Flow Cytometry

ZE5 Cell Analyzer and Everest Software Quick Guide

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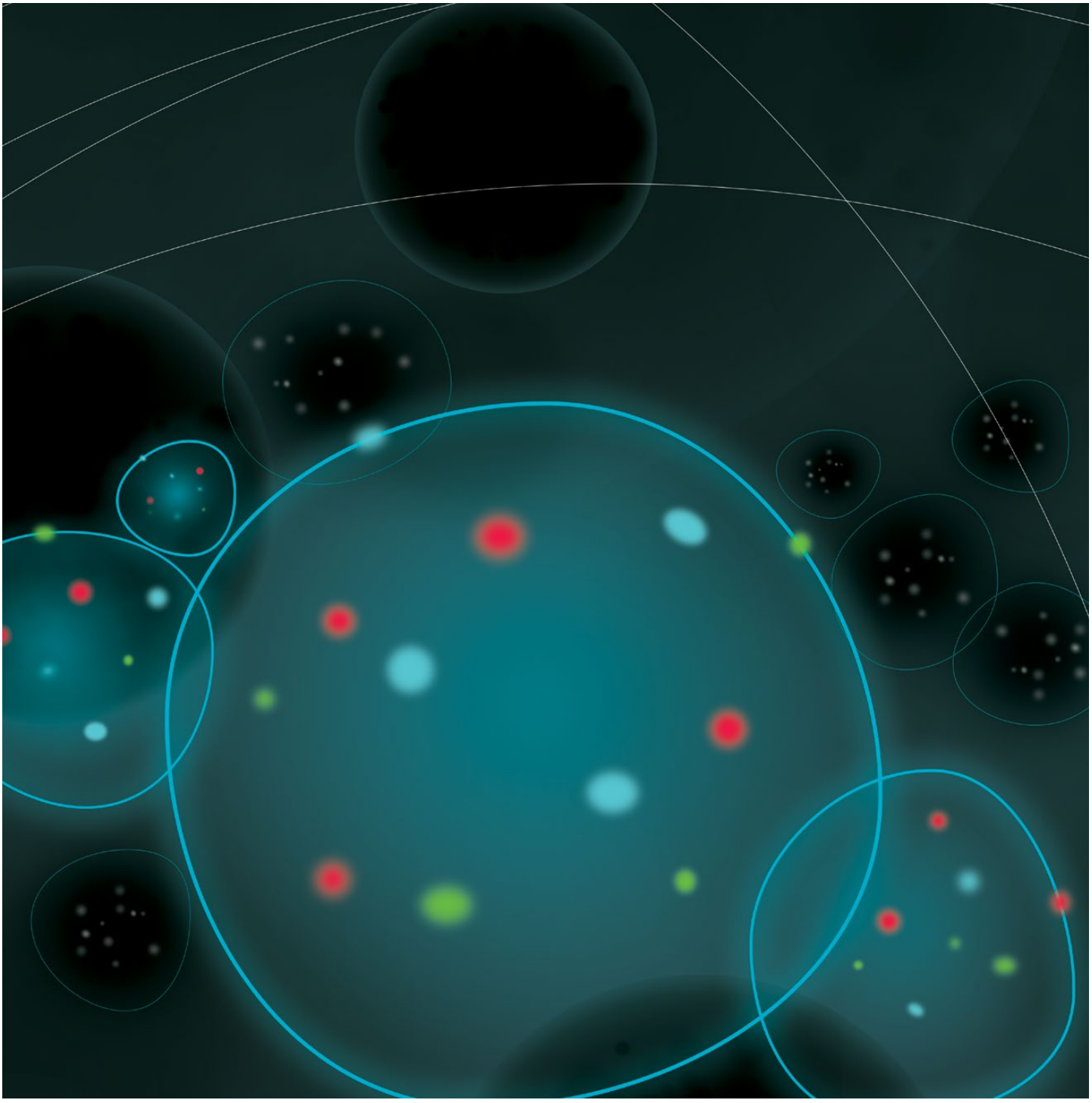

