Instructions for Use

CyAn ADP

High-speed Analyzer

BECKMAN
COULTER

CyAn ADP
Instructions For Use
Document 0000050 (November 2014)

Find us on the World Wide Web at:
www.beckmancoulter.com

This manual is not a substitute for the detailed operator
training provided by Beckman Coulter or for other
advanced instruction. Contact a Beckman Coulter
Representative in the event of instrument malfunction.

Revision History

Initial Issue
2004
Software Version Summit 4.1

Revision G, 06/2008
Software Version Summit 4.3
Change labeling to Beckman Coulter name and logo. Update consumables list, Laser Delay procedure, Summit Overview, Six-color compensation experiment, and document organization.

Revision H, 01/2009
Software Version Summit 4.3
Change reference to Worklist Builder Quick Start Guide.

Revision J, 05/2009
Software Version Summit 4.3
Note: We do not use the letters I or O for revisions because they can be confused with numbers.
Change reference to Windows XP.

Revision K, 11/2014
Software Version Summit 4.4
 Updates were made to the company corporate address.

Safety Notice

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting 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.

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 is not limited to, protective eyewear, gloves, and suitable laboratory attire when operating or maintaining this or any other mated laboratory analyzer.

Alerts for Warning and Caution

⚠ **WARNING**

WARNING indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury. May be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis (does not apply to all products).

⚠ **CAUTION**

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices. May be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis (does not apply to all products).

⚠ 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 broken 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.**
- **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 when troubleshooting.**

⚠ CAUTION

System integrity could be compromised and operational failures could 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 software 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.**

⚠ CAUTION

If you purchased this product from anyone other than Beckman Coulter or an authorized Beckman Coulter distributor, and, 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.

Instrument Safety Precautions

The CyAn high-speed cell analyzer has been engineered with safety as one of its primary features. Safety of the operator, field service personnel, bystanders, and of valuable samples, is paramount to Beckman Coulter's commitment to high performance design and engineering.

This section explains some general safety and hazard symbols and necessary precautions operators of the CyAn should follow during operation. Engineering controls have been put in place to protect the operator, and deliberate misuse of the instrument or its instructions may result in unintentional harm. Please follow all safety and hazard instructions as directed in this manual.

Symbols

Below are the symbols used and their corresponding meanings, which can be found on the instrument and throughout this manual.

Safety Symbols

Electrical Shock   Laser Irradiation   Biohazard   Caution
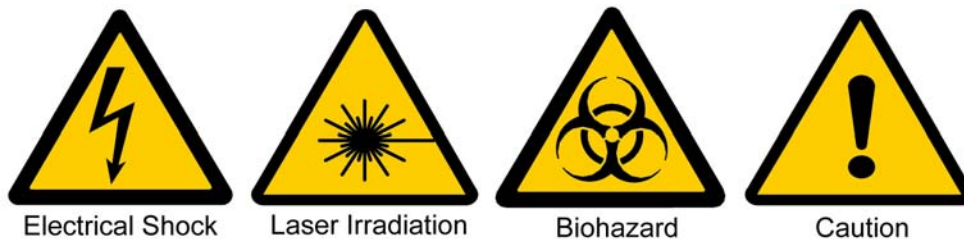
Electrical Shock — Risk of Electric Shock

Laser Irradiation — Avoid looking directly into laser, as it may cause permanent eye damage.

Biohazard — Biological Hazard/Risk

Caution — Important; Attention; Refer to Accompanying Documentation

Electrical Safety

The Beckman Coulter instrument product line conforms to international regulations encompassing the accessibility of high voltages by the user. In the United States, each flow cytometer is manufactured to comply with applicable regulations from the American National Standards Institute (ANSI), the Occupational Safety and Health Administration (OSHA), and various state regulatory organizations. Internationally, each CyAn ADP High-Performance Flow Cytometer is manufactured to comply with the International Electrotechnical Commission's Standard for Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use (IEC 61010-1) and subsequent amendments.

Optical/Laser Safety

Laser Product Hazard Classification

The intent of laser hazard classification is to provide clear distinction of the laser, or laser product properties, and the hazards to users so appropriate protective measures can be taken. CyAn ADP is a Class 1 laser product per 21 CFR 1040 and EN60825; meaning operators are not exposed to harmful levels of laser irradiation during normal operation. During times of service and/or repair, laser safety control measures for Class 3B and/or 4 lasers shall be followed.

NOTE Because the CyAn ADP has been designed to operate as a Class 1 Laser Device, the instrument must be operated with all light containment tubes in place and all protective light seals intact. Most laser components of the CyAn ADP flow cytometer are Class IIIB to Class IV. As such, these lasers have the potential to cause injury. There is one small viewing window inside the CyAn ADP, but its purpose is to be used only by trained Beckman Coulter personnel during service or repair. A safety interlock prevents operators from viewing laser light through this viewing window under normal operating conditions.

Certification of regulatory compliance of these lasers often requires significant involvement from the institutional or company laser-safety officer. Beckman Coulter does not provide regulatory certification for the individual lab, but can offer assistance in laser safety matters.

All access plates and other user accessible points of potential laser exposure are clearly designated on the CyAn ADP instrument. Interlocks are designed to prevent accidental irradiation of the operator. The user should not defeat these interlocks.

Any questions or concerns regarding laser safety of the CyAn ADP should be directed to Beckman Coulter Customer Care.

Laser Safety Label
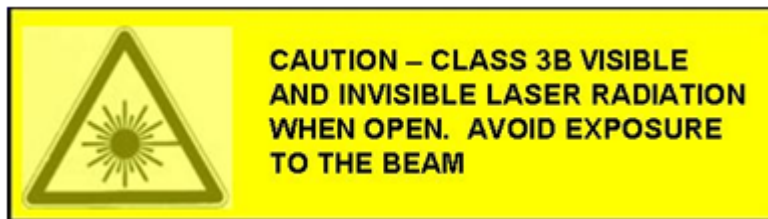
CyAn ADP Interior Laser Safety Label Locations
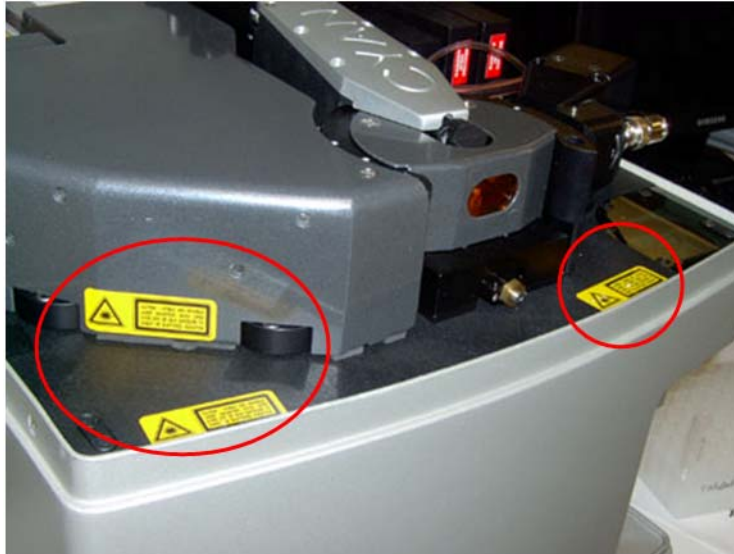
Biohazard Safety

IMPORTANT If any hazardous organism, material, or agent is used in the instrument, the site operator or Principal Investigator is responsible for informing Beckman Coulter in writing of those hazards before receiving service or repair. This includes a list of all pathogenic cell lines, hazardous reagents, radioactive material, or agents with a BSL Level II or higher. This information will be kept confidential and will be used to inform Beckman Coulter Field Service Representatives of any hazards prior to visiting any CyAn site. Failure to report this information may delay service on an instrument. Safety of the user as well as safety of Beckman Coulter employees is of overriding importance. Proper decontamination procedures must be followed for all applicable returned parts.

Universal precautions should be followed when handling samples and waste fluid containing samples:

- Gloves, a laboratory coat, and eye protection should be worn whenever handling samples.
- Waste fluid may contain hazardous levels of biological contamination. Gloves, a laboratory coat and eye protection should be worn whenever exposure to waste fluid is possible.
- To ensure inactivation of biological organisms in the waste tank, an appropriate type and quantity of an EPA registered disinfectant should be placed in the tank initially upon use, and every time the waste tank is emptied and reinstalled.

For additional information on laboratory biosafety, review the U.S. Department of Health and Human Services, Centers for Disease Control document, *Biosafety in Microbiological and Biomedical Laboratories.*

Waste Tank
The reusable waste tank is located on the right side of the SMS cart and is labeled with a large black and orange biohazard symbol on the front. Emptying waste fluid generated from the CyAn ADP could be a daily occurrence for some operators. It is expected that all operators have undergone proper blood-borne pathogens training provided by the institution of use, or similar authority, prior to using the CyAn. It is pertinent that the appropriate type and quantity of disinfectant is

placed in the waste tank to ensure effective inactivation of the biologics in use when the tank is full. Please see APPENDIX A, for a list of approved cleaners and disinfectants for use with the CyAn ADP. Please use caution when emptying the waste tank to avoid splashing of the waste fluid. Personal Protective Equipment (PPE) should be worn at all times when using any part of the CyAn ADP, including emptying the waste tank. Please contact Beckman Coulter Customer Care if there are any questions or concerns about handling waste fluid generated by the CyAn ADP.

Sample Waste

Exposure to sample waste fluid from clogs in the waste line may occur; however, this is rare. If a clog occurs, exposure to biohazards is likely. PPE should be worn at all times when using the CyAn ADP to protect the operator from unexpected hazards. If exposure to biohazardous material occurs due to a fault of the CyAn ADP, Beckman Coulter Customer Care should be contacted immediately.

Samples

Sample handling is a normal part of the use of the CyAn ADP. It is expected that all operators have undergone proper blood borne pathogens training provided by their institution, or similar authority, prior to using the CyAn ADP. PPE should be worn at all times when using any part of the CyAn ADP. Samples of any kind should not be handled without wearing gloves. Caution should be used when handling any biohazardous samples. Any reagents used on the instrument, or in the preparation of samples for use on the CyAn ADP, should be handled by professional users only.

Sample O-ring

There is an o-ring located in the top interior of the sample injection area. The purpose of this o-ring is to create a seal when a test tube is placed on the sample probe, which results in pressurization of the tube. Over time this o-ring may lose its elasticity or become damaged from repeated contact with polypropylene test tubes. Deterioration of this o-ring may cause test tubes to be loose when placed on the sample probe, leading to the potential for test tubes filled with biohazardous samples falling off, causing exposure to the operator or other laboratory personnel. This occurrence is not likely, but if an operator experiences this condition, Beckman Coulter Customer Care should be contacted immediately so the o-ring can be replaced.

Sample Waste Manifold

Waste fluid is continuously generated when running the CyAn ADP and is a normal function of this instrument. This waste fluid is routed through a manifold located within the SMS cart. If this part malfunctions, a backup of fluid may occur, resulting in waste fluid dripping through a small hole in the top corner of the sample injection area. Because this waste fluid may contain biohazardous material, caution should be used if this fault is identified; however, this occurrence is highly unlikely. PPE should be worn at all times when using any part of the CyAn ADP to ensure protection of the operator from all potential hazards. If an operator identifies such a leak, Beckman Coulter Customer Care should be contacted immediately.

Aerosols

The CyAn ADP is a flow cytometry analyzer and has no sorting capabilities. No aerosols are generated under normal operating conditions. In the event of extreme over pressurization of the system, there is a remote chance that sample and/or waste fluid could exit orifices at a rate which could potentially create aerosols; however, this has never been experienced. If at any time an operator identifies a potential over pressurization problem, Beckman Coulter Customer Care should be contacted immediately.

Sample Probe

The sample probe is constructed of a stainless steel tube with one end slightly squared off. Direct contact with the end of the probe could cause an abrasion or cut. Because biohazardous samples are run through the sample probe, there is a chance that if the skin is broken due to direct contact with the end of the sample probe, an operator could be directly exposed to biohazardous material. Use caution when loading and unloading samples on the probe. PPE should be worn at all times when using any part of the CyAn ADP. If injury related to the sample probe occurs, Beckman Coulter Customer Care should be contacted immediately.

General Safety

To protect the health, environment, and safety of CyAn ADP sites and their users, the following information should be reviewed by all operators.

- The CyAn ADP is intended for Professional Use Only. All operators should be trained on the proper use and limitations of the instrument prior to its operation.

- Wear appropriately sized gloves providing good manual dexterity to reduce the likelihood of skin pinches and abrasions.

- Protect the skin and eyes whenever handling chemicals of any kind, regardless of how benign they may appear.

- Summit workstations include a keyboard interface. Evaluate the ergonomic suitability of the location of the keyboard and the user to avoid injury.

- Cords and cables may be located on the floor around the unit, be aware that they can cause a tripping hazard. The Sheath Management System (SMS) is attached to the CyAn ADP head unit by an 8 foot (2.4 meters) umbilical cord. It is suggested that the SMS cart be placed underneath a laboratory bench to keep it out of the way of foot traffic. If this is not possible, it is recommended that the SMS be placed where it will not pose a tripping hazard. It is suggested that the umbilical cord be run behind the laboratory bench, if possible, to keep the cord from creating a tripping hazard. If the cord must be run down in front of the laboratory bench to the SMS, use caution when operating or walking past the CyAn ADP. It is suggested that hazard tape be placed on these items to make them highly visible.

- Always check with the site safety officer for correct disposal of waste products.

- Use proper lifting techniques, or seek assistance when handling full tanks. To reduce the likelihood of back injury, empty waste at least once per day.

- The lid of the CyAn ADP is on hinges that should keep the lid in the up position when open. It is recommended by Beckman Coulter that the lid not be left open for extended periods of time or be opened and closed repeatedly. Excessive wear on the hinges over time may cause the lid to fall unexpectedly. Use caution when the lid is open and do not leave the instrument unattended. If the lid of the CyAn ADP becomes loose and will not stay up on its own, contact Beckman Coulter Customer Care so it can be repaired.

Electromagnetic Information

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in its installation. This equipment

generates, uses, and can radiate radio frequency energy. If not installed and used in accordance with the instruction manual this equipment may cause harmful interference to radio communications. If this equipment does cause harmful interference the user will be required to correct the interference. This Class A digital apparatus complies with Canadian ICES-003.

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Introduction

How to Use Your Manual

Scope

The CyAn ADP Instructions for Use manual contains basic information regarding the use and operation of the CyAn ADP and assumes you have received basic training on the instrument. Please contact your Beckman Coulter Representative for information not provided in this manual. This manual does not provide instructions for the installation or upgrade of hardware because such actions must be provided by a Beckman Coulter Representative.

Intended Use

This instrument is intended for research use only.

Use the Instructions For Use manual for the day-to-day running of your instrument and workstation. Go through the detailed step-by-step procedures of startup, quality control (QC), running samples, analyzing data, printing reports, reviewing QC data, and shutdown before operating the instrument. This manual contains, safety and troubleshooting information, as well as procedures for cleaning the instrument and replacing some components.

About this Manual

The information in your Instructions for Use manual is organized as follows:

Chapter 1, Installation
Provides system specifications, lab environment requirements, and the instrument installation recommendation.

Chapter 2, System Overview
Provides an overview of CyAn ADP features, system architecture, and subsystems.

Chapter 3, Summit Software Overview
Provides basic information regarding the features in Summit software.

Chapter 4, Startup, Alignment and Shutdown
Provides information on starting, and shutting down the instrument.

Chapter 5, Performance Validation and Calibration
Contains an example validation and calibration procedure.

Chapter 6, Cleaning and Maintenance
Describes the cleaning procedures for daily cleaning as well as the biannual and yearly decontamination process.

Chapter 7, Troubleshooting Procedures
Provides a basic troubleshooting matrix and a list of possible error messages.

Appendix A, Approved Cleaners and Disinfectants
Contains a list of cleaners and disinfectants that can be used on the CyAn analyzer.

Appendix B, Consumables
Contains a list of consumables to be used with the CyAn analyzer.

Appendix C, Compensation Background Information
Provides information on how to resolve actual intensities from each antibody conjugate in a multicolored sample. Includes an example of compensation for a six-color experiment.

Appendix D, Laser Delay
Provides instructions on how to set Laser Delay.

Appendix E, Symbols
Defines the symbols used on the CyAn analyzer labels.

Conventions

This manual uses the following conventions:

- Bold font indicates instrument manual titles.
- Bold indicates a screen icon.
- Italics font indicates screen text displayed by the instrument.

IMPORTANT IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the IMPORTANT message adds benefit to the performance of a piece of equipment or to a process.

NOTE NOTE is used to call attention to notable information that should be followed during installation, use, or servicing of this equipment.

Installation

General Laboratory Information

IMPORTANT Your Beckman Coulter Representative is responsible for uncrating, installing, and initial setup of the CyAn ADP. Contact your Beckman Coulter Representative before relocating the instrument.

CyAn ADP Installation Specifications

Heating and air conditioning vents or fans are not recommended directly above the CyAn ADP because of the resulting temperature fluctuation, vibration, and possible dust.

Table 1.1 CyAn ADP General Requirements

Specification	Requirement
Power Requirements	100V - 230V, 600VA(W), 50/60 Hz
Lab Bench	Stable bench/table top to support the CyAn ADP, one monitor, keyboard, and a mouse pad
Service Access	45.7 cm (18 in.) minimum around instrument components
Phone	Location near CyAn ADP for contacting technical support
Installation Category	II
Pollution Degree	2
Internet Access	Internet Service Provider or LAN connection for downloading software updates
Dimensions (not including Auxiliary Components)	Height front cover closed - 39.1 cm (15.4 in.) front cover open - 72.1 cm (28.4 in.) Width - 33.3 cm (13.1 in.) Depth - 49.8 cm (19.6 in.) with clearance for cables - 62.5 cm (24.6 in.) Weight - 36.3 kg (80 lbs)

Table 1.2 Auxiliary Components

Component	Dimensions
Sheath Management System (with casters): Houses sheath container, waste container, cleaner fluid container, air compressor, and vacuum	Height - 61.3 cm (24.2 in.)
	Width - 73.3 cm (28.9 in.)
	Depth - 61.9 cm (24.4 in.)
	Weight - 22.6 kg (50 lbs)
Summit Workstation	Height - 42.9 cm (16.9 in.)
	Width - 19.1 cm (7.5 in.)
	Depth - 45.7 cm (18.0 in.)
	Weight - 10.5 kg (23 lbs)
Optional Uninterruptible Power Supply	Height - 20.3 cm (7.9 in.)
	Width - 14.7 cm (5.7 in.)
	Depth - 44.5 cm (17.5 in.)
	Weight - 20 kg (44 lbs)

Table 1.3 Operating Environment

Ambient Temperature	15 to 30°C (59 to 86°F)
	For optimum performance maintain at +/- 2°C
Relative Humidity	20 to 80% RH (non-condensing)

Figure 1.1 Power Configuration and Consumption

0000050K
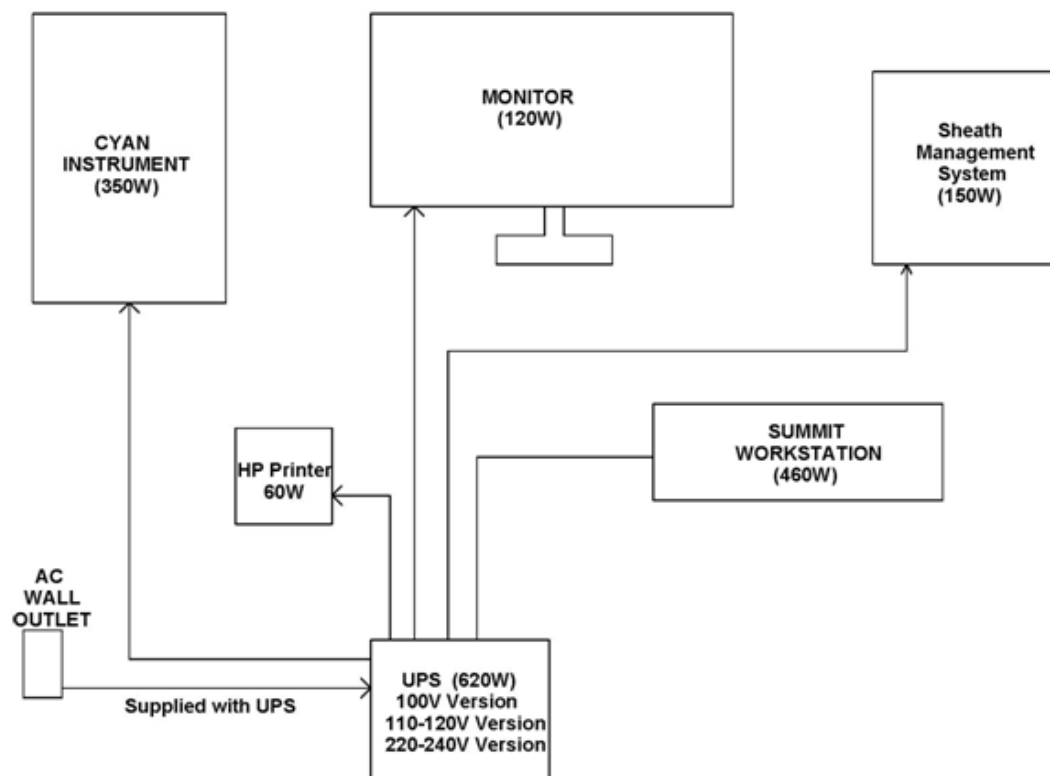
Technical and Instrument Specifications

The technical and instrument specifications for CyAn ADP are summarized in the following table.

Table 1.4 Instrument Performance

Maximum processing speed	Up to 70,000 events/sec.

Table 1.5 Excitation Optics

Optical parameters	2 scatter and 9 fluorescence
Beam geometry	Elliptical
Number of excitation lines	3
Laser options nominal operating output (See Coherent OPSL Operator's Manual for more information.)	488 nm (20 mW Semiconductor), 635 nm (25 mW semiconductor), 405 nm (25 mW semiconductor)
Laser maximum output value (Accessible in the interior of instrument)	488nm - 20mW 635nm - 27.5mW 405 nm - 27.5mW
Warm-up time	30 minutes

Table 1.6 Detectors and Filters (User Selected)

488 nm Excitation	FL1-530/40 nm, FL2-575/25 nm, FL3-613/20 nm, FL4-680/30 nm, FL5-750 nm
405 nm Excitation	FL6-450/50 nm, FL7-530/40
635 nm Excitation	FL8-665/20 nm, FL9-750 LP

Table 1.7 Signal Processing

Compensation	9 x 9 full matrix with compensation
Signal resolution	65536 (Summit software displays up to 4096 channels on all parameters)
Data acquisition channels	11
Sensitivity	MESF FITC < 100, MESF PE < 50

Table 1.8 Fluidics

Sheath Management System	Controls sheath, waste, and cleaning fluids. Software, hardware, and smart-sensor controls with RUN/PAUSE modes. Stable control of flow rates from 0 to 5 μm per second.
Sample flow rate	Up to 150 μL/min. (2.5μL/sec.)
Sheath fluid	Maximum 1.03 bar (15 psi), nominal 0.41 bar (6 psi)

Table 1.8 Fluidics

Quartz cuvette	UV-grade fused silica with 250 m square-sectioned internal channel
Noise level	< 70 dB

Table 1.9 Summit Workstation

Platform	Microsoft® Windows
Processor	> 2 GHz, AMD Athlon™, Intel® Pentium®
Memory	1 GB RAM (minimum)
Storage	160 GB hard drive (minimum), 16X DVD +/- CDRW
Monitor	24-inch LCD flat screen
Network	High-speed Ethernet 10/100 Mbps

Table 1.10 CyAn ADP Enclosure

|	
Type	Molded RIM polyurethane structural foam
Installation	Indoor only
Dimensions (not including Utility Cart or Summit Workstation)	Height 39.1cm (15.4 in.) front cover closed 72.1cm (28.4 in.) front cover open Width - 33.3cm (13.1 in.) Depth 49.8cm (19.6 in.) 62.5cm (24.6 in.) including clearance for cables. Weight - 36.3 kg (80 lbs)

Table 1.11 Auxiliary Components

Sheath Management System (with casters): Houses sheath container, waste container, cleaner fluid container, air compressor, laser power supply, and vacuum	Height - 61.3 cm (24.2 in.) Width - 73.3 cm (28.9 in.) Depth - 61.9 cm (24.4 in.) Weight - 52 kg (115 lbs)
Uninterruptible Power Supply (Optional)	Height - 20.3 cm (7.9 in.) Width - 14.7 cm (5.7 in.) Depth - 44.5 cm (17.5 in.) Weight - 20 kg (44 lbs)

Table 1.11 Auxiliary Components

Summit Workstation	Height - 42.9cm (16.9 in.)
	Width - 19.1 cm (7.5 in.)
	Depth - 45.7cm (18.0 in.)
	Weight - 10.5 kg (23 lbs)
External Transformer	Height - 19.1cm (7.5 in.)
	Width - 31.2 cm (12.3 in.)
	Depth - 19.1 cm (7.5 in.)
	Weight - 11.4 kg (25 lbs)

Table 1.12 Safety

Interlock	The front cover is protected with dual magnetic positive-break interlock switch. Operates dual spring solenoid shutter actuators on all laser paths.
Laser product class	Class 1 Laser Product
	IEC/EN 60825 - 1/A2:2001
Laser light leakage	Conforms to 21CFR1040.10 and 21CFR1040.11

Table 1.13 Operating Environment

Ambient temperature	15 to 30 C (59 to 86 F) For optimum performance maintain at +/- 2 C
Relative humidity	20 to 80% RH (non-condensing)

Table 1.14 System Utility Requirements and Fusing

Power, standard ADP	115 VAC +/- 10%, 60 Hz, single phase, 1.75A
Fuse	Type - 5x20mm, low breaking, high capacity, IEC. 115 VAC operation - 6.3A
Power, Sheath Management System	100-230 VAC, 50/60 Hz, single phase, 2A
Fuse, Sheath Management System	Type - 5x20mm, low breaking, high capacity, UL. 115 VAC operation - 3A
Power, Summit Workstation (not fused)	115 VAC +/- 10%, 60 Hz, single phase, 1A
Power, Summit Workstation Monitor (not fused)	115 VAC +/- 10%, 60 Hz, single phase, 2A
Power, External Transformer	Input: 230 VAC +/- 10%, 50/60 Hz, 5A
	Output: 115 VAC +/- 10%, 60 Hz, 8A
Fuse, External Transformer power	Type - 5x20mm, low breaking, high capacity, IEC, 115 VAC operation - 6.3A

System Overview

Overview of the CyAn ADP Analyzer

The CyAn ADP is a research-only flow cytometer engineered for precision analysis of cells, bacteria, and other similarly sized particles. CyAn ADP utilizes three excitation lines with independent, alignment-free focusing optics, simultaneous nine color and two scatter parameters, analysis rates of 70,000 events per second, a full 9 × 9 inter-laser compensation matrix, and high sensitivity. The instrument is optimized for cell cycle, kinetics, fluorescent protein work, and multi-color immunophenotyping. Rare-event analysis, such as MHC Dextramer studies, and no-lyse whole blood applications are easily performed on the CyAn ADP. The instrument also provides simplified compensation before, during, and after acquisition in all fluorescent parameters.

Figure 2.1 CyAn ADP Analyzer

CyAn ADP Features

Walk-up Operation

CyAn ADP offers walk-up operation. With no optical alignment necessary, the user places a 5 mL sample tube in the tube holder and chooses the desired protocol in Summit software. The software is used to control instrument settings.

Bench Top Configuration

The CyAn ADP measures 30 cm wide x 45 cm deep x 40 cm high.

Summit Software

Summit software, provides full control of all CyAn ADP functions, user- and application-specific data acquisition, analysis, storage, reduction, and retrieval. Summit software controls all instrument parameters using simple protocol-based menus. Summit software also provides high-content data acquisition, database capabilities, and open architecture for data exchange. Additional features include off-line analysis and easy page layout.

Safety

The CyAn ADP analyzer has been designed to incorporate the highest level of laboratory and operator safety. See Instrument Safety Precautions in the Safety chapter.

CyAn ADP Subsystems

CyAn ADP has four key subsystems that form a powerful research tool. The four subsystems are:

- Fluidics
- Optics
- Electronics
- Software

A functional block diagram for the CyAn ADP analyzer is shown in Figure 2.2. The fluidics system pressurizes the system and transports particles to the interrogation point. Lasers are used as excitation sources, and the emitted light, along with the ensuing light scatter and fluorescence, are directed within the optical system. Electronics are used to power and control the instrument functions. The software provides the user interface to control the fluidics, optical, and electronic hardware, as well as the functions to acquire, analyze, and store data associated with the particles.

Figure 2.2 CyAn ADP Functional Block Diagram
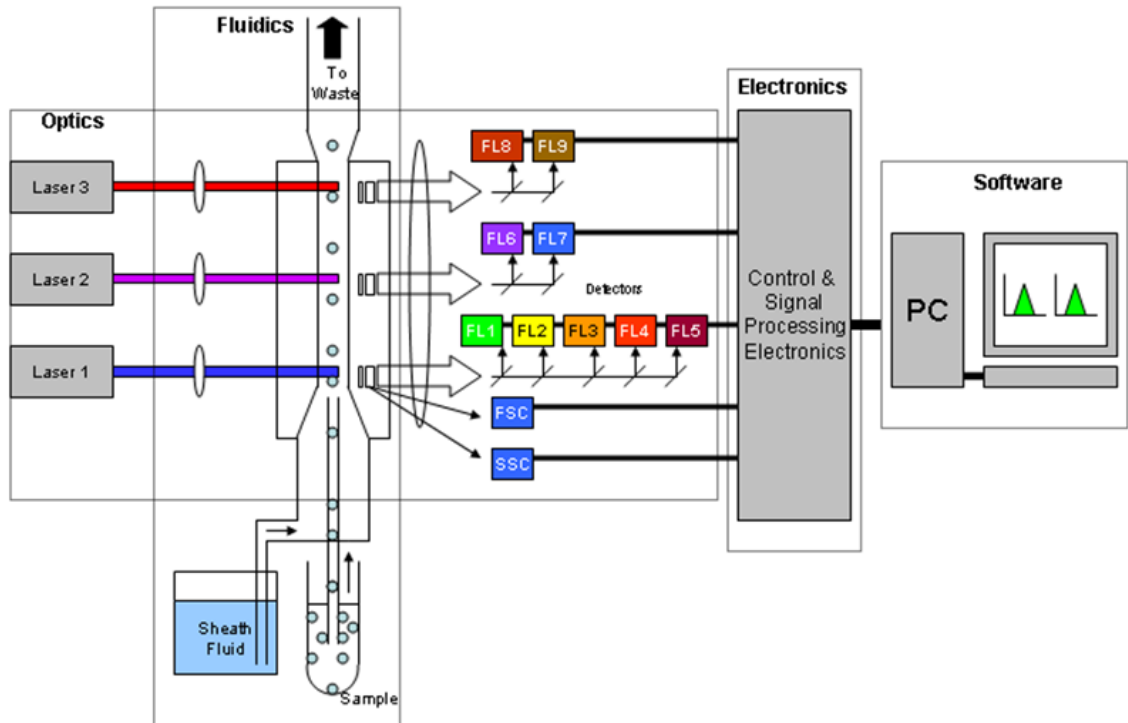
Fluidics Subsystem

The Sheath Management System controls the transfer of sheath fluid, waste, and cleaner fluid throughout the CyAn ADP system. Sheath fluid is housed in either a replaceable 20 liter cubitainer or 20 liter plastic carboy. Waste is contained in a 20 liter plastic carboy. Cleaner fluid is contained in a 5 liter cubitainer. The Sheath Management System provides sheath pressure stability and prevents bubbles from entering the system when the sheath container is changed. The built-in cleaner fluid cubitainer allows the user to easily clean and rinse the head-unit sheath path using Beckman Coulter LH Series Formaldehyde-Free Clenz and sheath or DI water as rinsing fluid. The 20 liter sheath and waste containers provide approximately 24 hours of system run time.

