

Application Note 1

Running Simultest™ Samples Using

the FACS Loader

Introduction

SimulSET™ software offers today's clinical laboratory a powerful analytical tool for the rapid enumeration of clinically relevant lymphocyte subsets in an easy-to-use format. Used with Simultest reagents, SimulSET automates lymphocyte gating, analyzes gate purity, positions markers, performs consistency checks, and precisely analyzes lymphocyte subsets.

This application note provides a set of instructions for acquiring Simultest samples using the FACS Loader with CELLQuest™ software, and batch analyzing the data files in SimulSET software. For detailed instructions on use, also refer to the appropriate Simultest reagent package insert.

These instructions are written with the assumption that you have a basic working knowledge of FACSCComp™, CELLQuest, WorklistManager, and SimulSET software programs. They provide the necessary information to:

- create a CELLQuest document for Simultest sample acquisition,
- acquire Simultest samples using the FACS Loader with CELLQuest and WorklistManager software programs, and
- batch analyze CELLQuest data using SimulSET software.

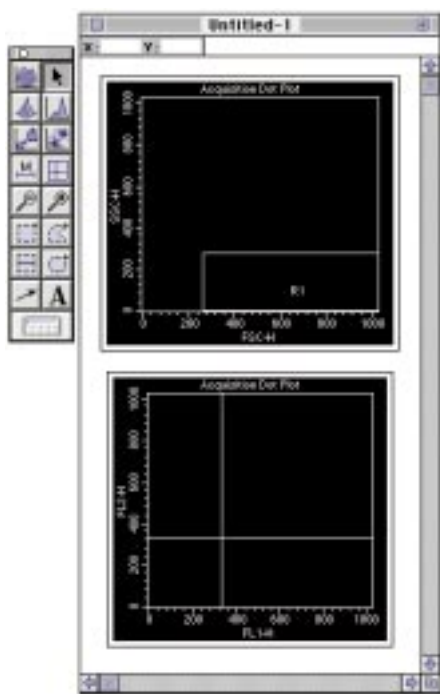


Figure 1 Dot plot set up for CELLQuest document.

Materials and Equipment

- FACSCalibur™, FACSort™, or FACScan™ flow cytometer
- FACStation™
- FACS Loader automated sample loader
- FACSComp software, version 1.0 or later
- CELLQuest software, version 1.2.1 or later
- WorklistManager software, version 1.0
- SimulSET software, version 1.0 or later

Instrument Setup

Refer to the *FACSComp Software Reference Manual* for information on running FACSComp software. For information on preparing CaliBRITE™ beads, refer to the *CaliBRITE Bead* package insert.

Set up the cytometer with FACSComp software using CaliBRITE beads. Be sure to select the Standard or Two-Color Standard option in FACSComp for Simultest samples.

Creating a CELLQuest Document for Simultest Samples

Refer to the *CELLQuest Software Reference Manual* for information on running CELLQuest software.

This section contains two parts. Part A describes the steps to create a CELLQuest document for Simultest samples, and Part B describes how to use the document to optimize the cytometer for acquiring Simultest samples.

A. Create a CELLQuest Document

1. Start CELLQuest software.
2. Create the following acquisition plots using the Plots menu:
 - Dot plot of FSC vs SSC
 - Dot plot of FL1 vs FL2
3. In the FSC vs SSC dot plot, set a region R1 as shown in Figure 1 using the Rectangular-Region tool in the tool palette.
4. In the FL1 vs FL2 dot plot, choose Format Dot Plot from the Plots menu. Choose G1 = R1 from the Gate pop-up menu. Set fluorescence markers using the Markers tool in the tool palette (see Figure 1).

- Choose Acquisition and Storage from the Acquire menu. Select Accept All in the Acquisition Gate. Set Collection Criteria to Event Count or Time. Stop acquisition when 3000 G1 = R1 events are counted, or after 420 seconds (flow rate on HI). Time Resolution will be set by the software. In the Storage Gate, set All events for data file. Set Resolution to 256. Click on the Parameters Saved button, select ONLY P1 : FSC - H, P2 : SSC - H, P3 : FL1 - H, and P4 : FL2 - H for Parameters Saved to the Data File. Click OK when done (see Figure 2).

