ZE5 Cell Analyzer and Everest Software

User Guide

Version 3.1

BIO-RAD

ZE5 Cell Analyzer and Everest Software

User Guide

Software Version 3.1

BIO-RAD

Bio-Rad Technical Support

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Legal Notices

Revision History

Document	Date	Description of Change
ZE5 Cell Analyzer and Everest Software User Guide	August 2021 (Ver G)	Update to include new and modified functionality in v3.1 Update with all environmental requirements and rewrite CSV app install section (web release only) (Doc DIR# 10000072647)
ZE5 Cell Analyzer and Everest Software User Guide	March 2021 (Ver F)	Update user guide to version 3.0 (Doc DIR# 10000072647)

Table of Contents

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.

Table 1. Explanations of safety warning labels, continued

Icon	Explanation
	**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.
	**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.

The ZE5 Cell Analyzer system requires three outlets on a common breaker, one for the ZE5 Cell Analyzer instrument, one for the computer, and one for the monitor. The outlets must be properly grounded to comply with local regulations and electrical safety standards.

Important: The ZE5 Cell Analyzer requires a dedicated circuit. Do not connect the ZE5 Cell Analyzer to a circuit shared with devices such as centrifuges or refrigerators.

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); see for operating power requirements.

Environmental and Safe Use Requirements

The ZE5 Cell Analyzer is designed to be safely operated within the environmental conditions specified in Table 2.

Table 2. ZE5 Cell Analyzer environment requirements

Parameter	Specification
Installation site	Indoor use only
Operating altitude	At or below 2,000 m
Operating temperature range	18 to 25°C (64–77°F) To operate the ZE5 Cell Analyzer, the ambient temperature must be stable within plus or minus 2°C (3.6°F).
Relative humidity	20-60% non-condensing
Mains electrical fluctuations	Plus or minus 10%
Operating power (instrument, computer, and monitor)	AC 96-264 V, 50-60 Hz ■ 500 W, maximum rated power (PSU efficiency typically 90%) ■ 360 W, Energy Star-compliant active PFC power supply (PSU efficiency typically 90%)
Overvoltage category	II
Pollution degree	2

…

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.

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

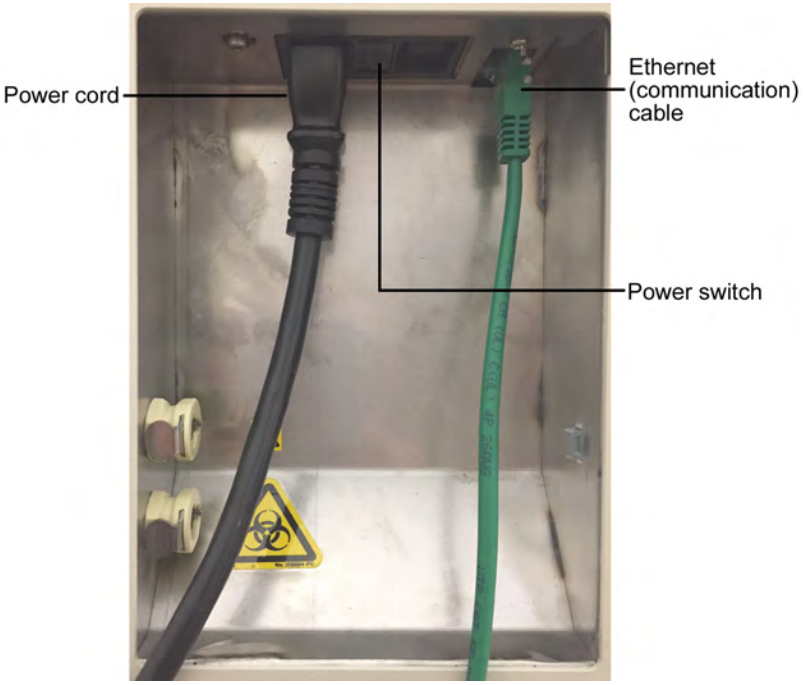
The ZE5 Cell Analyzer and associated accessories are covered by a standard Bio-Rad warranty. Contact your local Bio-Rad Laboratories office for details of the warranty.

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 188.

This area of the instrument is also available for optional connections for external DI water and external waste. For more information, see Chapter 14, Using External DI Water and Waste.

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 run design tabs. 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
- Audible alerts to indicate when the instrument has stopped automatically
- Ability to configure experiments in multiple panels on a single plate or tube layout
- Instrument settings library, where cytometer and compensation settings can be saved and then imported into experiments

- 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)
  - (12004279) 5 Laser
    355x5 / 405x7 / 488x4 / 561x7 / 640x4, 27 Color, 488 SSC FSC, 405 SPD
    Lasers: 355nm 50mW, 405nm 100mW, 488nm 100mW, 561nm 50mW, 640nm 100mW
    Detectors: 27 colors, one side scatter, two forward scatters
  - (12014135) 5 Laser 7 off UV Option A
    355x7 / 405x7 / 488x4 / 561x5 / 640x4, 27 Color, 488 SSC FSC, 405 SPD
    Lasers: 355nm 50mW, 405nm 100mW, 488nm 100mW, 561nm 50mW, 640nm 100mW
    Detectors: 27 colors, one side scatter, two forward scatters
  - (12014136) 5 Laser 7 off UV Option B
    355x7 / 405x7 / 488x4 / 561x5 / 640x4, 27 Color, 488 SSC FSC, 405 SPD
    Lasers: 355nm 50mW, 405nm 100mW, 488nm 100mW, 561nm 50mW, 640nm 100mW
    Detectors: 27 colors, one side scatter, two forward scatters
  - (12004278) 4 Laser
    405x7 / 488x6 / 561x7 / 640x4, 24 Color, 488 FSC SSC
    Lasers: 405nm 100mW, 488nm 100mW, 561nm 50mW, 640nm 100mW
    Detectors: 24 colors, one side scatter, one forward scatter
  - (12014138) 4 Laser with SPD
    405x7 / 488x6 / 561x7 / 640x4, 24 Color, 488 FSC SSC, 405 SPD
    Lasers: 405nm 100mW, 488nm 100mW, 561nm 50mW, 640nm 100mW
    Detectors: 24 colors, one side scatter, two forward scatters
  - (12004276) 3 Laser
    405x7 / 488x6 / 640x4, 17 Color, 488 FSC SSC
    Lasers: 405nm 100mW, 488nm 100mW, 640nm 100mW
    Detectors: 17 colors, one side scatter, one forward scatter
  - (12014141) 3 Laser with SPD
    405x7 / 488x6 / 640x4, 17 Color, 488 FSC SSC, 405 SPD

Lasers: 405nm 100mW, 488nm 100mW, 640nm 100mW
Detectors: 17 colors, one side scatter, two forward scatters

- (12004277) 3 Laser
  488x6 / 561x7 / 640x4, 17 Color, 488 FSC SSC
  Lasers: 488nm 100mW, 561nm 50mW, 640nm 100mW
  Detectors: 17 colors, one side scatter, one forward scatter
- (12014139) 3 Laser
  405x7 / 488x6 / 561x7, 20 Color, 488 FSC SSC
  Lasers: 405nm 100mW, 488nm 100mW, 561nm 50mW
  Detectors: 20 colors, one side scatter, one forward scatter
- (12014140) 3 Laser with SPD
  405x7 / 488x6 / 561x7, 20 Color, 488 FSC SSC, 405 SPD
  Lasers: 405nm 100mW, 488nm 100mW, 561nm 50mW
  Detectors: 20 colors, one side scatter, two forward scatters

- ZE5 Cell Analyzer Computer System
  - Computer with network adaptor
  - Wireless keyboard and mouse
  - Computer monitor, 29 in, 2560 x 1080
- ZE5 Accessory Kit

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

For information on consumables and accessories, see Appendix G, 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 3 lists the bench space required to install the ZE5 Cell Analyzer with and without the computer and monitor.

Table 3. 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.

To ensure all prerequisite requirements are met before the installation of the ZE5 Cell Analyzer in your laboratory, see the ZE5 Cell Analyzer Pre-Installation Guide.

Upgrading Everest Software and ZE5 Firmware

Important: Before upgrading Everest Software, ensure that no processes are running on the ZE5 instrument 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.

Upgrading Everest Software and your ZE5 instrument requires the following general steps:

- Upgrading Everest Software on the Everest computer
- Updating the ZE5 instrument firmware

Tip: You can upgrade Everest Software versions 1.2–3.0 to version2.0.

Upgrading Everest Software

Important: The software upgrade process removes the currently installed version of Everest Software. Before starting the installation process, ensure that no experiments are running on the instrument and that you have saved all data and exited the software.

Note: You must be an administrator on the Everest Software computer in order to upgrade the software. See your system administrator for more information.

To upgrade Everest Software

1. Verify that you have exited Everest Software.
2. From the ZE5 Cell Analyzer product page on the Bio-Rad website, locate the file Everest Setup zip file and download it to a folder on the Everest computer, for example the Downloads folder.
3. In Windows Explorer, navigate to and open the Downloads folder and double-click the Everest Setup zip file.
4. Extract the file Everest_Setup.exe.
5. Double-click Everest_Setup.exe to start the Everest installer.
6. You are prompted to install the ZE5 firmware and ZE5 service tool updates. Click Install.

   Tip: Installing the updates can take a few minutes. After the updates are installed you see the Everest Software installer wizard's Welcome window.
7. On the Welcome window, click Next.
8. Accept the license agreement and click Next.
9. Follow the remaining instructions in the wizard to install the software.
10. When the installation is complete, click Finish to exit the wizard.

    When completed, the Everest icon appears on the desktop.

Updating ZE5 Instrument Firmware

When you start Everest Software, the ZE5 Cell Analyzer detects the new firmware package and begins the update process.

Important: The firmware update process can take up to 5 min. The Everest Firmware Update Utility window and then the ZE5 Service Tool dialog box appear on the window. Do not close the dialog box or shut down the instrument during the update.

To update the ZE5 instrument firmware

1. Start Everest Software on the Everest computer.

   If the firmware update is required, Everest Software displays the ZE5 Firmware Update dialog box.

2. Click Update firmware.

   The Everest Firmware Update Utility window appears and then the ZE5 Service Tool launches. The firmware update begins.

   When the update is complete, the instrument starts and the software displays the login window.

3. Log in to Everest Software.

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 standard user. Bio-Rad recommends that at least two administrators be assigned per system.Table 4 on page 31 lists features available to administrators and standard users.

For more information on administrator privileges, see Managing Users on page 135 and Setting Preferences on page 123.

Table 4. Features available to administrators and users

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

Other Documentation

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

To access this user guide from Everest Software

▶ Click ☰ and 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.

Fluidics System

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.

Note: If you are using external fluidics, such as a house DI system or a fluidics cart, see Chapter 14, Using External DI Water and Waste for more information.

The ZE5 internal 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 consists of DI (deionized) water to which sheath additive is added. Laminar sheath flow carries the sample core through the center of the flow cell, where laser beams intercept the particles to be measured. For detailed information about the hydrodynamic focusing that occurs in the flow cell, see Flow Cell on page 40.

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 water; the two large bottles with red caps collect waste. Beneath the large bottles are small bottles containing system cleaner (blue cap) and sheath additive (white cap).

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 water and waste bottles, and displays an alert when less than 1 hr of run time remains.

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 the following:

- Replace both sheath and empty both waste containers at the same time.
- 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 188
- Cleaning Solutions on page 369

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 13, 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 cap and each holds 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.

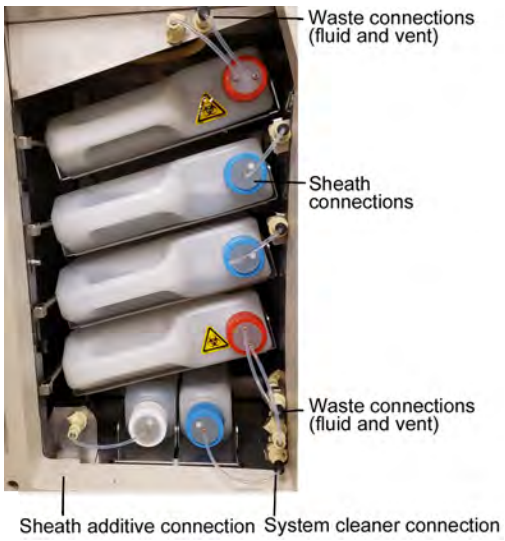
Caution: Biohazard! Consult with your local safety officer or review local, state, and federal regulations to ensure proper handling and disposal of biohazardous substances.

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.

Important: Replace both sheath and empty both waste containers at the same time.

Fluidics Connections

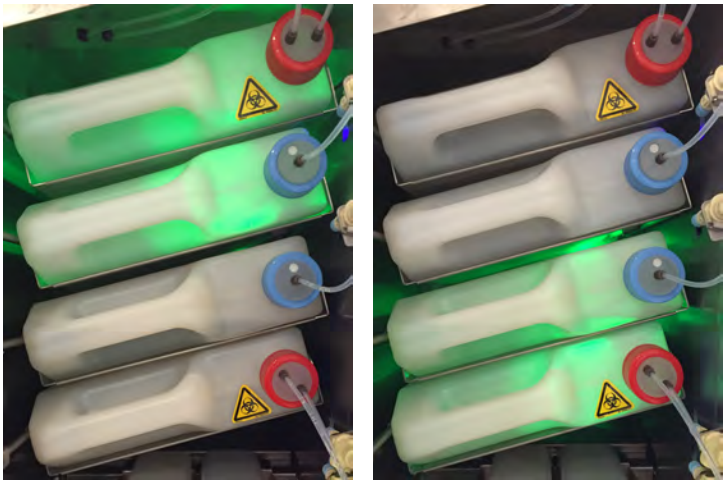
The ZE5 Cell Analyzer bulk fluidics chamber is located on the front of the instrument to the left of the loader. The fluidics chamber holds four large and two small bulk fluidics bottles. Connections to these bottles are depicted in the next figure.



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.

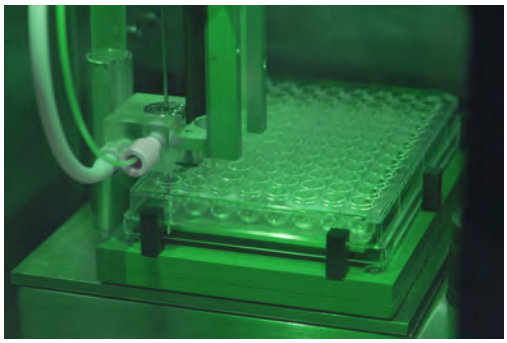
During system operation, the bottles illuminated in green are in use; unlit bottles are not in use and can be swapped. Examples are shown in the next figure.



For information about connecting your ZE5 Cell Analyzer to house DI or a fluidics cart to extend uninterrupted run time, see Chapter 14, Using External DI Water and Waste.

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 0.1–3.5 µl/sec (6–210 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 located 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 Instrument Tools dropdown located in the System section of the Everest Software toolbar.

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

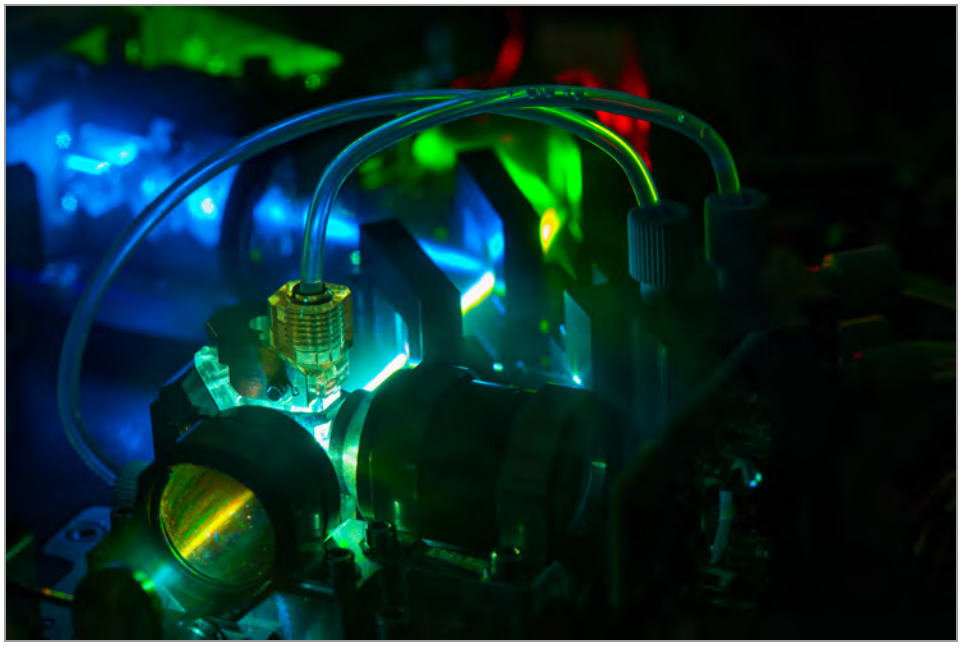
Caution: In addition to the loader door, there are two doors (filter access and bulk fluidics access) that require access on a regular basis. Use caution when opening and closing these doors and mechanisms to avoid pinching.

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 up to five lasers from the Coherent OBIS line of lasers.

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

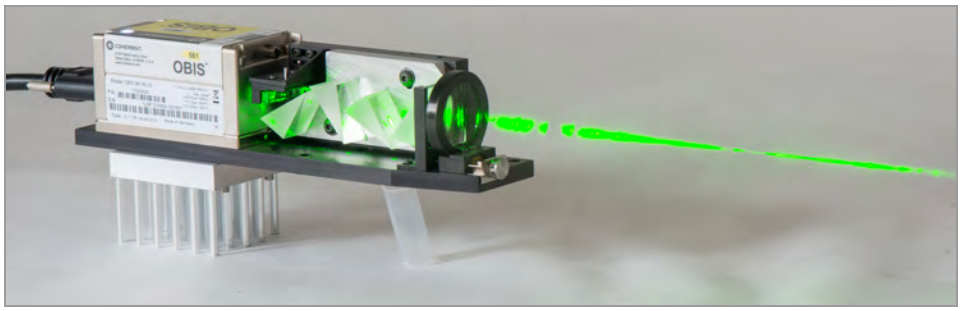
- 355 nm (UV) at 50 mW
- 405 nm (violet) at 100 mW
- 488 nm (blue) at 100 mW
- 561 nm (yellow green) at 50 mW
- 640 nm (red) at 100 mW

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 306.

Side Scatter

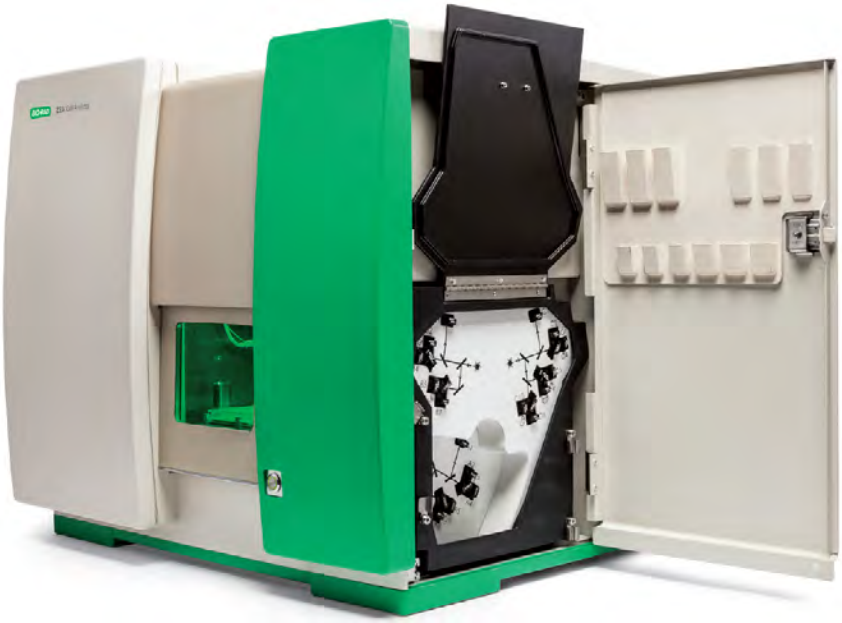
Light scattered by particles at an angle of about 90° to the laser beams is collected to indicate relative differences in particle complexity (for example, granularity, membrane structure, and cytoplasmic constituents). More complex particles usually reflect and refract more light than less complex particles, 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 50.

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 164. For information about installing other filter types, see Replacing Optical Filters on page 154.

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

Table 5. Optical filter sizes

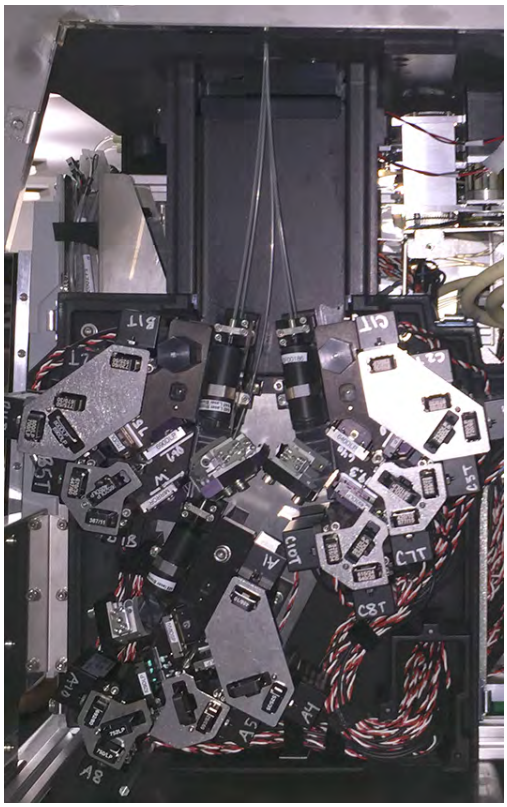
Filter type	Size (L* x W x thickness**)
Dichroic	17 x 15 x 1.05 mm
Bandpass	13 x 13 x 2 mm

* 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.



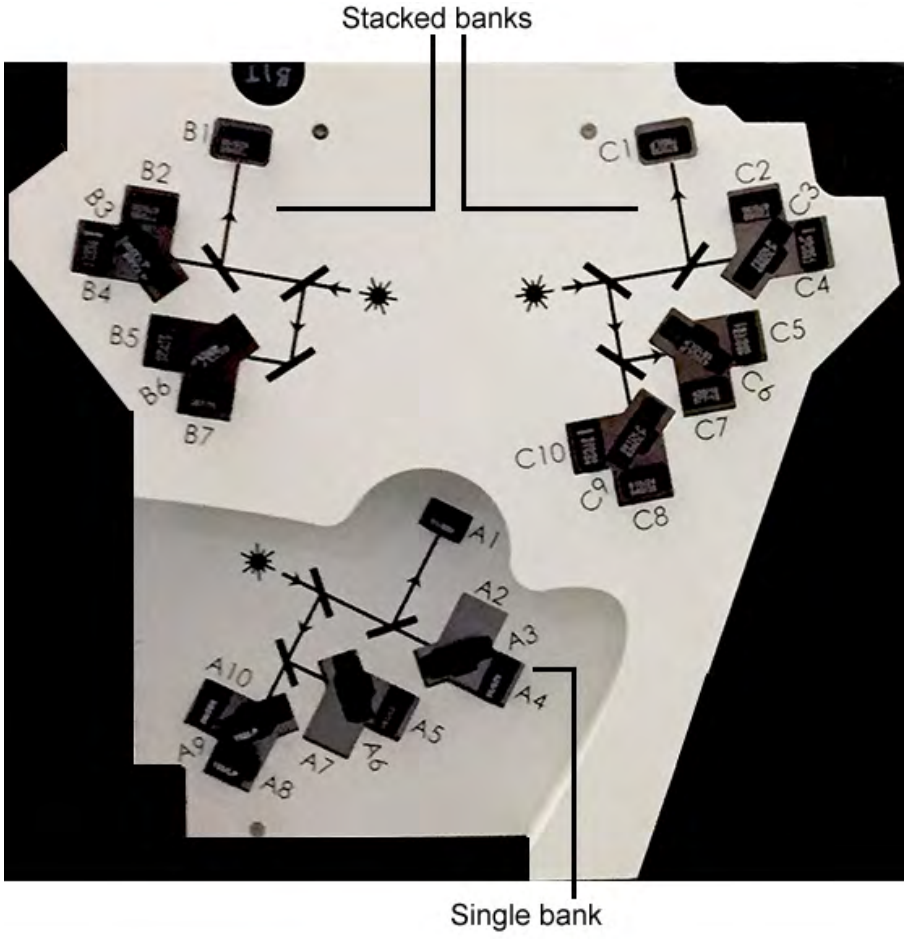
The ZE5 Cell Analyzer can be configured with up to five banks, one for each laser. The five banks are grouped into three regions: A, B, and C. Region A, located on the bottom, consists as a single bank, while regions B and C, located on the top left and top right, respectively, each contain two banks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

Table 6. Optical filter banks

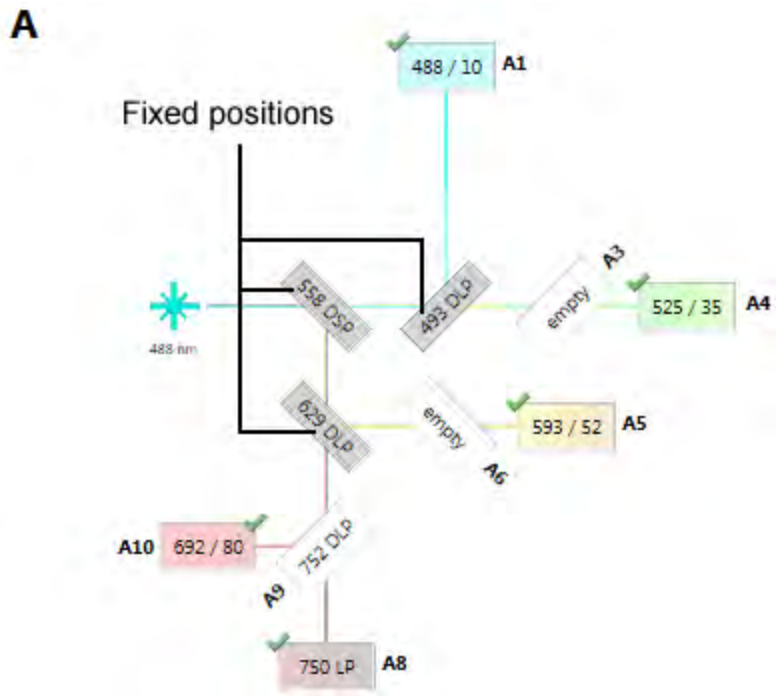
Bank	Laser	Total possible PMTs
A	Blue (488 nm)	7
B Top	Red (640 nm)	5
B Bottom	UV (355 nm)	5

Table 6. Optical filter banks, continued

Bank	Laser	Total possible PMTs
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.

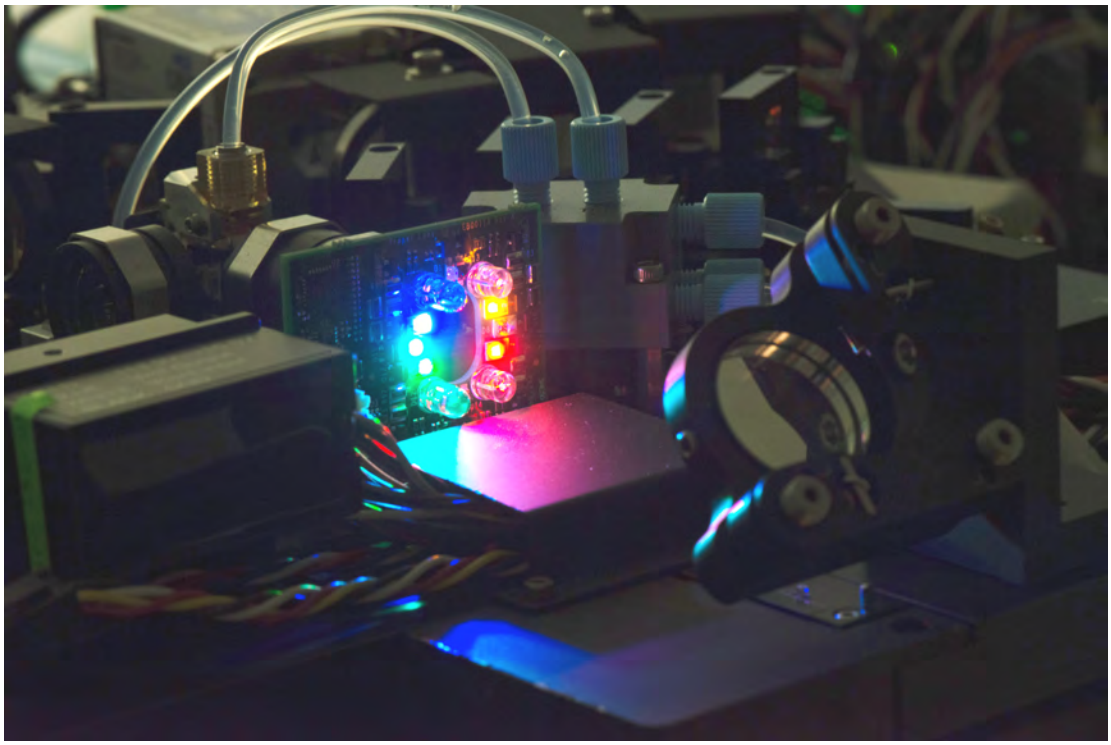


Filter holders contain either one or two mirrors/filters. Single filter holders are installed in region A, while double filter holders are installed in stacked banks (regions B and C). Examples of single and double filters are shown in the next figure.

For information about configuring filters, see Working with Optical Filter Configurations on page 141.

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 158.

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 158.

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 306.

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

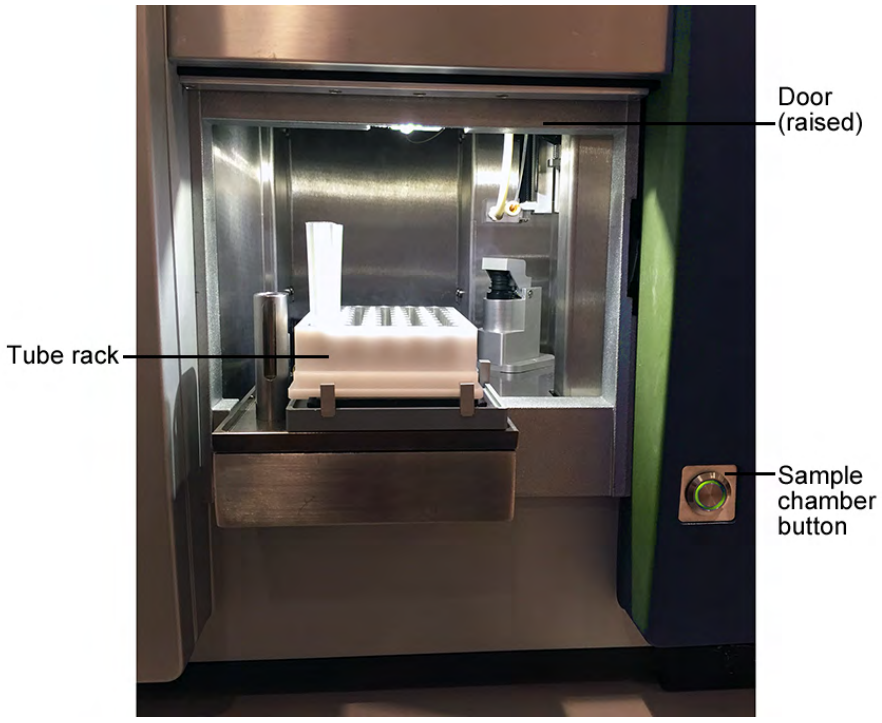
Analog-to-digital converters (ADCs) convert the electrical signal coming from the pre-amplifier into a digital signal and transfer that signal to the software for data visualization. The ZE5 Cell Analyzer is a fully digital instrument, transforming signals with 24-bit resolution for signal area and height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

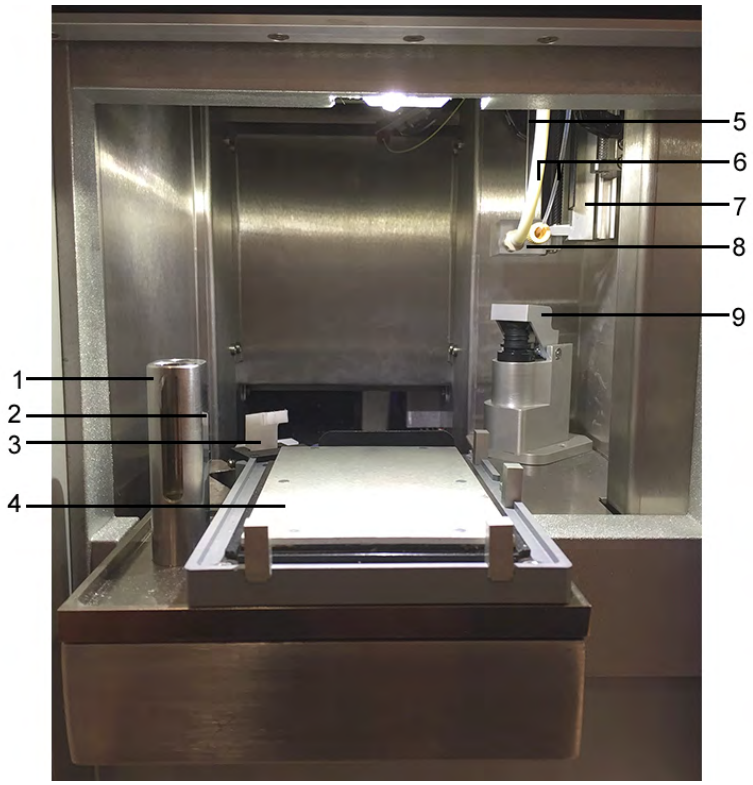


A wash module is integrated into the sample delivery system. The sample probe moves up and down through the wash station to expose contaminated regions of the probe to wash fluid. You can use Everest Software to control wash times for the inside of the sample line and the outside of the sample probe.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

For more information on wash cycles, see Wash Settings on page 95.

Loader Components

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



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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 removing clogs.
3	Clamp	Secures the tube rack or plate on the loader. Opens when loader is extended. Closes when loader is retracted.
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.

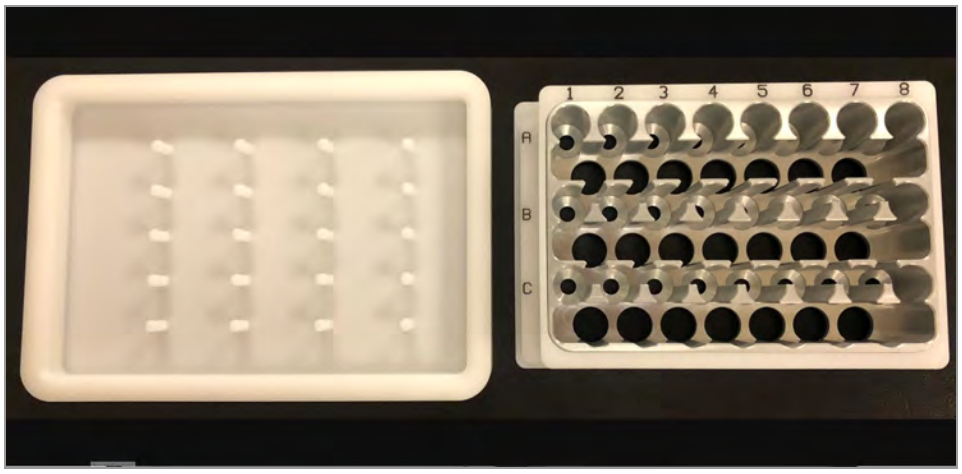
Media Types

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 below.



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.

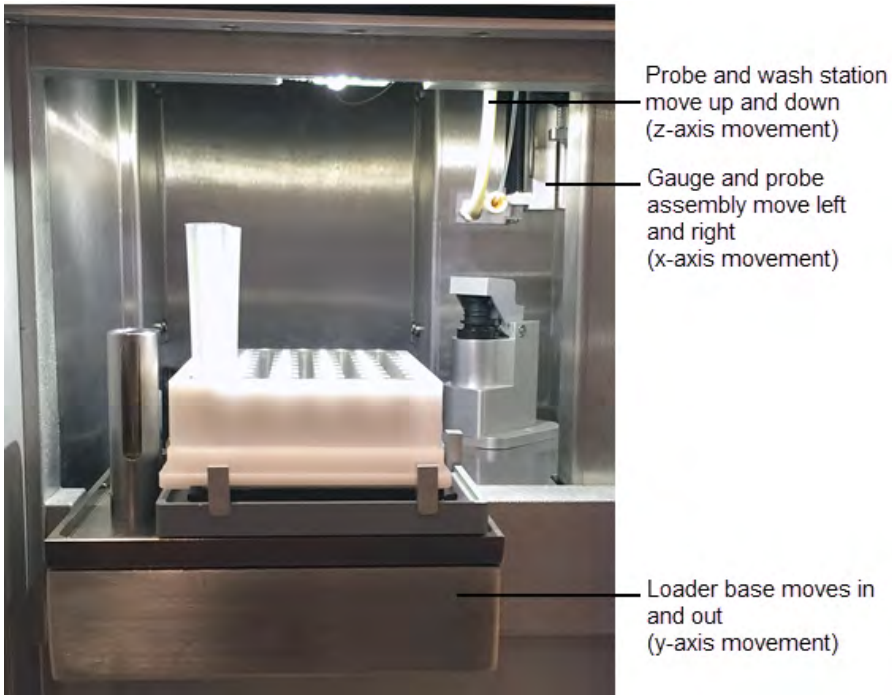
You can program washes into the sample run during run list setup, and you can specify both the inside and outside wash times. These washes use filtered (0.2 µm) sheath fluid. The outside of the sample probe is cleaned by moving the contaminated portion of the sample probe up and down through the wash 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 188.

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, run samples, and print reports.

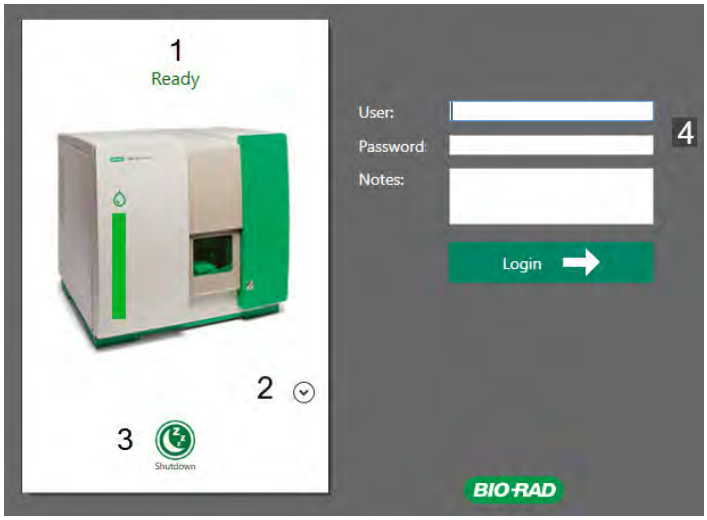
This chapter explains the main areas of Everest Software:

- Login window
- Home window
- Main menu
- Recent Experiments panel
- Experiment Builder panel and tabs
- Acquisition workspace
- Instrument Control panel
- Toolbar
- Status bar

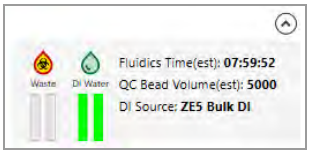
Some options in the Everest Software user interface are available only to system administrators. For information, see Administrator and User Rights on page 30 and Main Menu on page 62.

Login and System Status Window

The login window is divided to show system status on the left and the login pane on the right.



LEGEND

1	Displays the state of the instrument.	■ Off (shut down) ■ Starting up ■ Ready	■ Paused ■ Cleaning probe ■ Shutting down
2	Click the arrow icon to show or hide instrument actions		
3	Click the toggle icon to prompt the specified action. **Note:** You can start up or shut down the instrument without logging in. To shut down, ensure that samples are not actively running		**Shutdown** — appears after the system has been started up.  **Startup** — appears after the system has been shut down.
4	Log into Everest Software.	User name and password are provided by your system administrator. See Logging In on page 180.	

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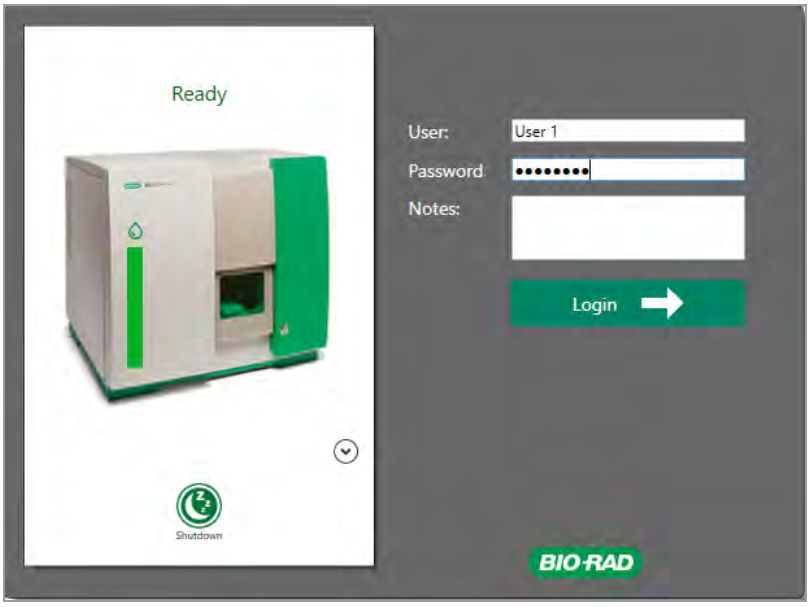
Logging Into Everest Software

To log into Everest Software

1. Double-click the Everest Software icon to open the software.

   The Login window appears.



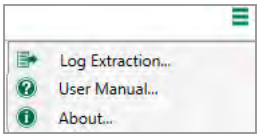
2. Enter your user name and password.
3. Click Login.

   Everest Software opens to the Home page, and your user name appears in the upper-right corner.

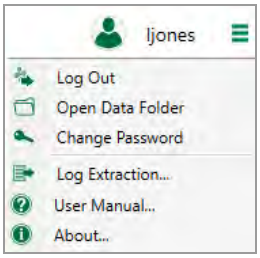
Main Menu

The main menu button ( ) is located in the upper-right corner of the software. It is available from all software windows, and includes a selection of the options described in Table 7. Users see only the options available for their user role (standard user or administrator).

Pre-login menu



Standard user menu



Administrative user menu

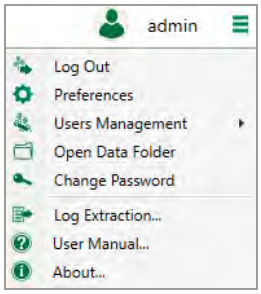


Table 7. Main menu options

Icon	Option	Available to
	Log Out — logs the current user out of the system.	Logged-in users
	Preferences — allows you to configure global settings for the instrument and software. See Setting Preferences on page 123.	Administrators only
	User Management — allows you to ■ Manage user accounts and access rights. ■ Open a report that tracks usage over time and includes session notes entered by logged in users. See Managing Users on page 135.	Administrators only
	Open Data Folder — opens the data folder associated with your user name. **Note:** An administrator enters the file path as part of your user account setup.	Logged-in users
	Change Password — allows you to reset your login password. See Changing Your Password on page 64.	Logged-in users

…

Table 7. Main menu options, continued

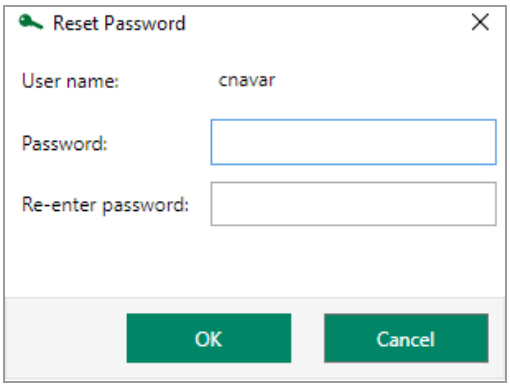
Icon	Option	Available to
You can access the following items at any time, including when no user is logged in.		
	**Log Extraction** — pulls system log files from the last 180 days, compresses them into a zip file, and saves the file on the computer's desktop. See Exporting and Viewing Log Files on page 403.	All users
	**User Manual** — opens and displays a PDF of this guide.	All users
	**About** — displays basic system information such as the instrument serial number, software version, and firmware version.	All users

Changing Your Password

You can change your password at any time after logging into Everest Software.

To change your password

1. Click the ☰ icon, and then select Change Password.
2. In the Reset Password dialog box, enter your new password twice and click OK.



Keyboard Shortcuts and Undoing Actions

Everest Software provides several keyboard shortcuts for common system actions.

Table 8. 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 Flow Cytometry Standard (FCS) file.
Ctrl + C	Copy plot.
Ctrl + V	Paste plot.
F5	Cycle data. See cycle mode under Setup Mode Controls on page 300.

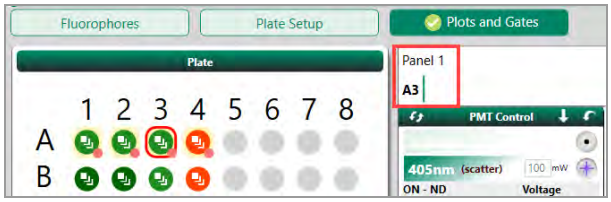
Everest Toolbars and Identifiers

Toolbar layouts for the associated functionality appear in the following windows:

- Plots and Gates
- Acquisition (after you apply the experiment)
- Analysis

Panel and Well Identifiers

As shown below, the selected panel and well are identified on the menu bar in the Plots and Gates tab.



After you apply the experiment, the selected panel and well are identified on the blue status bar. For information, see Status Bar on page 71.

Home

After you have applied your settings, the Home button appears on the far left side of the toolbar.

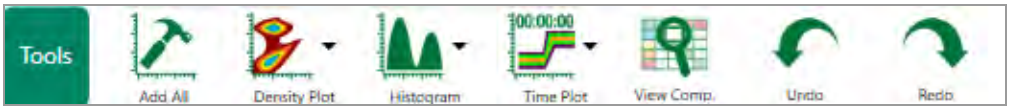
▶ Click the Home button to return to the corresponding Home window from the Acquisition or Analysis tabs.

- From the Acquisition tab, you can create a new experiment or load an existing experiment.
- From the Analysis tab, you can load a new experiment or resume analysis of a current experiment.

Acquisition Tools

The toolbars for the acquisition tools are slightly different before and after you apply your settings.

- Before you click Apply, you see the following tools:



  Before you apply settings, you can also use the Remove Plots button on the far-right to remove plots or histograms that exist in the workspace.

- After you click Apply, you see the following tools:

Note: Tools that appear in the Analysis toolbar are also identified. For more information on Analysis tools, see Analysis Toolbar on page 328.

Table 9. Acquisition Tools

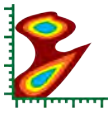
Button	Description
	**Advanced Plot Builder** — facilitates creation of histograms for all parameters, with constraints that you define. For more information, see Creating Histograms for All Channels on page 257. Available before and after settings are applied. Also available in the Analysis toolbar.
	**Create Density Plot** — creates a bivariate (two-parameter) density plot. For more information, see Creating Density Plots on page 246. Available before and after settings are applied. Also available in the Analysis toolbar.
	**Create Histogram** — creates a univariate (one-parameter) histogram. For more information, see Creating Histograms on page 257. Available before and after settings are applied. Also available in the Analysis toolbar.

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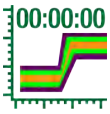
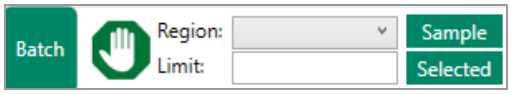
Table 9. Acquisition Tools, continued

Button	Description
	**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 248. Available before and after settings are applied. Also available in the Analysis toolbar.
	**Add Statistics** — opens a statistics window; in which 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. Available only after settings are applied, and also available in the Analysis toolbar. This window also displays the gating hierarchy. For more information, see Managing Plot Statistics on page 267, Viewing and Rearranging Plot Statistics on page 268, and Comparing Statistics on page 269.
	**View Compensation** — opens the compensation matrix in the workspace for viewing or editing. Available before and after settings are applied. Also available in the Analysis toolbar.
	**Undo** — reverses the last action taken. Applies only to creating, moving, resizing, or deleting a region or plot; and applying or removing a gate. Available before settings are applied.
	**Redo** — reverses the last Undo action. Available before settings are applied.
	**Optical Filter Configuration** — displays the current optical filter configuration for all detection banks. Also allows you to initiate the ZE5-EYE process. Avalable only after settings are applied.
	**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 a zip file. ■ Export run list to run list (RLST) format and export all FCS files for the current experiment. ■ Export full experiment, including list, telemetry, and all FCS files for each position, and compress to a zip file Available only after settings are applied.

Batch Tools

The batch tools appear in the toobar before settings are applied.



These tools allow you to set gate limits for compensation controls, all experimental samples, or for selected positions. Sampling occurs until the specified gate limits are reached.

Table 10. Batch toolbar tools and their functions

Tool	Function
Region	Specify the region to which the gate will apply.
Limit	Specify a gate limit.
Sample	Apply the gate limit to experimental samples, as opposed to compensation controls.
Selected	Apply the gate limit to the selected sample positions.

Quick Actions

A quick-action Analyze tool is available in the Acquisition toolbar, after you have applied your initial settings.

Table 11. Quick Actions buttons and functions

Button	Function
	**Library** — opens the Instrument Settings Library, where you can select and apply settings files that are compatible with you r experiment and instrument.
	**Analyze** — opens the most recent run list in the analysis tab. This is useful when you want to perform auto-compensation in the current run list.

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System Tools

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

Table 12. System tool buttons and their functions

Button	Function
	**QC** — initiates the QC process. See Running Quality Control on page 183.
	**Shutdown** — shuts down the system, if it has been started. See Shutting Down on page 188.
	**Startup** — starts up the system, if it has not yet been started. See Starting Up the System on page 177.
	**Instrument Tools** — includes tools for controlling and maintaining the system. **Note:** These tools are also available in the Home window.
	**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 185.
	**Decon** — initiates the Decontamination Wizard to decontaminate the system. See System Decontamination on page 373. *Available to administrative users only.*
	**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 369.
	**Clean** — runs cleaner through the probe and sample line. See Cleaning the Sample Line and Probe on page 371.
	**Pause System/Resume System** — pauses the sheath fluid flow and disables the lasers, or resumes the sheath flow and enables the lasers.

Table 12. System tool buttons and their functions, continued

Button	Function
	**Swap Beads** — 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 383.
	**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 168. For information on external fluidics, see Using External DI Water and Waste on page 387.
	**Low Fluidics Warning** — indicates that the level of bulk fluidics is low and should be replaced soon.
	**Extremely Low Fluidics Warning** — indicates that the bulk fluidics should be immediately replaced.
	**Swap Fluidics** — switches the active waste and sheath fluidics bottles. See Refilling Bulk Fluidics on page 170.
	**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.

Table 12. System tool buttons and their functions, continued

Button	Function
	**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.

Status Bar

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

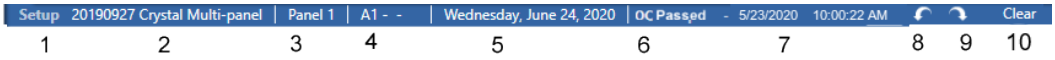
LEGEND

1	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.
2	Indicates that the instrument is directly connected to the computer.
3	Displays the system status.
4	Adds a window that displays a log of all error, warning, and information notifications that have been issued since the software was started. **Important:** This log window appears only in the Acquisition workspace.
5	Displays the most recently issued instrument status warning.

Table 13. 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.
Paused	The system is paused; lasers are turned off and sheath is not running.
Cleaning Probe	The system is running cleaner through the sample line and probe.
Decontaminating	The system is running the Decontamination process.
Shutting Down	The system is shutting down.
Off	The system is shut down.

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



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

LEGEND

1	Sampling mode: Setup or Acquisition
2	Experiment name
3	Currently selected panel
4	Currently selected sample position
5	Current date
6	QC status
7	Date of last QC run
8	**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.
9	**Redo** — reverses the last Undo action.
10	**Clear** — removes plots and histograms that have been added.

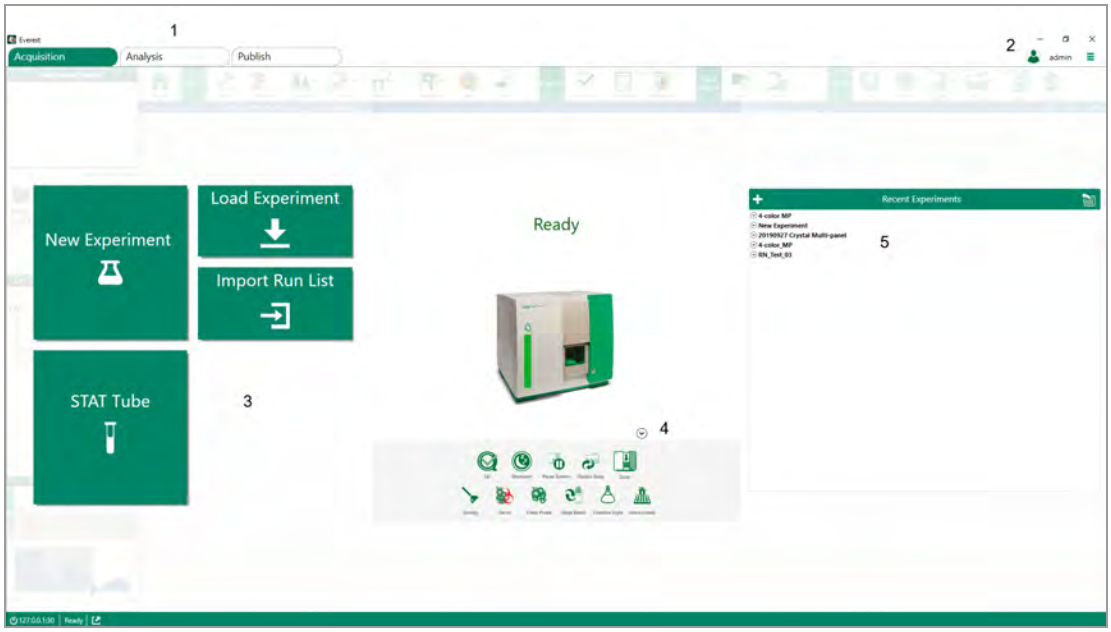
Other Toolbar Buttons

Buttons in the toolbar vary depending on the stage of experiment setup and the selected tab.

- For information about buttons for reports, see Reports Tools on page 341.
- For information about toolbar buttons in the Analysis tab, see Analysis Toolbar on page 328.

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 78, Creating Experiments on page 195, Acquiring Samples on page 299, and Analyzing, Saving, and Printing Data on page 325
2	Main menu and logged-in user name	Main Menu on page 62
3	Experiment buttons	Experiment Buttons on page 75
4	Instrument quick actions	Home Window Quick Access System Tools on page 77
5	Recent Experiment Sessions panel	Recent Experiment Sessions on page 76

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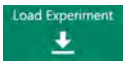
Experiment Buttons

Use the experiment buttons in the Home window to initiate experiments as described in Table 14.

Table 14. Home window experiment buttons and their functions

Button	Function
	Launches the Experiment Builder, which guides you through setup of fluorophores and corresponding detectors, samples, 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 (enabled PMTs) ■ Parameter names ■ Instrument settings ■ Plots ■ Compensation matrix **Note:** Sample positions are not imported, so you can import a run list configured for one method, such as tubes, and easily reconfigure it for another method, such as a plate.
	Appears if an experiment has been paused and the Home button has been clicked. Returns to the Acquisition 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.

Recent Experiment Sessions

Each time a user runs an experiment or edits an experiment, Everest Software creates and saves a separate session. The Recent Experiments window displays a list of recent experiment sessions that you can load into the Experiment Builder for modification. The number of files displayed is limited to the number specified in the Global System Preferences window. See Specifying UI Preferences on page 130.

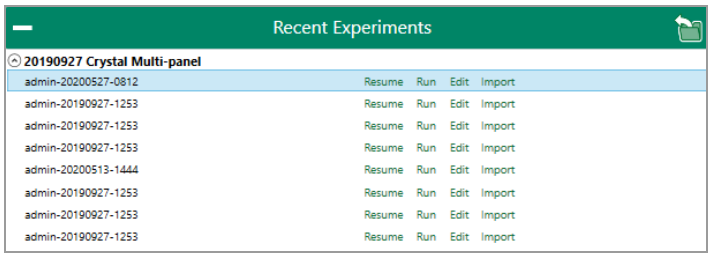


Table 15. Options for recent experiment sessions

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 to hide the experiment sessions. 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\<user name>). **Note:** You must know the folder path and the name of the RLST file that you seek.
Resume	Loads the run list, as it was last acquired, into the Acquisition 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. **Tip:** This is useful if acquisition was interrupted in the middle of the previous experiment.
Run	Loads a copy of the previously created run list into the Acquisition workspace.
Edit	Opens the run list in the Experiment Builder for editing.
Import	Copies fluorophores, parameter names, instrument settings, plots, and the compensation matrix from the run list into the new experiment. **Note:** The Import option does not copy sample positions.

Home Window Quick Access System Tools

After you log into Everest Software, the Home window includes a menu of system tools related to instrument operation and maintenance.

Tip: These tools are also available on the System toolbar, which is enabled after you have applied settings to an experiment.

Note: The decontamination (Decon) function is available only to administrators.

To show all tools

▶ Pause on the ellipsis to show all functional buttons.

For information on each function, see System Tools on page 69.

Experiment Builder

Clicking New Experiment starts the Experiment Builder, which is a flexible user interface for entering or modifying your experiment information. The Experiment Builder opens to the Media selection window, where you must select a plate or tube layout before proceeding. After selecting a media type, the Experiment Builder tab layout appears, where you can setup your experiment parameters.

Table 16. Experiment Builder tabs

Tab	Description
Fluorophores	Use the Fluorophores tab (default display) to select the fluorophores for your experiment.
Plate Setup	Use the Plate Setup tab to create a plate layout for your controls and samples. After selecting the Plate Setup tab, you can return to the Fluorophores tab after you identify at least one well.
Plots and Gates	Use the Plots and Gates tab to add and configure plots and regions before you apply the experiment and open the Acquisition window.. After selecting the Plots and Gates tab, you can return to the Plate Setup and Fluorophores tabs, and you can create a new panel.

You must follow the sequence described below to create a panel:

- You must select at least one fluorophore or detector before you can access the Plate Setup tab.
- You must select at least one well before you can access the Plots and Gates tab or return to the Fluorophores tab.
- Once you open the Plots and Gates tab, you can return to the Plate Setup and Fluorophores tabs, or you can start a new panel.

If a requirement is not met, Everest Software displays an advisory message when you select another tab.

For an illustration of the required task order, see Experiment Workflow on page 79.

For information on creating multiple panels, see Multipanel Experiments on page 114.

An Experiment pane also appears on the left, where you can name your experiment and panels, and navigate from one panel to another. For information, see Experiment Pane on page 81.

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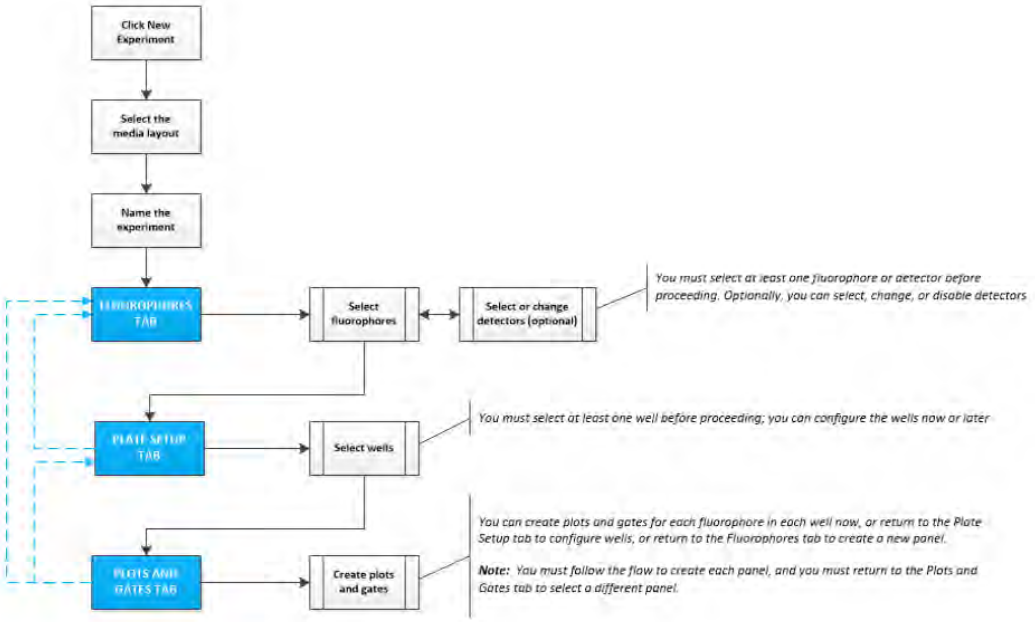
Experiment Workflow

The following workflow illustrates the flexibility of the Experiment Builder tab format.

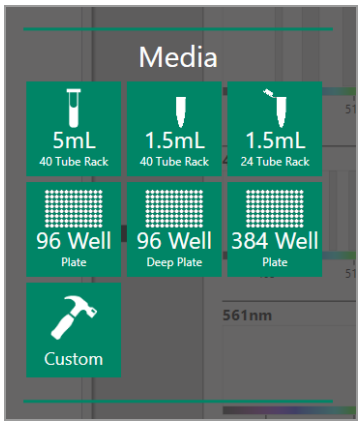


You can follow the logical path (solid lines) to create an experiment, or you can move back and forth between the tabs (dashed lines) to add, delete, or change information after the initial requirements have been met. The dashed lines show the points at which you can return to a previous tab.

- From the Fluorophores tab to Plate Setup tab:
  - You must select at least one fluorophore or detector.
- From the Plate Setup tab back to the Fluorophores tab or forward to Plots and Gates tab:
  - You must identify at least one well type.
- From the Plots and Gates tab, you can create plots and gates for the panel, or return to the Fluorophores or Plate Setup tabs without taking action. You can also create a new panel in the Experiment pane.

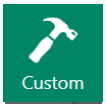
Media Selector

When you click New Experiment from the Home page, the Media selector appears and you are prompted to select a media layout.



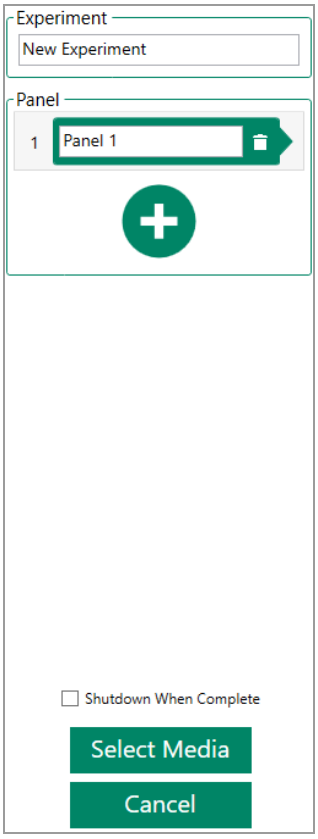
Important: You can change the media layout in the Experiment pane, but you must recreate your plate setup.

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 tubes)
	1.5 ml tubes (rack of up to 24 tubes)		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		

Experiment Pane

Use the Experiment pane to define elements of your experiment.



You can

- Name or rename your experiment.
- Create and name additional panels for wells in your plate or tube layout. You can create panels until the layout is full. For more information, see Multipanel Experiments on page 114. Panel 1 appears by default.