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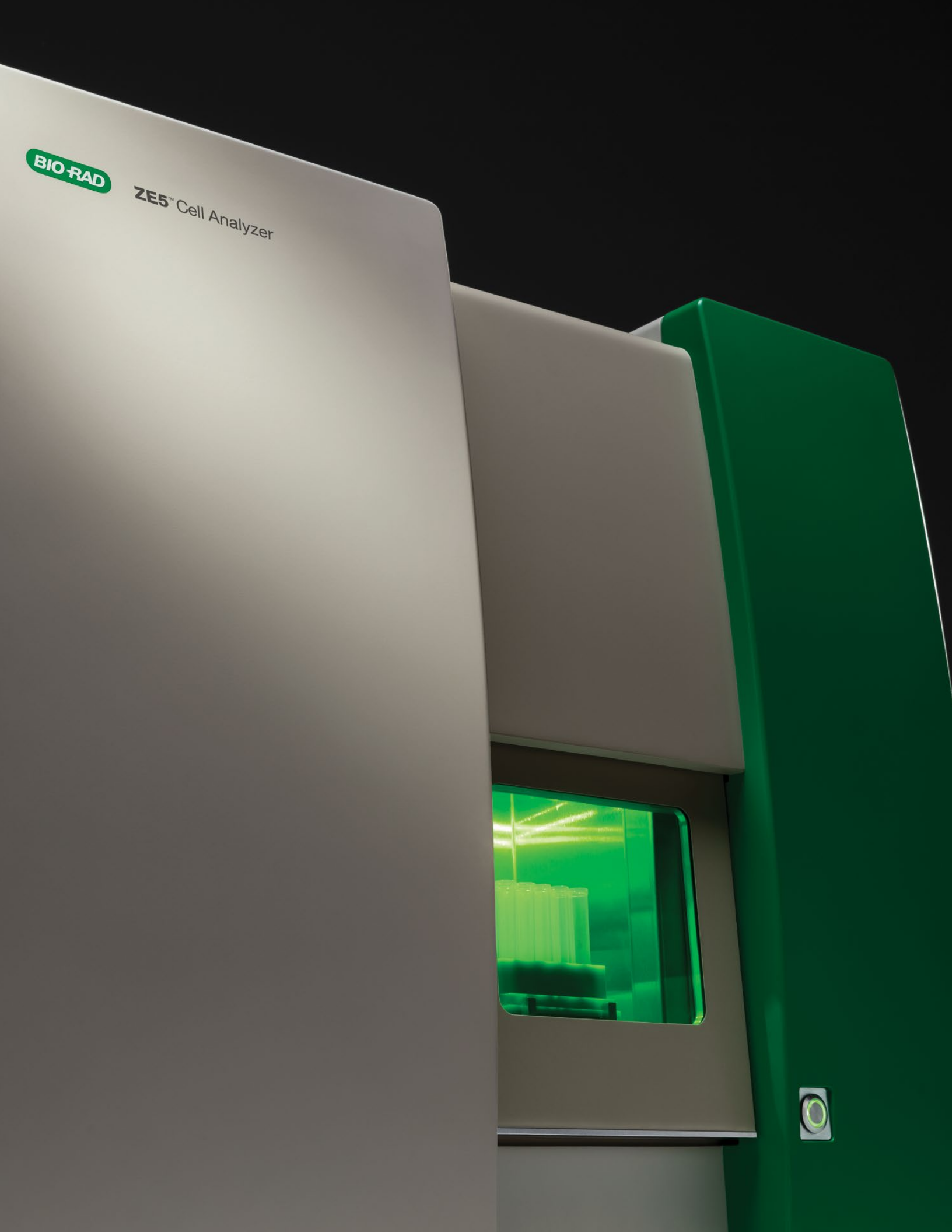
ZE5 Cell Analyzer and Everest Software Quick Guide


BIO-RAD
ZE5™ Cell Analyzer

Startup

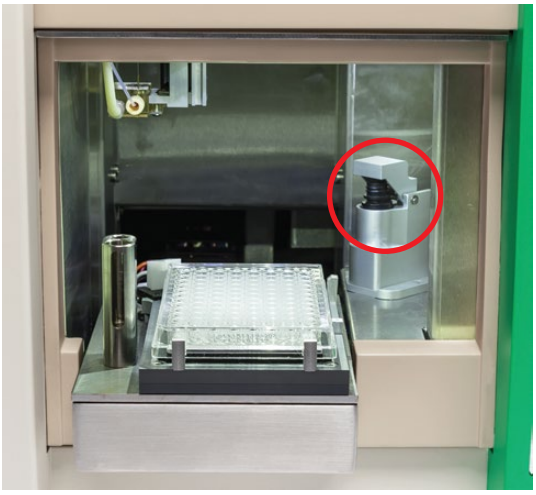
Before You Begin

1. Ensure that the instrument is plugged in, powered on, and connected to the computer.

   Tip: Once powered on, the ZE5 Cell Analyzer is designed to remain powered on. Do not power it down.

2. Ensure that an uncapped bottle of ZE-Series QC Beads is in place in the right side of the sample chamber. These beads must always be in place.

   Tip: One bottle will last for several months, depending on usage.

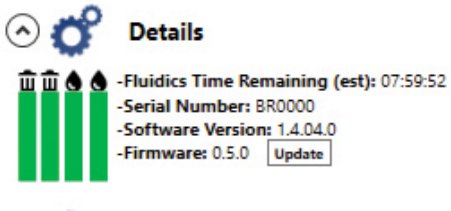


Start Up the Software

On the computer desktop, double click the Everest Software icon. The Everest login window displays instrument status and login text boxes.

Check Instrument Status

System status appears in the left pane. Status: OFF indicates that the instrument is plugged in, powered on, and shut down. Click the Details down arrow to view instrument status specifics. If necessary, fill the bulk fluidics containers and empty the waste containers.



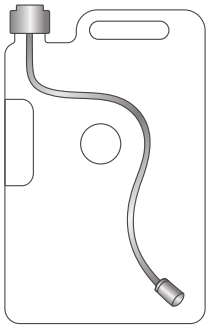
Check Fluidics

There are six bulk fluidics containers. If any of them need to be filled or emptied, use the quick-connect tabs to detach the containers from the instrument and access them.

1. Open bulk fluidics door.
2. Push the quick-connect tabs on the connections to disengage the tubes (one tube each for sheath fluid, additive, and cleaner containers, two for waste bottles) from the instrument.
3. Remove and uncap a container.
4. Refill, empty, or change container.
5. Recap container. Position the cap so the vent is at the 12 o'clock position to allow venting of the tank.
6. Place container back in the instrument and reattach tubing to the connectors.

Check Fluidics continued

Tip: When refilling or changing a sheath bottle, ensure the tubing and filter are reinstalled in the lower corner of the bottle, opposite the cap.



