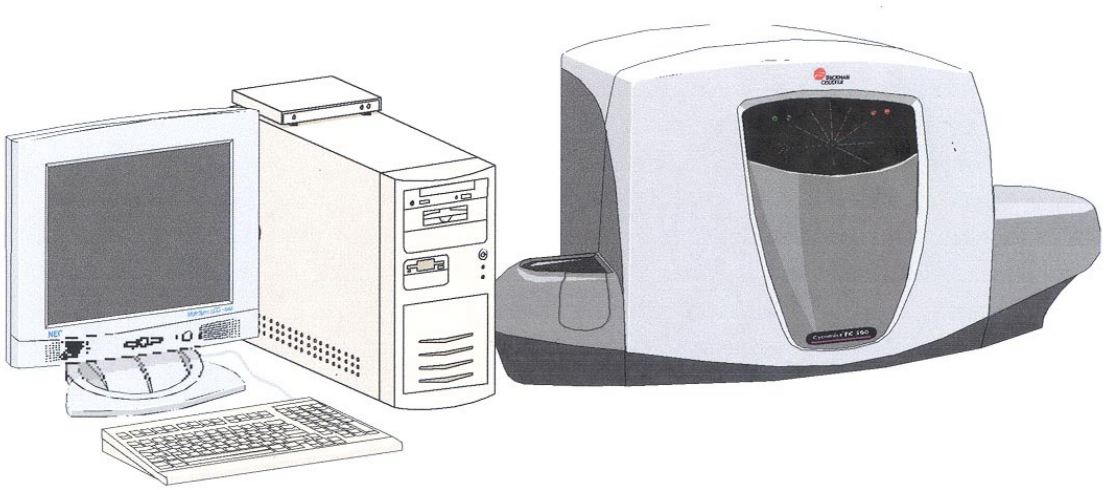
CYTOMICS FC 500 Flow Cytometer

Training Modules (CXP Software version 2.0)



PN 175306 Rev D (September 2004)
Beckman Coulter Inc.
Miami Education Center
Miami Lakes,

WARNINGS AND PRECAUTIONS

READ ALL PRODUCT MANUALS AND CONSULT WITH BECKMAN COULTER-TRAINED PERSONNEL BEFOREATTEMPTING TO OPERATE INSTRUMENT. DO NOT ATTEMPT TO PERFORM ANY PROCEDURE BEFORE CAREFULLY READING ALL INSTRUCTIONS. ALWAYS FOLLOW PRODUCT LABELING AND MANUFACTURER'S RECOMMENDATIONS. IF IN DOUBT AS TO HOW TO PROCEED IN ANY SITUATION, CONTACT YOUR BECKMAN COULTER REPRESENTATIVE.

HAZARDS AND OPERATIONAL PRECAUTIONS AND LIMITATIONS

WARNINGS, CAUTIONS and IMPORTANTS alert you as follows:

WARNING – Can cause injury.
CAUTION – Can cause damage to the instrument.
IMPORTANT – Can cause misleading results.

BECKMAN COULTER, INC. URGES ITS CUSTOMERS TO COMPLY WITH ALL NATIONAL HEALTH AND SAFETY STANDARDS SUCH AS THE USE OF BARRIER PROTECTION. THIS MAY INCLUDE, BUT IT IS NOT LIMITED TO, PROTECTIVE EYEWEAR, GLOVES, AND SUITABLE LABORATORY ATTIER WHEN OPERATING OR MAINTAINING THIS OR ANY OTHER AUTOMATED LABORATORY ANALYZER.

WARNING Risk of operator injury if:

- All doors, covers and panels are not closed and secured in place prior to and during instrument operation.
- The integrity of safety interlocks and sensors is compromised.
- Instrument alarms and error messages are not acknowledged and acted upon.
- You contact moving parts.
- You mishandle broke parts.
- Doors, covers and panels are not opened, closed, removed and/or replaced with care.
- Improper tools are used for troubleshooting.

To avoid injury:

- Keep doors, covers, and panels closed and secured in place while the instrument is in use.
- Take full advantage of the safety features of the instrument. Do not defeat safety interlocks and sensors.
- Acknowledge and act upon instrument alarms and error messages.
- Keep away from moving parts.
- Report any broken parts to your Beckman Coulter Representative.
- Open/remove and close/replace doors, covers and panels with care.
- Use the proper tools for troubleshooting.

CAUTION System integrity might be compromised and operational failures might occur if:

- This equipment is used in a manner other than specified. Operate the instrument as instructed in the Product Manuals.
- You introduce software that is not authorized by Beckman Coulter into your computer. Only operate your system's computer with software authorized by Beckman Coulter.
- You install software that is not an original copyrighted version. Only use software that is an original copyrighted version to prevent virus contamination.

IMPORTANT

If you purchased this product from anyone other than Beckman Coulter or an authorized Beckman Coulter distributor, and, if it is not presently under a Beckman Coulter service maintenance agreement, Beckman Coulter cannot guarantee that the product is fitted with the most current mandatory engineering revisions or that you will receive the most current information bulletins concerning the product. If you purchased this product from a third party and would like further information concerning this topic, call your Beckman Coulter Representative.

COURSE GUIDE

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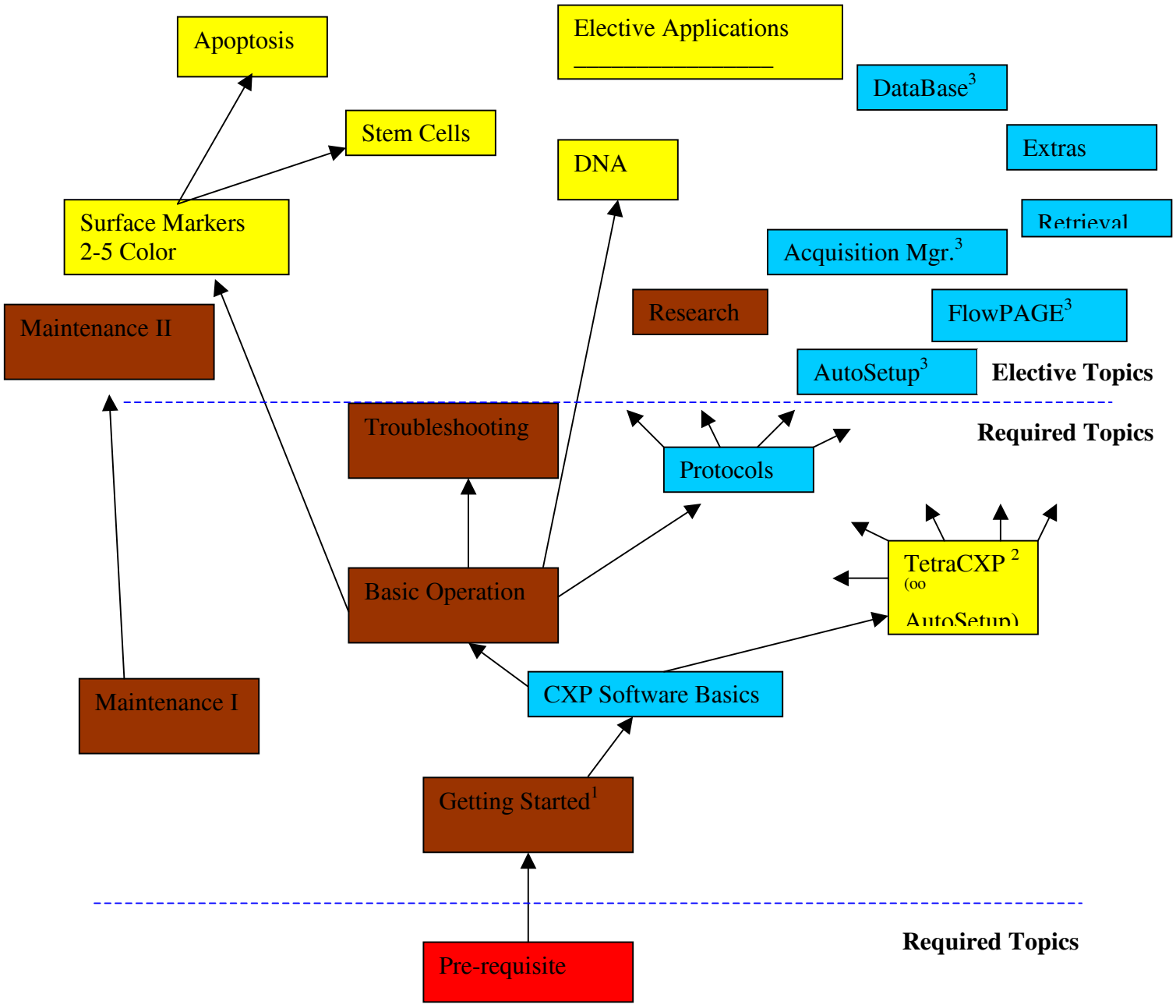
COURSE MAP



[1]Getting Started includes daily Startup and Shutdown procedures.
[2] The tetraCXP module provides an introduction to a number of options and tasks covered more completely in other modules.
[3]Quality Control System to output Data

PERSONAL PROGRESS SUMMARY Name _____

Pre-requisite		Date Achieved	Sign-Off
Flow Principles		_____	_____
Filter Concepts		_____	_____
Filter Selection		_____	_____
Compensation		_____	_____
Required Topics			
Getting Started		_____	_____
Basic Operation		_____	_____
Troubleshooting		_____	_____
Maintenance I		_____	_____
Clean air filters	_____		
Clean containers	_____		
Clean Samp. Sys	_____		
Clean head/probe	_____		
Replace Sheath filt.	_____		
Adj. Sys. Pressure	_____		
Change FS field pos.	_____		
Software Basics		_____	_____
TetraCXP or AutoSetup		_____	_____
Creating a Protocol		_____	_____
Elective Topics			
Maintenance II		_____	_____
Replace probe/tubing	_____		
Replace MCL head	_____		
Replace optical filter	_____		
Adjust HeNe Laser	_____		
Research		_____	_____
AutoSetup		_____	_____
FlowPAGE		_____	_____
Acquisition Manager		_____	_____
Retrieve and Analyze Data		_____	_____
Software Extras		_____	_____
Database		_____	_____
Cell Surface Markers 2 Color		_____	_____
Cell Surface Markers 3 Color		_____	_____
Cell Surface Markers 4 Color		_____	_____
Cell Surface Markers 5 Color		_____	_____
Stem Cells		_____	_____
Apoptosis		_____	_____
DNA		_____	_____
Other _____		_____	_____

HOW THIS COURSE WORKS

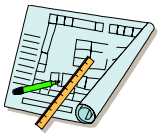
This course is probably unlike any other training you may have experienced, unless you have taken a criterion-referenced course before. What is criterion-referenced instruction? Simply put, it is self-paced learning of pre-specified performance criteria, which are verified using Skill Checks. You select the module you wish to work on based on the course map provided. The module provides information and guidance so you may practice the skills required to operate the system until you feel confident with them. A Skill Check determines whether you have achieved the stated objectives of each individual module.

The main features of this training are:

- Reviewing information and skills you already know so that you can do Skill Checks right away
- Learning only skills that are necessary for your job (however, make the best of your time while you are here so that it is most beneficial for you and your lab)
- Working at your own pace
- Practicing a new skill until you achieve competency

Let's look at the makeup of this course.

Course Map



The course map shows how each module of the course relates to other modules and to the course as a whole. It is a blueprint of the course. To use a course map, start at the bottom and work your way up to the top

- Complete the prerequisite topic before beginning to study a module. The Prerequisite topic is shown on the Course Map by arrows that lead into your chosen module.
- Where no sequence is shown (i.e., there are no arrows leading into a module), you are free to study the modules in any order you please.
- Complete the Prerequisite Topic and Required Topics.
- Choose Elective Topics based on your particular training needs and time remaining.

There is ample time to complete all Required Topics as well as some or all of the Elective Topics.

Modules

- Read the Objectives, What to Learn, or Skill Check requirements at the beginning of a new module.
- Complete the Skill Check for the module if you already know the task. It's a good idea to read through the module anyway to make sure there are no terminology or procedural surprises.
- Read the module and complete the practice exercises for new material presented in the module. Refer to additional resources provided for the module.
- Work through the module at your own pace. **This is not a race!**
- Within each module, you will see  icon in several places. This icon marks exercises you are to complete. You can keep track of your progress by checking the box next to the pencil as you finish the exercise.

Resources

- Each module lists the resources available to complete the topic.
- Consult any resource that is appropriate to your needs. You may use some or all of the resources.
- Additional resources are available on line through the CXP Software. These include product manuals and video clips illustrating certain procedures.
- The facilitator is always available to answer any questions you may have.

Skill Checks

- Each module has a criterion test called a Skill Check. You may complete a Skill Check when you feel ready. Before doing so, you will save yourself time if you first make sure you can answer "yes" to this question:

  ▸ Did I practice the skill(s) called for in the objective?

- If, after reading the module, you feel ready to complete the Skill Check without further study, do so.

- If your performance on a Skill Check is inadequate, you may, after further study and, at the facilitator's discretion, be asked to complete the same or similar Skill Check.
- When you complete a Skill Check, a facilitator or colleague (as indicated in the sign-off box) will check your work.

Personal Progress Summary

Ask whomever is certifying your work to date and initial your Personal Progress Summary next to the appropriate module.

Master Progress Chart

Facilitators use a Master Progress Chart to keep track of the progress of the entire class. When you are certified on a module, be sure the facilitator makes the proper entry on the Master Progress Chart. Until this entry is made, it is assumed that you have not yet mastered the module.

SCHEDULING YOUR TIME

The following discussions take place in the classroom/laboratory. Ending times noted are approximate.

Monday 8:15-10:00

- General Information
- Learn about each other
- Safety: Personal Protective Equipment and Fire Evacuation
- Three-part evaluation
- Introduction to the system, course, materials and equipment you will use.
- Location of additional reference materials
- Modules for today

_____

_____

_____

Tuesday 10:00 – 11:00

- Review and/or Basic Operation Discussion
- Class picture
- Modules for today

_____

_____

_____

Wednesday

- Troubleshooting Discussion
- Modules for today

_____

_____

_____

Thursday

- Modules for today

_____

_____

_____

Friday

- Modules and Applications for today

_____

_____

_____

- Evaluations
- Graduation

REMINDERS

You may take two fifteen minute breaks daily, one in the morning and one in the afternoon.

There will be 45 minutes for lunch.

Tuesday through Friday, remember to complete the getting started procedures: Startup, running Flow-Check, and Shutdown at the end of the day (about 10 minutes before leaving for the day).

Each day when you arrive, we will begin with time to answer any questions you may have and to make any neccessary announcements for the day.

OBJECTIVES AND SKILL CHECKS

Getting Started

Given an operational system, training materials, Getting Started manual, access to an Operator's Guide, and access to Help:

- Name the major components of the system.
- Startup the system as per the Operator's Guide.
- Log on to the software.
- Access the Startup and Shutdown Help screens and print them to be placed in this section of the training Guide.

Basic Operation

Given an operational system, training materials, access to an Operator's Guide, and access to online Help:

- Run and recognize good alignment check using Flow-Check™ Fluorospheres as a check on the fluidics and optics within the system.
- Apply basic troubleshooting steps to overcome poor alignment check data.
- Adjust the system to run a 2-color set of cell surface marker samples. Adjustments to include: gains, high voltage, discriminator, and compensation settings.
- Run a control for an application, determine the targeted values, compare these values to assayed results, and determine if the system is producing accurate results.
- Run Flow-Set™ Fluorospheres using the 2-color high voltage and gain settings and determine the target channel for these fluorospheres.

Troubleshooting

Given training materials, access to an Operator's Guide, and access to online Help:

- Recognize acceptable versus unacceptable data.
- When data is unacceptable, determine the possible causes and take appropriate action to correct the situation.
- Access and print the cytometer.log file and explain the meaning of each entry.
- Use the error message table to determine the cause of a particular error message and describe the action that should be taken to correct the situation.

Maintenance

Given an operational system, access to a Special Procedures and Troubleshooting Guide, and access to online Help:

- Clean and replace air filters.
- Clean the reagent and waste containers.
- Clean the sampling system.
- Clean the MCL sample head and the sample probe.
- Replace the sheath fluid filter.
- Adjust system pressure.

Advanced

- Replace the sample probe and sample pickup tubing.
- Replace the MCL sample head.
- Remove and replace optical filter plate and a filter.
- Adjust the HeNe laser.

Research

Raise the consciouness of the researcher as to how to generally proceed and solve problems related to research.

Software Basics

Given an operational flow cytometer, training materials, and access to online Help:

- Logon to the software.
- Identify screen layout.
- Use menus or toolbars to complete tasks.
- Access online Help screens.
- Access Workspace Preferences.

Creating a Protocol

Given an operating flow cytometer, prepared samples for a given application, or listmode data, training materials, and access to online Help:

- Create a protocol to run and/or analyze sample listmode data.
- Create an acquisition protocol in which parameters must be selected and the cytometer adjusted.

AutoSetup

Given an operational system, prepared samples for a given application, training materials, and access to online Help:

- Answer questions related to the concept of autostandardization.
- Create and/or modify an AutoSetup Application.
- Schedule a single and multiple applications for autostandardization.

FlowPAGE

Given an operational system, training materials, and access to online Help:

- Create a FlowPAGE to include arranged plots, statistics, text and images.
- Save the FlowPAGE as a separate entity and as part of a protocol.
- Print a FlowPAGE as a PDF file.

Acquisition Manager

Given an operational system, stored sample listmode data, training materials, and access to online Help:

- Setup the acquisition manager with the proper columns, labels, and desired listmode file labeling.
- Create a panel.
- Create a worklist.

Retrieve and Analyze Data

Given an operational system, stored sample listmode data, training materials, and access to online Help:

- Retrieve listmode data with the run time protocol.
- Retrieve listmode data with a new protocol.
- Retrieve multiple listmode files with a panel of protocols.
- Retrieve multiple listmode files with a single protocol.
- Create an overlay of several single parameter plots.
- Display an overlay as a gallery, angled or superimposed mode.
- Retrieve several sets of listmode files with a single protocol using the batch automator function.
- Publish data to Microsoft Excel.

CXP Software Extras

Given an operational system, training materials, and access to online Help:

- Set up the system to display colors on a dot plot in color blend, color precedence or advanced precedence modes.
- Access administrative functions and assign a new user, assign privileges to a user, create a workgroup, and track user usage of the system.
- Copy a plot and paste it into another Windows application.
- Overlay the outline of a previously run single parameter plot onto a new plot.

- Turn on or off the Baseline Offset option.
- Color compensate a listmode file.

Database

Given an operational system, training materials, and access to online Help:

- Edit the Quality Control product information.
- Access and evaluate the quality control reports.
- Modify a panel template.
- Create a tetraCXP and/or a LSA panel report.
- Edit the patient demographic information in the database.
- Access the Database Management portion of the database.

Set up Cell Surface Markers

Given an operational flow cytometer, proper reagents, blood samples, and access to reference materials and/or online Help:

- Perform an alignment check.
- Prepare samples using the TQ-Prep to run on the flow cytometer.
- Create a protocol to run 2, 3, or more color samples with proper gating and analysis.
- Establish standard reference values as per the guidelines in the appendix.
- Run quality control samples to set compensation values and verify instrument accuracy.
- Run prepared samples and generate sample reports.

TetraCXP and LSA

Given an operational flow cytometer, proper reagents, blood samples, and access to reference materials and/or online Help:

- Prepare and run tetraCXP samples
- Prepare and run LSA samples.

DNA

Given an operational system, available samples, training materials and access to online Help:

- Prepare a reference sample, control sample and at least one unknown DNA sample to run on the flow cytometer.
- Create a protocol to run the samples on the system to include parameters, gating and analysis similar to the examples illustrated in this module.
- Calculate a DNA Index on an abnormal DNA sample.
- Prepare a second set of DNA samples with at least one cell surface marker attached to the cells.
- Create a second protocol to run DNA and cell surface markers simultaneously illustrating marker gating and analysis similar to the examples shown in the DNA module.

Stem Cell CD34+ HPC Enumeration

Given an operational system, access to online Help, and required supplies:

- Perform Quality Control to verify instrument performance and reagent stability.
- Create a protocol following the ISHAGE guidelines
- Run and analyze CD34 stained samples and demonstrate an understanding of the ISHAGE gating strategy.

Apoptosis Detection Annexin V FITC

Given an operational system, access to online Help and required supplies:

- Perform Quality Control to verify instrument performance and reagent stability.
- Create a protocol to detect apoptosis following the package insert instructions.
- Run a set of ANNEXIN stained samples. Analyze the data and demonstrate an understanding of the results obtained.

OBJECTIVES

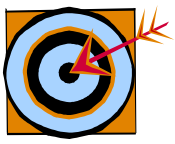
Given an operational system, training materials, Getting Started manual, access to an Operator's Guide, and access to online Help:

- Name the major components and indicators of the system.
- Start up the system as per the Operator's Guide.
- Log on to the software (User: guest, password: valued).
- Access and print the Startup and Shutdown Help screens.
- Shutdown the system.
- Log startup and shutdown procedures completed in the database log.

WHY IS IT IMPORTANT?

This module helps you to get started in your learning process. We are going to take a panoramic view of the instrument to learn the basic components. Next, we will overview the software you will use to operate the system. The purpose here is not to make you proficient in every aspect of this software but to gain a panoramic view of the layout as well as learn some of the basic mouse functions used consistently throughout. From this basic starting point, you can then proceed to the Quality Control module or perform maintenance on the system.

Skill Check Preview



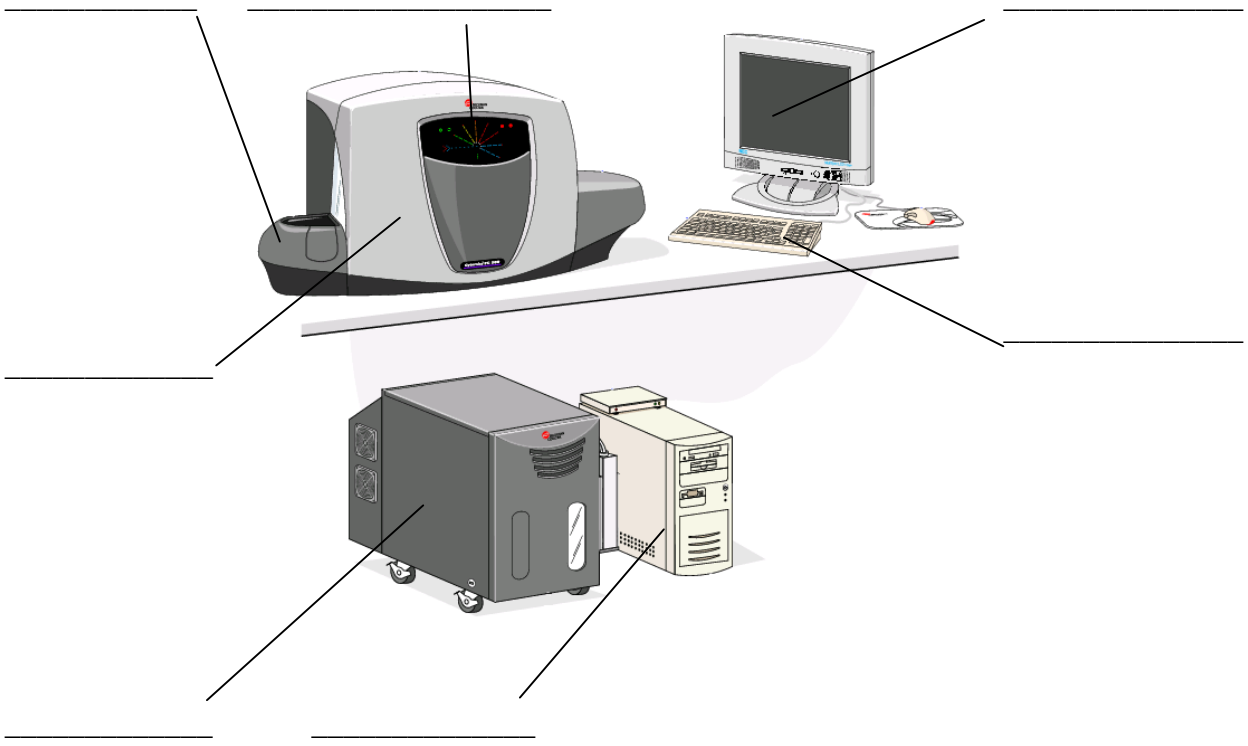
You have mastered the objectives when you can

- Name the major components and indicators of the system.
- Startup the system.
- Log On to the software (User: guest, password: valued).
- Access and print the startup and shutdown Help screens.
- Shutdown the system.
- Log startup and shutdown procedures completed in the database log.

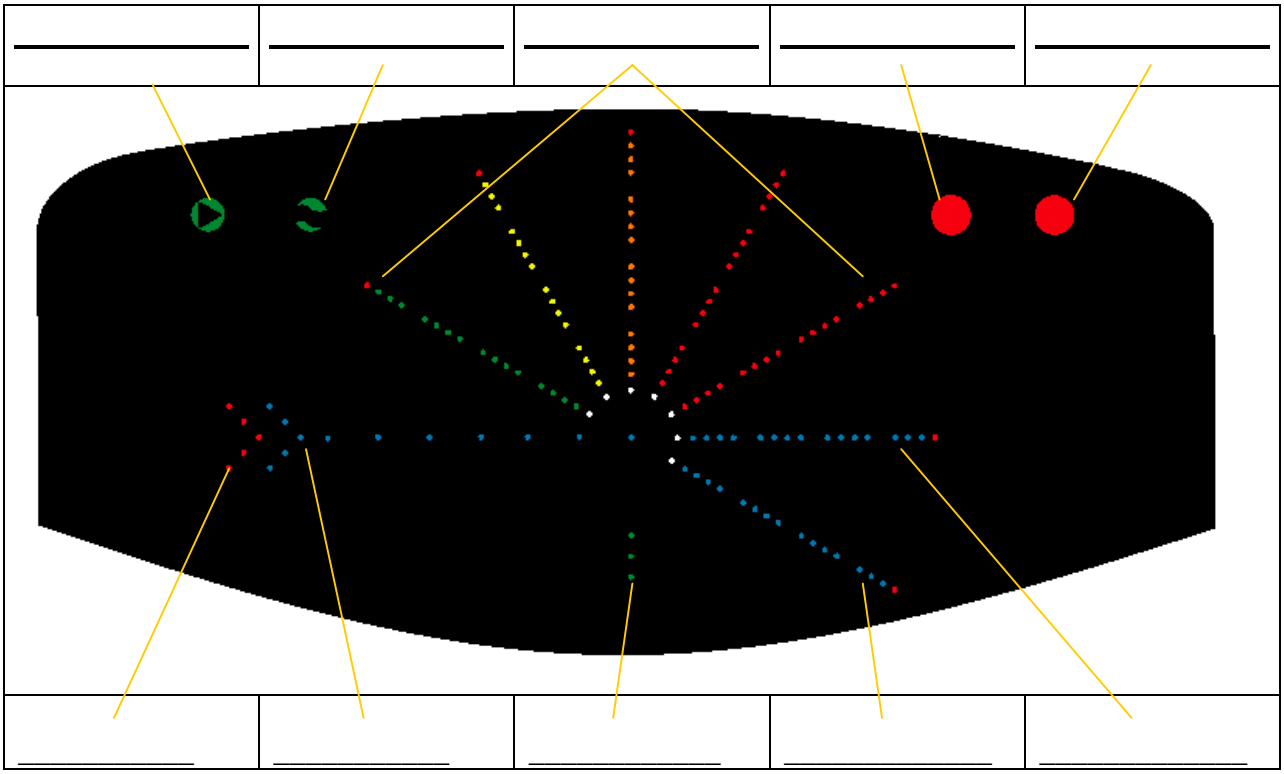
INSTRUMENT OVERVIEW

Take few minutes to read through the *Getting Started* booklet. Then fill in the blanks on the following worksheets.

Name the Major Components:



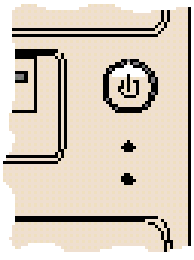
Name the Indicators:



INFORMATION / PRACTICE SECTION

Startup

Your instructor will have performed part of the Startup procedure so that you will initially only have to power up the system by pressing the Power on button on the computer. You will print the complete version of Startup later.



Log ON to the Software

1. Log ON to the software. You can find directions for logging on to the software in the:

   - Getting Started manual
   - **Software Basics** section of the **Procedure Guides** in this Training Guide

2. You may log on as a guest with "valued" as the password.

Access online Help

1. Select the  icon at the top of the screen to access online Help.
2. Select the + sign next to the Daily Routine option on the left side of the Help screen list.
3. Select Daily Startup.
4. Select the Print icon at the bottom of the Help screen.
5. Repeat the process for the Daily Shutdown procedure.
6. Place your printouts in this section of your Training Guide for reference.
7. (Optional) If you wish, explore other help topics or procedures on line.

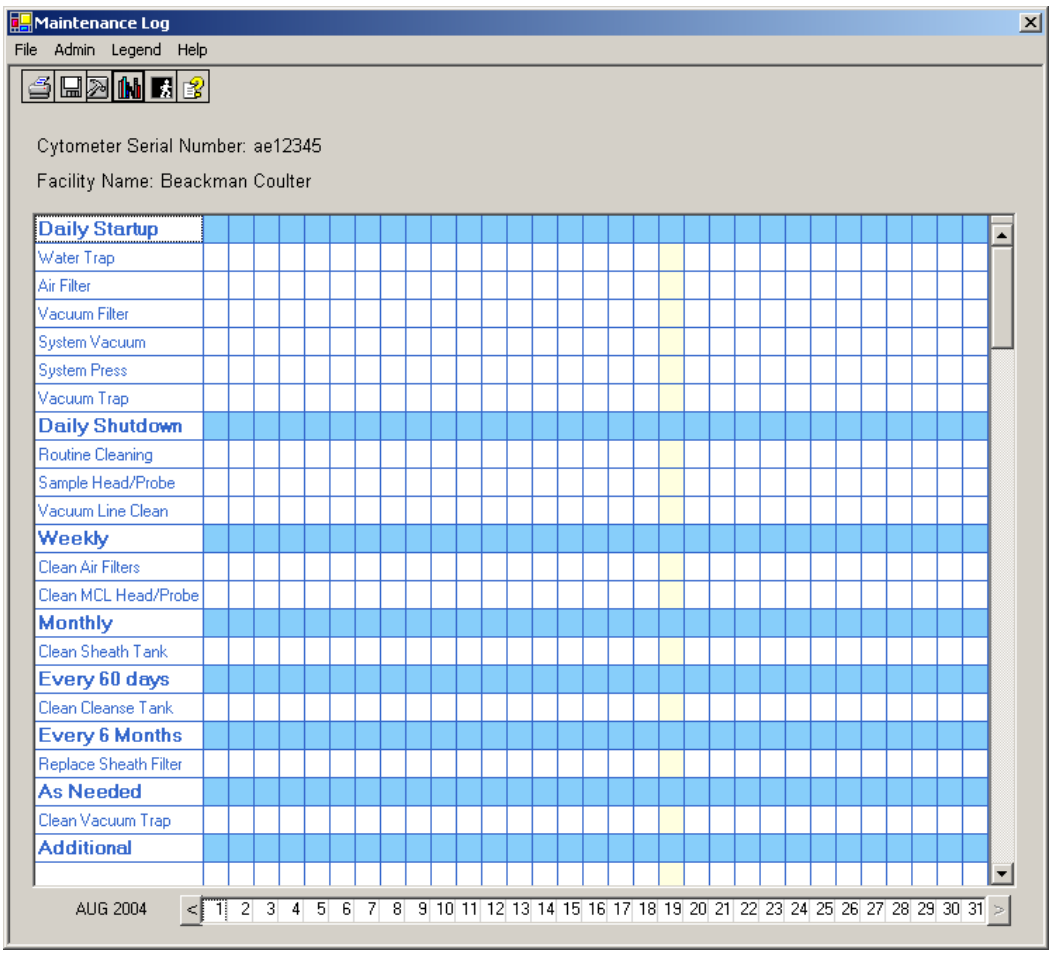
OPTIONAL: If you wish, you can set up your system to automatically startup the system. The procedure is in the online Help.

8. Select 6 Daily Routine
9. Select 6.5 Cytometer Auto Startup. We suggest that you print the procedure for your notes.

Logging Completed Startup and Shutdown Procedures

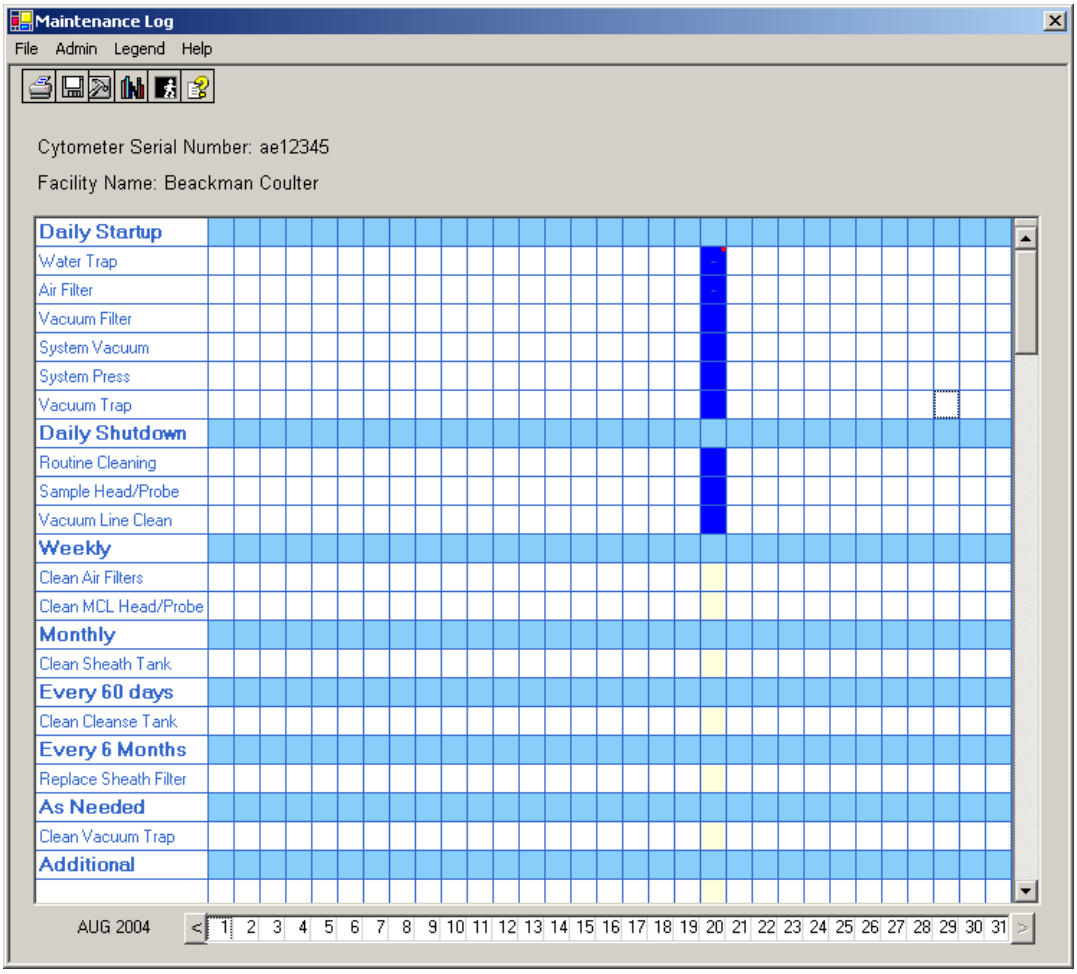
You can keep a record of completion of Startup and Shutdown procedures in a Maintenance log on the system. To do this:

1. Select the Report generator Toolbar (bottom of the screen) and then the  icon. The maintenance log will now display with today's cells shaded.



2. Double click on each box for todays date in the line for each completed procedure.
3. Right click on a box and select **Insert Comment**, if you wish to log any comments.

The system will highlight in color each box you select. Entries with comments will be indicated with a red dot within the box.



4. When you are finished Select **File** >> **Save** to save the updated log.

5. Click on the  icon to close the log.

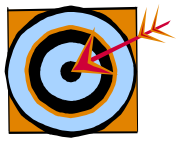
Table of Contents

OBJECTIVES

Given an operating flow cytometer, training materials, and access to online Help

- Logon and logoff to the software.
- Identify screen layout and screen icons.
- Use menus or toolbars to complete tasks.
- Access help screens
- Access Workspace Preferences.

Skill Check Preview



You will have mastered the tasks when you can

- Successfully logon and logoff to the software.
- Determine the menu and/or icon sequence required to accomplish some common tasks.
- Identify screen layout areas.
- Identify the screen icons.
- Demonstrate the ability to access and make changes to the Workspace Preferences.

Before you begin…

The purpose of this module is to get you familiar with the layout of the software and some common ways to accomplish tasks. We are not trying to make you proficient at this point. That will take some time. Some functions will be handled in more detail in other modules. Others you may not even be interested in. This module is just getting you started like in the overall structure of a house. The initial house layout is very rough but you can kind of see how the rooms will lie out even though they are not finished. As you proceed through other modules our "software house" will become more refined. Do not try to learn everything at once. Learn what you need to know to accomplish what you need to accomplish.

Tour Guides

We would suggest at this point that you use the Tour Guides to help familiarize yourself- with the software. To access the Tour Guides:

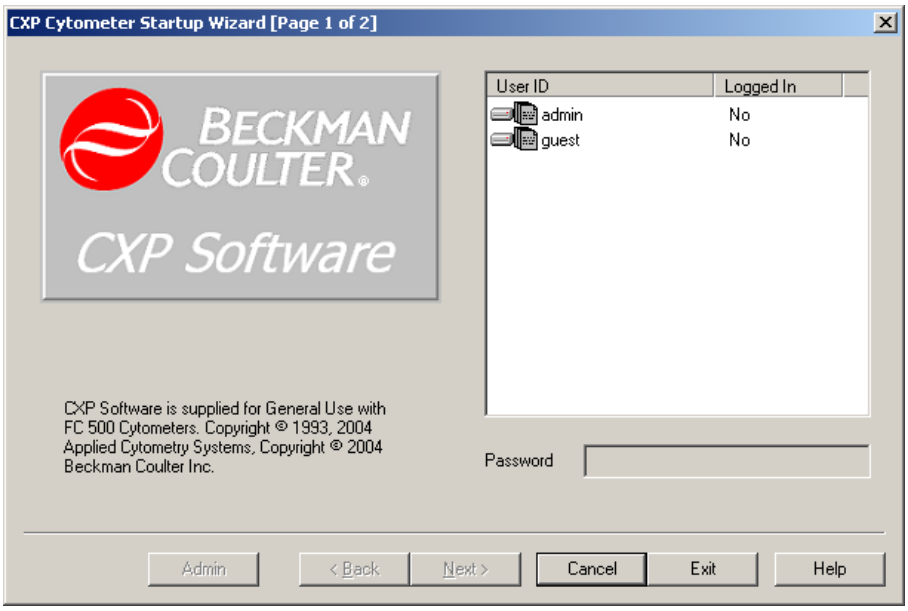
1. If there is a program running, minimize it by selecting  in the upper right corner of the application.

2. Place the Tour Guide disk into the CD Rom drive or if your facilitator has placed the Tour Guides on your hard drive, double click on the icon labeled Tour Guides. The system will start the program. A voice will come on and explain how to take the tours.

3. Take the Software Basics tours to familiarize you with the material in this module.

Log On (Cytomics CXP Analysis program)

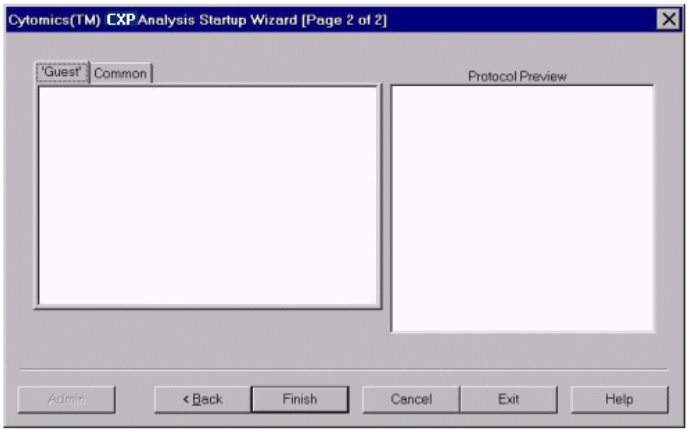
1. Select the CXP Analysis icon or Select **Start >> Cytomics CXP Analysis**.



2. Select the name (Guest) and then enter the password (Valued).

NOTE: The Administrator would set up each user and password.

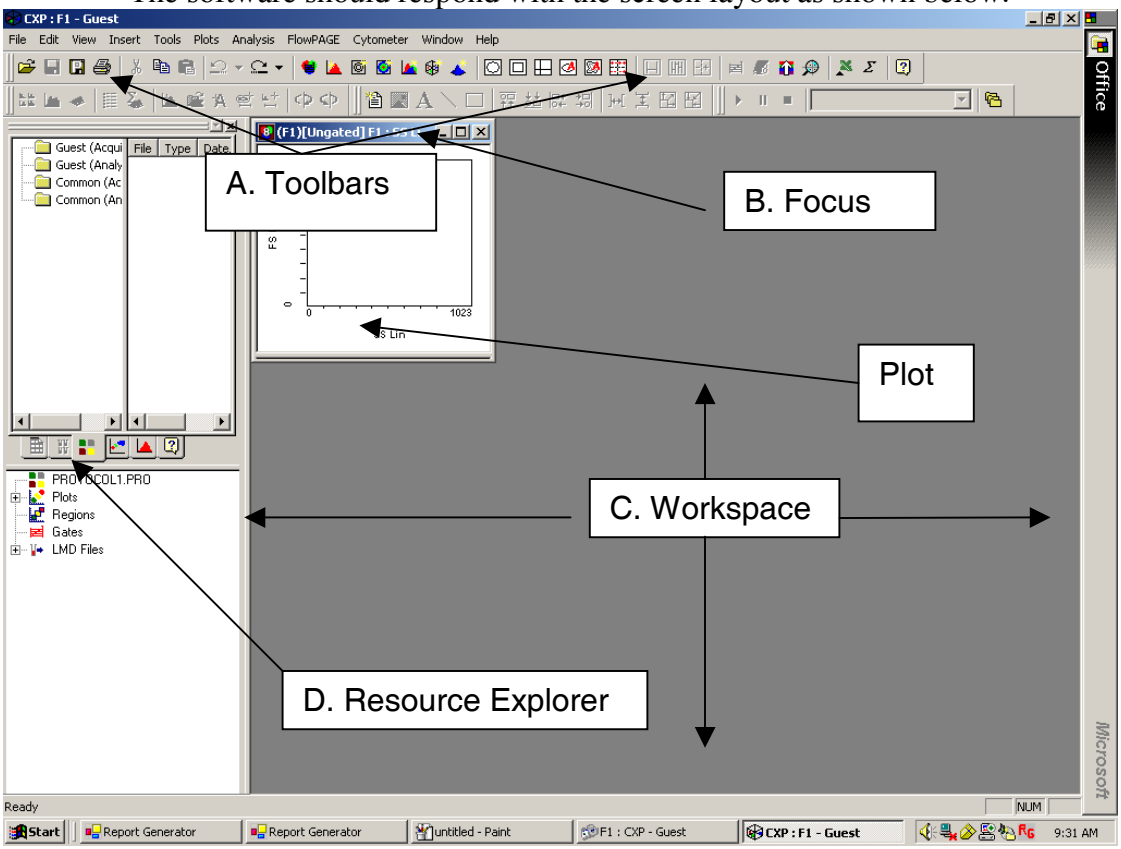
3. Select **Next**.



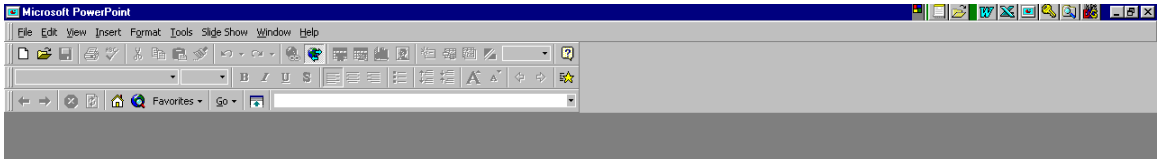
4. You can now select an established protocol and then **Finish** or just Finish to display the default. You will now be logged on to the Acquisition software.

Screen Layout

The software should respond with the screen layout as shown below.



The **Menu** options are similar in format to Microsoft Office programs (See the Power Point Menu options below).



A. Toolbars are shortcuts to various menu options.

B. When a particular **plot** is selected, the area at the top of the plot (called the **Focus**) highlights indicating the current window of interest.

C. The **Workspace** is really the "work area" of the screen where histograms (referred to as plots in this software) are displayed, manipulated, and analyzed.

D. The **Resource Explorer** allows you to access files and then bring them into the Workspace (typically with a drag and drop technique).

E. The **Acquisition Manager** (not shown - acquisition only) allows you to set up sample acquisition.

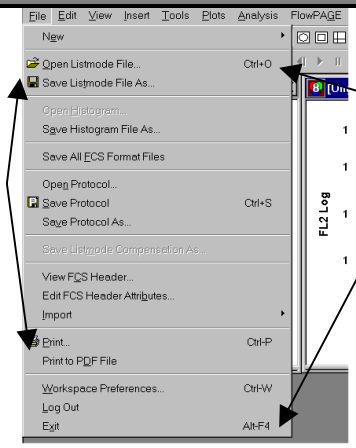
Use of Menus

NOTE: Each of the main menu options is shown in turn on the following pages with a brief description of its purpose. Note also on each menu the toolbar icons on the left side of the menu, which can be used in place of the menu options. In addition, the CTRL or ALT keys and an appropriate letter (shown on the right side of a menu option) can also be used to initiate an option. Options, which are not allowed for a particular screen setup, are grayed out.

1. Select **File**

The File menu opens or saves various types of files including listmode data, histogram data, protocols, header information (FCS files), and Workspace preferences.

Toolbar icons for these menu options.

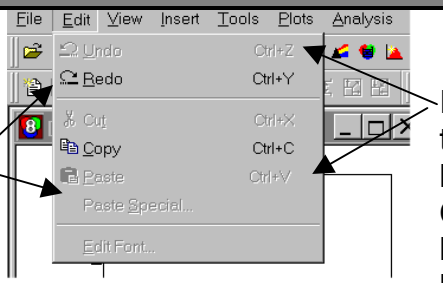


If you prefer to use the keyboard, CTRL or ALT key and letter shortcuts to the file menu options.

2. Select **Edit**

The Edit menu follows the typical Windows format options for editing the Workspace area including Undo (or Redo) a previous step, cut, copy or paste an object such as a plot, and font editing for a selected area of the workspace.

Toolbar shortcuts to the Edit menu options.

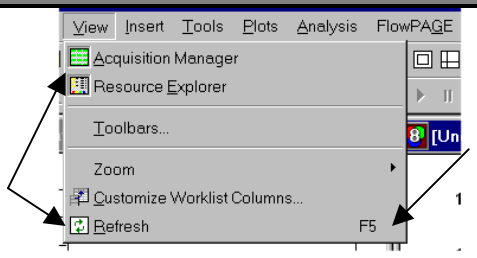


If you prefer to use the keyboard, CTRL or ALT key and letter shortcuts to the file menu options.

3. Select **View**

The View menu follows the typical Windows format options for displaying items on the screen including the Resource Explorer, in the acquisition program the Acquisition Manager, and Toolbars. The screen may also be zoomed for better viewing or refreshed if you suspect items are missing that should be there.

Toolbar shortcuts to the View menu options.

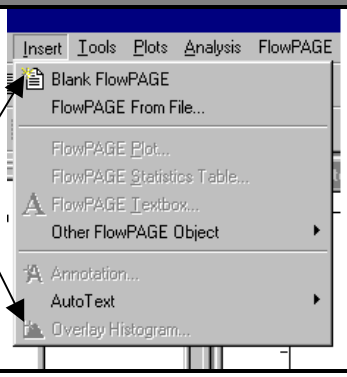


F5 can be used to refresh the screen.

4. Select **Insert**

The Insert menu is used to create a special report form called a FlowPAGE and then to insert items onto this page.

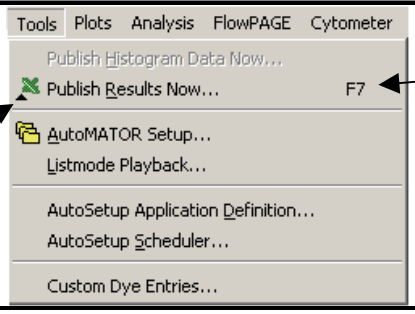
Toolbar shortcuts to the Insert menu options.



5. Select **Tools**

The Tools menu is used to open Microsoft Excel program and output statistics (Results) to this program. In addition, the software can be set up to retrieve multiple files, populate the Workspace plots and output data through AutoMATOR. This menu also provides aids to setting up applications through the AutoSetup and Custom Dye functions.

Toolbar shortcuts to the Tools menu options.



F7 can be used to Publish Results Now.

6. Select **Plots**

The Plots menu is used to create different types of plots on the Workspace.

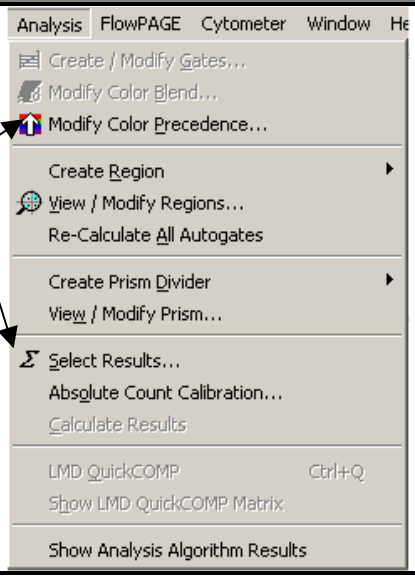
Toolbar shortcuts to the Plot menu options.

CTRL key options for initiating plots on the workspace

7. Select **Analysis**

The Analysis menu is used to create regions on the plots which then can be used for gating, coloring the plots, and generating statistics.

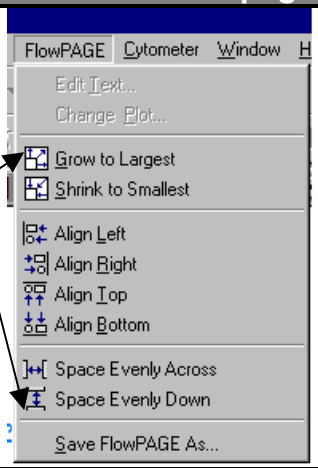
Toolbar shortcuts to the Analysis menu options.



8. Select **FlowPAGE**

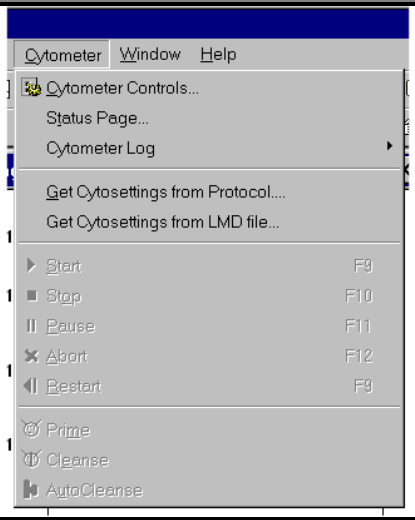
The FlowPAGE menu is only active when a FlowPAGE has been created and items on the page have been selected. This menu allows you to arrange the items on the page.

Toolbar shortcuts to the FlowPAGE menu options.



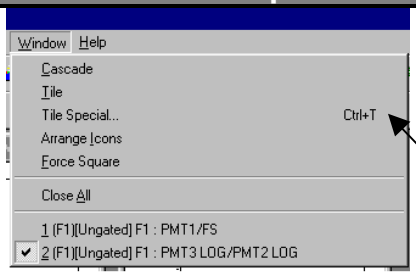
9. Select **Cytometer**

The Cytometer menu is only active in the Acquisition software and is used to control the cytometer settings and the sample acquisition.



10. Select **Window**

The Window menu provides standard Windows options for arranging the plots and other windows on the Workspace. In addition, the Tile Special option allows you to control the specific placement of plots on the Workspace.

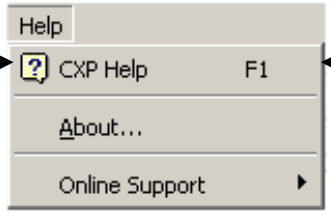


Use CTRL T to activate Tile Special option.

11. Select **Help**

The Help menu provides online support for various functions within the software including function explanations and how to perform certain tasks.

Use this icon to activate Help screen.



Use F1 to access general Help or if certain windows are displayed (e.g. Cytometer Control) Help information for that window.

NOTE: Throughout this guide, when describing a sequence of menus and sub menus, the individual menus will be separated by a >>.

For Example: **File>>New>>New Protocol** means select File, then New, and then New Protocol.

Standard Windows 2000 Functions

CXP Software operates with standard Windows-type functions. Methods, which you may already be familiar with from Microsoft Office programs, will work in a similar manner in this program. The following pages will highlight a few of these as examples.

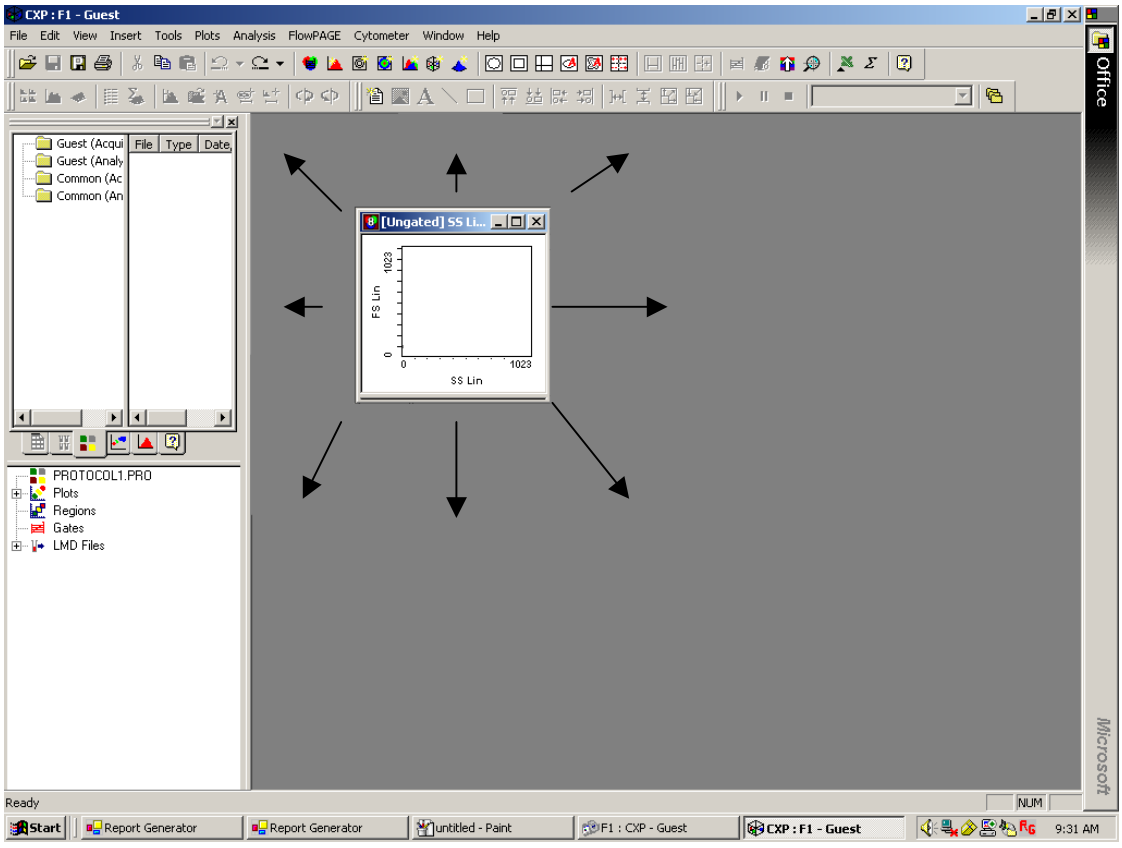
Drag and Drop

Items such as files or plots can often be selected and dragged to another location on the Workspace.

Exercise 1:

1. Select the plot (move the cursor to the bar on the plot then click and hold the left mouse button).
2. Move the mouse to move the plot to a new part of the screen and then release the left mouse button.

The plot should now be at a different location on the screen.



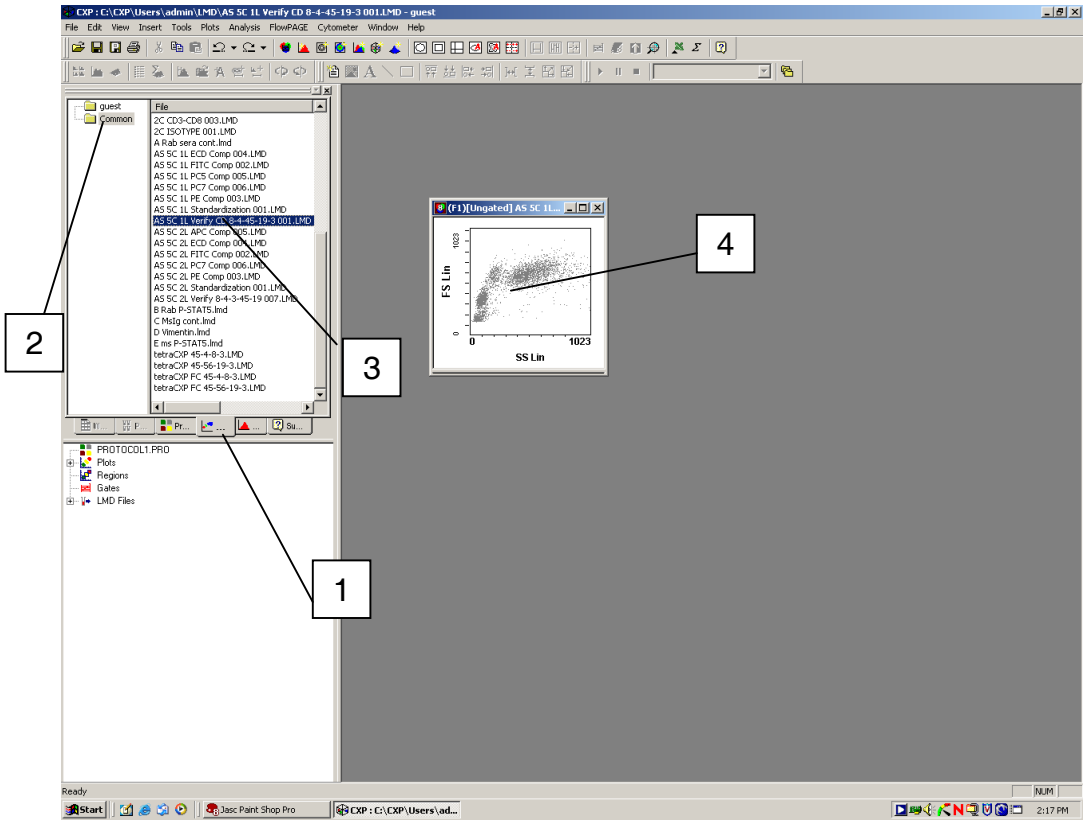
Exercise 2:

1. Select the  tab on The Resource Explorer (1).
2. Now select Common folder (2).

NOTE: The other tabs represent other files accessible through the Resource Explorer (see below).

3. Click left mouse button on AS 5C 1L Verify CD8-4-45-19-3 001.LMD listmode file (3). The file should highlight.
4. Hold the left mouse button and drag the file to the plot on the Workspace and then release the mouse button. The plot should now populate with data (4). An example is shown below.

Worklists (only available in acquisition)	Panels (only available in acquisition)	Protocols	Listmode files	Histogram files	Help
					

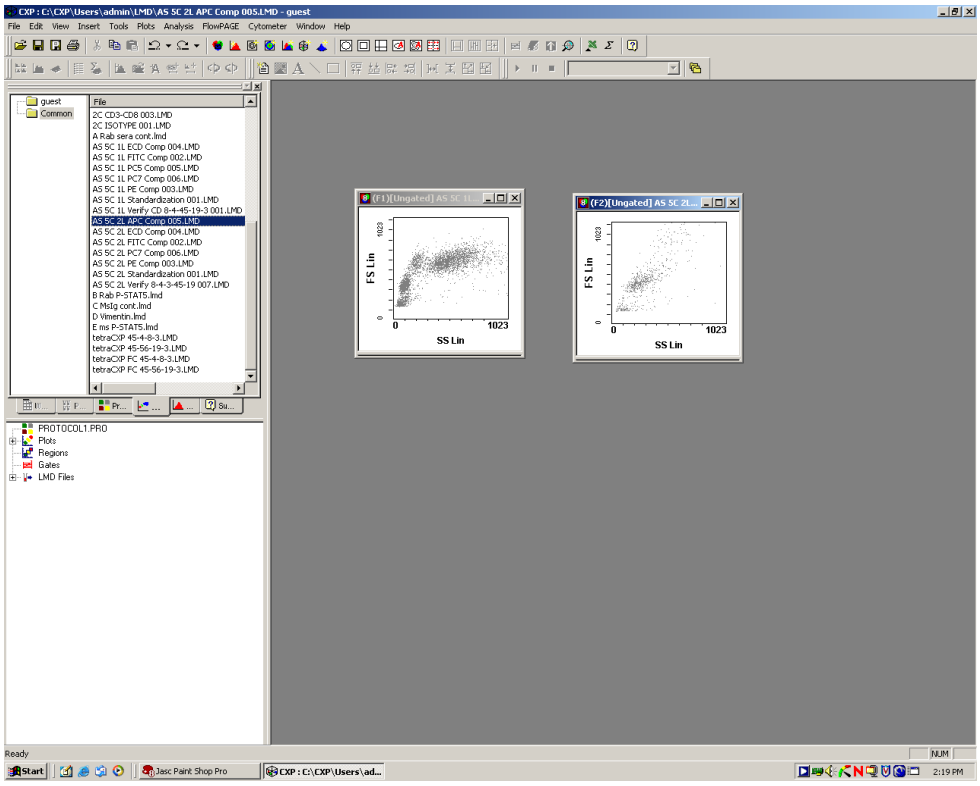


From here on, the action of clicking the left mouse button on an object, holding the left mouse button, moving the object to a new location, and then releasing the button will simply be referred to as "**Drag and Drop**".

Exercise 3:

1. Select the plot and then press **CTRL** and **D** at the same time. A duplicate plot should appear.
2. Move the second plot off to the side.
3. Select the next listmode file and drag it to one of the plots but before releasing the mouse button, press and hold the **CTRL** key. Only the plot selected should change.

NOTE: The cursor will change to a plus sign if you are doing it correctly.



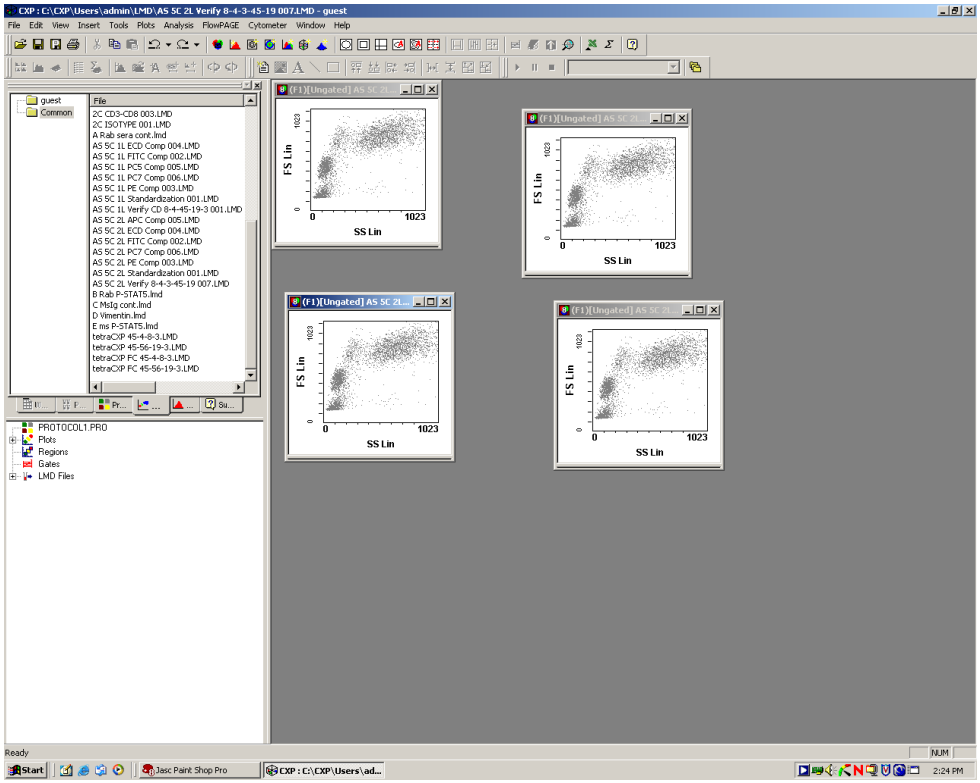
Other CTRL Key Functions

Press **CTRL** and then an appropriate letter to duplicate some of the menu functions.

Exercise 4:

1. Press **CTRL** and **N** together.
2. Select **No**. The screen should clear and bring up the default protocol.
3. Select the plot and then press **CTRL** key and the letter **D** three times. Three duplicates of your selected plot should appear in a cascade above the original plot.
4. Use a drag and drop technique to separate the plots.
5. Drag and drop AS 5C 2L Verify 8-4-3-45-19 to one of the plots. All plots should populate.

NOTE: When the **CTRL** key is depressed while dragging and dropping, only the single plot is changed (Analysis software only).



Minimizing, Expanding, Eliminating Windows

1. Select the underscore symbol (_) in the upper right corner of a plot to minimize the plot.

2. Select  in the upper right corner of the plot to display the plot on full screen.

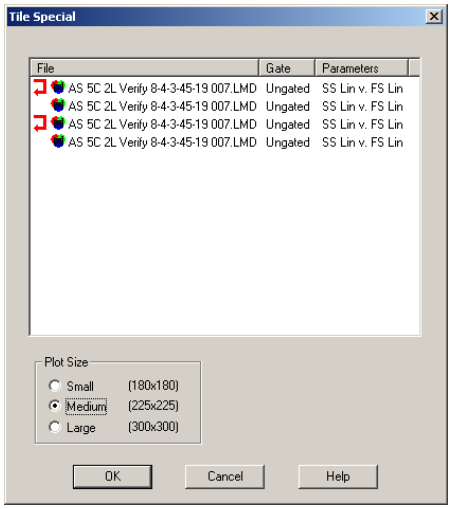
3. Select  in the upper right corner to return the display to its original size.

4. Select **X** in upper right corner of a plot to eliminate the plot.

Note: If the protocol is saved, the plot is permanently removed. If there was a region on the removed plot, the region remains.

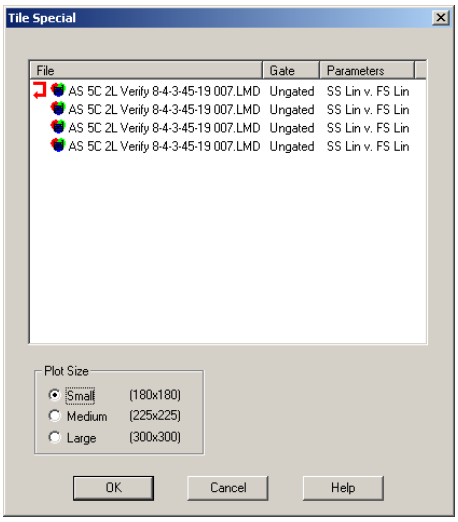
Arranging Windows

1. Place several plots on the Workspace.
2. Select **Window** >> **Tile Special…** (or press **CTRL T**). The screen responds as shown below:



3. Click on the second red () to delete it.
4. Select the small plot size. This window allows you to change the size and ordering of the plots.

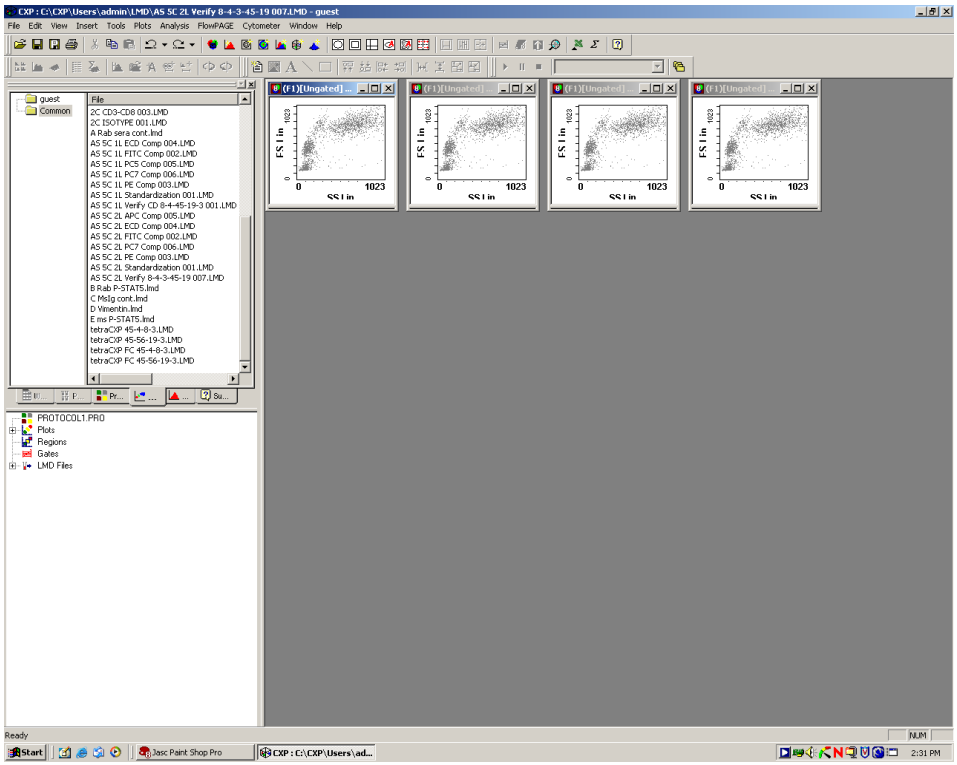
The screen responds:



Exercise 1:

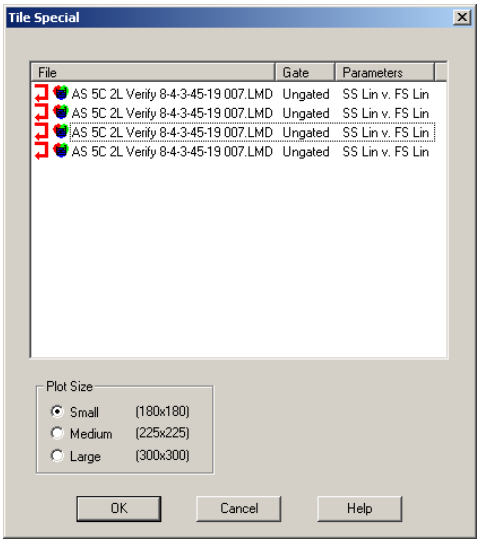
1. Select **OK**. The plots should now all be lined up horizontally across the Workspace.

If there are more plots horizontally then can be displayed, a scroll bar at the bottom of the Workspace appears to allow you to display the additional plots.

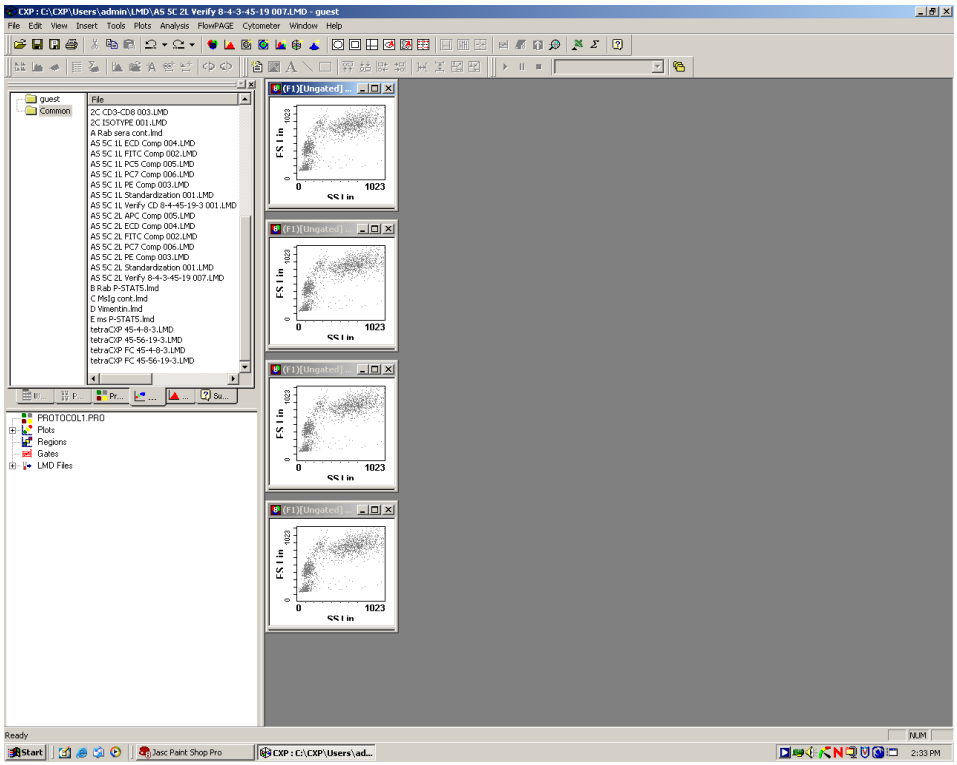


Exercise 2:

1. Press **CTRL T**. This time click next to each plot so that the top symbol is repeated next to each plot as shown below:

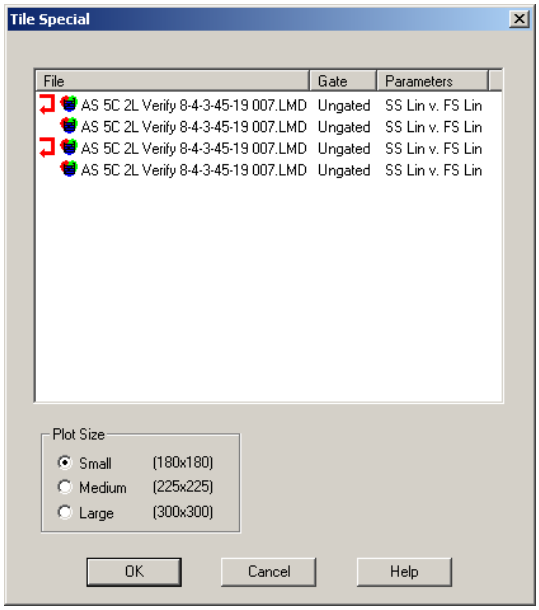


2. Now select **OK**. The plots should now be aligned vertically with the scroll bar on the right side of the Workspace.

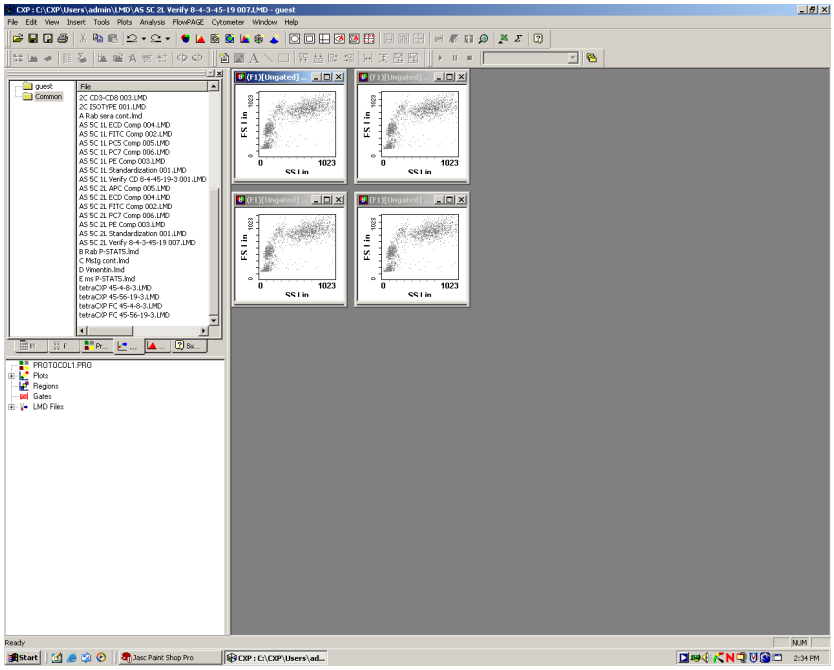


Exercise 3:

1. Press **CTRL T** again to bring up the Tile Special window
2. Deselect all but 2 of the ↵ symbols.



3. Select **OK**. The plots should now line up in two rows.

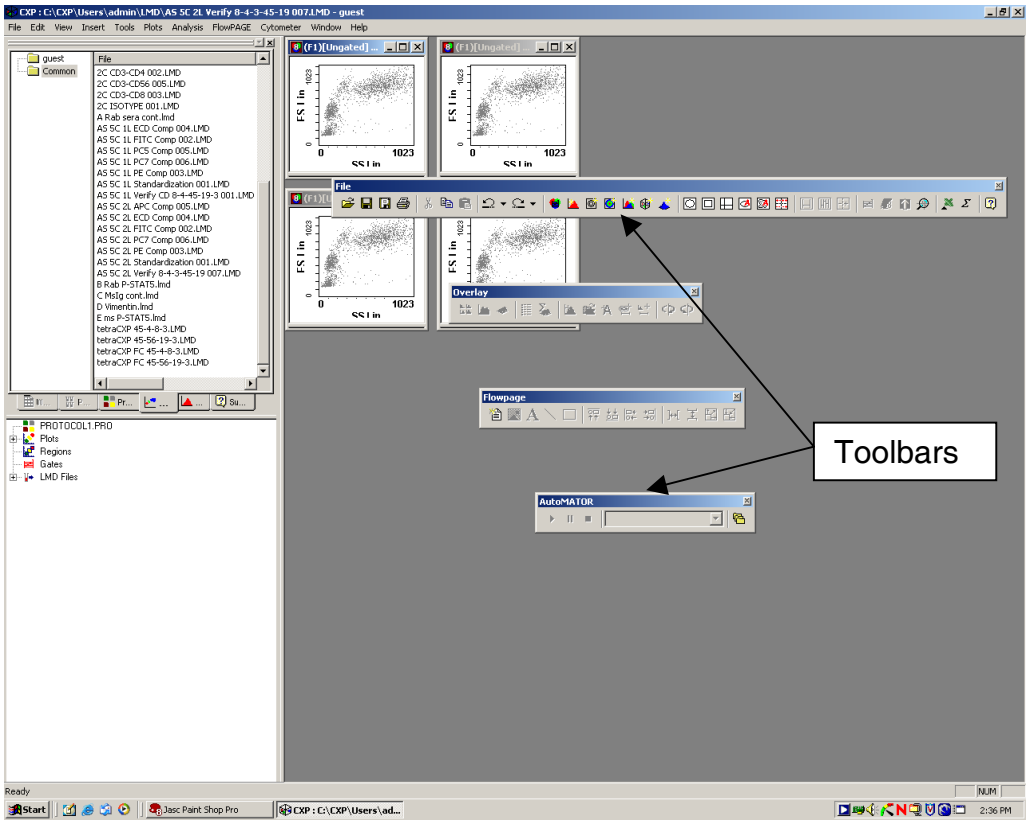


NOTE: You can also use the drag and drop technique on the Tile Special window to order the plots in any manner you desire. Then apply the ↵ symbol to set the desired rows. When you are finished, select **OK**.

Introduction to Toolbars

There are several Toolbars at the top of the screen below the menu. These can be separated and placed anywhere on the screen as follows:

1. Select the double vertical bar on the left side of the desired toolbar and use the drag and drop technique to move it to another part of the screen.
2. Repeat for each toolbar. Depending on where you drag them the icons may line up horizontally (as shown below) or vertically.



NOTE: Functions:

File deals with listmode, protocol and histogram or plot files.

Overlay deals with multiple single parameter plots.

FlowPAGE is used to arrange items on a FlowPAGE.

AutoMATOR sets up system to retrieve and analyze multiple listmode files.

Others not shown:

Cytometer controls the cytometer.

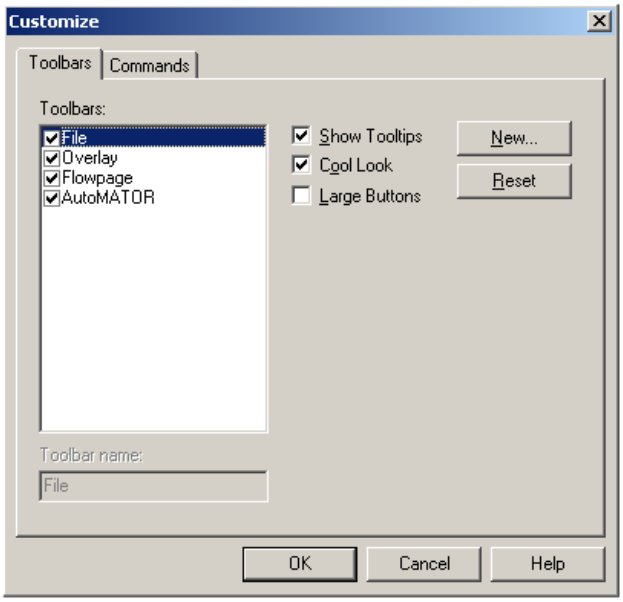
Acquisition Manager Tools adds worklist, tubes and panels to the Acquisition Manager defining future samples to be run on the system.

The Toolbars can also be placed back into their original positions. This action is referred to as "**docking**".

1. Double click on the top of each Toolbar in turn. Each should return to its original position below the menu.

If you do not wish to view all of the toolbars at the top of the screen:

1. Select **View** >> **Toolbars**.
2. Select only those bars you wish to view and then select **OK**. You can also reinstate the Toolbars the same way.



Toolbars may also be customized.

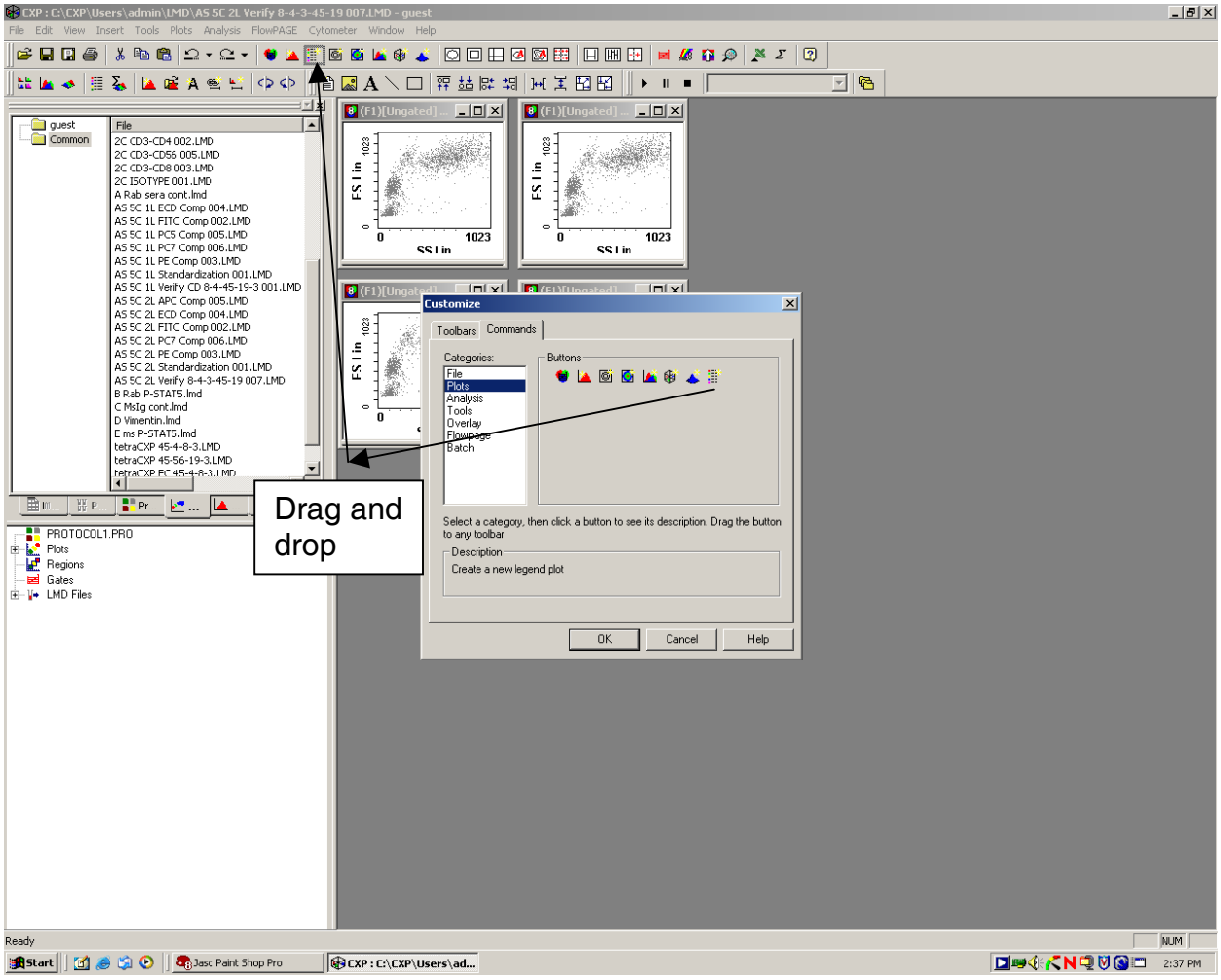
Optional Exercise:

You might wish to add a legend icon  on the File toolbar to create a legend window on the Workspace

To accomplish this:

1. Select **View** >> **Toolbars**.
2. Select Commands tab and then the Plots category. The buttons available on the Plots toolbar will be displayed.
3. Select  and drag and drop it onto the File toolbar in the plots section.

You can also rearrange the order of the buttons by dragging and dropping the buttons in turn to new locations on the bar.



4. If you drag and drop the button from a bar back to the Customize Command window, the button will be deleted from the Toolbar.
5. Select **OK** and the system will now remember your new customized toolbars.

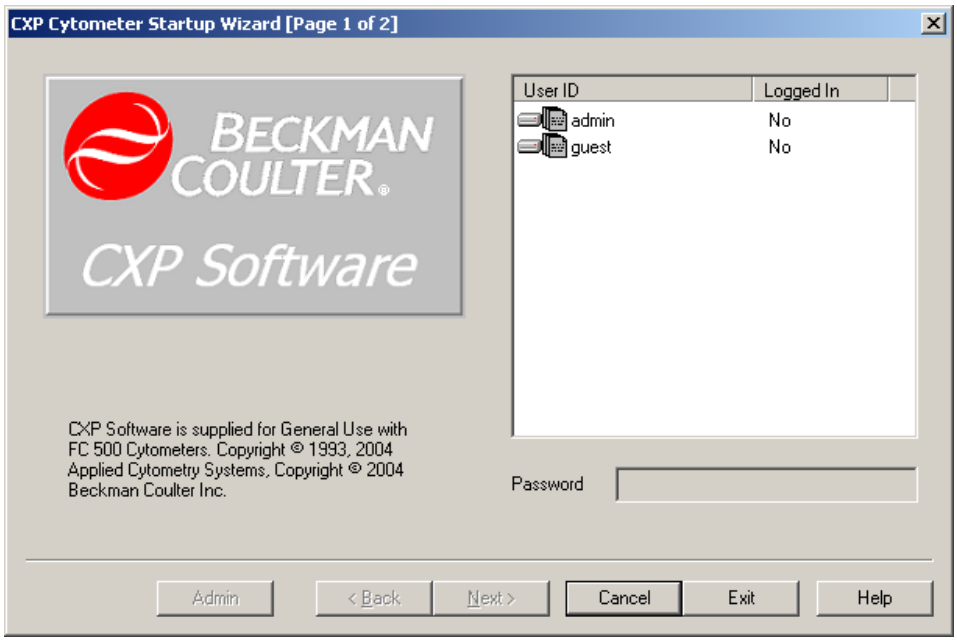
NOTE: Toolbars are global. Therefore the changes you make are going to be implemented for all users.

You have finished using the Analysis software for now. At this point we will close the Analysis software and prepare to logon to the Acquisition software.

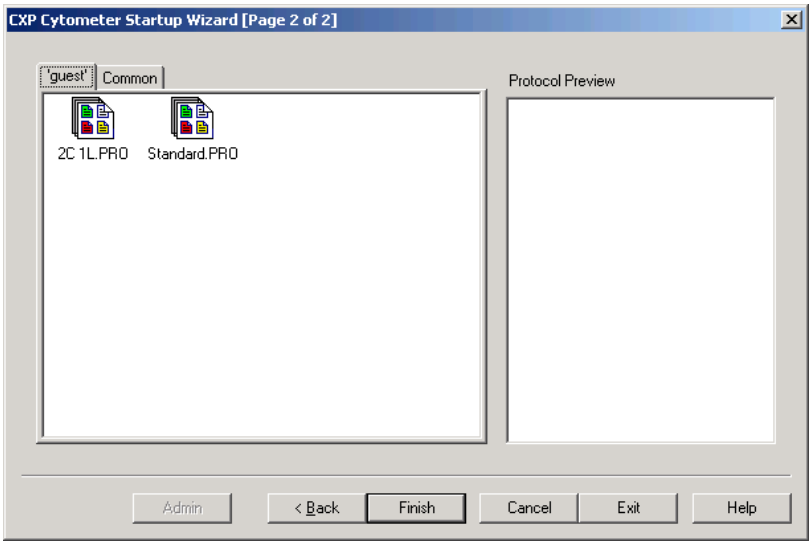
6. Select the ☒ in the upper right corner of the screen.
7. Select **No**.

Log on to the Acquisition Software

1. Select **Start** >> **CXP Cytometer**.



2. Select Guest and then enter the password (Valued).
3. Select **Next**.

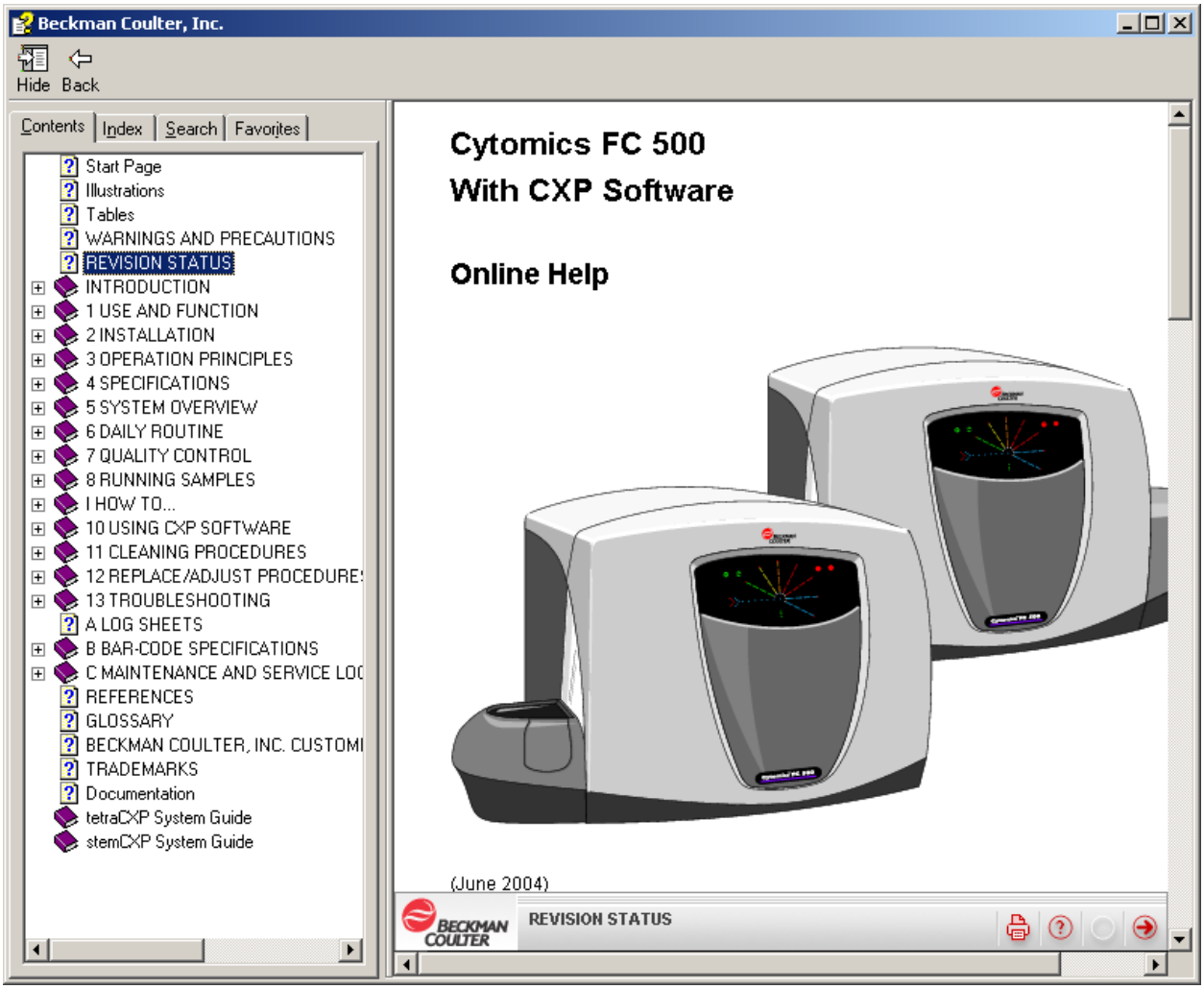


4. Select **Finish**.

Help Screens

NOTE: There is a comprehensive set of help screens built into the software. To access a general listing:

1. Select  on the File Toolbar. The Help window appears as below:

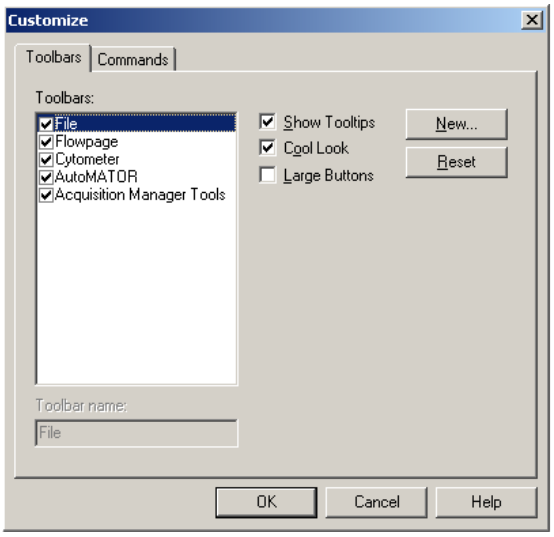


2. Click on the left listing to change the window on the right to supply information on any help topic. When you are finished, select the **X** in the upper right part of the Help window to close it.

NOTE: The Help screens are also context sensitive. If you are displaying a particular window or focused on a particular area of the screen, the help function may sense where you are and provide help on that particular item.

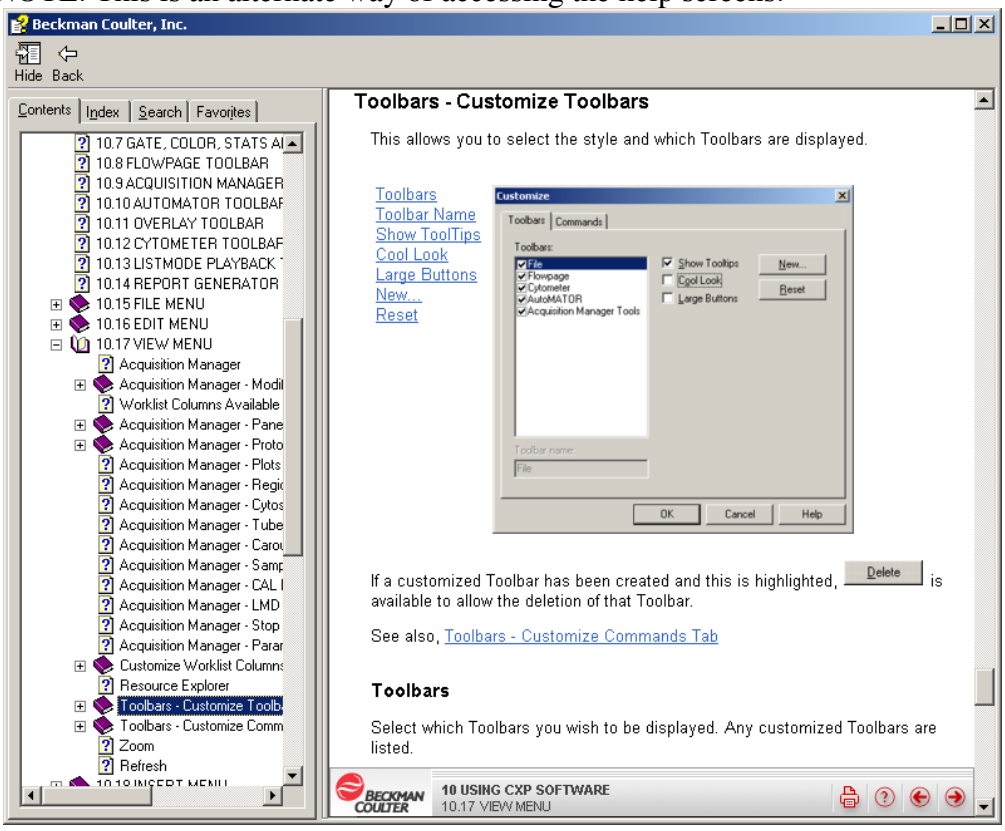
Example:

1. Select **View** >> **Toolbars**. The following window appears:



2. Press **F1**. The help screen for toolbars appears.

NOTE: This is an alternate way of accessing the help screens.



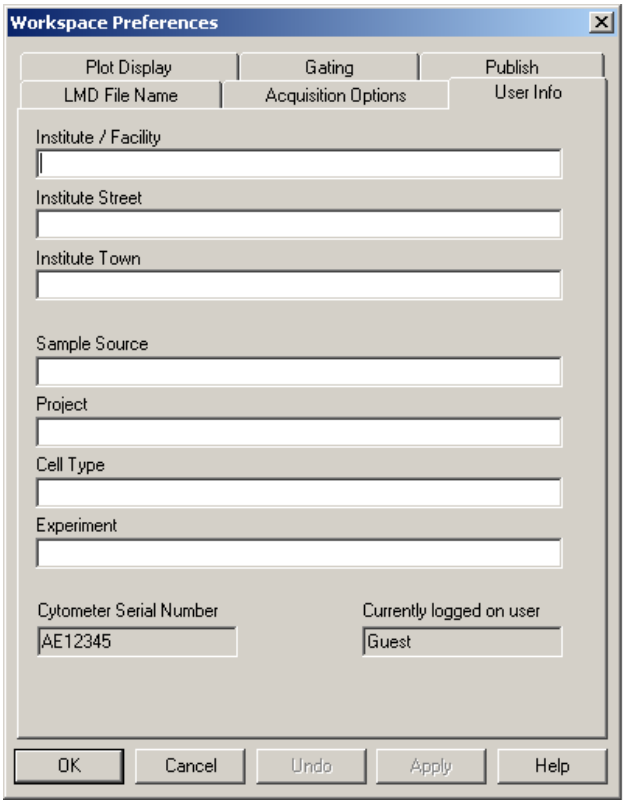
3. Select ☒ to close screen.

Accessing Workspace Preferences

Many screen display options, labeling options, print preferences and export preferences can be preselected. These items are called workspace preferences. The appearance of the screens, storage of data, and exporting of data would then be similar for different protocols and operators. If you wish to see demonstrations of some of these functions, go back and review the Basics, Workspace Preferences tours.

1. To access these options, select **File** >> **Workspace Preferences…** or press **CTRL + W**

2. To change user info, select User Info tab.



NOTE: This information will be stored as part of the FCS header in the listmode file. It will not be seen on the screen, but on all subsequent printouts. The Institute/Facility must be filled in to access any of the other tabs.

3. Select Plot Display tab.

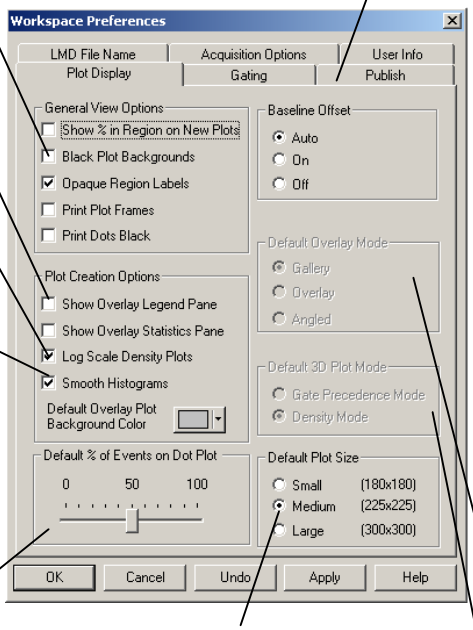
Use Opaque Region labels to display labels against a black plot background. Use Print Dots Black to eliminate all color on dual parameter plot printouts.

The Baseline Offset adds a random gaussian positive offset to log data to move very low channel signals up to higher channels (1st decade only). Auto mode selects based FCS header info.

The Overlay Legend or Statistics Pane adds a small label to the plot with the information requested.

Checking the Log Scale Density Plots will set the density levels for the plots based on a log scale. If not checked the density levels will be based on a linear scale.

Selecting Smooth Histograms reduces the roughness on single parameter plots.

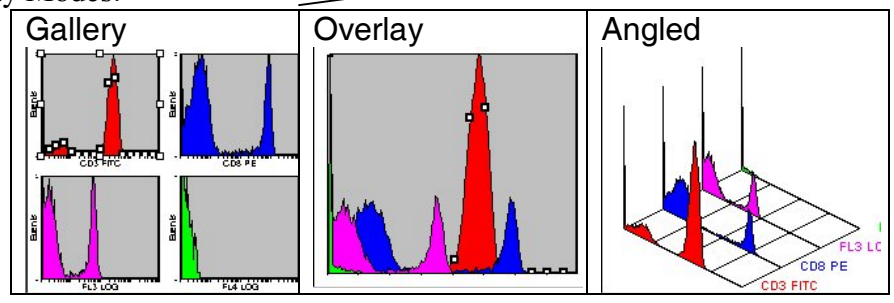


Decreasing the % of events displayed increases the speed of the software, increasing the % shows more of the data for viewing.

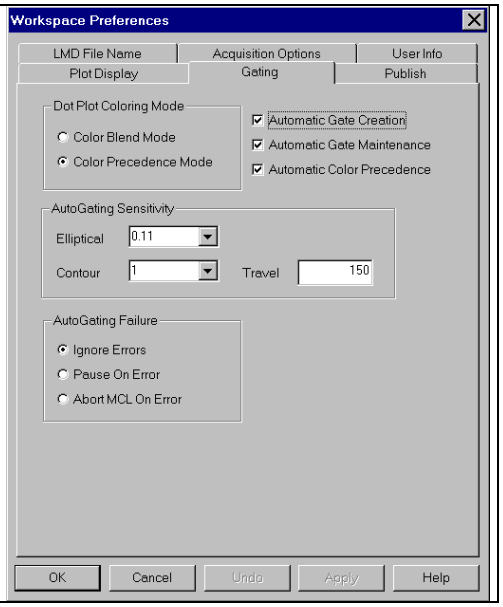
Preselect the default size of the plots when they are created.

Use Gate Precedence Mode to emphasize certain events over others (e.g. rare events) when coloring 3D plots. Use Density mode to specify up to 5 levels of color on 3D plots.

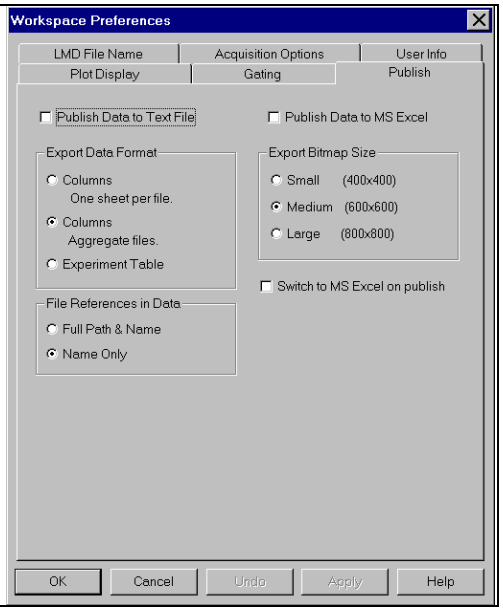
Overlay Modes:



4. To change gating preferences, select Gating tab.

Color Blend Mode chooses colors based on whether or not events fall within up to three gates. **Color Precedence Mode** chooses colors based on the color of the gate highest on a precedence list within which an event falls. **Automatic Gate Creation** makes every region a potential gate and sets up sequential gating. If **Gate Maintenance** is on, changing the gate region also sequentially changes the associated gate on any region drawn on the plot. **Color Precedence** places new gate at the top of the color precedence list with a new color. If this option is turned off, no color is assigned to the gate.	

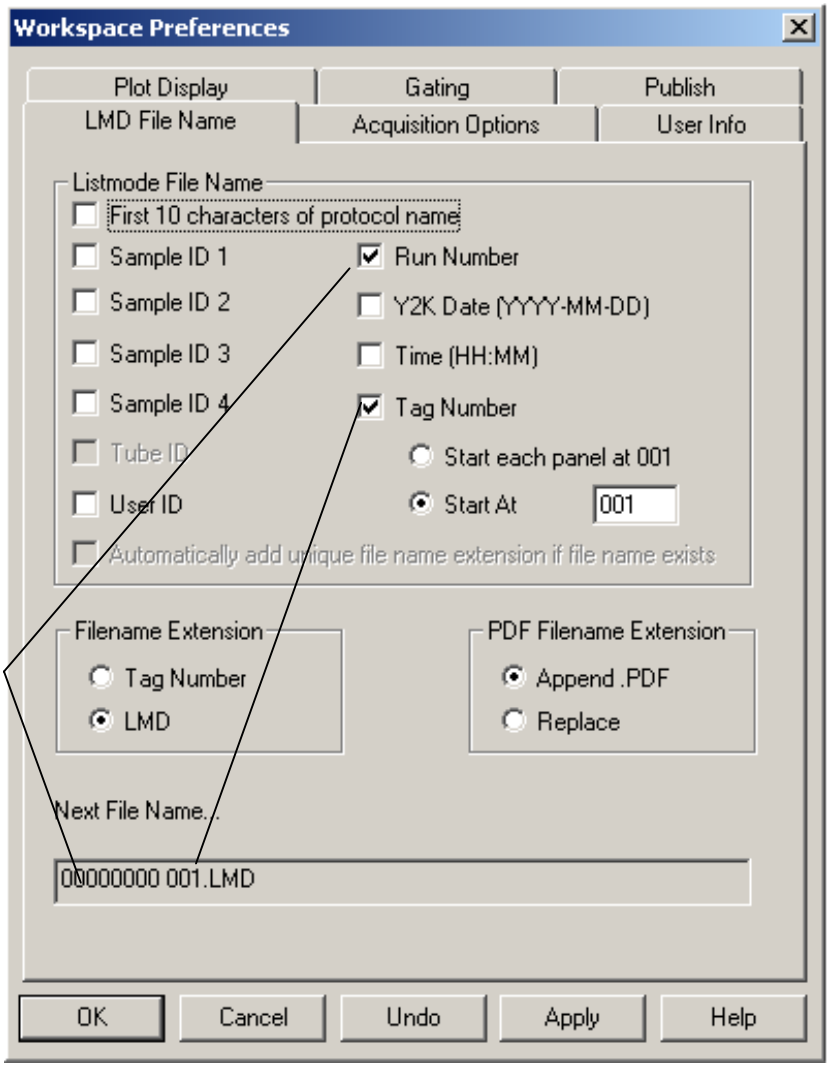
5. To select publishing preferences, select Publish.

Output data in either a text or an MS Excel format. **Columns Sheet per LMD** puts the data from one file on one worksheet. **Columns** put the data from many files on one worksheet. **Experiment Table** exports data in an experiment table format so that the data can be grouped based on some experiment criteria.	

Note: Also see step 7 with regards to setting the publishing feature on/off.

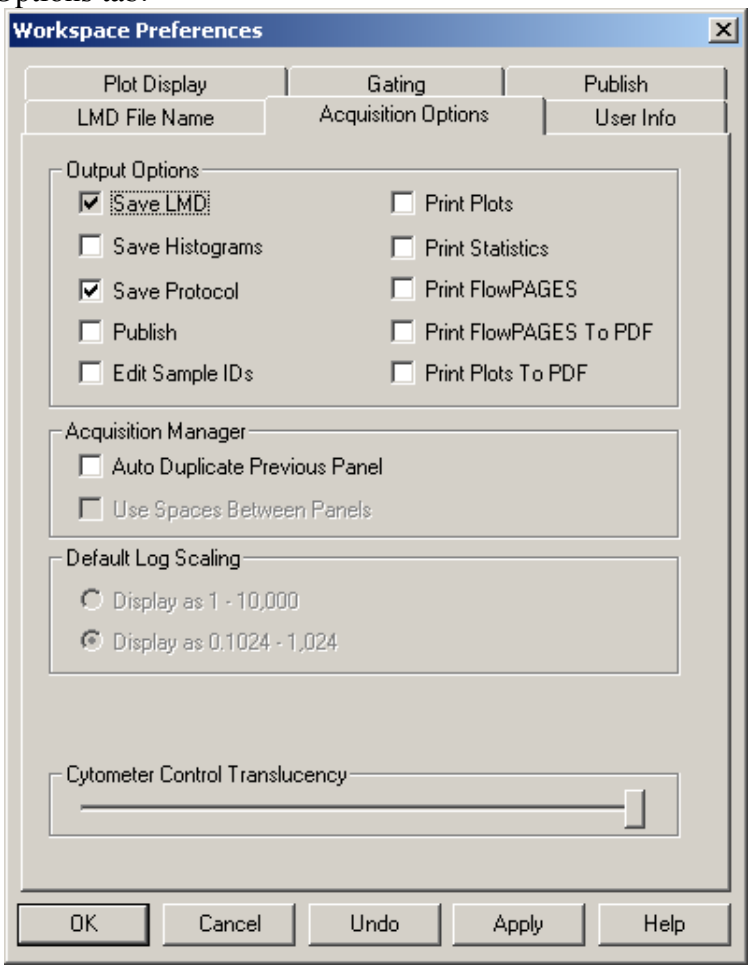
Acquisition Only Workspace Preferences

6. To change the format for the listmode file name, select LMD File Name tab.



NOTE: This window allows the operator to specify the naming scheme for storing listmode files. An example is shown in the Next File Name… box.

7. To specify automatic printing and output options select the Acquisition Options tab.



NOTE: This window controls the automatic saving, export (Publish) and printing of data. Panels can also be set to automatically duplicate with each new sample in a worklist in single tube mode.

NOTE: If you no longer wish to publish your data, you must de-select the Publish option, otherwise, all new data acquisitions will be published.

8. Select Cancel or  to close screen.

Quick Reference - Shortcuts

Drag and Drop

NOTE: Some items below you have not tried because they are options within the Acquisition software (e.g. Acquisition Manager, Worklists, Panels, etc.) or for the sake of time.

Drag From…	*Action*
Resource Explorer	
Worklist	Drag stored worklist to Acquisition Manager
Panels	Add a panel to the current Worklist
Protocols	Drag a protocol to the Workspace
Listmode Files	Drag a file to a series of plots or a plot (CTRL)
Histogram Files	Drag histograms to an overlay plot
Protocol Explorer	
Listmode Files	Drag a file to a series of plots or a plot (CTRL)
Gates	Drag a gate to all plots or a plot (CTRL)
AutoMATOR Setup	
Rearrange files	Reorder files for playback using AutoMATOR application
Modify Color Precedence	
Reorder Gates	Drag gates into the required precedence order
Plots	
Regions	Drag copies of regions from one plot to another, with CTRL Key gate logic is also copied
Plot Images	Drag the current plot image to a third party application
Overlay Plot	Reorder histograms in the overlay
Tile Special	
Plots	Reorder plots on the desktop
Cytometer Control (Cytometer Only)	
Parameters	Reorder parameters in the “Selected Signals” list
Acquisition Manager	
Worklist Columns	Reorder Worklist columns to any desired order

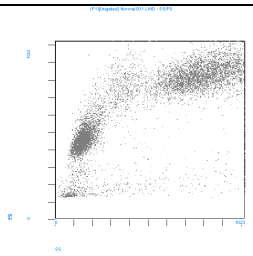
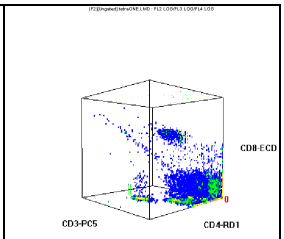
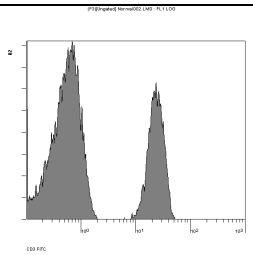
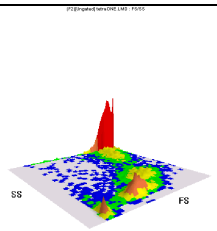
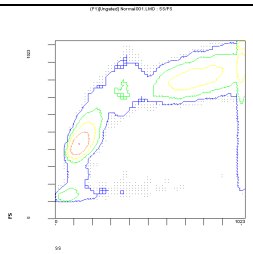
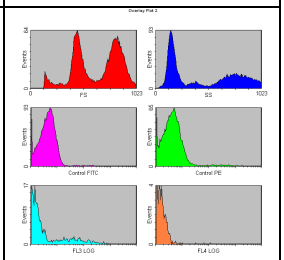
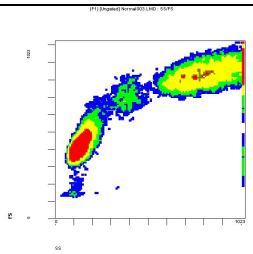
Keyboard Shortcuts in CXP Software

Hold the **CTRL** key and the desired letter or number together to initiate the shortcut.

