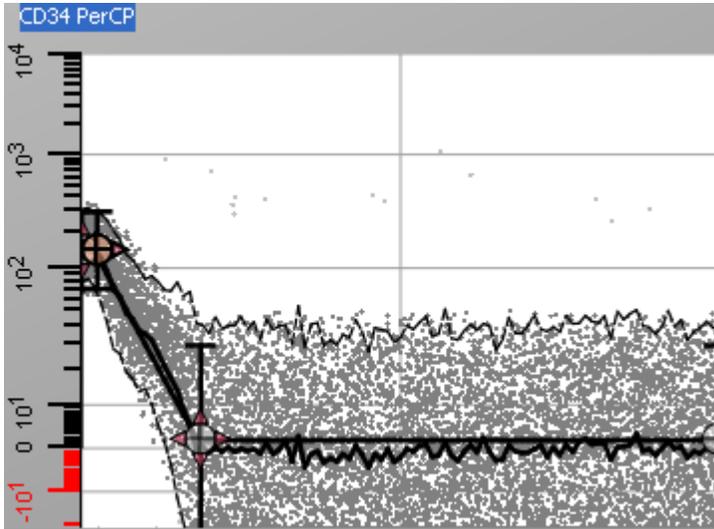
Click the **Choose Parameter** button in the Workspace toolbar, and select **CD34** in the list. The CD34-negative population is very broad and dim, and the positives at approximately 150 on the Y-axis. We will use a **Step-Down** profile for it. Right-click inside the **CD34** plot and from the menu, select **Choose Parameter Profile**. Choose the **Step Down** profile in the list.

Position the upper step at ~ 150 and the lower step at ~0 on the Y-axis. Next, change the **Variance Source** in the Parameter Profile panel to **Manual Point Entry**. This allows us to adjust the line-spread for the upper and lower steps separately.

Click the first point in the lower step and adjust the **Line-spread Value** to 10 or 11. Then, click **Estimate X-Positions** to activate the profile and allow GemStone to determine the best location horizontally.



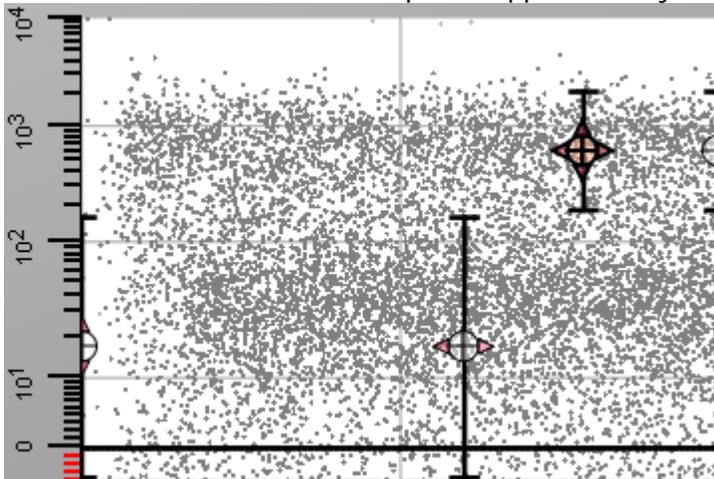
**Estimate X-Positions**



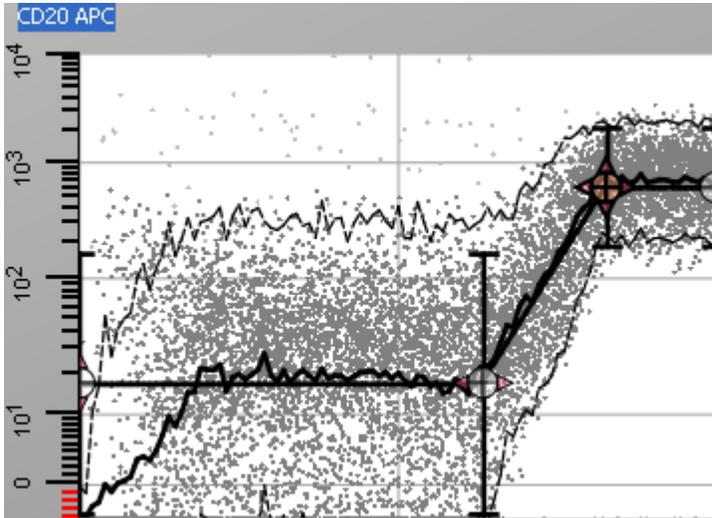
**Model Building Tip: Tweaking the Profile**  
When you are manually adjusting definition points and not using the 3 adjuster buttons, you can tweak the positions of the points and re-analyze to find optimal positions and line-spread settings. Try moving points up, down, and sideways just a little and reanalyzing. Then, use the **Estimate X-Positions** button to fine-tune it.

Let's move on to **CD20**. Click the **Choose Parameter** button in the Workspace toolbar, and select **CD20** in the list. We want to consider everything less than ~150 as negative, and the events centered at ~800 as positive. Right-click inside the **CD20** plot and from the menu, select **Choose Parameter Profile**. Choose the **Step Up** profile in the list.

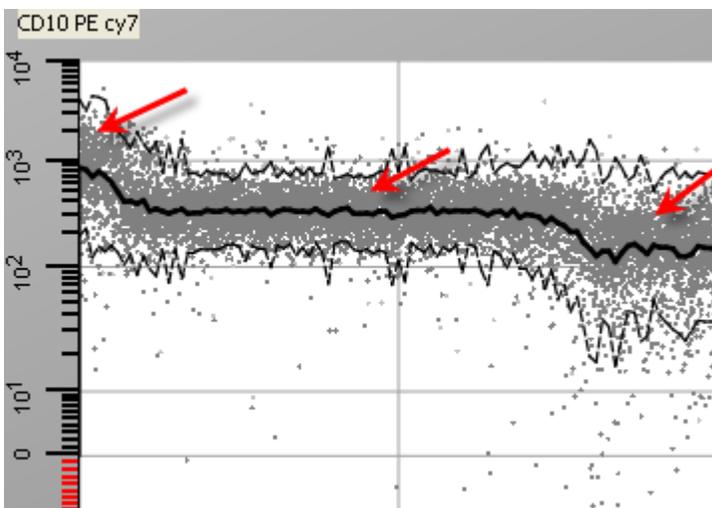
Position and size the definition points approximately as shown in the picture below.



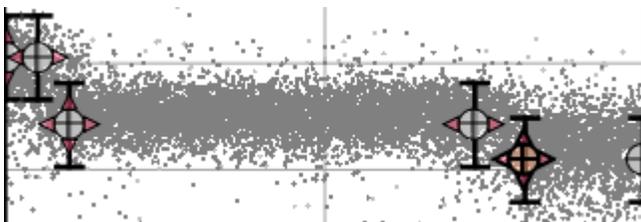
Trigger the **Classify Data** command from the menu, from the toolbar, or using the shortcut keys. Then, click the **Estimate X-Positions** button to allow GemStone to tweak the horizontal placement.



Click the **Choose Parameter** button in the Workspace toolbar, and select **CD10** in the list. With CD34 and CD20 defined, the expression characteristics of **CD10** become more obvious. We can easily see 3 levels of expression that we will model.



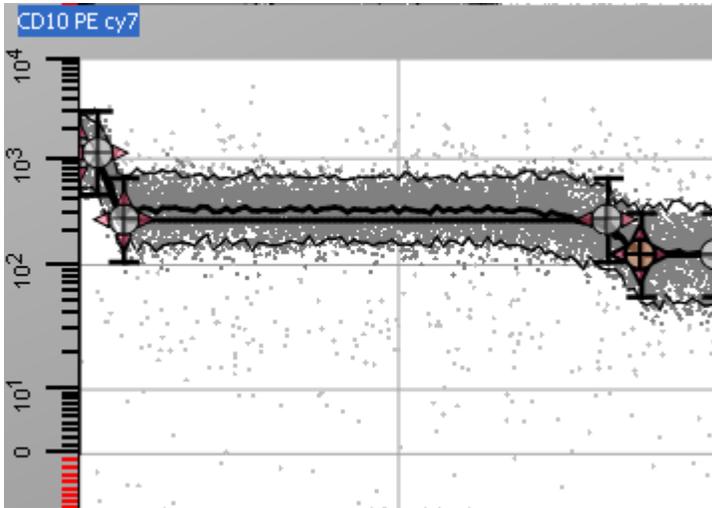
Right-click inside the **CD10** plot and from the menu, select **Choose Parameter Profile**. Choose the **Three Levels** profile in the list. Position the levels to match the transitions that appear in the distribution.



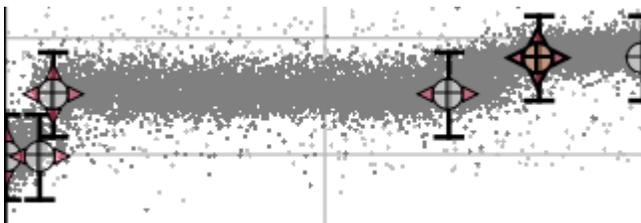
**Model Building Tip: 3-Levels or 4-Levels?**

We know that when B-cells become mature, they lose CD10. In this example, there are actually a few cells that have become fully mature and show virtually no CD10 expression. We could model this with a Four Levels profile, and set the last level very low at the end of the progression to capture those mature B-cells. For this tutorial, we'll keep it simple with Three Levels.

Click the **Estimate X-Positions** button to allow GemStone to tweak the CD10 definition points.



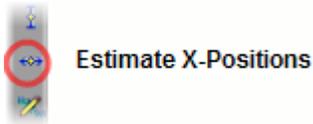
Now let's add **CD45** to the model. This marker starts low on early B-cells and elevates twice in the progression. Click the **Choose Parameter** button in the Workspace toolbar, and select **CD45** in the list. Right-click inside the **CD45** plot and from the menu, select **Choose Parameter Profile**. Choose the **Three Levels** profile in the list. For the initial placement of the definition points, use the graphic below as a guide.



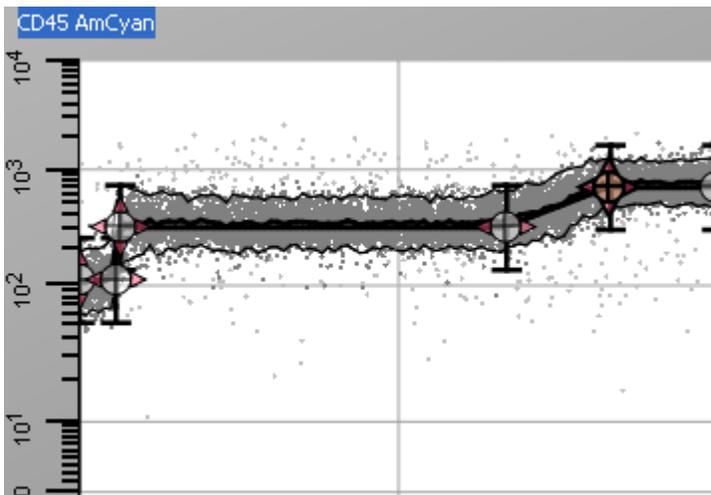
**Model Building Tip: Positioning Definition Points**

The horizontal spacing of Definition Points is based on the number of events that the points relate to. If there are many events that relate to a pair of points, there is more space between them on the X axis. If there are few events between the pair of points, the points are close together on the X axis.

Now use the **Estimate X-Positions** tool to fine-tune the positions and classify the data.



The adjusted profile will look similar to the image shown below.



At this point, our model is defined. We have modeled 6 parameters common to all tubes. Two parameters (CD19 and SSC) were used to select the B-cells. The other four parameters (CD34, CD20, CD10, and CD45) were used to define a scaffold for the progression.

We can now use **Auto Analysis** to optimize the fit. Click the **Auto Analysis** button.

### Adding the Remaining Parameters from Tube 1

We can now add the remaining parameters in tube 1. However, we will not need to do any modeling for these markers because the model is already well-defined. This is one of the benefits to using GemStone for the analysis: with a well-defined model, you can explore how other markers - even those with unknown expression characteristics.

Add **CD58** and **CD38** to the model.

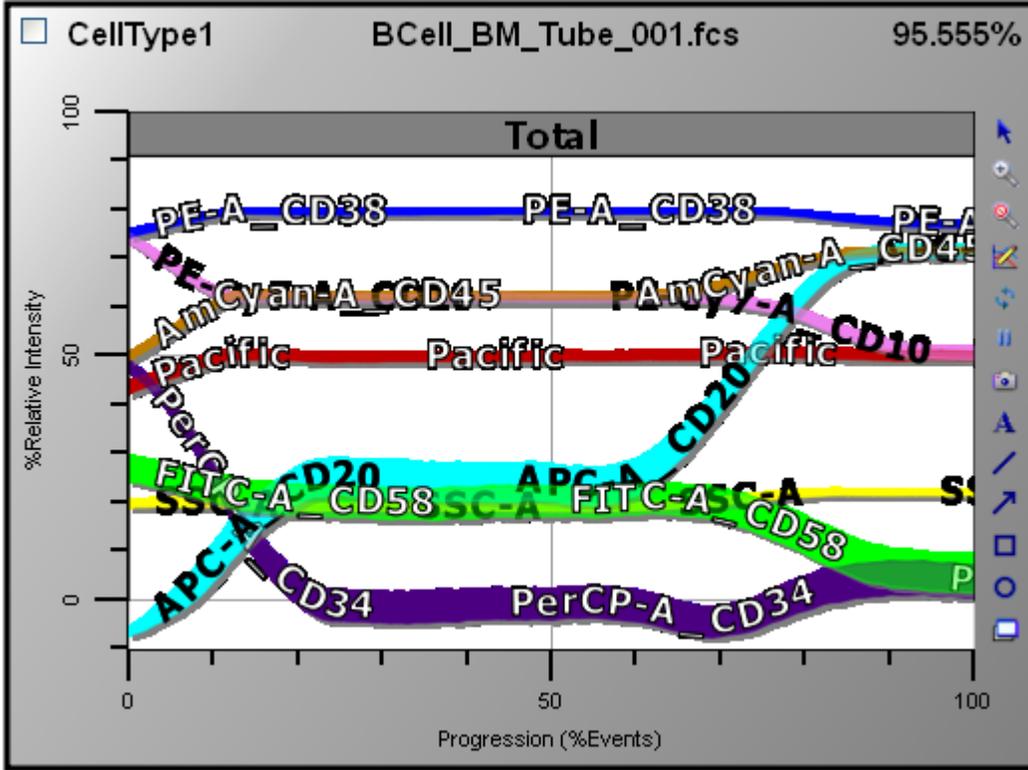
### Creating a Parameter Overlay Plot

Let's add a Parameter Overlay plot to the model, and then save the document.

Click the **Parameter Overlay** tool on the Workspace toolbar, hold the Shift key down and click the mouse on the canvas. The plot will be displayed.

Because the CD34 negatives are so dim, much of the CD34 ribbon in the plot is off scale. To adjust for this, we need to change the Y-axis scaling for the plot.

Double-click the axis ticks for the Y-axis to display the **Edit Properties for Y-Axis Area** dialog. Change the Axis Scaling Mode to **Fixed Scale**, and set the Axis Min Scale to **-10**. Click **OK**.



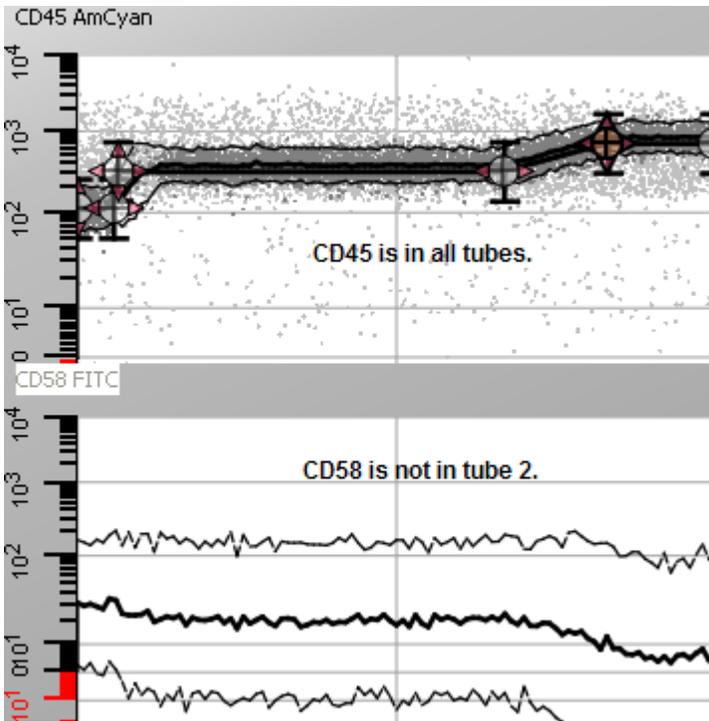
On the main toolbar, click the **Save Document** button, and save the model document as "BCell-Multi-Tube.gs".

### Analyzing Tube 2

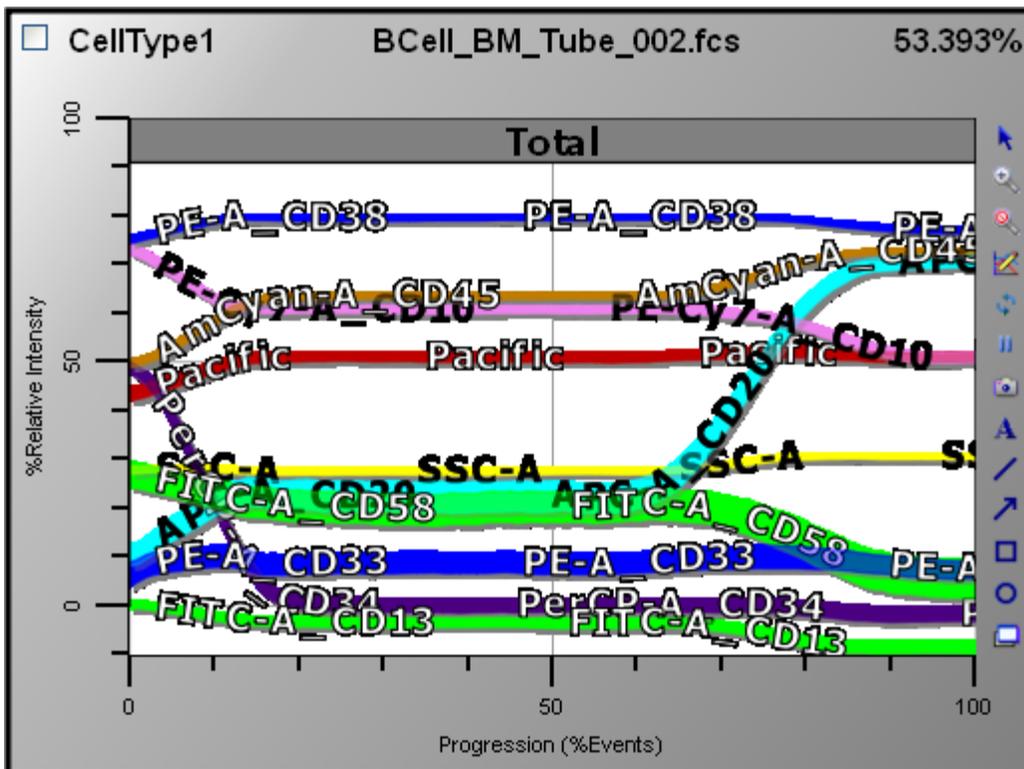
We are ready to open the next file in the panel. Click the **Next File** button on the batch toolbar to read it into the model.



The first things you will notice when the second file is loaded are that some of the plots now show no dots and that the Parameter Overlay plot did not appear to change. Here is why. The parameters that are common between tube 1 and 2 have been reanalyzed. The parameters that were in tube 1 and *not* in tube 2 still show the means and confidence limits for the analysis - but no dots. Those parameters can still be plotted on the Parameter Overlay plot based on the analysis from tube 1.



Let's add the parameters from tube 2 all at once. Click the **Choose Parameter** button in the Workspace toolbar, and select **Choose** in the list. The **Edit Parameters** dialog is displayed. Add **CD13** and **CD33** by double-clicking each one in the list on the left. Click **OK** to close the dialog.



We do not need to add Parameter Profiles for these markers because the model is already well defined. We can see the expected transitions for the markers, which come from the parts of the model that we *did* define. If the markers had characteristics that were not shown at this point, we probably would try to model them here.

**Model Building Tip: Watch the RCS when opening a new data file.**

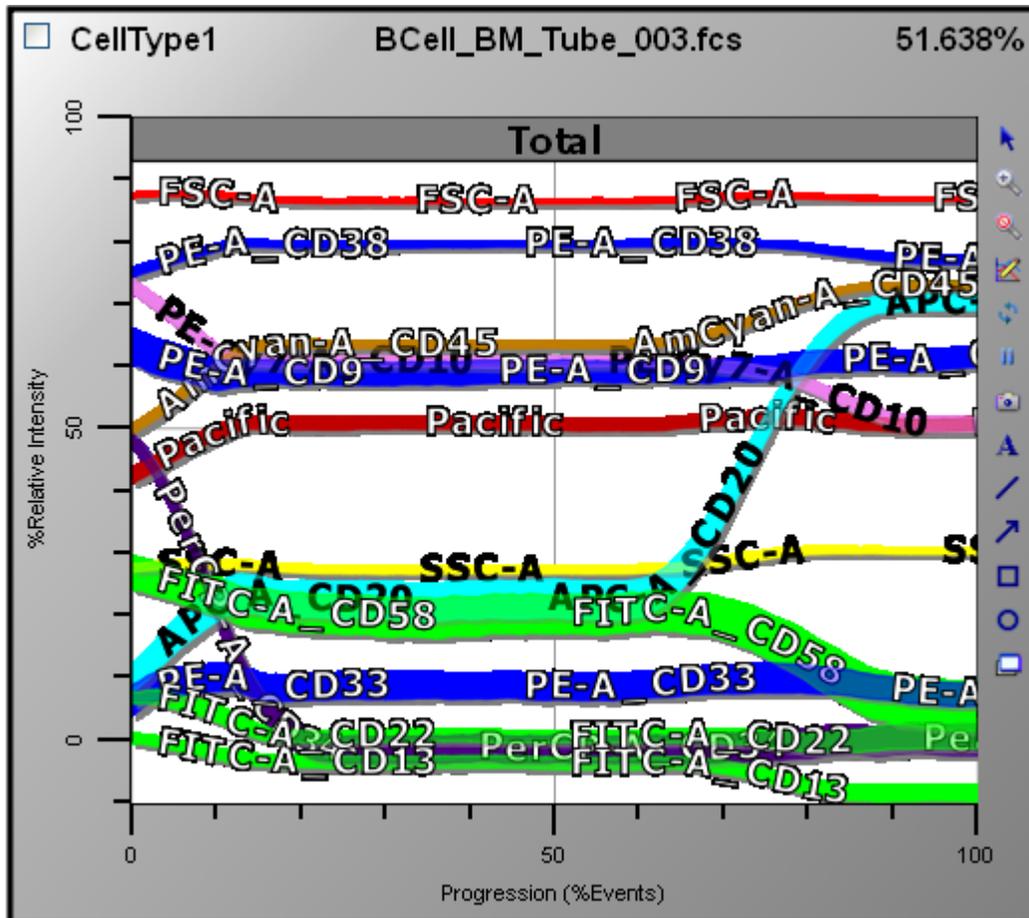
The RCS (Reduced Chi-Square) is a measurement of how well the model matches the data. With a multi-tube panel, it can also be used as a quality control tool. If the RCS changes much when a new tube is read into the model, there is a good chance that one of the common parameters has changed from the previous tube. If this occurs, you should stop and determine what has changed before moving on. GemStone has tools that can normalize parameter intensities from one file to the next.

Let's move on to tube 3.

### **Adding Tube 3**

Click the **Next File** button on the batch toolbar to read it into the model. Using any technique you prefer, add **CD22**, **CD9**, and **FSC-A** to the model.

Here again, if we needed to define any of these additional parameters, we could. It is not necessary in this case.



Our parameter overlay now shows us the correlated expression for all of the markers in the panel.

We would typically want to create some analysis zones for the model. That is left to the reader as an additional exercise, since it has been covered in earlier tutorials.

Let's save our model, now that it represents the entire panel of tubes. Click the **Save Document** button on the main toolbar. Type a name for the model and click Save.

### Summary

One of the most important aspects about multi-tube panels is the selection of *common parameters* that will be included in all tubes. These parameters are used by GemStone to create a backbone for the analysis, allowing correlations of markers in separate tubes to be explored as if they were in the same tube.

When creating a model for a panel of files, the common parameters are heavily relied upon. Markers that are found in only one tube are modeled when they provide key information to the progression. Once the model is fully defined with common parameters, GemStone can display and correlate parameters from different tubes.

**See also:**

[Tutorials and How To Section](#)  
[Parameter Profile Descriptions](#)

## Model Building 201

This tutorial introduces several advanced topics in model building. It assumes that you have worked through the Model Building 101, 102, 103, and 104 tutorials.

### Getting Started

In this section, you will learn some of the subtleties of parameter profiles. The most important controls are the *relations* that can exist between control points. These relations create dependencies to our models so that they can operate reliably with Auto Analysis.

To get started, just launch GemStone. We do not need to load a GemStone document or data file for this tutorial.

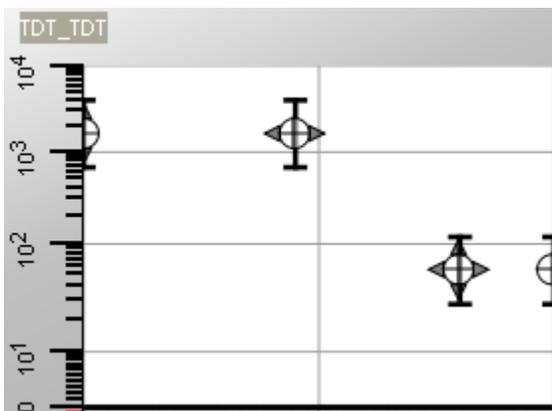
Click the **Choose Parameter** button in the **Workspace toolbar**, and then click **Choose** to display the Edit Parameters dialog.



Find and select **TDT** and **CD10** in the list. If these parameters are not listed, just choose two log parameters. Click **Add** and then **OK**.

### Control Points

Right click the **TDT** plot, and select **Choose Parameter Profile** from the context menu. Choose the **Step Down** profile for TDT.



Let's look carefully at the first Control Point on the left. There are arrow tips pointing up and down. This indicates that this control point can fit data up or down. Now, look at the second Control Point. It has left and right arrow tips that indicate that it can fit data to the right or left. The third Control Point can fit data in any direction. Finally, notice that the last Control

Point has no arrow tips, indicating that it does not fit data in any direction. Move each of these points to get a feeling for how they interact with each other.

**Model Building Tip: Arrows Indicate the Direction of Movement**

The arrow tips on a Control Point indicate the directions that the point can be moved by the program when it is fitting the model to the data. In many cases, you can manually move a point in other directions because it has a relation with another point.

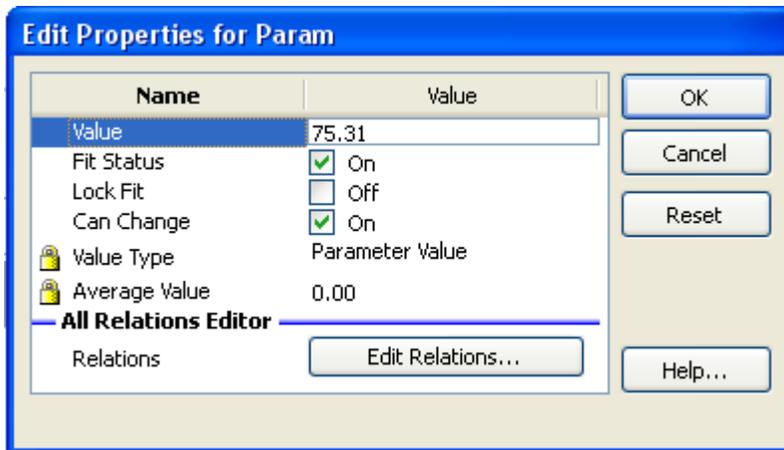
These attributes of the Control Point are editable, and for some modeling situations you have to make changes to achieve the behavior your model requires. Let's take a look at some of these important properties.

Double-click the first Control Point to display the editor.



We can edit characteristics related to the parameter intensity, state position, and standard deviation. **Param** controls properties related to the way the Control Point adjust on the parameter intensity axis. **State** controls the position on the progression, or state axis. **SD** relates to the line spread on the parameter intensity axis. Let's drill deeper.

With **Param** selected in the list, click the **Properties** button in the Param group box *at the bottom of the dialog*. This will display the **Param** property editor.



The **Value** property shows the current position of the point on the parameter intensity axis. GemStone's dynamic range for parameter values is a normalized scale of 0.0 to 100.0. We can directly edit the value here, but in most cases it's easier to drag the point with the mouse or use one of the left or right arrows on the keyboard.

The next property is the **Fit Status** property. If it is checked, the point's parameter value can be adjusted by GemStone's fitting routines. This property determines whether or not up and down arrow tips are displayed on the graphical control point.

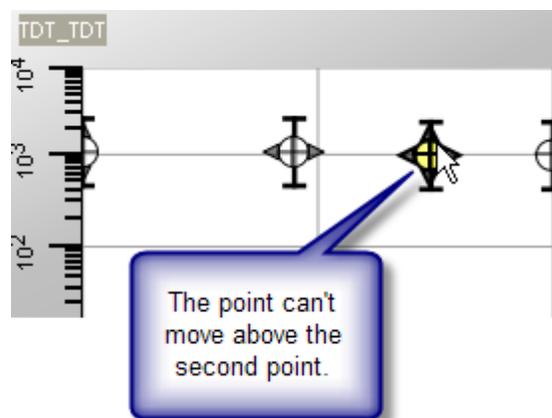
If the **Can Change** property is unchecked, you will not be able to move this CDP at all. Let's see how this affects this and the other points in the parameter profile.

Uncheck the **Can Change** property and click on **OK** twice.

Now, try to move the first Control Point. You can't.

Try to move the second point up or down. You can't move it either.

Now try moving the third Control Point up to the top of the plot. When it is almost as high as the second point, you can't move it any farther.



Why? The reason is that there is a *relation* between the third and second point. A *relation* defines a rule between two Control Points that restricts the movement in some way. In this case, there is a **Less Than** relation between the third point and the second. The third point must have a parameter intensity value that is less than the second point, and the system will not allow you to violate that relation. When you move the third point up, the system tries to

move the second point up. However, when we changed the **Can Change** property, we told it that it could not move. Relations can be quite intricate and ultimately allow us to create models with some amazing properties.

**Model Building Tip: Relations Can Restrict Movement**

A relation is a rule defined between two Control Points that restricts the movement in some way. Relations can be based on parameter intensity or position along the progression (state) axis. They can affect points in the same profile as well as points in other profiles.

Let's change our property back to what it was.

Double click on the first Control Point, select **Param** in the list, and click the **Properties** button at the bottom of the dialog.

Enable the **Can Change** property, and then click **OK** twice. The points can once again move as they had before.

Let's review what we have discovered about these Control Points. Each Control Point is composed of three parts: **Param**, **State**, and **SD**. **Param** controls the up and down movement of the Control Point, **State** controls the left and right movement, and the **SD** controls the line spread. The Fit Status property for Param and State allow or disallow the estimation and minimization routines to change these values.

If you want a Control Point to be set to a value and not change, uncheck the **Can Change** property.

These properties give you some sophisticated control over how Auto Analysis does its job. You can tell it what values to manipulate and which ones to leave alone.

**Model Building Tip: Use "Fit Status" and "Can Change"**

When the Fit Status property is checked, the automatic adjustment routines in GemStone have permission to adjust the value of the Control Point. Uncheck this property when you only want the user to manipulate the value. Uncheck the Can Change property when you do not want the user or the adjustment routines to change a value.

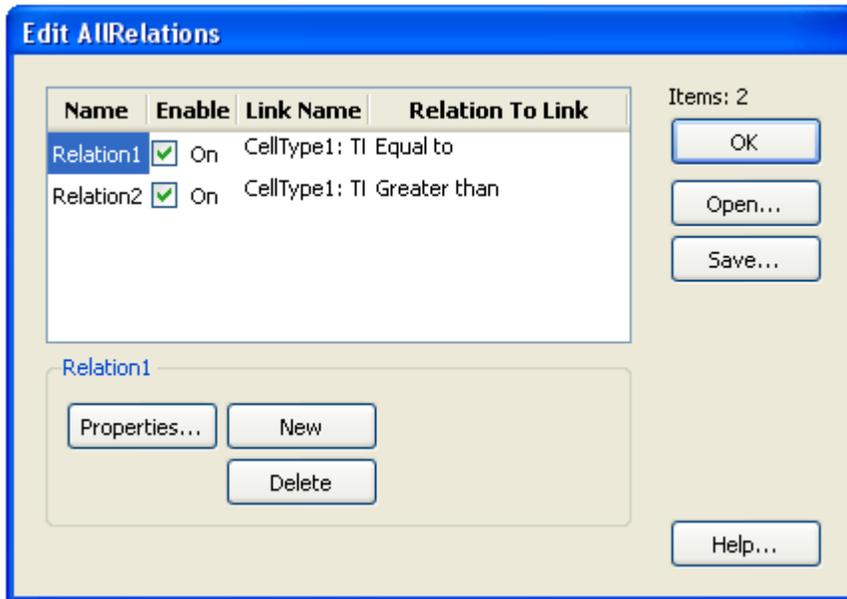
Let's now look at the relations in more detail.

**Relations**

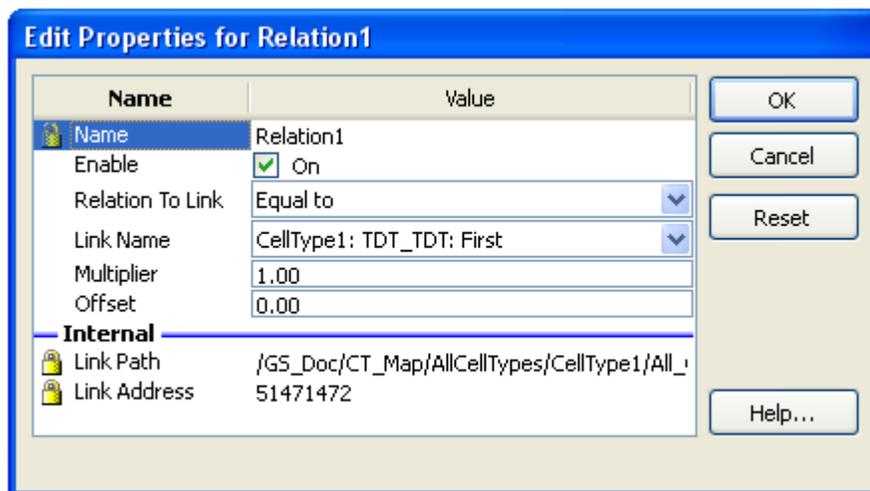
Slowly move the second Control Point down and watch what happens with the other points. Notice that the second point always moves vertically with the first. When the second point gets to the third point's vertical position, it pushes the third point down. As the third point

moves down, the fourth moves vertically with it. Let's see why the behavior of the Step Down profile works this way.

Double-click the second Control Point to display its editor. Now double click the **Param** entry to show its two, defined relations.

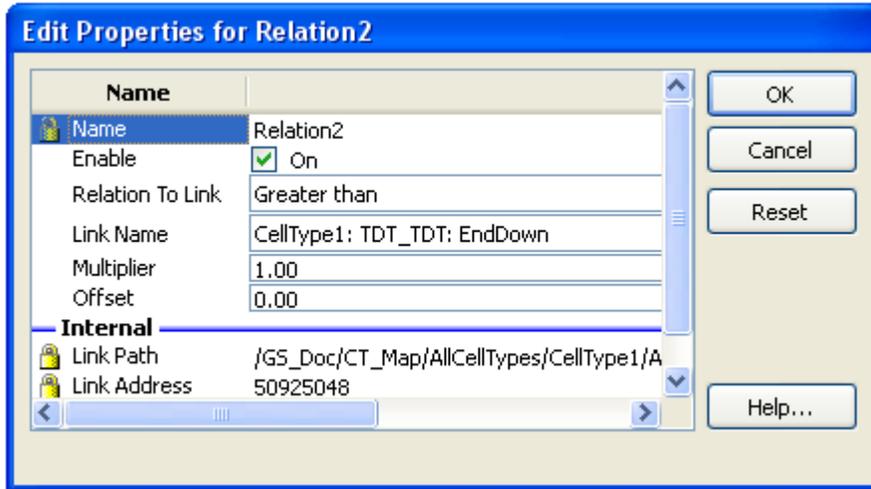


Double click **Relation1** to see its details.



Relation1 is enabled and related to **CellType1: TDT: First** with an **Equal to** relationship. This is why the first Control Point always accompanies the second one as we move it up and down.

Click **OK** to close the dialog, and then double click **Relation2** to see its properties.



The parameter intensity value for the second definition point has a **Greater than** relation with the third point, **CellType1: TDT: EndDown**. This relation is what forces the second point to be greater than the third point.

### Modifying a Relation

Suppose you wanted this point to remain greater than the second point by more than 20 units. Is it possible to create a relation with an offset of 20? Yes.

Type a value of **20** into the **Offset** property, and then click **OK** three times.

Now, move the second CDP down and watch what happens. As it approaches the third point, the third point starts moving down, too. If you move the third point up, you will see that the second point starts to move up. The two Control Points maintain a separation of more than 20 units (on a scale of 100).

The way to "read" the relation expression is represented by this equation:

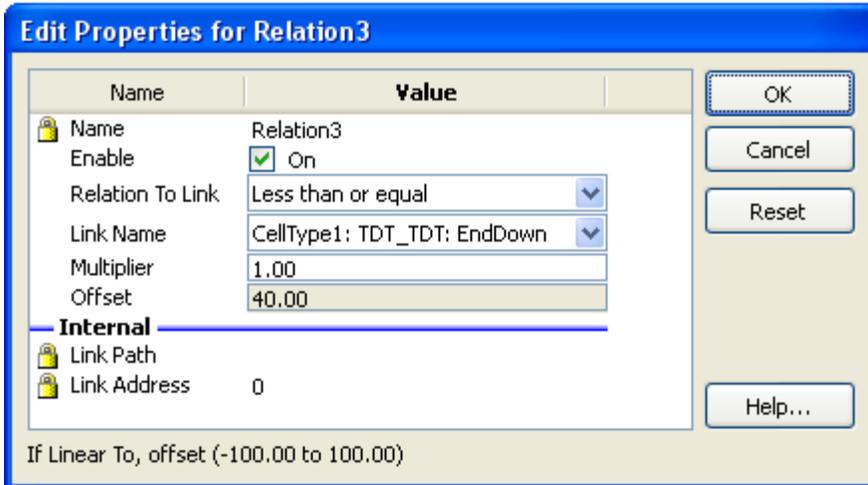
$$\text{BeginDown} > (\text{EndDown} + 20)$$

### Adding a Relation

Just to make sure you appreciate how sophisticated you can make these relations, let's augment what we have done so far. We have changed the **Greater than** relation between the second and third Control Point so that the second is always 20 units or more greater than the third. Suppose we also want the second point to be no more than 40 units away from the third. In order to do this, we will need to add an additional relation. Here is the expression:

$$\text{BeginDown} \leq (\text{EndDown} + 40)$$

Double click on the second point again to bring up the editor. Double click **Param** and click **New** to create a new relation and view its editor. Choose the **Less than or equal** relation. Then, choose **CellType1: TDT: EndDown** as the Link Name. Enter **40** for its offset.



Click **OK** three times to close all the editors. Now, move the second Control Point up and down to appreciate what we have done. We have established kind of window between these two points. The points can be no closer than 20 units, but no farther than 40 units from one another.

When we changed the second point's relations to the third, the system automatically made the reverse entries for the third point's relations. GemStone will always try to make these relations work bilaterally, which is normally what you want. If you need to have a unidirectional relationship, you can delete the relationships that are automatically added to the target point.