Figure 2.3 outlines the key fluidics components of CyAn ADP. In general, flow of air and fluids follows a path left to right in the diagram. An air compressor provides pressure for propulsion of the sample, cleaner, and sheath fluids. Air regulators condition and stabilize the pressure source prior to sheath and cleaner reservoirs and sample vessel. A number of electrically controlled valves control the flow state of the system and provide a means for cleaning and debubbling, in addition to routine flow for sample analysis. Sample is forced through tubing, is introduced to the flow cell, and is focused by sheath fluid. This hydrodynamic focusing effect causes individual particles to be introduced in single file to each of the sequential laser beams. While a pinch valve is used to allow sample to flow, the rate of sample flow is controlled by adjusting the over-pressure (differential pressure) of an electrically controlled air regulator relative to sheath pressure. Waste is drawn by a vacuum pump to a holding container for disposal.

Figure 2.3 CyAn ADP Fluidics Block Diagram
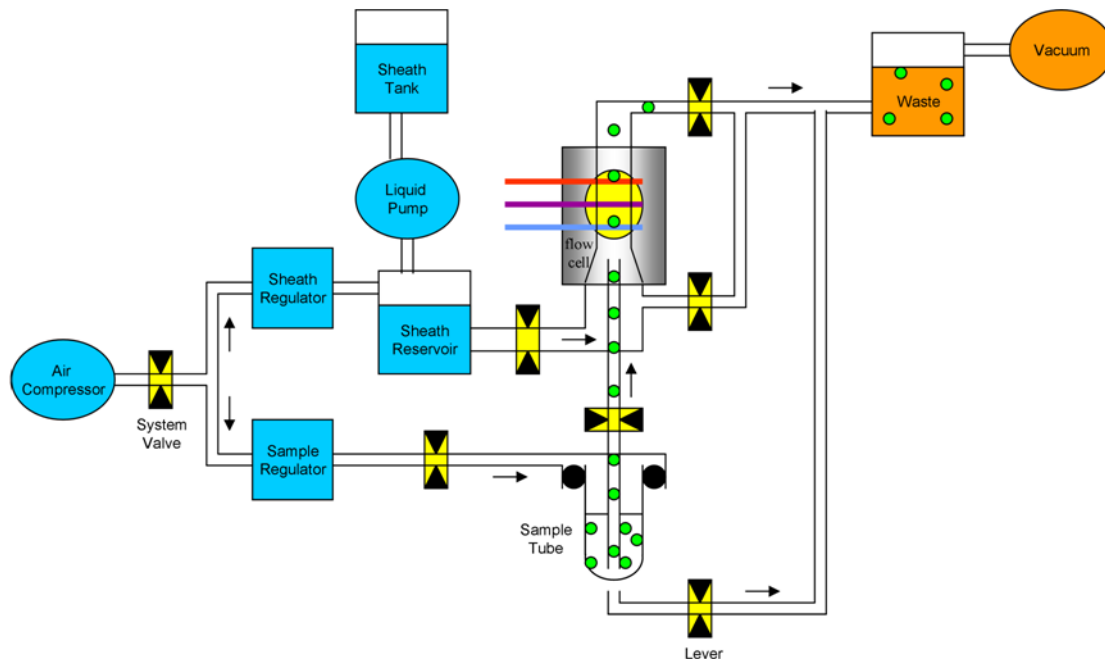
Sheath Management System (SMS) Indicators

The Sheath Management System provides 20 - 24 hours of sample run time. As the level of sheath fluid decreases and the level of waste fluid increases, both Summit software and Light Emitting Diodes (LED) indicators on the Sheath Management System front panel will provide fluid level status information and alerts to the operator.

SMS indicators can be viewed in the Sheath Management System section of the Instrument tab in the Instrument Control Panel. When all of the indicator lights are green, all components of the SMS are functioning properly.

Figure 2.4 SMS Section of the Instrument Control Panel
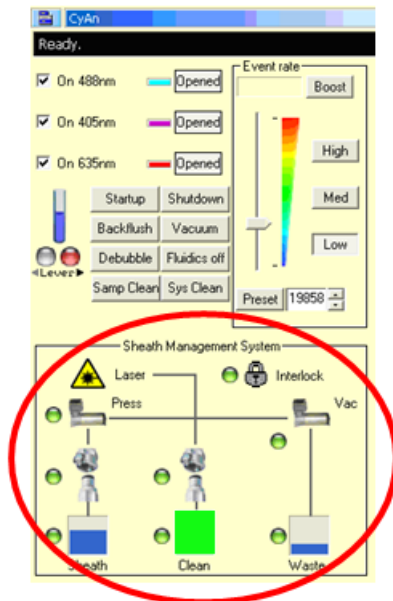
NOTE If an error condition occurs or status changes for a subsystem, an indicator light will change from green to amber or red. When you place the cursor over the indicator light, a message appears that provides a description of the warning and instructions for how to fix the problem. See CHAPTER 7, *Troubleshooting* for detailed information.

Optics Subsystem

Three excitation sources are included on the CyAn ADP optical bench. Each path has its own independent, steering and focusing elements to provide optimal excitation of particles at the interrogation point. Each laser beam is focused on the quartz flow cell, where particles are transported past the laser beams.

Figure 2.5 Optics Block Diagram
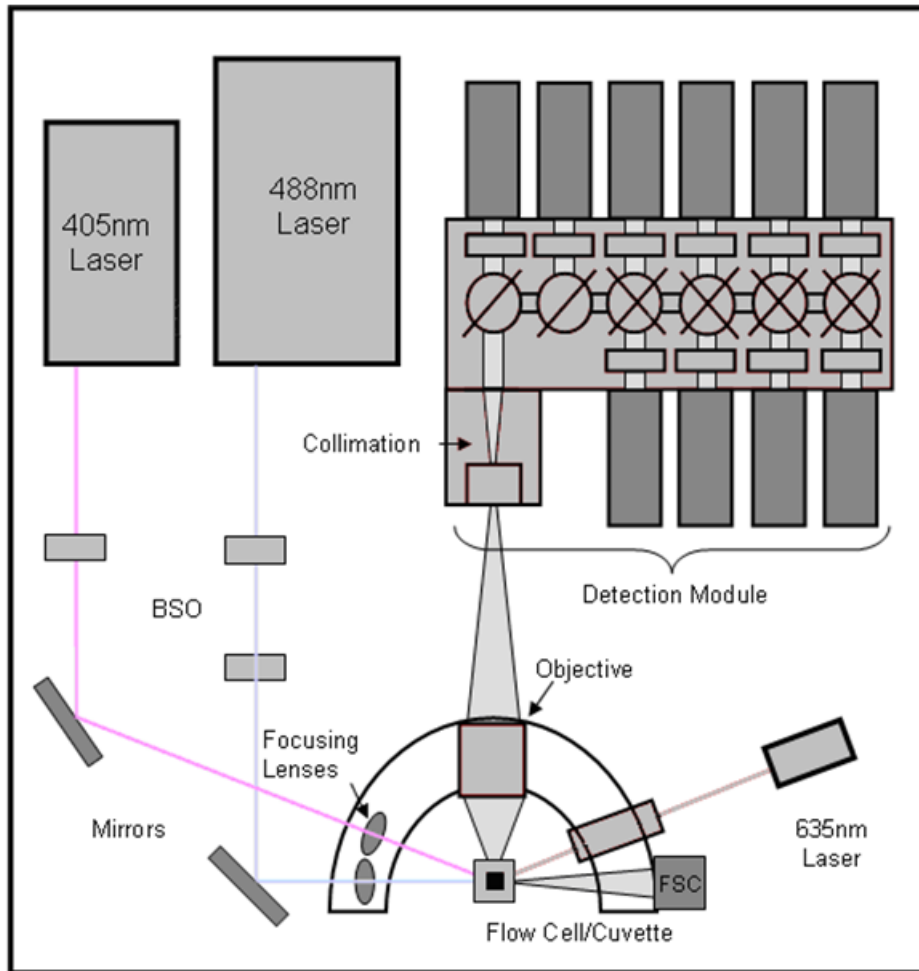
The focused beams are separated vertically to ensure minimal optical crosstalk between detection paths. Further, the signals that are detected as particles that traverse the beams are electronically gated to reduce unwanted signal interference between parameters. A high numerical aperture custom achromatic objective is used to collect scatter and fluorescence and to generate an image of the interrogation point for each of three apertures (spatial filters). The apertures improve the signal to noise ratio by preventing unwanted scatter around the excitation region from entering the detector block. Light that transmits through the spatial filters is collimated and spectrally filtered on its way to the appropriate photomultiplier tubes (PMTs) for detection.

Figure 2.6 CyAn Flow Cell Interrogation Point
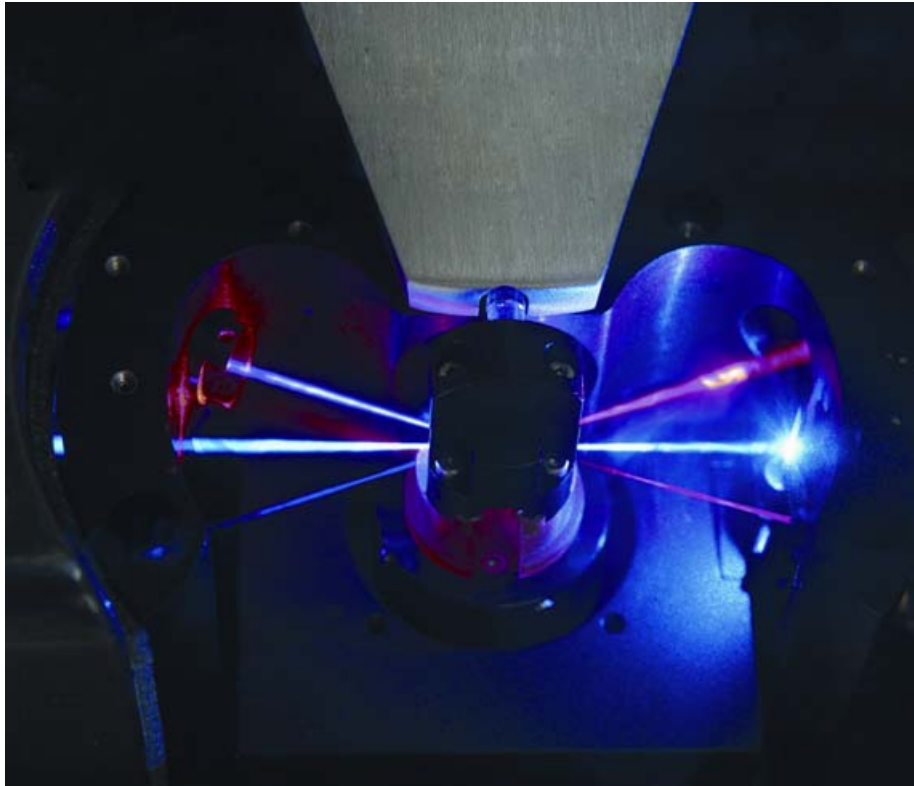
Figure 2.7 illustrates the location, position, and orientation of the optical filters and PMTs within the detection block assembly of the CyAn ADP. Dichroic filters are located at 45 degrees to the direction of light propagation, while emission filters are located at 90 degrees to the light path. Filter sticks (Figure 2.8) are interchangeable, thus allowing custom configurations to be implemented. Please contact Beckman Coulter to order dichroic filter sets.

Figure 2.7 Detector Block and Optical Filter Layout
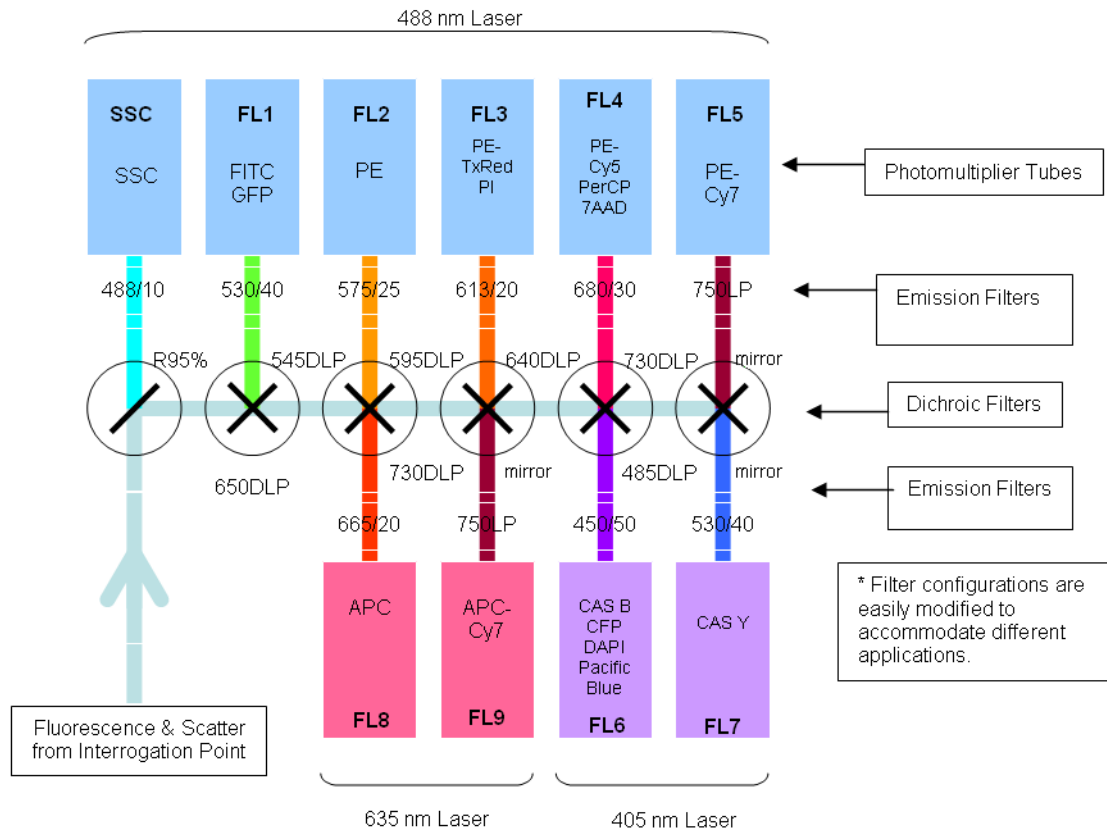
Figure 2.8 CyAn ADP Filter Stick

Electronics Subsystem

CyAn ADP electronic components include the PC workstation and the instrument electronic chassis. The PC workstation is used for instrument control, status, and data acquisition, and communicates with the CyAn ADP instrument through a high-speed serial link. Housed within the instrument is the electronic chassis that provides a communication backplane/bus with a number of available card slots to allow modular connection of key electronic control and sensing components. These components include trigger and signal processing, and multi-function signal input and output. Peripheral to the electronic chassis, but within the instrument are a number of devices that can be grouped as fluidics control and sense (pumps, regulators, valves, and sensors), laser/shutter control and sense, PMT voltage control, and PMT signal. The multifunction I/O card is used for controlling these peripheral devices and for sensing instrument status. The PMT and photodiode detectors convert light emitted from particles excited at the interrogation point, into electrical signals. These signals are input to the trigger and signal processing cards. Amplification, analog to digital conversion, and sampling techniques provide quantitative measurements including peak, area/integral, log, and pulse width for a given triggered particle event.

Peripheral Devices

The CyAn ADP includes the following peripheral devices: Summit Workstation with a printer, and the Sheath Management System (SMS). The SMS houses a sheath container, waste container, cleaner cubitainer, reusable plastic carboy, level indicators, in-line sheath filter, and a compressor/vacuum pump.

Software

CyAn ADP uses Summit software for instrument control, data acquisition, and data analysis.

Summit software offers users complete control of the CyAn ADP instrument at varying levels of complexity. Summit software, on CyAn ADP with all parameters enabled, has the capability to acquire, save, and analyze up to 10 million events. The instrument control panel (Figure 2.9) provides access to laser control, event rate settings, system maintenance functions such as clean and rinse, and Sheath Management System functions. The sample parameters panel (Figure 2.10) has software controls for adjusting parameter settings such as threshold, PMT voltage, and gain.

Figure 2.9 Instrument Control Panel
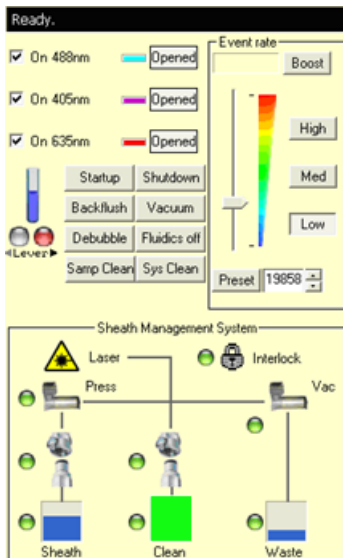
Figure 2.10 Sample Parameters Panel

Summit Features

Summit software offers compensation, flexibility, intelligence, organization, and ease of use.

Compensation
Summit software performs full inter-laser compensation of fluorescence parameters. Data saved as a sample file in Summit software will retain all compensation values as well as the uncompensated data for each parameter.

Flexibility
Summit software offers flexibility throughout the program to allow the data to be gathered and manipulated in ways that best suit the application and user. For example, most logical expressions can be defined using any number of regions in "AND" and "NOT" combinations for generating statistics, including non-rectangular regions with an almost limitless number of vertices. In addition, all parameters available with the CyAn ADP High-Performance Flow Cytometer can be selectively stored. It as possible to disable non-contiguous groups of parameters. Eliminating

unnecessary parameters increases the display performance during acquisition and reduces data storage needs.

Intelligence
While performing batch analysis, the intelligent parameter matching feature dynamically determines the correct parameter to display for a sample based on antibody, stain, name, parameter type, channel, or a combination of these.

Organization
The sample viewer tracks samples in user-definable folders. Tests and controls with different parameters can be grouped, and samples can be dragged and dropped between folders to help organize information. Statistics for each gate or region are automatically available under each histogram.

Summit Software Overview

Overview

Summit software is a Windows® based application that has a series of menus, hot keys, and buttons which allow you to acquire, sort, and analyze flow cytometry data then save the data in FCS format. With Summit software you can monitor and control the instrument, define protocols, configure compensation settings and workspaces, define batch protocol panels, reagents, and tubes, and auto-compensate data.

How to Open Summit Software

1 To open Summit software double-click the **Shortcut** icon on the Windows® desktop. The **Select database** dialog box appears.

Figure 3.1 Select a New Database
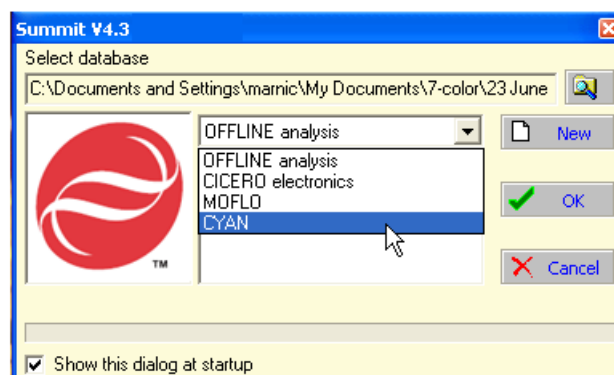
2 Select **CYAN** from the dropdown menu. This will allow you to interact with the instrument in real time. It is also possible to work with Summit software offline to analyze previously saved FCS data files.

Now you will either create a new database or open a previously saved database.

Summit Software Database

A Summit software database is a collection of protocols, samples, and data. After you open a new database a workspace appears in which to create histograms and dot plots. It is also possible to open existing protocol files that may already contain histogram and dot plot forms.

How to Create a New Database

1 Open Summit Software and click **New**.

Figure 3.2 Create a New Database
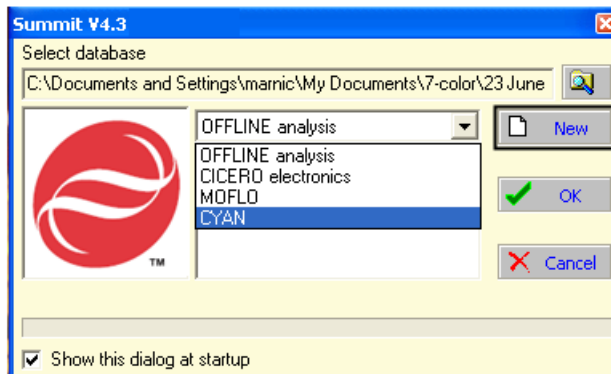
The **Create Database** dialog box appears.

2 Specify the folder in which you will save the database. Specify a name for the database and click **Save**. The main Summit software screen will appear.

How to Open an Existing Database

1 Open Summit Software.

Figure 3.3 Browse to an Existing Database

0000050K
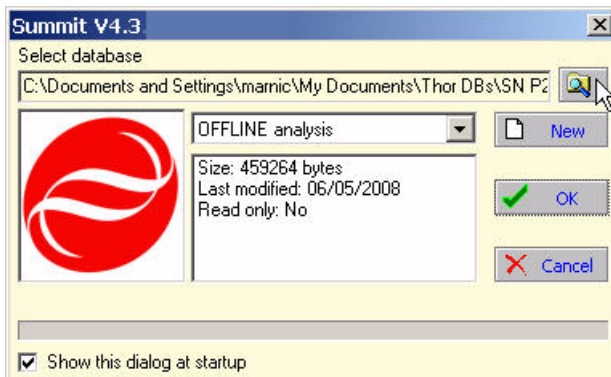
2 Browse to the folder that contains the database you would like to open, select the file and click **Open**.

Figure 3.4 Select an Existing Database
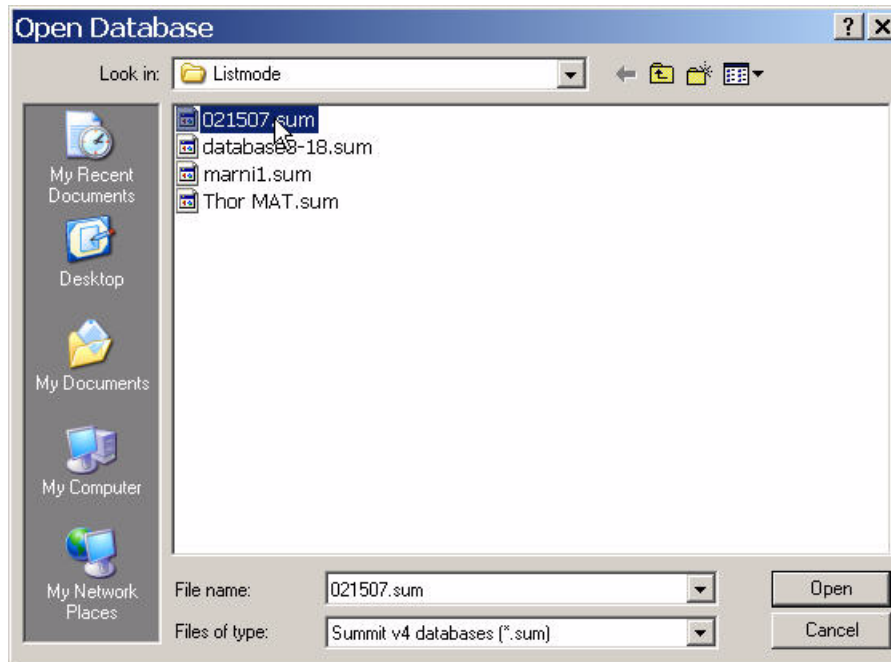
Summit Software Screen Overview

Figure 3.5 Summit Software Screen Overview
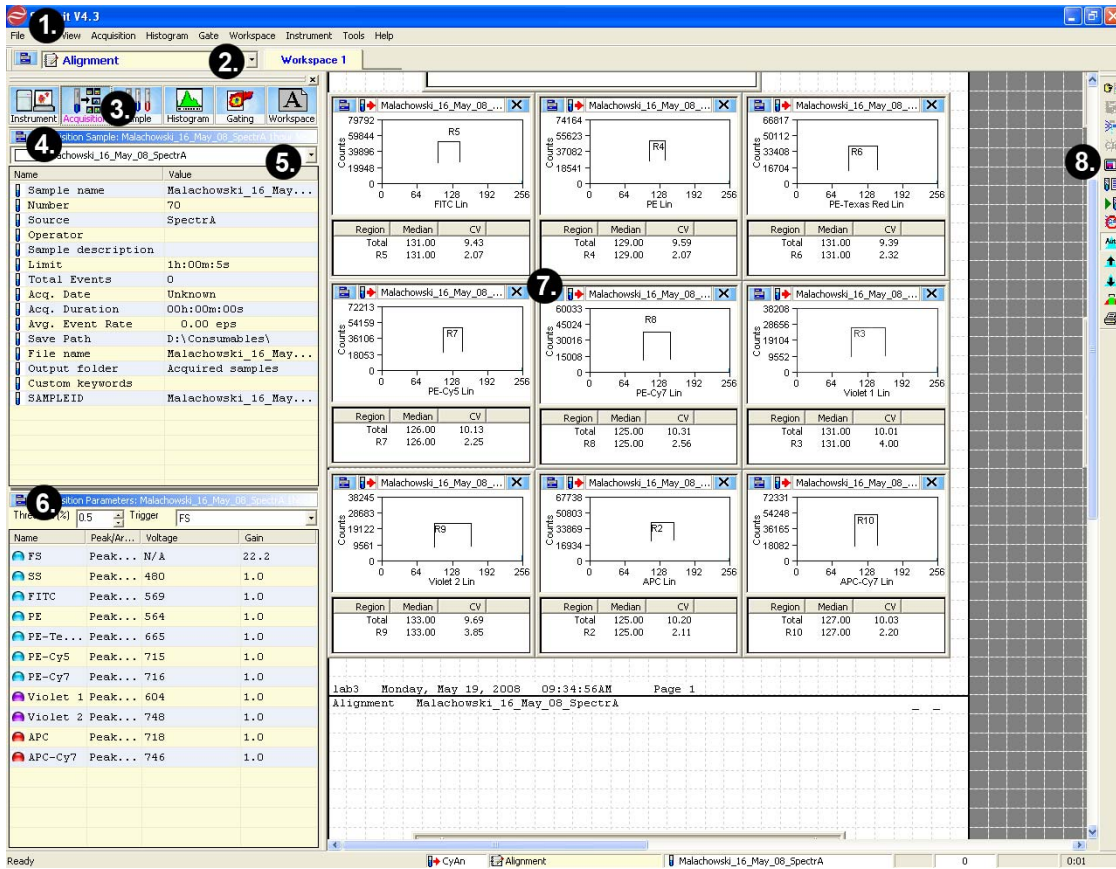
1. Summit software Main Menu	**5.** Sample List
2. Protocol List	**6.** Additional Menu
3. Summit Control Panel (to gain access to additional screens)	**7.** Workspace (with blank histograms)
4. Additional Menu	**8.** Toolbar Icons

Summit Software Control Panel

Most of the operations in Summit software can be accessed through the Summit Software Control Panel. The panel is located on the left side of the screen and has a series of buttons across the top. You can select each of these buttons to get information related to a particular topic. Each tab contains submenus that have options specific to that menu.

Figure 3.6 Summit Software Control Panel (see number 3. on Figure 3.5)
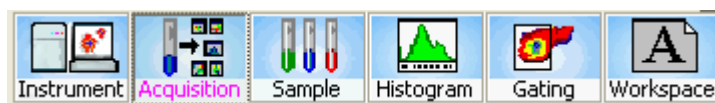
Acquisition Tab

The Acquisition tab allows you to set the threshold at which an event will be detected by the instrument, set the event triggering parameter, designate parameter names, specify the data types that will be collected, and set the voltage and gain to be applied to each parameter. From Acquisition tab you can also set up specific sample run information and view sample run statistics.

