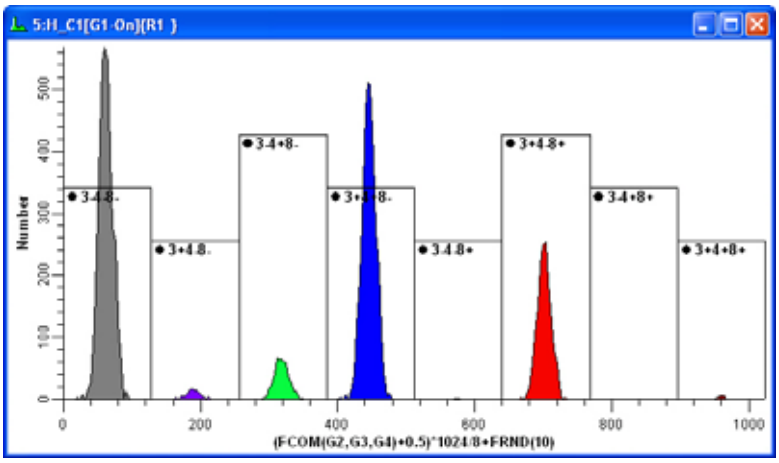
Using FCOM for Subset Analysis



What is FCOM?

FCOM is a calculated parameter that can be used to classify events based on combinations of selected gates. It is commonly used in phenotypic analysis.

How it Works

Since an event is either inside or outside a gate, its state can be represented by a single digit: 0=outside and 1=inside. The FCOM function assigns each event an integer number reflecting the gate combination for that event.

The histogram above shows the FCOM histogram from our experiment. This FCOM is the result of three gates, with the returned values of 0, 1, 2, 3, 4, 5, 6, or 7 representing each possible combination. The complete equation used for the FCOM seen above is (FCOM(G2,G3,G4)+0.5)*1024/8+FRND(10).

The equation multiplies the FCOM number by a scaling value so we will see a number of spikes with the FCOM parameter on the X-axis. The height of each spike tells us how many events satisfy that particular gate state.

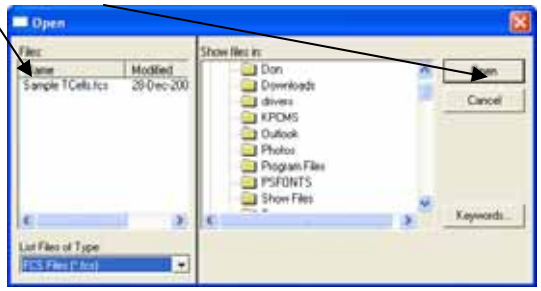
We add a small random number to each FCOM number (e.g. FRND(10)), so that instead of spikes we see Gaussian distributions which are more familiar to us.

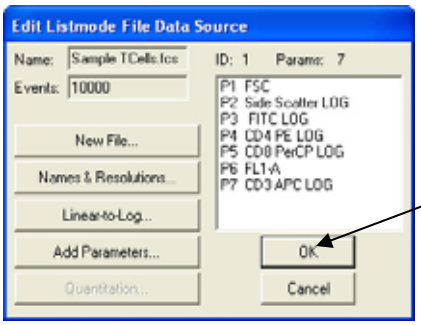
Example: Evaluate the percent of CD4 and CD8 cells as a percent of live T-cells.

In the following tutorial we will create three gates to define our populations, create an FCOM histogram and results based on these gates, and then gate the FCOM histogram on another population to determine the percent of CD4-CD8 relative to T-Cells.

1 Load data file, pass through Edit Data Source dialog and select histograms: FS vs SS, SS vs CD3, CD4 vs CD8.

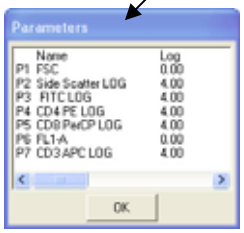
Select file, click Open button.





Click OK button.

