Enhanced Proliferation Analysis

Integration features in ModFit LT and WinList offer new ways to examine cell proliferation.

Overview

New features in ModFit LT and WinList provide unprecedented capabilities to analyze cell proliferation.

ModFit LT performs sophisticated non-linear least squares analysis in just a few clicks of a mouse and reveals the secrets of the sample: proliferation index, non-proliferative fraction, and precursor frequency, to name a few. A new feature allows ModFit's proliferation results to be exported so that other applications can extend the analysis.

Enter WinList, the premiere listmode analysis software. WinList's special NStat region can import ModFit LT's proliferation results and adjust itself to partition the generations according to the proliferation analysis. That simple enhancement sets the stage for exciting new analysis capabilities.
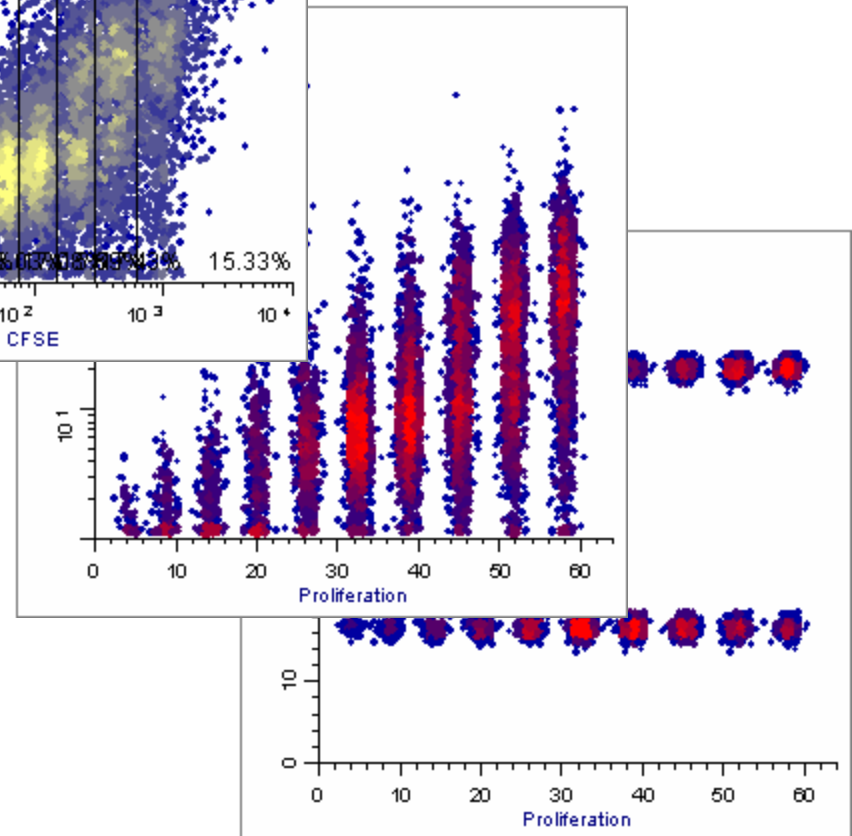
This tutorial will explore these new features to examine a CFSE-stained sample containing several other markers.

Where We're Going…

From the Proliferation
Wizard in ModFit LT…
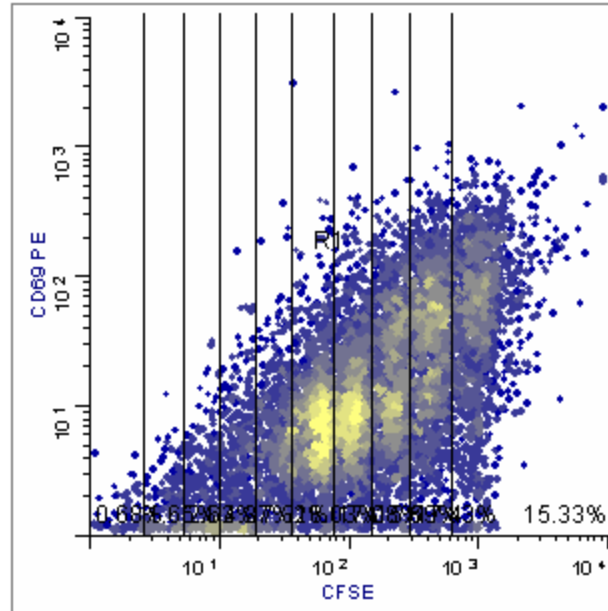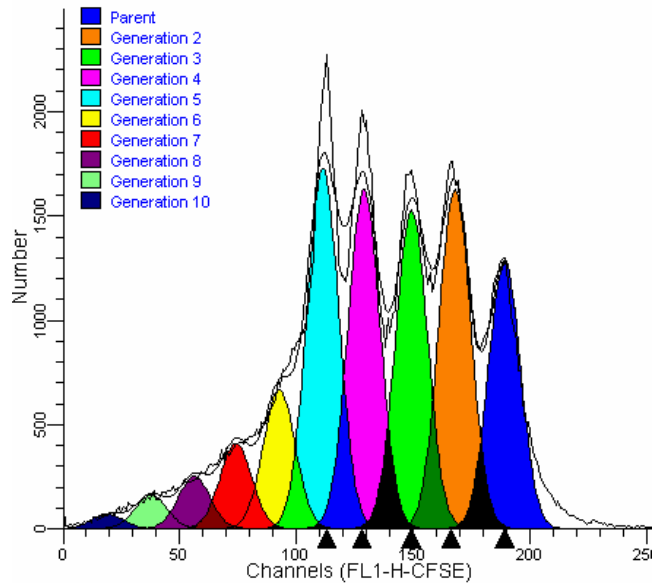
Into WinList for
exciting, new
explorations.

Getting Started

You will need both ModFit LT and WinList for this tutorial.

ModFit LT should be version 3.1 with a Build Date of 5 May 2003 or later.

WinList should be version 5.0 with a Build Date of 1 May 2003 or later.

• Launch WinList and pass through the opening dialogs.

• Launch ModFit LT and pass through the opening dialogs.

Part 1

In this section, you will perform a Proliferation Wizard analysis with ModFit LT and export its results. Then, you will set up WinList to import those results into an NStat region.
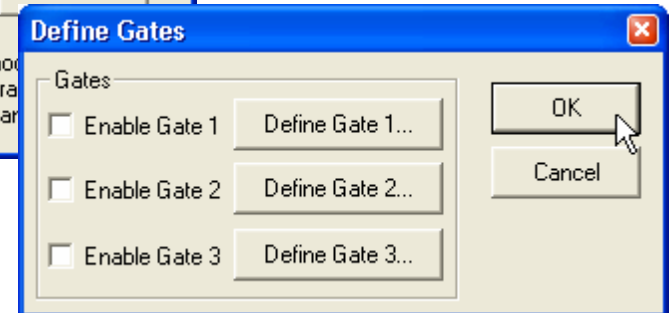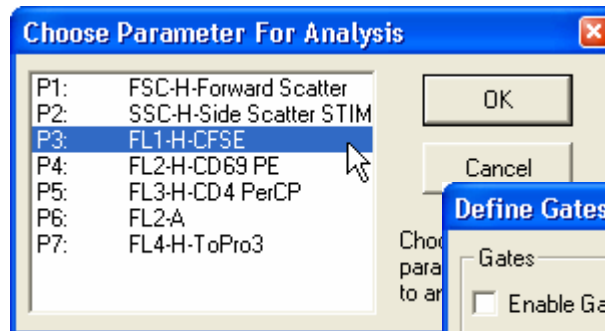
Open the Data File