Figure 3.7 Acquisition Tab
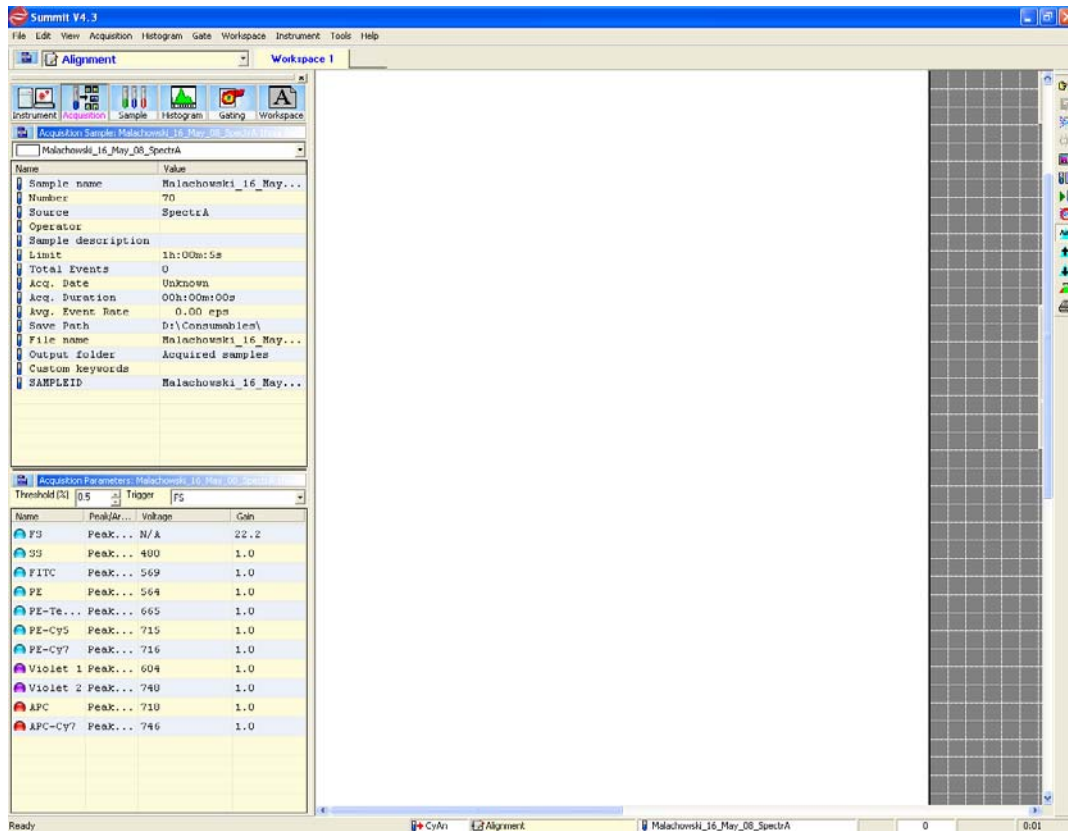
Acquisition Sample Panel

The Acquisition Sample Panel can be customized to display, and later save, information specific to a sample run.

Figure 3.8 Acquisition Sample Panel
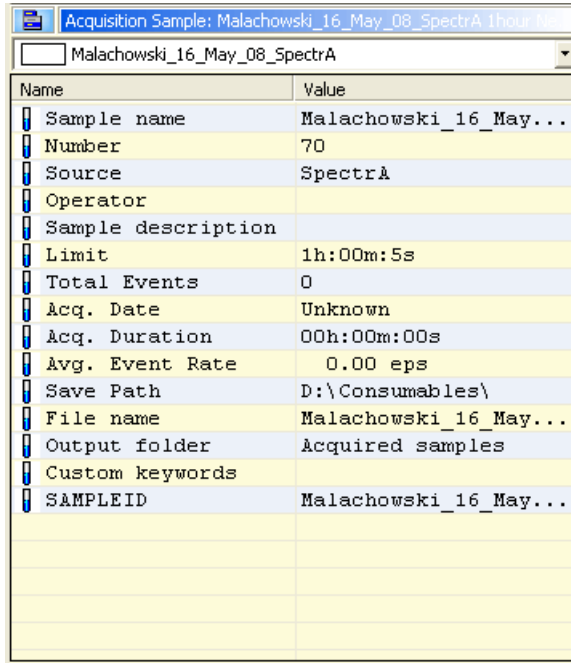
How to Edit Information Specific to a Sample Run

1 To view editable fields, click the blue additional menu icon and select **Edit View**. The fields that are editable will activate.

Figure 3.9 Acquisition Sample - Accessing Edit View
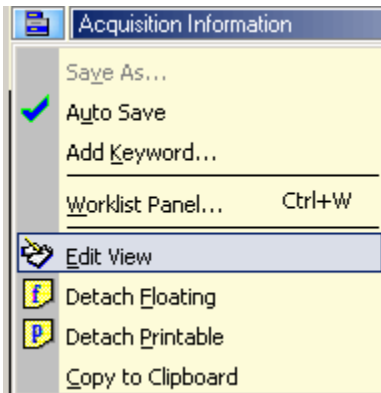
Figure 3.10 Acquisition Sample - Editing Example
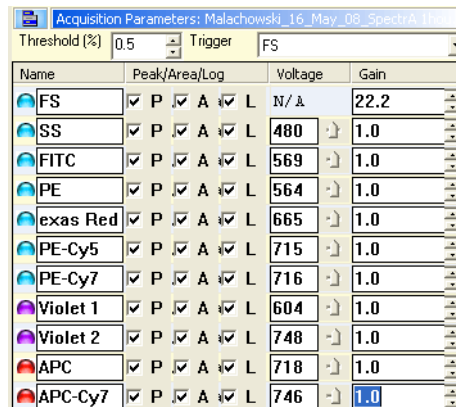
NOTE To return to the previous view click **Edit View** again.

2 Change the information in the **Value** fields as desired.

   NOTE To individually change a field, double-click in that field, enter the change, and click away
   from the field.

3 To add a new Name and Value to the panel select **Add Keyword**. The **Edit Keyword** dialog box
appears. For more information on keywords see page 3-19.

4 Enter the new information, and click **OK**.

How to Change Acquisition Settings

IMPORTANT Before data can be acquired, data type parameters must be enabled. See Figure 3.10

How to Change Acquisition Settings

1 To set the Threshold percentage at which an event will be detected by the instrument, click the
up or down arrows in the **Threshold %** field Figure 3.11. To change the event Trigger parameter,
choose an item from the **Trigger** dropdown list Figure 3.12.

Figure 3.11 Set Event Threshold Percentage
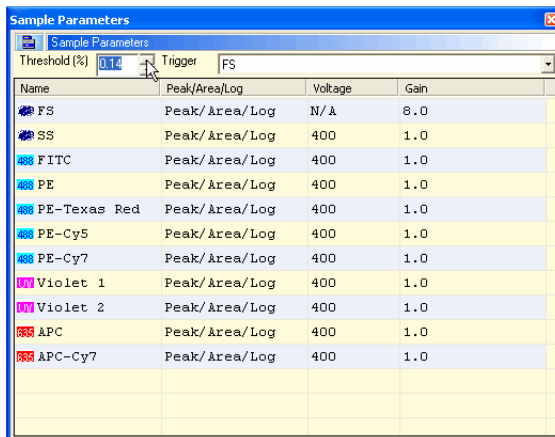
Figure 3.12 Set Event Trigger Parameter
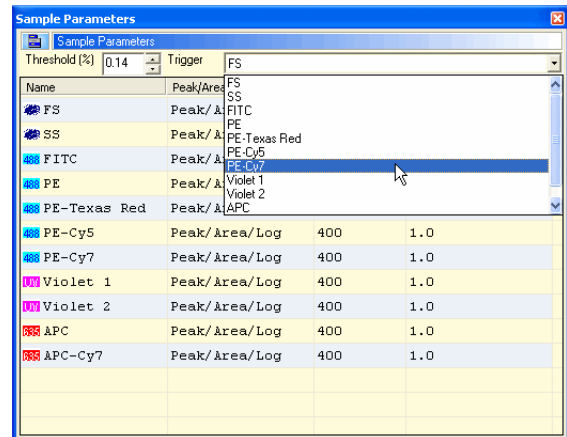
2 To change the name of a parameter, double-click the name of the parameter in the Name
column, type a new name Figure 3.13, and then press ENTER.

Figure 3.13 Change Parameter Name
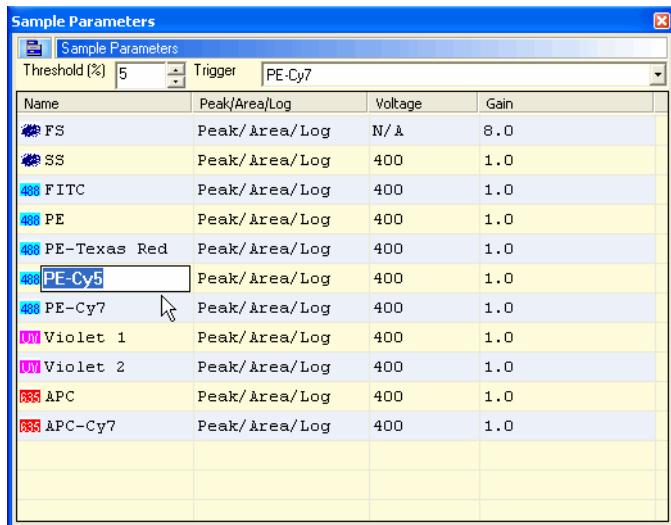
How to Enable Data Type Parameters
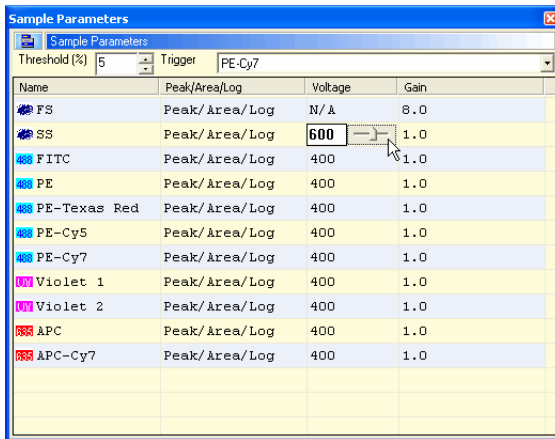
1 Click the **Acquisition** tab and locate the **Acquisition Parameters** panel. Double click in the **Peak/Area/Log** field. Select the check box that pertains to the data type you want to acquire. See Figure 3.10

NOTE This can also be done by entering edit mode. To enter edit mode, click on the menu icon in the acquisition parameters tab and select edit mode.

How to Change Voltage and Gain for a Parameter
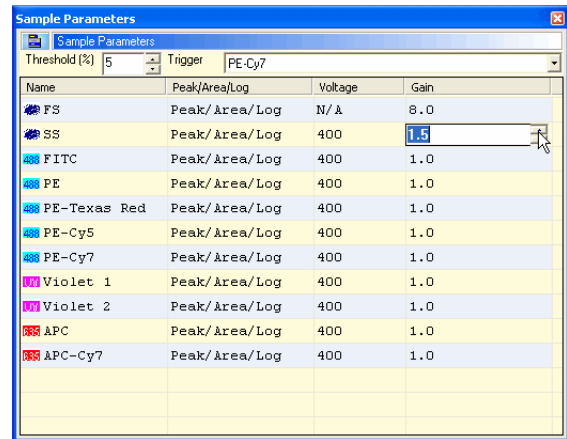
1 Click the **Acquisition** screen tab and locate the **Acquisition Parameters** panel. Double click in the **Voltage** field. Type a new value or click and drag the slider until the desired value is reached.

Figure 3.14 Set Voltage for a Parameter

Figure 3.15 Set Gain for the Voltage

Loading an Existing Protocol

How to Load an Existing Protocol

1 To load a previously saved protocol select **File > Protocol > Load**.

2 A list of previously saved protocol (.plo) files appears. Select the desired file and click **Open.**

Creating Protocols

When you open a new database there is a workspace in which to create histograms and dot plots. The histograms and dot plots that you create become Protocol 1. It is possible to create additional new protocols for this database, or load additional pre-existing protocols.

How to Create a New Protocol

1 To create a new protocol, go to the main menu and select **File > Protocol > New**. A new workspace appears in which to create dot plots and histograms for the new protocol.

2 Ensure that you have enabled the desired parameters. See figure .

3 Create dot plots and histograms. See page 3-21.

Switching Protocols

To change protocols in Summit software, go to the Protocols toolbar and select a new protocol from the drop down menu.

NOTE Only the protocols that you have loaded into the current database or that you have recently created will appear in this list.

Acquiring Data in Summit Software

When you have enabled parameters and created or loaded a protocol you may acquire data.

How to Start or Stop Data Acquisition

1 Click the Acquisition pull-down menu and select Start (or press F2).

Figure 3.16 Start Acquiring Data
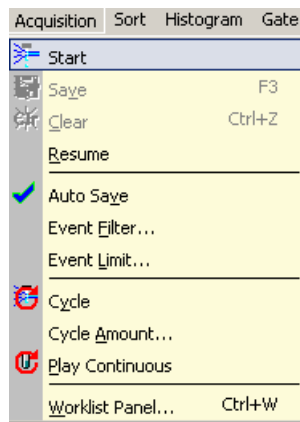
2 To stop data acquisition, click Stop (or press F2).

Saving Acquired Data

After you acquire data in Summit software you can save the information in FCS format.

How to Save Acquired Data

1 Click the Acquisition pull-down menu and select Save (or press F3).

2 Select a folder in which to save the data. Enter a file name and select an FCS file type.

3 Click **Save**.

Cycle Mode

The Cycle Mode cycles events through a buffer to display only the most recent data. This is useful during alignment activities. The number of data events displayed at any one time is adjustable.

How to Display the Most Recent Data During Alignment Activities

1 Click on the **Acquisition** pull-down menu.

2 Select **Cycle Amount**. Set the number of events that should be reached before the data cycles.

Figure 3.17 Setting the Cycle Amount for Cycle Mode
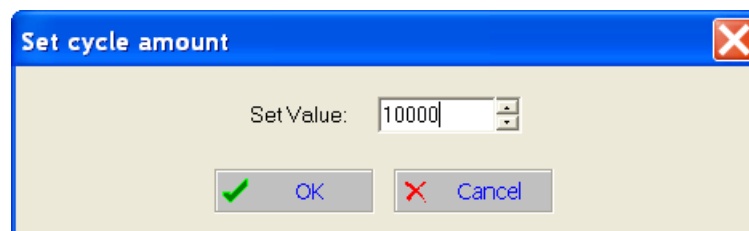
3 Click **OK**.

4 From the Acquisition pull-down menu, select **Cycle** or click the Cycle Mode icon on the right side of the screen.

Figure 3.18 Enabling Cycle Mode

---
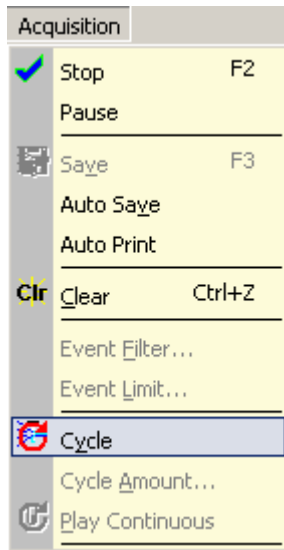
5 After you set the Cycle Mode, restart data acquisition for the Cycle Mode to become effective.

▶

Sample Tab

The Sample tab displays the parameters of the selected sample file, and allows you to change the list of parameters visible on screen. From this tab you can also compensate data.

Figure 3.19 Sample Tab
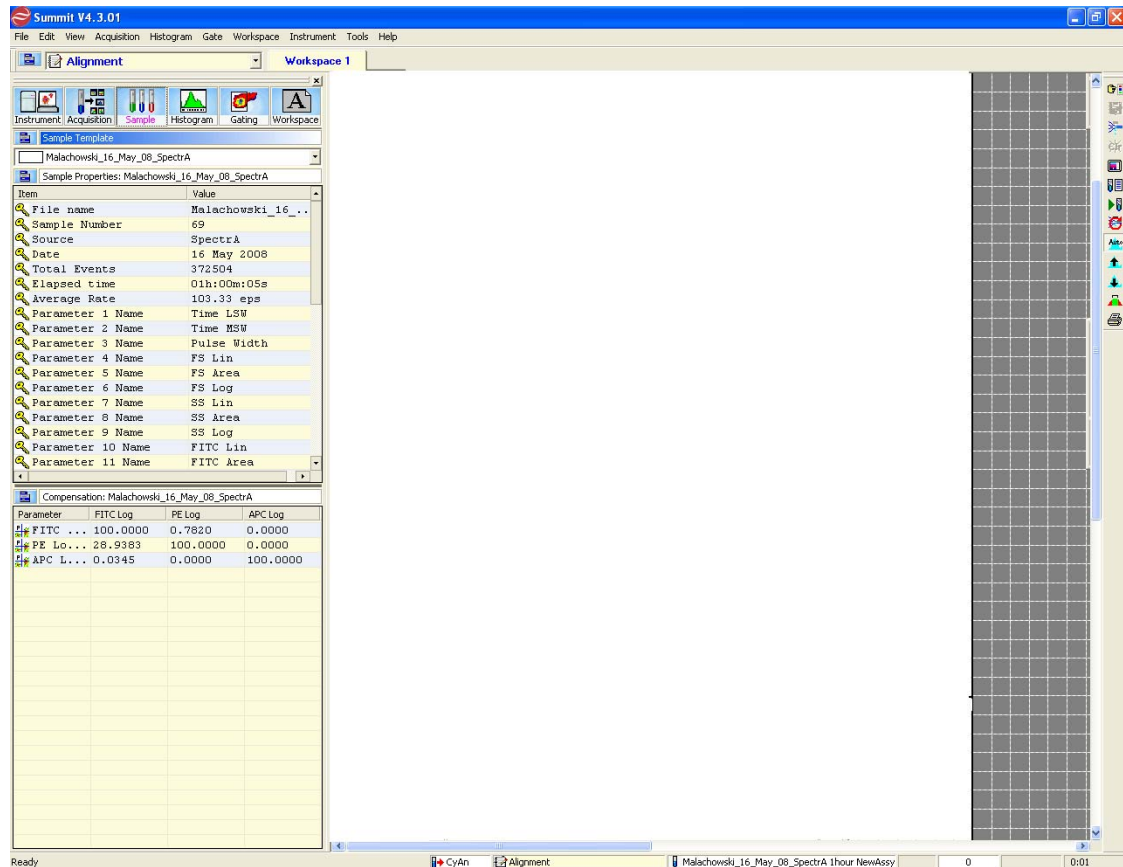
Auto Load from Sample

When you compensate data, the Compensation Matrix is established and can be saved with the FCS file. Selecting the **Auto Load from Sample** option located on the **Sample** tab, causes the Compensation Matrix to automatically display when you load a data file for analysis.

Figure 3.20 Auto Load Compensation Matrix from FCS File
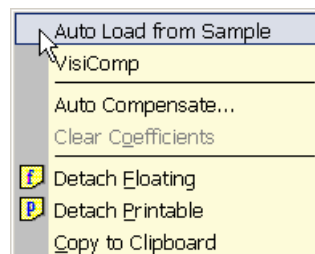
Auto Compensation Wizard for Single Positive Controls

Summit software provides an automatic method to obtain a full compensation matrix for multi color analysis. The compensation matrix is calculated from single stained controls by the auto-compensate function.

The following procedure describes how to use the Summit software Auto Compensation feature within single stained controls. Although this feature provides an automatic method to compensate data, compensation can still be adjusted, fine-tuned, or performed manually or via the compensation matrix.

NOTE Additional information regarding compensation can be found in APPENDIX C, *Compensation Background Information*.

How to use the Auto Compensation Wizard for a Single Stained Control

1 Acquire the first single-control sample required for your experiment. The first control sample should include an unstained or isotype control for which you will set PMT voltages. From the resulting dot plot, you can determine gating if required. Any gates that you want to use must be set before you apply Auto Compensation.

> **NOTE** During the Auto Compensation operation, adjustments to only the size and placement of regions are allowed.

2 Run the remaining single control samples and save the data files.

3 Load all control sample files into an experiment folder.

4 Click the **Sample** tab.

5 Identify or create a dot plot that will be used to adjust compensation. Ensure that the parameter for which you are compensating is on the x-axis.

6 In the **Sample Compensation** panel, click the small, blue icon in the upper-left corner and select **Auto Compensate** from the list.

Figure 3.21 Select Auto Compensate
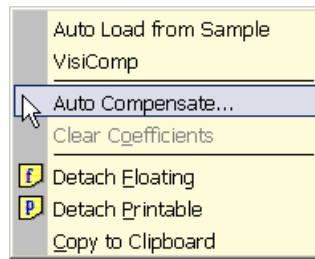
7 The **Auto Comp Sample** dialog box appears.

Figure 3.22 Auto Comp Sample Dialog
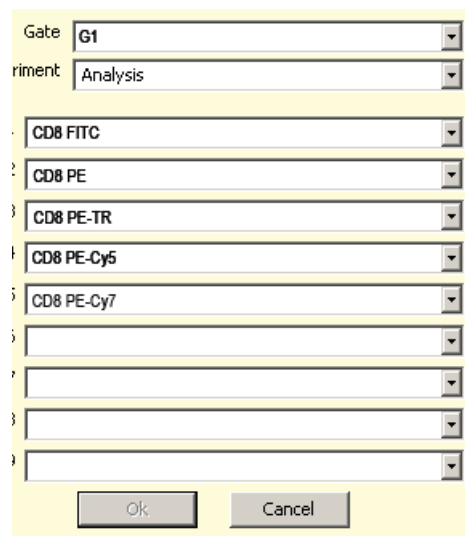
8 Select a gate from the Gate list, if applicable.

9 From the **Experiment** list, select the experiment folder that contains your control samples

10 Select all of the single control samples included in the experiment.

11 Click **OK**.

IMPORTANT If you click **Cancel** at any point in the auto compensation process, you will clear the compensation matrix and the **AutoComp Workspace.**

12 A new Workspace labeled **AutoComp** is created and the first set of dot plots is displayed. Each dot plot places the control parameter on the x-axis and a parameter to compensate against on the y-axis. Default auto compensation **Dim** and **Bright** regions are displayed and, if a gate was selected, it is applied to each dot plot. The **Auto Compensate** wizard appears.

Figure 3.23 Auto Compensate Wizard

Figure 3.24 Single Control Sample Dot Plots
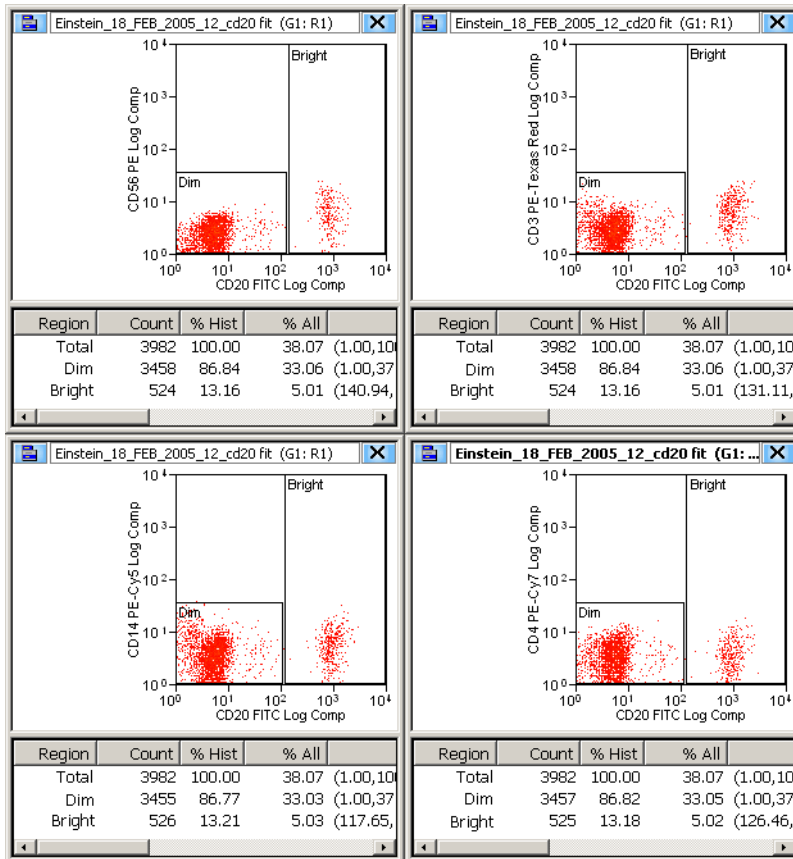
13 Examine the *% Hist* statistics for each histogram. If either the **Dim** or **Bright** region contains less than 5% of the data for the dot plot, click-and-drag the region until greater than 5% of the data appears in both the **Dim** and **Bright** regions.

14 When all regions on all plots contain greater than 5% of the data, Click **Next** on the **Auto Compensate** dialog box. The next set of dot plots will appear.

15 Repeat step 13 until all single-control samples have been compensated. When auto compensation is complete, the compensation matrix contains the appropriate values and the **AutoComp** workspace is removed.

▶

Applying VisiComp

To help you better visualize the results of compensation, Summit software includes a scaling algorithm called VisiComp that displays 0 and negative values. VisiComp provides a good way to verify the results of the Summit software Auto Compensation feature, and allows you to fine tune and make adjustments to compensation.

How to Use VisiComp to Visualize Compensation Results

1 Pre-load all necessary samples (Listmode.fcs files) that are required to perform compensation.

2 Create all plots, regions, and gates.

3 On the Sample tab, click the **Compensation** panel icon and select **VisiComp**.

Figure 3.25 Apply VisiComp
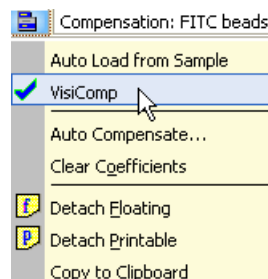
4 Use either the auto compensation wizard to set up plots, or manually set up all of the plots that you want to use for compensation analysis.

Figure 3.26 VisiComp Example
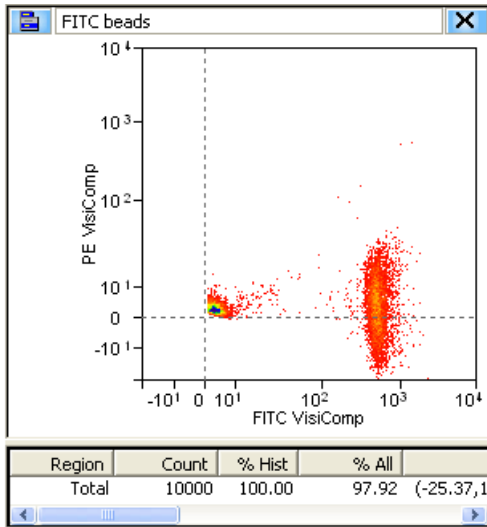
5 To adjust the width of the VisiComp linear region click the Sample icon in the upper left portion of the window and select **Adjust VisiComp**. Use the slider tool, or enter a specific value to complete the adjustment.

Figure 3.27 Adjust VisiComp
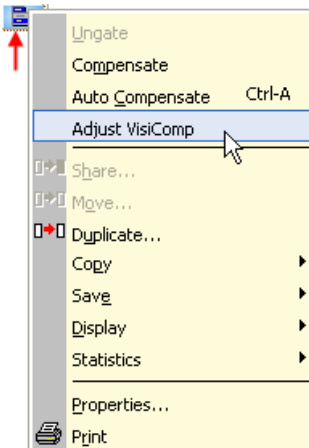
NOTE The adjusted width of the VisiComp linear region applies to all plots and histograms that display compensated parameters in any one sample template. Because of this, it is important to display all data before you adjust the width. What is ideal for one parameter pair may not be perfect for another. Therefore, adjust the width to display the best compromise across all plots.

IMPORTANT If you turn off VisiComp, any regions that extend into the negative area of the VisiComp scale will be moved where they can be displayed on the log scale. Any regions that were entirely in the negative area will have a 0 width and 0 height.

6 Create regions and gates to complete your analysis.

NOTE If you created regions and gates before you applied VisiComp, you will need to verify the location of the regions.

FCS Keywords

To customize your view of sample data you can add and remove Keywords.

How to Add or Remove Keywords

1 Click the blue menu icon and select **Add/Remove Keywords**.

Figure 3.28 Add/Remove Keywords 1
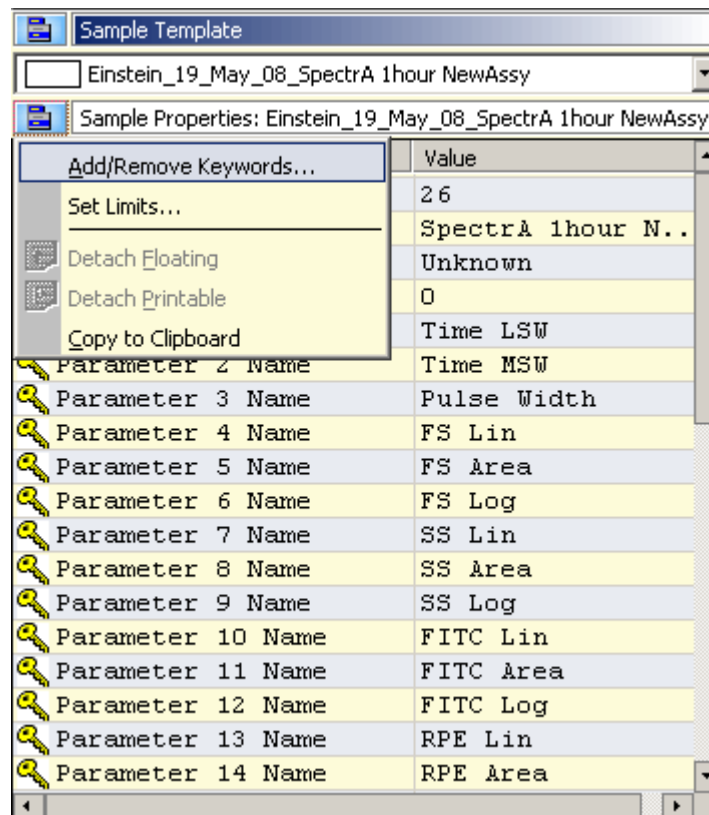
2 Select the check boxes next to the Keywords you would like to display, and click **OK**.