Full list of parameters with descriptive names is automatically



To select the histograms, do one of the following:

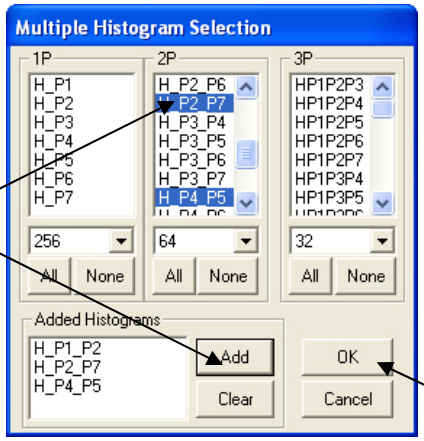
Select each parameter combination and click the Add button.

OR

Multiple select parameter combinations and click Add.

OR

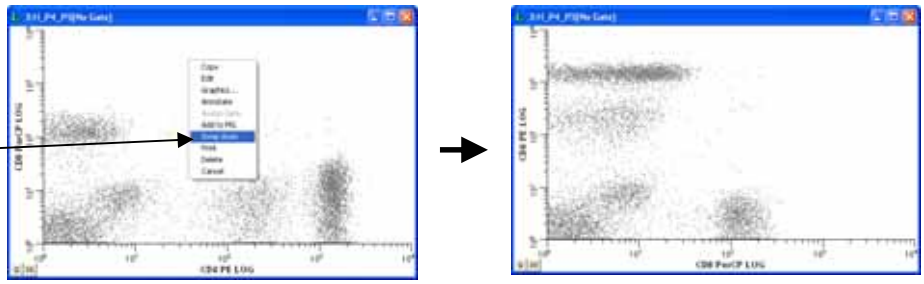
Double click on parameter combination.



Click OK button.

2 Orient histogram parameters to your preference.

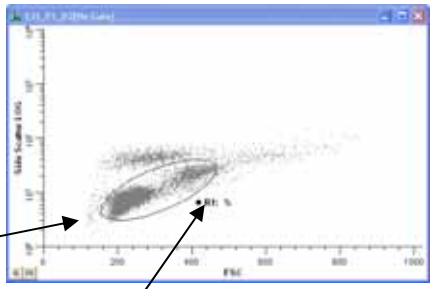
Click on the histogram with the right mouse button. Select Swap Axes.



The histogram will now show the axes in the opposite orientation.

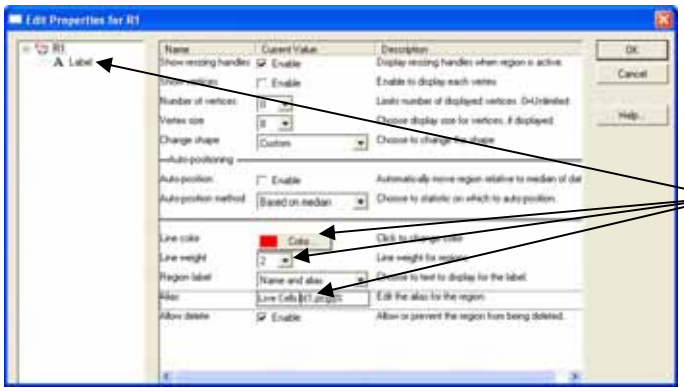
3 Create light-scatter and T-cell gates.

Click the Region Create button to enable region



Use the Ellipse tool to create the first region in the FSC vs SS histogram.

Double click on the region label, to edit properties

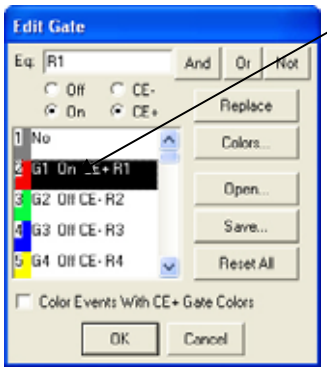


Change the region label color to RED, region line color to RED, set line weight to two, and add descriptive label to alias “Live cells”.

