ZE5™ Cell Analyzer and Everest™ Software

User Guide

Version 1.1

Catalog Number

17002031
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17002033
17002034

BIO-RAD

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BIO-RAD

Bio-Rad Technical Support Department

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Safety and Regulatory Compliance

For safe operation of the ZE5™ Cell Analyzer, Bio-Rad strongly recommends that you follow the safety specifications listed in this section and throughout this guide.

Safety Warning Labels

Warning labels posted on the instrument and **WARNING** and **Caution** notes listed in this guide warn you about sources of injury or harm. Review the meaning of each safety warning label.

Table 1. Explanations of safety warning labels

Icon	Explanation
	**Shock hazard!** This symbol draws attention to a possible injury or danger to life if the associated directions are not followed correctly. Only qualified, trained technicians should carry out service work on electronic components, due to potential shock hazard. **Risque d'électrocution!** Ce symbole met en garde contre les risque possibles de blessure ou un danger pour la vie si les instructions fournies ne sont pas suivies correctement. Seuls des techniciens qualifiés et formés peuvent effectuer des réparations sur des composants électroniques en raison du risque d'électrocution.
	**Risk of danger!** This symbol identifies components that pose a risk of personal injury or damage to the instrument if the associated directions are not followed correctly. Wherever this symbol appears, consult this guide for further information before proceeding. **Risque de danger!** Ce symbole identifie les composants qui présentent un risque de blessures ou de dommages pour l'instrument si les instructions ne sont pas suivies correctement. Lorsque ce symbole apparaît, consultez ce guide pour plus d'informations avant de continuer.
	**Laser hazard!** This symbol draws attention to a possible injury or danger to life due to laser radiation if the associated directions are not followed correctly. Do not remove system covers, which are in place for your safety. Only qualified, trained technicians should access exposed laser beams. **Risque laser!** Ce symbole attire l'attention sur un risque potentiel de blessure ou de danger pour la vie dû au rayonnement laser lorsque les instructions fournies ne sont pas suivies correctement. Ne retirez pas les caches de protection qui sont en place pour votre sécurité. Seuls des techniciens qualifiés et formés doivent accéder aux rayons laser exposés.

Table 1. Explanations of safety warning labels, continued

Icon	Explanation
	**Biohazard!** Biosafety is of utmost importance while operating this instrument. This symbol identifies components that may become contaminated with biohazardous material. When handling biohazardous samples or the ZE5 Cell Analyzer's waste container, adhere to the recommended precautions and guidelines in this guide, and comply with any local guidelines specific to your laboratory and location. **Risque biologique!** La biosécurité est d'une importance capitale lors de l'utilisation de cet instrument. Ce symbole identifie les composants qui peuvent être contaminés par des matières à risque biologique. Lorsque vous manipulez des échantillons biologiques ou le conteneur de déchets de l'analyseur de cellules ZE5, respectez les précautions et directives fournies dans ce guide et respectez les directives locales spécifiques à votre laboratoire et à votre site.
	**Consult documentation!** This symbol identifies components for which operating instructions must be followed to ensure safe and correct use. Wherever this symbol appears, consult this guide for information before using the instrument component. **Consultez la documentation!** Ce symbole identifie les composants pour lesquels les instructions d'utilisation doivent être suivies pour assurer une utilisation sûre et correcte. Chaque fois que cet instrument apparaît, consultez ce guide pour information avant d'utiliser l'instrument.

Safe Use Specifications and Compliance

Laser Product Hazard Classification

The intent of the laser hazard classification is to identify hazards to users posed by the laser and to provide appropriate protective measures. The ZE5 Cell Analyzer is a Class 1 laser product that complies with 21 CFR 1040.10 and 1040.11, except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007 stating that operators are not exposed to harmful levels of laser radiation during normal operation, maintenance, and/or service. During times of repair and/or major service by a trained technician, laser safety controls for Class 3B lasers must be followed.

WARNING! Use of controls, adjustments, or procedures other than those specified herein may result in hazardous laser radiation exposure.

Electrical Safety Information and Classification

The ZE5 Cell Analyzer conforms to international regulations encompassing the accessibility of high voltages by the user (IEC61010-1). Use all protective housings and shields as specified in this guide. Further information about specific electrical hazards is listed in the hardware description.

AC Fuse Requirements

Remove power cord before replacing fuses. Fuses are 5 x 20 mm and must be rated to AC250V, 4 A slow blow such as Schurter 0034.3123.

AC Power Cord Requirements

Power cord must be IEC 60320-1 compliant with a C13 plug on the instrument end. If the power cord must be replaced, replace it with only an adequately rated cord.

Position the instrument for easy access to the power switch and the power cord.

AC Outlet Requirements

Three outlets (instrument, monitor, computer), AC100–240V, 50–60 Hz, <500 W.

Regulatory Compliance

This instrument has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- IEC 61010-1:2010 (3rd Ed), EN61010-1:2010 (3rd Ed). Electrical Equipment for Measurement, Control, and Laboratory Use - Part 1: General Requirements
- UL/CSA 61010-1:2012 (3rd Ed), Standard for Safety Electrical Equipment for Electrical Safety (USA, Canada, NRTL)
- IEC 61010-2-081:2015, EN61010-2-081:2015. Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes (includes Amendment 1)
- IEC 60825-1:2014, EN 60825-1:2014. Safety of laser products - Part 1: Equipment classification and requirements
- Class 1 laser product per IEC 60825-1 and CDRH requirements and regulations
- EN 61326-1:2013 (Class A) Electrical equipment for measurement, control and laboratory use. EMC requirements, Part 1: General requirements
- IEC 61326-1:2012 (Class A) Electrical equipment for measurement, control and laboratory use. EMC requirements - Part 1: General requirements
- FCC Part 15 Subpart B Emissions (Class A)
- This ISM device complies with Canadian ICES-001
- Cet appareil ISM est conforme a la norme NMB-001

This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at their own expense.

Hazards

The ZE5 Cell Analyzer is designed to operate safely when used in the manner prescribed by the manufacturer. If the ZE5 Cell Analyzer or any associated component is used in a manner other than prescribed, or if modifications to the instrument are not performed by a Bio-Rad or other authorized agent, then the warranty on the system will be voided and the protection provided by the equipment might be impaired. Service of the ZE5 Cell Analyzer should be performed only by Bio-Rad personnel.

Biohazards

The ZE5 Cell Analyzer is a laboratory product. However, if biohazardous samples are present, adhere to the following guidelines and comply with any local guidelines specific to your laboratory and location.

General Precautions

- Always wear laboratory gloves, coats, and safety glasses with side shields or goggles.
- Keep your hands away from your mouth, nose, and eyes.
- Completely protect any cut or abrasion before working with potentially infectious materials.
- Wash your hands thoroughly with soap and water after working with any potentially infectious material before leaving the laboratory.
- Remove wristwatches and jewelry before working at the bench.
- Store all infectious or potentially infectious material in unbreakable leak-proof containers.
- Before leaving the laboratory, remove protective clothing.
- Do not use a gloved hand to write, answer the telephone, turn on a light switch, or touch anything that other people may touch without gloves.
- Change gloves frequently. Remove gloves immediately when they are visibly contaminated.
- Do not expose materials that cannot be properly decontaminated to potentially infectious material.
- Upon completion of the operation involving biohazardous material, decontaminate the work area with an appropriate disinfectant (for example, a 1:10 dilution of household bleach).

Disposal of Biohazardous Material

The ZE5 Cell Analyzer includes a waste container that may potentially contain hazardous biological materials, depending on the sample used. Dispose of the following potentially contaminated materials in accordance with laboratory, local, regional, and national regulations:

- Content in waste container
- Reagents
- Used reaction vessels or other consumables that may be contaminated

Chemical Hazards

The ZE5 Cell Analyzer includes a waste container that may potentially contain hazardous chemical materials, depending on the sample used.

Explosive or Flammability Hazards

The ZE5 Cell Analyzer system poses no uncommon hazard related to flammability or explosion when used in a proper manner as specified by Bio-Rad Laboratories.

Electrical Hazards

The ZE5 Cell Analyzer poses no uncommon electrical hazard to operators when installed and operated properly without physical modification and if connected to a power source of proper specification.

Transport

Moving the ZE5 Cell Analyzer is not recommended after installation. If the system must be moved, follow the decontamination procedure in this guide and remove all bulk fluidics. After a move, you must run the QC process to ensure that the instrument is functioning properly.

Caution: Lift the instrument with the inset handles on the base. To reduce the risk of personal injury or damage to the instrument, a minimum of two people must perform this task. Use caution to keep the instrument level, and handle the instrument gently.

Operating Conditions

The ZE5 Cell Analyzer must be operated under the following conditions:

- Temperature range 18–25°C
- Relative humidity 20–60%

Disposal

The ZE5 Cell Analyzer contains electronic or electrical materials; they should be disposed of as unsorted waste and must be collected separately, according to European Union Directive 2002/96/CE on waste and electronic equipment — WEEE Directive. Before disposal, contact your local Bio-Rad representative for country-specific instructions.

Warranty

The ZE5 Cell Analyzer and associated accessories are covered by a standard Bio-Rad warranty. Contact your local Bio-Rad Laboratories office for details of the warranty.

Chapter 1 Introduction

The ZE5™ Cell Analyzer is a compact benchtop flow cytometer that can characterize and measure cells and their properties by streaming a single-cell suspension through up to five spatially separated laser beams at varying wavelengths. It can be configured to use up to 30 detectors (photomultiplier tubes), including forward and side scatter detectors and an optional second forward scatter detector.

The integrated high-throughput sample loader can easily handle your samples in tube racks and Microtiter plates up to 384 wells, and allows custom media configuration. The innovative ZE5-EYE profiles the instrument with ten distinct LED wavelengths to verify the optical filter configuration and track detection performance over time.

Everest™ Software provides unattended startup and quality control, automated fluorescence compensation, a fluorophore selection panel, and a run list design wizard. The ability to analyze files while acquiring new data saves time and streamlines your workflow.

Key features include:

- Integrated, programmable wash station to reduce sampling time and sample carryover
- Onboard calibration beads for rapid QC without user intervention
- Volumetric sample uptake for absolute counting without beads
- Ability to add reagents to samples for kinetic experiments
- Bidirectional flow for built-in high-pressure unclogging
- Ability to return unused sample to tube or well
- Hot-swappable bulk fluidics bottles for uninterrupted system use
- Stat tube position for the flexibility to interrupt a plate and run a single sample
- Sample loader with plate shaker agitation and temperature control
- Threshold plot showing all data seen by electronics, enabling confidence in setting proper threshold
- Daily QC reporting and trending
- Custom heat map display for quick summary of experimental results

System Components

The ZE5 Cell Analyzer system includes the following components:

- ZE5 Cell Analyzer Instrument (one of the following)
  - ☐ ZE5 Cell Analyzer, 5 laser 27 color (355/405/488/561/640)
  - ☐ ZE5 Cell Analyzer, 4 laser 24 color (405/488/561/640)
  - ☐ ZE5 Cell Analyzer, 3 laser 17 color (405/488/640)
  - ☐ ZE5 Cell Analyzer, 3 laser 17 color (488/561/640)
- ZE5 Computer System
  - ☐ ZE5 Cell Analyzer Computer with Network Adaptor
  - ☐ ZE5 Cell Analyzer Computer Monitor, 29 in, 2560 x 1080
  - ☐ ZE5 Cell Analyzer Wireless Keyboard and Mouse
- ZE5 Accessory Kit

  The Accessory Kit includes consumables, documentation, and accessories for initial instrument startup.

For information on consumables and accessories, see Appendix E, Ordering Information.

Installation Requirements

The ZE5 Cell Analyzer should be installed by a Bio-Rad service engineer to ensure proper instrument operation and calibration. If any items are missing or damaged, contact your local Bio-Rad office.

Choose an appropriate site for ZE5 Cell Analyzer installation, such as a sturdy bench or tabletop, away from any other instruments that might interfere electrically, or mechanically by causing vibration. The bench or tabletop must accommodate 275 lb (125 kg), the approximate weight of the instrument, computer, and monitor. The area should be free of excessive dust or moisture. Do not place the instrument near air conditioning or heating vents, strong electrical fields, magnetic fields, or equipment that can produce vibrations, for example, bench centrifuges.

Table 2. Space required

Instrument only (W x D x H)	74 x 69 x 66 cm 29 x 27 x 26 in
Instrument with computer and monitor (W x D* x H**)	153 x 74 x 121 cm 60 x 29 x 48 in

* Maintain a 15 cm (2 in) space between the back of the ZE5 Cell Analyzer and any vertical surface to allow for cable connections.

** An additional 55 cm (22 in) of height clearance is needed for service.

Administrator and User Rights

Everest Software is the main interface for controlling the ZE5 Cell Analyzer. The software features available to you depend on whether you log in as an administrator or a nonadministrator user. Bio-Rad recommends that at least two administrators be assigned per system. Administrator and user rights are shown in Table 3.

For more information on administrator privileges, see Managing Users on page 85 and Setting Global Preferences on page 90.

Table 3. Administrator and user rights

Feature	Administrator	User
Start up	✓	✓
Shut down	✓	✓
Run QC and ZE5-EYE processes	✓	✓
Generate daily QC reports	✓	✓
Generate QC trending and EYE trending reports	✓	
Acquire samples	✓	✓
Generate analysis reports	✓	✓
Clean probe and sample line	✓	✓
Decontaminate system	✓	✓
Edit QC criteria	✓	
Configure global instrument and software preferences	✓	
Create users	✓	
Deactivate users	✓	
Edit users	✓	
Change user rights	✓	
Reset other users' passwords	✓	

Table 3. Administrator and user rights, continued

Feature	Administrator	User
Generate user reports	✓	
Change own password	✓	✓

Other Documentation and Training Materials

More information about the ZE5 Cell Analyzer and Everest Software is available from the following sources:

- ZE5™ Cell Analyzer and Everest™ Software Release Notes
- ZE5™ Cell Analyzer and Everest™ Software Quick Start Guide

Training modules are also available in Everest Software to help you become familiar with basic system functionality. These modules run your default browser, but Internet access is not required.

To access training modules

1. Click the Help and Information menu button in the upper right corner.
2. Select Training Module.
3. In the Help and Training dialog box, click the link to the training module you would like to view.
   The training module opens in the browser.

To access this user guide from Everest Software

1. Click the Help and Information menu button in the upper right corner.
2. Select User Manual to open the PDF.

To access the latest product documentation

▶ Visit the ZE5 Cell Analyzer product page on the Bio-Rad website (www.bio-rad.com/ZE5).

Chapter 2 Hardware Description

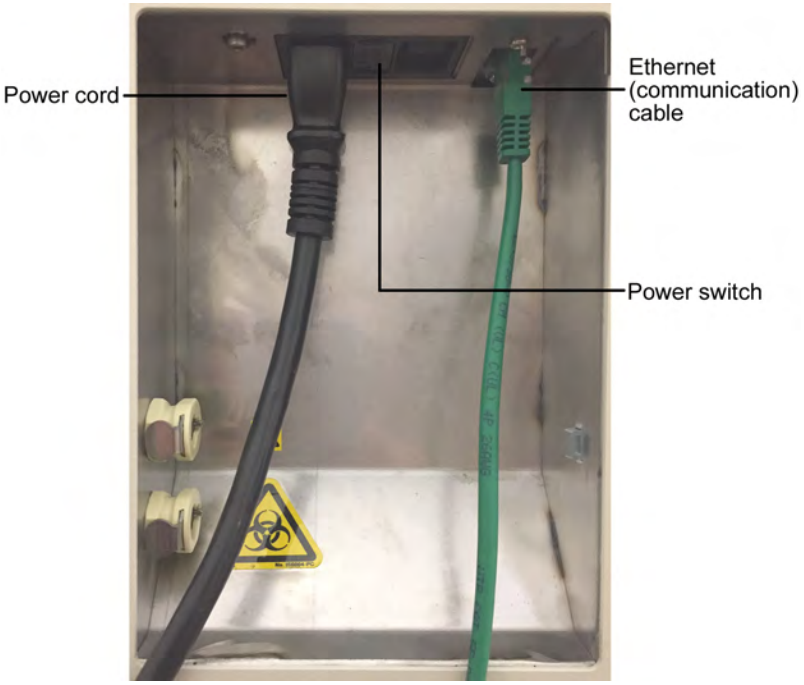
Read this section to understand the ZE5™ Cell Analyzer system hardware before operating the instrument.

System Overview

The ZE5 Cell Analyzer system consists of fluidics, optics, electronics, and software.

Power and Communication Connections

Power and communication connections are located on the back of the ZE5 Cell Analyzer.



The following features are located in this entry panel:

- Power cord (black) — IEC-compliant AC power cord.
- Ethernet cable (green) — connects the instrument to Ethernet port of the computer workstation. Used for data transfer and software control of the instrument.

  Caution: Be careful not to trip on the power cord or Ethernet cable when you walk around the instrument.

- Power switch — press the main power switch to turn on power to the system.

  Caution: Do not use the main power switch to shut down the system. Shut down the system using Everest™ Software. For more information, see Shutting Down on page 129.

This area of the instrument also contains optional connections for external DI water and external waste. For more information, see Appendix C, Optional External DI Water and Waste Ports.

Fluidics System

The ZE5 fluidics system consists of the bulk fluidics, sample loader, and flow cell. The system supplies sheath fluid and sample to the flow cell and collects the waste for proper disposal. Sheath fluid typically consists of DI water to which sheath additive is added; however, a saline solution can be used instead. Laminar sheath flow carries the sample core through the center of the flow cell, where the particles to be

measured are intercepted by laser beams. For detailed information about the hydrodynamic focusing that occurs in the flow cell, see Flow Cell on page 28.

Four large (4 L) and two small (450 ml) bottles are located in the instrument's bulk fluidics chamber. The two large bottles with blue caps contain DI (deionized) water; the two large bottles with red caps collect waste. Mounted below the four large bottles, the small bottle with the blue cap contains system cleaner, and the small bottle with the white cap contains sheath additive.

Caution: Biohazard! Maintain biosafety at all times while operating this instrument. Consult with your local safety officer or review local, state, and federal regulations to ensure proper handling and disposal of biohazardous substances.

The weight of each bottle is continuously monitored so the system can calculate the approximate remaining run time for each bottle. The system automatically switches between the two sets of sheath and waste bottles, and displays an alert when there is less than 1 hr of run time remaining.

Sheath Bottles

The two sheath bottles hold DI water for the system. This solution is also used for washing the sample probe between runs. These bottles have a blue cap. Each holds 4 L of fluid; together, they provide about 8 hr of continuous run time.

Note: When refilling these bottles, ensure that the tubing and filter are reinstalled so that they are in the lower corner of the bottle opposite the cap.

Sheath Additive Bottle

The sheath additive bottle, which has a white cap, contains 250 ml of concentrated balanced salts, antimicrobial/antifungal agent, and surfactant. This fluid is drip-fed into the internal sheath reservoir on a continuous basis while the system is running and helps prevent microbial buildup in the system. Microbes in the fluidic lines potentially increase background noise in the data, especially in the scatter channels, and can clog the lines or flow cell if the buildup becomes significant.

System Cleaner Bottle

The system cleaner bottle, which has a blue cap, contains a system cleaning reagent. During the shutdown process, cleaner is delivered to the portions of the fluidics system that are in contact with sample, such as the flow cell and sample line. This process minimizes sample buildup over time and preserves the integrity of the system.

For more information, see:

- Shutting Down on page 129
- Cleaning Solutions on page 260

Fluidics Filters

All of the onboard reagents are filtered through 0.2 µm capsule filters that remove particulates from the fluid before it is circulated through the system. This helps reduce background noise, especially in the scatter channels, and helps prevent microbes from entering a major portion of the fluidics path. These filters are mounted behind a panel on the right side of the instrument.

Important: Replace filter cartridges on a regular basis. Filter replacement is part of the annual Bio-Rad Service preventive maintenance visit. For additional information, see Chapter 12, Maintenance.

Waste Bottles

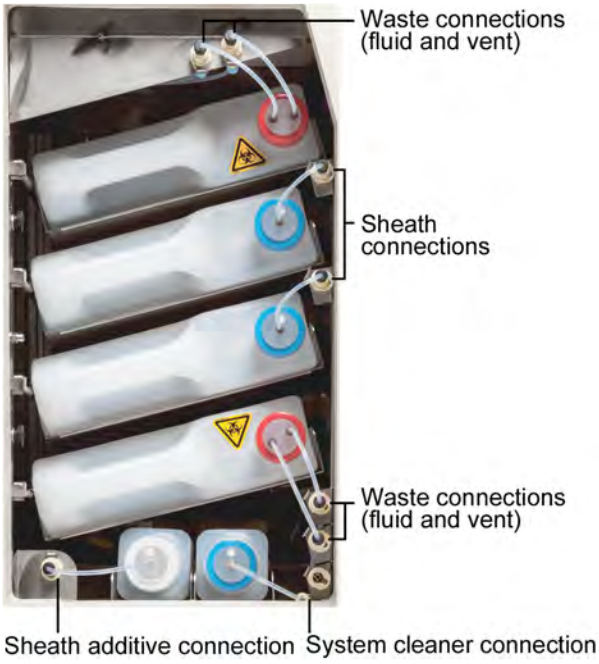
The waste bottles receive the system fluid after it has run through the flow cell and waste lines. The two waste bottles are distinguished by red caps and each hold 4 L; together, they provide about 8 hr of continuous run time. Decontaminate the fluid collected in the waste bottles in accordance with biohazard waste disposal guidelines.

Caution: Biohazard! Please contact your safety officer or local health and safety bodies regarding proper treatment and disposal of biohazardous waste.

You can add 400 ml of full-strength bleach to an empty waste bottle upon installation in the instrument for a final concentration of 10% in the full waste bottle. Alternatively, you can add full-strength bleach to a full waste bottle and allow it to sit to thoroughly decontaminate biohazardous material in the waste.

Fluidics Connections

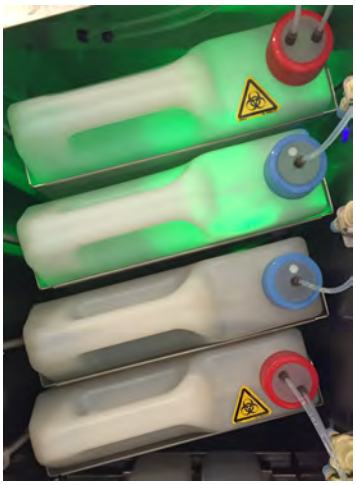
The ZE5 Cell Analyzer bulk fluidics chamber is located on the front of the instrument to the left of the loader. The fluidics chamber holds four large and two small bulk fluidics bottles. Connections to these bottles are depicted in the next figure.



Each waste bottle has two connections: one allows waste fluid to pass into the bottle, and the other allows air to flow out of the bottle when it is displaced by fluid. Each sheath (DI water) bottle has one connection to the instrument.

The connections for the Additive bottle and System Cleaner bottle are located at the bottom of the fluidics chamber next to each bottle.

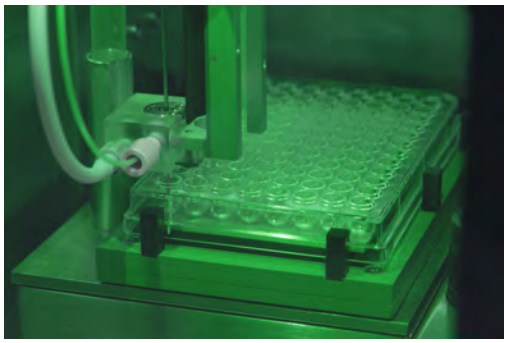
During system operation, the bottles illuminated in green are in use; unlit bottles are not in use and can be swapped. Examples are shown in the next figure.



For information about connecting the DI water and waste bottles to house DI water and waste, respectively, see Appendix C, Optional External DI Water and Waste Ports.

Sample Loader

The ZE5 Cell Analyzer features a sophisticated autoloader that can sample from a wide variety of media types, including racks of forty 5 ml tubes (12 x 75 mm), twenty-four 1.5 ml tubes, 96-well plates (deep-well or standard depth), and 384-well plates. A single sample position for a 5 ml tube is also provided for stat tube samples. Additionally, you can use Everest Software to set up custom sample input devices. The next figure shows the probe sampling a well of a 96-well plate.



A powerful agitator, modulated for each sample media type, is built into the autoloader and ensures that samples are adequately mixed. You can adjust the temperature range of the sample loading stage from 4–37ºC in 1ºC intervals to suit a variety of assay needs, for example, preserving cell viability.

Another feature of the ZE5 Cell Analyzer is the patented, custom-designed, dual-cam peristaltic sample pump that delivers sample to the interrogation points in the flow cell. Using Everest Software, you can adjust sample target flow rates within the range of 0.0025–3.5 µl/sec (0.15–250 µl/min). The pump can

also run in reverse to deposit sample back into a tube, to deposit reagent from another position into a sample position, or to clear blockages from the sample line.

An automated door slides up to provide access to the sample loading area. To open the door, briefly press the silver sample chamber button to the lower-right of the door. The button is shown in the next figure.

To illuminate the sample area, press and hold the silver sample chamber button or click the Sample Chamber Light button in the System Tools section of the Everest toolbar.

For more information about loading samples, see Chapter 3, ZE5 Loader.

Caution: In addition to the loader door, there are two doors (filter access and bulk fluidics access) that require access on a regular basis. Use caution when opening and closing these doors and mechanisms to avoid pinching.

Flow Cell

The flow cell is the heart of the system and is where sample interrogation occurs. It is composed of fused silica that surrounds a 145 x 265 µm channel through which sheath fluid flows and focuses the sample fluid.

Sample is drawn up into the sample line by the sample pump and is introduced into the flow cell through a sample introduction needle. Sheath fluid is introduced under pressure in an upward vertical flow at 10 psi (~8 m/sec). The faster flow of sheath fluid around the sample hydrodynamically focuses the sample into a narrow-diameter core stream. This allows cells to pass through at a high rate while maintaining the integrity of single-file particle flow through up to five spatially separated laser interrogation points. After cell interrogation, sheath fluid and sample exit the flow cell and are sent to a waste container.

Fluidics connections to the flow cell area are shown in the next figure.

The fluidics system allows sheath fluid flow within the flow cell to be reversed to help remove blockages. Cleaning fluid is introduced into the flow cell as part of the system shutdown process.

Optics

The ZE5 Cell Analyzer optics include lasers, mirrors, filters, lenses, and photomultiplier tubes (PMTs). The mirrors, filters, and lenses shape and guide the laser light to the interrogation points in the flow cell; they also focus and filter the laser light before it reaches the detectors. The PMTs detect scattered and fluorescent light signal.

Lasers

The ZE5 Cell Analyzer can be configured with a selection of up to five lasers from the Coherent OBIS line of lasers.

A typical configuration contains lasers of wavelengths and powers as follows:

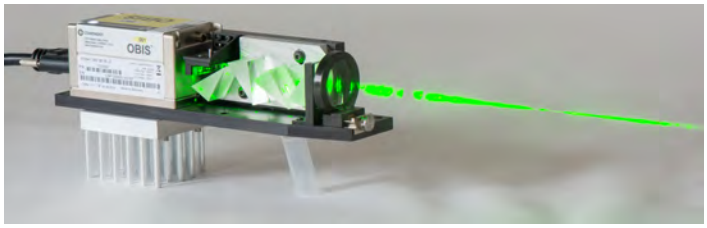
- 355 nm (UV) at 50 mW
- 405 nm (violet) at 100 mW
- 488 nm (blue) at 100 mW
- 561 nm (yellow green) at 50 mW
- 640 nm (red) at 100 mW

Caution: Laser irradiation can be hazardous. Do not remove system covers, which are in place for your safety. Only trained personnel should access the exposed laser beams.

The laser power and shutter can be controlled through Everest Software. This allows you to turn lasers on and off and to select different laser powers for different experiments.

Beam-Shaping Optics

Each laser includes an integrated beam shaping optic (BSO) assembly to ensure that each individual beam is delivered to the flow cell in the correct dimensions and focus. Consistent geometry between lasers optimizes illumination of each analyzed cell and maintains a high degree of measurement precision.



Interrogation

An interrogation point occurs where each laser beam intercepts the sample in the flow cell. The ZE5 Cell Analyzer system supports up to five spatially separated interrogation points along the core stream. Upon interrogation, the particles scatter the laser light and generate fluorescent signals.

Light Collection

Collected light can be categorized as either scattered or fluorescent. Scattered light is collected from two angles relative to the laser beam: immediately in front of the laser (forward) and at approximately 90° relative to the laser (side). Fluorescent light is collected only from the side.

Scattered light matches the wavelength of the laser light, which is deflected by the particles it encounters. Scattering depends on a particle's physical properties, such as size, shape, surface topography, and internal complexity.

Excitation by the laser light can cause particles to emit fluorescent light from three sources:

- added fluorochromes or dyes
- naturally occurring fluorescence
- biological structures such as mitochondria and lysosomes (autofluorescence)

Emitted fluorescent light is of lower energy (longer wavelength) than excitation light. Mirrors, optical filters, and lenses direct the fluorescent light to the detectors.

Forward Scatter

Laser light diffracted by particles in the forward direction (just off the axis of the laser beam) is collected to give an indication of relative differences in particle size. This forward-scattered light (FSC) is proportional to particle surface area or size. FSC can be used to distinguish debris from cells or other target particles; it can also be used to generate a doublet discrimination plot that distinguishes single particles from multiple particles passing through an interrogation point.

The ZE5 Cell Analyzer can include up to two FSC detectors. Both are highly sensitive PMTs with adjustable voltage.

The default FSC detector measures 488 nm light from 2–18° relative to the laser beam. It can resolve cells from debris and measure particles from 0.5–50 µm in diameter. Typical uses include generation of plots of lysed whole blood suspensions to resolve lymphocytes, monocytes, and granulocytes with high fidelity.

A second optional detector can be configured for small particle analysis or for measuring forward scatter generated by a different laser. The small particle option can resolve particles as small as 0.3 µm in diameter.

Each FSC detector can be associated with a mechanical, software-controlled 2.0 neutral density (ND) filter to alter the range of detection sensitivity. This filter can be enabled or disabled using the Everest PMT Control panel. See PMT and Laser Controls on page 208.

Side Scatter

Light scattered by particles at an angle of about 90° to the laser beams is collected to indicate relative differences in particle complexity (for example, granularity, membrane structure, and cytoplasmic constituents). More complex particles usually reflect and refract more light than less complex particles, which results in higher side scatter (SSC). The ZE5 Cell Analyzer is configured to collect SSC from the 488 nm laser.

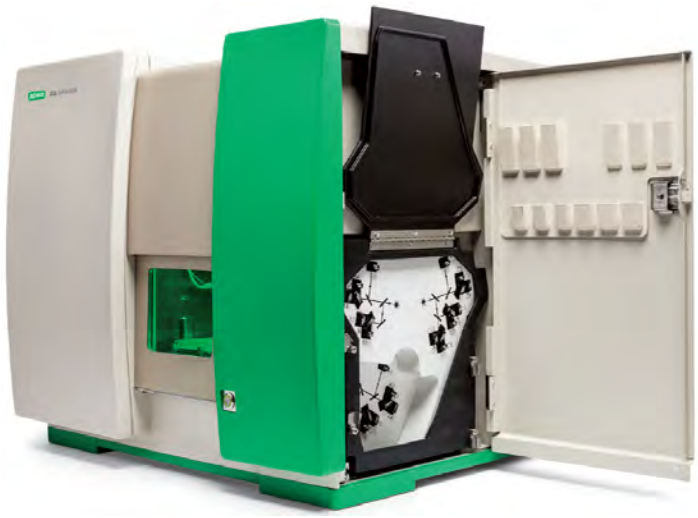
Optical Filters and Mirrors

The ZE5 Cell Analyzer's optical filters separate and direct fluorescent light to the PMTs for detection. The optical detection system is designed to be extraordinarily space-efficient and compact, allowing up to 27

fluorescence detectors to be installed. A unique feature of the optical system design is that each detector is an equal distance from the collection source, so that the system can collect every wavelength of light at the same focal distance. A single lens and steering mirror are used to optimize the collected light on all detectors, guaranteeing that the light efficiently reaches the final bandpass filter with no more than three interactions with dichroic filters.

Mirrors and filters permit multiparametric analysis. By partitioning the spectrum of collected light into specific ranges of wavelengths, each detector can be dedicated to the measurement of particular fluorophores.

The ZE5 Cell Analyzer's optical filters and detectors are arranged in banks dedicated to particular interrogation point/laser combinations, with one bank per laser. These banks are located behind the filter access door on the right side of the instrument. A black filter cover protects the optical detection system from ambient light. When lifted out of the way, the filter cover attaches to the instrument using a magnet, as shown in the next figure.



For more information about accessing filters, see Optical Filter Access Door on page 37.

Optical Mirror and Filter Types

Optical mirrors and filters are coated pieces of glass that are designed and configured in specific patterns to efficiently steer light to the correct detector. Mirrors direct all the light down the detection path and are not user changeable.

Longpass and shortpass filters can be dichroic or normal incidence. Dichroic filters reflect light that is not permitted to pass through them. They are typically placed at a 45-degree angle to the incident light and are used to direct light around the detection path.

Normal incidence longpass filters and bandpass filters typically absorb light that is not permitted to pass through them. They are placed directly in front of detectors to determine the specific range of wavelengths that the detector measures.

By analyzing the different detected bands of light, it is possible to examine multiple properties of each particle.

- Longpass filters allow light above a determined wavelength to pass through while reflecting the rest. For example, a 600 dichroic longpass (DLP) filter allows light with a wavelength longer than 600 nm to pass through, reflecting the rest.
- Shortpass filters allow light below a determined wavelength to pass through while reflecting the rest. For example, a 470 dichroic shortpass (DSP) filter allows light with a wavelength shorter than 470 nm to pass through, reflecting the rest.
- Bandpass filters allow light within a narrow wavelength range width to pass through while rejecting (attenuating) light of other wavelengths. For example, a 447/60 bandpass (BP) filter allows light from 417 nm to 477 nm through, absorbing the rest.
- Neutral density filters reduce or modify the intensity of all wavelengths of light equally by reflecting or absorbing a portion of it.

For experimental applications where events appear off scale when the detector is set at minimum gain, a neutral density filter can attenuate the signal and keep the events on scale. In the ZE5 Cell Analyzer, neutral density filters are used in front of the FSC detector. You also have the option to install neutral density filters in front of other detectors.

For information about installing neutral density filters in front of detectors other than the FSC detector, see Installing a Neutral Density Filter on page 113. For information about installing other filter types, see Replacing Optical Filters on page 105.

When replacing optical filters, note the following dimensions and tolerances.

Table 4. Optical filter sizes

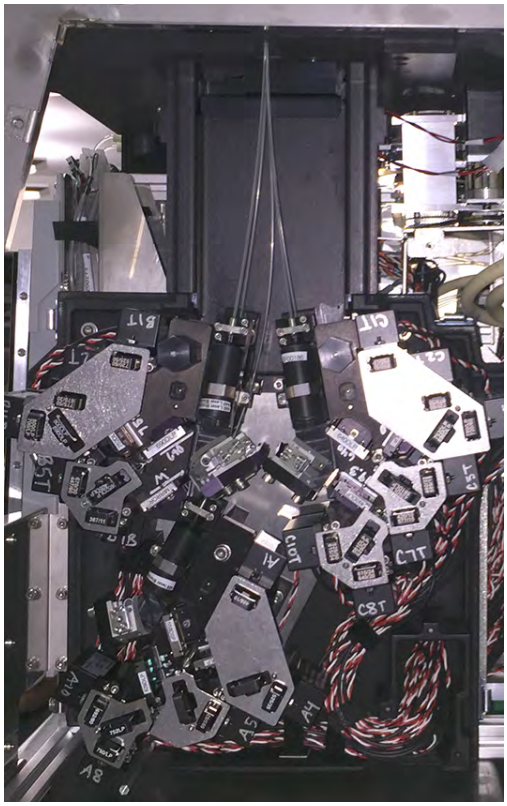
Filter type	Size (L x W x thickness)
Dichroic	17 x 15 x 1.05 mm
Bandpass	13 x 13 x 2 mm

Tolerance on length and width : +0.0 mm/–0.2 mm.

Tolerance on thickness : ±0.1 mm.

Optical Filter Banks

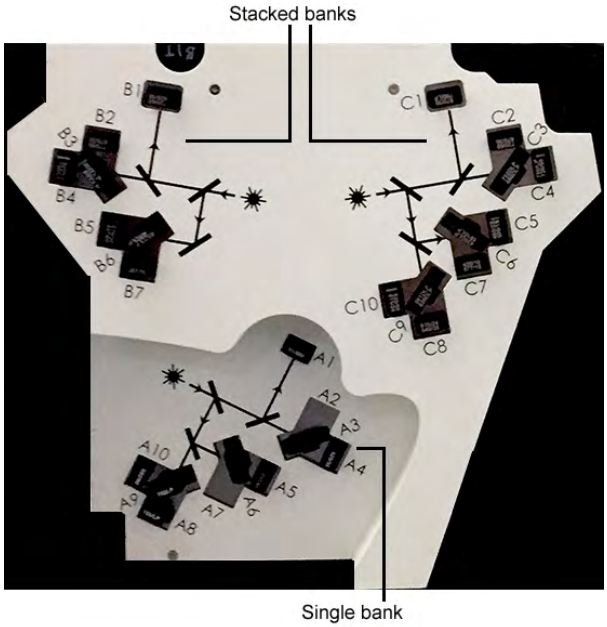
PMTs and associated mirrors and filters are organized into banks that are grouped by excitation source — each optical fiber directs light to a particular bank, as shown in the next figure.



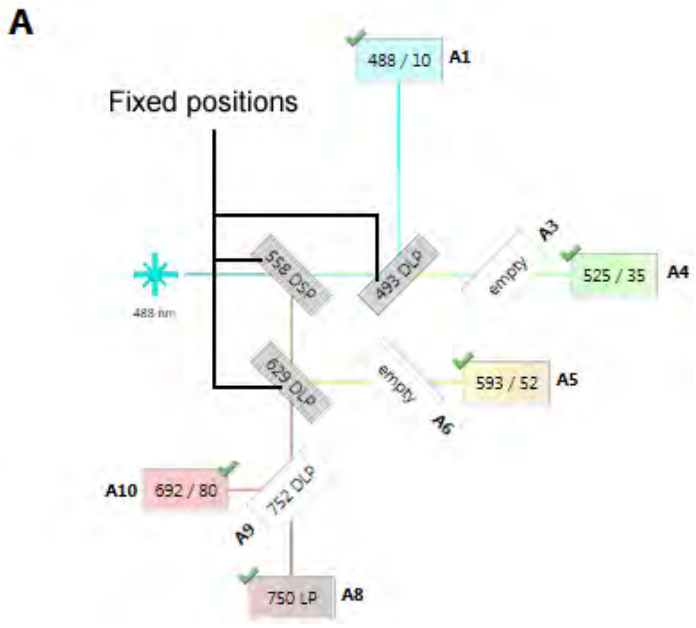
The ZE5 Cell Analyzer can be configured with up to five banks, one for each laser. The five banks are grouped into three regions: A, B, and C. Region A, located on the bottom, consists as a single bank, while regions B and C, located on the top left and top right, respectively, each contain two banks of PMTs.

Table 5. Optical filter banks

Bank	Laser	Total possible PMTs
A	Blue (488 nm)	7
B Top	Red (640 nm)	5
B Bottom	UV (355 nm)	5
C Top	Violet (405 nm)	7
C Bottom	Yellow Green (561 nm)	7



Optical mirrors and filters are configured in a combination of fixed and operator-changeable components. Each bank contains an initial array of fixed dichroic mirrors followed by replaceable dichroic mirrors and filters.

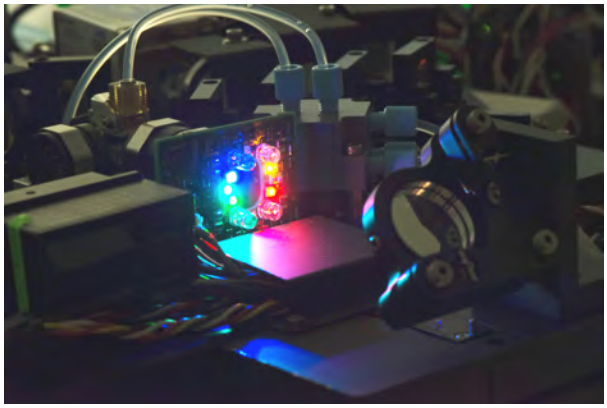


Filter holders contain either one or two mirrors/filters. Single filter holders are installed in region A, while double filter holders are installed in stacked banks (regions B and C). Examples of single and double filters are shown in the next figure.

For information about configuring filters, see Working with Optical Filter Configurations on page 97.

The ZE5-EYE

The ZE5-EYE is a hardware feature that verifies the configuration of the optical filter setup. Located in front of the detection paths, the ZE5-EYE uses multiple LEDs to pulse ten different wavelengths of light into the optical filters that lead to the detector banks. These LEDs are shown in the next figure.



The ZE5-EYE process is a component of the QC process and is run any time the QC process is initiated.

For more information, see Using the ZE5-EYE to Confirm Filter Choices on page 109.

Optical Filter Access Door

Extra optical filters can be stored in the slots in the filter access door. The filter door includes a sensor that communicates with Everest Software any time the door is opened for at least 5 sec.

The ZE5-EYE process runs in the background to check the detection paths any time the filter access door is opened and then closed. It notifies system operators if any filter has been changed, so that the filter configuration in the software can be updated accordingly.

For more information about the ZE5-EYE, see Using the ZE5-EYE to Confirm Filter Choices on page 109.

Photomultiplier Tubes

PMTs detect and amplify the scattered and fluorescent light signals produced by laser interrogation of the particles. Located behind the optical filters, the PMTs detect specific bands of fluorescent light based on the attached fluorochromes.

The ZE5 Cell Analyzer can be configured with a maximum of 30 PMTs that can be utilized simultaneously, including:

- Forward scatter (FSC) detector
- Optional second FSC detector
- Side scatter (SSC) detector
- 27 fluorescence detectors

Changing the voltage delivered to a PMT changes the PMT's signal amplification. Therefore, assays requiring a wide range of sensitivity can be carried out on the same instrument. The PMT voltages

optimized for a specific application are stored in the software within the experiment's run list for use when an assay is repeated. For more information, see PMT and Laser Controls on page 208.

Electronics

The ZE5 electronics process the PMT signals to deliver data to Everest Software for analysis.

WARNING! Shock hazard! Due to potential shock hazard, only qualified, trained technicians should carry out service work on electronic components.

Pre-Amplifiers

Pre-amplifiers boost the signals coming from the PMTs.

Analog-to-Digital Converters

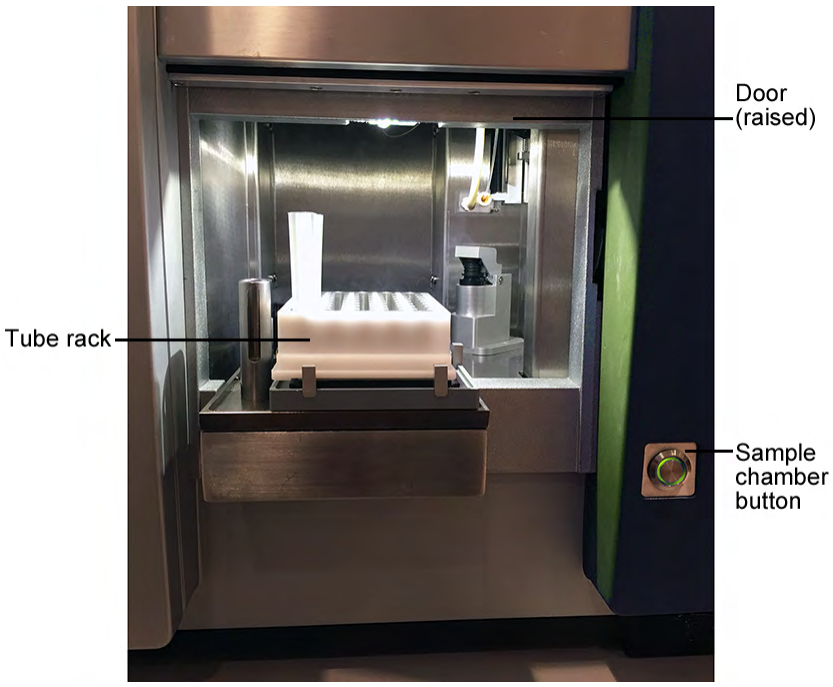
Analog-to-digital converters (ADCs) convert the electrical signal coming from the pre-amplifier into a digital signal and transfer that signal to the software for data visualization. The ZE5 Cell Analyzer is a fully digital instrument, transforming signals with 24-bit resolution for signal area and height as well as 17-bit resolution (using linear interpolation at half height) for signal width.

Chapter 3 ZE5 Loader

The ZE5™ Cell Analyzer loader provides built-in temperature control (from 4 to 37°C in 1° increments) as well as variable agitation. Agitation speed is preprogrammed for each media type to ensure that sample fluid does not spill.

Loader operation is primarily controlled by Everest™ Software. The run list (experiment), which is guided by the Experiment Builder, defines sample positions and sampling conditions such as agitation or wash.

To open the loader door and extend the loader, press the silver sample chamber button on the front of the instrument. The external light turns on when the loader door is opened. The sample chamber button also controls the inner loader chamber light when the door is closed. Press and hold the button to turn the internal illumination on or off.

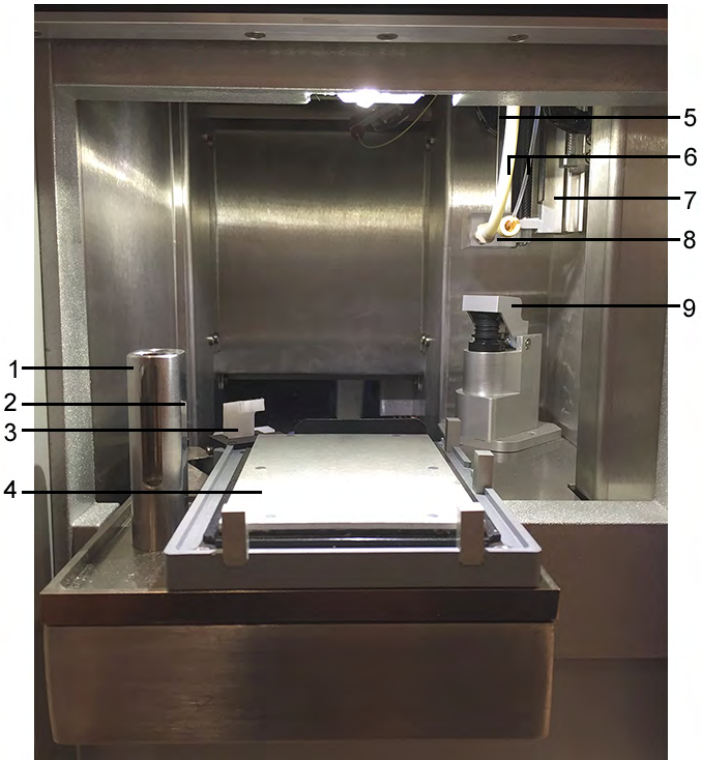


A wash module is integrated into the sample delivery system. The sample probe moves up and down through the wash station to expose contaminated regions of the probe to wash fluid. You can use Everest Software to control wash times for the inside of the sample line and the outside of the sample probe.

Washing also occurs during system shutdown. In high-throughput sampling mode, the sample pump runs continuously between samples, drawing air and wash fluid into the sample line between samples; these air bubbles and wash fluid segments serve as sample separators.

Loader Components

The various components of the loader are depicted in the next figure and described in the accompanying table.



LEGEND

1	Stat tube position	Allows for loading a single tube, rather than a rack of tubes, for quick sample acquisition without requiring experiment configuration in the Experiment Builder.
2	Waste overflow port (behind stat tube position)	Utilized for cleaning and wash station maintenance and for removal of clogs.
3	Clamp	Secures the tube rack or plate on the loader. Opens when loader is extended. Closes when loader is retracted.

LEGEND

4	Plate and tube rack position, agitation assembly, and temperature control	Accommodates tube racks and plates. Integrates agitation and temperature control.
5	Sample probe	Aspirates sample fluid and introduces it to the sample pump for delivery to the flow cell.
6	Rinse line and rinse waste line	Introduces sheath fluid to the wash station for cleaning the probe and sample line. During certain stages of instrument operation such as shutdown, cleaning fluid, rather than sheath fluid, can be used for washing.
7	Gauge	Supports the sample probe and the wash station.
8	Wash station	Facilitates probe and sample line cleaning.
9	Bead station	Houses beads used for quality control. Includes a cap to prevent evaporation of the bead suspension fluid. Integrated agitation ensures that beads are resuspended before the QC process begins.

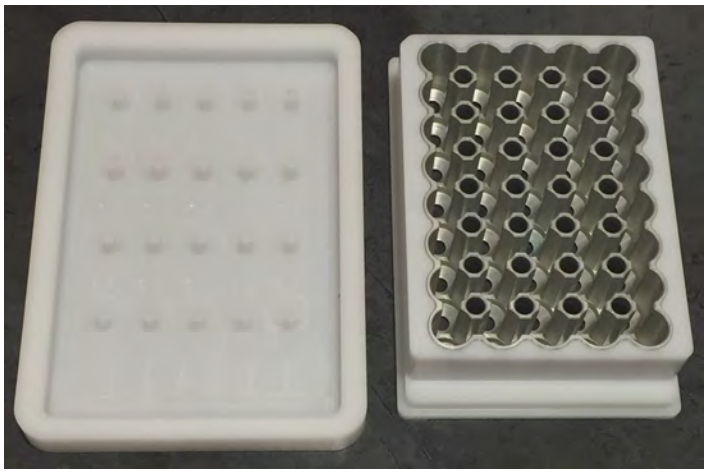
Media Types

The loader facilitates sampling from a variety of media types, including:

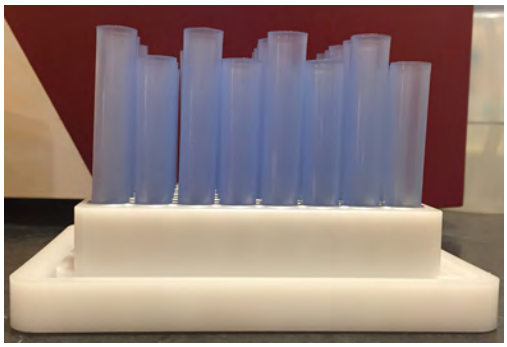
- 5 ml tubes (12 x 75 mm, 1–40 tubes per rack)
- 1.5 ml tubes (1–24 tubes per rack)
- 96-well plates
- 96 deep-well plates
- 384-well plates
- One 5 ml tube in the stat tube position (12 x 75 mm)

Everest Software allows definition of, and sampling from, custom media types, such as 48-well plates.

The ZE5 Cell Analyzer includes a bi-level tube lifter, shown in the next figure.



When a full 40-tube rack is placed on the tool, tubes in every other row are slightly raised, as shown in the next figure. This facilitates tube removal and replacement during sample preparation.



Probe Cleaning

The integrated sample line wash station is located at the bottom of the gauge. The wash station can introduce either sheath fluid (DI water with additive) or cleaning fluid (from the cleaner bottle) to the sample line, depending on the process and instrument state.

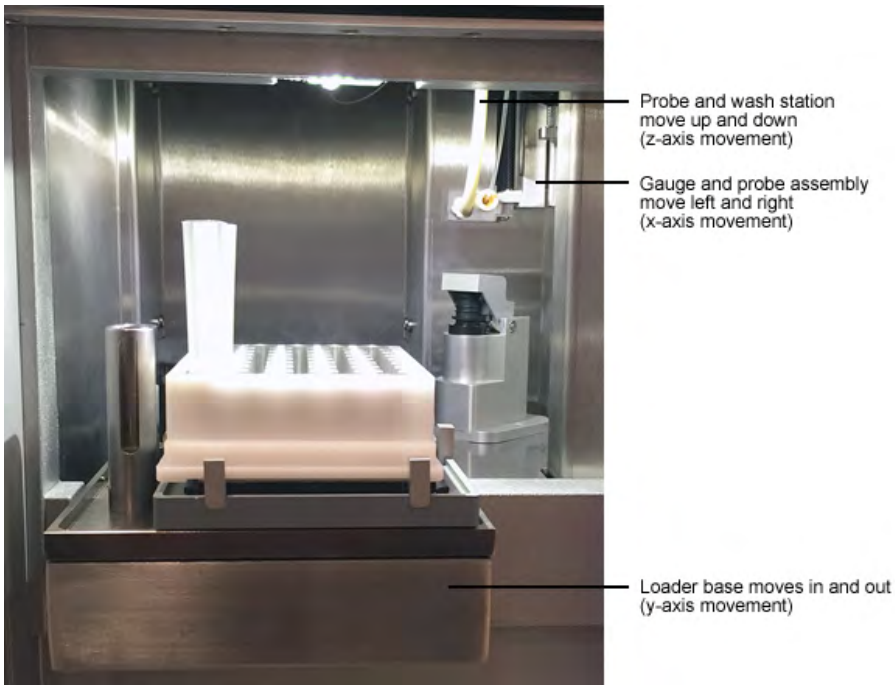
You can program washes into the sample run during run list setup, and you can specify both the inside and outside wash times. These washes use filtered (0.2 µm) sheath fluid. The outside of the sample probe is cleaned by moving the contaminated portion of the sample probe up and down through the wash station. The inside of the sample line is cleaned by moving the probe up and down through the wash station while the sample pump is running. This method introduces air and sheath segments into the sample line and pushes residual sample and debris off the walls and through to waste. This results in highly effective cleaning and minimal carryover between samples.

The shutdown process uses cleaning fluid to clean the probe and sample line. For more information, see Shutting Down on page 129.

Loader Movement

To sample from various types of media, the loader and probe must move in three directions:

- left-right (x-axis)
- in and out (y-axis)
- up and down (z-axis)



Combined with photo sensors in the loader, the gauge helps ensure that the vertical travel distance of the probe and wash station is appropriate for the media type.

Chapter 4 Everest Software

Everest™ Software is the main interface for controlling the ZE5™ Cell Analyzer, indicating overall system status, allowing you to calibrate the instrument and run samples, and providing reports.

This chapter provides descriptions of the following main areas of Everest Software:

- Login window
- Home window
- Help and Information menu
- Recent Experiment Sessions panel
- Experiment Builder — guides you through experiment setup using four sequential screens:
  - Name
  - Fluorophores
  - Samples
  - (Workspace) Settings
- Acquisition workspace
- Instrument Control panel
- Toolbar
- Status bar

For information about which sections of the Everest Software user interface are available only to system administrators, see Administrator and User Rights on page 19.

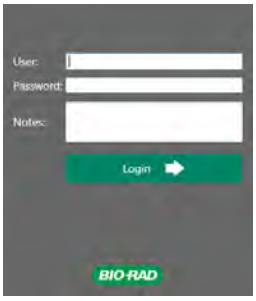
Login Window

The login screen is divided into two halves: Instrument Status and User Login.

Table 6. Instrument status items and their functions

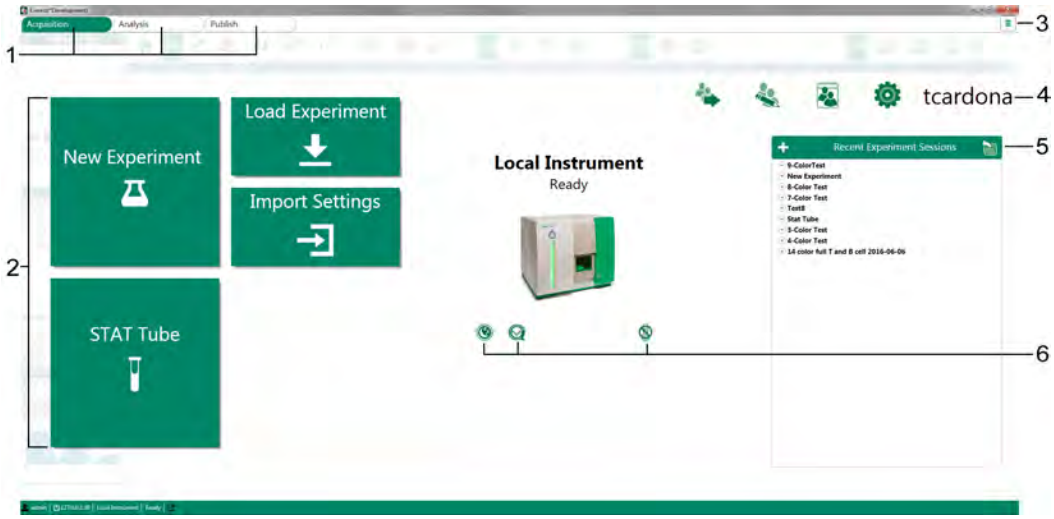
Instrument Status panel	Item	Function
	**Status**	Displays the state of the instrument. ■ Off (Shutdown) ■ Starting Up ■ Ready ■ Calibrating (Running QC) ■ Acquiring ■ Shutting Down
	User	If the system is running samples for a user, displays the logged-in user's name.
		**Details** — displays fluidics and system status, as well as software and firmware version information.
		**Experiment** — reserved for future use.
		**Shutdown** — displayed if the system has been started up. The instrument can be shut down without user login as long as samples are not actively running. See Shutting Down on page 129.
		**Startup** — displayed if the system has not yet been started up. The instrument can be started up without user login. See Starting Up the System on page 124.

Table 7. User login items and their functions

User Login panel	Item	Function
	**User**	Enter your user name as set up by your system administrator.
	Password	Enter the password for this user name.
	Notes	Enter any notes for this session. Notes appear in the user reports available to administrators.

Home Window

The Home window appears after you log in to the system.



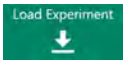
LEGEND		FURTHER INFORMATION
1	Acquisition, Analysis, and Publish tabs; after setting up an experiment and acquiring samples in the Acquisition tab, you can move data to the Analysis and Publish tabs	Experiment Builder on page 51, Chapter 7, Creating Experiments and Workspaces, Chapter 8, Acquiring Samples, and Chapter 10, Analyzing, Saving, and Printing Data
2	Experiment buttons	Experiment Buttons on page 48
3	Help and Information menu	Help and Information Menu on page 48

LEGEND		FURTHER INFORMATION
4	Home window tools and logged-in user name	Home Window Tools on page 49
5	Recent Experiment Sessions panel	Recent Experiment Sessions on page 49
6	Quick actions	Quick Actions on page 51

Experiment Buttons

This set of large buttons in the Home window allows you to initiate experiments in various ways.

Table 8. Home window experiment buttons and their functions

Button	Function
	Launches the Experiment Builder, which guides you through setup of fluorophores/detectors, samples, workspace, and plots.
	Skips the Experiment Builder and allows you to run a sample quickly from the single tube position in the loader. By default, all lasers and all parameters are active when acquiring sample in stat tube mode.
	Loads a previously generated experiment for use as a template for the current experiment. After the experimental file is opened, you can modify it before running it.
	Imports the following settings from the selected run list file: fluorophores (activated PMTs), parameter names, instrument settings, plots, and compensation matrix. **Note:** Sample positions are not imported, so, for example, you can import a run list configured for tubes and easily reconfigure it for a plate.
	Appears if an experiment has been paused and the Home button has been clicked. Returns to the workspace to continue acquiring from the paused experiment. Previously acquired positions are indicated in the plate map. If FCS files were acquired for a portion of the experiment, data from these files are included along with any new files acquired.

Help and Information Menu

The following items appear in the menu in the upper-right corner of the Everest Software window, so that you can access them at any time, including when no user is logged in.

Table 9. Help and Information menu buttons and their functions

Button	Function
	**Log Extraction** — pulls system log files from the last 180 days, compresses them into a ZIP file, and places the file on the workstation desktop.
	**Training Module** — displays a list of training videos that walk you through basic ZE5 Cell Analyzer system operations. These videos run in your default browser. Internet access is not required.
	**User Manual** — displays the user guide PDF.
	**System Info** — displays basic system information such as instrument serial number, software version, and firmware version.

Home Window Tools

The tools that appear above the Recent Experiment Sessions panel in the Home window depend on whether you have administrator privileges. The user name of the logged-in user appears to the right of the tools.

Table 10. Home window tools and their functions

Tool	Function
	**Log Out**— logs the current user out of the system.
	**Reset Password** — allows you to reset your login password. Available only to nonadministrators.
	**Manage Users** — allows you to manage login accounts and access rights. Available only to administrators.
	**User Report** — opens a report that tracks usage over time and includes session notes entered by logged-in users. Available only to administrators.
	**Global Preferences** — allows you to configure global settings for the instrument and software. Available only to administrators.

Recent Experiment Sessions

Each time a user runs an experiment or edits an experiment, a separate session is created and saved by Everest Software. The right side of the Home window contains a list of recent experiment sessions that you

can load into the Experiment Builder for modification.

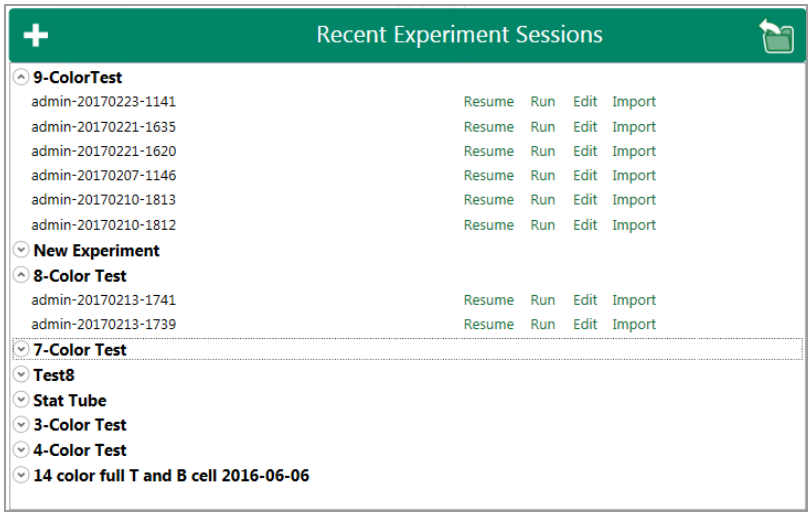


Table 11. Recent Experiment Sessions options and their functions

Option	Function
	**Expand All** — expands the entire experiment list to show the sessions listed for each experiment. You can also expand a single experiment by clicking the down arrow next to its name.
	**Collapse All** — collapses the entire experiment list so that the experiment sessions are hidden. You can also collapse a single experiment by clicking the up arrow next to its name.
	**Load Run List** — allows you to browse for and load experiment files that are stored in locations other than the default recent experiments folder (D:\EverestUsers\[username]). You must know the name of the .rlst file that you are seeking.
Resume	Loads the run list, as it was last acquired, into the workspace. Previously acquired positions are indicated in the plate map. If FCS files were acquired for a portion of the experiment, data from these files are included along with any new files acquired. This option is useful if acquisition was interrupted in the middle of the previous experiment.
Run	Loads a fresh copy of the previously created run list into the acquisition workspace.
Edit	Opens the run list in the Experiment Builder so that you can modify it.
Import	Copies fluorophores, parameter names, instrument settings, plots, and the compensation matrix from the run list into the new experiment. It does not copy sample positions.

Quick Actions

The Home window includes the following quick-action items that appear depending on the state of the instrument.

Table 12. Quick-action items and their functions

Quick-action area	Item	Function
		**Shutdown** — displayed if the system has been started up. See Shutting Down on page 129.
		**Startup** — displayed if the system has not yet been started up. See Starting Up the System on page 124.
		**QC** — initiates the QC process. See Running Quality Control on page 126.
		**Temperature Control** — enables and disables precise temperature control of the sample loader from 4 to 37°C.

Experiment Builder

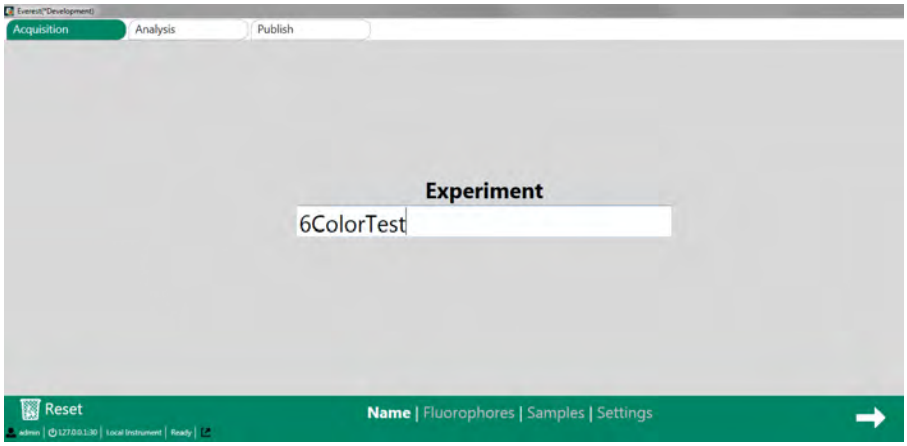
Clicking New Experiment starts the Experiment Builder, which guides you through experiment setup using four sequential screens.

Table 13. Experiment Builder screens

Screen	Description
Name	Allows you to assign a unique name to the experiment.
Fluorophores	Guides you in selecting fluorophores and choosing the appropriate detection channels.
Samples	Facilitates configuration of the sample media required for the experiment, as well as the acquisition settings for each control and sample.
(Workspace) Settings	Facilitates setup of the workspace, where you can create plots, enable lasers, adjust PMT voltages, and set the trigger/threshold as needed.

Name Screen

The Name screen allows you to assign a unique name to the experiment.



Fluorophores Screen

The Fluorophores screen allows you to select fluorophores and activate parameters (detectors) based on the instrument's laser and optical filter configuration.

