CELLQuest™ Analysis Tutorial

Introduction

This tutorial guides you through a CELLQuest Analysis run like the one demonstrated in the CELLQuest Analysis Movie on the FACStation™ Overview CD-ROM.

This tutorial covers:

Analyzing data using dot plots, contour plots and histograms
Creating regions and markers
Setting quadrants
Displaying and editing statistics

Before following this tutorial you will need:

1. CELLQuest Version 3.1 or higher

NOTE: Steps appearing in boldface are key instructions. Additional information is provided in plain text.

A. Launching CELLQuest

1. Choose CELLQuest from the Apple Menu.

B. Creating an Analysis Experiment Document

In CELLQuest, any Experiment document you set up with plots, regions, gates, statistics, markers, text and colors can be saved. The data files are not saved in the Experiment document. CELLQuest saves a reference to the data files that contains the information needed to recreate the plots and stats on the Experiment document. CELLQuest automatically creates a new Experiment document named Untitled-1 each time you launch the program by clicking the CELLQuest application icon.

1. Expand the Experiment window to full size by clicking the zoom box in the upper-right corner of the CELLQuest window.

C. Creating an Analysis Dot Plot

1. Select the Dot-Plot tool in the tool palette.

The Dot-Plot tool becomes highlighted.

2. Click in a clear area of the Experiment window and drag diagonally until the plot outline is the desired size.

The Dot Plot dialog box appears.

3. Click the Select File button.

The Open a Data File dialog box appears.

4. Click in the folder pop-up menu and choose Macintosh Hard drive. Click open

5. From the Hardrive selections, open the BD Applications folder; then open the CELLQuest Folder, and then open the Sample Files folder.

6. Click the isotype control data file, NORM001. Click Open

7. Change the color of the dots displayed by clicking on the white rectangle in the Color field. Choose a different color from the Select dot color dialog box that appears.

8. Click OK in the Dot Plot dialog box.

An FSC vs SSC dot plot of the subclass control appears. The X and Y parameters of the dot plot default to the first and second parameters.

9. Click the frame of the plot and move it away from the tool palette.

You have just selected the plot and moved it. When a plot or any view is selected, black handles appear on each corner. Selected views are affected by any command. Only selected views can be resized, deleted, and moved.

D. Defining a Lymphocyte Gate

Data analysis of a subpopulation is done by drawing a gate around the cells of interest. In this case, we wish to analyze lymphocytes only. The FSC vs SSC isotype control dot plot contains lymphocytes, monocytes, and granulocytes; therefore, in order to perform lymphocyte analysis, we must first draw a gate around the lymphocytes.

1. Click the Polygon-Region tool in the tool palette to select it.

The tool becomes highlighted.

2. Position the cursor on the dot plot and click around the lymphoctyes. Double-click to close the region.

If you make a mistake and want to draw another region, press the delete key. This will delete the region image from the plot. To completely delete a region , choose Region List from the Gates menu. Click the region on the list to select it, then press the delete key.
For information on editing, moving, or deleting regions,refer to your CELLQuest software manual.

E. Creating an FL1 vs FL2 Contour Plot

We will now view the gated subclass control data on a fluorescence plot.

1. Choose Contour Plot from the Plots menu.

The Contour Plot dialog box appears.

2. Click the Select File button.

The Open a Data File dialog box appears.

3. Click Open to select the isotype data file, NORM001.

The default folder should be the Sample Files folder. If it isn't, follow the same pathway as described in step 4 of section C.

4. Choose FL1 from the X parameter pop-up menu and FL2 from the Y parameter pop-up menu.

5. Choose G1=R1 from the Gate pop-up menu.

6. Click the Log Density radio button in the Scale field.

7. Change the Contour color by clicking one time on the white rectangle in the Colors field. Choose a different color.

8. Click OK to display the FL1 vs FL2 contour plot.

Notice that by choosing the Contour plot from the Plots menu, the plot size is the same as the original dot plot drawn from the tool palette.

9. Click the frame of the plot and drag it to a clear area of the Experiment window.

F. Defining Quadrant Markers and Displaying Statistics

Quadrant markers will be set around the gated data in the FL1 vs FL2 contour plot. The subclass control acts as a negative control in immunophenotyping. Quadrant markers are set using this sample to designate the areas of negativity and positivity on the plot.

1. Select the Quadrant Marker tool in the tool palette .

The tool becomes highlighted.

2. Click the FL1 vs FL2 plot and drag the handle of the markers so that the isotype control is in the lower-left quadrant.

Release the mouse to set the quadrants. To change the position of the markers, click the handle at their intersection and drag the markers to a new location. You can also delete the marker when it is selected. Click the plot outside the markers to deselect them and the handle disappears. Note that when you click on the contour plot, the frame turns gray, which means that the plot is active. To activate a plot or any view, click anywhere in the view except on the frame. Only one view can be active at a time and any command affects that view only.

