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## Release Notes for the ZE5 Cell Analyzer and Everest Software

### Version 3.2

2023

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## Introduction

The ZE5 Cell Analyzer is a compact benchtop flow cytometer that characterizes cells and their properties by streaming hydrodynamically focused cells through up to five spatially separated laser beams at varying wavelengths.

Everest Software is the comprehensive instrument control application for the ZE5 Cell Analyzer. Everest Software controls all functions of the ZE5 Cell Analyzer and provides accurate data acquisition and user-friendly data analysis.

## Supported Operating System

Bio-Rad supports Everest Software on the Windows 10 Pro and Windows 11 operating systems.

## Upgrading to New Versions

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

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

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

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. The software upgrade process removes the currently installed Everest Software version. If you need the installation file and instructions, contact Bio-Rad Technical Support.

## Additional Information

### New Fluorophores

Everest now includes the following StarBright Yellow (SBY) and StarBright Red (SBR) fluorophores:

SBY 575	SBR 670
SBY 605	SBR 715
SBY 665	SBR 775
SBY 720	SBR 815
SBY 800	

### Fixed Issues

Everest 3.2 includes the following fixes:

- Everest now correctly displays sample acquisition data in all plots when the plots workspace includes a time plot in log scale.
- Everest now correctly includes the cytometer serial number (\$CYTSN keyword) in exported FCS files generated from wells recorded in Setup mode, from a Stat Add on in a single sample experiment, and from stat tube experiments.
- Comp and Hyperlog buttons are removed from the FSC and SSC channels of the Density Plot builder, Histogram Plot builder, and the Edit Plot dialog of the Modify Plot Parameters function.

### Known Issues

You might encounter the following issues using Everest 3.2:

- Comp setting is enabled by default when a user creates a Histogram plot.  
**Workaround:** Open the Modify Plot Parameters function for the Histogram plot. In the Edit Plot dialog, deselect Comp, then click Apply.
- Current ZE5-EYE results are not automatically displayed in the EYE Trending report.  
**Workaround:** Open the QC report before opening the EYE Trending or QC Trending reports.
- The CSV Export function does not work correctly for multi-panel experiments.
- In multipanel experiments, Everest Software displays the correct PMT Control names for selected wells only in Panel 1.  
**Note:** This is a display issue only.
- If you select the High Throughput option for a sample well, and then reduce the sample volume for that well before you run the experiment, Everest Software displays the higher sample volume for the well in the Analysis module.  
**Note:** This is a display issue only. The correct sample volume is used.
- Adding or editing sample or panel settings causes existing compensation control plots to be reset to their default states.

- When you select a fluorophore, Everest Software removes its name from the Available Fluorophores list, but does not add the name to the Selected Fluorophores list.

**Workaround:** Restart Everest Software. If you continue to experience the issue, reinstall Everest Software.

- If you change the scale/bin for a density and histogram plot from 256 to 512, regions already drawn in the plot are not rescaled.

**Workaround:** Recreate the plots in a 512 resolution, and then add the regions.

- After deleting a region, gate assignments on remaining regions are lost (gate limit, hit detection, heat map).
- When you run QC, the QC Trending Report does not show the latest results if the end date specified is the current date.

**Workaround:** Restart Everest Software and reopen the QC Trending Report.

- The images reflecting fluidics levels in the fluidics stats drop-down menu can be inaccurate.

**Workaround:** Always empty both waste containers and fill both sheath containers *at the same time* to ensure the fluidics levels are displayed accurately.

- If you use the Drag and Drop feature to move sample well with custom labels assigned, the custom labels are lost.
- When you browse for an experiment, the Browse dialog box does not allow you to browse outside of the user folder.

**Workaround:** Restart Everest Software.

- In multipanel high-throughput experiments, data is not displayed during acquisition of some panels.

**Note:** This is a display issue only. The data collected is correct.

- When you open an existing experiment in Edit mode, Everest Software stops working.

