

GemStone User Guide

Updated 20 Dec 2019

This guide is intended for GemStone version 2.0 and minor version updates.

Copyright 2008-2019 by Verity Software House
All Rights Reserved.



Phone: (207) 729 6767

Email: verity@vsh.com

Web: www.vsh.com

Table of Contents

| | |
|---|----|
| Quick Start Guide..... | 3 |
| 1. Install and register your new software..... | 3 |
| 2. Take a quick look at the user interface and terminology..... | 3 |
| 3. Try out a tutorial or two..... | 3 |
| Further reading | 4 |
| License and Warranty..... | 5 |
| Latest Versions | 7 |
| New Features in GemStone..... | 9 |
| System Requirements..... | 11 |
| Installation and Setup of GemStone..... | 13 |
| Registering your new software | 15 |
| GemStone Terminology | 23 |
| GemStone User Interface | 23 |
| GemStone User Interface | 25 |
| The GemStone Workspace..... | 27 |
| What is a Project?..... | 28 |
| The Project Library | 29 |
| What is a Template Document? | 30 |
| Saving and Opening..... | 31 |
| Phenotype Naming..... | 34 |
| What is a Probability State Model? | 35 |
| Tutorials..... | 37 |
| Analyzing Files with an Existing Project..... | 37 |

Table of Contents

| | |
|--|-----|
| Analyzing Files with an Existing Project..... | 42 |
| Develop: Starting a New Project from Scratch | 47 |
| Develop: Adding Cell Types to the Project..... | 78 |
| Develop: Analyzing Subsets | 107 |
| Develop: Exploring with Cen-se' | 127 |
| Develop: Additional Exercises | 149 |
| Develop: Finishing the Project..... | 154 |
| Stain Assessment..... | 182 |
| Reference..... | 191 |
| Reference | 191 |
| File tab | 192 |
| Home tab..... | 209 |
| Insert tab..... | 225 |
| Options tab | 345 |
| Develop tab..... | 378 |
| Help tab..... | 450 |
| Batch Commands..... | 457 |
| Cell Type tabs | 472 |
| Users and Groups | 493 |
| Research and Compliance Modes | 493 |
| Research and Compliance Modes | 495 |
| Setting Up Compliance Mode | 497 |
| 21 CFR Part 11 Compliance | 501 |
| Index..... | 503 |

Quick Start Guide

1. Install and register your new software...

[Installation and Setup of GemStone](#)

[Registering your new software](#)

2. Take a quick look at the user interface and terminology...

[GemStone User Interface](#)

[The GemStone Workspace](#)

[What is a Project?](#)

[The Project Library](#)

[What is a Template Document?](#)

[Saving and Opening](#)

[Users and Groups](#)

3. Try out a tutorial or two...

[Analyzing Files with an Existing Project](#)

The Develop tutorials for creating your own projects:

[Starting a New Project from Scratch](#)

[Adding Cell Types to the Project](#)

[Analyzing Subsets](#)

[Exploring with Cen-se'](#)

[Additional Exercises](#)

[Finishing the Project](#)

[Stain Assessment](#)

Further reading

When you have a chance, review the Reference sections.

[Reference](#)

License and Warranty

License Agreement

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

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

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

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

Duplication of the manual is strictly prohibited without the expressed written permission of the copyright holder.

If you transfer the use of this software to another party, that party must accept the terms of this agreement, and you must transfer all copies of the software or destroy them.

Your license to use this software will terminate if you are found to be in violation of this agreement.

The laws of the State of Maine and the United States of America govern this license.

Limited Warranty

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

VSH makes no other warranties, either expressed or implied, with respect to this manual or with respect to the software described in this manual, its quality, performance, merchantability, or fitness for any particular purpose. VSH software is licensed "as is". The entire risk as to its quality and performance is with the buyer. Should the software prove defective following their purchase, the buyer (and not VSH, its distributor, or its retailer) assumes the entire cost of all necessary servicing, repair, or correction and any incidental or consequential damages. In no event will VSH be liable for direct, indirect, incidental, or consequential damages resulting from any defect in the software, even if VSH has been advised of the possibility of such damages. Some states do not allow the exclusion or limitation of implied warranties or liability for incidental or consequential damages, so some of the above limitations or exclusions may not apply to you.

Latest Versions

To find the latest versions for GemStone and the documentation, visit the Verity Software House web site at 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 verity@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.

The software also has a [Check for Updates](#) command in the Help menu. Use this command periodically to see if there are updates for your software. The command can download and install updates that are available, if you have administrative rights to your computer.

New Features in GemStone

GemStone 2.0 is a totally redesigned application. While it is based on the same Probability State Modeling engine introduced with GemStone 1.0, the user interface and workflows are entirely different.

And you will love the changes!

System Requirements

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

Minimum System Configurations

If you purchased a Windows version:

Windows 7, Windows 10 or greater, 3.40GHz CPU or better, 8GB RAM or greater, 8 or more logical processors, with 750MB free disk space for program files.

If you purchased a Mac version:

macOS 10.12 or greater, 3.40GHz CPU or better, 8GB RAM or greater, with 750MB free disk space for program files.

Optimal Configurations

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

Installation and Setup of GemStone

IMPORTANT NOTES:

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

Windows Version

Installation

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

Using the software

- Log on to your computer with your own user account. This should be the account that you normally use on the computer.
- Open the Start menu and navigate to the Verity folder. Choose **GemStone 2.0**.

Mac OS Version

Installation

- Log on to your computer with an account that has Administrator privileges. You *must* have administrative rights during installation.
- The installer is distributed as a disk image, or "DMG" file. Double-click the DMG file to mount and open the disk.
- A Finder window will display program icons and instructions for how to install the program. Follow those directions to complete the installation.
- See the section on [registering your software](#) for information on how to register your software.

Using the software

- Log on to your computer with your own user account. This should be the account that you normally use on the computer.
- In Finder, navigate to the Applications folder.
- If there is a **GemStone** shortcut in the Applications folder, double-click it to start the program.
- If there is no GemStone shortcut, open the **GemStone 2.0** folder, and then the Application subfolder. Double-click the **GemStone 2.0** application icon.

Registering your new software

Why You Should Register

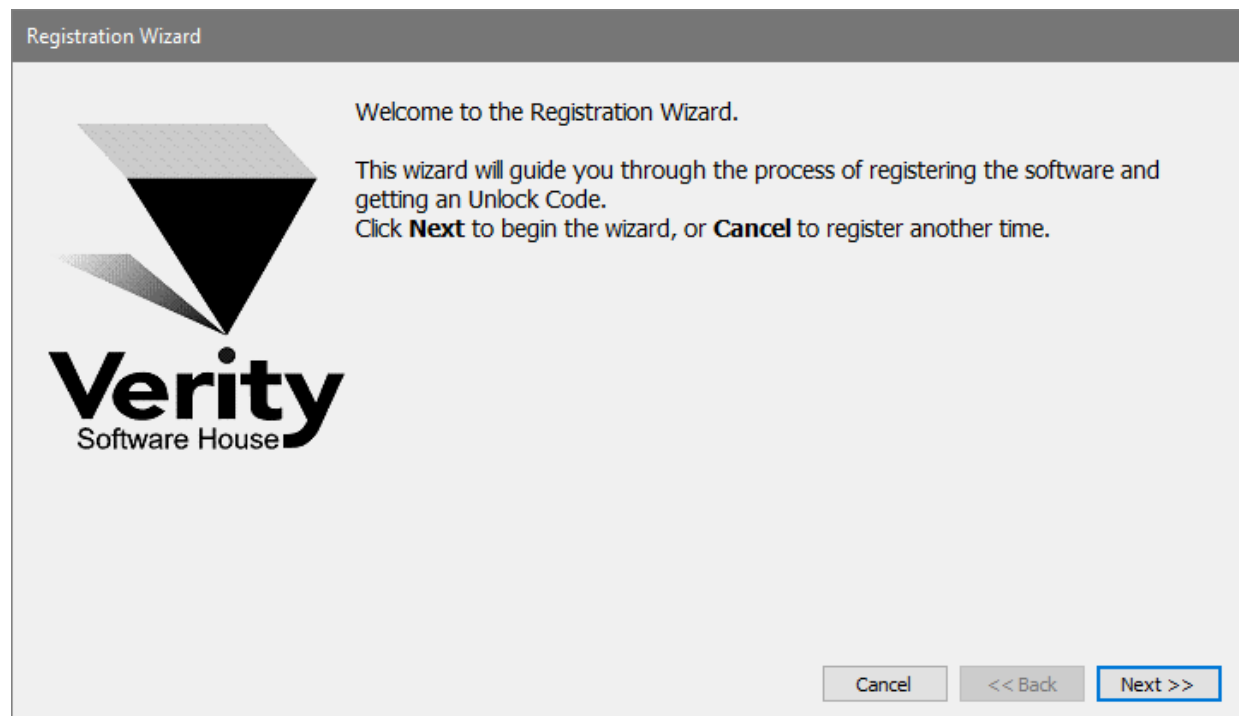
Registration is a key step in setting up the software to run on your computer. In fact, *your software will not be fully functional until you register*. Here's why.

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

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

How to Register

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



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

Registration Wizard

How would you like to do the registration?

- I want to register using the wizard.
- I want to register using my browser.
- I want to register by email.
- I want to use another computer to register.
Or I already have my Unlock Code.

Verity Software House

Cancel << Back Next >>

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

Registration Wizard

Please fill in the Serial Number and CD Key that came with the software, along with your email address.

Program: GemStone for Windows

Version: 2.0

Computer ID: AB1907FC233E

Computer Name(Optional): Ben-XPS

Serial Number: 12345

CD Key: M03FIYY

Your Email: yourEmail@yourDomain.edu

Descriptive Characters: Number 0; Letter 0; Number 1; Letter I

Print Cancel << Back 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 *Your 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 program box 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 your 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 not yet created a user account on vsh.com, a user account wizard is displayed. This step *only* appears if you have not yet created a user account.

Fill in all fields on the form and click **Next**.

Registration Wizard

We didn't find an account for **ben@gmail.com**.

- To create an account for ben@gmail.com, complete the form below.
- If ben@gmail.com is wrong, click Back to correct it.

Password: Confirm Password:

Password may contain: letters, numbers, dot, plus, minus, underscore, !, and @

First Name: Last Name:

Institution:

Address:


City: St./Prov.:

Country: Postal Code:

Phone:

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.

Registration Wizard



In the list below are the user accounts that matched your email address. Please select the user account that you would like to use to perform the registration.


Your Email:

Matching Web accounts:

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.

Registration Wizard



Congratulations, registration was successful.

Below is the Unlock Code used to register GemStone for Windows on this computer. Please keep this information for your records.

Web Account:

Serial Number:

CDKey:

Unlock Code:

Descriptive Characters: **Number 0; Letter O; Number 1; Letter I**

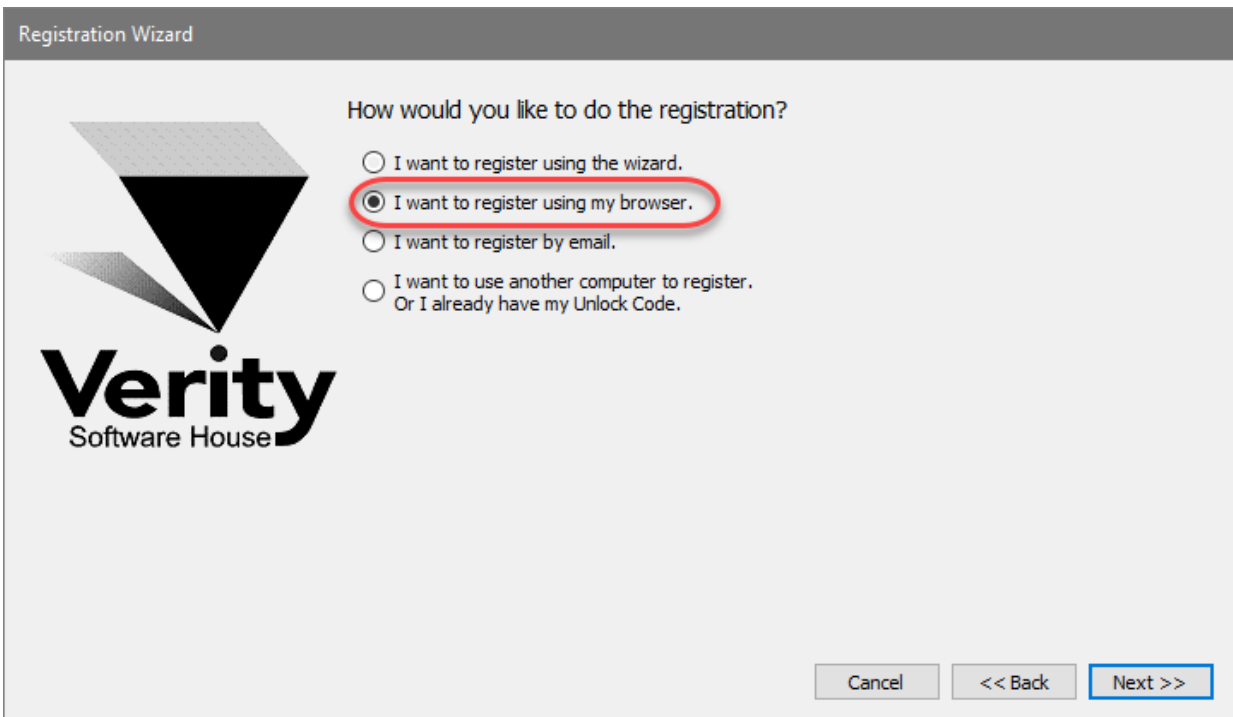
You can print the registration information by clicking the **Print** button. Click **Finish** to close the wizard and continue working with the program

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

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

If the registration wizard cannot connect to vsh.com

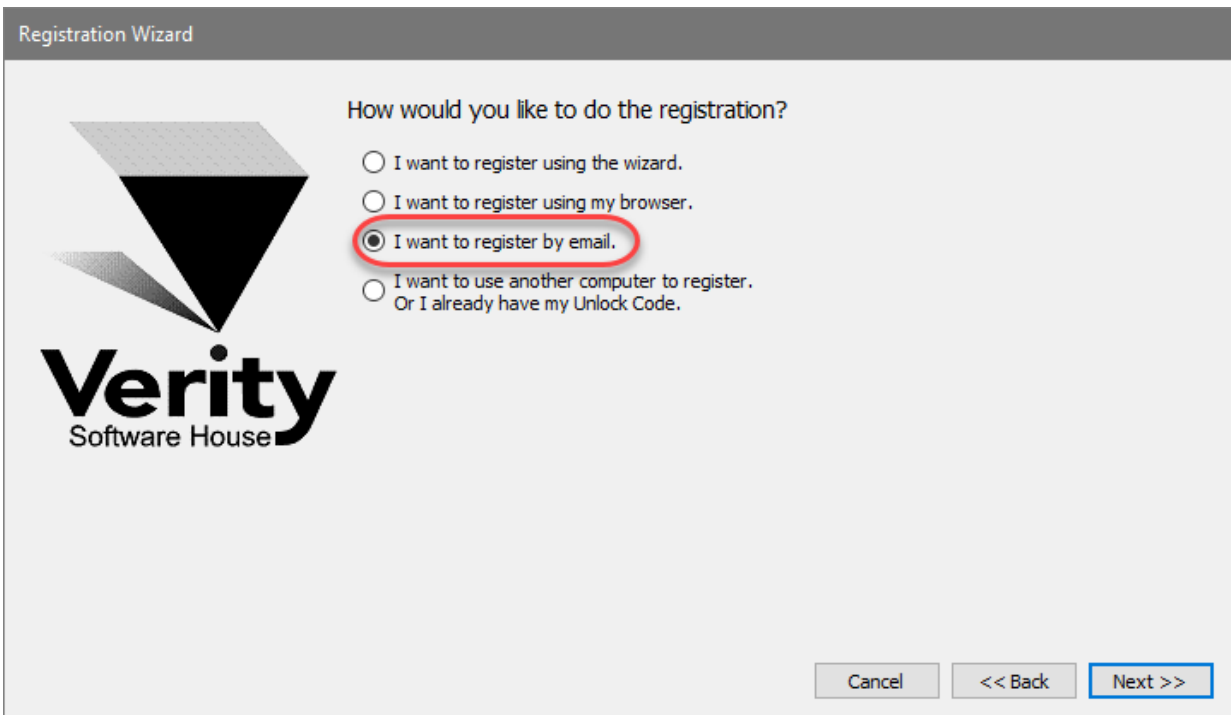
Sometimes firewalls can prevent the registration wizard from connecting to vsh.com. In this case, you can try registering with your browser. Choose the second option in the list and click **Next** to launch your browser with the correct registration information.



Follow instructions on screen to complete the process.

To register your software using email

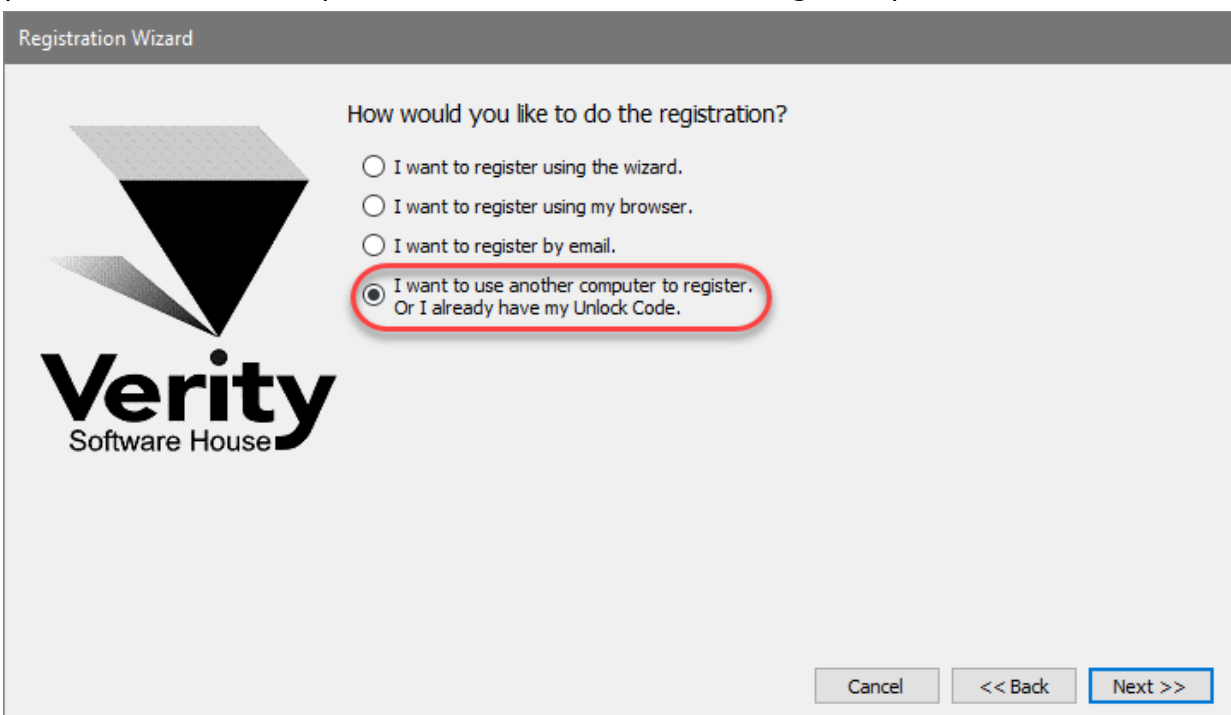
The registration wizard can help you send email to Verity Software House with your registration information. Choose the third option in the list and click **Next** to create an email message.



Follow instructions on screen to complete the process.

To register your software using another computer...

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



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

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

Click the **Register Software** link.

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

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

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

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

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

If you do not have Internet access

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

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

These fields are found in the Registration dialog box.

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

User Information

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

Fax:

Email:

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

Name to use on VSH web:

Password to use on VSH web:

A question we can ask to verify who you are:

The answer to the question to verify who you are:

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

Serial Number of previous version:

Contact Verity Software House

Verity Software House
PO Box 247, Topsham, ME 04086
USA

Email: verity@vsh.com

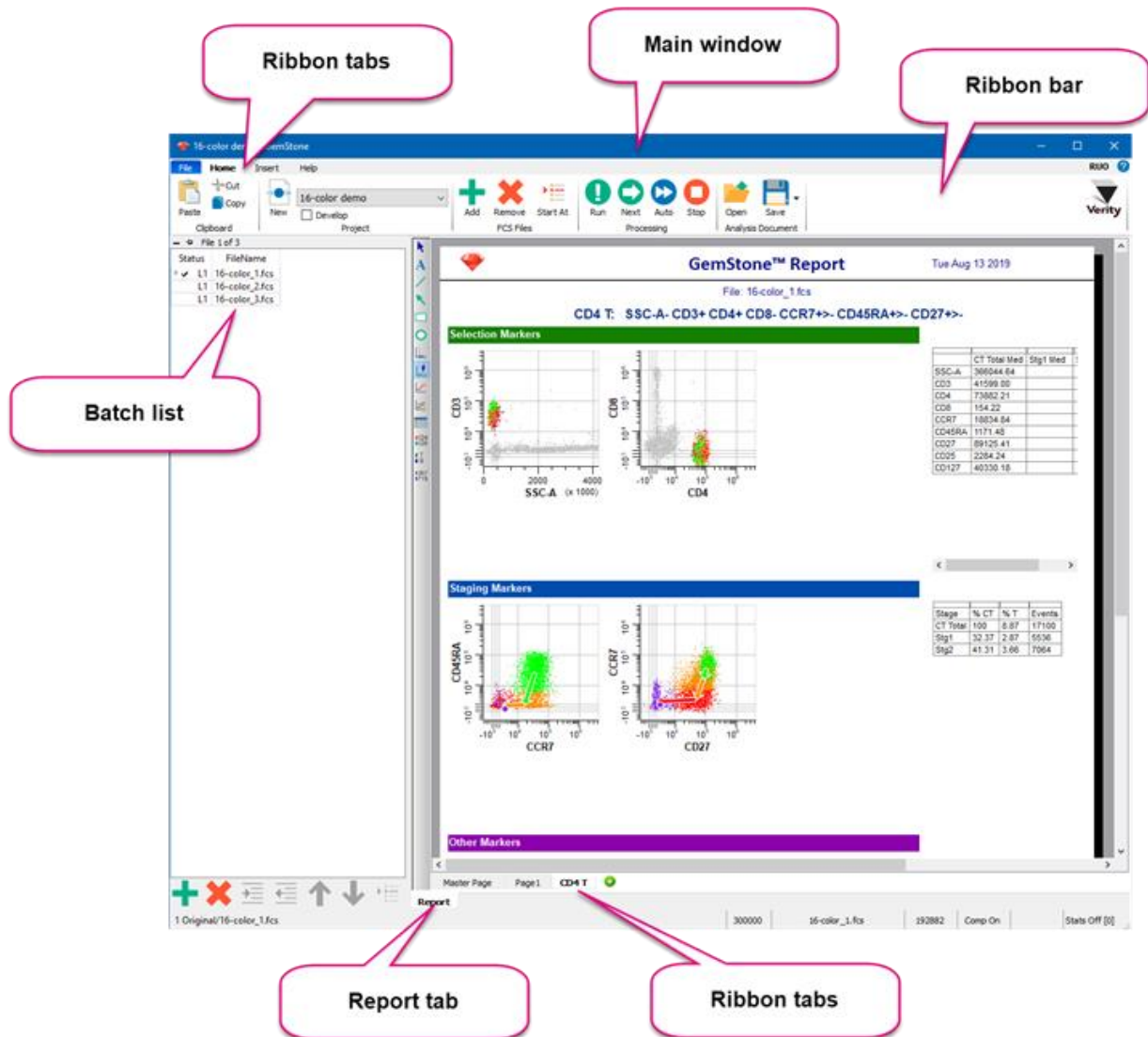
Web: www.vsh.com

Phone: (207) 729 6767

GemStone Terminology

GemStone User Interface

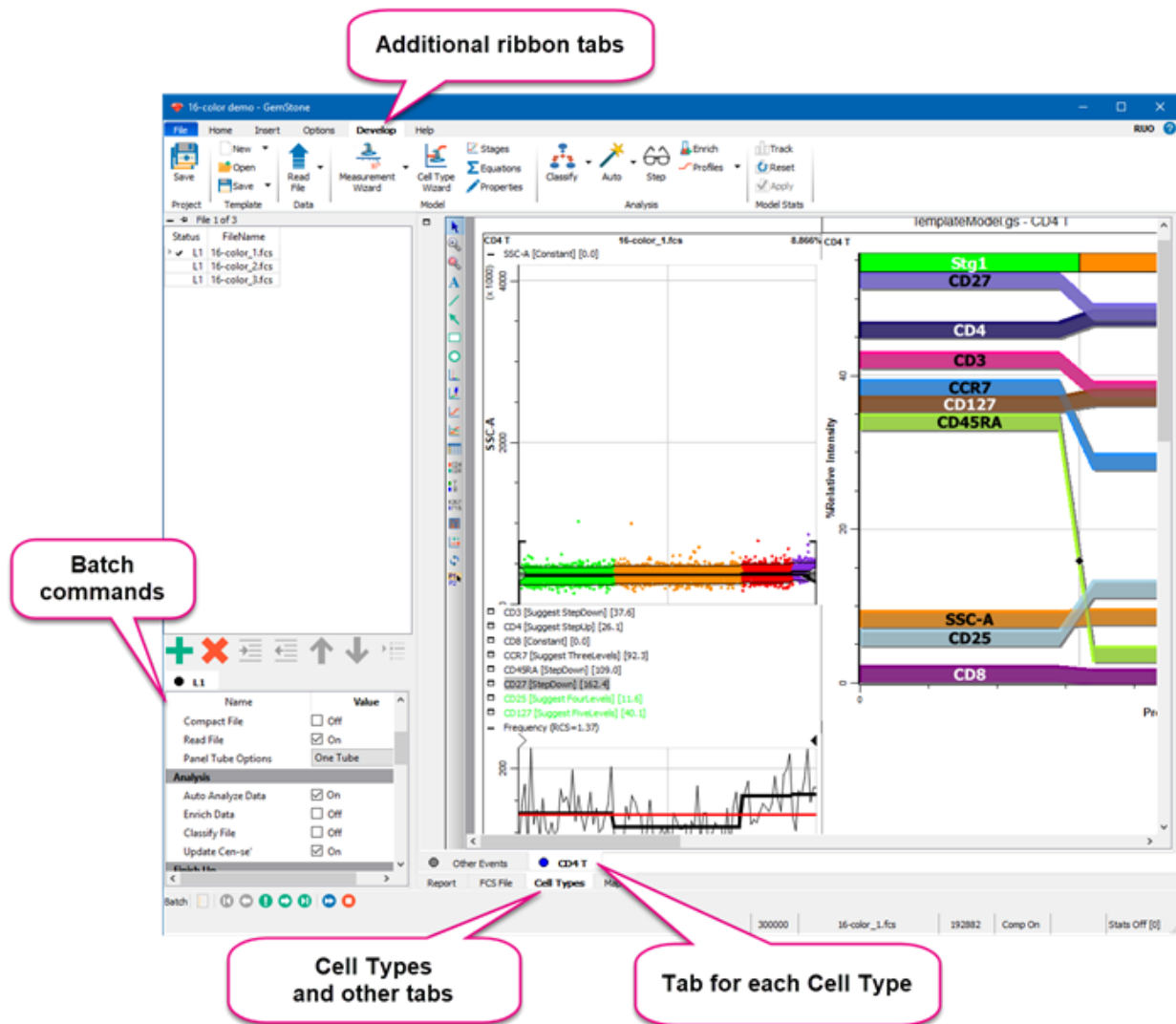
When GemStone starts-up, the main window is comprised of a ribbon bar at the top, a batch list on the left, and a report tab that contains tabs for each page in a report.



The ribbon bar contains most of the commands that you will use. The commands are divided into a series of ribbon tabs: [File tab](#), [Home tab](#), [Insert tab](#), [Options tab](#), [Develop tab](#), and the [Help tab](#). Some tabs are only visible when the [Develop](#) option is enabled.

Develop Mode

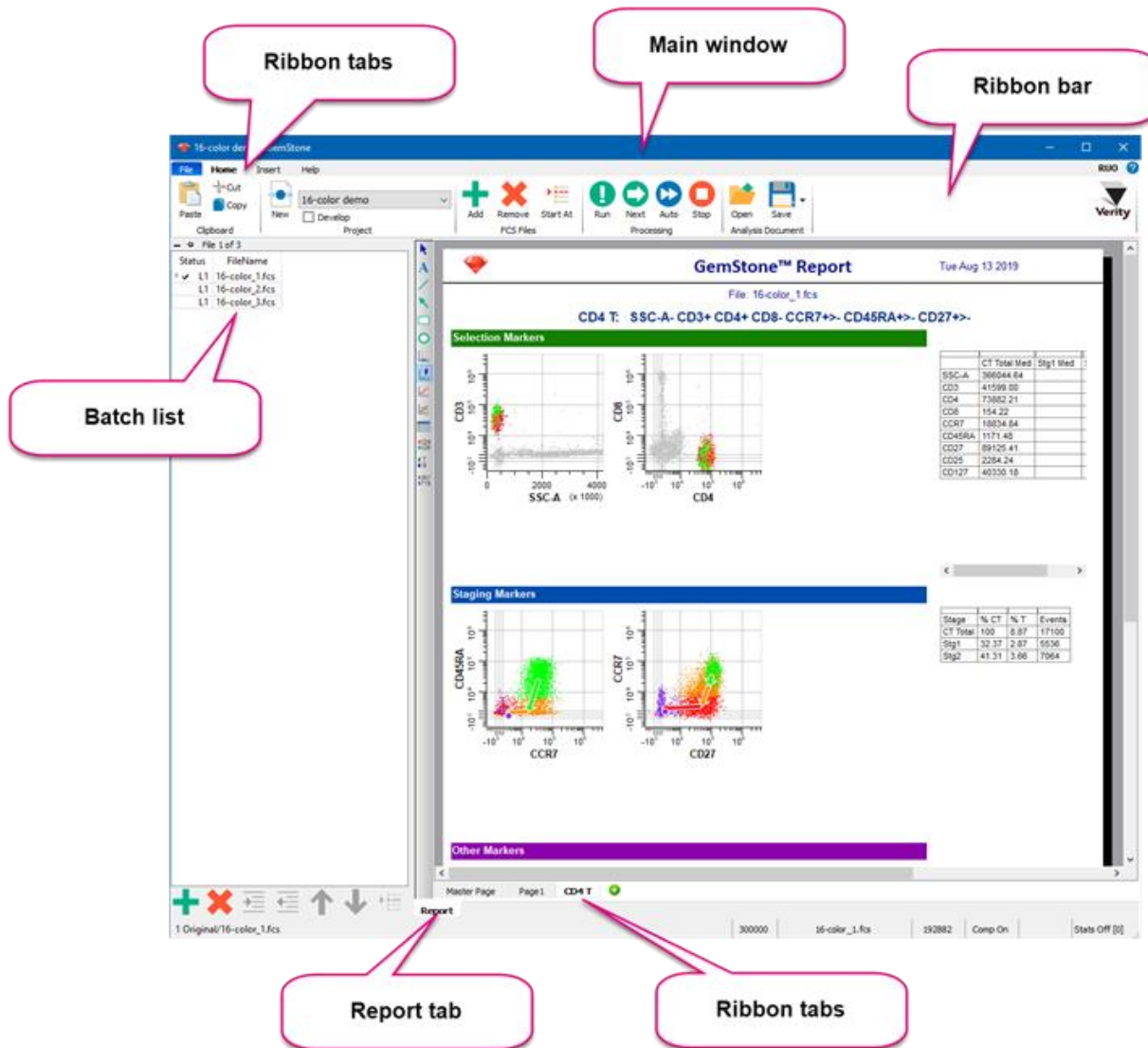
In Develop mode, additional elements are displayed:



Additional ribbon tabs are displayed in Develop mode. [Batch commands](#) determine what actions are performed on files in the batch. Additional tabs appear at the bottom of the window to select FCS File, Cell Types, and Maps view. With the Cell types view selected, a tab is shown for each cell type defined in the model template.

GemStone User Interface

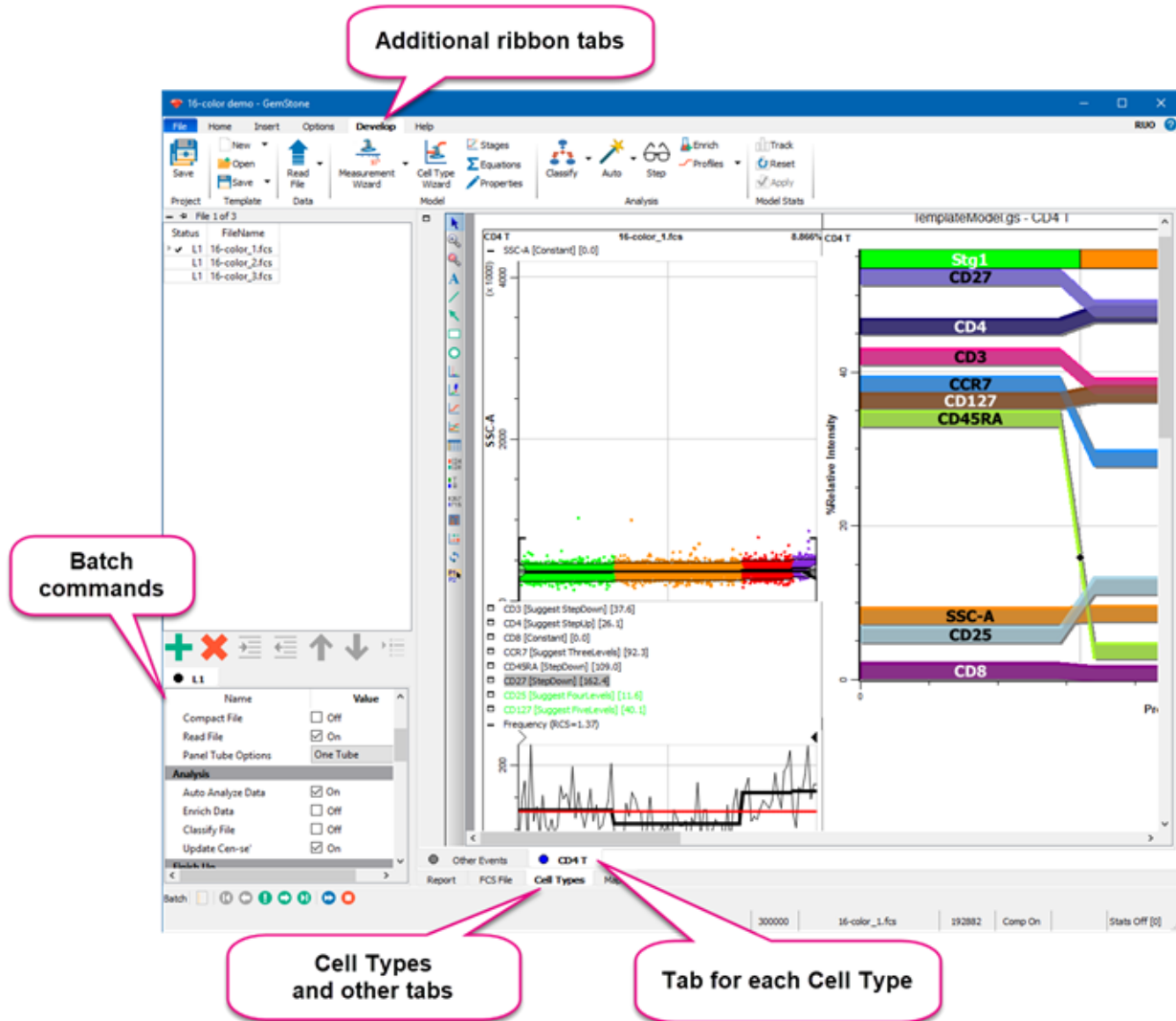
When GemStone starts-up, the main window is comprised of a ribbon bar at the top, a batch list on the left, and a report tab that contains tabs for each page in a report.



The ribbon bar contains most of the commands that you will use. The commands are divided into a series of ribbon tabs: [File tab](#), [Home tab](#), [Insert tab](#), [Options tab](#), [Develop tab](#), and the [Help tab](#). Some tabs are only visible when the [Develop](#) option is enabled.

Develop Mode

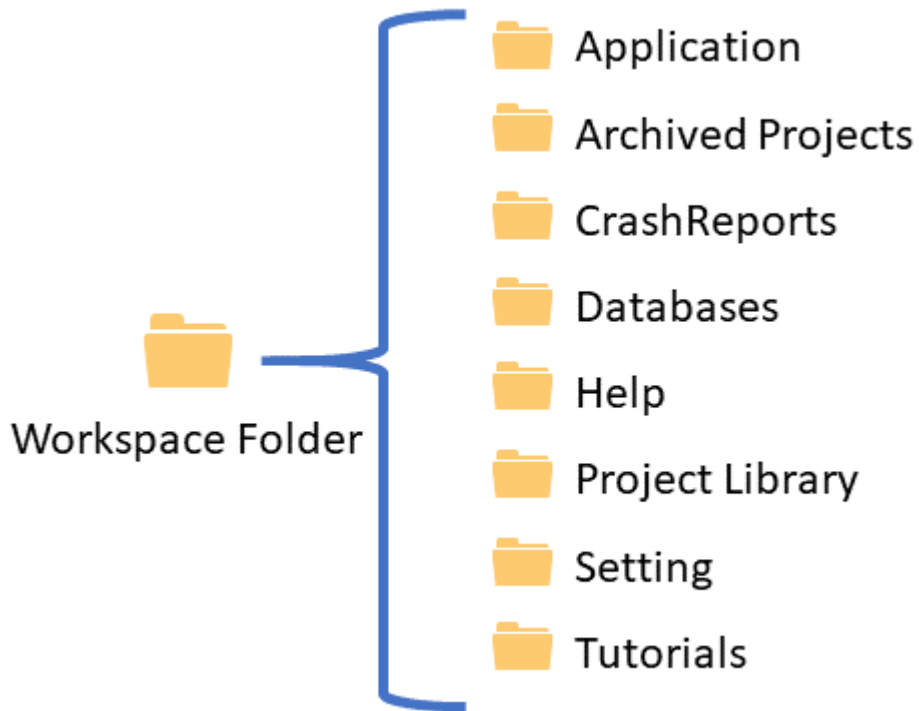
In Develop mode, additional elements are displayed:



Additional ribbon tabs are displayed in Develop mode. [Batch commands](#) determine what actions are performed on files in the batch. Additional tabs appear at the bottom of the window to select FCS File, Cell Types, and Maps view. With the Cell types view selected, a tab is shown for each cell type defined in the model template.

The GemStone Workspace

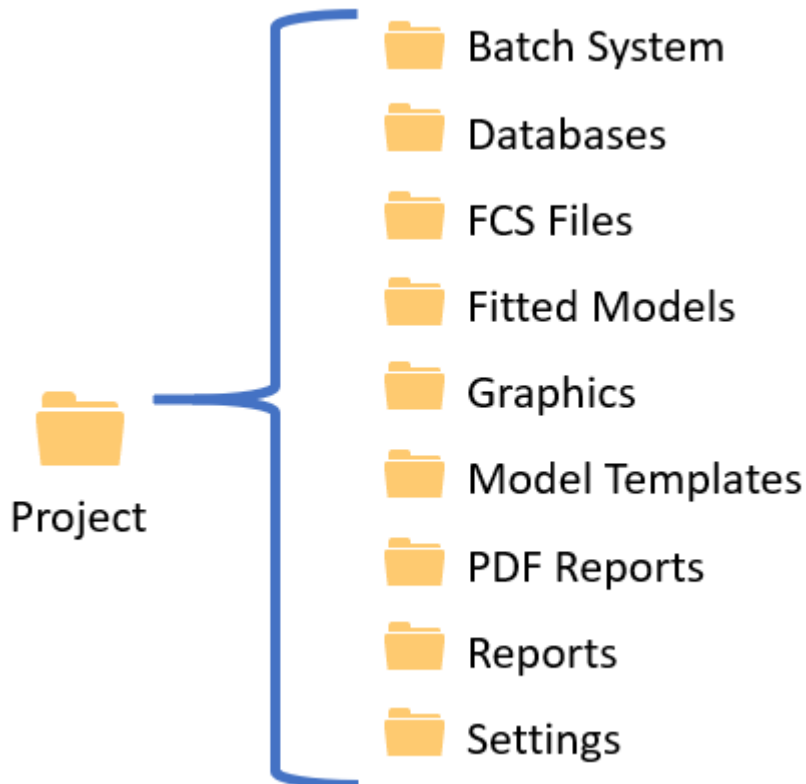
The GemStone workspace is a folder on disk where the software is installed. The folder contains key components of the program in sub-folders that store the program itself, the user guide, the Project library, and other important parts of the software.



What is a Project?

The term "Project" in GemStone refers to collection of files that determine an analysis approach. A Project includes one or more model templates that are used to analyze FCS files. It includes one or more batch scripts that determine how to load FCS files and model templates, what operations to perform on the FCS files, and the outputs of the analysis such as PDF files and databases. A Project contains the FCS files added to it, and the output files it generates.

Projects provide a simple way to design an analysis strategy and to manage the related files. On disk, a Project is organized as a set of folders and files.

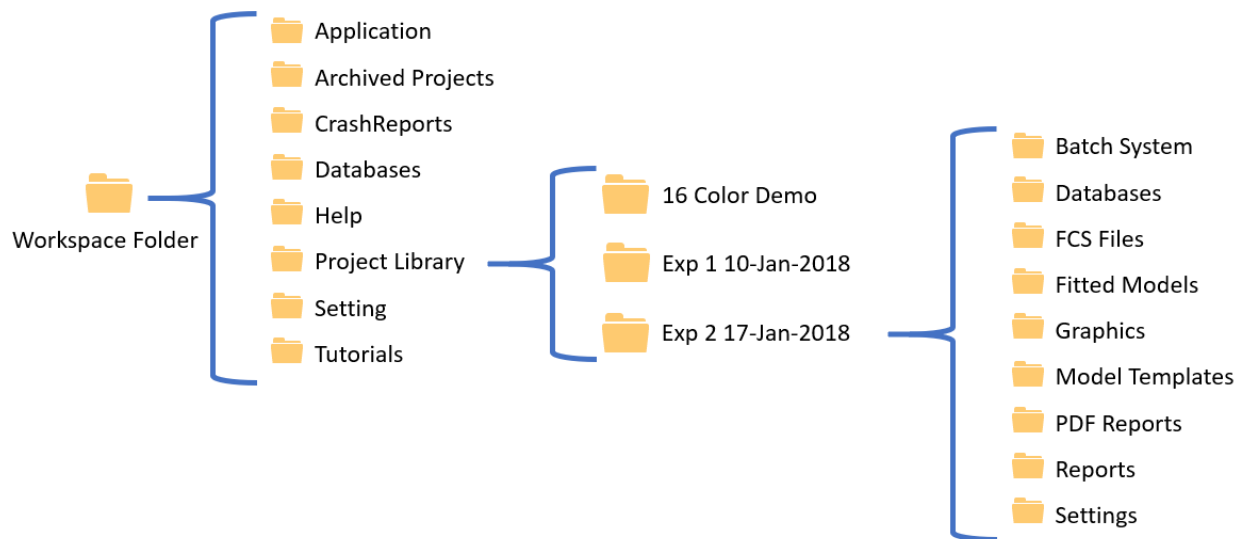


Project

The Project Library

The Project Library is part of the [GemStone Workspace](#) that contains the Projects that can be used for analysis, including those that you create. Each Project is a folder which contains additional folders of information.

The example below shows Projects that have been created: 16 Color Demo, Exp 1 10-Jan-2018, and Exp 2 17-Jan-2018. The folder structure of one of the Projects is also shown.



What is a Template Document?

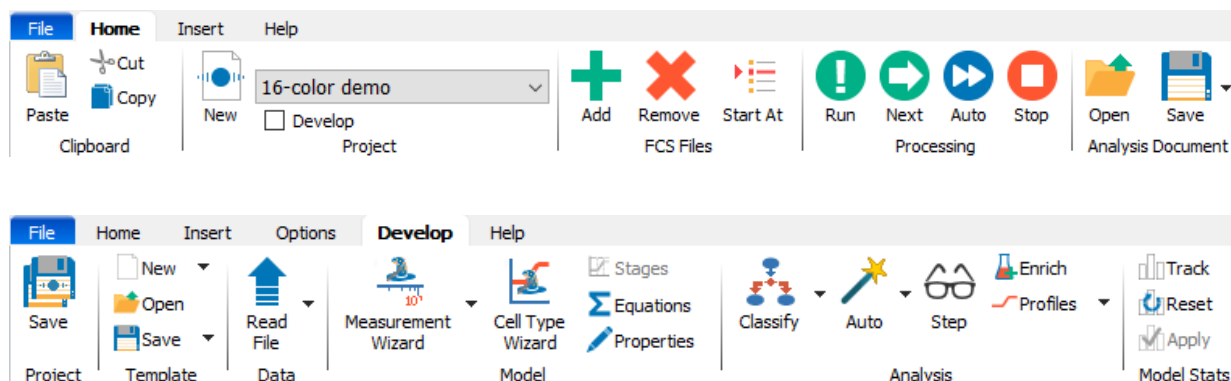
A template document contains the cell type definitions, expression profiles, report design, and objects on the cell type canvas. It is the underpinning of the analysis system - the [Probability State Model](#). This component of the software is only accessible in Develop mode.

A template document defines a set of cell types. Each cell type is defined by a set of expression profiles for the key markers of the cell type. For example, a CD8 cell type is typically defined with expression profiles for CD3, CD66b, CD19, CD8, CD4, and other markers. The cell types can include staging related to the cell type, as well as graphical elements like 1P and 2P plots, overlays, and more.

Saving and Opening

GemStone has many different types of items that can be saved and opened. Here's an explanation of the primary open and save operations that you will encounter in the software.

The [Home tab](#), [Develop tab](#), and [File tab](#) of the ribbon are used for saving and opening:



Projects

When you open or save a Project, the program opens or saves the [batch commands](#) and [database fields](#) for the method. The batch commands determine what template documents are used, what kinds of output to save (PDF, analysis documents, etc.), and what to print.

Command



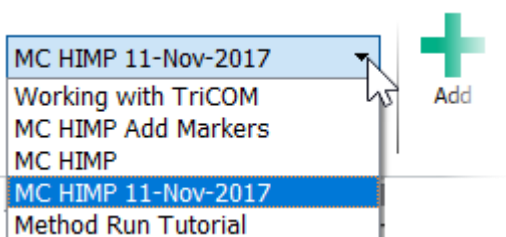
Save

Where Is It?

File menu, Develop tab

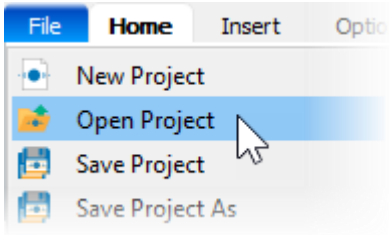
Function

Save the current method in the **Project Library** folder. In Develop mode, it will also prompt you to save the template document.



Home tab

This dropdown list displays all of the methods in the **Project Library**. It opens the selected method.

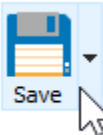
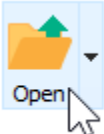


File menu

The Open Project command allows a method to be opened from outside of the **Project Library**.

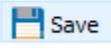
Analysis Documents

An Analysis document stores the analysis of an FCS file with a template document. Typically, this is saved automatically in the Fitted Models folder by the batch commands. The user can also manually save and open analysis documents for review.

| Command | Where Is It? | Function |
|---|--------------|--|
|  Save Analysis Document | Home tab | Saves the current analyzed document to the current method's Fitted Models folder. |
|  Open Analysis Document | Home tab | Opens an analysis document from the Fitted Models folder or other location. |

Template documents

A [Template document](#) stores the probability model definition. These files are only accessible in Develop mode. Templates are stored in the Model templates folder of the method. The Develop mode user can also manually save and open template documents.

| Command | Where Is It? | Function |
|--|--------------|--|
|  Save Template | Develop tab | Saves a template document to the current method's Model Templates folder. |

GemStone User Guide



Develop tab

Opens a template document from the **Model Templates** folder or other location.

Phenotype Naming

Each marker for each cell type has a Phenotype field. The field shows up in the [Cell Type Wizard](#) and on the [Expression Profile](#) properties. Use this field to describe the marker expression for the cell type.

The recommended syntax for this field is to use the short name of the marker (e.g. CD8), with one of the following appended:

| Symbol | | Meaning |
|---------------|---------------------------|---|
| + | plus symbol | Positive expression of the marker |
| - | minus symbol | Negative expression of the marker |
| dim | text | Dim expression of the marker |
| +>- | plus, greater than, minus | Transition of positive to negative expression of the marker |
| ->+ | minus, greater than, plus | Transition of negative to positive expression of the marker |

The values in the Phenotype field are used on report pages to show the full phenotype of a cell type. For example, the CD4 phenotype is shown as "CD4: CD66b-CD3+CD8-CD4+CD14-TCRgd-CCR7+>-CD27+>-CD45RA+>-"

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 drawn on plots are used to create a hierarchy of subsets by "gating" the events. The subsets are analyzed for frequency, intensity, and other common statistics to characterize the cells that were analyzed.

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:

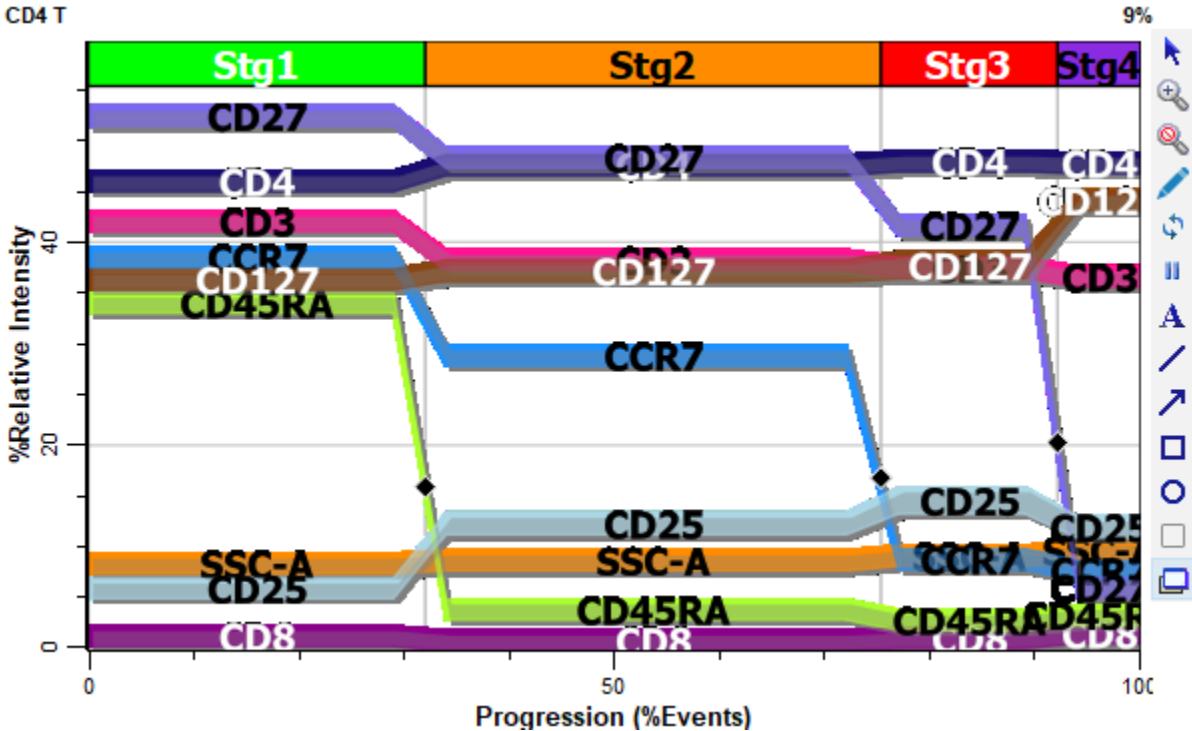
Probability: the statistical likelihood of a particular event occurring. A PSM classifies events using probabilities rather than gates. Gating is subjective, and relatively small errors made in drawing gates are compounded with each subsequent gate in the hierarchy. By using probability, a PSM actually *reduces* the error with each additional measurement 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 classifies events into a set of states, based on the characteristics defined for each measurement. GemStone uses the state as the common, X-axis for its expression plots. This allows all measurements to be compared on a common axis, making it possible to correlate *all measurements* in a single plot.

Model: a mathematical representation of a process. A PSM uses fitting routines to classify cells into the most probable states and cell types. Using this approach, GemStone can provide objective assessment 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 based on a model that we define. The model is made up of one or more *cell types*, where each cell type defines a subset that we want to analyze. We use what we know about the markers in our experiments to create a set of *expression profiles* for each subset. An expression profile uses a set of control points to define how the subset transitions over the state axis. The state 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.

With the PSM approach, 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.



Tutorials

Analyzing Files with an Existing Project

Overview

In this tutorial, you will use a previously-created GemStone Project to analyze FCS files from a 16-color immune profiling panel. The Project was designed to identify seven cell types and to present a report with a summary table and details for each cell type.

You will add files to this project and analyze them with just a few clicks. The results that you see can be reproduced if you or someone else runs the analysis again. The approach is simple, fast, and reproducible.

Let's give it a try!

Start GemStone

Start Gemstone by following the instructions in the [Installation and Setup of GemStone](#) section.

Create a New Project

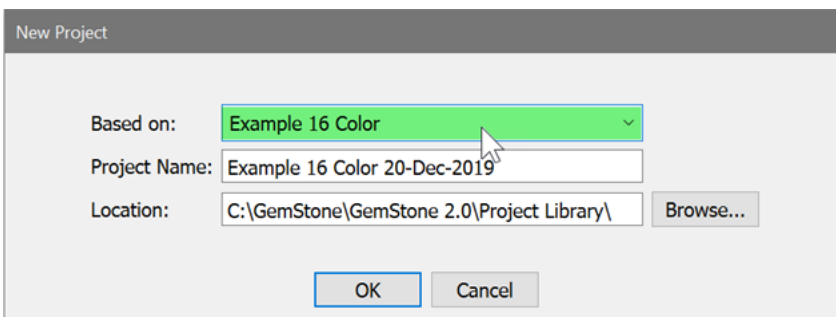
On the Home tab, click the [New Project](#) button to create a new [Project](#).



In the New Project dialog, select **Example 16 Color** in the **Based on** field. This will create a new project based on the selected project.

For **Project Name** you can leave the suggested name or type your own.

Click **OK**.

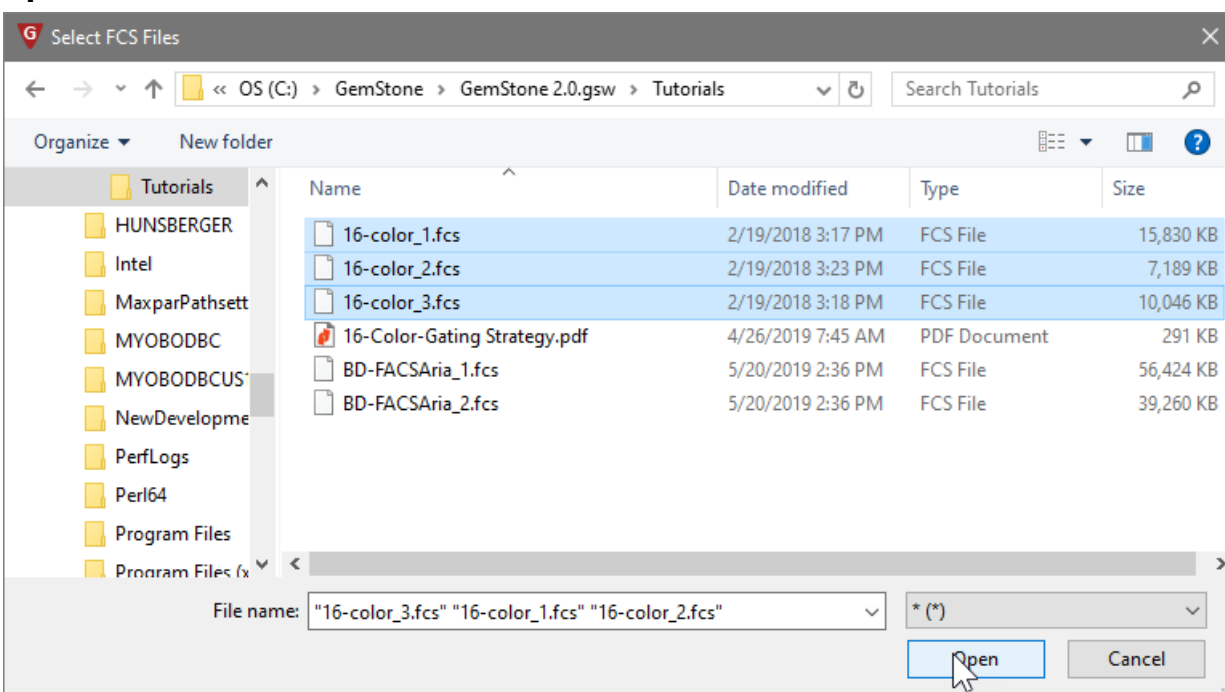
A screenshot of the "New Project" dialog box. The dialog has a title bar that says "New Project". It contains three main fields: "Based on:" with a dropdown menu showing "Example 16 Color" (highlighted in green), "Project Name:" with a text box containing "Example 16 Color 20-Dec-2019", and "Location:" with a text box containing "C:\GemStone\GemStone 2.0\Project Library\" and a "Browse..." button to its right. At the bottom of the dialog are two buttons: "OK" and "Cancel".

Add FCS Files

Next, you will add files to analyze. Click the [Add FCS Files](#) button on the Home tab.



In the Select FCS Files dialog, navigate to the Tutorials folder of the GemStone workspace. Select the three files 16-color_1.fcs, 16-color_2.fcs, and 16-color_3.fcs. Click **Open**.



The files are added to the batch list in the left panel of the program.

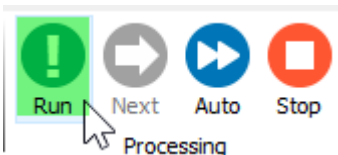
At this point, you are ready to analyze these files automatically with the example 16-Color immune panel project.

Analyze the Files

The First File

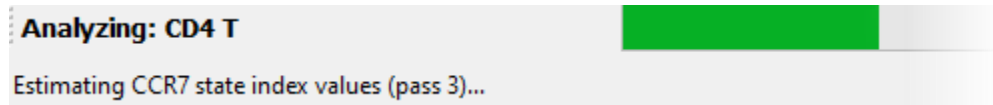
Let's analyze the first FCS file.

Click the **Run** button in the Processing group on the Home tab. This tells GemStone to follow the steps in the batch script to read and analyze the selected file.



The software first opens a *template document*, which defines the analysis for seven cell types, along with the plots and report pages. Once the template is opened, the FCS file is read into the template.

The auto-analysis step is next. In this step, each of the defined cell types is adjusted to the data in the FCS file. The status pane at the bottom of the main window indicates which step of the analysis is being performed.



When the process is complete, the summary page of the report presents a table of the key results of the analysis.

Verity GemStone™ Report Wed Dec 4 2019
File: 16-color_1.fcs

Summary

| | Count | % Cell Type | % Total |
|---------------|--------|-------------|---------|
| Intact Cells | 173039 | N/A | 89.71 |
| Lymphocytes | 29897 | N/A | 15.50 |
| CD3 T | 23066 | N/A | 11.96 |
| CD4 T | 17037 | 100 | 8.83 |
| Naive | 5396 | 31.67 | 2.80 |
| CM | 8634 | 50.68 | 4.48 |
| EM | 1907 | 11.19 | 0.99 |
| TE | 1100 | 6.46 | 0.57 |
| Tregs | 736 | 4.32 | |
| CD8 T | 5864 | 100 | 3.04 |
| Naive/CM | 2028 | 34.58 | 1.05 |
| EM | 2718 | 46.35 | 1.41 |
| TE | 1118 | 19.07 | 0.58 |
| Gamma Delta T | 165 | 100 | 0.09 |
| B Cells | 3173 | 100 | 1.65 |
| Naive | 2158 | 68.01 | 1.12 |
| Memory | 1015 | 31.99 | 0.53 |
| NK Cells | 3658 | 100 | 1.90 |
| Monocytes | 6457 | 100 | 3.35 |
| Classical | 5862 | 87.69 | 2.94 |
| Non-Classical | 795 | 12.31 | 0.41 |
| Granulocytes | 136685 | 100 | 70.86 |
| Other | 19843 | N/A | 10.29 |

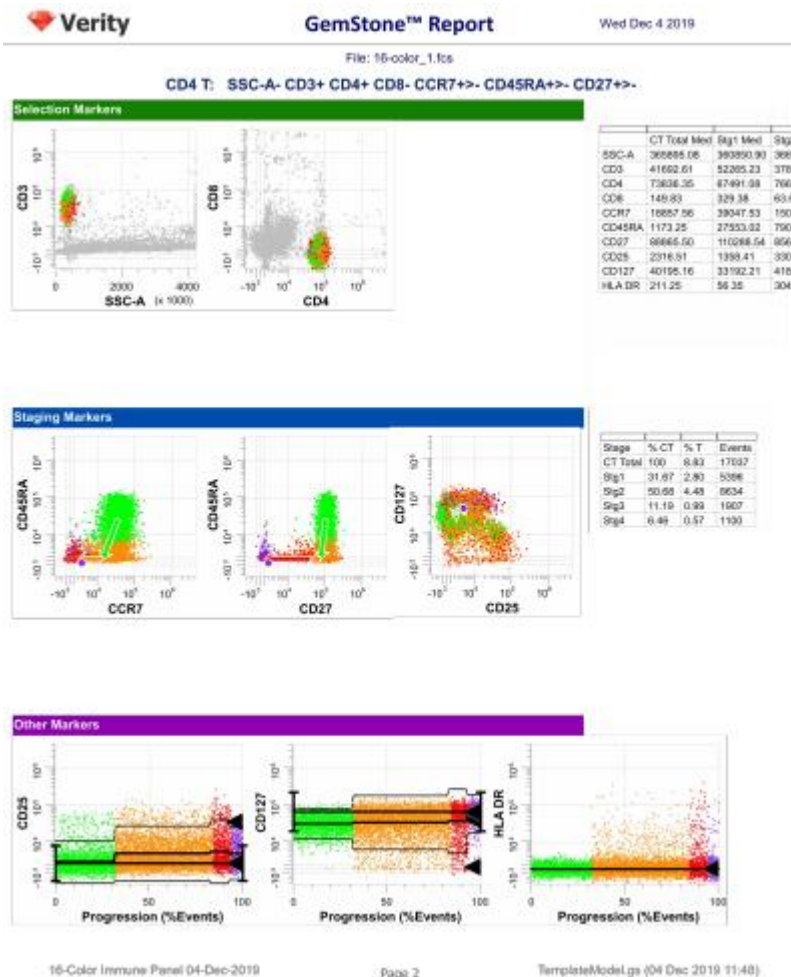
At the bottom of the main window, there are tabs for each page in the analysis report. To review a page, click the tab.



For example, click the **CD4 T** tab to review the analysis of CD4 T cells.

The report shows the name of the cell type and its phenotype at the top. The Selection Markers section shows several plots for the measurements that were used to identify CD4 T

cells, along with a table of intensity values. The Staging Markers section includes plots of markers that are used to identify the stages of CD4 T cell development and a table of frequencies for each stage.



The Rest of the Files

If you want to analyze and review each file one-by-one, you would use the [Next](#) button to read and analyze the next file. However, in this tutorial we want to analyze all of the files without intervention.

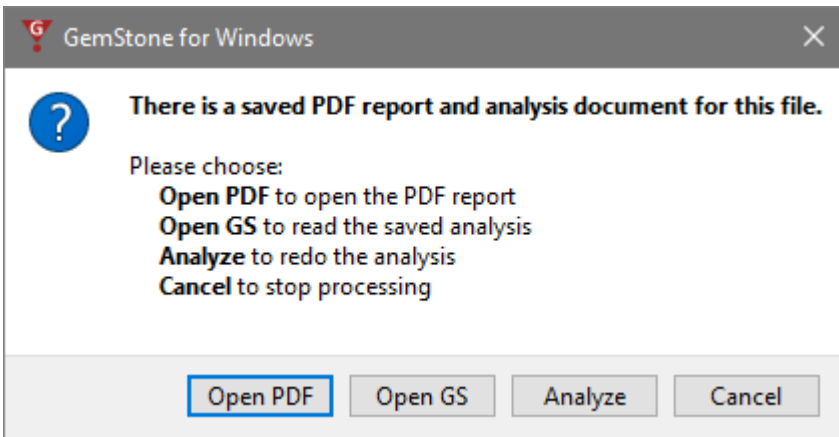
Click [Auto Advance](#) to process all of the files in the batch.



Review the Analysis

After analyzing each FCS file, GemStone saves the finished analysis so that it can be reviewed later. A PDF of the report is also generated for your records. You can easily re-open a saved analysis for the files in the project.

Double-click the second file in the batch list, 16-color_2.fcs. A prompt dialog will appear offering several choices of actions.



Choose **Open PDF** to review the PDF report of the analysis. **Open GS** will open the analysis document associated with the FCS file. Choose **Analyze** to re-analyze the file, or **Cancel** to close the dialog.

Summary

In this tutorial, you created a new Project based on an existing one designed to analyze the 16-color tutorial files. You added files to the new project and initiated the analysis of the files using the **Add**, **Run**, and **Auto-Advance** buttons on the Home tab of the ribbon.

GemStone automatically adjusted the *template document* to each of the FCS files, fitting the model to the peaks in each file. The analysis included a summary table of key results, along with a more detailed page for each of the seven cell types defined in the template document. A PDF and an analysis document was saved for each file.

You were able to analyze these files with just a few clicks. The approach is simple, fast, and reproducible.

Analyzing Files with an Existing Project

Overview

In this tutorial, you will use a previously-created GemStone Project to analyze FCS files from a 16-color immune profiling panel. The Project was designed to identify seven cell types and to present a report with a summary table and details for each cell type.

You will add files to this project and analyze them with just a few clicks. The results that you see can be reproduced if you or someone else runs the analysis again. The approach is simple, fast, and reproducible.

Let's give it a try!

Start GemStone

Start Gemstone by following the instructions in the [Installation and Setup of GemStone](#) section.

Create a New Project

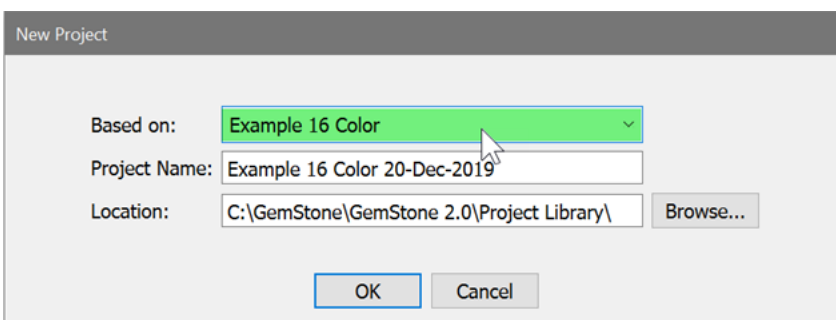
On the Home tab, click the [New Project](#) button to create a new [Project](#).



In the New Project dialog, select **Example 16 Color** in the **Based on** field. This will create a new project based on the selected project.

For **Project Name** you can leave the suggested name or type your own.

Click **OK**.

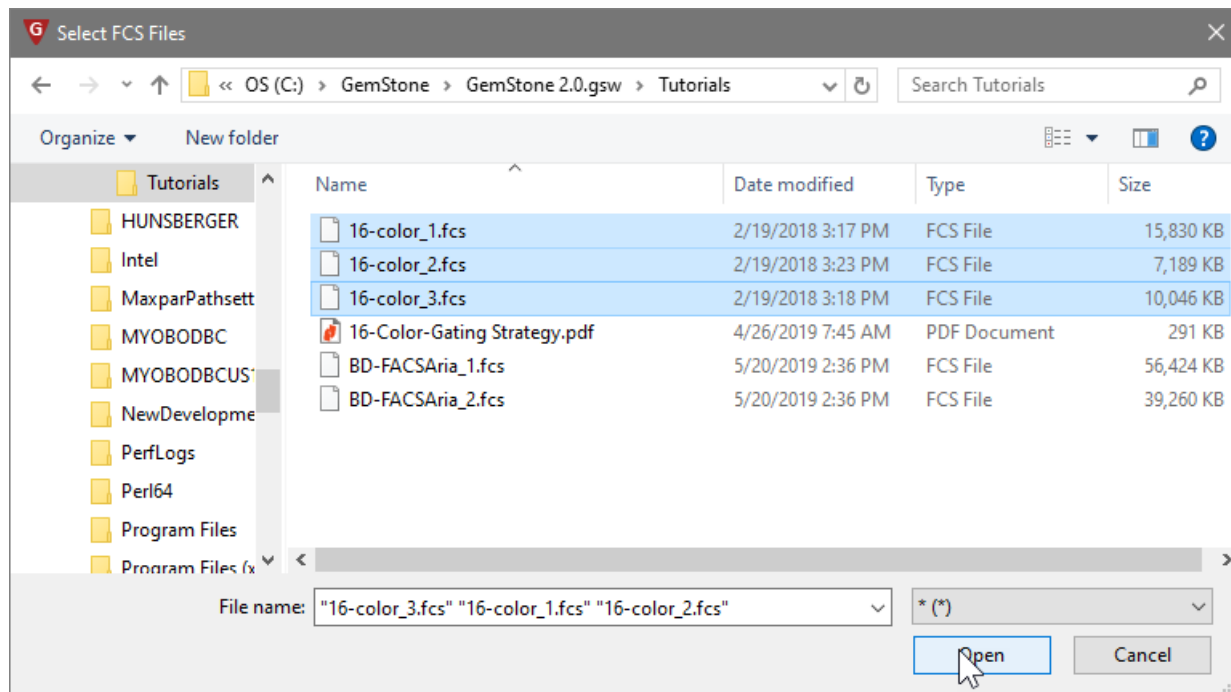


Add FCS Files

Next, you will add files to analyze. Click the [Add FCS Files](#) button on the Home tab.



In the Select FCS Files dialog, navigate to the Tutorials folder of the GemStone workspace. Select the three files 16-color_1.fcs, 16-color_2.fcs, and 16-color_3.fcs. Click **Open**.

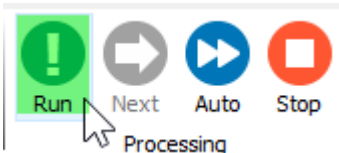


The files are added to the batch list in the left panel of the program. At this point, you are ready to analyze these files automatically with the example 16-Color immune panel project.

Analyze the Files

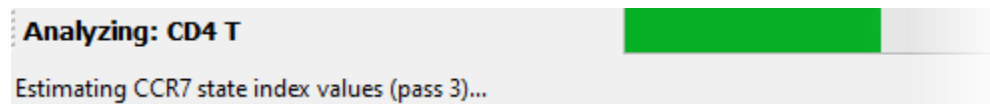
The First File

Let's analyze the first FCS file. Click the **Run** button in the Processing group on the Home tab. This tells GemStone to follow the steps in the batch script to read and analyze the selected file.



The software first opens a *template document*, which defines the analysis for seven cell types, along with the plots and report pages. Once the template is opened, the FCS file is read into the template.

The auto-analysis step is next. In this step, each of the defined cell types is adjusted to the data in the FCS file. The status pane at the bottom of the main window indicates which step of the analysis is being performed.



When the process is complete, the summary page of the report presents a table of the key results of the analysis.

Verity GemStone™ Report Wed Dec 4 2019
File: 16-color_1.fcs

Summary

| | Count | % Cell Type | % Total |
|---------------|--------|-------------|---------|
| Intact Cells | 173039 | N/A | 89.71 |
| Lymphocytes | 29897 | N/A | 15.50 |
| CD3 T | 23066 | N/A | 11.96 |
| CD4 T | 17037 | 100 | 8.83 |
| Naive | 5396 | 31.67 | 2.80 |
| CM | 8634 | 50.68 | 4.48 |
| EM | 1907 | 11.19 | 0.99 |
| TE | 1100 | 6.46 | 0.57 |
| Tregs | 736 | 4.32 | |
| CD8 T | 5864 | 100 | 3.04 |
| Naive/CM | 2028 | 34.58 | 1.05 |
| EM | 2718 | 46.35 | 1.41 |
| TE | 1118 | 19.07 | 0.58 |
| Gamma Delta T | 185 | 100 | 0.09 |
| B Cells | 3173 | 100 | 1.65 |
| Naive | 2158 | 68.01 | 1.12 |
| Memory | 1015 | 31.99 | 0.53 |
| NK Cells | 3658 | 100 | 1.90 |
| Monocytes | 6457 | 100 | 3.35 |
| Classical | 5862 | 87.89 | 2.94 |
| Non-Classical | 795 | 12.31 | 0.41 |
| Granulocytes | 136685 | 100 | 70.86 |
| Other | 19843 | N/A | 10.29 |

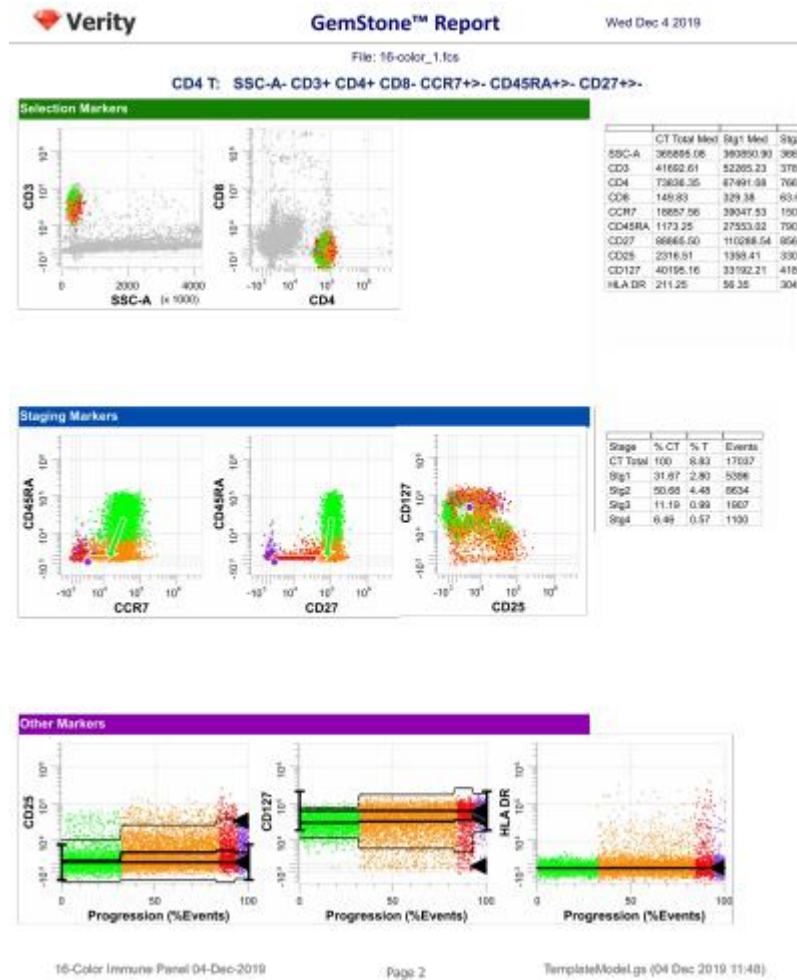
At the bottom of the main window, there are tabs for each page in the analysis report. To review a page, click the tab.



For example, click the **CD4 T** tab to review the analysis of CD4 T cells.

The report shows the name of the cell type and its phenotype at the top. The Selection Markers section shows several plots for the measurements that were used to identify CD4 T cells, along with a table of intensity values. The Staging Markers section includes plots of

markers that are used to identify the stages of CD4 T cell development and a table of frequencies for each stage.



The Rest of the Files

If you want to analyze and review each file one-by-one, you would use the [Next](#) button to read and analyze the next file. However, in this tutorial we want to analyze all of the files without intervention.

Click [Auto Advance](#) to process all of the files in the batch.

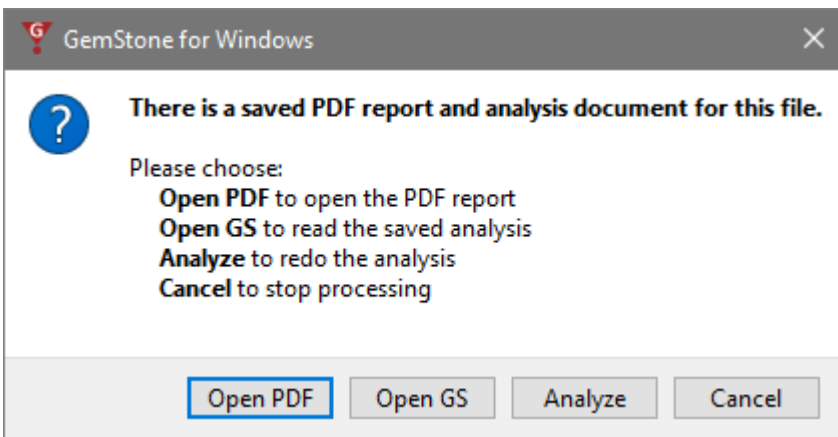


Auto

Review the Analysis

After analyzing each FCS file, GemStone saves the finished analysis so that it can be reviewed later. A PDF of the report is also generated for your records. You can easily re-open a saved analysis for the files in the project.

Double-click the second file in the batch list, 16-color_2.fcs. A prompt dialog will appear offering several choices of actions.



Choose **Open PDF** to review the PDF report of the analysis. **Open GS** will open the analysis document associated with the FCS file. Choose **Analyze** to re-analyze the file, or **Cancel** to close the dialog.

Summary

In this tutorial, you created a new Project based on an existing one designed to analyze the 16-color tutorial files. You added files to the new project and initiated the analysis of the files using the **Add**, **Run**, and **Auto-Advance** buttons on the Home tab of the ribbon.

GemStone automatically adjusted the *template document* to each of the FCS files, fitting the model to the peaks in each file. The analysis included a summary table of key results, along with a more detailed page for each of the seven cell types defined in the template document. A PDF and an analysis document was saved for each file.

You were able to analyze these files with just a few clicks. The approach is simple, fast, and reproducible.

Develop: Starting a New Project from Scratch

Overview

Any time you have a new panel to analyze with GemStone, you will create a new Project to setup the analysis for the panel. This tutorial shows how to create a new Project from scratch with a model template, a report, and a database of results. You will also set up batch options that define how each FCS file is read and analyzed.

That sounds like a lot of work, but you will leverage wizards and a streamlined interface to make quick work of it.

Start GemStone

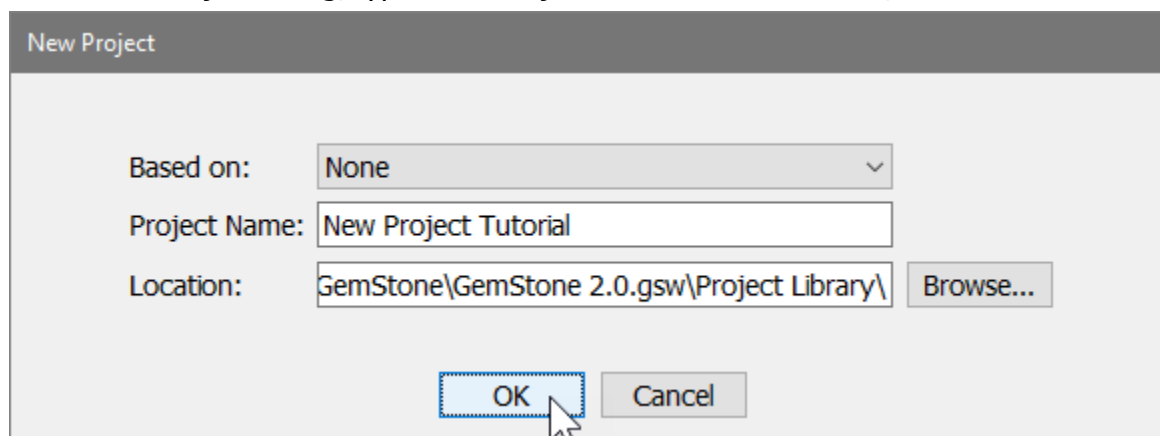
Start Gemstone by following the instructions in the [Installation and Setup of GemStone](#) section.

Create a New Project

On the Home tab, click the [New Project](#) button to create a new [Project](#).



In the New Project dialog, type "New Project Tutorial" for the name, and then click **OK**.

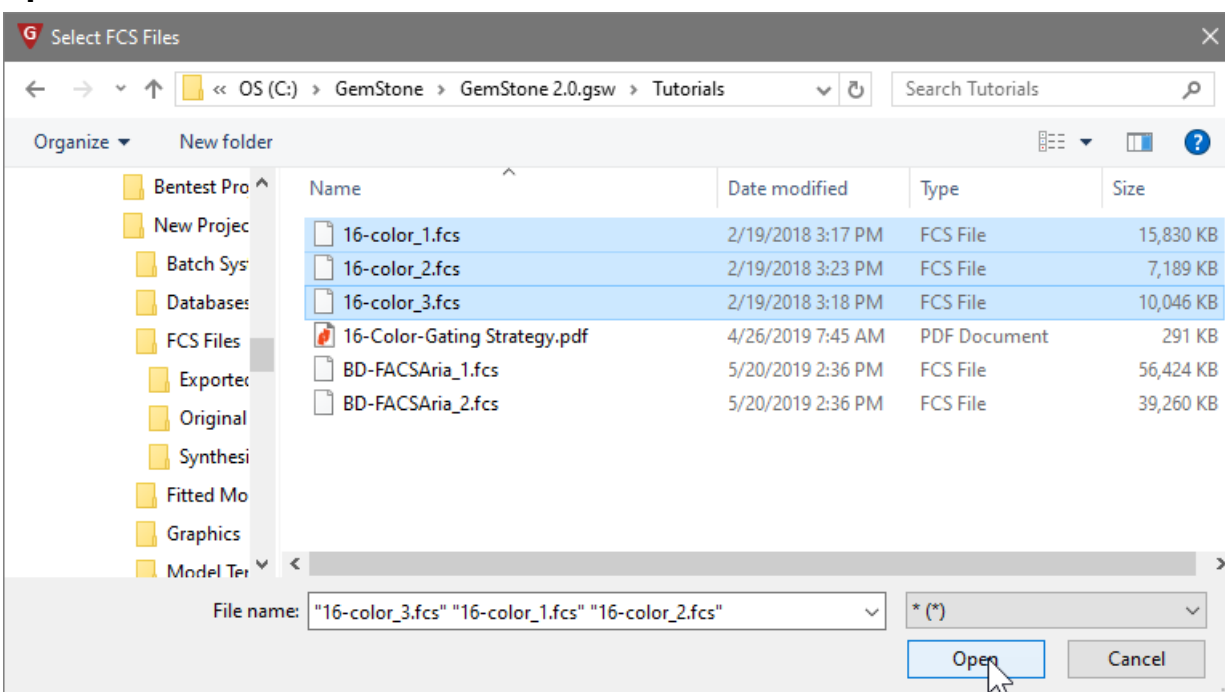


Add FCS Files

Next, you will add files to analyze. Click the [Add FCS Files](#) button on the Home tab.



In the Select FCS Files dialog, navigate to the Tutorials folder of the GemStone workspace. Select the three files 16-color_1.fcs, 16-color_2.fcs, and 16-color_3.fcs. Click **Open**.

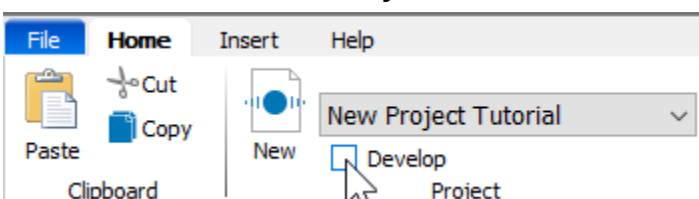


The files are added to the batch list in the left panel of the program.

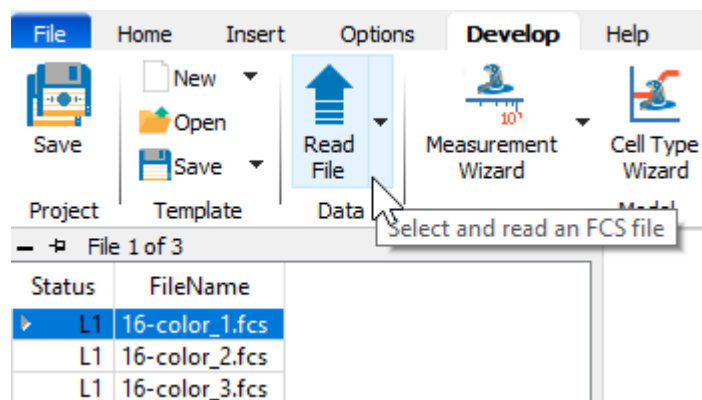
Using the Measurement Wizard

When you read files that are new to the program, it's a good idea to read one in and review the measurement names and transforms. You can edit the names and transforms with the Measurement Wizard.

Click the **Develop** checkbox to enable "Develop mode". This mode exposes additional tools that are used to build the Project.



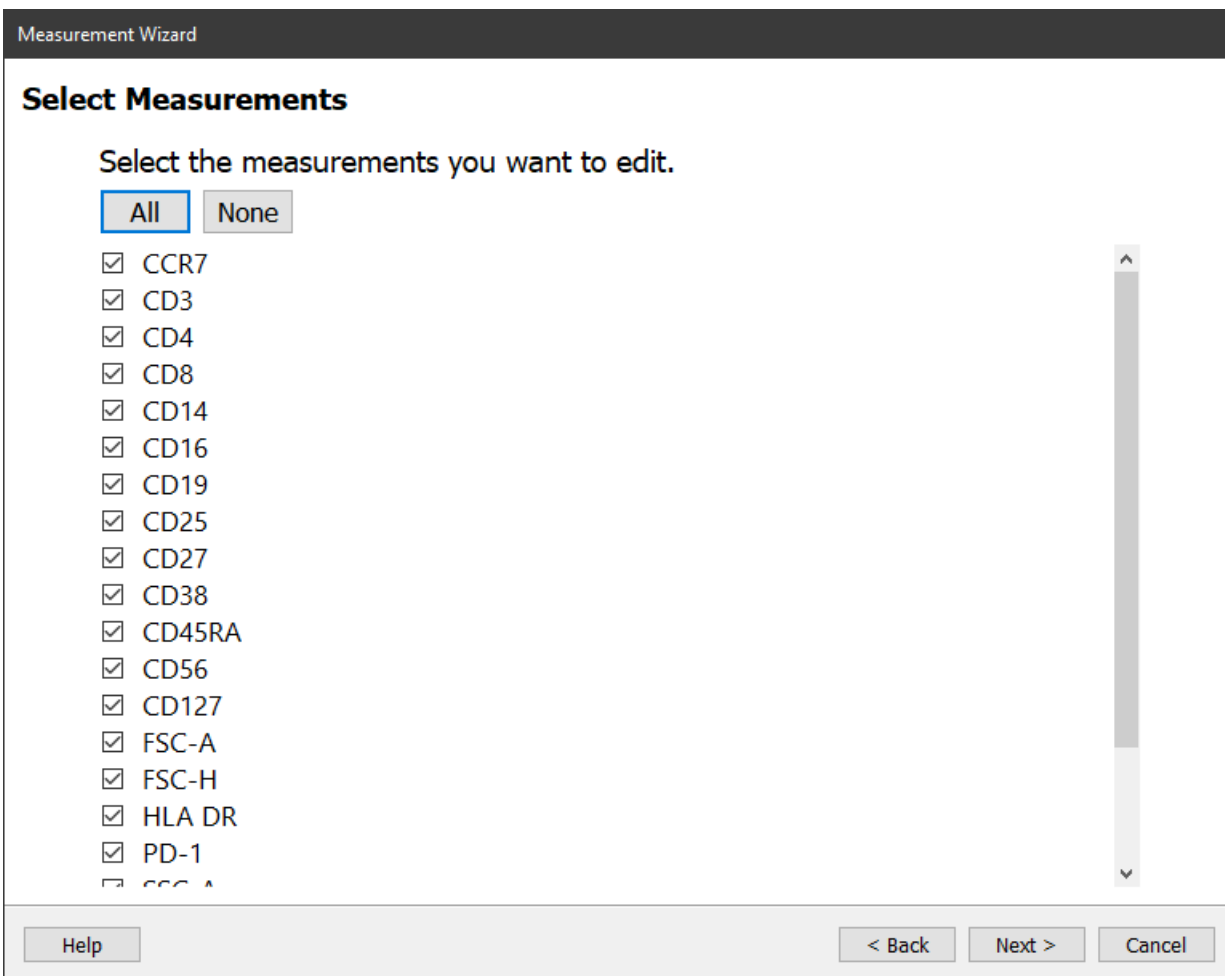
On the Develop tab, select the first file in the list and click the [Read File](#) button. This command will read the selected FCS file without performing any analysis.



Now click the [Measurement Wizard](#) button. This wizard is used to set up the names and transformations for the measurements in the FCS files.



Click the **All** button to select all of the measurements in the FCS file.

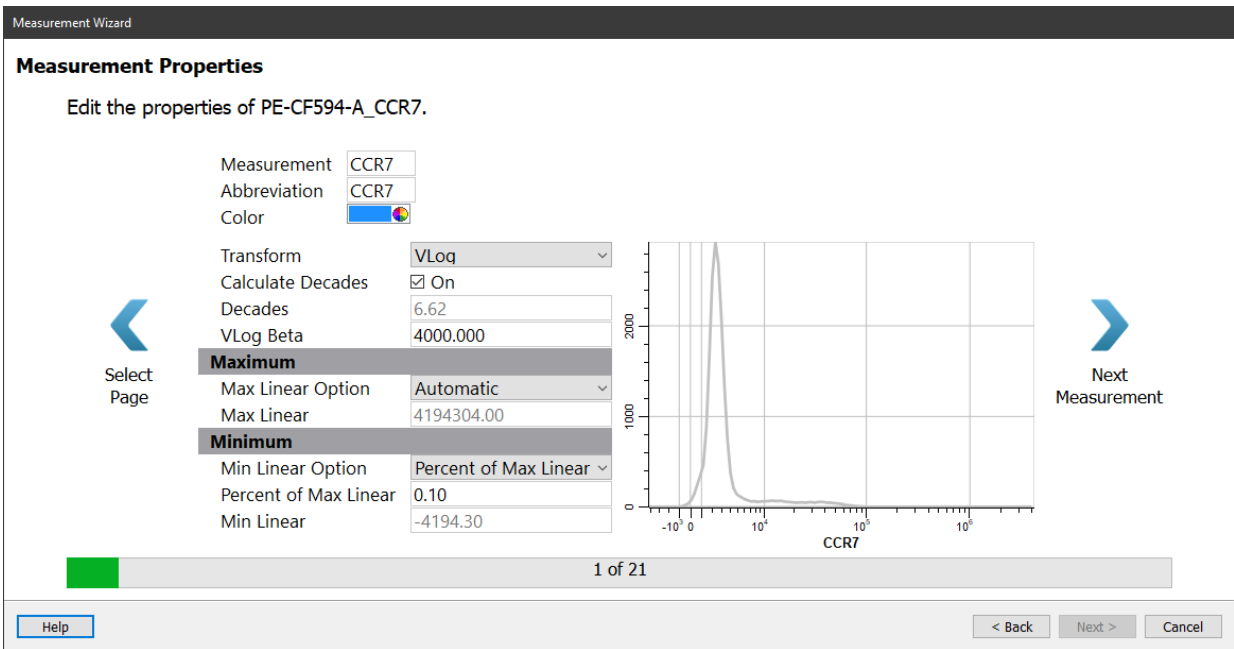


Click **Next**.

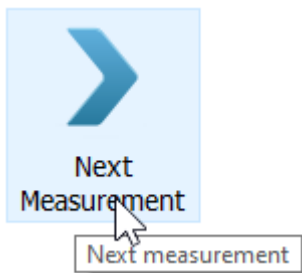
The properties for CCR7 are listed, and a histogram of the measurement provides a preview of the measurement with the properties applied to it.

In this dialog, you can edit the measurement name and abbreviation. By default, these will display the long name from the FCS file's keywords. You can also edit the transform and its properties.

No changes are needed for CCR7.



Click the **Next Measurement** button.



Review each of the remaining measurements in the same manner. There is only one change you will need to make.

When you reach the **Time** measurement, set the **Transform** to **Linear**.

Measurement Wizard

Measurement Properties

Edit the properties of Time.

| | |
|-----------------------|-----------------------|
| Measurement | Time |
| Abbreviation | Time |
| Color | |
| Transform | Linear |
| Maximum | |
| Max Linear Option | Automatic |
| Max Linear | 16777216.00 |
| Minimum | |
| Min Linear Option | Percent of Max Linear |
| Percent of Max Linear | 0.10 |
| Min Linear | -16777.22 |

21 of 21

Help < Back Next > Cancel

After setting the Time measurement, click **Next** and the Summary page is displayed. Enable the **Launch Cell Type Wizard** checkbox and then click **Finish**.

