Release Notes for the ZE5 Cell Analyzer and Everest Software

Version 3.0

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Contents
Introduction.................................................................................................................................................1
Supported Operating System.........................................................................................................................1
Upgrading to New Versions.............................................................................................................................2
New or Modified Features...............................................................................................................................2
Fixed Issues....................................................................................................................................................11
Known Issues................................................................................................................................................12
Documentation...............................................................................................................................................14
Contacting Technical Support.......................................................................................................................14
Legal Notices................................................................................................................................................15

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 Microsoft Windows 10 Pro operating system only.
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

If you are running an Everest Software version earlier than v2.5, Bio-Rad recommends upgrading your software to v2.5 before installing v3.0 to ensure the proper firmware is installed.

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.

New or Modified Features

More Flexible Experiment Builder with Tab Layout

The Experiment Builder has been updated with a new Experiment pane and tab layout, where you can select your fluorophores, set up your plate, and create plots and gates.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>New Experiment Builder Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component</td>
<td>Function</td>
</tr>
</tbody>
</table>
| Experiment Pane (static display) | Enter the Experiment name.  
Add panels and enter panel names.  
Change the media type.  
**Important:** Changing the media type discards all previous entries.  
Cancel the experiment. |
| Fluorophores Tab (default display) | Select the relevant fluorophores for the experiment.  
View the default detection filter corresponding to each fluorophore and modify if necessary. |
| Plate Setup Tab | Identify well types.  
Designate well settings.  
Use the Sample Naming templates for single and multiple names, keywords, and custom labels. |
| Plots and Gates Tab | Set up plots.  
Apply the settings. |
After you tap New Experiment in the Home window, Everest Software launches the new interface and prompts you to select a media (plate or tube) layout. Media layouts remain the same. After you identify your layout, the Experiment Builder opens and the Experiment pane, which is a static display, appears on the left.

- You can name your experiment and panels at any time before applying the experiment. For more information, see Experiment Names and Panel Names.
- As you begin building your experiment, you must satisfy certain requirements before you can move to the next tab, as shown in the following workflow.

*After you select your plate layout, the Fluorophores tab opens by default, where you can select fluorophores and available detectors. You can also rename the fluorophores in the Available Detection panel.*
When you select the Plate Setup tab, you can define actions for your plate, controls, and samples, and configure actions for one or more wells. The fluorophores you selected appear on the left. You can also use the Sample Naming panel for additional flexibility in sample identification.
When you select the Plots and Gates tab, you can configure plots and gates immediately or, if you are setting up a multipanel experiment, you can add a panel in the Experiment Pane. You are not required to change the default plots and gates settings before returning to an earlier tab, creating a new panel, or applying your experiment.

You can return to the Fluorophores tab and Plate Setup tab before entering information on the Plots and Gates tab.

You can apply an experiment with default Plots and Gates settings, and you can apply the experiment only from the Plots and Gates tab.

**Experiment Names**

In earlier versions of Everest Software, users were required to name the experiment immediately after clicking the New Experiment button. In the new Experiment Pane, Everest Software displays a default experiment name but you can change it at any time before you apply the experiment.

**Panel Names**

You can also name your panels at any time before applying your experiment. The panel name for a selected well (or multiple wells if on the same panel) appears in the following locations:

- Under the Panel heading in the Experiment pane
- Above PMT Control in the Plots and Gates tab
- After you apply the experiment and move to the Acquisition screen, in the blue bar above your plots
Adding More Panels to Your Plate

As long as each panel is configured on the same media layout, you can continue to add panels with different settings until the plate layout is full. When you have satisfied the requirements for your current panel, Everest Software enables the Add icon (+) and you can add a new panel.

- Panels appear in order of creation date/time, with the oldest panel at the top of the list and the newest at the bottom.
- Panel names also appear in the runlist tables to facilitate data identification.
- You can change the default panel names at any time before you apply the experiment.
- **Note:** Runlists continue to be sorted by location (well).
- You can navigate between panels during setup to add, delete, or modify information.
- New panels initially contain default cytometer settings.
- You can set up compensation controls for each panel.