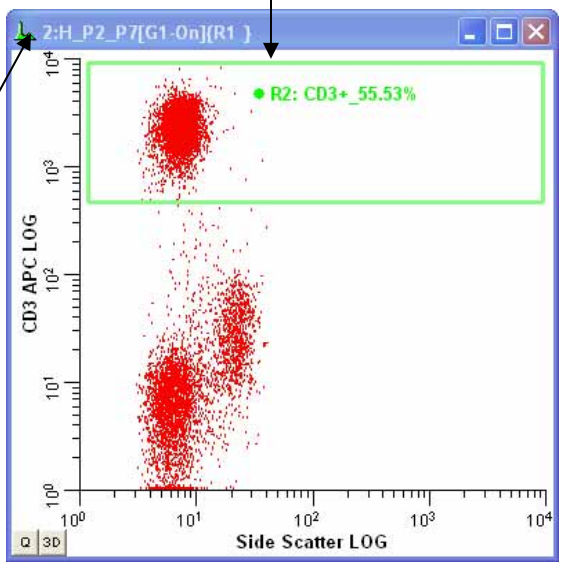
Create region 2 in SS vs CD3 histogram, then edit properties to change label and line color to green, weight 2 and edit alias to include “CD3+”.

Select the Edit Gate button.

Double click on G1 to activate Gate 1.

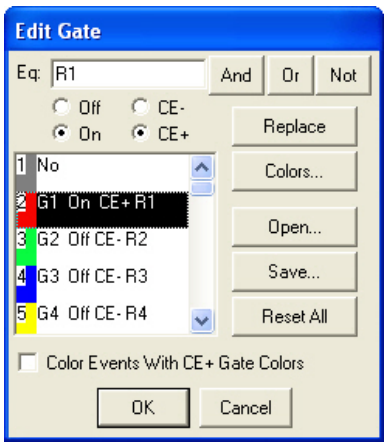


Double click on the title bar of the SS vs CD3 histogram to apply Gate 1 to histogram.

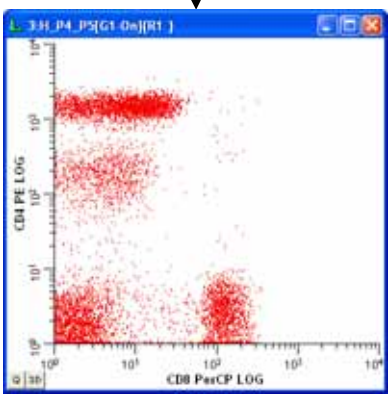


3 Create CD4 and CD8 gates.

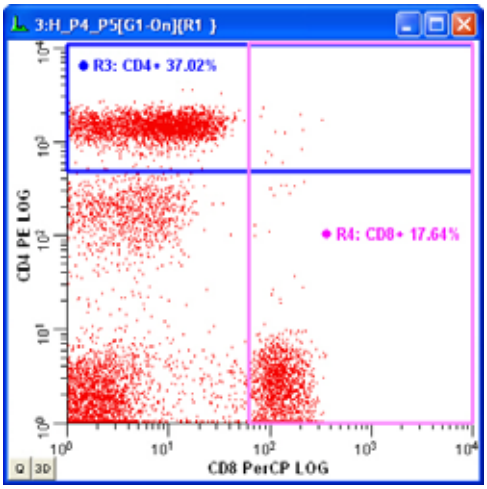
Click on G1.



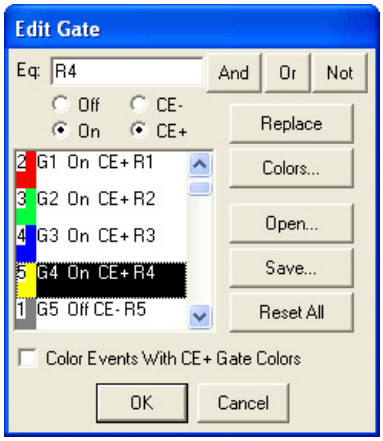
With G1 selected, apply gate 1 to the CD8 vs CD4 histogram by double clicking on the histogram title bar.



Create region 3 (R3) around the CD4 positive events, then edit properties to change label and line color to blue, weight 2 and edit alias to include "CD4+".