### Reading a Relation Expression

We can generalize the relation expression. Given:

**CDP\_a** is the Control Point we are editing

**relationship** is the relation to the other point, e.g. Greater than, Less than.

**CDP\_b** is the target Control Point in the Link Name field

**m** is the Multiplier value

**offset** is the Offset value

Then the general expression for the relation is:

**CDP\_a relationship (m \* CDP\_b) + offset**

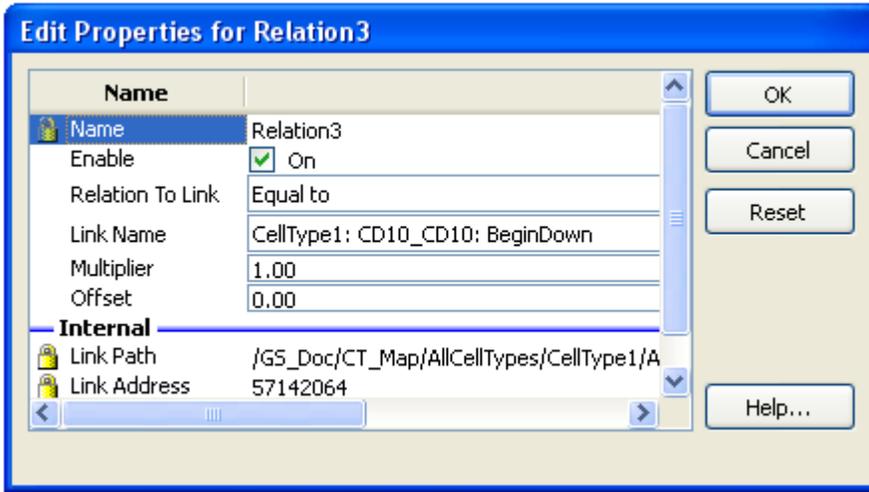
### Relations with Other Parameter Profiles

A Parameter profile can have sophisticated relations that constrain its shape and movement. You can also create relations with Control Points in any other Parameter Profile, even in other Cell Types. For example, suppose we wanted to create a model that coordinated the down regulation of TDT with the down regulation of CD10. Here is how you would do that.

Click on the **CD10** parameter plot to activate it. Then right click and select **Choose Parameter Profile**. Pick the **Step Down** parameter profile.

In the **TDT** plot, double click the second Control Point again. This time, double click the **State** item in the list and click **New**.

In the editor, choose **Equal to** for **Relation to Link**. Now click the **Link Name** and choose **Celltype1: CD10: BeginDown**. We are telling the GemStone that TDT and CD10 will begin the down transition at the same location on the progression (state) axis.



Click **OK** three times to close the editors.

Now move the second Control Point in the TDT profile back and forth and notice that the second point in CD10 moves back and forth as well. The two profiles are independent of one another in the vertical direction (the Param value).

### Saving Relations

How do you save all this information? The relations are stored in the model document when you use the [Save Document](#) command. However, you can also save your customized relations in your own Parameter Profile file. This allows you to reuse your work in another model document. To do this, right click the TDT parameter plot and choose **Edit Parameter** from the context menu. In the Edit Properties dialog, use the **Save** and **Open** buttons to store and read a customized Parameter Profile.

If you save these parameters to disk and then retrieve them, the system will automatically make all the hookups you have specified when they are both read into a model.

### Conclusions

Editing and creating relations is an advanced feature for GemStone. For some applications, it is important to add relations to ensure the system works reliably for complex analyses.

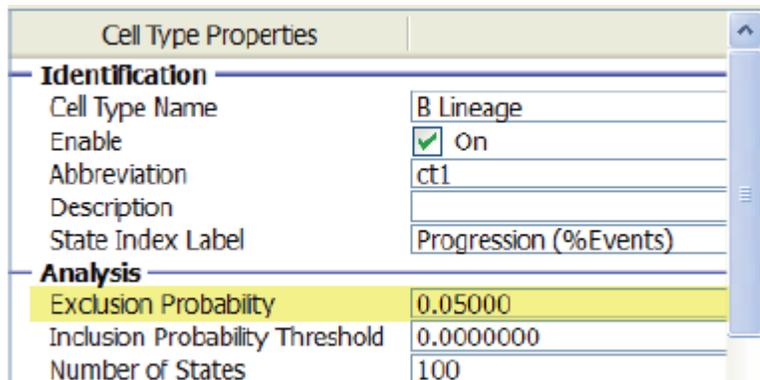
### See also:

[Tutorials](#)

## Heat Map and Animated Regions

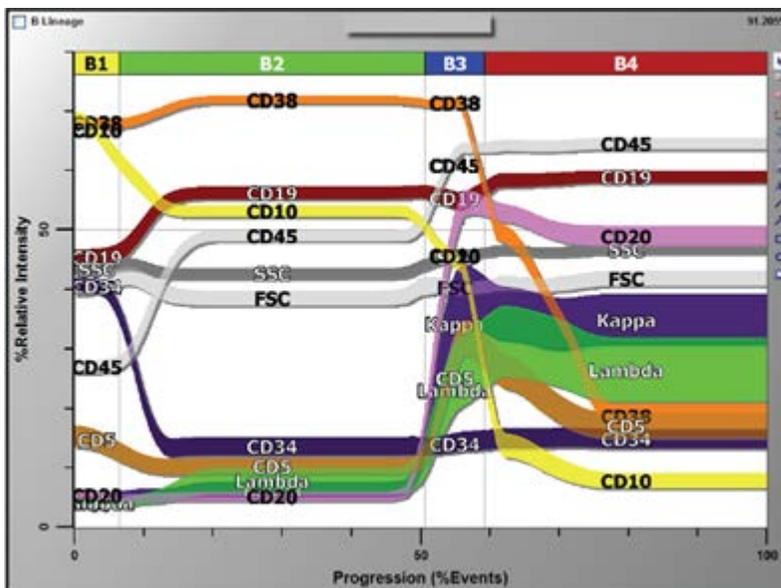
The Heat Map graphic allows the visualization and analysis of populations that statistically do not belong to the model. These aberrant populations appear in the heat map with unprecedented clarity and sensitivity. To use this new feature effectively, we need to examine how it works.

Normally when a GemStone Cell Type is created, the **Exclusion Probability** property is set to 0.05.



The property causes GemStone to exclude some fraction of outlier events from being classified by a Cell Type. If we did not have this option, unwanted events from nearby populations would become part of the Cell Type. For example, if we are modeling bone-marrow B-cells, we do not want myelo-monocytic events to contaminate our B-cell Cell Type. This property ultimately defines the extent of our Cell Type in any number of measurement dimensions.

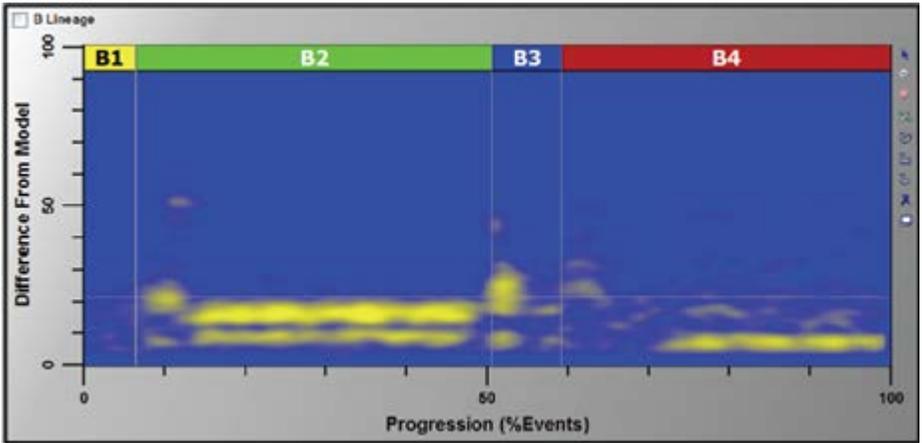
Let's suppose for this example that you have a progression similar to that shown below.



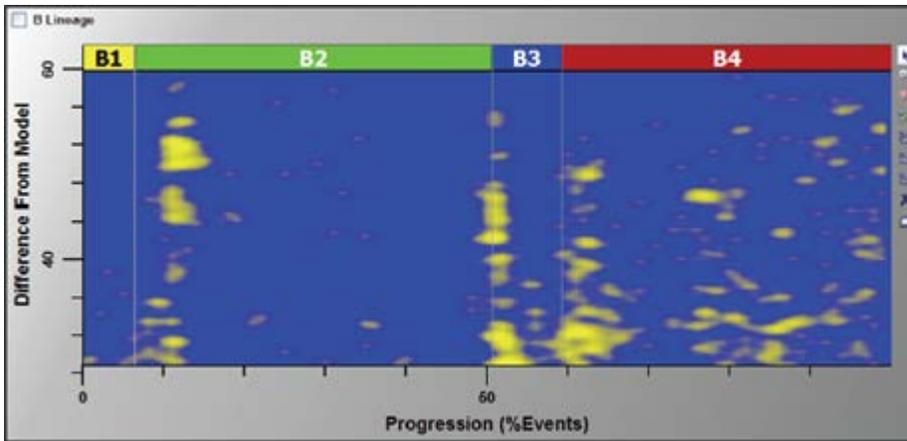
This model defines the B-cell progression probabilistically in all dimensions. If we had originally enriched our analysis packet with CD19 SSC B-cells, there could be events that for one reason or another do not belong to this normal progression. The Heat Map graphic is the best way to examine these events. Just click the Heat Map tool button and define the area where you want the graphic using your mouse.



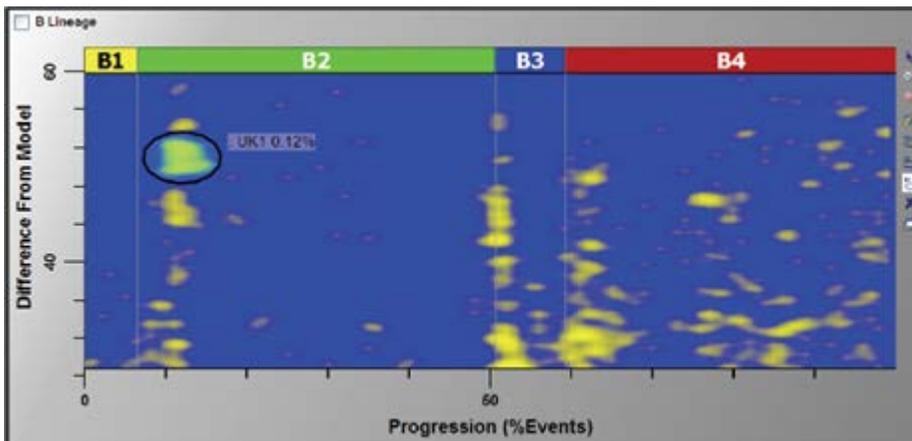
If you want to see all the events in the analysis packet, set your **Exclusion Probability** property to 0.00. This will cause every event to be classified by your Cell Type. Here is what the heat map looks like for this bone marrow B-cell example.



The X-axis represents our states in the B-cell progression. The Y-axis is the difference between the events and our model. The horizontal gray line is currently set to 0.01. Anything above this line has less than 1 out of 100 chance of being part of our cell-type. The higher the difference (higher on the Y-axis), the more likely the population is statistically different. We see quite a number of populations in this heat map. The long horizontal bands below the  $p=0.01$  line are presumably due to imperfections in the normal distributions of one or more parameters due to events piling up at the axis origin. The more interesting populations are further up on the Y-axis. If we zoom in on all the populations that are above the 0.01 p-value line, we can clearly see a number of small, outlier populations



What we really want to do here is to see other characteristics of these populations. For example, we might like to see where the cells fall on a CD38 vs. CD20 plot. We can do that very easily. Let's put a region around one of the populations.

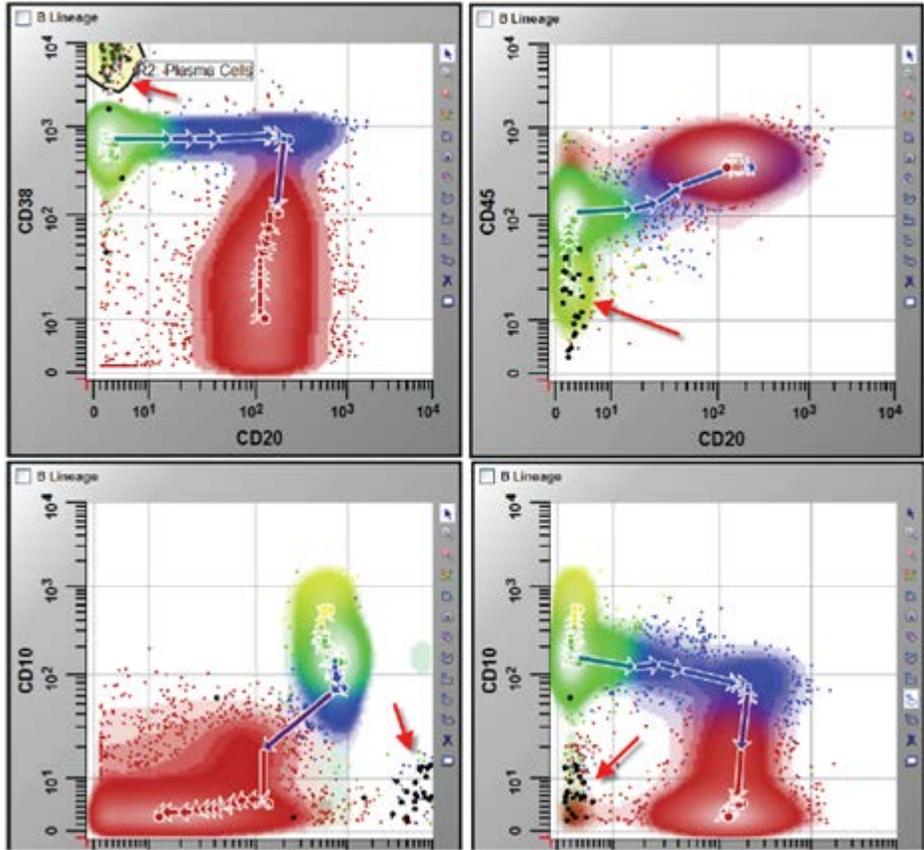


We will label this region as "Unknown 1" or "UK1". We can see that it comprises about 0.12% of our B-cells, or about 0.001% of the total events. To find out where these events are in all the other GemStone plots, we need to use another GemStone feature: Animated Regions.

### Animated Regions

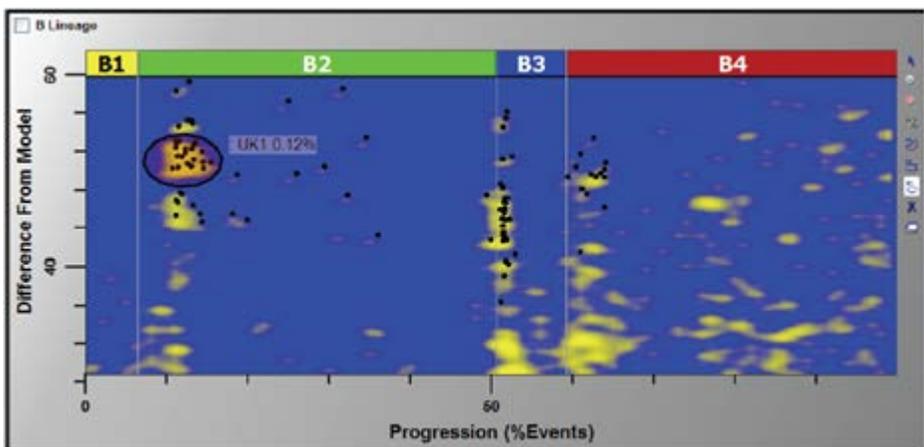
You can animate any region in GemStone. Simply right click the region and choose **Toggle Region Animation**. You can also double-click the region and check the **Animate Region** property.

What happens when you animate a region? Every graphic in GemStone shows where the region events are located by blinking those events with the region color. In dot plots, the animated dots are drawn larger than the other dots so it is easy to see the events.



The arrows in the figure above show where the UK1 events are in our example. These events seem to be plasma cells that have low CD45.

You can also use animated regions in reverse, to investigate where known populations fall on the heat map. For example, if we turn off animation for UK1 and turn on animation for R2: Plasma Cells region shown above, we can see the blinking dots in the heat map graphic:



In this example, we can see quite a few plasma cells in the heat map graphic, and they are not all in the same cluster on the heat map. One of the parameters that differentiates these cells is CD45. The plasma cells near B3 have higher CD45 than the ones near B1.

The Heat Map is a great exploratory tool. It can be used to find rare, malignant events in the marrow or to find new normal populations. If tumors have subtly different clones, the Heat Map will show them as separate spots. By using the Heat Map with animated regions together, we can understand characteristics in abnormal cells that were never possible before.

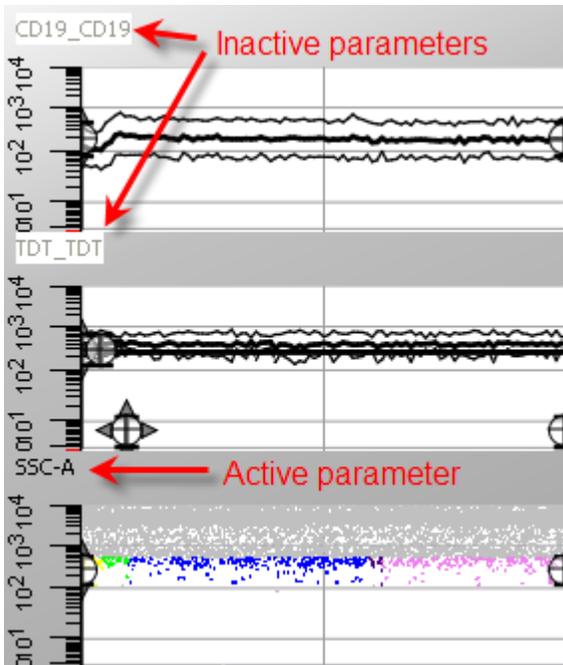
## How to Remap Parameters in a Model

### The Problem

Suppose that you have created a detailed model for one of your analysis applications, and you want to analyze a data file that was run on a different instrument. The new data file has the same markers that your model was built upon, but the parameter names are a little different. What happens when you read the data file into your model?

Nothing!

Some of the Parameter Profile plots show that the parameters are inactive. The label for the parameter is dimmed when the parameter is not found in the data file, and no events appear in the plot.



Does this mean that you can't analyze this data file with the model you worked so hard to create? Absolutely not. You just have some remapping to do, to tell GemStone which parameters in the file match up with the parameters in the model.

### Two Approaches

There are two ways to approach this: one that modifies the model to match the new parameters, and one that modifies GemStone's parameter database so that it can automatically remap the parameters for you. Modifying the model is quick and easy. The limitation with that approach is that you need to maintain a separate model for each of the naming variants that your data files use. Modifying the parameter database requires a little more effort, but it has the advantage of not requiring any changes to the model. We will look at both techniques in this *how to* section.

### Option 1: Remapping the Model Parameters

The first approach is to remap the parameters in the model to the appropriate parameters in the new data file. Here is how that is done.

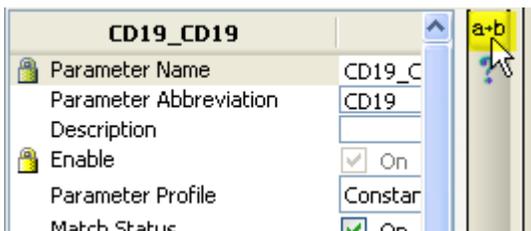
First, open the GemStone Model Document that you want to use. Then open the new data file that has different parameter names than those used in the model.

Now, show the Cell Type editors by clicking the Cell Type shrink tool.

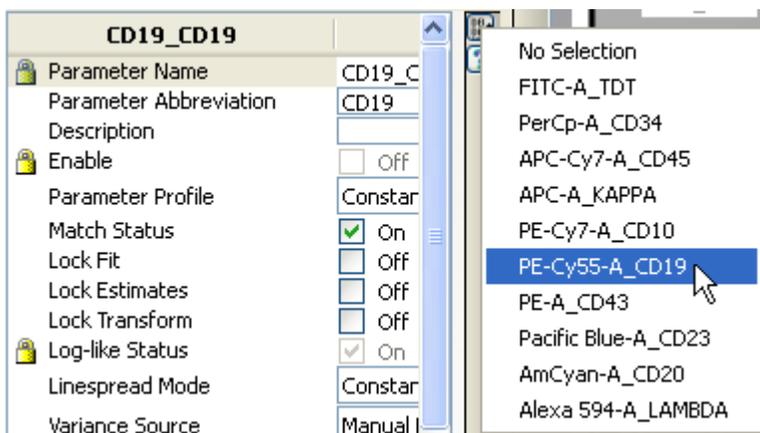


Next, click the label of the first Parameter Profile plot that you want to remap. For example, in the illustration above we would choose the **CD19\_CD19** plot label. This activates that plot and shows its properties in the Parameter Profile Properties panel.

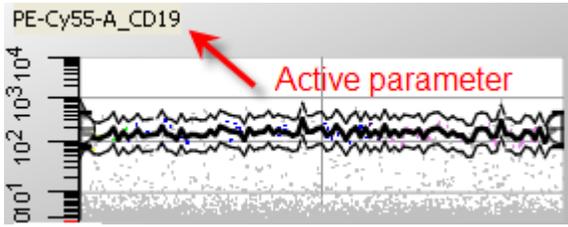
On the right edge of the Parameter Profile Properties editor is a toolbar. Click the button at the top of the toolbar, with the tooltip "Match to Database Parameter".



A list of parameters found in the data file appears. Choose the parameter in the list that you want to map to the model parameter. Using our example, we would choose **PE-Cy55-A\_CD19**.



As soon as we make the selection, GemStone fills the CD19 Parameter Profile with events from the data file and reactivates the parameter.



This process is repeated for each inactive parameter in the model. If the model has more than one Cell Type, you will need to repeat the process for each.

### **Saving the Changes to a New Model**

Once the model has been remapped for the new parameter names, it should be saved as a new model so that it can be used again with data files with this format for file names. The original model remains unchanged so that it works with the parameter names in the original data files.

### **Option 2: Using the Parameter Database**

The second approach is to make use of the Parameter Database to map the new file's parameters to existing parameters in the database that the model was designed for. Here is how that is done.

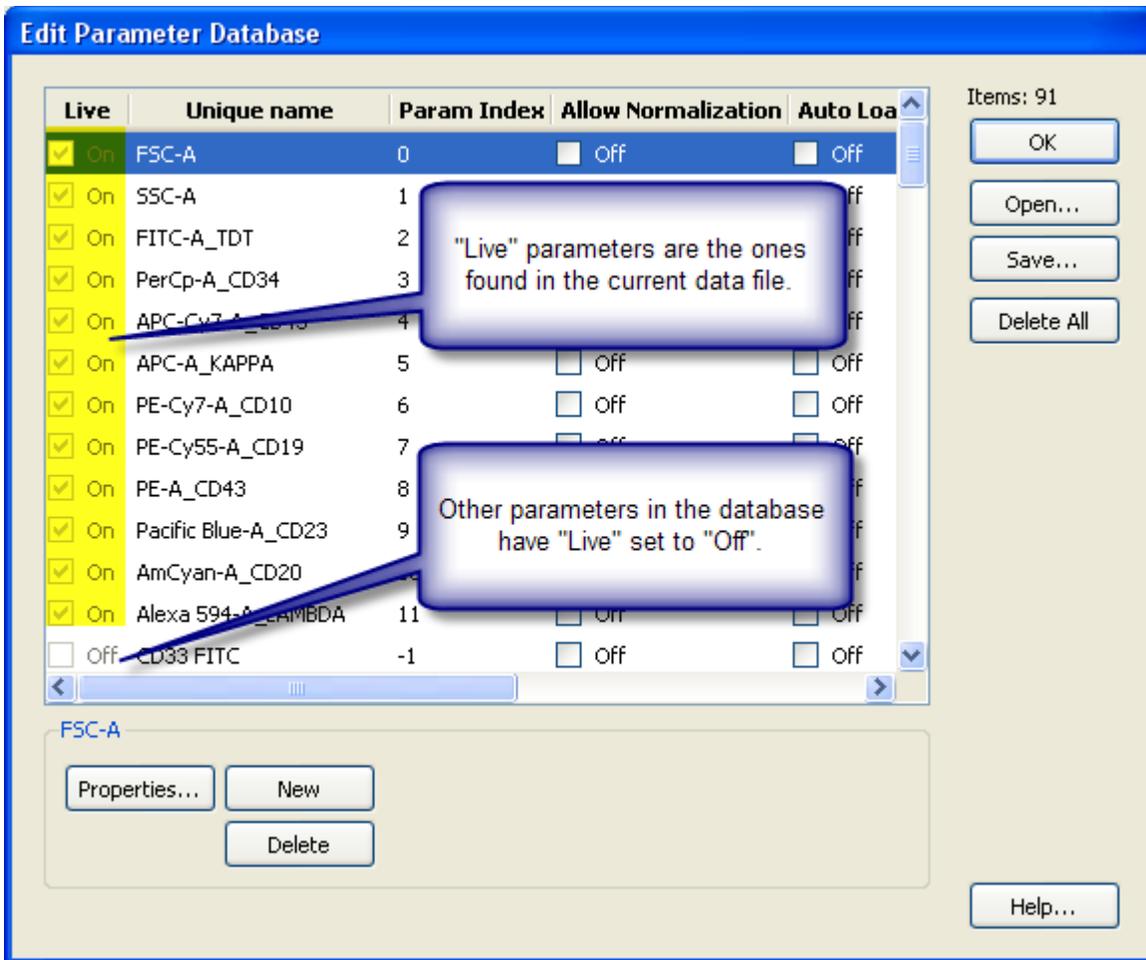
Once again, start by opening the GemStone Model Document that you want to use. Then open the new data file that has different parameter names than those used in the model.

As before, you will see some of the labels on the Parameter Profiles are dimmed, indicating that the parameters do not exist in the new data file.

### **Open the Parameter Database**

Click the **Parameter Database** button on the main toolbar. The **Edit Parameter Database** dialog is displayed.



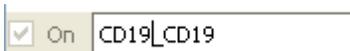


The "Live" column shows a value of "On" for the parameters that are found in the current FCS file. A value of "Off" means that the parameter is in the database, but not found in the current FCS file. Our job is to tell the database how to match the parameters in this new file with parameters that are already in the database.

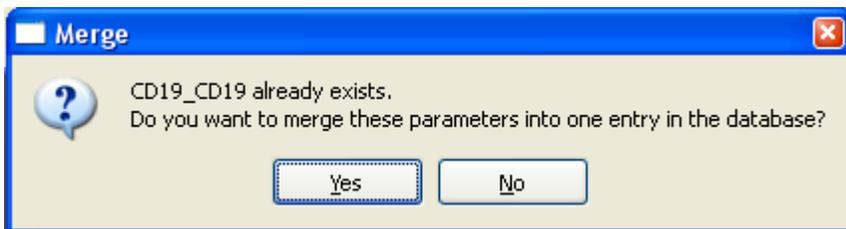
### Matching the Parameters

This technique requires us to edit the names of the "Live" parameters to match existing parameters. For example, in the dialog shown above, we see a parameter named "**PE-Cy55-A\_CD19**". Our model and the parameter database already know about a parameter named "**CD19\_CD19**". So, we need to edit the name of the Live parameter to match "**CD19\_CD19**".

Click on the label of a Live parameter that you want to edit. In our example, we will start with "**PE-Cy55-A\_CD19**". Edit the name to match the existing parameter, in this case "**CD19\_CD19**". Then, press the Tab key to finish the edit.



As soon as you press Tab, GemStone realizes that the name you typed matches a name it already has in the Parameter Database. It presents a "Merge" confirmation dialog, with a message similar to the one shown here:



Choosing "Yes" means that the program will merge the two parameter names into one entry in the database. It will treat the names as aliases for the same parameter, and our model will be able to work with either data file. Choosing "No" will allow us to choose a different name, in case we made the change by accident.

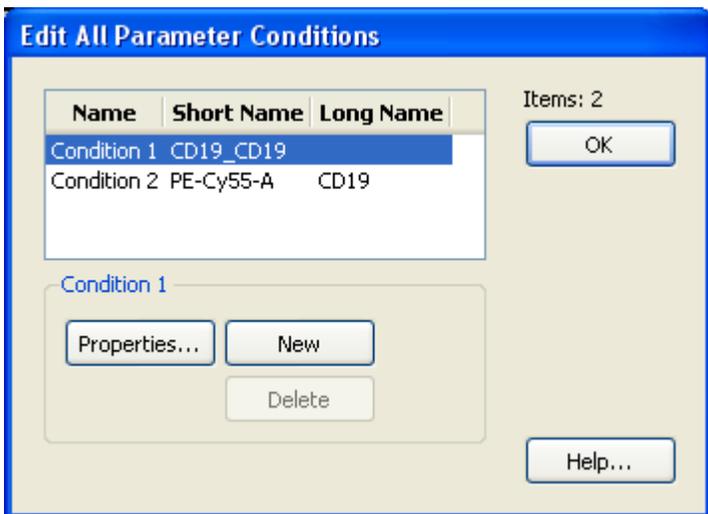
In this case, we want to choose "Yes". After we click "Yes", the entry we just edited is flagged to be deleted from the parameter database.



So if this entry is going to be deleted, how will the program keep track of the alias we just created? Let's take a look.

Scroll down in the list and you will eventually find another entry labeled "CD19\_CD19" that has "Live" set to "Off". Double-click this entry to display the **Edit Properties** dialog.

Click the **Edit All Conditions** button to display the **Edit All Parameter Conditions** dialog.



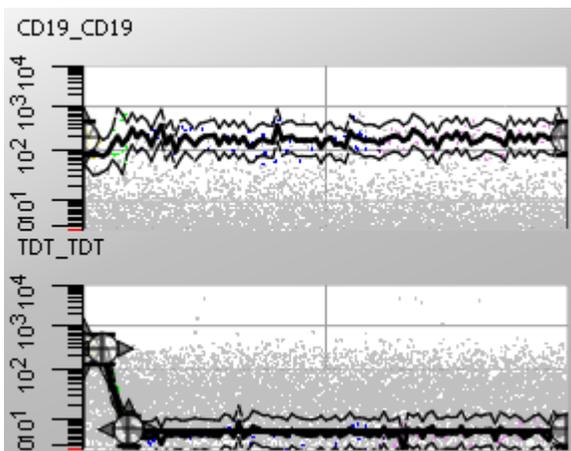
We see there are two entries in the condition list: one for "CD19\_CD19" as a short name, and another that has "PE-Cy55-A" for the short name and "CD19" for the long name. GemStone will consider parameters with these keyword values as equivalent. Note that you can manually create new conditions or remove existing conditions with this dialog. See [Edit Parameter Conditions](#) for details.

Click OK to close the dialog, and once again to close the Edit Properties dialog.

### Finishing Edits

Repeat this editing process for each of the parameters in the new data file until you have mapped each one to an existing entry in the parameter database. Take care to match the names with the names used by your model exactly.

When you finish your edits, click OK to close the Edit Parameter Database dialog. As soon as you close the dialog, the parameters in the model that had been inactive should activate and display events.



### Summary

We have looked at two methods for telling GemStone how to map parameters in a new data file to the parameters in an existing model. In the first case, we modified the model to use the parameters in the new file. This was very quick, but required us to create a second copy of the model to handle both of the file naming styles.

In the second approach, we used the Parameter Database to let GemStone map the parameters to the same database entry. We edited the new parameter names in the database to match existing parameters, and merged the parameters together. This created aliases for the parameter by defining "conditions". The advantage to this approach is that one model can now work with files that have different parameter names.

### See also:

[Edit Parameter Database](#)

## Batch Processing

In this tutorial, we will learn the basics of batch processing. Any time that you need to process a number of files using a repetitive pattern for the analysis, batch processing features in GemStone can save you time and provide consistency. The system is very flexible, allowing you to do different things to files in different groups. You can store your command scripts so that they can be reused the next time you need to repeat the process.

### Background of the Example

The example we will use in this tutorial is a model for a research project to monitor fetal red blood cells in cases of fetomaternal hemorrhage (FMH). The model was developed in conjunction with Dr. Bruce H. Davis of Trillium Diagnostics, LLC. See "Multicenter Clinical Experience With Flow Cytometric Method for Fetomaternal Hemorrhage Detection", *Cytometry* 50:285-290, 2002, published by Wiley-Liss, Inc for additional background.

The model uses three Cell Types, one for Adult RBCs, one for Adult F-Cells, and one for Fetal RBCs. We will set up batch processing for a set of files from different patients to quantify the relative percentages of each cell type.

### First Time Processing

#### Open the GemStone Model Document

In most cases, you will use one GemStone model document for a given batch process, but it is certainly possible to use a set of model documents, each applied to a different group of files. We will keep things simple for this tutorial.

Click the **Open Document** button on the main toolbar. Navigate to the folder in which GemStone was installed and find the **Model Examples** folder. Select "**FetalRBC.gs**" and click Open.

Activate the **Adult RBC** Cell Type by selecting its tab at the bottom of the Cell Type workspace.



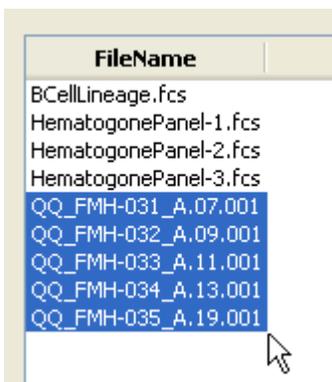
#### **Batch Processing Tip: Open the GemStone Model Document First**

It is a good idea to open the GemStone document that you are going to use for batch processing as your first step. This allows you to inspect the model before you begin batch processing with it.

### Select the Files to Analyze

Next, we select the FCS files that we want to analyze.

Click the **Select FCS Files** button on the main toolbar. Navigate to the folder in which GemStone was installed and find the **Sample Files** folder. Select the five "QQ" files in the list and click Open.

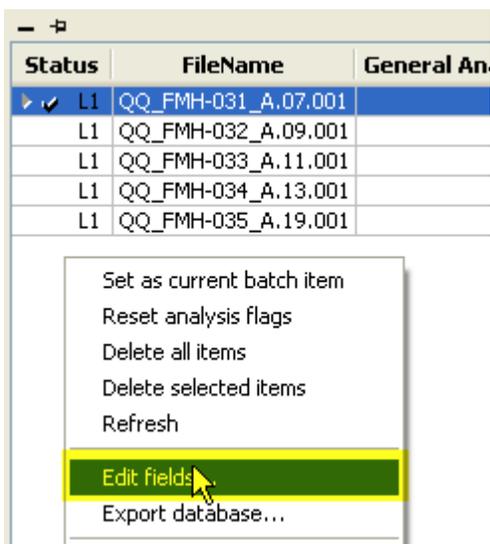


GemStone will add the files to the File Database panel and read the first file into our model.

### Edit the Fields for the Database

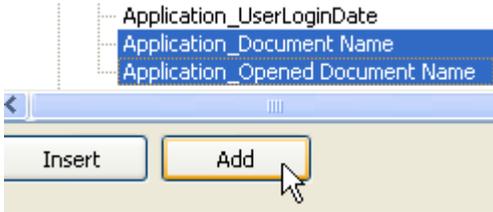
Since this is the first time we have done this analysis, we will need to set up our database fields so that we store the information we want. Once we select the fields, we will save them to a disk file so that we do not have to repeat the process next time.

Right click the File Database panel and choose **Edit Fields** from the menu.

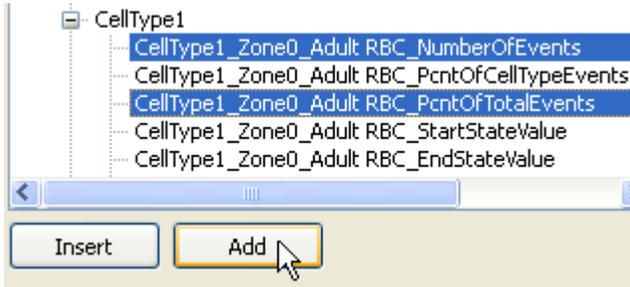


We want to define a new set of fields from scratch. In the Edit Columns dialog, click the **Delete All** button to remove any fields that might have been defined for another application. A few fields will be left in the list; these are fields that cannot be deleted. Now we are ready to select the new fields.

In the tree control on the left side of the dialog, you will see a section named **Application**. Within this section, find two fields: **Application\_Document Name** and **Application\_Opened Document Name**. Select these fields and click the **Add** button. These fields will store the name of the GemStone model document that we are using (FetalRBC.gs), as well as a GemStone document that we will save for each data file that we analyze.



Next, scroll down in the tree to the **CellType1** section. In this group, select **CellType1\_Zone0\_Adult RBC\_NumberOfEvents** and **CellType1\_Zone0\_Adult RBC\_PcntOfTotalEvents**. Click the **Add** button to add these fields to our database.



Scroll to **CellType2**, and select two fields: **CellType2\_Zone0\_Adult RBC\_NumberOfEvents** and **CellType2\_Zone0\_Adult RBC\_PcntOfTotalEvents**. Click the **Add** button.

Finally, scroll to **CellType3**, and select two fields: **CellType3\_Zone0\_Adult RBC\_NumberOfEvents** and **CellType3\_Zone0\_Adult RBC\_PcntOfTotalEvents**. Click the **Add** button.

Our database is set up to store the number of events and percentages for each of the cell types in the model. Let's save this database structure so that we can reuse it without having to find the results again in the tree.

Click the **Save** button on the right of the dialog. Navigate to a convenient location to store this file, type **FetalRBCFields** for the filename, and click **Save**.

**Batch Processing Tip: Save your Database Structure**  
Since you will probably do more than one kind of analysis with GemStone, the information you want to store in a database will change. Use the Save and Open buttons in the Edit Columns dialog to store and retrieve the appropriate database structure for the analysis you are doing.

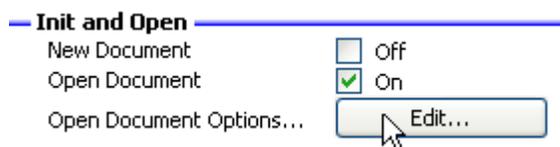
Click **OK** to close the Edit Columns dialog.

### Set the Batch Options

Now that we have selected the files and set up our database, we are ready to define what happens as each file is processed. For this, we use the Batch Options panel, found below the File Database panel on the left side of the GemStone workspace.

Here is what we want the batch to do. After it loads one of our data files, we want it to re-open the FetalRBC model document. We do this so that the model is in the same state when we begin our analysis. Then, we want GemStone to trigger the Auto Analysis command. And finally, we want the program to save the finished analysis in a separate GemStone model document.

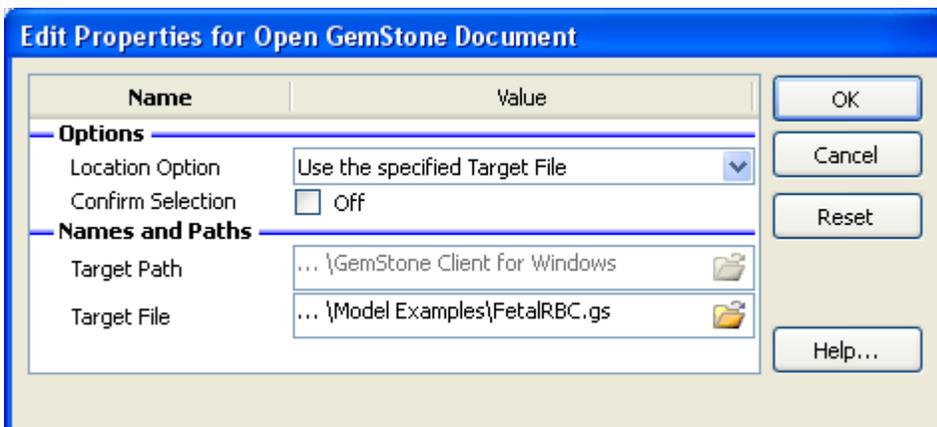
In the Batch Options panel, enable the **Open Document** command by checking it. Then, click the **Edit** button for **Open Document Options**.



In the dialog that is displayed, we will specify the GemStone document that we want to open.

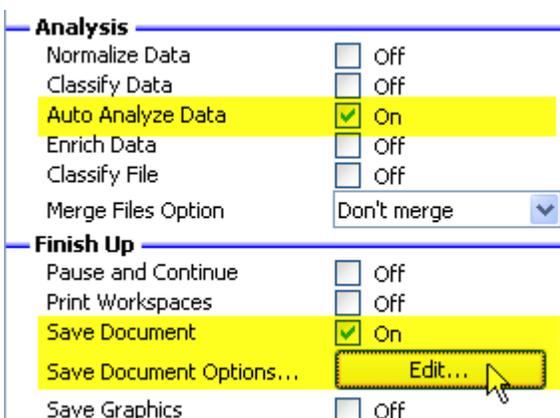
Set the **Location Option** to **Use the specified Target File**.

Click the **Target File** property, and in the Choose File dialog, select the **FetalRBC.gs** document and click Open. Click **OK** to close the document options dialog.