Measurement Wizard

Summary

CCR7
Abbreviation: CCR7
Transform: VLog

CD3
Abbreviation: CD3
Transform: VLog

CD4
Abbreviation: CD4
Transform: VLog

CD8
Abbreviation: CD8
Transform: VLog

CD14
Abbreviation: CD14
Transform: VLog

CD16

Finish Up

Add to Cen-se'

Launch Cell Type Wizard

Update Preferences

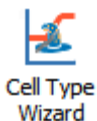
Launch the Cell Type Wizard to create or edit cell types

Help < Back Finish Cancel

Using the Cell Type Wizard

Now that the program knows about the measurements and transforms, let's start building the analysis using the [Cell Type Wizard](#). This wizard creates or edits the analysis of one cell type at a time, one measurement at a time. You will use it to create just about everything for analyzing this 16-color example.

If the wizard is not already showing, click the [Cell Type Wizard](#) button on the Develop tab.



On the Welcome page, choose **Create a new cell type** and click **Next**.

A screenshot of the "Cell Type Wizard" dialog box. The title bar says "Cell Type Wizard". The main content area has the heading "Welcome to the Cell Type Wizard" and the question "What would you like to do?". There are three radio button options: "Create a new cell type." (selected), "Duplicate the selected cell type. *" (with a dropdown menu and a checked "Link selection markers" checkbox), and "Edit the selected cell type. *" (with a dropdown menu). Below these is a note: "* Only cell types compatible with the Cell Type Wizard are available for duplicating and editing." There are two checked checkboxes: "Disable other cell types while editing" and "Create a report page". At the bottom left is an "Insert before" dropdown menu set to "None". At the bottom right are "Help", "Next >" (highlighted in blue), and "Cancel" buttons.

On this page, you will give the cell type a name.

Type "CD4 T" for the **Cell Type Name**.

This page also lets us enable the cell type, pick a color for it, and determine the classification type. For this tutorial, leave these options as they appear and click **Next**.

Cell Type Wizard

Cell Type Properties

Edit the properties for this cell type.

Cell Type Name

Description

Enable On

Color

Classification Type

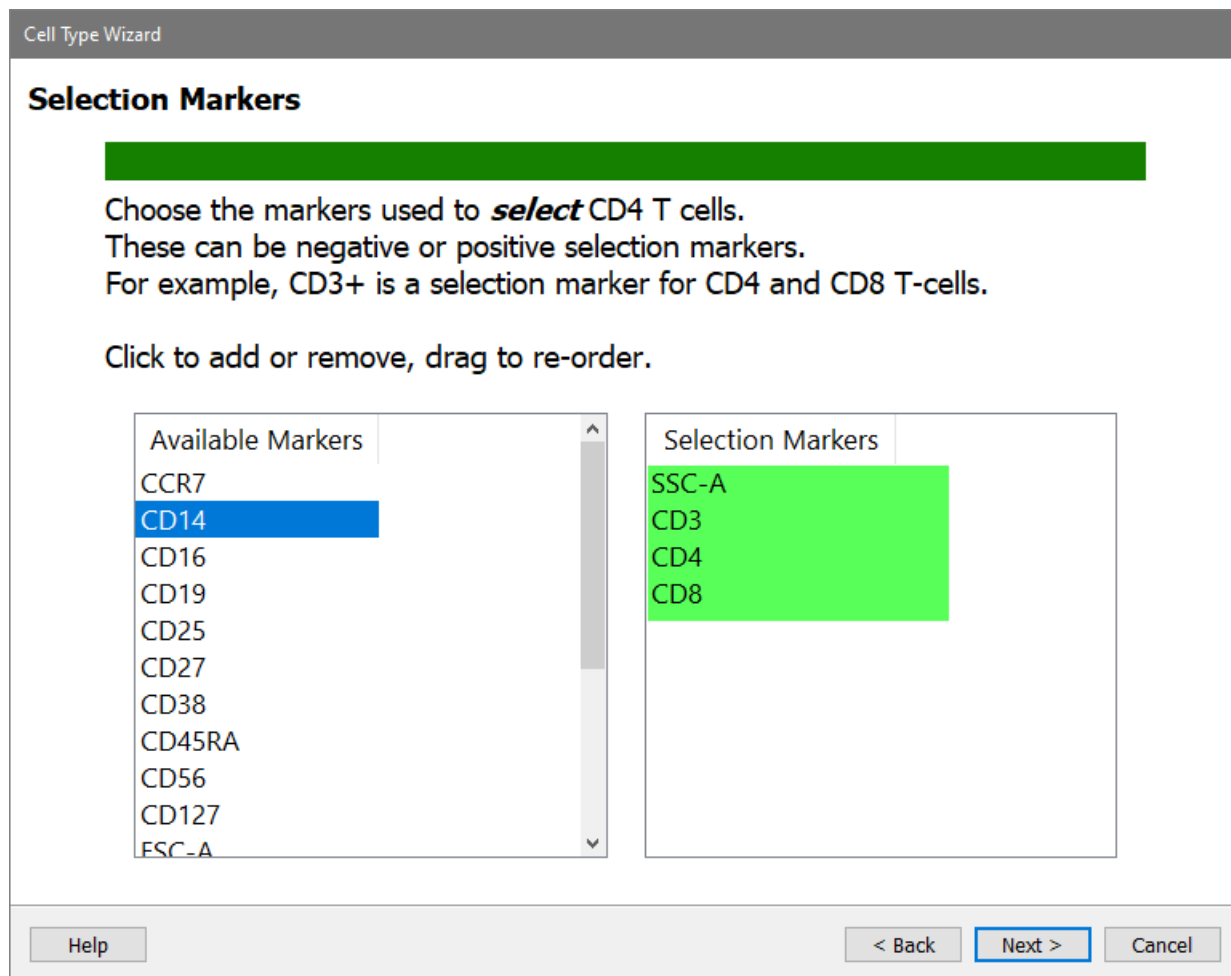
Help < Back Next > Cancel

Next choose the measurements that are used to identify the CD4 T cells. These are called Selection Markers. These can be "positive" markers, as well as markers that are "negative" for the cell type. In this tutorial, you'll just choose several markers that are positive for CD4 T cells.

In the Available Markers list, click each of these markers in this order: **SSC-A**, **CD3**, **CD4**, and **CD8**

The markers will be removed from the Available Markers list and added to the Selection Markers list.

If the markers are not in the correct order, you can drag the markers in the Selection Markers list to reorder them.



Click **Next**.

On the next page of the wizard, choose the Staging Markers for the CD4 T cells. These are markers that change expression levels within the cell type. They are used to identify different stages of CD4 T cells.

In the Available Markers list, click each of these markers in this order: **CCR7**, **CD45RA**, and **CD27**

Cell Type Wizard

Staging Markers

Choose the markers used to *stage* CD4 T cells.
Start with the simplest and best defined.
For example, CCR7 is a staging marker for CD4 and CD8 T-cells.

Click to add or remove, drag to re-order.

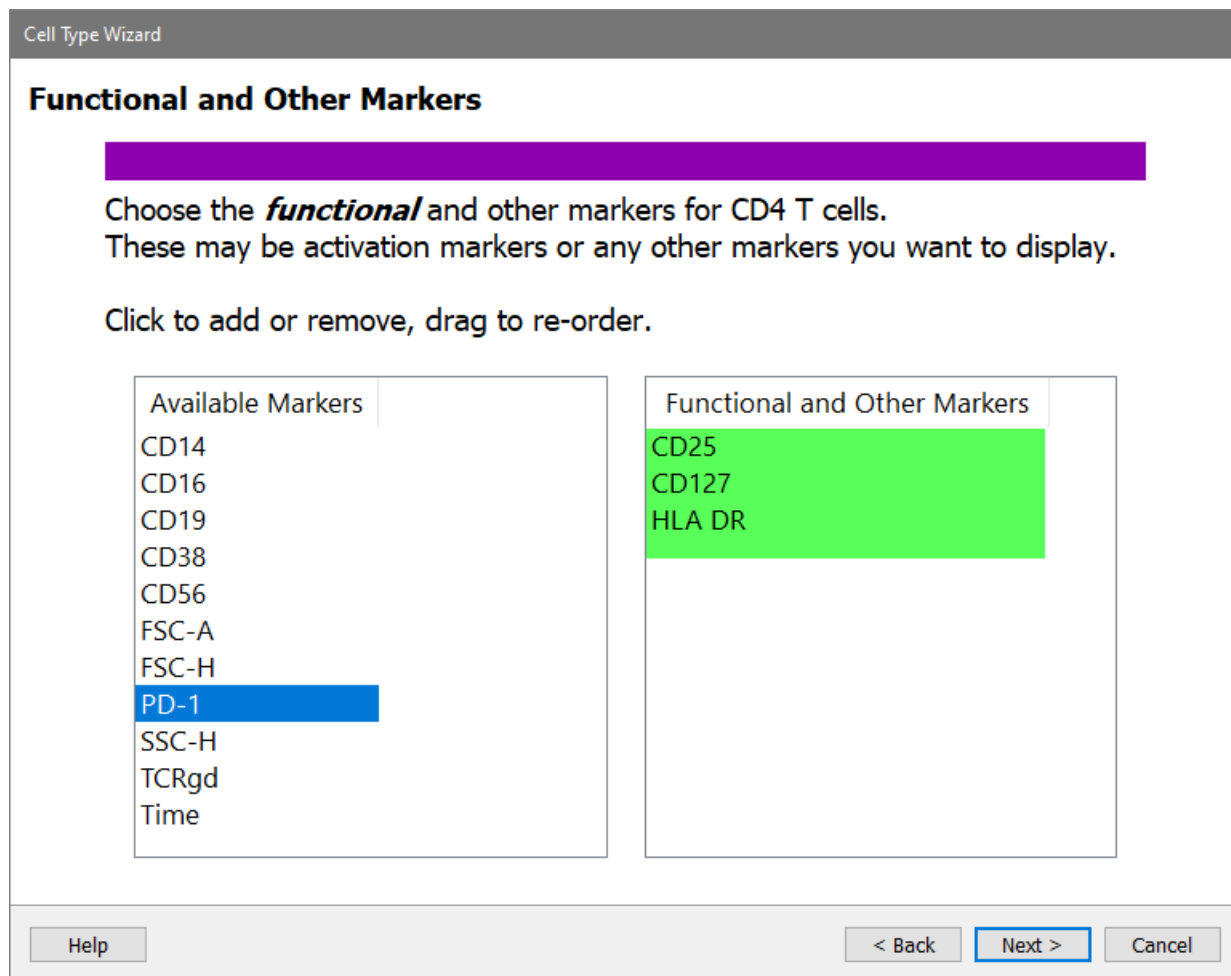
| Available Markers | Staging Markers |
|-------------------|-----------------|
| CD14 | CCR7 |
| CD16 | CD45RA |
| CD19 | CD27 |
| CD25 | |
| CD38 | |
| CD56 | |
| CD127 | |
| FSC-A | |
| FSC-H | |
| HLA DR | |
| PD-1 | |

Help < Back Next > Cancel

Click **Next**.

Next is the Functional and Other Markers page of the wizard. This is where you can pick other markers that you want to see in the CD4 T cell type, even if they are not used for selection or staging of the cells. Activation markers are a good example of what you would pick on this page.

In the Available Markers list, click each of these markers in this order: **CD25**, **CD127**, and **HLA DR**



Click **Next**.

Now that you have identified the markers to use for the CD4 T cells, the next task is to set up *expression profiles* for them.

GemStone uses expression profiles (EPs) instead of *gates* used in most cytometry analysis software. EPs come in different shapes, like constant, step-up, and step-down. They define a probability envelope, which does not have hard edges.

The wizard is showing the first Selection marker, SSC-A, with several properties and an unusual dot plot that may not make immediate sense to you.

Cell Type Wizard

Selection Profiles

Cell Type Events: 0

Define the expression of SSC-A in the CD4 T cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

SSC-A

Marker Page

←

| | |
|-------------------|--|
| Which Peak? | Brightest Peak |
| Distribution | Normal |
| Phenotype | SSC-A+ |
| Initial Intensity | 2556594.66 |
| Initial Width | 14.52 |
| Width from Peak | <input checked="" type="checkbox"/> On |
| Add to Report | <input checked="" type="checkbox"/> On |

Links...

Preview

→

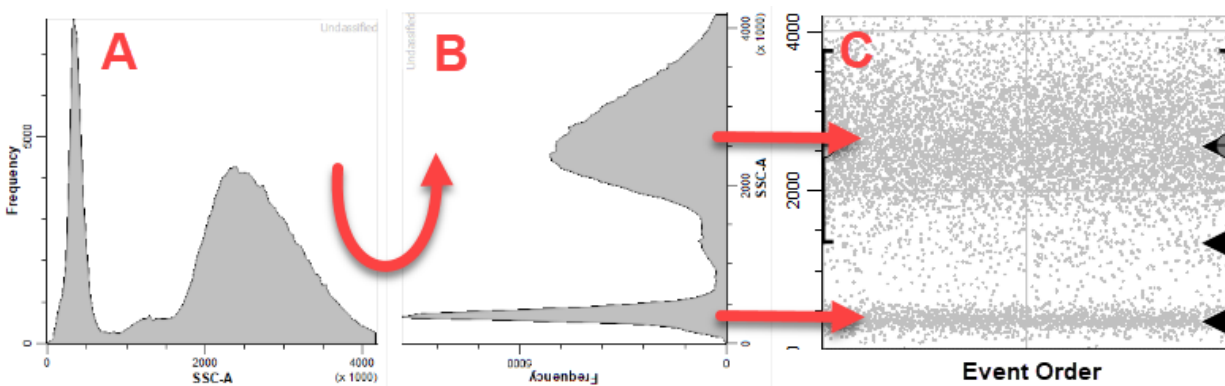
Next Profile

Disable Expression Profile

1 of 10

Help < Back Next > Cancel

Here's how to interpret the plot. Think of a typical histogram of SSC, as in panel A below. Now rotate that histogram 90 degrees (panel B). Now notice that the density of dots in panel C matches the peaks in panel B. The Y-axis of the dot plot is SSC-A, and the X-axis (at this point) is the order of the events in the FCS file. There are black triangles on the right edge to indicate locations where the program found a peak in the data.



The CD4 T cells have low SSC-A, and you can see a black triangle that identifies the dimmest peak of the SSC-A distribution. That's the one you want to capture with this expression profile.

Choose **Dimmest Peak** for **Which Peak**.

There are several options for the **Distribution**. Leave the Distribution set to **Normal**.

The Phenotype is automatically updated as you make choices. It should show "SSC-A-" at this point.

The **Initial Intensity** is currently set to the location of the peak that you linked the profile to. You do not need to change that.

The **Initial Width** is set to the width (SD) of the peak that was found. You don't want this expression profile to be quite that restrictive, so you'll make two changes.

Set **Initial Width** to **5** and uncheck **Width from Peak**. This will leave the width at an SD of 5 and maintain a generous width for SSC-A.

The screenshot shows the 'Cell Type Wizard' dialog box. At the top, it says 'Cell Type Wizard' and 'Cell Type Events: 0'. Below that, it says 'Selection Profiles' and 'Define the expression of SSC-A in the CD4 T cell type. Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.'

The main section is titled 'SSC-A'. It contains a list of settings:

- Which Peak? Dimmest Peak
- Distribution Normal
- Phenotype SSC-A-
- Initial Intensity 366186.87
- Initial Width 5.00
- Width from Peak Off
- Add to Report On

There are navigation arrows: a blue arrow pointing left labeled 'Marker Page' and a blue arrow pointing right labeled 'Next Profile'. A 'Links...' button is also present. A 'Preview' checkbox is checked. Below the settings is a 'Disable Expression Profile' checkbox, which is unchecked. A progress bar at the bottom indicates '1 of 10' profiles. At the very bottom, there are 'Help', '< Back', 'Next >', and 'Cancel' buttons.

Click the **Preview** checkbox on the right of the dialog to see what cells will be selected by this profile.

Cell Type Wizard

Selection Profiles

Cell Type Events: 40731

Define the expression of SSC-A in the CD4 T cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

SSC-A

Which Peak? Dimmest Peak

Distribution Normal

Phenotype SSC-A-

Initial Intensity 366186.87

Initial Width 5.00

Width from Peak Off

Add to Report On

Links... Preview

Marker Page

Next Profile

Disable Expression Profile

1 of 10

Help < Back Next > Cancel

Click **Next Profile**.

CD3 is next. We want the program to find the brightest CD3 peak, and it is already setup that way.

CD3

Which Peak? Brightest Peak

Distribution Normal

Phenotype CD3+

Initial Intensity 37730.45

Initial Width 4.34

Width from Peak On

Add to Report On

Links... Preview

Previous Profile

Next Profile

Disable Expression Profile

2 of 10

Click the **Preview** checkbox on the right of the dialog to see what cells will be selected by this profile.

CD3

Which Peak? **Brightest Peak** Preview

Distribution **Normal**

Phenotype **CD3+**

Initial Intensity **37730.45**

Initial Width **4.34**

Width from Peak On

Add to Report On

Disable Expression Profile

2 of 10

Click **Next Profile**.

CD4 is set up in the same way, tracking the brightest peak. You don't have to make any changes to this profile.

CD4

Which Peak? **Brightest Peak** Preview

Distribution **Normal**

Phenotype **CD4+**

Initial Intensity **71535.11**

Initial Width **2.35**

Width from Peak On

Add to Report On

Disable Expression Profile

3 of 10

Click **Next Profile**.

For CD8, choose **Dimmest Peak**.

For the Distribution, select **Low Pass**. A low pass distribution includes the events below the top of the expression profile. This will capture all of the CD8 negative events.

CD8

Which Peak? **Dimmest Peak** Preview

Distribution **Low Pass**

Phenotype CD8-

Initial Intensity 205.22

Initial Width 3.34

Width from Peak On

Add to Report On

Disable Expression Profile

Links...

Previous Profile Next Profile

4 of 10

Click **Preview** to see the events that are selected.

CD8

Which Peak? Dimmest Peak Preview

Distribution Low Pass

Phenotype CD8-

Initial Intensity 205.22

Initial Width 3.34

Width from Peak On

Add to Report On

Disable Expression Profile

Links...

Previous Profile Next Profile

4 of 10

Click **Next Profile**.

Now that you have set up the expression profiles for the selection markers, you are ready for the staging markers. For these, you will use profiles that have two or more levels of expression.

CCR7 is expressed on naive CD4 T cells, and it is down-regulated as the cells mature. The StepDown profile is designed for this type of expression.

NOTE: Depending on your monitor, you may need to resize this dialog to see the options in the property list.

For **Expression Profile Shape**, choose **StepDown**.

The other settings will update so that the First Level of the profile tracks the Brightest Peak and the Second Level tracks the Dimmest Peak.

Cell Type Wizard

Staging Profiles

Cell Type Events: 17001

Define the expression of CCR7 in the CD4 T cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

CCR7

Shape

Expression Profile Shape: StepDown

Phenotype: CCR7+ > -

First Level

Which Peak?: Brightest P

Initial Intensity: 12628.73

Initial Width: 11.77

Width from Peak: On

Disable Expression Profile

Links... Preview

5 of 10

Enable the **Preview** checkbox to see the StepDown profile results. Note that the dots representing the cells have been reordered so that the brighter CCR7 events are first and the dimmer ones are at the end. As soon as you enable a staging profile like this, the X-axis is no longer showing the order of events in the FCS file. Now, the X-axis is showing the progression of events, and this order is also enforced in the selection marker plots that you setup previously.

Define the expression of CCR7 in the CD4 T cell type.

Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

CCR7

Shape

| | |
|--------------------------|-----------|
| Expression Profile Shape | StepDown |
| Phenotype | CCR7+ > - |

First Level

| | |
|-------------------|--|
| Which Peak? | Brightest P |
| Initial Intensity | 12628.73 |
| Initial Width | 11.77 |
| Width from Peak | <input checked="" type="checkbox"/> On |

Disable Expression Profile

5 of 10

Click **Next Profile**.

You will setup CD45RA in a similar way with a StepDown profile.

Select **StepDown** for the Expression Profile Shape.

Because there is so much variability in the brighter level of CD45RA, you will set the **Initial Width** to **10** and **uncheck** the **Width from Peak** setting.

CD45RA

Shape

| | |
|--------------------------|-------------|
| Expression Profile Shape | StepDown |
| Phenotype | CD45RA+ > - |

First Level

| | |
|-------------------|------------------------------|
| Which Peak? | Brightest Peak |
| Initial Intensity | 31369.38 |
| Initial Width | 10.00 |
| Width from Peak | <input type="checkbox"/> Off |

Disable Expression Profile

6 of 10

Enable the **Preview** checkbox to see the StepDown profile results.

CD45RA

Previous Profile

| Shape | |
|--------------------------|------------------------------|
| Expression Profile Shape | StepDown |
| Phenotype | CD45RA+>- |
| First Level | |
| Which Peak? | Brightest Peak |
| Initial Intensity | 31369.38 |
| Initial Width | 10.00 |
| Width from Peak | <input type="checkbox"/> Off |
| Second Level | |

Links... Preview

Next Profile

6 of 10

Click **Next Profile**.

The program thinks there are three levels of expression for CD27, and this may in fact be the case. However, unless the levels are very clearly shown, it generally works better to use two levels for these distributions with wide variation.

CD27

Previous Profile

| Shape | |
|--------------------------|--|
| Expression Profile Shape | ThreeLevels |
| Phenotype | CD27 |
| First Level | |
| Which Peak? | Closest Peak |
| Initial Intensity | 97948.79 |
| Initial Width | 4.15 |
| Width from Peak | <input checked="" type="checkbox"/> On |
| Second Level | |

Links... Preview

Next Profile

7 of 10

Select **StepDown** for the Expression Profile Shape.

Set the **Initial Width** to **10** and **uncheck** the **Width from Peak** setting.

CD27

Previous Profile

| Shape | |
|--------------------------|------------------------------|
| Expression Profile Shape | StepDown |
| Phenotype | CD27+>- |
| First Level | |
| Which Peak? | Brightest Peak |
| Initial Intensity | 88077.61 |
| Initial Width | 10.00 |
| Width from Peak | <input type="checkbox"/> Off |
| Second Level | |

Disable Expression Profile

Links... Preview

Next Profile

7 of 10

Enable the **Preview** checkbox to see the StepDown profile results.

CD27

Previous Profile

| Shape | |
|--------------------------|------------------------------|
| Expression Profile Shape | StepDown |
| Phenotype | CD27+>- |
| First Level | |
| Which Peak? | Brightest Peak |
| Initial Intensity | 88077.61 |
| Initial Width | 10.00 |
| Width from Peak | <input type="checkbox"/> Off |
| Second Level | |

Disable Expression Profile

Links... Preview

Next Profile

7 of 10

Click **Next Profile**.

Next come the functional makers. For now, skip over these without setting up a profile.

Cell Type Wizard

Functional Profiles

Cell Type Events: 17037

Define the expression of CD25 in the CD4 T cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

CD25

Previous Profile

Expression Profile Shape: None

Which Peak?: User Estimate

Phenotype: CD25

Initial Intensity: 88077.61

Initial Width: 10.00

Width from Peak: Off On

Add to Report

Links...

Preview

Next Profile

Disable Expression Profile

8 of 10

Help < Back Next > Cancel

Click **Next Profile** for CD25.

The CD127 profile is shown.

CD127

Previous Profile

Expression Profile Shape: None

Which Peak?: User Estimate

Phenotype: CD127

Initial Intensity: 88077.61

Initial Width: 10.00

Width from Peak: Off On

Add to Report

Links...

Preview

Next Profile

Disable Expression Profile

9 of 10

Click **Next Profile** for CD127.

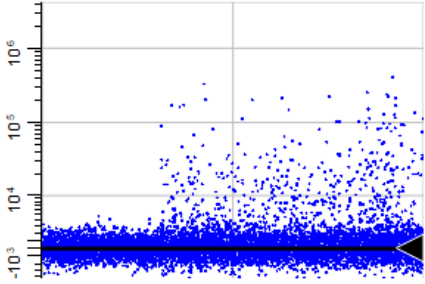
The HLA DR profile is shown.

HLA DR

Previous Profile

Expression Profile Shape: None
 Which Peak?: User Estimate
 Phenotype: HLA DR
 Initial Intensity: 88077.61
 Initial Width: 10.00
 Width from Peak: Off On
 Add to Report: On

Preview



Disable Expression Profile

10 of 10

Click the **Summary Page** button.

A Summary page is shown, allowing you to review the choices made in the wizard.

Make sure all the checkboxes are enabled, except for Do another cell type.

Cell Type Wizard

Summary

Cell Type Info

- CD4 T

Selection Markers

- 4 selection markers
- SSC-A (SSC-A-)
- CD3 (CD3+)
- CD4 (CD4+)
- CD8 (CD8-)

Staging Markers

- 3 staging markers
- CD3 (CD3+)

Finish Up

Do another cell type
 Update summary map

Save template model
 Add to Cen-se!

Add database fields

Click **Finish**.

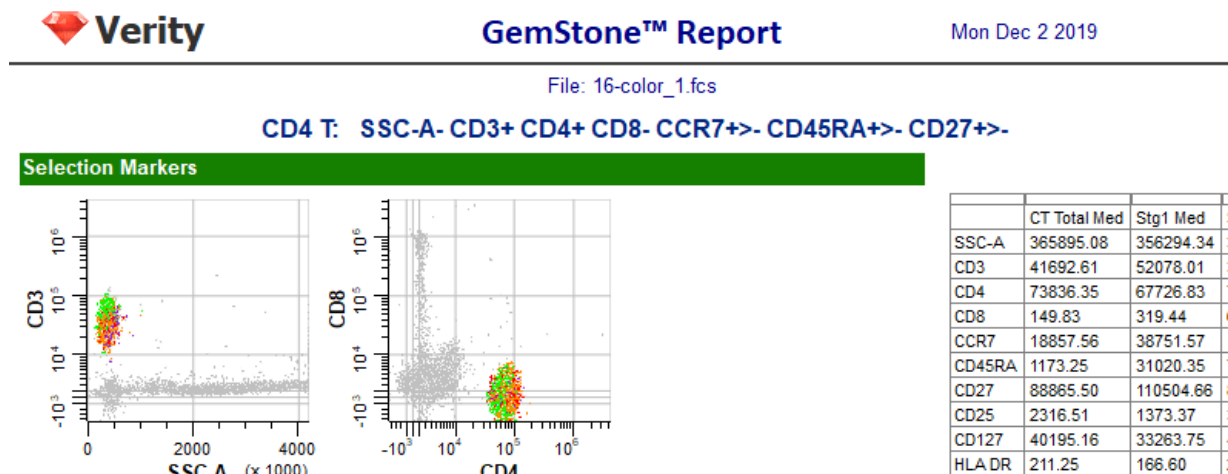
Congratulations! You've just created the CD4 T cell type analysis, complete with a report page. Your work was saved as a "template model" which can be applied to any FCS files that have the same markers and marker names.

Review the Cell Type Report

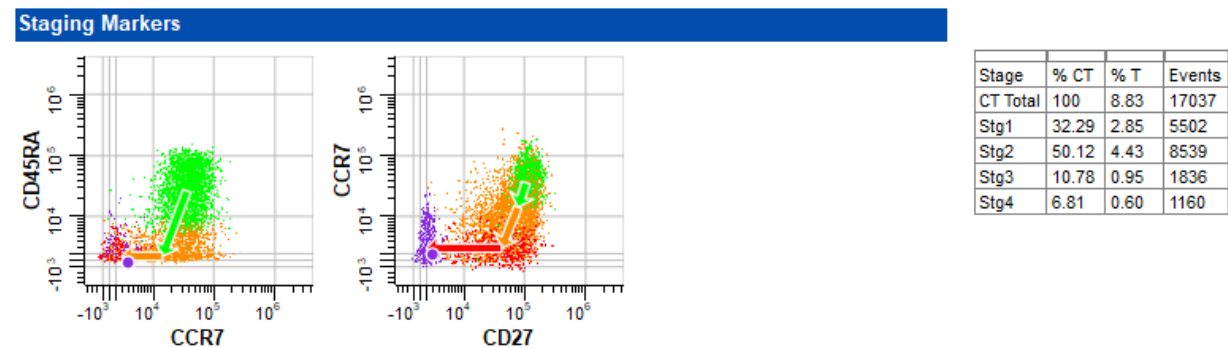
The wizard created a report page based on the choices you made for each marker. The page includes the current date, the name of the FCS file, and the cell type name and phenotype at the top.

Next, there is a section for the selection markers. Each marker is included in at least one dot plot. These plots can be changed to show different marker combinations, and you can add other plots as well with the tools on the Insert tab.

On the right is a table listing all of the markers you included in the cell type. For each marker, the table shows the median intensity for the whole cell type as well as for each stage. You would need to resize the table to see all of the columns, but leave that for later.

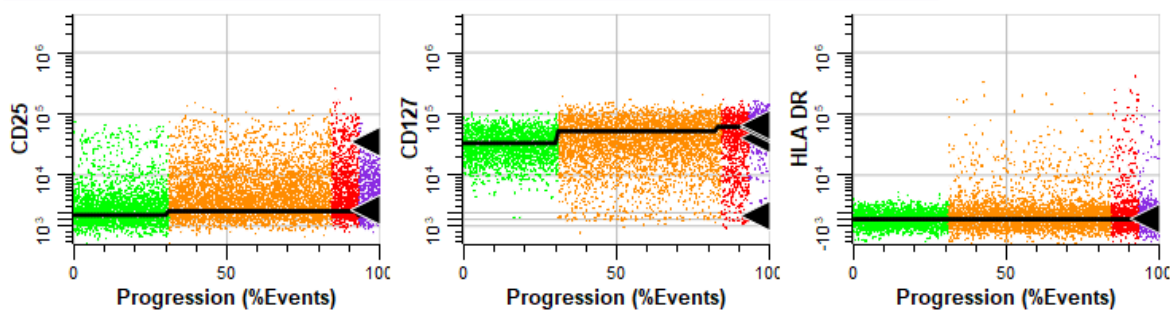


The next section in the report shows the staging markers. A table summarizing the percentages and number of events in each stage appears on the right. The wizard automatically determined the four stages of CD4 T cells based on when markers changed expression levels.



Finally, the other markers are shown, each with an expression profile plot. The footer of the report page shows the name of the Project, the page number, and the template model that was used for the analysis.

Other Markers



New Project Tutorial

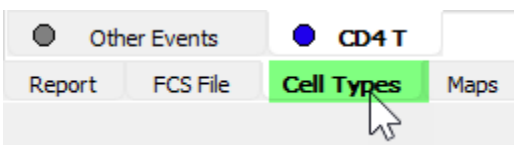
Page 2

TemplateModel.gs (02 Dec 2019 16:58)

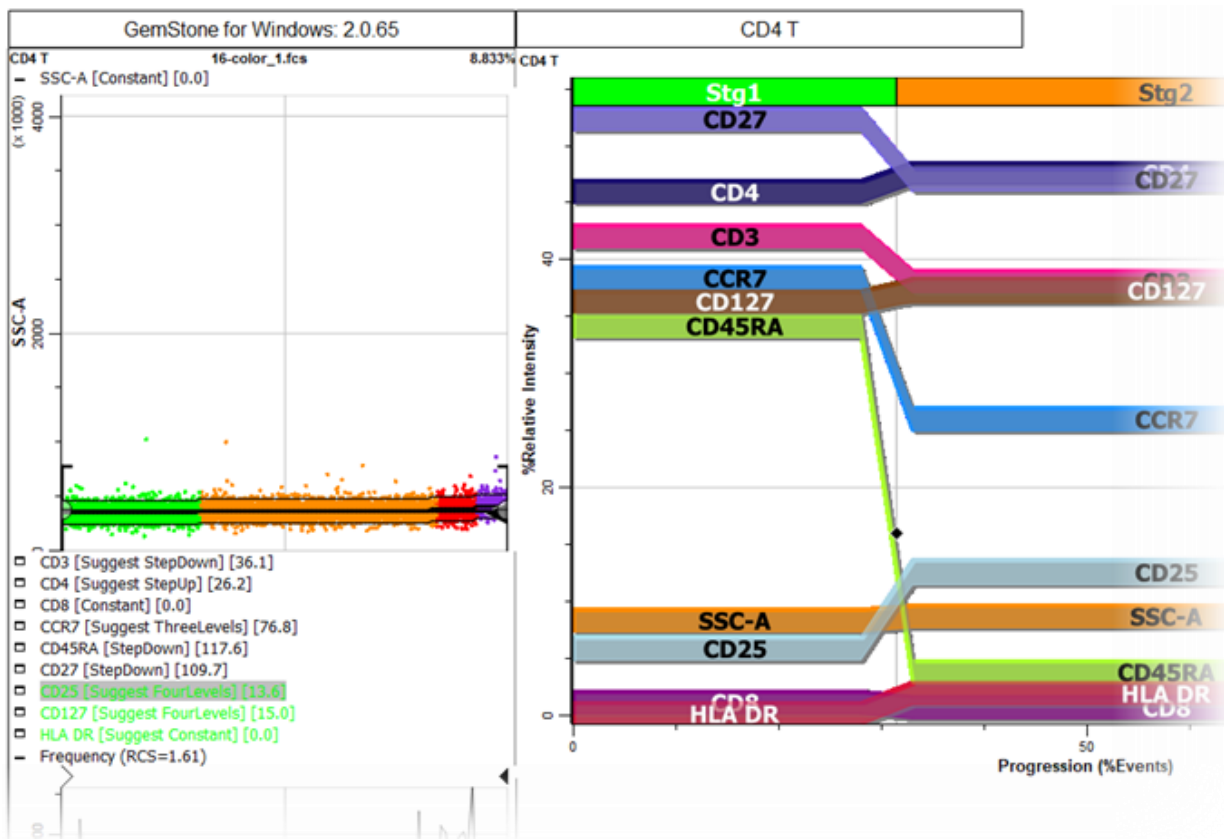
The report is well organized and shows the important information from the analysis. However, it is not showing the expression profiles that were set up in the Cell Type wizard. For that you need to switch to a different tab.

Review the CD4 Cell Type

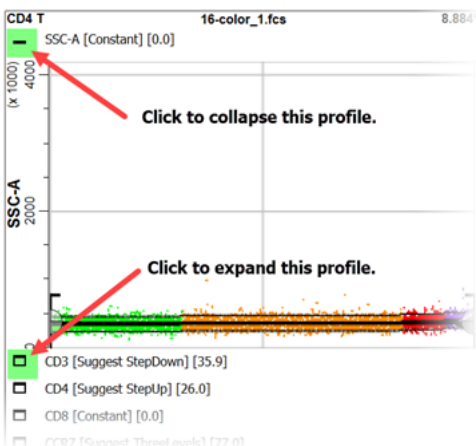
At the bottom of the program window, click the **Cell Types** tab. The program switches from showing the report pages to showing the cell type definitions.



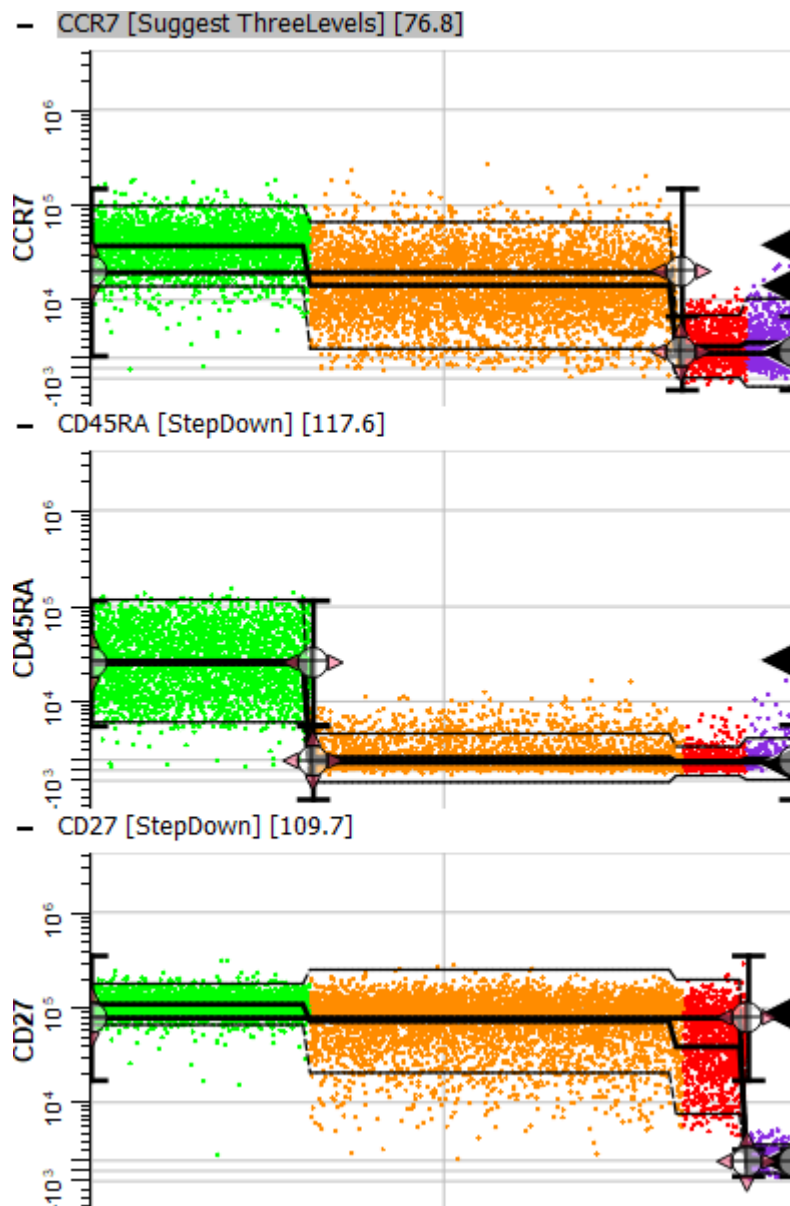
On the left of the CD4 T cell type tab is a stack of expression profiles. These are the same profile plots that were created in the wizard. There is also an [Overlay Plot](#), which shows all of the markers and stages on a single plot. This is a great plot for seeing how markers up- and down-regulate in a coordinated manner.



The icon next to the title of each expression profile is used to expand or collapse the profile.



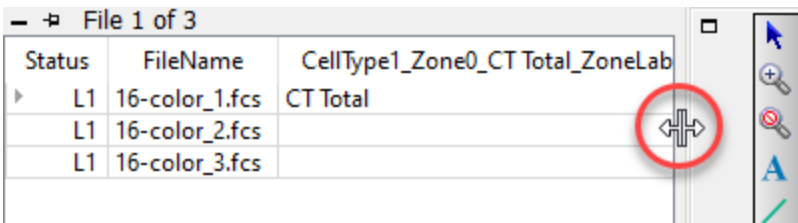
If you scroll down in the cell type window, you'll find the three staging markers that were set up with StepDown profiles. You can see that the first stage (green dots) ends when CD45RA has down-regulated. Stage two (orange) ends after CCR7 down-regulates. Stage three (red) ends after CD27 down-regulates, and stage four (purple) contains the events that have down-regulated for all three markers.



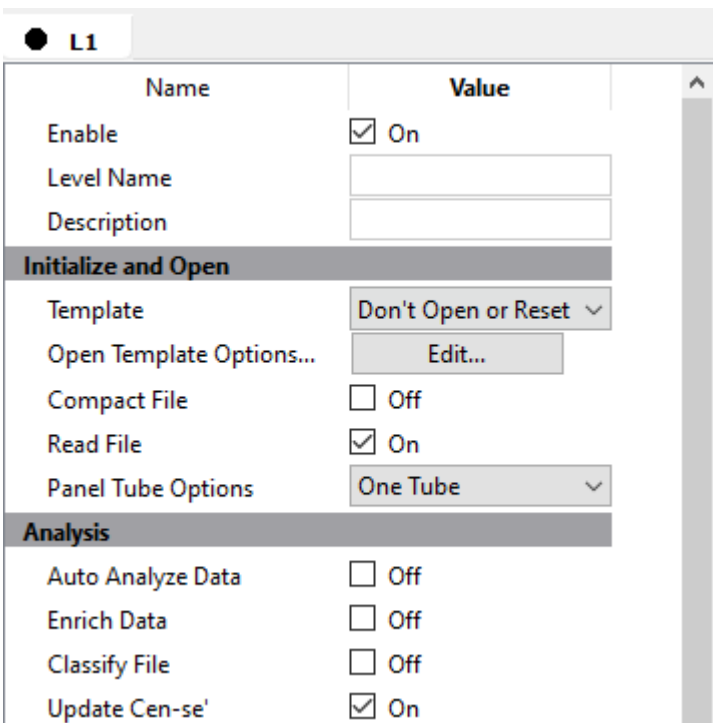
Setup the Batch Options

Now that you have the beginnings of an analysis approach, let's see how to set it up for automation.

You may need to resize the section to see the properties listed. You can also change the width of the batch list by adjusting the right edge as shown below.

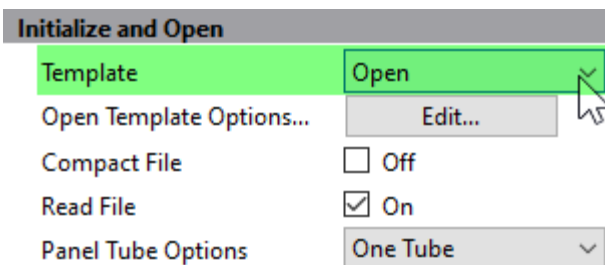


The lower left portion of the screen contains the settings that control batch processing.



The first thing the batch should do when analyzing a file is to open the template model that you just created with the wizard.

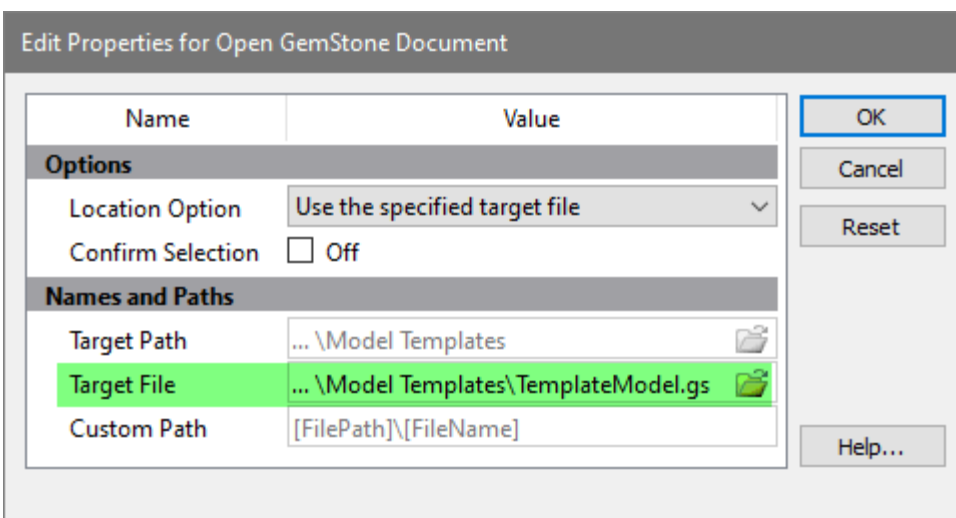
Choose **Open** for the **Template** property.



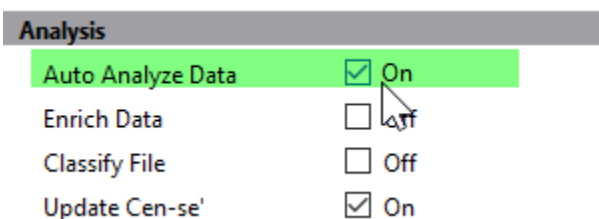
Next click the **Edit...** button for **Open Template Options**.

In the dialog that appears, make sure that **Target File** is set to **TemplateModel.gs**. If not, click the folder icon and select the template model to use.

Click **OK**.

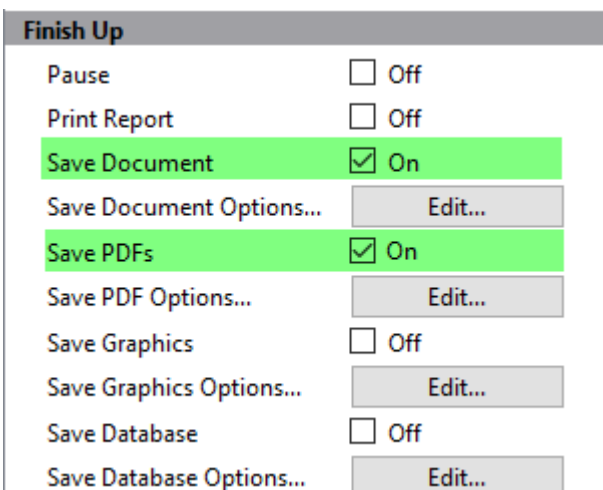


In the Analysis section, enable **Auto Analyze Data**. This tells the program to auto analyze the FCS file with the template model.

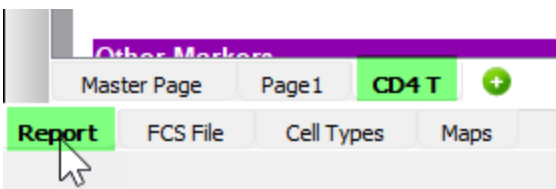


Once the analysis is complete, the Finish Up section determines what kinds of output to generate.

Enable the **Save Document** and **Save PDFs** properties.



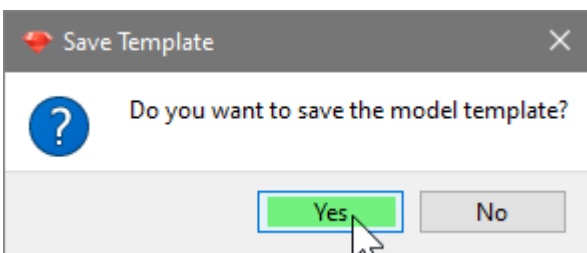
Now you're ready to save these changes. Before you do, let's make sure that the template model is showing the **Report** tab and the **CD4 T** report page. This will be the view you see when the analysis completes.



Click the [Save Project](#) button in the upper left of the Develop tab of the ribbon bar.



When prompted to save the model template, click **Yes**.

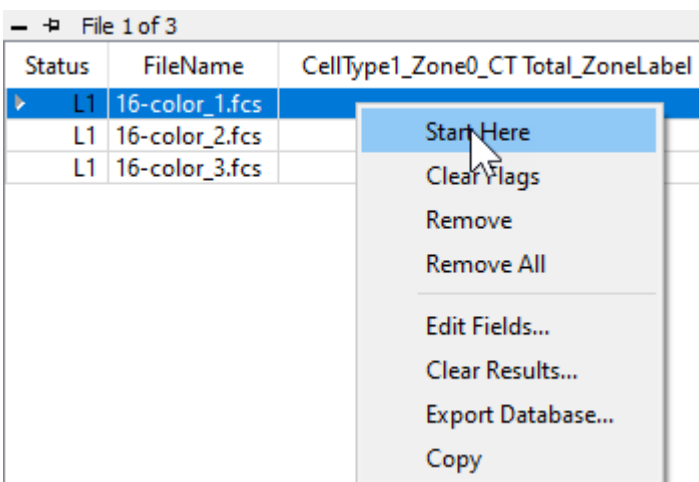


You now have created the model and defined the batch process.

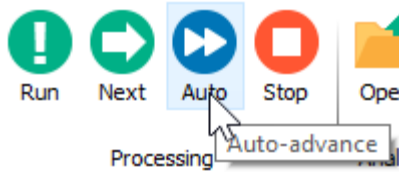
Try it out!

Let's see if everything works as expected.

Right-click the first file in the batch list and choose **Start Here** from the context menu.



On the Home tab, click the [Auto Advance](#) button. This tells the program to analyze all of the files in the batch without intervention.

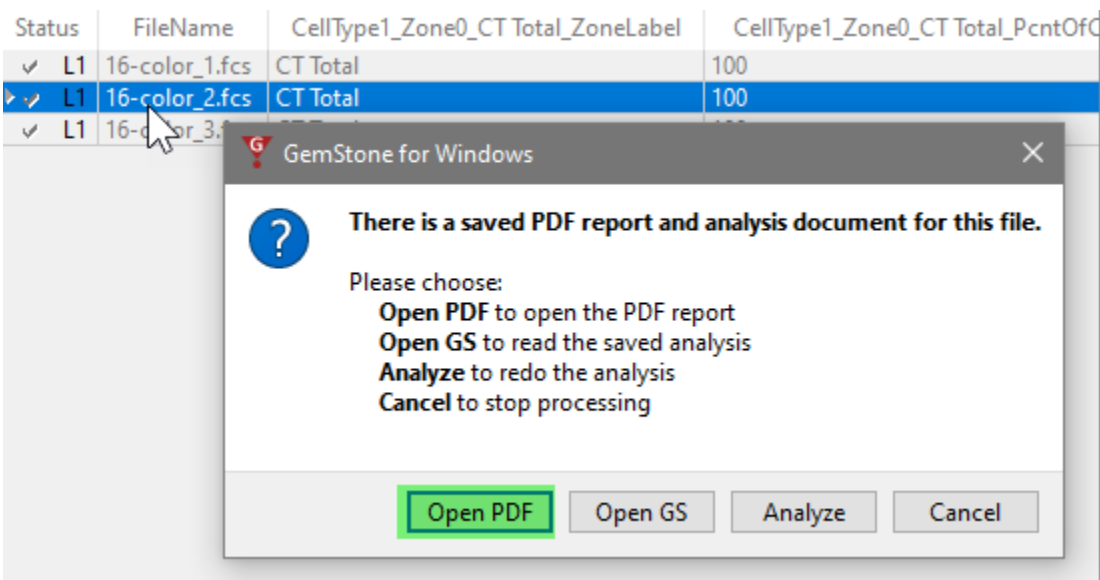


Our template model is pretty simple at this point, so the analysis will be quick. Once it has completed, you can review the results by scrolling horizontally in the batch list.

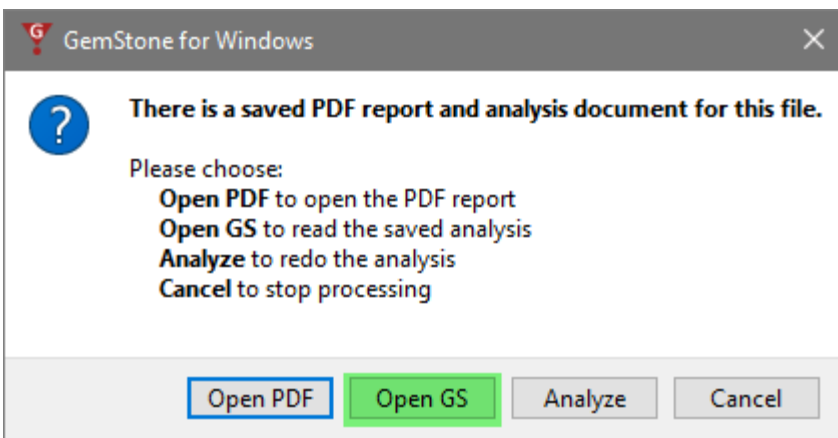
| Status | FileName | CellType1_Zone0_CT Total_ZoneLabel | CellType1_Zone0_CT Total_PcntOfCellTypeEvents | CellType1_Z |
|--------|----------------|------------------------------------|---|-------------|
| ✓ L1 | 16-color_1.fcs | CT Total | 100 | 8.83 |
| ✓ L1 | 16-color_2.fcs | CT Total | 100 | 10.52 |
| ✓ L1 | 16-color_3.fcs | CT Total | 100 | 7.39 |

If you want to review one of the analyzed files, **double-click** the file in the list. The program will ask what you want to do: open the PDF, open the saved analysis (Open GS), redo the analysis (Analyze), or cancel the dialog.

Choose **Open PDF** to view the report in PDF format. Note, at this point the first page in the report will be blank. We will use this blank page as a summary page in later tutorials.



Double-click the file again, and this time choose **Open GS**. This will open the finished analysis of the file. With this, you can make manual changes and review the details of each cell type. This is especially useful when creating new template models or trying to determine the cause of a problem.



Summary

In this tutorial you started a new project from scratch. You gave friendly names to the measurements and used the Cell Type wizard to create one cell type for CD4 T cells. In the process, you looked at the different ways to setup expression profiles and a few caveats. The wizard created a template model from our choices and a report page with plots and tables of statistics.

You learned how to set up the batch options to process a set of files through the template model, and then you tested it out by analyzing all of the files added to the project.

One thing to keep in mind is that you will not always have to start from scratch. You can reuse the template model for other files that were acquired with the same panel. If you extend that panel with additional markers, you can create a new Project based on this one so that you only need to define the new portions of the analysis.

The tutorials that follow will build upon what you have created in this one.

Develop: Adding Cell Types to the Project

Overview

In the previous tutorial, [Starting a New Project from Scratch](#), you set up the analysis of CD4 T cells and ended up with a nice report and database of results.

In this tutorial, you will add two more cell types to the analysis: CD8 T and NK cell types. You will use the Cell Type Wizard to add a cell type and test each cell type before moving on to the next one. This iterative process is the recommended approach, as it tests each enhancement incrementally.

Prerequisites

This tutorial continues to build the Project that was started in the following tutorial:

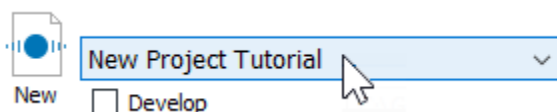
[Develop: Starting a New Project from Scratch](#)

Start GemStone

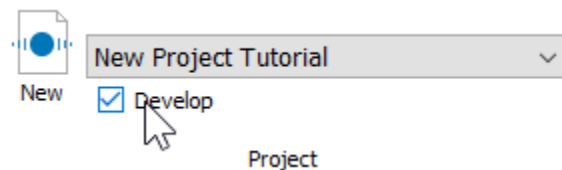
Start Gemstone by following the instructions in the [Installation and Setup of GemStone](#) section.

Open the Project

Open the "New Project Tutorial" Project that you created in the previous tutorial by selecting it in the Project dropdown list.

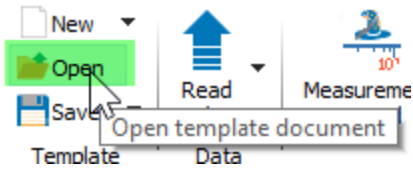


Click the **Develop** checkbox to enable develop mode.

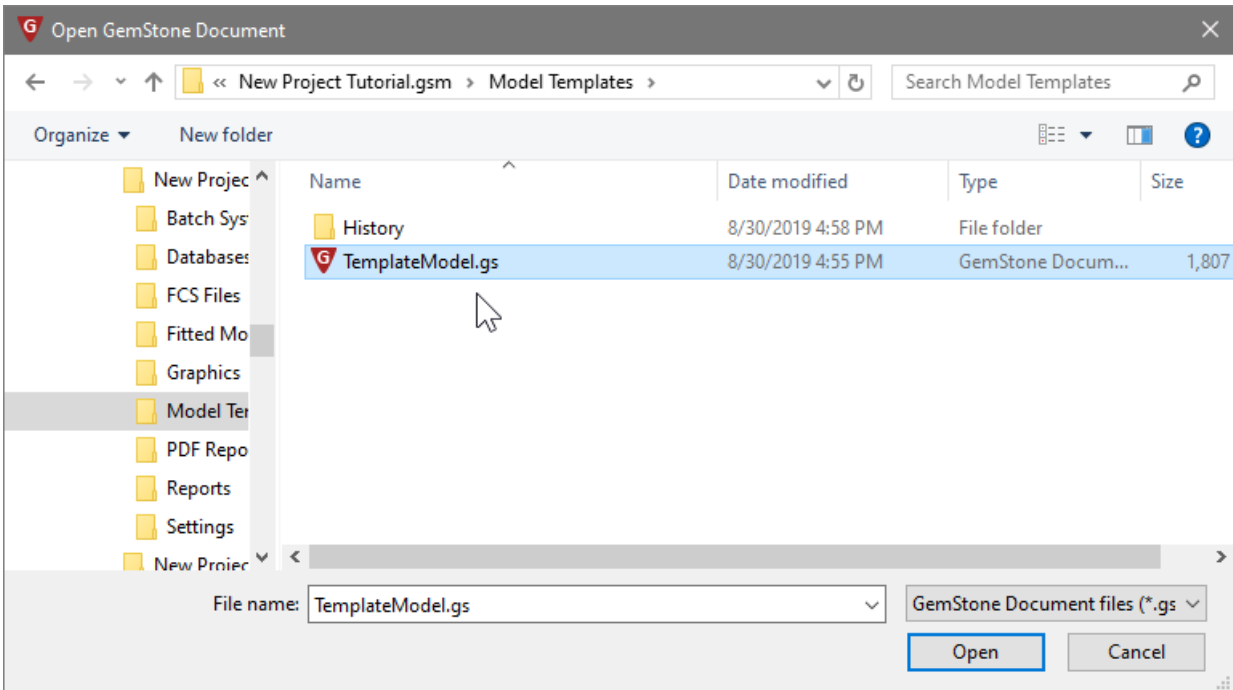


Open the Template

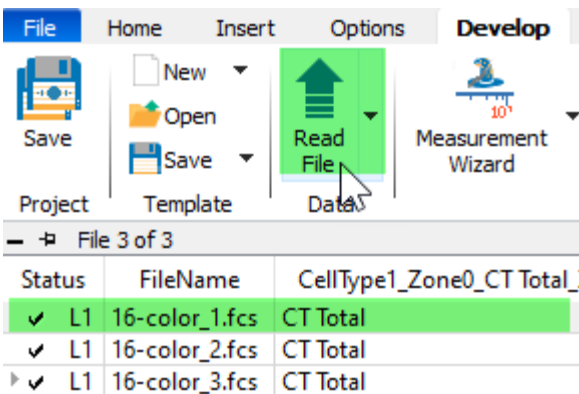
On the Develop tab, click the [Open Template](#) button.



Select "TemplateModel.gs" in the Open GemStone Document dialog and click Open.



Click the first file in the list, and then read the FCS file by clicking the [Read File](#) button. This will read the file into the template without auto-analyzing it.



Cell Type Wizard for CD8 Cell Type

Click the [Cell Type Wizard](#) button to start the wizard.



In the Cell Type Wizard dialog, select **Create a new cell type** and click **Next**.

Cell Type Wizard

Welcome to the Cell Type Wizard

What would you like to do?

Create a new cell type.

Duplicate the selected cell type. *

CD4 T Link selection markers

Edit the selected cell type. *

CD4 T

* Only cell types compatible with the Cell Type Wizard are available for duplicating and editing.

Disable other cell types while editing

Create a report page

Insert before None

Help Next > Cancel

For the Cell Type Name enter "**CD8 T**" and click **Next**.

Cell Type Wizard

Cell Type Properties

Edit the properties for this cell type.

Cell Type Name

Description

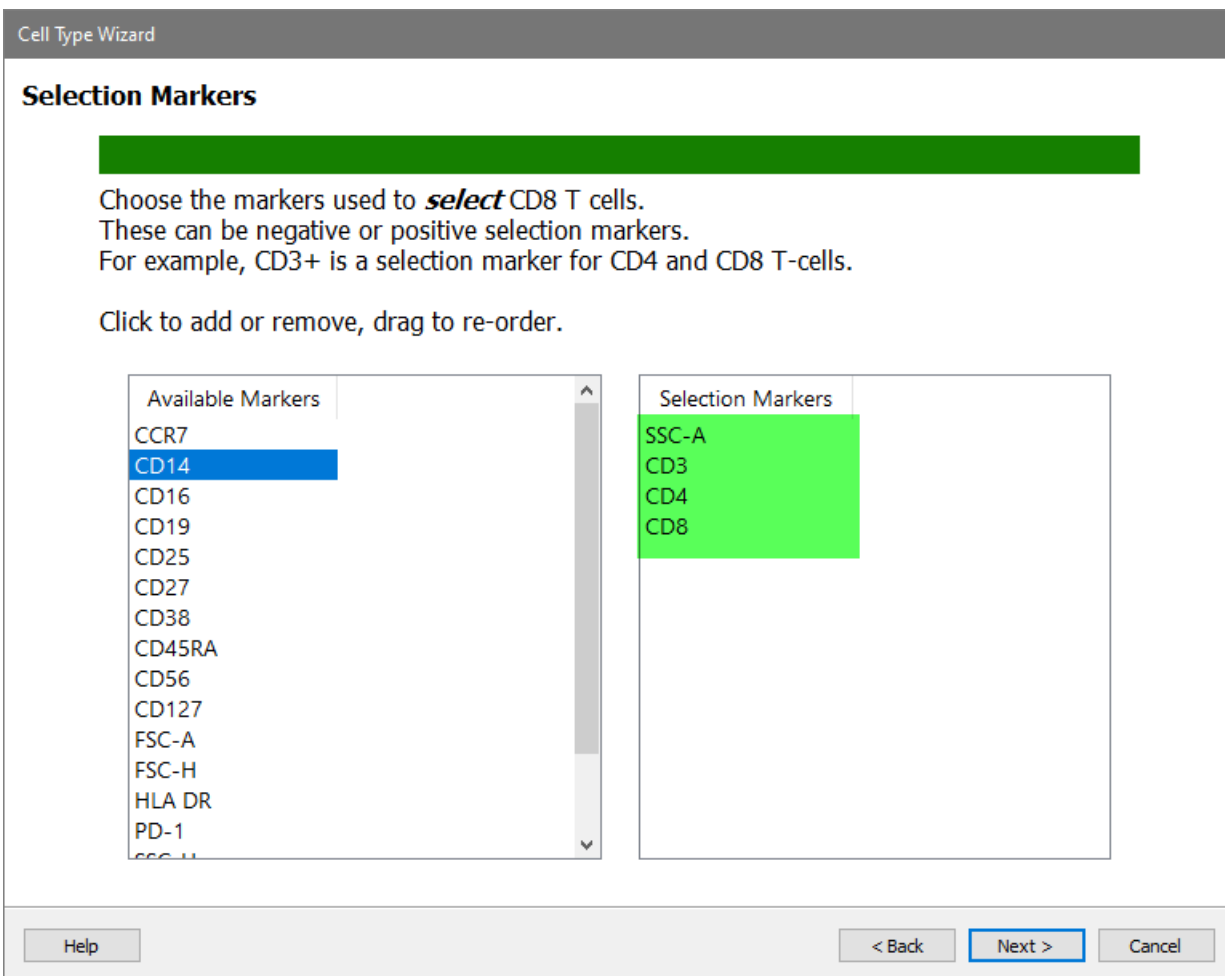
Enable On

Color

Classification Type

Selection Markers

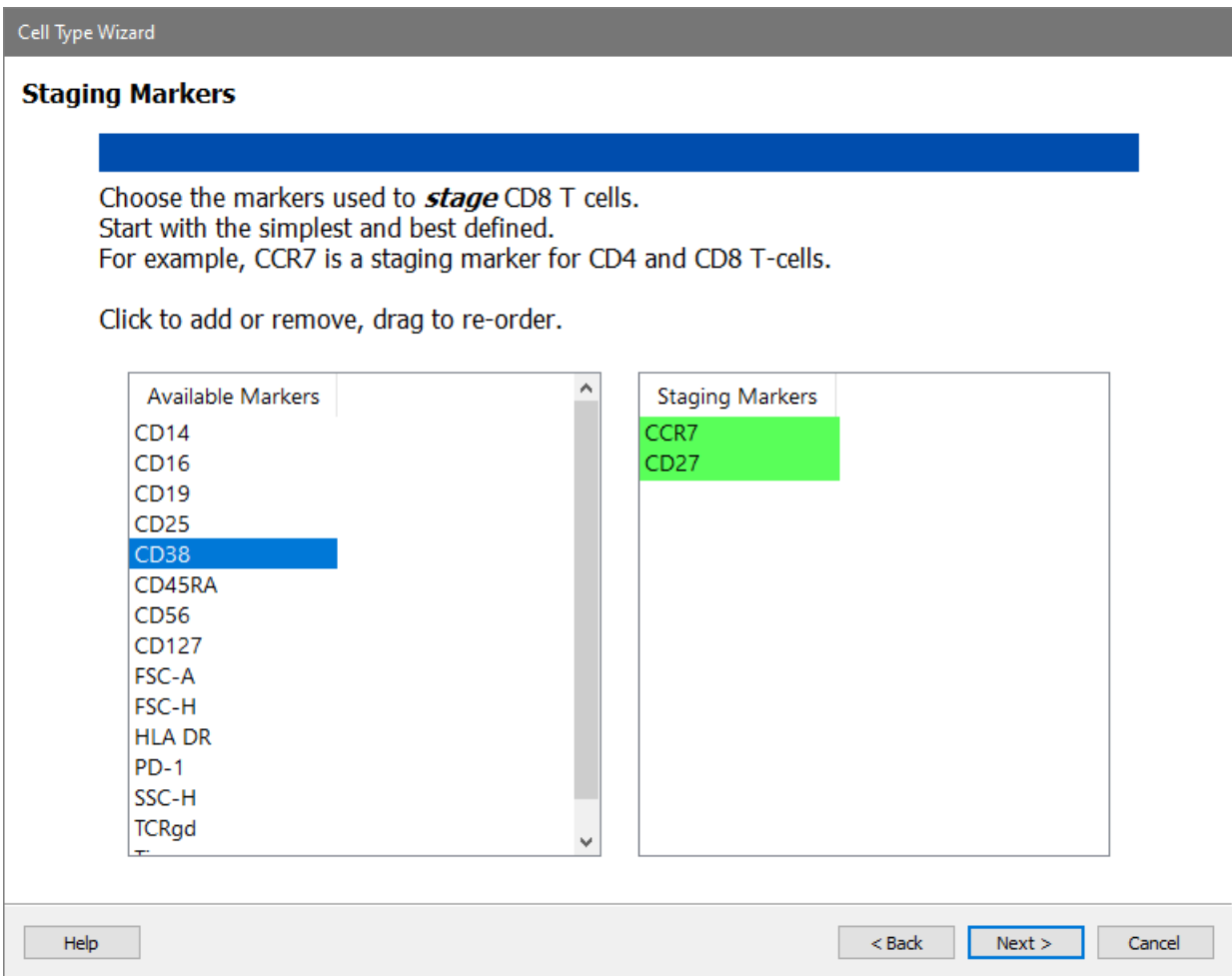
In the Available Markers list, click each of these markers in order: **SSC-A**, **CD3**, **CD4**, and **CD8**. The markers will be added to the Selection Markers list.



Click **Next**.

Staging Markers

In the Available Markers list, click each of these markers in order: **CCR7** and **CD27**. The markers will be added to the Staging Markers list.



Click **Next**.

Functional and Other Markers

In the Available Markers list, click each of these markers in order: **CD38** and **HLA DR**. The markers will be added to the Functional Markers list.

Cell Type Wizard

Functional and Other Markers

Choose the *functional* and other markers for CD8 T cells.
These may be activation markers or any other markers you want to display.

Click to add or remove, drag to re-order.

| Available Markers | Functional and Other Markers |
|-------------------|------------------------------|
| CD14 | CD38 |
| CD16 | HLA DR |
| CD19 | |
| CD25 | |
| CD45RA | |
| CD56 | |
| CD127 | |
| FSC-A | |
| FSC-H | |
| PD-1 | |
| SSC-H | |
| TCRgd | |
| Time | |

Help < Back Next > Cancel

Click **Next**.

Selection Marker Expression Profiles for CD8

SSC-A is the first selection marker, and you will use a new technique to set up its expression profile. When you setup the CD4 T cell type, you tracked the dimmest peak of SSC-A. That's what we want to do for the CD8 T cell type, too. When two cell types set up a marker in the same way, you can "link" them together. Linking expression profiles speeds up the analysis and ensures consistency in how the profiles are set up.

Click the **Links** button.

Cell Type Wizard

Selection Profiles

Cell Type Events: 0

Define the expression of SSC-A in the CD8 T cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

SSC-A

← Marker Page

Which Peak? Brightest Peak

Distribution Normal

Phenotype SSC-A+

Initial Intensity 2556594.66

Initial Width 14.52

Width from Peak On

Add to Report On

Links...

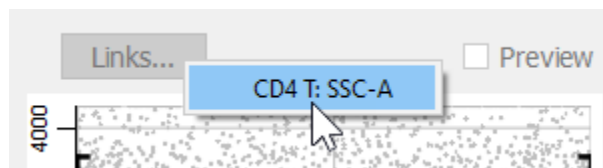
Preview

Disable Expression Profile

1 of 8

Help
< Back
Next >
Cancel

In the menu of choices, choose **CD4 T: SSC-A**.



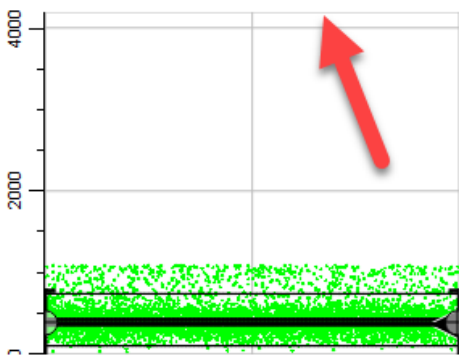
When the link is made, the properties for the CD8 T SSC-A measurement are set by the CD4 marker in the CD4 cell type. The profile switches into Preview mode automatically.

SSC-A

Which Peak? User Estimate ▾
 Distribution Normal ▾
 Phenotype SSC-A-
 Initial Intensity 366186.87
 Initial Width 5.00
 Width from Peak Off
 Add to Report On

Marker Page

Links... Linked to CD4 T Preview



Next Profile

Disable Expression Profile

1 of 8

Click **Next Profile**.

Use the same technique for CD3.

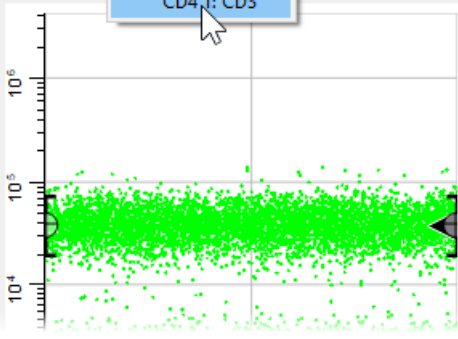
Click the **Links** button and select the "CD4 T: CD3" profile.

CD3

Which Peak? Brightest Peak ▾
 Distribution Normal ▾
 Phenotype CD3+
 Initial Intensity 37730.45
 Initial Width 4.34
 Width from Peak On
 Add to Report On

Previous Profile

Links CD4 T: CD3 Preview



Next Profile

Again, after the link is made the profile switches to Preview mode.

CD3

Previous Profile

Which Peak? User Estimate

Distribution Normal

Phenotype CD3+

Initial Intensity 37730.45

Initial Width 5.00

Width from Peak Off

Add to Report On

Links... Linked to CD4 T Preview

Next Profile

Disable Expression Profile

2 of 8

Click **Next Profile**.

For the CD4 marker in the CD8 T cell type, select **Dimmest Peak** and **Low Pass**. As we saw earlier, the Low Pass profile captures events that are below the top of the expression profile..

CD4

Previous Profile

Which Peak? Dimmest Peak

Distribution Low Pass

Phenotype CD4-

Initial Intensity 1126.40

Initial Width 1.42

Width from Peak On

Add to Report On

Links... Preview

Next Profile

Disable Expression Profile

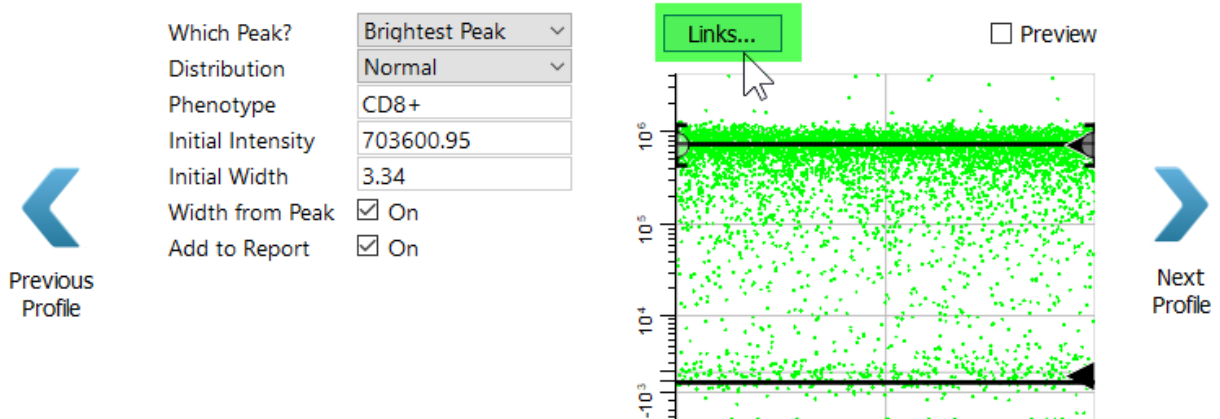
3 of 8

Click **Next Profile**.

For CD8 we are interested in the dim to bright CD8 events. Let's learn another trick to capture these events.

Click the **Links** button.

CD8



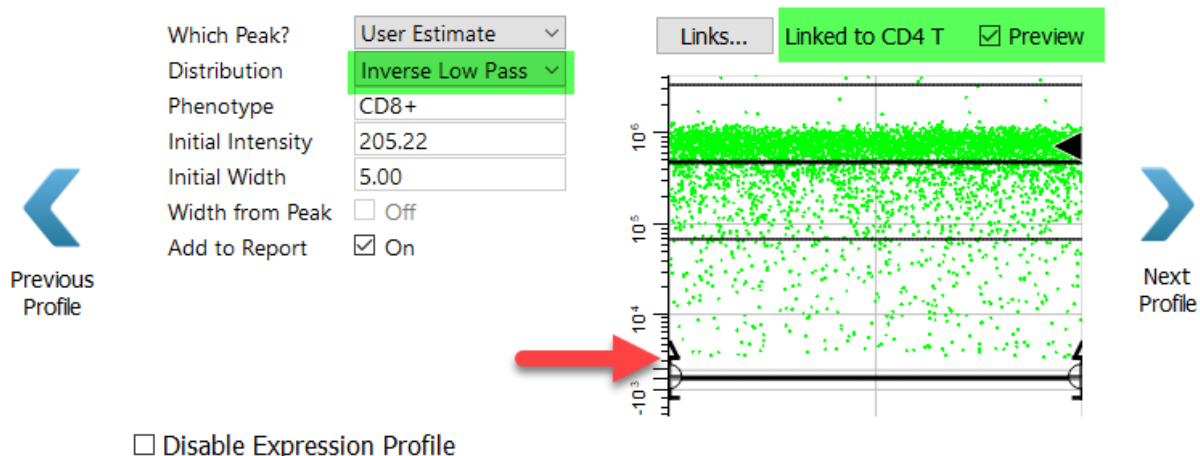
In the menu, select "CD4 T: CD8".



This may seem like a mistake at first, because the CD8 expression in the CD4 T cell type is *not* the expression level for the CD8 T cell type. In fact, it is the opposite. For the CD8 T cell type the cells of interest have dim to bright expression of CD8.

Change the Distribution to **Inverse Low Pass**. The Inverse Low Pass distribution selects the events *above* the top of the profile, which are the dim to bright events.

CD8



4 of 8

Click **Next Profile**.

Staging Marker Expression Profiles for CD8

Moving on to the staging markers for the CD8 T cell type, the first is CCR7. This marker is abundant on early CD8 T cells, and is lost as they mature.

Select **StepDown** for the Expression Profile Shape.

Enable the **Preview** checkbox to see how the StepDown profile fits the two levels of expression for CCR7.

Define the expression of CCR7 in the CD8 T cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

CCR7

| Shape | |
|--------------------------|--|
| Expression Profile Shape | StepDown |
| Phenotype | CCR7+>- |
| First Level | |
| Which Peak? | Brightest Peak |
| Initial Intensity | 47066.68 |
| Initial Width | 6.36 |
| Width from Peak | <input checked="" type="checkbox"/> On |
| Second Level | |
| Which Peak? | Dimmest Peak |
| Initial Intensity | 2082.75 |

Disable Expression Profile

Links... Preview

5 of 8

Click **Next Profile**.

The program suggests a ThreeLevels profile shape for CD27 because it detects three levels of expression. There may indeed be three levels of expression, but they are not clearly distinguishable in this data. We will choose a simpler profile shape.

CD27

| Shape | |
|--------------------------|--|
| Expression Profile Shape | ThreeLevels |
| Phenotype | CD27 |
| First Level | |
| Which Peak? | Closest Peak |
| Initial Intensity | 101758.60 |
| Initial Width | 3.77 |
| Width from Peak | <input checked="" type="checkbox"/> On |
| Second Level | |
| Which Peak? | Closest Peak |
| Initial Intensity | 40177.05 |

Disable Expression Profile

Links... Preview

6 of 8

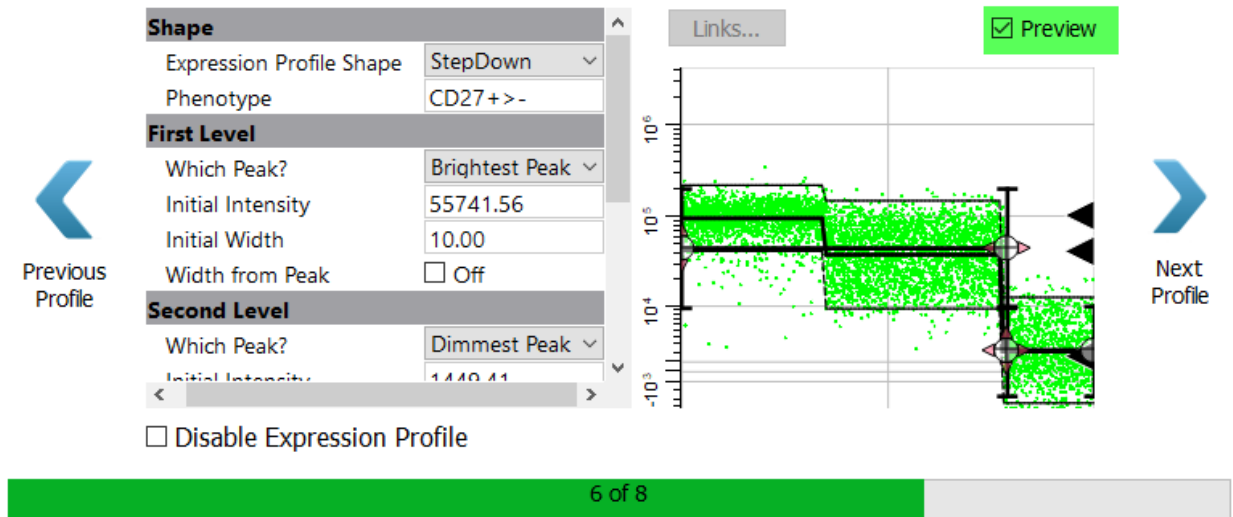
Select **StepDown** for the Expression Profile Shape. For the **Initial Width** type **10.00** and uncheck **Width from Peak**. Using a larger, fixed width for the bright peak will allow the program to capture the two apparent levels of bright expression.

CD27



Enable the **Preview** checkbox. Notice that even with the StepDown profile, we can appreciate three levels of expression in CD27.

CD27



Click **Next Profile**.

Functional Marker Expression Profiles for CD8

Next come the functional makers. For now, skip over these without setting up a profile.

Cell Type Wizard

Functional Profiles

Cell Type Events: 6031

Define the expression of CD38 in the CD8 T cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

CD38

Expression Profile Shape: Preview

Which Peak?:

Phenotype:

Initial Intensity:

Initial Width:

Width from Peak: Off On

Add to Report:

Disable Expression Profile

7 of 8

Click **Next Profile** to skip CD38.

The HLA DR profile is shown.

HLA DR

Expression Profile Shape: Preview

Which Peak?:

Phenotype:

Initial Intensity:

Initial Width:

Width from Peak: Off On

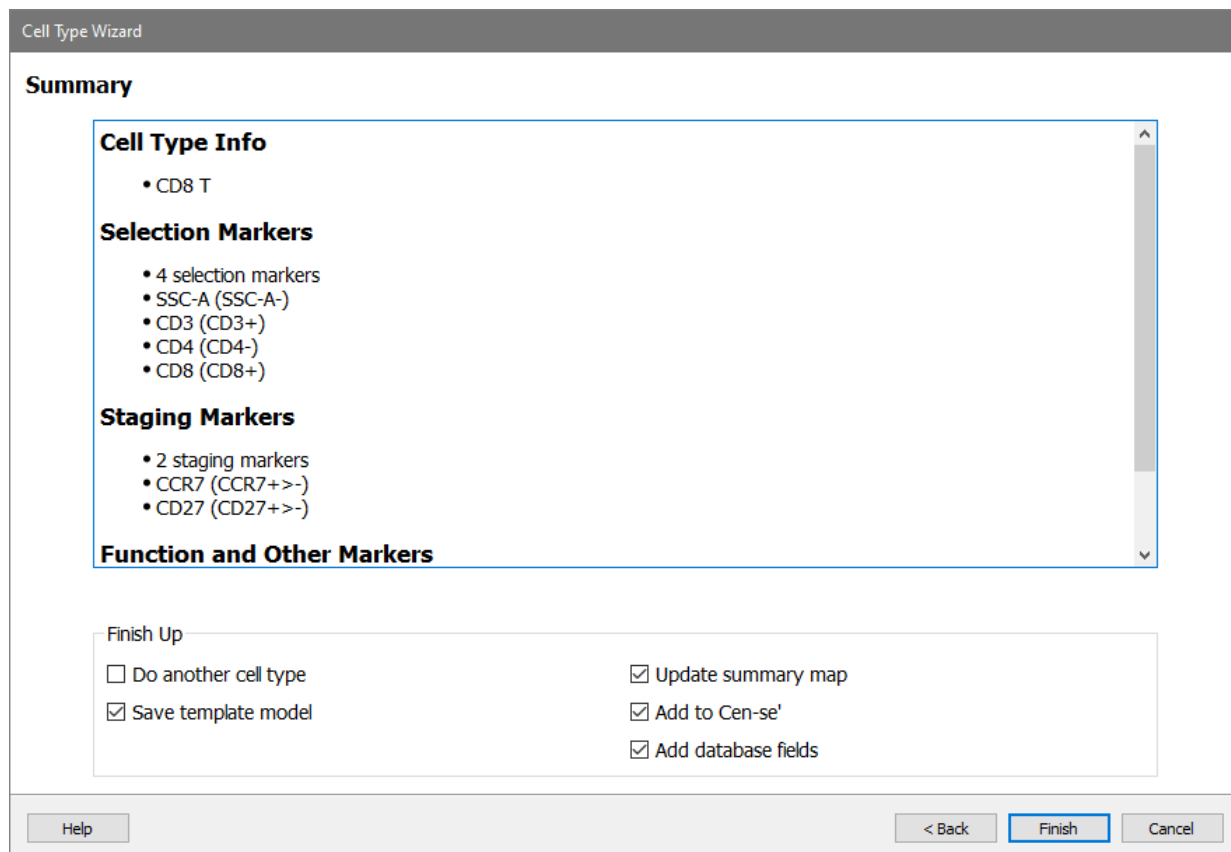
Add to Report:

Disable Expression Profile

8 of 8

Click **Summary Page** to skip HLA DR.

On the Summary page, review the summary information for the cell type. Make sure that **Save template model**, **Update summary map**, **Add to Cen-se'**, and **Add database fields** checkboxes are enabled.



Click **Finish**.

After the wizard closes, the changes you made to the model template will be saved and the wizard-created report page for CD8 will be displayed.

Review the CD8 Report

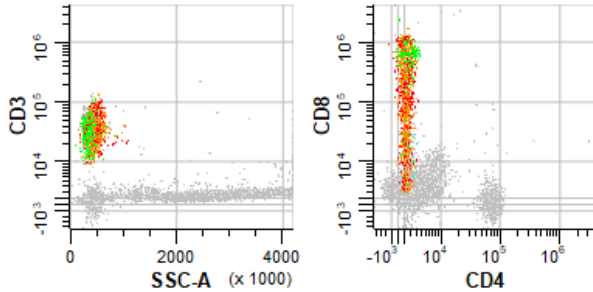
As we saw previously, the report page is divided into sections for Selection Markers, Staging Markers, and Other Markers. Plots in each section display the measurements that we added to the cell type. Tables are included to show median intensities for the markers and frequency information for the stages.



File: 16-color_1.fcs

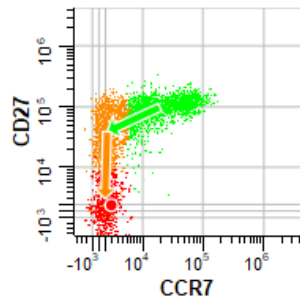
CD8 T: SSC-A- CD3+ CD4- CD8+ CCR7+>- CD27+>-

Selection Markers



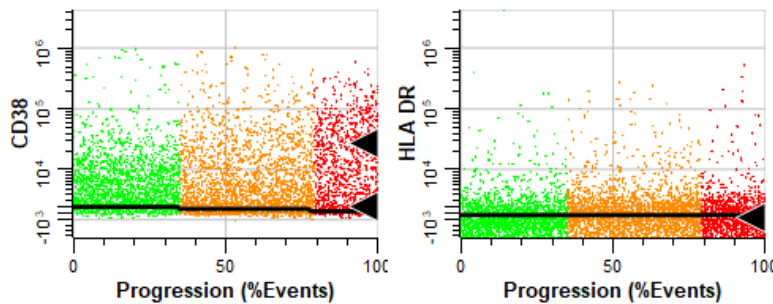
| | CT Total Med | Stg1 Med | S |
|--------|--------------|-----------|---|
| SSC-A | 424069.80 | 393435.20 | 4 |
| CD3 | 35137.47 | 35363.63 | 3 |
| CD4 | 1306.35 | 1373.13 | 1 |
| CD8 | 662251.62 | 679956.21 | 6 |
| CCR7 | 1865.58 | 18999.63 | 1 |
| CD27 | 50366.45 | 102469.74 | 3 |
| CD38 | 3306.32 | 5868.72 | 2 |
| HLA DR | -221.82 | -850.80 | - |

Staging Markers



| Stage | % CT | % T | Events |
|----------|-------|------|--------|
| CT Total | 100 | 3.13 | 6031 |
| Stg1 | 35.07 | 1.10 | 2115 |
| Stg2 | 45.23 | 1.41 | 2728 |
| Stg3 | 19.70 | 0.62 | 1188 |

Other Markers

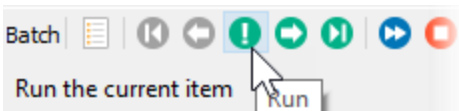


Test the Analysis with CD8 T and CD4 T

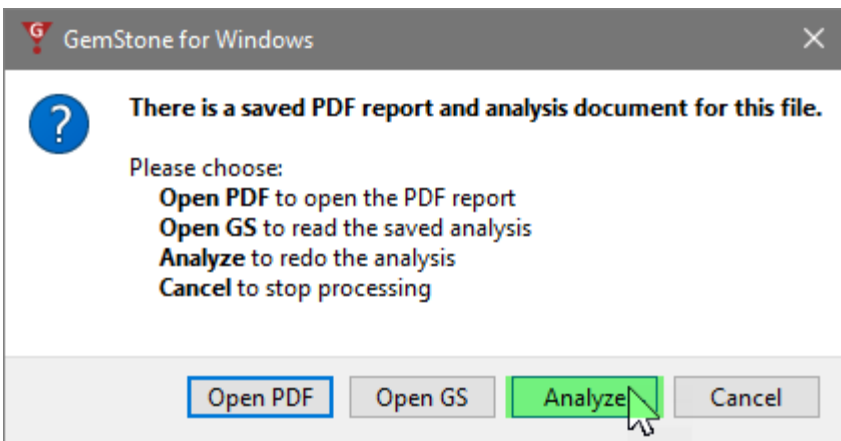
Each time a cell type is added or revised you should check how the new cell type affects other cell types in the model template.

The idea is to review each of the other cell types to make sure that nothing has changed dramatically.

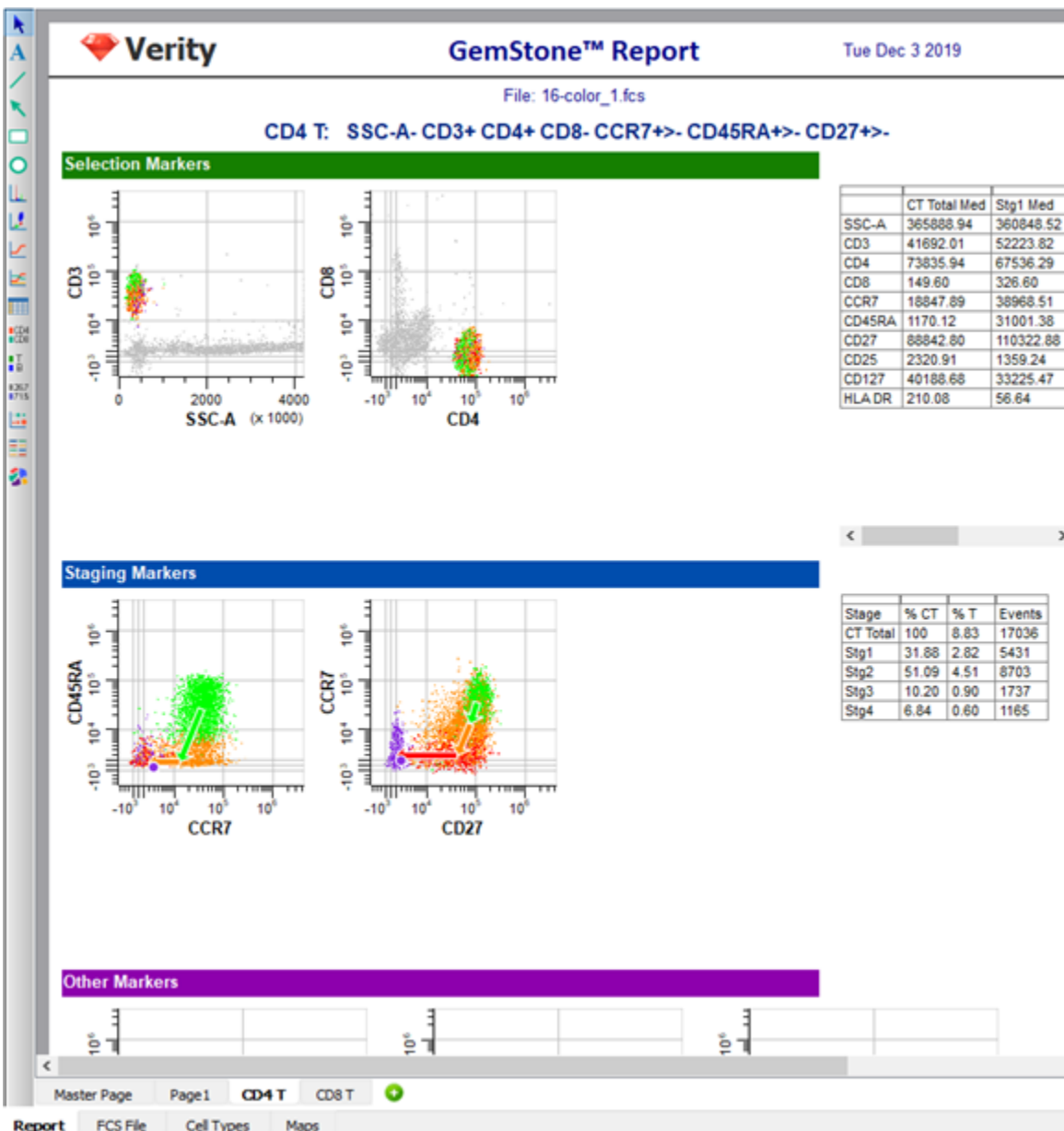
Click the [Run](#) button at the bottom of the batch list. This has the same function as the Run button on the Home tab, running the batch commands to process the selected FCS file.



When prompted, choose **Analyze** to re-run the analysis of this FCS file with the modified template.



When the analysis is complete, select the CD4 T cell type page of the report by clicking the CD4 T tab at the bottom of the report pages. The distributions in the CD4 T cell type are almost identical to what they were with the original model template. This suggests that adding the CD8 T cell type did not adversely affect the CD4 T cell type.



Excellent. Let's move on to the next cell type.

Cell Type Wizard for NK Cell Type

The NK cell type is a very simple one, so let's add that one now.

Click the [Cell Type Wizard](#) button.



Cell Type
Wizard

Choose **Create a new cell type** and click **Next**.

Cell Type Wizard

Welcome to the Cell Type Wizard

What would you like to do?

Create a new cell type.

Duplicate the selected cell type. *

CD4 T Link selection markers

Edit the selected cell type. *

CD4 T

* Only cell types compatible with the Cell Type Wizard are available for duplicating and editing.

Disable other cell types while editing

Create a report page

Insert before: None

Help Next > Cancel

Type **"NK"** for the Cell Type Name and click **Next**.

Cell Type Wizard

Cell Type Properties

Edit the properties for this cell type.