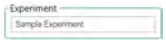
  Note: The + button is enabled after you complete the current workflow. See Experiment Workflow on page 79.
- Enter a unique name for each panel, or keep the default Panel <x> identifier.
- Click and drag to reorder panels.
- Instruct the instrument to shut down after the run is finished.
- Change the media type.

  Note: If you select a different media type, you must reconfigure your plate setup. See Changing the Media Type on page 205.
- Cancel the experiment setup.

Experiment Pane Fields and Buttons

Experiment Pane buttons are available in the Experiment pane and from each of the three tabs in the Experiment Builder.

Table 18. General Experiment Builder options

Button	Function
	Enter a name for your experiment.
	Adds a panel to the experiment, allowing you to configure your samples with different settings on the same plate
	Enter a panel name; a new field appears for each added panel. Pause on the number on the left to change the cursor and click and drag to reorder panels.
	Reopens the media selector, allowing you to change your media layout. **Important:** Switching to a different layout resets the entire plate setup.
	Cancels setup of this experiment and returns you to the Home window, where you can start a new experiment
	Applies the run list to the Acquisition workspace so that it can be run immediately
	Exports the run list to a file you can import into future experiments.
	From the Plots and Gates tab click this button to open the Instrument Settings Library, where you can import compatible compensation and cytometer settings into your experiment.

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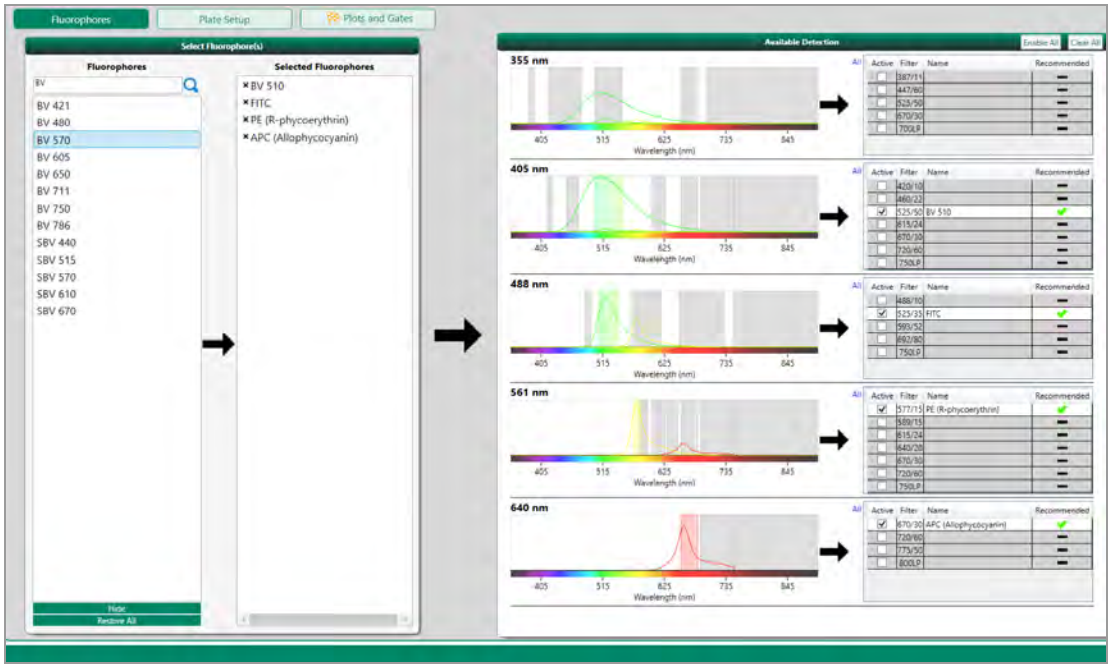
Fluorophores Tab

By default, the Fluorophores tab, where you select the fluorophores for your experiment, is the initial window display in the Experiment Builder after you select your media layout.

As shown in the following graphic, selecting a fluorophore automatically enables a corresponding detector based on the instrument's laser and optical filter configuration. Selected fluorophores are ordered by excitation emission range.

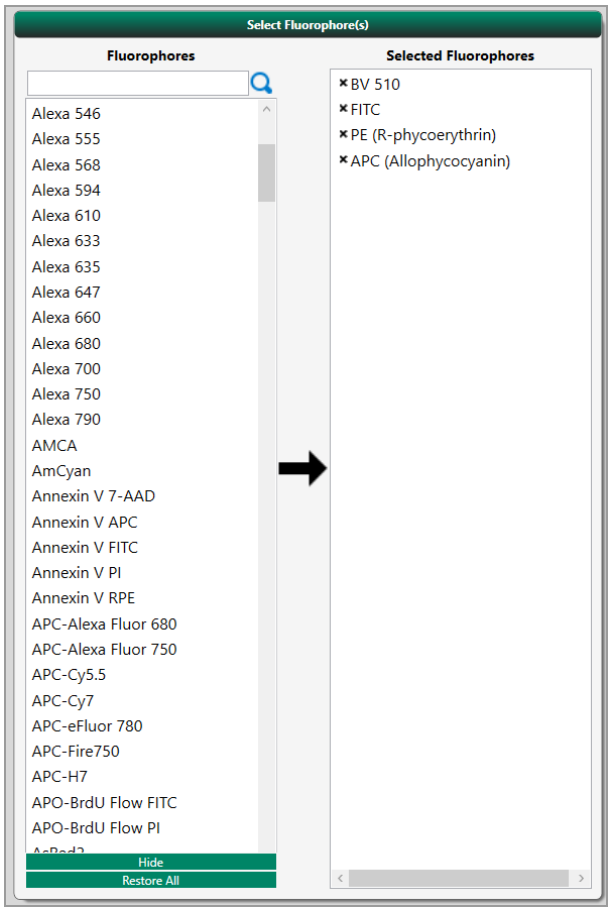


The Enable All and Clear All buttons are used to activate or deactivate all available detector, respectively. however, if the ZE5 Cell Analyzer is configured with a large number of PMTs, enabling all detectors can create large data files that can be difficult to handle when exporting to other software packages for analysis.

Important: Only experienced users should activate or deactivate individual default detectors.

Select Fluorophores Panel

Use the Select Fluorophores window to locate and select particular fluorophores.



You can scroll through the list or you can type the initial letters of a fluorophore name in the Search field, as shown below, to display all corresponding options. The list narrows to those fluorophores relevant to the search as you type more letters.

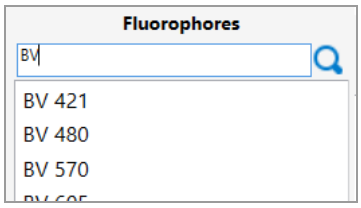


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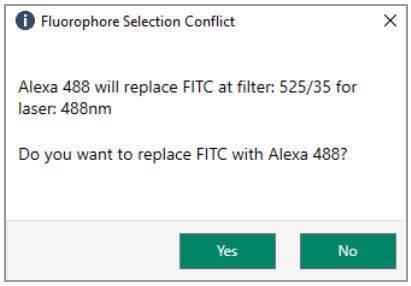
Table 19. Select Fluorophore(s) panel items and their functions

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

Conflicting Fluorophores

If you try to add a fluorophore that conflicts with one you have already selected, Everest Software displays an advisory message questioning whether you want to replace the existing fluorophore with the new fluorophore.

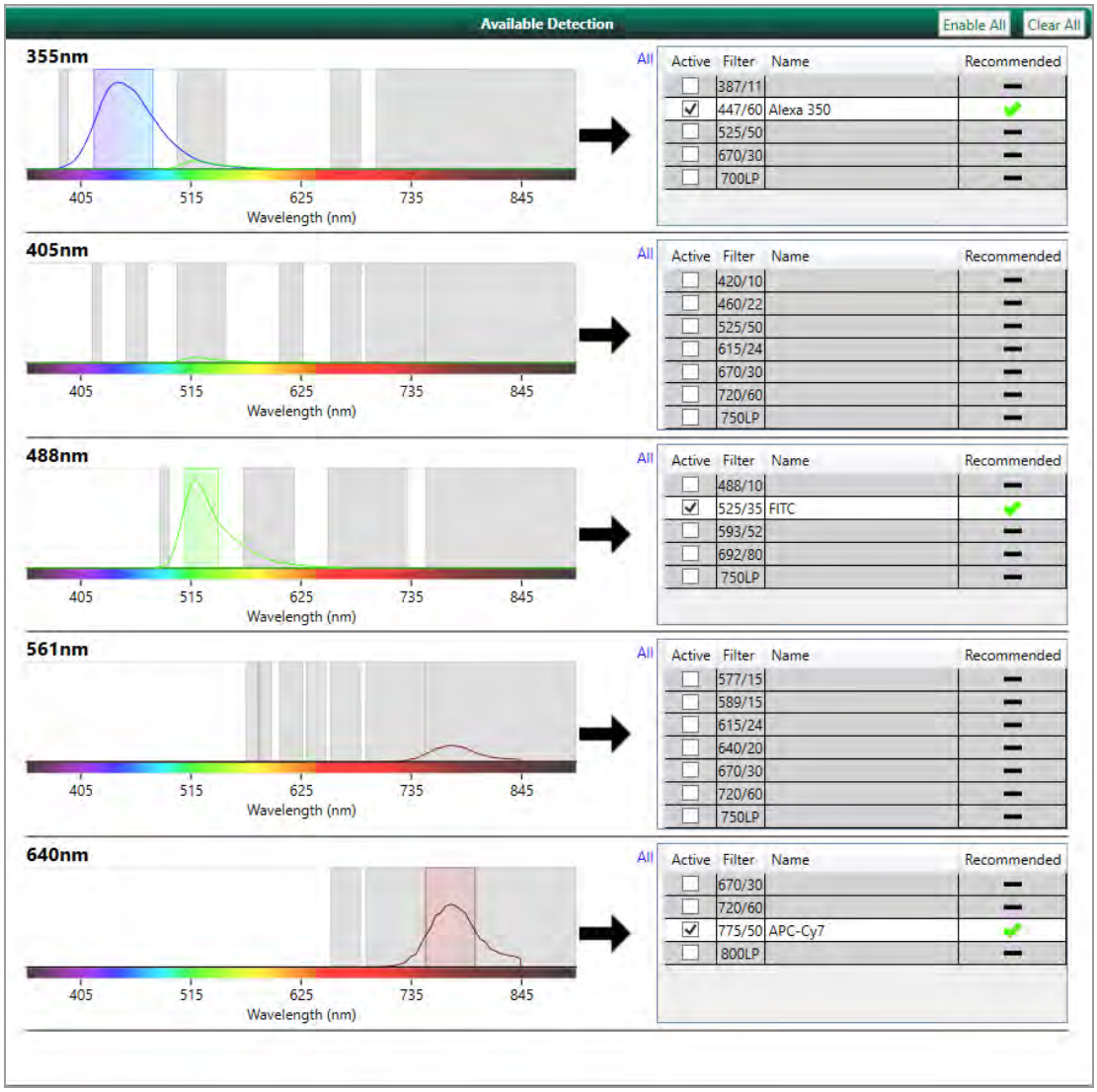


▶ Click Yes to accept the new fluorophore or No to keep the original fluorophore.

Available Detection Panel

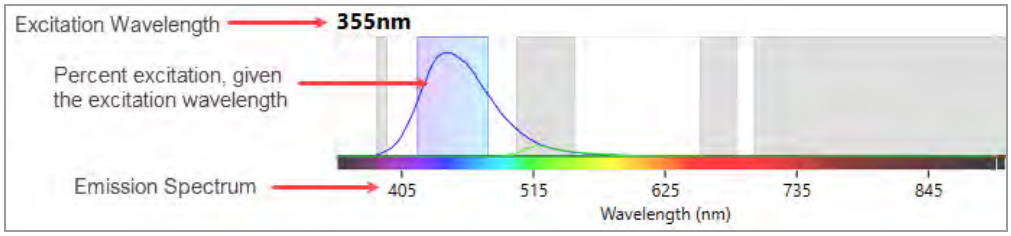
The Available Detection panel acts as a spectral layout that is specific to the local instrument configuration. Each laser shown on the left has a spectral graph associated with it. When you select a fluorophore, Everest Software automatically selects a corresponding detector to be used as an acquisition parameter. Changes must be made on this window; the checkboxes are disabled on the Plots and Gates and Acquisition windows.

Important: Bio-Rad recommends that only experienced users add or clear detectors.



…

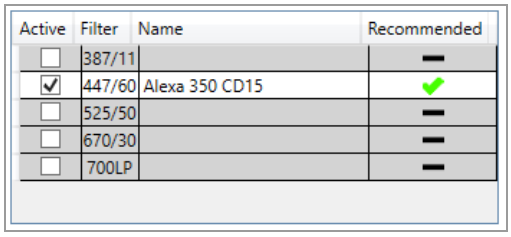
Because multiple dyes are excited with the same laser, spectra are overlaid on the same graph. When you select a fluorophore, its emission spectrum is shown in the graph associated with the laser that optimally excites it. Additionally, the gray regions in a graph represent the wavelengths allowed through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



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.

The software automatically populates the Name box with the fluorophore name. You can modify or overwrite the name in the Name column (for example, adding a cell marker as shown below), and you can also use the tabs in the Sample Naming pane on the Plate Setup tab for added flexibility. For information, see Sample Naming Panel on page 102.

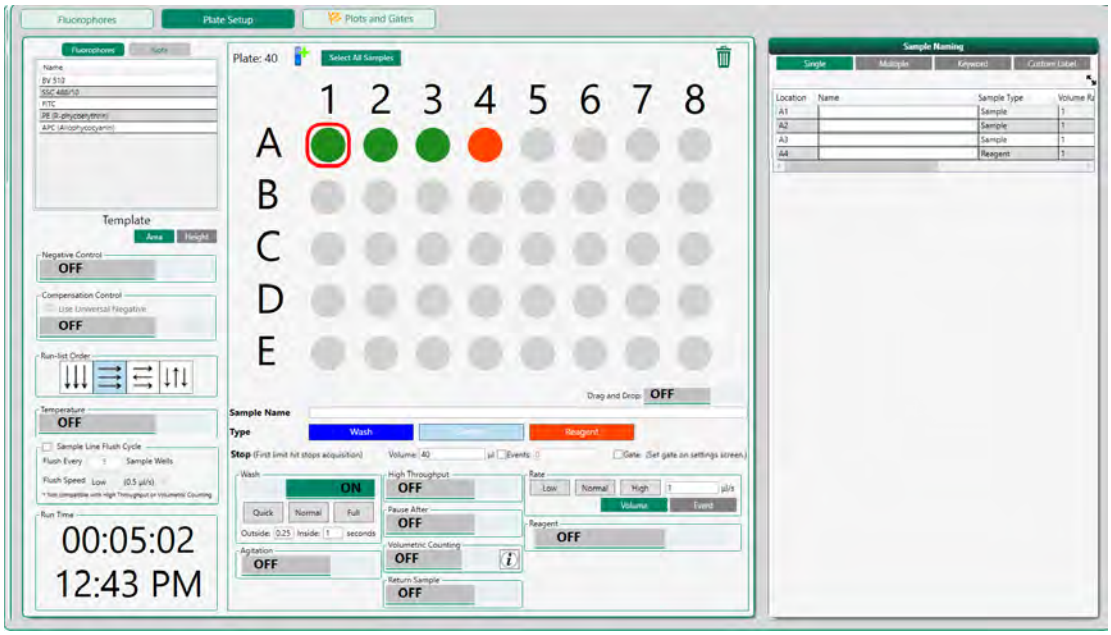
For multipanel experiments, the entry applies to the selected panel only.



Important: Each identifier you enter in the Name column must be unique. Everest Software does not accept duplicate names in the Available Detection Panel.

Plate Setup Tab

After you select your fluorophores you can select the Plate Setup tab, where you define the run list by specifying how samples are processed.



From the Plate Setup tab, you can

- View the selected fluorophores without returning to the Fluorophores window.
- From the panel on the left, you can choose the following settings for your plate:
  - Area or height pulse parameters
  - Negative and compensation controls

    Note: For multipanel experiments, these controls can be changed from panel to panel.
  - Run list order
  - Temperature
  - Sample line flushes

…

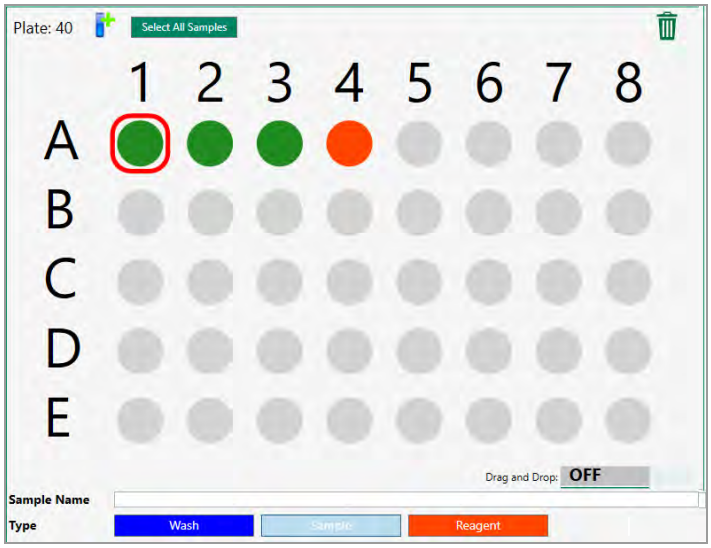
- Configure the following settings for selected wells:
  - Stop Limit
  - Wash
  - Agitation
  - High Throughput
  - Pause After
  - Volumetric Counting
  - Return Sample
  - Rate
  - Reagent

  Note: These settings are enabled after you identify the contents of at least one well in the panel.

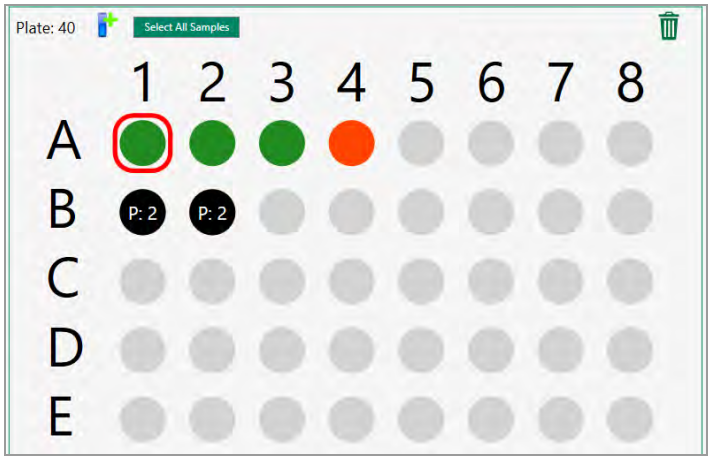
Media Layout

Use the media layout you selected to program each well or group of wells in the Plate Setup window with a set of run conditions. Selected wells are highlighted with a red border. Well types are identified by color; for more information, see Position Types on page 91.

You can also name each sample, and drag and drop samples to different wells.



Everest Software displays the plate in multipanel experiments as shown in the following graphic:



When you select a panel, Everest Software displays the active wells in color according to position type. The remaining panels are shown in black with the panel number displayed.

...

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 20. Position Type items and their functions

Button	Function	Plate map
Wash	Designates the position as wash, containing water or cleaning solution. No data acquisition occurs for this position type.	
Sample	Designates the position as a sample. Data acquisition occurs for this position type.	
Reagent	Designates the position as reagent that can be added to other wells/tubes. No data acquisition occurs for this position type.	
Not applicable	Compensation template wells are automatically added to the plate layout as setup wells when you enable negative control and/or compensation controls.	
Not applicable	Unassigned position.	

Position Images

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

Table 21. Images used on sample positions

Image	Description
	Appears on positions where agitation has been enabled.
	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.

Plate Control Settings

Controls that apply to the plate are located on the left side of the plate map.

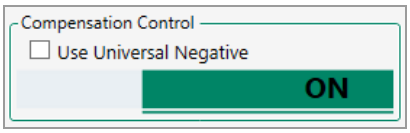
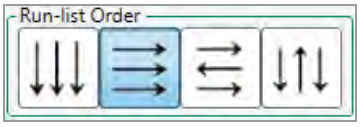
Notes: Negative and compensation controls can be applied to individual panels.

In the left panel, you can

- View the list of fluorophores on the Fluorophores tab
- Select the Note tab to enter pertinent notes about the experiment
- Select a pulse parameter for compensation (area or height),
- Designate control wells

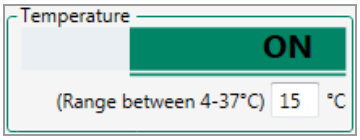
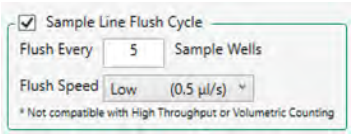
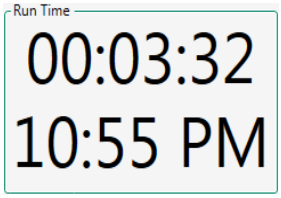
  Note: For multipanel plates, different controls can be applied to different panels.
- Change the run list order
- Prompt a flush of the sample line after a specified number of wells

Table 22. Plate Settings items and their functions

Item	Function
	Adds a negative control to the panel.
	Adds compensation controls to the panel. When the Use Universal Negative checkbox is checked, the data from the negative control sample is used as the negative population in each single color control, rather than a negative population that could be present in each single color control.
	You can select the route the sampler will take across the tube rack or plate:
	Runs each column in turn, starting with column 1.
	Runs each row in turn, starting with row A.

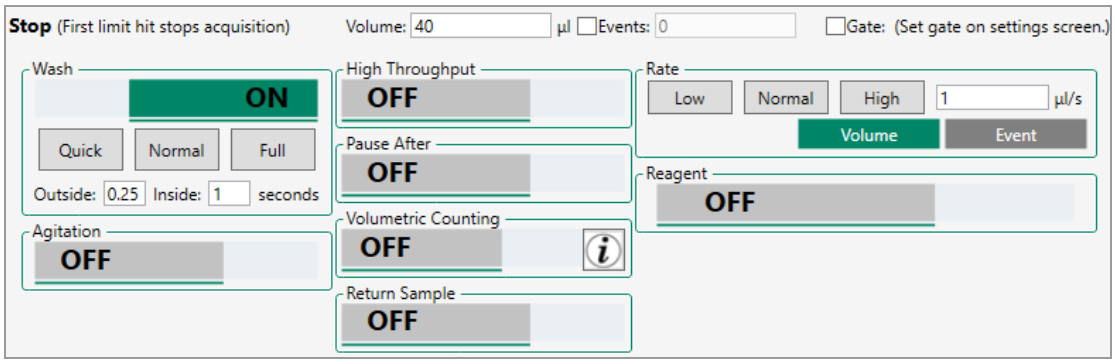
…

Table 22. Plate Settings items and their functions, continued

Item	Function
	Runs in serpentine fashion, row-by-row (A1 through A8, B8 through B1, C1 through C8, and so forth).
	Runs in serpentine fashion, column-by-column (A1 through E1, E2 through A2, A3 through E3, and so forth).
	When enabled, you can set the sample temperature from 4–37ºC, in 1º increments. The default is 15ºC. The actual temperature appears in the Acquisition workspace after the run list has been created.
	When enabled, you can configure incremental line flushes at a selected speed. The default is every 5 wells at low speed. **Important:** This function is not available if you selected High Throughput or Volumetric Counting.
	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. **Note:** These times are calculated using the settings (such as volume, target flow rate, washing, and agitation) currently programmed for the plate or tube rack. Event or gate limits are not considered, and maximum volume is always used for the calculations.

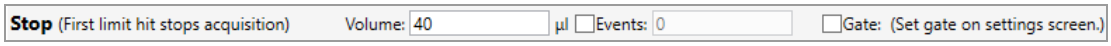
Position Control Settings

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:

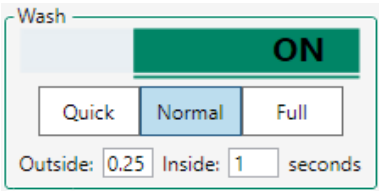
- 5 ml tubes: 4,000 µl
- 1.5 ml tubes: 1,200 µl
- 96 well plate: 200 µl
- 96-well deep-well plate: 1,000 µl
- 384-well plate: 100 µl

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

...

Wash Settings

Wash settings at normal speed are enabled automatically for active wells in your plate.



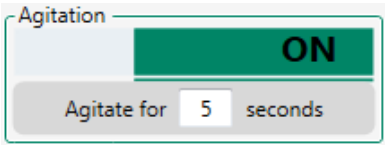
The wash controls allow you to specify both an outside and inside wash time. Bio-Rad strongly recommends that you leave it on for all positions, especially when using high-throughput sampling mode. Washing with sheath fluid occurs after the position's sample has been acquired. For information on configuring wash settings, see Configuring Wash Settings on page 225.

Important: If you are running the experiment in high-throughput mode, see High-Throughput Mode on page 98 for information on corresponding Wash settings.

Note: Post-workflow wash events automatically occur after stat tube experiments, QC events, and forced stops.

Agitation Settings

You can enable agitation for any position. The default and minimum time is 5 sec. The speed and radius of agitation automatically change with the sample media type.



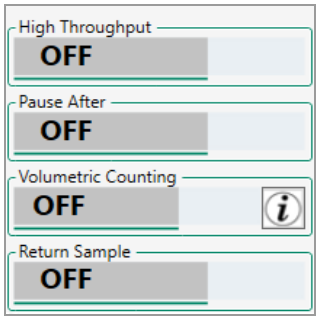
Tip: It might not be necessary to enable agitation for each well. For the highest throughput rate, it might be necessary to agitate only a few times within the plate.

Positions in the plate map in which agitation is enabled appear as

High Throughput, Pause After, Volumetric Counting, and Return Sample Settings

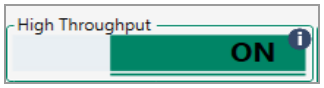
By default, all settings are turned off. You can apply any setting to individual sample wells on the plate.

Note: Pause After, Volumetric Counting, and Return Sample settings are available only when High Throughput mode is turned off.



High Throughput

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



For more information, see High-Throughput Mode on page 98.

Pause After

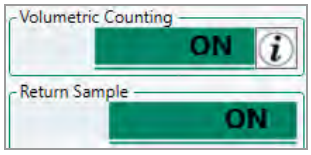
When Pause After is enabled, acquisition stops after the assigned sample position and the system waits for your input before continuing. This can be used to verify results achieved, or to set compensation values before moving on to the next series of samples.

Tip: You can enable 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.

Positions in the plate map in which Pause After is enabled appear as

…

Volumetric Counting



Important: Bio-Rad makes no claims of accuracy of the concentration statistic generated by the ZE5 Cell Analyzer. For information, click the *i* icon under Volumetric Counting.

When Volumetric Counting is enabled, the number of particles in a specified volume of each sample can be verified. In addition, the following events occur for each selected sample:

- The value for the Volume sample stop option changes from 40 µl to 10 µl.
- After sample boost, the software runs an additional 5 µl of sample to stabilize flow before acquisition begins.
- The probe remains in the sample while acquiring the target volume.
- Return Sample is automatically enabled. Excess sample in the line returns to the tube or well after target volume is acquired.

  Important: Users can set Return Sample to OFF if necessary. However, when using Volumetric Counting with Return Sample set to OFF, users will experience sample loss. If sample loss is a concern, run Volumetric Counting with Return Sample set to ON.

Return Sample

Unused sample in the sample line is returned to the tube or well after the instrument has collected the required number of events.

High-Throughput Mode

The ZE5 Cell Analyzer can run samples in standard mode or 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 enabled for selected positions, samples are aspirated continuously. Each sample is separated with a series of air and water boundaries, depending on the selected inside wash time.

High-throughput wash settings help to maintain a consistent separation between samples, and minimize carryover at the different flow rates. For best results, Bio-Rad recommends the following for high-throughput mode:

- Maximum speed of 2.5 µl/s.
- Minimum of 10 µl for the sample size.

Important: Recommended Wash settings for high-throughput mode are specified in Table 23 below.

Table 23. Flow Rate and Wash settings for high-throughput acquisition

Flow Rate	Wash
1.0 µl per sec	0.50s outside 2.00s inside
1.5 µl per sec	033s outside 1.67s inside
2.0 µl per sec	0.25s outside 1.25s inside
2.5 µl per sec	0.25s outside 1.00s inside

Note: The sample line flush cycle is not compatible with high-throughput mode.

High-throughput mode achieves 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, and 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.

You cannot enable high-throughput mode for setup (control) wells. You can use only sample volume limits (not event limits or gate limits) in high-throughput mode, and you can modify the volume rate (µl per second) but not the event rate (events per second). The Pause After, Volumetric Counting, and Return Sample options are available only in standard sampling mode.

Table 24. Differences between high-throughput and standard mode

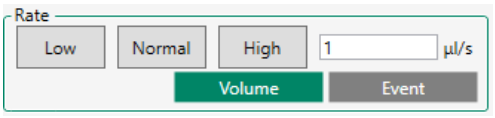
	High-throughput mode	Standard mode
Number of samples (tubes or wells) in sample line during run.	Multiple	One
Boost performed before sample acquisition?	No	Yes
Return Sample function allowed?	No	Yes
Pause (between-samples) function allowed?	No	Yes
Wash between samples allowed?	Yes	Yes
Agitation allowed?	Yes	Yes
Event limit allowed?	No	Yes
Gate limit allowed?	No	Yes
Volume limit allowed?	Yes	Yes
Can modify volume rate?	Yes	Yes
Can modify event rate?	No	Yes
Target flow rate can vary per sample?	No	Yes
Target flow rate range	0.5–2.5 µl/sec (30–150 µl/min)	0.1–3.5 µl/sec (6–210 µl/min)

For more information, see Selecting Standard or High-Throughput Acquisition on page 220, Pausing after a Tube or Well on page 221, and Returning Sample to a Tube or Well on page 223.

Flow Rate or Event Rate Settings

The rate controls allow you to specify a precise target flow rate in microliters per minute, or event rate in events per second, for each position, in µl/sec.

Flow Rate



Event Rate

These settings help to maintain a consistent separation between samples and minimize carryover at the different flow rates.

Note: In high-throughput mode, the Event Rate option is not available and all samples must be assigned the same flow rate. You can change the flow rate for all wells but not selected wells.

You can select from the following flow rate options:

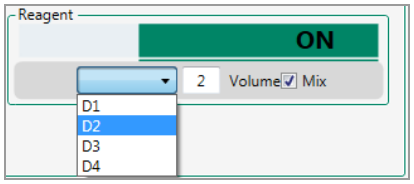
- **Low** — 0.5 µl/sec (30 µl/min)
- **Normal** — (default) 1.0 µl/sec (60 µl/min)
- **High** — 1.5 µl/sec (90 µl/min)

Bio-Rad recommends that you do not exceed 2.5 µl in high-throughput mode. For best results, a minimum of 10 µl is recommended for sample size. For information, see High-Throughput Mode on page 98.

During acquisition in standard mode, you can adjust the target flow rate manually (on the instrument) from 0.1 µl/sec up to 3.5 µl/sec. However, you cannot adjust the flow rate during acquisition in high-throughput mode.

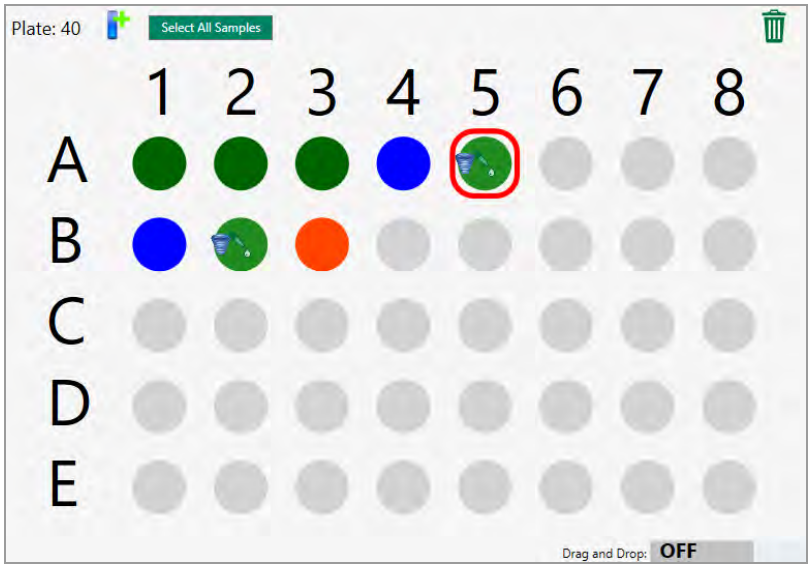
...

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 to the position to enhance mixing.

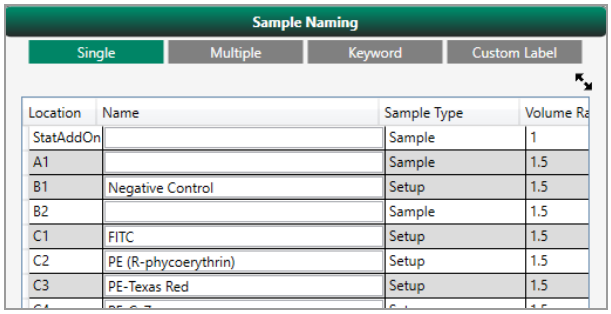
Positions in the plate map on which reagent will be added appear as



Sample Naming Panel

In Plate Setup, the Sample Naming pane appears on the right, and provides additional flexibility to associate sample and control wells with identifying information.

Note: If you click the double-arrows on the right to expand the grid, you can see all columns but cannot edit the Name column.



Each tab is briefly described in the following table.

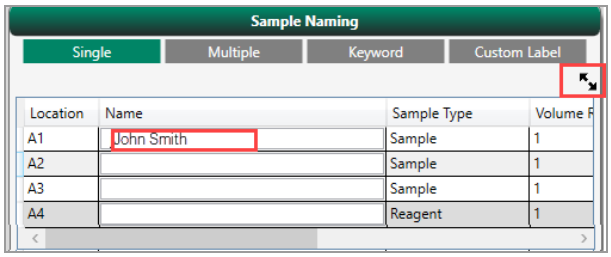
Table 25. Sample naming tabs

From this tab...	You can...
Single (selected by default)	Manually enter a unique identifier for each well
Multiple	Apply the same identifier to more than one well
Keyword	■ Import keywords and identifying values that are mapped to wells from a .csv file ■ Add keywords manually, and apply them to selected wells or all wells
Custom Label	■ Enter a label for each fluorophore (or detector) in the grid ■ Export a template of fluorophores and wells to a .csv file ■ Add labels to the template and then import the.labels in the csv template file into the grid

...

Single Tab

Under Single, you can enter names or identifiers for your wells in the Name column.

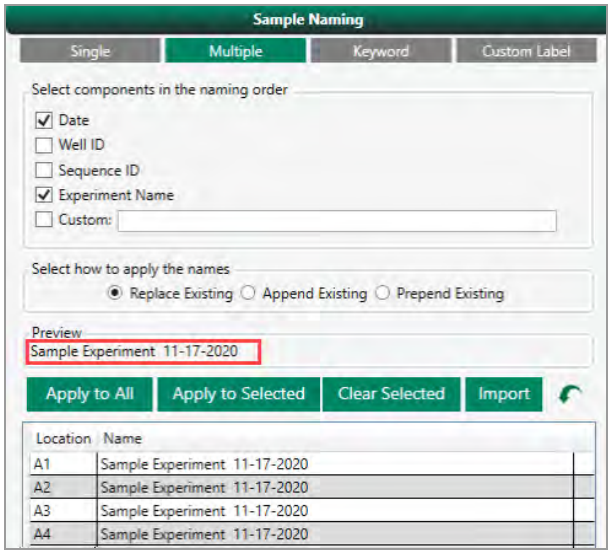


You can click the double arrow icon to expand the run list and display all columns. The expanded view shows the Run List Details table, which collects data in the following columns:

- Panel
- Name
- Volume Rate (µl)
- Max Volume (µl)
- Reagent
- Event Limit
- Agitage Time (sec)
- Probe Inside Wash Time (sec)
- Return Sample
- Location
- Sample Type
- Event Rate (e/s)
- Volume Limit (µl)
- Reagent Volume (µl)
- Gate LImit
- Probe Outside Wash Time (sec)
- High Throughput
- Has Gate LImit

Multiple Tab

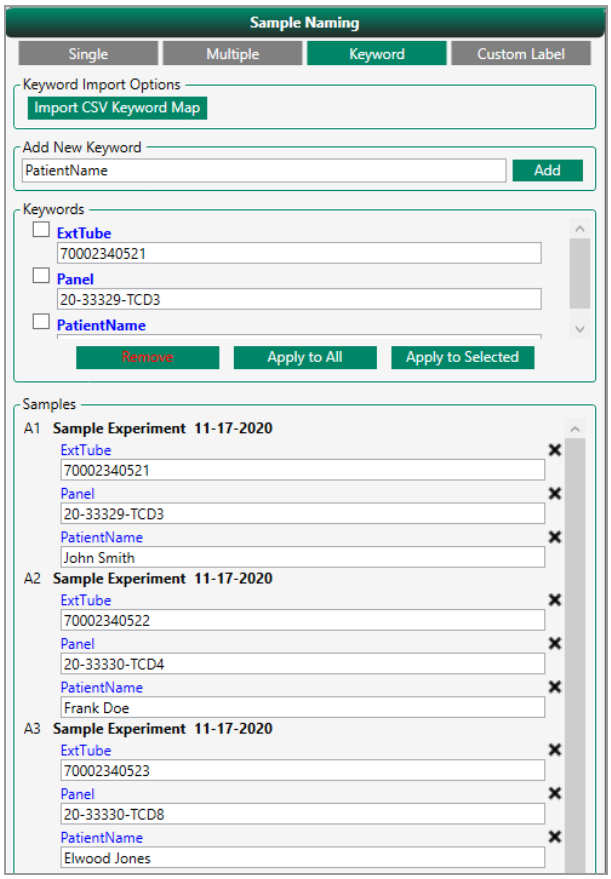
The Multiple tab facilitates naming multiple samples at a time using components including Date, Well ID, Sequence ID, Experiment Name, and Custom text. For more information, see Sample Naming—Labeling Positions Automatically on page 229.



...

Keyword Tab

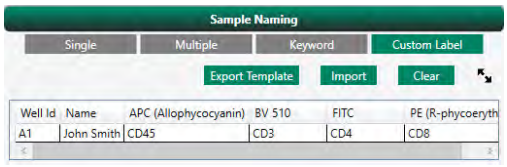
From the Keyword tab, you can create custom keyword/value pairs and associate them with sample positions. For example, keywords can identify a patient with a particular test panel. You can also import a set of keyword/value pairs from a .csv file. For more information, see Sample Naming—Setting Up Keywords on page 231.



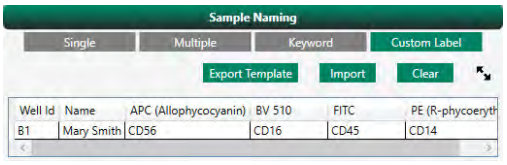
Custom Label Tab

Custom labels specify unique values to further identify your samples on your plots. Custom labels appear in separate views according to panel.

Panel 1



Panel 2



Note: If you entered names on the Single tab, the information also appears in the Name column of the Custom Label grid.

In the Custom Labels pane, you can

- For each panel, enter a label for each fluorophore in each well into a grid; panels appear in the expanded view.

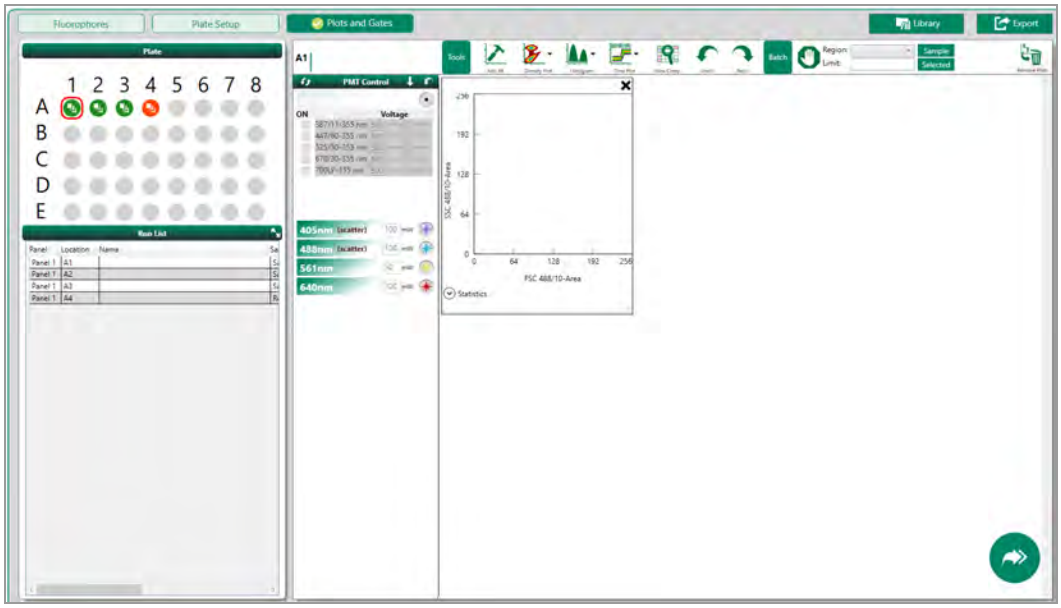
  Note: If you renamed the fluorophore in the Available Detection panel, the name appears as part of the heading, allowing you to enter other labels into the grid.

- Expand each grid to its full size to see wells associated with panels, fluorophores, and labels.
- Export the fluorophores for each panel to a template file for reuse
- Clear the existing entries

For information on adding custom labels, see Sample Naming—Creating Custom Labels on page 234.

Plots and Gates Tab

After you have configured your plate setup for the panel, you can select the Plots and Gates tab.



Important: The PMT Controls panel is disabled in the Plots and Gates tab. Adjusting instrument settings is now done exclusively from the Acquisition window after you apply the experiment.

From Plots and Gates, you can

- Create plots, regions, and gates for data collection and analysis in non-compensation wells
- For multipanel experiments, select a well in the plate map to enable the panel
- View the default plots for each well in the active panel

  Note: The FSC 488 plot appears by default. Plots also appear automatically if you enable Negative Control or Compensation Control.
- Create and assign additional regions for each well in the active panel
- Expand and view the run list, which summarizes the experiment settings by panel
- Return to the Fluorophores window or the Plate Setup window to make changes if necessary
- Add a new panel
- Import instrument settings from the Library
- Export the run list to a file

Plate Map

The plate map displays the layout of the plate before the run list has been applied.

Note: The plate map on the Plate Setup window displays different colors and symbols for each well position.

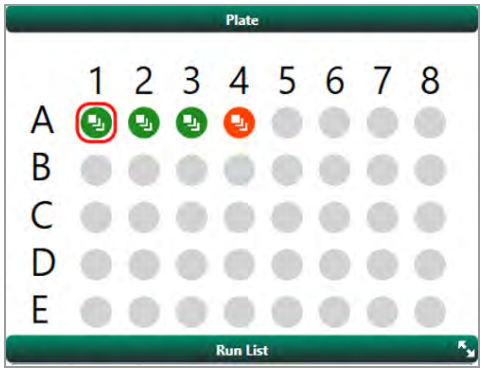


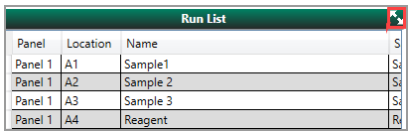
Table 26. Plate map symbols

Symbol	Description
	Selected position
	Setup position
	Sample position
	Reagent position
	Wash position
	Unassigned position

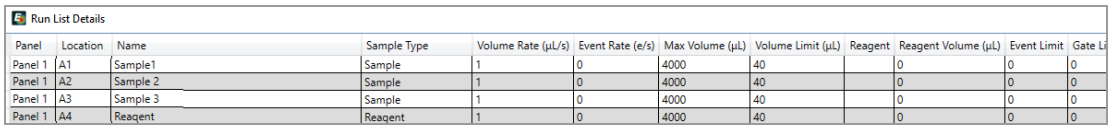
...

Run List

From the Plots and Gates window, you can expand the run list to view all of the setup information. Panels in the experiment are identified on the left.

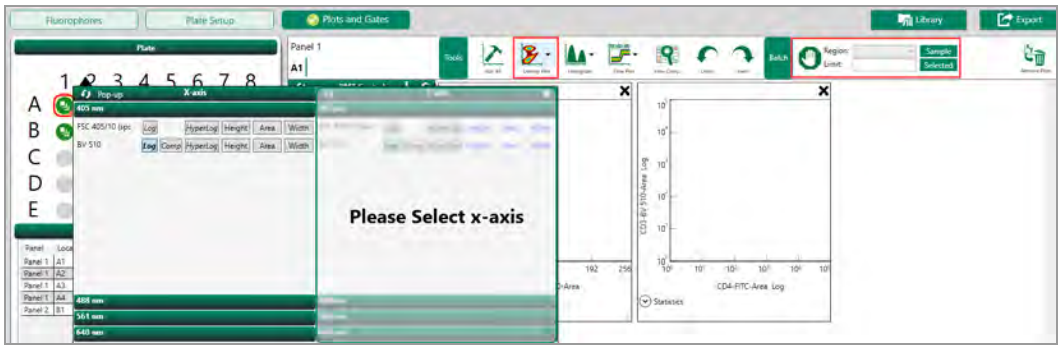


When you click the expand icon, the grid appears.



Creating Plots, Regions, and Limits

You can create initial plots from the Plots and Gates window, and define your regions and limits. For information, see Setting Up Plots and Gates on page 238.



Instrument Settings Library

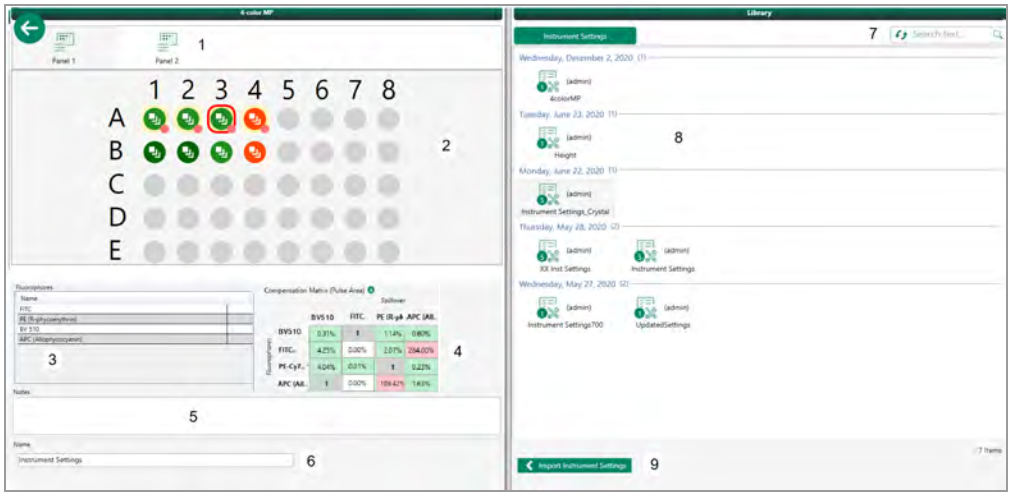
Everest Software features an instrument settings library, in which you can save and store instrument settings from a current experiment and then import them into future experiments. Each settings file represents your configuration for an individual panel.

You can open the Library from the following windows:

- Plots and Gates, where you can import settings into individual panels
- Acquisition, where you can manually modify and save your configurations

  Note: The Library icon on the Acquisition window is grayed out if the instrument is busy.

The plate layout, selected fluorophores, compensation matrix, and pertinent notes appear on the left and files in the Library appear on the right. Files are sorted by most recent date by default.



LEGEND

1	Icons representing each panel in your experiment
2	Plate layout, with active panel highlighted
3	Fluorophores list for the selected panel
4	Compensation matrix for the selected panel
5	Notes field, in which you can add notes for other users **Important:** You can add or edit notes only in the Acquisition window.
6	Name you specify for the instrument settings file
7	Search field, to filter files to a smaller group
8	Settings files, ordered by date of most recently used
9	Button to save or import a settings file: ■ From the Plots and Gates window, you can import settings from a settings file into one or more panels in your experiment. ■ From the Acquisition window, you can save the settings you have defined in the Experiment Builder.

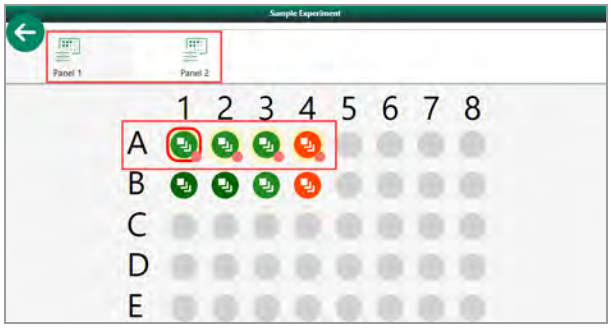
...

You can save and reuse the following settings:

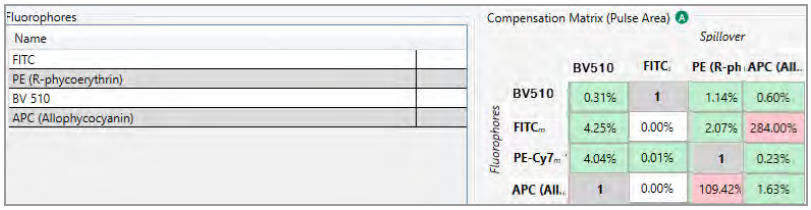
- Fluorophores
- PMT detectors
- Detector voltages
- Lasers
- Trigger and threshold values
- Compensation matrices

When you open the Library, an icon for each panel in your experiment appears above the plate map. The row corresponding to the selected panel is highlighted in yellow in the plate map, with a red dot in the lower-right corner.

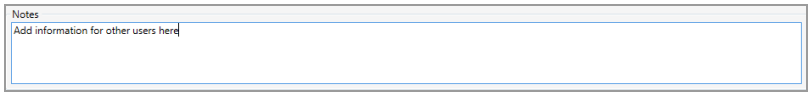
Note: Except for the Import or Save buttons, the display is identical from either access location.



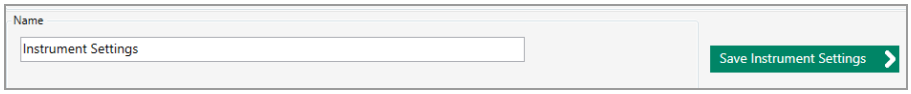
The fluorophores and compensation matrix for the selected panel appear below the plate map.



Notes you add form the Acquisition window before saving the file appear in the Notes field.

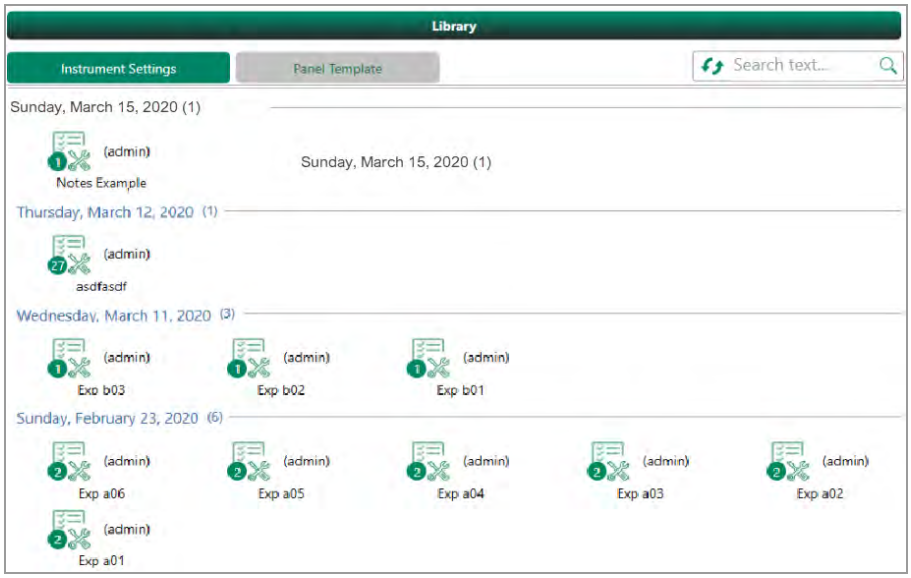


When you save the file, you must enter a new file name in the Name field to overwrite the default Instrument Settings name.



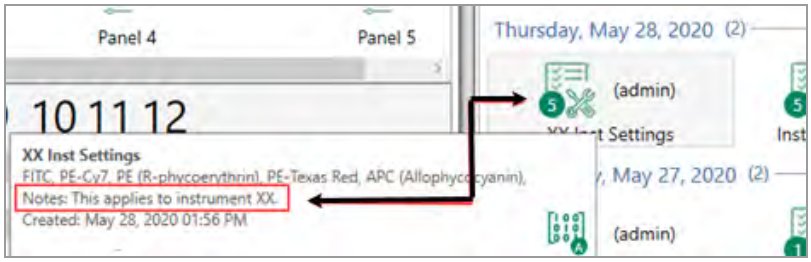
Note: Each file name in the Library must be unique. If you enter a duplicate name in the field, an error message appears and you must enter a new name.

By default, settings files in the Library are sorted by most recently used, but you can use the Search field to narrow the search and display a filtered set of files. You can search by owner name, experiment name, or content in the Notes field.



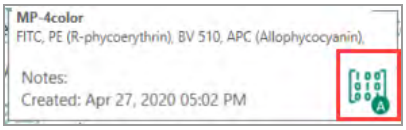
...

Before you import a file, you can pause on the file to view a tooltip containing the fluorophores and any pertinent notes. Any user can add or edit notes from the Acquisition window.



The tooltip also contains a compensation matrix icon, as follows:

- If the file was saved with compensation values, the icon is green. If the values were measured by area, an A appears in the icon; if measure by height, an H appears.



- If the file was saved without compensation values, the icon is grayed out.

Important: Files that you import must match the current experiment configuration and instrument settings. If they do not, an error message appears.

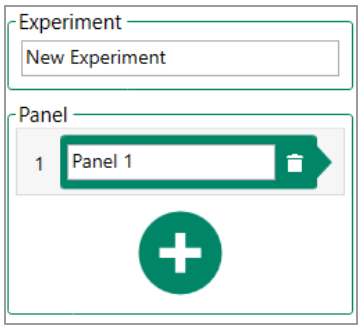
For information on saving an instrument settings file, see Saving Instrument Settings on page 310. For information on importing a settings file, see Importing Instrument Settings on page 240.

Multipanel Experiments

In Everest Software you can configure multiple panels for your controls and samples in a single plate or tube layout.

After you start an experiment and select a media layout, the Experiment Builder opens to the Fluorophores window by default. The Experiment pane, where you can rename your experiment and create new panels, appears on the left. The application creates the initial panel by default.

The + icon is disabled until you meet the sequential minimum requirements on each tab for the current panel. For more information, see Experiment Workflow on page 79. From the Plots and Gates tab in the Experiment Builder, Everest Software enables the + icon and you can create a new panel.



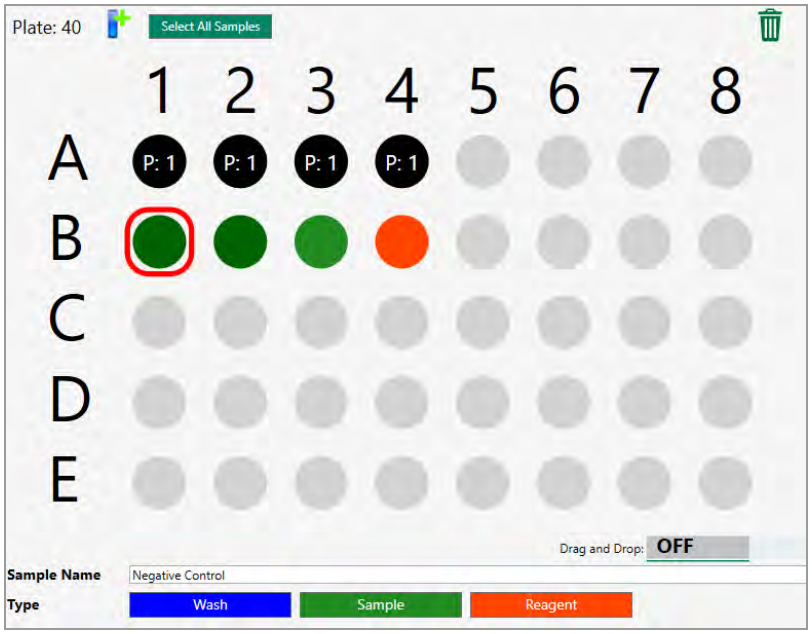
You can assign different fluorophores and detectors to each panel, and each group of positions that you define as a panel are uniquely programmed with settings and controls for those positions only.

Settings include

- Active fluorophores
- Parameter names (default and modified) in the Available Detection Panel
- Sampling settings, such as target flow rate or target event rate, washing, and agitation
- PMT voltages
- Triggers
- Thresholds

You can import different cytometer and compensation settings into each panel and you can add panels until your plate layout is full.

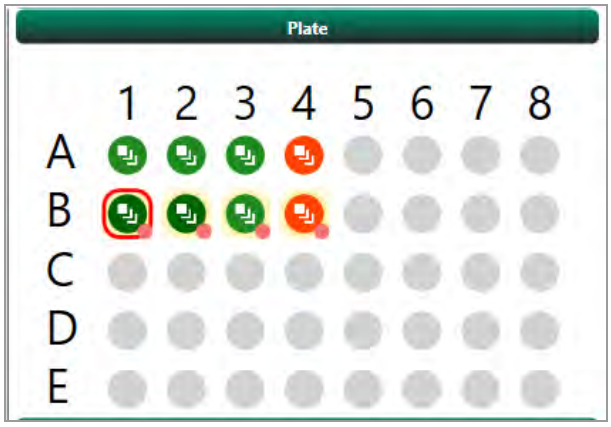
By default in the Plate Setup tab plate layout, the selected panel appears in color and the other panels are black and identified by panel number.



Panel identifiers are also reflected in the leftmost column of the run list. The default identifier is Panel <x>, but you can enter unique panel names in the panel fields in the Experiment pane and the names appear in the Panel column.

After you have created your panels and applied the experiment, you can make instrument adjustments on the Acquisition window and then save the settings for one or more panels to the Instrument Settings Library for reuse in other panels. For information, see Instrument Settings Library on page 109.

The Plots and Gates window also displays the plate layout as shown in the following graphic:



Note: Everest Software also displays this plate view in the Acquisition window after you apply the experiment.

The selected panel is identified by a highlighted background and a red dot, and is also identified above the PMT Control pane.

When you open the completed experiment in the Analysis window, the panel associated with the selected well is identified below the plate map.

In Analysis, you can

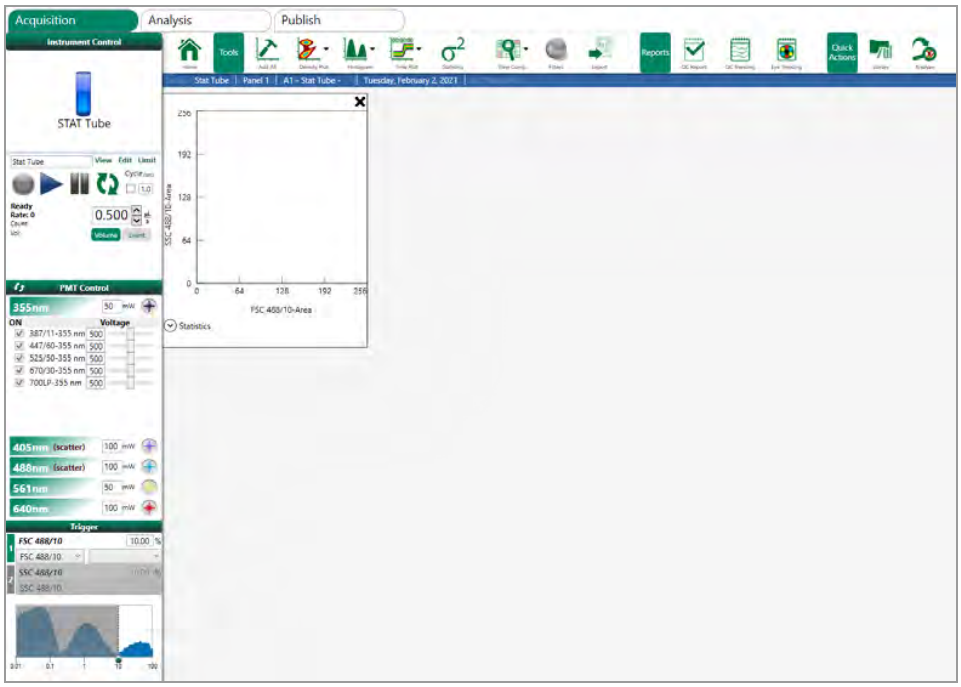
- View plots, histograms, and statistics
- Work with compensation data
- Export third-party software and run list files
- Export statistics and gates

For information, see Analyzing, Saving, and Printing Data on page 325.

Quick (Stat Tube) Experiments

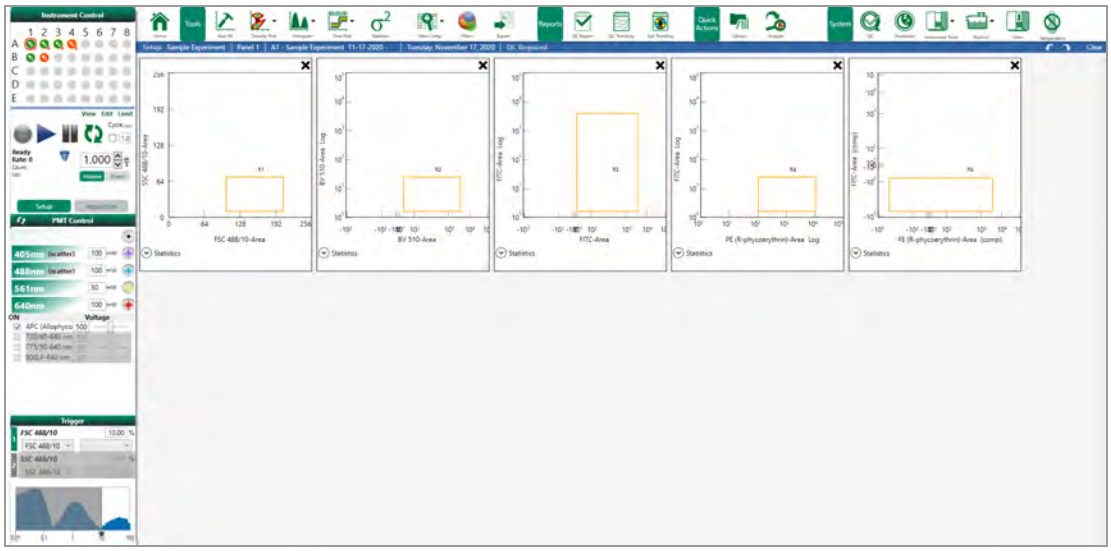
You can run an individual stat tube, where you bypass the Experiment Builder and go immediately to the Acquisition page, or you can add a stat tube to your plate layout in a larger experiment.

For information, see Running an Individual Stat Tube on page 295 and Adding a Stat Tube to a Panel in the Plate Layout on page 297.



Acquisition Workspace

After applying an experiment, the Acquisition workspace appears. By default, the experiment is in Setup mode.



From the Acquisition window you can perform the following tasks in Setup mode:

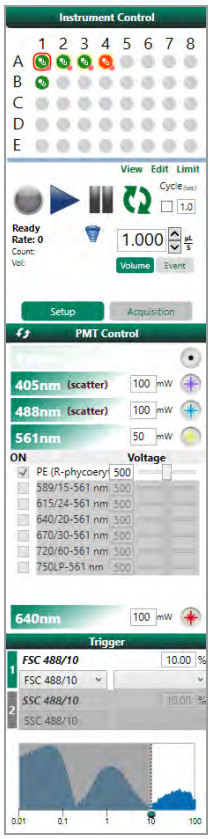
- Set PMT voltages, if known from a previous experiment or specifically for this particular sample type

  Important: You can adjust voltages in the PMT Control pane, but enabling or disabling PMTs can be done only from the Fluorophores tab.