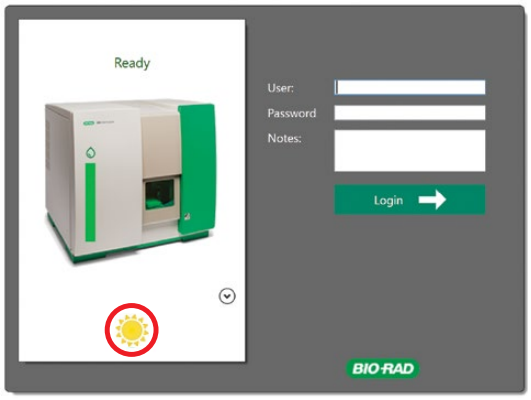
Tip: Double check all connectors to make sure none were accidently disconnected during refilling of containers.

Tip: Fluidics containers that are not in use can be exchanged during operation. Containers in use by the system are illuminated by a green light.

Note: You can refill fluids before logging in, but you must log in before you can manually run the quality control (QC) process or run samples. You cannot log in during the startup or QC process.

Start Up System and Log In

You may start up the instrument on the login page or on the Everest Software home page. When instrument startup is complete (~6 minutes), Instrument Status changes to Ready and the Shutdown icon replaces the Startup icon .



To start up on the login page:

1. Click the Startup icon.
2. Once the startup is completed, enter your user name and password and log in to the Everest Software.

To start up on the Everest home page:

1. Enter your user name and password and log in.
2. Click the Startup icon.

Run Quality Control

Run the automated QC process every day to ensure optimal system performance.

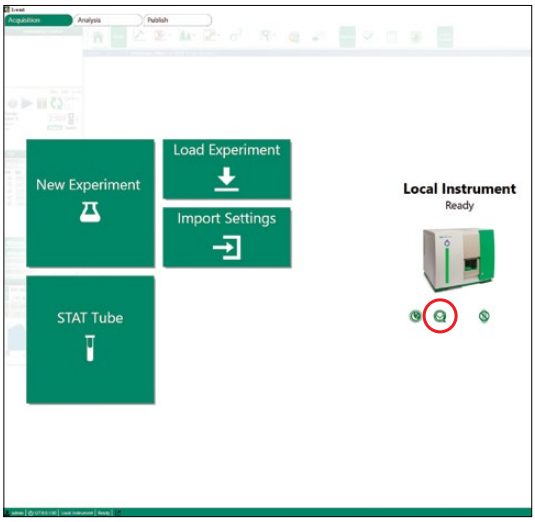
The QC process:

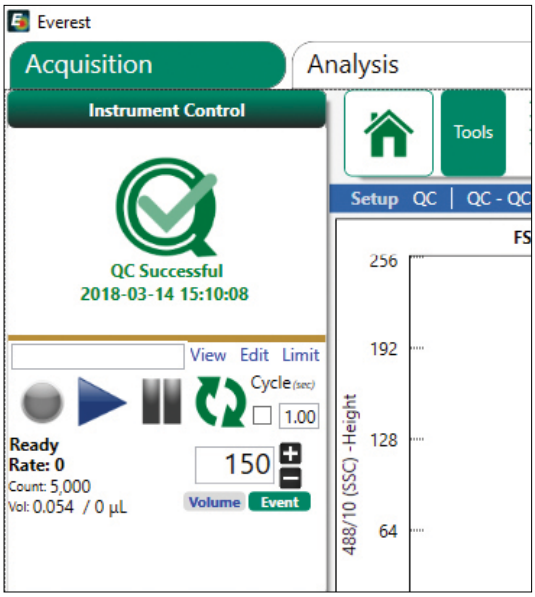
- Verifies the filter set
- Tests the event rate
- Adjusts laser delays
- Checks photomultiplier tube (PMT) voltages and bead CV for each channel
- Compares settings with QC criteria
- Provides Pass/Fail result
- Stores the results

To run the QC process:

1. In the Home window, click the QC icon . The Instrument Control panel opens and the automated QC process runs.
2. When a message in the Instrument Control panel notifies you that the process is finished, click the Home icon .

Tip: QC can also be run from the Acquisition tab by clicking the QC icon .



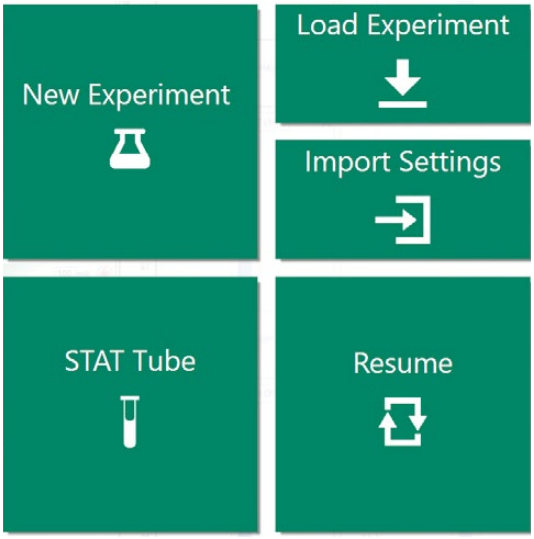


Choose What's Next

You can choose any of the following in the Home window:

- **New Experiment** — displays steps for setting up a new experiment in the experiment builder
- **STAT Tube** — runs a sample quickly from the single tube position in the loader with all the lasers and parameters enabled by default
- **Load Experiment** — loads a saved experiment, including fluorophores, parameter names, PMT voltages and other instrument settings, and compensation values, as well as samples and plots
- **Import Settings** — loads only these settings from a previously generated experiment: fluorophores, PMT voltages, triggers, and compensation values (if available)

Tip: You can apply imported settings to different sample sets. Refer to Chapter 7, Import settings, in the ZE5 Cell Analyzer and Everest Software User Guide.