**Workaround:** Restart Everest Software.

- In multi-panel experiments, Everest Software allows you to select and drag-and-drop wells from an unselected panel and the wells seem to disappear from the plate layout.
- In density plots in the Analysis module, if you apply a filter to a particular region, and then try to move the region, the data refresh is interrupted.

**Workaround:** Click Refresh Data to manually refresh the data.

- In the Analysis module, if you click a sample well while the auto-compensation process is running, the process is disrupted and cannot be canceled.
- When you reset the system preferences to default settings (current day), and enable Vacation mode with a future end date and 1-day intervals, and then prompt the Shutdown process, the system has issues with the specified days and immediately runs the Startup process after shutting down.

**Workaround:** You must always set both the start date and end date to the following day at the earliest.

- If you set the QC process End Date to the current date, and then run QC, when you open the QC Trending report, some data points appear outside of the plot.
- If you edit an experiment, add plots, and create a quadrant region in each plot, when you apply a filter, the list of regions do not appear in a logical order.

- If you rename a fluorophore and add a comma to the name, Everest Software considers the comma a separator and creates a column for each part of the name when using custom labels.
- When the computer clock is set to the 24-hour mode, issues arise when you use the up and down arrows to set the start and end times.
- In experiments with mixed sampling modes, Everest Software does not honor gate limits enabled on wells with standard sampling where a section of the plate is set to high throughput acquisition.
- In high throughput experiments with different volume limits in the wells, the displayed volumes collected are incorrect.
- If you cancel the QC process, the New QC Results window still appears.
- If Everest Software closes unexpectedly, or if you close it from the Windows Task Manager, the ZE5 does not perform some of the shutdown tasks, and if you restart Everest Software, an unhandled process exception error appears.

**Workaround:** Close and restart Everest Software. If the problem persists, use the Home command in the Service Tool to return the probe to its Home position in the ZE5.

- In multipanel experiments in which one panel contains a reagent well, Everest Software displays the reagent information when you select other panels. If you add reagent to another panel, or if you add a panel, error messages appear.
- For experiments that were created in a version earlier than 3.0, you must regenerate the list of fluorophores before you can add custom labels.
- In multipanel experiments that have the same fluorophore name under Available Detectors across different panels, Everest Software does not display a notification about the duplicate entries.
- If you create a new experiment and enter the same name for different fluorophores under Available Detectors, Everest Software allows you to select a different tab and displays a notification only once.
- In a high-throughput acquisition, the software displays the cumulative or total sampling volume count rather than the sampling volume for the current sample. However, the software correctly displays the actual sampling volume for each sample in Analysis. The correct values are also saved to the FCS file.
- The x-axis time scale does not update for continuous or sliding time plots after data are plotted for the default of 60 secs. Fixed time plots are not affected.
- Statistics for plots that do not contain gates cannot be exported to CSV.

**Workaround:** Before exporting statistics to CSV, create plots that contain gates, or add a gate to the plots for which you want to export the statistics to CSV.

- If multiple gates have the same name, some statistical data are mapped to the wrong column in the exported CSV file.

**Workaround:** Ensure that all gates have unique names.

## Documentation

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

- ZE5 Cell Analyzer and Everest Software User Guide
- ZE5 Cell Analyzer and Everest Software Quick Start Guide

### To access the ZE5 Cell Analyzer and Everest Software User Guide

1. In Everest Software, click the Main Menu button in the upper right corner.
2. Select User Manual to open the PDF.

### To access the latest product documentation

- ▶ Visit the ZE5 Cell Analyzer product page on the Bio-Rad website.

## Contacting Technical Support

The Bio-Rad Technical Support department in the U.S. is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific Time.

**Phone:** 1-800-424-6723, option 2

**Email:** Support@bio-rad.com (U.S./Canada Only)

For technical assistance outside the U.S. and Canada, contact your local technical support office or click the Contact Us link.

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For information on open source software used to develop Everest Software, see the ZE5 Cell Analyzer and Everest Software User Guide, Appendix D.

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