

FLOW CYTOMETRY

ZE5™ Cell Analyzer and Everest™ Software

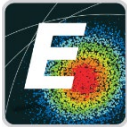
Quick Guide

Before You Begin

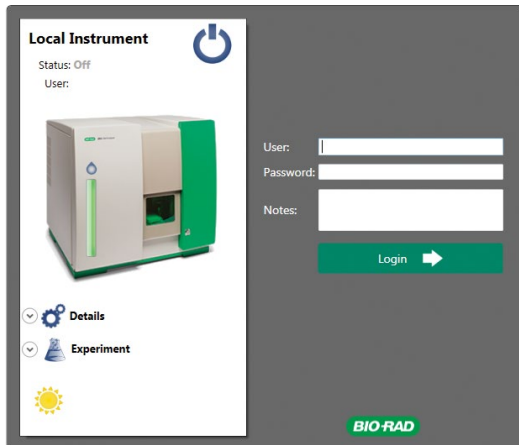
1. Ensure that the instrument is plugged in, powered on, and connected to the computer. Once powered on, the ZE5 Cell Analyzer is designed to remain powered on. Do not power it down.
2. Ensure that a bottle of ZE-Series QC Beads is in place in the right side of the sample chamber. These beads must always be in place. One bottle will last for several months, depending on usage.

Start Up the System

On the computer desktop, double click the Everest Software icon.



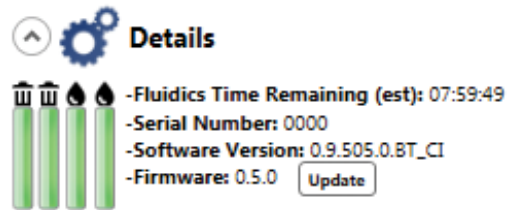
The Everest login window displays instrument status and login text boxes.



Check Instrument Status

System status appears in the left pane. Status: Off indicates that the instrument is plugged in, powered on, and shut down.

1. Click the Details down arrow to view instrument status specifics.



2. If necessary, fill the bulk fluids containers and empty the waste containers. Fluid containers can be exchanged during operation.

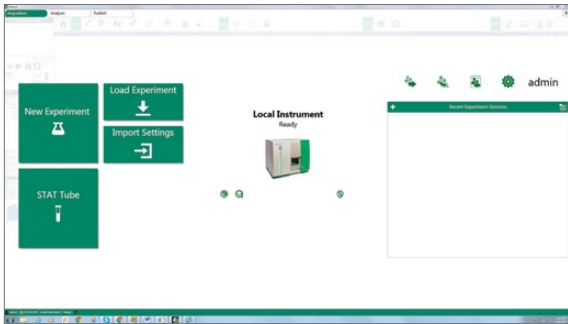
Note: You can refill fluids before logging in but you must log in before you can manually run the quality control (QC) process or run samples. You cannot log in during the startup or QC process.

Start Up and Log In

1. In the bottom left corner of the login window, click Startup ☀️. The instrument startup begins. Instrument Status changes to Ready and the Shutdown ⏻ icon replaces the Startup icon.



2. Enter your user name and password and click **Login**.
The Everest Home window appears.



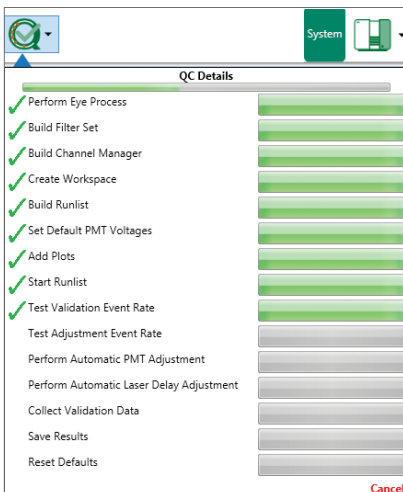
Run Quality Control

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 and photomultiplier tube (PMT) voltages, compares settings with QC criteria, acquires beads, and stores the results.

1. In the Home window, click QC . The Instrument Control panel opens and the automated QC process runs.



2. In the toolbar, click the QC down arrow to monitor progress in the QC Details box.



A message in the Instrument Control panel notifies you when QC finishes.