CTRL C = Copy	CTRL 3 = Create new Contour Plot
CTRL V = Paste	CTRL 4 = Create new Density Plot
CTRL X = Cut	CTRL 5 = Create new Overlay Plot
CTRL Z = Undo	CTRL 6 = Create new Tomogram Plot
CTRL Y = Redo	CTRL 7 = Create new Surface Plot
CTRL W = Workspace Preferences	CTRL 8 = Create new Prism Plot
CTRL T = Tile Special	Ctrl 9 = Create new Legend Plot
CTRL P = Print	Alt F4 = Exit CXP Software
CTRL N = New Protocol	F1 = Context Sensitive Help
CTRL S = Save Protocol	F5 = Refresh Screen
CTRL O = Open Listmode File	F7 = Publish to Excel (or text file)
CTRL F = Format Plot	F9 = Start Acquisition (Start MCL)
CTRL D = Duplicate Plot	F10 = Stop Acquisition
CTRL 1 = Create new Dot Plot	F11 = Pause Acquisition
CTRL 2 = Create new Histogram Plot	F12 = Abort Acquisition

Identify Icons

Plots

Dot Plot 		Tomogram (Analysis only) 	
Histogram Plot 		Surface Plot (Analysis only) 	
Contour Plot (Analysis only) 		Overlay Plot (Analysis only) 	
Density Plot 			

You have now completed this module. If you feel you can reasonably navigate around in the software and can perform some common types of operations (e.g. drag and drop, select a tool from a toolbar, etc.) then please complete the skill check.

OBJECTIVES

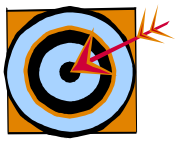
Given an operational flow cytometer, access to tetraCXP™ SYSTEM Guide, and required supplies:

- Schedule the tetraCXP TBNK FC-C and QC 1L FITC-PE-ECD-PC5 Flow Check (TM) applications.
- Prepare samples indicated in the carousel load list and run the samples as scheduled.
- Print out the QC 1L Flow Check (TM)_Align Levey-Jennings plot.
- Prepare 2 whole blood CD45-4-8-3 samples, one with Flow Count added and one without.
- Access the database per instructions in the module and enter a Sample ID1, WBC count and lymphocyte %.
- Run the tetraCXP 45-4-8-3 and tetraCXP 45-4-8-3 FC panels with the appropriate whole blood samples per instructions in the module.

WHY IS IT IMPORTANT?

This module provides step-by-step directions to set up and run a fully automated 4 color cell surface marker analysis using tetraCXP™ software for Cytomics FC 500 flow Cytometer systems CYTO-STAT tetraCHROME reagents. The total system automates the quality control process and the running of unknown samples. The process uses special protocols to standardize the system, set color compensation and verify reagent and system settings. The settings are then used to run all unknown samples.

Skill Check Preview



- You will have mastered the application when you can perform the procedures listed under objectives.
- Provide your facilitator with a hardcopy for all samples for skill check verification.

REFERENCES

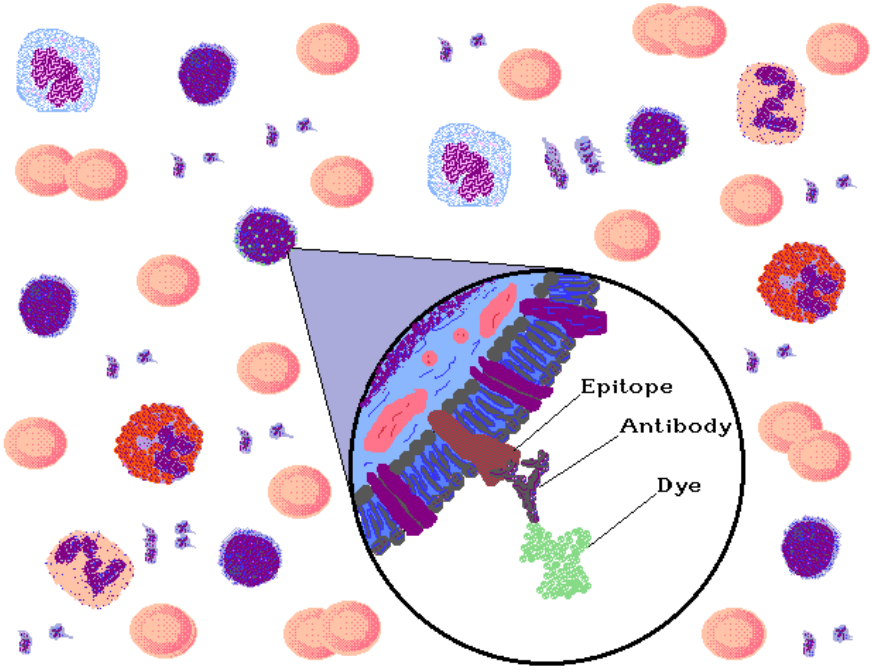
ON-line Help tetraCXP™ SYSTEM Guide.

CONCEPTS

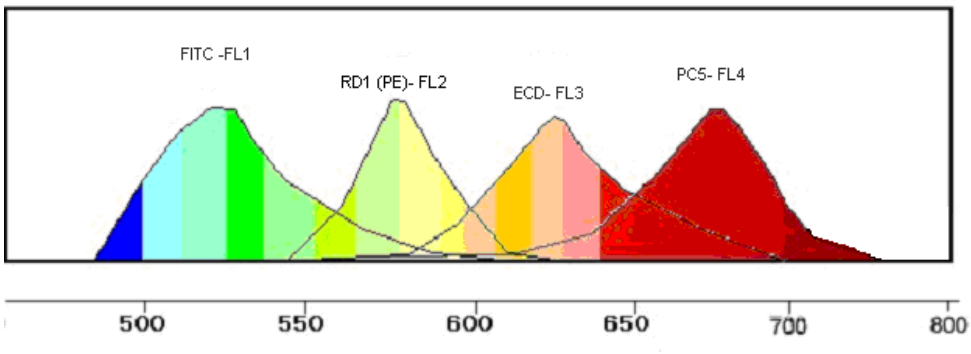
NOTE: The emphasis in this module is on the ease of running the tetraCXP application. This application will also be used to introduce some aspects of the CXP Software that will be covered in more detail in other modules. For these reasons the concept section is intended as a summary only. For a more detailed treatment, please refer to the On-line Help tetraCXP SYSTEM Guide.

The tetraCXP Software for the Cytomics FC 500 flow cytometer simplifies the process for setting up and running a set of four specific antibodies targeting lyphocyte subsets simultaneously. These antibodies are packaged as CYTO-STAT tetraCHROME™ CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and CYTO-STAT tetraCHROME CD45-FITC/CD56-RD1/CD19-ECD/CD3-PC5. An optional absolute count reagent can also be used to generate absolute counts.

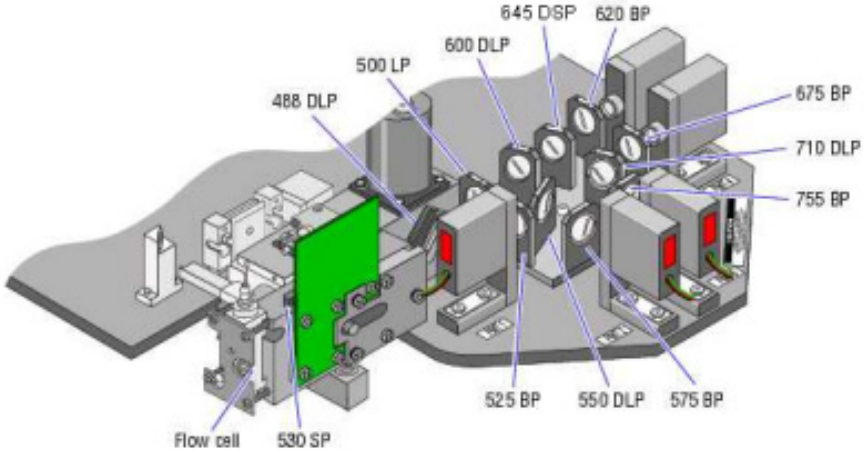
Each antibody carries a specific dye to a targeted site (epitope) on the cells as illustrated below.



The dye emission spectra and fluorescence PMTs are shown below.



The tetraCXP system requires the single laser filter block shown below. Your facilitator should have already installed the block for you. Directions on how to replace the filter block are located in the On-Line Help for the CXP Software. This task is one of the items to be completed as part of the Maintenance II module.

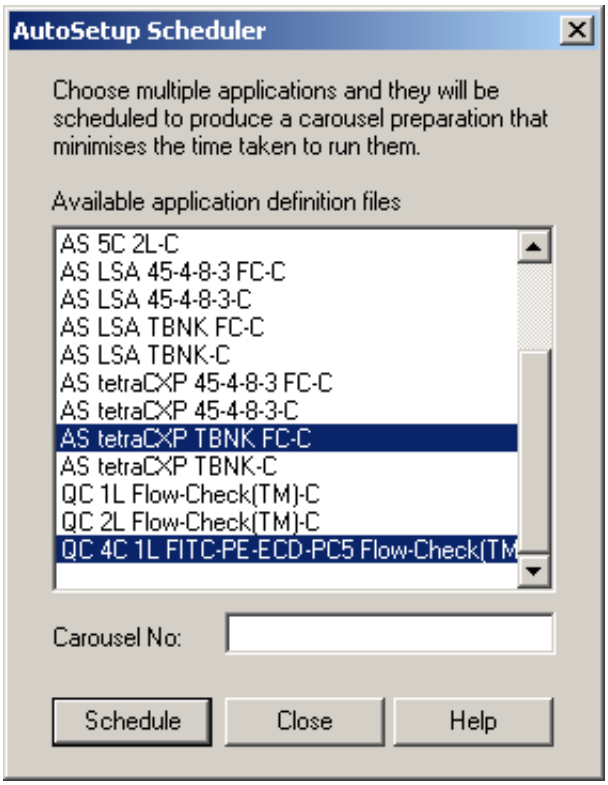


Each sample run on the system is run with a protocol. A ***Protocol*** is a complete set of instructions for generating data as well as instructions for displaying, gating and analyzing the data. The necessary protocols are included as part of the software and therefore there no need to create them. The tetraCXP protocols are enhanced with special algorithms to properly set gating and analysis. The tetraCXP application is scheduled to make sure the proper protocols are run in the correct sequence. These protocols and samples will automatically standardize the system and compensate where necessary for spectral overlap from one dye into another. Controls are also run to check for accuracy and the results can be sent to a Levey-Jennings plots and data table. Additionally, tetraCXP results from unknown samples are sent to a database that allows Patient Reports to be generated. Initially the tetraCXP databases will need to be activated. Your facilitator should already have done this for you. How to complete the task yourself is covered as part of the Database module.

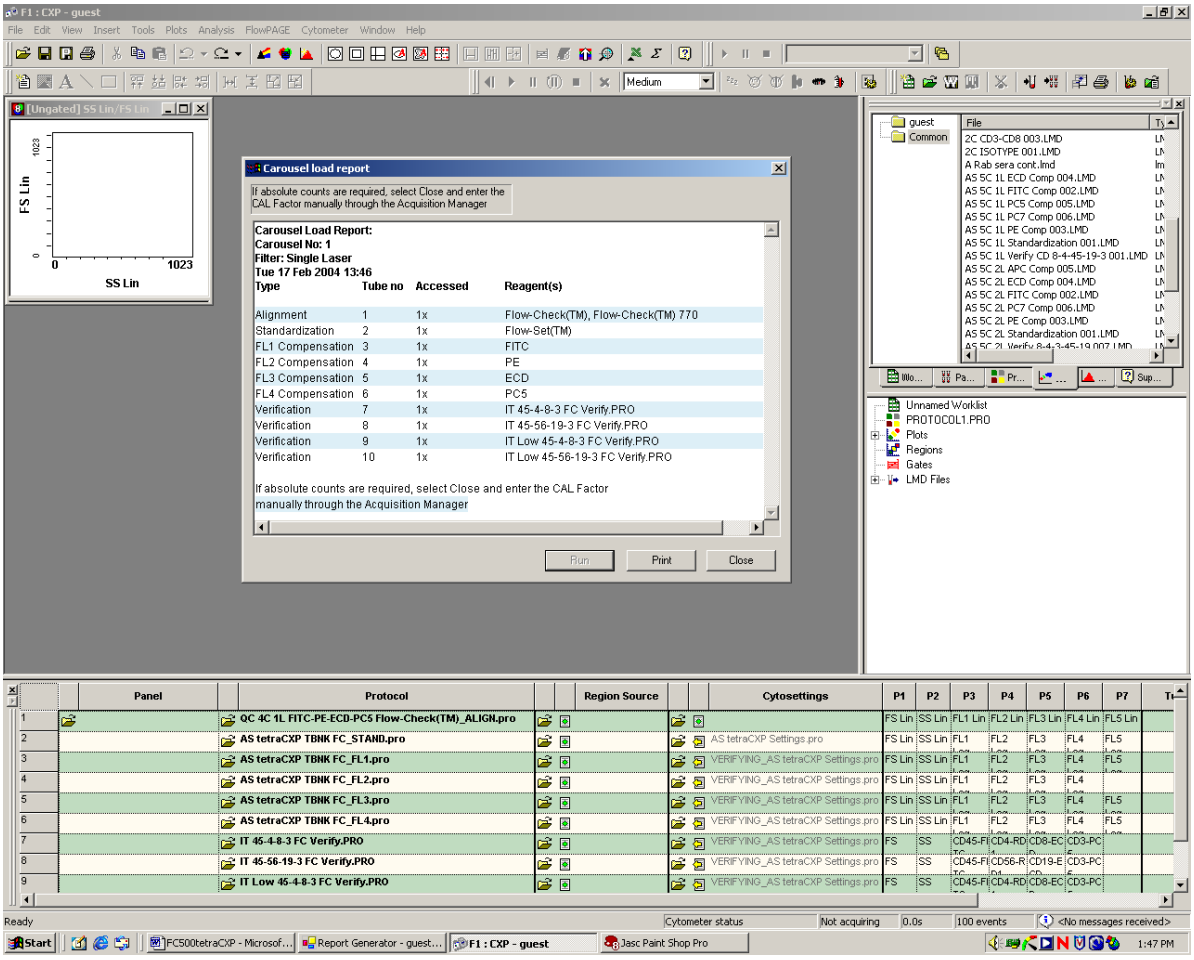
SCHEDULING

Scheduling selects the desired applications that you wish to run on the system. The appropriate protocols are then added to the Acquisition Manager in the proper sequence. The system also provides a Carousel Load report so that you will know exactly which tubes are to be placed in the carousel.

1. Select **Tools >> AutoSetup Scheduler**.
2. While holding down the Control key, select **AS tetraCXP TBNK FC-C** and **QC 4C 1L FITC-PE-ECD-PC5 Flow-Check(TM) - C.**



3. Enter the carousel number (located on the side of the carousel) and then select **Schedule.** The system lists the protocols in the proper sequence in the Acquisition Manager and the Carousel load report appears on the screen.



4. Select the Print button on the Carousel load report. You will use this list to keep track of the samples that you will need to prepare and where to place them in the carousel.
5. Select Close button.

SAMPLE PREPARATION

At this point, you will need to prepare the sample tubes for the carousel. Your facilitator will lay out the necessary reagents at the preparation area. Prepare the samples as follows:

1. Label a set of empty tubes per the Carousel load list.
2. Prepare each tube as indicated below.

   Tube 1 – Alignment – mix the bottle of Flow-Check™ fluorospheres and add 15 drops into the tube.

   Tube 2 – Standardization – mix the bottle of Flow-Set™ fluorospheres and add about 0.5 CC (about 15 drops) into the tube.

   Tubes 3-6 – Compensation –

   A. Add 20 uL of CD45 FITC into FL1 Compensation tube.
   B. Add 20 uL of CD45 PE into FL2 Compensation tube.
   C. Add 20 uL of CD45 ECD into FL3 Compensation tube.
   D. Add 20 uL of CD45 PC5 into FL4 Compensation tube.
   E. Add 100 uL of CYTO-COMP™ Cells into each of the compensation tubes.
   F. Incubate the tubes for about 10 minutes.
   G. Add 1 mL of PBS to each of the compensation tubes.

   Tubes 7-10 – Verification –

   A. Add 10 uL of tetraCHROME™ CD45-FITC/CD4-PE/CD8-ECD/CD3-PC5 to Verify tubes 7 and 9.
   B. Add 10 uL of tetraCHROME CD45-FITC/CD56-PE/CD19-ECD/CD3-PC5 to Verify tubes 8 and 10.
   C. Add 100 uL of IMMUNO-TROL™ Cells to tubes 7 and 8.
   D. Add 100 uL of IMMUNO-TROL Low Cells to tubes 9 and 10.
   E. Place the tubes in a carousel and then on the T-QPREP™ Workstation.
   F. Select the hour glass symbol on the T-QPREP.

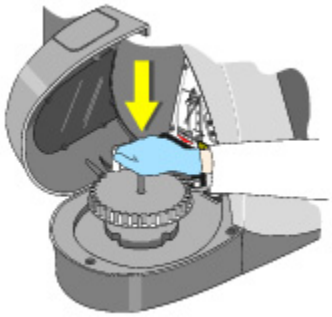
   Note: If you need help, ask your facilitator.

   G. After the T-QPREP has finished, add 100 uL of Flow-Count fluorospheres to tubes 7-10.

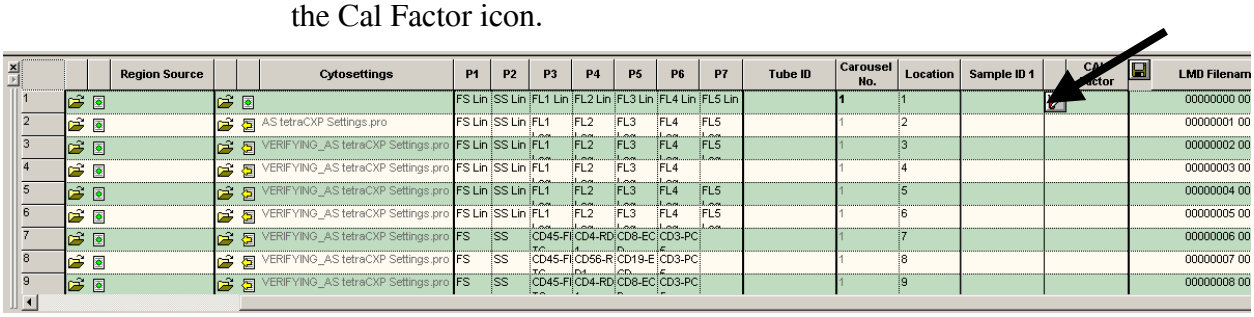
3. Place the finished tubes in the order listed on the Carousel load report in the carousel.

RUNNING SAMPLES

1. Open the MCL cover of the FC 500 and place the prepared carousel on the MCL.

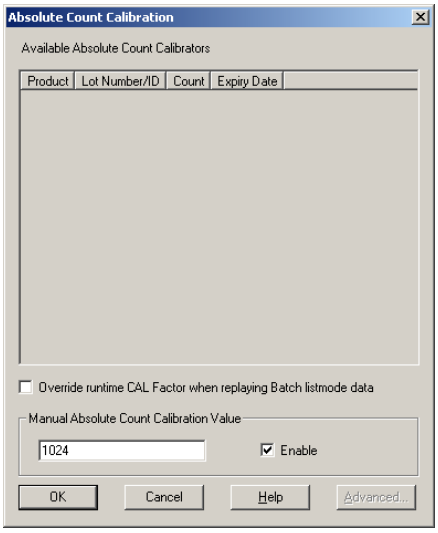


2. Close the MCL cover.
3. Obtain the Flow-Count calibration factor from your facilitator.
4. Scroll the Acquisition Manager (scroll bar below) to the right and select the Cal Factor icon.

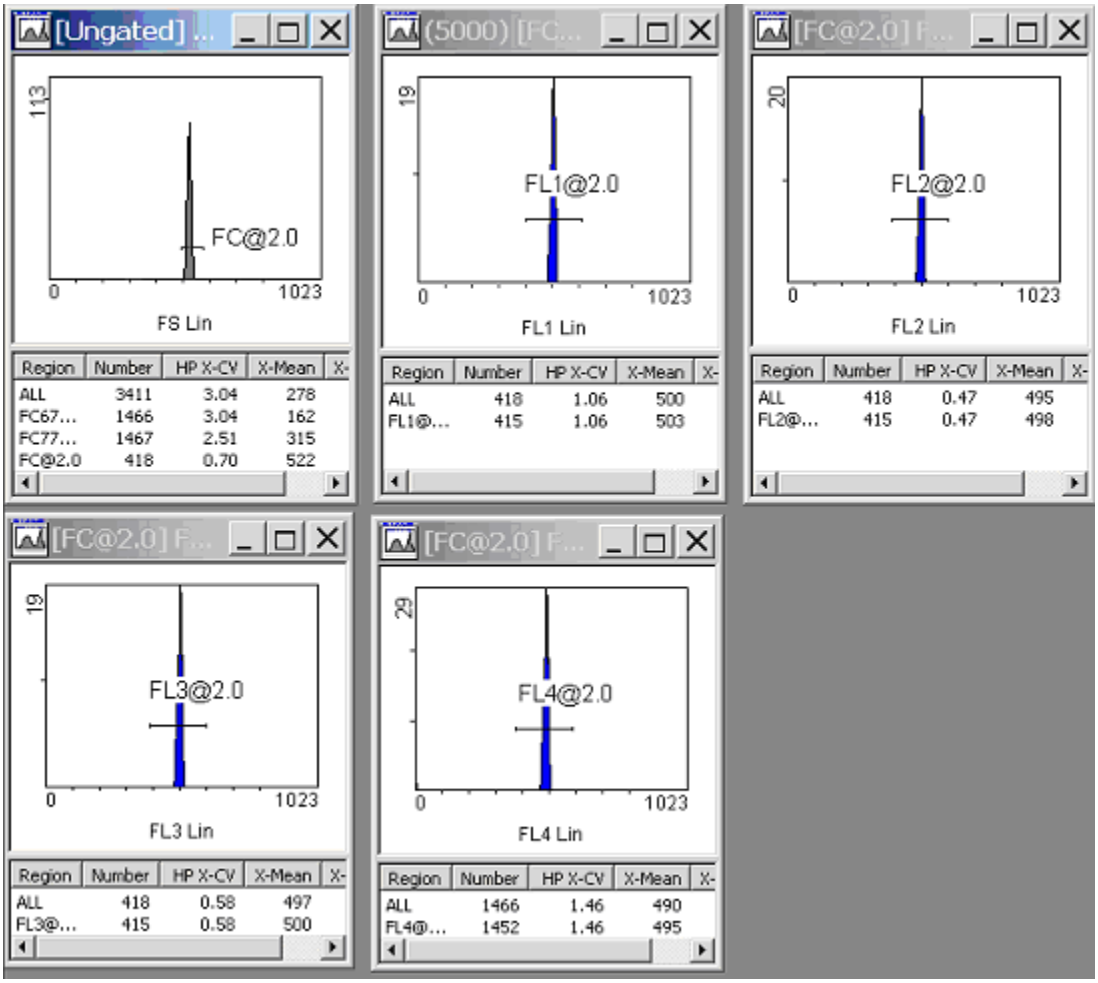


	Region Source	Cytosettings	P1	P2	P3	P4	P5	P6	P7	Tube ID	Carousel No.	Location	Sample ID 1	Cal Factor	LMD Filenam
1			FS Lin	SS Lin	FL1 Lin	FL2 Lin	FL3 Lin	FL4 Lin	FL5 Lin		1	1			00000000 00
2		AS tetraCXP Settings.pro	FS Lin	SS Lin	FL1	FL2	FL3	FL4	FL5		1	2			00000001 00
3		VERIFYING_AS tetraCXP Settings.pro	FS Lin	SS Lin	FL1	FL2	FL3	FL4	FL5		1	3			00000002 00
4		VERIFYING_AS tetraCXP Settings.pro	FS Lin	SS Lin	FL1	FL2	FL3	FL4			1	4			00000003 00
5		VERIFYING_AS tetraCXP Settings.pro	FS Lin	SS Lin	FL1	FL2	FL3	FL4	FL5		1	5			00000004 00
6		VERIFYING_AS tetraCXP Settings.pro	FS Lin	SS Lin	FL1	FL2	FL3	FL4	FL5		1	6			00000005 00
7		VERIFYING_AS tetraCXP Settings.pro	FS	SS	CD45-FI	CD4-RD	CD8-EC	CD3-PC			1	7			00000006 00
8		VERIFYING_AS tetraCXP Settings.pro	FS	SS	CD45-FI	CD56-R	CD19-E	CD3-PC			1	8			00000007 00
9		VERIFYING_AS tetraCXP Settings.pro	FS	SS	CD45-FI	CD4-RD	CD8-EC	CD3-PC			1	9			00000008 00

5. Type in the calibration factor and select **OK**. The system will fill in the Cal Factor in the Acquisition Manager.

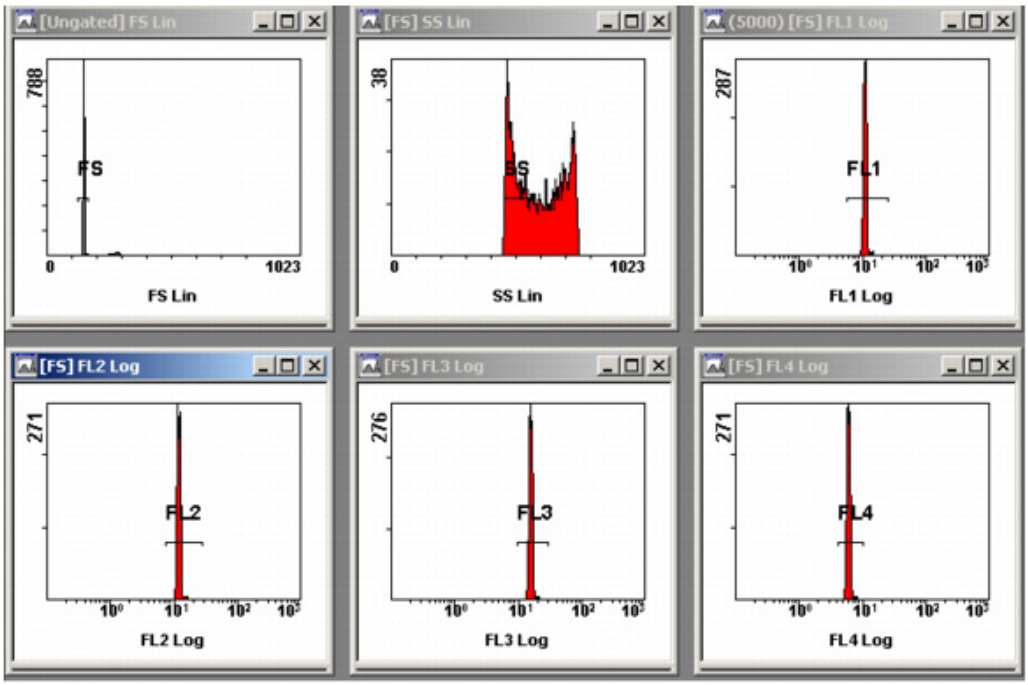


6. Select the  button on the Cytometer toolbar. The carousel will now move into position and begin running tube 1. The data will appear as shown below. Each tube in sequence will print a special report called a FlowPAGE.

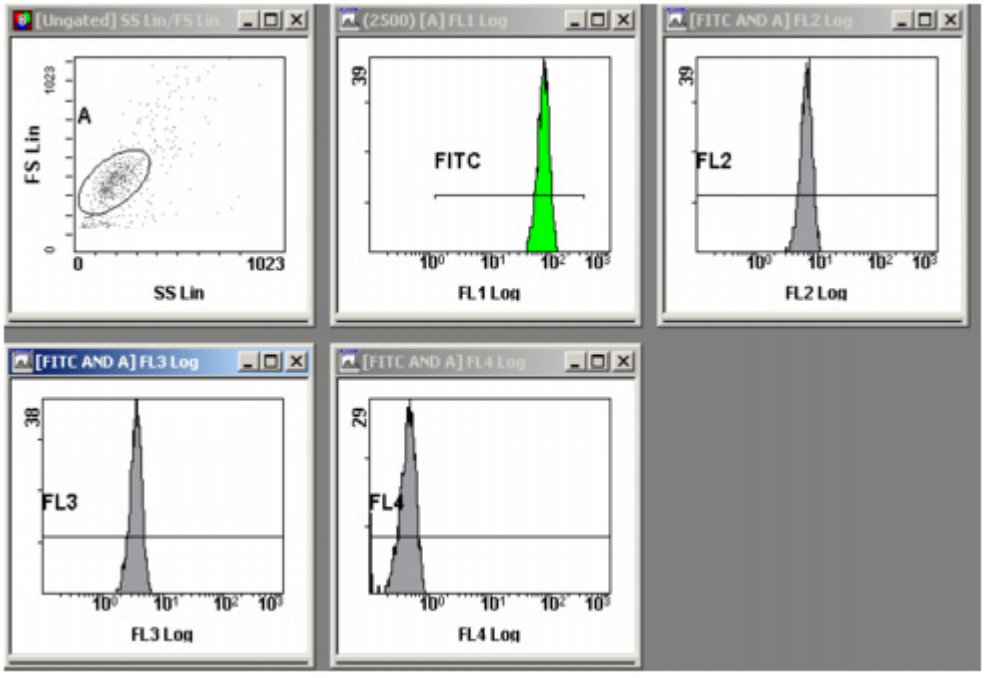


7. If the CV's are too high or the data has shifted, select the  button on the Cytometer toolbar. The system will prime the flow cell with sheath and then restart data collection. You can repeat the process a couple of times if necessary. If you still do not have good CV's, inform your facilitator. The Autosetup Wizard will prompt you to Rerun, Abort or Approve if the Flow-Check HPCVs exceed the limits (defined in the Region names). If the HPCVs are within the limits, Autosetup Wizard will carry on to the next tube in the carousel.

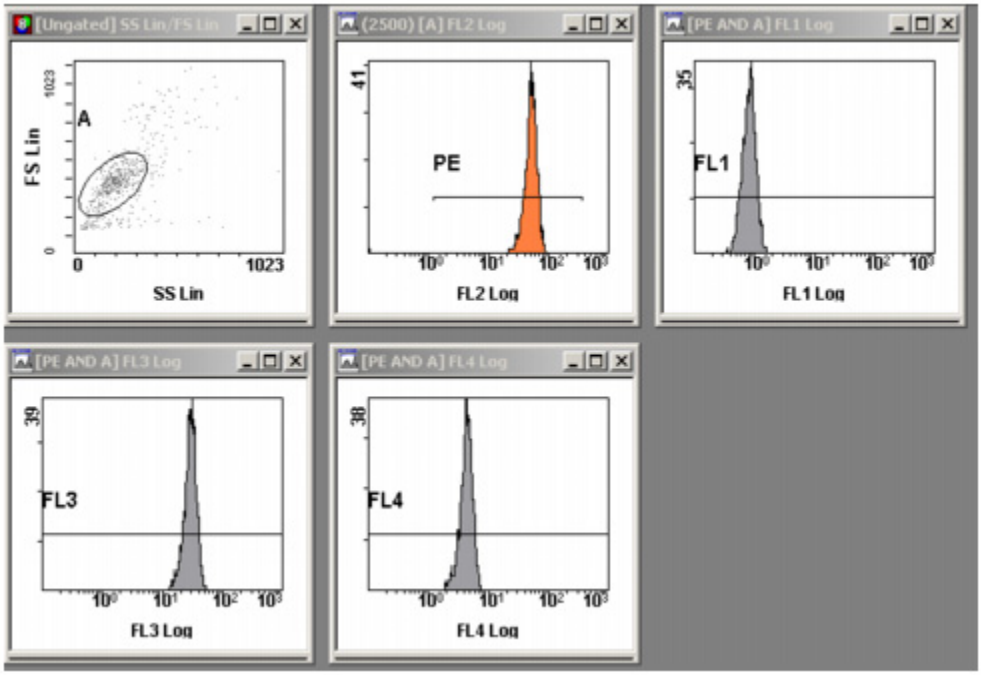
8. The system will now pickup tube 2, Flow-Set. Just watch. The system will adjust itself and the data will appear as below.



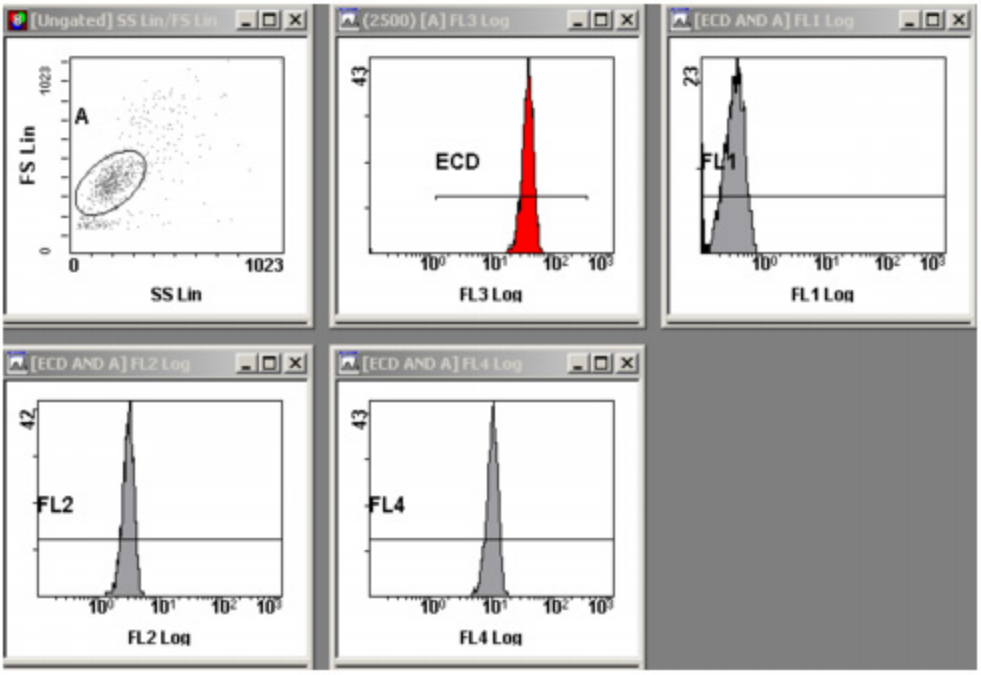
9. When finished with Flow-Set, the system will pickup tube 3 to measure the FITC dye interference into the other fluorescence channels. The data will appear as below.



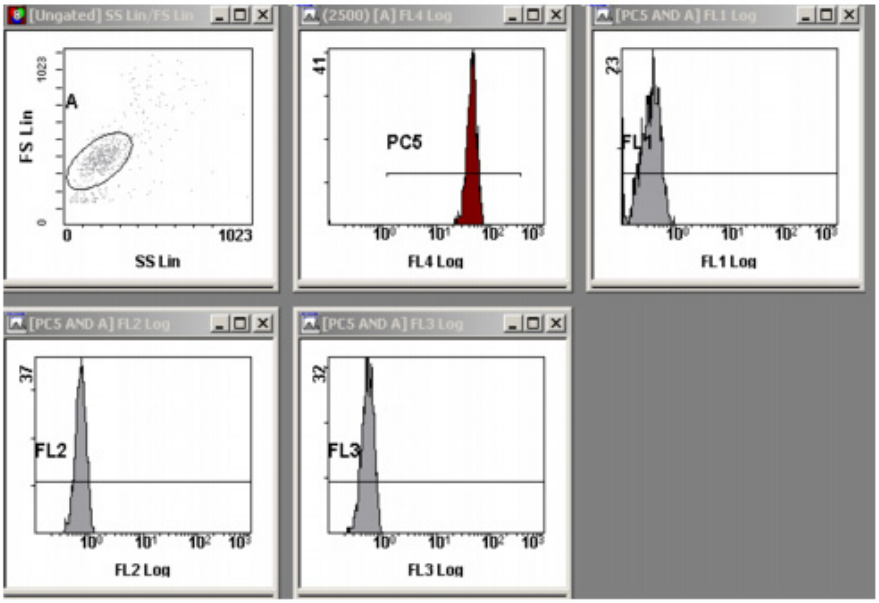
10. When the FITC tube is finished, the system will pickup tube 4 to measure the PE dye interference into the other fluorescence channels. The data will appear as below.



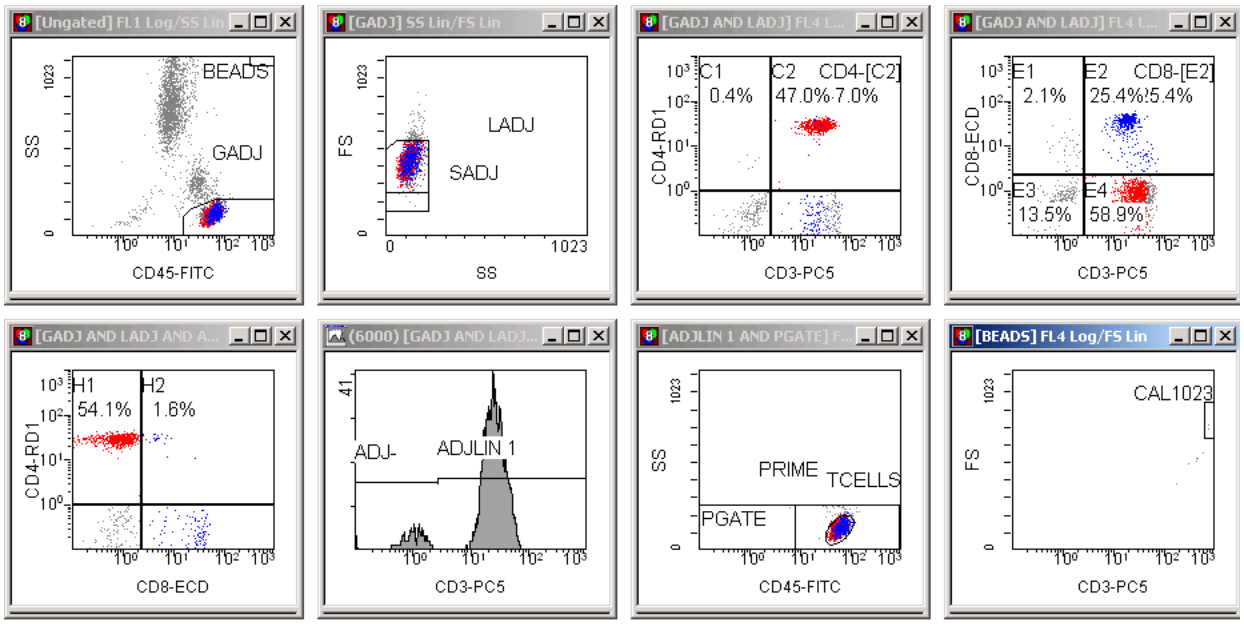
11. When the PE tube is finished, the system will pickup tube 5 to measure the ECD dye interference into the other fluorescence channels. The data will appear as below.



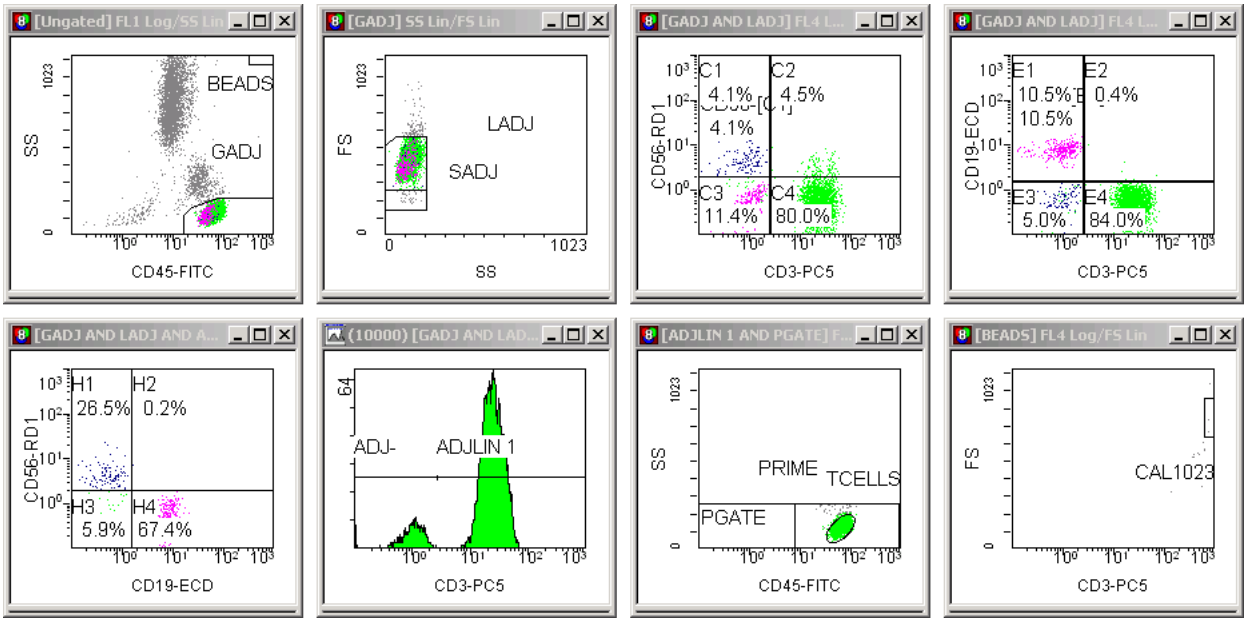
12. When the ECD tube is finished, the system will pickup tube 6 to measure the PC5 dye interference into the other fluorescence channels. The data will appear as below.



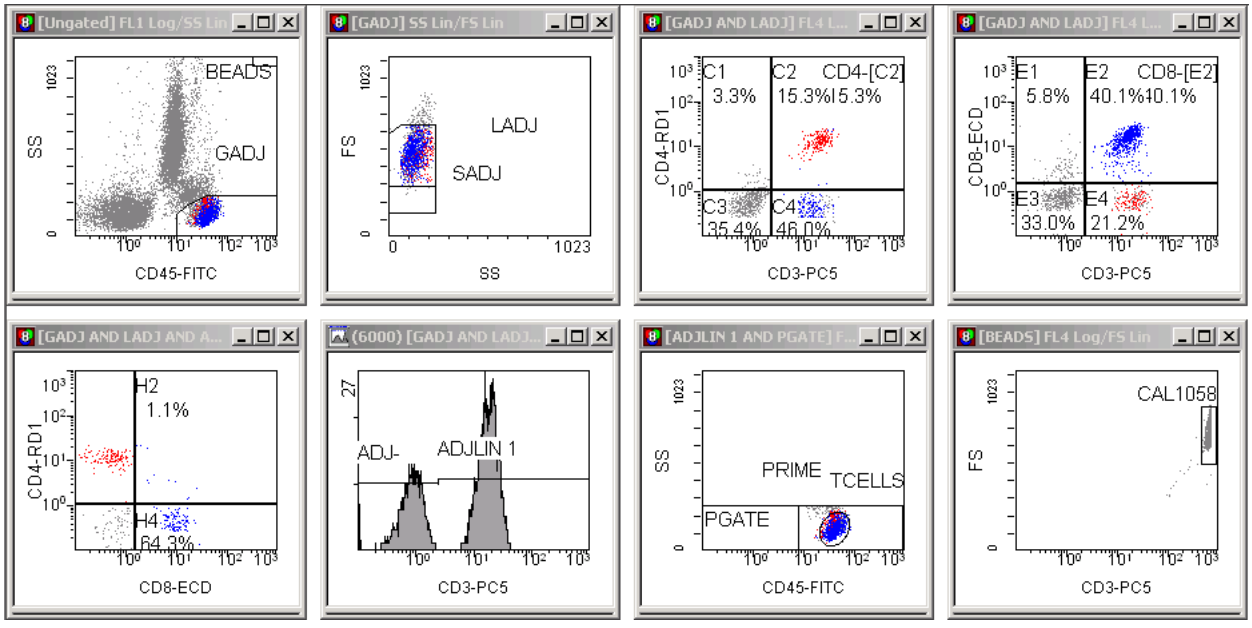
13. When the PC5 tube is finished, the system will pickup tube 7, the first of 4 IMMUNO-TROL samples. Just watch. The software calculates and sets the compensation values at this point. Then the system will gather some data and adjust the gating and analysis automatically. The data will appear as below.



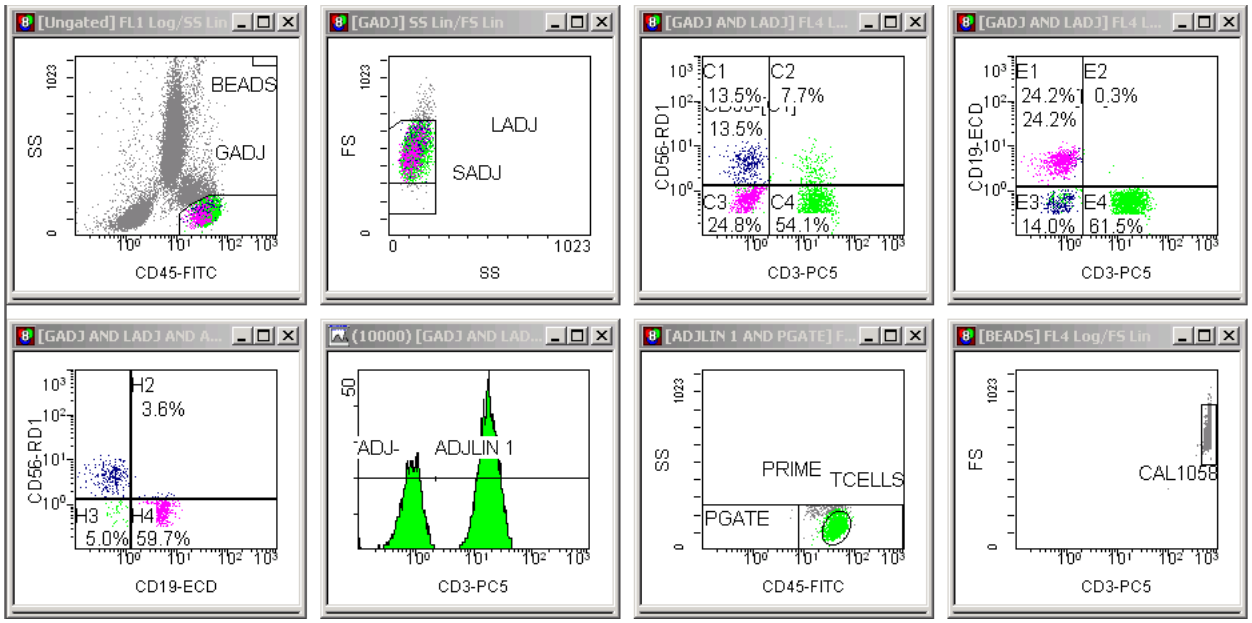
14. The second IMMUNO-TROL sample is picked up and run. The system again gathers some data and adjusts the gating and analysis automatically. The data will appear as below.



15. The third IMMUNO-TROL sample is picked up and run. The system again gathers some data and adjusts the gating and analysis automatically. The data will appear as below.



16. The fourth IMMUNO-TROL sample is picked up and run. The system again gathers some data and adjusts the gating and analysis automatically. The data will appear as below.



17. Gather your FlowPAGE printouts and place them at the end of this module as example data. Also ask your facilitator for the assay sheets for IMMUNO-TROL and IMMUNO-TROL Low. Compare your results against the assay values. Alert your facilitator for any that appear out of tolerance.

Note: The settings (e.g. High Voltage, Gains, and compensation) will now be stored in the AS tetraCXP Settings.pro. This protocol will be used later when you run patient samples.

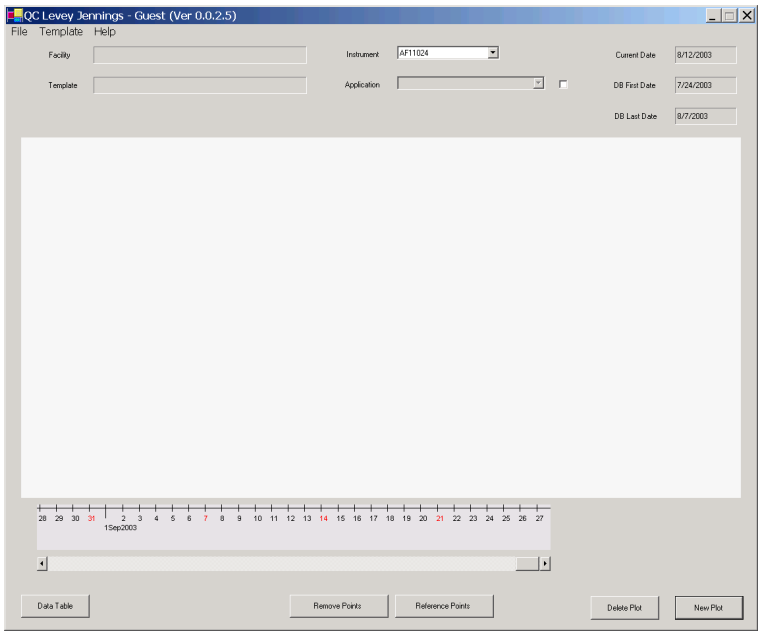
PREPARING PATIENT SAMPLES

1. Label 2 tubes: CD45/CD4/CD8/CD3 and CD45/CD4/CD8/CD3-FC.
2. Add 10 uL of tetraCHROME CD45/CD4/CD8/CD3 to each tube.
3. Add 100 uL of blood to each tube.
4. Place the tubes on the T-QPREP and select the hourglass icon (the T-QPREP waits 10 minutes and then completes the prep process.
5. After the T-QPREP has finished, add 100 uL of Flow-Count to the tube labeled CD45/CD4/CD8/CD3-FC. Note: You may go on and review the QC data in the next section while you are waiting for the T-QPREP tofinish.

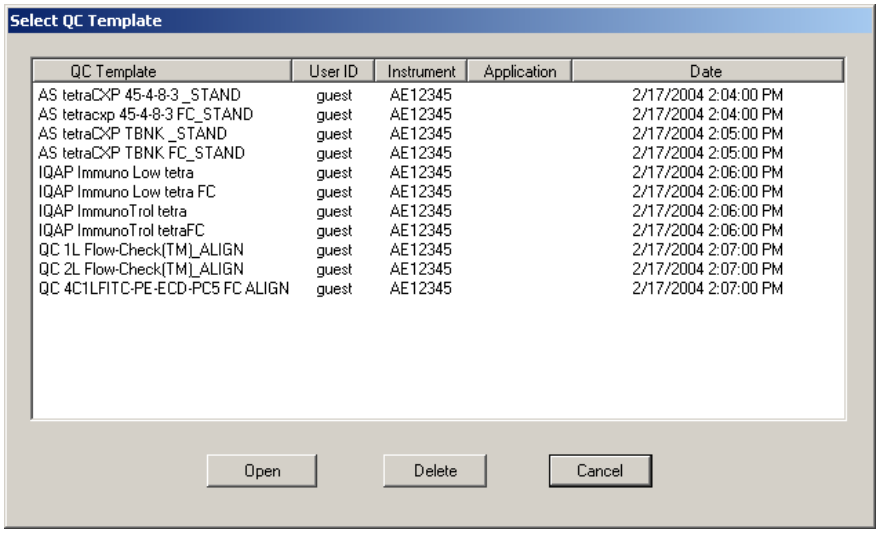
ACCESSING THE DATABASE

The system has already been set up properly and the appropriate Panel Report Templates in the database are activated (you will learn how to do this in the Database module), Flow-Check and IMMUNO-TROL results will be sent to their respective QC templates. You can then review the data as Levey-Jennings plots.

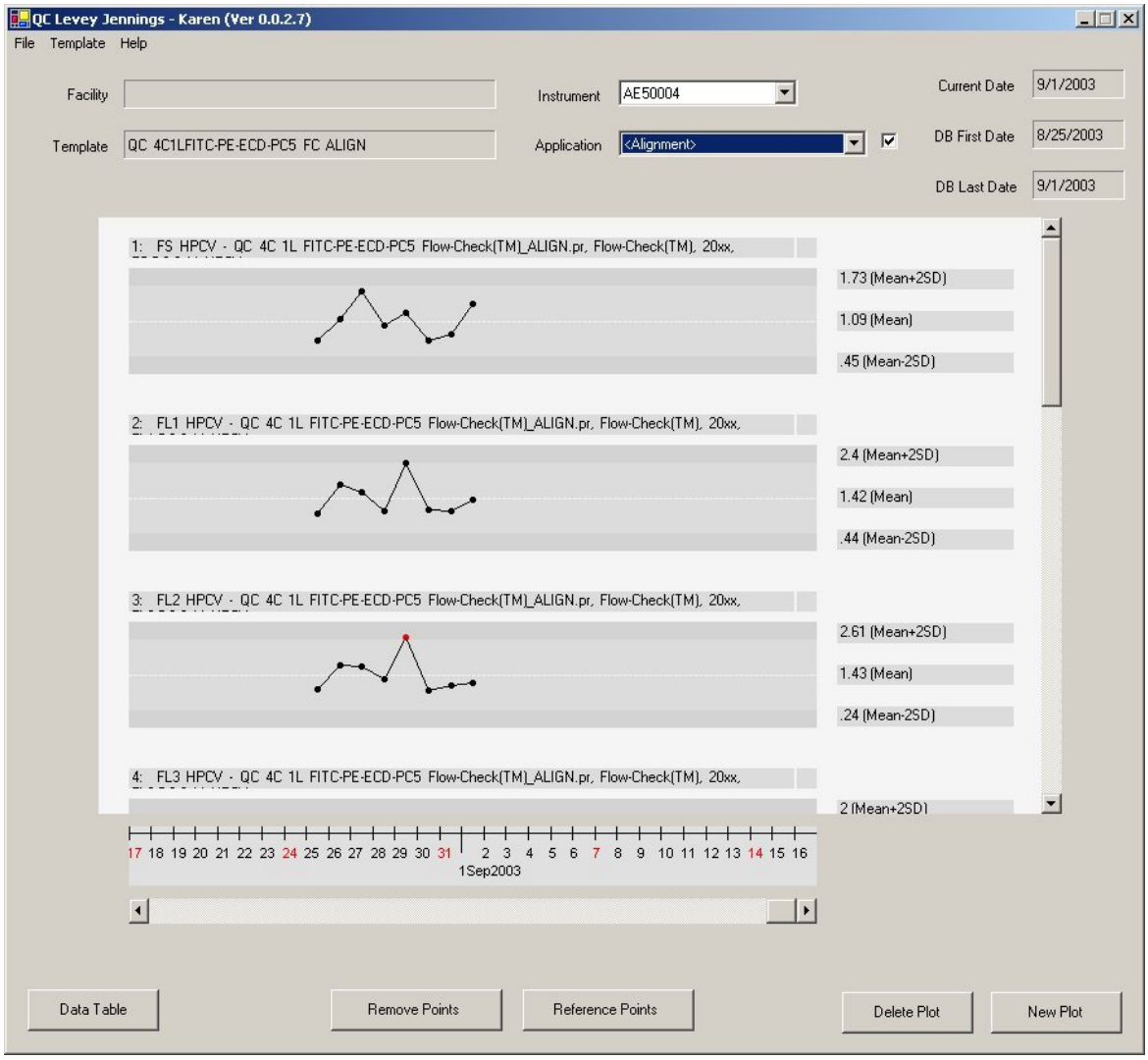
1. Select the Report Generator toolbar at the bottom of the screen and then the  icon. The screen will respond as below.



2. Click on **Template** >> **Select Template**. The screen will respond as below.



3. Select the QC 1L Flow-Check(TM)_ALIGN template and then **Open**. The screen will respond as below.

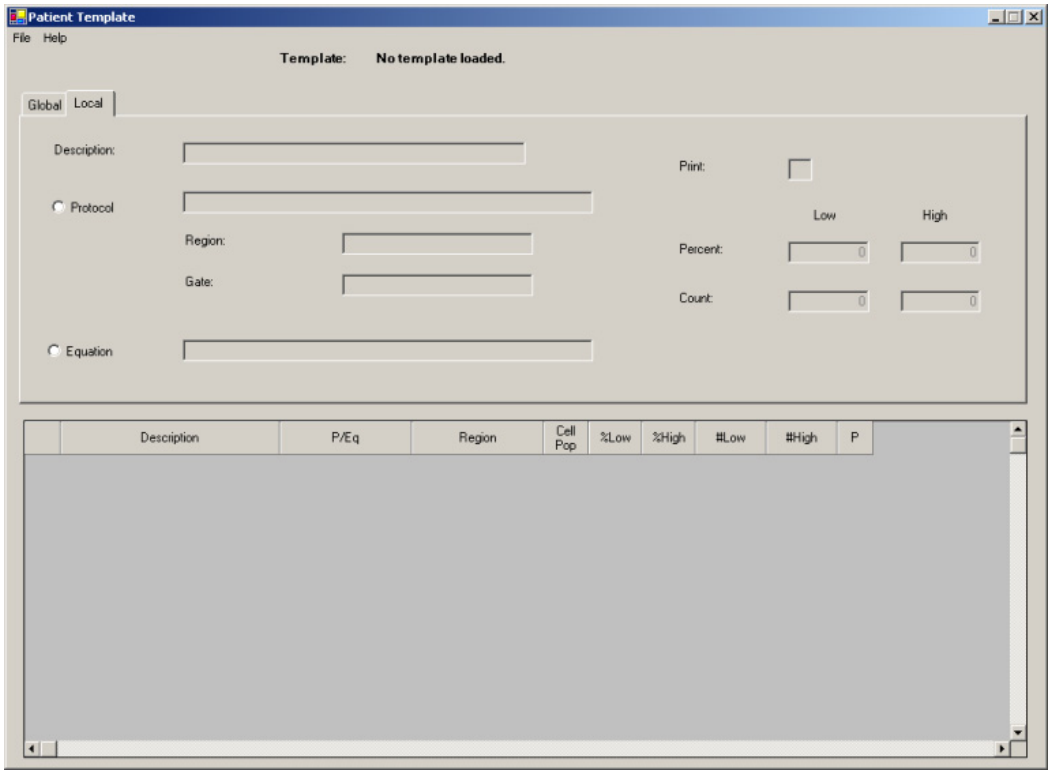


At this point you could review the QC data. You will cover the options in more detail in the Database module. For now, we are just introducing you to the basic sequence of events as you might interact with the system. You could also review the IMMUNO-TROL data in a similar fashion. You can spend a few minutes in the templates, if you wish, but do not spend a long time reviewing them. The Database module will cover the material as well as other options in more detail when you get to that module.

4. When you are finished, select the  button in the upper right corner of the window.

In preparation for running the samples you have prepared, you will now activate an automatic printing of the tetraCHROME blood sample results on a Panel report template. This template is located in another part of the database.

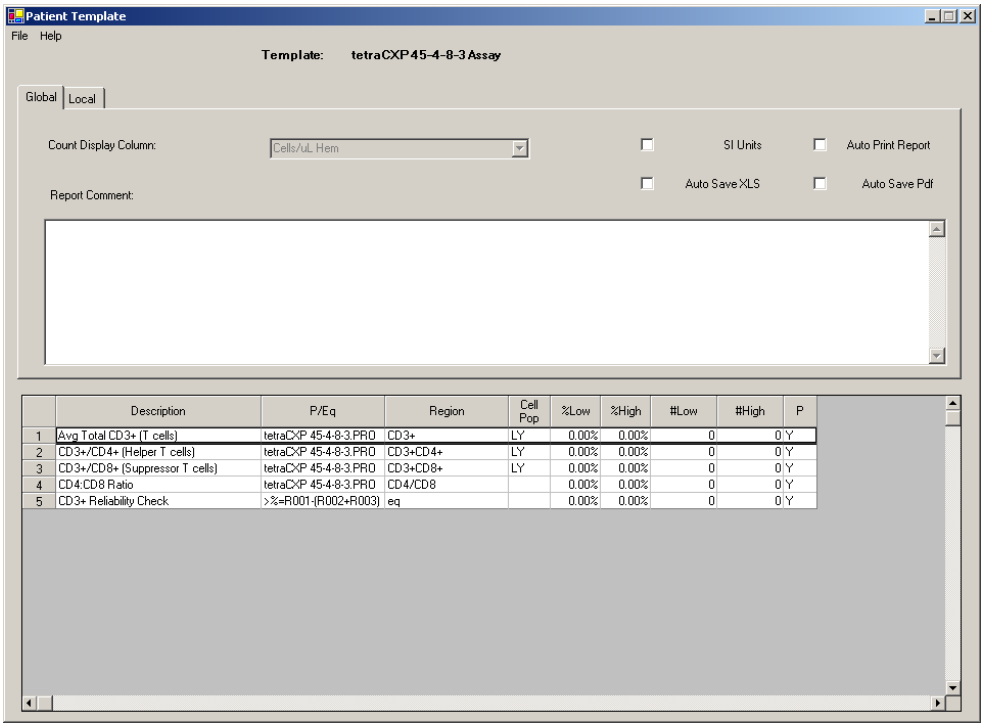
5. Select the  icon on the Report Generator. The screen will respond as shown below.



6. Select File >> Open. The screen lists the templates available in the current user directory (probably none).

7. Select the  icon twice. Now select the Admin folder, then the Panel folder, and then tetraCXP folder.

8. Select the tetraCXP 45-4-8-3 Assay and then **Open**. The screen will respond as below.



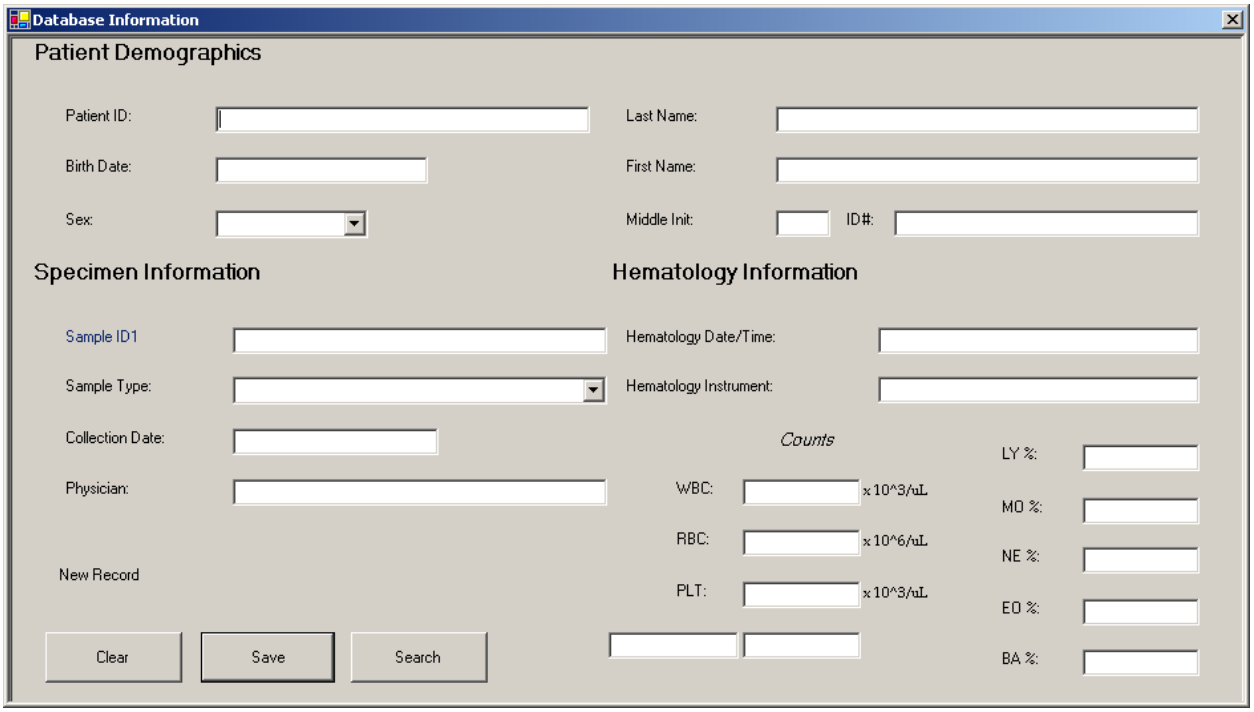
9. Select Auto Print Report. And then **File** >> **Save**. When you run your blood samples in the next section, the results will print according to the template.

10. Select **File** >> **Exit.**

Note: This step would need to be done one time, when initially setting up.

We will now do one more thing before you run your samples. Did you notice in your sample preparation earlier you prepared one sample with Flow-Count fluorospheres and the other without? Flow-Count is used in the protocol to help obtain an absolute count of sub-populations. You can also obtain an absolute count by going into the database and entering hematology data ahead of time so that the system can use this data to calculate the absolute count.

11. Select the  icon on the Report Generator toolbar. The screen will respond as below.

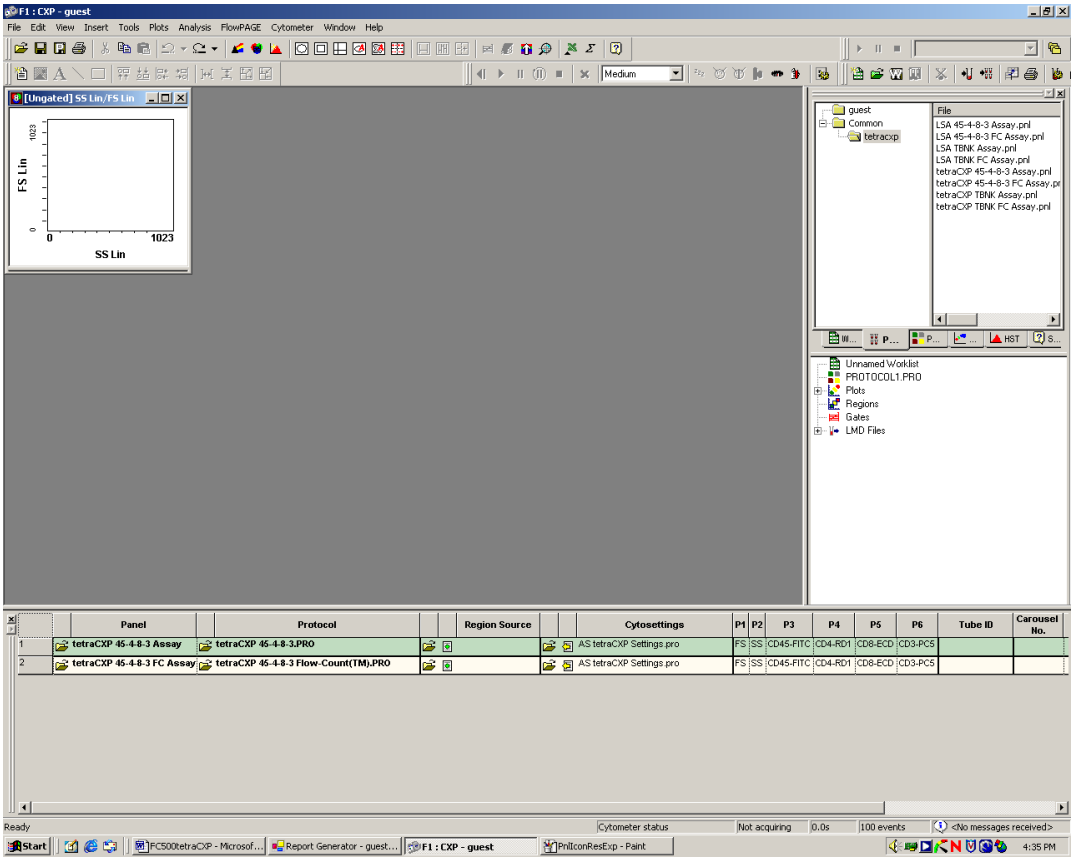


12. Type in a Sample ID1 and record your entry here (you will need it later) _____.
13. Type in a WBC count (ex. 5.0) and then a lymphocyte % (ex. 30).
14. You can enter in some other information, if you wish. When you are finished, select **Save**.
15. Select the  icon in the upper right corner and then minimize the Report Generator toolbar. The database options are covered in more detail in the Database module.

RUNNING THE BLOOD SAMPLES

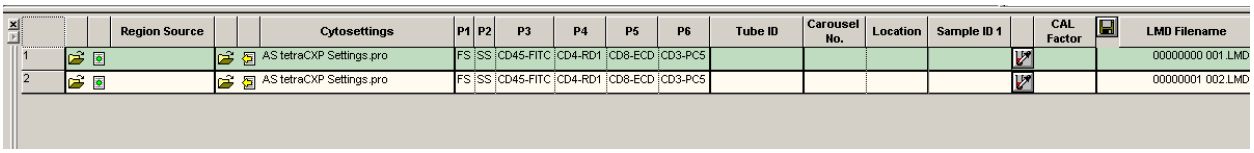
You will now run the samples you prepared earlier using the tetraCXP panels that come with the system. Panels consist of one or more protocols set up in a special way in the Acquisition Manager to optimize the running of samples. Creating the panels is covered in the Acquisition Manager module. For now we will use the ones that come with the tetraCXP application.

1. Select the  icon on the Acquisition Manager toolbar (top right part of the screen). The Acquisition Manager will clear of all previously run or programmed protocols.
2. Select the  tab on the Resource Explorer.
3. Select the + sign next to the Common folder on the Resource Explorer. The tetracxp folder will appear.
4. Select the tetraCXP folder. The tetraCXP panels will appear.
5. Drag and drop the tetraCXP 45-4-8-3 Assay panel to the Acquisition Manager.
6. Drag and drop the tetraCXP 45-4-8-3 FC Assay to the Acquisition Manager. Except perhaps for the Workspace, your screen will appear as below.



Note: The system standardized and stored the high voltage, gain, and compensation settings in the AS tetraCXP Settings.pro file when you scheduled and ran the tetraCXP samples earlier. Look at the Cytosettings column on the Acquisition Manager. The panels will retrieve these settings when they are run for use in the tetraCXP protocols.

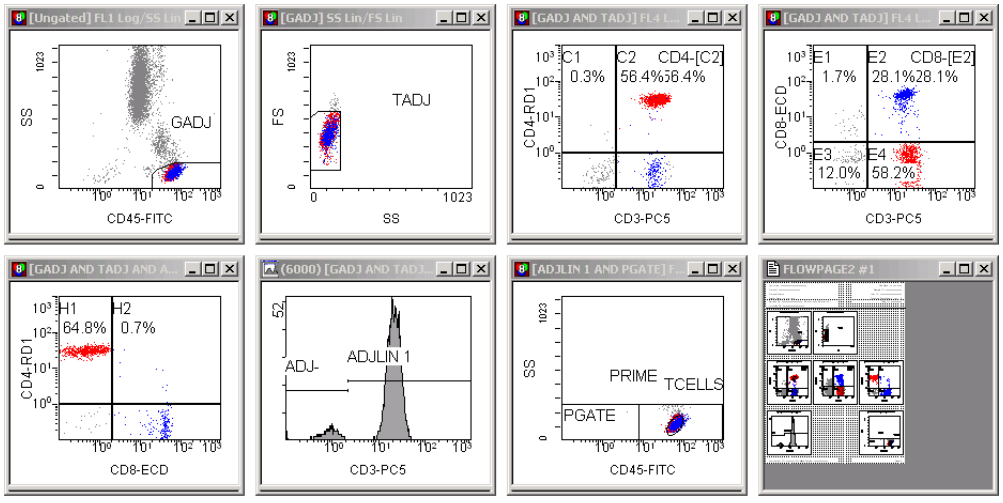
7. Scroll the Acquisition Manager to the right (lower right side) until the Sample ID1 and Cal Factor columns are visible as shown below.



	Region Source	Cytosettings	P1	P2	P3	P4	P5	P6	Tube ID	Carousel No.	Location	Sample ID 1	CAL Factor	LMD Filename
1		AS tetraCXP Settings.pro	FS	SS	CD45-FITC	CD4-RD1	CD8-ECD	CD3-PC5						00000000 001.LMD
2		AS tetraCXP Settings.pro	FS	SS	CD45-FITC	CD4-RD1	CD8-ECD	CD3-PC5						00000001 002.LMD

8. Type in the Sample ID1 you entered earlier in the Database (Step 12 of the previous exercise) for the first Sample ID. The system will use the ID to link the results from tube one to the hematology information you entered into the database to determine an absolute count.
9. Ask your facilitator for the Flow-Count Cal Factor and enter it into the Cal Factor column for the second tube. The system will use the Flow-Count fluorospheres you pipeted into the second tube and the Cal Factor to determine absolute counts for the second tube.
10. Click on the Carousel No. box for the first tube and enter the Carousel number. The tube location column will fill in automatically.
11. Place your labeled tubes into their proper positions on the carousel and place the carousel on the MCL.

18. Select the  button on the Cytometer toolbar. The system will begin running the tubes in turn with their respective panels. Just watch. The system gathers some data and adjusts the gating and analysis automatically. The data will appear as below.



If Flow-Count fluorospheres are present (2nd sample's tube), they will appear in the bead region on the first plot and be used to calculate the absolute count. The absolute count for the first tube will be calculated based on the hematology information you entered earlier in the database. Both tubes will automatically print Panel Reports.

You have now completed this module. The printouts from all of the samples is your proof and will be taken as the skill check. Before you leave this module, though, take a moment or two to read through the summary on the next page. By completing this module, you have been exposed to a little bit of the content in most of the other modules (with the exception of some applications). The idea is to give you a bigger picture of the instrument capabilities. You will fill in the gaps in your knowledge as you complete other modules on the map.

SUMMARY

At this point you will have completed several modules leading to the tetraCXP module:

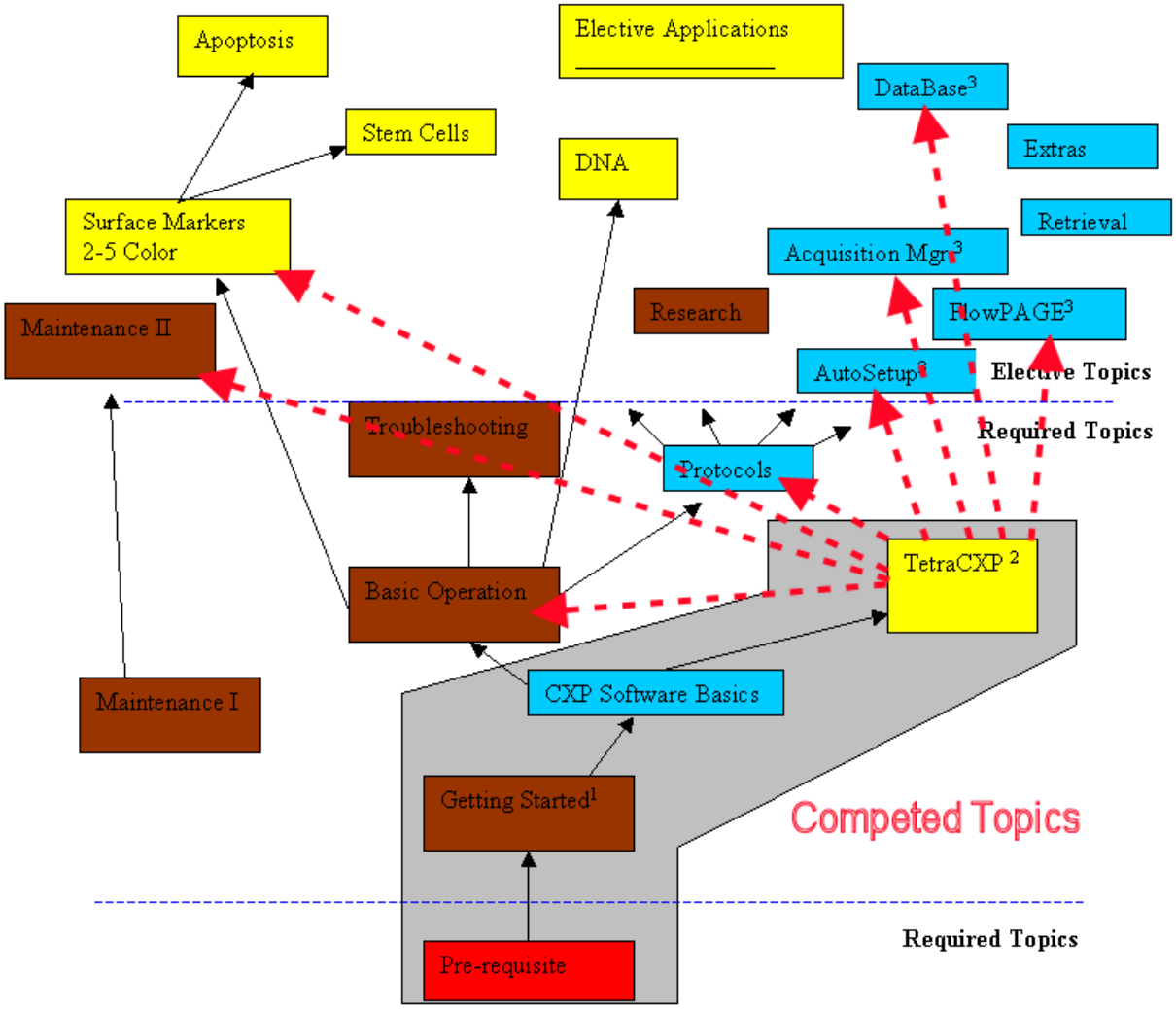
- ***Pre-requisite Flow Principles animation*** – These animations are intended to provide the basic concepts in flow cytometry and apply them to the FC 500 system. These concepts are foundational to your understanding of the system operation and your interaction with the system. Some students find it helpful to review these animations again after interacting more with the system.
- ***Getting Started*** – This module exposed you first to the instrument hardware. Then the module introduced some basic instrument and software procedures: startups, logon and shutdown.
- ***Software Basics*** – This module lays out the software screen and the basics on how to navigate through the various options. Some of the conventions were explained such as the Windows conventions, or the use of tools on toolbars as shortcuts to menu options, or the concept of dragging and dropping to accomplish a task. The module was not meant to make you proficient in every part of the software but rather to overview the software. Other modules will cover specific tasks in more detail.

In this tetraCXP module you have now set up, run, and printed out the results for an application. This application was chosen to run first because it is pre-programmed for ease of setting up, running, and analyzing the data. In doing so, you have practiced some software tasks and either touched on or accessed some of the software options. Many of these options are explained in more detail in other modules. For example:

- Cell Surface Marker concepts – explained more completely (including the concept of Flow-Count) in the Cell Surface Marker module.
- Filter block – replacement is covered as part of the Maintenance module.
- Application – Creating an application involves several tasks. You must know how to adjust the system such as high voltage and gains to establish the correct plot patterns as well as establish a mechanism for ensuring the patterns remain the same over time (Basic operation module). In addition, plots, regions, gating, and analysis as well as the instrument settings must be stored in a protocol (Protocol module).
- Scheduling applications – Entering application set up information and scheduling is explained in more detail in the AutoSetup module.
- Flow-Check – This sample checks the instrument and is always the first sample to be run. It is covered again in the Basic Operation module and examples of bad data is part of the discussion in the Troubleshooting module. Plotting and reviewing data is covered in more detail in the Database module.

- FlowPAGES – How to create these special report forms is covered in detail in the FlowPAGE module.
- Panels – How to create panels from protocols is covered in the Acquisition Manager module as well as creating longer worklists.
- TetraCXP reports – Modifying these reports is part of the Database module.

This one module provides an introduction to some of the tasks you may wish to learn. Not all of these tasks need to be mastered all at once. Like working with any other Windows programs (e.g. Microsoft™ Word, Excel, PowerPoint, etc.) you need to learn what you need to learn to do what you need to do. Other tasks, including retrieving data (Retrieval module) or occasionally used options (Extras module), can be learned as you need them. Ask your facilitator which module to cover next when you bring them the printouts from this module. They will suggest the best one for your needs.



OBJECTIVES

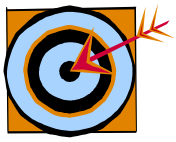
Given an operational system, training materials, access to an Operator's Guide, and access to online Help:

- Run and recognize a good alignment check using Flow-Check™, Flow-Check™ 770/488, and if needed, Flow-Check™ 675/633 Fluorospheres as a check on the fluidics and optics within the system.
- Apply basic troubleshooting steps to overcome poor alignment check data.
- Set the discriminator on a parameter to a reasonable level for the sample.
- Adjust the high voltage and gain settings to place data correctly on the plots as specified in the exercises.
- Run Flow-Set™ fluorospheres at the voltages and gain settings used for the sample and determine the mode values for the standard.
- Given a set of mode values for a standard calculate average mode values.
- Recognize incorrectly compensated samples when more than one dye is used and correctly set compensation settings.
- Run a 2 color verify tube with targeted assay values, and determine if the system is producing accurate results.
- Print all results
- Find and print log sheets from online Help.

WHY IS IT IMPORTANT?

Before you can ensure that patient or experiment results are accurate, several items must be checked. How do you know the system is operating properly? Are the settings adjusted properly? Are results accurate? These questions need to be answered. This module examines this process and reinforces the concepts covered in the pre-requisite CD.

Skill Check Preview



You will have mastered the application when you can show printed results meeting specifications and appropriate log sheets for each of the following:

- Flow-Check™, Flow-Check™ 770/488, and if needed, Flow-Check™ 675/633Fluorospheres

- Isotype whole blood controls illustrating proper settings as specified in the exercises.
- Flow-Set™, Flow-Set™ 675/633, Flow-Set™ 770/488 Fluorospheres (or equivalent for an application using a different standard).
- Log sheets for determining standard reference values for Flow-Set™ Fluorospheres.
- Compensated sample results.
- Immuno-Trol™ or Cyto-Trol™ sample results.
- Given a set of Flow-Set mode values, calculate the average mode values.

What to learn

The following sequence outlines the major steps used to determine that the system is operating properly, is adjusted properly, and is producing accurate results.

Daily

- Alignment check using Flow-Check™ Fluorospheres.
- Adjust the system to an isotype whole blood control.
- Run Flow-Set™ Fluorospheres at the settings determined for the isotype control to establish target channels for the fluorospheres.
- Compensate for spectral overlap when more than one dye is used.
- Run a control such as Immuno-Trol™ control cells (whole blood sample preparations) or Cyto-Trol™ control cells (non-whole blood sample preparations) to determine accuracy of results.

Sample Preparation

You will need to prepare the following samples to be used in this module:

Flow-Check™ Fluorospheres

1. Label a tube Flow-Check.
2. Mix a bottle of Flow-Check fluorospheres and place 10 drops into the labeled tube.
3. Mix a bottle of Flow-Check™ 770/488 Fluorospheres and add 5 drops to the tube in step 1.
4. If you are using a two-laser system, mix a bottle of Flow-Check™675/633 fluorospheres and add 5 drops to the same tube as in step 1.

5.

Flow-Set™ Fluorospheres

6. Mix a bottle of Flow-Set™ Fluorospheres and add about 15 drops to a tube. Label the tube Flow-Set.

Whole Blood and Immuno-Trol™ samples

7. Label 3 tubes respectively:

   Blood Isotype G1/G1

   Blood CD8/CD4

   Immuno-Trol or Cyto-Trol CD3/CD4.

8. Add 10 uL of the corresponding Cyto-Stat™ antibody into each tube (e.g. G1 FITC/G1 PE into Blood Isotype G1/G1, CD 8 FITC/CD 4 PE into Blood CD8/CD4, etc.).
9. Add 100 uL of whole blood into each of the tubes labeled blood.
10. Add 100 uL of Immuno-Trol into the tube labeled Immuno-Trol.
11. Ask your facilator to help you finish the prep process on the T-QPrep™.

Before you begin…

As you complete the module, you will be asked to run the prepared samples. To run each of these samples:

- Open the MCL cover and place a carousel on the system and then close the cover.
- Ensure the desired protocol, panel or application is selected.
- Select  on the toolbar. The system will move the carousel to place tube position 1 under the probe.
- Open the MCL Tube Access door on the carousel and place your sample in position 10.
- Select  on the toolbar. The carousel will rotate to place your sample (position 10) under the probe and run your sample.

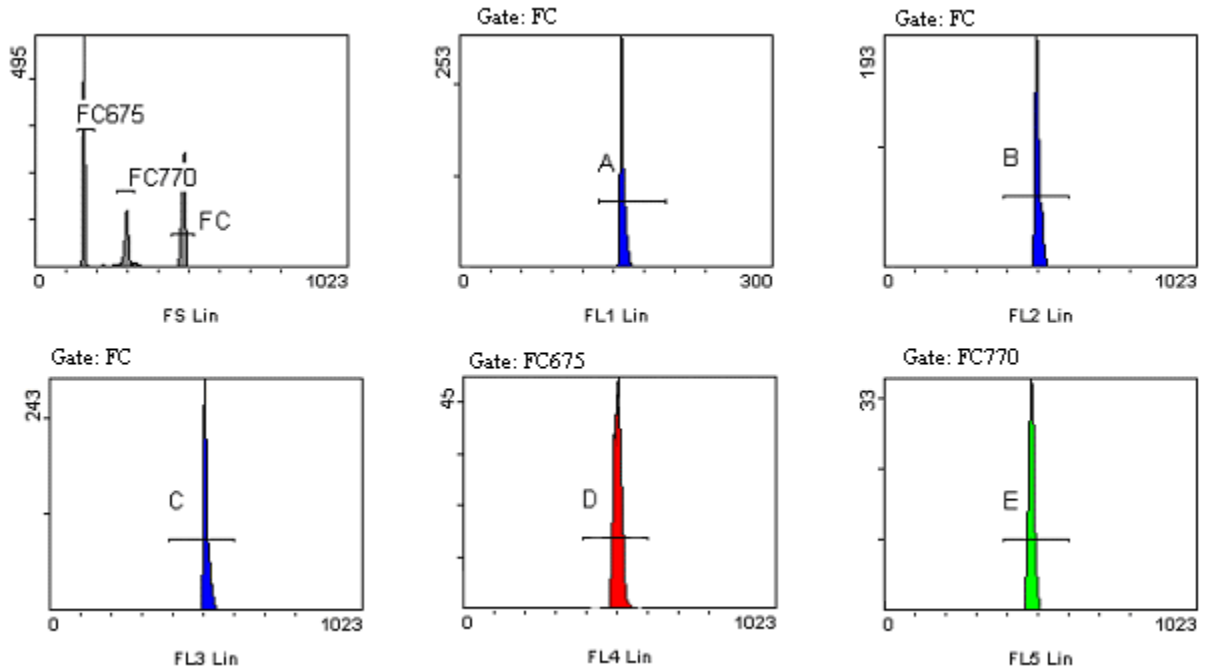
INFORMATION / PRACTICE SECTION

Alignment Check

Running a sample of Flow-Check™ Fluorospheres checks the fluidics and optics of the system. Flow-Check™ 675/633 checks the alignment of the HeNe laser and Flow-Check™ 770/488 checks the optimization of the 770 PMT. All three fluorospheres can be run together when the total system is to be checked. Sample particles must pass through the center of the laser beam for proper sensing. The fluidics align the particles to the center of the flow cell. The optics align the flow cell to the center of the laser beams and both beams and flow cell to the sensors. Poor results when running the fluorospheres is usually the result of a fluidic problem and rarely the result of an optical misalignment.

Results should appear as below when using QC 2L Flow Check.pro in the common protocol folder:

Note: On a single laser system, FC675 would be eliminated and FL4 Lin would be gated on Flow-Check.



All single parameter fluorescence histograms are gated on one of the FS Lin regions: FL1 through FL3 on Flow-Check, FL4 on FC675, and FL5 on FC770. HPCV's should be < 2% for FS and FL1-3, < 2.5 % for FL4, and < 4 % for FL5.

Exercise

1. Select **Tools >> AutoSetup Scheduler**.
2. Select the QC 2L Flow-Check(TM)-C application and then **Schedule**.
3. Do not save the old protocol.
4. Run the Flow-Check™ fluorospheres using a low flow rate.
5. If the results meet specifications, obtain a printout to prove completion of the task (you will need them later for your Skill Check).

If they do not meet specifications, try the following and re-run:

1. Select  to prime the system. If necessary, repeat.
2. If still out, repeat run.
3. If still out, press the black purge button next to the sheath filter, and prime again.
4. If still out, select the Cleanse  icon.
5. If still out, clean the system.

If the peaks are narrow but are not within the regions:

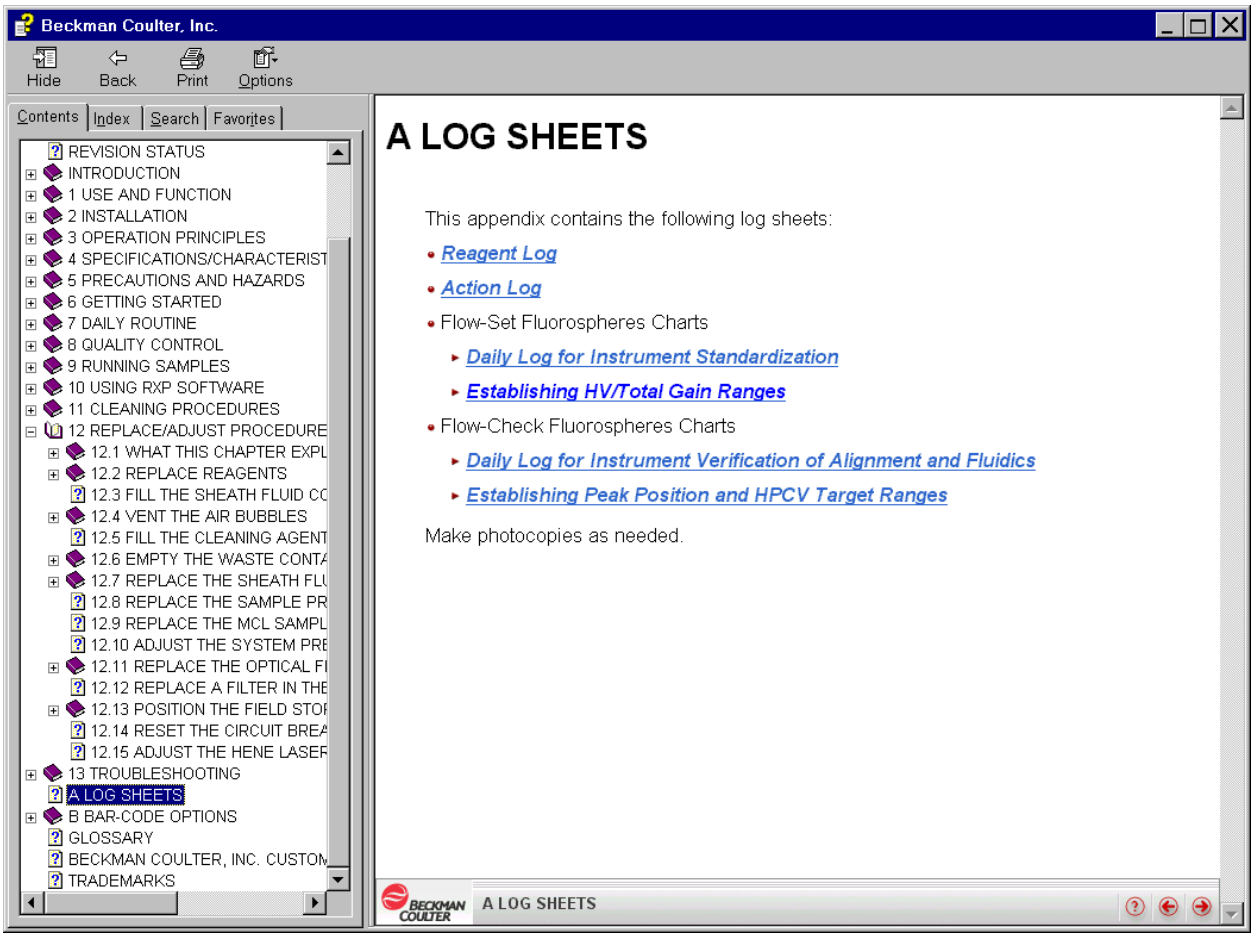
1. Select  from the toolbars at the top of the screen.
2. Select Setup mode and QuickSET.
3. Place the Flow Check sample on the carousel and select  icon.
4. Use the sliders on top of each plot to move data within the region.
   - Click the left or right of the slider bar to increase or decrease the high voltage by 10 volts
   - Click on the arrows to increase or decrease the high voltage by 1 volt.
   - For larger changes you may move the slider using a drag and drop technique.
5. When the peaks are within the regions, deselect Setup mode and QuickSET and accumulate data.

6. Select  icon to save the protocol.

Online Help Log Book Pages

In the exercises that follow, you will be shown examples of log book pages. Feel free to write on the ones in this training module. Additional copies (except for Log Amplifier Linearity Verification which comes packaged with the Immuno-Brite™ fluorospheres) can be obtained through online Help.

1. Select  on the toolbars.
2. Select 13 Troubleshooting >> A LOG SHEETS. The following screen appears.



3. Select and print the desired log sheet.

Logbook Alignment Check

The sheet below can be used to log the Flow-Check™ results if you are required to do so.

Note: Levey-Jennings plots are available to monitor QC. The QC is addressed in the Database module.

DAILY LOG FOR INSTRUMENT VERIFICATION OF ALIGNMENT AND FLUIDICS

Flow-Check™ Fluorospheres Lot Number _____ Expiration Date _____

Flow-Check™ 675 Fluorospheres Lot Number _____ Expiration Date _____

Flow-Check™ 770 Fluorospheres Lot Number _____ Expiration Date _____

Target Range	FS		FL1		FL2		FL3		FL4 Red Laser 488 ☐ ☐		FL5		
Run	Mode	HPCV	Mode	HPCV	Mode	HPCV	Mode	HPCV	Mode	HPCV	Mode	HPCV	Tech/Date
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
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21													
22													
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26													
27													
28													
29													
30													
31													

Cytometer Serial No: _____

Laboratory: _____

Cytomics FC 500

ESTABLISHING THE SETTINGS FOR A SAMPLE

The system must be set up to run each type of sample. The settings used to run the Flow-Check fluorospheres will likely not work for a whole blood sample or DNA sample or some other application. In this portion of the module, you will experience setting the discriminator, high voltage, gains, and compensation for a new sample. This section should reinforce what you have learned in the pre-requisite material. We will use your prepared whole blood samples to illustrate the setup process. The samples are examples but the process applies to any application.

Note: If the cell surface marker application is relatively new to you, we suggest you take a moment to read the first few pages of Concept section of the Cell Surface module. When you have finished reading, come back to this point and continue on.

We will examine 4 cytosettings:

- Discriminator
- High Voltage
- Gains
- Compensation

There is a tour in the Basic Operation section of the Tour Guides illustrating each of these adjustments. If you are new to the system or would just like to review the concepts, we suggest you take the tours first before attempting to complete the exercises.

Setting the Discriminator

To set the discriminator, you must first choose a parameter. It should be a particle measurement that produces much larger signals for the particles of interest than the noise and debris. The most common parameter used is forward scatter because often the particles (e.g. cells, fluorospheres, etc.) are much larger than the size of debris. This is not always the case, however. Bacteria, for example, are very small and would be hardly distinguishable from debris. Chromosomes and many marine specimens are additional examples. In these cases, the discriminator is often set on a fluorescence parameter in which the particles of interest fluoresce brighter than the debris and noise. In the example that follows, you will use forward scatter.

The second part of the process is to choose the discriminator level. Generally it should be a channel setting that eliminates most of the debris and noise but passes all of the particles of interest. This value can vary depending on the type of samples, the preparation process, and even the high voltage and gain settings you will set later. We are starting with the discrimnator because a parameter and value must be set to view any data. We may decide later to adjust the value, however, after finalizing the high voltage and gain settings.

1. Drag and drop the 2C 1L protocol to the WorkSpace.
2. Choose to save (if you have made changes, you may wish to save them) or not save the previous protocol.
3. Select **Cytometer >> Cytometer Control** or the  icon and the the Acq. Setup tab.
4. Select Setup Mode.

Note: Setup mode allows continuous acquisition of data from the cytometer giving continuous real-time feedback of instrument performance on the display plots. Any Stop & Save values are ignored in this mode. Display of data continues until Abort is selected or Setup Mode box is unchecked to begin normal acquisition. The plots are updated live time with only the most recently acquired events displayed.

5. Select FS and adjust the slider bar to set the discriminator to 100.

Note: This value is only a starting point. It can be set up or down from this value to eliminate most of the debris and noise but still pass all the cells of interest.

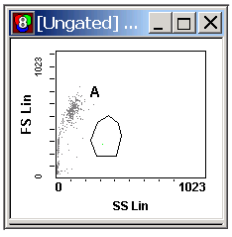
6. Open the MCL Tube Access door and place the Blood Isotype G1/G1 sample in position 10 and close the cover.

Note: If position 10 is not directly under the cover, select  on the toolbar.

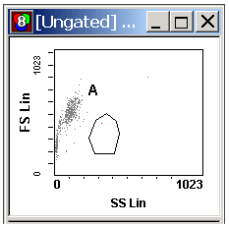
7. Select  on the toolbar. The carousel will rotate to place your sample (position 10) under the probe and run your sample.
8. Adjust the discriminator down until a lot of debris is seen on the SS versus FS plot. Then adjust the discriminator up to eliminate most of the debris without cutting off any cells.

Example:

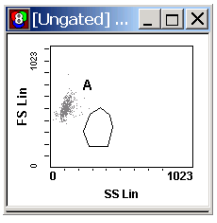
Too Low



Good



Too High



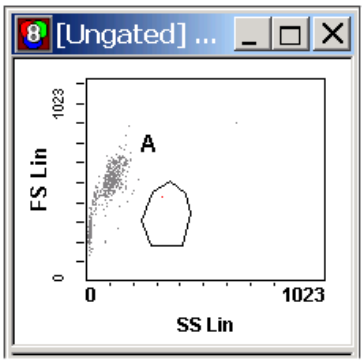
Setting Gains and High Voltage

Your cells at this point may not exhibit an optimal pattern on the SS versus FS plot. They could be too high or too low, too far to the left or to the right. Making the picture look better is the purpose of adjusting the gains and high voltage. We will begin with one of the parameters (e.g. FS) and adjust the gain and/or voltage to place the pattern midway along the FS axis. Then we will adjust the SS pattern. Finally, we will adjust the fluorescence voltages to bring out the cell background fluorecence to the end of the first decade. Our objective here is to insure that there will be good separation between the lymphs, monos and grans.

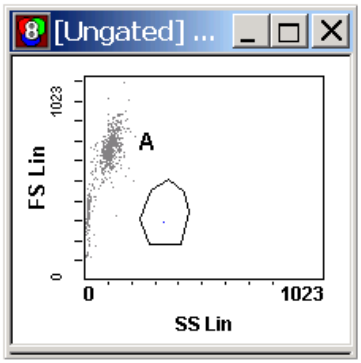
1. On the Cytometer Control dialog, select the Detectors tab.
2. If a large adjustment appears necessary for the FS parameter, select the Gain adjustment and increase or decrease as necessary to place the pattern midscale along the FS plot axis. For smaller adjustments, use the voltage adjustment in the same manner.

Example:

FS Too Low



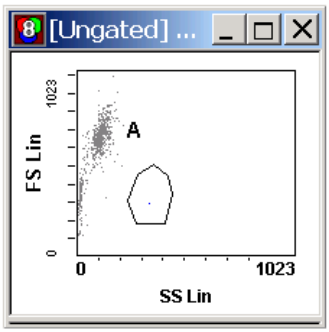
FS Good



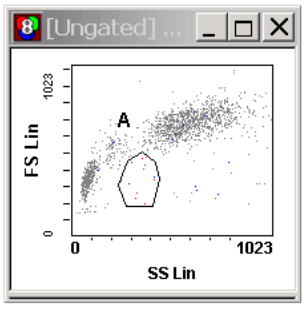
3. Repeat the process for the SS parameter.

Example:

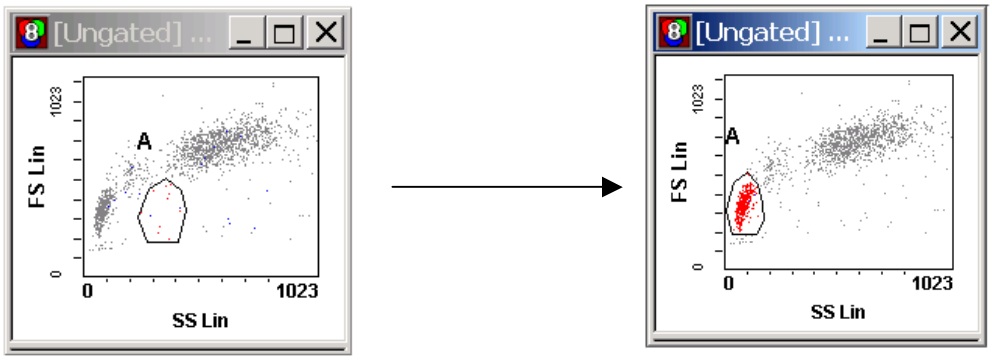
SS Too Low



SS Good



4. Select the A gate on the SS versus FS plot and adjust and move it around the lymphocytes as shown below.

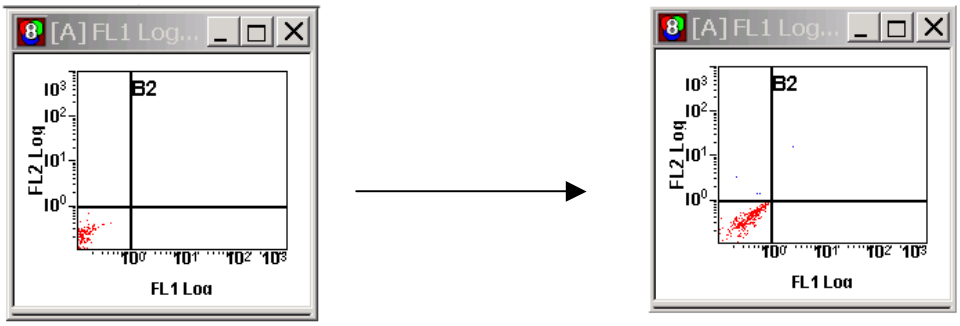


At this point we need to set the voltage for the FL1 and 2 Log parameters. Our purpose is to adjust until there is clear separation between those cells binding antibody (positives) and those that are not (negatives). This is usually achieved by adjusting so that the negative population ends at the end of the first decade. Weaker positives will generally fall in the second decade and the stronger positives in the 3rd and 4th decades.

5. Adjust the FL1 voltage until the data on the FL1 Log versus FL2 Log plot comes out to the end of the first decade along the FL1 Log axis.
6. Repeat the process for the FL2 voltage until the data comes out to the end of the first decade along the FL2 Log axis.

Example:

Too Low Good



Note: The high voltage necessary can vary depending on:

- the sample preparation
- The number of antibodies on the cells
- The type of cells

Etc.

For these reasons there can be different settings for different samples (e.g. 2, 3,4,and 5 colors).

7. Select **File** >> **Save Protocol** or the  icon to save the adjustments to the protocol.
8. Record the new settings below. We will use them later.

   FS Voltage _____ Gain ____ SS Voltage _____ Gain _____

   FL1 voltage _____ FL2 voltage _____
9. Select the Acq. Setup tab and deselect the Setup Mode.
10. Allow the data to collect about 10,000 events and select the stop  icon.
11. Print the results and save the printouts as proof of completion of this portion of the exercise.

Estabishing References

The initial settings can often be be reused again and again. There are, however, some things that can change the picture. These include:

- Components replaced in the system (e.g. PMTs, lasers, filters, etc)
- PMI's completed on the system
- Optical alignment
- Age of the system
- Changing to different wavelength optical filters

If changes have occurred, the same high voltage and gain settings may not yield the same picture. It is more important that the picture remain the same than that the settings remain the same. If the picture changes we cannot be sure that the change is due to an instrument or an actual change in the sample. Detecting the latter may be of great importance if we are monitoring samples over time. To make sure the picture remains the same, we need an arbitrator sample. This is a sample that does not change and that can be run at the initial settings to establish a pattern and then periodically to see if the instrument has changed (e.g. the pattern changes). If you are a clinician, the sample is referred to as a standard. If you are a researcher, it is a control for your experiment.

In the following exercise, we will establish the pattern for an example sample, Flow-Set™ Fluorospheres.

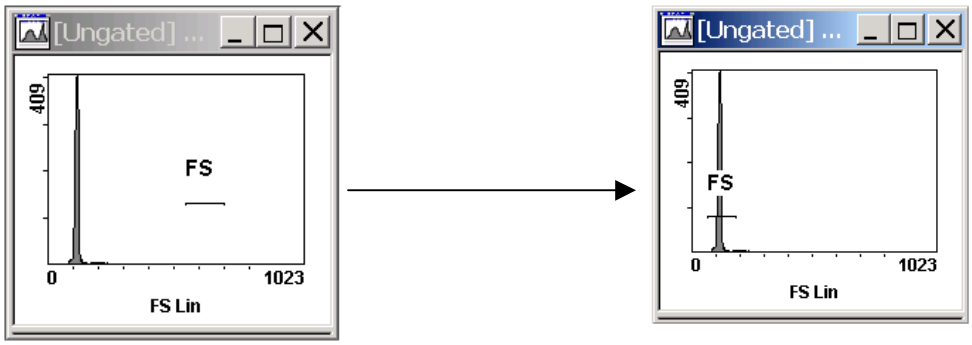
1. Drag and drop the Standard protocol to the Workspace.
2. Save the current protocol.
3. If the Cytometer Control window is not visible, select the  icon.
4. Select the Detectors tab.
5. Enter in the values you recorded earlier for FS, SS FL1 Log and FL2 Log voltages and gains into the system.
6. Save the protocol.
7. Mix and place the Flow-Set™ Fluorospheres into position 10 on the carousel.
8. Select  on the toolbar. Deselect the Auto Adj. Feature in the Wizard window.
9. Let the sample accumulate about 10,000 events and select  .

At this point, the regions on the plots may not be bracketing the data. In fact there may be no data appearing on the FL1 and 2 Log plots. The FL11 and 2 Log plots are gated on FS. If FS is not position on the data, there will be no data appearing in the fluorescence plots. You are going to move these regions beginning with the FS region to the present location of the beads.

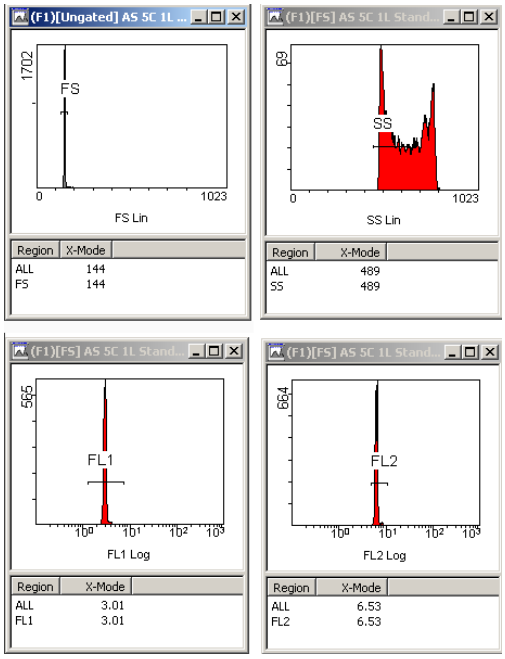
Note: You may need to lower the discriminator some if the FS data is low. Perhaps a value of 30 would work well.

10. Move your cursor over the FS region until  appears. Now use a drag and drop technique to drag the region to the data. Data should now appear on the fluorescence plots.

Example:



11. Repeat the process for SS (use the higher first peak, if there are two peaks), FL1 Log, and FL2 Log plots.



12. Record the X-mode channel for each of the parameters.

FS _____ SS _____ FL1 Log _____ FL2 Log _____

13. Print the results as proof of completion of task.

Note: If the mean channel is not visible, move the cursor to the lower left corner of the plot until the cursor changes to the T cursor and then click the left mouse button.

You have now "snapped" a picture of the standard at your original desired settings. The standard does not change over time. If we run a fresh preparation of this sample again a day, or week, or month from now, the standard should be in the same place unless the instrument has changed. If this is the case, the voltages and/or gains are adjusted to move the standard back within the regions. These regions are known as targets. It is important that the picture remains the same not the high voltage or gains. Doing this routinely ensures we are using the same fluorescence intensity and that changes seen in an unknown sample monitored over time are more the result of changes in the sample (or prep) and not the instrument.

Note: This whole process was done with one example whole blood sample which may not reflect the average population of samples. A more statistically valid method is to use a number of samples, establish the high voltage in each case, run the standard at those values, and average the positions to establish the target region position. This is discussed more in detail in the appendix section of the Surface Markers module.

Logbook Standardization

The sheet below can be used to log the Flow-Set™ fluorospheres if you are required to do so.

Application _____

Flow-Set™ Fluorospheres Lot Number _____ Expiration Date _____

Flow-Set™ 675 Fluorospheres Lot Number _____ Expiration Date _____

Flow-Set™ 770 Fluorospheres Lot Number _____ Expiration Date _____

Instrument HV/Total Gain Target Ranges:

FS _____ LOG FL1 _____ LOG FL3 _____

SS/LOG SS _____ LOG FL2 _____ LOG FL4 _____ LOG FL5 _____

	FS			SS or LOG SS			LOG FL1		LOG FL2		LOG FL3		LOG FL4		LOG FL5		
													HeNe ☐ 488 ☐				
Run	Mode	HV*	Gain	Mode	HV	Gain	Mode	HV	Mode	HV	Mode	HV	Mode	HV	Mode	HV	Tech/ Date
1																	
2																	
3																	
4																	
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Serial No. _____

Lab. _____

Cytomics™ FC 500

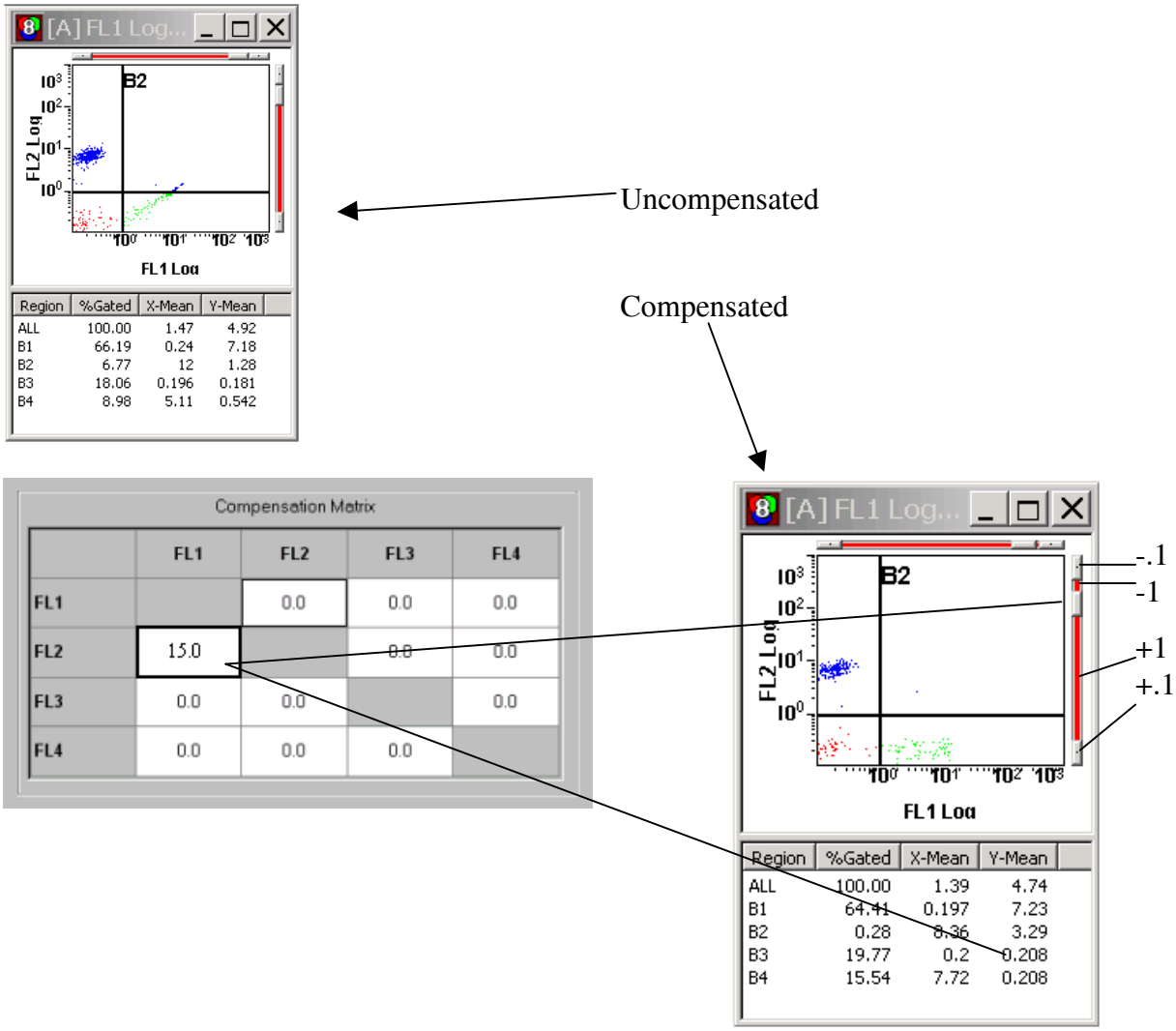
BECKMAN COULTER

7272013A

Compensation

After the fluidics, optics, and standardization have been performed, we need to compensate for any spectral overlap between multiple dyes which have not been removed by filters. There are two methods to perform Compensation: manually or automatically using the AutoSetup Wizard. The latter will be covered in the AutoSetup module.

In this method, a control or blood sample is prepared with two mutually exclusive positive antibodies (e.g. CD8 and CD4) bound to two different spectrally overlapping dyes. If data is seen in the quadrant 2 region when the sample is run (see below), the instrument requires compensation. Compensation is considered correct when the populations have been moved to the correct quadrant (1 or 4) and the X mean values for regions 1 and 3 and the Y mean values for regions 3 and 4 are the same within +/- 0.1. The process is repeated for each pair of dyes to be used. In some cases, the process is automated in the software.

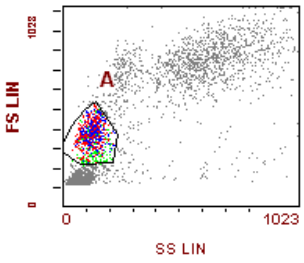


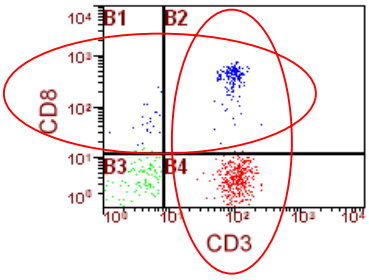
Exercise

1. If it is not already on the screen, drag and drop the 2C 1L.pro to the Workspace.
2. Select the  icon, the Acq. Setup tab, Setup Mode and QuickCOMP.
3. Run the Blood CD 8 /CD 4 sample and move the gate region, if necessary, to enclose just the lymphocytes.
4. Adjust the populations using the slider bars on the sides of the FL1 Log versus FL2 Log plot until the region 1 and 3 X mean channels and region 3 and 4 Y mean channels are the same within +/- .1.
5. Save the protocol .
6. Deselect QuickCOMP.
7. Print out plots and statistics to confirm manual compensation.