Cell Type Name

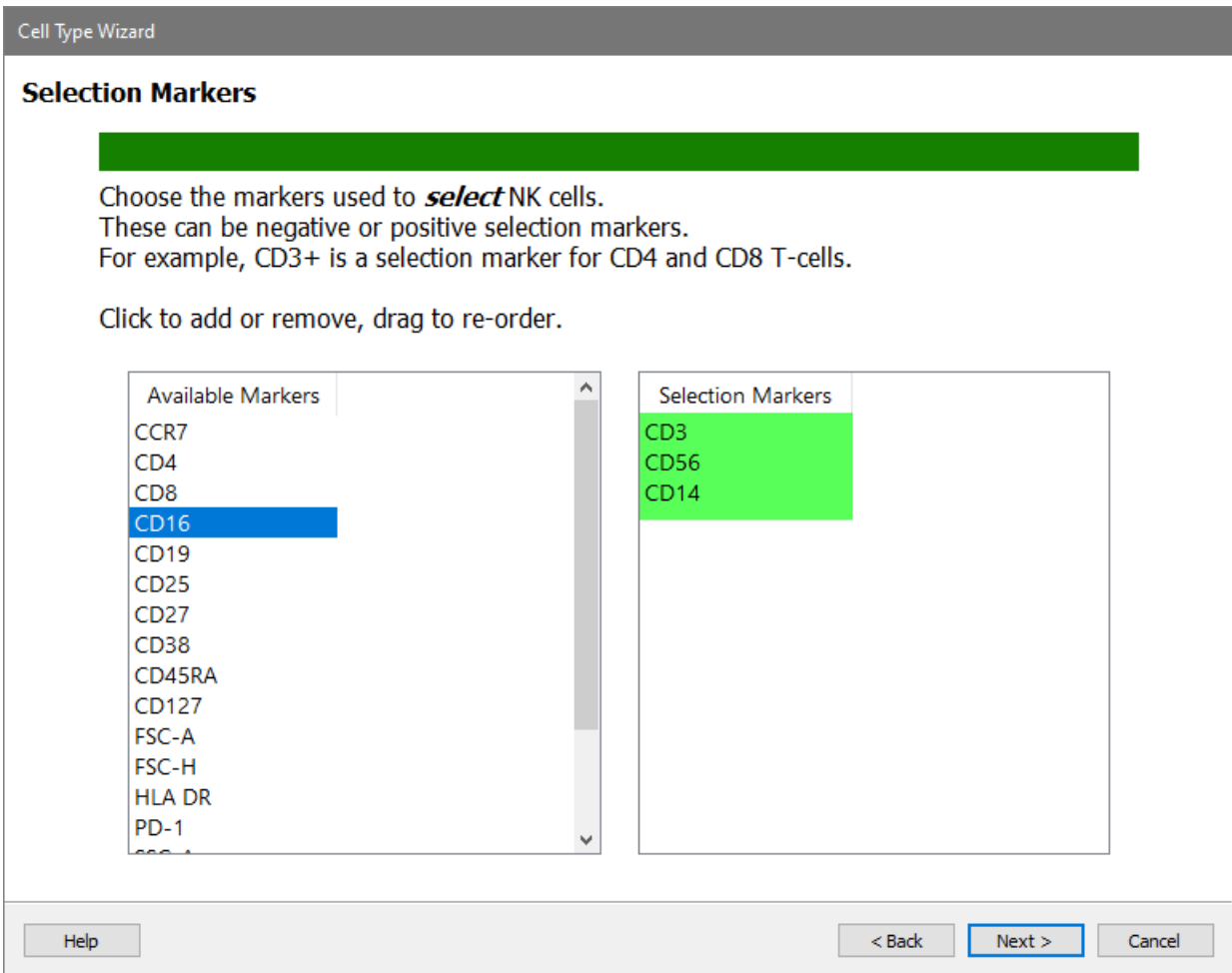
Description

Enable On

Color

Classification Type

Add three selection markers in order: **CD3**, **CD56**, and **CD14**.



Click **Next**.

This cell type will not have any staging or functional markers.
Skip Staging and Functional by clicking **Next** for each of these pages.

NK Selection Marker Expression Profiles

For CD3, choose **Dimmest Peak** and **Low Pass**.

Cell Type Wizard

Selection Profiles

Cell Type Events: 0

Define the expression of CD3 in the NK cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

CD3

Which Peak? **Dimmest Peak** ▾
 Distribution **Low Pass** ▾
 Phenotype CD3-
 Initial Intensity 1294.04
 Initial Width 2.20
 Width from Peak On
 Add to Report On

Disable Expression Profile

1 of 3

Marker Page Next Profile

Links... Preview

Help < Back Next > Cancel

Click **Next Profile**.

For CD56 we want the dim to bright events. The best way to capture them is with an Inverse Low Pass profile.

Choose **Dimmest Peak** and **Inverse Low Pass**.

Since the dim peak has a very small width, let's manually adjust the width so that we don't include the dimmest CD56 events.

Set the **Initial Width** to **5.0** and uncheck **Width from Peak**.

CD56

Which Peak?
Distribution
Phenotype
Initial Intensity
Initial Width
Width from Peak Off
Add to Report On

Preview

Previous Profile Next Profile

Disable Expression Profile

2 of 3

Enable the **Preview** checkbox to verify that the dim to bright events are selected.

Cell Type Events: 4919

Define the expression of CD56 in the NK cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

CD56

Which Peak?
Distribution
Phenotype
Initial Intensity
Initial Width
Width from Peak Off
Add to Report On

Preview

Previous Profile Next Profile

Disable Expression Profile

2 of 3

Click **Next Profile**.

For CD14, choose **Dimmest Peak** and **Low Pass**.ac

CD14

Which Peak? **Dimmest Peak** ▾

Distribution **Low Pass** ▾

Phenotype CD14-

Initial Intensity 714.12

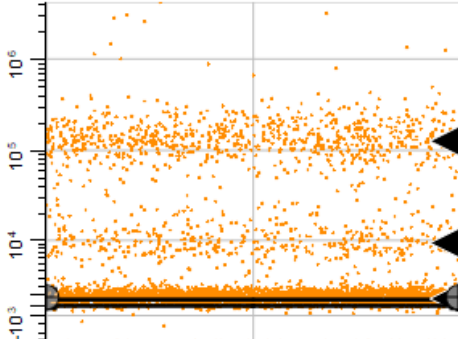
Initial Width 1.61

Width from Peak On

Add to Report On

Disable Expression Profile

Links... Preview



Previous Profile

Summary Page

3 of 3

Enable the **Preview** checkbox to verify the selection.

CD14

Which Peak? Dimmest Peak ▾

Distribution Low Pass ▾

Phenotype CD14-

Initial Intensity 714.12

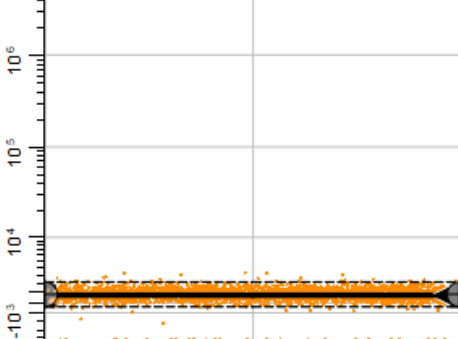
Initial Width 1.61

Width from Peak On

Add to Report On

Disable Expression Profile

Links... Preview



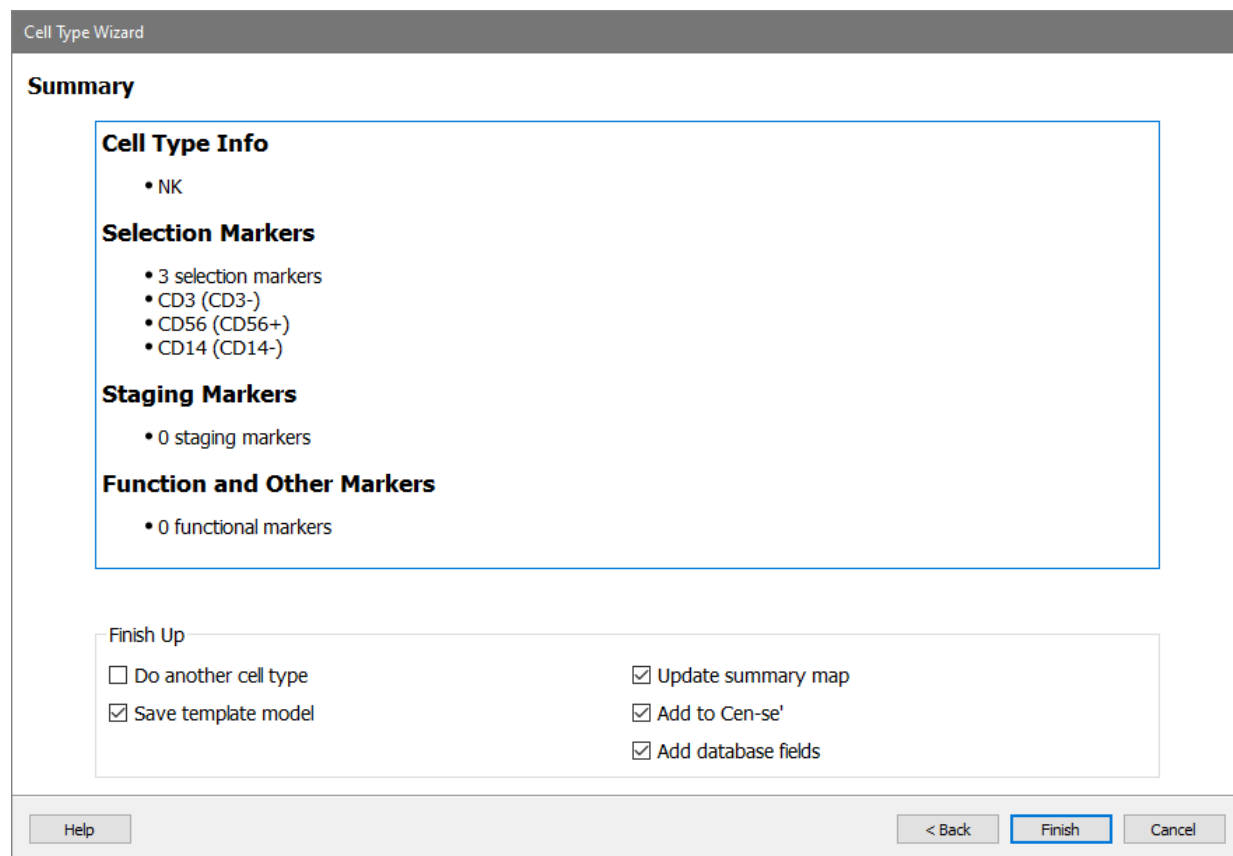
Previous Profile

Summary Page

3 of 3

Click **Summary Page**.

Review the cell type wizard summary and click **Finish**.



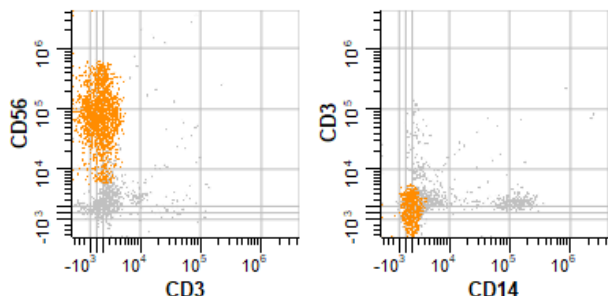
The report page for the NK cell type is very simple, with just the three selection markers.



File: 16-color_1.fcs

NK: CD3- CD56+ CD14-

Selection Markers



| | CT Total Med |
|------|--------------|
| CD3 | 631.28 |
| CD56 | 81410.43 |
| CD14 | 902.39 |

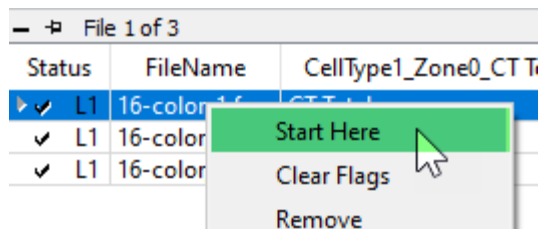
Staging Markers

| Stage | % CT | % T | Events |
|----------|------|------|--------|
| CT Total | 100 | 1.90 | 3664 |

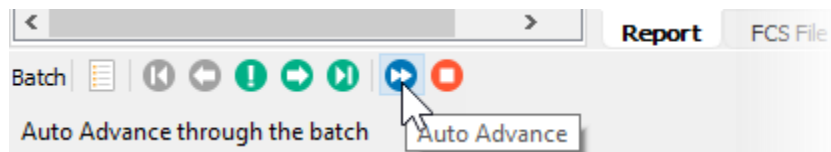
Test the Training Set with the Three Cell Type Model Template

The model template now has three cell types: CD4 T, CD8 T, and NK. Let's test it out by running the batch of FCS files in our training set. We'll review the reports for each of these to verify that all cell types are working properly.

Right-click the first file in the batch list and choose **Start Here**.

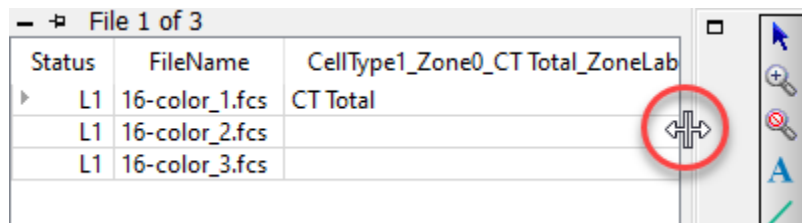


Click the [Auto Advance](#) button to automatically process all three files in the batch.

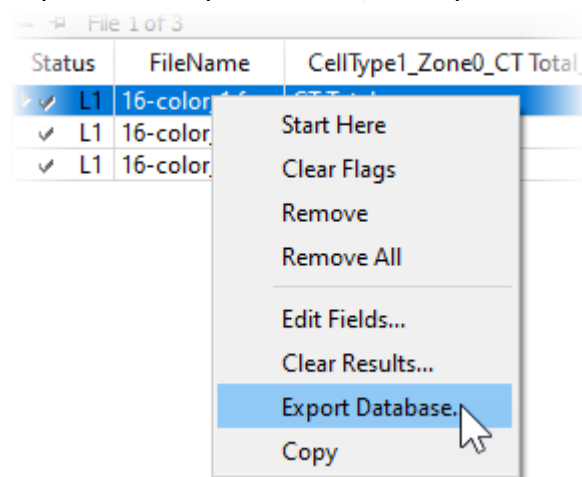


When the batch is finished, you can review the results by scrolling horizontally in the batch list, the part of the window where the files are listed. You can change the width of the batch

list by adjusting the right edge as shown below. Result for all three cell types will appear in the database.



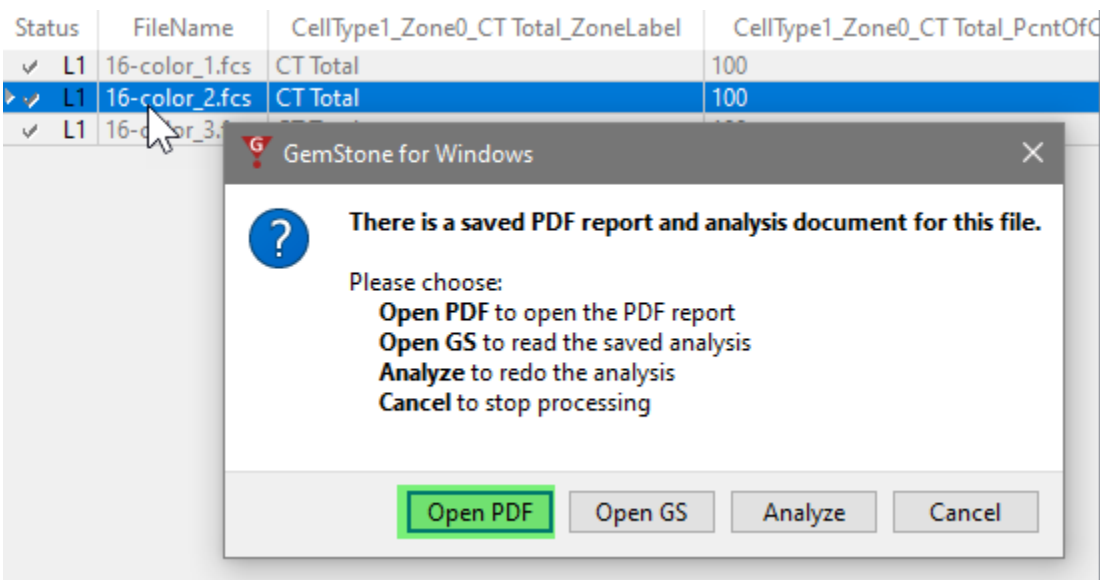
If you want to perform additional statistical analysis on the results in another program, you would right-click on the batch list and choose [Export Database](#). This option displays a file save dialog and allows you to save the database in tab-delimited format, which can be imported into spreadsheets, word processors, and lab information systems.



You can compare the report PDFs for each of the files. **Double-click** the file in the list. The program will ask what you want to do: open the PDF, open the saved analysis (Open GS), redo the analysis (Analyze), or cancel the dialog.

Choose **Open PDF** to view the report in PDF format. Note, at this point the first page in the report will be blank.

Repeat this for each of the files in the batch list.



Summary

In this tutorial you extended the model template by adding two more cell types: CD8 T and NK. You learned how to link expression profiles from one cell type to another. Linking profiles helps to speed up the processing and makes the model template more consistent and robust.

We introduced the use of Inverse Low Pass distributions for capturing the dim to bright distribution of the CD8 marker.

In the next tutorial you will learn how to analyze subsets in a cell type.

Develop: Analyzing Subsets

Overview

In this tutorial, you will learn how to extract information about subsets within a cell type. We use the term "subset" to refer to a group of cells within a cell type that have some common attributes, but they do not necessarily fall into a particular stage of development.

A good example of a subset is the Treg subset of CD4 T cells. These cells are positive for CD25, negative for CD127, and they can occur in any stage of CD4 T cells.

In this tutorial you will add to the Project we developed in the previous tutorials, [Starting a New Project from Scratch](#) and [Adding Cell Types to the Project](#). You will add a TriCOM object to quantify cells that are CD25+CD127- and add a table to the report to show Treg statistics.

Prerequisites

This tutorial continues to build the Project that was started in the following tutorials:

[Develop: Starting a New Project from Scratch](#)

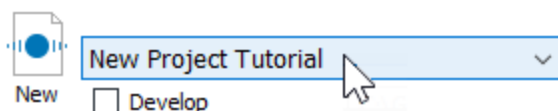
[Develop: Adding Cell Types to the Project](#)

Start GemStone

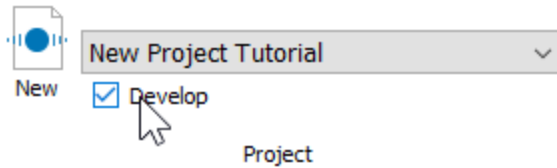
Start Gemstone by following the instructions in the [Installation and Setup of GemStone](#) section.

Open the Project

Open the "New Project Tutorial" Project that you created in the previous tutorials by selecting it in the Project dropdown list.

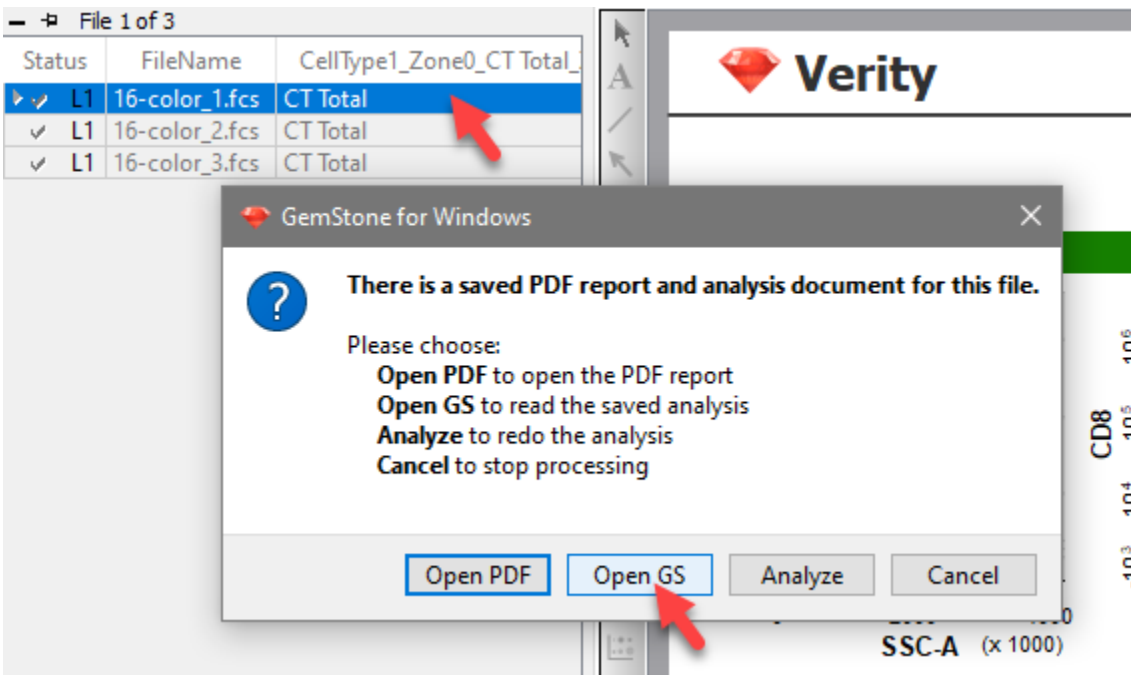


Click the **Develop** checkbox to enable develop mode.



Open a Saved Analysis Document

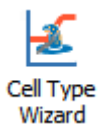
Double-click the first file in the batch list. A dialog of choices will be displayed. Click **Open GS** to open the saved analysis for the file.



Edit the Cell Type with the Cell Type Wizard

Next we need to make several edits with the Cell Type wizard. These edits will allow us to define the Treg subset of CD4 T cells.

Click the **Cell Type Wizard** button to initiate the wizard.



This time you will use the wizard to edit the CD4 T cell type.

Click the **Edit the selected cell type** option and select **CD4 T** from the list of choices and click **Next**.

Cell Type Wizard

Welcome to the Cell Type Wizard

What would you like to do?

Create a new cell type.

Duplicate the selected cell type. *

CD4 T Link selection markers

Edit the selected cell type. *

CD4 T

* Only cell types compatible with the Cell Type Wizard are available for duplicating and editing.

Disable other cell types while editing

Create a report page

Insert before None

Help Next > Cancel

We only need to edit the Functional markers, so we can skip past a number of pages in the wizard:

Click **Next** on the Cell Type Properties page.

Click **Next** on the Selection Markers page.

Click **Next** on the Staging Markers page.

Click **Next** on the Functional Markers page.

Most of the expression profiles do not need any edits:

Click **Next Profile** on the Profiles pages for **SSC-A, CD3, CD4, CD8, CCR7, CD45RA, and CD27**.

Set up the Functional Profiles

For CD25, set up the expression profile with the following:
Choose **Constant** for the shape and **Dimmest Peak** for the peak.

Cell Type Wizard

Functional Profiles

Cell Type Events: 17037

Define the expression of CD25 in the CD4 T cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

CD25

Expression Profile Shape: **Constant** (selected)

Which Peak?: **Dimmest Peak** (selected)

Phenotype: CD25

Initial Intensity: 1466.18

Initial Width: 7.24

Width from Peak: On

Preview

Links...

Previous Profile

Next Profile

Disable Expression Profile

8 of 10

Help < Back Next > Cancel

Enable the **Preview** checkbox to try out these settings.

Note the heavy black line above the expression profile. These identify the 95% confidence limits of the profile. Events that are above the top line have a low probability of being included in the profile. They are the events that will be considered "high" for CD25 as we define the Treg events.

If you wanted to include fewer events in the profile, you would make the Initial Width smaller and uncheck Width from Peak. Similarly, to include more events, you would make the Initial Width larger.

CD25

Expression Profile Shape: Constant
Which Peak?: Dimmest Peak
Phenotype: CD25
Initial Intensity: 1466.18
Initial Width: 7.24
Width from Peak: On

Links... Preview

Previous Profile  Next Profile

Disable Expression Profile

8 of 10

Click **Next Profile**.

For CD127, set up the expression profile with the following:

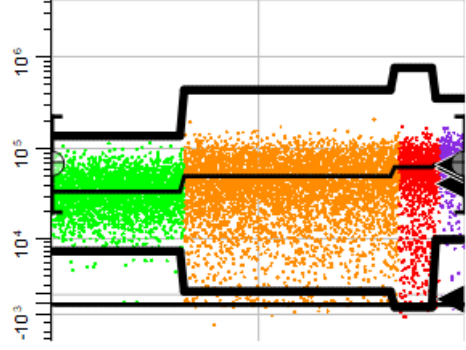
Choose **Constant** for the shape and **Brightest Peak** for the peak.

Set the **Initial Width** to 8.0 and uncheck the **Width from Peak** option.

CD127

Expression Profile Shape: Constant
Which Peak?: Brightest Peak
Phenotype: CD127
Initial Intensity: 66950.83
Initial Width: 8.00
Width from Peak: Off

Links... Preview

Previous Profile  Next Profile

Disable Expression Profile

9 of 10

Enable the **Preview** checkbox.

The Initial Width of 8 puts the lower side of the 95% confidence limit in a good position to separate positives and negatives. Events within the confidence boundaries will be considered "positive" for CD127, and those that fall below the lower boundary will be considered "negative" for CD127.

CD127

Expression Profile Shape: Constant

Which Peak?: Brightest Peak

Phenotype: CD127

Initial Intensity: 66950.83

Initial Width: 8.00

Width from Peak: Off

Disable Expression Profile

9 of 10

Click **Next Profile**.

Skip over the **HLA DR** profile for the time being. Click **Summary Page**.

Review the summary information. Make sure that **Save template model** and **Update summary map** options are checked.

Click **Finish** to close the wizard and save your changes.

Cell Type Wizard

Summary

Cell Type Info

- CD4 T

Selection Markers

- 4 selection markers
- SSC-A (SSC-A-)
- CD3 (CD3+)
- CD4 (CD4+)
- CD8 (CD8-)

Staging Markers

- 3 staging markers
- CCR7 (CCR7+>-)
- CD45RA (CD45RA+>-)
- CD27 (CD27+>-)

Finish Up

Do another cell type

Save template model

Update summary map

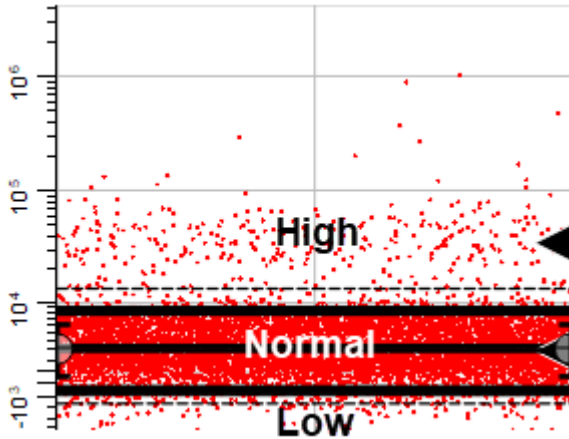
Add to Cen-se'

Add database fields

Help < Back Finish Cancel

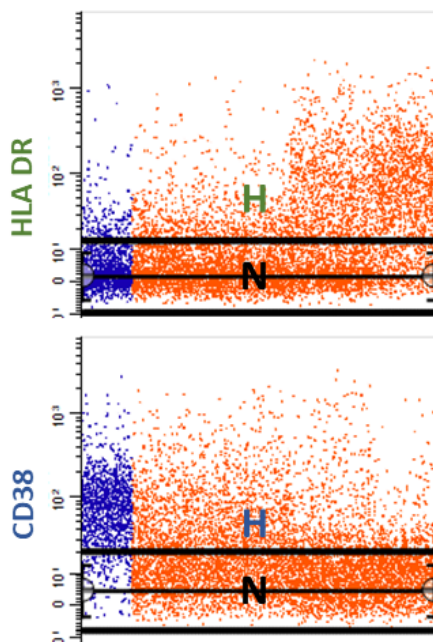
What is a TriCOM?

TriCOM is the tool that can identify subsets within a cell type. As its name implies, TriCOM can compute *three* states for the *combination* of measurements that we add to it. The three stats identify whether an event is within the 95% confidence limits of the expression profile (normal), below the lower confidence limit (low), or above the upper confidence limit (high).



In some cases, you may only be interested in whether the expression is high, as with CD25 in this tutorial. In other cases, only the low expression is of interest. TriCOMs can be set up for High-Only and Low-Only to accommodate these cases.

Here is an example that uses a High-Only TriCOM to quantify activated CD4 T cells. In the illustration, there are two expression profiles on the left, one for HLA DR and the other for CD38. These are activation markers for CD4 T cells. The plots show the high (H) and normal (N) expression levels. Also note that there are two stages: naive CD4 T cells are dark blue dots and memory cells are orange dots. A cartoon of a TriCOM is shown on the right. The bottom row of the TriCOM would show events that are in the normal 95% confidence limits, however this TriCOM is set up to show high events only. The next row up from the bottom quantifies events that are high for either HLA DR or CD38, but not both. For example, there are 2200 events that are high for HLA DR in the naive stage and 7000 events high for HLA DR in the memory stage. The top row contains events that are high for *both* HLA DR and CD38.

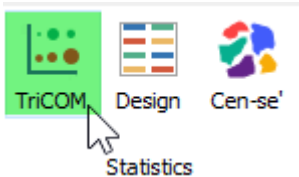


| | CD4 T naive | CD4 T memory |
|------------|-------------------|------------------|
| H H | 1800 | 1600 |
| H N N H | 2200 10000 | 1500 7000 |
| N N | | |

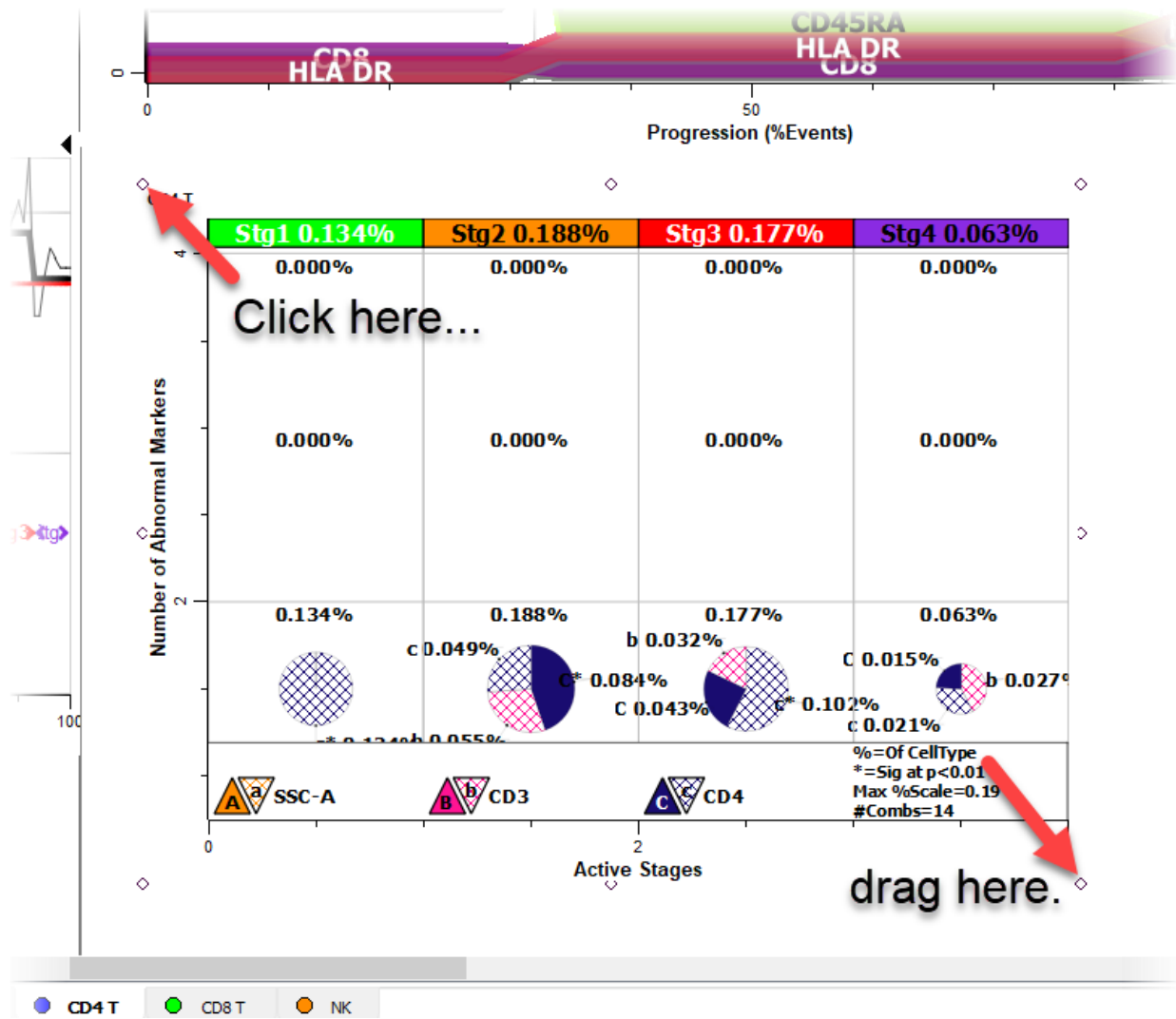
Let's create a TriCOM to see how it works.

Create a TriCOM

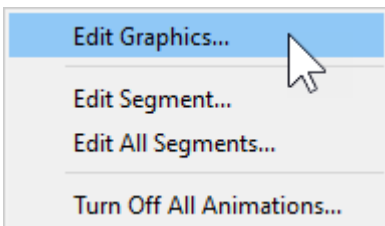
On the **Insert** tab of the ribbon, click the [TriCOM](#) button. There is also a TriCOM button on the cell type toolbar on the left edge of the Cell Type window.



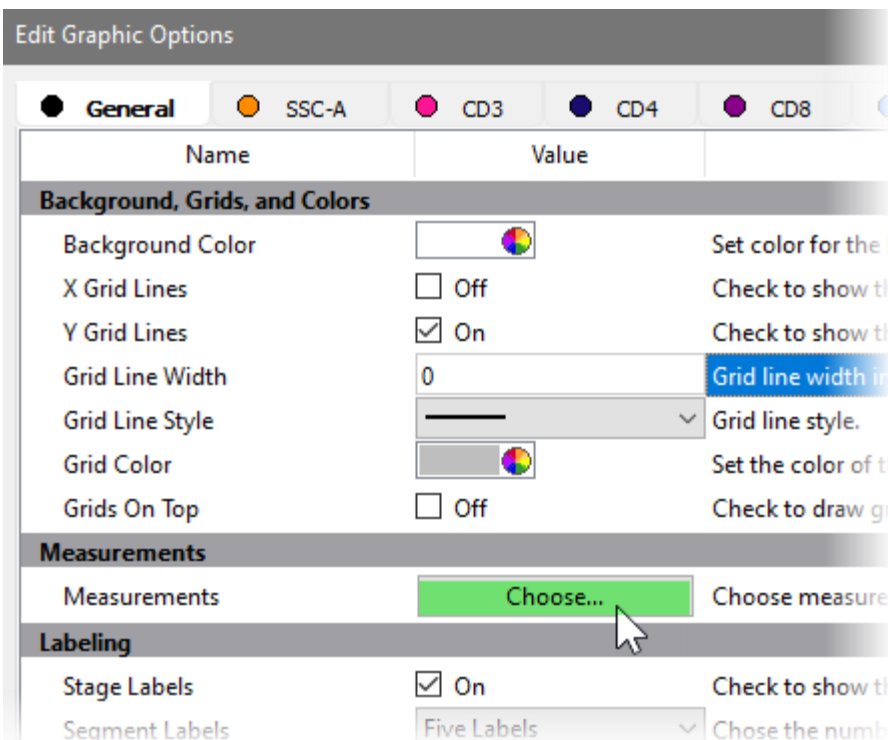
Scroll to find an empty place on the CD4 cell type, then **click and drag** to define the TriCOM location. A TriCOM with default measurements is created.



Right-click in the TriCOM and choose **Edit Graphics** from the context menu.



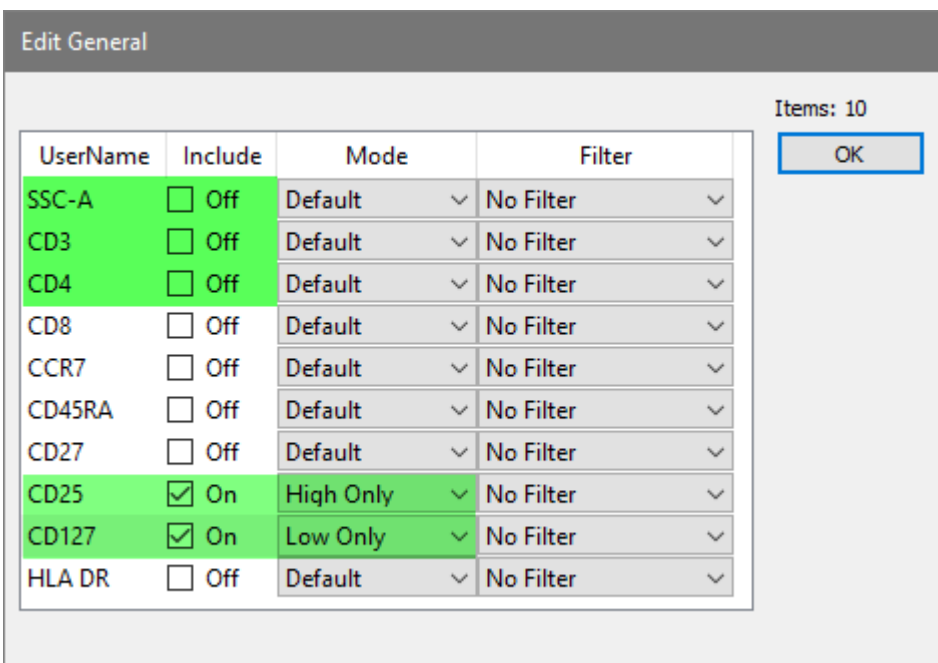
Find the **Measurements** section of the dialog and click the **Choose** button.



Uncheck the **SSC-A**, **CD3**, and **CD4** measurements Include property.

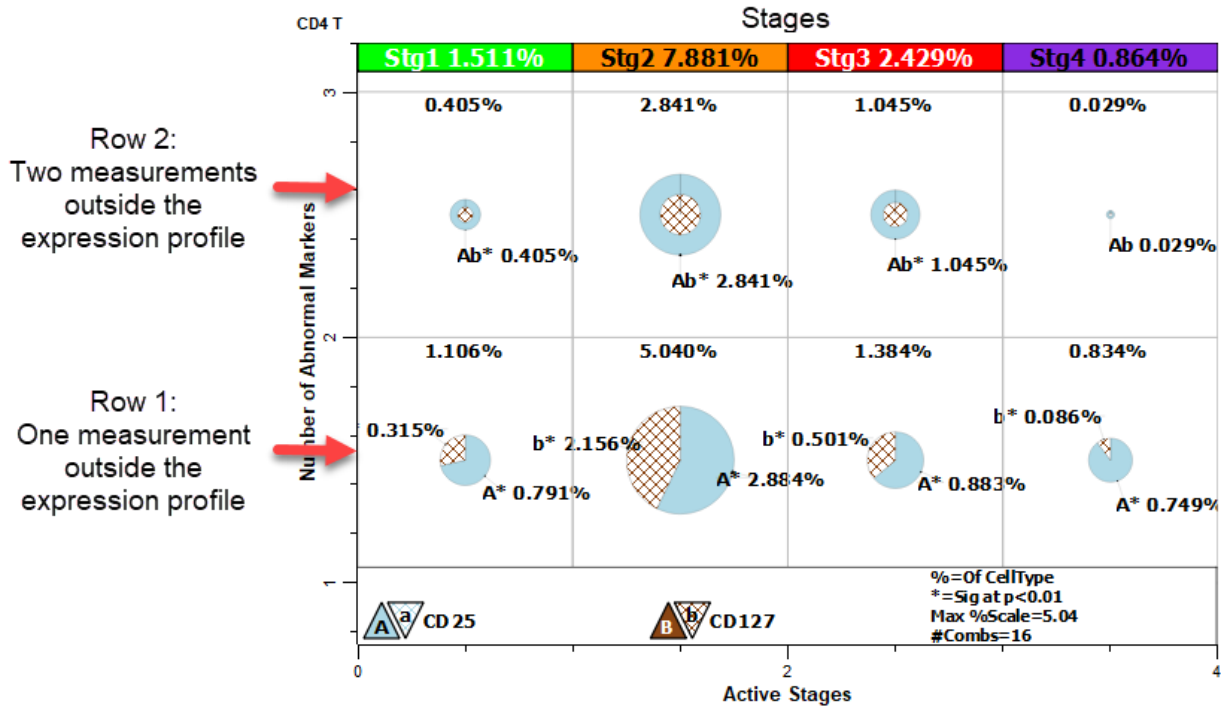
Check the **CD25** and **CD127** Include property.

Select **High Only** for **CD25** and select **Low Only** for **CD127**.



Click **OK**.

Click **OK** to close the Edit Graphic Options dialog.
 The TriCOM for CD25 and CD127 is displayed.



The TriCOM is divided into four vertical sections, one for each of the stages. The Y-axis shows the number of measurements that are outside the expression profiles. Each element in row one has *one* measurement outside the expected expression level, while row two has *two* measurements outside the expression profile. The key at the bottom indicates that **A** is CD25 and **B** is CD127. An uppercase letter indicates elevated expression, and a lowercase letter indicates low expression.

Notice the largest pie on the bottom row. All of the cells in this pie are in the **Stg2** stage of the progression. The **A** wedge represents cells that have elevated CD25 and normal, positive expression of CD127. The **b** wedge represents cells that have low expression of CD127 and normal, low expression of CD25.

Pies on the second row have the phenotype **Ab**, which corresponds to CD25+ CD127-. This is Treg phenotype. The size of the pies is proportional to percentage, so **Stg2** contains the largest fraction of the Tregs.

Naming Stages

Even though we will not rename the stages in this tutorial, it is useful to know that GemStone allows you to rename stages from the default stage names of Stg1, Stg2, etc. The [Stages](#) command on the Develop tab is used for this purpose. Some of the elements on reports need to be updated after stages are renamed, and the easiest way to update them is to use the Cell Type Wizard to edit the cell type and create a new report page.

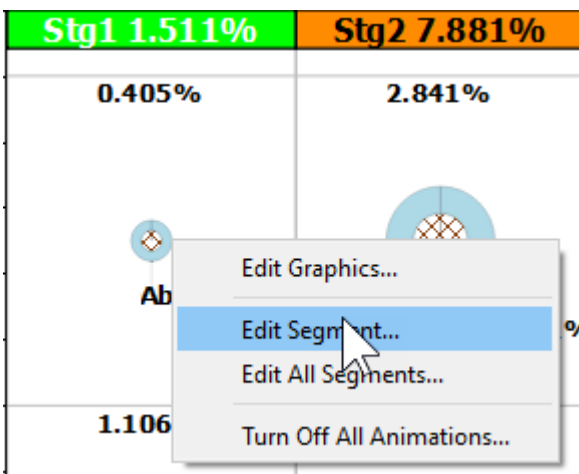
We will leave that for another tutorial.

Verify a TriCOM with Animation

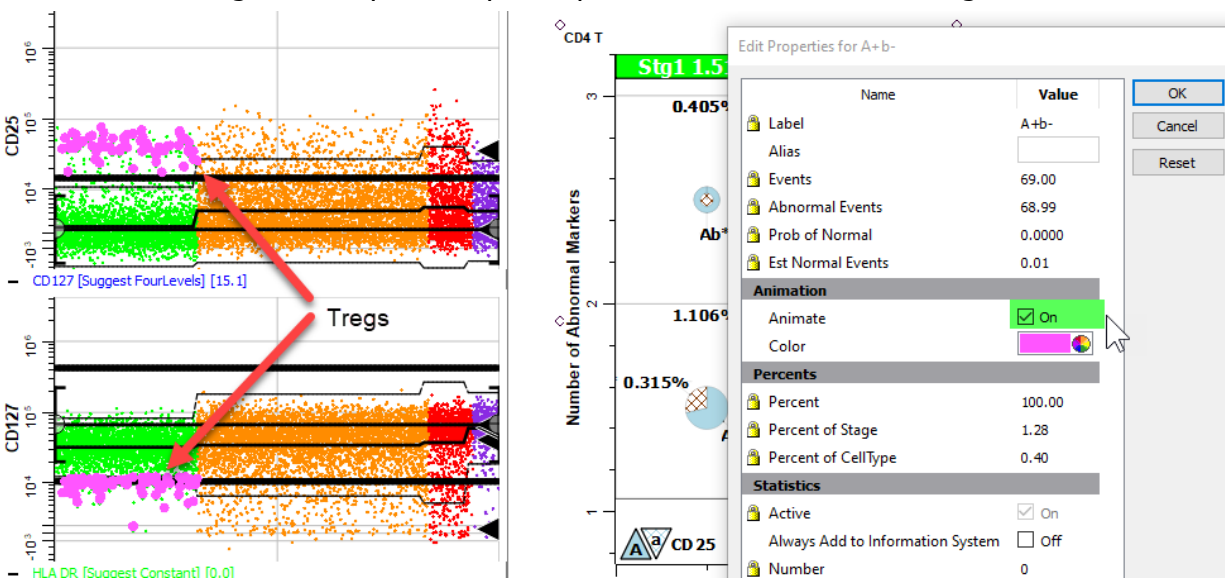
We can use animation to verify the Treg events. Animation will blink events in all of the plots for the pie segment that we select.

Right-click the pie for **Ab** events in the **Stg1** stage and choose **Edit Segment**.

Note: your statistics may differ from those shown in the images below.



In the Animation section, choose a bright color like pink and enable the **Animate** checkbox. Scroll the cell type window with this dialog open. Open the CD25 and CD127 profiles. Note the events blinking in the expression profile plots are CD25+ CD127- in Stg1.

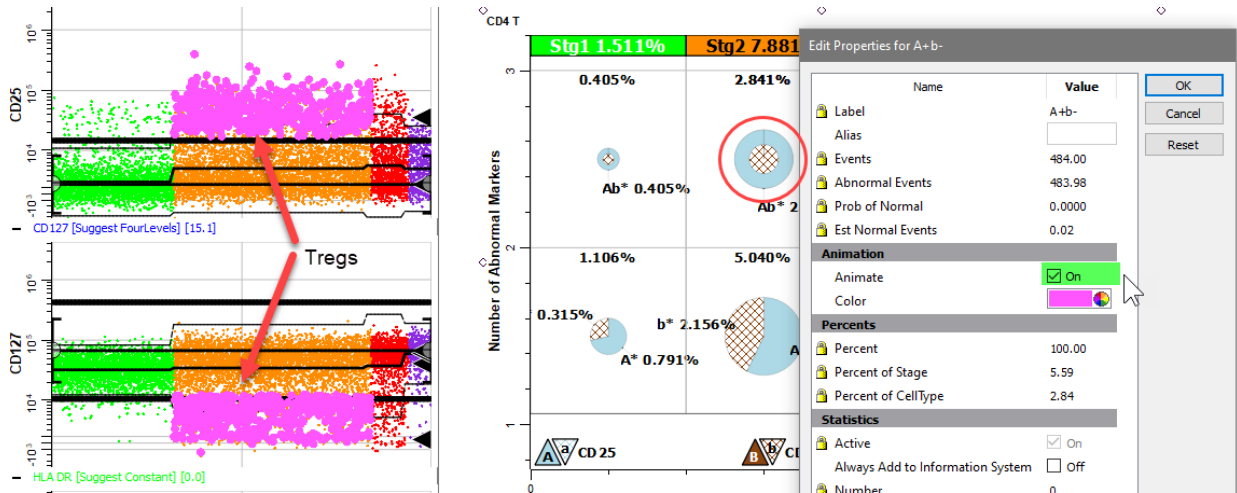


Uncheck the **Animate** checkbox and click **OK** to close the dialog.

Right-click the large **Ab** pie on the upper row in **Stg2** and choose **Edit Segment**.

Choose a bright color and enable the **Animate** checkbox.

Again the CD25+ CD127- events are blinking, this time in Stg2.



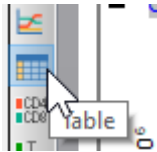
Animation makes it easy to verify the TriCOM segments.

Uncheck the **Animate** checkbox and click **OK** to close the dialog.

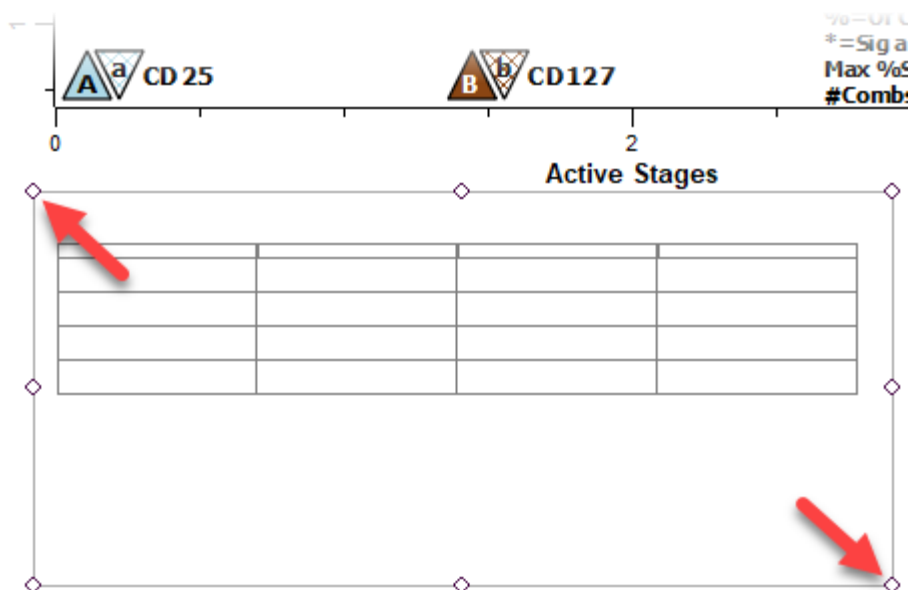
Create a Table

Let's create a table under the TriCOM to show the percentages for the Tregs in each stage, as well as the total percentage of Tregs.

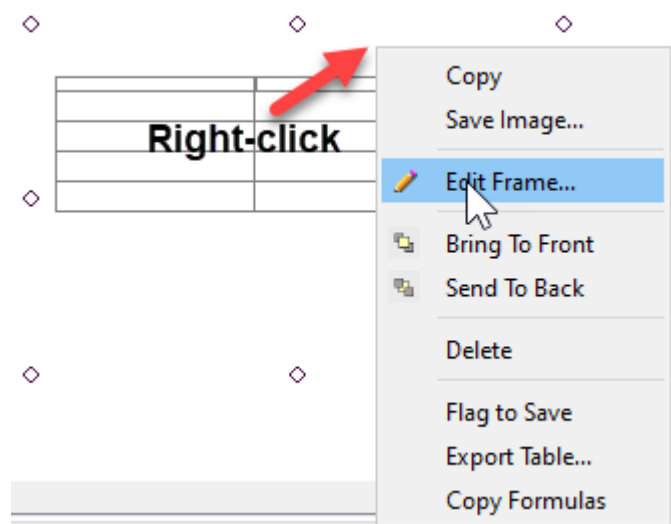
Click the **Table** tool on the Cell Type toolbar or on the Insert tab of the ribbon.



Below the TriCOM you just created, **click and drag** to define space for a table.

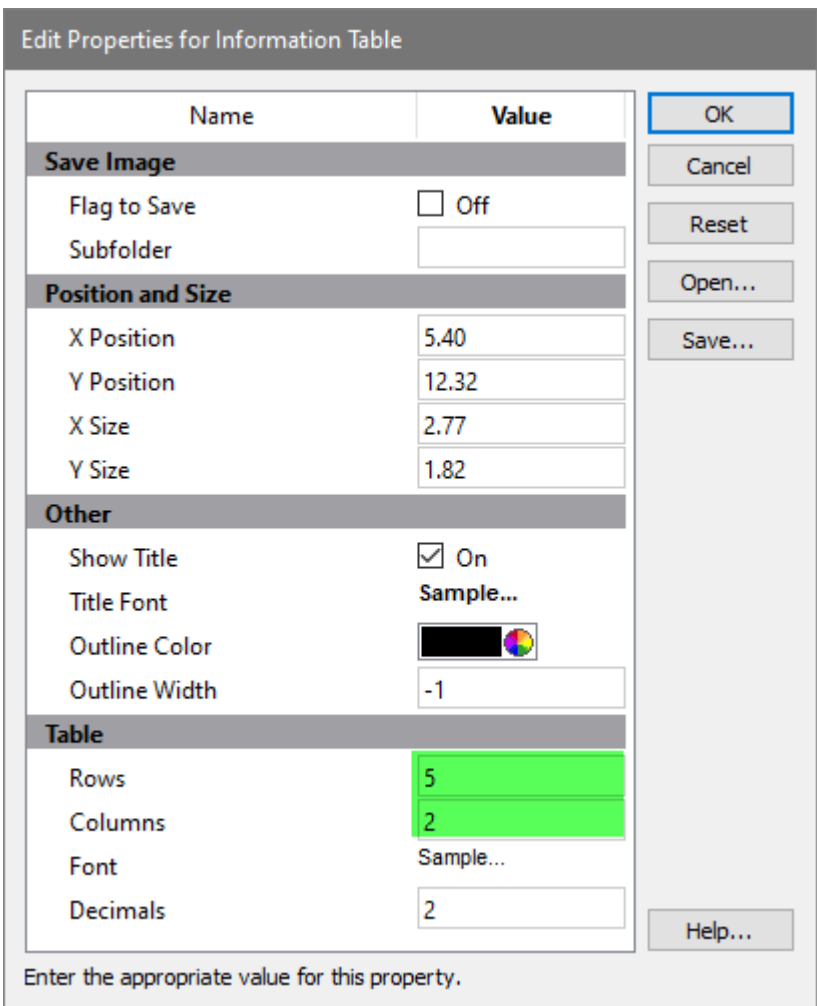


Right-click just above the table's grid and choose **Edit Frame** in the menu.



Edit the **Rows** to **5** and **Columns** to **2**.

Click **OK**.



In the first column, type the following text labels:

Naive Tregs, CM Tregs, EM Tregs, TE Tregs, and Total Tregs.

| | |
|-------------|--|
| | |
| Naive Tregs | |
| CM Tregs | |
| EM Tregs | |
| TE Tregs | |
| Total Tregs | |

Next you will add information fields that contain the percentages for the Treg subset.

Left-click the cell next to **Naive Tregs**, then right-click that cell.

| | |
|-------------|--|
| | |
| Naive Tregs | |
| CM Tregs | |
| EM Tregs | |
| TE Tregs | |
| Total Tregs | |

Right-click

The **Edit Selected Information Units** dialog is displayed. This dialog contains all of the information that GemStone has computed for this model template and FCS file, including the TriCOM information.

Note, you may need to resize this dialog and adjust column widths on some monitors to see the information presented below.

Scroll to the section labelled **CellType1_TriCOMGraph** and click the arrow next to the label to open the section.

Choose one or more information units to display.
If you choose more than one, the info keywords will be put in the rows directly under the one you are editing.

Select Items Below

| Name | Value |
|---|----------------------|
| Other Events | Other Events |
| CellType1_TriCOMGraph | |
| CellType1_TriCOMGraph_ParameterKey | A,a: CD25 B,b: CD127 |
| CellType1_TriCOMGraph_FilterKey | |
| CellType1_TriCOMGraph_CellTypeEvents | 17037 |
| CellType1_TriCOMGraph_CT Total_Abnormal... | 2160.98 |
| CellType1_TriCOMGraph_CT Total_NormalEv... | 14876.02 |
| CellType1_TriCOMGraph_CT Total_Degree0... | 14830 |
| CellType1_TriCOMGraph_CT Total_Degree0... | 14876.02 |
| CellType1_TriCOMGraph_CT Total_Degree0... | 87.32 |
| CellType1_TriCOMGraph_CT Total_Degree1... | 1471 |
| CellType1_TriCOMGraph_CT Total_Degree1... | 1425.02 |
| CellType1_TriCOMGraph_CT Total_Degree1... | 8.36 |
| CellType1_TriCOMGraph_CT Total_c-_Events | 75 |
| CellType1_TriCOMGraph_CT Total_c-_Adjust... | 52.10 |
| CellType1_TriCOMGraph_CT Total_c-_Percen... | 0.31 |

| Name | Description | SectionName |
|------|-------------|-------------|
|------|-------------|-------------|

Items: 0

Buttons: Insert, Add, Replace, Delete, Up, Down, OK, Delete All

Select the item named **CellType1_TriCOMGraph_Stg1_A+b-_PercentCellType** and click the **Add** button. The statistic is added to the list on the left of the dialog.

GemStone User Guide

Edit Selected Information Units

Choose one or more information units to display.
If you choose more than one, the info keywords will be put in the rows directly under the one you are editing.

Select Items Below

| Name | Value |
|--|-------|
| CellType1_TriCOMGraph__Stg1_a-b-_AdjustedEvents | 0 |
| CellType1_TriCOMGraph__Stg1_a-b-_PercentZone | 0 |
| CellType1_TriCOMGraph__Stg1_a-b-_PercentCellType | 0 |
| CellType1_TriCOMGraph__Stg1_A+b-_Events | 69 |
| CellType1_TriCOMGraph__Stg1_A+b-_AdjustedEvents | 68.99 |
| CellType1_TriCOMGraph__Stg1_A+b-_PercentZone | 1.28 |
| CellType1_TriCOMGraph__Stg1_A+b-_PercentCellType | 0.40 |
| CellType1_TriCOMGraph__Stg1_a-B+_Events | 0 |
| CellType1_TriCOMGraph__Stg1_a-B+_AdjustedEvents | 0 |
| CellType1_TriCOMGraph__Stg1_a-B+_PercentZone | 0 |
| CellType1_TriCOMGraph__Stg1_a-B+_PercentCellType | 0 |
| CellType1_TriCOMGraph__Stg1_A+B+_Events | 0 |
| CellType1_TriCOMGraph__Stg1_A+B+_AdjustedEvents | 0 |
| CellType1_TriCOMGraph__Stg1_A+B+_PercentZone | 0 |
| CellType1_TriCOMGraph__Stg1_A+B+_PercentCellType | 0 |
| CellType1_TriCOMGraph__Stg1_a-c-_Events | 0 |
| CellType1_TriCOMGraph__Stg1_a-c-_AdjustedEvents | 0 |

Items: 1

| Name | Value |
|--|-------|
| CellType1_TriCOMGraph__Stg1_A+b-_PercentCellType | Perce |

OK
Delete All

Replace Delete Up Down

Insert Add

Scroll down in the list and select **CellType1_TriCOMGraph__Stg2_A+b-_PercentCellType**. Click **Add** to add it to the list on the left.

Edit Selected Information Units

Choose one or more information units to display.
If you choose more than one, the info keywords will be put in the rows directly under the one you are editing.

Select Items Below

| Name | Value |
|--|--------|
| CellType1_TriCOMGraph__Stg2_a-b-_AdjustedEvents | 0 |
| CellType1_TriCOMGraph__Stg2_a-b-_PercentZone | 0 |
| CellType1_TriCOMGraph__Stg2_a-b-_PercentCellType | 0 |
| CellType1_TriCOMGraph__Stg2_A+b-_Events | 484 |
| CellType1_TriCOMGraph__Stg2_A+b-_AdjustedEvents | 483.98 |
| CellType1_TriCOMGraph__Stg2_A+b-_PercentZone | 5.59 |
| CellType1_TriCOMGraph__Stg2_A+b-_PercentCellType | 2.84 |
| CellType1_TriCOMGraph__Stg2_a-B+_Events | 0 |
| CellType1_TriCOMGraph__Stg2_a-B+_AdjustedEvents | 0 |
| CellType1_TriCOMGraph__Stg2_a-B+_PercentZone | 0 |
| CellType1_TriCOMGraph__Stg2_a-B+_PercentCellType | 0 |
| CellType1_TriCOMGraph__Stg2_A+B+_Events | 0 |
| CellType1_TriCOMGraph__Stg2_A+B+_AdjustedEvents | 0 |
| CellType1_TriCOMGraph__Stg2_A+B+_PercentZone | 0 |
| CellType1_TriCOMGraph__Stg2_A+B+_PercentCellType | 0 |
| CellType1_TriCOMGraph__Stg2_a-c-_Events | 0 |
| CellType1_TriCOMGraph__Stg2_a-c-_AdjustedEvents | 0 |

Items: 2

| Name | Value |
|--|-------|
| CellType1_TriCOMGraph__Stg1_A+b-_PercentCellType | Perce |
| CellType1_TriCOMGraph__Stg2_A+b-_PercentCellType | Perce |

OK
Delete All

Replace Delete Up Down

Insert Add

Next select **CellType1_TriCOMGraph__Stg3_A+b-_PercentCellType** and click **Add**.

Edit Selected Information Units

Choose one or more information units to display.
If you choose more than one, the info keywords will be put in the rows directly under the one you are editing.

Select Items Below

| Name | Value |
|---|--------|
| CellType1_TriCOMGraph_Stg3_Degree2_PcntOfZone | 9.20 |
| CellType1_TriCOMGraph_Stg3_Degree2_PcntOfCellType | 1.04 |
| CellType1_TriCOMGraph_Stg3_a-b-Events | 0 |
| CellType1_TriCOMGraph_Stg3_a-b-AdjustedEvents | 0 |
| CellType1_TriCOMGraph_Stg3_a-b-PercentZone | 0 |
| CellType1_TriCOMGraph_Stg3_a-b-PercentCellType | 0 |
| CellType1_TriCOMGraph_Stg3_A+b-Events | 178 |
| CellType1_TriCOMGraph_Stg3_A+b-AdjustedEvents | 178.00 |
| CellType1_TriCOMGraph_Stg3_A+b-PercentZone | 9.20 |
| CellType1_TriCOMGraph_Stg3_A+b-PercentCellType | 1.04 |
| CellType1_TriCOMGraph_Stg3_a-B+_Events | 0 |
| CellType1_TriCOMGraph_Stg3_a-B+_AdjustedEvents | 0 |
| CellType1_TriCOMGraph_Stg3_a-B+_PercentZone | 0 |
| CellType1_TriCOMGraph_Stg3_a-B+_PercentCellType | 0 |
| CellType1_TriCOMGraph_Stg3_A+B+_Events | 0 |
| CellType1_TriCOMGraph_Stg3_A+B+_AdjustedEvents | 0 |
| CellType1_TriCOMGraph_Stg3_A+B+_PercentZone | 0 |

Items: 3

| Name | Value |
|--|---------|
| CellType1_TriCOMGraph_Stg1_A+b-PercentCellType | Percent |
| CellType1_TriCOMGraph_Stg2_A+b-PercentCellType | Percent |
| CellType1_TriCOMGraph_Stg3_A+b-PercentCellType | Percent |

OK Delete All

Replace Delete Up Down

Insert Add

Select **CellType1_TriCOMGraph_Stg4_A+b-PercentCellType** and click **Add**.

Edit Selected Information Units

Choose one or more information units to display.
If you choose more than one, the info keywords will be put in the rows directly under the one you are editing.

Select Items Below

| Name | Value |
|---|-------|
| CellType1_TriCOMGraph_Stg4_Degree2_Events | 5 |
| CellType1_TriCOMGraph_Stg4_Degree2_AdjustedEvents | 5.00 |
| CellType1_TriCOMGraph_Stg4_Degree2_PcntOfZone | 0.48 |
| CellType1_TriCOMGraph_Stg4_Degree2_PcntOfCellType | 0.03 |
| CellType1_TriCOMGraph_Stg4_a-b-Events | 0 |
| CellType1_TriCOMGraph_Stg4_a-b-AdjustedEvents | 0 |
| CellType1_TriCOMGraph_Stg4_a-b-PercentZone | 0 |
| CellType1_TriCOMGraph_Stg4_a-b-PercentCellType | 0 |
| CellType1_TriCOMGraph_Stg4_A+b-Events | 5 |
| CellType1_TriCOMGraph_Stg4_A+b-AdjustedEvents | 5.00 |
| CellType1_TriCOMGraph_Stg4_A+b-PercentZone | 0.48 |
| CellType1_TriCOMGraph_Stg4_A+b-PercentCellType | 0.03 |
| CellType1_TriCOMGraph_Stg4_a-B+_Events | 0 |
| CellType1_TriCOMGraph_Stg4_a-B+_AdjustedEvents | 0 |
| CellType1_TriCOMGraph_Stg4_a-B+_PercentZone | 0 |
| CellType1_TriCOMGraph_Stg4_a-B+_PercentCellType | 0 |
| CellType1_TriCOMGraph_Stg4_A+B+_Events | 0 |

Items: 4

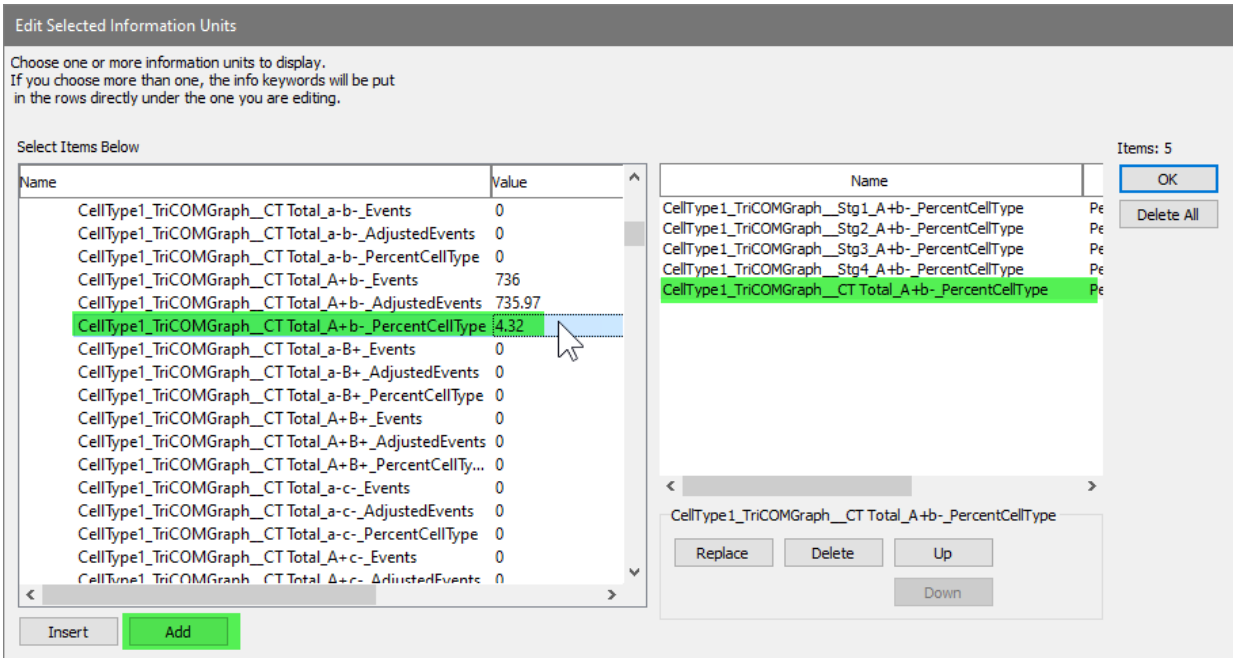
| Name | Value |
|--|---------|
| CellType1_TriCOMGraph_Stg1_A+b-PercentCellType | Percent |
| CellType1_TriCOMGraph_Stg2_A+b-PercentCellType | Percent |
| CellType1_TriCOMGraph_Stg3_A+b-PercentCellType | Percent |
| CellType1_TriCOMGraph_Stg4_A+b-PercentCellType | Percent |

OK Delete All

Replace Delete Up Down

Insert Add

Scroll back up to the CT total section and add the **CellType1_TriCOMGraph_CCTotal_A+b-PercentCellType**.



With these five statistics added to the list on the left, click **OK**.

The fields we picked fill down from the selected cell, and the five results are added to the table. You may need to tab out of the first cell to see the value.

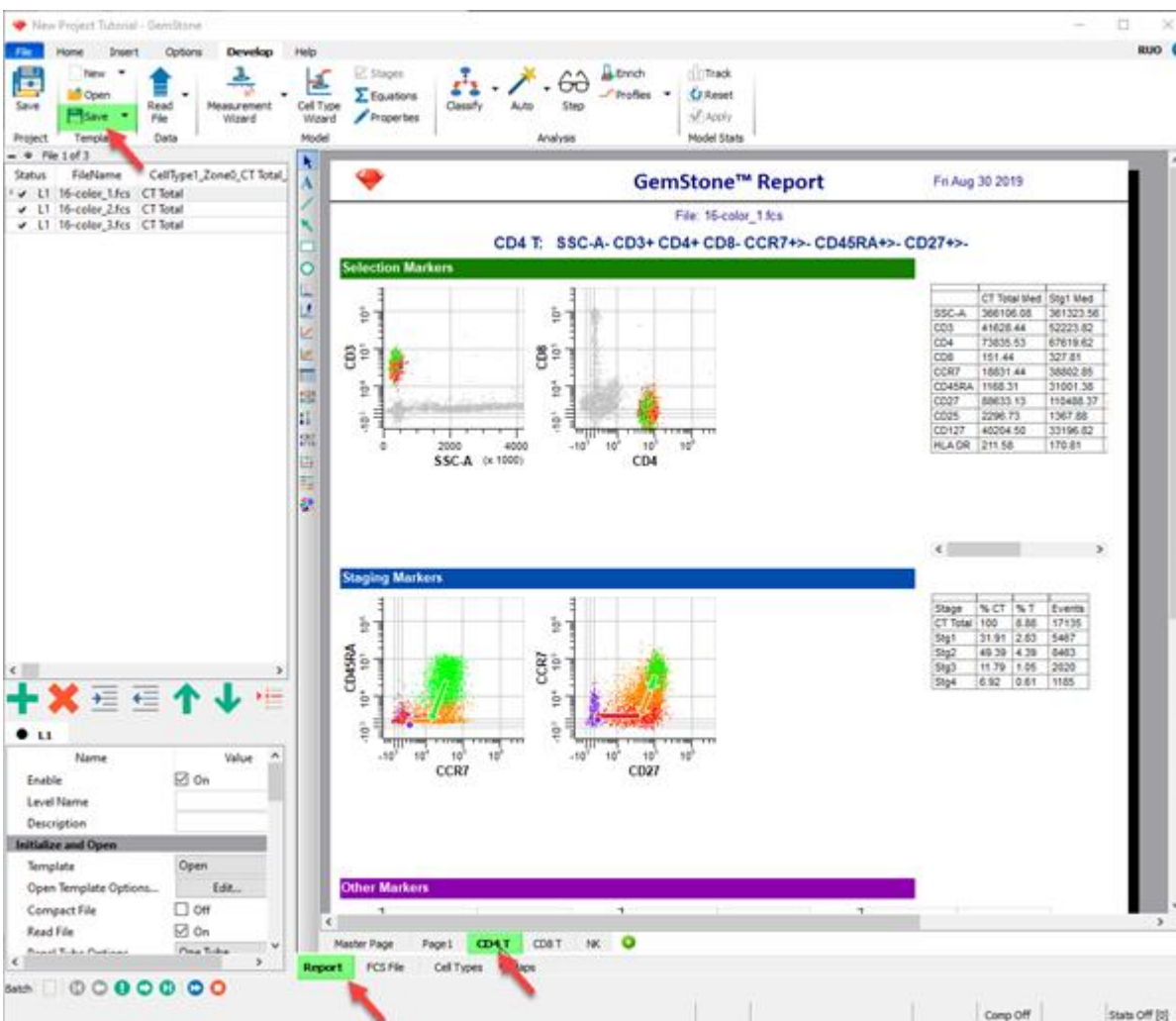
| | |
|-------------|------|
| Naive Tregs | 0.40 |
| CM Tregs | 2.84 |
| EM Tregs | 1.04 |
| TE Tregs | 0.03 |
| Total Tregs | 4.32 |

You can create tables on a report page using the same technique.

Save your Work

At this point you have a TriCOM for Tregs in the CD4 T cell type and a table to display the results. This is the approach used to quantify subsets within a cell type.

Before saving, switch back to the Report tab, display the CD4 T report page, and scroll it to the position you want to appear when this template is used. The template will remember this view when it is saved. Click **Save** in the Template section of the ribbon.



Summary

This tutorial introduced the TriCOM object, which you used to identify the Tregs subset of CD4 T cells. TriCOMs are associated with functional markers and identify elevated and diminished levels of expression. The Tregs TriCOM quantified high CD25 and low CD127 expression.

You animated segments of the TriCOM to see where the associated events appear in other plots. This technique was used to verify that the TriCOM was working correctly.

Once the TriCOM was created you created a table, added some text labels to it, and then selected the results to display in the table.

The next tutorial introduces the Cen-se' plot and how it is used to explore your data.

Develop: Exploring with Cen-se'

Overview

GemStone has two independent analysis systems. In the previous tutorials, you have worked with the Probability State Modeling (PSM) system to analyze the FCS files. Cen-se' is the second system, which can make use of PSM information to color and label cell type information.

Cen-se' is a fantastic tool for exploring your data. It statistically determines related events based on the measurements we select, and presents them in a bivariate plot. With Cen-se', we can assess how well our model is identifying cells in our cell types. We can also explore cells that do not yet belong to a cell type to find out what measurements identify them.

In this tutorial we extend the Project developed in the previous tutorials, [Starting a New Project from Scratch](#), [Adding Cell Types to the Project](#), and [Analyzing Subsets](#). We add a Cen-se' plot to a report page and used to explore our data.

Prerequisites

This tutorial continues to build the Project that was started in the following tutorials:

[Develop: Starting a New Project from Scratch](#)

[Develop: Adding Cell Types to the Project](#)

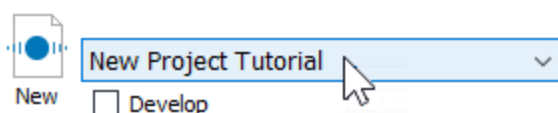
[Develop: Analyzing Subsets](#)

Start GemStone

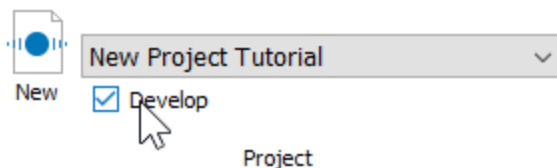
Start Gemstone by following the instructions in the [Installation and Setup of GemStone](#) section.

Open the Project

Open the "New Project Tutorial" Project that you created in the previous tutorials by selecting it in the Project dropdown list.

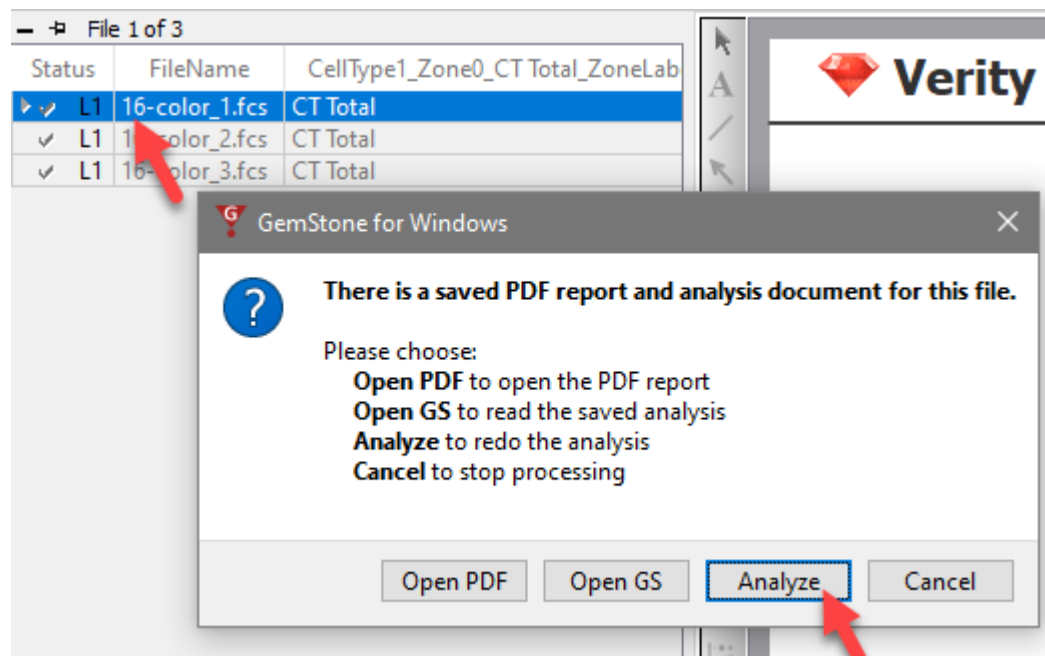


Click the **Develop** checkbox to enable develop mode.



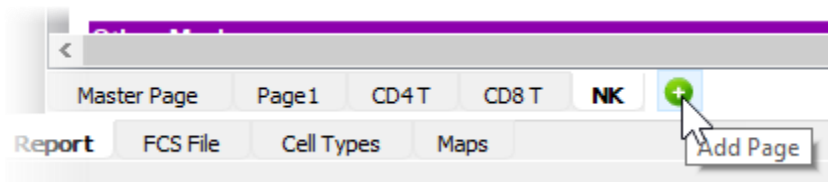
Analyze a File

Double-click the first file in the batch list. A dialog of choices will be displayed. Click **Analyze** to reanalyze the file with the latest model template revisions.

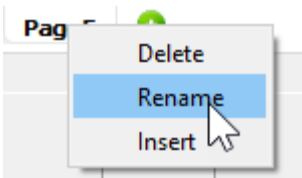


Adding a Cen-se' Page and Plot

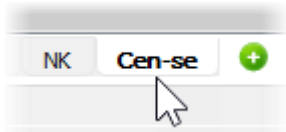
Click the **Add Page** button on the Report tab next to the NK cell type. This will create a new page for the report.



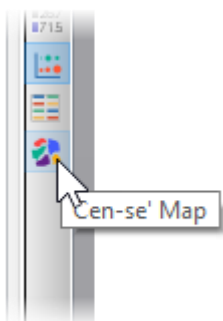
Right-click the new page and choose **Rename**.



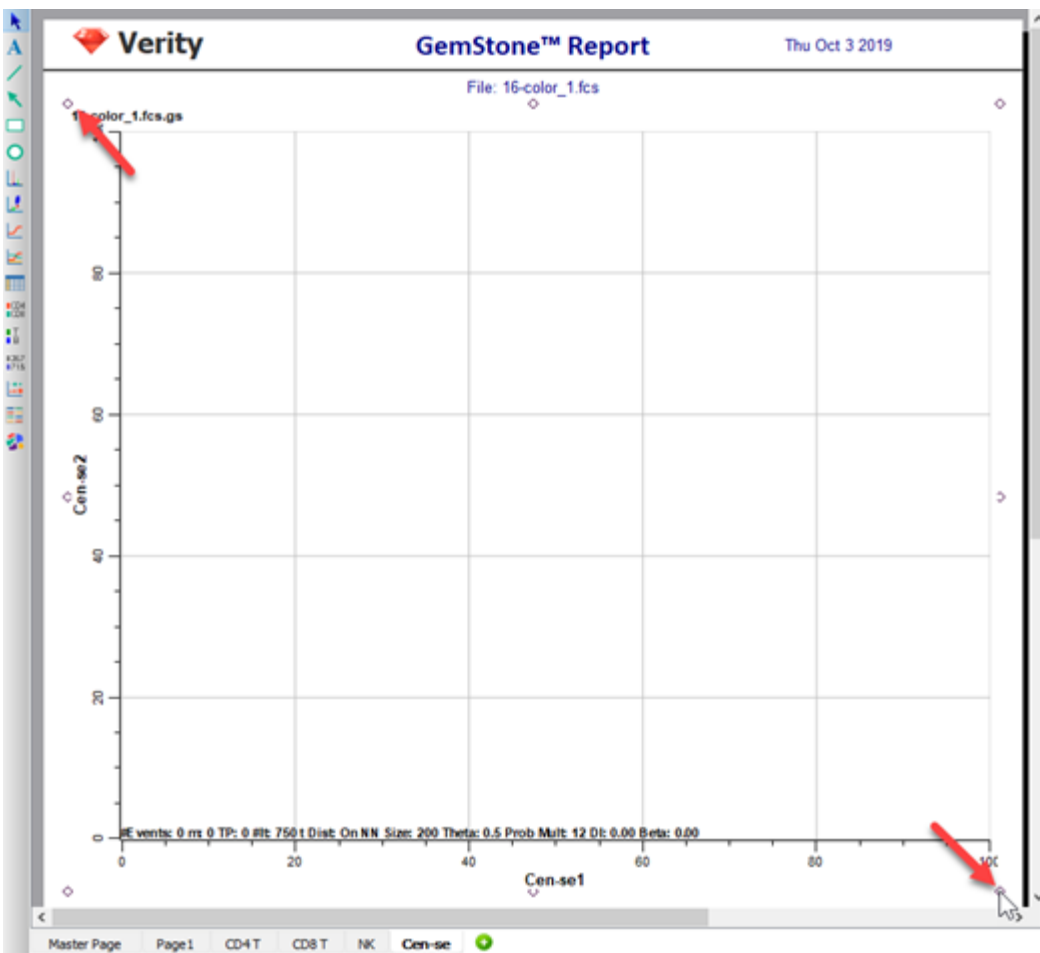
Edit the name to **Cen-se** and press Enter.



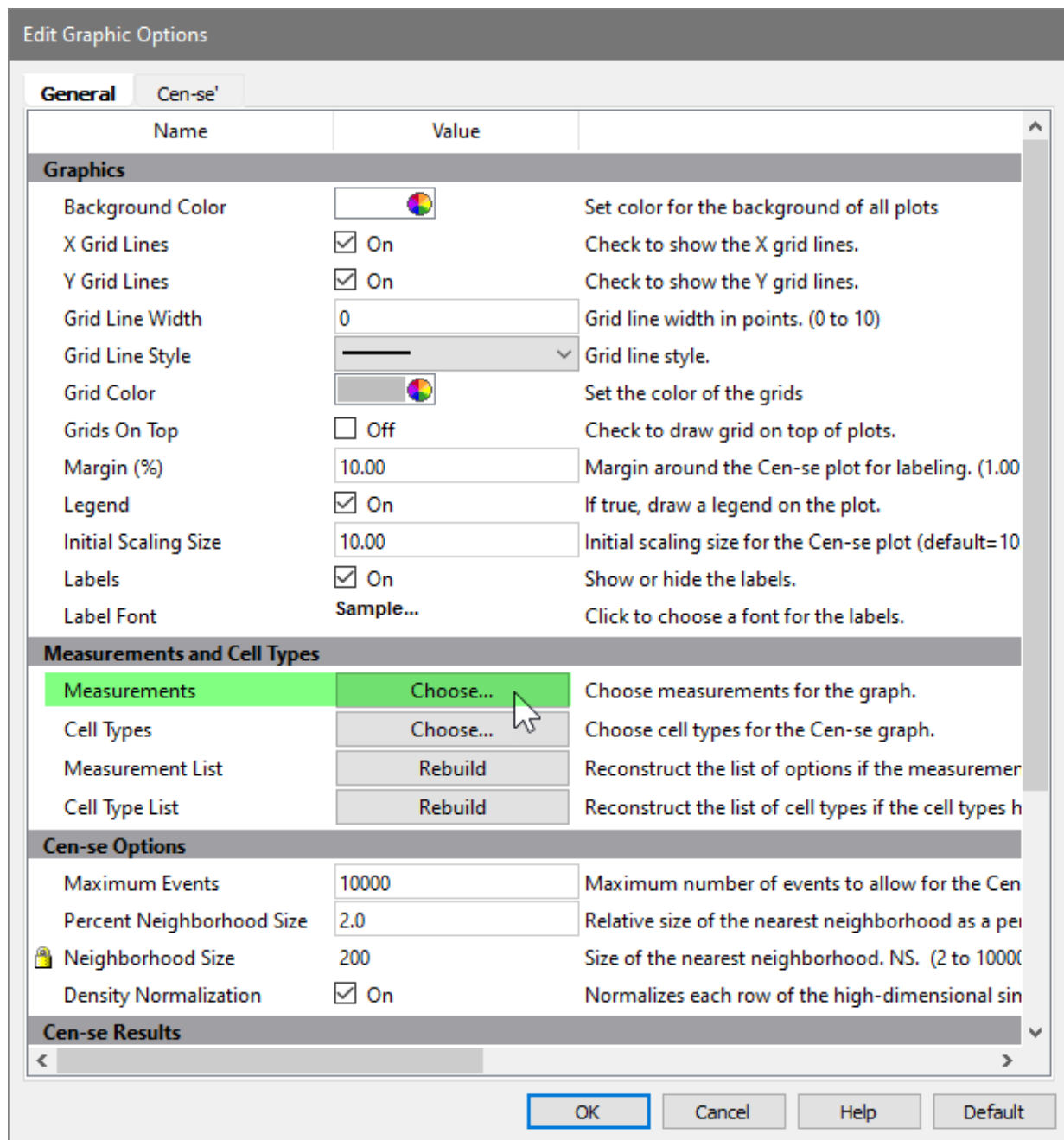
Select the [Cen-se'](#) tool on the report toolbar or from the Insert tab of the ribbon.



Click and drag to define space for a Cen-se' plot on the report page.



Double-click in the new Cen-se plot to display the **Edit Graphics Options** dialog. Click the **Choose** button for **Measurements**.



In the Choose Measurements dialog, enable all of the measurements *except* for **FSC-H**, **SSC-H**, and **Time**. We exclude these because FSC-A and SSC-A provide more information for the Cen-se, and Time does not help the Cen-se differentiate cell types.

Click **OK**.

Choose Measurements

Choose the measurements you want to include in the Cen-se' plot.

Items: 21

| Names | Include |
|--------|--|
| CCR7 | <input checked="" type="checkbox"/> On |
| CD3 | <input checked="" type="checkbox"/> On |
| CD4 | <input checked="" type="checkbox"/> On |
| CD8 | <input checked="" type="checkbox"/> On |
| CD14 | <input checked="" type="checkbox"/> On |
| CD16 | <input checked="" type="checkbox"/> On |
| CD19 | <input checked="" type="checkbox"/> On |
| CD25 | <input checked="" type="checkbox"/> On |
| CD27 | <input checked="" type="checkbox"/> On |
| CD38 | <input checked="" type="checkbox"/> On |
| CD45RA | <input checked="" type="checkbox"/> On |
| CD56 | <input checked="" type="checkbox"/> On |
| CD127 | <input checked="" type="checkbox"/> On |
| FSC-A | <input checked="" type="checkbox"/> On |
| FSC-H | <input type="checkbox"/> Off |
| HLA DR | <input checked="" type="checkbox"/> On |
| PD-1 | <input checked="" type="checkbox"/> On |
| SSC-A | <input checked="" type="checkbox"/> On |
| SSC-H | <input type="checkbox"/> Off |
| TCRgd | <input checked="" type="checkbox"/> On |
| Time | <input type="checkbox"/> Off |

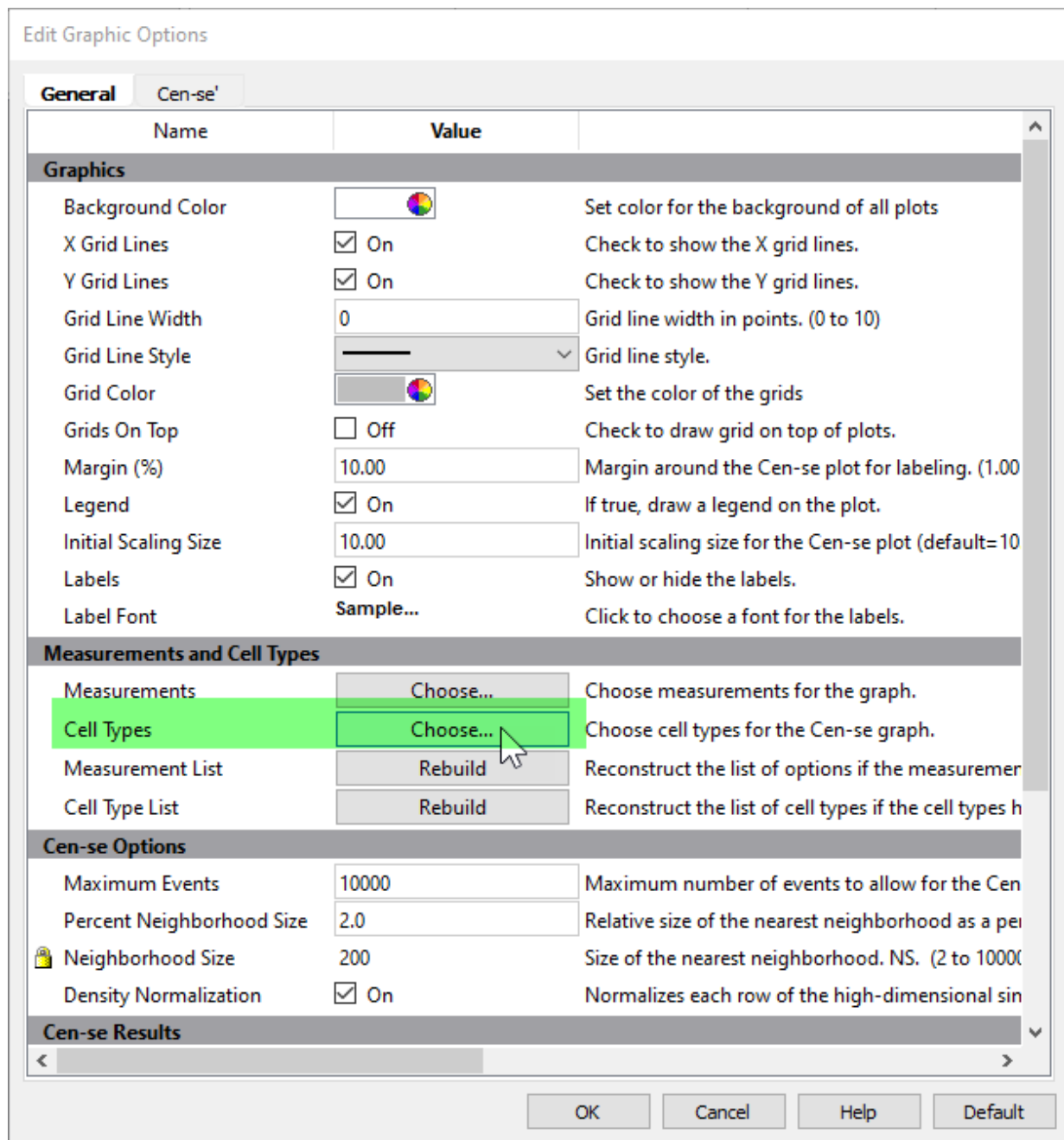
OK

Cancel

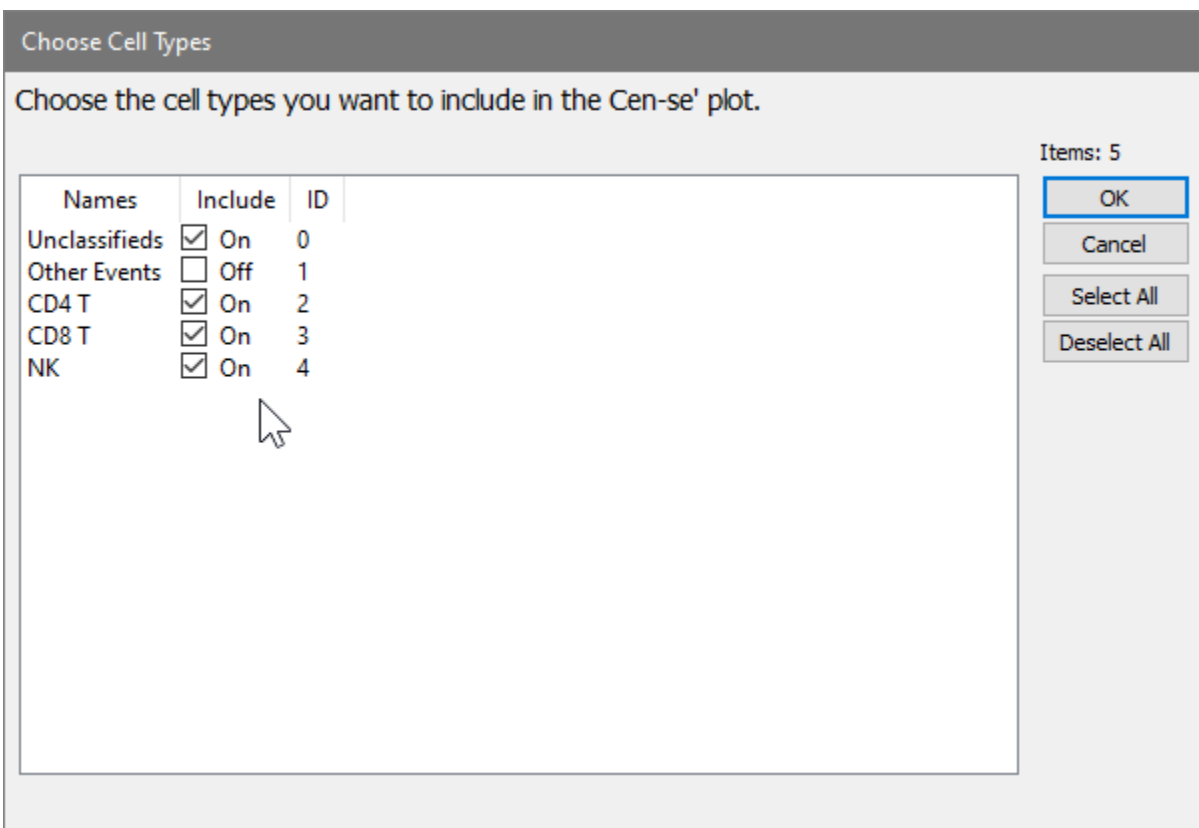
Select All

Deselect All

Click the **Choose** button for **Cell Types**.