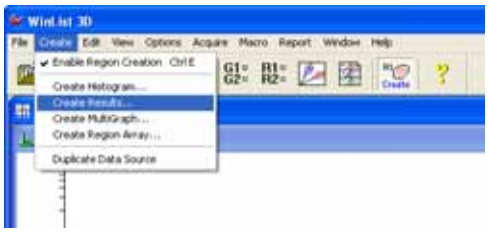
Create region 4 (R4) around the CD8 positive events, then edit properties to change label and line color to fuchsia, weight 2 and edit alias to include "CD8+".



Turn on Gate 2, Gate 3 and Gate 4 (associated with region 2, region 3 and region 4).

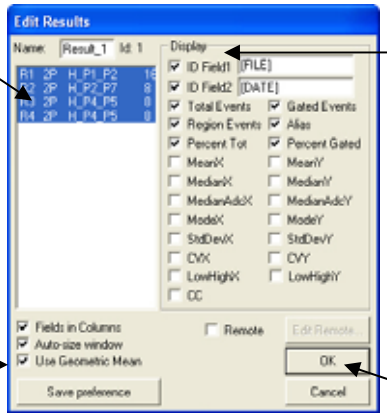
4 Display analysis results.

From the Create menu select Create Results.



Select the regions you want results for.

Other options are left in the default condition.



Select the results you want to display.

Click OK.

The results are displayed.

1:Result_1

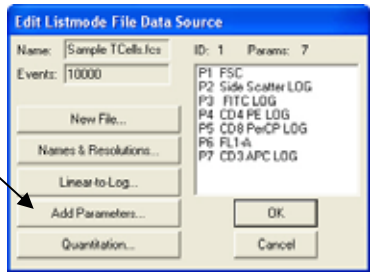
Id1: Sample TCells.fcs
Id2: 28Dec2004

	Alias	Total	Gated	Region	Percent	%Gate
R1	Live Cells 87.25%	10000	10000	8725	87.25	87.25
R2	CD3 + 54.68%	10000	8725	4771	47.71	54.68
R3	CD4+ 66.04%	10000	4771	3151	31.51	66.04
R4	CD8+ 31.92%	10000	4771	1523	15.23	31.92

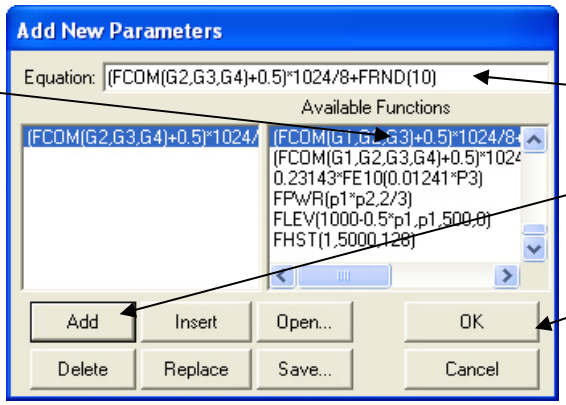
5 Create the FCOM parameter.

From the Edit menu select Edit Data Source.

Click "Add Parameters…" button.



Find the FCOM example that best matches the number of gates we want to use. In our case this will be a 3-gate FCOM.



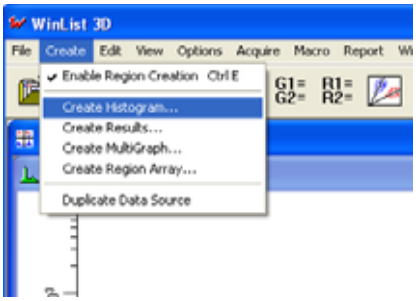
Edit the equation so the proper gates are used (G2, G3, G4).

Click Add button to add equation to parameter list.