NOTE: Make sure no other parameters are selected and the TIME parameter is deselected, otherwise SimulSET WILL NOT be able to read the files.

- Choose Edit Panels from the Acquire menu. Define your Simulset reagent panel. Refer to the *CELLQuest Software Reference Manual*.

- Choose Parameter Description from the Acquire menu. Check the panel box and select the desired panel. Verify individual tube information. Ignore the Sample ID, Patient ID, Folder, and File fields; you will enter this information later using the WorklistManager software (see Figure 3).

NOTE: If you are running more than one panel, you must create a separate CELLQuest document for each panel. This can be easily accomplished by using the first CELLQuest document you just created as a template, repeat steps 6 and 7, and then save the CELLQuest document under a different name to the WorklistManager folder: CELLQuest Experiments folder.

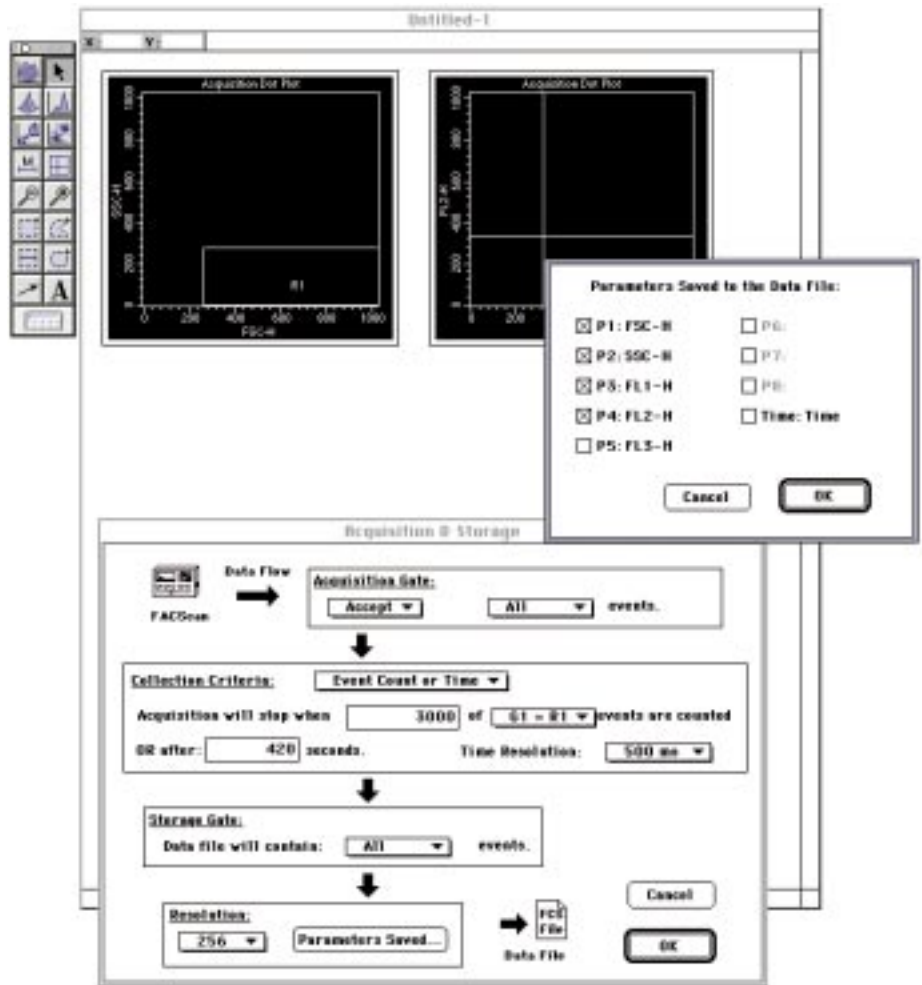


Figure 2 Acquisition and storage setup.