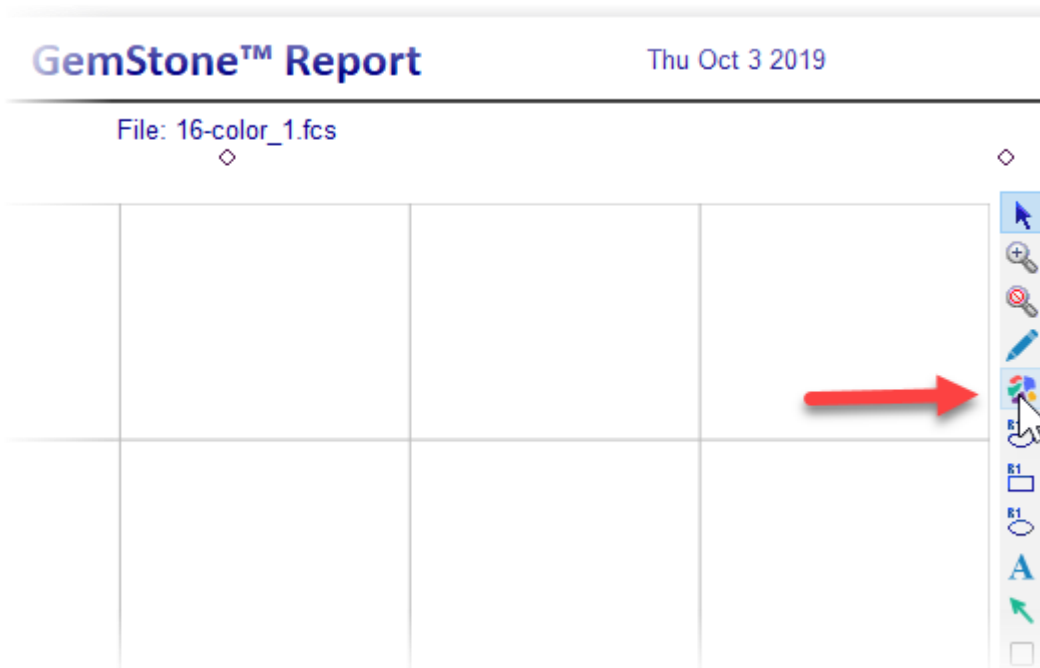


Make sure that all cell types are selected *except* for **Other Events**.
Click **OK**.



Click **OK** to close the Edit Graphic Options dialog.

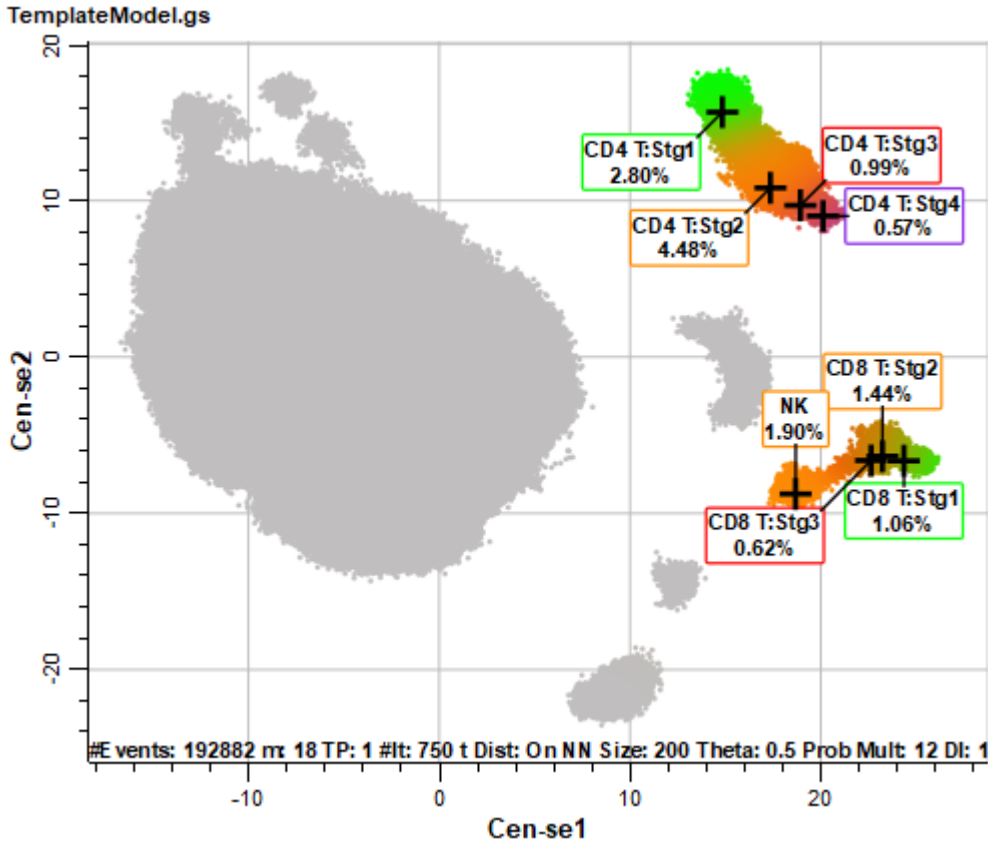
Move the mouse over the Cen-se plot to show its toolbar, then click the **Build Cen-se'** tool.



After a few moments, the Cen-se' plot will be displayed. The plot is a two-dimensional representation of the 21 measurements we selected in previous steps. Based on the popular t-SNE embedding algorithm (t-SNE © 2014 Laurens van der Maaten, Delft University of Technology), Cen-se' provides better separation of populations and computes much faster than t-SNE.

Notice that some of the populations are labeled with the cell type and stages created with the Cell Type wizard. This labeling is applied automatically for any of the cell types that we include in the Cen-se'. The coloring of the populations comes from the stage colors in this initial view of the plot. The gray dots represent populations that have not been defined in the template model yet.

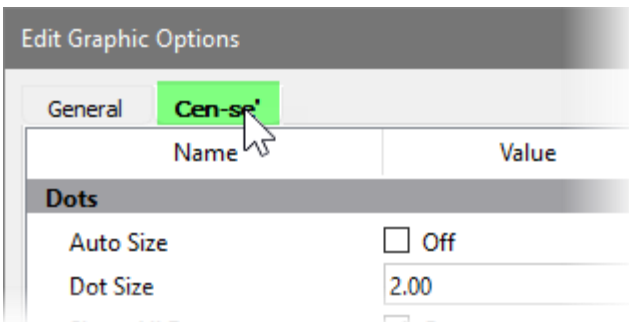
Note, your graphics and statistics may differ from those shown below.



Using the Intensity Heatmap

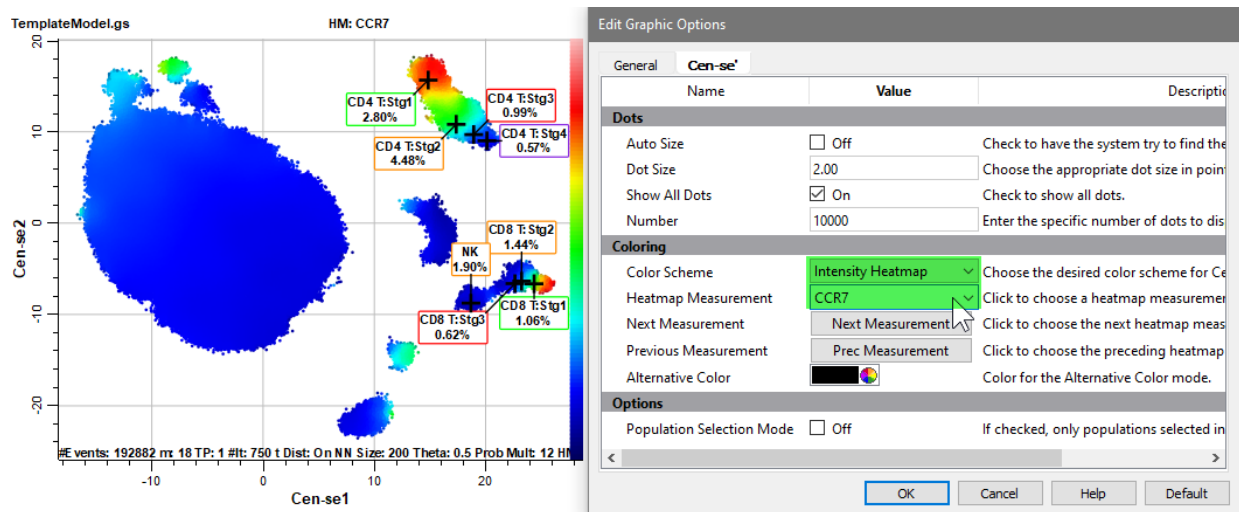
The CD4 T, CD8 T, and NK cell types are labeled, and the coloring from the cell types is applied to the events in the Cen-se' plot. There are also several populations of gray-colored events.

Double-click the plot to display the **Edit Graphic Options** dialog again. Move and size the dialog so that you can see the Cen-se' plot. Click the **Cen-se'** tab of the dialog.



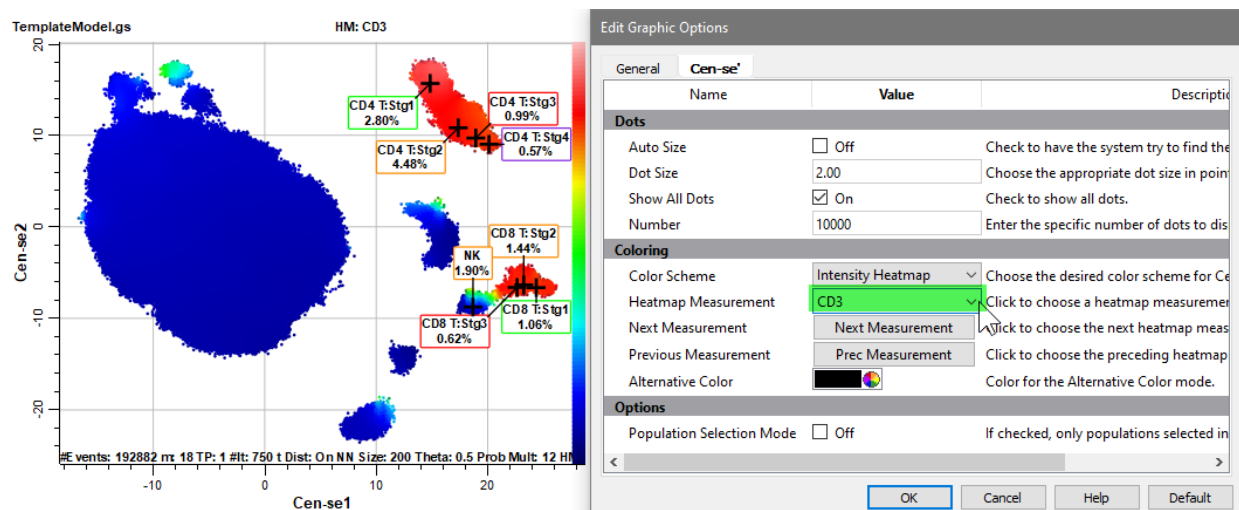
In the Coloring section, change **Color Scheme** to **Intensity Heatmap**.

Select **CCR7** for the Heatmap Measurement. The Naive (Stg1) CD4 T and CD8 T cells are brightest for CCR7, as shown with red dots. Events that do not express CCR7 are shown as dark blue dots. The intensity color scale is shown on the right-hand side of the Cen-se' plot.

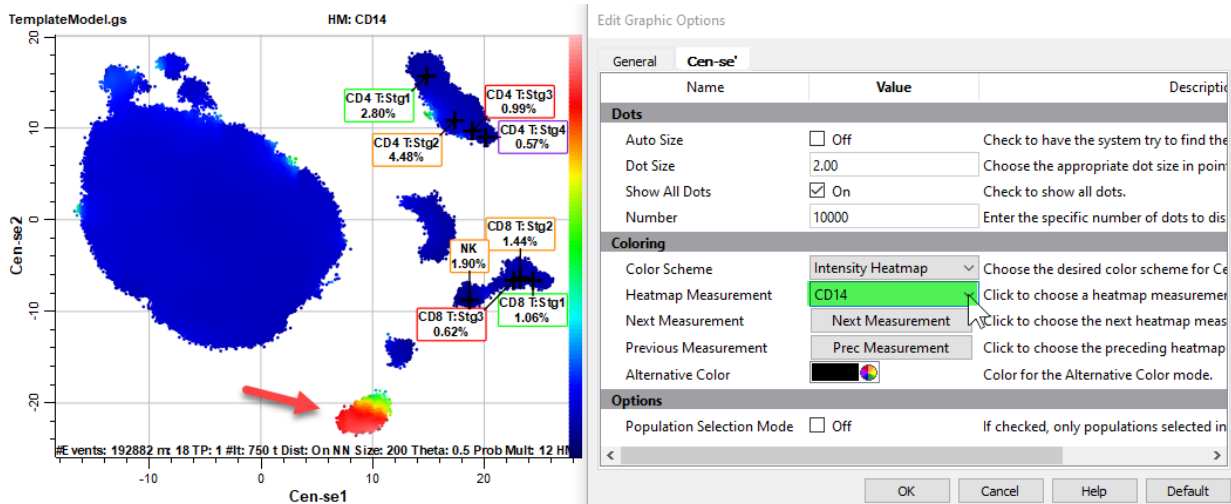


Change the Heatmap Measurement to **CD3**.

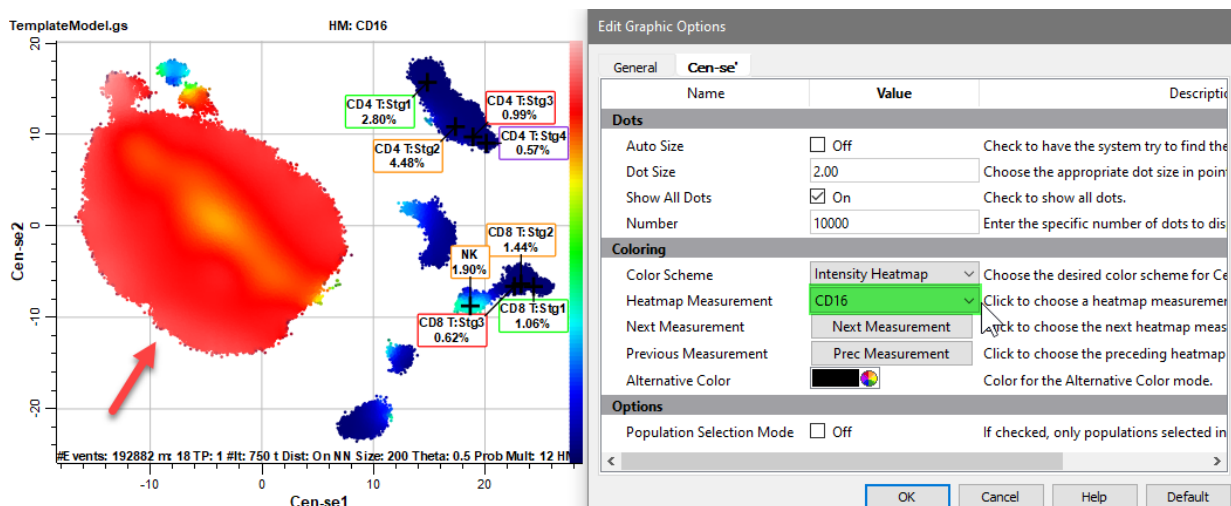
With CD3 selected, the CD4 T and CD8 T cell type show bright red dots.



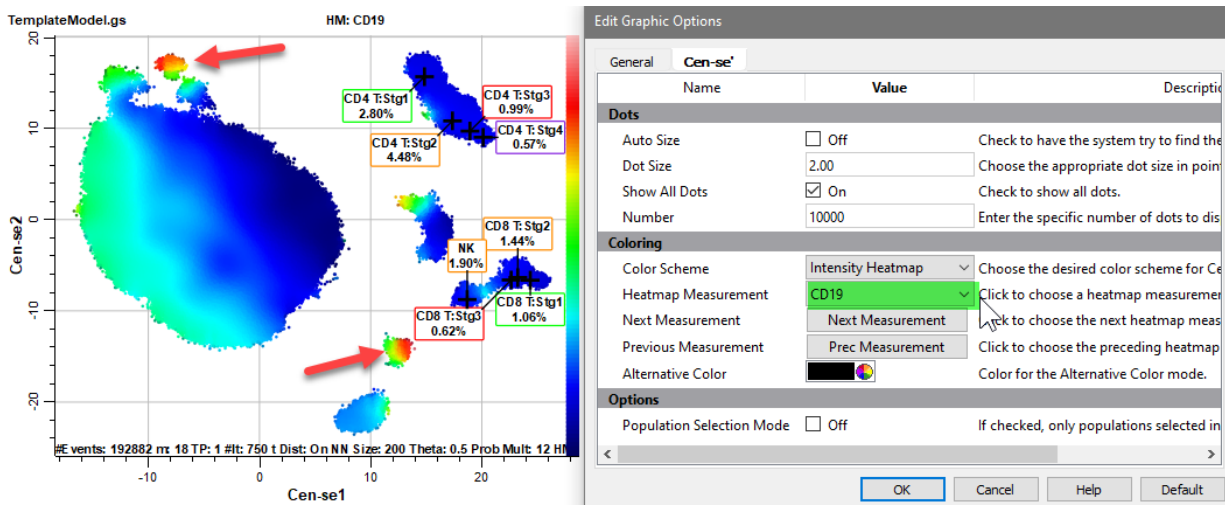
Select **CD14** for the Heatmap Measurement.
 One population is red, probably the monocytes.



Select **CD16** for the Heatmap Measurement.
 The CD16 events are likely neutrophils, which would explain the large fraction of events in this population.



Select **CD19** for the Heatmap Measurement.
 One of the CD19 positive populations is probably B cells.

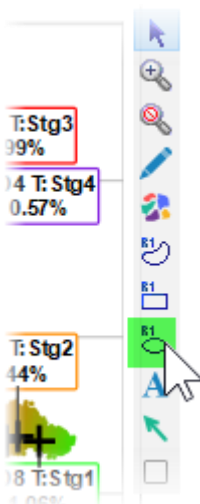


The Intensity Heatmap is a great way to explore and verify populations in the Cen-se plot. Set **Color Scheme** back to **Summary Map Colors**, and click **OK**.

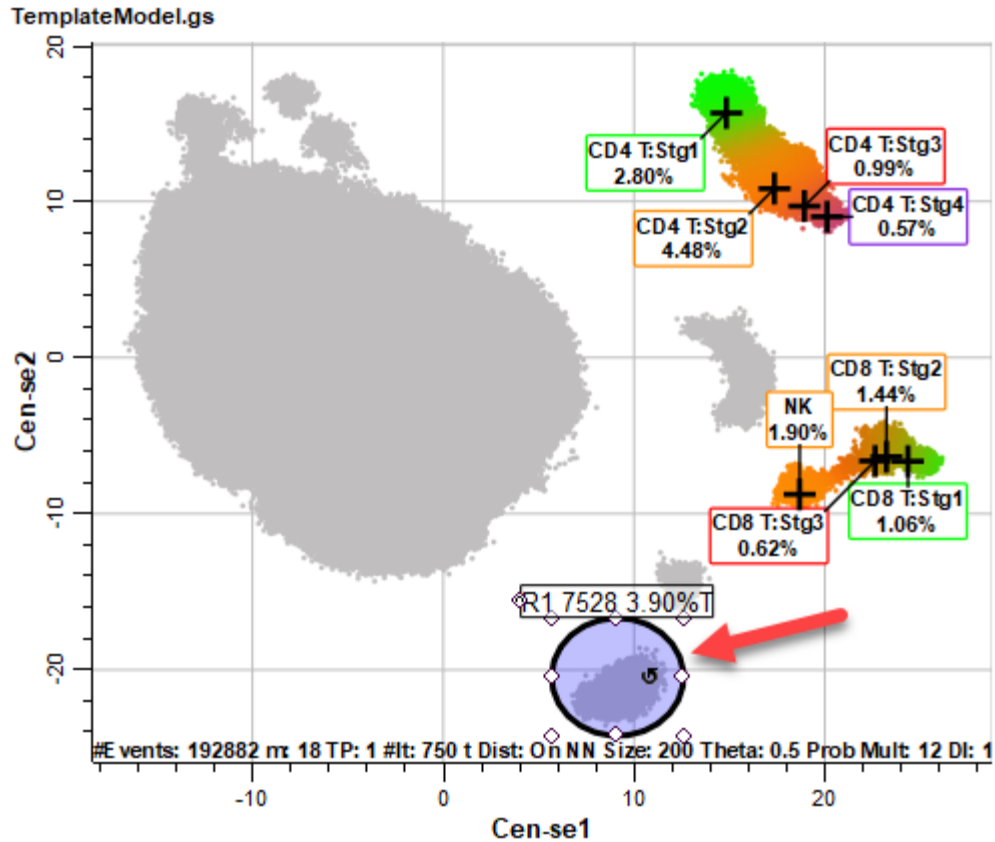
Exploring Unknowns with Cen-se'

We have seen how colors and labels from the template model can enhance the Cen-se' plot. We can also use the Cen-se' plot to explore populations in the template model. This tight integration of Cen-se' and the Probability State Model is unique to GemStone and may be one of its most powerful features. Let's try it out.

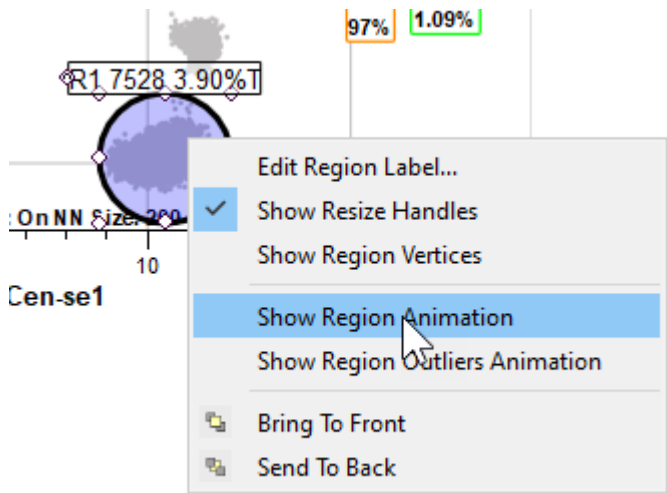
Select the **Ellipse Region** tool in the Cen-se toolbar on the right side of the Cen-se plot.



Draw an elliptical region around the population we identified as CD14+.



Right-click inside the region and choose **Show Region Animation**.



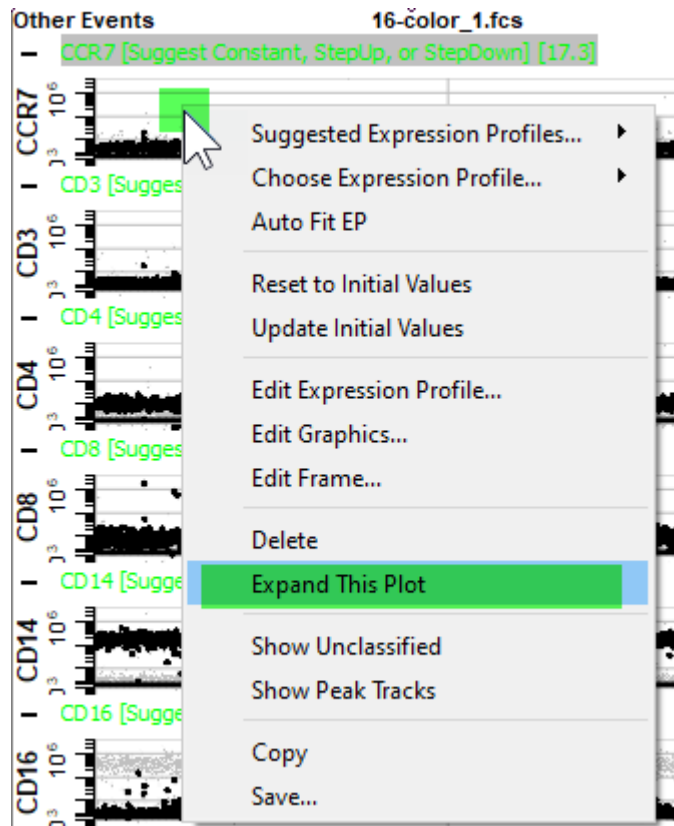
Click the **Cell Types** tab and select the **Other Events** cell type.



The Other Events cell type displays events that are not included in any of the other cell types defined in the model template.

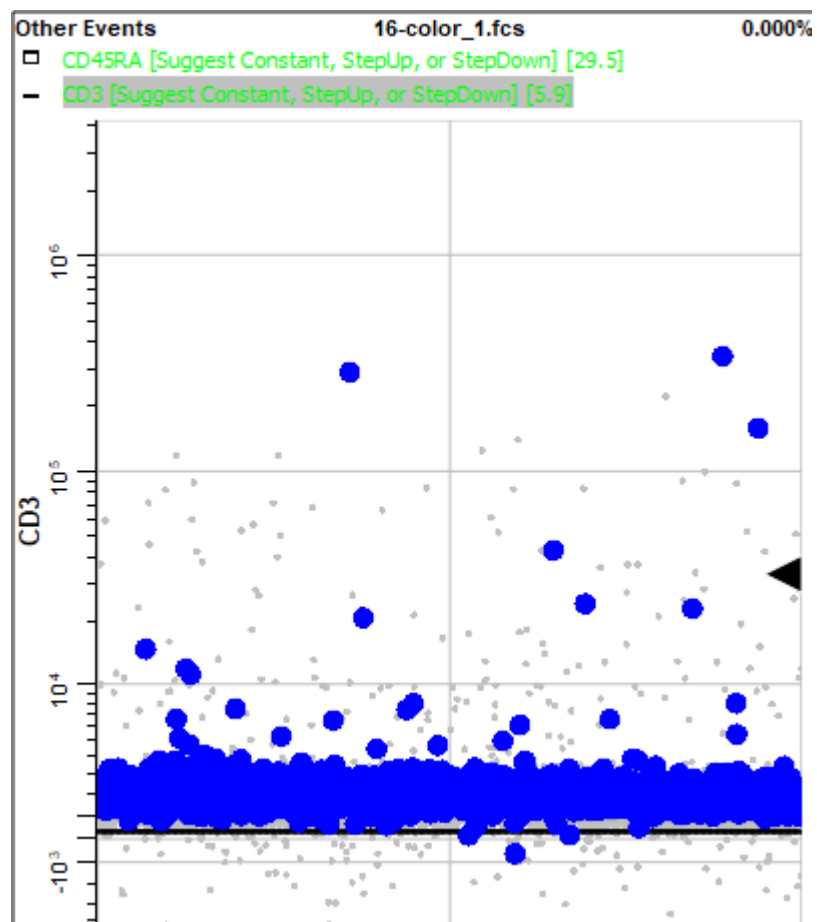
You may need to scroll the cell type window so that you can see the upper left corner.

Right-click the first plot and choose **Expand This Plot**.

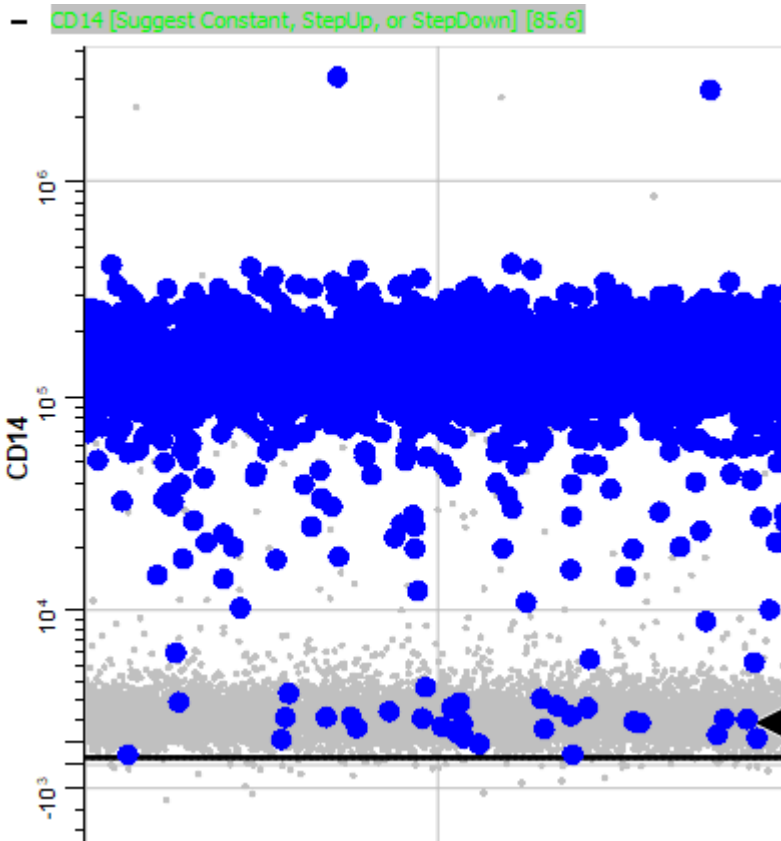


The dots that are blinking are the ones you surrounded on the Cen-se plot with the region. You can see that CCR7 is negative to dim for those events.

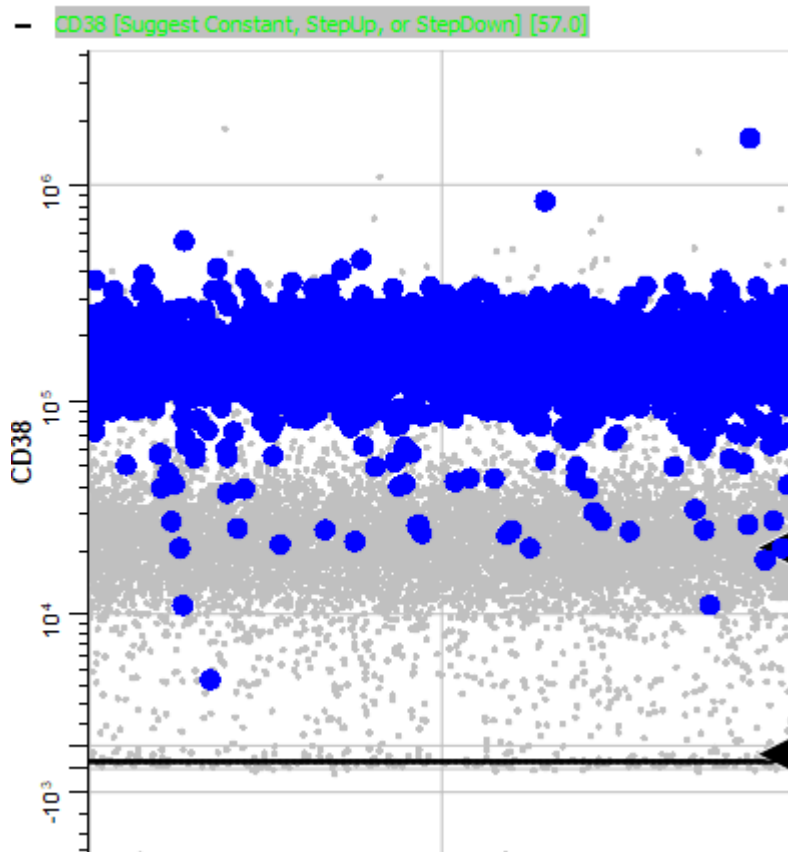
Use the **Page Down** key (PgDn) on the keyboard to view the next measurement, **CD3**. Most of the blinking events are negative for CD3.



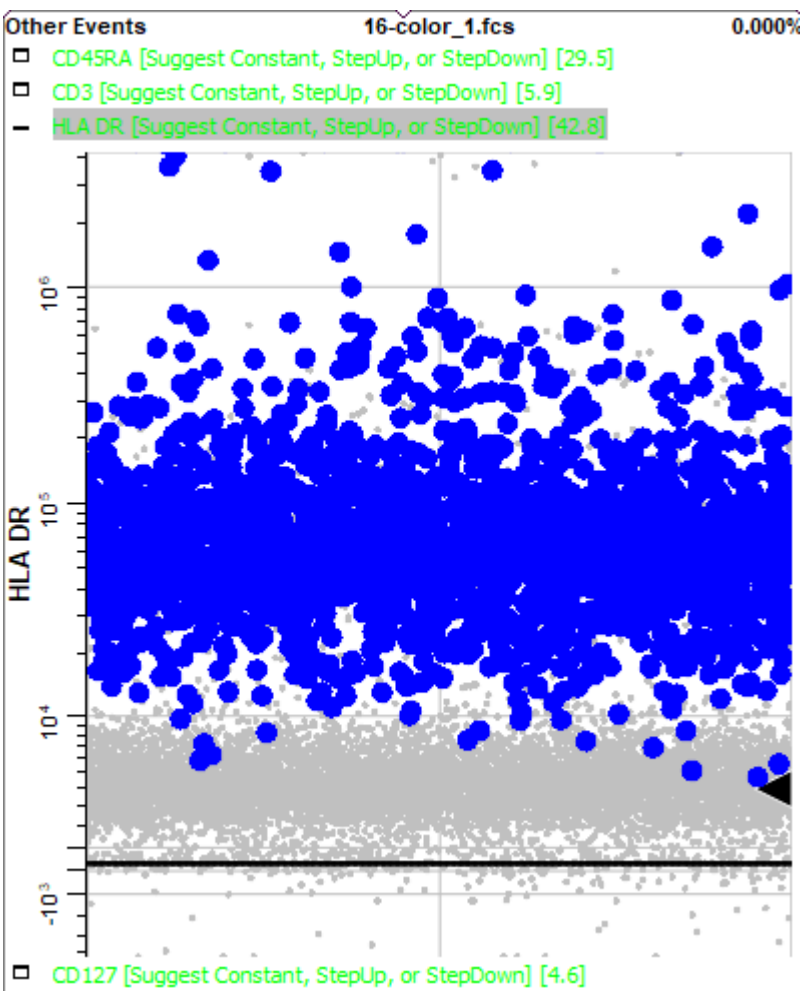
Page down until you find **CD14**. Note that most of the blinking events are positive for CD14.



Page down until you come to **CD38**. Notice that it is also positive.



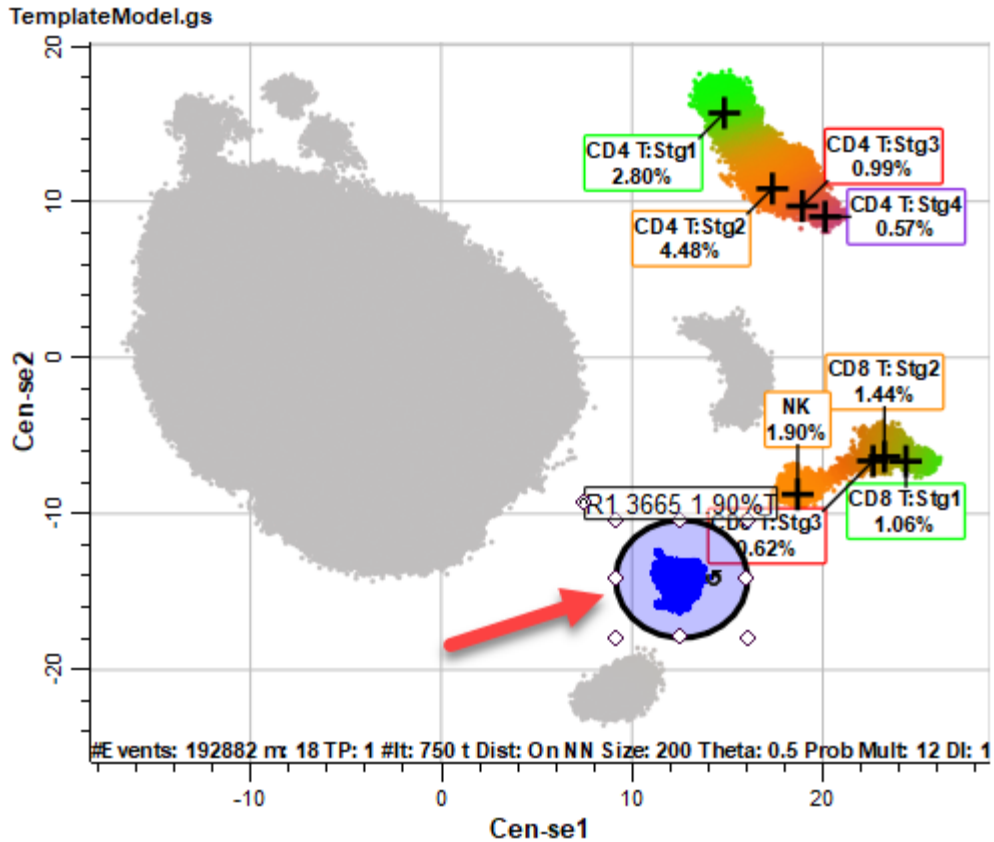
Page down to **HLA DR**. Note that this marker is positive for the animating events.



The phenotype of these blinking events, CD3- CD14+ CD38+ HLA DR+, points to this population being the monocytes. Using this information, you could use the Cell Type Wizard to create a new cell type for monocytes.

Return to the Cen-se' plot by selecting the **Reports** tab and choosing the **Cen-se** page.

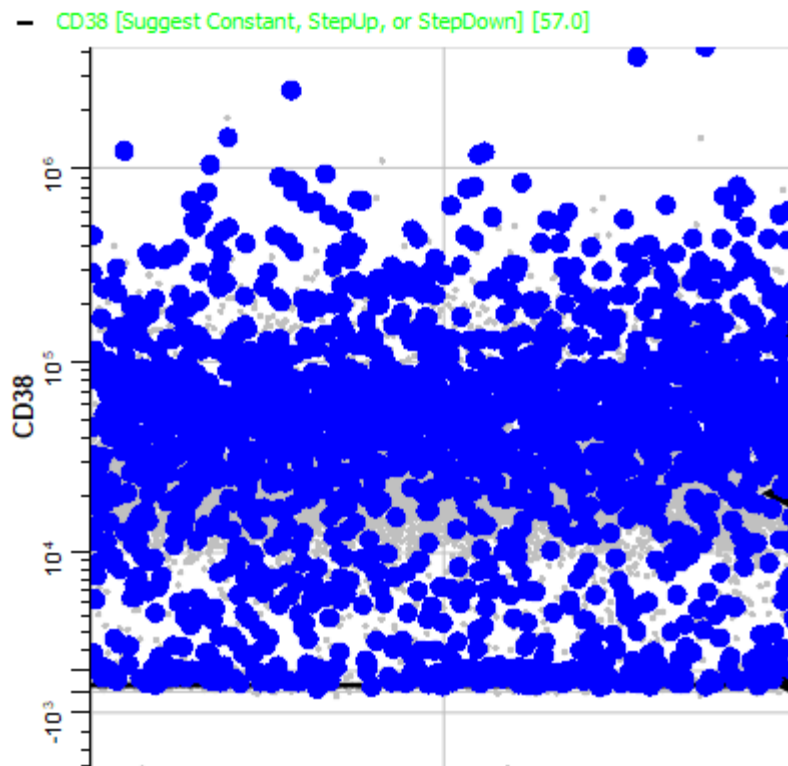
Move the region to the smaller population near the monocytes. This was CD19+ in the Intensity Heatmap, so it may be the B cell population.



Return to the **Other Events** cell type by selecting the **Cell Types** tab and choosing **Other Events**.

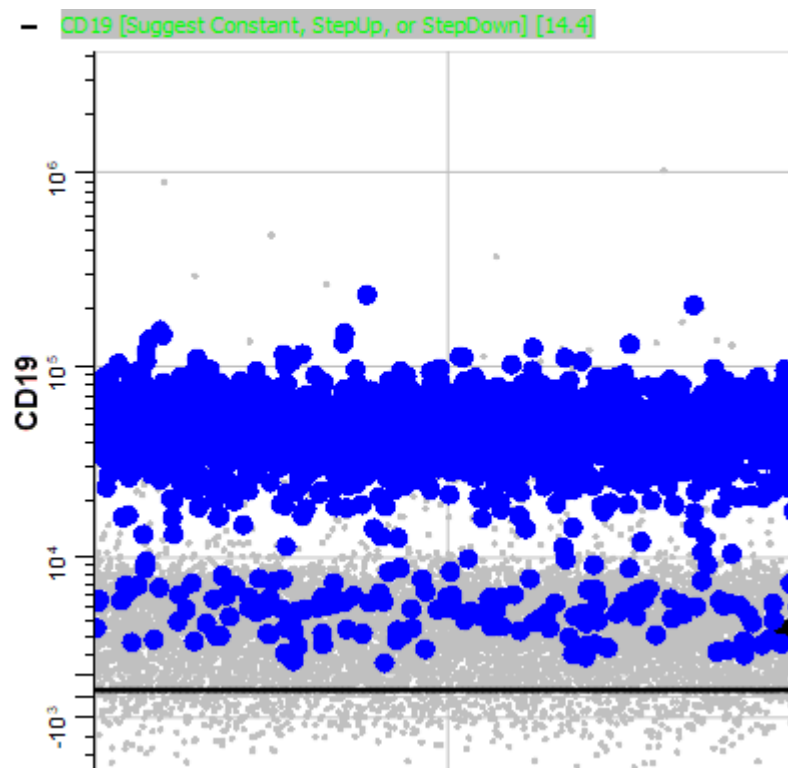
Find **CD38** and use the **Expand This Plot** command.

Note that all intensities of CD38 are blinking. Naive B cells express CD38, and CD38 is down-regulated as B cells mature.

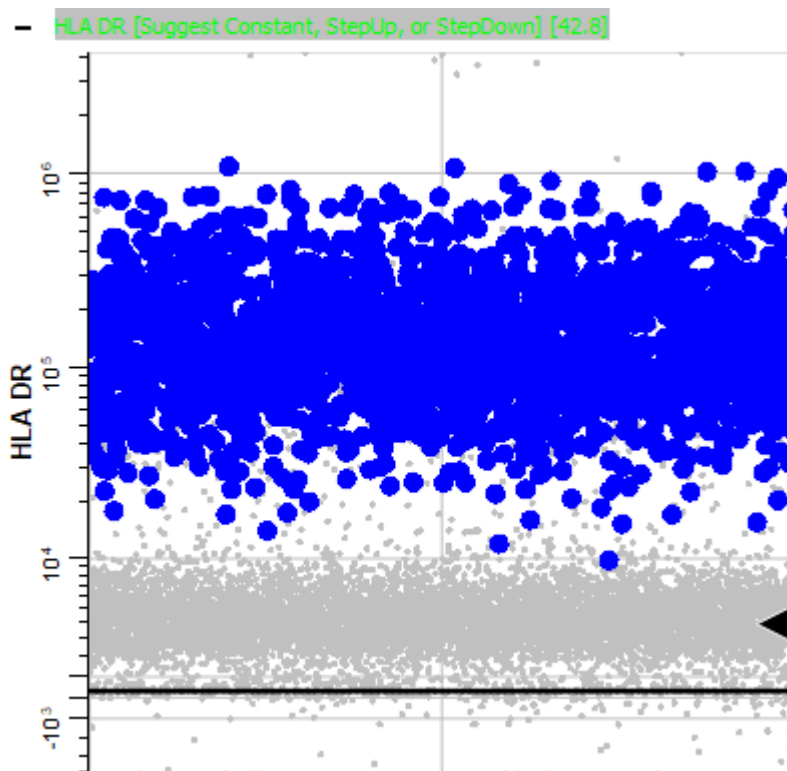


Expand the **CD19** expression profile.

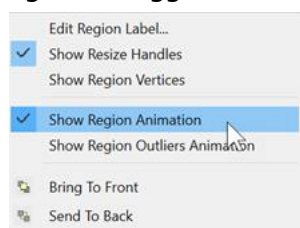
Consistent with B cells, the bright CD19 events are blinking, along with some dim CD19 events.



Finally, expand the **HLA DR** plot and notice the bright HLA DR events are animating. This confirms that this is the B cell population.



Back on the Cen-se plot, right-click the **R1** region and choose **Show Region Animation** again to toggle the animation off.



Using region animation, you can identify the key markers for unknown events that cluster together in Cen-se'. Often the markers will point to a known cell type that has not yet been created in the template model.

Save your Work

It is time to save the enhancements to our model template. Before saving, switch back to the Report tab, display the CD4 T report page, and scroll it to the position you want to appear when this template is used. The template will remember this view when it is saved.

Click **Save** in the Template section of the Develop tab on the ribbon.

Summary

In this tutorial, you create a Cen-se' plot on a report page. You selected the measurements and cell types that would be displayed in the Cen-se' and edited the graphic options.

The Intensity Heatmap display provided a tool for reviewing locations on the Cen-se' plot that expressed each of the markers, one at a time. It gave us clues about the phenotype of the events and where cell types were located on the Cen-se'.

You drew a region on the Cen-se' plot to animate events in clusters that were not identified by the model template. With the events animating, you reviewed the Other Events cell type to see which markers were positive and which were negative for the animating dots.

The next tutorial contains additional exercises to continue building this model.

Develop: Additional Exercises

In the previous tutorials, you created a template document that identifies three cell types: CD4 T cells, CD8 T, and NK cells. You added a TriCOM to identify the Treg subset of CD4 T, and then added a Cen-se' plot to the report.

There are several additional cell types that can be defined by the markers in the 16-color example file. These are left to the reader to add to the template model as additional exercises. Guidance on how to define these cell types is provided below.

Use the techniques illustrated in the previous tutorials to define each cell type.

Prerequisites

These exercises continues to build the Project that was started in the following tutorials:

[Develop: Starting a New Project from Scratch](#)

[Develop: Adding Cell Types to the Project](#)

[Develop: Analyzing Subsets](#)

[Develop: Exploring with Cen-se'](#)

Start GemStone

Start Gemstone by following the instructions in the [Installation and Setup of GemStone](#) section.

Open the Project and Analyze a File

Open the "New Project Tutorial" Project that you created in the previous tutorials by selecting it in the Project dropdown list.

Click the **Develop** checkbox to enable develop mode.

Double-click the first file in the batch list. A dialog of choices will be displayed.

Click **Analyze** to reanalyze the file with the latest model template revisions.

B Cells

B cell phenotype: SSC-A- CD3- CD14- CD56- CD19+ HLADR+ CD27->+

Use the [Cell Type Wizard](#) to create a new cell type named **B**.

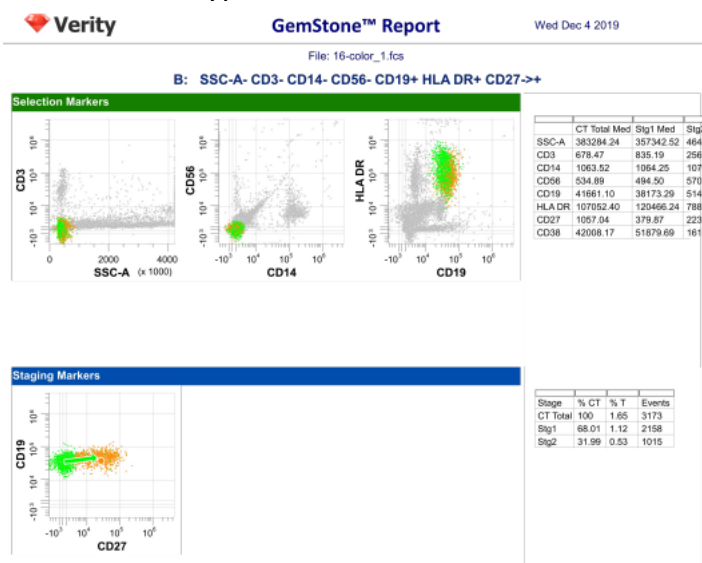
Selection markers: SSC-A- CD3- CD14- CD56- CD19+ HLADR+

Hint: For the negative markers, use Dimmest Peak and Low Pass. For positive markers, use Brightest Peak and Normal.

Staging markers: CD27 Step**Up**

Functional markers: CD38

The **B** cell report created by the wizard will look similar to the graphic below. Your results may differ. If you make a mistake or want to change the cell type, use the Cell Type Wizard to edit the cell type.



Monocytes

Monocyte phenotype: CD3- CD19- CD56- HLADR+ SSC-A CD14+>-

Use the [Cell Type Wizard](#) to create a new cell type named **Mono**.

Selection markers: CD3- CD19- CD56- HLADR+ SSC-A.

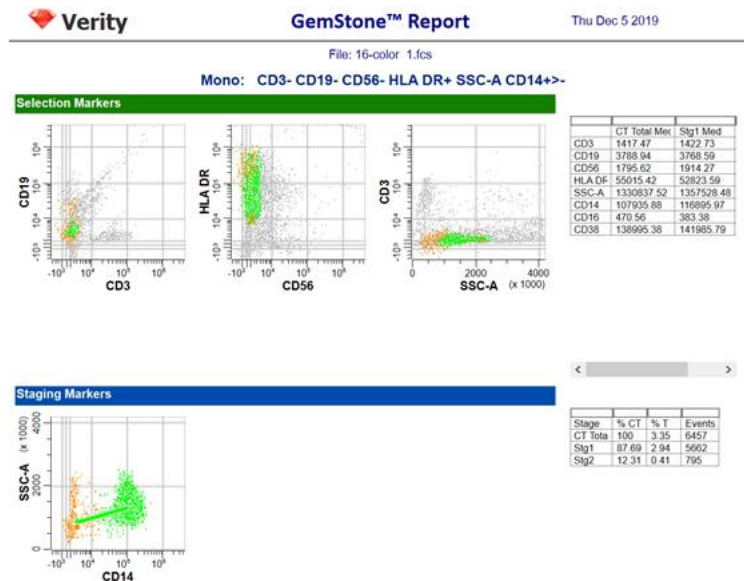
Hints: For the negative markers, use Dimmest Peak and Low Pass. For *most* of the positive markers, use Brightest Peak and Normal. **Use Inverse Low Pass on Dimmest Peak for HLA DR.** For SSC-A use Closest Peak, position the profile over the middle peak in SSC-A, and set the Initial Width to 5.

Staging markers: CD14 Step**Down**

Hint: Set the Initial Width for both levels to 8.0 and uncheck Width from Peak. This will help capture the transitional events between the bright and dim.

Functional markers: CD16, CD38

The **Mono** report created by the wizard will look similar to this. Your results may differ.



Gamma-Delta T

gd T phenotype: CD3+ CD4- CD14- CD8+ TCRgd+

Use the [Cell Type Wizard](#) to create a new cell type named **gd T**.

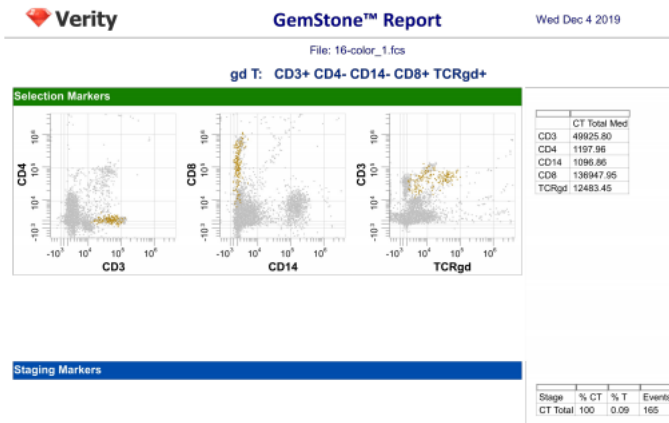
Selection markers: CD3+ CD4- CD14- CD8+ TCRgd+.

Hint: For CD3+, use Brightest Peak and Normal. For the negative markers, use Dimmest Peak and Low Pass. **Use an Inverse Low Pass on the Dimmest Peak for CD8 and TCRgd.**

Staging markers: none

Functional markers: none

The **gd T** report created by the wizard will look similar to this. Your results may differ.



Granulocytes

Granulocyte phenotype: CD3- CD14- CD56- CD16+

Use the [Cell Type Wizard](#) to create a new cell type named **Grans**.

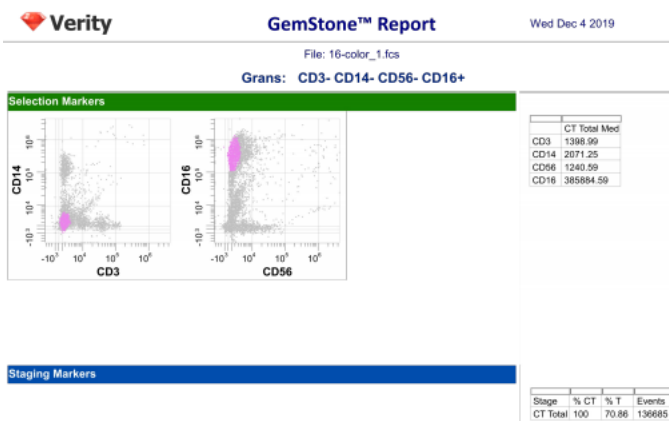
Selection markers: CD3- CD14- CD56- CD16+.

Hint: For the negative markers, use Dimmest Peak and Low Pass. For CD16+, use Brightest Peak and Normal.

Staging markers: none

Functional markers: none

The **Grans** report created by the wizard will look similar to this. Your results may differ.



Summary

The Cell Type Wizard is designed to make it quick and easy to define additional cell types. There are several strategies that we have used in these tutorials that are good to keep in mind.

- For negative selection markers, use a Low Pass profile on the Dimmest Peak.
- Positive selection markers are most often modeled with a Normal profile on the Brightest Peak.
- When positive peaks are not present, use Inverse Low Pass profiles on the Dimmest Peak.
- When peaks are highly variable in width, it can be helpful to manually set the Initial Width and uncheck Width from Peak.

Develop: Finishing the Project

Overview

This series of Develop tutorials introduced the key concepts needed to develop a new analysis project.

The Measurement wizard was used to review the names and transforms for the measurements in a file. The Cell Type wizard was used to define each cell type's selection markers, staging markers, and functional markers. The wizard also created a basic report page with graphics and statistic tables.

To identify additional subsets within a cell type, we created a TriCOM and set it up to quantify Tregs in the CD4 T cell type. We create a table to display the results Treg results from the TriCOM.

We added a Cen-se' plot to a report page and explored its display options. The Summary Map Coloring showed our cell type and staging colors on the Cen-se'. The Intensity Heatmap highlighted the portions of the Cen-se' plot that are hot spots for each marker we selected. We also used animated regions on the Cen-se' plot to explore unclassified events in the Other Events cell type. This allows us to locate the Monocytes and B cell populations.

In this last part of the tutorial, we'll add a summary page to the report and spruce up the other report pages.

Prerequisites

This tutorial continues to build the Project that was started in the following tutorials:

[Develop: Starting a New Project from Scratch](#)

[Develop: Adding Cell Types to the Project](#)

[Develop: Analyzing Subsets](#)

[Develop: Exploring with Cen-se'](#)

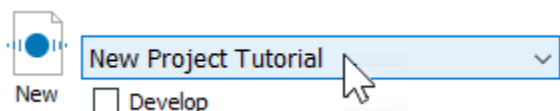
[Develop: Additional Exercises](#)

Start GemStone

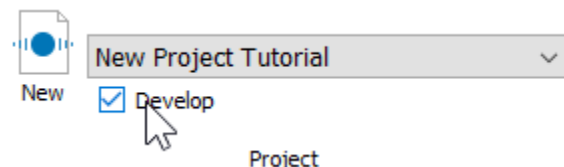
Start Gemstone by following the instructions in the [Installation and Setup of GemStone](#) section.

Open the Project

Open the "New Project Tutorial" Project that you created in the previous tutorials by selecting it in the Project dropdown list.

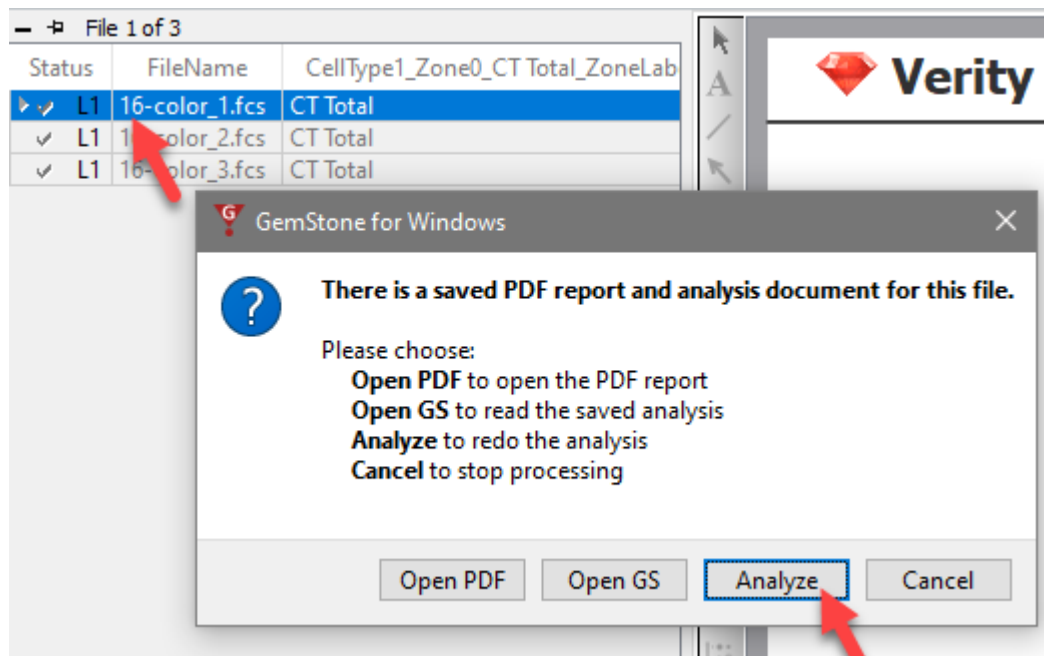


Click the **Develop** checkbox to enable develop mode.



Analyze a File

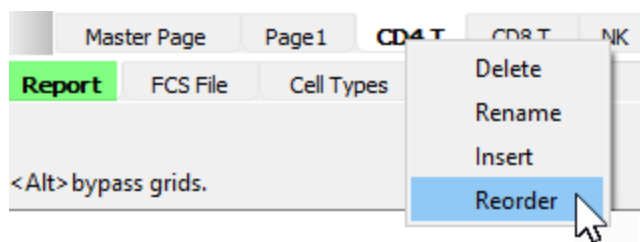
Double-click the first file in the batch list. A dialog of choices will be displayed. Click **Analyze** to reanalyze the file with the latest model template revisions.



Reorder the Report Pages

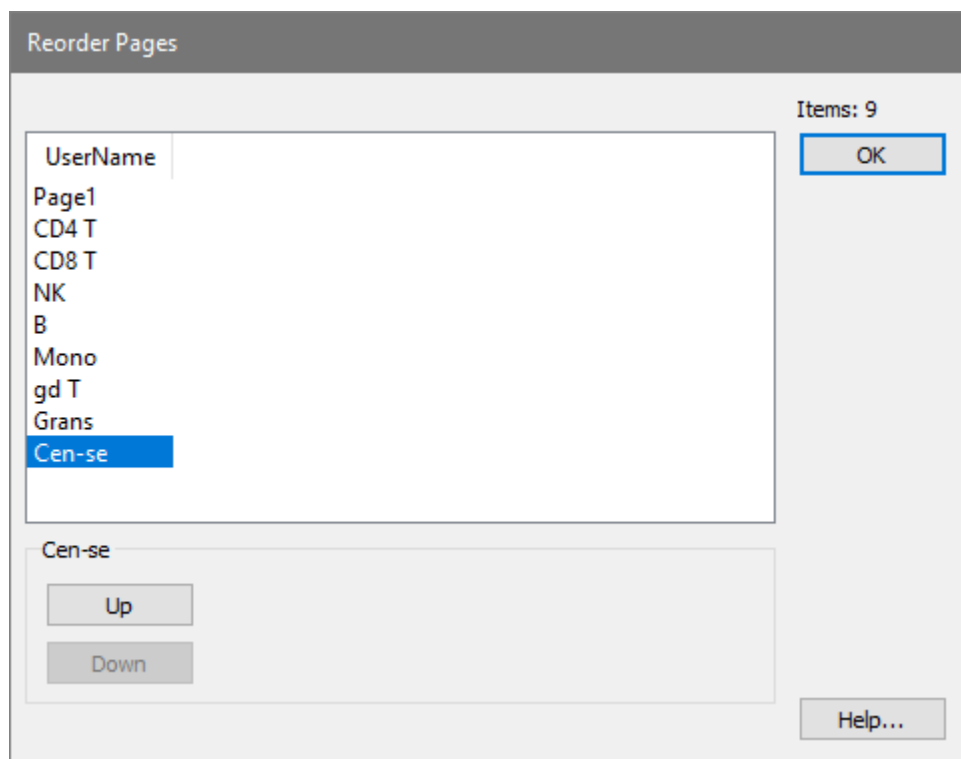
The report pages appear in the order in which they were created, putting the Cen-se' report page right in the middle of the pages. Let's change that so that Cen-se' comes at the end the cell type pages are in a better order, too.

Right-click one of the report page tabs and choose **Reorder** from the menu.



In the dialog, select the Cen-se page and use the **Down** button to move the pages into the order shown below.

Click **OK**.



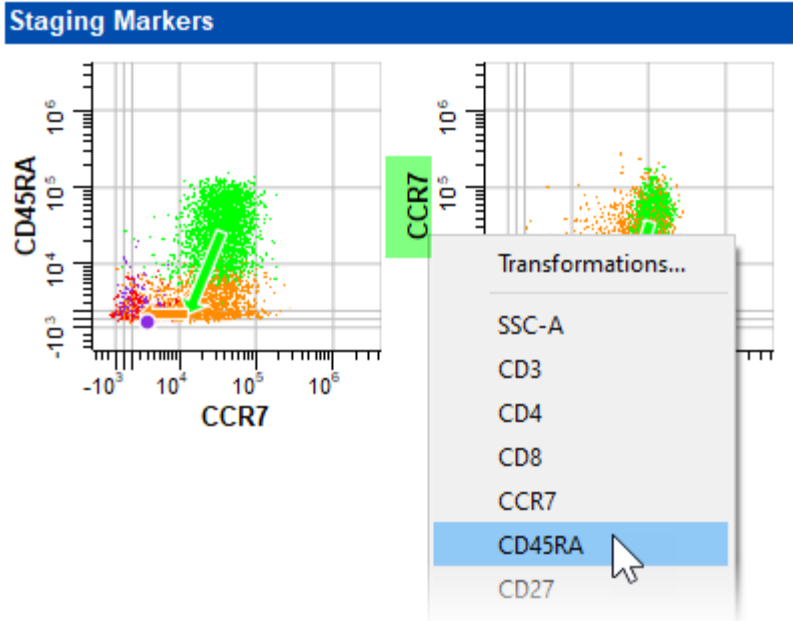
Graphic Improvements

The report pages created by the Cell Type wizard provide a good starting place, but the wizard doesn't know what combinations of measurements are most important. Let's review several cell type report pages to add and modify some of the plots.

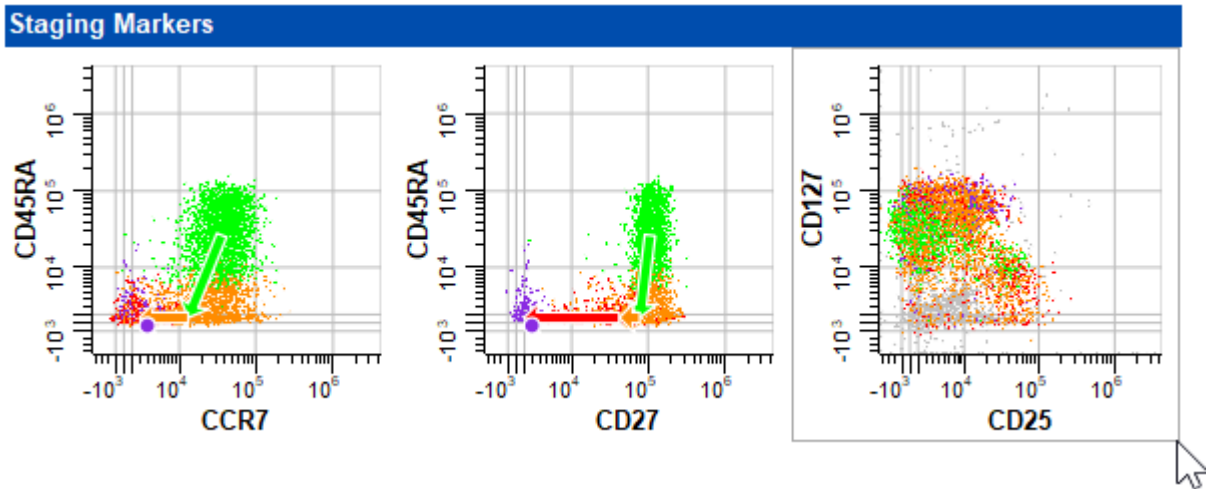
CD4 T Report Revisions

Select the **CD4 T** report page.

In the Staging Markers section, click the Y-axis **CCR7** label for the second plot and choose **CD45RA** instead.



Next, right-click the CD27 vs CD45RA plot and choose **Copy** from the menu. Right-click in the empty space next to the plot and choose **Paste**. Move the new plot next to the one you just edited. Select **CD25** and **CD127** for the X and Y axes.



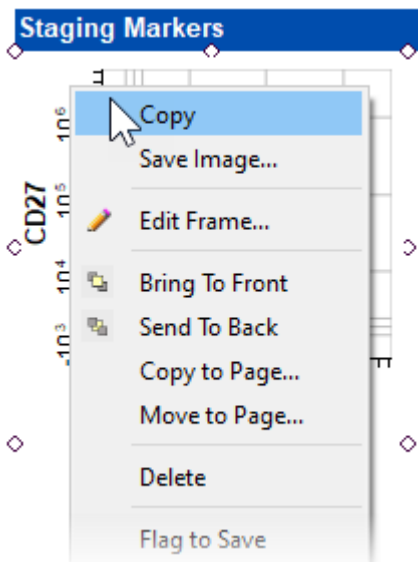
You can make other additions and edits if you choose.

CD8 T Report Revisions

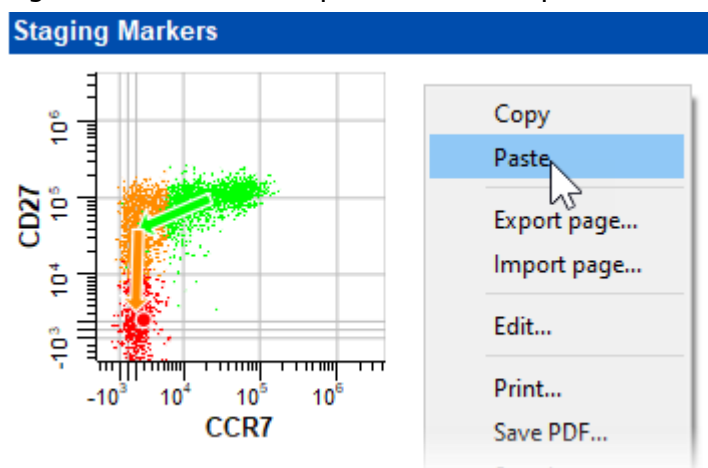
Select the **CD8 T** report page.

On this page let's revise the report to have three plots for the Staging Markers section: CCR7 vs CD27, CCR7 vs CD38, and CCR7 vs CD45RA. We'll use Copy and Paste to do this.

Right-click near the left corner of the CCR7 vs CD27 plot and choose **Copy**.



Right-click in the blank space next to the plot and choose **Paste**.



Drag the pasted plot next to the first plot.

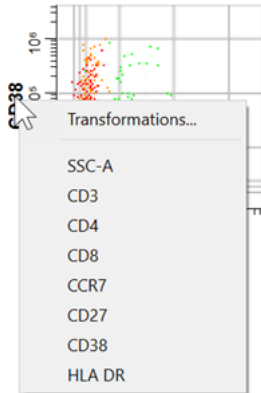
Click the Y-axis label and choose **CD38**.

Add Missing Markers to Display

Right-click and choose **Paste** again to paste another copy of the plot.

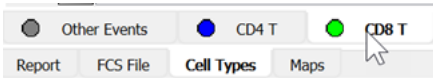
Drag to position the third plot next to the second.

We want to display CCR7 vs CD45RA, but CD45RA is not in the list of choices.

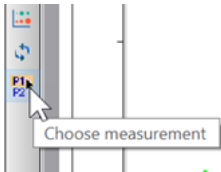


When selecting measurements to display in plots, only measurements that have been added to the cell type are shown. So when you want to display a marker in a plot, it must first be added to the cell type. This can be done with the Cell Type Wizard, adding the measurement as a Functional Marker. It can also be done with a tool on the Cell Type tab.

Click the **Cell Types** tab and select the **CD8 T** cell type.

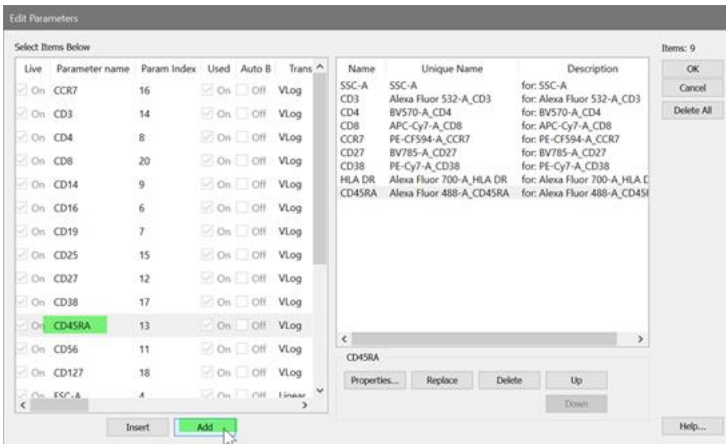


Click the Add Measurement tool in the cell type toolbar.



In the dialog that appears, select **CD45RA** in the list on the left and click **Add** to add it to the list on the right. CD45RA is added to the other measurements included in the cell type.

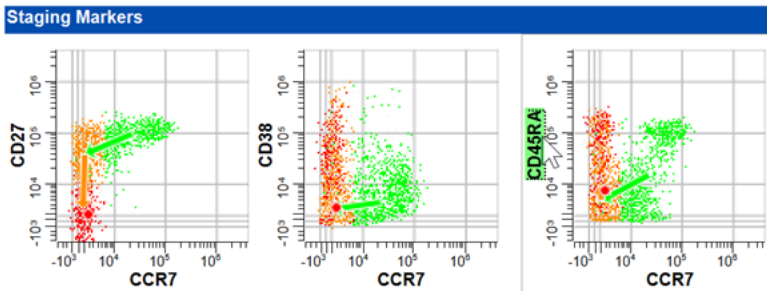
Click **OK**.



Note: You can use this technique to add other markers after defining the cell type with the Cell Type Wizard. It is not recommended to use this to add selection or staging markers, as it can make the cell type incompatible with the Cell Type Wizard.

Now that you have added CD45RA to the cell type, navigate back to the **Report** tab and select the **CD8 T** report.

Change the Y-axis of the third staging markers plot to **CD45RA**.

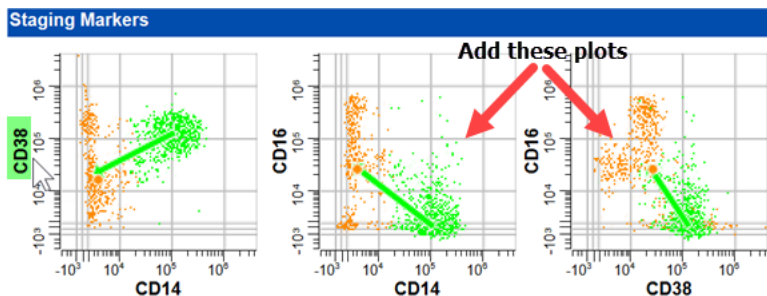


Mono Report Revisions

Select the **Mono** report page.

Change the Y-axis for the Staging Marker plot from **SSC-A** to **CD38**.

Add dot plots for **CD14 vs CD16** and **CD38 vs CD16**. Use the same copy-paste approach described for the CD8 revisions to create the plots.



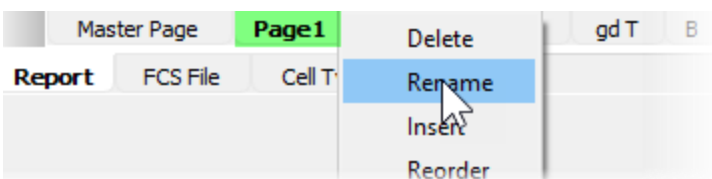
Feel free to make additional changes to any of the report pages.

Create a Summary Table

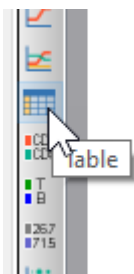
Right-click the **Page1** report tab.

Rename **Page1** to **Summary**.

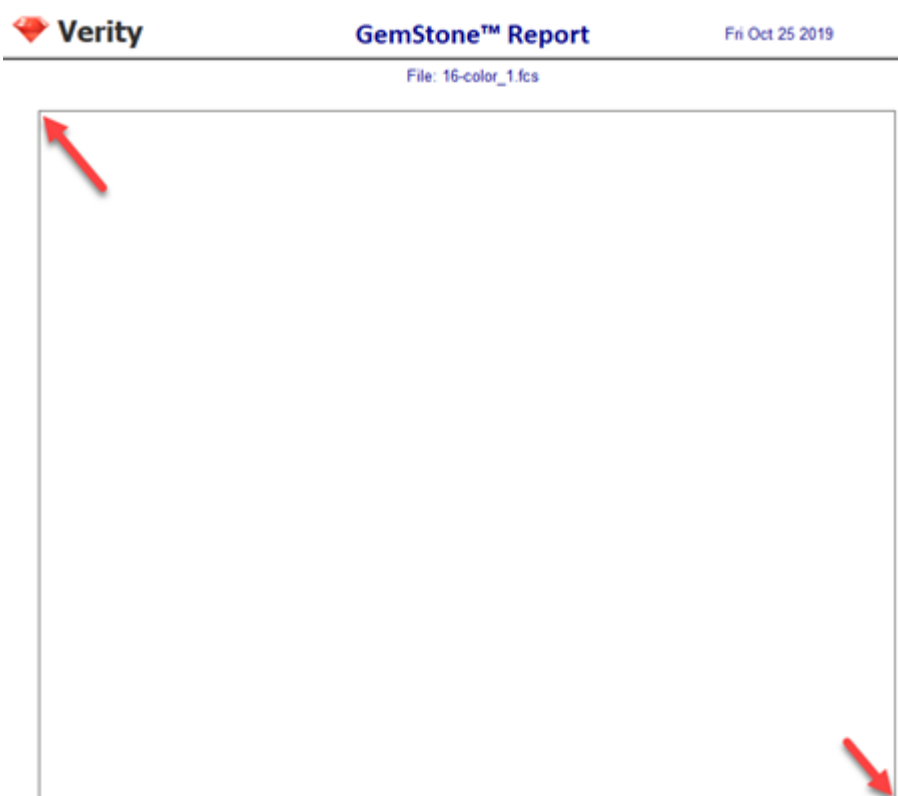
GemStone User Guide



Select the **Table** tool on the report toolbar.



Click and drag to define space for the table on the report page.



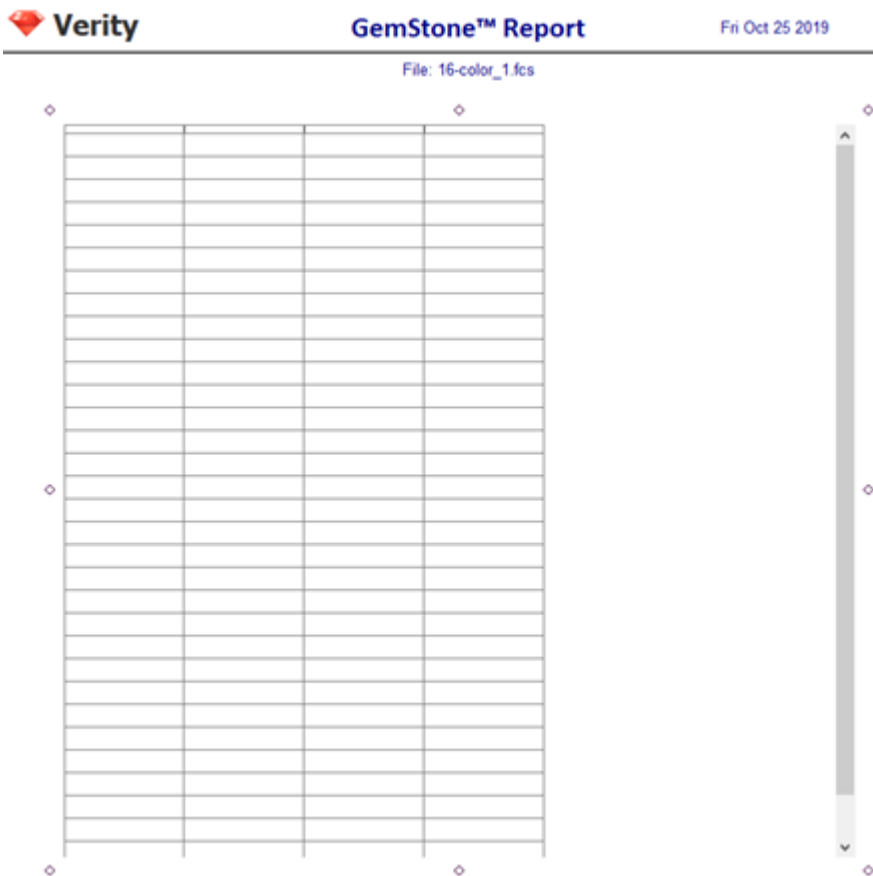
Right-click near the top of the table and choose **Edit Frame**.

Uncheck **Show Title**.

Set **Rows** to **33** and **Columns** to **4**.

Click **Font** and set the font size to **12** and click OK to close the Font dialog.

Click **OK**.



This table will contain a summary of population results for all cell types. You will start by adding labels in the top row and left column.

At the top of column two, three, and four, type **Count**, **% Cell Type**, and **% Total**.

| | Count | % Cell Type | % Total |
|--|-------|-------------|---------|
| | | | |

In the left column, enter the labels as shown. Indent the labels with spaces to show the relationship of the populations.

You may need to adjust the size of the table object to see all of the rows.

| |
|---------------|
| |
| Intact Cells |
| Lymphocytes |
| CD3 T |
| CD4 T |
| Naive |
| CM |
| EM |
| TE |
| |
| Tregs |
| |
| CD8 T |
| Naive/CM |
| EM |
| TE |
| |
| Gamma Delta T |
| |
| B Cells |
| Naive |
| Memory |
| |
| NK Cells |
| |
| Monocytes |
| Classical |
| Non-Classical |
| |
| Granulocytes |
| |
| Other |
| |

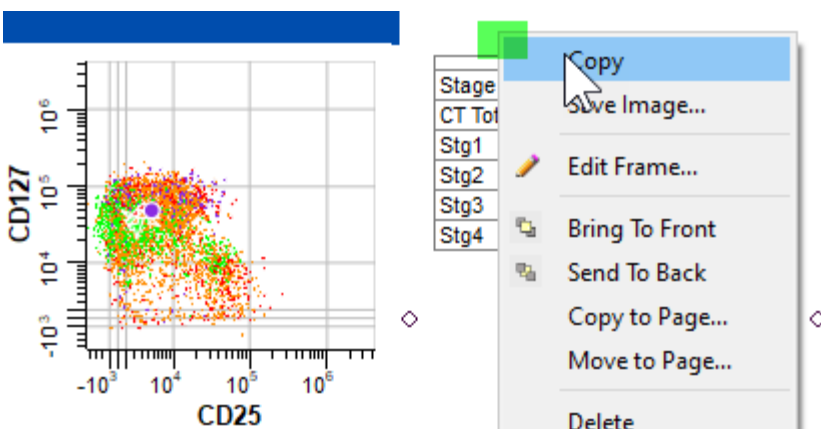
Copy and Paste Results

Most of the information for this table already appears in tables on the population report pages, so you can copy and paste results to fill in this table more quickly.

CD4 T results

Select the **CD4 T** page of the report.

Right-click the top of the population stage table and choose **Copy**.



Select the **Summary** page of the report.

Right-click the page and choose **Paste**. Move the pasted table next to the summary table.

| | Count | % Cell Type | % Total |
|--------------|-------|-------------|---------|
| Intact Cells | | | |
| Lymphocytes | | | |
| CD3 T | | | |
| CD4 T | | | |
| Naive | | | |
| CM | | | |
| EM | | | |
| TE | | | |
| Tregs | | | |
| CD8 T | | | |

| Stage | % CT | % T | Events |
|----------|-------|------|--------|
| CT Total | 100 | 8.83 | 17037 |
| Stg1 | 31.67 | 2.80 | 5396 |
| Stg2 | 50.68 | 4.48 | 8634 |
| Stg3 | 11.19 | 0.99 | 1907 |
| Stg4 | 6.46 | 0.57 | 1100 |

↑
**Pasted results from
CD4 T report.**

Next, you **copy** each statistic from the CD4 T table and **paste** it into the appropriate column of the summary table.

Click the first number in the **Events** column and press **Ctrl-C** on the keyboard to copy the content.

Click the **Count** column next to **CD4 T** and press **Ctrl-V** to paste into the cell.

In our summary table, we have given the CD4 T stages their common names. The results for **Stg1** will be copied into the table row for **Naive**. Similarly, **Stg2** is copied to **CM**, **Stg3** is copied into **EM**, and **Stg4** is copied into **TE**.

Repeat this process for each of the numbers in the CD4 T table, pasting them into the appropriate cell in the summary table.

| | Count | % Cell Type | % Total |
|--------------|-------|-------------|---------|
| Intact Cells | | | |
| Lymphocytes | | | |
| CD3 T | | | |
| CD4 T | 17137 | 100 | 8.88 |
| Naive | 5469 | 31.91 | 2.84 |
| CM | 8629 | 50.35 | 4.47 |
| EM | 1882 | 10.98 | 0.98 |
| TE | 1157 | 6.75 | 0.60 |

| Stage | % CT | % T | Events |
|----------|-------|------|--------|
| CT Total | 100 | 8.88 | 17137 |
| Stg1 | 31.91 | 2.84 | 5469 |
| Stg2 | 50.35 | 4.47 | 8629 |
| Stg3 | 10.98 | 0.98 | 1882 |
| Stg4 | 6.75 | 0.60 | 1157 |

Delete the CD4 T table that you pasted to this page; it is not needed.

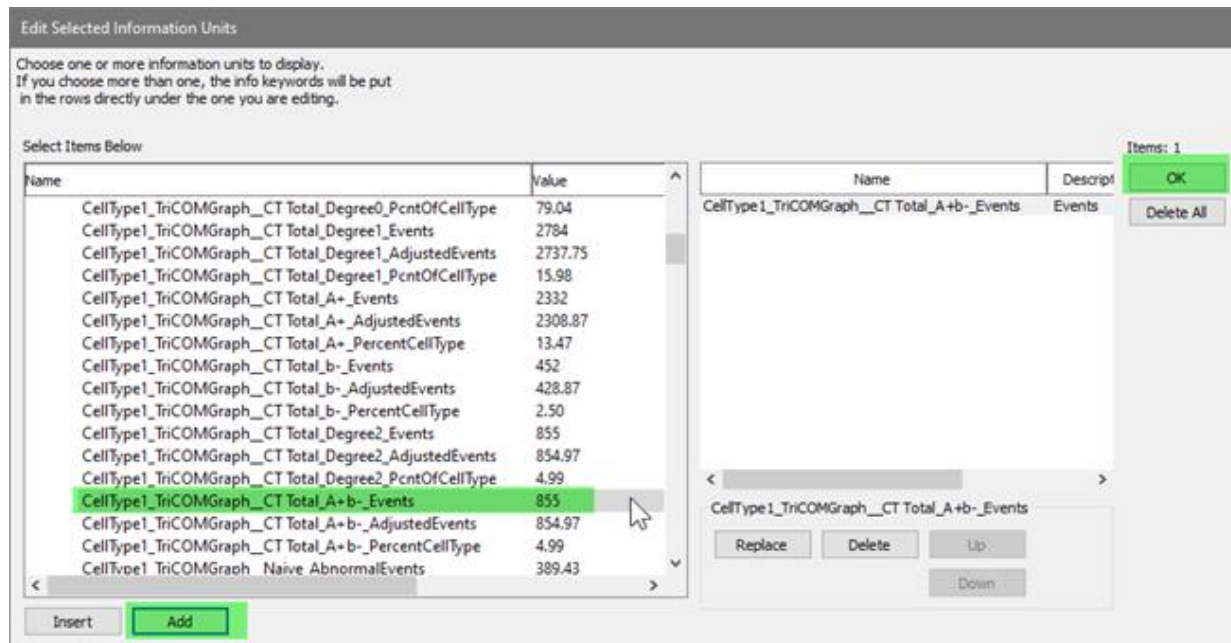
You may have noticed that you did not actually copy *numbers*. Instead, the content you have copied is a kind of formula that identifies each statistic for the CD4 T cells. This formula will allow the program to update the values when you change the analysis or read a new FCS file into this template.

To fill-in the results for the CD4 Tregs, let's find them in the information dialog.

Click the cell next to **Tregs** to activate it, then right-click in the cell.

In the **Edit Selected Information Units** dialog, select the **CellType1_TriCOMGraph** section and click the arrow to open it up.

Select **CellType1_TriCOMGraph_CT Total_A+b-_Events**, click **Add** and click **OK**.



Click the **Tregs** cell for %Cell Type to activate it, then right-click in the cell.

In the **Edit Selected Information Units** dialog, select the **CellType1_TriCOMGraph** section and click the arrow to open it up.

Select **CellType1_TriCOMGraph_CT Total_A+b-_PercentCellType**, click **Add** and click **OK**.

Note, your results may differ from the ones shown below.

| | | | |
|-------|-------|-------|------|
| CD4 T | 17037 | 100 | 8.83 |
| Naive | 5396 | 31.67 | 2.80 |
| CM | 8634 | 50.68 | 4.48 |
| EM | 1907 | 11.19 | 0.99 |
| TE | 1100 | 6.46 | 0.57 |
| | | | |
| Tregs | 736 | 4.32 | |

Results from the other cell types

Let's repeat this pattern with the remaining cell types to populate the summary table with information.

For each population:

Select the page for the next cell type of the report.

Right-click the top of the population stage table and choose **Copy**.

Select the **Summary** page of the report.

Right-click the page and choose **Paste**. Move the pasted table next to the summary table.

Copy and **paste** each result from the pasted table into the appropriate cell of the summary table.

Note that for cell types with staging, the order of stages in the summary table matches the order of stages in the cell type.

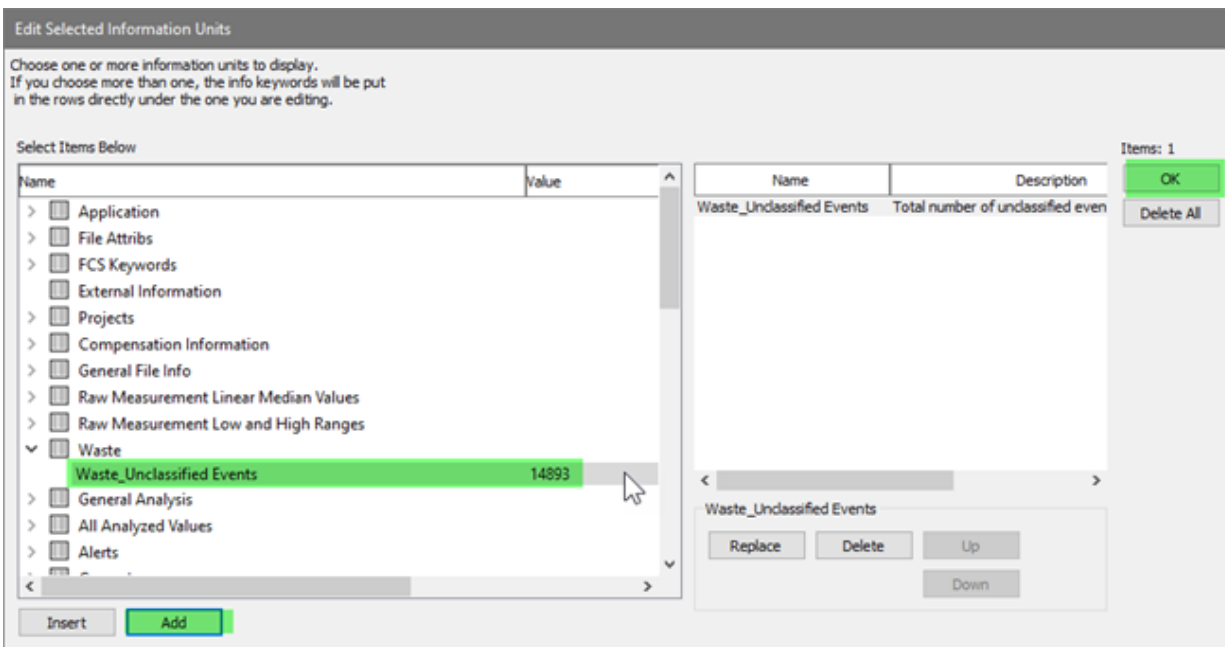
Delete the pasted table when you have finished copying results from it.

Add results for Intact Cells and Other

Move to the row for **Other** at the bottom of the table.

Click the cell for **Count** to select it, then right-click the cell.

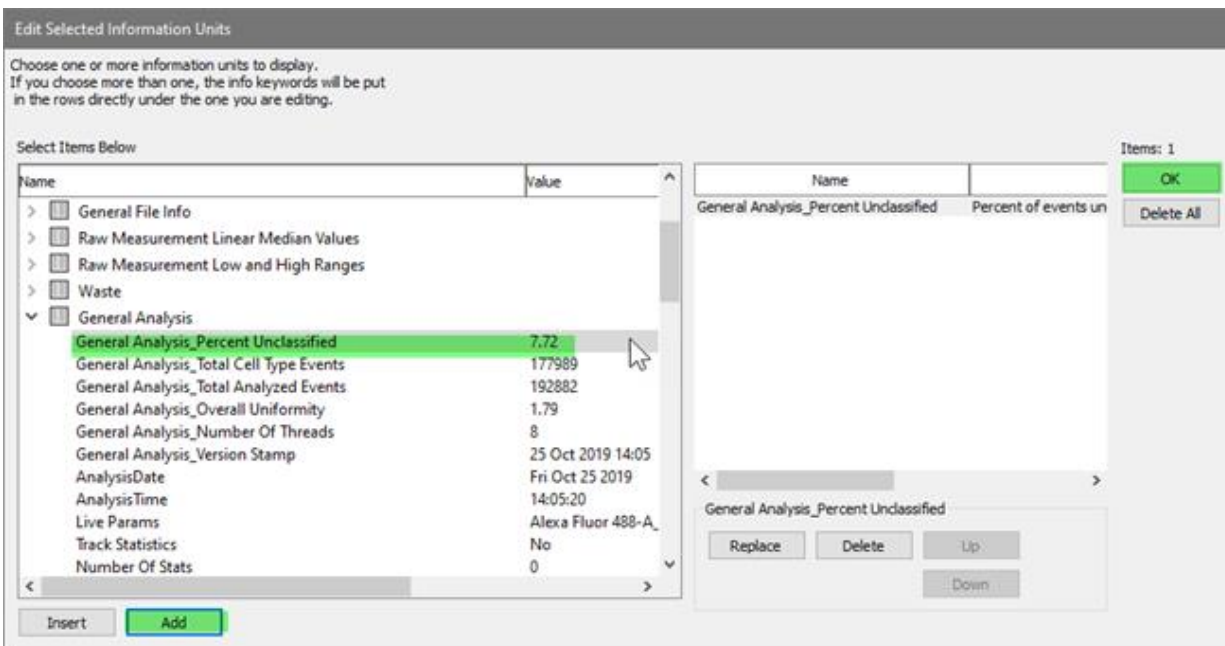
In the **Waste** section, select **Waste_Unclassified_Events**, click **Add** and click **OK**.



In the next column, type **N/A**.

Click the cell for **% Total** to select it, then right-click the cell.

In the **General Analysis** section, select **General Analysis_Percent Unclassified**, click **Add** and click **OK**.