3. Choose Quadrant Stats from the Stats menu.

The Quadrant Statistics view appears with descriptive and statistical information.

4. Click the Quadrant statistics view and choose Edit Quadrant Stats from the Stats menu.

Deselect all choices except the File Name, Percent of Gated, and Percent of Total. Also choose 1 column in the Header Columns field.

5. Click OK in the Edit Quadrant Stats view.

6. Click the border of the stats view and decrease the size.

Editing the stats view and decreasing its size allow for more room on the Experiment document.

G. Creating an Analysis Histogram Plot

1. Select the Histogram tool from the tool palette.

The Histogram tool becomes highlighted.

2. Click in a clear area of the Experiment window and drag diagonally until the plot outline is the desired size.

The Histogram dialog box appears.

3. Click the Select File button.

The Open a Data File dialog box appears.

4. Click Open to select the isotype data file, NORM001.

The default folder should be the Sample Files folder. If it isn't, follow the same pathway as described in step 4 Section C of this tutorial.

5. Choose FL1 from the Parameter pop-up menu.

6. Choose G1=R1 from the Gate pop-up menu.

7. Change the Line and Fill colors by clicking once in the rectangles in the Colors field.

Choose different Line and Fill colors.

8. Click OK to display the FL1 Histogram plot.

H. Defining Histogram Markers and Displaying Statistics

CELLQuest calculates statistics for the entire histogram or for sections of the histogram. The sections are defined by setting histogram markers. A marker consists of an upper and lower boundary connected by a horizontal line. A marker generates statistics for the events in a designated section of the histogram.

1. Select the Histogram Marker tool from the tool palette.

The tool becomes highlighted.

2. Click to position the left edge of the marker and drag to position the right edge of the marker. Draw the marker around the negative control population. Release the mouse button to complete the marker.

As the cursor passes over the plot, its shape changes to a crosshair to indicate that you can define histogram markers. The ribbon under the title bar displays the channel number or linear value of the x-axis (X) and the count (Y).

The marker appears with a handle at each endpoint.

3. Follow steps 1 through 3 and draw another marker from the upper boundary of marker 1 to the end of the histogram plot.

Any population inside marker 2 is FL1 positive.

4. Click the histogram plot.

5. Choose Histogram Stats from the Stats menu.

6. Click the border of the Histogram statistics view and position the view underneath the histogram plot.

The Histogram statistics view appears. Notice that the stat view editing and size is retained from the last stats view edited (in this case the Contour Plot Stats view).

7. Print one copy of the document by selecting Print One from the File menu.

The Printer dialog box is bypassed and one copy of the Experiment document is printed.

I. Saving an Experiment Document

You can save an Experiment document you have set up with plots, gates, markers, and statistics. The document can be opened later to resume your analysis or acquisition, or it can be used as a template for analyzing or acquiring future samples.

1. Choose Save from the File menu.

The Save dialog box appears.

2 . Type a name for your Experiment document

3. Click the folder pop-up menu and select Desktop.

4. Click the New Folder button and create a folder in which to save the Experiment document.

Type a folder name, click the Create button.

5. Click Save.

J. Batch Analyzing the Remaining Files in the Panel

Once you have set up the Experiment document with plots, gates, markers and statistics, CELLQuest can batch analyze subsequent files. The Batch analysis feature is based on the Next File command. For selected plots in the active Experiment document, the batch analysis will call the Next File command repeatedly until the plots can no longer advance to the next file in the folder. In addition to advancing the data files for the plots, batch analysis can provide automatic pausing after each execution. You can adjust markers or regions while the batch is paused. Batch analysis can also provide automatic printing of the document after each execution of the Next File command and automatically appends statsistics to an export file. For more information regarding batch analysis, refer to the latest version of your CELLQuest software manual.

1. Choose Setup from the Batch menu.

The Batch Setup dialog box appears.

2. Click the "All" radio button in the "Plots and Stats to Process" field.

All plots in the document will be selected each time the Next File command is executed.

3. Click the for [n] seconds radio button in the "Pause after each file increment" field and change the pause time to 3 seconds.

The batch will pause for 3 seconds after each execution of the Next File command.

4. Click in the "Print after each file increment "checkbox.

The Print One command is executed after each Next File command. The print will occur after the 3 second pause.

5. Set the File Increment to 1, and then click OK.

6. Choose Run from the Batch Menu.

Batch processing automatically starts and the Batch control floater appears. The floater has buttons in it that allow you to manually control the batch analysis. Refer to the CELLQuest software manual for more information about the Batch control floater.

Batch analysis of the files in the Samples Folder occurs. The batch finishes when the last plot has been analyzed. The batch floater closes automatically when the batch finishes and a dialog box appears with the message, *The batch has completed. All the plots are at the end of their folders*.

7. Click the OK button in the dialog box.

Batch Analysis is complete.

11-10900-00