Figure 3 Parameter Description window showing selected panel.

B. Sample Optimization

1. Choose Connect to Cytometer from the Acquire menu. The Acquisition Control window appears, and the Cytometer menu becomes enabled.
2. Be sure the setup box in the Acquisition Control window is checked.

3. Arrange the following windows on the screen so they are all accessible:

- Choose Detectors/Amps from the Cytometer menu
- Choose Compensation from the Cytometer menu
- Choose Threshold from the Cytometer menu

4. If you have just run FACSComp, go to step 5.

Choose Instrument Settings from the Cytometer menu. Click Open. Locate the Calib file in the System folder:

Preferences folder: BD

Preferences folder, and click Open.

The settings are now displayed. Click Set to send these instrument settings to the cytometer. Click Done.

5. Present a Simultest sample tube (CD3 FITC/CD19 PE is recommended) to the cytometer using the FACS Loader with the control keypad. Click Acquire in the Acquisition Control window. Optimize the cytometer by adjusting the FSC and SSC Amp Gain, and the FSC threshold. Adjust region R1 to include the lymphocyte population. Adjust fluorescence markers and check compensation. Click Pause and Abort when done. Refer to the *SimulSET Software User's Guide* on SimulSET two-color optimization (see Figure 4).
6. Choose Instrument Settings from the Cytometer menu. Save the instrument settings as Simultest Settings to the WorklistManager folder: CELLQuest Experiments folder.
7. Save the CELLQuest document to the WorklistManager folder: CELLQuest Experiments folder.
8. Exit CELLQuest software.

You have now created a CELLQuest document for your Simultest samples. You are ready to set up your FACS Loader for acquisition.

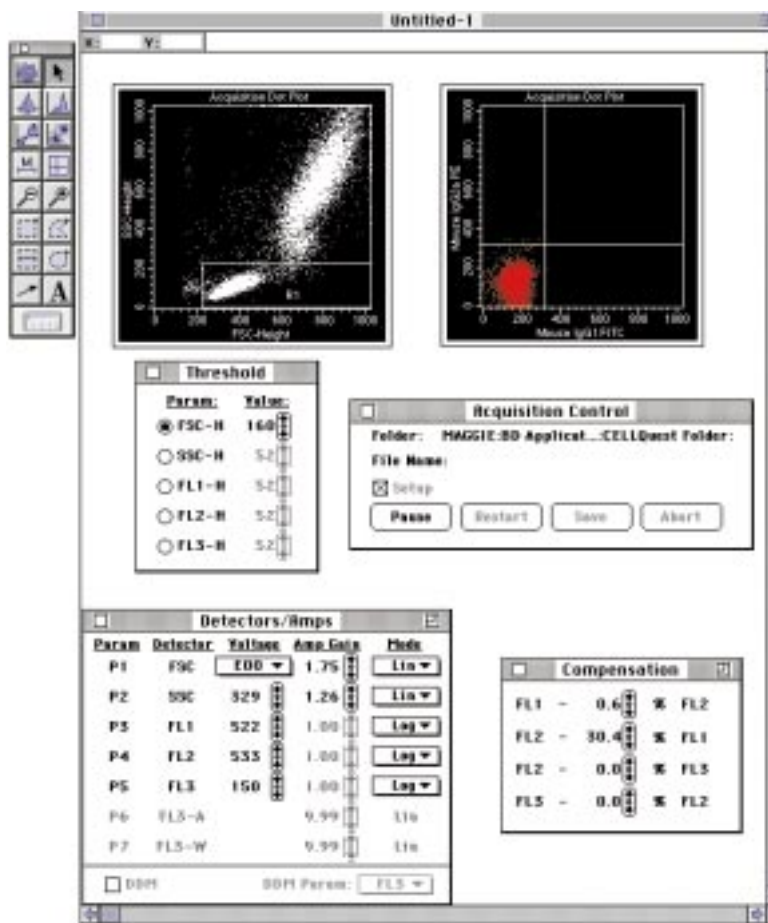


Figure 4 Acquisition display for sample optimization.