Figure 3.29 Add/Remove Keywords 2
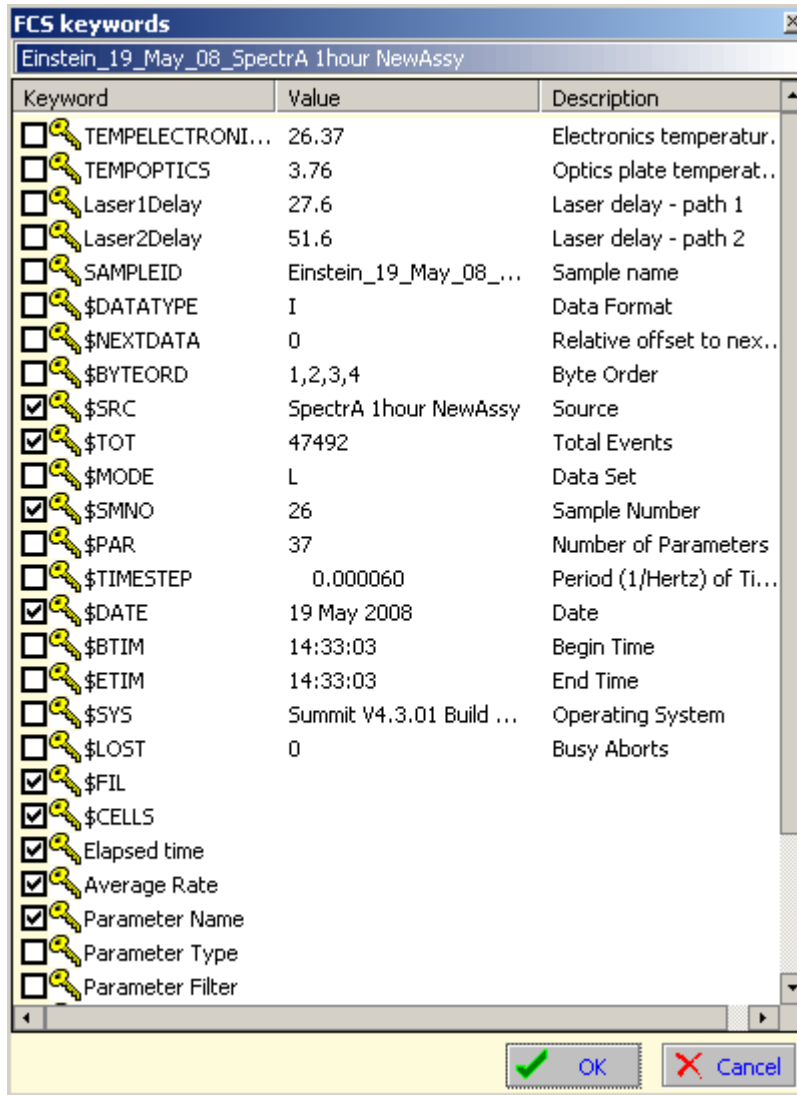
Histogram Tab

Histograms and dot plots (bivariate histograms) are created in the **Histogram** tab. The **Create Histograms** panel displays all of the parameters that are enabled in the **Acquisition** tab.

Figure 3.30 Histogram Tab
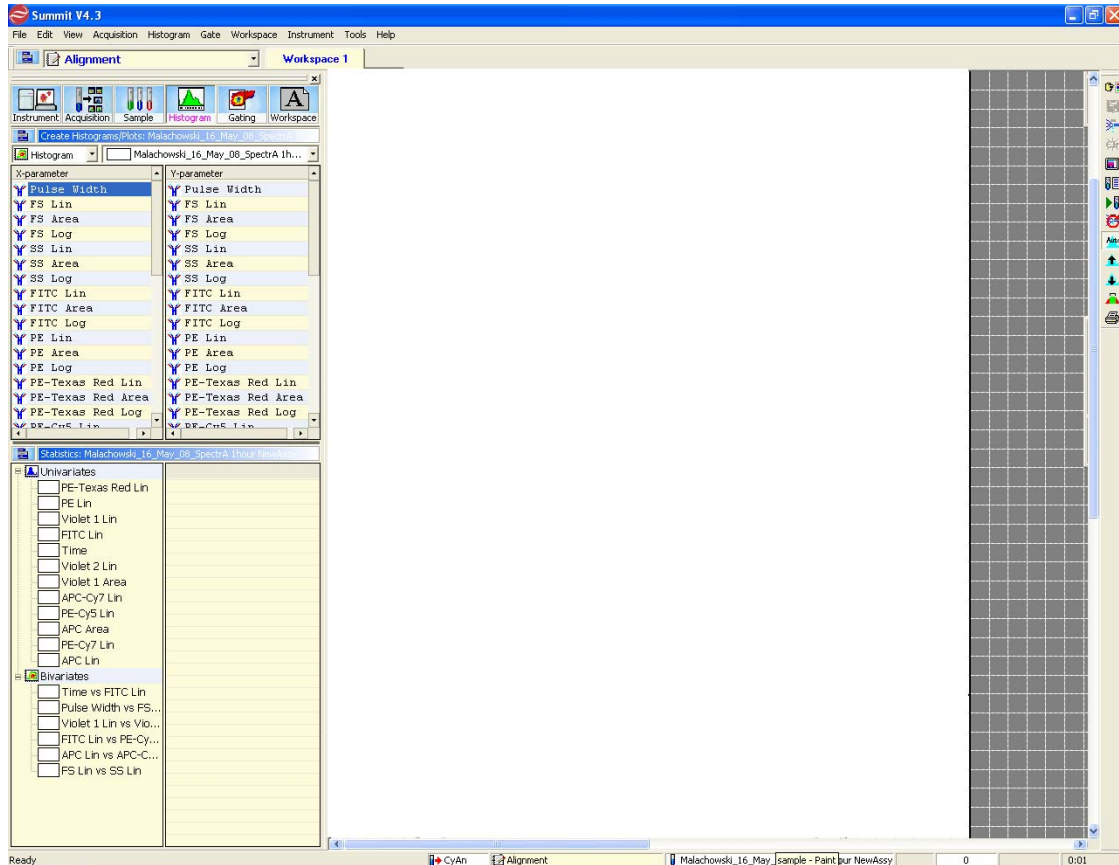
Creating Histograms and Dot Plots

You must create histograms and dot plots in order to display the data you acquire. Prior to creating dot plots and histograms you must enable the parameters you would like to collect. See page 3-7.

How to Create a Histogram or Dot Plot

1 Create dot plots and histograms by selecting the **Histogram** tab in the Summit **Software Control** panel (see #1. in Figure 3.31). The histograms and dot plots that you create will build a Protocol that you can elect to save.

Figure 3.31 Creating Histograms and Dot Plots
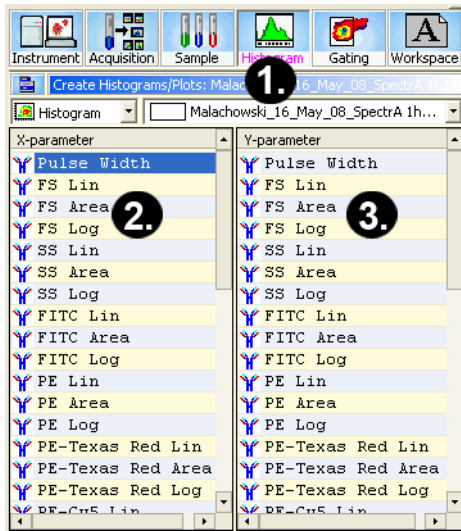
1. Histogram Tab
2. X-axis Parameters

3. Y-axis Parameters

2 Select one of the following:

- To create a single parameter histogram, double-click on the X-axis parameter for the histogram you would like to create. The frame for the histogram will appear in the Workspace on the right of the screen.

- To create a dual parameter dot plot, click once on the X parameter and twice on the Y parameter. The newly created frame for the dot plot will appear in the Workspace.

Maximize Dot Plots and Histograms

To maximize dot plots and histograms double-click on the title bar. This option is useful to better see the data, create regions, or set gates. Double-click the title bar again to restore the image.

Change Axis Parameters

To change the displayed parameter in a dot plot or histogram, right-click on the axis you want to change and select a new parameter from the menu. The options in the list include the parameters that were collected with the current data file.

Figure 3.32 Change Axis Parameters
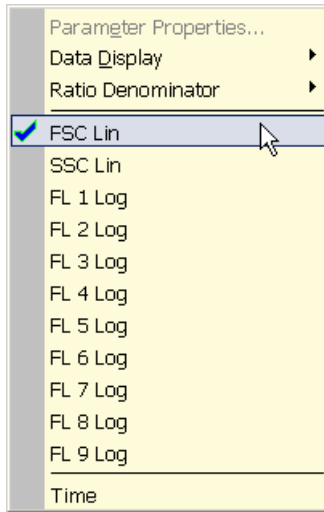
Change Axis Labels

To change the label that is displayed on the X or Y axis of a histogram or dot plot, right-click on the axis you want to change and select **Parameter Properties** from the menu. You can enter additional fluorochrome, marker, or antibody information in the field labeled **Long Name**. The information entered in this field will be the label displayed along the axis.

View Statistics in Histograms

To view statistics in histograms, click the additional menu icon within the histogram and select **Show**.

Figure 3.33 Show Statistics in Histograms
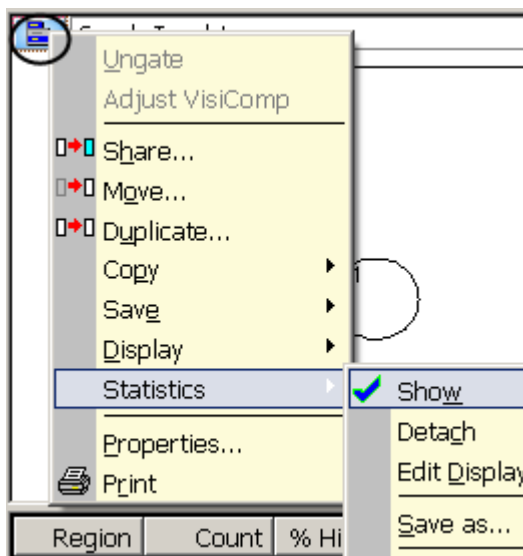
Edit Statistics Displayed in Histograms

To select the statistics that will be displayed in histograms, click the additional menu icon within the histogram and select **Edit Display**.

The check boxes that are selected will appear in the statistics window below the histogram or dot plot. You can also drag and drop to change the order of statistics. Click **OK** to apply these settings to all histograms by selecting the check box at the bottom of the dialog.

Figure 3.34 Edit Statistics Displayed in Histograms
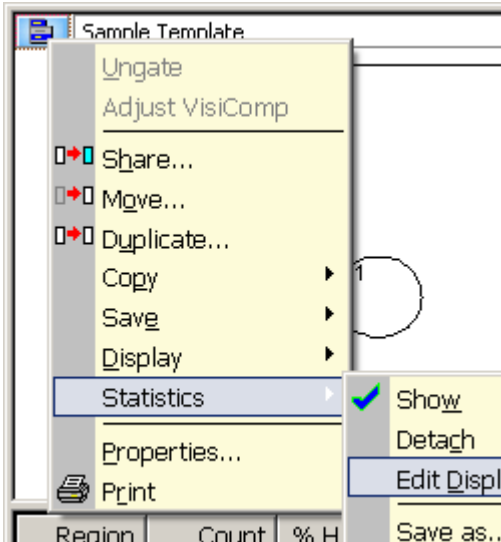
Figure 3.35 Edit Statistics Displayed in Histograms
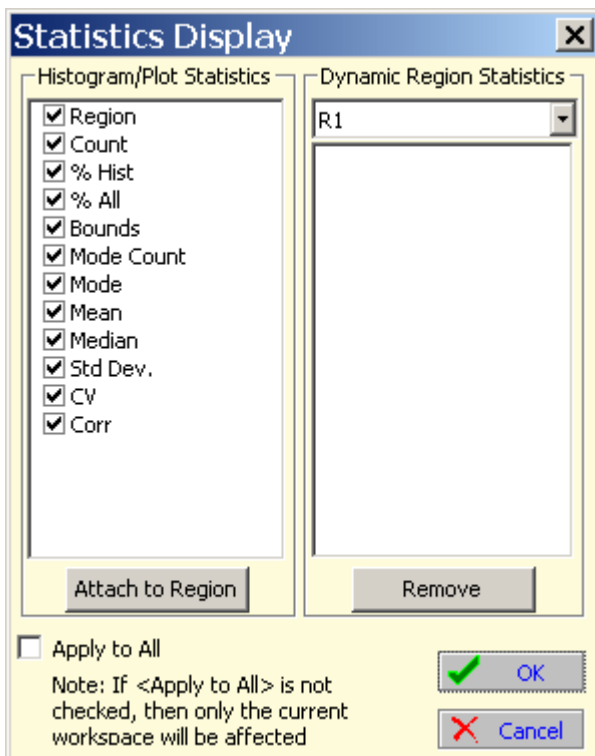
Create Regions in Histograms

To create bar regions in single parameter histograms right-click in the histogram and select **Bar** from the menu. In a dual parameter histogram, right click to create a rectangle, ellipse, polygon, or quadrants. Once created, you can click and drag to resize and reposition the region. Once you have created regions, the statistics for those regions will appear in the status window below the histogram. As you move regions the statistics will update in real time. To delete a region right-click and select **Delete**.

Figure 3.36 Create a Region in a Bivariate Histogram
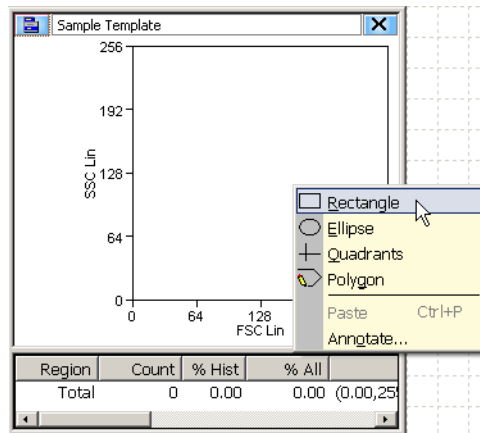
Renaming Regions

Regions can be renamed to reflect the population inside the region. To rename a region right-click in the region you want to rename and select **Properties**. A dialog box appears. Enter a new name for the region in the upper-left text field and click **OK**.

Copy and Paste Regions

You can copy all regions from histograms and dot plots and paste into another histogram or dot plot. Right-click inside the region and select **Copy**, go to the next histogram or dot plot, right-click and select **Paste**.

Manually Scaling Data

To manually rescale data within a dot plot or histogram, click the scale up or down buttons on the right side of the screen.

Figure 3.37 Manually Scaling Data

Contouring Data

To Enable Contouring, go to the main dot plot menu and select **Display > Contour**. Select the **Enable contours** check box. The dropdown menu directly below the check box lists the available contouring algorithms. The dialog box contains additional options for maximizing data and smoothing the contouring.

Exporting Histograms to Word

To export a dot plot or histogram to Word, go to the main histogram menu and select **Copy > Window as Bitmap**. Open Word and paste the histogram image into the document. The **Copy as Graphic** option does not include the histogram frame or statistics.

Multi File Display

- It is possible to display more than one data file or sample. Select the Sample tab in the Summit Software Control panel. Click the menu icon and select **Duplicate**. This will copy the existing dot plots and histograms in the protocol.

  NOTE All copied versions will be indicated with a different color.

- You can manually arrange the dot plots and histograms, or you can right-click on the white sheet, select **Arrange Windows** and select the desired option.

- To load additional samples, go to the Summit Software Main Menu and select **New > Samples**. Click on a sample name and drag and drop to load additional samples into the templates.

Create Overlays

Overlays are special histograms where you can display data from more than one sample within a single parameter or within a single histogram for one parameter.

How to Overlay Multiple Histograms

1 To create an overlay, select the Histogram tab from the Summit Software Control panel.

2 Click the drop down menu on left side and select **Overlay**. Double-click on the parameter you would like to use on the overlay.

3 To add data, go to the Main Overlay Menu and select **Add Data**. The curser will change.

4 Click on the histogram that contains the data you would like to add to the overlay.

5 To include additional sample data, go to the Summit Software Main Menu and select **New > Samples**.

6 Click on the sample of interest and drag and drop it on the overlay.

Gate Logic Tab

The Gate Logic tab allows you to view and adjust gate logic as well as apply color gating to histograms.

Figure 3.38 Gate Logic Tab Displaying an Alignment Protocol and Gating Scheme
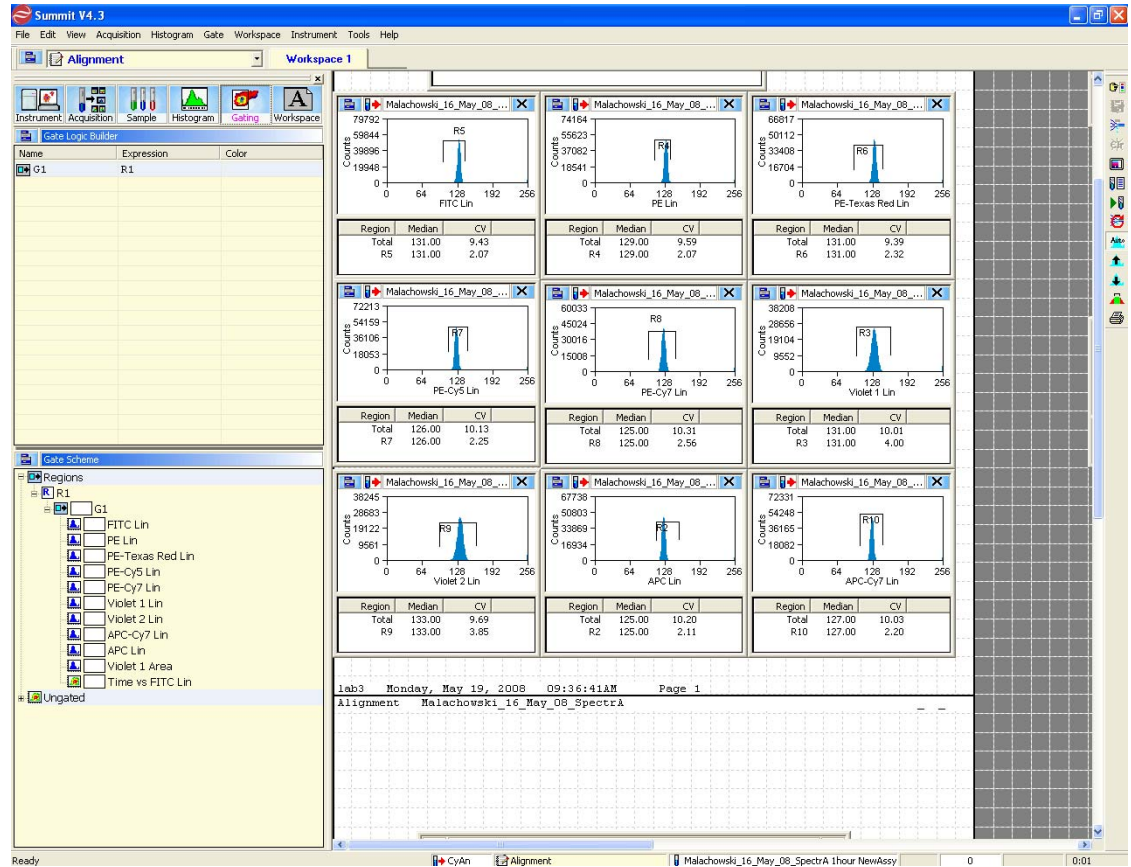
Setting a Gate from a Single Region

Gating on the main population in the FSC vs. SSC histogram will clean up the data and %CV values in other histograms. This eliminates bead or cell fragments and doublets from being considered in the alignment of fluorescent parameters. Gating can be done directly in dot plots and histograms in which regions have been created.

How to Gate One or More Histograms or Dot Plots

1   To gate one histogram or dot plot, right-click in the region from which you would like to gate, and select **Set Gates** from the menu. The appearance of the curser will change.

Figure 3.39 Set Gate 1

---
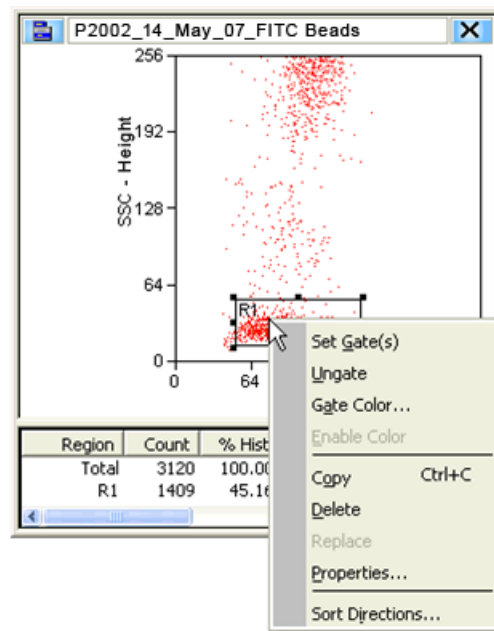
2 Use the newly changed curser to double-click in a histogram or dot plot in your protocol. Once the gate is applied there will be an annotation in the title bar to indicate a gate is applied.

Figure 3.40 Set Gate 2

---
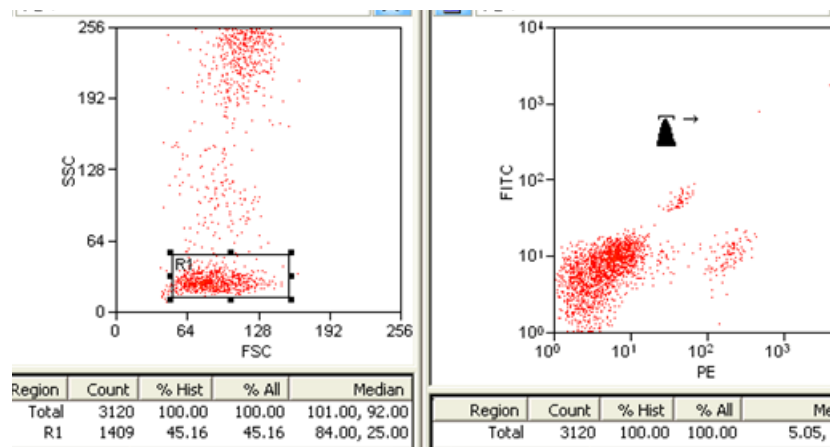
3 To gate more than one histogram or dot plot, right-click in the region from which you would like to gate, and select **Set Gates** from the menu. The appearance of the curser will change.

---

4 Use the newly changed curser to single-click in all of the histograms or dot plots in your protocol that you would like gated. When you come to the last histogram or dot plot, double-click to apply the gate.

5 To remove a gate, click the main histogram or dot plot menu icon and select **Ungate**.

Setting a Serial Gate

When a region in a dot plot or histogram is used for gating, the serial gate option works by automatically appending any gate regions applied to that plot to the new gate. For example, a dot plot is gated based on two regions (R1 & R2). If an R4 region is created in that plot and is used as a gate on another histogram or dot plot, the serial gate option defines the newly applied gate as (R1 & R2 & R4). Electing not to serial gate would apply a gate involving region (R4) only to the target histogram or dot plot.

How to Serial Gate Histograms or Dot Plots

1 Open a dot plot gated on region (R1).

Figure 3.41 Set Serial Gate 1
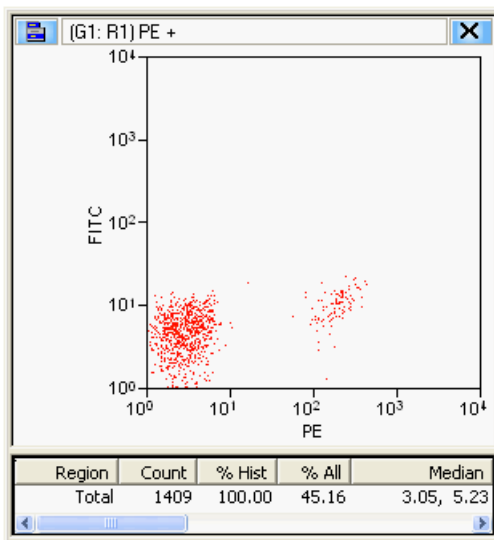
2 Create a region (R2) in the dot plot.

3 Right-click in region (R2) and then click **Set Gates**.

Figure 3.42 Set Serial Gate 2
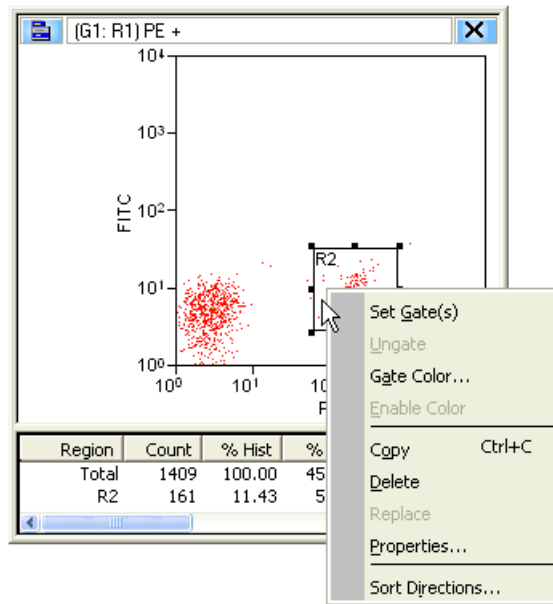
The **Combine region and gate?** Dialog box appears.

4 Click **Yes** to activate serial gating. Click **No** to gate using only the specified region.

Figure 3.43 Set Serial Gate 3
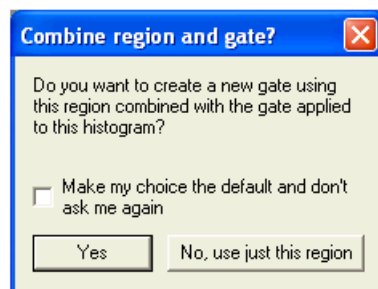
5 Check the **Make my choice the default and don't ask me again** check box to disable this dialog box in the future. If checked, this option is set as the default in Summit software. The default can be changed in the **Preferences** dialog box.

6 Single or double click in a histogram(s) to apply the gate.

Figure 3.44 Set Serial Gate 4
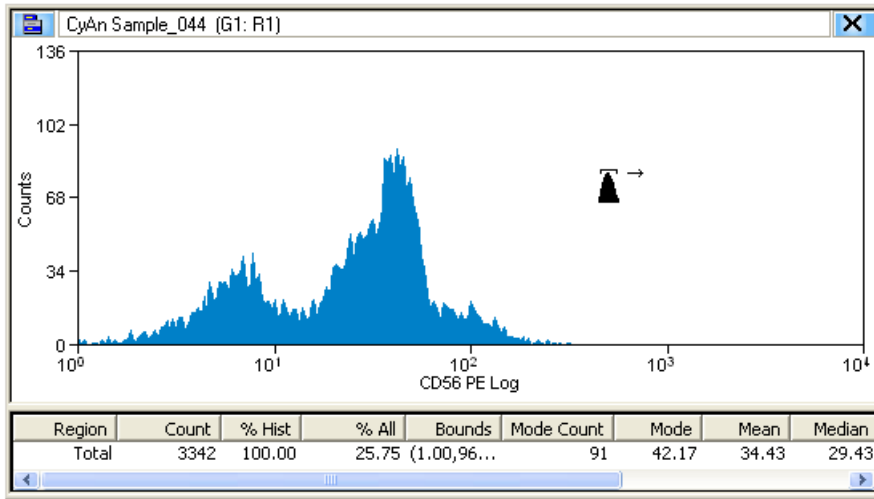
7   If you selected Yes in step 4, the serial (or sequential) gate logic is applied to the histogram or dot plot. The graphic below shows a gated histogram involving regions 1 and 2.

Figure 3.45 Set Serial Gate 5
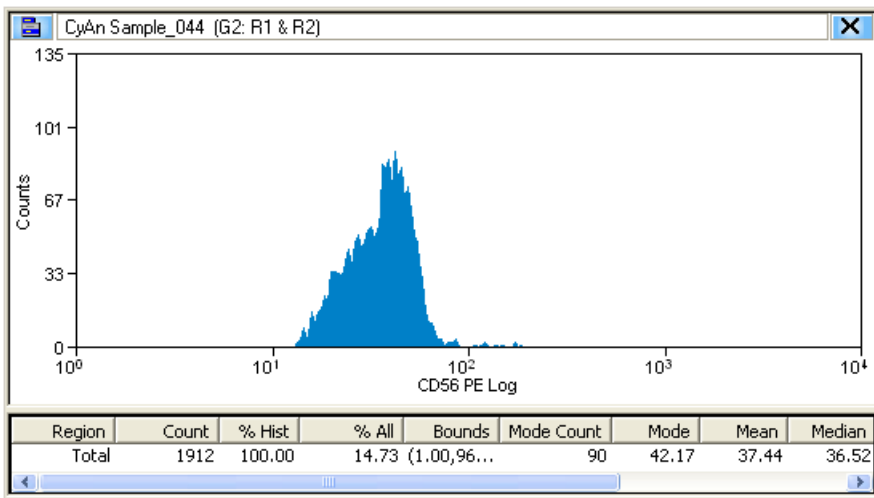
Gate Logic Builder

The **Gate Logic Builder**, located on the upper-left portion of the Gating tab, allows you to define gate logic and view it graphically.

How to Define (Edit) Gate Logic

1   Select the Gating tab in the **Summit Software Control Panel**.

2 Click the upper-left icon in the **Gate Logic Builder** panel and then click **New** to create a new gate.

Figure 3.46 Gate Logic Builder
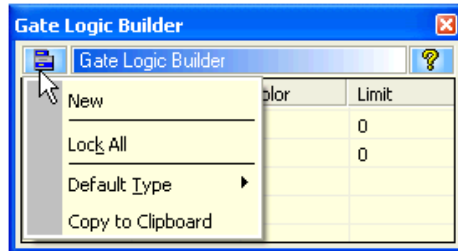
3 Double-click a text field in the **Expression** column. The **Edit gate expression** dialog box appears.

Figure 3.47 Edit Gate Expression 1
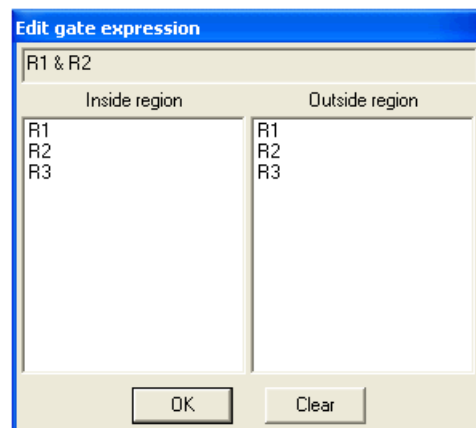
4 Select one or more regions to be included in the gate, and click **OK**. Gates can be defined to include those events that fall inside or outside specific regions. To clear all selected regions, click **Clear**.

Figure 3.48 Edit Gate Expression 2
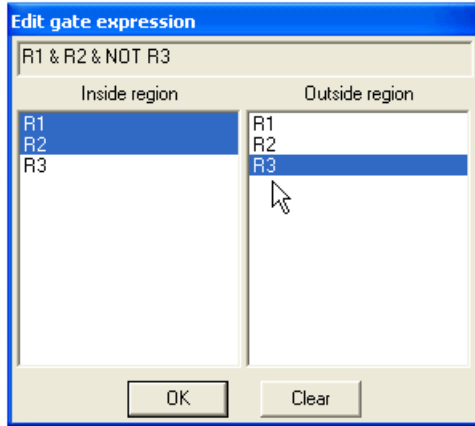
NOTE The number of regions available correlates to the number of regions created in the current
workspace.