Choose What's Next

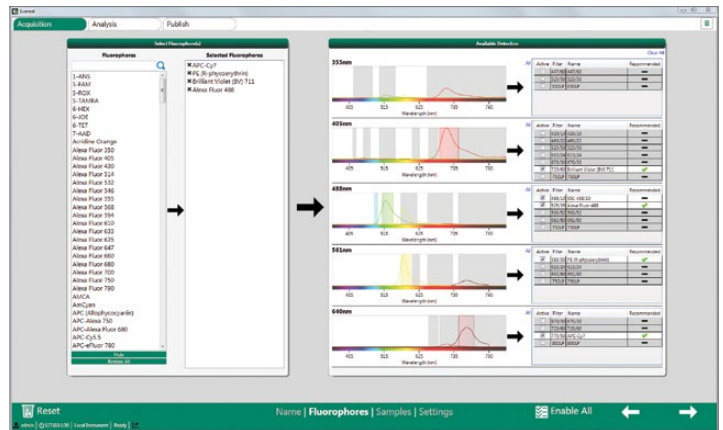
In the Home window, you can choose one of the following:

- **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
- **Load Experiment** — loads a saved experiment, including fluorophores, parameter names, PMT voltages and other instrument settings, compensation values, samples, and plots
- **Import Settings** — loads settings from a previously generated experiment, including fluorophores, voltages, and compensation values, if available

Tip: You can apply imported settings to different sample sets.

Set Up a New Experiment

1. In the Home window, click **New Experiment**.
2. In the Name box, enter an experiment name and click **Next**.
3. In the left pane of the Fluorophores screen, double click the name of each fluorophore you want to add to the experiment or click **Enable All** to select all fluorophores.

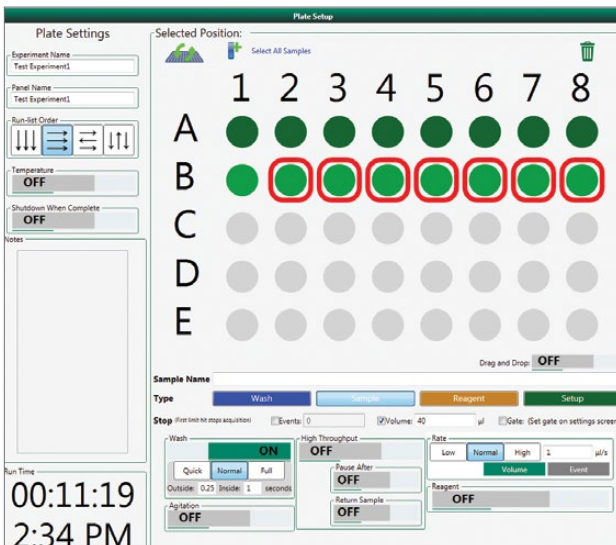


4. Click **Next**.

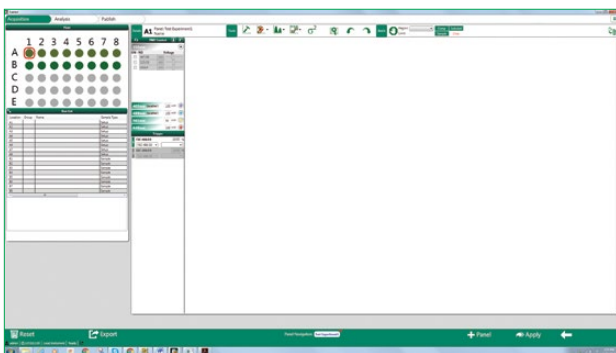
5. In the Plate Setup panel, select a media type.



6. In the Plate Setup panel, configure the tube rack or plate according to the requirements of your experiment. Click **Next**.



The Settings screen appears, in which you can configure the experiment workspace.



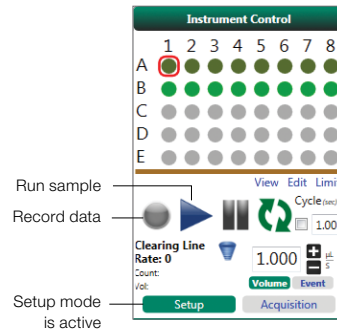
7. Create plots and regions for analysis and adjust instrument settings, such as thresholds and PMT voltages.

Acquire Data in an Experiment

1. In the experiment you set up in the Experiment Builder, click **Apply** in the lower right corner of the screen.



The configured workspace appears in setup mode; the Instrument Control panel displays setup options.



2. Click the Run sample icon.

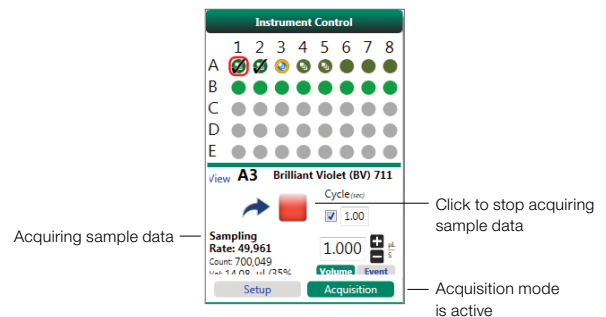
3. While acquiring samples in setup mode, you can configure the threshold and set PMT voltages for placing populations on scale in scatter and fluorescence channels.