Controls

At this point, all the cytosettings are in place. You now need to determine if the system will provide accurate answers with known samples. The control you use depends on the application. Some common controls might be Immuno-Trol™ cells (whole blood preparations) or Cyto-Trol™ (non-whole blood preparations) when running cell surface markers. The control has expected results and is run on the system to see if the system is able to reproduce those results. If the answer is yes, then you should feel more comfortable reporting unknown sample results. If the answer is no, you may need to troubleshoot the sample preparation process or the reagents used.





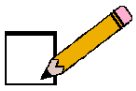
Region	%Gated	X-Mean	Y-Mean
B1	4.39	3.6	65.5
B2	19.74	97.4	428.8
B3	26.42	2.6	3.2
B4	49.46	116.0	3.7

Coulter® Immuno-Trol

Cell Lot No. 123456

Antibody	% Pos	Range +
T11(CD2)	83	2
CD3(IgG1)	72	5
T4 (CD4)	49	4
T1 (CD5)	74	5
T8 (CD8)	25	7
B4 (CD19)	13	4

NOTE: In the example above, the sum of B1 and B2 should be within the range for the % for CD8 and the sum of B2 and B4 should be within the range for the % for CD3.



Exercise

1. After compensation, run the CD3 FITC/CD4 PE (or CD3 FITC/ CD8 PE) Immuno-Trol sample using the 2C 1L.pro.
2. Obtain an assay sheet from your facilitator and compare your results to the assayed values.
3. Print the results as proof of completion of the task.

SUMMARY

You have now completed the process of setting up to run a sample. At this point, we supplied the protocol. The Creating a Protocol module later will take you through the process of creating one. The purpose in this module was to reinforce the concepts presented in the pre-requisite CD by giving you hands on experience in making cytometer adjustments. You now know how to:

- Drag and drop a protocol to the workspace to run a sample.
- Run Flow-Check™ fluorospheres to check out the instrument fluidics, optics, etc. and what to do if it is not good.
- Select the parameter and adjust the discriminator setting.
- Adjust the high voltage and gain settings to make the plot picture of a sample look right.
- Record the picture by running a standard (or research control) at the settings and establish target regions for the standard.
- Adjust the compensation settings.
- Verify instrument accuracy by running a know sample.

If you feel you are ready, please complete the Basic Operation Skill Check.

TABLE OF CONTENTS

OBJECTIVES

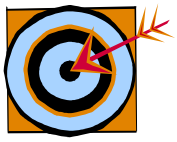
Given an operational flow cytometer, application samples or listmode data, training materials, and access to online Help

- Create a protocol to run and or to analyze sample listmode data.

  This protocol includes creating plots, arranging plots, creating gates, creating analysis regions and saving of the protocol.

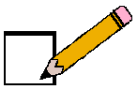
- Create an acquisition protocol in which parameters must be selected and the cytometer adjusted.

Skill Check Preview



You have mastered the objectives when you can

- Create two application protocols (one of which must be an acquisition protocol), which meet the criteria described in the application modules.
- Adjust the cytometer settings (including gains, high voltage, and compensation) to create fresh sample application plot patterns similar to the patterns shown in the application modules.



Tour Guides

If you have never created a protocol, we suggest you view the Creating a Protocol tour in the Tour Guides. To access the Tour Guides:

1. If there is a program running, minimize the screen by selecting  in the upper right corner of the application.

2. Place the Tour Guide disk into the CD Rom drive or if your facilitator has placed the Tour Guides on your hard drive, double click on the icon labeled Tour Guides. The system will start the program. A voice will come on and explain how to take the tours.

3. Take the Creating a Protocol tour.

INFORMATION / PRACTICE SECTION

Creating a Protocol Basics

DEFINITION: Protocol – A protocol is a complete set of instructions for generating data (Acquisition only), and/or displaying, gating, and analyzing the data.

There are 6 basic steps to creating a protocol:

- Select Parameters (In the Analysis software choosing the listmode sets the parameters and no parameter changes are permitted)
- Create Plots
- Create Regions
- Define Gates
- Analyze Data
- Save the Protocol

Each of these steps will be covered in more detail in the following sections. First, however you need to think through your protocol on paper. When you have mastered the software better, you will be able to think through what you need in the protocol and create it at the same time in the software. While you are learning, though, it may be easier to put your thoughts down on paper first and then take the paper version and implement it in the software. Let's suppose you wish to create a protocol to check out the linearity of the fluorescence log scales using the Immuno-Brite beads.

Step 1 – Select the parameters

In this case we might wish to use forward scatter (FS) as a discriminating parameter to eliminate debris. Forward (FS) and side scatter (SS) might be used for gating purposes and then observe the log fluorescence parameters. Our Parameter selection then would be:

FS (size)	FL3 Log (ECD)	Discriminator set on FS (e.g. 100)
SS (granularity)	FL4 Log (PC5 or APC – HeNe Laser)	
FL1 Log (FITC)	FL5 Log (PC7)	
FL2 Log (PE)		

Step 2 - Creating the plots

In this example, we might use a dotplot (2 parameters) based on side scatter (SS) versus forward scatter (FS) to identify our population of beads. The other plots would be histogram plots, one for each fluorescence signal.

The Immuno-Brite™ Fluorospheres are 10 um beads in 5 vials with 5 different levels of fluorescence brightness. All 5 levels are placed into one tube. The high voltage and gain settings will be adjusted to place the beads in the center of the SS versus FS dot plot. The fluorescence high voltages will be adjusted to spread the fluorospheres over all 4 decades of the log scales.

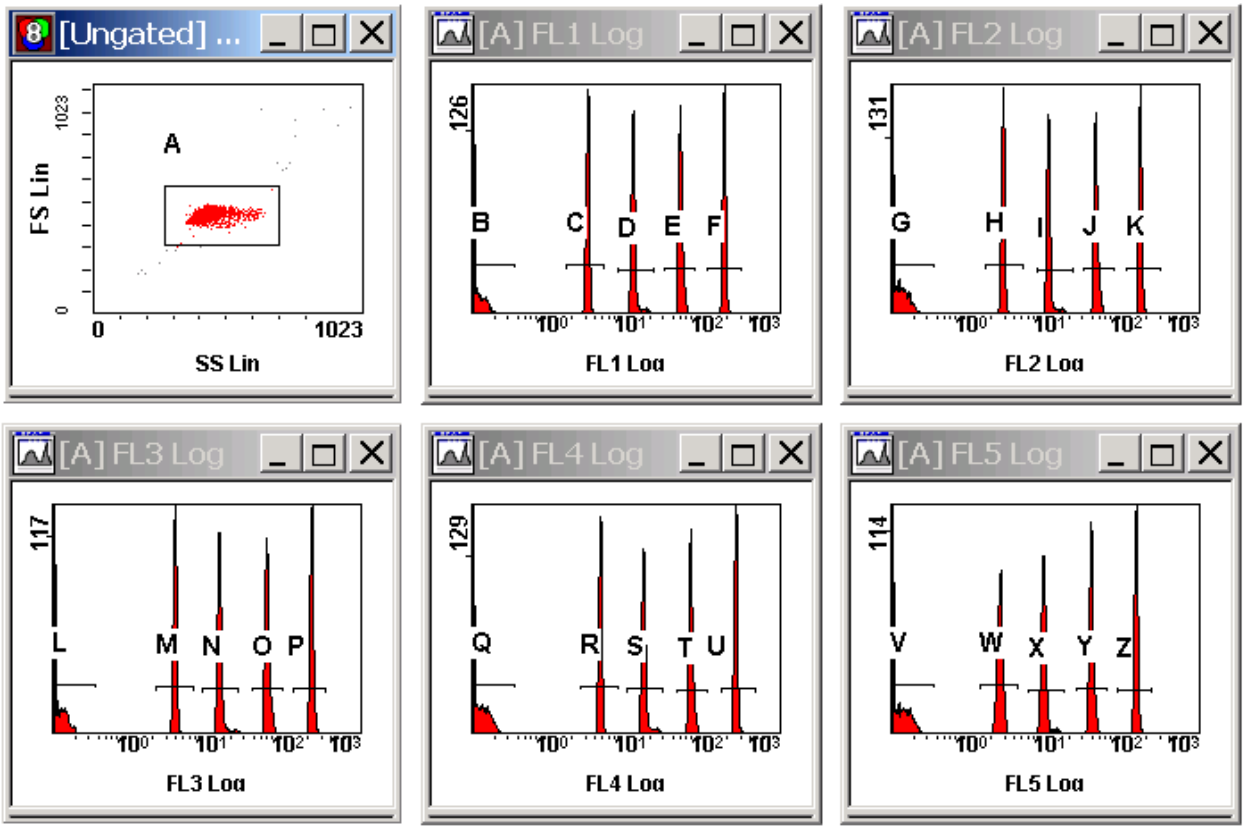
Step 3 – Create the regions

One region (polygon or rectangle) is created around the fluoresphere data on the SS versus FS plot and will be used to gate the fluorescence histograms. 5 linear regions (one for each fluorosphere level) are created on each fluoresence histogram to bracket each fluorescence level.

Step 4 – Gate the histograms

The polygon (or rectangle) is assigned to each of the fluorescence histograms.

The plots now look like:



Step 5 – Analyze the data

In this step, you determine the statistics required to analyze the data. Since we are only interested in the position of the fluorescence peaks to determine linearity we need to display only the X-Mean value. These values will be plotted on log graph paper to determine if the fluorecence parameter is linear.

Sample Preparation

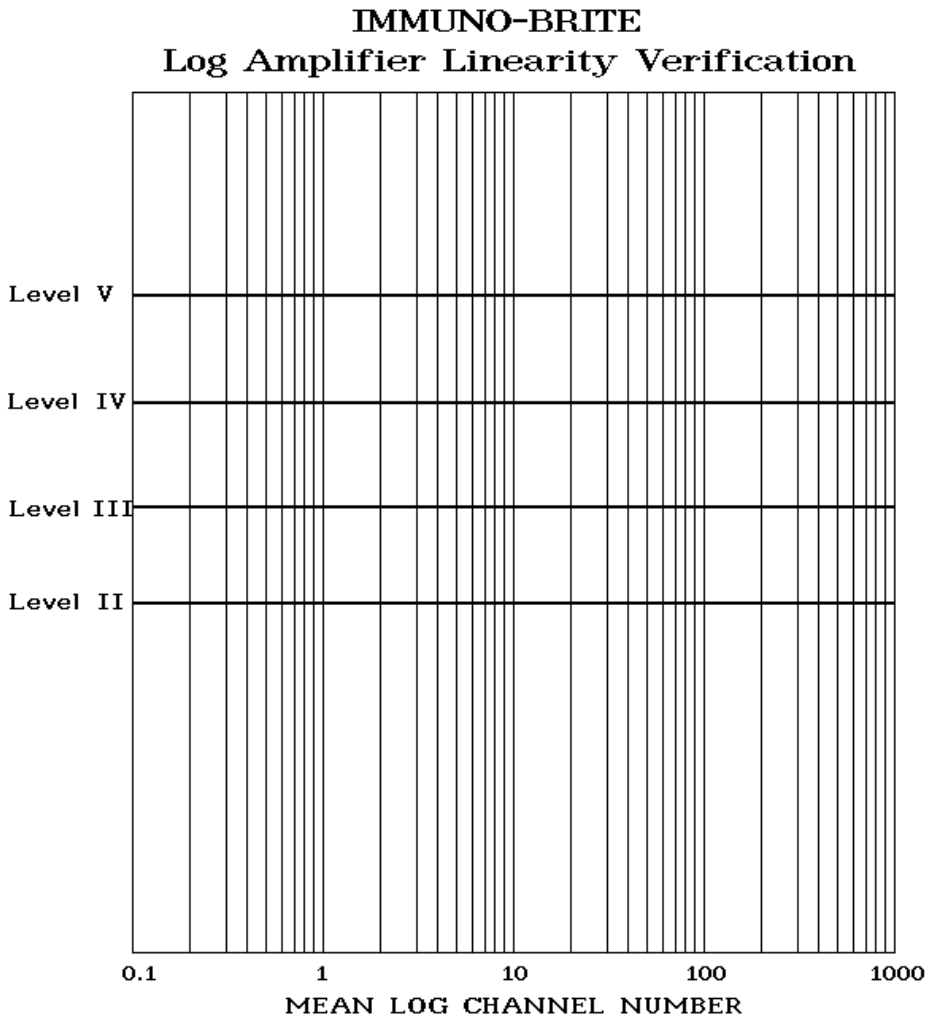
1. Obtain the box of Immuno-Brite™ Fluorospheres.
2. Mix each level in turn by rolling the vials several times in your hand.
3. Add 4-5 drops from each vial into the same tube. This will be your sample.

In the next section, you will create the protocol and run the Immuno-Brite fluorospheres. After you have run the fluorospheres and obtained the X-Mean values, the values are plotted on the graph below for levels 2 through 5. A straight line connecting the points indicates a linear system.

Log Scale Linearity Verification

Instrument: _____

Serial Number _____



NOTE: You may wish to remove pages 3 – 6 as a handy reference as you convert the protocol on paper into an actual protocol in the instrument in the following exercises.

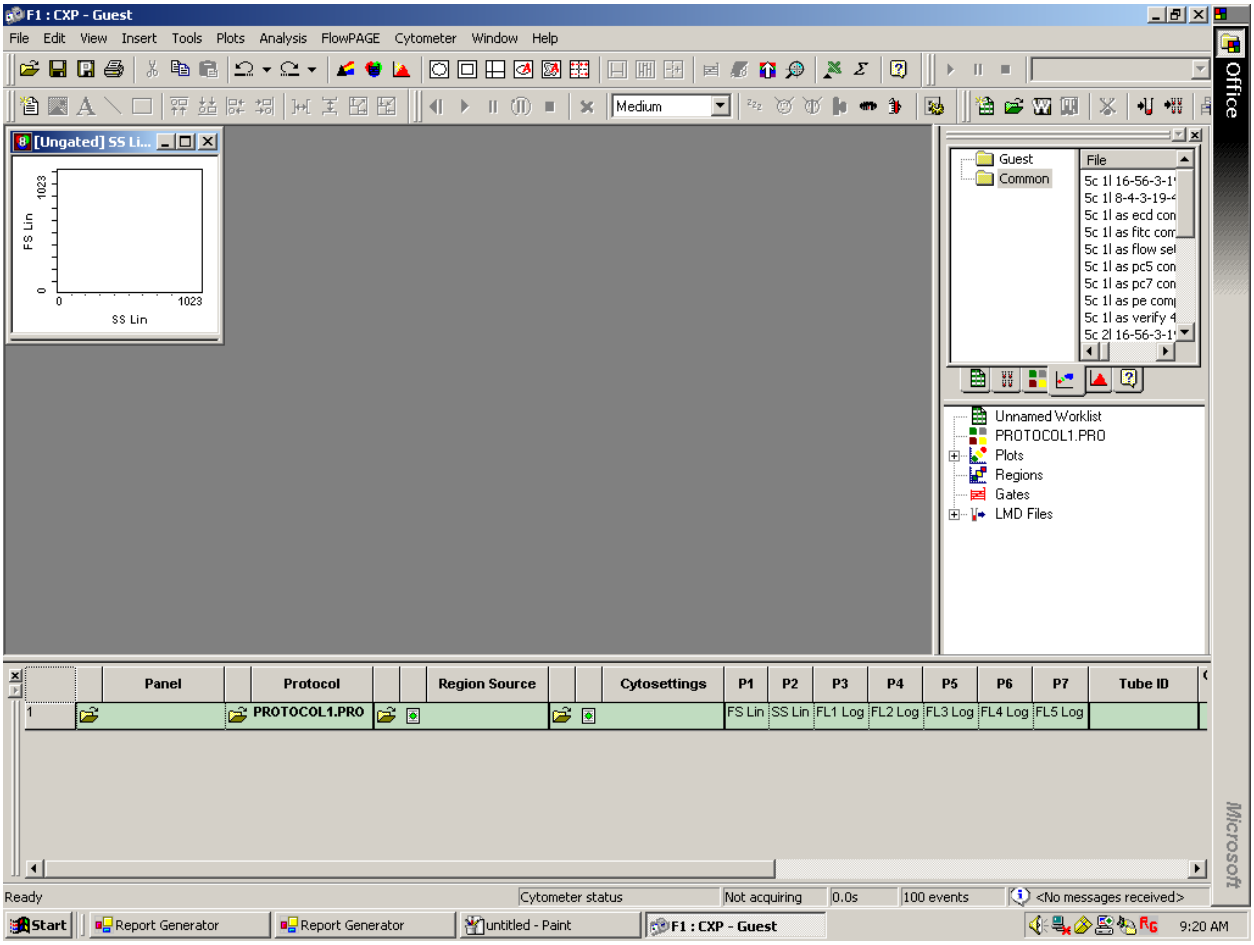
Clear the Workspace

NOTE: It is best to start fresh when creating a new protocol. Old regions may not be the same as the ones you will need in the new protocol. Clearing the Workspace deletes all the old regions and plots and makes it easier to create new ones. To accomplish this:

1. Press **CTRL N** or select **File >> New >> New Protocol**.

2. When prompted to save the current protocol, answer as desired (Yes or No). If no changes have been made to the present protocol, select No.

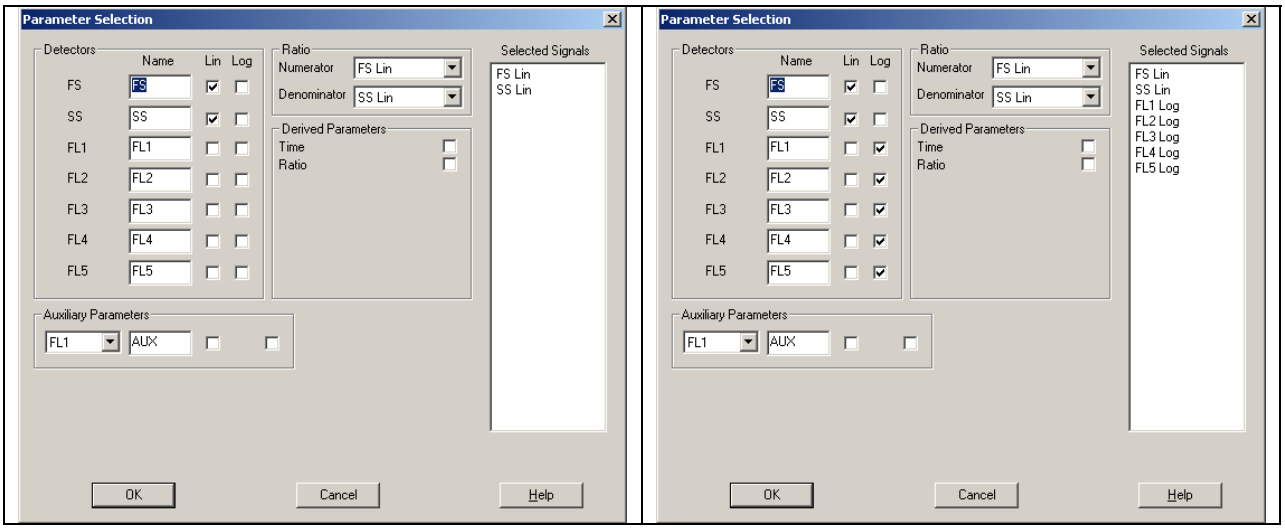
The default protocol appears.



Selecting Parameters (Acquisition Only)

1. Select **Cytometer >> Cytometer Controls** or the  icon.
2. Select Parameters button on the Cytometer Control Window.
3. Deselect undesired parameters and then select those you wish to include in the protocol.
4. You may reorder the parameters using a drag and drop technique on the parameter list.

Example: Immuno-Brite



NOTE: In the Analysis software, the parameters are the same as those in the chosen listmode file.

Creating Plots

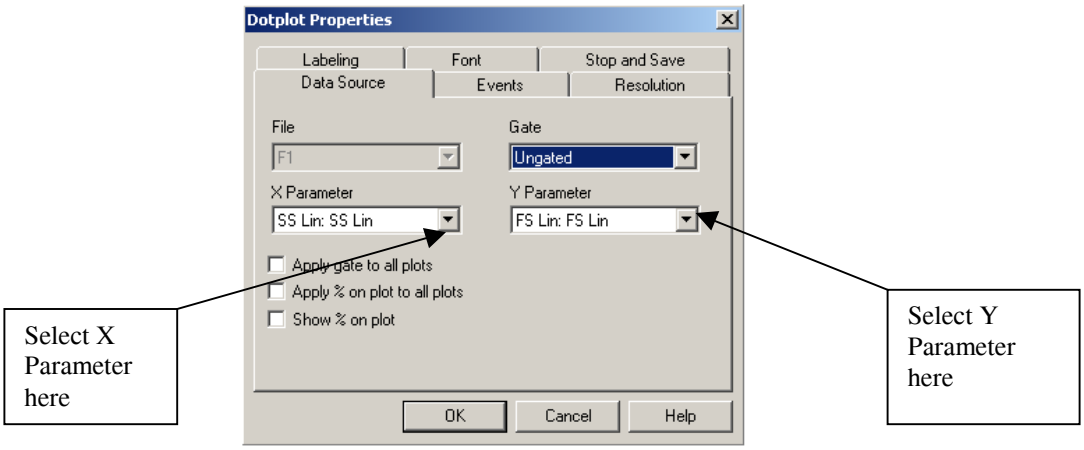
The following plots are common to the Acquisition and Analysis software:

Dot Plot		Histogram Plot		Density Plot NOTE: Not normally on the toolbar	

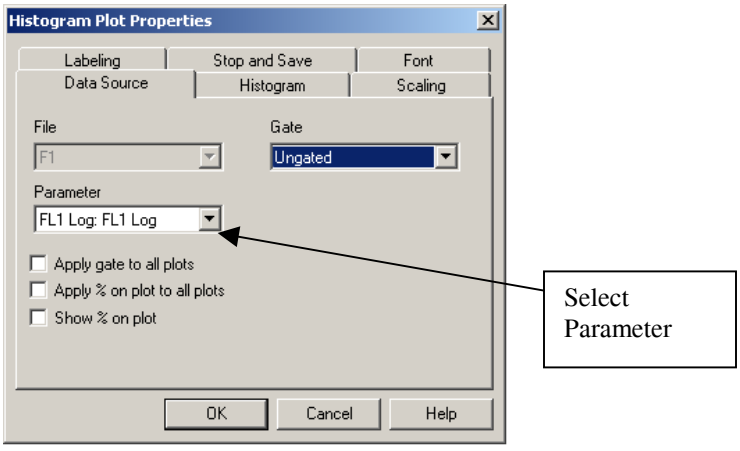
The default protocol contains a single dotplot (e.g. SS versus FS).

1. If this is not what you want, you can delete it by selecting  in the upper right corner of the plot or change it by moving the cursor to the lower right corner of the plot until the **>>>** appears. Clicking the left mouse button will open up the Dotplot properties window and allow you to change the parameters for the plot.

Example: Immuno-Brite – the default dotplot of SS versus FS is fine as is.



2. Select the  icon on the toolbar (or **Plots** >> **Histogram Plot**). The system places the plot on the screen and opens the Properties window.

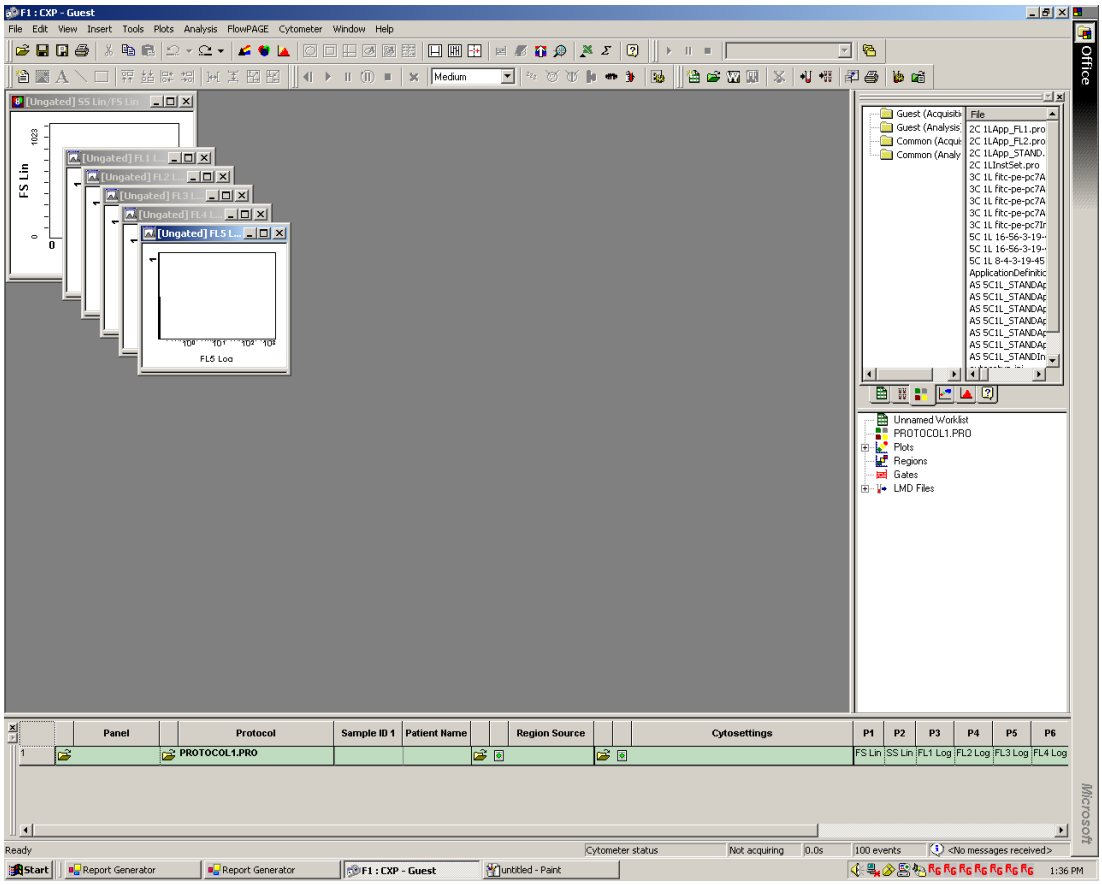


3. Select the desired parameter and then **OK**.

Example: Immuno-Brite – select FL1 Log

4. Repeat the process of selecting plots and parameters until all desired plots for the protocol have been placed on the screen.

Example: Immuno-Brite – there should be One SS versus FS dot plot and 5 Histogram plots with parameters FL1 Log, FL2 Log, FL3 Log, FL4 Log and FL5 Log respectively as shown below.



Arranging Plots

At this point, the plots are all on top of one another on the screen and it is better to arrange them horizontally and vertically on the screen. The general convention is to place the plot(s) that will be used to define the initial gating in the upper left corner of the workspace. Then the additional plots are placed from left to right top to bottom in rows, usually in the order you will work with or view them. For the Immuno-Brite example, the SS versus FS dotplot would be placed in the upper left corner and then the log fluorescence histograms from FL1 Log to FL5 Log. The plots can be moved individually by selecting the focus (top of the plot) and then using a drag and drop technique to move the plot to the desired position. There is an easier way to line them up shown below.

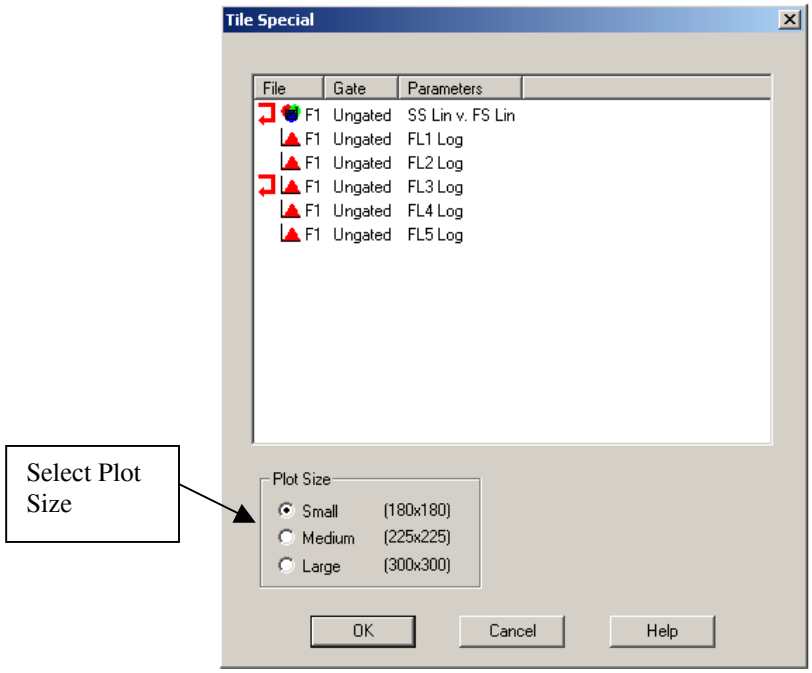
1. Press **CTRL T** to access the Tile Special window

2. Arrange the plots as desired (an example is illustrated) by dragging and dropping the plots into the desired order.

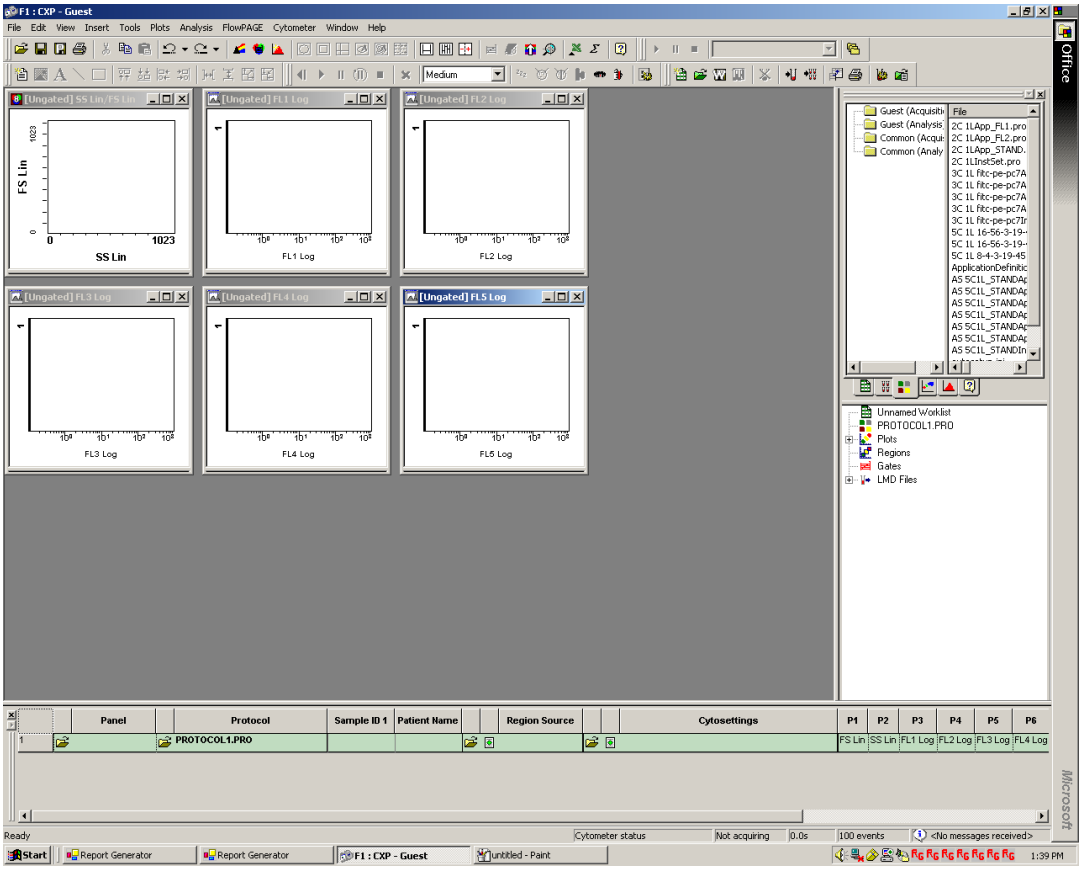
3. Select the new row symbol ( ) before each plot that starts a new row.

4. Select the desired plot size.

5. Select **OK**. In the example below, all of the plots will be displayed as small.



The plots now arrange themselves on the Workspace as requested. See the example below:



Running the Sample

Plots automatically populate by running new samples or by loading listmode files for post analysis. To load a single tube:

1. Open the MCL cover, place a carousel on the MCL and close the cover.

2. Select  on the toolbar. The carousel will align position 1 under the probe.

3. Open the MCL Tube Access door and place your tube in position 10 on the carousel. Close the cover.

4. Select  on the toolbar. The system will align position 10 under the probe and begin running your sample.

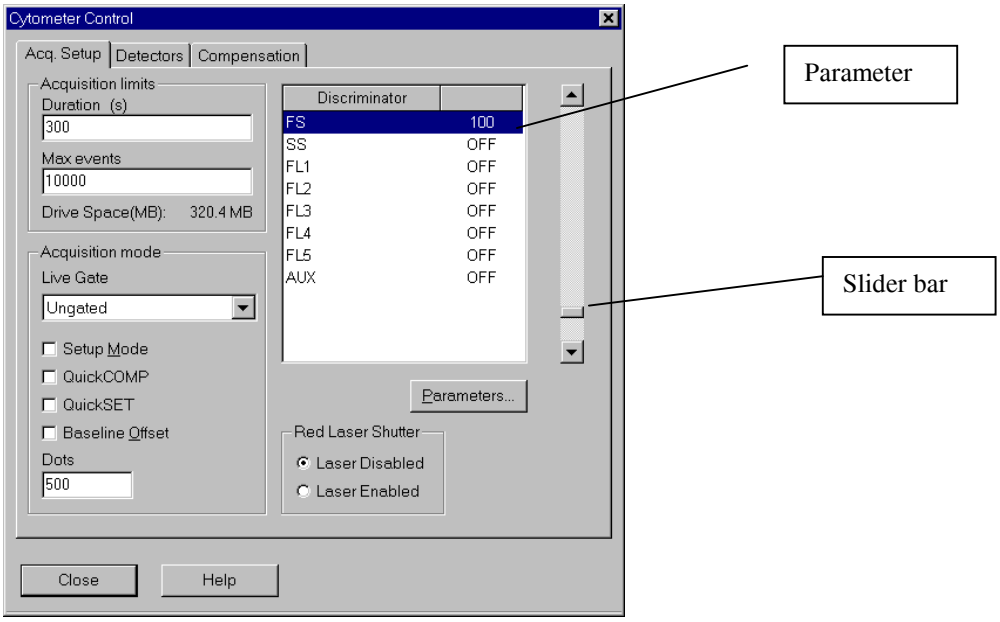
Adjusting CytoSettings (Acquisition Only)

NOTE: If you need a demonstration for adjusting the discriminator, gains or high voltage, you can find an example in the Acquiring Data Tour Guide.

Setting Discriminator

1. Select **Cytometer >> Cytometer Control** or the  icon and then the **Acq. Setup** tab.

2. Select the desired parameter and then use the slider bar to adjust the channel value.



Tips

- Set the discriminator on a parameter in which the signals for the cells of interest are significantly larger than the debris pulses and the noise.

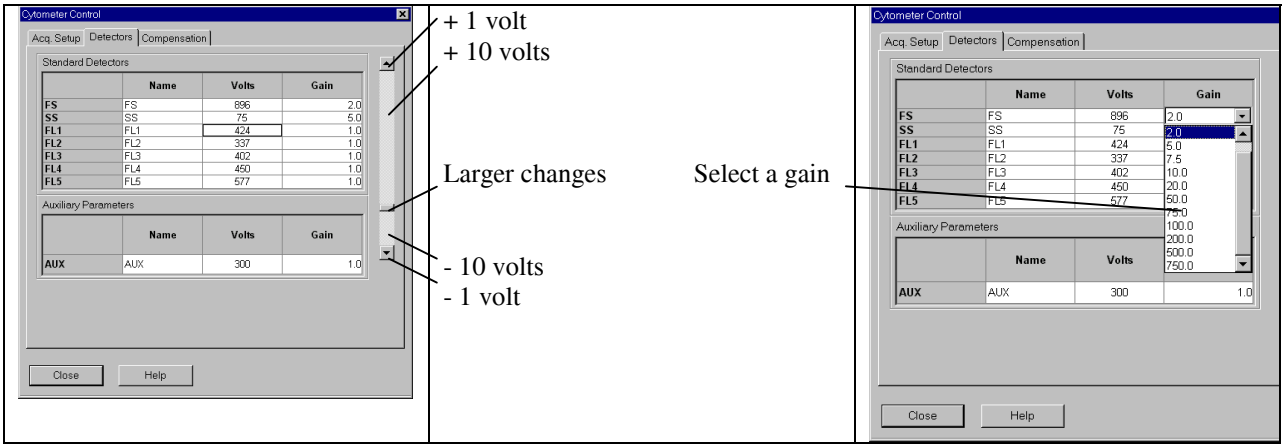
- Set the discriminator value to a channel, which will be above the noise and most of the debris is below the cells of interest.

Example: The Immuno-Brite fluorospheres are 10 um in diameter which is significantly larger than debris and noise. It makes sense, then, to set the noise discriminator on the FS signal. The choice of 100 is a starting point. The value could be adjusted later if debris or noise is still visible.

Note: the topic of Baseline offset is addressed in the Software Extras module.

Setting Gains and High Voltage

1. Select Setup Mode on the **Acq. Setup** tab window.
2. Select **Detectors** tab.
3. Place a sample on the system and select the  icon.
4. Adjust the high voltage or gain for a parameter while viewing the data on the plots to place the data in the desired position.

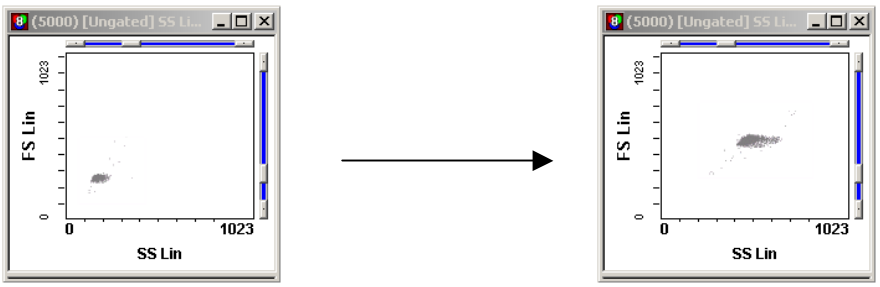


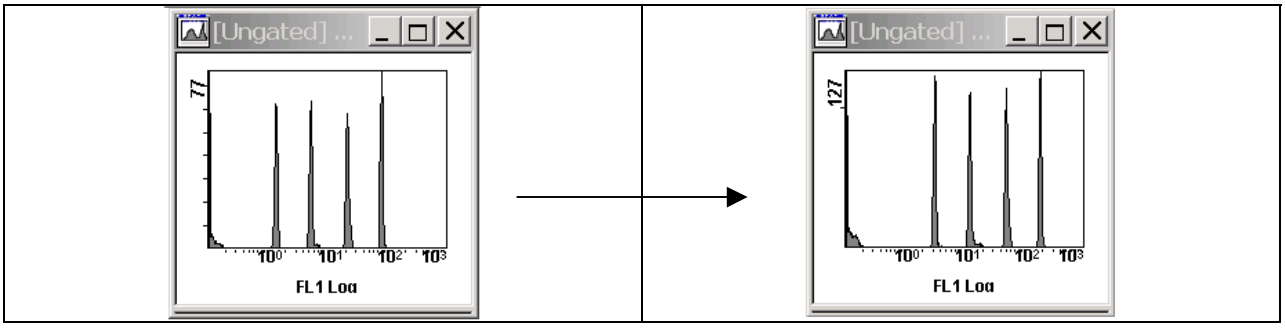
Tips

- If you do not know where to start, begin with the Flow-Check high voltage and gain settings. They are 10 um, bright fluorescing fluorospheres.
- 6-14 um cells will probably use the same forward scatter settings. Side scatter will need to be a bit higher.
- Fluorescence high voltage likely needs to be increased some.
- A measure of signal strength can be seen on the Cytometer Indicator Panel.

QuickSET

5. Select QuickSET on the **Acq. Setup** tab to visually adjust the High Voltage using slider bars on the plots.





NOTE: For the Immuno-Brite fluorospheres, repeat the adjustment for FL2 Log through FL5 Log.

Setting Compensation (If needed)

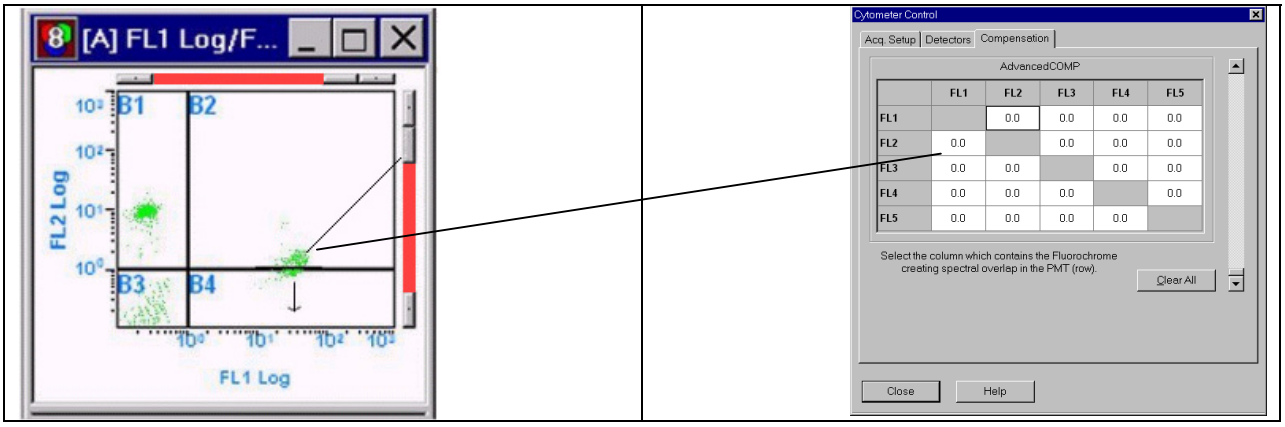
NOTE: Our Immuno-Brite example does not need compensation because there is only one dye. If there were multiple dyes then compensation would be adjusted as indicated below or you can take advantage of the autosetup option. The latter will be covered in a different module.

1. Select the Setup and QuickCOMP options on the **Acq. Setup** tab window.

2. Select the **Compensation** tab.

3. While running a compensation sample move the slider bars on the sides of the appropriate dot plot to adjust the dot plot data to the correct position.

NOTE: You can also see the desired compensation value on the Compensation tab window and set it by moving the slider bar on that window.

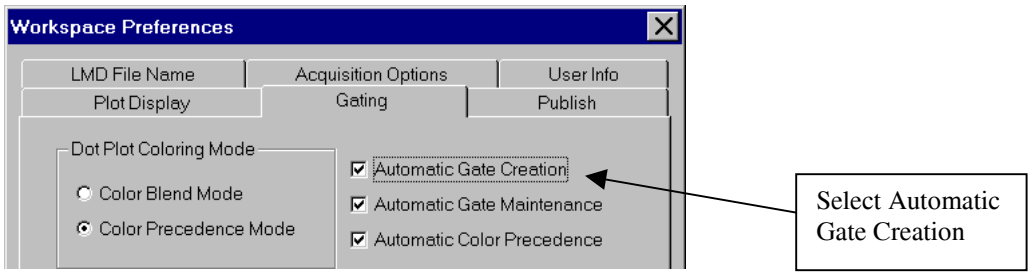


Creating Regions

Regions enclose a portion of the whole plot. They are used to generate statistics concerning that portion or can be used as gate criteria for other plots. If the latter use is desired, the Workspace Preferences should be set to allow new regions to be used as gates. They are best set with data on the screen to help you estimate their size and location.

Making every region a potential gate

1. Select **File** >> **Workspace Preferences** and then select the **Gating** tab. A window should appear as below:



2. Select **Automatic Gate Creation** if it is not already selected.

3. Select **OK**. This feature automatically places every region created (except Quadrant regions) onto the gate list.

Dot Plots

1. Click on the desired dot plot.

For our Immuno-Brite protocol, start with the SS versus FS plot and choose the polygon or rectangle.

NOTE: Notice that different region buttons are now highlighted on the File Toolbar (see below).

	Allows you to create a polygon around an area of the dot, contour, or density plot.
	Allows you to create a rectangular box around an area of a dot, contour, or density plot.
	Allows you to set two lines; one vertical and one horizontal, to divide a dot, contour, or density plot into four rectangular regions.
	Allows you to set an elliptical autogate region around a population.
	Allows you to set a contour autogate region around a population, which follows the outline of the population.

Polygon

2. Select the  button.

3. Move the cursor to the desired starting point to draw the polygon and then use a click, move, click, move technique to sketch the polygon around the desired population. To finish, move back inside the original starting box and click.

Rectangle

4. Select  box

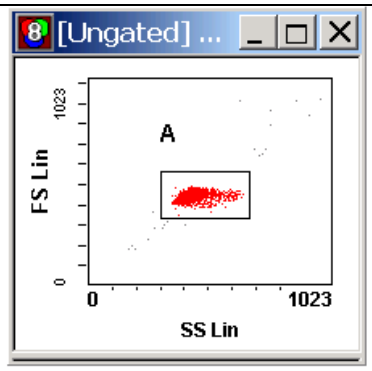
5. Move cursor to starting point and click and hold left mouse button.

6. Move mouse to stretch box to desired position.

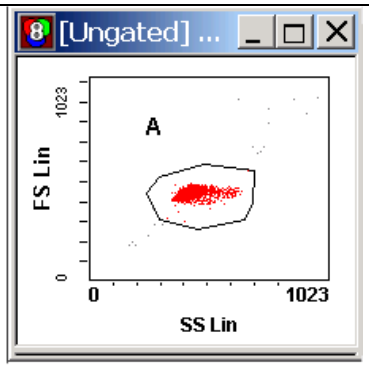
7. Release left mouse button.

NOTE: Notice the color appearing on the dot plots. Each region as it is created is assigned a color (e.g. green) if **Auto Color Precedence** is selected in the **Workspace Preferences**. Events within the region are colored on all dot plots; events outside the region remain gray.

Examples:



Rectangle



Polygon

Note: The rest of the regions shown for dotplots will not be needed for our Immuno-Brite example.

Quadrant Region

8. Select  box.

9. Move cursor to the plot and click the left mouse button.

10. Move the region to desired position and click left mouse button.

Elliptical and Contour Auto Gates

11. Select the desired option ( or ) and then click over the top of the population. The system automatically draws the elliptical or contour gate.

NOTE: If there is insufficient data or not a well-defined peak in the data, the elliptical autogate will draw a polygon in the shape of a rectangle. Each vertex can be edited to encompass the population.

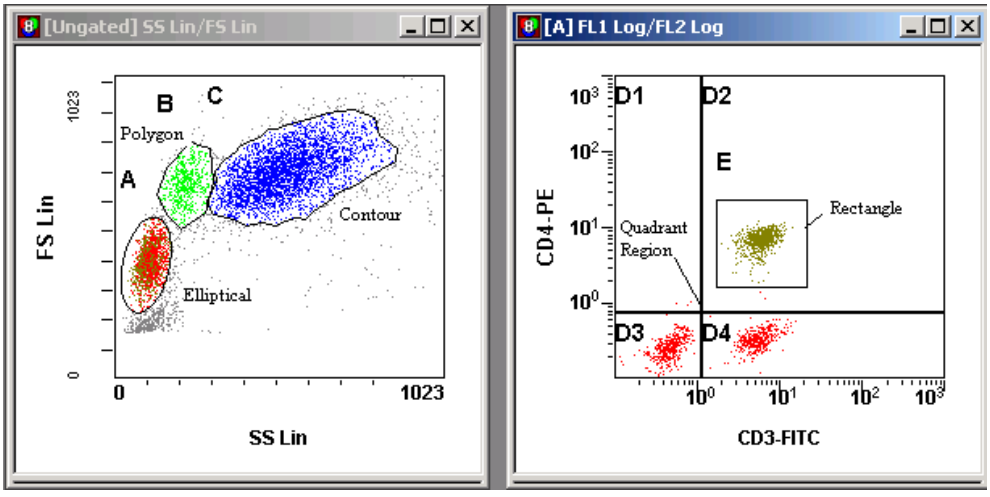
Having Trouble?

12. Press **Esc** key to stop.

13. Expand the plot to full screen using the  button and then try drawing the polygon again; it should be easier.

14. When you are finished, minimize the plot with the  button.

Other Examples



Common Editing Region Functions

A region can be changed (edited) if it is not exactly what you want.

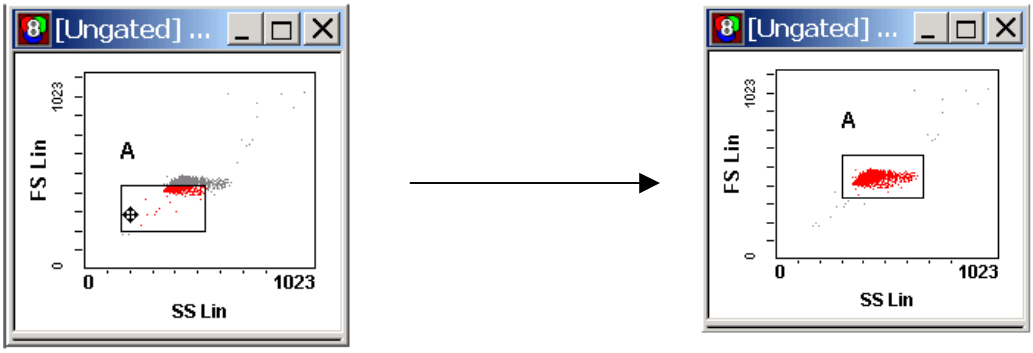
1. Select the letter (or name) of the region (except Quadrant in which you select the region itself). Region handles should appear.

2. Select a handle (vertex) to stretch the region in a direction or use the  symbol to move the entire region to a new location.

3. Click the mouse outside the region to finish and anchor the region.

4. To undo the move, select  or Edit >> Undo.

Example



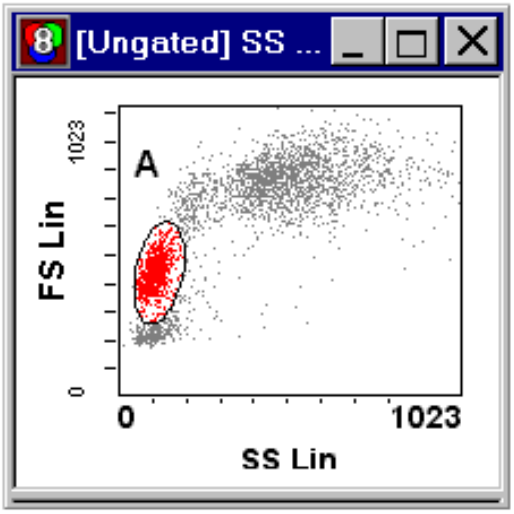
NOTE: For Quadrant regions, you can move just the horizontal line with an up/down arrow or the vertical line with a left/right arrow or click and drag individual lines.

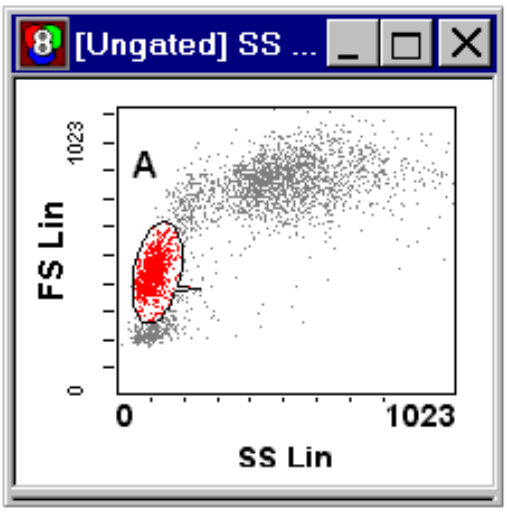
Additional Editing Functions for Polygon or autogate regions.

1. You can edit a single point by selecting that point and then drag and drop it to a new position.

2. To undo the change, select  or Edit >> Undo.

NOTE: The examples below illustrate a whole blood prep in which the red cells have been lysed and removed. Only the white cells remain.

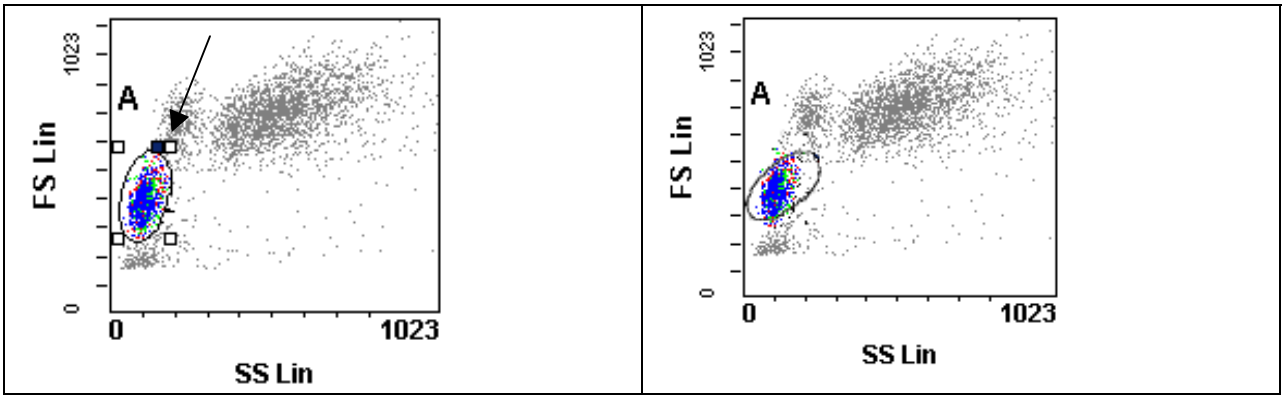




3. Select rotate handle (black box below) to rotate entire region.

4. To undo the rotation, select  or Edit >> Undo.

5. Click outside the region to eliminate the handles.



Single Histogram Plots

For the Immuno-Brite protocol, you will need 5 linear regions bracketing each of the five peaks on each log fluorescence histogram. They may be created individually (linear region) or using the multiple linear region option. The easiest way to accomplish this task is to create one set on FL1 log and then drag and drop copies of these regions from FL1 log to the other fluorescence plots. Later you can edit the regions to position them properly.

1. Select the desired single histogram plot (e.g. FL1 Log).

	Allows you to create a single linear region on a single parameter histogram plot.
	Allows you to create a series of linear regions, one right after the other on a single parameter histogram plot.

Single Linear Region

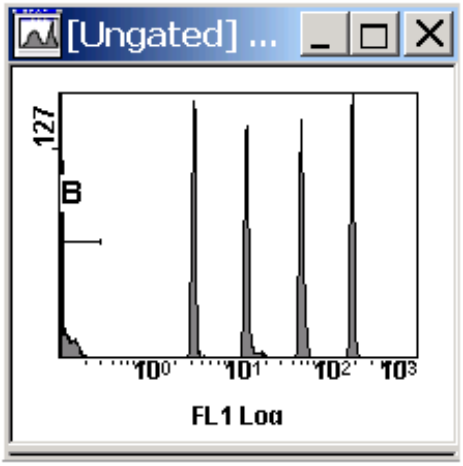
2. Select 

3. Move the cursor to the starting point and click the left mouse button.

4. Move the mouse to stretch the region to the desired position.

5. Click the left mouse button.

Example:



Multiple Linear Regions

6. Select 

7. Move the cursor to the far left starting point for the first region and click the left mouse button.

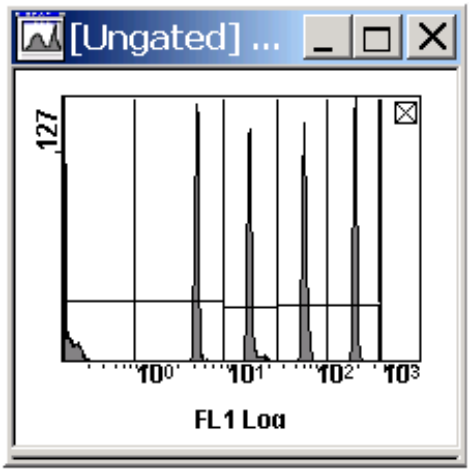
8. Move the mouse to stretch the region to the desired position.

9. Click the left mouse button.

10. Repeat steps 8 and 9 for each additional region.

11. Press **Esc** or click on the ☒ box at the upper right of the plot to end the process.

Example:



NOTE: When using multiple linear regions, you will likely have to edit the regions to place them in the proper position.

Tips

- When there a lot of similar regions on different plots (e.g. FL2 - 5 Log regions are similar to FL1 Log) you can use a drag and drop technique to copy regions to another plot. The system creates duplicate regions on a new plot with sequential letters. These new regions are independent of the previous regions.

- Multilinear regions cannot be used later as gates. If you wish to use a histogram region later as a gate, create it with the linear region tool.

Assigning Gates

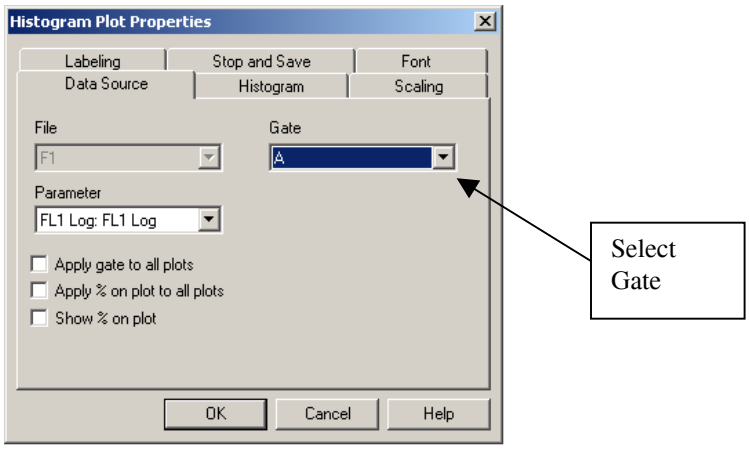
Gating is the use of some criteria that must be met first before data is used in a plot. Regions define the criteria but they must be assigned to a plot before gating can occur.

In our Immuno-Brite sample we wish to view the data only from the single fluorospheres and not doublets (two fluorospheres stuck together) or debris. We created a polygon or rectangle to define the single fluorospheres. Now we need to assign this region to the fluorescence histograms. To accomplish this:

1. Move the cursor to the lower right corner of the plot to be gated (e.g. FL1 Log) until the cursor changes to **>>>** and then click the left mouse button.

2. Select the gate region in the Gate box.

Example:



NOTE: If most of the plots are to be gated, you can reduce the number of steps by selecting the "Apply gate to all plots" option. Example: The Immuno-Brite plots FL1 Log through FL5 Log are all gated on A.

3. If you wish to display the % within a region on the plot or all plots, select the appropriate option.

4. Select **OK**.

5. Ungate the plot(s) you wish to remain ungated (ex. SS/FS plot) and select **OK**.

Analyze Data

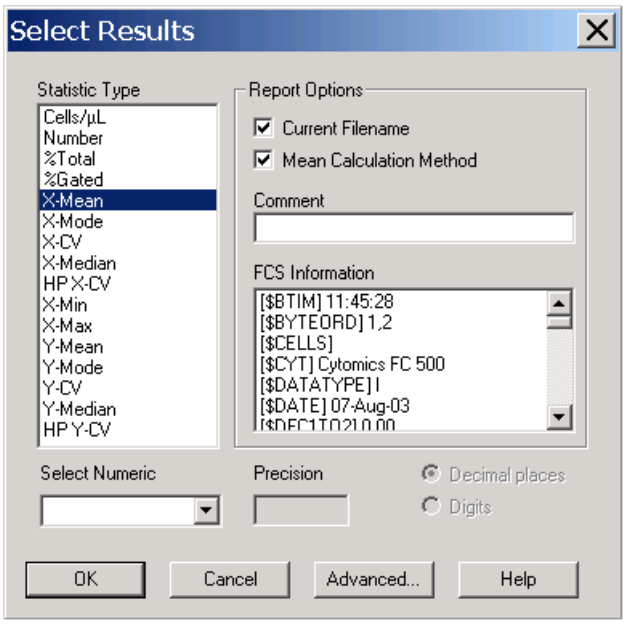
1. Select **Analysis >> Select Results…**

2. Deselect the statistics you do not wish to see within the Statistics box and then select the desired statistics.

3. When you are finished, select **OK**.

4. If you select more stats than can be displayed, a scroll bar will appear to allow you to scroll to the hidden ones.

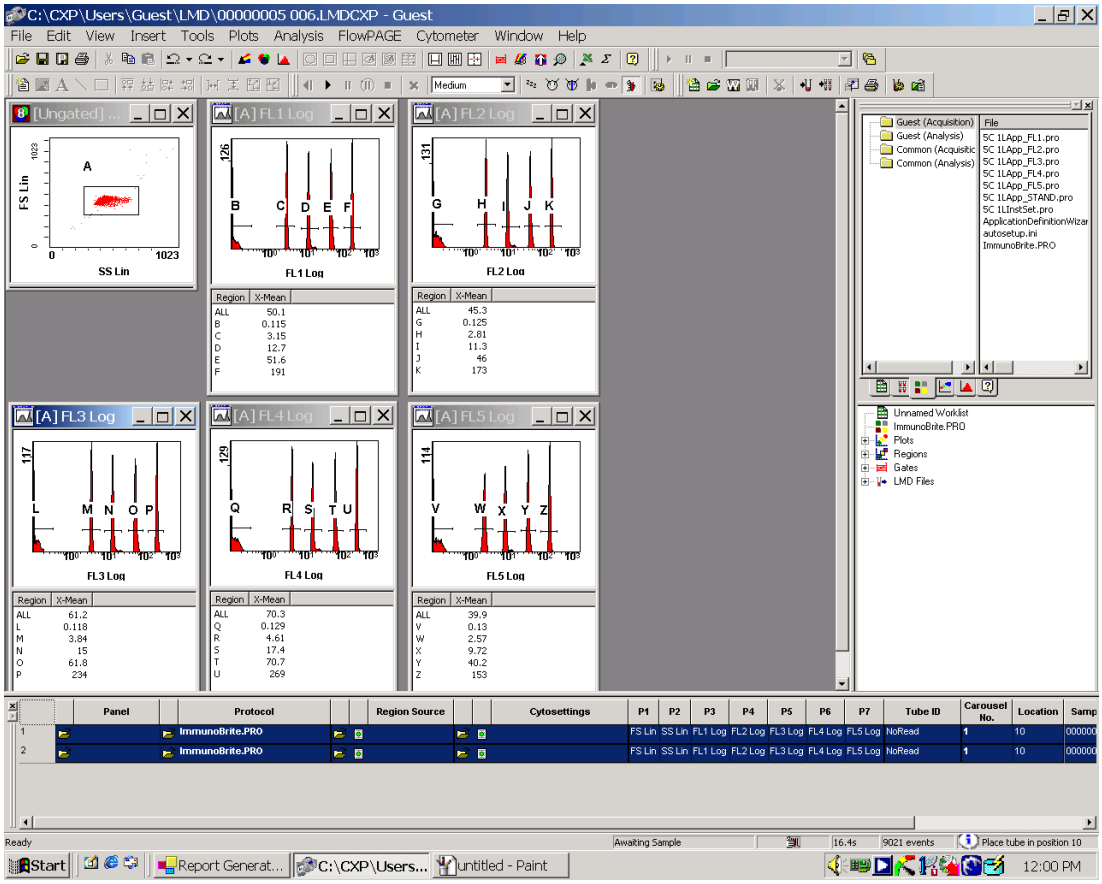
Example: For Immuno-Brite fluorospheres, you will need only the X-Mean.



5. Move the cursor to select the **T** cursor on the plot (lower left corner of the plot) to view the statistics.

NOTE: If you select more stats than can be displayed, a scroll bar will appear to allow you to scroll.

Example:



Optional: Add a FlowPAGE

The procedure will be covered in the Creating FlowPAGE module. You can add the FlowPAGE to the protocol later after you have completed that module.

Saving the Protocol

1. Select **File** >> **Save Protocol As…**
2. Type in a new name and select **Save**. A new protocol has now been added to your protocol list.

This protocol now contains: Plots, Regions, Gating, Color choices, Analysis, and FlowPAGES

NOTE: Print the final version of the protocol with the Immuno-Brite fluorosphere data as proof you have completed this practice session. You will be asked for this printout as part of your skill check.

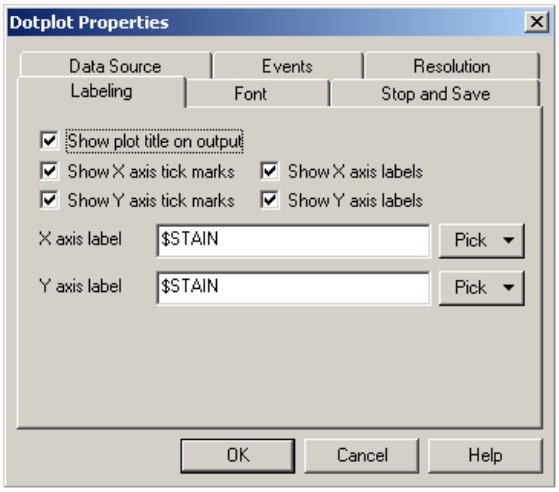
Additional Options

Not every protocol requires all the options. The following pages explain the various options available and you are encouraged to try some of them. For example, you might experiment with different fonts to improve the appearance of the plots.

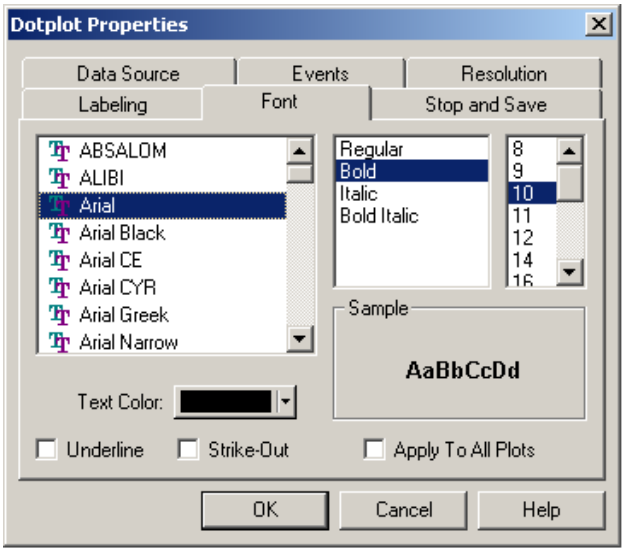
Plot Tab Options

Common Tabs:

1. Use the Labeling tab to place labels or tick marks on the plot.

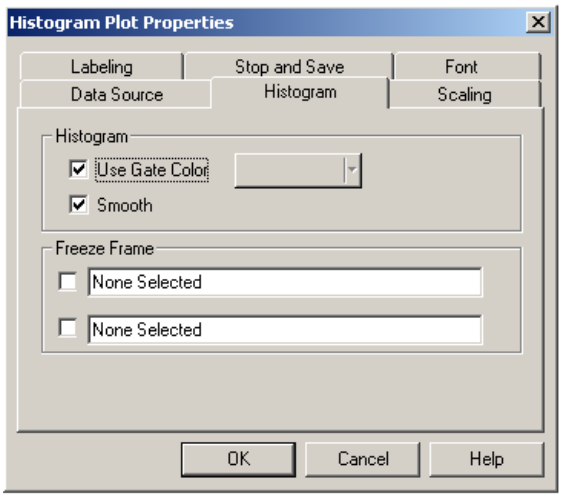


2. Use the Font tab to change the type, size and color of text.

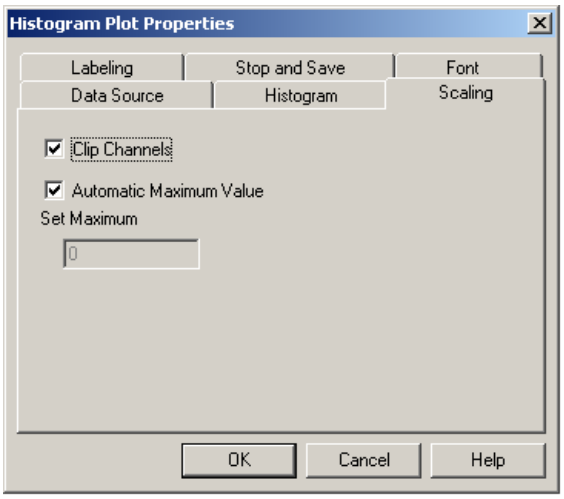


Histogram Tabs:

3. Use the histogram tab to Use Gate Color, or Smooth the data. Use Freeze Frame when samples are run or retrieved later. Freeze Frame is explained in more detail in the Extras module.

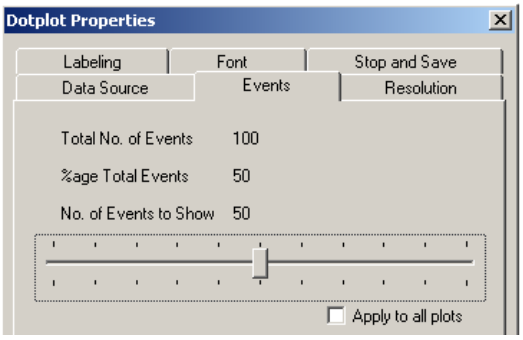


4. Use the scaling tab to exclude the first and last channels (Clip Channels selected) from the scaling process, set an automatic maximum value, or set the scale to a specific count.

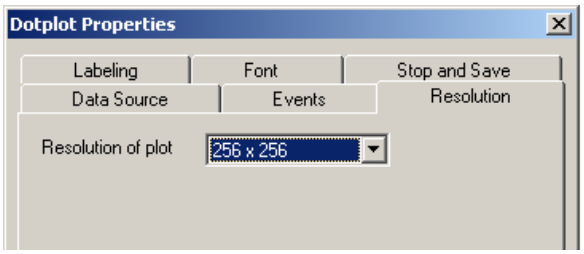


Dot Plot Properties Tabs:

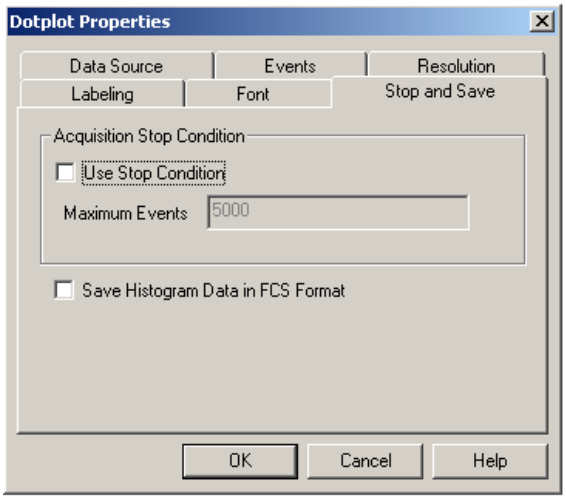
5. Select Events tab (if desired) to set the % of the events to be displayed. A higher % displays more of the data but will slow down the computer a bit when refreshing the screen.



6. Use the Resolution tab to change the number of channels for each axis.



7. Use the Stop and Save tab to set stop conditions and save data.



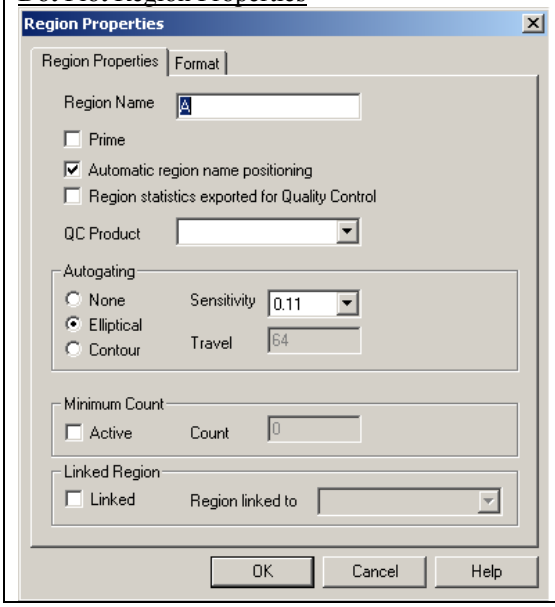
NOTE: When there are multiple stops set (e.g. time, total count, stop and save on a histogram, etc) the first stop reached stops data collection.

Additional Region Options

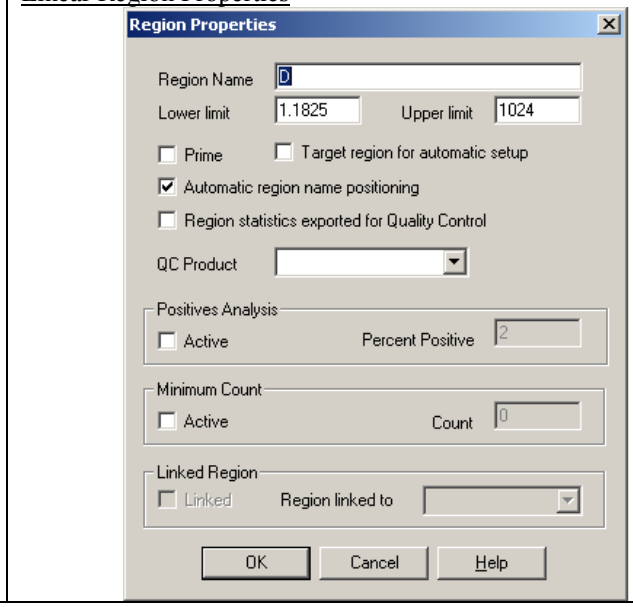
1. Click region to activate grab handles.

2. Right click to display **Region Properties** window.

Dot Plot Region Properties



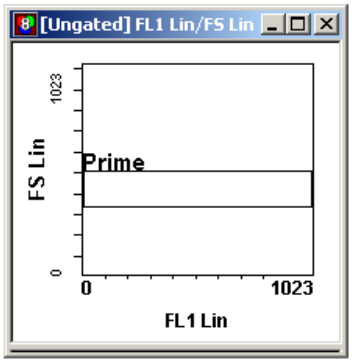
Linear Region Properties



Common Properties

3. Rename the region as desired.

4. Select **Prime** if you wish the system to activate a prime function whenever data falls outside the region.



5. Select **Automatic region name positioning** to position the region name at the upper left of the entire region on the plot automatically.

6. Select a region to automatically export data for quality control.

7. Select **Active** under **Minimum Count**. Set a minimum count value if you wish to continue collecting data beyond a set stop criteria until the minimum count in the region has been reached.

8. Link a region to another region. The regions must be a similar type (e.g. rectangle to rectangle, polygon to polygon, linear to linear, rectangle to one of the 4 quadrants in a quadstat region, etc.). The plots must be the same type with the same parameters. Note: On two parameter plots the parameters can be on opposite axes. Once linked, moving one region also moves the other one linked to it. When a polygon is linked to an autogate region, the polygon takes the shape of the autogate region.

Dot Plot Only Properties

9. Select **Autogating** if you wish the system to redraw the region automatically when new data is collected. Also set the sensitivity setting. There are 3 levels of sensitivity for elliptical autogate and 5 levels of sensitivity on contour autogate. The higher the number, the tighter the redraw of the ellipse from the center of the population.

NOTE: Use Travel to control the tightness of the redraw on contour autogates. Use lower numbers to tighten the pattern.

10. Use the **Format** tab to customize the color and width of the line.

NOTE: If the system is unable to autogate (for example, if there are insufficient data points), the system will draw the polygon as a rectangular shape. You may recalculate the autogate with a right mouse click on the region and select Re-calculate autogate.

Linear Region Only Properties

11. Enter a lower and upper limit if you wish to enter region boundaries numerically.

12. Select the **Target** region for automatic setup option to automatically adjust the population (e.g. Flow-Set™ Fluorospheres) to the region when a multibead Flow-Set preparation is used.

13. Activate the **Positives Analysis** and enter a Percent Positive value (usually 2.0 %) if you wish the system to automatically adjust the region lower boundary to place the requested percent of the data within the region.

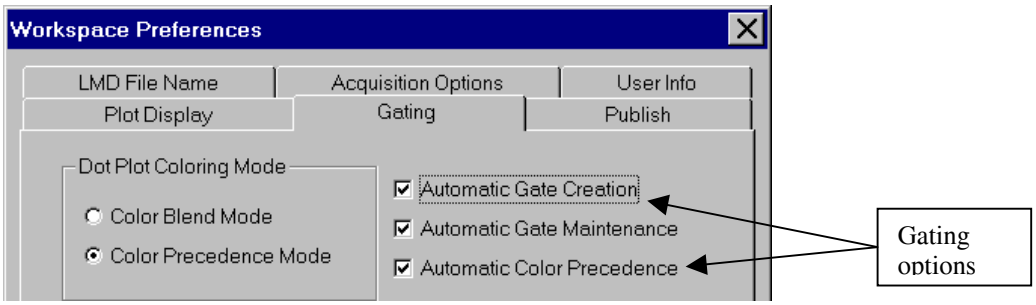
Quadrant Region Properties

14. Activate the **Positives Analysis** and enter a Percent Positive value if you wish the system to automatically adjust the region boundaries. The system will adjust quadrant 3 as close as possible to contain 100 – Percent Positive value you entered (example: enter 2 %, system adjusts to 100 – 2 or 98 %).

Additional Gate Options

Workspace Preferences

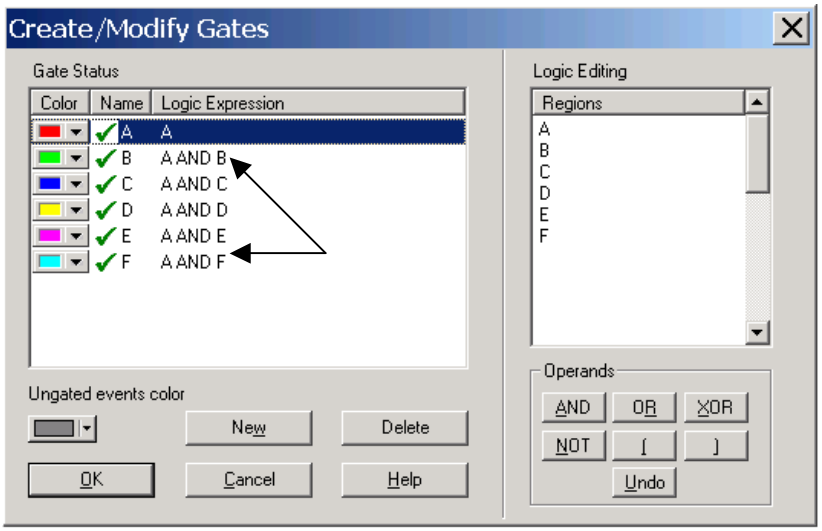
1. Press **CTRL** and **W**.
2. Select Gating Tab.
3. Select Automatic all three gating options and then **OK**. Each option will be explained in the following pages.



Automatic Gate Creation

NOTE: The **Automatic Gate Creation** also combines regions for you. Regions created on gated plots are combined with the gates for the plot. Suppose for example, the D region was created on a plot gated on region A. The system creates an A and D gate and calls it D. A new plot gated on D would really be gated on A and D. The title bar will show the true gating.

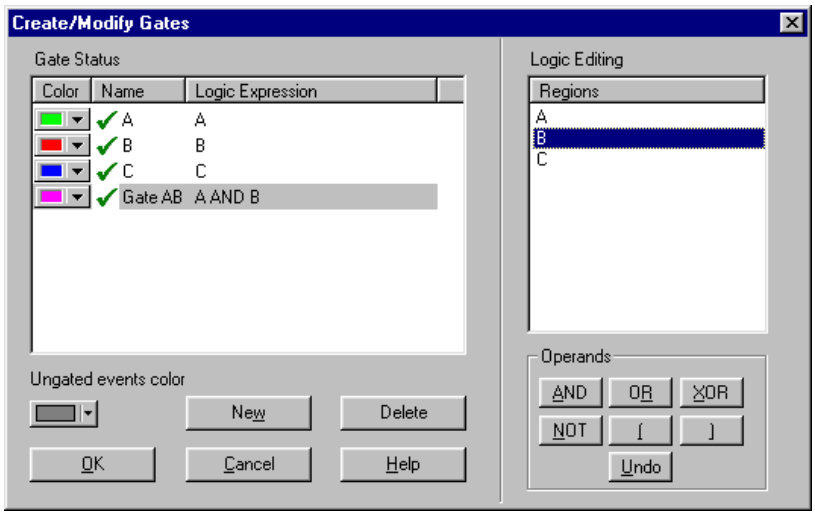
Example:



Creating Gating Strategies Using Boolean Logic

NOTE: The **Create/Modify Gates** window can also be used to create gates made up of multiple regions linked together in some logical order. In this case, all the criteria must be met before an event will be included in the gated plot.

1. Select  button and then the New button.
2. Type in desired name (e.g. GateAB) and then press **Enter**.
3. Now select in order the regions and logic linking them (e.g. A AND B).
4. Select **OK** to finish



Automatice Gate Maintenance

Automatic Gate Maintenance allows changes in the parent plot gating to be carried through to other plots. When Automatic Gate Maintenance is enabled, changing the gate on the parent plot (e.g. from A to B) changes the gate equation (from A and D to B and D). The new gate equation would be applied to every plot gated on D.

You now can make one change and have it applied to several plots at the same time.

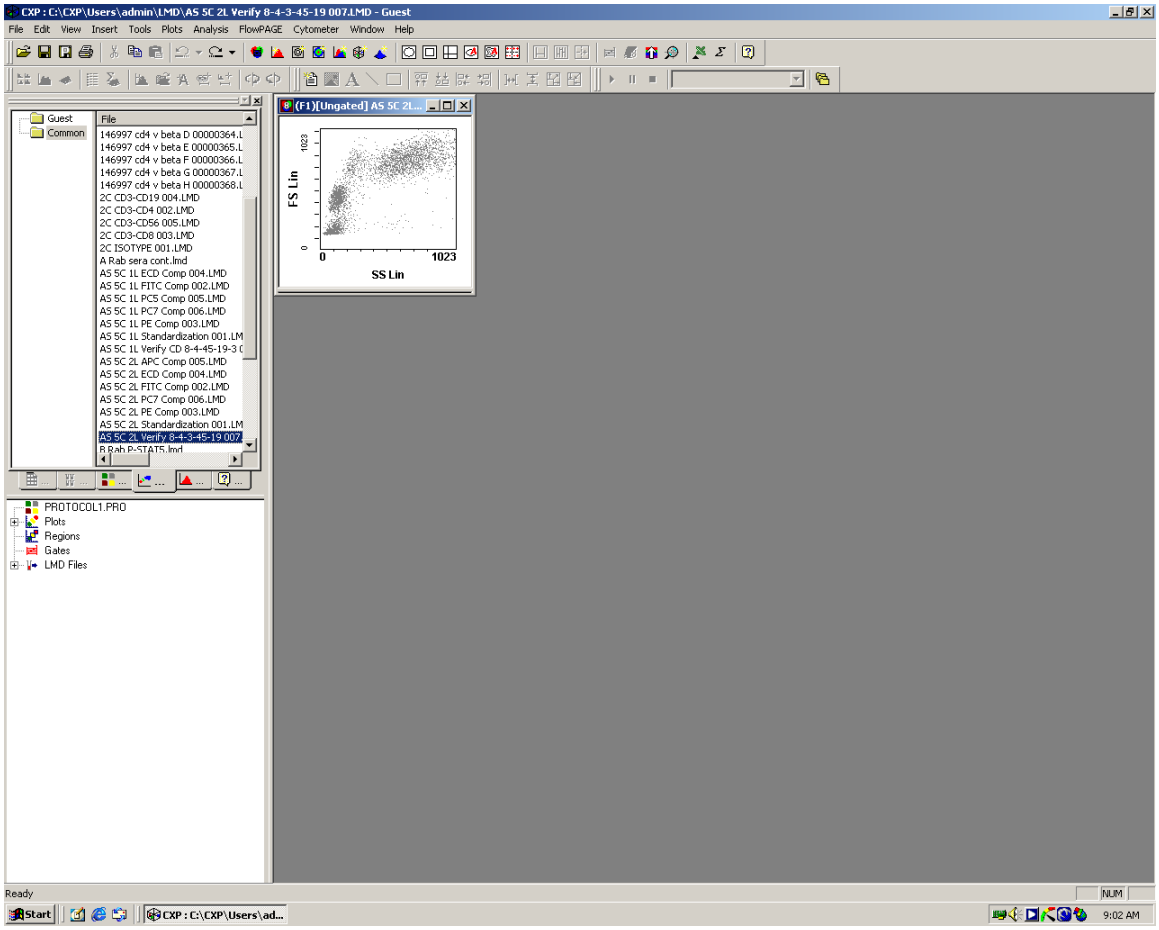
Automatic Color Precedence

NOTE: You can use either **Color Blend** or **Color Precedence** to assign gate colors to a dot plot. In color blend, a different color other than a region color is assigned when data falls in more than one region. With color precedence, one region color dominates (takes precedence) when data falls in multiple regions. The Extras module explains the concept in more detail.

Analysis Only Options

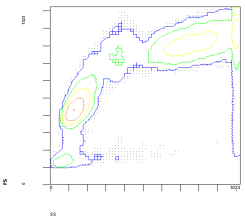
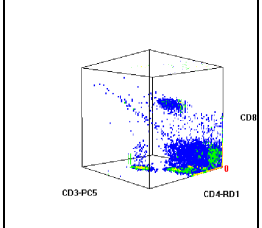
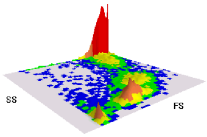
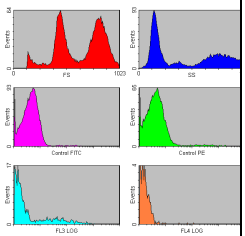
Log on to the Analysis Software

1. Minimize the Acquisition software.
2. Double click on the Analysis software icon on the desktop.
3. Select your user name (or Guest), enter your password, and select **Next**.
4. Select **Finish**.
5. Select the listmode file tab on the Resource Explorer, then the Common folder, and then AS 5C 2L Verify 8-4-3-45-19 data file.
6. Drag and drop this file onto the dotplot on the workspace. Your screen should now appear as below:



NOTE: This setup will be used to generate example analysis only plots.

Analysis Only Plots

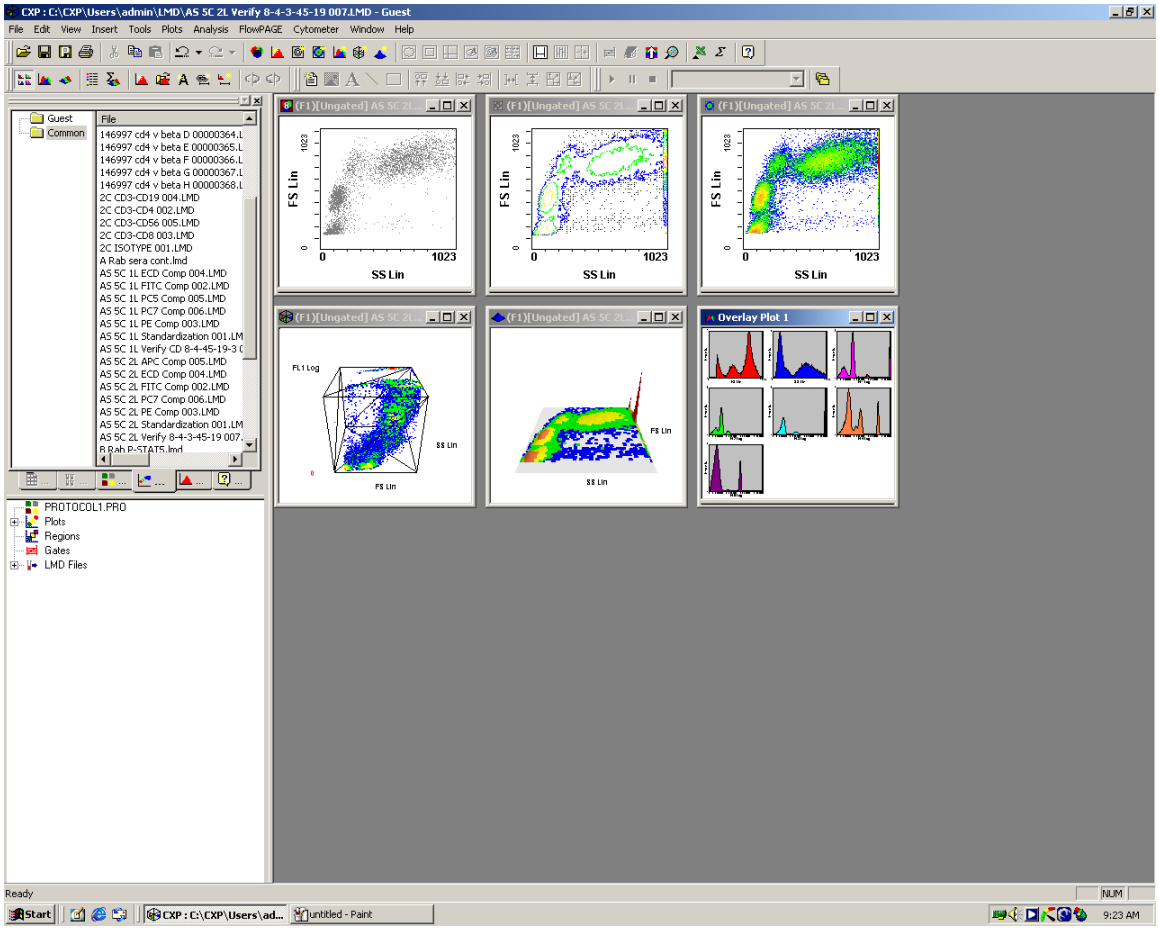
Contour Plot  (Analysis only)		Tomogram  (Analysis only)	
Surface Plot  (Analysis only)		Overlay Plot  (Analysis only)	

Creating and Populating the plots

NOTE: The following exercise provides example plots on the screen for you to view and change.

1. Select the  icon.
2. Select SS for the X-Axis and FS for the Y-Axis and then **OK**.
3. Select the  icon.
4. Select SS for the X-Axis and FS for the Y-Axis and then **OK**.
5. Select the  icon and then OK.
6. Select the Data Source tab, enter the desired X (e.g. SS), Y (e.g. FS), and Z (e.g. FL1 Log) parameters and then select **OK**.
7. Select the  icon, select the Data Source tab, enter the desired X (e.g. SS) and Y (e.g. FS) parameters, and then select OK.
8. Select the  icon.
9. Drag and drop the listmode file AS 5C 2L Verify 8-4-3-45-19 onto the new overlay plot to establish an example data overlay.
10. Press **CTRL** and **T**, order the plots into 2 rows and then select **OK**.

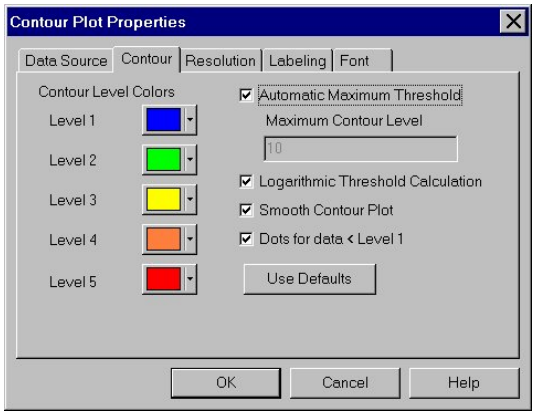
The screen should appear as below:



Plot Tabs

Contour, Density, Tomogram, and Surface Plots

11. Use the Contour and Density plot tab to set the level colors. There are also few other options. An example is shown below:



12. Select **OK** when finished.

NOTE: The other tabs are similar to the ones discussed earlier for a dotplot and histogram plot.

To change initial selections:

13. Move the cursor to the lower right corner of the new plot until the cursor changes to **>>>**. Now click the mouse. For the Tomogram and the Surface plots, you will need to right click the mouse button on the plot and select Format. The Properties window reappears.
14. Make the desired changes and then select **OK**.

NOTE: If you select a new listmode file, the parameters on some of the plots may change. If the Listmode file parameters are in a different order or are different than the selection order for the plot, the parameters will change. For this reason, you may want to populate the initial plot first to establish the parameter order and then create the other plots.

To rotate a tomogram or surface a plot

1. Move your mouse to the middle of the tomogram (or surface) plot. Now hold the left mouse button as you move the mouse. The plot rotates or flips depending on the mouse movement.

2. NOTE: If you move the mouse to the right and the plot rotates to the left, you have turned the plot upside down. Just move the mouse vertically if you wish to right the plot.

To resize tomogram or surface plot

1. Release the left mouse button and move the mouse wheel. The plot becomes larger or smaller depending on the direction you move the wheel.

2. You can right click on the Tomogram and/or Surface plots and select AutoRotate. The two plots now begin to spin.

Summary: Creating a Protocol

1. Think through your protocol on paper first. It will be easier.
2. Always start with the default protocol unless the new one is very similar to the present protocol.
3. Select the desired parameters. In the Analysis software, populating the default plot with a listmode selects the parameters.
4. Select the desired plots and arrange them on the screen.
5. Create the regions. We suggest you pay particular attention to those to be used for gating first.
6. Assign the gates to the plots.
7. Setup the rest of the regions to analyze the data.
8. Optional: You can add a FlowPAGE (details in the Creating FlowPAGE module).
9. Save the protocol.

You have now completed this module. If you feel you are ready, please complete the Creating a Protocol Skill Check.

OBJECTIVES

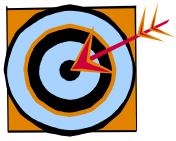
Given an operational system, all necessary tools, access to a Special Procedures and Troubleshooting Guide, and access to online Help

- Clean and replace air filters.
- Clean the reagent and waste containers.
- Clean the sampling system.
- Remove and replace the sheath filter.
- Adjust system pressure.
- Change field stop position for forward scatter.
- Replace the sample probe and sample pickup tubing.
- Replace the MCL sample head and associated tubing.
- Remove and replace the optical filter plate and a filter.
- Adjust the HeNe laser.

WHY IS IT IMPORTANT?

This module guides you through the cleaning and replacement procedures for the system. The Special Procedures and Troubleshooting manual outlines when each procedure is to be performed and how to accomplish each task. A properly operating system depends on faithfully performing these maintenance tasks.

Skill Check Preview



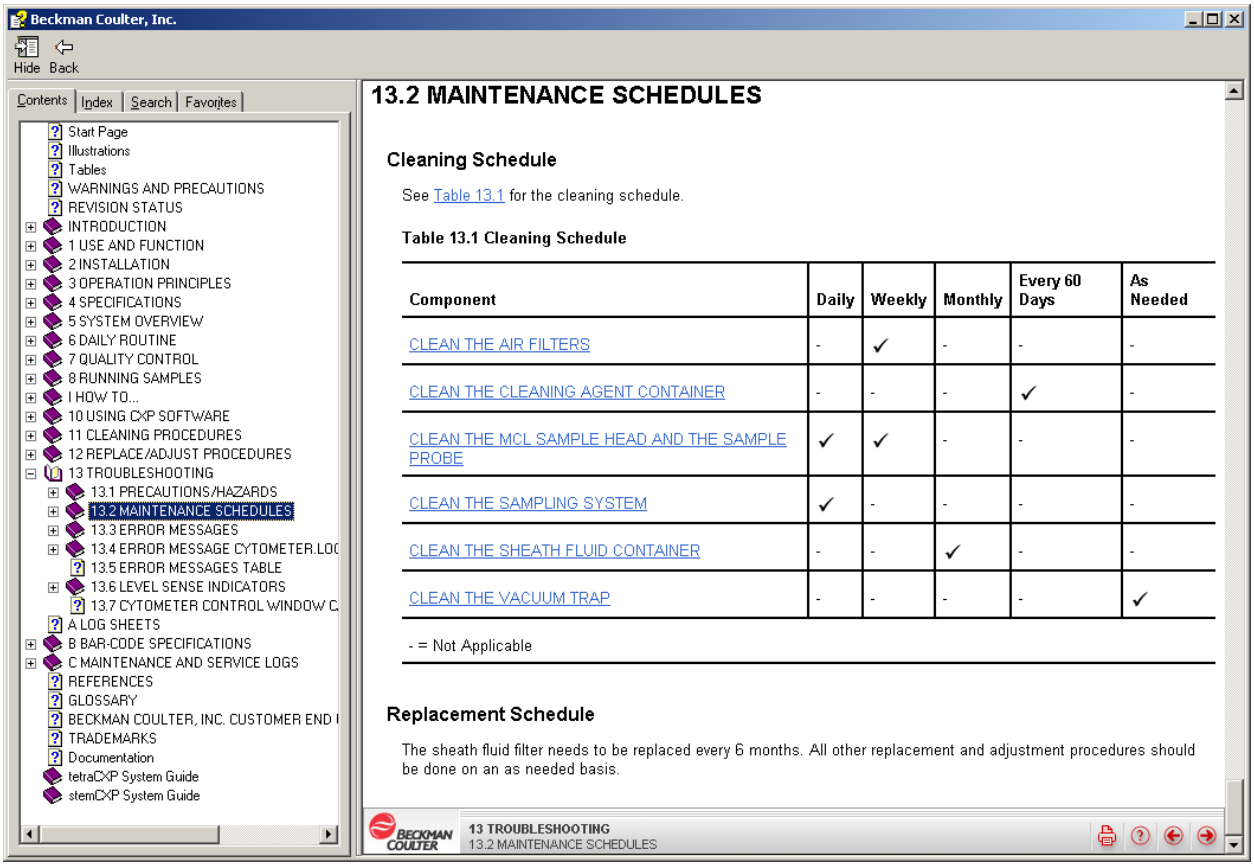
You will have mastered this maintenance module when you can perform each task listed in the objectives.

NOTE: Each individual task can be completed at different times. The whole module will be signed off when all tasks have been completed.

INFORMATION/PRACTICE SECTION

Before attempting any of the maintenance procedures, we suggest you use the online Help to view any available Show Me video clips and/or the step by step procedures. You can access the online Help:

1. Select the  on the toolbars.
2. Select 13.2 Maintenance Schedules. The following screen will appear.



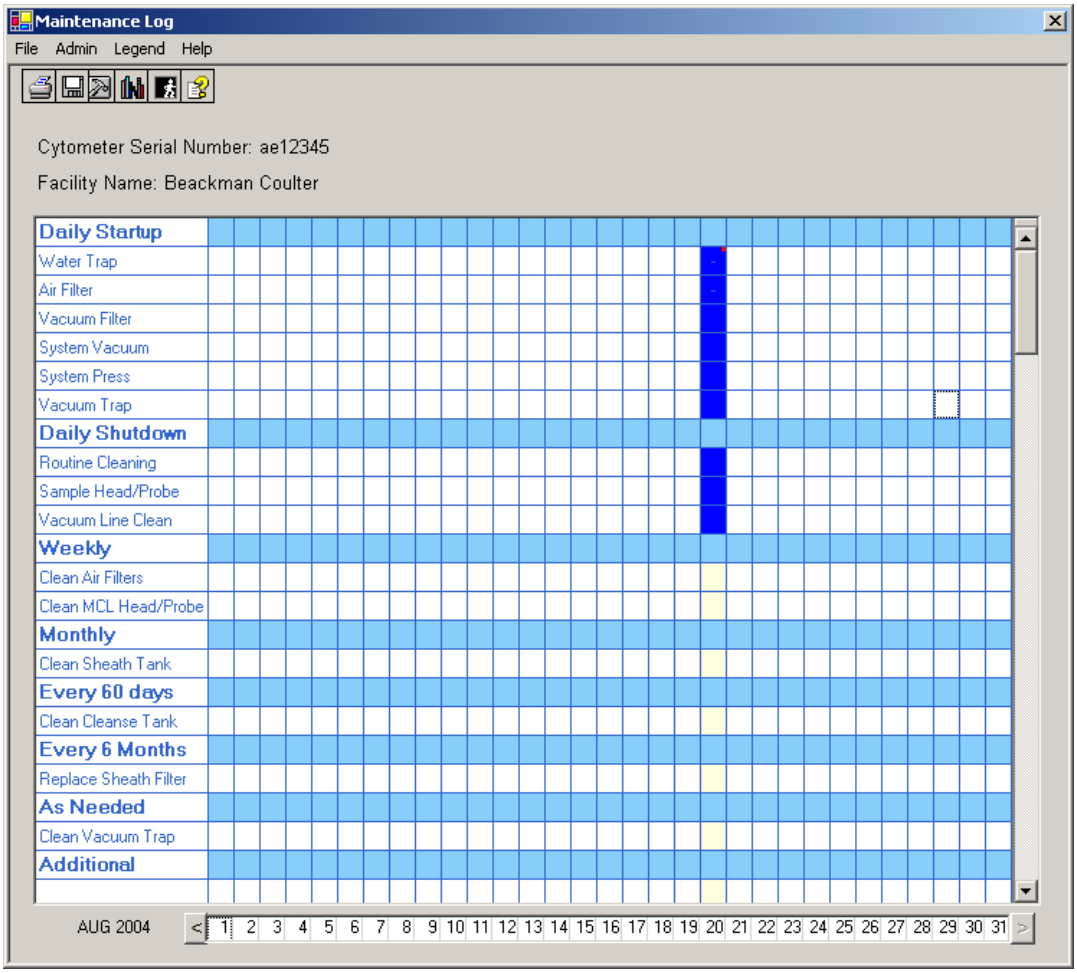
3. Select the desired procedure. If a blue Show Me appears, select it to view a video clip illustrating the procedure.

Get facilitator signoff as you complete each of the Maintenance procedures listed. Procedures may be performed in any order.

Remember to access and print the appropriate procedures from the HELP system.

You can also log any maintenance performed on the system in the Maintenance log.

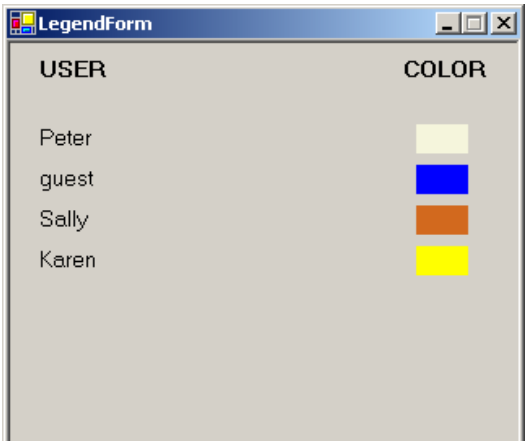
4. Select the Report Generator toolbar (bottom of the screen) and then the  icon. The screen displays the maintenance log.



5. Double click on the appropriate boxes on todays date to log completion of the tasks.

6. When you are finished, select the  icon to close the log.

Icons:

 - Print	Legend: 
 - Save	
 - Log on as administrator (the letter A will appear in entries entered by an administrator blue arrow above). The administrator can change any previously selected cell, any user, any date.	
 - User color legend	
 - exit	
	

OBJECTIVES

Given training materials, and access to online Help.

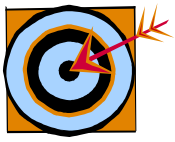
- Recognize acceptable versus unacceptable data.
- When data is unacceptable, determine the possible causes and appropriate action to correct the situation.
- Participate in the troubleshooting discussion.
- Access and view the cytometer.log file and explain the meaning of each entry.
- Use the error message table to determine the cause of a particular error message and describe the action that should be taken to correct the situation.
- Correctly answer 80% of the skill check troubleshooting questions per answer key.
- Print the Important Information file from the Resource Explorer.

WHY IS IT IMPORTANT

This module is part of a facilitator led discussion on troubleshooting. The pages that follow show example data illustrating situations that may be encountered when running quality control or application samples. As a group you will examine each data set and determine if the data is acceptable or not. If it is deemed unacceptable, you will be asked a series of questions leading to a correction of the situation. After all, correcting unacceptable situations is the essence of troubleshooting.

Skill Check

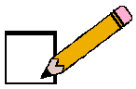
You will have mastered the troubleshooting module when you



- Recognize acceptable versus unacceptable data.
- Can determine possible causes for unacceptable data and appropriate actions to take.
- Have participated in the troubleshooting discussion.
- Can access and view the cytometer.log file and explain the meaning of each entry.
- Can use the error message table to determine the cause of a particular error message and describe the action that should be taken to correct the situation.

- Correctly answer 80 % of the Skill Check troubleshooting questions as determined against an answer key.

- Print out the Important Information file from the Resource Explorer.



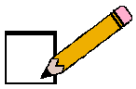
PROCEDURE

Exercises 1 - 21

In each of the following pages a set of data is displayed. You are to

- Determine if the data is acceptable or unacceptable.
- Explain your answer.
- Suggest causes for the unacceptable results.
- Suggest other observations that may confirm or eliminate a possible cause.
- Suggest a course of action to correct the situation.

Do not be concerned if you cannot answer all of the examples. There will be a group discussion scheduled (your facilitator will tell you when) in which each exercise will be discussed.



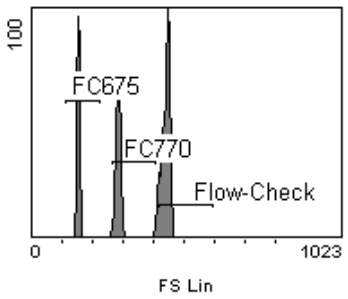
Cytometer.log file and Error Codes

- Follow the directions on cytometer.log file and Error Codes pages at the end of this section.

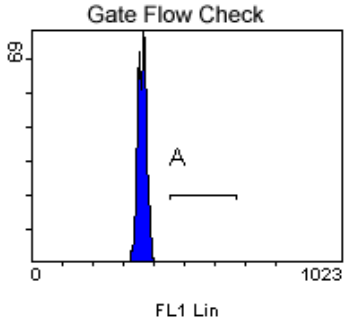
NOTE: It is suggested that you print the cytometer.log file and complete the Error Codes pages prior to attending the facilitator-led discussion session.