The newly defined gate expression is displayed in the **Expression** column of the **Gate Logic
Builder** column and the current gate scheme is displayed in the **Gate Scheme** panel.

Figure 3.49 Gate Logic Builder and Gate Scheme Panels
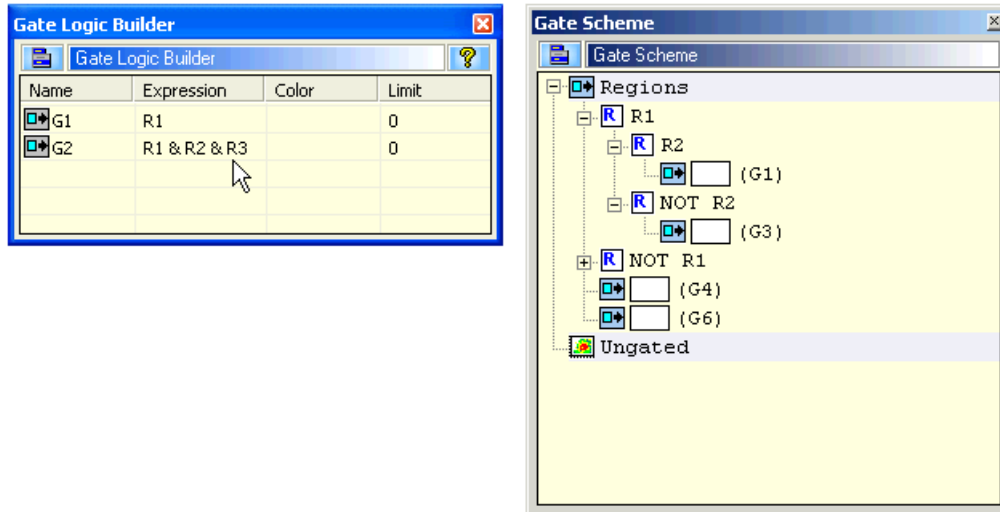
Color Gating

You can apply colors to gates to more easily distinguish gated populations. Double-click in the **Color**
column for a particular gate and select a color.

NOTE The order of the color dot plots and histograms correlates to the order of the gates listed in the **Gate
Logic Builder**. As an example, if you have a color gate applied to a rare population that is being hidden
in the background, you can reorder color to bring it to the front and more easily identify those rare events.

Layout Tab

The Layout tab assists you in manipulating the appearance of your Workspace as well as duplicate and share histograms, and print some or all of your Workspace.

Figure 3.50 Layout Tab Displaying an Alignment Protocol
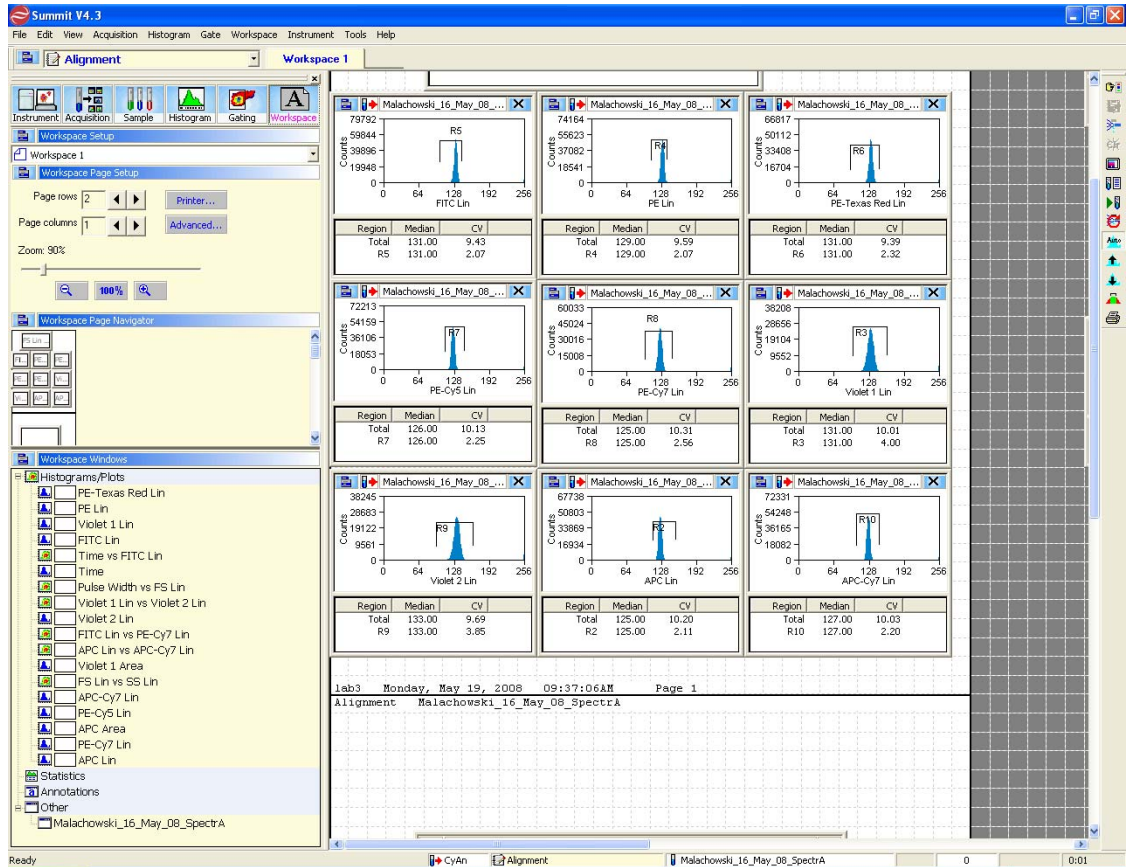
Workspace Page Setup

The Workspace Page Setup panel allows you to add and subtract pages from your Workspace, reduce or enlarge the layout, and print the layout.

Figure 3.51 Workspace Setup Panel
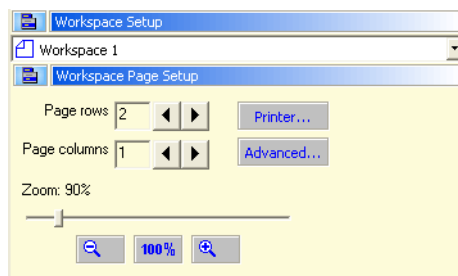
Workspace Page Navigator

The **Workspace Page Navigator** allows you to click and drag thumbnails of your histograms to reposition them on your layout. From **Workspace Page Navigator** you can also **Share**, **Move**, and **Duplicate** histograms between Workspaces.

Figure 3.52 Workspace Page Navigator
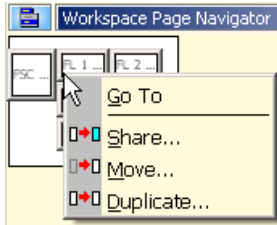
Go To
The Go To option activates the selected histogram.

Share
The **Share** option duplicates the histogram allows you to specify the Workspace where it will appear, and keeps the data in both histograms the same if changes are made.

Move
The **Move** option allows you to move a histogram from one Workspace to another.

Duplicate
The **Duplicate** option copies the histogram but does not link the data between the old and the new copy.

Shortcut Keys

The following list describes the keyboard shortcuts in Summit software.

Table 3.1 Shortcut Keys and Functions

Shortcut Key	Function
F1	Opens the Summit software online Help system.
F2	Starts/Stops acquiring events.
F3	Saves acquisition data to a drive (**C:**, **D:**, etc.), the network, disk, or CD.
F6	Opens the CyCLONE menu.
F7	Opens the **Create New Histograms** dialog box for the current workspace.
F8	Opens a window that displays all loaded sample files in the current database.
F9	Opens the **Drop Delay Wizard**.
Left arrow key	Sets the displayed sample in the current workspace or protocol as the first file in the **Database samples** window. If more than one file group is present in the sample window (that is, organized in multiple folders), the last sample in the currently selected group is displayed. Also repositions a region to the left when selected.

Table 3.1 Shortcut Keys and Functions *(Continued)*

Shortcut Key	Function
Right arrow key	Sets the displayed sample in the current workspace or protocol as the last file in the **Database samples** window. If more than one file group is present in the sample window (that is, organized in multiple folders), the last sample in the currently selected group is displayed. Also repositions a region to the right when selected.
Down arrow key	Sets the displayed sample in the current workspace or protocol as the next file in the **Database samples** window. If more than one file group is present in the sample window (that is, organized in multiple folders), the last sample in the currently selected group is displayed. Also repositions a region downward when selected.
Up arrow key	Sets the displayed sample in the current workspace or protocol as the previous file in the **Database samples** window. If more than one file group is present in the sample window (that is, organized in multiple folders), the last sample in the currently selected group is displayed. Also repositions a region upward when selected.
SHIFT + ARROW	Expands a region in the arrow direction (left, right, up, down) when selected.
CTRL + ARROW	Contracts a region in the arrow direction (left, right, up, down) when selected.
CTRL + O	Opens a dialog box to open one or more FCS Listmode files.
CTRL + S	Saves modified items on the Summit software desktop that have changed (or auto-save is invoked at periodic intervals and when Summit software is closed).
CTRL + P	Prints the current view of the Summit software desktop.
CTRL + C	Copies the selected region, which can be pasted into a histogram.
CTRL + V	Pastes the copied region into a histogram.
CTRL + D	Opens the **Sort Logic and Statistics** menu.
CTRL + G	Opens the **Gate Logic** menu.
CTRL + Z	Clears the event buffer of all acquired events.
CTRL + W	Opens the Worklist Panel.
SHIFT + F4	Starts a Hardware Sort.
ALT + F4	Exits Summit software.
+	Expands one node when viewing a folder list in a window (use + on the numeric keypad).
-	Collapses one node when viewing a folder list in a window (use - on the numeric keypad).
*	Expands an entire folder tree contained in a window (use * on the numeric keypad).

Startup and Shutdown

CyAn ADP Startup

NOTE The Quick Start Guide for the CyAn ADP (Document Number 0000023) is available as a quick reference for Startup and Shutdown procedures.

All CyAn ADP Operators

Refer to APPENDIX A, *Approved Cleaners and Disinfectants* for a list of reagents required for this Startup and Shutdown procedure.

How to Startup CyAn ADP

1 Double-click the Summit software icon on the desktop to launch the instrument control software.

2 Select an existing database or create a new one, and then click **OK**.

Figure 4.1 Open a Summit Database
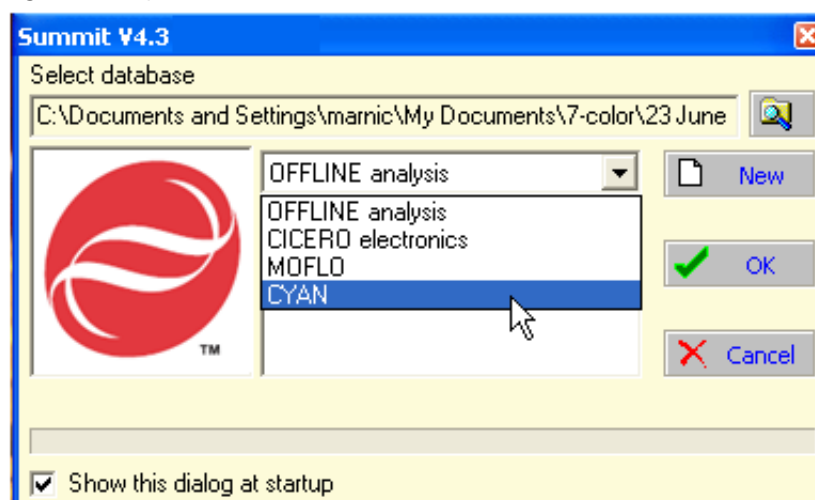
3 Click the **Instrument** tab to view the **CyAn Control Panel**.

Figure 4.2 CyAn Instrument Control Panel
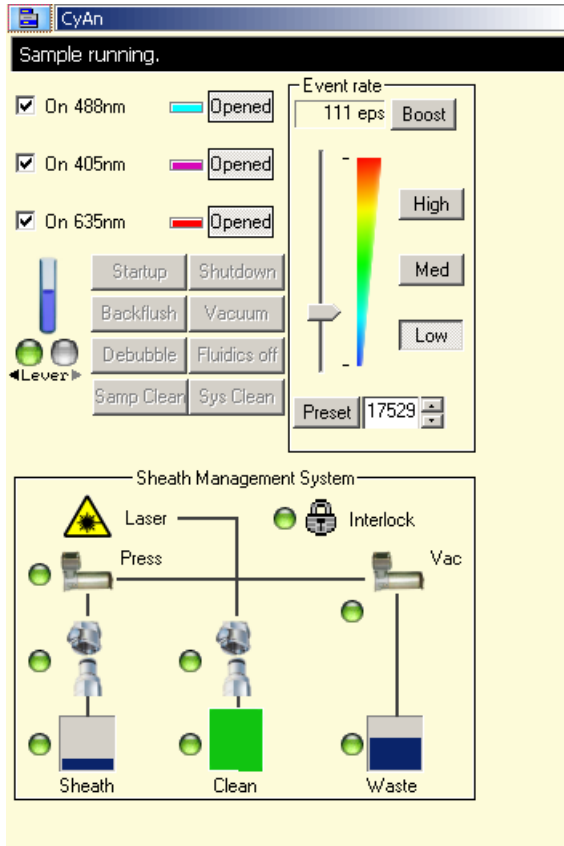
4 Check the status of the sheath container and the waste container by viewing the Sheath and Waste levels in the **CyAn Control Panel**. Make sure the sheath container is at least half full and the waste container is at least half empty. If necessary, fill the sheath container and empty the waste container as described in CHAPTER 6, *How to Change Sheath and Waste Containers*.

> ⚠ **CAUTION**
>
> **Remove the waste container and the sheath container from the cart before emptying or filling. Dispose of the contents of the waste container in accordance with local, state, and federal regulations.**
>
> **Do not drop the waste or sheath container on the Sheath Management System. Doing so may result in improper calibration of the load cells.**

5 Click **Startup** on the **Cyan Control Panel**.

6 On the **CyAn Control Panel**, make sure the desired lasers are checked on.

7 Open the required laser shutters by clicking the **Closed** buttons. These buttons should now read **Opened**.

8 Allow a minimum of 30 minutes for the lasers to stabilize.

9 It is recommended to perform a Clean and Rinse cycle while the instrument is warming up. Before running the Clean and Rinse cycle, make sure you have enough sheath fluid and cleaner fluid to complete the cycle.

- On the CyAn Control Panel, click the **Sys Clean** button and follow the on-screen prompts.
- A message box will warn you that the Clean and Rinse process will take 10-15 minutes. Click **OK** to continue. The clean cycle takes seven minutes to complete and the rinse cycle takes seven minutes to complete.

   NOTE The CyAn ADP lever should remain closed, and a tube should not be loaded.

10 Repeat the clean cycle using a tube of d.i. water.
- Slide the lever open.
- Load a sample tube of d.i. water over the probe and pressure fit the tube to the instrument.
- Slide the lever closed.

Figure 4.3 CyAn ADP Instrument with Sample Tube Loaded and Lever Open

1. Sample Probe with a Sample Tube Loaded   2. Lever

11 Open the QC file (protocol) created by your laboratory, place a tube with SpectrAlign beads (10^6 concentration) on the sample probe, and acquire $\boxed{F1}$ at a low event rate (~100 eps). Verify that the data is within your daily QC specification. Beckman Coulter recommends the following CV specifications for instrument quality control.

Table 4.1 Target CVs for CyAn ADP

Parameter	Target CV
FITC	< 3.0
PE	< 3.0
PE-Texas Red	< 3.5
PE-Cy5	< 4.5
PE-Cy7	< 6.5
Pacific Blue	< 6.0
Cascade Yellow	< 6.0
APC	< 6.0
APC-Cy7	< 6.0

12 If QC data is within specification, you are ready to run samples. If not, click **Debubble** on the CyAn Control Panel. Rerun the alignment beads. If necessary, repeat Debubble and then rerun QC.

IMPORTANT After the lasers have stabilized, do not power them down; leave them powered for the duration of CyAn ADP operations for the day. If the lasers are powered down, you must allow another 30-minute stabilization period before running samples again. To conserve sheath fluid, you can shut down the SMS by clicking the Fluidics off button in the CyAn Control Panel.

How to Perform a Daily Decontamination Procedure

Beckman Coulter recommends that you decontaminate the sample line daily.

1 Install a tube containing 3 mL of a decontamination solution and run for two minutes.

2 Remove the tube of decontamination solution, replace with a tube of deionized water, and rinse for two minutes.

3 Click the **Shutdown** button on the **CyAn Control Panel** and perform the steps as indicated in the software.

Shutdown Procedure

Beckman Coulter recommends that Sample Injection Clean and DI Water Clean are enabled prior to shutdown.

How to Enable Proper Settings for Shutdown

1 From the **Edit** menu in **Summit**, click Preferences.

2 Expand the **Instrument** list item by clicking the **+** sign.

Figure 4.4 Summit Preferences
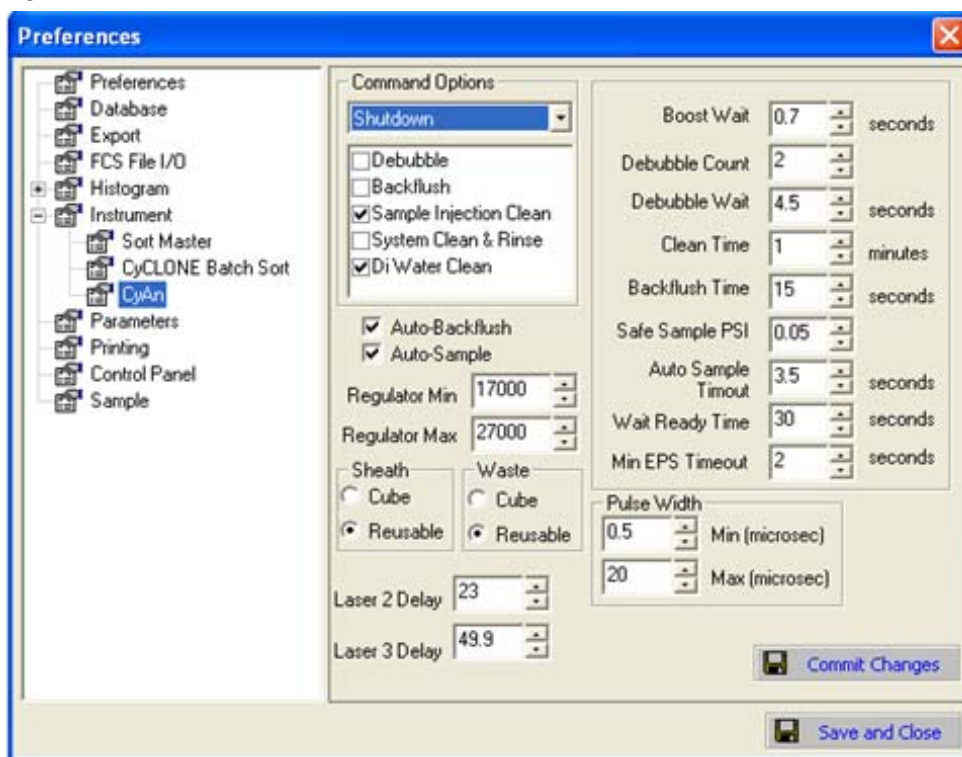
3 Click **CyAn**.

4 In the **Command Options** list, select **Shutdown**, check the **Sample Injection Clean** and **DI Water Clean** check boxes, and then click **Save** and **Close**.

5 Click **Shutdown** on the CyAn Control Panel. This will automatically shut down the lasers and close the laser shutters. When prompted, place a tube full of Beckman Coulter LH Series Formaldehyde-Free Clenz on the sample probe and slide the lever closed.

> **NOTE** If you are shutting down the instrument for the day, follow the instructions above, but use a decontamination solution instead of Beckman Coulter LH Series Formaldehyde-Free Clenz.

6 When prompted, remove the tube of cleaner, replace with a tube of deionized water, and move the sample lever in.

7 Check the status of the sheath container and the waste container by viewing the sheath and waste levels in the CyAn Control Panel. Make sure the sheath container is at least half full and the waste container is at least half empty. If necessary, fill the sheath container and empty the waste container as described in CHAPTER 6, *How to Change Sheath and Waste Containers*.

8 Fill the test tube with deionized water, place it on the sample probe and leave the lever out.

9 Close Summit software.

> **NOTE** Beckman Coulter recommends waiting 30 seconds after closing Summit software before logging off of the Summit Workstation in order to allow all data sources to close.

10 Log off the Summit workstation and turn off the computer and monitor.

Instrument Validation

CyAn Instrument Alignment Example

The following procedure is one example of an alignment procedure that can be used on the CyAn ADP analyzer. The actual validation protocol run by the customer will be selected and approved by customer.

How to Run a General Instrument Alignment Protocol

1 Start the CyAn ADP instrument and Summit workstation computer. See CHAPTER 4, *CyAn ADP Startup* for details.

2 Open the protocol approved by your laboratory. See CHAPTER 3, *Loading an Existing Protocol* for details.

Figure 5.1 Example Alignment Protocol
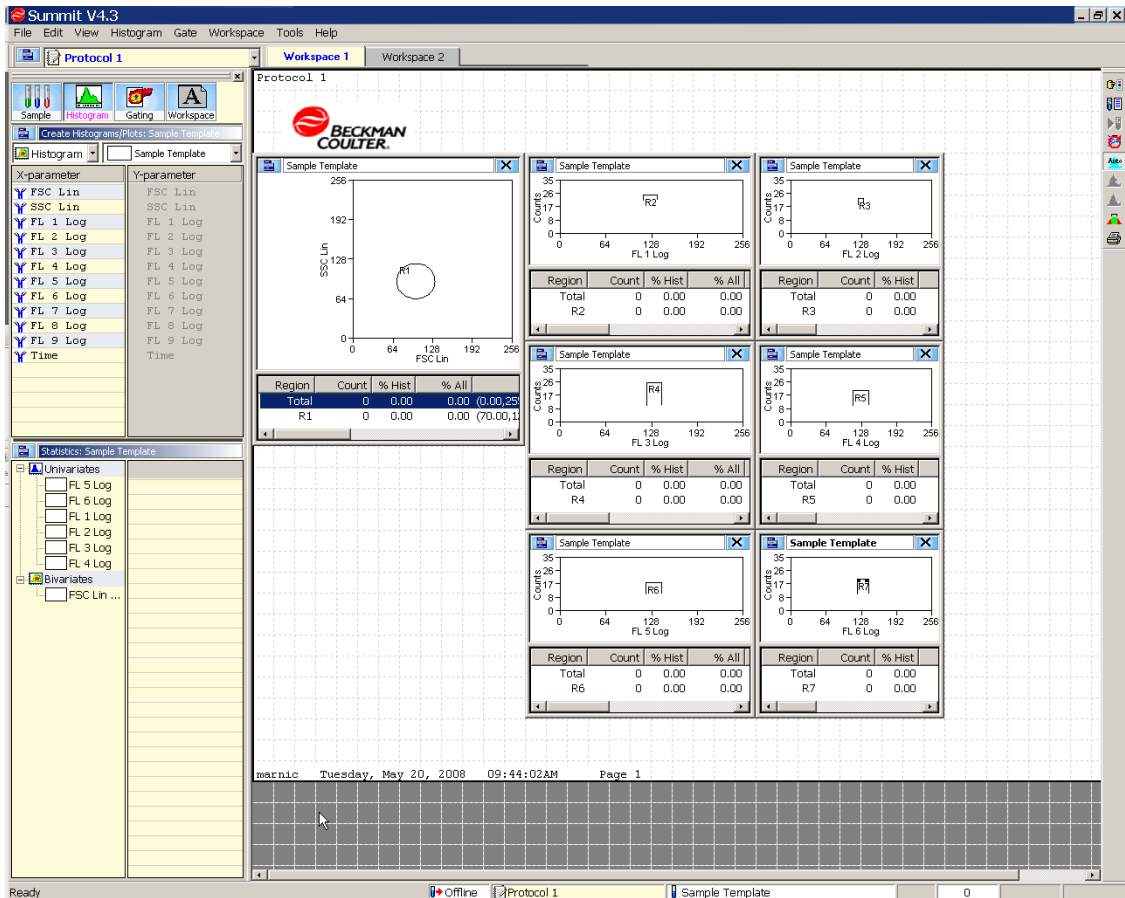
3 Prepare a sample of alignment beads. For example, two drops of (Spherotech SpectrAlign™ - 3.0 µm, 1 x 10^7/mL, 2 mL/vial RUO) in 1 mL deionized water.

4 Load the sample tube onto the CyAn and slide the lever closed. Data will begin to appear in the histograms.

> **NOTE** If data does not appear in the histograms, increase the event rate and/or click the **Boost** button on the CyAn Instrument Control panel. See Figure 5.2.

Figure 5.2 CyAn Instrument Control Panel
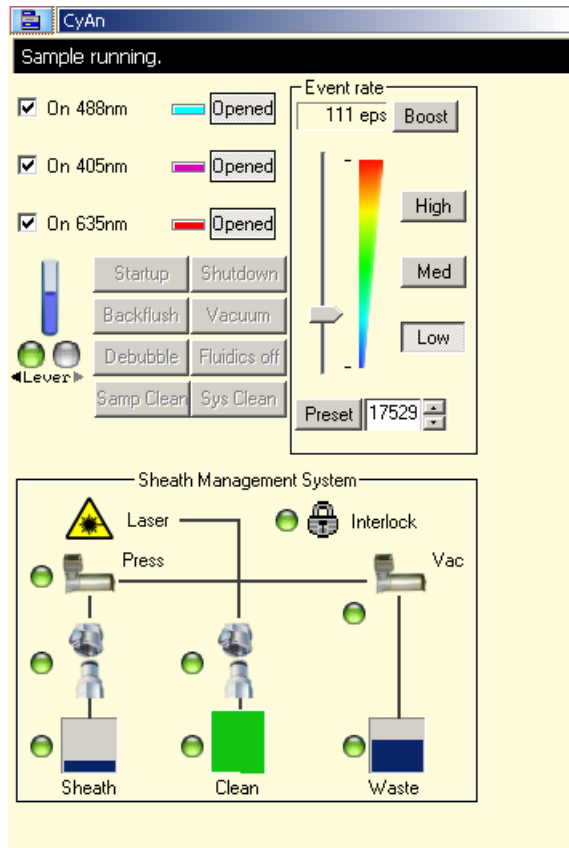
5 Click and drag the region (set as Gate 1) in the FSC and SSC histogram so that the gate comprises the greatest number of events.

6 Use the CyAn Instrument Control Panel to adjust voltages until the signal for each parameter is optimized to median 128 (+/- 4).

   NOTE (Ctrl) Z will refresh the events that appear on screen. Enabling Cycle Mode can be useful during this step. See Figure 3.18

7 Ensure that the Gate Limit is set at 5000. See Figure 3.8. Double-click in the field next to Limit and type 5000.

8 View the CVs at the bottom of the histograms to ensure that they fall within the recommended values. See Table 4.1, Target CVs for CyAn ADP.

   NOTE To view statistics in histograms see Figure 3.33.

9 If QC data is within specification, you are ready to run samples. If not, click **Debubble** on the CyAn Control Panel. Rerun the alignment beads. If necessary, repeat Debubble and then rerun QC.

10 Save the data. See CHAPTER 3, *Saving Acquired Data*.

11 Slide the sample lever open and remove the sample tube. Slide the lever closed to back flush the sample line.

12 Slide open the lever and place a tube of deionized water on the probe until you are ready to run samples. Do not slide the lever closed.

Cleaning and Maintenance

CyAn ADP Maintenance

Regular maintenance of the CyAn ADP instrument is recommended as described in this section. System integrity can be effectively maintained by assuring cleanliness of the fluidics. In addition to performing preventive maintenance procedures, we also recommend that you establish and perform other laboratory procedures for routine operations such as backing up your data and experimental protocols.

Daily Preventive Maintenance

Follow the cleaning steps outlined in CHAPTER 4, *Startup and Shutdown* on a daily basis.

Weekly Preventive Maintenance

The following steps should be performed on a weekly basis.

How to Maintain Reusable Sheath Containers

The reusable sheath carboy should be cleaned and disinfected once a week. Handle the container as aseptically as possible during cleaning. A location where a sink, hot water, counter space, and deionized water are available is required.

NOTE For institutions using disposable sheath cubitainers, no weekly maintenance of the sheath container is needed. See CHAPTER 6, *How to Change Sheath and Waste Containers*.

1 Wear gloves, a lab coat, and safety glasses.

2 Remove the cap from the container and place it on a clean paper towel.

3 Add a small amount of a phosphate-free laboratory grade soap and enough hot water to create a lather. Replace cap. Agitate or shake vigorously for 30 seconds.

4 Empty soap from the container and rinse with hot water until the soap is removed.

5 Rinse with d.i. water.

6 Use 70% Ethanol to spray the cap and threads on the container and the exterior of container and wipe clean.

7 Refill the carboy with sheath fluid.

How to Maintain the Head Unit and SMS Cart

The exterior surfaces of the CyAn ADP should be cleaned on a weekly basis. Any general-purpose surface cleaner/disinfectant can be used. All surfaces should be wiped down to remove dust, fingerprints, and any other soiling. The sample injection area should be thoroughly cleaned and disinfected as well. It is highly recommended that the computer keyboard and mouse are cleaned and disinfected on a weekly basis also.

How to Clean Fluidics Lines

SMS Clean and Rinse Cycle
Before performing the Clean and Rinse cycle on the CyAn ADP, ensure you have enough sheath fluid and cleaner fluid to complete the cycle.

1 On the CyAn Instrument Control Panel, click the **Sample Clean** button. See CHAPTER 5, *CyAn Instrument Control Panel*.

2 You will be prompted that the Clean and Rinse process will take 10-15 minutes. Click **OK** to continue. The clean cycle takes seven minutes to complete, followed by a rinse cycle that takes seven minutes to complete.

Monthly Preventive Maintenance

Performing this decontamination procedure is a necessary part of system maintenance and will ensure the instrument's longevity. To prevent the buildup of debris and potential contamination of the fluidics system on the CyAn ADP, the system must be decontaminated on a monthly basis. A disinfectant should be run through the system to eliminate any sample buildup or microorganisms that may have entered the system through normal everyday use. Only Beckman Coulter-approved disinfectants should be used. See APPENDIX A, *Approved Cleaners and Disinfectants*.

How to Perform System Decontamination

The following procedure must be followed once per month. A reusable sheath carboy is required.