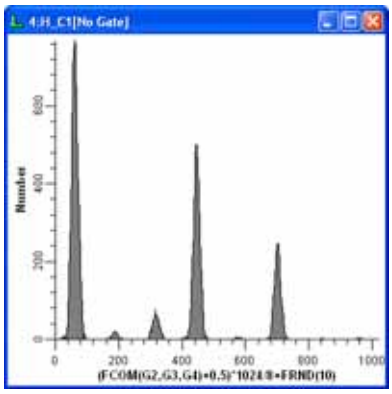
Click OK.

6 Display the FCOM histogram.

From the Create menu select Create Histogram.

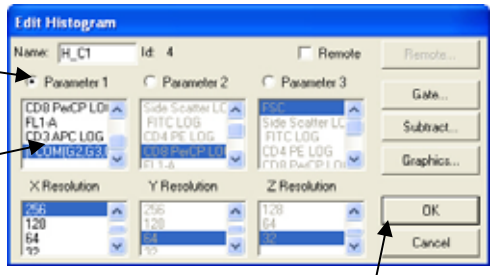


The new FCOM histogram will be displayed, containing eight populations.



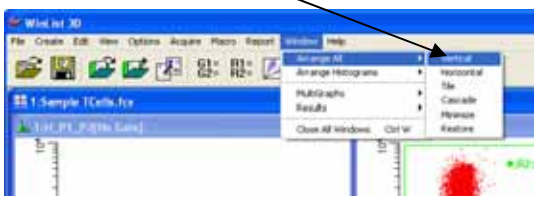
Select for a single parameter display

Select the FCOM parameter.



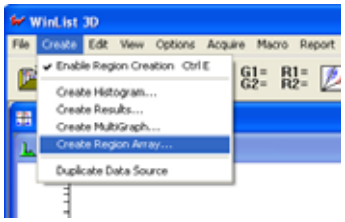
Click OK.

From the Window menu select Arrange All, Vertical.



7 Create a Region Array for the FCOM histogram

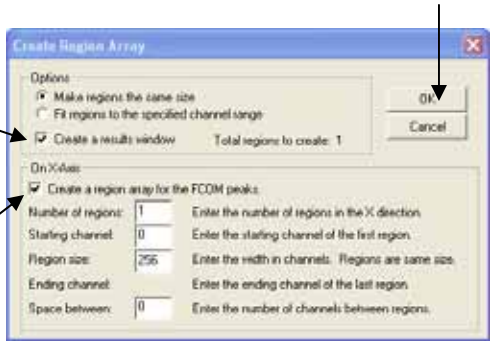
From the Create menu, select Create Region



Select "Create a results window" if not already selected.

Select "Create a region array for the FCOM peaks".

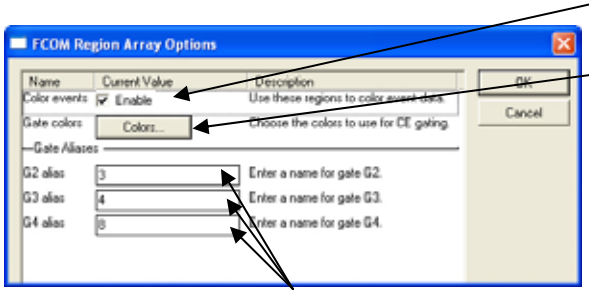
Click OK

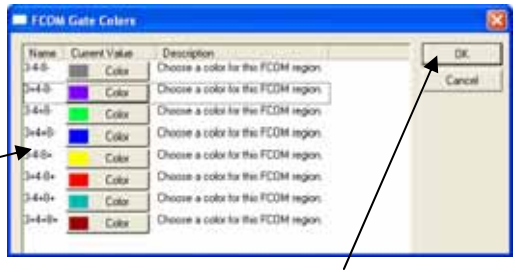


Check that the Color events option is enabled.

Click on the Color button.