In the **Analysis** section of the Batch Options panel, enable the **Auto Analyze Data** option, and uncheck any other options.

In the **Finish Up** section, enable the **Save Document** option and uncheck other options. Then, click the **Edit** button for **Save Document Options**.



In the dialog that is displayed, we will specify where we want GemStone to save our finished analysis and how to name it.

Set the **Location Option** to **Use the data file name and the Target Path**. This will name the GemStone document using the data file name that was analyzed.

Click the **Target Path** property, and in the Choose Folder dialog, choose a folder that you have write-access to and click **OK**. Click **OK** to close the document options dialog.

Let's save this set of batch options so that we can use the same commands again.

Just below the Batch Options panel, there are **Open** and **Save** buttons. Click **Save**, navigate to a convenient location, and type **FetalRBCBatchCommands** for the file name. Click **Save** to store the commands.

### Batch Processing Tip: Save your Batch Options

You can save time setting up for batch processing by saving your batch options to disk. The next time you perform the same analysis, open your saved batch options and you are ready to go.

## Process the Files

We are all set up and ready to go. The best approach for batch processing is to do the first file, make sure everything is working as planned, and then to let the batch process run automatically through the rest of the files.

Click the Redo button on the batch toolbar at the bottom of the GemStone workspace.



GemStone will open the first data file in the batch and then run the commands we selected. It opens the FetalRBC.gs model document, performs an Auto Analysis, and then saves a new GemStone model document based on the name of the data file we analyzed. We also see the analysis results in the File Database.

To automatically advance through the rest of the files, click the Auto Advance button on the batch toolbar.



As GemStone processes each file, it repeats the batch options that we selected and adds the analysis results to the File Database. Notice that the **Application\_Document Name** field shows the name of the document that was saved with the finished analysis, and the **Application\_Opened Document Name** shows the name of the original model document. These fields are very useful for keeping track of how files were analyzed and where the final analysis is stored.

### After the First Time

If we wanted to run this batch process again tomorrow, our task would be easier because we have saved the database fields and the batch commands. Our process for setting up would be simplified:

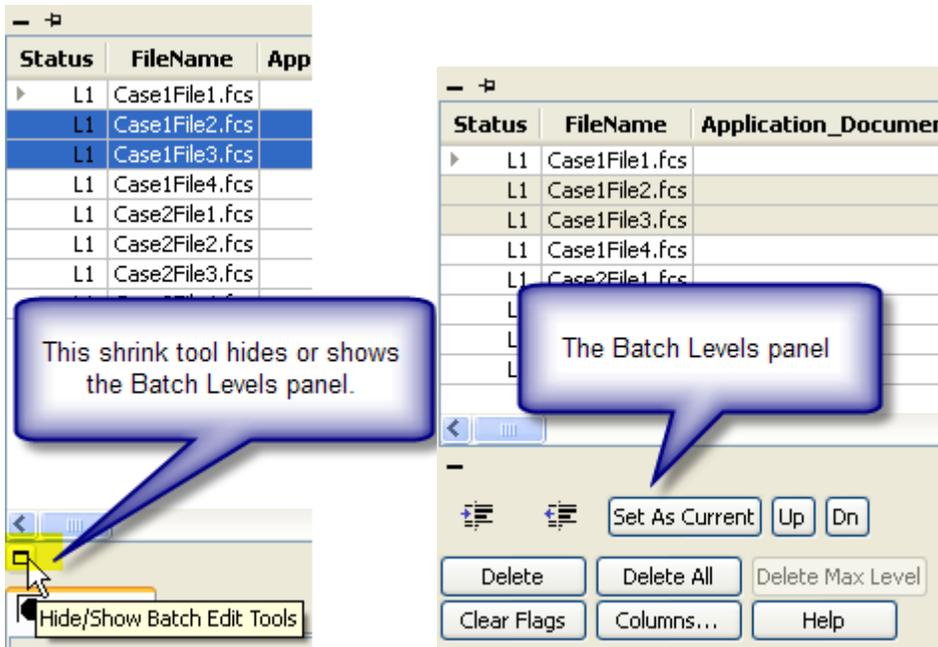
1. Open the GemStone model document.
2. Select the FCS files to analyze.
3. If the file database fields need to be set up, we use Edit Fields, and then Open the file we saved (FetalRBCFields.xml) with the database structure.
4. Use the Open command at the bottom of the Batch Options panel to read in our FetalRBCBatchCommands.xml file.
5. Process the first file by clicking the Redo button. Inspect to make sure everything is working OK.
6. Process the remaining files by clicking the Auto Advance button.

### Using Levels

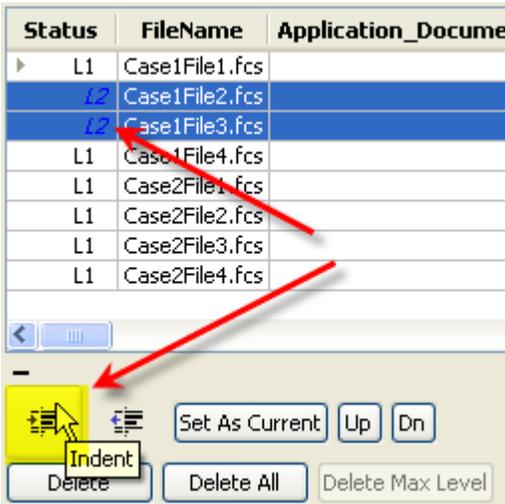
There are situations in batch processing where you need to treat some files differently than others in the batch. For example, suppose you have a GemStone model document that is designed to analyze 8 markers for a 4-tube panel. When the first file in the panel is read, you need GemStone to open the appropriate GemStone model document from disk and do an analysis. When the second and third files are read, you just want the program to analyze the data. And when the last file in the panel is read, you need to analyze the data, save the document, and print a report. Your analysis is not complete until you have read and analyzed all four tubes.

In a situation like this, you can use Batch Levels to perform different actions on certain files in the panel. Here is how you would handle the example described above.

After selecting the files for the cases you are going to analyze, expand the Batch Levels panel. This displays some additional controls that we will use to create the levels in our batch.



Next, you would select the second and third file in the list, and click the **Indent** button to indent the files to Level 2.



Similarly, you would select the fourth file in the list, and click the **Indent** button **twice** to indent the file to Level 3. If you have more than one case, you would repeat the process for the additional cases.

Now that you have 3 "levels" of files in the file database, you can set the batch options for each level separately. Notice that the Batch Options panel now shows a tab for each of the three levels.



To set the options for the Level 1 files, click the Level 1 tab. In our example, we would set the options on this tab to Open Document and Auto Analyze Data.

On the Level 2 tab, we would set the options to simply Auto Analyze Data.

And on the Level 3 tab, we would choose Auto Analyze Data, Print Workspaces, and Save Document.

As the files are processed, GemStone looks to see which level in the outline the files are on and selects the appropriate commands for that level.

This feature provides great flexibility for processing files automatically.

### Summary

Batch processing with GemStone simplifies the task of processing more than one file at a time. A batch process requires some initial setup to configure the database and the processing options you want to perform. Once the process is defined, files can be automatically analyzed and results stored.

The batch system allows more advanced processing through the use of outline "levels" in the file database. Files on different levels can be processed in different ways. Levels are useful for processing panels of files and other processes that require more than one analysis strategy for the files in the batch.

## **Batteries**

### **How to Work with Software Batteries**

This tutorial introduces the software battery concept and describes how to work with software batteries in GemStone.

### **Why Software Batteries?**

Software batteries provide a different form of software licensing than the typical commercial software license. The software battery is a per use style of license, which allows for much greater flexibility than other software license types. Since the battery charge can easily be transferred or shared the use of the software is highly portable. This allows you to install GemStone for use on any number of computers, allowing many individuals to utilize the application as needed. Software batteries eliminate the need to purchase licenses for each user who may use the application.

### **How do the Batteries Work?**

Each time a new FCS file is loaded into GemStone, the battery charge is decremented by one unit. When the battery charge is depleted GemStone will prevent you from opening additional FCS files. You will still be able to open any files that have previously been loaded.

When the battery is depleted, additional Battery charge can be obtained either via a purchase from Verity Software House or a request to a Battery Server for GemStone at your institution.

In addition to remembering the files that have been previously loaded, GemStone associates a set of files with each stored analysis or GemStone Document. The GemStone Document can be shared with others who will be able to load the associated files into that analysis without requiring any battery charge. This means that an analysis can be shared, adjusted, or modified by many users, or the same user on different computers, with only one charge per associated file.

Each user account on a computer will have its own software battery. This allows many users to use GemStone on the same computer without using up battery charges of another user.

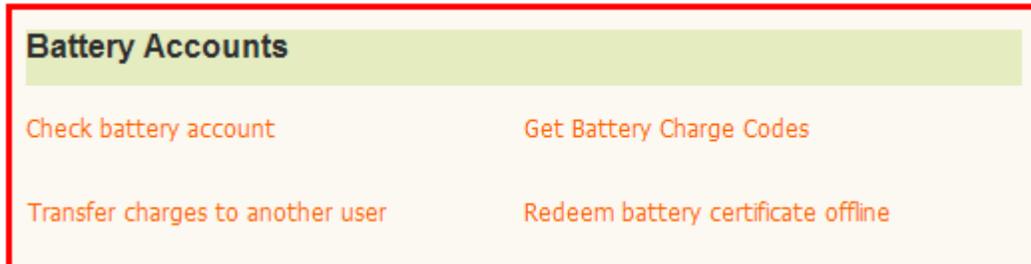
### **Recommendations for Battery Use**

In most cases it is recommended to leave most of your battery charge in your Web Battery Account. And, that you only transfer enough charge to your computer to accomplish the task at hand. Doing this will prevent loss of battery charge in the event that your computer's hard drive needs to be reformatted, replaced, or the OS needs to be reinstalled. Having the majority of your battery charge in your Web Battery Account also allows you to transfer charge easily to another computer or to another user. This enables you to quickly "activate" GemStone on another computer or for another user.

### **What is a Web Battery Account?**

A Web Battery Account is an account that can contain a number of battery charges for a specific application. Your Web Battery Account is associated with a Member Account on [www.vsh.com](http://www.vsh.com). Battery charges can be put into your account via a purchase, by uploading

remaining charge from an installation of GemStone, or via a transfer from another user's Web Battery Account. Battery charges are removed from your Web Battery Account when you download them to an installation of GemStone, or by transferring them to another user's Web Battery Account. When you login to your Member Account, you will see a number of actions that can be preformed on the Web Battery Account.



### Free Trial Battery

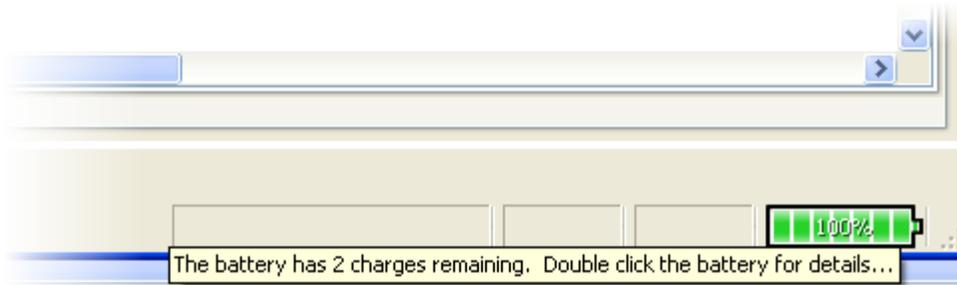
If you are new to GemStone you can request a free trial battery which will allow opening of your own data for evaluation. To request a free trial battery from GemStone you can click on the Free Trail button on the banner dialog that is displayed when GemStone first launches. This button will take you to Verity's website where you can fill in information needed to supply you with the trial battery.

### Working with Battery Charge

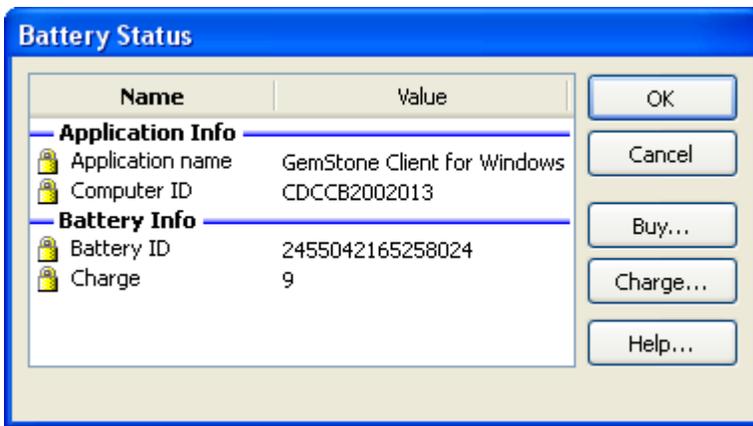
There are a number of actions that act upon GemStone's battery. If your computer is connected to the Internet, you can buy additional battery charge from [www.vsh.com](http://www.vsh.com), redeem a battery certificate, check the balance of your web account, download charges from your web account, or upload charges to your web account. If your computer is connected to an internal network that hosts a Battery Server for GemStone, you can request battery charge from the Battery Server. If you computer is not connected to a network you can use the offline transfer mechanism to obtain battery charge.

Some of the actions listed above have more than one way to access them. For our discussion, we will consider the use of the Battery Menu. Let's first introduce the other two locations of battery interaction.

The first is the Battery Meter, which is located in the lower right corner of the GemStone main window, and shown below. It will indicate the current percent of charge remaining in the battery. The tool tip displayed by holding the mouse over the Battery Meter will give the actual remaining charge amounts. The context menu, shown by right clicking the Battery Meter, contains a few shortcuts to actions also contained in the Battery Menu.



The second is the Battery Status dialog. The Battery Status dialog, shown below, can be accessed via the Battery Menu, the Battery button on the Banner dialog, or the context menu of the Battery Meter. The Battery Status dialog contains a few buttons that allow you to perform actions also available under the Battery Menu.



### Purchasing Battery Charge

If you need to purchase battery charge the process can be initiated by selecting the Buy Now option in the Battery Menu. You will be directed to the Verity Software House online store where the purchase will be completed.

### Charging the Software Battery

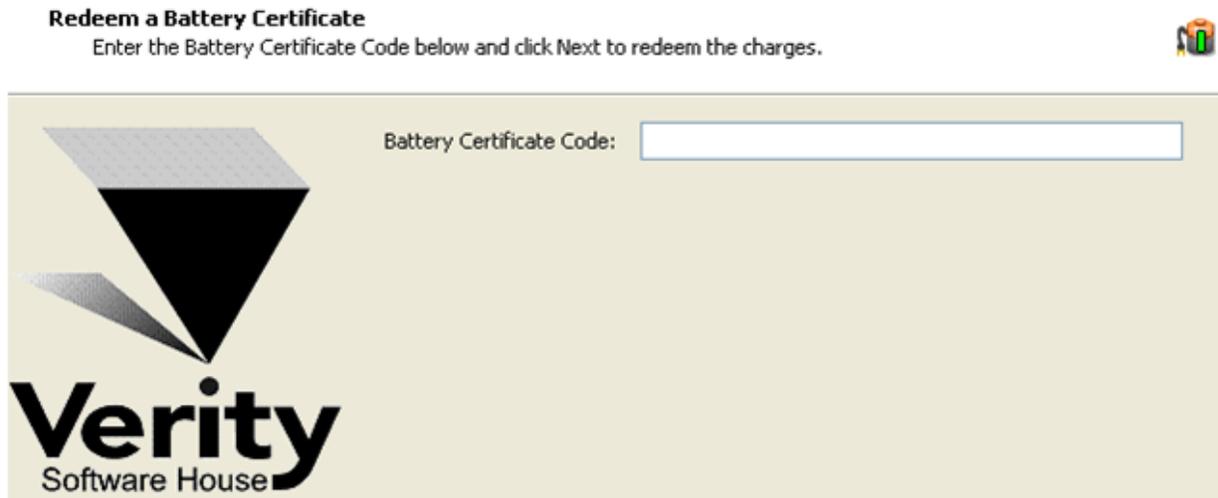
Selecting the Charge Battery command in the Battery Menu initiates the Charge Battery Wizard. The Charge Battery Wizard will guide you through the process of charging the battery.

There are three sources of battery charge that can add charge to GemStone's software battery: a Battery Certificate, a Web Battery account on Verity's web site, or a Battery Server on your local area network. For each of these sources, there are several methods for actually charging the software battery.

### Battery Charge Method 1: Redeem a Battery Certificate via the Wizard

If your computer is connected to the Internet you will be able to redeem a Battery Certificate directly using the wizard. Note that firewalls can sometimes interfere with this action. To redeem the certificate, select method 1 and click **Next**. The following page will be displayed.

**Redeem a Battery Certificate**  
Enter the Battery Certificate Code below and click Next to redeem the charges.



Enter the Battery Certificate Code in the field. When you click **Next** the wizard will attempt to redeem the certificate. The wizard will either move to a success or failure page when the request is completed. If this method fails you can redeem the certificate using method 2.

### **Battery Charge Method 2: Redeem a Battery Certificate using a web browser**

If your computer is not connected to the Internet you will need to use this method to redeem your Battery Certificate. Choose method 2 in the wizard and click **Next**. A page is displayed with a **Transaction Code** and your **Computer ID**. Take these codes and your Battery Certificate to a computer with internet access and visit [www.vsh.com](http://www.vsh.com). Click **Redeem a Battery Certificate** in the Quick Links section, enter the information and follow the instructions given.

The web site will provide you with several codes to complete the process. When you have the new codes, click **Next** in the wizard to move to the next page. On this page you will need to enter the three Battery Charge Codes generated by the website.

**Redeem a Battery Certificate using a Web Browser**

Please enter the three Battery Charge Codes from the website to complete the process.



Transaction Code: 2GEVGMM5KC2T962GLOG0T

Computer ID: CFAD6F184260

Code 1:

Code 2:

Code 3:

**Verity**  
Software House

After you enter the codes, click **Next**. The codes will be evaluated and the wizard will either move to a success or failure page. If this method fails please contact technical support at tech@vsh.com for assistance.

**Charge Battery Method 3: Download charges directly from your Web Battery Account**

If your computer is connected to the Internet, you can download charges directly from your Web Battery Account on the Verity web site. Note that firewalls can sometimes interfere with this action. To begin this method, select option 3 in the wizard and click **Next**. The following page will be displayed.

**Download Battery Charge**

Enter the needed information below. When you click Next the application will attempt to connect directly to your Web Battery Account on Verity's website to download the requested charge.



Web Login:

Password:

Remember Password

Download: All Charges

Specified Charge Amount: 50

**Verity**  
Software House

Enter your Verity Software House web account information and specify a number of charges to download. Click **Next** to initiate the download request. The wizard will either move to a success or failure page when the request is completed. The actual charge downloaded may be reduced if your web account has fewer charges than you have requested. If this method fails, you can download charges indirectly using method 4. The method will fail if the wizard

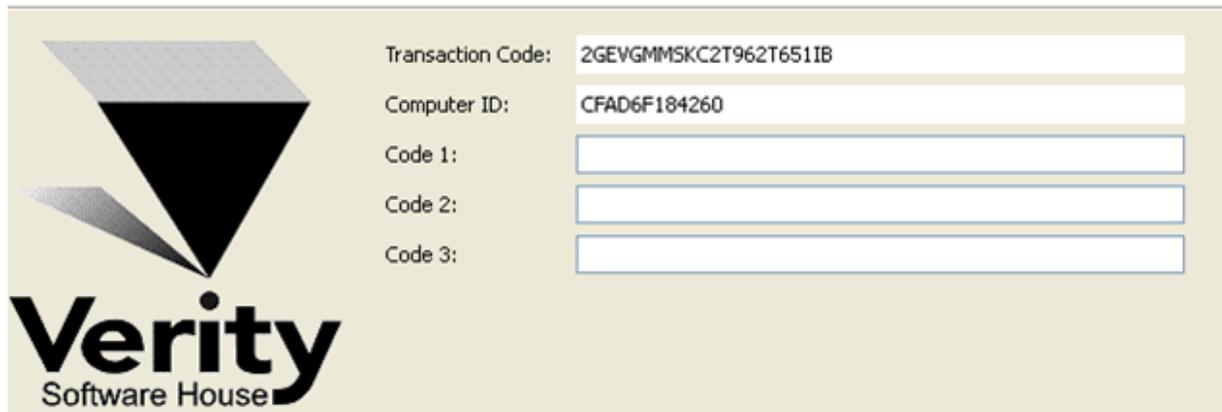
cannot connect with the Verity web site, the user account is not valid, or the account has no charges in it.

#### Charge Battery Method 4: Battery Charge Codes from a Web Battery Account

If your computer is not connected to the Internet, you can use this method to obtain battery charges from a Web Account on Verity's web site. Choose method 4 in the wizard and click **Next**. A page is displayed with a **Transaction Code** and your **Computer ID**. Take these codes to a computer with internet access and visit [www.vsh.com](http://www.vsh.com). Login to your Web Account and choose the **Get Battery Charge Codes** link. Enter the information and follow the instructions given.

The web site will provide you with several codes to complete the process. When you have the new codes, click **Next** in the wizard to move to the next page. On this page you will need to enter the three Battery Charge Codes generated by the website.

**Enter Battery Charge Codes**  
Please enter the three Battery Charge Codes generated for you either by [www.vsh.com](http://www.vsh.com) or by your Battery Server. 



Transaction Code:	2GEVGMMSKC2T962T651IB
Computer ID:	CFAD6F184260
Code 1:	<input type="text"/>
Code 2:	<input type="text"/>
Code 3:	<input type="text"/>

**Verity**  
Software House

After you enter the codes, click **Next**. The codes will be evaluated and the wizard will either move to a success or failure page. If this method fails please contact technical support at [tech@vsh.com](mailto:tech@vsh.com) for assistance.

#### Charge Battery Method 5: Request charges from a Battery Server

If your computer is connected to a local network that hosts a Battery Server for GemStone, you can request charges from the Battery Server. Note firewalls can sometimes interfere with this action. To begin the process, choose method 5 and click **Next**. The following page will be displayed.

### Request Charge from a Battery Server

Enter the needed information below. When you click Next the application will attempt to connect directly to the Battery Server to request the specified charge.



Battery Server:

Password:

Remember Password

Charge Requested:

**Verity**  
Software House

Enter the network name or the IP address of the Battery server, the password required by the Battery Server, and enter the number of charges requested. When you click **Next**, the request is sent to the Battery Server. The wizard will move either to a success or failure page depending on the result of the request. The actual charge downloaded may be reduced the Battery Server has fewer charges than you have requested or if there is a limit on how many charges you can requested at one time. If this method fails you can obtain charges form a Battery Server using method 6.

### Charge Battery Method 6: Battery Charge Codes from a Battery Server

If your computer cannot connect directly to your Battery Server, you can use this method to obtain battery charges. Choose method 6 in the wizard and click **Next**.

A page is displayed with a **Transaction Code** and your **Computer ID**. Take these codes to the Battery Server administrator. The administrator can enter the codes into the Battery Server to generate the Battery Charge codes you need to complete the process.

When you have the charge codes, click **Next** in the wizard to move to the next page. On this page you will need to enter the three Battery Charge Codes generated by the Battery Server.

### Enter Battery Charge Codes

Please enter the three Battery Charge Codes generated for you either by [www.vsh.com](http://www.vsh.com) or by your Battery Server.



Transaction Code: 2GEVGMMSKC2T962T651IB

Computer ID: CFAD6F184260

Code 1:

Code 2:

Code 3:

**Verity**  
Software House

After you enter the codes, click **Next**. The codes will be evaluated and the wizard will either move to a success or failure page. If this method fails please contact the Battery Server Administrator for assistance.

### Uploading Battery Charge to Your Web Account

If your computer is connected to the internet, you have the option of uploading all remaining battery charge to your Web Battery Account. Note, firewalls can sometimes interfere with this action. Uploading your remaining battery charges allows you to redistribute your remaining battery charge to other computers or users. You can also prevent the loss of your battery charge by uploading your battery charge before reformatting or replacing your hard drive or reloading your operating system. To upload the battery charge, choose the Upload Credits option from the Battery Menu. A dialog like the one shown below will be displayed. You will need to enter your web account login and password, and optionally choose to have the password remembered on this system. When you click OK the Upload will proceed and alert you as to the result, either success or a reason for failure.

Name	Value
Web Login	my account
Password	•••••••
Remember Password	<input type="checkbox"/> Off

Whether or not to remember the password.

### Checking the Balance of Your Web Battery Account

If your computer is connected to the Internet, you will be able to check the balance of your Web Battery Account directly from the application. Note, firewalls can sometimes interfere with this action. To check your Web Account Balance, choose the Check Balance option from the Battery Menu. Enter your web login and password and optionally choose to have the password remembered, in the dialog, shown below, that is displayed. When you click on OK, GemStone will attempt to check the balance of your Web Battery Account, and will either display the current balance or a reason the action failed.

### Summary

In this tutorial we have considered the why and how of software batteries. We have learned the benefits over other software license structures. We covered recommendations for using the battery system. We learned about the Web Battery Account. We then covered use of the battery system, and how to obtain the battery charge needed to complete analysis on a computer.

**See also:**

[Battery Menu](#)

[Server Menu](#)

[How to Work with Battery Server for GemStone](#)

## How to Work with Battery Server for GemStone

This tutorial covers the setup and use of the Battery Server for GemStone application. The Battery Server is used as a central hub on a network to distribute battery charges to client versions of GemStone. For more information on the use of software batteries see the tutorial [How to Work with Software Batteries](#).

### Setting up the Battery Server

Install the Battery Server on a computer that can be accessed by all of the GemStone Client computers. The client computers do not need to be in constant contact with the server; they periodically communicate with the server to get battery charges which allow the client software to read data files.

After installation of the Battery Server application is completed, there are a few steps necessary to prepare it to distribute charges:

1. Register the Battery Server.
2. Configure the Battery Server to distribute charges.
3. Charge the "software battery" for the server so that it can deliver charges to clients.
4. Optionally, configure the computer to automatically launch the Battery Server application.

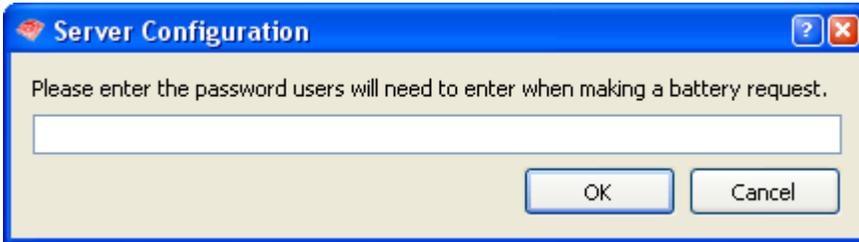
### Registering the Battery Server

When the application is first launched it will need to be registered. Read [Registering your new software](#) for instructions on how to register you software. After a successful registration, you are ready to configure the Battery Server.

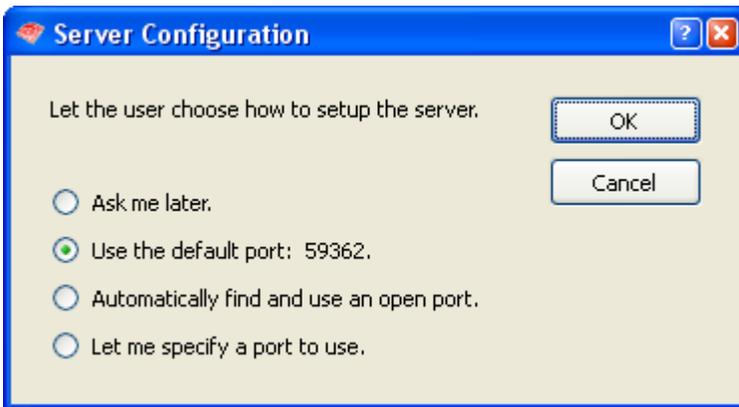


## Configure the Battery Server

You will be then presented with a couple dialogs that will assist in configuring the Battery server to distribute charges. The first dialog will ask you to enter a password. The Battery Server can be protected from unauthorized access by use of a password. Users will need to know this password when making battery requests.



The second dialog, will allow you to specify the port that the Battery Server will monitor for battery requests. In most cases, the default port selection should be used. If there is a conflict, a different port may be specified. If you do not use the default port, users making battery requests will need to know the port number you have chosen. They must modify the **Battery Server Port** specified in the **Miscellaneous** section of the **Preferences**. See [Edit Preferences](#) for additional details.



### Firewall Alerts!

When the Battery Server attempts to monitor the IP port selected, firewall software on the computer may display an alert. This is because the Battery Server is attempting to listen for incoming requests on an IP port - something that firewall software is designed to prevent.

The Battery Server needs to monitor a port in order to service requests from GemStone Client users. Firewall software settings need to be adjusted to allow this.

Once the port is selected, the Battery Server will begin monitoring the port for battery requests. You should see a message indicating success.



### Charging the Battery

The Battery Server's battery can be charged using any of the options covered in the tutorial [How to Work with Software Batteries](#). These options are found in the application's Battery menu. Choose the appropriate method and charge the battery. For new installations, use the "Battery Certificate" that was included with the software. The Battery Certificate encodes a set of charges for the Battery Server. When the battery has been successfully charged you should see a success message. The battery meter in the bottom right of the application window will show that it is fully charged.

At this point, the Battery Server is ready to fulfill battery requests from GemStone Client software on other computers. This can be tested by requesting a battery from the Battery Server itself. To do this "self-request", use the Request from Server option in the Battery Server's Battery Menu, and use **localhost** in the Battery Server Name field. You should again see a success message and have a full battery.

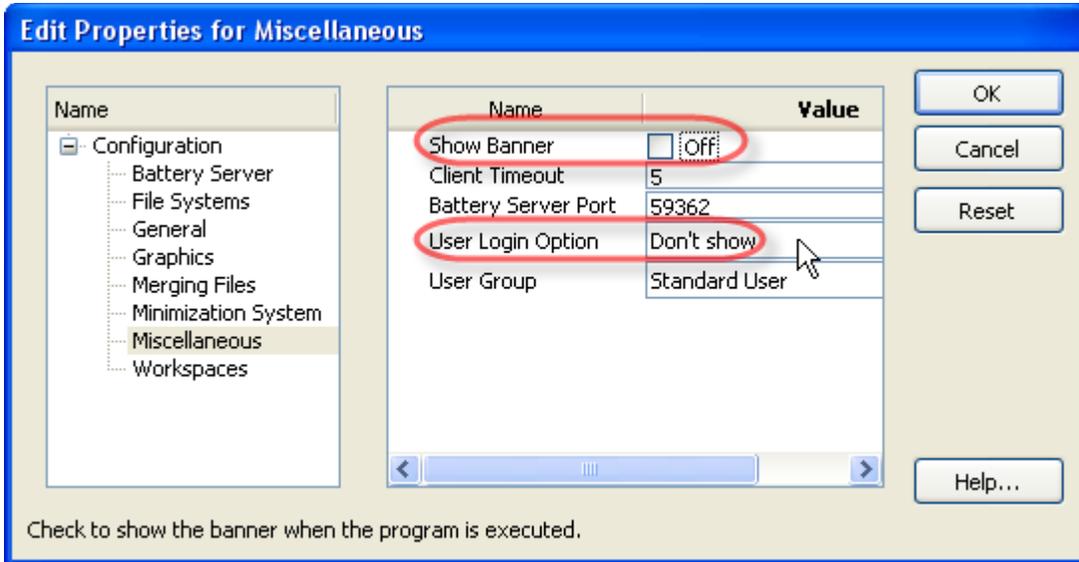
### Configuring the System to Automatically Run the Battery Server

The Battery Server should be available to GemStone Client installations at all times. The best way to ensure this is to set up the Battery Server to automatically launch when the computer is restarted. This allows the Battery Server to distribute charges without interaction on the part of the user. There are a few steps to set this up, depending on the operating system you are running.

### Common Steps to Automatically Run the Battery Server

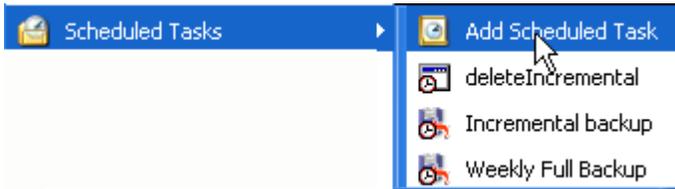
In order for the Battery Server to run automatically, the banner dialog and the user login need to be disabled. This can be done using the Preferences dialog. On the Mac, choose **Preferences** from the application menu. On Windows, choose **Preferences** from the Edit menu.

In the Edit Preferences dialog, choose the **Miscellaneous** section. Uncheck the **Show Banner** checkbox and choose **Don't Show** for the **User Login Option**.



### Automatically Run the Battery Server on Windows

If you are running Battery Server for GemStone on Windows, you will need to setup a scheduled task to launch GemStone each time the computer reboots. From the Control Panel, choose Scheduled Task, Add Scheduled Task.



This will start the Scheduled Task Wizard. Click Next.

Use the **Browse** button to navigate to the location where the Battery Server for GemStone was installed.

Choose the Battery Server for GemStone.exe application file. Note, you may or may not see the ".exe" extension. If you are unsure which entry is the application you can right click and choose Properties. The "Type of file" field for the application will specify Application.

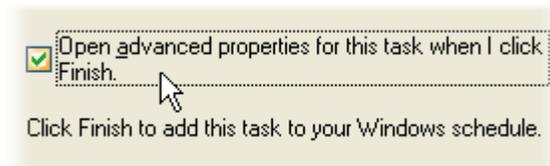
Select to run the application "When my computer starts" and click Next.

In the next pane of the wizard, you will need to enter a user account and password under which the Battery Server task will be run. Use the Windows OS user and password that was used to register Battery Server for GemStone. This is the user name and password that you use to login to your computer.

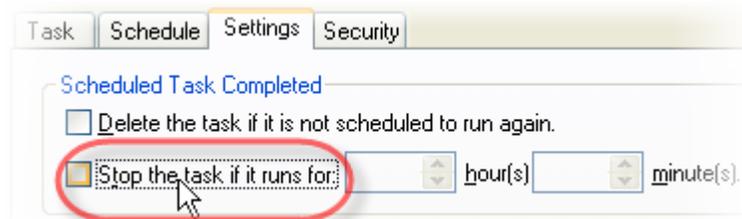


Click Next.

There are a few additional settings to configure, so enable the "Open the advanced properties for this task when I click Finish".



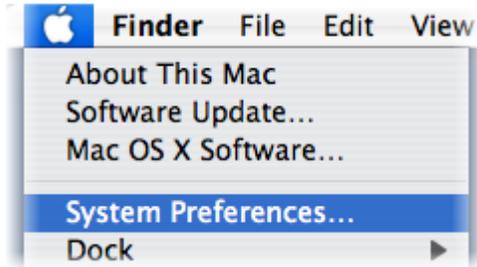
Select the **Settings** tab. Uncheck the Stop the task checkbox. Then click OK to finish the task.



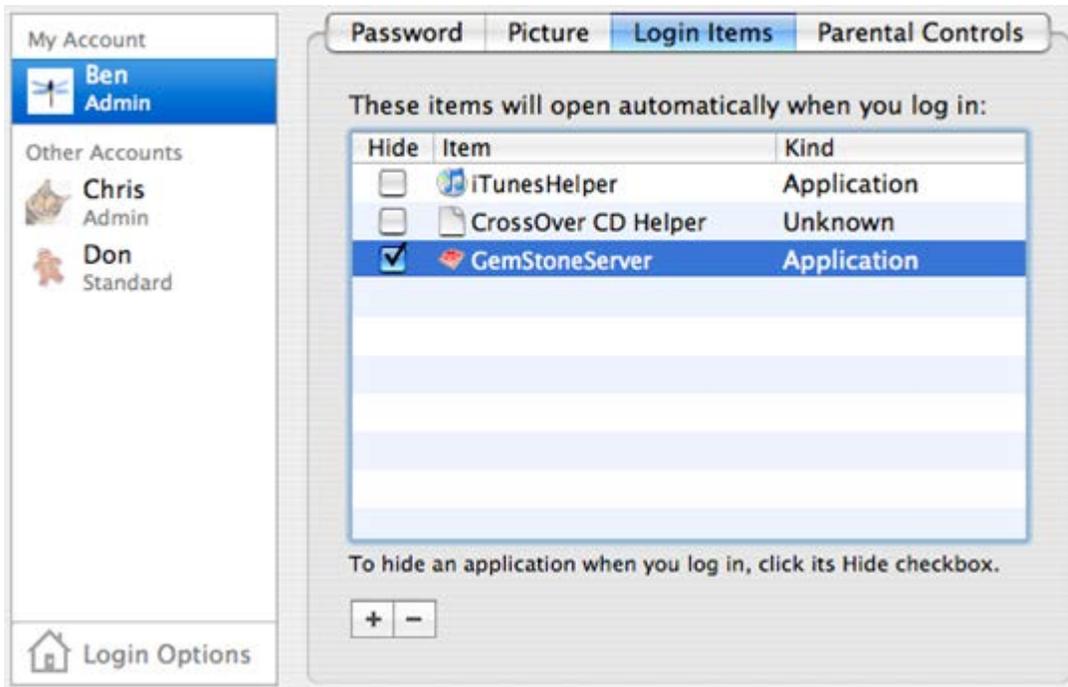
At this point, the Battery Server for GemStone should run in the background each time your computer starts up. It will monitor the IP port and be able to fulfill battery requests from GemStone Client users even if no user is logged in to the computer. To ensure that the Battery Server will always be available when needed, the system should also be configured not to sleep after a certain period of time, unless network activity can wake it up (Power Options).

### Automatically Run the Battery Server on Os X

If you are running Battery Server for GemStone on Macintosh OSX, you will need to add the Battery Server to a list of applications that start when a user logs in, and set that user to be automatically logged in when the system restarts. From the System menu choose **System Preferences**.



In the System Preferences, dialog click on the Accounts icon. From the list of users, select the user who preformed the Battery Server registration. Click the "Login Items" or "Startup Items" tab. Click the "+" button in the Login Items section, browse to and select the Battery Server for GemStone application file. If you want the Battery Server to run minimized check the Hide checkbox.



Now click on **Login Options** in the lower left. If the control is disabled you will need to click on the lock button in the lower left and authenticate to make the needed changes. In the Login Options section, check the **Automatically Login As** checkbox. Then choose the user that preformed the Battery Server registration. You may need to re-authenticate for this step as well.

At this point, the Battery Server for GemStone should run in the background each time your computer starts up. It will monitor the IP port and be able to fulfill battery requests from

GemStone Client users even if no user is logged in to the computer. To ensure that the Battery Server will always be available when needed, the system should also be configured not to sleep after a certain period of time, unless network activity can wake it up (Energy Saver Settings). Also the user should not be logged out after a period of inactivity (Security Settings).

### Using the Battery Server

When you need to interact directly with the battery server, you will need to log in to the operating system with the user login that was used to register the Battery Server.

If you are running the Battery Server on Windows and the server is configured to run automatically as outlined above, you will need to terminate the server background process, and then re-launch the Battery Server application directly after logging in to the operating system. To terminate the Battery Server process, you can run the "Stop\_Battery\_Server.bat" file that is installed in the same directory as the Battery Server for GemStone application.

You can view and adjust the server configuration using the **Properties** option in the Server menu. The server properties will show you the information needed by the user when making battery requests from the server. If changes need to be made to the Battery Server's configuration they can be made here. You can also view the Battery Creation Log, which shows information about the batteries that have been generated and distributed. From the Server Menu you can satisfy an offline transfer request from a user by choosing the Transfer Charge option. You will need enter the needed information from the user into the Transfer Charge dialog and return to the user the battery transfer codes that are generated. For more information on these options see the Server Menu section.

### Summary

In this tutorial we have considered the how to configure and use Battery Server for GemStone. This included registration, initial configuration, charging the battery, configuring the system to run the Battery Server automatically, and using the Battery Server directly after logging in to the computer on Windows.

### See also:

[Battery Menu](#)

[Server Menu](#)

[How to Work with Software Batteries](#)

[Tutorials](#)

## **Reference**

### **Reference Section**

The following sections document the features and functionality of all menus, dialogs, and functions of GemStone.

## Control Panels

## File Batch and Database panel

### File Database panel

This panel contains a list of FCS files that have been selected for analysis. The Status column on the left indicates the current file with a pointer icon. A check in the Status column indicates that the file has been analyzed. The batch level for the file is also shown, e.g. L1 is level 1.

Columns can be added to the list to display analysis results and keyword information from the files. This serves as the database of analysis information and can be exported for integration into other applications.