1 Ensure that the instrument is on and the software is open with at least one histogram created.

2 Wear gloves, safety glasses, and a laboratory coat. Shut down the fluidics on the CyAn ADP.

3 In a clean reusable sheath carboy make a minimum of two liters of diluted bleach solution. See APPENDIX A, *Disinfectants for Use in Sheath Line*.

4 Spray the quick connect with 70% Ethanol and hook up to sheath port on the SMS cart.*

5 Start up the fluidics. This will move the decontamination solution in the sheath tank through the system.

6 Place a test tube with 4mL of diluted bleach on the sample probe. See APPENDIX A, *Disinfectants for Use in Sample Line*

7 Press F2 to acquire. Sample flow rate should be on LOW (corresponding to ~ 10-20 µL/min.).

8 Let the decontamination solution run from both the sheath tank and the sample for 60 minutes.

9 After 60 minutes, click **Debubble** on the CyAn Control Panel. After this function is complete, remove the tube from the sample probe and back flush.

10 Shut down the fluidics.

11 Replace sheath with d.i. water. Install a disposable box of sterile d.i. water or thoroughly rinse the reusable carboy with d.i. water and then fill with 5 liters of fresh d.i. water.

12 Spray the quick connect with 70% Ethanol and attach it to the sheath port on the SMS cart.*

13 Start up the fluidics and place a tube with 4mL d.i. water on the sample probe.

14 Click F2 to acquire. Sample flow rate should be on LOW.

15 Rinse for a minimum of 90 minutes. Note: d.i. water may need to be refilled on the sample probe during the rinse time. Refill as necessary.

16 After 90 minutes have elapsed, click **Debubble** on the CyAn Control Panel. After this function is complete, remove the tube from the sample probe and back flush.

17 Perform an SMS Sys Clean Cycle. See CHAPTER 6, *Weekly Preventive Maintenance*.

The system is now fully decontaminated and ready to continue cellular analysis.

NOTE A sheath container with approximately 1L of fluid in it may alert the system that the tank is low and that it should be changed soon. The decontamination cycle will be able to run for the specified time without the instrument fluidics stopping. Contact Beckman Coulter Customer Care if any problems arise with performing this procedure.

How to Perform Laser Interlock Maintenance

1 Follow the instrument startup procedure. Make sure the lasers are on and the laser shutters are open.

2 Inspect the housing on the instrument to verify that panels or covers are fitted and tight so that all laser energy is contained in the interior.

3 Facing the instrument, lift the lid approximately one inch (2.54 cm). The LED light(s) on the right front of the cover should go out, verifying that the laser shutters are closed.

4 Close the lid.

5 If the LED light(s) did not go out when the lid was opened, contact Beckman Coulter Customer Care.

Biannual Maintenance Procedure

A Beckman Coulter Field Service Representative should perform a maintenance check on the CyAn ADP every six months. To schedule a six-month or annual maintenance service, contact Beckman Coulter Customer Care.

How to Change Sheath and Waste Containers

Use the shutdown fluidics procedure to fill and empty sheath and waste containers when running samples.

> ⚠️ **CAUTION**
>
> - **Remove the waste container and the sheath container from the cart before emptying or filling.**
> - **Dispose of the contents of the waste container in accordance with local, state, and federal regulations.**
> - **Do not drop the waste or sheath containers on the Sheath Management System. Doing so may result in improper calibration of the load cells.**

1 Click **Fluidics Off** on the CyAn Control Panel. See Figure 4.2.

2 Release the quick connect fittings from the waste container.

3 Remove the waste container from the cart, remove the lid, and empty the container. Dispose of the waste in accordance with local, state, and federal regulations.

4 Place an appropriate amount and type of disinfectant in the bottom of the waste tank to ensure effective killing action when the tank is full. All biological components introduced into the waste tank must be in contact with the disinfectant for a minimum of 10 minutes before being discarded. See APPENDIX A, *Disinfectants for Use in the Waste Tank*.

> **NOTE** If 200mL of regular household bleach (5.25% active chlorine) is placed in the container, this will provide approximately 500ppm available chlorine when the tank's full capacity of 20L has been reached. If the samples you are running will not be effectively killed by this concentration of sodium hypochlorite solution, an increased amount of bleach should be used to achieve effective disinfection concentration. Please keep in mind that diluted sodium hypochlorite solutions begin losing their effectiveness after 24 hours.

5 Replace the lid. Position the container on the cart and reconnect the waste container using the quick connect fittings.

6 Restore the sheath fluid by either replacing the entire sheath cubitainer or refilling the plastic carboy, depending on which type of sheath container is used with your Sheath Management System:

Disposable Sheath Cubitainer: Release the quick-connect fittings to separate the cubitainer from the cart. Dispose of the entire cubitainer in accordance with local, state, and federal regulations. Position a new sheath cubitainer on the cart. Locate the quick-connect fitting inside the box. Spray the quick-connect fitting with 70% ethanol. Attach the cubitainer to the SMS cart.

Plastic Carboy: Release the quick-connect fittings to separate the carboy from the cart. Remove the carboy from the cart, loosen the lid, and fill with particulate-free d.i. water or a suitable sheath fluid. Seal the lid, and place the container back in its position on the cart. Spray the quick connect with 70% ethanol. Reconnect the sheath container to the SMS cart using the quick-connect fittings.

7 Click **Startup** on the Cyan Instrument Control Panel. See Figure 4.2.

How to Replace Cleaner Fluid

1 Release the cleaner cubitainer quick-connect fitting.

2 Remove the cap from the empty cleaner cubitainer. Retain the cap for use on the new cubitainer. Do not place this cap and its attached tubing on the floor or other surface where it will be in contact with contaminants.

3 Dispose of the cleaner cubitainer in accordance with local, state, and federal regulations.

4 Remove the cardboard punch-out section on the new cleaner cubitainer. Hold the ring around the cap, pull up on the lid so that the lid extends up to the cardboard.

5 Remove the cap from the new cubitainer and replace it with the cap from the previous cubitainer.

6 Place the new cleaner cubitainer back in its position on the cart. Spray the quick connect with 70% ethanol. Reconnect the cubitainer to the cart.

Troubleshooting

CyAn ADP Instrument Troubleshooting

Contact Beckman Coulter Customer Care immediately for assistance with any instrument malfunction or service need.

> ⚠️ **WARNING**
>
> **Do not attempt any maintenance on the CyAn ADP laser components. Laser maintenance should only be performed by specially trained, certified Beckman Coulter Field Service Representatives.**

Table 7.1 CyAn ADP Troubleshooting Matrix

Problem	Action
Instrument has no power.	Ensure that the power plug is firmly attached to the wall and the power switch on the back of the instrument is on.
	There are no operator-replaceable fuses in the CyAn ADP
Acquiring data, but it does not display.	Ensure that the lasers are on. View the Instrument Control Panel in Summit and ensure that the laser shutters are open.
Indicator lights in the SMS portion of the Instrument Control Panel in Summit change from green to amber or red.	Move the cursor over the indicator light to see more information.
Data integrity - data looks questionable during acquisition.	Remove sample. Perform back flush and debubble. Check sample preparation. Re-run the sample. If the problem persists, check instrument alignment with beads. Perform another back flush and debubble.

Possible Error Conditions in the SMS

The following figures illustrate some of the possible error conditions in the SMS.

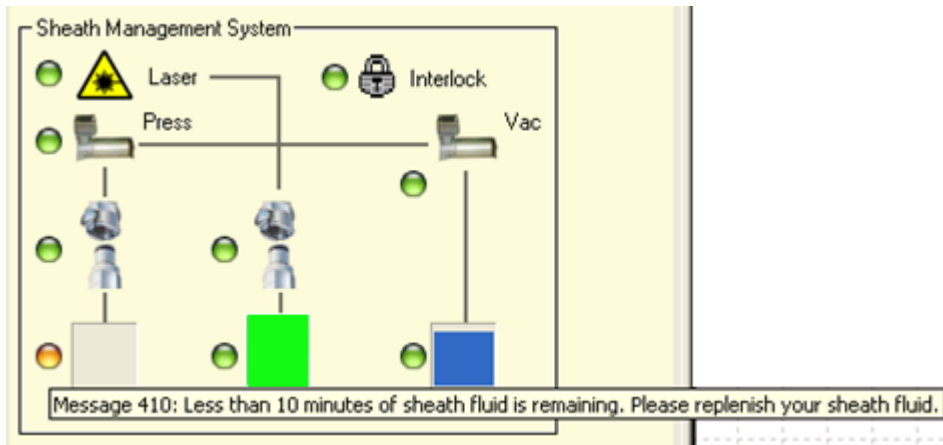**Figure 7.1** Low Sheath Fluid Warning

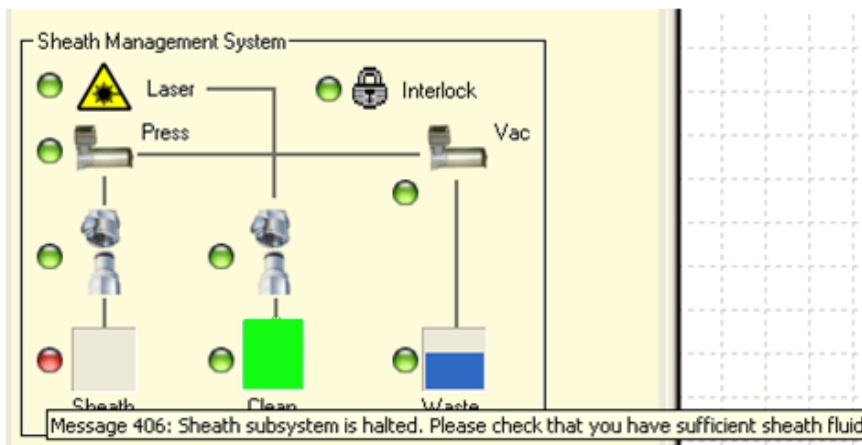
Figure 7.2 Empty Sheath Fluid Message

Figure 7.3 Quick Connect Error Message
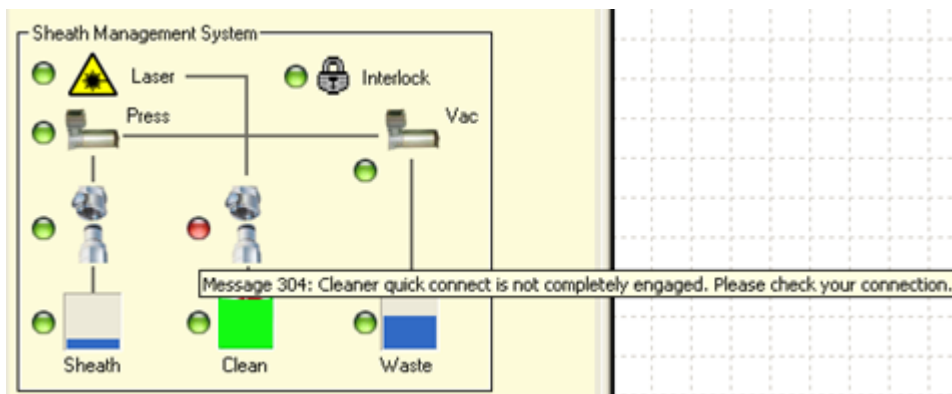
The LED indicators on the front panel of the Sheath Management System also change when fluid levels change in the SMS. Three fluid levels are monitored on the SMS panel: SHEATH LEVEL, CLEANER LEVEL, and WASTE LEVEL.

When all fluids are at suitable levels for proper operation of the CyAn ADP, the SHEATH LEVEL, CLEANER LEVEL, and WASTE LEVEL LEDs appear green beside the OK label:

Figure 7.4 SMS Fluid Levels are Suitable for Operation

When the sheath level or cleaner fluid level is low, the green OK LED changes to a flashing amber or a solid amber LED beside the LOW label:

Figure 7.5 SMS Low Sheath and Cleaner Levels

When the waste fluid level is high, the green OK LED changes to a flashing amber LED beside the HIGH label:

Figure 7.6 SMS High Waste Level

When the sheath or cleaner level changes from low to empty, the flashing amber LED changes to a red LED beside the EMPTY label:

Figure 7.7 SMS Sheath and Cleaner Empty

When the waste fluid level changes from high to full, the flashing amber LED changes to a red LED beside the FULL label:

Figure 7.8 SMS Waste Full

Table 7.2 CyAn ADP Instrument Control Panel Indicators in Summit
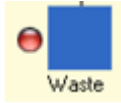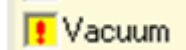
Panel Icon	Message Text
Interlock	Message 102: The CyAn ADP cover is open. The laser shutters have been closed and the fluidic system is shut down. Close the cover and restart the instrument.
Clean	Message 301: Cleaner fluid is low. Replace the cleaner solution and press the Startup button on the CyAn ADP Instrument Control Panel.
Clean	Message 302: Cleaner fluid is empty. Replace the cleaner solution and press the Startup button on the CyAn ADP Instrument Control Panel
	Message 304: Cleaner quick connect fitting is not securely attached. Check the connection.
Clean	Message 305: Internal reservoir is overfilled. This will not prevent the operation of the instrument, but may indicate the need for future service.

Table 7.2 CyAn ADP Instrument Control Panel Indicators in Summit

Panel Icon	Message Text
	Message 306: Cleaning subsystem is halted. Ensure that there is sufficient cleaner solution.
	Message 307: Cleaning subsystem switch error. This will not prevent the operation of the instrument, but may indicate the need for future service.
	Message 430: Less than 30 minutes of sheath fluid remains.
	Message 410: Less than 10 minutes of sheath fluid remains. Replenish the sheath fluid.
	Message 401: Internal sheath reservoir is low. CyAn ADP will soon stop. Replenish the sheath fluid.
	Message 402: Sheath fluid is empty. Replenish the sheath fluid and press the Startup button on the CyAn ADP Instrument Control Panel.
	Message 404: Sheath quick connect fitting is not securely attached. Check the connection.
	Message 405: Internal reservoir is overfilled. This will not prevent instrument operation but it may indicate the need for future service.
	Message 406: Sheath subsystem is halted. Ensure that the instrument has sufficient sheath fluid.
	Message 407: Sheath subsystem switch error. Service may be required.
	Message 705: Waste subsystem is halted. Check the fluid level in the waste tank.

Table 7.2 CyAn ADP Instrument Control Panel Indicators in Summit

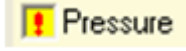| Panel Icon	Message Text
	Message 730: Less than 30 minutes of operation is possible before the waste container is full.
	Message 710: Less than 10 minutes of operation is possible before the waste container is full.
	Message 700: Waste container is full. Empty the waste container.
	Message 905: CyAn ADP has low sheath pressure. Secure the connection between the CyAn instrument and the SMS.
	Message 906: CyAn ADP has low vacuum. Secure the connection between the CyAn instrument and the SMS.
	Message 997: SMS has low sheath pressure.
\<Message in the top portion of the Control Panel\>	Message 999: SMS Power fault. Reset SMS to continue. If this problem reoccurs, call Beckman Coulter Customer Care.

Approved Cleaners and Disinfectants

Overview

The following list of cleaners and disinfectants can be used on the CyAn ADP. If products not specified on this list are used, it may cause damage to the system and void the warranty. Any questions or concerns regarding chemical usage on the CyAn ADP should be directed to Beckman Coulter Customer Service.

Cleaners

- Beckman Coulter LH Series Formaldehyde-Free Clenz
- 0.1% Triton-X100 in DI water
- 70% Ethanol in DI water

Disinfectants for Use in Sample Line*

- 70% Ethanol in DI water
- Bleach solution with a maximum concentration of 2000 ppm active chlorine (115 mL household bleach 2885 mL water, 1:25 dilution) The bleach solution may be diluted further depending on the pathogenicity of the sample.

Disinfectants for Use in Sheath Line†

- Bleach solution containing 400 ppm active chlorine (22 mL household bleach 2860 mL water, 1:130 dilution)

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* ANY disinfectant used on the sample probe must be rinsed with an equal amount of DI water.

† ANY disinfectant used in the sheath lines must be rinsed with DI water for a minimum of 90 minutes.

Disinfectants for Use in the Waste Tank

The following disinfectant types may be used in the waste tank. It is pertinent that the appropriate type and quantity of disinfectant is placed in the waste tank to ensure effective inactivation of the biologics in use when the tank is full. Please check compatibility of combined products before use.

- 10% Sodium hypochlorite or bleach
- Quaternary ammonium
- Thymols
- Phenols

Consumables

Consumables

Table B.1 Beads

Code	Product
URFP-30-2	SpectrAlign™ - 3.0 μm, 1 x 10^7/mL, 2 mL/vial (for installation only; RUO) Single population of beads exciting and emitting across 351 nm, 405 nm, 488 nm, and 635 nm.
RCP-30-5	FluoroSpheres – 6-peak, 3.2 μm, 1 x 10^7/mL, 40 tests
RCP-30-5A	FluoroSpheres – 8-peak, 3 μm, 1 x 10^7/mL, 100 tests

NOTE The items listed in Table E.1 must be purchased directly from Spherotech.

Table B.2 Sheath

Code	Product
8546859	IsoFlow Epics Sheath Fluid, 10 L
8546719	ISOTON II, 20 L
CY30230	Puraflow 8X Sheath Fluid, 24 L (6 L x 4 bags)

Table B.3 Maintenance

Code	Product
721542	Beckman Coulter Formaldehyde Free Clenz
	Bleach solution containing 2000 ppm active chlorine (115 mL household bleach 2885 mL water)

Compensation Background Information

Compensation

Compensation is the process of resolving the actual intensities from each antibody conjugate in a multicolored sample. Using single color staining or single color fluorochromes as a compensation control makes it possible to define the relative amount of light that ends up in another detector. For example, compensation can be used to determine how much light from FITC ends up in the FL2 detector and, conversely, how much light from PE ends up in the FL1 detector (see figure below). This percentage is defined as the spillover coefficient of FITC into FL2 and PE into FL1. By having these spillover coefficients defined for all colors and channels in an experiment, it is possible to calculate the compensation of data in a multi color sample using linear algebra. This is the way that compensation is performed mathematically in Summit software and other offline flow analysis software.

NOTE For instructions on how to use the Auto Compensation Wizard see CHAPTER 3, *Auto Compensation Wizard for Single Positive Controls*.

Figure C.1 FL1, FL2 Spectra Diagram
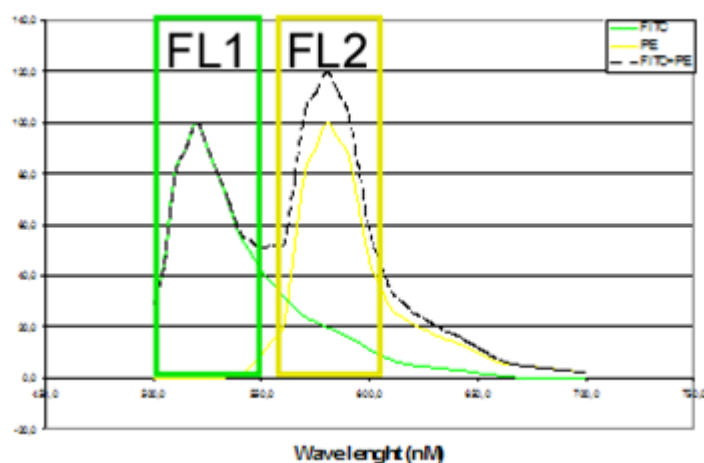
The amount of light that is detected in each fluorescence parameter (FL1, FL2) is the area under the curve of each spectra. If two fluorochromes are bound to the same cell, the sum of the light from each fluorochrome is detected. Knowing the spillover in both directions makes it possible to mathematically determine the light from each fluorochrome.

For two fluorochromes, the following math is used:

$FL1(FITC) = FL1(total) - a_{12}FL2(total)$

$FL2(PE) = FL2(total) - a_{21}FL1(total)$

Where a_{12} is the spillover of FITC into FL2 and a_{21} is the spillover of PE into FL1. The (total) is the signal measured on the dual stained cell in each channel.

Six-color Experiment Compensation

The following six-color experiment utilizes the Blue (488nm), Violet (405nm) and Red (635nm) lasers, uses the fluorochromes FITC, PE, PE-Cy5, Cascade Yellow, Pacific Blue and APC, and involves compensation. Whole blood is drawn from a donor and partitioned into eight tubes. Tube #1 is not stained with any fluorochrome (a no-stain control). Tubes #2-7 are each stained using one of the six colors. Cells within tube #8 are stained using all six fluorochromes. Below is a table listing the eight tubes, fluorochromes used for each, and the respective cell surface marker targeted.

Please note that in Tube #6 we have used CD3 Cascade Yellow and not CD45 Cascade Yellow as added in Tube #8. By using CD3 Cascade Yellow, an adequate number of positive and negative events will be seen, making compensation setup easier. In general, it is possible to use any fluorochrome antibody conjugate with the same fluorochromes to set compensation.

If the lymphocyte gate (R1) is set correctly, then the number of CD45 negative events seen will be minimal, making derivation of compensation settings difficult. In Tube #8 the combination of CD45 Cascade Yellow and Side Scatter can be used to set a more accurate lymphocyte gate by allowing for the exclusion of debris, basophils and monocytes.

Table C.1 Six-color Experiment Compensation

Sample	Target	Stain
Tube #1	None	None
Tube #2	CD4	FITC
Tube #3	CD56	PE
Tube #4	CD20	PE-Cy5
Tube #5	CD8	APC
Tube #6	CD3*	Cascade Yellow
Tube #7	CD3	Pacific Blue

Table C.1 Six-color Experiment Compensation

Sample	Target	Stain
Tube #8	CD4	FITC
	CD56	PE
	CD20	PE-Cy5
	CD8	APC
	CD45*	Cascade Yellow
	CD3	Pacific Blue

Setting up for Sample Acquisition

When performing a multi-color experiment, the forward and side light scatter parameters should be set to linear; all fluorescent parameters should be set to log. The range of expression of various antigens, e.g. CD3, CD4, CD8 etc., on a cell can be very large. This is why we need to use a logarithmic scale to ensure we can see the whole range of antigen expression from dim to very bright. When staining for DNA we are usually only looking for a doubling in fluorescence thus a linear scale is preferred.

Create a forward vs. side scatter dot plot and run the non-stained sample (Tube #1). This allows identification of the cell populations that will be used for gating. Below is a dot-plot showing unstained lysed and washed whole blood, using both the forward and side scatter parameters. The lymphocyte, monocyte, and granulocyte populations have been color coded for easy recognition.

Figure C.2 Unstained Sample Tube #1
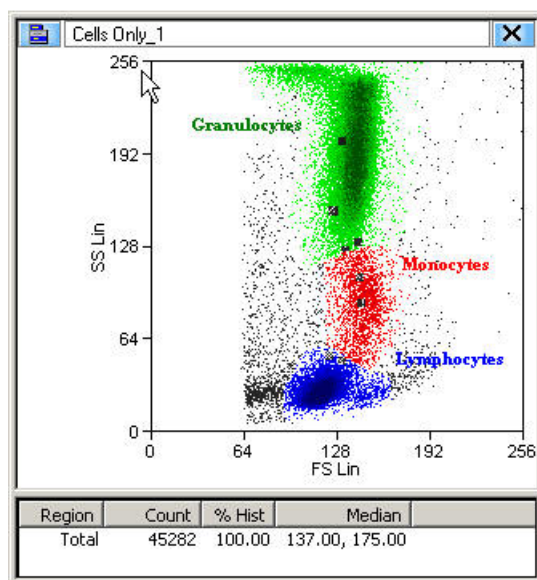
Within the forward-side scatter plot, create a tight region around the lymphocyte population (R1). This region will be used to gate the lymphocytes into additional histograms.

Figure C.3 Region R1 Around Lymphocytes
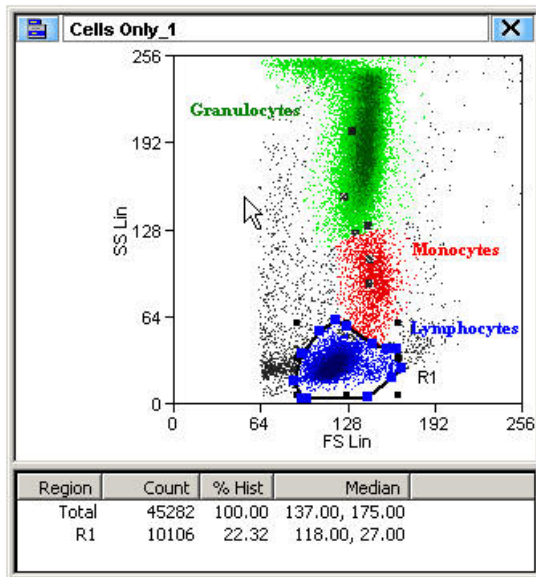
Now create a single or dual parameter histogram in log scale for each color of interest gated on the lymphocyte population from the FSC / SSC plot. Adjust the PMT voltage for each color so that the unstained lymphocyte population is situated within the first log decade for all colors. It is important the PMT voltage is set fairly high to prevent the median of the negative population becoming "1", because that will add uncertainty to manual compensation.

Acquire the single color compensation controls/tubes, as well as the multi color tube(s), with these PMT settings.

## Creating Histograms for Six-color Experiment Compensation

When performing a multi-color experiment, a non-stained sample (Tube #1) is run, followed by the first single-color stain to be analyzed (e.g. Tube #2 FITC). Create a series of plots such that this parameter is paired with each of the other colors in the experiment. For example, this six-color experiment involves FITC, PE, PE-Cy5, Cascade Yellow, Pacific Blue and APC. If FITC is the first single color to be run, five plots should be created: FITC vs. PE, FITC vs. PE-Cy5, FITC vs. APC, FITC vs. Cascade Yellow, and FITC vs. Pacific Blue. Typically, we recommend that you plot FITC along the X-axis and the other five colors along the Y.

Additionally, you will need to create quadrant regions within each of the dot plots except FSC vs. SSC. Statistics from the quadrant regions will be used in setting compensation from the control samples and identifying positive and negative populations in the all stains sample. In general, we recommend that you position the quadrants to encompass the first 1.5 log decades.

The graphic below shows five plots that have been created for the six-color data. Notice that FITC has been paired against the other five fluorochromes used in the experiment.

Figure C.4 Six-color Compensation Unstained Sample (Ungated)
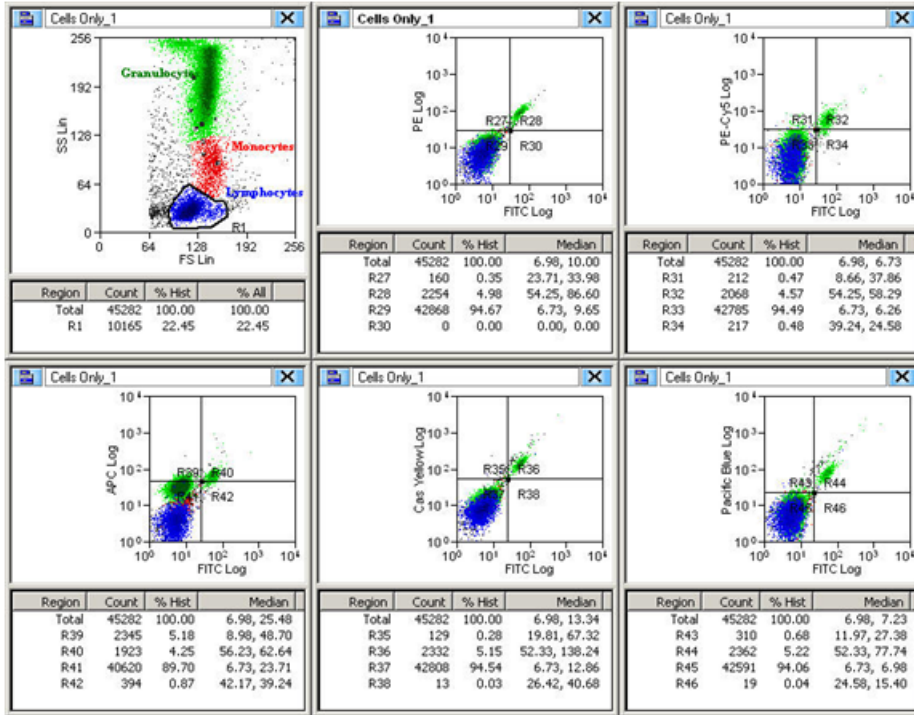
After creating the necessary histograms and quadrant regions, apply the gate from the lymphocyte population region (R1) to the five dot plots. See CHAPTER 3, *How to Gate One or More Histograms or Dot Plots*.

Figure C.5 Six-color Compensation Unstained Sample (Gate Applied)
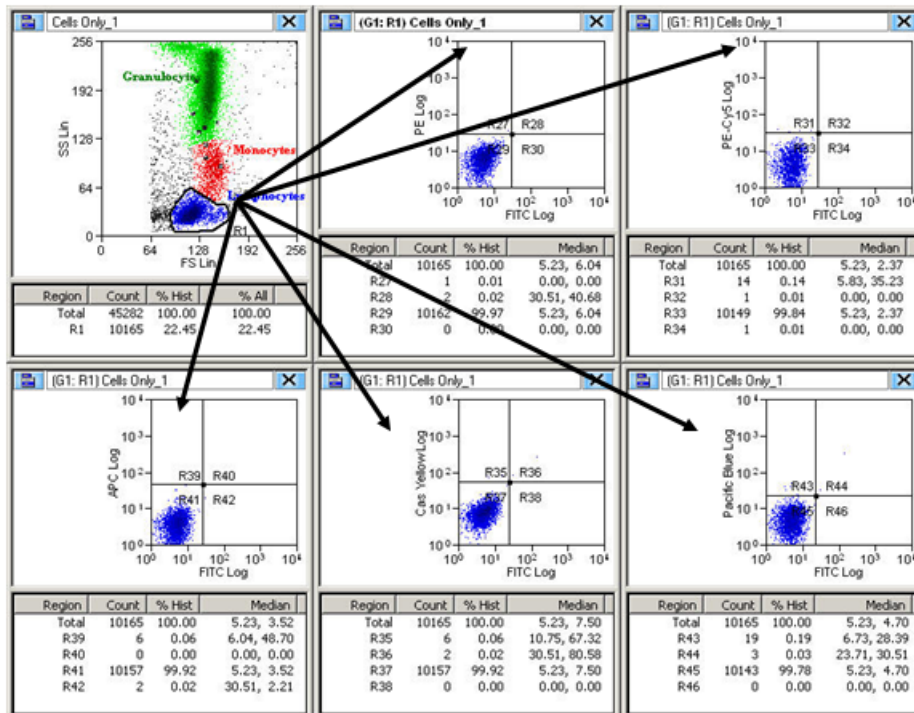
Load the non-stained sample Tube #1 to verify that the PMT settings were adjusted correctly during acquisition.