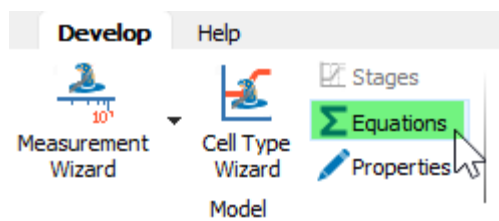
The summary table is now filled-in with most of the information we want to display.

| | Count | % Cell Type | % Total |
|---------------|--------|-------------|---------|
| Intact Cells | | | |
| Lymphocytes | | | |
| CD3 T | | | |
| CD4 T | 17037 | 100 | 8.83 |
| Naive | 5396 | 31.67 | 2.80 |
| CM | 8634 | 50.68 | 4.48 |
| EM | 1907 | 11.19 | 0.99 |
| TE | 1100 | 6.46 | 0.57 |
| Tregs | 736 | 4.32 | |
| CD8 T | 5864 | 100 | 3.04 |
| Naive/CM | 2028 | 34.58 | 1.05 |
| EM | 2718 | 46.35 | 1.41 |
| TE | 1118 | 19.07 | 0.58 |
| Gamma Delta T | 165 | 100 | 0.09 |
| B Cells | 3173 | 100 | 1.65 |
| Naive | 2158 | 68.01 | 1.12 |
| Memory | 1015 | 31.99 | 0.53 |
| NK Cells | 3658 | 100 | 1.90 |
| Monocytes | 6457 | 100 | 3.35 |
| Classical | 5662 | 87.69 | 2.94 |
| Non-Classical | 795 | 12.31 | 0.41 |
| Granulocytes | 136685 | 100 | 70.86 |
| Other | 19843 | N/A | 10.29 |

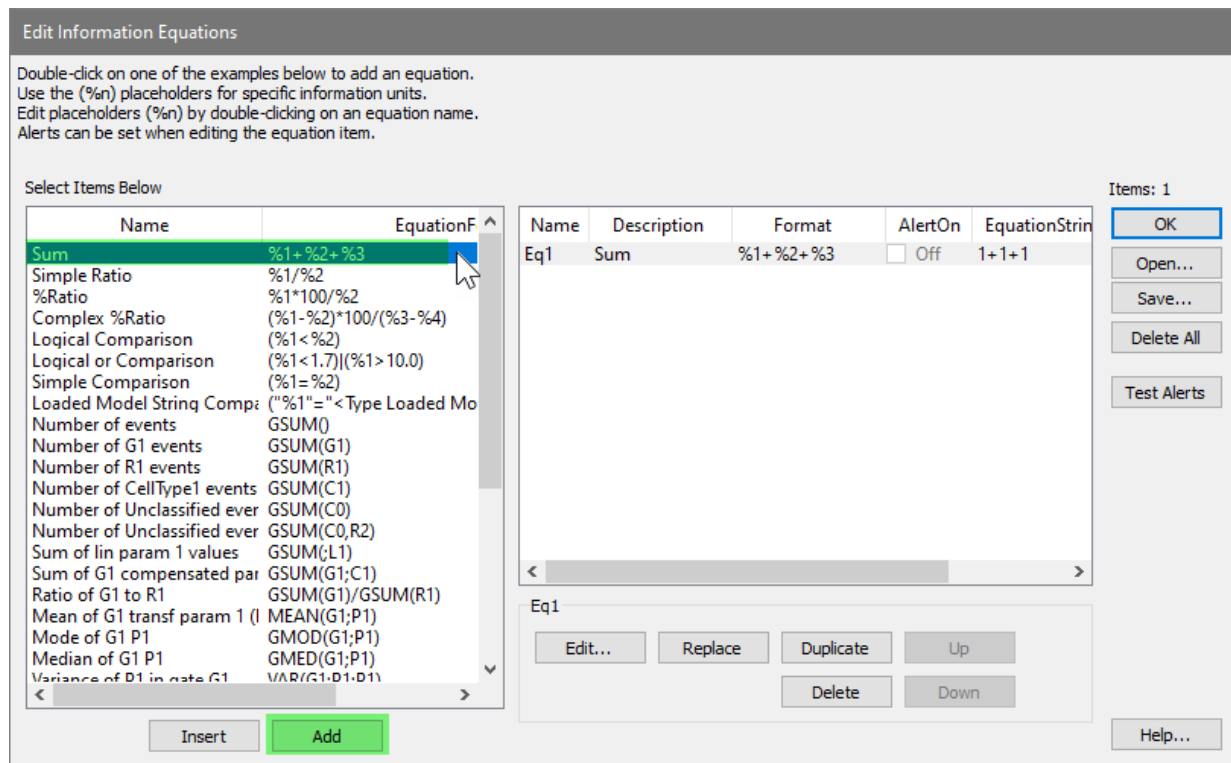
Create Equations for the Summary Table

The last statistics that we want to add do not exist yet. We did not create a cell type named "CD3 T" or "Lymphocytes", so there are not statistics for them. We need to create equations to compute these based on other statistics. GemStone has a tool for this purpose, which is discussed in detail in the [Equations](#) section. For this tutorial, we will only use the most common equation types: sums and percentages.

Click the **Equations** button on the Develop tab.



In the dialog that appears, click the first entry in the list on the left, **Sum**, and click the **Add** button.



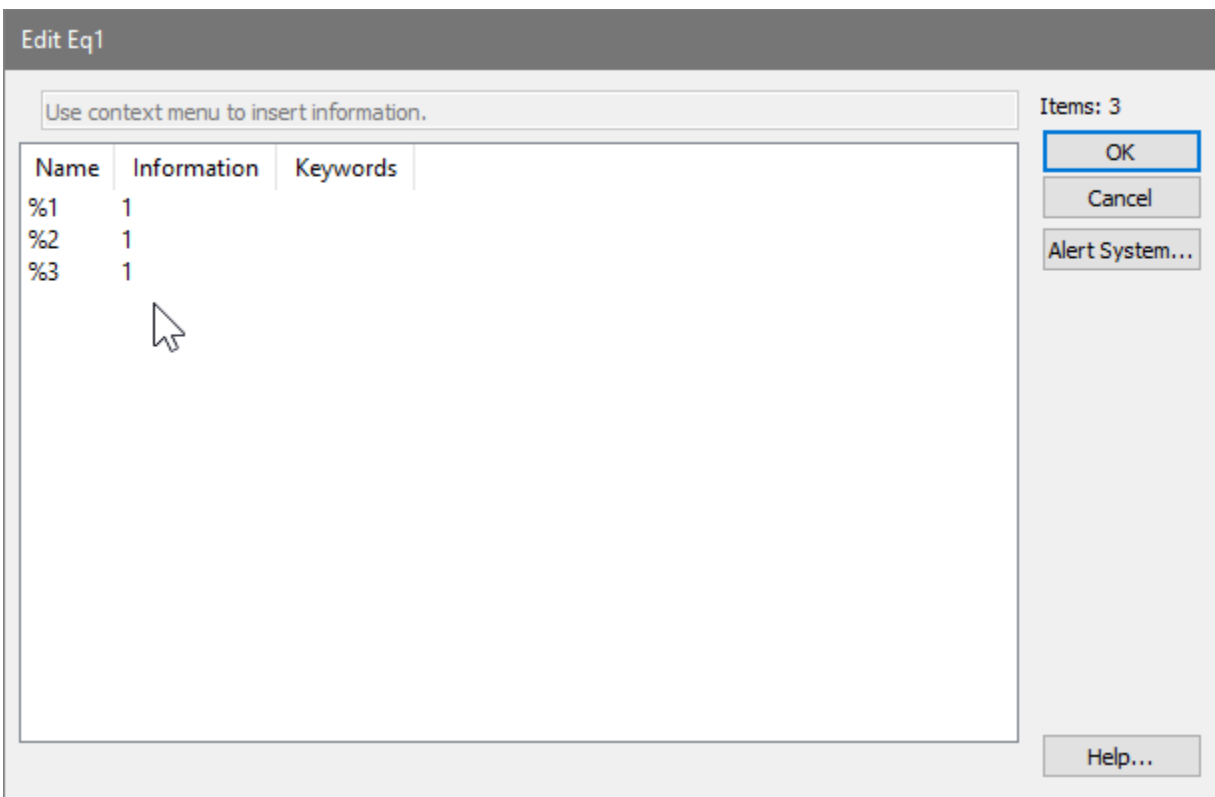
This adds an example Sum equation to the list. You will edit this equation so that it adds up the CD3 cell types.

Click the **Sum** text in the Description field and edit it to **CD3Events**.

| Name | Description | Format | AlertOn | EquationStrin |
|------|-------------|----------|------------------------------|---------------|
| Eq1 | CD3Events | %1+%2+%3 | <input type="checkbox"/> Off | 1+1+1 |

This equation is set up to add three numbers with the Format of **%1+%2+%3**. The parts of the equation that have % and a number are placeholders for some statistics that we want to substitute. In this case, we will substitute the CD4 T, CD8 T, and gd T events.

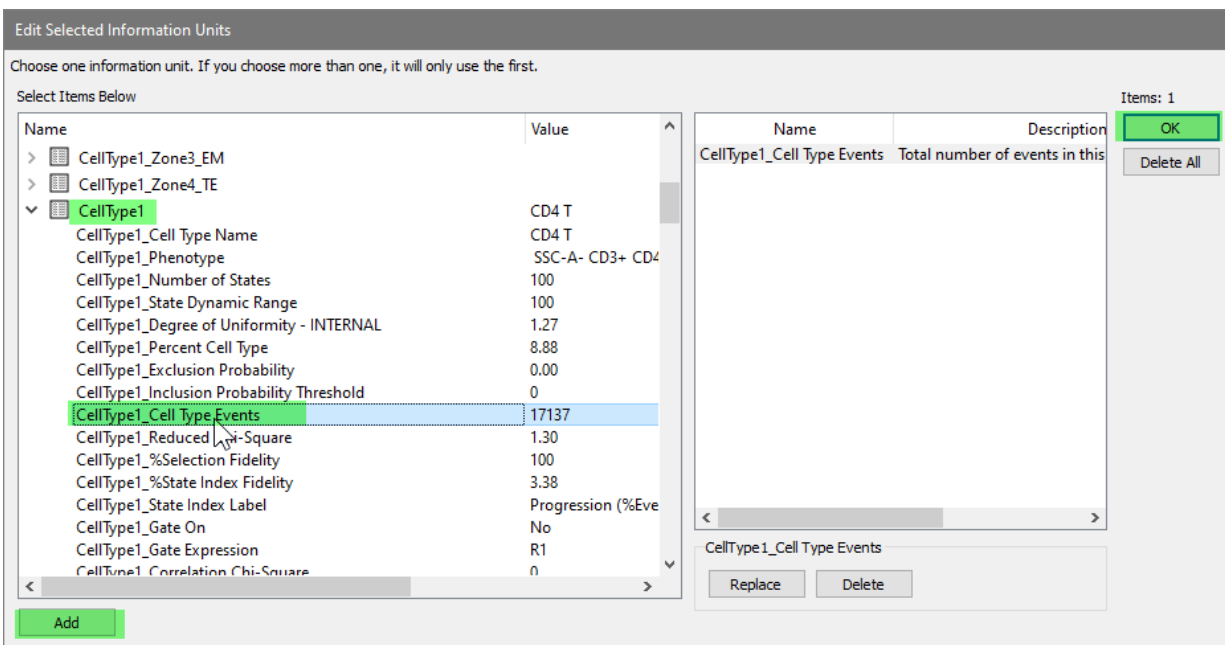
Double-click the **Eq1** name of the equation to edit what will be substituted for the placeholders.



We'll start with the CD4 T events.

Right-click the Information field next to **%1** to choose an information unit.

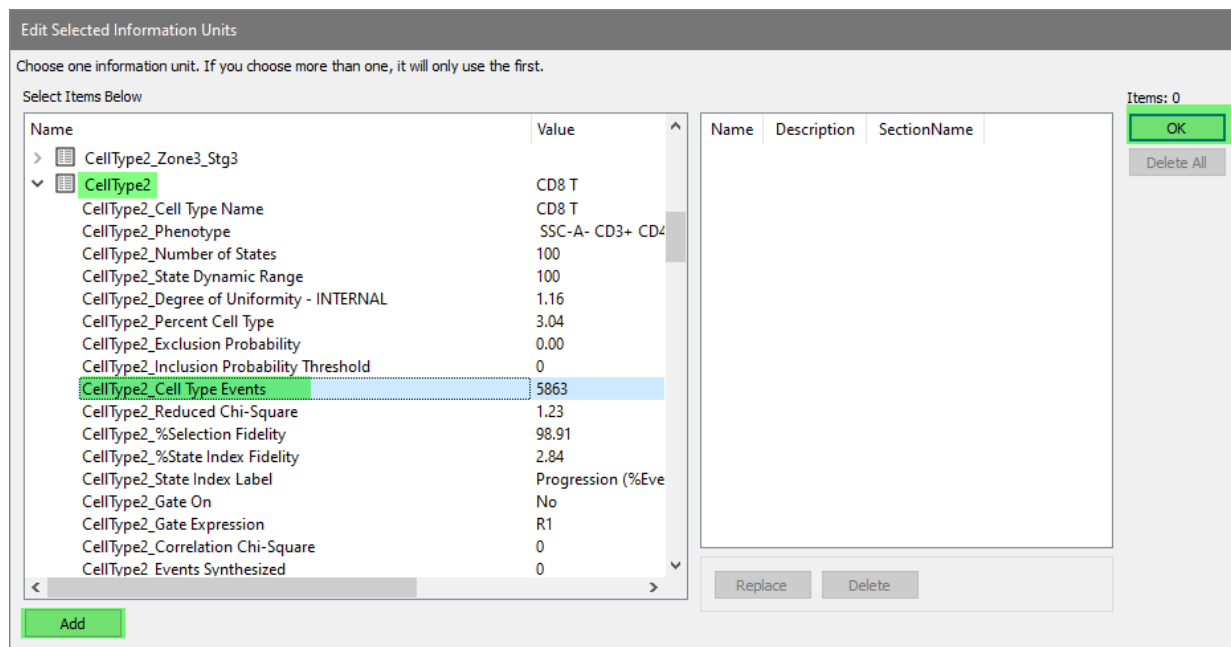
In the **Edit Selected Information Units** dialog, open the **CellType1** section, select **CellType1_Cell Type Events**, click **Add**, and click **OK**.



Next, let's add the CD8 T events.

Right-click the Information field next to **%2** to choose an information unit.

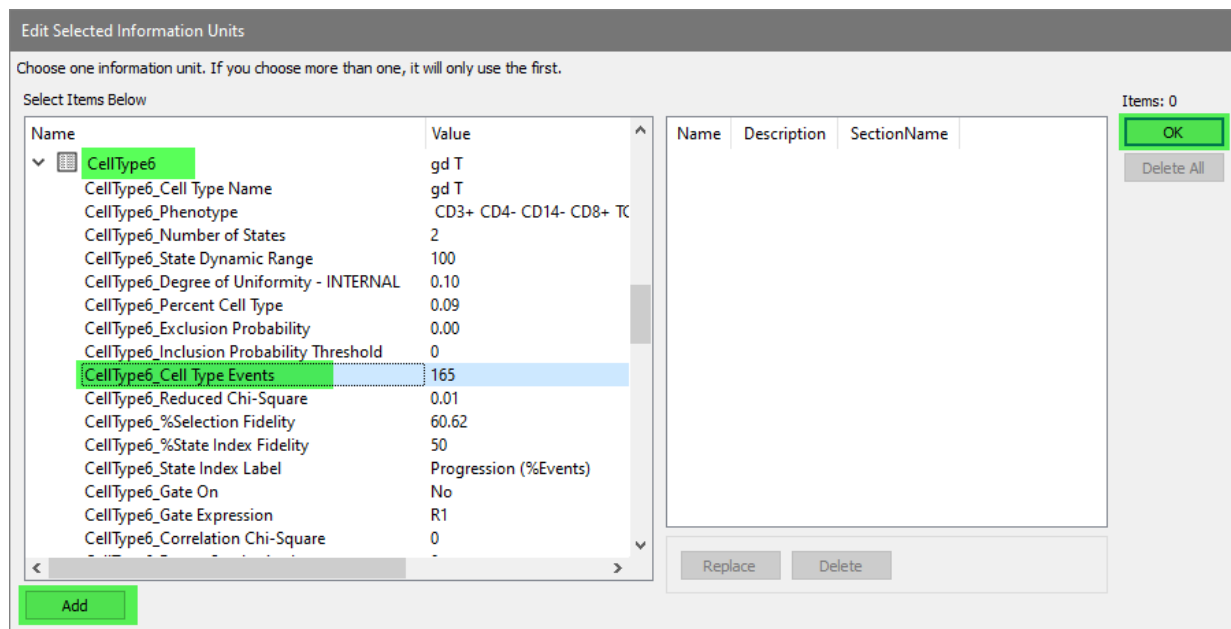
In the **Edit Selected Information Units** dialog, open the **CellType2** section, select **CellType2_Cell Type_Events**, click **Add**, and click **OK**.



Finally, we add the gd T events. CellType6 is the gd T cell type.

Right-click the Information field next to **%3** to choose an information unit.

In the **Edit Selected Information Units** dialog, open the **CellType6** section, select **CellType6_Cell Type_Events**, click **Add**, and click **OK**.



Click **OK** to close the Edit Eq1 dialog.

This equation now sums up the CD4 T, CD8 T, and gd T events so that we have a total of CD3 T events.

Let's do the same thing for the Lymphocytes. This will be another Sum type equation that will add CD3 T, B cells, and NK cells.

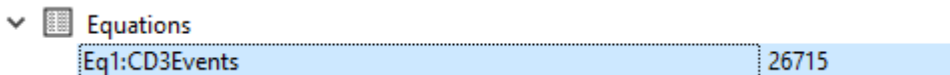
Select the **Sum** example equation in the left listbox and click **Add**.

Edit the Description to **Lymphocytes**.

Double-click the **Eq2** label to edit the placeholders.

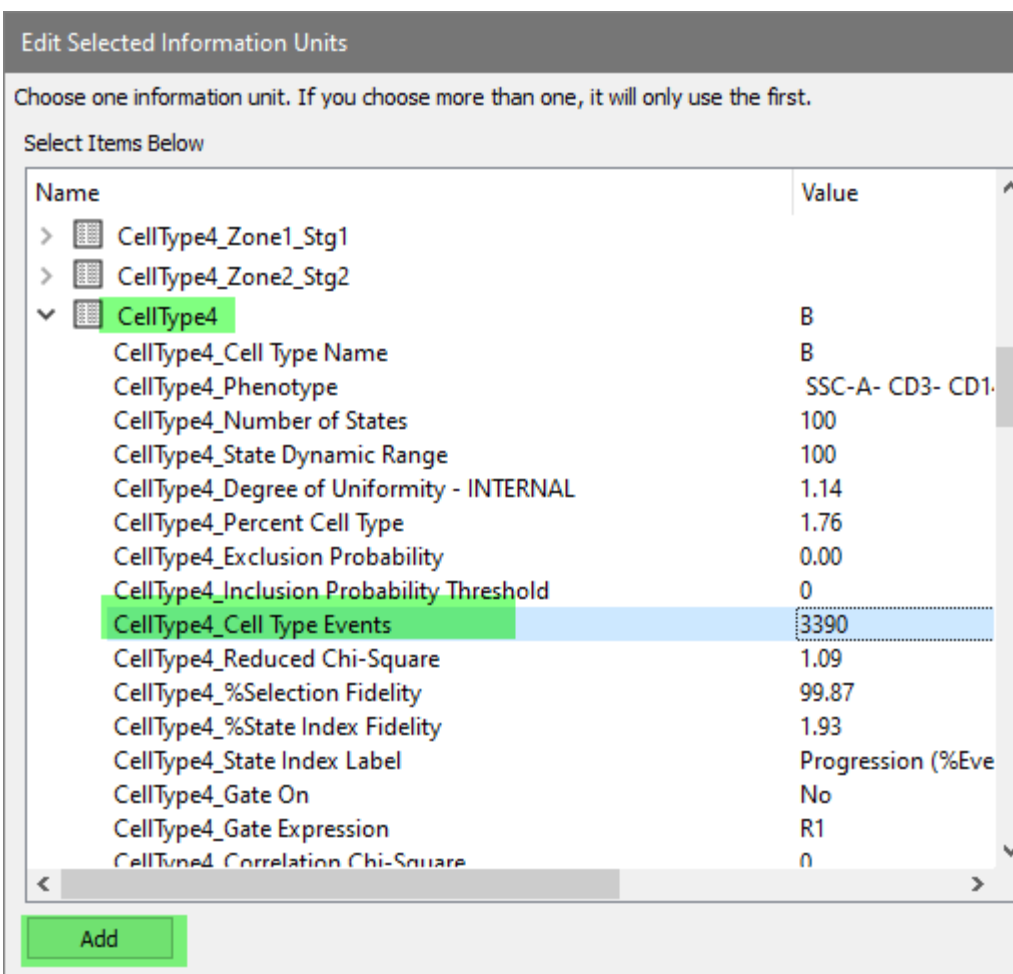
Right-click the **%1** Information field.

Open the **Equations** section and select **Eq1:CD3Events**. Click **Add** and click **OK** to use our equation for CD3 events for the %1 placeholder.



Right-click the **%2** Information field.

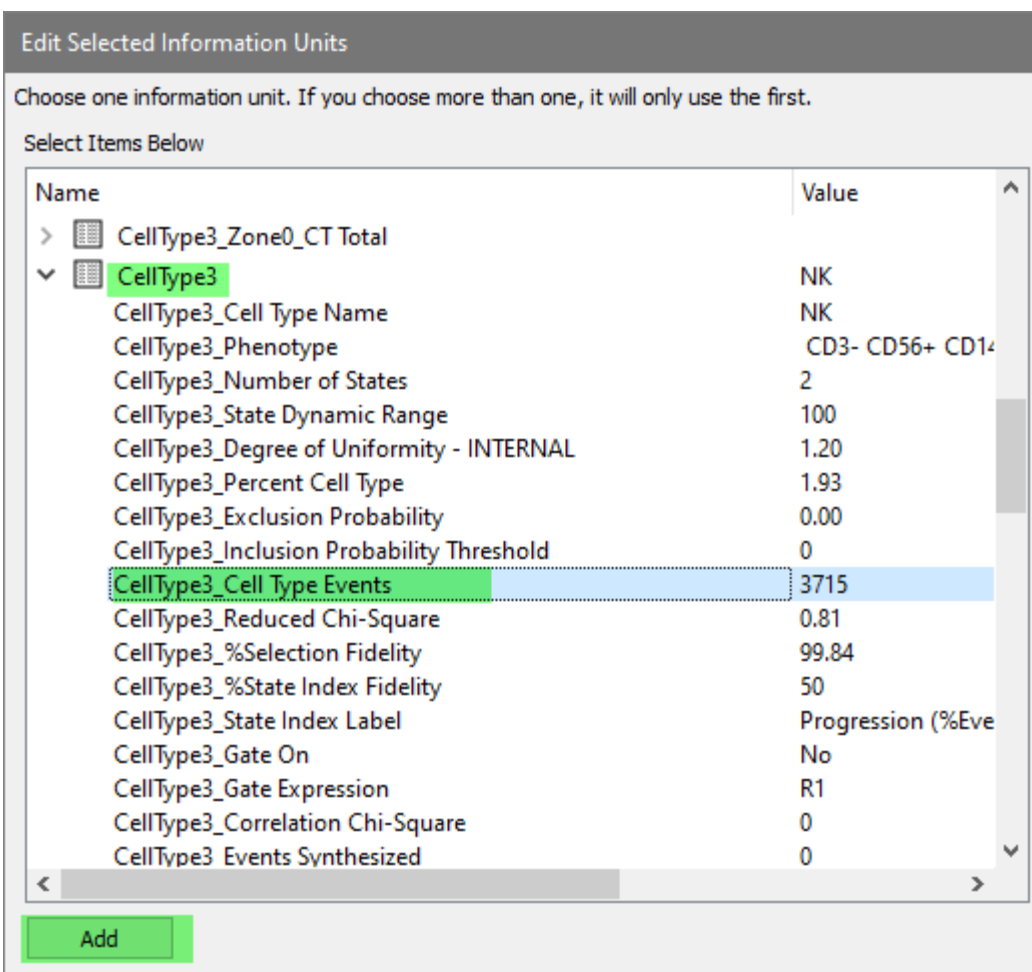
Select **CellType4_Cell Type_Events**, click **Add**, and click **OK** to use B cell events for %2.



Right-click the **%3** Information field.

Select **CellType3_Cell Type_Events**, click **Add**, and click **OK** to use NK cell events for %3.

Click **OK** to close the Edit Eq2 dialog.



There are three more equations to create: percentages for CD3 T, Lymphocytes, and Intact cells.

Select the **%Ratio** example equation from the list on the left and click **Add**.

Edit the **Description** to be **CD3Percent**.

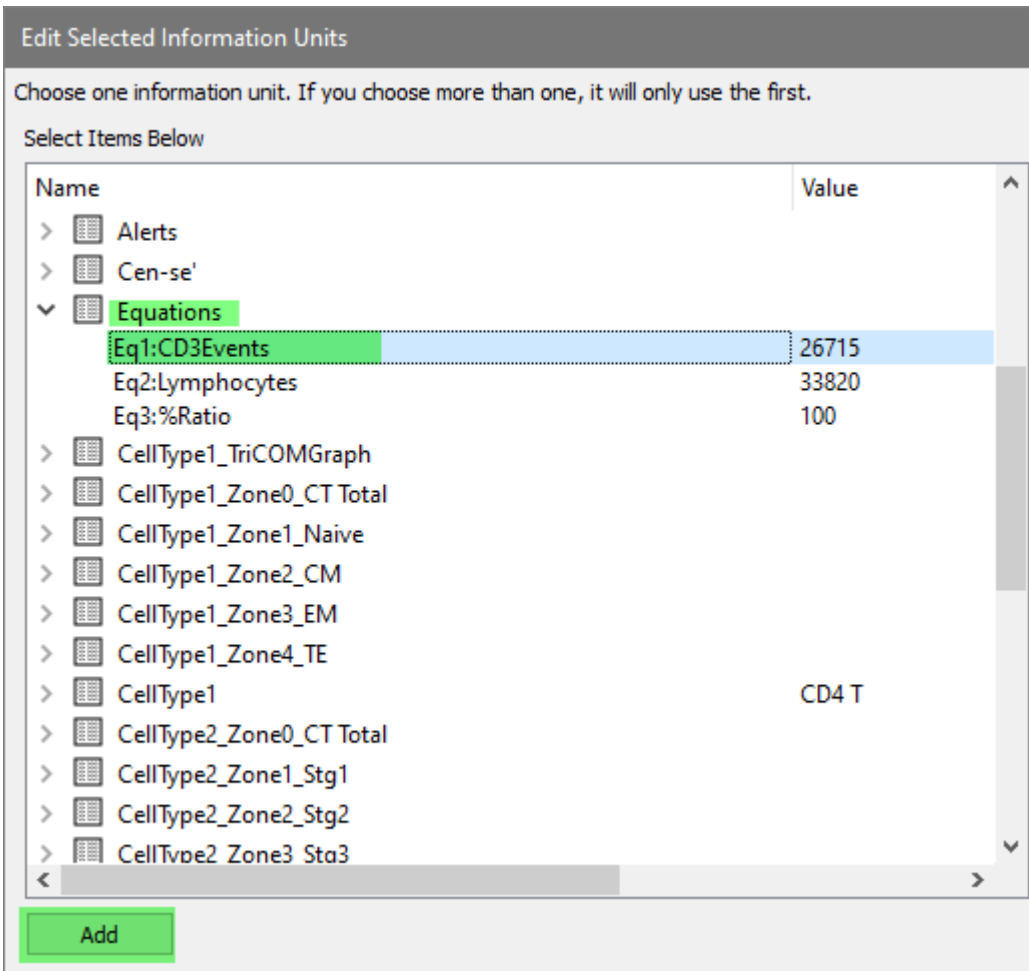
| Name | Description | Format | Alert | Equation |
|------|-------------|-----------|------------------------------|---|
| Eq1 | CD3Events | %1+%2+%3 | <input type="checkbox"/> Off | [CellType1_Cell Type Events]+[CellType2_Cell Type Eve |
| Eq2 | Lymphocytes | %1+%2+%3 | <input type="checkbox"/> Off | [Eq1]+[CellType4_Cell Type Events]+[CellType3_Cell Ty |
| Eq3 | CD3Percent | %1*100/%2 | <input type="checkbox"/> Off | 1*100/1 |

This equation's format is designed to compute a percentage of the first argument to the second. We will choose Eq1:CD3Events as the first argument and the total cells analyzed for the second.

Double-click **Eq3** to edit its placeholders.

Right-click the **%1** Information field.

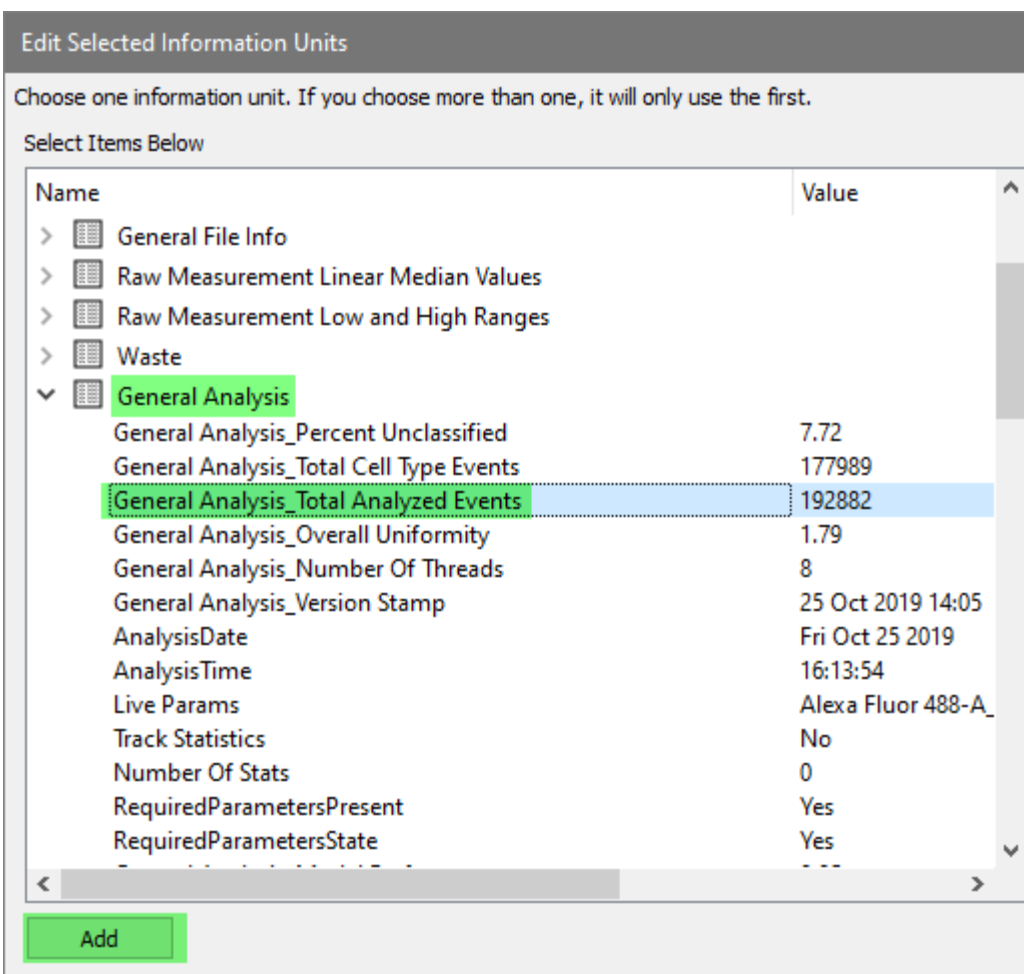
Open the **Equations** section and select **Eq1:CD3Events**. Click **Add** and click **OK** to use our equation for CD3 events for the %1 placeholder.



Right-click the **%2** Information field.

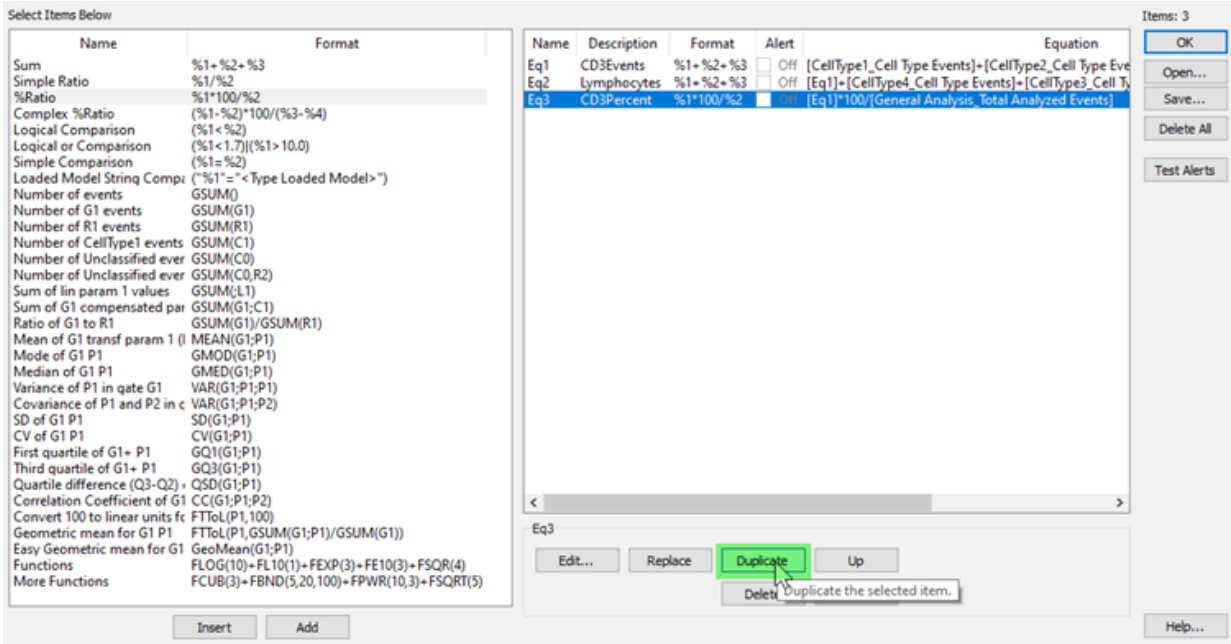
In the General Analysis section select **General Analysis_Total Analyzed Events**, click **Add**, and click **OK**.

Click **OK** to close the Edit Eq3 dialog.

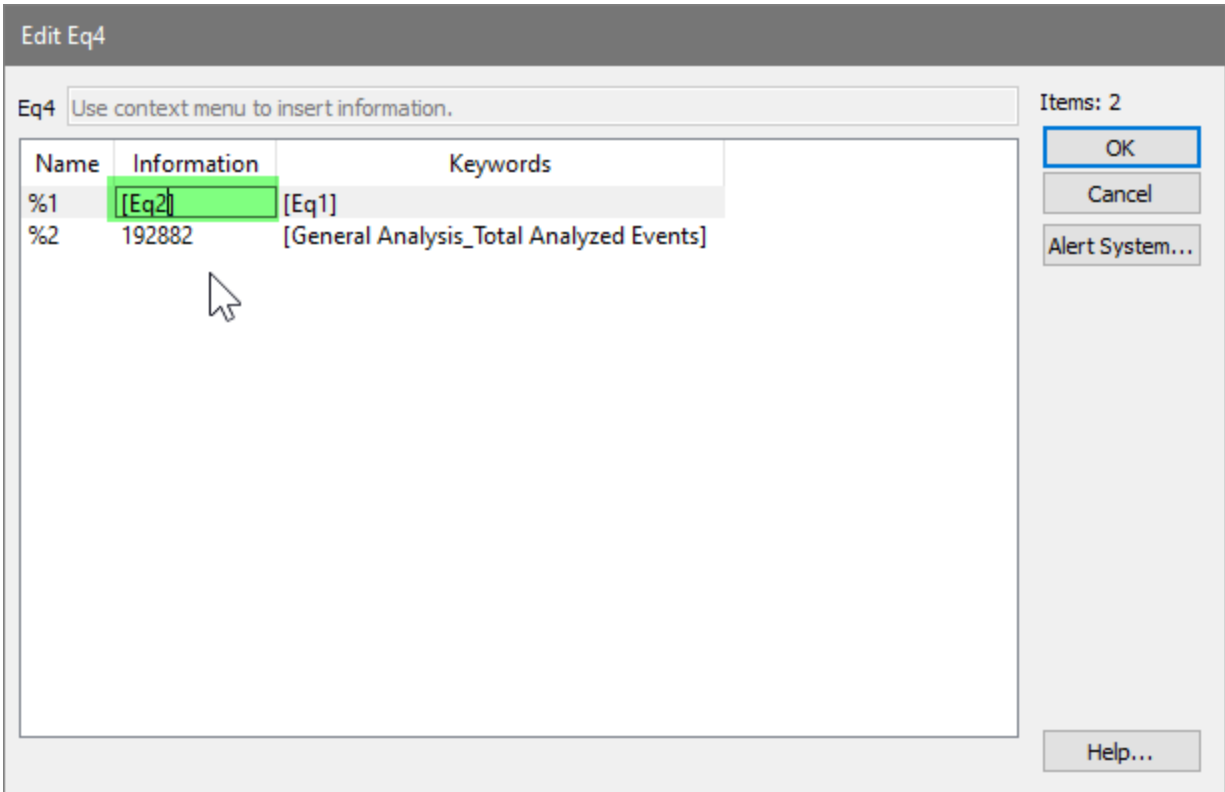


Since the next equation is so similar to the one we just finished, we'll duplicate it as a starting point.

Select **Eq3** in the list and click **Duplicate**.



Edit the Description to **LymphocytePercent**, and double click **Eq4** to edit its placeholders.
 We can directly edit the text of the Information column for **%1** placeholder.
 Click the Information column for **%1**.
 Edit the text to read **[Eq2]**. This is the Lymphocyte event count equation.
 Click **OK**.



Now for the last equation.

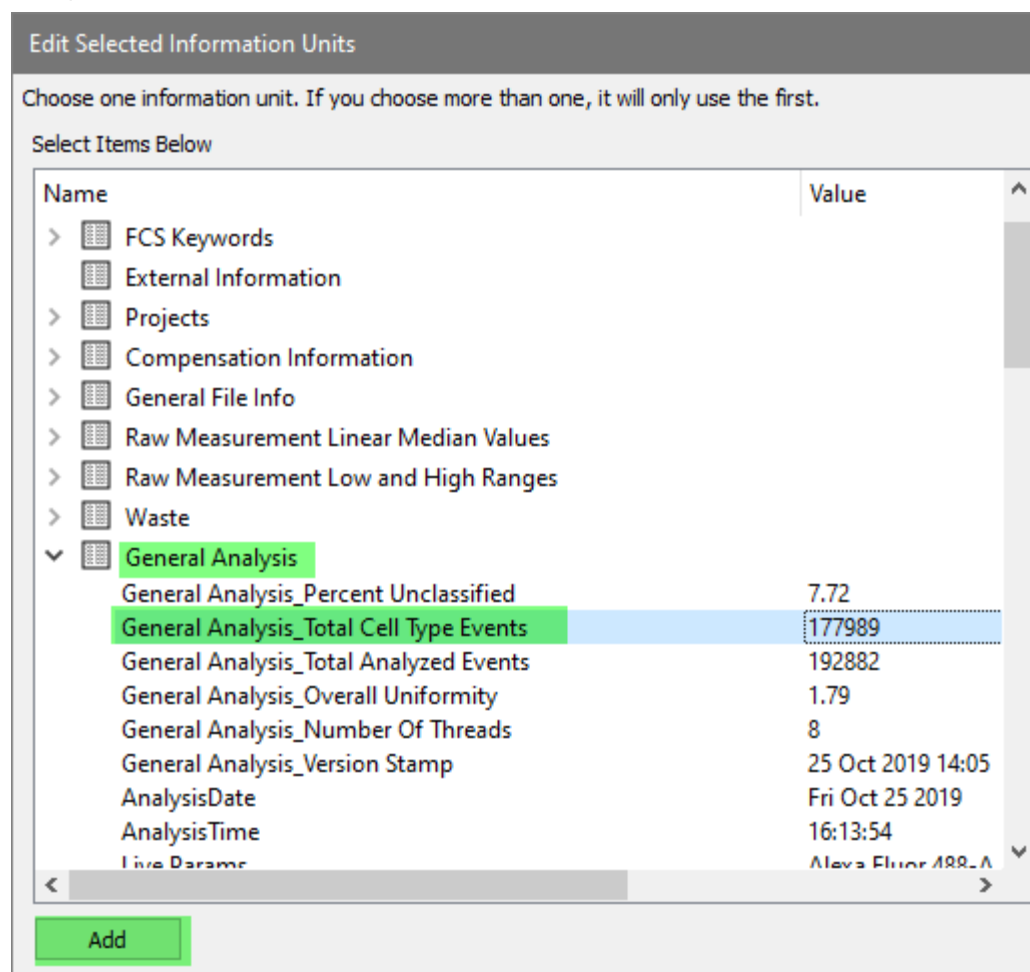
Select **Eq3** in the list and click **Duplicate**.

Edit the Description to **IntactPercent**, and double click **Eq5** to edit its placeholders.

We need to edit the first placeholder; the second one is correct.

Right-click the **%1** Information field.

In the General Analysis section select **General Analysis_Total Cell Type Events**, click **Add**, and click **OK**.



Click **OK** to close the Edit Eq5 dialog.

Click **OK** to close the Edit Information Equation dialog.

Yay! The equations we need are ready to go.

Finish the Summary Table

Let's finish the table with six more statistics.

Right-click the **Count** cell next to **Intact Cells**.

In the **General Analysis** section select **General Analysis_Total Cell Type Events**.

| | |
|---|--------|
| General Analysis_Percent Unclassified | 7.72 |
| General Analysis_Total Cell Type Events | 177989 |
| General Analysis_Total Analyzed Events | 192882 |

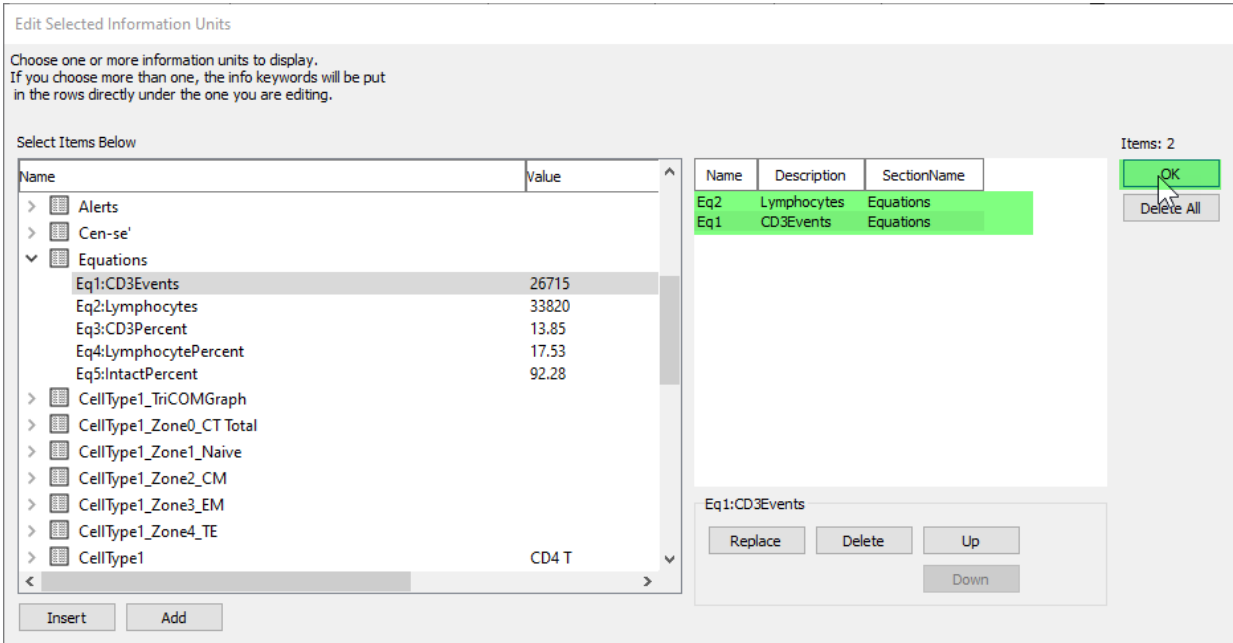
Click **Add** and click **OK**.

For the next two Count cells, we will use equations Eq2:Lymphocytes and Eq1:CD3Events. Since these two values will be in the next two rows, we can add them both at the same time.

Right-click the **Count** cell next to **Lymphocytes**.

In the **Equations** section select **Eq2:Lymphocytes** and click **Add**.

Select **Eq1:CD3Events** and click **Add**. Click **OK**.



In the **% Cell Type** column for the first three rows, type **N/A**.

The last three additions to the table are so simple you can just type them in!

In the **%Total** column for **Intact Cells**, type **[Eq5]**. This is the equation for Intact cells as a percent of total.

In the **%Total** column for **Lymphocytes**, type **[Eq4]**. This is the equation for Lymphocytes as a percent of total.

In the **%Total** column for **CD3 T**, type **[Eq3]**. This is the equation for CD3 T cells as a percent of total.

Again note, your results may differ from those shown.

| | Count | % Cell Type | % Total |
|--------------|--------|-------------|---------|
| Intact Cells | 177989 | N/A | 92.28 |
| Lymphocytes | 33820 | N/A | 17.53 |
| CD3 T | 26715 | N/A | 14.95 |
| CD4 T | 17137 | 100 | 8.88 |
| Naive | 5160 | 30.04 | 2.84 |




Well done, that's a lot of information!

Adjust the size and position of the table to be centered in the page. You can also adjust the width of the columns.

Use the **Text** tool to add a text object above the table, and edit it to **Summary**. Edit its properties to use an 18pt font and centered alignment.

Here's the finished summary page.


GemStone™ Report
Tue Dec 3 2019

File: 16-color_1.fcs

Summary

| | Count | % Cell Type | % Total |
|---------------|--------|-------------|---------|
| Intact Cells | 173039 | N/A | 89.71 |
| Lymphocytes | 33390 | N/A | 17.31 |
| CD3 T | 26559 | N/A | 13.77 |
| CD4 T | 17037 | 100 | 8.83 |
| Naive | 5396 | 31.67 | 2.80 |
| CM | 8634 | 50.68 | 4.48 |
| EM | 1907 | 11.19 | 0.99 |
| TE | 1100 | 6.46 | 0.57 |
| Tregs | 736 | 4.32 | |
| CD8 T | 5864 | 100 | 3.04 |
| Naive/CM | 2028 | 34.58 | 1.05 |
| EM | 2718 | 46.35 | 1.41 |
| TE | 1118 | 19.07 | 0.58 |
| Gamma Delta T | 165 | 100 | 0.09 |
| B Cells | 3173 | 100 | 1.65 |
| Naive | 2158 | 68.01 | 1.12 |
| Memory | 1015 | 31.99 | 0.53 |
| NK Cells | 3658 | 100 | 1.90 |
| Monocytes | 6457 | 100 | 3.35 |
| Classical | 5862 | 87.89 | 2.94 |
| Non-Classical | 795 | 12.31 | 0.41 |
| Granulocytes | 136685 | 100 | 70.86 |
| Other | 19843 | N/A | 10.29 |

New Project Tutorial
Page 1
TemplateModel.gs (03 Dec 2019 17:18)

Save your Work

Click the **Save** button in the Template section of the Develop tab.



Summary

In this last tutorial of the Develop series, you polished up the report with additional graphics and a summary page.

On the summary page you created a large table and typed in labels for the columns and rows. For each cell type, you copied a table from the cell type report page to the summary page, and then copied cells from that table into the summary table. You added a few other statistics by right-clicking a cell and picking the item from the Information tree.

In order to complete the table, you learned how to create equations. The equations added up several numbers to give us the totals for CD3 T events, Lymphocytes, and Intact cells. Next, you added equations that computed percentages for the newly-added summation equations.

Once the equations were created, you added them to the appropriate locations on the summary table.

This is now a well-developed Project template to analyze files like the ones you added to it. You can create new Projects based on this one so that you can leverage the work you have done building this.

You are now ready to start building for your own panels. Happy developing!

Stain Assessment

Overview

When you add a new measurement to your model, it is a good idea to evaluate how well the staining of the new marker is working. GemStone has a stain assessment tool to help assess the quality of a measurement, based on a cell type that is positive for the measurement and another cell type that is negative for the measurement.

In this tutorial, you will extend the project created in the Develop series of tutorials to include stain assessment for several measurements.

Prerequisites

This tutorial continues to build the Project that was started in the following tutorials:

[Develop: Starting a New Project from Scratch](#)

[Develop: Adding Cell Types to the Project](#)

[Develop: Analyzing Subsets](#)

[Develop: Exploring with Cen-se'](#)

[Develop: Additional Exercises](#)

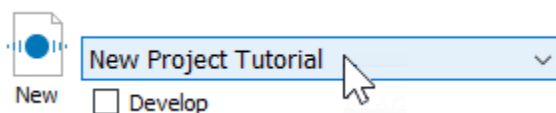
[Develop: Finishing the Project](#)

Start GemStone

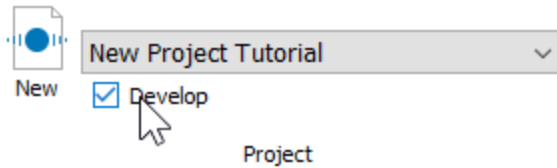
Start Gemstone by following the instructions in the [Installation and Setup of GemStone](#) section.

Open the Project

Open the "New Project Tutorial" Project that you created in the previous tutorials by selecting it in the Project dropdown list.

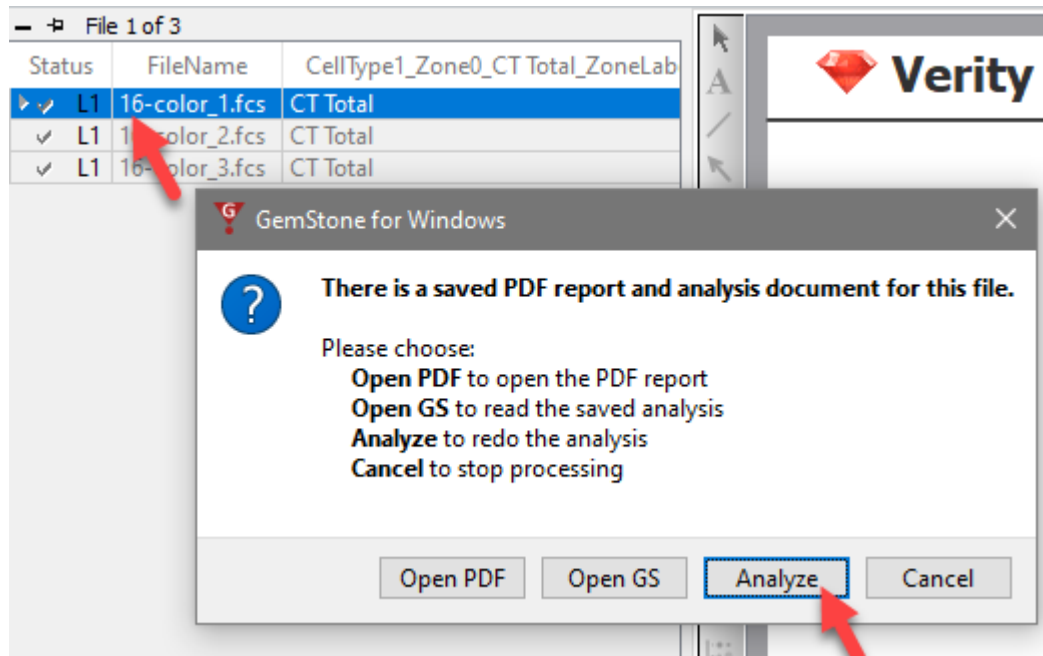


Click the **Develop** checkbox to enable develop mode.



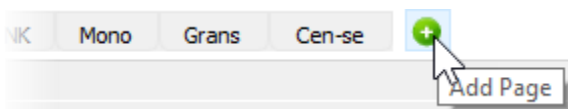
Analyze a File

Double-click the first file in the batch list. A dialog of choices will be displayed. Click **Analyze** to reanalyze the file with the latest model template revisions.

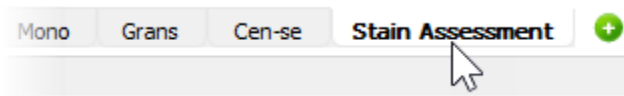


Create a Stain Assessment report page

Click the Add Page button next to the last page tab.



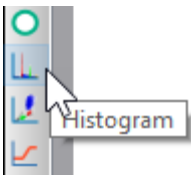
Right-click the new page and choose **Rename**. Type **Stain Assessment** for the page name.



Adding a histogram

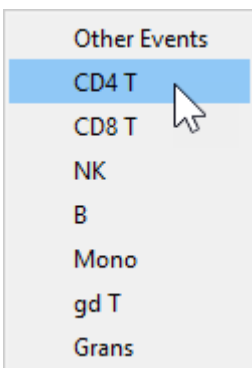
Let's add a 1P plot to the stain assessment page that will compare the CD3 staining between the CD4 T and B cell types.

Click the **Histogram** report tool button.



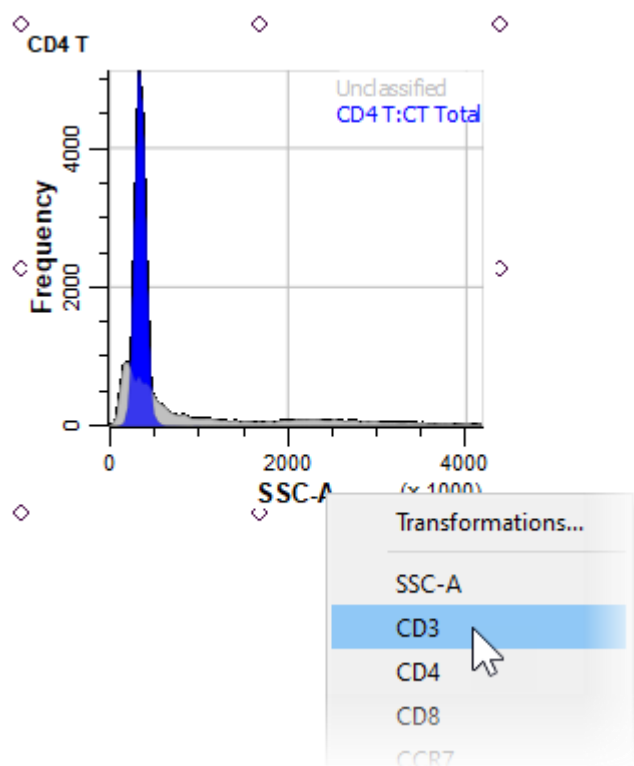
Click and drag on the report to define the histogram size and location. Alternatively, you can press Shift and click the mouse where you want to add the graphic on the report.

A menu of cell types will be displayed. Choose **CD4 T** for the cell type.



If you need to adjust the size or position of the histogram, click the white space of the axis area and use the handles to make adjustments.

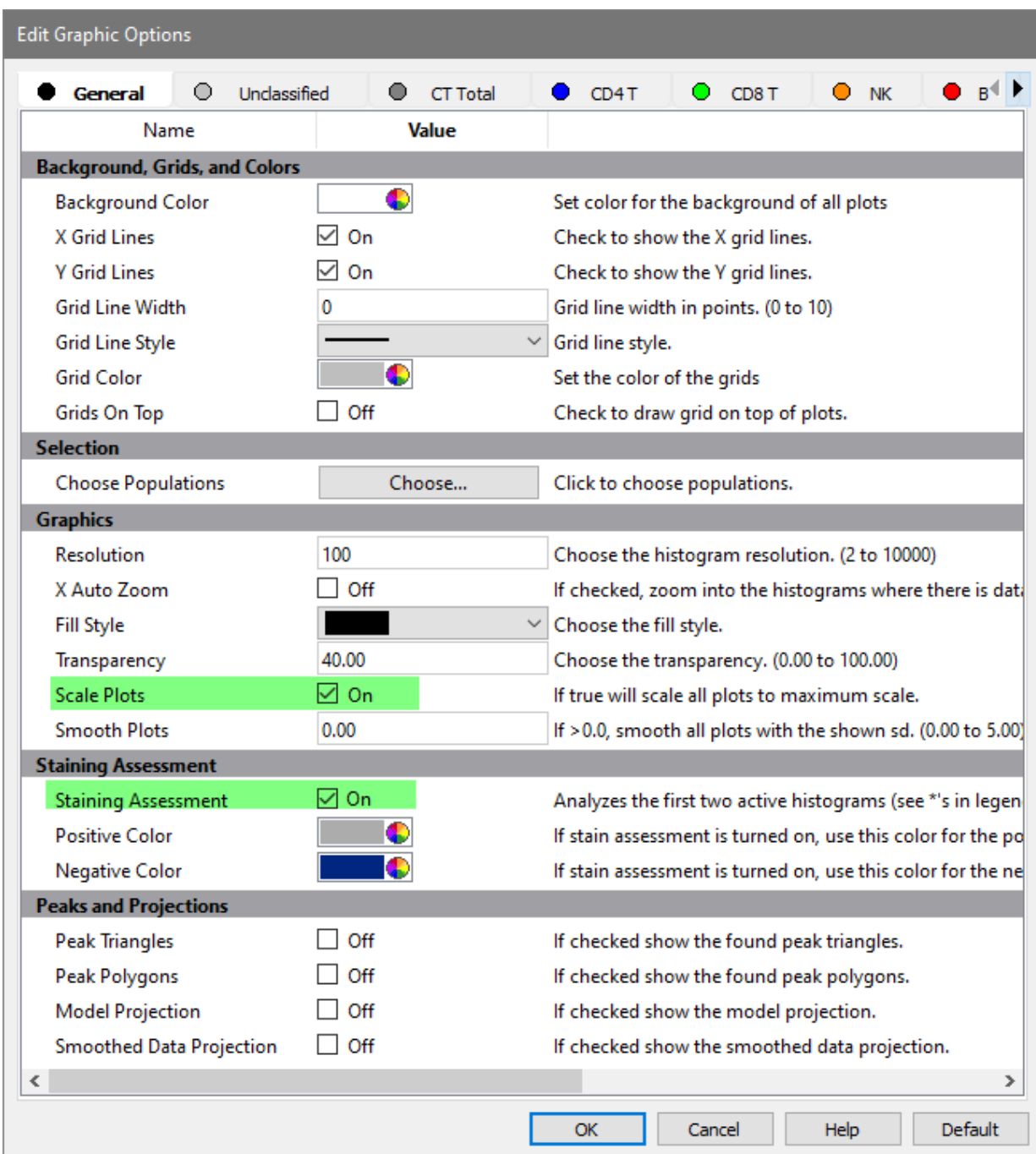
Click the X-axis label and choose **CD3**.



Overlaying Populations in the Histogram

We want to compare CD3 between the CD4 T and B cell type. The B cells should not express CD3, so it will serve as a good, negative control. The histogram can overlay populations from different cell types, so it will serve the purpose nicely.

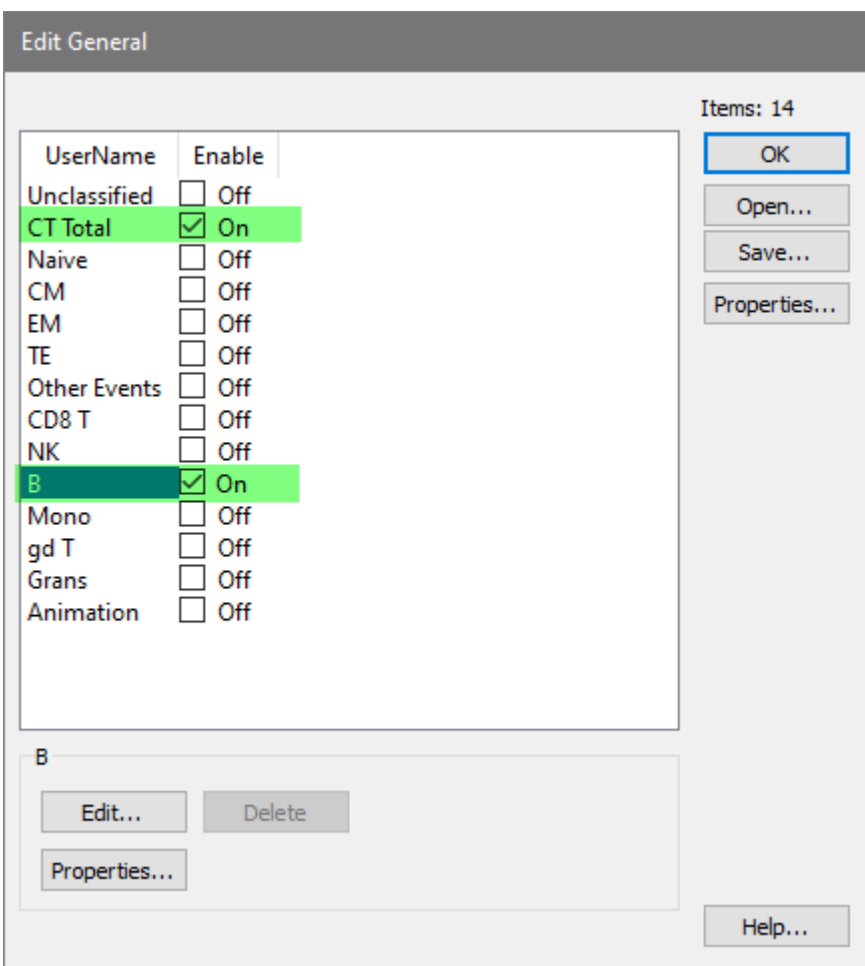
Double-click in the histogram plot area. The Edit Graphic Options dialog is displayed. Enable the **Scale Plots** and the **Staining Assessment** options.



Click the **Choose** button for Choose Population in the Selection section of the dialog.



Check **CT Total** and **B** cell type, and make sure all others are unchecked as shown below. CT Total in this case means the all events in the CD4 T cell type.



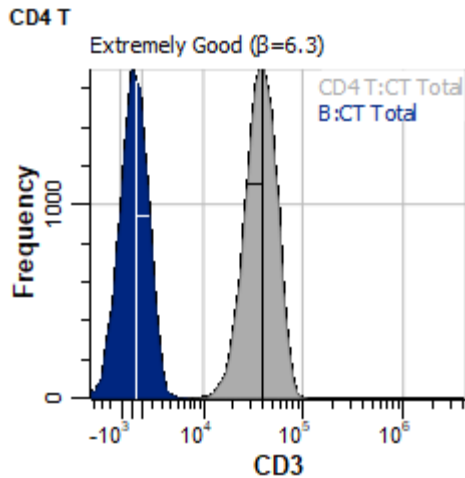
Click **OK**.

Click **OK** again to close the Edit Graphic Options dialog.

On the **Develop** tab, click **Classify** to reanalyze the data and perform the stain assessment.



At this point, the new histogram shows an overlay of CD4 T and B cells for the CD3 measurement.



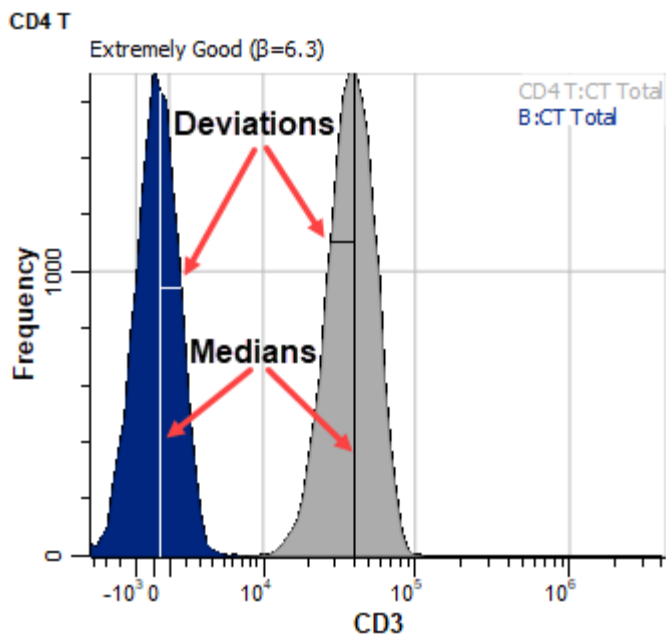
In the plot we can see that there is excellent separation between the positive and negative distributions. The text indicates "Extremely Good ($\beta=6.3$)". Your result may be slightly different. Let's take a look at what this assessment means.

Stain Assessment

When a histogram overlays a measurement from different cell types and you check the Stain Assessment option, GemStone performs additional calculations on the populations in the histogram. This system is based on a statistical approach to computing the difference between two populations, called **Strictly Standardized Mean Difference (SSMD)**. The SSMD can be estimated as

$$\hat{\beta} = \frac{\tilde{X}_P - \tilde{X}_N}{1.4826\sqrt{\tilde{s}_P^2 + \tilde{s}_N^2}},$$

where $\tilde{X}_P, \tilde{X}_N, \tilde{s}_P, \tilde{s}_N$ are the medians and median absolute deviations in the positive and negative controls, respectively. These are indicated in the graphical display.



The result of the equation is a unit-less number that expresses degree of difference. A look-up table is used to map the number to explanatory text, such as "Extremely Good". Values greater than 2.0 are strong, values between 2.0 and 1.0 are moderate, and values less than 1.0 are weak. With the CD3 measurement in this example, the staining is working very well to separate the positive and negative populations.

For more information on SSMD, review this Wikipedia article: https://en.wikipedia.org/wiki/Strictly_standardized_mean_difference

You can add as many stain assessment histograms as you need to your reports. For each measurement, choose a cell type that is the best "positive" and another cell type that should be "negative" for the measurement. In the absence of a negative cell type, you can use the Unclassified events as the negative population.

Save your Work

Click the **Save** button in the Template section of the Develop tab.



Summary

In this tutorial, you evaluated the quality of the CD3 staining using the histogram to overlay CD4 T and B cells. You enabled the stain assessment and selected a positive and negative population for the measurement.

For quality control purposes, you can create a stain assessment plot for each stain in your panels to verify that the stains are working correctly. The key for this approach is to identify one cell type that should be strongly positive for the stain and another that should be negative for the stain. Unclassified events can also be used in some cases as the negative population.

The stain assessment information can also be added to the database of results.

Reference

Reference

[File tab](#)

[Home tab](#)

[Insert tab](#)

[Options tab](#)

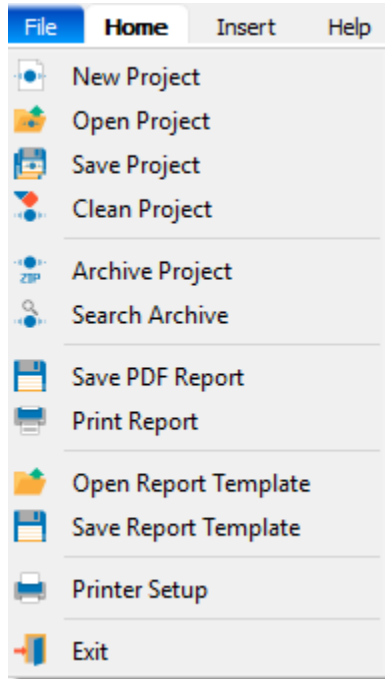
[Develop tab](#)

[Help tab](#)

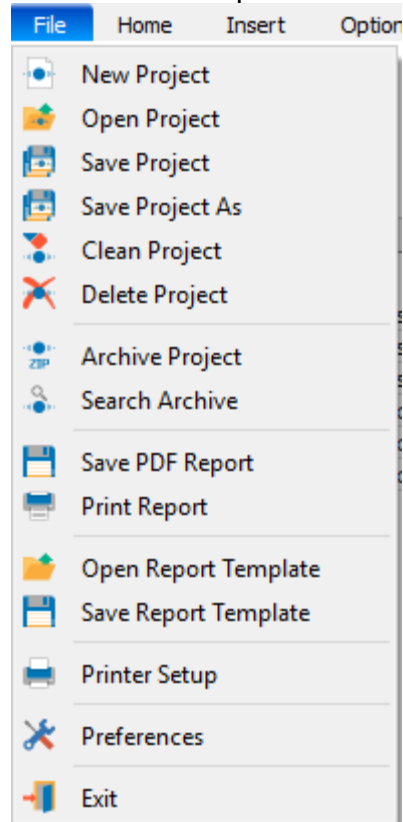
[Batch](#)

File tab**File Tab**

File tab in Run mode



File tab in Develop mode



[New Project](#)

[Open Project](#)

[Save Project](#)

[Save Project As](#)

[Clean Project](#)

[Delete Project](#)

[Archive Project](#)

[Search Archives](#)

[Save PDF Report](#)

[Print Report](#)

GemStone User Guide

[Open Report Template](#)

[Save Report Template](#)

[Printer Setup](#)

[Preferences](#)

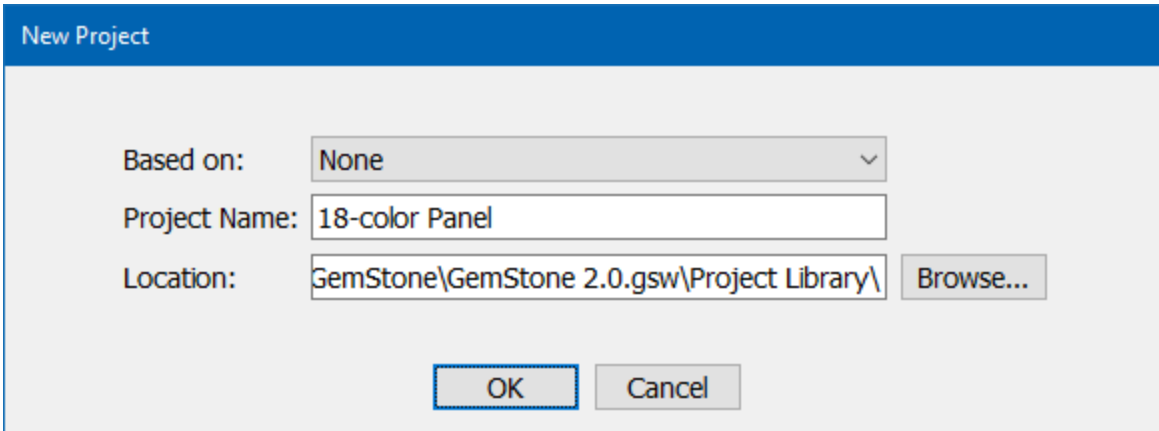
[Exit \(Quit\)](#)

New Project



This command creates a new Project. The command appears in the File menu and on the [Home](#) tab.

The New Project dialog is displayed.

A screenshot of the "New Project" dialog box. The dialog has a blue title bar with the text "New Project". Below the title bar, there are three input fields: "Based on:" with a dropdown menu showing "None", "Project Name:" with a text box containing "18-color Panel", and "Location:" with a text box containing "GemStone\GemStone 2.0.gsw\Project Library\" and a "Browse..." button to its right. At the bottom of the dialog, there are two buttons: "OK" and "Cancel".

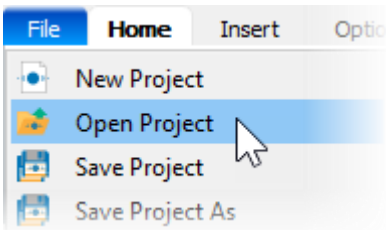
The new Project can be based on any existing Project in the Project Library, or it can be a completely new Project, not based on any Project. When you select a **Based on** Project, the suggested **Project Name** for the new Project will update.

The location for the Project can be changed with the **Browse** button. However, it is recommended to create methods in the Project Library folder, which is the default location.

See also:

[What is a Project?](#)

Open Project



This command opens a Project from disk. The command appears in the File menu. The standard file open dialog is displayed to choose a Project. Navigate to the Project folder with the dialog and select it. The Project will be opened.

See also:

[What is a Project?](#)

[Project List](#)

Save Project



Save

The **Save Project** command re-saves the current Project. The command appears in the File menu and on the [Develop](#) tab.

Use this command when you have changed the batch scripts, database format, or model template used in the Project.

The **Save Project As** command (Develop mode) displays a dialog that allows you to type a new name and location for the Project. All content from the original Project is copied to the new Project name and location.

See also:

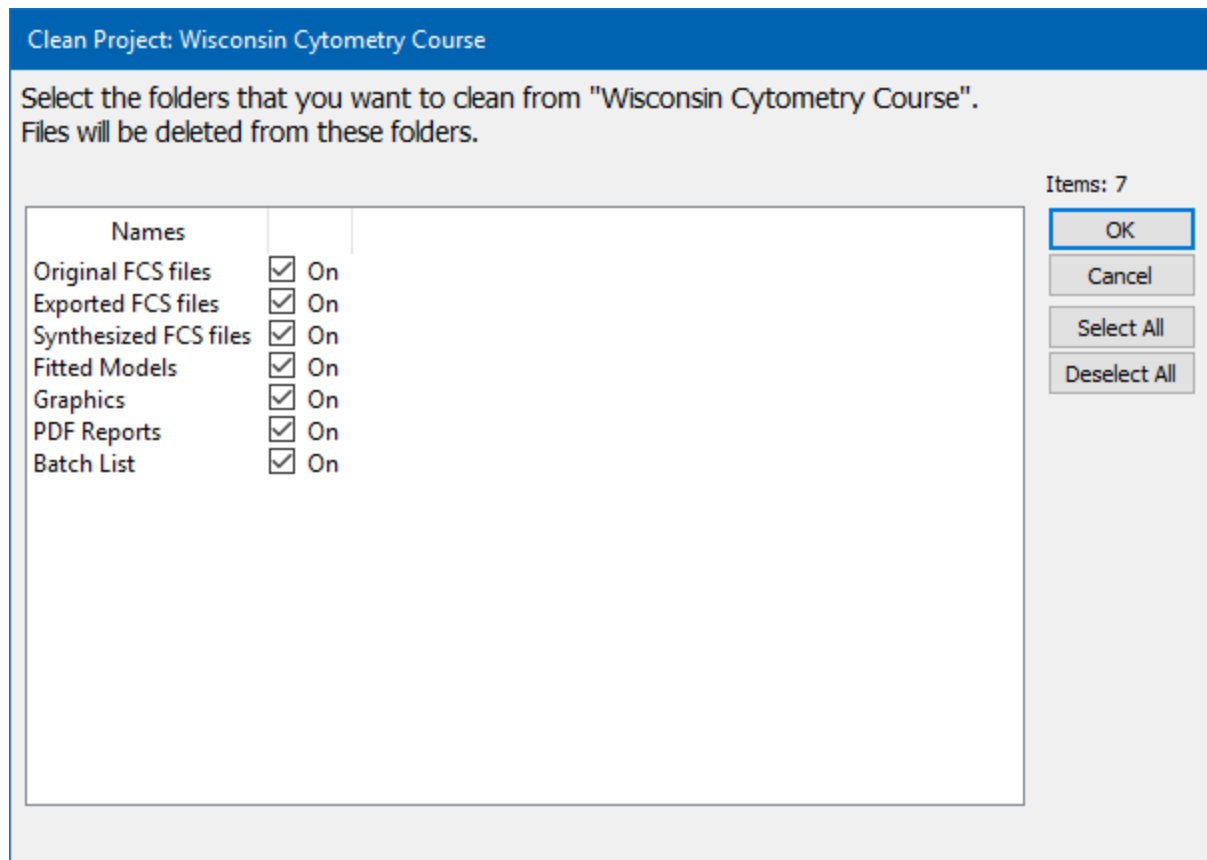
[What is a Project?](#)

Clean Project



This command cleans output files from the Project folders. The command appears in the File menu

The Clean Project dialog is displayed.



This command acts on files in the current Project, which is displayed in the title bar. Select the files that you want to remove from the Project using the check boxes in the list:

| Checkbox Option | Description | Location |
|-----------------------|---|------------------------------|
| Original FCS files | FCS files that were added to the Project for analysis. | FCS Files/Original folder |
| Exported FCS files | FCS files that created by a process in the Project (e.g. clean-up files). | FCS Files/Exported folder |
| Synthesized FCS files | FCS files that created by synthesized data in the Project. | FCS Files/Synthesized folder |

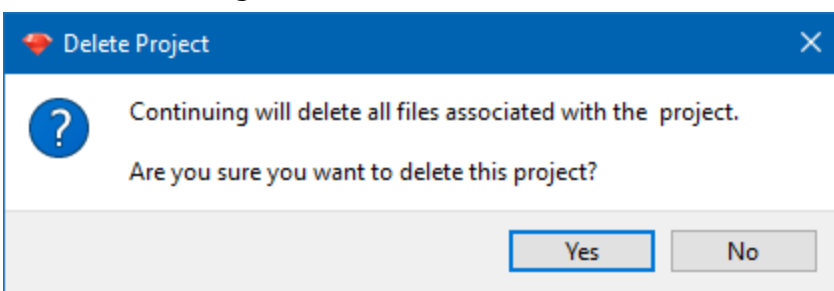
| | | |
|---------------|---|------------------------------------|
| Fitted Models | A fitted model can be saved for each FCS file analyzed. | Fitted Models folder |
| Graphics | Graphics can be saved for each FCS file analyzed. | Graphics folder |
| PDF Reports | PDF reports can be saved for each FCS file analyzed. | PDF Reports folder |
| Batch List | The entries in the batch file list on the left side of the main window. | Entries are removed from the list. |

After selecting the items you want to clean (remove), click the **OK** button to perform the operation.

Delete Project



The **Delete Project** command is only available in Develop mode. This command displays a confirmation dialog.



Choose **Yes** to confirm that you want to delete the Project and all of its content. Choose **No** to cancel the action.

Archive Project



This command allows you to archive a Project to a zip file on disk. The command appears in the File menu.

It displays the Archive Project dialog.

An archive is a Zip file that contains the entire Project folder and file structure. It is useful for storing an experiment prior to deleting it from the Project Library.

Select the Project you want to archive from the **Project to Archive** drop-down. The current Project is selected by default. When you select a Project, the suggested **Archive Name** will update.

The location for the Project can be changed with the **Browse** button. Be sure to choose a location that has enough disk space to store the archive.

Tags are words that you can enter to describe the experiment, the archive, or other elements you want to search for. Tags can be used in the [Search Archives](#) dialog to find a particular experiment that has been archived.

After filling in the information fields, click the **Save** button to initiate the archive operation. Depending on the size and number of files in the Project, it may take some time to complete.

See also:

[What is a Project?](#)

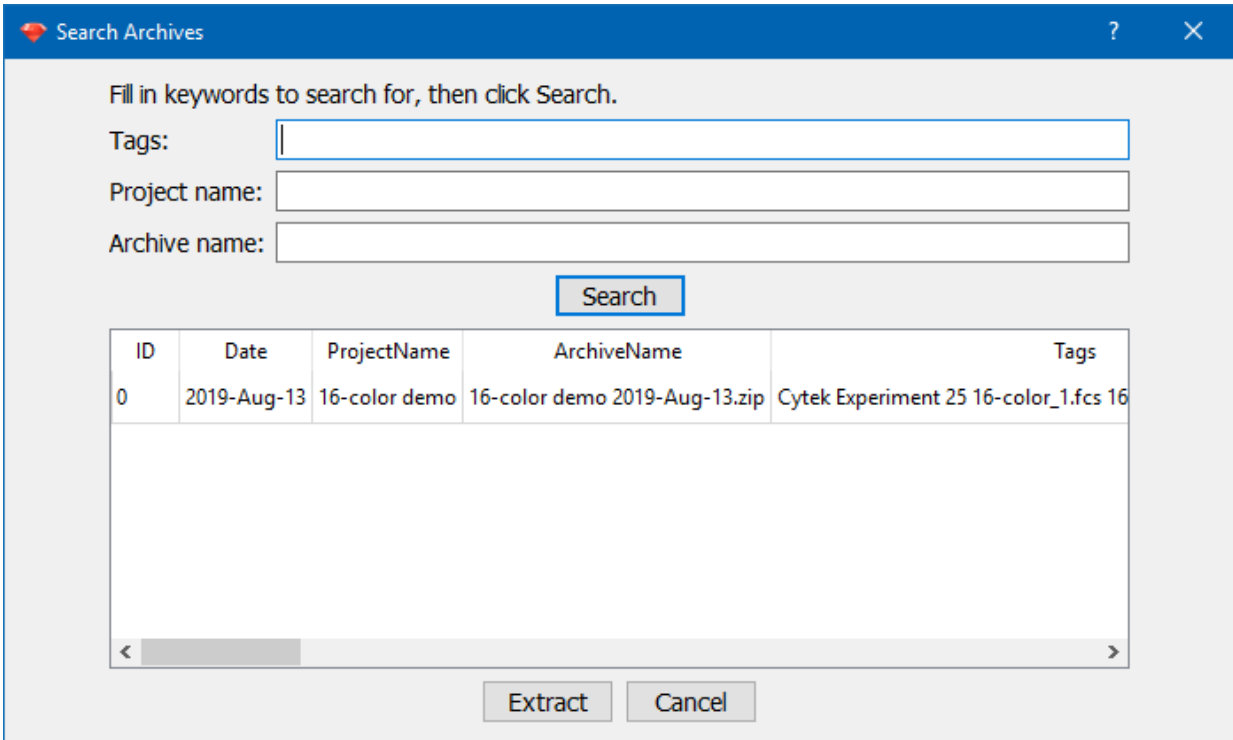
[Search Archives](#)

Search Archives



This command allows you to search for an archived Project based on file names and tags. The command appears in the File menu.

The Search Archives dialog is displayed.



This dialog allows you to search for archived Projects that match the criteria you enter.

Tags are words that you entered in the [Archive Project](#) dialog when the archive was created. You can also enter FCS file names as tags.

If you know the name of the Project that was archived, you can enter that in the **Project name** field. Similarly, you can enter the **Archive name** if you want to filter results on that.

After filling in the fields at the top of the dialog, click the **Search** button. The database of archived Projects will be search for matches, and matching results will be shown in the table.

Select an archive from the list of results and click **Extract** to extract the archive back into your Project Library folder. This will make the Project and all of its files available to the program again.

Click **Cancel** to close the dialog.

See also:

[What is a Project?](#)

[Archive Project](#)

Save PDF Report



This command allows you to save a PDF version of the report. The command appears in the File menu.

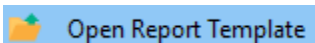
The command displays the standard file save dialog. Navigate to the location where you want to save the report, enter a name, and click the Save button to complete the command.

Print Report



This command allows you to print the report. The command appears in the File menu. The Print dialog is displayed. Select appropriate options for your printer, and click Print to complete the command.

Open Report Template

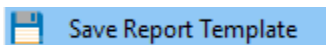


This command allows you to open a report template with a different report layout. The command appears in the File menu.

The command displays the standard file open dialog. Navigate to the location of the report template, select it, and click the Open button to complete the command.

Note, the report will become the current report until a new model template is opened, a new Project is selected, or the program is closed. To make the report template part of an analysis method, the model template must be resaved after opening the report template. This will make the *report* template part of the *model* template.

Save Report Template



This command saves the current report as a report template so that it can be used with a different model. The command appears in the File menu.

The command displays the standard file save dialog. Navigate to the location where you want to save the report template, enter a name, and click the Save button to complete the command.

Printer Setup



This command allows you to set up the printer that GemStone will use. The command appears in the File menu.

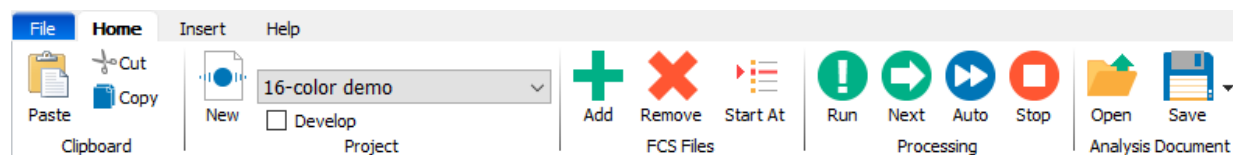
The command displays the standard printing dialog, which may vary in features and appearance depending on the operating system.

Exit (Quit)

This command closes the program. It is Exit on Windows versions, and Quit on the Mac. It appears in the File menu.

Home tab

Home Tab



| Clipboard | Project | FCS Files | Processing | Analysis Document |
|-----------------------|----------------------------------|----------------------------------|----------------------|--------------------------|
| Paste | New Project | Add FCS Files | Run | Open |
| Cut | Project List | Remove FCS Files | Next | Save |
| Copy | Develop Checkbox | Start At | Auto | |
| | | | Stop | |

* Some commands may be hidden when the software is running in [Compliance mode](#).

Paste



Paste

The command pastes text or graphics onto the current page of the report or cell type. It appears on the [Home Tab](#).

Cut



The command cuts the currently selected text or graphics from the report or cell type. It appears on the [Home Tab](#).

Copy

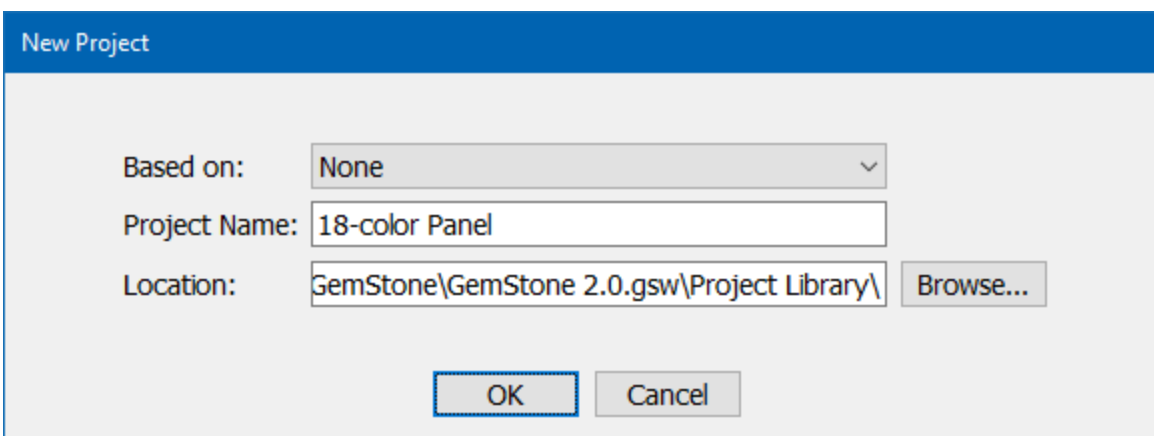
The command copies the currently selected text or graphics from the report or cell type to the clipboard. It appears on the [Home Tab](#).

New Project



This command creates a new Project. The command appears in the File menu and on the [Home](#) tab.

The New Project dialog is displayed.

A screenshot of the "New Project" dialog box. The dialog has a blue title bar with the text "New Project". Below the title bar, there are three input fields: "Based on:" with a dropdown menu showing "None", "Project Name:" with a text box containing "18-color Panel", and "Location:" with a text box containing "GemStone\GemStone 2.0.gsw\Project Library\" and a "Browse..." button to its right. At the bottom of the dialog, there are two buttons: "OK" and "Cancel".

The new Project can be based on any existing Project in the Project Library, or it can be a completely new Project, not based on any Project. When you select a **Based on** Project, the suggested **Project Name** for the new Project will update.

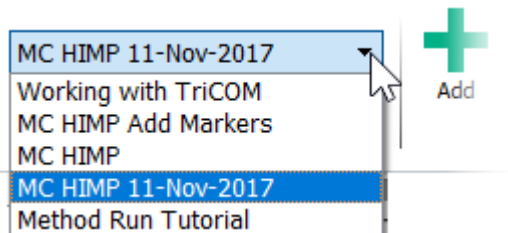
The location for the Project can be changed with the **Browse** button. However, it is recommended to create methods in the Project Library folder, which is the default location.

See also:

[What is a Project?](#)

Project List

This control is used to select and open a Project from the Project Library. It appears on the [Home](#) tab.



This is the quick and easy way to select a Project for analysis - simply open the list by clicking the down arrow and select the method you want. The Projects that are shown in the list are the ones that exist in your Project Library folder. If you need to open a Project that is not in the Project Library, use the [Open Project command](#).

Develop Check box



This control is used to enable development tools for Projects. The checkbox appears on the [Home](#) tab.

*Note, this option may not be available, depending on the user rights for your account and the version of the software that you have.

In Develop mode, GemStone displays additional ribbon tabs [Options](#) and [Develop](#) that are used for model design and developing new Projects.

Add FCS Files



This control is used to add FCS files to the batch to be analyzed by the current Project. The command appears on the [Home](#) tab.

The Select FCS Files dialog is displayed, which is a standard file open dialog. You can choose one or more files in the dialog, and then click Open to complete the command. The files will be validated as FCS files, and then added to the batch list.

Remove FCS Files



This control is used to remove files from the batch. The command appears on the [Home](#) tab. If files are selected in the batch list, those files will be removed from the batch. They will not be deleted from disk.

| | | |
|---|----|---|
| ✓ | L1 | 20170721 06 Blood 3 Lyo DOM20170720 H2 |
| ✓ | L1 | 20170728 04 Blood 1 Lyo DOM20170719 H2 |
| ✓ | L1 | 20170728 05 Blood 2 Lyo DOM20170724 H2 |
| ✓ | L1 | 20170728 06 Blood 3 Lyo DOM20170724 H2 |
| ✓ | L2 | 20170621 05 H215 Amazon Whole Blood in |
| ✓ | L2 | 20170630 05 Lyo in-house Whole Blood H2 |
| ✓ | L2 | 20170712 03 Blood 1 Lyo H215_01_0 (Dono |
| ✓ | L2 | 20170714 04 Blood 1 Lyo DOM20170623 H2 |
| ✓ | L2 | 20170714 05 Blood 2 Lyo DOM20170711 H2 |
| ✓ | L3 | 20170719 04 Lyo DOM 20170710 H215_01_1 |
| ✓ | L2 | 20170721 04 Blood 1 Lyo DOM20170711 H2 |
| ✓ | L2 | 20170721 05 Blood 2 Lyo DOM20170720 H2 |

If no files are selected in the batch list, *all* files will be removed from the batch. Files will not be deleted from disk.

| | | |
|---|----|---|
| ✓ | L1 | 20170728 04 Blood 1 Lyo DOM20170719 H2 |
| ✓ | L1 | 20170728 05 Blood 2 Lyo DOM20170724 H2 |
| ✓ | L1 | 20170728 06 Blood 3 Lyo DOM20170724 H2 |
| ✓ | L2 | 20170621 05 H215 Amazon Whole Blood in |
| ✓ | L2 | 20170630 05 Lyo in-house Whole Blood H2 |
| ✓ | L2 | 20170712 03 Blood 1 Lyo H215_01_0 (Dono |
| ✓ | L2 | 20170714 04 Blood 1 Lyo DOM20170623 H2 |
| ✓ | L2 | 20170714 05 Blood 2 Lyo DOM20170711 H2 |
| ✓ | L3 | 20170719 04 Lyo DOM 20170710 H215_01_1 |
| ✓ | L2 | 20170721 04 Blood 1 Lyo DOM20170711 H2 |
| ✓ | L2 | 20170721 05 Blood 2 Lyo DOM20170720 H2 |

Start At



Start At

This control is used to set the first file to be analyzed by the current Project. The command appears on the [Home](#) tab.

This is a convenience command that allows you to choose any file in the batch list as the starting point for processing. Any files before that item in the list will be ignored. Select the file you want to start with in the batch list, then click the Start At button to set it as the starting file.

Run



This control is used to run the analysis on the current file in the batch list. The command appears on the [Home](#) tab.

The command initiates the batch script associated with the current file in the file list. Typically, this involves opening a model template, opening the FCS file, and analyzing the file with the template. It may also include generating output, such as graphics and print outs.

Next

Next

This control is used to move to the next file in the batch list and analyze it. The command appears on the [Home](#) tab.

Auto Advance



This control is used to initiate auto-advancing through the batch list. The command appears on the [Home](#) tab.

This command is designed for unattended, unsupervised analysis of a batch of files. When auto-advancing, the program analyzes a file in the batch list according to its associated script, and then moves to the next file. It repeats this process until it reaches the end of the batch list, or the user clicks the [Stop](#) button.

See also:

[Stop](#)

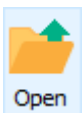
Stop



This control is used stop auto-advancing. It can also cancel any current analysis process. The command appears on the [Home](#) tab.

With this command it triggered, the program will stop its current analysis process as quickly as possible. This includes auto-advancing, analyzing a file, computing a Cen-se plot, and many other operations.

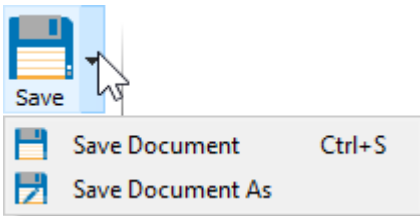
Open Analysis Document



This control is used to open a previous analysis that was saved as an analysis document. The command appears on the [Home](#) tab.

This command displays the file open dialog to select an analysis document to open.

Save Analysis Document



This control is used to Save an analysis document. The command appears on the [Home](#) tab.

Click the button to trigger the default action, or click the down arrow to see a list of Save actions.