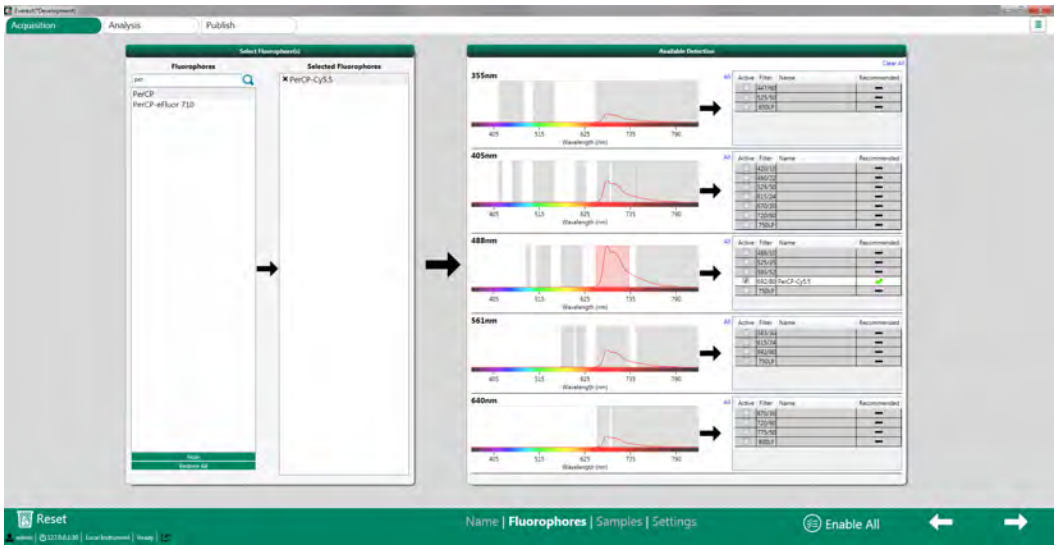
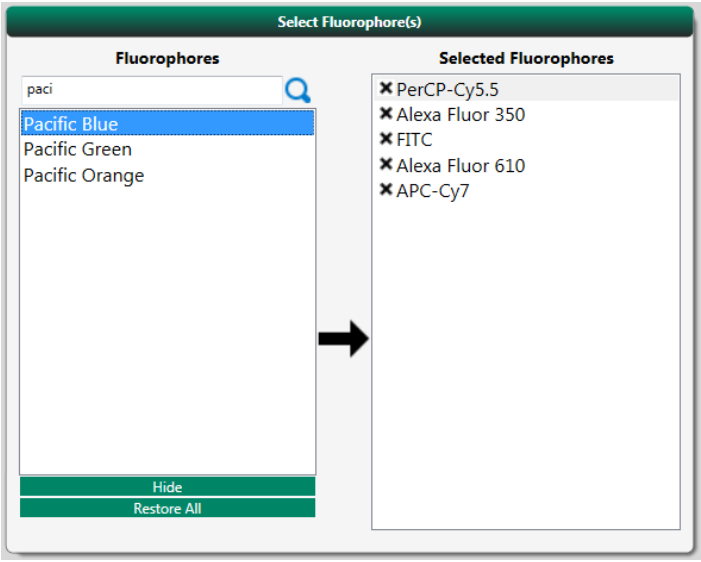


Table 14. Fluorophores screen items and their functions

Item	Function
	Activates all available detectors. **Note:** If the ZE5 Cell Analyzer is configured with a large number of PMTs, enabling all detectors can create large data files that can be difficult to handle upon export to other software packages for analysis.
	Cancels setup of this experiment. Returns you to the Home window, where you can start a new experiment.
	Returns to the previous (Name) screen.
	Moves to the next (Samples) screen.

Select Fluorophores Panel

In the Select Fluorophores panel, you can double-click a fluorophore to add it to the list of Selected Fluorophores. This list determines which PMTs and lasers are activated in the acquisition workspace.



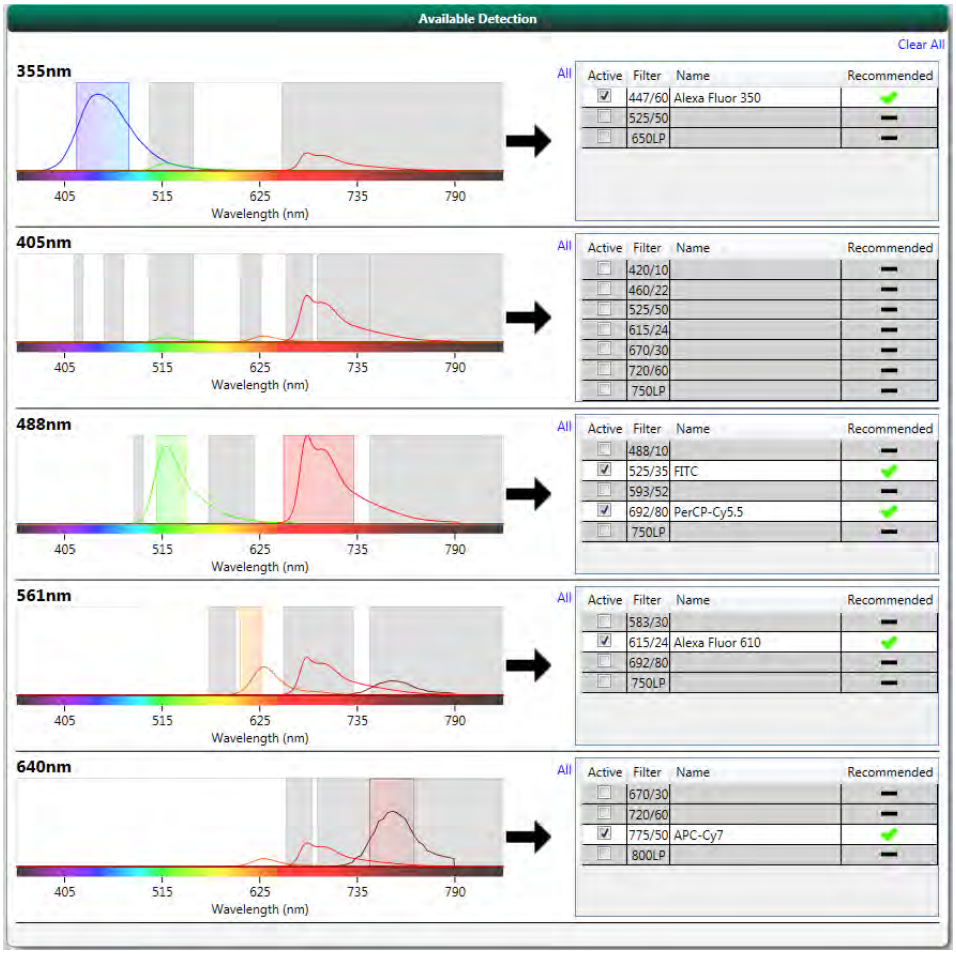
Tip: Use the search box to quickly find a fluorophore by typing in the first few letters of its name.

Table 15. Select Fluorophore(s) panel items and their functions

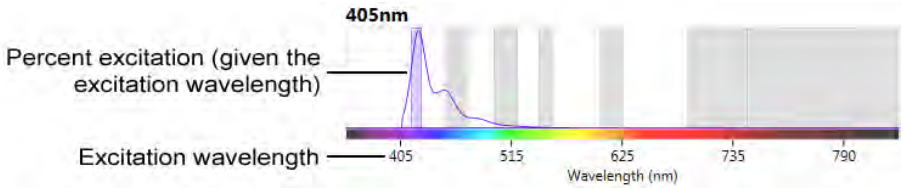
Item	Function
Hide	Hides fluorophores that are not used regularly. These settings apply to the logged-in user's profile; the selected fluorophores will not be hidden from other users.
Restore All	Restores the fluorophore list to the default list delivered with Everest Software.

Available Detection Panel

The Available Detection panel acts as a spectral layout that is specific to the local instrument configuration. Each laser has a spectral graph associated with it.



Because multiple dyes are excited with the same laser, spectra are overlaid on the same graph. When you select a fluorophore, its emission spectrum is shown in the graph associated with the laser that optimally excites it. Additionally, the gray regions in a graph represent the wavelengths allowed through by the bandpass filters located in the laser's fluorescence detection paths.



All

Active	Filter	Name	Recommended
☑	420/10	Brilliant Violet (BV) 421	✔
☐	460/22		—
☐	525/50		—
☐	615/24		—
☐	670/30		—
☐	720/60		—
☐	750LP		—

When you add a fluorophore to the Selected Fluorophores list, Everest Software activates a recommended detector to be used as an acquisition parameter. The software populates the parameter Name box with the fluorophore name. You can edit the name of the parameter. Any changes to the parameter names will carry through to the plot axes and filter names. You can make additional parameter selections if needed.

Tip: The only parameters that are activated are those for which fluorophores have been selected. Therefore, only data from the enabled (active) parameters will be collected during acquisition.

Height (24-bit resolution), area (24-bit resolution) and width (17-bit resolution using linear interpolation at half height) are collected for all active parameters. All signals are collected as raw digital values from the analog-to-digital converters (ADCs) in linear mode and are uncompensated. Logarithmic scaling is performed by Everest Software after the data have been received by the software.

Samples Screen

After you select the desired fluorophores and click the Next arrow, the Samples screen guides you through selection of sample input settings.

Table 16. Samples screen items and their functions

Item	Function
	Cancels setup of this experiment. Returns you to the Home window, where you can start a new experiment.
	Returns to the previous (Fluorophores) screen.
	Moves to the next (workspace Settings) screen.

Media Selector

Before you can set up the sample positions, you must select the type of media to contain the samples.

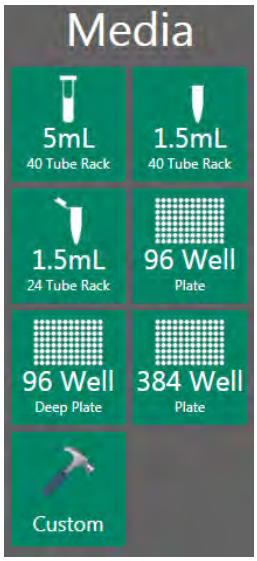
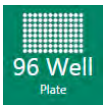
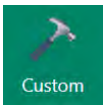
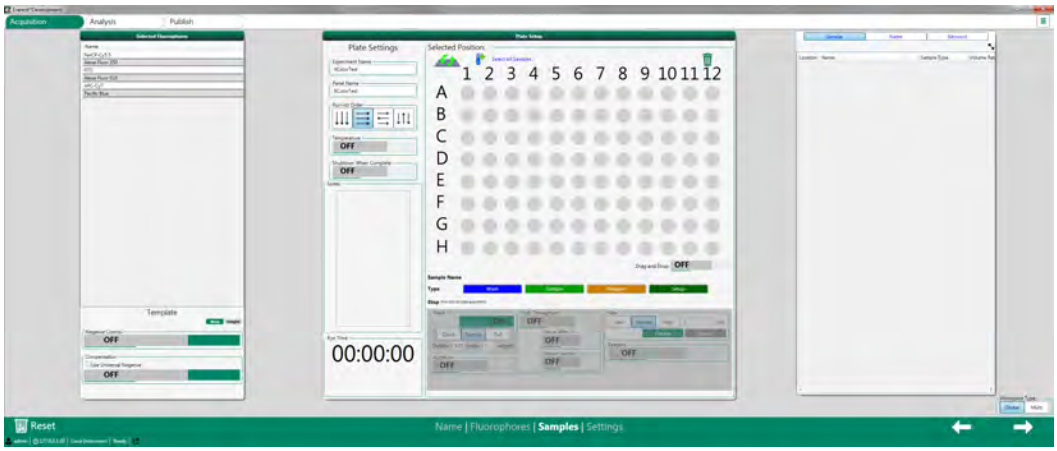


Table 17. Media selector buttons and their functions

Button	Function	Button	Function
	Rack of up to 40 12 x 75 mm 5 ml tubes in the ZE5 40-tube rack		1.5 ml tubes (rack of up to 40)
	1.5 ml tubes (rack of up to 24)		96-well plate
	96-deep well plate		384-well plate
	Configure a custom device from which sample can be acquired; can also be used to calibrate a standard plate		

After you select the media type, the full Samples screen appears.

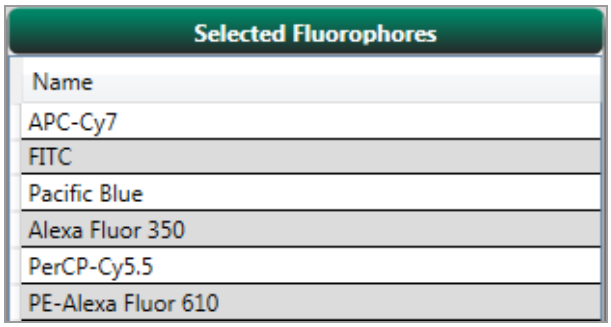


The Samples screen contains three panels:

- Selected Fluorophores
- Plate Setup
- Run List

Selected Fluorophores Panel

The left panel in the Sample screen, Selected Fluorophores, shows a list of the fluorophores that were selected on the previous screen.



The Template area of the Selected Fluorophores panel allows you to select a pulse parameter for compensation, add single-color compensation controls to the first wells or tubes, designate a negative control, and designate a universal negative control for the experiment.

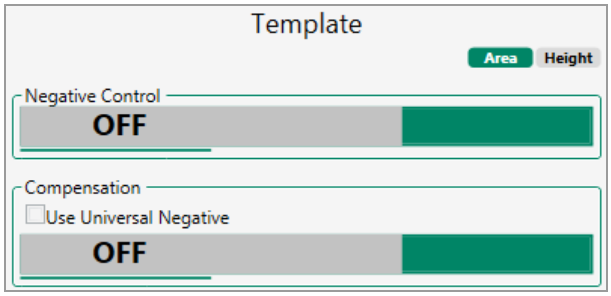
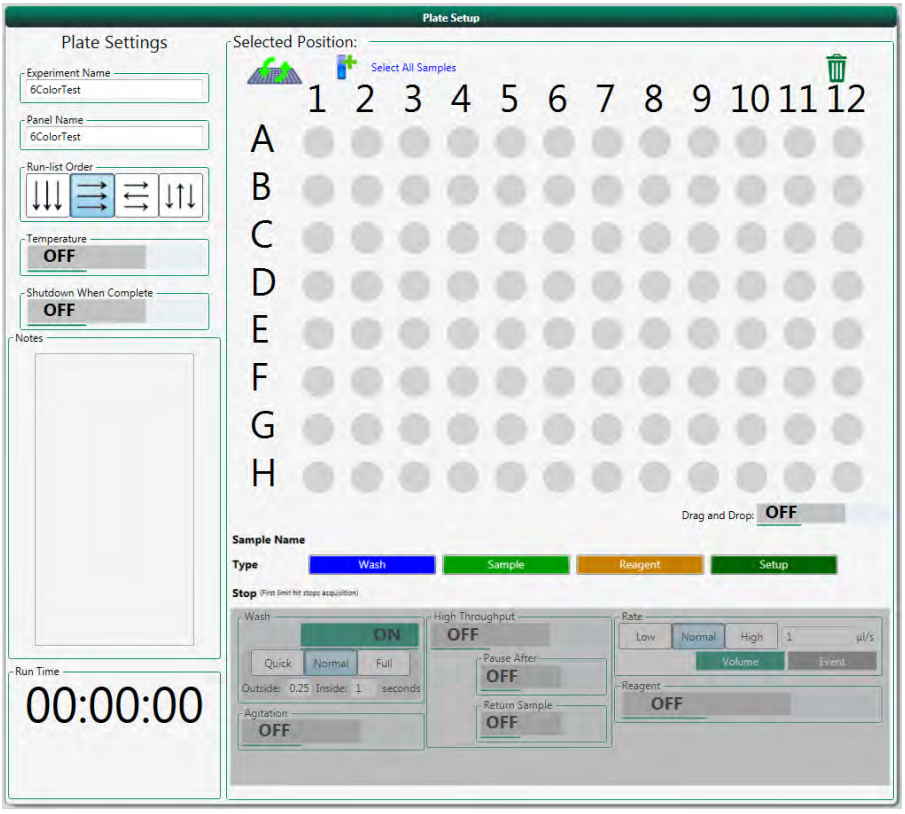


Plate Setup Panel

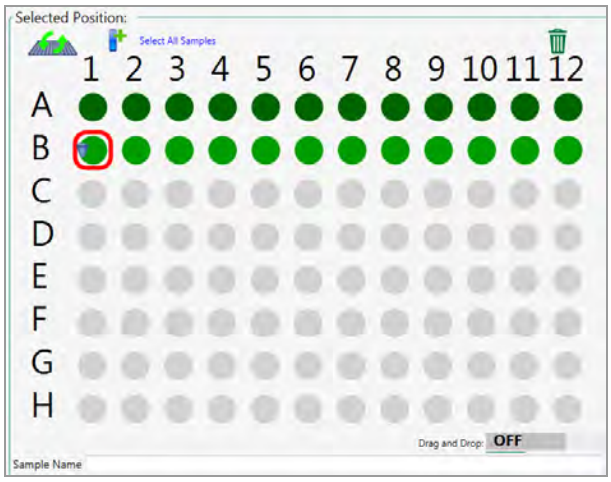
The Plate Setup panel contains Plate Settings on the left and Selected Position information on the right.



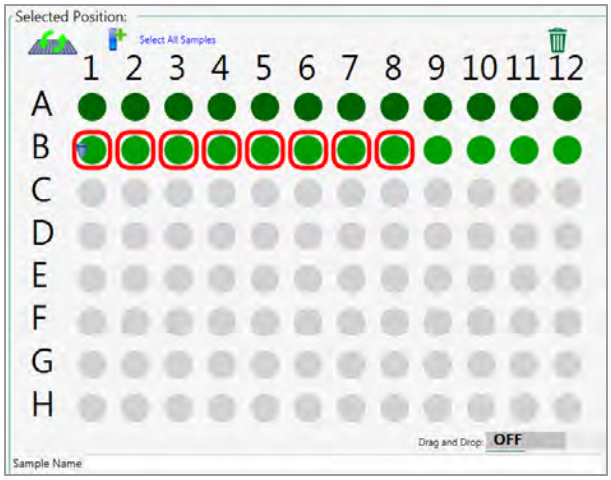
For information on controls in the Plate Settings area, see Plate Settings Controls on page 141. For information on controls in the Plate Setup area that are not covered in this chapter, see Position Settings Controls on page 152.

Selected Position

The Selected Position pane allows you to program each well or group of wells with a set of run conditions. The next figure shows the currently selected well highlighted with a red border.



If multiple wells are selected, the red border surrounds all of them, as shown in the next figure.



To select positions

▶ Do one of the following:

- To select a well, click it.
- To select multiple, adjacent wells, drag over them.
- To select multiple nonadjacent wells, press and hold Ctrl and click the wells.

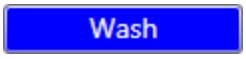
Table 18. Selected Position items and their functions

Item	Function
	Opens the media selector so that you can change the type of input device (for example, switching a 40-tube rack to a 96-well plate). **Note:** Switching to a different media type resets the run list order. For more information, see Plate Settings Controls on page 141.
	Removes the selected well(s) from the run list.
	Allows you to assign a name to a well. The FCS file that is saved for the well reflects this name. Displays the assigned name of the selected Plate Map position.

Position Types

Below the plate map, the Type setting allows you to designate a sample position or group of sample positions as Sample, Setup, Wash, or Reagent.

Table 19. Position Type items and their functions

Button	Function	Appears in plate map as
	Designates the position as a sample. Data acquisition occurs for this position type.	
	Designates the position as a setup or control sample. Controls generated using the compensation template are automatically designated as setup samples.	
	Designates the position as wash, containing water or cleaning solution. No data acquisition occurs for this position type.	
	Designates the position as reagent that can be added to other wells/tubes. No data acquisition occurs for this position type.	
Not applicable.	Unassigned position.	

Position Glyphs

The following table describes the glyphs (small images) that can appear on sample positions, depending on which sample parameters have been selected.

Table 20. Glyphs used on sample positions

Glyph	Description
	Appears on positions where agitation has been activated.
	Appears on positions where addition of reagent has been specified.
	Appears on positions after which the run list will be paused, allowing you time to perform tasks such as applying automatic compensation.

High-Throughput Mode

There are two ways to run samples: standard mode and high-throughput mode.

In the default standard (single sample) acquisition mode, each sample is acquired as an independent run. The sample is boosted to the flow cell before acquisition begins and there is only one sample in the sample line at any given time. Sampling continues until the event limit, volume limit, or gate limit is reached. If multiple limits are set, sampling stops when the first limit is reached. The sample pump runs backward to clear the line, then a wash occurs before the probe moves to the next position.

When high throughput is turned on for selected positions, samples are aspirated continuously and each sample is separated with a series of air and water boundaries depending on the selected inside wash time.

Select high-throughput mode to achieve the highest throughput with minimal time between each sample. After the probe finishes aspirating sample from a position, any programmed wash or agitation is performed, then the probe immediately moves to the next sample and begins aspirating. Samples are delivered to the flow cell at a constant rate and sample boost to the flow cell is not utilized.

High-throughput mode cannot be activated for setup (control) wells. Only sample volume limits (not event limits or gate limits) can be used in high-throughput mode. The Pause After and Return Sample options are available only in standard sampling mode.

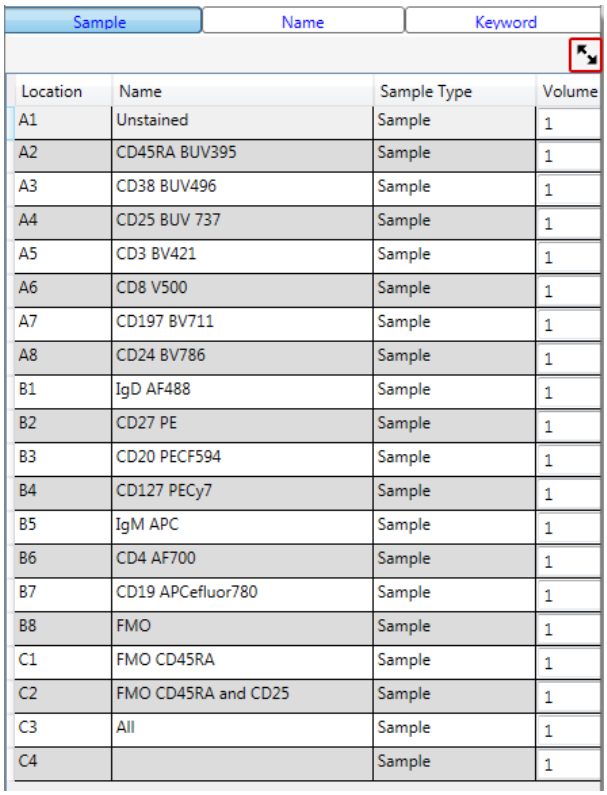
Table 21. Differences between high-throughput and standard mode

	High-throughput mode	Standard mode
Number of samples (tubes or wells) in sample line during run.	Multiple	One
Boost performed before sample acquisition?	No	Yes
Return Sample function allowed?	No	Yes
Pause (between-samples) function allowed?	No	Yes
Wash between samples allowed?	Yes	Yes
Agitation allowed?	Yes	Yes
Event limit allowed?	No	Yes
Gate limit allowed?	No	Yes
Volume limit allowed?	Yes	Yes
Target flow rate can vary per sample?	No	Yes
Target flow rate range	0.5–2.5 µl/sec (30–150 µl/min)	0.0025–3.5 µl/sec (0.15–250 µl/min)

For more information, see Selecting Standard or High-Throughput Acquisition on page 158, Pausing After a Tube or Well on page 159, and Returning Sample to a Tube or Well on page 159.

Run List Panel

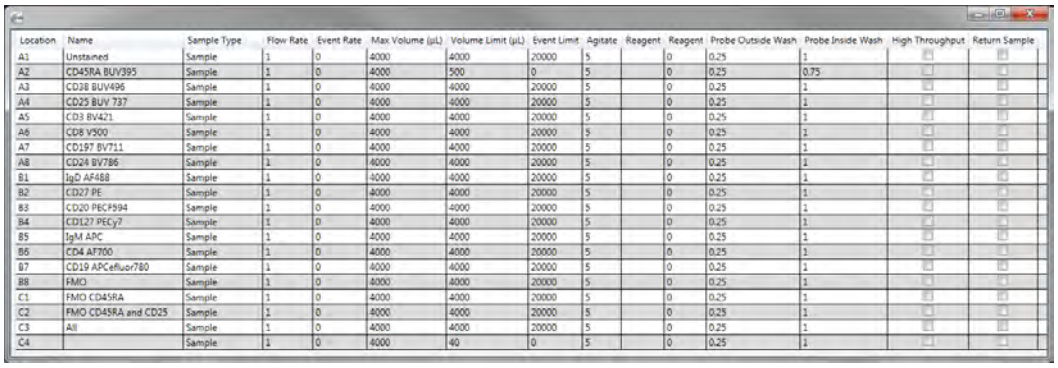
The panel on the right side of the Samples screen summarizes the run list created for the experiment. The collapsed view shows the Location on the plate, the well (sample) Name, the Sample Type, and Volume.



Sample | Name | Keyword

Location	Name	Sample Type	Volume
A1	Unstained	Sample	1
A2	CD45RA BUV395	Sample	1
A3	CD38 BUV496	Sample	1
A4	CD25 BUV 737	Sample	1
A5	CD3 BV421	Sample	1
A6	CD8 V500	Sample	1
A7	CD197 BV711	Sample	1
A8	CD24 BV786	Sample	1
B1	IgD AF488	Sample	1
B2	CD27 PE	Sample	1
B3	CD20 PECF594	Sample	1
B4	CD127 PECy7	Sample	1
B5	IgM APC	Sample	1
B6	CD4 AF700	Sample	1
B7	CD19 APCefluor780	Sample	1
B8	FMO	Sample	1
C1	FMO CD45RA	Sample	1
C2	FMO CD45RA and CD25	Sample	1
C3	All	Sample	1
C4		Sample	1

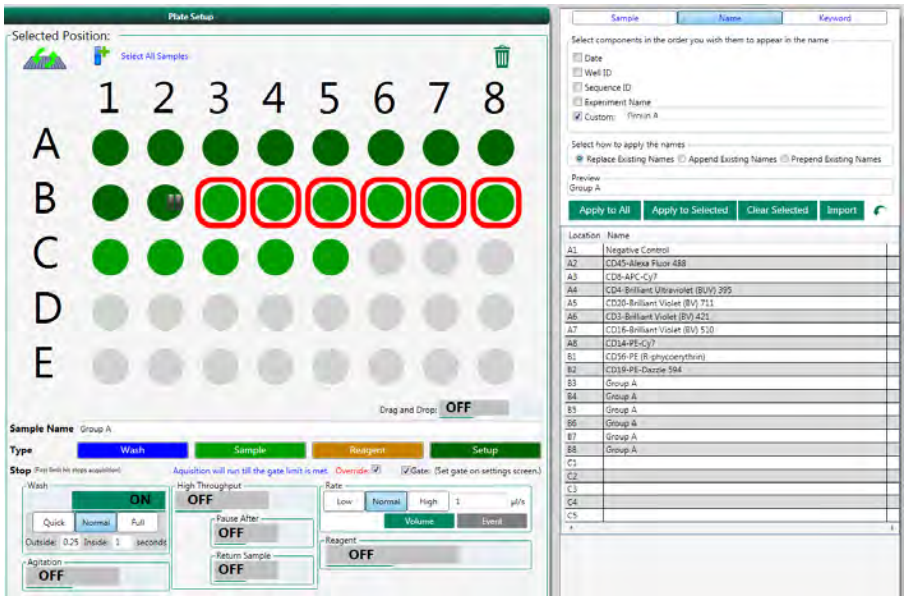
You can expand the run list using the button in the upper right corner.



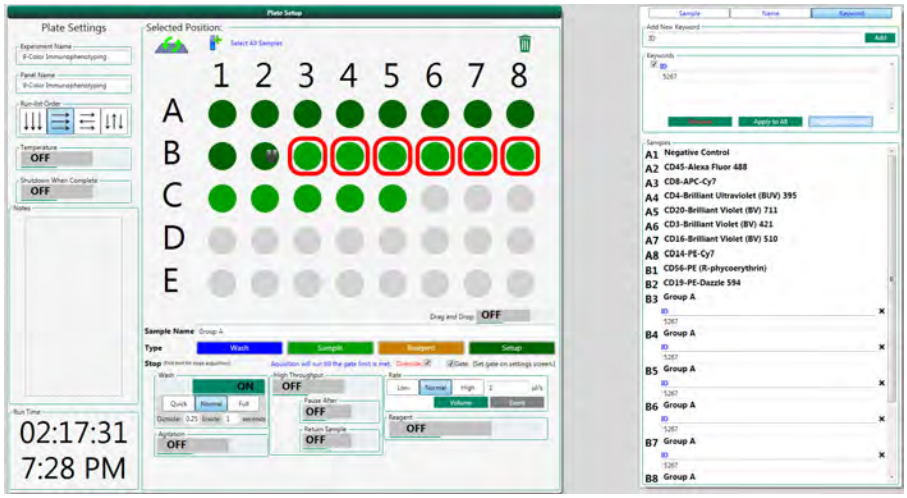
Location	Name	Sample Type	Flow Rate	Event Rate	Max Volume (µL)	Volume Limit (µL)	Event Limit	Agitate	Reagent	Reagent	Probe Outside Wash	Probe Inside Wash	High Throughput	Return Sample
A1	Unstained	Sample	1	0	4000	4000	20000	5		0	0.25	1		
A2	CD45RA BUV395	Sample	1	0	4000	500	0	5		0	0.25	0.75		
A3	CD38 BUV496	Sample	1	0	4000	4000	20000	5		0	0.25	1		
A4	CD25 BUV 737	Sample	1	0	4000	4000	20000	5		0	0.25	1		
A5	CD3 BV421	Sample	1	0	4000	4000	20000	5		0	0.25	1		
A6	CD8 V500	Sample	1	0	4000	4000	20000	5		0	0.25	1		
A7	CD197 BV711	Sample	1	0	4000	4000	20000	5		0	0.25	1		
A8	CD24 BV786	Sample	1	0	4000	4000	20000	5		0	0.25	1		
B1	IgD AF488	Sample	1	0	4000	4000	20000	5		0	0.25	1		
B2	CD27 PE	Sample	1	0	4000	4000	20000	5		0	0.25	1		
B3	CD20 PECF594	Sample	1	0	4000	4000	20000	5		0	0.25	1		
B4	CD127 PECy7	Sample	1	0	4000	4000	20000	5		0	0.25	1		
B5	IgM APC	Sample	1	0	4000	4000	20000	5		0	0.25	1		
B6	CD4 AF700	Sample	1	0	4000	4000	20000	5		0	0.25	1		
B7	CD19 APCefluor780	Sample	1	0	4000	4000	20000	5		0	0.25	1		
B8	FMO	Sample	1	0	4000	4000	20000	5		0	0.25	1		
C1	FMO CD45RA	Sample	1	0	4000	4000	20000	5		0	0.25	1		
C2	FMO CD45RA and CD25	Sample	1	0	4000	4000	20000	5		0	0.25	1		
C3	All	Sample	1	0	4000	4000	20000	5		0	0.25	1		
C4		Sample	1	0	4000	40	0	5		0	0.25	1		

In addition to Location, Name, and Sample Type, the expanded run list shows the following information for each position: (target) Flow Rate, (target) Event Rate, Max Volume for the media type, sample Volume Limit, Event Limit, Agitation Time, (added) Reagent Position, Reagent Volume, Probe Wash Times (outside and inside), acquisition mode (High Throughput versus Standard), and Return Sample.

The Name tab facilitates automated naming of samples using components including Date, Well ID, Sequence ID, Experiment Name, and Custom text. For more information, see Naming Positions Automatically on page 147.



The Keyword tab allows you to create custom keyword/value pairs and associate them with sample positions. For more information, see Setting Up Keywords on page 149.



Workspace Settings Screen

After you have configured your sample and controls in the Samples screen, you can configure other aspects of your experiment in the workspace Settings screen.

The Settings screen facilitates:

- Creation of plots, regions, and gates for data collection and analysis

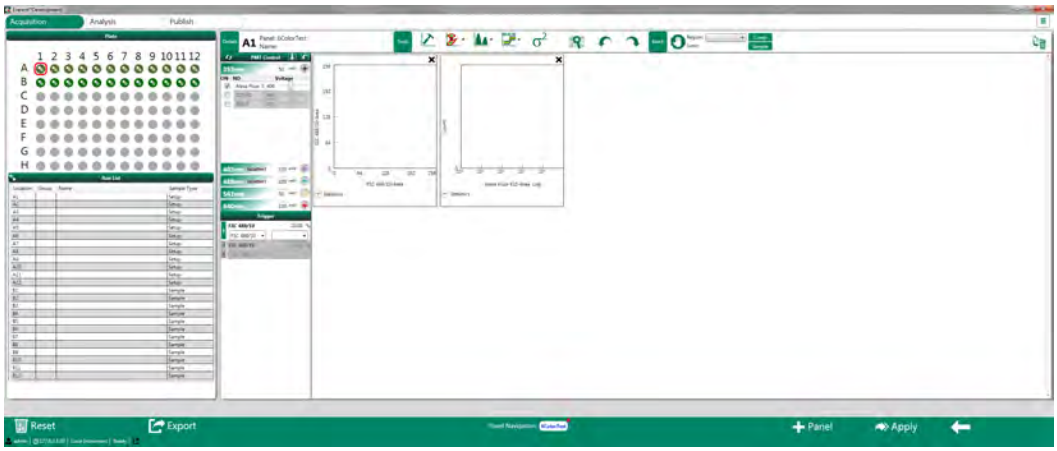
  For information about creating plots, see Creating Plots and Histograms on page 166. For information on adding regions and gates and using other plot tools for data collection and analysis, see Using Plot and Histogram Tools on page 172.

- Setting of initial PMT voltages, if known from a previous experiment or specifically for this particular sample type

  For information on setting PMT voltages, see PMT Control Panel on page 69 and Configuring Instrument Settings on page 206.

- Configuration of initial trigger and threshold values, if known from a previous experiment or specifically for this particular sample type

  For information on configuring trigger and threshold values, see Configuring Instrument Settings on page 206.



The Plate and Run List panes are similar to those that were set up in the Samples screen. These panes summarize the experiment in graphical and tabular format.

Table 22. Settings screen items and their functions

Item	Function
	Returns to the previous (Samples) screen.
	Allows you to configure multiple experiments on the same plate.
	Applies the run list to the acquisition workspace, so that it can be run.
	Exports the run list so that the experiment can be run at a later time.
	Cancels setup of this experiment. Returns you to the Home window, where you can start a new experiment.

Plate Map

The plate map in the workspace is a dynamic software element that provides real-time information on the progress of an experiment. It shows the total number of samples programmed to be run. If the run has already started, the plate map indicates the progress of the run by displaying different colors and symbols for each well position. The next three figures show plate maps for high-throughput, hit detection, and standard (single-sample) mode, respectively.

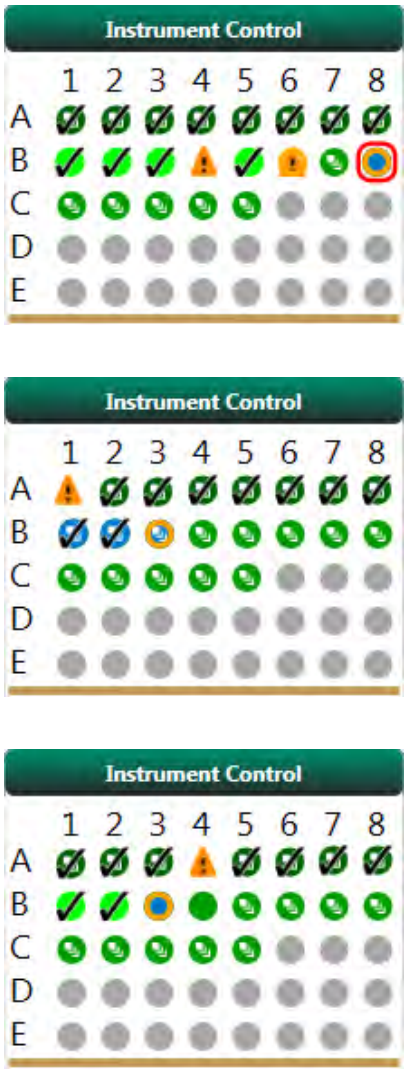


Table 23. Plate map symbols

Symbol	Description
	Control.
	Sample — acquisition has not yet begun.

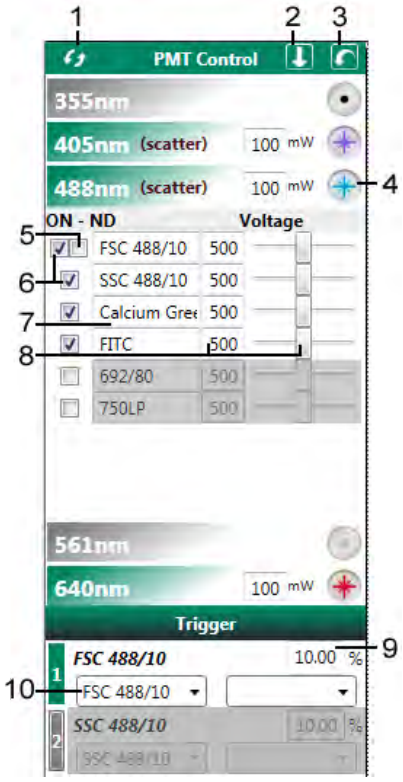
Table 23. Plate map symbols, continued

Symbol	Description
	Sample — acquisition has begun, but position has not yet been sampled.
	Sample — probe is currently sampling this position.
	Sample — sample from this position is currently passing through the interrogation point in the flow cell. The data presented in the workspace are from this sample position.
	Sample — (high-throughput) sample from this position is currently passing through the interrogation point in the flow cell. The data presented in the workspace are from this sample position.
	Sample — sample from this position has been acquired and an FCS file has been saved. (Hit detection mode only) this position has been acquired and has been classified as a hit.
	Sample — (hit detection mode only) this position has been acquired and an FCS file has been saved; position has not been classified as a hit.
	Sample — no events were detected. Check PMT voltages and sample setup. No FCS file saved for the position. This symbol appears on Wash positions.
	Reagent.
	Wash.
	Unassigned.

The white overlay on various types of positions indicates that the position is part of a Global workspace in which the plots, regions, and gates are replicated for each position. In a Multi workspace, the white overlay does not appear. For more information, see Selecting the Workspace Type on page 163.

PMT Control Panel

The PMT Control panel in the workspace Settings screens allows you to control initial settings related to lasers and detectors. These settings can be adjusted later after acquisition begins.



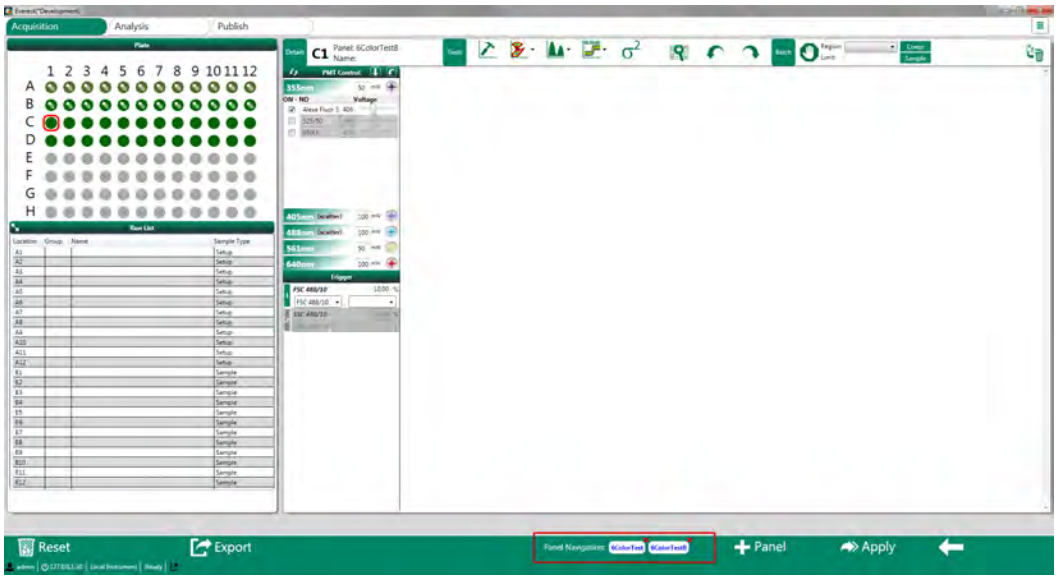
LEGEND

1	Toggle between detector filter numbers and detector names.
2	Load PMT voltages from a selected run list file.
3	Reset PMT voltages to default values.
4	Enable and disable lasers.
5	Enable and disable neutral density filter (FSC only).
6	Enable and disable detectors.
7	Modify detector names.
8	Modify PMT voltages (text entry, slider, or mouse scroll wheel).
9	Set threshold.
10	Set primary trigger (and optional secondary trigger).

For information about using this panel, see Configuring Instrument Settings on page 206.

Multipanel Experiments

You can configure a plate or tube rack with multiple experiments by adding panels in the Experiment Builder. Adding a panel returns you to the Experiment Builder Name screen. After assigning a unique name, you can set up a unique run list in a portion of the plate map. The name of each panel in the experiment is shown at the bottom of the Settings screen, as shown in the next figure.

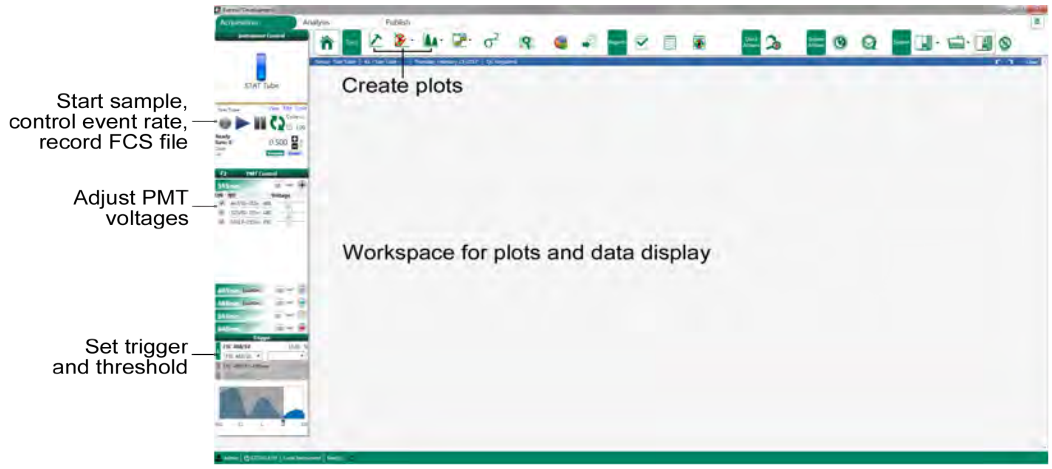


Groups of positions in the plate map can have uniquely defined settings and workspaces, including:

- Active parameters (fluorophores)
- Parameter names
- Sampling settings (such as target flow rate or target event rate, washing, and agitation)
- PMT voltages
- Trigger and threshold
- Global workspaces

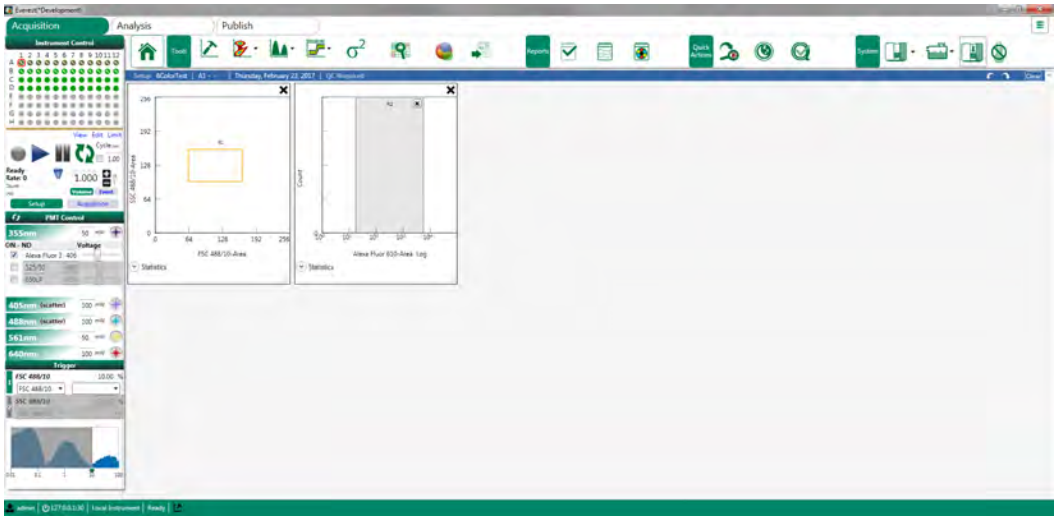
Quick (Stat Tube) Experiments

If you need to quickly run a single sample or a few samples, you do not need to set up an entire experiment. The STAT Tube option in the Home window bypasses the Experiment Builder and brings you directly to the workspace screen, where you can create plots, add gates, and adjust settings. You can acquire samples and save data directly from this screen. In stat tube mode, all parameters are activated by default and can be named by directly modifying the name in the parameter list.



Workspace

After applying an experiment, the acquisition workspace is displayed.

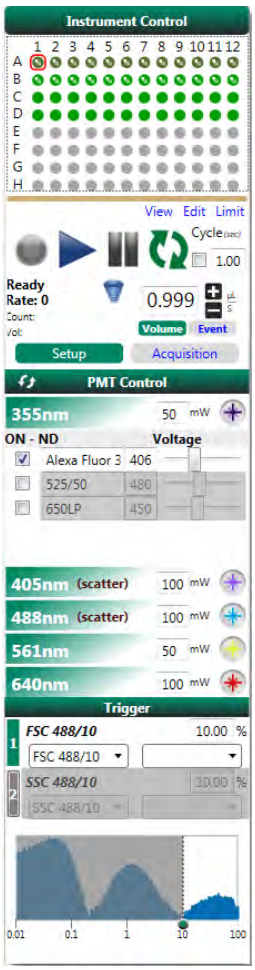


At the top of this screen there are three tabs:

- **Acquisition** — allows you to create experiments and run samples on the instrument.
- **Analysis** — allows you to load saved experiments and associated FCS files for data analysis. For more information, see Chapter 10, Analyzing, Saving, and Printing Data.
- **Publish** — provides tools for printing reports. Allows you to arrange plots so that you can export them to PDF or other applications such as PowerPoint. For more information, see Chapter 10, Analyzing, Saving, and Printing Data.

Instrument Control Panel

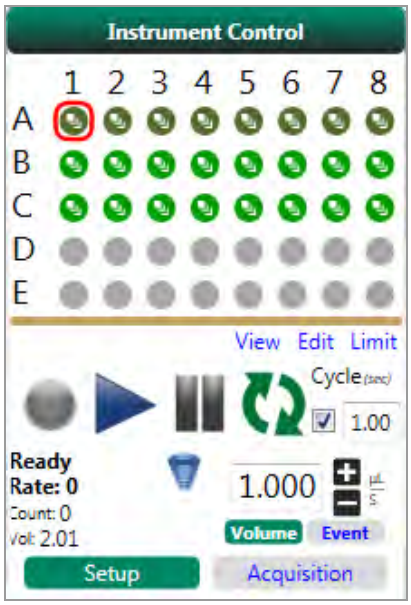
After you load an experiment into the workspace by clicking Apply, the view of the Instrument Control panel changes to include sampling controls for setup and acquisition modes.



The control panel provides the majority of controls and monitoring functions needed to operate the ZE5 Cell Analyzer system and run samples. For information on the PMT and trigger controls, see PMT Control Panel on page 69 and Configuring Instrument Settings on page 206.

There are two modes for running samples: setup and acquisition.

Setup Mode

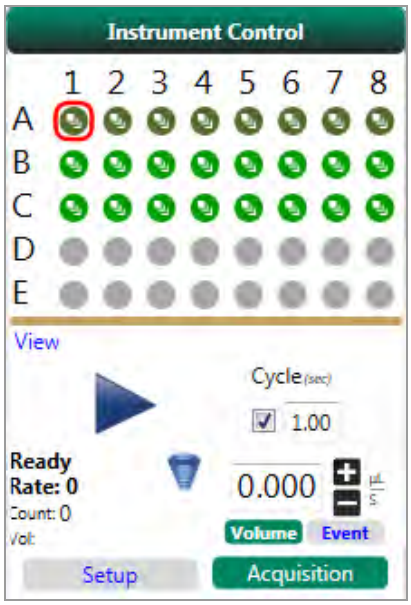


Setup mode has two primary uses:

1. You can acquire controls, on a single-sample basis, to optimize settings and set compensation. When you use setup mode in this way, sampling does not stop unless you instruct the instrument to stop.
2. You can use the Record function to record data files from samples; sampling stops when the preset limit is reached. This is useful if you need to acquire and save data from samples on a single-tube basis. You can use the Record function for some or all of the samples in the experiment.

For more information, see Setup Mode Controls on page 220 and Acquiring Initial Sample in Setup Mode on page 221.

Acquisition Mode



Acquisition mode measures and records data from samples in an automated manner, following the order specified in the Experiment Builder. This mode is typically used after optimizing settings in setup mode. After sample acquisition has been initiated in acquisition mode, the ZE5 Cell Analyzer proceeds to each position on the rack or plate, starts and stops sampling as programmed in the run list, and records data files. Acquisition mode requires minimal user intervention after acquisition has begun.

For more information, see Acquisition Mode Controls on page 223 and Running Samples in Acquisition Mode on page 225.

The Toolbar

The toolbar is displayed at the top of the workspace Settings screen and at the top of the Acquisition, Analysis, and Publish tabs in the workspace. The toolbar consists of various sections, depending on the context in which it is displayed.

Details

Details appear in the toolbar of the workspace Settings screen.

Table 24. Details toolbar items and their functions

Item	Function
Selected position	The position currently selected in the plate map.
Panel	The name of the experimental panel to which the selected position belongs.
Name	The sample name for the selected position.

Tools

The following tools appear in both the workspace Settings screen toolbar and the workspace Acquisition tab toolbar unless otherwise noted.

Table 25. Tools buttons and their functions

Button	Function
	**Home** — returns you to the Home window, where you can create a new experiment or open one that has already been created.
	**Advanced Plot Builder** — facilitates creation of histograms for all parameters, with constraints that you define. For more information, see Creating Histograms for All Channels on page 170.
	**Create Density Plot** — creates a bivariate (two-parameter) density plot. For more information, see Creating Density Plots on page 167.
	**Create Histogram** — creates a univariate (one-parameter) histogram. For more information, see Creating Histograms on page 168.
	**Create Time Plot** — creates a plot of time (x-axis) versus a selected parameter (y-axis). For more information, see Creating Time Plots on page 169.
	**Add Statistics** — opens a statistics window; in it, you can select the plot statistics to display for a particular filter (gate), such as concentration, count, CV, percent of total, maximum, mean, median, minimum, mode, percent of plot, standard deviation, and variance. This window also displays the gating hierarchy. For more information, see Managing Plot Statistics on page 176, Viewing and Rearranging Plot Statistics on page 177, and Comparing Statistics on page 178.
	**View Compensation** — opens the compensation matrix in the workspace for viewing or editing.

Table 25. Tools buttons and their functions, continued

Button	Function
	**Optical Filter Configuration** — displays the current optical filter configuration for all detection banks. Also allows you to initiate the ZE5-EYE process. Appears in the workspace toolbar, but not in the workspace Settings toolbar.
	**Export** — allows you to select from five export options: ■ Export FCS file for a single position. ■ Export all FCS files for the current experiment. ■ Export most recent FCS file for each position and compress to ZIP. ■ Export run list to RLST format and export all FCS files for the current experiment. ■ Export full experiment, including list, telemetry, and all FCS files for each position; compress to ZIP. Appears in the workspace toolbar, but not in the workspace Settings toolbar.
	**Undo** — reverses the last action taken. Applies only to creating, moving, resizing, or deleting a region; creating, moving, resizing, or deleting a plot; and applying or removing a gate.
	**Redo** — reverses the last Undo action.
	**Remove Plots** — removes any plots or histograms that have been added to the workspace. Appears in the workspace Settings toolbar, but not in the workspace toolbar.

Some of these tools also appear in the Analysis tab toolbar. For more information, see Analysis Toolbar on page 243.

Quick Action and System Action Tools

A quick-action tool is available in the workspace Acquisition tab toolbar.

Table 26. Quick Action button and its function

Button	Function
	**Analyze** — opens the most recent run list in the analysis tab. This is useful when you wish to perform auto-compensation in the current run list.

System actions tools are available in the workspace Acquisition tab toolbar; the items available depend on instrument status.

Table 27. System Action buttons and their functions

Button	Function
	**Shutdown** — shuts down the system, if it has been started up.
	**Startup** — starts up the system, if it has not yet been started up.
	**QC** — initiates the QC process.

System Tools

System tools are available in the workspace Acquisition tab toolbar. They include system and fluidics functions.

Table 28. System buttons and their functions

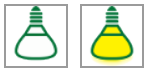
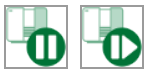
Button	Function
	**Instrument Tools** — includes tools for controlling and maintaining the system.
	**Sample Chamber Light** — turns the loader chamber light on and off.
	**Home Loader** — returns the loader to its home position. See Accessing the Loader on page 128.
	**Clean** — runs cleaner through the probe and sample line. See Cleaning the Sample Line and Probe on page 262.
	**Unclog** — moves the probe to the port behind the stat tube position in the sample loader and cycles through the unclog protocol. See Unclogging the Sample Line and Probe on page 261.
	**Bead Swap** — informs the system that you are replacing the calibration bead bottle and resets the volume to 5,000 µl. See Replacing the QC Beads on page 266.
	**Pause System/Resume System** — pauses the sheath fluid flow and disables the lasers, or resumes the sheath flow and enables the lasers.

Table 28. System buttons and their functions, continued

Button	Function
	**Decontamination** — initiates the Decontamination wizard.
 Fluidics Time(est): **07:59:53** QC Bead Volume(est): **4916**	**Fluidics** — displays status of fluidics bottles and calibration beads; allows you to swap the large fluidics bottles. Displays remaining fluidics run time. See Checking Fluidics Status on page 116.
	**Swap Fluidics** — switches the active waste and sheath fluidics bottles. See Refilling Bulk Fluidics on page 117.
	**Bottle Use Indicator** — indicates whether the top or bottom waste and DI water bottles are in use.
	**Waste Fluid Level** — indicates the level of fluid in the waste bottles.
	**DI Water Level** — indicates the level of fluid in the DI water (sheath) bottles.
	**Additive Fluid Level** — indicates the level of fluid in the sheath additive bottles.
	**Cleaner Fluid Level** — indicates the level of fluid in the system cleaner bottles.
	**Door Toggle** — indicates position of the loader door. Clicking this button opens and closes the loader door as long as the system is not actively acquiring.
	**Temperature Control** — displays the current temperature of the loader. The button enables/disables temperature control of the loader. The temperature can be set between 4–37°C in 1° increments. If a temperature is set in the Experiment Builder for a particular experiment, that value overrides any previous number set in the Home window.

Batch Tools

The batch tools appear in the toolbar of the workspace Settings screen. These tools allow you to set gate limits for compensation controls, all experimental samples, or selected positions. Sampling occurs until the specified gate limits are reached.

Table 29. Batch toolbar buttons and their functions

Button	Function
Region	Allows you to specify the region to which the gate will apply.
Limit	Allows you to specify a gate limit.
Comp	Apply the gate limit to compensation controls.
Sample	Apply the gate limit to experimental samples, as opposed to compensation controls.
Selected	Apply the gate limit to the selected sample positions.

Other Toolbar Buttons

Buttons in the toolbar vary depending on stage of experiment setup and which tab you have clicked.

- For information about buttons for reports, see Reports Tools on page 253.
- For information about toolbar buttons in the Analysis tab, see Analysis Toolbar on page 243.
- For information about toolbar buttons in the Publish tab, see Publish Toolbar on page 248.

Status Bar

At the bottom of the Everest Software window, the status bar shows important information about the system and its users.

LEGEND

1	Currently logged-in user.
2	IP address of the instrument that is connected to the computer. **Note:** Do not click the IP address link unless instructed to do so by Bio-Rad Technical Support. This link is used to disconnect Everest Software from the ZE5 Cell Analyzer and reconnect it for troubleshooting purposes.
3	Indicates that the instrument is directly connected to the computer.
4	Displays the system status.

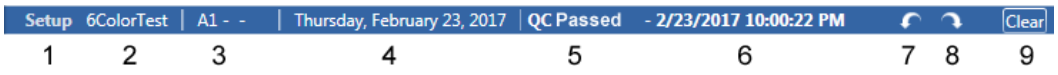
LEGEND

5	Adds a window displaying a log of all error, warning, and information notifications that have been issued since the software was started. This log window is displayed only in the acquisition workspace.
6	Displays the most recently issued instrument status warning.

Table 30. Possible system statuses

Status	Description
Starting Up	The system is performing the startup process.
Calibrating	The system is running the QC process.
Ready	The system is running and ready to acquire samples.
Acquiring	The system is currently acquiring sample.
Shutting Down	The system is shutting down.
Off	The system is shut down.

An additional workspace status bar appears at the top of the workspace after you apply a run list.



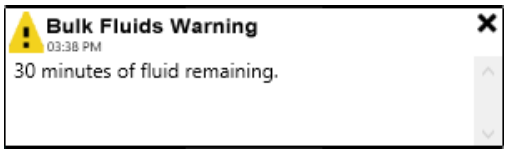
The workspace status bar turns green when sampling is performed in acquisition mode.

LEGEND

1	Sampling mode: Setup or Acquisition
2	Experiment name
3	Currently selected sample position
4	Current date
5	QC status
6	Date of last QC run
7	**Undo** — reverses the last action taken. Applies only to creating, moving, resizing, or deleting a region; creating, moving, resizing, or deleting a plot; and applying or removing a gate.
8	**Redo** — reverses the last Undo action.
9	**Clear** — removes plots and histograms that have been added to the workspace.

Notifications and System Logs

Everest Software displays error, warning, and information notifications in the lower right area of the screen after you log in. (Errors and warnings that occur before you log in are not displayed in this area, but are included in the notification window that you can add to the acquisition workspace after you log in.) For information about adding notifications to the workspace, see Status Bar on page 80.



Warning and information notifications close automatically after 30 sec. Error notifications persist until you dismiss them by clicking the X.

System logs capture notifications that appear in the software, as well as actions taken by users and instrument functions initiated by Everest Software. System logs can provide useful troubleshooting information needed by Bio-Rad Technical Support. You can use the Help and Information menu to extract detailed system log information to a ZIP file. For information about log files, see Exporting and Viewing Log Files on page 269.

Updating the Software and Firmware

Before updating Everest Software by using the installer, ensure that no processes are running on the ZE5 Cell Analyzer or in Everest Software. Stop any sample acquisition; do not open any large files; and ensure that any QC, startup, or shutdown processes have finished.

Firmware updates for the ZE5 Cell Analyzer are included in selected software releases. After running the installer, launch Everest Software. If a firmware update is required, the system notifies you, then installs the firmware automatically.

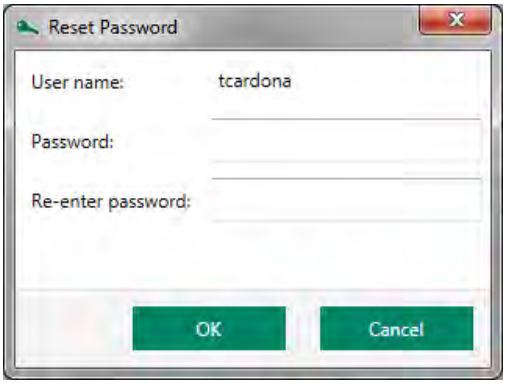
Resetting Your Password

If you do not have administrative rights, the Reset Password button appears in the Home window tools above the Recent Experiment Sessions panel.

To reset your password

1. Click Reset Password.

2. In the Reset Password dialog box, enter the new password twice and click OK.



Keyboard Shortcuts and Undoing Actions

Everest Software provides several keyboard shortcuts for common system actions.

Table 31. Keyboard shortcuts

Shortcut	Description
F2	Start acquisition. Stop acquisition if the system is currently acquiring data.
Ctrl + Z	Undo up to ten of the following actions: ■ Region — create/move/resize/delete ■ Plot — create/move/resize/delete ■ Gate — apply/remove
F3	Save FCS file.
Ctrl + C	Copy plot.
Ctrl + V	Paste plot.
F5	Cycle data. See cycle mode under Setup Mode Controls on page 220.

Chapter 5 Configuring the System

Before the ZE5™ Cell Analyzer is used for acquisition, an administrator must perform some initial system configuration using Everest™ Software. Administrative configuration tasks include:

- Setting up users and assigning them access rights
- Setting instrument default settings in the global preferences
- Editing the criteria used to determine whether the instrument passes or fails the quality control (QC) process
- Updating optical filter configuration settings in the software to reflect physical filter changes
- Reverting the optical filter configuration to factory settings

Both administrators and nonadministrators can change the physical optical filters.

Managing Users

As an administrator, you can use the Manage Users dialog box to create new users, make users inactive, manage user passwords, set user file storage directories, and set user privileges.

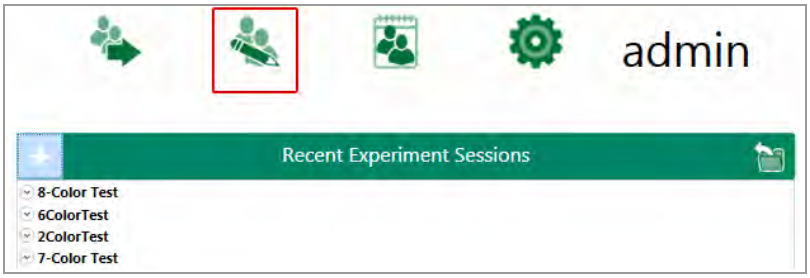
The User Management button is available in the Home window when a person with an Admin account is logged in to Everest Software.

Creating a New User

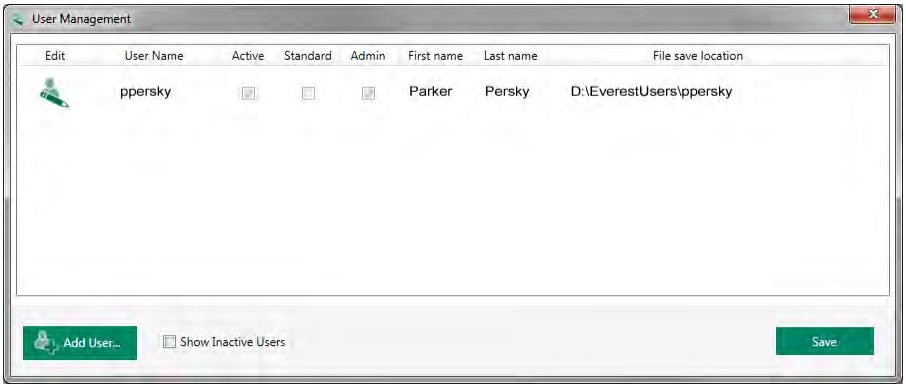
Create a new user account in Everest Software for each person who uses or configures the instrument, or for each group of people who use the instrument in a similar manner.

To add a user account

1. In the Home window, click Manage Users.

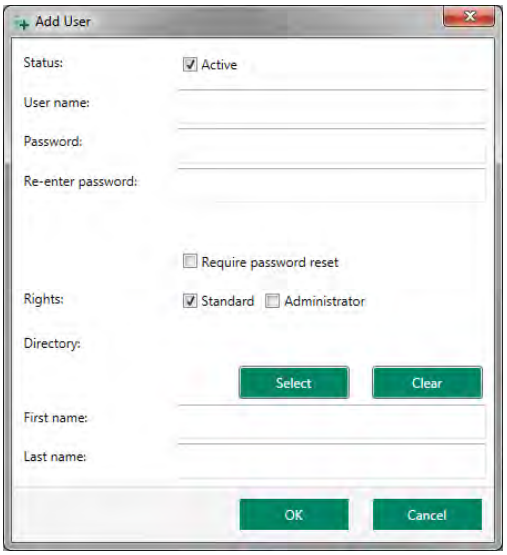


   The User Management dialog box opens.



2. Click Add User.

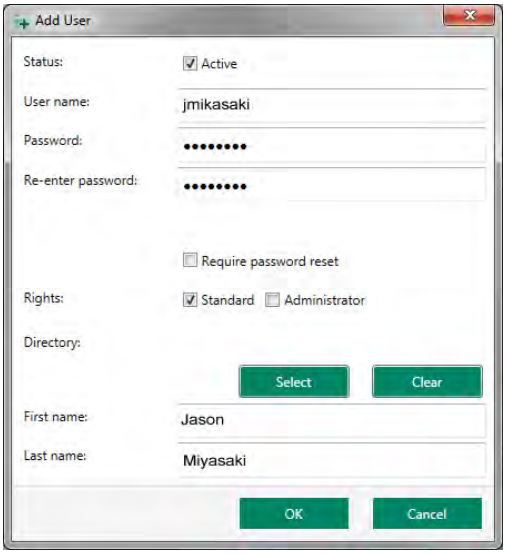
   The Add user dialog box opens.



3. Select the Active status checkbox.
4. Enter a user name.
5. Do one of the following:
   - To force the user to choose their own password upon initial login, enter a default password twice, select the Require password reset checkbox, and tell the user what the default password is.

     When logging in for the first time, the user must enter the default password before choosing their own password.
   - To assign a password to the user, enter it twice, and tell the user what their password is.
6. Select the Standard or Administrator checkbox to determine the account type.
7. In the Directory area, click Select to specify a folder in which the user's instrument data will be saved.

   Note: Any path specified here takes precedence over the default file save path set in the global preferences. For more information, see Setting File Save Parameters on page 93.
8. Enter the first and last name of the user.



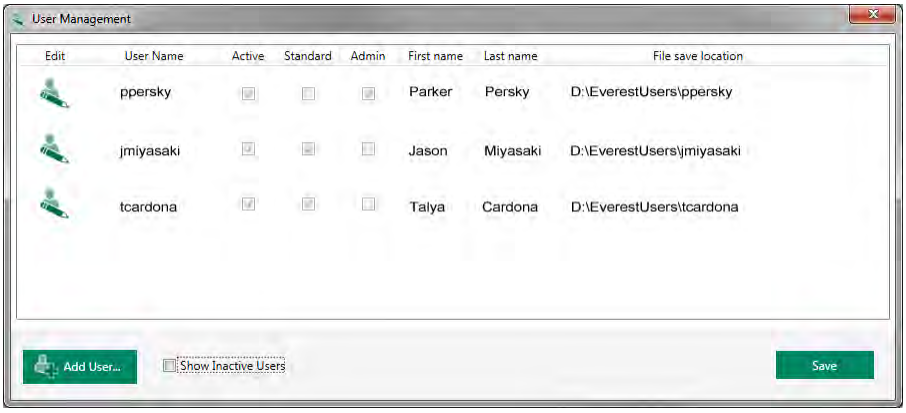
9. Click Save to save the new user account.