FLOW-CHECK™ FLUOROSPHERE DATA

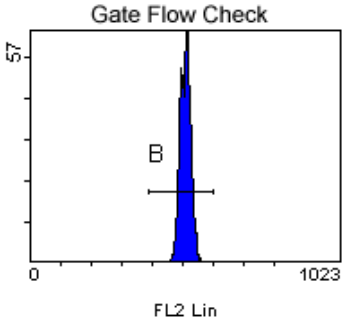
Exercise 1



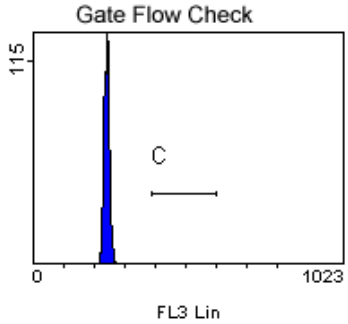
Re...	Num...	X-M...	HPXCV
ALL	5788	332.9	3.3
FC675	1296	149.4	3.3
FC770	1590	287.1	2.8
Flow...	2542	443.2	1.6



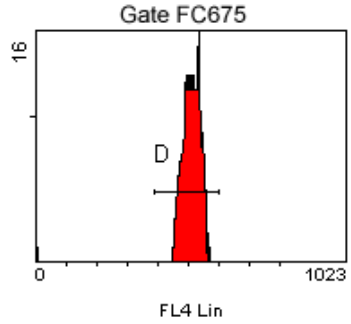
Re...	Num...	X-M...	HPXCV
ALL	2542	358.2	3.5
A	0	0.0	0.0



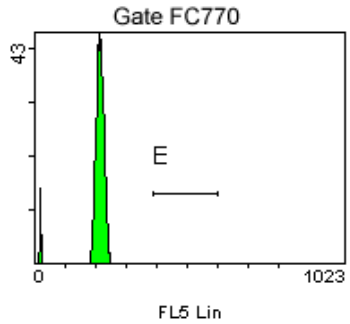
Re...	Num...	X-M...	HPXCV
ALL	2542	506.1	3.0
B	2521	509.0	3.0



Re...	Num...	X-M...	HPXCV
ALL	2542	236.6	3.4
C	2	477.0	0.2



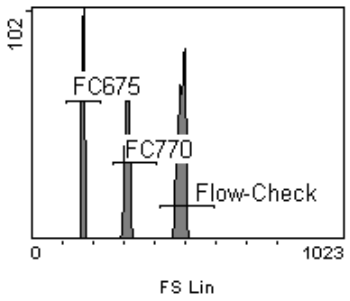
Re...	Num...	X-M...	HPXCV
ALL	1296	503.1	0.5
D	1265	510.0	0.5



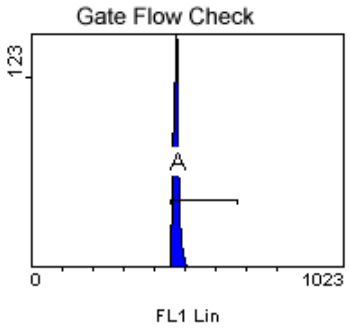
Re...	Num...	X-M...	HPXCV
ALL	1590	201.1	4.1
E	0	0.0	0.0

1. Acceptable or Unacceptable data? _____
2. Why? _____
   _____
3. If unacceptable, what may be the cause?
   _____
   _____
4. What should you do to correct the situation?
   _____
   _____

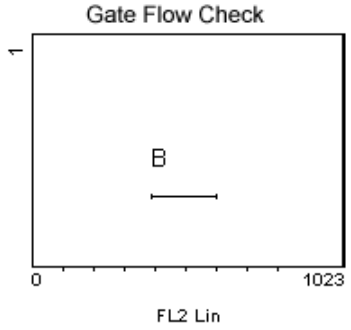
Exercise 2



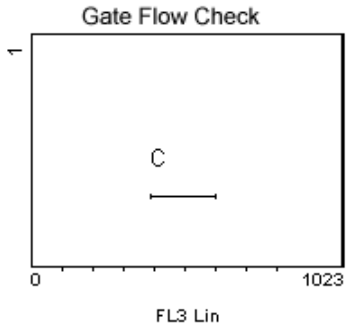
Re...	Num...	X-M...	HPXCV
ALL	5367	361.6	3.1
FC675	1284	163.3	3.1
FC770	1517	309.8	2.6
Flow...	2509	488.2	2.1



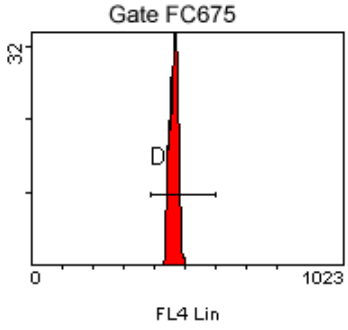
Re...	Num...	X-M...	HPXCV
ALL	2509	469.1	1.2
A	2467	471.7	1.2



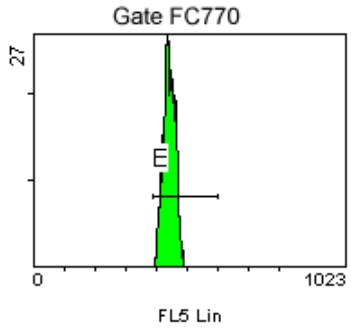
Re...	Num...	X-M...	HPXCV
ALL	2509	1018.8	0.0
B	3	527.0	0.2



Re...	Num...	X-M...	HPXCV
ALL	2509	1018.6	0.0
C	3	445.3	0.2



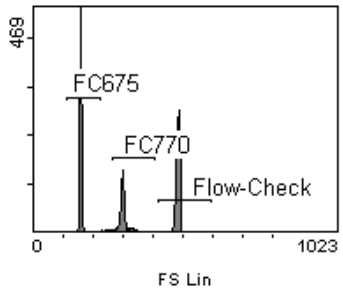
Re...	Num...	X-M...	HPXCV
ALL	1284	459.7	1.3
D	1272	463.9	1.3



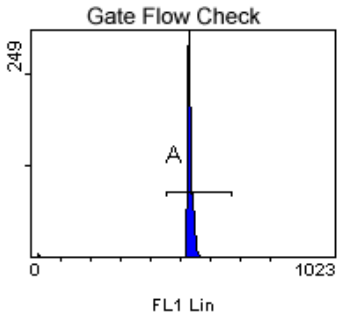
Re...	Num...	X-M...	HPXCV
ALL	1517	437.0	1.8
E	1493	441.7	1.8

1. Acceptable or Unacceptable data? _____
2. Why?
   _____
   _____
3. If unacceptable, what may be the cause?
   _____
   _____
4. Are there any additional observations you could make to confirm or eliminate a cause in the list. _____
   _____
5. What should you do to correct the situation?
   _____
   _____
   _____

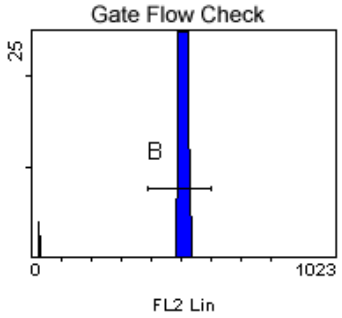
Exercise 3



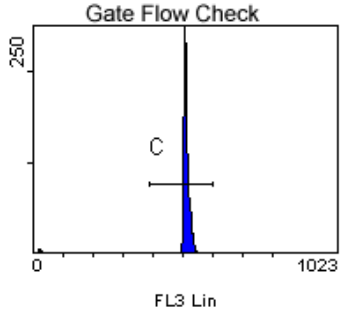
Re...	Num...	X-M...	HPXCV
ALL	10000	321.7	1.7
FC675	3765	155.2	1.7
FC770	2123	301.6	1.4
Flow...	3838	484.6	1.2



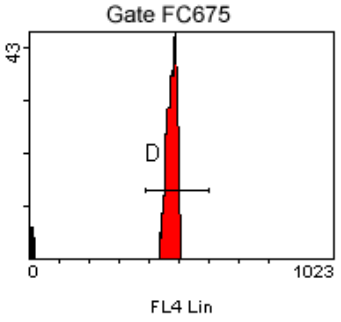
Re...	Num...	X-M...	HPXCV
ALL	3838	525.1	0.8
A	3779	532.2	0.8



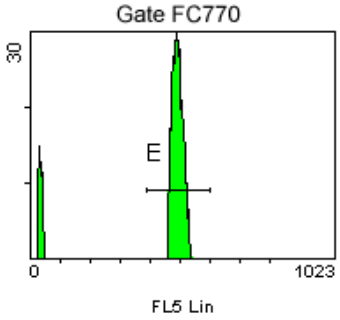
Re...	Num...	X-M...	HPXCV
ALL	3838	496.1	1.1
B	3780	502.7	1.1



Re...	Num...	X-M...	HPXCV
ALL	3838	505.8	0.8
C	3780	512.5	0.8



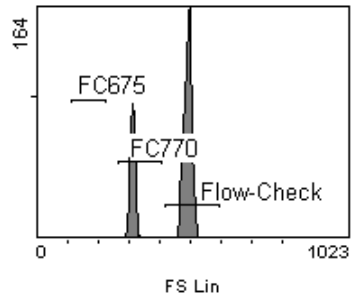
Re...	Num...	X-M...	HPXCV
ALL	3765	481.3	1.1
D	3632	497.2	1.1



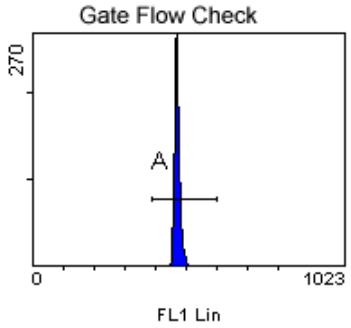
Re...	Num...	X-M...	HPXCV
ALL	2123	427.9	2.0
E	1830	490.5	2.0

1. Acceptable or Unacceptable data? _____
2. Why?
   _____
   _____
3. If unacceptable, what may be the cause?
   _____
   _____
4. What should you do to correct the situation?
   _____
   _____
   _____

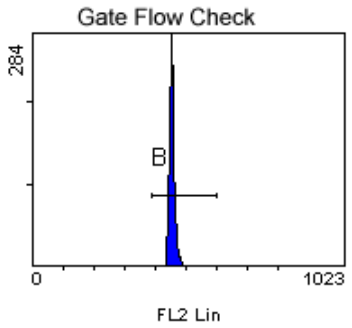
Exercise 4



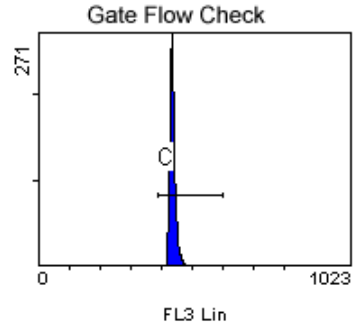
Re...	Num...	X-M...	HPXCV
ALL	7249	438.5	1.9
FC675	26	161.0	0.5
FC770	2130	309.9	2.8
Flow...	5000	491.4	1.9



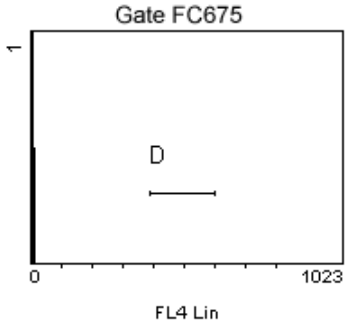
Re...	Num...	X-M...	HPXCV
ALL	5000	469.7	1.2
A	4984	471.0	1.2



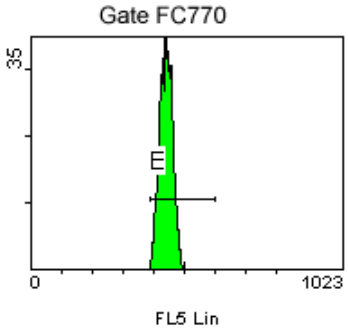
Re...	Num...	X-M...	HPXCV
ALL	5000	453.1	1.1
B	4983	454.2	1.1



Re...	Num...	X-M...	HPXCV
ALL	5000	434.8	1.2
C	4982	435.9	1.2



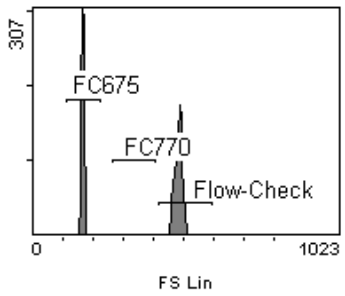
Re...	Num...	X-M...	HPXCV
ALL	26	2.7	###
D	0	0.0	0.0



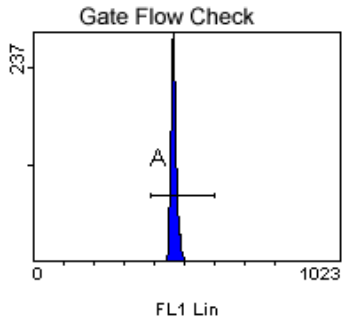
Re...	Num...	X-M...	HPXCV
ALL	2130	435.0	3.0
E	2088	440.9	3.0

1. Acceptable or Unacceptable data? _____
2. Why?
   _____
   _____
3. If unacceptable, what should you do to correct the situation?
   _____
   _____
   _____

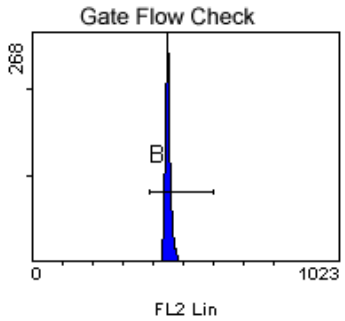
Exercise 5



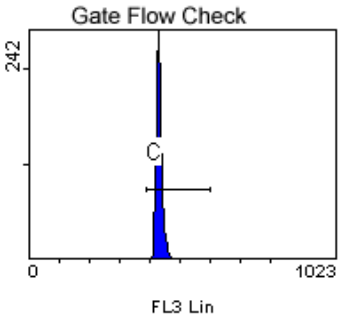
Re...	Num...	X-M...	HPXCV
ALL	9183	343.2	3.1
FC675	4060	161.8	3.1
FC770	25	326.0	0.2
Flow...	5000	483.4	1.6



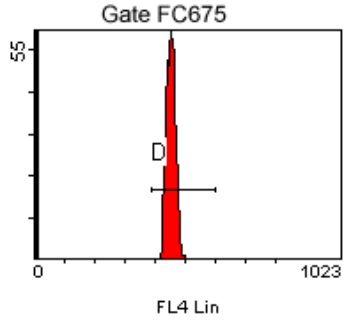
Re...	Num...	X-M...	HPXCV
ALL	5000	464.7	1.4
A	4994	464.9	1.4



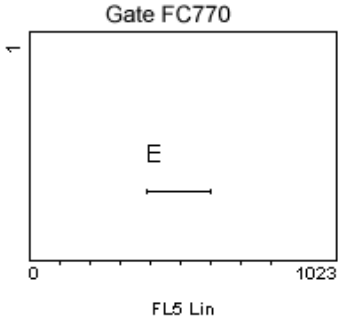
Re...	Num...	X-M...	HPXCV
ALL	5000	449.0	1.2
B	4993	449.2	1.2



Re...	Num...	X-M...	HPXCV
ALL	5000	430.1	1.5
C	4993	430.3	1.5



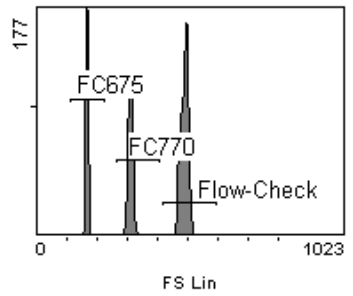
Re...	Num...	X-M...	HPXCV
ALL	4060	263.5	###
D	2361	452.4	3.0



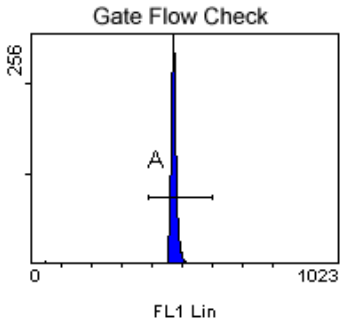
Re...	Num...	X-M...	HPXCV
ALL	25	82.4	6.5
E	0	0.0	0.0

1. Acceptable or Unacceptable data? _____
2. Why? _____
   _____
   _____
3. If unacceptable, what may be the cause?
   _____
   _____
   _____
4. What should you do to correct the situation?
   _____
   _____
   _____

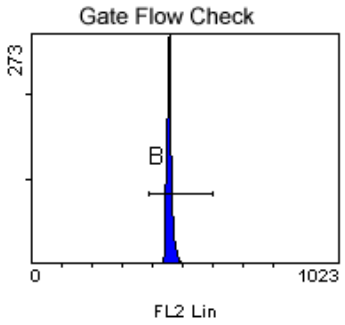
Exercise 6



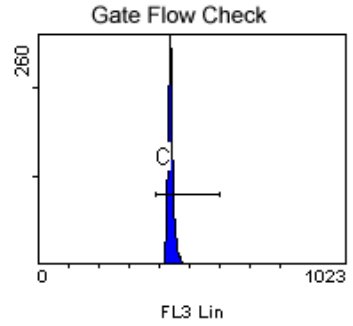
Re...	Num...	X-M...	HPXCV
ALL	10000	368.2	3.0
FC675	2217	162.5	3.0
FC770	2808	309.2	2.6
Flow...	4858	489.5	2.2



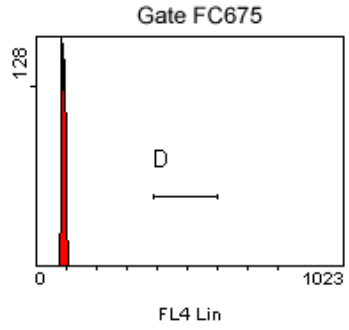
Re...	Num...	X-M...	HPXCV
ALL	4858	470.5	1.1
A	4831	472.8	1.1



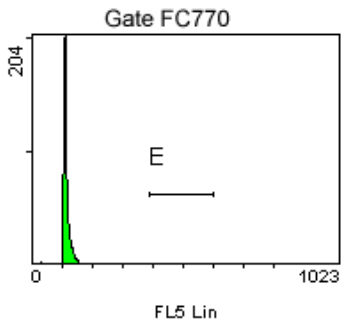
Re...	Num...	X-M...	HPXCV
ALL	4858	454.0	1.2
B	4831	456.1	1.2



Re...	Num...	X-M...	HPXCV
ALL	4858	435.2	1.5
C	4831	437.4	1.5



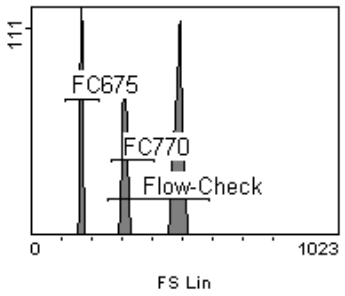
Re...	Num...	X-M...	HPXCV
ALL	2217	85.0	3.3
D	0	0.0	0.0



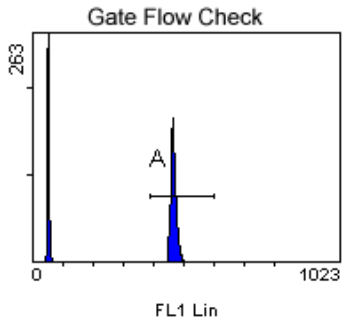
Re...	Num...	X-M...	HPXCV
ALL	2808	107.8	3.5
E	0	0.0	0.0

1. Acceptable or Unacceptable data? _____
2. Why? _____
   _____
   _____
3. If unacceptable, what should you do to correct the situation?
   _____
   _____

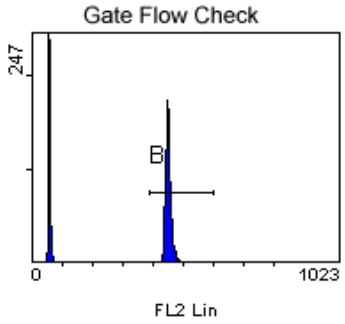
Exercise 7



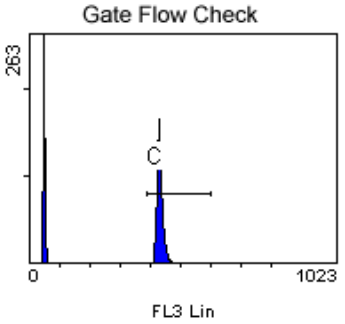
Re...	Num...	X-M...	HPXCV
ALL	6432	370.7	1.4
FC675	1369	161.7	2.9
FC770	1719	307.0	2.4
Flow...	5000	424.4	1.4



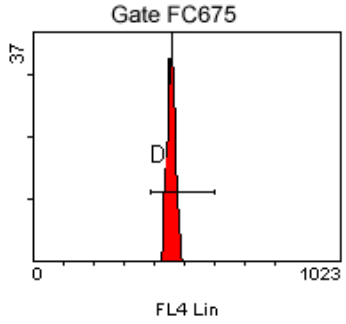
Re...	Num...	X-M...	HPXCV
ALL	5000	321.1	3.2
A	3268	466.5	1.3



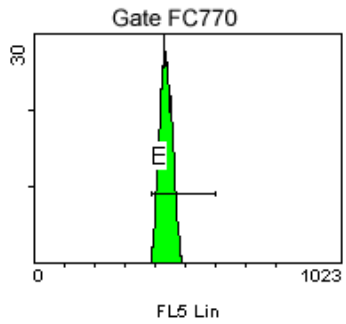
Re...	Num...	X-M...	HPXCV
ALL	5000	312.4	2.9
B	3267	450.4	1.2



Re...	Num...	X-M...	HPXCV
ALL	5000	297.2	3.4
C	3267	431.7	1.2



Re...	Num...	X-M...	HPXCV
ALL	1369	453.1	1.3
D	1352	456.6	1.3

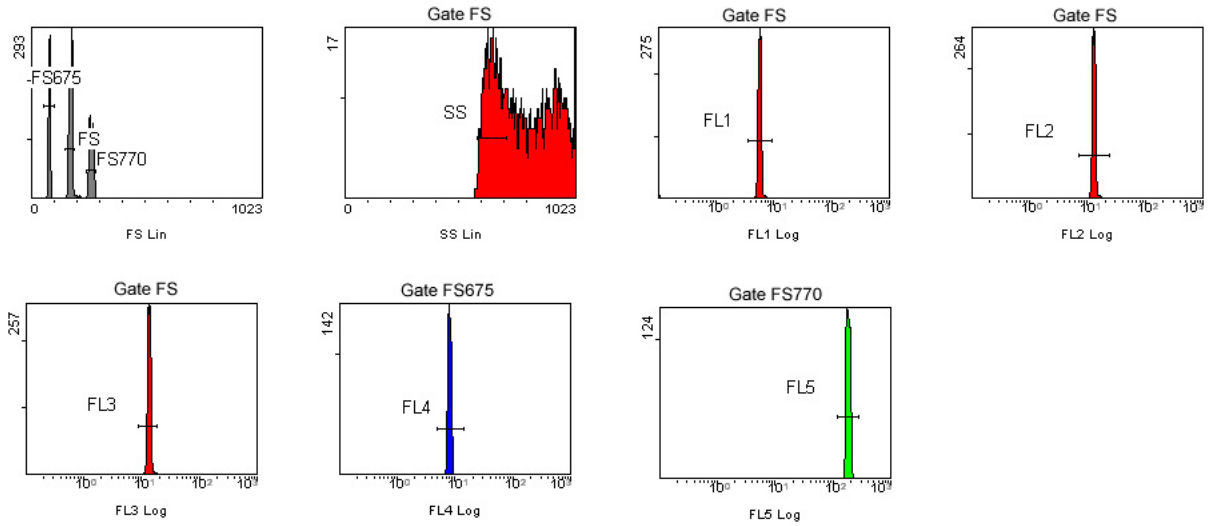


Re...	Num...	X-M...	HPXCV
ALL	1719	432.2	2.7
E	1684	436.4	2.7

1. Acceptable or Unacceptable data? _____
2. Why? _____
   _____
   _____
3. If unacceptable, what should you do to correct the situation?
   _____
   _____
   _____

STANDARDIZATION

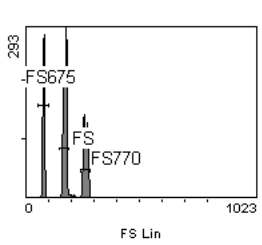
Exercise 8

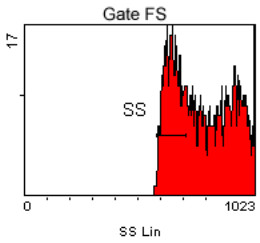


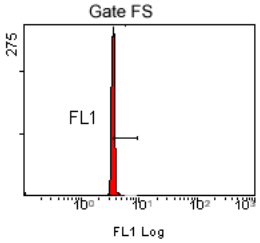
Note: The system adjusts the voltages to place the populations within regions FS, SS, and FL1 through FL5.

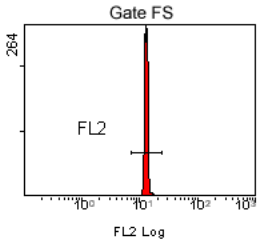
1. Acceptable or Unacceptable data? _____
2. Why? _____
   _____
   _____
3. If unacceptable, what should you do to correct the situation?
   _____
   _____
   _____

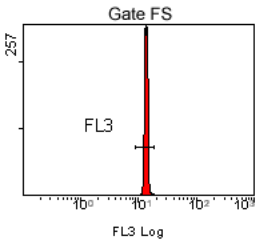
Exercise 9

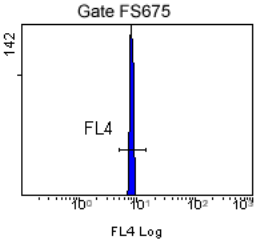


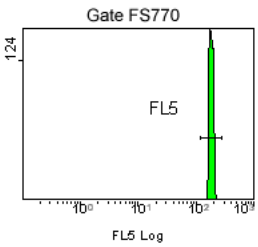






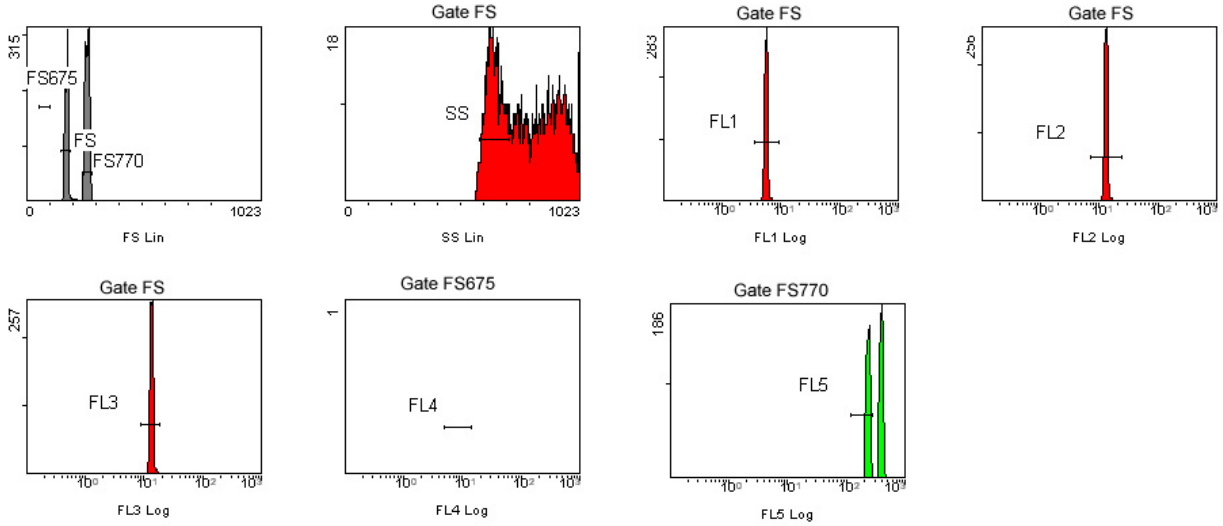






1. Acceptable or Unacceptable data? _____
2. Why? _____
   _____
   _____
3. If unacceptable, what may be the cause? _____
   _____
   _____
4. What will the system do to correct the situation?
   _____
   _____
   _____

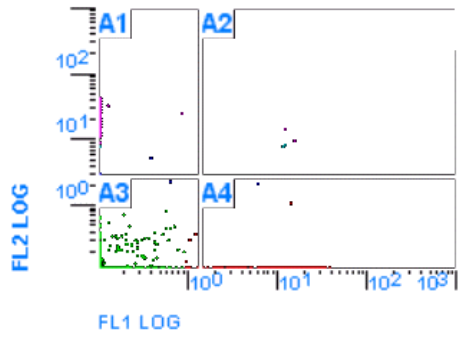
Exercise 10



1. The system fails to standardize the system. Why?
   _____
   _____
   _____
2. What may be the cause?
   _____
   _____
   _____
3. What should you do to correct the situation?
   _____
   _____

COMPENSATION

Exercise 11



Region	Number	%Gated	X-Mean	Y-Mean
A1	815	53.55	0.1	26.5
A2	4	0.26	13.1	9.6
A3	307	20.17	0.2	0.2
A4	396	26.02	18.2	0.1

Compensation Matrix

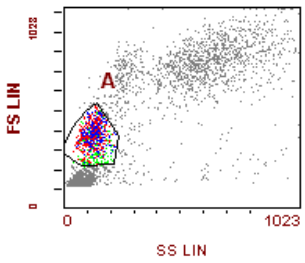
	FITC	PE	ECD	PC5
FITC		6.8	0.0	0.0
PE	29.6		0.0	0.0
ECD	0.0	0.0		0.0
PC5	0.0	0.0	0.0	

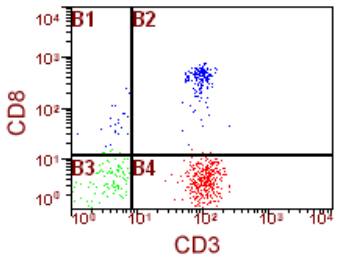
NOTE: Assume we are manually compensating in this example.

1. Acceptable or Unacceptable data? _____
2. Why?
   _____
   _____
   _____
3. If unacceptable, what should you do to correct the situation?
   _____
   _____

CONTROL

Exercise 12





Region	%Gated	X-Mean	Y-Mean
B1	4.39	3.6	65.5
B2	19.74	97.4	428.8
B3	26.42	2.6	3.2
B4	49.46	116.0	3.7

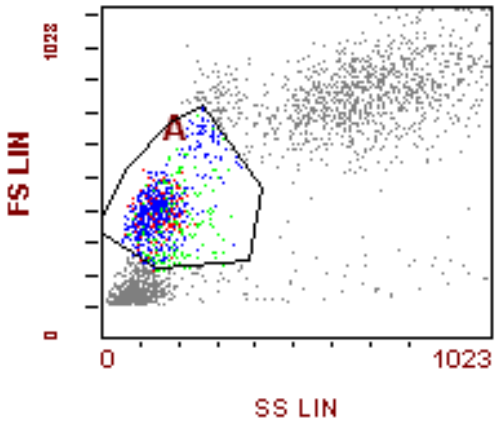
Coulter® Immuno-Trol

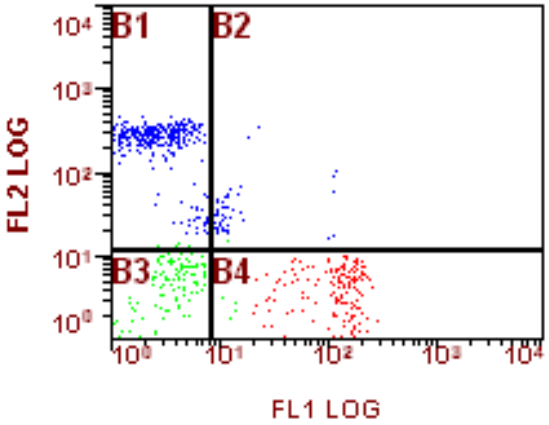
Cell Lot No. 123456

Antibody	% Pos	Range +
T11(CD2)	83	2
CD3(IgG1)	72	5
T4 (CD4)	49	4
T1 (CD5)	74	5
T8 (CD8)	25	7
B4 (CD19)	13	4

1. Acceptable or Unacceptable data? _____
2. Why? _____
   _____
3. If unacceptable, what should you do to correct the situation?
   _____
   _____

Exercise 13

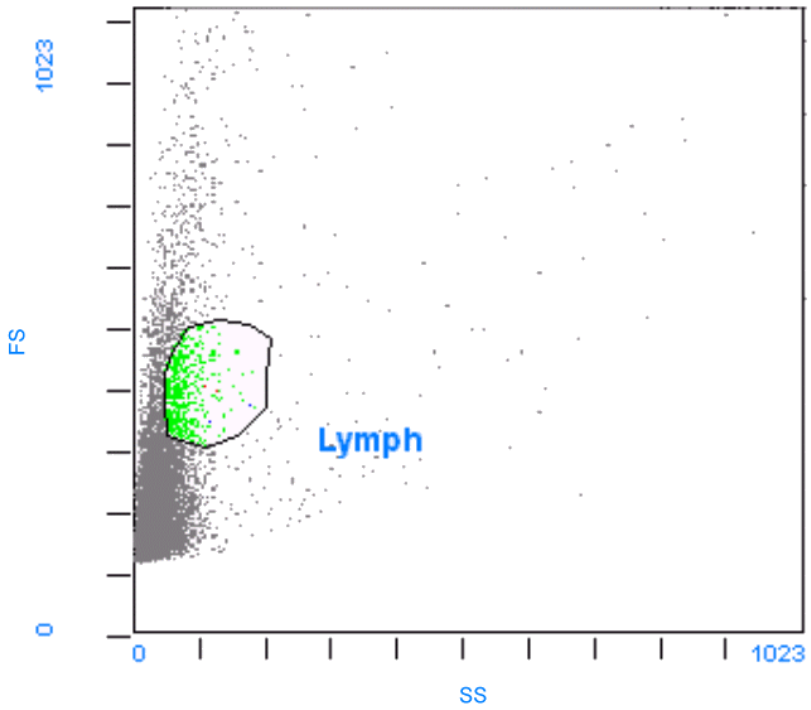




Region	%Gated	X-Mean	Y-Mean
B1	42.91	2.8	266.6
B2	6.63	23.6	50.9
B3	28.90	2.4	4.4
B4	21.56	123.9	4.1

1. Acceptable or Unacceptable data? _____
2. Why? _____
   _____
   _____
3. If unacceptable, what should you do to correct the situation?
   _____
   _____
   _____

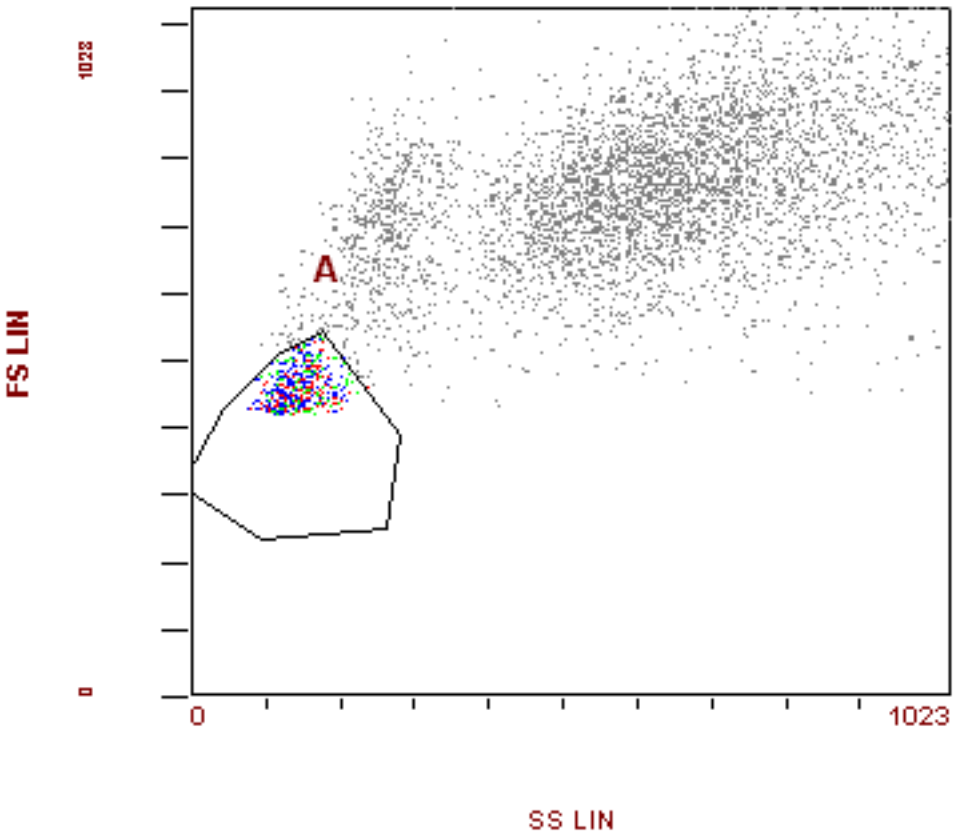
Exercise 14 Blood Sample



1. Acceptable or Unacceptable data? _____
2. Why?
   _____
   _____
3. If unacceptable, what may be the cause? _____
   _____
   _____
4. Are there any additional observations you could make to confirm or eliminate a cause in the list.
   _____
   _____
5. What should you do to correct the situation?
   _____
   _____

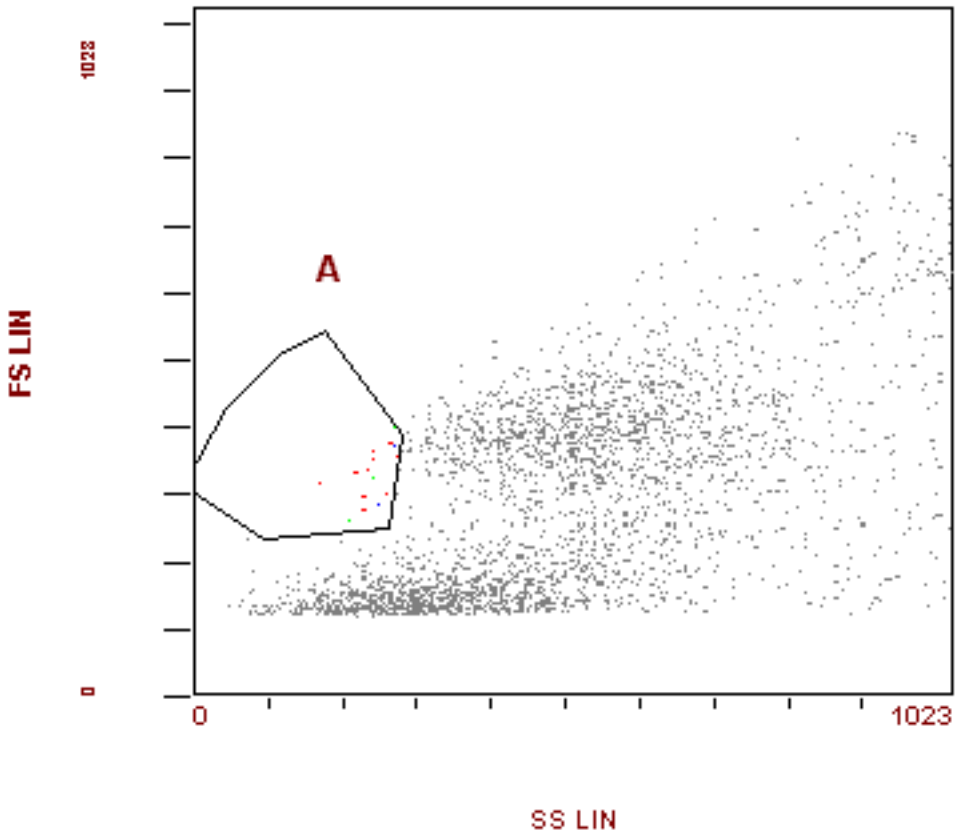
UNKNOWN SAMPLES

Exercise 15



1. Acceptable or Unacceptable data? _____
2. Why? _____
   _____
   _____
   _____
3. If unacceptable, what should you do to correct the situation?
   _____
   _____
   _____

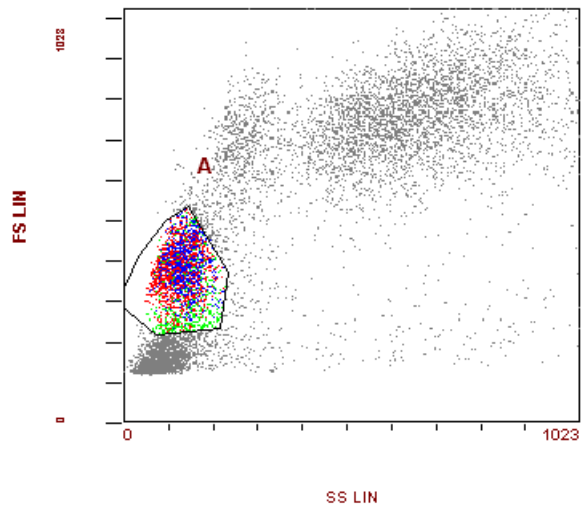
Exercise 16



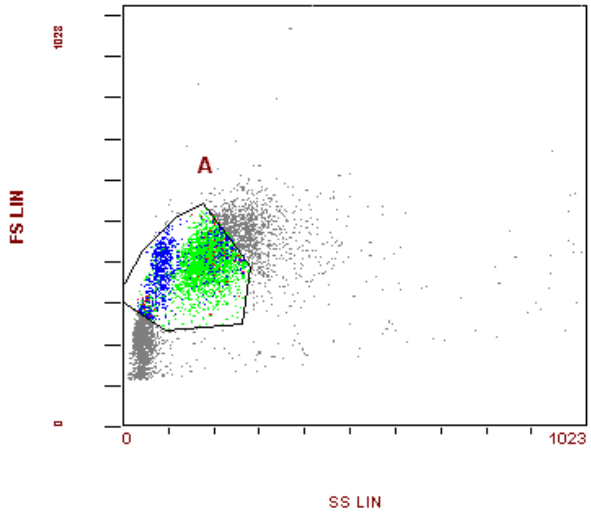
1. Acceptable or Unacceptable data? _____
2. Why?
   _____
   _____
3. If unacceptable, what should you do to correct the situation?
   _____
   _____
   _____

Exercise 17

Morning

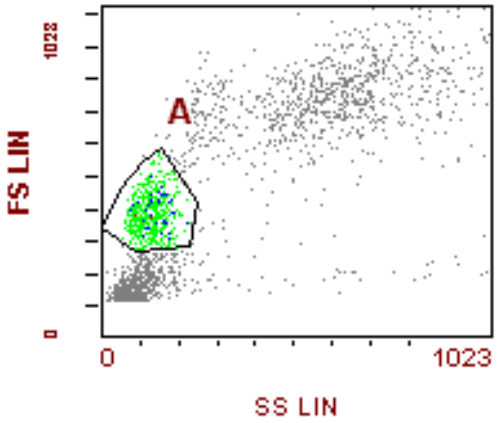


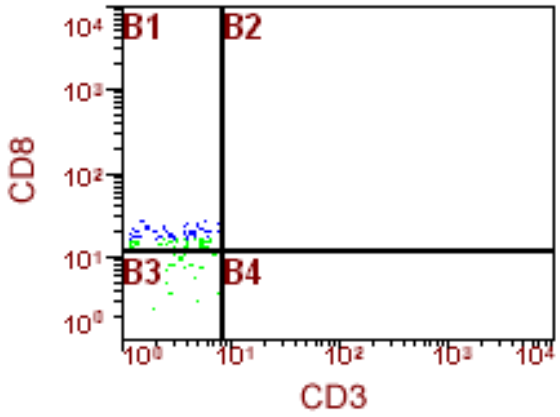
Afternoon



1. Acceptable or Unacceptable data? _____
2. Why? _____shifted data _____
   _____
   _____
3. If unacceptable, what may be the cause?
   _____
   _____
   _____
4. What should you do to correct the situation?
   _____
   _____
   _____

Exercise 18





Region	%Gated	X-Mean	Y-Mean
B1	13.67	2.0	17.7
B2	0.06	8.6	25.7
B3	82.59	1.8	1.5
B4	3.68	11.2	1.3

1. Acceptable or Unacceptable data? _____
2. Why? _____
   _____
   _____
3. If unacceptable, what may be the cause?
   _____
   _____
   _____
4. What should you do to correct the situation?
   _____
   _____
   _____

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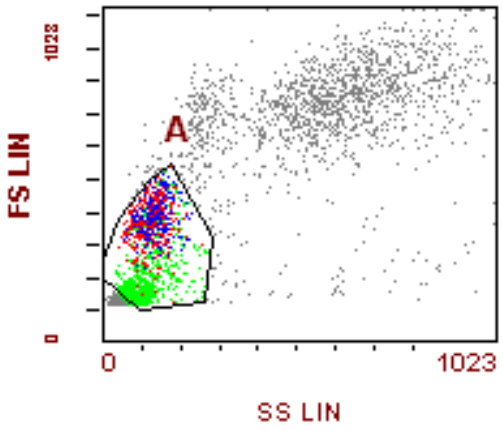
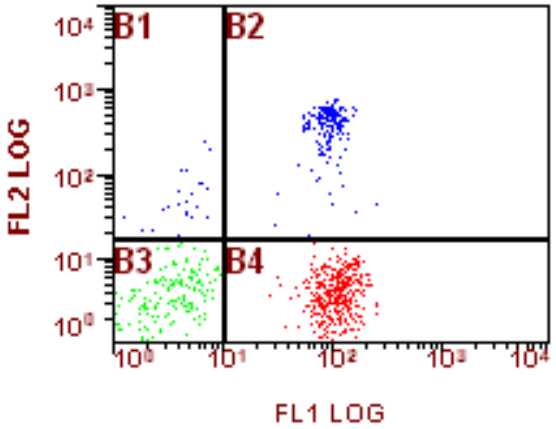
Exercise 19

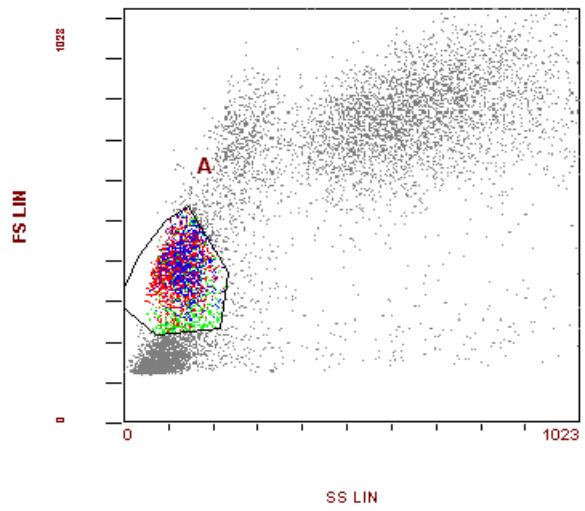
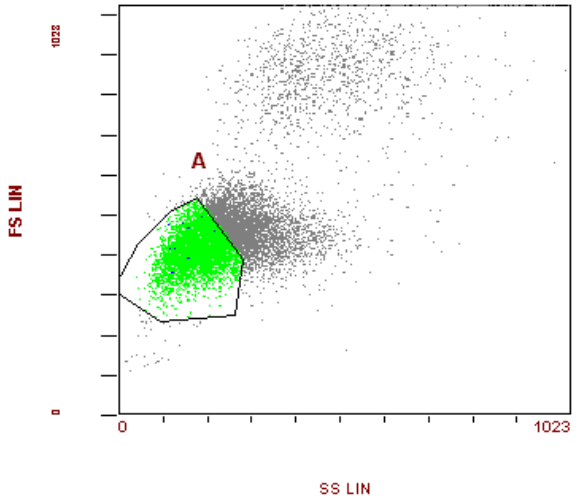




Region	%Gated	X-Mean	Y-Mean
B1	2.45	4.0	74.1
B2	11.17	100.0	439.4
B3	57.56	1.7	2.1
B4	28.81	117.0	3.8

1. Acceptable or Unacceptable data? _____
2. Why? _____
   _____
   _____
3. If unacceptable, what should you do to correct the situation?
   _____
   _____
   _____

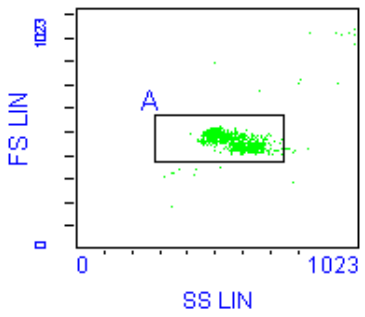
Exercise 20

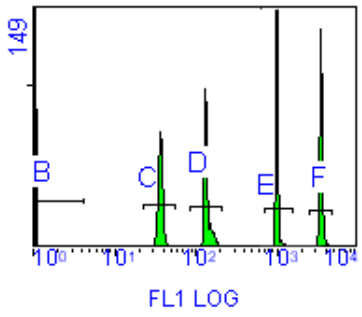
Previous sample – same patient	New sample – same patient
	

1. Acceptable or Unacceptable data? _____
2. Why?
   _____
   _____
3. If unacceptable, what may be the cause?
   _____
   _____
   _____
4. Are there any additional observations you could make to confirm or eliminate a cause in the list.
   _____
   _____
5. What should you do to correct the situation?
   _____
   _____
   _____

LINEARITY CHECK IMMUNO-BRITE™ FLUOROSPHERES

Exercise 21





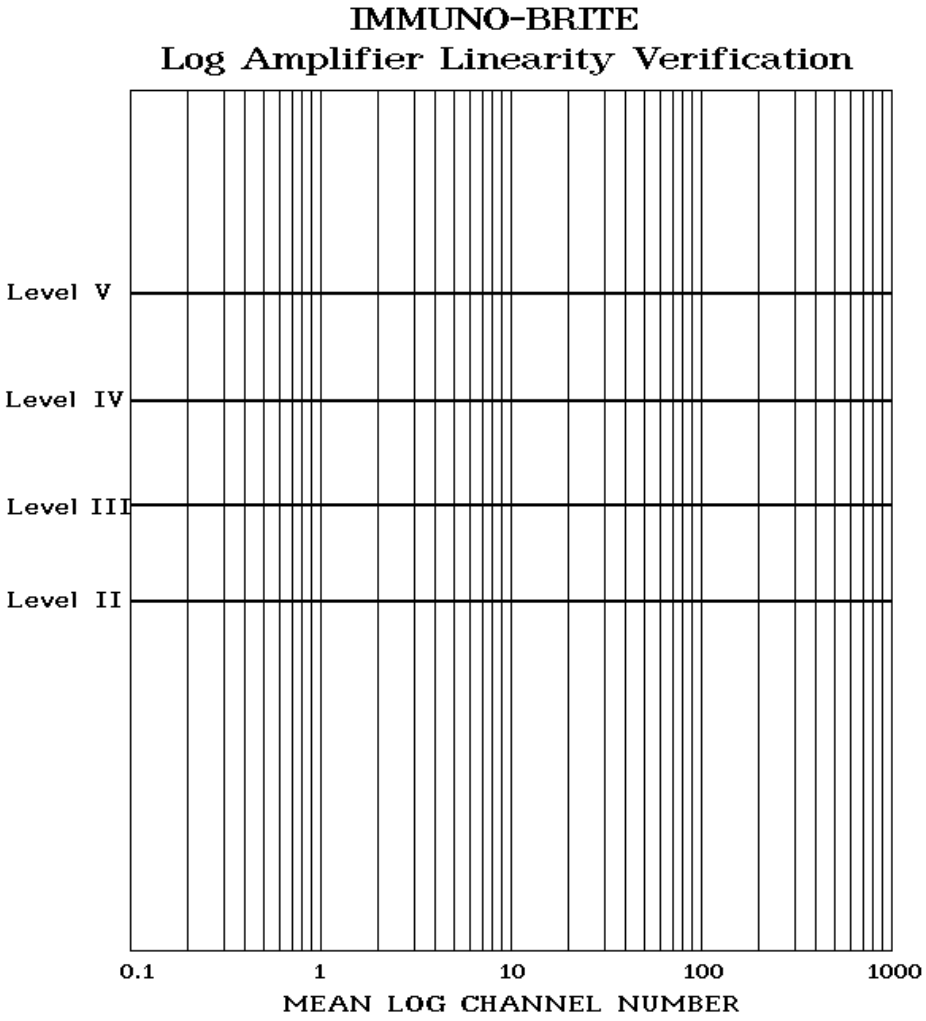
Region	X-Mean
B	0.0
C	3.8
D	14.3
E	110.1
F	399.4

1. Acceptable or Unacceptable data? _____
2. Why?
   _____
   _____
   _____
3. If unacceptable, what should you do to correct the situation?
   _____
   _____
   _____

Log Amplifier Linearity Verification

Instrument: _____

Serial Number _____

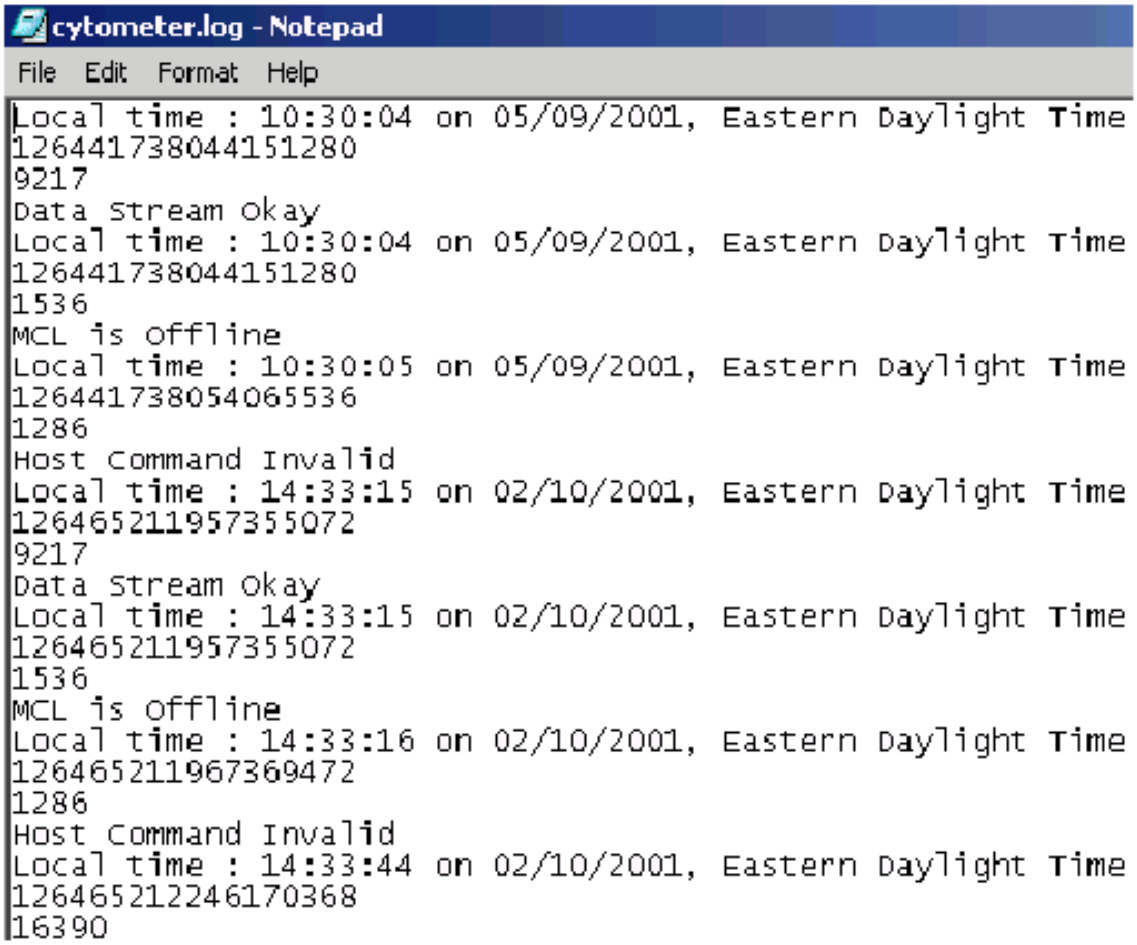


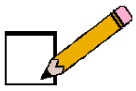
CYTOMETER.LOG FILE

The system keeps a record of all events occuring on the cytometer. This file, called the cytometer.log file, can be accessed and printed. It provides valuable information when troubleshooting and for this reason, you may be asked to print a copy for a service troubleshooter.

To View/Print the File

1. Select **Cytometer >> Cytometer Log >> View Log**. Messages remain for 30 days. After 30 days, the entries are moved to the cytometerarchive.txt file located in C:\Cytomics CXP subdirectory.





ERROR CODES

Use the OnLine Help to locate the 13.5 Error Messages table (under 13 Troubleshooting) and fill in the requested information for each error code listed. If you wish, you can print the file and highlight the error codes below in the file instead of rewriting the cause and action.

MCL Carousel In/Out Error

Cause _____

Action _____

_____

MCL Probe Up/Down Error

Cause _____

Action _____

_____

MCL Tube Load Error

Cause _____

Action _____

_____

MCL Tube Up/Down Error

Cause _____

Action _____

_____

Sample Pressure Error

Cause _____

Action _____

_____

System Vacuum Error

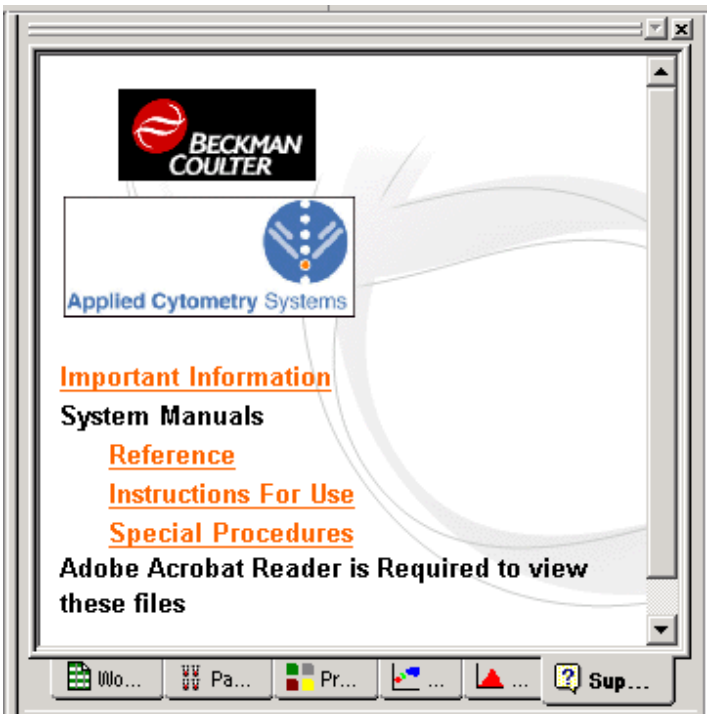
Cause _____

Action _____

_____

PRINT IMPORTANT INFORMATION FILE

1. Select the Sup... icon on the Resource Explorer. The screen reponds as below.



2. Select the Important Information file to view and print.

You have now completed this module.

TABLE OF CONTENTS

OBJECTIVES

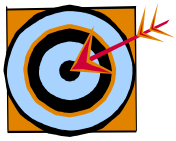
Given an operational system, training materials, access to an Operator's Guide, and access to online Help:

- Create a new application to include all pertinent information such as dyes, filters, and Flow-Set™ Fluorospheres target channels.
- Schedule several multiple color applications at one time.
- Prepare samples, load the carousel, and run a set of scheduled application autostandardization samples.

WHY IS IT IMPORTANT?

Before you can ensure that patient or experiment results are accurate, several items must be set up and checked. The system, for example, needs to be standardized for each application. When multiple dyes are used, compensation must be adjusted. These adjustments also depend on the standardization and therefore can be different with each application. We also should verify the accuracy of instrument results with known samples. All of this work can be very involved if you are running a number of different applications (e.g. 2 Color, 3 Color 1laser, 5 Color 1 laser, 5 Color 2 Laser, etc.). The process is made far easier by defining each application to the system using the Autosetup Apps option and letting the system schedule the tubes in the carousel using the Autosetup Scheduler option.

Skill Check Preview



You will have mastered the application when you can show printed results meeting specifications for each of the following:

- A new application summary created in the system using the Autosetup Apps option.
- A carousel load list illustrating multiple applications scheduled at the same time using the Autosetup Scheduler option.
- Results from tubes run using a multiple application worklist.

WHAT TO LEARN

In this module, you will learn:

- The basic concepts that allow the system to set up the proper high voltage, gain, and compensation settings for an application.
- How to define an application to the system using the Autosetup Apps option.
- Schedule multiple application setups in the most efficient manner that minimizes the number of tubes to be prepared and the time it takes to run them.

Tour Guides

We suggest you use the Tour Guides along with this module to provide you a virtual illustration of the concepts and the autosetup options. To access the Tour Guides:

1. If there is a program running, minimize the screen by selecting  in the upper right corner of the application.

2. Place the Tour Guide disk into the CD Rom drive or if your facilitator has placed the Tour Guides on your hard drive, double click on the icon labeled Tour Guides. The system will start the program. A voice will come on and explain how to take the tours.

3. Take the AutoSetup tours.

INFORMATION / PRACTICE SECTION

Advanced Digital Compensation (ADC)

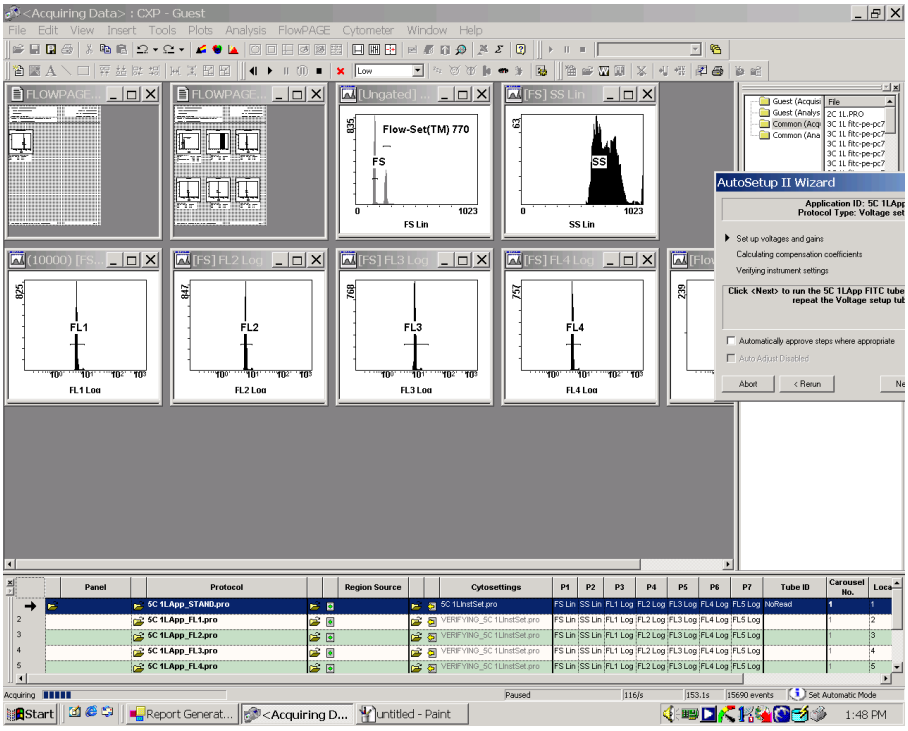
If the system is standardized by adjusting voltages and gains to place standards (e.g. Flow-Set™ fluorospheres) within target channels, then the compensation settings must also be checked. The ADC method determines the spectral overlap from each dye on an individual basis. The mean channel for each dye is measured along with the mean channels of the other fluorescence data. The results are then supplied to an algorithm to calculate the necessary compensations and these in turn are automatically entered into the system. Compensation is easy. Just run the samples with the appropriate CXP Software protocols.

A typical set of tubes might look like the following worklist (application 5 Color 1 Laser):

- Tube 1 – Standard (Flow-Set and Flow-Set 770)
- Tube 2 – CD45 FITC using Cyto-Comp cells
- Tube 3 – CD45 PE using Cyto-Comp cells
- Tube 4 – CD45 ECD using Cyto-Comp cells
- Tube 5 – CD45 PC5 using Cyto-Comp cells
- Tube 6 – CD45 PC7 using Cyto-Comp cells
- Tube 7 – Verify tube with various antibodies (ex. CD45 FITC, CD4 PE, CD8 ECD, CD3 PC5, CD19 PC7) using Cyto-Trol™ cells

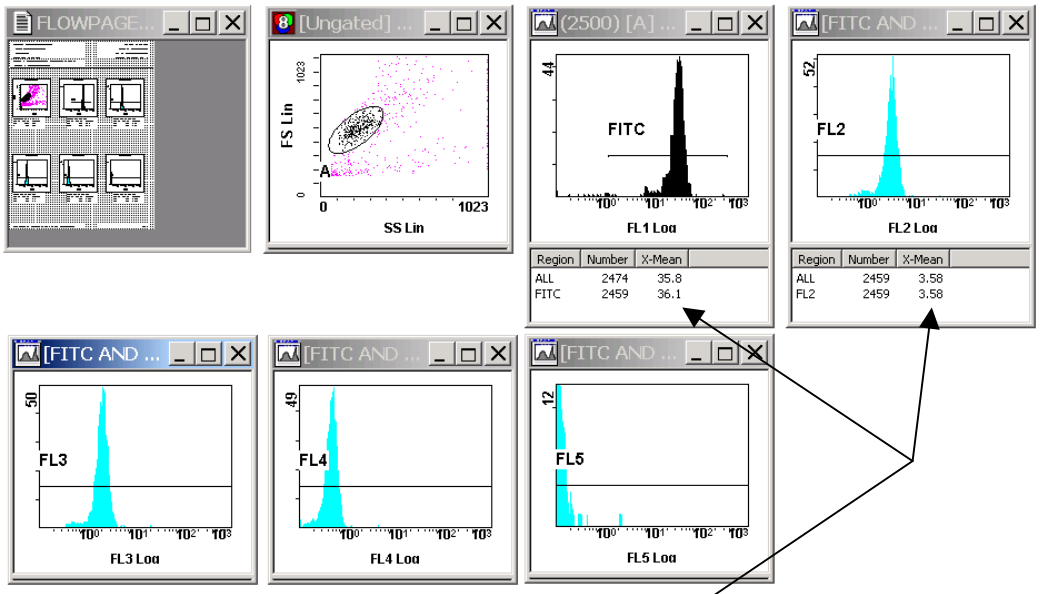
When tube 1 is run, the system attempts to adjust the high voltage and gains to place the standard at the target channels. These channels are determined for an application in a manner similar to the method you used in Basic Operation module.

Our 5 color 1 Laser data might look like:



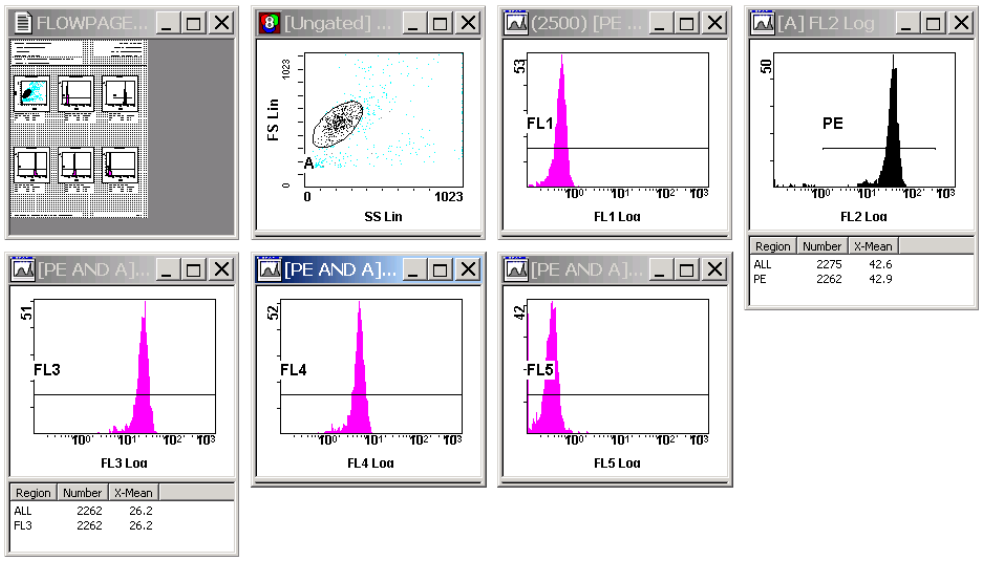
Now each of the 5 compensation tubes (CD45 FITC – PC7) are run in turn at the new high voltage and gain values. The mean channel of each dye is measured along with the mean channels of the other parameters. If there is spectral overlap into another parameter, the mean channel of the other parameter will increase. The plot data will move to the right. If there is no overlap, the data should be similar to the background fluorescence of an isotypic control. The compensation setting moves the data and therefore the mean channel back to the background position.

In the example below, CD45 FITC has been run and there is a fluorescence overlap into the PE parameter. The amount of compensation needed can be roughly estimated by dividing the mean channel of the PE data by the mean channel of the FITC data and multiplying by 100 to change the fraction to a percent (see below).

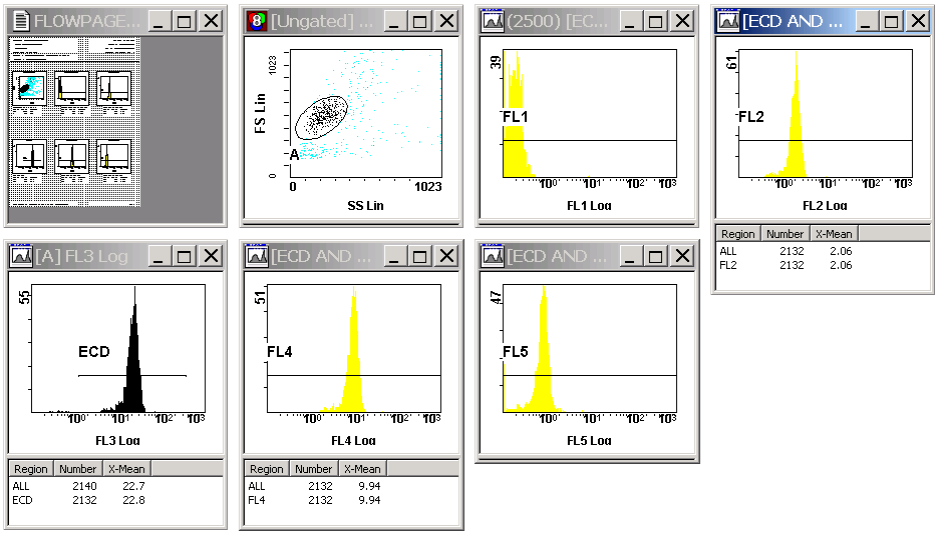


Example estimate: (3.58/36.1) X 100 = 9.92. The other tubes provide the same measurements in turn.

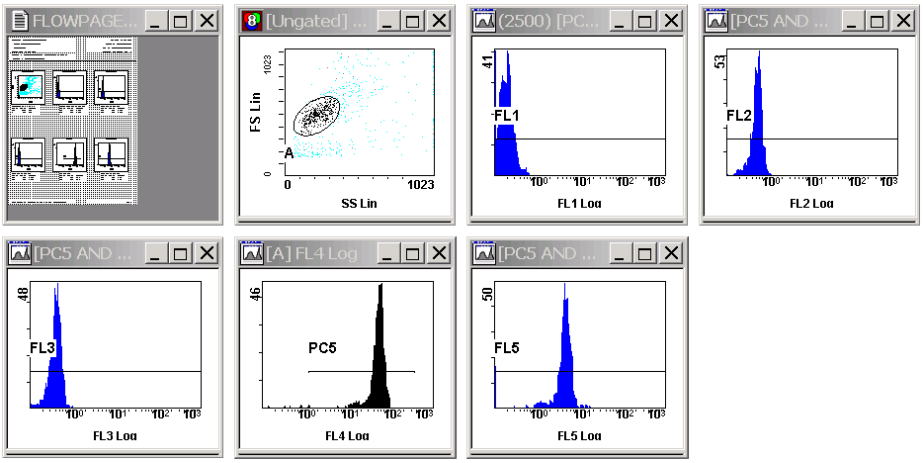
Tube 3 PE



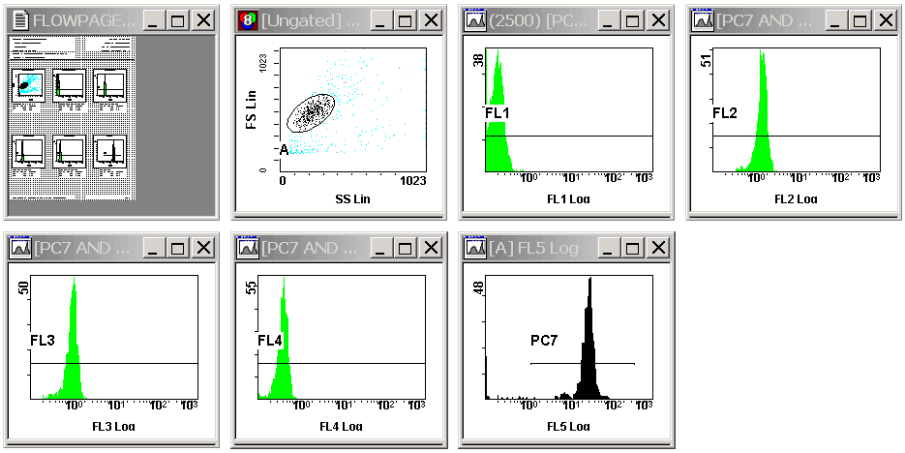
Tube 4 ECD



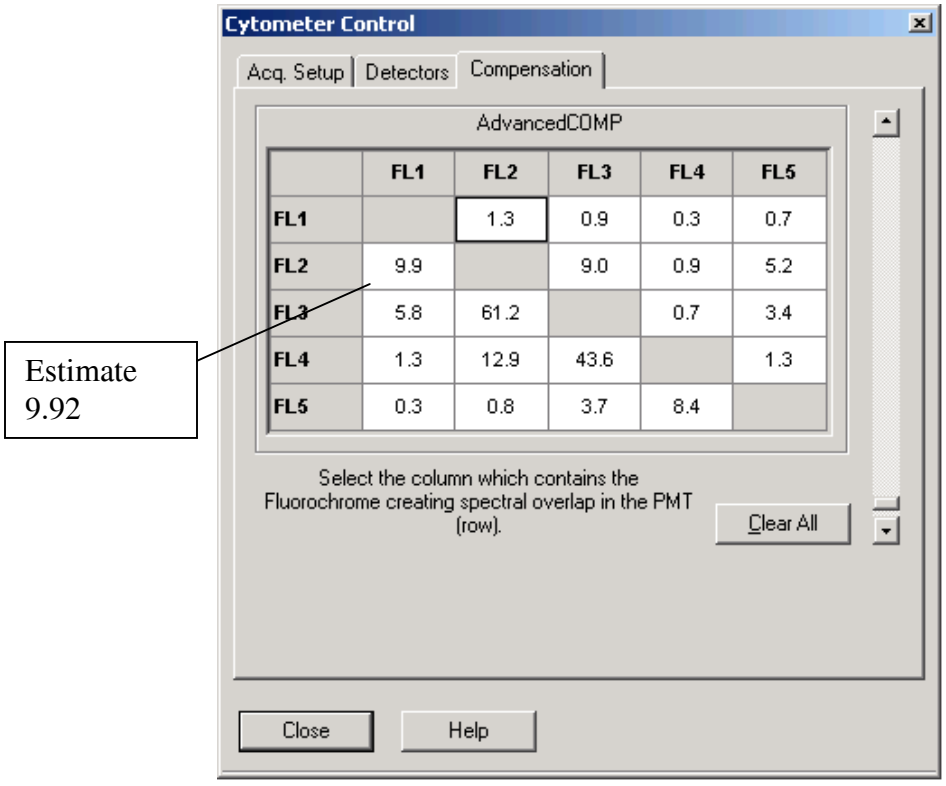
Tube 5 PC5



Tube 6 PC7



When a verification tube is run, the system sets the compensation settings as illustrated below.

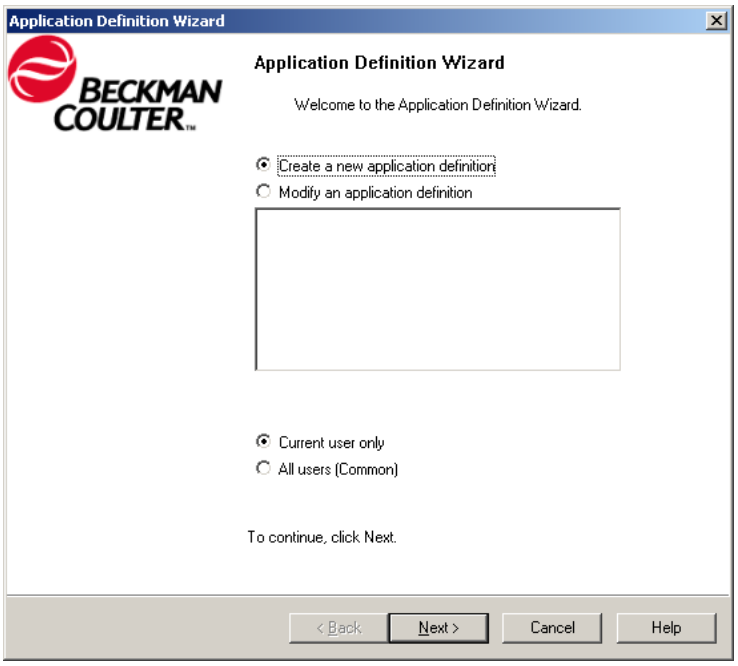


Since the compensation setttings depend on the initial high voltages to the system, the values can vary for different applications. A two color surface marker setup, for example, may be different than a 3, 4 or 5 color setup. The autostandardization would have to be run for each application. The AutoSetup Application Definition and the AutoSetup Scheduler options help minimize the time and the number of tubes needed to accomplish the task.

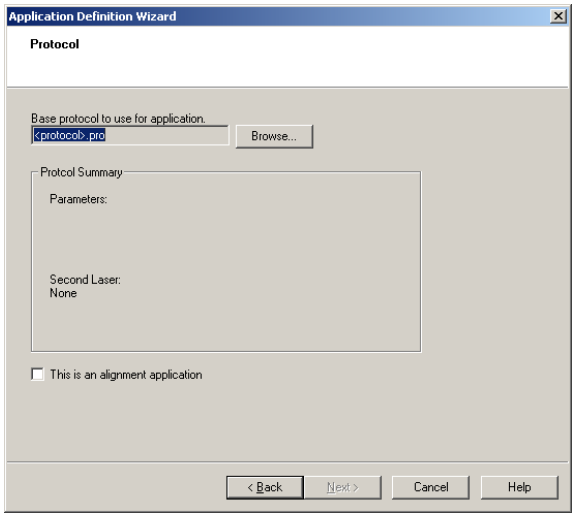
AutoSetup Application Definition

The operator tells the system in the Automatic Setup Application Definition option all pertinent information about an application (e.g. the parameters needed, dyes, lasers , filters, target channels, etc.). The Applications Definition Wizard allows the user to define the requirements for the application through a series of steps. In this exercise, you will enter the information for a two color application based on the work you did in the Basic Operation module.

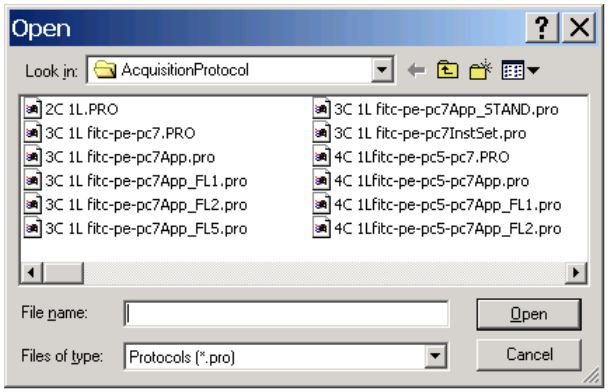
1. Select **Tools >> AutoSetup Appication Definition**. The screen responds as below.



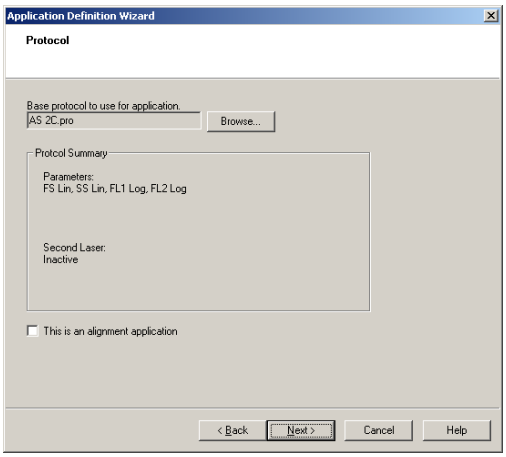
2. Make sure “Create a new application definition” is selected.
3. Select All users (Common) (if you want everyone to have access) as desired and then select **Next**.



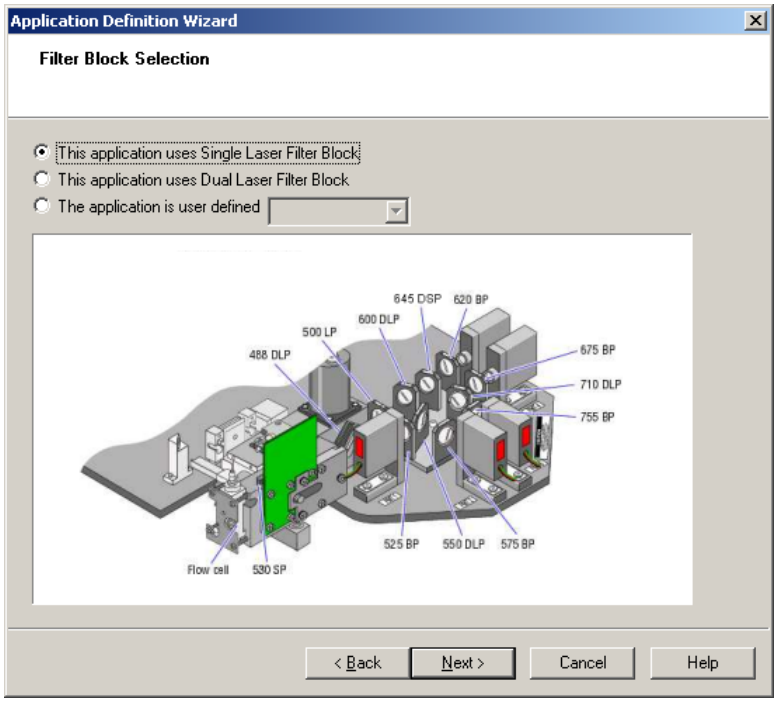
5. Select Browse button to select a protocol that will be used as the basis for the application.



6. Select AS 2C.PRO and then **Open**.

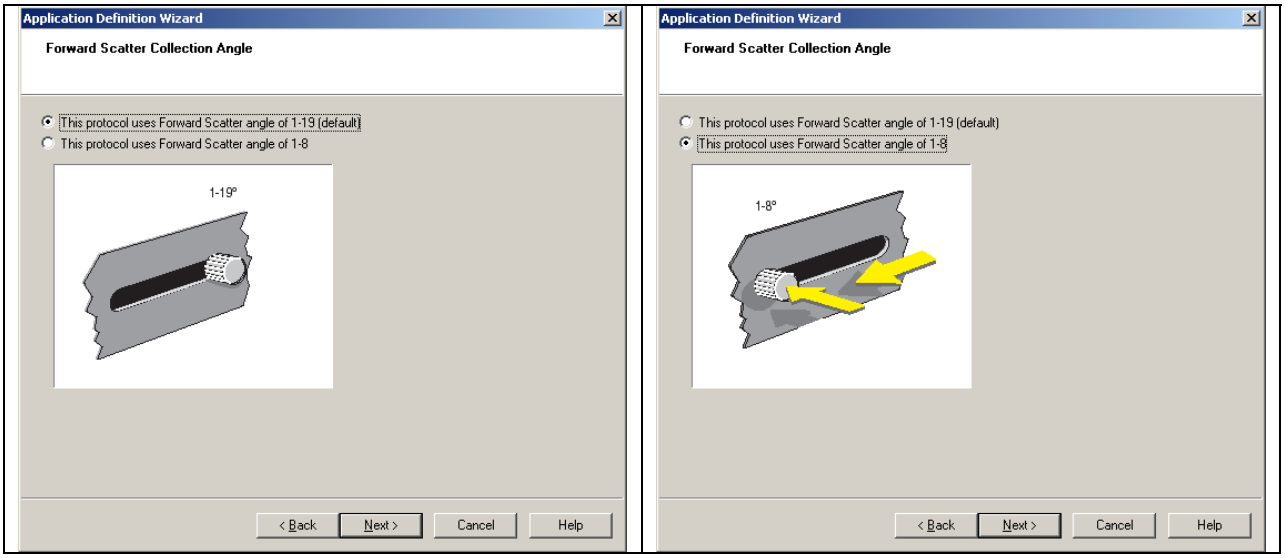


7. Ensure the parameters are correct and the second laser status is as desired. If they are not, you would need to select Cancel and correct the AS 2C.pro protocol. Incorrect parameters affects later screens.
8. Select **Next**.



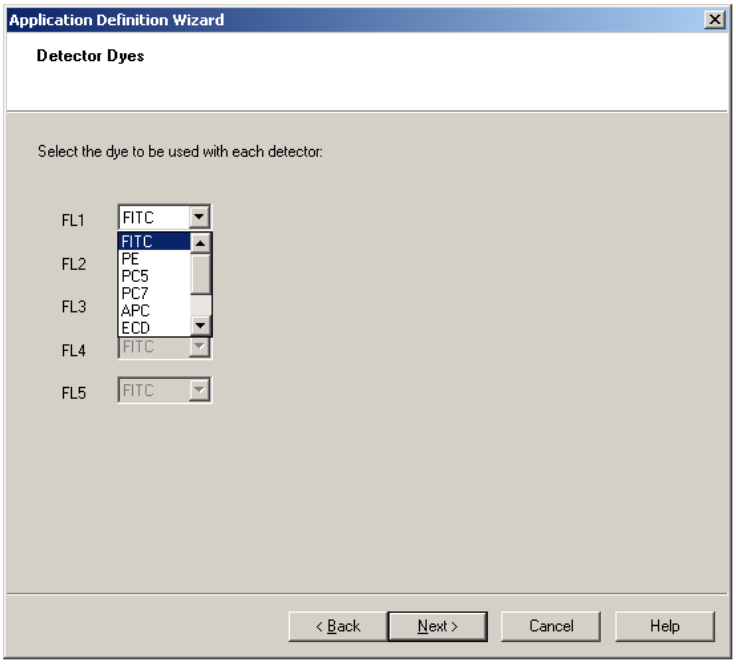
The block selection reminds the user that a different filter block assembly may have to be used for some applications. For the 2 color application, the initial filters are the same in both single and dual laser blocks.

9. Select desired block and then select **Next**.



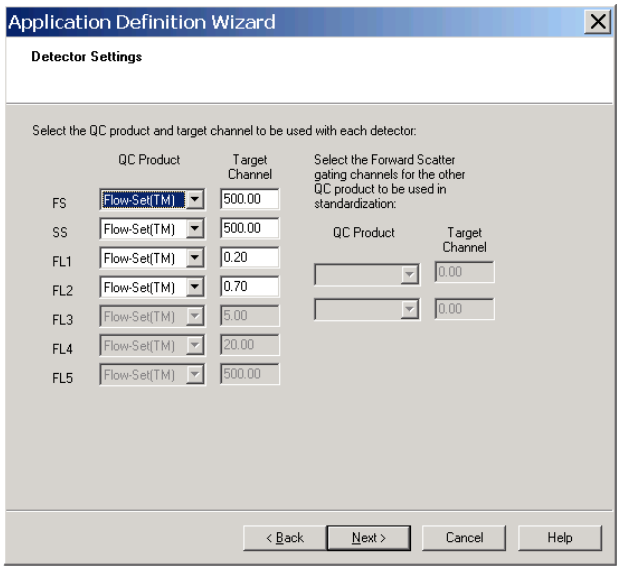
10. Select the forward scatter collection angle and then select **Next**.

Note: 1-19 degrees are the standard Coulter forward scatter collection angles. The 1-8 degree position simulates forward scatter patterns obtained on other manufacturer's instruments.



The system anticipates the dyes to be used which are correct for the two color application. If different dyes are to be used in the available slots, each parameter can be changed accordingly.

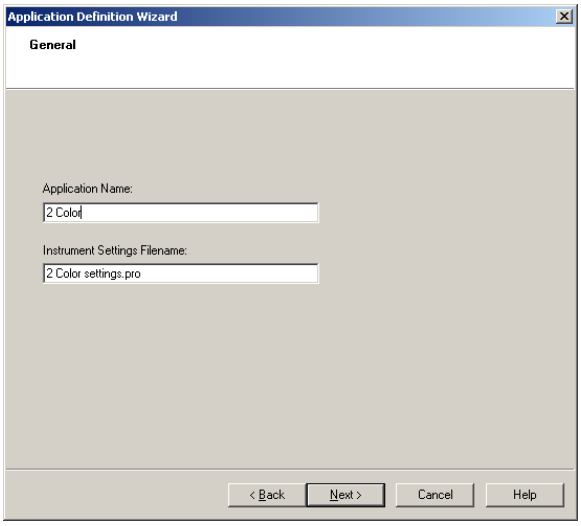
10. Select **Next**.



11. Retrieve the mean channels you recorded for the following parameters in the Basic Operation module and record them below:

FS _____ SS _____ FL1 Log _____ FL2 Log _____

12. Enter the target channels and select **Next**.

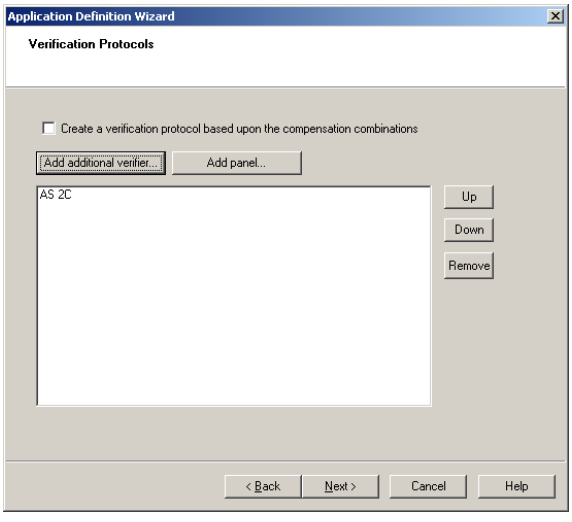


13. Enter the application name. The settings filename will autofill with the application name (Recommend AS 2Color Class to denote “Autosetup”.

14. If a different instrument settings file name is desired, enter it now.

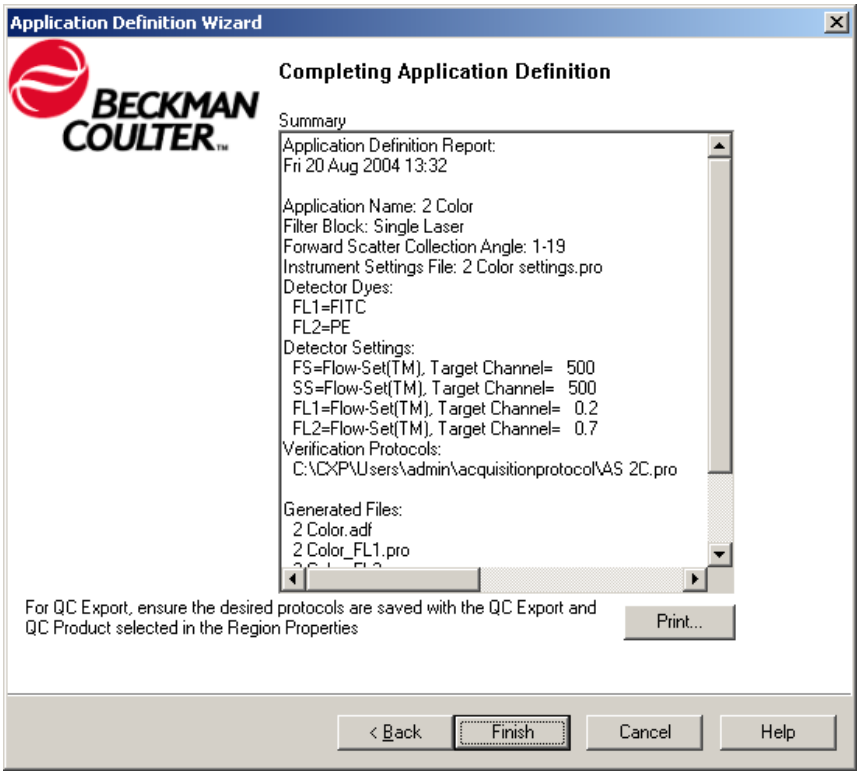
Note: This will be the protocol where all settings (e.g. high voltage, gains, compensation, etc.) will be stored after the system has been standardized and compensation settings determined. These settings should then be retrieved and used for the patient samples. This can be done directly into the protocol using the Cytometer menu Get Cytosettings from Protocol… option. These settings can also be used in a panel and will be covered in more detail in the Acquisition Manager module.

15. Select **Next**. The system now adds the verification protocol and allows you to add more or even a panel of protocols (an AS 2C protocol has been added below).



16. Select **Next**.

The software responds with a summary of your selections.



17. Print the report as proof of completion of this exercise and then select **Finish**.

The screen will go through a flurry of activity at this point to produce the protocol components needed to autostandardize the system for this application. This panel is virtual and will not be listed in the panel directory but will be available to the scheduler when you wish to run the application.

Other applications using more dyes will just have more entries in the report.

Use the Resource Explorer to drag and drop each of the application's protocols into the Workspace. Note the protocol naming convention is based on the name entered in:

*AS 2Color Class_stand.pro (Flow- Set for standardization)

AS 2Color Class_FL1.pro (FITC comp)

AS 2Color Class_FL2.pro (PE comp)

AS 2Color Class_Verify.pro (Verify protocol for control)

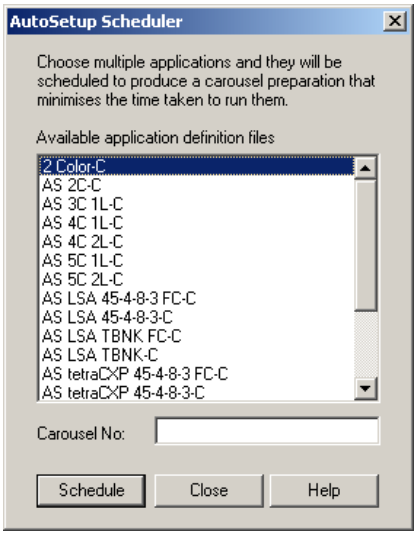
* Refer to the CXP Reference Manual for target region width details.

AutoSetup Scheduler

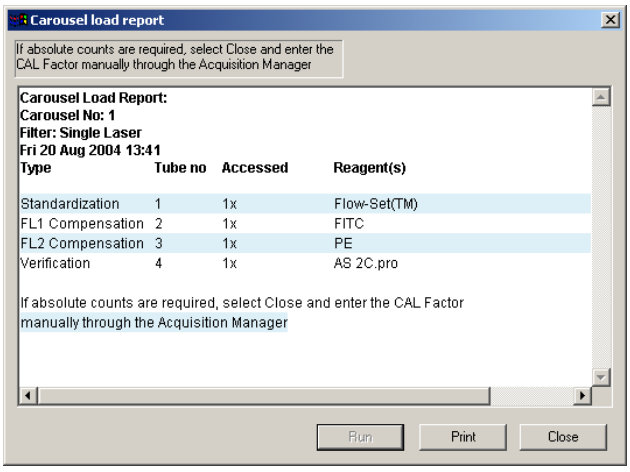
The Scheduler uses the application definition information to produce a load list for the carousel. If only one application is used, the list will be similar to the one described in the Advanced Digital Compensation (ADC) section of this module.

Let's schedule your 2 Color application to illustrate.

1. Select **Tools** >> **AutoSetup Scheduler**.



2. Select just the 2C 1L App and deselect all the others.
3. Enter a valid carousel number.
4. Select **Schedule**.



5. Print the report for your notes and then select **Close**. If cells/uL were not required, you could run the samples. The Acquisition Manager lists the tubes. If absolute counts (Flow-Count) are desired, you must add the Cal Factor in the Acquisition Manager before running the tubes.

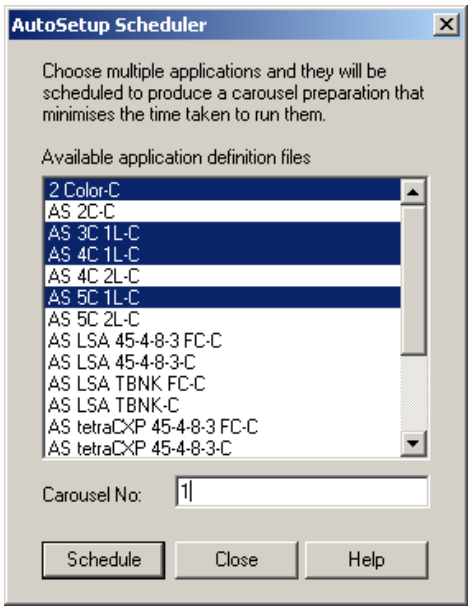
Mutiple Applications

If AutoSetup II uses the Auto Setup Scheduler to organize the carousel to run one or more applications. It optimizes the time for processing. As you will see, processing of multiple applications is streamlined. The Auto Setup Scheduler will analyze the selected application definition files and look for common optical configurations and reagents used between applications. It will then produce an optimal worklist sequence for running the tubes and instructions for preparing the carousel appropriately. Execution of the Auto Setup Schedule will endeavor to minimize the number of tube load operations by restarting acquisition with a protocol change on the Workspace rather than reloading the tube. The Scheduler will alert the user if non-compatible applications (different optical configurations) are selected.

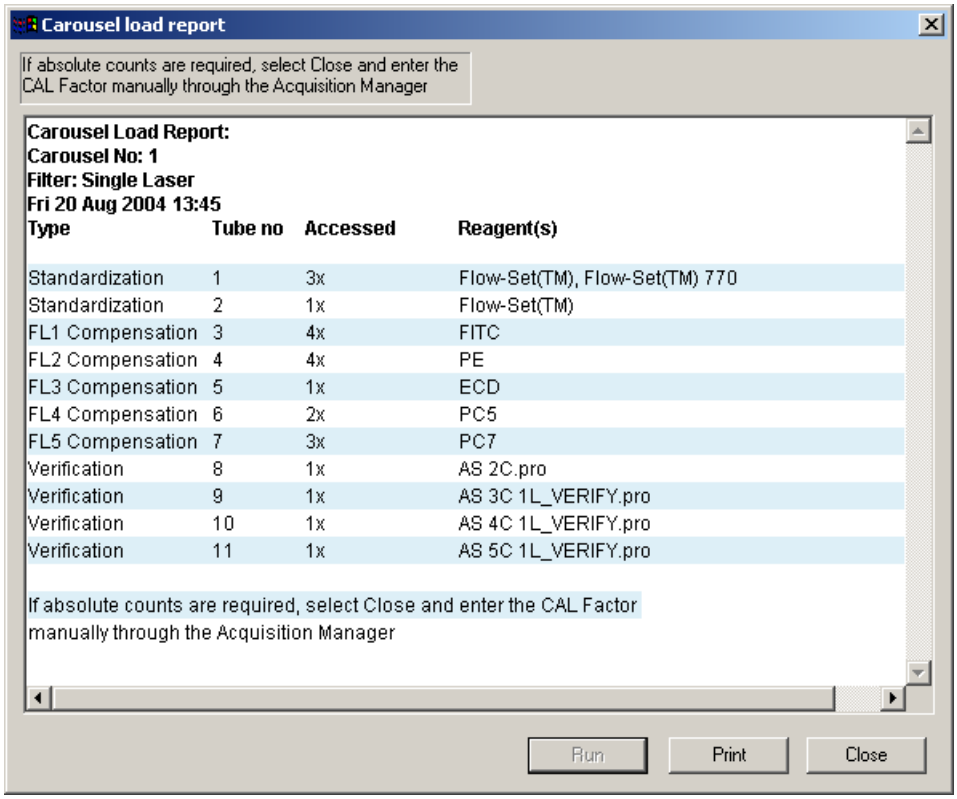
NOTE: You cannot co-mingle applications in which there are different physical instrument characteristics. For example, you cannot run 5 Color 1 Laser application using one filter block with a 5 color 2 laser application requiring a different filter block. The system will check and give you an error message.

Let's try scheduling the four different applications.

6. Select Tools >> AutoSetup Scheduler.
7. Select the applications as shown below. Use the (Ctrl) key to select multiple applications.



8. Enter a carousel number.
9. Select **Schedule**.



10. Print this report as proof of completion of this task.
11. Select **Close**. The Acquistion Manager lists the tubes.

This completes this module. If you feel you are ready, complete the Skill Check.

Table of Contents

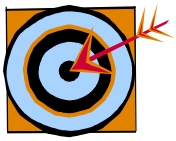
FlowPAGE

OBJECTIVES

Given an operational system, training materials, and access to online Help screens

- Create a flow page to include arranged plots, statistics, text and images.
- Save the FlowPAGE as a separate entity and as part of a protocol.
- Print a FlowPAGE as a PDF file.

Skill Check



You will have mastered these tasks when you can

- Print at least two different FlowPAGEs from different applications, which include arranged plots, statistics, text and images.
- Demonstrate a FlowPAGE saved with a protocol, saved separately and saved as a PDF file.

Tour Guides

If you would like to see a demonstration on how to create a FlowPAGE, we suggest you use the Tour Guides. To access the Tour Guides:

1. If there is a program running, minimize the screen by selecting  in the upper right corner of the application.

2. Place the Tour Guide disk into the CD Rom drive or if your facilitator has placed the Tour Guides on your hard drive, double click on the icon labeled Tour Guides. The system will start the program. A voice will come on and explain how to take the tours.

3. Take the FlowPAGE tour.

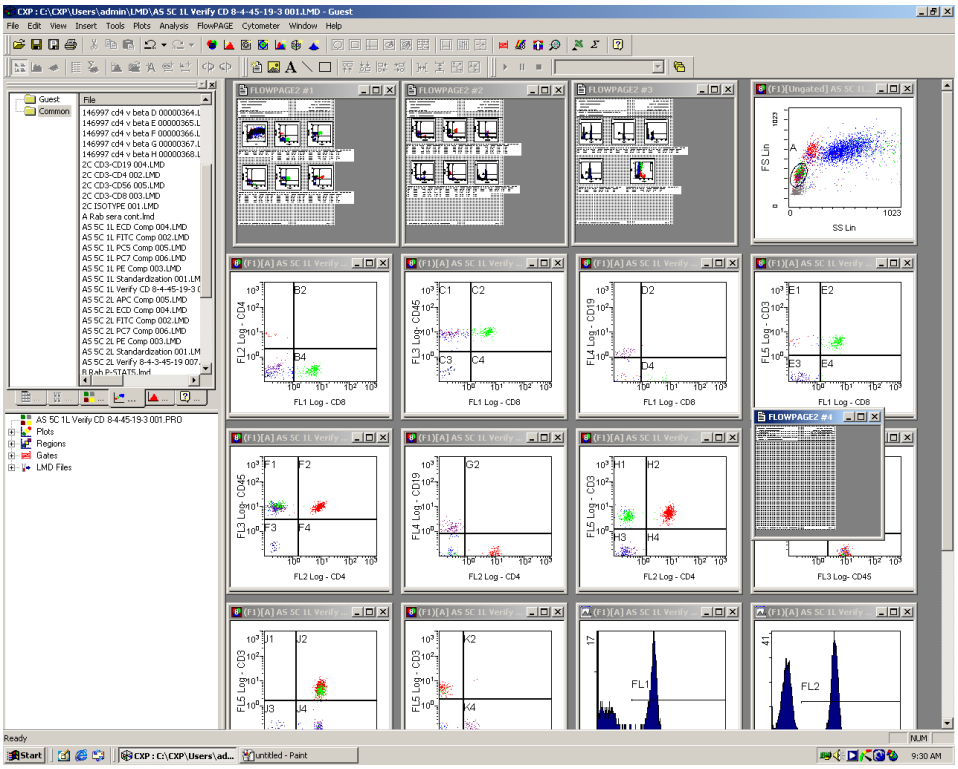
Creating a New FlowPAGE

A FlowPAGE is a customized report. This report can be saved with a protocol and printed automatically or manually.

1. Start by placing the desired protocol onto the Workspace. You can drag and drop one from the Resource Explorer. The examples that follow will use the 2C 1L.PRO from the Common folder populated with the AS 5C 1L Verify 8-4-45-19-3 listmode.

2. Now select **Insert >> Blank FlowPAGE**.

3. Move the new blank FlowPAGE to a convenient location on the screen.

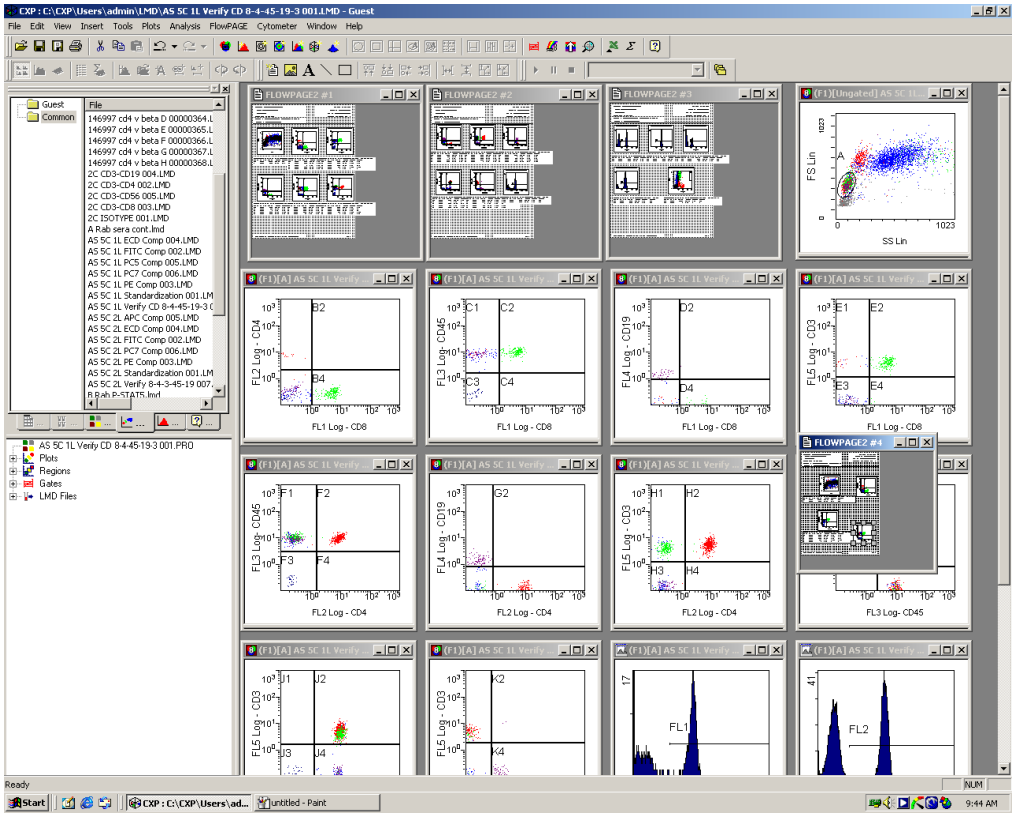


Adding Plots

4. Use a drag and drop technique to copy the plots onto the blank page.

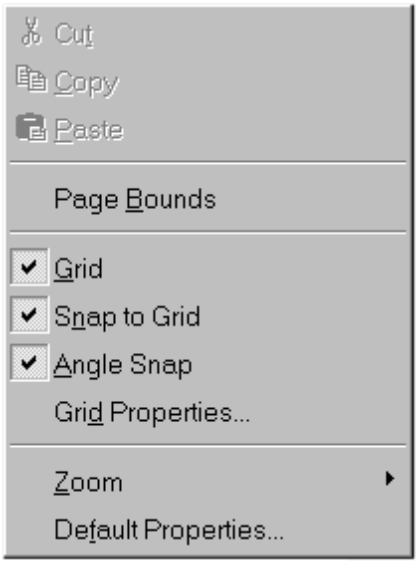
NOTE: Plots with a statistics box below them will be dragged as a unit to the page. You can also add a legend to the page in the same way. If the (Ctrl) key is pressed while dragging and dropping into the FlowPAGE, only the plot not the statistics will be dragged to the page.

Expand the size of the page by dragging one corner of the page to a new location or use the expand window option.



FlowPAGE Properties

5. Right click on the FlowPage to view the properties window.



Grid – sets up a dot grid on the page to aid in alignment.

Snap to Grid – aligns objects to a column or row of dots.

Angle Snap – Aligns to the nearest grid dot when plots are stretched or shrunk.

Arranging the plots

6. Select the ☐ symbol on the upper right corner of the FlowPAGE to display it full screen.

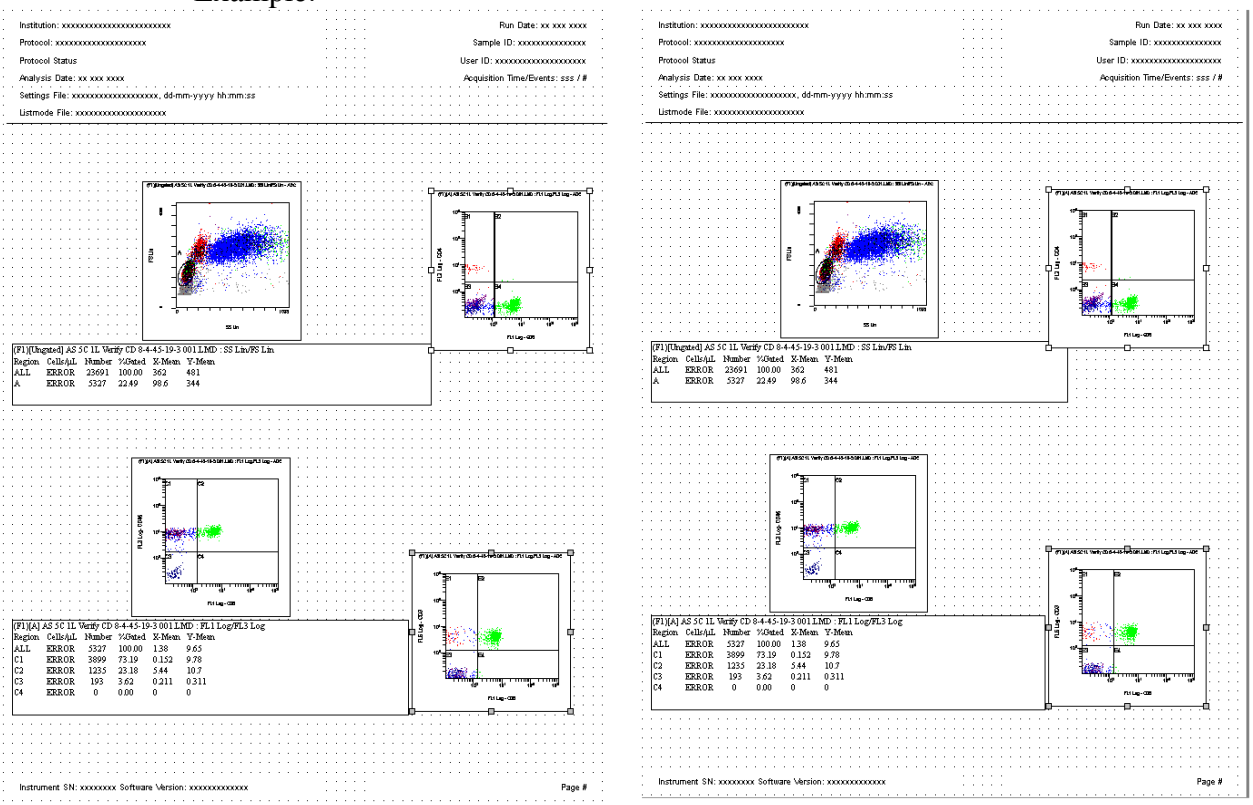
7. You can move each plot and statistics as a unit to anywhere on the screen. Take a moment to move them into place. If the grid is on, they should align to the grid.

8. You can align one to another by creating a box to include two of the plots (e.g. top two) and Stats.

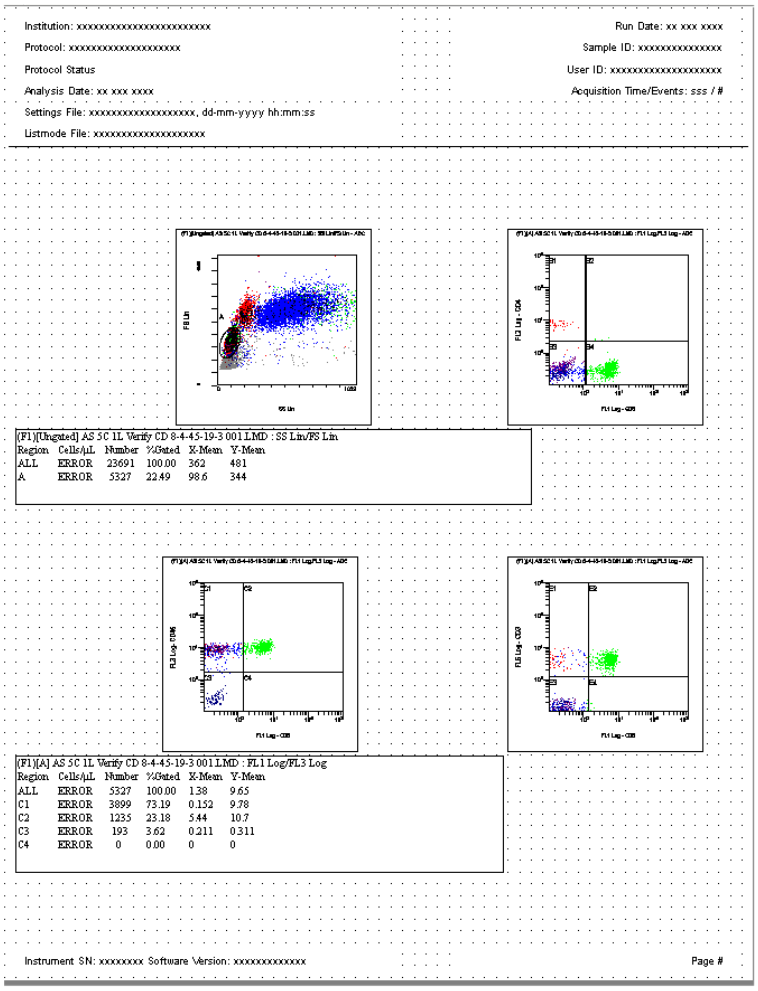
NOTE: The box you create must encircle all objects completely to be grabbed.

9. Now click on the appropriate align tool on the toolbar (e.g.  button) to align the two plots.

NOTE: The system will always align to the plot encircled with gray box handles. Example:



10. Repeat the process using any of the buttons you need to group the plots as you desire on the page. An example is shown below:



Take a moment to note the header information at the top. It includes:

Institution	Run Date / Time
Protocol Name	Sample ID
Protocol Status	User ID
Analysis Date / Time	Acq. Time/Events/Time Condition
Settings File	
Listmode File	

The Footer includes the instrument serial number and software version.

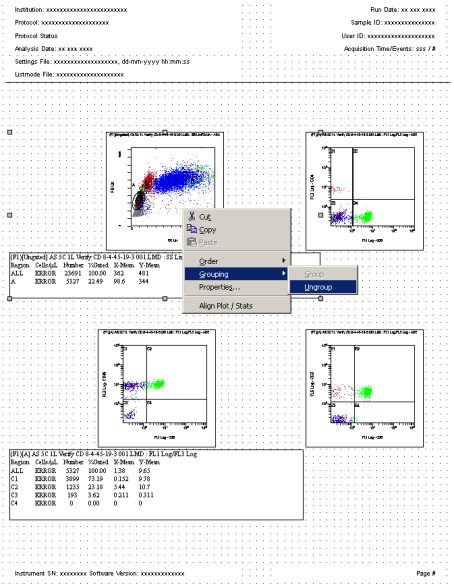
To Group or Ungroup objects

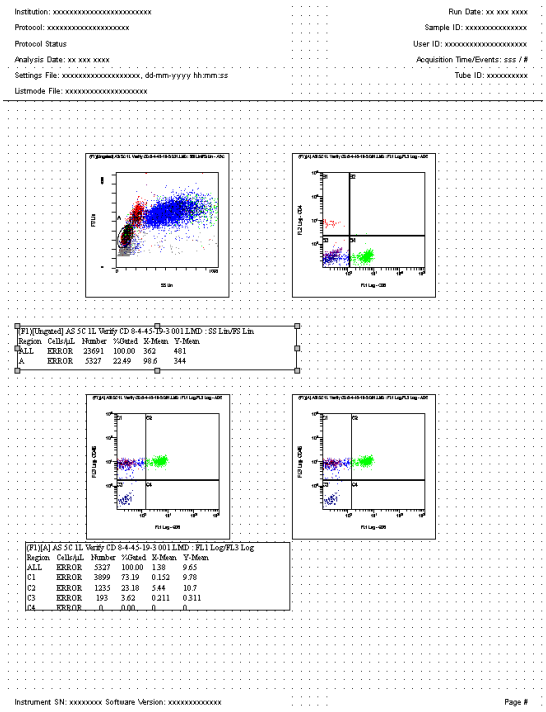
11. Encircle the Plot and statistics with a box and then right click when the cursor is on a plot.

12. Select **Grouping** and then **Ungroup**. The stats box can now be selected, resized, and moved independently of the plot.

13. Repeat the process with any other plots with stats.

14. Use a similar technique to group objects (e.g. all of the plots) so they can be moved as a unit.



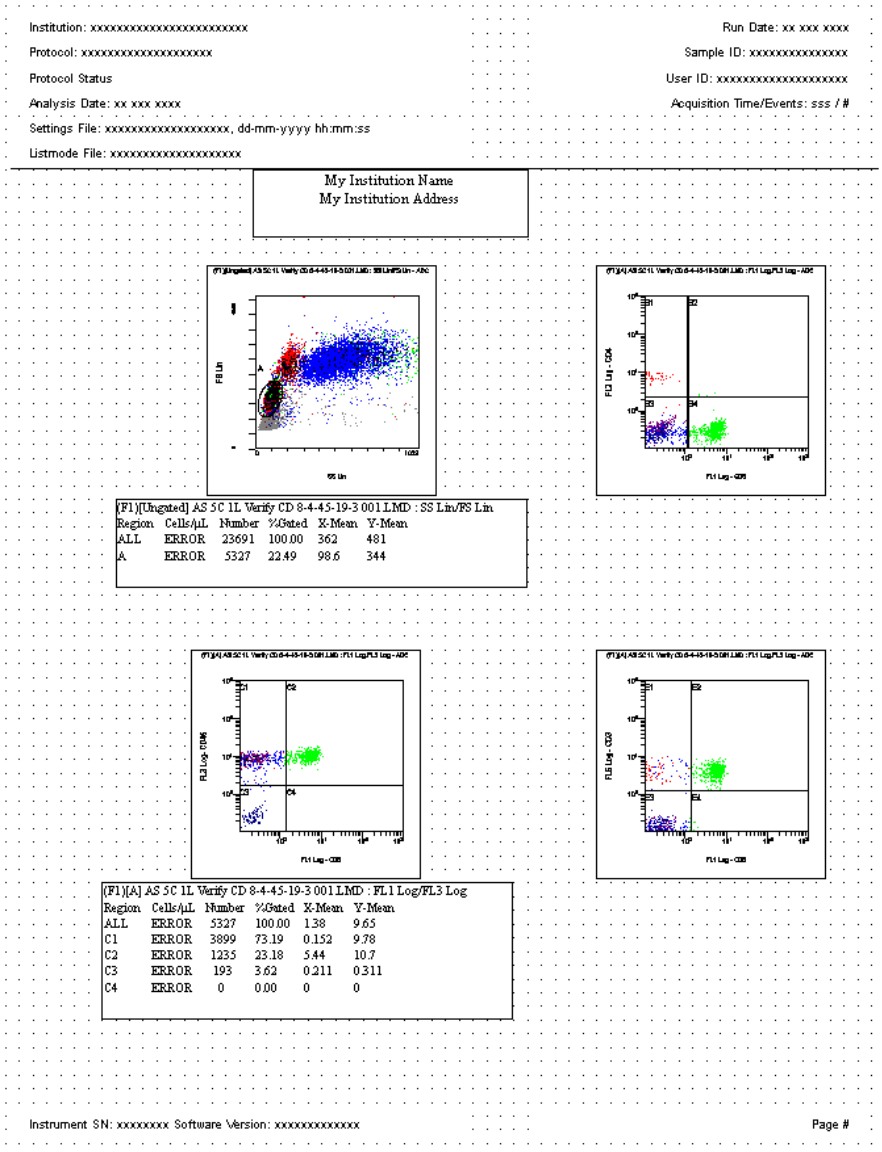


Adding Text

15. Select the **A** button on the FlowPAGE toolbar and then click on the page where you wish to place text. A text box will appear.

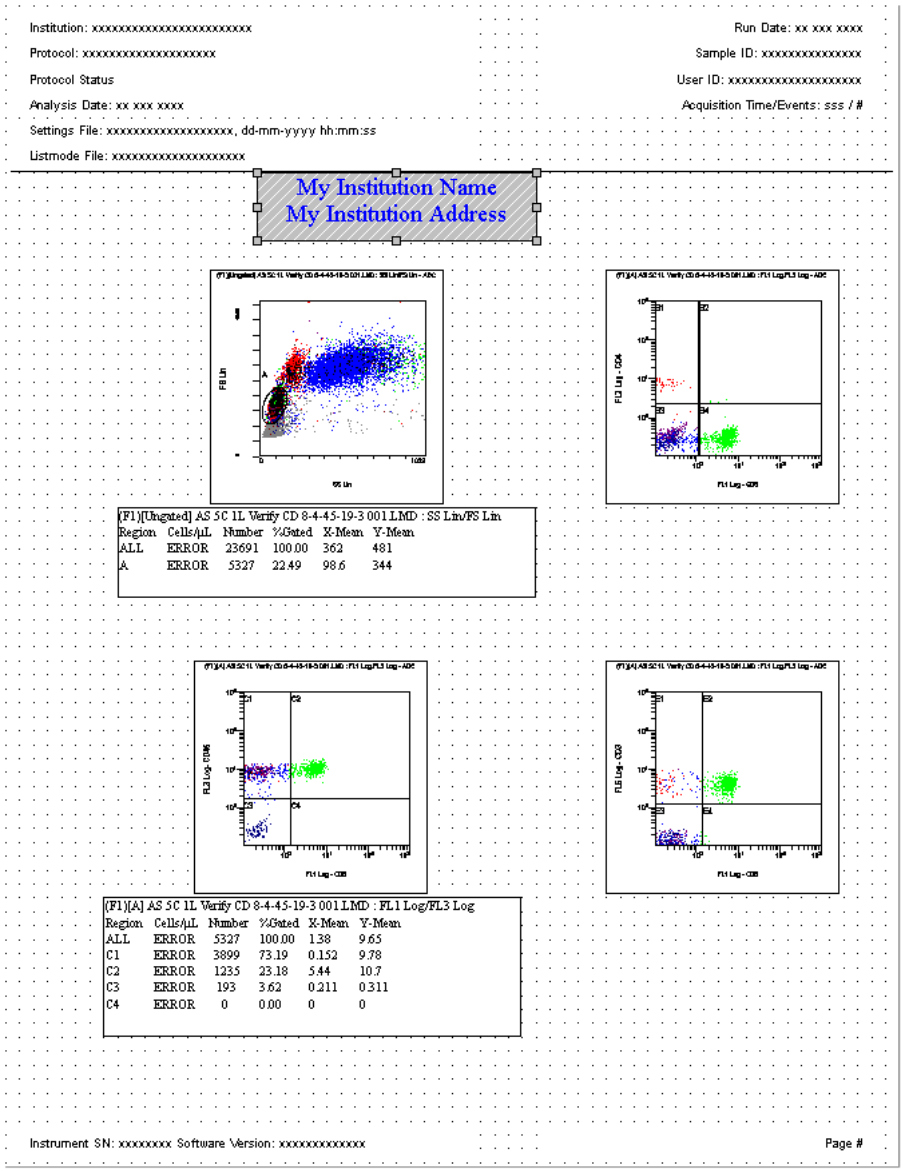
16. Type in the desired text and select **OK**. The text should appear.