Select your choice of colors for the



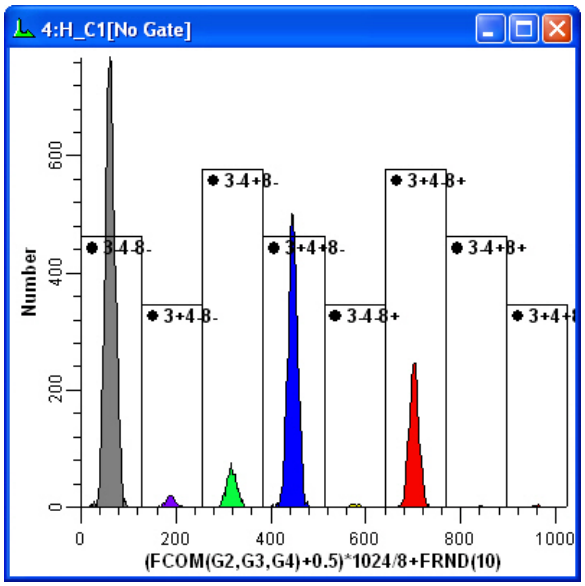


Edit the alias lines to give a better description of the population. Click OK to close dialog.

Click OK to close.

7 Create Region Array (cont)

You should now have a FCOM histogram with regions automatically placed about each population with each region displaying the proper alias identifier.



2:R5_H_C1

Id1: Sample TCells.fcs
Id2: 9Jan2005

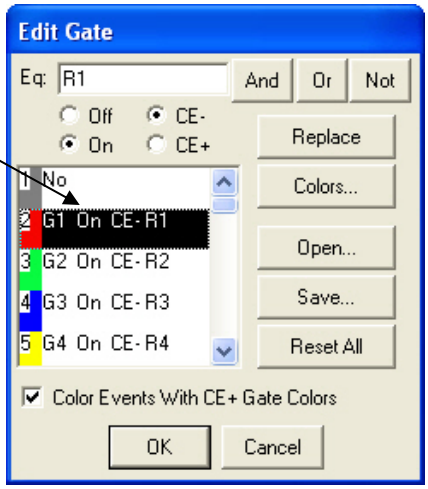
	Alias	Total	Gated	Region	Percent	%Gate
R5	3-4-8-	10000	10000	4990	49.90	49.90
R6	3+4-8-	10000	10000	158	1.58	1.58
R7	3-4+8-	10000	10000	66	0.66	0.66
R8	3+4+8-	10000	10000	3230	32.30	32.30
R9	3-4-8+	10000	10000	3	0.03	0.03
R10	3+4-8+	10000	10000	1537	15.37	15.37
R11	3-4+8+	10000	10000	0	0.00	0.00
R12	3+4+8+	10000	10000	16	0.16	0.16

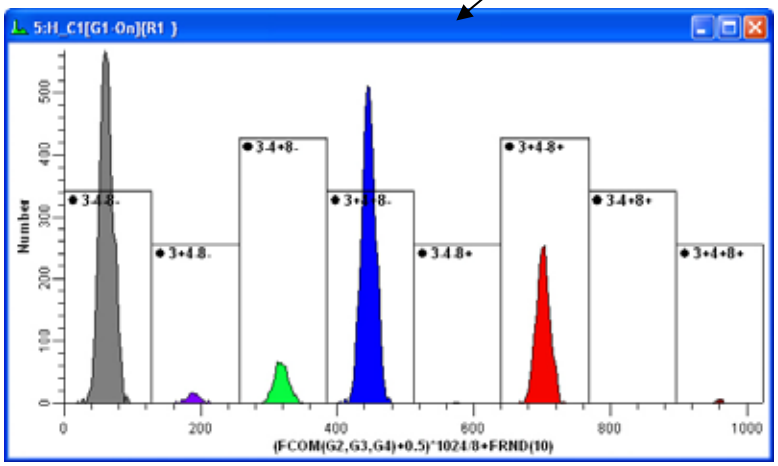
A new results window should have automatically been created and displaying the results for each population. (select different statistics if desired)

8 Gate the FCOM histogram on "Live" cells.

Click on Gate button.

Select Gate 1 (G1-Live Cells) then double click on the FCOM histogram title bar to apply the gate.