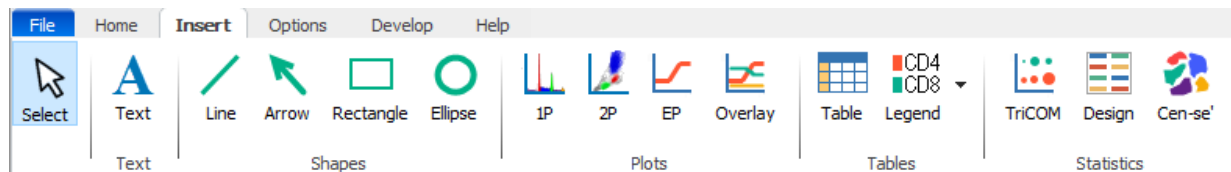
Save Document Saves the analysis document with the same name.

Save Document As Displays the file save dialog to save the document with a new name.

When you select a command from the down arrow menu, that command becomes the new default action for the button.

Insert tab

Insert Tab



Select
Selection
tool

Text
[Insert
Text](#)

Shapes
[Insert Line](#)
[Insert Arrow](#)
[Insert
Rectangle](#)
[Insert
Ellipse](#)

Plots
[Insert 1P Graph](#)
[Insert 2P Graph](#)
[Insert Expression
Profile Plot](#)
[Insert Overlay
Plot](#)

Tables
[Insert Table](#)
[Insert EP
Legend](#)
[Insert Cell
Type Legend](#)
[Insert Stage
Table](#)

Statistics*
[Insert
TriCOM](#)
[Insert
Design](#)
[Insert Cen-
se](#)

* Note, the Statistics panel is only displayed when the program is in [Develop mode](#). Develop mode is enabled from the [Home tab](#).

Text



This tool allows you to create text objects on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create a text object. Then edit the text on the object. Double-click the object to edit its properties.

Text Properties

| Name | Value |
|------------------|--|
| Label Text | This is a text box. |
| Text Color | |
| Label Font | Sample... |
| Alignment | <input checked="" type="checkbox"/> Centered |
| Word Wrap | <input checked="" type="checkbox"/> On |
| Text Margin | 3 |
| Rectangle | |
| Fill color | |
| Fill style | |
| Transparency | 50 |
| Pen color | |
| Pen style | |
| Pen width | 1 |
| Rotation Angle | 0.00 |

Buttons: OK, Cancel, Reset, Help...

Label Text

Text to be displayed by the label

Text Color

GemStone User Guide

Color of the label's text

Label Font

Font of the label's text

Alignment

Alignment of the label's text (top left or centered)

Word Wrap

Check to perform word wrapping.

Text Margin

Space between the edge of the object and the text. (0 to 10)

Rectangle Fill color

Color of the fill

Fill style

Style of the fill

Transparency

Amount of transparency (0 to 100)

Pen color

Color of the outline

Pen style

Style of the outline

Pen width

Width of the outline in pixels

Rotation Angle

Rotation angle, from -360.0 to 360.0 degrees

Line



This tool allows you to create line objects on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create a line object. Double-click the object to edit its properties.

Line and Arrow Properties

| Name | Value |
|-----------------|--------|
| Arrows | None |
| Arrow head size | 32 |
| Line width | 4 |
| Fill color | |
| Fill style | |
| Transparency | 0 |
| Pen color | |
| Pen style | |
| Pen width | 1 |
| Rotation Angle | 333.48 |

OK
Cancel
Reset
Help...

Arrows

Number of arrow heads: none, one, or two

Arrow head size

Size of the arrow heads. (0 to 100)

Line width

Width of the line in pixels. (0 to 100)

Fill color

Color of the fill

Fill style

Style of the fill

Transparency

Amount of transparency (0 to 100)

Pen color

Color of the outline

Pen style

Style of the outline

Pen width

Width of the outline in pixels

Rotation Angle

Rotation angle (-360.0 to 360.0 degrees)

Arrow



Arrow

This tool allows you to create arrows on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create an arrow object. Double-click the object to edit its properties.

Arrow properties are the same as [Line properties](#).

Rectangle



Rectangle

This tool allows you to create rectangles on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create a rectangle object. Double-click the object to edit its properties.

Rectangle and Ellipse Properties

| Name | Value |
|-----------------|--|
| Shape | Rectangle |
| Fill color | |
| Fill style | |
| Transparency | 50 |
| Pen color | |
| Pen style | |
| Pen width | 1 |
| Rotation Angle | 0.00 |
| RectangleCoords | Left: 89.00 Right: 308.00 Top: 457.00 Bottom: 590.00 |

Type of shape: Rectangle or Ellipse

Shape

Type of shape: Rectangle or Ellipse

Fill color

Color of the fill

Fill style

Style of the fill

Transparency

Amount of transparency (0 to 100)

Pen color

Color of the outline

Pen style

Style of the outline

Pen width

Width of the outline in pixels

Rotation Angle

Rotation angle (-360.0 to 360.0 degrees)

Ellipse



Ellipse

This tool allows you to create ellipse on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create an ellipse object. Double-click the object to edit its properties.

The properties for the ellipse are the same as the [Rectangle](#) properties.

1P Graph

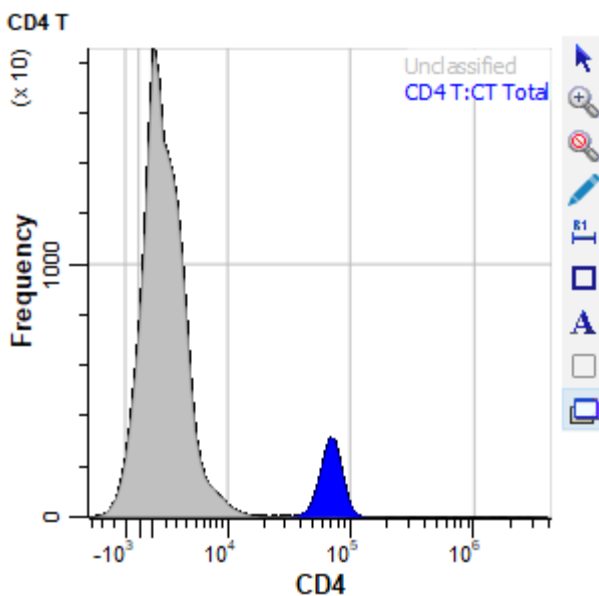
1P Graph



This tool allows you to create a 1P graph on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties.

The 1P graph displays a histogram for a selected measurement.



The name of the cell type is displayed at the top of the plot.

Click the X-axis label to select a different measurement; a menu of choices will be displayed.

Tools to manipulate the plot appear on the right edge of the plot window when the mouse is over the plot. See [1P Graphics toolbar](#) for a description of each tool.

Context menu

The context menu can be displayed by right-clicking on the object.

Edit Graphics

Displays the [Graphic Options for 1P Graphs](#) dialog.

Edit Frame

Displays the [Edit Frame](#) dialog.

Show Unclassified

Toggles showing/not showing the unclassified events in the plot

Copy

Copies the object to the clipboard.

Save

Displays the standard Save dialog to save the graphic object to disk.

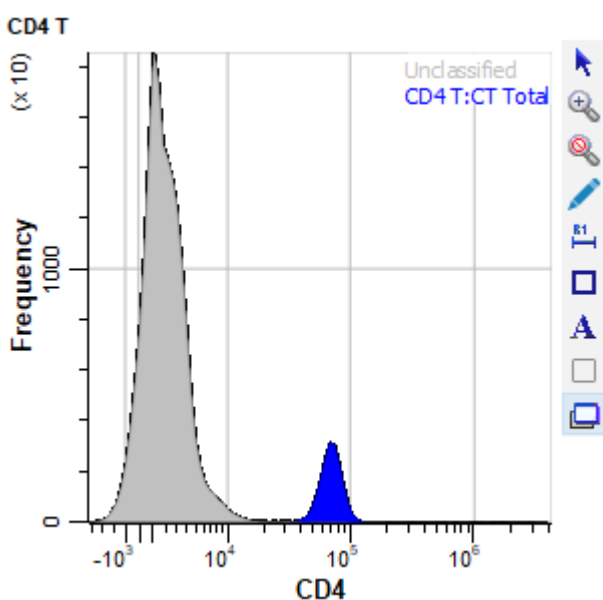
1P Graph



This tool allows you to create a 1P graph on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties.

The 1P graph displays a histogram for a selected measurement.



The name of the cell type is displayed at the top of the plot.

Click the X-axis label to select a different measurement; a menu of choices will be displayed.

Tools to manipulate the plot appear on the right edge of the plot window when the mouse is over the plot. See [1P Graphics toolbar](#) for a description of each tool.

Context menu

The context menu can be displayed by right-clicking on the object.

Edit Graphics

Displays the [Graphic Options for 1P Graphs](#) dialog.

Edit Frame

Displays the [Edit Frame](#) dialog.

Show Unclassified

Toggles showing/not showing the unclassified events in the plot

Copy

Copies the object to the clipboard.

Save

Displays the standard Save dialog to save the graphic object to disk.

Graphic Options for 1P Graphs

General Options tab

Background, Grids, and Colors

Background Color

Choose a color for the background of the plot.

X Grid Lines

Enable this option to show grids for the X-axis. Grid lines will appear for each major tic on the axis.

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.

Selection

Choose Populations

This option provides a quick way to select other cell types to overlay on this 1P graph. A dialog is displayed with a list of cell types, which can be enabled and disabled for the graph.

Graphics

Resolution

Enter the resolution for this histogram.

X Auto Zoom

When enabled, the x axis is zoomed to eliminate empty channels on the low and high ends of the plot.

Fill Style

Choose a fill style for the plot.

Transparency

Enter a transparency setting for the plot, where 0.00 is opaque and 100.00 is completely transparent.

Scale Plots

When enabled, all plots are scaled so that their highest values are full scale.

Smooth Plots

When enabled, all plots are smoothed.

Staining Assessment

Staining Assessment

When enabled, staining assessment information is displayed on the 1P graph, comparing the measurement in the selected cell type with the first overlaid cell type.

Positive Color

Select a color for the positive population in the stain assessment plot.

Negative Color

Select a color for the negative population in the stain assessment plot.

Peaks and Projections

Peak Triangles

Enable to show peak locations on the plot.

Peak Polygons

Enable to show peak polygons on the plot.

Model Projections

Enable to show model projections on the plot.

Smoothed Data Projections

Enable to show data projections on the plot.

Other Tabs

A tab is show for each Stage defined in the current cell type, as well as for each of the other cell types. These can be co-plotted on the plot.

Enable

Check this box to include the population on the plot.

Customize Properties

Customize

Enable this to customize fill and transparency for the object

Fill Style

Choose a fill for the population

Transparency

Choose a transparency for the population.

Animation

Enable

When this option is enabled and the Animate property is enabled for the cell type, this 1P graph will display animation cursors.

Animation 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.

Color

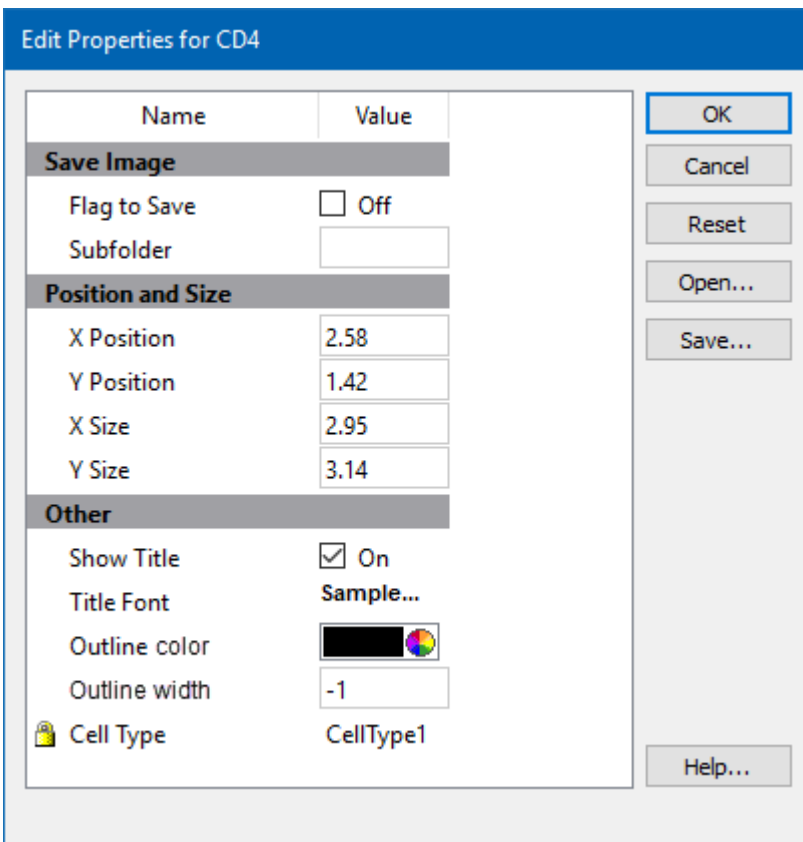
Choose a color for the animation cursors.

Line Width

Choose a line width for the animation cursors.

Edit Frame

This command edits properties of the object's frame.



Save Image

Flag to Save

Check to flag this object for saving its image when the file is analyzed.

Subfolder

Type in the subfolder name to store its image. Must be created in the canvas image folder.

Position and Size

X Position

Enter the x position in inches.

Y Position

Enter the y position in inches.

X Size

Enter the x size in inches.

Y Size

Enter the y size in inches.

Other

Show Title

Show the title of the object.

Title Font

Font of the title

Outline Color

Color of the object's border

Outline Width

Line width of the object's border(-1 to 20). A value of -1 turns off the border.

Cell Type

For objects that are associated with a specific cell type, this property shows the cell type.

Name

This is the internal name of the object that cannot be edited. It is used to uniquely identify the object.

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.

1P Graphics toolbar

The 1P Graphics toolbar is located on the right side of each 1P histogram object and is displayed when the mouse moves over the 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. |
|  | Rectangle | Use this tool to create a rectangle object on the histogram. |
|  | Label | Use this tool to create text on the histogram. |
|  | Flag to Save Graphic | Check this option to save the graphic after analysis. |
|  | View in dialog | Click this tool to view the object in a dialog window. |

2P Graph

2P Graph

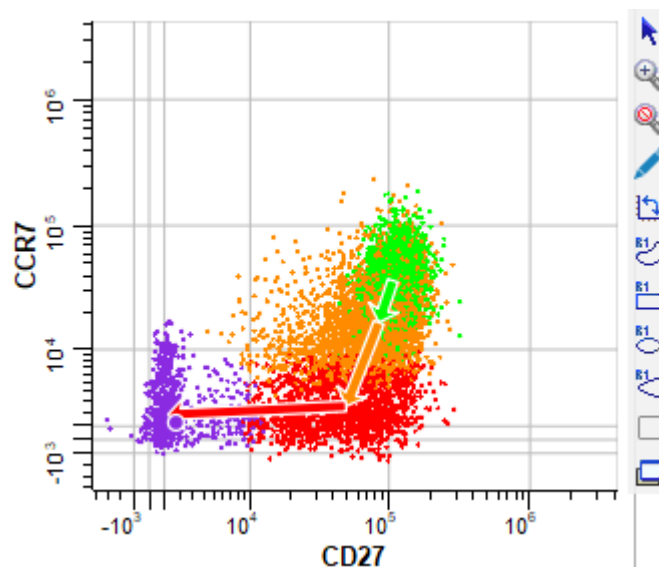


2P

This tool allows you to create a 2P graph on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties.

The 2P Graph displays a correlated plot for two parameters.



The X-axis of the plot shows intensity of a selected measurement, and the Y-axis shows the intensity of another.

Click the axis label to select a different measurement; a menu of choices will be displayed.

Tools to manipulate the plot appear on the right edge of the plot window when the mouse is over the plot. See [2P Graphics toolbar](#) for a description of each tool.

Context menu

The context menu can be displayed by right-clicking on the object.

Edit Graphics

Displays the [Graphic Options for 2P Graphs](#) dialog.

Edit Frame

Displays the [Edit Frame](#) dialog.

Auto Zoom

When enabled, the plot is zoomed to exclude empty channels.

Show Unclassified

Toggles showing/not showing the unclassified events in the plot.

Show State Vectors

Toggles showing/not showing the state vectors in the plot.

Show Surface

Toggles showing/not showing a surface plot.

Copy

Copies the object to the clipboard.

Save

Displays the standard Save dialog to save the graphic object to disk.

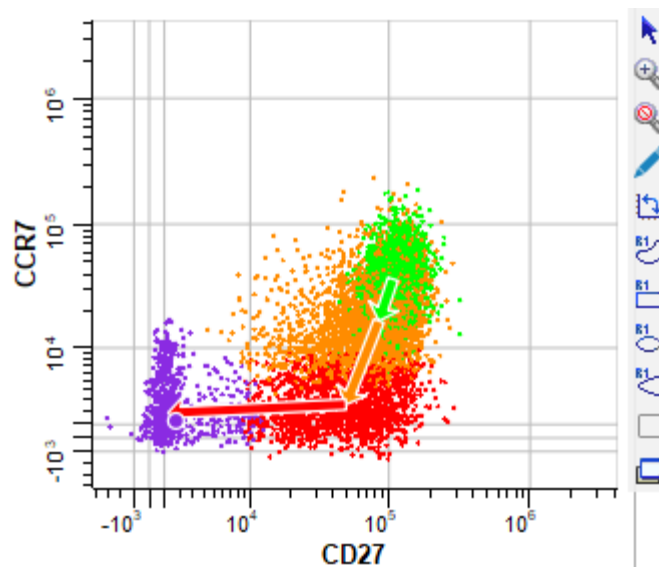
2P Graph



This tool allows you to create a 2P graph on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties.

The 2P Graph displays a correlated plot for two parameters.



The X-axis of the plot shows intensity of a selected measurement, and the Y-axis shows the intensity of another.

Click the axis label to select a different measurement; a menu of choices will be displayed.

Tools to manipulate the plot appear on the right edge of the plot window when the mouse is over the plot. See [2P Graphics toolbar](#) for a description of each tool.

Context menu

The context menu can be displayed by right-clicking on the object.

Edit Graphics

Displays the [Graphic Options for 2P Graphs](#) dialog.

Edit Frame

Displays the [Edit Frame](#) dialog.

Auto Zoom

When enabled, the plot is zoomed to exclude empty channels.

Show Unclassified

Toggles showing/not showing the unclassified events in the plot.

Show State Vectors

Toggles showing/not showing the state vectors in the plot.

Show Surface

Toggles showing/not showing a surface plot.

Copy

Copies the object to the clipboard.

Save

Displays the standard Save dialog to save the graphic object to disk.

Other Options for 2P Graphs

General Options tab

Background, Grids, and Colors

Background Color

Choose a color for the background of the plot.

X Grid Lines

Enable this option to show grids for the X-axis. Grid lines will appear for each major tic on the axis.

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.

Graphic Options for 2P Surface

This set of properties appears on the Surface tab in the dialog used for editing a 2P graph. You can view and edit these properties by double-clicking a plot or by right-clicking 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

Check to enable surface graphics.

Mixing Color

Choose the color to mix with the stage color to show cumulative probability.

Graphic Options for 2P Dots

This set of properties appears on the "Dots" tab in the dialog used for editing a 2P graph. You can view and edit these properties by double-clicking a plot or by right-clicking and choosing "Edit Graphics".

Enable

Check this option to display dots and other graphics from this tab.

Auto Size

Enable this option to allow the program to determine the best dot size, based on the current size of the plot 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 10.0 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.

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.

Maximum Number

This field sets a limit on the number of dots that will be drawn.

Auto Zoom

When enabled, the each axis is zoomed to eliminate empty channels on the low and high ends of the plot.

Surface Outliers

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.

Heat Map

When enabled, dots are colored to form a heat map of intensity.

Other Populations

Cell Types

This option allows dots from other cell types to be display in the plot.

Unclassified

Enable this option to display dots for unclassified events.

Graphic Options for 2P Vectors

This set of properties appears on the "State Vectors" tab in the dialog used for editing a 2P Graph. You can view and edit these properties by double-clicking a plot or by right-clicking and choosing "Edit Graphics".

State Vector arrows allow you to track the progression information in the context of two, correlated measurements. 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.

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.

Mode

Arrows can be drawn based on number of events or by stage boundaries.

Outline 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.

Shaft Height

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.

Other Populations

This value determines whether vectors from other cell types will be drawn on this plot.

2P Animation

This set of properties appears on the Animation tab in the dialog used for editing 2P Graphs. You can view and edit these properties by double-clicking a plot or by right-clicking and choosing "Edit Graphics".

When Animation is enabled for the cell type and for this 2P plot, the State Vector arrows will also animate.

Enable

Check this option to enable animation for this 2P histogram. When this option is enabled animation is turned on, this 2P histogram will display animation vectors.

Cell Type Animation

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.

Color

Choose the fill color for the vectors.

Outline 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.

Shaft 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 Animations

Dot Size

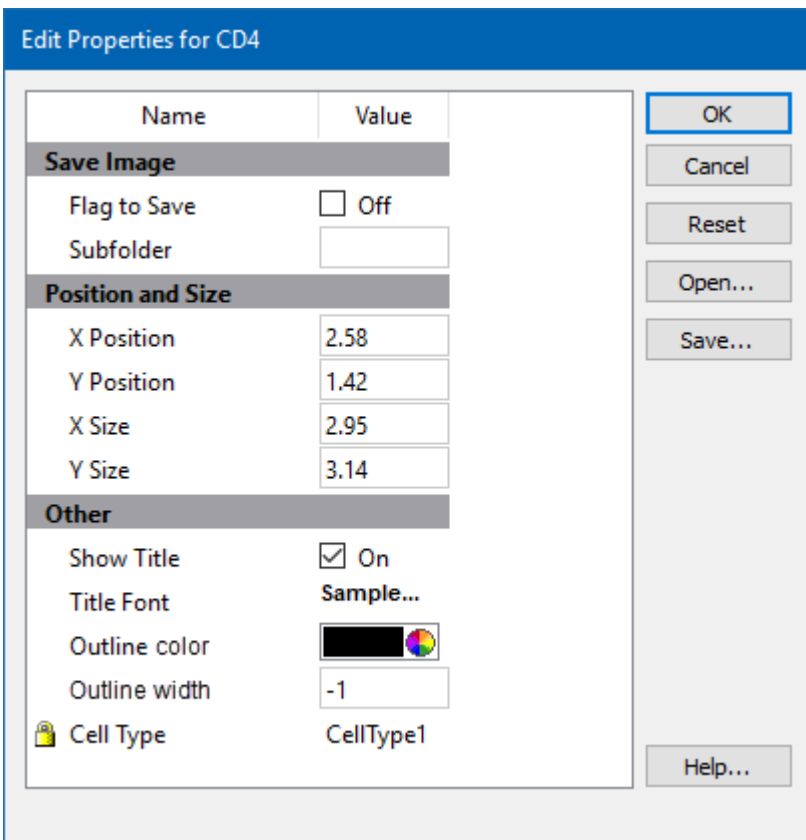
Sets the size of dots during region animation.

Number

Sets the maximum number of dots during region animation

Edit Frame

This command edits properties of the object's frame.



Save Image

Flag to Save

Check to flag this object for saving its image when the file is analyzed.

Subfolder

Type in the subfolder name to store its image. Must be created in the canvas image folder.

Position and Size

X Position

Enter the x position in inches.

Y Position

Enter the y position in inches.

X Size

Enter the x size in inches.

Y Size

Enter the y size in inches.

Other

Show Title

Show the title of the object.

Title Font

Font of the title

Outline Color

Color of the object's border

Outline Width

Line width of the object's border(-1 to 20). A value of -1 turns off the border.

Cell Type

For objects that are associated with a specific cell type, this property shows the cell type.

Name

This is the internal name of the object that cannot be edited. It is used to uniquely identify the object.

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.

2P Graphics toolbar

The 2P Graphics toolbar is located on the right side of each 2P histogram object and is displayed when the mouse moves over the 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 measurements on the X- and Y- axes. |
|  | Polygon 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. |
|  | Extruded circle 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. |
|  | Flag to Save Graphic | Check this option to save the graphic after analysis. |
|  | View in dialog | Click this tool to view the object in a dialog window. |

Expression Profile Plot

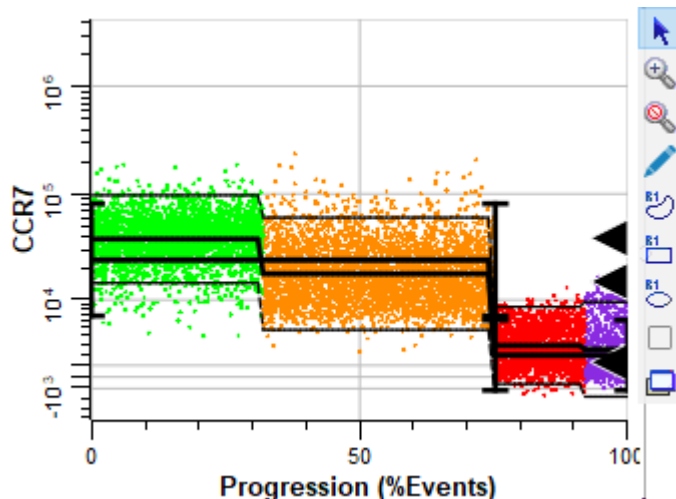
Expression Profile Plot



This tool allows you to create an expression profile plot on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties.

Expression profile plots are also contained in the [Cell Type Widget](#) and have additional properties in that case. The Cell Type Widget is only visible in [Develop mode](#).



The X-axis of the plot shows state or progression of a selected measurement, and the Y-axis shows the intensity.

Click the axis label to select a different measurement; a menu of choices will be displayed.

Tools to manipulate the plot appear on the right edge of the plot window when the mouse is over the plot. See [Expression Profile toolbar](#) for a description of each tool.

Context menu

The context menu can be displayed by right-clicking on the object.

Edit Graphics

Displays the [Edit Graphic Options](#) dialog.

Edit Frame

Displays the [Edit Frame](#) dialog.

Show Unclassified

Toggles showing/not showing the unclassified events in the plot.

Copy

Copies the object to the clipboard.

Save

Displays the standard Save dialog to save the graphic object to disk.

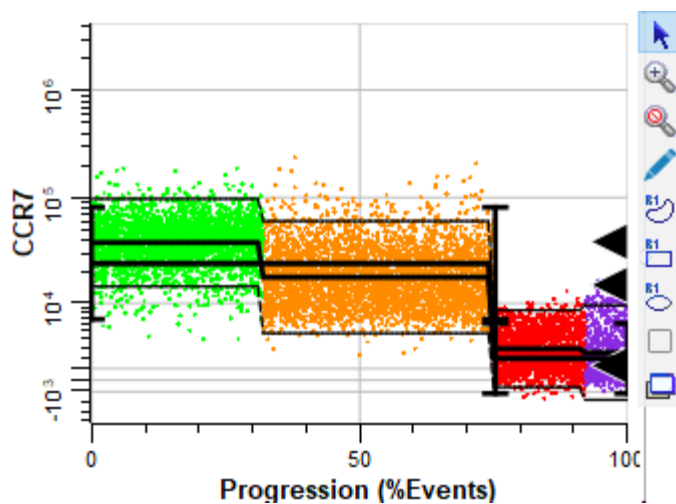
Expression Profile Plot



This tool allows you to create an expression profile plot on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties.

Expression profile plots are also contained in the [Cell Type Widget](#) and have additional properties in that case. The Cell Type Widget is only visible in [Develop mode](#).



The X-axis of the plot shows state or progression of a selected measurement, and the Y-axis shows the intensity.

Click the axis label to select a different measurement; a menu of choices will be displayed.

Tools to manipulate the plot appear on the right edge of the plot window when the mouse is over the plot. See [Expression Profile toolbar](#) for a description of each tool.

Context menu

The context menu can be displayed by right-clicking on the object.

Edit Graphics

Displays the [Edit Graphic Options](#) dialog.

Edit Frame

Displays the [Edit Frame](#) dialog.

Show Unclassified

Toggles showing/not showing the unclassified events in the plot.

Copy

Copies the object to the clipboard.

Save

Displays the standard Save dialog to save the graphic object to disk.

Graphic Options for EP Plots

General tab

Background, Grids, and Colors

Background Color

Choose a color for the background of the plot.

X Grid Lines

Enable this option to show grids for the X-axis. Grid lines will appear for each major tic on the axis.

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.

Graphic Options for EP Surface

This tab is part of the Edit Graphic Options for Expression Profiles. It configures the display of the Surface plot.

Enable

Check this option to display the surface graphics.

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.

Surface Mode

Choose between Smoothed Frequency and Model Probability modes.

Graphic Options for EP Dots

This tab is part of the Edit Graphic Options for Expression Profiles. It configures the display of dots.

Enable Dots

Check this option to display dots on the plot. (The plot can display other kinds of graphics, which are selected on other tabs in this dialog.)

Auto Dot Size

Enable this option to allow the program to determine the best dot size, based on the current size of the 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.

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.

Maximum Dot Number

This field sets a limit on the number of dots that will be drawn.

Auto Zoom

When enabled, the each axis is zoomed to eliminate empty channels on the low and high ends of the plot.

Surface Outliers

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.

Heat Map

When enabled, dots are colored as a probability heat map.

Other Dots

Cell Types

This option allows dots from one or all other cell types to be display in the plot.

Unclassified

Enable this option to display dots for unclassified events.

Graphic Options for EP Means

This tab is part of the Edit Graphic Options for Parameter Profiles. It configures the display of means of the classified events.

Enable

Check this option to display a line plotting the mean of classified events in the Parameter Profile.

Data Source

Choose the source of the data for drawing. Choices include Mean Data, Median Data, Peak Data, or Peak or Mean.

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.

Show Peaks

Shows the filtered peaks as triangles on the right edge of the plot.

Show All Peaks

Shows all peaks, including filtered peaks, on the right edge of the plot.

Graphic Options for EP Confidence Limits

This tab is part of the Edit Graphic Options for Expression 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.

Mode

Choose a method for visualizing 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.

Peak Tracks

Shows detected peaks as rectangles.

Low, Normal, High Regions

Shows all low, normal, and high regions in the data

Peak Search Range

Shows peak search range for the expression profile measurement.

Graphic Options for EP Model

This tab is part of the Edit Graphic Options for Expression Profiles. It configures the display of a line that connects each Control Point in the selected Expression Profile.

Enable

Check this option to display the model line on the Parameter Profile plot.

Color

Choose a color for the model drawing.

CDPs

Enable this option to show the control definition points of the related Expression Profile plot in the Cell Type widget.

Stage Labels

Enable this option to display stage labels at the top of the plot area.

Stage Label Font

Choose a font for the stage labels.

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.

EP Animation Options

This tab is part of the Edit Graphic Options for Expression Profiles.

The Expression 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

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 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.

Region

Dot Size

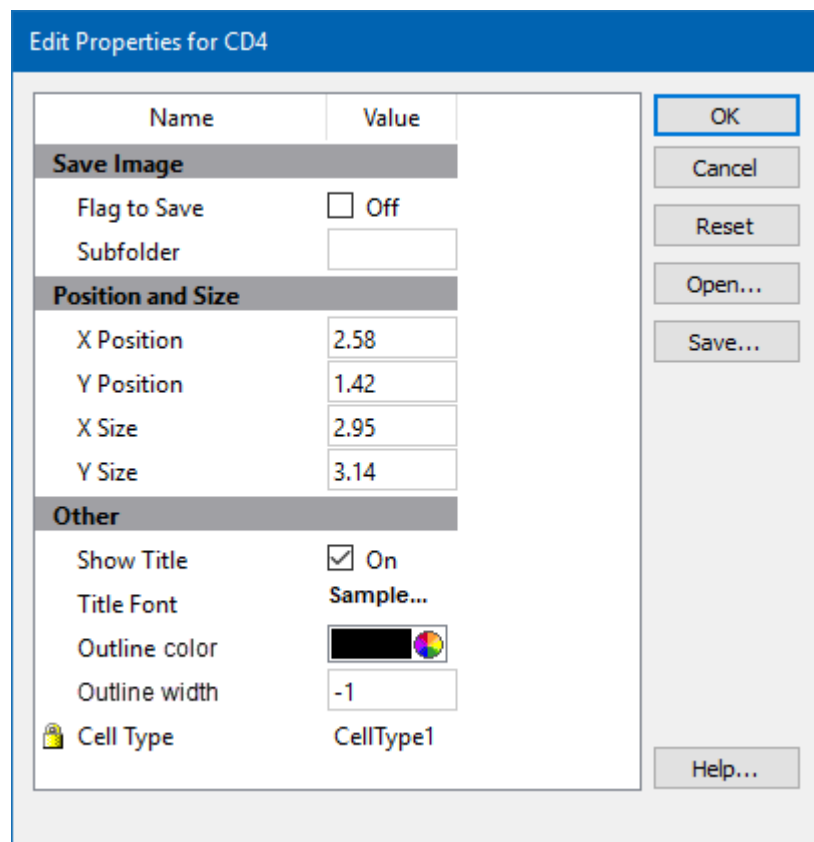
Sets the size of dots during region animation.

Number

Sets the maximum number of dots during region animation

Edit Frame

This command edits properties of the object's frame.



Save Image

Flag to Save

Check to flag this object for saving its image when the file is analyzed.

Subfolder

Type in the subfolder name to store its image. Must be created in the canvas image folder.

Position and Size

X Position

Enter the x position in inches.

Y Position

Enter the y position in inches.

X Size

Enter the x size in inches.

Y Size

Enter the y size in inches.

Other**Show Title**

Show the title of the object.

Title Font

Font of the title

Outline Color

Color of the object's border

Outline Width

Line width of the object's border(-1 to 20). A value of -1 turns off the border.

Cell Type

For objects that are associated with a specific cell type, this property shows the cell type.

Name

This is the internal name of the object that cannot be edited. It is used to uniquely identify the object.

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.

Expression Profile Plot Toolbar

The Expression Profile Plot toolbar is located on the right side of an [Expression Profile Plot](#) on the report and is displayed when the mouse moves over the object.

| | | |
|---|----------------------|--|
|  | Selection | Use this tool to select, move, and size objects in the object. |
|  | 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. |
|  | Edit Graphics | Edits the graphics settings for this object. |
|  | Polygon Region | Use this tool to define a free-hand region on the plot. 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 plot. 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 plot. 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. |
|  | Flag to Save Graphic | Check this option to save the graphic after analysis. |
|  | View in dialog | Click this tool to view the object in a dialog window. |

Overlay Plot

Overlay Plot

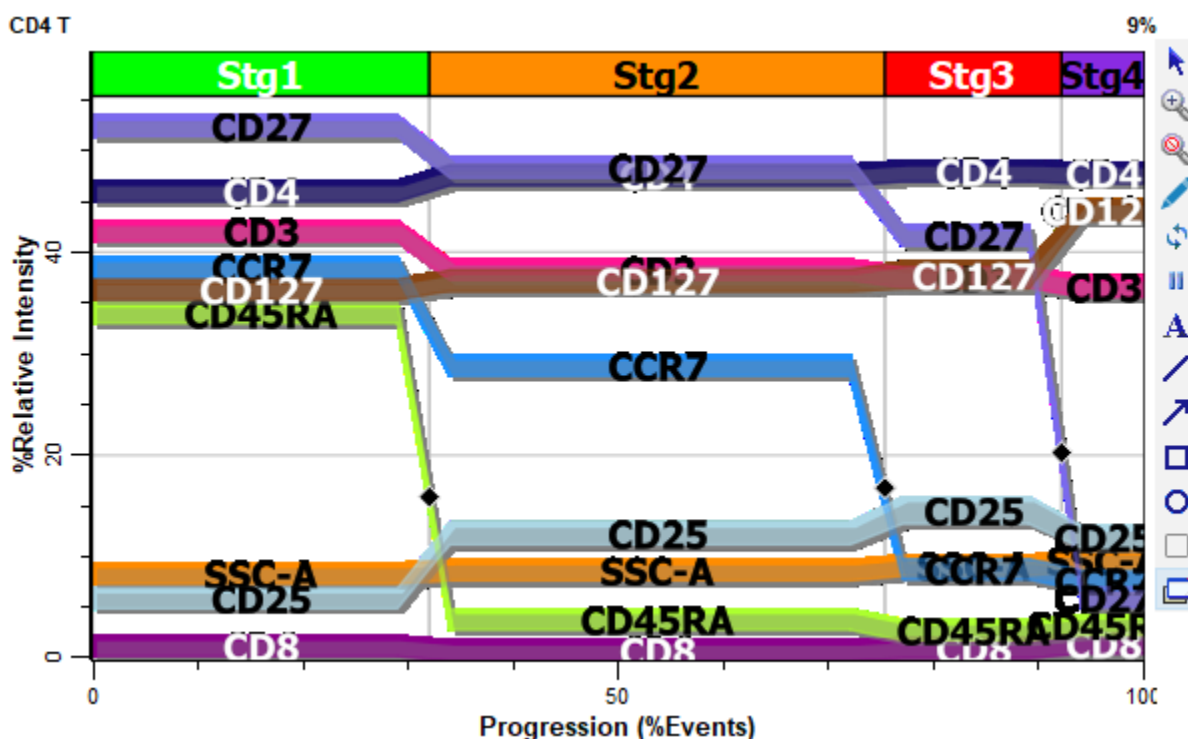


Overlay

This tool allows you to create an overlay plot on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties.

The Overlay plot displays the modeled progression for all measurements in a cell type simultaneously.



The X-axis of the plot shows state or progression of a selected measurement, and the Y-axis shows relative intensity of the measurements in the plot.

If stages have been defined, they are displayed along the top edge of the ribbon display area.

Each measurement appears as a "ribbon" in the plot, with text labels identifying the measurement name. The color for each ribbon is determined by the Color property in the [Expression Profile Properties panel](#).

Tools to manipulate the plot appear on the right edge of the plot window when the mouse is over the plot.

Context menu

The context menu can be displayed by right-clicking on the object.

Edit Graphics

Displays the [Graphic Options for Overlays Plot](#) dialog.

Edit Frame

Displays the [Edit Frame](#) dialog.

Copy

Copies the object to the clipboard.

Save

Displays the standard Save dialog to save the graphic object to disk.

Modeled Measurements Only

Enable this option to show only the modeled measurements - those with expression profiles - in the overlay plot.

Separate EPs

The command separates the expression profile ribbons to maximize the space on the overlay plot.

Reset EP separation

The command resets the separation performed with the Separate EPs command.

Overlay Plot

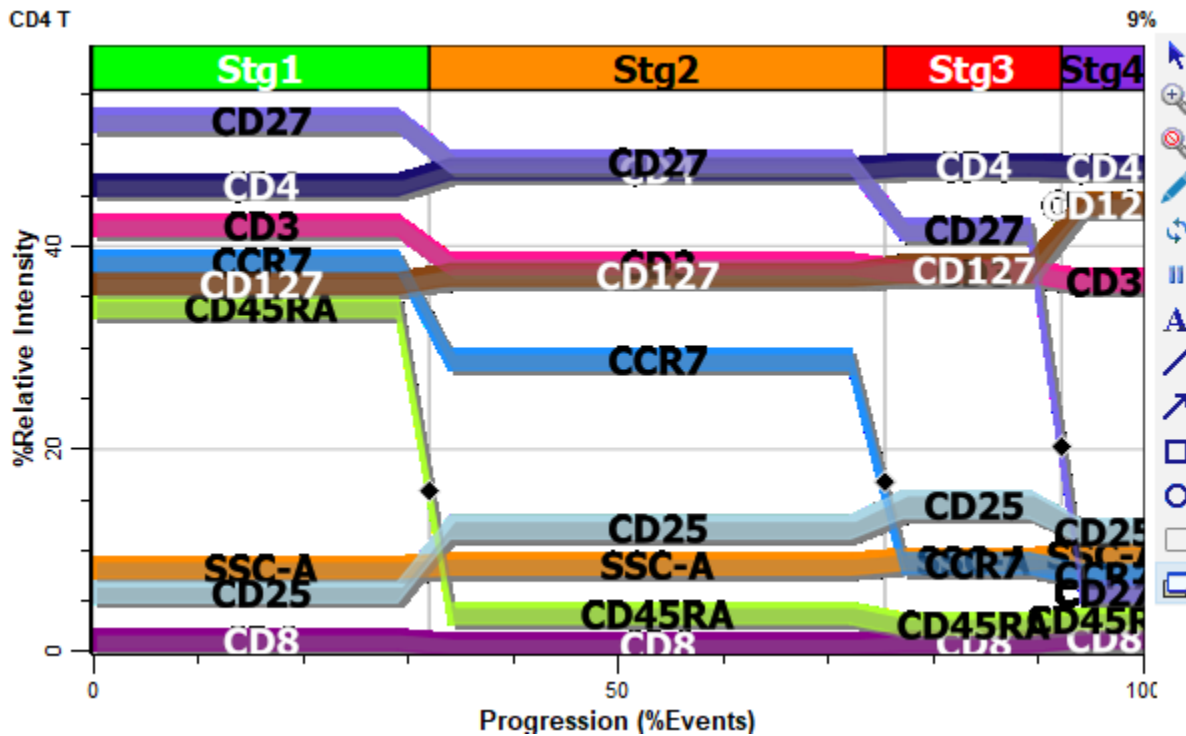


Overlay

This tool allows you to create an overlay plot on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties.

The Overlay plot displays the modeled progression for all measurements in a cell type simultaneously.



The X-axis of the plot shows state or progression of a selected measurement, and the Y-axis shows relative intensity of the measurements in the plot.

If stages have been defined, they are displayed along the top edge of the ribbon display area.

Each measurement appears as a "ribbon" in the plot, with text labels identifying the measurement name. The color for each ribbon is determined by the Color property in the [Expression Profile Properties panel](#).

Tools to manipulate the plot appear on the right edge of the plot window when the mouse is over the plot.

Context menu

The context menu can be displayed by right-clicking on the object.

Edit Graphics

Displays the [Graphic Options for Overlays Plot](#) dialog.

Edit Frame

Displays the [Edit Frame](#) dialog.

Copy

Copies the object to the clipboard.

Save

Displays the standard Save dialog to save the graphic object to disk.

Modeled Measurements Only

Enable this option to show only the modeled measurements - those with expression profiles - in the overlay plot.

Separate EPs

The command separates the expression profile ribbons to maximize the space on the overlay plot.

Reset EP separation

The command resets the separation performed with the Separate EPs command.

Graphic Options for Overlays Plot

Background, Grids, and Colors

Background Color

Choose a color for the background of the Overlay plot.

X Grid Lines

Enable this option to show grids for the X-axis. Grid lines will appear for each major tic on the axis.

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 profile ribbons. When the option is unchecked, grids are drawn behind the ribbons.

Measurements

Choose Measurements

Displays a dialog to select which measurements to include on the overlay plot.

Labeling

Type

Choose an option for labeling the parameter ribbons on the overlay plot.

| Option | Description |
|----------------|--|
| Wire Labeling | Labels follow the ribbon. |
| Stage Labeling | One label appears for each ribbon in each stage. |

Default Width Factor

This value controls the width of the profile ribbons in the overlay plot. The value represents the number of standard deviations from center of the ribbon. The default value is 0.10 SDs.

Font

Choose a font to use for the labels on the parameter profile ribbons.

Ribbons

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. |

Modeled Measurements Only

Enable this option to show only the modeled measurements - those with expression profiles - in the overlay plot.

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.

Drop Shadow

Enable this option to display a drop shadow under each parameter profile ribbon in the overlay plot.

Show Stages

Enable this option to display stages at the top of the overlay plot.

Show Stage Boundaries

Enable this option to display "grid lines" for the stage boundaries. Note, "Show Stages" must be enabled for this option to function.

Auto Zoom

Enable this option zoom to eliminate unused space at the top and bottom of the plot.

Statistics

Show Stage Percents

Choose an option for showing percentages of stages on the overlay.

Decimals for Percents

Set the number of decimal places for percentages.

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.

Graphic Options for Measurements

Each measurement in an Overlay Plot has graphic options that determine how it appears. One tab is displayed for each measurement in the cell type.

Show

Enable this option to include the measurement 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.

Fill Style

Select a style for the fill used for drawing the parameter ribbon in the Overlay Plot.

Width Factor

Choose the width factor to apply to the ribbon. 0: Use default. >0 Override default. 1.0 normally is the 95% confidence limits.

Color

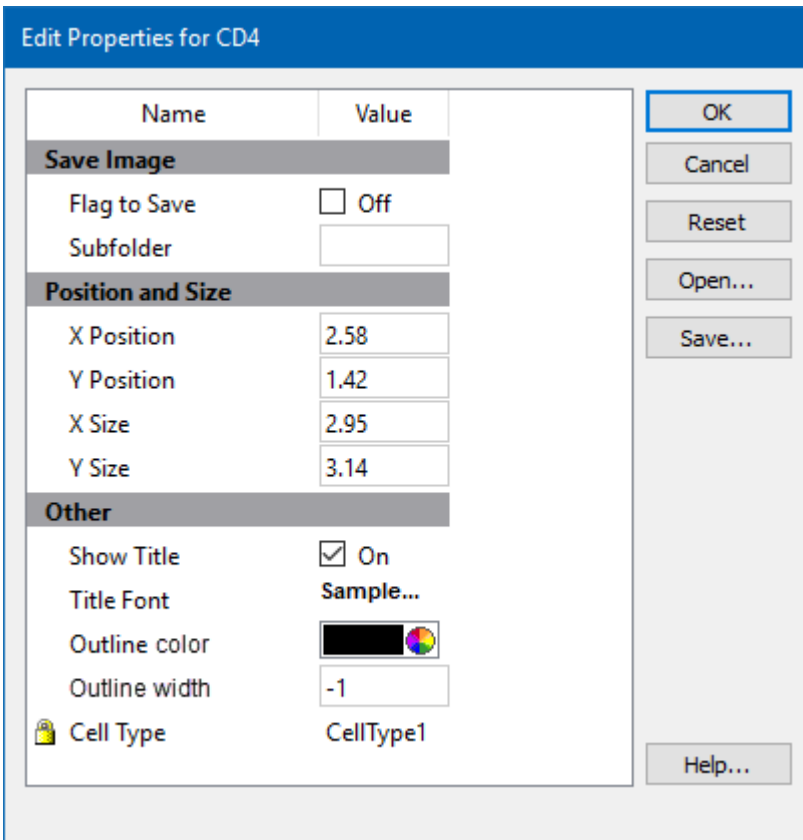
This property determines the color used for this parameter.

Plot Offset

Sets an offset for the ribbon.

Edit Frame

This command edits properties of the object's frame.



Save Image

Flag to Save

Check to flag this object for saving its image when the file is analyzed.

Subfolder

Type in the subfolder name to store its image. Must be created in the canvas image folder.

Position and Size

X Position

Enter the x position in inches.

Y Position

Enter the y position in inches.

X Size

Enter the x size in inches.

Y Size

Enter the y size in inches.

Other

Show Title

Show the title of the object.

Title Font

Font of the title

Outline Color

Color of the object's border

Outline Width

Line width of the object's border(-1 to 20). A value of -1 turns off the border.

Cell Type

For objects that are associated with a specific cell type, this property shows the cell type.

Name

This is the internal name of the object that cannot be edited. It is used to uniquely identify the object.

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.

Overlay Plot toolbar

The Overlay Plot toolbar is located on the right side of each parameter overlay object and is displayed when the mouse moves over the 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. |
|  | Animation Start | Use this tool to turn animation on or off for the overlay graph. 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. |
|  | Stop | 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. When the graph is animating, click the Pause button to pause an animation. When the graph is paused, click the Continue button to resume the animation. |
|  | 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. |
|  | Flag to Save Graphic | Check this option to save the graphic after analysis. |
|  | View in dialog | Click this tool to view the object in a dialog window. |

Table

Table



Table

Use this tool to create a table on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object.

Double-click the top of the object to edit its properties.

The Table object is used to display analysis results and keywords on the report.

| | Number | % Intact Live | Parent | % Parent |
|-------------------|----------|---------------|-------------------|----------|
| Intact Live Cells | 233850 | 88.36 | All events | 88.36 |
| Lymphocytes | 66905.00 | 25.28 | Intact Live Cells | 25.28 |
| CD3 T Cell | 49431.00 | 18.68 | Lymphocytes | 73.88 |
| CD8 T Cells | 7660 | 2.89 | CD3 T Cells | 15.50 |
| Naive | 842.00 | 0.32 | CD8 T Cells | 10.96 |
| Central memory | 809.23 | 0.31 | CD8 T Cells | 10.56 |
| Effector memory | 3164 | 1.20 | CD8 T Cells | 41.31 |
| Terminal effector | 2827 | 1.07 | CD8 T Cells | 36.91 |
| | | | | |
| CD4 T Cells | 38841 | 14.68 | CD3 T Cells | 78.58 |
| Naive | 5042 | 1.91 | CD4 T Cells | 12.98 |
| Central memory | 17762 | 6.71 | CD4 T Cells | 45.73 |
| Effector memory | 9290 | 3.51 | CD4 T Cells | 23.92 |
| Terminal effector | 6747 | 2.55 | CD4 T Cells | 17.37 |

A table has a user-defined number of rows and columns, which can be edited in the [Edit Properties for Information Table](#) dialog.

The cells in a table can contain text and keywords. Keywords must be surrounded with square brackets to be interpreted as keywords and evaluated.

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.

Click in a cell and type to enter a label.

| | Number | % Intact Live | Parent | % Parent |
|-------------------|-----------|---------------|-------------------|----------|
| Intact Live Cells | 288154 | 100.00 | All events | 96.05 |
| Lymphocytes | 139879.00 | 48.54 | Intact Live Cells | 48.54 |
| CD3 T Cell | 117834.00 | 40.82 | Lymphocytes | 84.10 |
| CD8 T Cells | 35899 | 12.48 | CD3 T Cells | 30.52 |
| Naive | 25089 | 8.71 | CD8 T Cells | 69.89 |
| Central memory | 4074 | 1.41 | CD8 T Cells | 11.35 |
| Effector memory | 3569 | 1.24 | CD8 T Cells | 9.94 |

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.

| | Number | % Intact Live |
|-------------------|-----------|---------------|
| Intact Live Cells | 288154 | 100.00 |
| Lymphocytes | 139879.00 | 48.54 |
| CD3 T Cell | 117834.00 | 40.82 |
| CD8 T Cells | 35899 | 12.48 |
| Naive | 25089 | 8.71 |
| Central memory | 4074 | 1.41 |
| Effector memory | 3569 | 1.24 |

Edit Selected Information Units

Choose one or more information units to display.
If you choose more than one, the info keywords will be put in the rows directly under the one you are editing.

Select Items Below

Name

- > Application
- File Attribs
- FCS Keywords
- External Information
- > Methods
- > CellType1_TriCOMGraph2
- > CellType1_TriCOMGraph
- > CellType1_TriCOMGraph

| Name | Description | SectionName |
|----------|-------------|-------------|
| Items: 0 | | |

Replace
Delete
Up

Down

Insert Add

The columns in the table will automatically size to fit the content. You can also set the column width manually with the mouse.

Context menu

There are 2 context menus for tables: the frame's context menu and the cell context menu.

Frame Context menu:

The context menu is displayed by right-clicking on the blank space above the table cells.

| | |
|----------------|--|
| Copy | Copies the table to the clipboard. |
| Save Image | Saves the table as a graphic to disk. |
| Edit Frame | Edits the properties of the table. |
| Bring to Front | Brings the object to the front of other objects. |
| Send to Back | Sends the object behind other objects. |
| Copy to page | Copies the object to another page. |
| Move to page | Moves the object to another page. |
| Delete | Deletes the object. |
| Flag to Save | Check this option to save the image after auto analysis. |
| Export Table | Exports the table in tab-delimited format. |
| Copy Formulas | Copies the table's formulas to the clipboard. |

Cell Context menu:

This menu is displayed when you right-click in a cell of the table.

| | |
|---------------|--|
| Insert Row | Inserts a row above the cell's row. |
| Insert Column | Inserts a column to the left of the cell's column. |
| Delete Row | Deletes the row the cell is in. |
| Delete Column | Deletes the column the cell is in. |

Table



Table

Use this tool to create a table on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object.

Double-click the top of the object to edit its properties.

The Table object is used to display analysis results and keywords on the report.

| | Number | % Intact Live | Parent | % Parent |
|-------------------|----------|---------------|-------------------|----------|
| Intact Live Cells | 233850 | 88.36 | All events | 88.36 |
| Lymphocytes | 66905.00 | 25.28 | Intact Live Cells | 25.28 |
| CD3 T Cell | 49431.00 | 18.68 | Lymphocytes | 73.88 |
| CD8 T Cells | 7660 | 2.89 | CD3 T Cells | 15.50 |
| Naive | 842.00 | 0.32 | CD8 T Cells | 10.96 |
| Central memory | 809.23 | 0.31 | CD8 T Cells | 10.56 |
| Effector memory | 3164 | 1.20 | CD8 T Cells | 41.31 |
| Terminal effector | 2827 | 1.07 | CD8 T Cells | 36.91 |
| CD4 T Cells | 38841 | 14.68 | CD3 T Cells | 78.58 |
| Naive | 5042 | 1.91 | CD4 T Cells | 12.98 |
| Central memory | 17762 | 6.71 | CD4 T Cells | 45.73 |
| Effector memory | 9290 | 3.51 | CD4 T Cells | 23.92 |
| Terminal effector | 6747 | 2.55 | CD4 T Cells | 17.37 |

A table has a user-defined number of rows and columns, which can be edited in the [Edit Properties for Information Table](#) dialog.

The cells in a table can contain text and keywords. Keywords must be surrounded with square brackets to be interpreted as keywords and evaluated.

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.

Click in a cell and type to enter a label.

| | Number | % Intact Live | Parent | % Parent |
|-------------------|-----------|---------------|-------------------|----------|
| Intact Live Cells | 288154 | 100.00 | All events | 96.05 |
| Lymphocytes | 139879.00 | 48.54 | Intact Live Cells | 48.54 |
| CD3 T Cell | 117834.00 | 40.82 | Lymphocytes | 84.10 |
| CD8 T Cells | 35899 | 12.48 | CD3 T Cells | 30.52 |
| Naive | 25089 | 8.71 | CD8 T Cells | 69.89 |
| Central memory | 4074 | 1.41 | CD8 T Cells | 11.35 |
| Effector memory | 3569 | 1.24 | CD8 T Cells | 9.94 |

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.

Right-click in a cell to select a keyword or result to display

| | Number | % Intact Live |
|-------------------|-----------|---------------|
| Intact Live Cells | 288154 | 100.00 |
| Lymphocytes | 139879.00 | 48.54 |
| CD3 T Cell | 117834.00 | 40.82 |
| CD8 T Cells | 35899 | 12.48 |
| Naive | 25089 | 8.71 |
| Central memory | 4074 | 1.41 |
| Effector memory | 3569 | 1.24 |

Edit Selected Information Units

Choose one or more information units to display. If you choose more than one, the info keywords will be put in the rows directly under the one you are editing.

Select Items Below

| Name | Description | SectionName |
|------------------------|-------------|-------------|
| Application | | |
| File Attribs | | |
| FCS Keywords | | |
| External Information | | |
| Methods | | |
| CellType1_TriCOMGraph2 | | |
| CellType1_TriCOMGraph | | |
| CellType1_TriCOMGraph | | |
| CellType4_TriCOMGraph1 | | |

Items: 0

OK Delete All

Replace Delete Up Down

Insert Add

The columns in the table will automatically size to fit the content. You can also set the column width manually with the mouse.

Context menu

There are 2 context menus for tables: the frame's context menu and the cell context menu.

Frame Context menu:

The context menu is displayed by right-clicking on the blank space above the table cells.

| | |
|----------------|--|
| Copy | Copies the table to the clipboard. |
| Save Image | Saves the table as a graphic to disk. |
| Edit Frame | Edits the properties of the table. |
| Bring to Front | Brings the object to the front of other objects. |
| Send to Back | Sends the object behind other objects. |
| Copy to page | Copies the object to another page. |
| Move to page | Moves the object to another page. |
| Delete | Deletes the object. |
| Flag to Save | Check this option to save the image after auto analysis. |
| Export Table | Exports the table in tab-delimited format. |
| Copy Formulas | Copies the table's formulas to the clipboard. |

Cell Context menu:

This menu is displayed when you right-click in a cell of the table.

| | |
|---------------|--|
| Insert Row | Inserts a row above the cell's row. |
| Insert Column | Inserts a column to the left of the cell's column. |
| Delete Row | Deletes the row the cell is in. |
| Delete Column | Deletes the column the cell is in. |

Edit Properties for Information Table

This command edits properties of the object's frame.

| Name | Value |
|--------------------------|--|
| Save Image | |
| Flag to Save | <input type="checkbox"/> Off |
| Subfolder | |
| Position and Size | |
| X Position | 6.38 |
| Y Position | 1.15 |
| X Size | 2.13 |
| Y Size | 3.30 |
| Other | |
| Show Title | <input checked="" type="checkbox"/> On |
| Title Font | Sample... |
| Outline | |
| Perimeter Line Width | -1 |
| Table | |
| Rows | 10 |
| Columns | 4 |
| Font | Sample... |
| Decimals | 2 |

Save Image

Flag to Save

Check to flag this object for saving its image when the file is analyzed.

Subfolder

Type in the subfolder name to store its image. Must be created in the canvas image folder.

Position and Size

X Position

Enter the x position in inches.

Y Position

Enter the y position in inches.

X Size

Enter the x size in inches.

Y Size

Enter the y size in inches.

Other

Show Title

Show the title of the object.

Title Font

Font of the title

Outline Color

Color of the object's perimeter

Outline Width

Line with of the object's perimeter (-1 to 20). A value of -1 turns off the border.

Rows and Columns

Rows

Enter the number of rows for this table.

Columns

Enter the number of columns for this table.

Font

Font for the table.

Decimals

Specify the number of decimal places for numbers in the table.

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.

EP Legend



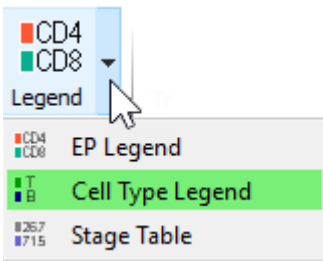
This tool allows you to create an expression profile legend on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties.

The expression profile legend is associated with a selected cell type when it is created. The legend shows the color of each measurement used in the cell type, along with other useful information about the measurement.

| | Color | Name | Metal/Fluor | Marker | Lookup Name |
|----|-------|--------|-------------|------------|----------------------|
| 1 | | CD66b | Yb172Di | CD66b | Yb172Di_172Yb_CD66b |
| 2 | | CD3 | Er170Di | CD3 | Nd150Di_150Nd_CD3 |
| 3 | | CD45 | Y89Di | CD45 | Y89Di_89Y_CD45 |
| 4 | | CD8 | Nd146Di | CD8a | Nd144Di_144Nd_CD8 |
| 5 | | CD4 | Nd145Di | CD4 | Nd143Di_143Nd_CD4 |
| 6 | | CD14 | Er168Di | CD14 | Sm154Di_154Sm_CD14 |
| 7 | | TCRgd | Dy164Di | TCRgd | Yb171Di_171Yb_TCRgd |
| 8 | | CD161 | Eu151Di | CD161 | Eu151Di_CD161 |
| 9 | | CCR7 | Er167Di | CD197_CCR7 | Gd160Di_160Gd_CCR7 |
| 10 | | CD28 | Gd160Di | CD28 | Er167Di_167Er_CD28 |
| 11 | | CD45RA | Nd150Di | CD45RA | Dy162Di_162Dy_CD45RA |

Cell Type Legend



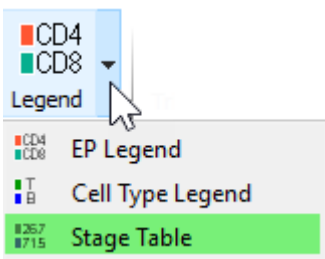
This tool allows you to create a cell type legend on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties.

The cell type legend shows a list of all defined cell types in the model. The color, name, and other attributes of the cell type can be displayed.

| | Color | Name | Enabled |
|----|-------|------------------|--|
| 1 | | CD8 | <input checked="" type="checkbox"/> On |
| 2 | | CD4 | <input checked="" type="checkbox"/> On |
| 3 | | GDs | <input checked="" type="checkbox"/> On |
| 4 | | MAITs NKTs | <input checked="" type="checkbox"/> On |
| 5 | | B | <input checked="" type="checkbox"/> On |
| 6 | | Monos | <input checked="" type="checkbox"/> On |
| 7 | | NKs | <input checked="" type="checkbox"/> On |
| 8 | | Basos | <input checked="" type="checkbox"/> On |
| 9 | | Neutrophils | <input checked="" type="checkbox"/> On |
| 10 | | CD66b- Neuts | <input checked="" type="checkbox"/> On |
| 11 | | Eos | <input checked="" type="checkbox"/> On |
| 12 | | pDCs | <input checked="" type="checkbox"/> On |
| 13 | | mDCs | <input checked="" type="checkbox"/> On |
| 14 | | Mo-MDSC | <input checked="" type="checkbox"/> On |
| 15 | | All Measurements | <input type="checkbox"/> Off |

Stage Table



This tool allows you to create a stage statistics table on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.

Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties.

The stage table is associated with a selected cell type when it is created. The table shows the name, color, and other useful information about the stages for the selected cell type.

| Name | Active | Color | Percent | %Total | Events | Mean | SD |
|----------|--|-------|---------|--------|--------|--------|------|
| Total | <input checked="" type="checkbox"/> On | | 100.00 | 2.89 | 7660 | 100.00 | 0.00 |
| T8 nv cm | <input checked="" type="checkbox"/> On | | 21.79 | 0.63 | 1669 | 2.79 | 0.00 |
| T8em | <input checked="" type="checkbox"/> On | | 41.31 | 1.20 | 3164 | 25.33 | 0.00 |
| T8te | <input checked="" type="checkbox"/> On | | 36.91 | 1.07 | 2827 | 42.52 | 0.00 |

The Active column can be used to turn stages on and off quickly. When a stage is active, statistics are computed and stage-coloring is applied to dots. When inactive, a stage is ignored by the analysis routines.

The Color for the stage is displayed and can be edited by clicking the color swatch.

Several statistics are displayed for each stage:

Percent The percentage of events relative to *this* cell type that are contained by the stage.

%Total The percentage of events relative to *all* cell types that are contained by the stage.

Events The number of events contained by the stage.

Mean When statistics are turned on, this shows the mean intensity value of the stage

SD When statistics are turned on, this shows the standard deviation of the stage.

TriCOM

TriCOM

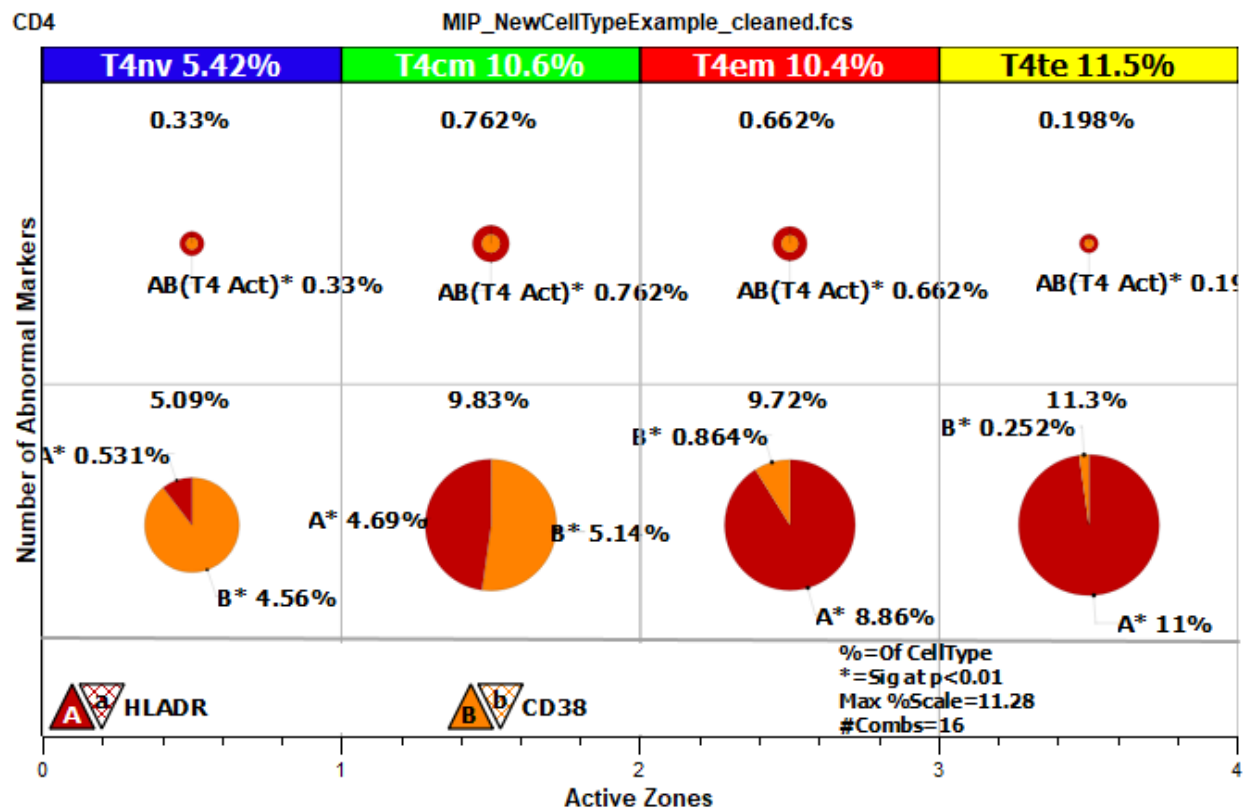
This tool allows you to create a TriCOM on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.



TriCOM

Once the tool is selected, click and drag on the report or cell type to create the object. The TriCOM will be created with default properties and a default set of markers. Double-click the object to edit its properties and select which markers to use with the TriCOM.

Tools to manipulate the plot appear on the right edge of the plot window when the mouse is over the plot.



The bottom of the TriCOM shows a legend of the markers that are being used. In the example above, the markers are HLADR and CD38. A letter is assigned to each marker. A capital letter is shown with a solid color and represents the above-normal expression of the marker. A lower-case letter is shown with a hatched color and represents below-normal

expression of the marker. In the example above, the letter A is assigned to HLADR and B is assigned to CD38.

If the TriCOM is created for a cell type that has stages defined, the TriCOM will have a column for each stage. In the example, there are 4 stages defined: T4nv, T4cm, T4em, and T4te.

The rows of a TriCOM contain pie charts of expression. The lowest row shows expression where only one of the markers is positive. The next row up shows 2 positive markers, and so on. In the example with two markers, there are two rows of expression pie charts: the first showing single-positive expression, and the second showing double-positives.

See the tutorial [Working with TriCOM](#) for additional explanation and examples.

Context menu

There are 2 context menus for TriCOM objects: the frame's context menu and the TriCOM plot context menu.

Frame context menu:

The context menu is displayed by right-clicking on the title area.

Copy

Copies the object to the clipboard.

Save Image

Saves the object as a graphic to disk.

Edit Frame

Displays the [Edit Frame](#) dialog.

Bring to Front

Brings the object to the front of other objects.

Send to Back

Sends the object behind other objects.

Delete

Deletes the object.

Flag to Save

Check this option to save the graphic after auto analysis.

TriCOM plot context menu:

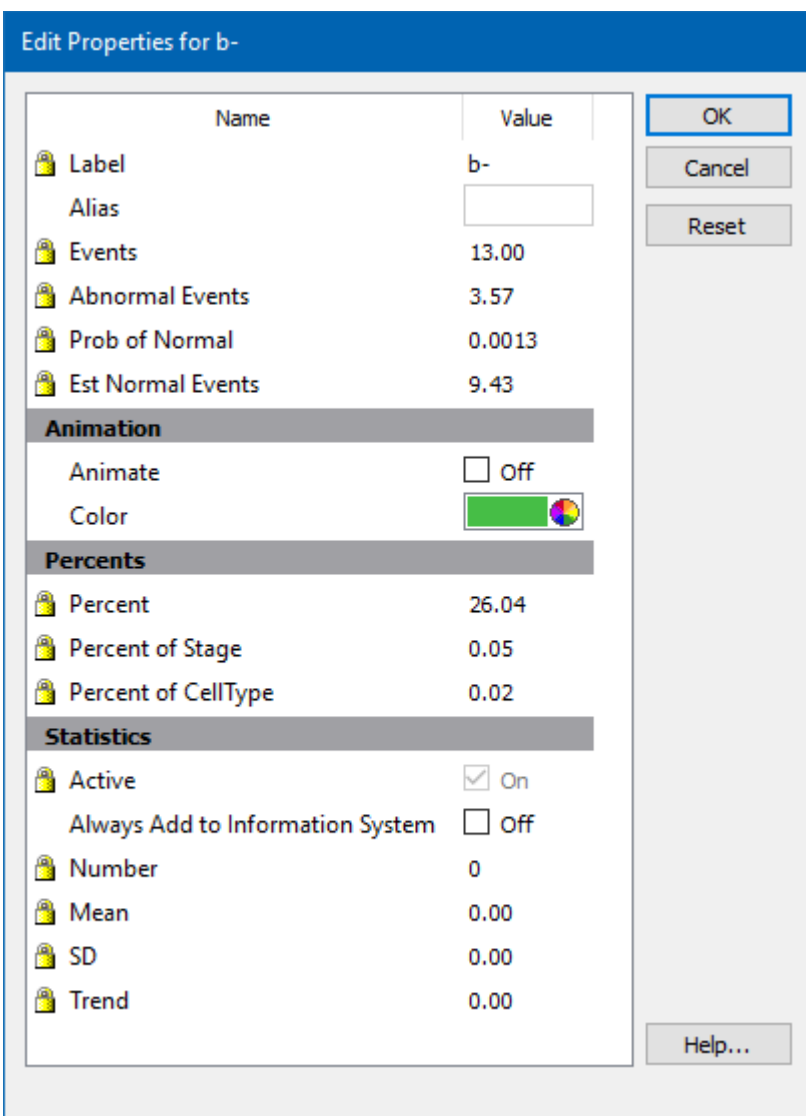
The context menu is displayed by right-clicking on the title area.

Edit Graphics

This command displays the [Graphic Options for TriCOM](#) dialog, used to select measurements for the TriCOM and adjust its display properties.

Edit Segment

This command allows you to edit the properties of the segment of the selected pie chart. Many properties are locked so that they cannot be edited. You can, however, edit the Alias, Animation, and several other properties of the segment with this dialog.



Edit All Segments

Edits all segments in all stages in the TriCOM. See the tutorial Working with TriCOM for additional explanation

Turn Off All Animations

Stops animations on all objects.

TriCOM

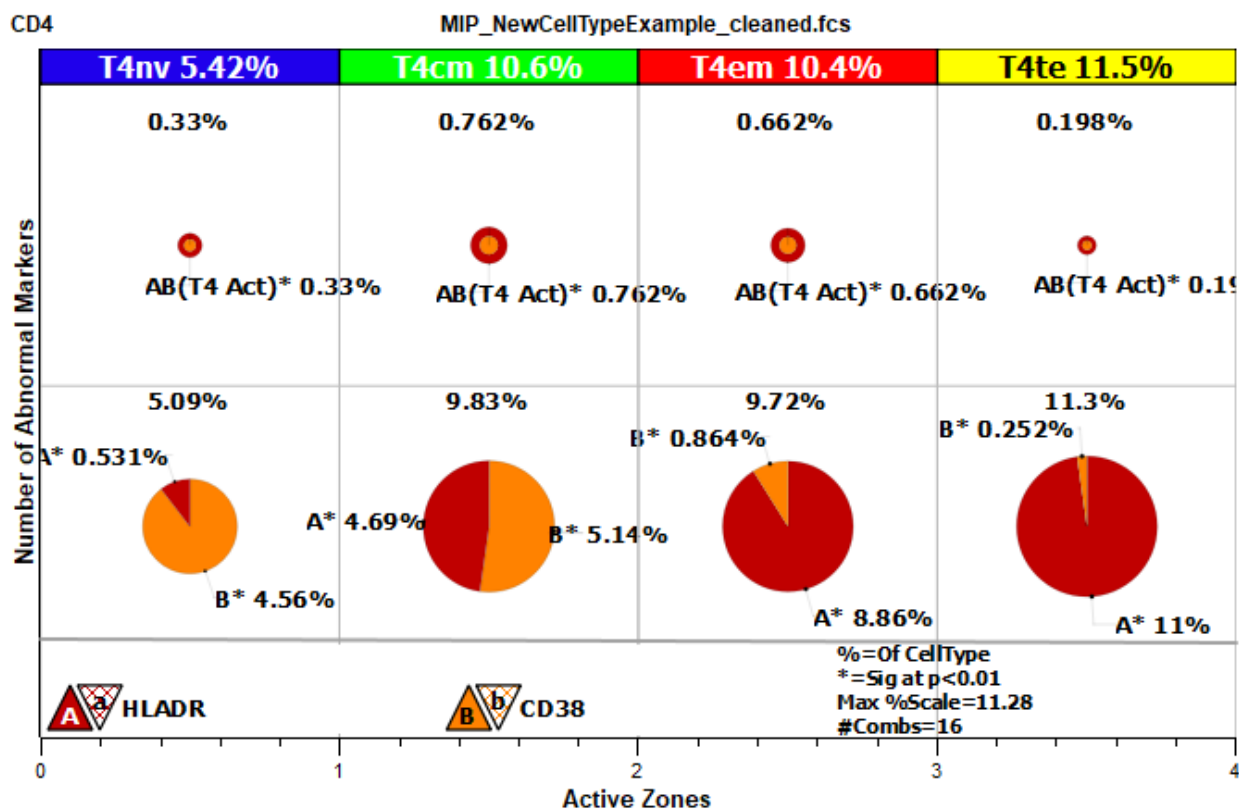
This tool allows you to create a TriCOM on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.



TriCOM

Once the tool is selected, click and drag on the report or cell type to create the object. The TriCOM will be created with default properties and a default set of markers. Double-click the object to edit its properties and select which markers to use with the TriCOM.

Tools to manipulate the plot appear on the right edge of the plot window when the mouse is over the plot.



The bottom of the TriCOM shows a legend of the markers that are being used. In the example above, the markers are HLADR and CD38. A letter is assigned to each marker. A capital letter is shown with a solid color and represents the above-normal expression of the marker. A lower-case letter is shown with a hatched color and represents below-normal expression of the marker. In the example above, the letter A is assigned to HLADR and B is assigned to CD38.

If the TriCOM is created for a cell type that has stages defined, the TriCOM will have a column for each stage. In the example, there are 4 stages defined: T4nv, T4cm, T4em, and T4te.

The rows of a TriCOM contain pie charts of expression. The lowest row shows expression where only one of the markers is positive. The next row up shows 2 positive markers, and so on. In the example with two markers, there are two rows of expression pie charts: the first showing single-positive expression, and the second showing double-positives.

See the tutorial Working with TriCOM for additional explanation and examples.

Context menu

There are 2 context menus for TriCOM objects: the frame's context menu and the TriCOM plot context menu.

Frame context menu:

The context menu is displayed by right-clicking on the title area.

Copy

Copies the object to the clipboard.

Save Image

Saves the object as a graphic to disk.

Edit Frame

Displays the [Edit Frame](#) dialog.

Bring to Front

Brings the object to the front of other objects.

Send to Back

Sends the object behind other objects.

Delete

Deletes the object.

Flag to Save

Check this option to save the graphic after auto analysis.

TriCOM plot context menu:

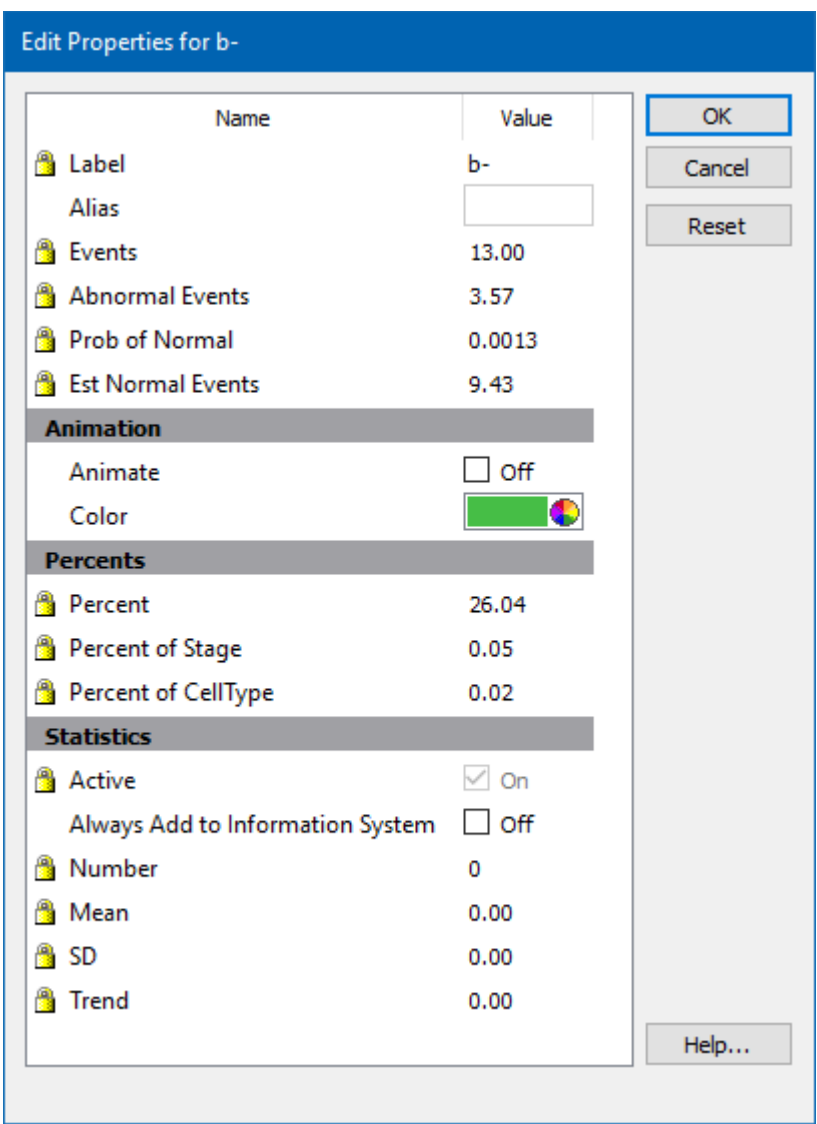
The context menu is displayed by right-clicking on the title area.

Edit Graphics

This command displays the [Graphic Options for TriCOM](#) dialog, used to select measurements for the TriCOM and adjust its display properties.

Edit Segment

This command allows you to edit the properties of the segment of the selected pie chart. Many properties are locked so that they cannot be edited. You can, however, edit the Alias, Animation, and several other properties of the segment with this dialog.



Edit All Segments

Edits all segments in all stages in the TriCOM. See the tutorial Working with TriCOM for additional explanation

Turn Off All Animations

Stops animations on all objects.

Graphic Options for TriCOM

General Options tab

Background, Grids, and Colors

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.

Quick Enable/Disable

Choose Measurements

Displays a dialog to select which measurements to include on the TriCOM.

Labeling

Show Stage Labels

Enable this option to show stage labels at the top of the TriCOM.

Show Key Labels

This option determines the number of labels to show on each pie chart segment.

Show Stage Boundaries

This option determines whether stage boundaries are drawn on the plot.

Stage Label Font

Choose a font to use for the labels.

Show Key

Displays a key of enabled EPs on the TriCOM.

Show Key Strings

Displays a key of additional information about the TriCOM.

Key Label Font

Choose a font to use for the keys.

Percent Precision

Choose the number of decimal places to show.

Percent Type

Choose the type of percentage computed.

Additional Properties

Default TriCOM Mode

Select the mode for measurements in this TriCOM. Choose from High and Low, High Only, and Low Only.

Show Significant Combinations

When checked, pie chart segments are only shown for combinations that are statistically significant.

Probability Threshold

When checked, the TriCOM will only include events with a probability of being defined by this cell type of less than or equal to this value. A value of 0.0 turns the feature off. A value of 0.01 shows only rare events.

TriCOM Count P Value

P value to use to determine whether a combination count is significant or not. (0.000 to 1.000)

Normal Adjustment

Check to automatically subtract the contributions from the normal events that happen to be outside of the normal boundaries.

Sort Percents

Check to sort the pie percents in descending order.

Scale to Counts

Check if you want to scale the pie areas to be proportional to their counts; otherwise, make pie charts the same size.

Maximum Scale Percent

Redefines the maximum scale percent if the graph is not zoomed and Scale To Counts is true. (0.00000 to 100.00000)

Show All Normals

Check to see all combinations, including those that are classified as "normal".

Graphic Options for Measurements

Each parameter in a TriCOM Plot has a set of graphic options that determine how it appears in the graphics. You can view and edit these properties by double-clicking a TriCOM plot, or right-clicking a TriCOM plot and choosing Edit Graphics.

Include Parameter

Check to include the parameter in the TriCOM analysis.

TriCOM Mode Override

Choose mode of the analysis. Overrides the main TriCOM editor mode.

Parameter Color

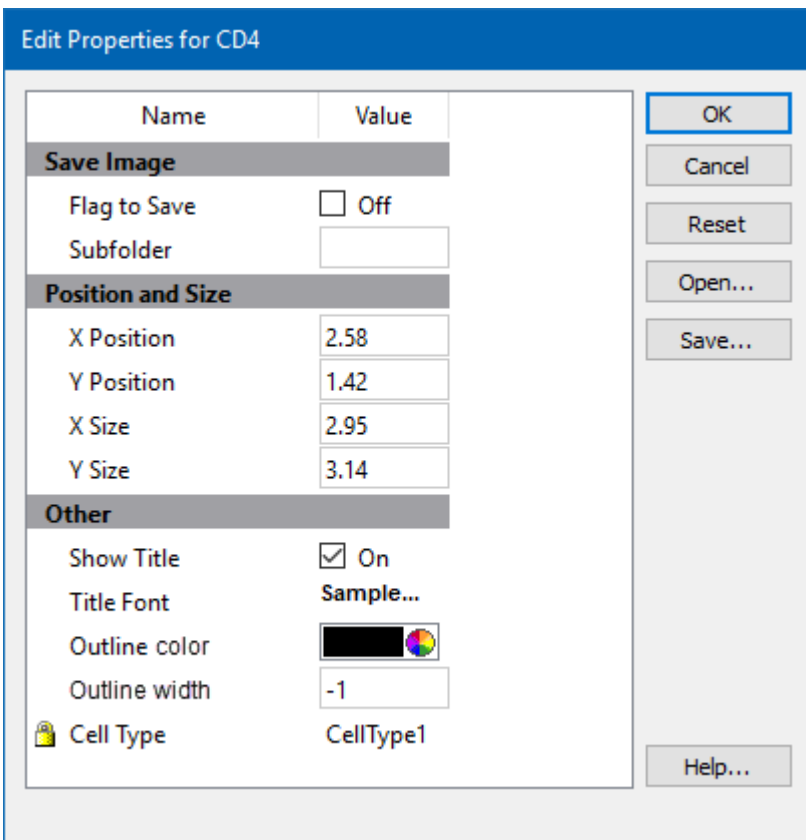
Show the parameter color. Defaults to the parameter profile color.

Filter

Choose which type of events to include in the TriCOM.

Edit Frame

This command edits properties of the object's frame.



Save Image

Flag to Save

Check to flag this object for saving its image when the file is analyzed.

Subfolder

Type in the subfolder name to store its image. Must be created in the canvas image folder.

Position and Size

X Position

Enter the x position in inches.

Y Position

Enter the y position in inches.

X Size

Enter the x size in inches.

Y Size

Enter the y size in inches.

Other

Show Title

Show the title of the object.

Title Font

Font of the title

Outline Color

Color of the object's border

Outline Width

Line width of the object's border(-1 to 20). A value of -1 turns off the border.

Cell Type

For objects that are associated with a specific cell type, this property shows the cell type.

Name

This is the internal name of the object that cannot be edited. It is used to uniquely identify the object.

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.

TriCOM toolbar

The TriCOM toolbar is located on the right side of each TriCOM object and is displayed when the mouse moves over the 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. |
|  | Flag to Save Graphic | Check this option to save the graphic after analysis. |
|  | View in dialog | Click this tool to view the object in a dialog window. |

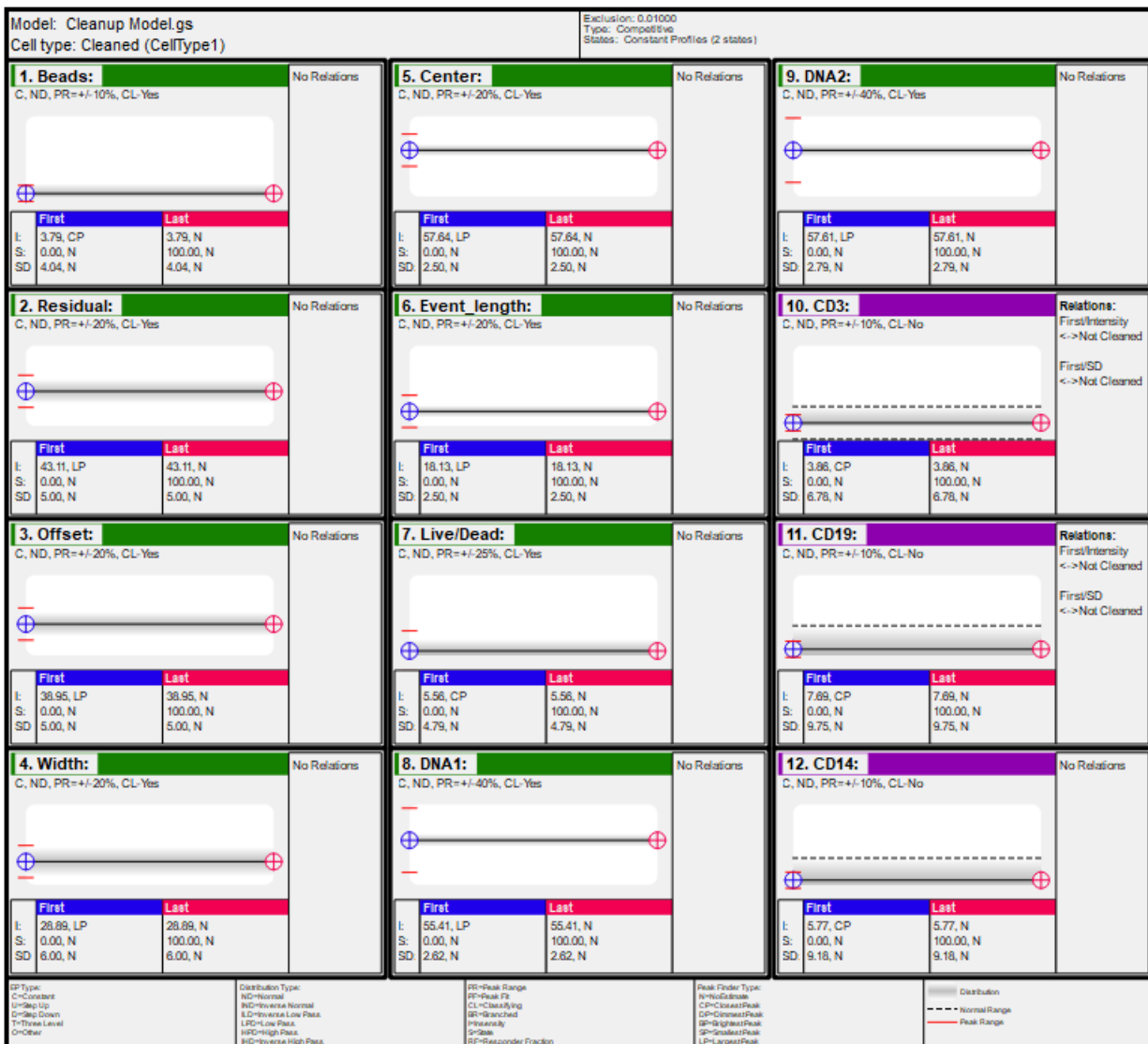
Design object

This tool allows you to create a Design object on the report or cell types. It is found on the [Insert tab](#) of the ribbon.



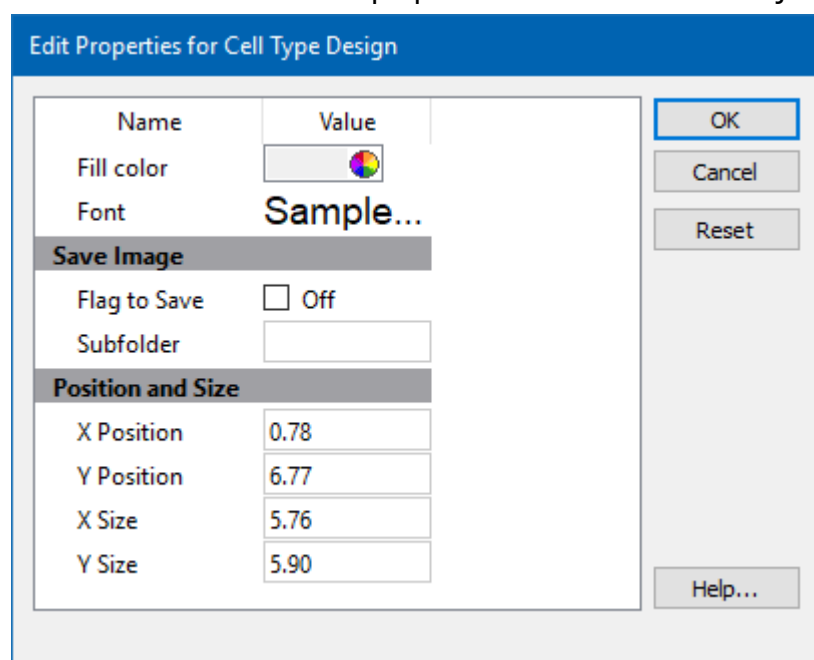
Design

The Design object displays a schematic diagram of the measurement expression profiles used in a cell type. It is a great way to document how the cell type is designed.



It works best when it is used on a report page that has nothing else on it, because it requires a log of space for its layout. The layout automatically adjusted to make best use of the space to show all of the profiles for the cell type.

Once the tool is selected, click and drag on the report create the object. The Design object will be created with default properties. Double-click the object to edit its properties.



Context menu

The context menu is displayed by right-clicking on the object.

Copy

Copies the object to the clipboard.

Save Image

Saves the object as a graphic to disk.

Edit

Displays the Edit Properties dialog shown above.

Bring to Front

Brings the object to the front of other objects.

Send to Back

Sends the object behind other objects.

Delete

Deletes the object.

Cen-se'

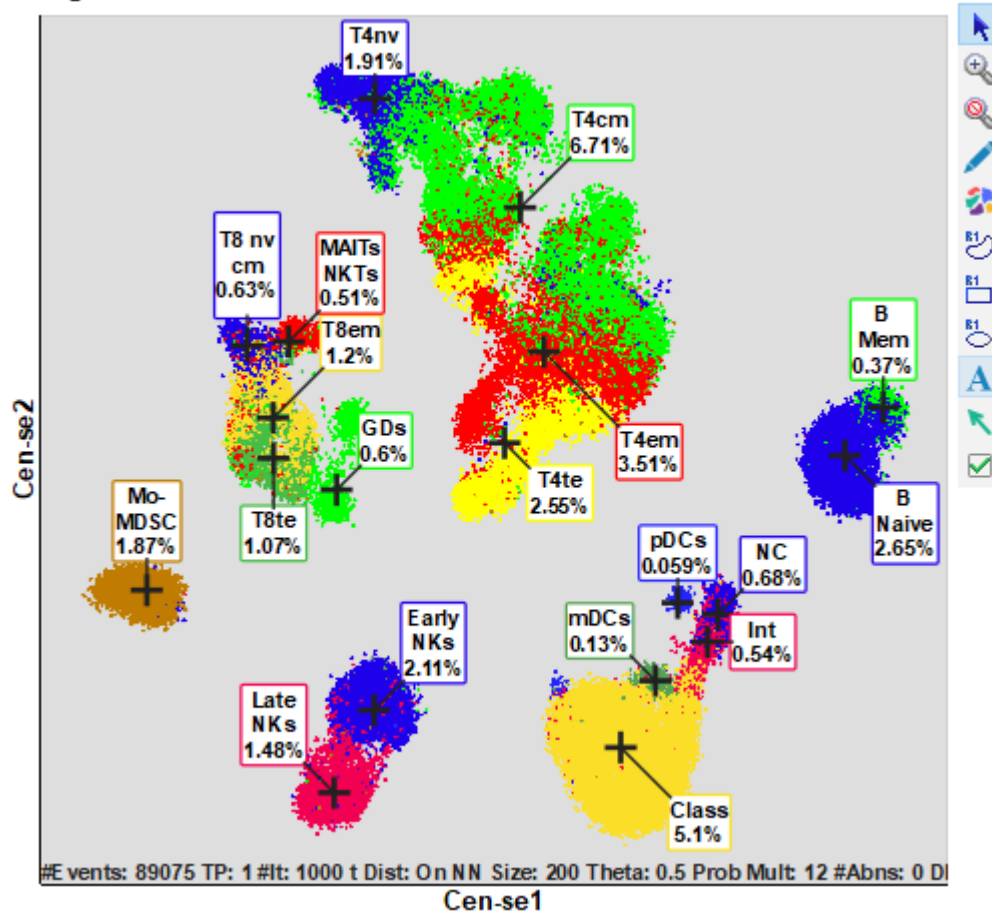
Cen-se

This tool allows you to create a Cen-se' plot on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.



Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties. The Cen-se' toolbar is displayed to the right of the object when the mouse moves over the plot.

MIP.gs



Context menu

There are 2 context menus for Cen-se' objects: the frame's context menu and the Cen-se plot context menu.

Frame context menu:

The context menu is displayed by right-clicking on the title area.

Copy

Copies the object to the clipboard.

Save Image

Saves the object as a graphic to disk.

Edit Frame

Displays the [Edit Frame](#) dialog.

Bring to Front

Brings the object to the front of other objects.

Send to Back

Sends the object behind other objects.

Copy to Page

Makes a copy of this object on another page.

Move to Page

Moves this object to another page.

Delete

Deletes the object.

Flag to Save

Check this option to save the graphic after auto analysis.

Cen-se' context menu:

The context menu is displayed by right-clicking on the title area.

Edit Graphics

This command displays the [Graphic Options for Cen-se](#) dialog, used to select cell types and measurements for the Cen-se' and adjust its display properties.

Edit Frame

Displays the [Edit Frame](#) dialog.

Rebuild Cen-se for All Events

Rebuild the Cen-se plot.

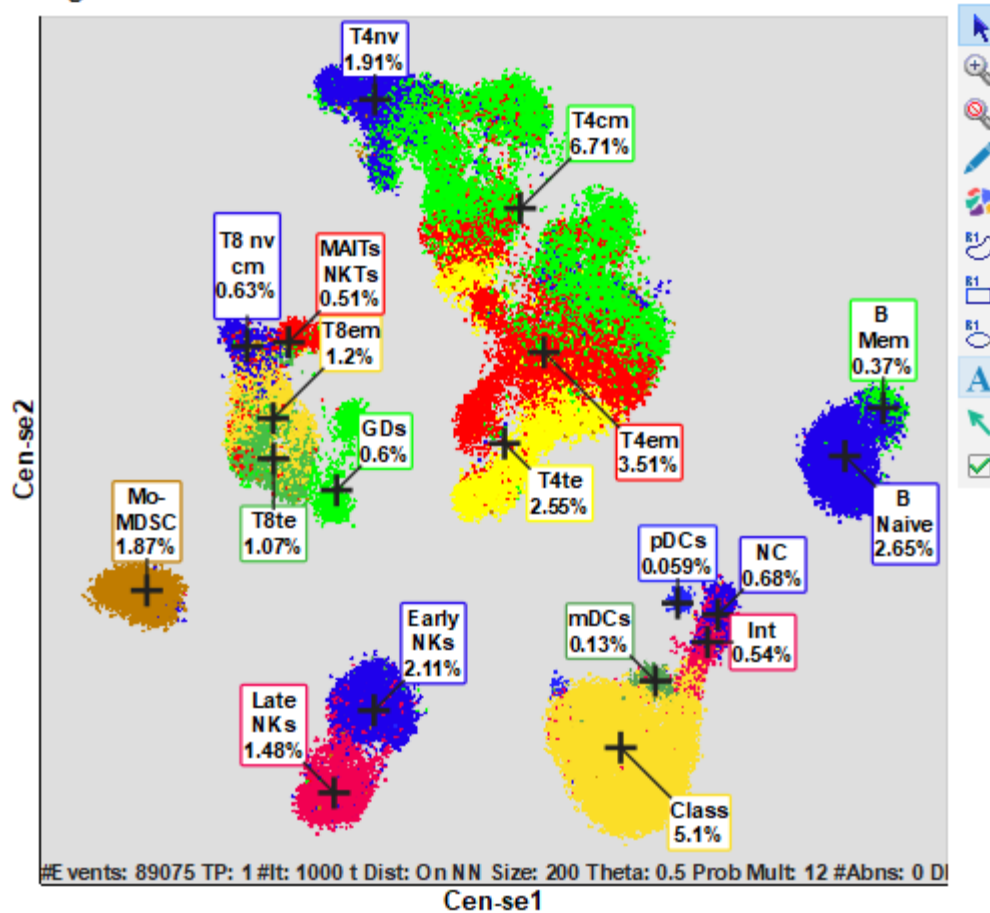
Cen-se

This tool allows you to create a Cen-se' plot on the report or cell types. It is found on the [Insert tab](#) of the ribbon, as well as the toolbar for the report and cell type.



Once the tool is selected, click and drag on the report or cell type to create the object. Double-click the object to edit its properties. The Cen-se' toolbar is displayed to the right of the object when the mouse moves over the plot.

MIP.gs



Context menu

There are 2 context menus for Cen-se' objects: the frame's context menu and the Cen-se plot context menu.

Frame context menu:

The context menu is displayed by right-clicking on the title area.

Copy

Copies the object to the clipboard.

Save Image

Saves the object as a graphic to disk.

Edit Frame

Displays the [Edit Frame](#) dialog.

Bring to Front

Brings the object to the front of other objects.

Send to Back

Sends the object behind other objects.

Copy to Page

Makes a copy of this object on another page.

Move to Page

Moves this object to another page.

Delete

Deletes the object.

Flag to Save

Check this option to save the graphic after auto analysis.

Cen-se' context menu:

The context menu is displayed by right-clicking on the title area.

Edit Graphics

This command displays the [Graphic Options for Cen-se](#) dialog, used to select cell types and measurements for the Cen-se' and adjust its display properties.

Edit Frame

Displays the [Edit Frame](#) dialog.

Rebuild Cen-se for All Events

Rebuild the Cen-se plot.

Graphic Options for Cen-se'

Graphics

Background Color

Choose a color for the background of the plot.

X Grid Lines

Enable this option to show grids for the X-axis. Grid lines will appear for each major tic on the axis.

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.

Margin

Margin as a percentage of the size.

Legend

Enable this option to show the legend on the plot.

Initial Scaling Size

Set the initial axis scaling for the Cen-se plot. This is recomputed as the plot is rebuilt.

Labels

Show or hide labels.

Label Font

Choose a font for the labels.

Measurements and Cell Types**Measurements**

Displays a dialog to select which measurements to include on the Cen-se.

Cell Types

Displays a dialog to select which cell types to include on the Cen-se.

Rebuild Measurements List

Rebuilds the list of measurements.

Rebuild Cell Type List

Rebuilds the list of cell types.

Cen-se Options**Maximum Events**

Maximum number of events to allow in the Cen-se when not in Template mode.

Percent Neighborhood Size

Relative size of the neighborhood used for each event, as a percentage of Maximum Events.

Neighborhood Size

Computed size of the neighborhood.

Density Normalization

Creates better-resolved plots. When unchecked, additional structure is shown within populations.

Cen-se Results

Info Loss

A measure of information loss from dimensionality reduction. The unit is nats.

%Information Lost

Information loss expressed as a percentage.

P Q R-Squared

Quantifies the degree of correlation between Q and P nearest neighbor matrices.

Total Time

Total time to build the Cen-se plot.

Interpolation Time

Interpolation time for Template mode.

Clustering Index

Index based on the Dunn index. Higher values indicate better clustering.

Beta Clustering Index

Index based on the SSMD beta index. Lower values indicate better clustering.

Cen-se Options

This tab is part of the [Edit Graphic Options](#) for Cen-se dialog and contains additional display options.

Dots

Auto Size

Enable this option to allow the program to determine the best dot size, based on the current size of the 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 10.0 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.

Number

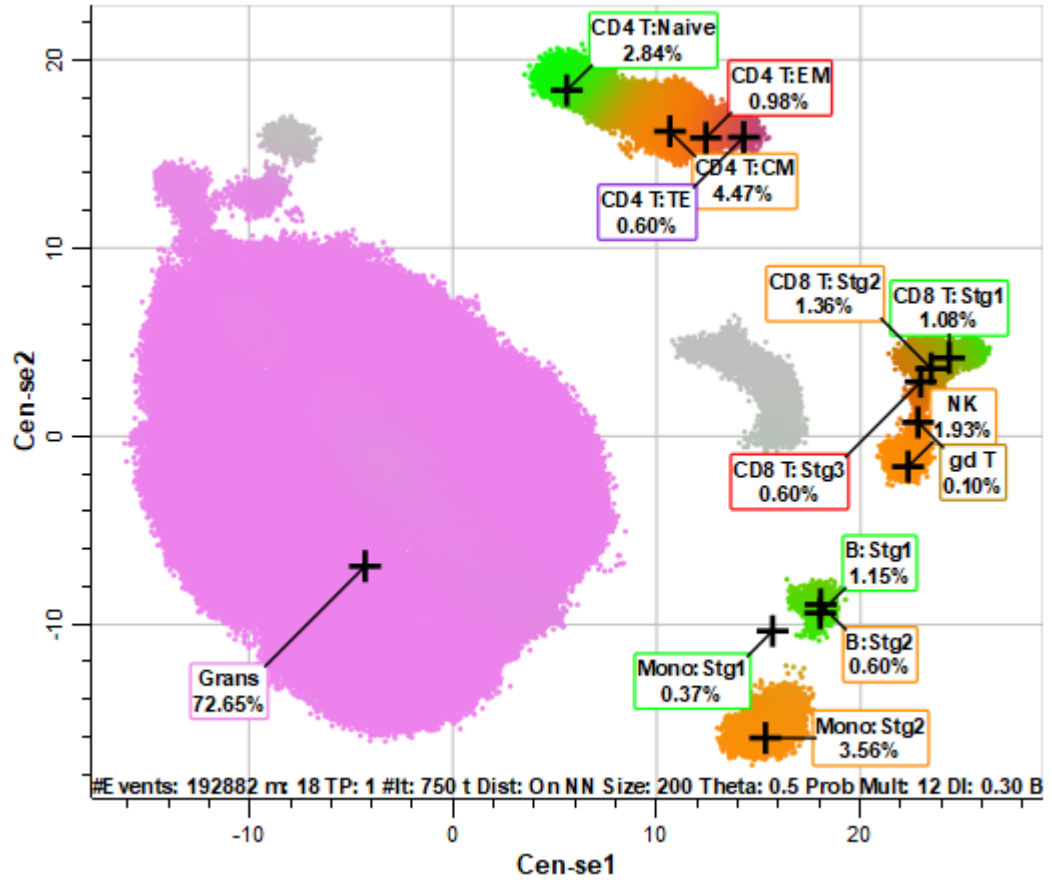
If Show All Dots is unchecked, the system will use this value to determine the number of dots to display in the plot.

Coloring

Color Scheme

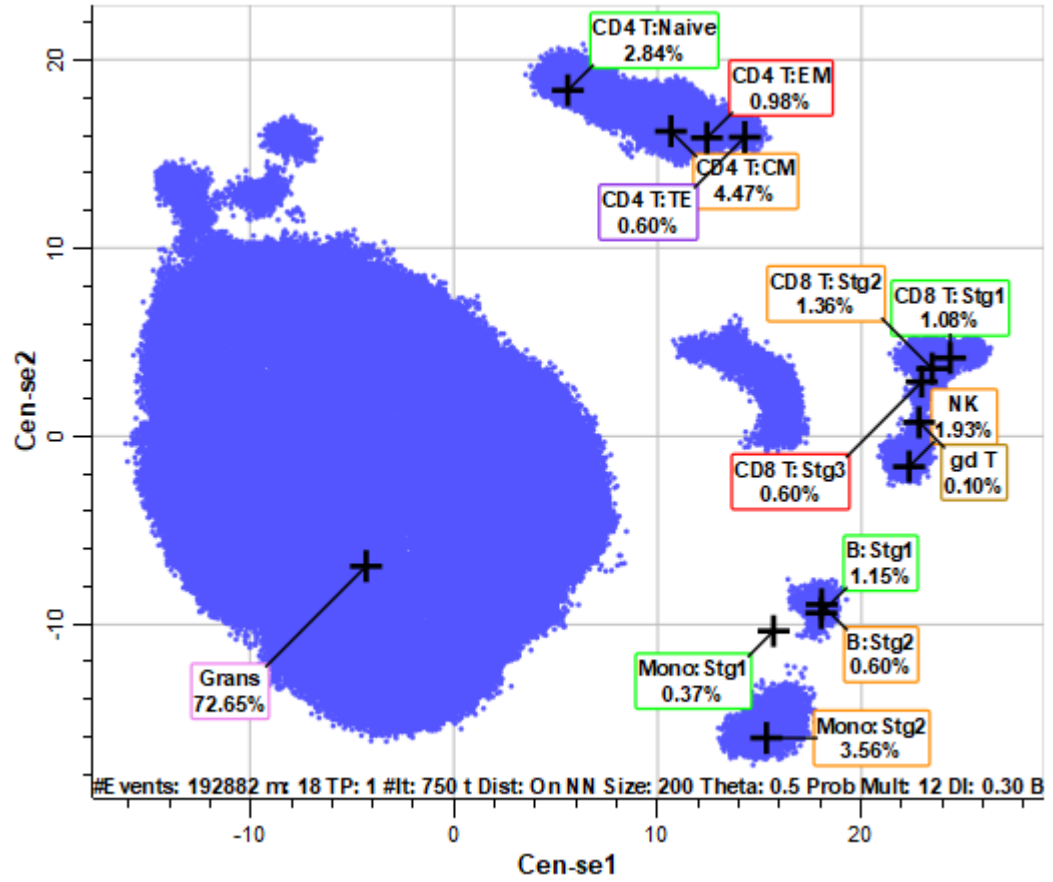
Choose the desired color scheme for Cen-se dots.

Summary Colors are based on cell type and staging colors.
Map
Colors



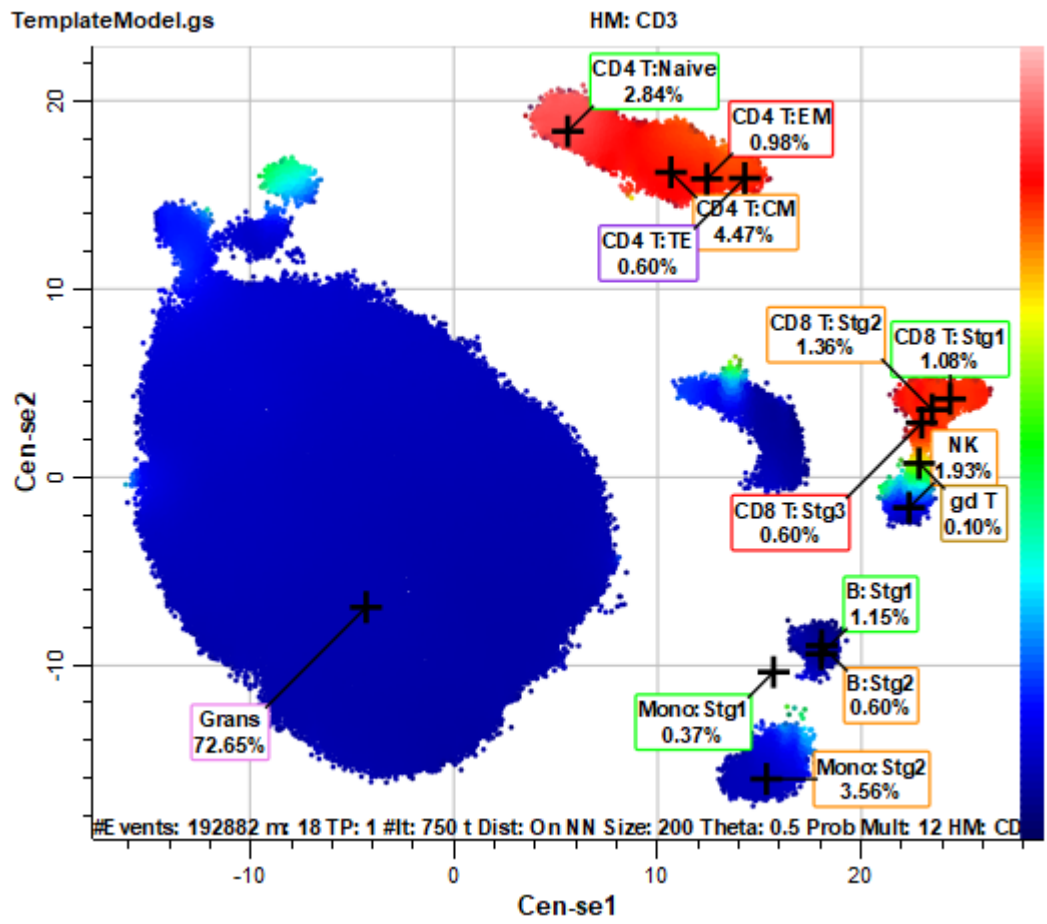
Alternative Color

All events are drawn with the Alternative Color selected.

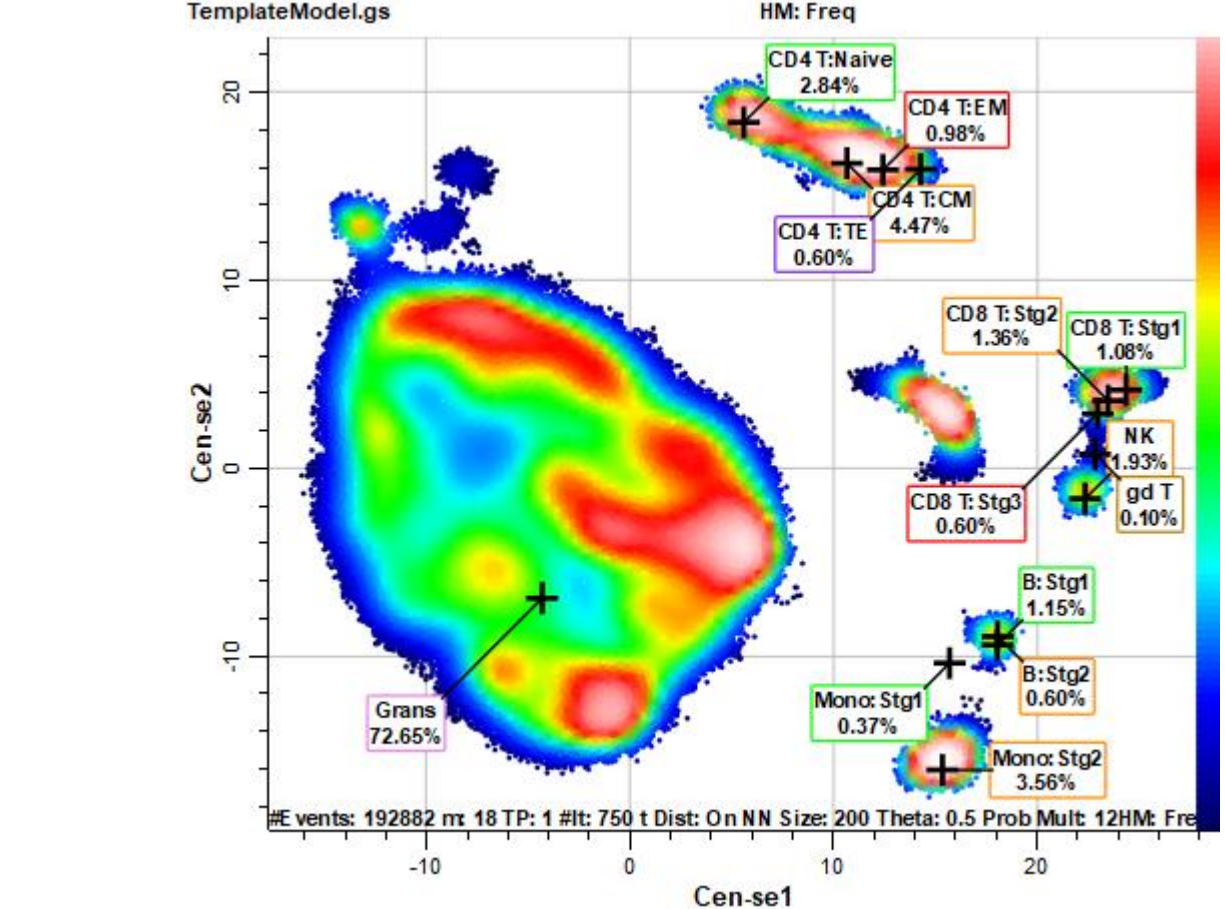


Intensity Heatmap

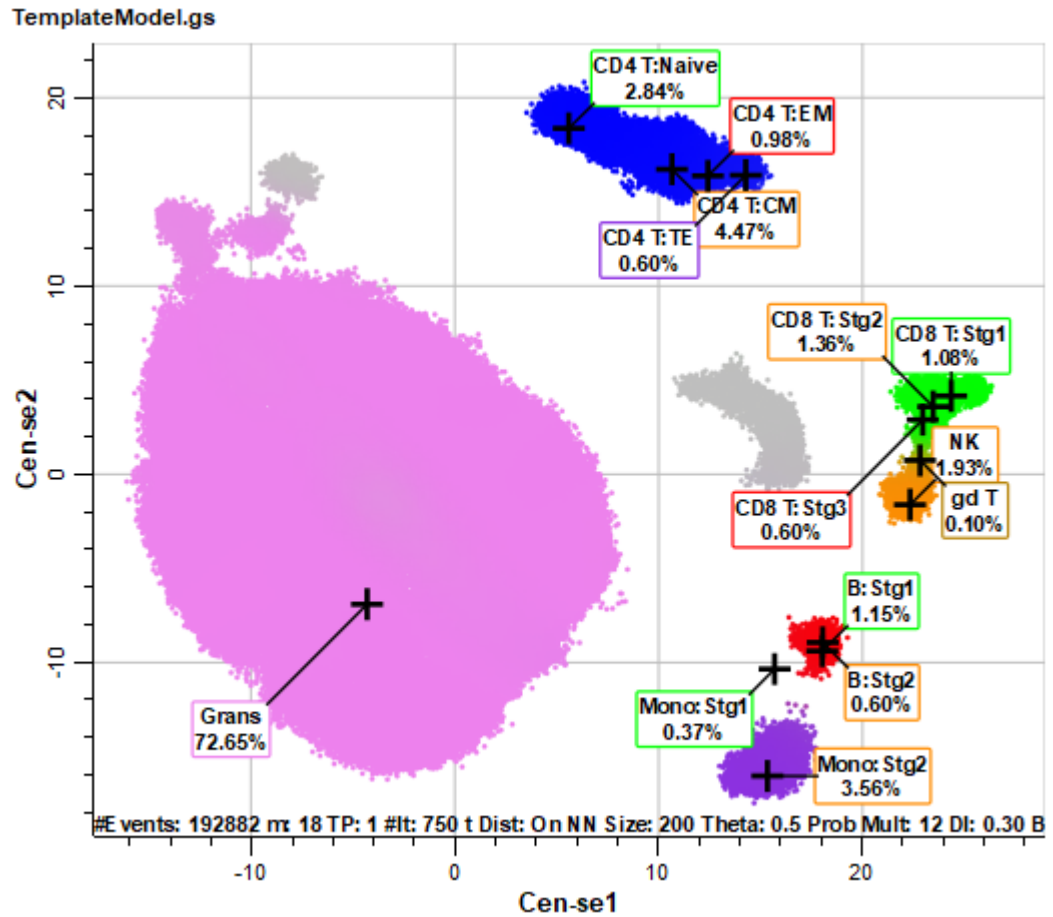
Heatmap colors are based on intensity of events for the selected measurement.



Frequency Heatmap colors are based on frequency of events.



Cell Type Colors are based on cell type colors.



Heatmap Measurement

Click to choose a measurement for the Intensity Heatmap.

Next Measurement

Selects the next measurement for Intensity Heatmap coloring.

Preceding Measurement

Selects the previous measurement for Intensity Heatmap coloring.

Alternative Color

This property defines the color used for Alternative Color coloring scheme.

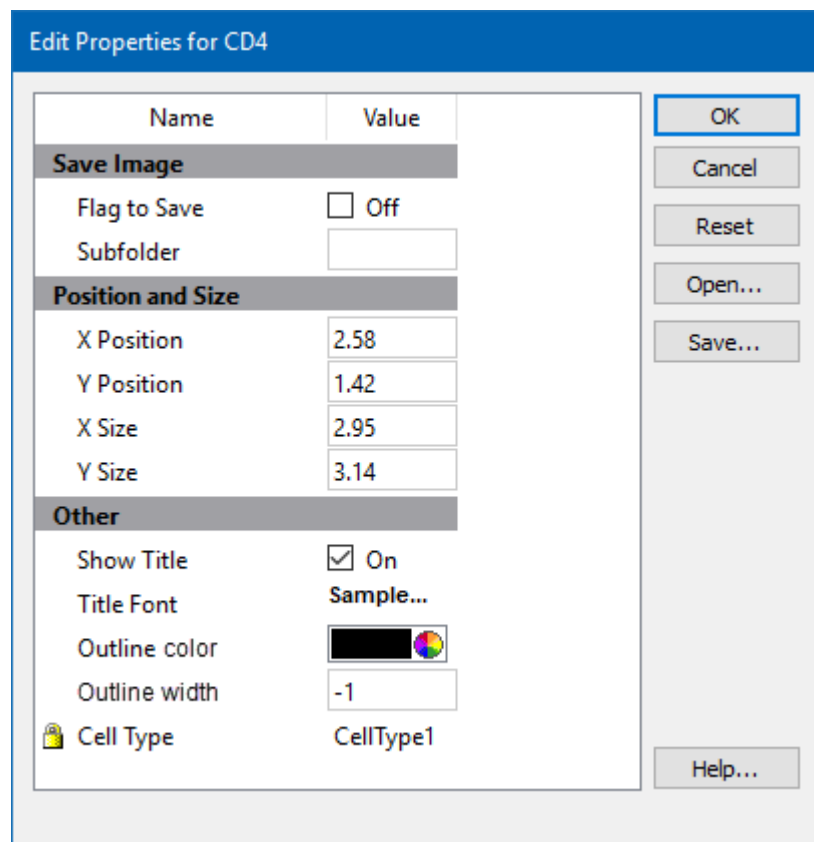
Options

Population Selection Mode

If checked, only populations selected in the Summary Map are drawn.

Edit Frame

This command edits properties of the object's frame.



Save Image

Flag to Save

Check to flag this object for saving its image when the file is analyzed.

Subfolder

Type in the subfolder name to store its image. Must be created in the canvas image folder.

Position and Size

X Position

Enter the x position in inches.

Y Position

Enter the y position in inches.

X Size

Enter the x size in inches.

Y Size

Enter the y size in inches.

Other**Show Title**

Show the title of the object.

Title Font

Font of the title

Outline Color

Color of the object's border

Outline Width

Line width of the object's border(-1 to 20). A value of -1 turns off the border.

Cell Type

For objects that are associated with a specific cell type, this property shows the cell type.

Name

This is the internal name of the object that cannot be edited. It is used to uniquely identify the object.

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.

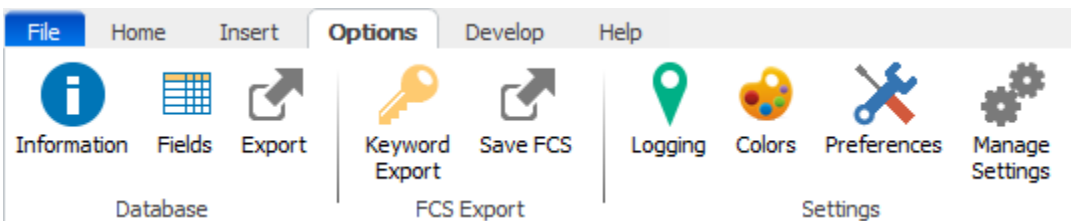
Cen-se' Toolbar

The Cen-se' toolbar is located on the right side of a [Cen-se' plot](#) on the report and is displayed when the mouse moves over the object.

| | | |
|---|----------------------|--|
|  | Selection | Use this tool to select, move, and size objects in the object. |
|  | 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. |
|  | Edit Graphics | Edits the graphics settings for this object. |
|  | Build Cen-se' | Click this button to rebuild the Cen-se plot. |
|  | Polygon Region | Use this tool to define a free-hand region on the plot. The region can be used to color events. After selecting this tool, click and drag on the plot to define the region. |
|  | Rectangle Region | Use this tool to define a rectangular region on the plot. The region can be used to color events. After selecting this tool, click and drag on the plot to define the region. |
|  | Ellipse Region | Use this tool to define an elliptical region on the plot. The region can be used to color events. After selecting this tool, click and drag on the plot to define the region. |
| <input checked="" type="checkbox"/> | Flag to Save Graphic | Check this option to save the graphic after analysis. |
| <input type="checkbox"/> | | |

Options tab

Options Tab



Database

[Information](#)
[Fields](#)
[Export](#)

FCS Export

[Keyword Export](#)
[Save FCS](#)

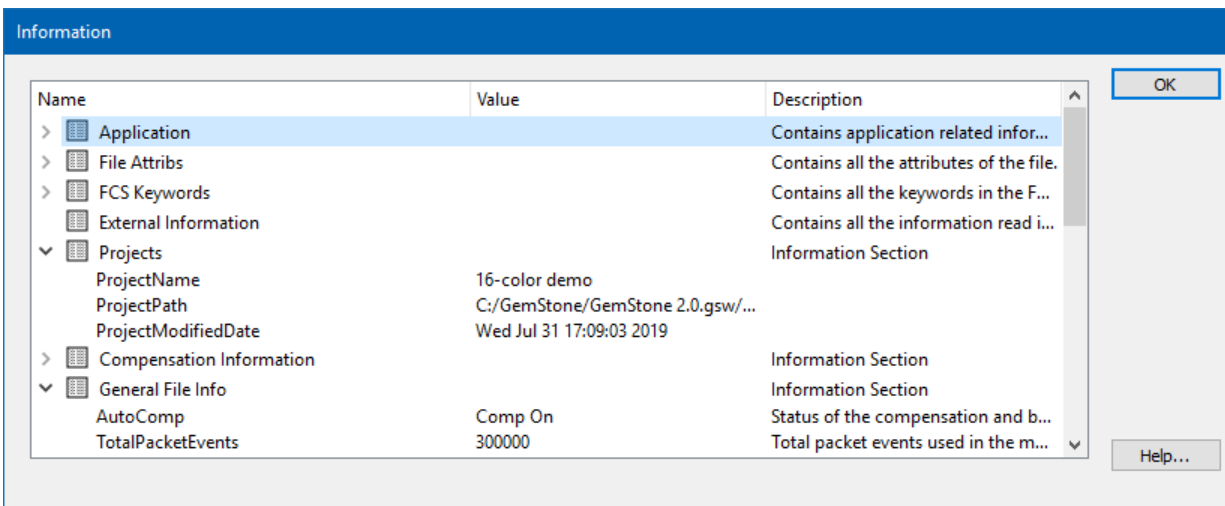
Settings

[Logging](#)
[Colors](#)
[Preferences](#)
[Manage Settings](#)

* Note, the Options tab is only displayed when the program is in [Develop mode](#). Develop mode is enabled from the [Home tab](#).

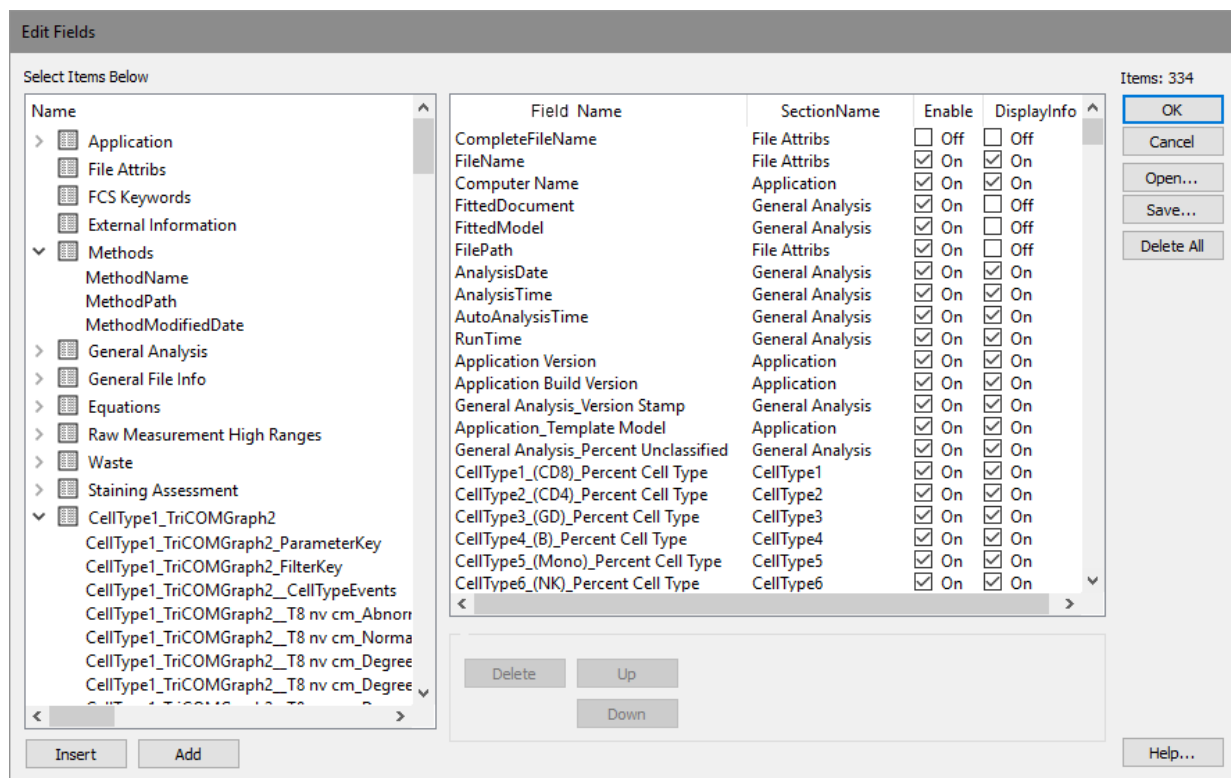
Information

This command displays a dialog that contains all information that is available to the database and reporting. The dialog information is read-only.



Edit Fields

This dialog is used to select the columns that are displayed in the [Batch List](#), 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 **Insert** or **Add** button.
- Remove fields Select one or more items from the list on the right and then click **Delete**.
- Change the order of columns Select an item in the list and use the **Up** or **Down** button.
- Change the name of a field Edit the text in the **Field Name** column.
- Show or hide a column Add the field to the list and check or uncheck the **Display** option.

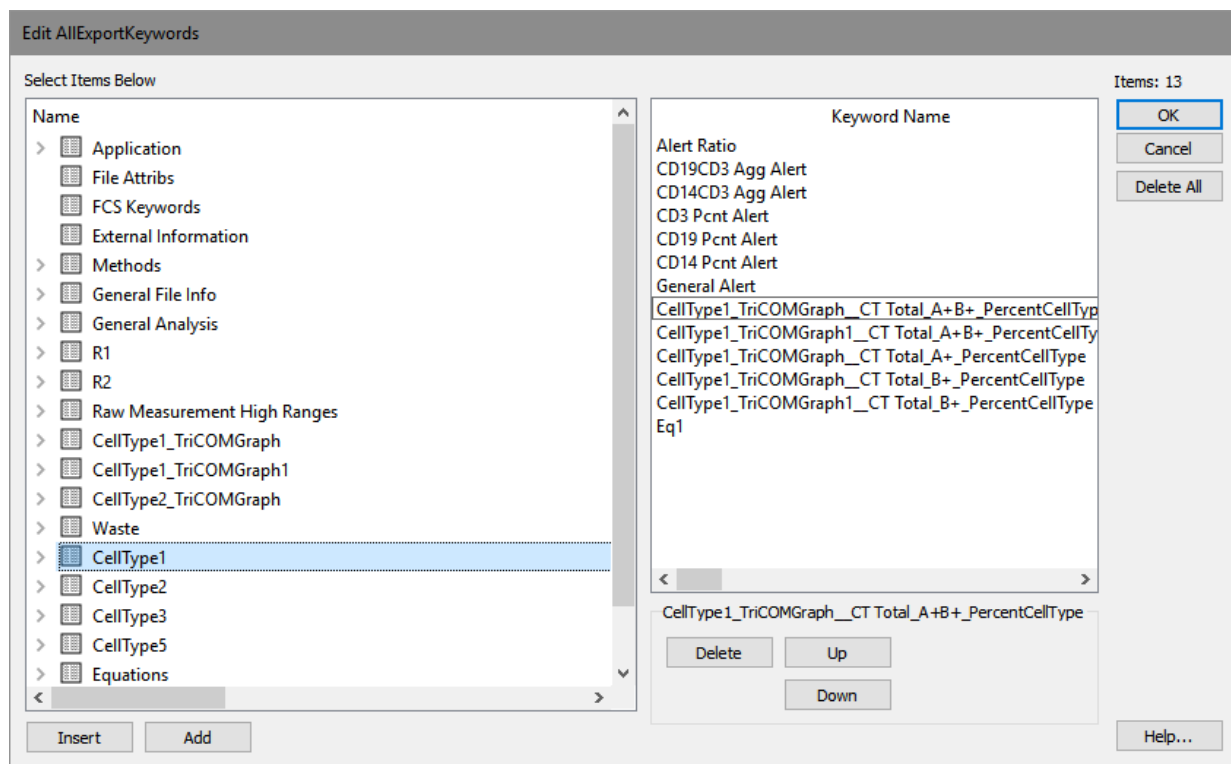
Export Database

Use this command to export the analysis information in the batch database to a tab-delimited text file. The command will display the standard Export Database 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.

Tab-delimited 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.

Keyword to Export

This command is used to set up keywords to export when the program creates FCS files with the Save FCS command or during batch processing. The keywords selected are saved in the model template, so when keywords are added or removed, the model template should be re-saved.



Add keywords from the information tree on the left side of the dialog. Once added, the Keyword Name can be edited to be concise and informative. Keywords selected will be added to any FCS files saved by that model template.

If you want to...

Add keywords

Select one or more items in the information tree on the left and then click the **Insert** or **Add** button.

Remove keywords

Select one or more items from the list on the right and then click **Delete**.

Change the order of keywords

Select an item in the list and use the **Up** or **Down** button.

Synthesize from Analysis

This command is used to generate data based on the current model *and* the FCS file that is currently analyzed with the model. See also the [Synthesize from Model](#) command.

Before choosing this command, construct or open a model to be used as a basis for the synthesized data and analyze an FCS file with the model.

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.

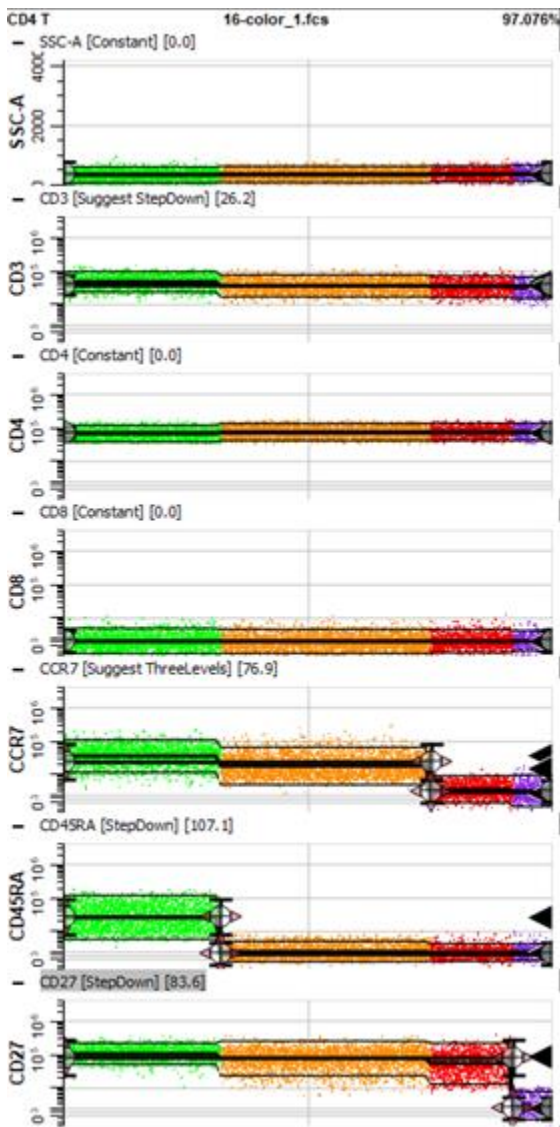
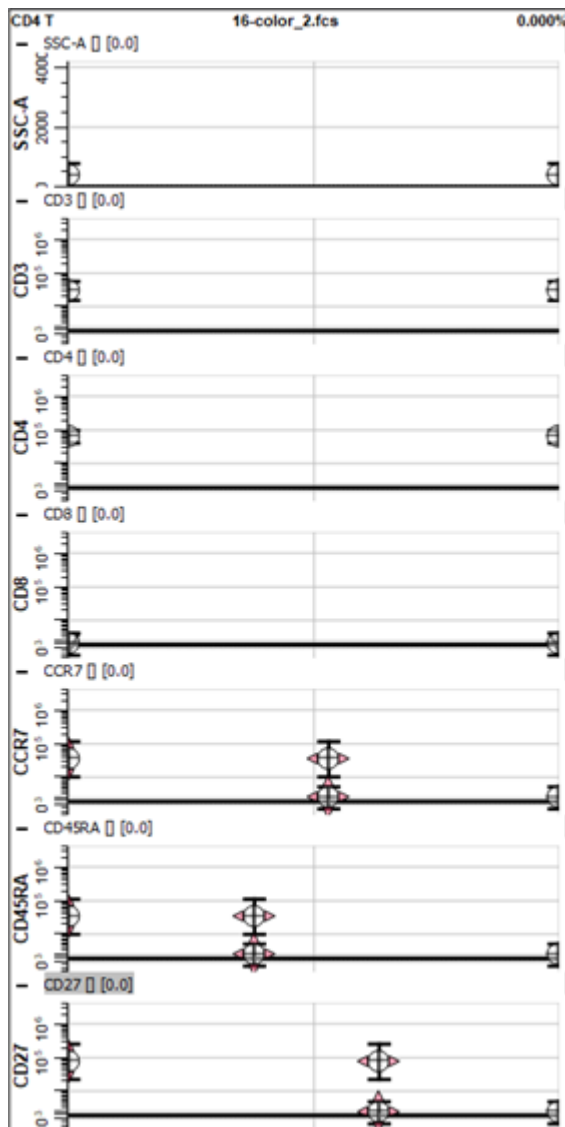
The model and the FCS file determine the characteristics of the synthesis process. The same probabilistic processes that are used for classifying real data with a model are put to use to synthesize events. The synthesized events will have a very realistic distribution, as the system takes into account the actual distributions in the FCS file data.

After synthesis, the generated data appears in the Expression Profile plots and other graphics. Statistics and stages are computed for the synthesized data.

The synthesized data can be exported to an FCS file using the [Save FCS](#) command.

Before

After



See also:

[Synthesize from Model](#)

[Save FCS](#)

Synthesize from Model

This command is used to generate data based on the current model. See also the [Synthesize from Analysis](#) command.

Before choosing this command, construct or open a model to be used as a basis for the synthesized data.

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.

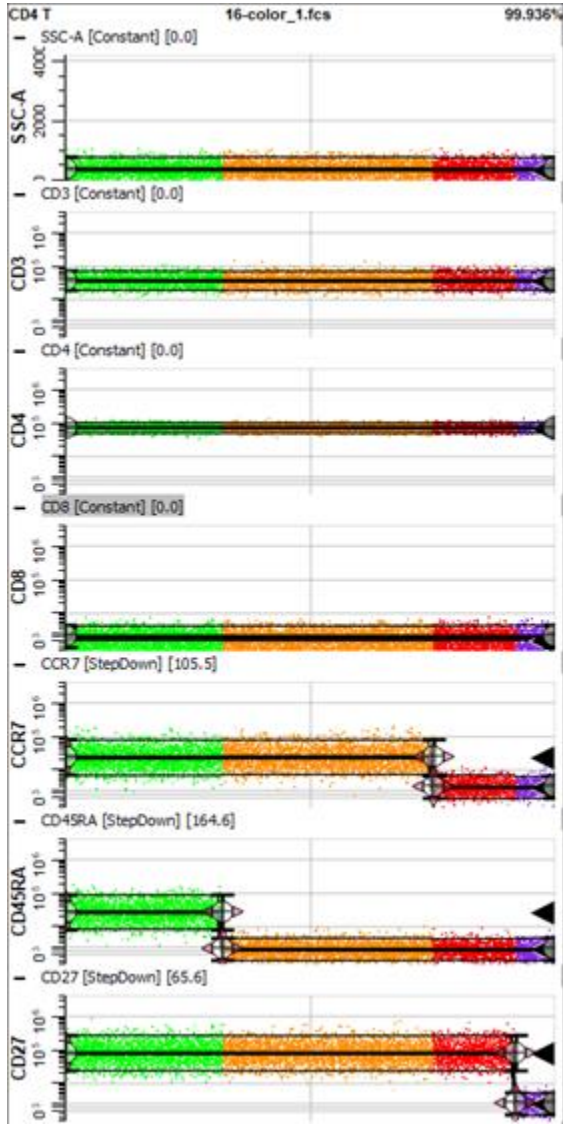
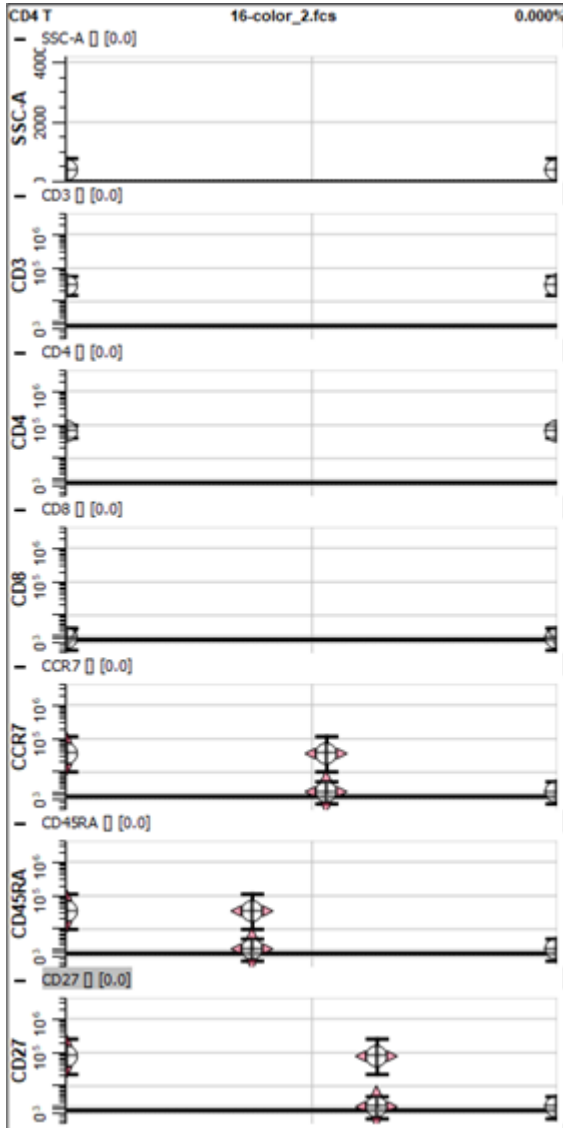
The model itself determines the characteristics of the synthesis process. The same probabilistic processes that are used for classifying real data with a model are put to use to synthesize events. If the model contains multiple cell types, the synthesis routines will apportion events accordingly. The better your model describes the real world process, the more realistic the synthesized data will appear.

After synthesis, the generated data appears in the Expression Profile plots and other graphics. Statistics and stages are computed for the synthesized data.

The synthesized data can be exported to an FCS file using the [Save FCS](#) command.

Before

After



See also:

[Synthesize from Analysis](#)

[Save FCS](#)

Save FCS

This command creates a new FCS file based on the modeled data.

The standard File Save dialog is displayed, allowing you to navigate to a location and enter a file name for the export. Files are stored in FCS file format.

Edit Logging

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. |

Properties for Logging System

This dialog is used to configure the logging system. It is accessed from the [Edit Logging System](#) dialog.

Active

Check this option to enable 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.

Log File

This read-only field shows the full path name for the logging file. This is useful if you need to send the log file to Verity Software House for technical support.

Logging Level

This field determines how much detail the log contains. With **Basic**, the system stores log entries for most user actions. **Detailed** stores additional lines, and **Comprehensive** store entries for many low-level functions in the program. Normally, **Basic** is an appropriate level. Technical Support may instruct you to use another level to help trouble-shoot a problem.

Maximum Log Size

This field displays the maximum for the log file before a new log file is created.

Full Error report

If the program crashes, it normally creates a basic error report to help trouble shoot the crash. Enable this option to create a comprehensive error report with more information in the event of a crash.

Edit Colors

This dialog is used to edit the colors that are used for measurement and stage 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.

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 for Color Palette

This dialog allows properties of the color palette to be edited. The palette contains a set of colors for parameter and stage coloring.

Number of Colors

This field determines the number of colors initially created in the palette.

Create Project

The field determines the method used to create the color palette: Cube Root, Rainbow, or High Contrast.

Background Color

Select the color for background. This is used as background for reports and cell types.

Preferences

Edit Preferences

This command displays a dialog that allows 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.

Edit Preferences

This command displays a dialog that allows 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

Packet Size

Enter the number of events from the FCS file to keep in memory.

Apply Dither

If enabled, a random dither is applied to the FCS data.

Auto Compensate

When enabled, GemStone will automatically decode compensation keywords in FCS files and apply the compensation described in the keywords. If an FCS file does not contain compensation information, this setting will have no effect.

Default Measurement Name

Choose how you want measurements to be named based on FCS keywords. The default is **Long Name (\$PnS)**, which typically is the antibody name. Other options include **Short Name (\$PnN)** and **Short and Long Name (\$PnN_\$PnS)**.

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**.

Range for Text Conversion

Enter the default range (\$PnR) to use when converting delimited text to FCS. (... to ...)

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.

Enhanced File Selection

Use an enhanced file selection dialog.

General Preferences

Precision

This property determines the number of decimal places that will be displayed for numbers in tables.

Specimen Keyword

This keyword is used on the Cell Type widget and other objects to identify the data file that is currently opened.

Export Classification

If true, export the classification information in Save FCS.

Synthesis Threshold

The number of TriCOM events required in order for it to be used in the Synthesize from Data command.

Parallel Processing

Select the number of threads to use for computation. Auto Determine Thread will use the processor's characteristics to determine the number of threads.

Graphics Preferences

These preferences are common to the graphic objects on the canvas and reporting areas.

PDF Output Quality

Sets the resolution for PDF output.

Cell Type Page Width

The width of the cell type page, in inches.

Cell Type Page Height

The height of the cell type page, in inches.

Unclassified Color

Color for the unclassified events.

Grid Snap

Enables grid snapping for object movement and sizing.

Grid Size

Sets the size of the snap grid.

Angle Snap

Enables angle snapping for object rotation.

Angle Snap Degrees

Sets the size of the snap grid for object rotation.

Copy/Save DPI

Sets the resolution for images that are copied to the clipboard or saved as images to disk.

Image Format

Sets the file format for images that are saved to disk.

Title Font

GemStone User Guide

Choose the title font for objects.

Outline Color

Choose the color of the perimeter for objects.

Outline Width

Choose the width in pixels of the perimeter for objects.

Background Color

Set color for the background of all plots

X Grid Lines

Check to show the X grid lines.

Y Grid Lines

Check to show the Y grid lines.

Grid Line Width

Grid line width in points.

Grid Line Style

Grid line style.

Grid Color

Set the color of the grids

Expression Profile Preferences

This dialog allows editing of Expression Profiles properties.

Auto-expand

This setting determines how Cell Type expression profiles are expanded during auto analysis.

Maximum Transition

Maximum transition allowed for the expression profile.

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 10.00)

Number

Enter the specific number of dots for this cell type to display. (0 to ...)

Unclassified

Check to show the unclassified events.

Auto Zoom

Check to automatically zoom in an area with data.

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.

1P Histograms Preferences

Default Width

Choose the default x size in inches.

Default Height

Choose the default y size in inches.

Resolution

Choose the number of channels to use for 1P plots.

Peak Triangles

Enable this option to show peak location triangles on 1P plots.

Fill Style

Choose the style for filling 1P plots.

Transparency

Enter a value between 0.0 and 100.0 for the amount of transparency for populations in a 1P plot.

X Auto Zoom

Enable this option to automatically zoom the X-axis to exclude channels with 0 events in them..

2P Plots Preferences

Default Width

Choose the default x size in inches.

Default Height

Choose the default y size in inches.

Auto Size

Check to enable auto-sizing of dots.

Dot Size

Choose the appropriate dot size in points.

Number

Enter the specific number of this cell type's dots to display.

Unclassified

Check to show the unclassified events.

Auto Zoom

Check to automatically zoom in an area with data.

Overlay Preferences

Default Width

Choose the default x size in inches.

Default Height

Choose the default y size in inches.

X Grid Lines

Check to show the X grid lines.

Auto Zoom

Check to show auto zoom the overlay.

Axis Preferences

This dialog allows axis preferences for the program to be edited.

Major Ticks

Enter the number of major ticks for all the axes.

Pen Weight

Enter the pen weight in pixels for the axes. (0 to 10)

Pen Color

Click to choose the color for the axis pen color.

Number Font

Click to choose the font for axis numbers.

Number Color

Click to choose the color for the axis numbers.

Label Font

Click to choose the font for the axis label.

Label Color

Click to choose the color for the axis label.

Show Ticks

Show or hide the axis ticks.

Show Numbers

Show or hide the axis numbers.

Show Label

Show or hide the axis label.

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.

Peak Finder Preferences

Auto Smoothing

Finds the optimal bandwidth to smooth the data prior to finding peaks.

Smoothing

Applies more or less smoothing to the data. (3 to 15)

Merge Peaks Below

Peaks below this negative threshold will be merged into one (default=1). (0 to 30)

Peak Separation

If $\text{abs}(\text{mean1}-\text{mean2})/(\text{sd1}+\text{sd2})$ is greater than this threshold, then the peaks are different. (0.10 to 1.00)

Fit Distance

Filters detected peaks where the fitted mean is too far from the peak's location. (1.00 to 4.00)

Peak Fitting Method

Choose peak fitting method to find peak location and standard deviation. Dimmer and brighter side use robust statistics instead of fitting for SD estimation.

Minimum Peak Height

Minimum peak height threshold in counts for a peak to be acceptable. (0.01 to 1000.00)

Minimum State Peak Height

Minimum state peak height threshold in counts for a peak to be acceptable. (0.01 to 1000.00)

Peak Deflection Threshold

Minimum peak deflection (vertical deflection*100/sd) for a peak to be acceptable. (0.10 to 50.00)

Region Preferences

This dialog allows editing of Regions properties.

Line Width

Line width in pixels (0 to 10)

Line Color

Line color for new regions.

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.

Information Table Preferences

This dialog allows all preferences for the program to be edited. The current section allows editing of Information Tables properties.

Number of Rows

Choose the number of rows in the table. (1 to 200)

Number of Columns

Choose the number of columns in the table. (1 to 200)

Information Table Font

Font to use for the information table

Number of Decimals

Number of decimals to show in the information table (0 to 6)

Transformation Preferences

This dialog allows all preferences for the program to be edited. The current section allows editing of Transformations properties.

Transform

Choose the desired transform as a default if possible.

Calculate Decades

Enable this option to compute the number of decades for a measurement based on its resolution.

Auto B

Check to automatically calculate B.

Min Linear Option

Select the option for computing the minimum value on the axis. Choice include **Percent of Max Linear**, **Percent of Area** and **User Defined**.

Percent of Area

If Min Linear Option is Percent of Area, this value determines the percentage to use for the computation.

Percent of Max Linear

If Min Linear Option is Percent of Max Linear, this value determines the percentage to use for the computation.

Negative Axis Fraction

This value determines the fraction of the axis that is devoted to values less than zero (0.0 - 1.0). The default value of 0.20 allows up to 20% of the axis to be devoted to negative channels and 80% to positive channels.

Manage Settings

Export Settings

This command displays the standard Save dialog box. Navigate to the location where you want to save the settings and enter a file name. The settings will be stored as a zip file.

Import Settings

This command displays the standard Open dialog box. Navigate to a previously-saved settings zip, select it, and the program will read the settings. You will be prompted that the program should restart.

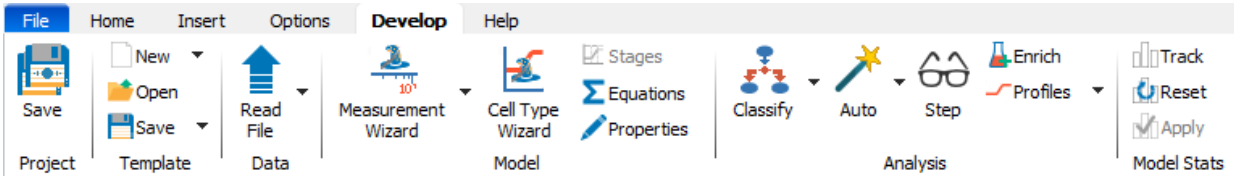
Reset Settings

A confirmation dialog is displayed to confirm that you want to reset the settings. Click OK to continue to reset the settings files, or Cancel to escape.

After resetting, the program will need to restart.

Develop tab

Develop Tab



| Project | Template | Data | Model | Analysis |
|----------------------|-------------------------|--------------------------|------------------------------------|---------------------------------|
| Save | New | Read FCS | Measurement Wizard | Classify |
| | Reset | | Cell Type Wizard | Classify File |
| | Open | | Stages | Auto Analyze |
| | Save | | Equations | CT Auto Analyze |
| | Save As | | Properties | Step Analysis |

* Note, the Develop tab is only displayed when the program is in [Develop mode](#). Develop mode is enabled from the [Home tab](#).

Save Project



The **Save Project** command re-saves the current Project. The command appears in the File menu and on the [Develop](#) tab.

Use this command when you have changed the batch scripts, database format, or model template used in the Project.

The **Save Project As** command (Develop mode) displays a dialog that allows you to type a new name and location for the Project. All content from the original Project is copied to the new Project name and location.

See also:

[What is a Project?](#)

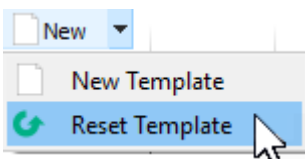
New Template



When selected, GemStone will create a new, empty model template.

A model template contains the cell type definitions, expression profiles, report design, and objects on the Cell Type canvas.

Reset Template



Use this command to put Expression Profile control definition points (CDPs) back to the positions they were in when the model template or analysis document was opened.

The command is typically used when creating new templates or modifying existing templates. Use the command before saving changes to the template if you want to use the CDP positions that were saved in the model.

Open Template



This command will display the standard File Open dialog to allow the user to select a previously saved GemStone model template file. GemStone will open the selected file and display the Cell Types, expression profiles, report, and other objects contained in the document.

Save Template



If the template has been saved previously, this command will resave it without displaying a dialog. The previous version of the document will be overwritten.

If the template has not been saved, this command performs identically to the Save Document As command.

Save Template As



When selected, this command will display the standard File Save dialog to allow the user to save the current GemStone model template to a file on disk. GemStone will save the Cell Types, expression profiles, report, and other objects contained in the document.

Read File



This command performs two actions:

1. It sets the Start At file to the file that is currently selected in the list.
2. It reads the FCS file into the current model template.

The command does *not* trigger the batch script processing that normally happens when an FCS file is loaded using the batch buttons. This allows a developer to read an FCS file without having the batch processing fire off.

Measurement Wizard

Measurement Wizard

The measurement wizard is used to set up names and transformations for new measurements, as well as to edit existing measurements. It is available in Develop mode only.



The wizard starts with the [selection page](#) for choosing the measurements you want to edit. The wizard then displays the [measurement properties page](#) for each measurement selected. It then allows the user to choose cell types that they want to add the measurement to. It concludes with a summary page.

Measurement Wizard

The measurement wizard is used to set up names and transformations for new measurements, as well as to edit existing measurements. It is available in Develop mode only.



The wizard starts with the [selection page](#) for choosing the measurements you want to edit. The wizard then displays the [measurement properties page](#) for each measurement selected. It then allows the user to choose cell types that they want to add the measurement to. It concludes with a summary page.

Measurement Wizard Matching Page

This page of the Measurement Wizard is displayed when there are measurements in the model that are not matched by measurements in the FCS file.

Measurement Wizard

Welcome to the Measurement Wizard

Match *FCS* measurements to the correct *Model* measurements.

Show Unmatched Show All

| FCS Measurements | | | Model Measurements | |
|------------------|-------------|------------|--------------------|-------------|
| FCS Name | FCS String | Matched To | Name | Matched To |
| BUV805-A | CD14 | | CCR7 | PE-Cy7-A_CC |
| BV421-A | CD57 | | CD3 | |
| BV480-A | CXCR3 | | CD4 | |
| BV605-A | CD95 | | CD8 | |
| BV650-A | HLA-DR | | CD14 | |
| BV711-A | CXCR5 | | CD16 | |
| BV750-A | CD20 | | CD19 | |
| BV786-A | CD25 | | CD25 | |
| BYG584-A | CCR6 | | CD27 | |
| IRD800-A | CD8 | | CD38 | |
| IRD820-A | LiveDead | | CD45RA | |
| PE-CF594-A | CCR4 | | CD56 | |
| PE-Cy5.5-A | CD279(PD-1) | | CD127 | |
| PE-Cy5-A | CD123 | | FSC-H | |
| PE-Cy7-A | CCR7 | CCR7 | HLA DR | |
| Time | | | PD-1 | |
| | | | TCRgd | |

Match Unmatch

Help Next > Cancel

The list on the left displays measurements in the FCS file. You can choose to **Show Unmatched** or to **Show All** measurements.

The list on the right shows measurements that are used in the Model.

Both lists have a column that indicates what the measurement is **Matched To** in the other list.

To match an FCS measurement to a model measurement, select the items that you want to match in each list and then click **Match**. You can also **Unmatch** measurements if they are incorrectly associated.

Click Next to move to the next page of the wizard, the [Measurement Wizard Selection Page](#).

Measurement Wizard Selection Page

This page of the measurement wizard is used to select measurements that you want to edit. By default, any new measurements that are not known to the model template will be selected.

Measurement Wizard

Welcome to the Measurement Wizard

Select the measurements you want to edit.

- BCKG190Di
- CCR6
- CCR7
- CD3
- CD4
- CD8
- CD11c
- CD14
- CD16
- CD19
- CD20
- CD25
- CD27
- CD28
- CD38
- CD45
- CD45RA
- CD45RO
- CD56
- CD57
- CD66b
- CD123_IL-3R
- CD127_IL-7Ra
- CD161
- CD194_CCR4
- CD294

Select measurements by checking the checkbox next to the measurement name. The All and None button will set or clear all check boxes.

Click Next to move to the next page of the wizard, the [Measurement Wizard Measurement Properties Page](#).

Click Previous to move to the [Measurement Wizard Matching Page](#).

Measurement Wizard Measurement Properties Page

This page of the measurement wizard is used to adjust the properties of a measurement. It is shown for each measurement selected on the [selection page](#) of the wizard.

Measurement Properties

Edit the **Measurement** and other properties below.
For example "FL1-A_FITC_CD4" might be shortened to "CD4".

| | |
|-----------------------|--|
| Measurement | CCR7 |
| Abbreviation | CCR7 |
| Color | |
| Transform | VLog |
| Calculate Decades | <input checked="" type="checkbox"/> On |
| Decades | 6.62 |
| VLog Beta | 4000.000 |
| Maximum | |
| Max Linear Option | Automatic |
| Max Linear | 4194304.00 |
| Minimum | |
| Min Linear Option | Percent of Max Linear |
| Percent of Max Linear | 0.10 |
| Min Linear | -4194.30 |

Graph: Y-axis (0 to 2000), X-axis (CCR7, -10⁰ to 10⁵). Peak at approximately 10⁰.

1 of 2

Buttons: Help, < Back, Next >, Cancel

Properties for the measurement are shown on the left, and a preview of the measurement using those properties appears on the right.

Use the wizard's Next and Previous buttons to navigate through the selected measurements. When all selected measurements have been edited, the **Next** button at the bottom of the wizard will enable to allow the wizard to move to the [Summary page](#).



Measurement properties

Measurement

Enter the name to use for this measurement.

Abbreviation

Enter an abbreviated form for the name.

Color

Select a color for the measure. This will be used in overlay plots.

Transformation Properties

Transform

Select a transform to use for the measurement.

Calculated Decades

When enabled, the program will compute the number of decades in the data. When disabled, the value in the Decades box is used.

Decades

When Calculated Decades is unchecked, this value can be adjusted to the desired number of decades. The program will scale data to the specified number decades.

VLog Beta

The Beta value for the VLog transform. Typically, this is 10.0.

Maximum

Max Linear Option

Choose **Automatic** to set the Max Linear value based on the resolution of the measurement. Choose **User Defined** to set a constant maximum value from file to file.

Max Linear

When Max Linear Option is set to User Defined, this value sets the maximum linear value for the measurement. When Max Linear Option is set to Automatic, this value is read-only, and shows the value being used as the maximum.

Maximum

Min Linear Option

This option determines how the the lowest value for the measurement is determined. Choose **Percent of Max Linear** to base the minimum value on the maximum value. Choose **Percent of Area** to based the value on a percentage of the area of the histogram. Or choose User Defined to set the Min Linear value directly.

Percent of Max Linear

Enter the percentage of the Max Linear value to use for the Min Linear value. Typically, this is 0.10%.

Percent of Area

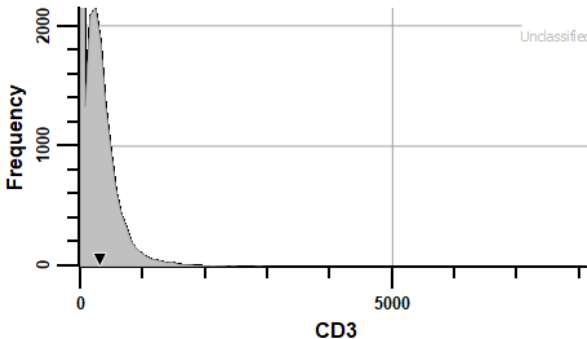
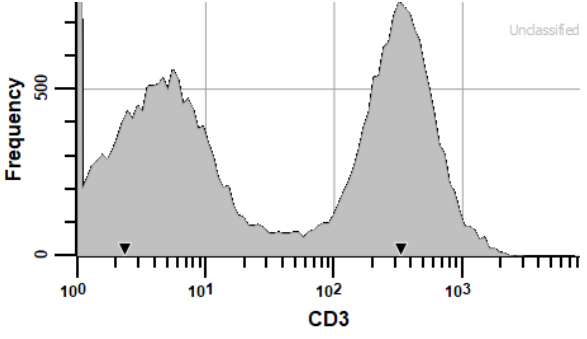
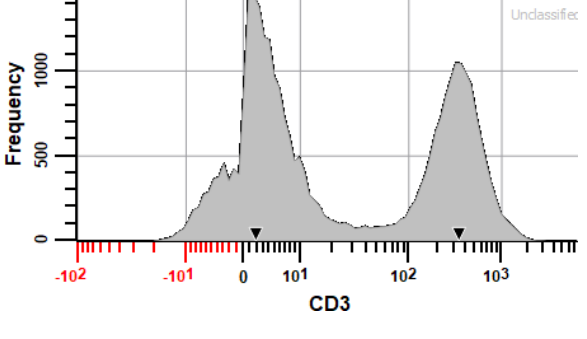
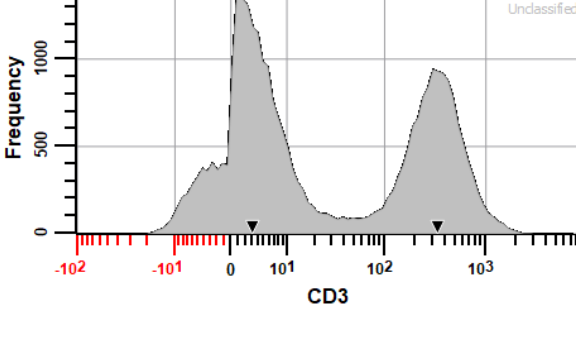
Enter the percentage of the area of the histogram to use to determine the value that will be used for the Min Linear.

Min Linear

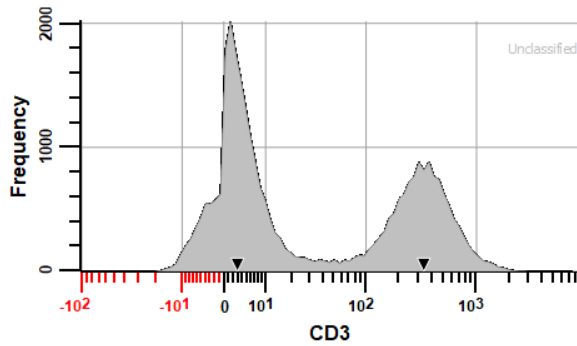
The minimum linear value for the measurement. This is dynamically computed if Min Linear Options is set to Percent of Max Linear or Percent of Area.

Transforms

GemStone offers a number of transforms for displaying FCS data in plots.

| Transform | Display | Description |
|-----------------|--|---|
| Linear |  | This is essentially "untransformed". Bins are evenly sized. |
| Log |  | Log compresses data as the intensity increases. It allows visualization of a wide dynamic range. Any event values less than 0 are set to be 0. |
| HyperLog |  | HyperLog is log-like, but it allows values less than 0. Decades may vary in width. The B coefficient is used to adjust the number of channels that are in the linear portion of the axis on either side of 0. In the example, B=10. |
| Hyperbolic Sine |  | Hyperbolic Sine is log-like, but it allows values ≤ 0 . Decades are the same width. The B coefficient is used to adjust the number of channels that are in the linear portion of the axis on either side of 0. In the example, B=10. |

VLog



VLog is log-like. It allows values ≤ 0 , and optimizes for consistent standard deviation across the dynamic range. The VLog transform stabilizes measurement variance arising from coefficient of variation or cv and background noise. The **VLog Alpha** coefficient adjusts for both cv and counting error, and the **VLog Beta** coefficient adjusts for cv and background. The **VLog Alpha** value is typically 1.0. The **VLog Beta** value is typically 10.0. It can be set to the approximate background intensity for the measurement. For mass cytometry data, the default value of 10 is recommended.

Measurement Wizard Summary page

This page of the measurement wizard summarizes the measurements that were edited.

Measurement Wizard

Summary

CCR7
Abbreviation: CCR7
Transform: VLog

CD4
Abbreviation: CD4
Transform: VLog

Finish Up

Add to Cen-se'

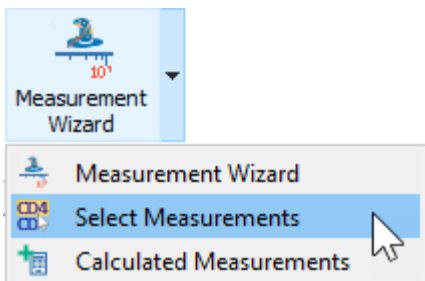
Launch Cell Type Wizard

Update Preferences

Help < Back Finish Cancel

Enable the [Launch the Cell Type Wizard](#) to initiate that wizard when this wizard finishes. Click the **Finish** button to complete the wizard and close the dialog. Click **Cancel** to close the dialog without saving any of the changes.

Select Measurements



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 measurement

Select the measurement in the list on the left of the dialog, and then click Add or Insert.

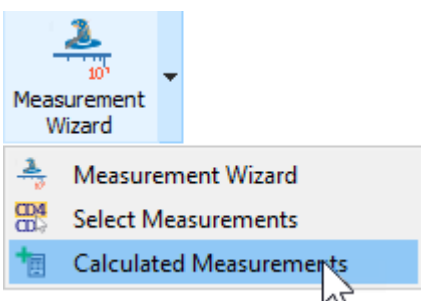
Remove a measurement from the cell type

Select the measurement in the list on the right, and then click Delete.

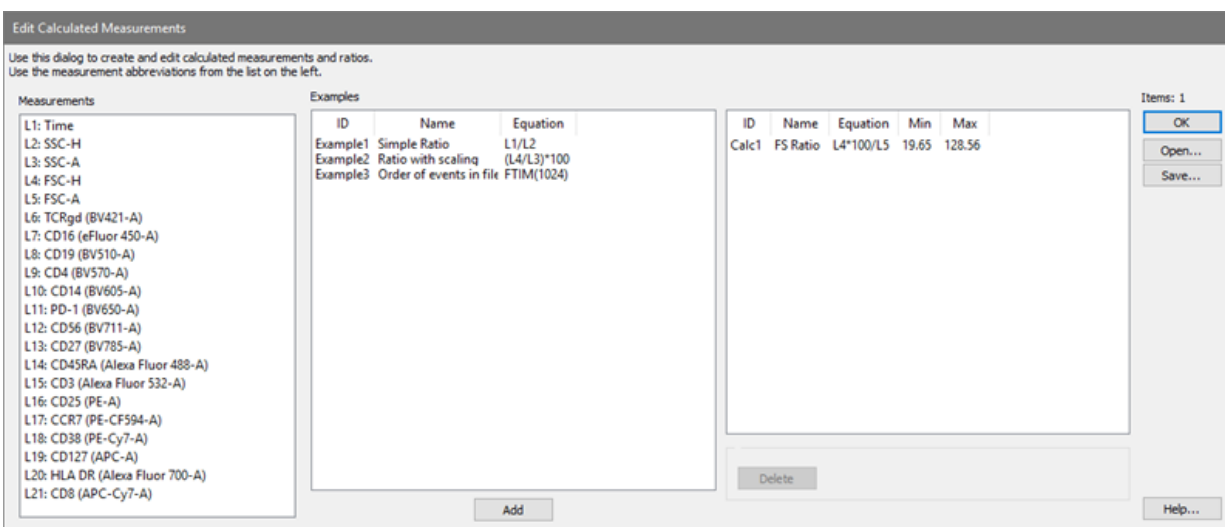
Remove all measurements in the cell type

Click the Delete All button.

Calculated Measurements



This dialog is used to create and edit calculated measurements, including ratios and other computed expressions. The resulting calculated measurements can be used just like any other measurement, in plots and expression profiles. They will be included if you use the [Save FCS command](#) or the batch Export FCS option to create a new FCS file.



The list on the left contains all measurements in the FCS file, prefixed with the abbreviation that can be used in the calculated expression (e.g. "L1").

The center list contains example expressions that can be used as a starting point for a new calculated measurement.

The list on the right contains all calculated measurements that have been created.

If you want to...

Create a new, calculated measurement

Select an example from the middle list and click Add. Edit the Name and Equation for your new measurement.

Remove a calculated measurement

Select the calculated measurement in the list on the right and click Delete.

Edit the **Name** field to be a simple but informative string. This field is concatenated with the **ID** field for the name of the measurement in lists.

Edit the **Equation** field to be the expression for your new, calculated measurement. Use the Measurement list on the left as a reference for the abbreviations to use for each measurement.

Cell Type Wizard

Cell Type Wizard

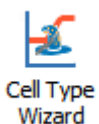
This wizard is used to simplify the process of creating a new cell type. It provides a guided approach to building the key aspects of a new cell type. It can also be used to edit an existing cell type. The feature is only available in Develop mode.



The wizard initially displays a [Welcome page](#) to with a choice of tasks you can perform. The next page defines the [properties of the cell type](#). Next you select the [selection markers](#), [staging markers](#), and [functional markers](#) that you want to analyze for this cell type. Once you have made these choices, the wizard guides you through the process of creating an [expression profile for each marker](#). Finally, a summary page is displayed.

Cell Type Wizard

This wizard is used to simplify the process of creating a new cell type. It provides a guided approach to building the key aspects of a new cell type. It can also be used to edit an existing cell type. The feature is only available in Develop mode.



The wizard initially displays a [Welcome page](#) to with a choice of tasks you can perform. The next page defines the [properties of the cell type](#). Next you select the [selection markers](#), [staging markers](#), and [functional markers](#) that you want to analyze for this cell type. Once you have made these choices, the wizard guides you through the process of creating an [expression profile for each marker](#). Finally, a summary page is displayed.

Cell Type Wizard Welcome

This page of the Cell Type Wizard is used to select the task you want to perform.

Cell Type Wizard

Welcome to the Cell Type Wizard

What would you like to do?

Create a new cell type.

Duplicate the selected cell type. *

 CD4 T Link selection markers

Edit the selected cell type. *

 CD4 T

* Only cell types compatible with the Cell Type Wizard are available for duplicating and editing.

Disable other cell types while editing

Create a report page

Insert before: None

Choose the task:

- Create a new cell type
- Duplicate a cell type *
You can duplicate existing cell types and then edit the new cell type.
- Edit a cell type *
You can edit an existing cell type with this option.

* Note, some cell types that have been customized outside of the wizard may not be editable in the wizard.

Disable other cell types

With this option enabled, the other cell types in the model will be disabled while the wizard is running. This allows you to build the new cell type as if it is the only cell type in the model.

With the option unchecked, the other cell types will be enabled while building the new cell type. This can be useful for cell types that have fewer events.

Create a report page

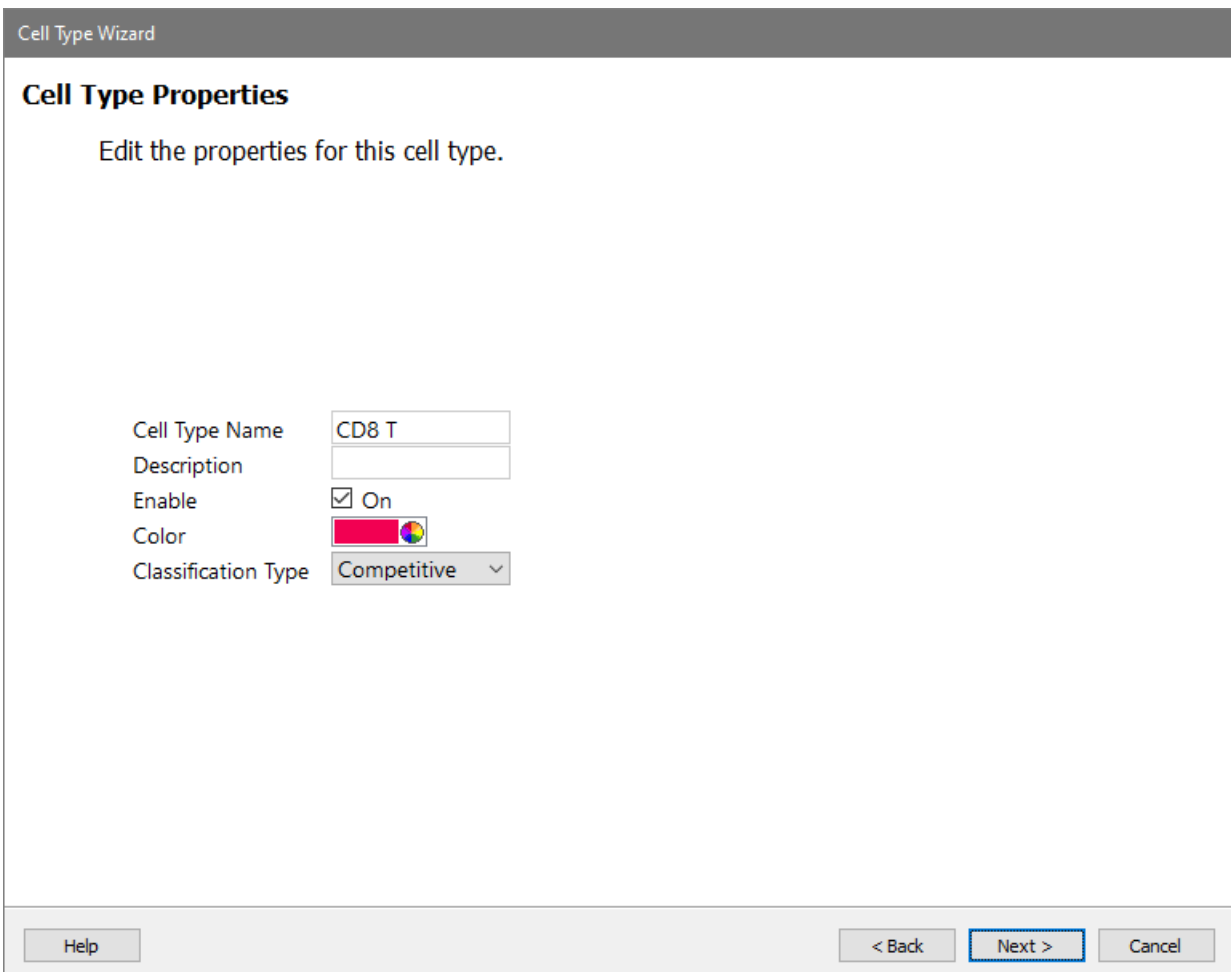
Enable this option to create a new report page for the cell type you are creating or editing. The wizard will create a page with plots and tables based on the choices made to create the cell type. The page can be edited and enhanced by the user after the wizard completes.

Insert before

Select where you want to insert the new cell type in the model. By default, a new cell type is appended to the list of cell types (i.e. Insert before "None").

Cell Type Wizard Properties

This page of the Cell Type Wizard is used to define several properties of the cell type.



The screenshot shows a dialog box titled "Cell Type Wizard" with a section titled "Cell Type Properties". Below the title is the instruction "Edit the properties for this cell type." The properties are:

- Cell Type Name: CD8 T
- Description: (empty text box)
- Enable: On
- Color: (red color swatch with a color picker icon)
- Classification Type: Competitive (dropdown menu)

At the bottom of the dialog box are four buttons: "Help", "< Back", "Next >" (highlighted with a blue border), and "Cancel".

Cell Type Name

Enter a name for the cell type. The name should be as concise as possible.

Description

Enter a helpful description of the cell type.

Enable

The cell type has to be enabled to be included in the modelling process.

Color

The color to use for this cell type. It is used in numerous graphic objects.

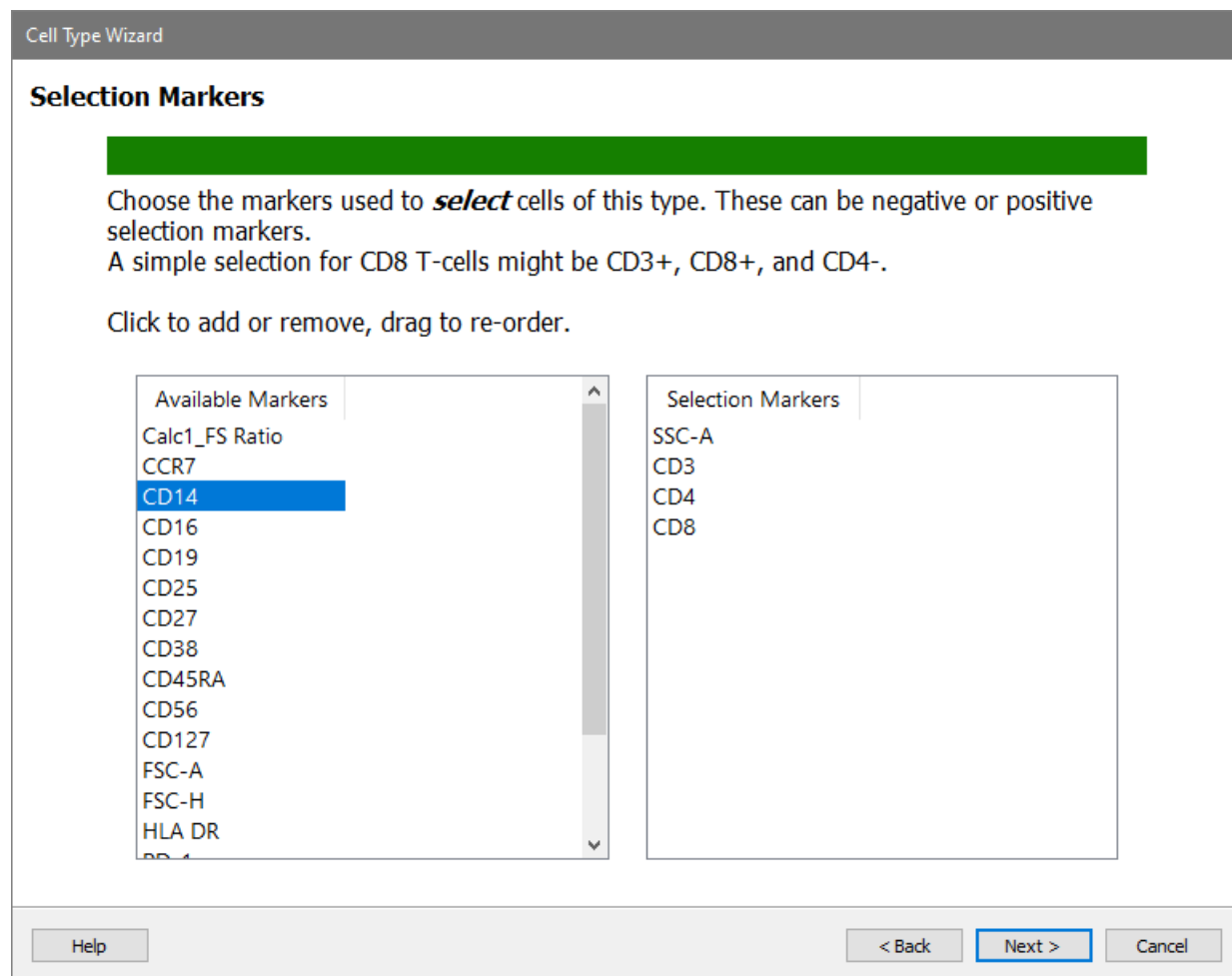
Classification Type

Choose the type of cell type that you want this to be:

| Type | Description |
|-----------------|--|
| Competitive | This is the most common type of cell type. The cell type will "compete" with other competitive cell types for events. Any given event can only belong to one of the competitive cell types. Use this for most cases. |
| Selective | This special cell type can be used as the <i>first</i> cell type in a model template, and there should be no more than one of them. The selective cell type selects events that will then be considered by the competitive cell types. It acts much like an initial gate to select cells of interest. This is not a commonly used type. |
| Non-competitive | A non-competitive cell type is typically the <i>last</i> cell type in a model template. It is designed to capture all of the events that are not assigned to any of the competitive cell types. It is useful for analyzing the unclassified events. |

Cell Type Wizard Selection

This page of the Cell Type Wizard is used to choose the Selection markers for the cell type. Selection markers use constant expression profiles to select a subset of events with a particular phenotype.



The screenshot shows the 'Cell Type Wizard' dialog box with the 'Selection Markers' tab selected. A green bar is at the top. Below it, the text reads: 'Choose the markers used to *select* cells of this type. These can be negative or positive selection markers. A simple selection for CD8 T-cells might be CD3+, CD8+, and CD4-.' Below this is the instruction: 'Click to add or remove, drag to re-order.'

There are two lists of markers:

- Available Markers:** Calc1_FS Ratio, CCR7, CD14 (highlighted in blue), CD16, CD19, CD25, CD27, CD38, CD45RA, CD56, CD127, FSC-A, FSC-H, HLA DR, and others.
- Selection Markers:** SSC-A, CD3, CD4, and CD8.

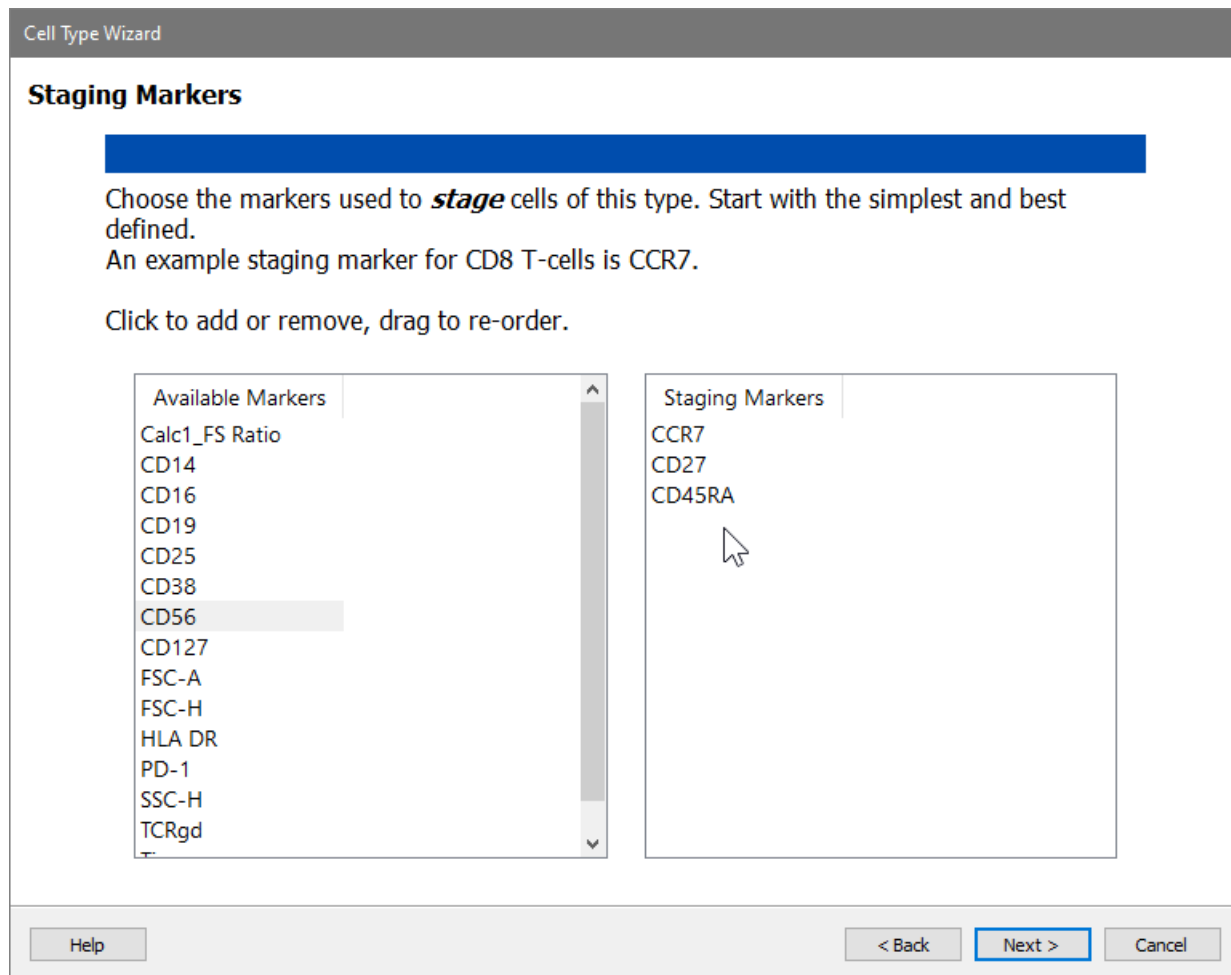
At the bottom, there are buttons for 'Help', '< Back', 'Next >' (highlighted in blue), and 'Cancel'.

To add a marker, find the marker in the **Available Markers** list and click it. The marker will be moved into the right-hand list of Cell Type Markers.

To remove a marker, find the marker in the **Cell Type Markers** list and click it. The marker will be removed from the list and put back into the Available Markers list.

Cell Type Wizard Staging

This page of the Cell Type Wizard is used to choose the Staging markers for the cell type. Staging markers use step-up, step-down, or three-level expression profile to define a marker's change in expression that occurs within the cell type.



To add a marker, find the marker in the **Available Markers** list and click it. The marker will be moved into the right-hand list of Cell Type Markers.

To remove a marker, find the marker in the **Cell Type Markers** list and click it. The marker will be removed from the list and put back into the Available Markers list.

Cell Type Wizard Functional

This page of the Cell Type Wizard is used to choose the Functional markers for the cell type. Functional markers use constant expression profiles or no profile at all. They may, for example, show activation of a marker.

Cell Type Wizard

Functional and Other Markers

Choose the *functional* and other unknown markers for this cell type. These markers might relate to activation, rare sub-populations, or have unknown characteristics.

Click to add or remove, drag to re-order.

| Available Markers | Functional and Other Markers |
|-------------------|------------------------------|
| Calc1_FS Ratio | CD38 |
| CD14 | HLA DR |
| CD16 | |
| CD19 | |
| CD25 | |
| CD56 | |
| CD127 | |
| FSC-A | |
| FSC-H | |
| PD-1 | |
| SSC-H | |
| TCRgd | |
| Time | |

Help < Back Next > Cancel

To add a marker, find the marker in the **Available Markers** list and click it. The marker will be moved into the right-hand list of Cell Type Markers.

To remove a marker, find the marker in the **Cell Type Markers** list and click it. The marker will be removed from the list and put back into the Available Markers list.

Cell Type Wizard Profiles

This page is shown for each of the selection, staging, and functional markers selected in previous pages of the Cell Type wizard. It is used to choose and set up the expression profile for the marker in the cell type being created or edited.

When this page is displayed, move the wizard so that you can see the expression profiles in the cell type widget. This allows you to preview your choices.

When navigating through the profiles, you will use the **Next Profile** button that appears on the right of the dialog and the **Previous Profile** button that appears on the left. If one is disabled, that indicates you've reached the beginning or end of the list of profiles.

Moving to previous profile



Previous
Profile

Moving to next profile



Next
Profile

Selection Profiles

Constant profiles are used for selection markers.