- Configure initial trigger and threshold values, if known from a previous experiment or specifically for this particular sample type
- Create or adjust plots and regions for sample wells
- Save settings to a file in the Instrument Settings Library. For information, see Saving Instrument Settings on page 310.

...

Instrument Control and PMT Control



The controls appear in the left panel of the Acquisition workspace.

- In the Instrument Control section, you can initially run each well in Setup mode to make adjustments, and then in Acquisition mode.
- in the PMT Control section, you can adjust voltages for the specified fluorophores and lasers.

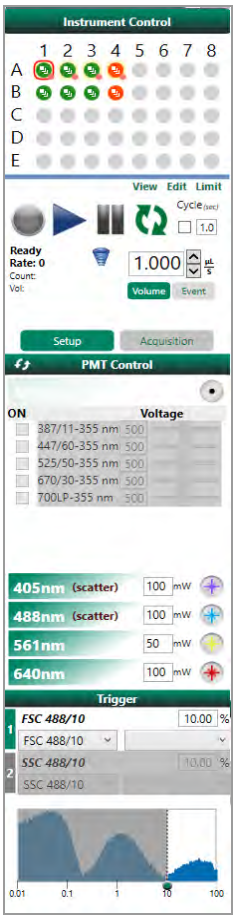
  Important: You can select additional fluorophores or filters from the Fluorophores tab only.
- In the Triggers section, you can specify the parameter or parameters to alert the system to the presence of an event over the threshold.

Note: For more information, see PMT and Laser Controls on page 306.

Setup Mode

Setup mode has two primary uses. You can do the following:

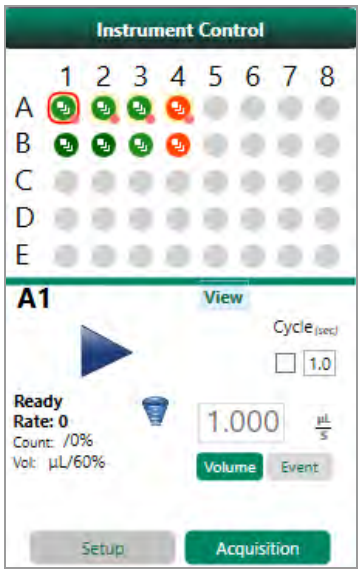
- Use the tool in the toolbar to optimize settings and set compensation on a single-sample basis. When you use Setup mode in this way, sampling does not stop until you manually stop the run.
- Use the Record function to record data files from samples; sampling stops when the preset limit is reached. This is useful if you need to acquire and save data from samples on a single-tube basis. These data are saved to an FCS file, which you can later open and review. You can use the Record function for some or all of the samples in the experiment.
- Set primary and optional secondary triggers.



For more information, see Acquiring Initial Sample in Setup Mode on page 302.

Acquisition Mode

Acquisition mode measures and records data from samples following the order specified in the Experiment Builder.

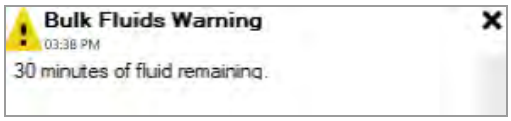


This mode is typically used after optimizing settings in Setup mode. After sample acquisition has been initiated in Acquisition mode, the ZE5 Cell Analyzer proceeds to each position on the rack or plate, starts and stops sampling as specified in the run list, and records data files. Acquisition mode requires minimal user intervention after acquisition has begun.

For more information, see Acquisition Mode Controls on page 311 and Running Samples in Acquisition Mode on page 312.

Notifications and System Logs

Everest Software displays error, warning, and information notifications in the lower right area of the window after you log in, and also during experiment setup and operations.



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 Acquisition workspace, see Status Bar on page 71.

Warning and information notifications close automatically after 30 sec. Error notifications persist until you close them.

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 Main menu to extract detailed system log information to a zip file. For information about log files, see Exporting and Viewing Log Files on page 403.

Chapter 5 Configuring the System

Before the ZE5 Cell Analyzer is used for acquisition, an administrator must configure the system 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 replicate the physical filter changes
- Reverting the optical filter configuration to factory settings

  Tip: Both administrators and nonadministrators can change the physical optical filters.

Setting Preferences

Administrators can set the following global preferences for the ZE5 Cell Analyzer and Everest Software:

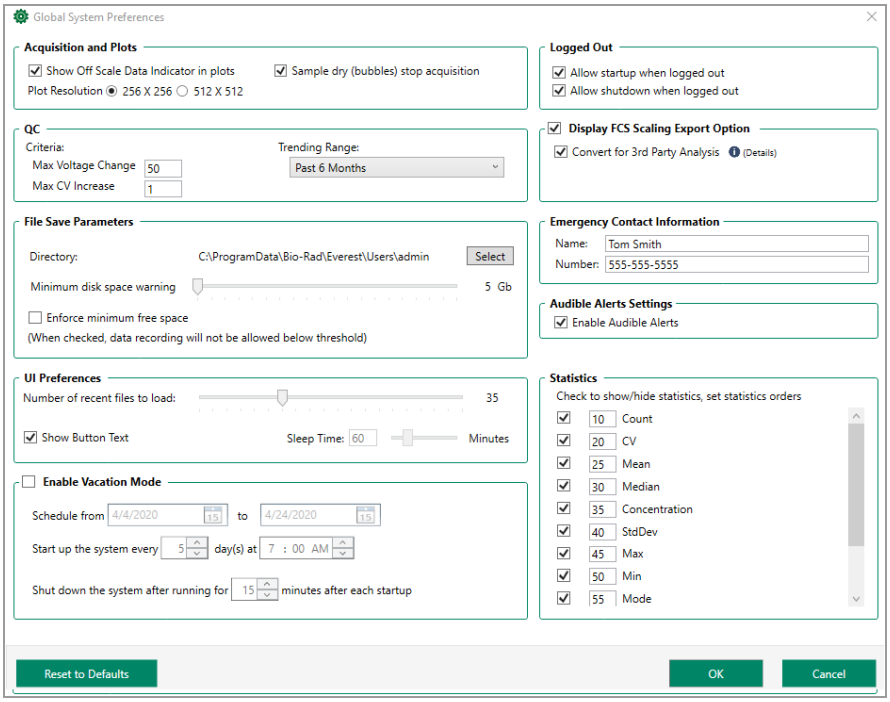
- Set data plot resolution
- Display or hide off scale data indicator in plots
- Specify that acquisition stops if sample runs out
- Set default QC criteria and trending range
- Set a default directory for user experiment data

  Note: Data folder locations specified for individual user accounts override this setting.
- Configure and enforce a warning regarding low disk space
- Specify number of experiment sessions to load
- Display or hide text below icons in the software interface
- Enable vacation mode
- Set permissions for startup and shutdown when a user is logged out

- Enable conversion of FCS files for analysis in third-party software
- Specify emergency contact information
- Specify how plot statistics are displayed
- Enable audible alerts when the instrument needs attention
- Reset the system to default settings

To access global preferences

▶ Click ☰ to open the main menu, and then click Preferences.



- Preferences that are 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.

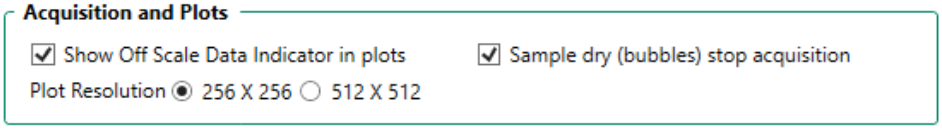
To avoid saving any unneeded changes made to the global preferences

▶ Click Cancel.

Setting Plot Display Defaults and Stopping Acquisition

Everest Software administrators can set preferences to

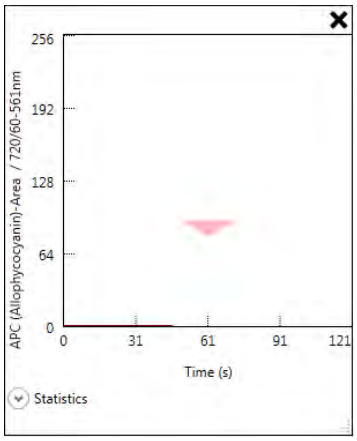
- Show a plot indicator for off-scale data
- Stop acquisition if Everest Software recognizes a dry sample
- Display plots at a pre-defined resolution



To set plot display defaults

1. Click ☰ to open the main menu, and then select Preferences.
2. In the Acquisition and Plots section:
   a. Select the Show Off Scale Data Indicator checkbox to display indicators when events fall on or below a plot axis.

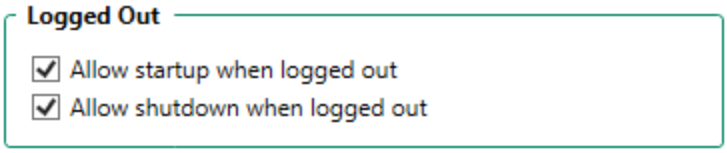
      Note: Such off-scale events, as shown in the next figure, might indicate the need to adjust instrument settings.



   b. Select a resolution setting for new plots.
   c. Select the Sample dry checkbox if you want to stop acquisition when bubbles occur, indicating that sample has run out.
3. Click OK to save the changes and close the Global System Preferences dialog.

Specifying Logged Out Settings

Everest Software administrators can set preferences to specify the actions permitted when no user is logged in to the system.

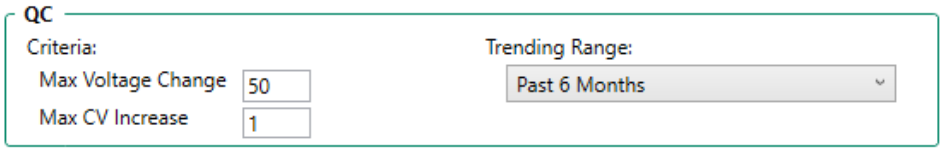


To specify logged out settings

1. Click ☰ to open the main menu, and then click Preferences.
2. In the Logged Out section, select the respective checkboxes to permit startup or shutdown when no user is logged into the system.
3. Click OK to save the changes and close the Global System Preferences dialog.

Editing QC Criteria and Trending Range

Administrators can set quality control criteria and a trending range timeframe.



- **Criteria** — criteria used to determine whether the instrument passes or fails quality control (QC). Changes to these criteria apply globally to the system.
  - **Max Voltage Change** — the maximum change in PMT voltage (measured from the baseline for each channel) required to bring the population to channel 128. The change in voltage required to place the population in the center of the histogram must be less than this value.
  - **Max CV Increase** — the maximum increase in the calculated coefficient of variation (CV) (measured from baseline for each parameter) must be less than this value.

    Caution: Adjusting QC criteria might affect overall system performance. Only experienced users who can evaluate potential effects on system performance should edit QC criteria.
- **Trending Range** — time frame to display in the QC Trends report, in increments of one, three, six, and nine months, and one full year.

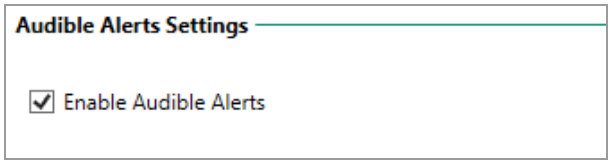
...

To edit the QC settings

1. Click ☰ to open the main menu, and then click Preferences.
2. In the QC section, adjust one or both of the following:
   - Under Criteria, adjust the values for QC Max Voltage Change and QC Max CV Increase.
   - Under Trending Range, click the drop-down arrow and select an interval.
3. Click OK to save the changes and close the Global System Preferences dialog.

Enabling Audible Alerts

Everest Software administrators can enable audible alerts in the Global Preferences dialog box.



When enabled, Everest Software prompts an alarm whenever the instrument stops automatically. This includes the following:

- Ordinary completion of experiments
- Run list paused or stopped due to error
- Failure to complete startup
- Failure to complete QC due to error

The alert consists of 5 beeps, and repeats every 3 seconds until you manually stop it in Everest Software. For more information, see Responding to Audible Alerts on page 188.

The alert does not sound or appear if you manually stop the instrument.

To enable audible alerts

1. Click the menu icon in the upper-right corner and select Preferences.
2. Under Audible Alert Settings, select the Enable Audible Alerts checkbox.
3. Click OK to save the setting.

Tip: You can adjust the alarm volume using the Windows 10 settings for system sound controls.

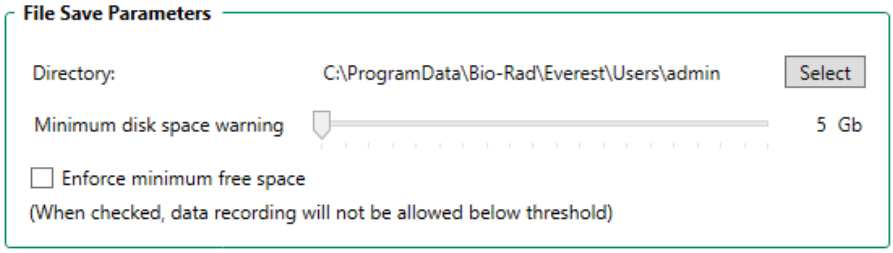
Setting File Save Parameters

Everest Software administrators can set preferences to

- Automatically save each FCS file to a default folder location for all users, after acquisition is complete or when acquisition is stopped by a user

  Tip: Administrators can also set a different file destination for a specific user, and the user setting takes precedence over the default save location. For more information, see Creating a New User on page 135 and Editing User Information on page 138.

- Specify whether users receive warning or error messages when they try to save files that exceed a minimum disk space threshold



To set file save parameters

1. Click ☰ to open the main menu, and then click Preferences.
2. In the File Save Parameters section, indicate a directory path:
   a. Click Select.
   b. Navigate to the target location for saving FCS files.
   c. Click OK.

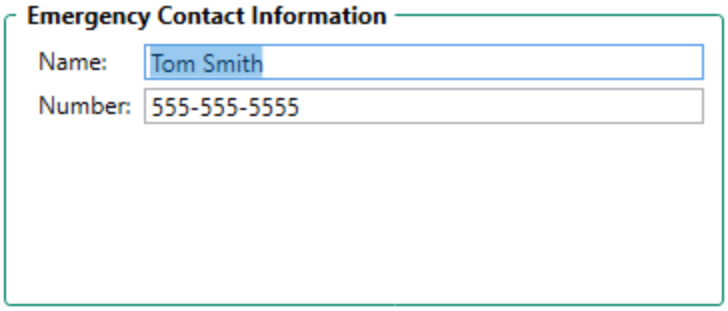
   The default file save location appears in the Directory Path.
3. Use the slider to specify the threshold for the Minimum disk space warning.

   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 changes and close the Global System Preferences dialog.

...

Setting Emergency Contact Information

Everest Software administrators can specify an emergency contact who can be contacted if issues arise with Everest Software or the ZE5 Cell Analyzer.



To set emergency contact information

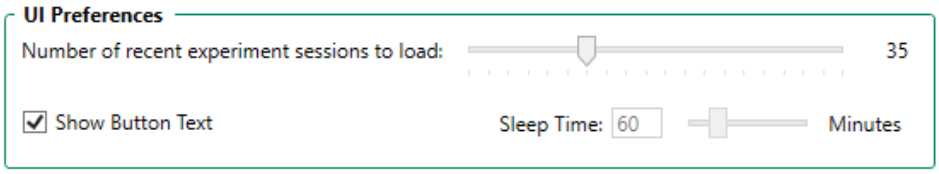
1. Click ☰ to open the main menu, and then click Preferences.
2. In the Emergency Contact Information section, enter a name and phone number for your emergency contact person.
3. Click OK to save the changes and close the Global System Preferences dialog.

Specifying UI Preferences

Everest Software administrators can set user interface (UI) preferences to

- Load a preset number of experiments in the Recent Experiments pane
- Show text identifying each toolbar button

Note: The Sleep Time function is not enabled in this version of Everest Software.



To specify UI preferences

1. Click ☰ to open the main menu, and then click Preferences.
2. In the UI Preferences section, use the slider to select the number of recent experiment sessions to load.

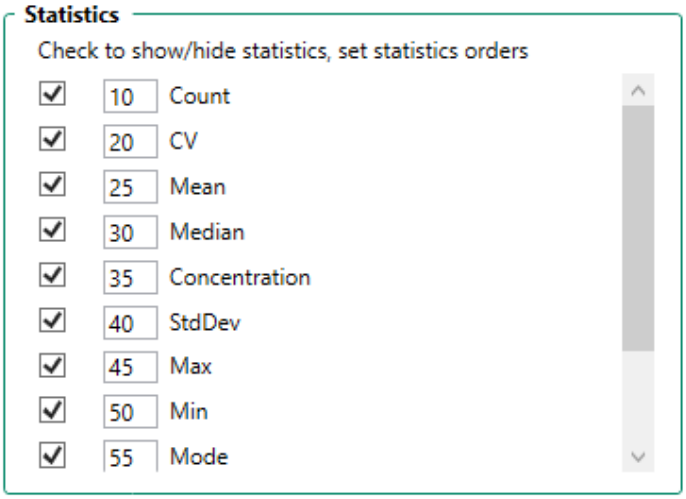
   If you select a very high number, it could take a while to fully load the list of recent experiment sessions.

   Tip: To display or hide all sessions for an experiment in the Recent Experiments list, click the arrow next to the experiment name in the Home window.

3. Select the Show Button Text checkbox to display the text for each toolbar button.
4. Click OK to save the changes and close the Global System Preferences dialog.

Specifying Statistics Preferences

Everest Software administrators can choose the statistics that appear in each plot and specify how the statistics columns are ordered in the table.



To specify statistics preferences

1. Click ☰ to open the main menu, and then click Preferences.
2. In the Statistics section, 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. The selected statistics appear in ascending order (those with higher numbers appear to the right of those with lower numbers).
4. Click OK to save the changes and close the Global System Preferences dialog.

Setting Up Vacation Mode

To maintain optimal system performance, Bio-Rad recommends that you run the ZE5 Cell Analyzer regularly, without long periods of rest. If your instrument will not be in use for up to 30 days, users with administrative access can enable Vacation Mode to schedule the automatic startup, idle period, and automatic shutdown processes. During the vacation period, the startup, idling, and shutdown processes occur at the specified times and intervals.

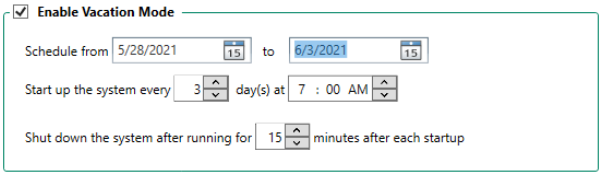
If your instrument will be unattended for up to a month, Bio-Rad recommends enabling Vacation Mode in Everest Software. Using Vacation Mode, you can schedule automatic startups and shutdowns on a defined schedule without an operator present in the lab. Upon startup, the instrument idles for the specified period of time, running sheath liquid as a maintenance process, before shutting down.

Note: If your instrument will be unattended for longer than a month, use the process defined for long-term storage.

Important: Ensure that bulk fluidics bottles contain enough fluids and space to support the number of runs occurring during the vacation period. Bio-Rad recommends a frequency of at least once per week, for 15 minutes of idling time. If you adhere to this recommendation, with fully filled DI water tanks and empty waste tanks, you can expect approximately 22 repeats of startup, idling, and shutdown.

To set up vacation mode

1. Log into Everest Software using administrator credentials.
2. Click ☰ in the upper-right corner to open the main menu, and then click Preferences.
3. Select the Enable Vacation Mode checkbox.



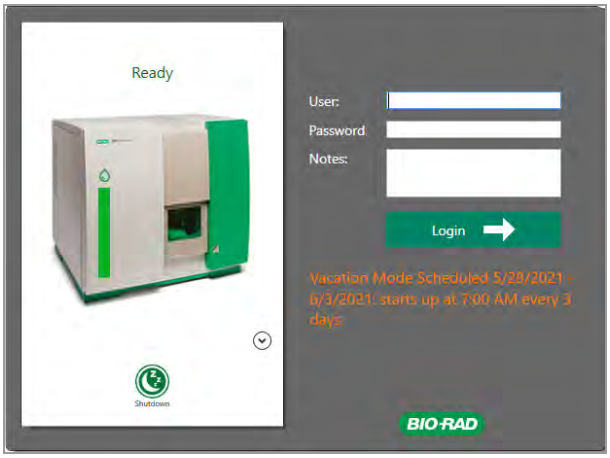
4. To set the vacation date range, click each calendar icon to set the start date and end date.

   Important: Always set the start date (Schedule from) and end date to the following day at the earliest. If the system finds the date range to be invalid, an error message appears.

5. Specify how often and at what time of day the system startup should occur.
6. Click OK to save the changes and close the Global System Preferences dialog.
7. Click OK to save the changes and close the Preferences dialog.

8. Log out of, but do not shut down, Everest Software.

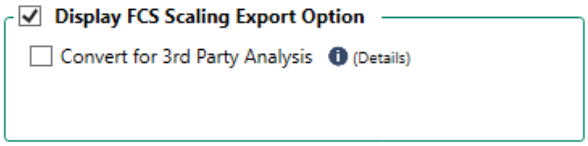
   Before and during the vacation period, a notification appears in the Login window.



   After the end of the vacation period, vacation mode automatically resets to off.

Allowing FCS File Conversion for Third-Party Software

Everest Software administrators can add an option to the Export Data dialog box that allows conversion of FCS files to a third-party analysis format.

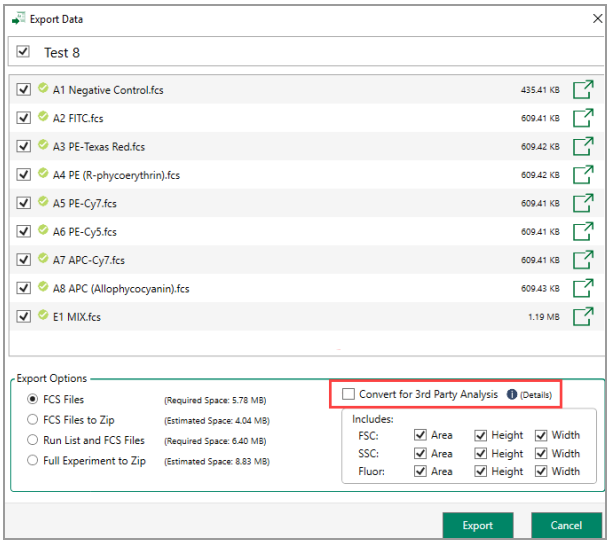


To allow file conversion

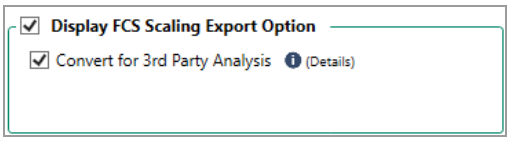
1. Click ☰ to open the main menu, and then click Preferences.
2. Select the Display FCS Scaling Export Option checkbox, and then select the Convert for 3rd Party Analysis.

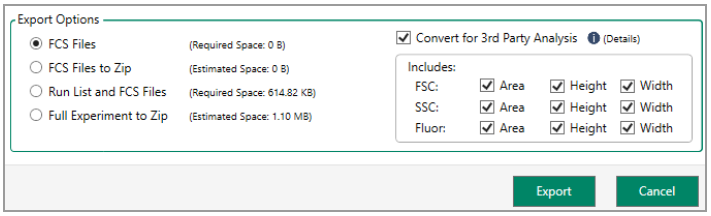
   When you select the Display FCS Scaling Export Option checkbox, Everest Software enables the Convert for 3rd Party Analysis checkbox.

3. Do one of the following:
   - To automatically convert all exported data for third-party analysis, select the Convert for 3rd Party Analysis checkbox.
   - If your users prefer to convert exported data on an as-needed basis, leave this checkbox clear. Users can optionally select this checkbox on the Export Data dialog box.
4. Click OK to save the changes and close the Global System Preferences dialog.



Tip: If your administrator selects the Convert for 3rd Party Analysis checkbox in Global Preferences, the same checkbox in the Export Data dialog box Options section is selected by default each time you open the dialog box.





Managing Users

The User Management option is available from the main menu to users who are logged into Everest Software with an administrative account.

As an administrator, you can use the Manage Users dialog box to

- Create new users
- Manage user passwords
- Set user file storage directories
- Set user privileges
- Deactivate existing users
- Display inactive users in the list
- Reactivate return users

Creating a New User

You can create a new user account in Everest Software for

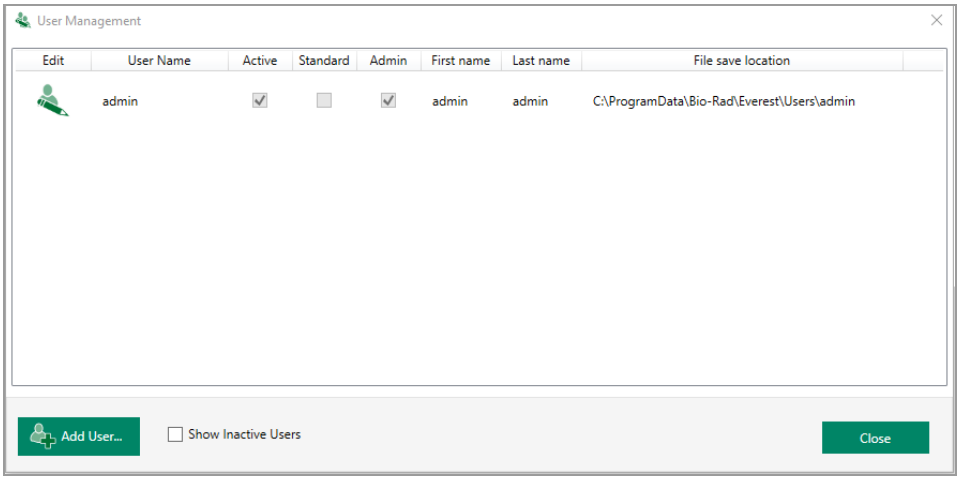
- Each person who uses or configures the instrument.
- A group of users who use the instrument in a similar manner

Tip: Users who log in with a group account can access the files created by other users who log in with the same account, since files created by that group account are stored in the same user data folder location.

To add a user account

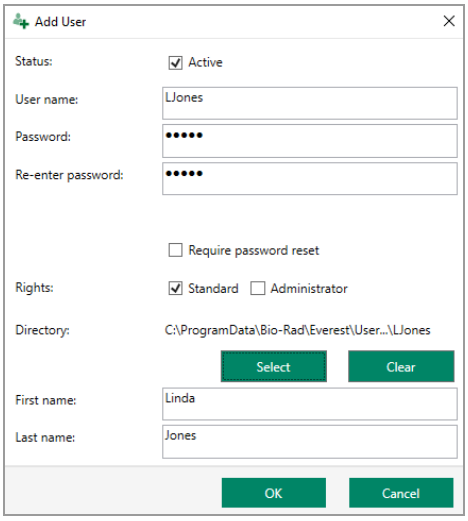
1. Click  to open the main menu.
2. Select User Management > Manage Users.

The User Management dialog box opens.



3. Click Add User.

   The Add user dialog box opens. By default, the Active checkbox is selected.



4. Verify that the Active status checkbox is selected.
5. Enter a user name.

...

6. For password setup, do one of the following:

   - Require that users set their own password:

     a. In the Password text boxes, enter and re-enter a default password. For example, Change Password.

     b. Select Require password reset, and then give the default password to the user.

     Note: When logging in for the first time, users must enter the default password. They will be prompted to change their password.

   - Assign a specific password to a user:

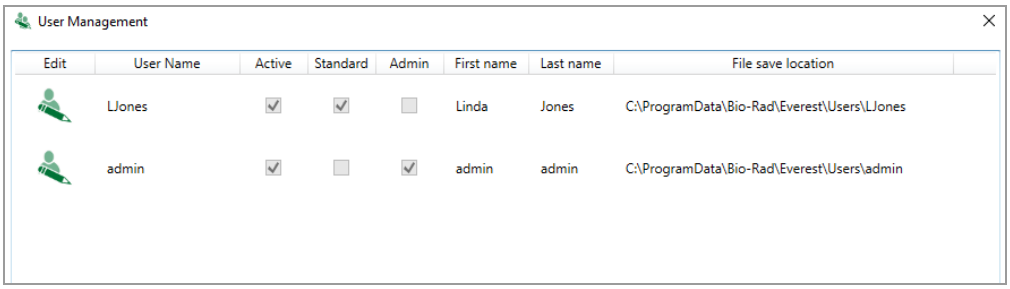
     a. In the Password text boxes, enter and re-enter a password.

     b. Give the password to the user.

7. Select Standard or Administrator to set the account type.
8. In the Directory area, click Select.
9. Browse to and select a folder in which the user's instrument data will be saved, and then click OK.

   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 128.

10. Type the first and last name of the user.
11. Click OK to save the new user account.



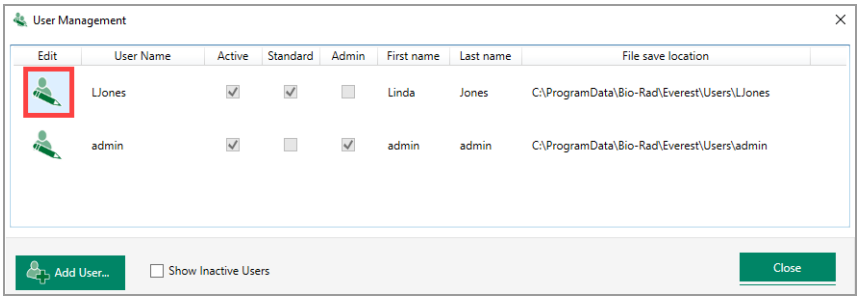
Editing User Information

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

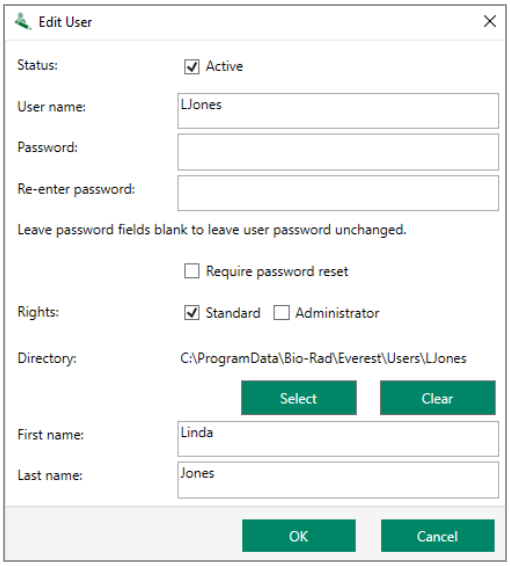
To edit user information

1. Click ☰ to open the main menu.
2. Select User Management > Manage Users.

   The User Management dialog box opens.
3. Click the Edit icon next to the applicable user.



The Edit user dialog box opens.



4. 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 128.

5. Click OK.

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

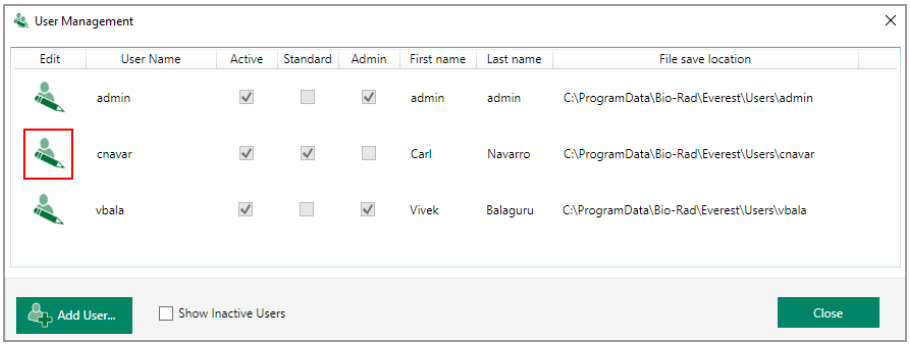
Managing User Account Access

As an administrator, you can deactivate any user who no longer works with Everest Software, reactivate return users, and you can change a user's access rights.

To manage user accounts

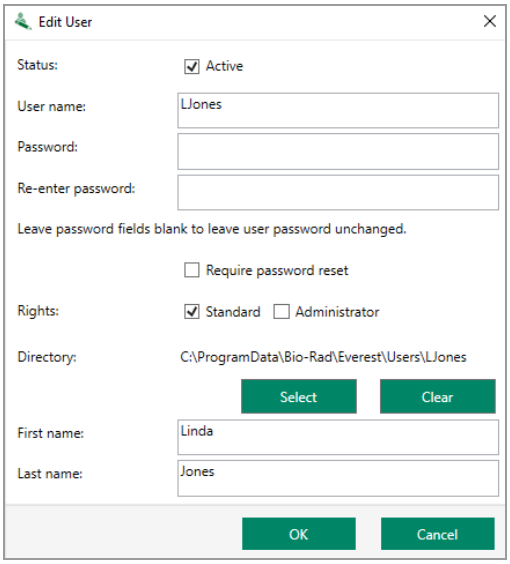
1. Click ☰ to open the main menu.
2. Select User Management > Manage Users.

   The User Management dialog box opens.



3. Click  .

The Edit User dialog box opens.



To require the user to change their password

▶ Select the Require password reset checkbox and click OK.

To change system access rights for a user

▶ Select Standard or Admin and click OK.

To deactivate a user

▶ Clear the Active check box and click OK.

To restore a user's access rights

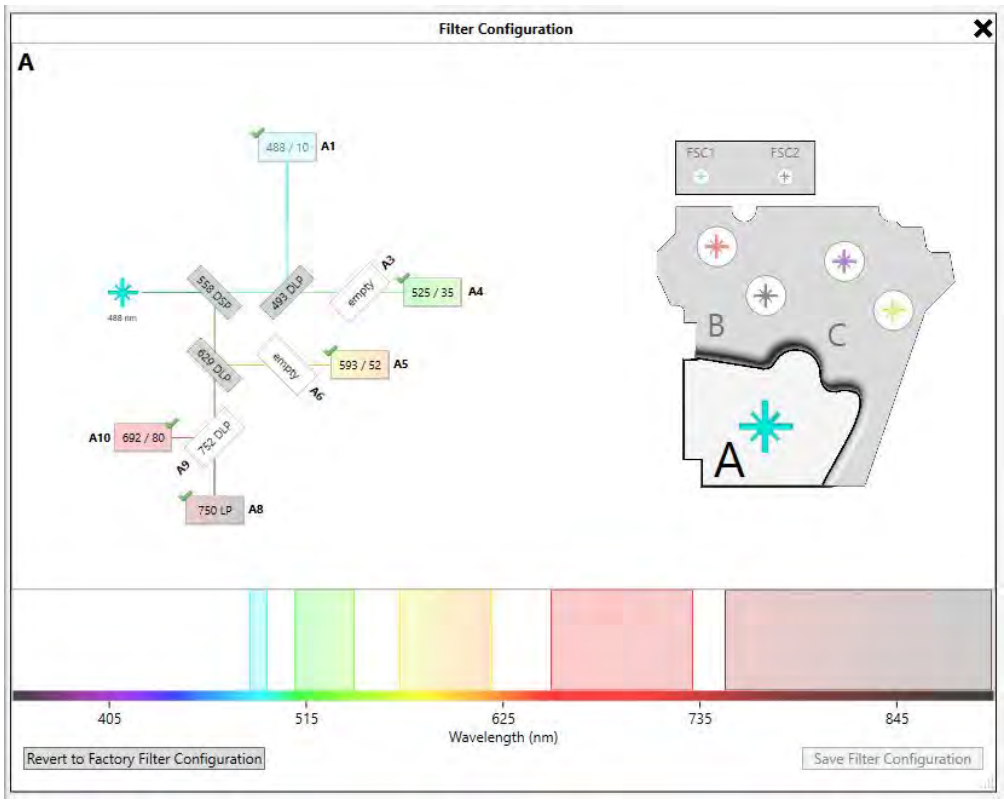
1. Select the Show inactive users checkbox to view inactive user accounts (clear it to hide inactive user accounts).
2. Select the Active checkbox.
3. Verify or modify the user's access level (standard or administrator).
4. Click OK.

...

Working with Optical Filter Configurations

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

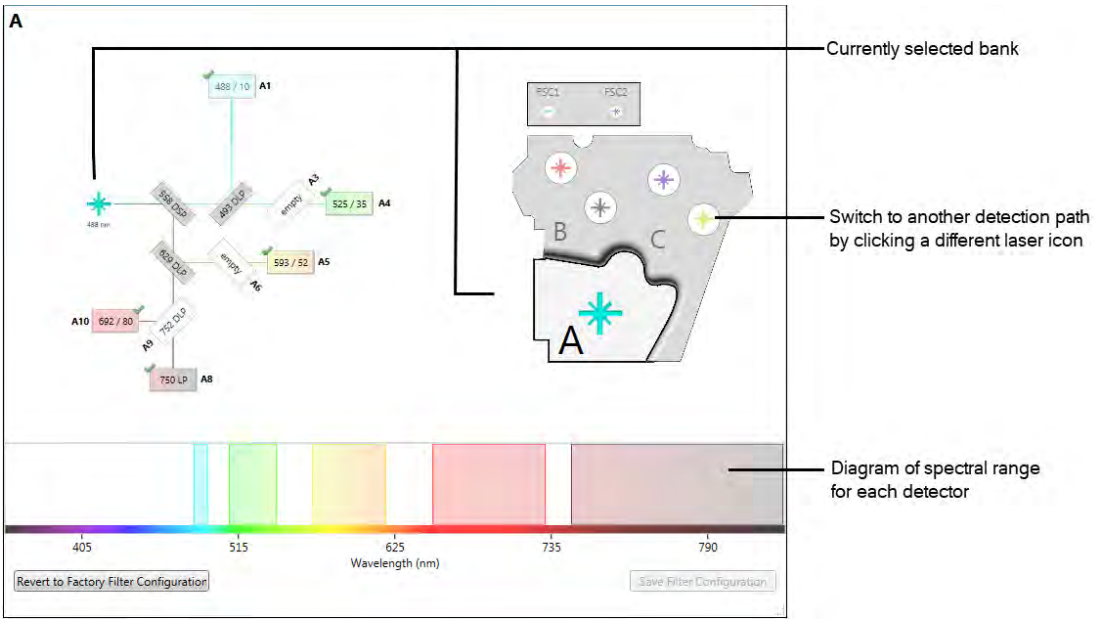
The layout of the detection path is shown on the left, reflecting the current configuration of the instrument. If you install a different physical filter, you must edit the corresponding mirror/filter position labels in this section of Everest Software (see Editing the Optical Filter Configuration on page 156).



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. 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 window, 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 window.



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.

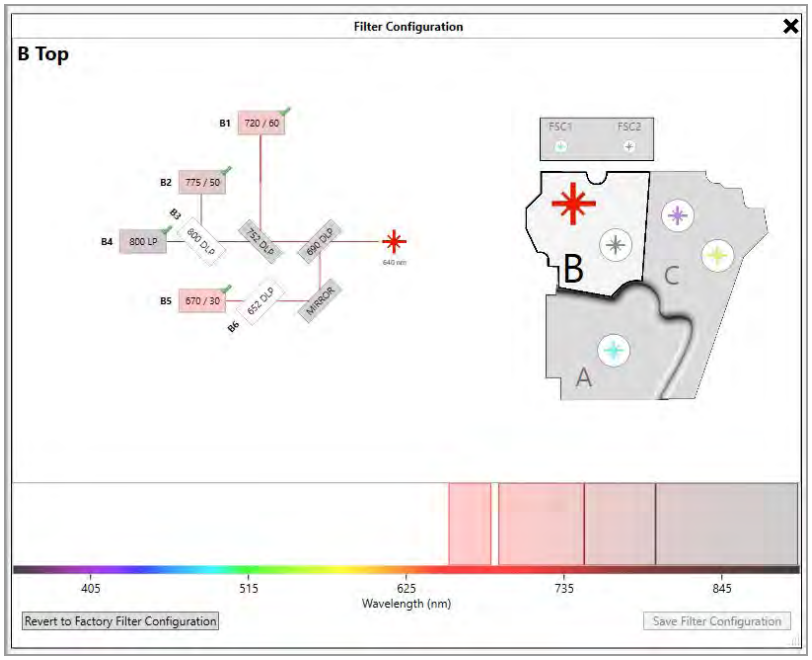
Note: After you save changes to the optical filter configuration in the software, the ZE5-EYE process runs to confirm that the software changes match the physical filter setup.

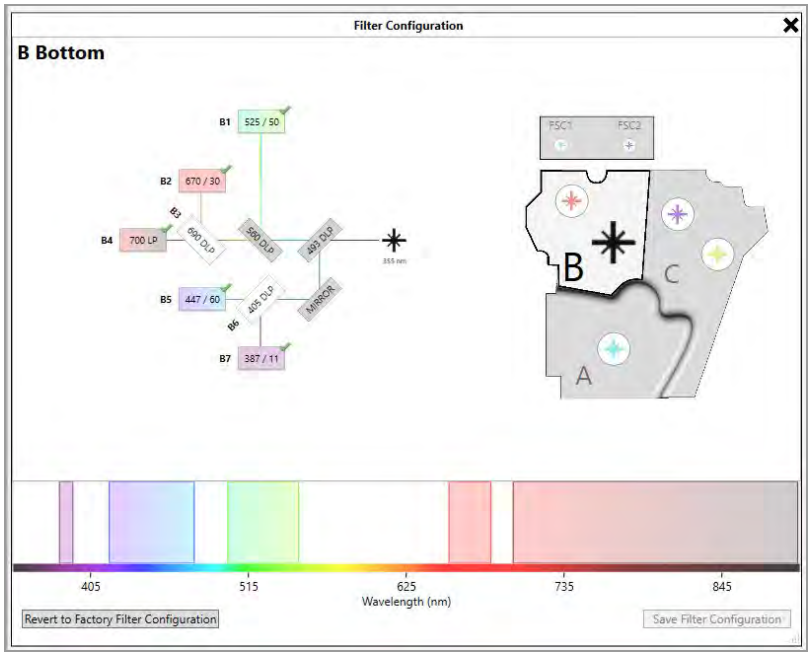
Depending on the laser setup of your instrument, the following detection paths in the banks can be edited from the Optical Filter Configuration dialog box:

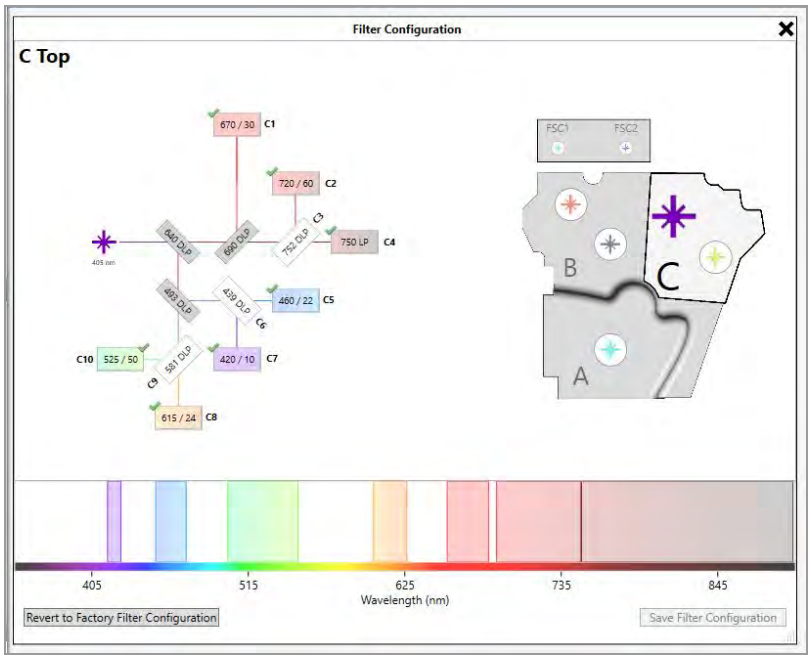
- A: 488 nm (blue) laser, up to seven PMTs
- B Top: 640 nm (red) laser, up to seven PMTs
- B Bottom: 355 nm (UV) laser, up to seven PMTs
- C Top: 405 nm (violet) laser, up to seven PMTs
- C Bottom: 561 nm (yellow green) laser, up to seven PMTs

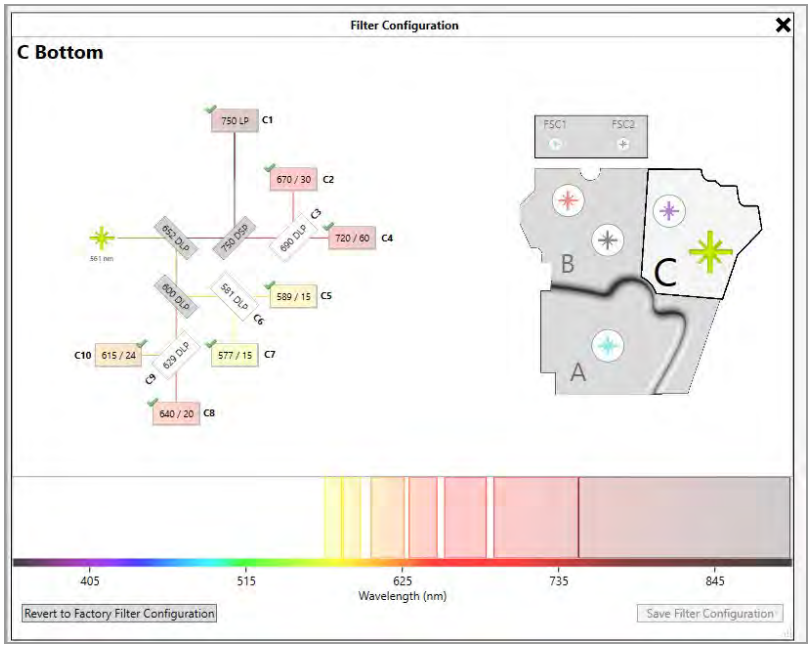
...

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









You can view the FSC1 and FSC2 detection paths from the Optical Filter Configuration dialog box, but you cannot edit them.

…

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	BUV 395 Indo-1 hi (Ca2+ saturated)
	405 DLP (changeable)	
	447/60	Alexa Fluor 350 DAPI PureBlue Hoechst 33342 Indo- 1 lo (Ca2+ free)
	493 DLP	
	525/50	BUV 496 BUV 563
	560 DLP	
	670/30	BUV 661 Hoechst 33342
	690 DLP (changeable)	
	700 LP	BUV 737

7 Detectors, Option A

UV 355 nm 50 mW	7 Detectors	Fluorophores
	387/11	BUV 395 Indo-1 hi (Ca2+ saturated)
	439 DLP (changeable)	
	509/24	BUV 496 DAPI
	560 DLP	
	577/15	BUV 563
	600 DLP (changeable)	
	615/24	BUV 615
	640 DLP	
	670/30	BUV 661
	690 DLP	
	747/33	BUV 737
	776 DLP (changeable)	
	780/LP	BUV 805

…

7 Detectors, Option B

UV 355 nm 50 mW	7 Detectors	Fluorophores
	387/11	BUV 395 Indo-1 hi (Ca2+ saturated)
	439 DLP (changeable)	
	460/22	DAPI
	493 DLP	
	509/24	BUV 496
	530 DLP (changeable)	
	577/15	BUV 563
	640 DLP	
	670/30	BUV 661
	690 DLP	
	747/33	BUV 737
	776 DLP (changeable)	
	780/LP	BUV 805

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 DAPI BD Horizon V450
	493 DLP	
	525/50	AmCyan BV 510 Cascade Yellow Pacific Orange
	581 DLP (changeable)	
	615/24	BV 605
	640 DLP	
	670/30	BV 650
	690 DLP	
	720/60	BV 711
	752 DLP (changeable)	
	750 LP	BV 786

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488 nm Laser Filter Choices

Blue 488 nm 100 mW	5-Detector Option	Fluorophores
	488/10	SSC on 488
	493 DLP	
	525/35	eGFP FITC
	558 DSP	
	593/52	PE (R-phycoerythrin)
	629 DLP	
	692/80	PE-Cy5 PerCP-Cy5.5
	752 DLP (changeable	
	750 LP	PE-Cy7

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

…

561 nm Laser Filter Choices

Yellow Green 561 nm 50 mW	**5 Detectors**	**Fluorophores**
	583/30	dTomato dsRed PE
	600 DLP (changeable)	
	615/24	PE-Texas Red PE-CF594 mCherry
	652 DLP	
	670/30	PE-Alexa Fluor 647 PE-Cy5
	690 DLP	
	720/60	mPlum
	752 DLP (changeable)	
	750/LP	PE-Cy7

Yellow Green **561 nm** **50 mW**	**7 Detectors**	**Fluorophores**
	577/15	dTomato dsRed PE
	581 DLP (changeable)	
	589/15	
	600 DLP	
	615/24	PE-Texas Red PI (bound to dead)
	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	APC Cy5 Alexa 647
	690 DLP	
	720/60	Cy5.5 Alexa 680 Alexa 700
	752 DLP	
	775/50	APC-Cy7 APC-H7 APC-Alexa Fluor 750
	800 DLP (changeable)	
	800 LP	

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 383 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.

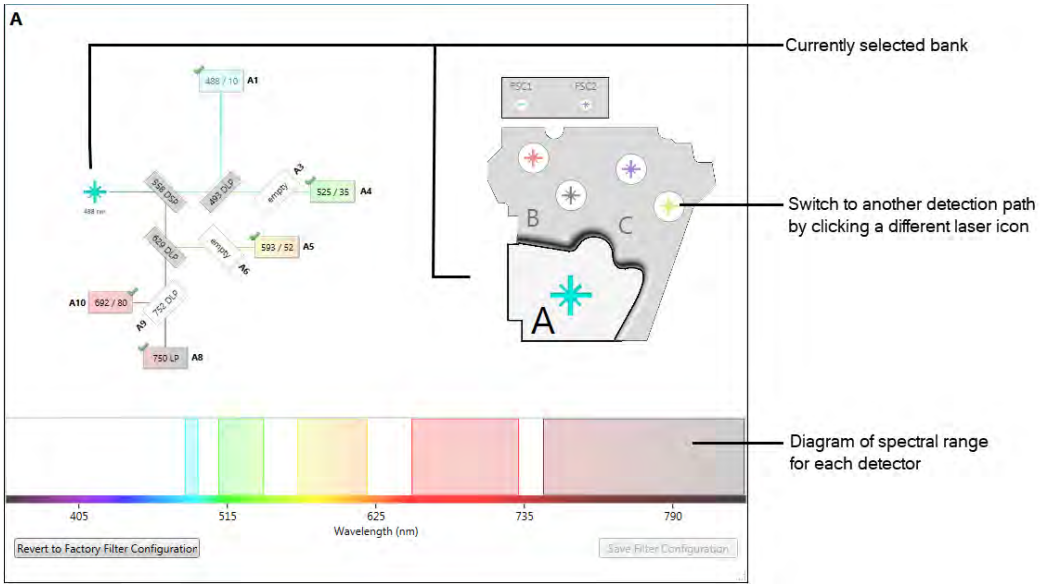
Editing the Optical Filter Configuration

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 on 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 PMT position or bandpass filter.

   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 PMT position or bandpass filter 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 for the current and future application sessions, click Save Filter Configuration.

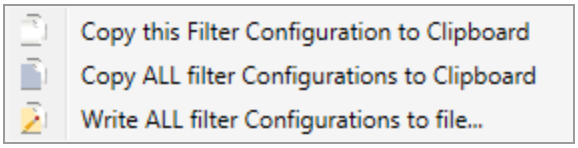
   The ZE5-EYE process runs automatically.

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. Do one of the following:

   - If you selected one of the Copy options, paste the graphic into the application of your choice.
   - 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 Everest Software. Before performing this task, ensure that the original filters are in their original slots.

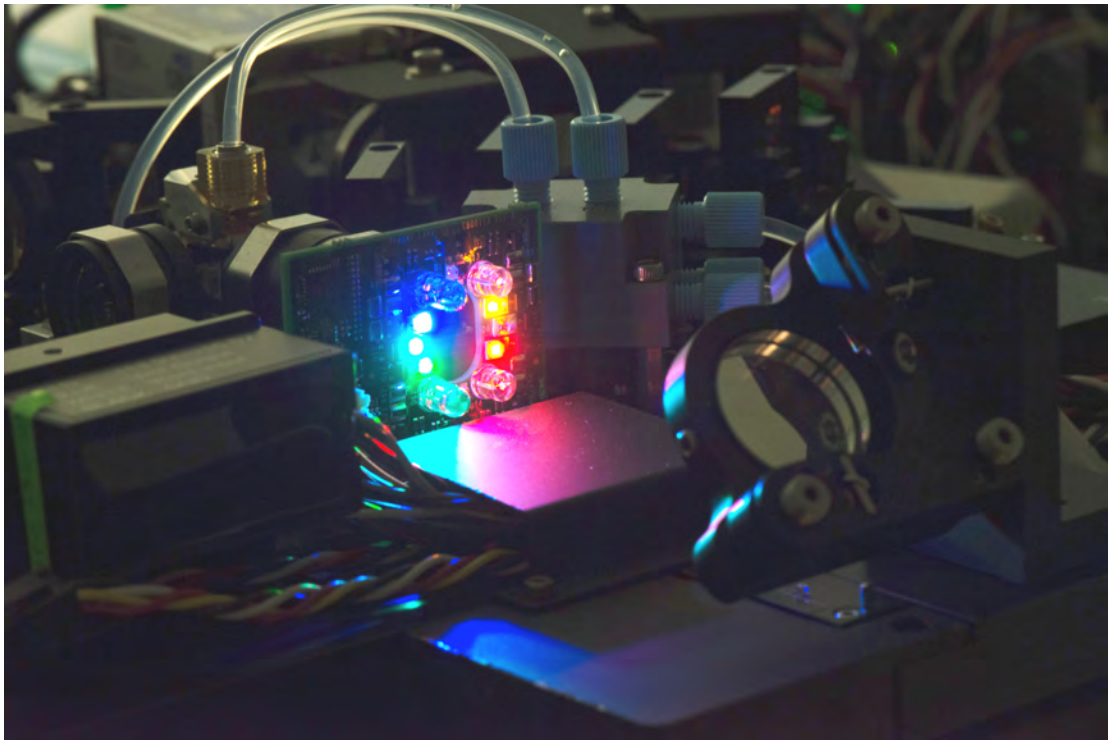
To revert to the default optical filter configuration

1. In the Filter Configuration dialog box, click Revert to Factory Filter Configuration.
2. Click OK.

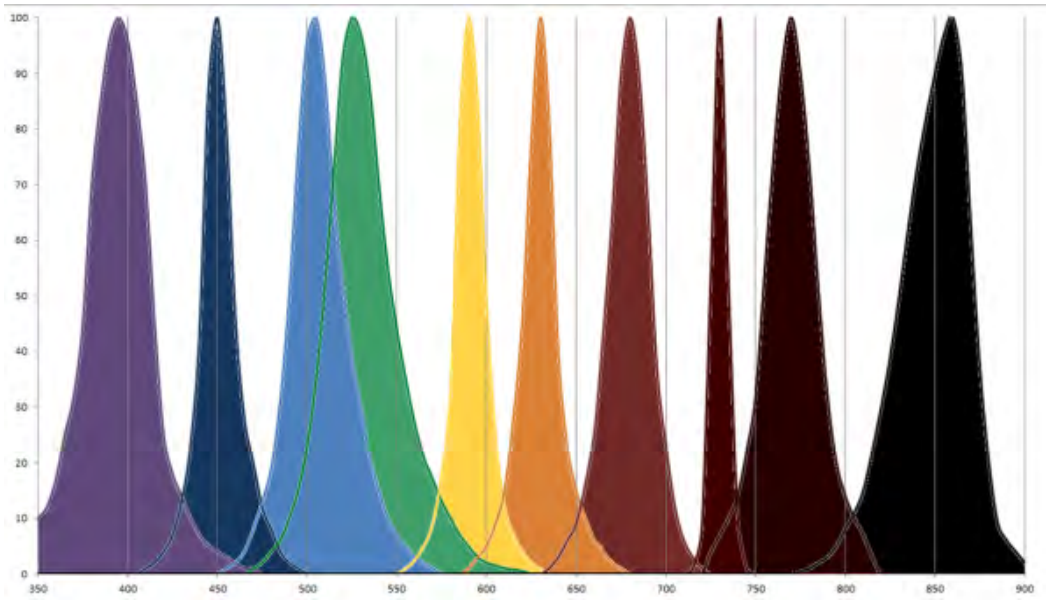
   The ZE5-EYE process runs and a success or failure message appears in the status bar.

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.

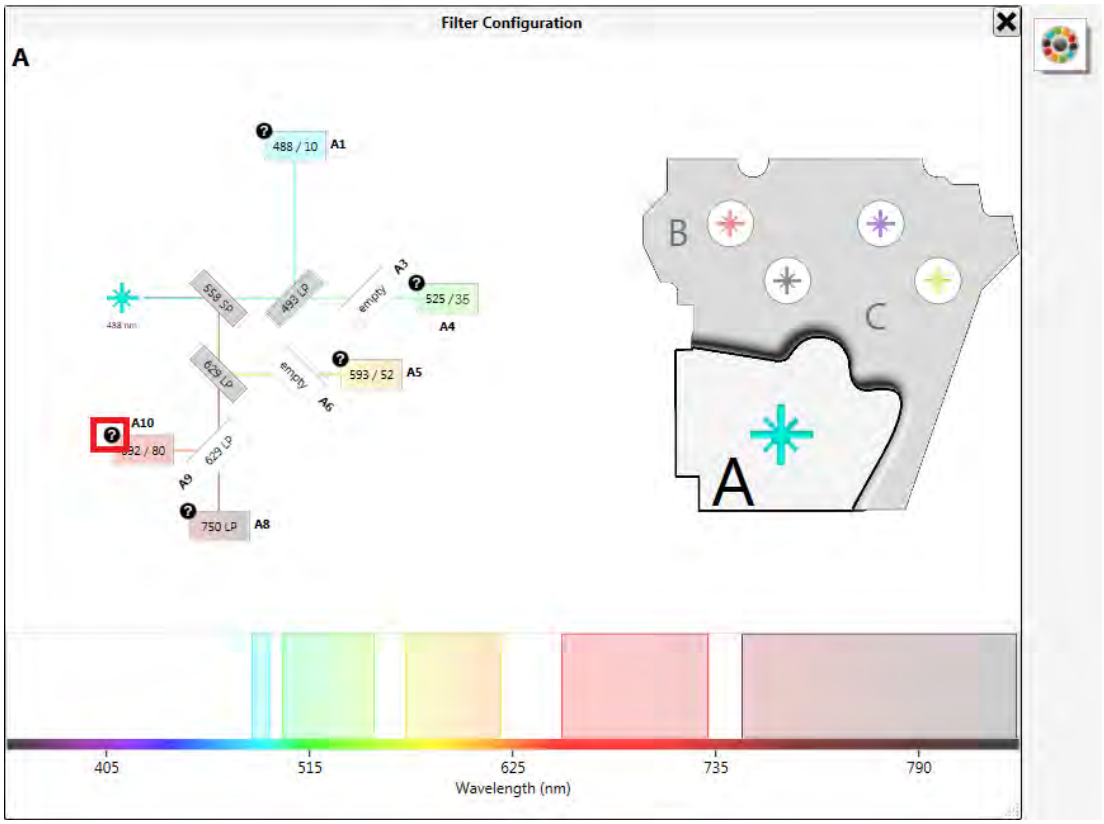


The ZE5-EYE runs automatically

- 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, data might appear in the plots.

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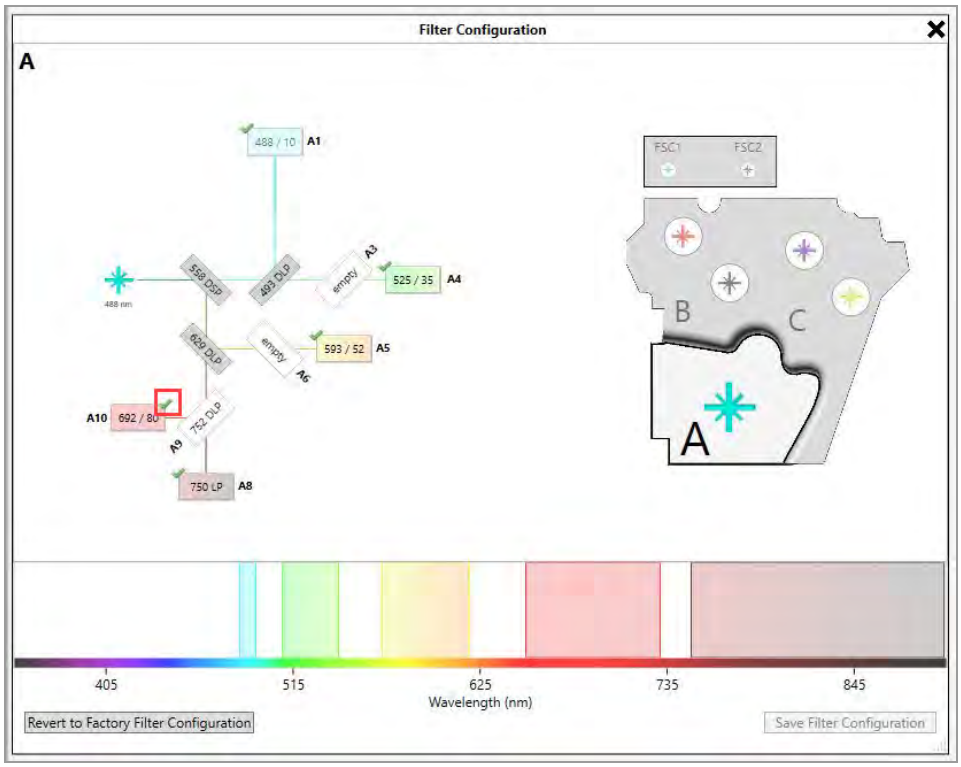
To confirm filter choices using the ZE5-EYE

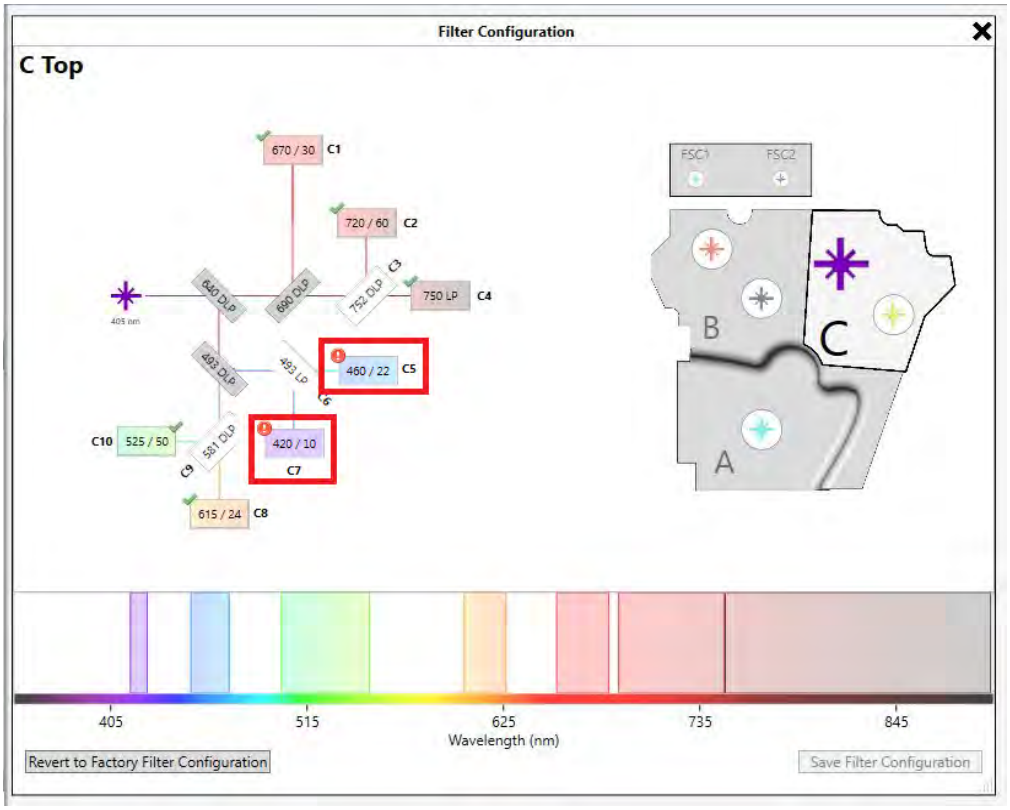
1. If the ZE5-EYE did not run automatically, click the ZE5-EYE button located to the right of the Filter Configuration dialog box, as shown in the next figure.