Sample Acquisition Using FACS Loader and WorklistManager

Refer to the *FACS Loader Reference Manual* for information on running WorklistManager software.

1. Start WorklistManager software.
2. At the Sign In view, enter the necessary information.
3. Create a discrete folder on the desktop for data storage.
4. At the Set Up view, enter the necessary information. Click on the CELLQuest Options button, choose your Simultest reagent panel/CELLQuest document from the panel pop-up menu. Choose Simultest Settings from the Instrument Settings pop-up menu. Click Save when done. Click Accept at the bottom of the view (see Figure 5).

NOTE: Figure 5 displays the default mixing conditions and Acquisition Start Delay setup that are recommended for Simultest samples.

5. Skip FACSComp.
6. At the Worklist view, enter the necessary information. Assign rack and print the rack manifest. Save the worklist.
NOTE: Sample Name and Sample ID in WorklistManager correspond to Patient Name and Patient ID in SimulSET.
7. Load sample tubes in a rack as described in the rack manifest. Place the rack on the Loader and close the Loader cover.
8. Click Run Tests.
9. Exit WorklistManager when acquisition is done.

You are now ready for data analysis.

Batch Analysis in SimulSET

Refer to the *SimulSET Software User's Guide*.

1. Start SimulSET software.
2. At the Sign In view, enter the necessary information.
3. At the Set Up view, select the desired choices. Select From Data Files for Data Source.
4. At the Test Prefs view, select the desired choices. Define the Simultest reagent panel that was used during acquisition.
5. At the Patients view, click Add Patient Files. Locate and open the folder where all the CELLQuest data are stored.

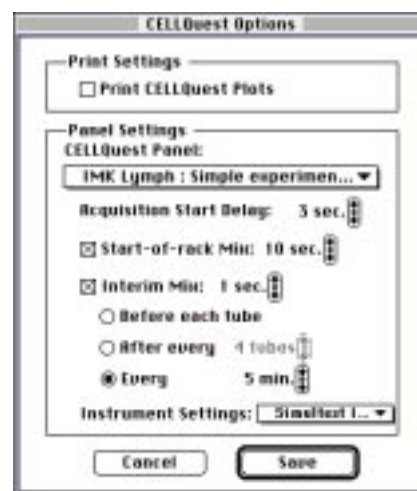


Figure 5 CELLQuest Options window showing selected panel and instrument settings.

BD publishes this method

as a service to investigators.

Detailed support for non-flow

cytometric aspects of this

procedure may not be

available from BDIS.

** This application is for*

research use only. Not

for use in diagnostic or

therapeutic procedures.

6. Select ONLY those files that correspond to the LeucoGATE™ sample for each patient (LeucoGATE is always the first tube in the panel and has a file name suffix .001. For example, Patient01.001, Patient02.001, Patient03.001, etc. See Figure 6). Click Add after selecting each LeucoGATE file. Repeat until all patients are selected, then click Done.

NOTE: Do not use Add All.

7. At the Patients view, select the proper panel for each patient. Enter values in WBC Count (x1000), Lymphs (%), and Lymphs (x1000) columns if desired.
8. Click Run Tests.

SimulSET batch analysis will proceed by automatically analyzing each patient file.

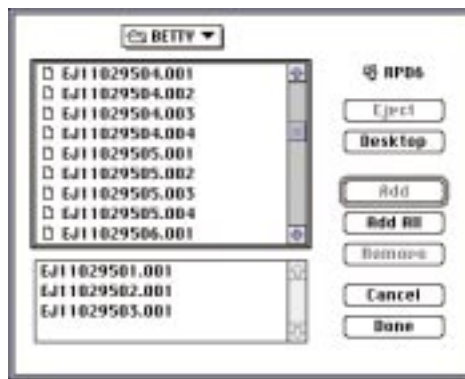


Figure 6 Patient files window showing selected LeucoGATE data files.

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