The [File Database context menu](#) can be displayed by right-clicking on the panel.

Status	FileName	TUBE NAME	State Index
▶ ✓ L1	HEMATAGONE-C-121307	HEMATAGONE-1213-	2.5311441122
✓ L1	Specimen_001_Tube_00	Tube_002	

### If you want to...

- |                                    |  |
|------------------------------------|--|
| Add files                          | Use the <a href="#">Select FCS Files</a> command from the File menu or the main toolbar. |
| Remove files                       | Use the mouse to select files, and then press the Delete key on the keyboard.            |
| Set the current file               | Right-click the file name and choose "Set Current Batch Item" from the context menu.     |
| Add or remove columns              | Right-click the list and choose <a href="#">Edit Columns</a> from the context menu.      |
| Export the database of information | Right-click the list and choose "Export Batch Database" from the context menu.           |

See also:

[File Database Context Menu](#)

[Edit Batch Columns](#)

## File Database Context Menu

This context menu appears when the mouse is right-clicked on the [File Database panel](#). On computers with a one-button mouse, it can be displayed by holding the Control key and clicking the mouse. The menu provides a number of actions that relate to the files in the batch.

### Set current batch item

Make the selected item the current batch item.

### Reset analysis flags

Set all files in the batch to "unanalyzed".

### Delete all items

Clear all files from the batch list and database.

### Delete selected items

Remove the selected file(s) from the batch list and database.

### Refresh

This command forces the file batch list to update.

### Edit Fields

Display the [Edit Batch Columns](#) dialog to choose columns for the batch and database.

### Export Database

Export the database to an ASCII text file. See [Export Database](#) for additional information.

### Delete maximum group level

Delete the deepest batch level in the in the batch, if there are no files on that level.

### Save batch list

Save the current state of the batch list. When GemStone starts up it restores the last state of the batch. When it exits, it saves the state of the batch automatically.

### Save batch list as

Displays the File Save dialog to save a batch list to disk. See [Save Batch List](#) for details.

### Open batch list

Displays the File Open dialog to opens a previously saved batch list. See [Open Batch List](#) for details.

## Edit Batch Columns

This dialog is used to select the columns that are displayed in the [File Database panel](#), as well as the fields that are included in the database of analysis results.

The tree on the left of the dialog contains all of the information available, including analysis results, FCS keywords, and other useful fields. The list on the right of the dialog shows the fields that have been selected either for display in the batch list, inclusion in the database, or both.

### If you want to...

Add columns or fields	Select one or more items in the information tree on the left and then click the <b>Insert</b> or <b>Add</b> button.
Remove fields	Select one or more items from the list on the right and then click <b>Delete</b> or <b>Delete All</b> .
Change the order of columns	Select an item in the list and use the <b>Up</b> or <b>Down</b> button.
Include a field in the database	Add the field to the list and make sure the <b>Enable</b> option is checked.
Show or hide a column	Add the field to the list and check or uncheck the <b>Display</b> option.

## **Export Database**

Use this command to export the analysis information in the batch database to a tab-delimited ASCII text file. The command will display the standard File Save dialog. Enter a file name for the database and navigate to the location where you want to store it. Then click the Save button to complete the export.

ASCII formatted text is one of the most universally accessible file formats available. It can be easily read and interpreted by database and spreadsheet applications, as well as most lab information systems.

### **Save Batch List**

Use this command to save the current files and database to disk. The standard File Save dialog will be displayed, allowing you to choose a name and location for the database file.

The [Open Batch List](#) command can be used to reload the database.

**Open Batch List**

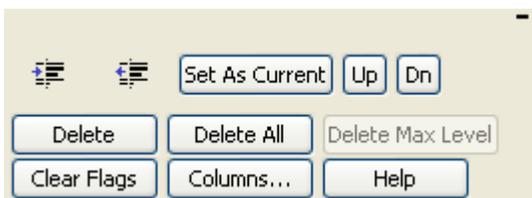
Use this command to open a previously saved database from a file on disk. The standard File Open dialog will be displayed, allowing you to choose a name and location of the database file.

Use the [Save Batch List](#) command to store a database on disk.

## Batch Levels panel

### Batch Levels panel

This panel is used to control the "batch level" of files in the [File Database panel](#).



GemStone uses "levels" to differentiate groups of files in the batch, and it allows different actions to be performed on each level. The level for each batch item is shown in the File Database panel; it appears in text form and as indentation in the Status column.

Status	FileName
✓ L1	HEMATAGONE-C-121307
▶ ✓ L1	Blymph_Sample1
L2	Blymph_Sample2
L3	D-908302.001V
L1	D-908302.002V

When GemStone opens a file in the batch list, it looks to see what level of indentation the file is on. It then finds the set of actions that relate to that level and executes them. The actions for each level can be edited in the [Batch Options panel](#). This provides a simple mechanism for creating sophisticated batch processing. For example, Level 1 could be used to identify the first file in a panel of related files to open a GemStone document and initialize the model, and then perform an Auto Analysis. Most other files in the panel could be assigned to Level 2, which would simply perform Auto Analysis. The last file in the panel could be assigned to Level 3, which would save the GemStone document and print the final results.

### If you want to...

- |   |   |
|---|---|
| Change the level  | Select the item and click  to increase or  to decrease the batch level. |
| Set the current batch item  | Click " <b>Set as Current</b> ".  |
| Move an item up or down   | Select the item and click <b>Up</b> or <b>Down</b> .  |
| Delete one or more items  | Select the items and click the <b>Delete</b> or <b>Delete All</b> button.   |
| Clear the analysis flags  | Select the items and click <b>Clear Flags</b> .   |
| Edit the columns that appear in the batch list and the fields that are included in the database | Click the <b>Columns</b> button.  |

**See also:**

[File Database panel](#)

[Batch Options panel](#)

## Batch Options panel

### Batch Options panel

This panel determines the actions that are performed when an FCS file in the batch is read into the program for analysis. When GemStone reads a file, it determines which "level" the file is on in the batch. Each batch level has a set of actions associated with it. For more on batch levels, see [Batch Levels panel](#) .

A tab will appear for each level that has been defined in the batch list. Select a tab to view or edit the actions for the associated batch level.



### Enable

Enable the actions defined on this tab.

### Custom

#### Custom Actions

Enable Custom Actions mode and the ability to create custom action scripts.

#### Advanced Editing

Edit the actions in the script. This is an advanced feature that provides access to additional scripting commands. See [Edit Actions](#) for more information.

### Init and Open

#### New Document

Create an empty model in a new document, clearing any previously opened document and graphics from memory.

#### Open Document

Open a specific GemStone document.

#### Open Document Options

Set options for opening the GemStone document, including the file name and path. See [Properties for File Open and Save](#) for additional details.

### Analysis

#### Normalize Data

Normalize intensity levels from one file to the next for any parameters enabled for normalization in the [Edit Parameter Database](#) dialog.

##### Normalize Data Options

Initialize                      Clears any normalization factors and target mean intensity values.

	This is the default option.
Set targets	Stores the linear mean values of parameters flagged for normalization. The mean values will be used as target mean values for subsequent files when Use Targets is selected.
Use targets	Parameters flagged for normalization are normalized using the current Target Mean Intensity. Graphics and results for these parameters will reflect the normalized data.

### Classify Data

Initiate the [Classify Data](#) command after the file is read.

### Auto Analyze Data

Initiate the [Auto Analysis](#) command after the file is read.

### Enrich Data

Initiate the [Enrich Data](#) command after the file is read.

### Classify File

Initiate the [Classify File](#) command after the file is read.

### Merge Options

Choose an option for file merging. File merging allows a set of related files (e.g. a panel of tubes) to be merged into one file containing the union of all parameters in the set. The program synthesizes data for missing parameters.

#### Merge Options

Don't merge	Disables file merging.
Start merge	Creates a new file to start merging data.
Add to merge	Adds to a merge file. This option is only valid after a "Start merge" action and before a "Finish merge" action.
Finish merge	Finishes the file merging and closes the output merge file.

### Finish Up

#### Pause and Continue

Enable this option to pause batch processing before printing, saving, and continuing to the next file. When this command is triggered, a dialog is displayed indicating that processing is paused. The dialog shows a count-down timer and will automatically close and continue after the timer runs out. If the user clicks anywhere on the GemStone workspace during the pause, the timer is shut off and the program will wait until the user clicks the Continue button before it advances.

#### Print Workspaces

Print the workspaces for all cell types.

### **Save Document**

Save the GemStone document after the analysis.

### **Save Document Options**

Set options for saving the GemStone document, including the file name and path.

### **Save Graphics**

Save graphics for objects that have been selected.

### **Save Graphic Options**

Set options for saving graphics, including the file name and path. See [Properties for File Open and Save](#) for additional details.

### **Stop Auto Advance**

Stop the Auto Advance of the batch processor.

### **Open and Save**

These buttons allow you to save and re-open batch scripting setups. This makes it easy to store scripts for different purposes and reuse them without having to reset all of the options.

## Properties for File Open and Save

These dialogs are displayed from the [Batch Options](#) panel to set options for opening and saving GemStone documents and other file-based actions. The dialogs are very similar; the Save dialog has one additional property.

### Options

#### Location Option

Choose the naming option to use for the open or save operation.

#### Options

Use the data file name and path	With this option selected, the GemStone document name and location are based on the name of the FCS data file that is currently opened. For example, if the FCS file is "C:\Data\Experiment123.fcs", the document that the program attempts to open will be "C:\Data\Experiment123.gs".
Use the data file name and the Target Path	With this option selected, the GemStone document name is based on the name of the FCS data file that is currently opened, but the location of the document is defined by the <b>Target Path</b> property below.
Use the specified Target File	With this option selected, the GemStone document name and location are defined by the <b>Target File</b> property below.

#### Confirm Selection

When this option is enabled, the File Open or Save dialog will be displayed so that the file name and location can be verified or changed by the user.

#### Replace Existing File (Save Document dialog only)

Check this option to overwrite a file of the same name without prompting for confirmation.

### Names and Paths

#### Target Path

When "Use the data file name and the Target Path" is selected as the Location Option, this field sets the path that is used. For other Location Options, this field is ignored.

#### Target File

When "Use the specified Target File" is selected as the Location Option, this field sets the path and file name that is used. For other Location Options, this field is ignored.

## Edit Actions

This dialog provides custom editing for batch processing scripts. *It is an advanced option and is not recommended for most users.*

The actions listed in this dialog are intentionally left undocumented. If you need to create a customized action sequence, you will need to contact Verity Software House Technical Support for assistance.

The dialog displays a list of available actions on the left. The list on the right of the dialog displays the current script of actions. Additional actions can be added or inserted in the script, and actions in the script can be edited, reordered, or deleted.

A set of buttons on the right of the dialog allows scripts to be saved to and read from disk. Additional buttons allow the script to be executed for testing purposes.

### If you want to...

Add or insert a new action	Select the action(s) in the list on the left of the dialog, and then click Add or Insert.
Delete an action from the script	Select the action in the list on the right, and then click Delete.
Delete all actions in the script	Click the Delete All button.
Edit the parameters for an action in the script	Select the action in the list on the right, then click the Properties button. You can also directly edit the Return, Object, and Parameters columns in the list.
Save a script to a file on disk	Click the Save button.
Open a script from disk	Click the Open button.
Run the script	Click the Run button.
Pause a script that is running	Click the Pause button.
Run the script one line at a time	Click the Step button to advance the script one line.
Run the script to the selected line	Click the Run Until button.

## **Global Properties panel**

### **Global Properties panel**

This panel contains properties that apply to all cell types in the current GemStone model.

### **Specimen keyword**

Set the keyword that is used to identify the specimen represented by the current FCS file. Enclose the keyword with square brackets. FCS file keywords as well as internal keywords can be used.

### **Color by Gate Combinations**

When enabled, this option applies coloring to *unclassified events* based on the combination of gates (i.e. regions) that each event is contained by.

### **Document Name**

The name of the GemStone document currently opened.

### **Document Path**

The path to the FCS file currently opened.

### **Total Analyzed Events**

The number of events in the FCS file analyzed by the model.

### **Percent Unclassified**

The percentage of events that were not classified by the model.

### **Model Author**

This field can be used to enter the name of the person who created the model.

### **Saved Program Version**

This field displays the program version that was used to save the model.

### **Synthesize Data Using Model**

When checked, the model's means and line spreads are used when synthesizing data. If unchecked, the observed means and SDs of the existing data are used.

## Cell Type Properties panel

### Cell Types

A GemStone model can define one or more "cell types", with each cell type describing the characteristics of one subset of cells. When the model analyzes the events in an FCS file, each event ("cell") is classified into one cell type or it is considered an "unclassified" event that does not match any of the cell type definitions.

General characteristics for a cell type are defined in the [Cell Type Properties panel](#). The model definition for a cell type is defined using the [Cell Type widget](#) and [Parameter Profile Properties panel](#).

#### If you want to...

Add a new cell type	In the Cell Type Properties panel, click the Utility Actions dropdown and select Add Cell Type.
Duplicate a cell type	In the Cell Type Properties panel, click the Utility Actions dropdown and select Duplicate Cell Type.
Delete a cell type	In the Cell Type Properties panel, click the Utility Actions dropdown and select Delete Cell Type.
Select a cell type	At the bottom-left of the Cell Type workspace, click the tab for the cell type that you want to view or edit.

#### See also:

[Cell Type Properties panel](#)

[Cell Type widget](#)

[Parameter Profile Properties panel](#)

## Cell Type Properties panel

This panel contains properties and actions that relate to the current Cell Type tab. A GemStone model can contain any number of Cell Types. Each Cell Type has its own collection of parameter profiles that define the attributes of events that will be selected into that Cell Type. For example, it is common practice to create a Kappa Cell Type and a Lambda Cell Type for a model designed for Leukemia/Lymphoma analysis. Each event (cell) can be classified into one of the Cell Types or assigned as an "unclassified" event. An event cannot be assigned to more than one Cell Type.

By default, there are 100 states defined for each Cell Type, but each Cell Type can use a different number of states. If the progression is a very simple and rapid transition, a lower value can be used and the classification process will be faster. In some cases, it may be necessary to increase the number of states for a Cell Type in order to resolve finer, more gradual transitions in the progression.

### Identification

#### Cell Type Name

Enter a name for the Cell Type.

#### Enable

Enable or disable classification of events for this Cell Type.

#### Abbreviation

Enter the short name for this Cell Type.

#### Description

Enter the description of the Cell Type.

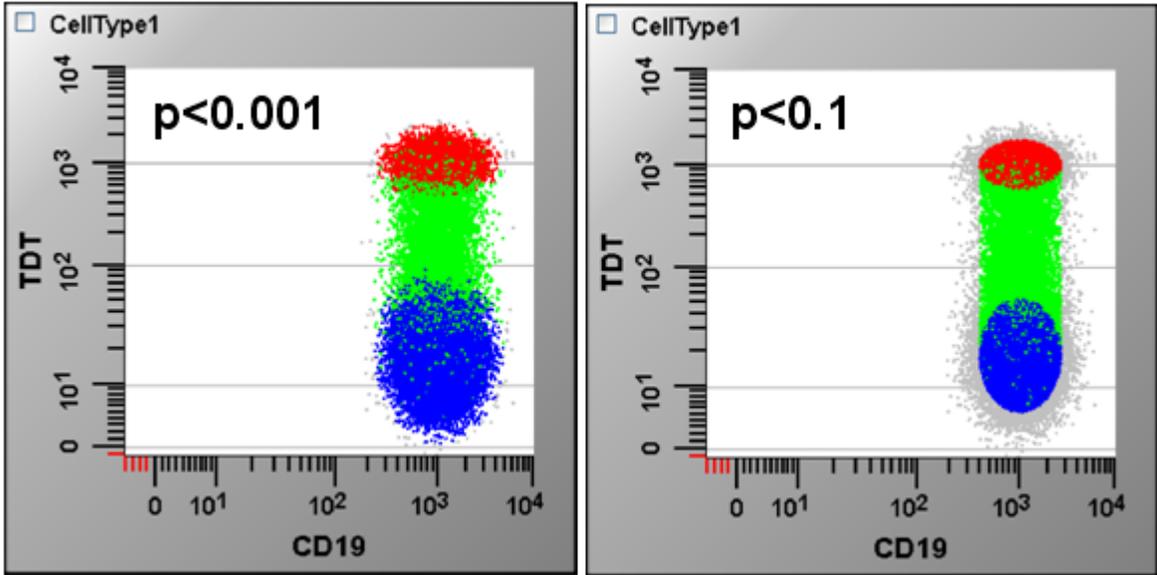
#### State Index Label

Enter the label that will be used on the X-axis (the state index axis).

### Analysis

#### Exclusion Probability

This value determines the probability at which an event will be excluded from a Cell Type during the classification process. The value ranges from 0.0 to 1.0. More events are excluded as the value approaches 1.0. For example, a value of 0.1 will exclude 10% of the events from the Cell Type. The events excluded will be the ones with the lowest probability of matching the Cell Type.



**Inclusion Probability**

This is an additional filtering option that can restrict the events that are included by a Cell Type. It sets the minimum probability for events that are to be included in the Cell Type. Normally it is set to 0.0. Its range is 0.0 to 1.0. As the value approaches 1.0, fewer events are included in the Cell Type.

**Number of States**

Enter the number states to define for the progression of events in this Cell Type. The default value is 100. Smaller values improve analysis speed and can be used when transitions are distinct and dramatic. Larger values take more time to process, but may be useful for gradual or complex transitions in the progression.

**Gate On**

Enable or disable the gating expression by toggling this option. See [Gating and Gate Expressions](#) for additional details.

**Gate Expression**

Enter a gating expression, if desired, to apply to events in the listmode file prior to the classification process. The gate is only applied if the Apply Gate option below is enabled. See [Gating and Gate Expressions](#) for additional details.

**Statistics**

**Cell Type Events**

The number of events classified by this Cell Type.

**Reduced Chi-Square (RCS)**

The goodness-of-fit for events classified into this Cell Type. Well-defined models produce RCS values between 0.0 and 3.0. Values between 3.0 and 5.0 are acceptable for many models. Complex models with fine distinctions between states may produce even higher RCS values.

**% State Index Fidelity**

The field shows the average probability that the classification made by the Probability State Model is correct, expressed as a percentage. This can be useful when building a model to determine where the model is well-defined and where it is not well defined.

## Editors

### Select Parameters

Click the Edit button to edit the parameters that are selected for analysis in the current Cell Type. The [Edit Parameters](#) dialog is displayed.

### Edit Zones

Click the Edit button to create and edit Zones defined for this Cell Type. The [Edit Zones](#) dialog is displayed.

## Utility Actions

### Actions

Update all parameters from database	Updates all parameter profiles in the cell type from the parameter database.
Deselect definition points	Remove the selection of all definition points.
Link selected control points	Links the set of selected control points so that they maintain the same relative positions on the state index scale. This forces the control points to maintain the relationship during Auto Analysis and other fitting operations.
Unlink selected control points	Removes the linkage relationship previously established with the "Link selected control points" action.
Expand parameter graphs	Expands all parameter profiles plots in the cell type.
Shrink parameter graphs	Shrinks all parameter profiles plots in the cell type.
Show definition points	Shows the definition points for all parameter profiles in the cell type.
Hide definition points	Hides the definition points for all parameter profiles in the cell type.
Fidelity Analysis	Performs a Fidelity Analysis on the cell type. See <a href="#">Fidelity Analysis</a> for additional details.

## Graphics

### Show Parameter X-Axes

Toggle this option to show or hide the state index axis for each Parameter Profile plot.

## **Animation Controls**

### **Animate**

Toggle animation on or off. Animation appears on Parameter Overlay plots as well as 2P histogram plots.

### **Animation Speed**

Set the speed of the animation in milliseconds.

### **Animation Pause**

Set the number of milliseconds to pause at the end of the progression before the animation returns to the beginning of the progression.

### **Marker Separation**

Set the separation between the start and end markers for animation. The value is a percentage of the state resolution scale.

## **Other**

### **Relative Events to Synthesize**

When using the Synthesize Data into Packet action in batch processing (see [Batch Options panel](#)), this field controls how many events of this cell type are generated into the output. Each cell type can use a different value for this field, so that the mixture in the output file has a known composition.

For example, with 2 cell types, if you were to set this field to 200 for Cell Type 1 and 100 for Cell Type 2, GemStone would attempt to create a 200-to-100 mixture (or 2-to-1) for Cell Type 1 and 2 respectively in the synthesized file.

## Edit Parameters

This dialog is displayed to select the parameters that are included in a cell type for analysis or display.

The list on the left side of the dialog displays all of the parameters that exist in the file, as well as other parameters that GemStone has seen in other files. The parameters in the current file display "On" in the "Live" column.

The list on the right shows the parameters that have been selected for analysis or display.

### If you want to...

Add or insert a new parameter	Select the parameter(s) in the list on the left of the dialog, and then click Add or Insert.
Remove a parameter from the cell type	Select the parameter in the list on the right, and then click Delete.
Remove all parameters in the cell type	Click the Delete All button.

### See also:

[Parameter Profile Properties panel](#)

## Edit Zones

This dialog allows zones to be added, deleted, and modified. Zones, in GemStone, are defined on the Progression (State Index) axis and provide statistics and coloring for portions of the progression.

Zones are most often defined using Definition Points in the Parameter Profiles for a given Cell Type. This allows the boundaries of the zone to automatically adjust as the Definition Points are adjusted by the user or by a process in the program. However, it is also possible to define Zones using percentage values. For example, a zone could be defined so that it started at 50% of the progression and ended at 75% of the progression.

Each zone has a user-defined name and color. The color is used when drawing dots that fall within the zone's boundaries and along the top of Parameter Overlay plots when zones are displayed. If overlapping zones are defined, the color of the last zone containing an event is used to color dots for that event.

A [Zone Statistics Table](#) object appears on each Cell Type canvas to provide statistics for the zones. The table shows the name, color, active state, and statistics for each zone.

See [Edit Properties for Zone](#) for additional details on zone properties.

### If you want to...

Add a new zone	Click the New button and fill in the <a href="#">Edit Properties for Zone</a> dialog.
Delete a zone	Select the zone in the list, and then click Delete.
Change the order of zones	Select a zone, and then use the Up or Down button to move it in the list of zones.
Save a set of zones	Click the Save button. The standard File Save dialog will be displayed, allowing you to navigate to a location and name the file.
Open a set of previously saved zones	Click the Open button. Navigate to the zone file on disk and choose Open. The zones in the file on disk will replace any zones already defined in the list.

### See also:

[Edit Properties for Zone](#)

[Zone Statistics Table](#)

## **Edit Properties for Zone**

This dialog displays all of the properties for a single zone. Zones are defined on the Progression (or State Index) axis and provide statistics and coloring for portions of the progression.

### **Name**

Type a name for the zone. Short names are usually recommended so that they can be read when displayed on Parameter Overlay plots.

### **Active**

Check this option to enable the zone for coloring and analysis. If this option is unchecked, GemStone will ignore the zone; dots will not be colored with the zone and statistics will not be computed.

### **Show in Graphics**

When this option is checked, the zone is displayed in the Frequency plot at the bottom of the Cell Type widget.

### **Color**

Choose a color for the zone. The color is applied to dots in Parameter Profile plots and 2P histograms. It is also used as a background for the zone label on Parameter Overlay plots.

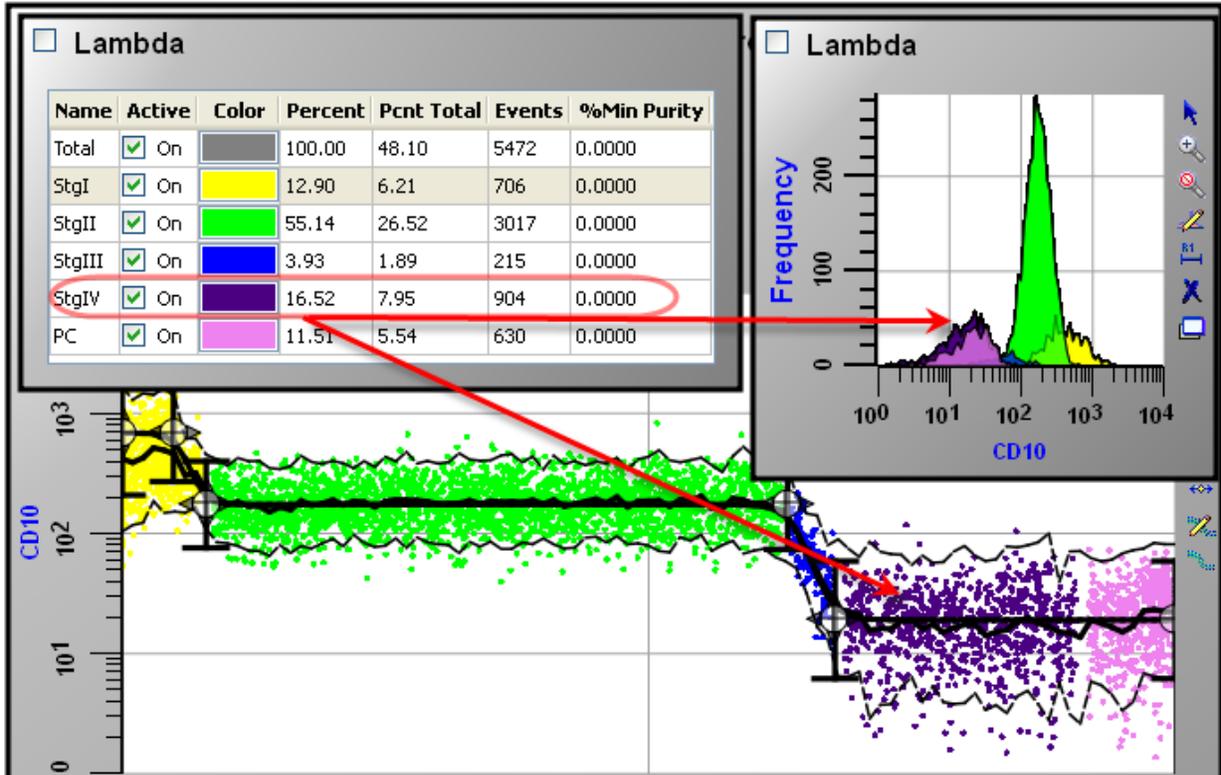
### **Parameter Intensity Stats**

When this option is enabled, GemStone computes intensity statistics within the zone for each parameter. The statistics are available for databasing and display on reports.

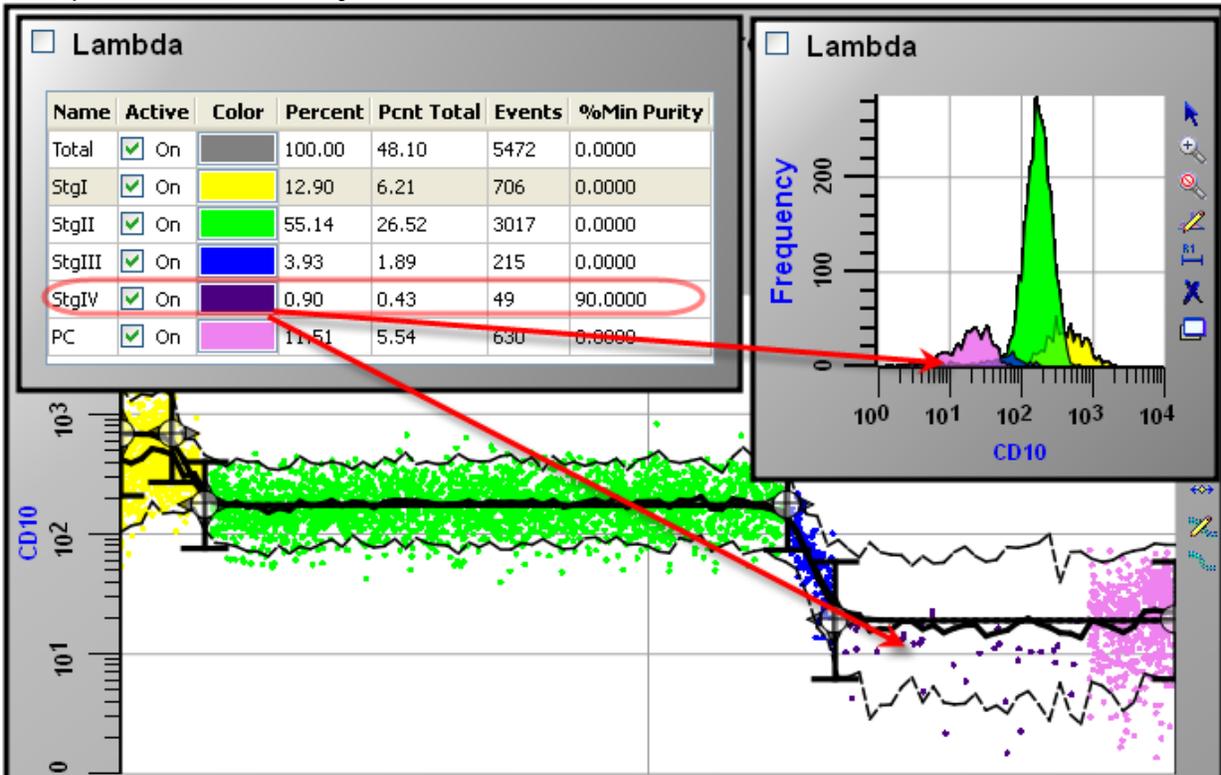
### **% Minimum Purity**

This field adjusts a probability filter for including events within the zone. By default, the value is 0.0, and any events that fall within the zone boundaries are counted as "in" the zone. A positive value for this field will eliminate events from the zone based on the standard deviation of events for a given state index location. For example, if "90.0" is entered in this field, GemStone will only include events that is determines with 90.0% certainty to fall within the zone boundaries.

Example of Minimum Purity set to 0.0



Example of Minimum Purity set to 90.0



Link

**Start Link**

Select a Control Point to link to for the *start* of the zone. Each Definition Point in the list is named based on the parameter and the position in the Parameter Profile that owns the point. Select "None" if you want to enter a fixed value for the starting location of the zone in the Start State Value field.

**Start State Value**

If the Start Link field is set to "None", enter a number between 0.0 and 100.0 to define the starting location for the zone. The value represents a percentage of the Progression axis for the starting location of the zone.

If a Definition Point is selected for Start Link, this field is disabled. It shows the location of the selected point.

**End Link**

Select a Control Point to link to for the *end* of the zone. Each Definition Point in the list is named based on the parameter and the position in the Parameter Profile that owns the point. Select "None" if you want to enter a fixed value for the ending location of the zone in the Start State Value field.

**End State Value**

If the End Link field is set to "None", enter a number between 0.0 and 100.0 to define the ending location for the zone. The value represents a percentage of the Progression axis for the ending location of the zone.

If a Definition Point is selected for End Link, this field is disabled. It shows the location of the selected point.

**Label****Vertical Location**

This option sets the vertical position of the label in the Frequency plot at the bottom of the Cell Type widget.

**Font**

This option sets the font of the label in the Frequency plot at the bottom of the Cell Type widget.

**Statistics****Percent**

This read-only field displays the percentage of events in this Cell Type that fall into this zone.

**Pcnt Total**

This read-only field displays the percentage of all events that fall into this zone.

**Events**

This read-only field displays the number of events that fall within the boundaries of this zone.

## Gating and Gate Expressions

Each Cell Type can define a gate expression that can be used to filter the events that will be considered for classification by the Cell Type's model. If gating is enabled for a Cell Type, the events that satisfy the gate are the only ones that the Cell Type examines for possible inclusion.

Edit and enable gating using the [Cell Type Properties panel](#). This panel contains a "Gate" checkbox to enable or disable gating, and a "Gate Expression" edit box to enter the gate expression.

A gate expression is a Boolean expression that can reference any regions that have been created. The regions do not need to be in the same Cell Type to be referenced by a gate expression. If the expression references a region that does not exist, the gate is effectively disabled. To avoid unexpected gating behavior, make sure that gate expressions only reference valid regions.

Expressions can contain parentheses for grouping. Regions are referenced by the region labels, e.g. "R1". The table below shows the valid elements for gating expressions.

Symbol	Name	Use
Rn	Region	Identifies a region, where <i>n</i> is the region ID. For example, R1 identifies the first region, R2 identifies the second region, etc.
(	Open parenthesis	Begin a grouping expression
)	Close parenthesis	End a grouping expression
&	Boolean "AND" is represented by the ampersand.	Events must satisfy the expressions on the left and right sides of the "&" symbol to pass the gate.
	Boolean "OR" is represented by the piping symbol.	Events can satisfy either the expression on the left or the expression on the right of the " " symbol to pass the gate.
!	Boolean "NOT" is represented by the exclamation point.	This operator reverses the result of the expression to its right. In other words, if the expression to the right evaluates to TRUE, the "!" operator changes the result to FALSE.

### Examples

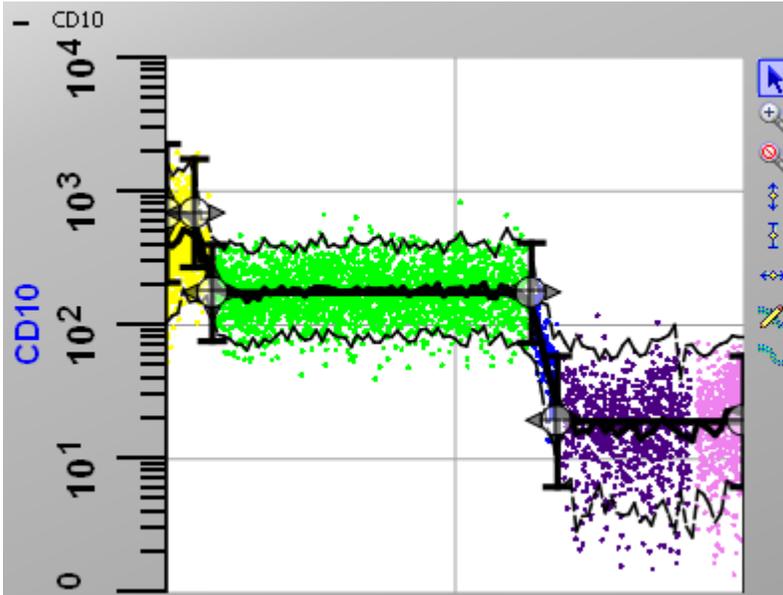
R1	Select events that fall within region R1.
R1&R2	Select events that fall within <i>both</i> R1 and R2
R1!R2	Select events that are in R1 but <i>not</i> in R2

$R1 \& \!(R2 | R3)$  Select events that are in  $R1$ , but *not* in either  $R2$  or  $R3$ .

## Parameter Profile Properties panel

### Parameter Profile

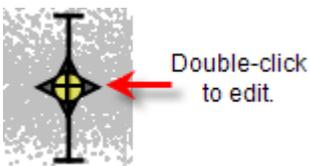
Each parameter that is added to a Cell Type for display or modeling creates a Parameter Profile in the [Cell Type widget](#).



If a Parameter Profile is selected for a parameter, this plot displays a set of **Control Points** that are used to define the profile. Also called Control Definition Points, the Control Points can be moved and have defined relationships with one another, depending on the selected profile. See [Parameter Profile Descriptions](#) for a complete list of available profiles.

When you add a parameter to a Cell Type, the parameter becomes available for display in 1P and 2P histograms, and in Overlay plots. It also appears in the Cell Type widget, where it can be simply displayed or it can be modeled.

Properties for each Control Point can be edited by double-clicking the point in a Parameter Profile plot.



### See also:

[Parameter Profile Properties panel](#)

[Parameter Profile Descriptions](#)

[Edit Control Point](#)

## Parameter Profile Properties panel

This panel is used to define the parameter profile for a parameter.

### Parameter Name

This field shows the name of the parameter associated with this parameter profile.

### Parameter Abbreviation

Enter an abbreviation to use for the parameter. This is used on parameter overlay plots, among other places in the program.

### Description

Enter a description or strategy for this parameter profile. For example, a CD10 profile for a B-cell model could be described as "Elevated in early stage, moderate in mid stage, and low in late stage" to indicate the expected progression of CD10 for normal B-cells.

### Live

If the parameter associated with this parameter profile exists in the current FCS file, this option will be checked. If unchecked, the parameter is not found in the current FCS file.

### Parameter Profile

Choose the type of profile to use for modeling this parameter. An extensive set of options is provided. Each option defines a set of Control Points with interdependencies to constrain the relative positions of the points. When modeling a parameter, choose the simplest option that describes the progression for the parameter in your experiment.

### Enable Profile

Check this option to include this parameter profile in the GemStone modeling process.

### Lock Fit

If this option is checked, the Control Points for the parameter profile will be ignored by Optimize Model, Auto Analysis, and other routines that attempt to adjust definition points to fit the data. Control Points can still be moved manually by the user.

### Lock Estimates

If this option is checked, the estimates for peaks and standard deviations for the parameter will not be modified by Auto Analysis.

### Lock Transform

Check this option to prevent the transformation used by this parameter from changing when a new file is opened.

### Line-spread Mode

This option is only enabled if the parameter is using a linear transformation.

Note: The control is disabled for HyperLog and log transformations. Constant SD mode is always selected for log-like transformations.

## Options

**Constant CV** In this mode, the line spread estimation uses Coefficient of Variation (CV). CV is similar to Standard Deviation (SD), but it is normalized on the intensity. In other words, while the SD generally increases with the intensity measurement, the CV does not.

**Constant SD** In this mode, the line spread estimation uses the Standard Deviation (SD).

### Variance Source

Select the method for estimating the variance for the parameter profile Control Points.

The variance provides a constraint on the intensity axis (Y-axis) of the parameter profile. Smaller variance values constrain the model and select a more restricted set of matching events. Larger values allow the definition points more freedom to match events. For the most accurate modeling, the goal is to match the variance with the uncertainty of the data at each Control Definition point.

Sequence is important in choosing a Variance Source. Always start with "Estimated Line Spread" when creating a parameter profile. This is the best way to provide initial estimates to the modeling system. Once the model has classified data, other options for Variance Source can be considered.

### Variance Source Options

**Estimated Line Spread** Uses the Line-spread Value as the estimated variance for all Control Points in the parameter profile.

**Observed Line Spread** Uses the last calculated variance of the data at each Control Point in the parameter profile. This variance is an average variance of definition points surrounding each Control Point.

*This option should not be selected until the model has analyzed the data at least once.*

**Manual Point Entry** Allows each definition point's variance to be adjusted independently. When this option is selected, each definition point must be selected and adjusted. This can be done one-by-one or by multiple selection of Control Points.

**Observed SDs** Uses the last calculated variance of the data at each state index in the parameter profile.

*This option should not be selected until the model has analyzed the data at least once.*

### Line-spread Value

When either Estimated Line Spread or Manual Point Entry is selected as the Variance Source, this control adjusts the variance for the Control Points. The control is disabled if the Variance Source is either Observed Line Spread or Observed SDs.

## Utility Actions

This list contains useful commands that act on the parameter profile.

### Action

Hide All Definition Points	Hides the Control Points for the parameter profile.
Show All Definition Points	Shows the Control Points for the parameter profile.
Update from Parameter Database	Takes all properties from the associated parameter in the parameter database and updates the parameter profile with them. This includes the transformation used to display the parameter.
Open...	Opens a previously saved parameter profile from disk.
Save...	Saves a parameter profile to disk.
Export model point values	Exports information about all of the model's Control Points and state indices to a text file. The file includes fields for state index, mean, SD, observed mean, and observed SD.
Edit Parameter Transformation	Select this option to display the <a href="#">Properties for Linear Transformation</a> dialog, which allows you to edit the transformation used by the parameter.
Update Parameter Transformation	If the parameter is using the HyperLog transformation, this command will re-evaluate the B-coefficient and the negative percentage based on the current data. Use this command after opening a new data file that has different population distributions.  The command has no effect on linear and log transformations.
Distribute Control Points...	[TODO]
Level Control Points...	[TODO]

## Custom Properties

### Cell Tracking Intensity Shift

For Cell Tracking parameter profiles, this option defines the magnitude of the intensity shift from one generation to another. The value is normalized to a 0 to 100 scale, so a value of 10 means that the intensity drops 10% from one generation to the next.

### Estimation Range

This value restricts the estimation routine that adjusts Control Points on the parameter intensity (Y-axis) scale. The value is normalized to a 0 to 100 scale, so a value of 10 means that the estimation routine will consider moving a Control Point as much as 10% up or down if it finds a peak within that range.

### Parameter Color

Choose a color to use when displaying this parameter in Parameter Overlay plots.