Using ModFit LT's Open Data command, navigate to the Samples\Proliferation folder in the application folder.
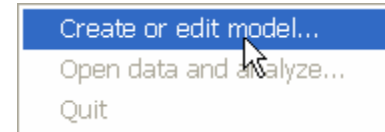
Select "CFSE sample.fcs" and click Open.
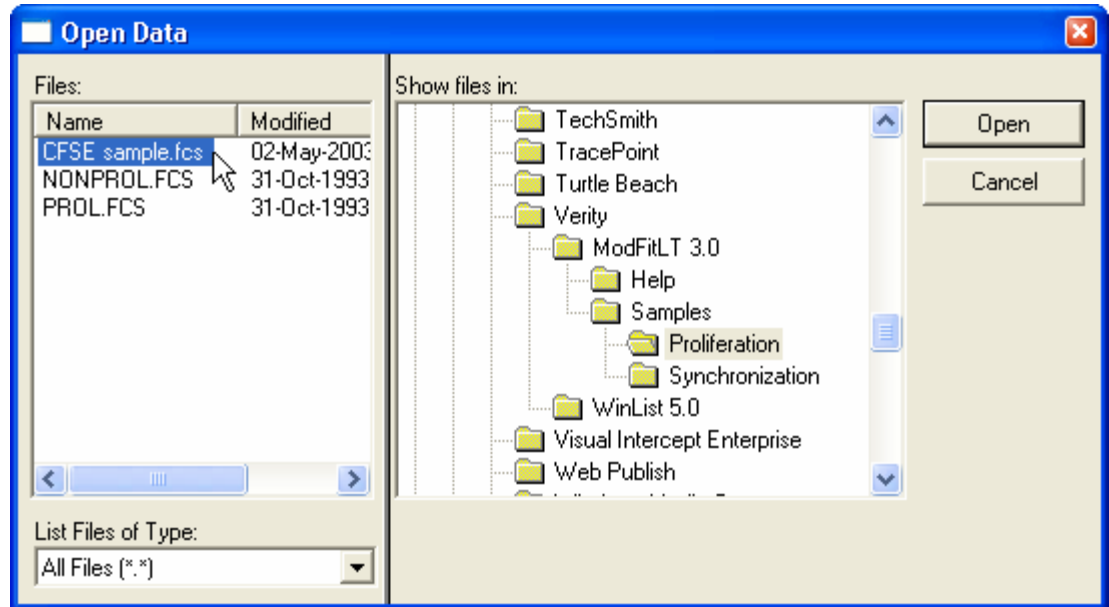
Choose "P3: FL1-H-CFSE" from the parameter list, and click OK.

Leave the Gate checkboxes unchecked and click OK.
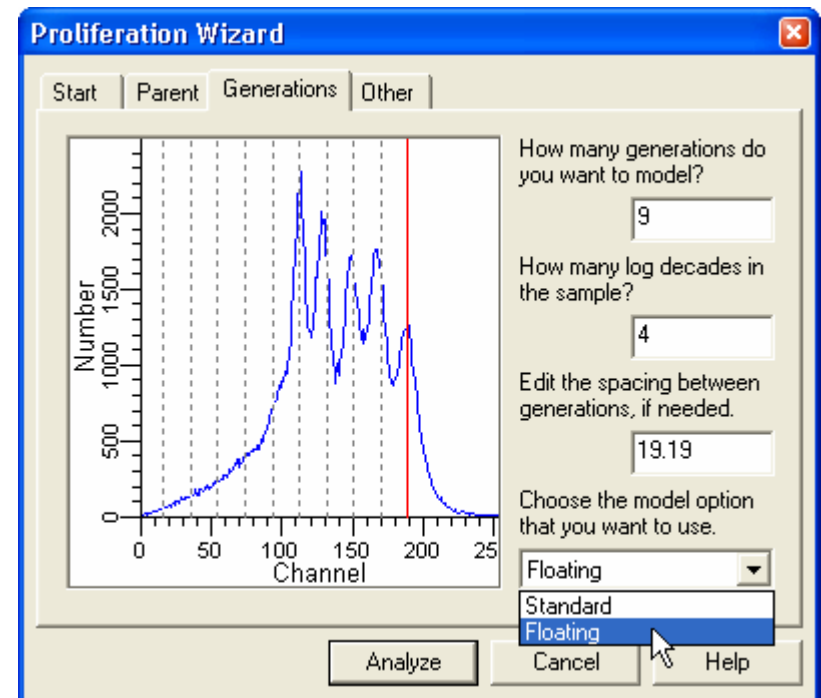
Using the Proliferation Wizard

From the Analysis menu,
choose Proliferation Wizard, and then
Create or edit model…

In the Proliferation Wizard dialog, click
the Generations tab.

Choose the "Floating" model option
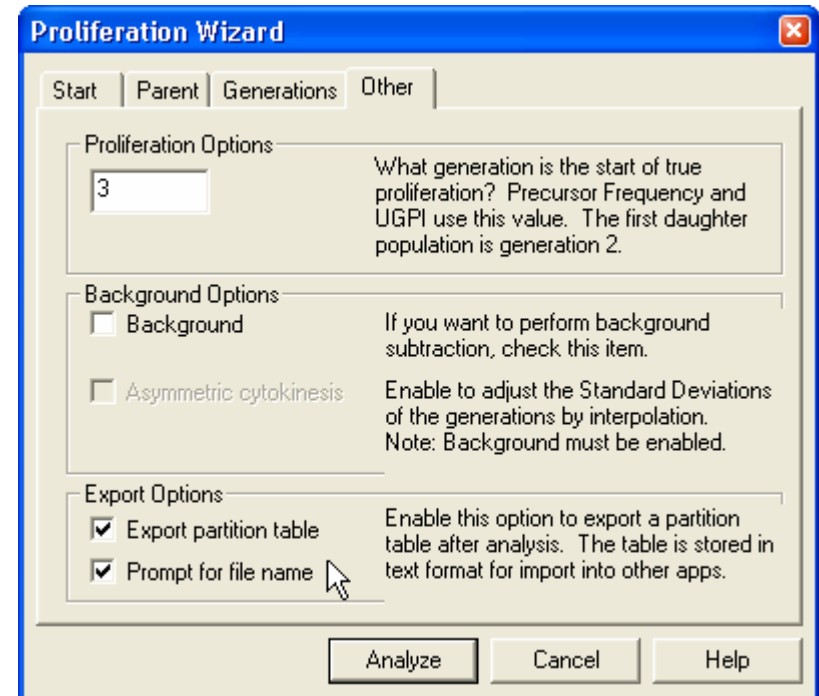from the drop-down list.

Using the Proliferation Wizard

In the Proliferation Wizard dialog, click the Other tab.

Enable the Export options checkboxes.