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.



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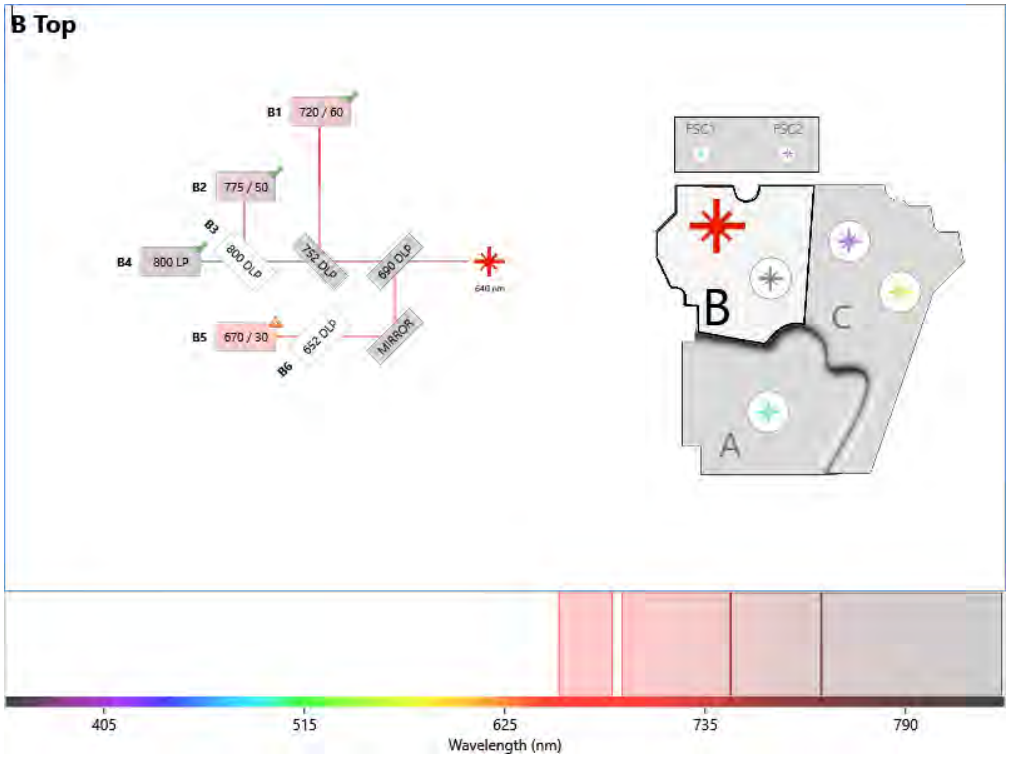
- If the voltage change required to center the peak around channel 128 exceeds the value set in the QC criteria, an error appears in the status bar and an orange triangle with an exclamation point appears next to the final filter position for the detector. For more information, see Editing QC Criteria and Trending Range on page 126.



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 44.

Ensure the following when handling the filter stick:

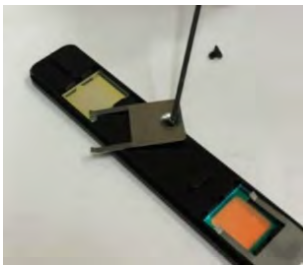
- Wear gloves
- Handle filters by the edges
- Do not touch any filter surfaces

To insert a neutral density filter into a filter stick

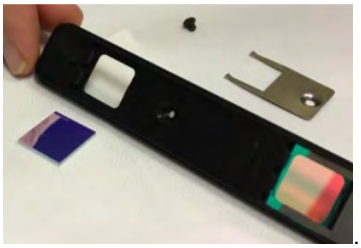
1. Remove the filter stick from its slot in the instrument's filter bank.
2. Locate the screw on the back of the filter holder.

Important: If you see white material (Loctite, not pictured) on top of the screw, do not remove it. Use a blank filter stick instead.

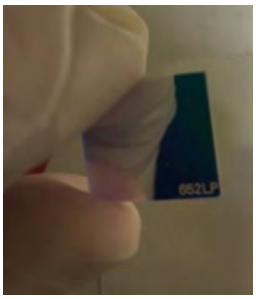
3. If the screw is clean, use a 1.5 mm Allen wrench to remove it.
4. Remove the metal clip that holds the bandpass filter.



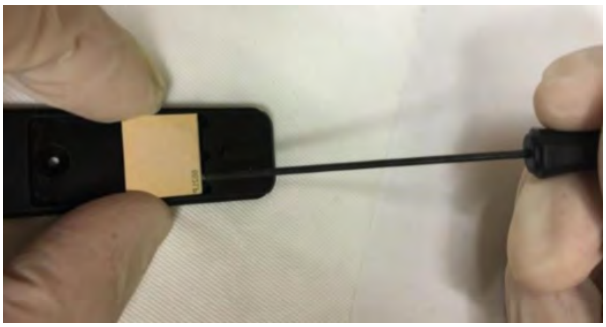
5. Empty the filter glass onto a clean, lint-free material.



The filter glass has a marking etched into the coating.



6. Place the filter glass into the filter glass pocket, making sure the side of the filter glass with the marking is facing down.



7. Re-install the metal clip and tighten the screw with the Allen wrench.
8. 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 any 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 306.

Chapter 6 Daily Routine

This chapter explains 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
- 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 23. For information on shutting down Everest Software, see Shutting Down on page 188.

Starting Everest Software

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

Checking Fluidics Status

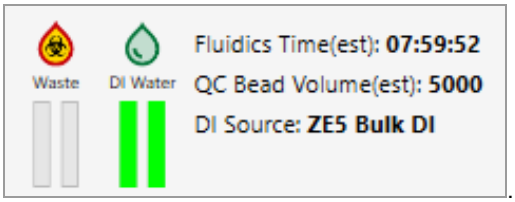
Six fluidics bottles are located behind the fluidic door on the left front of the instrument:

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

Important: Be sure to empty waste and fill sheath concurrently to ensure correct time displays and reporting.

▶ Before logging in, click the Details down arrow to examine the fluidics status.

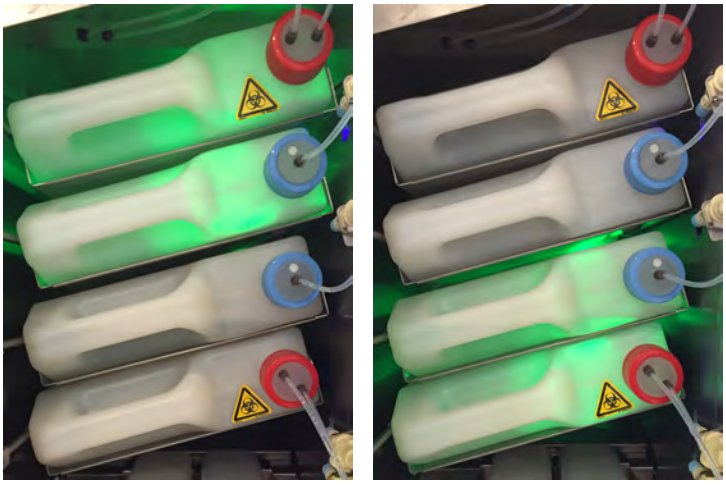
In the Details window, you can view the estimated remaining run time and the current bottle levels.



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 69.

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 the system can transition to them.



To identify decreasing sheath and increasing waste, Everest Software displays status messages at 60, 30, and 10 minutes of remaining runtime. You can also check the individual bottles to ensure that the waste bottles are sufficiently empty and the sheath fluid bottles are sufficiently full, but not overfull.

Refilling Bulk Fluidics

Important: Bio-Rad recommends changing waste and sheath bottles at the same time to ensure correct time displays and reporting.

- 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 10 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 10 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.

Note: After logging in, you can also force the system to switch to the upper or lower pair of bottles by clicking Fluidics in the toolbar, and then clicking the Fluidics Swap icon, as shown in the following graphic.

Emptying Waste Bottles

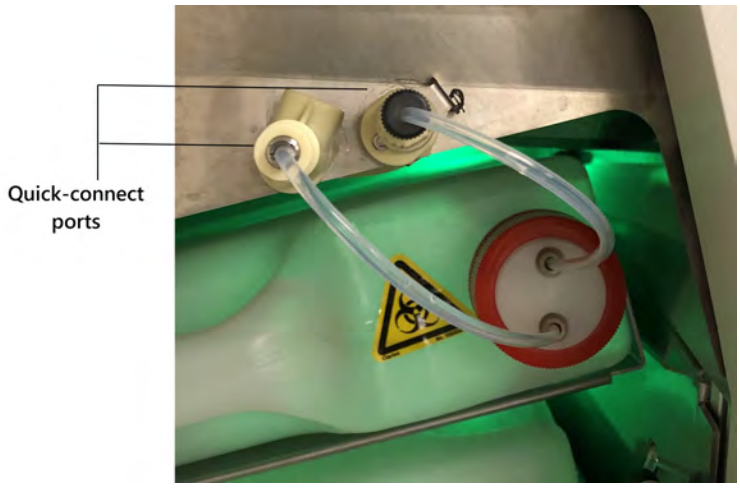
Each waste bottle has two connections to the instrument. One connection is a fluidics connection, and the other is an air vent connection to ensure that air is properly displaced as fluid is pumped into the bottle. Any air vented from the waste bottle passes through a 0.2 µm filter. The waste fluidics 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: Empty both waste and replace both sheath containers at the same time. 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.

Replacing Sheath Bottles

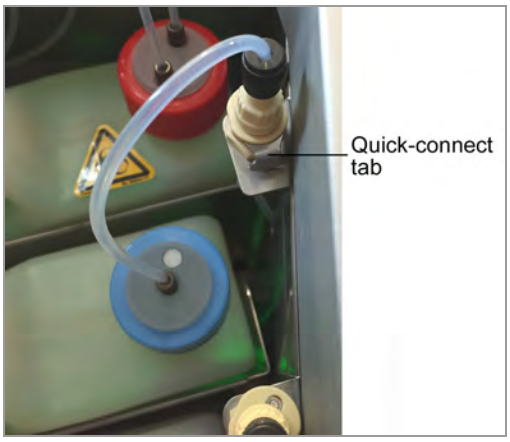
Each sheath bottle has a single connection to the instrument. Waste and sheath bottle connections are specifically keyed to their respective ports and cannot be connected to the wrong ports.

Important: When refilling the sheath fluid bottles:

- Ensure you replace both sheath and empty both waste containers at the same time.
- 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 bottles.
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.

Replacing Sheath Additive and Cleaner Bottles

The sheath additive and cleaner bottles are located at the bottom of the fluidics chamber. They can be refilled or replaced at any time other than during shutdown.

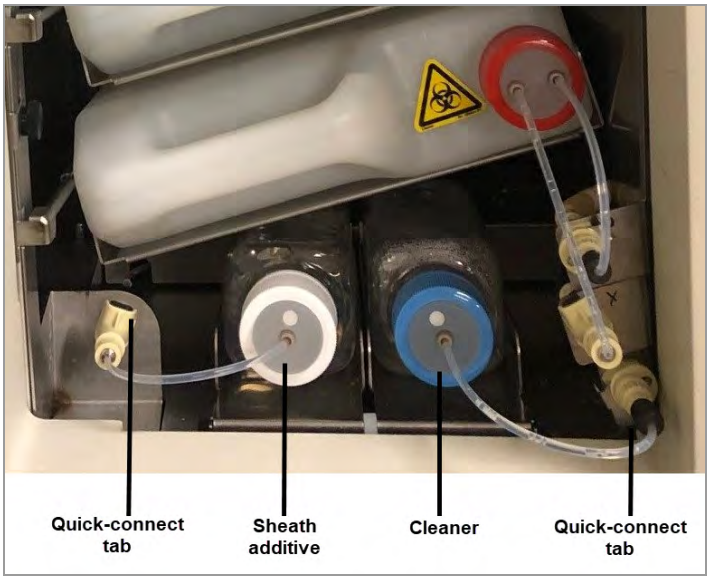
Sheath additive and cleaner bottle connections are specifically keyed to their respective ports and cannot be connected to the wrong ports: the white-capped sheath additive bottle always goes on the left and the blue-capped cleaner bottle always goes on the right.

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.

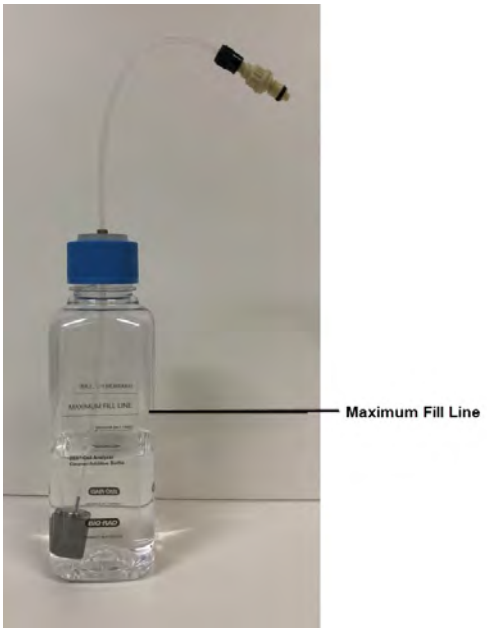
To refill an additive or cleaner bottle

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 you do not hear a click, salt might have built up on the connector. Remove salt using a paper towel moistened with DI water.

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

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

The startup process turns on the lasers, pressurizes the internal sheath reservoir, 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 369
- Using the ZE5-EYE to Confirm Filter Choices on page 158
- Running Quality Control on page 183
- Specifying Logged Out Settings on page 126

To start up the instrument

1. Click Startup.
2. To view the sequential startup steps as they occur, click the down arrow..

3. To view the full list of startup tasks, click ⌄.

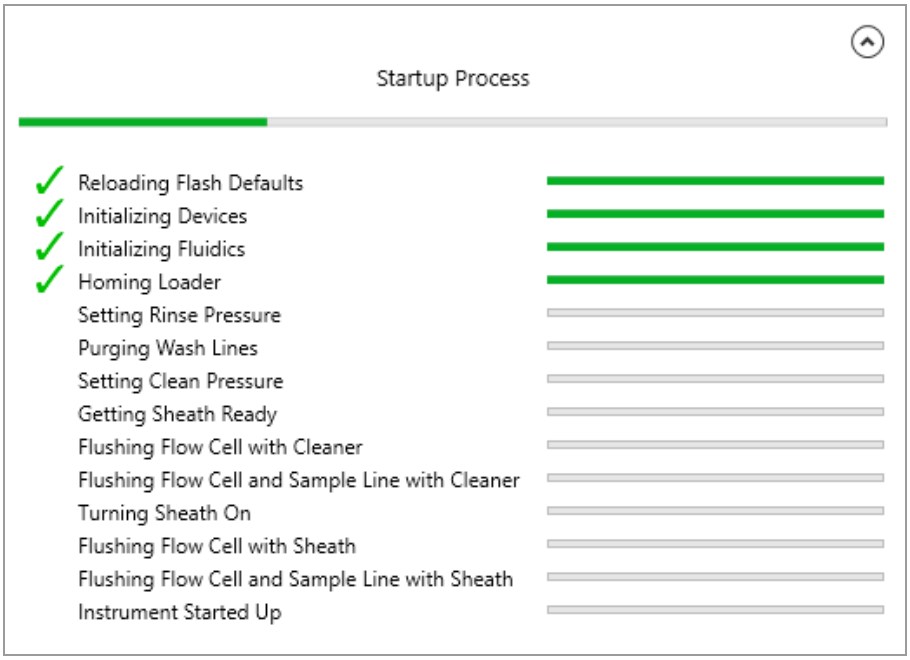

Table 27 on page 179 describes each process step.

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Table 27. Steps in the startup process

Reloading Flash Defaults	Loads instrument-specific default settings from onboard flash memory to Everest Software. This includes the instrument's serial number, channel and pinhole configuration, as well as settings specific to the loader, probe, sampling rate, sample pump, and bulk fluidic sensors.
Initializing Devices	Initializes the ZE5 instrument and subcomponents for startup.
Initializing Fluidics	Initializes the fluidics system by starting the cleaner, water, waste, waste evacuation, air, and cooling pumps. The air pump begins to slowly pressurize an internal air bottle from 0 to 10 psi, which in turn regulates sheath pressure in the internal sheath reservoir (separate from the bulk sheath tanks).
Homing Loader	Homes the loader, probe, and wash station to a known position to ensure all three are in the correct starting position and ready to run samples.
Setting Rinse Pressure	Sets rinse pressure to 12 psi for subsequent steps using cleaner (approximately 5 ml of cleaner is used in the startup process).
Purging Wash Lines	Runs deionized water through wash lines.
Setting Clean Pressure	Sets clean pressure to 12 psi for subsequent cleaner steps.
Getting Sheath Ready	Waits for the air pump to reach a sheath pressure of 10 psi, and if necessary waits for the internal sheath reservoir to fill to approximately 160 ml (or a level of 40mm).
Flushing Flow Cell with Cleaner	The flow-cell cleaner port opens and the cleaner pump fills the flow cell with cleaner.
Flushing Flow Cell and Sample Line with Cleaner	The sample pump runs backward to move cleaner backward through flow cell and sample lines.
Turning Sheath On	The sheath pump turns on to fill the flow cell with sheath.
Flushing Flow Cell with Sheath	The sheath pump fills the flow cell with sheath.
Flushing Flow Cell and Sample Line with Sheath	The sample pump runs backward to move sheath backward through flow cell and sample lines.
Instrument Started Up	The instrument is ready to use.

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.

Logging In

The login window is divided into two panes: Instrument Status and User Login, as described in Login and System Status Window on page 60.

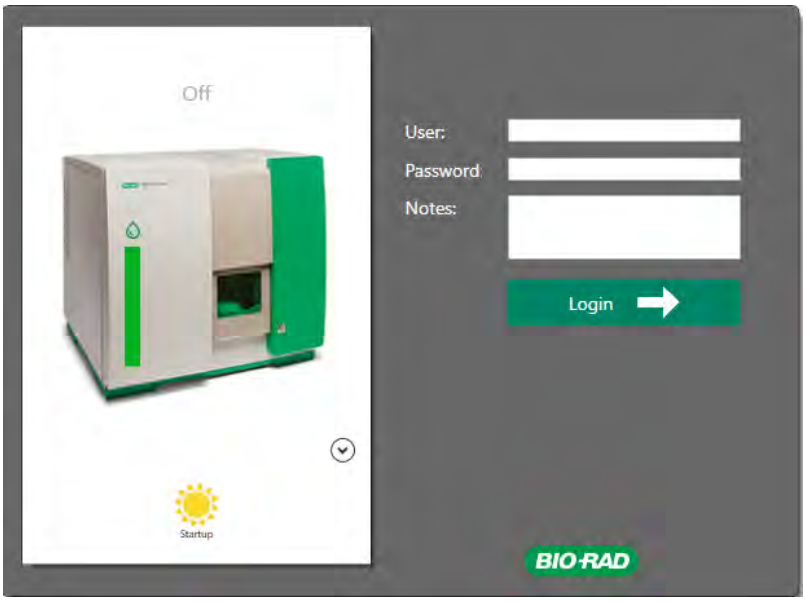
If the software is connected to an instrument, an instrument picture appears in the Instrument Status pane. Before proceeding, check the instrument status:

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

You can refill fluidics before logging in; you must log in before you can manually run the QC process or run samples.

To log in to Everest Software

1. Enter your user name and password.



2. (Optional) Add relevant notes for your session in the Notes textbox. User session notes are saved to the user log and can be viewed in the user reports available to administrators. For more information on user reports, see Generating User Reports on page 342.
3. Click Login.

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

Quality Control Process

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

- Home window
- Tools section on the Acquisition workspace 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.

Note: See Replacing the QC Beads on page 383 for information on swapping the QC beads.

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 K 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.

Important: While QC is in progress, the following functions are disabled:

- Returning to the Home window
- Initiating the ZE5-EYE process
- Sending the run list to the local instrument (performed in the Analysis workspace)
- In the Instrument tools:
  - Returning sample probe to home position
  - Cleaning the sample line and probe
  - Unclogging the sample line, probe, and flow cell
  - Swapping QC beads
  - Pausing the sheath fluid and disabling the lasers
- Opening the sample loader door

The software enables these functions when the QC process completes.

Running Quality Control

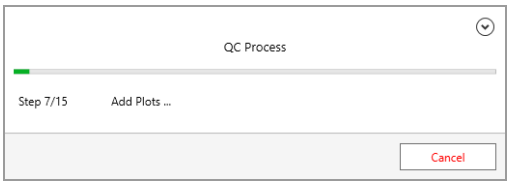
To initiate the QC process

1. In the System toolbar, click QC.

Note: You can also initiate QC from the Home window.

2. While QC is in progress, click the down arrow.

The QC Process window appears, providing real-time updates on each portion of the process.



3. To see the full list of sequential steps, click ⌄.

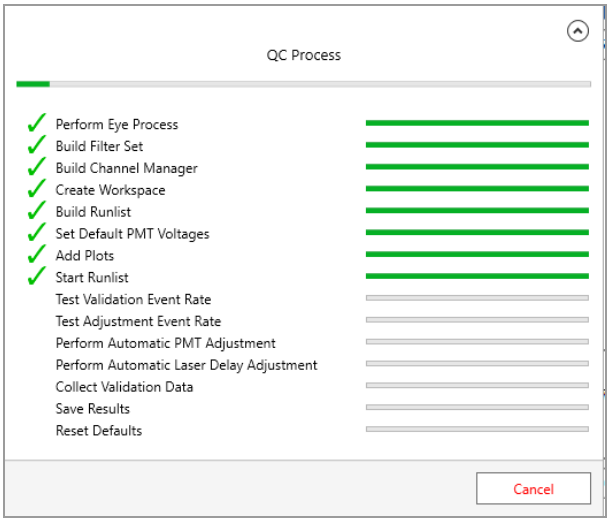


Table 28. Steps in the QC process

Perform 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 bead swap (instrument baseline) 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.
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 Acquisition workspace. When QC completes, the software remains in the Acquisition workspace. To return to the Home window, click the Home button.

Note: The Home button on the toolbar is disabled during the QC process. You can return to the Home window after the QC process completes.

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 341.

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 System toolbar.

The external light turns on when the door is open.

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 on the front of the instrument.

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 Chapter 12, 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. While the system is paused, the following functions are disabled:

- All functions in the Instrument Control, PMT Control, and Trigger panels
- Initiating the ZE5-EYE process
- Running the QC process
- Shutting down Everest Software and the ZE5 Cell Analyzer
- In the Instrument tools:
  - Cleaning the sample line and probe
  - Unclogging the sample line, probe, and flow cell
  - Swapping QC beads

The software enables these functions when the system is restarted.

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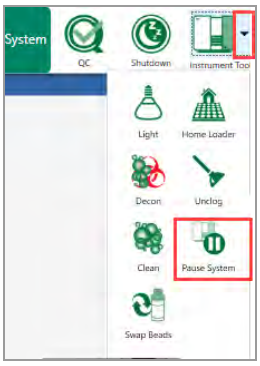
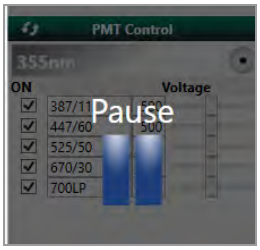
To pause the system

▶ In the System toolbar, click the Instrument Tools down arrow, and then click Pause System.

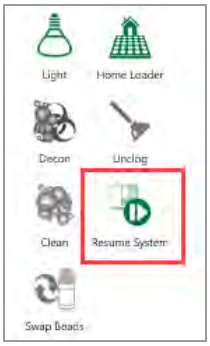


A message in the left panel indicates that the system is paused.



To resume the system

▶ In the System toolbar, click the Instrument Tools down arrow, and then click Resume System.

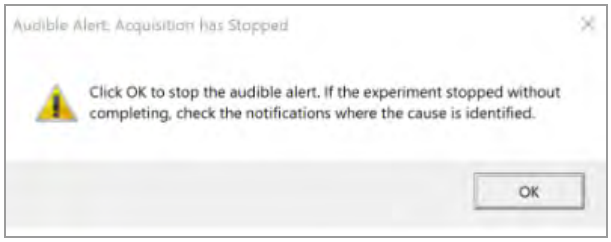


Responding to Audible Alerts

If you have enabled audible alerts in the Preferences window, Everest Software prompts an repeating alarm whenever the following occurs:

- Ordinary completion of experiments
- Run list paused or stopped due to error
- Failure to complete startup
- Failure to complete QC due to error

The audible alert dialog box appears over all other open windows until you acknowledge the alert.



To stop the alert

▶ Click OK to stop the alarm and close the dialog box.

If a warning or error triggered the alert, resolve the issue and continue.

Tip: You can adjust the alarm volume using the Windows 10 settings for system sound controls on the Everest Software PC.

Shutting Down

Important: Do not press the main power switch to shut down the ZE5 Cell Analyzer Refer to System Power on page 167 for more information.

Using Everest Software, 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 interaction.

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 is located

- In the Home window
- In the System toolbar

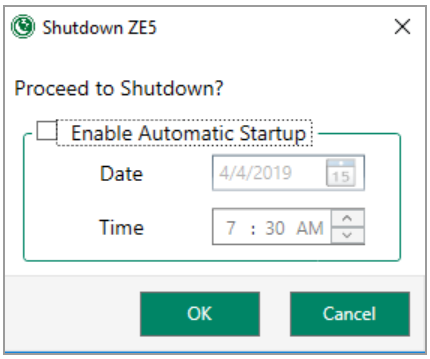
It can also be found in the Login window, if your system administrator has allowed shutdown to occur when no user is logged in. For more information, see Specifying Logged Out Settings on page 126.

Note: 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 following dialog box appears.

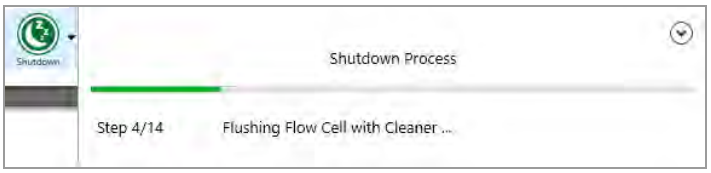


3. Do one of the following:
   - To begin the shutdown process immediately, click OK.
   - To restart the instrument on a particular date and time, select Enable Automatic Startup and click OK. For more information, see Scheduling Automatic Startups on page 192.

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

   Important: Everest Software must be running for a scheduled automatic startup to be performed. Leave the software running if an automatic startup is scheduled. The sheath bottles must be sufficiently full and the waste bottles 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.

4. Empty the waste bottles and refill the sheath bottles if needed.
5. To view the sequential shutdown steps as they occur, click the down arrow in the upper-right corner.
6. To view shutdown details, click the down arrow next to the icon on the left.



7. To view the full list of shutdown tasks, click ⌄.

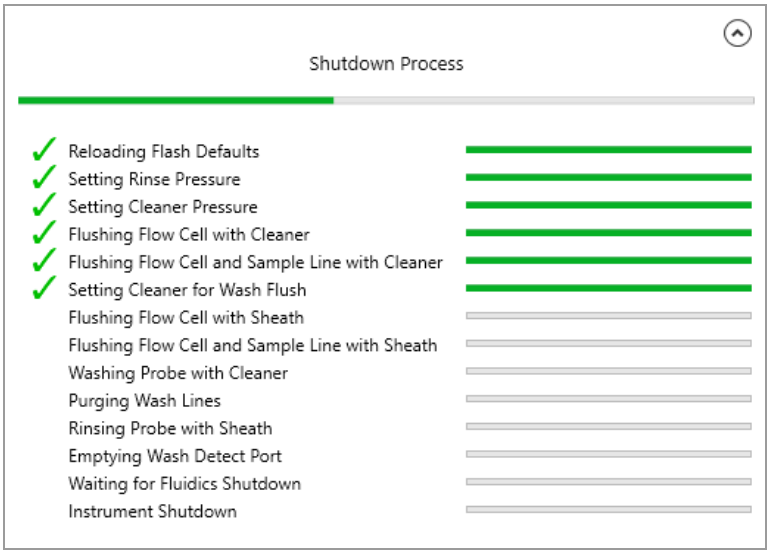


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Table 29. Steps in the shutdown process

Reloading Flash Defaults	Turns off the loader, and reloads the instrument-specific configuration settings from the onboard flash memory to Everest Software. Settings include ■ Instrument serial number ■ Hardware configuration ■ Channel ■ Pinhole configuration ■ Loader ■ Sample pump ■ Bulk fluidics ■ standard fluidics ■ Flow rate ■ Volume calibration
Setting Rinse Pressure	Sets rinse pressure to the configuration value (typically 10 or 12 psi) for subsequent steps.
Setting Cleaner Pressure	Sets cleaner pressure to 12 psi for subsequent steps using cleaner. Approximately 7.5 ml of cleaner is used in the shutdown process.
Flushing Flow Cell with Cleaner	Homes the loader; the flow-cell cleaner port opens and the cleaner pump fills the flow cell with cleaner.
Flushing Flow Cell and Sample Line with Cleaner	The sample pump runs backwards to move cleaner through the flow cell and sample lines.
Setting Cleaner for Wash Flush	Sets cleaner pressure to the same as the rinse pressure (typically 10 or 12 psi).
Flushing Flow Cell with Sheath	Flushes the flow cell with DI for 10 sec.
Flushing Flow Cell and Sample Line with Sheath	The sample pump runs backwards to flush the flow cell and sample line with cleaner for 20 sec.
Washing Probe with Cleaner	Moves the loader to a safe position, moves the sample probe up and down between home and the maximum position through the wash station several times to clean the exterior of the probe with cleaner. Probe crash is also checked during this step.
Purging Wash Lines	Purges wash line with DI for 20 sec.

Table 29. Steps in the shutdown process, continued

Rinsing Probe with Sheath	Moves the loader to safe position, moves the sample probe up and down between home and the maximum position through the wash station several times to clean the exterior of the probe with DI. Probe crash is also checked during this step.
Emptying Wash Detect Port	Turns on the sample waste detect valve, empties the wash detect port. Waste pump evacuates fluid from the wash station. Cleaner and air pump run backwards to reduce cleaner and sheath pressure.
Waiting for Fluidics Shutdown	Depressurizes sheath pressure to 0 psi, turns off all the pumps, empties the waste detect in case of drips, waits for fluidics to shut down in 4 min.
Instrument Shutdown	Turns off the LEDs and the loader, ZE5 Cell Anaylzer instrument shuts down.

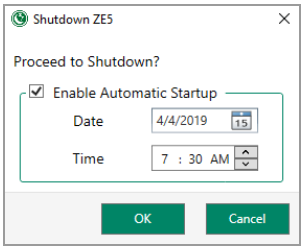
Scheduling Automatic Startups

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.

To schedule an automatic startup

▶ Select the Enable Automatic Startup checkbox, and then enter a date and time.

Tip: You can also click the date icon and the time up and down arrows.



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

Important: Scheduled automatic startups do not occur if Everest Software is not running, Ensure the software is running if automatic startup is scheduled. If the system automatically starts up and no user logs in within 1 hr, the system shuts down.

Exiting Everest Software

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

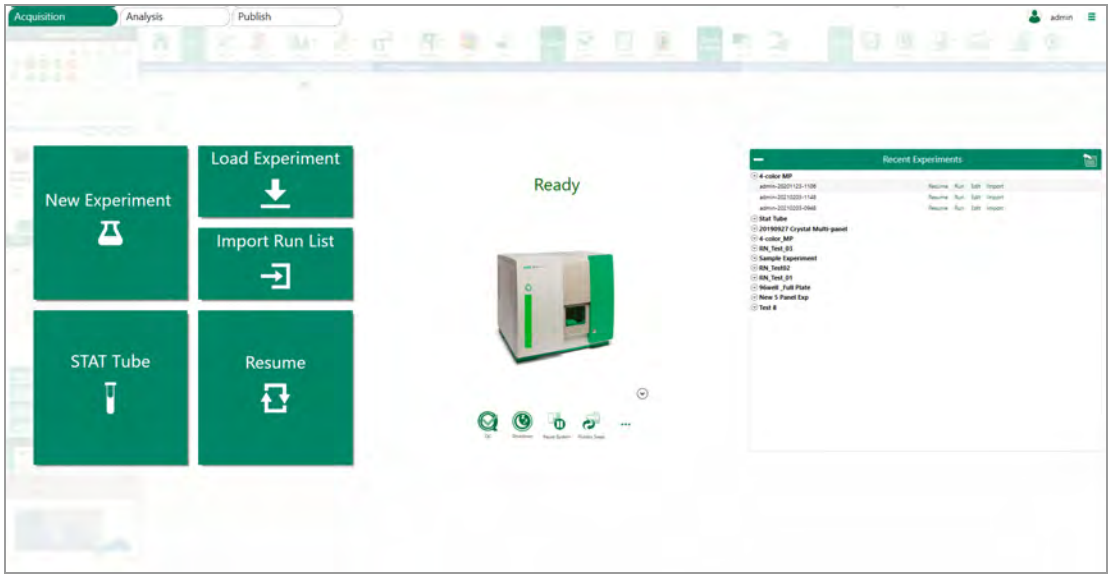
To exit Everest Software

▶ Click Close in the upper right corner.

Note: If the software cannot exit immediately, Bio-Rad recommends waiting rather than forcing the software to exit.

Chapter 7 Creating Experiments

The chapter explains how to create or edit experiments using a plate or tube layout, or a single stat tube.



Creating or Editing an Experiment

To create a new experiment

▶ On the Home window, do one of the following:

- Click New Experiment and continue to Selecting the Media Type on page 198.
- Click Import Run List and continue to Importing a Run List on page 197.

To edit an existing experiment

1. Do one of the folloing:
   - In the Recent Experiments pane, select an experiment and click Edit.
   - On the Home window, click Load Experiment and select an experiment.

     The Experiment Builder opens to the Fluorophores window.

   Tip: You can also select a file in the Recent Experiments pane, and click Edit.
2. Continue to Working in the Experiment Pane on page 204.

Importing a Run List

When creating a new experiment, you can import a subset of experiment settings (run list) from a previous experiment. Doing so imports the following items:

- Fluorophores (enabled PMTs)
- Parameter names
- Instrument settings
- Compensation matrix
- Plots

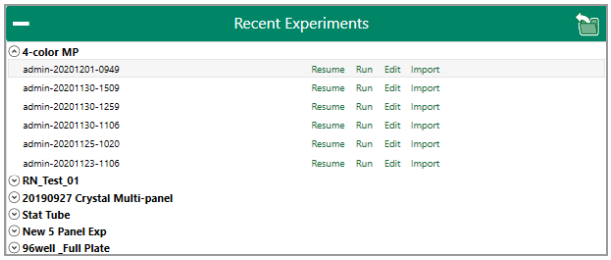
Sample positions are not imported. This allows you to apply the run list settings to different media and different configurations of samples.

Important: You cannot import a multipanel run list.

To import the run list settings

▶ Do one of the following:

- In the Home window, click import Run List, and then locate and select the run list file that contains the desired setting 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.

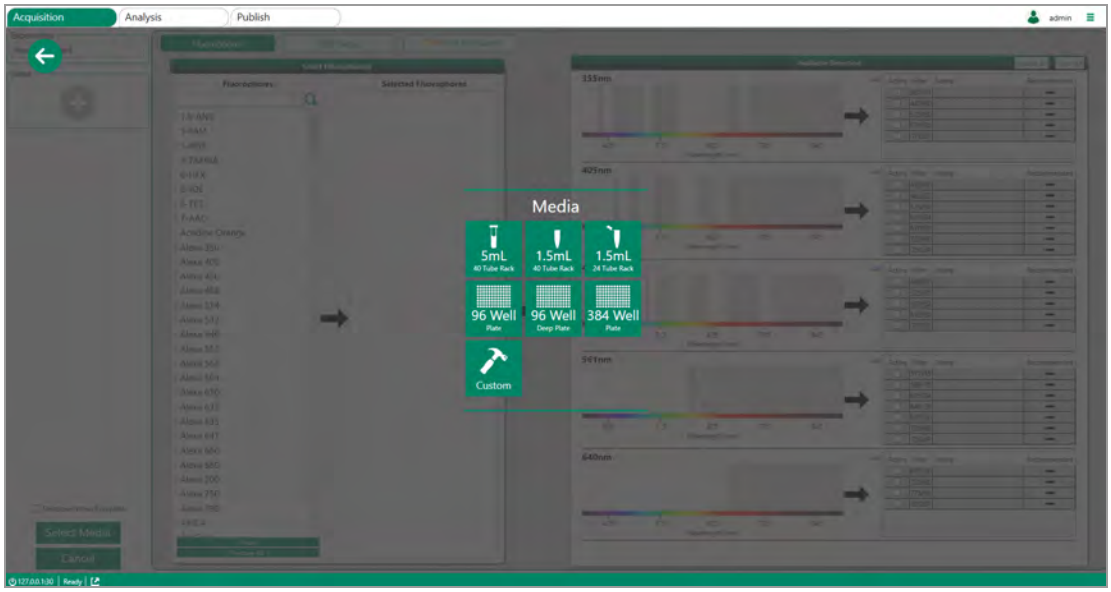


The Experiment Builder opens to the Fluorophores window. You can use the imported fluorophore and detection settings as-is, or you can modify them.

Plate settings are not imported with the run list, so you must set up controls and samples for the experiment on the Plate Setup tab. If you used the compensation template in the previous experiment, the same compensation template will be applied.

Selecting the Media Type

After you start the Experiment Builder, the Media selection window opens.



You can select from the predefined plate or tube layouts, or you can create a custom layout. For more information about media types, see Media Selector on page 80. You must select a media type or click the Back arrow in the upper-left corner to return to the Home window.

Note: You can also change the media type in the Experiment pane of the Experiment Builder. See Changing the Media Type on page 205.

To select the media type

▶ Under Media, do one of the following:

- To use a standard rack or plate type, click the appropriate button. The corresponding plate layout appears when you select the Plate Setup tab.
- To create a custom rack or plate type, click Custom. For information on creating a custom plate layout, see Creating a Custom Media Type on page 199.

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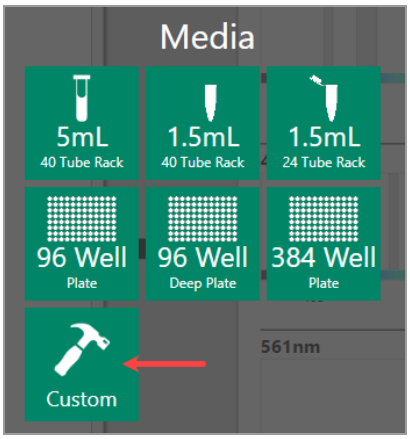
Creating a Custom Media Type

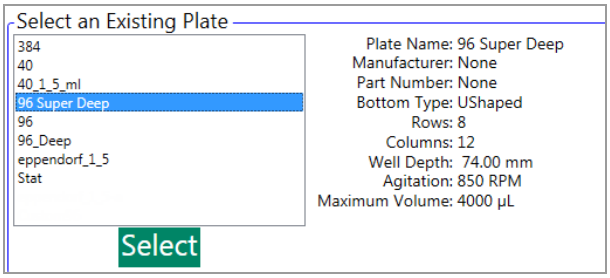
If none of the six predefined media types match the device you intend to use, you can create a custom media type.

To create a custom media type

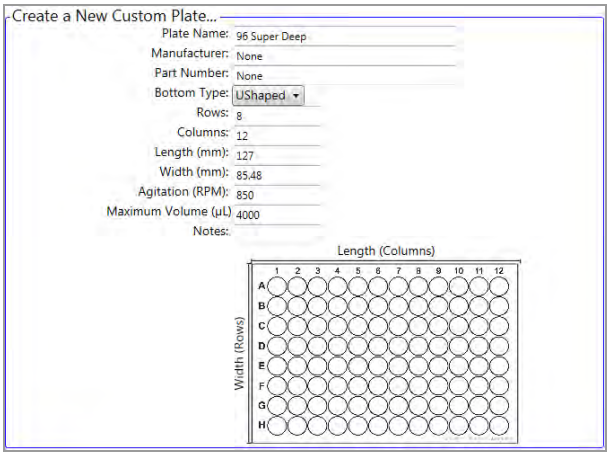
1. In the Media list, click Custom.



2. (Optional) If your custom device is similar to an existing plate (device), select it to use as a template.



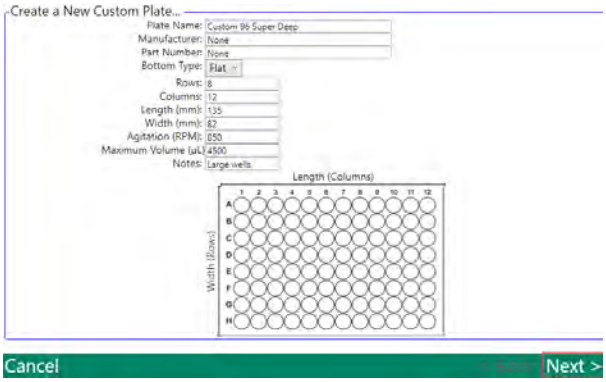
The settings for the existing plate populate the settings for the custom device.



3. In the Create a New Custom Plate area, type a name for the new custom media.

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

4. Edit plate settings as necessary and then click Next.



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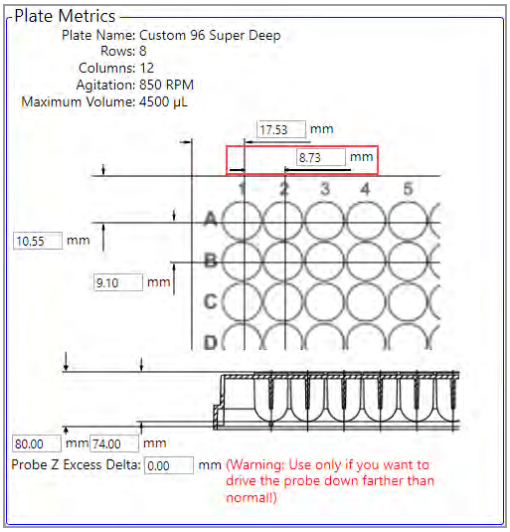
5. In the Plate Metrics area, click inside each text box to change the value within.

   The value in each box corresponds to the horizontal or vertical distance between the arrows nearest the box. For example, the highlighted arrows in the image that follows indicate the horizontal distance between the centers of two adjacent wells (A1, A2), which is 8.73 mm.



   Note: If the probe does not drop low enough into a well, enter a value for Probe Z Excess Delta to drive the probe down lower. If the probe hits the well bottom, this information is used in calibrating the probe Z value.

6. Edit the dimensions as needed and click Next.
7. Place the custom device into the sample loader and press Continue.

8. 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.

9. 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.

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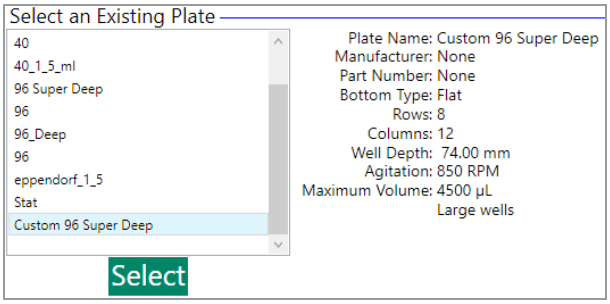
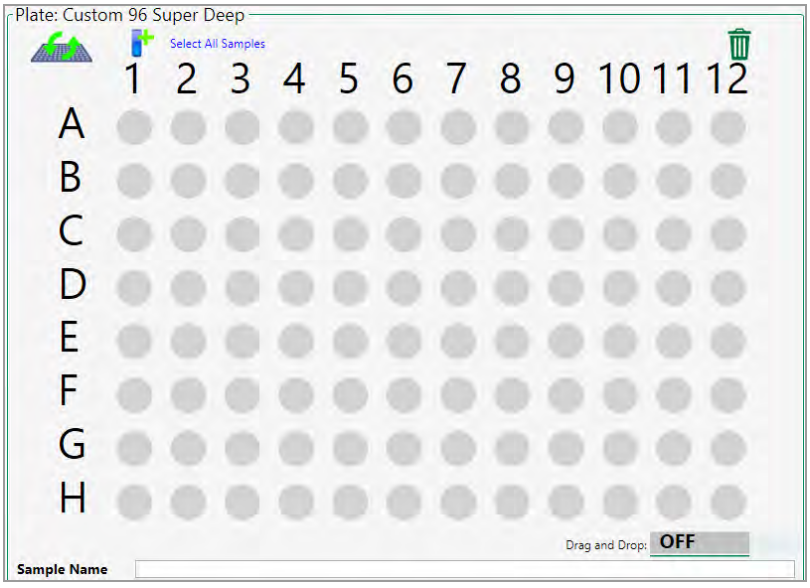
To select a custom plate

1. In the Media list, click Custom.
2. Select the custom plate from the list of plates and then click Select.

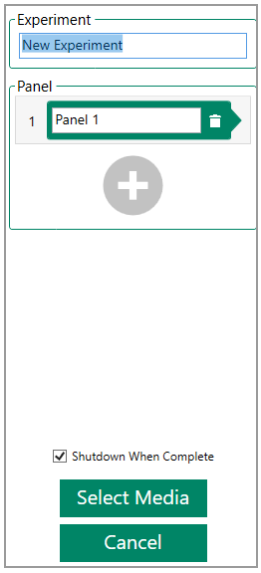


The layout of the custom device appears in the Plate Setup panel.



Working in the Experiment Pane

After you select the media type the Experiment Builder opens to the Fluorophores window, and the Experiment pane appears on the left..



From the Experiment pane, you can

- Replace the default experiment and panel names
- Add or delete panels

  Note: The + button to add a panel is enabled when you navigate to the Plots and Gates window. You must follow the experiment workflow before you can add a new panel or edit an existing panel. For information, see Experiment Workflow on page 79.
- Specify instrument shutdown
- Select a different plate layout
- Cancel the experiment and return to the Home window

…

To name the experiment and panels

1. Under Experiment, replace the default entry with a name for your experiment.

   Note: This name serves as the umbrella name for all associated panels. If you skip this step, Everest Software uses the default New Experiment as the experiment name. You can enter or change your experiment name at any time before you apply the experiment.

2. Under Panel, enter a name to replace the default entry. If you are editing an existing experiment, change the panel name for any panel.

   Note: You can enter panel names for subsequent panels as you add them to the experiment.

3. (Optional) To select a different media layout, click Select Media. For information, see Changing the Media Type on page 205.

4. (Optional) To cancel the experiment and return to the Home window, click Cancel.

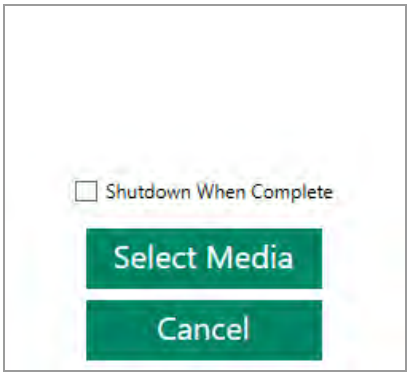
Changing the Media Type

You can select a different media type while you are setting up your experiment in the Experiment Builder.

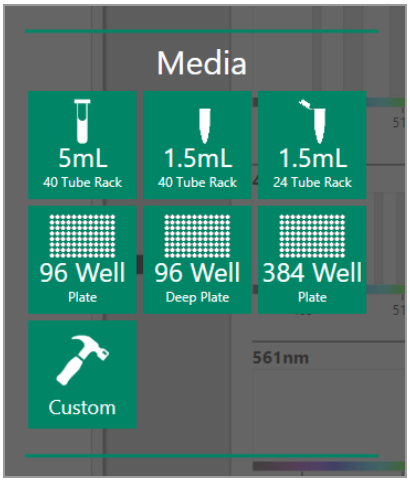
Important: Your selected fluorophores remain, but you must reconfigure your plate and plots if you change the media. You cannot change the media type if you have configured more than one panel.

To change the media type

1. In the Experiment pane, click Select Media.



The Media Selector appears.



2. Select a different media layout.

   A warning message appears, telling you that you will lose your current plate setup.

3. To proceed, click Continue.

   Note: To return to the Experiment Builder without changing the media layout, click Cancel.

   The Fluorophores window reappears, showing the fluorophores you selected.

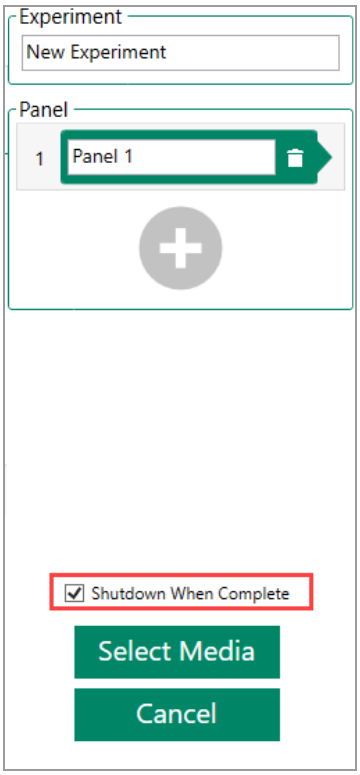
4. Select the Plate Setup tab and reconfigure your plate.

Specifying Shutdown Upon Completion

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

To specify shutdown upon completion

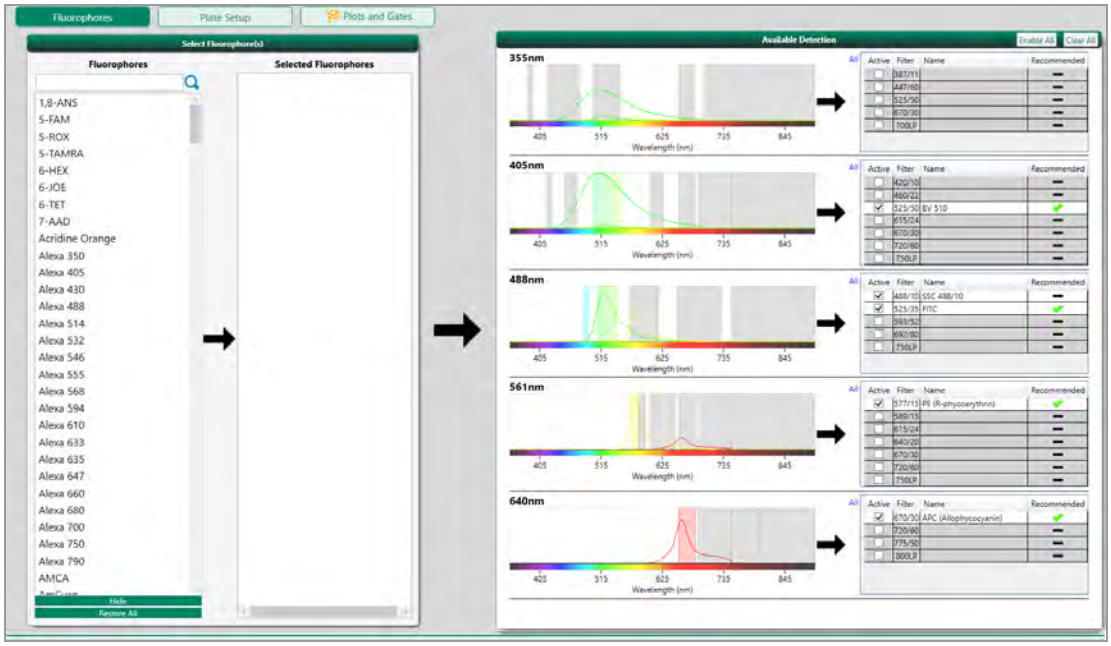
▶ In the Experiment pane, select the Shutdown When Complete checkbox.



Selecting Fluorophores

The Fluorophores tab displays all available fluorophores on the left in alphabetical order. The tab is selected by default when the Experiment Builder opens. You must select at least one fluorophore or detector before you can select the Plate Setup tab.

Note: If you are creating an experiment with more than one panel, you must complete the workflow for the first panel before you can create a second panel. For information, seeExperiment Workflow on page 79 and Multipanel Experiments on page 114.



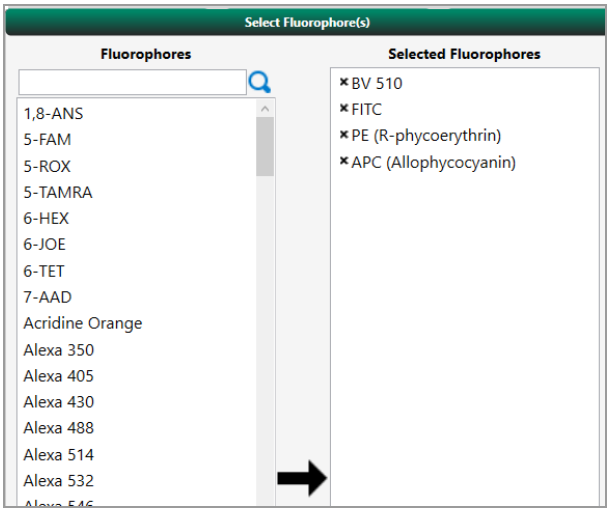
To select fluorophores

1. Scroll through the list on the left or use the Search field to locate each fluorophore.

   Tip: You can filter to specific fluorophores by entering letters or numbers in the Search field.

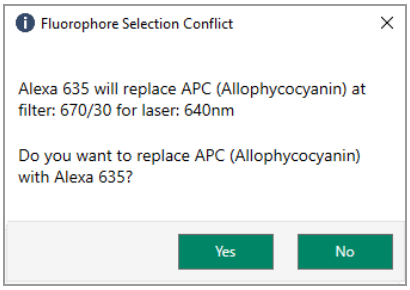
...

2. Under Fluorophores, double-click each fluorophore to add to the experiment.



Each fluorophore moves to the Selected Fluorophores list. Selected fluorophores are ordered by excitation emission range.

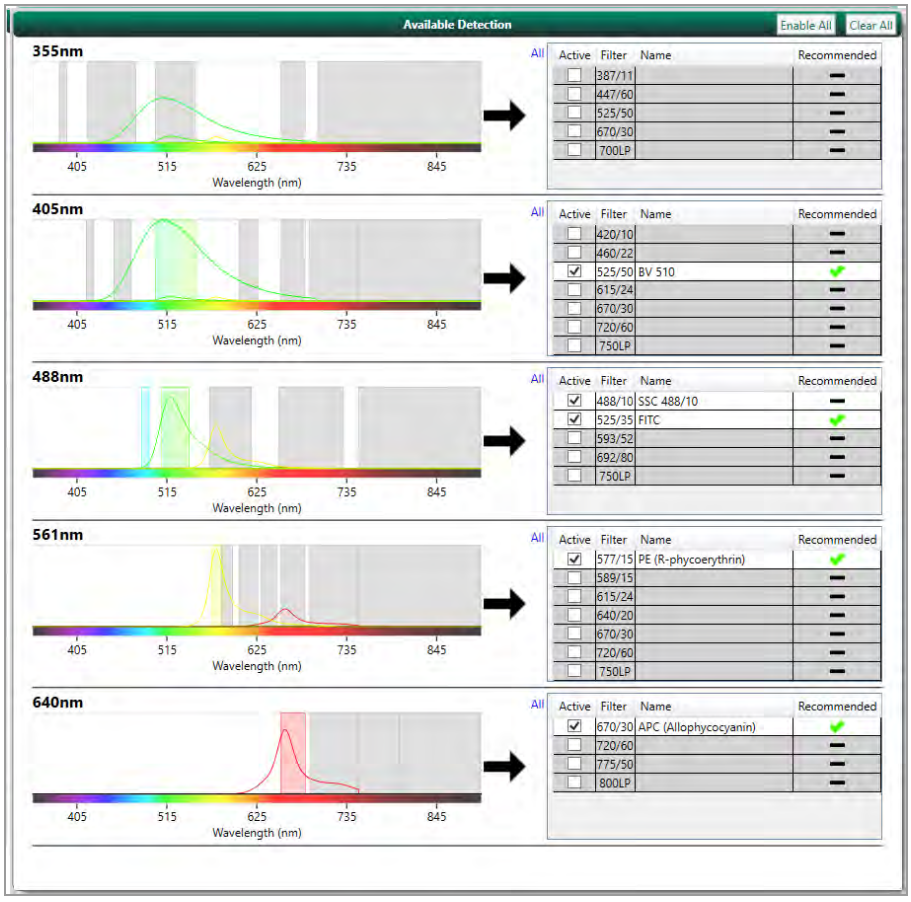
Note: If you select a fluorophore that conflicts with a fluorophore you have already chosen, an advisory message appears. You can click Yes to replace the existing fluorophore with the new one, or No to keep the existing fluorophore.



Activating Available Detectors

As you add fluorophores, the following occurs in the Available Detection panel:

- The emission spectra of each fluorophore appear on the emission plot associated with the optimal excitation laser line of the fluorophore.
- 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.
- You can add information, such as cell marker identifiers, next to the fluorophore name and the names are appended to the headings of the custom labels grid.

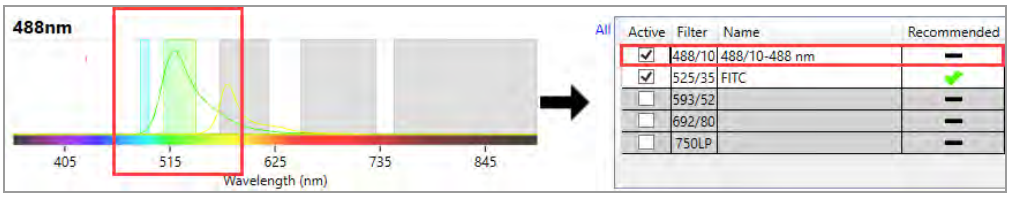


In a spectral graph containing multiple spectra, you can click on a point in the spectral line to view the name of the corresponding fluorophore. Experienced users can also manually enable detectors.

An error message appears if all detectors are removed.

To manually enable a detector

1. Select the Active checkbox for the detector.



2. (Optional) Do any of the following:

   - To enable all detectors for a particular laser, click All for that laser.

     Note: You can also enable all detectors for all lasers, but this is not recommended.

   - To clear all detectors, click Clear All.

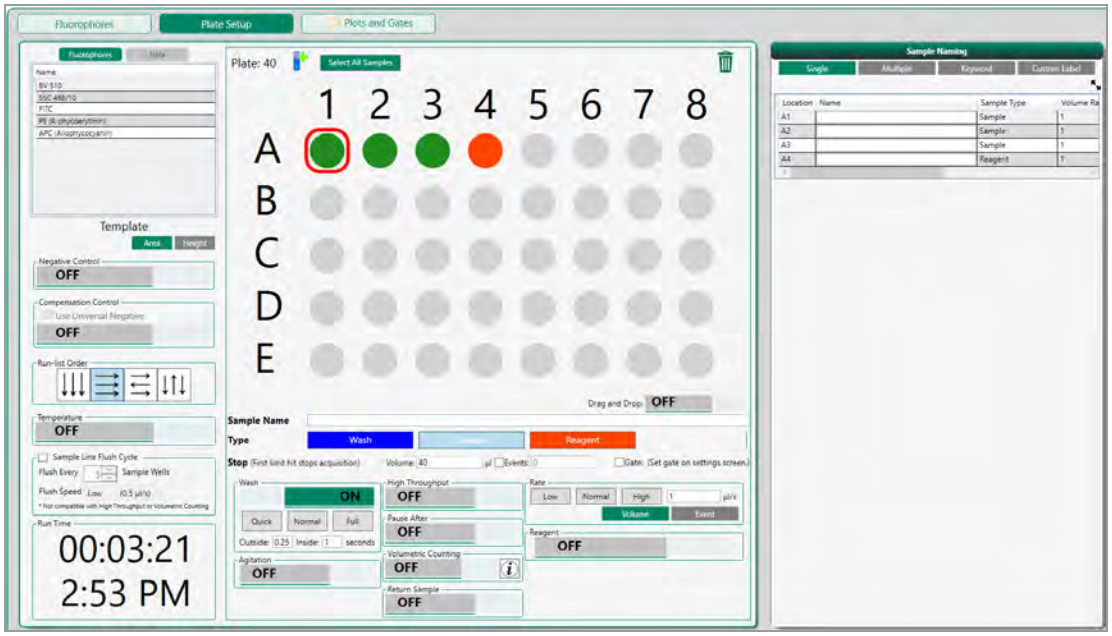
   Important: Manually adding or changing detectors can be done from the Fluorophores window only.

3. (Optional) Edit the parameter Name to include more descriptive information for each detector, such as target (for example, CD19).

   Note: Changes in the Name column apply to the selected panel only.

Configuring the Plate

Use the settings in the Plate Setup window to identify well contents and configure your controls and samples. Use the Sample Naming pane to update sample information, add keywords, or set up custom labels.



Important: If you are setting up an experiment with multiple panels, you must select the Plots and Gates tab before you can add a new panel.

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Setting Up Compensation Controls

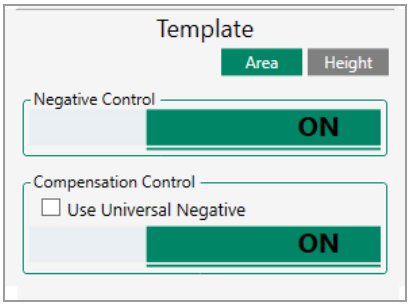
To use automatic compensation in Everest Software, you must enable the compensation template. This tool creates control positions that integrate with the automatic compensation process and are automatically added to the plate map.

Notes: For multipanel experiments, you can set up different compensation controls in each panel that you configure.

To set up compensation controls

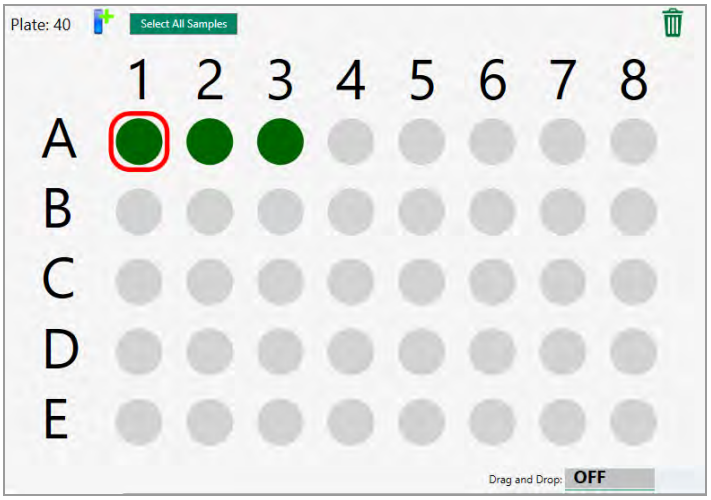
1. In the Template area of the Selected Fluorophores panel, select the pulse parameter (Area or Height) that you want to compensate. Area is selected by default.
2. (Optional) To choose the Height parameter, click Height before you continue.

   Important: Neither button is selectable after you enable one or both controls in steps 3 and 4.



3. To include a negative control tube or well in the experiment, click the Negative Control toggle to the ON position.
4. To use this negative control as a universal negative in automatic compensation calculations, select the Use Universal Negative checkbox.
5. To automatically create compensation control positions in the plate setup, click the Compensation toggle to the ON position.

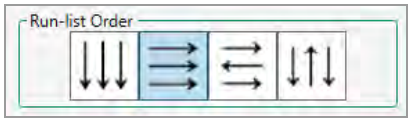
In the plate map, positions are filled with controls starting with the first available well.



Setting the Run List Order

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

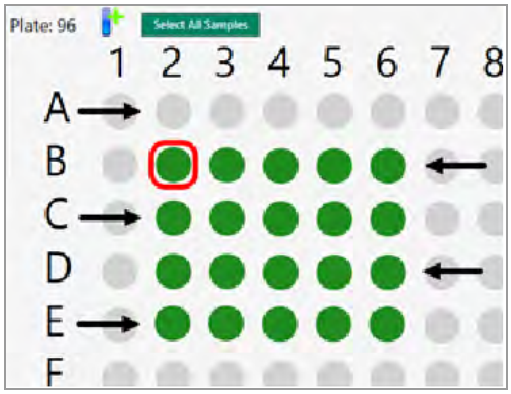
The default setting is left-to-right, as shown below.