### **Allow Transitions**

Enable this option to force smooth, sigmoidal transitions in the modeled data surrounding the Control Points. The option applies to Step-Up, Step-Down, Three-Level, Four-Level, Five-Level, and the Constant Increase parameter profiles. The option requires additional processing, so analysis will take longer with the option enabled. Also note that each Control Point has a property that determines whether it supports transition logic or not. This allows some portions of the parameter profile to show smooth transitions, while other show sharp transitions.

When this option is unchecked, the transitions at Control Points are sharper and more abrupt. The default for this option is unchecked, providing faster analysis results.

### **Allow Interpolation**

Normally, the sloped portions of a parameter profile are straight lines. When this option is enabled for a parameter using a log or HyperLog transform, the sloped portions of the profile will be linearly interpolated between the control points.

### **Block Initialization**

Enable this option to prevent the Auto Analysis system from initializing this parameter profile fit information.

### **Store Definition Points**

Enable this option to store the positions of the Control Points for this parameter profile in the information system. Once stored, the positions become available to the database and tables for reporting.

### **Auto Analysis**

#### **Pause and Continue**

Enable this option to force Auto Analysis to pause after it adjusts the profile for this parameter. This allows you to review the changes Auto Analysis makes and to make other adjustments before it continues to the next parameter.

#### **Paused Delay**

Enter the number of seconds to pause.

### **Read-only properties**

#### **Average Obs SD**

This displays the average of the observed standard deviations.

#### **Lookup Param Name**

This displays the name that was used to find the parameter in the Parameter Database.

### **See also:**

[Parameter Profile](#)

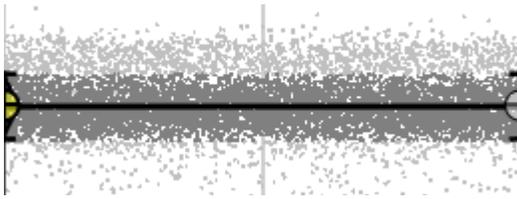
[Parameter Profile Descriptions](#)

## Parameter Profile Descriptions

The Parameter Profiles available in GemStone provide great flexibility for modeling different intensity characteristics for parameters in the progression being modeled.

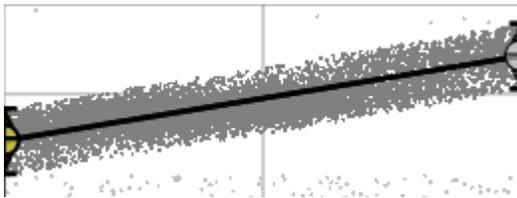
The general rule for choosing a profile is to choose the simplest one that describes the intensity changes you expect to find in your samples. Start with a simple profile, and then as the model becomes well-defined and additional structure appears, a more appropriate profile can be selected.

### Constant



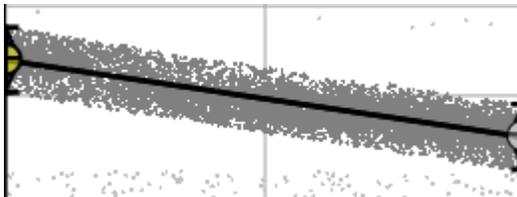
This profile has 2 Control Points that are linked to the same intensity. It is most often used to select events for a subset.

### Increasing



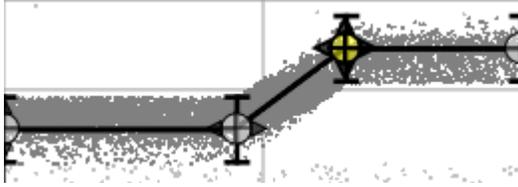
This profile has 2 Control Points. The first point is forced to have a lower intensity than the second. It can be used on Time parameters to order events based on acquisition time.

### Decreasing



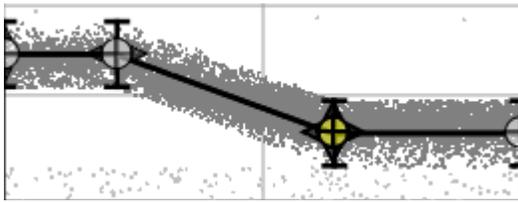
This profile has 2 Control Points. The first point is forced to have a higher intensity than the second.

### Step Up



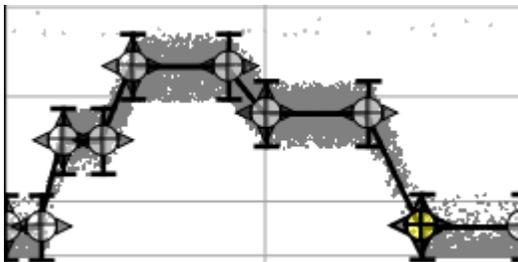
The Step Up profile has 4 Control Points. The first 2 points are linked to have the same intensity values. The last 2 points are also linked, and are forced to have a *higher* intensity than the first two. It is used for parameters that are up-regulated in the progression being modeled.

### Step Down



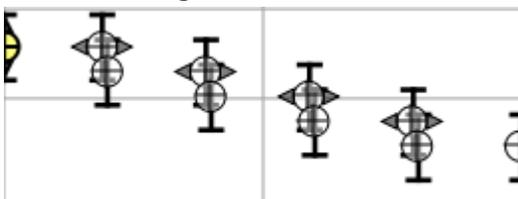
The Step Down profile has 4 Control Points. The first 2 points are linked to have the same intensity values. The last 2 points are also linked, and are forced to have a *lower* intensity than the first two. It is used for parameters that are down-regulated in the progression being modeled.

### Three, Four, Five Levels



These profiles are very flexible, used to define 3, 4, or 5 levels of intensity. For each level, there are 2 Control Points that are linked to the same intensity for the parameter. Each of the levels has no dependencies on the other levels. In other words, the next level can be higher, lower, or even the same intensity as its neighboring level.

### Cell Tracking 5, 6, 7, 8

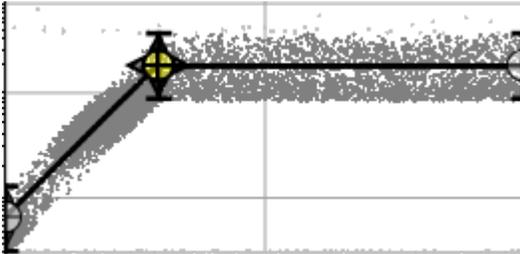


These profiles are used for modeling cells stained with cell-tracking dyes, like CFSE

and PKH-26. With these dyes, there is a well-defined relationship of intensity from one level to the next. When the cells divide, each daughter cell takes half of the cell-tracking dye. Therefore, the intensity drops by 50% from one generation to the next.

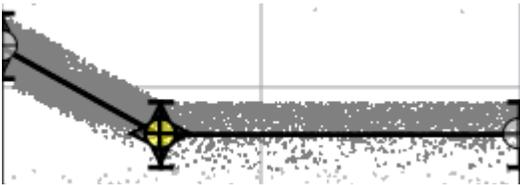
When choosing between profiles for 5, 6, 7, and 8 generations, choose the one that will accommodate the divisions in the full course of the experiment.

### Increase and Level out



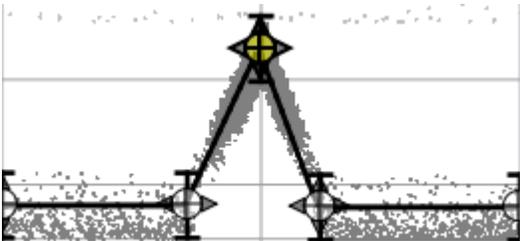
This profile has 3 Control Points. The first point is forced to have a lower intensity than the others. The second and third points are forced to have the same intensity values. It is used for parameters that up-regulate at the beginning of the progression, and then level off.

### Decrease and Level out



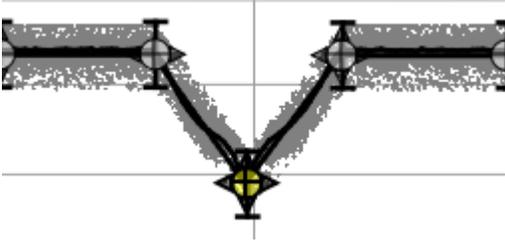
This profile has 3 Control Points. The first point is forced to have a higher intensity than the others. The second and third points are forced to have the same intensity values. It is used for parameters that down-regulate at the beginning of the progression, and then level off.

### Up Pulse



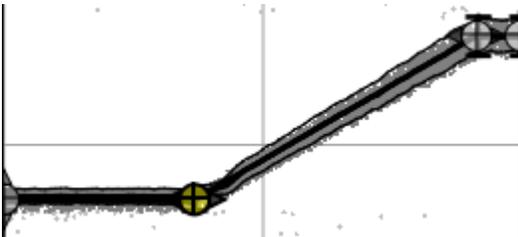
This profile has 5 Control Points that are used to model an up- and down-regulation in the progression. The first 2 points are forced to be on the same intensity level. The third point is forced to be higher than the others. The last two points are locked to the same intensity level, but the level can be different than the first level.

### Down Pulse



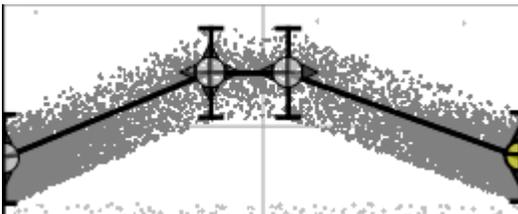
This profile has 5 Control Points that are used to model a down- and up-regulation in the progression. The first 2 points are forced to be on the same intensity level. The third point is forced to be lower than the others. The last two points are locked to the same intensity level, but the level can be different than the first level.

### Constant Increase Two-fold Constant (DNA)



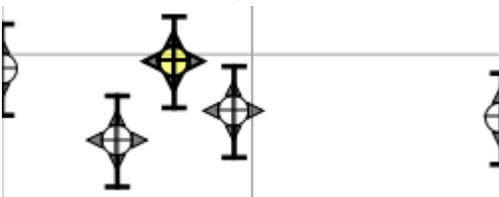
This profile is similar to the Step Up profile, but is specifically designed to be used with linear parameters that have a 2-fold increase in the progression. It is most commonly used for DNA parameters, where the G2-M intensity is twice that of the G0-G1 intensity.

### Up Constant Down



This profile uses 4 Control Points to model an up-regulation, leveling off, and ending with a down-regulation.

### Custom 2 through 10 Point



These profiles are extremely flexible. There are no linkages or dependencies on the points, so the shapes they can model are very open ended. In most cases, it is best to choose a simpler, more constrained Parameter Profile instead of a Custom profile. In

general, simpler profiles create more robust models.

## Edit Control Point

This dialog is displayed by double-clicking a Control Point on a Parameter Profile plot. It is used to view and edit properties of the Control Point.



Each Control Point has 3 editable characteristics: its parameter intensity value (Param), its state index value (State), and its standard deviation value (SD). Each of these has a value and a Fit Status flag that indicates whether the automatic adjustment routines like Auto Analysis can adjust the setting. Additional settings can be edited by selecting the item and clicking the Properties button below the list. This displays the [Properties for Param, State, and SD](#) dialog box.

This dialog also provides a convenient way to create Zones based on this Control Point. Use the **Create Zone** buttons to create a zone between the current Control Point and the end of the previous zone or to the beginning of the next zone.

### See also:

[Parameter Profile](#)

[Properties for Param, State, and SD](#)

[Zone Statistics Table](#)

## Properties of Control Point

This dialog exposes properties for a Control Point. It is accessed through the [Edit Control Point dialog](#).

### **Allow To Transition**

Enabling this property causes GemStone to use a sigmoidal transition between this Control Point and the next. If this property is disabled, the transition is linear from one Control Point to the next.

### **Linearize the Next Transition**

Enabling this option causes GemStone to linearize the transition between this and the next Control Point.

## **Properties for Param, State, and SD**

This dialog allows properties for one of the Control Point characteristics to be edited. The same dialog is used to edit the point's parameter intensity value (Param), its state index value (State), or its standard deviation value (SD).

### **Value**

This displays the current value of the object being edited. If it is a Param object, it shows the intensity (y-axis) position of the control point in the parameter. If it is a State object, it shows the state index (x-axis) position of the control point. If the object is an SD, it shows the current standard deviation of events in the state index where the control point is located.

### **Fit Status**

If checked, the point's value can be adjusted by GemStone's fitting routines

### **Can Change**

Uncheck this property to prevent the value from changing by any means.

### **Value Type**

This field describes the type of value represented.

### **Average Value**

This field shows the average value for events falling in the same state index. The value will always be 0.00 when editing State.

### **Edit Relations**

Click this button to display the Edit Relations dialog. This dialog is used to define relationships between control points.

## Edit Relations

This dialog is used to define relationships between control points.

Note of caution: This is considered an advanced feature. Because any definition point can have a relationship with any other point, relationship trees can be very deep and even circular. Only create or edit relations when you have a clear understanding of the existing relations of control points.

Relations between points are used to provide restrictions on movement of the points. The relationships can affect a point's frequency, state index, or standard deviation. Typically, the relationships are relative to other definition points in the same parameter profile. However, this is not a requirement; relationships can span profiles and even cell types to coordinate the position and size of control points.

### If you want to...

Edit an exiting relation	Select the relation in the list, and then click the Properties button. This will display the <a href="#">Edit Properties for Relation</a> dialog.
Create a new relation	Click the New button. When the new Relation object is created, click the Properties button to edit the relation's properties.
Delete a relation	Select the relation in the list, and then click the Delete button.
Save a set of relations	Click the Save button. The standard File Save dialog will be displayed, allowing you to navigate to the desired location and save the file.
Open a set of relations	Click the Open button. The standard File Open dialog is displayed, allowing you to select the previously saved relation file.

### See also:

[Edit Properties for Relation](#)

## Edit Properties for Relation

This dialog allows editing of a relationship between one control point and another. It is accessed from the [Edit Relations](#) dialog.

Note of caution: This is considered an advanced feature. Because any definition point can have a relationship with any other point, relationship trees can be very deep and even circular. Only create or edit relations when you have a clear understanding of the existing relations of control points.

The relationship for the control point is an expression that involves the properties of this dialog:

This point's value must be **[Relation To Link] [Link Name] times [Multiplier] plus [Offset]**.

For example:

This point's value must be **less than CellType1:SSC:BeginUp times 1.0 plus 0.0**.

### Name

This is a read-only field containing the name of the relation. It is automatically assigned by the system when the relation is created.

### Enable

The relation can be enabled and disabled using this checkbox. This allows relations to remain in the list but disabled.

### Relation to Link

Choose the relationship from the list of options.

### Link Name

Choose the definition point with which to define a relationship.

### Multiplier

By default, this value is 1.0. If a value other than 1.0 is entered, the multiplier is applied to the value of the link prior to making the comparison.

### Offset

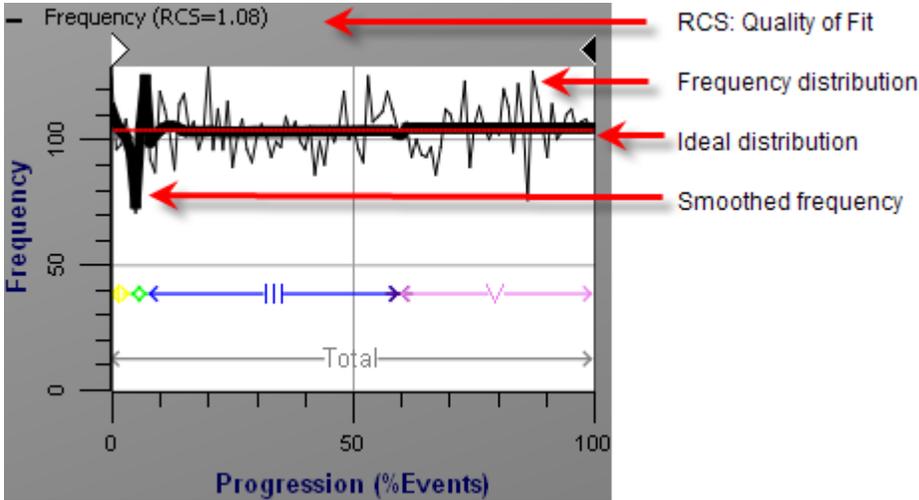
By default, this value is 0.0. If a value other than 0.0 is entered, the offset is added to the value of the link after any Multiplier and prior to making the comparison.

### See also:

[Edit Relations](#)

## Frequency Plot

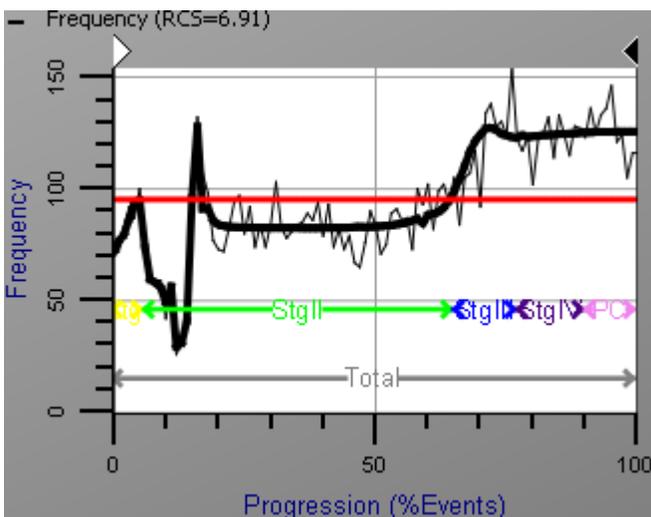
The Frequency Plot appears at the bottom of the Cell Type widget. It provides information about how well the model is fitting the data.



At the top of the plot, the RCS value is a measure of quality of fit. Generally, values between 1 and 7 are pretty good, and higher values indicate that the model is not properly characterizing the data.

The number of events classified into each state of the progression is plotted as the **frequency distribution**, which will typically bounce above and below the **ideal distribution** shown as a straight, red line. The **smoothed frequency distribution** shows an averaged distribution.

The goal is for the model to evenly distribute events in all states along the progression axis. Therefore, if the model is fitting properly, the frequency distribution will closely follow the ideal distribution horizontally. In case where the model does not match as well, the frequency distributions will show areas where they differ from the ideal significantly.



Also shown on the Frequency plot are zone boundaries for all active zones in the cell type.

**See also:**

[Properties of Frequency Plot](#)

## Properties of Frequency Plot

This dialog is displayed to edit the graphics for the [Frequency Plot](#).

### Frequency

#### Show

Enable this option to display the frequency line.

#### Line width

Choose a line width.

#### Line Style

Choose a line style.

### Smooth Frequency

#### Show

Enable this option to display the smooth frequency line.

#### Line width

Choose a line width.

#### Line Style

Choose a line style.

### Average Frequency

#### Show

#### Show

Enable this option to display the average frequency line.

#### Line width

Choose a line width.

#### Line Style

Choose a line style.

### State Index Fidelity

#### Show

Enable this option to display the state index fidelity.

#### Line width

Choose a line width.

#### Line Style

Choose a line style.

**Scale Factor**

Select a scaling factor for the fidelity line.

**Fidelity Color**

Select a color for the fidelity line.

**State Index Uniqueness**

**Show**

Enable this option to display the uniqueness plot.

**Model View**

Enable this option to use the model's values for the line. Uncheck to use the data values.

**Uniqueness Line Width**

Choose a line width.

**Uniqueness Line Style**

Choose a line style.

**Uniqueness Scale Factor**

Select a scaling factor for the uniqueness line.

**Uniqueness Color**

Select a color for the uniqueness line.

**Edit Zone**

**Show**

Enable this option to display zone boundaries on the plot.

**Edit Zones**

Click this button to edit the zones.

## Graphic Objects

### Cell Type Canvas

The Cell Type canvas is the workspace that contains GemStone graphical objects, like the Cell Type Widget, 1P and 2P histograms, and the Zone Statistics table. Objects can be moved and resized on the canvas, and reports are generated from the objects that reside on it.

#### See also:

[Cell Type Canvas Context Menu](#)

## Cell Type Canvas Context Menu

This menu is displayed when the mouse is right-clicked on the [Cell Type Canvas](#).

### **Edit Canvas Properties**

This command displays a dialog with properties of the canvas. Use the dialog to control grids, conform mode, zooming, and how the object is copied and saved to disk. See [Edit Canvas Properties Dialog](#) for details.

### **Edit Background Page Properties**

This command displays a dialog with properties of the background page. You can set the page size, margins, and a number of other background page properties. See [Edit Background Page Properties Dialog](#) for details.

### **Copy Page to Clipboard**

This command copies the entire canvas to the clipboard as a bitmap. Use Edit Canvas Properties to determine the size and resolution of the bitmap.

### **Paste**

This command pastes an object from the clipboard onto the canvas.

### **Save Page to Disk**

This command saves the canvas to disk in the format specified in Edit Canvas Properties.

### **Copy Selected Item to Clipboard**

This command copies the selected object on the canvas to the clipboard as a bitmap. Use Edit Canvas Properties to determine the size and resolution of the bitmap.

### **Save Selected Item in Standard Format**

This command saves the selected object on the canvas to disk in the format specified by Edit Canvas Properties.

### **Print Selected Item**

This command prints the selected object on the canvas to the printer.

### **Print**

This command prints the entire canvas to the printer.

### **Save Image Flag**

If this option is enabled, the canvas will be saved to disk automatically during batch processing.

## **Edit Canvas Properties Dialog**

This dialog edits the properties of the canvas. Use the dialog to control grids, conform mode, zooming, and how the object is copied and saved to disk.

### **Grid Snap**

When enabled, objects on the canvas move and size based on the grid. This makes it easier to align objects.

### **Grid Size**

This property determines the size of each grid element, in pixels. It is only used when Grid Snap is enabled.

### **Angle Snap**

When enabled, objects rotate on fixed increments to assist in alignment.

### **Angle Snap Size**

This property determines the number of degrees of rotation when Angle Snap is enabled.

### **Zoom Percent**

This command adjusts the zooming of the canvas. Choose a size from the list, or enter a custom zoom value by typing.

### **Conform Mode**

When enabled, some objects on the canvas will conform to the edges of other objects when they are moved. This is most useful for regions on 2P plots, where it is desirable to have adjacent analysis areas.

### **Bitmap Operations**

#### **Copy DPI**

Type the number of dots per inch (DPI) to use when copying and saving graphic objects. Higher values produce finer graphics, but also require more memory. The default value is 300.

#### **X and Y Screen DPI**

These fields show the DPI for the screen. They are not editable.

#### **Saving Format**

Choose a graphic file format to use when saving graphics to disk. All supported formats appear in the list.

#### **Saved Image Path**

Choose a location on disk to use for saving graphics on this canvas.

#### **Save Image**

Enable this property to flag this object to be saved to disk during batch processing.

## **Edit Background Page Properties Dialog**

This dialog edits the properties of the background page. You can set the page size, margins, and a number of other background page properties.

### **Measurement Units**

Choose English or Metric units.

### **Percent Zoom**

This field displays the current zoom of the canvas.

### **Page Size**

#### **Page Width and Height**

Set the overall width and height of the page. These settings do not have to match the paper size for your printer. The program will scale the page size to the actual paper size when you print.

### **Margins**

#### **Show Mat**

Enable this option to see the mat area surrounding the page. This is useful if you want to drag some objects off the page so that they are not printed.

#### **Mat Left, Right, Top and Bottom Margin**

Set the margins to use for the mat. These values are added to the page size to determine the actual working area on the screen.

### **Colors**

#### **Page Color**

Select a color for the page.

#### **Mat Color**

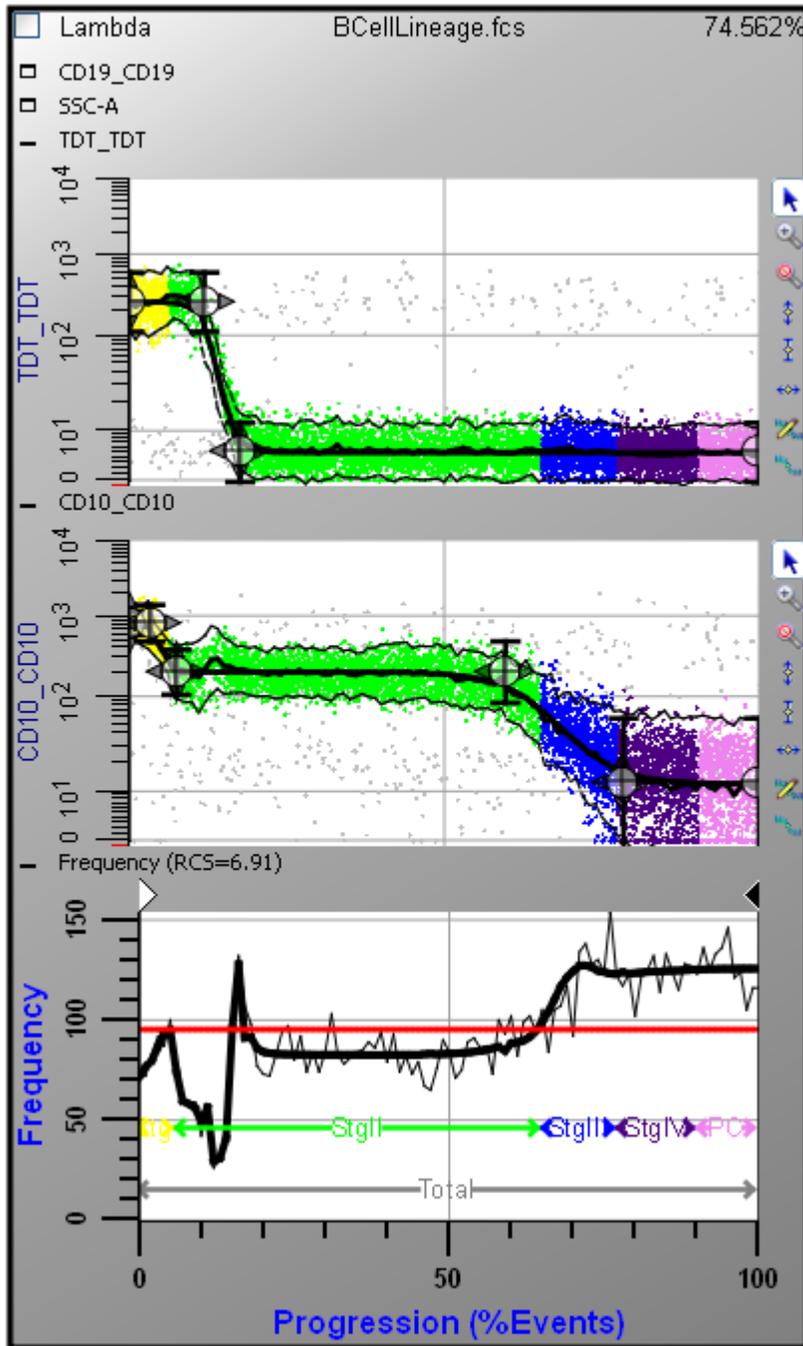
Select a color for the mat.

#### **Shadow Color**

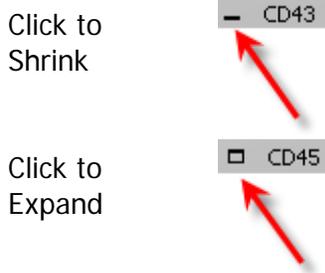
Select a color for the shadow.

## Cell Type Widget

The Cell Type widget contains the set of Parameter Profile plots that represent one Cell Type in the model. In addition, a Frequency Plot appears at the bottom of the widget to provide information about how well the cell type is being modeled.



You can expand and collapse parameter profiles within the widget for better visualization of the data. To collapse a profile that is showing, click the "shrink" icon next to the parameter name on the left edge. To expand a profile that is hidden, click the "expand" icon next to the parameter name.



When you click on a parameter profile within the collection of profiles, the profile becomes the active profile and its properties appear in the Parameter Profile Properties panel. See [Parameter Profile](#) and [Parameter Profile Properties panel](#) for more about parameter profiles.

After you click on a parameter profile in the Cell Type Widget, you can use the **PgUp** and **PgDn** keys on the keyboard to move to the next or previous parameter profile. This method automatically opens the next (or previous) profile and activates it.

The Frequency Plot provides valuable information for understanding how well the model for this cell type is working. The Y-axis shows frequency of events, and the X-axis shows the Progression axis (normally 100 states). A bold red horizontal line shows the optimal frequency across the progression axis. The optimal frequency is computed by dividing the number of events by the number of states on the Progression axis. In other words, the ideal model would put the same number of events into each state of the progression.

The jagged black line shows the actual number of events that have been assigned to each state for this cell type. For a model that matches the data well, the black line will follow the red line, bouncing slightly over and under the red line. In places where the black line is significantly *above* the red line, there are too many events being assigned to those states. Conversely, where the black line falls well *below* the red line, there are not enough events assigned. This relationship helps identify which Parameter Profiles need to be adjusted. In general, when there are too many or too few events in a portion of the Frequency Plot, look for Parameter Profiles with control points and transitions in those states. Examine those profiles to be sure the control points are properly positioned and the other characteristics of the Parameter Profile are appropriate for the model.

The Frequency Plot also shows the Reduced Chi Square (RCS) of the model and the zones that have been defined.

## Properties of Cell Type Widget

This dialog is displayed when the [Cell Type Widget](#) is double-clicked. It displays a number of properties for the object that can be edited.

### Save Image

If this property is enabled, the Cell Type Widget will be saved to disk as an image file when any Save Graphics command is triggered. Save Graphics is an option in batch processing, and is also available in the [Workspace toolbar](#).

### Position and Size

#### X Position, Y Position, X Size, Y Size

These fields show the position and size of the object, in inches. The values can be directly edited. They are also updated when the object is moved or resized using the mouse.

### Other Properties

#### Background

Select a background style for the Cell Type widget.

#### Title Font

Select a title font for the Cell Type widget

### See also:

[Cell Type Widget](#)

### **Graphic Options for Parameter Profile: Surface**

This tab is part of the Edit Graphic Options for Parameter Profiles. It configures the display of the Surface plot.

#### **Enable Surface**

Check this option to display the surface graphics.

#### **Probability Mixing Color**

Choose a color that will be used to for the densest portions of the surface plot (the highest probability). The default color is white.

## Graphic Options for Parameter Profile: Dots

This tab is part of the Edit Graphic Options for Parameter Profiles. It configures the display of dots.

### Enable Dots

Check this option to display dots on the plot. (The 2P histogram can display other kinds of graphics, which are selected on other tabs in this dialog.)

### Auto Dot Size

Enable this option to allow GemStone to determine the best dot size, based on the current size of the histogram object and other factors.

### Dot Size

This field displays the current size of dots in points; a point is 1/72 inches. To set a specific size for the dots, uncheck the Auto Dot Size option and enter a value between 0.0 and 5.0 for Dot Size.

### Show All Dots

Enable this option to display dots for all events. If this option is unchecked, the system will use the value in Desired Dot Number.

### Desired Dot Number

If Show All Dots is unchecked, the system will use the value in Desired Dot Number to determine the number of dots to display in the plot.

### Probability of Inclusion

This setting determines how dots are display when showing Surface plots and Dots simultaneously. With the default setting of 0.05, only the 5% outlier dots will be drawn. This setting allows the Surface plot to highlight the dense portions of the plot.

### Other Dots

#### Show Other Types

This option allows dots from one or all other cell types to be display in the plot.

#### Show Unclassified Events

Enable this option to display dots for unclassified events.

## **Graphic Options for Parameter Profile: Means**

This tab is part of the Edit Graphic Options for Parameter Profiles. It configures the display of means of the classified events.

### **Enable Means**

Check this option to display a line plotting the mean of classified events in the Parameter Profile.

### **Line Width**

This option controls the thickness of the line used to plot the mean. Enter a value from 0 to 10 inclusive.

### **Line Style**

Choose a line style for the mean line.

## Graphic Options for Parameter Profile: Confidence Limits

This tab is part of the Edit Graphic Options for Parameter Profiles. It configures the display of confidence limits for the classified events.

### Enable

Check this option to display confidence limit lines on the Parameter Profile plot.

### Line Spread Mode

Choose a method for visualizing line spread.

### Line Spread Mode Options

Model SD Bars

Displays the standard deviation bars at each Control Point.



Model SD Envelope

Displays the standard deviation of the model as an envelope.



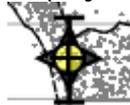
Observed SD Envelope

Similar to Model SD Envelope, except that the standard deviation of the actual data values is used.



SD Bars and Observed Envelope

Displays the Model SD bars and the Observed Envelope.



### Number of SDs

This option controls the number of standard deviations (SDs) used for the display. The default value is 1.96, equivalent to 95% confidence limits.

### Line Width

This option controls the thickness of the line. Enter a value from 0 to 10 inclusive.

### Line Style

Choose a line style for the line.

### Color

Choose a color for the line.

### **Graphic Options for Parameter Profile: Model**

This tab is part of the Edit Graphic Options for Parameter Profiles. It configures the display of a line that connects each Control Point in the selected Parameter Profile.

#### **Enable Model**

Check this option to display the model line on the Parameter Profile plot.

#### **Line Width**

This option controls the thickness of the line. Enter a value from 0 to 10 inclusive.

#### **Line Style**

Choose a line style for the line.

## Graphic Options for Parameter Profile: Animate

This tab is part of the Edit Graphic Options for Parameter Profiles. It configures the animation options for the Parameter Profile.

The Parameter Profile Plot can display additional information when animation is enabled in the [Cell Type Properties panel](#). When Cell Type animation is enabled, the plot will display two vertical cursors that move along the progression axis.

### Enable Animation

Check this option to enable animation for the Parameter Profile plot.

### Line Width

This option controls the thickness of the line. Enter a value from 0 to 10 inclusive.

### Line Style

Choose a line style for the line.

### Color

Choose a color for the line.

### Animation Data Source

When Animation is enabled, it can position its cursors based on either Mean Data or Model Data. Mean Data uses the mean for the classified events at each state index. Model Data uses the model's position based on Control Points at each state index.

## **Other Graphic Options**

This set of properties appears on the "Other Options" tab in the dialog used for editing a 1P or 2P histogram. You can view and edit these properties by double-clicking a histogram or by right-clicking a histogram and choosing "Edit Graphics".

Select the "Other Options" tab to view these properties.

### **Background Color**

Choose a color for the background of the plot.

### **Show X Grid Lines**

Enable this option to show grids for the X-axis. Grid lines will appear for each major tic on the axis.

### **Show Y Grid Lines**

Enable this option to show grids for the Y-axis. Grid lines will appear for each major tic on the axis.

### **Grid Line Width**

Enter a value for the width of the grid lines. The default, 0, creates the narrowest possible line that the screen (or printer) can draw.

### **Grid Line Style**

Choose a line style for grid lines.

### **Grid Color**

Choose a color for grid lines.

### **Grids On Top**

Enable this option to draw grids on top of the other graphics in the plot. When the option is unchecked, grids are drawn behind the other graphics.

## **Background Image**

A plot can display a background image, on top of which the other graphics are displayed. This can be useful, for example, to present a comparison of the current analysis with a previous analysis that was saved as an image to disk.

### **Image Transparency**

Enter a value between 0.0 and 100.0 to define how transparent a background image appears. Higher values produce more transparency.

### **Open Image**

Click this button to select an image file from disk. This file will be opened and displayed as the background image for the plot.

### **Clear Image**

If an image has been opened using the Open Image option, the Clear Image option removes the images from memory.

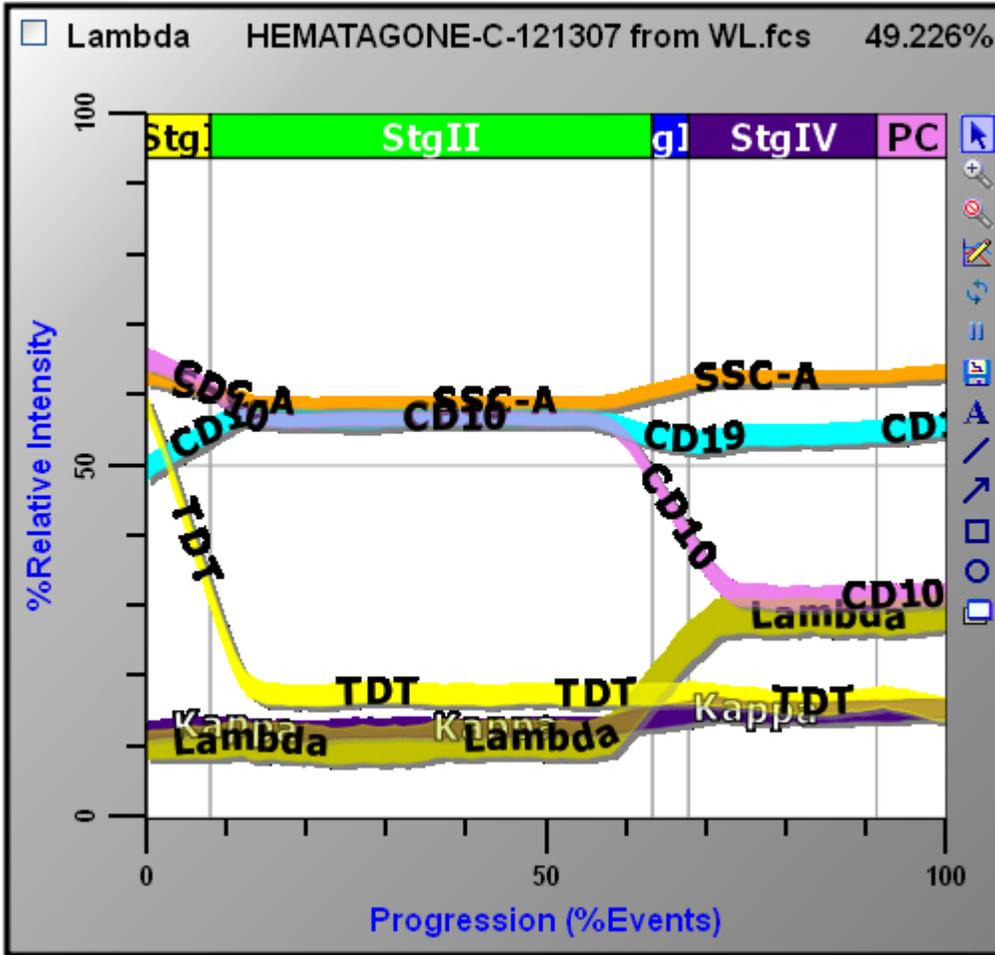
**See Also:**

[1P Histogram](#)

[2P Histogram](#)

### Parameter Overlay Plot

The Parameter Overlay Plot displays the modeled progression for all parameters simultaneously.



The cell type, file name, and percentage of events in the cell type are displayed in the area at the top of the plot. Each cell type can have its own Parameter Overlay plots, but it can also include parameters from other cell types, too.

The checkbox at the top left of the plot is the "save graphic" checkbox. Check this box if you want the graphics from the overlay plot to be saved when you click the Save Graphics button on the [Workspace toolbar](#).

The X-axis of the plot is the Progression axis, or "state index" axis. The Y-axis is a relative intensity scale from 0 to 100, which allows linear, log, and HyperLog transformed parameters to be compared on the same plot.

If zones have been defined, they are displayed along the top edge of the ribbon display area.

Each parameter appears as a "ribbon" in the plot, with text labels identifying the parameter name. The color for each ribbon is determined by the Parameter Color property in the [Parameter Profile Properties panel](#).

Tools to manipulate the plot appear on the right edge of the plot window. See [Overlay Graphics toolbar](#) for a description of each tool.

**If you want to...**

Create a new Parameter Overlay Plot	Click the Overlay Plot button in the <a href="#">Workspace toolbar</a> , and then click and drag to define the area where the plot should be displayed.
Edit the properties of the Parameter Overlay Plot	Double-click within the ribbon display area, or right-click and choose Edit Graphics.
Delete the Parameter Overlay Plot	Select the plot and press the Delete key.
Create a free-floating window with the Parameter Overlay plot	Click the Create Dialog button in the <a href="#">Overlay Graphics toolbar</a> .

**See also:**

[Graphic Options for Parameter Overlay Plots](#)

[Other Options for Parameter Overlays](#)

[Workspace toolbar](#)

[Overlay Graphics toolbar](#)

## Graphic Options for Parameter Overlay Plots

Each parameter in a Parameter Overlay Plot has a set of graphic options that determine how it appears. You can view and edit these properties by double-clicking a Parameter Overlay plot, or right-clicking a Parameter Overlay plot and choosing "Edit Graphics".

### Show Parameter

Enable this option to include the parameter in the Overlay Plot. This does not affect whether or not the parameter is included in the modeling process.

### Line Style

Select a style for the line that borders the parameter ribbon in the Overlay Plot.

### Brush Style

Select a style for the fill used for drawing the parameter ribbon in the Overlay Plot.

### OK to Smooth

Enable this option to apply smoothing to graphics. Uncheck this option to display the unsmoothed values from the model.