Removing Panels

You can remove one or more panels from your experiment by clicking the Remove Panel icon (-) next to the panel name. An experiment must have at least one panel. Wells for a removed panel become available for use again, and can be added to a different panel.

Changing the Media Type

You must select a media type before proceeding to the Fluorophores tab, but you can change the media type in the Experiment pane at any time before you set up your wells on the Plate Setup tab.

**Important:** Changing the media type discards all plate settings.

Importing Settings

You can continue to import the following settings from earlier experiments:

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

Loading Run Lists from Earlier Versions

Runlists created in earlier versions of Everest Software map correctly to the new Experiment Builder layout. However, mapping of multipanel experiments created in version 2.3 or earlier is not supported in version 3.0.
**Instrument Settings Library**

Everest Software features a library in which you can store and access files containing cytometer and compensation (instrument) settings, such as fluorophores, lasers, PMT detectors, detector voltages, trigger and threshold values, and the compensation matrix that you can reuse in future experiments. As you define these settings in individual experiments, you can save them to a file and then import the settings to another panel with the same fluorophores.

To access the Library, click the Library button in the Plots and Gates tab or the Library icon in the Acquisition screen.

If the instrument is busy, the Library icon in the Acquisition screen is grayed out.

To be busy, an instrument is starting up, running QC, performing sampling, unclogging, cleaning the sample probe, decontaminating, pausing, and shutting down.

Using the Instrument Settings Library, you can do the following:

- From the Acquisition screen, you can save the settings for an individual panel to a file in the Library.
- From the Plots and Gates tab in the Experiment Builder, you can import settings from a file to individual panels as you edit your experiment.
- For multipanel experiments, you can select individual panels in which to import settings. An icon for each panel in the experiment appears above the plate map.
  - To select all wells in the panel, click the panel icon.
The selected panel is highlighted in the plate map and the first well in the panel is selected.

- Fluorophores are listed below the plate map, on the left.
- The compensation matrix is displayed below the plate map, on the right. When you select a panel, the associated matrix appears.
- You can save notes applicable to your settings before you save them to the instrument settings file.
  - Before you save the settings, type your notes into the Notes field in the bottom-left corner.
- After the file is saved, you can pause on the file in the Library to view notes and other details in a tooltip.

- By default, the tooltip contains the file name, fluorophores, any notes added by you, and the compensation matrix icon in the lower-right corner.
- A green compensation matrix icon indicates the file was saved with compensation values – by area (A) or height (H) – and a gray icon indicates the file was saved without compensation values.

- You can enter a unique instrument settings file name in the field below the Notes. If you enter a duplicate name, you are prompted to change it.
- Files are automatically sorted by most recently used.
- Using the library Search field, you can search by owner name, experiment name, or content in the Notes field to locate files.
- You can save your settings for an individual panel to an instrument settings file from the Acquisition screen.
• You can import settings from the Plots and Gates tab.

• When you import a settings file, a confirmation message appears.
  
  **Important:** If you try to import settings that do not match your experiment or instrument configuration (for example, a fluorophore or detector mismatch is identified), an error message appears.

**Enhanced Multipanel Functionality**

• You can add different cytometer and compensation settings to different panels from a file in the Instrument Settings Library.

• You can change triggers and threshold settings for each panel.

• Each panel has independent cytometer settings and analysis objects.

**Panel Identification**

The active panel in a multipanel experiment appears as follows:

• In the Plate Setup plate map, the wells are green for the active panel, and wells in the other panels are black and display the panel number.

![Plate Setup](image)

• On the Plots and Gates tab, the active panel is displayed above the PMT controls.

![Plots and Gates](image)

• Panels are also identified in the run list table below the plate layout.