Editing User Information

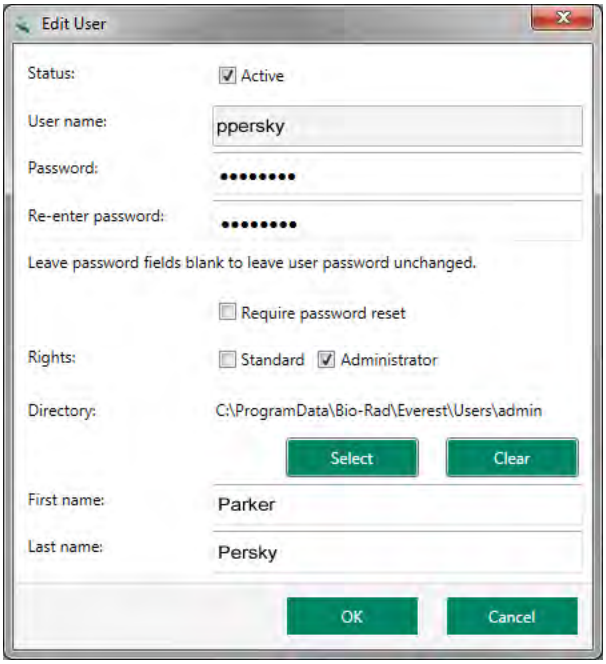
After a user has been created, you can edit details such as password, email address, default file directory, and user rights.

To edit user information

1. In the User Management dialog box, click the pencil button for the user.



   The Edit user dialog box opens.



2. Modify the information in the Edit User dialog box.

Note: Any path specified in the Directory area takes precedence over the default file save path set in the global preferences. For more information, see Setting File Save Parameters on page 93.

3. Click Save.

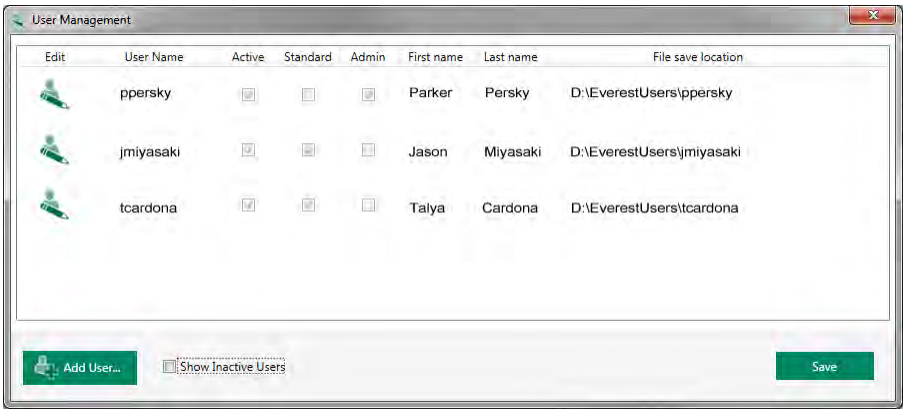
Tip: If all administrator passwords are lost or forgotten, call Bio-Rad Technical Support to receive a temporary administrator password.

Managing User Accounts

As an administrator, you can deactivate any user who no longer needs to work with Everest Software, and you can assign access rights to users.

To manage user accounts

1. In the User Management dialog box, select or clear the Active checkbox in a user row to activate or deactivate a user, respectively.



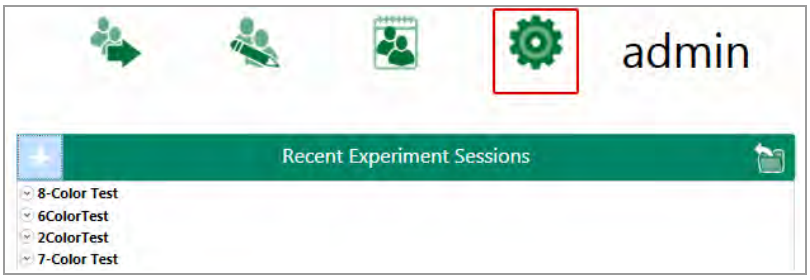
2. To change system access rights for a user, select the Standard or Admin checkbox.
3. Select the Show inactive users checkbox to view inactive user accounts; clear it to hide inactive user accounts.
4. Click Save.

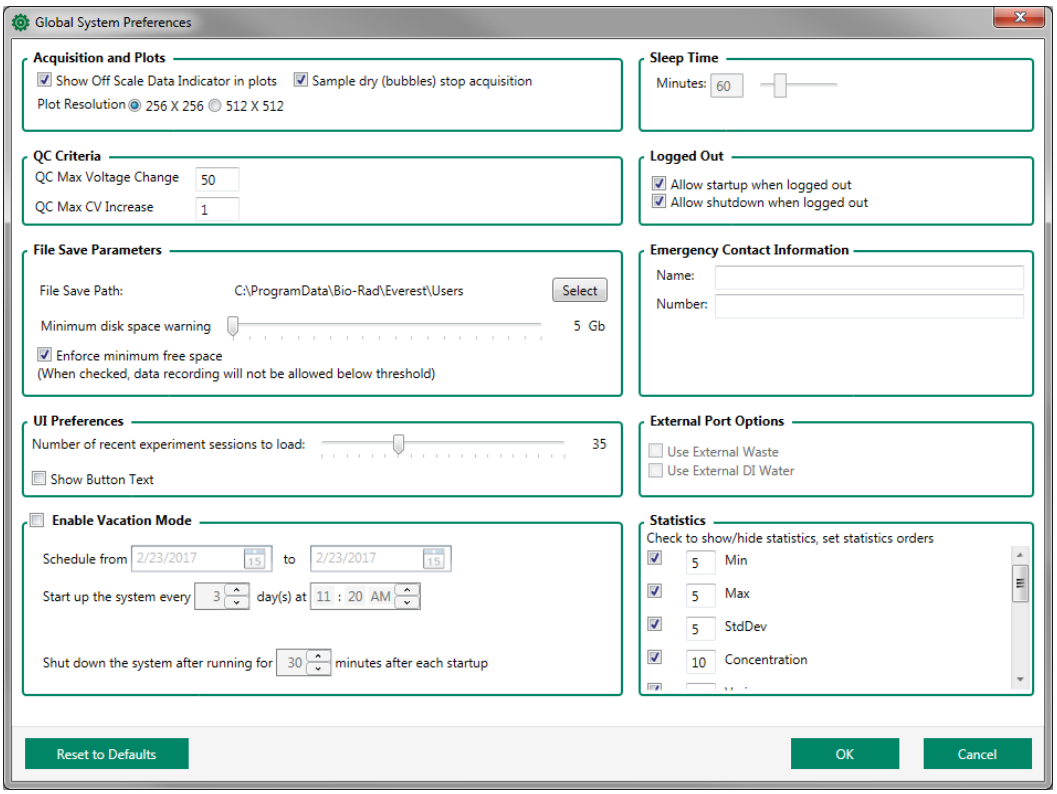
Setting Global Preferences

Administrators have the ability to set Global Preferences for the ZE5 Cell Analyzer and Everest Software. The global preferences button is available in the Home window when a person with an Admin account is logged in to the software.

To access global preferences

▶ In the Home window, click Global Preferences.





Administrators can:

- Configure settings for data plots
- Specify that acquisition stops if sample runs out
- Set default QC criteria
- Set permissions for startup, shutdown, and QC when a user is logged out
- Set default file save parameters
- Specify number of experiment sessions to load
- Display or hide button text in the software interface
- Enable vacation mode
- Specify how plot statistics are displayed
- Reset the system to default settings

Preferences specific to plots take effect the next time you create a new plot. Preferences related to vacation mode take effect as soon as the current user logs out. All other global preference changes take effect as soon as they are saved. To avoid saving any unneeded changes made to the global preferences, click Cancel.

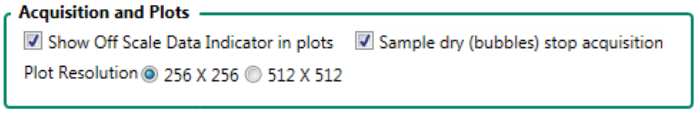
Note: For information on external DI water and waste settings, see Specifying External Port Use on page 301. System sleep time settings are reserved for future use.

Setting Plot Display Defaults and Stopping Acquisition

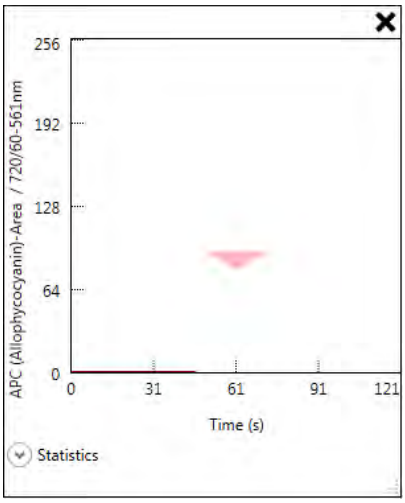
In the Acquisition and Plots area of the global preferences, you can determine the size of plots added to the workspace, specify whether off-scale data indicators are shown in plots, and tell the system to stop acquisition when sample runs dry.

To set plot display defaults

1. In the Home window, click Global Preferences.
2. In the Acquisition and Plots area, select the Show Off Scale Data Indicator checkbox to display indicators when events fall on or below a plot axis.



Such off-scale events, as shown in the next figure, might indicate the need to adjust instrument settings.



3. Select a resolution setting for new plots.
4. Select the Sample dry checkbox if you want to stop acquisition when bubbles occur, indicating that sample has run out.
5. Click OK to save the global preference changes.

Editing QC Criteria

Administrators can edit the criteria used to determine whether the instrument passes or fails quality control (QC). Changes to these criteria apply globally to the system.

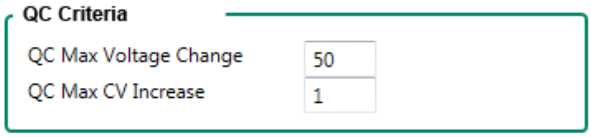
Caution: Adjusting these values might affect overall system performance. Only experienced users who can evaluate potential affects on system performance should edit QC criteria.

An administrator can set two criteria:

- **Max Voltage Change** — the maximum change in PMT voltage (measured from the baseline for each channel) required to bring the population to channel 128. In other words, the change in voltage required to place the population in the center of the histogram must be below this set value.
- **Max CV Increase** — the maximum increase in the calculated coefficient of variation (CV) (measured from baseline for each parameter) must be under this set value.

To edit the QC criteria

1. In the Home window, click Global Preferences.



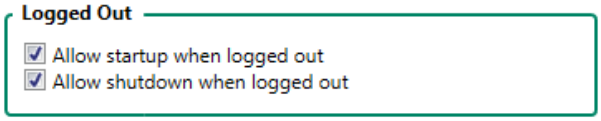
2. In the QC Criteria area, adjust the values for QC Max Voltage Change and QC Max CV Increase.
3. Click OK to save the global preference changes.

Specifying Logged Out Settings

You can specify what actions are permitted when no user is logged in to the system.

To specify logged out settings

1. In the Home window, click Global Preferences.
2. In the Logged Out area, select the respective checkboxes to permit startup or shutdown when no user is logged into the system.



3. Click OK to save the global preference change.

Setting File Save Parameters

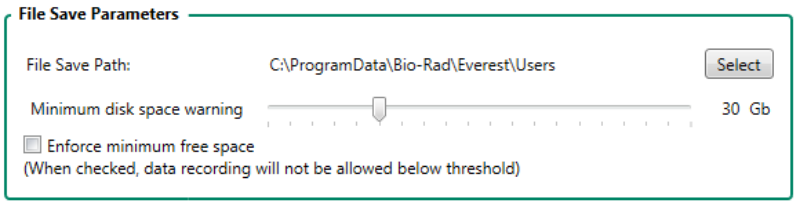
Everest Software can autosave each FCS file after acquisition is complete or when acquisition is stopped by a user. As an administrator, you can specify the default save location for all users. You can also set a different file destination for a specific user; this setting takes precedence over the default save location. For more information, see Creating a New User on page 85 and Editing User Information on page 88.

You can also specify whether users receive warning or error messages when they try to save files that exceed a certain threshold.

To set file save parameters

1. In the Home window, click Global Preferences.
2. In the File Save Parameters area, click Select, navigate to the desired location for saving FCS files, and click OK.

   The location appears in the File Save Path.



3. Use the slider to specify the threshold for the Minimum disk space warning. If the specified minimum disk space exceeds the space available on the hard drive, Everest Software displays a disk space warning to the user before acquisition begins.
4. To prevent Everest Software from recording data when the specified minimum disk space exceeds the space available on the hard drive, select the Enforce minimum free space checkbox.
5. Click OK to save the global preference changes.

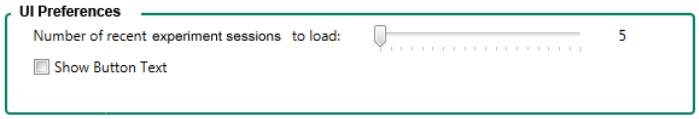
Specifying UI Preferences

You can specify display preferences for various items in the Everest user interface, for example, the number of recent experiment sessions displayed, or whether button tooltip text is displayed.

To specify UI preferences

1. In the Home window, click Global Preferences.
2. In the UI Preferences area, use the Number of recent experiment sessions slider to select a number.

   Note: If you select a very high number, it could take a while to fully load the list of recent experiment sessions. To display all sessions for an experiment in the Recent Experiment Sessions list, click the down arrow next to the experiment name.



3. Select the Show Button Text checkbox to display button text; clear it to hide button text.
4. Click OK to save the global preference change.

Setting Up Vacation Mode

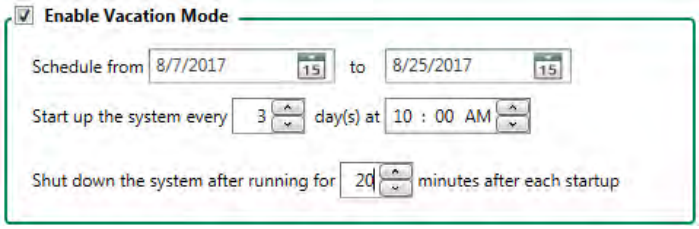
To maintain optimal system performance, Bio-Rad recommends that you run the ZE5 Cell Analyzer regularly without long periods of disuse. If nobody will be running your instrument for an extended period, you can use the Everest Software vacation mode to schedule automatic startup and perform the QC process regardless of whether an operator is in the lab.

During the vacation period, if system startup or QC is not initiated by a user, the following processes occur on the specified interval: startup, laser warmup, QC and ZE5-EYE processes, recording of QC results, running for the specified time, and shutdown. If the system is used on a vacation period day on which no startup is scheduled, this resets the intervals, resulting in a new start date. If the system is used on a vacation period day on which startup is scheduled, the vacation interval schedule is not affected.

Important: Ensure that there is sufficient fluid in the bulk fluidics bottles to support the number of runs that will occur during the vacation period.

To set up vacation mode

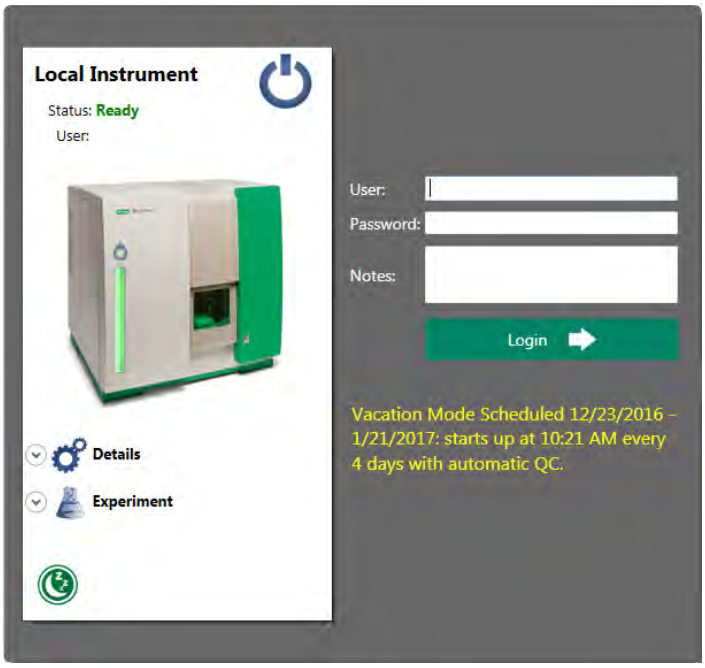
1. In the Home window, click Global Preferences.
2. Select the Enable Vacation Mode checkbox.



3. Set the vacation date range by selecting dates from the start date and end date calendars.

   After the end of the vacation period, vacation mode automatically resets to off.
4. Specify how often the system startup should occur and at what time of day startup should occur.
5. Specify how after long startup the system should shut down.
6. Click OK to save the global preference changes.

   During the vacation period, a vacation notification appears in the Login window, as shown in the next figure.

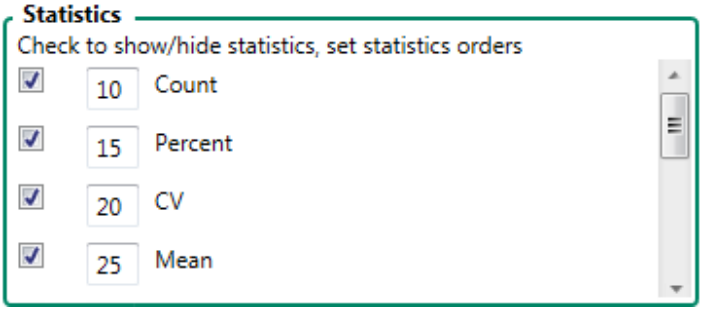


Specifying Statistics Preferences

You can specify which statistics are shown in plots, and the order in which they are shown.

To specify statistics preferences

1. In the Home window, click Global Preferences.
2. In the Statistics area, select the checkbox for each statistic that you want to show, and clear the checkbox for each statistic that you want to hide.

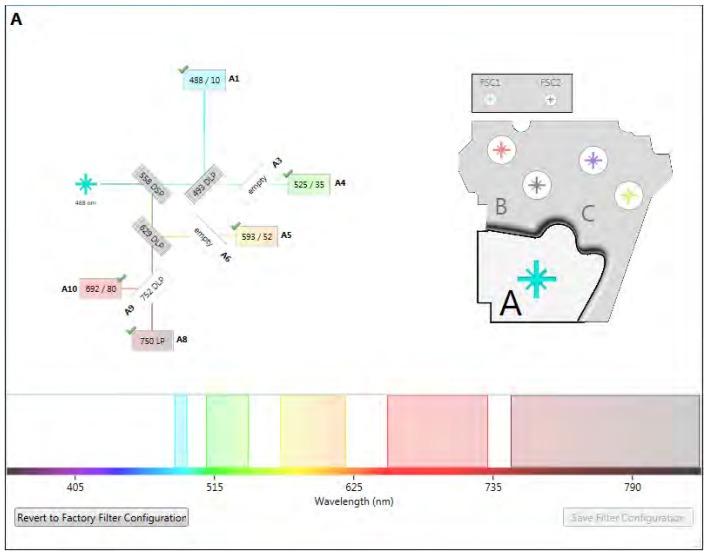


3. Assign a unique number to each statistic to specify the display order. Statistics with higher numbers are shown to the right of statistics with lower numbers.
4. Click OK to save the global preference change.

Working with Optical Filter Configurations

To view and edit the optical filter configuration, click Display Optical Filter Configuration in the toolbar.

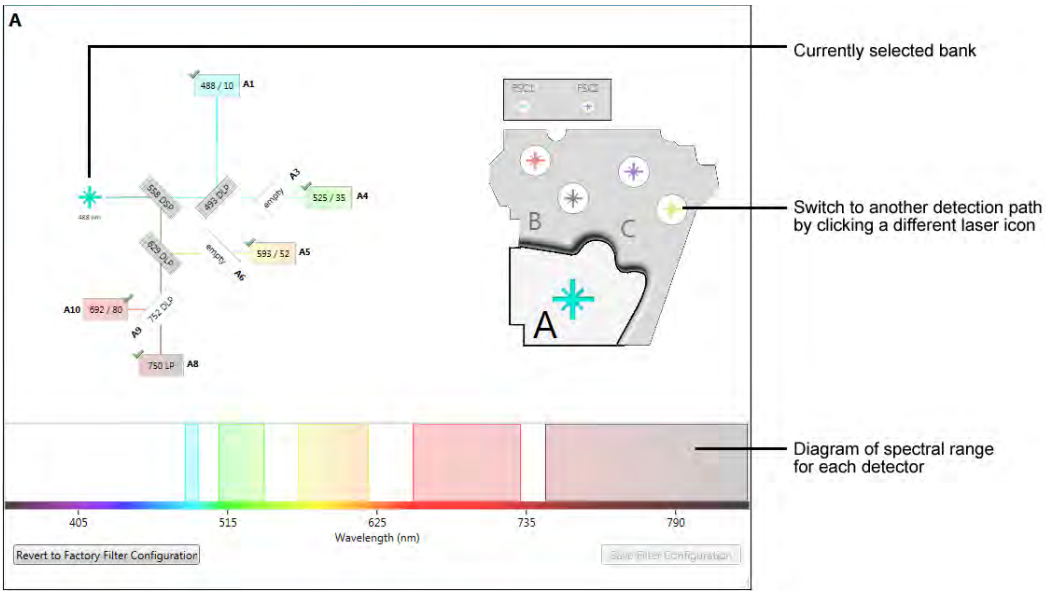
The layout of the detection path is shown on the left, reflecting the current configuration of the instrument. If you install a different physical filter, edit the corresponding mirror/filter position labels in this section of Everest Software.



A green check mark on each final position in each optical detection path indicates that the ZE5-EYE process has verified that the filter listed in Everest Software is currently installed in the instrument. The ZE5-EYE relies on the filter labels used in the software, so if you change a filter label without changing the physical filter, the ZE5-EYE process fails and Everest Software warns you that the correct filter is not installed.

For reference, the light wavelengths allowed through by the filters associated with each PMT are shown at the bottom of the filter configuration screen, overlaid on a spectral plot.

To view the detection layouts for the other filter banks, click the laser symbol in the bank diagram on the right side of the screen.



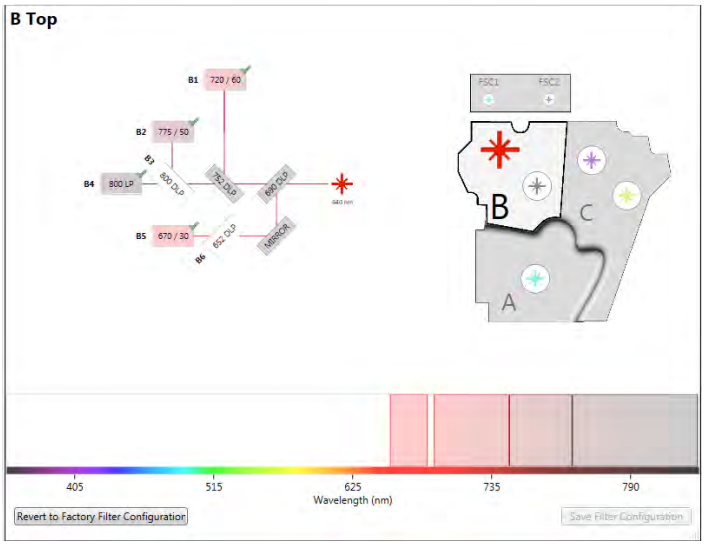
Clicking on the blue laser symbol in the filter bank diagram displays the configuration of the 488 nm laser. Labels A1 through A9 show the filters that you can change. Any changes that you make to the labels here will propagate to the parameter labels in other parts of Everest Software.

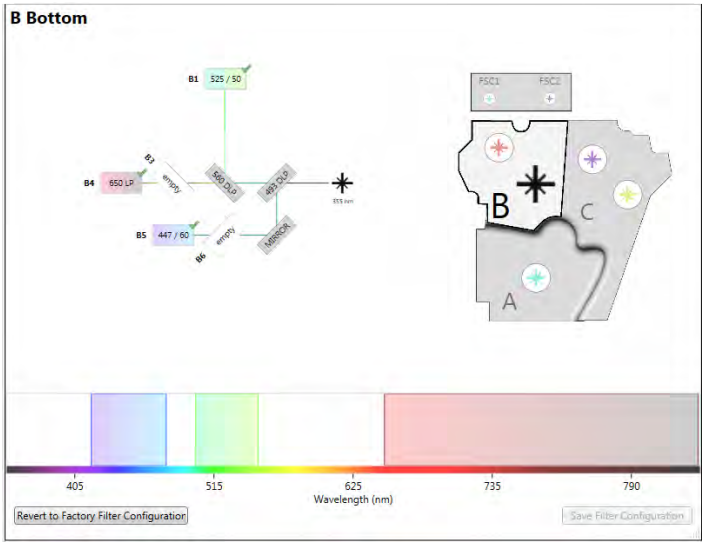
Note: After you save changes to the optical filter configuration in the software, the ZE5-EYE process runs to confirm that the software changes match the physical filter setup.

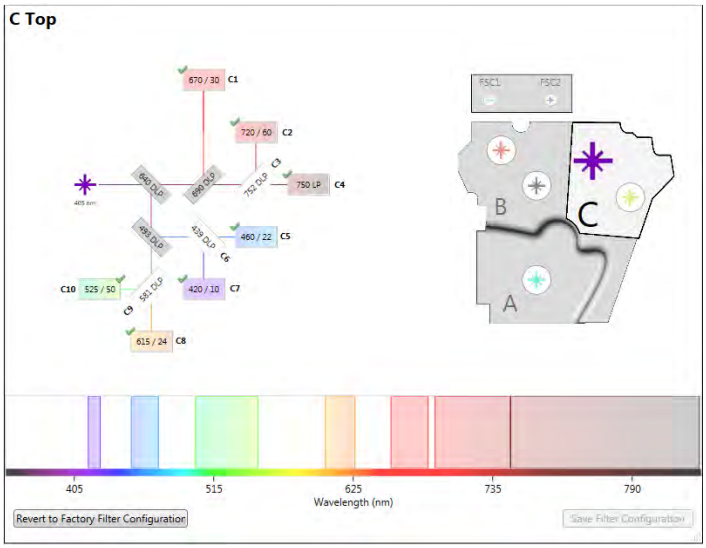
Depending on the laser setup of your instrument, the following detection paths in the banks can be edited from the Optical Filter Configuration dialog box:

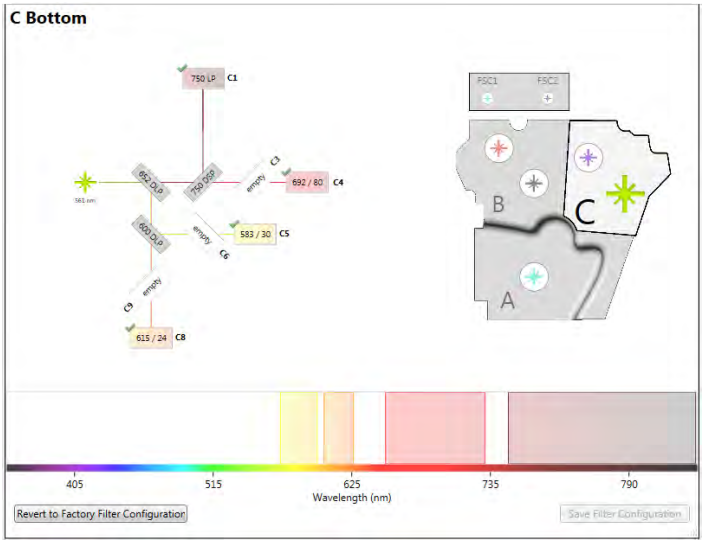
- A: 488 nm (blue) laser, up to seven PMTs
- B Top: 640 nm (red) laser, up to five PMTs
- B Bottom: 355 nm (UV) laser, up to five PMTs
- C Top: 405 nm (violet) laser, up to seven PMTs
- C Bottom: 561 nm (yellow green) laser, up to seven PMTs

Detection paths for banks B and C are shown in the next four figures.


B Top
B1
720 / 60
B2
775 / 50
B3
B4
800 LP
B5
670 / 30
B6
752 DLP
690 DLP
652 DLP
MIRROR
FSC1
FSC2
B
C
A
405
515
625
735
790
Wavelength (nm)
Revert to Factory Filter Configuration
Save Filter Configuration


B Bottom
B1
525 / 50
B2
empty
B3
B4
650 LP
B5
447 / 60
B6
empty
560 DLP
493 DLP
MIRROR
FSC1
FSC2
B
C
A
405
515
625
735
790
Wavelength (nm)
Revert to Factory Filter Configuration
Save Filter Configuration





The following detection paths can be viewed from the Optical Filter Configuration dialog box, but not edited:

- FSC1
- FSC2

Standard Filter Combinations

The ZE5 Cell Analyzer can be ordered with a variety of filter combinations depending on your requirements for number of lasers and combinations of fluorophores. The following sections show some standard combinations of optical elements that are available.

355 nm (UV) Laser Filter Choices

UV 355 nm 50 mW	5 Detectors	Fluorophores
	387/11	BUV395
	405 DLP (changeable)	
	447/60	Alexa Fluor 350 DAPI Hoechst-Blue Indo-hi
	493 DLP	
	525/50	BUV496 Indo-lo
	560 DLP	
	670/30	BUV661 PI
	690 DLP (changeable)	
	700 LP	BUV737 Hoechst-Red

405 nm Laser Filter Choices

Violet 405 nm 100 mW	7 Detectors	Fluorophores
	420/10	Cascade Blue BV421
	439 DLP (changeable)	
	460/22	Pacific Blue BV450
	493 DLP	
	525/50	AmCyan BV510 Cascade Yellow Pacific Orange
	581 DLP (changeable)	
	615/24	BV605
	640 DLP	
	670/30	BV650
	690 DLP	
	720/60	BV711
	752 DLP (changeable)	
	750 LP	BV786

488 nm Laser Filter Choices

Blue 488 nm 100 mW	5-Detector Option	7-Detector Option	Fluorophores
	488/10	**488/10**	SSC on 488
	493 DLP	493 DLP	
	525/35	**509/24**	eGFP FITC
		530 DLP (changeable)	
		549/15	eYFP
	558 DSP	558 DSP	
	593/52	**583/30**	PE (R-phycoerythrin)
		600 DLP (changeable)	
		615/24	PE-Texas Red
	629 DLP	629 DLP	
	692/80	**692/80**	PE-Cy5 PerCP-Cy5.5
	752 DLP (changeable	752 DLP (changeable)	
	750 LP	**750 LP**	PE-Cy7

561 nm Laser Filter Choices

Yellow Green 561 nm 50 mW	7 Detectors	Fluorophores
	577/15	PE
	581 DLP (changeable)	
	589/15	dTomato DsRed
	600 DLP	
	615/24	PE-Texas Red
	629 DLP (changeable)	
	640/20	mPlum
	652 DLP	
	670/30	PE-Alexa Fluor 647 PE-Cy5
	690 DLP (changeable)	
	720/60	PE-Cy5.5
	750 DSP	
	750 LP	PE-Cy7

640 nm Laser Filter Choices

Red 640 nm 100 mW	4 Detectors	Fluorophores
	670/30	SSC
	690 DLP	
	720/60	APC Cy5 Alexa Fluor 647
	752 DLP	
	775/50	Cy5.5 Alexa Fluor 680 Alexa Fluor 700
	800 DLP (changeable)	
	800 LP	APC-Cy7 APC-H7 Alexa Fluor 750

Replacing Optical Filters

Important: Only authorized and trained personnel should access or modify the optical configuration of the ZE5 Cell Analyzer system. The coated pieces of glass are delicate; handle them with care. Any scrape or scratch on the surface could significantly affect the light passing through. Always wear gloves when removing or replacing filters to avoid depositing smudges and fingerprints on the glass surfaces. See Cleaning the Optical Filters on page 266 for specific instructions on cleaning optical filters.

To replace optical filters

1. Open the outer optical filter access door.
2. Lift the black optical filter cover and attach it to the side of the instrument using the built-in magnet.

3. Remove existing optical filters by pulling the filter sticks out of their slots in the filter bank, as shown in the next figure.

4. Insert replacement filter sticks into empty slots as needed.

   Tip: Store extra optical filter sticks in the slots in the filter access door.

5. Lower the black optical filter cover.
6. Close the outer optical filter access door.

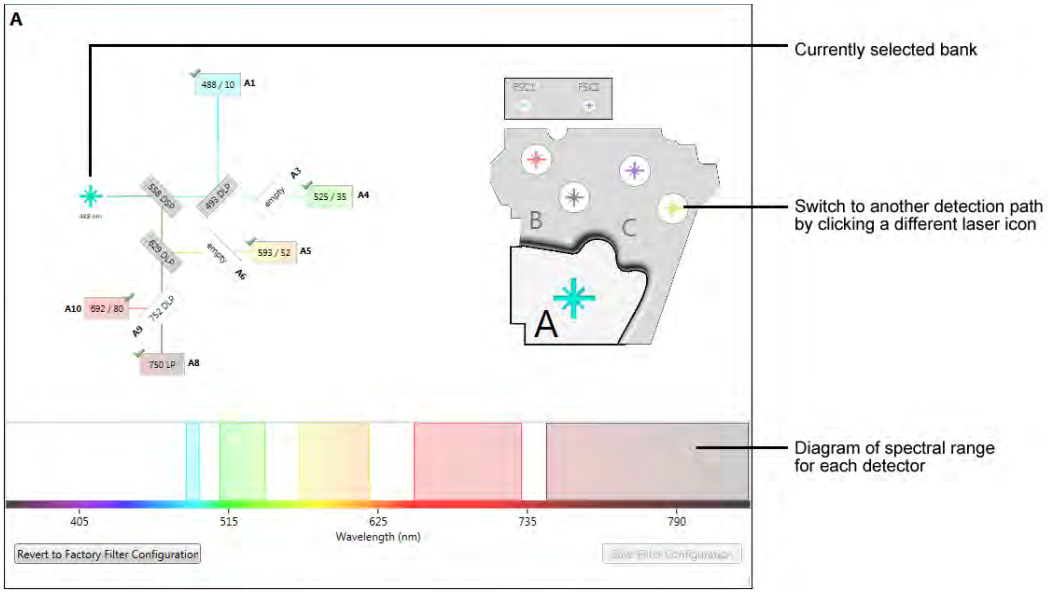
Editing the Optical Filter Configuration

If you make any changes to the physical optical filters, an administrator can use Everest Software to reflect these changes in the optical filter configuration. Any changes made to the software configuration will be automatically saved for the current application session.

To view and edit the optical filter configuration

1. Click Display Optical Filter Configuration in the toolbar.

The Filter Configuration dialog box opens.



2. In the filter bank diagram on the right, click the laser symbol for the laser that corresponds to the filter that you are replacing.

   The detection paths for the laser appear on the left.

3. Click a final pathway position.

   In the spectral plot at the bottom, this highlights the range of wavelengths allowed through the entire detector path.

4. Select the label in the final pathway position and replace it with the label for the new filter.

   Tip: Notice how the path's allowed wavelength range changes in the spectral plot.

5. Do one of the following:
   - To save the changes only for the current application session, close the Filter Configuration dialog box.
   - To save the changes across application launches, click Save Filter Configuration.

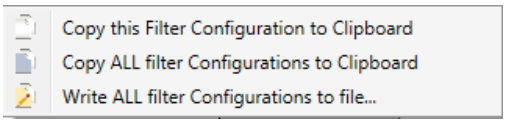
   The ZE5-EYE process runs automatically.

Exporting Optical Filter Configurations

You can copy a single filter configuration or all filter configurations to the clipboard. The copied item, in BMP graphic format, can then be pasted into another application. You can also write all filter configurations to a single BMP file.

To export optical filter configurations

1. Right-click the detection path diagram or the filter bank diagram in the Filter Configuration dialog box.
2. Select an option from the menu that appears.



3. If you selected one of the Copy options, paste the graphic into the application of your choosing.
4. If you selected the Write option, select a location for the file and click Save.

Reverting to the Default Optical Figure Configuration

Administrators can revert to the original filter configuration settings in the software. Before performing this task in the software, ensure that the original filters are all in their original slots.

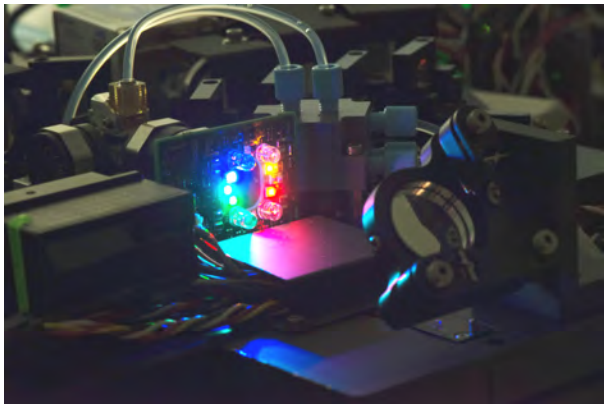
To revert to the default optical filter configuration

1. In the Filter Configuration dialog box, click Revert to Factory Filter Configuration.
2. Click OK when you are asked to confirm.

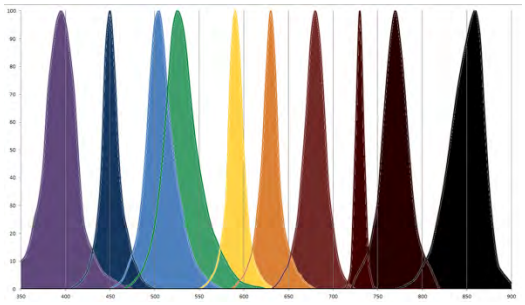
   The ZE5-EYE process runs and a success or failure message appears in the status bar.

Using the ZE5-EYE to Confirm Filter Choices

The ZE5-EYE, shown in the next figure, uses multiple LEDs to pulse ten different wavelengths of light through the instrument's optical detection paths.



The next figure shows a graph of these light wavelengths.

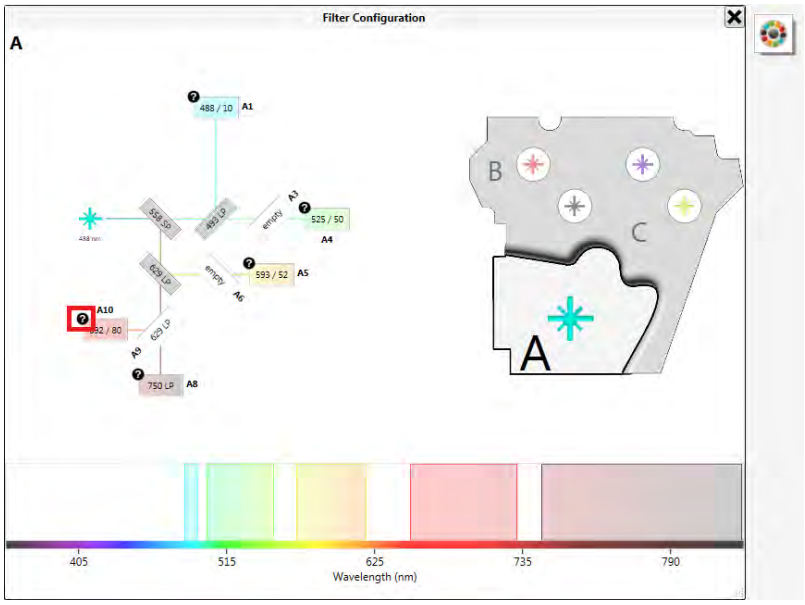


The ZE5-EYE runs automatically at these times:

- as part of the Startup and QC processes
- when the optical filter access door is opened for at least 5 sec and then closed
- when an optical filter configuration is saved in the software
- when the optical filter settings are reverted to the factory settings

Administrators can edit the optical filter configuration to correct problems detected by the ZE5-EYE process.

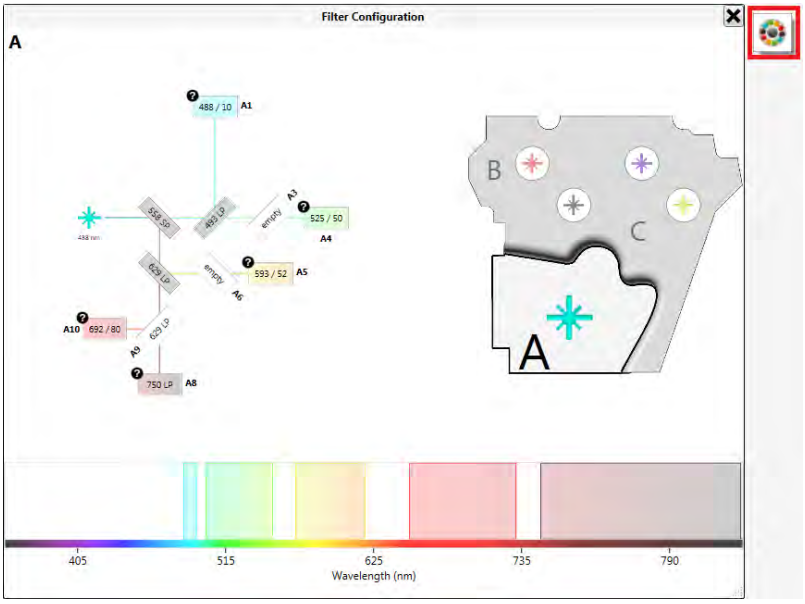
Before the ZE5-EYE process runs, a black circle with a question mark appears next to each detector, as shown in the next figure.



Note: If the ZE5-EYE runs while you have plots open in an experiment workspace, data might appear in the plots.

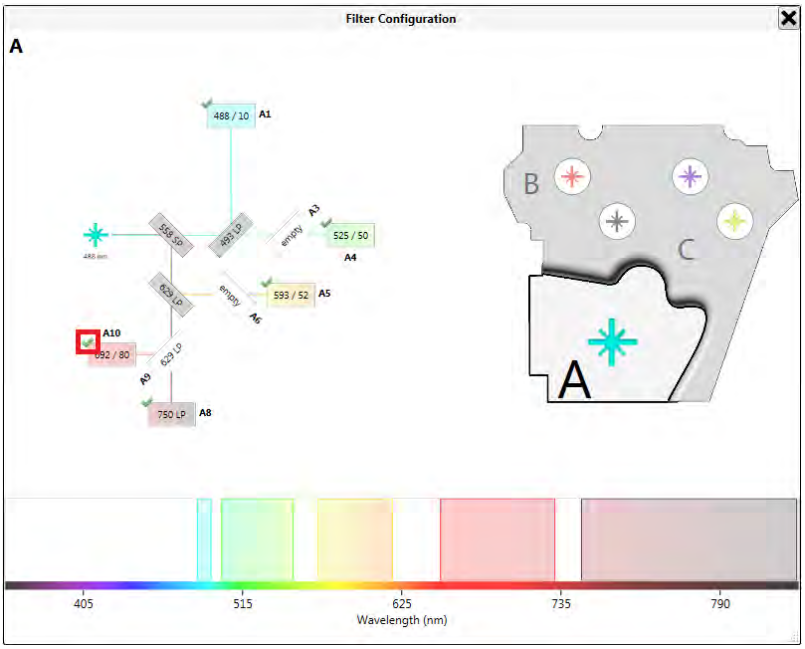
To confirm filter choices using the ZE5-EYE

1. If the ZE5-EYE did not run automatically, click the ZE5-EYE button located to the right of the Filter Configuration dialog box, as shown in the next figure.

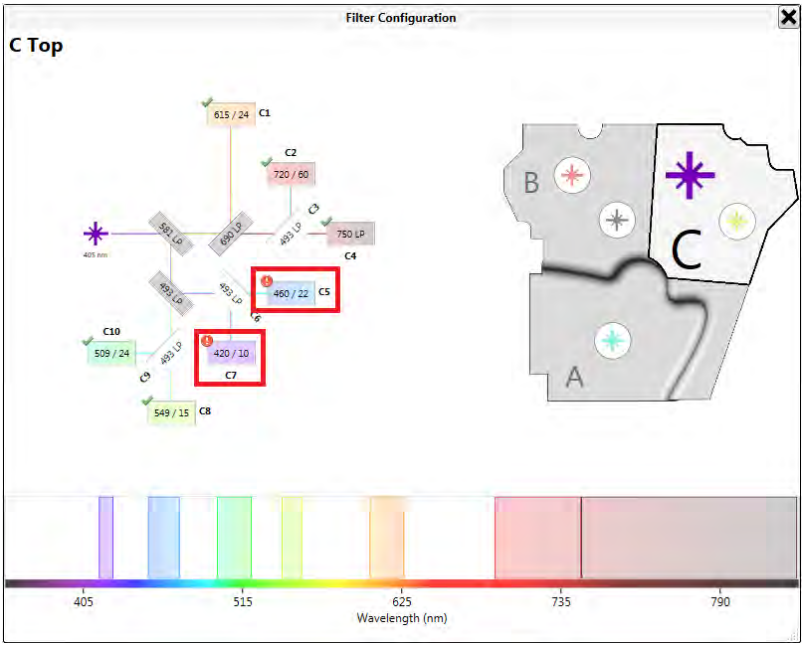


ZE5-EYE success or failure is shown as follows:

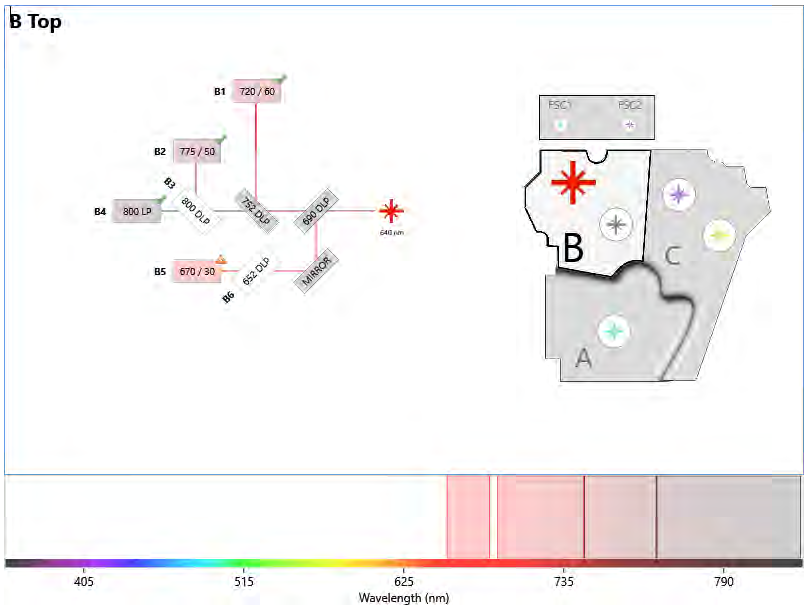
- If the ZE5-EYE runs successfully, a success confirmation message appears in the status bar and a green check mark appears next to the final filter position for each detector.



- If a filter has been changed without a corresponding filter label modification in the software, an error appears in the status bar and a red circle with an exclamation point appears next to each position that caused the ZE5-EYE process to fail.



- If the voltage change required to center the peak around channel 128 exceeds the value set in the QC criteria, an error appears in the status bar and an orange triangle with an exclamation point appears next to the final filter position for the detector. For more information, see Editing QC Criteria on page 92.



2. To resolve the ZE5-EYE failure, ensure that the correct optical filter is placed in its slot, and edit the optical filter configuration to reflect this.

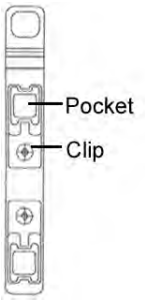
Installing a Neutral Density Filter

To alter the range of detection sensitivity of an SSC or fluorescence detector, you can replace a bandpass filter with a neutral density filter. For more information about neutral density filters, see Optical Mirror and Filter Types on page 32.

Tip: When handling the filter stick, wear gloves, handle filters by the edges, and be careful not to touch any filter surfaces.

To insert a neutral density filter into a filter stick

1. Remove the filter stick from the instrument, if needed.



2. Remove the metal clip that holds the bandpass filter.
3. Lift the bandpass filter out of the filter pocket.
4. Place the neutral density filter into the pocket.
5. Replace the bandpass filter over the neutral density filter, preserving its original orientation.
6. Re-install the metal clip.
7. Re-insert the filter stick into the instrument.

Note: Installing a neutral density filter causes a reduction in light detection, and can cause the affected filter position to be flagged by the ZE5-EYE. This ZE5-EYE failure serves as a reminder to remove the neutral density filter at the end of the experiment that requires it.

For information about using Everest Software to enable and disable the neutral density filters located in front of the FSC detectors, see PMT and Laser Controls on page 208.

Chapter 6 Daily Routine

This chapter covers the tasks that you must perform either on a daily basis or on each day that the ZE5™ Cell Analyzer system is used. Ensure that an administrator has already set up the system according to the instructions in Chapter 5, Configuring the System.

Although the daily startup, quality control, and shutdown procedures can be automated, Bio-Rad recommends that you familiarize yourself with the ZE5 Cell Analyzer and Everest™ Software by reading the following chapters before you begin to use the instrument:

- Chapter 2, Hardware Description
- Chapter 3, ZE5 Loader
- Chapter 4, Everest Software
- Chapter 7, Creating Experiments and Workspaces
- Chapter 8, Acquiring Samples
- Chapter 10, Analyzing, Saving, and Printing Data

System Power

Do not turn off the ZE5 Cell Analyzer system by using the main power switch. The system is safe in standby mode after a software shutdown. This facilitates quick or automated startup. If you do need to turn instrument power off, shut down Everest Software first.

If the instrument is shut off using the main power switch, always power the instrument on before starting Everest Software. The instrument must be running so that Everest Software can communicate with it.

Important: Powering the system on or off incorrectly can cause the instrument to malfunction.

For information on the power switch, see Power and Communication Connections on page 21. For information on shutting down Everest Software, see Shutting Down on page 129.

Starting Everest Software

Double-click the Everest Software icon on the desktop to launch the software.

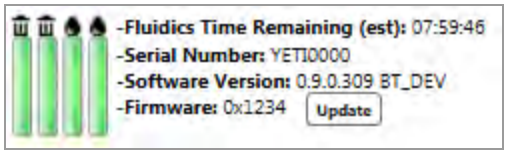
Checking Fluidics Status

Six fluidics bottles are located behind the fluidic door on the left front of the instrument:

- Two sheath/DI water bottles
- Two waste bottles
- One cleaner bottle
- One additive bottle

These bottles require maintenance on a regular basis.

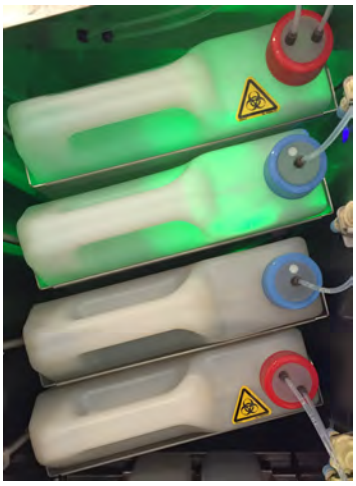
Before logging in, click the Details down arrow to examine the fluidics status, including the estimated remaining run time with the current bottle levels. Ensure that the waste bottles are sufficiently empty and the sheath fluid bottles are sufficiently full, but not overfull.



After logging in, you can check fluidics status from the toolbar. For more information, see the list of fluidics status items in System Tools on page 78.

The pair of bottles illuminated by the green LEDs in the bulk fluidics chamber is in use, as shown in the next figure. The pair that is not illuminated is the pair that can be emptied of waste or filled with DI water during operation.

Important: Ensure that replacement bottles are securely connected so that the system can transition to them.



Refilling Bulk Fluidics

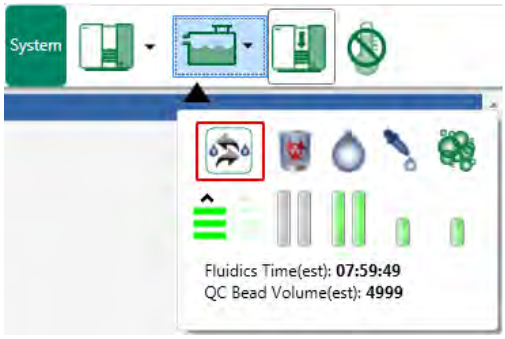
Important: Follow these guidelines when working with fluidics bottles:

- When handling bottles, always wear gloves and minimize air exposure to help avoid contamination.
- When transferring a cap assembly to a new bottle, avoid touching the exterior bottle surface with the cap assembly. If you need to set a cap assembly down, place it on a sterilized surface.
- For safety, treat all waste as biohazardous.

Waste bottles are sealed with red caps. Each waste bottle has a run time of about 4 hr and must be emptied when full. Everest Software provides warnings 1 hr, 30 min, and 5 min before both waste bottles are nearly full, allowing you to swap the fluidics. If the waste bottles are not changed before they are full, the system shuts down to avoid overfilling.

The sheath/DI water bottles are sealed with blue caps. Each sheath bottle has a run time of about 4 hr and must be filled when empty. Everest Software provides warnings 1 hr, 30 min, and 5 min before the sheath bottles are nearly empty, allowing you to swap the fluidics. To avoid running the system dry, shutdown occurs when 5 min of sheath fluid remain.

After the first pair of bottles has been fully utilized, the system automatically switches to the second pair. Additionally, you can force the system to switch to the upper or lower pair of bottles by clicking Switch the Active Sheath and Waste Bottles in the System section of the toolbar, as shown in the next figure.



Emptying Waste Bottles

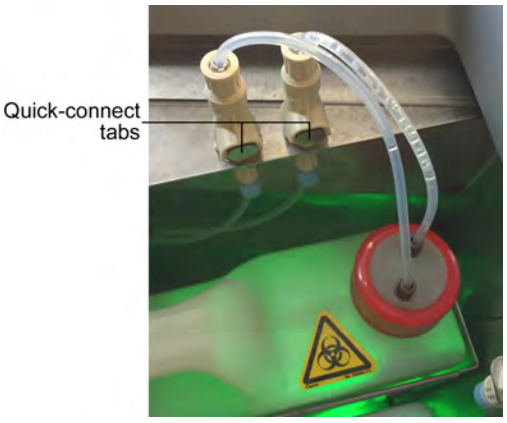
Each waste bottle has two connections to the instrument. One connection is a fluidics connection, and the other is an air vent connection to ensure that air is properly displaced as fluid is pumped into the bottle. Any air vented from the waste bottle passes through a 2 µm filter. The waste fluidics and air vent connections are interchangeable. Waste and sheath bottle connections are specifically keyed to their respective ports and cannot be connected to the wrong ports.

Important: When removing the waste bottle, ensure that the bottle cap is firmly tightened; otherwise, leakage might occur.

Caution: Biohazard! Please contact your safety officer or local health and safety bodies regarding proper treatment and disposal of biohazardous waste.

To empty the waste bottles

1. Push the quick connect tabs on both connections to disengage the tubes from the instrument.



2. Pull the waste bottles out of the instrument.
3. Unscrew the cap assemblies and remove them from the bottles.
4. Empty the bottles following lab procedures for liquid biohazardous and chemical waste removal.

5. Carefully place the cap assemblies onto the emptied waste bottles and tighten the caps.
6. Place the waste bottles back into the instrument.
7. Attach the cap assembly tubing to the connectors on the instrument.

   Tip: An audible click indicates that the tubing is connected.

Refilling Sheath Bottles

Each sheath bottle has a single connection to the instrument. Waste and sheath bottle connections are specifically keyed to their respective ports and cannot be connected to the wrong ports.

Important: When refilling the sheath fluid bottles:

- Ensure that the filter and tubing are not contaminated when you remove the cap assemblies from the sheath bottles.
- Ensure that fluid reaches no higher than the fill line, as shown in the next figure, so that fluid does not leak from the air vent port in the cap.

To refill the sheath bottles

1. Push the quick connect tabs on the connections to disengage the tubes from the instrument.

2. Pull the sheath bottles out of the instrument.
3. Unscrew the cap assemblies, remove them from the bottles, and pull the tubing out of the bottles.

   Notice the 90 µm filter at the bottom of the fluidics line.

4. Refill the bottles with DI water or replace them with new ones.
5. Carefully place the cap assemblies onto the bottles and tighten the caps.

   Important:

   - Ensure that the filter falls to the corner of the bottle diagonally opposite from the cap, so that the entire contents of the bottle can be drawn into the instrument without risking introduction of air.
   - Ensure that the bottle cap is firmly tightened; otherwise, leakage might occur.
6. Place the sheath bottles back into the instrument.
7. Attach the cap assembly tubing to the connectors on the instrument.

   Tip: An audible click indicates that the tubing is connected.

Refilling Sheath Additive and Cleaner Bottles

The sheath additive and cleaner bottles are located at the bottom of the fluidics chamber. They are hot-swappable at any time other than during shutdown.

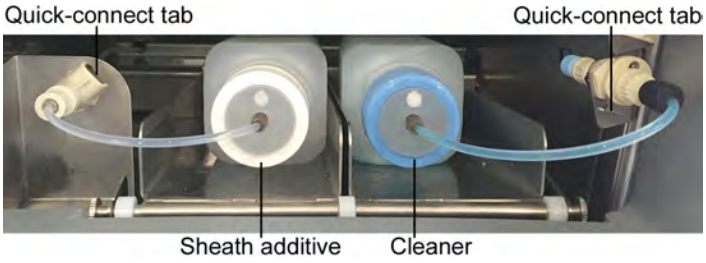
Sheath additive and cleaner bottle connections are specifically keyed to their respective ports and cannot be connected to the wrong ports: the white-capped sheath additive bottle always goes on the left and the blue-capped cleaner bottle always goes on the right.

Important: When filling the sheath additive or cleaner bottles:

- Fill the bottle only to the fill line to ensure that liquid does not leak from the vent in the cap.
- Ensure that the tubing that extends into the bottle is not contaminated during the refill procedure.

To refill an additive or cleaner bottle

1. Push the quick connect tab on the connection to disengage the tube from the instrument.



2. Pull the bottle out of the instrument.
3. Unscrew the cap assembly and remove it from the bottle.
4. Refill the white-capped bottle with sheath additive, refill the blue-capped bottle with cleaner, or replace a bottle with a new one.

5. Carefully place the cap assembly onto the bottle and tighten the cap.
6. Place the bottle back into the instrument.
7. Attach the cap assembly tubing to the connector on the instrument.

Tip: An audible click indicates that the tubing is connected. If no click is audible, salt might have built up on the connector. Salt can be removed using a paper towel moistened with DI water.

Important: Ensure that the bottle cap is firmly tightened; otherwise, leakage might occur.

Logging In

The login window is divided into two panes: Instrument Status and User Login, as described in Login Window on page 46.

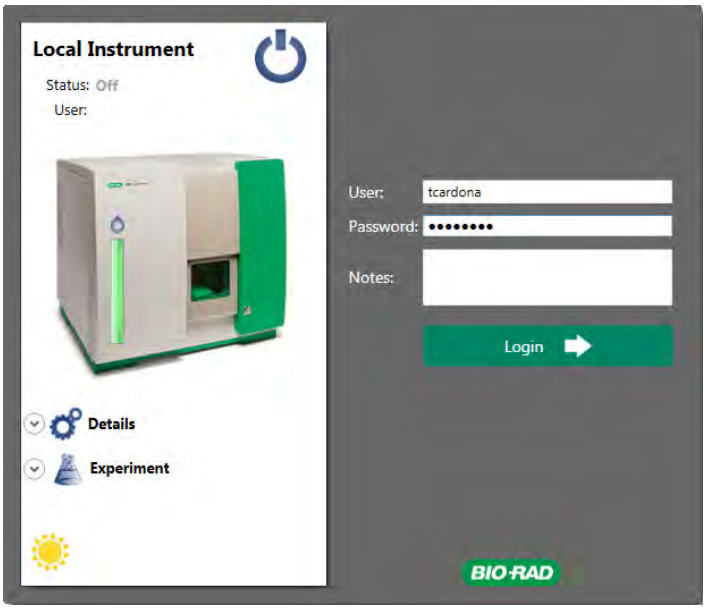
If the software is connected to an instrument, an instrument picture appears in the Instrument Status pane. Before proceeding, check the instrument status:

- A status of Ready indicates that the system has been started up and is running.
- A status of Off indicates that the instrument has been shut down and has not yet been started up.

You can refill fluidics before logging in; you must log in before you can manually run the QC process or run samples. You cannot log in during the startup or QC process.

To log in to Everest Software

1. Enter your User name and Password.



2. (Optional) If needed, add notes in the Notes box. These user session notes are saved to the user log and can be recalled by generating administrative user reports. For more information on user reports, see Generating User Reports on page 257.
3. Click Login.

Note: After you log in, your usage time is recorded in the user database.

Starting Up the System

Before running samples, the instrument must be powered on and Everest Software must perform system startup. Bio-Rad recommends that you leave the system powered on at all times and that you perform the shutdown procedure in Everest Software at the end of each day.

Note: In Everest Software, a system status of Off indicates that the ZE5 Cell Analyzer is shut down but not powered off. If the ZE5 Cell Analyzer is powered off, a Check Instrument message appears in the software Login window.

Important: Powering the system on or off incorrectly can cause the instrument to malfunction.

The startup process turns on the lasers, pressurizes the internal sheath tank, and initiates sheath fluid flow through the flow cell. The unclog and ZE5-EYE processes run immediately after the startup process.

You can run the startup process after logging into the system. If your system administrator has allowed it, you can also run the startup process before logging into the system.

For more information, see Unclogging the Sample Line and Probe on page 261, Using the ZE5-EYE to Confirm Filter Choices on page 109, Running Quality Control on page 126, and Specifying Logged Out Settings on page 93.

To start up the instrument

1. Click Startup.
2. To view startup details, click the down arrow.

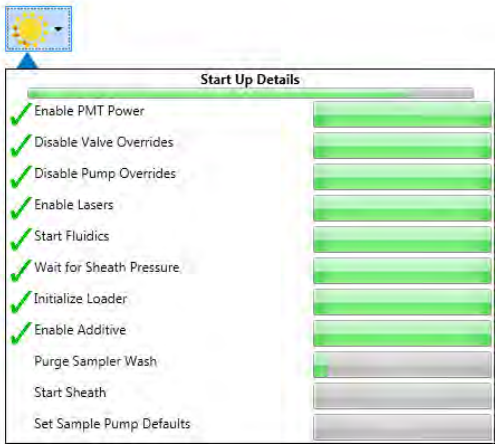
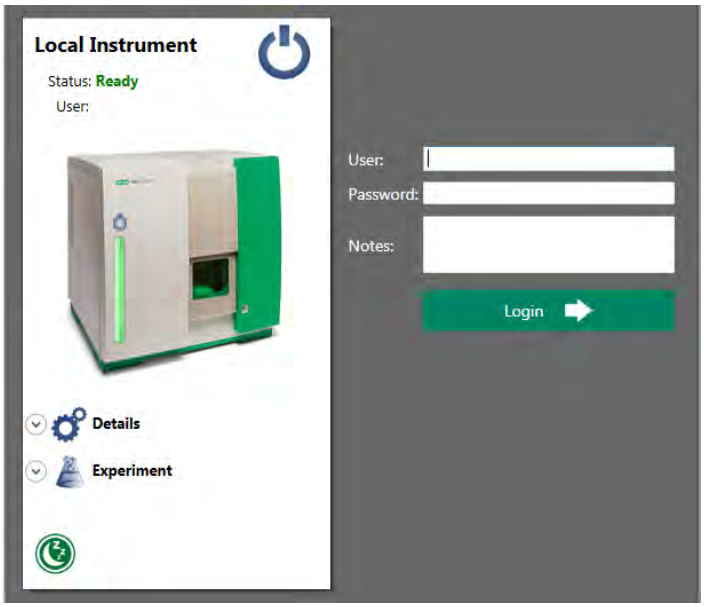


Table 32. Steps in the startup process

Enable PMT Power	Turns on power to the PMTs.
Disable Valve Overrides	Places all valves in proper states.
Disable Pump Overrides	Places all pumps in proper states.
Enable Lasers	Turns on and enables the lasers so they are ready to deliver light to the flow cell when the QC process is initiated.
Start Fluidics	Initializes the fluidics system.
Wait for Sheath Pressure	Starts the air pump so that it pushes air into the internal sheath reservoir and waits until the pressure reaches 10 psi.
Initialize Loader	Homes and sets up the loader so it is ready to run samples.
Enable Additive	Enables the valve to begin the process of introducing additive into the internal sheath reservoir.
Purge Sampler Wash	Prepares the wash station.
Start Sheath	Starts sheath fluid flowing through the system.
Set Sample Pump Defaults	Enables the sample pump.

After startup, the status changes to Ready and the Shutdown button appears in the Login window (if you have not logged in yet) or in the Home window, if you have logged in.



After logging in, run the Quality Control process.

Running Quality Control

The Quality Control (QC) process can be activated in the following places in Everest Software:

- Home window
- Tools section of the Toolbar

The QC process utilizes single-peak beads conveniently located on board. The beads contain a mixture of dyes that can be excited by all lasers installed on the ZE5 Cell Analyzer and that can emit in every fluorescence channel. The level of QC beads is monitored and displayed along with the fluidics status information.

The process first builds a QC workspace and begins acquiring beads. It then ensures that the system can acquire the beads at both 150 events per second and 500 events per second. Then, at 500 events per second, the system automatically adjusts laser delays and PMT voltages so that the mean intensity of the bead population falls into data channel 128 in all fluorescence channels. CV increases and PMT voltage changes are compared against the QC criteria. Finally, a 5,000-event file is collected at 150 events per second and voltages and CVs are stored for reporting purposes. These values can be displayed, based on the date the process was run, in daily QC reports and QC trending reports.

While QC is in progress, click the down arrow to display the QC Details window, which provides real-time updates on each portion of the process.