### Show All Cell Types

With this option enabled, this overlay plot will display one ribbon for the selected parameter for each of the cell types that have been defined. To assist with identification, the cell type abbreviation is used as a prefix for the ribbon labels. For example, if you wanted to view a "CD10" parameter ribbon for two cell types, "Kappa" and "Lambda", you would enable this option. One ribbon would be labeled "Kappa-CD10" and the other "Lambda-CD10".

### Parameter Color

This read-only property shows the color used for this parameter. To edit the color, use the "Parameter Color" property in the [Parameter Profile Properties panel](#).

## Other Options for Parameter Overlays

This set of properties appears on the "Other Options" tab in the dialog used for editing a Parameter Overlay Plot. You can view and edit these properties by double-clicking a Parameter Overlay plot, or right-clicking a Parameter Overlay plot and choosing "Edit Graphics".

Select the "Other Options" tab to view these properties.

### Background Color

Choose a color for the background of the Parameter Overlay plot.

### Show X Grid Lines

Enable this option to show grids for the X-axis. Grid lines will appear for each major tic on the axis.

### Show Y Grid Lines

Enable this option to show grids for the Y-axis. Grid lines will appear for each major tic on the axis.

### Grid Line Width

Enter a value for the width of the grid lines. The default, 0, creates the narrowest possible line that the screen (or printer) can draw.

### Grid Line Style

Choose a line style for grid lines.

### Grid Color

Choose a color for grid lines.

### Grids On Top

Enable this option to draw grids on top of the parameter profile ribbons. When the option is unchecked, grids are drawn behind the ribbons.

### Background Image

An overlay plot can display a background image, on top of which the parameter ribbons are displayed. This can be useful, for example, to present a comparison of the current analysis with a previous analysis that was saved as an image to disk.

### Image Transparency

Enter a value between 0.0 and 100.0 to define how transparent a background image appears. Higher values produce more transparency.

### Open Image

Click this button to select an image file from disk. This file will be opened and displayed as the background image for the Overlay Plot.

### Clear Image

If an image has been opened using the Open Image option, the Clear Image option removes the images from memory.

## Labeling

### Type of Labeling

Choose an option for labeling the parameter ribbons on the overlay plot.

Option	Description
Wire Labeling	Labels follow the ribbon.
Zone Labeling	One label appears for each ribbon in each zone.

### Number of SDs

This value controls the width of the parameter profile ribbons in the overlay plot. The value represents the number of standard deviations from center of the ribbon (either the mean of classified events or the value from the model). The default value is 0.20 SDs.

### Label Font

Choose a font to use for the labels on the parameter profile ribbons.

### Show Abbreviation

Enable this option to use the abbreviation for the parameter labels on the ribbons. If the option is not enabled, the full parameter names are used.

## Additional Properties

### Data Type

Option	Description
Model +/- SD	The parameter ribbon is based on the model's Y-axis position at each state in the progression. This value approximately matches the Control Point locations for the Parameter Profile of each parameter, with interpolation between control points. The width of the ribbon is controlled by the Number of SDs property.
Mean +/- SD	The parameter ribbon is based on the mean intensity of the data at each state in the progression. The width of the ribbon is controlled by the Number of SDs property.

### Transparency

Enter a value between 0.0 and 100.0 to control the transparency of the parameter profile ribbons. Higher values make the ribbons more transparent.

### Smooth Data

If "Mean +/- SD" is selected for Data Type, this option will smooth the data displayed in the overlay plot. The option has no effect if "Model +/- SD" is selected for Data Type.

### Smooth Label

For wire labeling, smooths the angles for the labels.

### **Drop Shadow**

Enable this option to display a drop shadow under each parameter profile ribbon in the overlay plot.

### **Show Zones**

Enable this option to display zones at the top of the overlay plot.

### **Show Zone Boundaries**

Enable this option to display "grid lines" for the zone boundaries. Note, "Show Zones" must be enabled for this option to function.

## **Animation**

The Overlay Plot can animate the process of drawing the parameter profile ribbons. The settings below control that animation.

### **Seconds**

This option controls the number of seconds each parameter profile ribbon is displayed when animation is enabled in the overlay plot. Animation is turned on and off using tools in the [Overlay Graphics toolbar](#).

### **One at a Time**

With this option enabled, each parameter ribbon is displayed by itself during animation. If this option is unchecked, each ribbon remains on the plot until all ribbons have been drawn. Then the plot is cleared and drawing starts again.

### **Cell Type Animation**

The Overlay Plot can display additional information when animation is enabled in the [Cell Type Properties panel](#). When Cell Type animation is enabled, the overlay will display two vertical cursors that move along the progression axis.

### **Show Animation**

Enable this option to display a pair of moving cursors when the cell type is animating. The cursors define a window on the progression, and many graphical objects make use of these cursors to show animation for the cell type.

### **Line Width**

Choose a width for the animation cursors.

### **Line Style**

Choose a line style for the animation cursors.

### **Color**

Choose a line color for the animation cursors.

## **Smoothing**

If the Smooth Data option is enabled, these properties define characteristics of the smoothing algorithm.

**Number of Points**

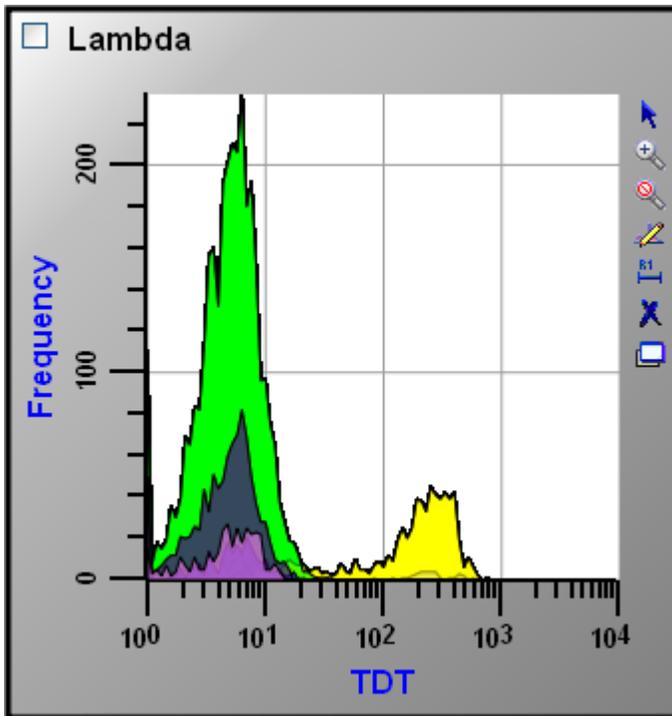
This value controls the degree of smoothing by determining how far to the left and the right of a given state to include in the smoothing routine. Larger values result in greater smoothing. The default value is 6.

**Polynomial Number**

This is the order of the polynomial to use for smoothing. The default is 1, and the range is 1 to 4.

## 1P Histogram

The 1P Histogram plot displays a histogram for a selected parameter. Any number of 1P Histograms can be created.



The name of the cell type is displayed in the area at the top of the plot. Each cell type can have any number of 1P histograms, but it can also include parameters from other cell types, too.

The checkbox at the top left of the plot is the "save graphic" checkbox. Check this box if you want the graphics from the histogram to be saved when you click the Save Graphics button on the [Workspace toolbar](#).

The X-axis of the plot shows the selected parameter in the transformation selected. The Y-axis is frequency of events. Click the X-axis label to select a different parameter; a menu of available parameters will be displayed.

Tools to manipulate the plot appear on the right edge of the plot window. See [1P Graphics toolbar](#) for a description of each tool.

### If you want to...

Create a new 1P Histogram

Click the 1P Histogram button in the [Workspace toolbar](#), and then click and drag to define the area where the histogram should be displayed.

Edit the properties of the 1P Histogram

Double-click within the histogram display area, or right-click and choose Edit Graphics.

Delete the 1P Histogram

Select the plot and press the Delete key.

Create a free-floating window  
with the 1P Histogram plot

Click the Create Dialog button in the [1P Graphics toolbar](#).

**See also:**

[Graphic Options for 1P Histograms](#)

[1P Animation Options](#)

[Other Graphic Options](#)

[Workspace toolbar](#)

[1P Graphics toolbar](#)

## Graphic Options for 1P Histograms

This set of properties appears on the "Histograms" tab in the dialog used for editing a 1P Histogram. You can view and edit these properties by double-clicking a histogram or by right-clicking a histogram and choosing "Edit Graphics".

### Graphics

#### Resolution

Choose a resolution for the histogram. The default value is 100. Lower values produce smoother histograms with less detail; higher values reveal more detail in the distribution.

#### Fill Style

Choose the fill style to use for the histogram.

#### Transparency

Choose a transparency value between 0.0 and 100.0. A value of 0.0 is opaque; a value of 100 is completely transparent. The transparency setting is most useful when Zones have been defined and the histogram is displaying a set of overlapping sub-populations.

### Cell Types

#### Show Unclassified Events

Enable this option to display a polygon for the unclassified events, along with polygon(s) for classified events.

#### Show All Cell Types

When this option is enabled, additional polygons are displayed representing the selected parameter in each of the other cell types.

### Peaks

#### Show Peaks

When this option is enabled, a small triangle is displayed on the histogram for each of the statistically significant peaks. These peaks are used by several of the automatic positioning routines for Control Points

#### Peak P-value

This value is a filter used by the peak-finding routine to decide whether or not a peak is real. The p-value is the probability that the peaks represent statistical noise. The default value of 0.0010 means that there is a 1 out of 1000 change that the peak is due to statistical noise. Higher values allow more peaks to pass the filter (i.e. it is more likely that the peaks are due to statistical noise).

#### See also:

[1P Histogram](#)

[1P Animation Options](#)

[Other Graphic Options](#)

## 1P Animation Options

This set of properties is displayed on the Animation tab of the 1P Histogram [Edit Graphics](#) dialog. It contains properties that relate to animation features of GemStone.

### Enable

When this option is enabled *and* the Animate property is enabled in the [Cell Type Properties panel](#), this 1P histogram will display animation cursors.

### Animation Data Source

When Animation is enabled, it can position its cursors based on either Mean Data or Model Data. Mean Data uses the mean for the classified events at each state index. Model Data uses the model position based on Control Points at each state index.

### Animation Color

Choose a color for the animation cursors.

### Line Width

Choose a line width for the animation cursors.

### See also:

[1P Histogram](#)

[Graphic Options for 1P Histograms](#)

[Other Graphic Options](#)

## **Other Graphic Options**

This set of properties appears on the "Other Options" tab in the dialog used for editing a 1P or 2P histogram. You can view and edit these properties by double-clicking a histogram or by right-clicking a histogram and choosing "Edit Graphics".

Select the "Other Options" tab to view these properties.

### **Background Color**

Choose a color for the background of the plot.

### **Show X Grid Lines**

Enable this option to show grids for the X-axis. Grid lines will appear for each major tic on the axis.

### **Show Y Grid Lines**

Enable this option to show grids for the Y-axis. Grid lines will appear for each major tic on the axis.

### **Grid Line Width**

Enter a value for the width of the grid lines. The default, 0, creates the narrowest possible line that the screen (or printer) can draw.

### **Grid Line Style**

Choose a line style for grid lines.

### **Grid Color**

Choose a color for grid lines.

### **Grids On Top**

Enable this option to draw grids on top of the other graphics in the plot. When the option is unchecked, grids are drawn behind the other graphics.

## **Background Image**

A plot can display a background image, on top of which the other graphics are displayed. This can be useful, for example, to present a comparison of the current analysis with a previous analysis that was saved as an image to disk.

### **Image Transparency**

Enter a value between 0.0 and 100.0 to define how transparent a background image appears. Higher values produce more transparency.

### **Open Image**

Click this button to select an image file from disk. This file will be opened and displayed as the background image for the plot.

### **Clear Image**

If an image has been opened using the Open Image option, the Clear Image option removes the images from memory.

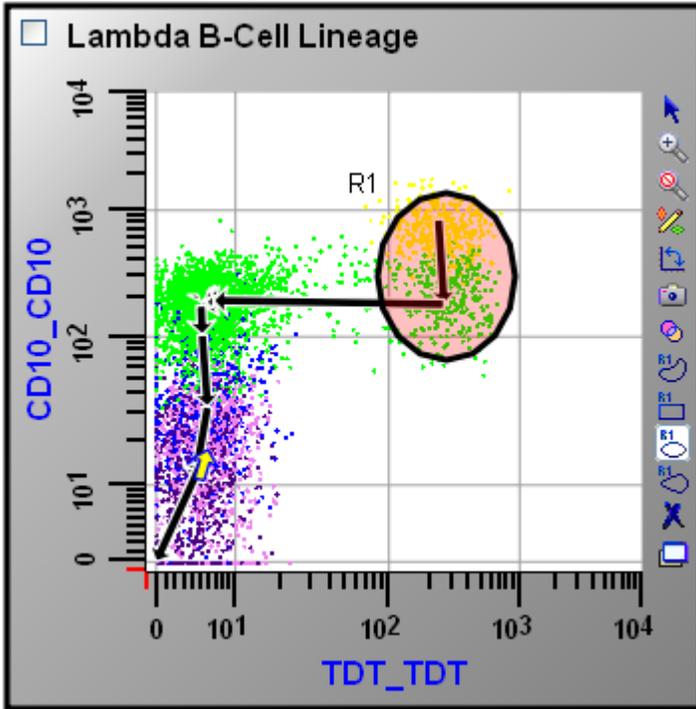
**See Also:**

[1P Histogram](#)

[2P Histogram](#)

## 2P Histograms

The 2P Histogram plot displays a correlated plot for two parameters. Any number of 2P Histograms can be created.



The name of the cell type is displayed in the area at the top of the plot. Each cell type can have any number of 2P histograms, but it can also include parameters from other cell types, too.

The checkbox at the top left of the plot is the "save graphic" checkbox. Check this box if you want the graphics from the histogram to be saved when you click the Save Graphics button on the [Workspace toolbar](#).

The X-axis of the plot shows intensity of a selected parameter in the transformation selected. Similarly, the Y-axis shows the intensity of another parameter. Click an axis label to select a different parameter; a menu of available parameters will be displayed.

Tools to manipulate the plot appear on the right edge of the plot window. These include region drawing tools, which can be used to create gates and to color events. See [2P Graphics toolbar](#) for a description of each tool.

### If you want to...

Create a new 2P Histogram      Click the 2P Histogram button in the [Workspace toolbar](#), and then click and drag to define the area where the histogram should be displayed.

Edit the properties of the 2P      Double-click within the histogram display area or

Histogram

right-click and choose Edit Graphics.

Delete the 2P Histogram

Select the plot and press the Delete key.

Create a free-floating window  
with the 2P Histogram plot

Click the Create Dialog button in the [2P Graphics toolbar](#).

**See also:**

[Graphic Options for 2P Dots](#)

[Animation for 2P Histograms](#)

[Other Graphic Options](#)

[Workspace toolbar](#)

[2P Graphics toolbar](#)

## **Properties for 2P Surface**

This set of properties appears on the Surface tab in the dialog used for editing a 2P Histogram. You can view and edit these properties by double-clicking a histogram or by right-clicking a histogram and choosing Edit Graphics.

Surface graphics offer a smooth, color transition that helps visualize the density of events, similar to color density or color gradient plots.

### **Enable Surface**

Check to enable surface graphics.

### **Probability Mixing Color**

Choose the color to mix with the zone color to show cumulative probability.

### **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.

#### **Reset**

Click this button to reset the object's properties to default values.

## Graphic Options for 2P Dots

This set of properties appears on the "Dots" tab in the dialog used for editing a 2P Histogram. You can view and edit these properties by double-clicking a histogram or by right-clicking a histogram and choosing "Edit Graphics".

### Enable Dots

Check this option to display dots and other graphics from this tab. (The 2P histogram can display other kinds of graphics, which are selected on other tabs in this dialog.)

### Auto Dot Size

Enable this option to allow GemStone to determine the best dot size, based on the current size of the histogram object and other factors.

### Dot Size

This field displays the current size of dots in points; a point is 1/72 inches. To set a specific size for the dots, uncheck the Auto Dot Size option and enter a value between 0.0 and 5.0 for Dot Size.

### Show All Dots

Enable this option to display dots for all events. If this option is unchecked, the system will use the value in Desired Dot Number.

### Desired Dot Number

If Show All Dots is unchecked, the system will use the value in Desired Dot Number to determine the number of dots to display in the plot.

### Probability of Inclusion

This value controls how dots are included for display in the plot. Enter 1.0 to include all dots. Enter 0.05 to include only the outermost 5% of dots. This is most useful when plotting [Surface](#) plots along with dots.

### Other Dots

#### Show Other Types

This option allows dots from one or all other cell types to be display in the plot.

#### Show Unclassified Events

Enable this option to display dots for unclassified events.

### Types of Dots

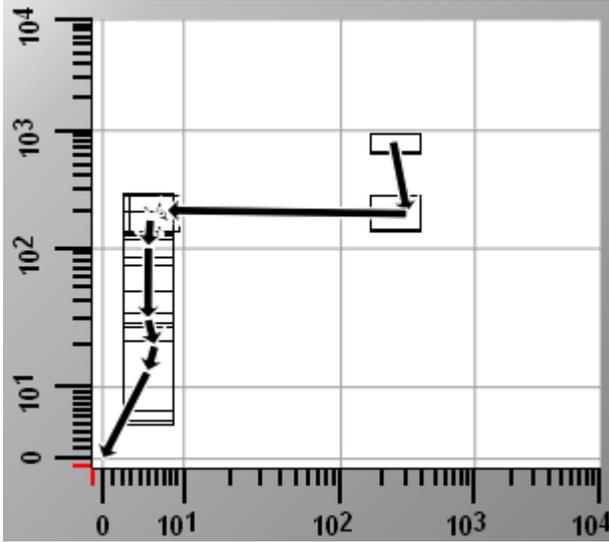
#### Show Parameter Dots

Enable this option to display dots based on parameter event values as a traditional dot plot.

#### Show Model Values

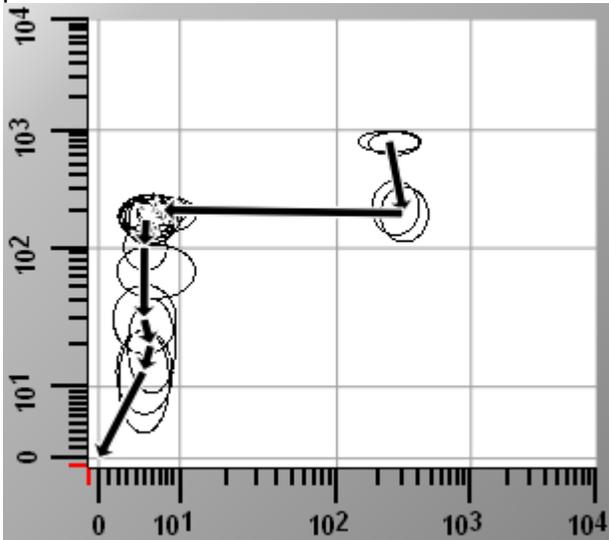
When this option is enabled, a rectangular region is displayed for each state index. The position of each rectangle is based on the model's value for the parameters at each state

index. The size of each rectangle is based on the Number of SDs option and the standard deviation of the parameter in the model at that state index.



### Show Mean Values

When this option is enabled, an elliptical region is displayed for each state index. The position of each ellipse is based on the mean value for the parameters at each state index. The size of each ellipse is based on the Number of SDs option and the standard deviation of the parameter in the model at that state index.

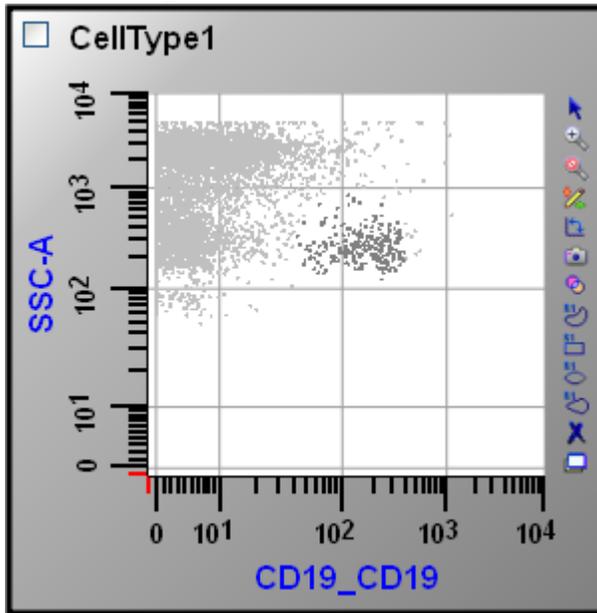


### Show Calculated Dots

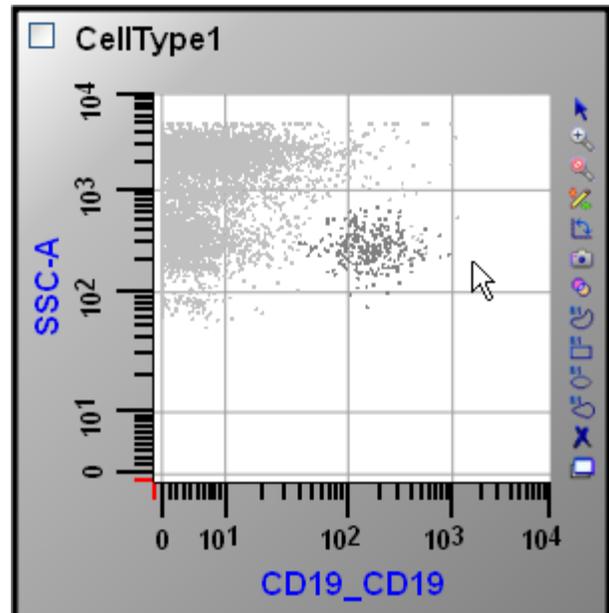
This option works in conjunction with the Show Parameter Dots option. When both options are enabled, dots for classified events are displayed using the calculated mean and standard deviation from the model to display a Gaussian-like distribution.

The option is useful for comparing modeled vs. actual event data.

Dots showing actual data values



Calculated Dots



### Number of SDs

Enter the number of Standard Deviations to use for several of the plotting options (Show Model Values, Show Mean Values, etc.) The default value is 1.0.

### See also:

[Graphic Options for 2P Vectors](#)

[Animation for 2P Histograms](#)

[Other Graphic Options](#)

[Workspace toolbar](#)

[2P Graphics toolbar](#)

## Graphic Options for 2P State Vectors

This set of properties appears on the "State Vectors" tab in the dialog used for editing a 2P Histogram. You can view and edit these properties by double-clicking a histogram or by right-clicking a histogram and choosing "Edit Graphics".

State Vector arrows allow you to track the progression information in the context of two, correlated parameters. Each arrow represents the same number of states in the progression, so the length of arrows give an impression of the relative speed of transitions. Longer arrows indicate more rapid state transitions; shorter arrows suggest that there is not much change occurring.

### Enable

Check this option to display arrows showing the state progression in the 2P histogram.

### Number

Enter the number of state vectors to display.

### Arrow Data Source

Choose "Mean Data" to position the state vectors based on the mean of the classified events. Choose "Model Data" to position the vectors based on the model positions.

### Perimeter Color

Choose the color for the border of the vectors.

### Line Width

Enter the line width to use for the perimeter of the arrow.

### Head Height

Enter the height of the arrow head.

### Width

Enter the width of the arrow.

### Head Proportion

Enter the proportion of the arrow head height to head width. Values greater than 1.0 produce elongated arrow heads.

### Angle Indent

This value controls the angle for the base of the arrow head. Enter 0.0 for a square base.

### See also:

[Graphic Options for 2P Dots](#)

[Animation for 2P Histograms](#)

[Other Graphic Options](#)

[Workspace toolbar](#)

[2P Graphics toolbar](#)

## Animation for 2P Histograms

This set of properties appears on the Animation tab in the dialog used for editing 2P Histograms. You can view and edit these properties by double-clicking a histogram or by right-clicking a histogram and choosing "Edit Graphics".

When Animation is enabled for the cell type and for this 2P histogram, the State Vector arrows will also animate.

### Enable

Check this option to enable animation for this 2P histogram. When this option is enabled *and* the Animate property is enabled in the [Cell Type Properties panel](#), this 2P histogram will display animation vectors.

### Animation Data Source

Choose "Mean Data" to position the state vectors based on the mean of the classified events. Choose "Model Data" to position the vectors based on the model positions.

### Animation Color

Choose the fill color for the vectors.

### Perimeter Color

Choose the color for the border of the vectors.

### Line Width

Enter the line width to use for the perimeter of the arrow.

### Head Height

Enter the height of the arrow head.

### Width

Enter the width of the arrow.

### Head Proportion

Enter the proportion of the arrow head height to head width. Values greater than 1.0 produce elongated arrow heads.

### Angle Indent

This value controls the angle for the base of the arrow head. Enter 0.0 for a square base.

## Region/Gate Animations

### Dot Size

### See also:

[Graphic Options for 2P Dots](#)

[Graphic Options for 2P Vectors](#)

[Other Graphic Options](#)

[Workspace toolbar](#)

[2P Graphics toolbar](#)

## Region Label Properties

The properties of a region label can be edited by double-clicking the region label.

### Name and Alias

#### Show Region Name

Show/hide region name

#### Region Name (Read only)

Name of the region

#### Show Region Alias

Show/hide region alias

#### Region Alias

Alias for the region

### Statistics

#### Show Region Counts

Show/hide region counts in the region label

#### Show Region Percent

Show/hide region percent in the region label

#### Show Region Percent Total

Show/hide region percent total in the region label

#### Decimal Digits

Enter the number of decimal digits to display. (0 to 6)

### Color and Font

#### Text Color

Color of the label's text

#### Label Font

Font of the label's text

### Background Properties

#### Fill color

Color of the fill

#### Fill style

Style of the fill

#### Transparency

Amount of transparency (0-100%) (0 to 100)

**Pen color**

Color of the outline

**Pen style**

Style of the outline

**Pen width**

Width of the outline in pixels (0 to ...)

**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.

**Reset**

Click this button to reset the object's properties to default values.

## Heat Map

A Heat Map object can be created on the canvas to display a plot of how well events fit the model. The Heat Map tool in the Workspace toolbar is used to create the graph.

### Save Image Flag

#### Save Image

Check to flag this object for saving its image.

### Position and Size

#### X Position

Enter the x position in inches. (0.00 to 100.00)

#### Y Position

Enter the y position in inches. (0.00 to 100.00)

#### X Size

Enter the x size in inches. (0.25 to 100.00)

#### Y Size

Enter the y size in inches. (0.25 to 100.00)

### Other Properties

#### Background

Choose the wrapper or background for the object.

#### Title Font

Font of the title

### 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.

#### Reset

Click this button to reset the object's properties to default values.

## **Edit Graphics for Heat Map: Surface**

This set of properties appears on the Surface tab in the dialog used for editing a model state graph. You can view and edit these properties by double-clicking a histogram or by right-clicking a histogram and choosing Edit Graphics.

### **Enable Model Surface**

Check to enable surface graphics for the model.

### **Surface Color**

Choose the color that represents abnormal populations.

### **Base Color**

Choose the color that represents very few abnormal populations.

### **Sensitivity Threshold**

Lower values has higher sensitivity and false positives. (0.01 to 1.00)

### **Smoothing SD**

Controls the smoothing of the model difference distribution. (0.00 to 5.00)

### **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.

#### **Reset**

Click this button to reset the object's properties to default values.

**Edit Graphics for Heat Map: Dots**

This tab is part of the Edit Graphic Options for Model Graph. It configures the display of dots.

**Enable Dots**

Check to enable or disable showing dots.

**Auto Dot Size**

Check to have the system try to find the best size of the dots.

**Dot Size**

Choose the appropriate dot size. (0.00 to 5.00)

**Show All Dots**

Check to show all the dots in the listmode buffer.

**Desired Dot Number**

Enter the specific number of dots to display. (0 to ...)

**Probability of inclusion**

Enter 1.0 for all dots, 0.05 for outermost 5% of dots. (0.00 to 1.00)

**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.

**Reset**

Click this button to reset the object's properties to default values.

## **Edit Graphics for Heat Map: Zones and P-Values**

This set of properties appears on the Zones et al. tab in the dialog used for editing a model state graph. You can view and edit these properties by double-clicking a histogram or by right-clicking a histogram and choosing Edit Graphics.

### **Zones**

#### **Show Model Zone Labels**

Check to show the zone labels for the celltype.

#### **Show Model Zone Boundaries**

Check to show the zone boundaries for the celltype.

#### **Label Font**

Choose the font to use for the zone labels.

### **P-Values**

#### **Show P-Value 1**

Check to show the first p-value for the celltype.

#### **P-Value 1**

Choose the first p-value grid line to display. (0.00000 to 1.00000)

#### **Show P-Value 2**

Check to show the second p-value for the celltype.

#### **P-Value 2**

Choose the second p-value grid line to display. (0.00000 to 1.00000)

### **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.

#### **Reset**

Click this button to reset the object's properties to default values.

## **Edit Graphics for Heat Map: Animation**

This set of properties appears on the Animation tab in the dialog used for editing the model heat map system. You can view and edit these properties by double-clicking or by right-clicking in the plot area and choosing Edit Graphics.

### **Enable**

Enables animation for the Heat Map.

### **CellType**

### **Line Width**

Choose line width for animation. (0 to 10)

### **Line Style**

Choose the desired line style.

### **Color**

Choose the color.

### **Region/Gate**

### **Dot Size**

Choose the appropriate dot size in points for region/gate animations. (0.00 to 6.00)

### **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.

#### **Reset**

Click this button to reset the object's properties to default values.

## **Region Label Properties**

The properties of a region label can be edited by double-clicking the region label.

### **Name and Alias**

#### **Show Region Name**

Show/hide region name

#### **Region Name (Read only)**

Name of the region

#### **Show Region Alias**

Show/hide region alias

#### **Region Alias**

Alias for the region

### **Statistics**

#### **Show Region Counts**

Show/hide region counts in the region label

#### **Show Region Percent**

Show/hide region percent in the region label

#### **Show Region Percent Total**

Show/hide region percent total in the region label

#### **Decimal Digits**

Enter the number of decimal digits to display. (0 to 6)

### **Color and Font**

#### **Text Color**

Color of the label's text

#### **Label Font**

Font of the label's text

### **Background Properties**

#### **Fill color**

Color of the fill

#### **Fill style**

Style of the fill

**Transparency**

Amount of transparency (0-100%) (0 to 100)

**Pen color**

Color of the outline

**Pen style**

Style of the outline

**Pen width**

Width of the outline in pixels (0 to ...)

**Actions**

**OK**

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

**Cancel**

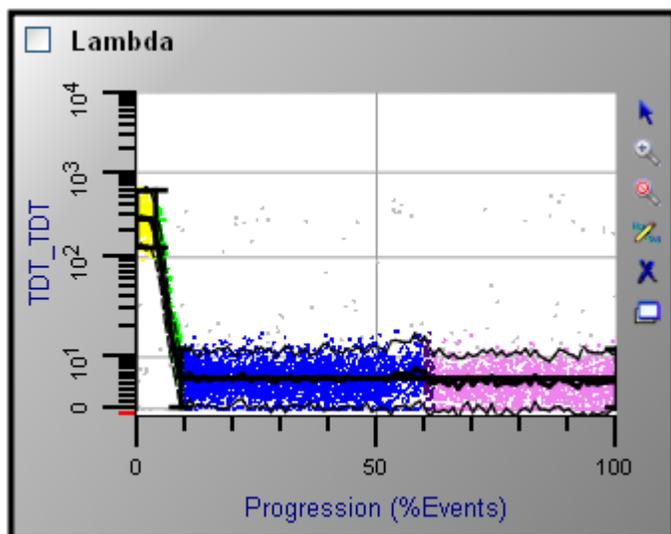
Click this button to discard any changes and close the dialog box.

**Reset**

Click this button to reset the object's properties to default values.

## Parameter Graph

A Parameter Graph object can be created on the canvas to display a single Parameter Profile plot. The Parameter Graph tool in the Workspace toolbar is used to create the graph.



The plot shows the same information that appears in the Parameter Plots in the Cell Type widget. The difference is that as a separate Parameter Graph object, the plot is not subjected to the automatic sizing and hiding logic that apply to the Cell Type widget plots. This makes the Parameter Graph useful for visualization of important parameters that you always want or need to see.

## Zone Statistics Table

Each Cell Type in a GemStone model contains a Zone Statistics Table in the workspace. The table contains a row of information for each "zone" that has been defined in the Cell Type. For details on how to define and edit zones, see [Edit Zones](#).

At the top of the object, the name of the cell type is displayed.

A checkbox in the upper-left corner of the object is used to flag the object to be saved as a graphic image file. For additional information on saving graphics, see [Batch Options panel](#).

Lambda B-Cell Lineage

Name	Active	Color	Percent	%Total	Events	%Minimum Purity
Total	<input checked="" type="checkbox"/> On		100.00	45.64	5260	0.0
StgI	<input checked="" type="checkbox"/> On		6.35	2.90	334	0.0
StgII	<input checked="" type="checkbox"/> On		58.95	26.91	3101	0.0
StgIII	<input checked="" type="checkbox"/> On		11.16	5.09	587	0.0
StgIV	<input checked="" type="checkbox"/> On		14.43	6.59	759	0.0

The Name of each zone is displayed in the left column.

The Active column can be used to turn zones on and off quickly. When a zone is active (checked), statistics are computed and zone-coloring is applied to dots. When inactive, a zone is ignored by the analysis routines.

The Color for the zone is displayed and can be edited by clicking the color swatch.

Several statistics are displayed for each zone:

Percent: the percentage of events relative to *this* cell type that are contained by the zone.

% Total: the percentage of events relative to *all* cell types that are contained by the zone.

Events: the number of events contained by the zone.

% Minimum Purity: a probability value between 0.0 and 100.0 that the events are actually contained by the zone. See for [Edit Zones](#) details.

### See also:

[Batch Options panel](#)

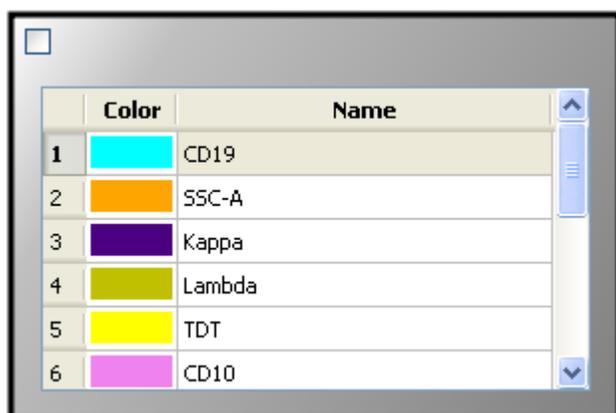
[Edit Zones](#)

## Legend

The Legend object displays a list of the parameters in a model for one Cell Type. The color associated with each parameter is displayed next to the parameter name.

The object can be added to a Cell Type workspace by clicking the Legend tool on the [Workspace toolbar](#), and then clicking and dragging to define an area where the legend should be displayed.

A checkbox in the upper-left corner of the object is used to flag the object to be saved as a graphic image file. For additional information on saving graphics, see [Batch Options panel](#).



The screenshot shows a legend window with a checkbox in the top-left corner. It contains a table with two columns: 'Color' and 'Name'. The table lists six parameters with their corresponding colors:

	Color	Name
1	Cyan	CD19
2	Orange	SSC-A
3	Purple	Kappa
4	Olive Green	Lambda
5	Yellow	TDT
6	Pink	CD10

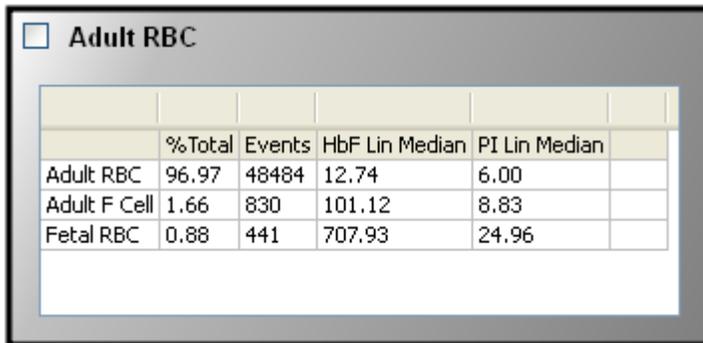
### See also:

[Workspace Names and Overview](#)

[Workspace toolbar](#)

## Table

The Table object is used to display analysis results and keywords on the report. It is created using the Table tool on the [Workspace toolbar](#).



	%Total	Events	HbF Lin Median	PI Lin Median
Adult RBC	96.97	48484	12.74	6.00
Adult F Cell	1.66	830	101.12	8.83
Fetal RBC	0.88	441	707.93	24.96

A table has a user-defined number of rows and columns, which can be changed by double-clicking the object and editing the fields.

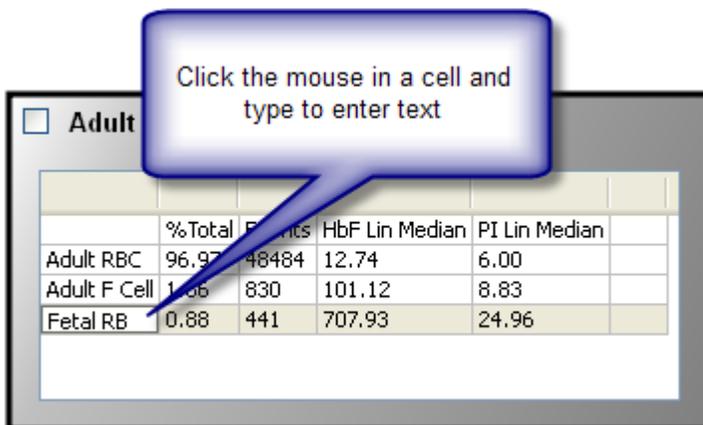
The cells in a table can contain text and keywords. Keywords must be surrounded with square brackets to be interpreted as keywords and evaluated.

### Decimal Places

You have control over the number of decimal places displayed by numeric keyword results that you add to a table. Use the **Edit** menu **Preferences** command to display the Preferences dialog. Decimal places are set in the **General** section.

### Adding Text

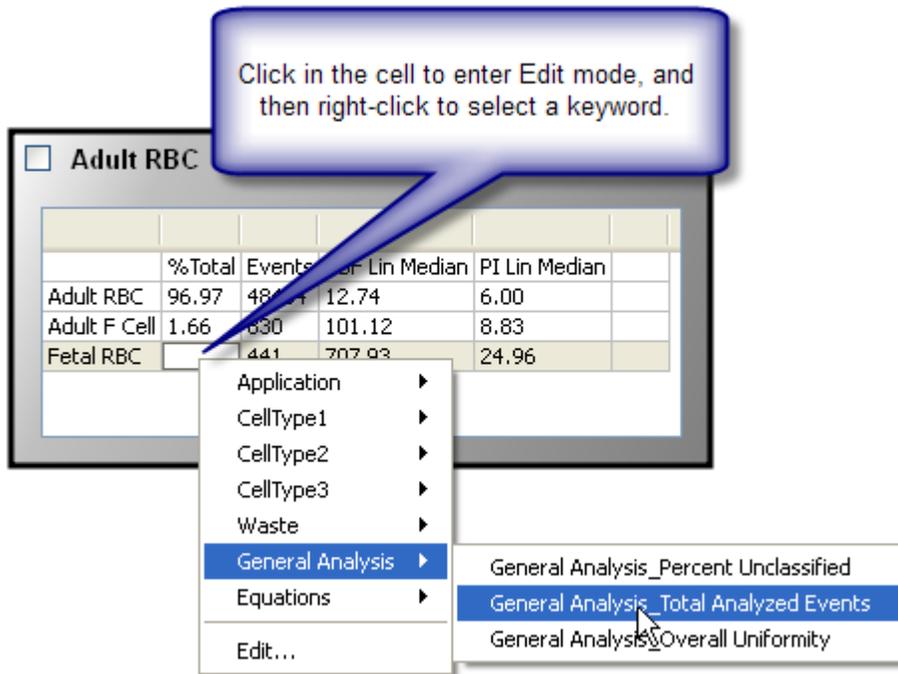
Text can be typed directly into a cell in the table. Simply click the mouse in a cell and type the text. Typical text editing keys can be used to delete, copy, and paste text.