These options will cause the program to create the export file after analysis and allow you to enter a name for the export file.
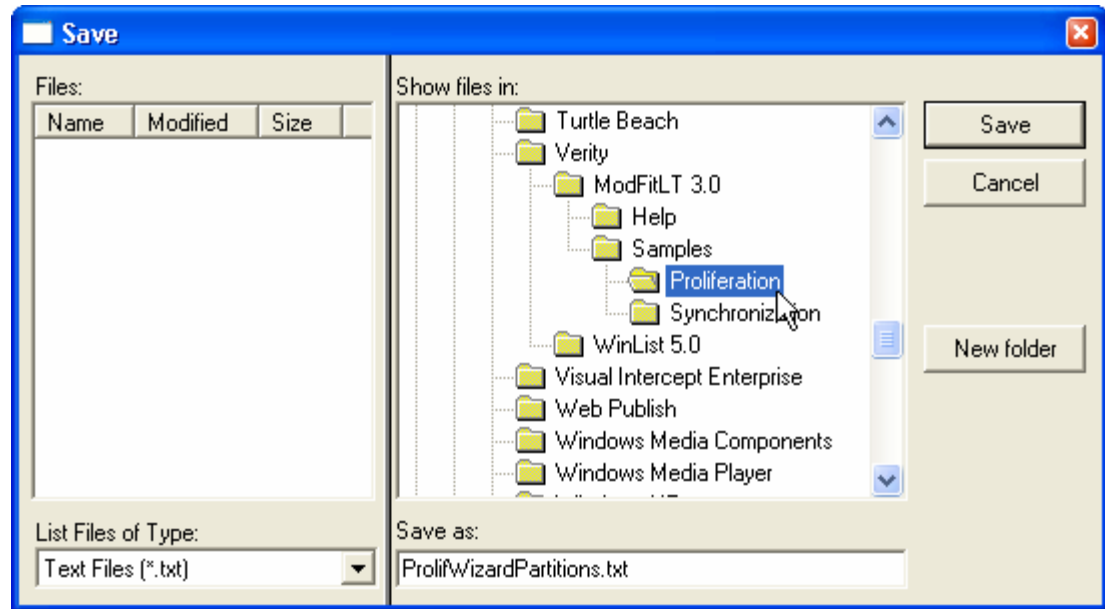
Finally, click the Analyze button to perform the analysis.

Proliferation Wizard

Start | Parent | Generations | Other

Proliferation Options

3

What generation is the start of true proliferation? Precursor Frequency and UGPI use this value. The first daughter population is generation 2.

Background Options

☐ Background

If you want to perform background subtraction, check this item.

☐ Asymmetric cytokinesis

Enable to adjust the Standard Deviations of the generations by interpolation. Note: Background must be enabled.

Export Options

☑ Export partition table
☑ Prompt for file name

Enable this option to export a partition table after analysis. The table is stored in text format for import into other apps.
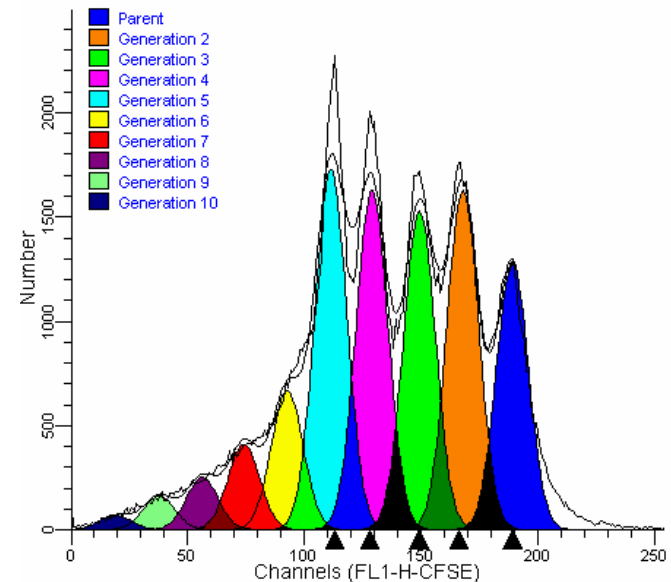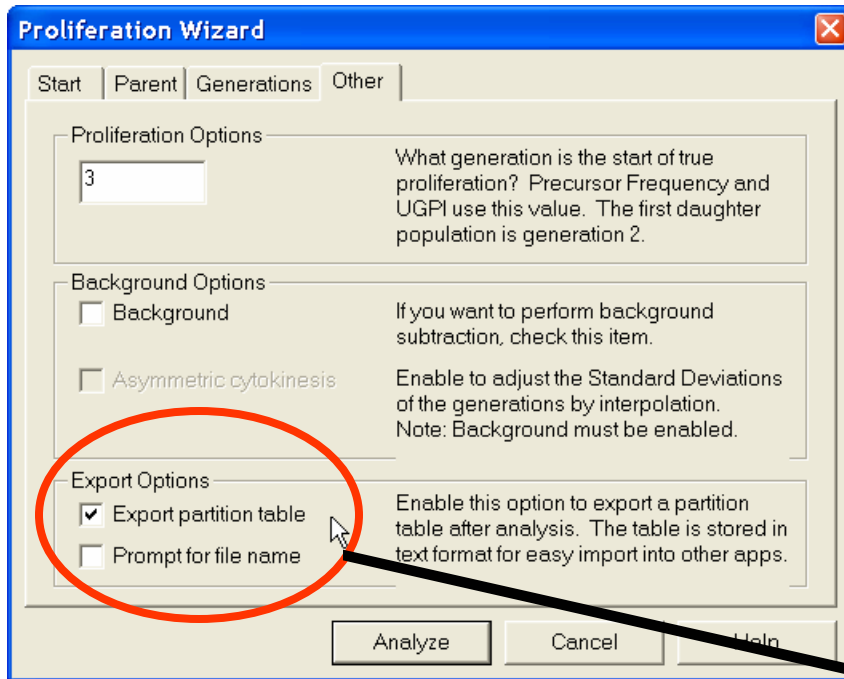
[Analyze] [Cancel] [Help]

Exporting the Analysis

When the analysis is complete, the Save dialog will be displayed.

You can save the file to any location. For this tutorial, it is best to save the export file in the same folder as the data file.

What was Exported?

Proliferation Wizard

Start | Parent | Generations | **Other**

Proliferation Options

`3`

What generation is the start of true proliferation? Precursor Frequency and UGPI use this value. The first daughter population is generation 2.

Background Options

☐ Background

If you want to perform background subtraction, check this item.

☐ Asymmetric cytokinesis

Enable to adjust the Standard Deviations of the generations by interpolation. Note: Background must be enabled.

Export Options

☑ Export partition table
☐ Prompt for file name

Enable this option to export a partition table after analysis. The table is stored in text format for easy import into other apps.

Analyze | Cancel | Help

```
Prolif Wizard Partitions
File    CFSE sample.fcs
Regions 10
Scale   0        256
Gen10   0.00     26.20
Gen9    26.20    45.80
Gen8    45.80    63.82
Gen7    63.82    82.06
Gen6    82.06    99.51
Gen5    99.51    120.15
Gen4    120.15   139.11
Gen3    139.11   158.28
Gen2    158.28   178.87
Parent  178.87   256.00
```

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

Setting Up WinList

Activate WinList and click the Open Data icon.

Navigate to the ModFit LT folder, and then to the Samples\Proliferation folder.