4. Optional: Select the Cycle checkbox and adjust the flow rate, if necessary.



5. After you optimize settings, you can acquire data from all samples automatically in acquisition mode.

6. In the Instrument Control panel, click **Acquisition** and then click Run List ▶ to begin sampling. Run List changes to Stop Sample ■.

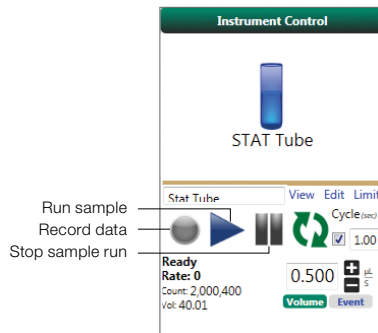


In the upper left corner of the workspace, a plate map monitors sampling progress in real time. If you set limits in setup mode, sample data collection stops when the first limit is reached.

Acquire Data from a QUICK (STAT) Sample

Without building an experiment, you can run a single sample quickly.

1. In the Home window, click **STAT Tube**. The workspace displays Stat Tube settings.



2. Create plots and regions for analysis and adjust instrument settings, such as thresholds and PMT voltages.
3. Click the Play icon to start running the sample.
4. Adjust settings, plots, and regions as appropriate.
5. When you are ready to save data, click the Record icon.
6. When you are finished, click the Stop icon.

Analyze the Data

After acquiring samples, you can analyze the data in the Analysis tab workspace.

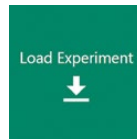
Analyzing Data Just Acquired

1. Click **Analyze** in the Acquisition tab.
2. Create plots and regions as necessary for analysis.



Analyzing Saved Data No Longer in the Workspace

1. Click the **Analysis** tab.
2. Click **Load Experiment**.



3. In the Recent Experiment Sessions panel, browse to the folder where your data are saved, select an experiment session, and click **OK**.
4. In the Analysis tab workspace, create plots and regions as necessary for analysis.

Shut Down the ZE5 Cell Analyzer

1. In the Acquisition tab toolbar, click Shutdown
2. Optional: Schedule automatic startup.
3. Click the checkmark in the automatic startup dialog box.
4. Shutdown begins. The system automatically does the following:
 - Turns off all lasers
 - Rinses the system with cleaner
 - Depressurizes the fluidics
 - Enters a sleep state until startup is run

Find Out More

More information about the ZE5 Cell Analyzer and Everest Software is available in the Everest Help menu.

- ZE5 Cell Analyzer and Everest Software User Guide
- Video training modules that walk you through basic system operations. These modules run in your default browser. Internet access is not required

Also, see the ZE5 Cell Analyzer and Everest Software Release Notes, which are delivered with Everest Software.

Visit bio-rad.com/web/ZE5Analyzer for more information.

Alexa Fluor, FAM, HEX, JOE, ROX, TAMRA, and TET are trademarks of Life Technologies Corporation. BD Horizon Brilliant Violet is a trademark of Becton, Dickinson and Company. Cy is a trademark of GE Healthcare. eFluor is a trademark of eBioscience, Inc.

BIO-RAD

**Bio-Rad
Laboratories, Inc.**

Life Science
Group

Web site bio-rad.com **USA** 1 800 424 6723 **Australia** 61 2 9914 2800 **Austria** 43 1 877 89 01 177 **Belgium** 32 (0)3 710 53 00 **Brazil** 55 11 3065 7550
Canada 1 905 364 3435 **China** 86 21 6169 8500 **Czech Republic** 420 241 430 532 **Denmark** 45 44 52 10 00 **Finland** 358 09 804 22 00
France 33 01 47 95 69 65 **Germany** 49 89 31 884 0 **Hong Kong** 852 2789 3300 **Hungary** 36 1 459 6100 **India** 91 124 4029300 **Israel** 972 03 963 6050
Italy 39 02 216091 **Japan** 81 3 6361 7000 **Korea** 82 2 3473 4460 **Mexico** 52 555 488 7670 **The Netherlands** 31 (0)318 540 666 **New Zealand** 64 9 415 2280
Norway 47 23 38 41 30 **Poland** 48 22 331 99 99 **Portugal** 351 21 472 7700 **Russia** 7 495 721 14 04 **Singapore** 65 6415 3188 **South Africa** 27 (0) 861 246 723
Spain 34 91 590 5200 **Sweden** 46 08 555 12700 **Switzerland** 41 026 674 55 05 **Taiwan** 886 2 2578 7189 **Thailand** 66 2 651 8311
United Arab Emirates 971 4 8187300 **United Kingdom** 44 020 8328 2000