Cell Type Wizard

Selection Profiles

Cell Type Events: 40731

Define the expression of CD3 in the CD8 T cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

CD3

Which Peak? Preview

Distribution

Phenotype

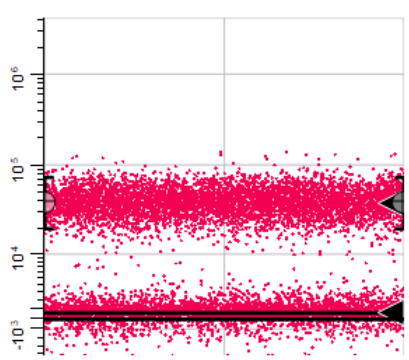
Initial Intensity

Initial Width

Width from Peak On

Add to Report On

Previous Profile



2 of 9

Help < Back Next > Cancel

The **Links** button allows this profile to be linked to another profile. For example, it is common to link the CD3+ profile for CD4 cell type to the CD3+ profile of the CD8 cell type. The **Preview** checkbox shows the profile in its unmodelled (unchecked) or modelled (checked) state.

The properties for selection profiles are listed below.

Which Peak?

Choose which peak in the marker the expression profile should track.

| | |
|---------------|---|
| User estimate | The marker doesn't use a peak. It uses the estimates for Intensity and SD to position and size the profile. |
| Closest | Find the closest peak to the starting location of the profile and use its peak estimates. |
| Dimmest | Find the dimmest peak (lowest intensity) and use its peak estimates. |
| Brightest | Find the brightest peak (highest intensity) and use its peak estimates. |
| Smallest | Find the smallest peak (lowest number of events) and use its peak estimates. |
| Largest | Find the largest peak (largest number of events) and use its peak estimates. |

Distribution

Choose the type of distribution to use for this profile.

| | |
|---------------------|--|
| Normal Distribution | The control definition points define the mean of a normal distribution. |
| Inverse Normal | The profile is the inverse the Normal Distribution. |
| Inverse Low Pass | The profile is the inverse of the Low Pass distribution. |
| Low Pass | The control definition points define a probability "edge". Events below the CDPs are included. |
| High Pass | The control definition points define a probability "edge". Events above the CDPs are included. |
| Inverse High Pass | The profile is the inverse of the High Pass distribution. |

Phenotype

This field is automatically filled in when possible. Enter a short phenotype description for the selection. This is used on report pages.

Initial Intensity

This intensity estimate can be edited when the Which Peak value is set to User Estimate or Closest.

Initial Width

This intensity estimate can be edited when the Which Peak value is set to User Estimate or Closest.

Width from Peak

If this option is enabled, the program will adjust the profile's width based on the peak estimates.

Add to Report

If this option is enabled, a graph of the measurement will be included in on the report page.

Staging Profiles

Staging profiles can be StepUp, StepDown, or ThreeLevels. Choose the one that best matches the change in expression for that marker in the cell type being edited.

Cell Type Wizard

Staging Profiles

Cell Type Events: 6026

Define the expression of CCR7 in the CD8 T cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

CCR7

Shape

Expression Profile Shape: StepDown

Phenotype: CCR7+ > -

First Level

Which Peak?: Brightest Peak

Initial Intensity: 47036.80

Initial Width: 6.20

Width from Peak: On

Second Level

Which Peak?: Dimmest Peak

Initial Intensity: 2686.22

Initial Width: 8.97

Links... Preview

5 of 9

Help < Back Next > Cancel

The properties of the staging profile are the same as for Selection, except they are repeated for each level of the profile.

Functional Profiles

Function markers use constant profiles or no profile at all.

Cell Type Wizard

Functional Profiles

Cell Type Events: 5535

Define the expression of CD56 in the CD8 T cell type.
Use the *Next Profile* and *Previous Profile* arrows to set up each expression profile.

CD56

Previous Profile

Expression Profile Shape: Constant

Which Peak?: Dimmest Peak

Phenotype: CD56

Initial Intensity: 196.17

Initial Width: 2.73

Width from Peak: On

Links...

Preview

Next Profile

8 of 9

Help < Back Next > Cancel

The properties of the functional profile are the same as for Selection, except they are repeated for each level of the profile.

Cell Type Wizard Summary

The summary page is the final page in the Cell Type wizard, providing a summary of the selections made in the wizard.

Cell Type Wizard

Summary

Cell Type Info

- CD8 T

Selection Markers

- 4 selection markers
- SSC-A (SSC-A-)
- CD3 (CD3+)
- CD4 (CD4-)
- CD8 (CD8+)

Staging Markers

- 3 staging markers
- CCR7 (CCR7+>-)
- CD27 (CD27+>-)
- CD45RA (CD45RA+>-)

Finish Up

Do another cell type

Save template model

Update summary map

Add to Cen-se'

Add database fields

Help < Back Finish Cancel

Enable **Do another cell type** to run the wizard again after finishing the current cell type.

Enable **Save template model** to save the changes made by the wizard in the current template model file.

Enable **Update summary map** to tell Cen-se' plots and the summary map about the new cell type.

Enable **Add to Cen-se'** if you have a Cen-se' plot on a report page and want to include this cell type in the Cen-se'.

Enable **Add database fields** to append statistics for this cell type to the database.

Edit Stages



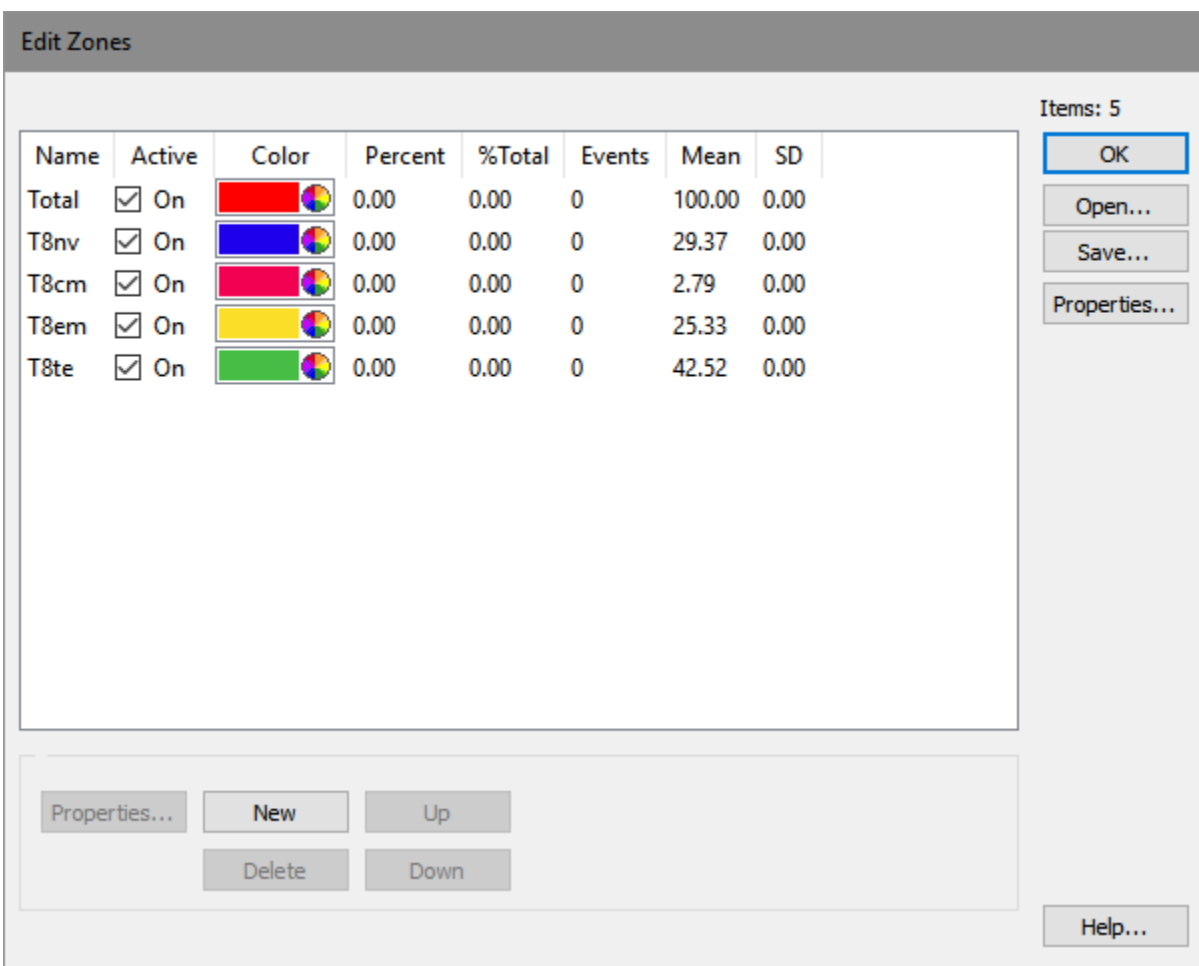
This dialog allows stages to be added, deleted, and modified. Stages are defined on the Progression (State Index) axis and provide statistics and coloring for portions of the progression.

Stages are most often defined using Definition Points in the Expression Profiles for a given Cell Type. This allows the boundaries of the stage to adjust automatically as the Definition Points are adjusted.

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

A Stage Table object appears on each Cell Type to provide statistics for the stages. The table shows the name, color, active state, and statistics for each stage.

See [Edit Properties for Stage](#) for additional details on stage properties.



If you want to...

Add a new stage

Click the New button and fill in the [Edit Properties for Stage](#) dialog.

Delete a stage

Select the stage in the list, and then click Delete.

Change the order of stages

Select a stage, and then use the Up or Down button to move it in the list of stages.

Edit Properties for Stage

This dialog displays all of the properties for a single stage. Stages 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 stage. Short names are usually recommended so that they can be read when displayed on Overlay plots.

Active

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

Show in Graphs

When this option is checked, the stage is displayed in the plots.

Color

Choose a color for the stage. The color is applied to expression profile plots, overlays, and 2P histograms.

Intensity Stats

When this option is enabled, the program computes intensity statistics within the stage for each parameter.

Start

Start CDP

Select a Control Point to link to for the *start* of the stage. Each Definition Point in the list is named based on the parameter and the position in the profile that owns the point.

Select "None" if you want to enter a fixed value for the starting location of the stage in the Start State Value field.

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 stage. The value represents a percentage of the Progression axis for the starting location of the stage.

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

End

End CDP

Select a Control Point to link to for the *end* of the stage. Each Definition Point in the list is named based on the parameter and the position in the profile that owns the point.

Select "None" if you want to enter a fixed value for the ending location of the stage in the Start State Value field.

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 stage. The value represents a percentage of the Progression axis for the ending location of the stage.

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

Equations

Equations



Equations are user-defined expressions that can be evaluated and stored in the information database and displayed on reports.

The equations you write are stored as part of the model template. You can store a set of equations using the Save button and then import those equations into another template 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.

Edit Information Equations

Double-click on one of the examples below to add an equation.
 Use the (%n) placeholders for specific information units.
 Edit placeholders (%n) by double-clicking on an equation name.
 Alerts can be set when editing the equation item.

Select Items Below

| Name | Format |
|------------------------------|--------------------------------|
| Sum | %1+ %2+ %3 |
| Simple Ratio | %1/%2 |
| %Ratio | %1*100/%2 |
| Complex %Ratio | (%1- %2)*100/(%3- %4) |
| Logical Comparison | (%1 < %2) |
| Logical or Comparison | (%1 < 1.7) (%1 > 10.0) |
| Simple Comparison | (%1 = %2) |
| Loaded Model String Comparis | ("%1" = " <Type Loaded Model > |

| Name | Description | Format | |
|------|---------------------------|----------------|-----------|
| Eq1 | CD3 Events | %1+ %2+ %3+ %4 | [CellType |
| Eq2 | Lymphocyte Events | %1+ %2+ %3 | [Eq1]+ [C |
| Eq3 | Dendritic Events | %1+ %2 | [CellType |
| Eq4 | Intact Live % Intact Live | %1*100/%2 | [General. |
| Eq5 | Lymphs % Intact Live | %1*100/%2 | [Eq2]*10C |
| Eq6 | CD3 % Intact Live | %1*100/%2 | [Eq1]*10C |
| Eq7 | DCs % Intact Live | %1*100/%2 | [Eq3]*10C |
| Eq8 | Lymphs % Intact Live | %1*100/%2 | [Eq2]*10C |
| Eq9 | CD3 % Lymphs | %1*100/%2 | [Eq1]*10C |
| Eq10 | CD8 % CD3 | %1*100/%2 | [CellType |
| Eq11 | CD4 % CD3 | %1*100/%2 | [CellType |
| Eq12 | GD % CD3 | %1*100/%2 | [CellType |
| Eq13 | NKT % CD3 | %1*100/%2 | [CellType |
| Eq14 | B % Lymphs | %1*100/%2 | [CellType |
| Eq15 | Monos % Lymphs | %1*100/%2 | [CellType |
| Eq16 | pDC % DCs | %1*100/%2 | [CellType |
| Eq17 | mDC % DCs | %1*100/%2 | [CellType |

Items: 69

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 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*. Functions 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 Stage 1 and Stage 2, we would tell GemStone to replace %1 with the Stage 1 events and %2 with Stage 2 events. This is done by selecting the equation in the list and then clicking the **Edit** button.

In the Edit Equation dialog, **right click** the **Information** field to display a dialog of choices available to fill-in the placeholder. In our example, you would choose CellType1_Stage1_NumberOfEvents for %1 and CellType1_Stage2_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 Stage1 by 4.56, and then divides by the mean of another parameter in Stage1. 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 <i>format</i> 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 equations | Click the Save button, navigate to a location on disk, and save the 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](#)

Equations



Equations are user-defined expressions that can be evaluated and stored in the information database and displayed on reports.

The equations you write are stored as part of the model template. You can store a set of equations using the Save button and then import those equations into another template 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.

Double-click on one of the examples below to add an equation.
Use the (%n) placeholders for specific information units.
Edit placeholders (%n) by double-clicking on an equation name.
Alerts can be set when editing the equation item.

Select Items Below

| Name | Format |
|------------------------------|------------------------------|
| Sum | %1+ %2+ %3 |
| Simple Ratio | %1/%2 |
| %Ratio | %1*100/%2 |
| Complex %Ratio | (%1-%2)*100/(%3-%4) |
| Logical Comparison | (%1<%2) |
| Logical or Comparison | (%1<1.7) (%1>10.0) |
| Simple Comparison | (%1=%2) |
| Loaded Model String Comparis | ("%1"="<Type Loaded Model>") |

| Name | Description | Format | |
|------|---------------------------|----------------|-----------|
| Eq1 | CD3 Events | %1+ %2+ %3+ %4 | [CellType |
| Eq2 | Lymphocyte Events | %1+ %2+ %3 | [Eq1]+ [C |
| Eq3 | Dendritic Events | %1+ %2 | [CellType |
| Eq4 | Intact Live % Intact Live | %1*100/%2 | [General |
| Eq5 | Lymphs % Intact Live | %1*100/%2 | [Eq2]*100 |
| Eq6 | CD3 % Intact Live | %1*100/%2 | [Eq1]*100 |
| Eq7 | DCs % Intact Live | %1*100/%2 | [Eq3]*100 |
| Eq8 | Lymphs % Intact Live | %1*100/%2 | [Eq2]*100 |
| Eq9 | CD3 % Lymphs | %1*100/%2 | [Eq1]*100 |
| Eq10 | CD8 % CD3 | %1*100/%2 | [CellType |
| Eq11 | CD4 % CD3 | %1*100/%2 | [CellType |
| Eq12 | GD % CD3 | %1*100/%2 | [CellType |
| Eq13 | NKT % CD3 | %1*100/%2 | [CellType |
| Eq14 | B % Lymphs | %1*100/%2 | [CellType |
| Eq15 | Monos % Lymphs | %1*100/%2 | [CellType |
| Eq16 | pDC % DCs | %1*100/%2 | [CellType |
| Eq17 | mDC % DCs | %1*100/%2 | [CellType |

Items: 69

OK
Open...
Save...
Delete All

Edit... Replace Duplicate Up
Delete Down

Insert Add Help...

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 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*. Functions 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 Stage 1 and Stage 2, we would tell GemStone to replace %1 with the Stage 1 events and %2 with Stage 2 events. This is done by selecting the equation in the list and then clicking the **Edit** button.

In the Edit Equation dialog, **right click** the **Information** field to display a dialog of choices available to fill-in the placeholder. In our example, you would choose CellType1_Stage1_NumberOfEvents for %1 and CellType1_Stage2_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 Stage1 by 4.56, and then divides by the mean of another parameter in Stage1. 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 <i>format</i> 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 equations

Click the Save button, navigate to a location on disk, and save the 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.

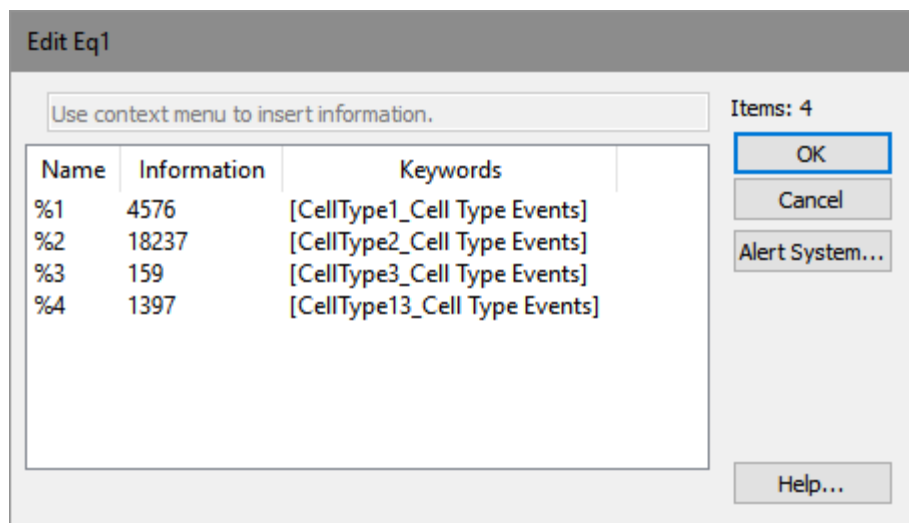
See also:

[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 keyword for a placeholder, **right click** the **Information** field. A dialog of the available items in the information system will appear. 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 **Listmode Parameter** field in the Expression Profile properties.

 Listmode Parameter

14

For example, if the Listmode Parameter is 14, we would add one for an index of 15 when using it in equations.

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 measurements 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. Internal floating point scale of 0.0 to 100.0. |
| Transformed | P | 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](#)

Model Properties

This panel contains properties that apply to all cell types in the current model. It is located on the left side of a [cell type tab](#).

Names and Paths

Document Name

The name of the GemStone document currently opened.

Template Model

The name of the template model that was used.

Version stamp

The version of the template model that was used.

Stats

Total Events In File

The number of events in the FCS file.

Total Analyzed Events

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

Total Cell Type Events

The number of events in the Cell Type.

Total Unclassified

The number of unclassified events.

Percent Unclassified

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

Author

Model Author

Optional, the name of the creator of the model.

Lock Model

When enabled, the program will prompt for a password from the author, and then lock the model from user edits. To edit the model, the Lock Model option must be unchecked and the correct password entered.

Auto Analysis**Auto Analysis Mode**

Choose one of the options.

Sequential mode enables the cell types as they are auto-analyzed, and each cell type remains enabled as other cell types are analyzed.

Independent mode enables one cell type, perform auto-analysis on that cell type, then disables that cell type before moving to the next.

Model Performance**Model Performance Table**

Typically, this is set not to show a performance table, also known as a *contingency table*. Select *Show Table* after an analysis to display the table for the current analysis.

Model Performance Table

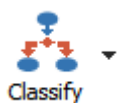
A well-constructed model will have values near 1.0 on the diagonal. Cells show probabilities of correctly assigning events to that cell type.

| | CD8 | CD4 | GDs | MAITs NKTs | B | Monos | NKs | Basos | Neutrophils | CD66b- Neuts |
|--------------|--------|--------|--------|------------|--------|--------|--------|--------|-------------|--------------|
| CD8 | 0.9997 | 0.0000 | 0.0026 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| CD4 | 0.0000 | 0.9999 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0036 | 0.0000 | 0.0000 |
| GDs | 0.0003 | 0.0000 | 0.9822 | 0.0031 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| MAITs NKTs | 0.0000 | 0.0000 | 0.0153 | 0.9969 | 0.0000 | 0.0000 | 0.0000 | 0.0007 | 0.0000 | 0.0000 |
| B | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.9989 | 0.0000 | 0.0082 | 0.0000 | 0.0000 | 0.0000 |
| Monos | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.9860 | 0.0030 | 0.0000 | 0.0000 | 0.0092 |
| NKs | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0010 | 0.0016 | 0.9833 | 0.0000 | 0.0000 | 0.0009 |
| Basos | 0.0000 | 0.0001 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.9850 | 0.0000 | 0.0000 |
| Neutrophils | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0006 | 0.9999 | 0.0031 |
| CD66b- Neuts | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0021 | 0.0000 | 0.0000 | 0.0000 | 0.9858 |
| Eos | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0101 | 0.0001 | 0.0010 |
| pDCs | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| mDCs | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0104 | 0.0032 | 0.0000 | 0.0000 | 0.0000 |
| PB | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0022 | 0.0000 | 0.0000 | 0.0000 |

Total Performance: 0.9732

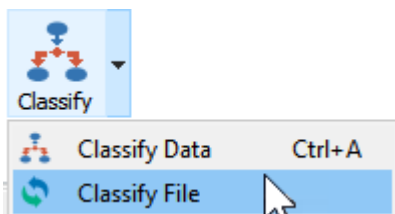
When the model is working well, the elements on the diagonal will be close to 1.0. When values are less than 1.0 on the diagonal, the other values in the same column will indicate other cell types that are "overlapping" in some way with the primary cell type for that row. For example in the table above, the element on the **GDs** row-column shows a value of 0.9822. This indicates that the events in the GDs cell type have a 98% probability of being GDs. Some of the GDs are being captured by the MAITs_NK cell type (0.0031) and CD8 cell type (0.0003).

Classify Data



Use the Classify Data command to initiate the model's classification routines on the data in memory. This command is typically used when opening a new FCS file into a model template to apply the model to the new FCS data. A related command, Classify File, performs the same functions but on the entire FCS file.

Classify File



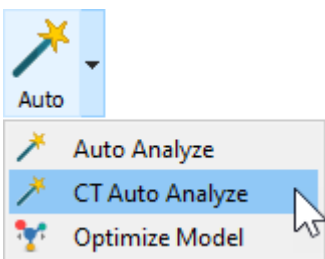
Use the Classify File command to model the all of the data in the file with the Probability State Model. A related command, Classify Data, performs the same functions but only on the data in memory.

Auto Analyze



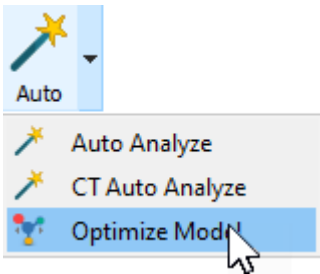
Auto Analysis automatically adjusts the positions of control points to the data. It affects all expression profiles in all cell types.

Auto Analyze Cell Type



Auto Analysis Cell Type (or CT Auto Analyze) automatically adjusts the positions of control points to the data for the current cell type only. It is a handy tool when building new cell types, allowing you to test the auto analysis of just the cell type you are working on.

Optimize Model



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.

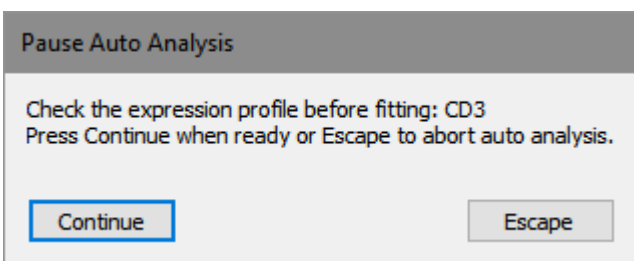
Optimize Model can be time-consuming. A progress meter is displayed on the status bar to let you see how the optimization is proceeding. You can halt the process by pressing the Esc key on the keyboard.

Step



Step

Step is a toggle state command. When enabled, the auto analysis process is performed one step at a time to allow review of each step. A dialog appears after each step, allowing you to review the step before moving on to the next. You can also stop the auto analysis process by clicking Escape.



Enrich

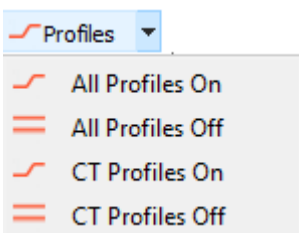


This command is typically used during the model-building process to select events from a data file that match the model. Before using the command, you should make sure that the only parameters with Match Status enabled are those that use "Constant" profiles. Constant profiles are sometimes referred to 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.

Profiles On and Off

These commands are useful when trouble-shooting or building models. They allow you to turn on or off all Expression Profiles in the model or in the current cell type.



All Profiles On

Turn ON all expression profiles in all cell types in the model.

All Profiles Off

Turn OFF all expression profiles in all cell types in the model.

CT Profiles On

Turn ON all expression profiles in the current cell type.

CT Profiles Off

Turn OFF all expression profiles in the current cell type.

Model Stats

Model Stats

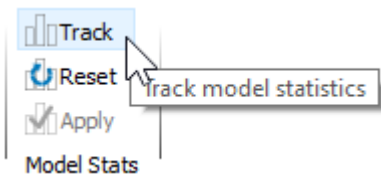
One way to improve the performance of a model template is to adjust its Expression Profiles (EPs) to the average intensity and width of the peaks for a training set of files. By moving the starting location for an EP to the average intensity, the EP is more likely to find the right peak. Similarly, setting the initial width to the average width based on files in the training set, the EP is in its best starting condition.

GemStone has a built-in feature that makes this a very simple process. The [Track](#) command is used to initiate the process of tracking statistics for files in the training set. Once the files have all been analyzed, the [Apply](#) command is used to update the model template with the average intensities and SDs for the files.

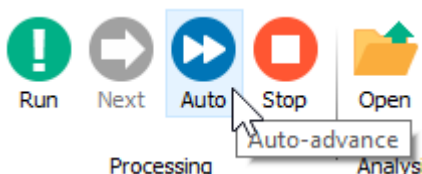
The easiest way to understand the process is with an example. Suppose that you have a model template that is initially set up with intensities and widths using whole numbers. The model has CD3 set with 34,000 for the intensity and a width of 5, and it is set to adjust the width based on the peak. Similarly, SSC is set for 360,000 initial intensity, a width of 5, and it is *not* adjusting the width based on the peak.

| | CD3 Expression Profile | SSC Expression Profile |
|--------------------|---|--|
| Before Track Stats | Which Peak? <input type="text" value="Brightest Peak"/> | Which Peak? <input type="text" value="Dimmest Peak"/> |
| | Distribution <input type="text" value="Normal"/> | Distribution <input type="text" value="Normal"/> |
| | Phenotype <input type="text" value="CD3+"/> | Phenotype <input type="text" value="SSC-A-"/> |
| | Initial Intensity <input type="text" value="34000.00"/> | Initial Intensity <input type="text" value="360000.00"/> |
| | Initial Width <input type="text" value="5.00"/> | Initial Width <input type="text" value="5.00"/> |
| | Width from Peak <input checked="" type="checkbox"/> On | Width from Peak <input type="checkbox"/> Off |

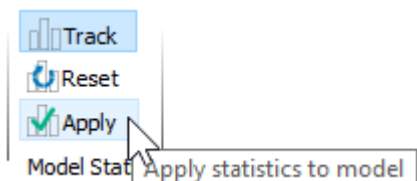
Turn on the Model Stats system by clicking the [Track](#) command in the Model Stats group on the Develop tab.



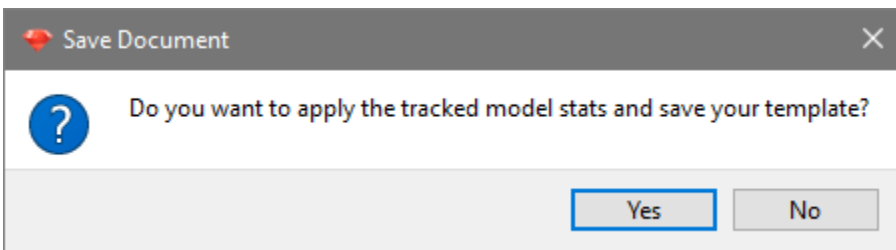
Use the batch processing Auto Advance tool to analyze all files in the training set.



When the process is finished, use the [Apply](#) command to apply the average statistics to the model template.



A confirmation dialog is displayed to confirm that you want to apply the stats and save the updated model template.



After applying the statistics, the initial intensity and width for CD3 have been adjusted to the average intensity and width of the files in the training set. SSC's initial intensity has been adjusted, but not the initial width. This is because Width from Peak is not checked for SSC, so its initial estimate will not be changed.

| | CD3 Expression Profile | SSC Expression Profile |
|-------------------|------------------------|--|
| After Apply Stats | Which Peak? | Brightest Peak |
| | Distribution | Normal |
| | Phenotype | CD3+ |
| | Initial Intensity | 34111.78 |
| | Initial Width | 4.65 |
| | Width from Peak | <input checked="" type="checkbox"/> On |
| | Which Peak? | Dimmest Peak |
| | Distribution | Normal |
| | Phenotype | SSC-A- |
| | Initial Intensity | 364061.23 |
| | Initial Width | 5.00 |
| | Width from Peak | <input type="checkbox"/> Off |

Use this system to polish a well-developed model that is close to production use.

See also:

[Track Stats](#)

[Reset Stats](#)

[Apply Stats](#)

Track Stats



This command is part of the Model Stats system, used for finding average intensities and widths for Expression Profiles. The command turns the system on and accumulates intensity and peak width information for a set of training files.

See [Model Stats](#) for a detailed explanation.

See also:

[Model Stats](#)

[Reset Stats](#)

[Apply Stats](#)

Reset Stats



This command is part of the Model Stats system, used for finding average intensities and widths for Expression Profiles. This command is used to reset the accumulated statistics that have been gathered since the [Track Stats](#) command was initiated.

See [Model Stats](#) for a detailed explanation.

See also:

[Model Stats](#)

[Track Stats](#)

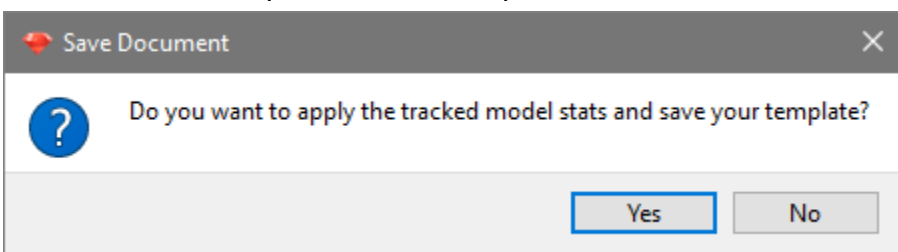
[Apply Stats](#)

Apply Stats



This command is part of the Model Stats system, used for finding average intensities and widths for Expression Profiles. This command is used to apply the average intensity and peak width values to the Expression Profiles in the model template.

When the command is applied, a dialog is displayed to confirm that you want to apply the stats and save the updated model template.



See [Model Stats](#) for a detailed explanation.

See also:

[Model Stats](#)

[Reset Stats](#)

[Apply Stats](#)

Help tab

Help Tab



Online

- [Updates](#)
- [Website](#)
- [Contact](#)
- [Support](#)
- [Make a Suggestions](#)
- [Report a Problem](#)

Learn

- [App Info](#)
- [User Guide](#)
- [About](#)

Updates

This command launches the VSH Updater application, which can check for program updates. In order to function correctly, the updater needs access to the Verity Software House web site (www.vsh.com) and administrative rights to some areas on your hard drive. You may need to provide authorization credentials for it to run. Follow instructions provided by the updater to perform the check and update your software.

Support

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.

Make a Suggestion

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.

Report a Problem

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.

User Guide

This option displays this 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 features and functions.

About

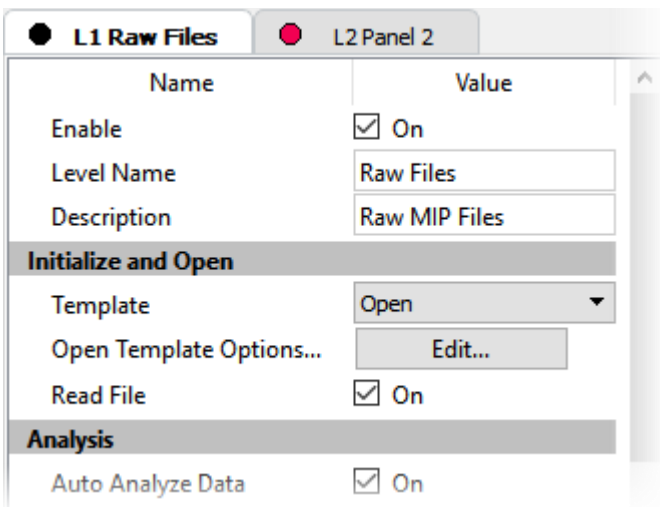
This command displays the application banner dialog box. The dialog contains information about the application. It also provides access to the Registration or Battery system, depending on the type of installation you have.

The phone number for Verity Software House is shown, as are hyperlinks to www.vsh.com and the general email account.

Batch Commands

Batch Commands

This panel determines the actions that are performed when an FCS file is read into the program for analysis. There is a tab for each "level" in the batch, so that you can set up different actions for different kinds of files.



Select a tab to view or edit the actions for the associated batch level.

Commands that appear on each tab

Enable

Enable the actions defined on this tab.

Level Name

A name for this level

Description

A description for this level

Initialize and Open

Template

Open a specific GemStone model template.

Open Template Options

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

Compact File

For FCS files from Fluidigm instruments, this option will re-write the FCS file eliminating extraneous segments from the file. It will have no effect on other FCS file types.

Read File

Read the FCS file associated with this batch item.

Panel Tube Options

Select the option that describes the files on this level of the batch:

Options

- | | |
|-----------------|--|
| One Tube | This file represents the only tube in the panel. |
| First Tube | This is represents the first tube in a multi-tube panel. |
| Subsequent Tube | This is represents the second or greater tube in a multi-tube panel. |

Analysis

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.

Update Cen-se'

This command updates the Summary map and the Cen-se' plot to display named populations and cell types.

Finish Up

Pause

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 away during the pause, the timer is shut off and the program will wait until the user clicks the Continue button before it advances.

Print Report

Print the report.

Save Document

Save the analysis document after the analysis.

Save Document Options

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

Save PDFs

Save PDFs for reports.

Save PDF Options

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

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.

Save Database

Save the database after each analysis.

Save Database Options

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

Synthesize Data

Synthesize Data

If enabled, new data values will be synthesized based on the selected options. This option is typically used along with the Export FCS options to create and export synthetic data for experimentation.

Synthesize Data Options

This command displays the Properties for Synthesize Data dialog. This dialog is used to set how much to vary the intensities of populations when synthesizing data. The range of intensities is defined by **Low Intensity Factor** and **High Intensity Factor**. A value between these two factors is randomly selected for each synthesis.

Export and Merge FCS

Export FCS

If enabled, an FCS file representing the result of the analysis is exported.

Export FCS Options

Set options for exporting FCS files, including the file name and path.

Merge FCS Option

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. |

Advanced Options

Stop Auto Advance

Stop the Auto Advance of the batch processor.

Use Custom Actions

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

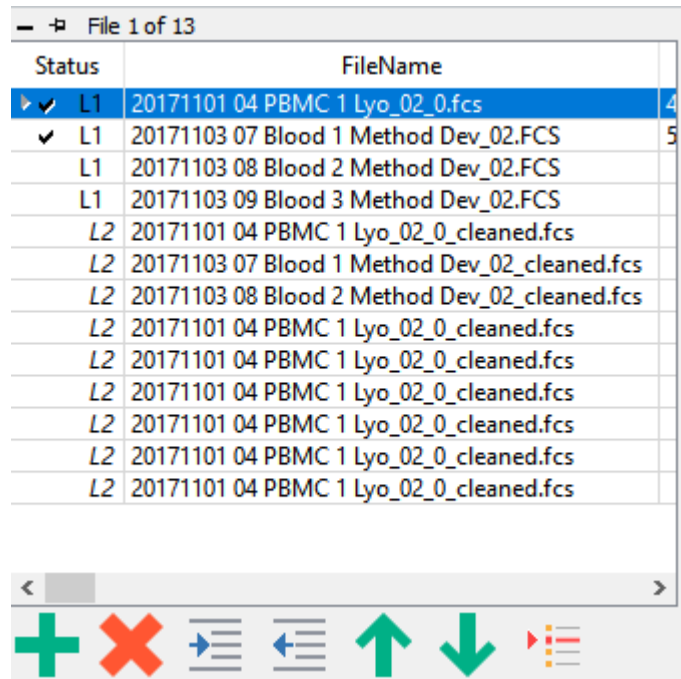
Custom Actions

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

Batch List

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 outline level for the file is also shown, e.g. L1 is level 1.

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



The screenshot shows a window titled "File 1 of 13" containing a table with two columns: "Status" and "FileName". The first row is selected and highlighted in blue, showing a pointer icon in the Status column, "L1" as the status, and "20171101 04 PBMC 1 Lyo_02_0.fcs" as the file name. The second row has a checkmark in the Status column, indicating it has been analyzed. The table lists several files, including original and cleaned versions. Below the table is a toolbar with icons for adding (+), removing (X), indenting (right arrow), outdenting (left arrow), moving up (up arrow), moving down (down arrow), and a list icon.

| Status | FileName |
|--------|---|
| ▶ ✓ L1 | 20171101 04 PBMC 1 Lyo_02_0.fcs |
| ✓ L1 | 20171103 07 Blood 1 Method Dev_02.FCS |
| L1 | 20171103 08 Blood 2 Method Dev_02.FCS |
| L1 | 20171103 09 Blood 3 Method Dev_02.FCS |
| L2 | 20171101 04 PBMC 1 Lyo_02_0_cleaned.fcs |
| L2 | 20171103 07 Blood 1 Method Dev_02_cleaned.fcs |
| L2 | 20171103 08 Blood 2 Method Dev_02_cleaned.fcs |
| L2 | 20171101 04 PBMC 1 Lyo_02_0_cleaned.fcs |
| L2 | 20171101 04 PBMC 1 Lyo_02_0_cleaned.fcs |
| L2 | 20171101 04 PBMC 1 Lyo_02_0_cleaned.fcs |
| L2 | 20171101 04 PBMC 1 Lyo_02_0_cleaned.fcs |
| L2 | 20171101 04 PBMC 1 Lyo_02_0_cleaned.fcs |
| L2 | 20171101 04 PBMC 1 Lyo_02_0_cleaned.fcs |

The controls at the bottom relate to files in the batch list:

- add and remove files
- indent and outdent files to different levels
- move files up or down in the batch
- set the starting file

The outline level that a file is on determines how it will be processed. Each level has its own script of actions, which are edited and control on the [Batch Commands](#) panel that appears below the Batch List when in Develop mode.

See also:

[Batch List Context Menu](#)

[Batch Controls toolbar](#)

Batch List 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.

Start here

Make the selected item the current batch item.

Clear flags

Set all files in the batch to "unanalyzed".

Remove

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

Remove all

Clear all files from the batch list and database.

Edit Fields

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

Clear Results

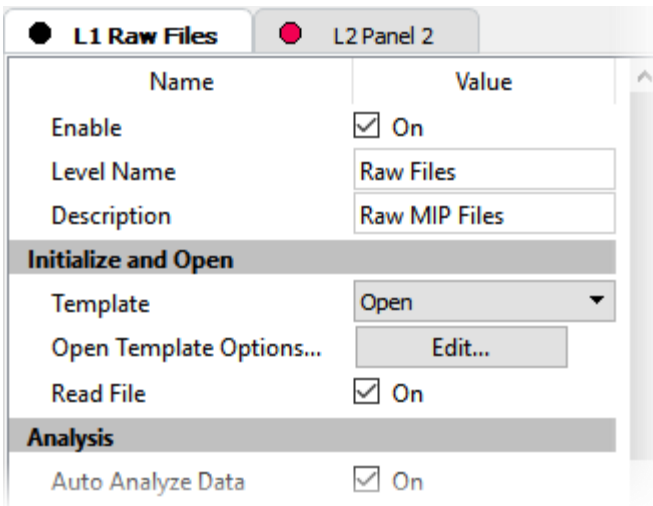
Clears the results for the selected entries in the database.

Export Database

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

Batch Commands

This panel determines the actions that are performed when an FCS file is read into the program for analysis. There is a tab for each "level" in the batch, so that you can set up different actions for different kinds of files.



Select a tab to view or edit the actions for the associated batch level.

Commands that appear on each tab

Enable

Enable the actions defined on this tab.

Level Name

A name for this level

Description

A description for this level

Initialize and Open

Template

Open a specific GemStone model template.

Open Template Options

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

Compact File

For FCS files from Fluidigm instruments, this option will re-write the FCS file eliminating extraneous segments from the file. It will have no effect on other FCS file types.

Read File

Read the FCS file associated with this batch item.

Panel Tube Options

Select the option that describes the files on this level of the batch:

Options

- | | |
|-----------------|--|
| One Tube | This file represents the only tube in the panel. |
| First Tube | This is represents the first tube in a multi-tube panel. |
| Subsequent Tube | This is represents the second or greater tube in a multi-tube panel. |

Analysis

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.

Update Cen-se'

This command updates the Summary map and the Cen-se' plot to display named populations and cell types.

Finish Up

Pause

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 away during the pause, the timer is shut off and the program will wait until the user clicks the Continue button before it advances.

Print Report

Print the report.

Save Document

Save the analysis document after the analysis.

Save Document Options

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

Save PDFs

Save PDFs for reports.

Save PDF Options

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

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.

Save Database

Save the database after each analysis.

Save Database Options

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

Synthesize Data

Synthesize Data

If enabled, new data values will be synthesized based on the selected options. This option is typically used along with the Export FCS options to create and export synthetic data for experimentation.

Synthesize Data Options

This command displays the Properties for Synthesize Data dialog. This dialog is used to set how much to vary the intensities of populations when synthesizing data. The range of intensities is defined by **Low Intensity Factor** and **High Intensity Factor**. A value between these two factors is randomly selected for each synthesis.

Export and Merge FCS

Export FCS

If enabled, an FCS file representing the result of the analysis is exported.

Export FCS Options

Set options for exporting FCS files, including the file name and path.

Merge FCS Option

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. |

Advanced Options

Stop Auto Advance

Stop the Auto Advance of the batch processor.

Use Custom Actions

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

Custom Actions

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

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.



Choose a file in the batch.



Go to the first batch file.



Go to the previous batch file.



Run

Run the analysis script on the current batch file.



Next

Go to the next batch file and analyze.



Go to the last batch file.



Auto

Auto-advance and analyze through the batch.



Stop

Stop auto-advancing.

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 Target Path property below. |
| Use the specified Target File | With this option selected, the GemStone document name and location are defined by the Target File 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.

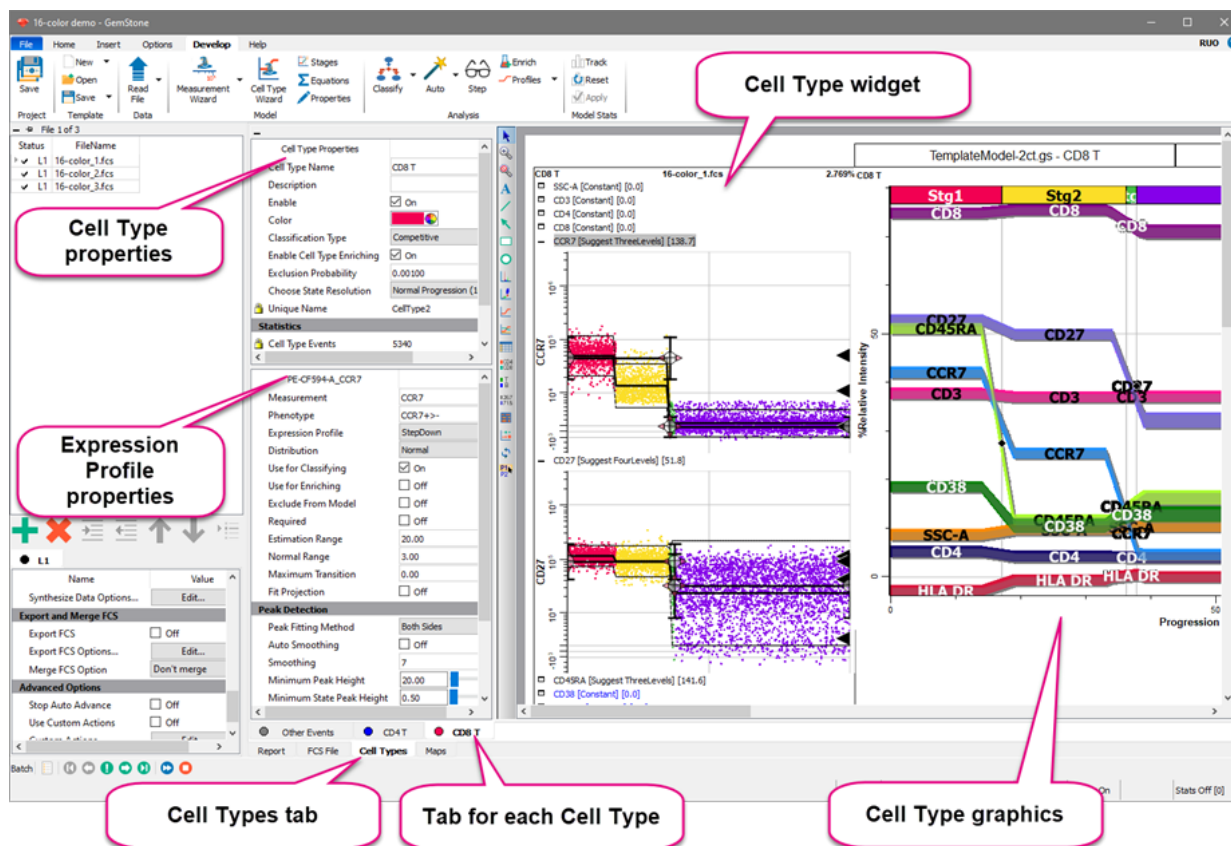
Target File

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.

Cell Type Tabs

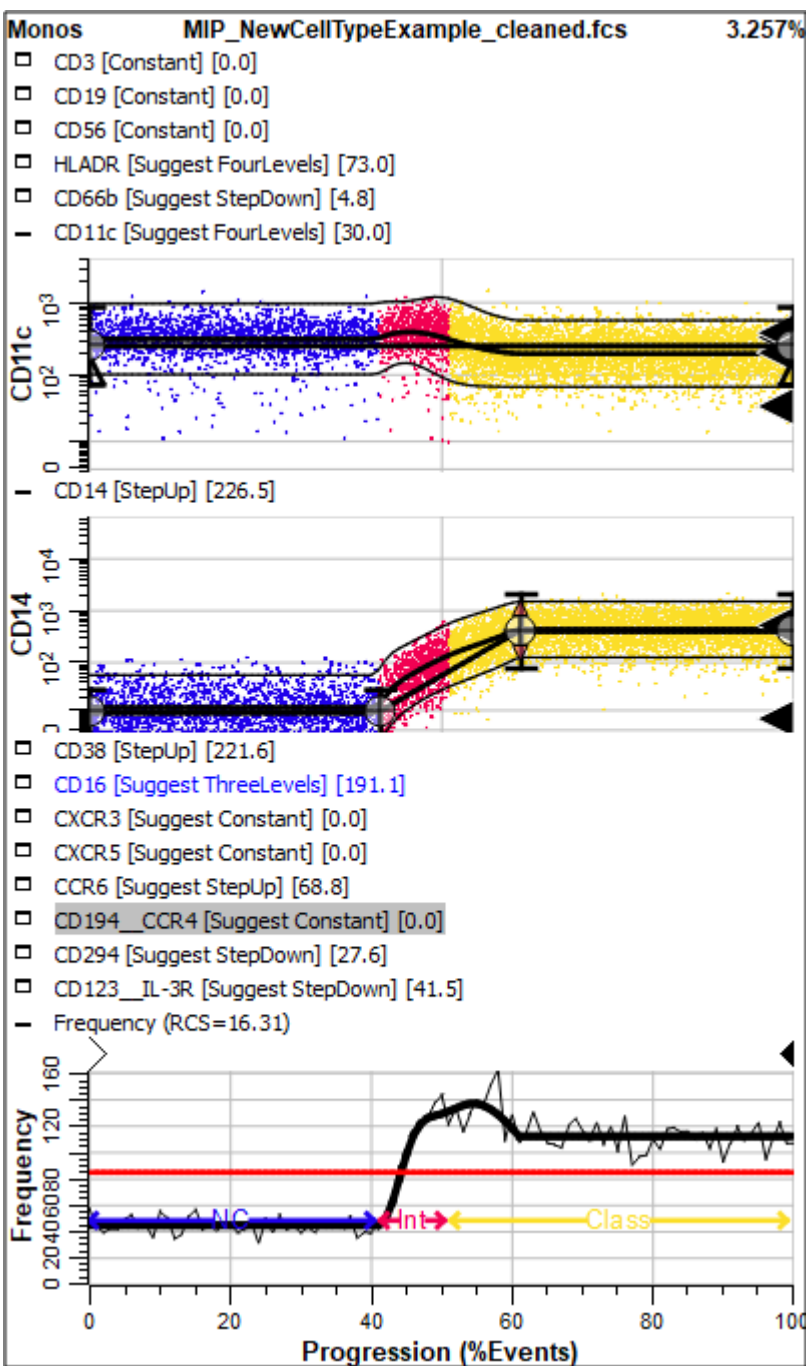
In [Develop mode](#), the program shows a **Cell Types** tab at the bottom of the main window. This is where you will find many of the tools for developing a model. This tab contains a set of tabs, one for each Cell Type that has been defined in the model. Each Cell Type tab has a [Cell Type Widget](#) that includes all of the [Expression Profiles](#) used for that Cell Type. The tab can also include other graphics and tables added by the user.

On the left side of the tab area, a collapsible section includes [Cell Type Properties](#) and [Expression Profile Properties](#).



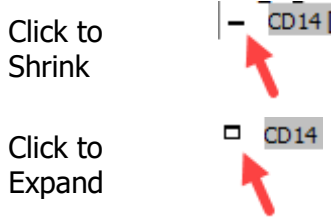
Cell Type Widget

The Cell Type widget contains the stack of [Expression Profile plots](#) that define a cell type in the model.



You can expand and collapse expression profiles within the widget for better visualization of the data. To collapse a profile that is showing, click the "shrink" icon next to the

measurement name on the left edge. To expand a profile that is hidden, click the "expand" icon next to the measurement name.



When you click on an expression profile, the profile becomes the active profile. You can edit its properties in the [Expression Profile Properties panel](#), or by right-clicking and choosing [Edit Expression Profile](#) to edit the most common properties.

















The Frequency Plot provides information about how well the model is working. The Y-axis shows frequency of events, and the X-axis shows the Progression axis. 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 Expression Profiles need to be adjusted. In general, when there are too many or too few events in a portion of the Frequency Plot, review the Expression Profiles with control points and transitions in those states. Examine those profiles to be sure the control points are properly positioned.

The Frequency Plot also shows the Reduced Chi Square (RCS) of the model and the stages that have been defined.

Cell Type toolbar

This toolbar appears on the left edge of the Cell Type tab.

| | | |
|--|------------------|---|
|  | 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 | Text | Click and drag to define an area for the text. A dialog will be displayed to input the text. |
|  Line | Line | Click and drag to define the end points of the line. |
|  Arrow | Arrow | Click and drag to define the end points of the arrow. |
|  Rectangle | Rectangle | Click and drag to define the outline of the rectangle. |
|  Ellipse | Ellipse | Click and drag to define the bounding rectangle for the ellipse. |
|  1P | 1P Histogram | Click and drag to define the bounding rectangle for the 1P histogram. |
|  2P | 2P Histogram | Click and drag to define the bounding rectangle for the 2P histogram. |
|  EP | Parameter Graph | Click and drag to define the bounding rectangle for a parameter graph. |
|  Overlay | Overlay plot | Click and drag to define the bounding rectangle for the overlay plot. |
|  Table | Table | Click and drag to create a table object. |
|  | Legend | Click and drag to define the bounding rectangle for the legend. |
|  | Cell Type Legend | Click and drag to define the bounding rectangle for the Cell Type legend. |
|  | Stage Table | Click and drag to define the bounding rectangle for the Stage Table. |

GemStone User Guide



Heat Map
plot

Click and drag to define the bounding rectangle for the heat map plot.



TriCOM

Click and drag to define the bounding rectangle for the TriCOM plot.

TriCOM



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.

Cell Type Properties panel

This panel contains properties that relate to the current [Cell Type tab](#). It is located on the left side of a [cell type tab](#).

Identification

Cell Type Name

Enter a name for the Cell Type.

Description

Enter a description for the Cell Type.

Enable

Enable or disable classification of events for this Cell Type.

Color

Choose the color for this Cell Type.

Classification Type

Select the type of classification to use for this cell type.

| | |
|-----------------|---|
| Competitive | The cell type will compete with other cell types to capture events |
| Selective | The cell type will be used to select events for all cell types that follow it in the model. |
| Non Competitive | The cell type includes events that have not been classified by other cell types. |

Enable Cell Type Enriching

This checkbox determines whether the Cell Type will be enriched by the [Enrich](#) command.

Exclusion Probability

The value determines the fraction of events that will be excluded from the Cell Type after initially being selected into it. A value of 0.001 will exclude one tenth of a percent of the events that would have been selected.

State Resolution

Select the resolution to use for the cell type. 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. The default is Normal Progression (100 states).

Unique Name

Enter the number states to define for the progression of events in this Cell Type.

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.

Expression Profile Properties Panel

This panel is used to define the expression profile. It is located on the left side of a [cell type tab](#).

Measurement

Enter a short name to use for the profile.

Phenotype

Enter a short phenotype description to use for the profile.

Expression Profile

Choose the type of profile to use for modeling this measurement. See [Expression Profile Descriptions](#) for a list of profile types.

Distribution Type

Choose the type of distribution to use for this profile. See [Expression Profile Distribution Types](#) for details.

| | |
|---------------------|---|
| Normal Distribution | The control definition points define the mean of a normal distribution. |
| Inverse Normal | The profile is the inverse the Normal Distribution. |
| Inverse Low Pass | The profile is the inverse of the Low Pass distribution. |
| Low Pass | The control definition points define a probability "edge". Events <i>below</i> the CDPs are included. |
| High Pass | The control definition points define a probability "edge". Events <i>above</i> the CDPs are included. |
| Inverse High Pass | The profile is the inverse of the High Pass distribution. |

Use for Classifying

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

Use for Enriching

Check this option to include this profile when using the [Enrich](#) command.

Exclude from Model

Enable this option to exclude the profile from the modeling process. This is a convenient way to temporarily disable a profile without removing it.

Required

If this option is enabled, the measurement *must* be included in the FCS file. If it is missing, the modeling process will terminate and the file will not be analyzed.

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.

Normal Range

This value is the number of Standard Deviations that define the normal range when used with inverse profiles and TriCOMs.

Maximum Transition

This value sets a limit on the amount of transition in a staging profile like a StepUp or StepDown.

Fit Projection

If this option is checked, the modeling process will attempt to fit the projections in order to optimize the fit.

Color

Select a color for this measurement. This color is used in Tri-COMs and overlay plots.

Peak Detection

Peak Fitting Method

Choose the method to use for fitting peaks for this measurement. The default, Both Sides, uses the full peak to estimate position and SD. Steeper side, Dimmer side, and Brighter side use only one side of the peak for the estimates.

Auto Smoothing

Enable this option to automatically determine the best smoothing bandwidth.

Smoothing

Enter a smoothing value. Lower numbers result in less smoothing. Default value is 7.

Minimum Peak Height

The lowest height that is considered a peak.

Minimum State Peak Height

When Fit Projection is enabled, the lowest height that is considered a peak.

Peak Deflection Threshold

The minimum change between a low and high value to consider the segment a peak..

Merge Peaks Below

Peaks below this value will be into one peak.

Peak Separation

If $\text{abs}(\text{mean1}-\text{mean2})/(\text{sd1}+\text{sd2})$ is greater than this threshold, then the peaks are different.
(0.10 to 1.00)

Fit Distance

Filters detected peaks where the fitted mean is too far from the peak's location. (1.00 to 4.00)

Edit Expression Profile dialog

This dialog edits the most common properties for an expression profile. See [Edit Profile Properties Panel](#) for the full set of properties and descriptions.

| Name | Value |
|---------------------------|--|
| Expression Profile | |
| Measurement | CD4 |
| Expression Profile | Constant |
| Distribution | Normal |
| Color | |
| Use for Classifying | <input checked="" type="checkbox"/> On |
| Use for Enriching | <input type="checkbox"/> Off |
| Estimation Range | 20.00 |
| Normal Range | 3.00 |
| Low, Normal, High Regions | <input type="checkbox"/> Off |
| Peak Search Range | <input type="checkbox"/> Off |

Measurement

Enter a short name to use for the profile.

Expression Profile

Choose the type of profile to use for modeling this measurement. See [Expression Profile Descriptions](#) for a list of profile types.

Distribution Type

Choose the type of distribution to use for this profile. See [Expression Profile Distribution Types](#) for details.

Color

Select a color for this measurement. This color is used in Tri-COMs and overlay plots.

Use for Classifying

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

Use for Enriching

Check this option to include this profile when using the [Enrich](#) command.

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.

Normal Range

The number of SDs that define the normal range. This is used with inverse distribution types and TriCOMs.

Low, Normal, High Regions









Shows all low, normal, and high regions in the data

Peak Search Range

Shows peak search range for the expression profile measurement.

Expression Profile toolbar

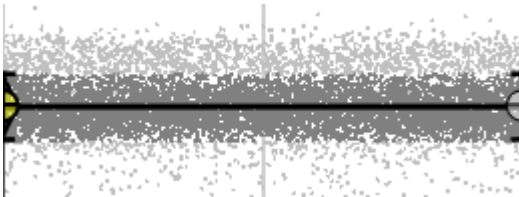
The Expression Profile toolbar is located on the right side of each Expression Profile in the Cell Type widget. It appears when the mouse pointer is moved over the profile plot.

| | | |
|---|---------------------------|---|
|  | 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. |
|  | Edit Graphics | Edit the graphics from this profile. |
|  | Edit Expression Profile | Edit the properties of the profile |
|  | Select Profile | Choose a profile for the measurement. |
|  | Auto-analyze profile | Analyze this profile in the same way the Auto-Analysis analyzes the entire model. |
|  | Toggle Expression Profile | This button toggles the profile on or off. When enabled (on), the profile is an active part of the model and becomes part of the selection process for events in the cell type. |

Expression Profile Descriptions

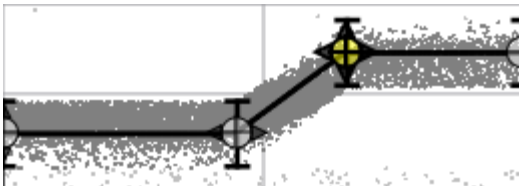
Expression Profiles provide great flexibility for modeling different kinds of measurement expressions. This page describes the types available in the software. See [Expression Profile Distribution Types](#) for related information about types of distributions.

Constant



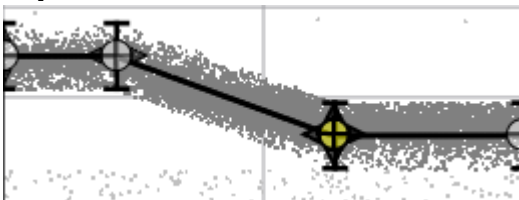
This profile has two Control Points that are linked to the same intensity. It is used to select events for a cell type.

Step Up



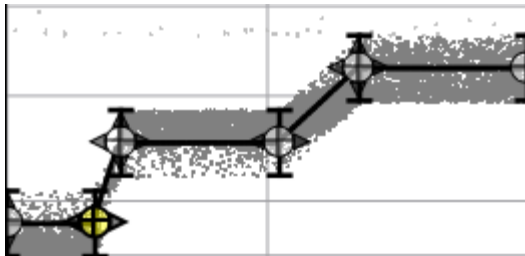
The Step Up profile has four Control Points. The first two points are linked to have the same intensity values. The last two points are also linked, and are forced to have a *higher* intensity than the first two. It is used for measurements that are up-regulated in the cell type.

Step Down



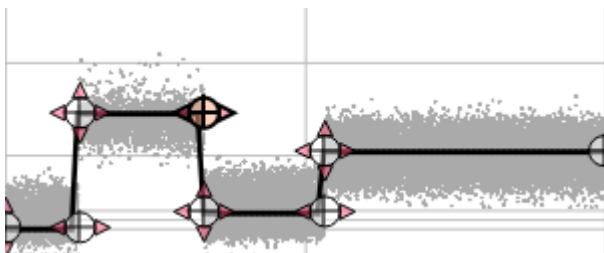
The Step Down profile has four Control Points. The first two points are linked to have the same intensity values. The last two points are also linked, and are forced to have a *lower* intensity than the first two. It is used for measurement that are down-regulated in the cell type.

Three Levels



This profile defines three levels of intensity. For each level, there are two 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.

N-Levels

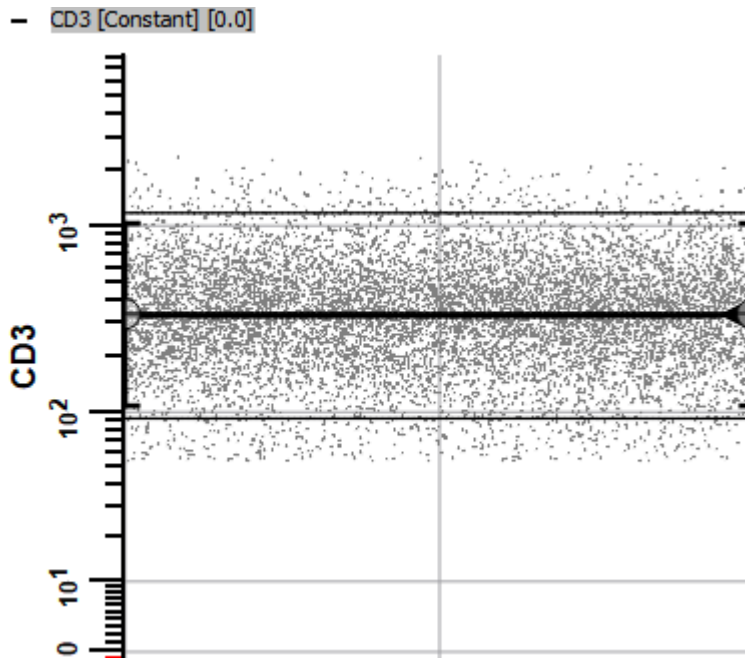


This profile has a user-defined number of levels. Each level has two Control Points, one that defines the beginning of the level, and one that defines the end.

Expression Profile Distribution Types

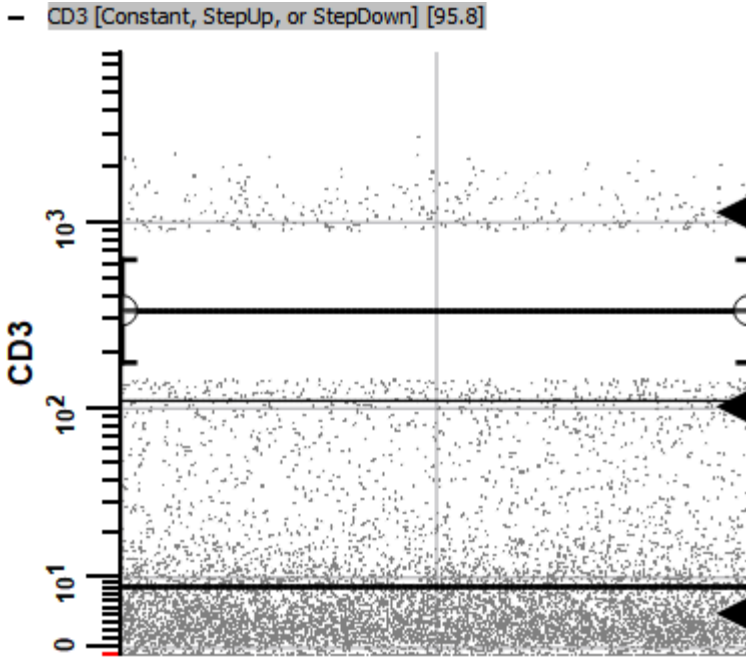
Expression Profiles can be set up with a number of different types of distributions. This page describes the types available in the software. See [Expression Profile Descriptions](#) for related information.

Normal



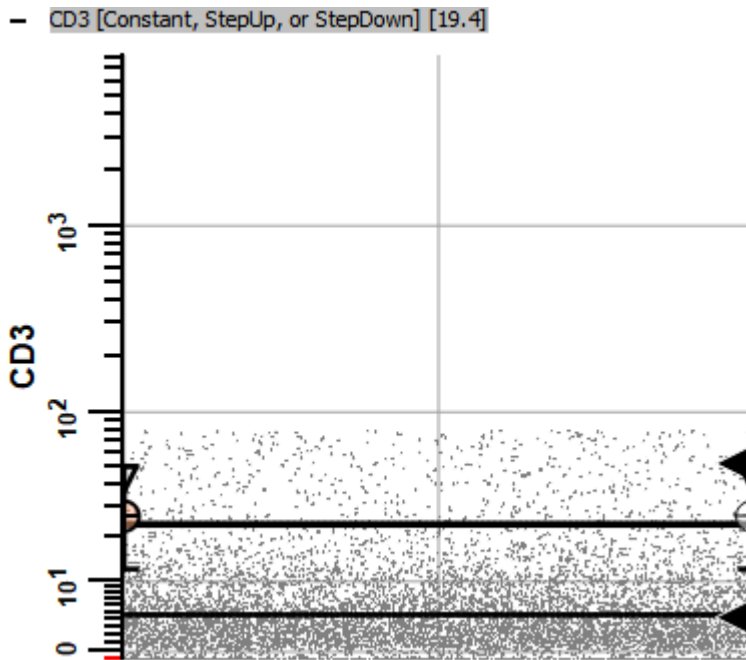
Normal distributions are typically used when the events of interest are in an intensity band that relates to a peak. A normal distribution is also called a Gaussian distribution.

Inverse Normal



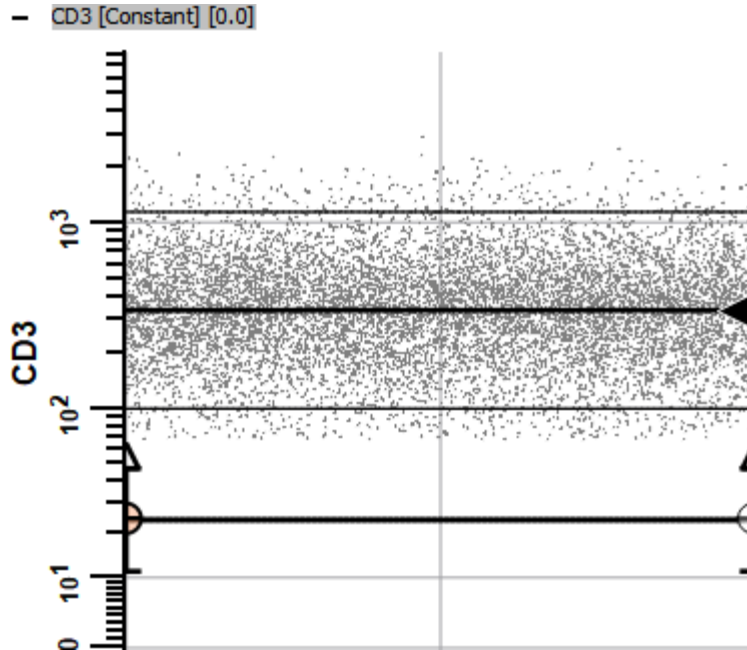
This is a rarely used distribution type. It selects the events that are *not* in the normal distribution.

Low Pass



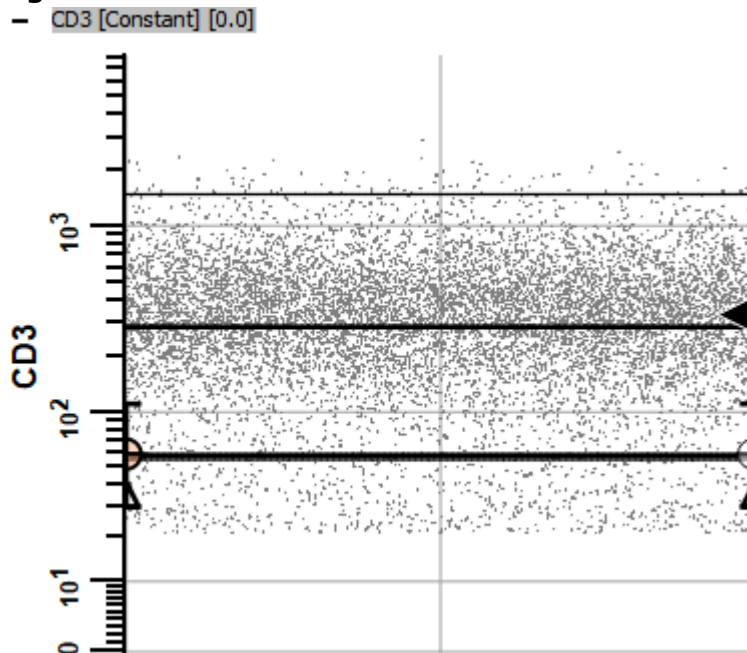
This selects events in and below the normal distribution defined by the profile. It is commonly used for negative selection markers.

Inverse Low Pass



This selects events that are above the normal distribution defined by the profile. It can be used, for example, when there is a well-defined low peak that contains events that are *not* of interest.

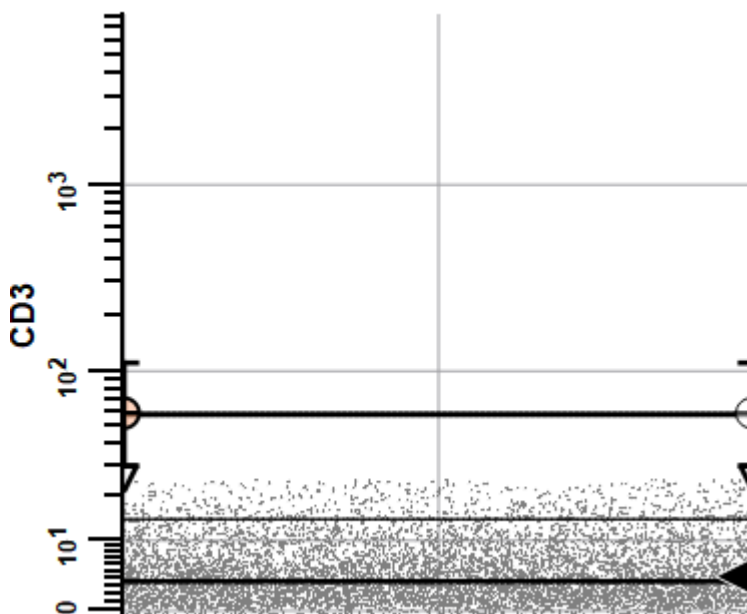
High Pass



This selects events that are in and above the normal distribution defined by the profile. It can be used, for example, when a peak defines the lower area of interest, and you want to include all events above that.

Inverse High Pass

- CD3 [Constant] [0.0]



This selects events that are below the normal distribution defined by the profile. It can be used, for example, when a peak defines the lower area of interest, and you want to include only events below that.

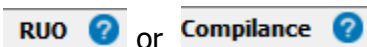
Users and Groups

Research and Compliance Modes

GemStone can be set up to run in either a Research-Use-Only mode or in Compliance mode. When the program is in Compliance mode, you have the ability to control which users can do different operations. Here are some differences between RUO and Compliance modes:

- Compliance mode makes use of your operating system's user groups to control the level of access to program features. Research mode allows any user to perform any operation.
- Compliance mode displays a login dialog when the program is starting up to verify the user and determine the group to which the user belongs.
- Compliance mode enables logging so that there is a record of the activities of each user. Research mode can be run with logging enabled or disabled.
- Compliance mode is designed to be part of a compliance plan for the US-FDA's 21 CFR Part 11 regulations. Research mode does not provide compliance with 21 CFR Part 11.
- Both modes use the same intuitive interface and analysis engine.

The program displays the mode of operation in the upper right corner of the ribbon:



Set Up Research Use Only Mode

After initial installation, GemStone will automatically be in Research (RUO) mode. No further configuration is required.

To revert to Research mode after setting up Compliance mode, the user groups that were created for Compliance mode must be removed. Once the user groups are removed, the program will revert to Research mode.

Set Up Compliance Mode

Setting up Compliance mode should be performed by IT personnel.

While it is not a complicated task, it requires administrative access rights and a clear understanding of user groups and permissions. It is therefore something that we strongly recommend be performed by the IT team at your institution. The task involves creating several GemStone user groups for your computer or domain, and then adding users to those groups. Once the groups are defined, the program automatically operates in Compliance mode. [Click here for details.](#)

21 CFR Part 11 Compliance Matrix

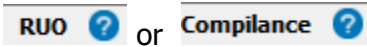
This matrix provides a simple summary of how GemStone in Compliance mode helps you with 21 CFR Part 11 compliance. [Click here for details.](#)

Research and Compliance Modes

GemStone can be set up to run in either a Research-Use-Only mode or in Compliance mode. When the program is in Compliance mode, you have the ability to control which users can do different operations. Here are some differences between RUO and Compliance modes:

- Compliance mode makes use of your operating system's user groups to control the level of access to program features. Research mode allows any user to perform any operation.
- Compliance mode displays a login dialog when the program is starting up to verify the user and determine the group to which the user belongs.
- Compliance mode enables logging so that there is a record of the activities of each user. Research mode can be run with logging enabled or disabled.
- Compliance mode is designed to be part of a compliance plan for the US-FDA's 21 CFR Part 11 regulations. Research mode does not provide compliance with 21 CFR Part 11.
- Both modes use the same intuitive interface and analysis engine.

The program displays the mode of operation in the upper right corner of the ribbon:



Set Up Research Use Only Mode

After initial installation, GemStone will automatically be in Research (RUO) mode. No further configuration is required.

To revert to Research mode after setting up Compliance mode, the user groups that were created for Compliance mode must be removed. Once the user groups are removed, the program will revert to Research mode.

Set Up Compliance Mode

Setting up Compliance mode should be performed by IT personnel.

While it is not a complicated task, it requires administrative access rights and a clear understanding of user groups and permissions. It is therefore something that we strongly recommend be performed by the IT team at your institution. The task involves creating several GemStone user groups for your computer or domain, and then adding users to those groups. Once the groups are defined, the program automatically operates in Compliance mode. [Click here for details.](#)

21 CFR Part 11 Compliance Matrix

This matrix provides a simple summary of how GemStone in Compliance mode helps you with 21 CFR Part 11 compliance. [Click here for details.](#)

Setting Up Compliance Mode

Setting up Compliance mode should be performed by your IT team. It requires administrative rights and a clear understanding of your network operating system.

The task involves creating several GemStone user groups for your computer or domain, and then adding users to those groups. Once one or more of the groups are defined, the program automatically operates in Compliance mode.

User Groups and Permissions

There are two (2) user groups for Compliance mode: **GemStoneAdmins** and **GemStoneUsers**.

The table below shows the kinds of features that group members can access.

| Feature | GemStoneUsers | GemStoneAdmins | RUO Mode All users |
|-------------------------------|---------------|----------------|-----------------------|
| Edit Preferences | No | Yes | Yes |
| Create and Edit Models | No | Yes | Yes |
| Develop new analysis Projects | No | Yes | Yes |
| Project Develop | No | Yes | Yes |
| Run Projects | Yes | Yes | Yes |
| Select Projects | Yes | Yes | Yes |
| Add files to batch | Yes | Yes | Yes |
| Run a batch of files | Yes | Yes | Yes |
| Log and Preference folder | Shared | Shared | Shared |

Creating User Groups

The program requires at least one of the GemStone user groups to be defined in order to run in Compliance mode. GemStone user groups can be defined for your computer or for your entire domain using tools provided by the operating system. This is typically done by information technology personnel because it requires administrative rights.

The advantage of defining the user groups at the domain (network) level is that the groups are defined *for all computers* on the domain at the same time. If you define the user groups on an individual computer, those groups are available *only on that computer*.

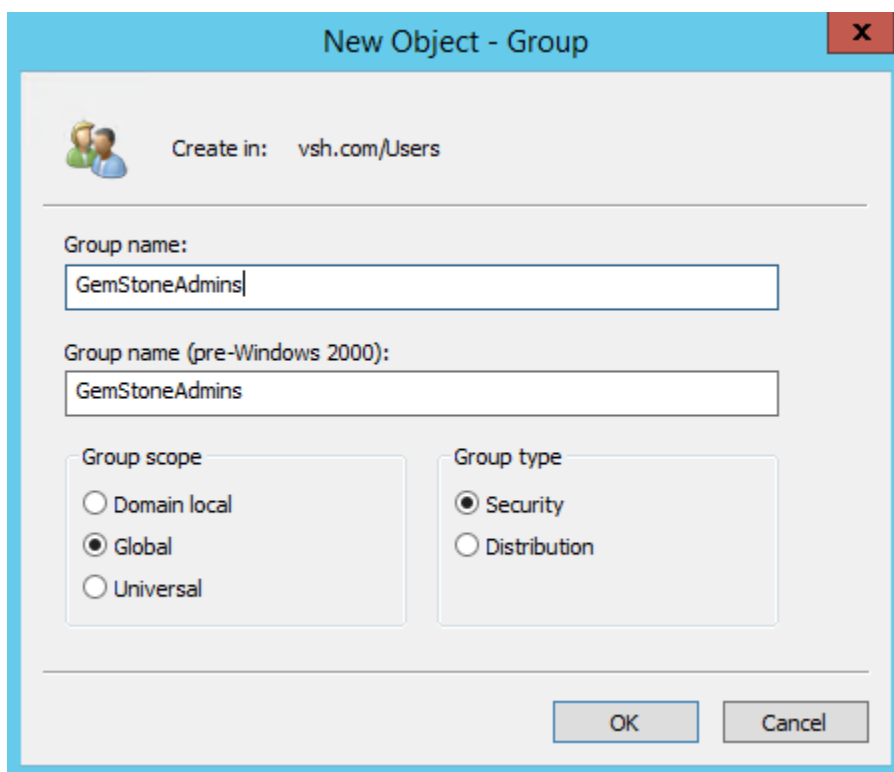
Below we describe how to create user groups on a Windows server or on a Windows computer, as examples. The specific methods you need to use may differ depending on the version of the operating system you are working with.

After you have defined the user groups and added users to them, GemStone will detect the groups when it starts up and operate in Compliance mode automatically.

Windows Server User Groups

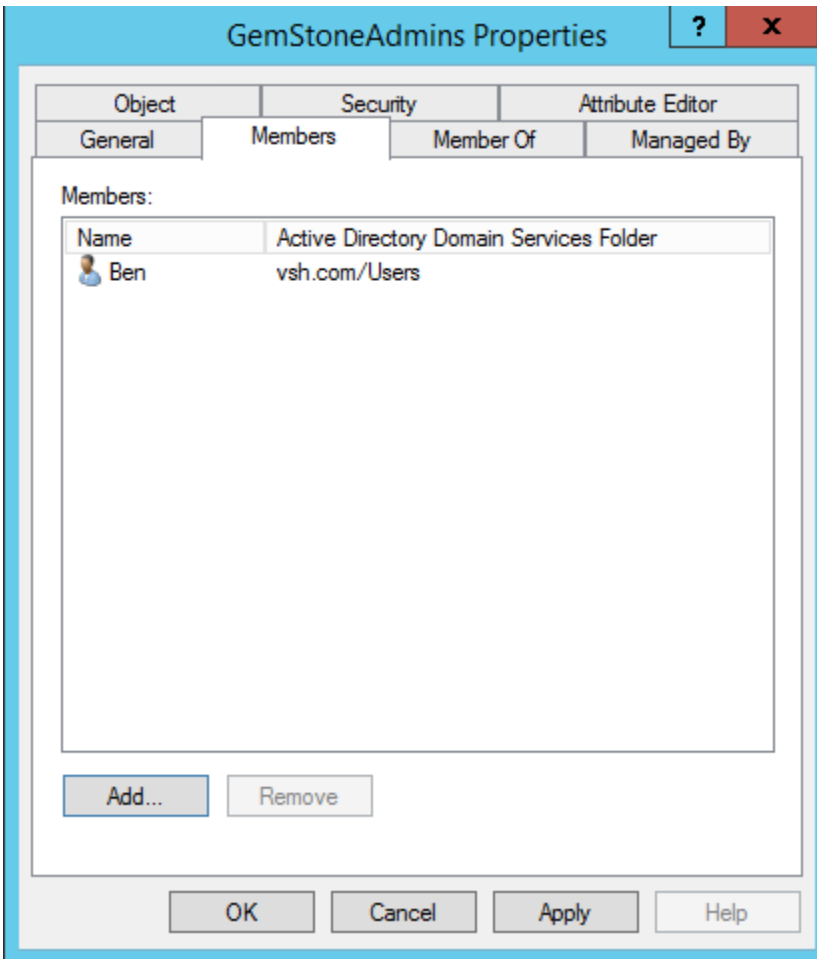
Follow these steps to define user groups for an entire network or domain. **Note: if you use this technique, you do not need to define user groups on each individual computer.**

- On a domain server, launch the "Active Directory Users and Computers" administrative tool.
- Right click the "Users" folder in the left panel and choose New>Group. The New Group dialog is displayed.



The screenshot shows the "New Object - Group" dialog box. At the top, it says "Create in: vsh.com/Users". Below that, there are two text boxes for "Group name:" and "Group name (pre-Windows 2000):", both containing "GemStoneAdmins". Under "Group scope", the "Global" radio button is selected. Under "Group type", the "Security" radio button is selected. At the bottom, there are "OK" and "Cancel" buttons.

- Type **GemStoneAdmins** for the group name and click OK.
- Once the group is created, double-click the group in the list to display the Properties for the group.
- Click the **Members** tab and click **Add** to select members for the GemStoneAdmins group. You should have at least one user in the GemStoneAdmins group. Members can be added by typing user names, or by using the dialog's search features.



- Repeat this process for the **GemStoneUsers** group.
- Once the groups are created and users are added to them, the program will automatically use Compliance mode when it starts up and is connected to the domain.
- Any user who is not in a defined user group will be prevented from using the software.

Mac OS X User Groups

Follow these steps to define user groups for an individual Mac OS computer.

- On a Mac OS computer, launch System Preferences. Choose "Accounts" or "Users and Groups", depending on the version of the OS.
- Click the lock icon to authenticate to make changes. This will require administrative rights.
- Click the "+" button under the list of users and groups. The "New Account" dialog is displayed.
- Change the New Account type to "Group".
- Type **GemStoneAdmins** for the group name and click "Create Group".

- Select the users you want to be members of the **GemStoneAdmins** group. You should have at least one user in the **GemStoneAdmins** group.
- Repeat this process for the **GemStoneUsers** group.
- GemStone will run in Compliance mode the next time it starts.

See also:

[21 CFR Part 11 Compliance](#)

21 CFR Part 11 Compliance

GemStone can be part of your lab's compliance strategy for 21 CFR Part 11 when it is setup and operated in Compliance mode. It has been developed under a quality management system and validated with a suite of quality assurance tests. The table below summarizes how the key elements of Part 11 relate to GemStone and its use in Compliance mode settings.

| Section | Summary of Rule | Compliance Strategy |
|-----------|------------------------------------|---|
| 11.10 | Controls for closed systems | GemStone can be accessed only through the user interface; it is a closed system. |
| 11.10 (a) | Validation and accuracy. | GemStone uses externally-generated data to validate its results and employs proprietary binary format, static PDF, and/or CRC methods to ensure valid files. |
| 11.10 (b) | Record generation and readability | GemStone generates CRC-checked log files and static PDF reports that are human-readable and machine-readable. |
| 11.10 (c) | Record protection and retrieval | Record retention and retrieval plan is managed by the laboratory's file management practices. |
| 11.10 (d) | Authorized access | GemStone uses the operating system's user login validation, and user group membership determines permissions within the software. |
| 11.10 (e) | Audit trails | GemStone employs a CRC-checked logging system with time stamps, and log files are preserved. Log files cannot be deleted by ordinary means. |
| 11.10 (f) | Operational system checks | GemStone enables and disables portions of the UI to enforce permitted sequencing of steps and events. |
| 11.10 (g) | Authority checks | GemStone uses the operating system user groups and login validation to assure authorized access. |
| 11.10 (h) | Device checks and validity | GemStone performs validation checks on the FCS data files. |
| 11.10 (i) | Education, training and experience | GemStone provides a User Guide, technical support, and tutorial videos to assist with the proper, intended use of the program. Specific on-site training may be arranged. |
| 11.10 (j) | Electronic signatures | This is an individual laboratory requirement beyond the scope of use of GemStone. |
| 11.10 (k) | Systems documentation | GemStone version releases are accompanied by an updated User Guide. Verity employs document control procedures. |
| 11.30 | Controls for open systems | GemStone is designed as a closed system. |
| 11.50 | Signature manifestations | GemStone does not require or offer electronic signatures. |

| | | |
|-----------------|--------------------------------|---|
| 11.70 | Signature linking | GemStone does not require or offer electronic signatures. |
| 11.100 (a) | Unique electronic signatures | Your laboratory must ensure that electronic signatures are unique to each user on the network. |
| 11.100 (b) | Identity verification | Each laboratory must verify its own employment records and IDs. |
| 11.100 (c) | Signature verification | Each laboratory must verify electronic signature eligibility with the FDA. |
| 11.200 (a)(1) | Two-component signing | GemStone does not require or offer electronic signatures. |
| 11.200 (a)(2,3) | Authenticity and falsification | GemStone does not require or offer electronic signatures. |
| 11.200 (b) | Biometric signatures | GemStone does not utilize biometric IDs. |
| 11.300 (a) | Controls for identification | GemStone uses the operating system user groups and login validation to assure unique user identities. |
| 11.300 (b) | Identification aging | The individual laboratory IT structure must control password checks and aging requirements. |
| 11.300 (c) | External ID validation | Not applicable. GemStone does not utilize external tokens, cards, RFID, or other physical identity-confirming devices. |
| 11.300 (d) | Unauthorized use | The individual laboratory IT structure must control login authenticity and manage safeguards to prevent unauthorized use. |
| 11.300 (e) | External device testing | Not applicable. GemStone does not utilize external tokens, cards, RFID, or other physical identity-confirming devices. |

Index

1

1P Graph 234, 236

1P Histogram 182, 245

2

2P Graph 246, 248, 252, 256

2P Histogram 261

A

Add Files 78, 216, 497

Adding 78

 New Cell Type 78, 400, 401

 New Measurement 386, 387

Adding a New Cell Type 78, 182, 400, 401

Adding a New Measurement 182, 386, 387

Alternative Color 334

Analysis Document 223, 224

Analyze 221

Animation 9, 238, 256, 274, 285, 293, 306,
 310, 478

Archive Method 200, 201

Arrow 230

Auto Advance 221

Auto Analysis 438, 439

Auto Analyze 438, 439

B

Batch Commands 457, 464

Batch Controls toolbar 469

Batch List 23, 25, 216, 219, 221, 462

Batch List Context Menu 463

C

Cell Type Legend 304

Cell Type Tabs 472, 473

Cell Type toolbar 476

Cell Type Widget 273, 474

Cell Type Wizard 78, 400, 401, 402, 404,
 406, 407, 408, 409, 414

Cell Type Wizard Profiles 409

Cell Types 9, 23, 25, 30, 35, 78, 210, 211,
 212, 226, 228, 230, 231, 233, 234, 236,
 238, 246, 248, 252, 254, 256, 262, 264,
 268, 273, 274, 279, 282, 285, 289, 294,
 297, 303, 304, 305, 306, 310, 314, 322,
 325, 328, 350, 358, 363, 380, 382, 384,
 400, 401, 402, 404, 406, 407, 408, 409,
 415, 417, 426, 433, 438, 439, 440, 457,
 464, 472, 473, 474, 476, 478, 485, 486

Cen-se 182, 325, 328, 331, 334, 344

Cen-se Options 331, 334

Cen-se plot 78

Check for Updates 451
 Classify Data 436
 Classify File 437
 Clean Method 197
 Color Scheme 334
 Control Definition Points 273
 Control Points 483
 Copy 212
 D
 Delete Method 199
 Design object 322
 Develop 23, 25, 30, 31, 78, 215, 225, 262, 264, 345, 378, 386, 387, 400, 401, 472, 473, 497, 501
 Develop Check box 215
 Develop Tab 378
 Distribution Type 483
 Dots 252, 369
 E
 Edit Colors 357
 Edit Equation 419, 422, 425
 Edit Fields 347
 Edit Frame 242, 258, 275, 290, 318, 341
 Edit Graphic Options 78, 267, 268, 271, 273
 Edit Graphics 245, 251, 252, 261, 293, 317, 321
 Edit Logging 355
 Edit Preferences 359, 360
 Edit Profile 485
 Edit Zones 415, 478
 Ellipse 233, 261
 Enrich 442
 Entropy Heatmap 334
 EP Animation Options 274
 EP Legend 303
 Equation Functions 426
 Equations 419, 422
 Estimation Range 483
 Export Database 348
 Expression profile 30, 35, 78, 262, 264, 267, 268, 271, 273, 274, 279, 282, 285, 289, 303, 322, 366, 380, 381, 382, 384, 406, 407, 408, 409, 415, 417, 426, 438, 480, 483, 485, 486, 488
 Expression Profile Descriptions 486
 Expression Profile Distribution Types 488
 Expression Profile Plot 262, 264, 273
 Expression Profile Plot Toolbar 278
 Expression Profile Properties 480
 Expression Profile toolbar 485

- F
 - Interface 3, 23, 25, 245, 261, 293, 476, 493, 495, 501
- FCS file 354, 385
- File Tab 192
- Fitted Documents 31
- Frequency Heatmap 334
- Functional Markers 78, 408, 409
- G
 - Global Properties panel 433
 - Graphic Options for 2P Vectors 254
 - Graphic Options for EP Means 270
 - Graphic Options for Measurements 289
 - Graphic Options for Overlays Plot 285
- H
 - Heat Map 252
 - Help Tab 450
 - Home Tab 209
- I
 - Information 346
 - Information Table Preferences 375
 - Initialize and Start Stats 448
 - Insert Tab 225
 - Installation and Setup 13
 - Installation and Setup of GemStone 13
 - Intensity Heatmap 334
- K
 - Keyword Export 349
- L
 - License Agreement 5
 - Line 228
 - Logging 356
- M
 - Manage Settings 377
 - MC HIMP 29, 30, 78
 - Measurement 35, 78, 234, 236, 238, 246, 248, 254, 262, 264, 271, 279, 282, 285, 289, 303, 306, 310, 314, 317, 322, 386, 387, 390, 391, 426, 480, 486
 - Measurement Wizard 386, 387, 390, 391, 396
 - Method 9, 27, 28, 29, 30, 31, 78, 194, 195, 196, 197, 200, 201, 205, 209, 213, 214, 215, 216, 218, 271, 358, 366, 378, 379, 474, 480, 497, 501
 - Method Library 27, 29, 31, 194, 200, 201, 213, 214
 - Method List 214
 - Minimum System Configurations 11
 - Model Templates 23, 25, 28, 31, 78, 196, 205, 219, 349, 379, 380, 381, 382, 384, 385, 390, 404, 419, 422, 436, 457, 464

N

New Cell Type 78, 400, 401, 402

New measurement 386, 387, 390

New Method 194, 213

New Template 380

Next 220

Node Colors 334

Normal Range 480, 483

O

Open 223

Open Analysis Document 223

Open Last Document 223

Open Method 195

Open Recent Document 223

Open Report Template 205

Open Template 382

Optimize Model 440

Options Tab 345

Overlay Plot 279, 282

P

Paste 210

Phenotype 34

Phenotype Naming 34

Print Report 204

Printer Setup 207

Probability State Model 35, 437, 478

Properties for Color Palette 358

PSM 35

Q

Quick Start Guide 3

R

Read FCS 385

Rectangle 231, 261

Register Software 15

Registering 15

Remove FCS Files 217

Report tab 23, 25

Research and Compliance Modes 493, 495

Ribbon bar 23, 25

Run 209, 219

S

Save Analysis Document 224

Save Document As 383

Save FCS 354

Save Method 196, 379

Save PDF Report 203

Save Report Template 206

Save Template 383

| | | |
|---------------------------------------|-------------------|---|
| Save Template As | 384 | T |
| Saving | 31 | Table 294, 297, 300 |
| Search Archives | 201 | Template Document 30, 31, 380 |
| Selection Markers | 78, 406, 409, 488 | Template Mode 78, 331, 334 |
| Software Registration | 15 | Text 226 |
| SOP | 3 | Track Stats 447 |
| SSMD | 182 | Transformation Preferences 376 |
| Staging Markers | 78, 407 | Transformations 386, 387 |
| Stain Assessment | 182 | Transforms 394 |
| Start At | 218 | TriCOM 78, 306, 310, 314, 317, 321, 480 |
| Step Review | 441 | TriCOM toolbar 321 |
| Stop | 222 | W |
| Strictly Standardized Mean Difference | 182 | Warranty 5 |
| Synthesize from Analysis | 350 | Z |
| Synthesize from Model | 352 | Zone Table 305 |
| | | Zones 273, 417 |