Select the CFSE sample.fcs file and click Open.
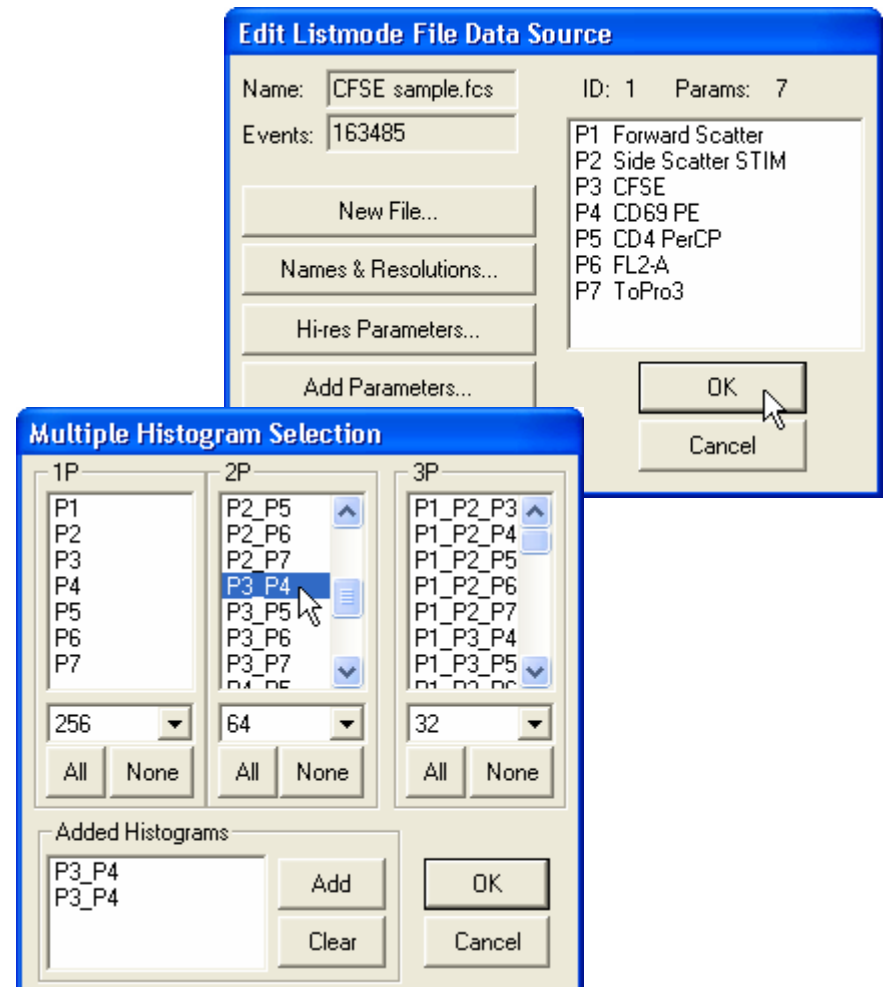
Setting Up WinList

Click OK to close the Edit Listmode dialog.

In the Multiple Histogram Selection dialog, select P3_P4 in the 2P listbox.

Click the Add button twice. This will create two copies of CFSE vs. CD69 PE.
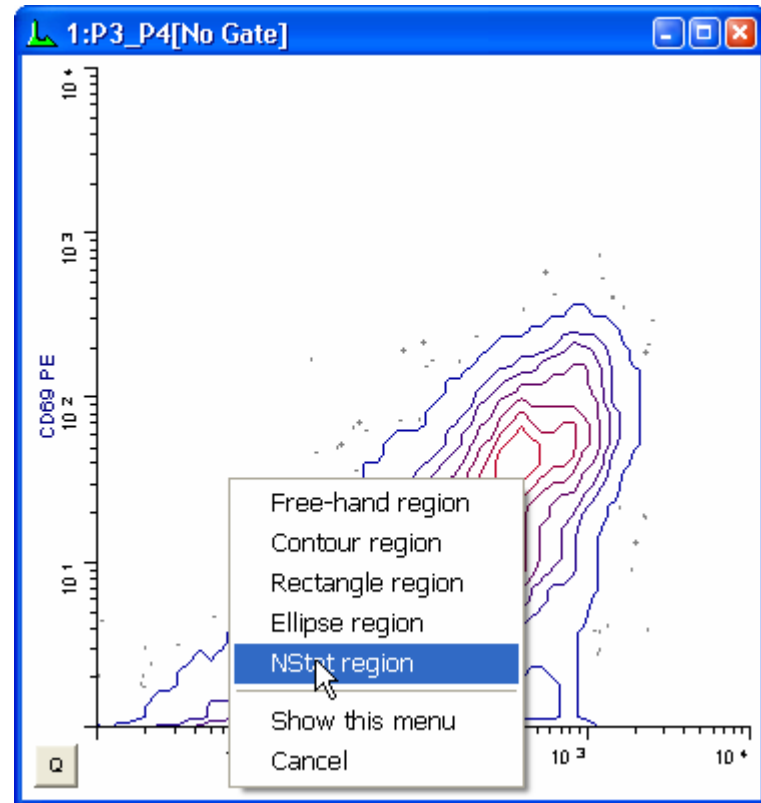
Click OK to close the dialog.

Setting Up WinList

Enable region creation by clicking the Region Creation button on the toolbar.

Depending on your configuration settings for Regions, either click or Shift-click inside the first histogram to display the region creation menu.

Choose NStat region from the menu.

Setting Up WinList

In the NStat Properties dialog, click the Import button.

Navigate to the folder in which the Proliferation export file was saved.

Select ProlifWizardPartitions.txt and click Open.

After the import, the number of columns is automatically changed to 10.

Edit the NStat rows to 1.

Click OK.
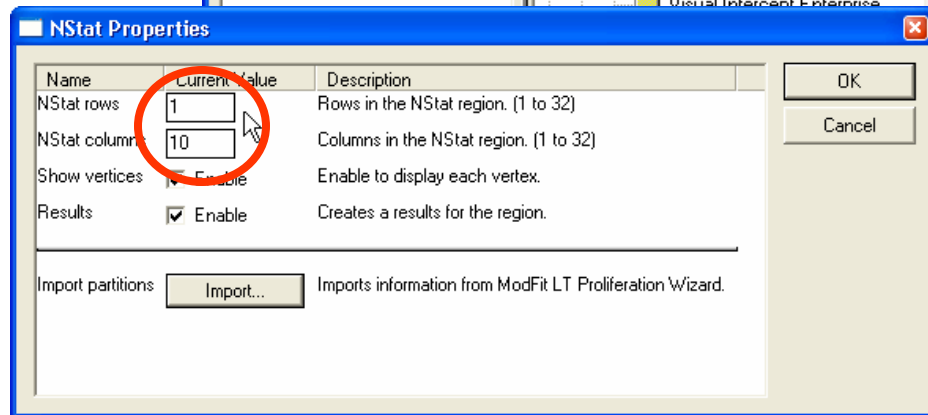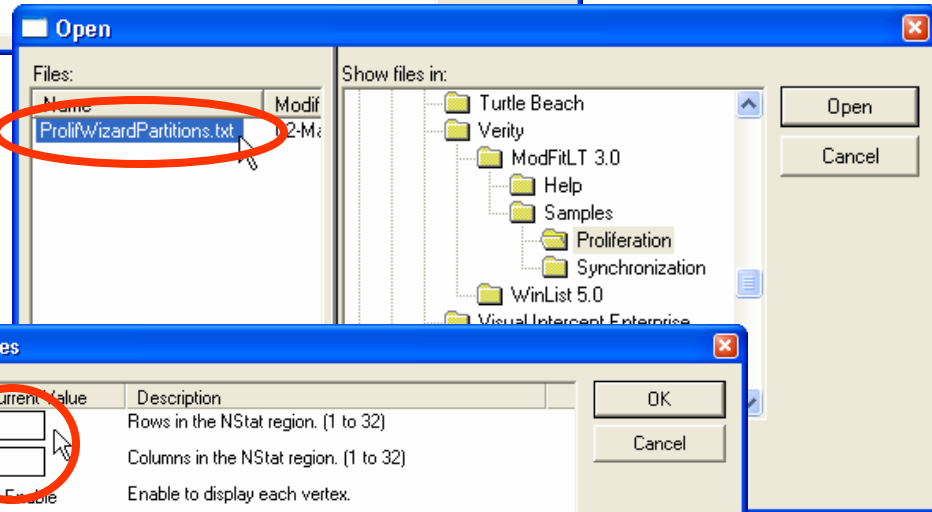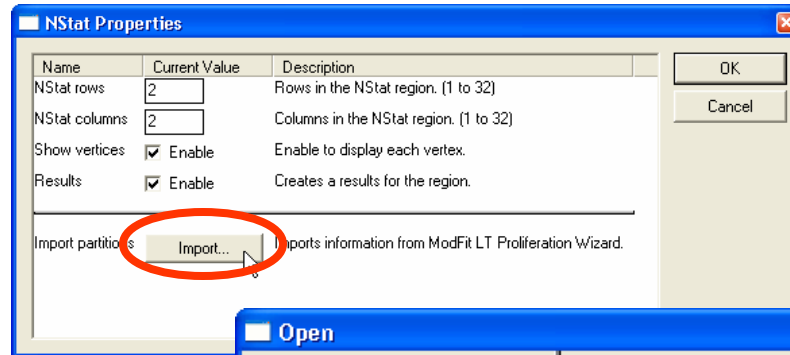
Setting Up WinList