	%Total	Events	HbF Lin Median	PI Lin Median
Adult RBC	96.97	48484	12.74	6.00
Adult F Cell	1.66	830	101.12	8.83
Fetal RB	0.88	441	707.93	24.96

### Adding Keywords

The easiest way to add keywords to the table is to select keywords from the list of available information. This is done by clicking the cell to enter Edit mode, and then right-clicking to display a menu of categories. Within the category sub-menus you will find all of the available keywords that can be included.



The columns in the table will automatically size to fit the content.

**See also:**

[Table Context Menu](#)

## **Table Context Menu**

The Table Context menu appears when you right-click the title area of a table object.

### **Edit Properties**

This command displays the Edit Properties dialog for the table, used to set the number of rows and columns in the table.

### **Bring To Front**

Move the table to the front of all graphic objects.

### **Send To Back**

Move the table to the back of all graphic objects.

### **Delete**

Delete the table.

### **Export Table**

Save the table to disk. The standard File Save dialog is displayed to allow you to select a location and name for the file. The file is saved in tab-delimited ASCII format.

### **Copy Table to Clipboard**

Copy the table as tab-delimited text to the clipboard.

## Toolbars and Shortcuts

### Main toolbar

The main toolbar contains a set of buttons that provide commonly used application actions.



Displays the [Open Document](#) dialog to open a GemStone document from disk.



Displays the [Save Document](#) dialog to save the GemStone document to disk.



Displays a dialog to [select FCS](#) files for analysis.



Displays the [Edit Parameter Database](#) dialog



[Classifies](#) the current data with the current model.



[Optimizes](#) the control points for all parameter profiles in the model to best fit the current data.



[Auto-analyzes](#) the data.



[Enriches](#) the data in the packet to select for events that are classified by the model.



[Classifies](#) the data in the entire FCS file with the current model.



Launches the [online help](#) system.

## Batch Controls toolbar

The batch toolbar contains a set of buttons to navigate through the selected batch of files. The toolbar appears in the lower left of the GemStone workspace.

-  Edit properties for batch processing.
-  Choose a file in the batch.
-  Go to the first batch file.
-  Go to the previous batch file.
-  Redo the current batch file.
-  Go to the next batch file.
-  Go to the last batch file.
-  Auto-advance through the batch.
-  Stop auto-advancing.

### See also:

[Overview: Batch Processing](#)

[Overview: Workspace Names and User Interface](#)

## Workspace toolbar

The workspace toolbar appears on the left edge of the Cell Type canvas. It contains buttons to create and manipulate objects in the Cell Type canvas.

	Selection	Use this tool to select, move, and size objects in the canvas.
	Zoom	Use this tool to define a portion of the canvas to zoom-in on. Click and drag to surround the area that you want to zoom.
	Cancel zoom	Click this tool to cancel out of zoom and return to normal viewing.
	Text	Click and drag to define an area for the text. A dialog will be displayed to input the text.
	Line	Click and drag to define the end points of the line.
	Arrow	Click and drag to define the end points of the arrow.
	Rectangle	Click and drag to define the outline of the rectangle.
	Ellipse	Click and drag to define the bounding rectangle for the ellipse.
	1P Histogram	Click and drag to define the bounding rectangle for the <a href="#">1P histogram</a> .
	2P Histogram	Click and drag to define the bounding rectangle for the <a href="#">2P histogram</a> .
	Parameter Graph	Click and drag to define the bounding rectangle for a <a href="#">parameter graph</a> .
	Overlay plot	Click and drag to define the bounding rectangle for the <a href="#">overlay plot</a> .
	Heat Map plot	Click and drag to define the bounding rectangle for the <a href="#">heat map plot</a> .
	Legend	Click and drag to define the bounding rectangle for the legend.
	Table	Click and drag to create a table object.
	Save Graphics	Use the tool to save graphic images for objects that are enabled for saving. To enable an object for saving, check the box in the upper-right corner of the object. To save graphics, click the Save Graphics tool button.
	Delete object	Click this button to delete the selected objects from the canvas. Note: Some objects are not user-deletable.
	Utility Actions	Click this tool button to select one of the utility actions from a list.
	Choose Parameter	Use this tool to select a parameter to include in the current Cell Type. Once a parameter is selected, it appears in the Cell Type widget for the Cell Type. It can also be displayed in 1P and 2P histograms.

**See also:**

[Overview: Workspace Names and User Interface](#)

## Parameter Profile toolbar

The Parameter Profile toolbar is located on the right side of each Parameter Profile in the Cell Type widget. It provides tools useful for working with the parameter profile.

	Selection	Use this tool to select, move, and size objects in the Parameter Profile.
	Zoom	Use this tool to define a portion of the object to zoom-in on. Click and drag to surround the area that you want to zoom.
	Cancel zoom	Click this tool to cancel out of zoom and return to normal viewing.
	Estimate Y Positions	When this tool is clicked, GemStone uses a peak-finding routine to analyze the parameter. It then adjusts the parameter value (Y-axis) for each Control Point in the parameter profile.
	Estimate Line Spread	When this tool is clicked, GemStone adjusts the line-spread for each Control Point in the parameter profile.
	Estimate X Positions	When this tool is clicked, GemStone adjusts the state index (X-axis position) of each Control Point in the parameter profile. The positions are adjusted to provide the best fit of the data currently displayed.
	<a href="#">Edit Graphics</a>	Click this tool to edit the graphics settings for the Parameter Profile.
	Enable Profile: On	This button toggles the Enable Profile property for the parameter profile. When enabled (on), the parameter profile is an active part of the model and becomes part of the selection process for events in the cell type.
	Enable Profile: Off	Turn Enable Profile property off (disabled) to eliminate the parameter profile from the model selection process.

### See also:

[Overview: Workspace Names and User Interface](#)

[Cell Type Properties panel](#)

## 1P Graphics toolbar

The 1P Graphics toolbar is located on the right side of each 1P histogram object. It provides tools useful for working with the object.

	Selection	Use this tool to select, move, and size objects in the object.
	Zoom	Use this tool to define the portion of the object to zoom-in on. Click and drag to surround the area that you want to zoom.
	Cancel zoom	Click this tool to cancel out of zoom and return to normal viewing.
	Edit Graphics	Edits the graphics settings for this object.
	Create 1p Region	Use this tool to define a 1P region on the histogram. The region can be used for gating or to color unclassified events. After selecting this tool, click and drag on the histogram to define the region.
	Delete object	Click this tool to delete the histogram.
	Create dialog	Click this tool to create a floating dialog copy of the histogram object. The dialog will create an independent duplicate of the histogram in a floating window that can be moved outside of the workspace.

### See also:

[Overview: Workspace Names and User Interface](#)

[1P Histogram](#)

## 2P Graphics toolbar

The 2P Graphics toolbar is located on the right side of each 2P histogram object. It provides tools useful for working with the object.

	Selection	Use this tool to select, move, and size objects in the object.
	Zoom	Use this tool to define the portion of the object to zoom-in on. Click and drag to surround the area that you want to zoom.
	Cancel zoom	Click this tool to cancel out of zoom and return to normal viewing.
	Edit Graphics	Edits the graphics settings for this object.
	Swap axes	Click this tool to reverse the parameters on the X- and Y- axes.
	Save graphics	Use this tool to save the graphics to disk in one of the graphic formats available. The standard File Save dialog will be displayed. Enter a file name and choose a graphics format, then click Save.
	Conform mode On	This tool toggles "Conform mode" on and off. By default, Conform mode is off.
	Conform mode Off	When Conform mode is on, a region that is being moved will "conform" its shape to the boundary of other region(s) that it bumps into. There are several important considerations when using Conform mode. 1. You must not move the region by the region's label in this mode. Instead, drag the region by dragging its interior. 2. Conform mode works best with free-hand regions that are created with many vertices.
	Free-hand Region	Use this tool to define a free-hand region on the histogram. The region can be used for gating or to color unclassified events. After selecting this tool, click and drag on the histogram to define the region.
	Rectangle Region	Use this tool to define a rectangular region on the histogram. The region can be used for gating or to color unclassified events. After selecting this tool, click and drag on the histogram to define the region.
	Ellipse Region	Use this tool to define an elliptical region on the histogram. The region can be used for gating or to color unclassified events. After selecting this tool, click and drag on the histogram to define the region.
	Bike-chain Region	Use this tool to define a bike-chain region on the histogram. The shape of this region resembles the chain of a bicycle, with one end larger and the other smaller. The region can be used for gating or to color unclassified events. After selecting this tool, click and drag on the histogram to define the region.



Delete  
object

Click this tool to delete the histogram.



Create  
dialog

Click this tool to create a floating dialog copy of the histogram object. The dialog will create an independent duplicate of the histogram in a floating window that can be moved outside of the workspace.

**See also:**

[Overview: Workspace Names and User Interface](#)

[2P Histogram](#)

## Overlay Graphics toolbar

The Overlay Graphics toolbar is located on the right side of each parameter overlay object. It provides tools useful for working with the object.

	Selection	Use this tool to select, move, and size objects in the object.
	Zoom	Use this tool to define the portion of the object to zoom-in on.
	Cancel zoom	Click and drag to surround the area that you want to zoom. Click this tool to cancel out of zoom and return to normal viewing.
	Edit Graphics	Edits the graphics settings for this object.
	Animation Start	Use this tool to turn animation on or off for the overlay graph.
	Stop	When animation is started, a parameter "ribbon" is shown for several seconds, and then it is hidden and the next is shown. This continues for each parameter, and the process repeats starting with the first parameter again. When animation is stopped, all parameter ribbons are shown at the same time.
	Pause or Continue Animation	This tool only has an effect when Animation is on for the overlay graph.
	Pause	When the graph is animating, click the Pause button to pause an animation.
	Continue	When the graph is paused, click the Continue button to resume the animation.
	Save graphics	Use this tool to save the graphics to disk in one of the graphic formats available. The standard File Save dialog will be displayed. Enter a file name and choose a graphics format, then click Save.
	Text	Click and drag to define an area for the text. A dialog will be displayed to input the text.
	Line	Click and drag to define the end points of the line.
	Arrow	Click and drag to define the end points of the arrow.
	Rectangle	Click and drag to define the outline of the rectangle.
	Ellipse	Click and drag to define the bounding rectangle for the ellipse.
	Create dialog	Click this tool to create a floating dialog copy of the overlay graph object. The dialog will create an independent duplicate of the overlay in a floating window that can be moved outside of the workspace.

**See also:**

[Overview: Workspace Names and User Interface](#)

[Parameter Overlay Plot](#)

## Shortcuts

GemStone supports many keyboard shortcuts for commands and other actions. The table below describes these and their uses.

Shortcut	Available	Description
Ctrl+N (PC) Command+N (Mac)	Always	Creates a new Model Document. See <a href="#">New Document</a> for details.
Ctrl+O (PC) Command+O (Mac)	Always	Opens a Model Document. See <a href="#">Open Document</a> for details.
Ctrl+S (PC) Command+S (Mac)	Always	Saves a Model Document. See <a href="#">Save Document</a> for details.
Ctrl+F (PC) Command+F (Mac)	Always	Displays the Select FCS Files dialog to choose data files to open. See <a href="#">Select FCS Files</a> for details.
Ctrl+Y (PC) Command+Y (Mac)	Always	Synthesizes data. See <a href="#">Synthesize Data</a> for details.
Ctrl+X (PC) Command+X (Mac)	Always	Exports Model Data. See <a href="#">Export Model Data</a> for details.
Alt+F4 (PC) Command+Q (Mac)	Always	Exit the program.
Ctrl+D (PC) Command+D (Mac)	Always	Displays the Edit Parameter Database dialog. See <a href="#">Edit Parameter Database</a> for details.
Ctrl+A (PC) Command+A (Mac)	Always	Classifies data. See <a href="#">Classify Data</a> for details.
Ctrl+B (PC) Command+B (Mac)	Always	Optimizes the model. See <a href="#">Optimize Model</a> for details.
Ctrl+E (PC) Command+E	Always	Enriches for data that matches the model. See <a href="#">Enrich Data</a> for details.

(Mac)		
Ctrl+R (PC) Command+R (Mac)	Always	Classify the entire file. See <a href="#">Classify File</a> for details.
Esc	Always	Interrupts the current command process.
PgUp/PgDn	When a Parameter Profile is selected	Opens and activates the previous or next Parameter Profile in the Cell Type Widget.

## **File Menu**

### **New Document**

File menu

When selected, GemStone will discard the current model and GemStone document from memory and create a new, empty GemStone document.

A GemStone document contains the Cell Types, parameter profiles, and objects on the Cell Type canvas.

## Open Document

### File menu

When selected, this command will display the standard File Open dialog to allow the user to select a previously saved GemStone document file. GemStone will open the selected file and display the Cell Types, parameter profiles, and other objects contained in the document.

## **Save Document**

File menu

If the document has been saved previously, this command will resave it without displaying a dialog. The previous version of the document will be overwritten.

If the document has not been saved, this command performs identically to the Save Document As command.

### **See also:**

[Save Document As](#)

## Save Document As

File menu

When selected, this command will display the standard File Save dialog to allow the user to save the current GemStone document to a file on disk. GemStone will save the Cell Types, parameter profiles, and other objects contained in the document.

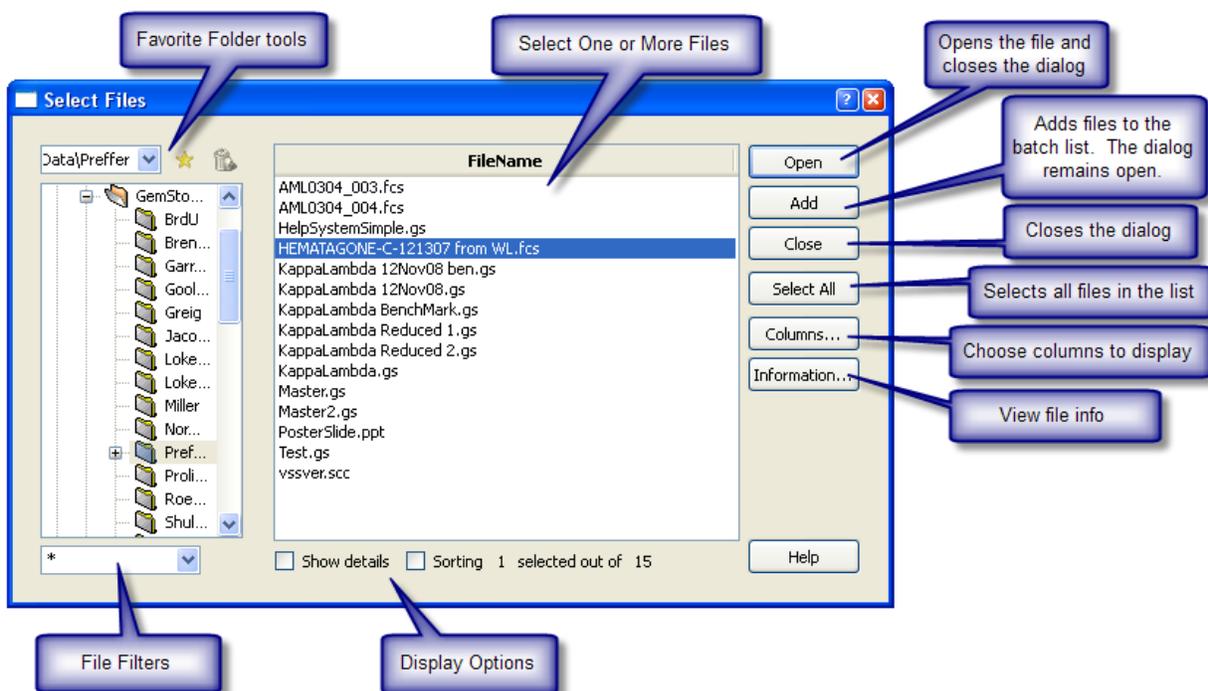
**Error! Hyperlink reference not valid.**

**Error! Hyperlink reference not valid.**

## Select FCS Files

File menu

This command allows the user to select data files to be analyzed by GemStone. The Select Files dialog is displayed. This is a special File Open dialog designed to make selection of FCS files efficient and easy.



### Favorites

A group of controls at the top left of the dialog allows you to store and select file folders that you frequently visit as "favorites". Use the drop-down list to select a folder you have previously stored as a favorite. Use the star button to store a folder as a favorite, and use the trash can button to delete a path from the favorites list.

### File Folder Tree

A tree control on the left of the dialog allows you to navigate to the FCS files that you want to analyze. Double-click a folder in the tree or click the "+" symbol next to it to expand its contents. The files contained in the selected folder will be displayed in the file list.

### Files of Type

This dropdown control displays the current file selection filter. Click the down-arrow to view and select another filter, or type a selection filter into the control directly. Use "\*" to display all files in the selected folder.

### File List

The list in the center of the dialog displays the files in the selected file folder that match the selection filter in the Files of Type control. Normally, the list shows only the names of the

files. However, you can use the **Columns** button to select additional information to display in the list. The **Show Details** option must be enabled in order to view the additional columns of information.

### **Show Details**

When this option is enabled, the **File List** will display additional columns of information about the files. Use the **Columns** button to select the information you want to display.

### **Sorting**

When this option is enabled, you can click on the labels at the top of columns in the **File List** to sort the files based on the information in that column. When the option is unchecked, files are listed alphabetically by file name.

### **Open**

This command adds the selected file(s) to the [File Batch](#) and closes the dialog.

### **Add**

This command adds the selected file(s) to the [File Batch](#) and keeps the dialog open for further file selection.

### **Close**

This command closes the dialog.

### **Select All**

Click this command to select all of the files in the File List.

### **Columns**

This command displays the [Edit Batch Columns](#) dialog to choose the columns of information you want to display in the File List.

### **Information**

This command displays the [Edit FCS Information](#) dialog, which displays keyword information from the selected FCS file.

### **See also:**

[File Database panel](#)

[Edit Batch Columns](#)

[Edit FCS Information](#)

## **Edit FCS Information**

This dialog displays information about an FCS file. It can be displayed by selecting a file in the [Select FCS Files](#) dialog and clicking the Information button.

### **See also:**

[Select FCS Files](#)

**Close FCS File**

File menu

Use this command to close the current FCS file. The command closes the file and removes its keywords from memory. This is useful when constructing models that will be used to [synthesize experimental data](#).

## Synthesize Data

### File menu

Models are most often used to analyze datasets. However, one of the best ways to evaluate a model is to use it to synthesize an experimental dataset. The Synthesize Data command is used to generate data based on the current model.

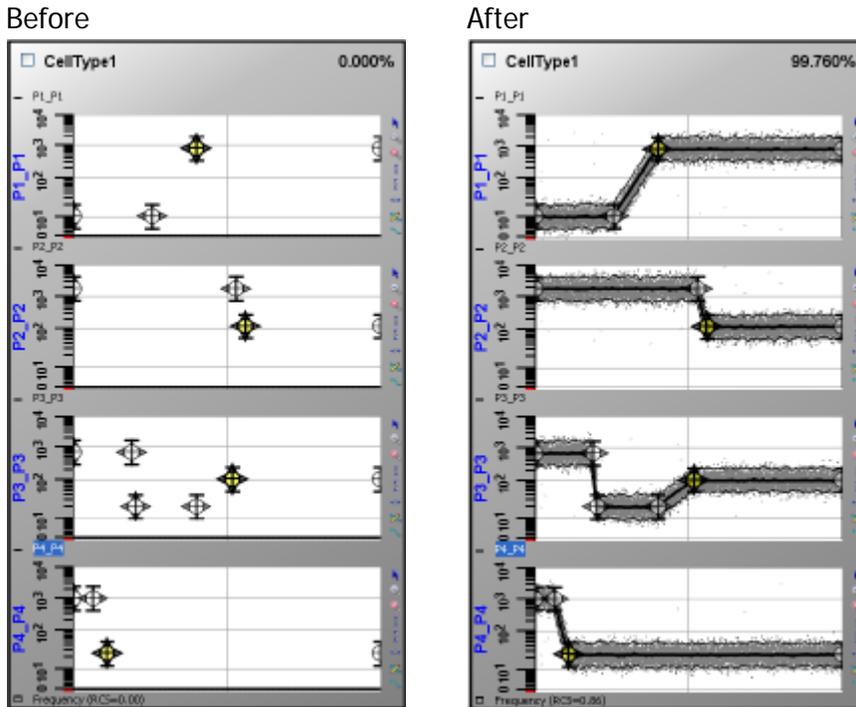
Before choosing this command, construct a model or open one from disk. This model will be used as a basis for the synthesized data.

When the command is executed, a dialog is displayed to allow you to choose the number of events to synthesize. Enter the value and click OK to synthesize the data.

The model itself determines the characteristics of the synthesis process. The same probabilistic processes that are used for classifying real data with a model are put to use to synthesize events. If the model contains multiple cell types, the synthesis routines will apportion events accordingly. The better your model describes the real world process, the more realistic the synthesized data will appear.

After synthesis, the generated data appears in the model's Parameter Profile plots and other graphics. Statistics and zones act on the synthesized data, too.

The synthesized data can be exported to an FCS file using the [Export Data](#) command.



See also:

[Export Data](#)

## Export Model Data

File menu

This command exports the current packet of FCS data in memory to a new FCS file. It is most useful in conjunction with the Synthesize Data command to store experimental datasets created by GemStone for other software to read and interpret.

Only those parameters displayed in one or more Cell Type objects will be included in the exported file.

The standard File Save dialog is displayed, allowing you to navigate to a location and enter a file name for the export data. Files are stored in FCS file format.

## Manage Settings

File menu

This command displays the Settings Manager dialog, which can be used to import, export, and clear settings for GemStone.

### Pathways

#### Import or Export Settings Path

This path is the location that Export Settings or Import Settings will act on. Click the path to choose a different location.

#### Recent Paths

This control stores the most recent locations used for importing or exporting settings. Selecting a path from this list will set the Import/Export Settings Path.

### Settings to Act On

This section displays a list of settings that can be imported or exported. The table below lists each setting and a description. Check the box next to the settings that you want to import or export before using the Export, Import, or Clear Settings buttons.

Setting	Description
All Application Settings	All configuration files and settings. This option includes a number of settings that are not listed as individual options in the list below. When this option is selected, all other options are disabled.
Preferences	User preferences set up with the Edit->Preferences dialog.
Color Palette	Palette of colors set up with Edit->Color Palette.
User Commands	Custom commands created with the Advanced Editing option in the Batch Options panel.
Batch Files	Files in the batch list.
Batch Columns	Columns for the batch list database.
Parameter Database	Database of parameters that GemStone has read.
FCS Selected Information	Fields in the Select FCS Files dialog.

### Actions

#### Export Settings

After checking the settings you want to saved, click Export Settings to store the settings on disk. The Choose Folder dialog will appear to allow you to select a location for the settings. A confirmation dialog is displayed after the settings have been exported.

#### Import Settings

After checking the settings you want to import, click Import Settings to read the settings from disk. The Choose Folder dialog will appear to allow you to select a location to read the settings from. A confirmation dialog is displayed after the settings have been imported.

**Clear Settings**

After checking the settings you want to clear, click the Clear Settings button to delete the settings file(s) from disk. A confirmation dialog is displayed to confirm that you really want to clear the settings. Click OK to continue to clear the settings files, or Cancel to escape.

After clearing settings, GemStone will close to complete the action.

## **Print All Cell Types**

File menu

This command prints the Cell Type canvas for each Cell Type defined by the model. The canvas is scaled to fit on the current printer page.

The standard Print dialog is displayed to allow the selection of print options.

## **Exit (Quit)**

File menu

This command closes the program. It is Exit on Windows versions, and Quit on the Mac.

## **Edit Menu**

### **Edit Parameter Database**

#### Edit menu

This command displays the Edit Parameter Database dialog, used to edit properties of FCS file parameters and to control how GemStone interprets parameters.

Each time GemStone reads an FCS file, it interacts with the Parameter Database. If a file contains a parameter that GemStone has never seen before, a record is added to the Parameter Database with information from the file. When GemStone reads that parameter from another file, it will use the record from the Parameter Database to determine how the parameter will be interpreted and displayed. Records in the database can be edited to control what the parameter display name will be, what color and transformation it will use, and much more. The database even allows you to map any number of FCS parameter names to a single record in the database. See [How to Remap Parameters in a Model](#) for a discussion on this.

#### **Fields**

##### **Live**

This column displays "On" for parameters that are contained in the FCS file that is currently opened. It displays "Off" for parameters in the database that are not present in the current FCS file.

##### **Unique Name**

This editable field is used as the display name for the parameter. By default, it shows the \$PnS keyword value, but it can be edited to any text you want to use for the parameter name.

Each record in the database must have a unique name. If you type the same name as another existing record, GemStone will ask if you want to merge the information from the record you are editing into the existing record. This is an easy way to map more than one FCS parameter to the same record in the database.

##### **Param Index**

If the parameter is "Live", this is the ordinal index of the parameter in the FCS file. If the parameter is not contained in the current FCS file, this field displays -1.

##### **Allow Normalization**

Normalization is a process that spans files that the program reads. It allows GemStone to adjust the mean linear intensity for selected parameters to be approximately equivalent across files. There are two aspects to normalizing parameters. First, in the Batch Options panel, an option other than Initialize needs to be selected. This tells GemStone that you want to normalize or set normalization targets. Secondly, enable the Allow Normalization flag for each parameter that you want to normalize. Use the [Edit Properties for Parameter](#) dialog to view or edit the Target Mean Linear Intensity.

##### **Auto Load**

When this option is enabled, GemStone will automatically add a Parameter Profile to the model to display this parameter when it is found in an FCS file.

**Transform**

Select the transformation that should be used to display the parameter: Linear, Log, or HyperLog. Additional properties for the transformation are available by clicking the **Properties** button.

**Abbreviation**

Enter an abbreviation to use for the parameter. The abbreviation is used as a label on the parameter ribbons in Parameter Overlay plots.

**Color**

Choose a color to use for this parameter. The color is used, by default, for the parameter when displayed in Parameter Overlay plots.

**Description**

This option field can be used to add notes or to describe the parameter.

**Actions****Properties**

Click this button to display additional properties for the selected parameter record. The [Edit Properties for Parameter](#) dialog is displayed.

**New**

Click this button to create a new parameter entry manually. The system will prompt for the Unique Name and other information about the parameter.

**Delete**

This action will delete the selected record from the database.

**OK**

Click this button to close the dialog.

**Open**

Use this action to open a previously saved Parameter Database file from disk. The standard File Open dialog is displayed. Navigate to the file and click Open. The selected database file replaces the database in memory.

**Save**

Use this action to save the current Parameter Database to a file on disk so that it can be read at a later time or by other users. The standard File Save dialog is displayed. Navigate to the desired location, enter a file name, and click Save to store the file.

**Delete All**

This action removes all records from the database.

**See also:**

[Edit Properties for Parameter](#)

[How to Remap Parameters in a Model](#)

## **Edit Properties for Parameter**

This dialog is displayed to edit the properties for a parameter record in the Parameter Database dialog. It provides access to all user-editable properties and displays a number of read-only properties for the parameter. To display the dialog, select a parameter in the [Edit Parameter Database](#) dialog, and then click the Properties button at the bottom of the dialog.

### **Name**

This field displays the name that will appear on axis labels and other locations in the program. This field can be edited to any text you want to display.

### **Abbreviation**

The abbreviation for the parameter is used in Parameter Overlay plots to identify parameter ribbons. In general, it should be a short, concise name.

### **Allow Normalization**

Enables normalization for this parameter. Normalization adjust the intensity of the parameter in subsequent data files to be approximately equivalent to the target mean intensity.

Normalization is a process that spans files that the program reads. It allows GemStone to adjust the mean linear intensity for selected parameters to be approximately equivalent across files. There are two aspects to normalizing parameters. First, in the Batch Options panel, an option other than Initialize needs to be selected. This tells GemStone that you want to normalize or set normalization targets. Secondly, enable the Allow Normalization flag for each parameter that you want to normalize.

### **Target Mean Linear Intensity**

When the Allow Normalization option is enabled, this field is used to specify the mean intensity that the parameter should be normalized to when a new file is read.

### **Auto Load**

When this option is enabled, GemStone will automatically add a Parameter Profile to the model to display this parameter when it is found in an FCS file.

### **Auto Zoom**

When this option is enabled, GemStone will automatically zoom-in on the parameter when it is displayed. The auto-zoom will set the lower end of the parameter display to the first channel which has events, and the upper end of the display to the last channel that has events.

### **Always Update Transform**

When this option is enabled and the HyperLog transform is used, GemStone will adjust the B-coefficient and the negative boundary of the HyperLog transformation whenever a file is read. The data in the file are used to determine the best negative boundary and B-coefficient for the transformation.

### **Color**

Choose a color to use for this parameter. The color is used, by default, for the parameter when displayed in Parameter Overlay plots.

**Fluorochrome**

This combo box provides another mechanism for assigning a color to the parameter. If you select a fluorochrome from the list, GemStone will set the Color field to the emission wavelength color of the fluorochrome. This is a quick way to match parameter colors to the fluors they represent.

**SD for Log**

This value is used as the default Line-spread Value for this parameter profile for parameters that use a Log or HyperLog transform.

**%CV for Linear**

This value is used as the default Line-spread Value for this parameter profile for parameters that use a Linear transform.

**Description**

This option field can be used to add notes or to describe the parameter.

**Status****Name**

This field displays the internal name used to identify the parameter. It is based on keyword values from the FCS file.

**Live**

This field displays "On" for parameters that are contained in the FCS file that is currently opened. It displays "Off" for parameters in the database that are not present in the current FCS file.

**Param Index**

If the parameter is "Live", this is the ordinal index of the parameter in the FCS file. If the parameter is not contained in the current FCS file, this field displays -1.

**Mean Transform Intensity**

This is the mean intensity of the parameter on a scale of 0.0 to 100.0, referred to as Transform units.

**Mean Linear Intensity**

This is the mean intensity of the parameter in linear units.

**Short Name**

This is the value of the \$PnN keyword in the FCS file.

**Long Name**

This is the value of the \$PnS keyword in the FCS file.

**Lookup Param Name**

This is the name that is used to look up the parameter in the parameter database.

**Editors**

**Edit Transformation**

Click this button to display the Edit Properties for Linear Transformation dialog. This dialog allows selection of a transformation, and setting of properties related to the transformation.

**Edit Conditions**

Click this button to view or edit conditions used to select this parameter. Conditions can be created to map different parameter names to a common, database parameter record.

**OK**

Click the OK button to accept changes and close the dialog.

**Cancel**

Click the Cancel button to cancel changes and close the dialog.

**Reset**

Click the Reset button to set all properties back to default values.

**See also:**

[Parameter Database](#)

## Properties for Linear Transformation

This dialog is used to select a transformation to be used when displaying a parameter. Properties related to the transformation can also be set. The dialog is accessed from the [Edit Properties for Parameter](#) dialog box, via the [Edit Parameter Database](#) dialog.

### Editable Settings

#### Transform

Choose Linear, Log, or HyperLog. The selected transform is used whenever this parameter is displayed: in Parameter Profile plots, in 1P and 2P histograms, and in Parameter Overlay plots.

#### Calculated Decades

When this option is enabled, GemStone will calculate the number of decades displayed by Log and HyperLog transformations based on the dynamic range of the parameter. For example, if a parameter has a range of 0 – 262144, GemStone would calculate the number of decades as  $\log(262144)$ , or approximately 5.42 decades.

#### Decades

If the Calculated Decades option is not enabled, this value is used for the number of decades in the Log and HyperLog transformations. Data values are scaled to this setting.

#### HyperLog B Coefficient

The B Coefficient defines the size of the linear portion of the HyperLog transform. A value of 10, for example, indicates that there will be 10 linear channels above and below 0. Best visual results are obtained by using multiples of 10 for the B Coefficient. See also the Always Update Transform property in the [Edit Properties for Parameter](#) dialog, which tells the program to recalculate the B Coefficient each time a file is read.

#### Max Linear Range

This field normally displays the dynamic range of the linear parameter values, based on the \$PnR keyword for the parameter. When Linear is selected for the Transform, this field determines what the high value on the axis is set to. Data values are scaled to this new range, not clipped.

#### Negative Percent

This value determines how much of the HyperLog axis will be apportioned to negative values.

### Zooming Settings

#### High Linear Range

This field sets the high linear value used for zooming. By default, it is the full dynamic range.

#### Low Linear Range

This field sets the low linear value used for zooming. By default, it is the lowest value that can be displayed on the axis.

### Current Settings

#### Min Linear Range

This field displays the lowest linear value for the current transformation.

**Normalization Factor**

This field displays the normalization factor that was applied to this parameter. Normally, it is 1.00. However, if Allow Normalization is enabled for the parameter in the [Edit Properties for Parameter](#) dialog, the value will show the actual factor used to normalize the data to the target linear mean intensity.

**See also:**

[Edit Properties for Parameter](#)

## Edit Parameter Conditions

This dialog is used to set up conditions for mapping more than one FCS parameter name to a common, database parameter. This allows files with different parameter names to be analyzed by the same model, and for the parameter axis label and transformations to be common across the files.

There is one entry in the list for each parameter name variant that will be mapped to this database record. You can create new conditions by clicking the New button, or remove a condition by selecting it in the list and clicking the Delete button.

To view or edit details for one of the conditions, select the entry in the list and click the Properties button. This will display the Edit Properties of Parameter Condition dialog.

### See also:

[Edit Properties for Parameter](#)

[Edit Properties of Parameter Condition](#)

## **Edit Properties of Parameter Condition**

This dialog is used to edit the Short Name and Long Name for a parameter that is to be mapped to a common, database parameter.

GemStone allows files with different parameter names to be analyzed by the same model, and for the parameter axis label and transformations to be common across the files. This is accomplished by creating "conditions" for each of the parameter names that will map to a common parameter. The conditions simply define two keyword values from the file that will be used to identify the parameter and map it correctly.

### **Short Name**

The field should contain the value for the \$PnS keyword for this parameter in the FCS file.

### **Long Name**

The field should contain the value for the \$PnN keyword for this parameter in the FCS file.

### **See also:**

[Edit Properties for Parameter](#)

[Edit Properties of Parameter Condition](#)

## **Edit Color Palette**

### **Edit menu**

This dialog is used to edit the colors that are used by GemStone for parameter and zone coloring. You can define the number of colors in the palette, and select the colors and color names you want to use. The colors in the palette are used when initially creating objects. In most cases, you can choose any color you want for an object after you have created it.

The list contains the colors that have been already defined in the palette. Colors and color names can be directly edited in the list. Simply click the color wheel next to a color to select a new color. Click on a color name to edit the text.

### **Edit**

This command edits the properties of the selected color in the list.

### **New**

Use this command to create a new entry in the color list. Once the entry has been added, the color and color name can be edited.

### **Delete**

Use this command to delete the selected color from the color palette.

### **OK**

Click OK to accept the changes and close the dialog.

### **Open**

Use this command to open a color palette file from disk, previously saved using the Save command. The standard File Open dialog is displayed, allowing you to select the file you want to open.

### **Save**

Use this command to save the color palette file to disk, so that it can be shared with others or re-opened at another time. The standard File Save dialog is displayed, allowing you to navigate to the appropriate location and to enter a name for the file.

### **Properties**

Use this command to edit properties of the palette. The Properties for Color Palette dialog is displayed.

## **Properties for Color Palette**

This dialog allows properties of the color palette to be edited. The palette contains a set of colors that are used by GemStone for parameter and zone coloring.

### **Number of Colors**

This field determines the number of colors initially created in the palette.

### **Create Method**

The field determines the method used to create the color palette: Cube Root or Rainbow.

### **See also:**

[Edit Color Palette](#)

## Edit Logging System

Edit menu

This command displays a dialog to view the application log and to edit properties of the logging system.

When the logging system is enabled, GemStone records log entries for the operations that it performs. It is most often used to trouble-shoot a problem, but it can also provide a record of user interactions with the program.

### **If you want to...**

- |                |  |
|----------------|--|
| Enable logging | Click the Properties button to display the Edit Properties dialog. |
| Clear the log  | Click the Delete All button.                                       |

## **View All Application Information**

Edit menu

This command displays a dialog that contains all information that is available to the database and reporting. The dialog information is read-only.

## Edit Information Equations

### Edit menu

Information Equations are user-defined mathematical expressions that can be evaluated and stored in the information database that GemStone maintains. Equations allow you to generate additional statistics to extend the information that can be extracted and stored by the program. Any equations that you create are available to the database and to reporting objects like the table through the information system.

The equations you write are stored as part of the GemStone model document, so they are unique to each model. You can store a set of equations using the Save button and then import those equations into another model using the Open button.

A dialog is displayed, showing a set of example equations on the left, and the list of equations already defined on the right.

### Anatomy of an Equation

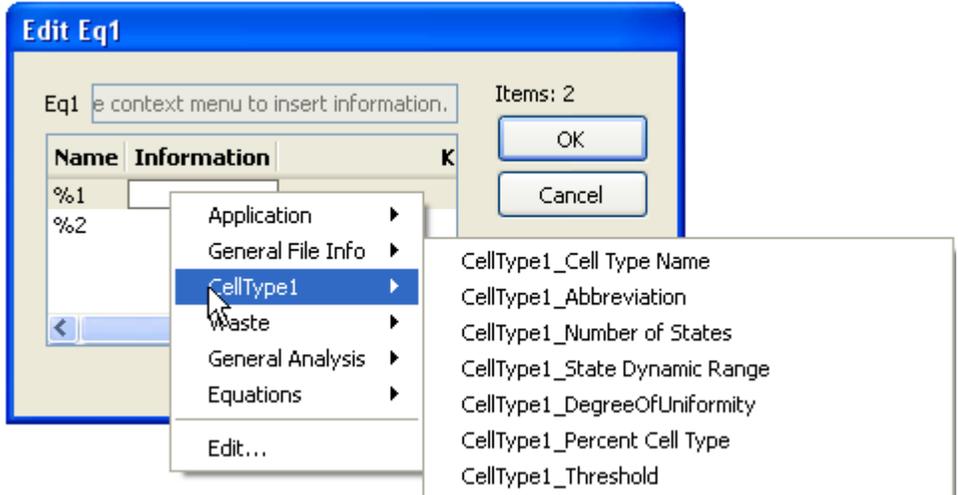
Each equation has at least two fields that can be edited: **Description** and **Format**. In addition, the equation will have additional **Properties** that can be edited if the equation contains placeholders for some of its arguments (e.g. "%1"). The placeholders in an equation are used to reference keywords and other elements in the information system.

For example, let's examine the "Simple Ratio" equation that is the first in the example list. It has a **Description**, "Simple Ratio", which can be edited to be anything you want. To edit the description, click the mouse in the Description field, and edit the text.

The **Format** portion of the equation is where the equation expression is actually defined. The format can contain numbers and mathematical operators, but it can also contain special *functions* and *placeholders*. Function are found in the examples in the tree on the left of the dialog and listed in [Equation Functions](#). Placeholders are the parts of the equation format that use the percent symbol (%) and a number.

In the example equation, the Format field uses two placeholders: %1 and %2. As the name implies, a placeholder identifies part of the equation that will be replaced by an element that GemStone has read from a data file or that it has computed. For example, with our Simple Ratio equation, if we want to create a ratio between the number of events in Zone 1 and Zone 2, we would tell GemStone to replace %1 with the Zone 1 events and %2 with Zone 2 events. This is done by selected the equation in the list and then clicking the **Edit** button.

In the Edit Equation dialog, **right click** the **Information** field to display the menu of choices available to fill-in the placeholder. In our example, you would choose CellType1\_Zone1\_NumberOfEvents for %1 and CellType1\_Zone2\_NumberOfEvents for %2.



Each placeholder in an equation must be associated with an information field in this manner before the equation can be properly evaluated.

Suppose that you want to create a custom equation that multiplies the mean of a parameter in Zone1 by 4.56, and then divides by the mean of another parameter in Zone1. The Format field would look like this:

$$\%1*4.56/\%2$$

After typing the equation into the Format field, you would click the Edit button and select the statistics to fill-in for %1 and %2.

Suppose that you wanted to use a function in this equation, to take the square root of the result. The format field would look like this:

$$FSQRT(\%1*4.56/\%2)$$

For a list of functions, see [Equation Functions](#).

**If you want to...**

- Add a new equation                      Select an example equation from the tree on the left and click Insert or Add to copy the example to the equation list. Edit the *format* field to define the equation expression. Then use the **Edit** button to select fields that will be evaluated by the equation (see [Edit Equation](#)).
- Edit placeholders in an equation      Select the equation in the list on the right and click the **Edit** button. In the Edit Equation dialog, right click in the **Information** column to display the choice of items. Select the keyword or statistics you want from the choices shown.
- Remove an equation                      Select the equation in the list on the left and click the Delete button.
- Save a set of                              Click the Save button, navigate to a location on disk, and save the

equations

equation file.