Note: The run list order you select applies to all panels you configure in your plate.

If you select a serpentine run list order (or), the ZE5 Cell Analyzer recognizes the initial rows or columns in its acquisition pattern, whether or not you have filled those wells with sample.

For example, when the back-and-forth pattern is selected, the ZE5 moves left to right in Row A, even though no wells are actually acquired, and then begins acquiring wells in Row B from right to left.



The run list for the configuration shown above appears in the following order:

B6-B5-B4-B3-B2
C2-C3-C4-C5-C6
D6-D5-D4-D3-D2
E2-E3-E4-E5-E6

If you select up-and-down for the same well configuration, the run list appears as shown below, going past the wells in column 1 in a downward direction, and acquiring wells in an upward direction starting at well E2.

E2-D2-C2-B2
B3-C3-D3-E3
E4-D4-C4-B4
B5-C5-D5-E5
E6-D6-C6-B6

To set up the run list order

▶ Click the Run-list Order button that displays 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.

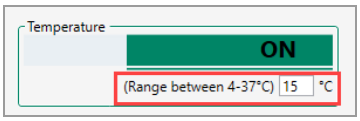
Activating Temperature Control

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

Temperature activation and setting apply to the entire plate.

To enable temperature control

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



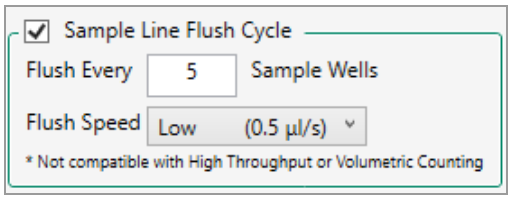
Note: Allow the ZE5 Cell Analyzer 5 min to reach the target temperature after activating temperature control.

Prompting Periodic Sample Line Flushes

To reduce occurrences of clumping, you can configure your plate settings to prompt a sample line flush after a selected number of wells have been processed, and at a selected speed.

To begin the wash step, the sample probe moves to the Stat Tube position and runs the cleaning solution at the selected speed. After the wash, the probe moves to the wash station, where a rinse is completed at the same speed. When the process concludes, the instrument restarts the run with the next well in the sequence.

Note: This option is disabled if you are using high-throughput or volumetric counting.



To define a flush cycle during your experiment

1. Select the Sample LIne Flush Cycle checkbox.
2. In the Flush Every ___ Sample Wells field, enter a flush point after a certain number of wells. The maximum number is 384.
3. Click the Flush Speed dropdown arrow and select a flush speed (low, normal, or high).

Selecting or Moving Wells in the Plate Layout

You can select a single well or multiple wells in the plate layout.

- Single well
- Multiple adjacent wells
- Multiple non-adjacent wells

To select wells

▶ Do one of the following:

- To select a well, click it.

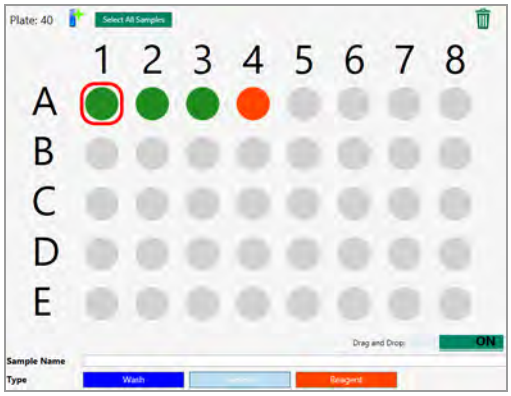
- To select multiple, adjacent wells, click the first well and then drag to select additional wells. All selected wells are surrounded by a red border.

- To select multiple nonadjacent wells, press and hold Ctrl and click the wells.

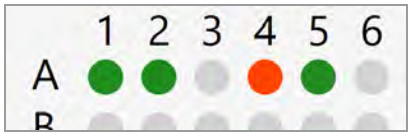
To move wells

▶ To change the location of a single well or multiple sequential wells:

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



b. Drag one or more adjacent filled positions to empty ones.



c. Click Drag and Drop to turn off the feature.

Tip: You can set up a single plate or tube rack with multiple experimental panels. For more information, see Multipanel Experiments on page 114 and Setting Up Multiple Panels on page 285.

Assigning Position Types

Use these instructions to assign a position type to each well.

Note: To add a stat tube to the plate layout, see Adding a Stat Tube to a Panel in the Plate Layout on page 297.

To assign position types

▶ Select one or more wells and click one of the Type buttons (Wash, Sample, or Reagent).

Selected positions are highlighted with a red border.

Note the following:

- Wash positions contain wash solution.
- Reagent positions contain reagent that can be added to other positions in the plate map.
- If you enabled negative and compensation controls, the setup positions are already configured.

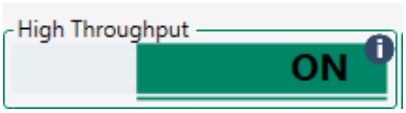
Selecting Standard or High-Throughput Acquisition

In the default standard (single sample) Acquisition mode, there is only one sample in the sample line at any given time. When high-throughput mode is enabled, samples are aspirated continuously; the sample line contains multiple samples, each sample is 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 98.

To select high-throughput mode

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



3. (Optional) Click the information icon, which appears when you enable high throughput. The message contains suggested Wash settings for the different flow rates. For more information, see High-Throughput Mode on page 98.

Note: In high-throughput Acquisition mode, the Pause After, Volumetric Counting, and Return Sample functions are disabled, and you can change the volume rate but not the event rate. High-throughput mode does not support event limits or gate limits; only volume limits can be used. All samples in a high-throughput run must use the same target flow rate.

Pausing after a Tube or Well

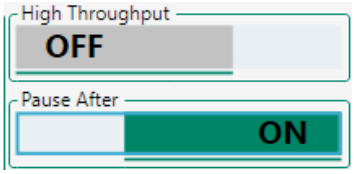
Note: Pause After is disabled when high-throughput mode is enabled.

Pause After can be useful when applying automatic compensation. You can enable 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.

You can pause after any well in any panel.

To pause after a tube or well

1. In the plate map, select the relevant positions.
2. Click the Pause After button.



The run list pauses after the applicable position has been acquired. Positions for which pause after is enabled are marked with an image in the plate map. For more information, see Position Images on page 91.

Setting Volumetric Counting on Samples

You can enable Volumetric Counting to verify the number of cells in a specified volume of each sample. Activating Volumetric Counting automatically enables Return Sample, where excess sample in the line returns to the tube or well after target volume is acquired.

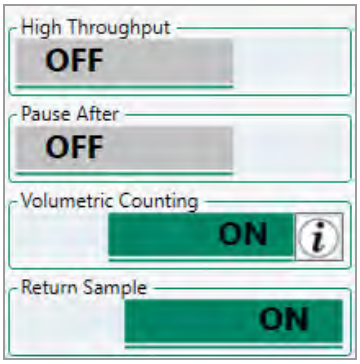
Important: Bio-Rad makes no claims of accuracy of the concentration statistic generated by the ZE5 Cell Analyzer. For information, click the *i* icon under Volumetric Counting.

In multipanel experiments, volumetric counting is enabled or disabled by panel rather than for the entire plate.

Note: You can set Return Sample to OFF if necessary. However, when using Volumetric Counting with Return Sample set to OFF, you might experience sample loss. If sample loss is a concern, run Volumetric Counting with Return Sample set to ON.

To set volumetric counting

1. In the plate map, select the relevant positions.
2. Click the Volumetric Counting button.

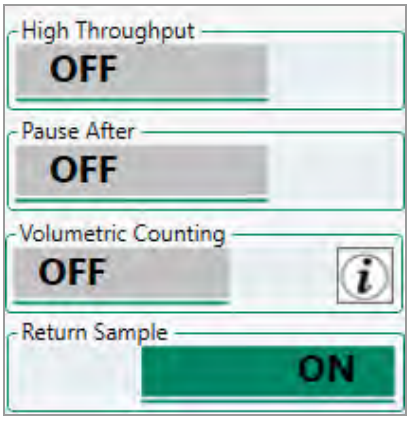


Returning Sample to a Tube or Well

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

To return sample

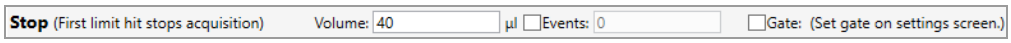
1. In the plate map, select the relevant positions.
2. Click the Return Sample button.



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 you select more than one type of limit, acquisition stops when the first limit is reached. For information about maximum volumes for various media types, see Stop Settings on page 94.



You can set different stop conditions for different panels.

Note: If high-throughput mode or volumetric counting has been enabled for a position, you can use only a volume limit.

To set stop conditions

1. In the plate map, select the relevant position.
2. Select one or more of the following checkboxes and enter the appropriate limit:
   - **Volume** — acquisition stops when the total acquisition volume reaches the specified value.

     Note: Default volume settings are based on the media type and whether events are also selected.
   - **Events** — acquisition stops when the total event count reaches the specified number.
   - **Gate** — sets the volume limit to the maximum for the position. You can configure gate limits in the next window (Settings) and change the volume to reflect what is in the tube or well if needed.
3. To set different limits for other positions, repeat Steps 1 and 2 for other positions in the run list.

Configuring Wash Settings

By default, all positions are configured to include a 0.25 sec outside wash and a 1.0 sec inside wash, but you can choose an automatic wash option or configure the wash manually. You can configure different wash settings on different panels.

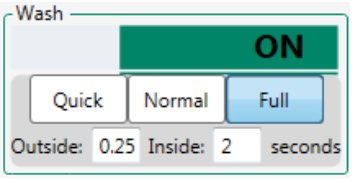
Bio-Rad highly recommends that you keep the enabled wash step for all positions, especially when using high-throughput sampling mode.

To configure wash settings

1. In the plate map, select the relevant positions.

   Note: Washes are performed after acquisition of the position.

2. To change the wash time, click the appropriate wash option.



   - **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

   Important: For information on specifying Wash settings in high-throughput mode, see High-Throughput Mode on page 98.

Activating Agitation

For information on agitation settings, see Agitation Settings on page 95. You can enable agitation on wells on any of your panels.

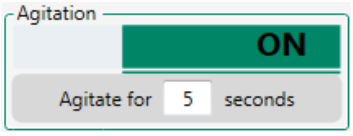
To enable agitation

1. In the plate map, select the relevant positions.

   Note: Agitation is performed before acquisition of a position.

2. Click the Agitation button.

3. Enter the agitation time.



   Positions for which agitation is enabled are marked with an image in the plate map. For more information, see Position Images on page 91.

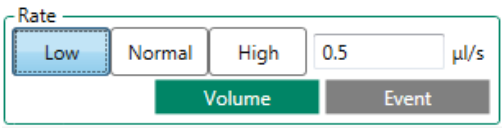
Specifying Flow Rate or Event Rate

You must assign a volumetric target flow rate or target event rate for all samples. By default, a rate of 1 µl/sec is assigned when positions are enabled. You can set a different flow rate for each panel.

Note: In a high-throughput run, the Event Rate option is not available, and all samples must use the same target flow rate. You can change the flow rate for all wells but not selected wells.

To specify a target flow rate

1. In the plate map, select the relevant positions.
2. In the Rate area, verify that Volume is selected.
3. To change the target flow rate, do one of the following:
   - Click the appropriate flow rate option.
   - Manually enter the flow rate into the Rate box.



To specify a target event rate

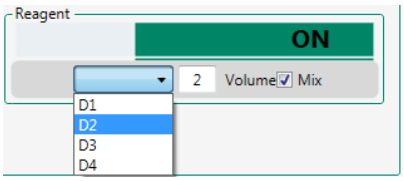
1. In the plate map, select the relevant positions.
2. In the Rate area, click Event.
3. Enter the target event rate into the Rate box.

Adding Experimental Reagents

For information about experimental reagents, see Reagent Settings on page 101. You can add reagent to wells in any of your panels.

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 add reagent.
3. Click the Reagent button.
4. Select the position from which to add reagent in the dropdown list.
5. Specify the reagent volume in the Volume box.
6. 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 when Mix is selected. Positions to which reagent will be added are marked with an image in the plate map. For more information, see Position Images on page 91.

Sample Naming—Labeling Positions Manually

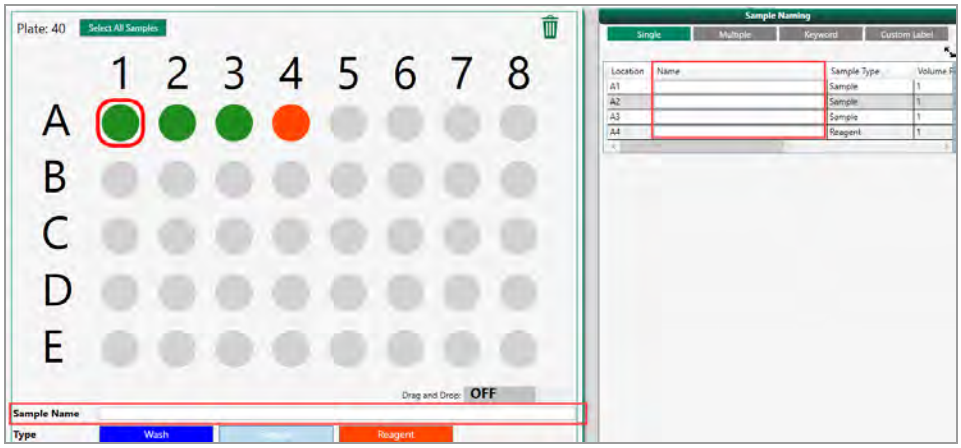
The Single tab in the Sample Naming panel is selected by default, and allows you to label individual positions one at the time. Names are written to the FCS file associated with each position.

Note: If you enabled Compensation, the compensation template automatically names each Setup position based on the corresponding fluorophore name.

To label positions manually

1. In the plate map, click the sample to label.
2. Do one of the following:
   - Type a name in the Sample Name box below the plate map.

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



   - Type a name for the selected location in the Single tab on the Sample Naming window.

     Tip: Press Enter to move to the next row.

…

Sample Naming—Labeling Positions Automatically

The Multiple tab in the Sample Naming panel allows you to label multiple positions at the same time using naming components. You can label several positions with the same naming components, or the whole plate. As with single naming, the names are written to the FCS file associated with each position.

To name positions automatically

1. In the plate map, select the positions to label.

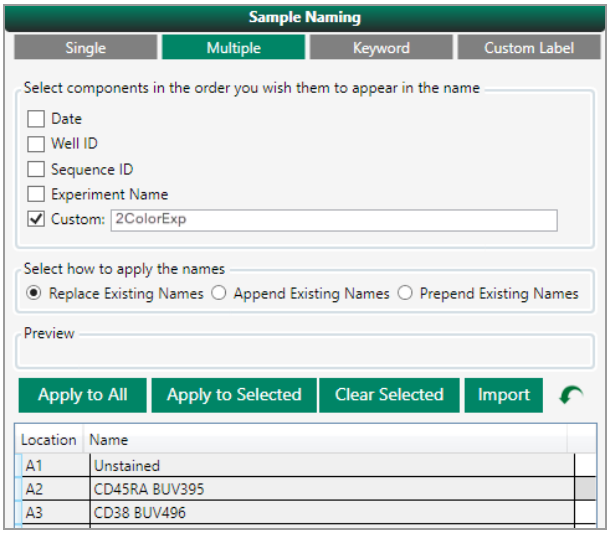
   Tip: If you plan to use the same name components on all positions, you can skip this step.

2. Select the Multiple tab in the Sample Naming panel.

   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** — name of the experiment from the Experiment pane
   - **Custom** — allows you to create your own prefix or suffix

3. Select the checkbox for the components to include in the name.
4. To use the Custom name component, type a value in the Custom box.



5. Specify whether the name components should
   - Replace existing names
   - Be added to the end of existing names (append)
   - Be added to the beginning of existing names (prepend)
6. Do one of the following:
   - Click Apply to All to apply the name to all positions.
   - Click Apply to Selected to apply the name to the selected 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.

Setting a Default Spreadsheet Application

To use the import features for keywords and custom labels, you must ensure a default spreadsheet application is installed and associated as the default application to open CSV files.

To verify or set a default app

1. In the Windows toolbar at the bottom of the window, click the search icon and enter Default Apps.
2. When the window appears, click Default apps to display the Default apps window.
3. Scroll down and click Choose default apps by file type.
4. Scroll down the list on the left until you see .csv.
5. Do one of the following:
   - If an app is already associated with the .csv file type, close the window.
   - If Choose a default with a + sign appears, click Choose a default and select a spreadsheet application such as Microsoft Excel or LibreOffice, and then close the window.

     Note: LibreOffice is already installed on the Everest computer. However, if you want to use a different application , then you might need to install it and then repeat this procedure.

Sample Naming—Setting Up Keywords

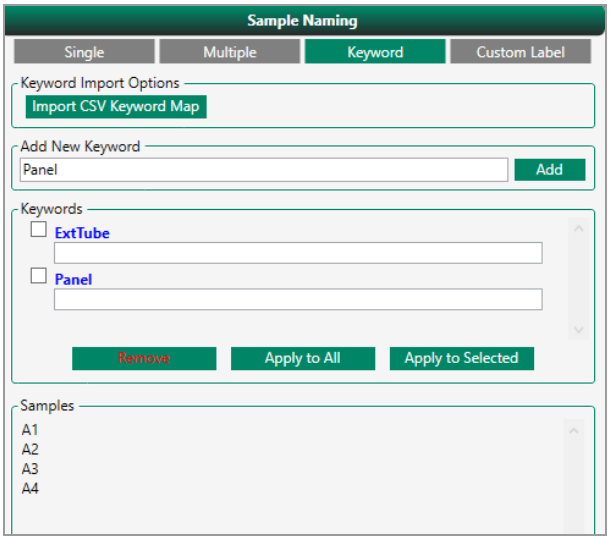
You can manually define or import keywords for additional sample naming flexbility. Keywords are written to the FCS file during acquisition and can be used for batch processing in third-party analysis software.

For information on importing keywords, see Importing Keywords and Values on page 232.

To manually create keywords and values

1. In the Sample Naming panel, select the Keyword tab.
2. Type a keyword in the Add New Keyword box and click Add.

   The keyword appears under Keywords.



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

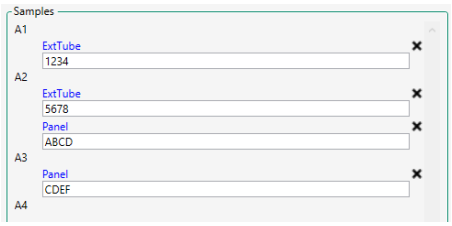
To assign keywords and values to wells

1. Select one or more wells in the plate.
2. Under Keywords, select the checkboxes for the keywords to associate with the selected wells.
3. Click Apply to Selected.

   Tip: To apply the selected keywords to all wells, click Apply to All.

   The applied values appear in the Samples area in the Keyword tab.

4. In each field below the keyword, enter the appropriate value for the keyword.



To remove a keyword from the keyword list

1. In the Keywords area, select the checkbox for keywords to remove.
2. Ensure the checkboxes are cleared for keywords to keep.
3. Click Remove.

To remove a keyword from a sample position

▶ In the Samples area in the Keyword tab, select the applicable wells, and click the x to the right of the keyword.

Importing Keywords and Values

You can import keywords and their corresponding values from a CSV spreadsheet file created and edited in third-party software (such as Microsoft Excel).

Note: Commas cannot be used as part of the keywords or values.

Important: Before you begin, you must confirm or identify a default app to open .csv files. For information, see Setting a Default Spreadsheet Application on page 230.

The spreadsheet must be set up in the format shown below, with the Well IDs corresponding to the wells being run in the plate.

	A	B	C	D
1	**Well ID**	**Keyword 1**	**Keyword 2**	**Keyword 3**
2	A1	K1_Value	K2_Value	K3_Value
3	A2	K1_Value	K2_Value	K3_Value
4	A3	K1_Value	K2_Value	K3_Value

Keywords with at least one value can define any part of your experiment parameters. For example, a keyword might be PatientName, with separate values for each well as shown below.

	A	B	C	D
1	**Well ID**	**ExtTube**	**Order_Panel**	**PatientName**
2	A1	70002340521	20-33329_TBBS	John Smith
3	A2	70002340522	20-33330_TCD4	Wilma Flintstone
4	A3	70002340523	20-33331_TBTXC	Betty Rubble

...

You can add keywords to the spreadsheet as needed – for example, PatientAddress or PatientPhone, to identify and organize the value categories. Existing keyword values are overwritten for wells where new data is imported from the spreadsheet.

To import keywords and their associated values from a .csv file

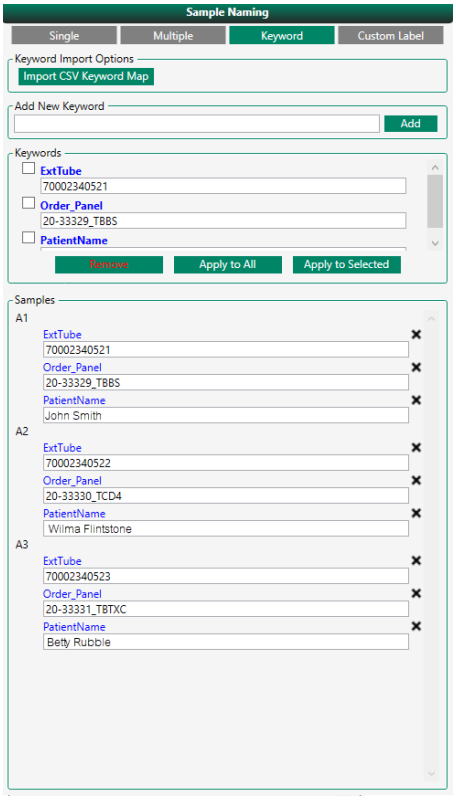
1. In the Sample Naming panel on the Plate Setup window, click Keyword.
2. Click Import CSV Keyword Map.
3. Navigate to the CSV file stored on the instrument computer and click Open.

   Note: By default, Everest Software opens the following path:

   C:\Program Data\Bio-Rad\Everest

   If you change the path, then Everest Software uses the new path until it is manually changed or Everest Software is restarted.

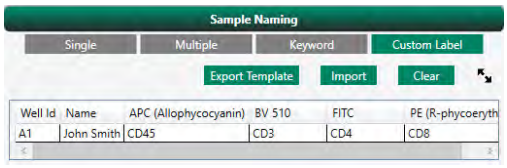
   The data from the file appears in the Keyword display.



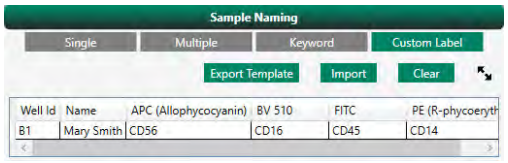
Sample Naming—Creating Custom Labels

You can specify a custom label for each fluorophore in each sample well. Everest Software displays each fluorophore in the heading row by default. If you modified or overwrote the fluorophore in the Name column of the Available Detection Panel (Fluorophores tab), that information appears in the heading row for added flexibility.l

Panel 1

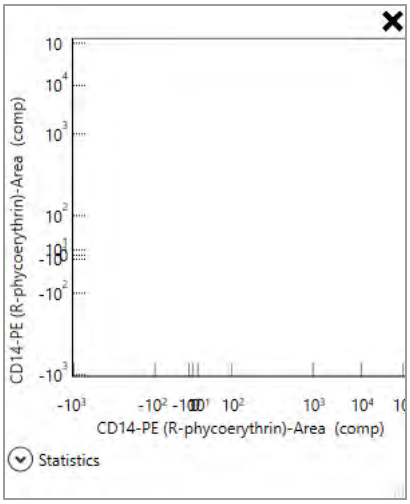


Panel 2

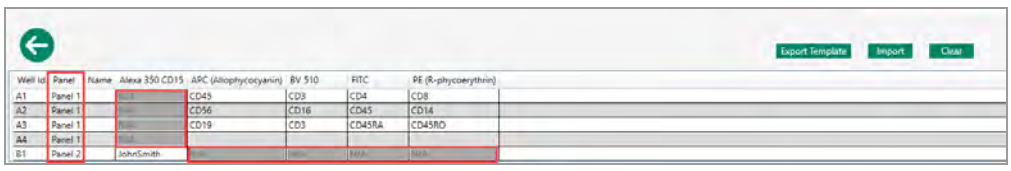


1. For each well, enter a value for the fluorophore in each cell.
2. Repeat for each panel.

   Applicable labels appear before fluorophore names in your plots.



If you expand the Custom Labels grid the panel associated with each well is also displayed.. A fluorophore that is not applicable to a well is grayed out, as shown in the following graphic.



...

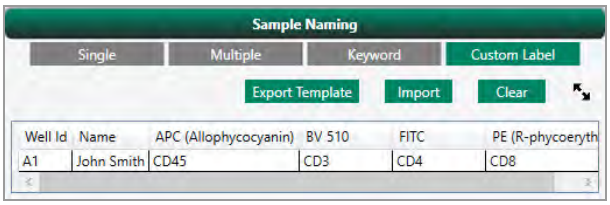
Exporting and Importing Custom Labels

You can export the structure of fluorophores and wells for each panel to a .csv file template, and then add custom labels you will reuse to the template before importing the content into the custom label grid.

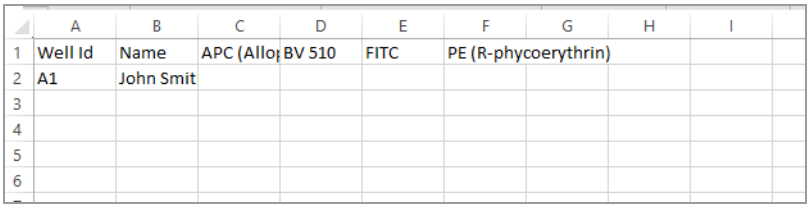
Important: Before you begin, you must confirm or identify a default app to open .csv files. For information, see Setting a Default Spreadsheet Application on page 230.

To export the wells and fluorophore structure to a template

1. Click Export Template.

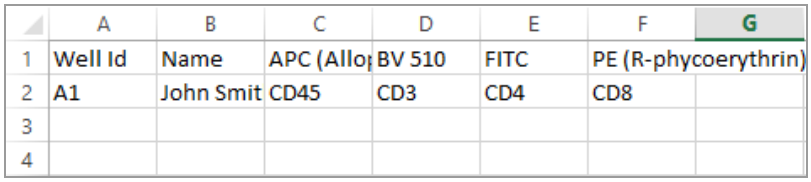


   Everest Software exports the fluorophore and well layout for the selected panel to a .csv file that opens automatically in the default app, as shown below. Fluorophores are ordered alphabetically in the heading row.



2. Do one of the following:

   - If the structure is static but labels will change, you save the structure as a template. Go to step 3.
   - If structure and labels are static, add the labels to the template file before going to step 3.



3. To save the file for reuse, enter a new file name and click Save.

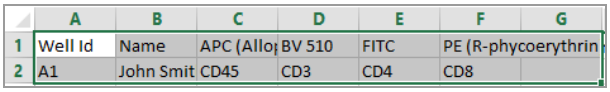
   Note: The default file name is Custom Labels.

Files are automatically saved to C:\Everest\Users\<user name>\LabelExportTemplate.

…

To import the custom labels from the .csv file

1. If necessary, open the applicable .csv file.
2. Select the grid contents for all wells, and then right-click and select Copy.

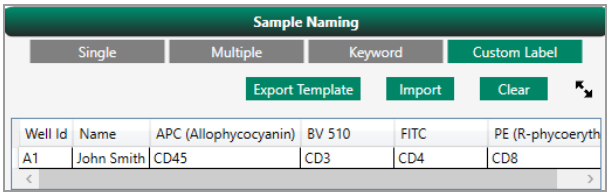


	A	B	C	D	E	F	G
1	Well Id	Name	APC (Allo	BV 510	FITC	PE (R-phycoerythrin	
2	A1	John Smit	CD45	CD3	CD4	CD8	

Note: The row and column headers (A, B, C, etc. and 1, 2, 3, etc.) should be automatically selected; if not, repeat Step 2.

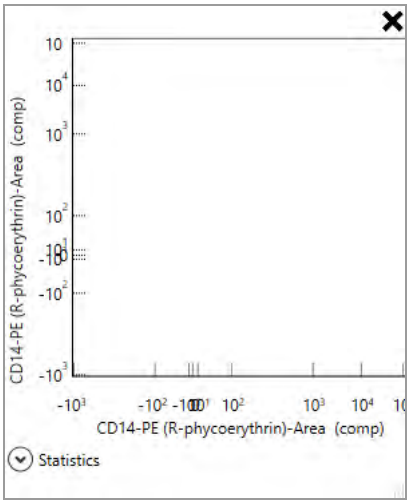
3. In the Custom Label pane for the selected panel, select the first row and click Import.

   The grid is populated with the contents from the template.



4. Repeat for each panel.

   Applicable labels appear before fluorophore names in your plots.



Setting Up Plots and Gates

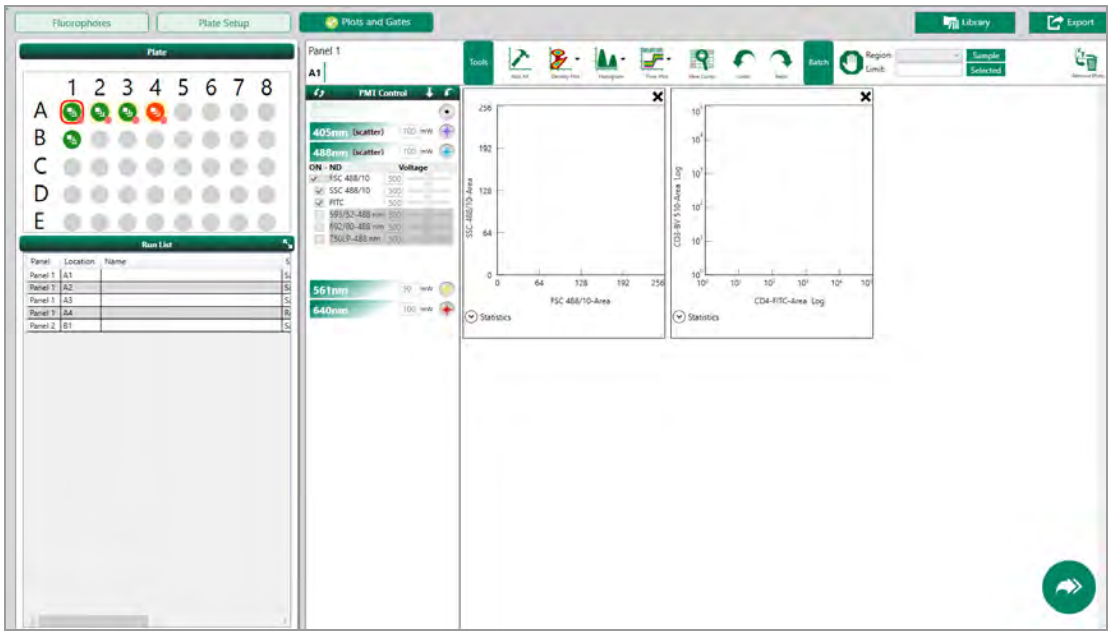
From the Plots and Gates tab, you can

- Create density and time plots, and histograms for your sample wells
- Set up regions and limits
- Export the run list to a file
- Access the instrument settings library to import instrument settings into panels
- Add a panel (returns you to the Fluorophores window)

Important: You can add detectors from the Fluorophores tab only, and you can modify voltages only on the Acquisition window.

For multipanel experiments, note the following:

- You can switch between panels in the plate layout on the Plots and Gates tab.
- When you select one ore more wells, the panel is highlighted in the plate layout, showing red dots on the bottom of the related wells.



Reviewing or Exporting the Run List

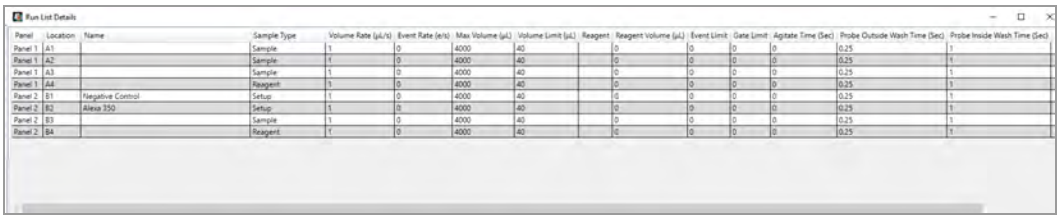
When you select the Plots and Gates tab, the run list appears below the plate layout.

To review the run list and go to experiment settings

1. To display the full run list, click  in the upper right corner

   Note: You can also expand the run list in the Sample Naming panel on the Plate Setup tab.

   The run list details appears in a separate window.



   If you have configured multiple panels, each panel is identified in the column on the left.

2. Inspect each tube or well to verify that all settings are correct.
3. To export the run list, click Exort in the upper-right corner.
4. Close the Run List Details window.

Importing Instrument Settings

From the Plots and Gates tab, you can import compatible compensation and controls settings into individual panels in your experiment. For information, see Instrument Settings Library on page 109.

Note: To create a settings file, complete the elements of your experiment, and then make adjustments on the Acquisition window after you apply the experiment. When the settings are correct, you can save them to the Instrument Settings Library. See Configuring Instrument Settings on page 306.

To import compatible settings into your experiment

1. From the Plots and Gates tab, select a panel in the plate layout.
2. Click the Library button.

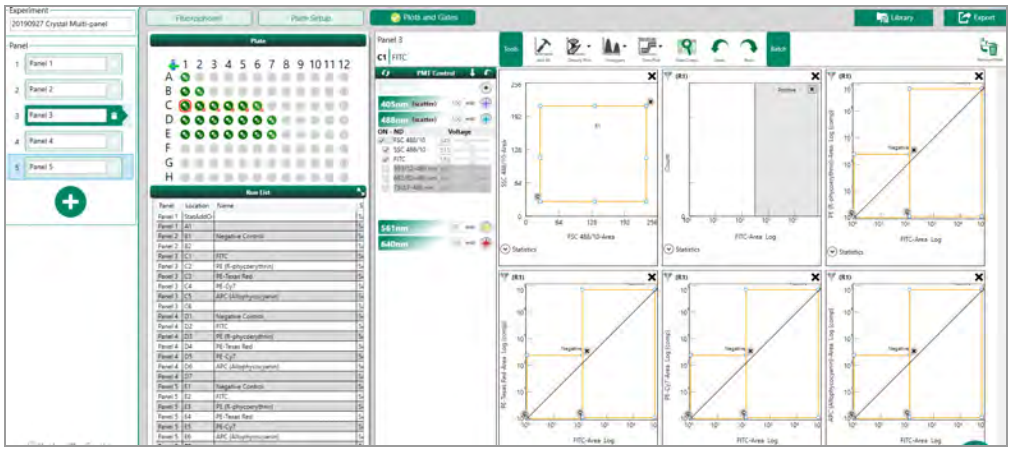
3. (Optional) Enter search criteria into the Search field and click the Search icon to narrow the number of displayed settings files.

   Filtered files are sorted by most recent save date.
4. Select a file and then click Import Instrument Settings.
5. If the file is compatible, Everest Software imports the settings into the selected panel and displays a confirmation message.

   Note: If you try to import a settings file that contains mismatched fluorophores, or is incompatible with your instrument, an error message appears.

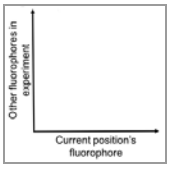
Plots Created by the Compensation Template

If you turned on the Negative Control and/or Compensation Control options in the Template area of the Plate Setup window, Everest Software automatically creates the necessary plots. The negative control position is associated with a forward scatter-by-side scatter plot. Each compensation control is associated with its own plot 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 for a compensation control 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, you must create plots for compensation controls manually.

Creating Plots and Histograms

You can create and modify plots and histograms using the tools that appear when you pause on 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.

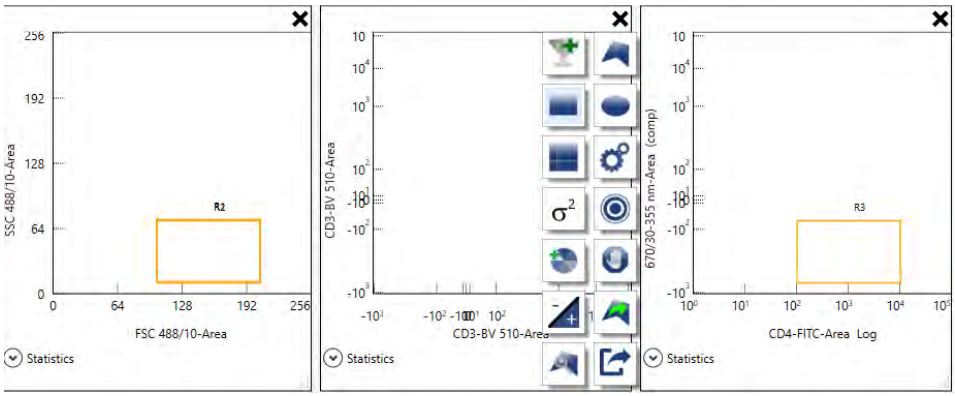


Table 30. Plot and histogram tools and their functions

Tool	Function	Further information
	**Add Polygon Region** — 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 249
	**Add Rectangle Region** — adds a rectangle region to the plot. Applies only to density and time plots.	Adding Regions to Density Plots and Time Plots on page 249
	**Add Ellipse Region** — adds an ellipse region to the plot. Applies only to density and time plots.	Adding Regions to Density Plots and Time Plots on page 249
	**Add Quadrant Region** — adds quadrant regions to the plot. Applies only to density plots.	Adding Regions to Density Plots and Time Plots on page 249
	**Add Bar Region** — adds a bar region to the plot. Applies only to histograms.	Adding Bar Regions to Histograms on page 259

Table 30. Plot and histogram tools and their functions, continued

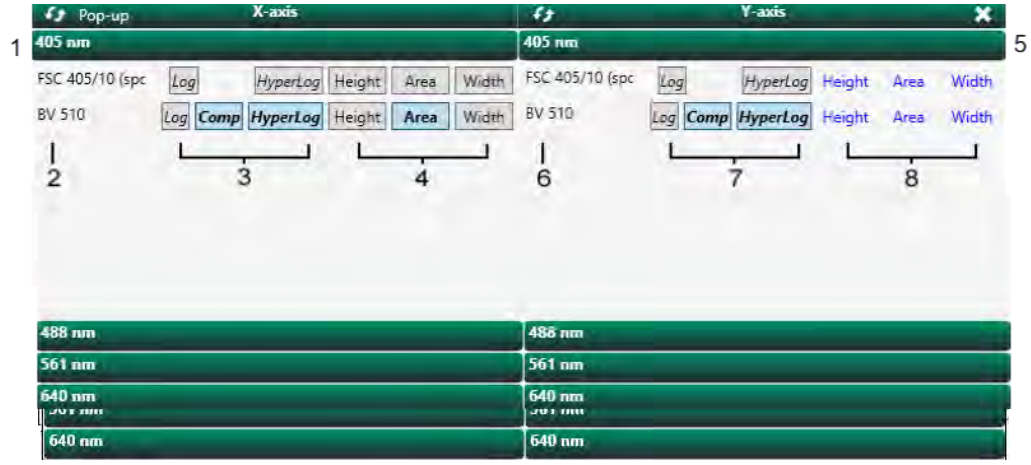
Tool	Function	Further information
	**Modify Plot Parameters** — allows you to modify plot parameters such as fluorophore, compensation, height, width, area, and bin count.	Modifying Plot Parameters on page 266
	**Manage Statistics** — allows you to select the plot statistics to display, such as count, percent, mean, max, min, mode, median, standard deviation, variance, and CV.	Managing Plot Statistics on page 267
	**Assign Hit Regions** — allows you to specify a hit detection region. Applies only to density plots and histograms.	Configuring Hit Detection on page 281
	**Assign Heat Map Region** — allows you to add regions to a heat map.	Applying Heat Maps on page 279
	**Assign Gate Limit Regions** — 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 271
	**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 283
	**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.	Applying a Region to All Plots on page 265

Table 30. Plot and histogram tools and their functions, continued

Tool	Function	Further information
	**Assign Data Track Regions** — 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 277
	**Apply Filter** — applies a gate assignment to the plot.	Applying Filters (Gates) on page 271
	**Remove Filter** — removes a gate assignment from the plot.	Applying Filters (Gates) on page 271
	**Export to PNG** — saves a PNG of the plot to a location that you specify.	Exporting Plots and Histograms on page 284
	**Add Annotation** — allows you to add a note to the plot.	Adding Annotations to Plots on page 263

To visualize data, you can also add density plots, time plots, and histograms manually, or you can let Everest Software automatically create plots.

The following graphic 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 enabled in the Experiment Builder Fluorophores window. 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.

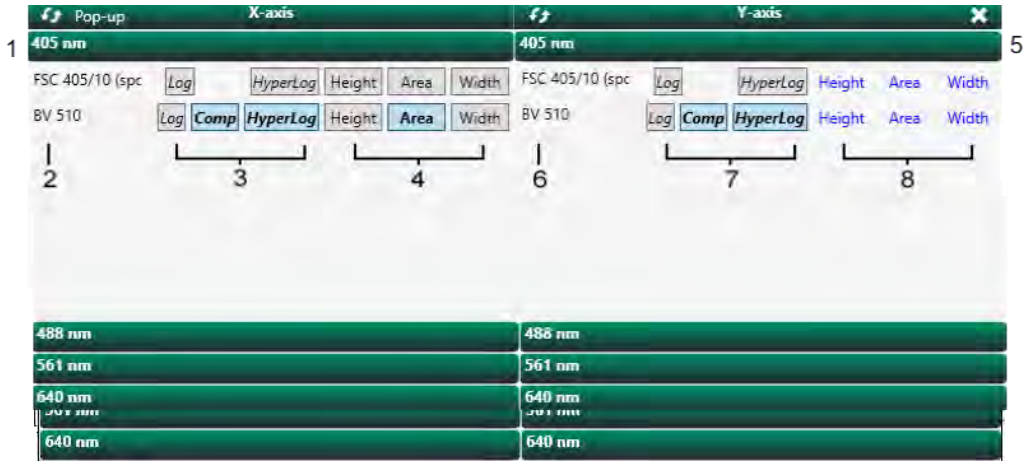
For more information, see Creating Density Plots on page 246, Creating Histograms on page 257, and Creating Time Plots on page 248.

Creating Density Plots

The density plot builder displays the parameters that were enabled in the Experiment Builder Fluorophores window. The parameters that appear in the plot builder reflect those that were enabled in the Experiment Builder Fluorophores window. 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.

Note: When you click Area, the Comp and Hyperlog buttons are selected by default.

The following graphic 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

…

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, the Log is cleared by default..
   - For log scale —Click the log button.
   - Hyperlog scale is selected by default.
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.

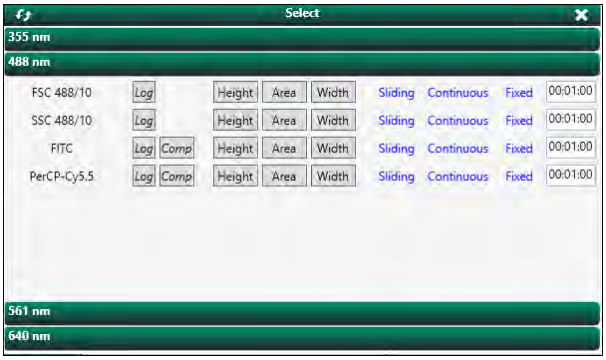
   Everest Software addsThe plot.
7. Click the X or click outside the plot creation dialog box to close it.

Creating Time Plots

The time plot builder displays the parameters that were enabled in the Fluorophores window.

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 31. 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.

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 plot and histogram toolbar appears.

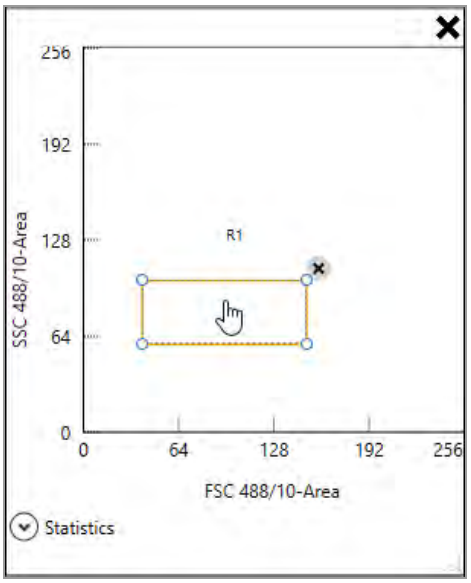
To add a rectangle region to a plot

1. In the plot and histogram toolbar, click Add Rectangle Region.

   A rectangle is added to the plot.

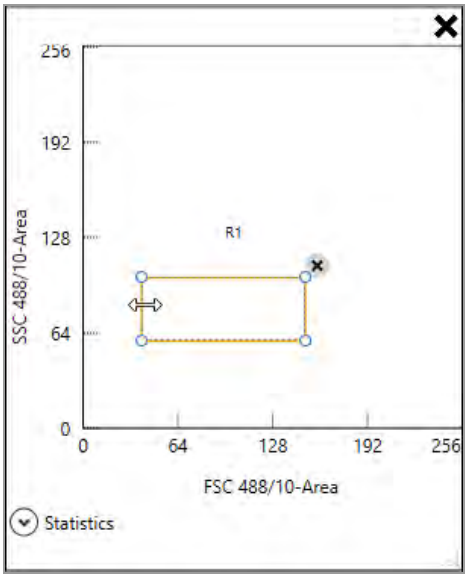
2. To move the rectangle to another part of the plot, point inside the region.

   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.



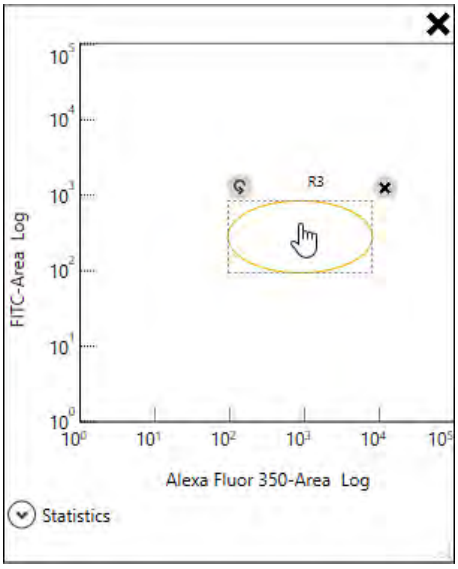
To add an ellipse region to a plot

1. In the plot and histogram toolbar, click Add Ellipse Region.

   An ellipse is added to the plot.

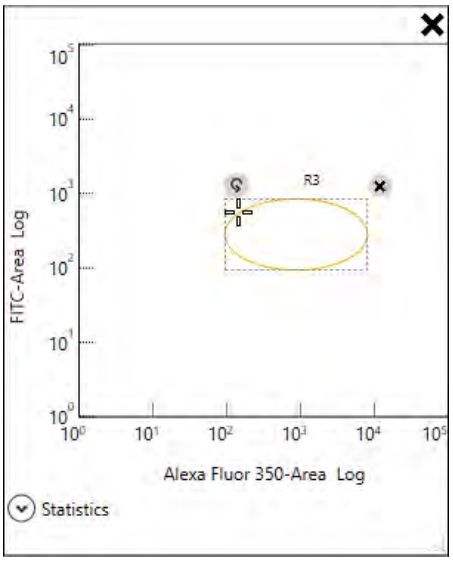
2. To move the ellipse to another part of the plot, point inside the region.

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



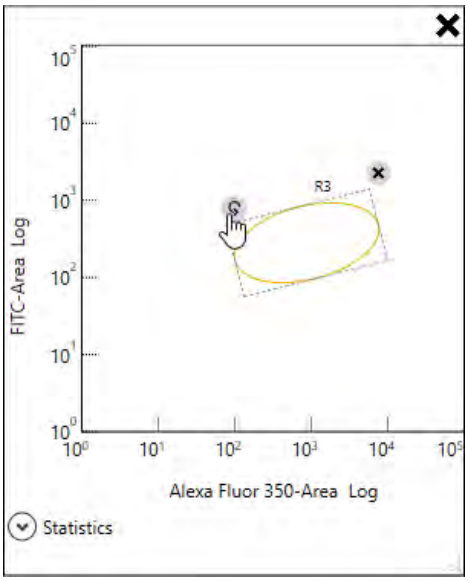
3. To resize the ellipse, point to its outline.

   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

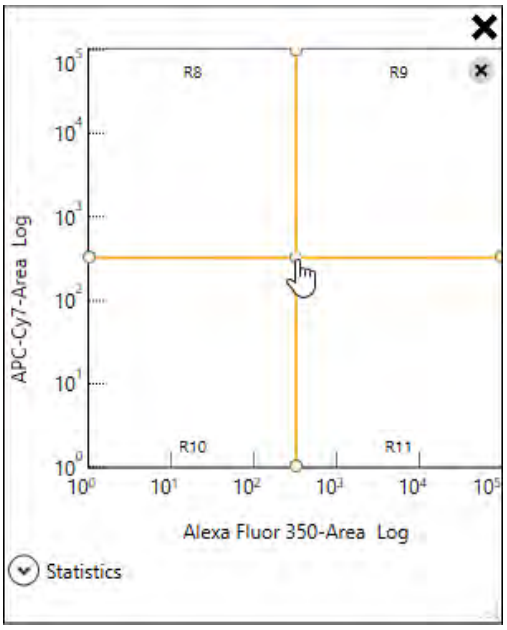
Note: Quadrants apply only to density plots.

1. In the plot and histogram toolbar, click Add Quadrant Regions.

   Quadrants are added to the plot.

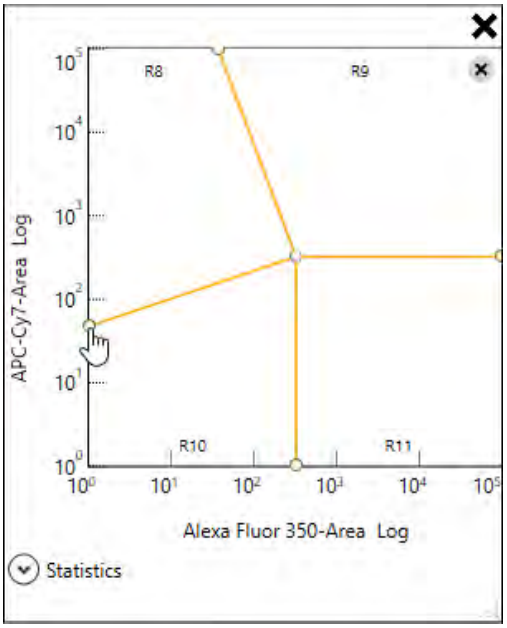
2. To move the quadrant dividers, point to the center circle.

   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.

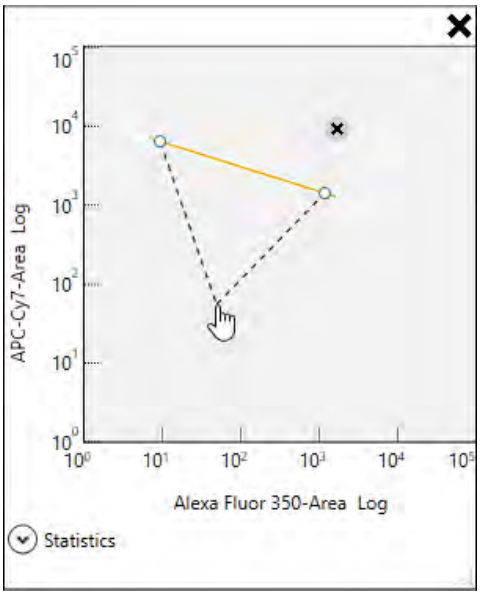
When the pointer changes to a hand, use it to drag the circle along the axis. This changes the shape of two of the quadrants at the same time.



...

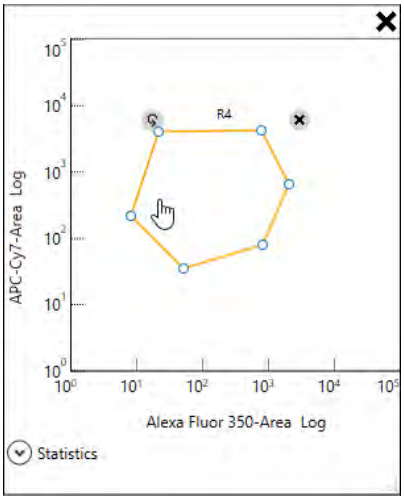
To add a polygon region to a plot

1. In the plot and histogram toolbar, click Add Polygon Region.
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.

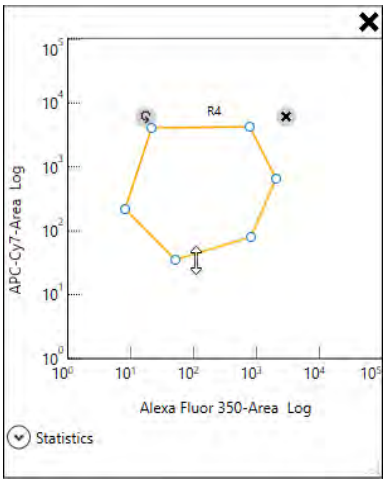


6. To rotate the polygon, point inside the region.

   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 on the upper right of the region.

Creating Histograms

The histogram builder displays the parameters that were enabled in the Experiment Builder Fluorophores window.

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. The Comp and Hyperlog buttons are selected by default. For Log scale, click Log.
4. Specify whether to display compensated data for the parameter.
5. Select the pulse measurement (height, area, or width) for the parameter.

   Everest Software adds the histogram.
6. Click the X or click outside the histogram creation dialog box to close it.

Creating Histograms for All Channels

You can create histograms for all parameters, and do the following in the plot (histogram) builder:

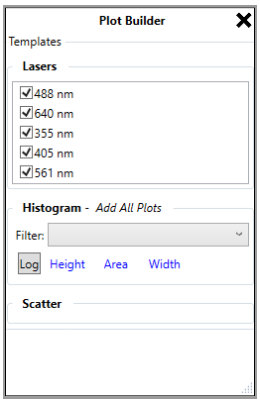
- Apply filters (gates) to all histograms
- Specify whether histogram data are displayed in linear, log, or hyperlog scale

- Specify the pulse measurement (area, height, or width).

To create histograms for all channels

1. Click Advanced Plot Builder in the toolbar.

   The plot (histogram) builder opens.

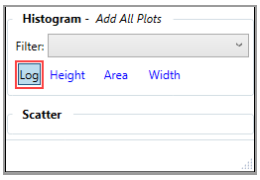


2. Clear the checkbox for any laser detection path that you do not want to include.
3. Apply a filter, if needed.

   Note: You can apply a filter only if you have set filters (or regions) on plots that you previously created in the current experiment.

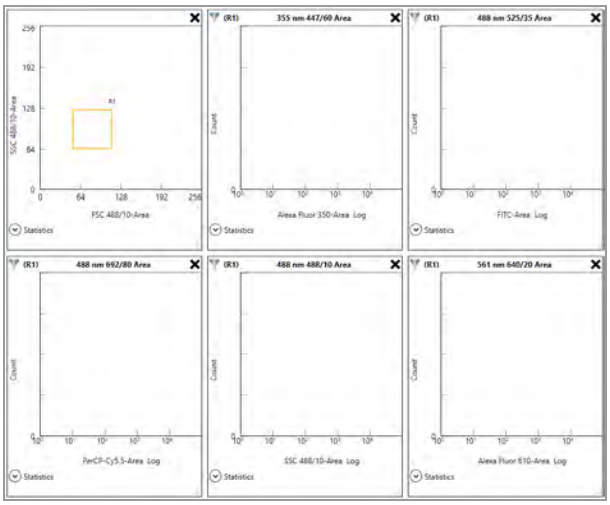
4. Specify linear or log scale.

   Note: The default scale is linear although Log appears to be selected. To specify log scale, click Log until the box appears blue. For example:



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

   Everest Software adds the histograms.



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.

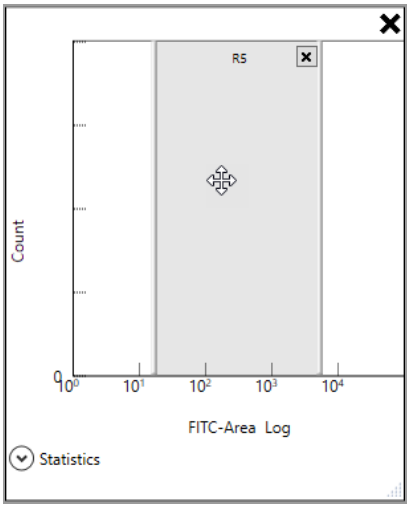
   The plot and histogram toolbar appears.

2. In the plot and histogram toolbar, click Add Bar Region.

   A bar region is added to the histogram.

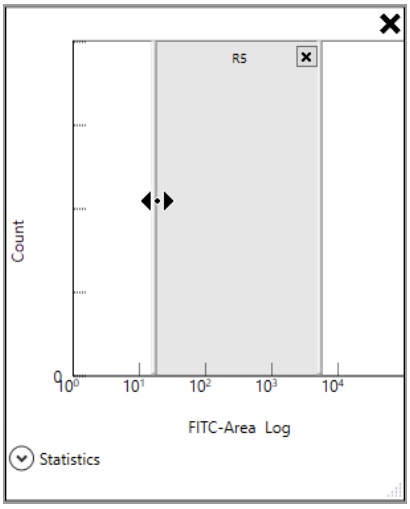
3. To move the bar region to the left or right, point inside the region.

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.

Using Plot Ratios

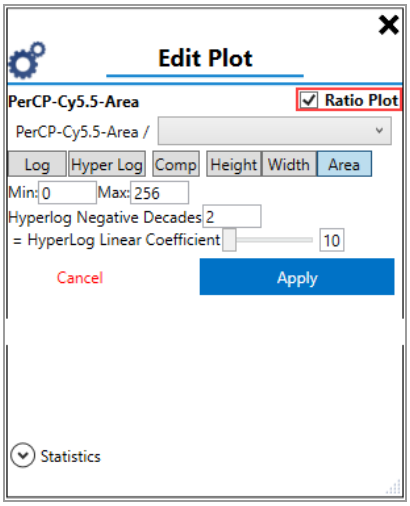
You can select one parameter as the numerator of a ratio, and a different parameter as the denominator of the ratio. You can then plot this signal ratio against time; this can be useful in time-based assays. For more information about time plots, see Creating Time Plots on page 248.

To use a ratio in a time plot

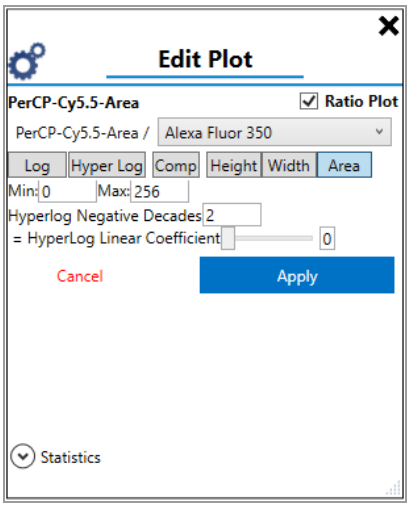
1. Decide which parameter you want to use as the numerator in the ratio, and which parameter you want to use as the denominator.
2. Click Create Time Plot in the Settings window toolbar.
3. Specify axis scaling, display of compensated data, pulse measurement, and time range as needed.

4. Click Modify Plot Parameters in the plot and histogram toolbar for the time plot.

5. Select the Ratio Plot checkbox.

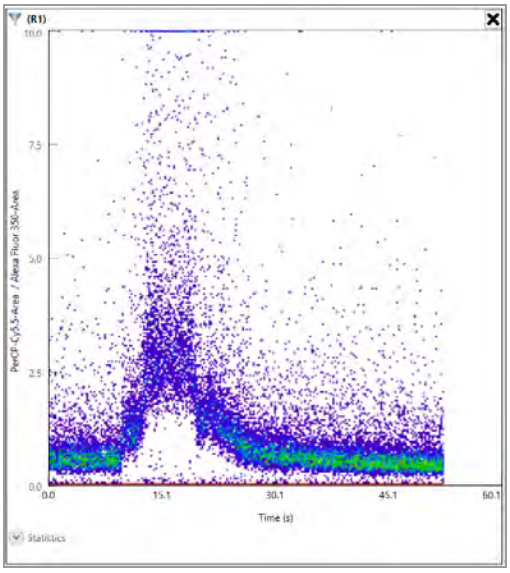


6. Select a second parameter to use for the ratio denominator.



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.

Adding Annotations to Plots

You can annotate a plot or histogram directly.

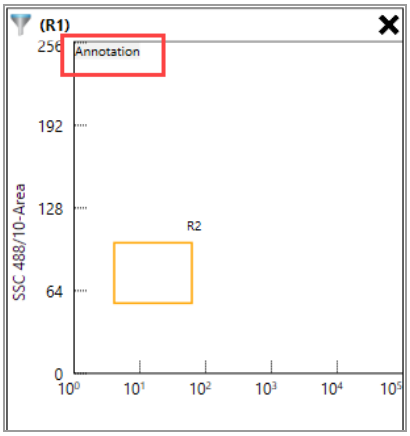
To add an annotation to a plot or histogram

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

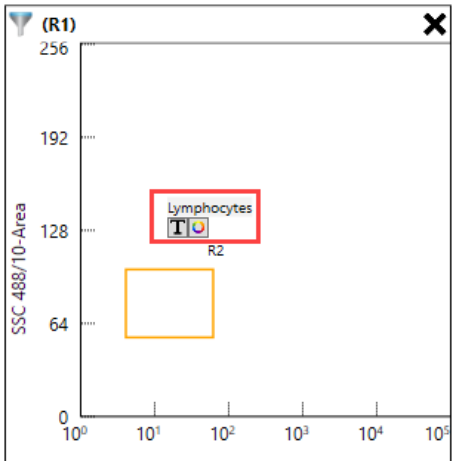
   The plot and histogram toolbar appears.

2. Click Add Annotation.

A text block containing the word Annotation appears 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 toolbar that appear. 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.

Applying a Region to All Plots

Everest Software offers several options to apply regions to other plots or histograms:

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

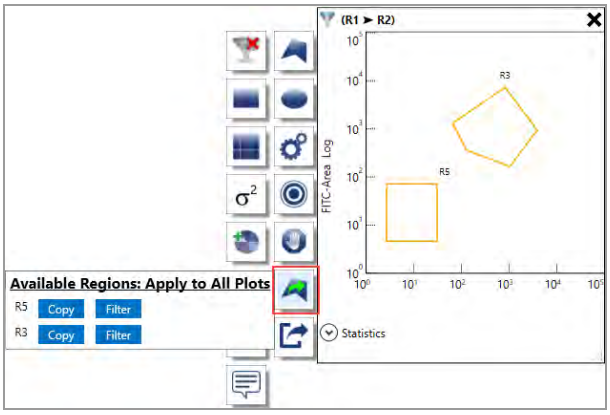
Note: The change applies to all plots or histograms in the experiment.

To apply a region to all plots

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

   The plot and histogram toolbar appears.

2. Click Apply Gate to all Plots.



   - 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.

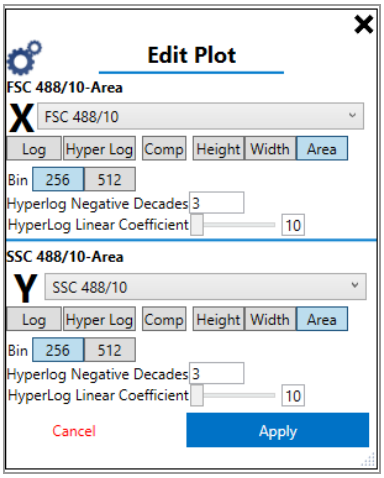
3. Repeat Step 2 for other regions as needed.
4. Point outside the plot or histogram to save your changes.