In the second histogram, create another NStat region.

For this one, set NStat rows to 2, NStat columns to 1, and uncheck other options.

Click OK.
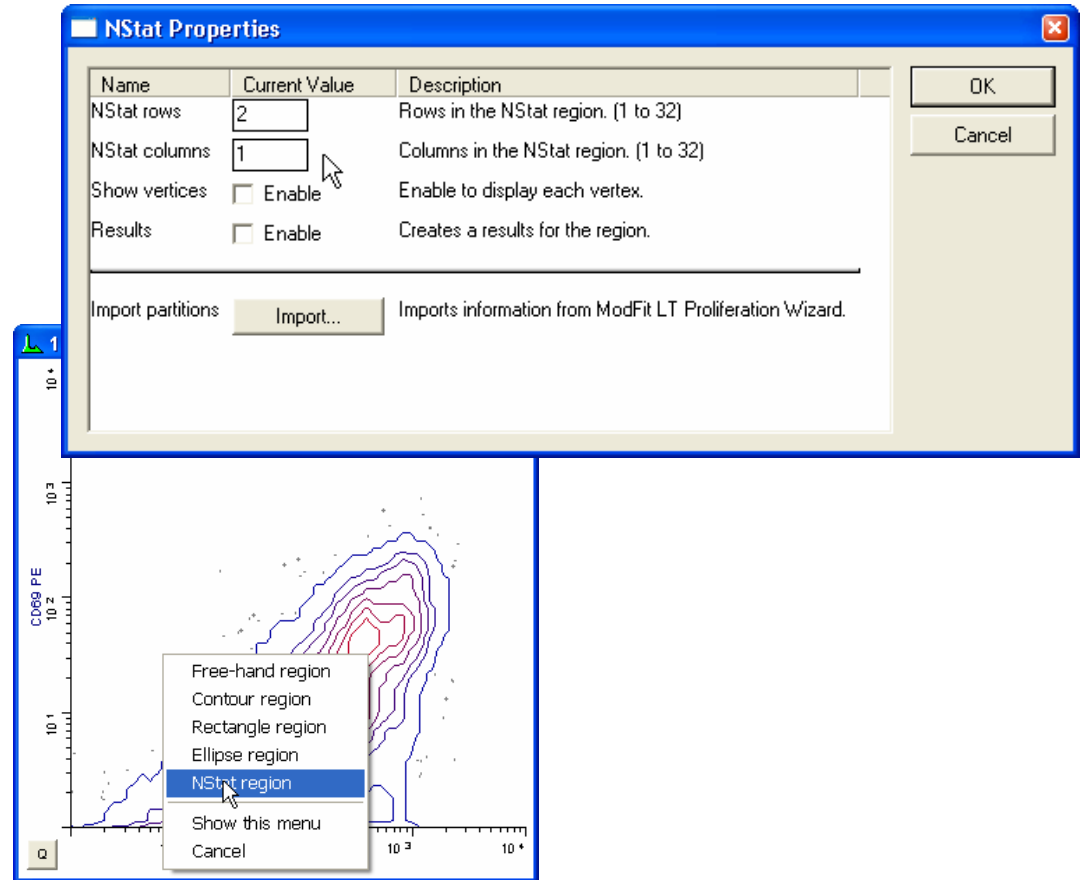
Setting Up WinList

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

Adjust the second NStat so that it approximately divides the two major populations in the second histogram, as shown.

At this point, you have completed the basic import process.
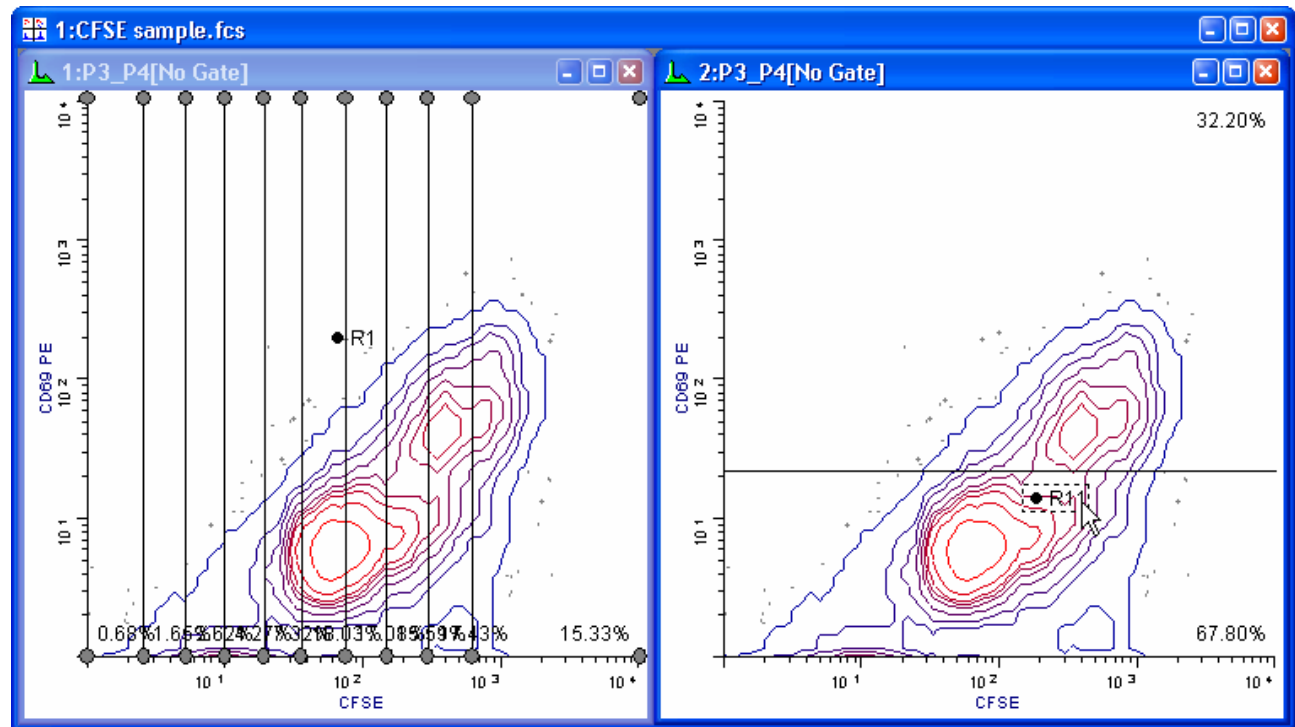
Part 2

In this section, you will extend the WinList analysis to explore other markers as a function of the proliferation analysis.