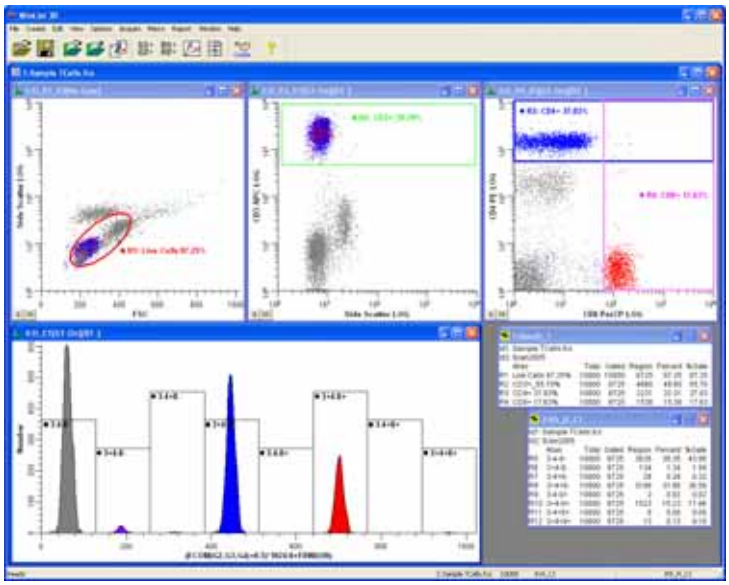




Note that the results automatically update.

9 Adjust analysis display and save protocol.

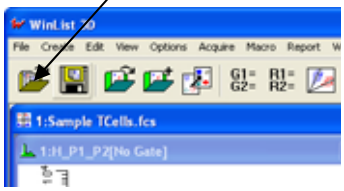
Adjust the FCOM histogram size and position to make the populations easy to see. Move the results window.



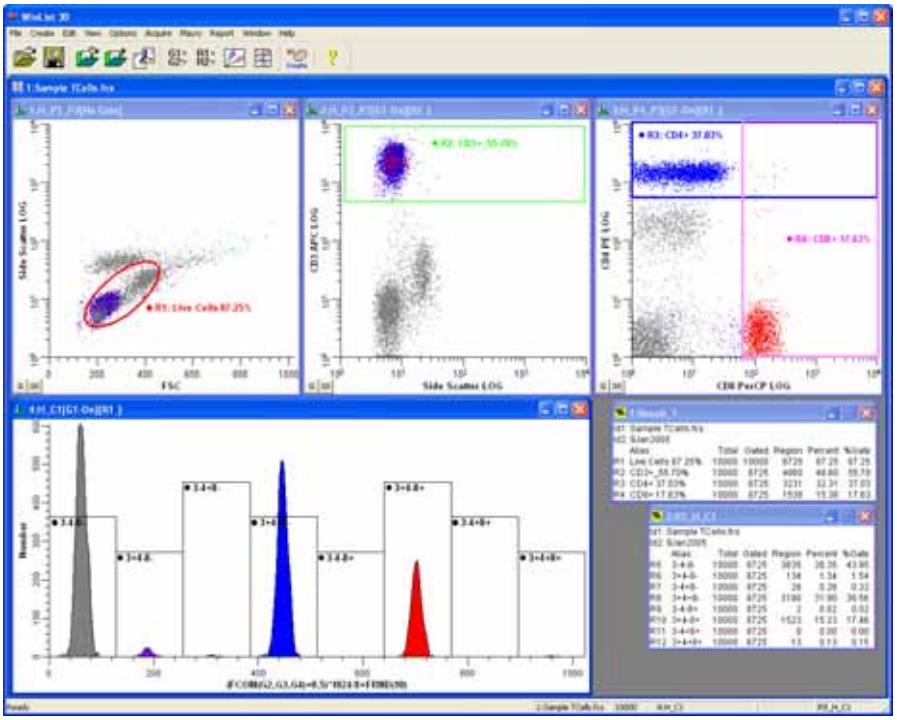
Click the Save Protocol button. Save the protocol file to a location of your choice.

10 To use the protocol, click the Open Protocol button.

Click the Open Protocol button. Navigate to where you saved the protocol. Click Open.