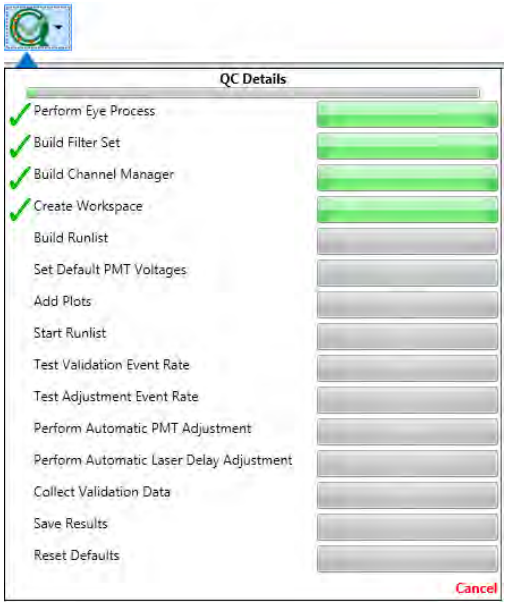


Table 33. Steps in the QC process

Perform ZE5-EYE Process	Checks the physical optical filter configuration in the instrument against the software.
Build Filter Set	Configures acquisition according to the filter set currently installed in the instrument.
Build Channel Manager	Configures channels for acquisition.
Create Workspace	Creates a workspace containing a FSC x SSC plot and a univariate histogram for each channel.
Build Runlist	Builds a run list (including loader movement, accessing the QC bead bottle, and bead agitation) for the QC process.
Set Default PMT Voltages	Sets PMT voltages to the values determined at the end of the last successful QC process.
Add Plots	Adds plots to the workspace.
Start Runlist	Initializes and begins acquisition.
Test Validation Event Rate	Tests whether the system can acquire beads at 150 events per second.

Table 33. Steps in the QC process, continued

Test Adjustment Event Rate	Tests whether the system can acquire beads at 500 events per second.
Perform Automatic PMT Adjustment	Adjusts PMT voltages so that the mean value of the bead population in all fluorescence channels falls into data channel 128.
Perform Automatic Laser Delay Adjustment	Automatically sets laser delays so that the relevant signals are coordinated and attributed to the correct cells.
Collect Validation Data	Adjusts the event rate to 150 events per second and collects a 5,000-event file.
Save Results	Stores data, voltages, and CVs.
Reset Defaults	Resets all voltages so that you can proceed with your experiment.

The QC workspace loads automatically regardless of whether it was initiated from the Home window or Workspace screen. If you are currently in the Home window, click Resume to access the workspace.

After the QC process is complete, you can access the QC report from the Reports section of the toolbar.

For more information about reports, see Quality Control and ZE5-EYE Reports on page 253.

Accessing the Loader

To open the loader door and extend the loader

▶ Do one of the following:

- Press the silver sample chamber button on the front of the instrument.
- Click the Door toggle in the toolbar.

The external light turns on when the door is opened.

The button also controls the inner chamber light when the door is closed.

To turn the internal illumination on and off

▶ Press and hold the silver sample chamber button.

Running Experimental Samples

At this point in the daily routine, you can run experimental samples.

- For information on applying compensation, see Chapter 9, Applying Fluorescence Compensation and Appendix B, Example 9-Color Immunophenotyping Experiment.
- For information on acquiring experimental samples, see Chapter 8, Acquiring Samples.

Pausing the System

To conserve bulk fluidics and prolong laser lifetime, you can pause the system.

To pause the system

▶ In the System section of the toolbar, click Pause System.

A message in the Control Panel indicates that the system is paused.

To restart the system

▶ In the System section of the toolbar, click Pause System again.

Shutting Down

Important: Do not shut down the ZE5 Cell Analyzer using the main power switch. Refer to System Power on page 115 for more information.

Shut down the ZE5 Cell Analyzer system at the end of each day of use. This turns off all lasers; cleans the sample line, probe, and flow cell with cleaner; depressurizes the fluidics; and logs you out of the system. The shutdown process is entirely automated and does not require user intervention.

Important: Always follow the personal protective equipment (PPE) guidelines relevant to your laboratory's safety procedures for dealing with ethanol or bleach.

The Shutdown button can be found in the following locations:

- Toolbar (Quick Actions section)
- Home window

It can also be found in the Login window, if your system administrator has allowed shutdown to occur when no user is logged in. For more information, see Specifying Logged Out Settings on page 93.

Shutdown cannot be performed if acquisition is in progress.

To shut the system down at the end of the day

1. Close the loader door.
2. Click Shutdown.

   The system asks you whether you want to schedule an automatic startup.
3. Do one of the following:
   - Select No to skip scheduling of an automatic startup.
   - Select Yes to schedule an automatic startup. Specify a restart date and time.
4. Click the check mark.

   The Shutdown Progress symbol appears in the Toolbar. If you scheduled an automatic startup, the countdown to startup begins.

   Important: If Everest Software is closed, any scheduled automatic startup will not be performed. Leave the software open if an automatic startup is scheduled. The sheath bottles must be sufficiently full and the waste bottles must be sufficiently empty to allow 2 hr of run time. If the system automatically starts up and no user logs in within 1 hr, the system shuts down.
5. Empty the waste bottles and refill the sheath bottles if needed.
6. To view shutdown details, click the down arrow.

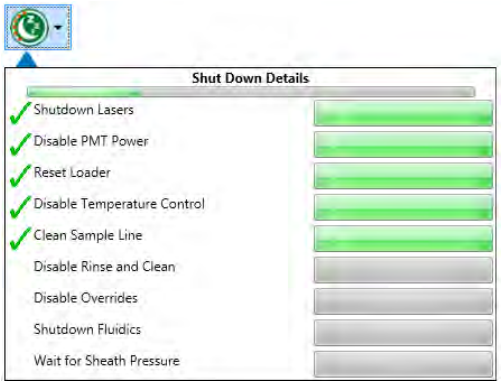


Table 34. Steps in the shutdown process

Shutdown Lasers	Turns off the lasers.
Disable PMT Power	Turns off power to the PMTs.
Reset Loader	Homes the loader so that it is in the appropriate position for the shutdown process.
Disable Temperature Control	Turns off the temperature control device (TEC).
Clean Sample Line	Performs rigorous cleaning of the sample line and probe using both cleaner and sheath fluid.
Disable Rinse and Clean	Lowers rinse and clean pressure and puts valves and pumps into their shutdown states.
Disable Overrides	Puts all pumps and valves in their proper states.
Shutdown Fluidics	Configures the fluidics system for shutdown. Begins lowering sheath fluid pressure.
Wait for Sheath Pressure	Waits until sheath fluid pressure reaches ~1 psi. At that point, Shutdown ends and you are logged out of Everest Software.

Scheduling Automatic Startups

When you perform a shutdown, you can schedule a date and time to restart the ZE5 instrument and Everest Software automatically. The sheath bottles must be sufficiently full and the waste bottles must be sufficiently empty to allow 2 hr of run time.

After the Auto Startup function is set, the countdown to startup begins.

Important: If Everest Software is closed, any scheduled automatic startup will not be performed. Leave the software open if an automatic startup is scheduled. If the system automatically starts up and no user logs in within 1 hr, the system shuts down.

Stopping Everest Software

Important: Do not exit the software during sample acquisition or during the startup, shutdown, decontamination, or QC process.

To exit Everest Software

▶ Click Close in the upper right corner.

Note: If the software cannot close immediately, Bio-Rad recommends waiting for the software to finish closing, rather than forcing a close by clicking I Can't Wait.

Chapter 7 Creating Experiments and Workspaces

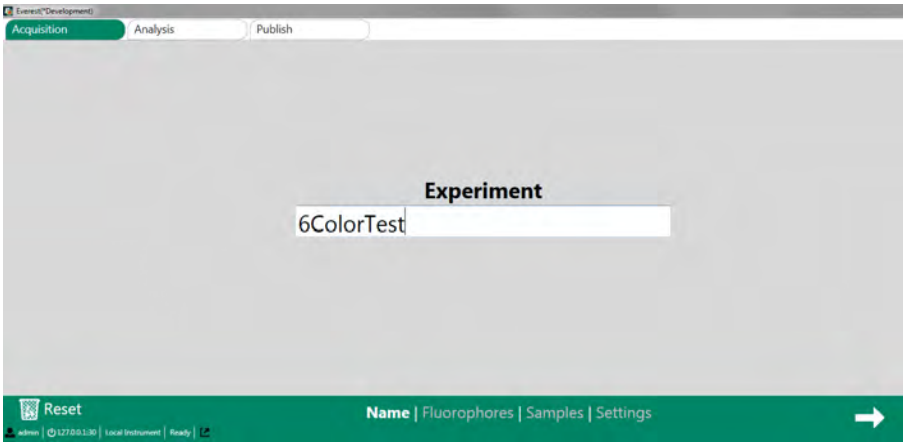
The following section guides you through the processes of creating a new experiment, loading an existing experiment, and resuming a paused experiment. It also shows you how to set up stat tubes, so that you can run single samples quickly, or add a single sample to an experiment that uses a tube or rack.

Creating a New Experiment

In the initial screen of the Experiment Builder, you assign an experiment name. The experiment name also becomes the default name of the first panel in the experiment. Experiment and panel names can be changed on later screens.

To create a new experiment

1. After logging in, click New Experiment on the Home window.
2. Enter a name for the experiment and click the Next arrow.



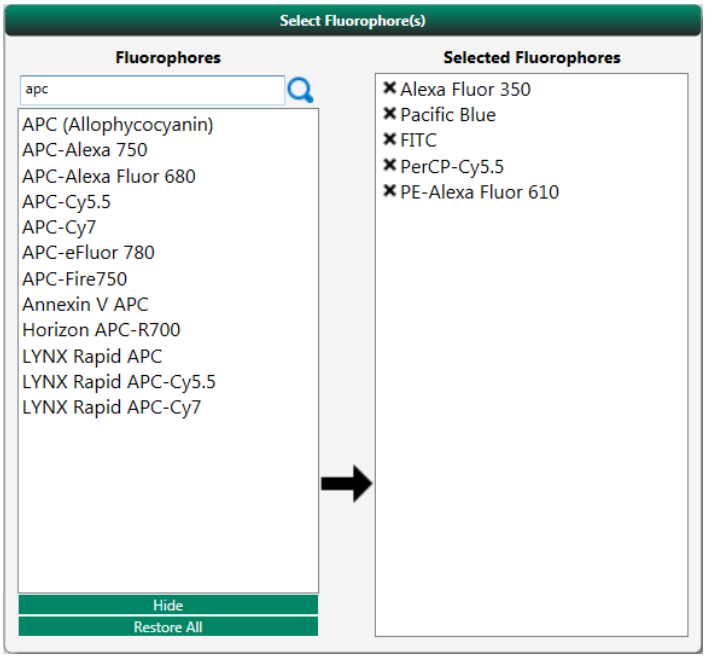
Tip: If needed, you can set up a single plate or tube rack with multiple experimental panels. For more information, see Multipanel Experiments on page 71 and Setting Up Multiple Panels on page 210.

Selecting Fluorophores

Everest™ Software makes it easy to find and select the fluorophores needed for your experiment.

To select fluorophores

1. Begin finding the fluorophores being utilized for the experiment from the list of fluorophores. Use the Search box to type at least the first few letters of the fluorophore in question. The list narrows to those fluorophores relevant to the search.

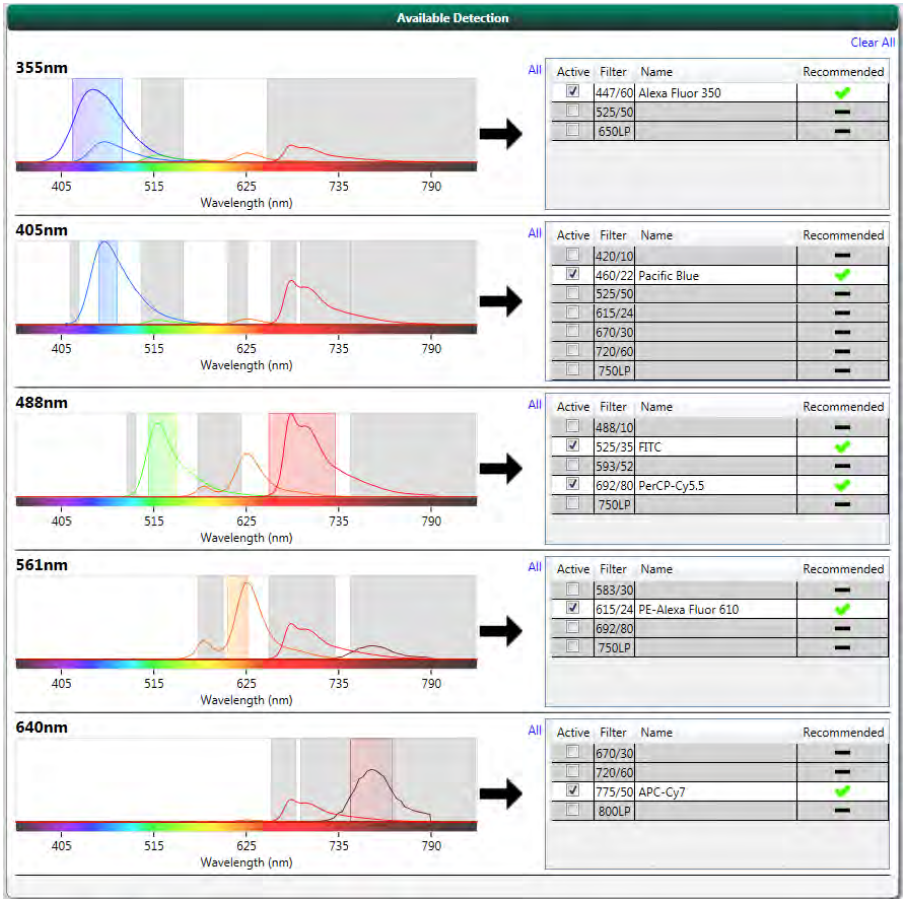


2. Double-click the fluorophore that you want to add.

   The fluorophore is moved to the Selected Fluorophores list.

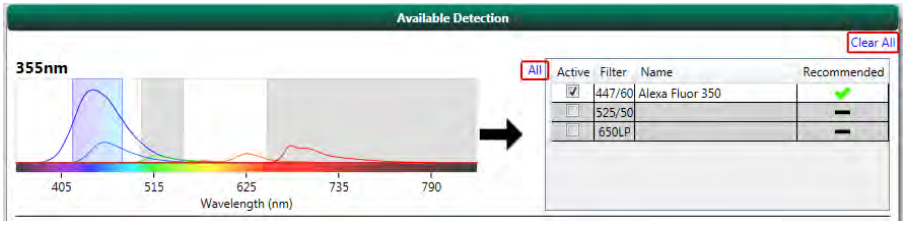
   In the Available Detection panel, the following happens:

   - As fluorophores are added, the emission spectra of each fluorophore appear on the emission plot associated with the fluorophore's optimal excitation laser line.
   - The fluorophore is automatically assigned the most appropriate detector, based on the instrument's current configuration.
   - The Active checkbox is selected.
   - The Name column is populated with the fluorophore name.



Tip: In a spectral graph containing multiple spectra, point to a spectral line to view the name of the corresponding fluorophore.

3. To activate all detectors for a particular laser, click All for that laser.



4. To clear the entire list of selected fluorophores and active detectors, click Clear All in the upper right of the Available Detection pane.
5. If necessary, edit the parameter Name to include more descriptive information for each detector, such as target (for example, CD19).

6. If needed, activate detectors manually.
    a. Select the Active checkbox for the detector.
    b. Rename the detector in the Name box.
7. Click the Next arrow to go to the Samples screen.

Setting Up the Run List

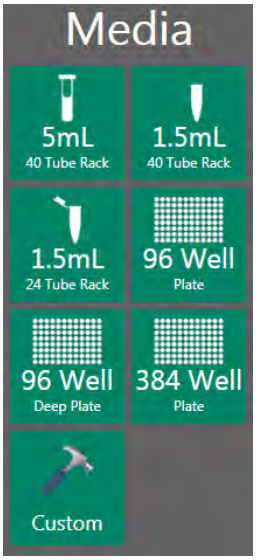
In the Samples screen, you define the run list by specifying how samples are processed. Settings include media type, well/tube positions, sample names, compensation controls, volume, limit, target flow rate or target event rate, sampling speed, wash, agitation, temperature, addition of reagent, and pauses.

Selecting the Media Type

You can select from six predefined media (sample input device) types or create a custom media type. For more information about media types, see Media Selector on page 56.

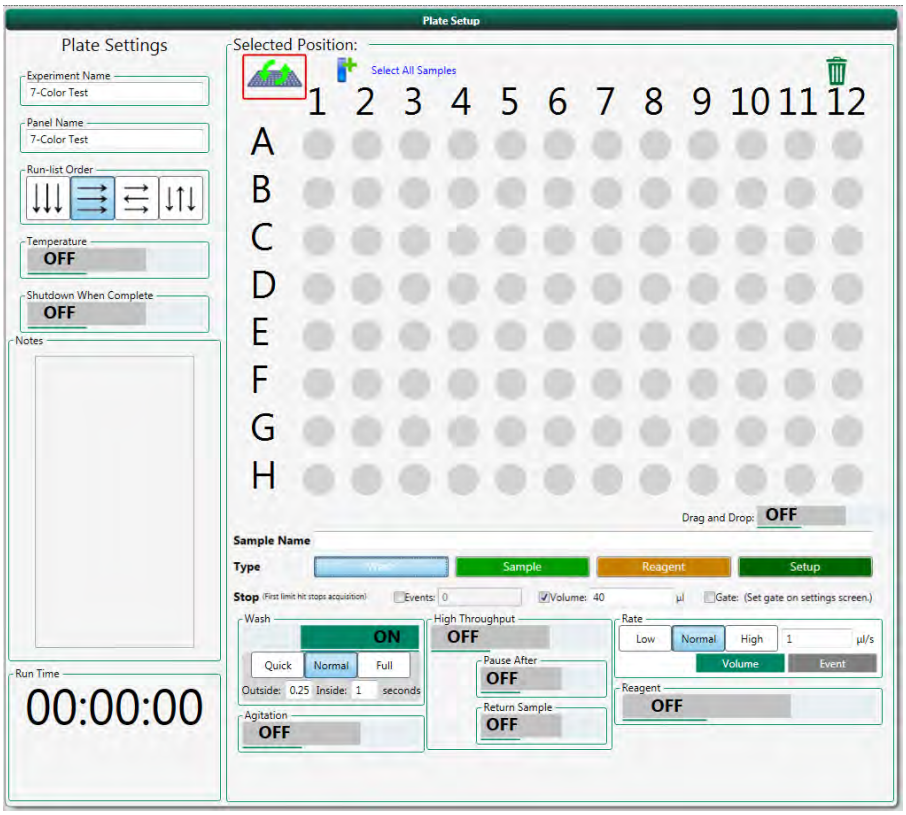
To select the media type

▶ Click the appropriate button in the Media list.



The plate setup changes to reflect the selected media.

Tip: If you make a mistake and need to change the selected media type, click Switch Media in the upper left corner of the Selected Position panel.



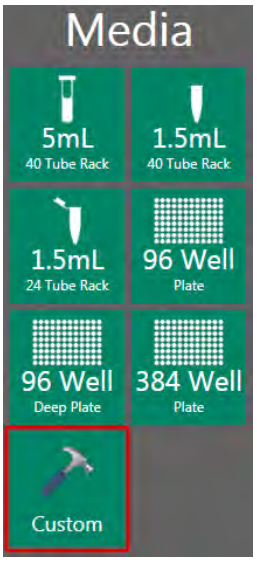
The Experiment Name reflects the name entered in the Name screen, and can be modified here if needed.

Creating a Custom Media Type

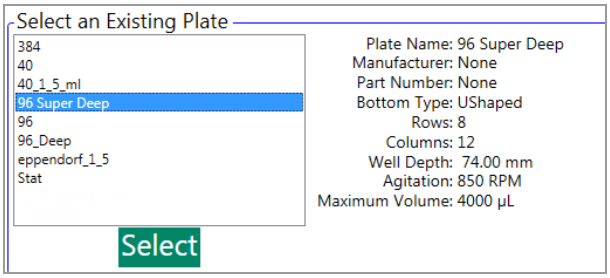
If none of the six predefined media types match the device you intend to use, you can create a custom media type.

To create a custom media type

1. Select the Custom device type.

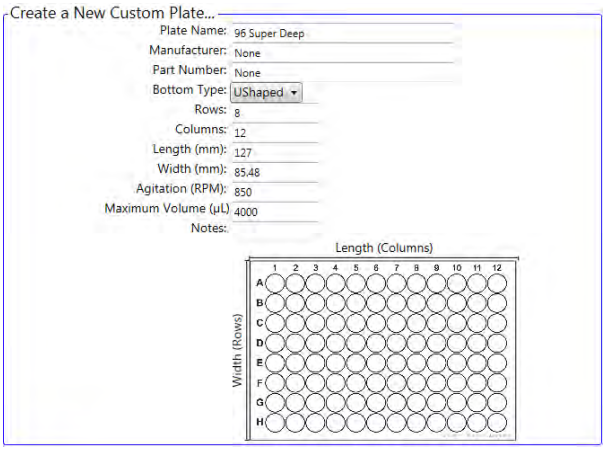


2. If your custom device resembles a type of existing plate (device), select it to use it as a template.



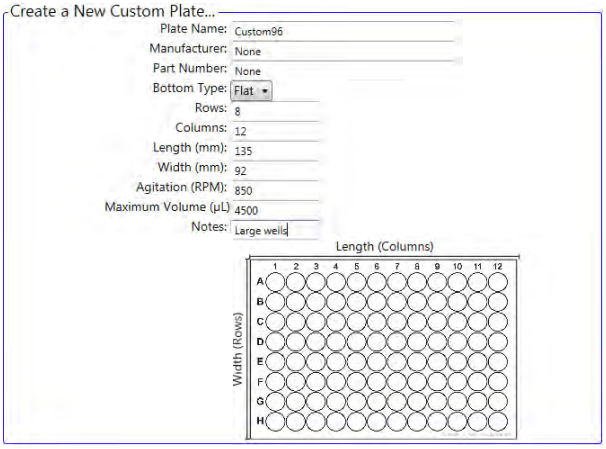
3. Click Select.

   The settings for the existing plate pre-populate the settings for the custom device.



4. In the Create a New Custom Plate area, edit plate settings as needed, taking into account any differences between your custom device and the one selected as a template.

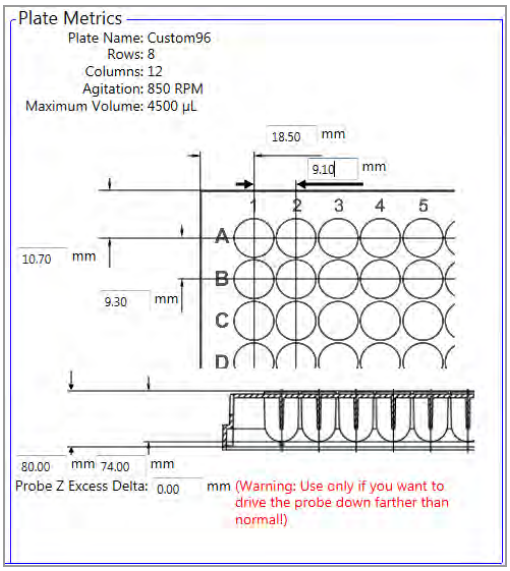
   Note: If you select an existing device as a template but you do not change the Plate Name value, the calibration for the existing device will be modified.



5. Click Next.

6. In the Plate Metrics area, click inside each text box.

   The bold arrows indicate the dimensions that correspond to the text box, for example, the horizontal distance between the centers of two wells, shown as 9.10 mm in the next figure.



Note: If the probe is not dropped low enough into a well, entering a value for Probe Z Excess Delta drives the probe down lower. If the probe hits the well bottom, this information is used in calibrating the probe Z value.

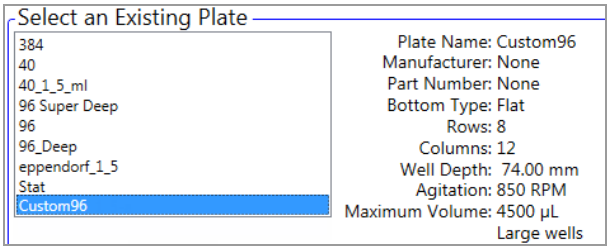
7. Edit the dimensions as needed and click Next.
8. Place the custom device into the sample loader and press Continue.
9. Determine whether the probe has entered the first position on the custom device.
   - If the probe is centered in the first position, click Yes.
   - If the probe is not centered in the first position, click No, use the arrows to adjust it to the center of the well, and click Accept Current Location. Repeat this process as needed.

10. Determine whether the probe has entered the last position on the custom device.
    - If the probe is centered in the last position, click Yes.
    - If the probe is not centered in the last position, click No, use the arrows to adjust it to the center of the well, and click Accept Current Location. Repeat this process as needed.

    The layout of the custom device appears in the Plate Setup panel.

To select a custom plate

1. Select the Custom device type from the media selector.



2. Select the created device in the list of plates.

3. Click Select.
4. The layout of the custom device appears in the Plate Setup panel.

Plate Settings Controls

The Plate Settings controls apply to the entire plate or tube rack, with the exception of panel names.

Table 35. Plate Settings items and their functions

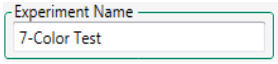
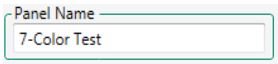
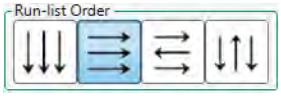
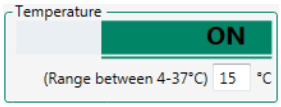
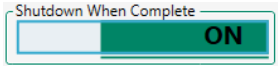
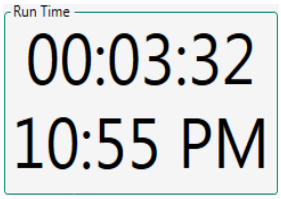
Item	Function
	Displays the name of the currently loaded experiment.
	Displays the name of a panel (group of samples) within the experiment that has uniquely configured parameters, parameter names, or settings.
	Shows the route the sampler will take across the tube rack/plate.
	Runs each column in turn, starting with column 1.
	Runs each row in turn, starting with row A.
	Runs in serpentine fashion, row by row. Runs A1 through A8, B8 through B1, C1 through C8, and so forth.
	Runs in serpentine fashion, column-by-column. Runs A1 through E1, E2 through A2, A3 through E3, and so forth.
 	Allows you to set the sample temperature from 4–37ºC, in 1º increments. The default is 15ºC. The actual temperature is shown in the workspace after run list creation is complete.
	When turned on, the system shut downs automatically after the entire run list has been acquired.

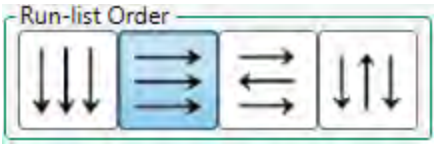
Table 35. Plate Settings items and their functions, continued

Item	Function
	The first row displays the total time required for the run list when volume limits are chosen. The second row displays the estimated time at which run list acquisition would be completed if it were started immediately. These times are calculated taking into account the settings (such as volume, target flow rate or event rate, washing, and agitation) currently programmed for the plate or tube rack. However, event or gate limits are not considered; maximum volume is always used for the calculations.

Setting the Run List Order

You can choose the order in which rows and columns of tubes or wells are sampled.

To set up the run list order



▶ Click the Run-list Order button that reflects the order in which tubes or wells should be sampled.

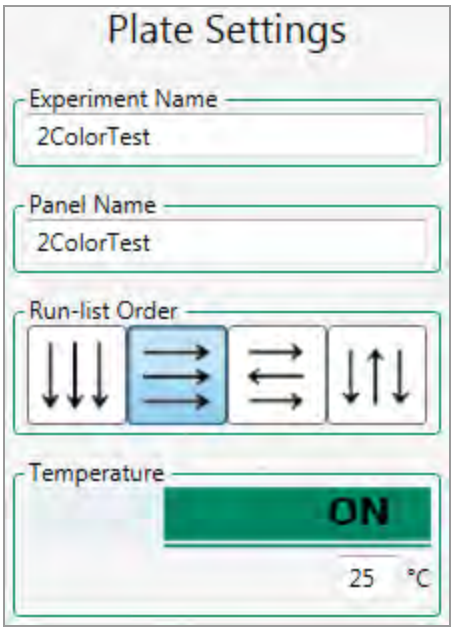
Note: Ensure that any inserted tube rack or plate is oriented correctly, with position A1 in the front left corner.

Activating Temperature Control

When temperature control is enabled, the specified temperature applies to all positions in the plate or tube rack.

To activate temperature control

1. In the Plate Settings area, click the Temperature toggle.
2. Enter the desired temperature.



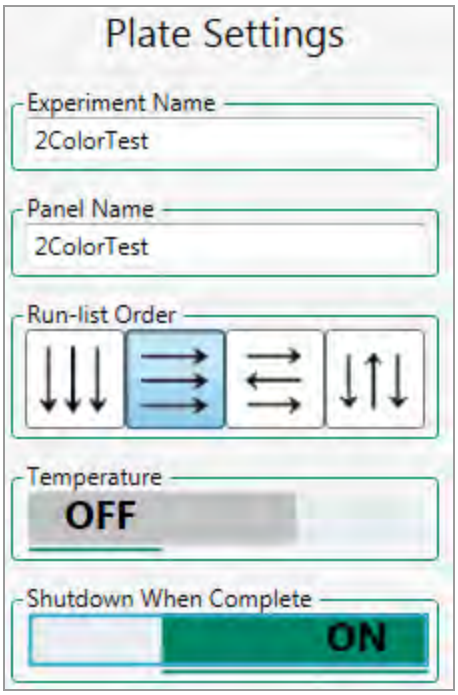
Note: Allow the ZE5™ Cell Analyzer 5 min to reach the desired temperature after activating temperature control.

Specifying Shutdown Upon Completion

You can instruct the system to shut down when run list acquisition is complete.

To specify shutdown upon completion

▶ In the Plate Settings area, click the Shutdown When Complete toggle.



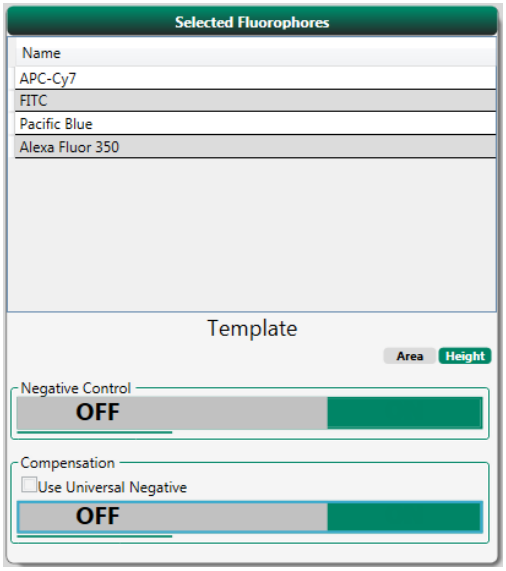
Setting Up Compensation Controls

To use automatic compensation in Everest Software, activate the compensation template. This tool creates control positions that integrate with the automatic compensation process. These control positions are automatically added to the plate map.

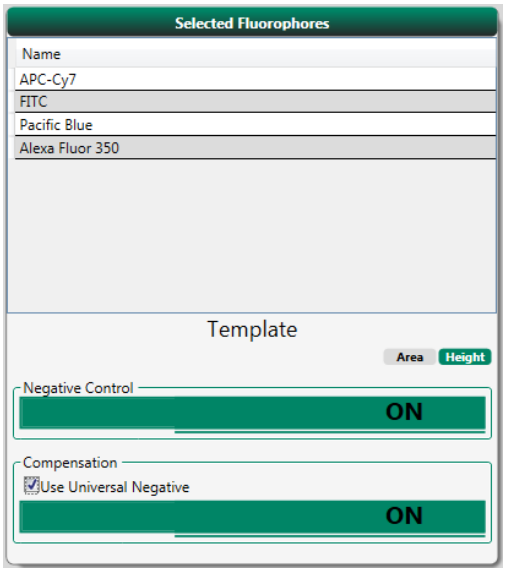
To set up compensation controls

1. In the Template area of the Selected Fluorophores panel, select the pulse parameter (Area or Height) that you want to compensate.

   Note: You must select this parameter first; the Area and Height buttons are disabled after the compensation template is activated.



2. To include a negative control tube or well in the experiment, click the Negative Control toggle so that it is ON.



3. To use this negative control as a universal negative in automatic compensation calculations, select the Use Universal Negative checkbox.

4. To automatically create compensation control positions in the plate setup, click the Compensation toggle so that it is ON.

   In the plate map, positions are filled in with controls starting with A1; each control has a designated workspace.

Naming Positions Manually

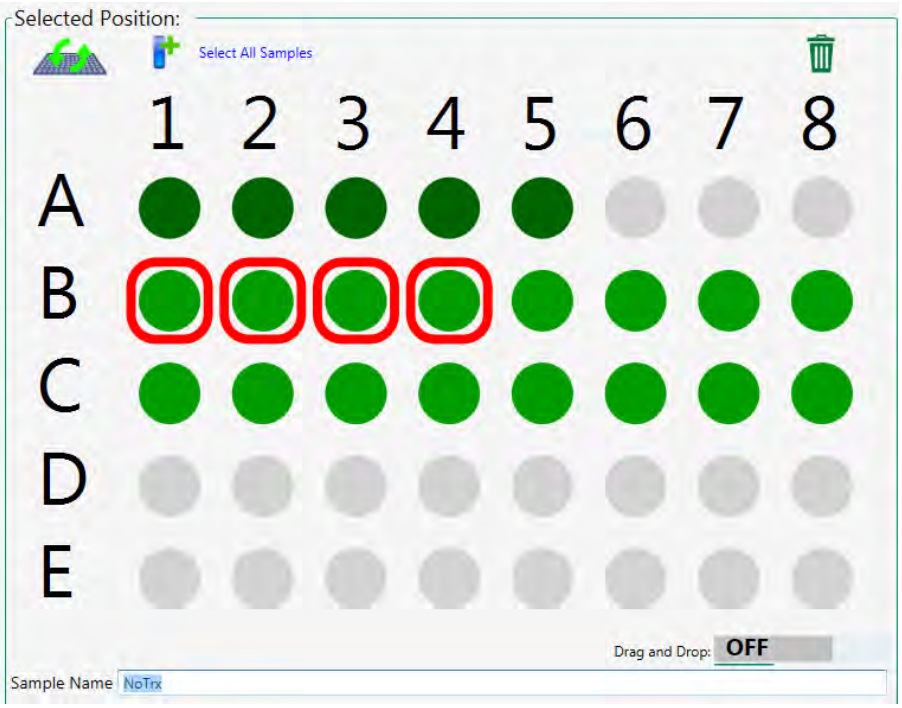
Name positions as needed. Names are written to the FCS file associated with each position.

Note: If you turned on Compensation in the Selected Fluorophores panel, the compensation template automatically names each Setup position based on the corresponding fluorophore name.

To name positions manually

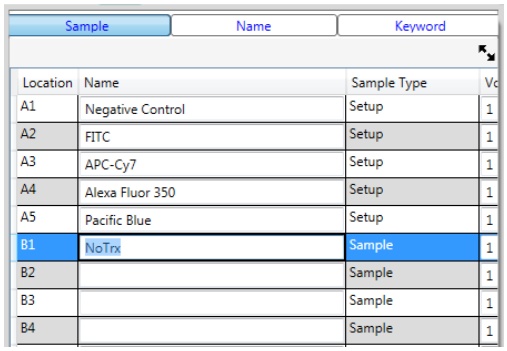
1. In the plate map, click the sample to be named.
2. Do one of the following:
   - Enter a name in the Sample Name box under the plate map.

     Tip: To assign the same name to multiple samples, select them in the plate map.



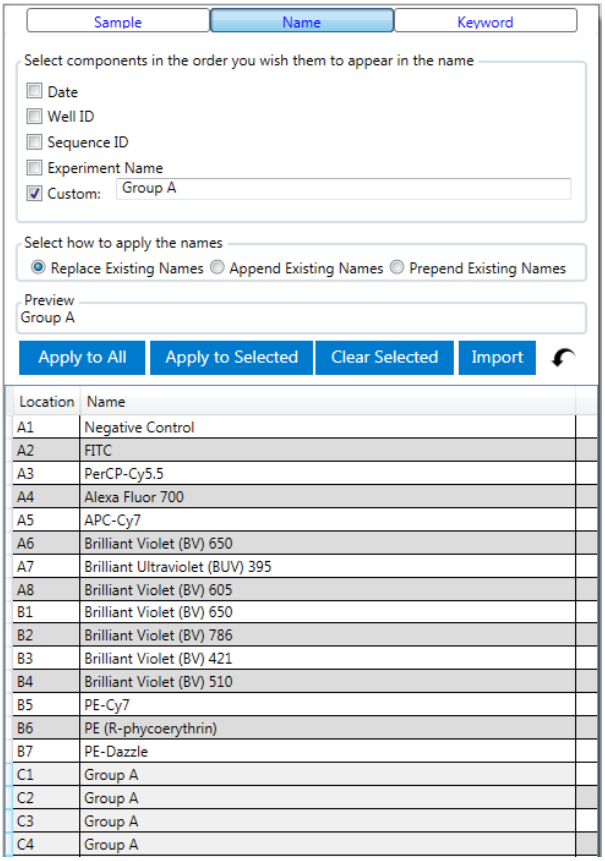
- Enter a name in the Run List on the right side of the Samples screen.

  Tip: You can quickly name samples in the Run List by pressing Enter to move to the next row.



Naming Positions Automatically

The Name tab in the Run List panel allows you to create sample names using name components. For information on the Run List panel, see Run List Panel on page 64.



To name positions automatically

1. Click the Name tab in the Run List.

   There are five available name components:

   - **Date**
   - **Well ID** — well position (for example, A1, B5)
   - **Sequence ID** — order in which positions will be acquired
   - **Experiment Name**
   - **Custom** — allows you to create your own prefix or suffix

2. In the plate map (not the Run List), select the positions to be named.
3. If you want to use a custom name component, enter a value in the Custom box and then select the Custom checkbox.
4. In the Name tab, select the checkboxes for other desired name components.
5. Specify whether the name components should replace existing names, be added to the end of existing names, or be added to the start of existing names.
6. Do one of the following:

   - Click Apply to Selected to apply the name to the selected positions.
   - Click Apply to All to apply the name to all positions.
   - Click Clear Selected to remove names from the selected positions.
   - Click Undo to reverse the last naming action.

To change the Custom component of a name

1. Modify the value in the Custom box.
2. Clear the Custom checkbox and then select it again before applying the change.

Importing Names from a Spreadsheet

The Name tab in the Run List also allows you to import names from a spreadsheet.

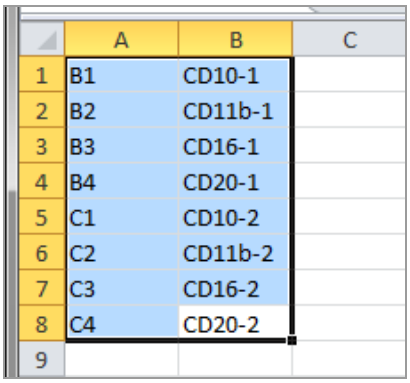
To name positions using a spreadsheet

1. Set up an external spreadsheet with two columns:

   a. Populate column 1 with position IDs (for example, B1, B2, B3) corresponding to the samples in the tube rack or plate.

   b. Populate column 2 with name values.

2. In the spreadsheet, select the values in column 1 and column 2. Do not select any column headings.

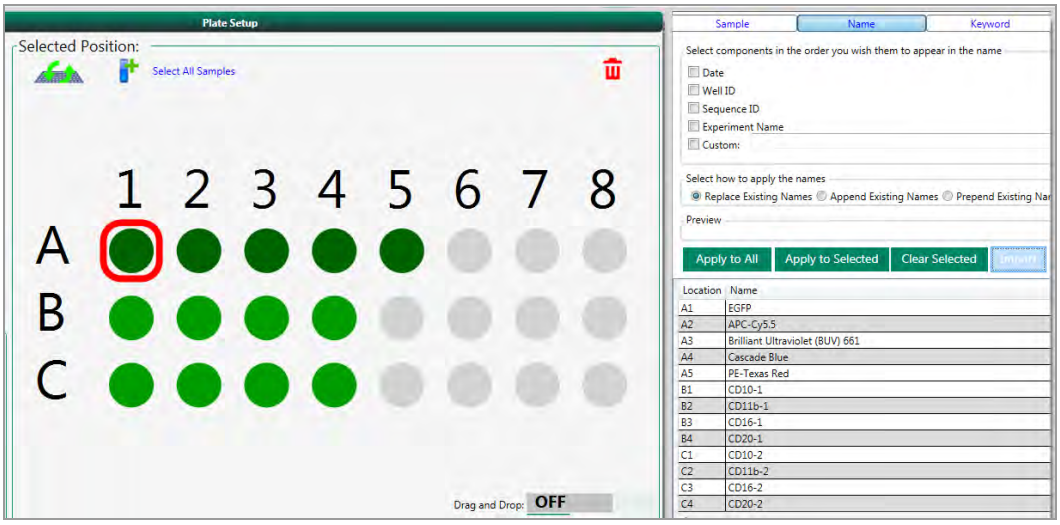


	A	B	C
1	B1	CD10-1	
2	B2	CD11b-1	
3	B3	CD16-1	
4	B4	CD20-1	
5	C1	CD10-2	
6	C2	CD11b-2	
7	C3	CD16-2	
8	C4	CD20-2	
9			

3. Ensure that all positions selected in column 1 of the spreadsheet have been assigned as Sample in the plate map.

4. Click Import.

   The names are imported into the positions selected in column 1 of the spreadsheet.

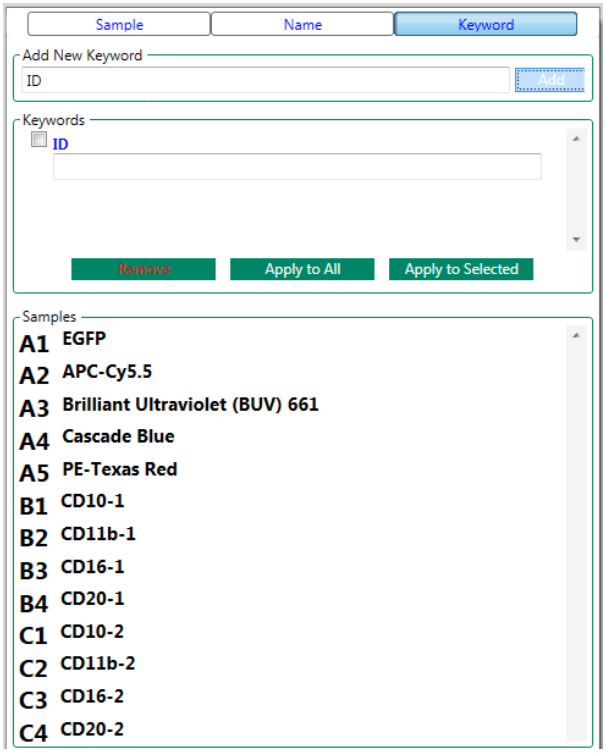


Setting Up Keywords

Everest Software also includes custom keyword functionality. These keywords are written to the FCS file during acquisition and can be used for batch processing in third-party analysis software.

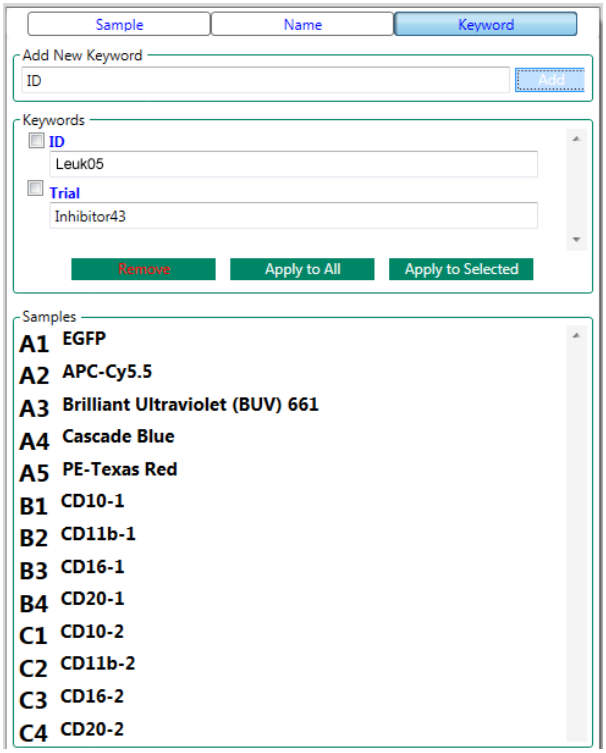
To set up keywords

1. In the Run List, click the Keyword tab.
2. Enter a keyword in the Add New Keyword box and click Add.



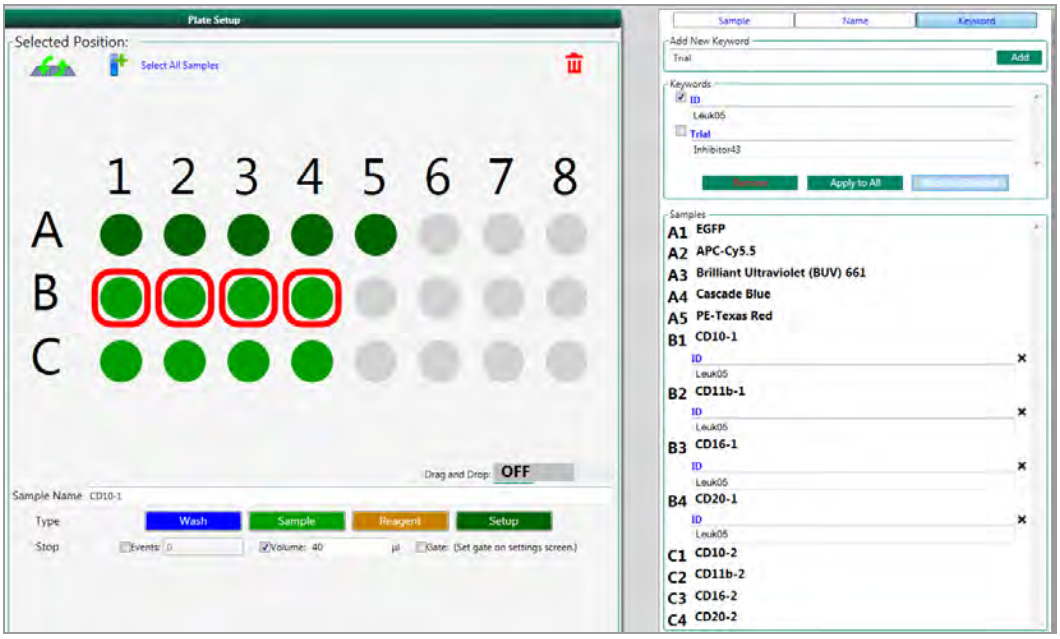
3. Repeat Step 2 for any additional keywords that you want to add.

4. Assign a value to each keyword in the box below the keyword.



5. In the plate map, select the samples to which you would like to apply a keyword.
6. In the Keywords area, select the checkbox for each keyword that you want to apply.
7. Click Apply to Selected.

   The applied values appear in the Samples area of the Run List.



To remove a keyword from the keyword list

1. In the Keywords area, select the checkbox for the keyword.
2. Click Remove.

To remove a keyword/value pair from a sample position

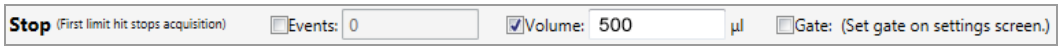
▶ In the Samples area, click the x to the right of the keyword box.

Position Settings Controls

Controls that apply to selected positions are located below the plate map in the Plate Setup panel.

Stop Settings

Sample stop conditions are determined based on event limit, volume limit, or gate limit. The volume value is used to calculate total plate run time.



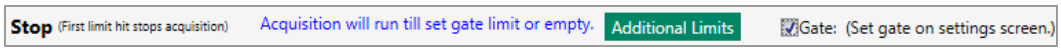
If you select the Events or Gate option, the Volume value automatically defaults to the maximum capacity for the chosen media type:

- 5 ml Tubes: 4,000 µl
- 1.5 ml Tubes: 1,200 µl

- 96 well Plate: 200 µl
- 96-well Deep-Well Plate: 200 µl
- 384-well Plate: 100 µl

Note: If more than one type of stop limit is selected, acquisition for the sample stops when the first stop condition is reached.

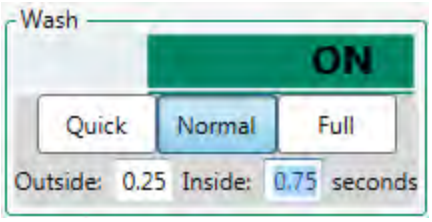
The Gate checkbox adjusts the volume limit to the well or tube maximum. Gate limits are set on the Settings screen of the Experiment Builder. If you want to use a gate limit, select the Gate checkbox on the Samples screen, and specify the actual gate limit on the Settings screen. When you select the Gate checkbox, this hides the Events and Volume settings.



If you want to set an event or volume limit in addition to a gate limit, click Additional Limits to reveal the Events and Volume checkboxes and text boxes.

Wash Settings

If wash is turned on for a position, washing with sheath fluid occurs after the position's sample has been acquired. The wash controls allow you to specify both an outside and inside wash time.



The following quick-set buttons are also provided:

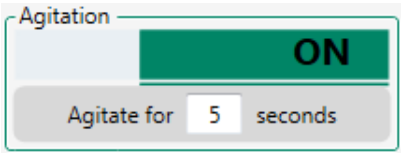
- **Quick** — outside wash 0.25 sec; inside wash 0.5 sec
- **Normal** (default) — outside wash 0.25 sec; inside wash 1.0 sec
- **Full** — outside wash 0.25 sec; inside wash 2.0 sec

The wash times associated with the Quick, Normal, and Full buttons are not editable. Clicking one of the wash buttons causes this box to be populated by the values associated with the button.

Important: Wash is turned on by default, and it is highly recommended to leave it included for all positions, especially when using high-throughput sampling mode.

Agitation Settings

If agitation is turned on for a position, agitation is activated. The default and minimum time is 5 sec. The speed and radius of agitation automatically change with the sample media type.

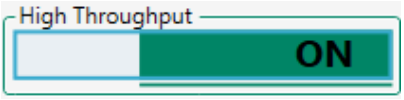


Tip: It might not be necessary to activate agitation for each well. For the highest throughput rate, it might be necessary to agitate only a few times within the plate.

Positions where agitation is activated appear in the plate map as shown in the next figure.

High-Throughput, Pause, and Return Sample Settings

When high-throughput mode is activated for selected positions, samples are aspirated continuously and each sample is separated with a series of air and water boundaries depending on the selected inside wash time.

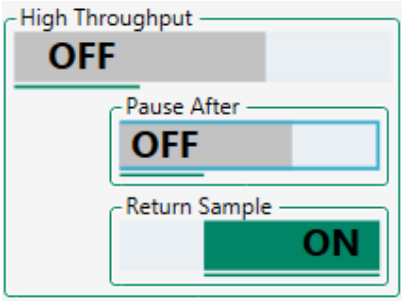


The following options are available only when high-throughput mode is off:

- **Pause After** — inserting a pause step causes acquisition to stop after the assigned sample position and the system waits for your input before continuing. This can be used to verify results achieved, or to wait for you to take action such as setting compensation values before moving on to the next series of samples.

  Tip: Activate Pause After at the last compensation control position so that compensation can be calculated before returning to sample recording. The resulting compensation matrix is written to the FCS files.

- **Return Sample** — if there is sample available in the sample line after the required number of events has been acquired, this portion of the sample can be returned to the tube/well.

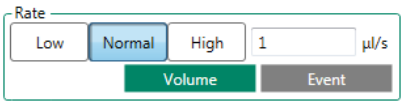


Positions where Pause After is activated appear in the plate map as shown in the next figure.

For more information about high-throughput mode, see High-Throughput Mode on page 62.

Flow Rate or Event Rate Settings

The flow rate controls allow you to specify a precise target flow rate for each position, in µl/sec.



The following quick-set buttons are also provided:

- **Low** — 0.5 µl/sec (30 µl/min)
- **Normal** — 1.0 µl/sec (60 µl/min) (default)
- **High** — 1.5 µl/sec (90 µl/min)

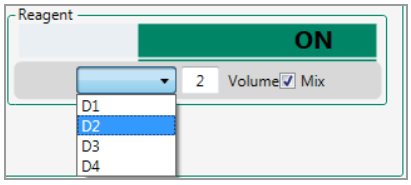
The flow rates associated with the Low, Normal, and High buttons are not editable. Clicking one of the flow rate buttons causes this box to be populated by the value associated with the button.

Alternatively, you can specify a target event rate, in events/sec.

If you are present at the instrument during acquisition in standard mode, you can adjust the target flow rate manually, from 0.1 µl/sec up to 3.5 µl/sec. However, you cannot adjust the flow rate during acquisition in high-throughput mode.

Note: In a high-throughput run, all samples must use the same target flow rate.

Reagent Settings

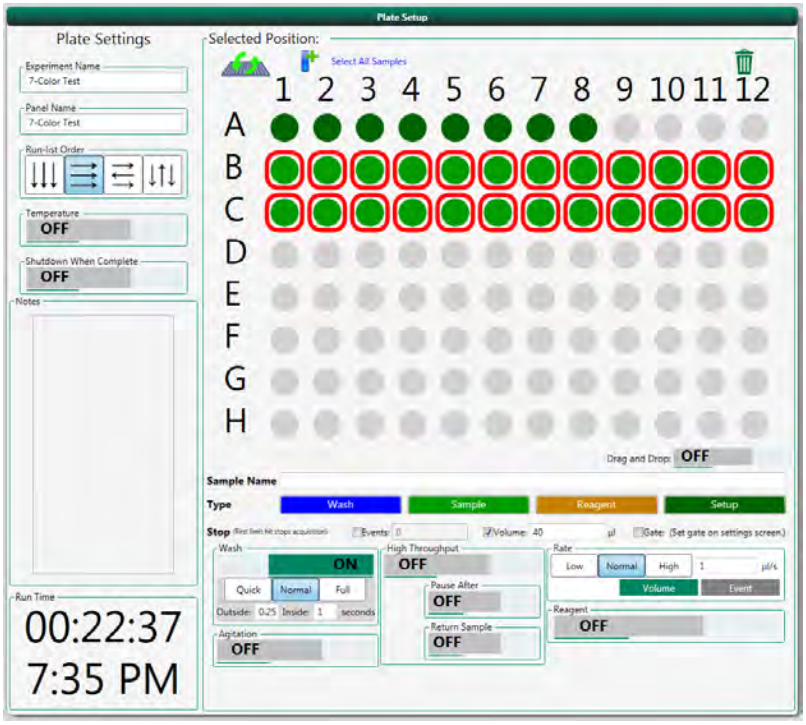


Before you can specify addition of reagent to any samples, first designate one or more positions in the plate map as reagent positions. Then, for a selected sample position, you can select a reagent position from the dropdown list and enter the required volume of reagent to add to the sample, in the range of 2–20 µl. You can instruct the system to mix the reagent after it is added by aspirating and dispensing the combined sample and reagent several times. You can also choose to add an agitation step on the position to enhance mixing.

Positions where reagent will be added appear in the plate map as shown in the next figure.

Assigning Position Types

For detailed information on position types and selecting positions, see Position Types on page 61 and Selected Position on page 59, respectively.



To assign position types

1. Assign positions based on their type: Wash, Sample, Reagent, or Setup (control). Select positions by dragging over the appropriate region of the plate or tube rack.

   Selected positions are highlighted with red boxes. If you turned on Compensation in the Selected Fluorophores panel, Setup positions will have already been configured.

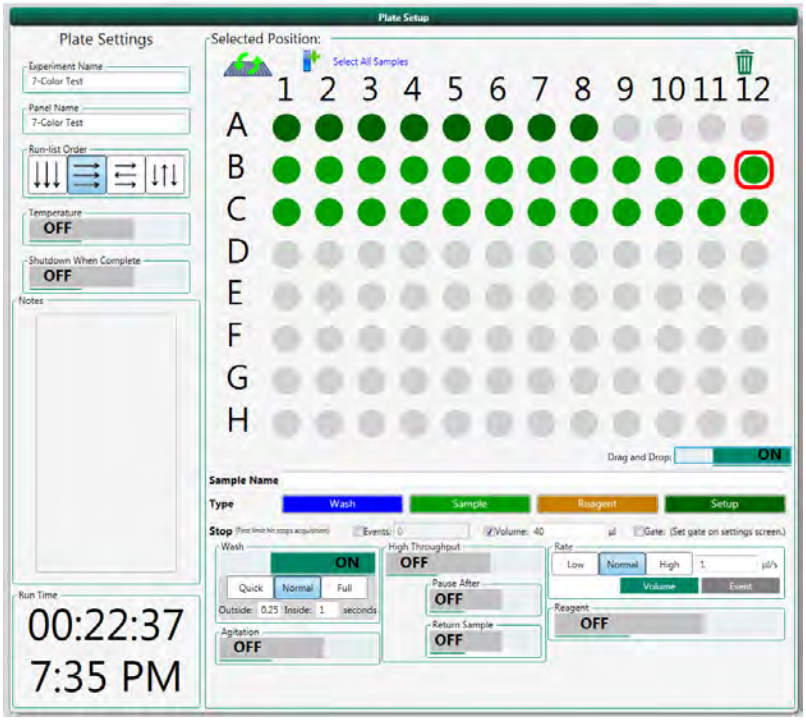
   a. Assign samples to the desired positions in the tube rack/plate. Highlight the appropriate positions and click the Samples button.

   b. Assign Wash and Reagent positions as needed.

      Note: Wash positions contain wash solution. Reagent positions contain reagent that can be added to other positions in the plate map.

2. To change the location of any assigned position, do the following:

   a. Click the Drag and Drop toggle in the lower right of the plate map.



b. Drag a filled position to an empty one.

c. Click the Drag and Drop toggle again.

Tip: This procedure shows how to set up positions for a single-panel experiment. If needed, you can set up a single plate or tube rack with multiple experimental panels. For more information, see Multipanel Experiments on page 71 and Setting Up Multiple Panels on page 210.

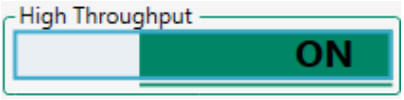
Selecting Standard or High-Throughput Acquisition

In the default standard (single sample) acquisition mode, there is only one sample in the sample line at any given time. If high-throughput mode is activated, samples are aspirated continuously; the sample line contains multiple samples, each sample separated by a boundary of air and wash fluid.

Select high-throughput mode to achieve the highest possible sample throughput. For more information about high-throughput mode, see High-Throughput Mode on page 62.

To select high-throughput mode

1. In the plate map, select the positions to sample in high-throughput mode.
2. Click the High Throughput toggle.



Note: In high-throughput acquisition mode, the Pause After and Return Sample functions are disabled, and only volume limits can be used. All samples in a high-throughput run must use the same target flow rate. Standard mode supports event limits and gate limits as well as volume limits.

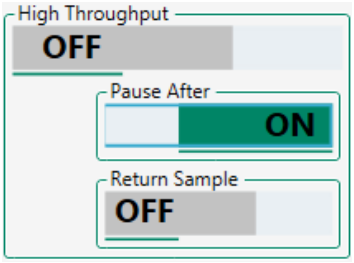
Pausing After a Tube or Well

If high-throughput mode has not been activated, you can use the Pause After function.

Tip: Pause After can be useful when applying automatic compensation. Activate Pause After on the last compensation control position in the experiment. Acquisition automatically stops after the file for that control is recorded. You can then navigate to the Analysis module to perform automatic compensation and then return to the experiment to acquire and record sample files along with the compensation matrix, which are exported in the FCS file for analysis in third-party software.

To pause after a tube or well

1. Select the relevant position(s) in the plate map.
2. Click the Pause After toggle.



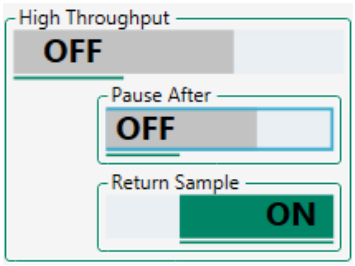
The run list pauses after the applicable position has been acquired. Positions where the system will pause are marked with a glyph. For more information, see Position Glyphs on page 62.

Returning Sample to a Tube or Well

If high-throughput mode has not been activated, you can use the Return Sample function to conserve sample.

To return sample

1. Select the relevant position(s) in the plate map.
2. Click the Return Sample toggle.



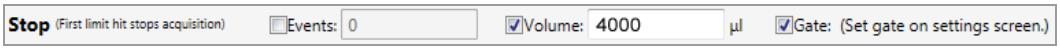
After the acquisition limit has been reached for the assigned positions, the sample pump runs backwards to return any unused sample to its tube or well.

Setting Stop Conditions

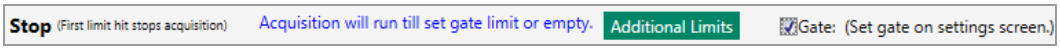
You can set an event limit, volume limit, or gate limit for each position. If more than one type of limit is set, acquisition stops when the first limit is reached. If high-throughput mode has been activated for a position, you can use only a volume limit. For information about maximum volumes for various media types, see Stop Settings on page 152.

To set stop conditions

1. Select the relevant position(s) in the plate map.
2. Select one or more of the following checkboxes:
   a. **Events** — acquisition stops when the total event count reaches the specified number.
   b. **Volume** — acquisition stops when the total acquisition volume reaches the specified value.
   c. **Gate** — sets the volume limit to the maximum for the position. You can configure gate limits in the next screen (Settings) and change the volume to reflect what is in the tube or well if needed.



Note: If you select the Gate checkbox, the Events and Volume limit controls are hidden, but values entered in them are still applicable. To reveal the Events and Volume limit controls, click Additional Limits.



3. If you selected Events, enter an event limit.
4. If you selected Volume, enter a volume limit.
5. To set different limits for other positions, repeat Step 1 through Step 4 for other positions in the run list.

Configuring Wash Settings

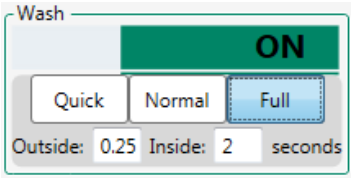
Configure washes as needed. By default, all positions are configured to include a 0.25 sec outside wash and a 1.0 sec inside wash. See Wash Settings on page 153 for descriptions of the quick-set Wash buttons.

To configure wash settings

1. Select a relevant position in the plate map.

   Note: Washes are performed after acquisition of the position.

2. Change the wash time by clicking one of the quick-set buttons or by manually entering the wash times into the Outside and Inside boxes.



Important: It is highly recommended to include a wash step for all positions, especially when using high-throughput sampling mode.

Activating Agitation

For information on agitation settings, see Agitation Settings on page 154.

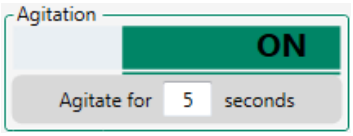
To activate agitation

1. Select a relevant position in the plate map.

   Note: Agitation is performed before acquisition of a position.

2. Click the Agitation toggle.

3. Enter the agitation time.



   Positions with agitation enabled are marked with a glyph. For more information, see Position Glyphs on page 62.

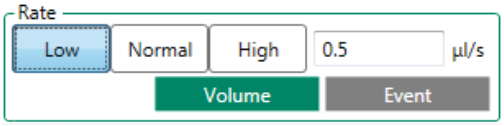
Specifying Flow Rate or Event Rate

A volumetric target flow rate or target event rate must be assigned for all samples. By default, a rate of 1 µl/sec is assigned when positions are activated. See Flow Rate or Event Rate Settings on page 155 for descriptions of the quick-set Flow Rate buttons.

Note: In a high-throughput run, all samples must use the same target flow rate.

To specify a target flow rate

1. Select the relevant position or positions in the plate map.
2. Change the target flow rate by clicking one of the quick-set buttons or by manually entering the target flow rate into the Rate box.



To specify a target event rate

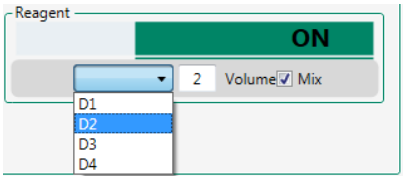
1. Select the relevant position or positions in the plate map.
2. In the Rate area, click Event.
3. Enter the target event rate into the Rate box.

Adding Experimental Reagents

For information about experimental reagents, see Reagent Settings on page 156.

To add reagents to samples

1. Ensure that at least one position in the plate map is designated as a Reagent position.
2. Select the sample position to which you wish to add reagent.
3. Click the Reagent toggle.
4. Select the position from which to add reagent in the dropdown menu.
5. Specify the reagent volume in the Volume box.
6. To instruct the system to mix the reagent by aspirating and dispensing the combined sample and reagent several times, select the Mix checkbox.



Reagent is not added to sample in setup mode. When the run list proceeds to the assigned position in acquisition mode, the instrument aspirates the specified volume of reagent from the reagent position and adds it to the selected position, mixing afterward if programmed. Positions to which reagent will be added are marked with a glyph. For more information, see Position Glyphs on page 62.

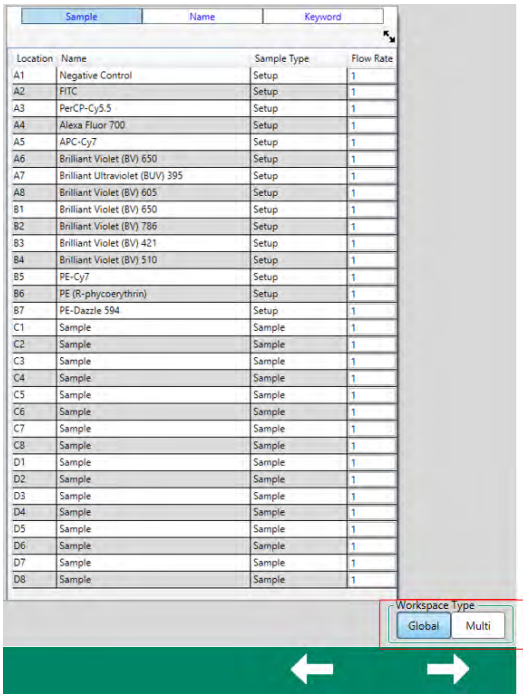
Selecting the Workspace Type

Everest Software offers two options for setting up workspaces.