17. You may size the box by grabbing a handle and stretching or shrinking the box to the desired size.



18. Right click in the box and select **Properties**. You may now make changes to the text using the appropriate tab. Try changing the font or fill.

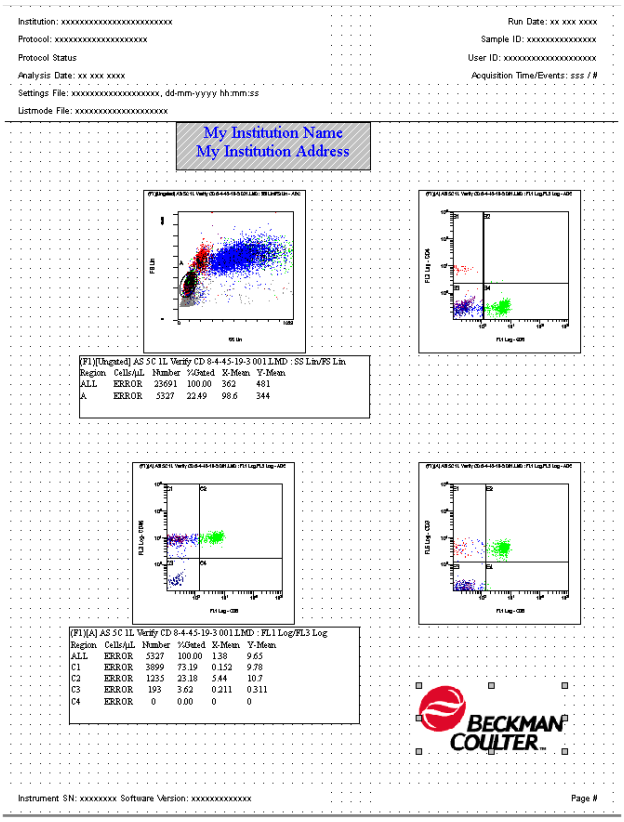
Example:



Adding Images

Images such as photos, logos, or clipart can be added to the page. Produce a bitmap image of your art and then it can be added to the page. We will insert a Beckman Coulter logo in the following example.

1. Select the  button on the toolbar.
2. Locate the directory where the desired image is stored.
3. Select the desired image and then **Open**.
4. Move the cursor to the desired position on the page and click the mouse.
5. Use the handles to size or move the image. When finished, click on another part of the page.



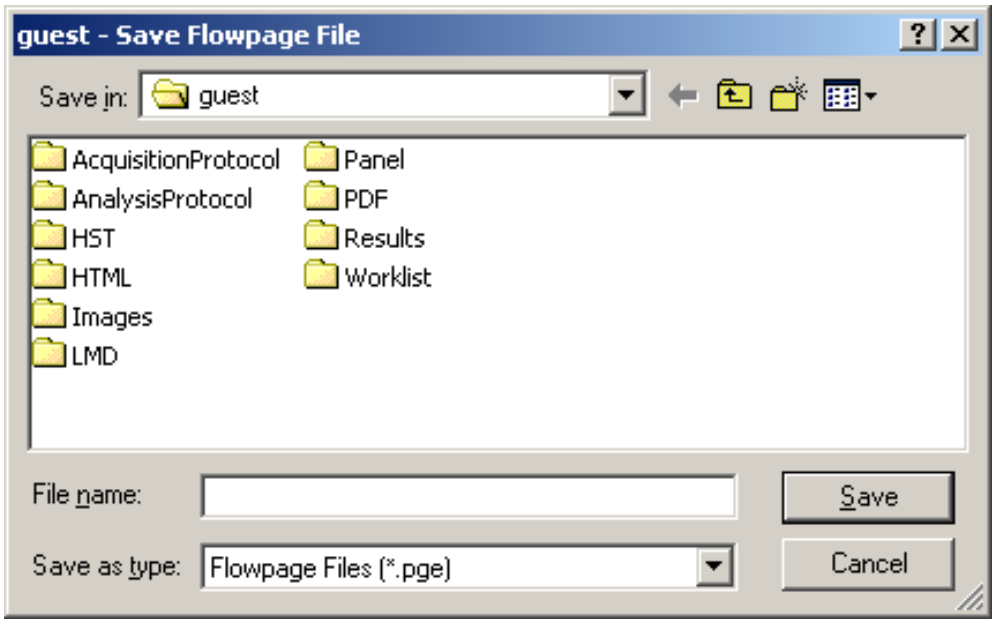
NOTE: You could also add some lines or boxes if you wish. You can also create additional FlowPAGES using the same process perhaps to illustrate additional information such as a graph.

Saving the FlowPAGE as Part of the Protocol

1. Select **File** >> **Save Protocol** or select the  .

Saving the FlowPAGE

1. Select **FlowPAGE** >> **Save FlowPAGE As**.
2. Select the desired folder and enter a name for your FlowPAGE and then select **Save**.

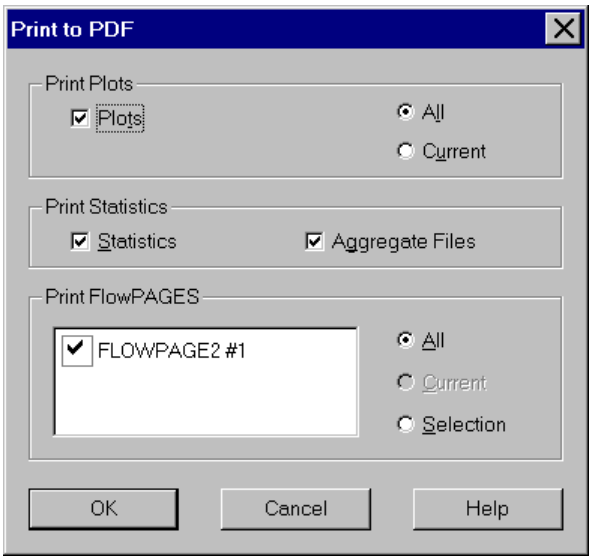


To Insert a Saved FlowPAGE

1. Select **Insert** >> **Blank FlowPAGE from file ….**
2. Navigate to the folder containing the desired FlowPAGE.
3. Select the *.pge file and then select **Open**. The FlowPAGE displays inserted into the protocol.

Printing to PDF File

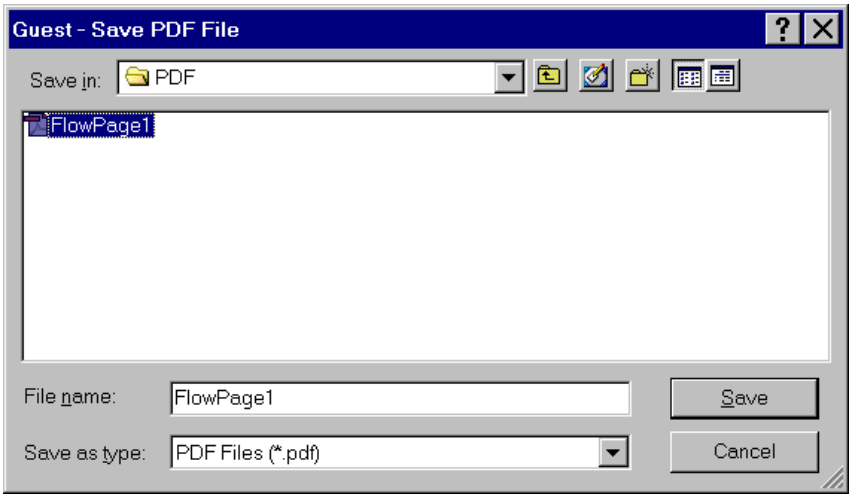
1. Select **File** >> **Print to PDF File**.



2. Select desired options and then select **OK**.

3. Type in a file name and select **Save**.

Note: The pdf filename will autofill with the Lmd filename if a listmode is displayed. If print to PDF is selected, when a protocol is displayed with no listmode, the pdf filename will autofill with the protocol name. In either case, the pdf filename can be edited by the user.



The system saves the plots, stats, and FlowPAGES as a PDF file which can be read by Adobe® Acrobat® Reader.

You have now completed this module. If you feel you are ready, please complete the Reports skill check.

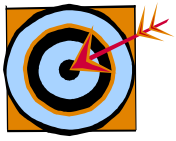
Table of Contents

OBJECTIVES

Given an operational system, application samples, training materials, and access to online Help

- Create a panel of at least 3 protocols to run application samples using the Acquisition Manager. The panel must reflect the loading of cytosettings from a properly updated settings file for the first tube that is carried over to the other tubes, first tube regions carried over to the other tubes, re-labeling of parameters, and Sample ID1 and 2 included in the sample name.
- Prepare and run the samples required for the panel (including autostandardization)
- Create at least a 20-tube worklist of protocols and panels. The worklist should reflect at least one two color and one three color panel with at least 3 patients, rinse tubes between patients, parameters labeled according to the antibodies run, at least one panel to provide absolute count data, and Sample ID1 and date included in the listmode filename.

Skill Check Preview



You will have mastered these tasks when you can perform each task listed in the objectives.

Tour Guides

If you would like to see a demonstration on how to create a panel, we suggest you use the Tour Guides. To access the Tour Guides:

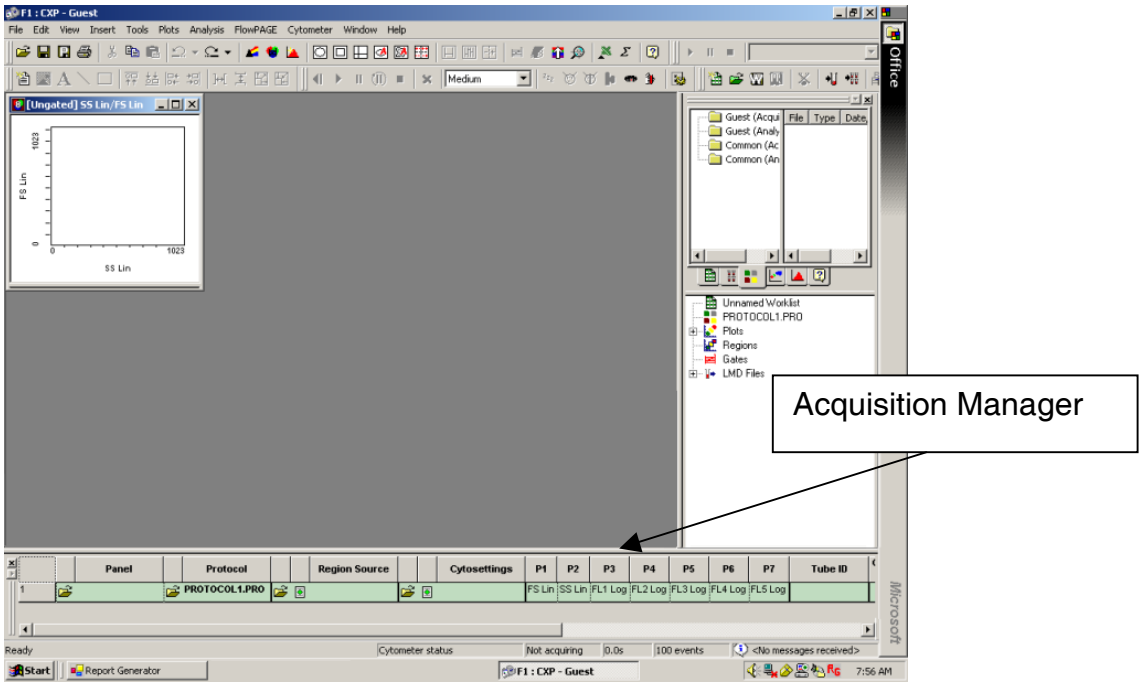
1. If there is a program running, minimize the screen by selecting  in the upper right corner of the application.

2. Place the Tour Guide disk into the CD Rom drive or if your facilitator has placed the Tour Guides on your hard drive, double click on the icon labeled Tour Guides. The system will start the program. A voice will come on and explain how to take the tours.
3. Take the Acquisition Manager tours.

Acquisition Manager

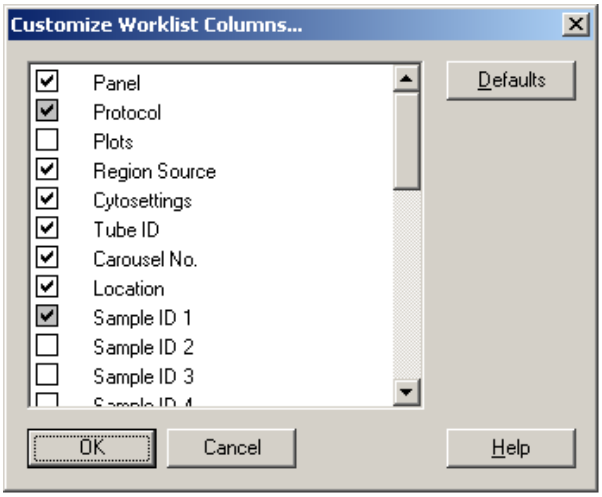
NOTE: If you are in the CXP Acquisition software, you have an additional viewing option called the Acquisition Manager. This resource displays a list of samples to be run and allows the user to enter information about each sample before they are run.

1. To view the Acquisition Manager if it is not on the screen, select **View** >> **Acquisition Manager**.



To Specify Columns in the Manager Area

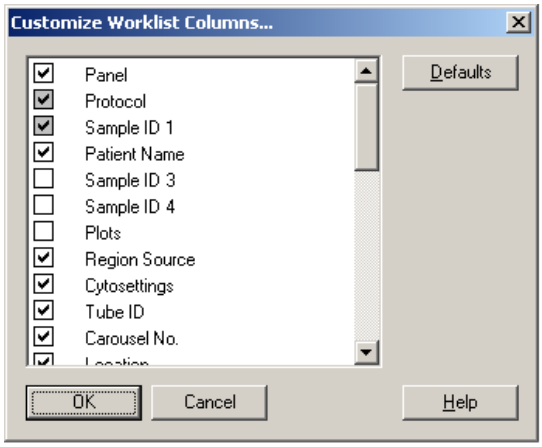
1. Select **View >> Customize Worklist Columns…**or  .



2. Check each item you wish to be included in the Acquisition Manager area and then select **OK**. Grayed items are always selected. Other selected items depend on your personal choices. If for example, you wish to use Sample ID 2 to identify the patient and include the entry in the listmode file name then Sample ID 2 should also be selected to appear on the Acquisition Manager.

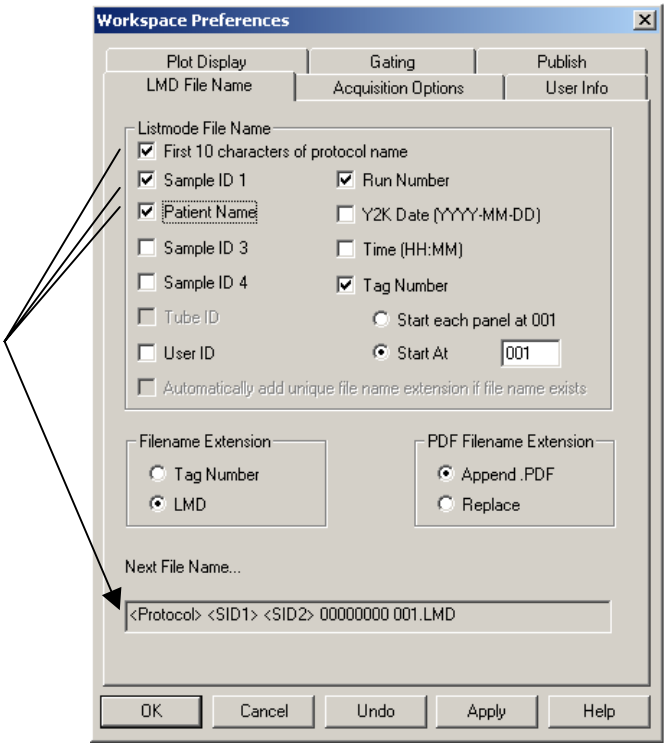
To Edit the Column Name

3. Double click on the name you wish to change (e.g. Sample ID 2).
4. Type in a new name (e.g. Patient Name) and press **Enter**. The Customized Worklist Columns should now appear as below.



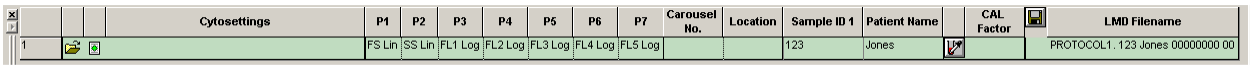
To make Column Entries part of the Listmode File Name:

5. Press **CTRL** and **W** at the same time and then select the LMD Name tab.
6. Select the column entries on the Acquisition Manager to be included in the Listmode File Name. In our example, the window may appear as below.



5. When you are finished, select OK.
6. Select the boxes in turn below the Sample ID and Patient Name columns on the Acquisition Manager, type in something (at least 3 characters) and press Enter. The Listmode file name should reflect your entries.

Example:



To Move Columns in the Acquisition Manager:

7. Click on the column name you wish to move. The Name box should change color.
8. Drag and drop the column to the new location on the Acquisition Manager. The column or group of columns should move.

Example:

Create A Panel Using the Acquisition Manager

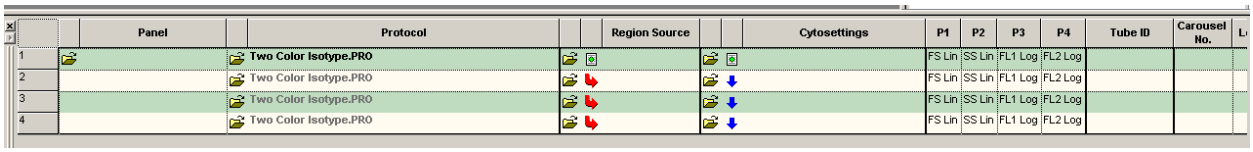
NOTE: A panel is a list of protocols to be run on the system in sequence. Using Panels allows regions and/or cytosettings to pass from protocol to protocol.

Before you Begin

1. Drag and drop the Two Color protocol from the Resource Explorer (Guest) to the Workspace.
2. Select **No**.
3. Select **File > Save Protocol As**, type in the name Two Color Isotype and then select **Save**. You will use this protocol in your panel.

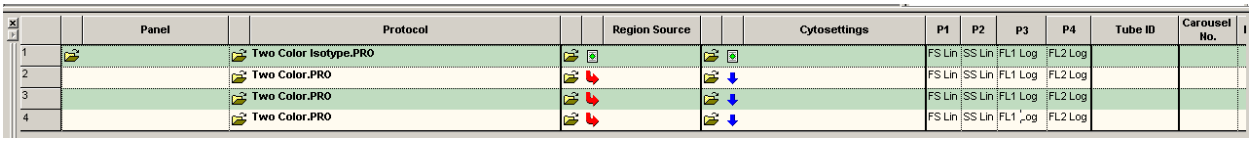
Create Panel

1. Select New Worklist button  .
2. Drag and drop a desired protocol (e.g. Two Color Isotype.PRO) from the Resource Explorer to the Acquisition Manager.
3. Select Insert Tube button  several times. Several lines with your chosen protocol should appear on the Acquisition Manager.



	Panel	Protocol	Region Source	Cytosettings	P1	P2	P3	P4	Tube ID	Carousel No.
1		Two Color Isotype.PRO			FS Lin	SS Lin	FL1 Log	FL2 Log		
2		Two Color Isotype.PRO			FS Lin	SS Lin	FL1 Log	FL2 Log		
3		Two Color Isotype.PRO			FS Lin	SS Lin	FL1 Log	FL2 Log		
4		Two Color Isotype.PRO			FS Lin	SS Lin	FL1 Log	FL2 Log		

4. If you wish to change a protocol on a line, select the Protocol file folder  on Acquisition Manager, select desired protocol and then the **Open** button.
5. Repeat the process for each line.



	Panel	Protocol	Region Source	Cytosettings	P1	P2	P3	P4	Tube ID	Carousel No.
1		Two Color Isotype.PRO			FS Lin	SS Lin	FL1 Log	FL2 Log		
2		Two Color.PRO			FS Lin	SS Lin	FL1 Log	FL2 Log		
3		Two Color.PRO			FS Lin	SS Lin	FL1 Log	FL2 Log		
4		Two Color.PRO			FS Lin	SS Lin	FL1 Log	FL2 Log		

To Set the Regions

6. Use the  icon to use the regions within the protocol, the  icon to use the regions from a different specified protocol, or the  icon to carry regions from one protocol to another in the panel.

To Load Instrument Settings:

7. Use the  icon to use the instrument settings within the protocol, the  icon to use the settings from a different specified protocol (e.g. AS 2C1L Settings.PRO), or the  icon to carry settings from one protocol to another in the panel.

Note: If you are retrieving Instrument Settings from an external file, you must have the Cytosettings column selected (from the Customize Worklist Column dialog).

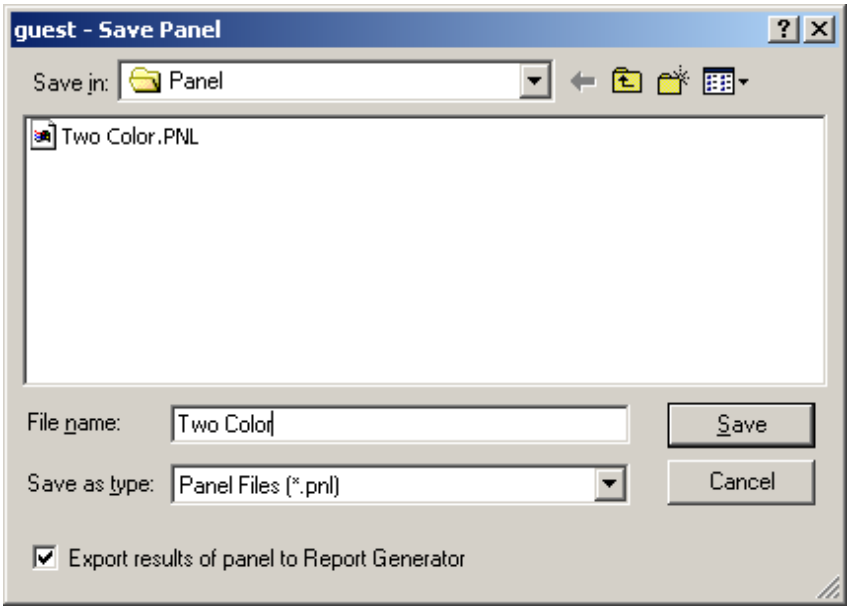
To Change Parameter Names

8. Select each box under the parameter in turn, type in a new name and press **Enter**.

	Panel	Protocol	Region Source	Cytosettings	P1	P2	P3	P4	Tube ID	Carousel No.
1		Two Color Isotype.PRO		AS 2C Settings.pro	FS Lin	SS Lin	G1 FITC	G1 PE		
2		Two Color.PRO			FS Lin	SS Lin	CD3-FITC	CD19-PE		
3		Two Color.PRO			FS Lin	SS Lin	CD3-FITC	CD4-PE		
4		Two Color.PRO			FS Lin	SS Lin	CD3-FITC	CD8-PE		

To Save Panel

9. Right click on the Panel folder  button and select **Save as Panel**.



10. Type in a new name (e.g. Two Color) and select Export results of panel to Report Generator.

Note: The export option creates a blank template for reporting your data in the database. In the Database module, you will see how to define exactly what you wish to print from the panel. For now, just continue with this module.

11. Select **Save**. You have now created a new panel.

Example:

	Panel	Protocol	Region Source	Cytosettings	P1	P2	P3	P4	Tube ID	Carousel No.
1	Two Color.PNL	Two Color Isotype.PRO		AS 2C Settings.pro	FS Lin	SS Lin	G1-FITC	G1-PE		
2	Two Color.PNL	Two Color.PRO			FS Lin	SS Lin	CD3-FITC	CD19-PE		
3	Two Color.PNL	Two Color.PRO			FS Lin	SS Lin	CD3-FITC	CD4-PE		
4	Two Color.PNL	Two Color.PRO			FS Lin	SS Lin	CD3-FITC	CD8-PE		

Note: Panels may also be created using Panel Wizard. Use the online Help for instructions on Panel Wizard.

Create a Worklist in Acquisition Manager

NOTE: A Worklist is a list of protocols and panels to be run on the system in sequence.

1. Select New Worklist button  .
2. Select the  tab on the Resource Explorer.
3. Select a protocol and drag and drop it onto the Acquisition Manager.
4. To select a panel, select panel  tab on the Resource Explorer, then a panel, and the drag and drop it to the Acquisition Manager.
5. Continue adding as many protocols and/or panels to the Acquisition Manager as you desire.

Editing the Acquisition Manager Worklist

NOTE: Individual fields within the Acquisition Manager Worklist can be edited. The parameter names can be changed, for example, or sample ID's added and so forth.

6. Select desired box.
7. Type in desired information.

Note: Sample ID1 entered for tube 1 in a panel will autofill for the remaining tubes in the panel and cannot be edited. Sample ID1 is the linking identifier for a panel of tubes for panel reports.

Example:

	Panel	Protocol	Sample ID 1	Sample ID 2	Region Source	Cytosettings	P1	P2	P3	P4	Tube ID
1	Two Color.PNL	Two Color Isotype.PRO	123	Jones		AS 2C Settings.pro	FS Lin	SS Lin	G1 FITC	G1 PE	
2	Two Color.PNL	Two Color.PRO	123	Jones			FS Lin	SS Lin	CD3 FITC	CD 19 PE	
3	Two Color.PNL	Two Color.PRO	123	Jones			FS Lin	SS Lin	CD3 FITC	CD4 PE	
4	Two Color.PNL	Two Color.PRO	123	Jones			FS Lin	SS Lin	CD3 FITC	CD8 PE	
5		cleanse water.pro					FS Lin	SS Lin	TIME		
6	Two Color.PNL	Two Color Isotype.PRO	456	Smith		AS 2C Settings.pro	FS Lin	SS Lin	G1 FITC	G1 PE	
7	Two Color.PNL	Two Color.PRO	456	Smith			FS Lin	SS Lin	CD3 FITC	CD 19 PE	
8	Two Color.PNL	Two Color.PRO	456	Smith			FS Lin	SS Lin	CD3 FITC	CD4 PE	
9	Two Color.PNL	Two Color.PRO	456	Smith			FS Lin	SS Lin	CD3 FITC	CD8 PE	
10		cleanse water.pro					FS Lin	SS Lin	TIME		
11	Two Color.PNL	Two Color Isotype.PRO	789	Gonzalez		AS 2C Settings.pro	FS Lin	SS Lin	G1 FITC	G1 PE	
12	Two Color.PNL	Two Color.PRO	789	Gonzalez			FS Lin	SS Lin	CD3 FITC	CD 19 PE	
13	Two Color.PNL	Two Color.PRO	789	Gonzalez			FS Lin	SS Lin	CD3 FITC	CD4 PE	
14	Two Color.PNL	Two Color.PRO	789	Gonzalez			FS Lin	SS Lin	CD3 FITC	CD8 PE	
15	Cleanse.PNL	Cleanse Bleach.PRO					FS Lin	SS Lin	TIME		
16	Cleanse.PNL	Cleanse Water.PRO					FS Lin	SS Lin	TIME		
17	Cleanse.PNL	Cleanse Water.PRO					FS Lin	SS Lin	TIME		
18	Cleanse.PNL	Cleanse Water.PRO					FS Lin	SS Lin	TIME		

Assigning a Carousel Number and Tube Location

8. Type in a carousel Number in the first box of the Carousel No. Column and press **Enter**. The same number appears on all the tubes in the worklist. The system also assigns the tube locations beginning with 1.
9. Some carousel positions can be used more than once in a worklist. For example, you may wish to run water in between each panel. The water tube is placed in a specific carousel position but every time you wish to use it a new line is added to the worklist. The tube location, however, is the same. Assign the other positions accordingly.

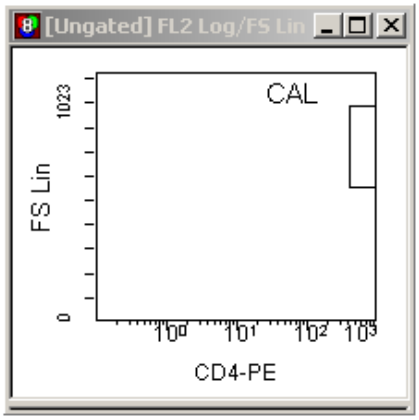
Example:

	Sample ID 1	Sample ID 2	Region Source	Cytosettings	P1	P2	P3	P4	Tube ID	Carousel No.	Location	CAL Factor	LMD Filename
1	123	Jones		AS 2C Settings.pro	FS Lin	SS Lin	G1 FITC	G1 PE		1	1		00000000 001.LMD
2	123	Jones			FS Lin	SS Lin	CD3 FITC	CD 19 PE		1	2		00000001 002.LMD
3	123	Jones			FS Lin	SS Lin	CD3 FITC	CD4 PE		1	3		00000002 003.LMD
4	123	Jones			FS Lin	SS Lin	CD3 FITC	CD8 PE		1	4		00000003 004.LMD
5					FS Lin	SS Lin	TIME			1	5		00000004 005.LMD
6	456	Smith		AS 2C Settings.pro	FS Lin	SS Lin	G1 FITC	G1 PE		1	6		00000005 006.LMD
7	456	Smith			FS Lin	SS Lin	CD3 FITC	CD 19 PE		1	7		00000006 007.LMD
8	456	Smith			FS Lin	SS Lin	CD3 FITC	CD4 PE		1	8		00000007 008.LMD
9	456	Smith			FS Lin	SS Lin	CD3 FITC	CD8 PE		1	9		00000008 009.LMD
10					FS Lin	SS Lin	TIME			1	5		00000009 010.LMD
11	789	Gonzalez		AS 2C Settings.pro	FS Lin	SS Lin	G1 FITC	G1 PE		1	10		00000010 011.LMD
12	789	Gonzalez			FS Lin	SS Lin	CD3 FITC	CD 19 PE		1	11		00000011 012.LMD
13	789	Gonzalez			FS Lin	SS Lin	CD3 FITC	CD4 PE		1	12		00000012 013.LMD
14	789	Gonzalez			FS Lin	SS Lin	CD3 FITC	CD8 PE		1	13		00000013 014.LMD
15					FS Lin	SS Lin	TIME			1	14		00000014 015.LMD
16					FS Lin	SS Lin	TIME			1	15		00000015 016.LMD
17					FS Lin	SS Lin	TIME			1	16		00000016 017.LMD
18					FS Lin	SS Lin	TIME			1	17		00000017 018.LMD

Absolute Count

10. If the Cal Factor column is not present on the Acquisition Manager, select **View >> Customize Worklist Columns…**, select Cal Factor, and then **OK**.

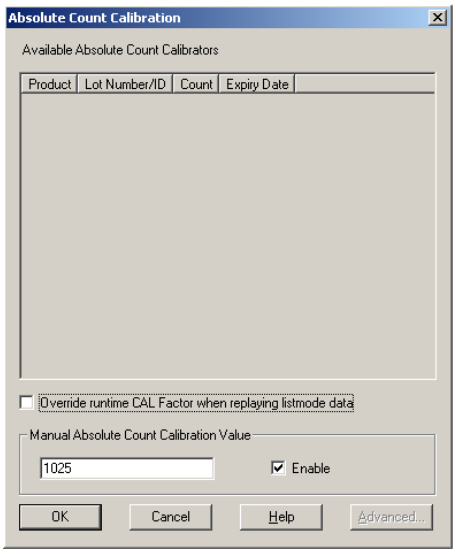
NOTE: In order for absolute counts to be generated, the sample must have Flow-Count beads in it and the protocol must have a CAL region where only the beads will accumulate (see below). A calibration factor must also be included in the Acquisition Manager worklist. The analysis should also include cells/uL.



11. Select the  icon in the Cal Factor column, enter a calibrator value and select **OK**. Repeat for each applicable panel.

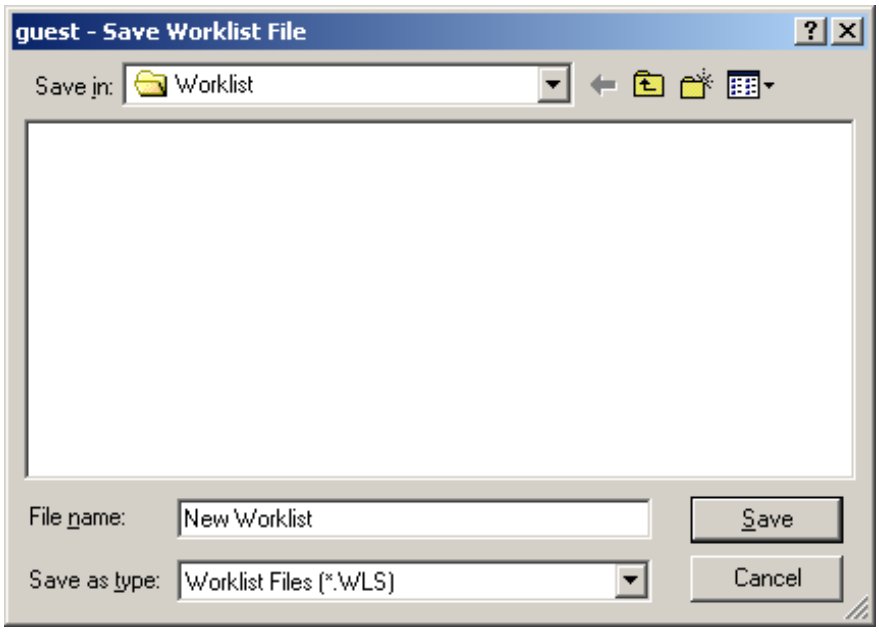
Note: To autofill all Worklist entries with the same CAL factor selection, hold the (Shift) key when selecting the  icon.

Example:



Saving a Worklist

12. Select  on the Acquisition Manager toolbar, type in a name, and select the **Save** button.



You have now completed this module. If you feel you are ready, please complete the Acquisition Manager skill check.

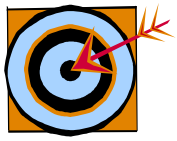
Table of Contents

OBJECTIVES

Given an operational system, stored sample data, training materials, and access to online Help screens

- Retrieve listmode data with the run time protocol.
- Retrieve listmode data with a new protocol.
- Retrieve multiple listmode files with a panel of protocols (Analysis only).
- Retrieve several sets of listmode files with a single protocol using the batch AutoMATOR function.
- Create Linked regions.
- Create an overlay of several single parameter plots which include stats, a legend, regions, annotation and data analyzed using the Overton or Kolmogorov-Smirnov modes (Analysis only).
- Publish data to Microsoft Excel.

Skill Check Preview



You will have mastered these tasks when you can

- Show printouts of data retrieved using the runtime protocol and a new protocol.
- Show a panel of printouts of specified retrieved listmodes.
- Show a set of printouts illustrating at least two sets of listmode files retrieved with one protocol using the batch automator.
- Show a set of linked regions.
- Print an overlay plot of at least 3 single parameter plots, with stats, a legend, regions, annotation, and data analyzed with either Overton or Kolmogorov-Smirnov modes.
- Print data published to Microsoft Excel.

Tour Guides

If you would like to demonstrations related to retrieving data, we suggest you access the Tour Guides. To access the Tour Guides:

1. If there is a program running, minimize the screen by selecting  in the upper right corner of the application.

2. Place the Tour Guide disk into the CD Rom drive or if your facilitator has placed the Tour Guides on your hard drive, double click on the icon labeled Tour Guides. The system will start the program. A voice will come on and explain how to take the tours.

3. Take the Data Retrieval tours.

Log On to Analysis Software

This module is to be completed using the Analysis software and **not the Acquisition software**. Some functions illustrated on the following pages do not work in the Acquisition software. If you are presently logged on to the Acquisition software:

1. Minimize the Acquisition software screen by selecting  in the upper right corner of the screen or log off by selecting  and save the current protocol if you have made changes.

2. Double click on the Cytomics CXP Analysis icon or select **Start** >> **Cytomics CXP Analysis**.

3. Select your log on name (you can use Guest).

4. Enter your password ("valued" for Guest) and select **Next**.

5. Select **Finish**. The Analysis screen will appear with the default protocol.

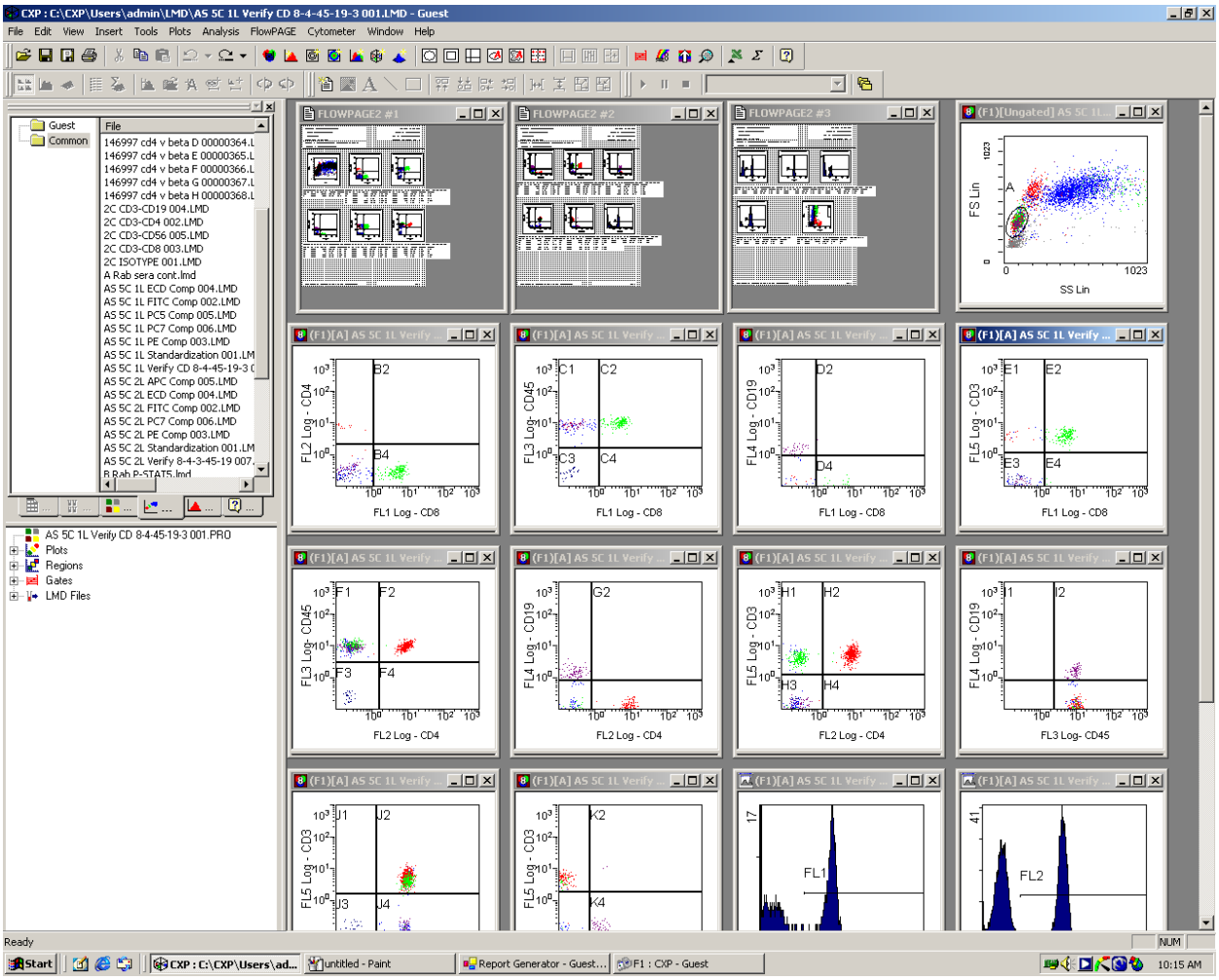
Retrieving Protocol and Listmode Files

Retrieval with Runtime Protocol

1. Select the  on the Resource Explorer and then the desired file folder (e.g. Common).

2. Select the desired file (e.g. AS 5C 1L Verify 8-4-45-19-3) and drag and drop it onto the Workspace (not into a plot). The system should ask if you wish to save the current protocol. The listmode will play through the protocol that was used to acquire the data.

NOTE: You may need to use the slider bar at the bottom of the screen to see the rest of the plots.



Retrieval with a Different Protocol

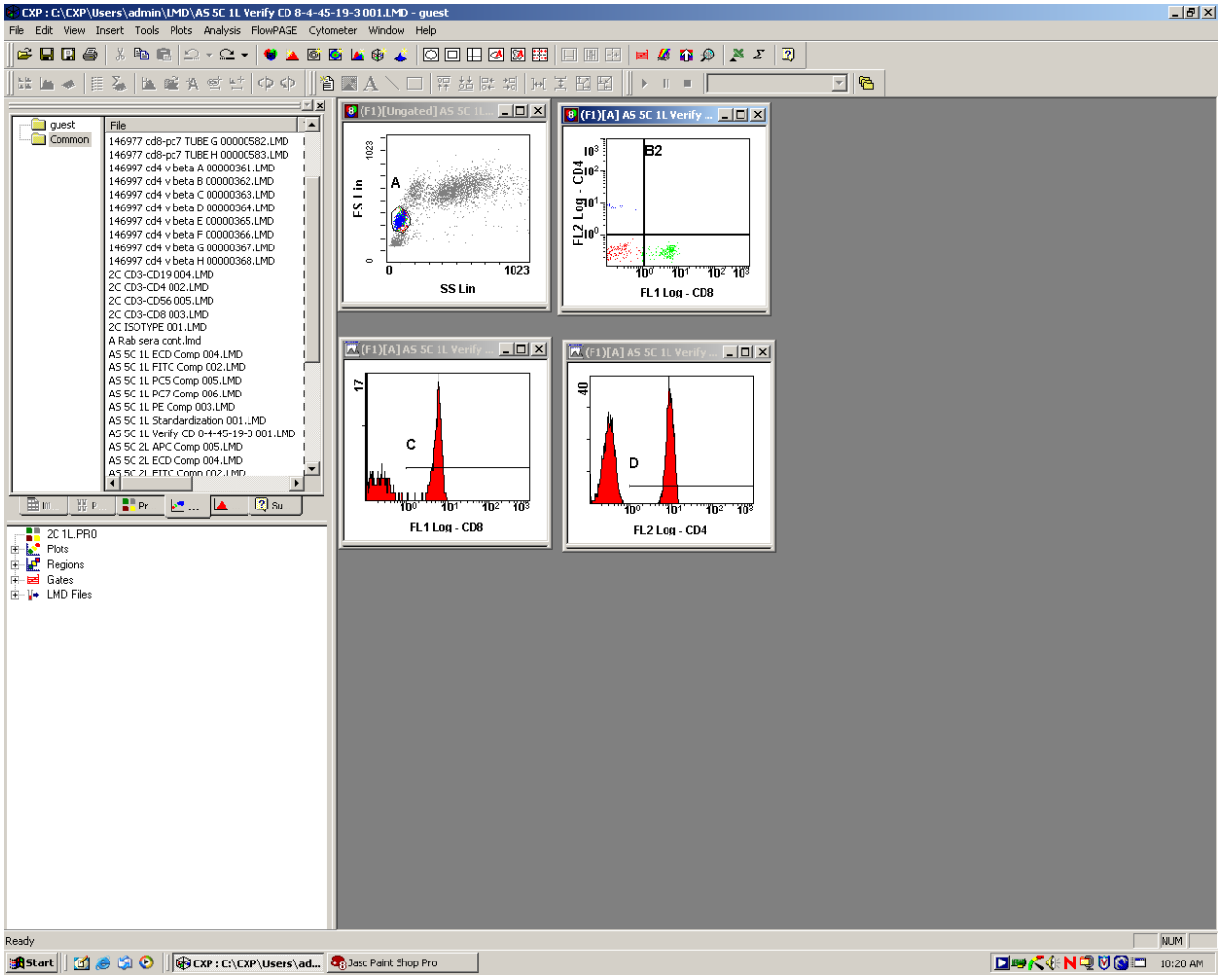
1. Select the  button and then drag and drop the desired protocol onto the Workspace.

2. Now select the  button and then the desired file folder.

3. Select the desired file and drag and drop it onto one of the plots.

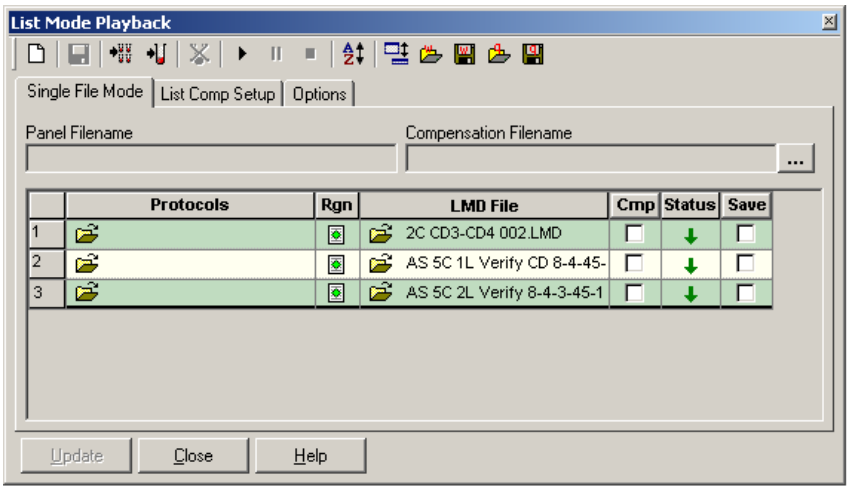
NOTE: If the parameters of the listmode file are different than the protocol, the system will prompt you to continue or abort. If you wish to display the file anyway, select Continue.

4. Adjust the gate regions to place them around the correct populations. An example is shown below:

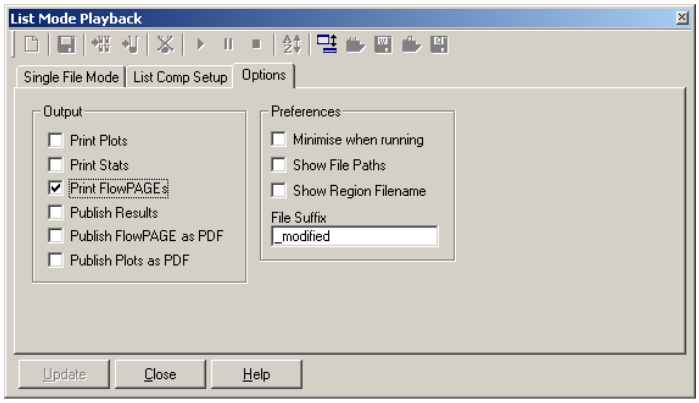


Retrieving Multiple Listmode Files with Different Protocols

1. Select **Tools >> Listmode Playback**.
2. Right click on  and select New Worklist.
3. Select  icon 3 times.
4. Select the folder icon in the first line of the LMD file column. And select a LMD file (ex. 2C CD3-CD4 002.LMD).
5. Repeat the process for lines 2 and 3 with different LMD files (ex. AS 5C 1L Verify CD 8-4-45-19-3 and AS 5C 2L Verify 8-4-3-45-19).

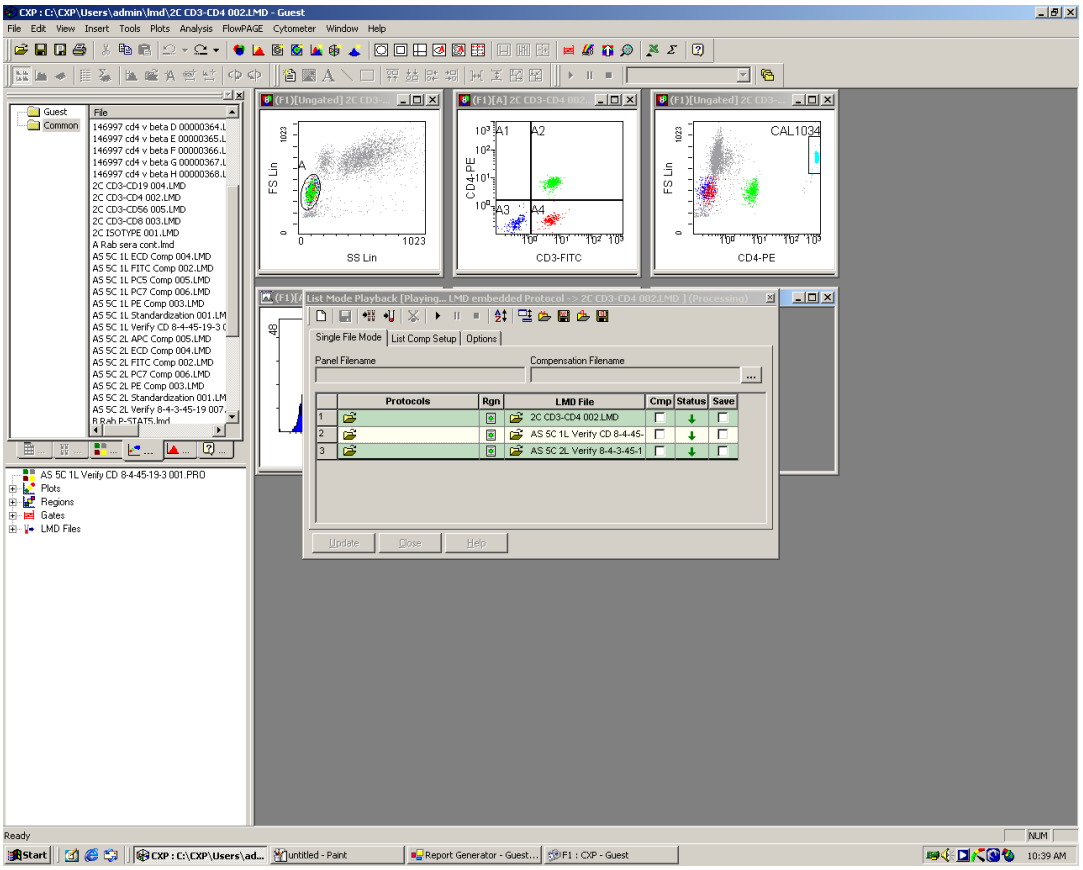


6. Select the Options tab and select the desired output options.



7. Select the Single File Mode tab.
8. Select desired Status for each listmode file (hourglass – pause, hand – stop, or green arrow – continue).

9. Select  icon. The system should play each listmode in turn with the runtime protocol.

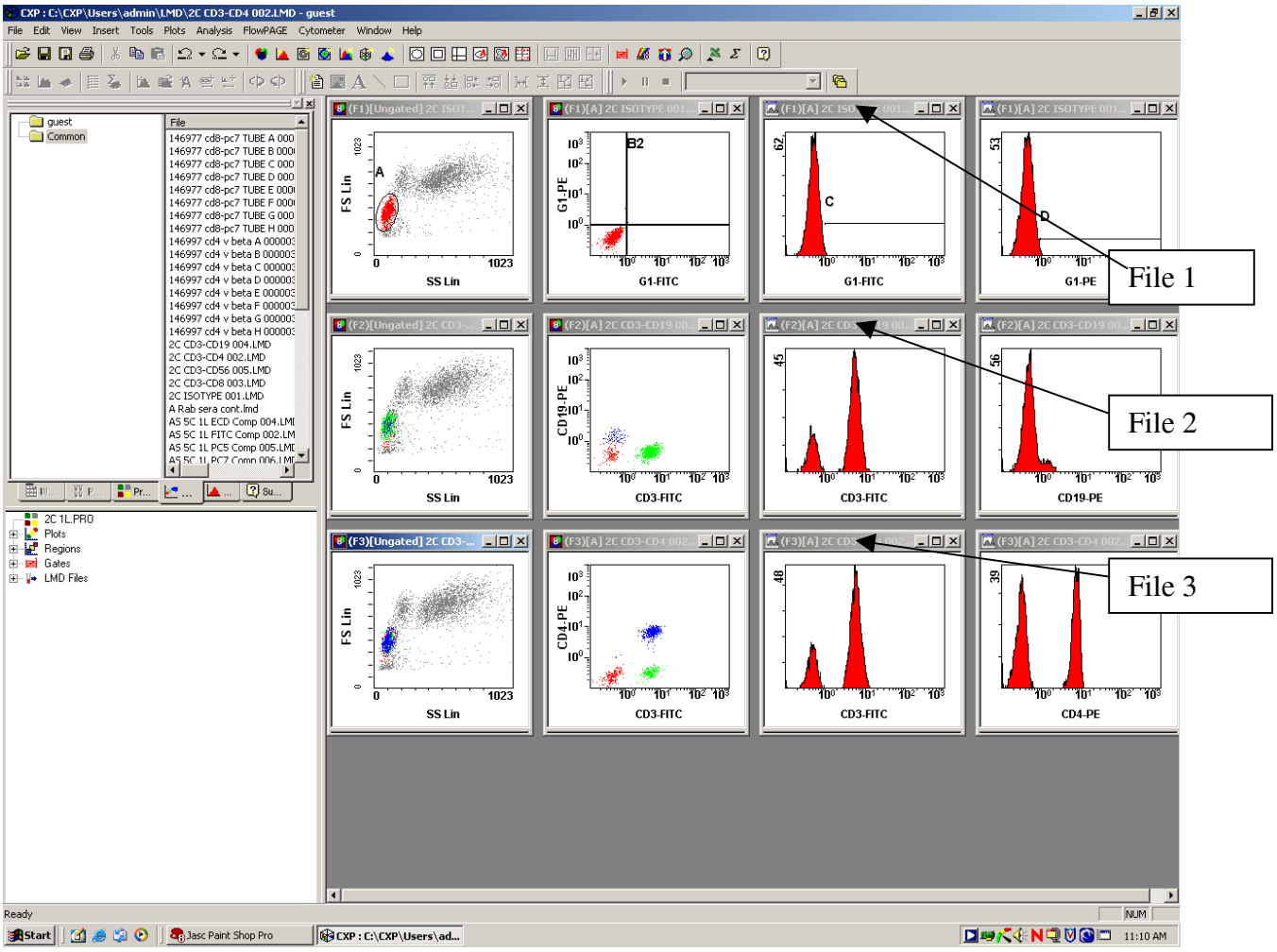


For additional information, select  at the top of the screen. Then select 10 USING CXP SOFTWARE >> 10.16 TOOLS MENU >> Listmode Playback.

Retrieving Multiple Listmode Files into one Protocol

1. Drag and Drop the desired protocol onto the workspace or create one.
2. Duplicate each plot for the number of listmode files (select desired plot and then Press **CTRL D**).
3. Move the plots roughly below each other (drag and drop). Do not worry about lining them up exactly yet.
4. Now Press **CTRL T,** select desired size, and then **Enter**. The plots should change size and line up.
5. Select the first listmode file and Drag and Drop it onto one of the plots. All the plots should populate.
6. Select the second listmode file.
7. While holding the **CTRL** key, drag and drop this file to the first plot on the second row. Repeat the process for all the rest of the plots in the second row.
8. Now repeat the process for all the plots in the next row using the third listmode file.
9. Repeat the process until all plots have been populated with desired listmode.

NOTE: If you made a mistake either correct it by dragging the correct file onto a plot with the CTRL key or drag the first list mode to any plot without the CTRL key and start over. A 3 Listmode example is shown on the following page.



Copying and Creating Linked Regions

NOTE: The gating is being carried to the new plots but not the regions. To duplicate the regions:

1. Drag from anywhere on the first dot plot to the one below it. A new region (or regions) will be created on the new plot with a different letter name.

2. Repeat the process for each plot in the second row and then the third row.

NOTE: These new regions are entirely independent of the old ones. If you hold the CTRL key while dragging regions, the region will also be placed on the gate list. This is true for all regions except Quadrant regions.

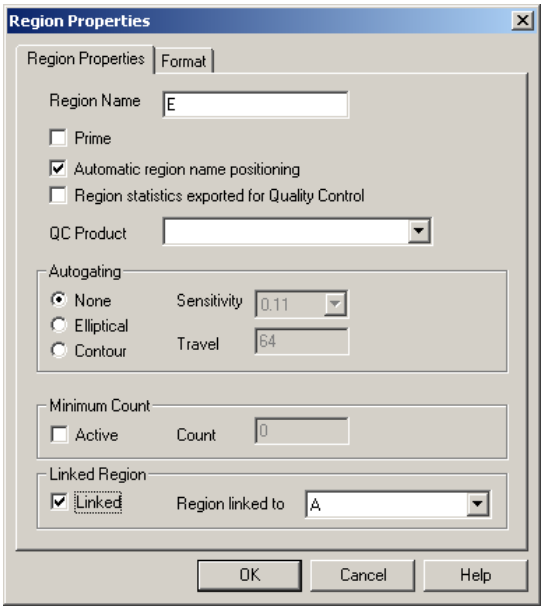
3. If you wish, you can gate the fluorescence plots in row 2 on region E and the fluorescence plots in row 3 on region I.

To Link Regions:

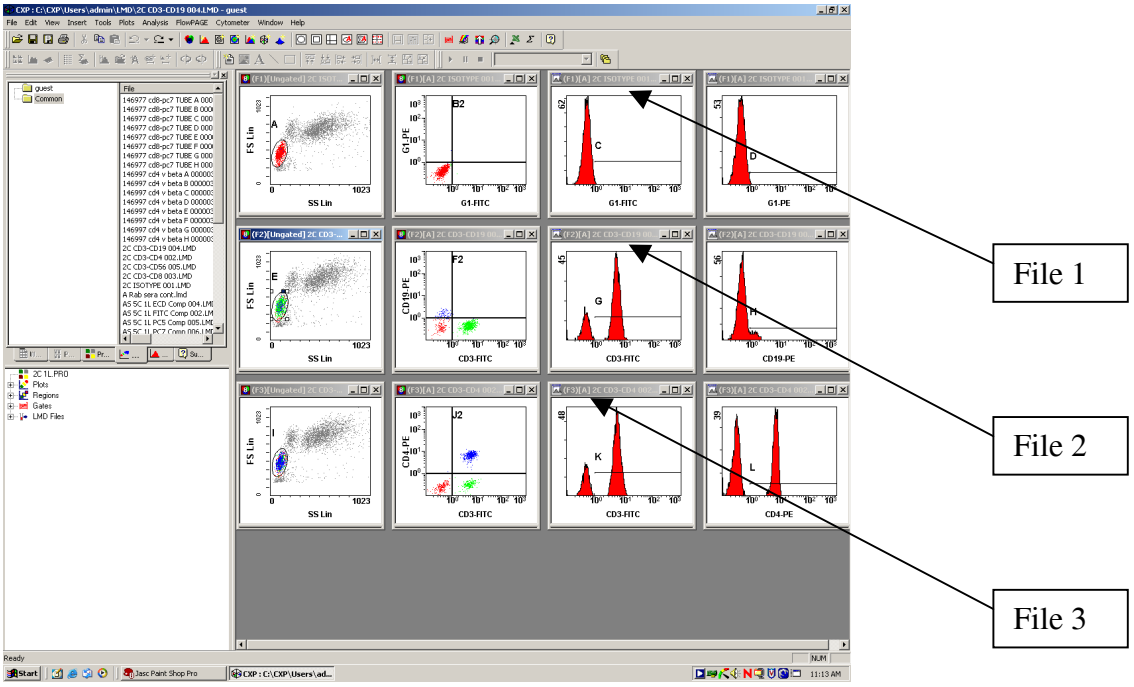
Regions can be linked between plots that are the same type and have the same parameters. These can include the same type of region and rectangular to quadrant. The regions can be linked even if the parameters are on opposite axes.

4. Select the region, right click and select Region Properties.

5. Select Linked, the region to link to, and then OK.



6. Save the protocol (i.e. 2C 3File.pro).



Batch AutoMATOR

Batch Automator allows for a batch of LMD files to be replayed through an individual protocol open in the Workspace.

Before you complete this part of the module, you may wish to view the Tour Guide called Automator.

NOTE: After you have a set of files on the Workspace, you can re-populate the files with a new set of files.

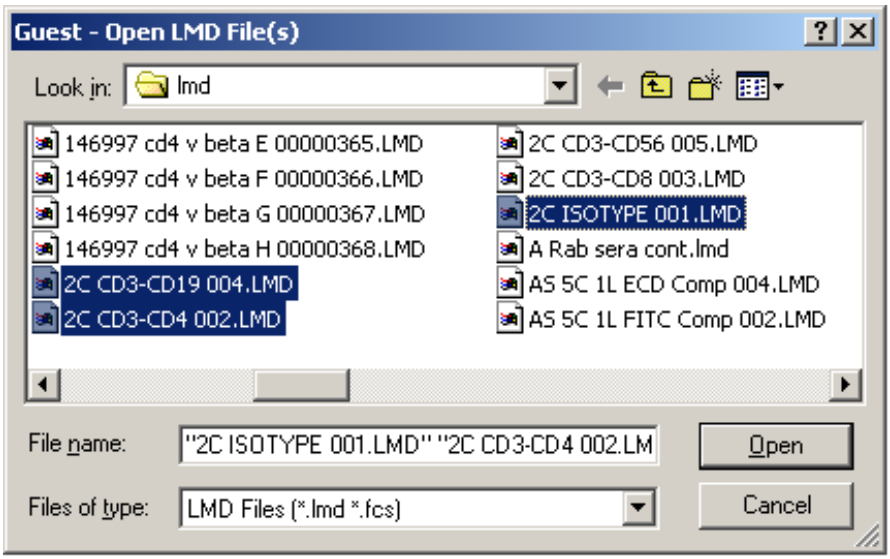
Setup

1. Select **Tools** >> **AutoMATOR Setup…** or the  button.

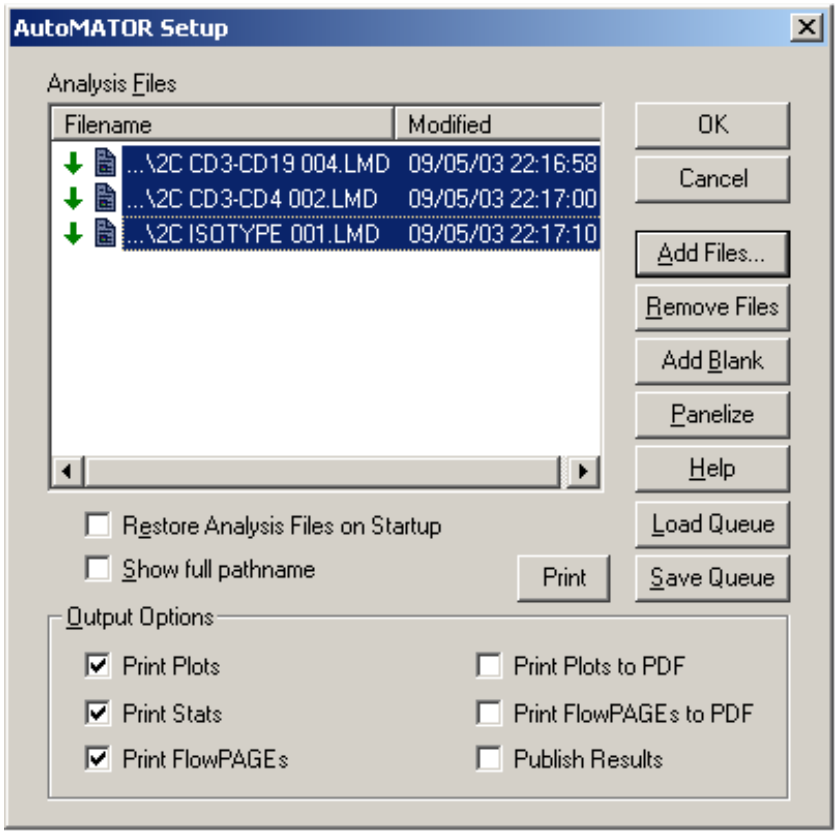
NOTE: Some files may be listed at this point but may not be in the order you wish to process them. To remove the existing files if present:

2. Select the files (use the Shift key to select them all) and then the **Remove Files** button.

3. Select **Add Files** button and select the desired sets of files and then select the **Open** button. An example is shown for 3 sets of 3 listmode files.



4. Your AutoMATOR Setup screen should look like the following (If not just repeat the process):



NOTE: The icons on the left side can be toggled through three positions:

pass through without stopping.

pause before continuing.

stop.

To Change the order of the files

5. Select the file and then drag and drop it to a new position.

To change all at the same time:

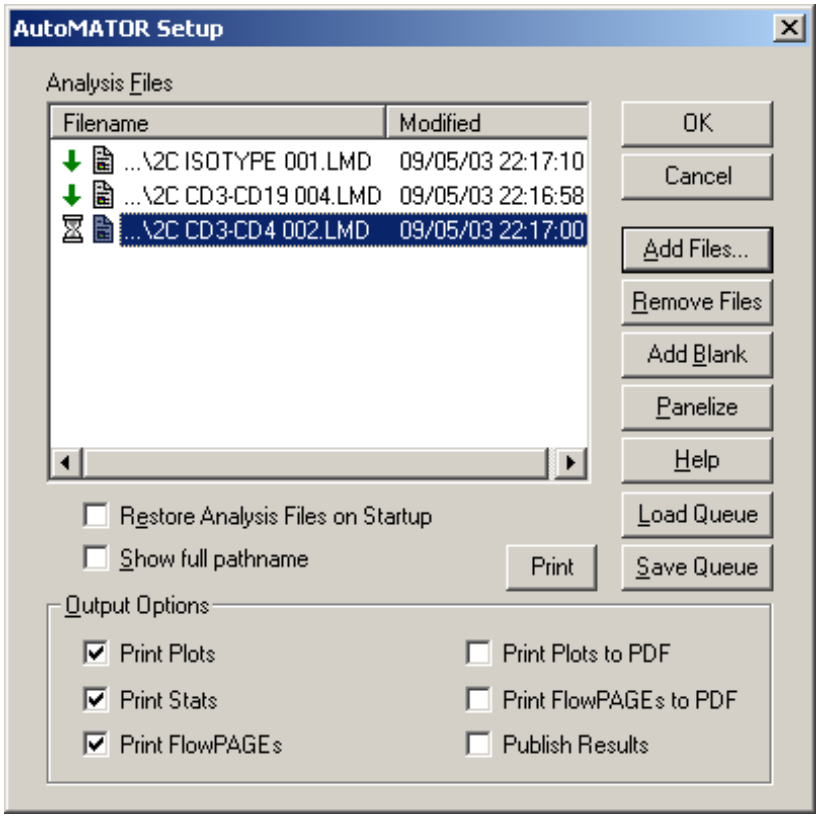
6. With all files highlighted click several times on one of the left icons make the changes then return back to the ↓ icon.

To change only one file icon:

7. First select one of the listmode files to remove the window selection of all files and then toggle the icons you wish to change.

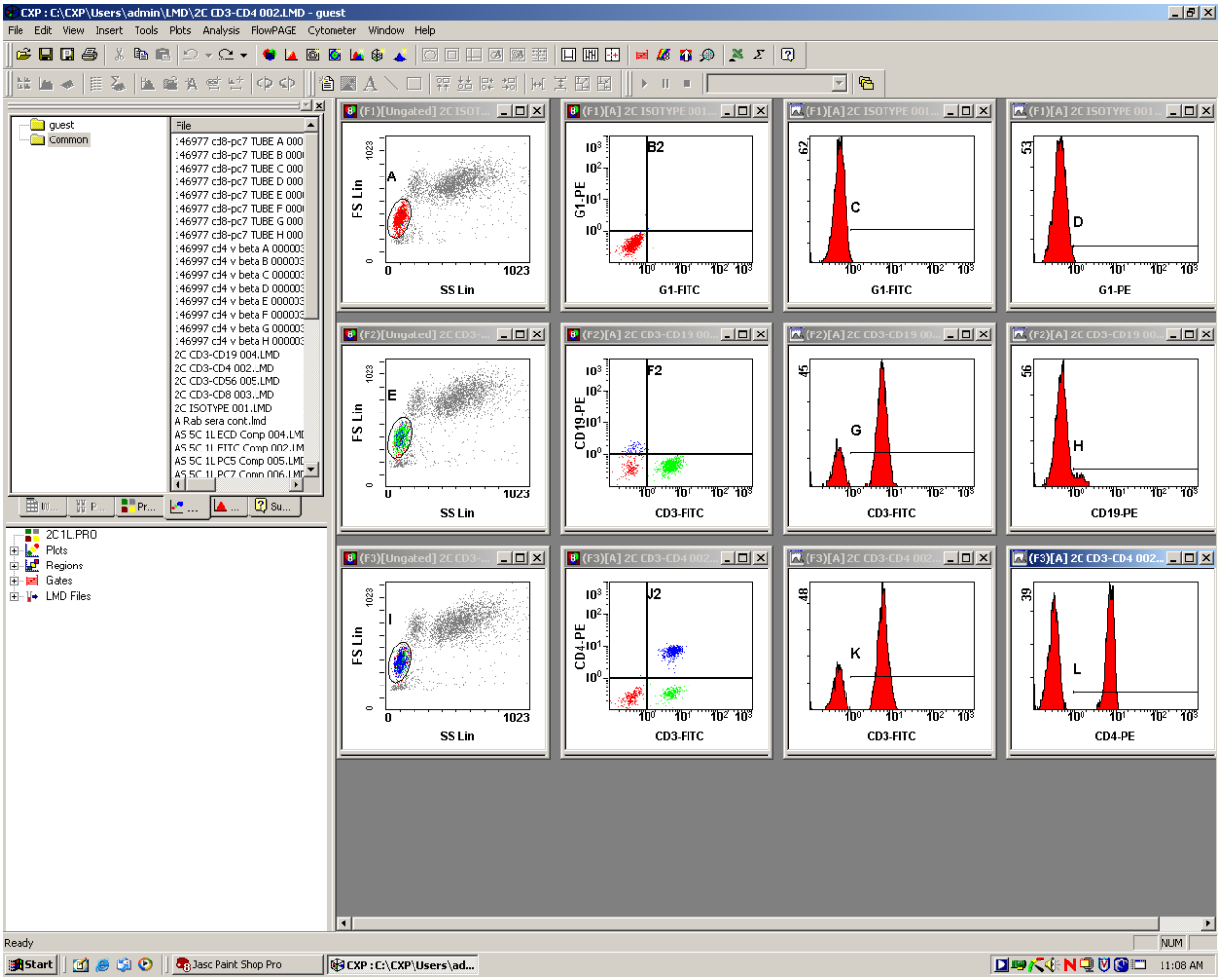
NOTE: The icon indicates output will occur after this file.

8. Select the desired output option and then **OK**.



9. Select the  button on the AutoMATOR. The system should populate the Workspace with the first set of files and then Pause for 15 seconds (if this icon has been selected) to give you time to make changes.

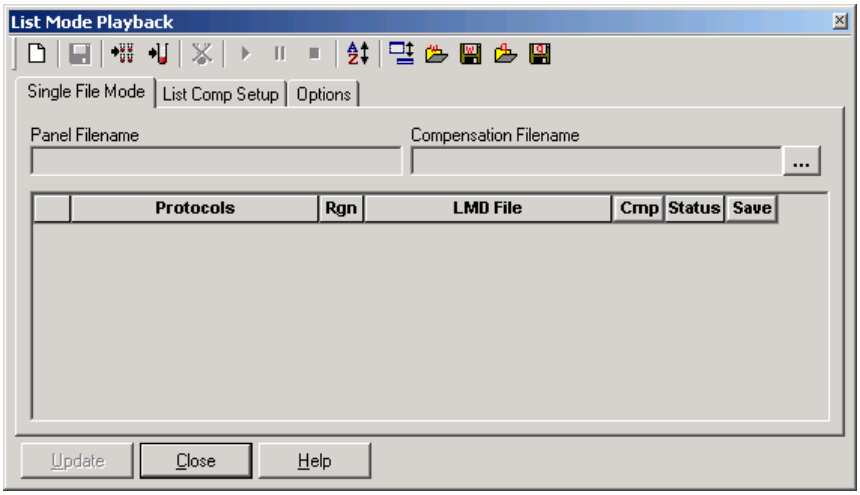
10. You can wait or select the  button to continue. Once all files in the first set have been replayed, the system should print the results (if you requested this option) and repeat the process with the next set of files. The system continues until all files have been processed. The last set remains visible on the screen.



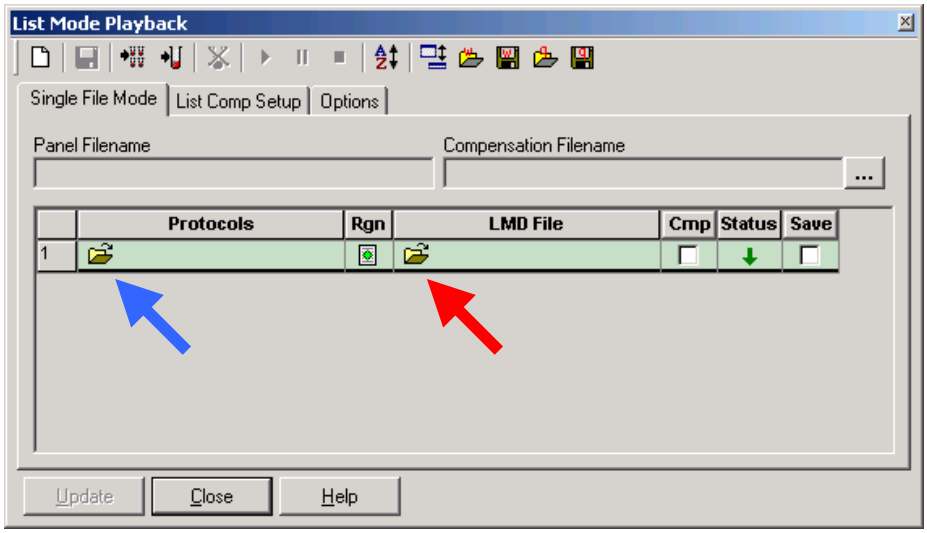
Listmode Playback Tool

The listmode playback tool allows you to playback a listmode file with the runtime protocol/panel (or with a new protocol/panel). The tool also allows you to make changes, such as change the CAL factor or compensation settings or replay a panel to produce a panel report (The latter option will be demonstrated in the Database module).

1. Select **Tools** >> **Listmode Playback**. The listmode playback tool window appears.

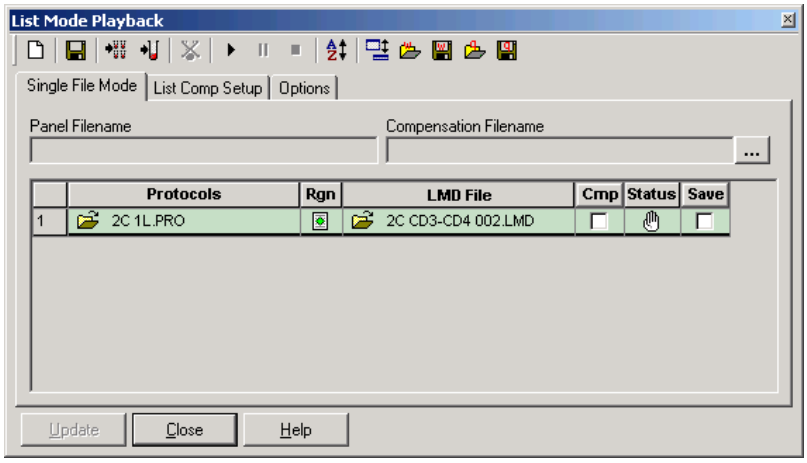


2. Select either the protocol icon  or the panel icon  (the tube icon is shown below).

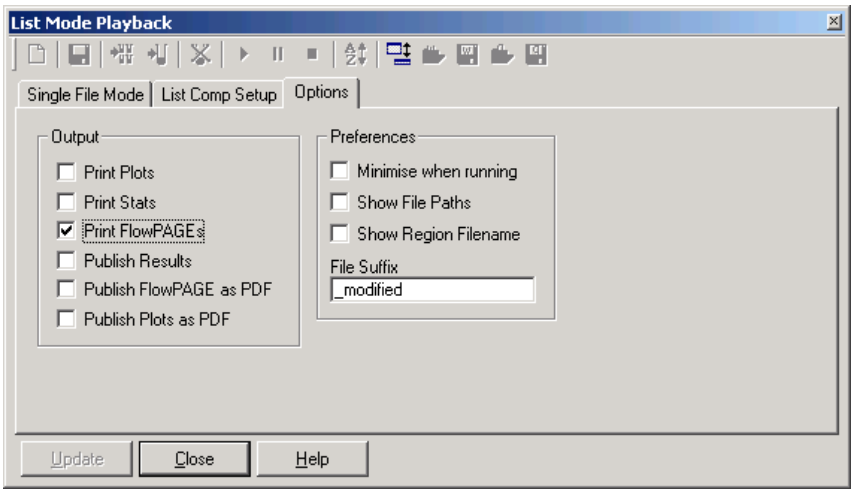


3. Select the File tab for the listmode (red arrow) and then the desired listmode file). If you also select the protocol file folder (blue arrow) and a protocol, the system will play the file through a new protocol otherwise it will play the file with the runtime protocol.

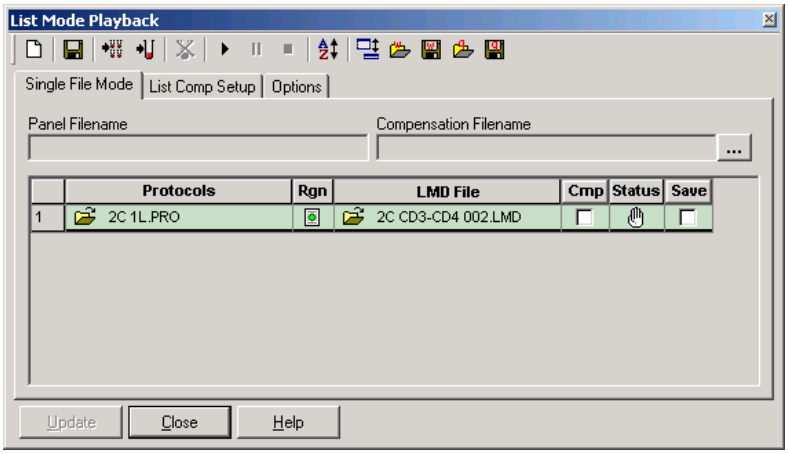
4. The window will now appear as below.



5. Select the Options tab and choose the output options.



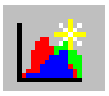
6. Select the Single File Mode tab and then the Run icon  to play the file. The file will playback according to your setup. For more information, refer to the Online Help.



Setting Up Overlay Plot

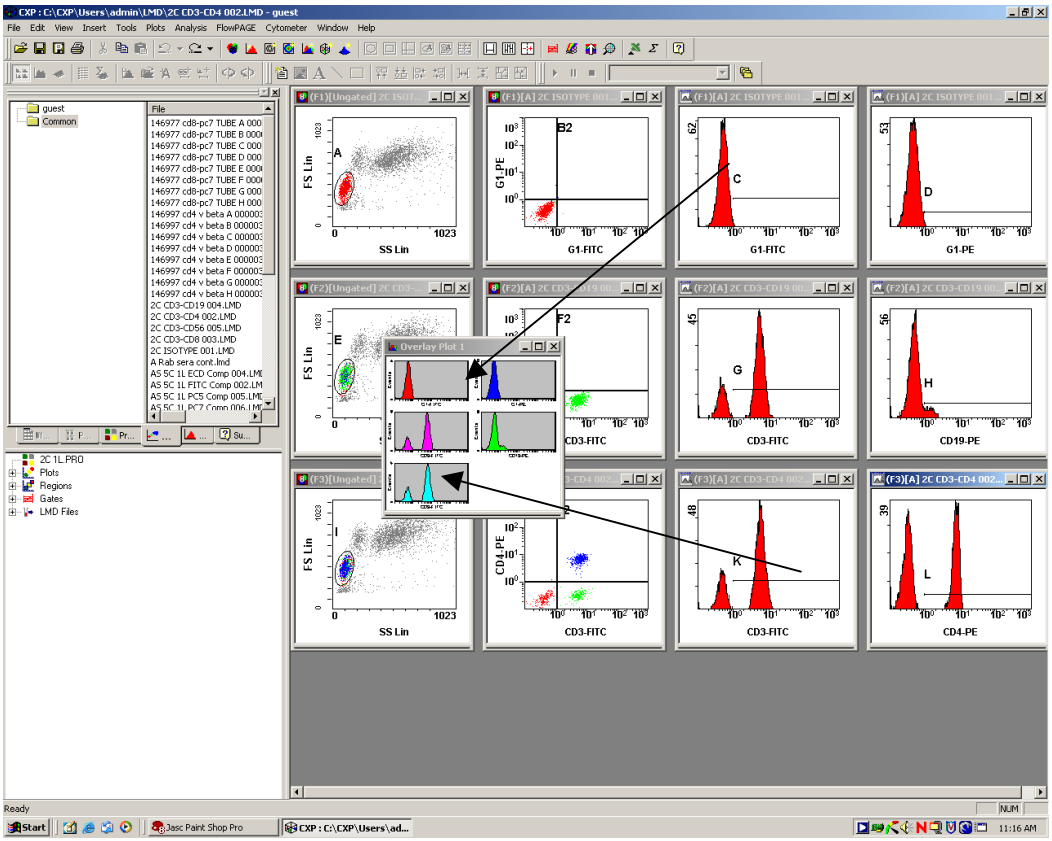
NOTE: Before you complete this part of the module, you may wish to view the Tour Guide called Overlay.

Populating overlay from Workspace

1. Select the  button on the toolbar.

2. Move the plot to a convenient location on the screen.

3. If you already have histogram plots on the Workspace, you can drag and drop them onto the Overlay Plot window.



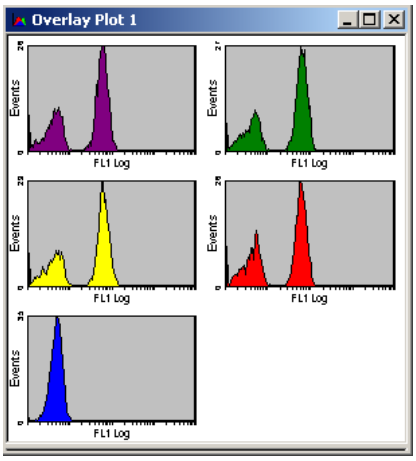
To Delete Plots on the Overlay

1. Click on the plot within the overlay window and press the **Delete** key.

2. You can delete them all by first selecting the Overlay window, then Pressing **CTRL A**, and then pressing the **Delete** key.

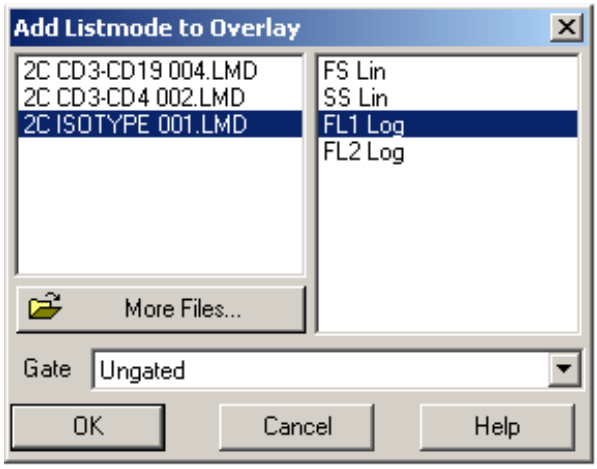
Populating Overlay from Histogram files

1. Select the tab on the Resource Explorer.
2. Select Desired path to histogram files (e.g. Common, Guest etc).
3. Select desired file or use the **Shift** key to select several in a row or Ctrl key to select individual files.
4. Drag and Drop Files onto the overlay plot.

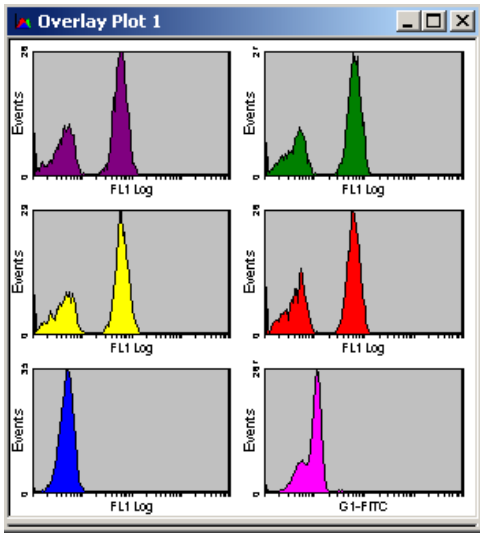


Populating Overlay from Listmode Files

1. Select the button on the Overlay toolbar. If all the listmode files are not visible, select the More Files… button.
2. Use the Shift key as you select several files. A list of parameters common to all the files appears.



3. Select one or more parameters (e.g. FL1 LOG), and then OK. The system adds these files to the Overlay plot.

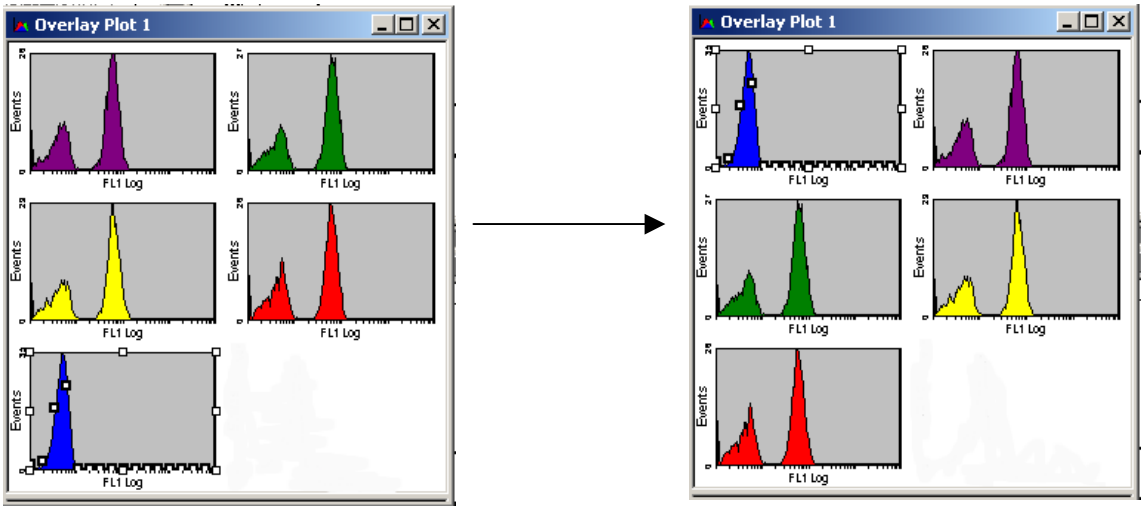


Arranging Plots in an Overlay

Moving Individual Plots in the Overlay Window

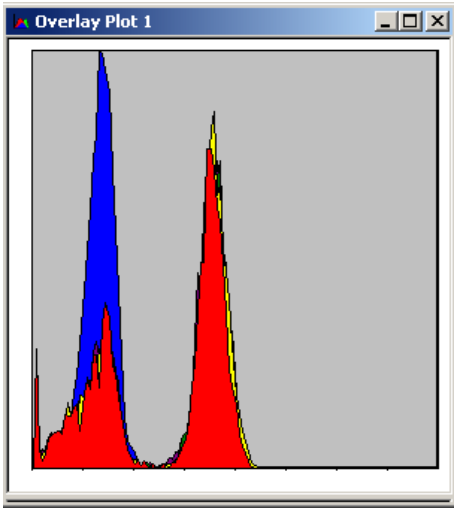
1. Click on a plot you wish to move. Boxes should appear around the plot.

2. Drag and drop the plot to the new location.



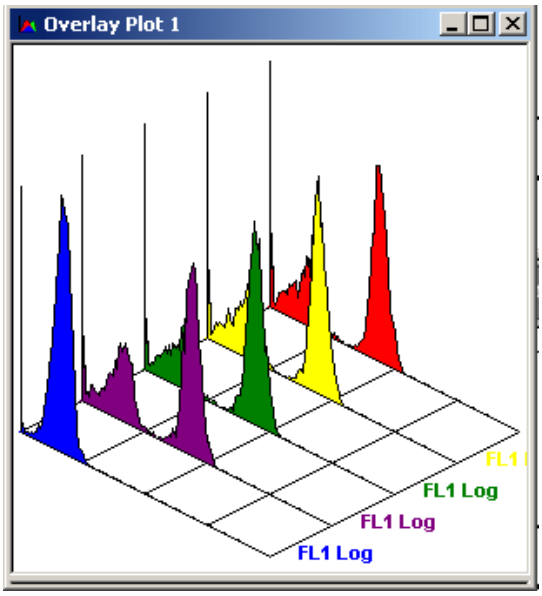
Superimposing Plots

1. Select the  button. The plots should superimpose on top of each other.



To Angle Plots

1. Select the  button. The plots should display angled.

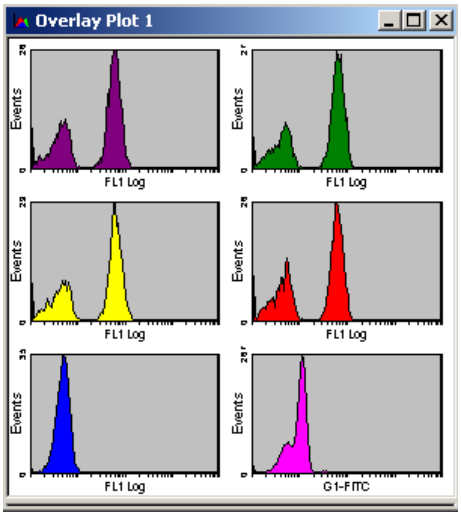


NOTE: The angled mode actives the rotation option.

2. Select either one of the  buttons. The whole angled plot should rotate in the direction indicated on the button.

To Display in Gallery Mode

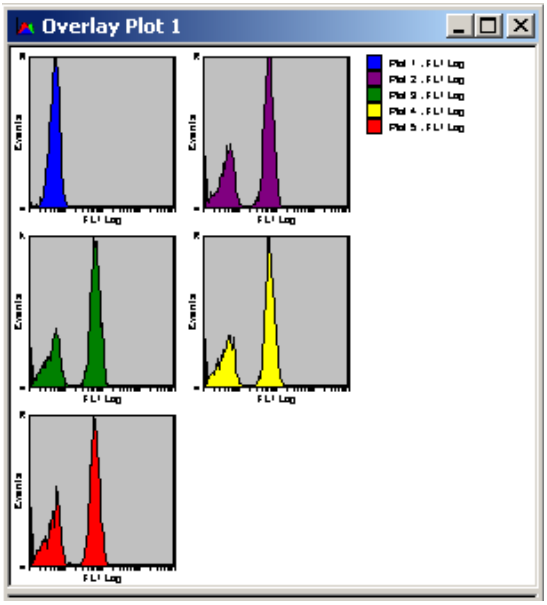
1. Select the  button. The window should now separate out the individual plots as before.



Additions to the Overlay

To Add a Legend

1. Select the  button. A Legend should be added to the plot.

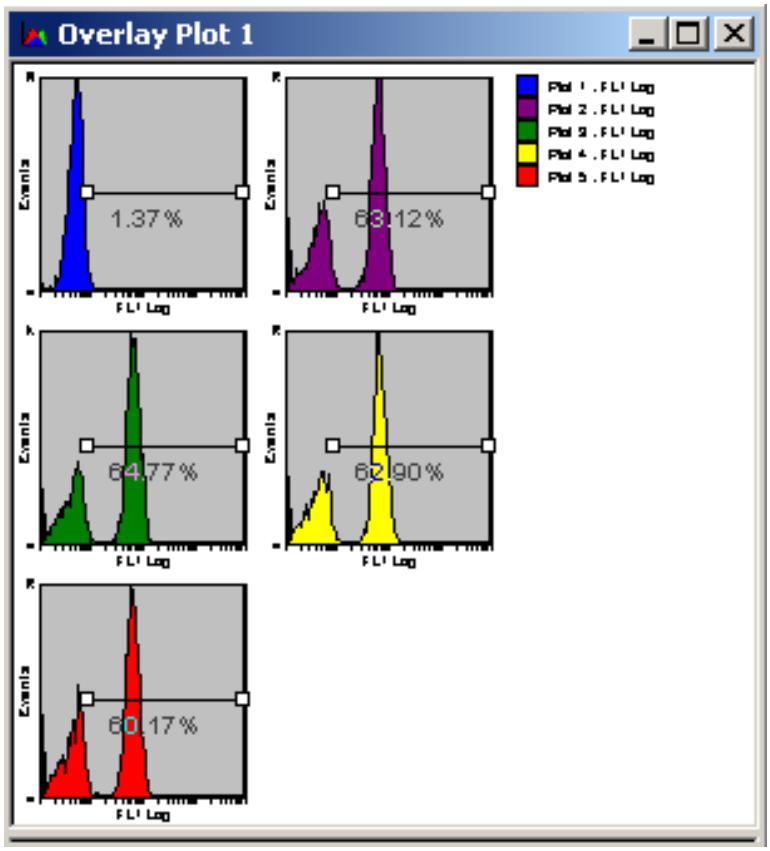


To Add Regions to the Plot

1. Select the  button. Linear regions appear on each of the plots within the Overlay window. They are all linked together.

2. Use the handles on either end of one of the regions to move and stretch the region to the desired position.

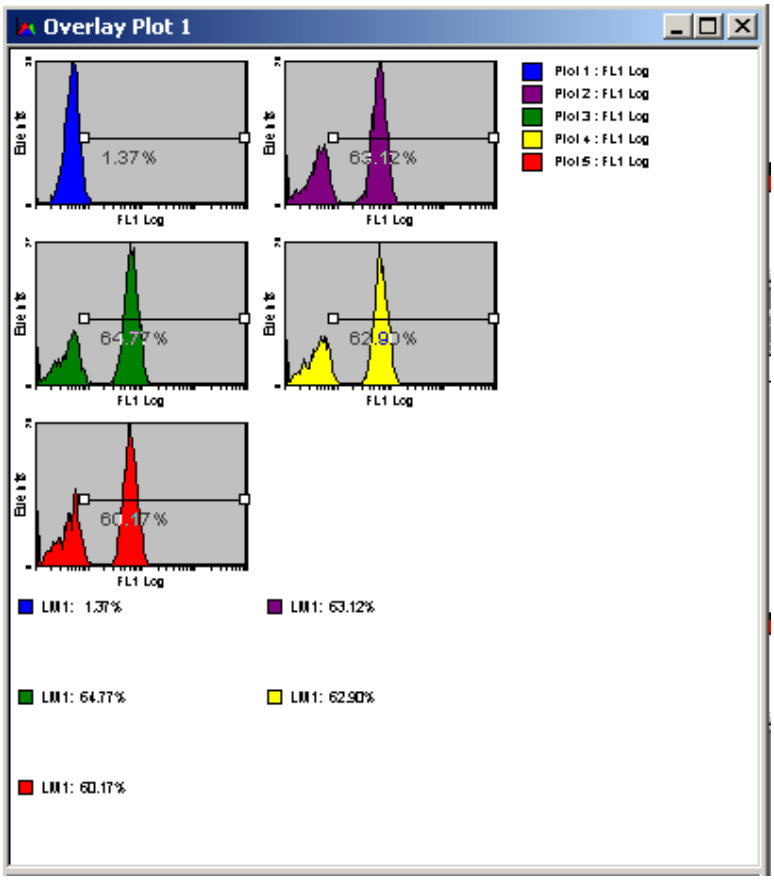
NOTE: Notice that all the regions on all the plots within the overlay move as well because they are linked together.



3. The  button can be used to create a single region on one plot.

Adding Statistics

1. Select the  button. The stats are now displayed below the plots.



You can also arrange plots by deviation (lowest % to the highest), by stain, or by parameter.

1. Right click on the Overlay plot and then select **Arrange**.

2. Select desired option.

To Add Annotation

1. We suggest maximizing the plot image (use the  button on the Overlay window) before continuing.

2. Select the  button.

3. Move the cursor to the desired position and click. The annotation box appears.

To Change the Box Size

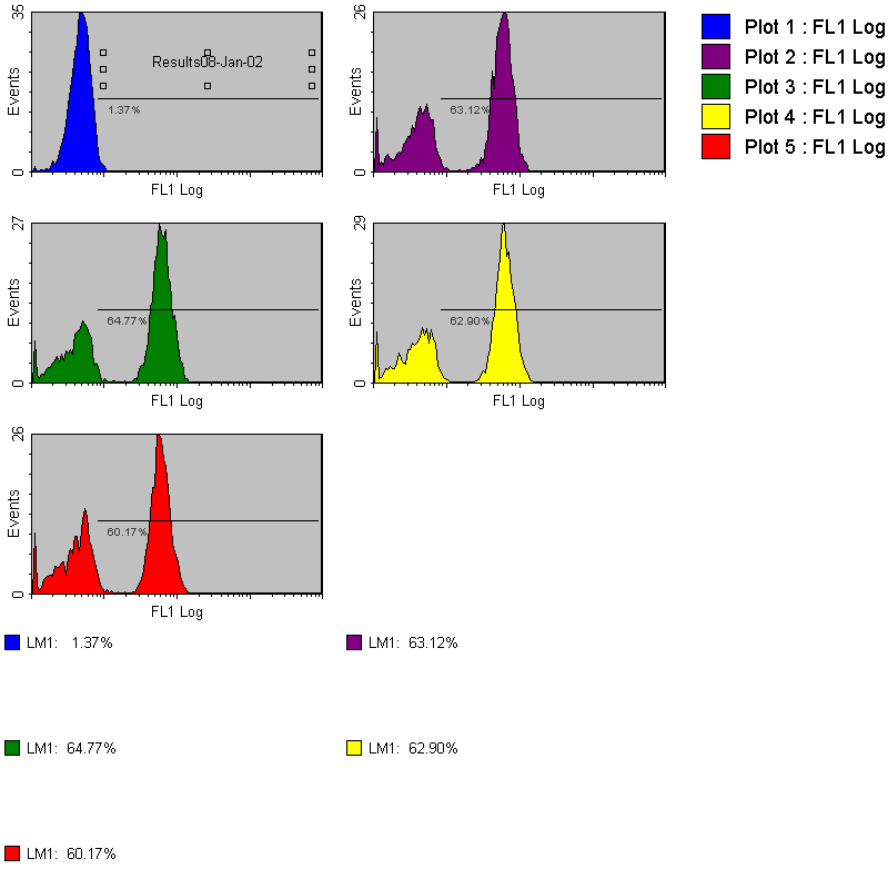
1. Click on the annotation until the handle boxes appear around it. You may now grab one of the handles and stretch the box to the desired size.

To Add Text

1. Click until the text cursor appears within the annotation box and then edit the text within the box (example: change from annotation to Results).

2. Now add the $ sign after the word. A list of possible values will appear. Select the desired information to be added to the text.

3. Double Click outside the annotation box to close it and display the text.

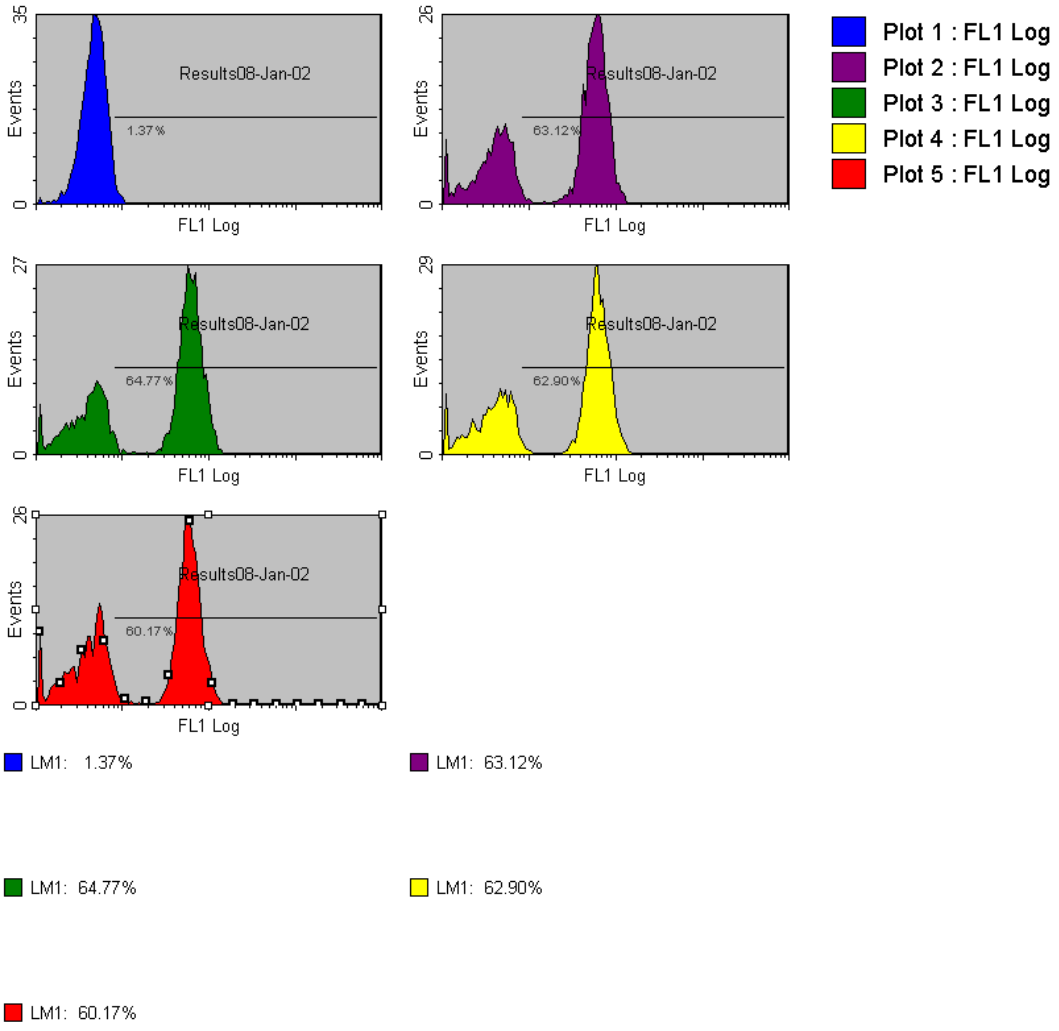


NOTE: If the entire text is not seen, you can click on the annotations box. Handles should appear which can be adjusted to resize the box.

To Copy the Annotations box to other plots

1. Select the annotations box. The adjustment handles appear.
2. Now right click outside the box and then Cut. The box disappears.
3. Press CTRL A, right click, and select Paste. The results should now appear on all the plots.

NOTE: If you wish to apply the same annotations box to only some but not all the plots, use the Copy option after selecting the annotation and the Paste option after selecting the destination plot.

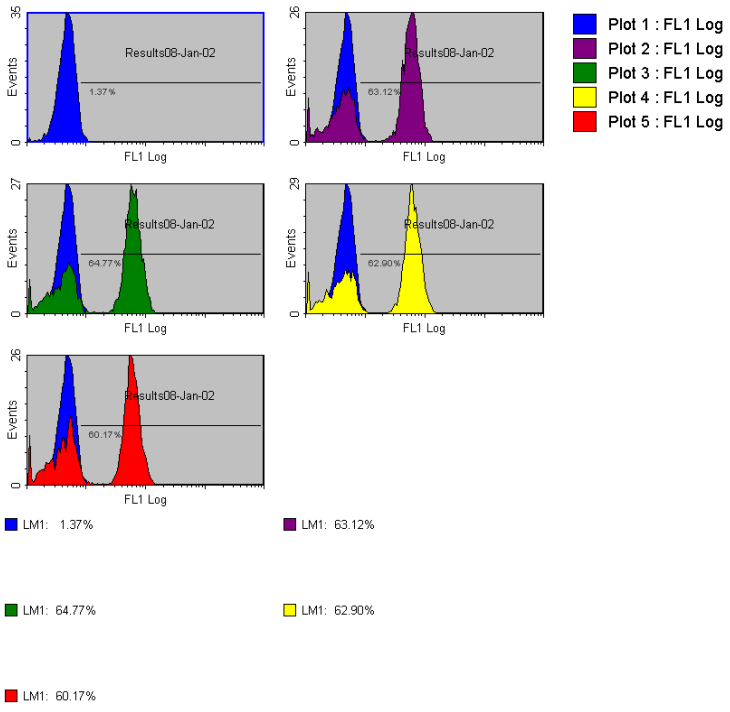


Comparing to a Control Plot

Assigning the Control Plot

1. Click on the control plot within the overlay.

2. Right click the mouse on the Overlay Plot and select **Set As Control** option. The control plot now is superimposed on top of all the other plots.



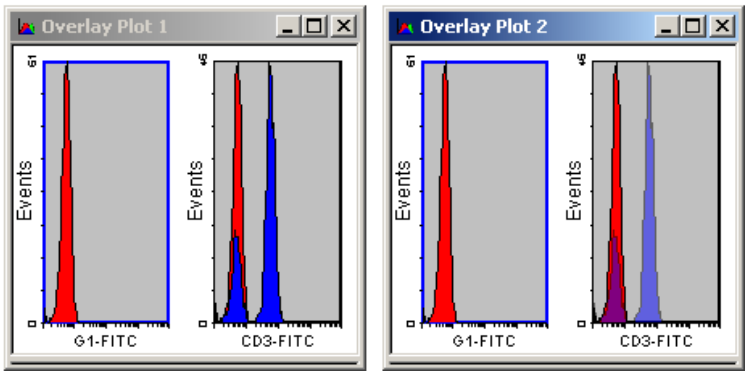
NOTE: In a similar manner, the control can also be removed or placed on top or behind the other plot.

To Move the Control to the Front

Click on the control plot, then right click, and then select **Control in front**. The control should now be on top. Deselect the option to place the test on top.

To Apply Translucency

Click outside of the plots to deselect plots, then right click, and then select Translucency. The coloring changes. Right click again and deselect the option to turn it off.



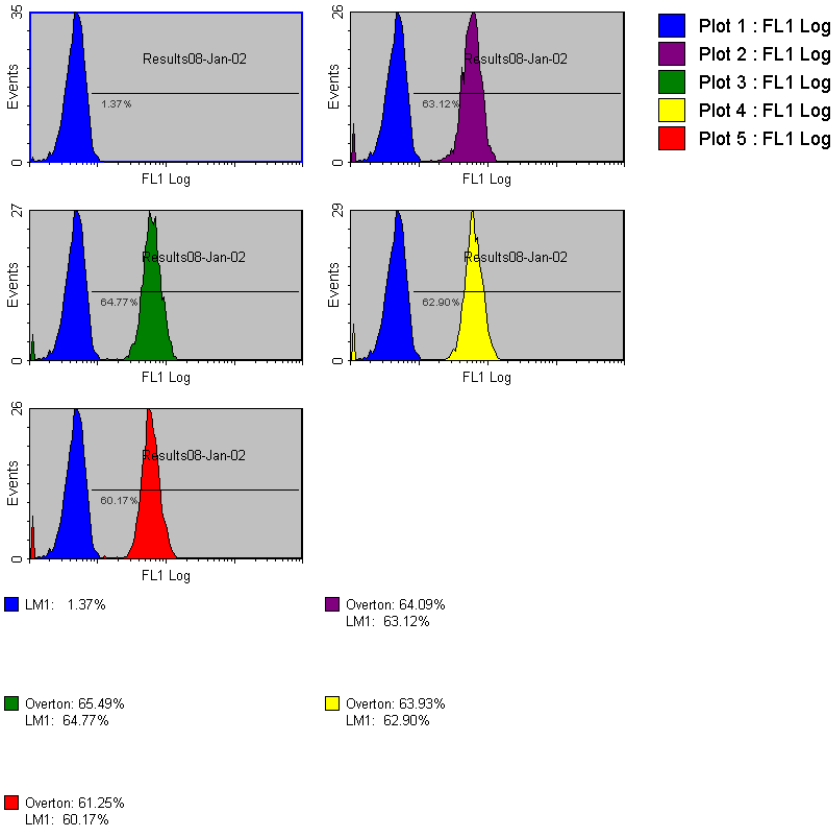
To Apply Overton Mode Subtraction

This mode calculates the percent positives by subtracting an isotypic control data from the test data.

1. Without any plots selected, right click on the Overlay Plot.

2. Now select **Overton Mode**.

3. Select the  button. The system subtracts the control plot from all the others per the Overton subtraction technique and displays the results underneath the plots.



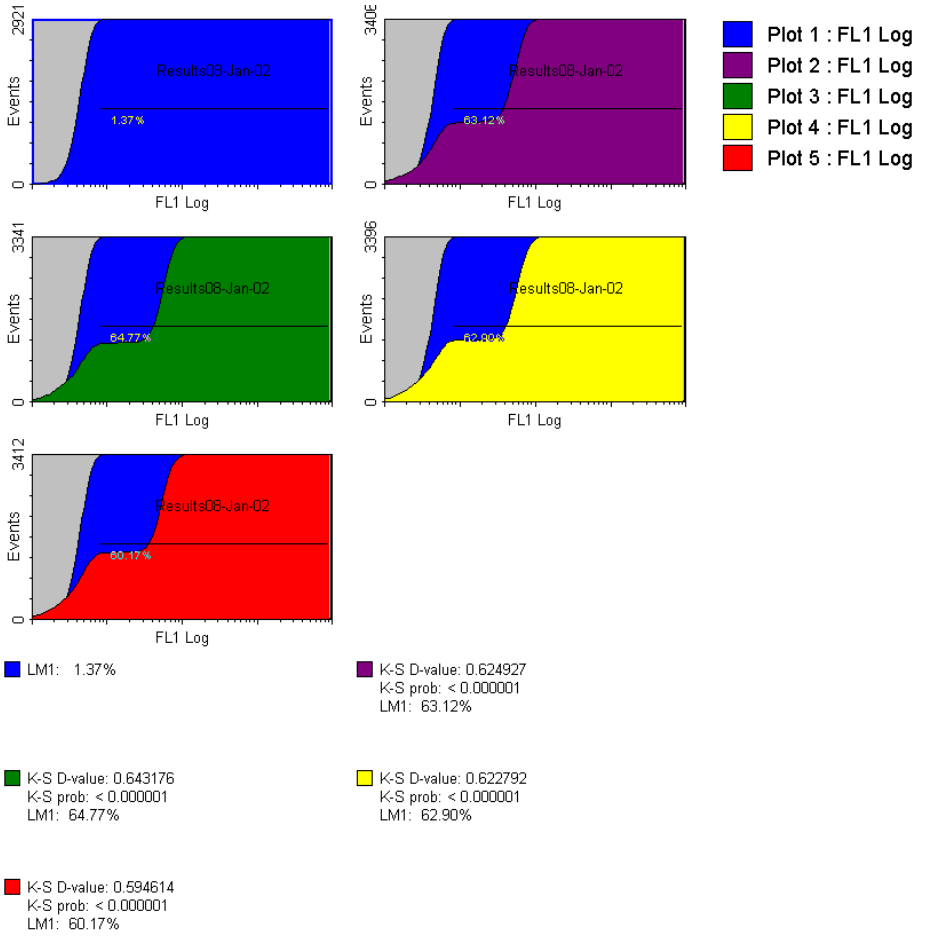
To Apply Kolmogorov-Smirnov Mode Comparison

This mode compares the data to a control. It might be used, for example, to get results of a kappa/lambda comparison.

7. Without any plots selected, right click on the Overlay Plot.

8. Now select the **Kolmogorov-Smirnov Mode**.

9. If the  button has not been selected, select it. The results of the Kolmogorov-Smirnov test should appear underneath the plots. For more information, select On-line Help.



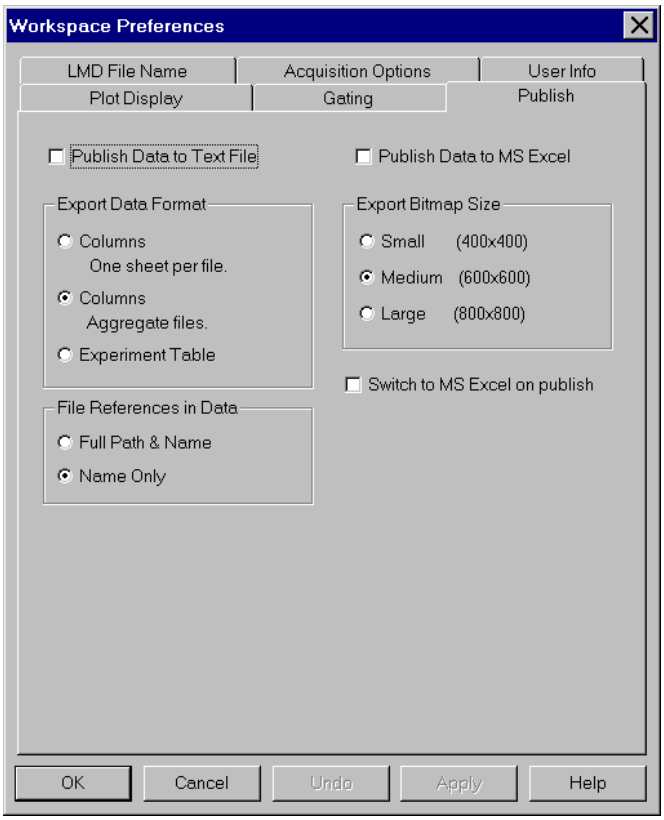
Publishing

The CXP software has been set up to output data directly to Microsoft Excel and as a *.txt file, which can be read by the Excel program. Microsoft Excel must be loaded on the computer to perform the following exercises.

NOTE: Before completing this part of the module, you may wish to view the Tour Guide called Publishing.

Setting Preferences

1. Press **CTRL W** and then select the Publish tab.



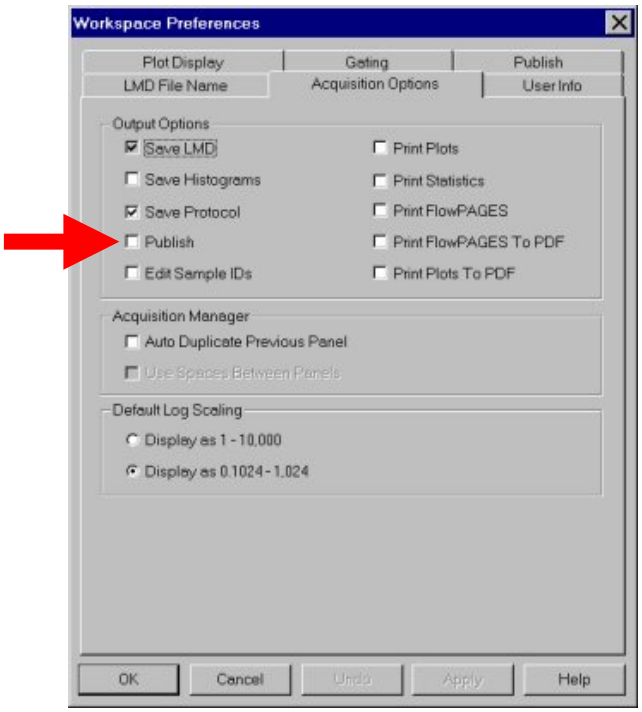
2. Set the system to publish the results to a text file (Publish Data to Text File) and open the Excel program and display the results (Publish Data to MS Excel).