Figure C.6 Unstained Sample to Verify PMT Settings
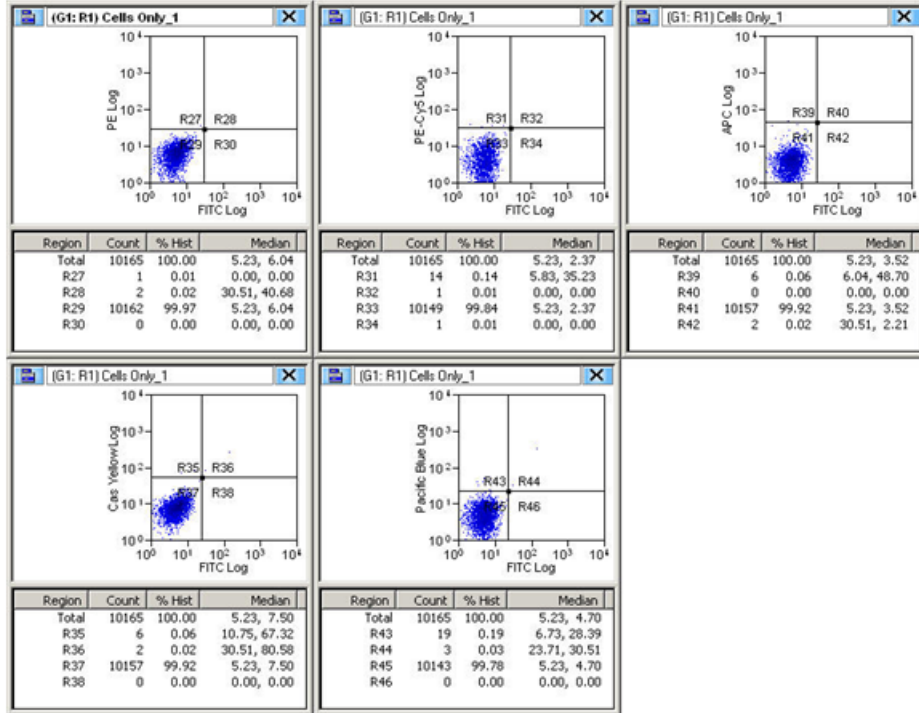
Manual Compensation

After gating the dot plots for the lymphocyte population within region (R1) in the FSC vs. SSC dot plot, load the first single-color sample. In theory, it does not matter which of the six single-color samples you begin with, although this example starts with FITC.

You will notice that in the FITC vs. PE plot, the positive population is shifted into the upper right quadrant. This means the signal from the FITC population is also being detected by the PE parameter. The degree to which signal is being detected for the PE parameter represents the percent spillover of PE into the FITC signal. See Figure C.7.

Figure C.7 Single-stain Sample FITC Uncompensated
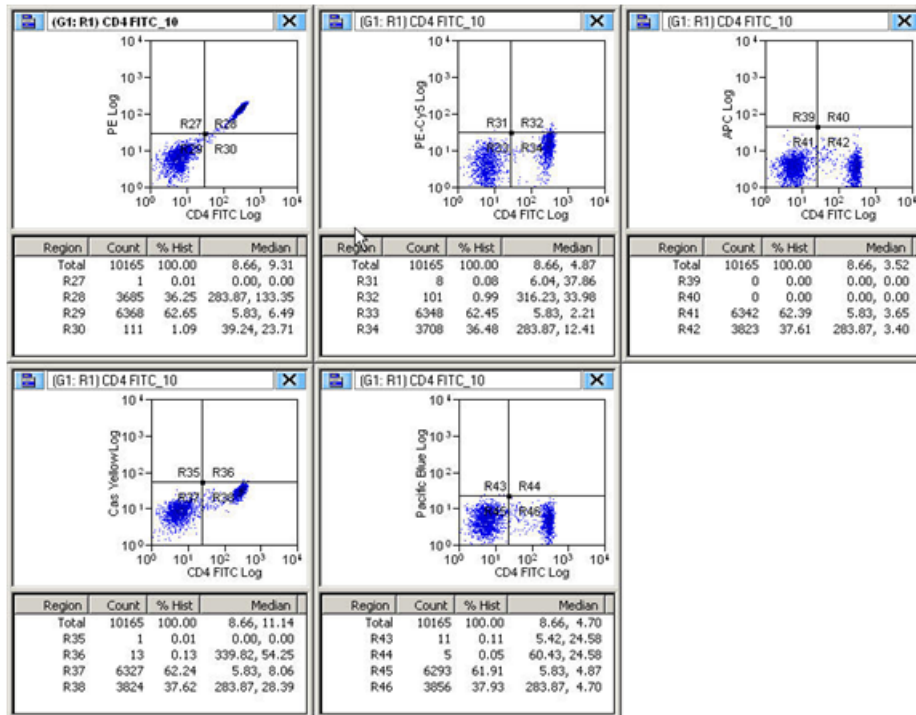
Setting Spillover Percentage for Compensation

For proper compensation, adjust the spillover percentage so that the Y-axis median fluorescence value for the positive population (lower right hand quadrant) is equivalent to that seen with the negative population (lower left hand quadrant). A range of ± 1.0 is usually acceptable.

By adjusting the spillover percentage value, the negative population might obtain a value of 1 before the median of the positive and negative population are aligned (thus the high PMT settings as previously explained). If this happens, you might need to adjust the spillover percentage without having the negative to align to, which can result in overcompensation. Overcompensation in general is to be avoided, as this can result in populations of interest disappearing when analyzing the multi color samples.

Figure C.8 Single-stain Sample FITC Compensated
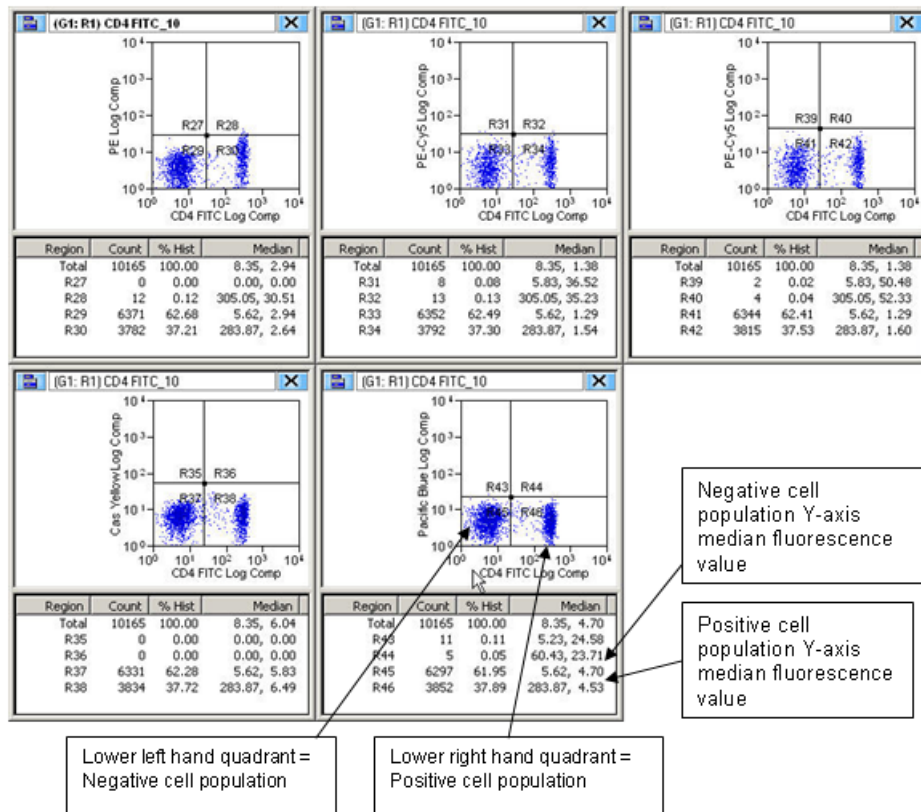
Running the Second Single-color Sample

Next, load the second single-color sample. Before doing this, change the parameters of each of the histograms such that the second color (in this case PE) is plotted against the other five colors: PE vs. FITC, PE vs. PE-Cy5, PE vs. APC, PE vs. Cascade Yellow and PE vs. Pacific Blue. Two populations will be delineated, one being PE negative and the other positive.

Figure C.9 Single-stain Sample PE Uncompensated
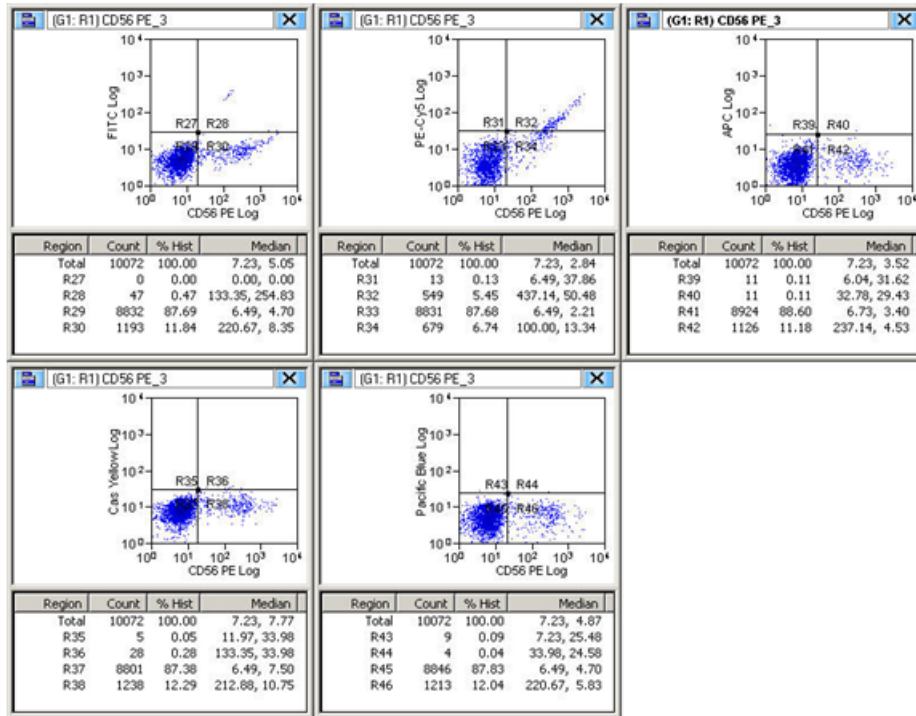
Compare the Y-axis median fluorescence between the lower left and lower right quadrants, and adjust compensation as needed.

Figure C.10 Single-stain Sample PE Compensated
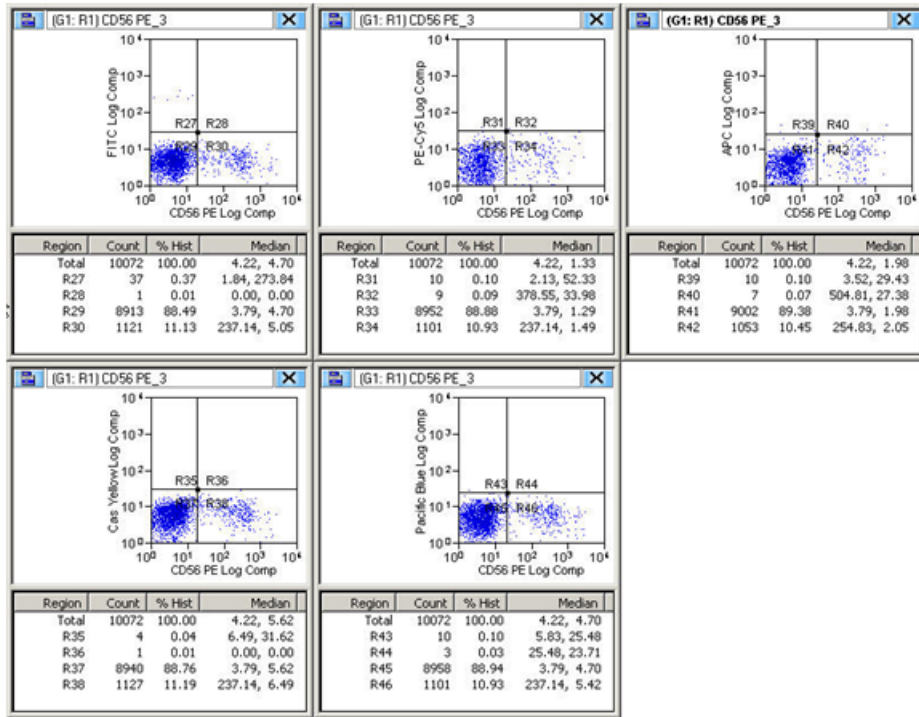
Why adjust compensation for the PE vs. FITC plot when FITC vs. PE was compensated for the FITC sample? This is because the % fluorescent spillover occurs both ways, FITC into the PE parameter and PE into the FITC parameter. Look at the spectral emission curves for both fluorochromes. You will notice that the degree of spillover between the two fluorochromes actually differs. This is why compensation must be adjusted based on both the FITC and PE single-color samples.

Figure C.11 Spectral Emission Curves for FITC and PE
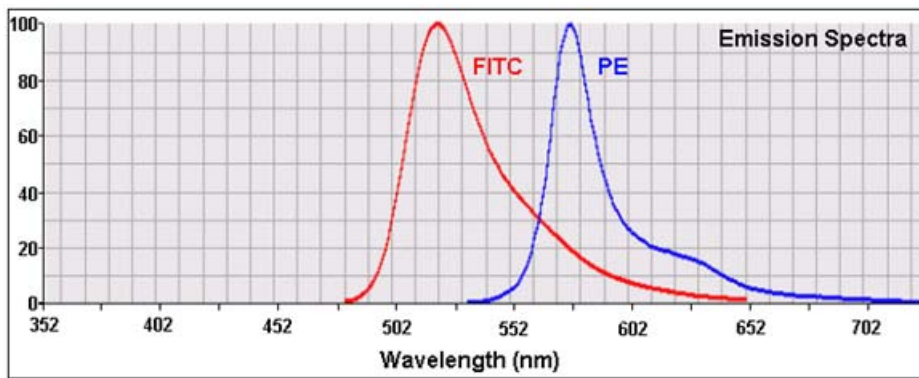
Running the Remaining Samples

Now run the third single-color sample, in this case PE-Cy5. Remember to reset the parameters along the dot plot axes so that PE-Cy5 is plotted against the other five colors.

NOTE These steps will be repeated for the remaining single-color samples.

Figure C.12 Single-stain Sample PE-Cy5 Uncompensated
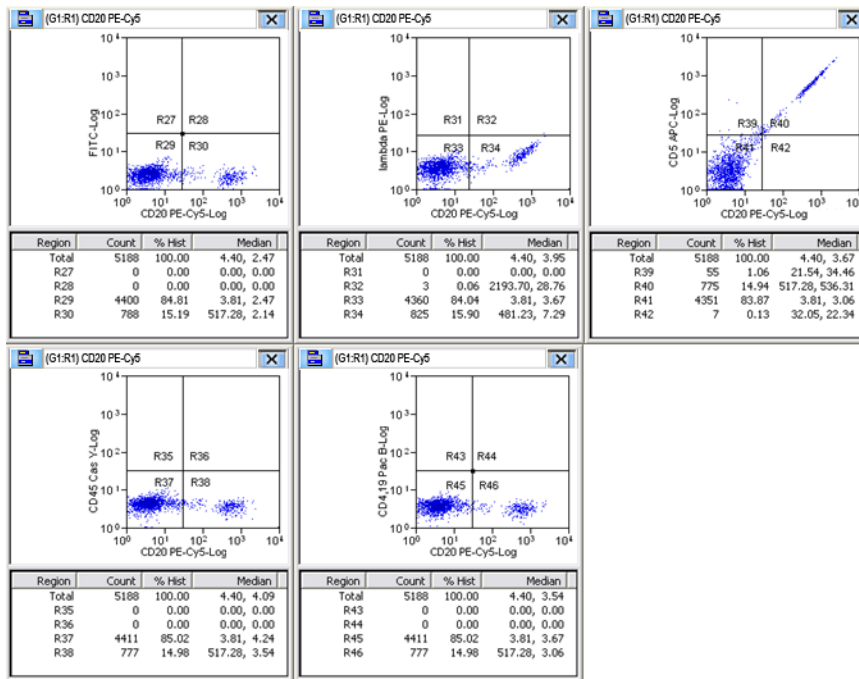
Where necessary, adjust compensation to align the Y-axis median fluorescence (relative to the other five colors) between the lower left and lower right quadrants.

Figure C.13 Single-stain Sample PE-Cy5 Compensated
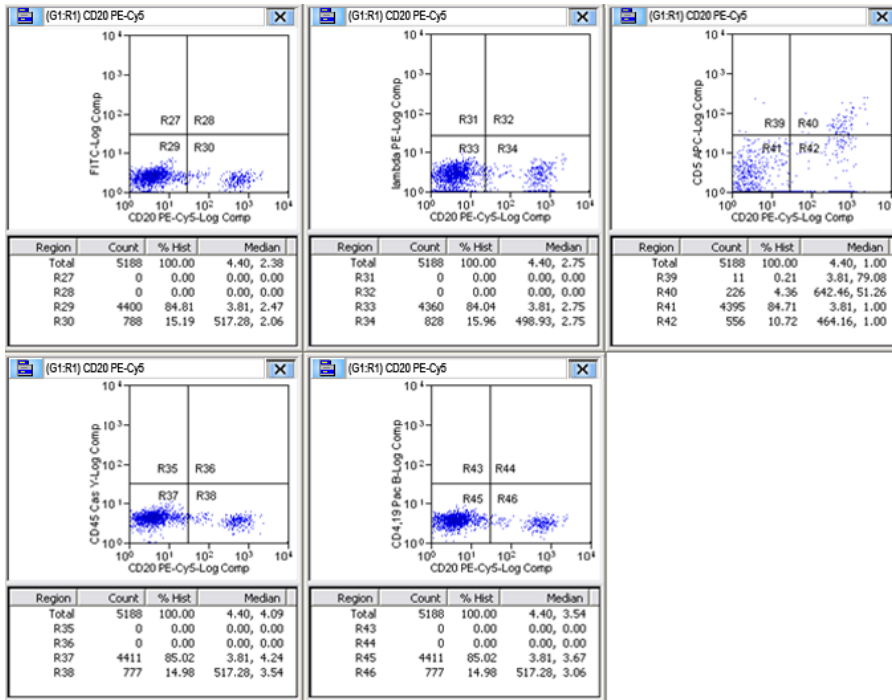
Run the fourth single-stain sample.

Figure C.14 Single-stain Sample APC Uncompensated

Where necessary, adjust compensation to align the Y-axis median fluorescence (relative to the other five colors) between the lower left and lower right quadrants.

Figure C.15 Single-stain Sample APC Compensated

Run the fifth single-stain sample.

Figure C.16 Single-stain Sample Cascade Yellow Uncompensated

Where necessary, adjust compensation to align the Y-axis median fluorescence (relative to the other five colors) between the lower left and lower right quadrants.

Figure C.17 Single-stain Sample Cascade Yellow Compensated

Run the sixth single-stain sample.

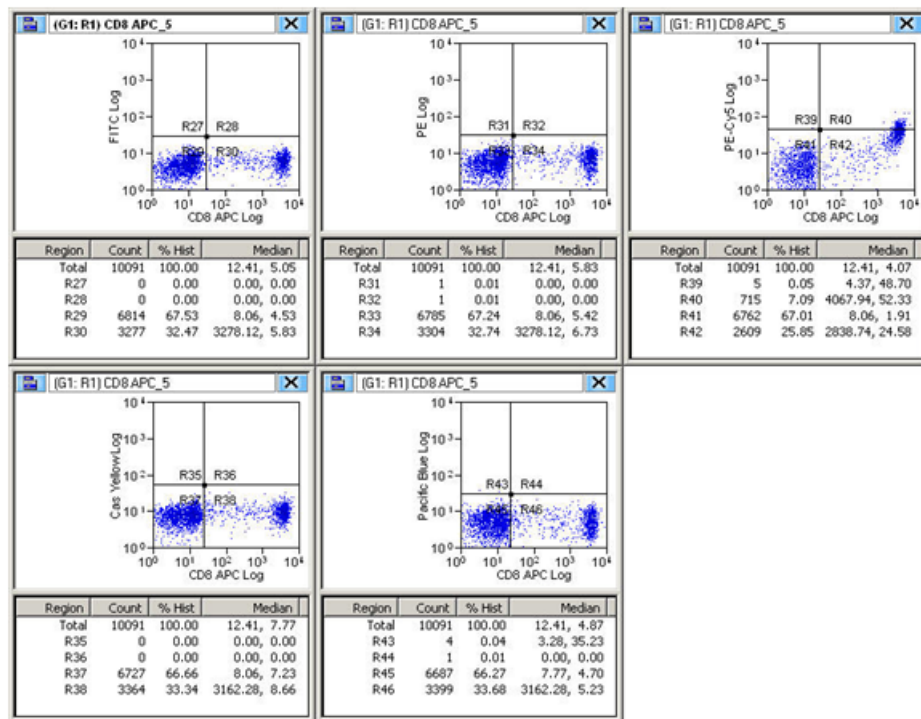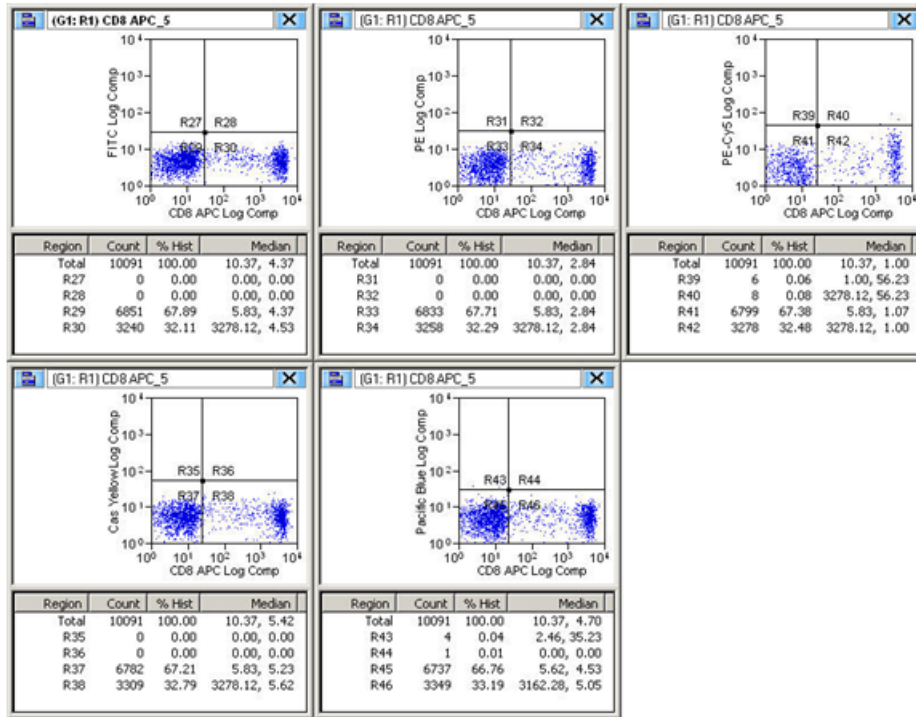**Figure C.18** Single-stain Sample Pacific Blue Uncompensated
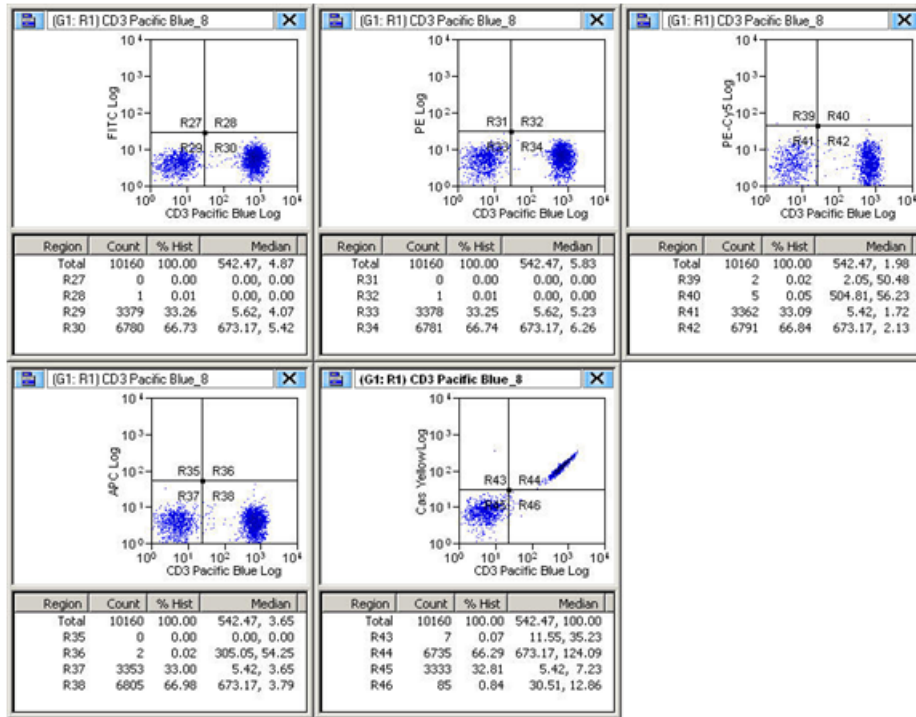
Where necessary, adjust compensation to align the Y-axis median fluorescence (relative to the other five colors) between the lower left and lower right quadrants.
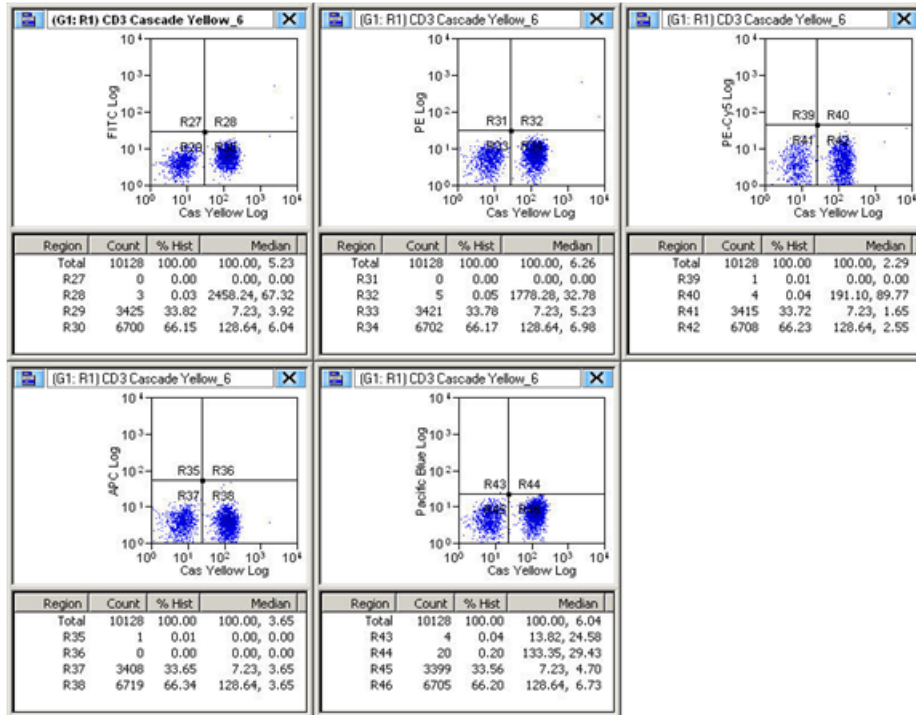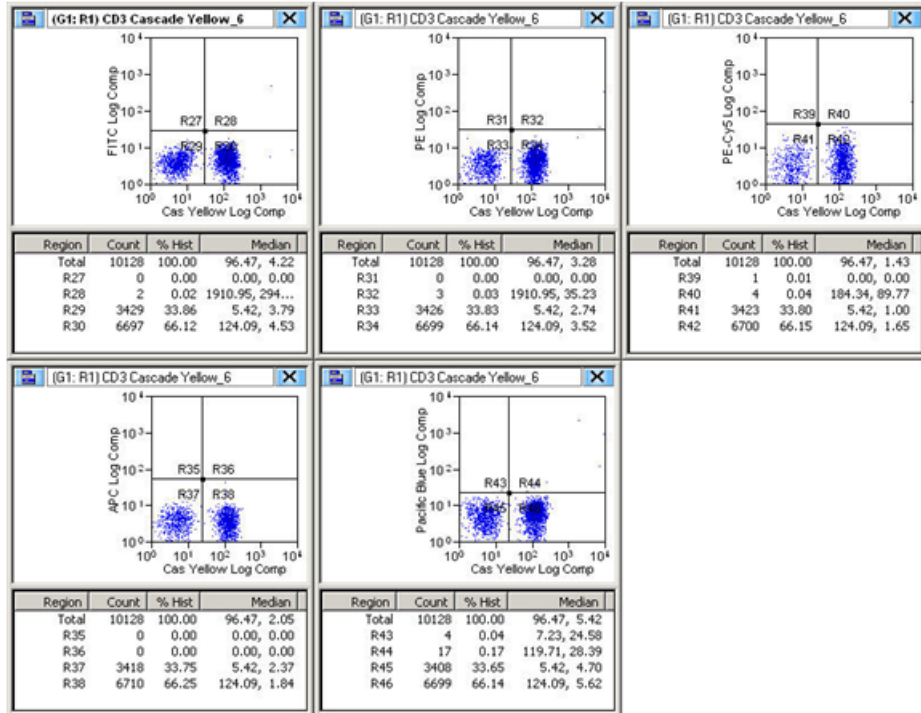
Figure C.19 Single-stain Sample Pacific Blue Compensated
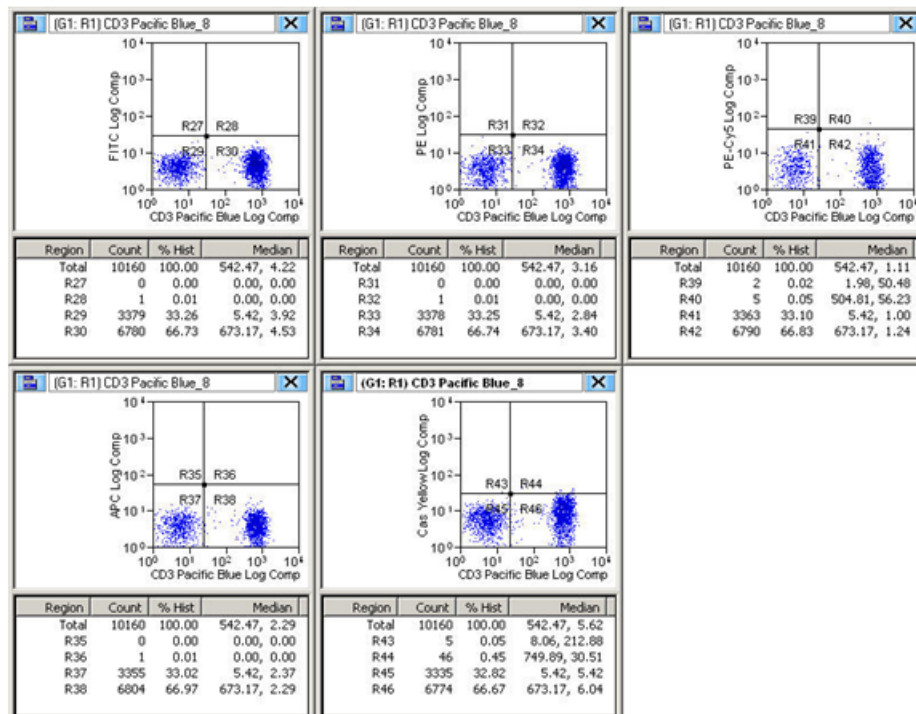
Now the spillover percentage is defined for all the combinations of colors and the multicolored sample can be analyzed.