Table 36. Workspace types

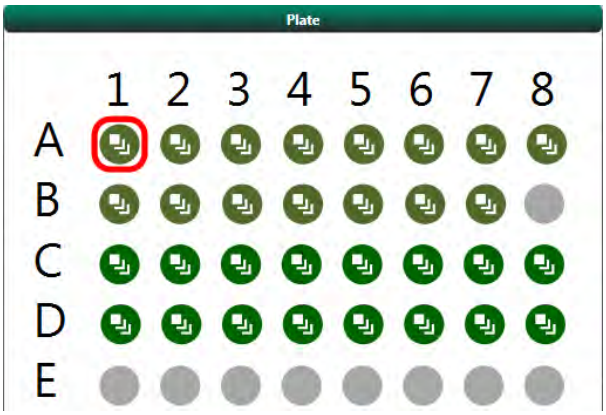
Workspace type	Description
Global	The workspace is identical for positions designated as Sample within an experimental panel. All plots, regions, and gates, including stopping gates and hit detection regions, are replicated for each sample position. Use this option for faster experiment building.
Multi	Each Sample position can be configured with its own set of plots, regions, and gates. A workspace must be created individually for each position, so this option takes more time.

To select the workspace type

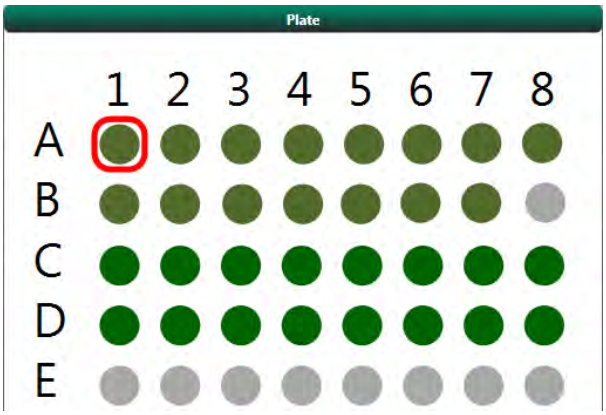


▶ Click Global or Multi in the Workspace Type area in the lower right corner of the Samples screen.

When you add plots in the workspace Settings screen, symbols in the plate map indicate the type of workspace selected. The next figure shows the Global workspace type, in which the plots, regions, and gates are replicated for each position.



The next figure shows the Multi workspace type, in which the plots, regions, and gates must be created individually for each position.



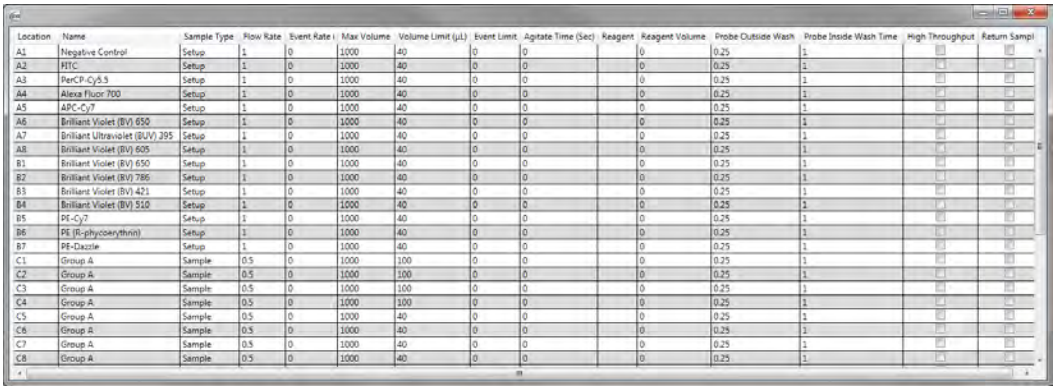
Reviewing the Run List

After you have applied the settings for all of the samples included in the experiment and selected the workspace type, review the run list.

Important: After configuring settings in the Plate Setup panel, examine the expanded Run List panel to ensure that each sample position has been configured as needed.

To review the run list and go to experiment Settings

1. Expand the Run List panel.



Location	Name	Sample Type	Flow Rate	Event Rate	Max Volume	Volume Limit (µL)	Event Limit	Agitate Time (Sec)	Reagent	Reagent Volume	Probe Outside Wash	Probe Inside Wash Time	High Throughput	Return Sampl
A1	Negative Control	Setup	1	0	1000	40	0	0		0	0.25	1		
A2	FITC	Setup	1	0	1000	40	0	0		0	0.25	1		
A3	PerCP-Cy5.5	Setup	1	0	1000	40	0	0		0	0.25	1		
A4	Alexa Fluor 700	Setup	1	0	1000	40	0	0		0	0.25	1		
A5	APC-Cy7	Setup	1	0	1000	40	0	0		0	0.25	1		
A6	Brilliant Violet (BV) 650	Setup	1	0	1000	40	0	0		0	0.25	1		
A7	Brilliant Ultraviolet (BUV) 395	Setup	1	0	1000	40	0	0		0	0.25	1		
A8	Brilliant Violet (BV) 605	Setup	1	0	1000	40	0	0		0	0.25	1		
B1	Brilliant Violet (BV) 650	Setup	1	0	1000	40	0	0		0	0.25	1		
B2	Brilliant Violet (BV) 786	Setup	1	0	1000	40	0	0		0	0.25	1		
B3	Brilliant Violet (BV) 421	Setup	1	0	1000	40	0	0		0	0.25	1		
B4	Brilliant Violet (BV) 510	Setup	1	0	1000	40	0	0		0	0.25	1		
B5	PE-Cy7	Setup	1	0	1000	40	0	0		0	0.25	1		
B6	PE (R-phycoerythrin)	Setup	1	0	1000	40	0	0		0	0.25	1		
B7	PE-Dazzle	Setup	1	0	1000	40	0	0		0	0.25	1		
C1	Group A	Sample	0.5	0	1000	100	0	0		0	0.25	1		
C2	Group A	Sample	0.5	0	1000	100	0	0		0	0.25	1		
C3	Group A	Sample	0.5	0	1000	100	0	0		0	0.25	1		
C4	Group A	Sample	0.5	0	1000	100	0	0		0	0.25	1		
C5	Group A	Sample	0.5	0	1000	40	0	0		0	0.25	1		
C6	Group A	Sample	0.5	0	1000	40	0	0		0	0.25	1		
C7	Group A	Sample	0.5	0	1000	40	0	0		0	0.25	1		
C8	Group A	Sample	0.5	0	1000	40	0	0		0	0.25	1		

2. Inspect each tube or well and its associated settings, ensuring that all settings are correct.
3. Click the Next arrow to go to the Settings screen.

   In the Settings screen, you can create plots for analysis and adjust instrument settings such as thresholds and PMT voltages. You can also add experimental panels to a single plate or rack, if needed.

Creating Plots and Histograms

To visualize data in the workspace, you can add density plots, time plots, and histograms manually, or you can let Everest Software automatically create plots. The next figure shows the controls for configuring a density plot.

LEGEND

1	X-axis laser selection	**5**	Y-axis laser selection
2	X-axis parameters	**6**	Y-axis parameters
3	X-axis scaling and compensated data display	**7**	Y-axis scaling and compensated data display
4	X-axis pulse measurement	**8**	Y-axis pulse measurement

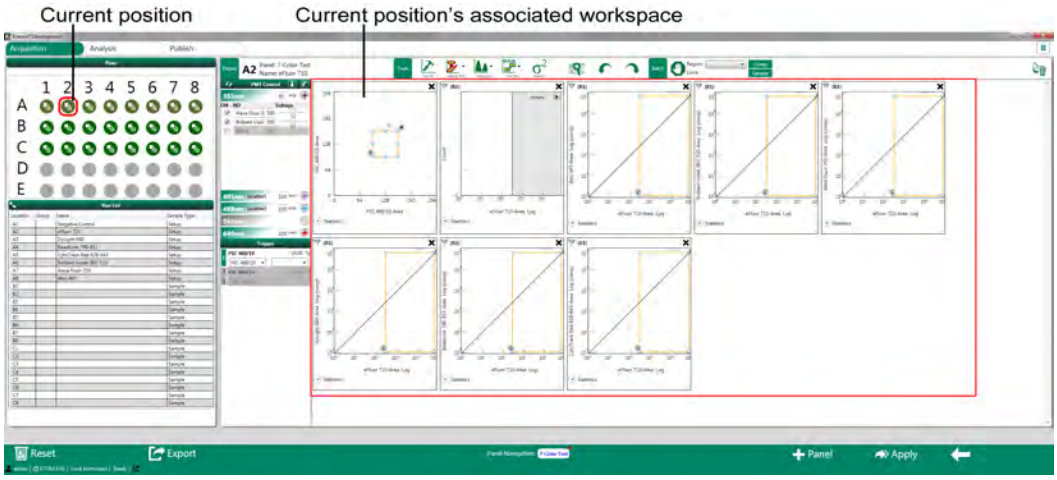
The parameters that appear in the plot builder reflect those that were activated in the Experiment Builder Fluorophores screen. You can specify linear, log, or hyperlog scaling for each axis; specify the pulse measurement (area, height, or width); and enable display of compensated data as needed.

For more information, see Creating Density Plots on page 167, Creating Histograms on page 168, and Creating Time Plots on page 169.

Plots Created by the Compensation Template

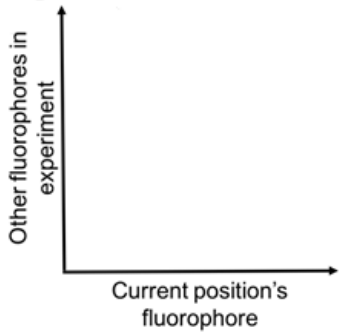
If you turned on the Negative Control option in the Template area of the Samples screen, Everest Software automatically creates the necessary plots. The negative control position is associated with a forward scatter-by-side scatter plot and a plot for each fluorophore; these plots can assist you in setting voltages.

If you turned on Compensation in the Template area of the Samples screen, Everest Software automatically creates the necessary plots. Each compensation control is associated with its own set of plots to facilitate configuration and assessment of compensation.



Each single-color compensation control position contains:

- A forward scatter-by-side scatter plot
- A histogram for the control's channel that includes a region for identifying the positive population
- A plot for each other fluorophore in the experiment so that spillover of the position's fluorophore into each other detector can be assessed and corrected



Each fluorescence density plot in a compensation control workspace is configured with the current fluorophore's detector on the x-axis and the other fluorophore on the y-axis.

If you did not use the compensation template in the Samples screen, you must create plots for compensation controls manually.

Creating Density Plots

In the density plot builder, the displayed parameters reflect those that were activated in the Experiment Builder Fluorophores screen.

To create a density plot

1. Click Create Density Plot on the toolbar.
2. Locate a parameter to assign to the x-axis. Parameters are listed under each laser.

3. Set the axis scaling:
   - For linear scale — if the Log button is blue, click it so that it is no longer blue.
   - For log scale — if the Log button is blue, leave it selected; if the Log button is not blue, click it.
   - For hyperlog scale — leave Log selected and click HyperLog so that both the Log and HyperLog buttons are blue.
4. Specify whether to display compensated data for the parameter.
5. Select the pulse measurement (height, area, or width) for the parameter.
6. Repeat Step 2 through Step 5 for the y-axis.

   The plot is added to the workspace.
7. Click the X or click outside the plot creation dialog box to close it.

Creating Histograms

In the histogram builder, the displayed parameters reflect those that were activated in the Experiment Builder Fluorophores screen.

To create a histogram

1. Click Create Histogram in the toolbar.
2. Locate a parameter to assign to the x-axis. Parameters are listed under each laser.

3. Set the y-axis scaling:
   - For linear scale — if the Log button is blue, click it so that it is no longer blue.
   - For log scale — if the Log button is blue, leave it selected; if the Log button is not blue, click it.
   - For hyperlog scale — leave Log selected and click HyperLog so that both the Log and HyperLog buttons are blue.
4. Specify whether to display compensated data for the parameter.
5. Select the pulse measurement (height, area, or width) for the parameter.

   The histogram is added to the workspace.
6. Click the X or click outside the histogram creation dialog box to close it.

Creating Time Plots

In the time plot builder, the displayed parameters reflect those that were activated in the Experiment Builder Fluorophores screen.

To create a time plot

1. Click Create Time Plot in the toolbar.
2. Locate a parameter to assign to the y-axis. Parameters are listed under each laser.

3. Set the y-axis scaling:
   - For linear scale — if the Log button is blue, click it so that it is no longer blue.
   - For log scale — if the Log button is blue, leave it selected; if the Log button is not blue, click it.
4. Select the pulse measurement (height, area, or width) for the y-axis.
5. Select a time range for the x-axis.

Table 37. Time range options

Time range option	Description
Sliding	As time progresses, display range remains the same but slides to reflect current time.
Continuous	As time progresses, time range increases to reflect the entire acquisition time.
Fixed	Time range reflects only a fixed maximum, depending on the value that you enter into the box.

   The plot is added to the workspace.
6. Click the X or click outside the plot creation dialog box to close it.

Creating Histograms for All Channels

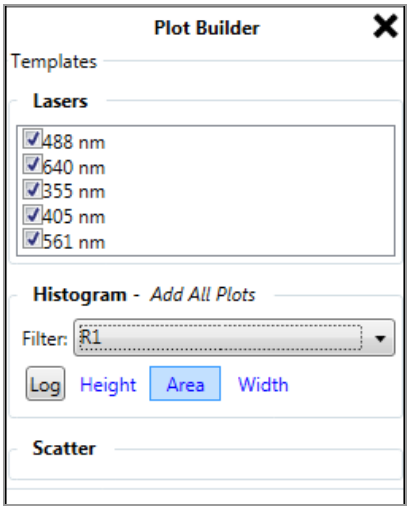
Everest Software allows you to automatically create histograms for all parameters. In the plot (histogram) builder, you can apply filters (gates) to all histograms; specify whether histogram data are displayed in linear, log, or hyperlog scale; and specify the pulse measurement (area, height, or width).

Tip: Histograms are created only for parameters that are activated in the workspace. Checkboxes next to each laser allow you to specify histogram creation for a particular detection path.

To create histograms for all channels

1. Click Advanced Plot Builder in the toolbar.

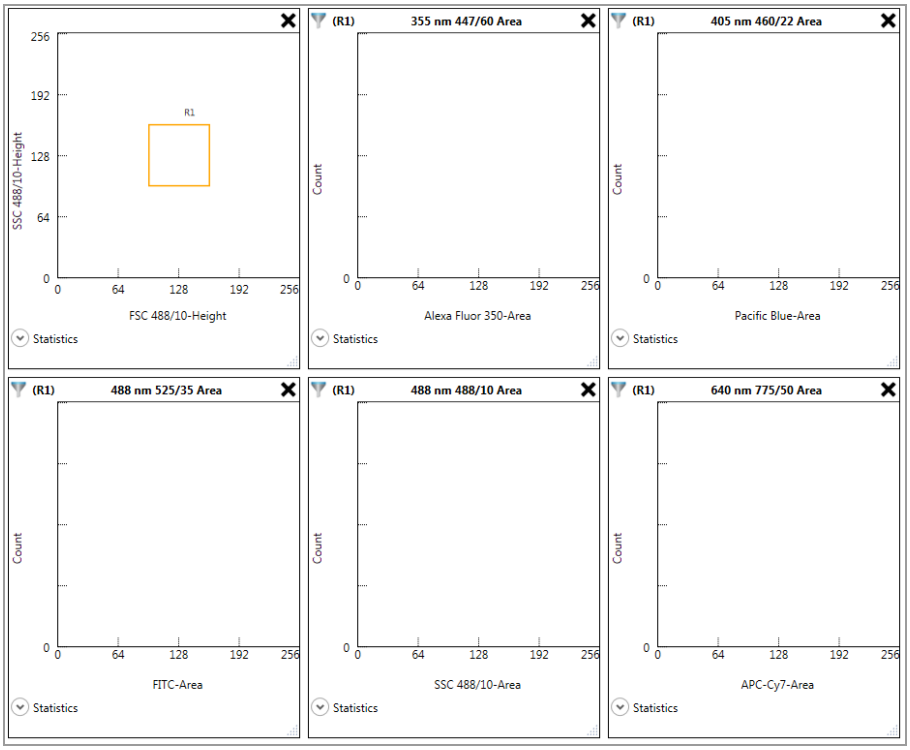
   The plot (histogram) builder opens.



2. Clear the checkbox for any laser detection path that you do not want to include.
3. Apply a filter, if needed.
4. Specify linear or log scale.

5. Select the pulse measurement (height, area, or width) for the parameter.

   The histograms are added to the workspace.



Using Plot and Histogram Tools

After creating a plot or histogram, you can modify it using the tools that appear on the right when you point at the plot or histogram. Different tools are available for bivariate density plots (next figure, left) versus univariate histogram plots (next figure, right). Tools for time plots are similar to those for density plots.

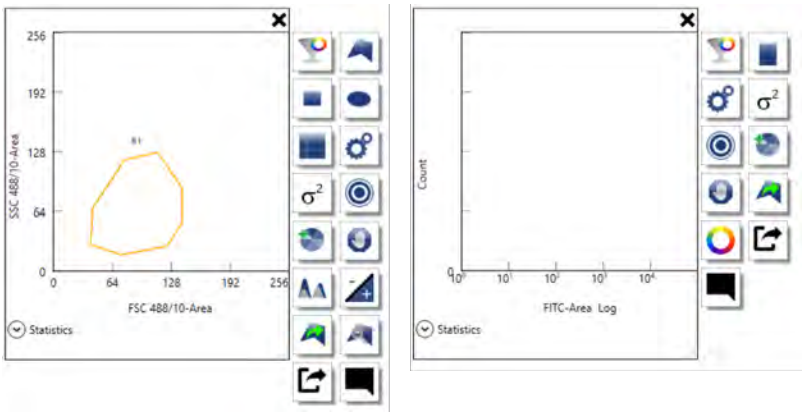


Table 38. Plot and histogram tools and their functions

Tool	Function	Further information
	**Apply a Color Filter** — allows you to select a color. Events in this region appear in the selected color in all other plots. Color filters are also known as color gates.	Adjusting Plot Color on page 191
	**Add Polygon** — adds a polygon region to the plot; click each point to create the shape and double-click to close. Applies only to density and time plots.	Adding Regions to Density Plots and Time Plots on page 180
	**Add Rectangle** — adds a rectangle region to the plot. Applies only to density and time plots.	Adding Regions to Density Plots and Time Plots on page 180
	**Add Ellipse** — adds an ellipse region to the plot. Applies only to density and time plots.	Adding Regions to Density Plots and Time Plots on page 180
	**Add Quadrant** — adds quadrant regions to the plot. Applies only to density plots.	Adding Regions to Density Plots and Time Plots on page 180
	**Add Bar Region** — adds a bar region to the plot. Applies only to histograms.	Adding Bar Regions to Histograms on page 185
	**Modify Plot Parameters** — allows you to modify plot parameters such as fluorophore, log decade, compensation, height, width, area, and bin count.	Modifying Plot Parameters on page 175

Table 38. Plot and histogram tools and their functions, continued

Tool	Function	Further information
	**Manage Statistics** — allows you to select the plot statistics to display, such as count, percent, mean, max, min, mode, median, standard deviation, variance, and CV.	Managing Plot Statistics on page 176
	**Hit Region Assignment** — allows you to specify a hit detection region. Applies only to density plots and histograms.	Configuring Hit Detection on page 198
	**Heat Map Region Assignment** — allows you to add regions to a heat map.	Applying Heat Maps on page 195
	**Gate Limit Assignment** — allows you to assign a gate limit to a region so that acquisition stops when the event count in the region reaches the specified limit.	Applying Filters (Gates) on page 187
	**Export to PNG** — saves a PNG of the plot to a location that you specify.	Exporting a Plot as a Graphic on page 205
	**Add Diagonal Separation** — adds a diagonal line to the plot. This graphical element can aid you in setting PMT voltages for compensation. Applies only to density plots.	Adding Diagonal Separators to Plots on page 199
	**Apply Gate to All Plots** — allows you to either apply a gate (region) to all plots or copy a region and create an identical one in all other plots of the same type in the workspace.	Applying a Region to All Plots on page 200
	**Track Region Assignment** — allows you to specify target event percentage for a region within a plot. If the event percentage within the region does not reach the target, acquisition pauses. Useful as a clog-detection tool when utilized with the scatter gate. Applies only to density plots.	Assigning Data Track Regions on page 194

Table 38. Plot and histogram tools and their functions, continued

Tool	Function	Further information
	**Adjust Plot Decorations** — Select a color to represent the displayed data. Applies only to histograms.	Adjusting Plot Color on page 191
	**Add Filter** — applies a gate assignment to the plot.	Applying Filters (Gates) on page 187
	**Remove Filter** — removes a gate assignment from the plot.	Applying Filters (Gates) on page 187
	**Add Annotation** — allows you to add a note to the plot.	Adding Annotations to Plots on page 201

Modifying Plot Parameters

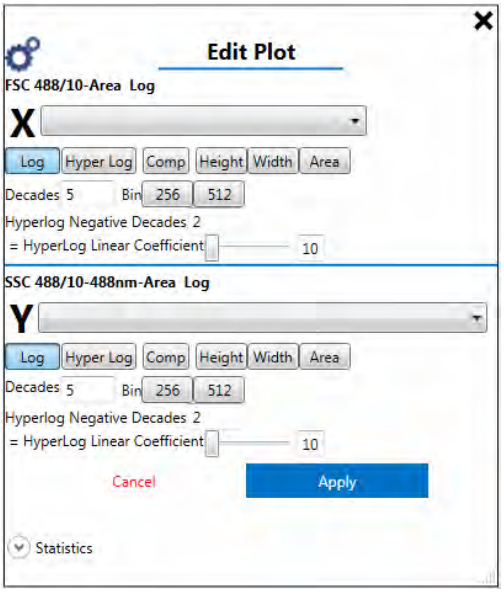
For each axis of a plot or histogram, you can customize parameters such as selected fluorophore, log decade, pulse parameter, and bin count.

1. Point to a plot or histogram for which you want to modify parameters.

   The pop-up toolbar appears to the right of the plot or histogram.

2. Click Modify Plot Parameters.

3. In the Edit Plot pop-up dialog box, specify settings for each axis:



a. Select a fluorophore from the dropdown list.

b. Specify linear, log, or hyperlog scaling (for hyperlog, both Log and Hyper Log must be selected).

c. Specify display of compensated data.

d. Select the pulse parameter (height, width, or area).

e. Enter the number of log decades to display.

f. Specify 256 x 256 or 512 x 512 plot resolution (bin).

g. Enter the number of hyperlog decades to display.

h. Use the slider to select a hyperlog linear coefficient.

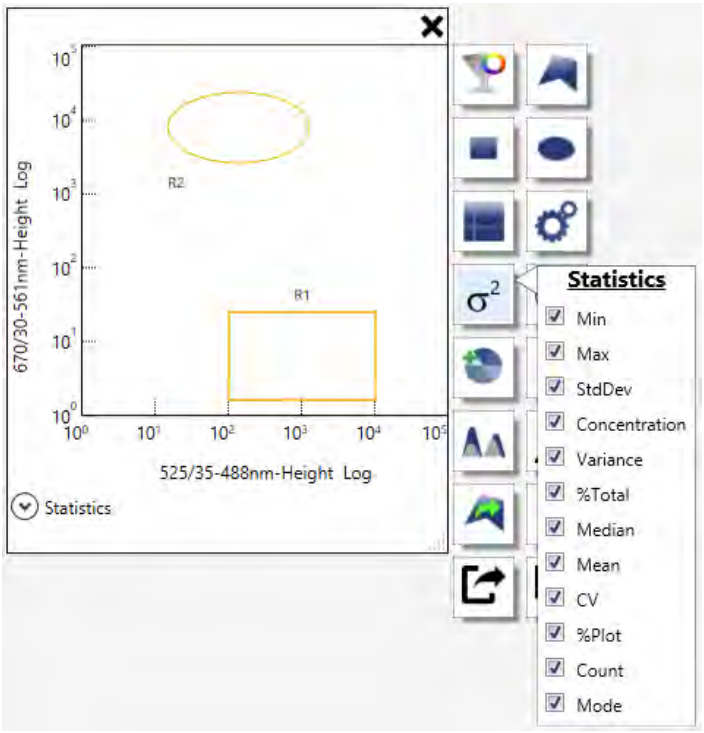
4. Click Apply to save the changes.

Managing Plot Statistics

You can select specific statistics to view in each plot or histogram.

To manage plot statistics

1. Point to a plot or histogram for which you want to manage statistics.
   The pop-up toolbar appears to the right of the plot or histogram.
2. Click Manage Statistics.
3. In the Statistics pop-up window, select the items you want to view and clear the items you want to hide.



4. Point outside the plot to save your changes.

Viewing and Rearranging Plot Statistics

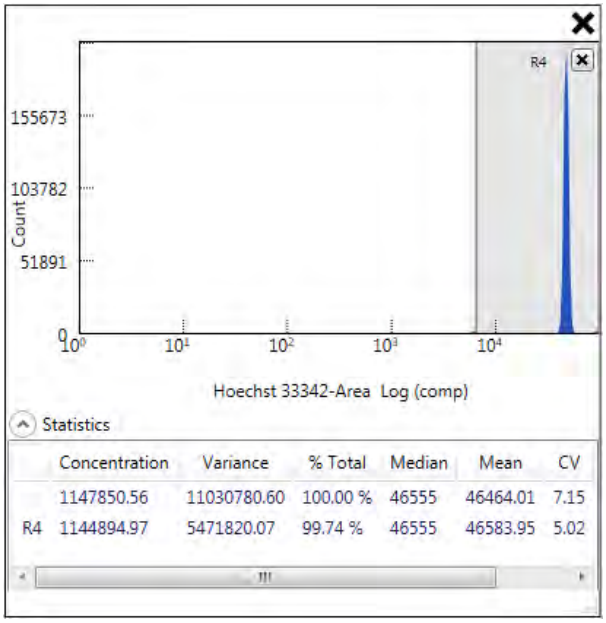
For each plot and histogram, you can view statistics for the plot and for each region in the plot. You can also specify the order in which statistics are displayed.

The master list of statistics is controlled in the global preferences. For more information, see Specifying Statistics Preferences on page 96. If you do not see the statistics that you want to display, contact your system administrator.

To view plot statistics

Note: If you plan to add any regions to a plot or histogram, add them before viewing statistics.

1. Click the Statistics down arrow.
2. Resize the plot window if needed.



To rearrange plot statistics

▶ In the Statistics area of a plot or histogram, drag a statistic's column heading to a new position.

Comparing Statistics

You can display statistics for selected filters (gates) and compare them to each other as well as to all events. The statistics can be viewed in real time as acquisition occurs. Use this feature after applying an experiment to a workspace.

To compare statistics

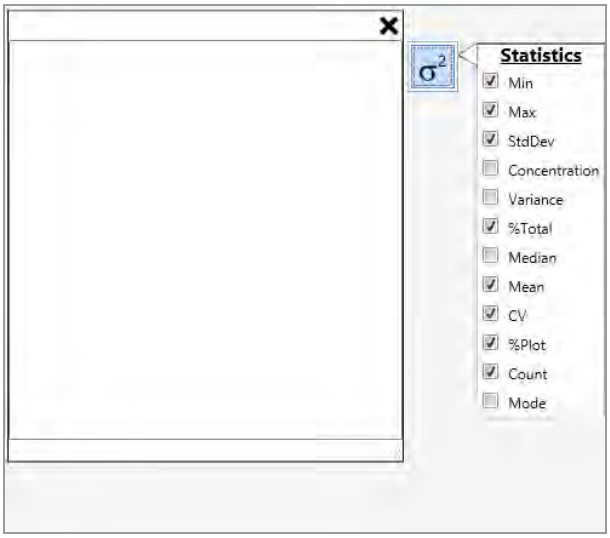
1. In the Tools area of the toolbar, click Statistics.

   A statistics window is added to the workspace.

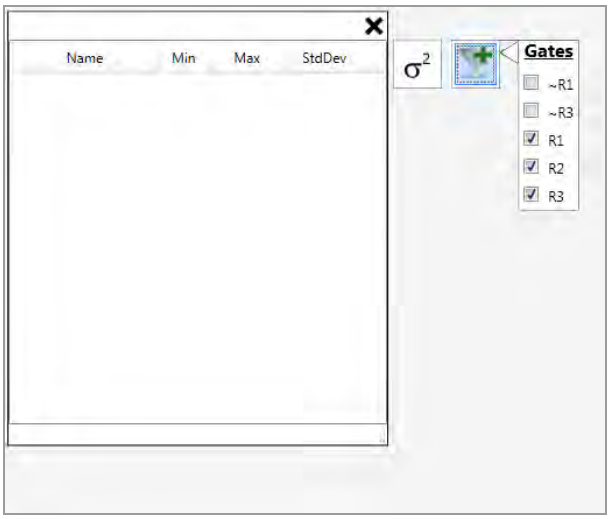
2. Point to the statistics window.

   The pop-up toolbar appears to the right of the window.

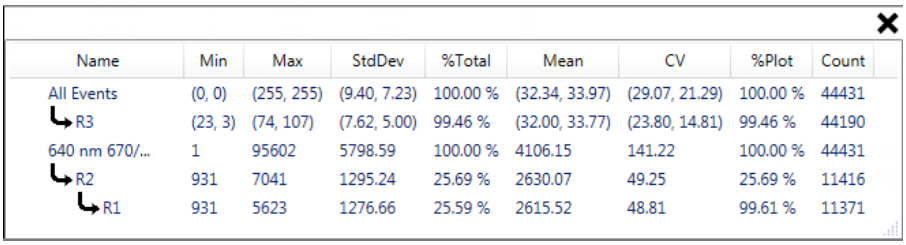
3. Click Select statistics.



4. In the Statistics pop-up window, select the items you want to view and clear the items you want to hide.
5. In the pop-up toolbar, click Select gates for statistics window.



6. In the Gates pop-up window, select the filters (gates) for which you want to compare statistics.
7. Point outside the Statistics window to save your changes.



Name	Min	Max	StdDev	%Total	Mean	CV	%Plot	Count
All Events	(0, 0)	(255, 255)	(9.40, 7.23)	100.00 %	(32.34, 33.97)	(29.07, 21.29)	100.00 %	44431
R3	(23, 3)	(74, 107)	(7.62, 5.00)	99.46 %	(32.00, 33.77)	(23.80, 14.81)	99.46 %	44190
640 nm 670/...	1	95602	5798.59	100.00 %	4106.15	141.22	100.00 %	44431
R2	931	7041	1295.24	25.69 %	2630.07	49.25	25.69 %	11416
R1	931	5623	1276.66	25.59 %	2615.52	48.81	99.61 %	11371

The statistics for the selected gates are displayed, along with comparisons to all events in the gated plot. The gating hierarchy is shown, and for gates on density plots, values for the x-axis and y-axis are shown.

Adding Regions to Density Plots and Time Plots

The steps to follow when adding regions depend on the region type.

To display density plot and time plot region tools

▶ Point to the plot.

The pop-up toolbar appears to the right of the plot.

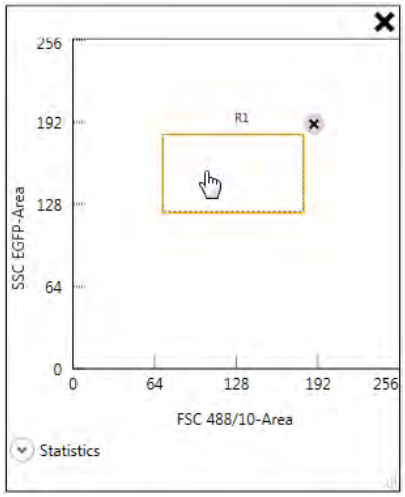
To add a rectangle region to a plot

1. Click Add Rectangle.

   A rectangle is added to the plot.

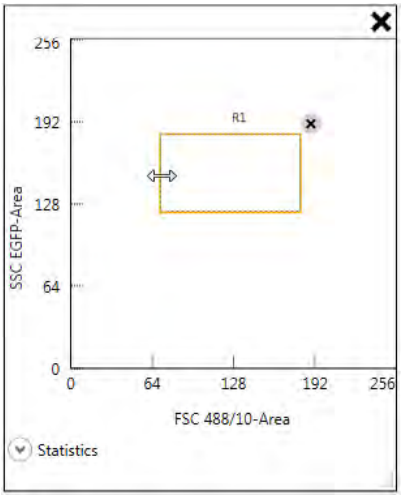
2. To move the rectangle to another part of the plot, point inside the region.

   When the pointer changes to a hand, use it to drag the rectangle.



3. To resize the rectangle, point to its outline.

When the pointer changes to a double-headed arrow, use it to drag an edge of the rectangle.



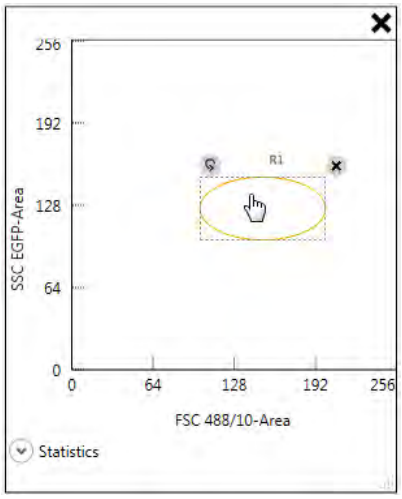
To add a ellipse region to a plot

1. Click Add Ellipse.

   An ellipse is added to the plot.

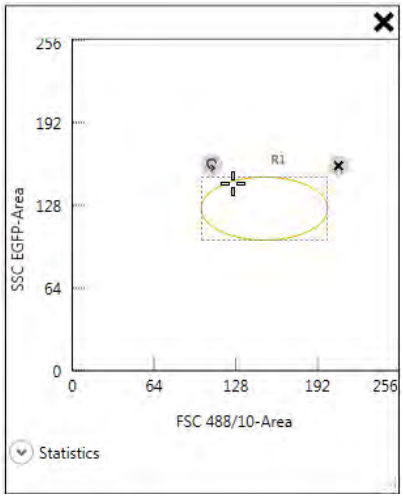
2. To move the ellipse to another part of the plot, point inside the region.

   When the pointer changes to a hand, use it to drag the ellipse.



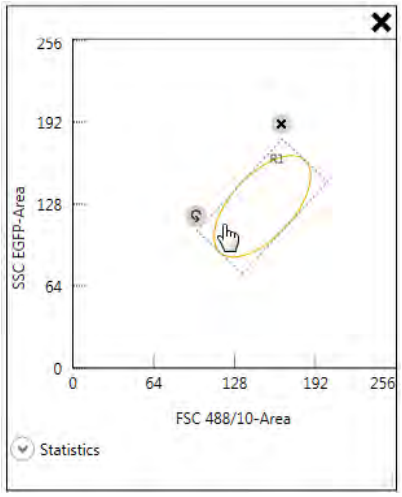
3. To resize the ellipse, point to its outline.

When the pointer changes to a hollow plus sign, use it to drag an edge of the ellipse.



4. To rotate the ellipse, point inside the region.

   When the rotation arrow appears on one corner, drag it to rotate the ellipse.



To add a quadrant region to a plot

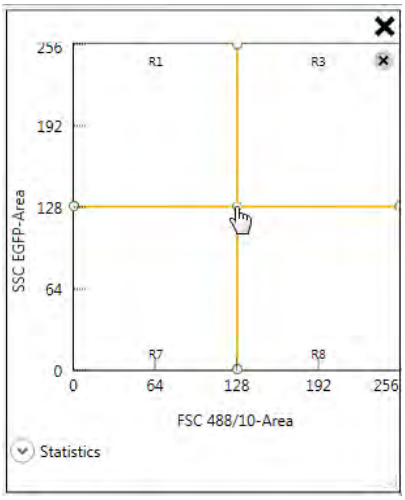
Note: Quadrants apply only to density plots.

1. Click Add Quadrant Region.

   Quadrants are added to the plot.

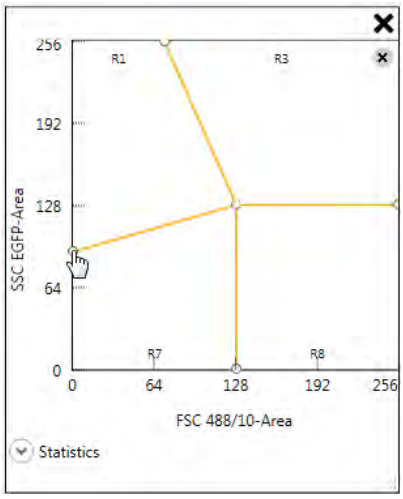
2. To move the quadrant dividers, point to the center circle.

When the pointer changes to a hand, use it to drag the horizontal divider up or down, or use it to drag the vertical divider to the left or right.



3. To skew the quadrant dividers, point to a circle along a plot axis.

   When the pointer changes to a hand, use it to drag the circle along the axis. This changes the shape of two of the quadrants at the same time.

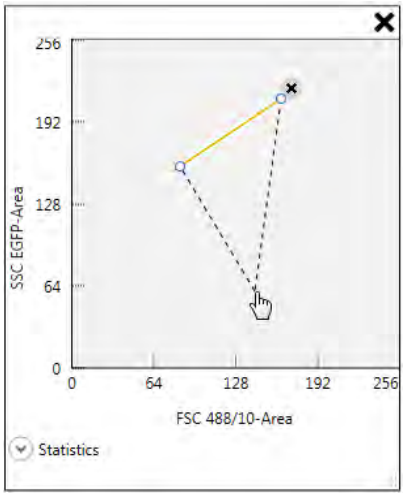


Note: Do not apply hit detection, data tracking, or heat maps to quadrant regions in the workspace Settings screen; wait until you have applied the experiment to the workspace.

To add a polygon region to a plot

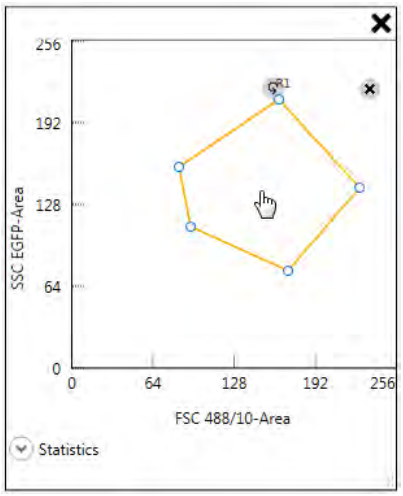
1. Click Add Polygon.

2. Click inside the plot where you want the first corner of the polygon.



3. Move the pointer and click again to create more corners of the polygon.
4. Double-click to finish drawing the polygon.
5. To move the polygon to another part of the plot, point inside the region.

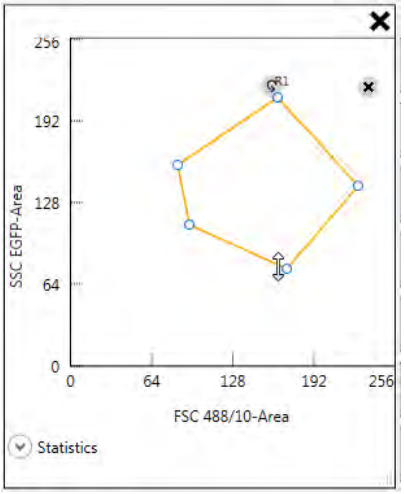
   When the pointer changes to a hand, use it to drag the polygon.



6. To rotate the polygon, point inside the region.

   When the rotation arrow appears on one corner, drag it to rotate the polygon.
7. To resize the polygon, point to its outline.

When the pointer changes to a double-headed arrow, use it to drag an edge of the polygon.



To delete any type of region from a plot

▶ Click the x that appears to the upper right of the region.

Adding Bar Regions to Histograms

The only type of region you can add to a histogram is a bar region, also known as a range.

To add a bar region to a histogram

1. Point to the histogram.

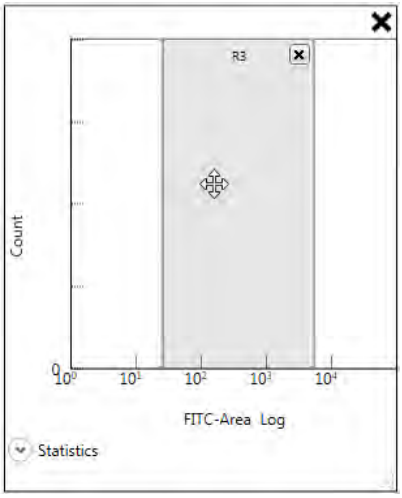
   The pop-up toolbar appears to the right of the histogram.

2. Click Add Bar Region.

   A bar region is added to the histogram.

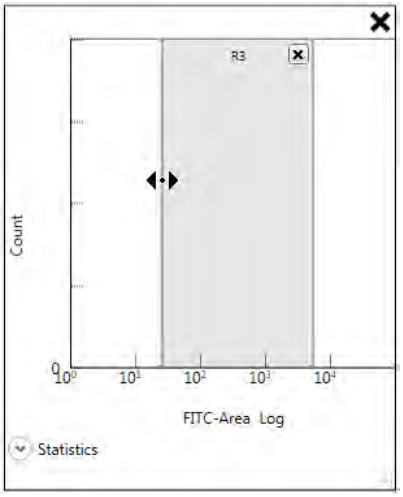
3. To move the bar region to the left or right, point inside the region.

When the pointer changes to a four-headed arrow, use it to drag the bar region.



4. To resize the bar region, point to its left or right edge.

   When the pointer changes to a black double-headed arrow, use it to drag an edge of the bar region.



To delete a bar region

▶ Click the x that appears in the upper right of the region.

Renaming Regions

You can rename regions created in plots or histograms. Bio-Rad recommends that you rename regions before you base gates on the regions.

To rename a region

1. Double-click the region name.

   The region name is selected.

2. Enter the replacement region name.

Tip: If a region name overlaps another plot feature such as a rotation arrow or gate limit symbol, you can drag the region name to move it.

Applying Filters (Gates)

In Everest Software, a gate is a type of filter that is derived from another region. After creating regions, you can apply gates to select a specific area of a plot or histogram. This allows you to restrict analysis to a particular population within a sample and exclude the rest.

Note: When you create a rectangle, ellipse, or polygon region, Everest Software creates a NOT gate that includes events that fall outside of the region.

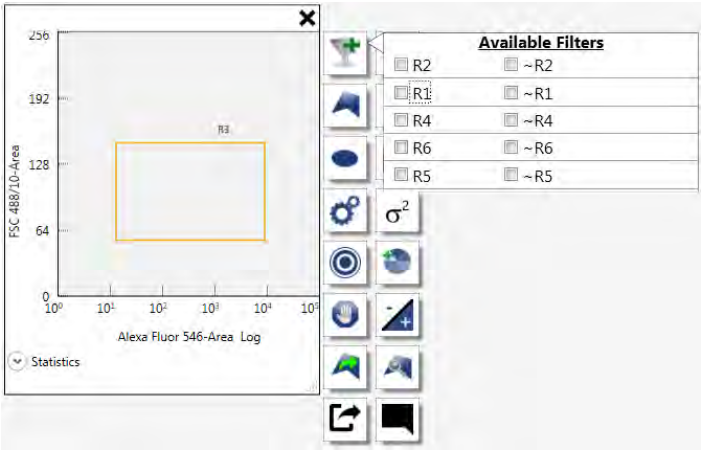
To apply a filter to a plot or histogram

1. Point to the plot or histogram that you want to filter.

   The pop-up toolbar appears to the right of the plot or histogram.

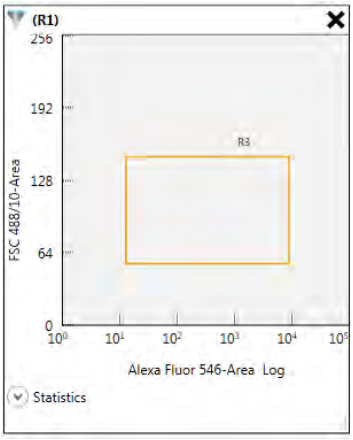
2. Click Apply Filter.

   A list of available filters appears.



3. Select the checkbox for the filter that you want to apply.

   The applied region is shown in the upper left corner of the plot or histogram.

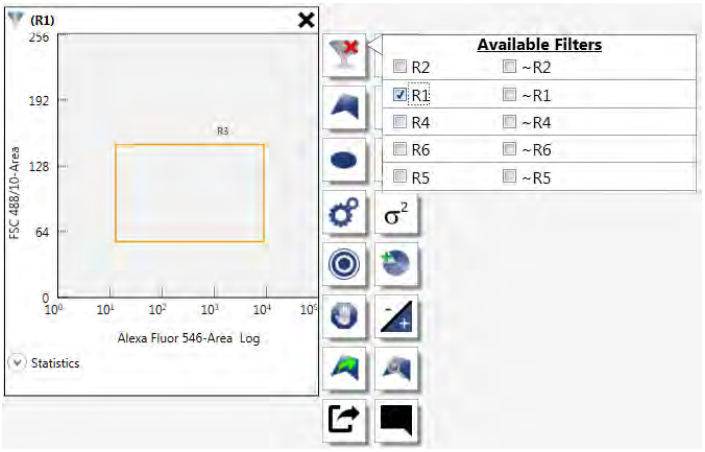


4. The reconfigured plot will now show only the data that fall into the region applied in the filter.

To remove a filter from a plot or histogram

1. Point to the plot or histogram.

   The pop-up toolbar appears to the right of the plot or histogram.



2. Click Remove Filter.

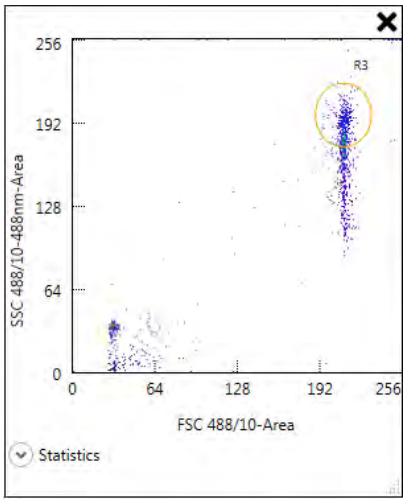
   The filter is removed form the plot or histogram.

Applying Filters (Gates) Using Multiple Regions

By applying filters sequentially, you can create a filter (gate) that uses multiple regions.

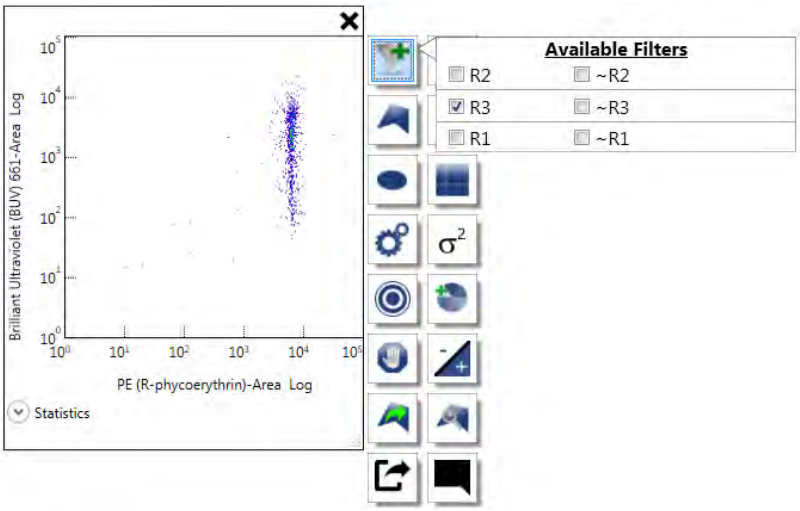
To apply sequential filters to a plot or histogram

1. Create a region on a plot or histogram.



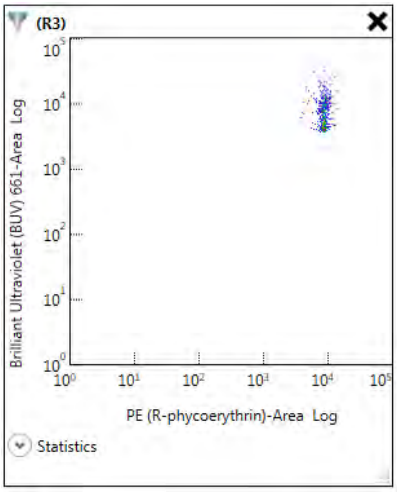
2. Point to a second plot and click Apply Filter in the pop-up toolbar.

   A list of available filters appears.

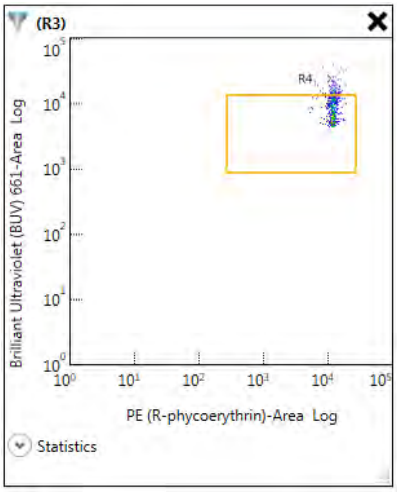


3. Select the region that you created in the first plot.

The applied region is shown in the upper left corner of the plot or histogram.

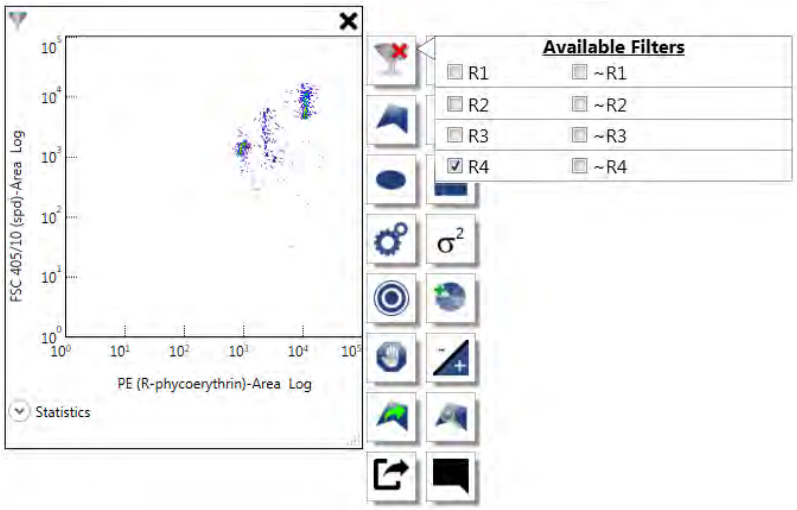


4. Add a region to the second plot.

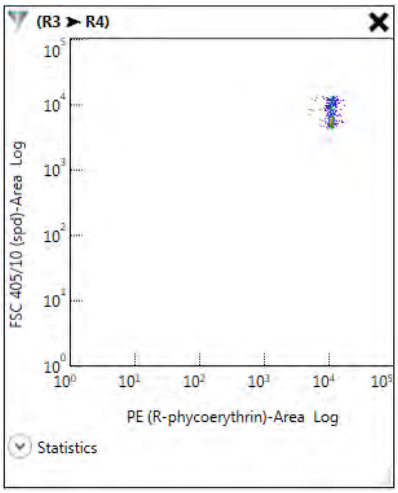


5. Point to a third plot and click Apply Filter in the pop-up toolbar.

6. Select the region that you created in the second plot.



The filter in the upper left corner of the plot or histogram indicates that the plot shows the subset of data from the first region (R3) that also appear in the second region (R4).



Adjusting Plot Color

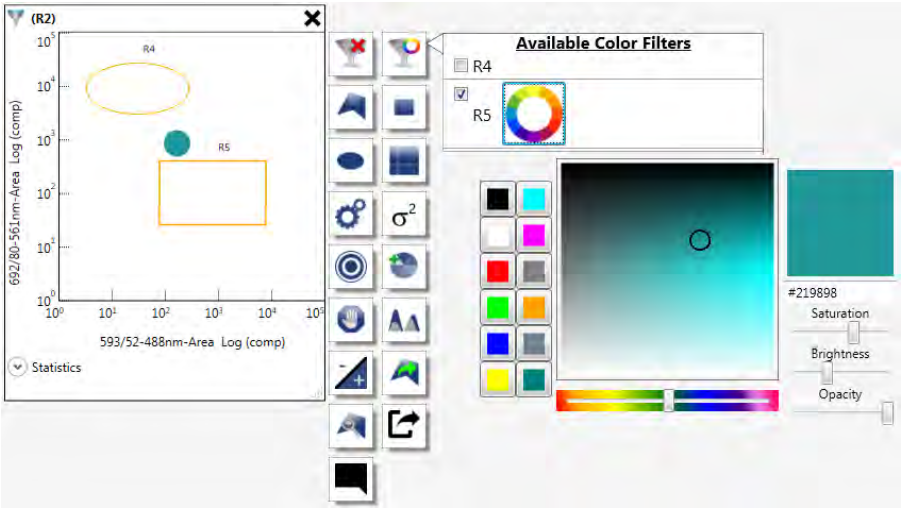
Using color filters (color gating) you can assign a color to a plot region. The population defined by that region will appear in the assigned color in all other plots in the workspace. If the workspace type is Global, the change applies to all plots in the experiment.

To adjust plot color

1. Point to a plot or histogram that contains the region(s) you want to use as a color filter.

   The pop-up toolbar appears to the right of the plot or histogram.

2. Click Apply Color Filter.
3. Select the checkbox for any region that needs a color filter assignment.



4. Click the color wheel.
5. Click a color swatch in the palette on the left or enter the hex number of the color you want.

   Shades of the selected color appear in the selection area.
6. Adjust the color by doing one or more of the following:
   - Use the slider at the bottom.
   - Use the Saturation, Brightness, and Opacity sliders.
   - Drag the circle to a different part of the color selection area.

   The selected color appears to the upper left of the region in the plot.
7. Repeat Step 4 through Step 6 for other regions as needed.
8. Point outside the plot to save your changes.

Applying Gate Limits

Use the Gate Limit tool in the workspace to apply a limit to a region. Acquisition stops when the specified number of events has accumulated in the region or when sample is depleted.

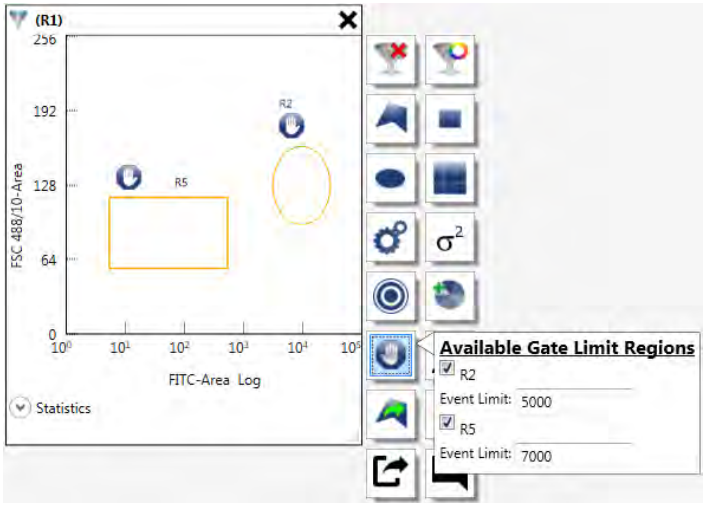
To apply a gate limit to a region

1. Point to a plot or histogram that contains the region(s) that you want to gate.

   The pop-up toolbar appears to the right of the plot or histogram.
2. Click Gate Limit Assignment.

3. In the Available Gate Limit Regions area that pops up, select the checkbox for each region that you want to gate.
4. If needed, change the Event Limit value for each gated region.
5. Point outside the plot or histogram to save your changes.

   A gate symbol appears to the upper left of the region(s), indicating that a gate limit has been applied.



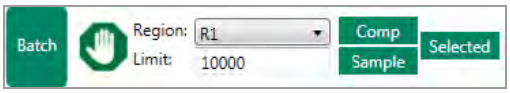
To remove a gate limit

1. Point to a plot or histogram that contains the gated region(s).

   The pop-up toolbar appears to the right of the plot or histogram.
2. Click Gate Limit Assignment.
3. In the Available Gate Limit Regions area that pops up, clear the checkbox for each region where you want to remove a gate.
4. Point outside the plot or histogram to save your changes.

   The gate symbol is removed from the region(s) and the gate limit no longer applies.

Tip: You can also assign gate limits using the Batch section of the toolbar in the Samples screen of the Experiment Builder. This applies the specified limit to the specified gate in Setup positions (compensation controls), Sample positions, or selected positions.



For more information on using this tool, see Applying Gates and Creating Plots on page 286.

Assigning Data Track Regions

You can use data track regions to monitor for clogs or sample disturbances during acquisition. When you assign data tracking to a region in a density plot or histogram, you specify a target percentage. During acquisition, if the percentage of events drops below the target, acquisition pauses and you are notified so that you can determine what sort of problem might be causing unexpected results. Data tracking is especially useful in high-throughput sampling mode.

Note: When assigning data tracking to quadrant regions, wait until the experiment has been applied to the workspace.

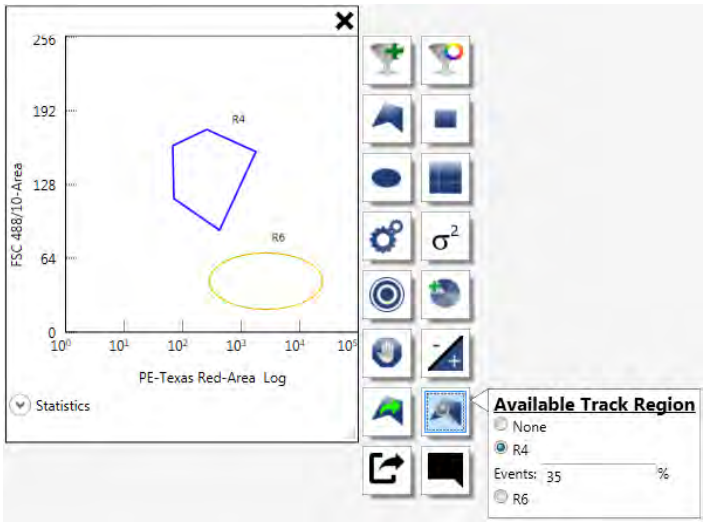
To assign data tracking to a region

1. Point to a density plot or histogram that contains the region where you want to use data tracking.

   The pop-up toolbar appears to the right of the plot or histogram.

2. Click Track Region Assignment.
3. In the Available Track Region area that pops up, select the region where you want to use data tracking.
4. If needed, modify the target percentage in the Events box. The default value is 20%.

   The tracked region is outlined in blue.



5. Point outside the plot or histogram to save your changes.

Note: When specifying the target percentage, leave some room for margin of error, to avoid pausing acquisition for samples with regions that come very close to meeting the target percentage.

To remove data tracking from a region

1. Point to a density plot or histogram that contains the region that uses data tracking.

   The pop-up toolbar appears to the right of the plot or histogram.

2. Click Track Region Assignment.
3. Select None.
4. Point outside the plot or histogram to save your changes.

   The region outline reverts to yellow and the region is no longer used for data tracking.

Applying Heat Maps

You can set up heat maps to determine which samples are rich in one region versus another. You can specify multiple regions for the comparison, using regions from any type of plot or histogram. The heat map regions do not have to be set up in the same plot or histogram.

During acquisition and in the Analysis tab, pointing at a position in the plate map displays a heat map represented as a pie chart. Colors and color gradients provide visual indicators of relative richness. The color intensity of a region's pie slice corresponds to the percentage of the sample's events coming from that region:

- Low percentages appear lighter and gray.
- High percentages appear darker and red.

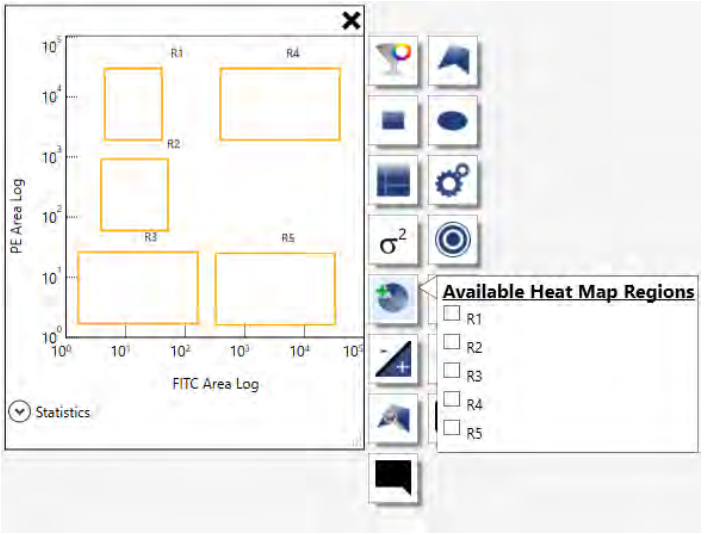
Note: When applying heat maps to quadrant regions, wait until the experiment has been applied to the workspace.

To apply regions to a heat map

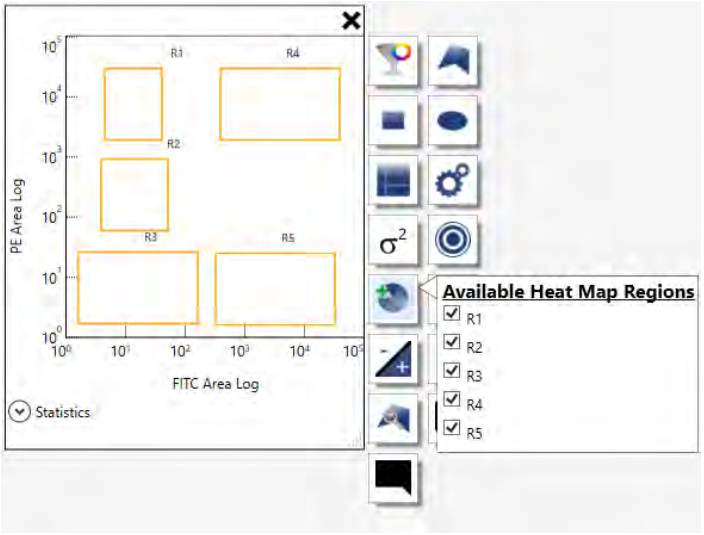
1. Point to a plot or histogram that contains the region(s) that you want to apply to a heat map.

   The pop-up toolbar appears to the right of the plot or histogram.

2. Click Heat Map Region Assignment.

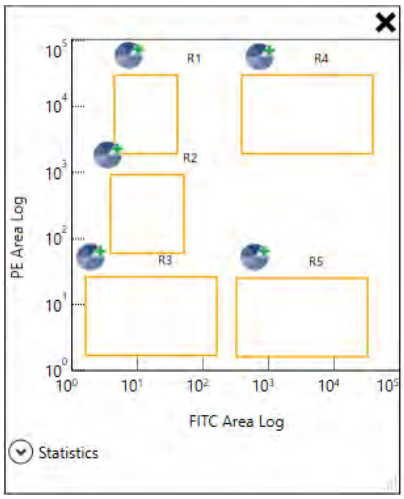


3. In the Available Heat Map Regions area that pops up, select the checkbox for each region that you want to apply to the heat map.



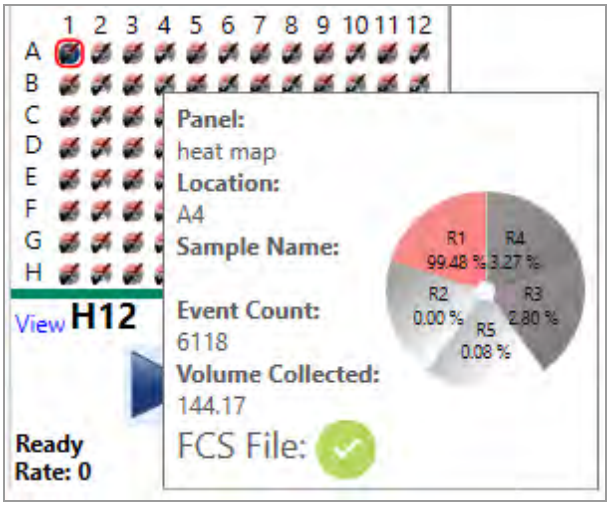
4. Point outside the plot or histogram to save your changes.

   Heat mapping is indicated with a pie chart symbol above the region.



To compare samples using heat maps

1. During acquisition or analysis, point to a position on the plate map.



2. View the heat map that appears for the position.

   The percentage for the region is shown on each pie slice.

To remove regions from a heat map

1. Point to a plot or histogram that contains the region(s) applied to the heat map.

   The pop-up toolbar appears to the right of the plot or histogram.

2. Click Heat Map Region Assignment.

3. In the Available Heat Map Regions area that pops up, clear the checkbox for each region that you want to remove from the heat map.
4. Point outside the plot or histogram to save your changes.

   The pie chart symbol is removed from the region and the region is no longer applied to the heat map.

Configuring Hit Detection

In hit detection mode, Everest Software provides real-time feedback regarding whether a population is present or absent in a given region for each tube or well. You can use hit detection to classify sample positions as hits only if events in a particular region exceed a threshold that you specify. The region is assigned as a "hit region." This is helpful if you are trying to determine which wells or tubes contain positive populations or cells that are of interest.

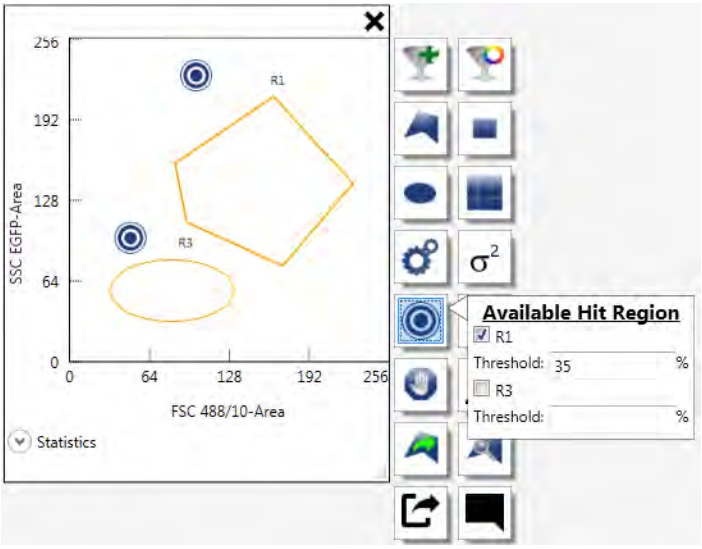
Note: When configuring hit detection using quadrant regions, wait until the experiment has been applied to the workspace.

To configure hit detection

1. Point to a plot or histogram that contains the region where you want to use hit detection.

   The pop-up toolbar appears to the right of the plot or histogram.
2. Click Hit Region Assignment.
3. In the Available Hit Region area that pops up, select the checkbox for the region where you want to use hit detection.
4. If needed, modify the threshold percentage for hit detection in the Threshold box. The default value is 15%.