3. Select the desired Export Data Format (examples are shown on the following pages).

4. Select the desired File References option and then select **OK**.

5. Optional: Select Acquisition Options tab and click on Publish. The system will automatically publish the results of all samples when the data collection is stopped.

NOTE: If you do not wish to publish, turn this option off, otherwise all data, including the cleaning panel, will be published.

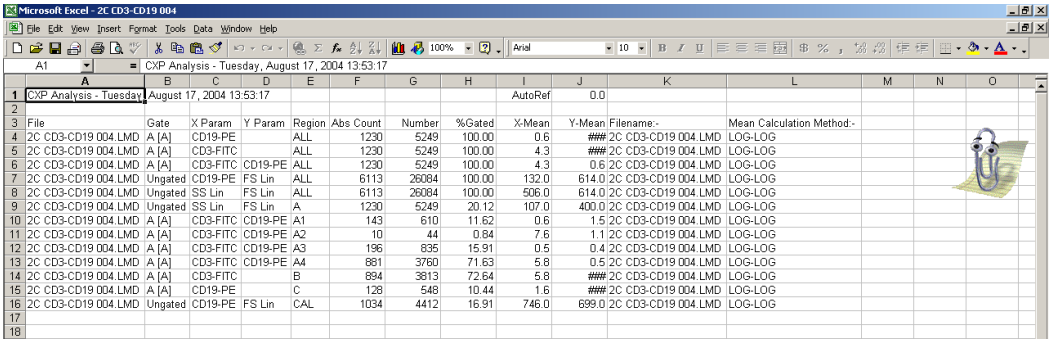


Publishing to Excel

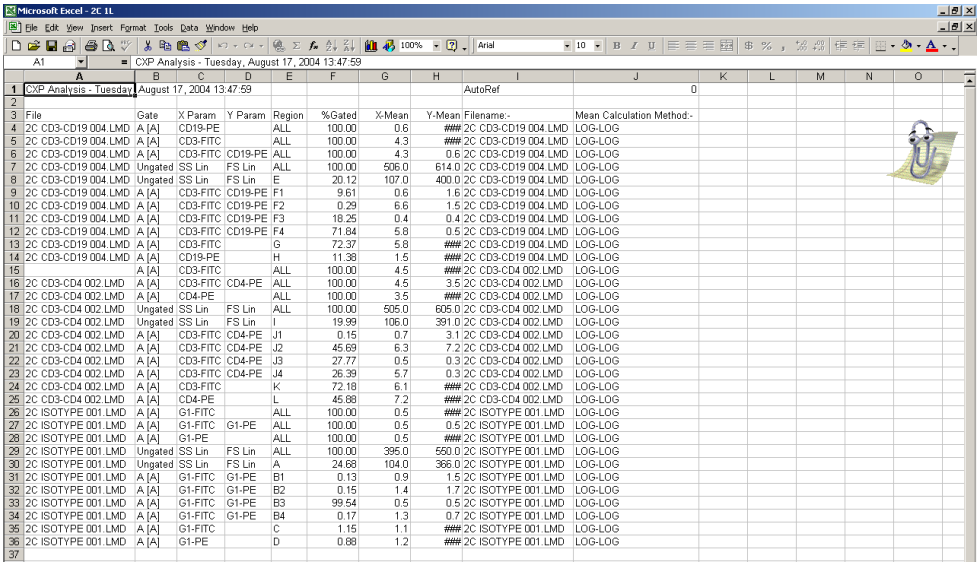
1. Select **Tools** >> **Publish Results Now…** or select the  button or press **F7**.

2. Type in a name for the file and then select **Save**. The system opens MS Excel and displays the data in the export format you requested.

One Sheet Per File

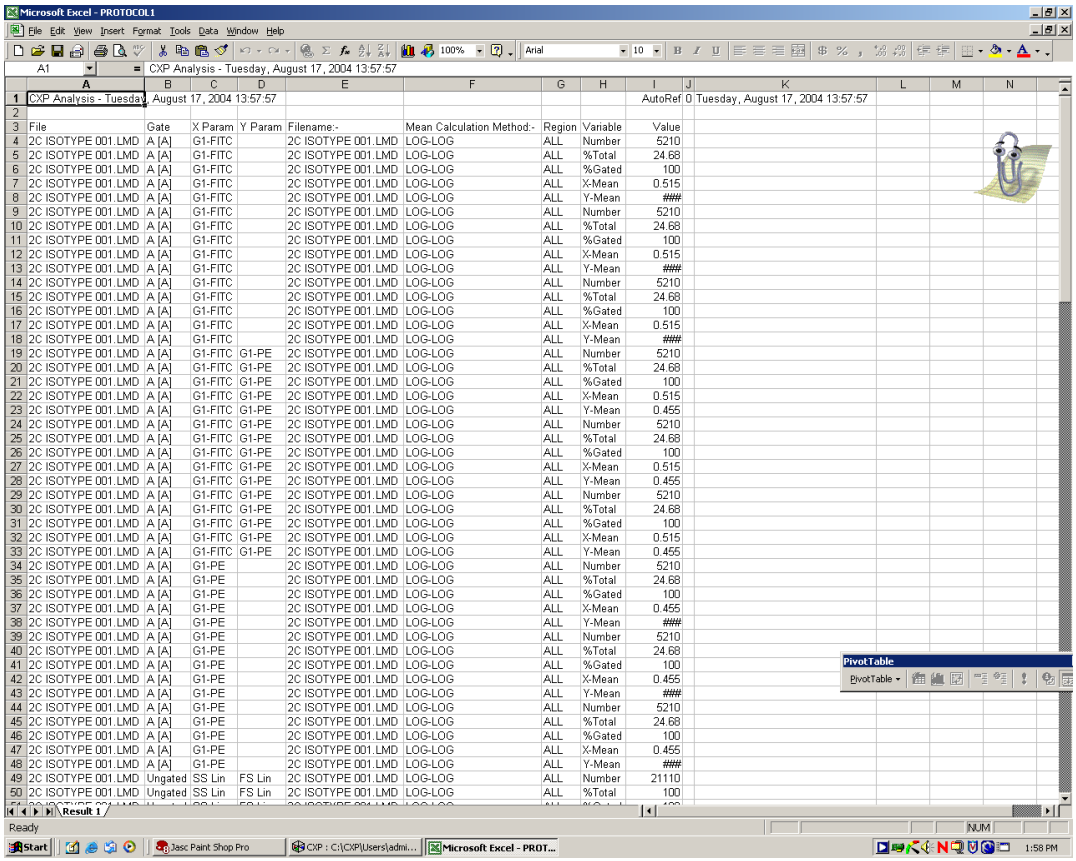


Columns Aggregate Files



	A	B	C	D	E	F	G	H	I	J
1	CXP Analysis - Tuesday	August 17, 2004 13:47:59							AutoRef	0
2										
3	File	Gate	X Param	Y Param	Region	%Gated	X-Mean	Y-Mean	Filename:-	Mean Calculation Method:-
4	2C CD3-CD19 004.LMD	A [A]	CD19-PE		ALL	100.00	0.6	###	2C CD3-CD19 004.LMD	LOG-LOG
5	2C CD3-CD19 004.LMD	A [A]	CD3-FITC		ALL	100.00	4.3	###	2C CD3-CD19 004.LMD	LOG-LOG
6	2C CD3-CD19 004.LMD	A [A]	CD3-FITC	CD19-PE	ALL	100.00	4.3	0.6	2C CD3-CD19 004.LMD	LOG-LOG
7	2C CD3-CD19 004.LMD	Ungated	SS Lin	FS Lin	ALL	100.00	506.0	614.0	2C CD3-CD19 004.LMD	LOG-LOG
8	2C CD3-CD19 004.LMD	Ungated	SS Lin	FS Lin	E	20.12	107.0	400.0	2C CD3-CD19 004.LMD	LOG-LOG
9	2C CD3-CD19 004.LMD	A [A]	CD3-FITC	CD19-PE	F1	9.61	0.6	1.6	2C CD3-CD19 004.LMD	LOG-LOG
10	2C CD3-CD19 004.LMD	A [A]	CD3-FITC	CD19-PE	F2	0.29	6.6	1.5	2C CD3-CD19 004.LMD	LOG-LOG
11	2C CD3-CD19 004.LMD	A [A]	CD3-FITC	CD19-PE	F3	18.25	0.4	0.4	2C CD3-CD19 004.LMD	LOG-LOG
12	2C CD3-CD19 004.LMD	A [A]	CD3-FITC	CD19-PE	F4	71.84	5.8	0.5	2C CD3-CD19 004.LMD	LOG-LOG
13	2C CD3-CD19 004.LMD	A [A]	CD3-FITC		G	72.37	5.8	###	2C CD3-CD19 004.LMD	LOG-LOG
14	2C CD3-CD19 004.LMD	A [A]	CD19-PE		H	11.38	1.5	###	2C CD3-CD19 004.LMD	LOG-LOG
15		A [A]	CD3-FITC		ALL	100.00	4.5	###	2C CD3-CD4 002.LMD	LOG-LOG
16	2C CD3-CD4 002.LMD	A [A]	CD3-FITC	CD4-PE	ALL	100.00	4.5	3.5	2C CD3-CD4 002.LMD	LOG-LOG
17	2C CD3-CD4 002.LMD	A [A]	CD4-PE		ALL	100.00	3.5	###	2C CD3-CD4 002.LMD	LOG-LOG
18	2C CD3-CD4 002.LMD	Ungated	SS Lin	FS Lin	ALL	100.00	505.0	605.0	2C CD3-CD4 002.LMD	LOG-LOG
19	2C CD3-CD4 002.LMD	Ungated	SS Lin	FS Lin	I	19.99	106.0	391.0	2C CD3-CD4 002.LMD	LOG-LOG
20	2C CD3-CD4 002.LMD	A [A]	CD3-FITC	CD4-PE	J1	0.15	0.7	3.1	2C CD3-CD4 002.LMD	LOG-LOG
21	2C CD3-CD4 002.LMD	A [A]	CD3-FITC	CD4-PE	J2	45.69	6.3	7.2	2C CD3-CD4 002.LMD	LOG-LOG
22	2C CD3-CD4 002.LMD	A [A]	CD3-FITC	CD4-PE	J3	27.77	0.5	0.3	2C CD3-CD4 002.LMD	LOG-LOG
23	2C CD3-CD4 002.LMD	A [A]	CD3-FITC	CD4-PE	J4	26.39	5.7	0.3	2C CD3-CD4 002.LMD	LOG-LOG
24	2C CD3-CD4 002.LMD	A [A]	CD3-FITC		K	72.18	6.1	###	2C CD3-CD4 002.LMD	LOG-LOG
25	2C CD3-CD4 002.LMD	A [A]	CD4-PE		L	45.88	7.2	###	2C CD3-CD4 002.LMD	LOG-LOG
26	2C ISOTYPE 001.LMD	A [A]	G1-FITC		ALL	100.00	0.5	###	2C ISOTYPE 001.LMD	LOG-LOG
27	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	ALL	100.00	0.5	0.5	2C ISOTYPE 001.LMD	LOG-LOG
28	2C ISOTYPE 001.LMD	A [A]	G1-PE		ALL	100.00	0.5	###	2C ISOTYPE 001.LMD	LOG-LOG
29	2C ISOTYPE 001.LMD	Ungated	SS Lin	FS Lin	ALL	100.00	395.0	550.0	2C ISOTYPE 001.LMD	LOG-LOG
30	2C ISOTYPE 001.LMD	Ungated	SS Lin	FS Lin	A	24.68	104.0	366.0	2C ISOTYPE 001.LMD	LOG-LOG
31	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	B1	0.13	0.9	1.5	2C ISOTYPE 001.LMD	LOG-LOG
32	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	B2	0.15	1.4	1.7	2C ISOTYPE 001.LMD	LOG-LOG
33	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	B3	99.54	0.5	0.5	2C ISOTYPE 001.LMD	LOG-LOG
34	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	B4	0.17	1.3	0.7	2C ISOTYPE 001.LMD	LOG-LOG
35	2C ISOTYPE 001.LMD	A [A]	G1-FITC		C	1.15	1.1	###	2C ISOTYPE 001.LMD	LOG-LOG
36	2C ISOTYPE 001.LMD	A [A]	G1-PE		D	0.88	1.2	###	2C ISOTYPE 001.LMD	LOG-LOG
37										

Experiment Table



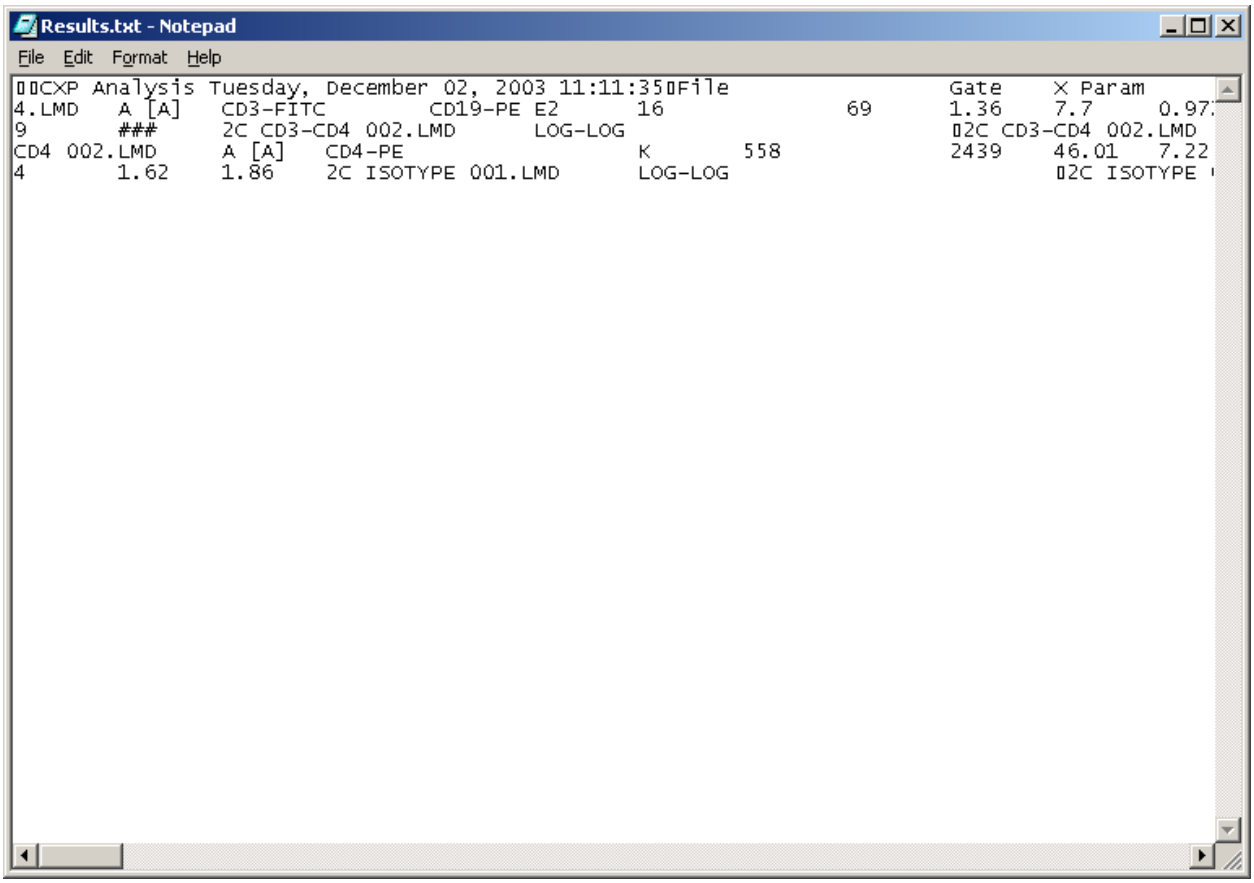
	A	B	C	D	E	F	G	H	I	J	K
1	CXP Analysis - Tuesday	August 17, 2004 13:57:57							AutoRef	0	Tuesday, August 17, 2004 13:57:57
2											
3	File	Gate	X Param	Y Param	Filename:-	Mean Calculation Method:-	Region	Variable	Value		
4	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	Number	5210		
5	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Total	24.68		
6	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Gated	100		
7	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	X-Mean	0.515		
8	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	Y-Mean	###		
9	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	Number	5210		
10	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Total	24.68		
11	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Gated	100		
12	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	X-Mean	0.515		
13	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	Y-Mean	###		
14	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	Number	5210		
15	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Total	24.68		
16	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Gated	100		
17	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	X-Mean	0.515		
18	2C ISOTYPE 001.LMD	A [A]	G1-FITC		2C ISOTYPE 001.LMD	LOG-LOG	ALL	Y-Mean	###		
19	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	Number	5210		
20	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Total	24.68		
21	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Gated	100		
22	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	X-Mean	0.515		
23	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	Y-Mean	0.455		
24	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	Number	5210		
25	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Total	24.68		
26	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Gated	100		
27	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	X-Mean	0.515		
28	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	Y-Mean	0.455		
29	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	Number	5210		
30	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Total	24.68		
31	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Gated	100		
32	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	X-Mean	0.515		
33	2C ISOTYPE 001.LMD	A [A]	G1-FITC	G1-PE	2C ISOTYPE 001.LMD	LOG-LOG	ALL	Y-Mean	0.455		
34	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	Number	5210		
35	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Total	24.68		
36	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Gated	100		
37	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	X-Mean	0.455		
38	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	Y-Mean	###		
39	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	Number	5210		
40	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Total	24.68		
41	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Gated	100		
42	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	X-Mean	0.455		
43	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	Y-Mean	###		
44	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	Number	5210		
45	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Total	24.68		
46	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Gated	100		
47	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	X-Mean	0.455		
48	2C ISOTYPE 001.LMD	A [A]	G1-PE		2C ISOTYPE 001.LMD	LOG-LOG	ALL	Y-Mean	###		
49	2C ISOTYPE 001.LMD	Ungated	SS Lin	FS Lin	2C ISOTYPE 001.LMD	LOG-LOG	ALL	Number	21110		
50	2C ISOTYPE 001.LMD	Ungated	SS Lin	FS Lin	2C ISOTYPE 001.LMD	LOG-LOG	ALL	%Total	100		

Refer to the MS Excel literature for more information on how to query, display, or manipulate the results with MS Excel.

FCS Keywords can also be exported to Excel. Go to Analysis >> Select Results and choose the desired FCS information to be added as a column in Excel.

NOTE: Any changes to the protocol will cause a new sheet to be created in Excel.

Example Text File Opened with WordPad



You have now completed this module. When you feel you are ready, please complete the CXP Retrieve and Analyze Data skill check.

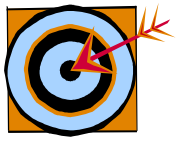
Table of Contents

OBJECTIVES

Given an operational system, training materials, and access to online Help screens

- Set up the system to display colors on a dot plot in color blend or color precedence modes based on 3 positive regions.
- Access administrative functions and assign a new user, assign privileges to a user, and create a workgroup.
- Track user usage.
- Copy a plot and paste it into a different Windows application.
- Create a Freeze Frame example of at least one single plot overlaid on another.
- Turn the Baseline Offset option on or off.
- Color compensate a listmode file.

Skill Check



You will have mastered these tasks when you can

- Print a color blend and color precedence example data.
- Create a workgroup of your classmates with privileges to access other files, overwrite other data files and add absolute calibration to batches.
- Track user usage for the week.
- Paste a plot to PowerPoint, Word or a Paint program.
- Create a Freeze Frame example of at least one single plot overlaid on another.
- Print two dual parameter and single parameter data sets, one illustrating Baseline Offset on and the other off.
- Show to the facilitator your ability to color compensate a listmode file.

Tour Guides

If you would like to see a demonstration on how to use several of the functions listed in this module, we suggest you use the Tour Guides. To access the Tour Guides:

1. If there is a program running, minimize the screen by selecting  in the upper right corner of the application.

2. Place the Tour Guide disk into the CD Rom drive or if your facilitator has placed the Tour Guides on your hard drive, double click on the icon labeled Tour Guides. The system will start the program. A voice will come on and explain how to take the tours.
3. Select the Extras tours. All of these tours can be viewed at one time or you can view each section as you cover that section in the training modules.

Before You Begin

1. We suggest you create a protocol to include dot plots FS/SS and FL1 Log/FL2 Log and Histogram plots FL1 Log and FL2 Log.
2. Then drag and drop a listmode file (e.g. AS 5C 1L Verify 8-4-45-19-3 will work) onto the plots. This will allow you to try the exercise in the next section.

Assigning Color to Dot Plots

NOTE: The original assigning of color results from enabling the Automatic Color Precedence option.

To enable:

1. Press **CTRL W** and then select the Gating tab (if it is not already displayed).

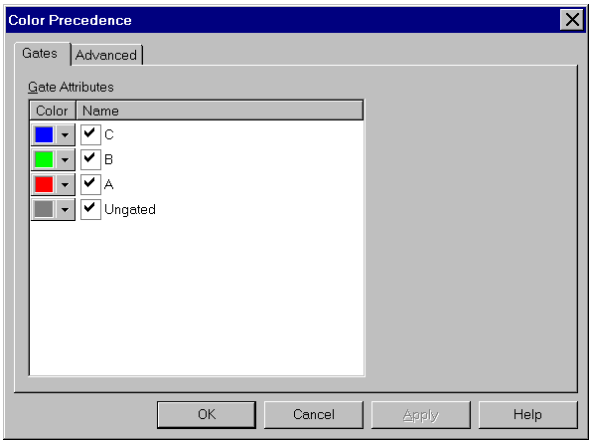
2. Select the Automatic Color Precedence and then select **OK**.

NOTE: Each new gate region is assigned its color in turn and added to the top of a precedence list. The last region created then, is given the highest precedence.

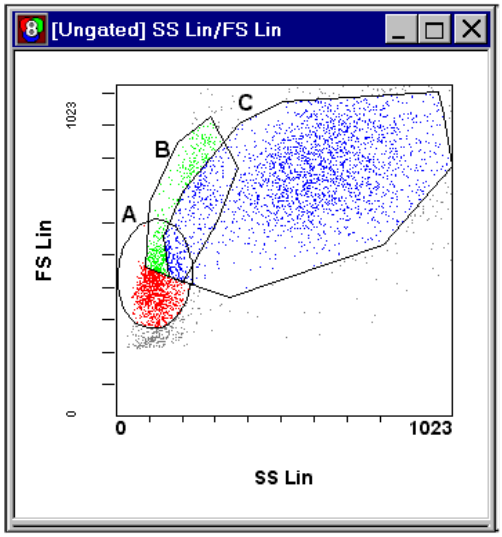
To see the list:

Select the  button. The Color Precedence window is displayed.

NOTE: Where events meet the criteria of more than one gate, the gate highest on the list takes precedence to color the event on a dot plot.



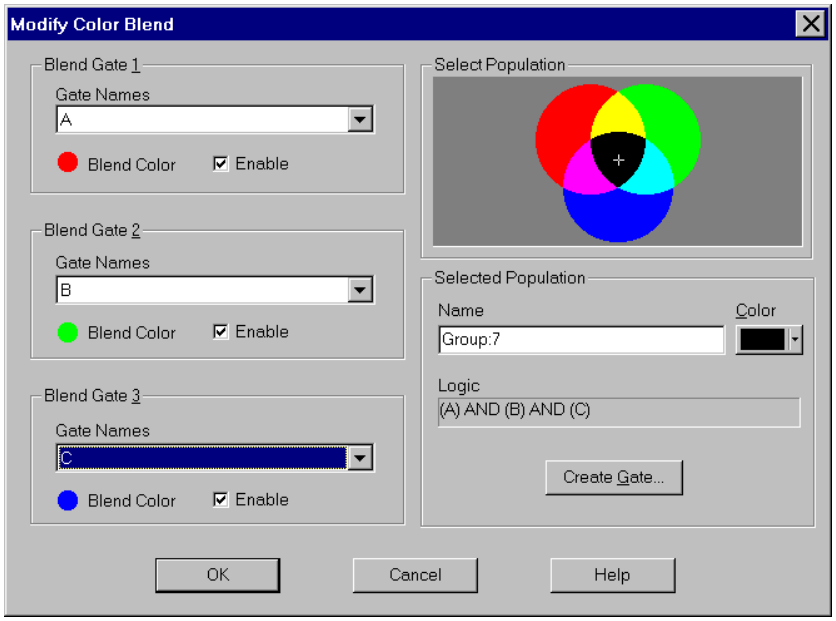
Example:



Color Blending

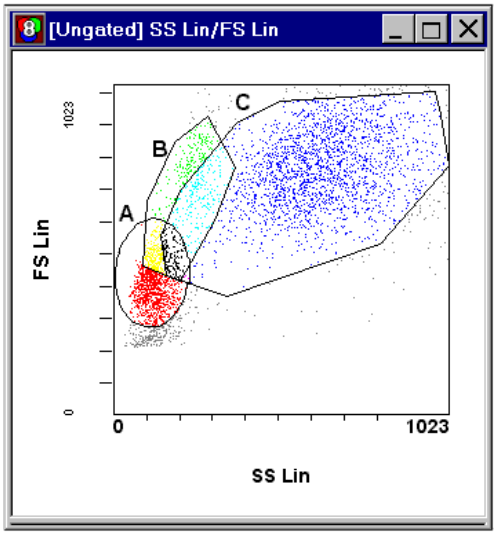
A second method of coloring the plots is called color blending.

1. Press **CTRL W**, select the Gating Tab and then select Color Blend Mode.
2. Select **OK.**
3. Select the  button on the toolbar. The following screen should appear:
4. Select 3 different regions for Blend Gate 1, 2, and 3.



Example:

Events only in region A but not B or C will be colored red; those in B but not A or C will be colored green; those in both A and B but not C will be colored yellow and so forth. Take a moment to study the screen.

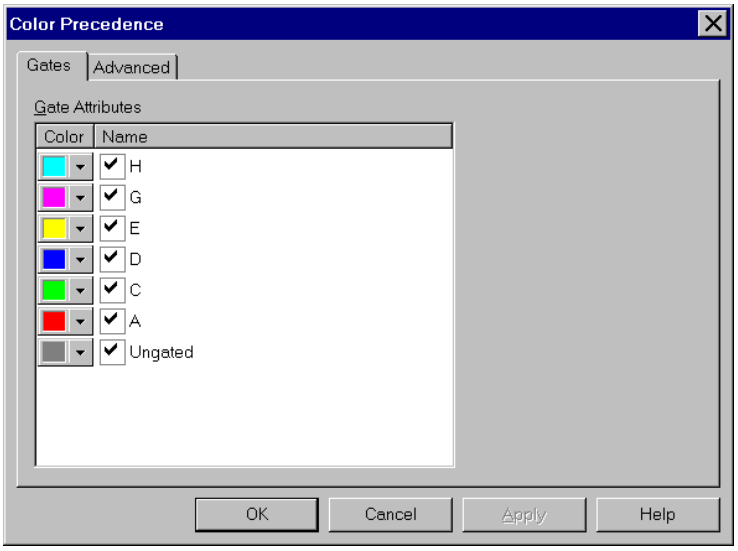


To Set Advanced Precedence

NOTE: Use advanced precedence when multiple plots are to be color gated on completely different criteria. Different plots can be made to express color precedence from different sets of gates.

1. First, create all regions to be used as color gates.

2. Second, check all regions to be used on the base screen and assign all colors.

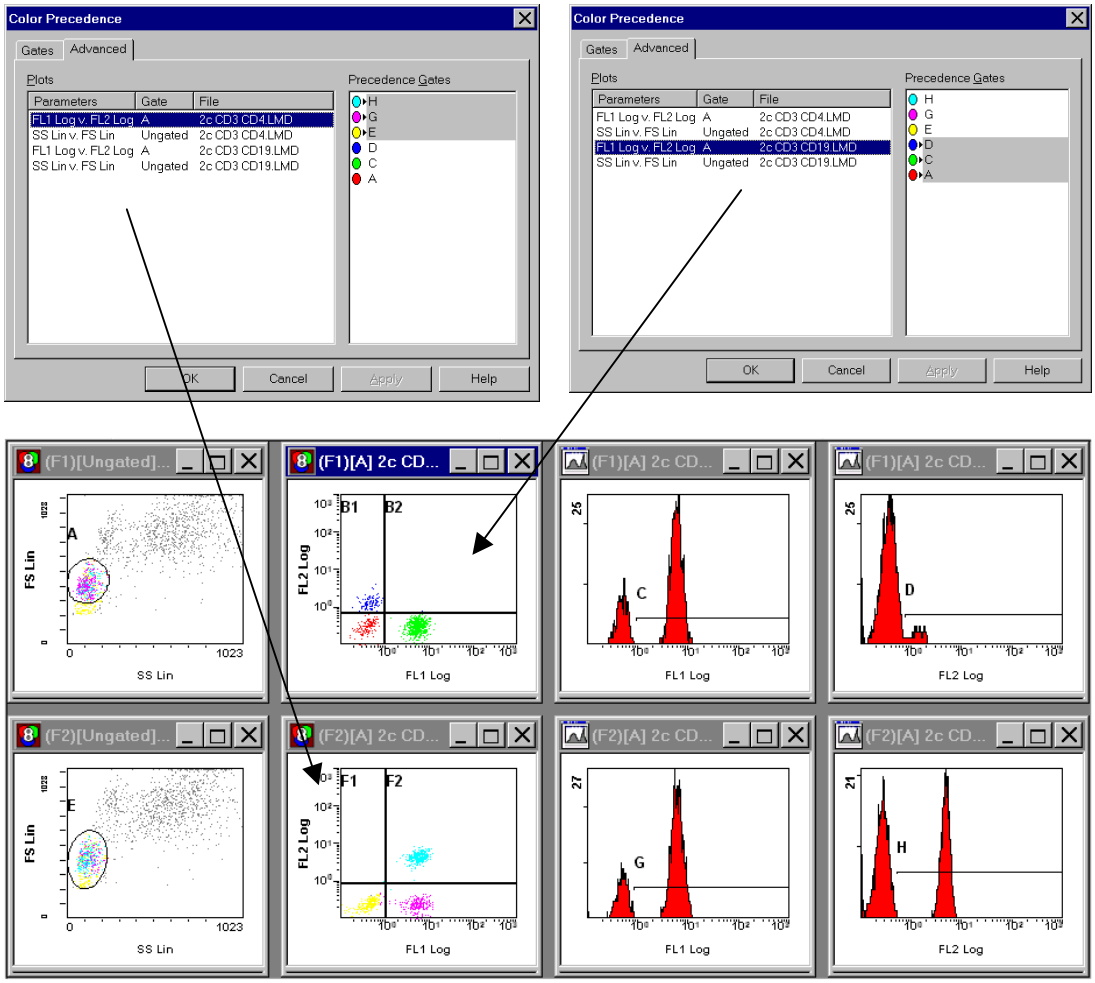


3. Highlight the plot desired to assign colors and select the Advanced tab.

4. Select plot on advanced screen and then the gates to be displayed on that plot.

5. Repeat for each plot and then select **OK**.

The system will now display different color precedence schemes on different plots. An example is shown below:



If you would like more information on Workspace Preference Options, you can access online Help. To access online Help

1. Select  at the top of the screen or **Help >> Cytomics CXP Help**.
2. Select **10 USING CXP SOFTWARE >> 10.15 FILE MENU >> Workspace Preferences- Gating**.

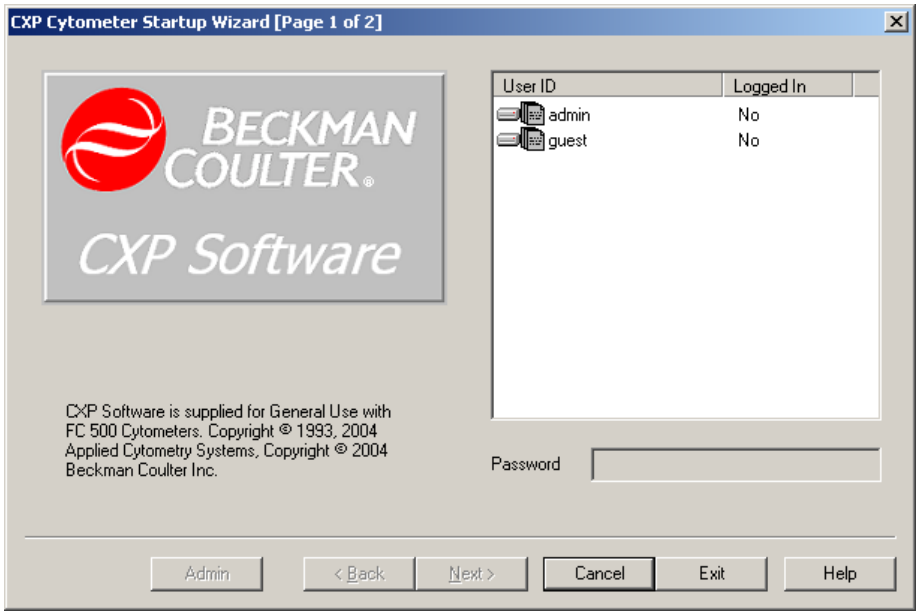
Tour Guides

1. We suggest you maximize the Tour Guides and take the Administration tour before completing this section.
2. When you are finished, minimize the Tour Guides.

Administration Functions

There are several functions allowing the administrator of the system to:

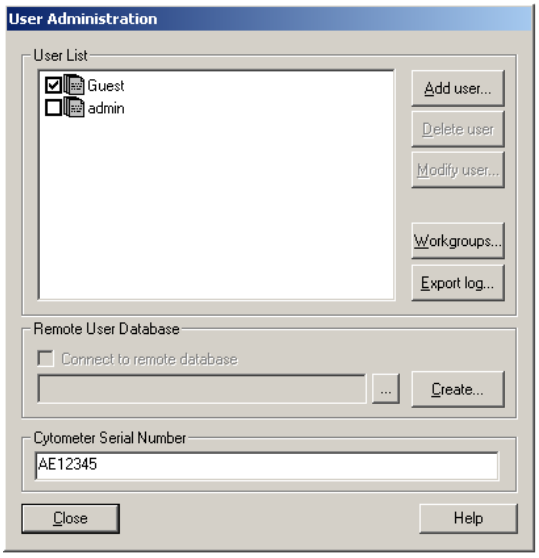
- Limit those allowed to use the system.
- Set up specific pathways for each user.
- Grant privileges to view or change other user files.
- Group users into workgroups with common access to certain files.
- Track user usage of the system, for example, for billing purposes.



1. Select Admin as a user.

Note: The initial password for admin is the word “Password”.

2. Select Admin button at the lower left of the Startup Wizard (Page 1 of 2) dialog.



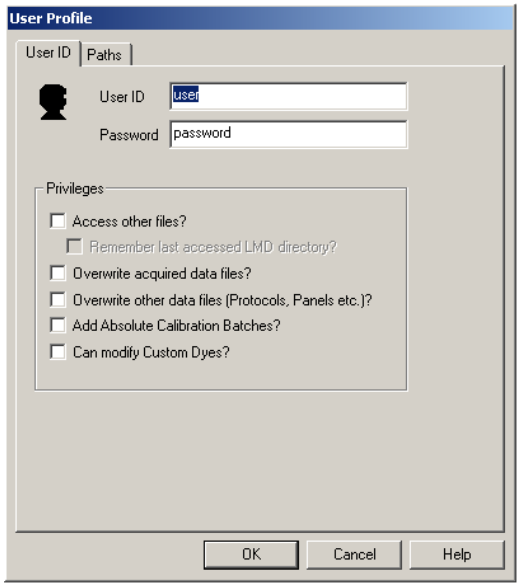
To Add User

1. Select **Add user…**

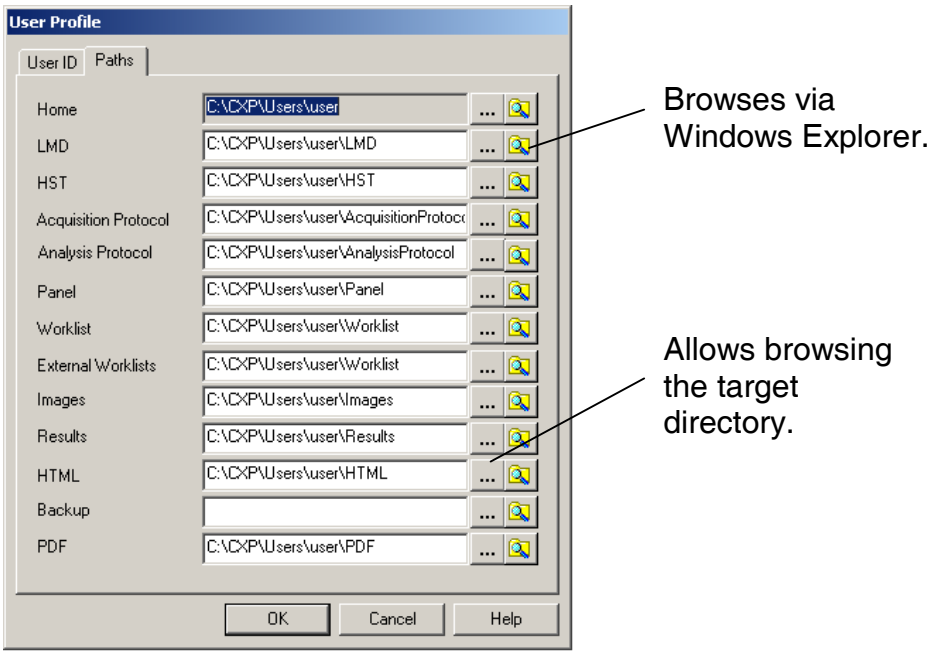
2. Enter new user name and password (minimum 6 characters).

3. Select desired privileges.

NOTE: For more information on user privileges, use the on-line help.



4. Select Paths tab if you wish to change the automatically created subdirectories for storing and retrieving user files.

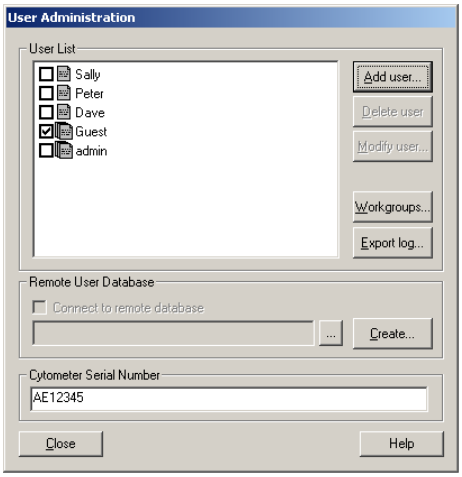


Note: If a backup filepath is identified, upon logoff allows data files to be automatically copied.

5. Select **OK** twice to return to User Administration window and repeat the process for each new user.

To Group Users Into Workgroups

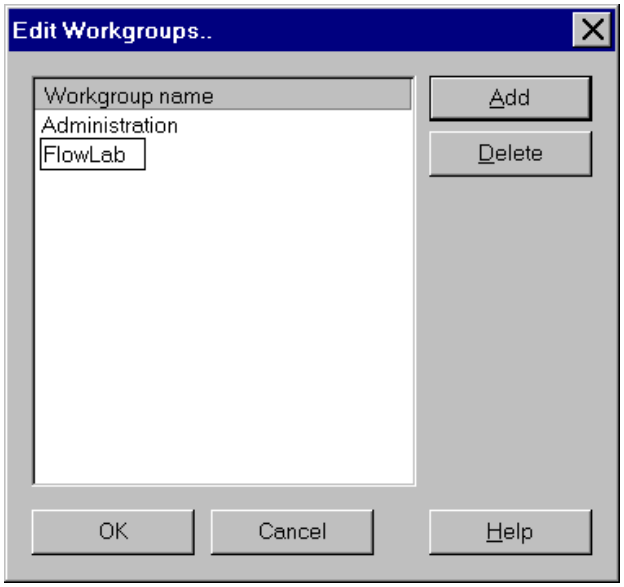
NOTE: A workgroup is a set of users with common privileges to create, access, or change files.



1. Select **Workgroups…**

2. Select **Edit** and then select **Add**.

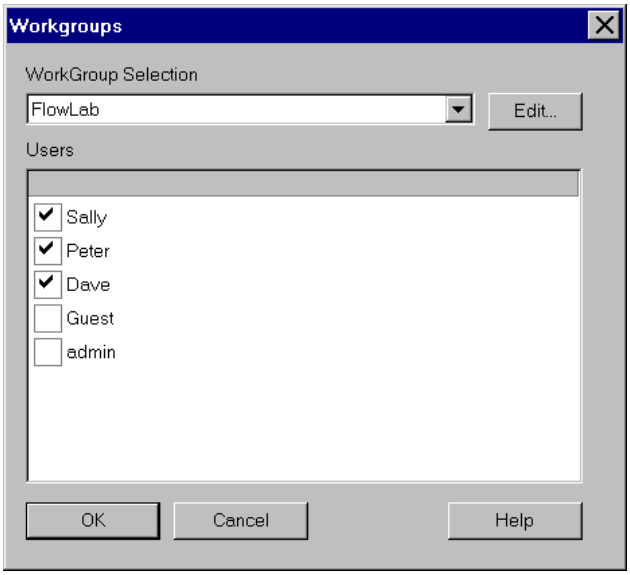
3. Rename your workgroup as desired. Note: add as many workgroups as you desire before leaving this screen.



4. Select **OK**.

5. Select Workgroup at the top of the window using the drop down menu.

6. Select all users to be included in the workgroup.



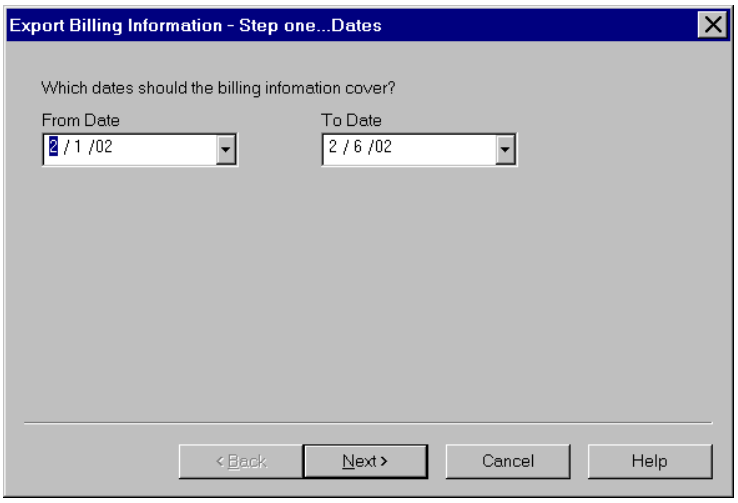
7. Select **OK**.

These users now have access (according to privileges given) to the files of anyone within the workgroup.

To Track User Usage of the System

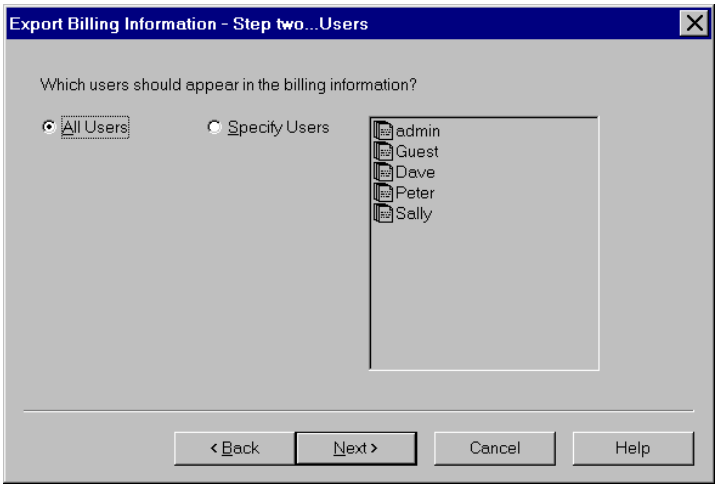
1. Select **Export log**.

2. Enter range of dates desired (e.g. beginning to the end of the month).



3. Select **Next**.

4. Select All or individual user to be included in the billing file.

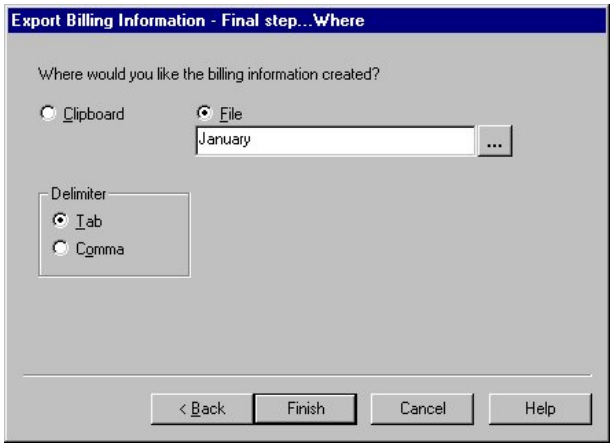


5. Select **Next**.

6. Now specify whether the information is to be used immediately (Windows Clipboard) or stored as a file for later retrieval.

7. Enter file name if you select File.

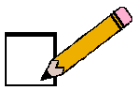
8. Select a Delimiter.



9. Select **Finish** and then **Close**.

NOTE: A summary of the user usage is now either on the Windows clipboard or in a file. The information can then be viewed and manipulated, for example, for billing purposes.

10. From the Windows desktop, select either Excel or Notepad.
11. Select **Edit** >> **Paste** or navigate to the file created in step 7 and select Open. Follow the prompts of the Windows application (Excel or Notepad) to view the Export log.



Tour Guides

3. We suggest you maximize the Tour Guides and take the Copy/Paste tour before completing this section.
4. When you are finished, minimize the Tour Guides.

Copy and Paste

Items on the Workspace can be copied to the Windows Clipboard for downloading into other programs. This action is called Copy and Paste.

To Copy a Plot to Windows Clipboard

1. Select the plot.
2. Select **Edit** >> **Copy** or select  button, or right click on the plot and select **Copy Plot Image**.

To Paste a Plot Into Another Program

1. Open the desired program (e.g. Powerpoint, Word, Paint program, etc.).
2. Select desired area to copy image.
3. Select **Edit** >> **Paste** or use the appropriate icon in the program.

Tour Guides

5. We suggest you maximize the Tour Guides and take the Freeze Frame tour before completing this section.
6. When you are finished, minimize the Tour Guides.

To Freeze Single Parameter Plots

NOTE: Up to three different listmode single parameter plots may be overlaid onto each other. In Acquisition mode, each sample is run and frozen for overlay onto the next sample plot. In Analysis mode, the listmode files are retrieved in sequence and each single parameter is frozen for overlay onto the next plot.

1. Either run a sample using the corresponding protocol (Acquisition mode) or retrieve a listmode with the corresponding protocol (Analysis mode).

2. Select **>>>>** on a single histogram plot you wish to freeze.

3. Select Histogram tab and then first Freeze frame.

The software places a ---- line around the histogram.

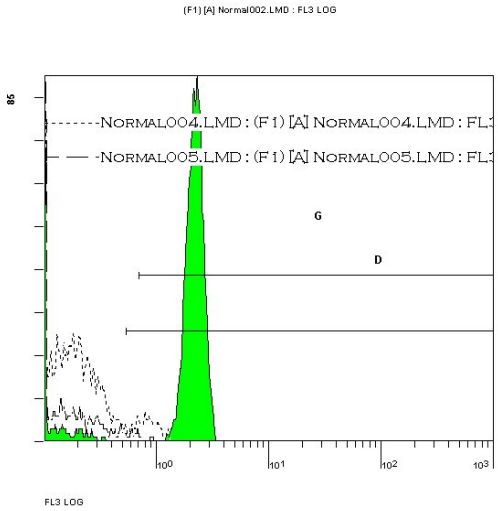
4. Run another sample (Acquisition) or retrieve another file. You can retrieve a file by either:

   A. Selecting **File** >> **Open Listmode File** and then desired file, or

   B. Selecting a file from the Resource Explorer and dragging it onto the plot.

NOTE: A dashed line representing the previous plot is overlaid on top of the new plot.

5. Repeat the process to overlay 2 frozen plots onto a new plot.



Baseline Offset

NOTE: The baseline offset option is used with log data to move very low signals up from low numbered channels by adding a randomize gaussian positive offset. This option is more of an individual preference and alters the display of data in the first log decade only.

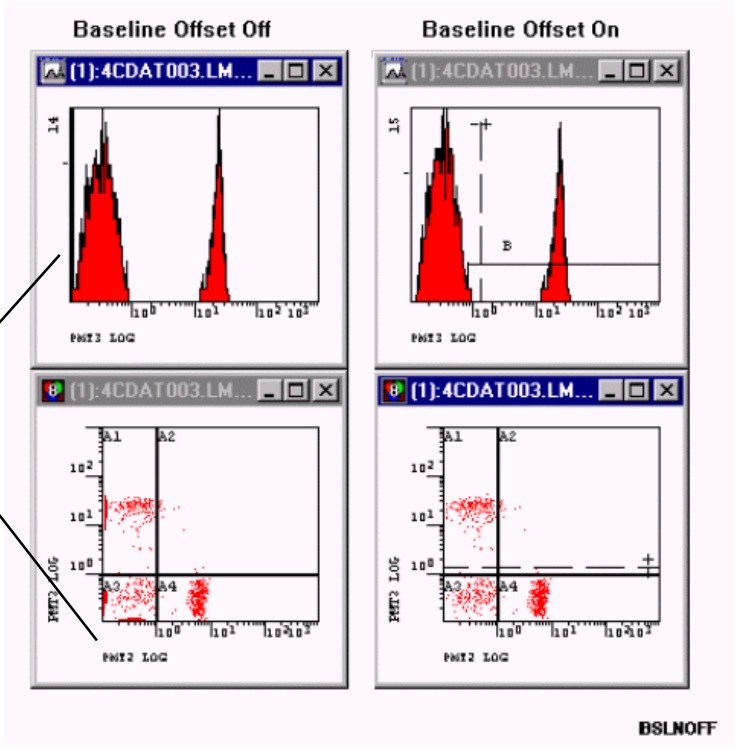
If you wish, you can see the effect of Baseline offset demonstrated by maximizing the Tour Guides and then selecting the Workspace Preference Plot Display tour and then the Baseline Offset box.

1. Select **File >> Workspace Preferences...**

2. Select Plot Display tab.

3. Select On to turn it on or Off to turn it off. Auto applies the baseline offset according to the FCS header stored with the listmode file. If it was on during Acquisition, it replays on and conversely, if it was off during Acquisition, it replays off.

The data display will change as in the example below:



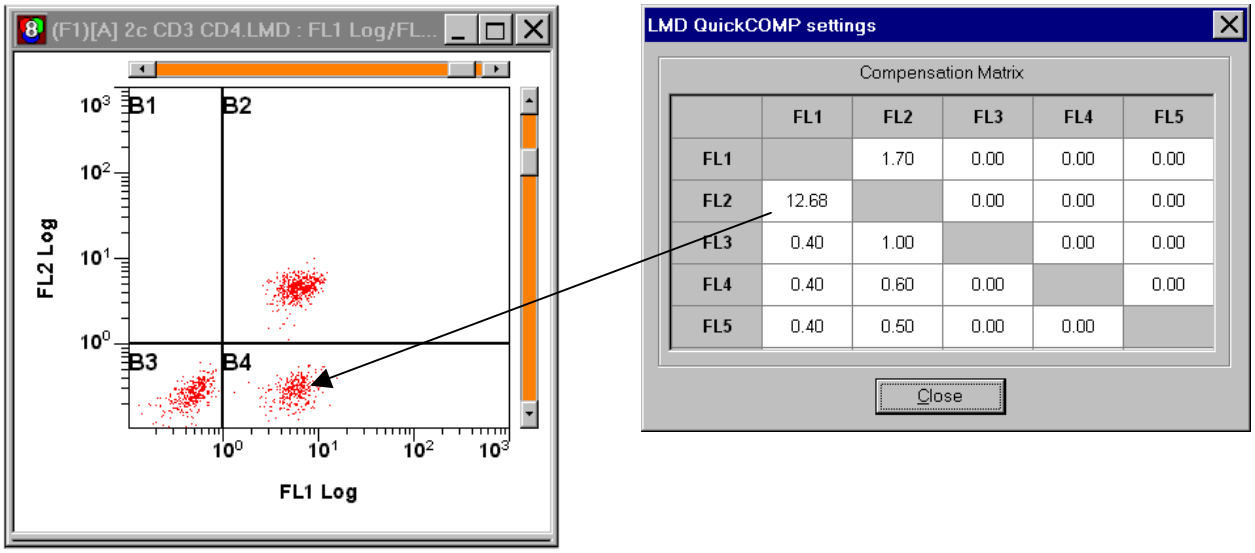
CAUTION: Always view the data first with baseline offset off to make sure the overall results of the assay are not significantly affected by turning it on. Also never use baseline offset when initially setting up voltages and compensation.

Listmode Compensation Playback

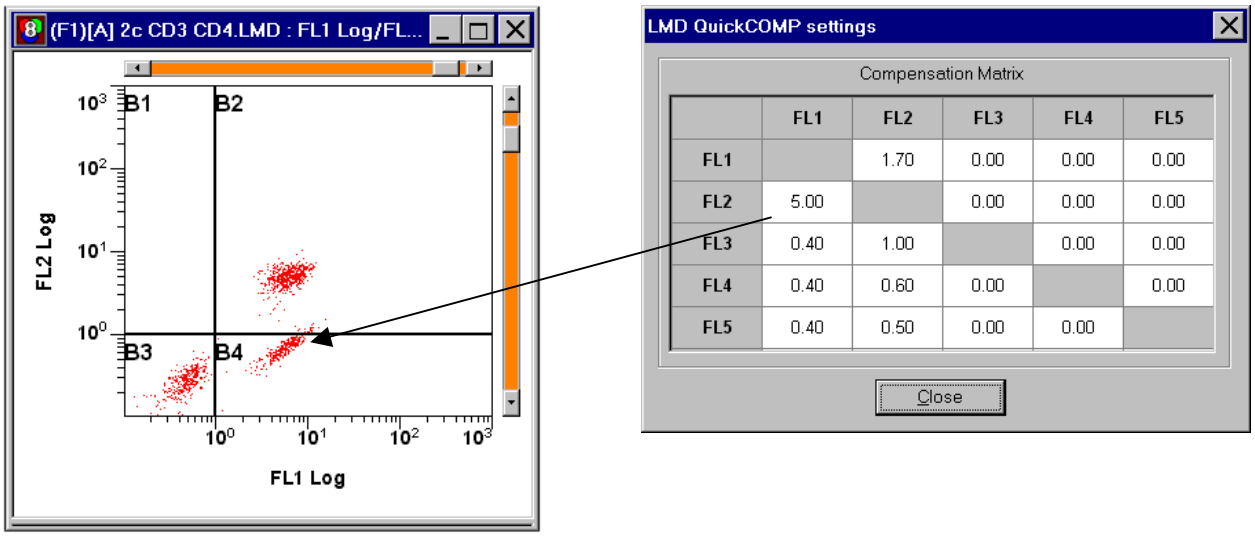
The software can apply color compensation to listmode file data and then, if desired, store the compensated data as a new listmode file.

1. Select a listmode file from the Resource Explorer and drag and drop it to the Workspace.
2. Select **Analysis >> LMD Quick COMP**.
3. Move the slider bars on either the top or side of the fluorescence dot plots to adjust compensation.

Example:



	FL1	FL2	FL3	FL4	FL5
FL1		1.70	0.00	0.00	0.00
FL2	12.68		0.00	0.00	0.00
FL3	0.40	1.00		0.00	0.00
FL4	0.40	0.60	0.00		0.00
FL5	0.40	0.50	0.00	0.00	



	FL1	FL2	FL3	FL4	FL5
FL1		1.70	0.00	0.00	0.00
FL2	5.00		0.00	0.00	0.00
FL3	0.40	1.00		0.00	0.00
FL4	0.40	0.60	0.00		0.00
FL5	0.40	0.50	0.00	0.00	

4. You can save the corrected file by selecting File >> Save As and give it a new name.

You have now completed the Software Extras module. When you feel you are ready, please complete the skill check.

TABLE OF CONTENTS

OBJECTIVES

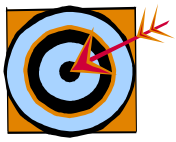
Given an operational system, training materials, access to an Operator's Guide, and access to online Help:

- Enter a new QC product into the databasewith all fields filled in.
- Print out either a QC Levey-Jennings plot or Data Table for a current QC product (e.g. Flow-Check).
- Create a new panel template to include stats, an equation, flagging limits, and plots.
- Enter patient demographics (minimum name, WBC count, and Lymphocyte %).
- Printout a patient panel report.
- Remove or restore database table entries.
- Input maintenance log information.
- Input service log information.

WHY IS IT IMPORTANT?

The Database stores quality control and panel report results. The QC results can be in a tabluar or plotted format and provides you with an on-line method of monitoring your system operation. You can also keep track of your quality control product lot numbers and descriptions. In addition, you can retrieve TetraCXP and Lymphocyte Subset Analysis patient reports, any export panel results, and, if you wish, enter patient demographics.

Skill Check Preview



You will have mastered the application when you can show printed results meeting specifications for each of the following:

- Enter a new QC product into the databasewith all fields filled in.
- Print out either a QC Levey-Jennings plot or Data Table for a current QC product (e.g. Flow-Check).
- Print a new panel report to include stats, an equation, flagging limits, plots, and patient demographic information.
- Remove/ restore database records.
- Print out both a maintenance log and service log both with new logged entries.

WHAT TO LEARN

In this module, you will learn:

- How to enter quality control product information such as the lot number and expiration date.
- How to access the database to retrieve and modify the stored quality control and panel report and/or patient demographics.
- How to modify an existing Panel Report template for outputing patient tetraCXP and LSA results.
- How to enter patient demographics.
- How to schedule removal/restore of old database records.

TOUR GUIDES

We suggest you use the Tour Guides along with this module to provide a visual illustration of the concepts and the autosetup options. To access the Tour Guides:

1. If there is a program running, minimize the screen by selecting  in the upper right corner of the application.

2. Place the Tour Guide disk into the CD Rom drive or if your facilitator has placed the Tour Guides on your hard drive, double click on the icon labeled Tour Guides. The system will start the program. A voice will come on and explain how to take the tours.

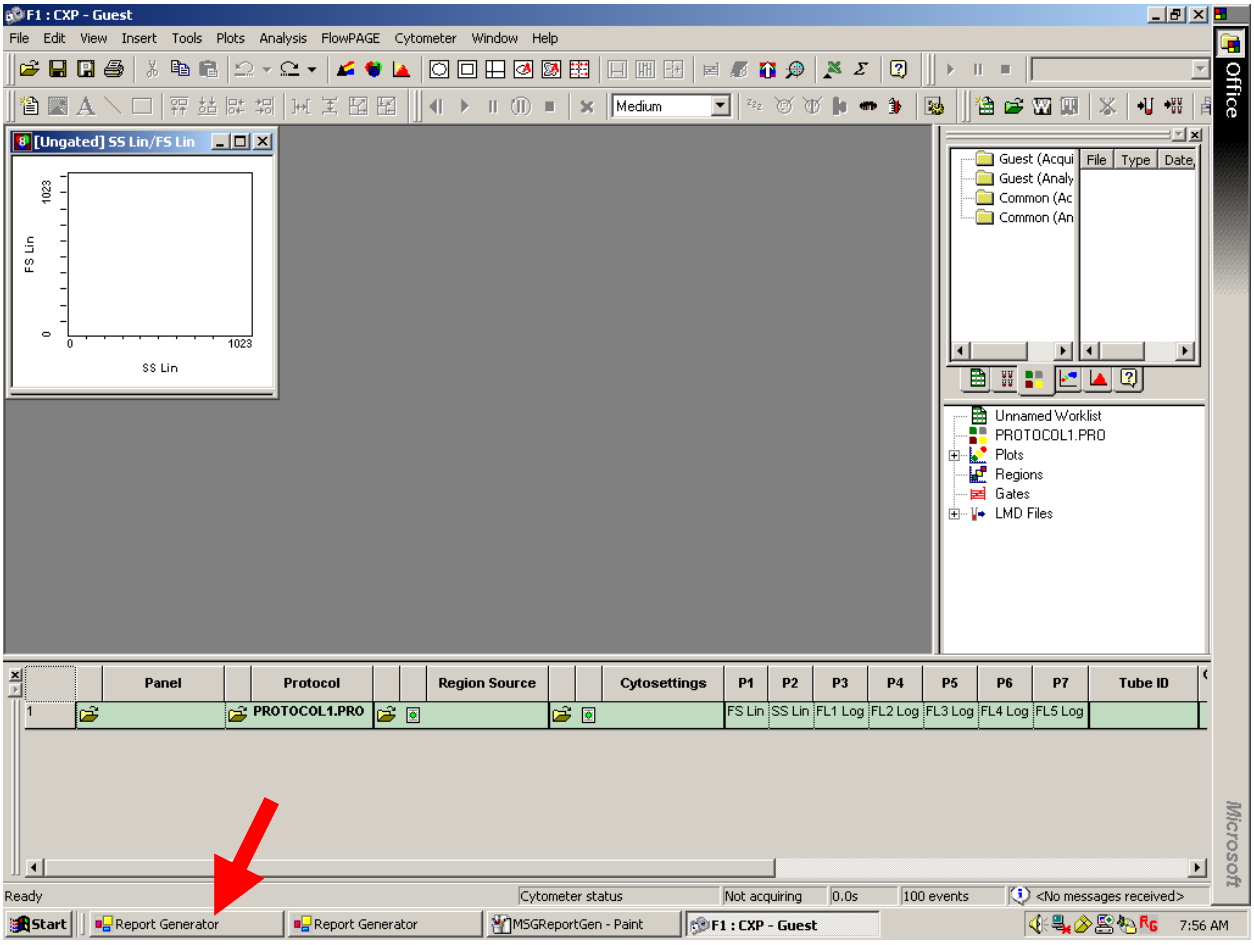
3. Take the Database tours.

INFORMATION / PRACTICE SECTION

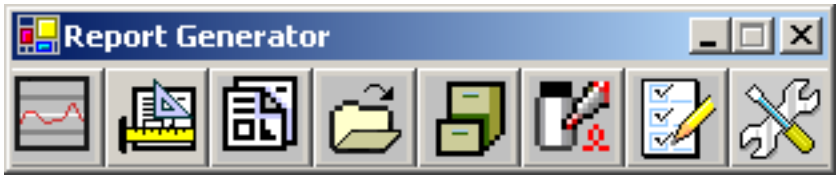
Quality control results, Panel Report templates, Panel Report results, patient demographics, and quality control product information are all stored with the database. The database can be accessed to retrieve, modify, and output this information.

To access the database:

1. Select the Report Generator button at the bottom of the screen.



The screen responds with the toolbar shown below:



Each icon is briefly described below:

 ***Quality Control Report*** – Use this icon to access quality control data, such as Flow-Check HPCV's. The data can be displayed as a Levey-Jennings plot or in a tabular display.

 ***Panel Template*** – Use this icon to create or retrieve and modify an existing Panel Report template.

 ***Panel Report*** – Use this icon to retrieve and output Panel Reports.

 ***Database Entry*** – Use this icon to enter, retrieve, and modify patient demographic information.

 ***Data Management*** – Use this icon to archive/retore database table QC Records or Panel Report Records.

 ***QC Product Editor*** – Use this icon to access and add new or modify quality control product information

 ***Maintenance*** – Use this icon to log completion of maintenance procedures.

 ***Service*** – Use this icon to log conditions noted and actions taken on your system.

NOTE: In the sections that follow, we will examine each section in more detail. When viewing a section, the toolbar may be hidden from view. It can always be retrieved by selecting the Report Generator button at the bottom of the screen.

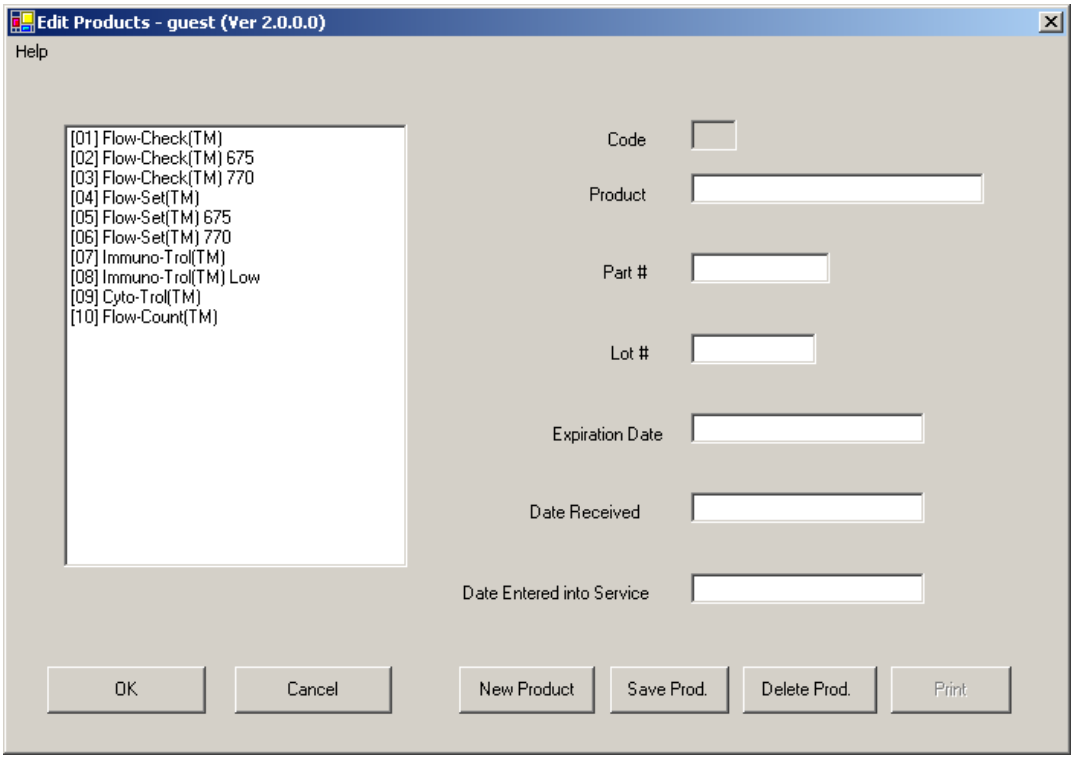
NOTE: To minimize the toolbar, select  . If you select  and close the toolbar, you will see an R/G icon at the far right of the taskbar. Right click on R/G and select "Show".

QC PRODUCT EDITOR

The QC Product Editor allows you to modify existing quality control product information page or setup or enter a new product and its associated information. Each product entered can also be setup to generate data for a QC Control Report. In this section we will first look at editing an existing product, such as Flow-Check™ Fluorospheres and then we will enter a new product.

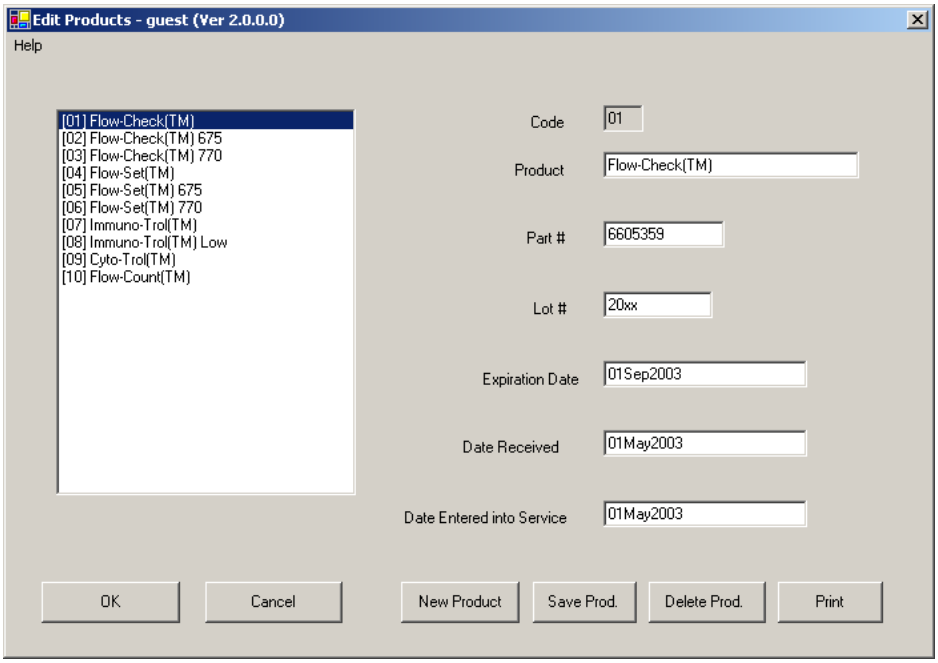
Editing an Existing Product

1. Select the  icon. The screen responds as below.



2. Select the desired product you wish to edit.

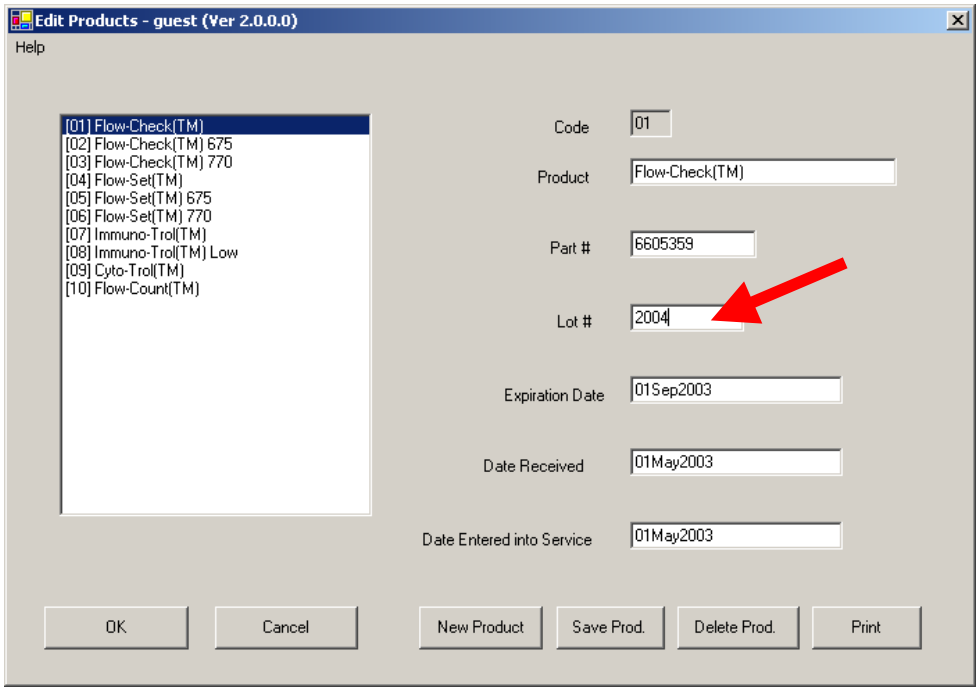
The right side of the screen reponds with the existing information about that product.



3. Select the desired information field to edit (ex. Lot #) and enter a new value.

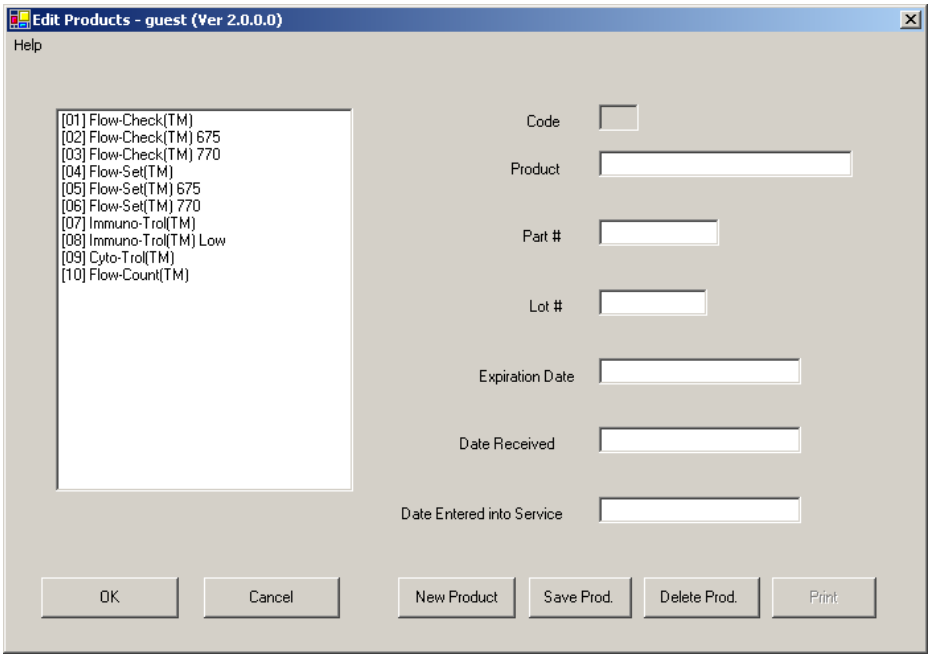
NOTE: Any existing QC template data with a previous lot # should be printed prior to editing the lot #.

4. Repeat the process for each field and then select **OK**. The system saves the new data.

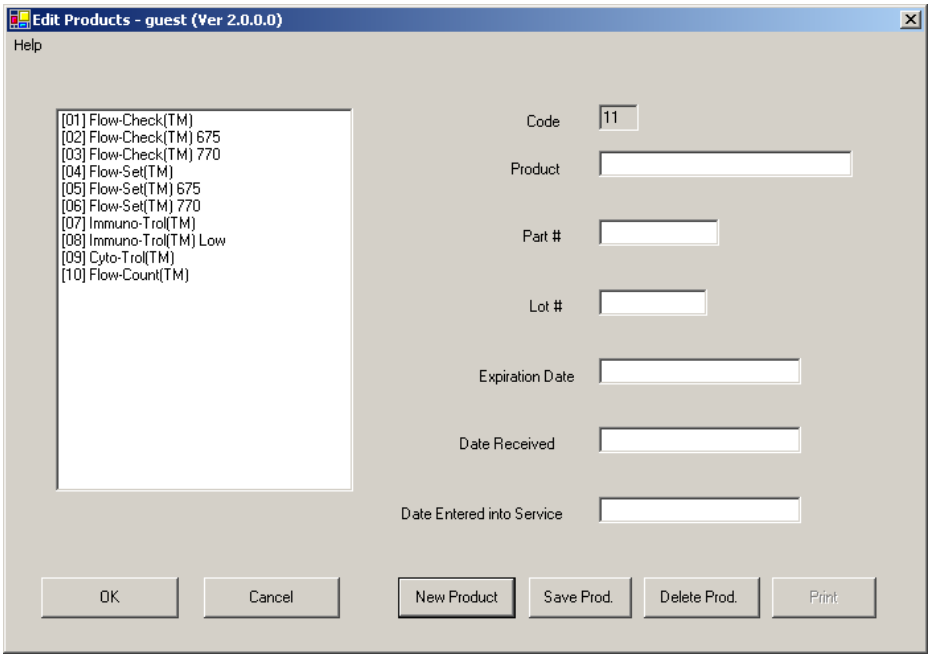


Entering a New QC Product

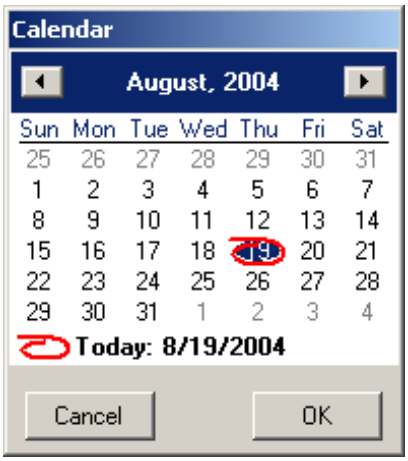
1. Select the  icon. The screen responds as below.



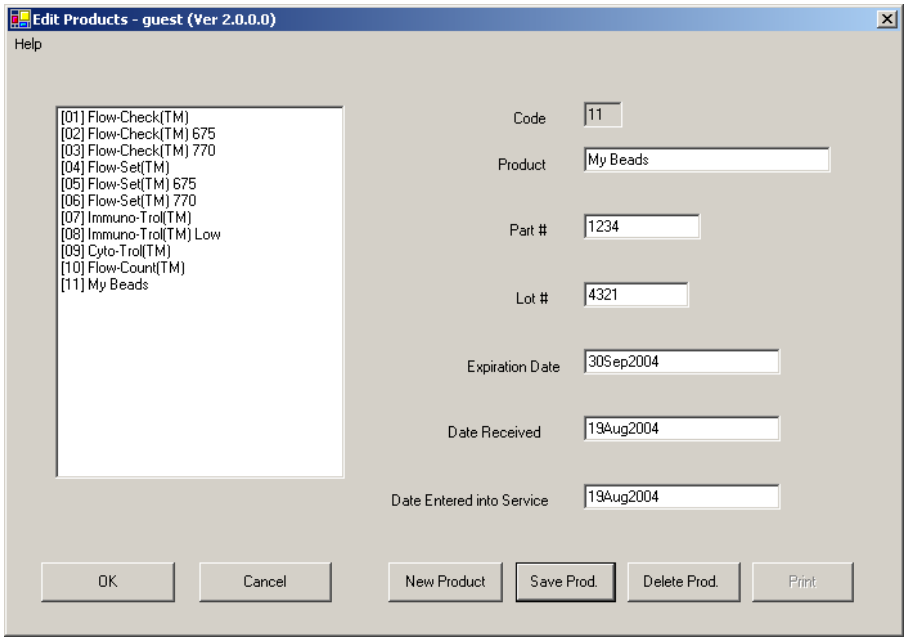
2. Select the New Product button. The screen enters the next available code.



3. Select each field in turn and enter the desired information about the product.
   - Note: When entering a date, a calendar will appear. Use the arrows at the top to change to the correct month, select a day and then **OK**.



2. After all entries have been made, select **Save Prod**. The screen responds as below.



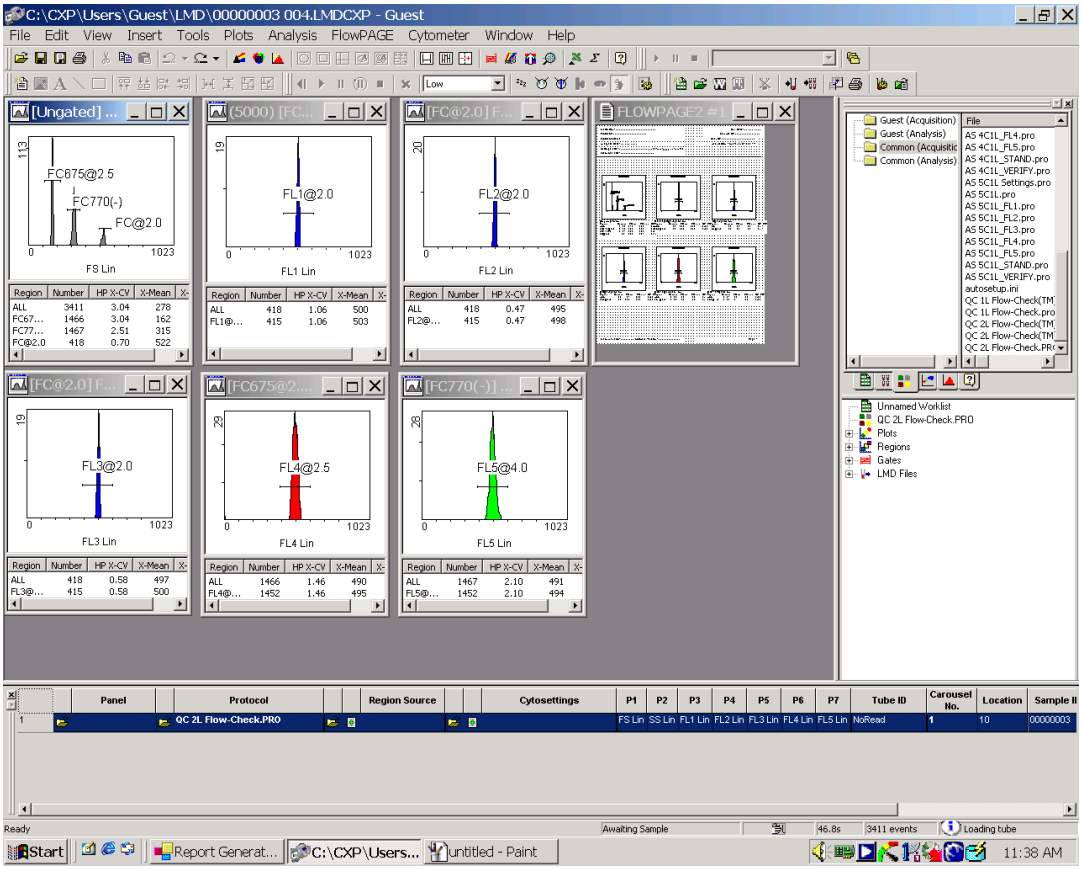
3. Select **OK** when finished.

QUALITY CONTROL REPORT

Save QC Data to the Database

QC data is saved to the database by running samples with protocols (ex. Flow-Check™) that have regions selected for QC export and a QC product selected. The regions within these protocols are set with the region properties to export for quality control.

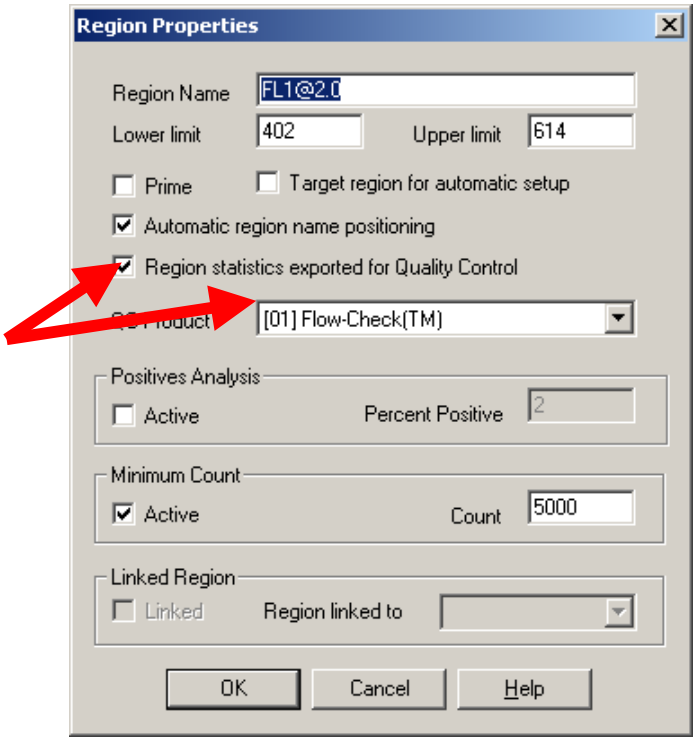
For example, the following screen is a run of Flow-Check™ fluorospheres.



1. Select a region (ex.FL1) and then right click the mouse button. The menu below will appear.

2. Select **Region Properties**.

The Region Properties window will appear.



When the "Region statistics exported for Quality Control" option is selected and the QC product has been assigned, the system will send that region's results to the assigned database everytime this QC product is run.

3. Select **OK**.

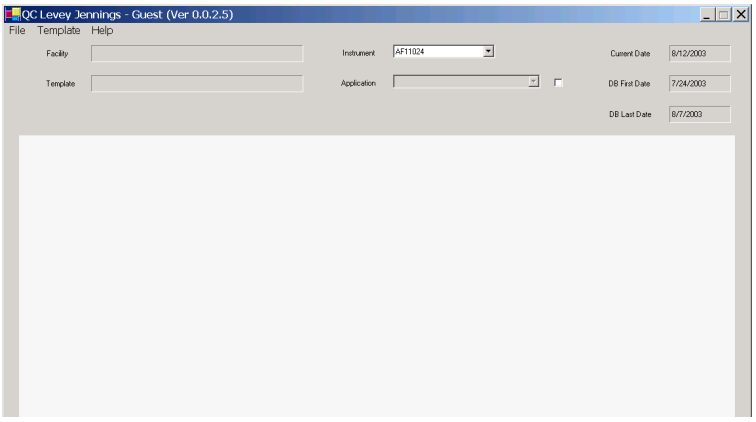
NOTE: The Standard QC protocols found in the Common (Admin) folder should already be assigned to a QC product.

Retrieving the Quality Control Data

Initial Setup

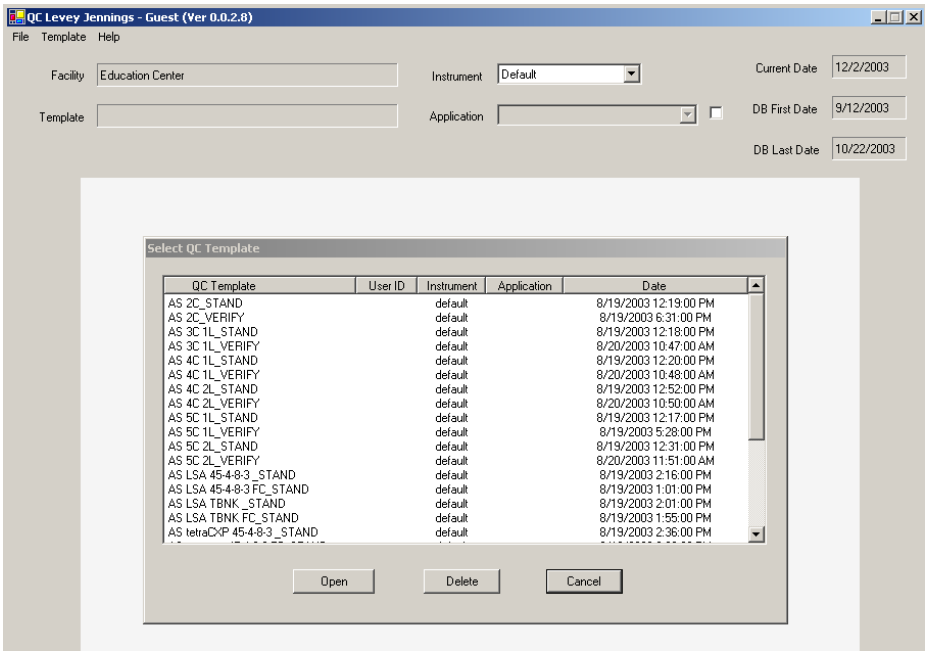
When the system is first installed, the default QC templates you intend to use need to be assigned to your unit serial number. This number is entered by the administrator the first time they log on. To accomplish this task:

1. Select the  icon. The system responds as below.



Note: Make certain that Application is **not** checked. If it is, deselect it.

2. Change the instrument to Default.
3. Select **Template** >> **Select Template**.

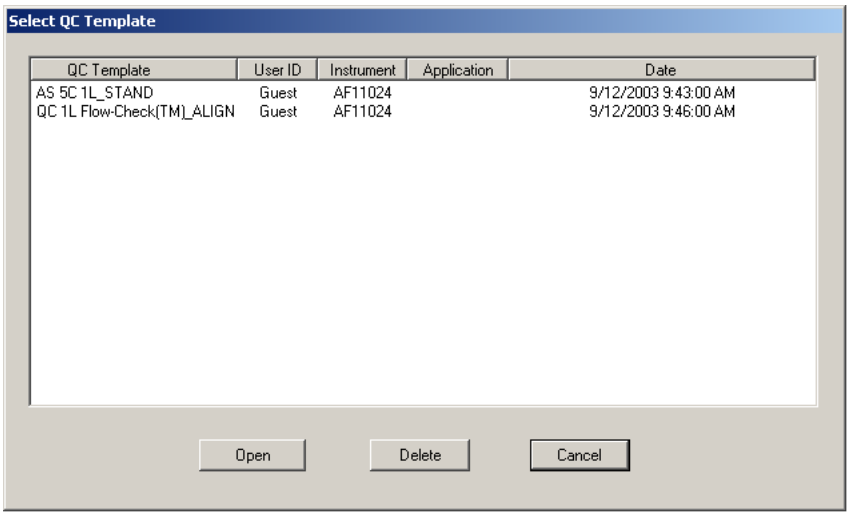


4. Select the desired Template from the list and then **Open**.

5. To save the template, select **Template** >> **Save Template**. The system will save the default template with the serial number and this template can now be accessed. Do these steps for each template you wish to use.

Selecting a Template

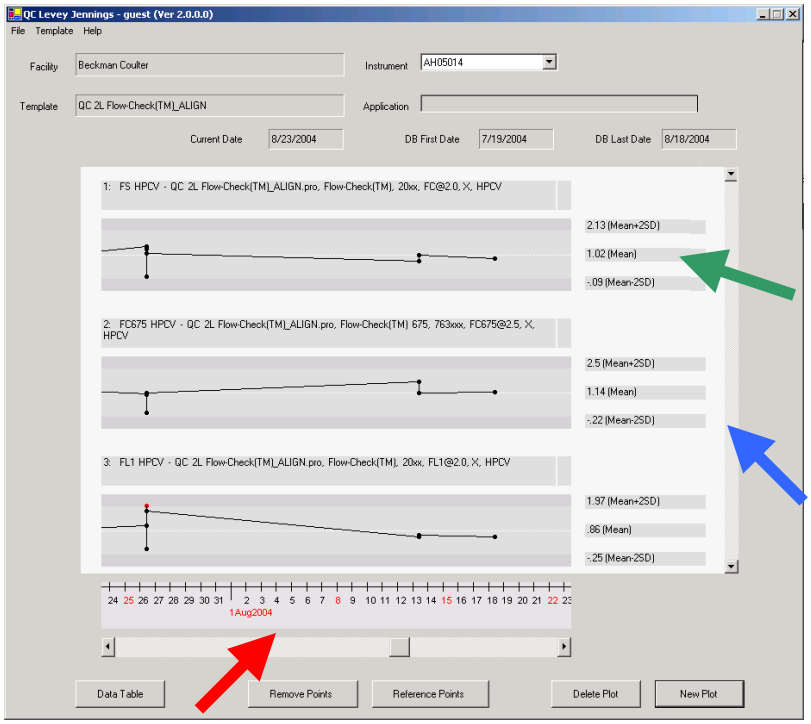
6. Click on **Template** >> **Select Template**. The screen responds as below.



7. Select the desired template (ex. QC 1L FlowCheck™_Align) and then **Open**.

Note: If the Application Checkbox is selected first and then an application, only templates and data from the selected application is displayed.

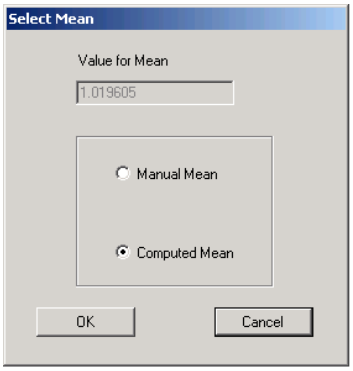
The screen responds as below.



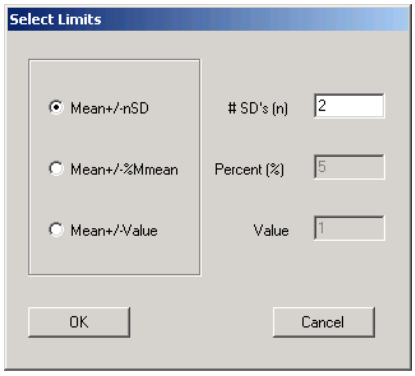
NOTE: If there are no data points and you are sure you have run samples, click the top line on one of the plots. This action forces the system to update the screen and new points will appear.

Additional Options to try:

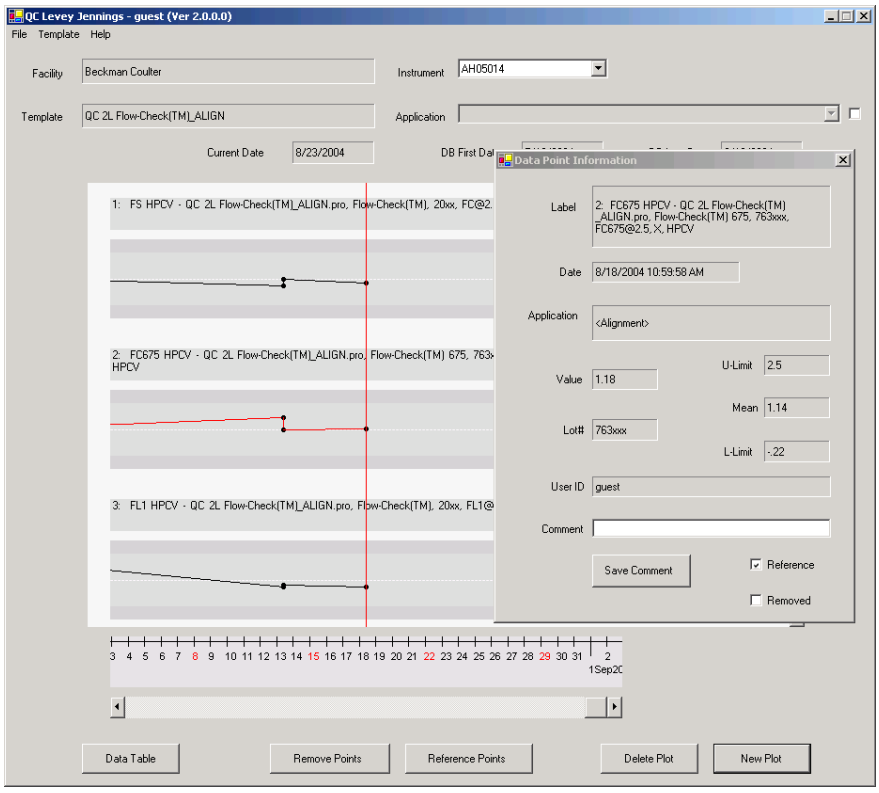
- You can right click on the time line (red arrow) to change the time line to day, week, month, or year.
- The scroll bar on the right (blue arrow) scrolls down to other plots.
- A left click on the Mean field (green arrow) accesses the Select Mean Window and allows you to select a manual mean, enter a value, and select **OK**.



- A left click on the upper or lower limits field accesses the Select Limits window. Select the type of limit desired and then **OK**.



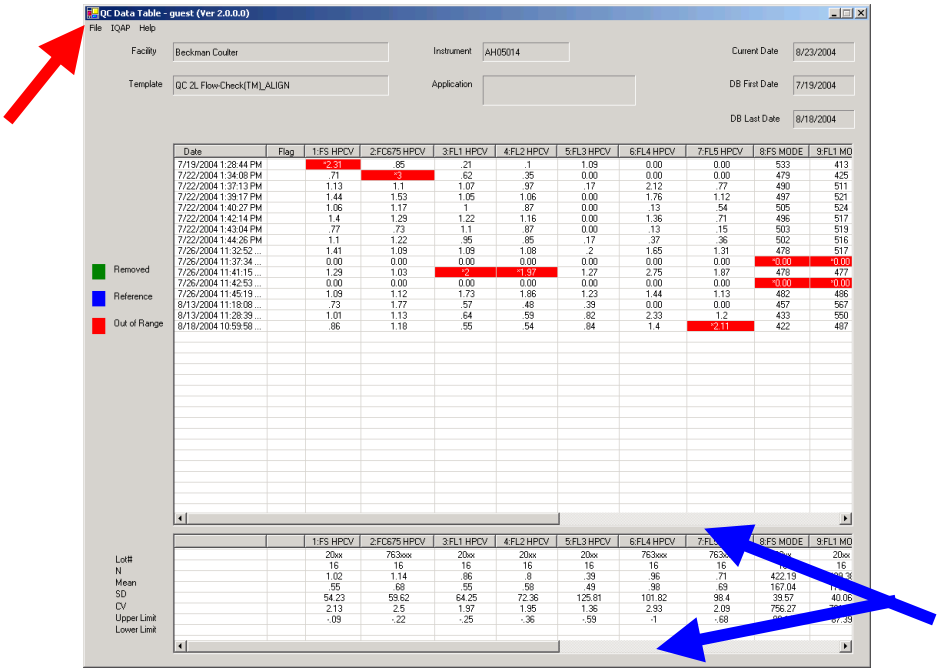
- Click on a point on the plot. A red line and a Datapoint information dialog will appear as illustrated below.



- From the DataPoint Information, select a point as a reference (illustrated above). The point color changes to blue and this point will be used to determine reference values for the data set.
- Select to remove a point. The point color changes to green but does not disappear. The point is removed from the statistics only.

Note: The Data Table will also show the data that the poin(s) represented in green to reflect this action.

- If you desire to select several points as reference (or removal), select the Reference Points (or Remove Points) button at the bottom of the Levey-Jennings window. Use a left click to mark the first data point and a right click for the last data point. The system colors all the points in between blue (or green) to reflect your actions.
- If several samples have been run on a day, the points will overlap each other. Right click the mouse to scroll through the points in sequence.
- Outliers will be red; points within limits will be black.
- You can also enter a comment about the point and then save all changes made.
- When printing to a black and white printer, the blue reference data points print as triangles and the green removed points print as diamonds.
- Select the Data Table button to display the data as below:



- Use the Scroll bars (blue arrow) to scroll the tables.
- To change Interlaboratory Quality Assurance Program (IQAP) information, select IQAP (red arrow) >> Edit IQAP. This program allows you to send in quality control data to be compared to other laboratories running the same QC products. For more information, contact the IQAP department:

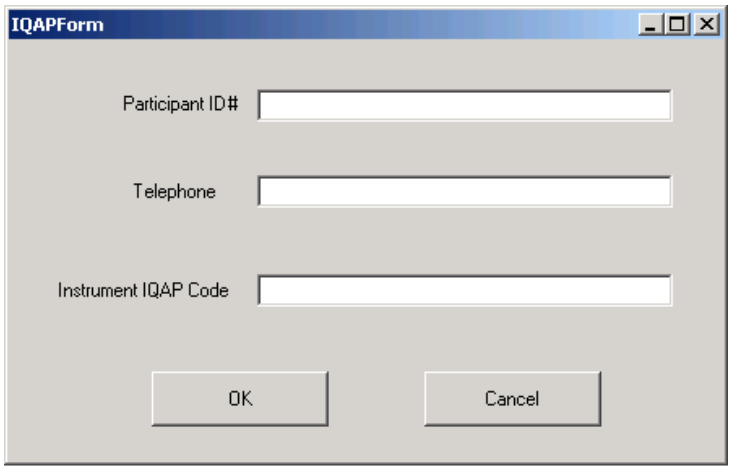
Beckman Coulter Corporation

IQAP (M/C) 31-BO4

P.O. Box 169015

Miami, FL 33116-9015

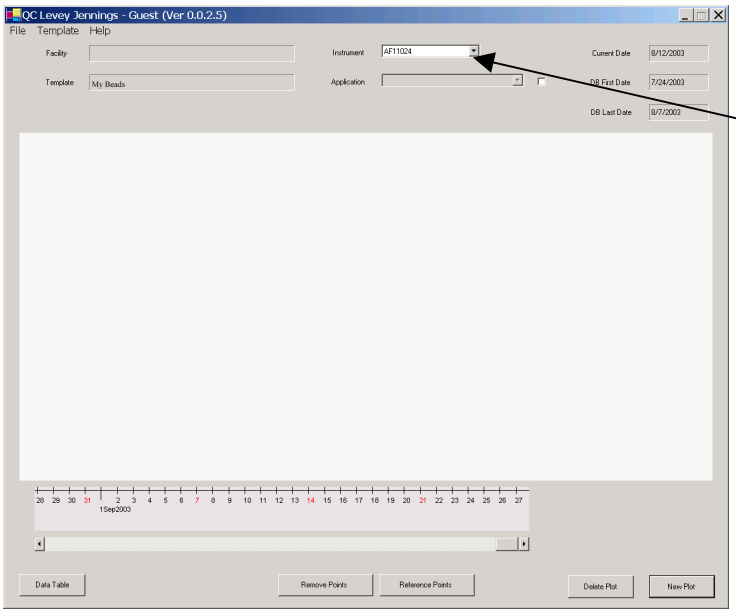
- Edit the information in each field and select OK.



NOTE: When changing Lot Numbers, be sure all data has been printed and copied to disk for IQAP before editing the QC products Lot #. If your laboratory overlaps lot numbers, you may choose to create a product and protocol for the "New Lot Number". After a sufficient number of data points have been accumulated, print out the QC data for both the old and new lot numbers. Be sure the old lot number has been printed and copied to disk for IQAP submission before editing the existing product with the new lot number informattion.

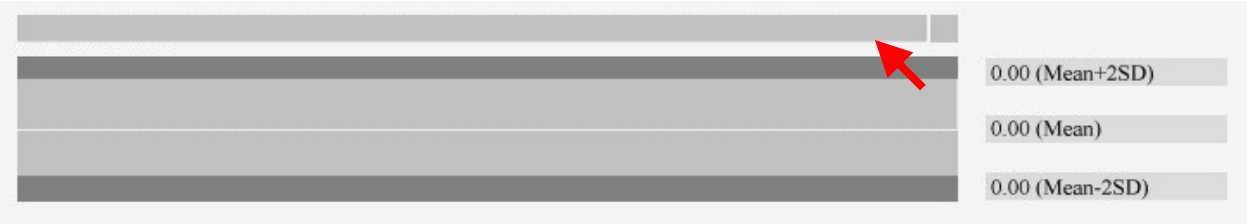
Creating a New QC Template

1. Start by entering a new QC product using the Edit Product screen.
2. Create a new QC protocol (create a Protocol module) with some of the regions assigned to your QC product using the Region Properties window.
3. Run the protocol at least once (more than once is recommended) to establish some points in the database.
4. Select the  icon.
5. Select **Template** >> **Clear template**.

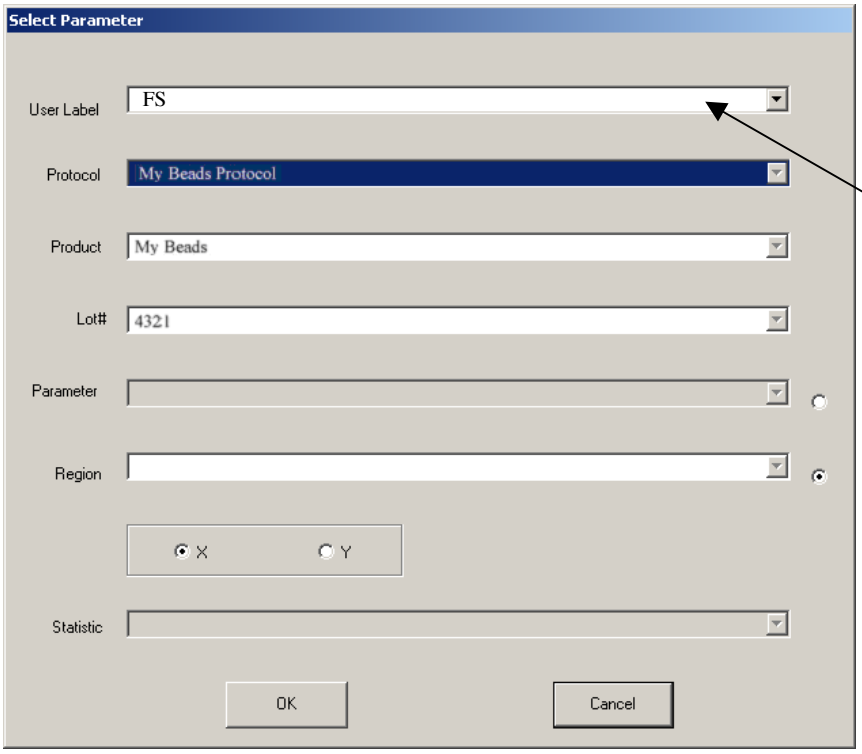


Note: Make certain you are not in Default. If you are, click on drop down arrow and change selection.

6. Select **New Plot** button. A blank plot will display.

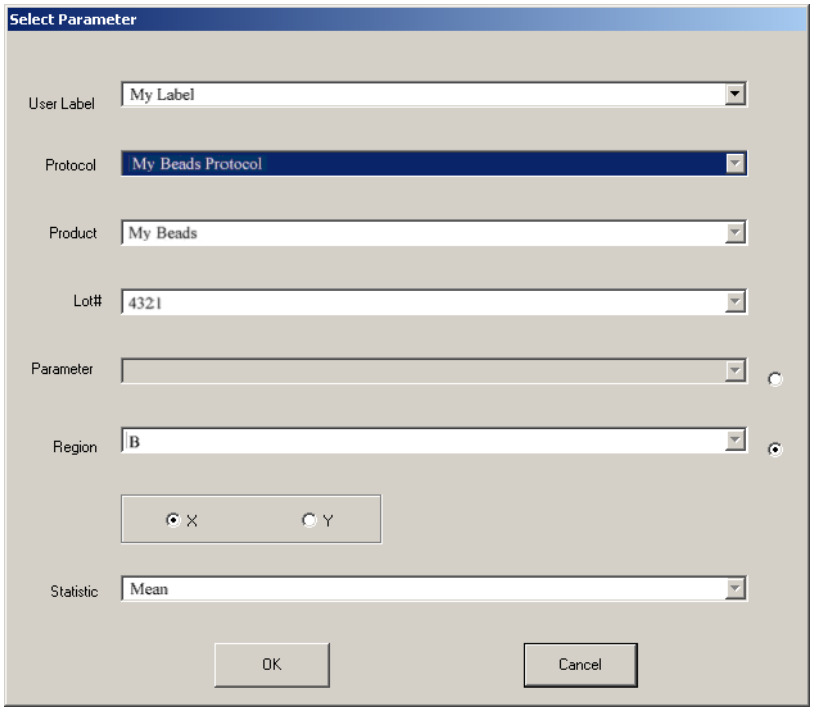


7. Click on header portion of the plot (red arrow).

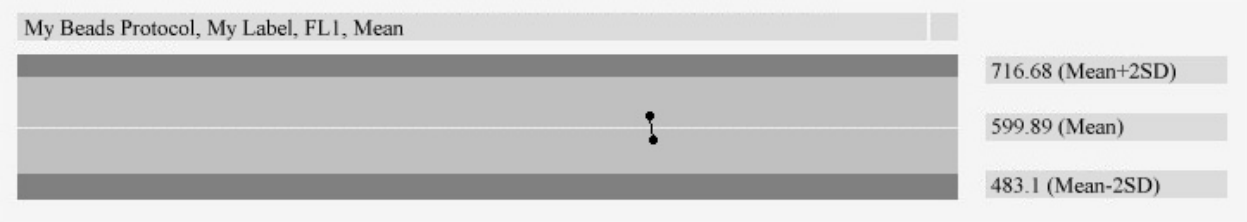


8. You can now enter a user label, select a parameter or a region, select the X or Y axis, and the statistic that will be plotted.

The Select Parameter window will appear as below when you are finished.



5. When you are finished, select **OK**. A new plot should appear with the points from the sample runs you ran earlier.



6. Create additional plots in the same manner.

To Delete plots:

7. Click on the box in the upper right corner of each plot to be deleted. An X appears.
8. Select the **Delete Plot** button and answer **Yes**.

Save Template:

9. Select **Template** >> **Save As** and enter a template name.

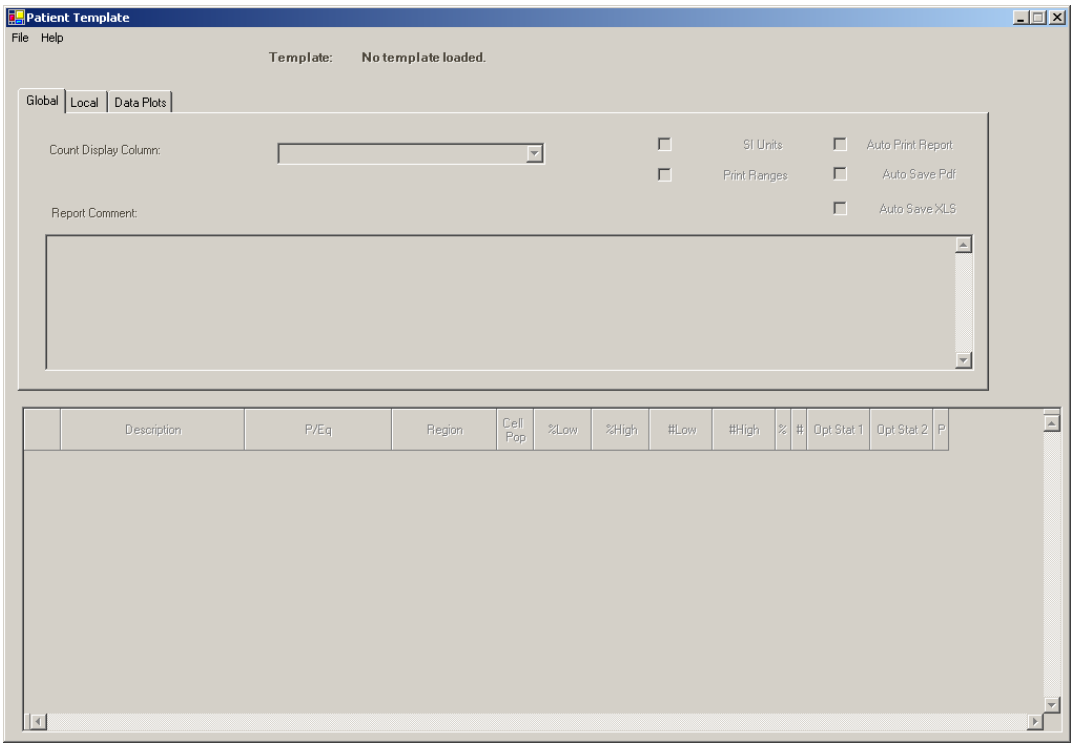
Note: If the protocol is run as part of an application, save the template with the application box selected and the application chosen. The template will be easier to access later.

PANEL TEMPLATE

The panel template allows you to create templates for reporting panel data. If tetraCXP and LSA (Lymphocyte Subset Analysis) have been loaded on the system, predefined panel templates are also loaded. Once templates are established, the results are automatically exported to the database. These templates tell the system how to report out the results.

To access a template:

1. Select the  icon. The screen responds as below.

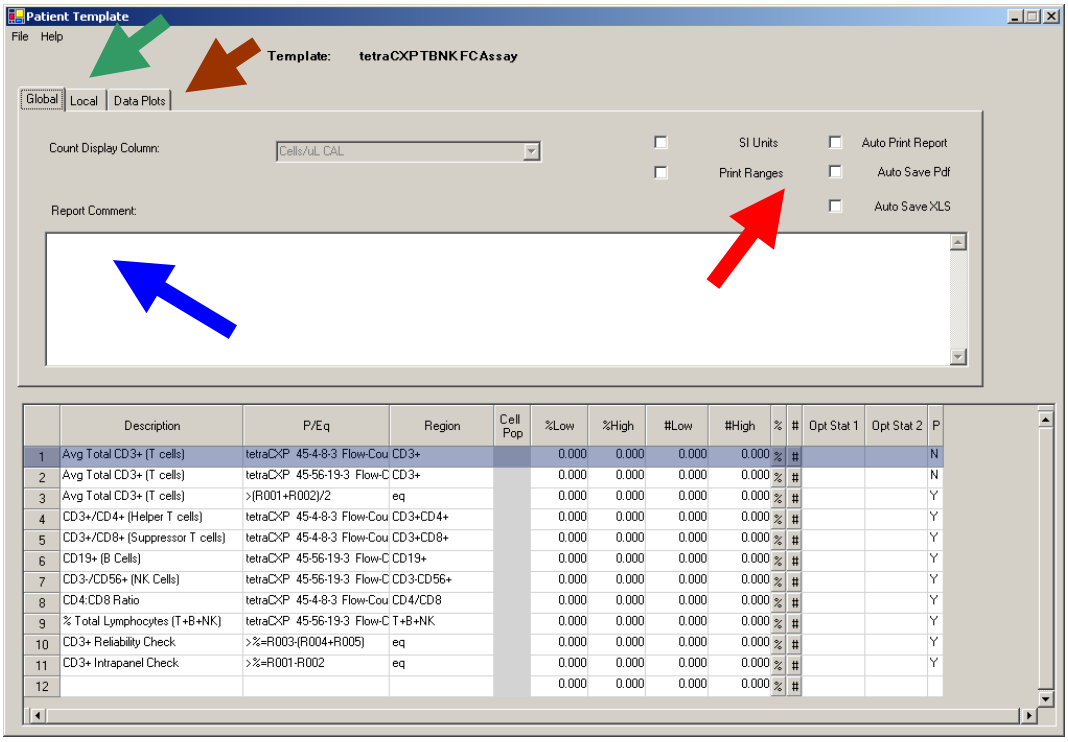


2. Select **File >> Open**. The screen list the templates available in the current user directory.

3. If you wish to select from the common directory, select the  icon twice. Now select Admin folder, then Panel folder, and then tetraCXP folder.

4. Select the desired template (i.e. tetraCXP TBNK FC Assay) and then Open. The screen reponds with the template name.

Example:



	Description	P/Eq	Region	Cell Pop	%Low	%High	#Low	#High	%	#	Opt Stat 1	Opt Stat 2	P
1	Avg Total CD3+ (T cells)	tetraCXP 45-4-8-3 Flow-Cou	CD3+		0.000	0.000	0.000	0.000	%	#			N
2	Avg Total CD3+ (T cells)	tetraCXP 45-56-19-3 Flow-C	CD3+		0.000	0.000	0.000	0.000	%	#			N
3	Avg Total CD3+ (T cells)	>(R001+R002)/2	eq		0.000	0.000	0.000	0.000	%	#			Y
4	CD3+/CD4+ (Helper T cells)	tetraCXP 45-4-8-3 Flow-Cou	CD3+CD4+		0.000	0.000	0.000	0.000	%	#			Y
5	CD3+/CD8+ (Suppressor T cells)	tetraCXP 45-4-8-3 Flow-Cou	CD3+CD8+		0.000	0.000	0.000	0.000	%	#			Y
6	CD19+ (B Cells)	tetraCXP 45-56-19-3 Flow-C	CD19+		0.000	0.000	0.000	0.000	%	#			Y
7	CD3-/CD56+ (NK Cells)	tetraCXP 45-56-19-3 Flow-C	CD3-CD56+		0.000	0.000	0.000	0.000	%	#			Y
8	CD4:CD8 Ratio	tetraCXP 45-4-8-3 Flow-Cou	CD4/CD8		0.000	0.000	0.000	0.000	%	#			Y
9	% Total Lymphocytes (T+B+NK)	tetraCXP 45-56-19-3 Flow-C	T+B+NK		0.000	0.000	0.000	0.000	%	#			Y
10	CD3+ Reliability Check	>%=R003-(R004+R005)	eq		0.000	0.000	0.000	0.000	%	#			Y
11	CD3+ Intrapanel Check	>%=R001-R002	eq		0.000	0.000	0.000	0.000	%	#			Y
12					0.000	0.000	0.000	0.000	%	#			

5. From the Global tab, you can select whether to print out results in SI units, print ranges, and/or auto save to pdf or xls or autoprint functions (red arrow). You can also add a global comment if you wish (blue arrow). The comment added will print globally on all patient reports run with the same panel.
6. If you wish to enter flagging limits, select the Local tab (green arrow). The system will flag the printout when these ranges are exceeded.
7. If you wish to add plots to the template, select the Data Plots tab (brown arrow).