Modifying Plot Parameters

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

To modify plot parameters

1. Point to a plot or histogram for which you want to modify parameters. The plot and histogram toolbar appears.
2. Click Modify Plot Parameters.
3. In the Edit Plot 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. Select Comp to display compensated data and then sSelect the pulse parameter (height, width, or area).
   d. Specify 256 x 256 or 512 x 512 plot resolution (bin).
   e. Enter the number of hyperlog negative decades to display.
   f. Use the slider to select a hyperlog linear coefficient.
4. Click Apply to save the changes.

Important: If you click the X in the upper right corner of the Edit Plot dialog box, Everest Software removes the plot.

Managing Plot Statistics

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

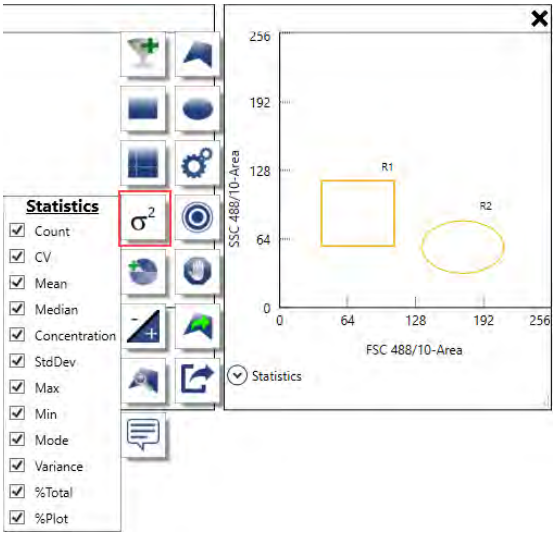
To manage plot statistics

1. Point to a plot or histogram for which you want to manage statistics.

   The plot and histogram toolbar appears.

2. Click Manage Statistics.

3. In the Statistics dialog box, 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 131. If you do not see the statistics that you want to display, contact your system administrator.

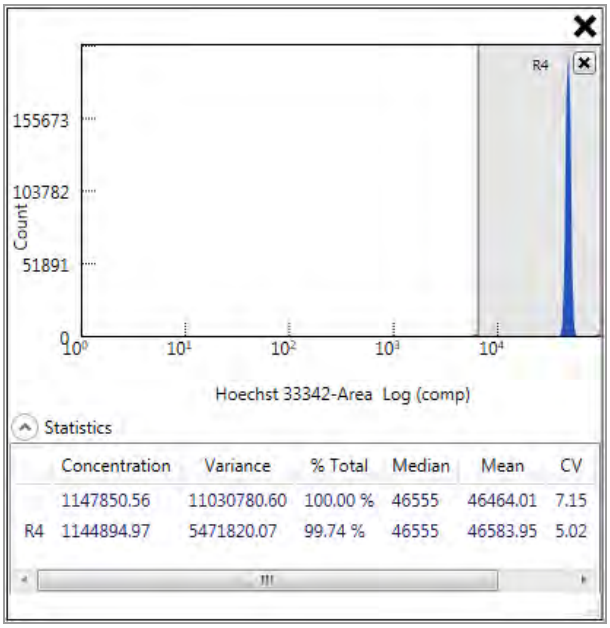
Note: If you plan to add any regions to a plot or histogram, add them before viewing statistics.

To view plot statistics

1. On the plot, click the Statistics down arrow.

   The plot and region's statistics appear in a table within the plot.

2. Resize the plot window if needed to view all the contents of the table.



To rearrange plot statistics

▶ In the Statistics table, drag a 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 compare statistics

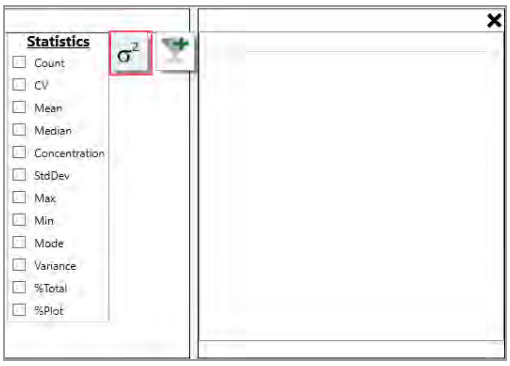
1. In the Tools area of the toolbar, click Add Statistics.

   A statistics window appears.

2. Point to the statistics window.

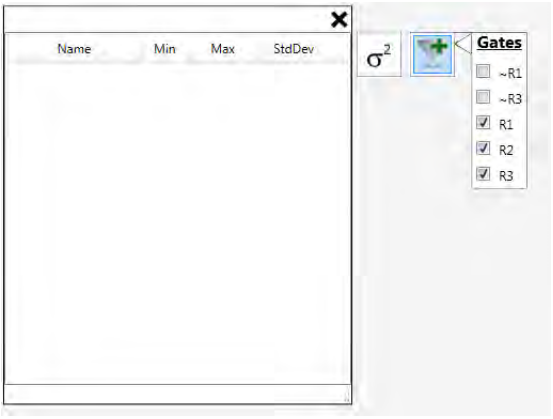
   The Select Statistics and Select Gates toolbar buttons appear.

3. Click Select Statistics.



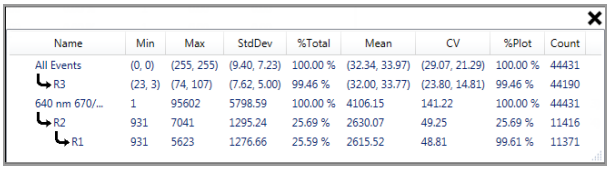
4. In the Statistics dialog box, select the items you want to view and clear the items you want to hide.

5. Click the Select Gates toolbar button for statistics window.



6. In the Gates dialog box that appears, 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 appear, 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.

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. Type new name and press Enter.

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.

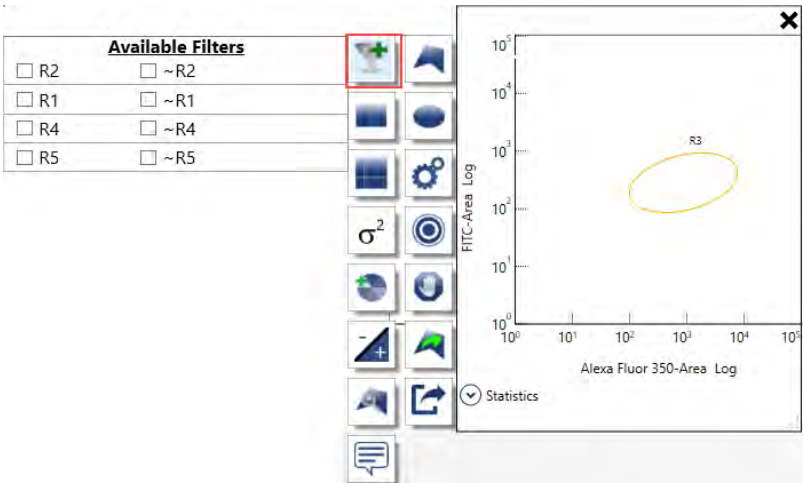
To apply a filter to a plot or histogram

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

   The plot and histogram toolbar appears.

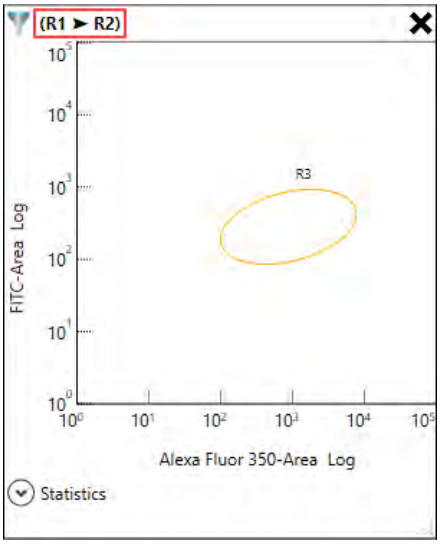
2. Click Apply Filter.

   A list of available filters appears.



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

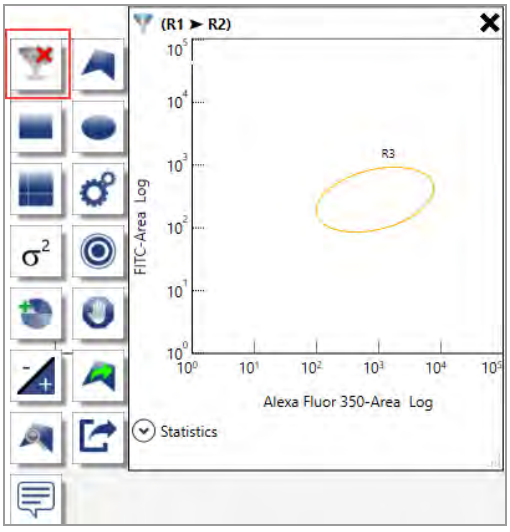
The applied region is shown in the upper left corner of the plot or histogram.



4. The reconfigured plot displays 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.
2. Click Remove Filter in the plot and histogram toolbar that appears.



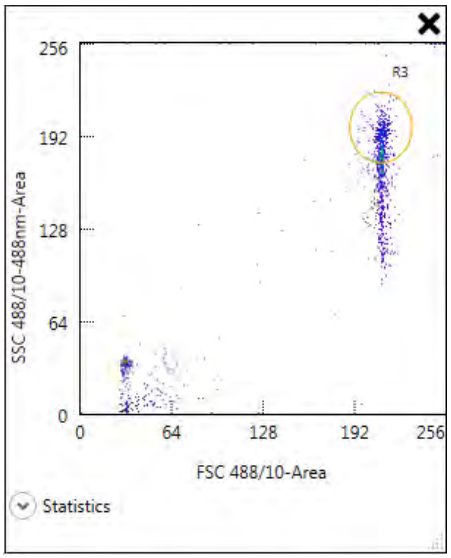
The filter is removed form the plot or histogram.

Applying Filters (Gates) Using Multiple Regions

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

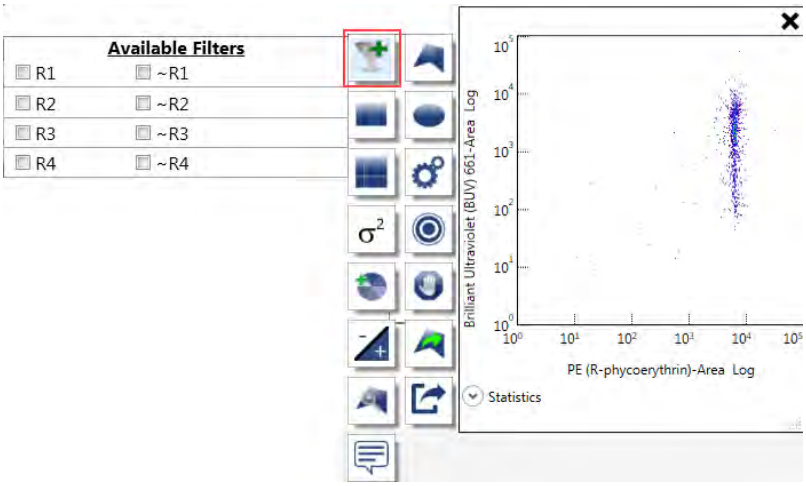
To apply sequential filters to a plot or histogram

1. Create a region on a plot or histogram.



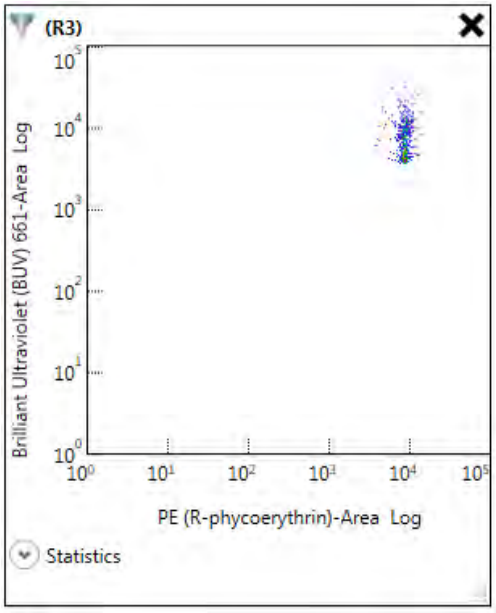
2. Point to a second plot and click Apply Filter in the plot and histogram toolbar.

   A list of available filters appears.

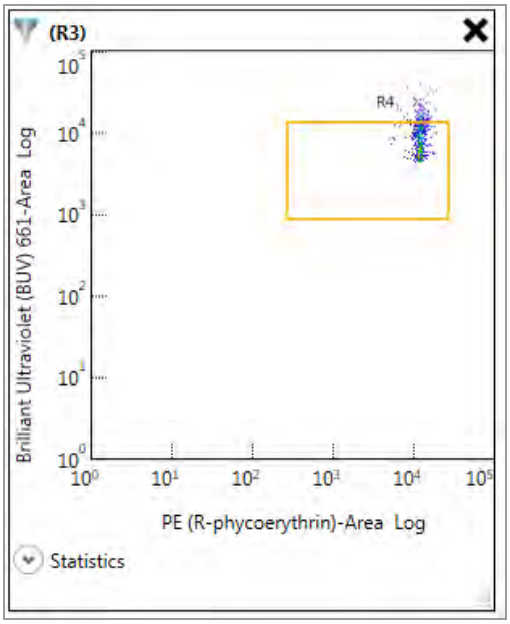


3. Select the region that you created in the first plot.

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

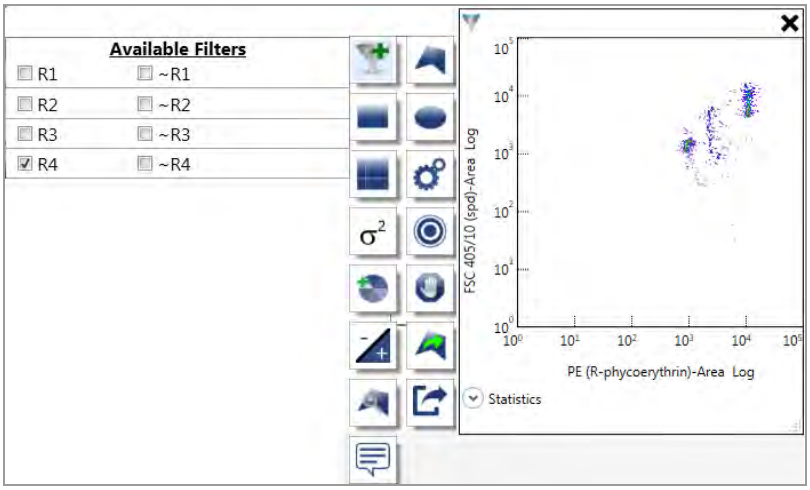


4. Add a region to the second plot.

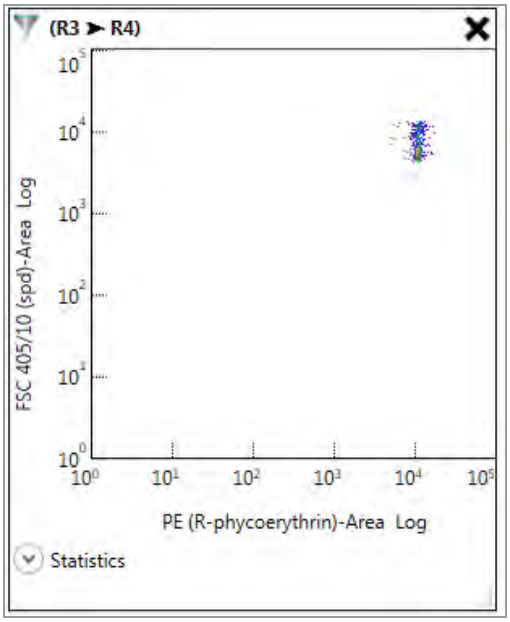


...

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).



Applying Gate Limits

Use the Gate Limit tool 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.

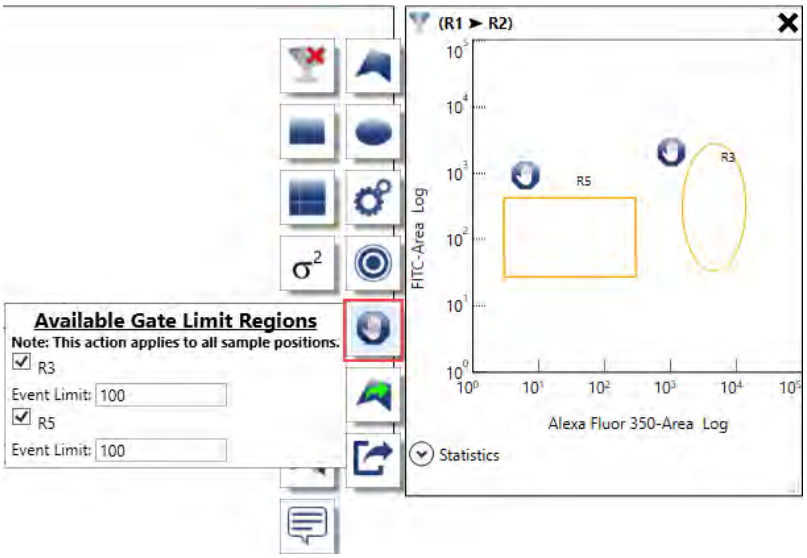
To apply a gate limit to a region

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

   The plot and histogram toolbar appears.

2. Click Assign Gate Limit Regions.
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 plot and histogram toolbar appears.

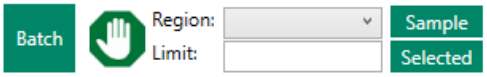
2. Click Assign Gate Limit Regions.

3. In the Available Gate Limit Regions area that appears, clear the checkbox for each region from which 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 Settings window. 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 Creating Plots for the Experimental Sample on page 354.

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, 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 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 after you apply the experiment.

To assign data tracking to a region

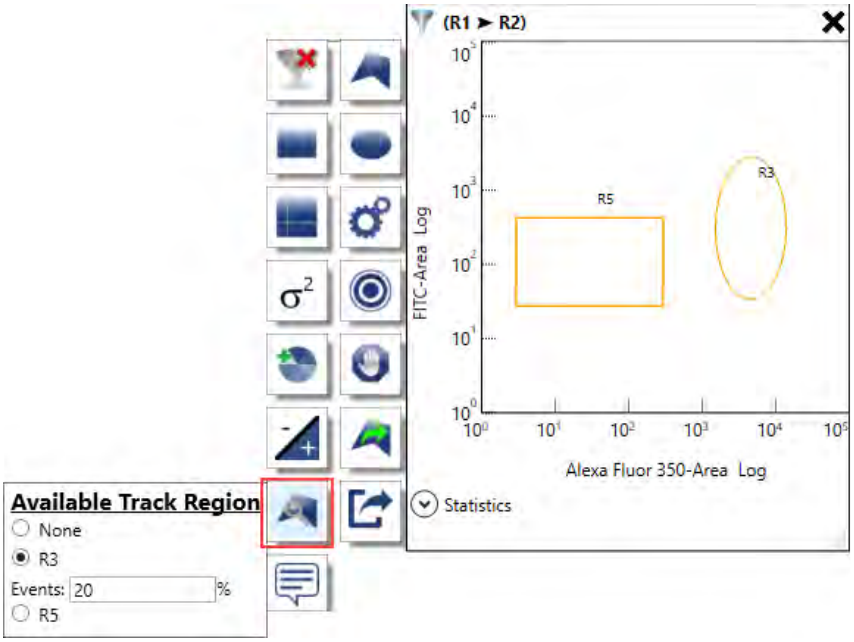
1. Point to a density plot that contains the region where you want to use data tracking.

   The plot and histogram toolbar appears.

2. Click Assign Data Track Regions.

3. In the Available Track Region area that appears, select the region where in which to track data.

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 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 that contains the region that uses data tracking.

   The plot and histogram toolbar appears.

2. Click Assign Data Track Regions.
3. Select None.
4. Point outside the plot 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:

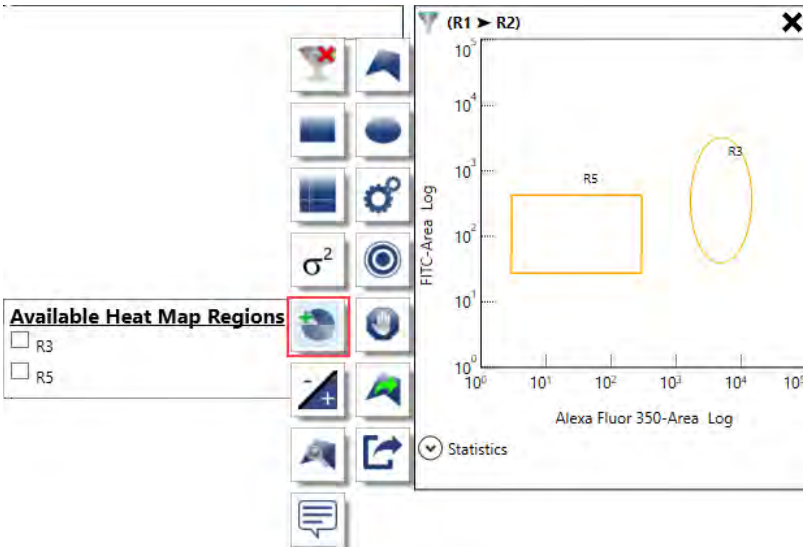
- Low percentages appear lighter and gray.
- High percentages appear darker and red.

To apply regions to a heat map

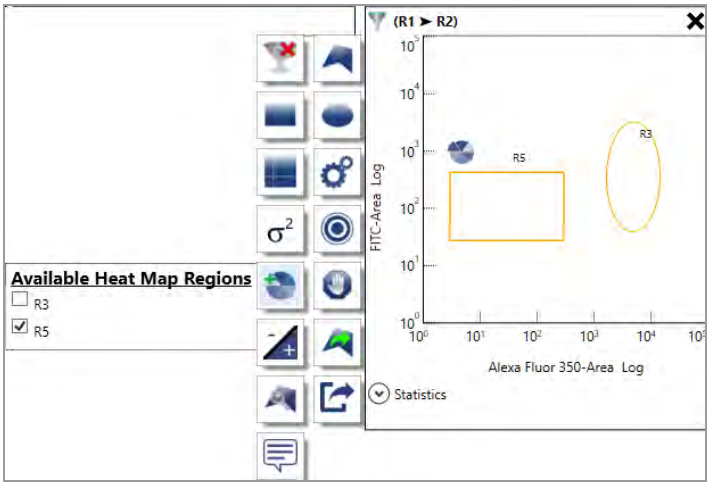
1. Point to a plot or histogram that contains the region(s) that you want to apply to a heat map.

   The plot and histogram toolbar appears.

2. Click Assign Heat Map Region.



3. In the Available Heat Map Regions dialog box that appears, select the checkbox for each region that you want to apply to the heat map.

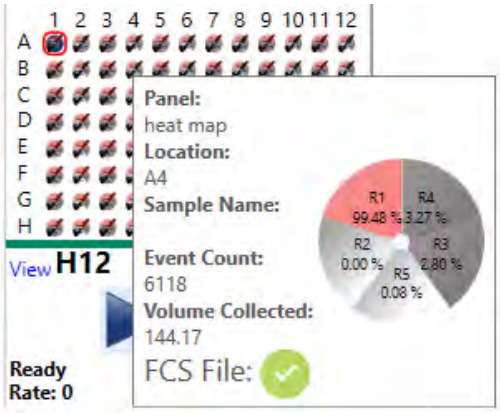


4. Point outside the plot or histogram to save your changes.

   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 the plot or histogram that contains the region(s) assigned the heat map.

   The plot and histogram toolbar appears.

2. Click Assign Heat Map Region.
3. In the Available Heat Map Regions dialog box, 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.

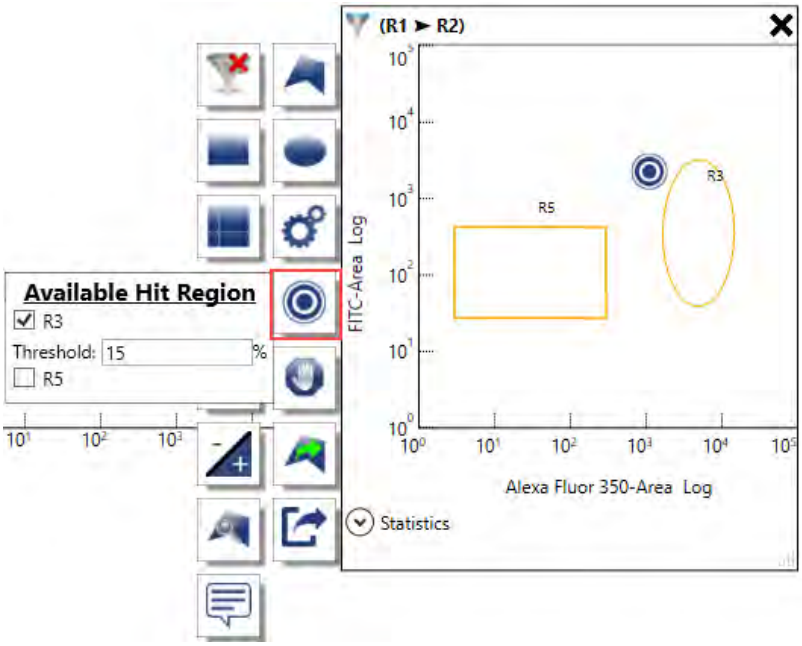
To configure hit detection

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

   The plot and histogram toolbar appears.

2. Click Assign Hit Regions.
3. In the Available Hit Region dialog box, 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 plot and histogram toolbar appears.

2. Click Assign Hit Regions.
3. In the Available Hit Region dialog box, clear the checkbox of the region for which you want to remove hit detection.
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 above 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.

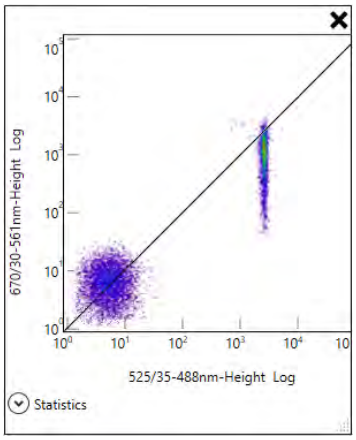
To add a diagonal separator

1. Point to the plot to which you want to add diagonal a separator.

   The plot toolbar appears.

2. Click Add Diagonal Separation.

   A diagonal line appears on the plot.



To remove a diagonal separator

1. Point to the plot that contains the separator.

   The plot toolbar appears.

2. Click Add Diagonal Separation.

   The separator is removed.

Exporting Plots and Histograms

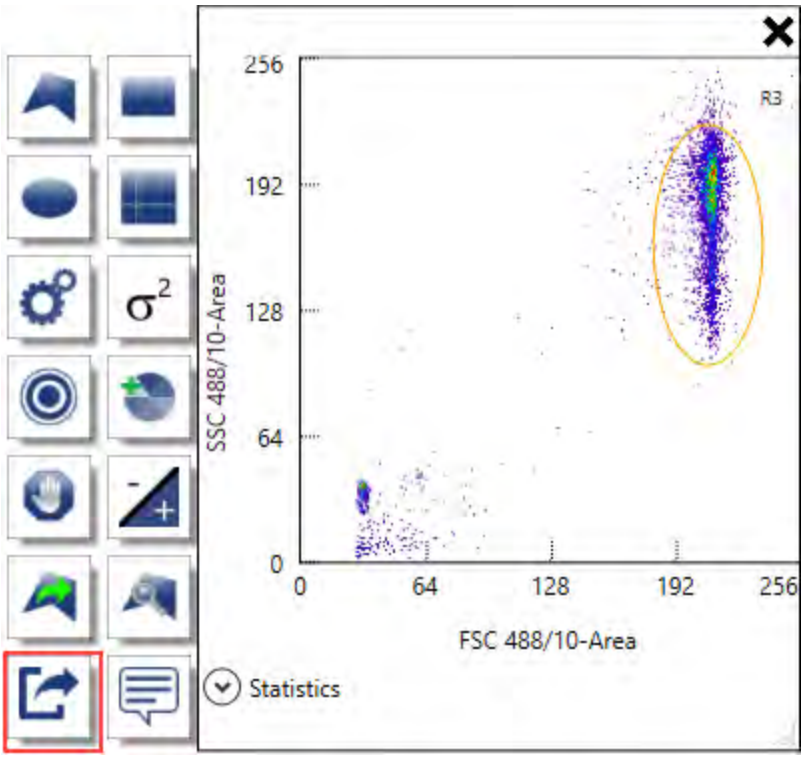
You can export any plot or histogram as a PNG file.

To export a plot as a graphic

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

   The plot and histogram toolbar appears.

2. Click Export to PNG.



3. In the Save As dialog box, browse to a location in which to save the file.
4. Type a name for the file and click Save.

   The plot or histogram is exported as a PNG file.

...

Setting Up Multiple Panels

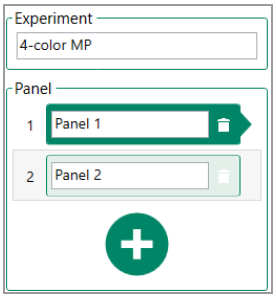
Everest Software allows you to set up unique panels, with different fluorophores, samples, and settings, on a single plate or tube rack. for more information, see Multipanel Experiments on page 114.

You must follow the experiment workflow before you can add a new panel from the Plots and Gates window. You can add panels until the plate layout is full.

To add a new panel

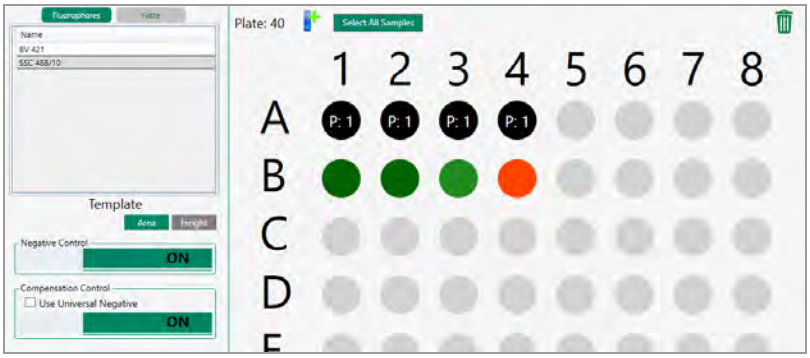
1. Click the + icon in the Experiment pane.

   A new panel row appears, and the Fluorophores window appears on the right.



2. Type a name for the new panel. If you do not replace the name, the experiment uses the default panel name (Panel <x>).
3. Select fluorophores for the new panel. See Selecting Fluorophores on page 208.
4. Select the Plate Setup tab.

   Positions programmed for the previous panel are shown in black, with the panel number identified.



5. Select and configure one or more wells for the new panel. For information, see Configuring the Plate on page 212.
6. Select the Plots and Gates tab.
7. (Optional) Import instrument settings from the instrument settings library. See Instrument Settings Library on page 109.
8. Draw plots and regions and create gates for the panel. For information, see Setting Up Plots and Gates on page 238 and Creating Plots and Histograms on page 242.
9. Repeat steps 1 through 8 until all panels are created or modified.
10. Click Apply to apply the experiment. All panels are applied.

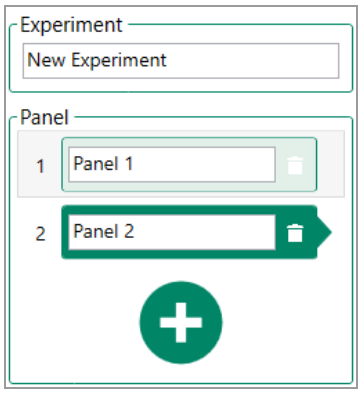
    The Acquisition window appears. See Working on the Acquisition Screen on page 289.
11. Run Setup mode to adjust voltages for each laser and set the trigger. See Acquiring Initial Sample in Setup Mode on page 302.
12. Save instrument settings to the instrument settings library. see Configuring Instrument Settings on page 306.
13. Run Acquisition mode. Running Samples in Acquisition Mode on page 312.

    In Acquisition mode, the ZE5 Cell Analyzer acquires the whole plate or set of tubes in a single run and will adhere to the settings for each panel in Setup mode. Therefore, you do not need to reoptimize settings between the panel runs.

For information on analyzing data in multiple panels, see Analyzing, Saving, and Printing Data on page 325.

Editing Panel Information and Settings

You can edit panels in an experiment before and after it has been run.



To edit panels in a multipanel experiment

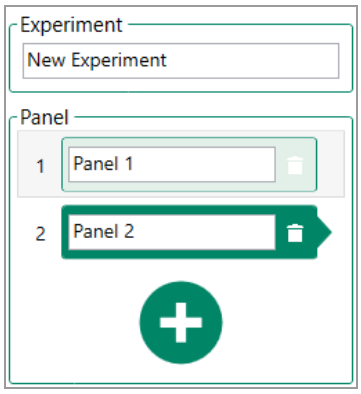
1. In the Experiment pane, select a panel.

   The panel opens to the Fluorophores tab.

2. (Optional) Edit the panel name in the Experiment pane.
3. For the selected panel, do any of the following:
   - Edit the fluorophores in the panel.
   - Select the Plate Layout tab and edit information and settings shown below the plate layout.
   - Select the Plots and Gates tab, and change plots and PMT controls for the panel.
4. Navigate to a different panel and repeat steps 1 and 2.

Deleting Panels

You can delete panels that are no longer needed in your experiment.



To delete a panel from the experiment

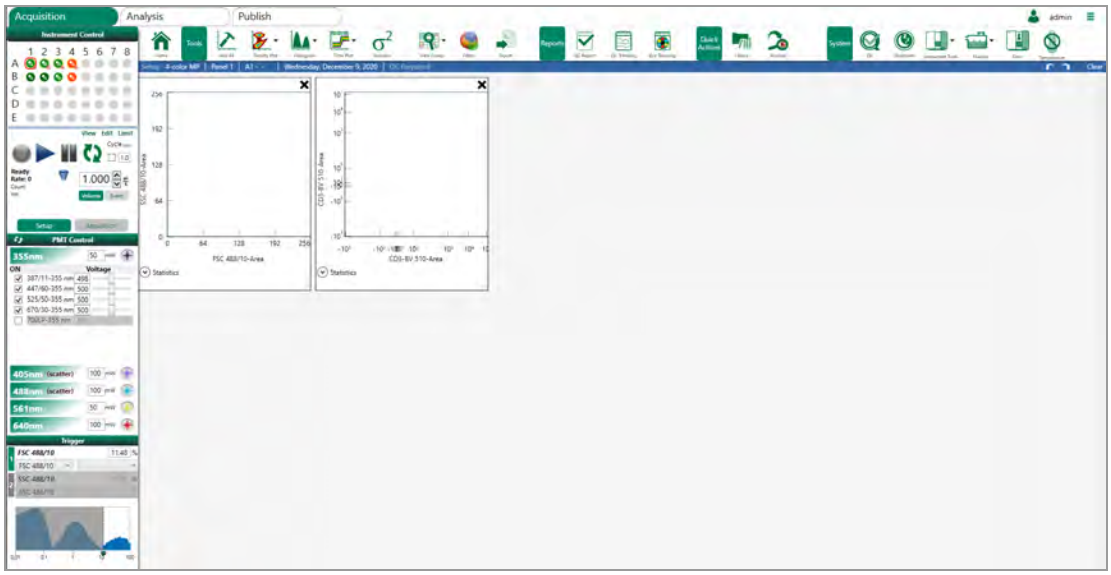
1. In the Experiment pane, click the trash can icon to the right of the panel.

   A message appears, asking you to confirm the deletion on the panel.

2. Click Yes to delete the panel and then select the Plots and Gates tab. Everest Software confirms the deletion..

Working on the Acquisition Screen

When you apply the experiment from the Plots and Gates window, the Acquisition window appears.

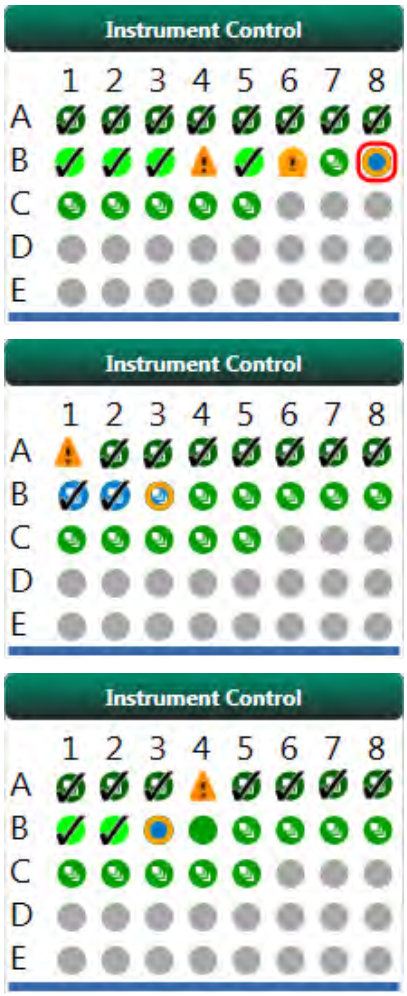


You can make further adjustments, and add or edit plots and histograms, while in Setup mode. For information, see Creating Plots and Histograms on page 242.

Note: When you select one or more wells in a multipanel experiment, the corresponding panel is identified on the blue information bar. You can only select wells in one panel at a time.

Plate Map in the Instrument Control Panel

The plate map in the Acquisition workspace displays 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.



Note: To identify the panels in multipanel experiments, Everest Software highlights the wells in the selected panel with a red dot in the lower-right corner of each well. For more information on multiple panels in an experiment, see Setting Up Multiple Panels on page 285.

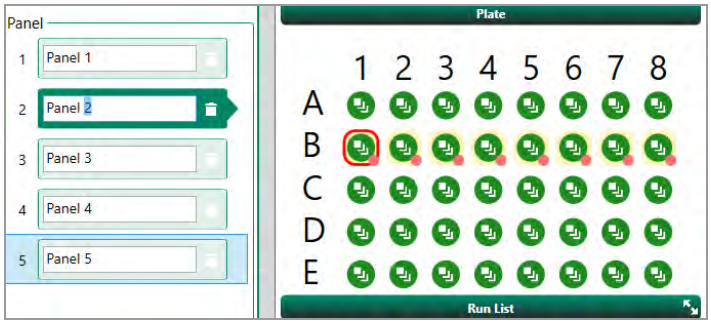


Table 32. Plate map symbols

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

Table 32. Plate map symbols, continued

Symbol	Description
	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.

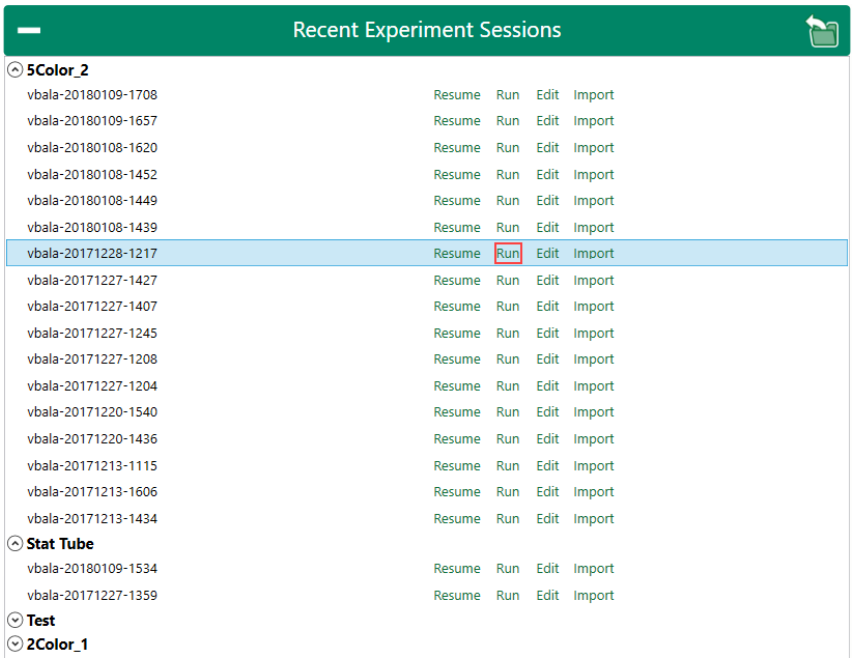
The white overlay on various types of positions indicates that the plots, regions, and gates are replicated for each position in the panel.

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

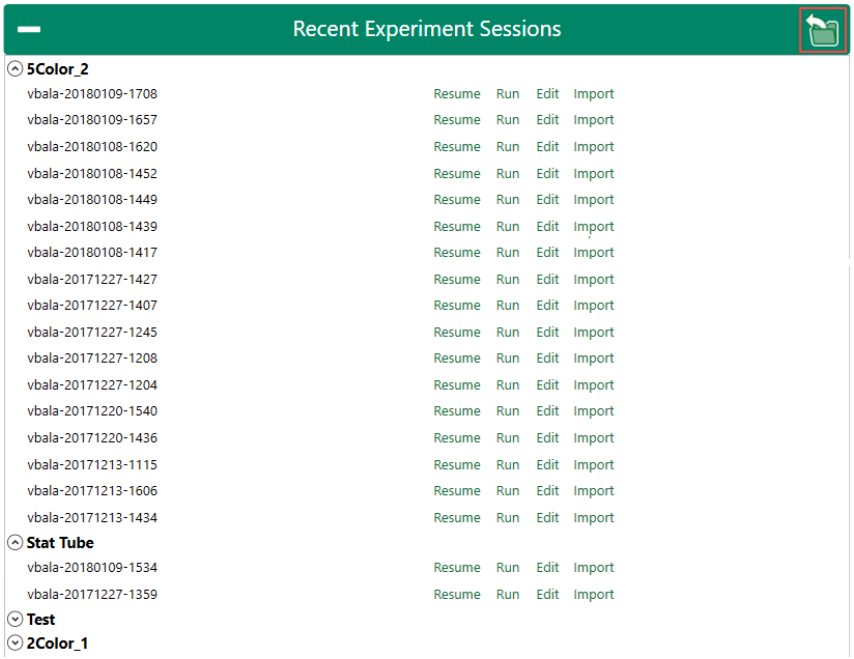
1. Return to the Home window.
2. In the Recent Experiment Sessions panel, expand the list of recent experiments.
3. Do one of the following:
   - If the experiment session that you want to run is in the list of recently displayed items, click Run for the experiment session.



Everest Software applies the experiment session.

- 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.

For more information, see Recent Experiment Sessions on page 76.

Running Stat Tubes

If you need to quickly run a single sample or a few samples, you can run a single stat tube using the Stat Tube option in the Home window, or you can attach a stat tube to a panel in your experiment.

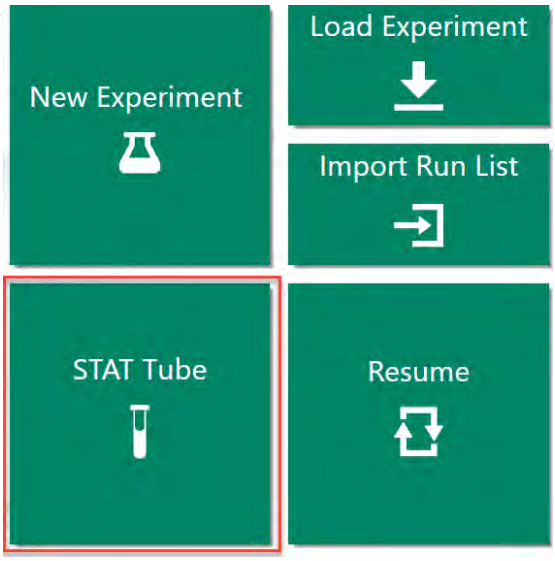
Sample acquisition and data recording are available directly from this window. When you run a stat tube, all parameters are enabled by default and can be named by editing the name in the parameter list.

Running an Individual Stat Tube

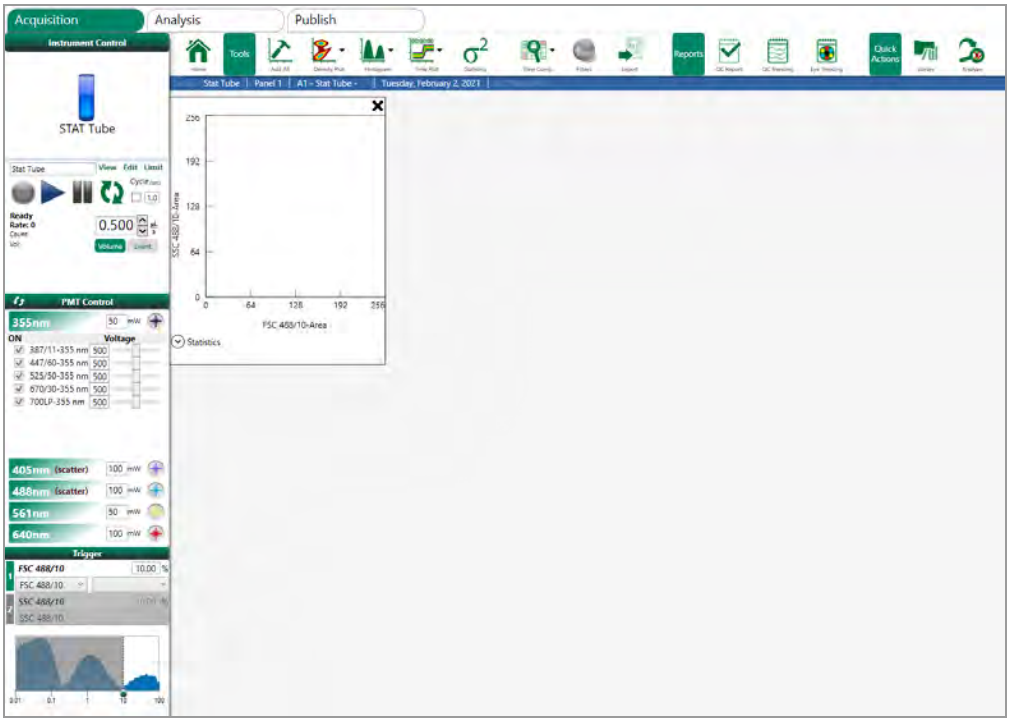
When you run an individual stat tube, the Acquisition workspace opens immediately, where you can create plots, add gates, and adjust settings. You can acquire samples and save data directly from this window. In stat tube mode, all parameters are enabled by default and can be named by directly modifying the name in the parameter list.

To run a single Stat Tube

1. in the Home window, click Stat Tube.



The Acquisition window appears.



2. Configure plots using options on the Tools toolbar.

See the following sections for more information:

- PMT and Laser Controls on page 306
- Creating Plots and Histograms on page 242
- Chapter 8, Acquiring Samples

...

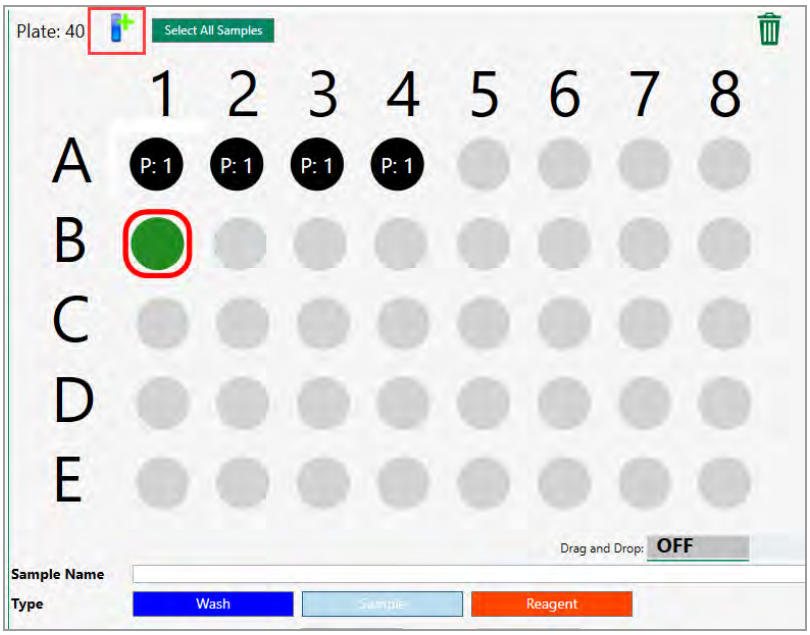
Adding a Stat Tube to a Panel in the Plate Layout

You can add a single stat tube to a panel in your experiment. An added stat tube can be assigned to one panel only, for the Sample position type.

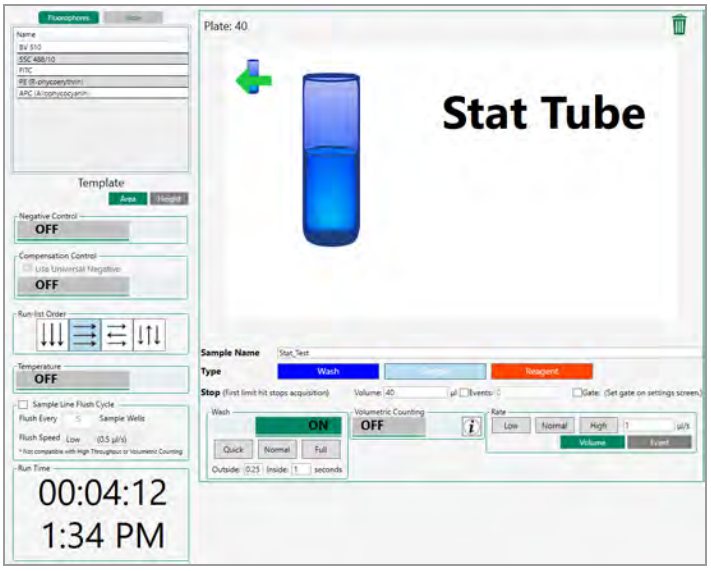
Note: High-throughput settings are not applicable to stat tubes.

To add a stat tube to an experiment

1. In the plate layout, click the Add Stat Tube icon ( ).



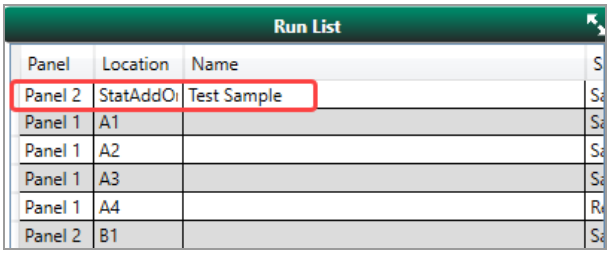
2. (Optional) Type a sample name for the stat tube.



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 the stat tube icon again to return to the plate layout.

   The added stat tube is indicated with a symbol in the upper left area of the plate map.

   The stat tube also appears at the top of the run list, regardless of the panel number.



Chapter 8 Acquiring Samples

For information about the types of files produced during acquisition, see File Types in Everest Software on page 325.

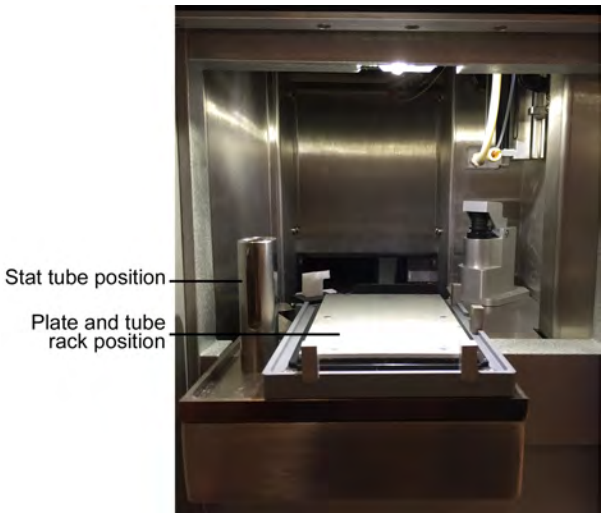
Loading Sample Media into the ZE5 Cell Analyzer

After configuring your experiment in the Everest Software 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 according to the experiment setup..

   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.

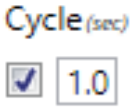
Tip: To edit an experiment, navigate to the Instrument Control panel in Setup mode and click Edit.

Table 33. 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.

...

Table 33. Setup Mode Control Items, continued

Item	Function
	**Stop** — ends data acquisition and turns off the sample pump. Replaces the Play button when sampling begins.
	**Pause Run List** — pauses sampling. Time gaps that correspond to pause times appear in the data file.
	**Resume Run List** — resumes sample flow. Replaces the pause button when sample is paused.
	**Clear Data** — clears the data displayed in plots on the Acquisition window. Does not clear data being stored.
	**Cycle Mode** — when enabled, 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. **Tip:** 0.1 µl is the slowest flow rate.

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 306 to fine-tune instrument settings while in Setup mode.

Note: Event, volume, and gate limits apply only when recording data in Setup mode.

Important: While the system is acquiring sample in Setup mode, the following functions are disabled:

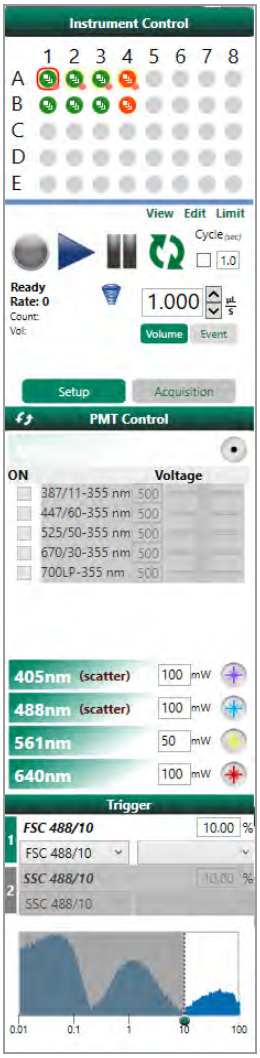
- Initiating the ZE5-EYE process
- Shutting down Everest Software and the ZE5 Cell Analyzer
- In the Instrument tools:
  - Returning sample probe to home position
  - Cleaning the sample line and probe
  - Unclogging the sample line, probe, and flow cell
  - Swapping QC beads
  - Pausing the sheath fluid and disabling the lasers
- Opening the sample loader door

Everest Software enables these functions when sample acquisition is complete.

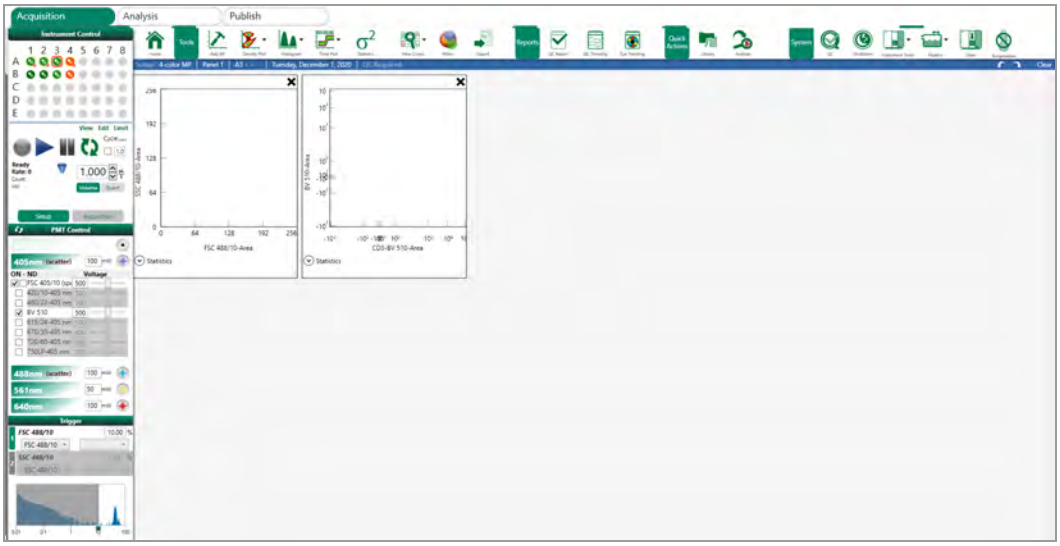
...

To acquire 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.

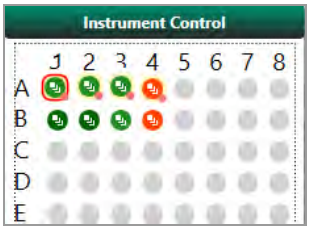


Note: The status bar displays the current mode on the left. For a single stat tube, the Setup button does not appear; sampling occurs in Setup mode by default.



4. In the plate map, click the position from which to start sampling.

   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 sampling begins.

6. Adjust the flow rate by setting a target volume 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.

   Important: PMT voltage and trigger threshold values reset to default for each new experiment. To save settings for Reuse, click Library in the Quick Actions menu, and then click Save Instrument Settings.

   Tip: enable 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 sampling or record a data file.
   - To stop sampling, click Stop.
   - To record, first ensure that the limits are set as needed. 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.

     a. Adjust limits as needed.

     b. Click Record. Data are collected until the specified limit is reached. To stop acquisition, click Stop.

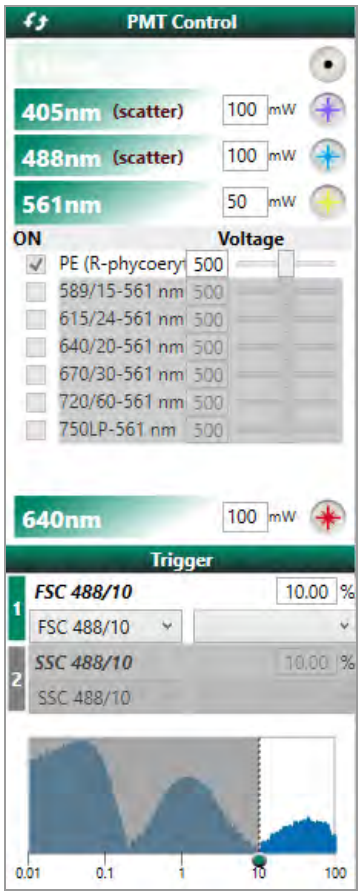
     c. After data have been recorded for a tube or well, the position in the plate map is indicated by a black check mark. For information about symbols shown in the plate map, see Plate Map in the Instrument Control Panel on page 290.
9. After the first position has been sampled from and/or recorded, sampling automatically starts in the next tube in the plate map and proceeds 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.

Configuring Instrument Settings

From the Acquisition window, you can enter instrument settings such as PMT voltages, laser power, and primary and secondary trigger and threshold. The default PMT voltage values are those set at the most recent QC baseline. The default threshold value for both FSC and SSC triggers is 10%.

Note: In multipanel experiments, instrument settings are saved for the selected panel only.



PMT and Laser Controls

In this section of the Instrument Control panel, you can modify settings related to lasers and their associated detectors.

Note: You can modify from the Acquisition window only.

…

Table 34. PMT and Laser Control Items

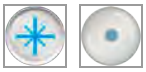
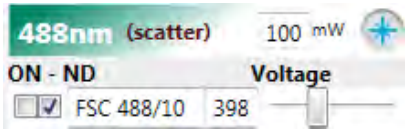
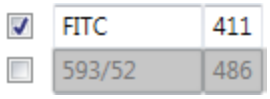
Item	Function
	**Display filter numbers/detector names** — toggles between detector filter numbers and detector names for each of the lasers in the system.
	**Load voltages** — loads PMT voltages from a run list file that you browse to and select.
	**Reset voltages** — resets PMT voltages to default 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 44.
	**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 windows, and the Filter Configuration dialog box.

Table 34. PMT and Laser Control Items, continued

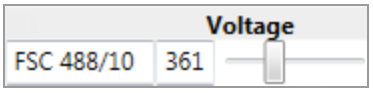
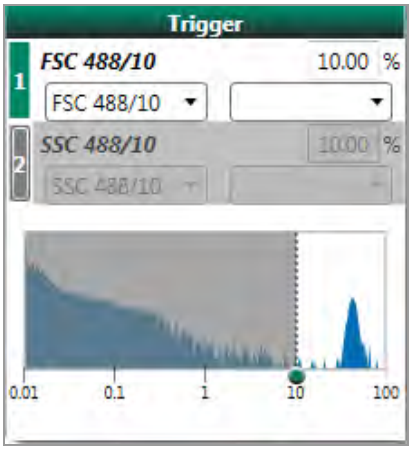
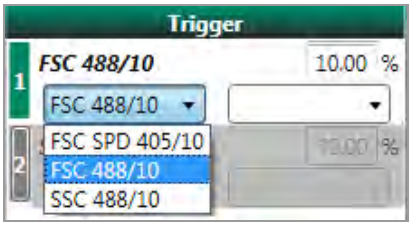
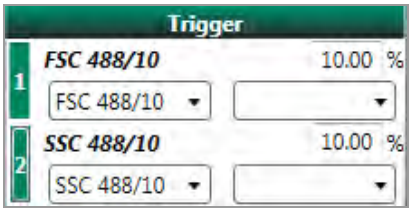
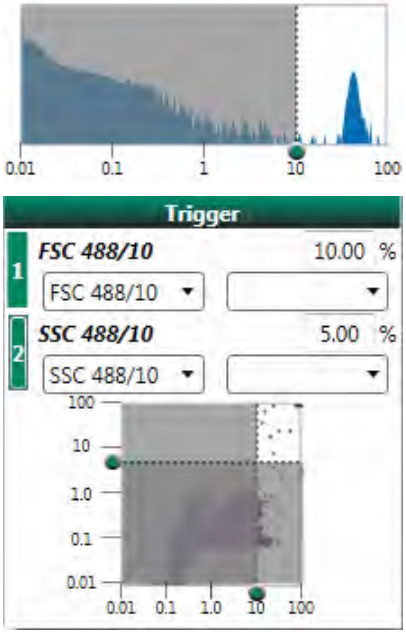
Item	Function
	**PMT voltage** — allows you to adjust the PMT voltage for the selected detector, either by entering a value or moving the slider. **Important:** The voltage adjustments are saved only for the current experiment. For each new experiment the values are reset to default, which are set at the most recent QC baseline. The adjusted PMT voltage values are included in the FCS file.
	**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** — the default secondary trigger is side scatter (488 nm laser). The secondary trigger is disabled by default. Click the row number to enable and add a secondary trigger/threshold channel.

Table 34. PMT and Laser Control Items, continued

Item	Function
	**Adjust threshold setting** — the primary and secondary trigger threshold is a percentage of the signal in the trigger's detector. The range that can be entered is 0.01 to 100%. The default value is 10.00%. **Important:** The threshold adjustments are saved only for the current experiment. The default threshold value for the primary trigger, which is enabled for each new experiment, is 10%. The default threshold value for the secondary trigger, which is disabled for each new experiment, is 10% when the trigger is enabled. The adjusted threshold values are included in the FCS file. **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.
	**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.

Saving Instrument Settings

You can save your configured settings for reuse while in Setup mode on the Acquisition window. You can save settings for only one panel at a time.

To save your settings

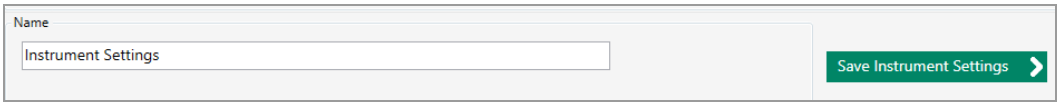
1. After you have completed your configuration, click (Apply).
2. In the Acquisition window, run Setup on your plate configuration and adjust voltages as necessary.
3. For multipanel experiments, select a panel.
4. From the Quick Actions toolbar, click Library.

Note: If the instrument is busy, the icon is grayed out.

5. In the lower left corner, replace the default Instrument Settings in the Name field with a unique name for the settings file.

Tip: Use the search field to locate specific file names or name sets. Filtered files are sorted by most recent save date.

Note: If you save the experiment with a duplicate name, an error message appears.



6. Click Save Instrument Settings.

Everest Software saves and stores the settings in the Library. Settings are ordered by most recently used or saved date.

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Acquisition Mode Controls

Use Acquisition mode after establishing settings in Setup mode. This automatically completes the run as defined in the Experiment Builder.

Note: Acquisition mode does not apply to single stat tubes.