Your fully formatted and gated analysis window should be reconstructed for you and be ready to open new data files.



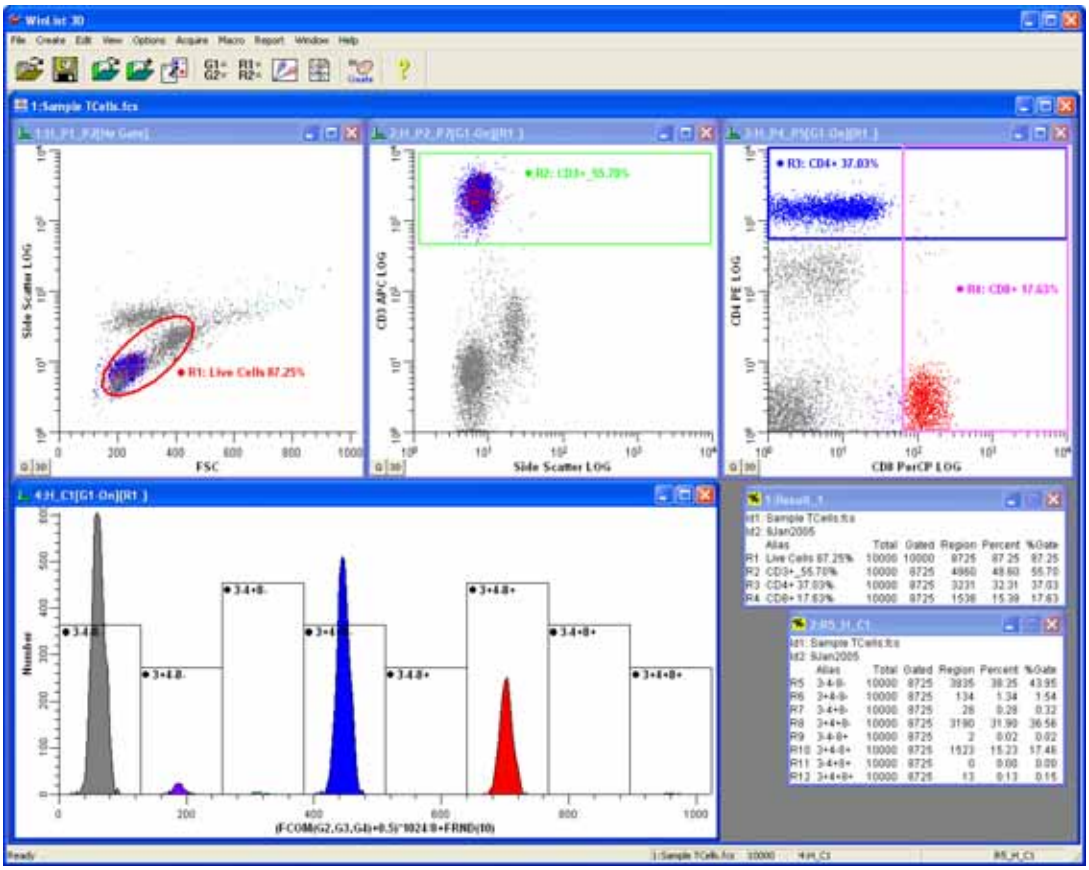
Summary:

This tutorial's primary goal was to show you how to use FCOM to obtain the percent of CD4+ T-cells and CD8+ T-cells within "Live" cells of a sample.

The process started with loading the file and displaying the native parameters. We created regions and gates to identify the particular populations we wanted to evaluate (Live cells, CD3+ cells, CD4+ cells and CD8+ cells). We then displayed some analysis values for these individual populations.

The last section of the tutorial described how to create a FCOM parameter, and to use the WinList Region Array option to identify and obtain results for each population. The last step in the FCOM section was to apply a gate (Live cell gate) to the FCOM so all FCOM peaks and results would be based only on the "Live" cells.

After some minor adjustments to the location and size of the histograms and results, we used the Save Protocol option to create an easy way to reproduce this analysis for future use.



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