Note: In the next section, you will create a complete template based on a new panel. The complete instructions for each of these tabs will be presented at that time.

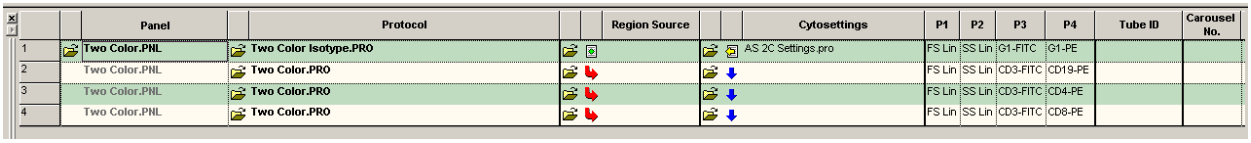
8. If changes are made, select **File** >> **Save** to save them.
9. If you wish a printout of the template, select **File** >> **Print**.
10. When you are finished, select **File** >> **Exit**.

CREATING A NEW PANEL TEMPLATE

Creating a panel report begins by first creating an export panel. The Acquisition Manager module provides directions on how to complete this task. The completed panel can then be set up to produce a report.

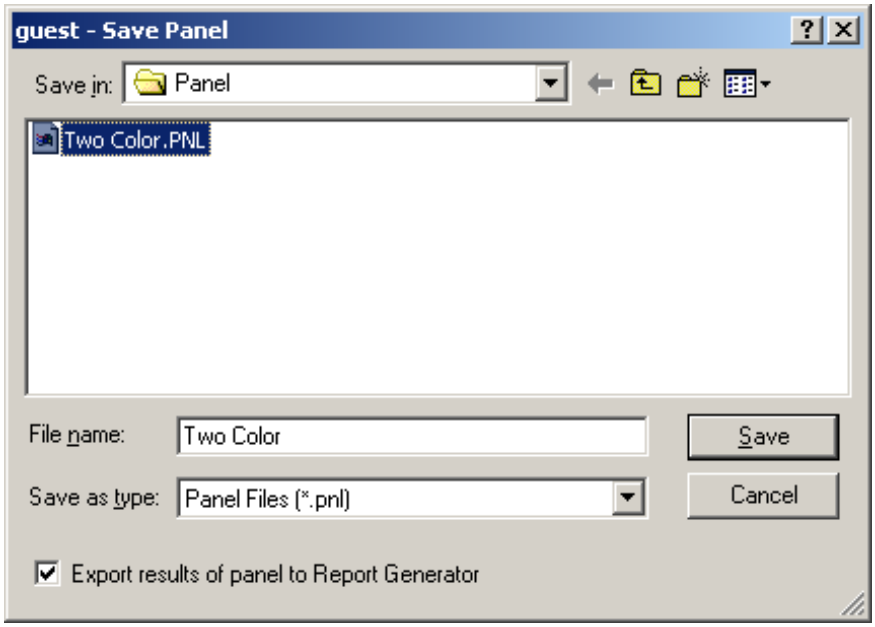
Creating a Blank Template

1. Select the  icon to clear the worklist.
2. Now drag and drop the panel to the Acquisition Manager. An example is shown below.



	Panel	Protocol	Region Source	Cytosettings	P1	P2	P3	P4	Tube ID	Carousel No.
1	Two Color.PNL	Two Color Isotype.PRO		AS 2C Settings.pro	FS Lin	SS Lin	G1-FITC	G1-PE		
2	Two Color.PNL	Two Color.PRO			FS Lin	SS Lin	CD3-FITC	CD19-PE		
3	Two Color.PNL	Two Color.PRO			FS Lin	SS Lin	CD3-FITC	CD4-PE		
4	Two Color.PNL	Two Color.PRO			FS Lin	SS Lin	CD3-FITC	CD8-PE		

3. Right click anywhere in the panel column and then select **Save as Panel**.

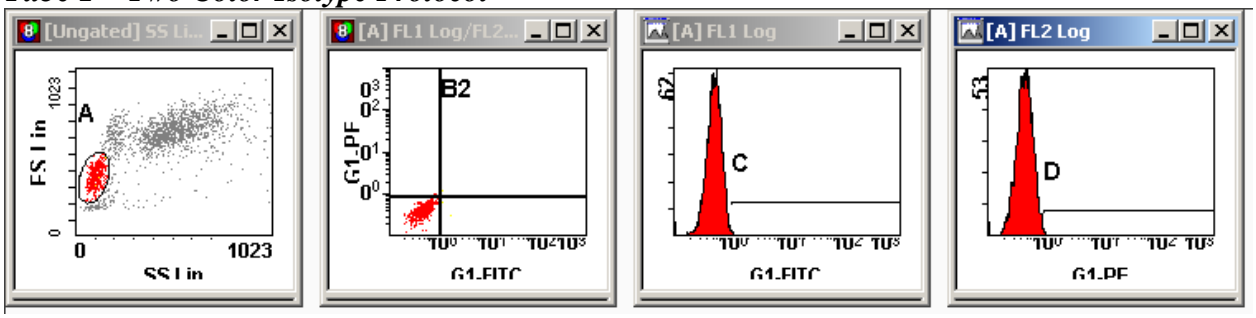


4. Select the Export results of panel to Report Generator box and then **Save**.
5. Select **Yes** to update the panel. The system now creates a blank template that you can now customize.

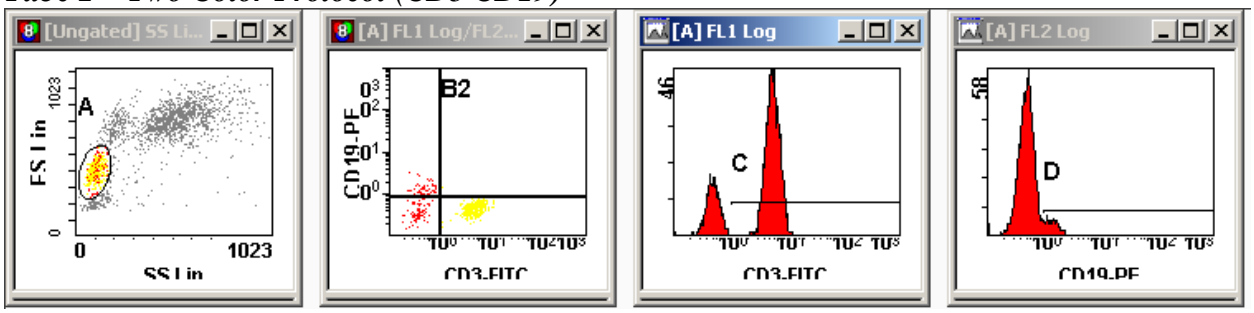
Example Panel Data

The Panel Report Template tells the system which information from which plots to report. It is important then to know exactly how the data appears within each protocol within the panel. You can gather this information from individual printouts. The Two Color Panel is summarized below. This information will be critical especially when the panels are more complex.

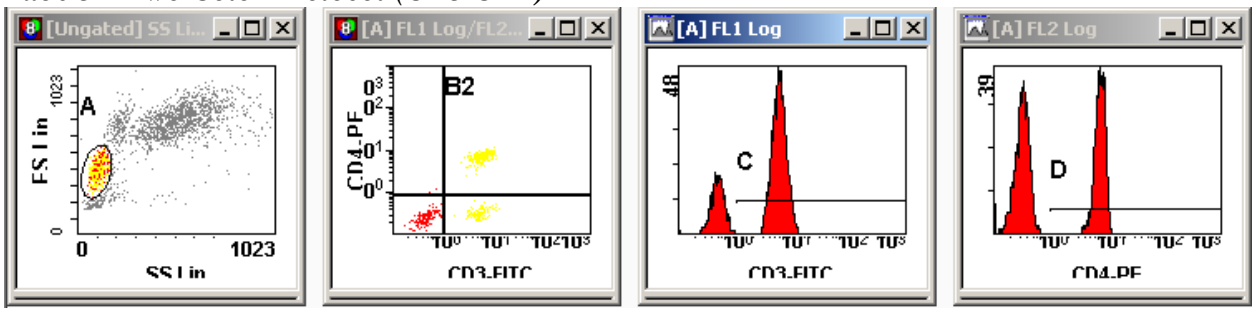
Tube 1 – Two Color Isotype Protocol



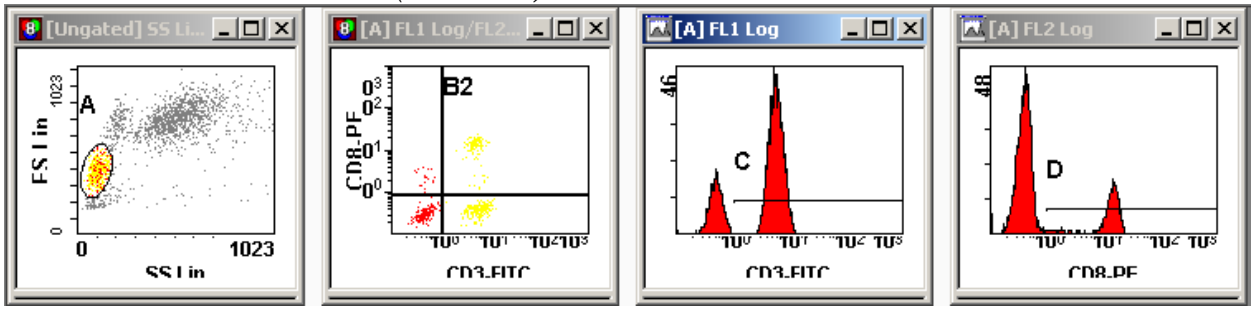
Tube 2 – Two Color Protocol (CD3 CD19)



Tube 3 – Two Color Protocol (CD3 CD4)



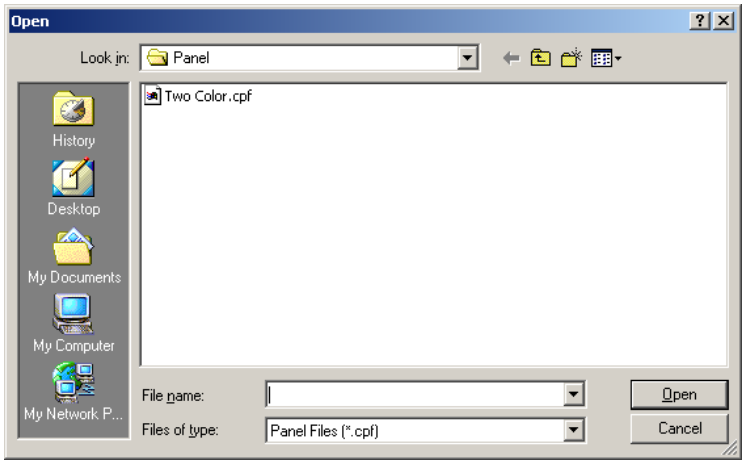
Tube 4 – Two Color Protocol (CD3 CD8)



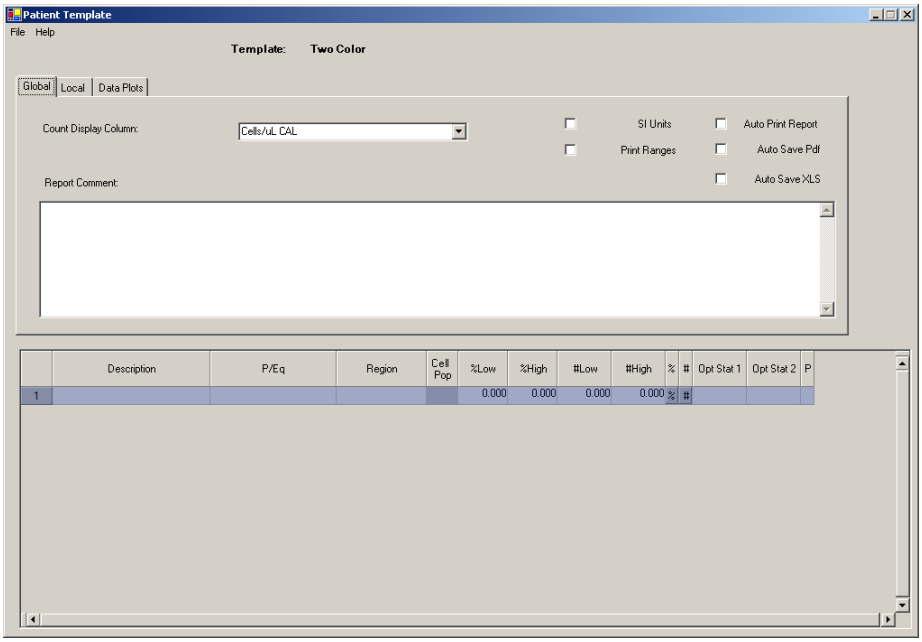
Note: You may wish to remove this page as you complete the next sections.

Reporting Results

1. Select the  icon on the Report Generator toolbar.
2. Select **File** >> **Open**. The screen should respond with a list of templates in the user subdirectory (including the Two Color blank template created in the previous exercise).

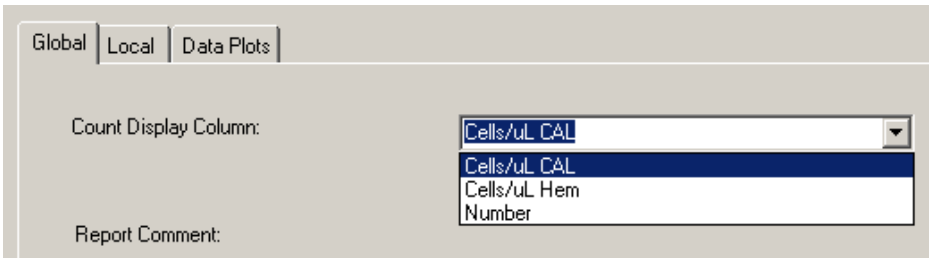


3. Select the desired template (e.g. Two Color.cpf) and then **Open**.



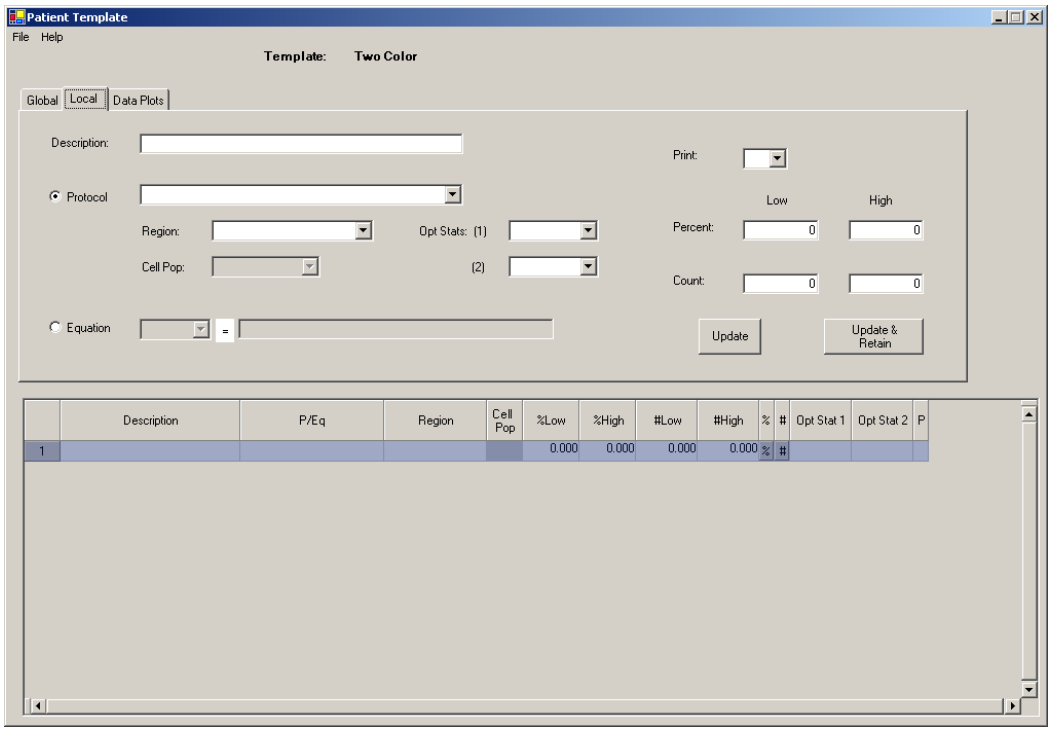
4. Select the desired global options for the entire report (e.g. Auto Print Report – automatically prints the report after the panel samples have been run).

5. Add a comment, if you wish, to be printed on every report.
6. Select desired Count Display Column option.



- Cells/uL CAL – absolute counts to be determined using Flow Count.
- Cells/uL Hem – absolute counts to be calculated using hematology values entered into the database.
- Number – report the raw counts in the regions.

6. Select the Local tab.

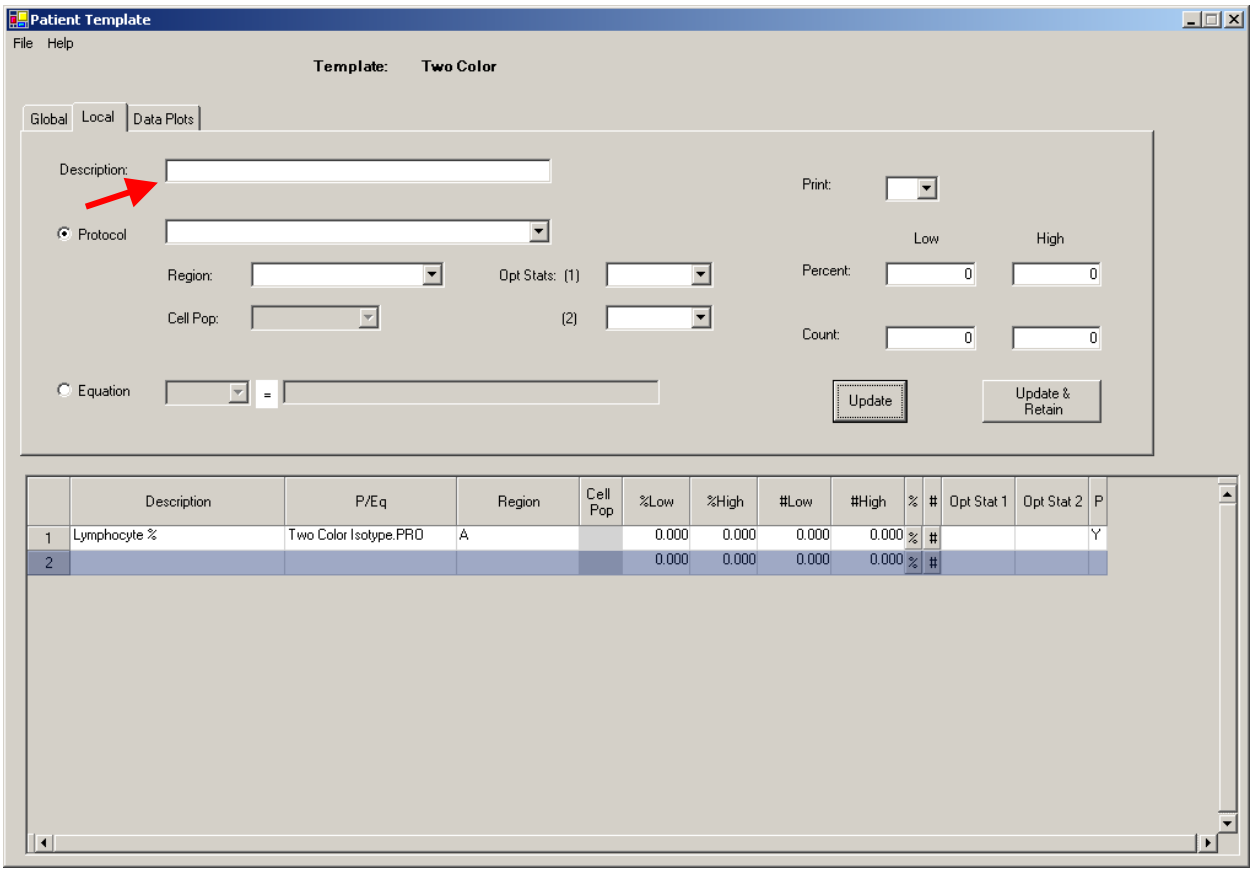


Now enter line by line, the data to report. We will do a line or two to give you the idea and then display a complete list.

7. In the Description field (red arrow), type in a descriptor describing what you wish to report (e.g. Lympocyte %). The Report line will print exactly as entered here.
8. Select the Protocol field and select the first protocol (e.g.Two Color Isotype.PRO).
9. Select Region and the region from which you wish to report data (e.g. A).
10. Select Print and whether you wish to print the result as part of the report (e.g. Y).

Note: B produces a blank line.

11. Select Update. The new line appears as shown below on the template.



Note: Update and Retain adds the new line but does not blank out the previously data entered fields. Use this option to save steps when similar information is going to be used in each line. You can edit the fields later.

Note: Cell Pop is blanked out because Cells/uL CAL was chosen on the Global screen. If Cells/uL Hem was chosen, this would be a viable option with the choices: LY, MO, NE, EO, BA, OTH, WBC.

12. Enter new description for line 2 (e.g. CD3).
13. Select protocol (e.g. first Two Color.PRO).

    Note: If the same protocol is used multiple times in a panel, the protocol list will have the same name more than once. The list will be in order, however, of the panel. This is the reason that it is a good idea to have printouts of your panel illustrating example data. That way you can more easily see what to print. In this example, we will set up to print the total CD3 and CD19 from the first Two Color.PRO, the CD3+CD4+ results from the second Two Color.PRO, and the CD3+CD8+ results from the third Two Color.PRO.

14. Select the Region (e.g. C).
15. Select the Opt Stats (e.g. X Mean).

    Note: 2 Opt Stats/region (Row) may be selected. The Opt Stats listed come from any of the following 10 selected in the protocol:

    X/Y Mean

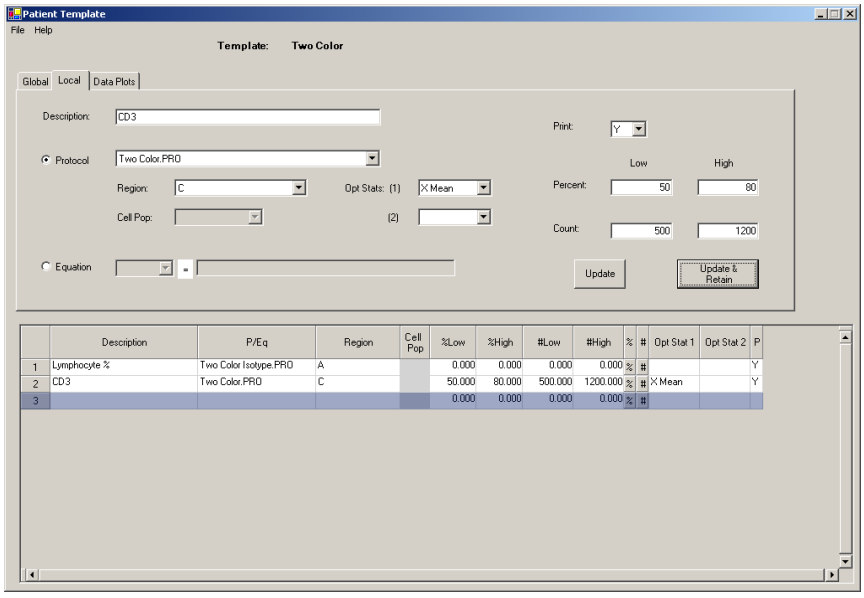
    X/Y Mode

    X/Y Median

    X/Y CV

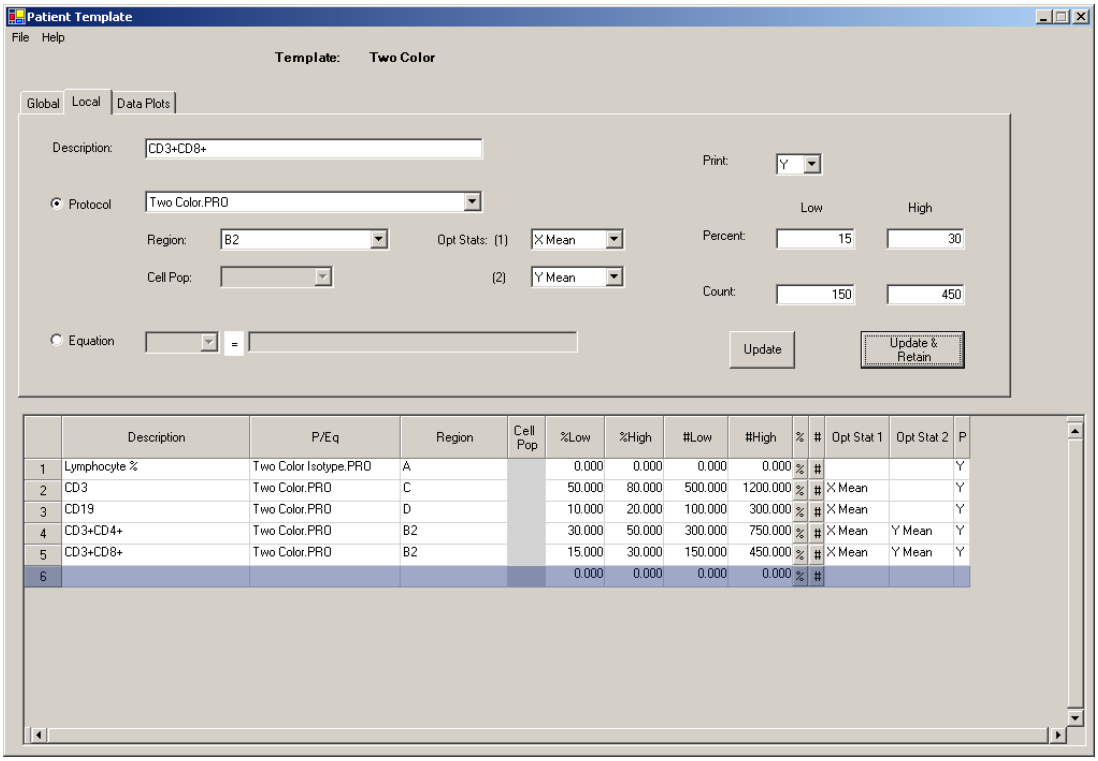
    X/Y HPCV

16. Select Print option (e.g. Y).
17. Enter, if desired, low and high per cents and low and high absolute counts.
18. Select Update and Retain. The screen should appear as below.



Note to User: List differences of update and Update & Retain. Why is one used over another?

After adding a few more lines, the template might now appear as below.



Adding Equations

Equations can also be added but they must follow a specific format. Rows are specified as R001, R002, R003, etc. Each operation must be enclosed within parentheses (). The common math operators are shown below.

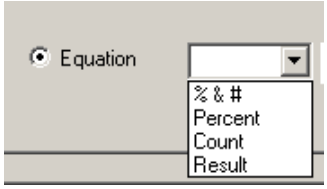
/ division

* multiplication

\+ addition

\- subtraction

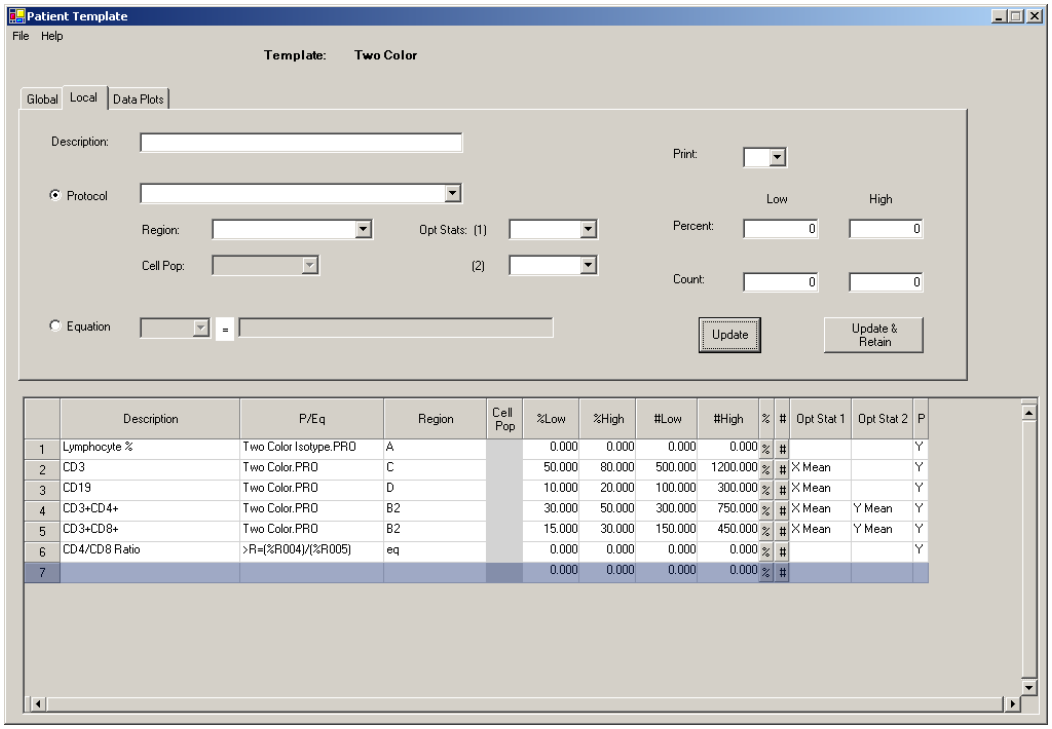
1. Enter description as before.
2. Select Equation. The Protocol option blanks out.
3. Select equation choice (e.g. calculate a ratio use Result, calculate an average, you might use any of the other three).



4. Now type in the equation. For example, if you wish to do a CD4/CD8 Ratio, you can use the result in line 4 expressed as: (R004) for CD4 and the result in line 5 expressed as: (R005) for CD 8. The equation should be entered as below:

   (%R004)/(%R005)

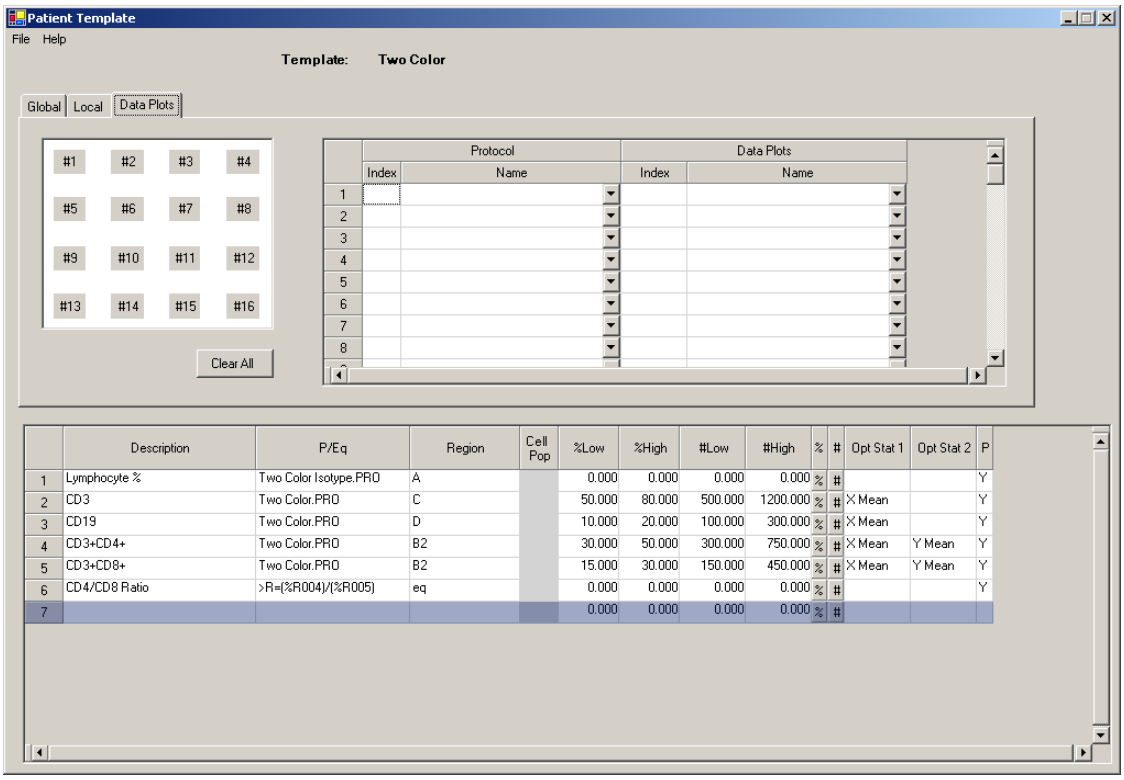
5. Now select Update. Your template should appear as below.



	Description	P/Eq	Region	Cell Pop	%Low	%High	#Low	#High	%	#	Opt Stat 1	Opt Stat 2	P
1	Lymphocyte %	Two Color Isotype.PRO	A		0.000	0.000	0.000	0.000	%	#			Y
2	CD3	Two Color.PRO	C		50.000	80.000	500.000	1200.000	%	#	X Mean		Y
3	CD19	Two Color.PRO	D		10.000	20.000	100.000	300.000	%	#	X Mean		Y
4	CD3+CD4+	Two Color.PRO	B2		30.000	50.000	300.000	750.000	%	#	X Mean	Y Mean	Y
5	CD3+CD8+	Two Color.PRO	B2		15.000	30.000	150.000	450.000	%	#	X Mean	Y Mean	Y
6	CD4/CD8 Ratio	>R=(%R004)/(%R005)	eq		0.000	0.000	0.000	0.000	%	#			Y
7					0.000	0.000	0.000	0.000	%	#			

Adding Plots

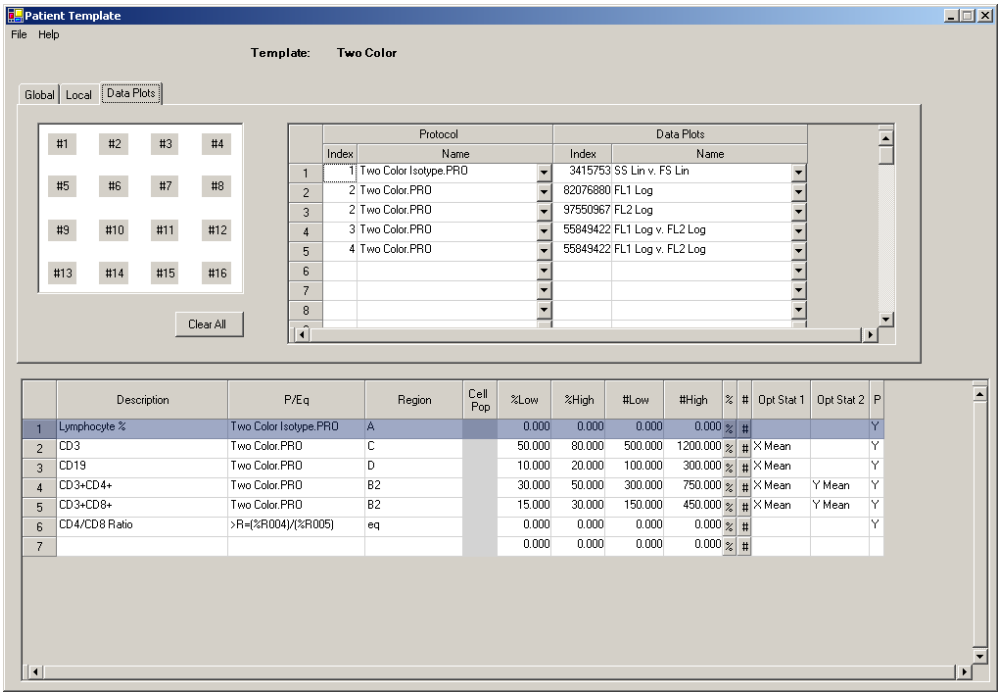
1. Select the Data Plots tab to add plots to your template. The screen appears as below.



At this point you really need to think through what needs to be on the template. You may not want to have every plot in the panel on the template. The gating plot, for example, may be the same for each protocol. Other plots may be used as visual checks while the sample is running but may not be important to a final summary of the data. In our two color example, we might wish to display one of the gating plots and then each plot from which the final data was taken. This list is shown below:

- Two Color Isotype.PRO – SS/FS dot plot
- 1st Two Color.PRO – Both Single histogram plots
- 2nd Two Color.PRO – FL1 Log/FL2 Log dot plot
- 3rd Two Color.PRO – Fl1 Log/FL2 Log dot plot

5. Select first block under Protocol Name and then the first protocol (e.g. Two Color Isotype.PRO).
6. Select the first block under Data Plot Name and then the desired plot (e.g. SS Lin vs FS Lin). The system now adds this plot to the template.
7. Select the second block under Protocol Name and select the next protocol (e.g. 1st Two Color.PRO).
8. Select the second block under the Data Plot Name and the desired plot (e.g. 1st single plot).
9. Repeat the process until all plots have been selected. The set of plots for our Two Color Template might appear as below.



Note: If you make a mistake and wish to start over, just click on the Clear All button.

Save Template

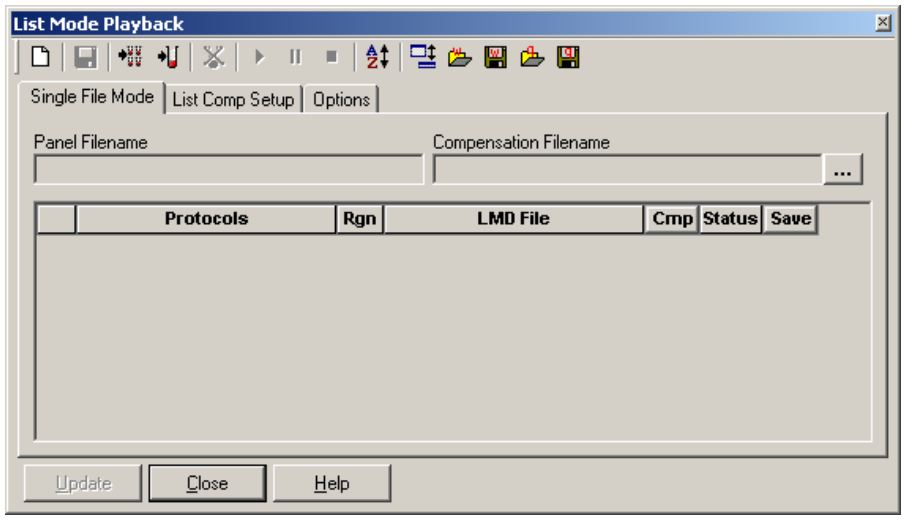
1. Select **File > Save** to save all your work. A message that the panel was saved successfully will appear. You will see the finished result when you generate a report in the next section.

PANEL REPORT

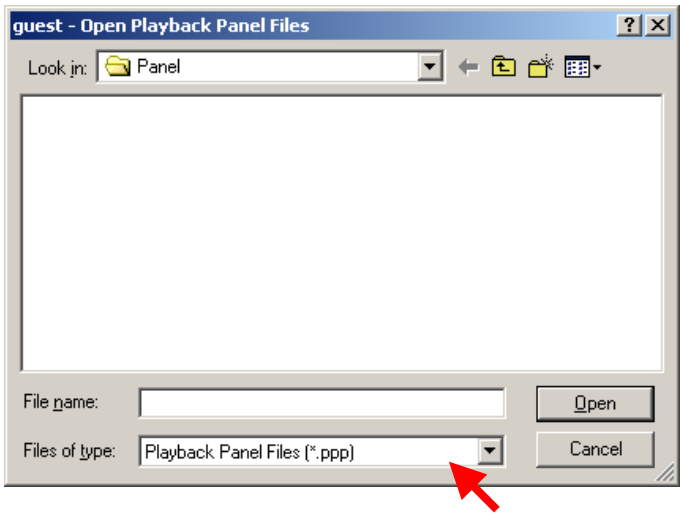
After samples have been run the report can be printed. This action is automatic if the template has been set to autoprint. You can also access the report and print it yourself. If you have not yet run any panels, you can generate a panel report by replaying listmode data with the following exercise.

Using Listmode Playback to Generate Panel Data

1. Select **Tools** > **Listmode Playback**. The screen responds as below.

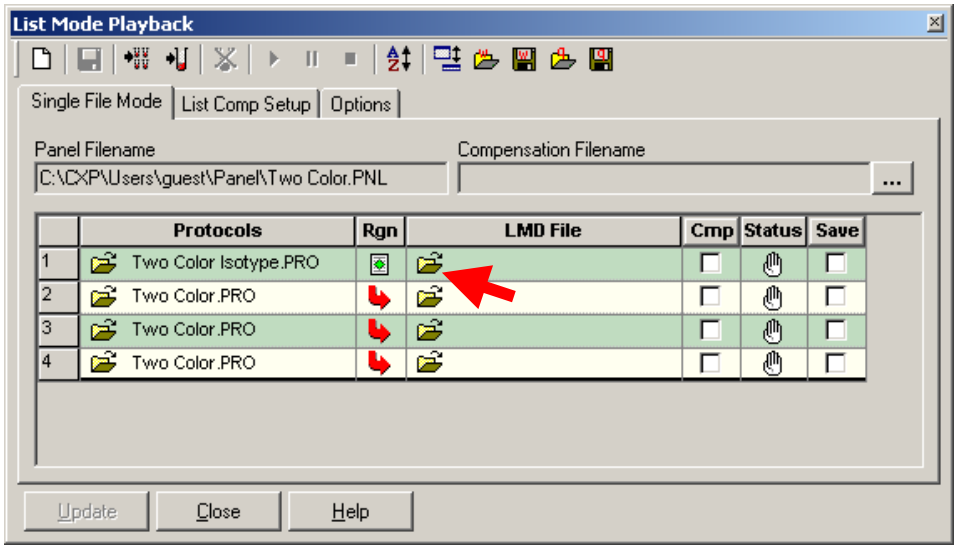


2. Select  icon. The screen will respond as below.

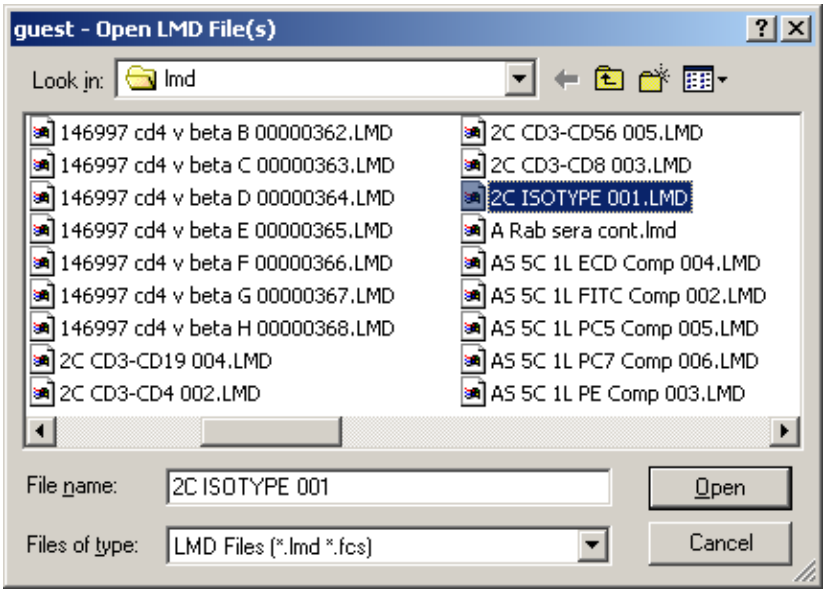


3. Select the Files of Type box and change the file type to PNL. The Two Color panel should now appear.

4. Select the Two Color.PNL and then the Open button. The screen will respond as below.

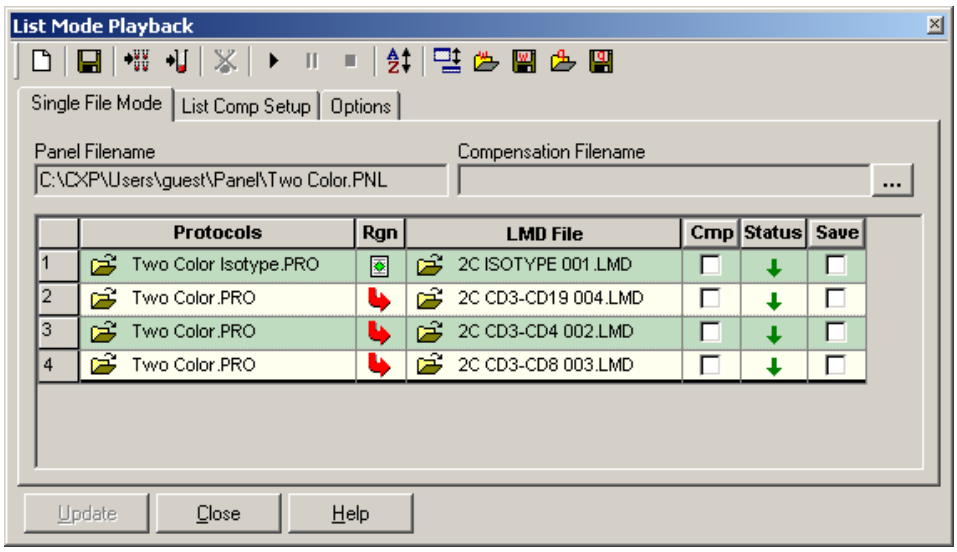


5. Change the Status icon to a green arrow by clicking on each line (Refer to Online Help for details on the Listmode Playback).
6. Now select the first LMD File (red arrow above) and then the 2 Color Isotype listmode file (see below) and then select **Open**.
7. Repeat the process for the other listmode files selecting in turn: 2C CD3-CD19 004.LMD, 2C CD3-CD4 002.LMD, and 2C CD3-CD8 003.LMD.

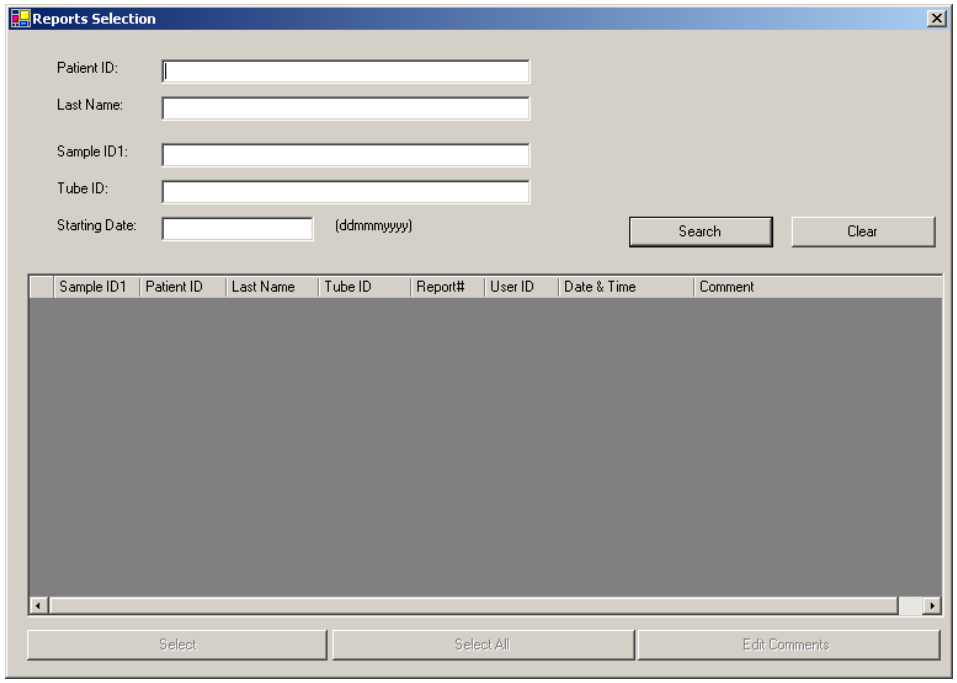


Note: You can select all the listmode files at one time using the Ctrl key. If you select the first file, then hold down the Shift key and select the last file, all the files from the first to the last will highlight.

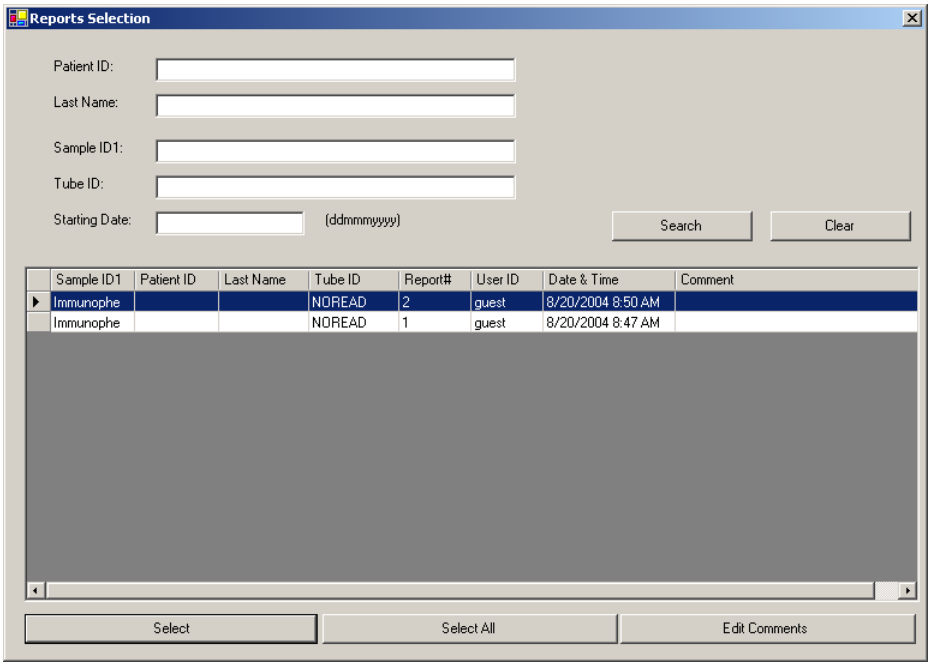
8. Repeat the process for the other listmode files in turn so that the final Listmode playback appears as below.



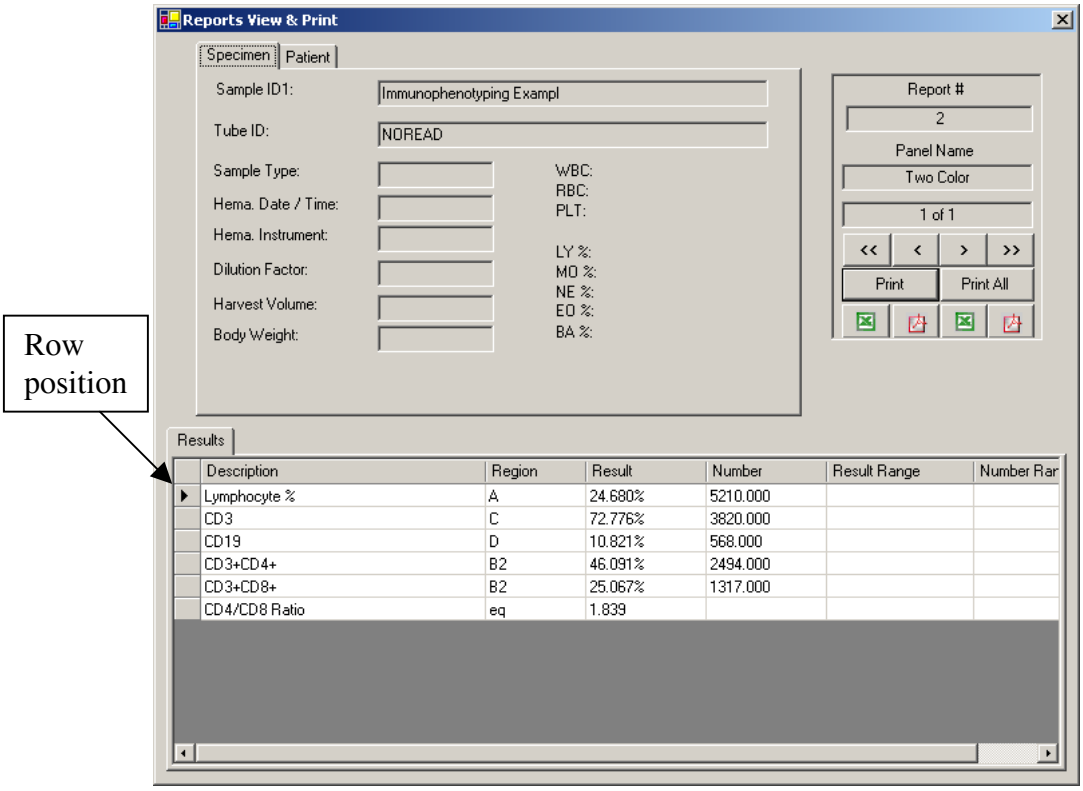
9. Select the  tool to play the listmodes through the panel and output for the report.
10. Select the **Close** button.
11. Select the  icon on the Report Generator Toolbar. The screen responds as below.



12. If you know the sample information, you can enter the information and then select Search or leave all fields blank and select Search to get a global list. The screen responds with a list.



13. Select an entry by its row position (e.g. the most recent) and then the **Select** button. The screen reponds with the report.



14. Select Print button to print the result. Your Two Color panel will appear as below.

CXP Panel Report: **Beackman Coulter**

8/20/2004 8:51:50AM Listmode Playback

Sample ID1: Immunophenotyping Exampl Name:

Panel Name: Two Color Patient ID: D.O.B.:

Panel Complete: Y Match: Y FC 500 SN: AE50004

LMD File Name(s): CXP v 2.0:

C:\CXP\Users\admin\lmd\2C ISOTYPE 001.LMD

C:\CXP\Users\admin\lmd\2C CD3-CD8 003.LMD

Collection Date: User ID: guest

Analysis Date / Time: 20Aug2004 8:50 am Tube ID: NOREAD

Sex: ID#: Hematology Date /Time:

Physician: Hematology Instrument:

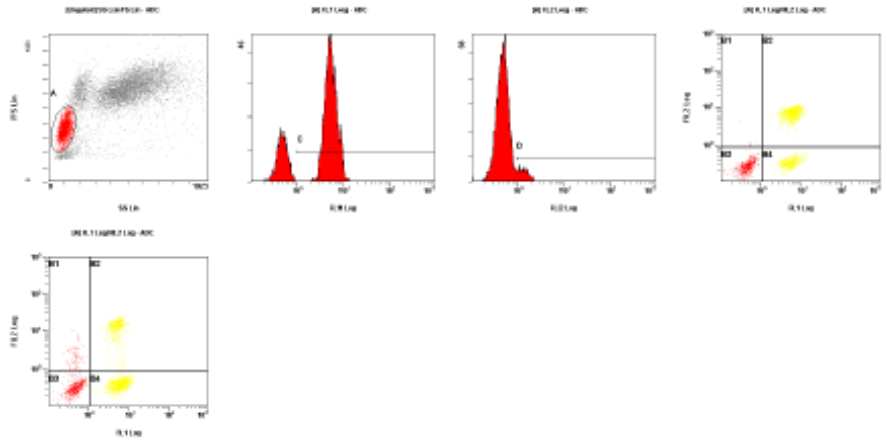
Sample Type: WBC: LY %:

Dilution Factor: RBC: MO %:

Harvest Volume: PLT: NE %:

Body Weight: EO %:

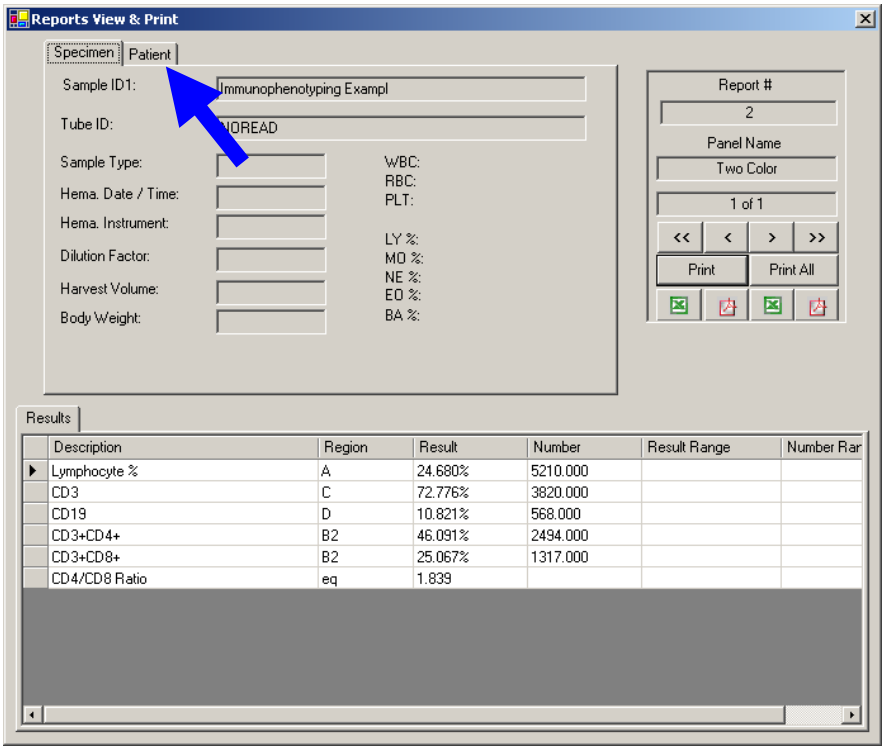
BA %:



Description	Region	Result	Number	Opt Stat 1	Opt Stat 2
Lymphocyte %	A	24.680%	5210.0		
CD3	C	72.776%	3820.0	XMean:5.77	
CD19	D	10.821%	568.0	XMean:1.56	
CD3+CD4+	B2	46.091%	2494.0	XMean:6.33	YMean: 7.26
CD3+CD8+	B2	25.067%	1317.0	XMean:5.21	YMean: 13.0
CD4/CD8 Ratio	eq	1.839			

Page 1 of 2

Signature _____



15. You can select the Patient tab (blue arrow) to view any patient information previously entered into the database.

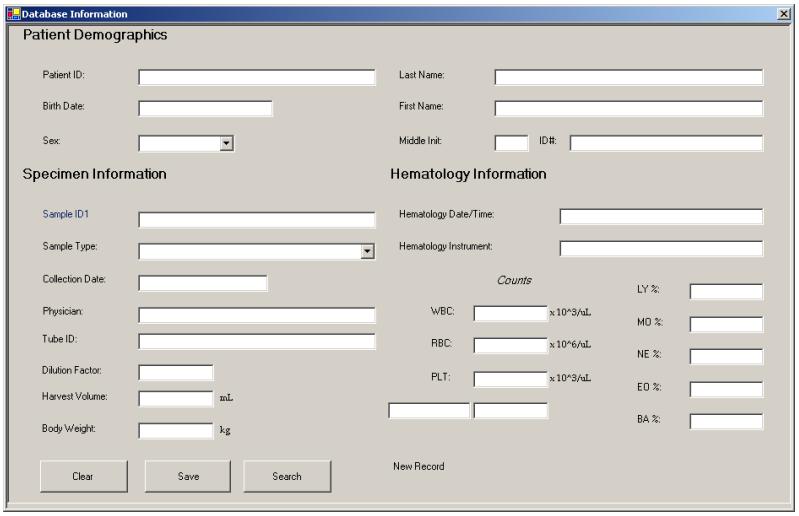
Additional Notes:

- Use the <<, <, >, >> options to change to a different report when multiple reports have been selected.
- Use the  button to select to print to a PDF file.
- Use the  button to export the file as an .xls file.

DATABASE ENTRY

This option allows you to enter patient demographic information prior to acquisition or listmode replay which the system will add to the patient report before printing.

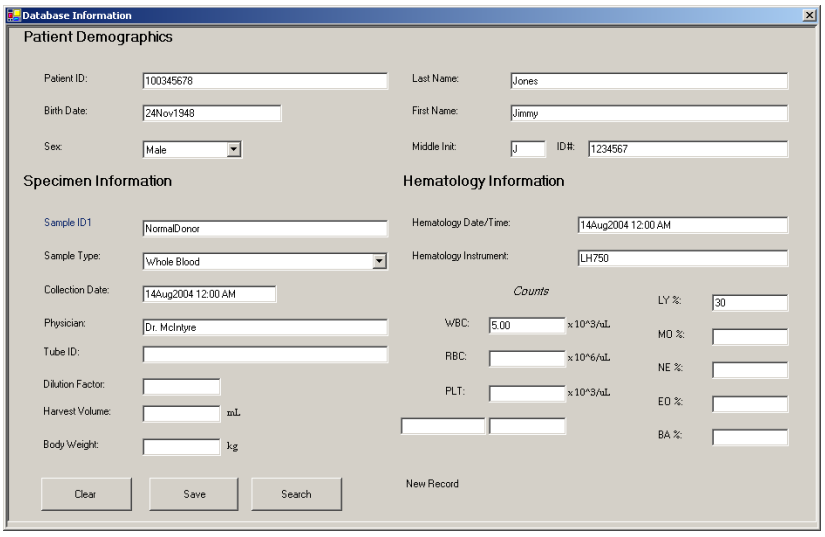
1. Select the  icon. The system responds as below.



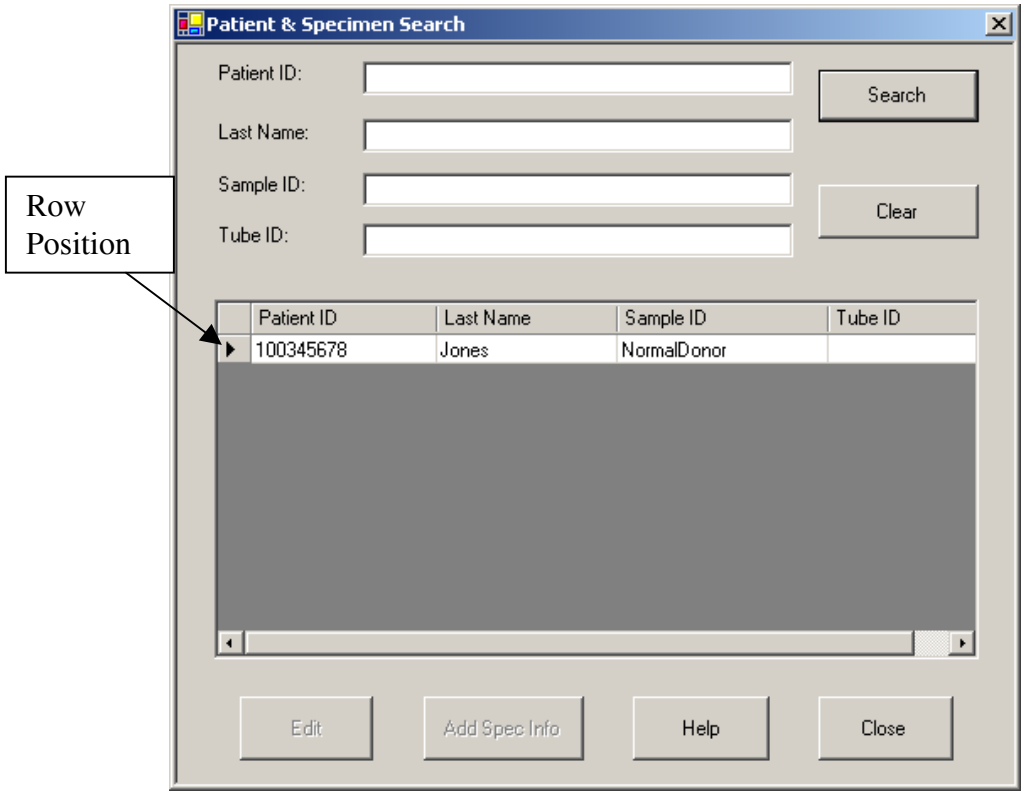
2. You can now enter information into any of the fields you desire.

Note: The Sample ID is a required field for entry. This field links to the Sample ID1 entered in the Acquisition Manager. If they do not match, the system will be unable to use the entries in a report or compute cells/uL

Example:



3. If you wish to add information to a sample already in the data base, select **Search**. The system lists the patients in the database.



4. Select the desired patient by its row position and then **Edit**. The system displays the Database Information window.

Note: If you select Add Spec Info button instead of Edit, only the Patient Demographics will be retained. The Specimen Information will clear to allow the entry of new information (Refer to the tetra System Guide or online Help).

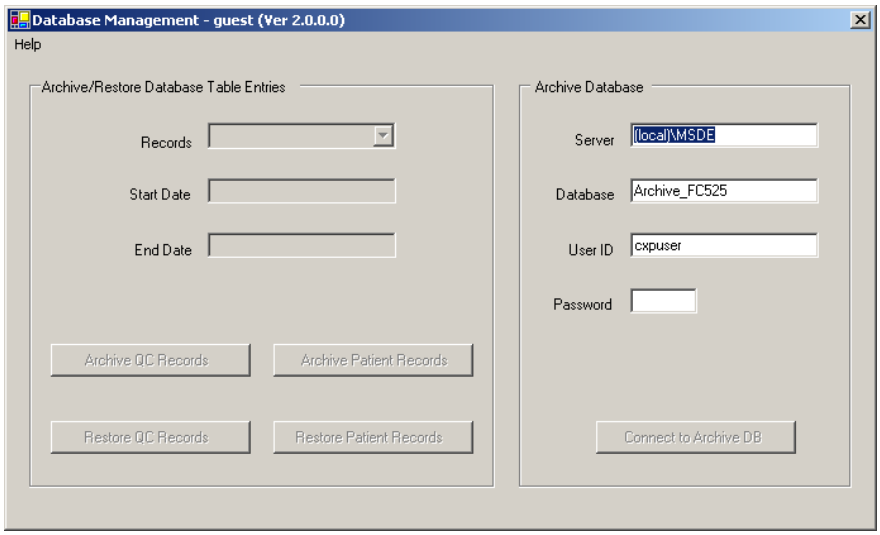
5. You can now enter additional information and select **Save**. The system saves the information. If the sample is now run or replayed, the information will appear in the report.

Note: you must run the sample or replay the listmode files through the panel using the Listmode playback tool to generate a new report that includes the added demographic/hematology information.

DATABASE MANAGEMENT

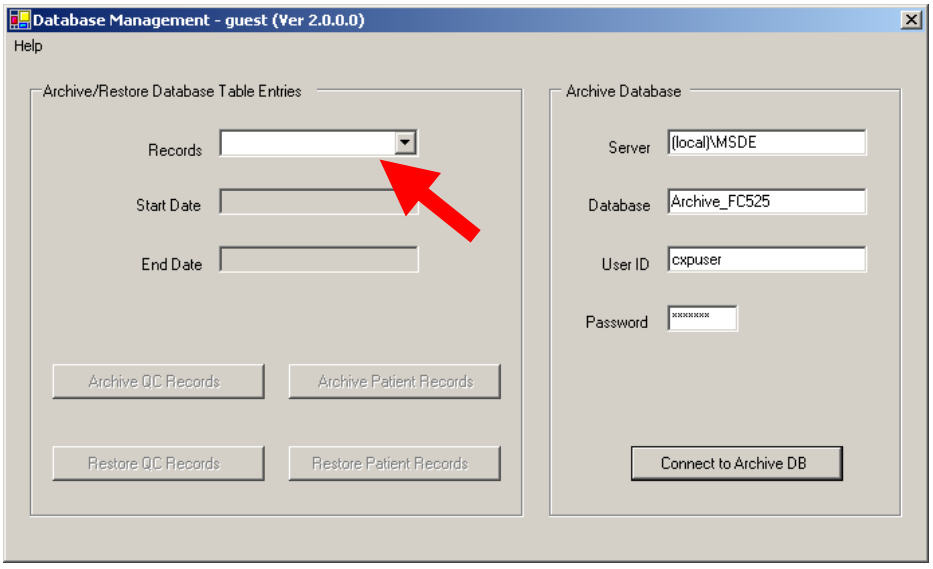
The CXP software has the ability to archive and restore patient records and QC records. The system defaults to a local archive, but in networked systems the server could be changed.

1. Select the  button. The system responds as below.

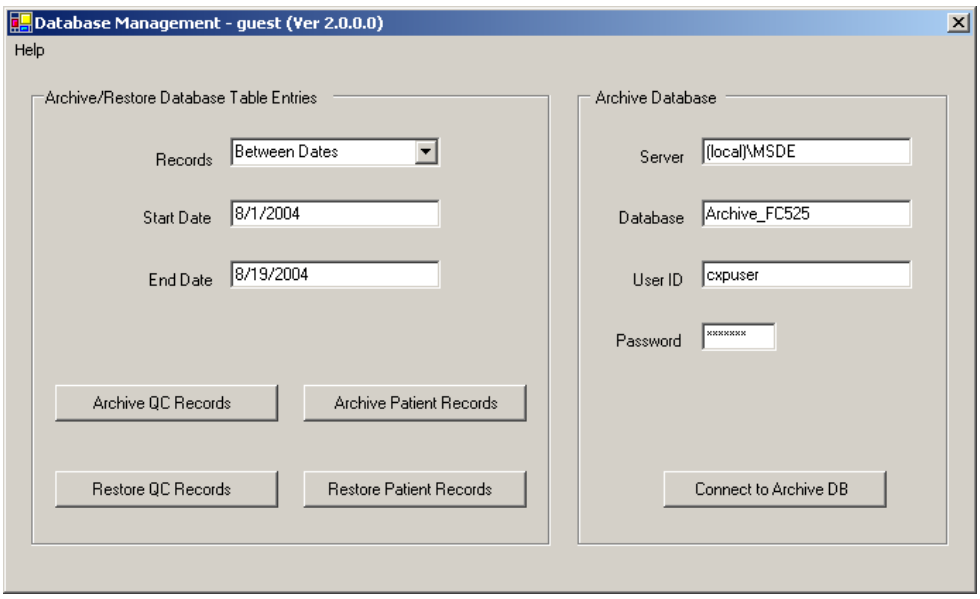


The system defaults to a local server. If you are on a network, this could be changed to the network server.

2. Enter “cxppass” as the password.
3. Select **Connect to Archive DB** button and then **OK**.

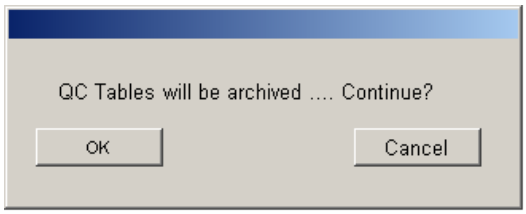


4. Select Records and then either Before Date or Between Dates (red arrow).
5. File in the Start Date and if needed the End Date.



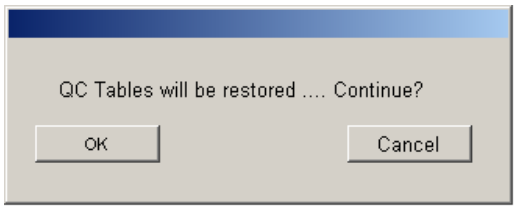
6. Now select either Archive QC Records or Archive Patient Records and then **OK**.

Example:



7. To verify the records are achived, attempt to search for them using the Panel Report option.

8. If you wish to restore the records, select the desired option button and then **OK**.



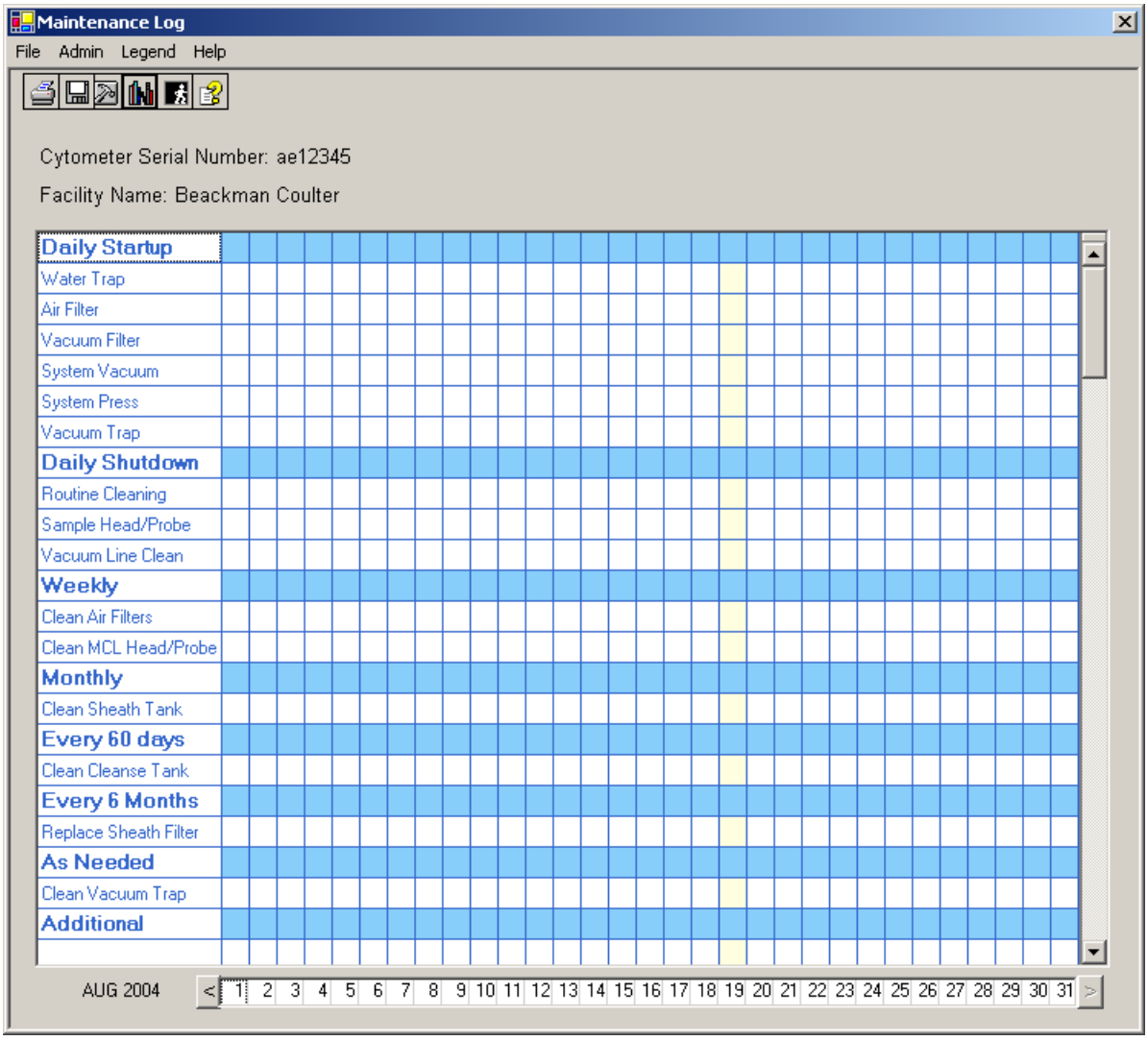
9. To verify the records are restored, search for them using the Panel Report option.

MAINTENANCE LOG

Use this option to log all maintenance procedures completed on the system.

1. Select the  icon on the Report Generator toolbar. The system responds as below:

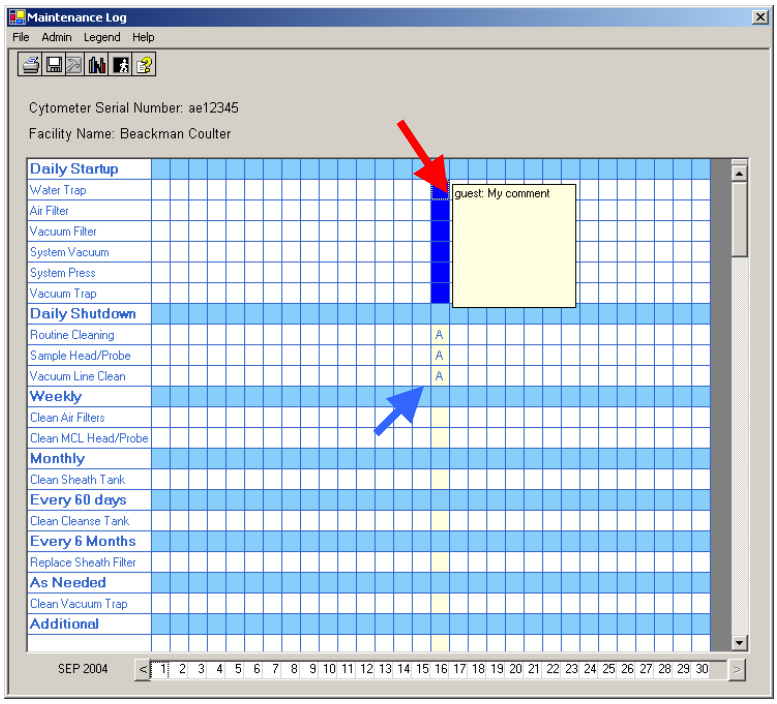
Note: Today's date column will highlight in yellow.



2. Double click on each block to log completion of the task for the appropriate date. The box will change to the operator color (e.g. blue for Guest).
3. If you right click, you can insert a comment, delete, or save an entry.

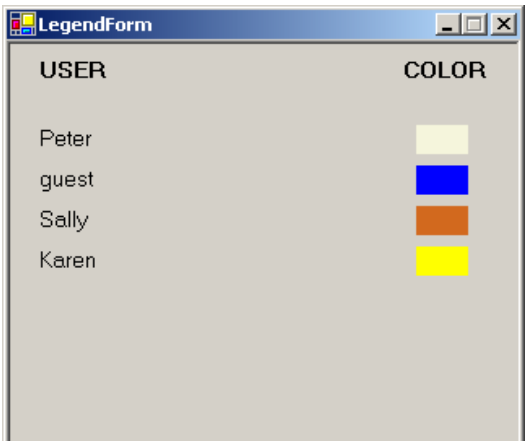
Your log might appear as below.

Note: The red dot on one of the boxes (red arrow) indicates there was a comment entered. A left click on the box will reveal the comment.



4. Select **File** > **Print** for a hardcopy.

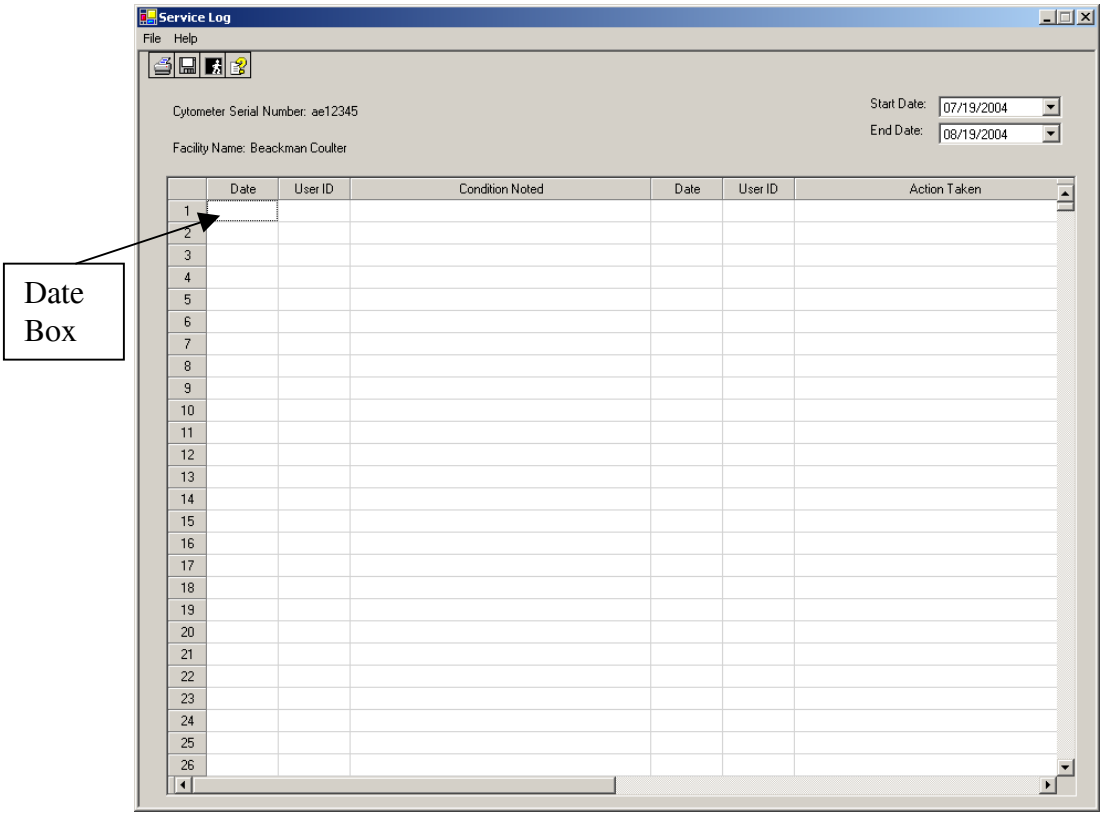
Icons:

 - Print	Legend: 
 - Save	
 - Log on as administrator (the letter A will appear in entries entered by an administrator blue arrow above). The administrator is able to change any previously selected cell, any user, any date.	
 - User color legend	
 - exit	
	

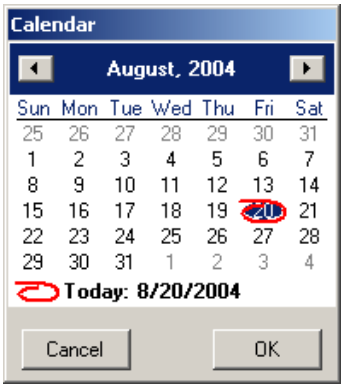
SERVICE LOG

Use this option to log conditions noted on the system and any actions taken.

1. Select the  icon on the Report Generator toolbar. The system responds as below:

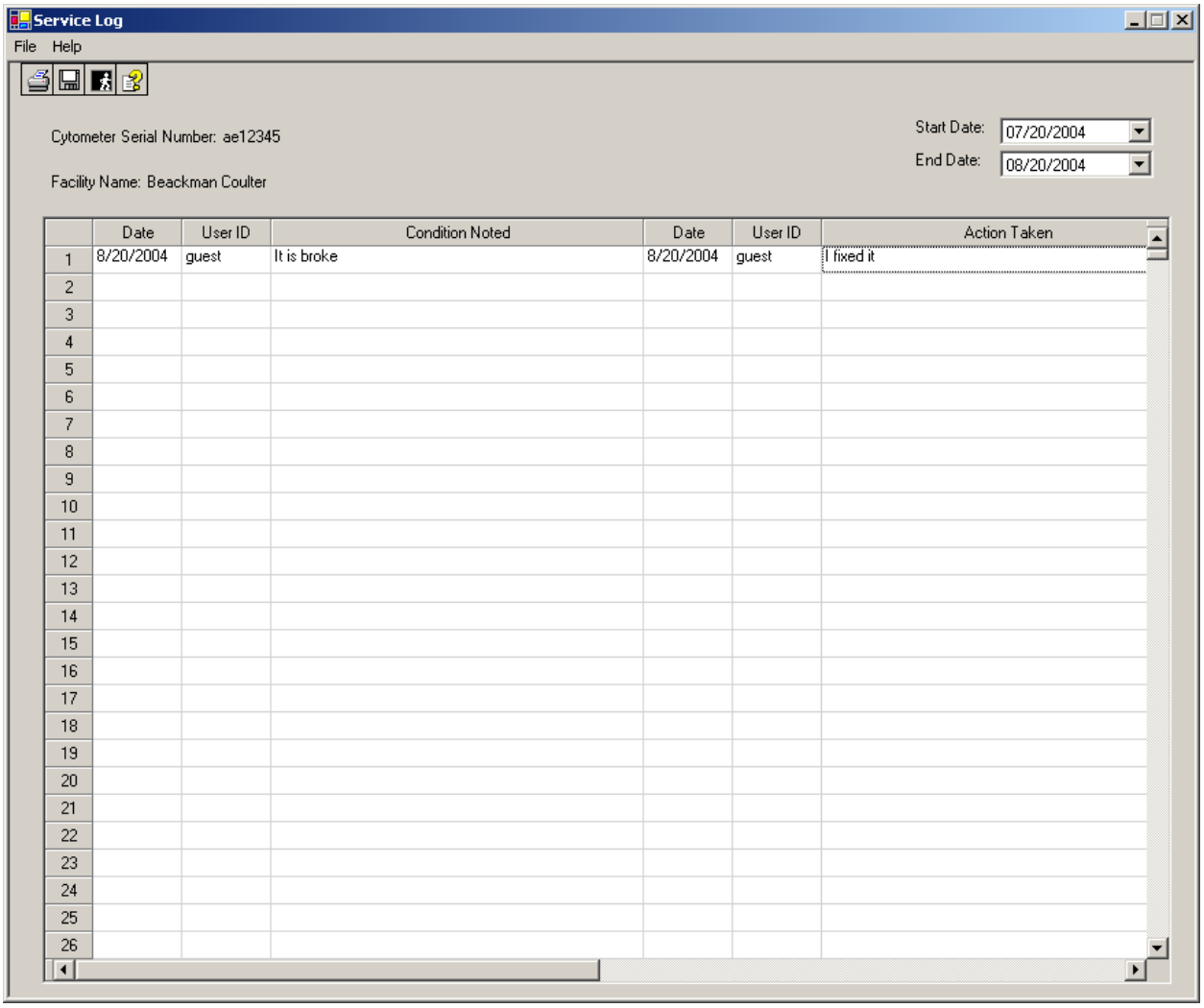


2. Double click on the Date box. A calendar appears.



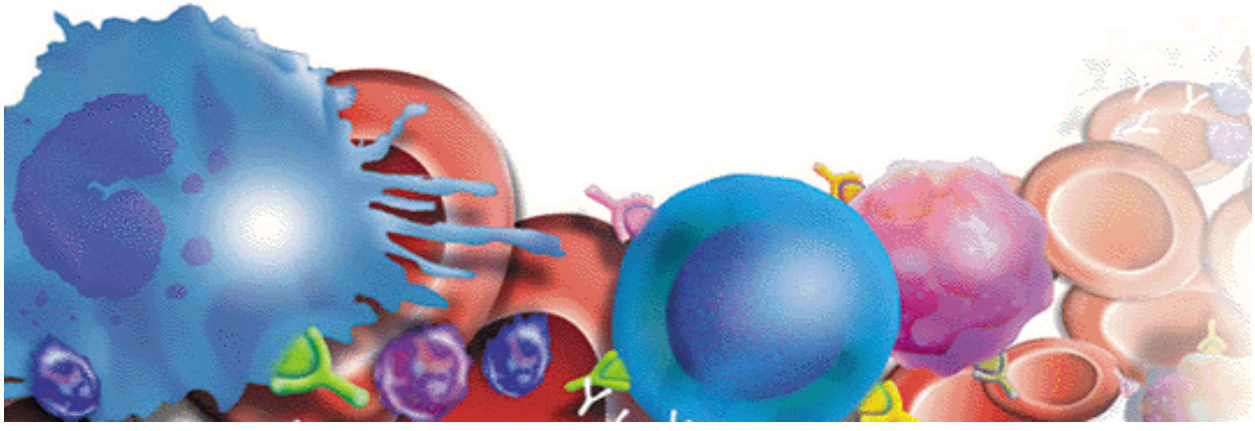
3. Select date or accept todays date and then select **OK**. The system enters the present logged on user under the User ID column.

4. Double click on the box under Condition Noted or Action Taken to type in text. An example entry is shown below.
5. Select **File** > **Print** for a hardcopy.



You have now completed this module. When you think you are ready, please complete the Database Skill Check for this module.

Table of Contents



OBJECTIVES

The following objectives can be completed for 2 Color, 3 Color, 4 Color, or 5 Color. The objectives can be met in 4 stages with credit for each stage or if you feel you are able, stages can be skipped (ex. 2 Color) and a higher level completed (ex. 3 Color). If you successfully complete a greater number of colors, credit will also be given for fewer colors (ex. Complete 3 Color, credit will also be given for 2 Color). For each application given an operational flow cytometer, proper reagents, blood samples, and access to reference materials and/or online Help

- Schedule the appropriate application (e.g. 2 Color, 3 Color, 4 Color 1 Laser, etc.) to generate the settings to be used for running unknown samples.
- Prepare the samples indicated by the carousel load list and run the samples.
- Create an application protocol (e.g. 2, 3, or more color samples) using the instrument settings generated by the scheduled application with proper gating and analysis.
- Prepare and run at least 3 samples with the created protocol.

WHY IS IT IMPORTANT?

This module examines the cell surface marker application with an emphasis on whole blood samples. It introduces the concepts as well as guides you in setting up the application on a system. Experienced instrument operators may be able to translate the general instrument protocols into specific protocols for any flow system. Instrument specific procedure guides are also available for those who are new to a system.

Skill Check Preview

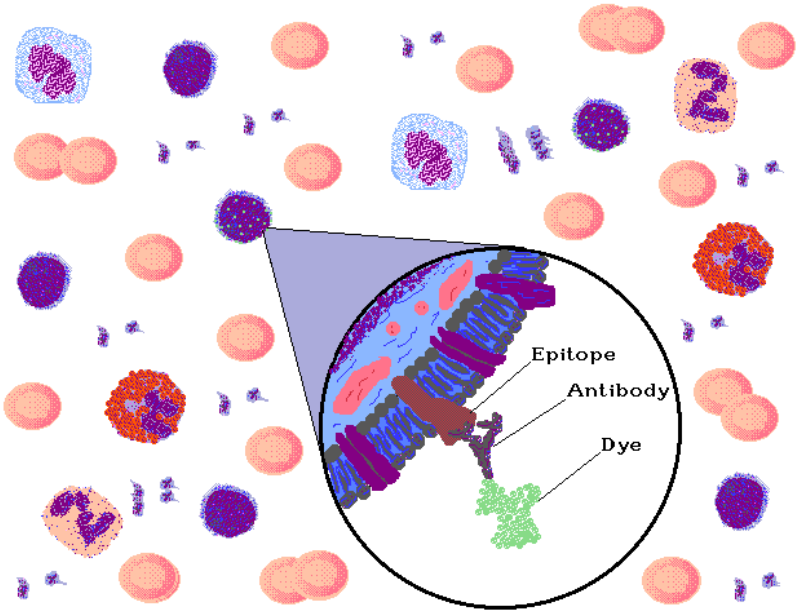
You will have mastered the application when you can

- Run and print results obtained by running a scheduled application (e.g. 2 Color, 3 Color, 4 Color 1 Laser, etc.).
- Create an application protocol (2 Color, 3 Color, etc.) and demonstrate to you facilitator that you are using the settings established from running the scheduled appropriate application (e.g. 2 Color, 3 color, etc.)
- Run and print at least 3 samples run with a created protocol illustrating proper gating and analysis.

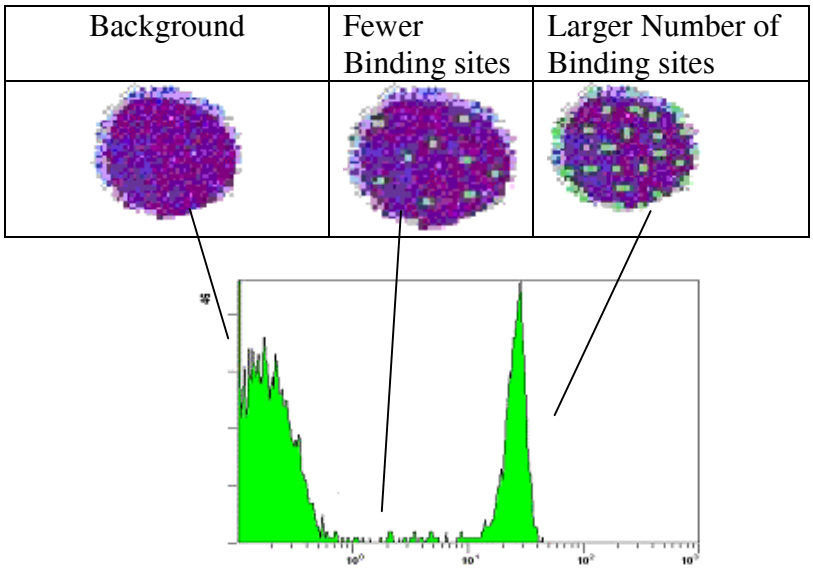
INFORMATION / PRACTICE SECTION

Concepts

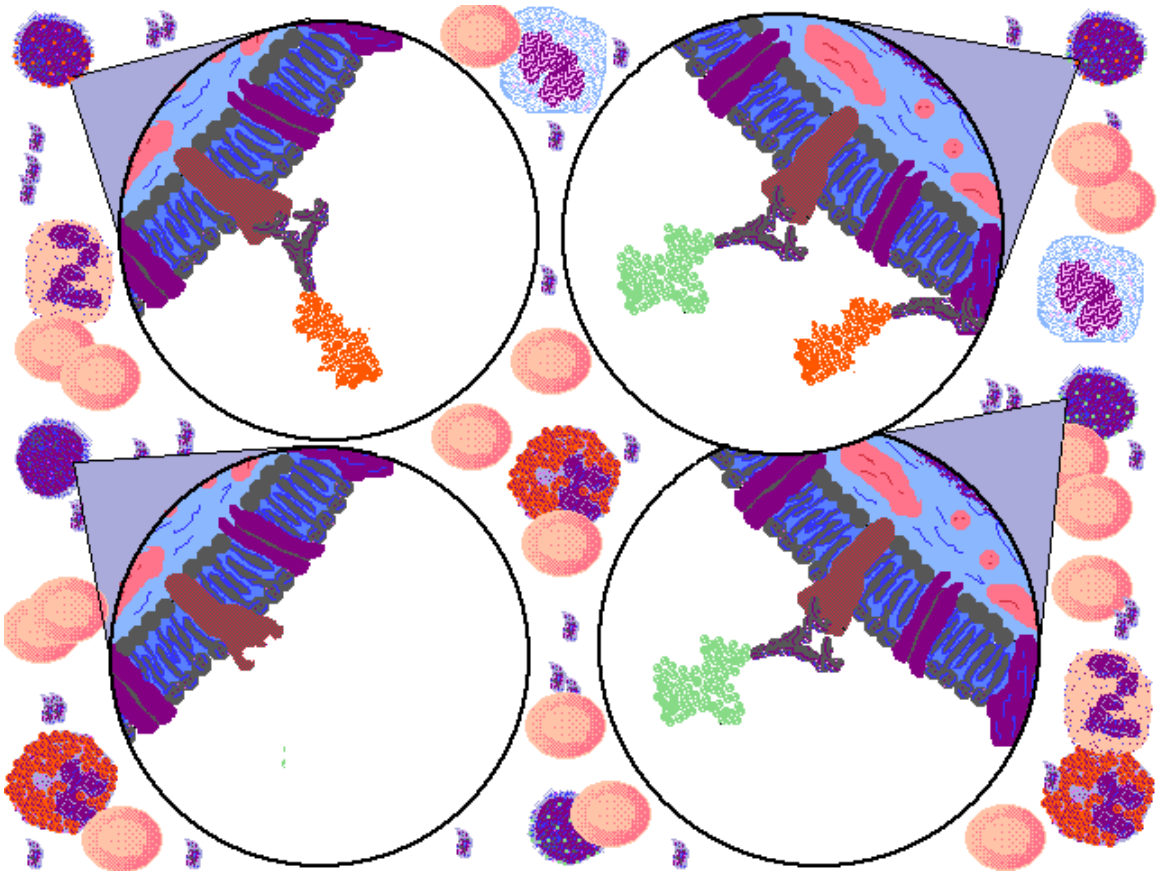
In this application, a fluorescent dye is bound to an antibody and the antibody binds to specific targeted cells. Cells binding antibody fluoresce brighter than those not binding antibody. The fluorescence intensity also indicates the number of antibodies binding to the cell.



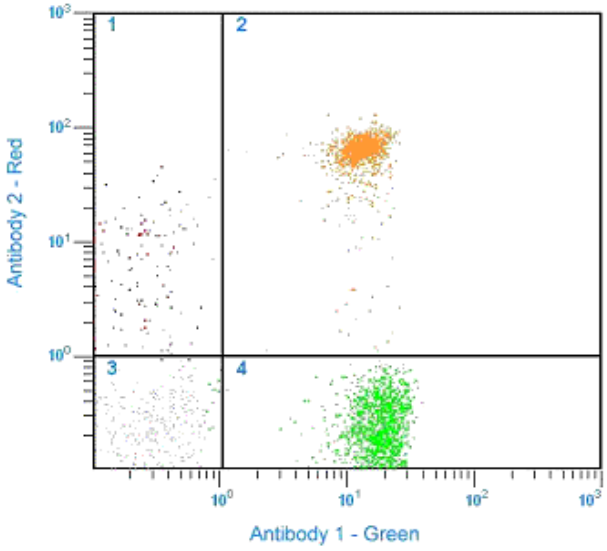
A single fluorescence histogram might appear as below:



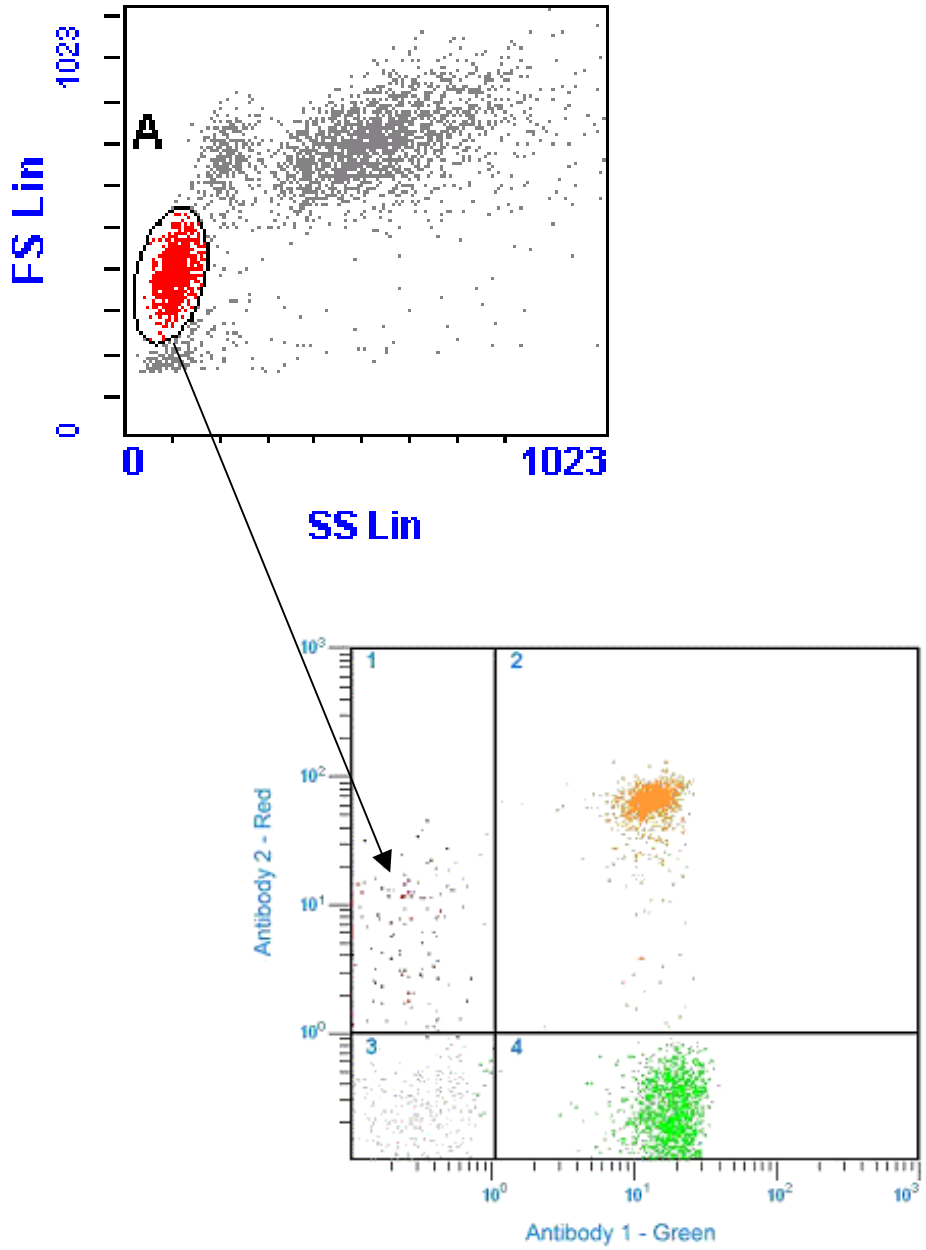
Multiple antibodies, each with a different dye, may also be added to cells to provide more information. Two antibodies are illustrated below:



The resulting two color histogram might appear as below:



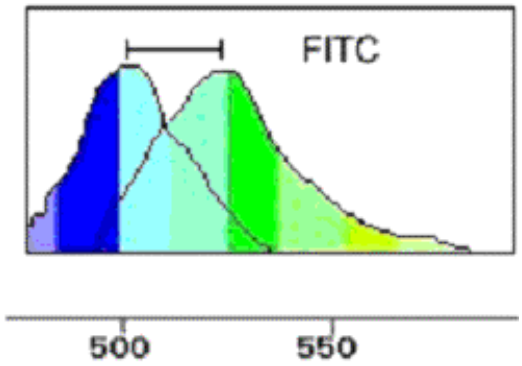
Other measurements, such as forward and side scatter, may be used to isolate a particular population for antibody analysis.

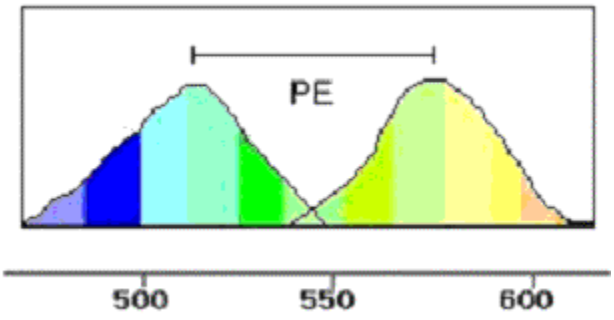


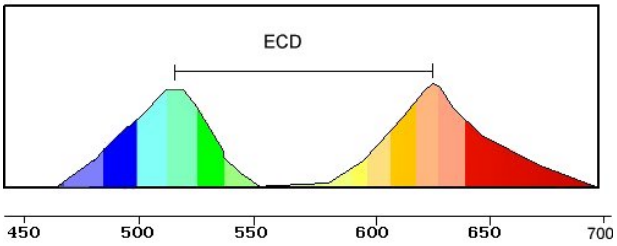
Additional antibodies and dye combinations can be used to increase the number of simultaneous measurements.

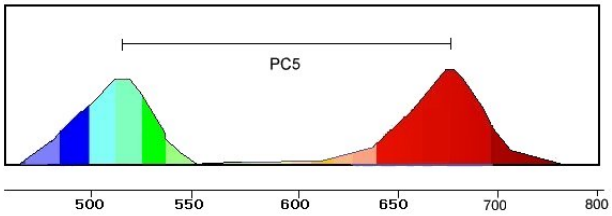
Dyes

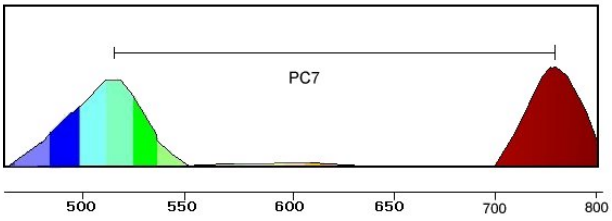
Some of the more common dyes which can be bound to antibodies are illustrated below. All dyes except APC can be excited by the Argon 488 nm laser. APC excites by a HeNe laser at 633 nm. In addition, the HeNe can excite Cy5 (not shown).

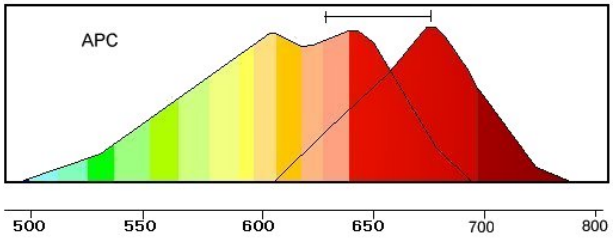


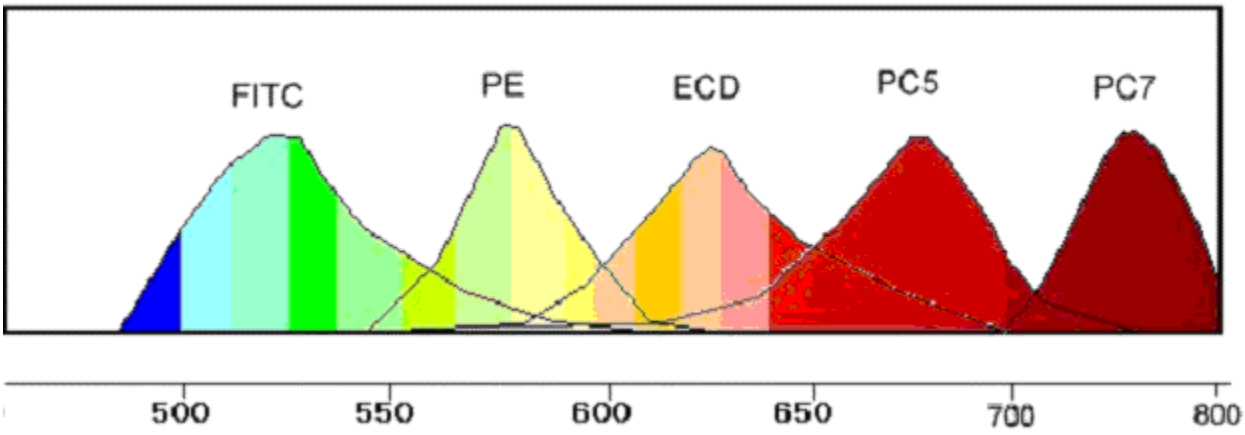








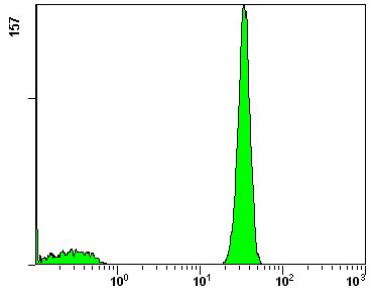




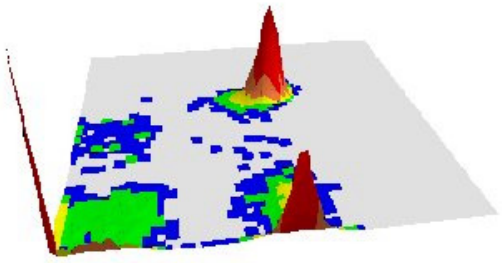
Prism

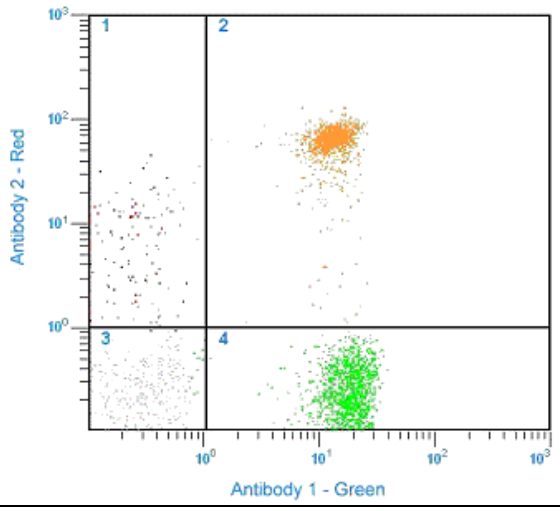
Single color and two color histograms are easily represented as shown below. However, multiple colors beyond two cannot be easily represented in two dimensional space.

Single Color



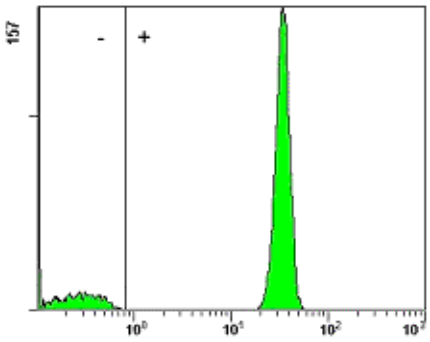
Two Color





3, 4, 5 or more Colors?

If we observe each antibody binding individually, a dividing channel can be determined separating the antibody binding cells (positives) from those that do not bind antibody (negatives). See below:

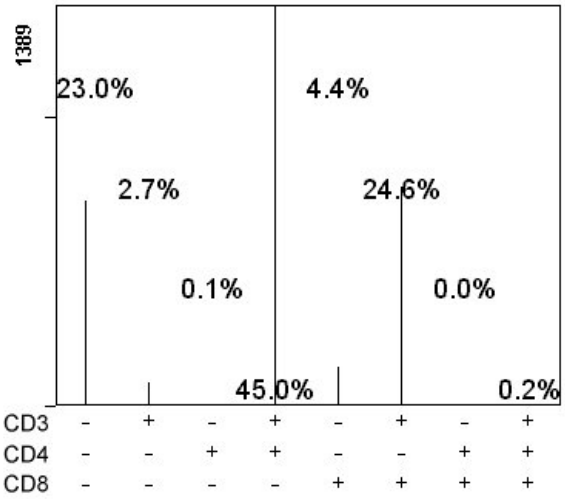


When the parameters to be considered and the corresponding dividing channels are specified, the system can then keep track of the number of cells binding each possible combination of antibodies. See example below:

3 Color Example

Phenotype	Antibody 1 (CD3)	Antibody 2 (CD4)	Antibody 3 (CD8)	Count
A	-	-	-	710
B	+	-	-	83
C	-	+	-	1
D	+	+	-	1389
E	-	-	+	136
F	+	-	+	759
G	-	+	+	0
H	+	+	+	6

The data can then be plotted as a bar graph known as a PRISM histogram. This histogram, while only displaying 8 channels of data, can be gated like any other histogram. Multiple colors beyond 3 would simply increase the number of channels collecting data. The system is capable of handling up to 7 colors.



NOTE: To set up the Prism plot

- Select the  to create the plot, select desired gating, and **OK**.
- Use the  (histogram plots) or the  (dot plots) to set the divider channels for each fluorescence parameter.

ABSOLUTE COUNTS

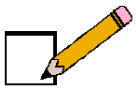
Absolute counts can be determined by adding a specific volume (100ul) of a known concentration of Flow-Count ™ Fluorospheres to a sample. These fluorospheres are detected using an extra dot plot (Log FL1-4 vs FS). The assayed concentration is entered into the software. The system can then calculate and report the absolute count for any desired population using the formula shown below.

$$\text{Absolute Count (cells/uL)} = \frac{\text{Total Number of Cells Counted}}{\text{Total Number of Fluorospheres Counted}} \times \text{Assayed Concentration}$$

Example:

$$\frac{\text{3000 cells}}{\text{2040 Fluorospheres}} \times 1020\ /\text{uL} = 1500 \text{ absolute count}$$

PRACTICE



Sample Preparation

COULTER® Q Prep™ and TQ-Prep™ Workstations

1. Add appropriate mAB combination to the sample tube per package inserts.
2. Add 100 µl of blood (if WBC count is believed to be abnormal, verify with hematology counter and refer to package insert for handling).
3. Vortex the tube.
4. Incubate per package inserts.
5. Prep on the COULTER TQ-Prep workstation or related multi-tube system.
6. If absolute counts are to be determined, add 100 uL of Flow-Count ™ Fluorospheres (make sure the bottle has been vortexed 10-12 seconds) to each tube, post lyse.

Whole Blood Prep and other Preps

1. Refer to antibody package inserts.

QUALITY CONTROL

1. Run Flow-Check Fluorospheres to check fluidics and optics

2. Run Flow-Set Fluorospheres to standardize the system. The bead peak channel should fall within the range set for your system (See the Appendix for information on how to determine a range). If they do not, adjust the high voltage to move the bead peak channel to the reference value. Use these high voltage values on all unknown samples.

NOTE: If the reference values have not been determined, see Establishing Standard Reference Values in the Appendix.

3. Run biological sample(s) to:

   - Adjust fluorescence compensation when two or more dyes are used and fluorescence overlap exists.

Manual ADC Compensation	**Automated Advanced Digital Compensation**
▪ Use dual color mutually exclusive populations (i.e. CD4/CD8) ▪ Look for mean equivalency between two negative populations	▪ Single tube methodology to determine true fluorescence bleed over (i.e. CD45 in each fluorescence tube) ▪ Measure single tube and bleed over into other PMTs (i.e. CD45 FITC) ▪ Most accurate form of color compensation available.
Refer to the Quality Control module for details on how to compensate.	

   - Check instrument accuracy using pre-assayed cells (e.g. Cyto-Trol or Immuno-Trol) with known antibodies.

TYPICAL PROTOCOLS

NOTE: Refer to your software Creating a Protocol procedure guide for details on how to create the protocol within your software. The appropriate sections discussing each step will be labeled as the headings below.

Selecting Parameters

FS, SS (or Log SS), and log signals for each dye emission color.

Creating Plots

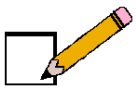
- A dot plot for gating purposes such as SS vs FS or CD45 vs SS
- Dot plots illustrating all antibody-dye combinations
- Single histogram plots where desired
- A Prism plot if more than two colors are represented.

Creating Regions

- Polygon used for gating purposes
- Quad-Stat and linear regions for analysis

Defining Gates

Based on SS vs FS or an antibody (e.g. CD45) vs SS plot isolating a particular population for analysis. Other histogram plots are gated on the isolated population.