Set Up a New Experiment

In the Home window, click **New Experiment**.

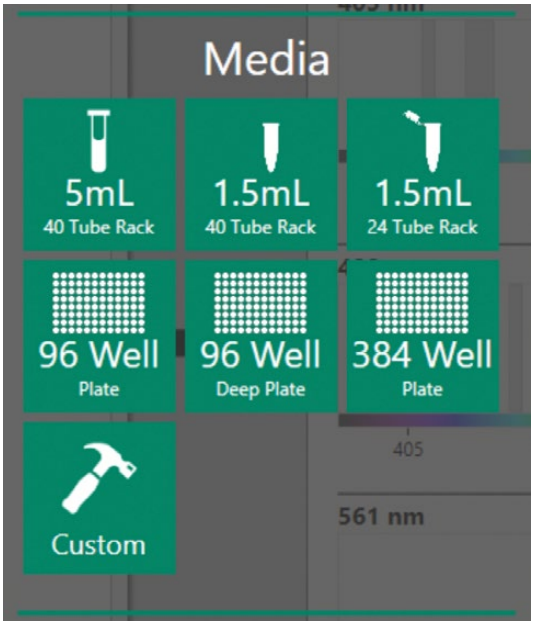
Select media type

Select the media in which samples are placed and determine the controls and sample locations, along with their running parameters.

- The 5mL and 1.5mL tube selections are designed to correlate with their associated racks
- The 96 Well (Plate or Deep Plate) and 384 Well (Plate) selections are designed to correlate with flat bottom plates

Tip: Use the Custom option to customize for other plates. See the ZE5 Cell Analyzer and Everest Software User Guide for details.

Tip: You can change the media selection by using the Switch Media button.



Set Up a New Experiment continued

1. Name the Experiment 1.
2. Name the Panel 2.

Select fluorophores

Use the Fluorophores screen to select the fluorophores and channels required for the experiment. When you select a fluorophore, its emission spectrum is shown in the Available Detection panel in the graph associated with the laser that optimally excites it.

To select fluorophores:

1. Double click a name in the Fluorophores column to add it to the Selected Fluorophores column.

> **Tip:** To find a fluorophore quickly, type the name into the search bar 3.

2. Select **Enable All** to activate all the channels.

> **Tip:** Select **Clear All** 4 to deselect the channels and restart the fluorophore list.

> **Tip:** Parameter names can be edited. For example, Brilliant Violet (BV) 711 can be changed to CD3 Brilliant Violet (BV) 711 5.

3. Click **Plate Setup** [Plate Setup] to advance.

> **Tip:** You can change the media selection by using the Select Media button [Select Media].

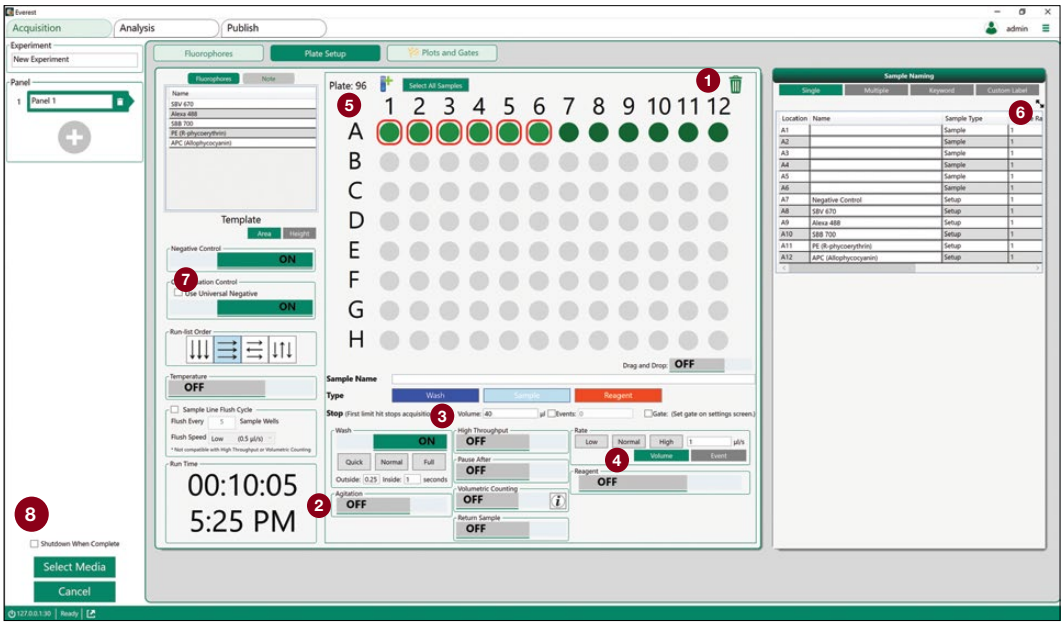
Set Up a New Experiment continued

To configure samples in the tube rack or plate: For a single sample, click a well to select it. For multiple samples, click and drag across wells to select them.

4. Click **Sample**.
5. To remove a sample, select the well and click the trash icon at the upper right of the Plate Setup window 1.
6. If agitation is required, select wells to be agitated and toggle the Agitation button to ON 2.
7. Select acquisition limit criteria, **Events** or **Volume** 3.
8. Select sample flow rate 4.

Tip: To add a STAT tube to the plate layout to help with optimization, select the STAT tube icon at the top of the plate layout 5.