Open a set of  
equations

Click the Open button, navigate to the location where the equation file  
was previously saved on disk, and open the equation file.

**See also:**

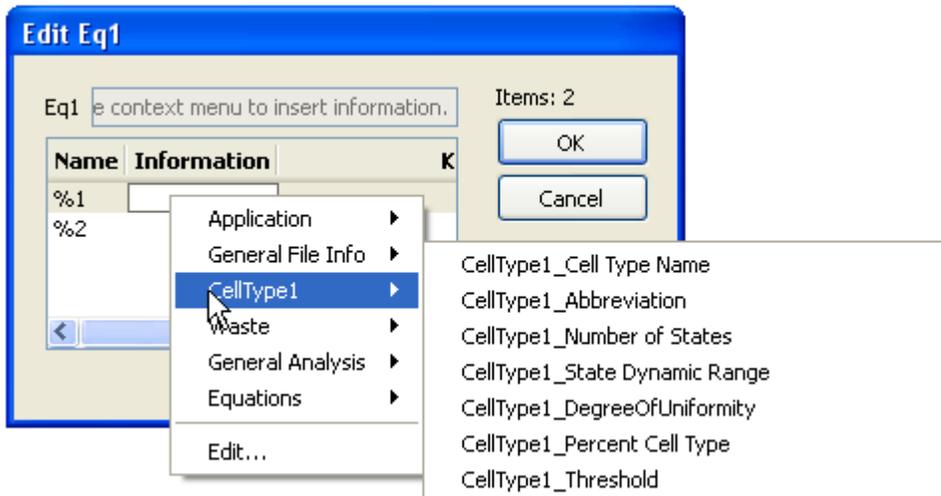
[Edit Equation](#)

[Equation Functions](#)

## Edit Equation

This dialog is used to edit the *placeholders* in an equation. Placeholders are the parts of the equation format that use the percent symbol (%) and a number. Each placeholder must be associated with a piece of information, which is used when the equation is evaluated.

To select the information associated with a placeholder, **right click** the **Information** field to display the menu of choices available to fill-in the placeholder. An expanding menu of choices is displayed. Choose the category to expand the appropriate submenu, then select the specific item from the choices.



Each placeholder in an equation must be associated with an information field in this manner before the equation can be properly evaluated.

### See also:

[Edit Information Equations](#)

[Equation Functions](#)

## Equation Functions

These functions can be used when writing equations using the [Edit Information Equations](#) dialog.

### Indexing

Indexes for FCS parameters start at 1 and represent the parameter order in the FCS file. For example, if a file has six parameters, they are referenced as P1 through P6 in equation functions. Parameters do not need to be included in the model to be referenced by an equation. The best way to determine the parameter index for a specific parameter is to **add one** to the **Associated Parameter** field in the Parameter Profile properties panel.



For example, if the Associated Parameter is **4**, the parameter indexing would **add one** for an index of **5**.

Indexing for gate and regions starts at 1. The expression "G1" refers to the gate for Cell Type 1, and region R1 is the first region index.

Indexes for the Cell Types defined in a model also start at 1. However, there is a hidden Cell Type that contains all unclassified events. This is referred to as Cell Type 0, and it can be referenced in equation functions as "C0".

### Parameter Scales

GemStone keeps track of several different scales for parameters at the same time. The table below describes each scale and gives the symbol to use to access the scale in equation functions.

Scale	Symbol	Description
Linear	L	Raw, linear data values from the data file for the parameter. These values are in the ADC range of the instrument.
Compensated	C	Compensated values, on the same scale as Linear.
Transformed	P	Internal floating point scale. Log or HyperLog transforms have been applied, if the parameter is displayed using one of these transforms. If compensation is defined for the parameter, the Transformed value is in compensated data space. Linear value 0.0 maps to Transformed value 0.0. Linear max value maps to Transformed 100.0.

### Functions

This system is designed to provide a flexible but efficient framework to calculate any kind of statistic we want from the data. This ambitious goal is achieved by an extensive syntax.

Let's begin with the simplest of the summation functions, the gated sum:

**GSUM({GIDn{,Cq}}{;IDm{;IDp}})**

where

{ } indicate an optional argument

GIDn = Rn or Gn

n represents a gate or region index, starting with 1

C = cell type

q = 0 (unclassified), 1, 2, ..., number of cell types

ID = L, C, P

m and p represent a parameter index, starting with 1.

The number of IDn arguments limited to two.

The IDm and IDp are optional.

With no arguments, GSUM becomes a simple count.

The first optional argument to the GSUM function, GIDn, identifies a region or gate. If a gate or region ID is specified, only the events in that gate or region are considered. If the gate or region ID is omitted, then all events are considered in the sum.

For example:

**GSUM()**

returns the number of events

**GSUM(G1)**

returns the number of events in gate G1

**GSUM(R1)**

returns the number of events in region R1

A semi-colon (;) is required between the optional gate and the next argument.

The GSUM function can have up to two ID arguments that identify a parameter of interest. There are 3 scales for parameter data: L for linear, C for compensated, and P for transformed. These are described in the Parameter Scales section above. For example, L1, C1, and P1 all related to parameter 1 in the data file. The scaling is different for each of these representations of parameter 1.

**GSUM(;L1)**

returns the sum of linear intensity values for parameter 1

**GSUM(;C1)**

returns the sum of compensated intensity values for parameter 1

**GSUM(;P1)**

returns the sum of transformed intensity values for parameter 1

If two parameter ID arguments are specified in the GSUM function, the result is the products of the event values for the specified parameters. For example,

**GSUM(R3;P3;P3)**

finds the sum of the square of transformed parameter 3 values that are in region R3.

Gated cross products (cc) are computed in this manner:

**GSUM(R3;P3;P4)**

returns the correlation coefficient for parameters 3 and 4 events that are in region R3.

A simple linear mean is evaluated as,

**GSUM(G3;L1)/GSUM(G3)**

returns the linear mean for parameter 1 events that are in gate G3.

Other types of intensity related summation functions compute the mode and median:

**GMOD(;P2)**

computes the mode for parameter 2 in transformed space.

**GMED(G3;P2)**

computes the median for parameter 2 events that are in gate G3. The result is in transformed space.

Quadrant statistics include

**GQ1(G3;P2)**

returns the location at which 25% of parameter 2 events gated on G3 fall below and 75% fall above.

**GQ3(G3;P2)**

returns the location at which 75% of parameter 2 events gated on G3 fall below and 25% fall above.

Percentage calculations are trivial. For example, would be given by

**GSUM(G3)\*100/GSUM()**

returns G3 events as a percent of total events

**GSUM(R3)\*100/GSUM(G2)**

returns R3 events as a percentage of G2 events.

Any expression can be transformed between linear units and transformed units:

**FLToT(Pn,<Exp>)**

converts a value on the Linear scale to a value on the Transformed scale for the specified parameter *n*.

**FTToL(Pn,<Exp>)** (Transformed to linear)

converts a value on the Transformed scale to a value on the Linear scale for the specified parameter *n*.

For example, suppose P2 is a parameter displayed on a log scale. Then,

**FTToL(P2,GSUM(G3;P2)/GSUM(G3))**

finds the geometric mean for parameter 2 gated on G3. The result is expressed in linear units that relate to the log axis displayed for the parameter.

Some of the more commonly used functions are also included.

**GeoMean(G3;P2)**

is a convenient short-hand for  $FTToL(P2,GSUM(G3;P2)/GSUM(G3))$

**LinMean(G3;P2)**

is equivalent to  $GeoMean(G3;P2)$

**LinMedian(G3;P2)**

is short-hand for  $FTToL(P2,GMED(G3;P2))$

**LinMode(G3;P2)**

Is short-hand for  $FTToL(P2,GMOD(G3,P2))$

**VAR(G3;L3;L3)**

computes the variance of parameter 3 in linear space.

**VAR(G3;L3;L4)**

computes the covariance of parameters 3 and 4 in linear space.

**SD(G3;L3)**

computes the standard deviation for parameter 3 events in gate G3 in linear space.

**QSD(G3;P2)**

computes the quadrant variance for parameter 2 events in gate G3 in transformed space.

**CV(G3;L3)**

computes the coefficient of variation for parameter 3 gated on G3 in linear space.

Additional Examples	
GSUM()	Total number of events
GSUM(G1)	Number of events in gate G1
GSUM(R1)	Number of events in region R1
GSUM(C1)	Number of events in CellType 1
GSUM(C0)	Number of unclassified events (Cell Type 0 is the Unclassified Cell Type).
GSUM(C0,R2)	Number of unclassified events in region R2
GSUM(;L1)	Sum of linear intensity values for parameter 1
GSUM(G1;L1)	Sum of linear intensity values for parameter 1 that are in gate G1
GSUM(G1)/GSUM(R1)	Ratio of the sum of gate G1 events to R1 events
MEAN(G1;P1)	Mean of parameter 1 events that are in gate G1
GMOD(G1;P1)	Mode of parameter 1 events that are in gate G1
GMED(G1;P0)	Median of parameter 1 events that are in gate G1
VAR(G1;P1)	Variance of parameter 1 events that are in gate G1
SD(G1;P1)	SD of parameter 1 events that are in gate G1
CV(G1;P1)	CV of parameter 1 events that are in gate G1
GQ1(G1,P1)	First quartile of parameter 1 events that are in gate G1
GQ3(G1,P1)	First quartile of parameter 1 events that are in gate G1
CC(G1;P1,P2)	Correlation Coefficient of P1 vs. P2 events that are in gate G1
FLtoT(parameter, value)	Converts linear value 100 into transformed units for parameter 1
FLtoT(P1,100)	Converts linear value 100 into transformed units for parameter 1
FTtoL(P1,GSUM(G1;P1)/GSUM(G1))	Geometric mean for parameter 1 events that are in gate G1
GeoMean(G1;P1)	Simple form geometric mean for parameter 1 events in gate G1
FLOG(10)	Natural log of 10
FL10(1)	Log base 10 of 1

FEXP(3)	Exponent of 3
FE10(3)	Exponent base 10 of 3
FSQR(4)	Square of 4
FCUB(3)	Cube of 3
FBND(arg,lower,upper)	Evaluates the first argument to see if it is between lower and upper. Returns the first argument if it is between the others, returns lower if arg is less then lower, or returns upper if arg is greater than upper.
FPWR(10,3)	Returns 10 raised to the 3rd power.
FSQRT(5)	Returns the square root of 5.

**See also:**

[Edit Information Equations](#)

[Edit Equation](#)

## Show Tool Button Labels

Edit menu

This command toggles the display of text labels for the main toolbar on and off.

Labels On



Labels Off



## **Edit Preferences**

Edit menu on PC, Application menu on Mac

This command displays a dialog that allows all preferences for the program to be edited.

Select an item in the tree on the left side of the dialog to display the properties for that object in the pane on the right. Preferences are automatically saved as defaults for new objects, and they are remembered after the program quits.

[File System Preferences](#)

[General Preferences](#)

[Graphics Preferences](#)

[Cell Type Graphics Preferences](#)

[Control Point Graphic Preferences](#)

[2P Graphics Preferences](#)

[1P Graphic Preferences](#)

[Merging File Preferences](#)

[Minimization Preferences](#)

[Miscellaneous Preferences](#)

[Parameters Preferences](#)

[Workspace Preferences](#)

## Preferences

### File System Preferences

#### Packet Size

Enter the number of events from the listmode file to keep in memory.

#### Auto Compensate

When enabled, GemStone will automatically decode compensation keywords in FCS files and apply the compensation described in the keywords. Currently, files created by BD Diva software, Accuri software, and Beckman Coulter files are supported. If an FCS file does not contain compensation information, this setting will have no effect.

#### Hardware Compatible Comp

If compensation is applied by the Auto Compensate logic, this option forces the compensation to be a subtraction similar to hardware compensation.

#### Read All Keywords

Enable this option to read keywords from all datasets.

#### Desired Dataset

If the FCS files you work with contain more than one dataset, this value allows you to select which dataset to read. If you want the program to prompt you to select a dataset when there is more than one, set Desired Dataset to **0**. To automatically select a specific dataset, enter the 1-based dataset index. For example, to read the 2nd dataset in a file, enter the value **2**.

#### Dataset Descriptive Keywords

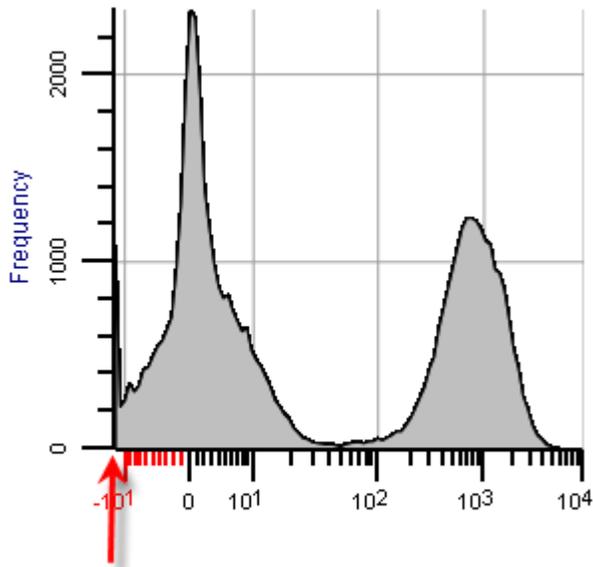
Enter keywords that you want the program to read and present to you from each data source to help select the one you want to open. Use commas between keywords.

## HyperLog

### Percent Negative Boundary

This value controls how GemStone determines the negative boundary for the HyperLog transform. Using the default value of 3.0 as an example, the program finds the channel at which 3.0% of events are in lower channels. If the channel at that location is a negative channel, the channel is used as the lower boundary of the histogram. If the channel is positive, a secondary calculation is used to determine the lower boundary.

Smaller values allow more negative channels to be shown.



3% of events fall below this channel and are clipped from the display.

### Negative To Positive Ratio

This ratio controls the relative portions of the axis that are devoted to negative and positive channels. The default value of 0.20 allows up to 20% of the axis to be devoted to negative channels and 80% to positive channels.

## **General Preferences**

### **Specimen Keyword**

This keyword is used on the Cell Type widget and other objects to identify the data file that is currently opened.

### **Number of decimals**

This property determines the number of decimal places that will be displayed for numbers in tables.

## **Graphics Preferences**

These preferences are common to the graphic objects on the canvas and reporting areas.

### **Background for Drag Objects**

Select the background to use for objects on the canvas.

### **Title Font for Drag Objects**

Select the font used for titles.

### **Axis Num Major Tics**

Select the number of major tics for axes.

### **Axis Pen Weight**

Enter a pen weight for the axes.

### **Axis Pen Color**

Select a color for the axes.

### **Axis Number Font**

Select a font for numbers on the axes.

### **Axis Number Color**

Select a color for the numbers on the axes.

### **Axis Label Font**

Select a font for the axis labels.

### **Axis Label Color**

Select a color for axis labels.

### **Background Color**

Select a background color for plots.

### **Show X Grid Lines**

Enable to show X grids.

### **Show Y Grid Lines**

Enable to show Y grids.

### **Grid Line Width**

Enter a line width.

### **Grid Line Style**

Choose a line style for grids.

### **Grid Color**

Choose a color for grids.

### **Unclassified Color**

Select a color for unclassified events.



## **Cell Type Graphics Preferences**

### **Enable Dots**

Check this option to enable dots in the cell type parameter plots.

### **Dot Size**

Type a default dot size.

### **Show All Dots**

Enable to show dots for all events in the packet.

### **Desired Dots**

Select a desired number of dots to display. If Show All Dots is enabled, this setting is ignored.

### **Show Unclassified Events**

Enable this option to show unclassified events in addition to the classified events.

### **Show Fidelity Plot**

Enable this option to show the state index fidelity plot by default.

## **Control Point Graphic Preferences**

### **Size**

Set the size in pixels for control points.

### **Pen Width Selected**

Select a pen width for control points that are selected.

### **Pen Width Not Selected**

Select a pen width for control points that are not selected.

### **Pen Color**

Select a pen color for control points.

### **Fill Color Selected**

Select a fill color for control points that are selected.

### **Fill Color Not Selected**

Select a fill color for control points that are not selected.

### **Tip Color**

Select a fill color for the triangular tips of control points.

### **Transparency**

Choose a transparency setting for the control point fill.

## **2P Graphics Preferences**

### **Enable Dots**

Check this option to enable dots in the 2P plots.

### **Dot Size**

Type a default dot size.

### **Show All Dots**

Enable to show dots for all events in the packet.

### **Desired Dot Number**

Select a desired number of dots to display. If Show All Dots is enabled, this setting is ignored.

### **Show Unclassified Events**

Enable this option to show unclassified events in addition to the classified events.

### **Enable State Vectors**

Enable the display of state vectors.

### **Enable Surface**

Check this option to display the surface graphics.

### **Probability Mixing Color**

Choose a color that will be used to for the densest portions of the surface plot (the highest probability). The default color is white.

### **Number of Vectors**

Select a desired number of state vectors to display.

## 1P Graphic Preferences

### Fill Style

Choose a fill style for 1P histograms.

### Transparency

Enter a transparency for filled 1P histograms (0 = solid, 100=transparent)

### Resolution

Enter the number of channels for 1P histograms.

### Show Peaks

Enable this option to show the peaks that were found by the peak finding routine. Peaks are used by optimization routines to locate populations and position control points.

### Peak P-Value

Enter a P-value filter to apply to the set of peaks that were found for the parameter.

## **Overlay Graphic Preferences**

### **Show X Grid Lines**

Check to show the X grid lines.

### **Show Zone Boundaries**

Check to show the zones boundaries on the overlay.

## **Parameter Graphics Preferences**

### **Enable Surface**

Check this option to display the surface graphics.

### **Probability Mixing Color**

Choose a color that will be used to for the densest portions of the surface plot (the highest probability). The default color is white.

## **Heat Map Preferences**

This dialog allows all preferences for the program to be edited. The current section allows editing of Heat Map Graphics properties.

### **Chi Multiplier**

This value is used to scale the Y-axis distribution of the Heat Map over 100 units. The value is 5. (1.00 to 20.00)

### **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.

#### **Reset**

Click this button to reset the object's properties to default values.

## **Merging File Preferences**

### **Auto Create Model Resolution**

User higher values for more parameters. Higher values slow down the merging process. (2 to ...)

### **Auto Create Model Threshold**

Controls the spot size for the merging process. A normal value is close to 1. (0.00 to 10.00)

### **Auto Calculate**

If true, will automatically calculate the resolution and threshold to satisfy the desired yield.

### **Desired Yield**

If Auto Calculate is true, finds the threshold and resolution to give a desired yield. (0.00 to 100.00)

### **Maximum Auto Create Resolution**

Maximum resolution to try for the Auto Create Model option. (2 to ...)

## **Minimization Preferences**

### **Delta Param**

Choose the fraction of the parameter to change.

### **Termination Criteria**

When the response function changes by less than this value, the system terminates the minimum search routine.

### **Max Function Evaluations**

Choose the maximum number of function evaluations.

## Miscellaneous Preferences

### Client Timeout

Enter the number of seconds to wait until a battery request times out.

### Battery Server Port

Enter the port number used to connect to the battery server. This value must match the port that the battery server is configured to use.

### User Login Option

Select the user login option you want to use when the program starts up.

### User Group

Select the default user group for the user login dialog. The user group controls access to program features.

### Show Banner

Enable this option to show the banner dialog when the program is launched.

## **Parameters Preferences**

### **Estimated %CV for Linear Parameters**

Enter the default estimated %CV for linear parameters.

### **Estimated SD for Log Parameters**

Enter the default estimated SD for log parameters (e.g. 10.0). (0.10 to 50.00)

### **Estimate Min Response Change**

Enter the estimated minimum response changes. Lower values produce more accurate estimates.

## **Workspace Preferences**

### **Grid Snap**

Enable this option to snap drag objects to the grid.

### **Grid Size**

Set the size of grid elements, in pixels.

### **Angle Snap**

When rotating, snap objects to increments of the specified angle.

### **Angle Snap Size**

Set the angle snap, in degrees.

### **Copy DPI**

Choose a desired number of dots per inch (DPI) for copying graphics to the clipboard.

### **Saving Format**

Select a file format to use when saving the graphics to disk.

### **Default Page Width**

Enter a size for the canvas in inches.

### **Default Page Height**

Enter a size for the canvas in inches.

### **Auto-expand Parameter Plots**

Select the number of parameter plots to keep open as new plots are added to the model.

## Analysis Menu

### Classify Data

Analysis menu

Use the Classify Data command to model the *data in memory* with the Probability State Model. This command is typically used when opening a new FCS file into a GemStone document to apply the model to the new FCS data. A related command, Classify File, performs the same functions but on the entire FCS file.

When classifying data, GemStone uses the Probability State Model to assign each event to a Cell Type, and within a Cell Type, to a particular state on the progression axis. Virtually every decision in the classification process is probabilistic.

#### See also:

[What is a Probability State Model?](#)

[Auto Analyze](#)

[Batch Processing](#)

## Optimize Model

### Analysis menu

Use the Optimize Model command to adjust the positions and line-spreads of Control Points based on the FCS data that is currently displayed. This is a useful for making small adjustments to the model due to slight intensity shifts from one file to another.

The process makes adjustments to all Parameter Profiles in all Cell Types. GemStone uses peak-finding routines to determine the best intensity positions for the Control Points. It performs statistical fits on peaks to determine line-spread adjustments.

Optimize Model can be time-consuming. A progress meter is displayed on the status bar to let you see how the optimization is proceeding. You can halt the process by pressing the Esc key on the keyboard.

### See also:

[What is a Probability State Model?](#)

[Auto Analyze](#)

[Classify File](#)

[Batch Processing](#)

## **Auto Analyze**

### Analysis menu

Auto Analysis is a more sophisticated version of Optimize Model. It is used to automatically tune the positions of Control Points to the data file that is currently opened. Like Optimize Model, it affects all Parameter Profiles in all Cell Types.

Auto Analyze simulates the approach that you might take to adjust a model manually. It starts by turning off all of the Parameter Profiles so that nothing is being fit by the model. Next, it searches for Parameter Profiles that are using the "Constant" profile, and it optimizes the position and line spread for each. Once all Constant profiles are adjusted, it uses the Enrich Data command to select as many events as possible into the model. Then, one by one it visits the remaining Parameter Profiles and optimizes the positions of the Control Points. Once it has completed these steps, the complete model is analyzed once again with all profiles active.

### **See also:**

[What is a Probability State Model?](#)

[Classify Data](#)

[Classify File](#)

[Batch Processing](#)

## Enrich Data

### Analysis menu

This command is typically used during the model-building process to select events from a data file that match the model. Before using the command, you should make sure that the only parameters with Match Status enabled are those that use "Constant" profiles. Constant profiles are sometimes referred to as "selection" profiles, because they are used to select subsets of events from the data file. They are the most useful for the Enrich Data because they are so simple and robust.

The Auto Analyze command uses this command as part of its analysis process.

### See also:

[What is a Probability State Model?](#)

[Auto Analyze](#)

[Batch Processing](#)

## **Classify File**

Analysis menu

Use the Classify File command to model the *all of the data in the file* with the Probability State Model. A related command, Classify Data, performs the same functions but only on the data in memory.

When classifying data, GemStone uses the Probability State Model to assign each event to a Cell Type, and within a Cell Type, to a particular state on the progression axis. Virtually every decision in the classification process is probabilistic.

### **See also:**

[What is a Probability State Model?](#)

[Auto Analyze](#)

[Classify Data](#)

[Batch Processing](#)

## Fidelity Analysis

Cell Type Properties panel, Utility Actions

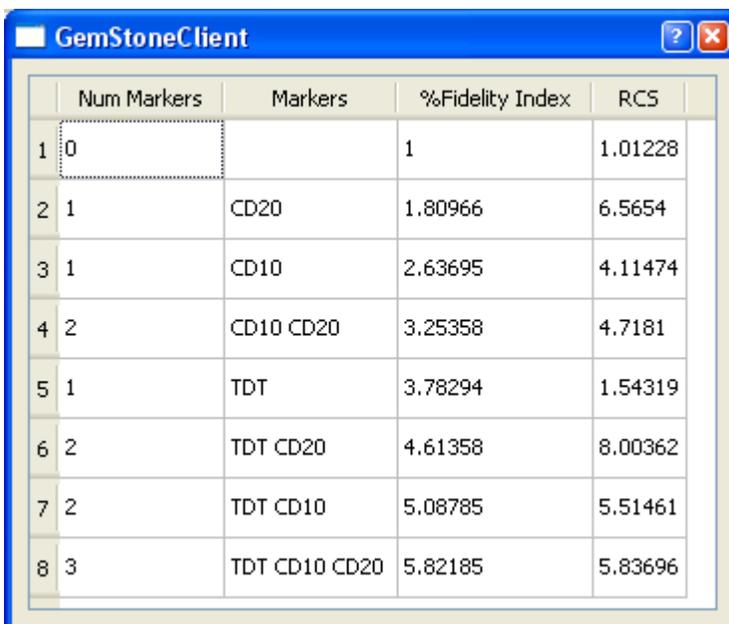
For each event it analyzes, GemStone keeps track of the probability that the event has been correctly classified. This allows it to evaluate the classifications for an entire Cell Type and to report an additional statistic: **State Fidelity Index**. The State Fidelity Index is the average probability for all events in all states that the events are correctly classified, expressed as a percentage. This value is shown in the Cell Type Properties panel. The closer the value is to 100, the more likely it is that events are correctly classified.

The State Fidelity Index is more than a fancy statistic, though. It is the basis for an objective assessment of how well the markers in the model are working to define the progression. The **Fidelity Analysis** is that assessment, and with it we can determine which markers are the most important and which are least important. We now have a means for objectively determining the best markers to use in a panel. In this case, "best" means the markers that allow the model to produce the highest State Fidelity Index for each cell type.

The Fidelity Analysis command is found in the Cell Type Properties panel's Utility Actions. To perform the analysis, simply select the command from the list of Utility Actions.

Here is what the Fidelity Analysis does. First, it determines which Parameter Profiles in the Cell Type are active and use something other than a Constant profile. Then, it methodically evaluates all combinations of these non-constant profiles, performing a Classify Data command on each combination. It stores the State Fidelity Index for each of these combinations.

When the analysis is complete, it presents a table of the results. The table is organized starting with each Parameter Profile alone, then every combination of two parameters, and so on. The rows are sorted from lowest State Fidelity Index to highest. This allows you to evaluate the relative importance of each parameter in each combination. From this, you can determine which parameters are most vital to your panel of markers.



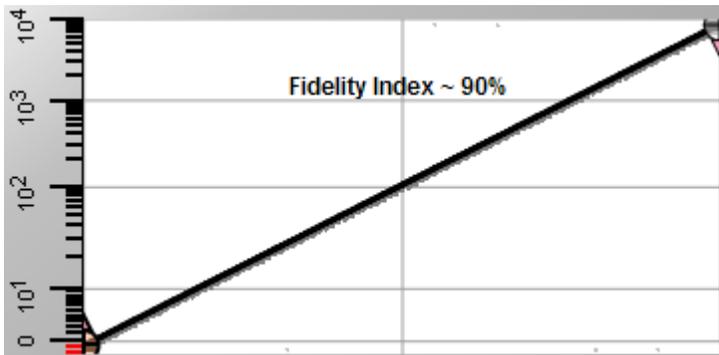
	Num Markers	Markers	%Fidelity Index	RCS
1	0		1	1.01228
2	1	CD20	1.80966	6.5654
3	1	CD10	2.63695	4.11474
4	2	CD10 CD20	3.25358	4.7181
5	1	TDT	3.78294	1.54319
6	2	TDT CD20	4.61358	8.00362
7	2	TDT CD10	5.08785	5.51461
8	3	TDT CD10 CD20	5.82185	5.83696

In the example results shown above, we can see that the best State Fidelity Index our model achieves is 5.82, using TDT, CD10, and CD20. Notice, that TDT alone is reaching a higher index than CD10 and CD20 together. This shows us that TDT is by far the most important marker for this panel and this model.

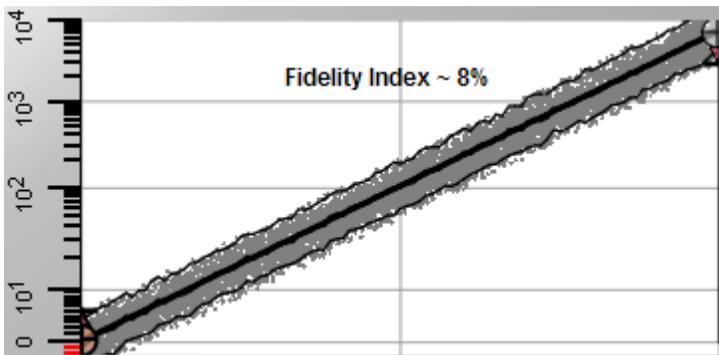
When you close the window, GemStone allows you to save the table of results to a text file on disk. The text file is a simple, tab-delimited file that can be imported into spreadsheets, statistic software, and databases for further analysis and storage.

As you might guess, the parameter profile selected for each parameter has an impact on how important the parameter is in the Fidelity Analysis. In general, the more the profile changes over the progression, the more important it is. To illustrate, here are a few profiles with synthesized data. We analyzed each one separately to determine its relative Fidelity Index.

For a profile that increases or decreases continuously over the progression and varies over the full dynamic range for the parameter, we see a very high Fidelity Index of about 90%. In our example, we created the profile with a very small line-spread, which also improves the fidelity score.

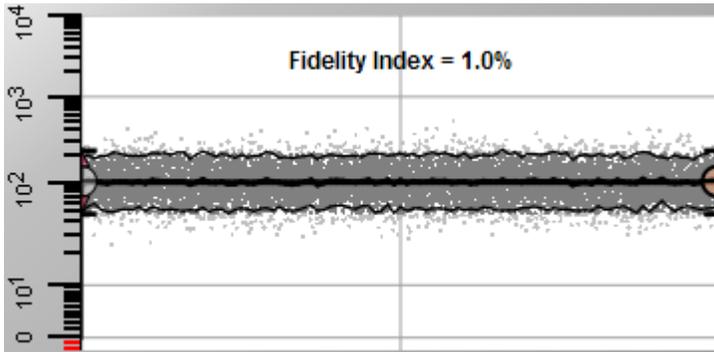


If we increase the line-spread of this parameter profile to 5.0, the score drops all the way to 8%. So, not surprisingly, we see that line-spread plays an important roll in the likelihood that we are correctly classifying the events. Larger line-spread means more uncertainty.

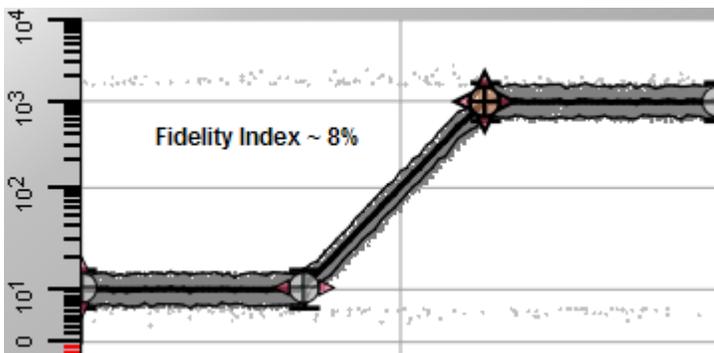


At the other extreme, a constant parameter profile always yields a Fidelity Index of 1.0. Since all events in the progression have the same likelihood of falling into any of the states on the

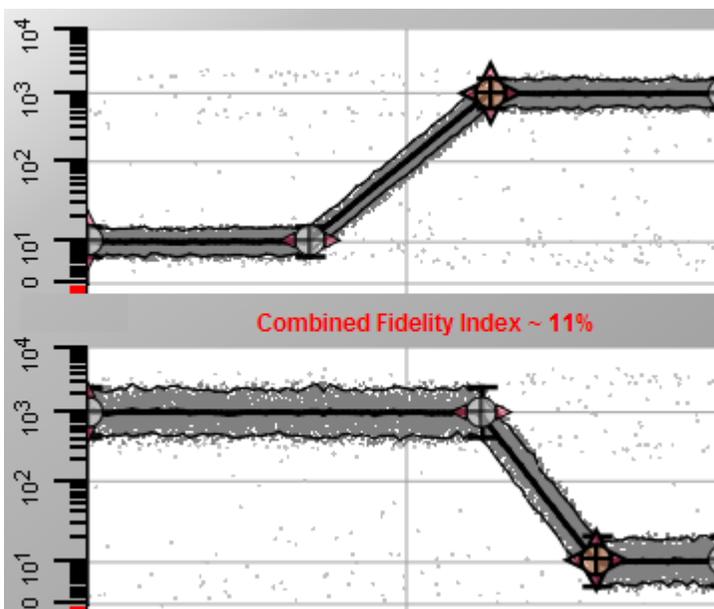
progression, we have virtually no confidence that any given event is classified into the correct state.



A typical Step-Up profile with a line-spread of 3.0 produces a fidelity index of around 8%.



If we combine two profiles that have transitions in different portions of the progression, the Fidelity Index of the model as a whole improves to ~11. This illustrates the synergistic nature of parameter profiles in defining the progression.



Use the Fidelity Analysis feature to determine objectively which markers are really adding information in your panel.

## Battery Menu

### Battery Menu

The Battery menu is used to manage the "software batteries" that GemStone uses. Software batteries encode a certain amount of "charge". With each charge, you can open one data file for analysis.

The program keeps track of the files you have opened and allows you to re-open files without using a battery charge. You can purchase additional charges when you need to open additional data files, and you can transfer charges to other users.

#### **See also:**

[Overview: Software Batteries](#)

[How to Work with Software Batteries](#)

## **Battery Status**

Battery menu

This dialog displays the status of the software battery currently installed for GemStone. See [Battery Menu](#) for a description of the battery system.

### **Application Info**

#### **Application name**

This field displays the name of the application.

#### **Computer ID**

This field displays the computer ID. The battery system uses the Computer ID when ordering or requesting batteries to identify the computer that the battery is for.

### **Battery Info**

#### **Battery ID**

This field displays the ID of the current battery.

#### **Charge**

This field displays the charge remaining on the current battery.

#### **Install-by date**

This field displays the date by which the battery had to be installed. Batteries must be installed prior to their Install-by date in order to operate correctly.

#### **OK**

Click this button to close the dialog and accept changes.

#### **Cancel**

Click this button to cancel the dialog.

#### **Buy**

Click this button to order additional software battery charges from the Verity Software House web site. See [Buy Now](#) for details.

#### **Charge**

Click this button to download prepaid battery credits from the Verity Software House web site. See [Charge Battery](#) for details.

## **Buy Now**

### Battery menu

Use this command to order additional software battery charges from the Verity Software House web site.

In order to use it, your computer and GemStone need to have internet access. Firewalls can sometimes interfere with this function.

## Charge Battery

Battery menu

Use this command to add charges to your GemStone software battery. The command initiates the [Charge Battery Wizard](#), which will guide you through the process of getting charges for your battery.

### See also:

[Overview: Software Batteries](#)

[How to Work with Software Batteries](#)

## Charge Battery Wizard

This wizard is displayed by selecting [Charge Battery](#) from the Battery menu of the application. The wizard guides you through the process of getting charges for your software battery.

For a discussion of how to use the wizard, see [How to Work with Software Batteries](#),

### See also:

[Overview: Software Batteries](#)

[Check Balance](#)

[Upload Credits](#)

## **Check Balance**

### Battery menu

This option requires that you have a member account on [www.vsh.com](http://www.vsh.com). Visit the web site to create a member account if you have not already done so.

This command allows you to check to see how many battery charges remain in your Verity Software House web account. In order to use it, your computer and GemStone need to have internet access. Firewalls can sometimes interfere with this function.

### **Web Login**

Enter the login name for your Verity Software House web account.

### **Password**

Enter the password for your Verity Software House web account.

### **Remember Password**

Enable this option if you want the password to be remembered on this machine.

Click the OK button to initiate the balance check. The program will communicate with [www.vsh.com](http://www.vsh.com) to determine the balance and display the results.

Use the [Charge Battery](#) command to download some or all of the available charges.

### **See also:**

[Upload Credits](#)

## Upload Credits

### Battery menu

This option requires that you have a member account on [www.vsh.com](http://www.vsh.com). Visit the web site to create a member account if you have not already done so.

This command uploads battery credits *from* your local battery *to* the Verity Software House web site. In order to use it, your computer and GemStone need to have internet access. Firewalls can sometimes interfere with this function.

If the command is successful, *all* battery charges on the computer will be uploaded to the specified user account on the Verity Software House.

This command is useful for several purposes. When credits are uploaded to Verity Software House, you can easily transfer them to another user or download them to another computer that you are using. You can use the Upload Credits command *before* reformatting your hard drive or reload your operating system to preserve your available charges. After reformatting, you can download the credits using the [Charge Battery](#) command to restore charges on your computer.

### Web Login

Enter the login name for your Verity Software House web account.

### Password

Enter the password for your Verity Software House web account.

### Remember Password

Enable this option if you want the password to be remembered on this machine.

When you click OK, the program will communicate with [www.vsh.com](http://www.vsh.com) to upload the credits. The results of the upload will be displayed.

### See also:

[Check Balance](#)

[Charge Battery](#)

## **Server Menu**

### **Server Menu**

This menu provides commands for Battery Server functions. It only appears in Battery Server versions.

## Properties for Battery Server

Server menu

This dialog is informational and does not provide any editing of properties. It is most useful for viewing the server name, IP, and port address.

### Server host name

This is the network name of the server computer.

### Server IP Address

This is the IP address of the server computer.

### Server Port

This field specifies the IP Port that the battery server uses to communicate with client applications. The default value is generally not used by other well-known applications. If there is a port conflict with another application, a value between -1 and 65535 can be entered. Use -1 to allow the program to find an available port.

Client applications need to know the port that is used by the server. It must be entered in the Preferences dialog.

### Server Password

Enter a password that client applications will use to authenticate their requests for battery charges.

### Maximum Battery Request

Enter the maximum number of charges that can be requested at a time by a client application.

### Server Timeout

This field sets the number of seconds that the server will wait before communication with a client times out. Use a higher value to allow more time for slower network connections.

## **Transfer Charge**

Server menu

This command is used to fulfill an offline battery request from a client application. The information used to fill in this dialog comes from the client computer and is displayed to the user in the [Charge Battery](#) dialog box.

Fill in the fields with the information from the client computer, and then click OK to generate a set of codes that the user will use to add the charges to the client computer.

### **Program**

Choose the program that the user has installed on the client computer.

### **Transaction Code**

Enter the transaction code from the client computer Initiate Offline Transfer dialog.

### **Computer ID**

Enter the computer ID from the client computer Initiate Offline Transfer dialog.

### **Charge To Transfer**

Enter the amount of charge to transfer to the client computer.

### **User (optional)**

Enter the name of the user, or leave blank. This field will be stored in the transaction log.

### **OK**

When you click OK, the program will attempt to draw the requested number of charges from the server battery. If charges are available, a dialog will be displayed containing a set of codes. Print or send these codes to the user, who will then need to enter them in the [Charge Battery](#) dialog.

### **Cancel**

The button closes the dialog without taking any action.

### **See also:**

[Charge Battery](#)

**Help Menu**

**Help Index**

Help menu

This option displays this online User Guide in your browser. The User Guide is fully indexed and searchable, and contains tutorials and reference sections to provide a comprehensive explanation of GemStone features and functions.

## **Ask Tech Support**

### Help menu

This command interacts with your email application to create a new message to Verity Software House Tech Support. A new message is created with the appropriate "To" 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 question(s) you want to ask. A member of the Tech Support team will respond to your question as quickly as possible.

### **See also:**

[Make a Suggestion](#)

[Report a Problem](#)

## **Make a Suggestion**

Help menu

This command interacts with your email application to create a new message to Verity Software House Tech Support. A new message is created with the appropriate "To" 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.

### **See also:**

[Ask Tech Support](#)

[Report a Problem](#)

## **Report a Problem**

### Help menu

This command interacts with your email application to create a new message to Verity Software House Tech Support. A new message is created with the appropriate "To" 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.

### **See also:**

[Make a Suggestion](#)

[Ask Tech Support](#)

## **Application Info**

Help menu

This command displays a dialog that contains information about the program and its installation. It is useful when interacting with tech support.

## **About**

Help menu on PC or Application menu on Mac

This command displays the application banner dialog box. The dialog contains information about the application. It also provides access to the Registration or Battery system, depending on the type of installation you have.

The phone number for Verity Software House is shown, as are hyperlinks to [www.vsh.com](http://www.vsh.com) and the general email account.

### **See also:**

[Registering your new software](#)

[Battery Menu](#)



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