Isolating Generation Populations

To better analyze and visualize the generations, we will create a calculated parameter using the FCOM function. This function creates a new parameter that will allow us to look at each population independently. It uses gates as input, so we start by enabling gates for the NStat regions we just created.

Display the Edit Gate dialog by clicking the Gate button on the toolbar.

Double-click the G1 entry in the list to turn the gate on.

In the same manner, double-click on G2 through G11.

Click OK to close the Edit Gate dialog.
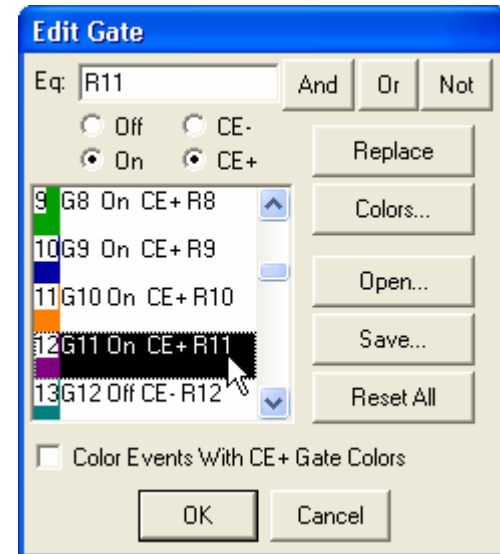
Isolating Generation Populations

Choose Edit Data Source from the Edit menu.

Click Add Parameters.
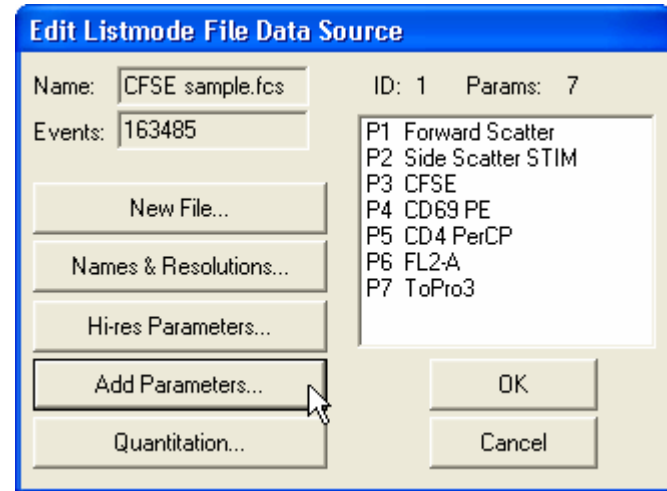
Now for the fun part. Type this expression into the Equation edit box:
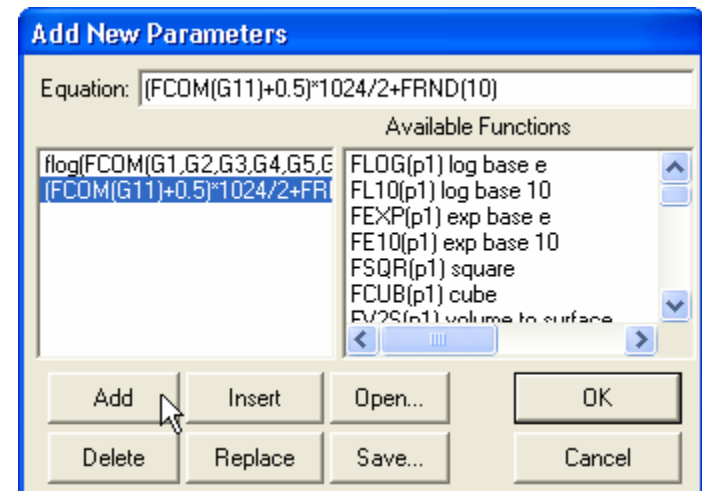flog(FCOM(G1,G2,G3,G4,G5,G6,G7,G8,G9,G10)+0.5)*1024/flog(1024)+FRND(10)

Click the Add button.
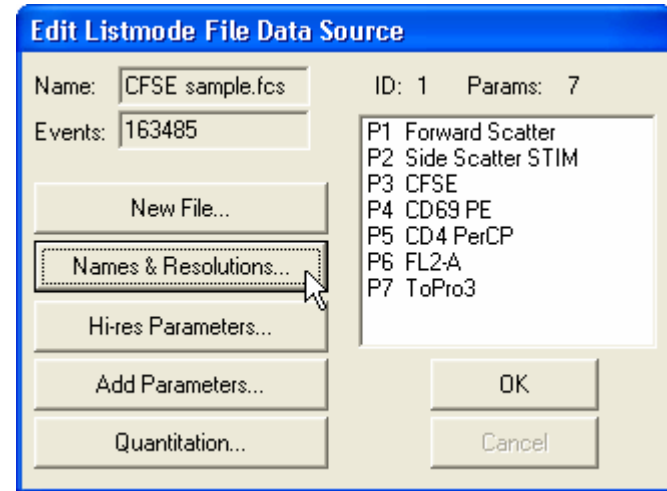
Type this expression:
(FCOM(G11)+0.5)*1024/2+FRND(10)

Click the Add Button.

Click OK.

Isolating Generation Populations
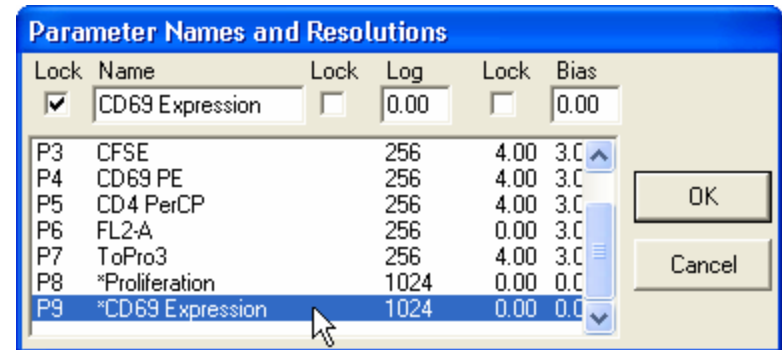
Let's create more explanatory names for the two equations we just created.

Click Names and Resolutions.

Click on P8 in the listbox.

In the Name edit box, type Proliferation.

Click on P9 in the listbox.

In the Name edit box, type CD69 Expression.

Click OK.

Click OK to close the Edit Data Source dialog.

CD69 as a function of Proliferation

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

From the Create menu, choose Create Histogram.

In the first listbox, choose Proliferation.

In the second listbox, choose CD69 PE.

Click the Graphics button.

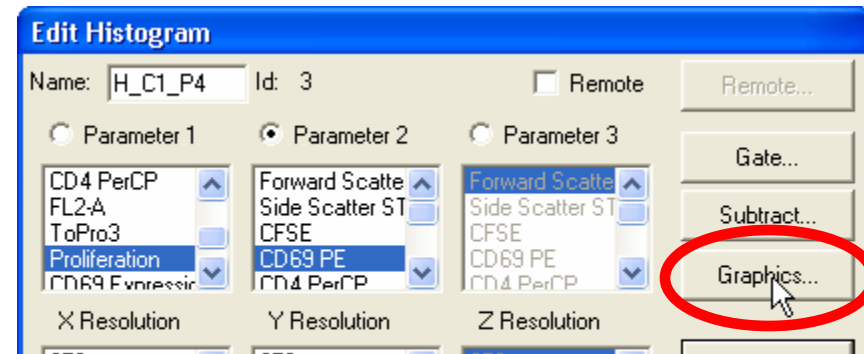For this display, dots will work better than contours.