   The hit region is indicated with a target symbol.



5. Point outside the plot or histogram to save your changes.

To remove hit detection from a region

1. Point to a plot or histogram that contains the region that uses hit detection.

   The pop-up toolbar appears to the right of the plot or histogram.

2. Click Hit Region Assignment.
3. Select None.
4. Point outside the plot or histogram to save your changes.

   The target symbol is removed from the region and the region is no longer used for hit detection.

Adding Diagonal Separators to Plots

A diagonal separating line in a density plot can assist you in determining fluorescence overlap and in setting PMT voltages for a compensation control. In a typical scenario, the parameter to be compensated is shown on the x-axis (as in the plots that Everest Software creates automatically for compensation controls). If a positive population falls below the diagonal line, this indicates that the control sample is brighter in the intended channel than in the channel shown on the y-axis. Thus, the required compensation will be less than 100%. If a positive population falls below the diagonal line, this indicates that the control sample is brighter in a channel other than the intended one and will need more than 100% compensation.

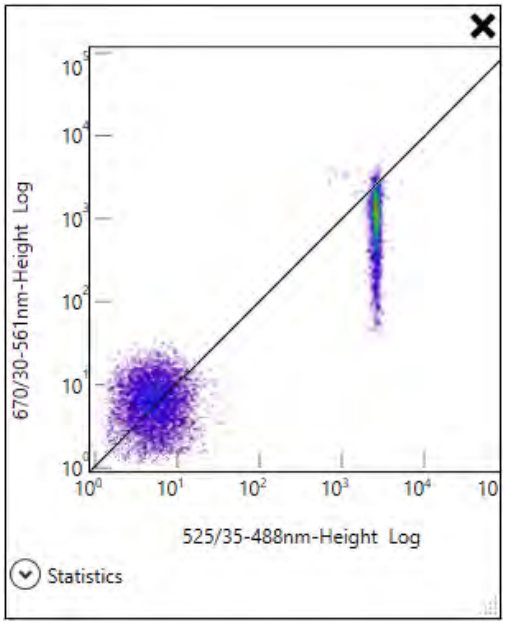
To add a diagonal separator

1. Point to a plot.

   The pop-up toolbar appears to the right of the plot.

2. Click Add Diagonal Separation.

A diagonal line appears on the plot.



To remove a diagonal separator

1. Point to the plot that has the separator.

   The pop-up toolbar appears to the right of the plot.

2. Click Add Diagonal Separation.

   The separator is removed.

Applying a Region to All Plots

You can apply regions to other plots or histograms in the following ways:

- After setting up a rectangle, ellipse, or polygon region on a plot, you can copy this region to all other plots in the workspace.
- After setting up a bar region on a histogram, you can copy this region to all other histograms in the workspace.
- After setting up a region on a plot or histogram, you can apply this region as a filter on all other plots or histograms in the workspace.

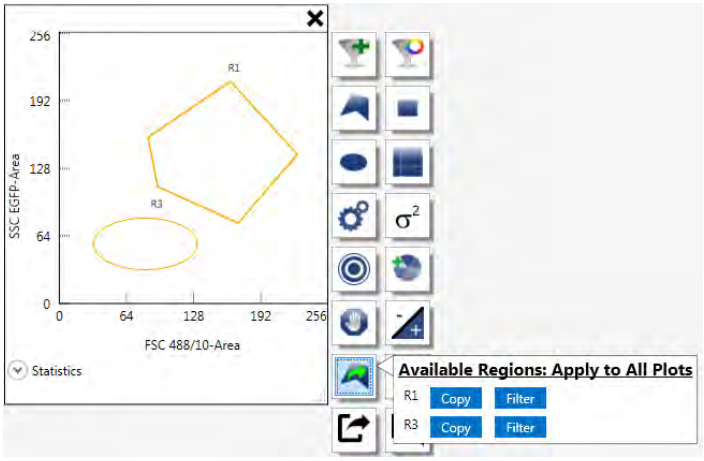
If the workspace type is Global, the change applies to all plots or histograms in the experiment.

To apply a region to all plots

1. Point to the plot that contains the region(s) you want to use.

   The pop-up toolbar appears to the right of the plot or histogram.

2. Click Apply Gate to all Plots.



3. Do one of the following:

   - Click Copy to copy the region to other plots of the same type.
   - Click Filter to apply the region as a filter on all other plots.

4. Repeat Step 3 for other regions as needed.

5. Point outside the plot or histogram to save your changes.

Adding Annotations to Plots

You can annotate a plot or histogram directly.

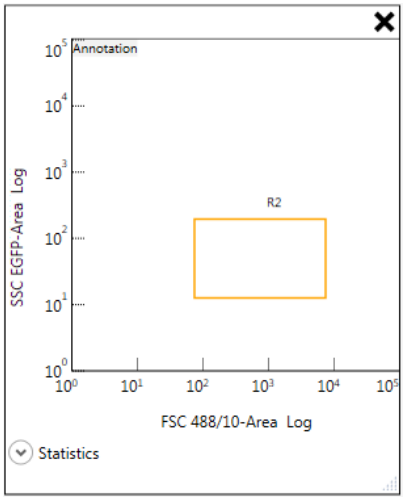
To add an annotation to a plot or histogram

1. Point to the plot or histogram that you want to annotate.

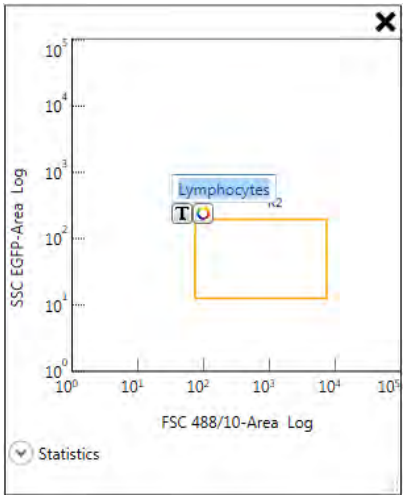
   The pop-up toolbar appears to the right of the plot or histogram.

2. Click Add Annotation.

   A text block containing the word “Annotation” is added to the upper left area of the plot or histogram.



3. Double-click the text to select it; replace it with your annotation.
4. To change the font size, double-click the text and click the T symbol in the pop-up toolbar that appears below the annotation. Use the slider to increase or decrease the size.



5. To move the annotation, drag it to another part of the plot or histogram.
6. To remove the annotation, double-click it and press Delete.

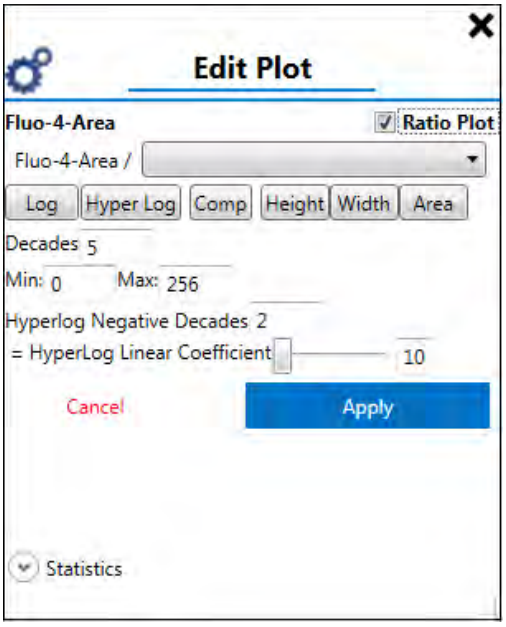
Using Plot Ratios

Everest Software allows you to select one parameter as the numerator of a ratio, and a different parameter as the denominator of the ratio. You can then plot this signal ratio against time; this can be useful in time-based assays. For more information about time plots, see Creating Time Plots on page 169.

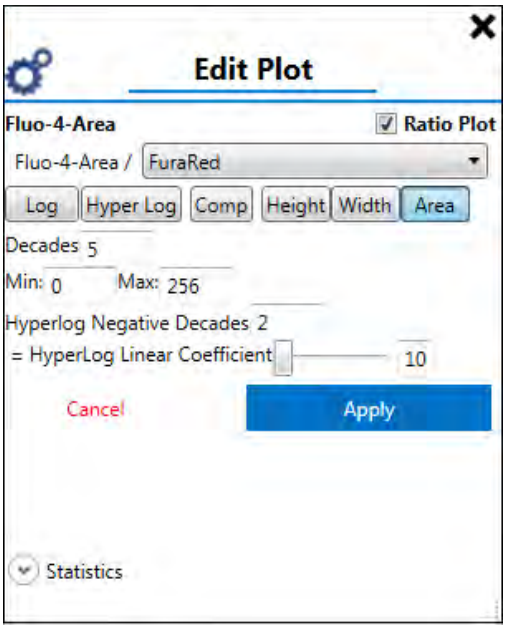
To use a ratio in a time plot

1. Decide which parameter you want to use as the numerator in the ratio, and which parameter you want to use as the denominator.
2. Create a time plot, choosing the numerator as the parameter for the y-axis.
3. Specify axis scaling, display of compensated data, pulse measurement, and time range as needed.

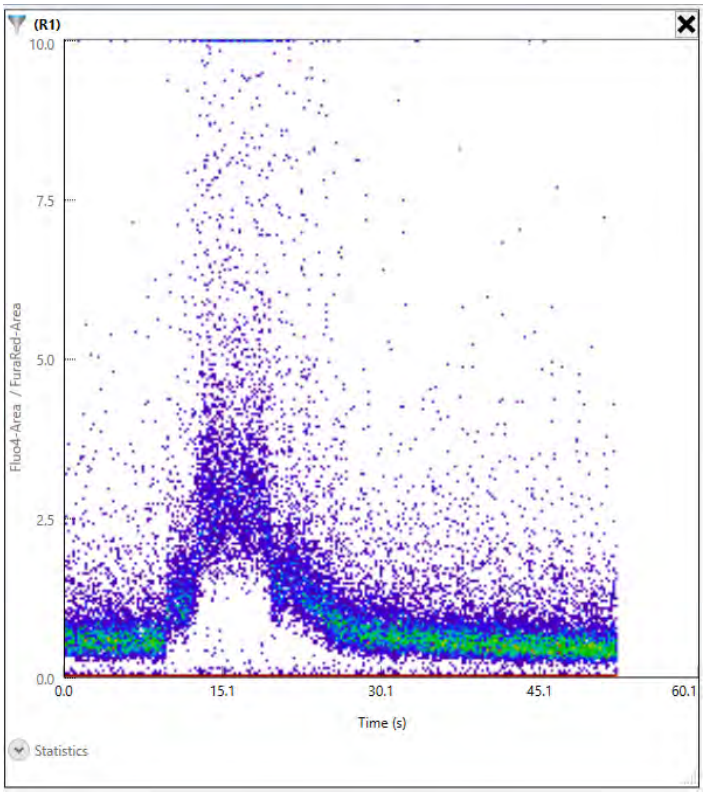
4. Click Modify Plot Parameters in the pop-up toolbar for the time plot.



5. Select the Ratio Plot checkbox.
6. Select a second parameter to use for the ratio denominator.



7. Specify axis scaling, display of compensated data, and pulse measurement as needed.
8. Click Apply.



The modified time plot appears. The y-axis uses the ratio of the first parameter to the second parameter.

Exporting a Plot as a Graphic

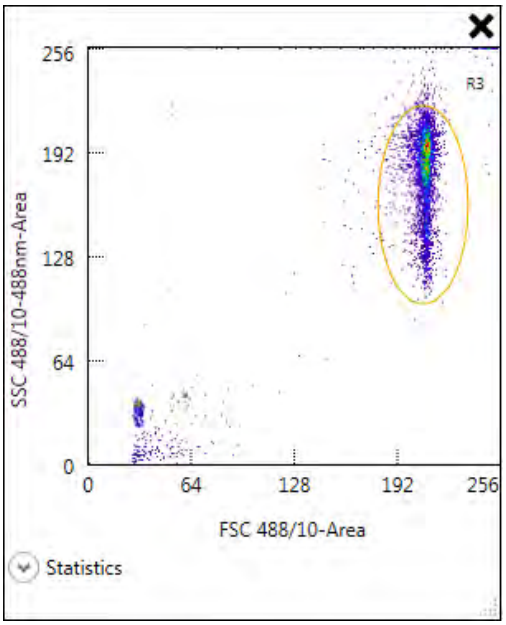
You can export any plot or histogram as a graphic in PNG format.

To export a plot as a graphic

1. Point to the plot or histogram that you want to save as a graphic.

   The pop-up toolbar appears to the right of the plot or histogram.

2. Click Export to PNG.

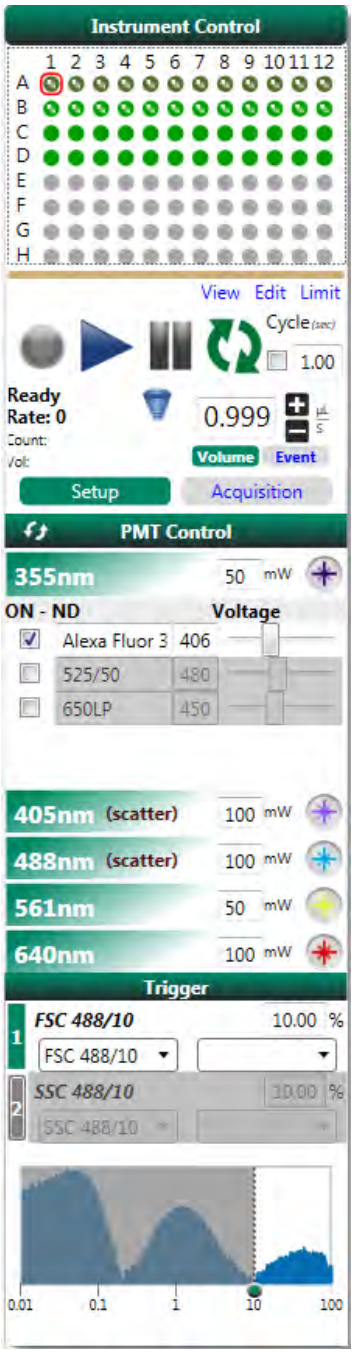


3. In the Save As dialog box, browse to the location where you want to save the graphic.
4. Give the graphic a name.
5. Click Save.

   The graphic is exported as a PNG file.

Configuring Instrument Settings

In addition to setting up plots in the workspace, you can use the Settings screen of the Experiment Builder to enter initial instrument settings such as PMT voltages, laser power, and primary and secondary trigger and threshold.



Note: The default instrument settings are those used for the previous experiment.

PMT and Laser Controls

In this section of the Instrument Control panel, you can modify settings related to lasers and their associated detectors.

Table 39. PMT and Laser Control Items

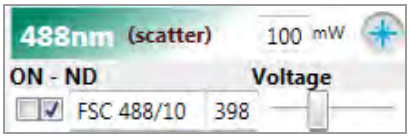
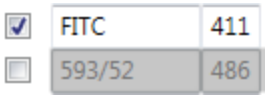
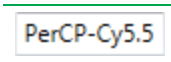
Item	Function
	**Display filter numbers/detector names** — toggles between detector filter numbers and detector names for each of the lasers in the system.
	**Load voltages** — loads PMT voltages from a run list file that you browse to and select.
	**Reset voltages** — resets PMT voltages to default values.
	**Open/close laser shutter** — controls the laser shutter. When this button is gray, the laser is not delivering light to the flow cell. **Note:** The shutter must be open to allow parameter selection from that laser. The shutter automatically closes if no parameters for the laser have been selected in the Experiment Builder.
	**Edit laser power** — allows you to specify power settings for individual lasers, in steps, from 10 mW to the maximum power output.
	**Neutral density checkboxes** — if selected, a 2.0 ND filter is moved in front of the forward scatter detector. For more information, see Optical Mirror and Filter Types on page 32.
	**Select parameter for acquisition** — allows you to select and name acquisition parameters. If a parameter is not selected, the box remains unselected, appears gray, and is not acquired or included in the FCS data file.
	**Editable parameter name** — each parameter name is editable and is saved with the acquired FCS data. When changed, this name propagates to plot and histogram axes, compensation screens, and the Filter Configuration dialog box.

Table 39. PMT and Laser Control Items, continued

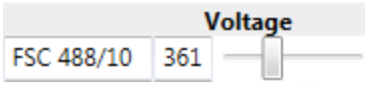
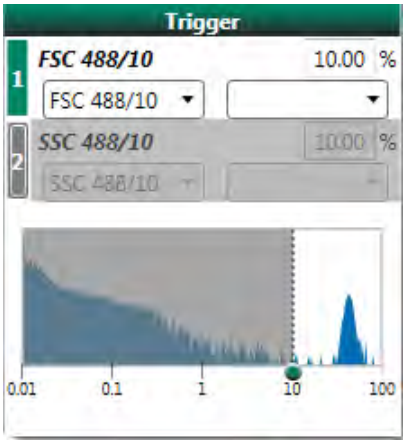
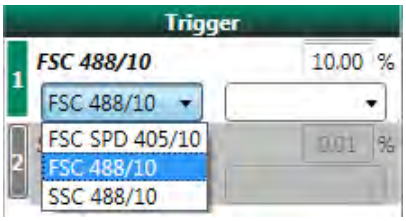
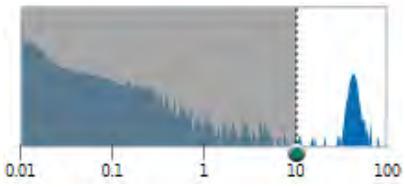
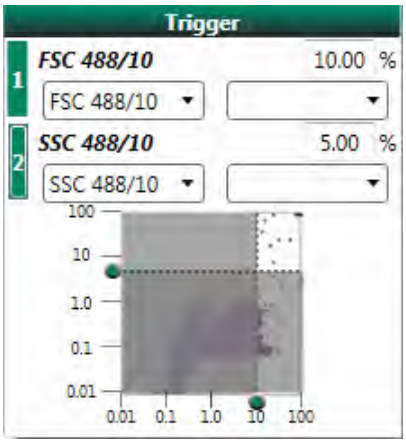
Item	Function
	**PMT voltage** — allows you to adjust the PMT voltage for the selected detector, either by entering a value or moving the slider.
	**Trigger** — use the trigger parameter or parameters to alert the system to the presence of an event over the threshold. The trigger plot represents what the electronics are detecting; it uses log scaling on the x-axis and data in the gray region are excluded. Raising or lowering the threshold allows you to exclude unwanted data from acquisition. Data below the threshold are not saved as part of the FCS file; only events at or above the threshold are saved.
	**Select trigger parameter** — the default trigger is forward scatter (488 nm laser). Everest Software enables data triggering by up to two unique parameters, either scatter or fluorescence. The threshold is set using the selected trigger parameter.
	**Secondary trigger channel** — click the row header to add a secondary trigger/threshold channel. This row is grayed out if it is not in use.
	**Adjust threshold setting** — threshold is a percentage of the signal in the trigger detector. The range that can be entered is 0.01 to 99.99%. The default value is 10.00%. **Tip:** When utilizing a trigger parameter in log display, set a threshold value of less than 1% to allow display of data in the lower log decades.

Table 39. PMT and Laser Control Items, continued

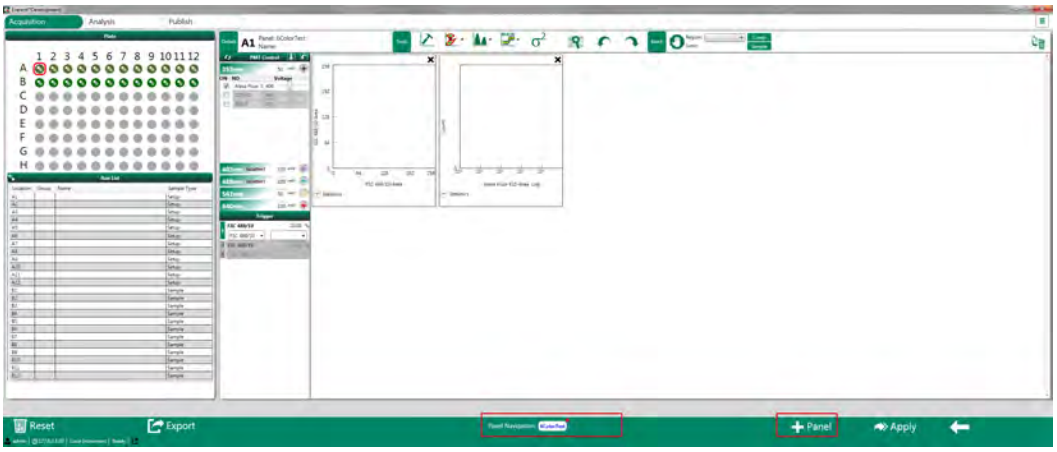
Item	Function
 	**Current threshold position** (threshold plot) — this live data plot shows all the data seen by the acquisition electronics in the trigger parameter. The current threshold position is indicated by the dotted line. Although the threshold plot shows every event measured in this detector, events below the threshold are not saved in the data file. If two triggers are selected, the primary trigger threshold is indicated by the vertical dotted line and the secondary trigger threshold is indicated by the horizontal dotted line.

Setting Up Multiple Panels

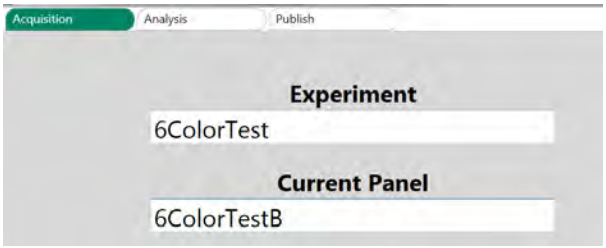
You can use the Panels feature in Everest Software to set up multiple experimental panels on a single plate or tube rack. The panels within an experiment are displayed at the center bottom of the Settings screen. For more information about multipanel experiments, see Multipanel Experiments on page 71.

To set up a multiple panels

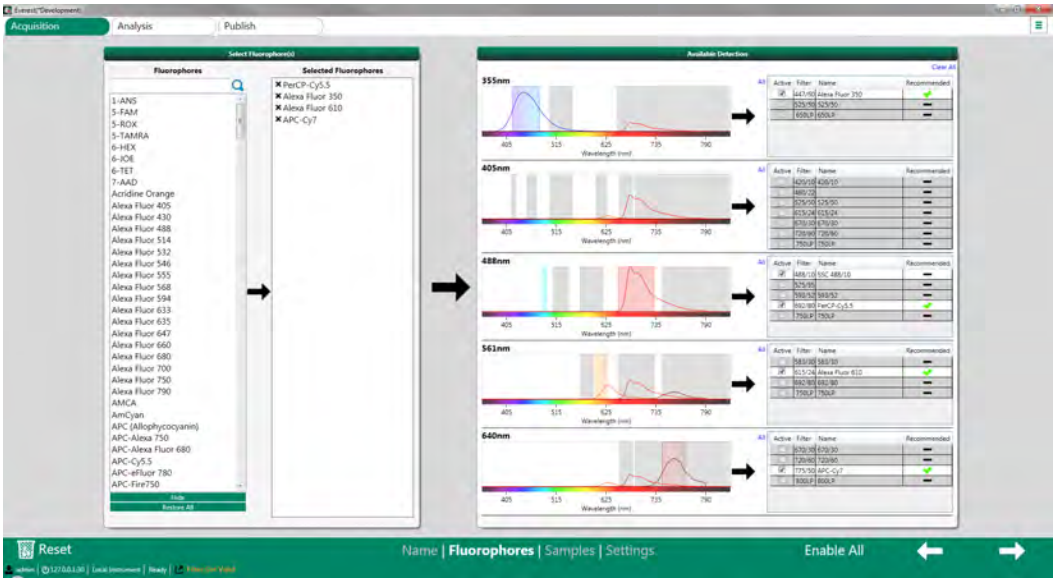
1. After defining the first panel, click +Panel in the Settings screen.



2. In the Name screen, enter a name for the new panel in the Current Panel box.

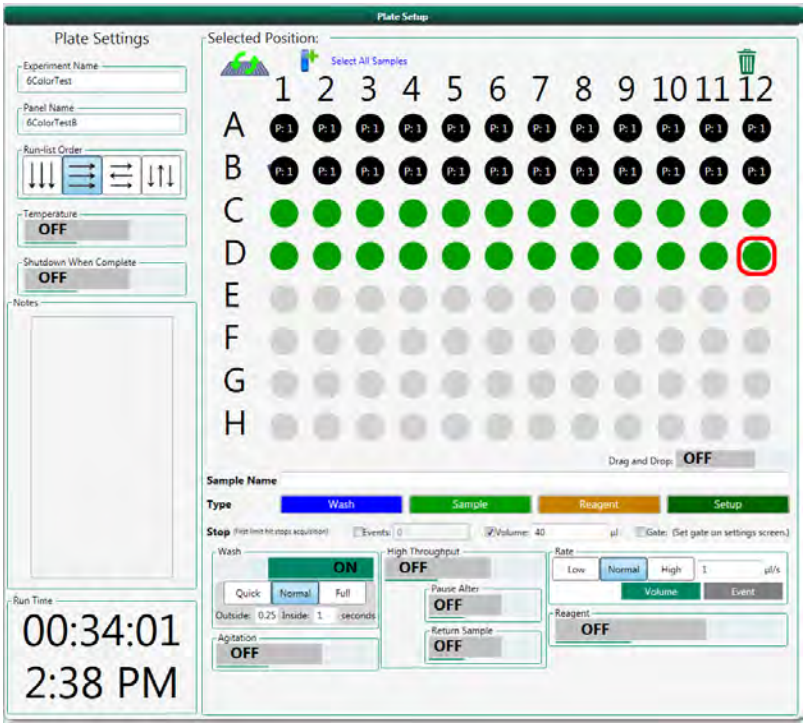


3. Click the Next arrow to go to the Fluorophores screen.
4. Define a new set of activated parameters and parameter names.



5. Click the Next arrow to go to the Samples screen.

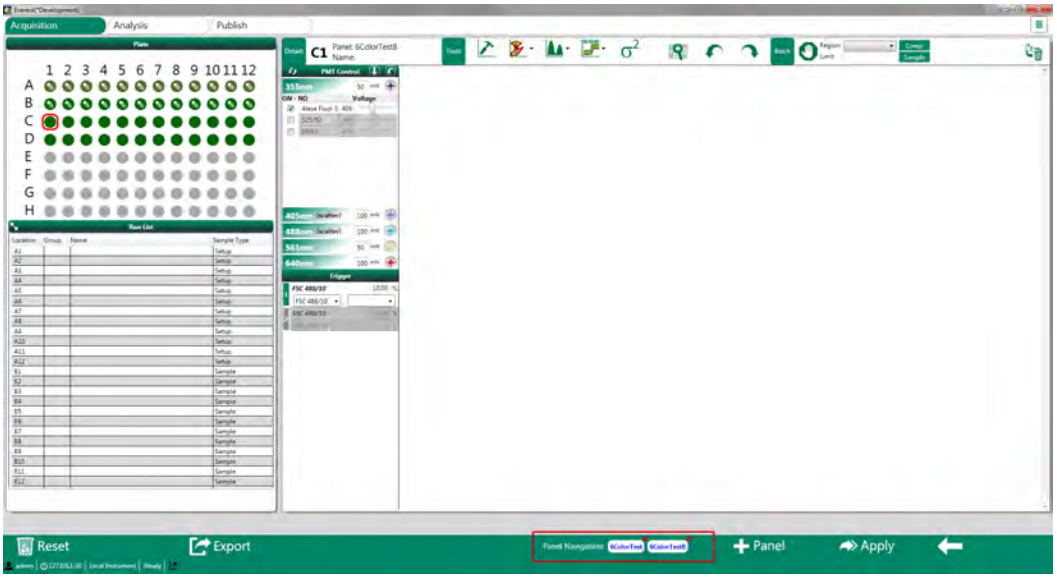
Positions programmed for the previous panel are shown in black; any positions created for the new panel appear as usual.



6. Using the Selected Fluorophores, Plate Setup, and Run List panels, configure the new panel as needed.

   Note: If settings for the new panel need to differ from settings for the previous panel, ensure that you change them.

7. Click the Next arrow to go to the Settings screen. Note the names of the panels at the bottom center of the screen.



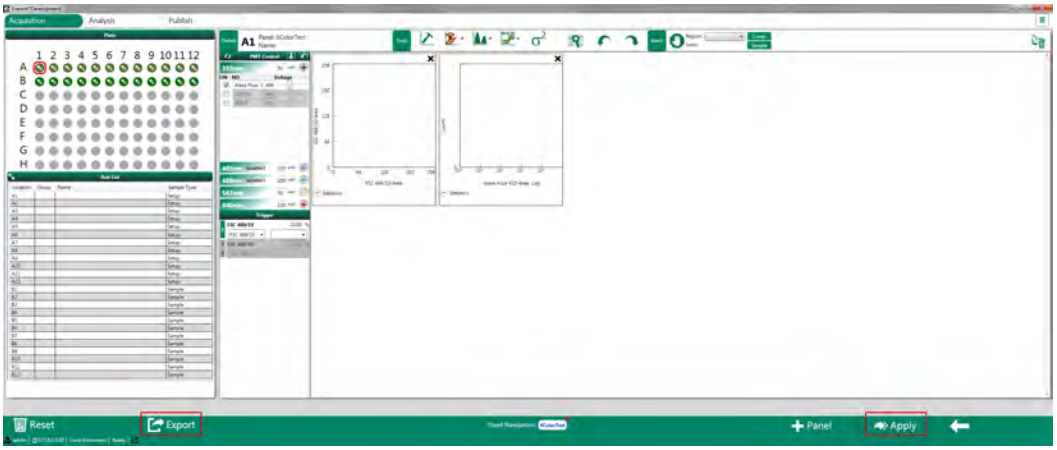
8. Draw plots and regions and create gates for the new panel.
9. Click Apply to apply the experiment, including all of the panels, to the workspace.

Tip: To adjust settings, including PMT voltages, in a multipanel experiment, adjust for each panel individually in setup mode. Point to the positions in the plate map to view their panel assignments. When you move to acquisition mode, the ZE5 Cell Analyzer will acquire the whole plate or set of tubes in a single run and will respect the settings adjustments optimized for each panel in setup mode; there is no need to reoptimize settings between the panel runs.

Running or Saving the Experiment

After configuring the workspace and initial instrument settings in the Settings screen of the Experiment Builder, you are ready to begin acquiring your samples, as described in Chapter 8, Acquiring Samples.

To run or save the experiment

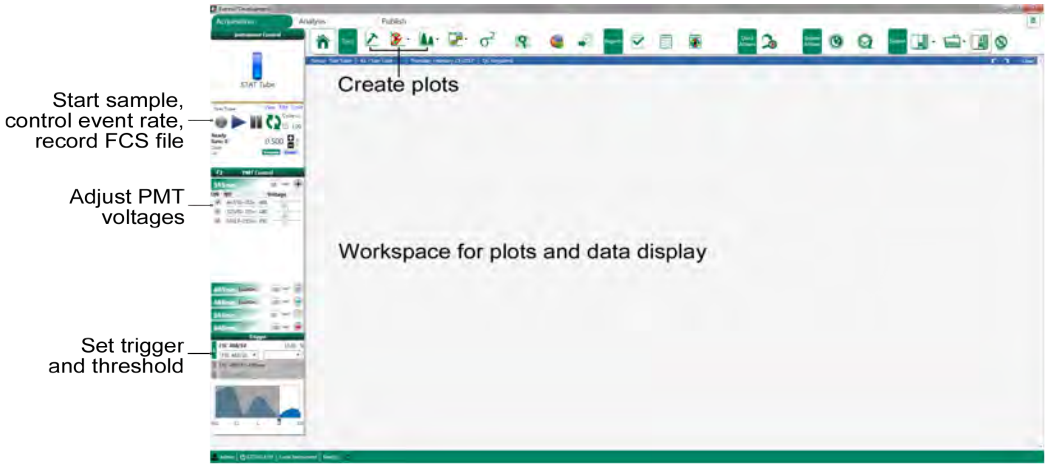


▶ Do one of the following:

- To load the completed run list, click Apply.
- To save the run list for future use, click Export.

Running a Quick (Stat) Sample

If you need to quickly run a single sample or a few samples, you can bypass the Experiment Builder. The stat tube option in the Home window brings you directly to the Workspace screen, where you can create plots, add regions, apply filters, and adjust settings. Sample acquisition and data recording are available directly from this screen. When you run a stat tube, all parameters are activated by default and can be named by editing the name in the parameter list.



See the following sections for more information:

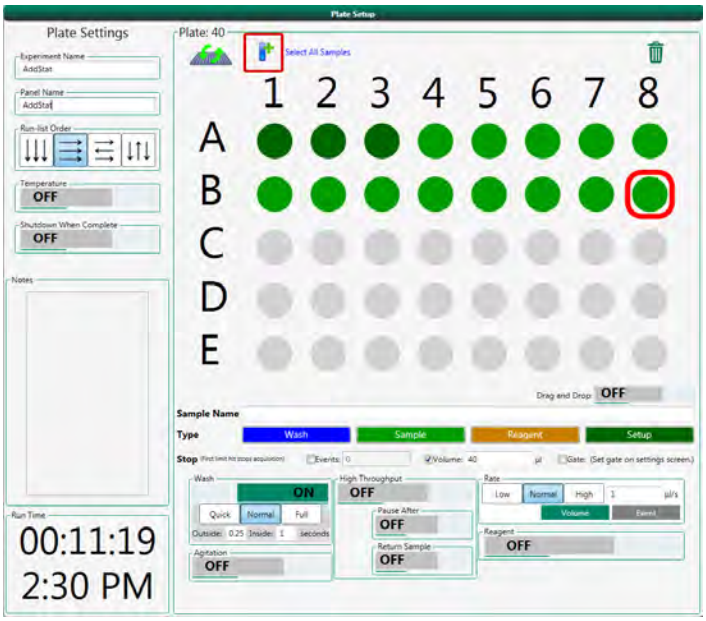
- Chapter 8, Acquiring Samples
- PMT and Laser Controls on page 208
- Creating Plots and Histograms on page 166

Adding a Stat Tube to a Plate or Rack

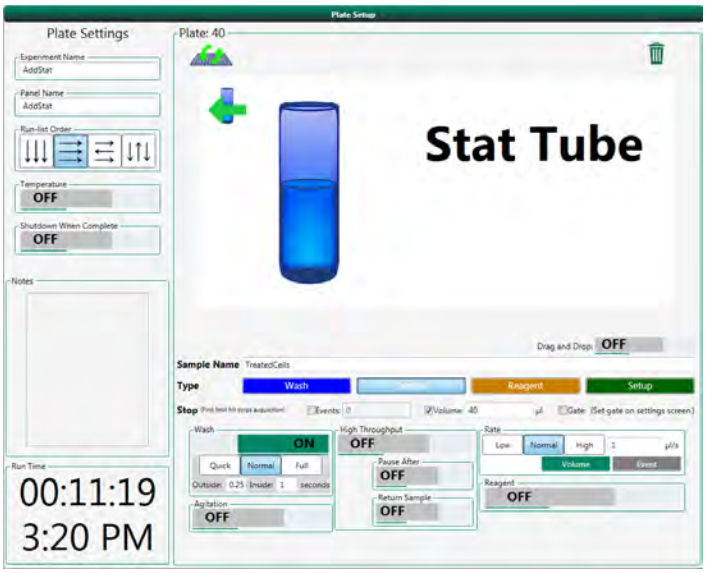
To accommodate large volumes of sample, you can add a single stat tube to an experiment that is based on a plate or tube rack. An added stat tube can be assigned only the Sample position type; it cannot be assigned as Wash, Reagent, or Setup. Agitation, temperature, and high-throughput settings are not applicable to stat tubes.

To add a stat tube to an experiment

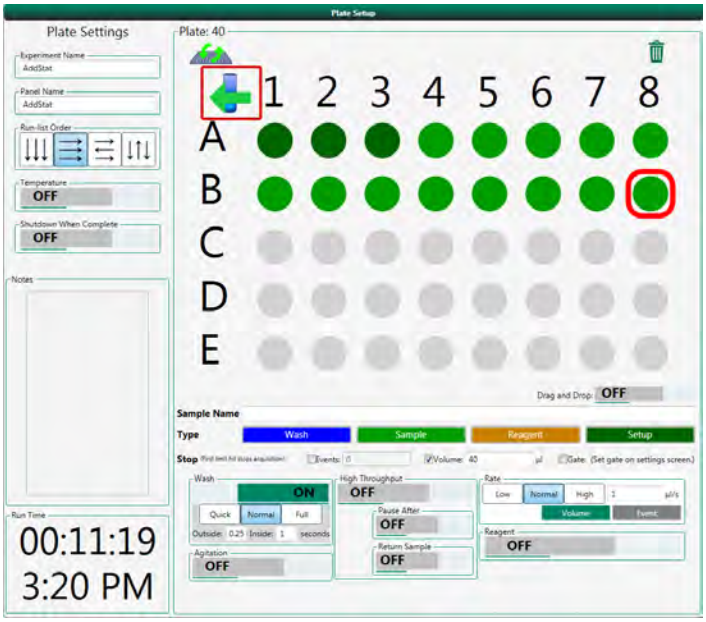
1. Click Add Stat Tube.



2. (Optional) Enter a sample name for the stat tube.



3. Click Sample to select the Sample position type for the stat tube.
4. Specify other settings for the stat tube such as stop limits and target event or flow rate.
5. Click Plate View to return to setting up the plate or rack.



The added stat tube is indicated with a symbol in the upper left area of the plate map.

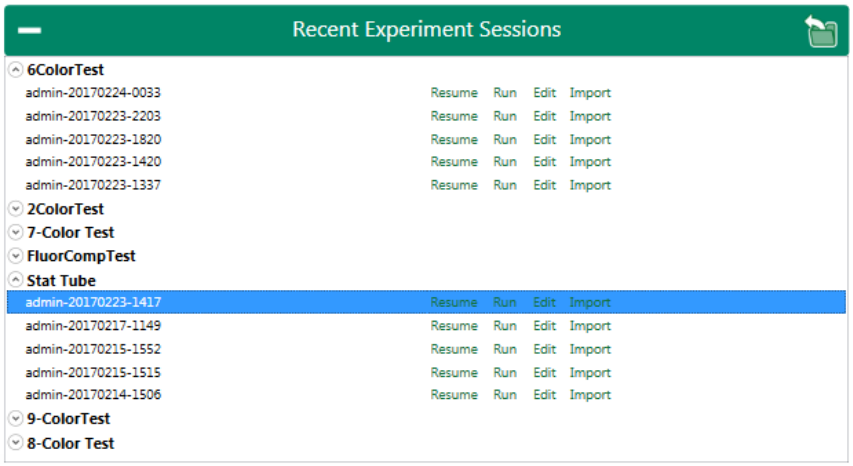
Running an Existing Experiment

The Recent Experiment Sessions panel displays the number of recent sessions specified in the global preferences. If the existing experiment that you want to run is relatively recent, it might appear in the list. If it is not recent, you can browse to find it.

To run an existing experiment

1. Return to the Home window.
2. In the Recent Experiment Sessions panel, expand the list of recent experiments.
3. Do one of the following:

   - If the experiment session that you want to run is in the list of recently displayed items, click Run for the experiment session.



     The experiment session is applied to the workspace.

   - If the experiment session that you want to run is not in the list of recently displayed items:

     a. Click Load Run List in the upper right.

     b. Browse to find the experiment that you want to run.

     c. Select the .rlst file and click Open.

     The experiment opens in the Experiment Builder, where you can make any needed adjustments before applying it to the workspace.

For more information, see Recent Experiment Sessions on page 49.

Importing Settings

To speed up creation of a new experiment, you can import a subset of experiment settings from a previous experiment. Importing brings in the following items:

- Fluorophores (activated PMTs)
- Parameter names
- Instrument settings
- Compensation matrix
- Plots

Sample positions are not imported. This allows you to apply the settings to different media and different configurations of samples.

To import settings

▶ Do one of the following:

- In the Home window, click Import Settings. Find the run list file that contains the desired settings, select it, and click Open.
- In the Home window, expand the list in the Recent Experiment Sessions panel. For the experiment session that contains the desired settings, click Import.

The Experiment Builder opens at the Name screen, where you can assign a name for the new experiment that uses the imported settings. In the Fluorophores screen, you can use the imported fluorophore and detection settings as-is, or you can modify them. In the Samples screen, you will need to select a media type and set up controls and samples for the experiment, because these settings are not imported. If you used the compensation template in the previous experiment, the same compensation template will be applied.

Chapter 8 Acquiring Samples

For information about the types of files produced during acquisition, see File Types in Everest Software on page 239.

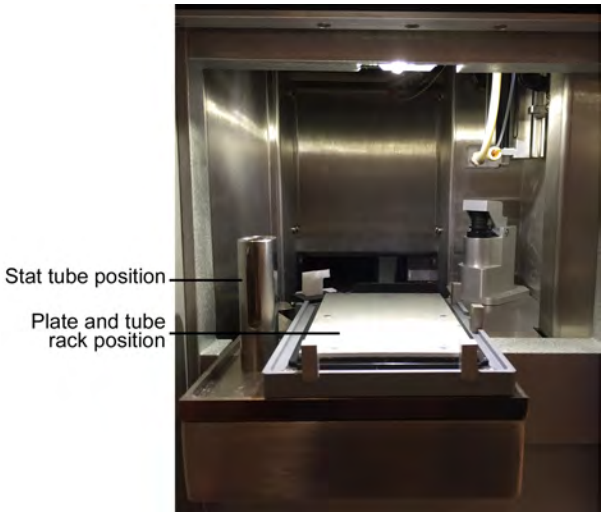
Loading Sample Media

After configuring your experiment in the Everest™ Experiment Builder and applying it, load your samples into the instrument.

To load a sample

1. Press the silver sample chamber button on the front of the instrument to extend the loader.
2. Install a tube rack, plate, or stat tube on the loader in the appropriate position, depending on the workspace that has been created for the experiment.

   Note: Ensure that any inserted tube rack or plate is oriented correctly, with position A1 in the front left corner.



3. Press the silver sample chamber button to retract the loader.

Setup Mode Controls

Use setup mode to determine optimal instrument settings for sample acquisition. You can modify acquisition parameters (including target flow rate or event rate, stop conditions, agitation, and wash settings) from what was defined in the Experiment Builder. In setup mode, you can adjust the threshold and PMT voltages and create gates. You can also use the Record function to manually save data to files. This gives you more control over when data are saved during the run. Data are only saved after you click Record. Recording stops after the stop conditions have been met or when you manually stop recording.

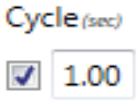
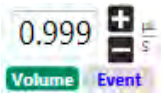
For a stat tube, the Setup button does not appear; sampling occurs in setup mode by default.

Tip: To edit an experiment, navigate to the Instrument Control panel in setup mode and click Edit.

Table 40. Setup Mode Control Items

Item	Function
View	**View Run List** — expands the run list to display settings for the current experiment.
Edit	**Edit Experiment** — guides you through the Experiment Builder to modify the experiment as needed.
Limit Event: 0 Volume: 20	**Acquisition Limit for Record Function** — allows you to specify an event or volume limit for recording data in setup mode.
	**Record** — appears after acquisition begins. Starts saving data to an FCS file.
	**Play** — moves the probe to the selected position, lowers it, and turns on the sample pump to run sample. Begins streaming data to Everest Software.
	**Stop** — ends data acquisition and turns off the sample pump. Replaces the Play button when sampling begins.

Table 40. Setup Mode Control Items, continued

Item	Function
	**Pause Run List** — pauses sampling. Time gaps that correspond to pause times appear in the data file.
	**Pause Run List** — resumes sample flow. Replaces the pause button when sample is paused.
	**Clear Data** — refreshes data displayed on plots in the workspace. Does not clear data being stored.
	**Cycle Mode** — when activated, plots display only a certain number of events, based on the time period specified in this box. After the interval is reached, data are cleared from the plots and newly acquired events appear.
	**Event Rate and Volume** — during sampling, displays the triggered event rate, the event count, and the volume that has been acquired from the sample during the current run, along with target volume.
	**Agitate** — allows you to apply agitation to a sample that has been sitting for an extended period. Click to turn on agitation at the default speed for the media type. Click again to turn off agitation.
	**Flow Rate** — flow rate can either be determined by a target volume rate or by a target event rate. To switch between volume control and event rate control, click the corresponding button. Change the value in the box by clicking the plus and minus buttons (for volume rate only) or by entering it directly.

Acquiring Initial Sample in Setup Mode

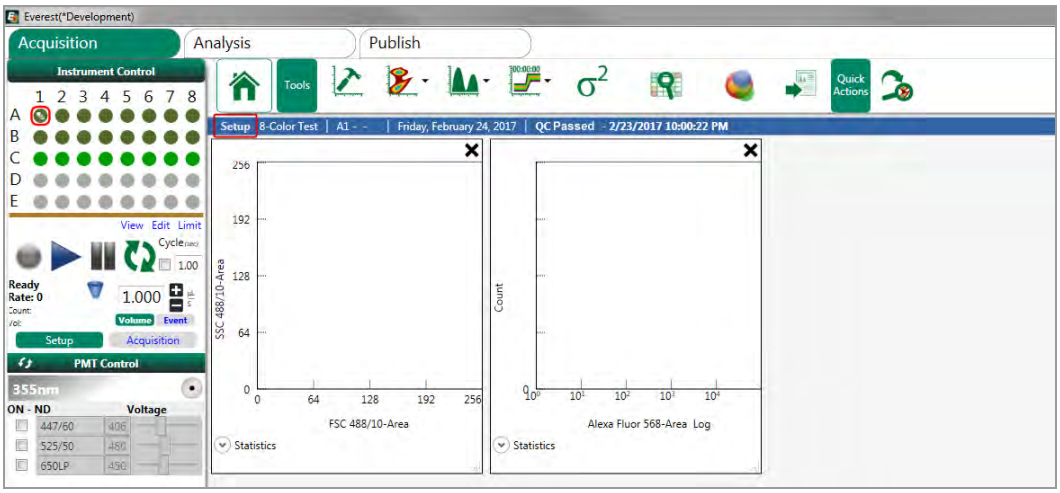
Before proceeding through these steps, ensure that you have set up an experiment using the Everest Experiment Builder (unless you are running a stat tube, which does not require experiment setup). Use the information in Configuring Instrument Settings on page 206 to fine-tune instrument settings while in setup mode.

Note: Event, volume, and gate limits apply only when recording data in setup mode.

To sample in setup mode

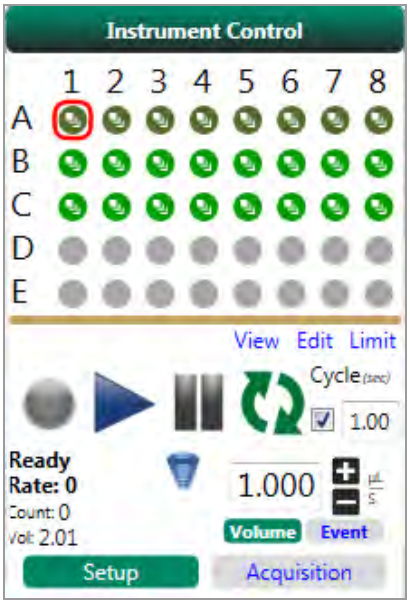
1. Ensure that the correct experiment has been created and loaded.
2. Load the sample.
3. Click Setup in the Instrument Control panel.

Note: The current mode is displayed in the workspace status bar. For a single stat tube, the Setup button does not appear; sampling occurs in setup mode by default.



4. In the plate map, click the position from which sampling should begin.

   The current position is outlined in red.



5. Click Play to initiate sampling. The probe moves to the selected position, sample is boosted to the flow cell, and acquisition begins.

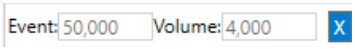
6. Adjust the flow rate by setting a target volume rate or event rate as needed.

7. Configure the settings for the current sample by selecting the trigger, setting the threshold, and adjusting the PMT voltages.

   Tip: Activate cycle mode when adjusting settings so that the data displayed in the plots are up to date and reflect any changes that have been made.

8. After optimizing the settings, either stop acquisition or record a data file.

   - To stop sampling, click Stop.
   - To record, first ensure that the limits are set as needed. The default limits are those that have been set in the Experiment Builder. To change the limit, click Limit in the Instrument Control panel.

     You can use event, volume, and gate limits simultaneously. During recording, acquisition stops when the first limit is reached. When you enter an event limit, the volume limit is automatically set to the maximum for the media type.

     a. Adjust limits as needed.

     b. Click Record. Data are collected until the specified limit is reached. To stop sampling, click Record again.

     c. After data have been recorded for a tube or well, the position in the plate map is indicated by a black check mark. For information about symbols shown in the plate map, see Plate Map on page 67.

9. After the first position has been sampled from and/or recorded, select the next tube by clicking its position in the plate map and proceeding as described above.

10. Continue sampling from tube/well positions in setup mode as needed.

    You can acquire data and record data for your entire experiment in setup mode. If you have finished optimizing settings in setup mode, you can switch to acquisition mode to continue acquiring sample in a more automated fashion. If you want to record data from a stat tube, you must record it in setup mode; acquisition mode does not apply to single stat tubes. In acquisition mode, data are not acquired from a stat tube added on to the run list for a plate or tube rack.

Acquisition Mode Controls

Use acquisition mode after establishing settings in setup mode. This automatically completes the run as defined in the Experiment Builder and does not require any intervention on your part. Acquisition mode does not apply to single stat tubes.

Table 41. Acquisition Mode Control Items

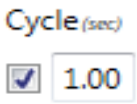
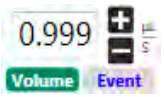
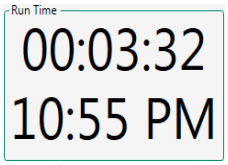
Item	Function
View	**View Run List** — expands the run list to display settings for the current experiment.
A1	**Sample Position** — during acquisition, the position currently being acquired.
	**(Start) Run List** — initiates the experiment as defined in the Experiment Builder.
	**(Stop) Run List** — stops the experiment that is currently in progress.
	**Cycle Mode** — when activated, plots display only a certain number of events, based on time period specified in this box. After the interval is reached, data are cleared from the plots and newly acquired events appear.
	**Event Rate and Volume** — during sampling, displays the triggered event rate, the event count, and the volume that has been acquired from the sample during the current run, along with the percentage of target volume acquired.
	**Agitate** — allows you to apply agitation to a sample that has been sitting for an extended period. Click to turn on agitation at the default speed for the media type. Click again to turn off agitation.
	**Flow Rate** — flow rate can either be determined by a target event rate or by a target volume rate. To switch between event rate control and volume control, click the corresponding button. Change the value in the box either by clicking the plus and minus buttons (for volume rate only) or by entering it directly.

Table 41. Acquisition Mode Control Items, continued

Item	Function
	**Run Time** — displays the elapsed experiment time and the projected time remaining until experiment completion.

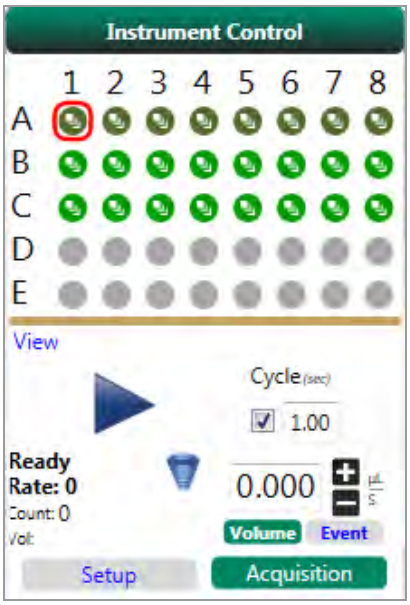
Running Samples in Acquisition Mode

After optimizing settings in setup mode, use acquisition mode to acquire samples in the run list that you set up in the Everest Experiment Builder.

Note: Data are not acquired from any stat tube that has been added on to the run list for a plate or tube rack.

To sample in acquisition mode

1. Switch to acquisition mode by clicking Acquisition in the Instrument Control panel.



2. To review the sampling configuration, click View in the Instrument Control panel.

   The run list opens, summarizing the experimental setup. Click the X in the upper right corner to close it.

3. Click (Start) Run List to begin sampling.

The instrument acquires samples as determined in the Experiment Builder. If multiple limits are set, acquisition stops when the first limit is reached.

Tip: By default, sampling begins at position A1 in the plate map, but you can instruct the instrument to begin acquisition mode from any sample position. Click a position in the plate map and click (Start) Run List. The run list proceeds as programmed in the Experiment Builder, starting from the selected position.

4. After a position has been sampled, its position on the map is indicated by a black check mark. For information about symbols shown in the plate map, see Plate Map on page 67.
5. To stop the run list before acquisition is complete for all positions, click (Stop) Run List.

Using High-Throughput Mode

In high-throughput mode, acquisition time is greatly reduced. Wells are sampled continuously with no boosting to the flow cell in between. In this mode, multiple samples occupy the sample line at the same time.

To use high-throughput mode, you must use the Experiment Builder to program it into your run list. If samples have not yet been assigned to high-throughput mode, you can click Edit to modify the run list.

To sample in high-throughput mode

1. Ensure that instrument settings have been configured properly for the experimental setup and sample type by acquiring control positions in setup mode as described in Acquiring Initial Sample in Setup Mode on page 221.
2. Click Acquisition in the Instrument Control panel to switch to acquisition mode.
3. Click (Start) Run List.

   Acquisition progress is indicated on the positions on the plate. For information about symbols shown in the plate map, see Plate Map on page 67.

   Note: Because the sample line contains multiple samples and sample is not boosted to the flow cell, there is a delay between sample aspiration and the time that sample reaches the flow cell; this delay depends on the flow rate used in the experiment.
4. Acquisition proceeds until the last programmed well has been acquired.
5. To stop acquisition at any time, click (Stop) Run List.

Pausing, Stopping, and Resuming Samples and Experiments

There are different ways to pause or stop sample acquisition and experiments, depending on when you plan to resume.

Note: Pausing or stopping during high-throughput sample runs is not recommended; any samples in the line that have not yet been analyzed will be backflushed to waste. If a pause or stop is necessary, the ideal time is between samples in standard sampling mode.

Pausing Sample Acquisition

Pausing sample acquisition pauses the sample pump. The probe stays in the sample. If recording is taking place, the FCS data file is not closed. You can use this method if you are making adjustments but have limited sample; you can pause while trying to figure out gating, for example, then resume to complete the data file. This option is available only when acquiring in setup mode.

To pause sample acquisition

▶ In the Instrument Control panel, click Pause Run List.

To resume sample acquisition after a pause

▶ In the Instrument Control panel, click Pause Run List again.

Stopping Sample Acquisition

Stopping sample acquisition stops the sample pump. The probe exits the sample and is washed, if wash has not been disabled for the sample position. If recording is taking place, the FCS data file is closed.

To stop sample acquisition

▶ In the Instrument Control panel, click Stop.

To restart sample acquisition after a stop

▶ In the Instrument Control panel, click Play.

Pausing an Experiment

You can build a planned pause step into the run list; this stops sample acquisition until you are ready to restart it manually. You can use this method to pause at a certain point, for example, after running compensation controls, or to verify results obtained so far.

To plan an experiment pause

▶ Add a Pause After step to a sample position. For more information, see High-Throughput, Pause, and Return Sample Settings on page 154.

To resume acquisition after a planned pause

1. In the Instrument Control panel, ensure that acquisition mode is active.
2. Select the position to restart from.

3. Click (Start) Run List to restart sampling.

Stopping an Experiment

You can use this method if you need to leave for the day or if you want to run a different set of samples before completing the current experiment. Resuming a stopped experiment loads the experiment run list, as it was last acquired, into the workspace. If files were acquired for a portion of the experiment, these files appear in the plate map and are exported along with any new files acquired.

To stop an experiment

1. In the Instrument Control panel, click (Stop) Run List.
2. Allow acquisition of the sample to finish.
3. In the toolbar, click Home.

To resume an experiment that you just stopped

1. In the Home window, click Resume.
2. In the Instrument Control panel, select the first sample that does not have a check mark.
3. Click (Start) Run List to restart sampling.

To resume a previously stopped experiment

1. Expand the list in the Recent Experiment Sessions panel.
2. For the experiment session that you want to restart, click Resume.
3. In the Instrument Control panel, select the first sample that does not have a check mark.
4. Click (Start) Run List to restart sampling.

Exporting Data to FCS and RLST

You can export FCS files for analysis in third-party software. Everest Software also allows you to export other associated data files such as run list and telemetry files.

To export data to FCS and RLST

1. Click Export Data in the Tools section of the Acquisition toolbar or the Share section of the Analysis toolbar.

2. To export data for a single position:

   a. Click the export button for the position's row.

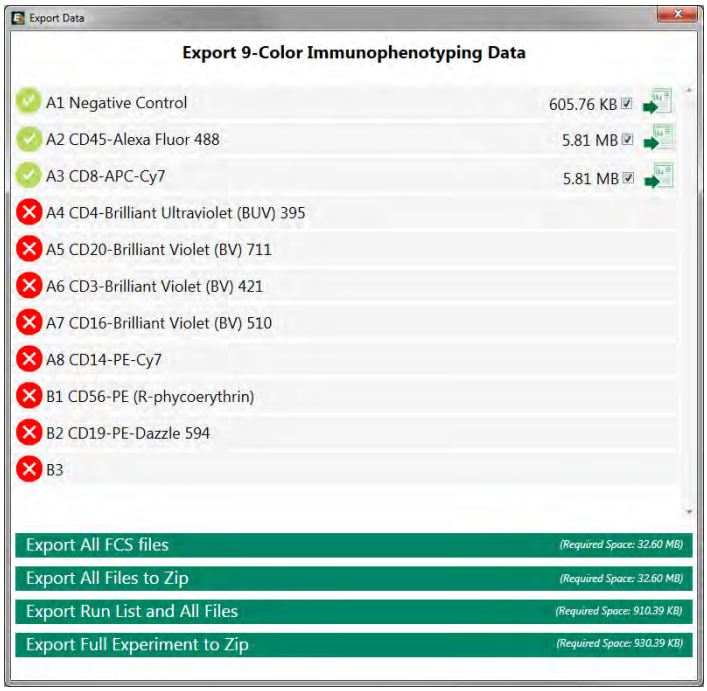
   b. In the Save As dialog box, browse to a location, specify a file name, and click Save.

3. To export data for multiple positions, clear checkboxes for any positions that you want to exclude. Then, choose one of the following options:

   - **Export All FCS Files** — export all FCS files for the current experiment.
   - **Export All Files to Zip** — export most recent FCS file for each position and compress to ZIP.
   - **Export Run List and All Files** — export run list to RLST format and export all FCS files for the current experiment.
   - **Export Full Experiment to Zip** — export full experiment, including run list, telemetry, and all FCS files for each position; compress to ZIP.

   In the Save As dialog box, browse to a location, specify a file name, and click Save.

If data have not been acquired and saved for a position, the position is indicated with an X rather than a check mark, and the option to export for the individual position does not appear.



Chapter 9 Applying Fluorescence Compensation

Everest™ Software offers simplified workflows for both manual and automatic compensation. To perform automatic compensation, acquire single-color controls using the Everest compensation template. You can then adjust regions to define the positive populations in all controls' channels and initiate the automatic compensation calculation. Everest Software also offers automatic region determination, so that you do not need to manually adjust the gates to define the positive populations.

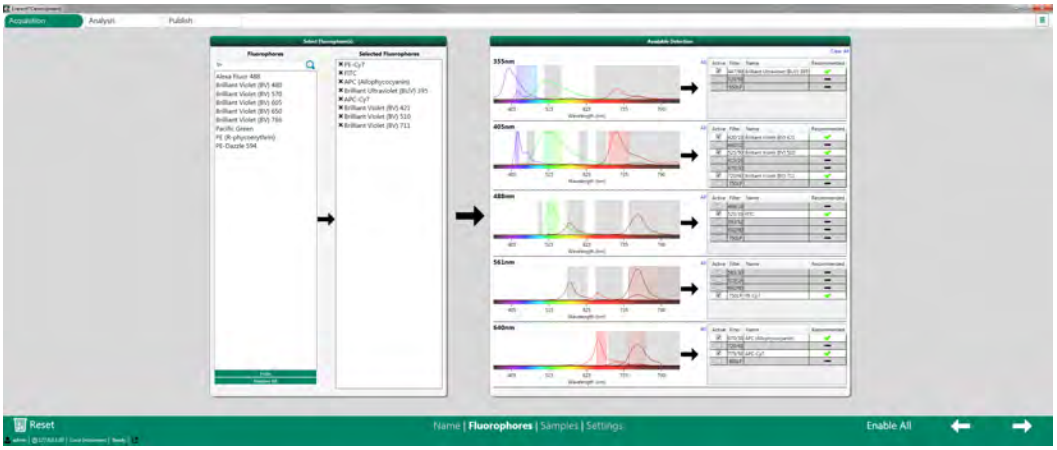
Tip: Everest Software permits visualization of both compensated and uncompensated data in the same workspace. Be sure to select the Comp parameter for plot axes so that the compensation matrix is applied to the data.

For information on applying compensation to multicolor experiments, see Appendix B, Example 9-Color Immunophenotyping Experiment.

Setting Up the Experiment

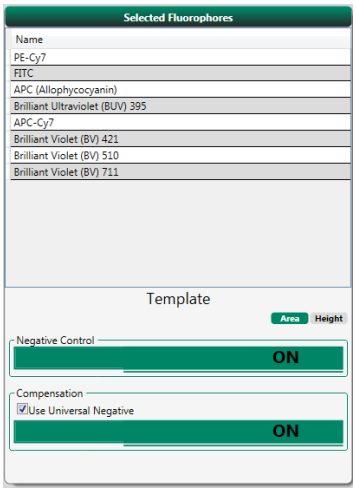
Before applying compensation in Everest Software (either automatically or manually), set up the workspace to streamline the compensation process. For information about the Compensation template, see Setting Up Compensation Controls on page 144.

1. Set up an experiment by selecting fluorophores and activating detectors as described in Chapter 7, Creating Experiments and Workspaces.

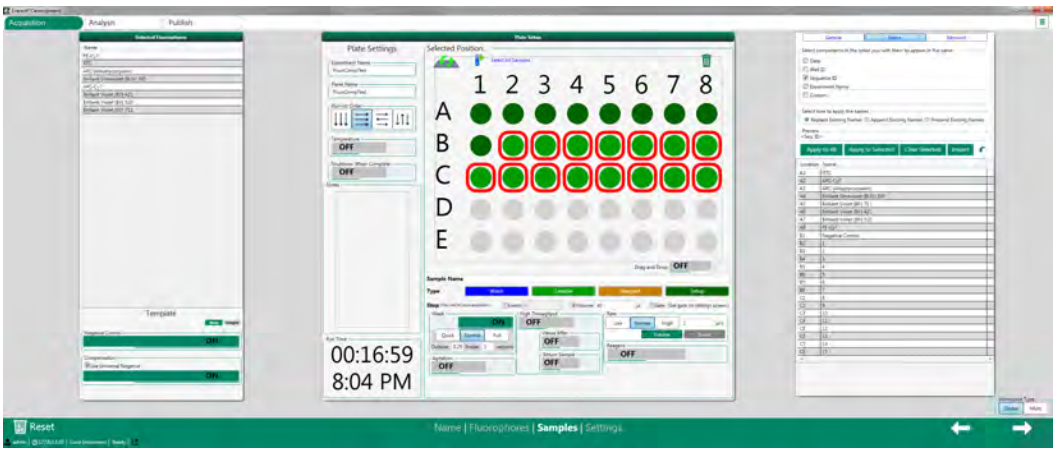


2. In the Samples screen, select a media type.

3. In the Selected Fluorophores panel, enable the Negative Control if your experiment includes one.
4. Enable Compensation to automatically set up fluorophores as controls.
5. To use the negative control as a universal negative in automatic compensation calculations, select the Use Universal Negative checkbox.



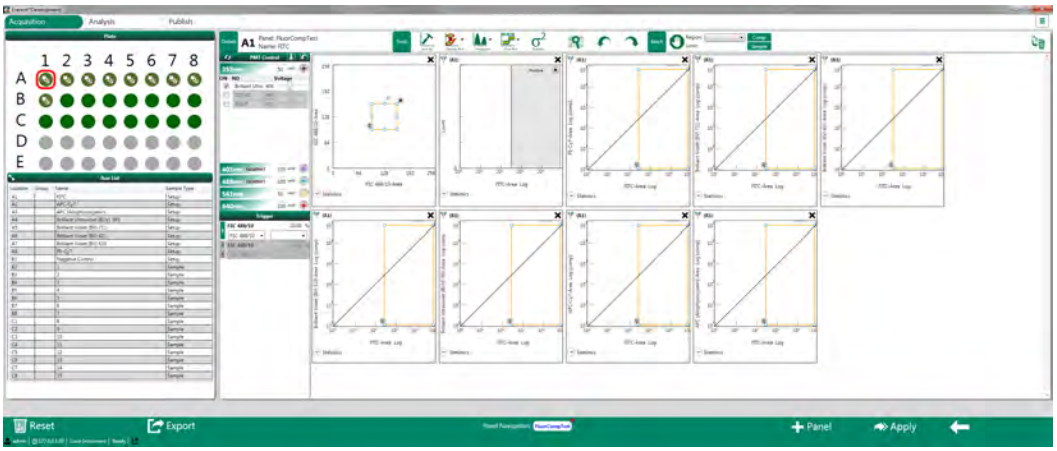
6. Set up samples.



