CELLQuest™ Acquisition Tutorial

Introduction

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

This tutorial covers:

Setting up an Acquisition Experiment document.
Optimizing samples using CELLQuest Acquisition features.
Setting up for sample acquisition.
Acquiring data.

Before following this tutorial you will need:

1. CELLQuest Version 3.1 or greater
2. To view the CELLQuest Analysis Movie and complete the CELLQuest Analysis tutorial.
3. The Analysis document that you created in the CELLQuest Analysis tutorial.
4. A FACScan™, FACSort™, FACSCalibur™, or FACS Vantage™ flow cytometer.
5. A panel of lysed whole blood tubes stained with the following SimulTEST™ reagents: LeucoGATE™, IgG1/IgG2 isotype control, CD3/CD19, CD3/CD4, CD3/CD8, CD3/CD16+CD56

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

A. Launching CELLQuest

1. Launch CELLQuest by double-clicking the CELLQuest analysis Experiment document that you created in the analysis tutorial.

CELLQuest is launched and the analysis Experiment document appears with the data, plots, regions, and statistic views that were last saved.

B. Creating An Acquisition Experiment Document

Although you're going to acquire data, there is no difference between documents created for acquisition or for analysis. You can have an acquisition document, an analysis document, or a combination of both plot types within one document.

1. Click the FSC vs SSC dot plot.

2. Choose Format Dot Plot from the Plots menu.

The Format Dot Plot Dialog box appears.

3. Choose Acquisition from the Plot Source pop-up menu.

4. Click OK.

An FSC vs SSC acquisition plot appears.

5. Choose Save As from the File menu.

The Save As dialog box appears. Save As allows you to save the Experiment document as an Acquisition document while the Analysis document remains intact.

6. Name your document *Acquisition 1* and navigate to your folder on the desktop; then click Save.

Your acquisition document is saved in the same folder as your Analysis document.

7. Click the frame of the FL1 vs FL2 contour plot.

8. Delete the contour plot by choosing Clear from the Edit menu or press the delete key.

Notice that the statistics view was deleted also.

9. Delete the histogram plot by repeating steps 7 and 8.

C. Optimizing Samples Using CELLQuest Acquisition Features

Before data is collected, the electronics are optimized for your samples. The settings can be optimized with cytometer controls accessed from CELLQuest software. The optimization sequence for lymphocytes in lysed whole blood is as follows: Adjust FSC and SSC detectors and FSC threshold, gate on the lymphocyte population; adjust FL1 and FL2 detectors; and adjust fluorescence compensation.

1. Choose Connect to Cytometer from the Acquire menu.

The Acquisition Control window appears. Click and drag the window to a clear area of the screen.

2. Choose Detectors/Amps from the Cytometer menu.

The Detectors/Amps window appears. Click and drag the window to a clear area of the screen.

3. Choose Threshold from the Cytometer menu.

The Threshold window appears. Click and drag the window to a clear area of the screen.

D. Adjusting the FSC and SSC Detectors and FSC Threshold Setting

The FSC Amp Gain, SSC voltage, and FSC threshold will be adjusted to appropriately display the scatter properties of the lysed whole blood sample. The FSC Amp Gain and SSC voltage are used to place the population of interest on scale. The FSC Threshold is adjusted to exclude any unwanted debris and noise. Refer to the the optimized FSC vs SSC acquisition plot in the CELLQuest Acquisition Movie for reference.

1. Place the flow cytometer in RUN mode. Place the isotype control tube on the Sample Injection Port (SIP).

2. Click Acquire in the Acquisition Control window.

Note that the Setup check box in the Acquisition Control window is checked. This allows you to click Acquire and view real-time acquisition display without saving a data file.

3. Choose Lin from the Mode pop-up menu for FSC.
Verify the FSC voltage is E00 in the Detectors/Amps window.

4. Adjust the FSC Amp Gain. Click the up or down arrow of the FSC Amp Gain.

5. Choose Lin from the Mode pop-up menu for SSC. Adjust the SSC voltage in the Detectors/Amps window by clicking the the icon between the up or down arrows to display a slider.

6. Move the slider to the appropriate voltage and release the mouse.

7. Adjust the FSC threshold in the Threshold window. Set the threshold to remove most of the debris without cutting off the lymphocytes.

8. Click the up or down arrow of FSC threshold while pressing the Option key. The number is incremented or decremented by ten.

9. If necessary, double-click the polygon outline (the vertices become handles), and click and drag the region so that it is surrounds the lymphocyte population.

E. Adjusting the FL1 and FL2 Detectors

The FL1 and FL2 Detectors may need adjustment on the gated lymphocytes of the isotype control. These cells will be placed in the lower left-hand corner of the FL1 vs FL2 plot. Refer to the optimized FL1 vs FL2 acquisition plot in the CELLQuest Acquisition Movie for reference.

1. Choose Dot Plot from the Plots menu.

The Dot Plot dialog box appears.

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

3. Choose Gate G1=R1 form the gate pop-up menu.

4. Click OK.

The FL1 vs FL2 dot plot appears.