Table 35. Acquisition Mode Control Items

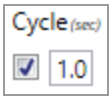
Item	Function
View	**View Run List** — expands the run list to display settings for the current experiment.
A1	**Sample Position** — during acquisition, the position currently being acquired.
	**(Start) Run List** — initiates the experiment as defined in the Experiment Builder.
	**(Stop) Run List** — stops the experiment that is currently in progress.
	**Cycle Mode** — when enabled, 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. **Note:** Event Rate is not available in high-throughput mode.

Table 35. Acquisition Mode Control Items, continued

Item	Function
	**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. **Tip:** 0.1 µl is the slowest flow rate.

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 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.

Important: While the system is acquiring sample in Acquisition mode, the following functions are disabled:

- Initiating the ZE5-EYE process
- Shutting down Everest Software and the ZE5 Cell Analyzer
- In the Instrument tools:
  - Returning sample probe to home position
  - Cleaning the sample line and probe
  - Unclogging the sample line, probe, and flow cell
  - Swapping QC beads
  - Pausing the sheath fluid and disabling the lasers
- Opening the sample loader door

The software enables these functions when sample acquisition in complete.

Note: The panel is identified for each well as it is acquired.

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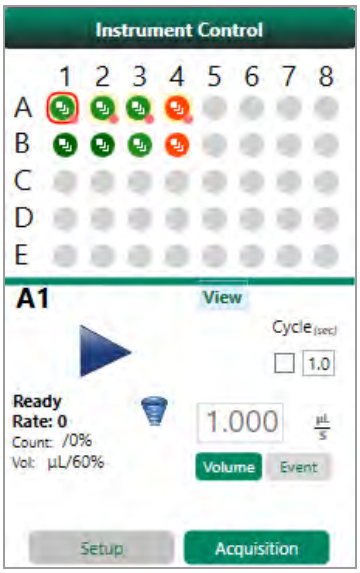
To run samples in Acquisition mode

1. Click Acquisition in the Instrument Control panel to change to Acquisition mode.



2. To review the sampling configuration, click View in the Instrument Control panel.

   The run list opens, summarizing the experiment setup. Click the X in the upper right corner to close it.

3. (Optional) Click Library in the Quick Actions menu to save the settings before you start the run.

4. 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.

   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 in the Instrument Control Panel on page 290.

   Tip: By default, sampling begins at position A1 in the plate map, but you can begin acquisition from any sample position. Click a position in the plate map and click (Start) Run List. The run list proceeds as determined in the Experiment Builder, starting from the selected position.

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.

Some options available in standard mode are not available in high-throughput mode: For example:

- Because multiple samples occupy the sample line, you cannot enable Return Sample when using high-throughput mode.
- You can define volume limits in high-throughput mode, but not event limits.

.For information, see High-Throughput Mode on page 98.

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 experiment setup and sample type by acquiring control positions in Setup mode as described in Acquiring Initial Sample in Setup Mode on page 302.
2. Click Acquisition in the Instrument Control panel.
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 in the Instrument Control Panel on page 290.

   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

Note: This option is available only when acquiring in Setup mode and for stat tubes.

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.

To pause sample acquisition

▶ In the Instrument Control pane in Setup mode, click the Pause Run List () button.

To resume sample acquisition after a pause

▶ In the Instrument Control panel in Setup mode, click the Resume Run List () button.

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 the Stop () button.

To restart sample acquisition after a stop

▶ In the Instrument Control panel, click the Play () button.

Using Pause After in an Experiment

In Acquisition mode, to pause sample acquisition at a certain point in the experiment, you can insert a pause step into the run list. Sample acquisition stops until you are ready to restart it manually. You can use this method to pause after running compensation controls, or to verify results obtained so far.

To insert a pause step into a run list

- Add a Pause After step to a sample position. For more information, see High Throughput, Pause After, Volumetric Counting, and Return Sample Settings on page 96.

To resume acquisition after a pause step

1. In Acquisition mode, select the position from which to restart the experiment in the Instrument Control panel.
2. Click (Start) Run List to resume sampling.

Stopping and Resuming 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 Acquisition 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. On 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 resume sampling.

To resume a previously stopped experiment

1. In the Home window, expand the list in the Recent Experiment Sessions panel.
2. Locate the target experiment in the list and 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 resume sampling.

Chapter 9 Applying Fluorescence Compensation

Everest Software offers simplified workflows for both manual and automatic compensation. To perform automatic compensation, you can acquire single-color controls using the Everest Software 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. 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 Chapter 12, Example 9-Color Immunophenotyping Experiment.

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.

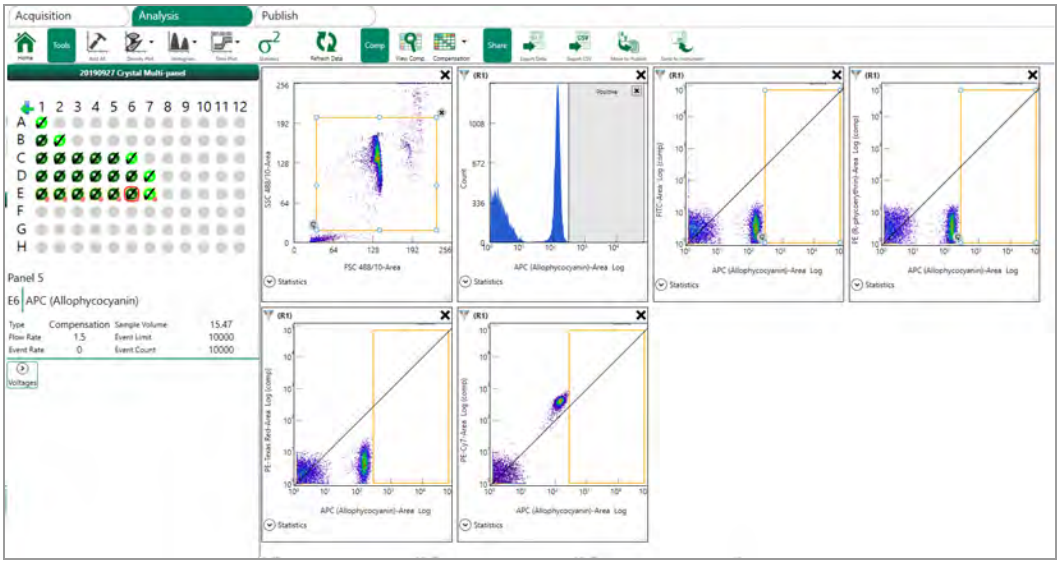
Note: Wells in each panel share the same compensation matrix.

To automatically calculate compensation

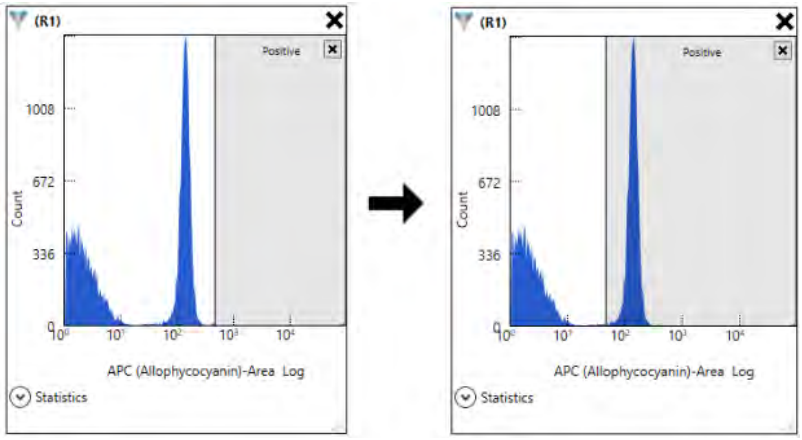
1. Record all control positions using one of the following methods:
   - Using the Record button in Setup mode
   - Running the control samples in Acquisition mode

   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 on the toolbar to move the run list and data to the Analysis tab.

Everest Software displays the Analysis workspace and all plots for the selected well.

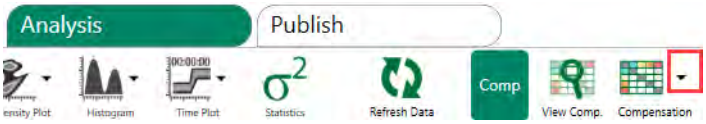


3. Click the position for the first control, in this case B1, and if necessary, adjust the region in the histogram so that it includes the stained population, as shown in the next figure.

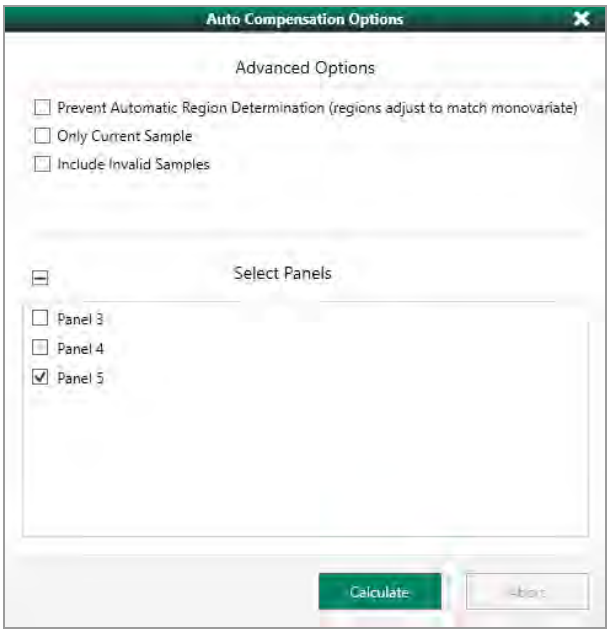


4. Repeat step 3 for all the control positions, adjusting the gate in the parameter's histogram if needed.

5. Click the down arrow next to the Compensation button,



The Auto Compensation Options dialog box opens.



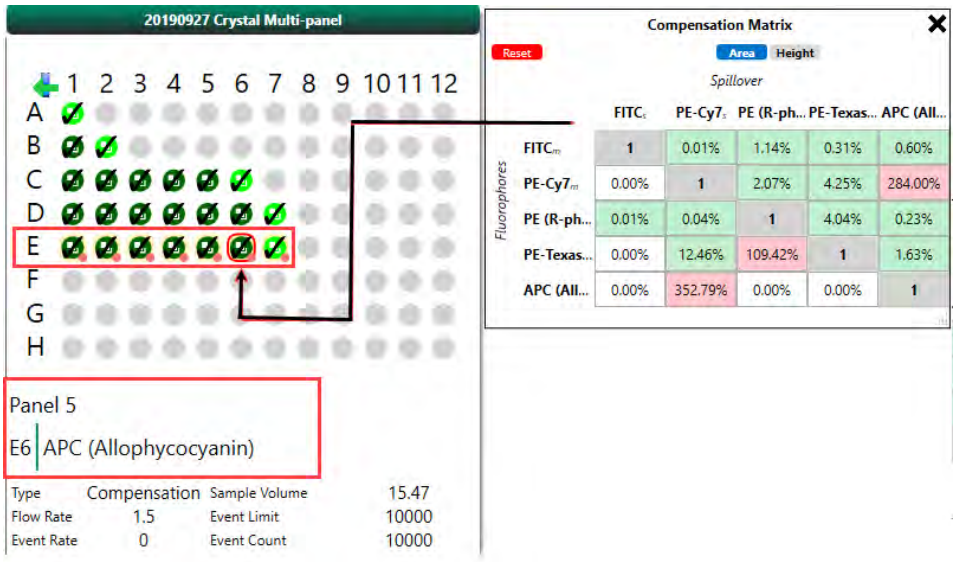
6. (Optional) Under Advanced Options, select one or more checkboxes.

   Selected options are applied to enabled wells in the panels you select in step 7. Each option is briefly described below.

   - **Prevent Automatic Region Determination** — 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 density plots for each control to match your regions. If this option is not selected, Everest Software performs automatic region determination and adjusts the regions in all plots for each control automatically.
   - **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. Under Select Panels:
   - To clear all selected checkboxes, click the ▬ checkbox next to Select Panels.
   - To select all checkboxes, click the empty checkbox .
   - To select or clear checkboxes manually, click individual checkboxes.
8. Click Calculate to initiate automatic compensation for each panel selected in the dialog box.
9. After the compensation process finishes, select a well in the plate layout, and then open the compensation matrix to check the values.

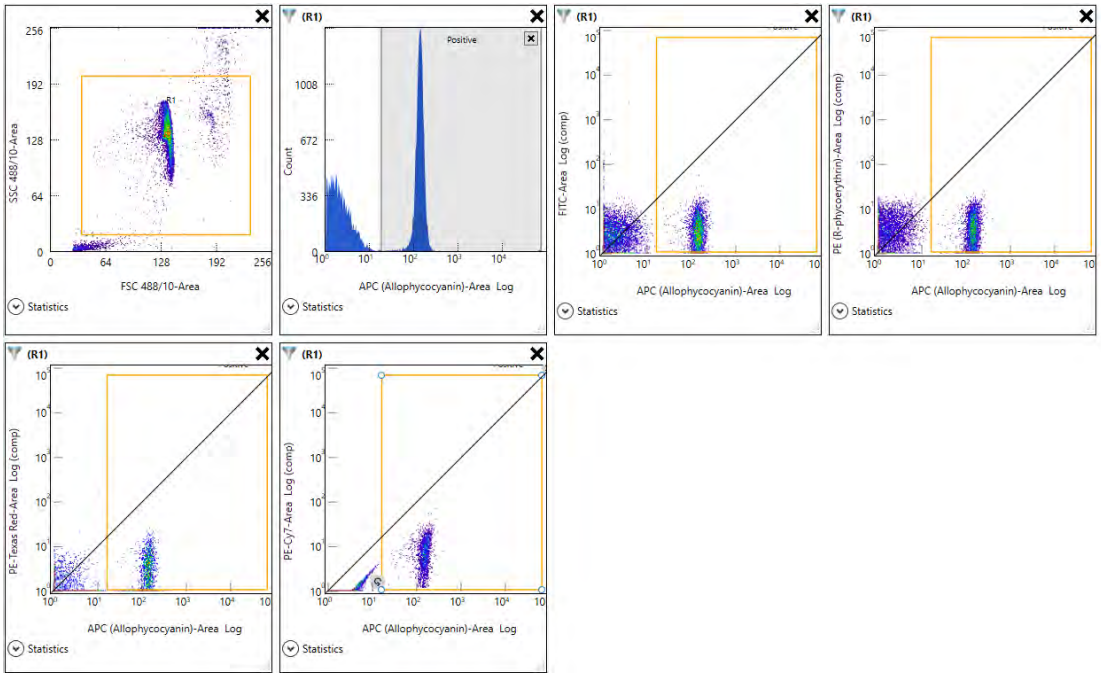


Each row in the matrix corresponds to a channel that contributes spillover signal to other detectors.

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.

...

10. Review the compensated control plots for the selected well.



11. To continue running samples, click Send to Instrument to send the calculated compensation values back to the instrument.

12. 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

Everest software offers two options to manually adjust compensation

- Dragging populations
- Editing the compensation matrix directly

Note: Unless you know how to perform statistically correct compensation adjustments for all fluorophores used in your experiments, Bio-Rad recommends that you use automatic compensation.

Dragging Populations

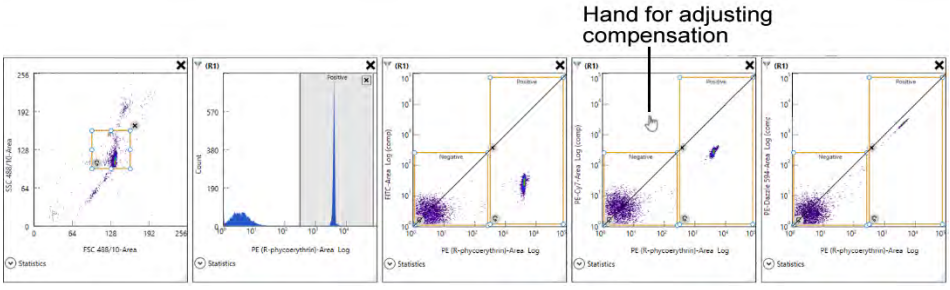
You can adjust compensation by dragging populations in plots either in the Acquisition tab or in the Analysis tab.

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

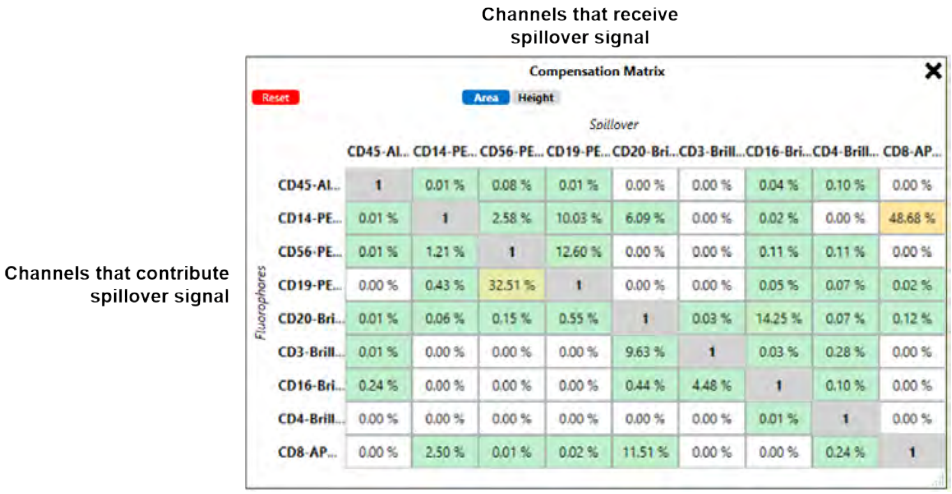
You can adjust compensation by editing the compensation matrix either in the Acquisition tab or in the Analysis tab.

Note: If you plan to perform manual compensation, you should understand how to match 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 Compensation Matrix in the Comp section 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 on the Acquisition window and then sending it to the Analysis window, 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 Analysis 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. 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.

In the Analysis Recent Experiments window, a green check mark () indicates the session contains an FCS file.

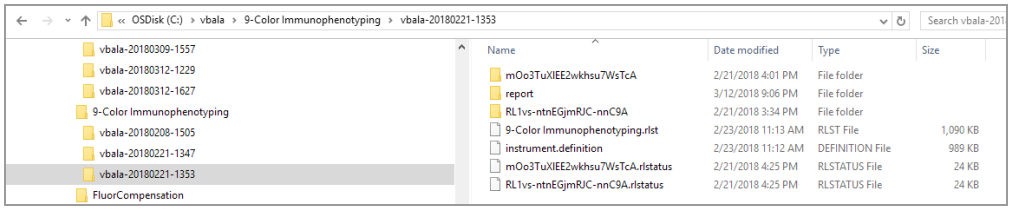
Run list files are used to

- Open previously created experiments for re-running or editing
- Load experiments in the Analysis tab

File Structure of Saved Data

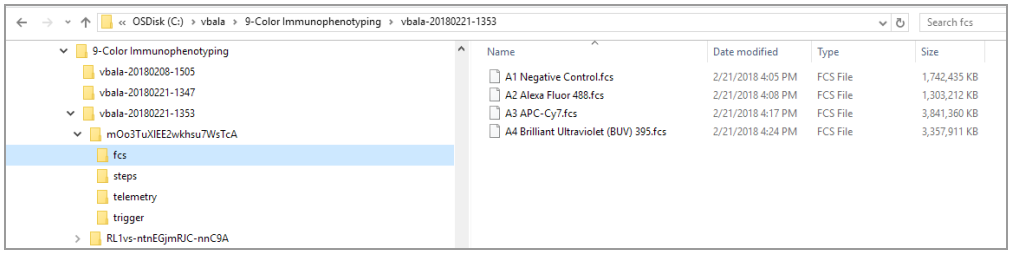
Tip: This section explains where to find saved data, but the best way to obtain FCS files for analysis in third-party software is to load the experiment and export the data. For more information, see Exporting Third Party and RLST Data on page 334.

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 global unique identifier (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 on the Acquisition window, you can analyze it on the Analysis window.

Note: Wait for acquisition to complete or stop acquisition before moving data to analysis.

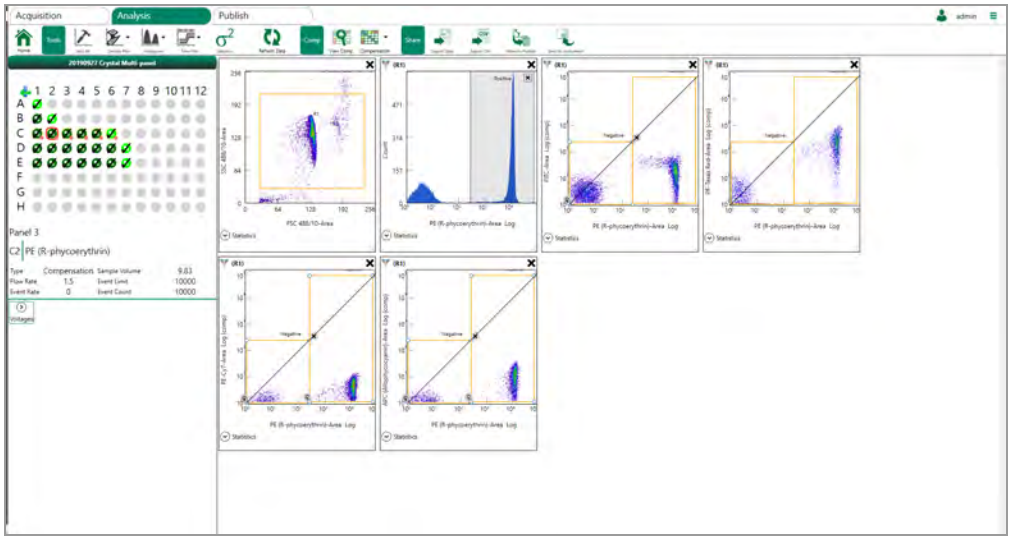
To access the Analysis tab

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.

The Analysis window appears.



Analysis Toolbar

In the Analysis tab, the toolbar contains three sections of tools to assist in performing data analysis: Tools, Comp, and Share.

Table 36. Analysis toolbar buttons and their functions

Button	Function
Tools	
	**Home** — returns to the Analysis start window, 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 257.
	**Create Density Plot** — creates a bivariate (two-parameter) density plot. For more information, see Creating Density Plots on page 246.
	**Create Histogram** — creates a univariate (one-parameter) histogram. For more information, see Creating Histograms on page 257.
	**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 248.
	**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. For more information, see Managing Plot Statistics on page 267, Viewing and Rearranging Plot Statistics on page 268, and Comparing Statistics on page 269.
	**Refresh** — refreshes data displayed on plots on the Analysis window.
Comp	
	**View Compensation Matrix** — opens the compensation matrix for viewing or editing.
	**Auto Compensation** — automatically compensates data displayed in plots. Available only in the Analysis tab.

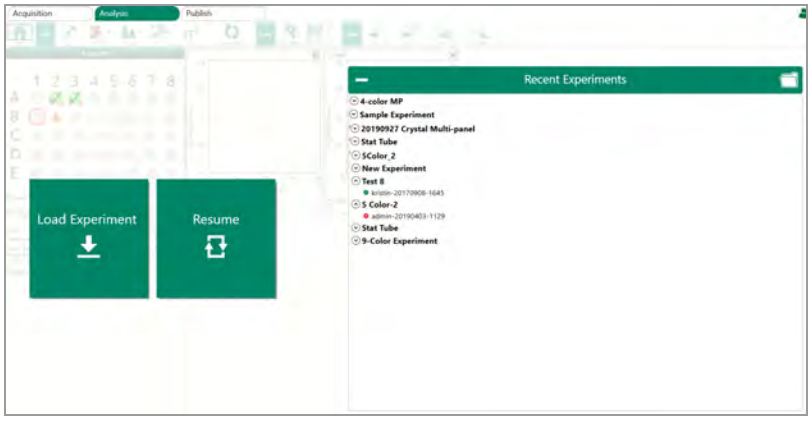
Table 36. Analysis toolbar buttons and their functions, continued

Button	Function
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 file. ■ Export run list to RLST format and export all FCS files for the current experiment. ■ Export full experiment, including run list, telemetry, and all FCS files for each position; compress to zip file.
	**Export Data to CSV** — allows you to select statistics and gates, and then 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 Run List to Local Instrument** — send updated run list back to instrument for acquisition (for example, after compensation is applied).

Loading Previous Experiments

In the Analysis tab, you can view data from previous experiments.

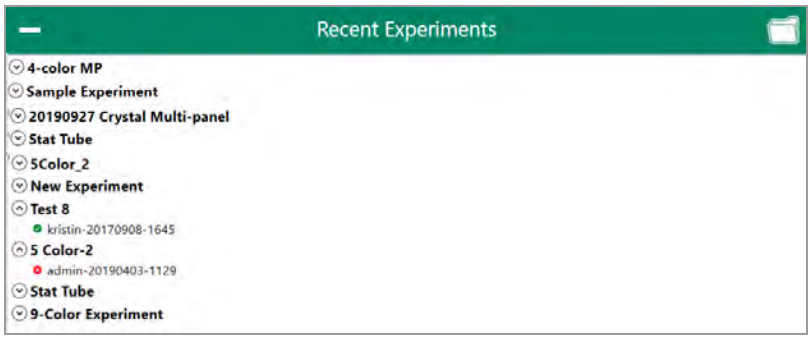
Tip: Sessions displayed in the Recent Experiments pane with a green check mark, contain FCS files.



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.

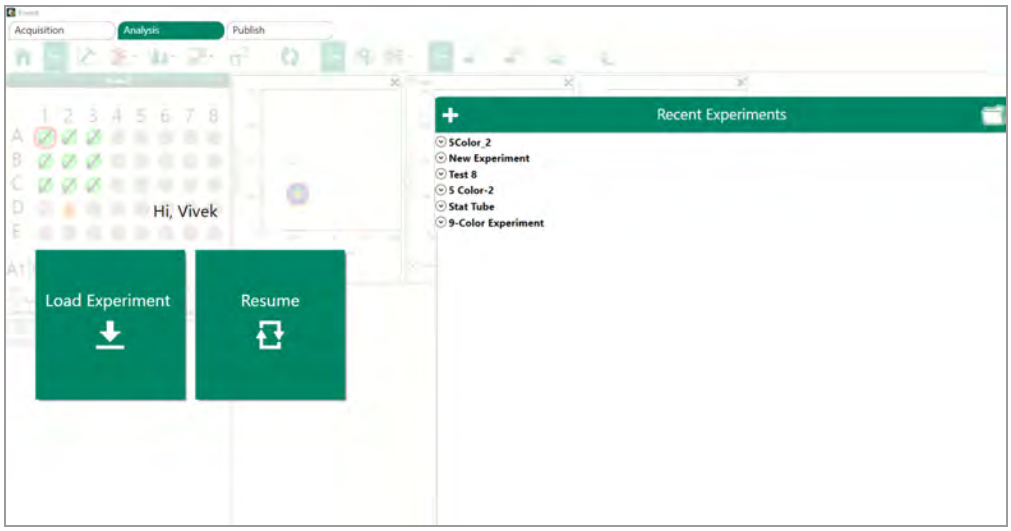
   The experiment session opens in the Analysis workspace.

   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 window. 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.



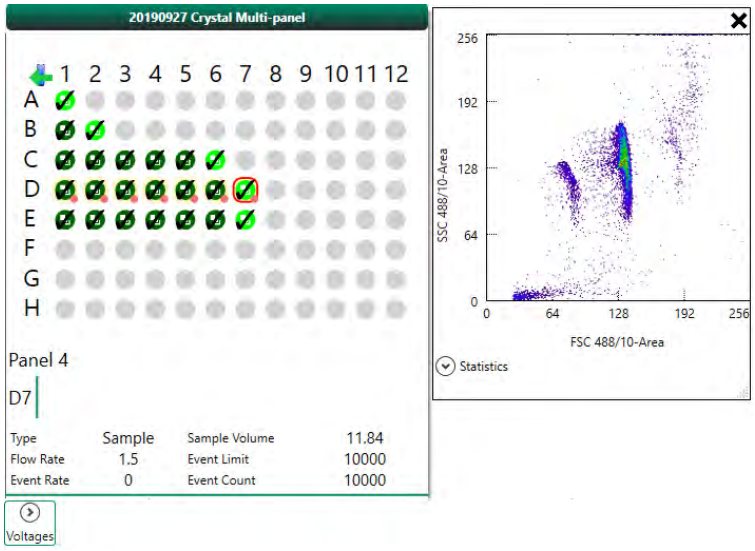
Working with Plots and Statistics in the Analysis Tab

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 242
- Working on the Acquisition Screen on page 289
- Managing Plot Statistics on page 267

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 322.

Click Refresh 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:

- Panel identifier
- 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 339.

Working with Compensation in the Analysis Tab

In the Analysis tab, you can either view and manually edit the compensation matrix for each panel, 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 Chapter 12, Example 9-Color Immunophenotyping Experiment.

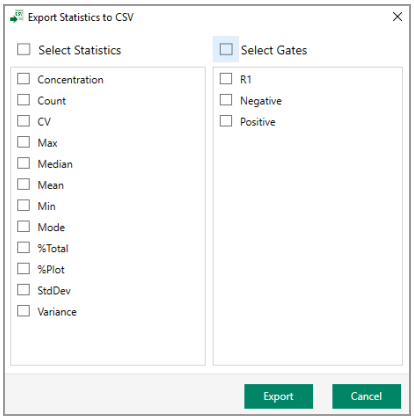
Exporting Statistics and Gates to CSV

You can export statistical and gate data for all experiment sample positions to a comma-delimited file.

Note: All panels are exported to the same file.

To export data to CSV

1. Click Export CSV on the toolbar.
2. Select the checkbox for each statistic and gate that you want to include. You must select at least one of each.



3. Click Export.
4. In the Save As dialog box, click Save.

   The data are saved to a CSV file.

For information on exporting data to FCS and RLST files, see Exporting Third Party and RLST Data on page 334.

Exporting Third Party and RLST Data

Everest Software allows you to export

- FCS files for analysis in third-party software, for a single position on the plate, multiple positions, or all positions.
- Other associated data files such as run list and telemetry files

Tip: To configure Everest Software for exporting FCS files into third-party software, see Allowing FCS File Conversion for Third-Party Software on page 133.

Note: Everest Software can export files up to 55 GB in size. Use Windows Explorer to copy files exceeding 55 GB to the target destination. Exported FCS files over 5 GB, and exported zip files over 6 GB, include all data parameters and the original compensation matrix.

Everest Software creates a folder in the default D:\EverestUsers folder labeled Export into which all files are saved. The files are saved into a subfolder named using the following convention:

username-experiment name_session date-revision number

For example:

D:\EverestUsers\Export\vbala-5Color_2-20180125-1421

Note: Because all sessions are included when exporting the full experiment to a compressed zip file, the software labels the file with only the user name and experiment name. For example:

D:\EverestUsers\Export\vbala-5Color_2.zip

Important: Exported data that do not include the full experiment (including the run list, telemetry, and all FCS files) do not appear in the Recent Experiments panel on the Home window.

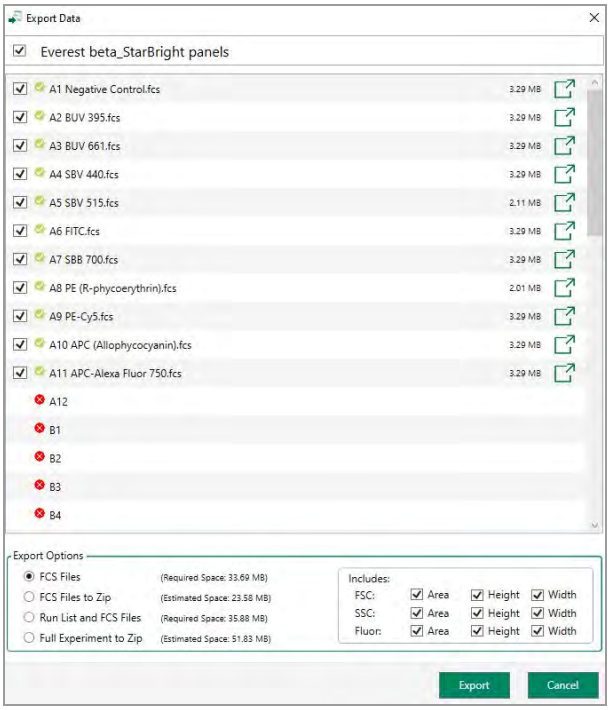
Exporting the FCS Data File for a Single Position

This procedure explains how to export only the FCS file for a single position on the plate. You can also export the FCS and related data files for a single position. See Exporting FCS and RLST Data Files on page 336.

To export the FCS file for a single position on the plate data to FCS and RLST

1. Click Export Data on the Acquisition toolbar or the Analysis toolbar.

The Export Data dialog box appears.



Positions for which data have not been acquired and saved are indicated with a red circle rather than a check mark, and the option to export does not appear.

2. To export data for a single position, click the export button for the position in the right column.
3. In the Browse For Folder dialog box that appears, do one of the following:
   - Click OK to accept the default location (D:\EverestUsers\Export).
   - Click Make New Folder to create a new folder in the \Export folder, or browse to another location, rename the new folder and click OK.

   Everest Software saves the FCS file for that position.

Exporting FCS and RLST Data Files

This procedure explains how to export the FCS and other data files for a single position, multiple positions, or the complete experiment.

To export data the FCS and RLST data files

1. Click Export Data on the Acquisition toolbar or the Analysis toolbar.

   The Export Data dialog box appears.

2. (Optional) In the Export Data dialog box, clear the checkbox for any position that you want to exclude.

3. In the Export Options section, choose one of the following:

   - **FCS Files** — exports all FCS files for the each selected position.
   - **FCS Files to Zip** — exports all FCS file for each selected position and compress to zip file.
   - **Run List and FCS Files** — exports run list to RLST format and export all FCS files for the selected positions.
   - **Full Experiment to Zip** — exports full experiment, including run list, telemetry, and all FCS files for the full experiment, including all sessions, and compress to zip file.

4. By default, each export option includes the FSC, SSC, and fluorophore information including the area, height, and width for the position.

   To exclude any data parameter, clear its checkbox.

5. Click Export.

6. In the Browse For Folder dialog box that appears, do one of the following:

   - Click OK to accept the default location (D:\EverestUsers\Export).
   - Click Make New Folder to create a new folder in the \Export folder, or browse to another location, rename the new folder and Click OK.

   Everest Software saves the FCS file for that position.

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 Run List to Local Instrument in the Share section of the toolbar.

   The experiment run list opens 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.

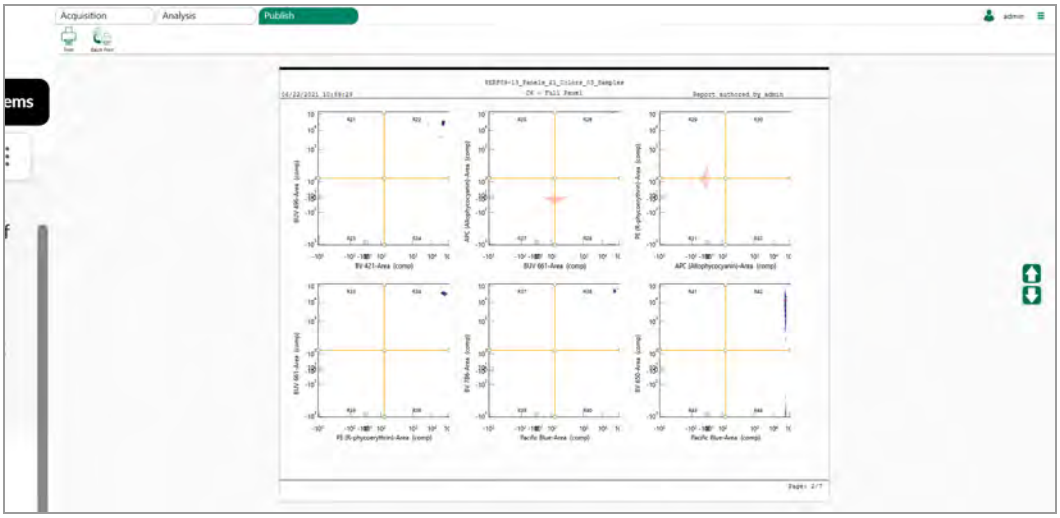
To access the Publish tab

1. Select a sample position in the Analysis tab plate map.
2. Do one of the following:
   - On the Analysis window, click Move to Publish in the Share section of the toolbar.

   - Select the Publish tab.

   The report for the selected position opens in the Publish tab.

3. Do one of the following:
   - Click Print to print the set of pages for the selected well.
   - Click Batch Print to create and print a report for all wells.

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.

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 window.

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.

Quality Control and ZE5-EYE Reports

Three report tools are available to track performance of the ZE5 Cell Analyzer: daily QC reports, QC trending reports, and ZE5-EYE trending reports.

Reports Tools

The buttons available to you in the Reports section of the toolbar depend on whether you have administrative rights.

Table 37. Reports toolbar buttons and their functions

Button	Function
	**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.
	**ZE5-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.

Generating User Reports

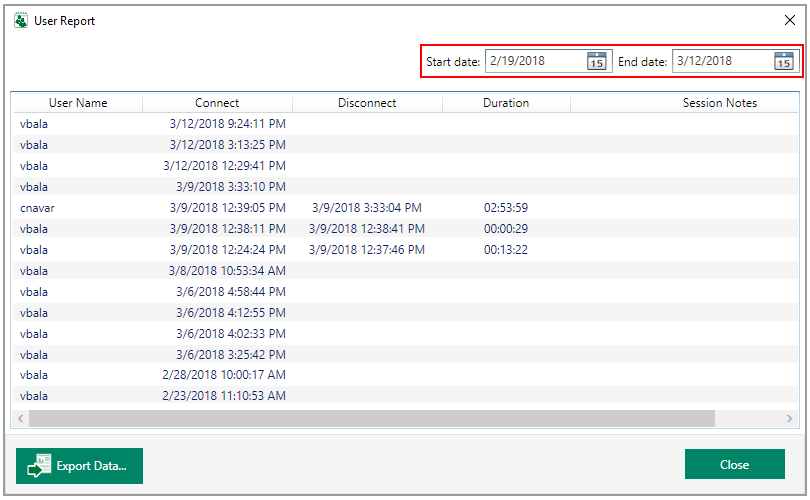
Administrators can view and save user reports for billing purposes and to analyze system usage. These reports track usage over time and include session notes entered by logged-in users. These reports can be printed or exported for reference.

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 Data.

...

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.

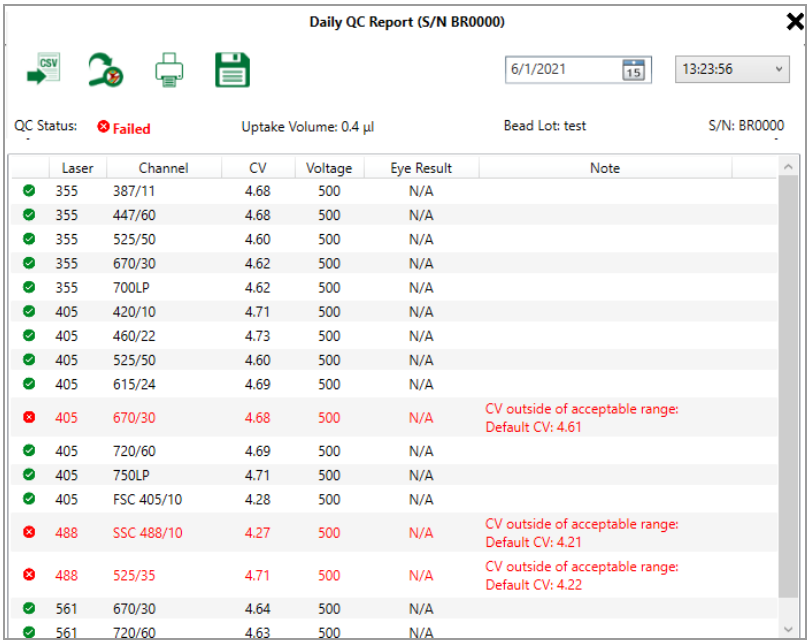
To view the most recent daily QC report

▶ In the Reports section of the toolbar, click QC Report.

The most recent daily QC report appears.

A green checkmark appears next to those detectors whose CV, PMT voltage, and ZE5-EYE values pass the QC criteria. When all values for all detectors are green, the QC status is Passed.

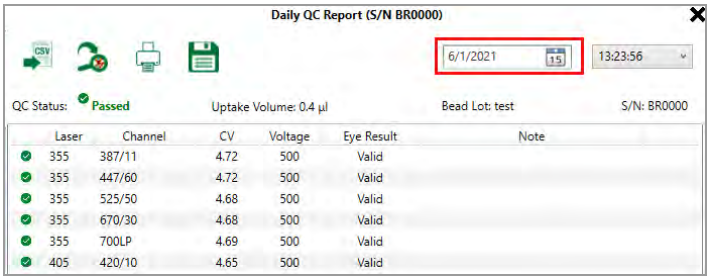
A red x appears next to those detectors whose values do not pass the QC criteria and the software displays the reason for the failure in the Note column. In this case, the QC status is Failed, for example:



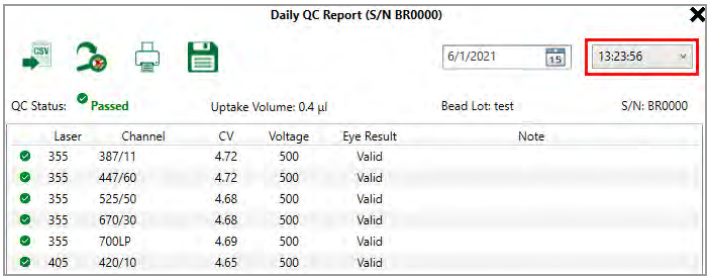
Laser	Channel	CV	Voltage	Eye Result	Note
355	387/11	4.68	500	N/A	
355	447/60	4.68	500	N/A	
355	525/50	4.60	500	N/A	
355	670/30	4.62	500	N/A	
355	700LP	4.62	500	N/A	
405	420/10	4.71	500	N/A	
405	460/22	4.73	500	N/A	
405	525/50	4.60	500	N/A	
405	615/24	4.69	500	N/A	
405	670/30	4.68	500	N/A	CV outside of acceptable range: Default CV: 4.61
405	720/60	4.69	500	N/A	
405	750LP	4.71	500	N/A	
405	FSC 405/10	4.28	500	N/A	
488	SSC 488/10	4.27	500	N/A	CV outside of acceptable range: Default CV: 4.21
488	525/35	4.71	500	N/A	CV outside of acceptable range: Default CV: 4.22
561	670/30	4.64	500	N/A	
561	720/60	4.63	500	N/A	

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.



3. If more than one report exists for a date, click the time selector and select a time.



To perform other actions on QC reports

▶ Point to the report and select an option from the buttons that appear.

Button	Function
	Export the report to CSV.
	Move the data file to analysis.
	Print the report.
	Save the report.

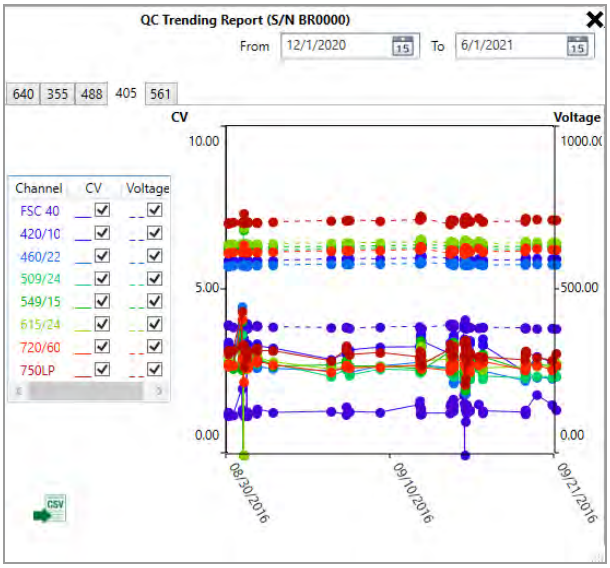
...

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.



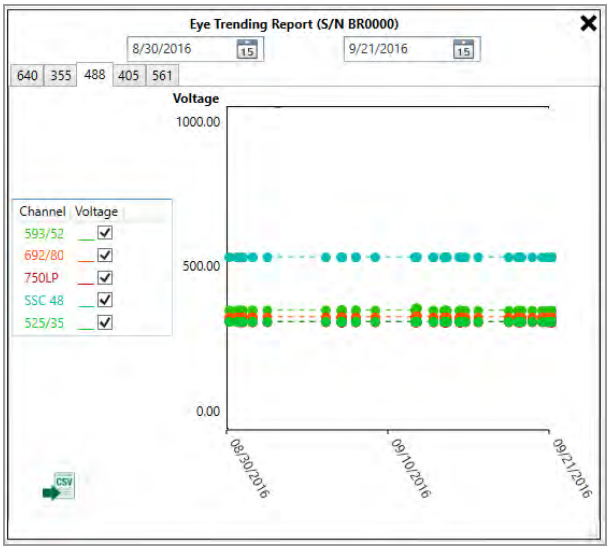
4. To view a QC trending report for a different laser, click its tab.
5. To export the QC trending report to a comma-delimited file:
   a. Click Export to CSV.
   b. Specify a file name and location.
   c. Click Save.

Generating ZE5-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 49 and Using the ZE5-EYE to Confirm Filter Choices on page 158.

To access the EYE trending report

1. In the Reports section of the toolbar, click ZE5-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 ZE5-EYE trending report for a different laser, click its tab.
5. To export the ZE5-EYE trending report to a comma-delimited file:
   a. Click Export to CSV.
   b. Specify a file name and location.
   c. Click Save.

Chapter 12 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:

- BUV 395
- BV 421
- BV 510
- BV 711
- Alexa 488
- PE (R-phycoerythrin)
- PE-Dazzle 594
- PE-Cy7
- APC-Cy7

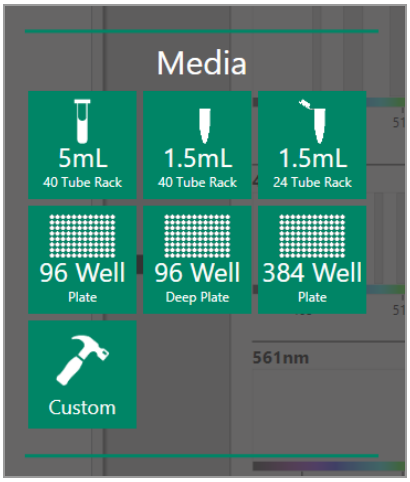
Preparing Controls and Samples

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.

Creating the New Experiment and Selecting the Media Layout

1. Open Everest Software and log in.
2. From the Home window, select New Experiment.
3. Select a media layout.

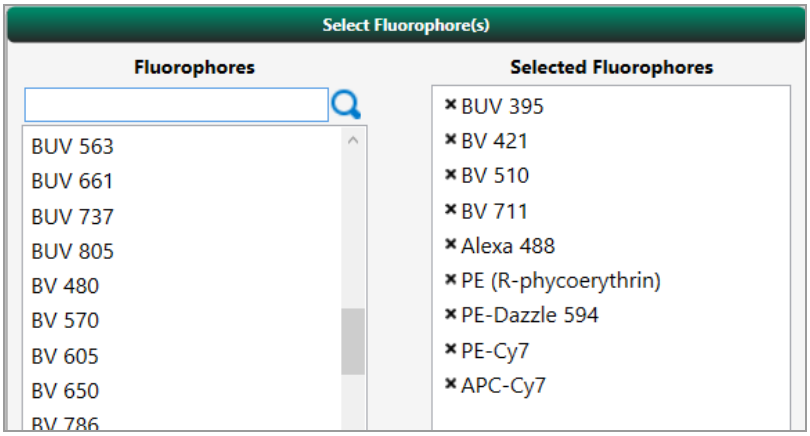


When you click a layout, the Fluorophores window in the Experiment Builder opens.

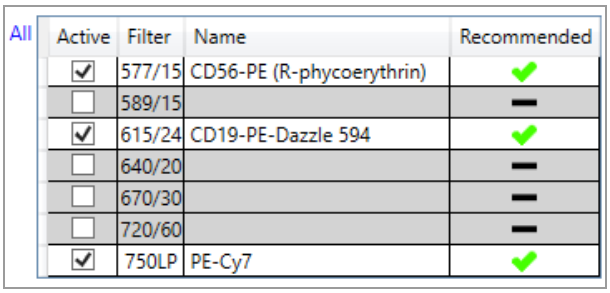
…

Selecting Fluorophores and Detectors

1. In the Select Fluorophores window, double-click fluorophore names in the Fluorophores list to add them to the Selected Fluorophores list.



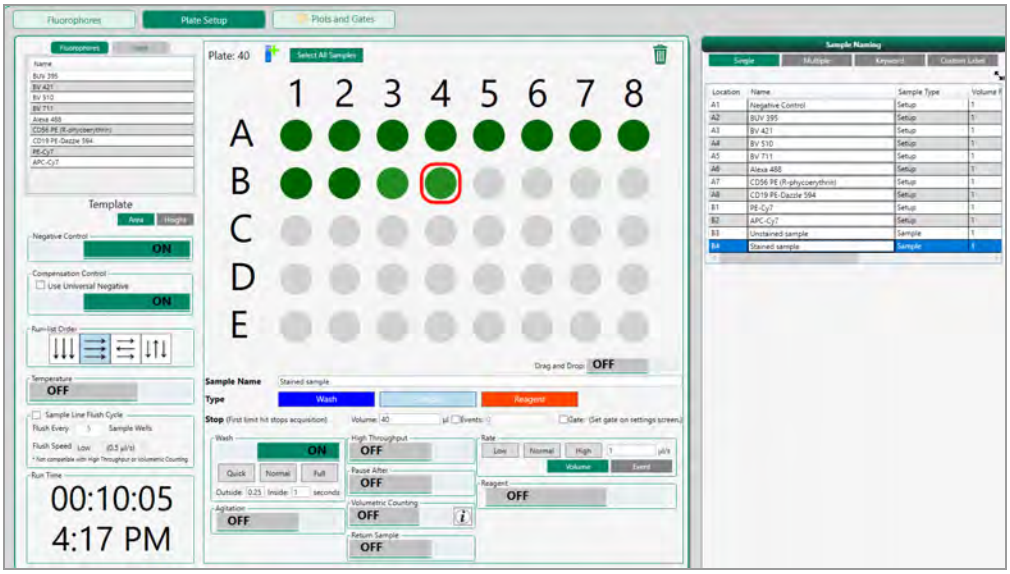
2. In the Available Detection panel, add to the parameter names as applicable; for example, change the parameter names to reflect the cell markers used in the experiment.



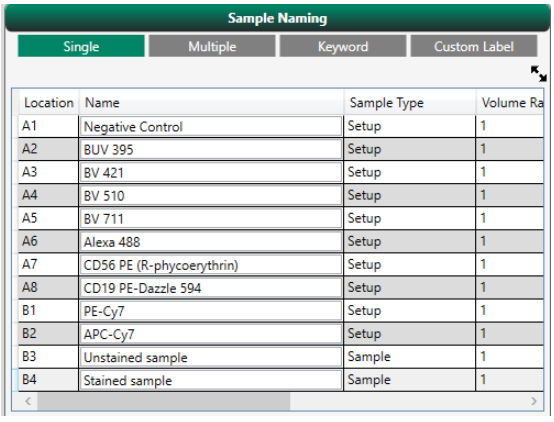
Configuring the Controls and Sample in the Plate Layout

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. In the left panel, enable negative and compensation controls.



2. In the Plate Setup pane, select two unused positions and then click Sample for the position type.
3. (Optional) To change the sample name, click the name box in the Sample Naming pane and type a new name.



Sample Naming

Single | Multiple | Keyword | Custom Label

Location	Name	Sample Type	Volume Ra
A1	Negative Control	Setup	1
A2	BUV 395	Setup	1
A3	BV 421	Setup	1
A4	BV 510	Setup	1
A5	BV 711	Setup	1
A6	Alexa 488	Setup	1
A7	CD56 PE (R-phycoerythrin)	Setup	1
A8	CD19 PE-Dazzle 594	Setup	1
B1	PE-Cy7	Setup	1
B2	APC-Cy7	Setup	1
B3	Unstained sample	Sample	1
B4	Stained sample	Sample	1

...

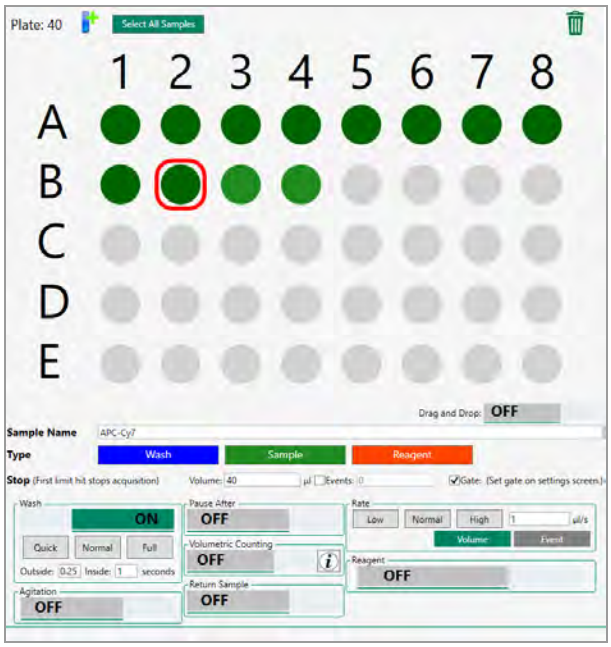
4. Apply sampling parameters to all positions at the same time:

   a. Select all enabled wells.

   b. Select the Gate checkbox.

      Note: The volume defaults to the maximum volume for the media type you are using. You will set sample and control gate limits after completing the plate setup.

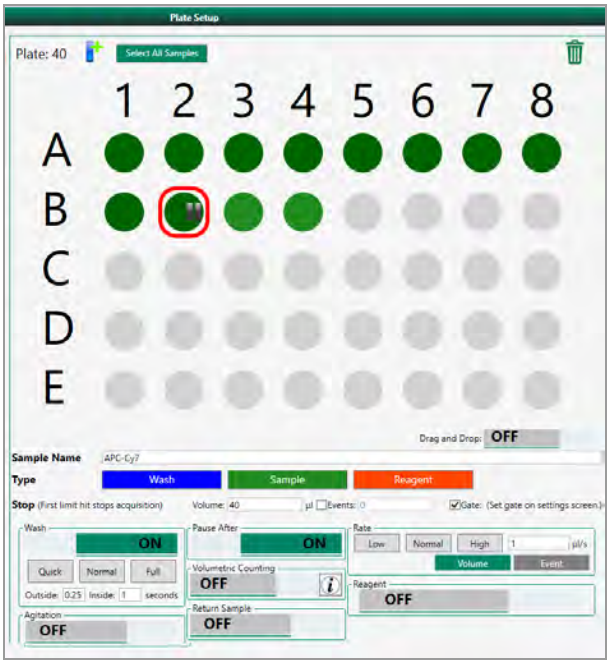
   c. In the Volume box, enter the actual volume used in the tubes for this experiment.



   d. Specify any other sampling parameters to apply to these positions, such as agitation or target flow rate or target event rate.

5. Click the position for the last compensation control.
6. Click Pause After to instruct the run list to pause after the last compensation control is acquired.

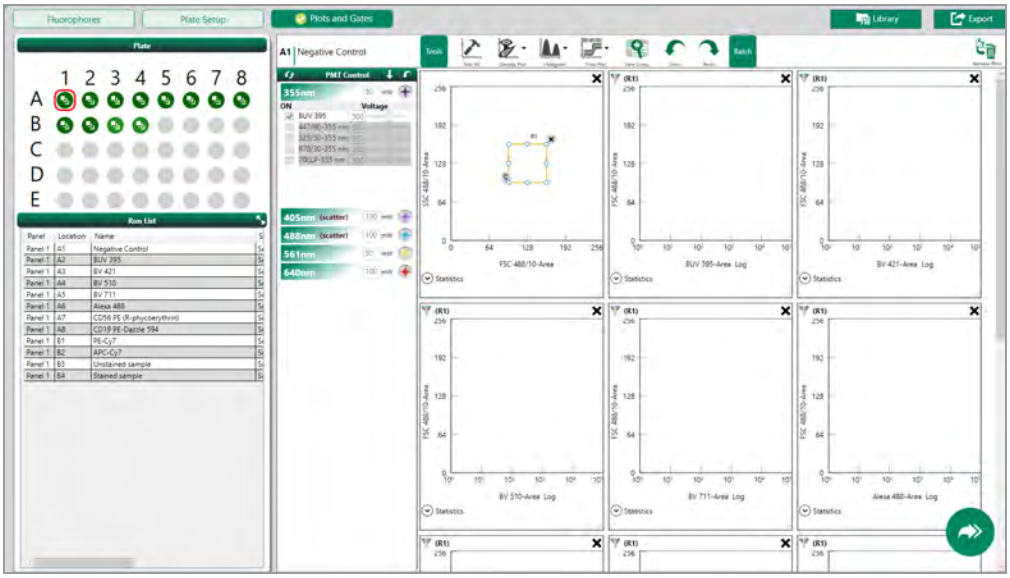
   Note: This allows you to perform automatic compensation before running the fully stained sample. A pause image appears on the selected position, for example:



   Tip: For more information about images, see Position Images on page 91.

...

7. When sample setup is complete, select the Plots and Gates tab.

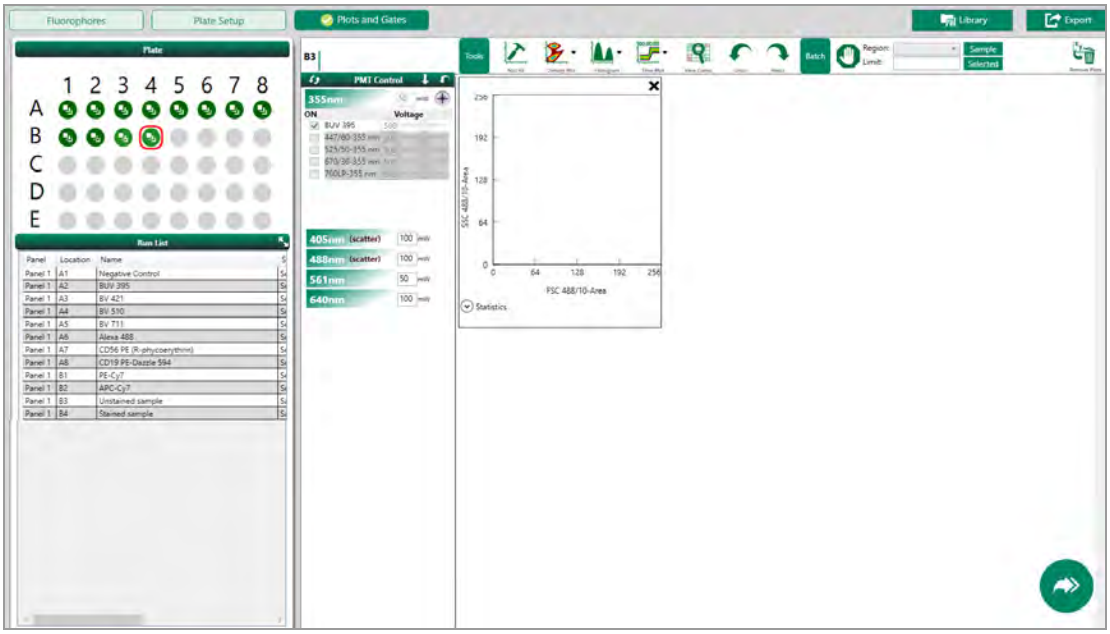


Note: Plots are created automatically for the controls.

Creating Plots for the Experimental Sample

1. Click the sample position in the plate map, and then click Create Density Plot.

2. Create a forward scatter versus side scatter plot (FSC/SSC) for the 488 laser.



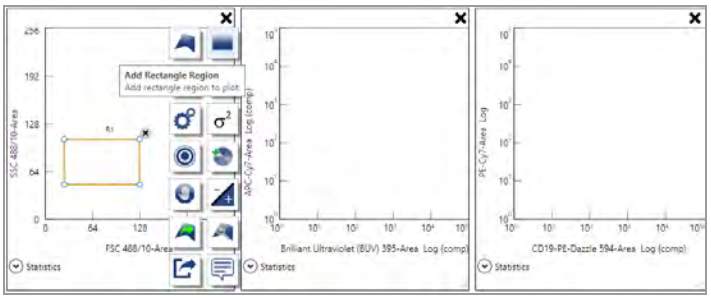
...

3. Create plots for the remaining parameters in the experiment.

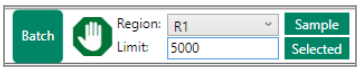
   Important: For each plot, click Comp so that the plots will display compensated data.

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.



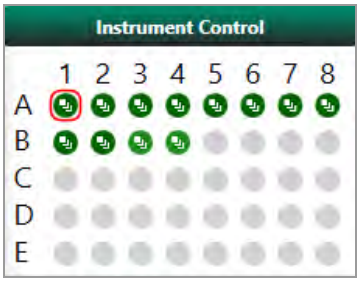
   This gate limit will be the stop limit for sample acquisition.

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 plate onto the instrument.
2. In the Plots and Gates tab of the Experiment Builder, click Apply.
3. Click the first position in the plate map.

   This is the position from which the instrument will start sampling.

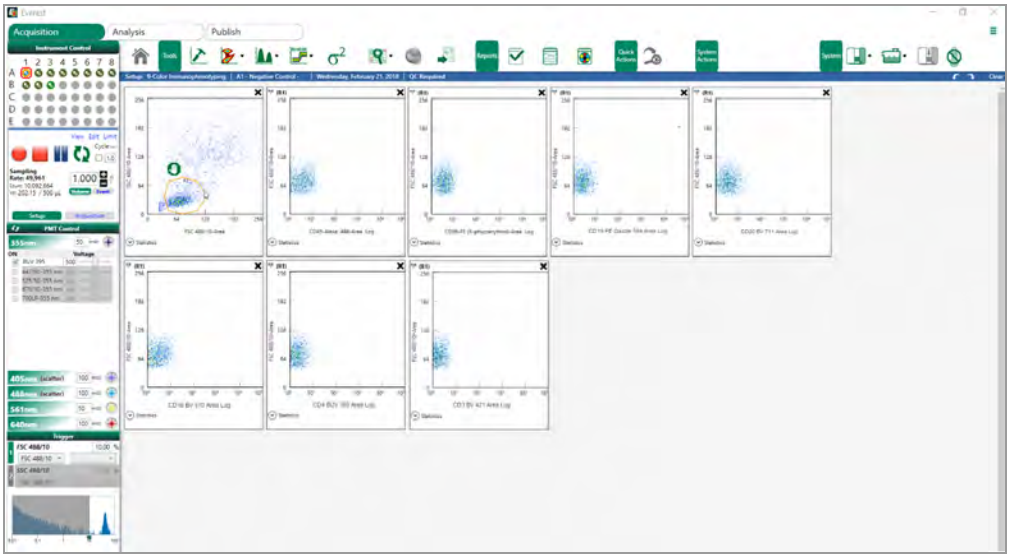


...

4. In the Instrument Control panel, ensure that Setup mode is active.

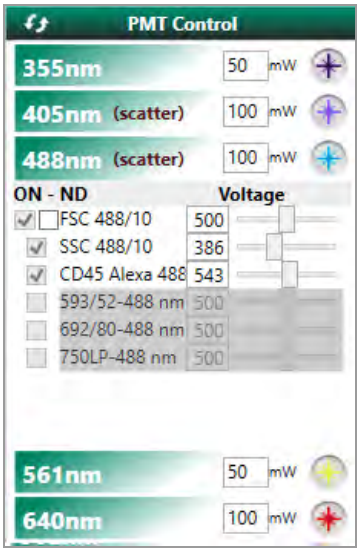
5. Click Play ( ).

   The system displays plots in the Acquisition 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.