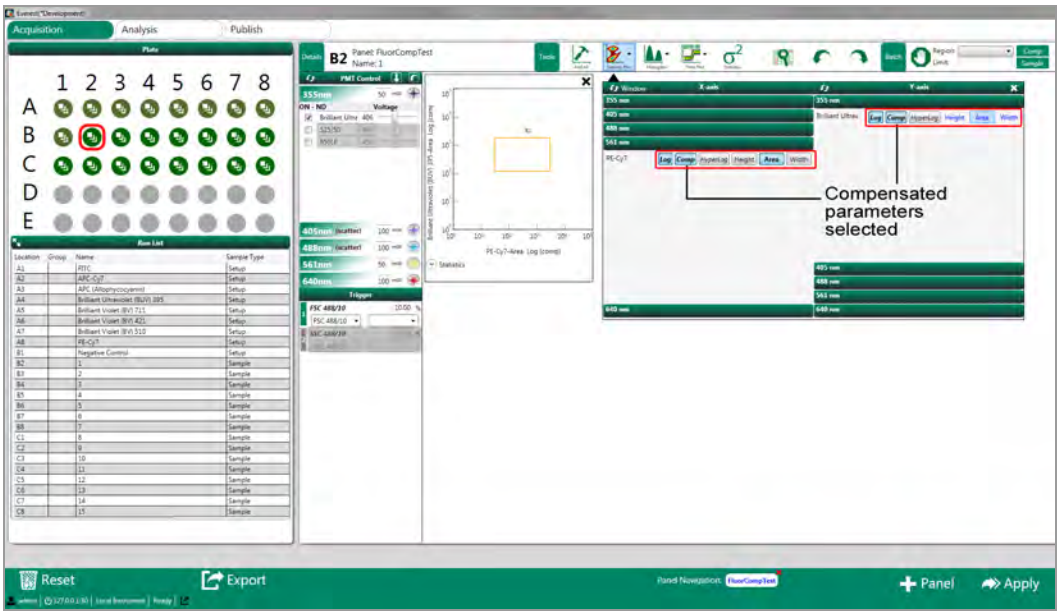
Tip: You can activate the Pause After function at the last control tube position so that acquisition stops after this tube and compensation can be calculated before the sample positions are acquired. For more information, see High-Throughput, Pause, and Return Sample Settings on page 154.

7. Proceed to the workspace Settings screen.

Everest Software sets up a unique workspace, including plots, for each compensation control.



Tip: When creating plots for the samples, be sure to select the Comp option for each axis in the plot builder to show compensated parameters on the plots.



8. Apply the experiment to the workspace by clicking Apply.

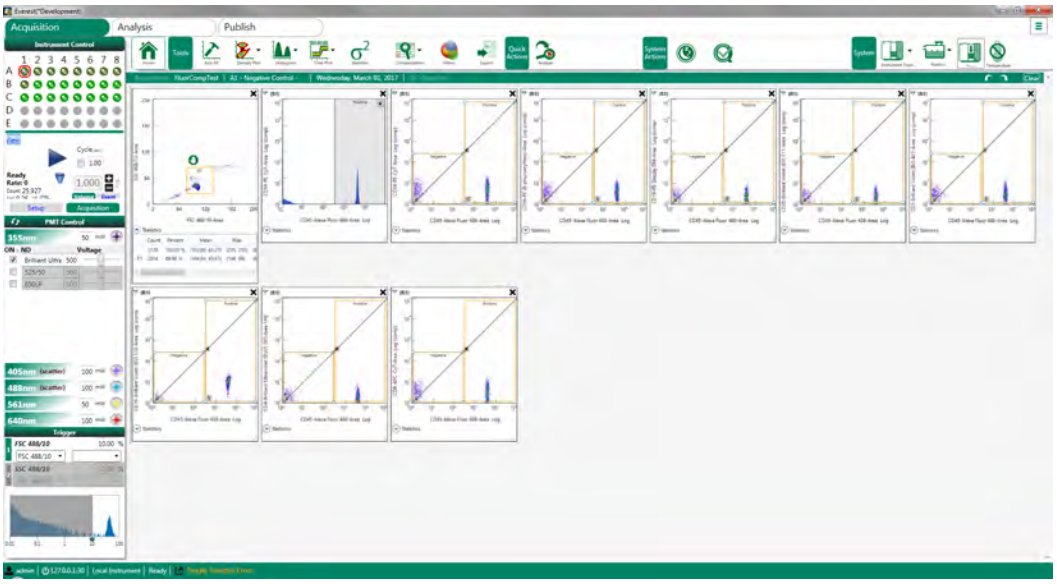
Adjusting Compensation Automatically

For automatic compensation, you must use the Compensation template so that Everest Software can reliably attribute signals to the proper detector and accurately calculate compensation.

To automatically calculate compensation

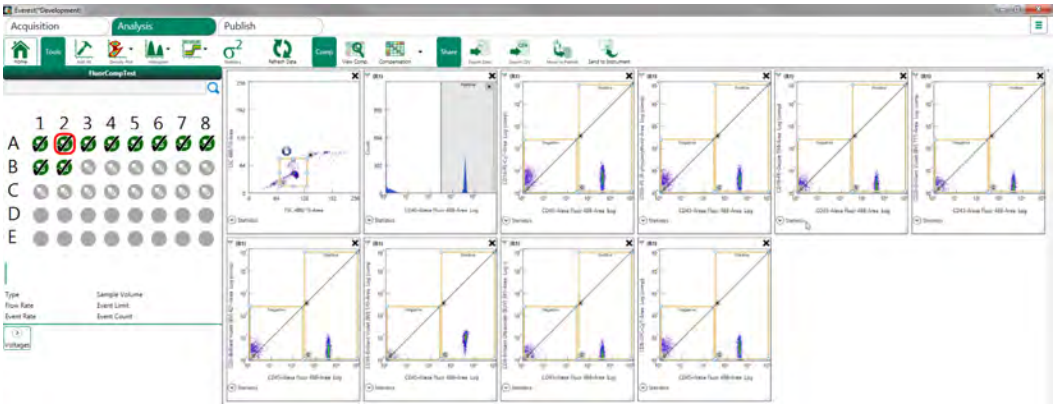
1. After setting up the workspace using the Compensation template, record all of the control positions either by using the Record button in setup mode or running the control samples in acquisition mode.

   Tip: To export the relevant compensation matrix with each experimental FCS data file, ensure that compensation has been calculated before running experimental samples.

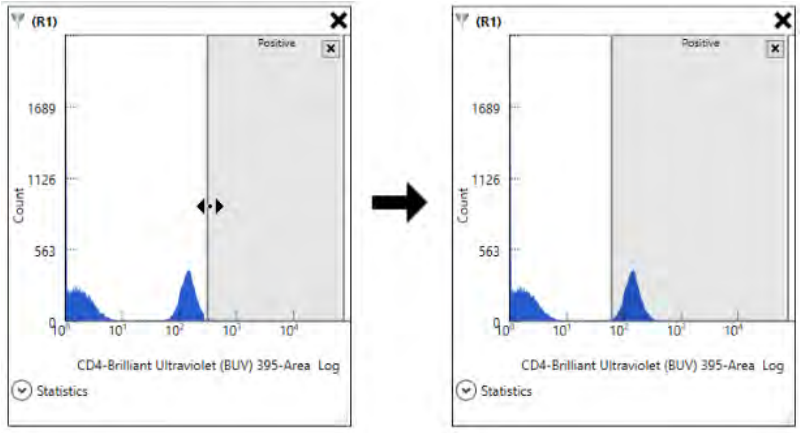


2. After acquiring controls, click Analyze in the Quick Actions area of the toolbar to move the run list and data to the Analysis tab.

   Everest Software displays the workspace and all plots for all data files.



3. Click the position for the first control, in this case A2, and adjust the region in the histogram so that it includes the stained population, as shown in the next figure.



4. Repeat step 3 for all the control positions, adjusting the gate in the parameter's histogram if needed.

5. Click the down arrow next to the Compensation button.

6. Select options as needed:

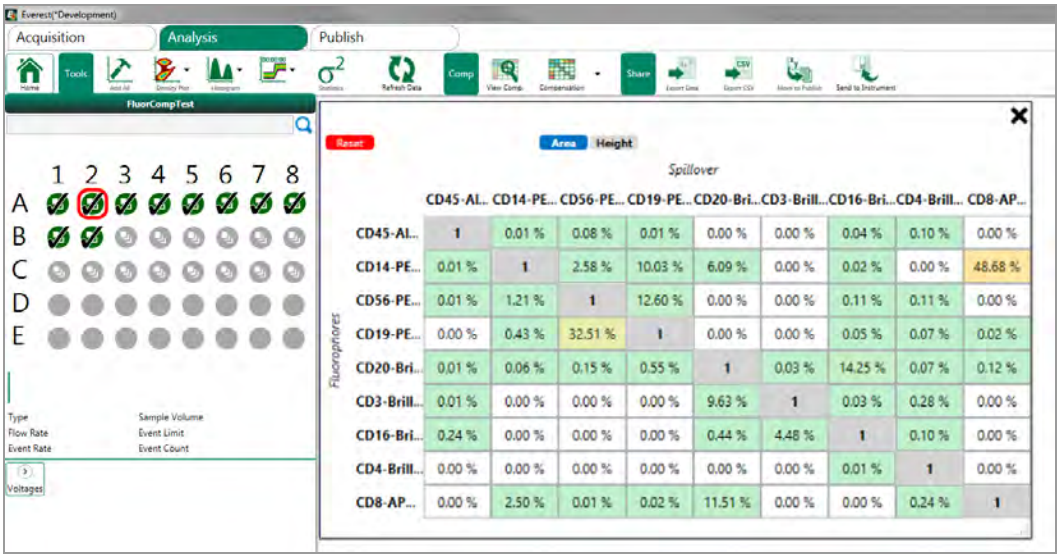
   - **Prevent Automatic Region Determination** — If this option is not selected, Everest Software performs automatic region determination and adjusts the regions in all plots for each control automatically. If this option is selected, Everest Software respects the regions that you configured in the parameters' histograms to determine positive and negative populations for compensation. It adjusts the regions in the other plots for each control to match your regions.

   - **Only Current Sample** — If this option is selected, Everest Software performs compensation only for the currently selected sample. Spillover of this channel into all other channels will be corrected only for this sample.

   - **Include Invalid Samples** — Everest Software evaluates the quality of compensation controls during the automatic compensation process. If this option is selected, Everest Software includes controls found to be invalid according to the algorithms that segment the positive population from the negative population. If this option is not selected, Everest Software excludes them. Excluding invalid controls might be necessary if there was no clear separation between positive and

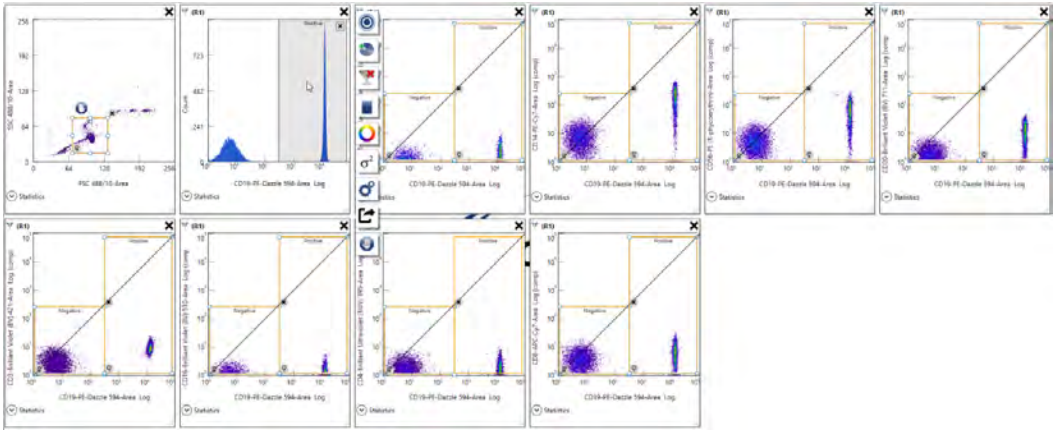
negative populations, or if a sufficient number of events could not be obtained for a particular single color control.

7. Click Calculate to initiate automatic compensation.
8. After the compensation process finishes, open the compensation matrix to check the values.



Each row in the matrix corresponds to a channel that contributes spillover signal to other detectors. Each column in the matrix corresponds to a channel that receives spillover signal from other detectors. Cells in the matrix are shaded green, yellow, and red to indicate increasing percentage values.

9. Review the compensated control plots.



10. To continue running samples, click Send to Instrument to send the calculated compensation values back to the instrument.

11. In the Acquisition tab, proceed with acquisition as necessary, selecting the position in the plate map at which you want to start.

Tip: When viewing compensated data, ensure that the Comp option has been selected for the plot axes; otherwise non-compensated data are displayed.

Adjusting Compensation Manually

You can manually adjust compensation in either of two ways: by dragging populations or by directly editing the compensation matrix.

Note: Unless you know how to perform statistically correct compensation adjustments for all fluorochromes used in your experiments, using automatic compensation is recommended.

Dragging Populations

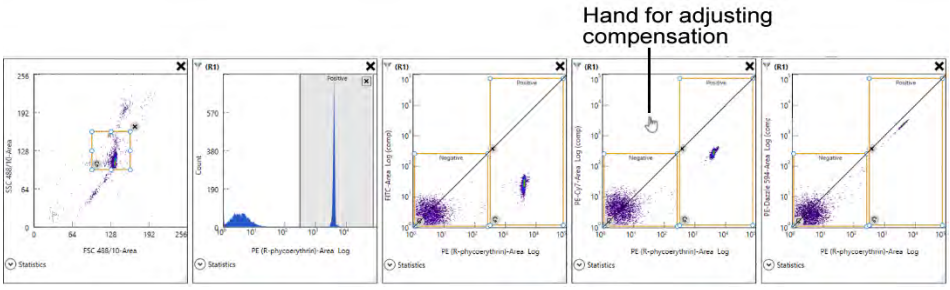
You can adjust compensation by dragging populations in plots either in the Acquisition tab or in the Analysis tab.

Tip: If you did not use the Everest compensation template to set up compensation control plots, ensure that y-axis of the control plot is designated as Comp, but that the x-axis is not. For experimental sample plots, both axes should be designated as Comp if you want to view compensated data.

To perform manual compensation by manipulating the plots directly

1. Point to one of the compensation plots.
2. When the pointer changes to a hand, drag it over the positive population to increase compensation and move the population out of the spillover channel. (Drag the population towards the x-axis so that the median matches that of the negative population on the y-axis.)

   Tip: If the hand is in a region, it will drag the region instead of the population. Ensure that the hand is outside of a region before dragging it.



3. Repeat Step 1 and Step 2 for each single-stained control.

Editing the Compensation Matrix

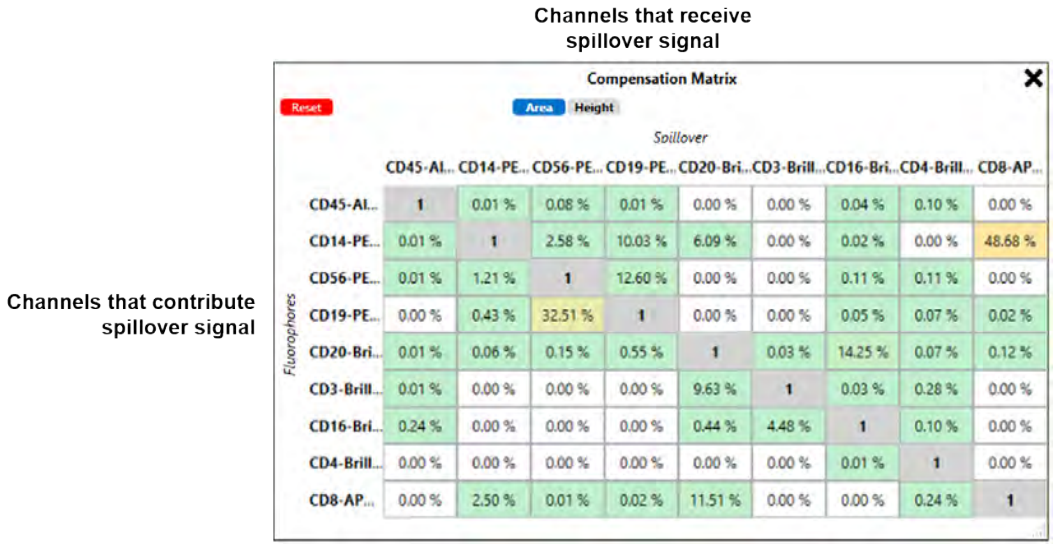
You can adjust compensation by editing the compensation matrix either in the Acquisition tab or in the Analysis tab.

Note: If you plan to perform manual compensation, you should be versed in matching medians to ensure that you apply accurate compensation values.

To adjust compensation by editing the compensation matrix

1. Open the compensation matrix by clicking View Comp on the toolbar.

2. Select the appropriate pulse parameter (area or height).
3. Directly adjust the spillover values by editing values in the matrix.



   Each row in the matrix corresponds to a channel that contributes spillover signal to other detectors. Each column in the matrix corresponds to a channel that receives spillover signal from other detectors. Cells in the matrix are shaded green, yellow, and red to indicate increasing percentage values.

4. Save the adjusted compensation values by clicking the X in the upper right corner.

Tip: When viewing compensated data, ensure that the Comp option has been selected for the plot axes; otherwise non-compensated data are displayed.

Chapter 10 Analyzing, Saving, and Printing Data

You can access the Everest™ Software Analysis tab by first acquiring data in the Acquisition tab and then sending it to the Analysis tab, or you can directly click the Analysis tab and load a previously acquired experiment session.

The Analysis tab allows you to create new plots for viewing data, view the PMT voltages that were set when the data were acquired, view and edit the existing compensation matrix, or run automatic compensation. From this tab, you can export CSV, FCS, RLST, and ZIP files.

When data analysis is complete, you can transfer the workspace view to the Publish tab, where you can continue to fine-tune experimental data for presentation.

File Types in Everest Software

There are two main types of files created by Everest Software during sample acquisition: FCS and RLST files.

FCS files are formatted using the 3.1 standard and thus can be analyzed using compatible third-party applications such as FlowJo, WinList, and Kaluza. Run list (RLST) files are specific to Everest Software. They contain all of the information related to an experiment, including fluorophores, samples, sampling settings, instrument settings, plots, and the compensation matrix.

Run list files are used to:

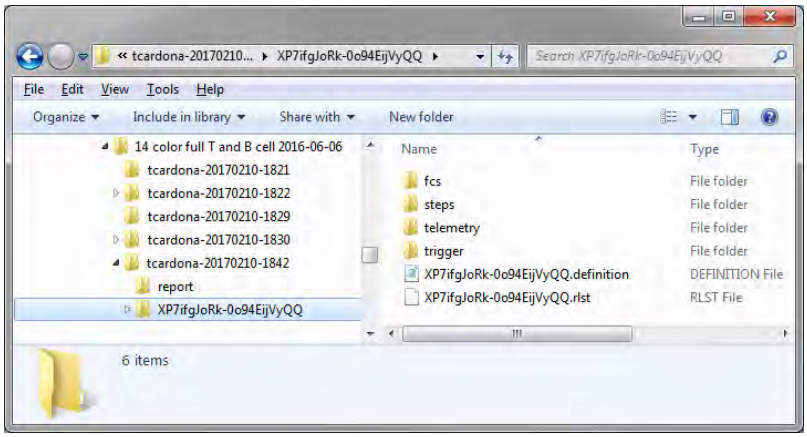
- Open previously created experiments for re-running or editing
- Load experiments in the Analysis tab

File Structure of Saved Data

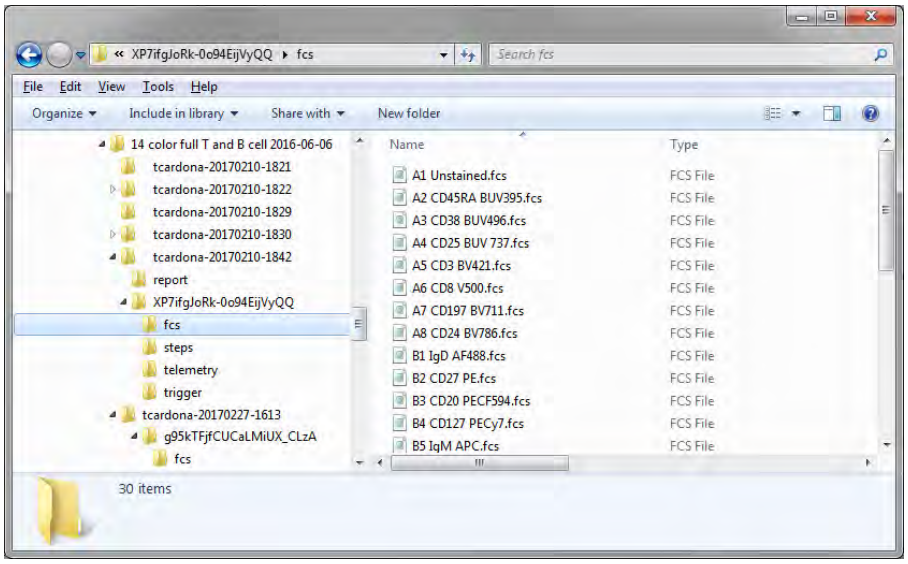
Tip: This section describes where to find saved data, but the best way to obtain FCS files for analysis in third-party software is to load the experiment and export the data. For more information, see Exporting Data to FCS and RLST on page 228.

The folder for each saved experiment contains a folder for each experiment session. The session folder contains the run list file and instrument definition file for the session, as well as subfolders containing detailed experiment data.

Multiple subfolders are created when sample acquisition is paused (or stopped) and resumed within an experiment session. Each subfolder is named with a 22-character GUID, and can contain a folder containing FCS data.



The FCS folder appears only if the session included sample acquisition with data recording. It contains an FCS file for each position.



Analyzing Data

After running an experiment in the Acquisition tab, you can analyze it in the Analysis tab.

Note: Wait for acquisition to complete or stop acquisition before moving data to analysis.

To access the Analysis tab

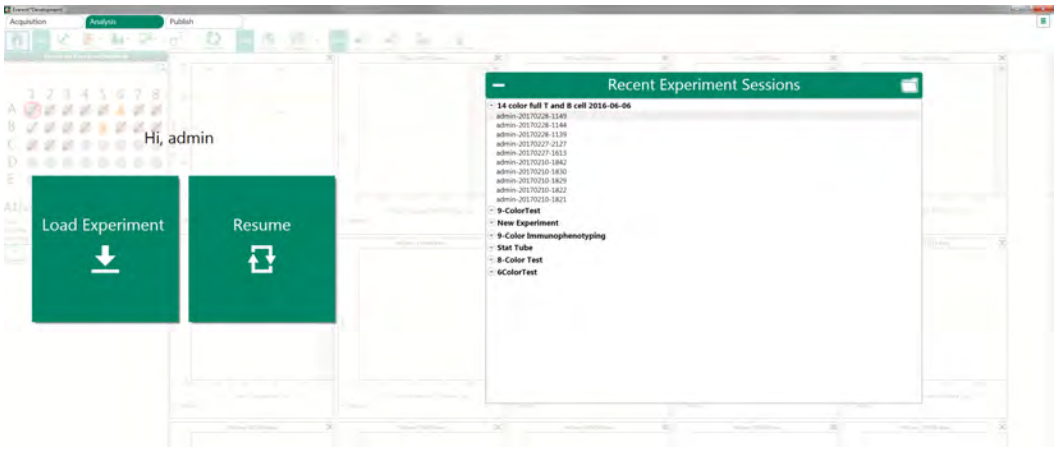
1. Acquire data in the Acquisition tab.
2. Do one of the following:
   - Click Analyze in the Quick Actions section of the toolbar.

   - Click the Analysis tab.

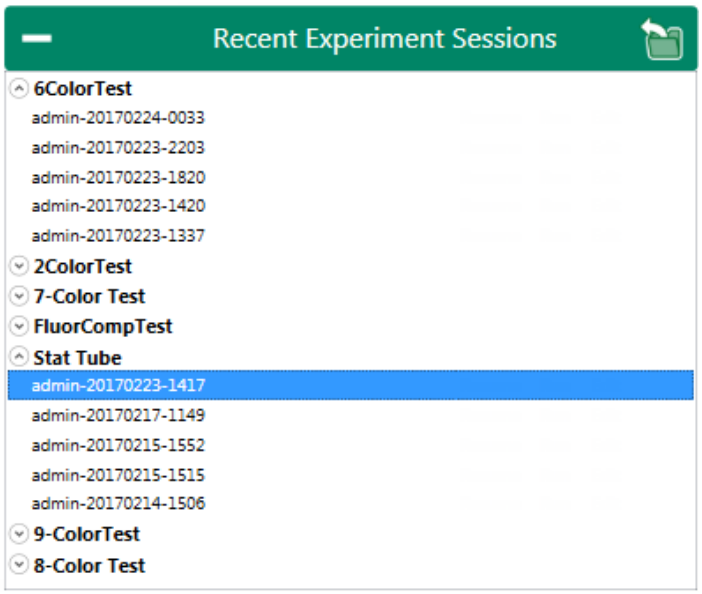
Loading Previous Experiments

In the Analysis tab, you can view data from previous experiments.



To load an experiment in Analysis

1. Click the Analysis tab.
2. In the Recent Experiment Sessions panel, expand the list of recent experiments.
3. Do one of the following:
   - If the experiment session that you want to analyze is in the list of recently displayed items, double-click it.



- If the experiment session that you want to analyze is not in the list of recently displayed items:

  a. Click Load Experiment.

  b. Browse to find the experiment that you want to analyze.

  c. Select the desired session and click OK.

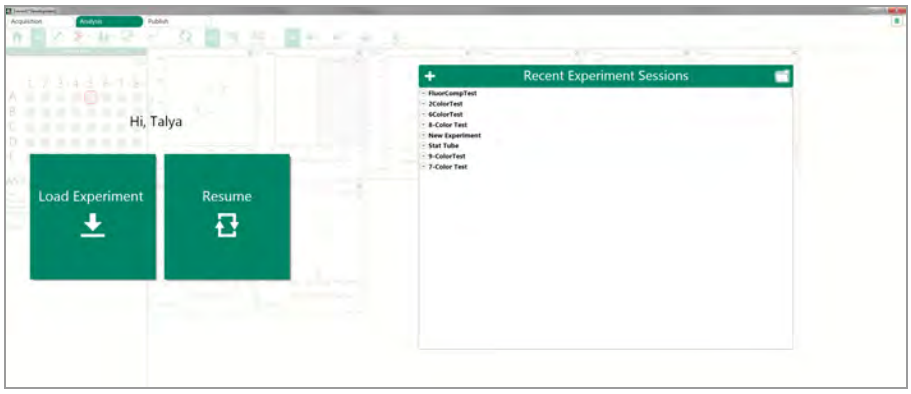
  The experiment session opens in the Analysis tab workspace.

  Note: For either method, the data (FCS) files are stored separately from the RLST file, so those must be present within the folder as well.

4. Click a sample position in the plate map to view data specific to that sample.

Resuming Experiment Analysis

During analysis, you can click Home to return to the Analysis start screen. From here, you can either click Resume to resume analysis of the previous experiment or click Load Experiment to load a different experiment for analysis.



Analysis Toolbar

In the Analysis tab, the toolbar contains three sections of tools to assist in performing data analysis: Tools, Comp, and Share.

Table 42. Analysis toolbar buttons and their functions

Button	Function
Tools	
	**Home** — returns to the Analysis start screen, where you can load a new experiment to analyze or resume analysis of the current experiment.
	**Advanced Plot Builder** — facilitates creation of plots for all parameters, with constraints that you define. For more information, see Creating Histograms for All Channels on page 170.
	**Create Density Plot** — creates a bivariate (two-parameter) density plot. For more information, see Creating Density Plots on page 167.
	**Create Histogram** — creates a univariate (one-parameter) histogram. For more information, see Creating Histograms on page 168.
	**Create Time Plot** — creates a plot of time (x-axis) versus a selected parameter (y-axis). For more information, see Creating Time Plots on page 169.
	**Add statistics window** — opens a statistics window; in it, you can select the plot statistics to display for a particular filter (gate), such as concentration, count, CV, percent of total, maximum, mean, median, minimum, mode, percent of plot, standard deviation, and variance. For more information, see Managing Plot Statistics on page 176, Viewing and Rearranging Plot Statistics on page 177, and Comparing Statistics on page 178.
	**Refresh Display** — refreshes data displayed on plots in the Analysis workspace.
Comp	
	**View Compensation** — opens the compensation matrix in the workspace for viewing or editing.
	**Compensation** — automatically compensates data displayed in plots. Available only in the Analysis tab.
Share	
	**Export Data** — allows you to select from five export options: ■ Export FCS file for a single position. ■ Export all FCS files for the current experiment. ■ Export most recent FCS file for each position and compress to ZIP. ■ Export run list to RLST format and export all FCS files for the current experiment.

Table 42. Analysis toolbar buttons and their functions, continued

Button	Function
	■ Export full experiment, including run list, telemetry, and all FCS files for each position; compress to ZIP.
	**Export CSV** — allows you to select statistics and export them to a comma-delimited file.
	**Move to Publish** — transfer the experiment to the Publish tab and display a report for the selected position.
	**Send to Instrument** — send updated run list back to instrument for acquisition (for example, after compensation is applied).

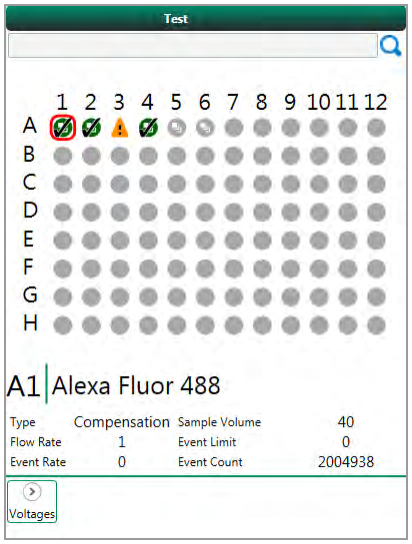
Working with Plots and Statistics in the Analysis Tab

In the Analysis tab, you can continue to modify experiment plots and statistics using the same controls that were available on the Acquisition tab. For more information, see these sections:

- Creating Plots and Histograms on page 166
- Using Plot and Histogram Tools on page 172
- Managing Plot Statistics on page 176

Note: You can adjust compensation by dragging outside a region in a compensation plot. When dragging items in plots, ensure that you do not adjust compensation unintentionally. For more information, see Dragging Populations on page 237.

Click Refresh Data in the Tools section of the toolbar to update the displayed data.



Below the plate map, the following information about the selected position is displayed:

- Sample position
- Sample name
- Sample type (Sample, Compensation/Control, Wash, or Reagent)
- Target Flow Rate (µl/sec) or Target Event Rate (events/sec)
- Sample Volume (µl)
- Event Limit
- Event Count

Below this area, you can click the Voltages arrow to display or hide PMT voltage conditions at the time of data acquisition.

Tip: If you want to add plot annotations or otherwise modify plots for multiple sample positions for presentation purposes, make these modifications in the Analysis tab, then use the Batch Print feature of the Publish tab to save the report to PDF or print it. For more information, see Printing a Report for All Positions on page 251.

Working with Compensation in the Analysis Tab

In the Analysis tab, you can either view the compensation matrix and edit it manually, or you can apply automatic compensation.

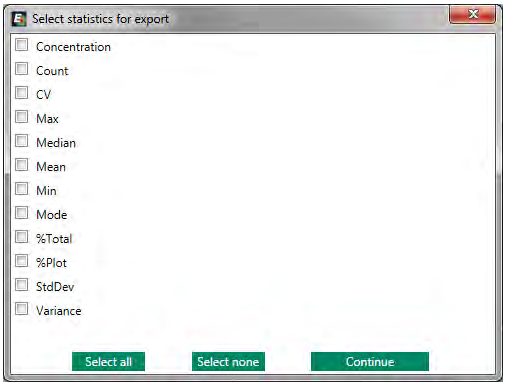
For detailed information on applying compensation automatically or manually, see Chapter 9, Applying Fluorescence Compensation. For an example experiment utilizing compensation, see Appendix B, Example 9-Color Immunophenotyping Experiment.

Exporting Data to CSV

You can export data for all experiment sample positions to a comma-delimited file.

To export data to CSV

1. Click Export CSV in the Share section of the toolbar.
2. Select the checkbox for each statistic that you want to include.



3. Click Continue.
4. In the Save As dialog box, browse to a location, specify a file name, and click Save.

   The data are saved to a CSV file.

For information on exporting data to FCS and RLST files, see Exporting Data to FCS and RLST on page 228.

Sending Analysis Settings to Acquisition

After applying compensation or making other changes in the Analysis tab, you can resubmit an experiment run list to be reacquired. This allows the calculated compensation to be applied during acquisition and saved within the resulting FCS file.

To send analysis settings to acquisition

1. Click Send to Instrument.

   The experiment run list is opened in the Acquisition tab.
2. Acquire the samples again.

Publishing Data

After analyzing experiment data in the Analysis tab, you can create and print reports in the Publish tab.

To access the Publish tab

1. Select a sample position in the plate map on the Analysis tab.
2. Do one of the following:
   - Click Move to Publish in the Share section of the toolbar.

   - Click the Publish tab.

   The report for the selected position opens in the Publish tab.

Note: If you modify the report for a position in the Publish tab, return to the Analysis tab, and use Move to Publish again, the report for the position is reset. To preserve such report modifications, click the Publish tab instead.

Publish Toolbar

In the Publish tab, the toolbar contains tools to assist in preparing data for presentation.

Table 43. Publish toolbar buttons and their functions

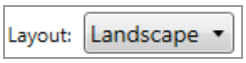
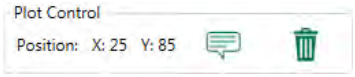
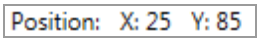
Button	Function
	**Print Current Well** — for the sample position that was selected in the Analysis tab, saves the analyzed data to a PDF file or sends it to the printer that you specify.
	**Batch Print** — for all sample positions in the experiment, saves the analyzed data to a PDF file or sends it to the printer that you specify.
	**Print Size** — allows you to specify Letter or Ledger paper size for printing.
	**Print Layout** — allows you to specify portrait or landscape orientation for PDF generation or printing.
	**Annotation** — allows you to add annotations to the workspace for inclusion in the published output.

Table 43. Publish toolbar buttons and their functions, continued

Button	Function
	**Plot Control** — provides controls for a selected plot.
	**Position** — provides x- and y-coordinates to assist with alignment of plots as you move them.
	**Add annotation to selected plot** — allows you to add annotations to the plot.
	**Delete selected plot** — removes the selected plot from the report.
	**Reset** — resets the plot data to match what came from the Analysis tab; in other words, it undoes any annotations and plot rearrangement/deletion that you have made to the report in the Publish tab.

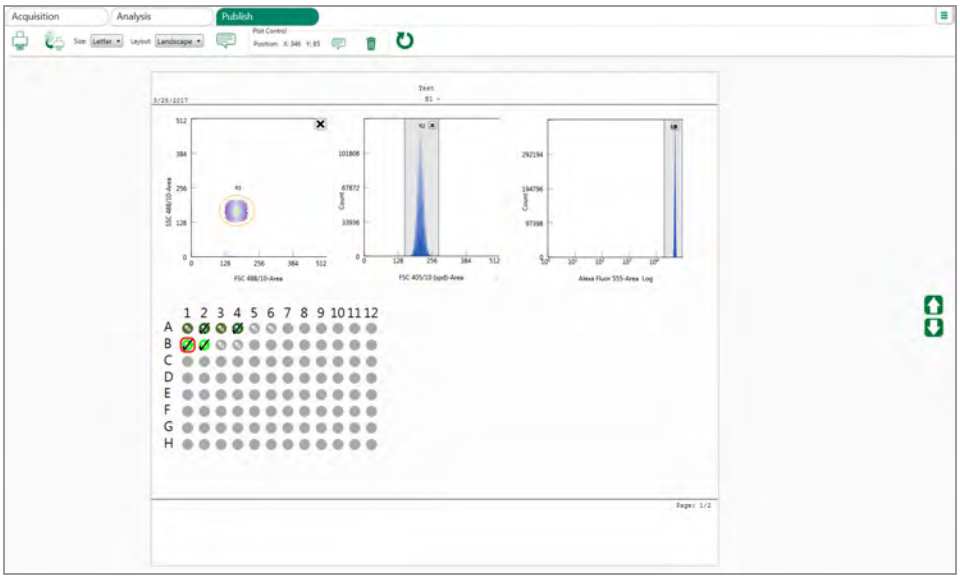
Preparing Reports

In the Publish tab, you can prepare a detailed report for a selected sample position by rearranging or deleting plots and adding annotations to plots or the workspace.

To rearrange plots

▶ Drag a plot to another position in the workspace.

Tip: To help you align items in the workspace, X and Y coordinates are provided in the Plot Control section of the Publish toolbar.



To delete a plot

▶ Do one of the following:

- Click the X in the upper right corner of the plot.
- Click the plot, then click Delete selected plot in the Plot Control section of the Publish toolbar.

To add annotations

▶ Do one of the following:

- Click Annotation on the Publish toolbar.
- Click a plot, and then click Add annotation to selected plot in the Plot Control section of the Publish toolbar.
- Right-click a plot, and then select Add Annotation from the pop-up menu.

To reset the report

▶ Click Reset on the Publish toolbar.

This discards any modifications that you have made to the report.

Printing a Report for the Selected Position

You can either send a report for one position directly to a printer, or save it as a PDF for later printing or viewing.

To print a report for one position

1. Select the position in the plate map of the Publish tab.
2. In the Publish toolbar, click Print.
3. Do one of the following:
   - To send the report to a printer:
     a. Select a printer on your network.
     b. Click Print.
   - To generate a PDF for the report:
     a. Select Adobe PDF.
     b. Click Print.
     c. In the Save PDF As dialog box, browse to a location, specify a file name, and click Save.

Printing a Report for All Positions

You can either send a report for all tubes/wells directly to a printer, or save it as a PDF for later printing or viewing.

To print a report for all positions

1. In the Publish toolbar, click Batch Print.

   Everest Software prepares the report and displays a status bar near the bottom of the screen.

2. Do one of the following:

   - To send the report to a printer:

     a. Select a printer on your network.

     b. Click Print.

   - To generate a PDF for the report:

     a. Select Adobe PDF.

     b. Click Print.

     c. In the Save PDF As dialog box, browse to a location, specify a file name, and click Save.

Chapter 11 Reports

Everest™ Software reports help you monitor instrument performance and usage. QC reports are available to all users. QC trending reports, EYE trending reports, and user reports are available only to administrators. You can export reports to CSV so that Bio-Rad Technical Support can assist you with troubleshooting.

Reports Tools

The buttons available to you in the Reports section of the toolbar depend on whether you have administrative rights.

Table 44. Reports toolbar buttons and their functions

Button	Function
	**Daily QC Report** — opens the most recent daily QC report. You can also view reports from previous dates and times. Available to all logged-in users.
	**QC Trending Report** — opens the QC Trending report. You can specify a date range for data display. Available to logged-in users who have administrative rights.
	**Eye Trending Report** — opens the EYE Trending report. You can specify a date range for data display. Available to logged-in users who have administrative rights.

Quality Control and ZE5-EYE Reports

Three reports are available to track performance of the ZE5™ Cell Analyzer: daily QC reports, QC trending reports, and EYE trending reports.

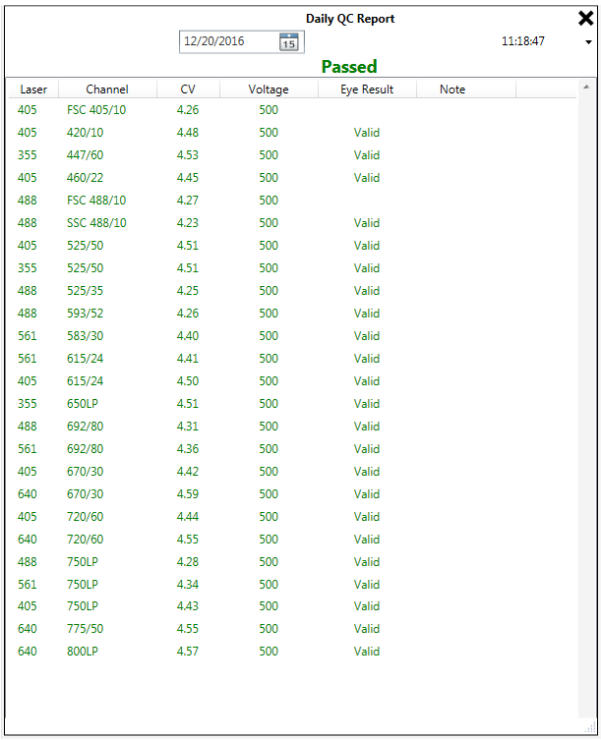
Generating Daily QC Reports

The daily QC report shows pass/fail information for the selected QC run, along with CV, PMT voltage, and ZE5-EYE result information.

To view the most recent daily QC report

▶ In the Reports section of the toolbar, click QC Report.

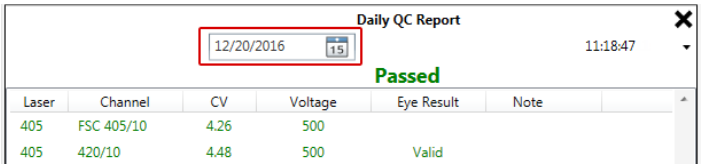
The daily QC report appears.



Laser	Channel	CV	Voltage	Eye Result	Note
405	FSC 405/10	4.26	500		
405	420/10	4.48	500	Valid	
355	447/60	4.53	500	Valid	
405	460/22	4.45	500	Valid	
488	FSC 488/10	4.27	500		
488	SSC 488/10	4.23	500	Valid	
405	525/50	4.51	500	Valid	
355	525/50	4.51	500	Valid	
488	525/35	4.25	500	Valid	
488	593/52	4.26	500	Valid	
561	583/30	4.40	500	Valid	
561	615/24	4.41	500	Valid	
405	615/24	4.50	500	Valid	
355	650LP	4.51	500	Valid	
488	692/80	4.31	500	Valid	
561	692/80	4.36	500	Valid	
405	670/30	4.42	500	Valid	
640	670/30	4.59	500	Valid	
405	720/60	4.44	500	Valid	
640	720/60	4.55	500	Valid	
488	750LP	4.28	500	Valid	
561	750LP	4.34	500	Valid	
405	750LP	4.43	500	Valid	
640	775/50	4.55	500	Valid	
640	800LP	4.57	500	Valid	

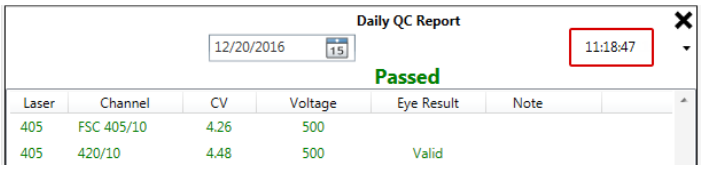
To view a QC report from a previous date

1. In the Reports section of the toolbar, click QC Report.
2. Click the calendar button to select a previous date.



Laser	Channel	CV	Voltage	Eye Result	Note
405	FSC 405/10	4.26	500		
405	420/10	4.48	500	Valid	

3. If more than one report exists for a date, click the time selector and select a time.



Laser	Channel	CV	Voltage	Eye Result	Note
405	FSC 405/10	4.26	500		
405	420/10	4.48	500	Valid	

To perform other actions on QC reports

▶ Point to the report and select an option from the buttons that appear to the right of the report.

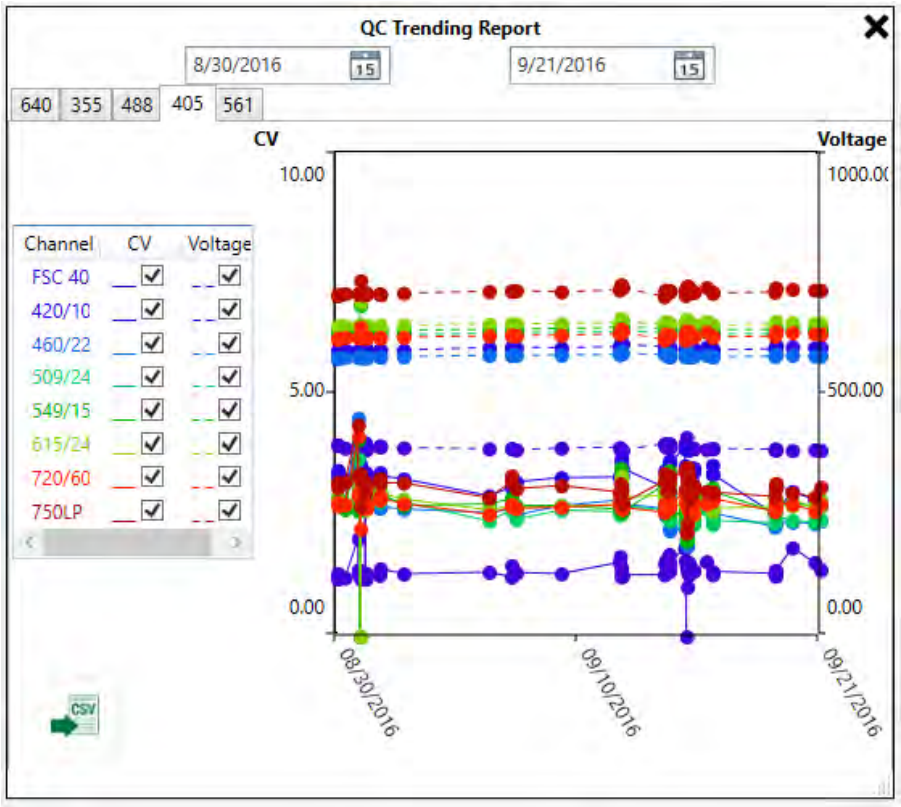
Button	Function
	Export the report to CSV.
	Move the data file to analysis.
	Print the report.
	Save the report.

Generating QC Trending Reports

The QC trending report facilitates visualization of QC data (CVs and PMT voltages) over time. Data are organized by laser. Each detection channel is represented by a unique color and can be shown or hidden as needed. Each data parameter is represented by a different line style (solid versus dotted).

To access a QC trending report

1. In the Reports section of the toolbar, click QC Trending Report.
2. Set the date range for the report by selecting dates from the Start date and End date calendars.
3. Select the parameters for which you want to view trends.



4. To view a QC trending report for a different laser, click its tab.
5. To export the QC trending report to a comma-delimited file:
    a. Click Export to CSV.
    b. Specify a file name and location.
    c. Click Save.

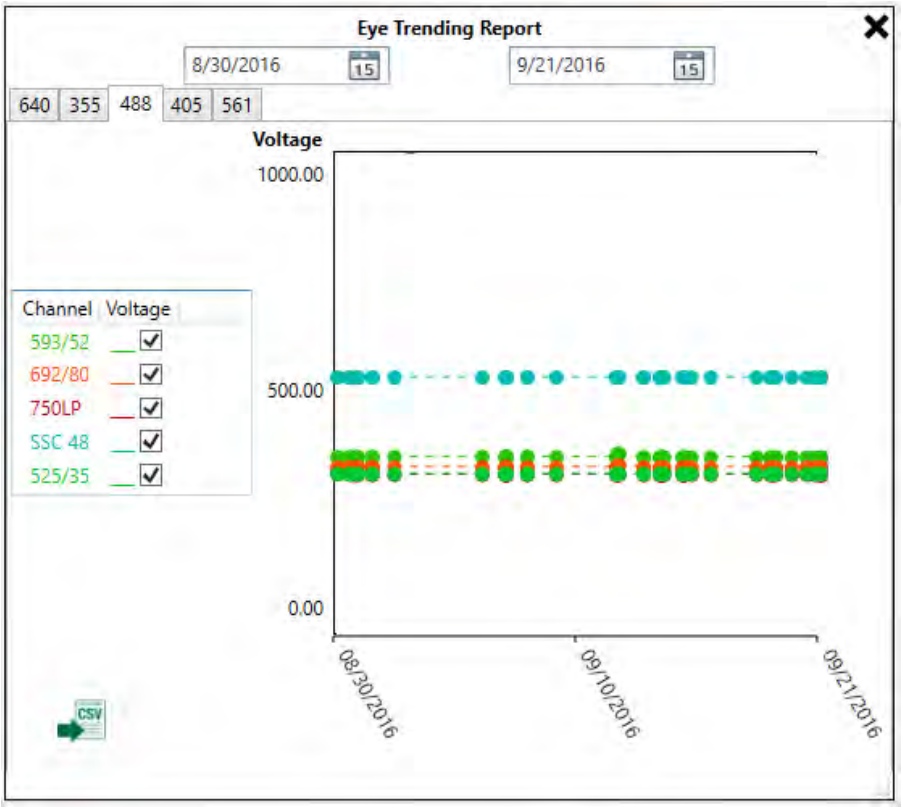
Generating EYE Trending Reports

The EYE trending report facilitates visualization of ZE5-EYE data (PMT voltages) over time. Data are organized by laser. Each detection channel is represented by a unique color and can be shown or hidden as needed. For more information about the ZE5-EYE, see The ZE5-EYE on page 36 and Using the ZE5-EYE to Confirm Filter Choices on page 109.

To access the EYE trending report

1. In the Reports section of the toolbar, click Eye Trending Report.
2. Set the date range for the report by selecting dates from the Start date and End date calendars.

3. Select the channels for which you want to view trends.



4. To view a EYE trending report for a different laser, click its tab.
5. To export the EYE trending report to a comma-delimited file:
   a. Click Export to CSV.
   b. Specify a file name and location.
   c. Click Save.

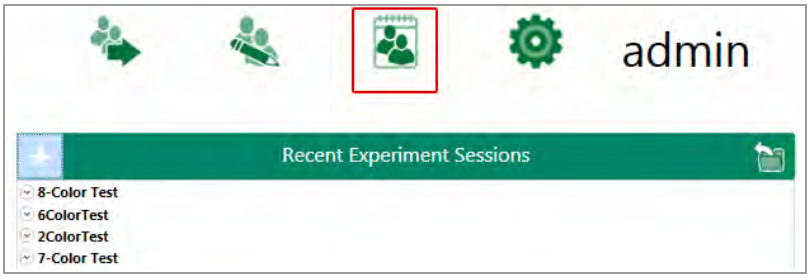
Generating User Reports

Administrators can view and save user reports for billing purposes and to analyze system usage. These reports track usage over time and include session notes entered by logged-in users. These reports can be printed or exported for reference.

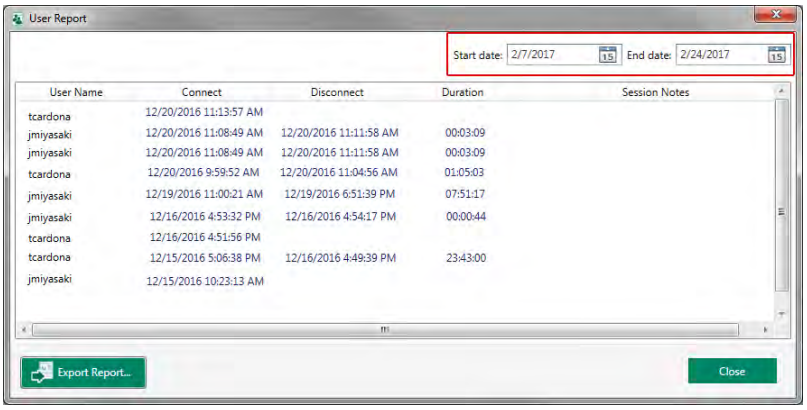
The User Reports button is available in the Home window when a person with an Admin account is logged in to Everest Software.

To generate a user report

1. In the Home window, click User Reports.



2. Set the date range for the report by selecting dates from the Start date and End date calendars.



3. To export the report to a CSV, click Export Report.

Chapter 12 Maintenance

To ensure reliable operation of the ZE5™ Cell Analyzer and accuracy of experimental results, perform preventive maintenance regularly.

Important: Always follow the PPE guidelines relevant to your laboratory's safety procedures for dealing with the chemicals recommended in this section and for any biohazards encountered during instrument maintenance, including the waste bottles.

Recommended Maintenance Schedule

Daily

The system must be shut down through the software on a daily basis. If the system is not shut down properly at the end of each day, it might be prone to contamination.

During the shutdown procedure, the sample line, probe, and flow cell are cleaned automatically with the onboard cleaner. If additional cleaning is desired, it can be run prior to the shutdown process. See Cleaning the Sample Line and Probe on page 262. See Cleaning Solutions on page 260 for details regarding approved cleaners for the system tubing.

To perform the system shutdown procedure

1. Ensure that the onboard cleaner bottle contains sufficient fluid.
2. Click the moon button on the Home screen to initiate the automatic shutdown process. For more information, see Shutting Down on page 129.

Weekly

Each week, wipe down the system with a mild disinfectant. Clean any debris or buildup on the loader stage and surrounding area. Inspect the bulk fluidics area for any drips or buildup and clean the area.

Inspect the levels of cleaner and sheath additive to ensure that they are sufficient for system usage. When you fill the cleaner and additive bottles, the fluid level must not exceed the half-full mark on the bottles.

Monthly

Fill the additive and cleaner bottles to the half-full mark on a monthly basis.

Remove bulk fluid bottles, disinfect if necessary, and wipe down the trays to remove any fluid or buildup.

Yearly

Inline sheath filter replacement is performed as part of the regular preventative maintenance service visit.

Bio-Rad recommends that you purchase the annual preventative maintenance (PM) plan offered with the ZE5 Cell Analyzer. The PM plan includes but is not limited to an annual onsite visit by a Bio-Rad Service engineer to:

- Replace peristaltic pump heads (6)
- Replace sample cartridge
- Clean the overflow sensor
- Replace the sample probe
- Replace the sheath, cleaner and additive filter cartridges (3)
- Replace the bulk fluid filters (4)
- Replace the bulk fluid connectors (8 sets)
- Replace the disk filters (2)
- Replace the fan air filter
- Clean optics
- Fill coolant

Cleaning Solutions

Important: Always follow the PPE guidelines relevant to your laboratory's safety procedures for dealing with the following recommended disinfectants.

Disinfectants for Use in Sample Line

- 70% ethanol in DI water
- Bleach (sodium hypochlorite) solution with a maximum concentration of 5,800 ppm active chlorine (a 1:10 dilution, which is roughly equivalent to 10% active chlorine). You can dilute the bleach solution further depending on the pathogenicity of the sample.

Disinfectants for Use in Sheath Line

Use a bleach solution containing 580 ppm active chlorine (a 1:100 dilution, which is roughly equivalent to 1% active chlorine). This solution can be used for running the Decontamination wizard. See Decontaminating the System on page 263.

Disinfectants for Use in the Waste Bottles

Use a 1:10 bleach dilution in the waste bottles. Place an appropriate quantity of disinfectant in the waste bottle to ensure effective inactivation of biologics that enter the bottle. Check compatibility of combined disinfectant products before use.

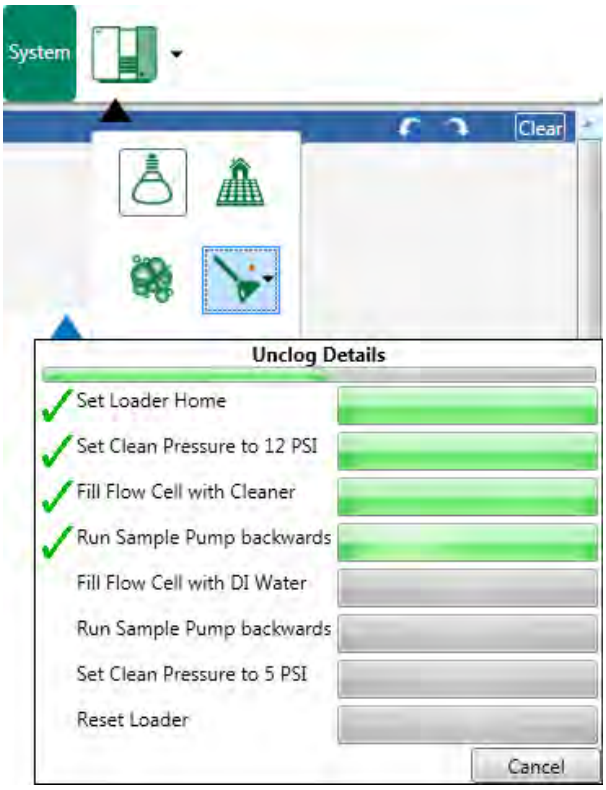
Unclogging the Sample Line and Probe

Everest™ Software includes an option to unclog the sample line, probe, and flow cell using system cleaner and DI water. This process moves the probe to the port behind the stat tube and cycles through the unclog process.

To unclog the sample line and probe

1. In the System section of the toolbar, click Unclog Sample Line.

2. To view unclog details, click the down arrow.



The system is ready for use when this process is complete, but you can choose to run the QC process to confirm that the clog has been cleared.

Important: To avoid clogs when working with cells, resuspend cells into single cell suspension and use a 40 µm or smaller filter prior to cell analysis.

Cleaning the Sample Line and Probe

Everest Software includes an option to initiate a sample line and probe clean cycle. This process runs the onboard system cleaner through the sample line and probe. You can also perform the cleaning using a tube of ethanol or bleach solution. See Cleaning Solutions on page 260 for recommended cleaning solutions to use in the sample line.

To clean the sample line and probe

1. In the System section of the toolbar, click Clean the Sample Probe.

2. Follow the directions that appear on screen.

   The system is ready for use when this process is complete, but you can choose to run the QC process afterward.

Decontaminating the System

You can use the Decontamination wizard to completely decontaminate the system using 1% filtered bleach solution (30 ml bleach and 2,970 ml water, equivalent to 580 ppm active chlorine). See Cleaning Solutions on page 260 for details. Periodic decontamination of the entire ZE5 Cell Analyzer fluidics system is recommended to ensure that lines, bottles, and valves are free of microbial growth.

Use the Decontamination wizard at least once every six months, or as often as every month. Use this wizard if there is a noticeably high background level of particles in the acquired data. The source could be within the fluidics path. Bacteria or fungi can grow in the lines if samples are not handled using basic cellular sterile techniques. The bulk fluidics can also contribute to contamination, despite having internal filters built into the lines. To test for contamination, disconnect the waste bottle cap, collect fluid in the waste input line, and culture it.

The wizard guides you through the necessary steps to complete the decontamination process, which takes about 2 hr. It leaves the system ready for running samples, but you can choose to run the QC process afterward.

Before running the wizard, ensure that the waste bottles are empty. The bottles containing the bleach solution must be filled no more than halfway.

Important: When handling sheath fluid and DI water bottles, always wear gloves and minimize air exposure to help avoid contamination.

To decontaminate the system

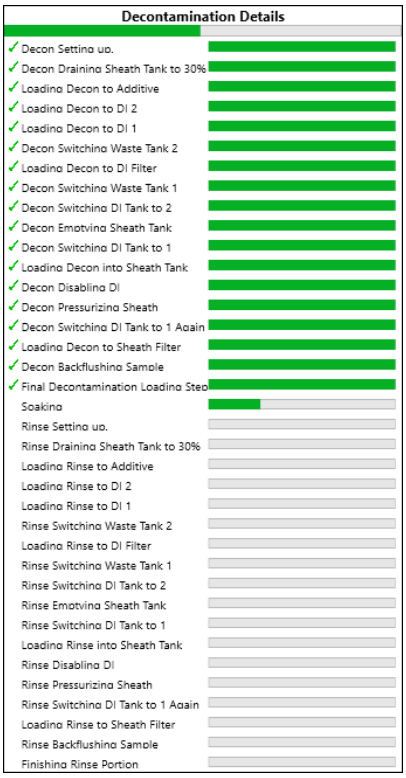
1. In the System section of the toolbar, click Decontamination.

2. Follow the instruction to replace the DI water bottles with bottles containing bleach solution.

3. Click the check mark.

   The Decontamination process begins.

4. To view decontamination progress details, click the down arrow.



5. When the Decontamination process is complete, the system prompts you to replace the bulk fluidics bottles with fresh DI water and waste bottles.

6. Click the check mark to exit the wizard.

Cleaning the Optical Filters

The optical filters used in the ZE5 Cell Analyzer lose performance when dirt, dust, or fingerprints are present on the glass surface. Regularly inspect and clean these optical components to maintain high system performance. See Replacing Optical Filters on page 105 for information on accessing the filters.

Important: These coated pieces of glass are delicate; handle them with care. Any scrape or scratch on the surface could significantly affect the light passing though. When handling filters, always wear gloves to avoid depositing oils and particles on the filter surface.

To clean an optical filter

1. Remove the filter from the instrument.
2. Gently spray compressed air on the surfaces of the filter to remove any large debris particles.
3. Using a lint-free wipe (such as Kimwipes or camera lens paper) or swab moistened with isopropyl alcohol, gently wipe the surfaces of the filter.
4. Inspect the filter by holding it up to a light to ensure that all debris particles have been removed.
5. Place the filter back into the instrument.

Replacing the QC Beads

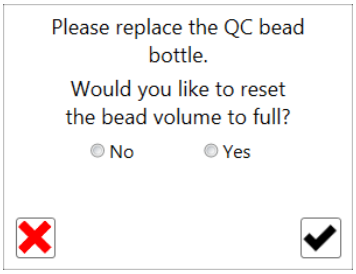
When the QC calibration bead volume is low (less than 150 µl), as indicated in the fluidics status, replace the bottle. If the fluid level in the calibration bead bottle drops too low, the system displays air bubble warning messages.

Important: Do not mix the contents of QC bead bottles. When replacing QC beads, do not add drops from the previous bottle to the new bottle.

To replace the beads

1. In the System section of the toolbar, click Swap Beads.

2. Replace the QC bead bottle with a full bottle.



3. When asked if you want to reset the bead volume to full, answer Yes and click the check mark to finish.

Maintaining the System During Periods of Disuse

Flow cytometry instruments have fewer performance issues if they are run and maintained regularly with no long periods of disuse. Everest Software includes a vacation mode that allows the you to schedule automatic startup and QC on the instrument regardless of whether an operator is in the lab. For more information, see Setting Up Vacation Mode on page 94.

Appendix A Troubleshooting

This appendix provides information on exporting system log information that can be used for troubleshooting purposes. It also lists potential problems and suggested solutions for the ZE5™ Cell Analyzer and Everest™ Software.

You can obtain more information about your system by visiting the Flow Cytometry area of the Bio-Rad website (www.bio-rad.com/flowcytometry) and the ZE5 Cell Analyzer product page (www.bio-rad.com/ZE5).

Exporting and Viewing Log Files

Everest system log files contain information that is helpful in troubleshooting problems with the system. Bio-Rad Technical Support might ask you to provide these files so that they can better assist you in resolving problems.

The Everest Help and Information menu provides a quick way to export all logs generated by the system in the last 180 days.

To export and view system log files

1. Click the Help and Information menu button in the upper right corner.
2. Select Log Extraction.

   Everest Software extracts the system log files, compresses them into a ZIP file, and places the file on the workstation desktop.
3. To view the log information, unzip the ZIP file and view the log files in a text editing or word processing program.
4. Provide the system log ZIP file to Bio-Rad Technical Support if needed.

Fluidics Issues

Error	Possible causes	Troubleshooting steps
System suddenly shuts down	Bulk fluidics bottles empty	Empty the waste and refill DI water bottles as necessary. See Refilling Bulk Fluidics on page 117.
	DI water uptake line and filter in wrong position	Ensure that the filter on the DI water uptake line falls into the lower corner of the bottle on the side away from the cap. Refer to the figures in Refilling Sheath Bottles on page 120.

Acquisition/Event Issues

Error	Possible causes	Troubleshooting steps
Acquisition pauses with track region set	Clog causing events to fall below specified track region percentage	Follow these steps, testing after each to determine whether the problem has been resolved: 1. Run the unclog process. See Unclogging the Sample Line and Probe on page 261. 2. Run the cleaning process. See Cleaning the Sample Line and Probe on page 262.
Air bubble warning	Probe too high and not in sample fluid	Follow these steps, testing after each to determine whether the problem has been resolved: 1. Check the level of QC calibration beads and replace the bead bottle if needed. See Replacing the QC Beads on page 266. 2. Recalibrate the probe, using custom media settings if needed. See Media Selector on page 56.
Data suddenly disappear from the plots in the workspace and threshold plot, and the event rate drops to 0 during acquisition	Trigger channel voltage too low	Increase the trigger channel voltage until data start to appear in the threshold plot. Then, adjust the trigger voltage and threshold value until data appear as expected in the plots in the workspace. See Configuring Instrument Settings on page 206.

Error	Possible causes	Troubleshooting steps
	Excessive light leaking into trigger channel	This typically results in a BLR (baseline restoration) fault. Excessive light being introduced into the trigger channel causes the baseline in that detector to move up, which can drown out signal in that channel. This can be caused by high background fluorescence in the sample. Run the cleaning process, using bleach. See Cleaning the Sample Line and Probe on page 262. If this does not resolve the problem, contact Bio-Rad Technical Support for assistance.
	Flow cell dirty	Ensure that the flow cell is clean. Built-up cellular material on the cuvette walls can cause unexpected light scatter, which can then be introduced into the scatter detector inappropriately. Run the unclog process in Everest Software to clean the flow cell. See Unclogging the Sample Line and Probe on page 261.
Dramatic change in PMT for one single channel	Improper bandpass filter installed	Run the ZE5-EYE process and inspect filters. See Using the ZE5-EYE to Confirm Filter Choices on page 109.
	Improper dichroic filter placement	Run the ZE5-EYE process and inspect filters. See Using the ZE5-EYE to Confirm Filter Choices on page 109.
	PMT malfunctioning	Contact Bio-Rad Technical Support for help.
	Mirror or filter scratched	Inspect and replace if necessary. See Working with Optical Filter Configurations on page 97.
	Contamination of flow cell (if PMT change is seen in FSC channel)	Follow these steps, testing after each to determine whether the problem has been resolved: 1. Run the cleaning process. See Cleaning the Sample Line and Probe on page 262. 2. Run the Decontamination wizard. See Decontaminating the System on page 263.

Error	Possible causes	Troubleshooting steps
Dramatic change in PMTs for all channels	Incorrect beads used	Ensure single peak ZE-Series QC Beads are being used. ProLine™ Rainbow Beads or ProLine™ Universal Calibration Beads do not fluoresce in certain channels, especially for the UV and violet lasers.
	Flow cell clogged	Run the unclog process. See Unclogging the Sample Line and Probe on page 261.
	Flow cell dirty	Follow these steps, testing after each to determine whether the problem has been resolved: 1. Run the cleaning process. See Cleaning the Sample Line and Probe on page 262. 2. Run the Decontamination wizard. See Decontaminating the System on page 263.
Event rate decreases unexpectedly	Sample has run out	Stop sample acquisition and check the tube/plate to see whether sample has in fact run out.
	Sample has settled	Modify the run list to include an agitation step or use the manual agitation option in the workspace to resuspend the sample. Note that settled samples might require a longer agitation than usual. See Activating Agitation on page 161, Setup Mode Controls on page 220, and Acquisition Mode Controls on page 223.
Event rate lower than expected based on sample concentration	Flow cell is clogged	Run the unclog process. See Unclogging the Sample Line and Probe on page 261.
	Miscalibration of media (sample probe not going into sample fully)	Recalibrate the probe, using custom media settings if needed. See Media Selector on page 56.
	Sample has settled	Modify the run list to include an agitation step or use the manual agitation option in the workspace to resuspend the sample. Note that settled samples might require a longer agitation than usual. See Activating Agitation on page 161, Setup Mode Controls on page 220, and Acquisition Mode Controls on page 223.