9. Check details of the run list by selecting the double arrow above the list 6.
10. Click **Plots and Gates** Plots and Gates to advance.



Tip: Use the Template section of the Fluorophores panel of the Plate Setup screen 7 to set up compensation controls. For details on compensation, see page 8.

Tip: Check the Shutdown When Complete box when you need to leave prior to completing the acquisition of the samples 8.

Setting Up the Workspace

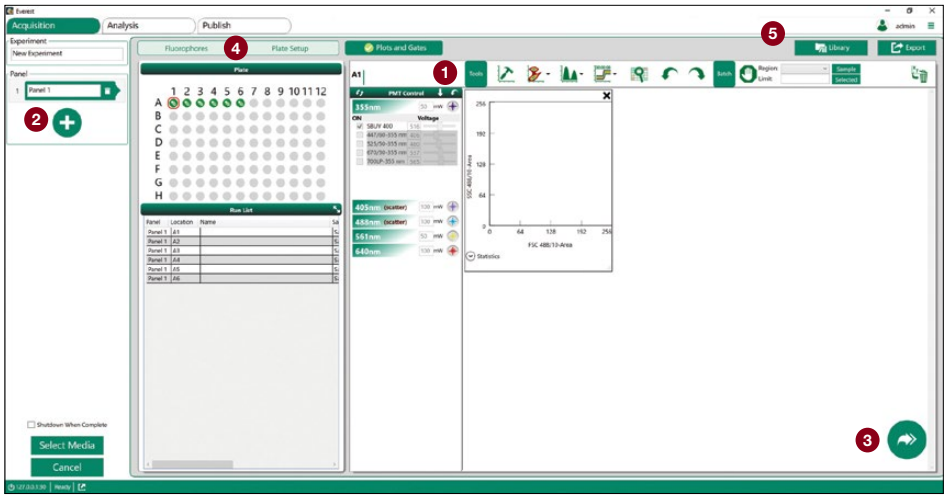
Use the Plots and Gates screen to configure the workspace and to set up predesigned plots and gates and a hierarchical structure. Plots for the negative control and compensation samples are predetermined.

To create a plot and region for analysis using the Tools menu 1:

1. Select a sample well.
2. Select the laser and channel of interest for the x-axis.
3. Select the laser and channel of interest for the y-axis.
4. To add a different panel, select the **+** button in the panel menu 2.
5. Click the forward arrow 3 to complete building your experimental run list. The configured workspace appears in Setup mode and the Instrument Control panel displays setup options.

Tip: Click the menu buttons 4 at the top of the page to edit previous pages.

Tip: Access the instrument settings library 5 to import instrument settings into panels. See Importing Panel Settings on page 10 (black) and Saving Panel Settings on page 9.



Tip: To view compensated data, select **Comp** before selecting **Height**, **Area**, or **Width**. Hyperlog is automatically selected. To display data in linear scaling, deselect **Log**.

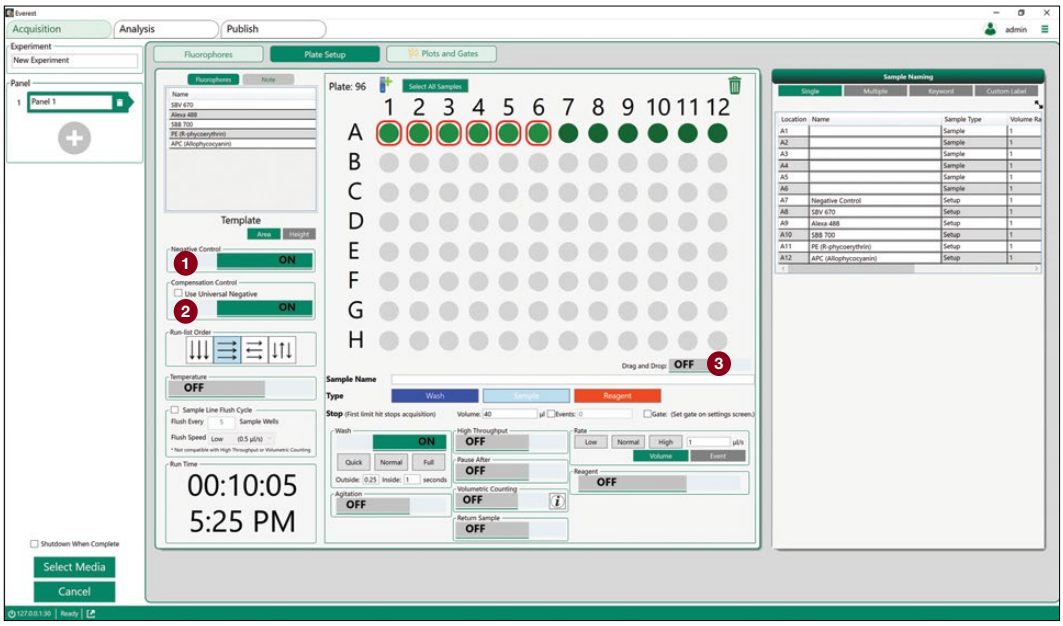
Compensation Setup

This setup is used with single-color controls to negate emission spillover.

Note: If single-color controls do not have positive and negative populations, use a universal negative (negative control). Enable it by selecting the checkbox under Compensation Control in the Select Fluorophores panel.