Enable Show all dots and Color density.

Disable Contours.

Click OK.

Click OK to close the Edit Histogram dialog.

A new histogram is displayed.
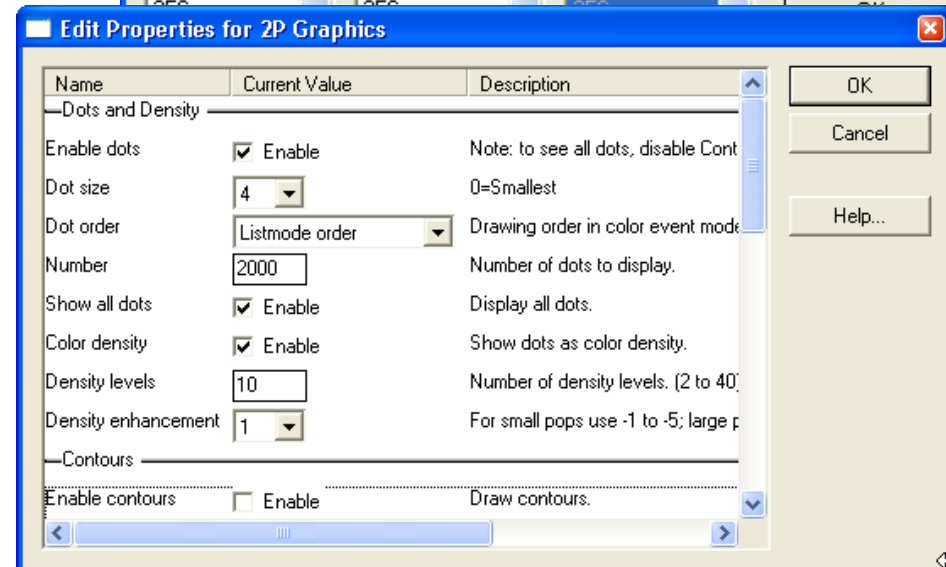
CD69 as a function of Proliferation

Let's create another histogram to isolate the CD69 Positives and Negatives.

From the Create menu, choose Create Histogram.

In the first listbox, choose Proliferation.

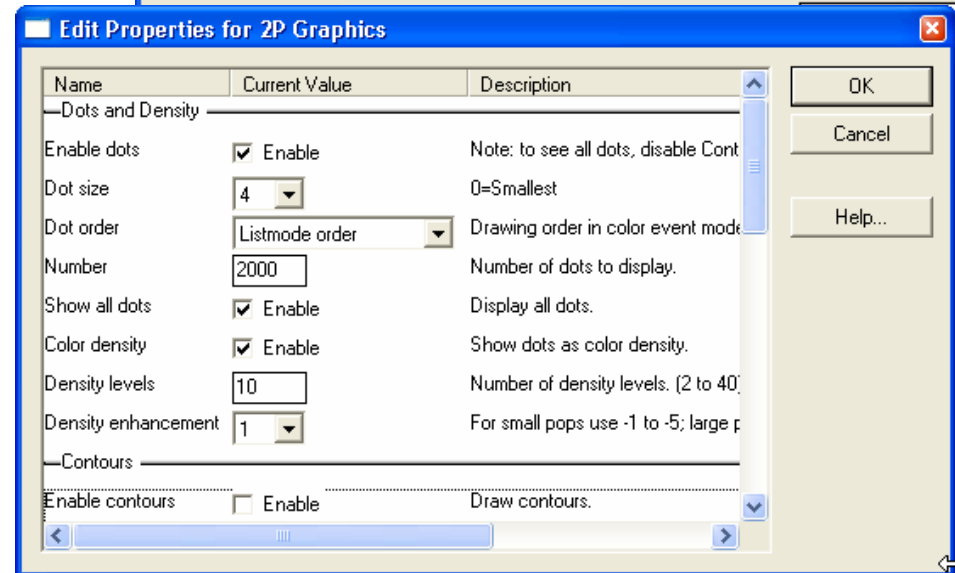In the second listbox, choose CD69 Expression.
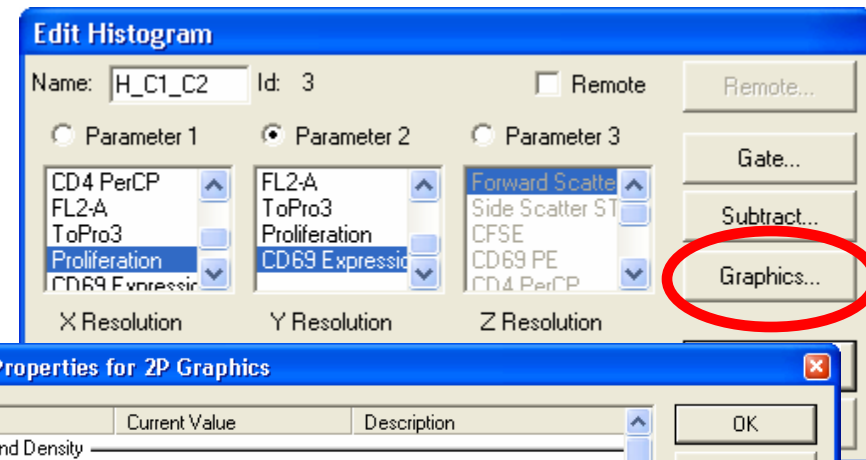
Click the Graphics button.

Choose the options you want for this display.

Click OK.

Click OK to close the Edit Histogram dialog.

Another new histogram is displayed.

From the Window menu, choose Arrange All, then choose Tile. This will arrange the histograms so that all 4 can be seen.