This experiment has demonstrated offline compensation; however, compensation can be applied during acquisition as well.

Running the All-stains Sample

Finally, run the sample tube that contains all of the stains. This sample can be analyzed using dot plots containing any combination of the six parameters: FITC, PE, PE-Cy5, APC, Cascade Yellow and Pacific Blue. If compensation has been properly set, the populations will be aligned so that the positive and negative populations can be easily identified.

When performing Lymphocyte sub set analysis, it is preferable to use CD45 vs. SSC to locate the lymphocyte population than FSC vs. SSC. CD45 vs. SSC gating allows for the determination of a more precise lymphocyte population by excluding other contaminating cell populations from the gate. The plots shown below illustrate this. If the lymphocyte gate is set using FSC vs. SSC it is likely to include some debris (Blue), basophils (Green) and some monocytes (Gray). These contaminating populations can be easily identified and excluded using a CD45 vs. SSC plot.

Figure C.20 All-stains Sample
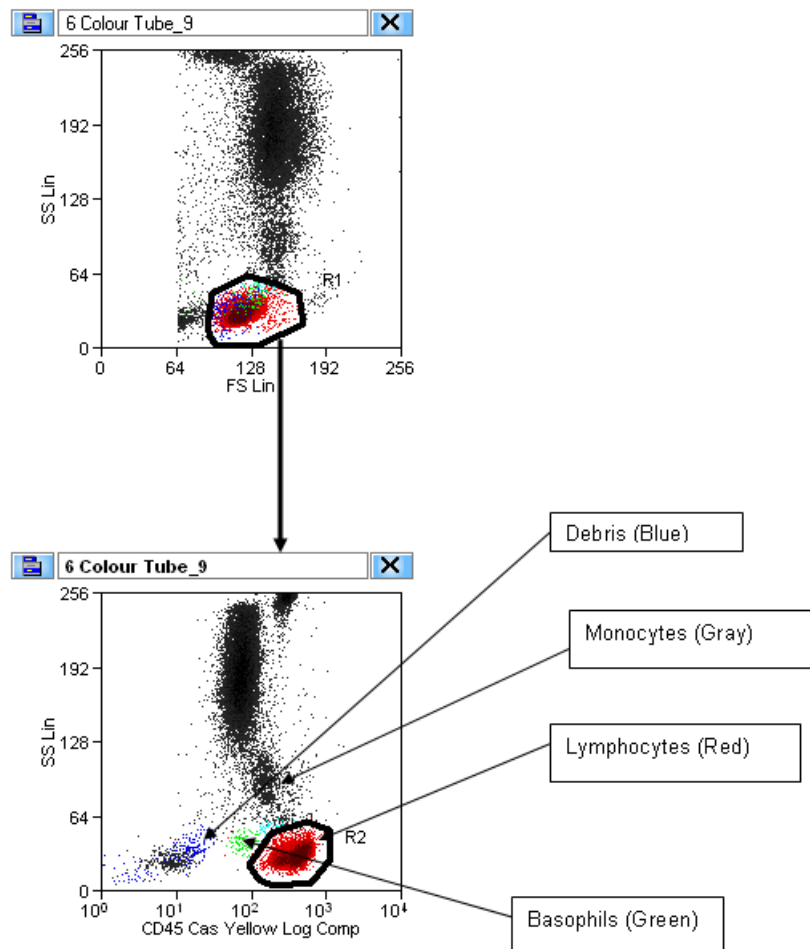
Create a plot showing CD45 (Cascade Yellow) vs. SSC and draw a region enclosing the lymphocyte population that has low SSC and bright CD45 staining, taking care to exclude the basophils and monocytes. Apply this gate to the rest of the plots.

Putting Compensation Into Practice

This tutorial has demonstrated how to perform a six-color experiment involving compensation. However, after running the all-stains sample, how does one make sense of and use the compensated data from the all-stains sample? The following histograms illustrate the different cell populations that can be identified, analyzed, and/or sorted based on the six-color experiment. For each of the cell populations below, identification is based upon the presence or absence of specific cell surface markers. To recall which marker correlates to which fluorochrome; refer to the original tube setup table Table C.1.

CD3 and T-Lymphocytes

Create a dot plot showing compensated CD3 Pacific Blue vs. compensated CD20 PE-Cy5 gated through the lymphocyte gate R1.

The CD3+ T-Lymphocyte population appears in the lower right hand quadrant (R42 in the example shown). So in this case the CD3+ T-Lymphocyte population constitutes 69% of the total lymphocyte population.

Figure C.21 Compensated CD3 Pacific Blue vs. Compensated CD20 PE-Cy5 Gated Through R1
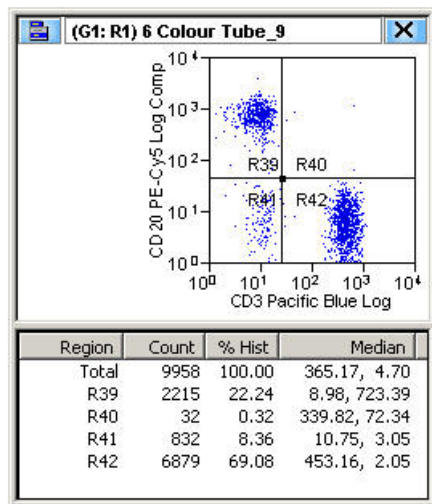
CD20 and B-Lymphocytes

Using the compensated CD3 vs. compensated CD20 plot, the CD20+ B-Lymphocytes are found in the upper left quadrant (R39).

CD4+/CD3+ T-Lymphocytes (T-Helper cells)

Create a dot plot showing compensated CD3 Pacific Blue vs. compensated CD4 FITC.

The CD4+/CD3+ T-Lymphocytes are found in the upper right quadrant (R44). Events found in the upper left quadrant (R43) that are CD4 dim and CD3 negative are contaminating monocytes in the lymphocyte gate.

Figure C.22 Compensated CD3 Pacific Blue vs. Compensated CD4 FITC
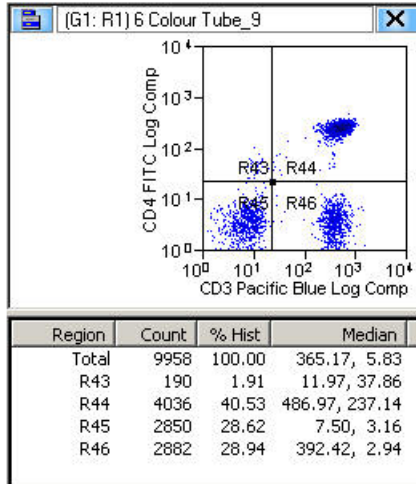
CD8+/CD3+ T-Lymphocytes (T-Cytotoxic/Suppressor cells)

Create a dot plot showing compensated CD3 Pacific Blue vs. compensated CD8 APC.

The CD8+/CD3+ T-Lymphocytes are found in the upper right quadrant (R36). Events found in the upper left quadrant (R35) that are CD8dim and CD3 negative are sub set of Natural Killer lymphocytes.

Figure C.23 Compensated CD3 Pacific Blue vs. Compensated CD8 APC
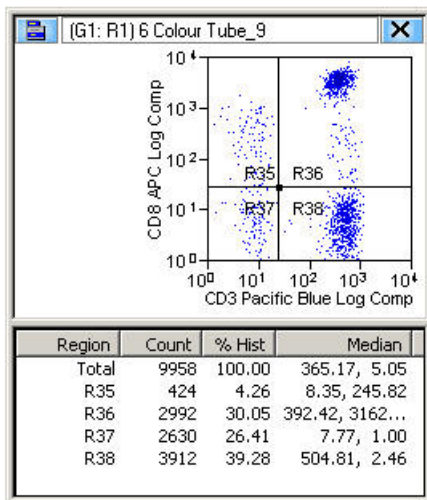
Natural Killer Lymphocytes

Create a dot plot of compensated CD3 Pacific Blue vs. compensated CD56 PE.

Natural Killer lymphocytes are found in the upper left quadrant (R31). Occasionally a small population of T-Lymphocytes can be seen which also express CD56. These "Natural killer like" lymphocytes can be distinguished from "true" Natural Killer lymphocytes because they are also positive for CD3. (See upper right quadrant R32).

Figure C.24 Compensated CD3 Pacific Blue vs. Compensated CD56 PE
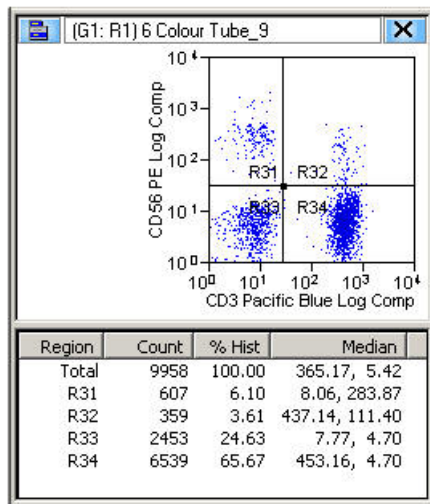
Laser Delay

Setting Laser Delay

IMPORTANT This procedure assumes that the CyAn ADP has been installed and aligned by a Beckman Coulter Service representative and the laser beams are already directed correctly to the pinholes. This procedure also assumes that the CyAn ADP is triggering Forward Scatter off of the default laser, which is typically the 488 nm.

When the CyAn ADP detects a triggering event from the 488 nm (blue) laser path it allows an interval of time to pass and then looks for events from the 405 nm (violet) laser path. Another interval is allowed to pass and then the CyAn looks for events from the 635 nm (red) laser path. The interval between detection points is referred to as Laser Delay.

You will need to reset laser delay after you adjust and align CyAn ADP lasers.

1 Perform the Startup procedure, CHAPTER 4, *How to Startup CyAn ADP*, and the alignment procedure CHAPTER 5, *How to Run a General Instrument Alignment Protocol*.

2 Create area histograms for at least one parameter on each laser line.

3 Set cycle mode to 100 events, and ensure that all signals are appear in the 128 median on the histograms.

4 Press CTRL , ALT , DELETE to display the Laser Delay window.

Figure D.1 Laser Delay Adjustment Window
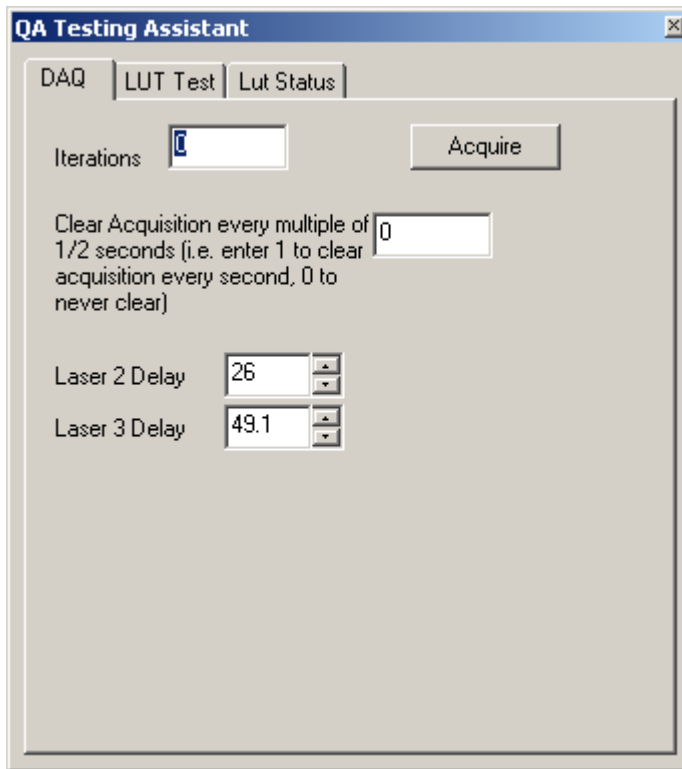
5 Click the *Laser Delay 2* field to activate it. Ensure that sample is running at 100 -120 EPS in cycle mode and that data appears in the histograms.

6 View the histograms that pertain to the secondary laser (usually the violet laser) while adjusting the Laser Delay value.

> **NOTE** Using the scroll button on the mouse is the most efficient way to adjust the value.

Figure D.2 Secondary Laser Signal Prior to Laser Delay Adjustment
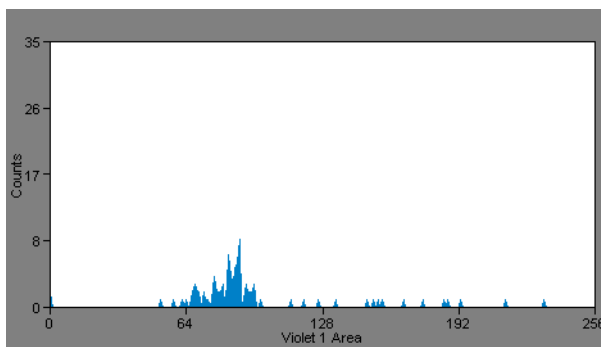
7 Maximize the signal in the 128 median to appear as high and tight as possible. See Figure D.3.

Figure D.3 Maximized Signal for Secondary Laser

---
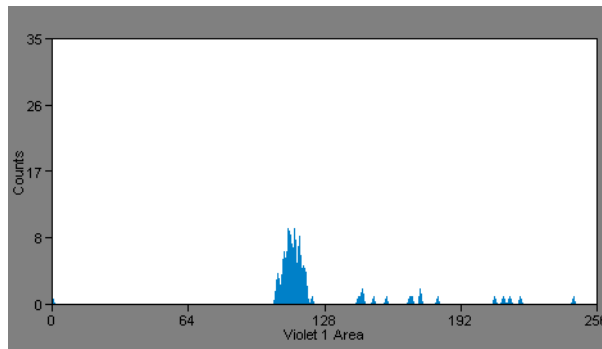
8 Click the *Laser Delay 3* field to activate it.

---

9 View the histograms that pertain to the tertiary laser (usually the red laser) while adjusting the Laser Delay value.

Figure D.4 Tertiary Laser Signal Prior to Laser Delay Adjustment

---
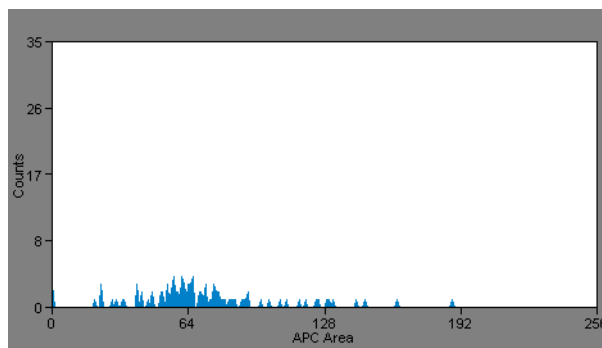
10 Maximize the signal in the 128 median to be as high and tight as possible. See Figure D.5.

Figure D.5 Maximized Signal for Tertiary Laser
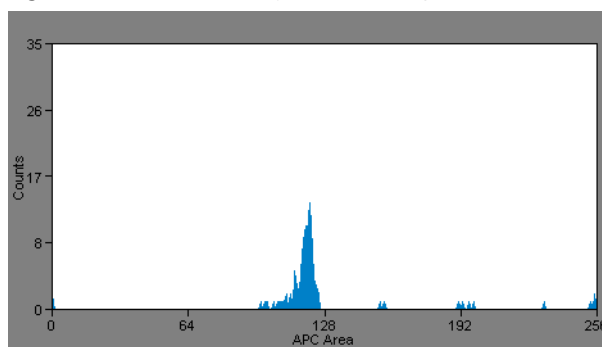
11 Close the Laser Delay Adjustment window.

APPENDIX E

Symbols

Symbol Definitions

Table E.1 Symbol Definitions

Symbol	Definition
SN	Instrument Serial Number
REF	Beckman Coulter Model Number
	Date of Manufacture
~	Alternating Current Input
	Fuse
⚠	Caution, Consult Accompanying Documents

Table E.1 Symbol Definitions *(Continued)*

Symbol	Definition
	Identification of Manufacturer
	Correct Disposal of this Product (According to Directive 2002/96/EC on Waste Electrical and Electronic Equipment [WEEE] applicable in the European Union and other European countries with separate collection systems.)
	Contact a Beckman Coulter Representative for disposal of the equipment at the end of its working life. This product should not be mixed with other commercial waste for disposal.

Abbreviations

The following list is a composite of the symbols, abbreviations, acronyms, and reference designators either used in this manual or related to the information in it. When the same abbreviation (or reference designator) is used for more than one word (or type of component), all meanings relevant to this manual are included, separated by semicolons.

° — degree (as in angle or temperature)

> — greater than

< — less than

≥ — greater than or equal to

≤ — less than or equal to

— number, as in number of events

& — and

~ — approximately

% — percent

+ — plus

- — minus

± — plus or minus

°C — degrees Celsius

°F — degrees Fahrenheit

® — registered trademark

™ — trademark

μ — micron

μL — microliter

μm — micrometer

μs — microsecond

A — ampere; linear area (regarding data type parameter selection

ADC — analog-to-digital conversion

ANSI — American National Standards Institute

baud — bits per second

BIOS — basic input/output system

BSL — biosafety level

BSO — beam shaping optics

C — centigrade

CD-ROM — compact disk - read only memory

CFR — Code of Federal Regulations

cm — centimeter

COM — serial communications port

CPU — Central Processing Unit

CV — coefficient of variation

DAC — digital-to-analog converter

dB — decibels

dc — direct current

DLP — dichroic long pass

DSP — dichroic short pass

DVM — digital volt meter

EC — European community

EMI — electromagnetic interference

EN — standard governing the safety of laser products in Europe

EPS — events per second

ESD — electrostatic discharge

F — fahrenheit; function key

FCC — Federal Communications Commission

FCS — flow cytometry standard

FITC — fluorescein isothiocyanate

FL — fluorescent light; fluorescent light signal

FSC — forward scatter

ft — foot; feet

g — grams

G — gate

GA — gauge

GB — gigabytes

gal. — gallon

GND — ground

H — linear height (regarding data type parameter selection)

Hz — Hertz

i.d. — internal diameter

IEC — standard governing the safety of laser products internationally

IECS — Integrated Environmental Control System

in. — inches

I/O — input/output

IT — information technology

IVD — In Vitro Diagnostics

K — 1000

kg — kilogram

kPa — kilopascal

L — liter; left; log height (regarding data type parameter selection)

LA — log area (regarding data type parameter selection)

lb — pound

LCD — liquid crystal display

LED — light emitting diode

M — mega

m — meter

max — maximum

MB — megabyte

mbar — mean bar

MHz — megahertz

min — minimum

mL — milliliter

mm — millimeter

ms — millisecond

N/A — not applicable

nm — nanometer

no. — number

ns — nanosecond

OS — operating system

PC — personal computer

PCB — printed circuit board

PE — phycoerythrin

PI — principal investigator

plo — file extension for protocol files

PMI — preventative maintenance inspection

PMT — photomultiplier tube

ppm — parts per million

psi — pounds per square inch

QC — quality control

R — region; right

RAM — random access memory

RG — regulator

RH — relative humidity

ROM — read only memory

RPE — phycoerythrin

RUO — Research Use Only (Not for Use in Diagnostic Procedures)

SS — side scatter

SD — standard deviation

sec — second

UL — Underwriter's Laboratory

UV — ultraviolet

V — volts

Vac — volts alternating current

vac — vacuum

Vdc — volts direct current

W — watt; pulse width (regarding data type parameter selection)

WEEE — Waste Electrical and Electronic Equipment

References

Researchers around the world and across many scientific disciplines rely on the CyAn ADP High-Performance Flow Cytometer. A partial list of peer-reviewed journal articles highlighting some of their science on the CyAn ADP instrument follows.

Bacteria and other microorganisms
With superior resolution for small particles, the CyAn ADP High-Performance Analyzer offers a fast, versatile, high-resolution platform for evaluating and manipulating bacteria, viruses, fungi, protists, and other microorganisms.

University of Georgia — Martin, D.L. and R.L. Tarleton. 2005. Antigen-specific T cells maintain an effector memory phenotype during persistent *Trypanosoma cruzi* infection. *J Immunol 174(3): 1594-1601.*

Colorado State University — Bosio, C.M., A. Goodyear, and S. Dow. 2005. Early interaction of *Yersinia Pestis* with APCs in the lung. *J Immunol 175(10): 6750-6756.*

Cancer
Although cancer, is a complex disease process involving genetic, environmental and behavioral factors, and can stem from a vast range of cell types, it displays the common characteristic of uncontrolled cell division resulting in abnormal tissue growth. By enabling researchers to obtain specific cell cycle, phenotypic, and functional information about the mechanism in which cells become malignant and the body's response, the CyAn ADP High-Performance Analyzer ultimately contributes to the development of cancer treatments and novel vaccines.

ARNAS Civico-Benfratelli, Palermo — Gervasi, F., R. Lo Verso, C. Giambanco, G. Cardenale, C. Tomaselli, G. Pagnucco.2004. Flow cytometric immunophenotyping analysis of patterns of antigen expression in non-Hodgkin's B cell lymphoma in samples obtained from different anatomic sites. *Ann NY Academy Sci 1028(1): 457-462.*

Northwestern University Medical School — Goolsby, C., M. Paniagua, M. Tallman, R.B. Gartenhaus. 2005. Bcl-2 regulatory pathway is functional in chronic lymphocytic leukemia. *Cytometry Part B: Clin Cytometry. 63B(1): 36-46.*

Jacobberger, JW, R.M. Sramkoski, P.S. Frisa, P.P. Ye, M.A. Gottlieb, D.W. Hedley, T.V. Shankey, B.L. Smith, M. Paniagua, C.L. Goolsby. 2003. Immunoreactivity of Stat5 phosphorylated on tyrosine as a cell-based measure of Bcr/Abl kinase activity. *Cytometry. 54A(2): 75-88.*

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Dendritic cells
Dendritic cells - the most potent antigen-presenting cells in the immune system - typically comprise less than two percent of lymphoid organs. The CyAn ADP High-Performance Analyzer rapidly characterizes this complex population.

Colorado State University — Perry, J.A., A. Rush, R.J. Wilson, C.S. Olver, A.C. Avery. 2004. Dendritic cells from malaria-infected mice are fully functional APC. *J Immunol 172(1): 475-482.*

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Children's Hospital of Philadelphia — Wang, L., R. Han, I. Lee, A. Hancock, G. Xiong, M. Gunn and W. Hancock. 2005. Permanent survival of fully MHC-Mismatched islet allografts by targeting a single chemokine receptor pathway. *J. of Immunol 175(10): 6311-6318.*

Wistar Institute — Hensley, S., W. Giles-Davis, K. McCoy, W. Weninger, and H. Ertl. 2005. Dendritic cell maturation, but not CD8+ T Cell induction, is dependent on type I IGN signaling during vaccination with adenovirus vectors. *J. of Immunol 175(9): 6032-6041.*

Fluorescent proteins

In the rapidly evolving proteomics era, applications for fluorescent proteins, such as CFP, GFP, YFP and BFP, continue to grow. Whether as simple indicators of gene expression levels or as tools in fluorescence resonance energy transfer (FRET), the CyAn ADP High-Performance Analyzer is an ideal platform for detecting these proteins and isolating cells with desired expression patterns.

Trudeau Institute — Mayer, K. D., K. Mohrs, S.R. Crowe, L.L. Johnson, P. Rhyne, D.L. Woodland, and M. Mohrs. 2005. The functional heterogeneity of type 1 effector T cells in response to infection is related to the potential for IFN-gamma production. *J Immunol 174(12): 7732-7739.*

Mayer, K.D. Mohrs, K., S.R. Crowe, L.L. Johnson, P. Rhyne, D.L. Woodland, and M. Mohrs. 2005. The functional heterogeneity of type 1 effector T cells in response to infection is related to the potential for IFN-gamma production. *J Immunol. 2005. 174(12): 7732-7739.*

Kemp, R.A., T.J. Powell, D.W. Dwyer, R.W. Dutton. 2004. Cutting edge: Regulation of CD8+ T cell effector population size. *J. Immunol 173(5): 2923-2927.*

High-throughput screening

Interest is growing in the use of flow cytometers to screen cell- or bead-based combinatorial libraries. Increasingly, flow cytometric assays are used to detect molecules that bind to a target protein in vitro or exhibit a particular activity in a cell-based assay. Flow cytometry also enables screening of protein libraries expressed in cells or displayed on the surface of bacteria or beads. A flow cytometer, for instance, can detect modulation of a signal transduction pathway by a particular small molecule and identify proteins with a particular binding specificity, enzymatic activity, expression level and stability. The CyAn ADP High-Performance Analyzer is finding wide application in this arena.

Trudeau Institute — Mayer, K. D., K. Mohrs, et al. 2005. The functional heterogeneity of type 1 effector T cells in response to infection is related to the potential for IFN-gamma production. *J Immunol 174(12): 7732-7739.*

MHC multimer assays

Fluorescent major histocompatibility (MHC) class I- and class II-peptide multimers are finding increasing application in the study of antigen-specific T cells. The CyAn ADP High-Performance Analyzer allows rapid detection of these cells, which typically comprise less than one percent of peripheral blood mononuclear cells.

Memorial Sloan-Kettering Cancer Center — Dao, T., D. Guo, A. Ploss, A. Stolzer, C. Saylor, T.E. Boursalian, J.S. Im, D.B. Sant'Angelo. 2004. Development of CD1d-restricted NKT cells in the mouse thymus. Eur *J Immunol 34(12): 3542-3552.*

Technical University of Munich — Huster, K.M., V. Busch, M. Schiemann, K. Linkemann, K.M. Kerksiek, H. Wagner, and D.H. Busch. 2004. Selective expression of IL-7 receptor on memory T cells identifies early CD40L-dependent generation of distinct CD8+ memory T cell subsets. *PNAS 101(15): 5610-5615.*

University of Pittsburgh School of Medicine — Meidenbauer, N., T.K. Hoffmann, A.D. Donnenberg. 2003. Direct visualization of antigen-specific T cells using peptide-MHC-class I tetrameric complexes. *Methods 31(2): 160-171.*

Stem cells
Both hematopoietic and non-hematopoietic stem cells continue to garner increasing attention in laboratories around the world. Studies continue to define phenotypic markers, in vivo reconstitutional activity, and creation of model systems for stem cell development, multi-drug resistance (mdr) and use in genetic therapies. The CyAn ADP High-Performance Analyzer is ideal for identifying and isolating populations of these valuable cells from a variety of sample sources.

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Yuan, Y., H. Yu, M. Boyer, X. Song, S. Cao, H. Shen, and T. Cheng. 2005. Hematopoietic stem cells are not the direct target of spontaneous leukemic transformation in p18INK4C-Null reconstituted mice. *Cancer Research 66(1): 343-351.*

John Radcliffe Hospital — Sternberg, A., S. Killick, T. Littlewood, C. Hatton, A. Peniket, T. Seifl, S. Soneji, J. Leach, D. Bowen, C. Chapman, E. Massey, G. Standen, L. Robinson, B. Vadher, R. Kaczmarski, R. Janmohammed, K. Clipsham, A. Carr, and P. Vyas. 2005. Evidence for reduced B-cell progenitors in early (low-risk) myelodysplastic syndrome. *Blood 106(9): 2982-2991.*

Oxford University — North, T.E., T. Stacy, C.J. Matheny, N.A. Speck, M.F.T.R. de Bruijn. 2004. Runx1 is expressed in adult mouse hematopoietic stem cells and differentiating myeloid and lymphoid cells, but not in maturing erythroid cells. *Stem Cells 22: 158-168.*

University of York — Etheridge, S.L., G.J. Spencer, D.J. Heath, P.G. Genever. 2004. Expression Profiling and functional analysis of Wnt signaling mechanisms in mesenchymal stem cells. *Stem Cells 22(5): 849-860.*

T - and B-cells
Classic subjects for flow cytometric analysis, T and B lymphocytes play a central role in the function of the immune system. The CyAn ADP High-Performance Analyzer enables efficient, multiparametric identification, and isolation of the almost infinite number of subsets of these and other immune cell types, revealing the complexities of cellular and humoral immune response.

Memorial Sloan-Kettering Cancer Center — Dao, T., D. Guo, A. Ploss, A. Stolzer, C. Saylor, T.E. Boursalian, J.S. Im, and D.B. Sant'Angelo. 2004. Development of CD1d-restricted NKT cells in the mouse thymus. *Eur J Immunol 34(12): 3542-3552.*

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Mohrs, K, D. Harris, F. Lund, M. Mohrs. 2005. Systemic dissemination and persistence of Th2 and type 2 cells in response to infection with a strictly enteric nematode parasite. *J of Immunol 175 (8): 5306-5313.*

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Papasavvas, E., J. Sun, Q. Luo, E. Moore, B. Thiel, R. MacGregor, A. Minty, K. Mounzer, J. Kostman, and L. Montaner. 2005. IL-13 acutely augemtns HIV-specific and recall responses from HIV-1 infected subjects in vitro by modulating monocytes. *J. of immunol 175(8): 5532-5540.*

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Related Documents

- CyAn ADP Quick Start Guide
- Summit 4.3 Online Help

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