1. Turn Negative Control **ON** 1. Everest designates a negative control setup well in the first available well.
2. Turn Compensation Control **ON** 2 to designate a setup well in the next available well for each selected channel/fluorophore. If necessary, drag and drop samples into the correct order.
   a. Toggle the Drag and Drop button 3 to **ON**.
   b. Click sample to select.
   c. Drag selected sample to an empty well.
   d. Continue to select and drag samples to align with sample order on the sample plate/rack.
   e. Toggle the Drag and Drop button to **OFF** when reordering is complete.

Tip: To avoid using the Drag and Drop function, create your worklist first and then put your samples in the order generated by Everest Software.



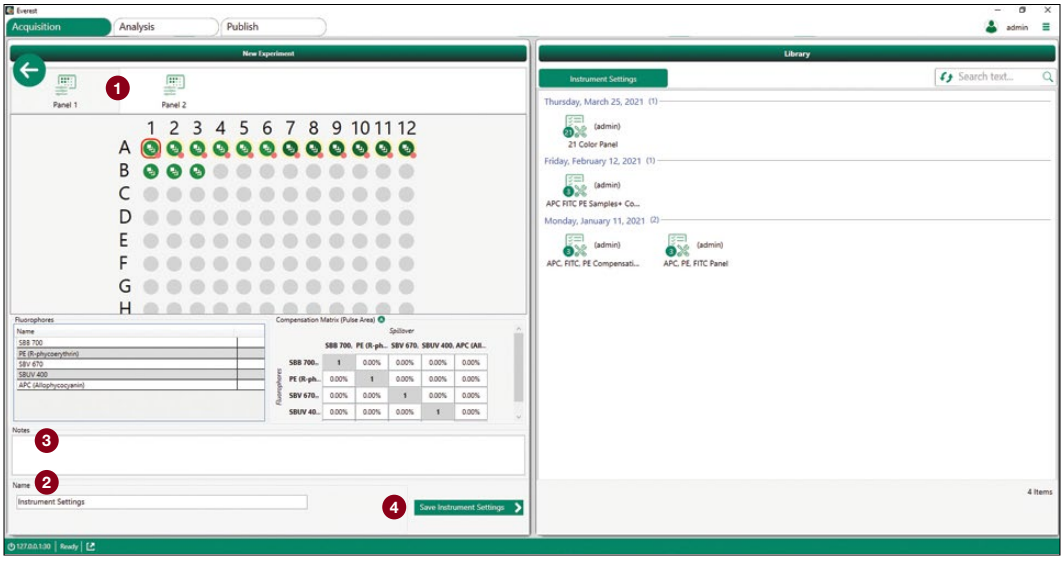
Saving Panel Settings

Following adjustment of voltages and calculation of compensation, select the Library button in the Acquisition window.

1. Select the panel for which you are saving the settings 1.
2. Name the settings you wish to add 2.

> **Tip:** Add notes to the settings if desired 3.

3. Save the instrument settings 4.



Importing Panel Settings

In the Plots and Gates screen, select the Library button. 

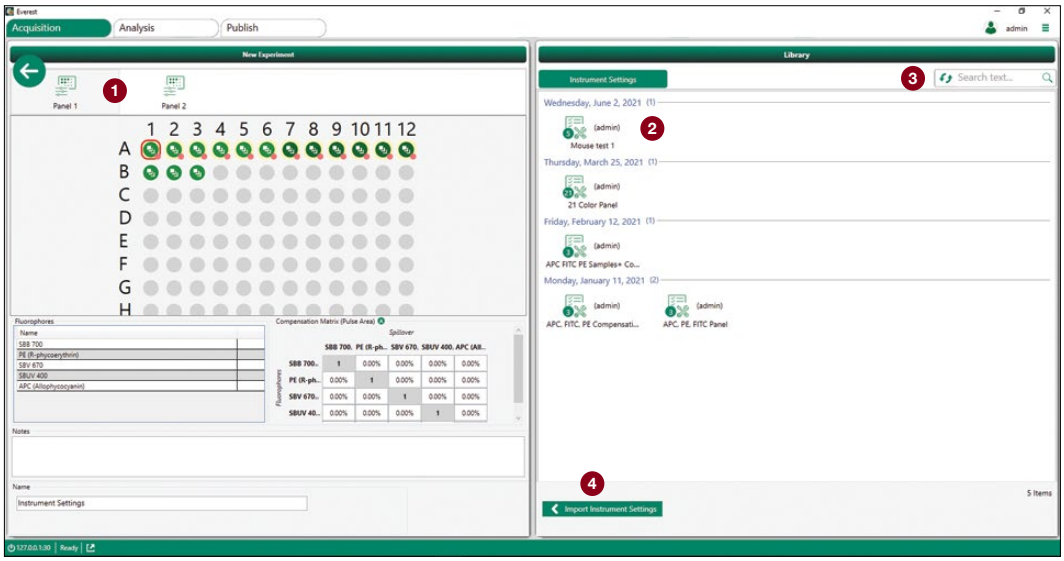
1. Select the panel for which you are saving the settings 1.
2. Select the settings you wish to add 2.

Tip: Settings are listed by the date when they were last used.

Tip: Settings are searchable using the search box 3.

Tip: The names in the Name list in the Fluorophore window must exactly match those listed for the settings.

3. Import the instrument settings 4.



Set Sample Parameters

If loading a previous experiment or importing settings from a prior experiment setup, verify that populations fall into the correct plots and gates. If starting a new experiment, optimize the setup to place populations in the correct plots and gates.