CD69 as a function of Proliferation

Here, we can see the generations separated into discrete populations along the X-axis. The Y-axis shows the continuous CD69 parameter in the lower-left histogram and discrete subsets of CD69 positives and negatives in the lower-right histogram. Regions can be created to quantify the subsets.
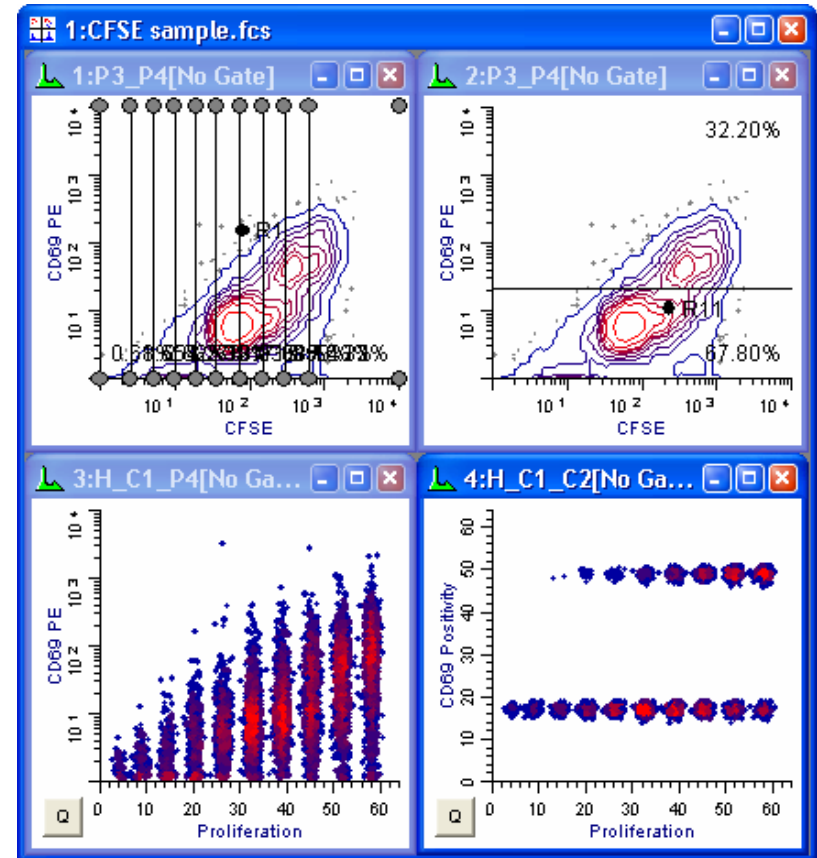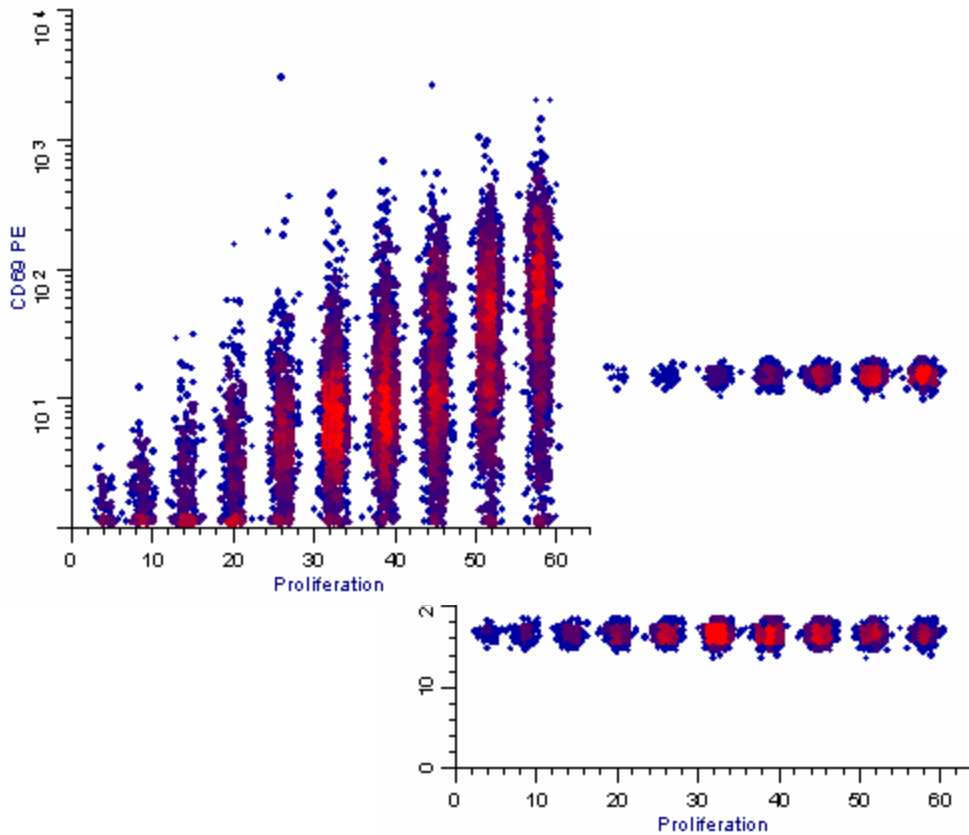
Other Parameters as a function of Proliferation

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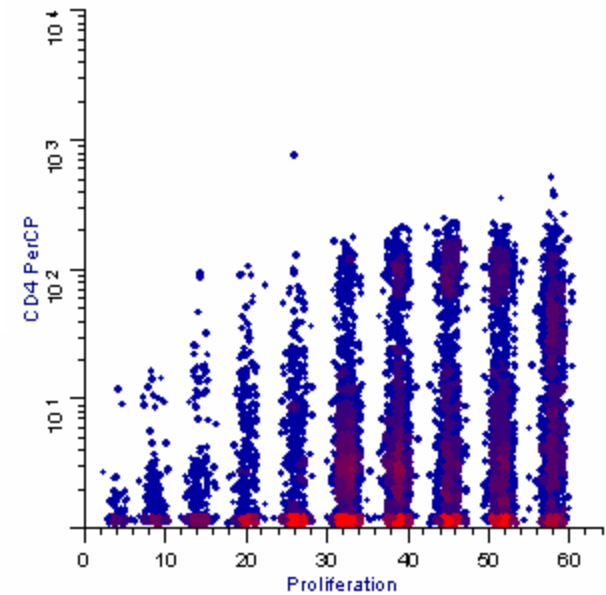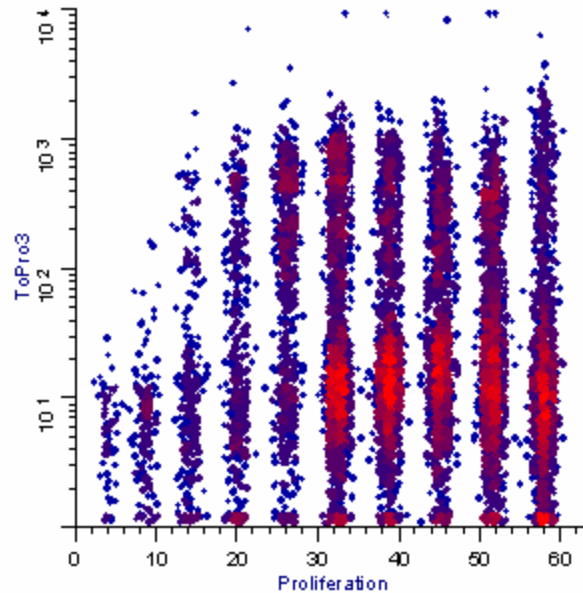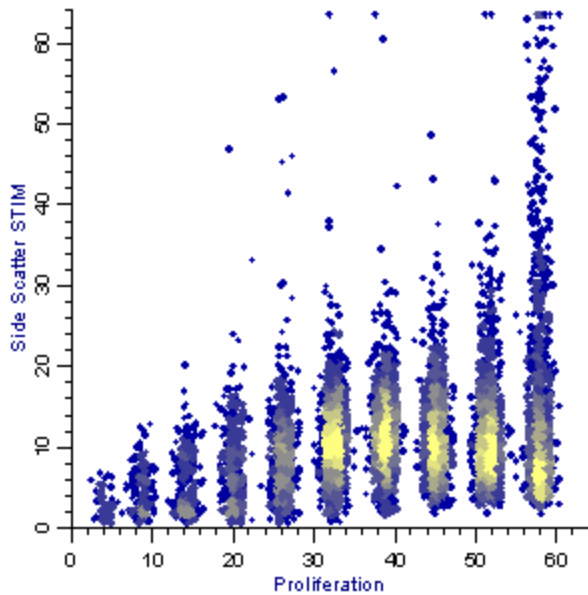
Making Life Easier

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

When you analyze new data files with ModFit LT's Proliferation Wizard, you can export new partition files. Then, open the same data file into your WinList protocol and re-import the partitions into the NStat region. All of the calculated parameters will update based on the new data and partitions.