5. Click the frame of the FL1 vs FL2 plot and drag it to the right of the FSC vs SSC plot.

6. If necessary, adjust the FL1 and FL2 voltages in the Detector Amps window.

Verify that the FL1 and FL2 Modes are set to LOG.

7. Click the up or down arrow of the FL1 or FL2 voltage while pressing the Option key.

The voltage number is incremented or decremented by 10.

8. Click the Quadrant Marker tool in the tool palette to select it.

The tool becomes highlighted. Quadrant markers are set around the gated isotype control data on the FL1 vs FL2 dot plot. These markers will designate areas of negativity and positivity.

9. Click in the FL1 vs FL2 plot and drag the handle of the markers so that the cross-hairs are at 10 and 10 on the X and Y scales respectively. Release the mouse to set the quadrants.

10. Choose Quadrant Stats from the Stats menu. Reposition the statistics view to you can see it on the page.

11. Click Pause in the Acquisition Control window.

12. Remove the isotype control from the SIP.

13. Click the close boxes of the Detectors/Amps and Threshold windows to remove the windows from the screen.

F. Adjusting Fluorescence Compensation

The last step in the optimization process is adjusting for spectral overlap. You will adjust the FL1–%FL2 and FL2–%FL1 compensation networks using the CD3 FITC/CD19 PE sample. Refer to the compensated FL1 vs FL2 acquisition plot in the CELLQuest Acquisition Movie for reference.

1. Choose Compensation from the Cytometer menu.

The Compensation window appears.

2. Click and drag the window to a clear area of the screen.

3. Place the CD3 FITC/ CD19 PE tube on the SIP.

4. Click Restart in the Acquisition Control window.

5. If necessary, adjust FL1–%FL2 by clicking the up or down arrow to place the PE population in the upper-left quadrant. Align the X mean of this FL2 positive population with the X mean of the negative population.

6. If necessary, adjust FL2–%FL1 by clicking the up or down arrow to place the FITC population in the lower-right quadrant. Align the Y mean of this FL1 positive population with the Y mean of the negative population.

7. Click the close box of the Compensation window to remove the window from the screen.

8. Click Pause and then Abort in the Acquisition Control window.

9. Remove the CD3 FITC/CD19 PE tube from the SIP.

10. Place a tube of DI water on the SIP.

11. Place the cytometer in STANDBY mode.

G. Setting Up the Acquisition and Storage Window.

The Acquistion and Storage window is the place where you input the number of events to acquire and the number of parameters saved.

1. Click the Acquire menu and choose Acquistion and Storage.

Verify that the event count is 10,000. This is the number of events that will be saved in your data file.

2. Click the Parameters saved button and verify that all enabled parameters are checked. Click OK twice

All checked parameters will be saved in your data file.

H. Setting Up the Parameter Description Window

File Storage and parameter labels are defined in the Parameter Description window.

1. Choose Parameter Description from the Acquire menu.

The Parameter Description window appears.

2. Click the Folder button.

The Destination Folder dialog box appears.

3. Click the destination folder pop-up menu.

4. Choose Desktop.

5. Click the same folder that contains the Acquisition 1 Experiment document; then click the Select button at the bottom of the dialog box.

The dialog box closes automatically.

6. Click the File button.

The File Name Editor window appears.

7. Type a file name in the highlighted Custom Prefix field.

8. Choose File Count from the File Name Suffix pop-up menu.

9. Click OK.

10. Click the Panel checkbox in the Parameter Description window.

CELLQuest allows you to label parameters any way you choose. You can type in your own label parameters, define reagents and use them to label your samples, or combine a series of reagents into a panel. When you acquire samples using a panel, the parameter labels are automatically updated after each tube is acquired.

11. Choose the IMK-Lymphocyte panel in the Panel pop-up menu.

12. Choose Save from the File menu.

The Acquistion Document is saved.

I. Acquisition of Two-Color Data

At this point, the electronics have been optimized for your cells, and file storage has been defined. The samples can now be acquired.

1. Deselect the Setup check box in the Acquisition Control window.

2. Place the flow cytometer in Run mode.

3. Install the LeucoGATE tube on the SIP. Immediately place the support arm under the sample tube.

This is Tube #1 in your reagent panel.

4. Wait approximately 5 seconds before clicking Acquire.

During this brief period, the sample tube is being pressurized.

5. Click Acquire.

The live quadrant statistics will be displayed as the data is acquired. The system alert sounds at the completion of acquisition.

6. Remove the LeucoGATE tube after the beep.

7. Place the next tube (isotype control) on the SIP.

This is Tube #2 in your reagent panel.

8. After waiting approximately 5 seconds, click Acquire, and remove the tube after the beep.

9. Repeat steps 7 and 8 for the remaining tubes in your panel:

Tube #3 CD3 FITC / CD19 PE
Tube #4 CD3 FITC / CD4 PE
Tube #5 CD3 FITC / CD8 PE
Tube #6 CD3 FITC / CD 16+CD56 PE

10. Place a tube of DI water on the SIP.

11. Place the flow cytometer in STANDBY mode.

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