Error	Possible causes	Troubleshooting steps
High event rate	Trigger channel voltage too high	Decrease the trigger channel voltage until data start to appear in the threshold plot. Then, adjust the trigger voltage and threshold value until data appear as expected in the trigger channel plot in the workspace. See Configuring Instrument Settings on page 206.
High voltage present in certain channels during QC process	Incorrect beads used	Ensure single peak ZE-Series QC Beads are being used. ProLine™ Rainbow Beads or ProLine™ Universal Calibration Beads do not fluoresce in certain channels, especially for the UV and violet lasers.
High CV in data plots	Poor sample preparation	Prepare a new sample.
	Dirty or clogged flow cell	Follow these steps, testing after each to determine whether the problem has been resolved: 1. Run the unclog process. See Unclogging the Sample Line and Probe on page 261. 2. Run the cleaning process. See Cleaning the Sample Line and Probe on page 262. 3. Run the Decontamination wizard. See Decontaminating the System on page 263. If these steps do not resolve the issue, contact Bio-Rad Technical Support.
	Air in system	Stop sample acquisition and run a stat tube filled with at least 500 µl DI water.
	Dirty optical filters	Inspect and clean filters. See Cleaning the Optical Filters on page 266.
	Improper laser delay	Run the QC process again. See Running Quality Control on page 126.
	Beads have gone bad or expired	Install a new bottle of QC beads. See Replacing the QC Beads on page 266.
	Incorrect optical filter in place	Run the ZE5-EYE to ensure that installed filters match the filter configuration in Everest Software. See Using the ZE5-EYE to Confirm Filter Choices on page 109.

Error	Possible causes	Troubleshooting steps
High fluorescence	Antibody concentration in sample too high	Prepare a new sample, ensuring that antibody titration is correct.
	Inadequate cell preparation or washing	Prepare a new sample.
	Cells have naturally high auto fluorescence	Adjust PMT voltages to place cells on scale. See Configuring Instrument Settings on page 206.
	Poor compensation	Run the compensation process. See Adjusting Compensation Automatically on page 233.
	Bacterial contamination causing autofluorescence	Run the Decontamination wizard. See Decontaminating the System on page 263.
	Secondary antibody cross-reacting with cells	Evaluate sample preparation.
No events present during acquisition	Probe too high and not in sample fluid	Recalibrate the probe, using custom media settings if needed. See Media Selector on page 56.
	Laser or lasers off	Turn on the relevant lasers in software. See Configuring Instrument Settings on page 206. Modify the experiment in the Experiment Builder so that required fluorophores are activated to ensure that lasers are on when the run list is initiated. See Selecting Fluorophores on page 133.
	Laser or lasers not functioning properly	Contact Bio-Rad Technical Support for help.
	Events below threshold or threshold set too high	Adjust the trigger PMT voltage or decrease the threshold percentage. See Configuring Instrument Settings on page 206.
	Threshold not set correctly	Change threshold value. See Configuring Instrument Settings on page 206.
	PMTs set too high or too low to see data	Edit PMT voltages in setup mode or change trigger threshold in order to visualize data. See Configuring Instrument Settings on page 206.

Error	Possible causes	Troubleshooting steps
	Clog in system	Follow these steps, testing after each to determine whether the problem has been resolved: 1. Run the unclog process. See Unclogging the Sample Line and Probe on page 261. 2. Run the cleaning process. See Cleaning the Sample Line and Probe on page 262.
	Sample not aspirated	Open the loader door and check the sample chamber for leaks. Ensure that the probe is moving correctly. Contact Bio-Rad Technical Support for assistance with checking for leaks in the sample line connection.
	Incorrect optical filter in place	Run the ZE5-EYE process to ensure that all filters are correct and in the right locations. See Using the ZE5-EYE to Confirm Filter Choices on page 109.
	Sample too dilute	Recreate the experiment with a more concentrated sample.
	Sample has run out	Stop the acquisition and check the tube/plate to see whether sample has in fact run out.
	Plots created in the workspace do not match the enabled parameters	Create new plots that match the enabled parameters (see Plots Created by the Compensation Template on page 166), or enable parameters that match the plots (see Selecting Fluorophores on page 133).
	Loader door open	Stop the acquisition process, close the loader door, and proceed with running the experiment.
No events present during QC process	Probe position not sufficient to aspirate low fluid levels in QC bead bottle	Contact Bio-Rad Technical Support for assistance with recalibrating the probe position for the bead station.
	Bead bottle empty	Replace bead bottle. See Replacing the QC Beads on page 266.
	Bead bottle has been diluted	Replace bead bottle. See Replacing the QC Beads on page 266.

Error	Possible causes	Troubleshooting steps
Noisy threshold plot	Debris in sample line	Follow these steps, testing after each to determine whether the problem has been resolved: 1. Run the cleaning process. See Cleaning the Sample Line and Probe on page 262. 2. Run the Decontamination wizard. See Decontaminating the System on page 263.
	Debris in sheath or DI water bottles	Follow these steps, testing after each to determine whether the problem has been resolved: 1. Run the cleaning process. See Cleaning the Sample Line and Probe on page 262. 2. Run the Decontamination wizard. See Decontaminating the System on page 263.
	Dead cells in sample	Adjust gates (see Applying Filters (Gates) on page 187) or repeat experiment with fresh cells.
Two or more populations are present when expecting one	Gating inaccurate	Adjust gates. See Applying Filters (Gates) on page 187.
	Target protein expressed on multiple cells	Evaluate sample preparation and experiment setup.
	Inadequate cell preparation	Ensure adequate cell separation and preparation because multiple cell types or debris could be present in a sample.
	Large number of doublets in sample	Adjust the flow rate down.
Unexpected fluorescence signal	Free dye accumulating in sample line	Run the cleaning process. See Cleaning the Sample Line and Probe on page 262.
Weak or no fluorescence	Not enough antibody used during sample preparation	Verify antibody titrations and prepare a new sample.
	Intracellular targets insufficiently labeled	Ensure correct techniques are used to fix cells.
	Incorrect fluorophore selection	Evaluate sample preparation and experiment setup.

Error	Possible causes	Troubleshooting steps
	Poor compensation	Run the compensation process. See Adjusting Compensation Automatically on page 233.
	Reagent old or degraded	Antibody might not have been stored in the proper conditions (refrigerated and kept in the dark).
	Antibodies are not compatible	Verify that the secondary antibody used has been grown against the species in which the primary antibody has been grown.
	Lasers turned off	Turn on lasers in software. See Configuring Instrument Settings on page 206.
	Lasers misaligned	Contact Bio-Rad Technical Support.

Software Issues

Error	Possible causes	Troubleshooting steps
Unable to click the Play button	Sample back flushing or previous run not complete	Wait for the process to complete.
	Run list error	Validate that everything has been properly selected when setting up a run list.
	Loader door open or sample plate inserted incorrectly	1. Stop the acquisition process. 2. Open the loader door (if it is not already open). 3. Ensure that the sample tube rack or plate is seated correctly. 4. Close the loader door. 5. Proceed with running the experiment.

Hardware/Electronics/Laser Issues

Error	Possible causes	Troubleshooting steps
Loader comes back out after trying to close door before a run	Incorrect plate inserted	Ensure that the plate fits correctly on the loader platform.
Low signal	Laser power not set correctly	Check the laser power settings. See PMT and Laser Controls on page 208.
Probe is lowered into a tube or well when a run list starts but the run list immediately stops	Probe crash	Check the position of sample device. Ensure that tubes are set properly in the rack and that the rack or plate is flush against the loader. Recalibrate the probe, using custom media settings if needed. See Media Selector on page 56.
	Incorrect plate inserted	Place the sample into a plate that is compatible with the ZE5 Cell Analyzer.

Appendix B Example 9-Color Immunophenotyping Experiment

This section provides an example of how to set up a typical multicolor experiment using the ZE5™ Cell Analyzer and Everest™ Software. It shows how to set up compensation controls using the compensation template and how to apply auto compensation. The sample experiment uses the following fluorophores:

- Alexa Fluor 488
- Brilliant Ultraviolet (BUV) 395
- APC-Cy7
- PE (R-phycoerythrin)
- PE-Cy7
- PE-Dazzle 594
- Brilliant Violet (BV) 421
- Brilliant Violet (BV) 510
- Brilliant Violet (BV) 711

Preparing Controls and Samples

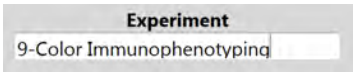
First, prepare the compensation controls and samples to be placed in your medium of choice, such as a 40-tube rack.

1. Prepare a compensation control for each fluorophore to be used in the experiment.
2. Prepare an unstained cell sample.
3. Prepare a fully stained test sample.

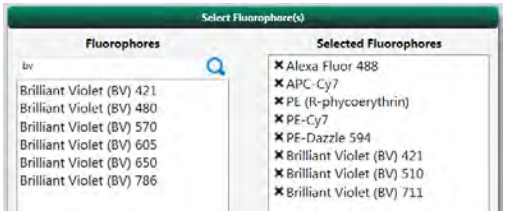
Setting Up Fluorophores and Controls

After you set up the fluorophores, Everest Software helps you quickly assign corresponding control positions.

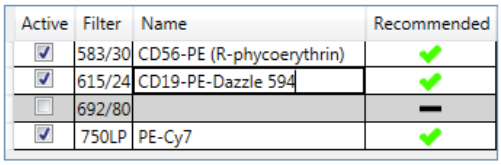
1. In the Home window, click New Experiment.
2. Enter the name for the experiment and click the Next arrow.



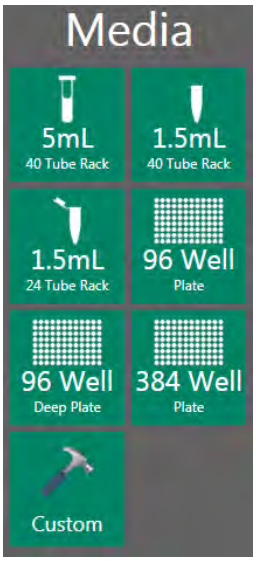
3. In the Fluorophores screen, double-click fluorophore names in the Optimal Fluorophores list to add them to the Selected Fluorophores list.



4. In the Available Detection panel, change the parameter names to reflect the cell markers used in the experiment.

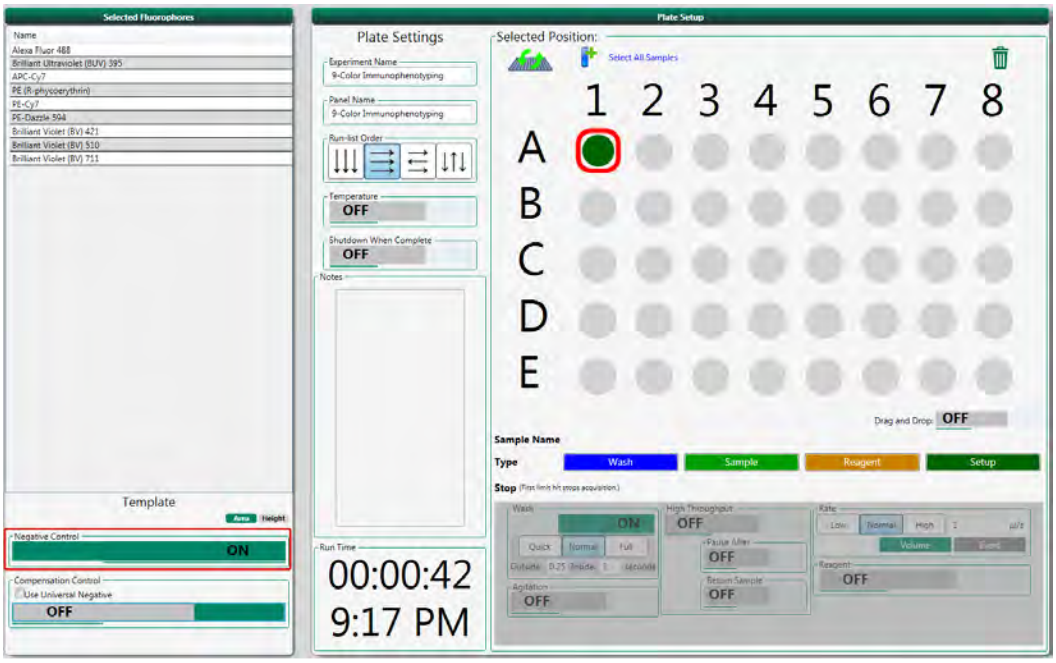


5. Click the Next arrow.
6. Select the Media type, for example, 40 Tube Rack.



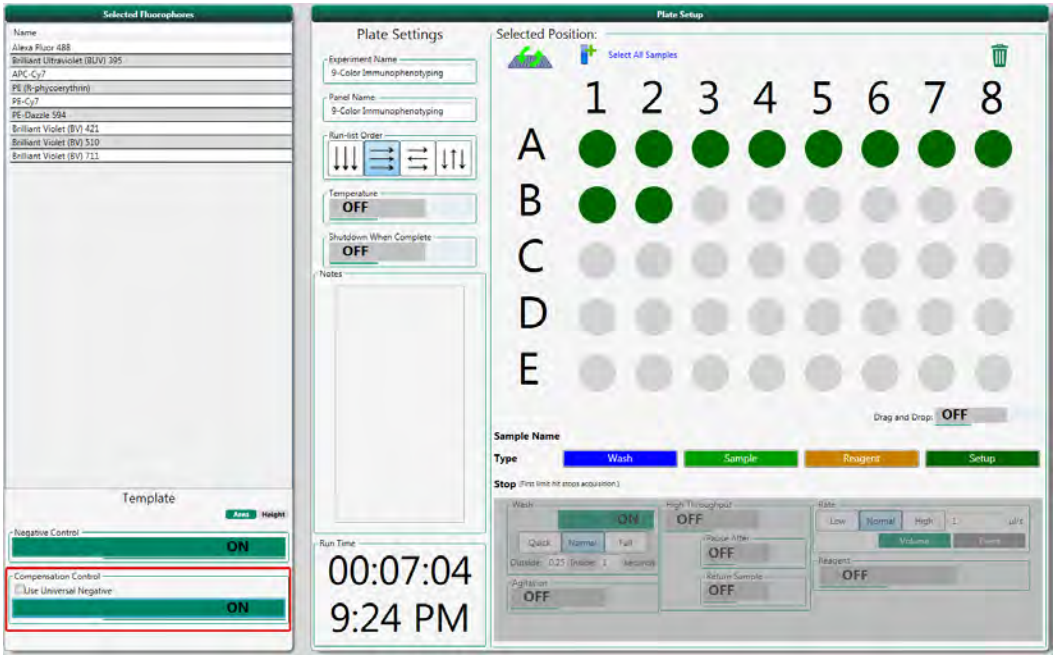
7. In the Template area of the Selected Fluorophores panel, click the Negative Control toggle to indicate that the experiment will use a negative control.

   The first position in the compensation template is automatically assigned to the negative control, as shown in the next figure.



8. In the Template area of the Selected Fluorophores panel, click the Compensation toggle to automatically add compensation positions for each fluorophore in the experiment.

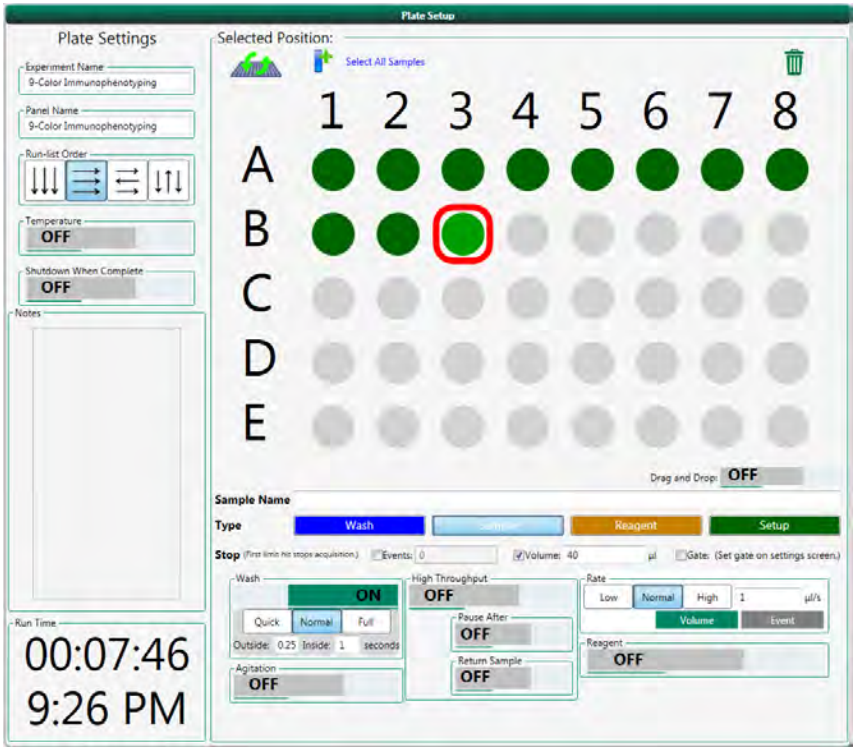
   The next nine positions in the compensation template are automatically assigned to the fluorophores, as shown in the next figure.



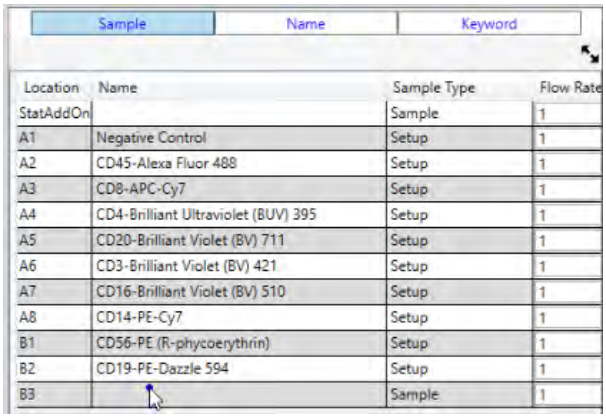
Setting Up the Sample and Sampling Parameters

Next, set up a fully stained sample and specify settings such as gate limits, agitation time, and target flow rate or target event rate.

1. Set up a sample tube by clicking an unused position and then selecting Sample for the position type.



2. To change the sample name, click the name box in the Run List pane and type a new name.

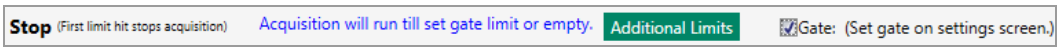


3. Drag around positions in the template to select them.

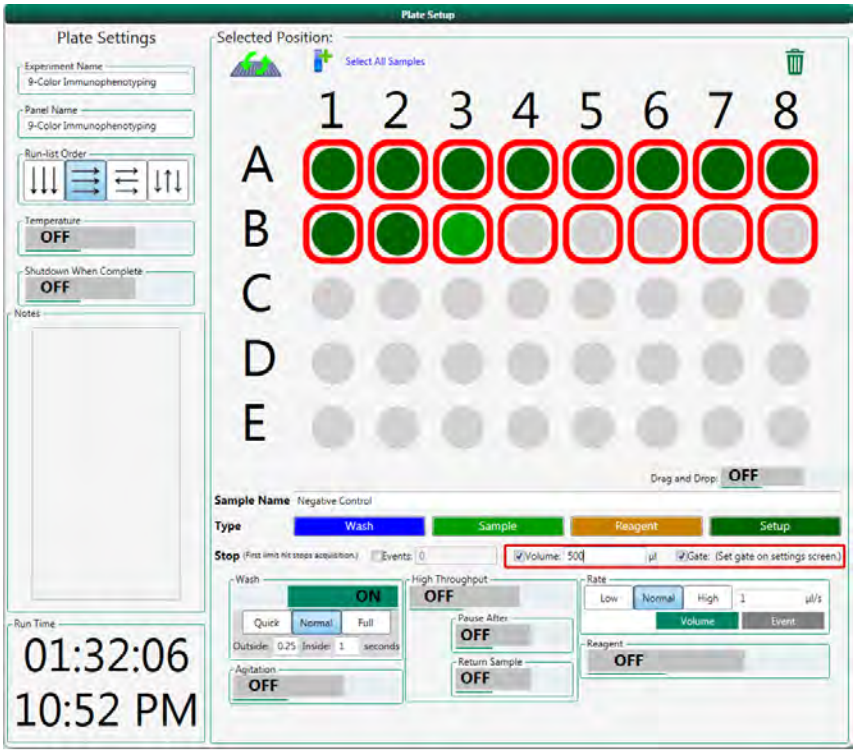
   After selecting positions, you can apply sampling parameters to all of the positions at once.

4. Select the Gate checkbox.

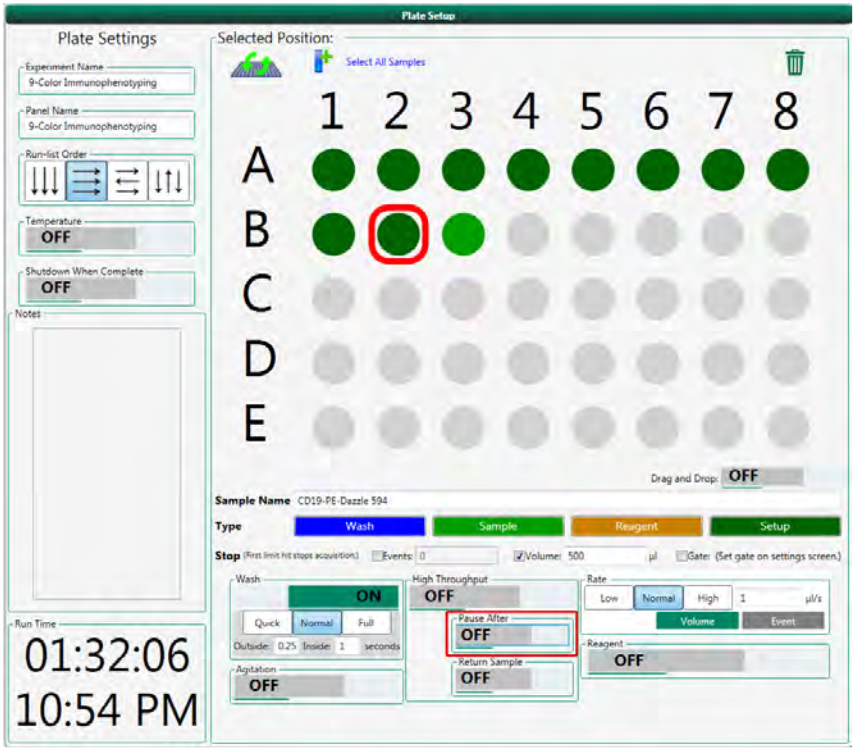
   The volume defaults to the maximum volume for the media type you are using and the Events and Volume limit controls are hidden. You will set sample and control gate limits in the Settings screen after completing the plate setup.



5. Click Additional Limits to reveal the Events and Volume limit controls.
6. In the Volume box, enter the actual volume used in the tubes for this experiment.

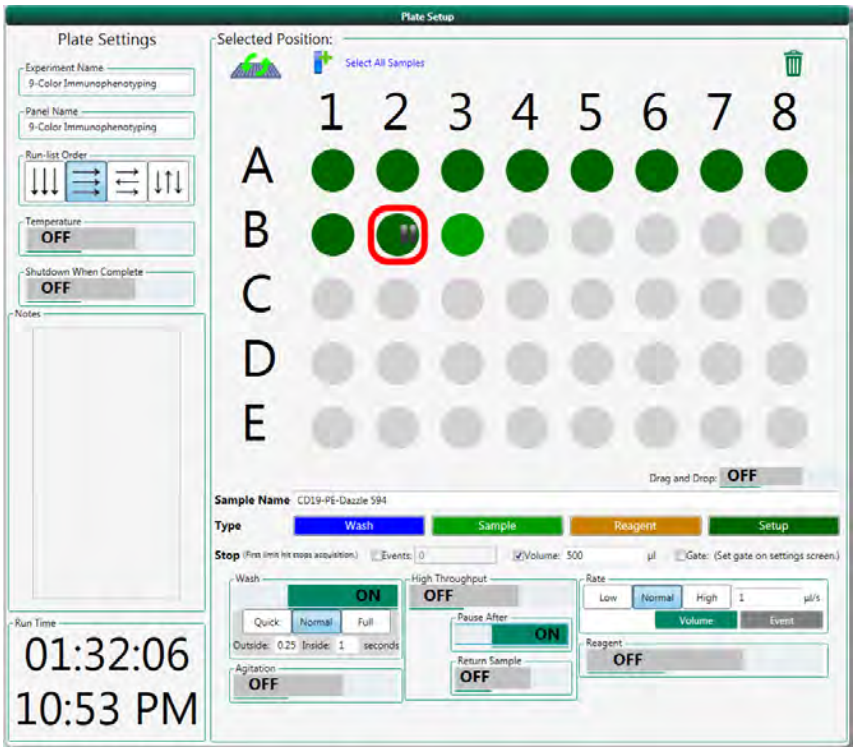


7. At the bottom of the Plate Setup panel, specify any other sampling parameters to apply to these positions, such as agitation and target flow rate or target event rate.
8. Click the position for the last compensation control.



9. Click the Pause After toggle to instruct the run list to pause after the last compensation control is acquired.

   This allows you to perform automatic compensation before running the fully stained sample. A pause glyph appears on the selected position, as shown in the next figure. For more information about glyphs, see Position Glyphs on page 62.



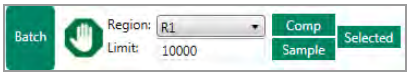
10. When sample setup is complete, click the Next arrow.

    Plots are created automatically for the controls.

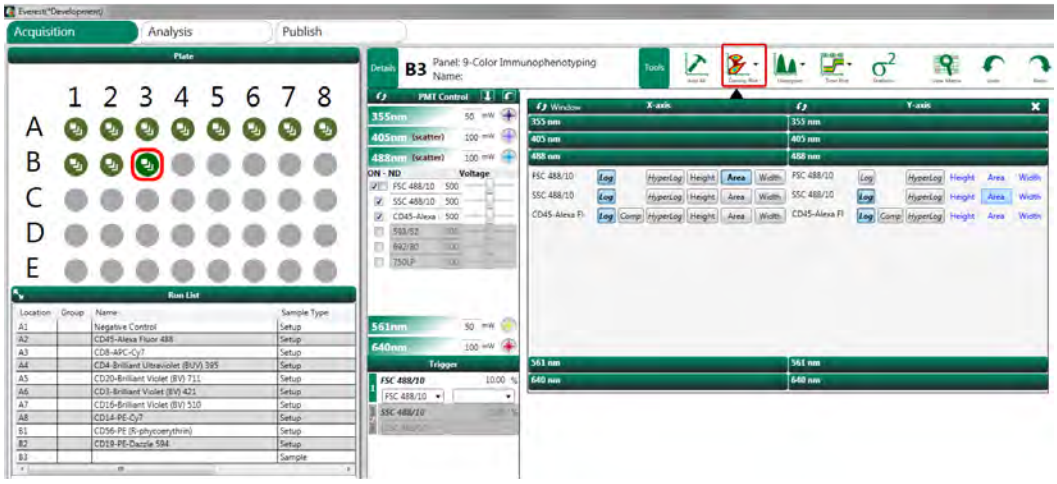
Applying Gates and Creating Plots

Set gate limits for the controls and samples. Sampling will occur until the specified gate limits are reached.

1. In the Plate map, select all controls.
2. In the Batch area of the toolbar, apply a gate limit to the compensation controls by typing a value in the Limit box and then clicking Comp.

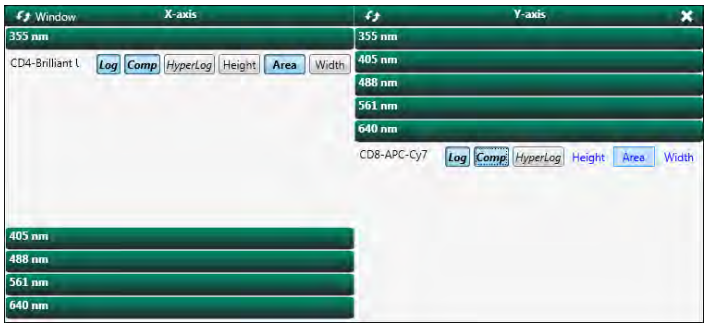


3. Create plots for the fully stained experimental sample.
    a. Click the sample position in the plate map and click Create Density Plot.



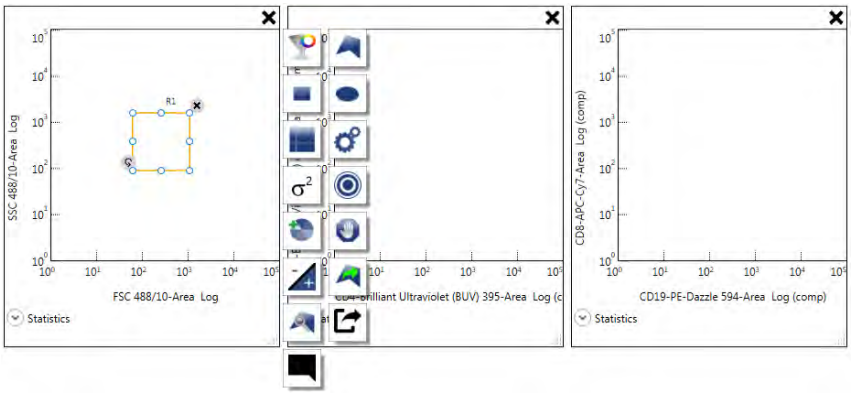
b. Create a forward scatter versus side scatter plot (FSC/SSC) for the 488 laser.

c. Create plots for the remaining parameters in the experiment. For each plot, click Comp so that the plots will display compensated data.

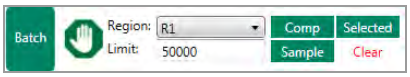


4. Create a region in the FSC/SSC plot.

This region will be used to apply a gate limit to the other plots.



5. In the Gate area of the toolbar, apply a gate limit to the sample by typing a value in the Limit box and then clicking Sample.



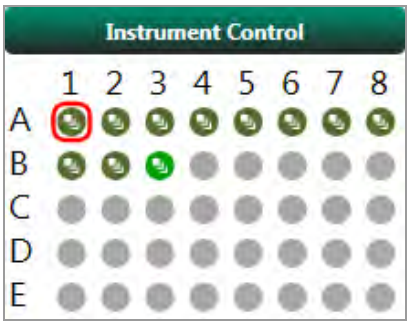
This gate limit will be the stop limit for sample acquisition.

Acquiring Initial Data

After you create the plots, acquire some data in setup mode so that you can adjust PMT voltages. Events viewed in setup mode will not be saved to FCS files. After making adjustments, you can proceed to acquisition mode.

1. Load the controls and samples onto the instrument.
2. Click Apply.
3. Click the first position in the template.

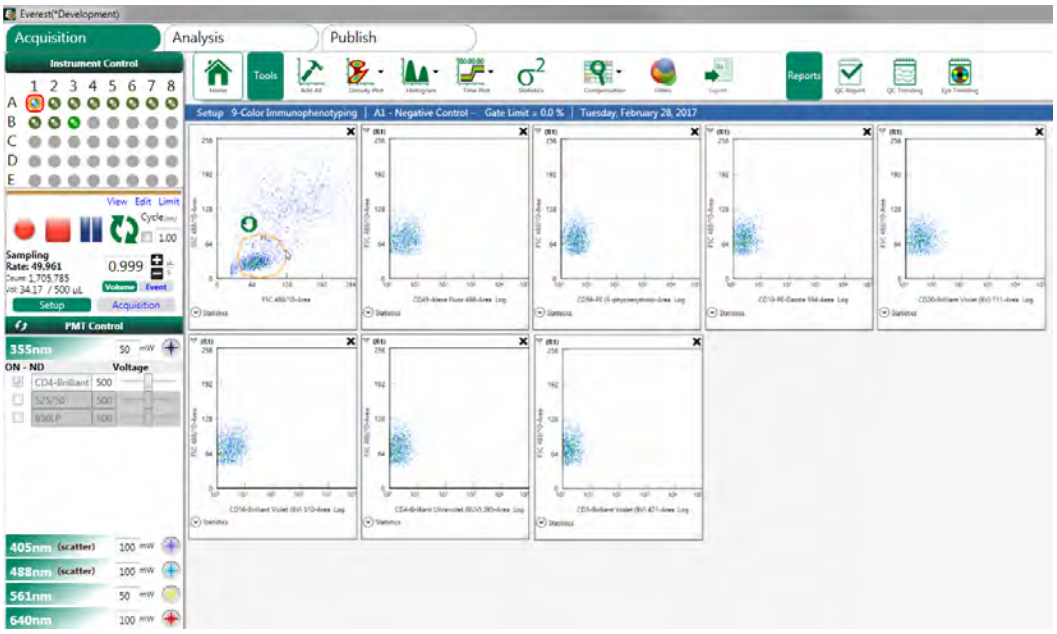
   This is the position from which the instrument will start sampling.



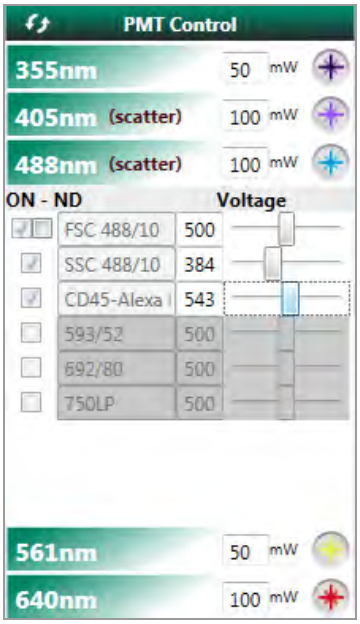
4. In the Instrument Control panel, ensure that setup mode is active.

5. Click Play.

   The system displays plots in the workspace.



6. In the FSC/SSC plot, position the gate over the relevant part of the sample population.
7. In the PMT Control panel, adjust PMT voltages to position the population as desired.



8. After setting up gates and voltages, click Record to save data for the sample.

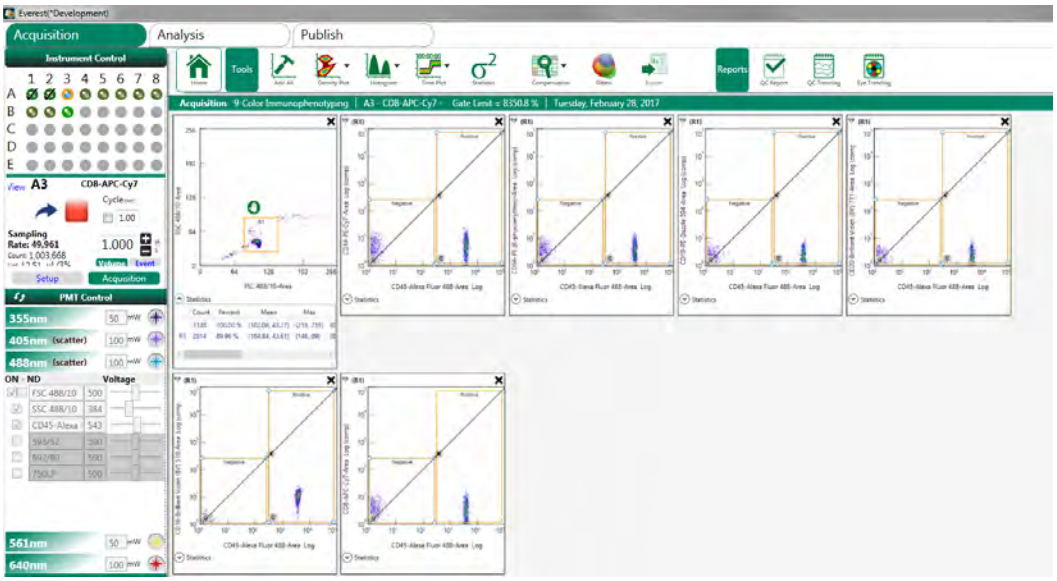
   This clears existing data, disables PMT adjustment, and starts saving a data file. Data are acquired until the set limit is reached or until you click Stop; sampling does not proceed automatically to the

next position.

9. Repeat Step 5 through Step 8 for other samples as needed.
10. When you are satisfied with the setup plots, click Acquisition to put the system in acquisition mode.

11. Click Play to start acquiring data.

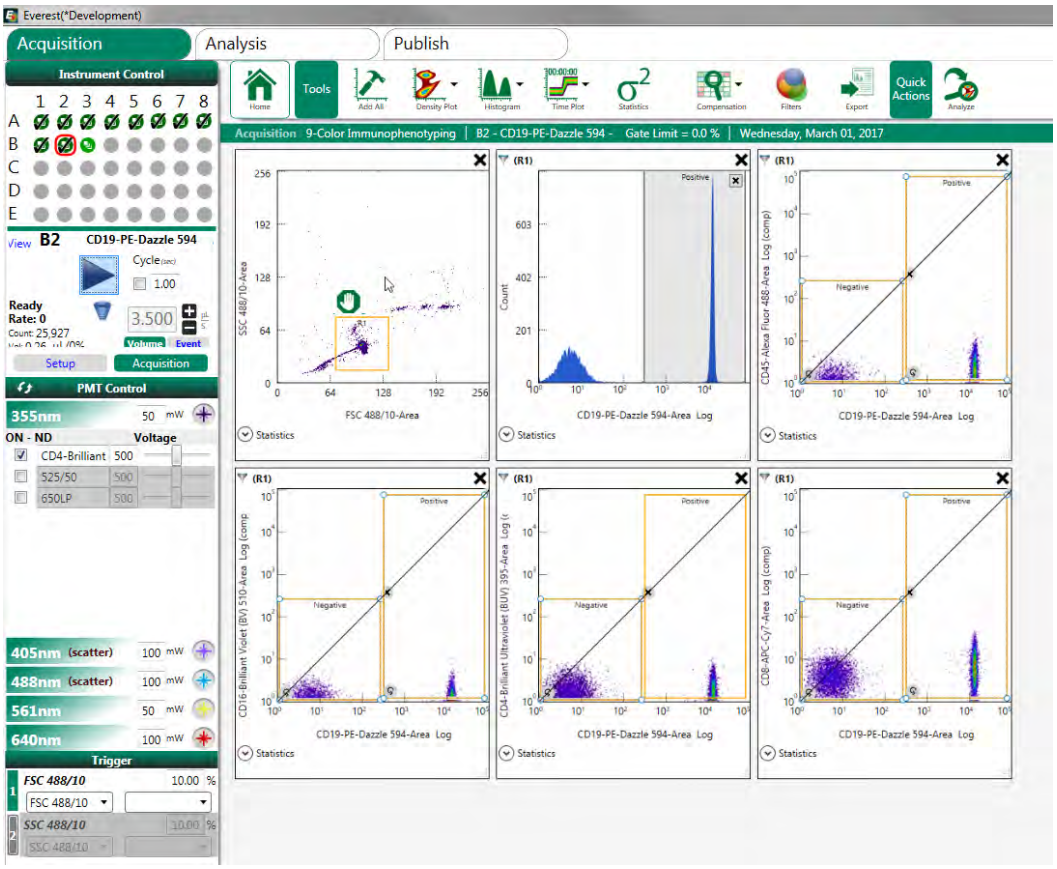
    The run list automatically advances to the next sample when the gate limit is reached.



12. To adjust a gate during the run, click inside the region and drag the gate to include the relevant part of the sample.

    Because Pause After was selected, after acquiring data for the controls, the instrument pauses to allow you to apply auto compensation before running the fully stained sample.

    Note: A check mark glyph appears on the control positions in the compensation template to show that they have been acquired.



13. On the toolbar, click Analyze to move data to the Analysis tab.

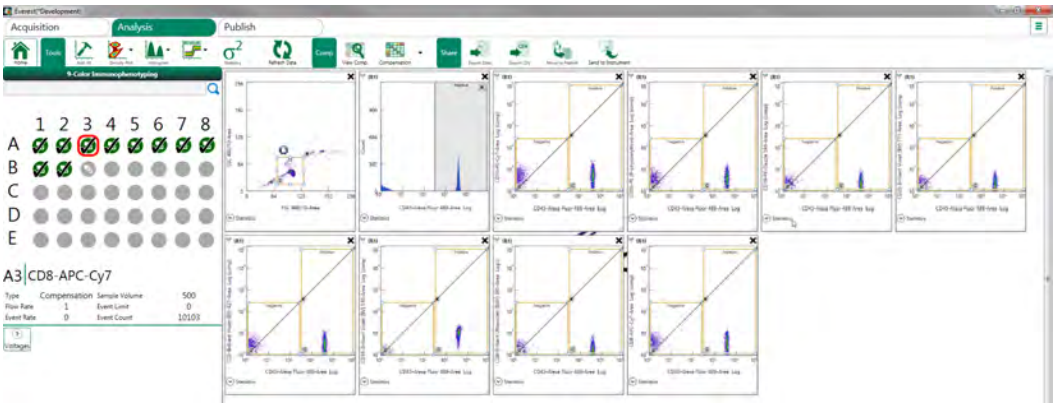
Performing Initial Data Analysis

When applying automatic compensation, you have two options:

- Manually adjust regions to include stained populations and instruct the automatic compensation process to include these manually adjusted regions.
- Let the automatic compensation process perform region determination; manually adjust the regions afterward if needed.

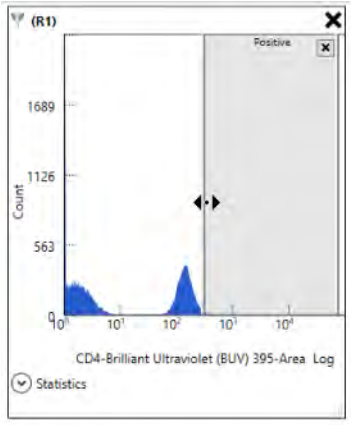
The following steps illustrate the first option.

1. Click a control position to view the data from that control.

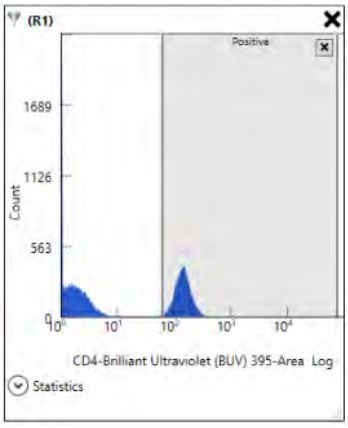


2. For each control, ensure that the positive and negative populations are identified properly (for example, two populations should appear and the scatter gate set should be set correctly).

   The PMT voltage is low for the parameter in this example, so the region must be adjusted to encompass the stained population.



3. Drag to reposition the region to include the positive population.



Note: If compensation is performed in the Analysis tab and those settings are moved back to the instrument, compensation settings will be saved within the FCS files for any samples that are subsequently acquired. If all sample acquisition is done before compensation is performed, compensation settings will not be saved within the FCS files.

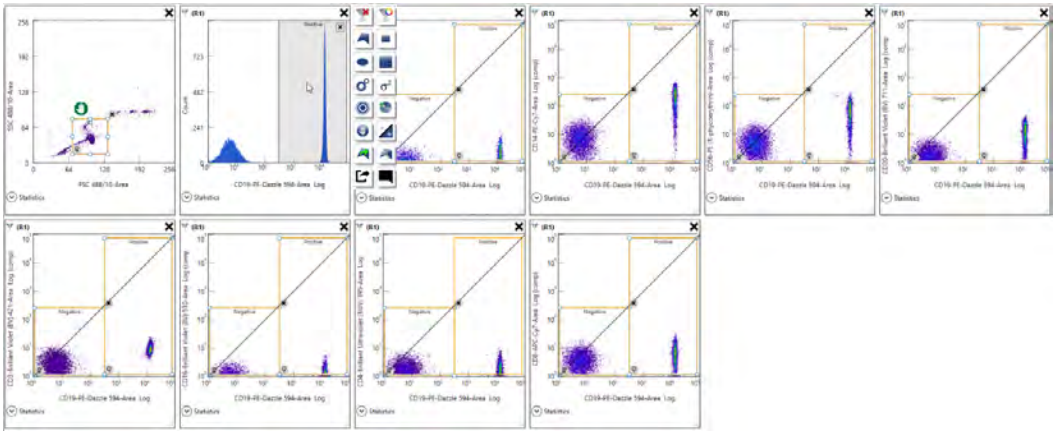
Performing Automatic Compensation

Before running the fully stained sample, perform automatic compensation. The FCS file for the fully stained sample will be exported with the compensation matrix. Data are exported in accordance with FCS 3.1 standards and can be analyzed using third-party software.

1. Click the down arrow on the Compensation button to select auto compensation options.

2. Select the Prevent Automatic Region Determination checkbox to include any regions that you adjusted manually.
3. Click Calculate.

   This applies auto compensation using the data from the compensation controls and refreshes the plots to display compensated data.

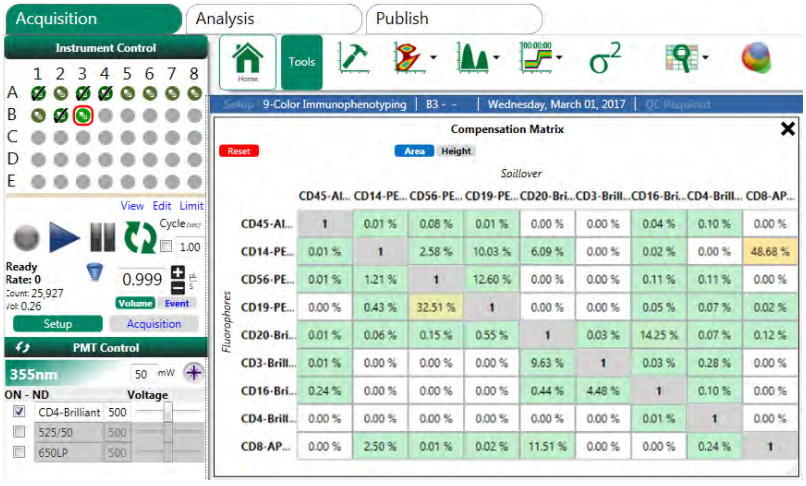


4. Click Send to Instrument to send the calculated compensation values back to the local instrument.

This returns you to the Acquisition tab.

5. On the Acquisition tab toolbar, click View Comp.

The compensation matrix shows the calculated compensation values.

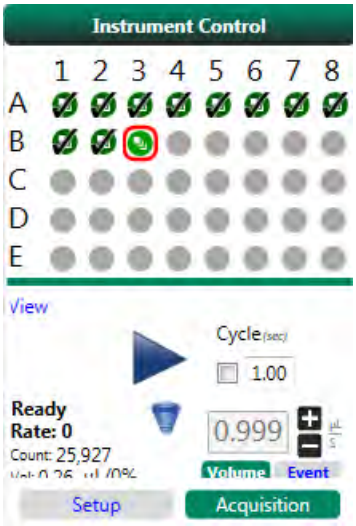


6. In the plate map, click the sample position from which acquisition should resume.

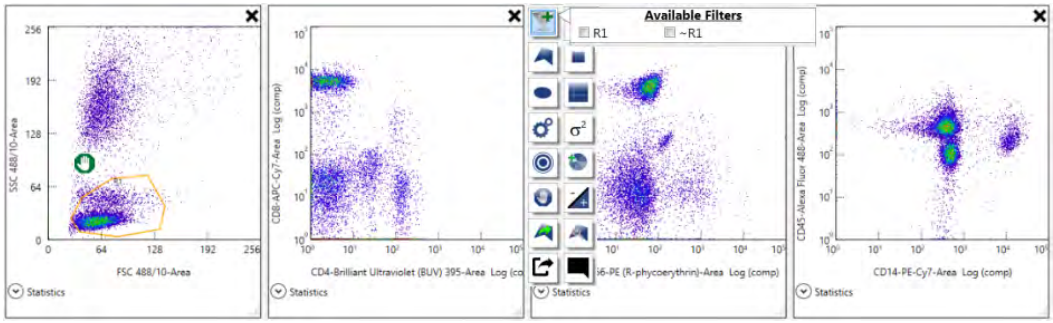
Resuming Acquisition

After applying compensation, acquire sample again.

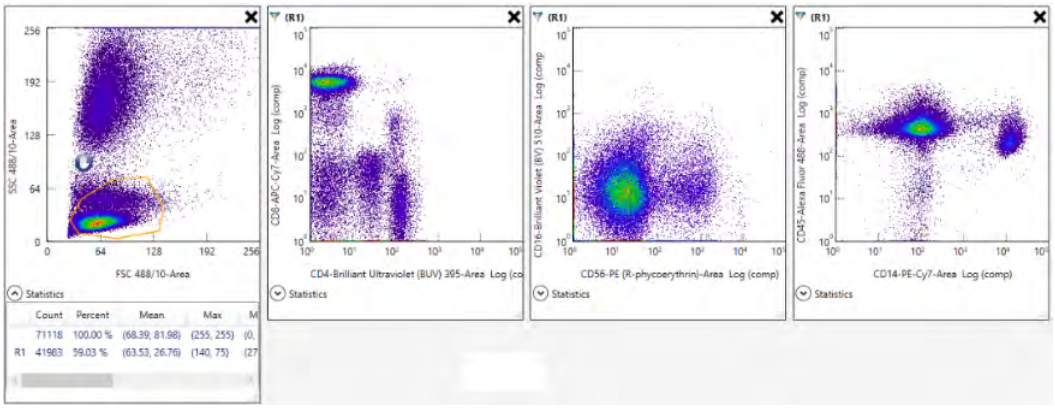
1. In the Instrument Control panel, ensure that Acquisition Mode is active.



2. Click Play to start acquiring data from the fully stained sample.
3. To ensure that the gate is applied to all of the plots, click the filter tool in each plot and select R1.



The filter is applied to each plot.



Analyzing or Exporting Final Data

From the Analysis tab, you can export data for analysis in third-party software.

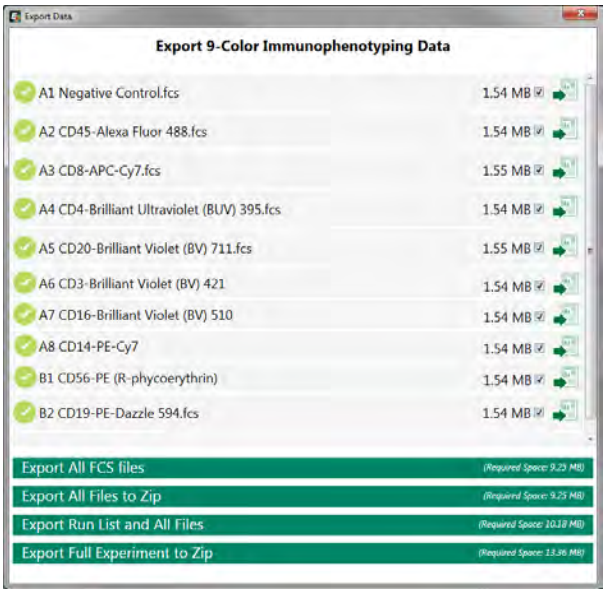
1. Click Analyze.

2. Click the Analysis tab.
3. If you want to analyze data using third-party software, click Export.

Tip: You can also export data from the Local Instrument tab.

4. Select an export option.

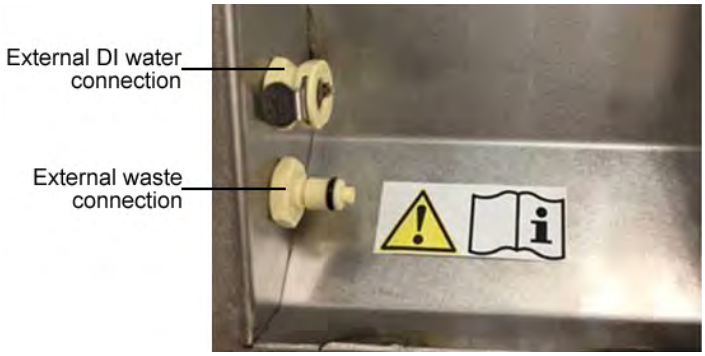
   Everest Software exports one FCS file per acquired control or sample.



5. Select a location for the exported FCS files and click Save.
6. Close the export dialog box when the export is complete.

Appendix C Optional External DI Water and Waste Ports

Ports for connecting the ZE5™ Cell Analyzer to house DI water and house waste are located on the instrument back panel. Contact Bio-Rad Technical Support if you plan to use either of these ports.



Before putting the instrument into external DI water mode or external waste mode, a qualified, trained technician must hook up your house DI water or waste line to the instrument.

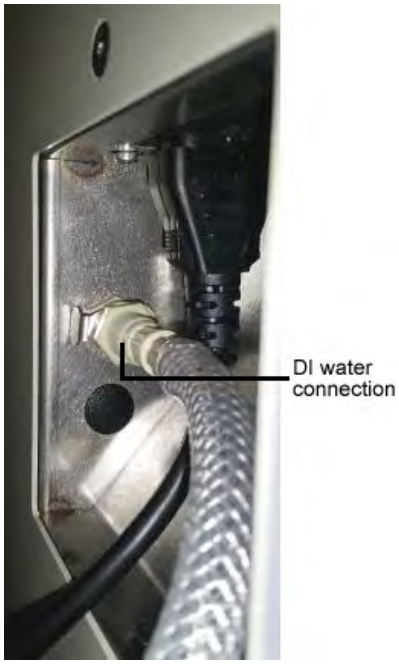
Caution: Only Bio-Rad Service personnel are permitted to connect the ZE5 Cell Analyzer to external DI water or waste lines.

Connecting External DI Water

When the instrument is connected to external DI water and is put into external DI water mode, the volume in the top DI water bottle will drop to around 1/4 full while sheath fluid is flowing, then the bottle will automatically refill to around 3/4 full. The bottom DI bottle is not used in this mode. For information about external DI water mode, see Specifying External Port Use on page 301.

Caution: Only Bio-Rad Service personnel are permitted to install and connect the External Deionized Water Supply kit. The water supply must be of a pressure between 10 and 20 psi, filtered by a 1 µm or better filter, and be of pure deionized water with at least 10 megaohm-cm resistivity.

The next two figures show a DI water hose connected to the instrument's external DI water port and the DI water hose connected to a house DI water filter system, respectively.



Connecting External Waste

When the instrument is connected to an external waste line and is put into external waste mode, waste enters the top waste bottle and drains from there into the external waste line. The bottom waste bottle is not used in this mode.

Caution: Biohazard! Only Bio-Rad Service personnel are permitted to install the External Waste kit and connect the ZE5 Cell Analyzer to external DI water or waste lines. The waste contains samples that are run through the instrument and thus could contain biohazardous materials. Consult with your local safety officer or review local state and federal regulations to ensure proper handling and disposal of biohazardous substances.

Specifying External Port Use

After the instrument has been connected to an external DI water or waste line, a Bio-Rad service engineer must configure your system to use the external port.

Appendix D ZE5 Cell Analyzer Specifications

The specifications for the ZE5™ Cell Analyzer are shown in Table 45.

Table 45. ZE5 Cell Analyzer specifications

Category	Description	Specification
System	Fluorescence sensitivity	<100 MESF (molecules of equivalent soluble fluorochrome) for FITC, PE, APC
	Scatter sensitivity	<0.5 µm FSC resolution with standard FSC detector <0.3 µm FSC resolution with small particle detection module
	Loader	Integrated sample loader with agitation Sample and collection temperature control from 4–37°C, Peltier solid state system Media types: ■ 5 ml tubes (12 x 75 mm, 1–40 tubes per rack) ■ 1.5 ml tubes (1–24 tubes per rack) ■ 96-well plates ■ 96 deep-well plates ■ 384-well plates ■ Stat tube position for single tube, 5 ml (12 x 75 mm)

Table 45. ZE5 Cell Analyzer specifications, continued

Category	Description	Specification
Optics	Excitation lasers	Up to five spatially separate lasers. Standard options include: ■ 355 nm 50 mW ■ 405 nm 100 mW ■ 488 nm 100 mW ■ 561 nm 50 mW ■ 640 nm 100 mW
	Detection	Up to 30 detectors (PMTs) including: ■ Forward scatter (FSC) detector ■ Optional second FSC detector ■ Side scatter (SSC) detector ■ 27 fluorescence detectors
	Cuvette	Fused silica with 145 x 265 µm channel
Electronics	Speed	>100,000 events per second with all parameters enabled
	Data processing	Simultaneous measured peak, area, and width for every channel 24-bit data for peak and area 17-bit data for width with high-resolution linear interpolation at the half height
Fluidics	Bulk fluids	Four 4 L bulk fluid bottles onboard for sheath fluid and waste Onboard sheath additive concentrate and cleaner Optional kit for connecting to house DI water and waste (field upgrade)
	Sample flow rates	0.0025–3.5 µl/sec (0.15–250 µl/min) (standard mode) 0.5–2.5 µl/sec (30–150 µl/min) (high-throughput mode)

Table 45. ZE5 Cell Analyzer specifications, continued

Category	Description	Specification
Software/computer system	Workstation	Operating system: Windows 10 Pro CPU: Intel Core i7-6700 Quad Core 3.4 GHz RAM: 8 GB DDR4 2133 MHz Storage: 1 TB 7200 RPM hard drive Networking: Dual gigabit network interface card, 802.11ac
	Program	Everest™ Software
	Flow Cytometry Standard (FCS) format	FCS 3.1
	QC	Automated quality control with onboard calibration beads
	Monitor	29" LCD; 2560 x 1080 resolution
	Printer	Optional
Installation	Power	AC 96–264 V, 50–60 Hz, <500 W
	Dimensions (instrument only) (W x D x H)	29 x 27 x 26 in 74 x 66 x 69 cm
	Weight (instrument only)	<260 lb <118 kg
	Temperature	Ambient temperature range 18–25°C
	Relative humidity	20–60%
	Air and vacuum supply	Included, onboard

Table 45. ZE5 Cell Analyzer specifications, continued

Category	Description	Specification
Regulatory	Electromagnetic compatibility	EN 61326-1:2013 Class A ICES-001 Issue 4 Class A FCC Part 15 Subpart B Class A
	Environmental	EN 50581:2012
	Laser	IEC 60825-1:2014, EN 60825-1:2014 Class 1 laser product per IEC 60825-1 and CDRH requirements and regulations
	Safety	IEC 61010-1:2010, EN61010-1:2010 IEC 61010-2-081:2015, EN61010-2-081:2015 UL/CSA 61010-1:2012
	Use	For research use only

Appendix E Ordering Information

Ordering information for the ZE5™ Cell Analyzer, accessories, and replacement parts is shown in Table 46.

Table 46. Ordering information

Catalog #	Description
Instrumentation	
17002031	ZE5 Cell Analyzer, 5 laser 27 color (355/405/488/561/640)
17002032	ZE5 Cell Analyzer, 4 laser 24 color (405/488/561/640)
17002033	ZE5 Cell Analyzer, 3 laser 17 color (405/488/640)
17002034	ZE5 Cell Analyzer, 3 laser 17 color (488/561/640)
Computer System	
17002096	Includes: ■ ZE5 Cell Analyzer Computer with Network Adaptor ■ ZE5 Cell Analyzer Computer Monitor, 29 in, 2560 x 1080 ■ ZE5 Cell Analyzer Wireless Keyboard and Mouse
Accessory and Replacement Parts	
12004273	ZE5 Cell Analyzer Accessory Kit
12004397	ZE5 DI Water Container with Tubing, blue cap, 4 L
12004418	ZE5 DI Water Container, 4 L, pack of 2
12004396	ZE5 Waste Container with Tubing, red cap, 4 L
12004402	ZE5 Waste Container, 4 L, pack of 2
12004395	ZE5 Cleaner Bottle with Tubing, blue cap, 450 ml
12004404	ZE5 Additive Bottle with Tubing, white cap, 450 ml

Catalog #	Description
12004444	ZE5 Cleaner/Additive Bottle, 450 ml
12005002	Bi-level Tube Lifter for 40 x 5 ml tube rack
12005163	Replacement Filter, single bandpass 525/35, position A4
GF00175	Neutral Density Filter, 2.0, 11 mm square 400–650 nm
12004445	ZE5 Tube Rack, 40 x 5 ml
12004446	ZE5 Tube Rack, 24 x 1.5 ml
12004389	ZE5 Webcam, Logitech C310 with USB cable
Consumables	
12004274	Test Tubes, 5 ml, 12 x 75 mm, pack of 25
12004272	Cytometer Cleaner, 1 L
12004271	ZE5 Additive, 4 x 300 ml
12004403	ZE-Series QC Beads, 5 ml, pack of 3
12005773	96-well Microplates, polystyrene, U-bottom, clear, 10 pieces/bag
1451083	ProFlow™ Sort Grade Water 5 x 4 L
1451085	ProLine™ Rainbow Beads, 5 ml (a mixture of beads dyed with 8 different fluorescent intensities for excitation at wavelengths 365–650 nm)

Appendix F References

The following references and resources are useful for learning more about flow cytometry and its applications.

- Shapiro HM (2003). Practical Flow Cytometry: Fourth Edition (Hoboken: John Wiley & Sons).
- Cytometry: Part A; Journal of the International Society for the Advancement of Cytometry. Wiley.

  http://onlinelibrary.wiley.com/journal/10.1002/%28ISSN%291552-4930
- Purdue University Cytometry Laboratories: cytometry and confocal microscopy education and research material, cytometry email archive, and links to cytometry web sites and suppliers worldwide.

  http://www.cyto.purdue.edu/

Glossary

acquisition	Process in which sample data are gathered from the PMTs, processed, and sent to and displayed in Everest™ Software.
acquisition mode	In this mode, samples are processed following the programmed steps in the run list. This mode is typically used after running samples in setup mode.
area	The area under the curve of the pulse, as analyzed by the electronics.
bandpass filter	An optical component, usually placed in front of a detector, that allows passage of wavelengths of light within a specified range while absorbing the rest.
compensation	A mathematical method used to correct the emission overlap from one fluorophore into the emission channel of another fluorophore. Compensation can be applied manually or automatically.
cycle mode	Shows a current “snapshot” of data defined within the time (in seconds) specified by the user. Data are automatically refreshed. Cycle mode is primarily used while adjusting PMT voltages, regions, and gates in setup mode.
dichroic filter	An optical component, usually part of the detection path, that allows passage of a range of wavelengths of light while reflecting the rest. Dichroic filters can either be longpass or shortpass.
event	Any signal pulse with an intensity above the threshold in the trigger parameter(s). See trigger on page 313 and threshold on page 313.
event rate	The number of independent electronic events above the threshold in the trigger parameter. The event rate is dictated by the number of particles that pass through the interrogation point per second; however, it is not always equal to cells per second — debris, cell clumps, and other material also contribute to the event rate as they pass through the laser.
experiment	Another name for a run list.
EYE	See ZE5-EYE on page 313.
flow cell	The fused silica chamber through which sample flows to be interrogated. Also referred to as a cuvette.

heat map	A graphical representation of data where the individual values are represented as colors or color shades.
height	The height of the pulse, as analyzed by the electronics.
high-throughput mode	A sampling mode in which the probe moves from sample to sample and continuously aspirates sample, with programmed washes in between, resulting in multiple samples in the sample line at the same time. In this mode, samples cannot be returned to positions and reagent cannot be added to samples.
hydrodynamic focusing	In the ZE5™ Cell Analyzer, the sample is injected into the middle of the sheath fluid flow at the base of the cuvette just before the interrogation point. The two fluids form a two-layer stable flow, centering the sample in the middle of the sheath (carrier) fluid without mixing of the fluids.
interrogation point	The spot at which the lasers intersect the sample core inside the flow cell.
longpass filter	An optical component, usually placed in front of a detector, that allows passage of a range of wavelengths of light above a specified wavelength while absorbing or reflecting the rest.
neutral density filter	An optical filter that reduces or modifies the intensity of all wavelengths of light equally by reflecting or absorbing a portion of it.
PMT	Photomultiplier tube. An extremely sensitive vacuum phototube that detects and amplifies the scattered and fluorescent light signals produced by laser interrogation of particles.
pulse	The signal coming from a single particle in a single channel as it passes through the interrogation point. The pulse is amplified by the PMT and processed by the electronics.
pulse width	The width of the pulse, as analyzed by the electronics.
run list	A set of instructions — including media type, sample well/tube positions, sample volume, limits, speed, wash and agitation, sample names, enabled parameters, plots, and voltages — that allows the ZE5 Cell Analyzer system to acquire a set of samples. Also referred to as an experiment.
setup mode	Typically used during PMT voltage adjustment and setting up regions and gates, setup mode can be used to run any sample continuously without requiring adherence to the limits set in the run list.
sheath fluid	A carrier fluid used to hydrodynamically focus the sample stream for proper interrogation by the laser beam(s). For the ZE5 Cell Analyzer, this is typically DI water containing a sheath additive, but a saline solution can also be used.

shortpass filter	An optical component, usually placed in front of a detector, that allows passage of a range of wavelengths of light below a specified wavelength while absorbing or reflecting the rest.
stat tube	A single tube (as opposed to a rack of tubes) that can be used for quick sample acquisition without requiring experiment configuration in the Everest Experiment Builder.
threshold	The threshold is set using the trigger parameter(s) and is the level above which signal must fall to be classified as an event. The value is reported as a percentage of the total range of signal intensities in that detector. It is not efficient to analyze every single particle that passes through the interrogation point — samples contain debris that would inundate the dataset and drown out the population(s) of interest. Additionally, the threshold is helpful in eliminating irrelevant optical noise from the data.
time (parameter)	Each event is time-stamped when analyzed. All events can be viewed with respect to when they occurred during the sample acquisition.
track region percentage	A percentage that can be assigned to a region in a plot. During acquisition, if the percentage of events drops below the specified number, acquisition pauses and the user is notified. This feature is useful in monitoring for clogs or sample disturbances during acquisition.
trigger	The trigger parameter is the initial parameter of detection that signals the system that a particle of interest is present. The trigger, combined with the threshold setting, defines real events that should be detected and analyzed. Typically, a scatter parameter is selected for the trigger because it identifies all particles above a given size regardless of the fluorescent signal. However, any parameter or combination of parameters can be used as a trigger.
width	See pulse width on page 312.
workspace	A set of plots and regions that facilitate data analysis and interpretation.
ZE5-EYE	A ZE5 hardware feature that verifies the configuration of the optical filter setup by using multiple LEDs to pulse ten different wavelengths of light into the optical filters that lead to the detector banks.

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