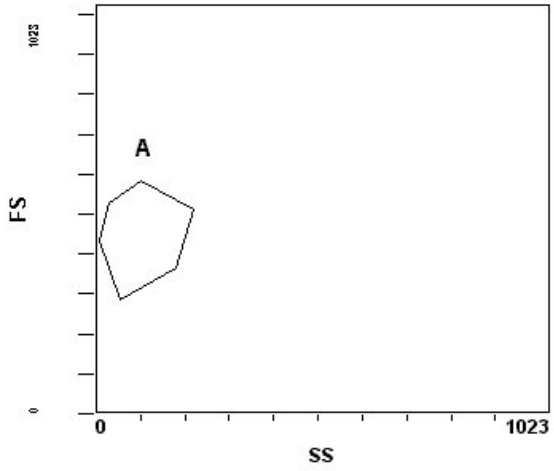


Analyze Data

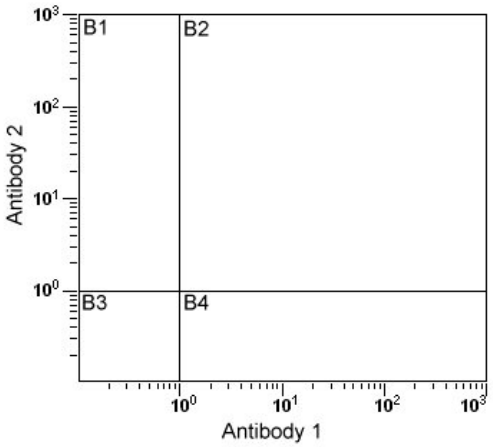
Quadrant regions set on dot plots and single regions set on single parameter plots to determine the % positive cells and the mean fluorescence channels. When more than two colors are assayed, % positives may be determined using a Prism histogram plot. Some software allows the operator to choose the desired statistics.

Examples

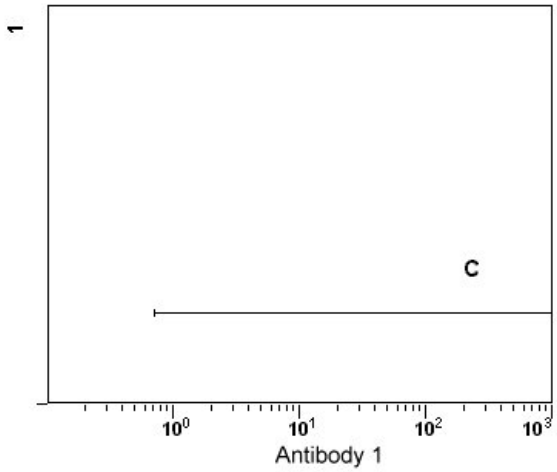
Two Color example



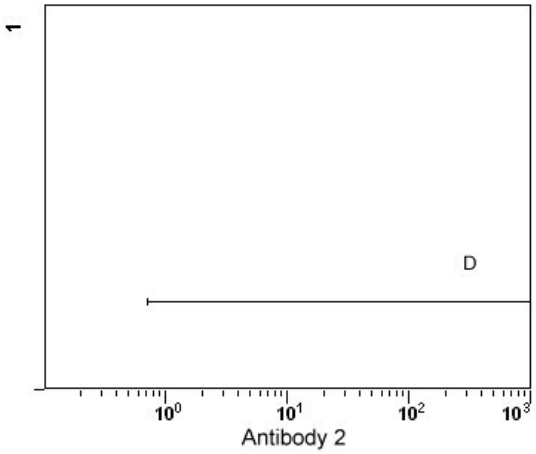
Gate on Region A



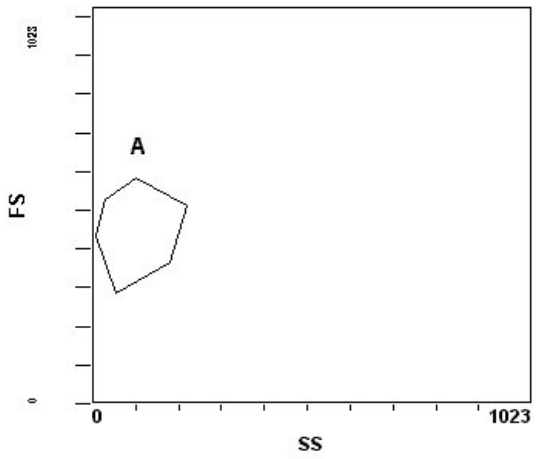
Gate on Region A



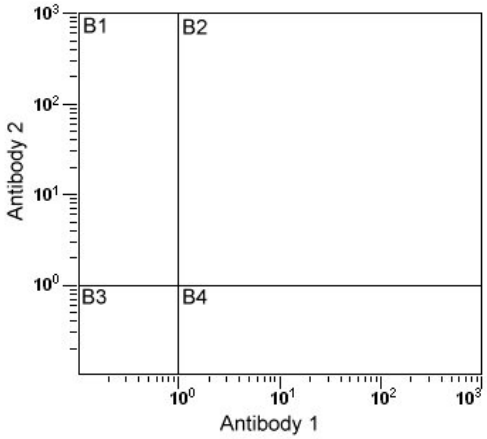
Gate on Region A



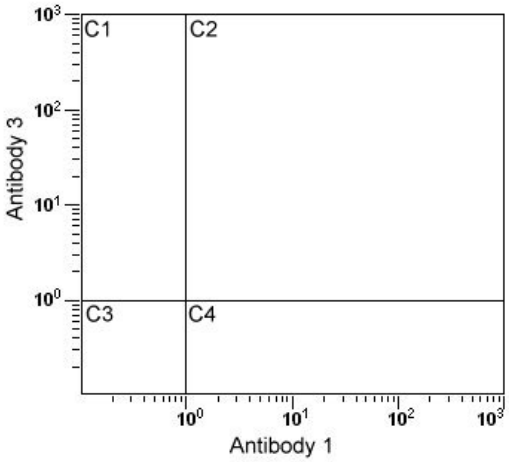
Three Color example



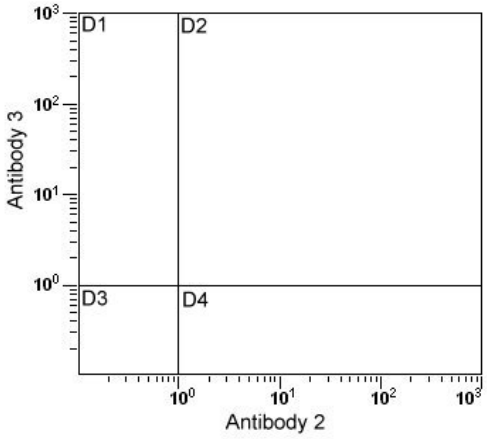
Gate on Region A



Gate on Region A

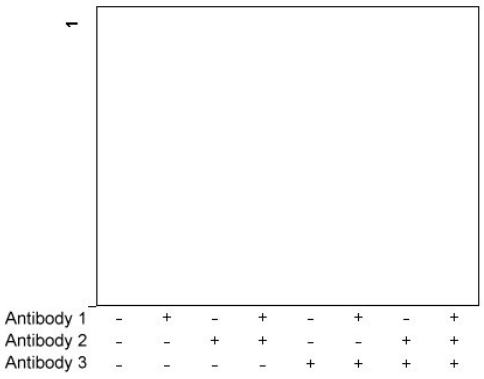


Gate on Region A

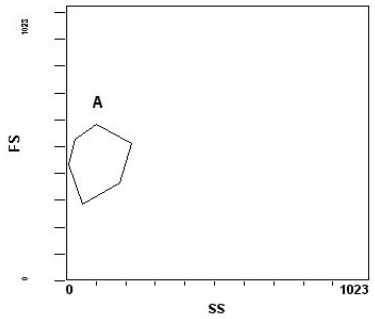


The Two Color and Prism histograms should be gated on A. Divider channels would be set approximately where the Quadrant regions are located. If desired, the single parameter histograms may also be generated.

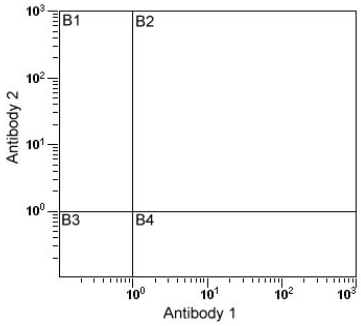
Gate on Region A



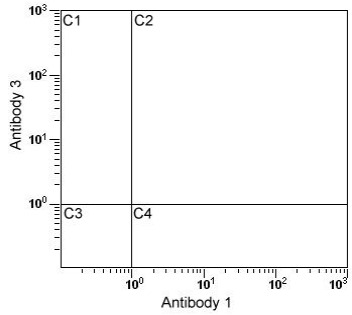
Four Color example



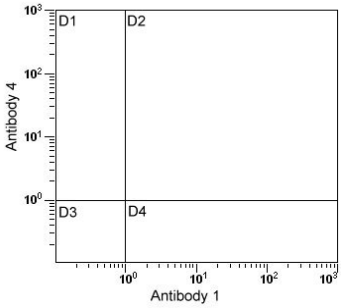
Gate on Region A



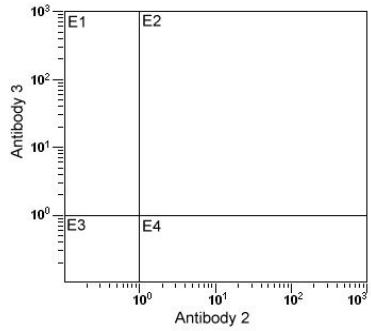
Gate on Region A



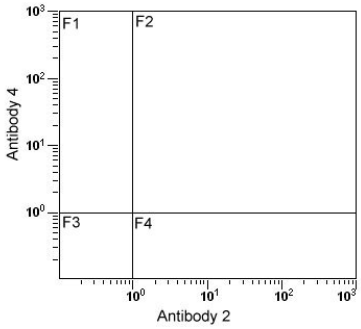
Gate on Region A



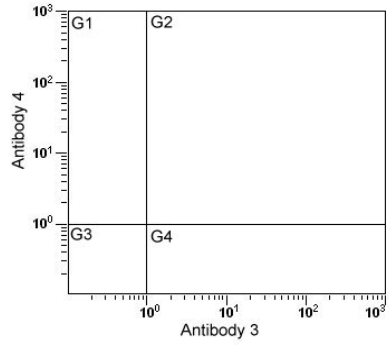
Gate on Region A



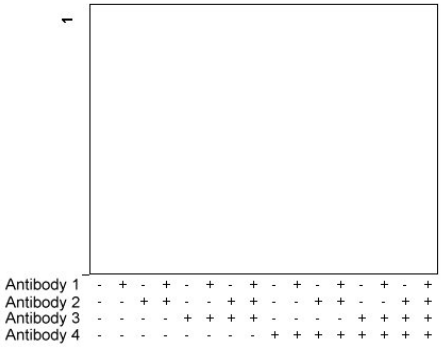
Gate on Region A



Gate on Region A



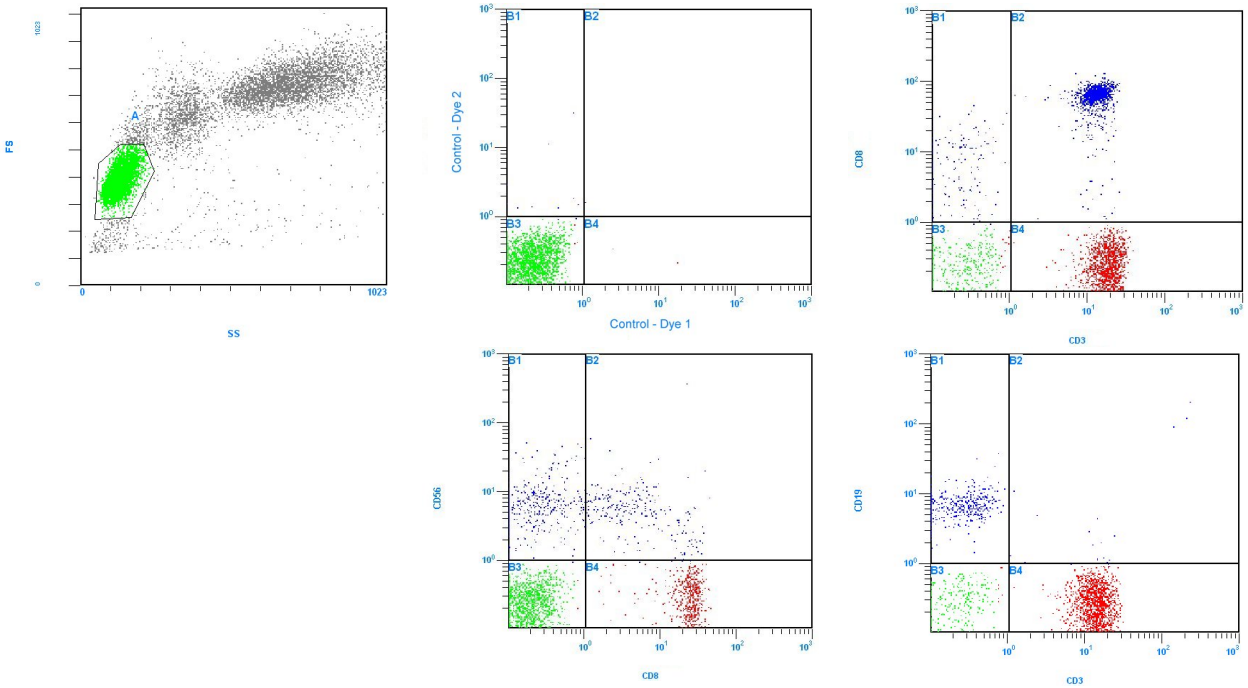
Gate on Region A



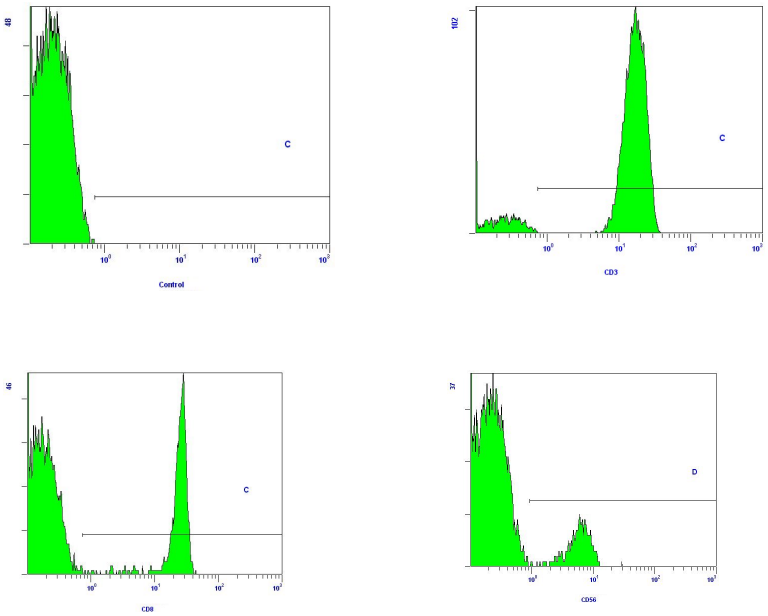
The Two Color and Prism histograms should be gated on A. Divider channels would be set approximately where the Quadrant regions are located.

TYPICAL HISTOGRAMS

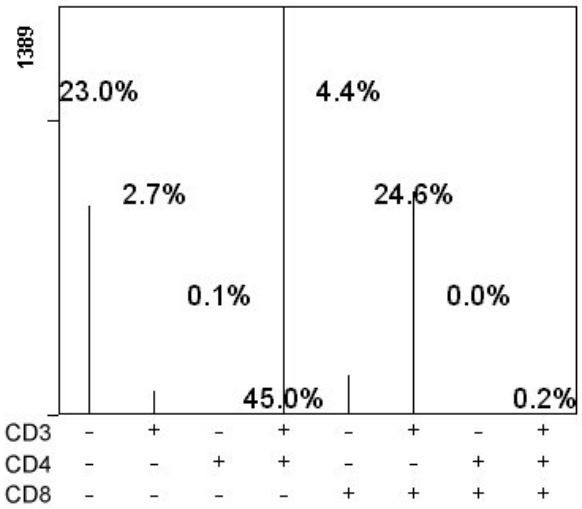
Two Parameter Dot Plots

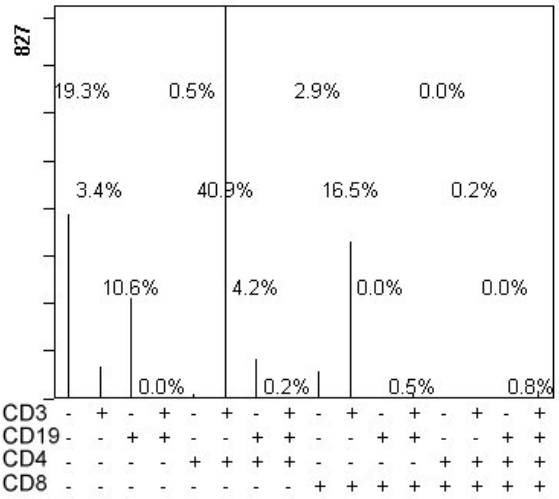


Single Parameter Histograms



Prism Histograms



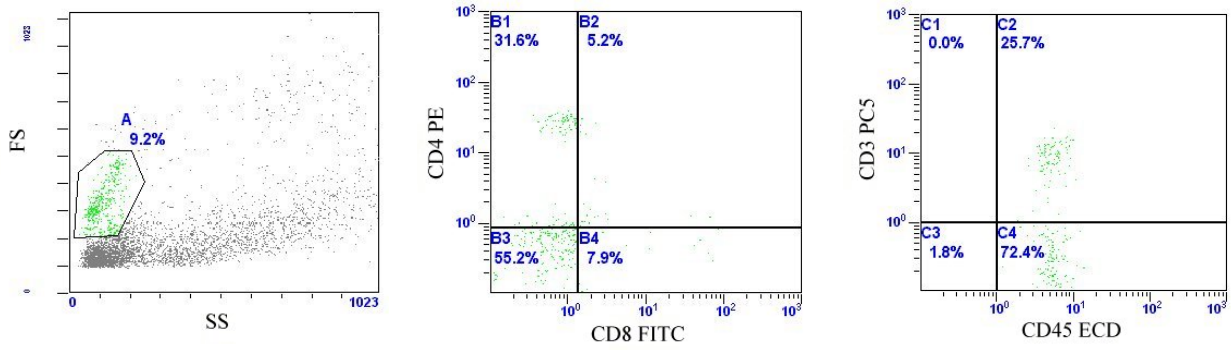


ATYPICAL HISTOGRAMS

The following example data sets were collected from customer accounts. We are grateful to all who sent us examples even if we do not use all of the data in this presentation.

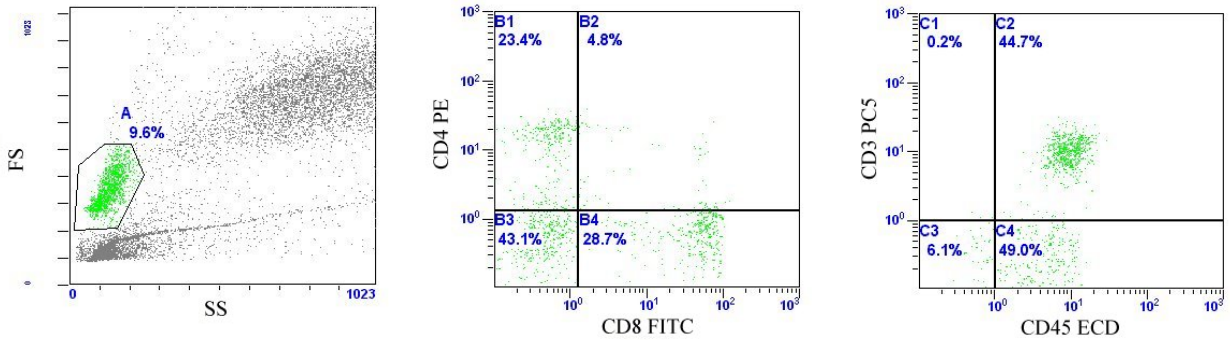
These histograms are examples only. Only a few antibodies are shown. For these and other reasons, the data should not be taken to represent the indicated diagnosed disease state.

Example1



Disease state: Leukemia, Acute Lymphocytic

Example2

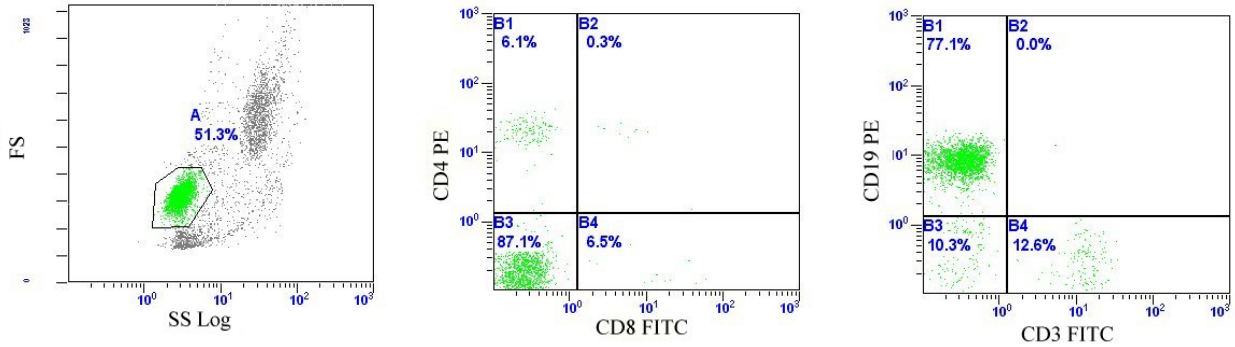


Disease state: Leukemia, Acute Lymphocytic

Example3

Tube 1

Tube 2

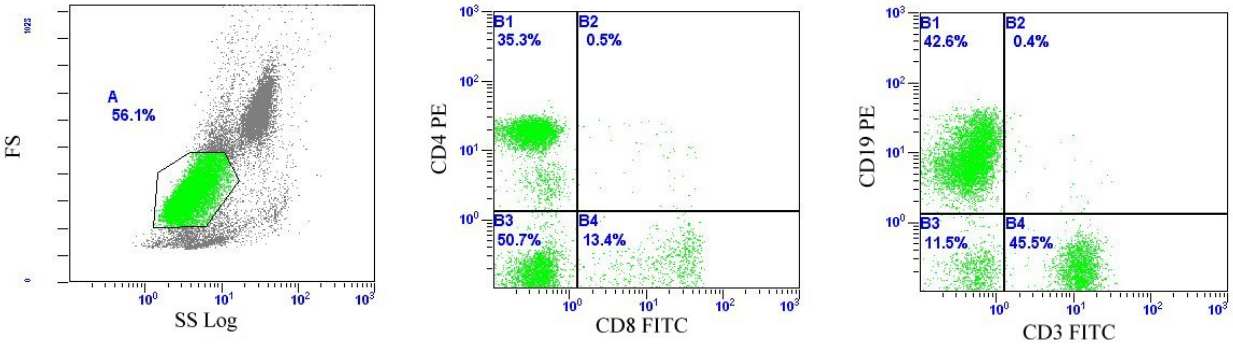


Disease state: Leukemia, CLL

Example4

Tube 1

Tube 2



Disease state: Leukemia, Hairy Cell

GLOSSARY

Antibody

a molecule produced by a B cell or B cell hybridoma which will bind to specific binding sites on the surface of a cell.

APC

allophycocyanin - an orange exciting (heNe laser), red fluorescing dye (peak detected at 675 nm) binding to protein. This dye cannot be used with PC5.

CYTO-TROL Control Cells

control cells with assayed values for certain antibodies which can be used as part of a quality control program for the cell surface marker application.

ECD

energy coupled dye - a tandom dye exciting at 488 nm and emitting at the orange end of the spectrum (peak detected at 620 nm) used with cell surface markers.

Emission curve

a plot of the relative fluorescent light intensity from a dye versus the wavelength of the light.

Epitope

a binding site on the surface of a cell

FITC

fluorescein isothiocyanate - a 488 nm excitable dye fluorescing in the green end of the spectrum (peak detected at 525 nm) used primarily for cell surface marker applications.

Flow-Check Fluorospheres

a 10 µm bead with a bright imbedded dye used to check the alignment of a flow system.

Flow-Set Beads

a 3.6 µm bead product with an imbedded dye used as a fluorescece standard for cell surface marker type applications.

Fluorescence

the property of emitting electromagnetic radiation usually as visible light resulting from and occurring only during the absorption of radiation from some other source

Fluorescence compensation

a correction to a dye fluorescence signal to eliminate the increase in the signal due to interference from another dye.

Forward scatter

light primarily from the surface of a particle as it passes through a laser beam deflected at low angles and traveling in the same direction as the beam. The amount of scattered light is generally proportional to the size of the particle.

Gating

the use of some criteria that must be met first before events will be included in a specific histogram.

Histogram

the plot of the count of the number of pulses versus the corresponding channel heights (single parameter) or the plot of count of the number of events versus two channel height measurements simultaneously (two parameter).

Linear region

a channel range identifying a portion of a single parameter histogram to be used for analysis, gating or sorting purposes.

Mean

average channel value.

PE

phycoerythrin - a dye exciting at 488 nm and emitting in the yellow end of the spectrum primarily used in cell surface marker applications.

Prism

to separate out: A feature which separates multiple cell surface marker measurements into the possible marker phenotypes.

Protocol

a set of instructions; e.g. a set of instructions to a computer on how to run a sample.

Quadrant Region

a set of two cursors dividing a two parameter histogram into four sections or quadrants.

Rectangular region

a box with four sets of channel coordinates describing a portion of a two parameter histogram to be used for analysis, gating or sorting.

Side scatter

light measured at 90 degrees to the laser beam and generally originates from the internal structure with a particle.

REFERENCES

Refer to Product Information inserts for each antibody or the antibody catalog for more specific information concerning each antibody.

APPENDIX

Establishing Standard Reference Values

A standard (e.g. Flow-Set) should be run daily to detect instrument changes that might affect results. Since the daily standard results are compared to reference values for the standard, it is important to establish these reference values.

The values are established after creating the cell surface marker protocol and represent the way the system views an average sample in your patient population. We suggest you use 20 patients over a 5 day period to represent this average population.

General Procedure

- Perform an alignment check first.
- Prepare an isotypic control sample for each sample (4 per day for 5 days).
- Run each patient and adjust the system for a good scatter and fluorescence pattern.
- Record the high voltage and gains required to produce the desired patterns.
- Run the standard at the same high voltage and gain settings and record the mode channels produced.
- Average the mode to establish the reference values.

Sample preparation

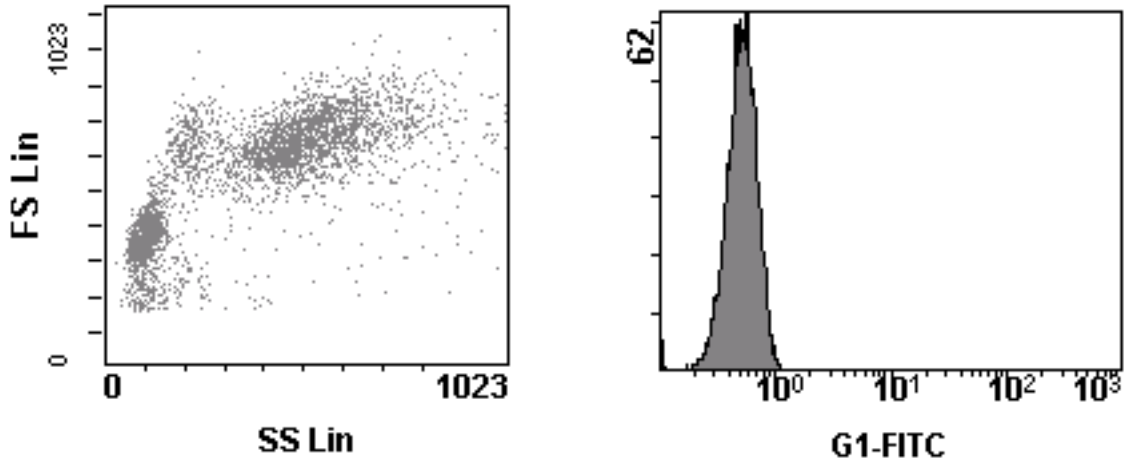
Prepare an Isotypic control from 4 different patients each day for 5 days using the preparation method you will use in your laboratory

Running patients

Run each patient as follows:

- Open or create a marker protocol (e.g. 2 Color, 3 Color, 4 Color, etc.).
- Place first sample on the system and adjust the gains and high voltage to produce the desired light scatter pattern (see below).
- Gate the fluorescence histograms on the desired scatter population.

- Adjust the fluorescence high voltage to extend the negative population to the end of the first decade for each fluorescence to be measured (see below).



- Record the high voltage and gain settings
- Repeat for each patient (4 per day over 5 days)

Run the standard (e.g. FLOW-SET, etc.)

- Run the standard at the first set of high voltage and gain settings.
- Record the mode for all measurements.
- Repeat for each set of high voltage and gain settings (4 per day over 5 days).
- Average the mode values to establish the reference values.

Standardization Worksheet

		FS		SS		PMT Voltages				Mode Channel					
Day	Don.	Att.	Gain	HV	Gain	FL1	FL2	FL3	FL4	FS	SS	LFL1	LFL2	LFL3	LFL4
1															
2															
3															
4															
5															
								Mean							
								1 S.D.							

Example:

		FS		SS		PMT Voltages				Mode Channel					
Day	Don.	Att.	Gain	HV	Gain	FL1	FL2	FL3	FL4	FS	SS	LFL1	LFL2	LFL3	LFL4
1	**1**					725						**35.4**			
	2					**710**						**32.6**			
	3					**740**						**38.2**			
	4					**725**									

.

. . .

	19					**715**						**33.2**			
	20					**735**						**37.6**			
								Mean				**35.4**			
								1 S.D.				**2.0**			

TABLE OF CONTENTS

OBJECTIVES

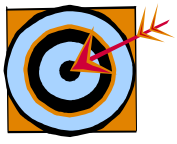
Given an operational flow cytometer, available samples, training materials and access to online Help

- Prepare a reference sample, control sample and at least one unknown DNA sample to run on the flow cytometer.
- Create a protocol to run the samples on the system to include parameters, gating and analysis similar to the examples illustrated in this module.
- Calculate a DNA Index on an abnormal DNA sample.
- Optional: Prepare a second set of DNA samples with at least one cell surface marker attached to the cells.
- Optional: Create a second protocol to run DNA and cell surface markers simultaneously illustrating marker gating and analysis similar to the examples shown in this module.

WHY IS IT IMPORTANT?

This module is intended to present basic techniques for DNA analysis on the flow cytometer. A brief review of the cell cycle in terms of DNA content will be presented followed by information on sample preparation, protocol creation, standardization, quality control and sample analysis. This kit and all results generated from it are "**For Research Use Only. Not for Use in Diagnostic Procedures."**

Skill Check Preview



You will have mastered the application when you can

- Print out the results of each sample set run on your created protocols illustrating proper gating and analysis per this modules illustrated guidelines.
- Calculate the DNA indexes for all abnormal samples run.

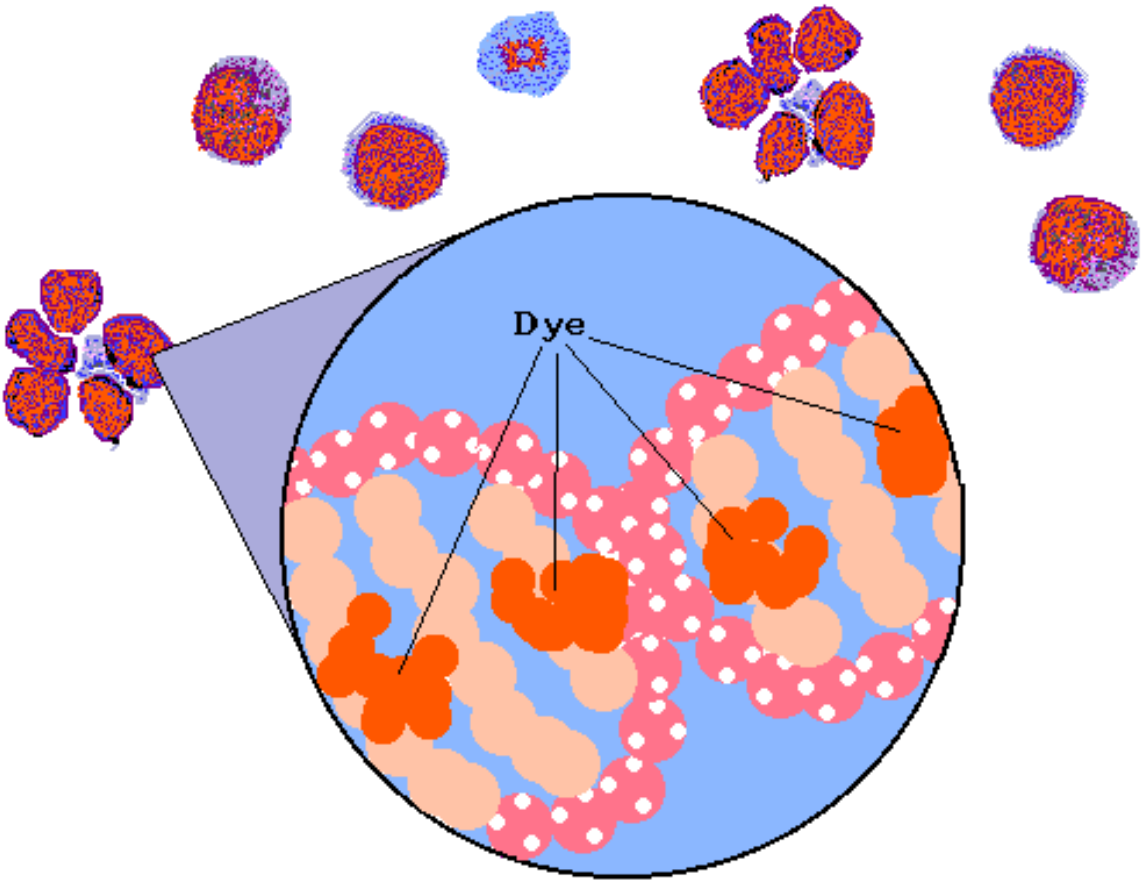
INFORMATION / PRACTICE SECTION

Concepts

Dyes

In this application, a fluorescent dye is bound directly to the DNA in the nucleus of cells. Measuring the fluorescence provides a measure of the amount of dye taken up by the cell and indirectly the amount of DNA content.

The most common dye used is the intercalating dye Propidium Iodide (PI – emits at 610 nm). FL 3 will be used to measure the fluoresence.

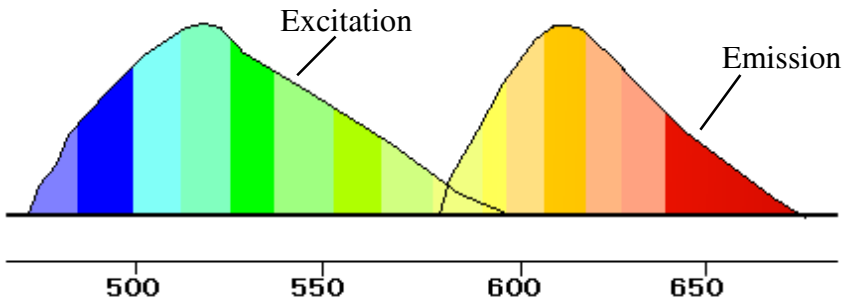


Other potential dyes:

- Ethidium Bromide
- Acridine Orange

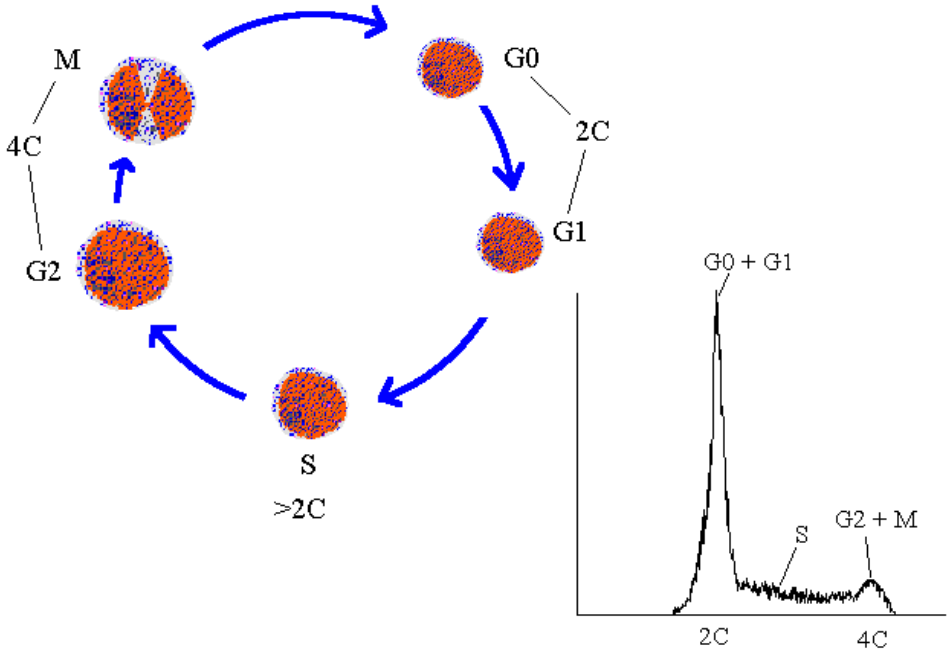
PI Excitation and Emission

In this exercise you will use propidium iodide (PI) to bind to the DNA. The diagram below illustrates the excitation and emission curves for this dye:



The Cell Cycle

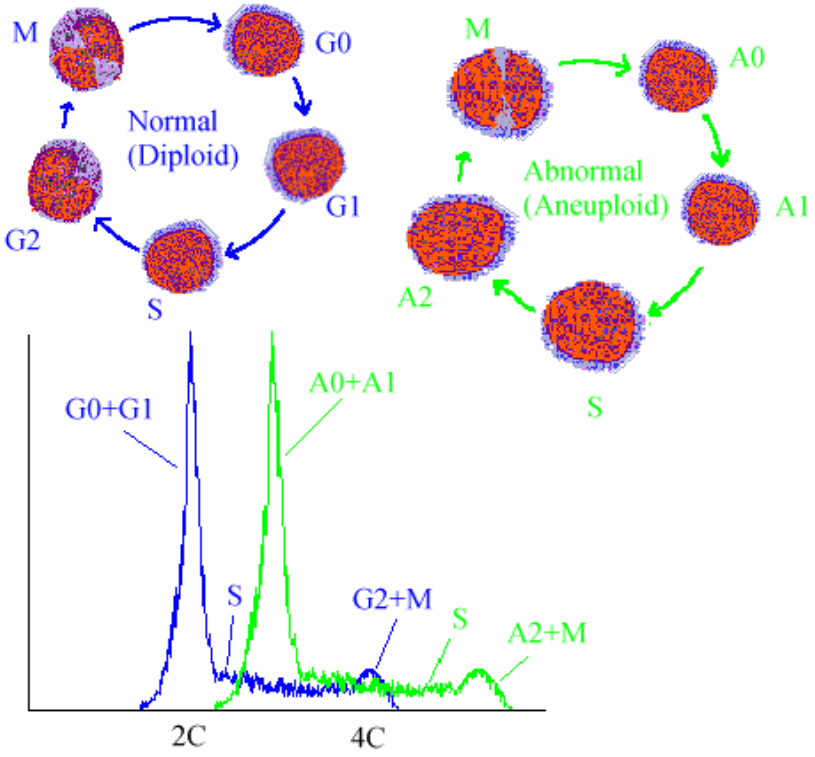
Cells that are reproducing will be going through phases to increase their DNA content to twice the original amount. These cells will eventually divide into two new cells with the original DNA content. This cell cycle is illustrated below for human cells with an initial 2C DNA content. The typical DNA histogram representing the number of cells of varying DNA content is also shown.



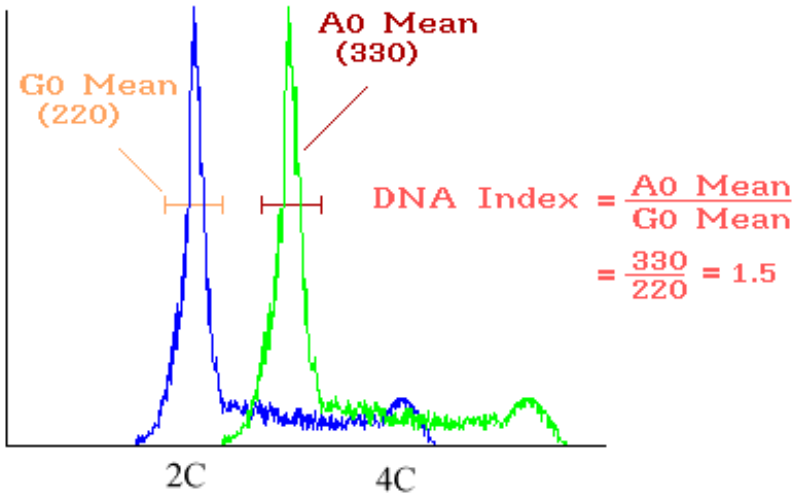
NOTE: If there is no variability in the staining, cells, dye, or measurement, the DNA histogram would place all the G0 + G1 cells in one channel, the G2 + M cells in another channel twice as far out and all the S phase cells would fall in between.

Analysis

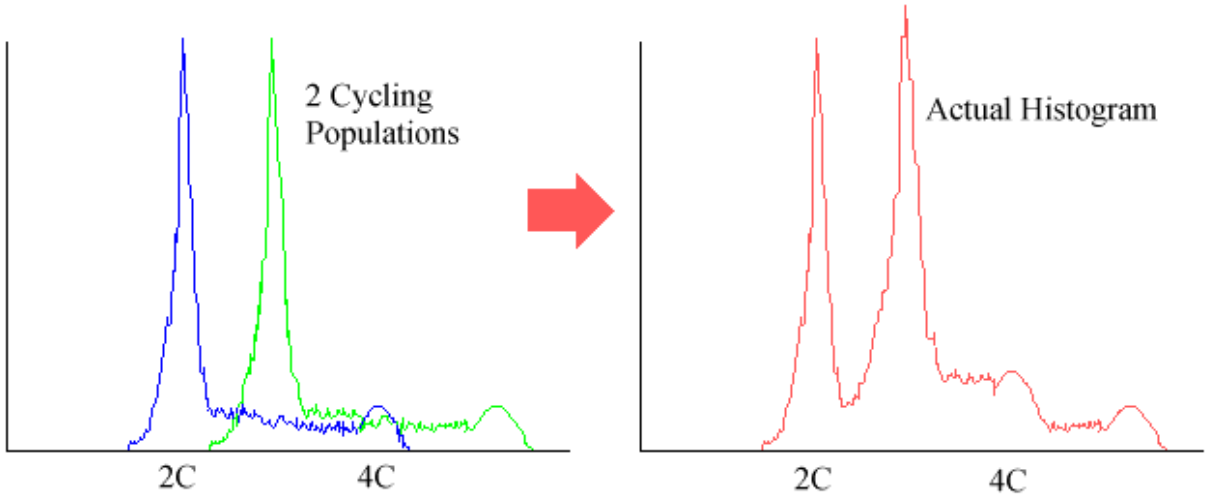
Abnormal cells may have an abnormal quantity of DNA (most often increased) and samples may have a mixture of such cells. Normal human DNA content of 2C is referred to as diploid and the abnormal as aneuploid. An example is shown below:



A measure of abnormal DNA content can be obtained by comparing the G0 + G1 mean channels for the aneuploid and diploid peaks as shown below:

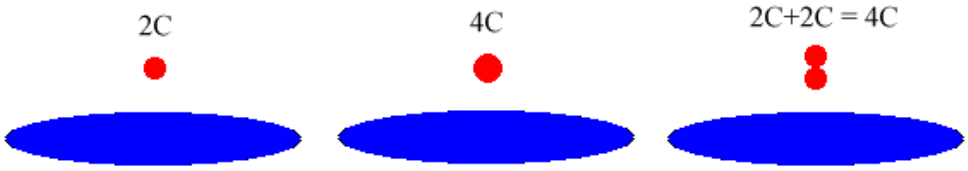


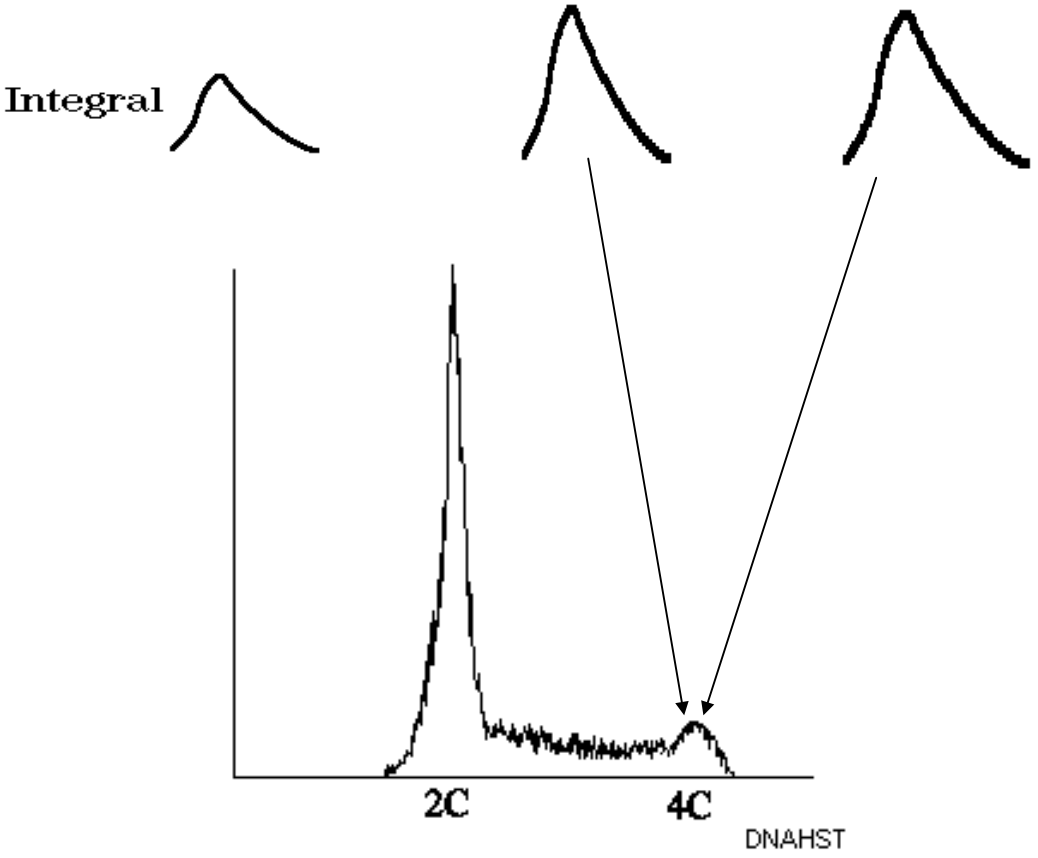
The histogram does not really show the individual cycling populations but rather adds the cycles together to form a combined histogram (see below). This combined histogram complicates analysis. Many prefer to use special DNA analysis software to determine the DNA index and also the percent of cells in each of the phases of each of the cycling populations.



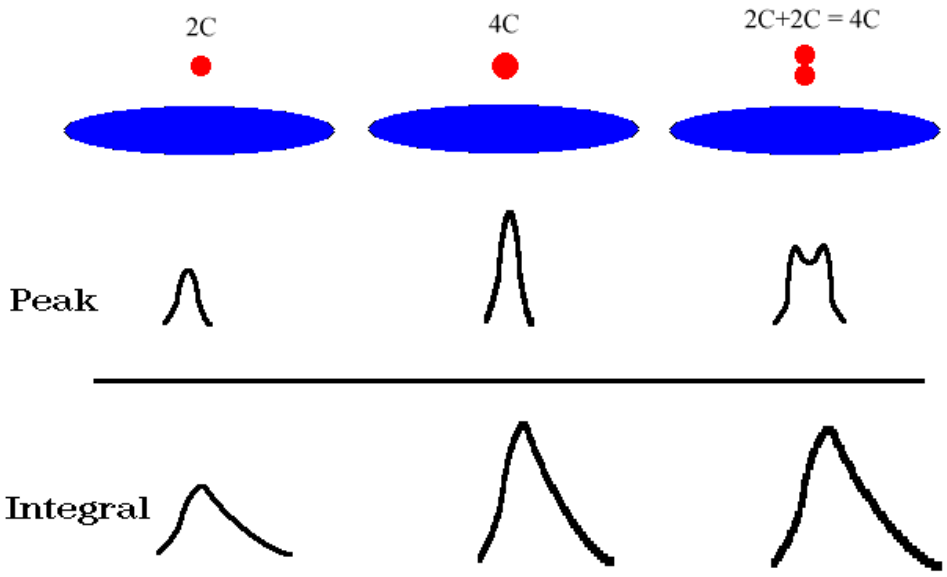
Gating Out Doublets

If a doublet passes through the laser beam, the system may see it as a single particle with an increased amount of DNA. The resulting DNA histogram will be incorrect and percent determinations for each phase of the cell cycle will be inaccurate.



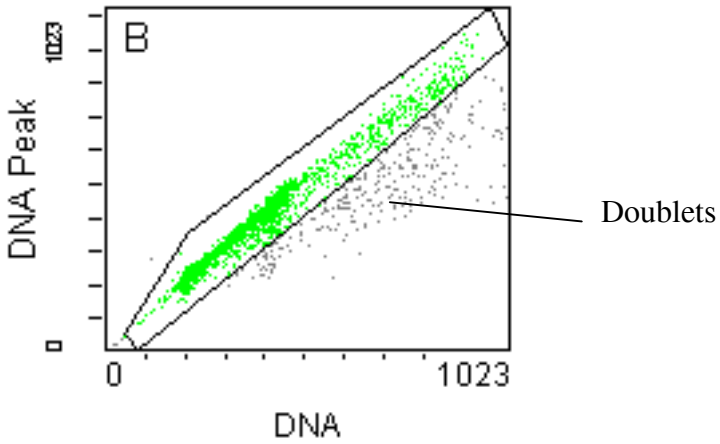


One solution to the problem is to detect and gate out the doublets from the DNA histogram. All detection schemes are based on the peak pulse.

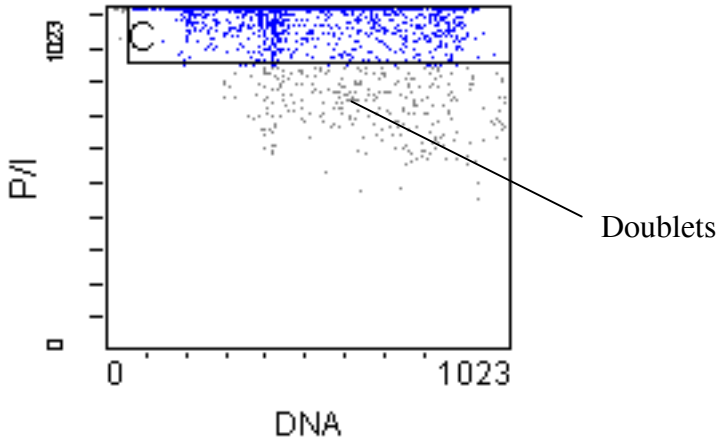


For single particles, the peak and integral pulses are set using the gain adjustments for equal pulse heights. Doublets often exhibit a lower peak pulse height as compared to the integral. These characteristics have generated two methods of gating out the doublets:

- **Peak versus Integral *(*Classic method*)*** - This is the oldest method in use and is based on a peak versus integral two-parameter histogram. Single particles fall along the long diagonal of the histogram; the doublets tend to fall away from the diagonal.



- **Ratio of Peak versus Integral** - The ratio of the single particles is close to one and falls along the edge of a Ratio versus integral histogram. The doublets with a lower ratio fall away from the single particles.



PRACTICE

Sample Preparation

There are a number of different preparation procedures depending on the type of sample, kinds of measurements desired, and the experience of the investigator. All procedures, however, must accomplish the following:

1. Dissociate the cells and filter the sample to remove clumps.

2. Obtain a cell count.

3. If surface markers are to be run as well, mark the cells before the next step.

4. Add RNAse to eliminate competing strands of RNA.

5. Make the cell membrane permeable and suspend the cells in the dye (e.g. PI) solution to the desired concentration and allow the dye to bind to the DNA.

6. Incubate for the desired amount of time.

The following samples are usually prepared and run:

- A DNA reference used to adjust the fluorescence high voltage and gains

  Example: Chicken (rooster) erythrocytes (about 35 % of normal human DNA content)

- A Control to establish the normal diploid population

  Example: Lysed Whole Blood

- Unknown samples

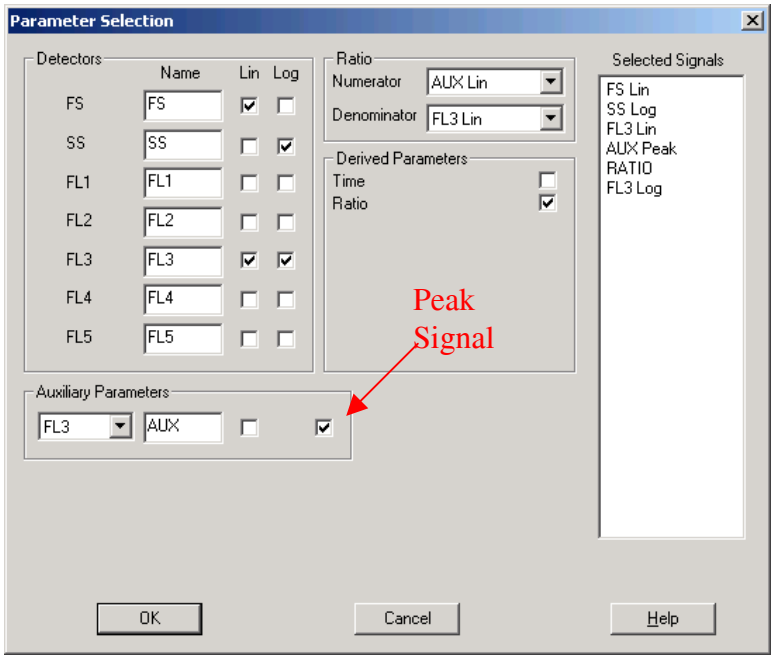
QUALITY CONTROL

1. Run Flow-Check™ Fluorospheres to check fluidics and optics.
2. Run the "Reference" sample (rooster erythrocytes) with the DNA protocol and adjust the system to place the peak fluorescence at the reference channel (suggestion: 78 +/- 2 channels).
3. Run a known DNA control at the same voltages to check the accuracy of the process.

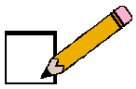
TYPICAL PROTOCOL

NOTE: Refer to your software Creating a Protocol procedure guide for details on how to create the protocol within your software. The appropriate sections discussing each step will be labeled as the headings below.

Selecting Parameters



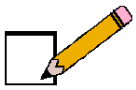
NOTE: Set the discriminator on FL3 Peak, suggested value of 30.



Creating Plots

A dot plot of SS (or Log SS) vs FS which can be used for gating, a dot plot of DNA vs Peak DNA, DNA vs Ratio (DNA Peak/DNA), or Time of Flight vs DNA for gating purposes and the final single DNA histogram.

There also may be additional plots if other measurements are made in addition to DNA.



Creating Regions

A polygon based on a FS/SS Log dot plot and either a polygon based on a DNA Peak/DNA dotplot or a rectinear based on a Ratio (DNA Peak/DNA)/DNA dot plot are used as gates.

Linear regions based on the DNA Histogram are used for analysis.

Defining Gates

Typically based on SS (or Log SS) vs FS and a region set on the main population based on DNA vs DNA Peak or DNA vs Ratio (DNA Peak/DNA).

Additional gating may be added if there are additional measurements.

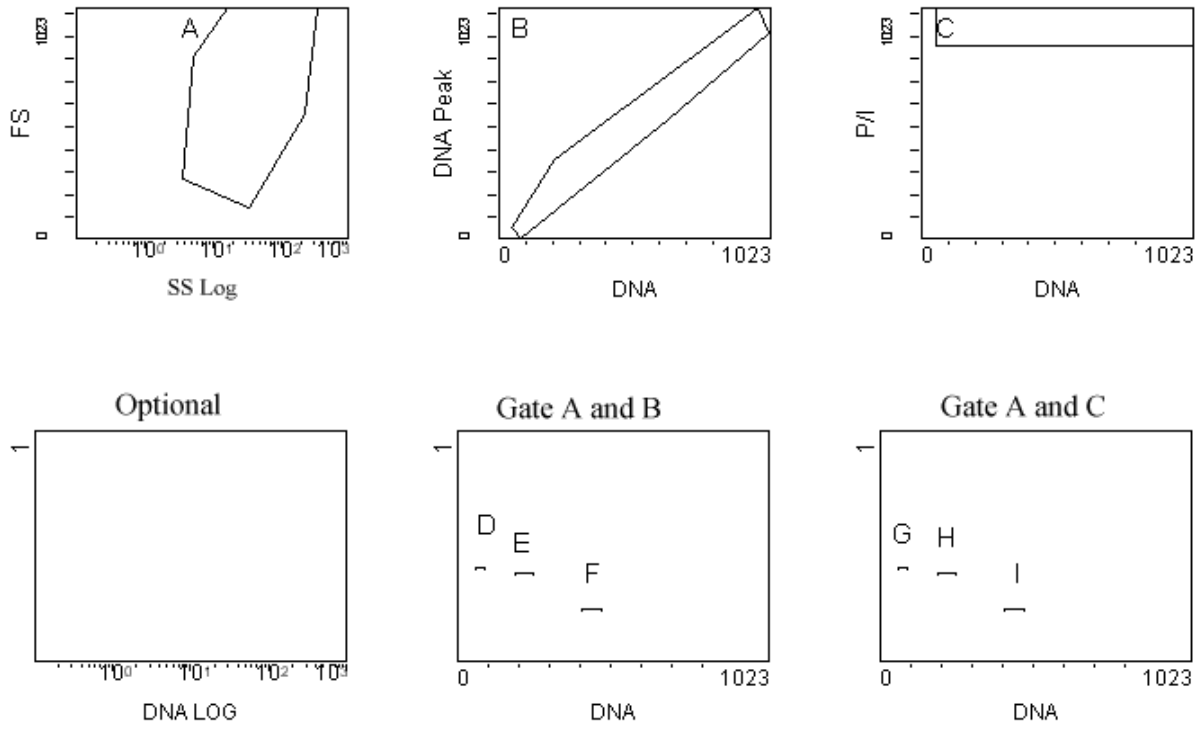
Analyze Data

Regions are set on the G0+G1 peaks to determine the DNA index as compared to the diploid peak.

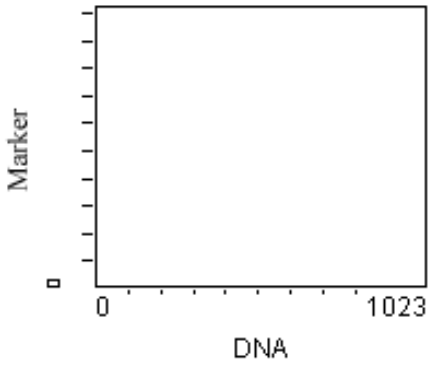
Analyze the DNA histogram using a DNA analysis program such as MulticycleAV™ or ModFit™ to determine percents. Additional measurements can make it easier to separate sub-populations.

Example

The following diagram illustrates the two doublet gating schemes. Typically only one of the two is used. The choice is an individual preference. The DNA Log histogram is an extra histogram added by some to see if there is any additional data further out.

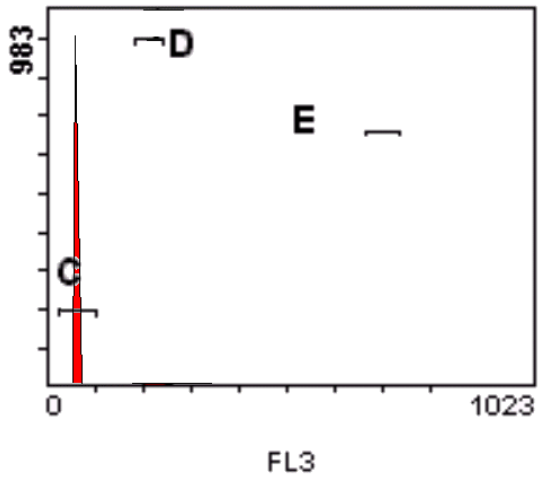


NOTE: You may also add addditional histograms when making multiple measurements as below:

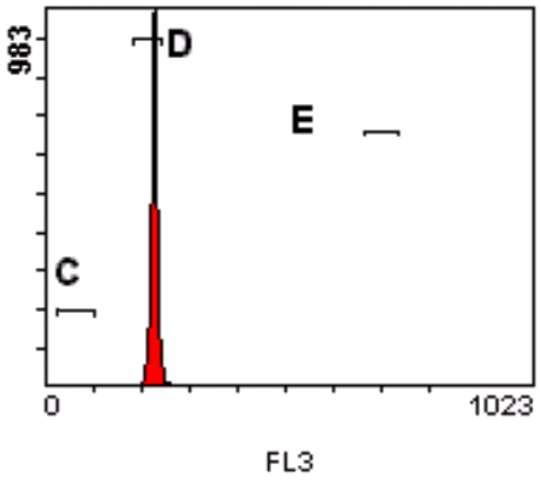


TYPICAL HISTOGRAMS

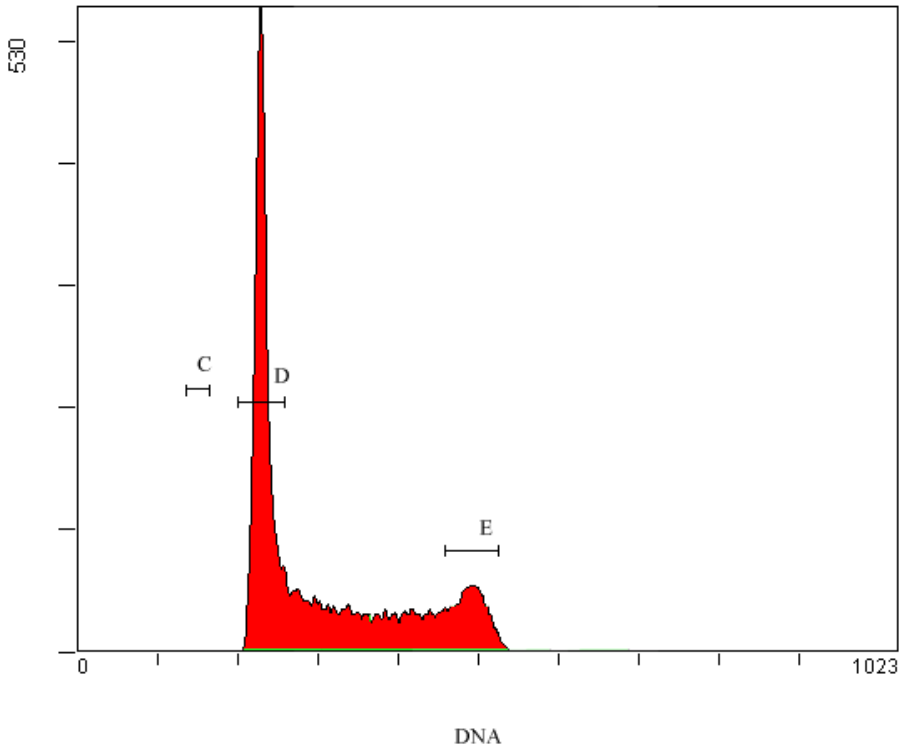
Reference: Gate on A and either B or C



Control: Gate on A and either B or C

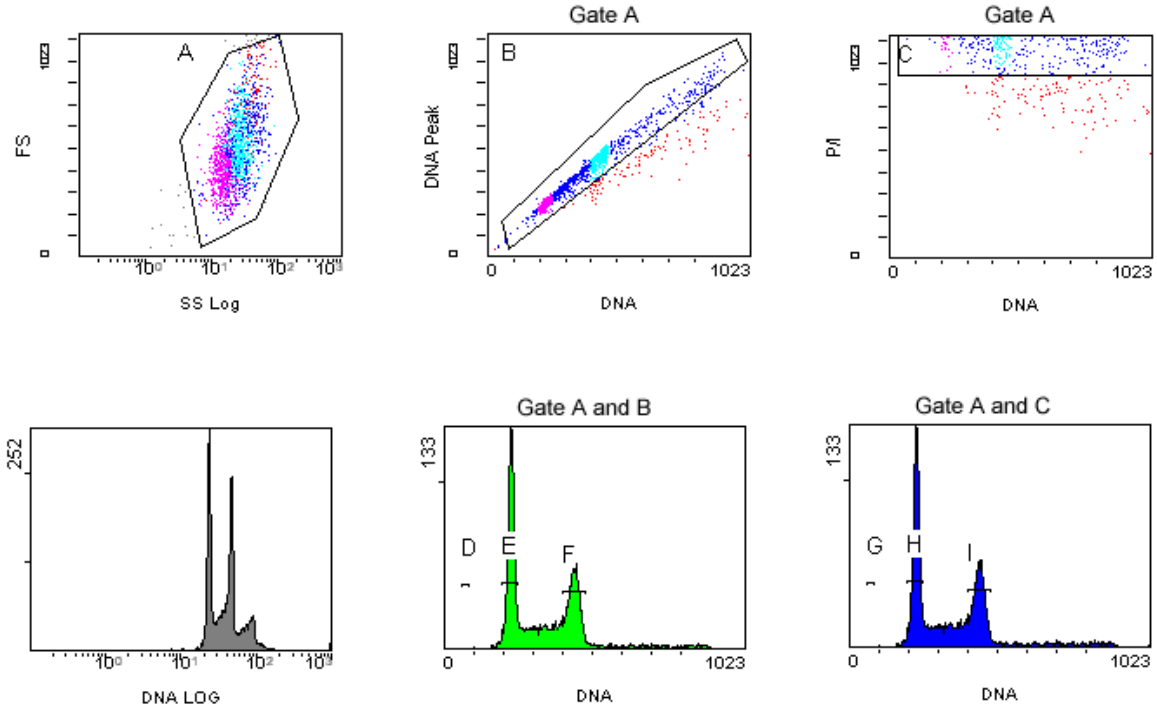


Example:

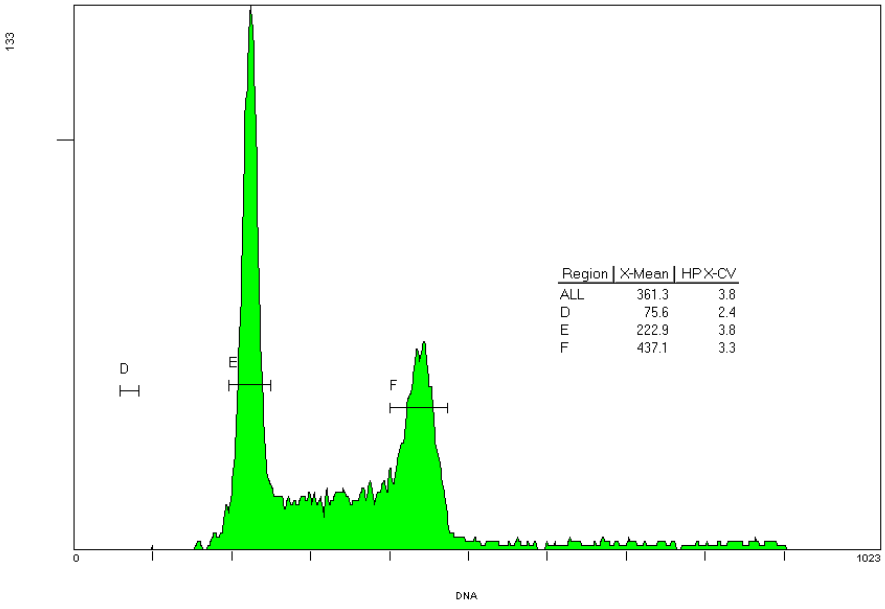


ATYPICAL HISTOGRAMS

CEM Cell Line – Acute Lymphoblastic Leukemia



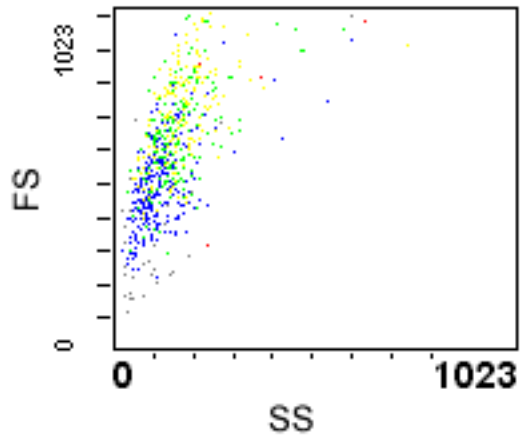
CEM Cells

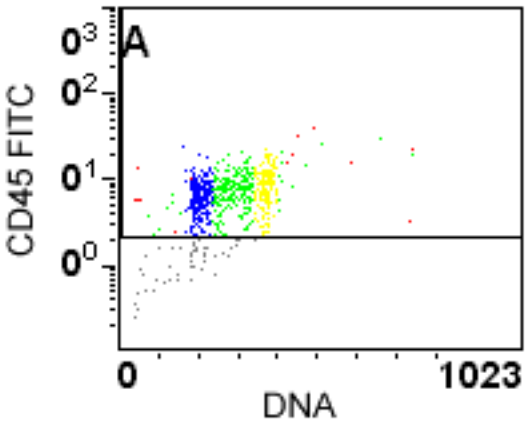


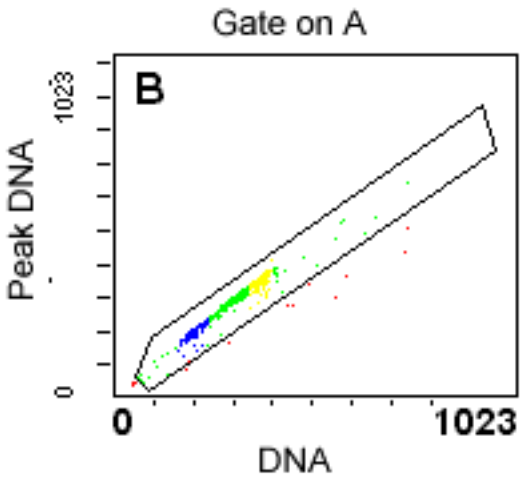
Dna Index 1= Mean F/Mean E = 437.1 / 222.9 = 1.96

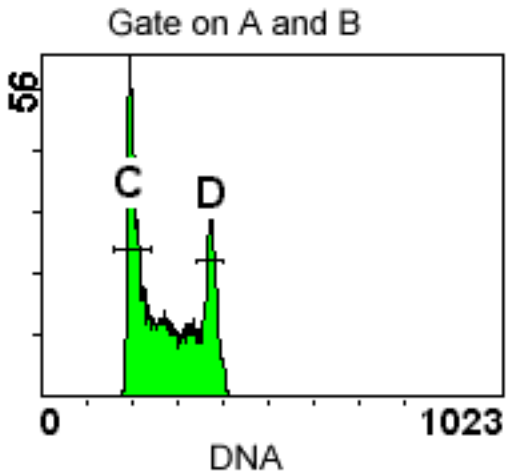
Example 2 Color:

Doudi cells









Region	X-Mean
ALL	277.5
C	203.3
D	371.4

GLOSSARY

Acridine Orange

A DNA/RNA dye which excites at 488 nm and fluoresces green when bound to DNA and orange-red when bound to RNA.

Aneuploid

A term describing a sample with an abnormal DNA content.

DAPI

4',6-Diamidino-2-phenylindole – an ultra violet excitable DNA binding dye which fluoresces in the blue portion of the spectrum.

Diploid

A term refering to a normal quantity of human DNA content.

DNA

Deoxyribonucleic acid.

DNA Index

Aneuploid mean channel divided by the Diploid mean channel.

Ethidium Bromide

A DNA and double stranded RNA intercalating dye which excites in the ultra violet range (320-360 nm) or in the blue-green range (480-550 nm) and fluoresces in the orange-red end of the spectrum.

G0

Gap 0 – refers to the resting phase in which the cell is not going through the cell cycle.

G1

Gap 1 - refers to the initial proliferative phase of the cell cycle in which cells have the same DNA content as the resting phase.

G2

Gap 2 – refers to the phase just prior to mitosis in which a cell has twice the DNA content but has not yet indicated it is ready to divide.

Hoechst 33342

An A-T specific DNA dye exciting with ultra violet light and fluorescing blue often used as a vital stain.

M

Mitosis phase – in which cells are dividing.

PI

Propidium Iodide – A blue exciting orange-red intercalating dye which binds to DNA and double stranded RNA.

RNA

Ribonucleic acid.

S

S Phase – refers to the proliferative phase of the cell cycle in which cells are increasing their DNA content.

Tetraploid

Four ploid – refers to cells containing twice the normal DNA content.

TOF

Time of Flight – A measurement of the peak pulse width indicating the time it takes to move ("fly") through the beam.

REFERENCES

Shapiro Howard M.: *Practical Flow Cytometry Third Edition*. New York, Wiley-Liss, 1995.

Technical Monograph: DNA Preparation Techniques - PN 9600029

ST STEM CELL CD34+ HPC ENUMERATION

OBJECTIVES

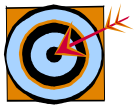
Given an operational flow cytometer, access to StemCXP™ SYSTEM Guide and/or Stem Kit™ package insert, and required supplies:

- Perform Quality Control to verify instrument performance and reagent stability.
- Set up the system to run samples per the instructions.
- Run samples and analyze the data per the instructions.

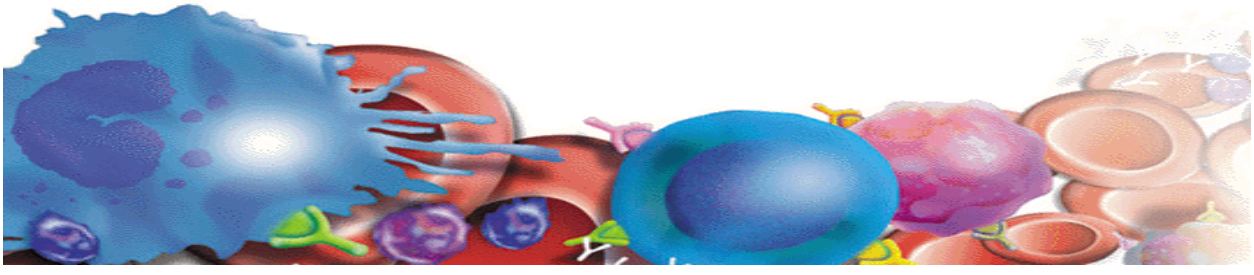
WHY IS IT IMPORTANT?

This module presents basic techniques for CD34+ HPC enumeration on a Flow Cytometer. The StemCXP SYSTEM Guide and/or Stem kit package insert guides the user through the process.

Skill Check Preview



- You will have mastered the application when you can perform the procedures listed under objectives.
- Provide your facilitator with a hardcopy for all samples for skill check verification.



REFERENCES

StemCXP™ SYSTEM Guide (PN 627260A) for Cytomics FC 500 Flow Cytometer.

Stem Kit™ CD34+ HPC Enumeration Kit package insert PN IM3630.

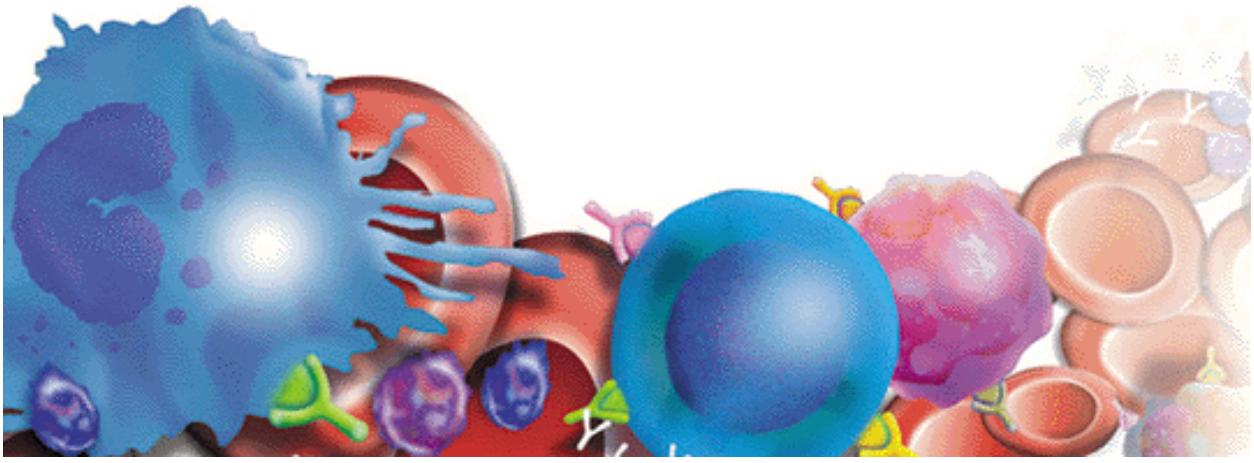
INFORMATION/PRACTICE SECTION

Please refer to the StemCXP SYSTEM Guide or the Stem Kit package insert for discussion and instruction for performing stem cell analysis on the FC500 Flow Cytometer.

After you have read through the SYSTEM Guide and/or package insert and you believe you are ready, complete the Skill Check.

AP APOPTOSIS DETECTION ANNEXIN V FITC

Table of Contents



OBJECTIVES

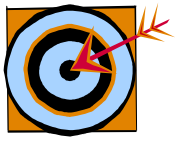
Given an operational flow cytometer, access to online Help, and required supplies:

- Perform Quality Control to verify instrument performance and reagent stability.
- Create a protocol to detect apoptosis following the package insert instructions.
- Obtain an apoptosis listmode file from your facilitator and populate your plots or if possible prepare and run a set of ANNEXIN stained samples.
- Analyze the data per the module guidelines and print the results.

WHY IS IT IMPORTANT?

This module presents basic techniques for detection of apoptosis of cell suspensions on a Beckman Coulter Flow Cytometer. A brief review of the current usage of this type of analysis will be presented followed by information on sample preparation, protocol creation, quality control, sample analysis and data interpretation. Samples will be prepared using the ANNEXIN V FITC Kit$^{\Pi}$ from Beckman Coulter-Immunotech. This kit and all results generated from it are **"For Research Use Only. Not For Use In Diagnostic Procedures."**

Skill Check Preview



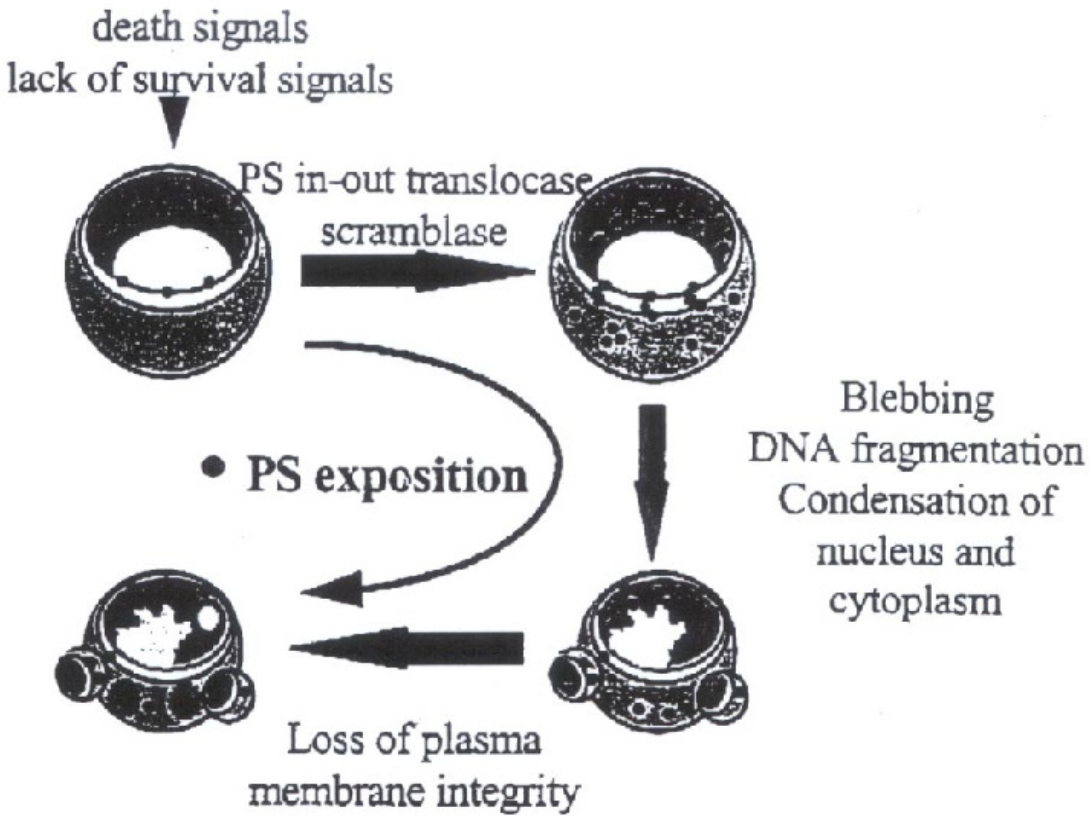
- You will have mastered the application when you can perform the procedures listed under objectives.
- Provide your instructor with a hardcopy of the apoptosis protocol for skill check verification.
- Analyze the annexin stained samples and print results for skill check verification

INFORMATION/PRACTICE SECTION

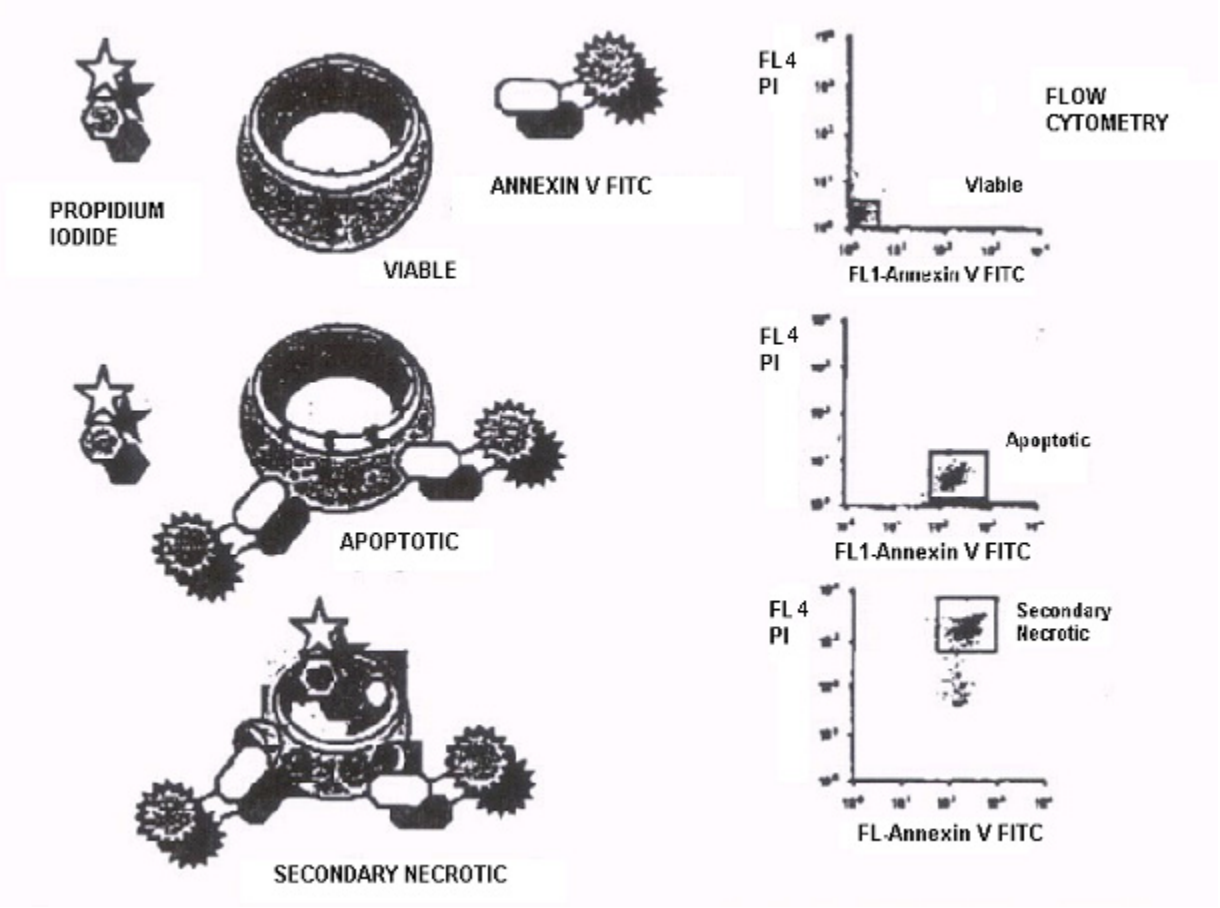
Concepts

The different stages of apoptosis are characterized by the cell surface exposure of PS (Phosphatidylserine). Apoptosis in vitro leads to a loss of plasma membrane integrity during the late phase. This stage is designated as secondary necrotic.

PS Exposure During Apoptosis



Flow Cytometric Result of Analyzing Cells With ANNEXIN V FITC and Propidium Iodide



FITC-conjugated annexin V binds to phosphatidylserine (PS). To discriminate between the different stages of apoptosis, the vital dye PI is added to the assay mixture. The combination of Annexin V FITC and PI yields three distinct phenotypes. ANNEXIN V FITC and PI yield three distinct phenotypes, 1) The viable population, 2) the apoptotic population and 3) the secondary necrotic population.

PRACTICE

Sample Preparation

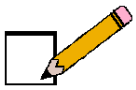
Preparation of reagents:

1. Pepare a 1: 10 dilution of Binding Buffer with distilled water. (ie: 1.5 mL Binding Buffer + 13.5 mL distilled water)
2. Dissolve the 250 νg Propidium Iodide in 1 mL of diluted binding buffer.

Procedure:

1. Wash the cell samples with ice-cold PBS. Centrifuge for 5 minutes at 500 X g at 4±C. Discard supernatant.
2. Resuspend the cell pellet in ice-cold diluted binding buffer to 10^5 – 10^6 cells/ mL. Keep the tubes on ice or refrigerated.
3. Add 5 νL of ANNEXIN V FITC solution, and 5 νL dissolved PI to 490 νL of the prepared cell suspension.

Follow product package insert for more detailed information



QUALITY CONTROL

Verify Fluidics and Optics

1. Run Flow-Check™ Fluorospheres and verify HPCVs. Refer to the Quality Control module and/or Flow-Check Fluorospheres package insert.

Standardizing PMT Voltages

2. Run Flow-Set™ Fluorospheres per the Flow-Set Package insert.
3. Use the gains and high voltage used to adjust the Flow-Set to the target channels when running unknown samples.
4. No fluorescence compensation is required for this application.

Positive Control

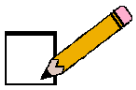
5. The ANNEXIN V FITC Kit package insert includes a detailed procedure to induce cell apoptosis providing a positive quality control of the system.

TYPICAL PROTOCOL

NOTE: Refer to your software Creating a Protocol procedure guide for details on how to create the protocol within your software. The appropriate sections discussing each step will be labeled as the headings below.

Selecting Parameters

FS, SS, FL1 LOG and FL4 LOG. Label the parameters with the appropriate name: FL1 LOG: Annexin Fitc, FL4 LOG: PI.



Creating Plots

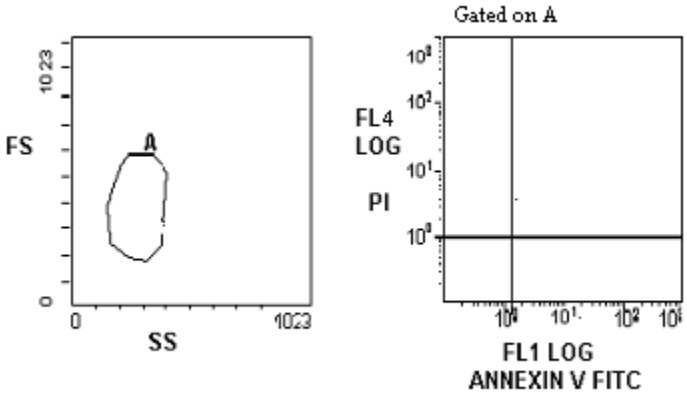
A dot plot/ 2 parameter histogram FS vs SS for gating purposes, and a dot plot / 2 parameter histogram FL4 LOG vs FL1 LOG

Creating Regions

A polygon is used as a gate region based on a FS/SS dot plot and Quadrant region is used for analysis based on FL1 Log/FL4 Log dot plot.

Defining Gates

Based on FS vs SS isolating a particular population for analysis. The FL1 LOG vs FL4 dot plot is gated on the isolated population.

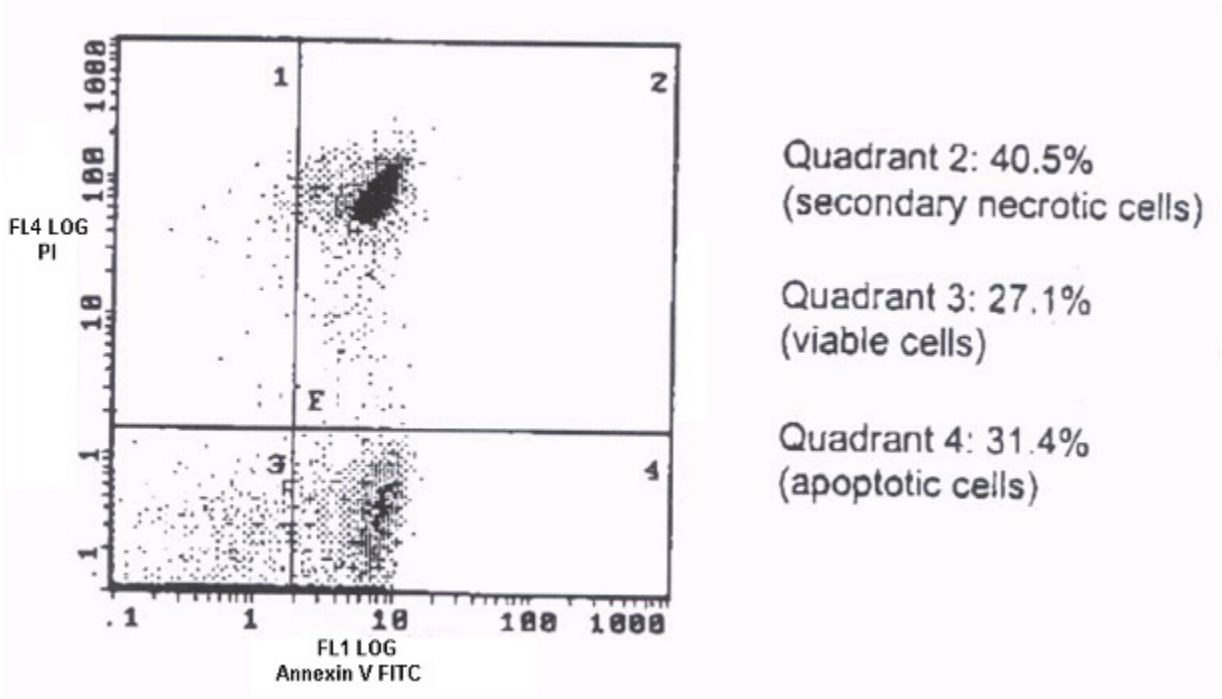


Analyze Data

The flow cytometer is set such that the mean fluorescence intensity of the annexin V- negative population is in the first decade. Optimal parameter settings are found using a positive control (refer to the package insert).

TYPICAL HISTOGRAMS

Flow cytometric analyis of apoptotic Jurkat cells after staining by ANNEXIN V FITC/PI. Jurkat cells have been treated with 100 ng/mL of anti-Fas/CD95 antibody for 6 hours.



GLOSSARY

Annexin V A Ca2+ dependent phospholipid-binding protein with high affinity for Phospatidylserine.

Apoptosis An active, physiologic cascade of events, characterized by cell shrinkage due to dehydration, membrane blebbing, nuclear chromatin condensation and fragmentation of the nucleus and the cell.

Blebbing Cell membrane distortion or blebbing is a result of the breakdown of structural proteins as part of the cascade of events in apoptotic cells.

Necrosis A passive, degenerative, non-specific pathway to death induced by acute injury or extremes in the external environment, usually characterized by cell and mitochondrial swelling, metabolic collapse, flocculation of chromatin, rupture of the nuclear and cytoplasmic membrane resulting in dispersal of the cell contents.

Phosphatidylserine (PS) A phospholipid component of the cell membrane which is translocated from the inner side of the membrane to the outer layer during the early events of the apoptotic process (prior to loss of cell membrane integrity).

Propidium Iodide (PI) A DNA-binding dye which is often used to detect loss of cell membrane integrity.

SKILL CHECK

GETTING STARTED

1. Review answers on pages GS-2 and GS-3 with your facilitator.
2. Startup the system.
3. Perform a shutdown on the system per the printed procedures. Call your facilitator when you are ready to observe the implementation of the cleaning tube procedure. Be prepared to
   - Show them your Startup and Shutdown printouts.
   - Answer a few questions on indicators and basic system components.
4. Enter the completion of the startup and shutdown into the database maintenance log.

❑ Facilitator sign off

_____

BASIC OPERATION

Provide printouts for each exercise in the Basic Operation module which include:

1. Flow-Check™ Fluorosphere results demonstrating HPCV's < 2 % for FS and FL1-3.
2. A properly set 2 Color isotype control.
3. Flow-Set™ Fluorosphere 2 color results run at high voltage set for isotype control illustrating mode channels.
4. Properly compensated 2 color CD8/CD4 sample.
5. 2 Color CD3/CD4 Immuno-Trol (or Cyto-Trol) sample within assay guidelines.
6. Print out the logbook pages for Flow-Check and Flow-Set (Help Screen) and enter the results of your system quality control checks.
7. Ask your facilatator for a set of Flow-Set raw data from which to determine the reference mode channels. Calculate the average values and record the answers below:
8. Given a set of mode values for a standard calculate average mode values.

   FS _____ SS _____ FL1 Log _____ FL2 Log _____

   FL3 Log _____ FL4 Log _____ FL5 Log _____
9. Answer the following questions:

   - What should you do if the Flow-Check HPCV's are high ?

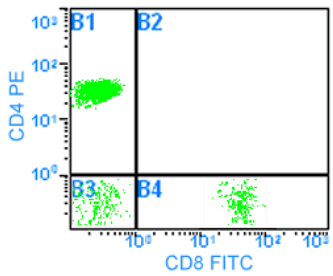
     _____

     _____

   - Is the data below acceptable? _____
     Why or why not?

     _____

     _____



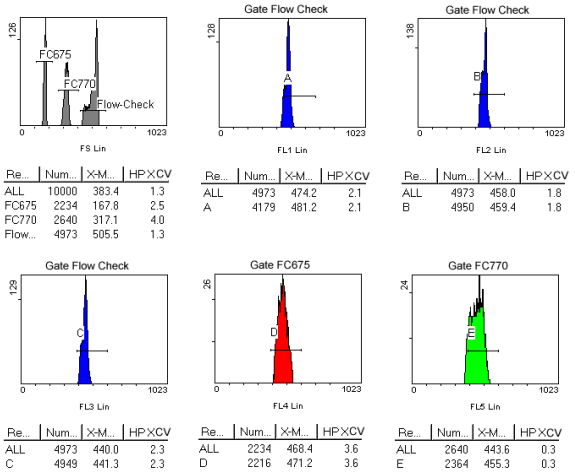
	X Mean	Y Mean
B1	.3	14.3
B2	1.1	1.4
B3	.3	.4
B4	32.1	.7

TROUBLESHOOTING

1. Display the cytometer.log file from the system.
2. Complete the Error Codes exercsize at the end of the module.
3. In each of the examples that follow, indicate whether the data is acceptable or not and if not, what you should do about it.

- Example 1 Flow-Check _____

_____



Re...	Num...	X-M...	HP×CV
ALL	10000	383.4	1.3
FC675	2234	167.8	2.5
FC770	2640	317.1	4.0
Flow...	4973	505.5	1.3

Re...	Num...	X-M...	HP×CV
ALL	4973	474.2	2.1
A	4179	481.2	2.1

Re...	Num...	X-M...	HP×CV
ALL	4973	458.0	1.8
B	4950	459.4	1.8

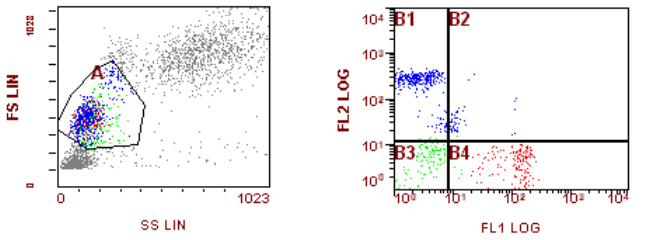
Re...	Num...	X-M...	HP×CV
ALL	4973	440.0	2.3
C	4949	441.3	2.3

Re...	Num...	X-M...	HP×CV
ALL	2234	468.4	3.6
D	2216	471.2	3.6

Re...	Num...	X-M...	HP×CV
ALL	2640	443.6	0.3
E	2364	455.3	0.3

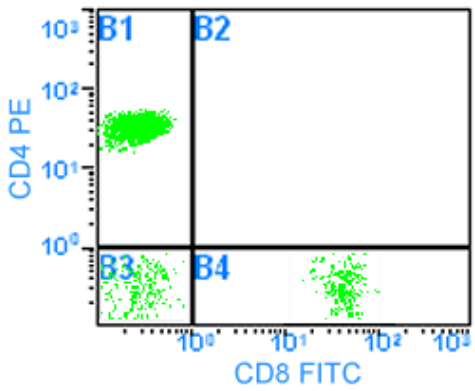
- Example 2 _____

_____



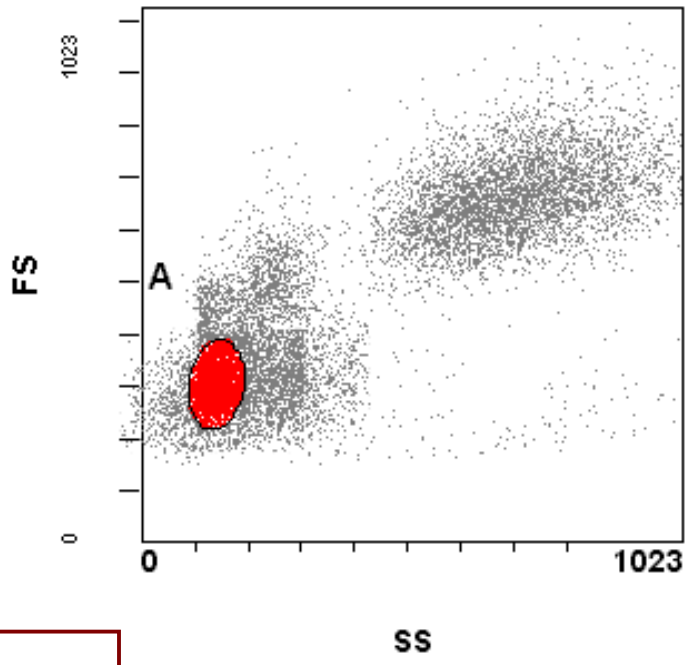
Region	%Gated	X-Mean	Y-Mean
B1	42.91	2.8	266.6
B2	6.63	23.6	50.9
B3	28.90	2.4	4.4
B4	21.56	123.9	4.1

- Example 3 _____
  _____



	X Mean	Y Mean
B1	.3	14.3
B2	1.1	1.4
B3	.3	.4
B4	32.1	.7

- Example 4 _____
  _____



- ❑ Facilitator sign off
  ___

SKILL CHECK

MAINTENANCE

Maintenance I Procedures	*Facilitator sign-off*
Clean and replace air filters	
Clean reagent and waste containers	
Clean the sampling system	
Remove and replace sheath fluid filter	
Adjust system pressure ▪ Perform adjustment in presence of facilitator	
Change field stop position for forward scatter	

MAINTENANCE I SKILL CHECKS

Final Sign Off_____

Maintenance II Procedures	*Facilitator sign-off*
Remove and replace the sample probe and sample pickup tubing. DO NOT REMOVE TUBING ▪ Remove the probe and tubing ▪ Show to facilitator ▪ Replace sample probe and tubing	
Remove and replace MCL sample head and associated tubing. DO NOT REMOVE TUBING ▪ Remove MCL sample head ▪ Show to facilitator ▪ Replace MCL sample head	
Remove and replace optical filter plate and a filter	
Adjust the HeNe laser ▪ Discuss with facilitator before performing procedure	

MAINTENANCE II SKILL CHECKS

Final Sign Off_____

SKILL CHECK

CXP SOFTWARE BASICS

1. Demonstrate logging on/off the software.

Please answer the following questions

2. How would you complete each of the following using the menus and/or shortcuts. Where appropriate, list both methods.

- Save a protocol _____
  _____
- Workspace Preferences for gating _____
  _____
- Display the Acquisition Manager _____
  _____
- Insert a blank FlowPAGE _____
  _____
- Create a dot plot _____
  _____
- Display high voltage and gain adjustments _____
  _____
- Arrange plots on the workspace _____
  _____
- Duplicate a plot on the Workspace _____
  _____

3. How would you populate a plot with a listmode file from the Resource Explorer? _____
   _____
   _____

4. How could you add the Create a new FlowPAGE icon to the FlowPAGE toolbar (assume it did not already exist)? _____
   _____
   _____

5. How could you change the listmode filename to include Sample ID 1 and 2? _____
   _____
   _____

5. Place the letter describing the icon meaning next to each icon below.

____ 	A. Save protocol
____ 	B. Print
____ 	C. Worklists
____ 	D. Color dot plot
____ 	E. Protocols tab
____ 	F. Start collecting data
____ 	G. Help
____ 	H. Cytometer Control
____ 	I. Listmode files icon
____ 	J. Histogram plot

6. Print out 7.2 QC Processes from the Help screen.

❑ Facilitator sign off

_____

SKILL CHECK

CXP SOFTWARE CREATING A PROTOCOL

1. Show facilitator a printout of the Immuno-Brite practice protocol you created illustrating meeting the Worksheet guidelines.
2. Create a protocol for an application per guidelines within the application modules or outside information source (ex. Journal Cytometry) or a two color protocol using the Worksheet guidelines.
3. Print the protocol with data to illustrate proper parameter selection, plot selection, gating and analysis.

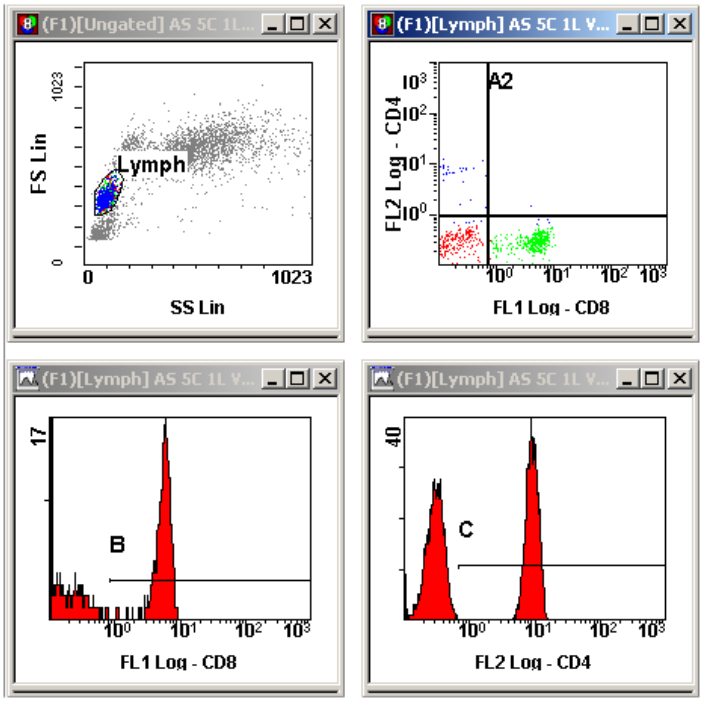
WORKSHEET – 2 COLOR PROTOCOL

Selecting Parameters

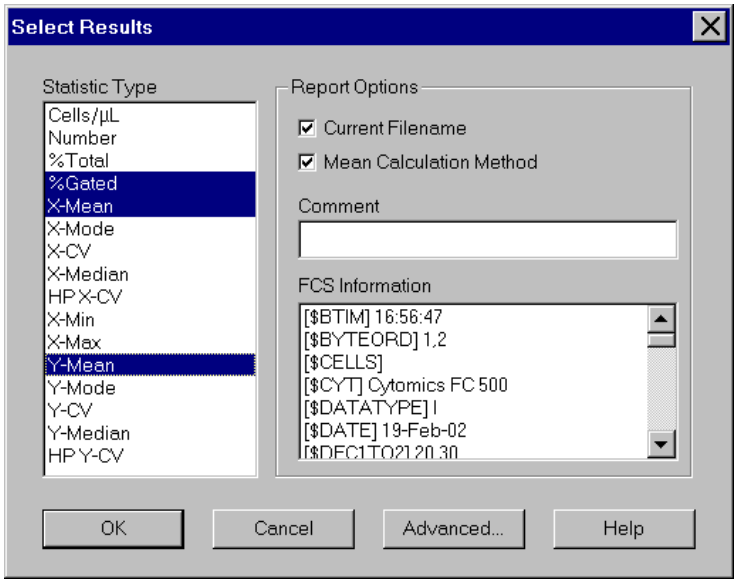
Drag and Drop the AS 5C 1L Verify 8-4-45-19-3 listmode file (or equivalent) in your Common folder onto your plots and use this data to adjust the regions.

The parameters are automatically entered when the listmode file is dragged to a plot.

Creating Plots, Creating Regions, Defining Gates



Analyze Data



- ❑ Facilitator sign off

_____

SKILL CHECK

CXP SOFTWARE AUTOSETUP

1. Create a new application to include all pertinent information such as dyes, filters, and Flow-Set™ Fluorospheres target channels. You may create one of your choice or use the guidelines below:

   5C 2 L, 1-19 Forward scatter angle, dual laser filter block

   Obtain target values for the parameters below from your facilitator.

   FS _____ FL2 _____ FL3 _____

   SS _____ FL4 (FS 675) _____ FS675 _____

   FL1 _____ FL5 (FS 770) _____ FS770 _____

   Name the application AS Skill Check. Ensure the Flow-Set and Verify protocols are set up to export to QC.

2. Schedule multiple application autostandardization and print the load list.

3. Prepare the samples called for on the load list, place them in the carousel in the positions indicated in the load list, and run the samples on the system. Save all printouts as proof of completion of the task.

- Facilitator sign off

_____

SKILL CHECK

CXP SOFTWARE FLOWPAGE

1. Create a FlowPAGE with actual data with the following characteristics:
   - Contains a hospital name and address.
   - Contains at least 4 arranged sets of plots and statistics.
   - Contains a bitmap image provided by your facilitator
2. Store the FlowPAGE as a separate page.
3. Print the page as proof.
4. Print the page as a PDF file.

❑ Facilitator sign off
_____

SKILL CHECK

CXP SOFTWARE ACQUISITION MANAGER

1. Create a panel of at least 3 protocols to run Cell Surface Markers using the Acquisition Manager to include:.
   - Completion of autosetup appropriate scheduled application (ex. 2 Color panel use 2C 1L application) and cytosettings loaded from appropriate settings file (ex. 2 Color, use AS 2C Settings.pro).
   - The isotype control sample should be run first, regions adjusted, and plots and regions carried to the other protocols.
   - Parameters should be labeled for each protocol according to the antibodies in the tube.
   - Set up the Workspace Preferences to use Sample ID 1 and 2 in the listmode file name and display these columns on the worklist. Use the Sample ID 1 to label the patient name (e.g. John Doe). Use the Sample ID 2 to label the antibodies run in each tube (e.g. CD8/CD4).
   - Save the panel, run the samples and print the results of the sample runs.

   Note: If you have trouble deciding what markers to use, ask your facilitator for help.
2. Create a Worklist to include:
   - At least 20 tube positions on the carousel.
   - At least one two color panel and one three color panel.
   - At least 3 different patient samples plus the setup panel (you may use the auto setup panels)
   - Transfer of settings from the autosetup {settings} protocol to the other panels.
   - A rinse tube between each patient.
   - All parameters labeled according to antibodies run in each tube.
   - Include sample ID and date in the listmode file name and enter a specific name for each patient.
   - Set up at least one panel to provide absolute count data.

❑ Facilitator sign off

_____

SKILL CHECK

CXP SOFTWARE RETRIEVE AND ANALYZE DATA

1. Retrieve a listmode file with the Runtime protocol and print the results as proof.
2. Retrieve a listmode file with a new protocol (ex. Retrieve a 3 or more color sample with a 2 color protocol) and print the results as proof.
3. Retrieve multiple files with a panel of protocols and print the reuslts as proof.
4. Retrieve at least 2 sets of listmode files with a single protocol using batch AutoMATOR function and print the results as proof.
5. Create a set of linked regions and demonstrate to your facilator that they are linked (if you move the linked region, the others linked to it should also change).
6. Create an Overlay plot of at least 3 single parameter plots which include stats, a legend, regions, annotation, and data analyzed using either the Overton or Kolmogorov-Smirnov modes.
7. Publish data to Microsoft Excel and print the results as proof.

❑ Facilitator sign off

_____

SKILL CHECK

CXP SOFTWARE EXTRAS

1. Retrieve a 3 color sample and set up a dot plot to display in color blend or color precedence mode. Print the results (if a color printer is available) or show your results to the facilitator if not.
2. Access administrative functions and setup a new user with all assigned privileges and assign the user to a workgroup.
3. Create a user usage file.
4. Copy and paste a single parameter plot to another Windows application (e.g. Microsoft™ PowerPoint, Word, etc.) and print the results.
5. Create a freeze frame with at least one single parameter plot overlaid on another and print the results as proof.
6. Demonstrate to your facilitator your ability to turn Baseline Offset option on or off. You can accomplish this task by printing two sets of plots, one with Baseline offset on and the other with Baseline offset off.
7. Call your facilitator and demonstrate your ability to adjust compensation on a listmode file and save the compensated file as a new listmode.

❑ Facilitator sign off

_____

SKILL CHECK

CXP SOFTWARE DATABASE

1. Enter a new QC product into the databasewith all fields filled in. Print the result as proof.
2. Print out either a QC Levey-Jennings plot or Data Table for a current QC product (e.g. Flow-Check).
3. Create a new panel template to include stats, an equation, flagging limits, and plots. You can show the final product to your facilitator or create a printout by either running the panel or using the listmode playback tool to repaly data as a panel.
4. Enter patient demographics (minimum name, WBC count, and Lymphocyte %). Show your final result to your facilitator.
5. Printout a patient panel report.
6. Demonstrate to your facilitator your ability to remove or restore database table entries.
7. Input maintenance log information. Print the log as proof.
8. Input service log information. Print the log as proof.

❑ Facilitator sign off

_____

SKILL CHECK

CELL SURFACE MARKERS

The following skills apply for 2 Color, 3 Color, 4 Color, or 5 Color applications. Each application can be met in 4 separate skill checks beginning with 2 Color and proceeding through 3 Color, 4 Color and 5 Color. If you feel you are able, applications can be skipped (ex. 2 Color) and a higher level completed (ex. 3 Color). If you successfully complete a greater number of colors, credit will also be given for fewer colors (ex. Complete 3 Color, credit will also be given for 2 Color).

For each application (e.g. 2 Color, 3 Color, 4 Color or 5 Color):

1. Schedule the appropriate application (ex. 2C 1L for 2 Color, 3C 1 L for 3 Color, etc.).
2. Prepare all samples indicated in the carousel load list, run the samples, and print the results as proof of completion.
3. Create an application protocol (e.g. 2 Color for 2 color samples, 3 Color for 3 color samples, etc.) to run the samples.
4. Load the instrument settings from the updated application settings.pro (ex. 2 Color use settings from AS 2C Settings.pro, 3 Color 1 laser use settings from AS 3 C 1L Settings.pro, etc.) into the created protocol.
5. Prepare and run at least 3 samples with the created protocol.

Example samples (There may be others you can choose from):

2 Color:	2 Color Isotype
	CD3FITC/CD4PE
	CD3FITC/CD8PE
	CD3FITC/CD19PE
3 Color 1L	3 Color Isotype
(CytoStat)	CD3FITC/CD4PE/CD8PC5
	CD45FITC/CD19PE/CD3PC5
	CD45FITC/CD56PE/CD3PC5

❑ Facilitator sign off

_____

PN 175306 D (September 2004)
Miami Education Center

3 Color 1 L	Isotype
(IOT)	CD8FITC/CD4PE/CD3ECD
	CD16FITC/CD56PE/CD3ECD

Note: For 4 Color and 5 Color, there are many possibilities. You can create these by adding a single dropin (or two single color dropins) to a 3 color antibody sample. Ask your facilitator for the possible reagents available to produce at least 3 different samples for your skill check. Write these combinations in the space provided below.

4 Color __L _____

_____

_____

5 Color __L _____

_____

_____

- ❑ Facilitator sign off

2 Color _____
3 Color _____
4 Color _____
5 Color _____

SKILL CHECK

TETRACXP

1. Show printouts for all parts of the tetraCXP module to your facilitator including:
   - Scheduled application.
   - QC 1L Flow ChecK (TM) _Align Levey-Jennings plot.
   - Panel FlowPAGES
   - Two Panel Reports illustrating cells/uL values one obtained by Flow Count and the other by entering WBC count and lymphocyte % values into the database.

❑ Facilitator sign off
_____

DNA

1. Prepare a reference sample, control sample and at least one unknown DNA sample to run on the flow cytometer using the DNA Prep.
2. Create a protocol to run the samples on the system to include parameters, gating and analysis similar to the examples illustrated in this module.
3. Run the samples per the guidelines in this module and print the results.
4. Calculate a DNA Index on an abnormal DNA sample.

5. Optional: Prepare a second set of DNA samples with at least one cell surface marker attached to the cells.
6. Optional: Create a second protocol to run DNA and cell surface markers simultaneously illustrating marker gating and analysis similar to the examples shown in this module.
7. Optional: Run the samples and print the results.

❑ Facilitator sign off

_____

SKILL CHECK

STEM CELL CD34+ HPC ENUMERATION

1. Perform Quality Control to verify instrument performance and reagent stabilty.
2. Create an ISHAGE protocol to assay stem cells.
3. Obtain a stem cell listmode file from your facilitator and populate your plots or if possible, prepare a set of stem cell samples and run it to populate the plots.
4. Adjust the regions per the ISHAGE guidelines and print your results as proof ot task completion.

- Facilitator sign off

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SKILL CHECK

APOPTOSIS DETECTION ANNEXIN V FITC

1. Perform Quality Control to verify instrument performance and reagent stability.
2. Create a protocol to run apoptosis samples per the module guidelines.
3. Obtain an apoptosis listmode file and populate your plots or if possible, prepare apoptosis samples to populate the plots.
4. Adjust the regions per the module guidelines and print results as proof of task completion.

❑ Facilitator sign off

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Access Online Help

1. Select  on the Toolbars.
2. Select desired subsection(s). The information will appear to the right of the Contents list.
3. You may also print a subsection.

Access Online Help

1. Select  on the Resource Explorer.
2. Select desired manual. Acrobat Reader will now display the manual in a new window.
3. You may now select the desired subsection(s) for display or print all or any part of the manual.

Important Information

Online Manuals Available:

Reference

Instructions For use

Special Procedures