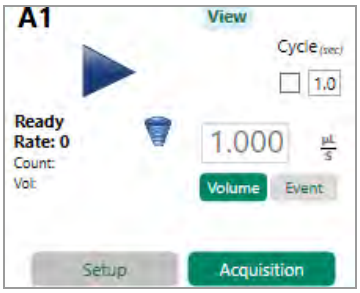


8. After setting up gates and voltages, click Record to save data for the sample.

   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.

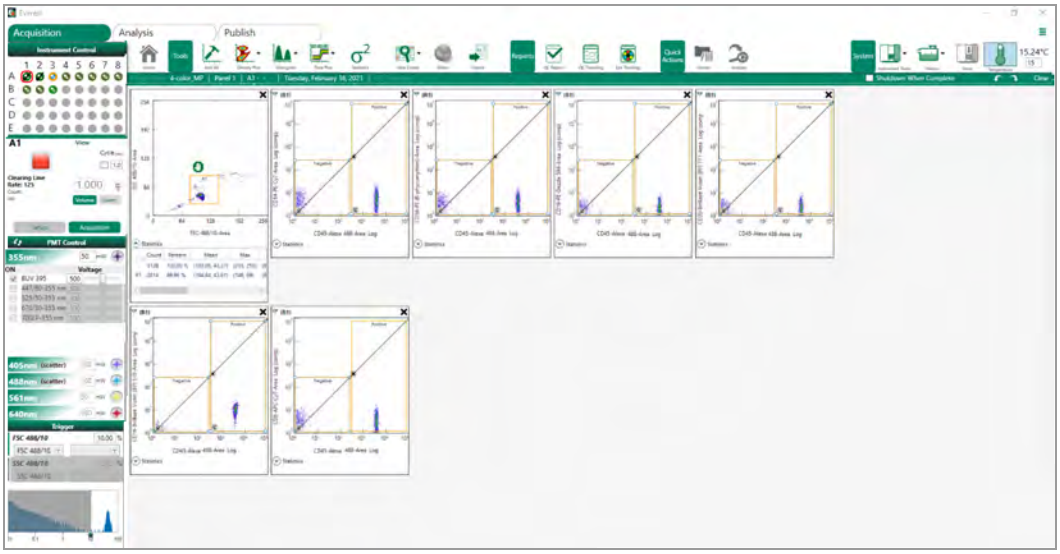
10. When you are satisfied with the setup plots, click Acquisition to put the system in Acquisition mode.



...

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 appears on the control positions in the Instrument Control panel to show that they have been acquired.

13. After acquisition is complete, click Analyze on the toolbar to move data to the Analysis tab.

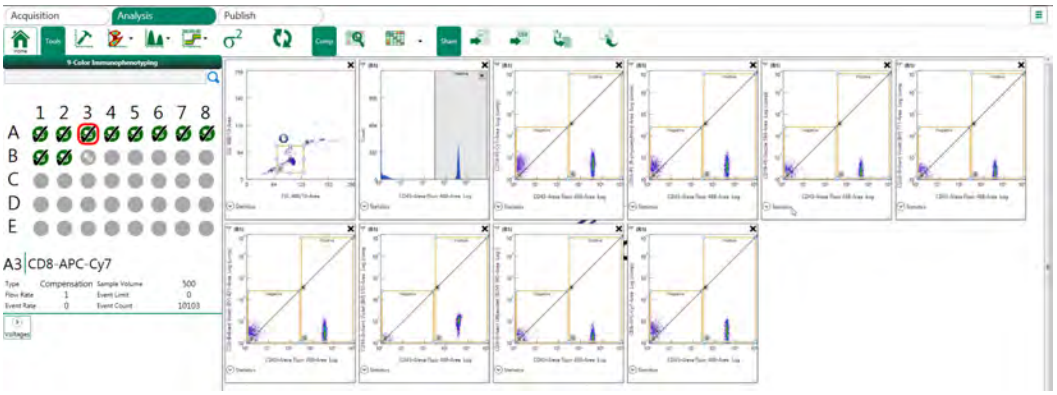
Performing Initial Data Analysis

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.

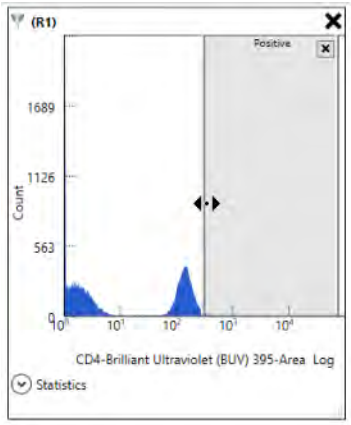
The following steps illustrate the first option.

1. Click a control position to view the data from that control.

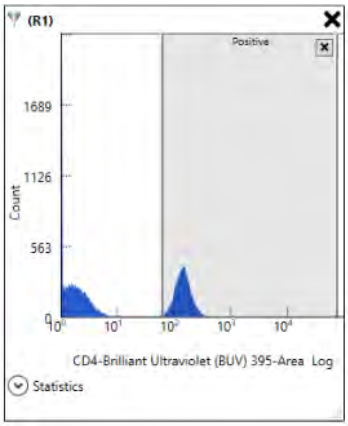


2. For each control, ensure that the positive and negative populations are identified properly (for example, two populations should appear and the scatter gate set should be set correctly).

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.

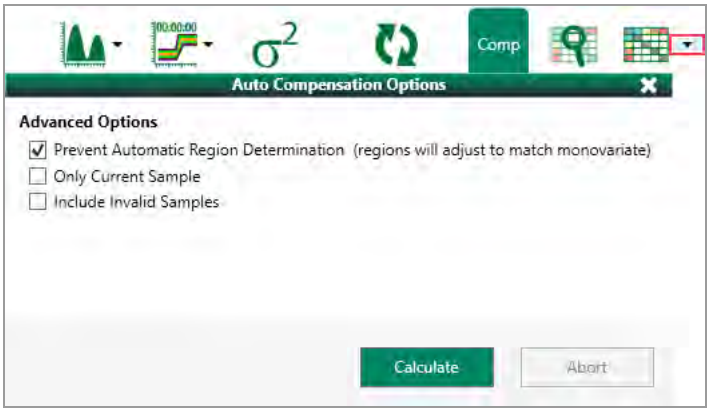


Note: If compensation is performed in the Analysis tab and those settings are moved back to the instrument, compensation settings will be saved within the FCS files for any samples that are subsequently acquired. If all sample acquisition is done before compensation is performed, compensation settings will not be saved within the FCS files.

Performing Automatic Compensation

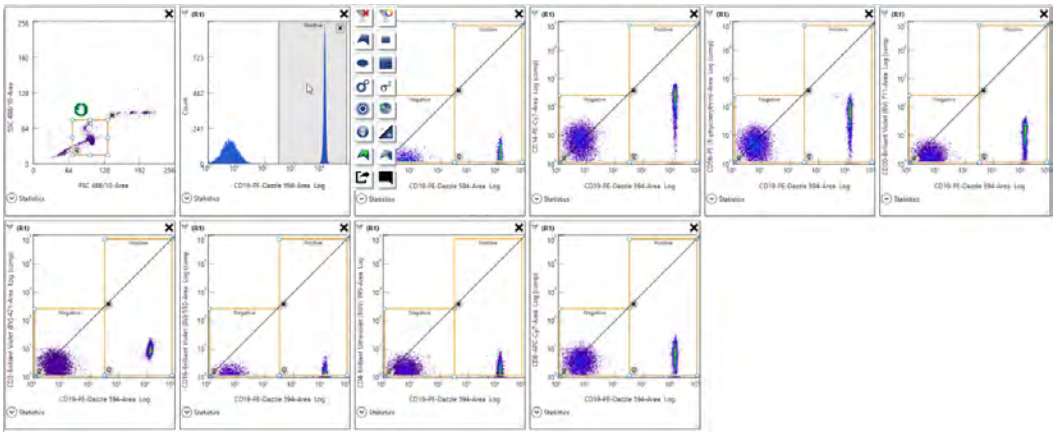
Before running the fully stained sample, perform automatic compensation. The FCS file for the fully stained sample will be exported with the compensation matrix. Data are exported in accordance with FCS 3.1 standards and can be analyzed using third-party software.

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.

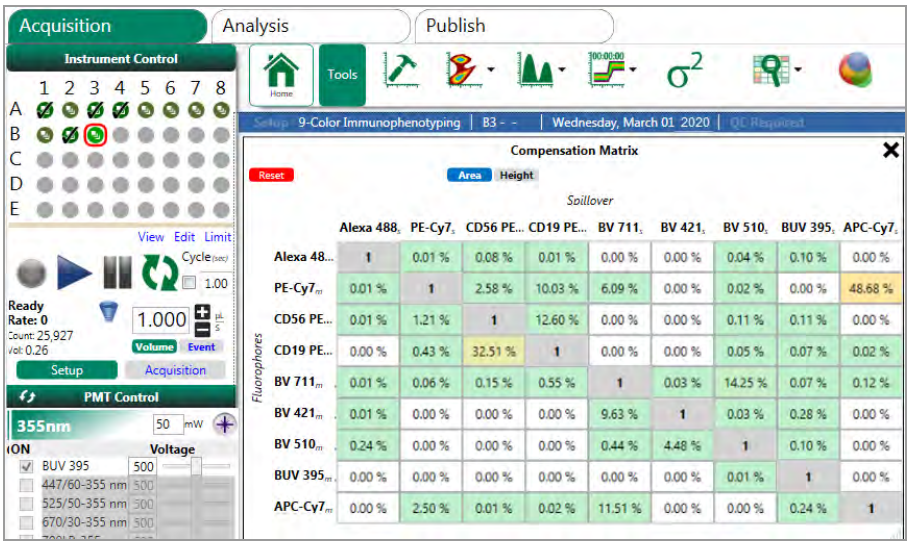


4. Click Send to Instrument to send the calculated compensation values back to the local instrument.

Everest software displays the contents on the Acquisition tab.

5. On the Acquisition toolbar, click View Comp. (View Compensation Matrix).

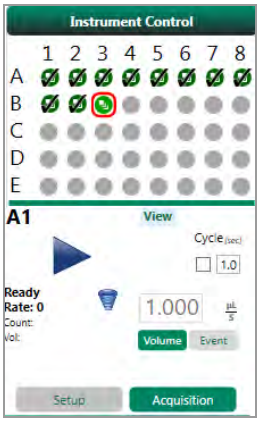
The compensation matrix displays the calculated compensation values.



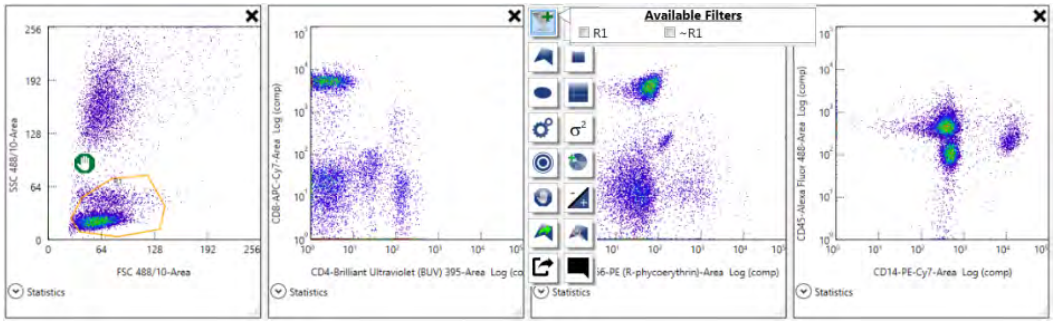
Resuming Acquisition

After applying compensation, acquire sample again.

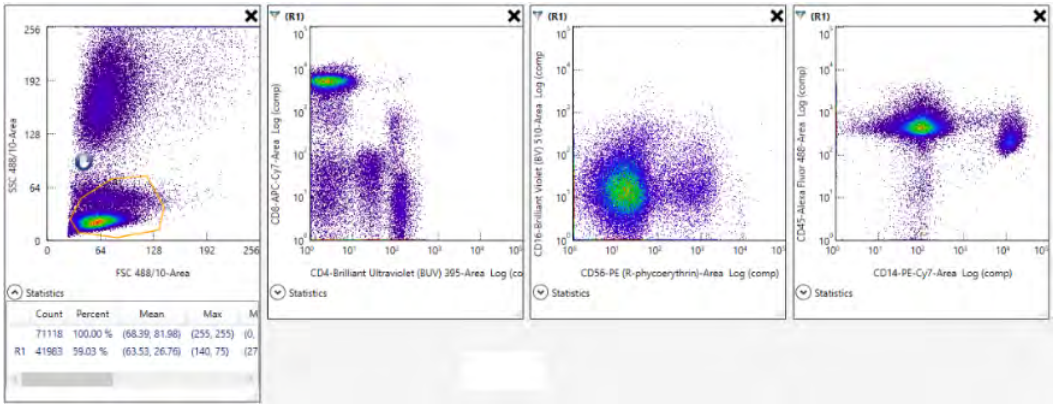
1. In the Instrument Control panel, ensure that Acquisition Mode is active.



2. In the plate map, click the sample position from which acquisition should resume.
3. Click Play to start acquiring data from the fully stained sample.
4. 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. On the Analysis tab, click Export analyze data using third-party software.

Tip: You can also export data from the Local Instrument tab.

3. Select an export option and click Export.

   Note: Everest Software exports one FCS file per acquired control or sample.

   For more information about exporting data files, see Exporting Third Party and RLST Data on page 334.

4. In the Browse For Folder dialog box that appears, accept the default Export folder or navigate to another location and click OK.
5. Close the export dialog box when the export is complete.

Chapter 13 Maintenance

To ensure reliable operation of the ZE5 Cell Analyzer and accuracy of experimental results, perform preventive maintenance regularly.

Important: Always follow the personal protective equipment (PPE) guidelines relevant to your laboratory's safety procedures for handling 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 needed, it can be run prior to the shutdown process. See Cleaning the Sample Line and Probe on page 371. See Cleaning Solutions on page 369 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 Shutdown in the Home window to initiate the automatic shutdown process. For more information, see Shutting Down on page 188.

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 the 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 the optics
- Fill the coolant

Cleaning Solutions

Important: Always follow the PPE guidelines relevant to your laboratory's safety procedures for handling 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 System Decontamination on page 373.

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.

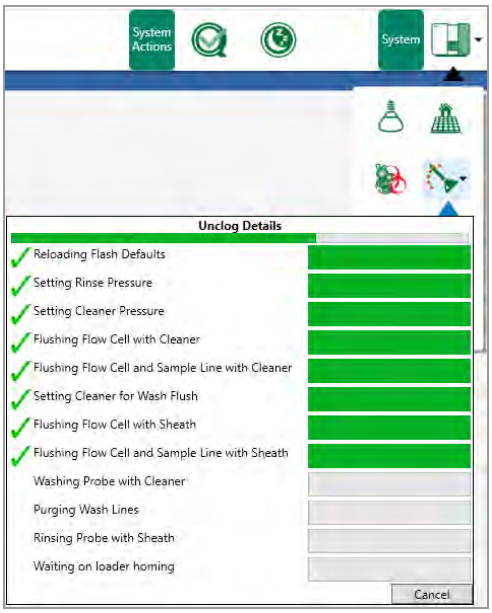
Unclogging the Sample Line and Probe

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 in the Instrument Tools dropdown list.

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 369 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 in the Instrument Tools dropdown list..

2. Follow the directions that appear on window.

   The system is ready for use when this process is complete, but you can choose to run the QC process afterward.

Preparing the ZE5 Cell Analyzer for Long Term Storage

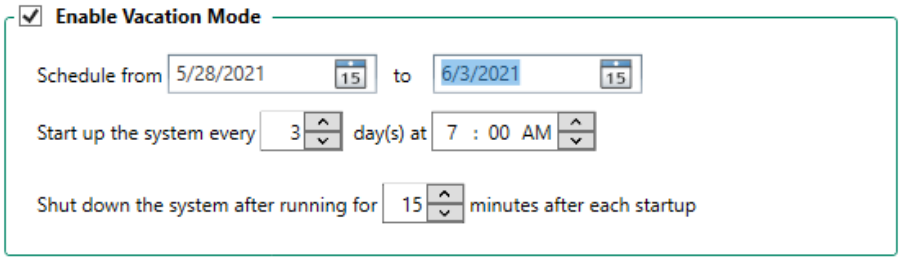
If your ZE5 Cell Analyzer will be unused for a month or longer, Bio-Rad recommends that you follow this procedure to protect the operation of your instrument.

To set up your ZE5 Cell Analyzer for long-term storage

1. Follow the decontamination procedure in Decontaminating the System on page 378, which provides instructions for decontaminating both the fluidics line and sample path.
2. After system decontamination is finished, close Everest Software and power off the computer.
3. Power off the ZE5 instrument using the power button located on the back of the instrument.
4. If the ZE5 Cell Analyzer is not connected to an uninterrupted power supply (UPS), remove the plug from the wall outlet (otherwise, leave it plugged in).

To set up vacation mode

1. Click ☰ to open the main menu, and then click Preferences.

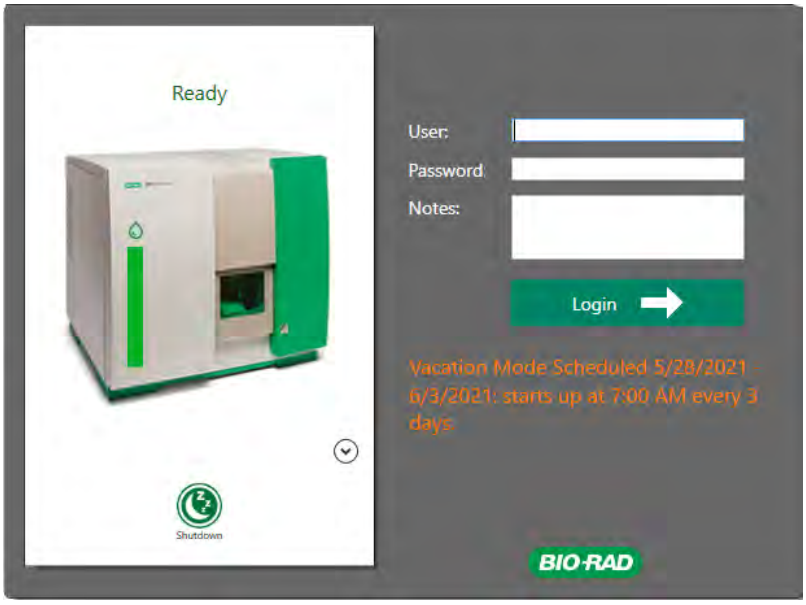


2. Select the Enable Vacation Mode checkbox.
3. To set the vacation date range, click each calendar icon to set the start date and end date.

   Important: You must set the Vacation Mode start date to the following day at the earliest.

   Note: After the end of the vacation period, vacation mode automatically resets to off.
4. Specify how often and at what time of day the system startup should occur.
5. Specify how many minutes the system should run after each startup.
6. Click OK (lower right corner) to save the changes and close the Global System Preferences dialog box.

During the vacation period, a notification appears in the Login window.



System Decontamination

Bio-Rad strongly recommends that your preventive maintenance program include decontamination of the entire ZE5 Cell Analyzer fluidics system. You can use the Decontamination wizard to completely decontaminate the system using 1% filtered bleach solution (25 ml household bleach and 2.475 L filtered water). See Cleaning Solutions on page 369 for details.

Note: Only administrative users can run the Decontamination wizard.

Regular system decontamination 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 necessary; for example, 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.

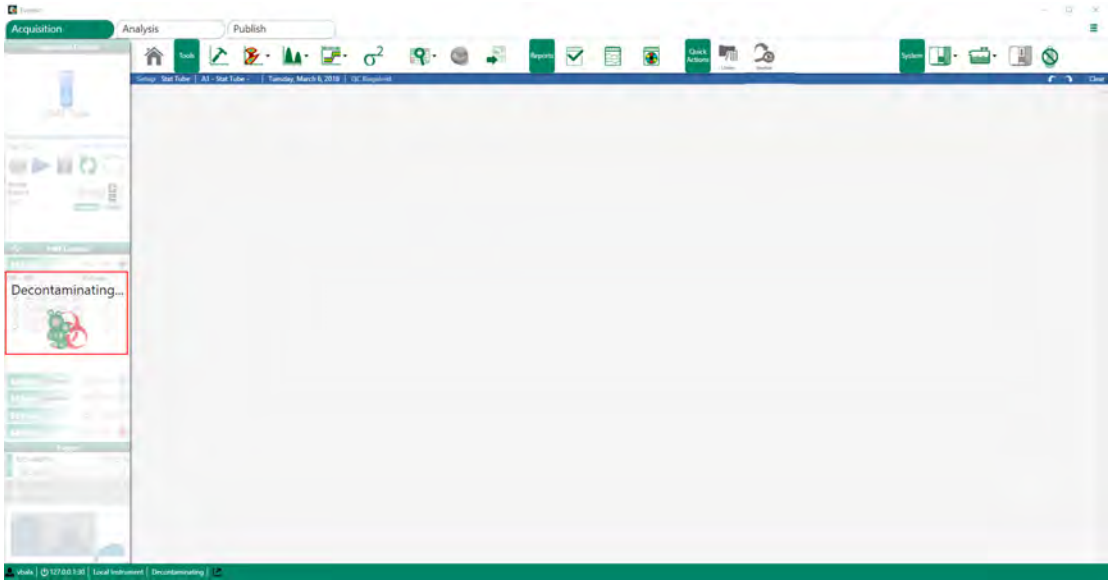
The wizard guides you through the necessary steps to complete the decontamination process, which takes about 4 hr. When the process completes, the system is ready to run samples, although you can run the QC process afterward.

Note: The Decontamination wizard uses the internal fluidics system for its process, even if you normally use an external source for DI water, such as house DI or a fluidics cart. However, as soon as the decontamination is complete, use of external fluidics is resumed.

Important: While decontamination is in process, the following functions are disabled:

- Returning to the Acquisition window
- Viewing the optical filter configuration
- In the Instrument tools:
  - Returning the sample probe to home position
  - Unclogging the sample line, probe, and flow cell
  - Cleaning the sample line and probe
  - Pausing the sheath fluid and disabling the lasers
  - Swapping QC beads
- Opening the sample loader door
- Running the QC process
- Sending the run list to the local instrument
- Shutting down the instrument
- Closing Everest software

The system displays a message indicating that decontamination is in progress.



Note: If Everest Software detects an instrument error, it stops the decontamination process immediately and displays an advisory message. Follow the instructions in the message and contact Bio-Rad Technical Support for assistance.

Required Materials

To complete the decontamination process you will need the following materials.

- Four extra sheath bottles
- Two extra additive bottles
- Two extra cleaner bottles
- Two standard fluorescence-enabled cell sorting (FACS) tubes (12 x 75 mm, 5.0 ml)
- 12 L filtered DI water
- 100 ml filtered bleach (filtered through a 0.2 µl filter flask or syringe filter)

Preparing for the Decontamination Process

Important: When handling sheath fluid and DI water bottles, always wear gloves and minimize air exposure to help avoid contamination.

Before beginning the decontamination process you must

- Decontaminate the outside of the probe with 10% bleach solution.
- Prepare bleach and rinse bottles for decontamination.
- Replace both sheath bottles with bottles filled with 2.5 L diluted 1% bleach.
- Replace the additive and cleaner bottles with bottles filled with 200 ml diluted 1% bleach.

This section explains how to decontaminate the probe and prepare the bleach solution.

Tip: You can prepare the bottles while the system is decontaminating the probe.

To decontaminate the probe and sample lines

1. Prepare 100 ml of filtered bleach solution using a 0.2 µl filter flask or syringe filter.
2. Prepare 10 ml of 10% vol bleach solution in filtered water by adding 1 ml filtered bleach to 9ml filtered DI water.
3. Fill a stat tube with 3.5 ml of the 10% filtered bleach solution and insert it into the stat tube position.
4. On the Home window of Everest Software, click STAT Tube to start acquisition.
5. Run the stat tube acquisition with the filtered bleach solution for 5 min at 1 µl/sec.
6. After 5 min, stop the stat tube acquisition and remove the stat tube.
7. Insert a stat tube containing 4.0 ml of DI water into the stat tube position.
8. Run the stat tube acquisition with DI water for 5 min at 1 µl/sec to remove any bleach from the outside of the probe.

To prepare the bleach bottles for decontamination

1. Obtain the following materials:
   - Two 4 L sheath bottles
   - One additive bottle
   - One cleaner bottle
   - 0.2 µm filtered bleach
   - Filtered DI water
2. Prepare each 4 L sheath bottle:
   a. Add 25 ml of filtered bleach solution to each bottle.
   b. Add 2.475 L of filtered DI water to each bottle.
   c. Ensure that the liquid solution in each bottle is thoroughly mixed.
3. Prepare the additive and cleaner bottles:
   a. Add 2 ml of filtered bleach to each bottle.
   b. Add 198 ml of filtered DI water to each bottle.
   c. Ensure that the liquid solution in each bottle is thoroughly mixed.

To prepare the rinse bottles for decontamination

1. Obtain the following materials:
   - Two 4 L sheath bottles
   - One additive bottle
   - One cleaner bottle
   - Filtered DI water
2. Add 2.5 L filtered DI water to each 4 L sheath bottle .
3. Add 200 ml filtered DI water to the additive bottle and the cleaner bottle.

   Tip: If you do not have additional bottles to use as rinse bottles, you can use the sheath, additive and cleaner bottles that you prepared with bleach. When you are prompted to replace the bleach bottles, remove the bottles from the ZE5 Cell Analyzer system. Empty the bottles of any remaining bleach solution. Rinse the bottles with DI water and then fill the bottles half full with DI water.

To prepare the system for decontamination

▶ In the instrument's fluidics chamber, replace both sheath bottles and the additive and cleaner bottles with those containing the filtered bleach solution.

See Replacing Sheath Bottles on page 173 and Replacing Sheath Additive and Cleaner Bottles on page 175 for information about removing and replacing the bottles.aQ`

Decontaminating the System

The decontamination process takes approximately 4 hr to complete. During the process, you will be prompted to

- Replace both sheath bottles approximately 50 min into the procedure with sheath bottles filled with 2.5 L of DI water.
- Replace the additive and cleaner bottles filled with 200 ml of DI water.
- Replace both sheath bottles again approximately 190 min into the procedure with the default (original) sheath bottles filled to the Fill Line with sheath fluid.
- Replace the additive and cleaner bottles with the default (original) additive and cleaner bottles filled to the Fill Line with the appropriate solutions.

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.

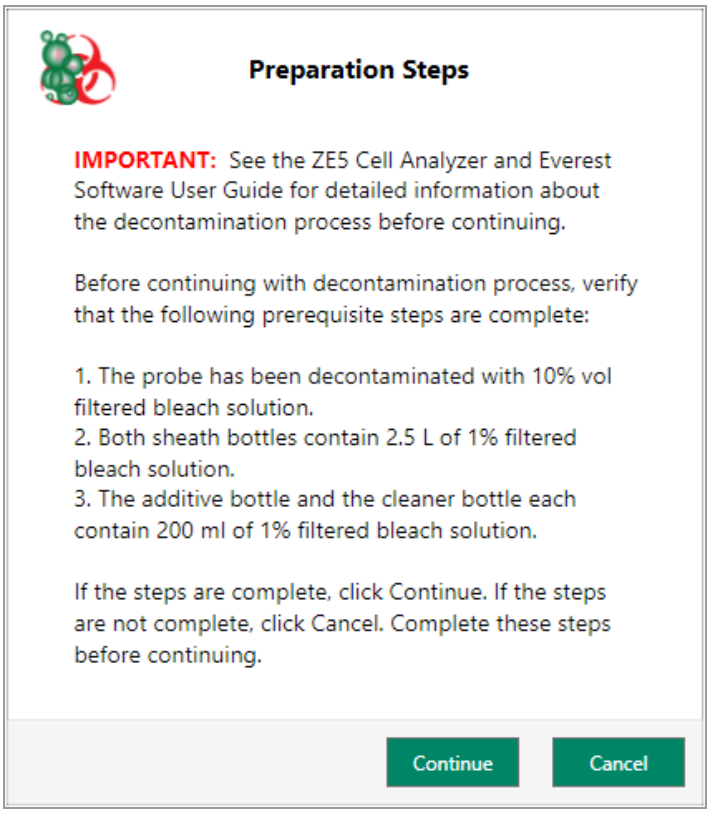
To decontaminate the system

1. Before starting the procedure, ensure that you have performed the steps in Preparing for the Decontamination Process on page 376.

   The Decontamination wizard prompts you to replace the existing bottles.

2. In the System section of the toolbar, click Decontamination in the Instrument Tools dropdown list.

The Decontamination wizard starts.

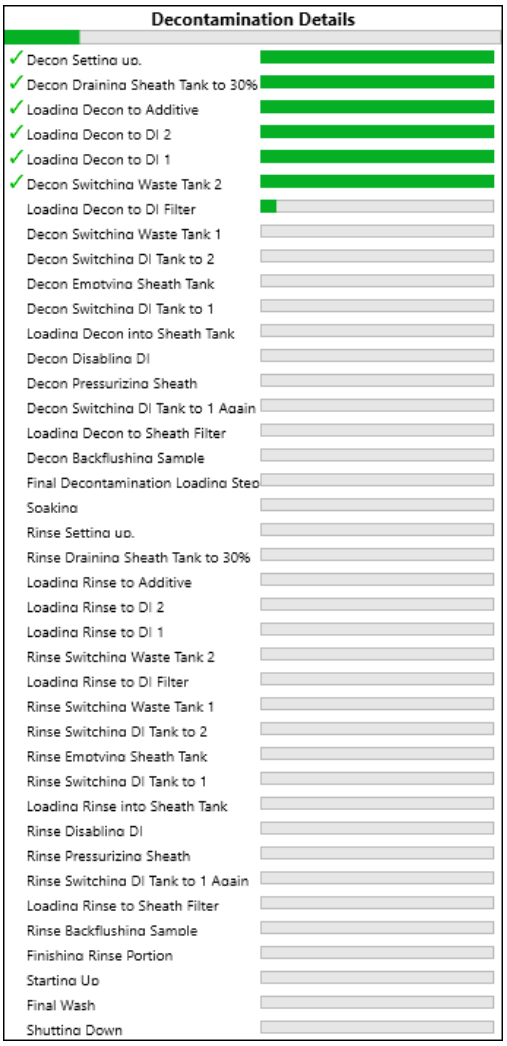


3. Carefully read the contents of the window. Verify that you have completed the preparation steps and click Continue to proceed with the Decontamination process.

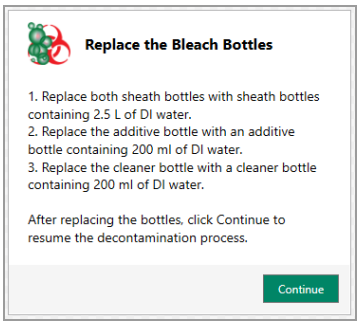
   If you have not completed the preparation steps, click Cancel to stop the Decontamination wizard and perform the steps in Preparing for the Decontamination Process on page 376 before continuing.

4. To view the decontamination progress details, click the down arrow on the Decontamination button.

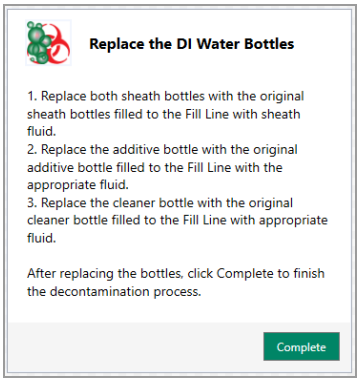
   Important: If at any point during the decontamination Everest Software detects an instrument error, it stops the decontamination process immediately and displays an advisory message. Follow the instructions in the message and contact Bio-Rad Technical Support for assistance.



5. Approximately 50 min into the procedure, the software prompts you to replace both sheath bottles with bottles containing 2.5 L of DI water, and replace the additive and cleaner bottles with bottles containing 200 ml of DI water.



6. After replacing the bottles, click Continue.
7. Approximately 190 min into the procedure, the system prompts you to replace both sheath bottles with bottles filled to the Fill Line with sheath fluid, and replace the additive and cleaner bottles with the default (original) bottles filled to the Fill Line with the appropriate solutions.



8. After replacing the bottles, click Complete.

   Everest Software initiates a fluidics startup and then a fluidics shutdown to complete the process. This can take an additional 12 min.

   After decontamination completes, the user login window appears.

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 154 for information on accessing the filters.

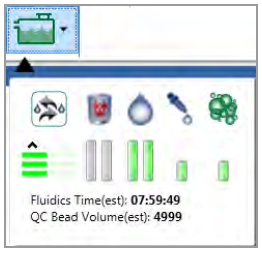
Important: These coated pieces of glass are delicate; handle them with care. Any scrape or scratch on the surface could significantly affect the light passing though. When handling filters, always wear gloves to avoid depositing oils and particles on the filter surface.

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

Bio-Rad suggests that you replace the QC beads when the QC calibration bead volume is low (less than 150 µl), as indicated in the fluidics status. If the fluid level in the calibration bead bottle drops too low, the system displays air bubble warning messages.



Replacing the QC beads requires the system to re-baseline. Re-baselining establishes a new median range for CV and PMT voltages in each channel for the new bead lot. Dyed beads have inherent variation, so calibration must be performed for each lot. The system automatically recalibrates the instrument after the bead swap is initiated.

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. Do one of the following:

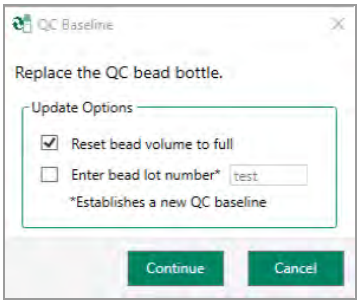
   - On the Home window, click the down arrow below the in strument, and then select Swap Beads.

   - In the System section of the toolbar, click Instrument Tools to display the dropdown list, and then click Swap Beads.

   The QC Baseline dialog box appears.

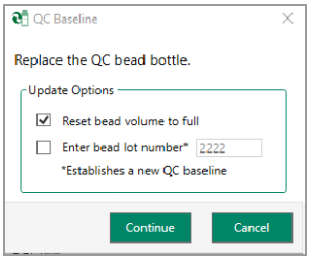
2. In the ZE5 Cell Analyzer, replace the QC bead bottle.

...

3. In the dialog box, select the appropriate update options:

   - If you replace the QC bead bottle with a bottle that is not full, clear the Reset bead volume to full checkbox. If the bottle you insert is full, ensure the checkbox is selected (the default setting).
   - If you replace the QC bead bottle with a bottle from another bead lot, select Change bead lot number and enter the number in the text box.

   Note: In order for the next QC calibration to become the new QC baseline, you must select the Change bead lot number checkbox and provide the new bead lot number, for example:

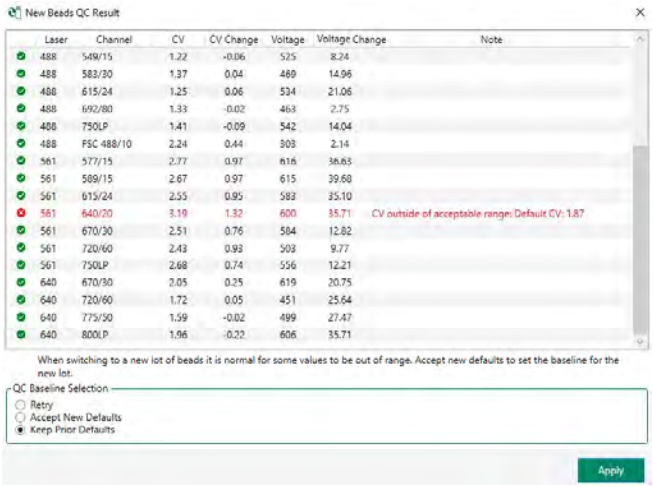


4. Click Continue.

   The recalibration process verifies the new median range for CV and PMT voltages in each channel for the new bead lot against the previous QC baseline set by the administrator.

   One of the following occurs:

   - If the instrument recalibration with new bead lot passes, no report appears. The system is ready to use.
   - If the instrument recalibration with the new bead lot fails, a New Bead QC report appears, and a red x appears next to those channels whose values do not pass the QC criteria and the software displays the reason for the failure in the Note column..



5. In this case, in the QC Baseline Selection section, click one of the following:

   - Retry and click Apply to retry the calibration.
   - Accept New Defaults and click Apply.

     The new CV and PMT voltage values for all parameters become the new baseline.
   - Keep Prior Defaults and click Apply.

     The previous CV and PMT voltage baseline values are retained for all parameters.

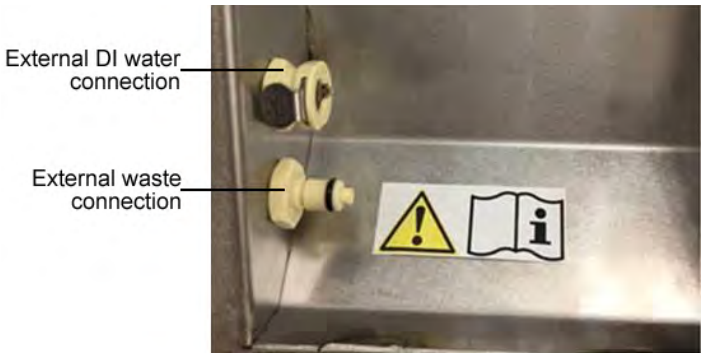
Maintaining the System When Not in Use

Flow cytometry instruments have fewer performance issues if they are run and maintained regularly with no long periods between usage. Everest Software includes a vacation mode, which you can use to schedule regular and 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 132.

Chapter 14 Using External DI Water and Waste

Optionally, you can have your ZE5 Cell Analyzer upgraded to use external fluidics, which can increase the instrument's uninterrupted run time for up to 22 hours.

Note: To use external fluidics, version 2.3 (or higher) of Everest Software must be installed on any computers that connect to the ZE5 Cell Analyzer, and you must purchase the appropriate upgrade kits from Bio-Rad. For information, see Appendix G, Ordering Information (External Fluidics section).



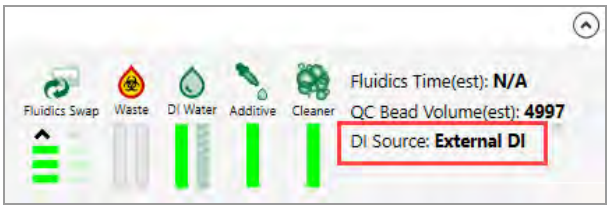
Special ports are installed on the instrument, and connect to the fluidics sources described below:

- External deionized (DI) water (pressurized house DI **or** fluidics cart DI water tank)
- External waste (fluidics cart waste tank)

Important: Only trained Bio-Rad service engineers are qualified and authorized to reconfigure the ZE5 Cell Analyzer and connect it to external water and waste sources. To request an external fluidics installation, contact Bio-Rad Technical Support.

To set up your system, the Bio-Rad service engineer

- Installs the water and waste ports on the ZE5 Cell Analyzer
- Connects them to the external sources
- Configures each fluidics cart (if you are using one or both tanks)
- Changes the DI Source entry in Everest Software to External DI



Note: If you are using external fluidics and prompt a decontamination of the system, the Everest Software Decontamination wizard uses the ZE5 Cell Analyzer's internal fluidics for the process, and shows ZE5 Bulk fluidics as the DI Source rather than External DI. The wizard automatically returns the system to external fluidics as soon as the decontamination is finished. For more information on decontaminating the instrument, see System Decontamination on page 373.

This appendix contains information on

- Required changes to the internal fluidics system
- House DI water
- Fluidics cart DI water and waste tanks
- Operating the fluidics carts
- Servicing fluidics cart DI water and waste tanks

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Changes to Internal Fluidics

External fluidics are routed through the ZE5 Cell Analyzer internal bulk fluidics chamber using the upper DI water container and both waste containers, as well as the sheath additive and system cleaner containers. The instrument maintains an internal DI water level equating to approximately two hours of runtime, and switches to internal fluidics if the external DI water source is identified as empty or the external waste container is full.

All containers in the internal fluidics chamber must be connected to the corresponding quick-connect ports, as shown in the following graphic.

Before each run using external fluidics, ensure the following:

- All internal fluidics containers are installed and connected to the quick-connect ports.

  Note: The lower DI water container must remain connected, but it is not used with external fluidics.

- Both waste containers are empty.
- The upper DI water container is filled with 2.5 to 3L of DI water and the lower container is empty.

  Important: More than 4 L in the upper water container can trigger a system error and put the instrument into Safe Mode. Less than 2 L can trigger an Unable to Fill system warning.

  Note: Use the circular depression on the container as a fill guide, and fill to the top edge of the circle.

- The sheath additive and system cleaner bottles are filled.

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House DI Water

Using the ZE5 External House DI Water Upgrade Kit, a Bio-Rad service engineer can conect your ZE5 Cell Analyzer to your pressurized house DI water source.

ZE5 Cell Analyzer

House DI water system

To collect waste while running house DI water, you can either use the instrument's internal waste system, or the instrument can be connected to the larger fluidics cart waste tank. For information, see Fluidics Cart and Carboy Tanks on page 393.

Tip: For kit catalog numbers corresponding to the House DI and Waste Carboy Upgrade Kits, see Appendix G, Ordering Information.

Important: After you contact Bio-Rad with your order, you will receive a checklist of prerequisites that must be met before the installation. Only Bio-Rad service personnel are qualified and authorized to install the upgrade kits.

Requirements for the water itself are:

- Tap water to DI water conversion process must be in place and functioning.
- House DI water should be Type II pure deionized water, with better than 10 mega-ohm (MΩ) resistivity.

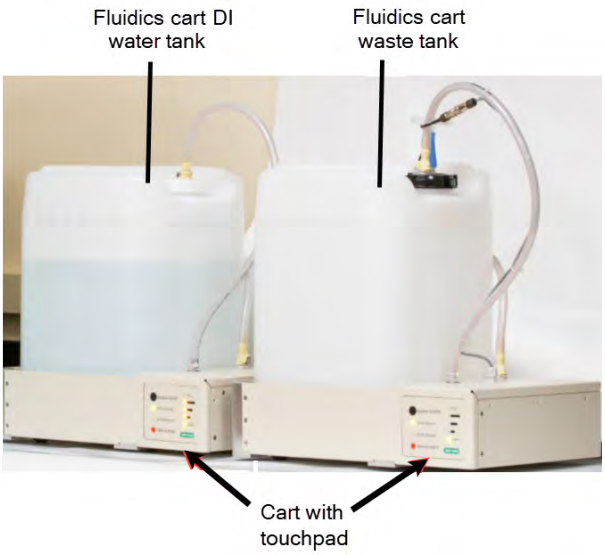
  Note: Bio-Rad recommends purchasing the Evoqua Adsorber II and Research II cartridges, as well as the Evoqua wall bracket and fittings. Each item is listed in Appendix G, Ordering Information, and also in the pre-installation checklist.
- Incoming water pressure from the source water system must be between 20 and 50 psi (between 138 and 345 kPa).
- House DI water outlet must be 1/4" OD (outer diameter) metal (non-copper) or plastic tubing.

Ensure all requirements on the checklist are met before the installation.

Chapter 15 Fluidics Cart and Carboy Tanks

Using the ZE5 External Carboy Upgrade Kits, a Bio-Rad service engineer can connect your ZE5 Cell Analyzer to fluidics cart DI water and waste tanks. Your instrument can also be connected to a fluidics cart waste tank if you are using house DI water.

Each kit includes one 20L (approximately 5 gallons) Carboy tank and one dedicated fluidics cart to which the tank is connected. For kit catalog numbers, see Appendix G, Ordering Information.



On the front of each fluidics cart is a touchpad that controls the following:

- Start and stop capability
- Tracking the DI water level from full to empty
- Tracking the waste level from empty to full
- Alarm capability to notify when liquid levels approach full or empty.

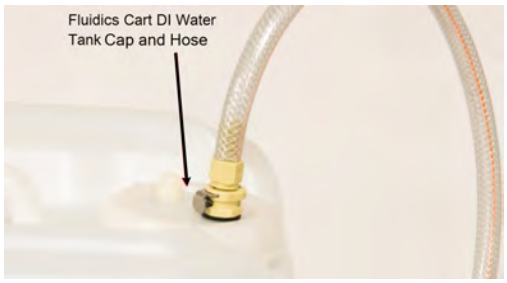
For information on using the touchpad, see Operating the Fluidics Carts on page 395.

Note the following:

-  **Caution: Biohazard!** If your samples 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, including waste generated from the sample run.
- If a spill occurs in the waste fluidics cart, do not use the cart as a secondary form of containment, as it is not designed to contain waste liquid.
- When the DI water tank is identified by the fluidics cart as empty or the waste tank as full, the system switches to internal fluidics, which can run for approximately two hours.
- After switching to internal fluidics, Everest Software displays incremental fluidics level warnings based on the liquid levels in the internal fluidics chamber.
- You can add DI water and empty waste while the system continues to run. For information, see Servicing Carboy DI Water and Waste Tanks on page 397.

After your ZE5 Cell Analyzer is connected to each fluidics cart, you can install each tank in its cart. For information, see Setting Up the Fluidics Cart and Carboy DI Water Tank on page 394 and Setting Up the Fluidics Cart and Carboy Waste Tank on page 395.

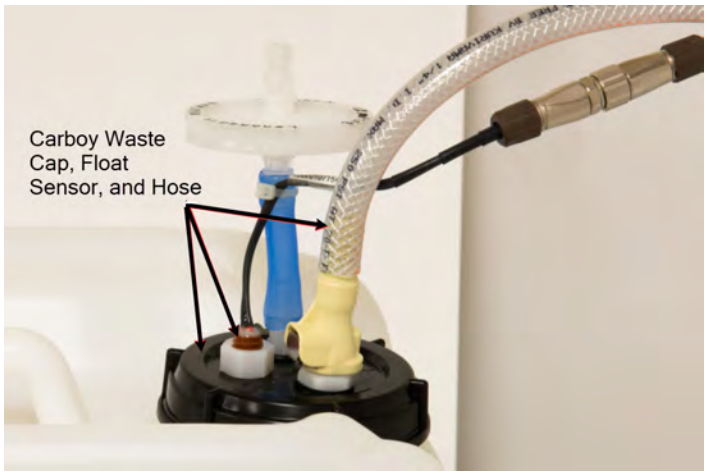
Setting Up the Fluidics Cart and Carboy DI Water Tank



To set up the fluidics cart and Carboy DI water tank

1. Fill the Carboy DI water tank and close it with the supplied white cap.
2. Place the Carboy tank carefully onto the DI water fluidics cart.
3. Connect the hose to the quick-connect port on the cap.
4. Connect the supplied power cord to the fluidics cart and then to the electrical outlet.

Setting Up the Fluidics Cart and Carboy Waste Tank



To set up the fluidics cart and Carboy waste tank

1. Ensure the Carboy waste tank is empty, and close it with the supplied black cap.
2. Place the Carboy waste tank carefully on the waste fluidics cart.
3. Connect the hose to the quick-connect port on the cap.
4. Connect the float sensor.
5. Connect the supplied power cord to the fluidics cart and then to the electrical outlet.

Operating the Fluidics Carts

The fluidics cart touchpad is both a prompt mechanism and an indicator.

The following subsections explain each function.

System On or Off

To power the system on or off

▶ Press the System On/Off button to display one of the following indicators:

- The System Running light is green when the cart is powered on.
- The System Stopped light is red when the cart is powered off.

Fluidics Status

During the installation, one fluidics cart is calibrated for DI water and the other is calibrated for DI waste. The lights on the right side of the touchpad indicate incremental DI water tank levels:

	The tank is full. ■ For DI water, the light is green. ■ For waste, the light is flashing red and the alarm is triggered.
	The tank is three-quarters full. ■ The light is yellow for either DI water or waste.
	The tank is half full. ■ The light is yellow for either DI water or waste
	The tank is one-quarter full. ■ The light is yellow for either DI water or waste.
	The tank is empty. ■ For DI water the light is flashing red and the alarm is triggered. ■ For waste, the light is green.

Note: When the fluidics cart Carboy tanks require attention (added water, emptied waste), the ZE5 Cell Analyzer switches to the internal fluidics containers to continue operating. Use the information in Changes to Internal Fluidics on page 389 to ensure that DI water, waste, sheath additive, and system cleaner containers are filled to the designated levels and connected appropriately.

Fluidics Alarm

An audible system alarm is triggered if the

- DI water tank is removed from the fluidics cart while the cart is powered on
- DI water tank in the fluidics cart is empty
- Waste tank in the fluidics cart is full

If the alarm sounds

▶ Press Silence Alarm once to stop the alarm, and immediately address the problem.

To adjust the alarm volume

The default alarm volume is High.

▶ To reset the volume level, press the Silence Alarm button for a duration indicated below:

- Medium — press the button for just over two seconds.
- Low — press the button again for just over two seconds.

Servicing Carboy DI Water and Waste Tanks

Carboy t tanks are hot-swappable, which means that when the DI water tank approaches empty, or the waste container approaches full, you can change the containers without shutting down the ZE5 Cell Analyzer.

When the DI water Carboy tank is empty, Everest Software displays an error message, advising that approximately 2 hours of run time remains, because the system still has two liters of DI water in the internal DI water container. If the fluidics issue is not addressed during the 2-hour window, Everest Software (v2.3 forward) continues to warn the user that the DI water level is low at 60, 30, and 10 minutes of run time remaining.

If you do not fill up the DI water tank at the 10-minute warning, the ZE5 Cell Analyzer goes into Safe Mode shortly afterward and Everest Software displays a “sheath level low” error message.

To service the Carboy DI water tank

1. Press the System On/Off button on the fluidics cart touchpad.

   The System Stopped light turns red.

2. Disconnect the hose from the Quick connect port on the cap.

3. Remove the Carboy tank from the cart.
4. Fill the Carboy tank with DI water.
5. Carefully place the filled tank in the fluidics cart.
6. Reconnect the hose to the cap.
7. On the fluidics cart touchpad, press the System On/Off button.

   The System Running light turns green.

   Note: When refilling the Carboy DI water tank, the ZE5 Cell Analyzer continues to run using internal bulk DI water.

To service the DI waste tank

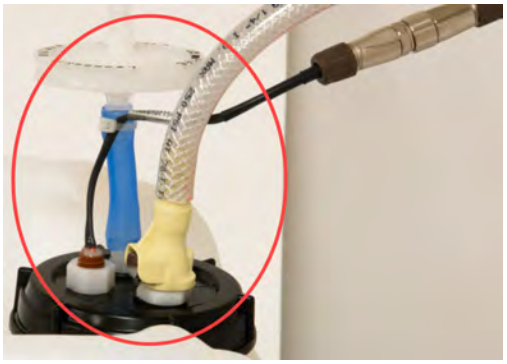
Caution: Biohazard! If your samples contain biohazardous materials, consult with your local safety officer or review local, state, and federal regulations to ensure proper handling and disposal of biohazardous substances, including waste generated from the sample run.

If a spill occurs in the fluidics cart, do not use the cart as a secondary form of containment, as it is not designed to contain waste liquid.

1. Press the System On/Off button on the fluidics cart touchpad.

   The System Stopped light turns red.

2. Disconnect the float sensor, and then disconnect the hose from the Quick connect port on the cap.



3. Remove the Carboy waste tank from the cart.
4. Empty the waste tank per safety regulations.
5. Add 2 L of household bleach (5% Sodium Hypochlorite) to the empty Carboy tank.
6. Carefully place the Carboy tank in the cart.
7. Reconnect the hose and float sensor to the cap.
8. On the fluidics cart touchpad, press the System On/Off button.

   The System Running light turns green.

   Note: When emptying the waste tank, the ZE5 cell analyzer continues to run using internal bulk waste containers.

Appendix A Viewing Everest Software FCS Files in FlowJo Software

FlowJo Software does not automatically recognize FCS files imported from Everest Software. To view Everest Software FCS files in FlowJo, you must configure the cytometer settings in FlowJo Software to recognize the ZE5 instrument.

To configure FlowJo to recognize Everest Software FCS files

1. Open FlowJo Software and import an Everest FCS file.
2. On the FlowJo toolbar, click Instrumentation. You should see the ZE5 Cell Analyzer instrument listed as ZE5.
3. On the FlowJo toolbar, click Preferences.
4. In the Preferences window, select Cytometers.
5. In the left column, select GENERIC (ZE5).
6. (Optional) In the Cytometer Identification section, change GENERIC to BIORAD_ZE5.
7. In the Parameter Scale Settings section, clear the Custom log scaling and Custom linear scaling checkboxes if they are selected.
8. Enter the following parameters:
   - For Custom log scaling, set Min. value to 1, Max. value to 214748, and Divider to 10000.
   - For Custom linear scaling, set Min. value to 0, Max. value to 214748, and Divider to 10000.
9. In the Transformation Settings section, select Enable Transforms and keep the default value (-10).
10. Click OK to save the changes.

Appendix B 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 Software 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 Software Main 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 Main 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.

Deleting Acquired Data Files

Everest Software saves the acquired data files for each experiment session. The data files remain on the Everest Software computer after exporting them. Users might find it necessary to remove the data files from the Everest Software computer but want to retain the experiment's setup parameters in order to run the experiment again. Deleting the experiment in the D:\EverestUsers\<user name> folder deletes the complete experiment, including the setup parameters, run list, and telemetry files.

This procedure explains how to delete only the acquired data files for an experiment while retaining its setup parameters. Users can then rerun the experiment and collect data in future sessions.

Important: Everest Software does not back up data or run list files. Bio-Rad strongly suggests that you use the Export Run List and FCS Files option to export the experiment's run list and all associated FCS files before performing this procedure. Consider using the Export Full Experiment to Zip option to ensure that you have safely exported and saved all run list, telemetry, and FCS files that you want to retain. See Exporting Third Party and RLST Data on page 334 for more information.

To delete acquired data files

1. In the Recent Experiment Sessions panel in the Home window, expand the experiment that contains the data you want to delete, for example 5Color_2.
2. In the expanded experiment, locate and note the name of the specific session. By default, the session's name includes the user's name, the year, month, and date of the last edit, and the session number, for example vbala-20180125-1421.
3. Log out of Everest Software.
4. Open Windows Explorer and navigate to the user's file folder. This is the folder into which all experiment data are saved, and was specified during user setup by the administrator. By default, this folder is located in the following directory:

   D:\EverestUsers\<user_name>\
5. In the user's folder, locate and open the target experiment's folder, for example <user_name>\5Color_2.

   The experiment's folder contains folders of all experiment sessions. You might have many subfolders within the experiment's folder.

   Each session folder is labeled the same as the session name (in this example, vbala-20180125-1421).

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6. Locate and open the session folder that contains the data that you want to delete.

   This folder contains

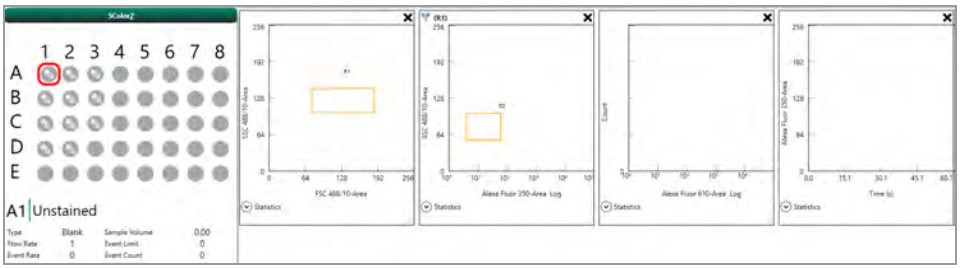
   - At least one folder labeled with a global unique identifier (GUID)
   - The experiment's run list (.rlst) file (5Color_2.rlst in this example)
   - A run list status (.rlstatus) file for each GUID folder
   - The instrument.definition file

   The GUID folder contains the FCS data that you recently acquired for the session.

7. If you have more than one GUID folder, open each folder and locate those that contain a folder labeled fcs.
8. Delete the GUID folder or folders that contain an fcs folder.
9. Log in to Everest Software.

   Your experiment appears in the Recent Experiments panel. You can edit and run the experiment, and acquire data again.

   Note: Because you deleted the acquired data, Everest Software does not display data for the experiment in Analysis mode, for example:



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 170.
	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 Replacing Sheath Bottles on page 173.

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.

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 369. 2. Run the cleaning process. See Cleaning the Sample Line and Probe on page 371.
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 383. 2. Recalibrate the probe, using custom media settings if needed. See Media Selector on page 80.
Data suddenly disappears 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. See Configuring Instrument Settings on page 306.

Error	Possible causes	Troubleshooting steps
Data suddenly disappears from the plots (continued)	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 371. 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 369.

Error	Possible causes	Troubleshooting steps
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 158.
	Improper dichroic filter placement	Run the ZE5-EYE process and inspect filters. See Using the ZE5-EYE to Confirm Filter Choices on page 158.
	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 141.
	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 371. 2. Run the Decontamination wizard. See System Decontamination on page 373.
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 369.
	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 371. 2. Run the Decontamination wizard. See System Decontamination on page 373.

Error	Possible causes	Troubleshooting steps
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 to resuspend the sample. Note that settled samples might require a longer agitation than usual. See Activating Agitation on page 226, Setup Mode Controls on page 300, and Acquisition Mode Controls on page 311.
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 369.
	Miscalibration of media (sample probe not going into sample fully)	Recalibrate the probe, using custom media settings if needed. See Media Selector on page 80.
	Sample has settled	Modify the run list to include an agitation step or use the manual agitation option to resuspend the sample. Note that settled samples might require a longer agitation than usual. See Activating Agitation on page 226, Setup Mode Controls on page 300, and Acquisition Mode Controls on page 311.
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. See Configuring Instrument Settings on page 306.
High voltage present in certain channels during QC process	Incorrect beads used	Ensure single peak 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.

Error	Possible causes	Troubleshooting steps
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 369. 2. Run the cleaning process. See Cleaning the Sample Line and Probe on page 371. 3. Run the Decontamination wizard. See System Decontamination on page 373. 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 383.
	Improper laser delay	Run the QC process again. See Running Quality Control on page 183.
	Beads have gone bad or expired	Install a new bottle of QC beads. See Replacing the QC Beads on page 383.
	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 158.

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 306.
	Poor compensation	Run the compensation process. See Adjusting Compensation Automatically on page 317.
	Bacterial contamination causing autofluorescence	Run the Decontamination wizard. See System Decontamination on page 373.
	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 80.

Error	Possible causes	Troubleshooting steps
	Laser or lasers off	Turn on the relevant lasers in software. See Configuring Instrument Settings on page 306. Modify the experiment in the Experiment Builder so that required fluorophores are enabled to ensure that lasers are on when the run list is initiated. See Selecting Fluorophores on page 208.
	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 306.
	Threshold not set correctly	Change threshold value. See Configuring Instrument Settings on page 306.
	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 306.

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 369. 2. Run the cleaning process. See Cleaning the Sample Line and Probe on page 371.
	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 158.
	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 do not match the enabled parameters	Create new plots that match the enabled parameters (see Plots Created by the Compensation Template on page 241), or enable parameters that match the plots (see Selecting Fluorophores on page 208).
	Loader door open	Stop the acquisition process, close the loader door, and proceed with running the experiment.

Error	Possible causes	Troubleshooting steps
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 383.
	Bead bottle has been diluted	Replace bead bottle. See Replacing the QC Beads on page 383.
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 371. 2. Run the Decontamination wizard. See System Decontamination on page 373.
	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 371. 2. Run the Decontamination wizard. See System Decontamination on page 373.
	Dead cells in sample	Adjust gates (see Applying Filters (Gates) on page 271) or repeat experiment with fresh cells.

Error	Possible causes	Troubleshooting steps
Two or more populations are present when expecting one	Gating inaccurate	Adjust gates. See Applying Filters (Gates) on page 271.
	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 371.
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.
	Poor compensation	Run the compensation process. See Adjusting Compensation Automatically on page 317.
	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 306.
	Lasers misaligned	Contact Bio-Rad Technical Support.

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 306.
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 80.
	Incorrect plate inserted	Place the sample into a plate that is compatible with the ZE5 Cell Analyzer.

Appendix C ZE5 Cell Analyzer Specifications

The specifications for the ZE5 Cell Analyzer are shown in Table 38.

Table 38. 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 38. 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	0.1–3.5 µl/sec (6–210 µl/min) (standard mode) 0.5–2.5 µl/sec (30–150 µl/min) (high-throughput mode)

Table 38. 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 and operation	Site	For information, see Environmental and Safe Use Requirements on page 18
	Operating altitude	
	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
	Power	For information, see Environmental and Safe Use Requirements on page 18
	Overvoltage degree	
	Pollution degree	
	Air and vacuum supply	

Table 38. 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 D Bio-Rad Free and Open-Source Notices for Cell Analyzer Products

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Download the product at https://github.com/blackchair/netdata-json/blob/master/LICENSE.md

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Appendix E 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/

Appendix F 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 432 and threshold on page 431.
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 432.

FCS	Flow cytometry standard file type.
FSC	Forward scatter in plots.
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.

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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 DI water containing a sheath additive.
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.
SSC	Sid scatter in plots.
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 430.

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.

Appendix G Ordering Information

See theinformation below to order accessories and replacement parts for the ZE5 Cell Analyzer.

Table 39. Ordering information

Catalog #	Description
Instrumentation	
3 Laser	
12004276	488x6 / 561x7 / 640x4, 17 Color, 488 FSC SSC
12004277	488x6 / 561x 7 / 640 x4, 17color 488 SSC, FSC
12014141	With SPD, 405x7 /488x6 / 640x4, 17 Color, 488 FSC SSC, 405 SPD
12014139	405x7 /488x7 / 561x7, 20 Colors, 488 FSC SSC
12014140	With SPD, 405x7 / 488x7 / 561x7, 20 Color, 488 FSC SSC, 405 SPD
4 Laser	
12004278	405x7 / 488x6 / 561x7 / 640x4, 24 Color, 488 FSC SSC
12014138	With SPD, 405x7 / 488x6 / 561x7 / 640x4, 24 Color, 488 FSC SSC, 405 SPD
5 Laser	
12004279	355x5 / 405x7 / 488x4 / 561x7 / 640x4, 27 Color, 488 SSC FSC, 405 SPD
12014135	With 7 off UV Option A, 355x7 / 405x7 / 488x4 / 561x5 / 640x4, 27 Color, 488 SSC FSC, 405 SPD
12014136	With 7 off UV Option B, 355x7 / 405x7 / 488x4 / 561x5 / 640x4, 27 Color, 488 SSC FSC, 405 SPD
Computer System	

Catalog #	Description
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
FCS Software	
12013092	FCS Express for academic customers
12013095	FCS Express for non-academic customers
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
12004444	ZE5 Cleaner/Additive Bottle, 450 ml, pkg of 2
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

Catalog #	Description
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)
External Fluidics	
12009651	ZE5 External House DI Water Upgrade Kit
12009707	ZE5 External DI Water Carboy and Fluidics Cart Upgrade Kit, includes DI water fluidics cart with power supply, DI water Carboy (20L), DI water Carboy cap (vented with integrated replaceable filter and leak-free Quick Connect/Disconnect)
12009734	ZE5 External Waste Carboy and Fluidics Cart Upgrade Kit, includes external waste fluidics cart with power supply, waste Carboy (20L), waste Carboy cap (with overflow sensor, leak-free Quick Connect/Disconnect, and filtered vent)
12009675	House DI replacement wrench spanner for 10” filter housing
12009682	House DI replacement filter, 4”x10”, pleated cellulose, 1μm
12010480	ZE5 Waste Carboy with biohazard labels, includes ZE5 waste Carboy with standard Carboy cap
12010521	ZE5 DI water Carboy with labels, includes ZE5 DI water Carboy with standard Carboy cap
12010522	ZE5 External Waste Carboy cap with Quick Connect/Disconnect, with overflow sensor, leak-free Quick Connect/Disconnect, and filtered vent
12010523	ZE5 External DI water Carboy cap with Quick Connect/Disconnect, vented with integrated replaceable filer and leak-free Quick Connect/Disconnect
12010524	Tubing for ZE5 External waste fluidics cart
12010525	Tubing for ZE5 External DI water fluidics cart

Catalog #	Description
W3T184483	Evoqua cartridge adsorber II for house DI
W3T185125	Evoqua cartridge research II for house DI
W3T184340	Evoqua duplex IWT wall bracket with fitting for house DI

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