• On the Acquisition screen, when you select a well in the plate map, the active panel name appears in the blue status bar.

![Acquisition](image)
• In the Analysis screen, the active panel name appears below the plate map.

![Plate Map with Panel 3 and C4 PE-Cy7 highlighted.](image)

**Import Settings Button Label**

The button on the Home screen now reads Import Run List.

![Home screen with New Experiment, Load Experiment, Import Run List, STAT Tube, and Resume options.](image)

**Editing PMT Controls**

PMT control editing is disabled on the Plots and Gates tab. You can continue to

• Add or delete fluorophores, and activate, deactivate, and rename detectors on the Fluorophores tab.
• Change voltages, enable or disable the ND filters, and enabling or disabling lasers in the Acquisition screen.
Sample Line Flush Interface

When you select the Sample Line Flush Cycle checkbox, you can enter the flush increment directly. Your entry in the Flush Every ___ Sample Wells field can range from 5 to 384.

Custom Labels

Custom labels specify unique values to further identify your samples on your plots. For example, you can specify different cell markers for each fluorophore in each well, as shown in the following graphic.

If you rename a fluorophore in the Available Detectors grid (Fluorophores tab), you can use the Custom Label grid to enter additional values.

The custom label structure lists fluorophores for all panels, in alphabetical order, and does not distinguish between panels.

In the Custom Label pane, you can
- Enter a label for each fluorophore in each well into the grid
- Click the double-arrow in the upper-right corner to expand the grid to its full size
- Export the well and fluorophore layout to a template file for reuse
- Clear the existing entries

Additional Information

Bio-Rad recommends the following:
- In multipanel experiments, you can add negative and compensation controls to one panel only.
  Tip: After you apply the experiment and adjust the compensation settings on the Acquisition screen, Bio-Rad recommends saving the settings for the panel to assign to panels in future experiments.
- If you need to delete panels, return to the Fluorophores screen and delete the applicable panels, and then navigate to the Plots and Gates screen to add panels.
- Whenever you replace the bulk fluidics in the ZE5 Cell Analyzer, you must empty both waste containers and fill both sheath containers concurrently to ensure that the fluidics levels shown in Everest Software are accurate.
Fixed Issues

- When a new quadrant region was added over an existing rectangle, ellipse, and polygon region, the quadrant region blocked the other regions and user could not drag, resize, or delete the non-quadrant regions.
- In rare occurrences, Everest Software became non-responsive at the beginning of the Clean Probe process, forcing the user to close, and then reopen, the application.
- When the default volume limit was changed for a particular well, Everest Software changed the limit for adjacent wells instead of the selected well.
- When the default event limit was changed for a particular well, Everest Software changed the limit for adjacent wells instead of the selected well.
- For PMT Controls, when a detector was enabled for an inactive laser, the laser did not appear to be activated.
- The names of the DAPI and Horizon V450 fluorophores were displayed incorrectly.
- Invalid well settings occurred when high throughput mode was enabled on all selected wells, including wells that were unassigned.
- Loading any experiment, and then acquiring for the first time, caused the secondary trigger and threshold values to be ignored.
- If you launched the Everest application, the first experiment that was loaded did not correctly retain the secondary trigger.
- Users were sometimes unable to create a new folder from the Browse window because the export process would hang.

Known Issues

You might encounter the following issues using Everest 3.0:

- 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 and fill both sheath containers at the same time to ensure the fluidics levels are displayed accurately.

• When you create a new panel, and before you select wells in the plate layout, the well order shown in the Custom Labels grid is be incorrect. The order is corrected when you select one or more wells.

• 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 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
- Everest Software training modules, which help you become familiar with basic system functionality. These modules run in your default browser, but Internet access is not required.

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 training modules
1. In Everest Software, click the Main Menu button in the upper right corner.
2. Select Training Module.
3. In the Help and Training dialog box, click the link to the training module you want to view.
4. The training module opens in your default browser.

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 at www.bio-rad.com.
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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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