To optimize experimental settings

To optimize a negative sample in Setup mode using an added Stat tube (see Tip under step 8 on page 6):

1. Select **Setup** in the Instrument Control panel. The Acquisition button will turn gray and the Setup button will go green.
2. Click the STAT tube icon 1 for optimization.
3. Load the STAT tube position with a tube containing negative sample.
4. Click **Play** to run negative sample (no data are collected at this point).
5. Adjust flow rate to low (100–300 events/sec) by clicking **Event** 2 and typing a flow rate value between 100 and 300 in the flow rate field.
6. While previewing negative sample, set PMT voltages and configure the threshold in order to place populations on the scale in the scatter and fluorescence channels.

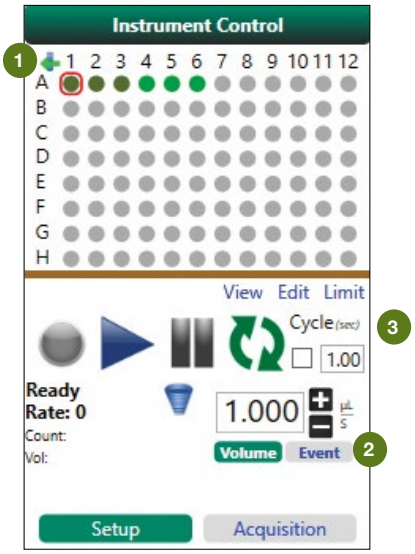
   Note: See page 12 for instructions to change the voltages and threshold.

7. Once settings are optimized for negative sample, click **Stop** .

To quickly ensure that stained populations are on scale:

1. Switch negative sample for a fully stained multicolor sample in the STAT tube position.
2. Click **Play** to run multicolor sample (no data are collected at this point).
3. Adjust flow rate to low (100–300 events /sec) by clicking **Event** 2 and typing a flow rate value between 100 and 300 in the flow rate field.
4. While previewing multicolor sample, configure PMT voltages and review populations' locations in the scatter and fluorescence channels.
5. Once settings are optimized for the multicolor sample, click **Stop** .

Optional: If necessary, select the Cycle checkbox 3 and adjust seconds per cycle for optimal visualization.



Set Sample Parameters continued

Return to **Plate View** once settings are optimized.

Tip: Confirm positive population in single-color control is under the diagonal line before calculating compensation. If positive population is not under the line, then increase voltage in primary channel (x-axis) and/or decrease voltage in spillover channel (y-axis).

To change the voltages

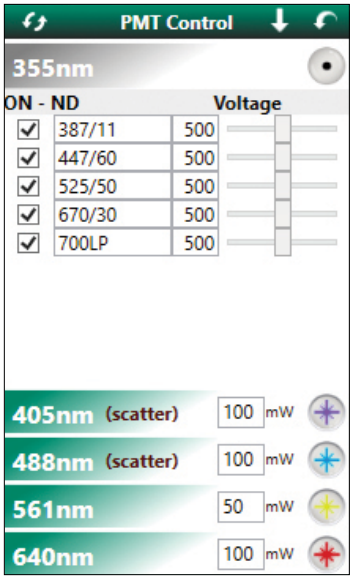
PMT voltages are modified in the PMT Control window. Active channels will be checked and inactive channels will be grayed out.

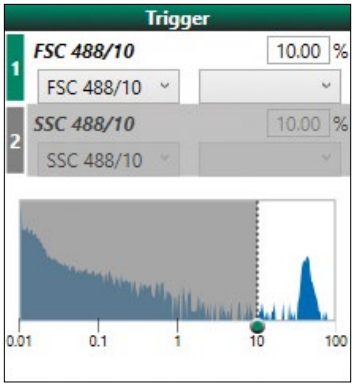
There are four ways to change the voltage:

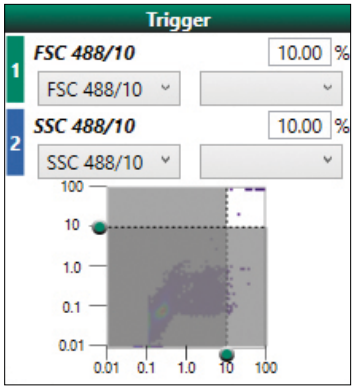
- Type in a specific voltage number
- Move the pointer over the voltage number and use the mouse wheel to scroll up and down
- Drag the slider next to the number
- Click and hold the bar in which the slider moves for finer adjustment

To change the threshold

Thresholds trigger data acquisition. Modify the threshold in the Trigger window by dragging the bar or entering a percentage value. Use the dropdown menus to select any parameter as a trigger. Everest Software supports two triggers. Click **2** to activate the second trigger. The button will switch from gray to blue.







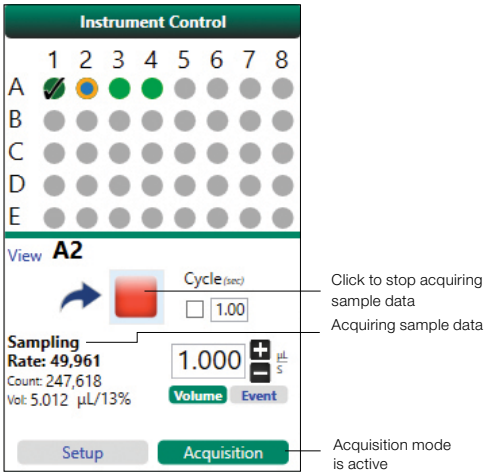
Acquire Data

Make sure plate or tube rack is loaded and instrument door is closed.

1. Select **Acquisition** in the Instrument Control panel. The Acquisition button will turn green and the Setup button will go gray.
2. Select the first control or sample well to be acquired.
3. Click **Play** to begin acquiring data using the experimental run list.
4. Click **Stop** to stop the run list at any time.
5. Click **Skip to Next Sample** to advance to next sample.

Acquisition of samples will stop at the end of the run list.

Note: In the upper left corner of the workspace, a plate map monitors sampling progress in real time. See Appendix for plate map symbols.

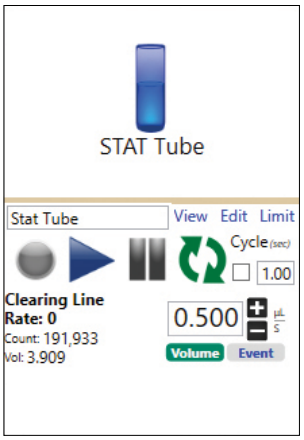


To edit run list

Select **Setup** in the Instrument Control window. Click **Edit**. Follow instructions for a new experiment to edit details as needed (page 4).

To save compensation settings with FCS data files

To save compensation settings, add a **Pause** on the last compensation sample. When the ZE5 Cell Analyzer pauses acquisition, click the **Send to analysis** icon . This allows you to begin auto compensation in the current run list by selecting the **Auto compensation** icon . When auto compensation is completed, click the **Send runlist to local instrument** icon .



To acquire data in STAT tube (optional)

You can run a single sample quickly without building an experiment.

To run individual sample:

1. Select **STAT Tube** from the Home window.
2. Click **Play** .
3. Adjust flow rate to low (100–300 events /sec) by clicking **Events** and typing a flow rate value between 100 and 300 in the flow rate field.
4. Select a laser and channel of interest for the x and y axes from the Tools menu to create plots and regions for analysis.
5. While previewing a multicolor sample, configure PMT voltages and review populations' locations in the scatter and fluorescence channels (see pages 11–12).
6. Once settings for multicolor sample are optimal, click **Record** to acquire sample data.

Optional: Select the Cycle checkbox and adjust seconds per cycle for optimal visualization, if necessary.

7. Click **Stop** when finished.

Analyze Data Just Acquired

Going to the Analysis tab through the Acquisition tab allows direct access to data from the just completed run. Go to Analysis to create plots and regions for analysis after acquiring samples.

1. In the Acquisition tab click the Analyze icon.
2. Create plots and regions as necessary.

Analyze Data No Longer in Workspace

From the Analysis tab you can load an experiment one of two ways.

Option 1:

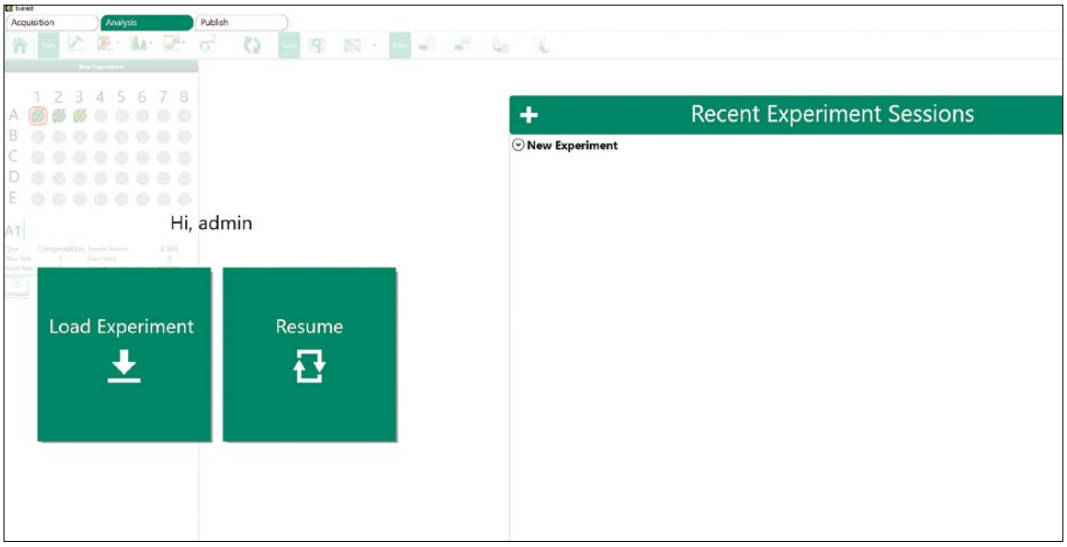
1. Click **Load Experiment**.
2. Find experiment name in the Experiment list.
3. Click experiment name to open its Experiment session.
4. Find specific experiment in the Experiment session.
5. Click **OK**.

Option 2:

1. In the Recent Experiment Sessions panel, browse to the folder containing the experiment you want to analyze.
2. Click experiment session name.
3. Find the specific experiment in the Experiment session.

Tip: Experiments containing data are marked by a green check mark. Experiments without data have a red X next to them.

4. Double click specific experiment.



Shutdown

Shut Down

In the Acquisition tab toolbar or on the Home screen, click **Shutdown**  .

Shutdown begins. The system automatically:

- Turns off all lasers
- Rinses the system with cleaner and DI water
- Depressurizes the fluidics
- Enters a sleep state until startup is run

Optional: To schedule automatic startup, click the Yes checkbox in the automatic startup dialog box before shutting down.

Tip: Before shutting down the instrument, it is recommended to clean the probe with bleach and water.

Refer to the ZE5 Cell Analyzer and Everest Software User Guide for a description of the icons used in the plate map (Chapter 7, Creating Experiments and Workspaces).

Appendix

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

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