Instruction For Use

Vi-CELL XR

Cell Viability Analyzer

PN 383674BE
October 2017

Beckman Coulter, Inc.
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BECKMAN COULTER

Vi-CELL XR Cell Viability Analyzer
Instructions for Use
PN 383674BE (October 2017)

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If you have any questions, contact our Customer Support Center.

- Worldwide, find us via our website at www.beckmancoulter.com/customersupport/support.
- In the USA and Canada, call us at 1-800-369-0333.
- Outside of the USA and Canada, contact your local Beckman Coulter Representative.

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Original Instructions

Revision History

Initial Issue, 4/03
Software version 2.01

Revision B, 01/08
Software version 2.03
Additions and changes were done on these pages: 3-1 through 3-10, 4-1, 4-3, 4-5, 4-6 and 8-6.

Issue BA, 10/11
Updates were made to the company corporate address.

Issue BB, 04/16
Updates were made to the following sections:

Safety
- RoHS Notice
- Safety Symbols

Chapter 1
- System Overview
- Daily Verification

Chapter 2
- Setting Up Preferences
- Daily Verification

Chapter 4
- Types of Users

Chapter 7
- File History
- Applying Electronic Signatures

Issue BC, 02/17
Software version 2.06
Complete revision for 21 CFR 11 compliance.

This document applies to the latest software listed and higher versions. When a subsequent software version affects the information in this document, a new issue will be released to the Beckman Coulter Web site. For labeling updates, go to www.beckmancoulter.com and download the latest version of the manual or system help for your instrument.

Issue BD, 06/17
Software version 2.06.1
Updates were made to the following sections:

Safety
- Safety Precautions
- Safety Symbols

Chapter 1
- System Overview
- How Viability is Determined
- System Components

Chapter 2
- Starting the Software
- Connecting the Hardware

Chapter 3
- Getting Started
- Managing the Autosampler Queue
- Viewing Data

Chapter 4
- Vi-CELL XR Software Menus
- File Menu
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- Help
- Navigation Bar

Chapter 5
- Control Feature
- Creating And Managing Cell Types

Chapter 7
- Establishing an Electronic Record

Appendix A
- Data Acquisition
- Cell Viability/Concentration/Cell Count

Appendix E
- Decontamination Procedure

Issue BE, 10/17
Software version 2.06.2
Updates were made to the following sections:

CHAPTER 4, Software Menus
Types of Users

This document applies to the latest software listed and higher versions. When a subsequent software version affects the information in this document, a new issue will be released to the Beckman Coulter Web site. For labeling updates, go to www.beckmancoulter.com and download the latest version of the manual or system help for your instrument.

Safety Notice

Read all product manuals and consult with Beckman Coulter-trained personnel before attempting to operate instrument. Do not attempt to perform any procedure before carefully reading all instructions. Always follow product labeling and manufacturer's recommendations. If in doubt as to how to proceed in any situation, contact us.

Beckman Coulter, Inc. urges its customers to comply with all national health and safety standards such as the use of barrier protection. This may include, but is not limited to, protective eyewear, gloves, and suitable laboratory attire when operating or maintaining this or any other automated laboratory analyzer.

Alerts for Warning and Caution

Throughout this manual, you will see the appearance of these alerts for Warning and Caution conditions:

⚠ WARNING

WARNING indicates a potentially hazardous situation, which, if not avoided, could result in death or serious injury. May be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis.

⚠ CAUTION

CAUTION indicates a potentially hazardous situation, which, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices. May be used to indicate the possibility of erroneous data that could result in an incorrect diagnosis.

Safety Precautions

⚠ WARNING

California Proposition 65: This product can expose you to chemicals including phthalates, which are known to the State of California to cause cancer and birth defects or other reproductive harm. For more information go to www.P65Warnings.ca.gov.

⚠ WARNING

Risk of operator injury if:

- All doors covers and panels are not closed and secured in place prior to and during instrument operation.
- The integrity of safety interlocks and sensors is compromised.
- Instrument alarms and error messages are not acknowledged and acted upon.
- You contact moving parts.
- You mishandle broken parts.
- Doors, covers and panels are not opened, closed, removed and/or replaced with care.
- Improper tools are used for troubleshooting.

To avoid injury:

- Keep doors, covers and panels closed and secured in place while the instrument is in use.
- Take full advantage of the safety features of the instrument.
- Acknowledge and act upon instrument alarms and error messages.
- Keep away from moving parts.
- Report any broken parts to your Beckman Coulter Representative.
- Open/remove and close/replace doors, covers and panels with care.
- Use the proper tools when troubleshooting.

⚠ CAUTION

System integrity could be compromised and operational failures could occur if:

- This equipment is used in a manner other than specified. Operate the instrument as instructed in the product manuals.
- You introduce software that is not authorized by Beckman Coulter into your computer. Only operate your system's computer with software authorized by Beckman Coulter.
- You install software that is not an original copyrighted version. Only use software that is an original copyrighted version to prevent virus contamination.

⚠ CAUTION

If you purchased this product from anyone other than Beckman Coulter or an authorized Beckman Coulter distributor, and, it is not presently under a Beckman Coulter service maintenance agreement, Beckman Coulter cannot guarantee that the product is fitted with the most current mandatory engineering revisions or that you will receive the most current information bulletins concerning the product. If you purchased this product from a third party and would like further information concerning this topic, call your Beckman Coulter Representative.

Electrical Safety

To prevent electrically related injuries and property damage, properly inspect all electrical equipment prior to use and immediately report any electrical deficiencies. Contact us for any servicing of equipment requiring the removal of covers or panels.

High Voltage

This symbol indicates the potential of an electrical shock hazard existing from a high-voltage source and that all safety instructions should be read and understood before proceeding with the installation, maintenance, and servicing of all modules.

Do not remove system covers. To avoid electrical shock, use supplied power cords only.

Disposal of Electronic Equipment

It is important to understand and follow all laws regarding the safe and proper disposal of electrical instrumentation.

The symbol of a crossed-out wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates:

- That the device was put on the European Market after August 13, 2005 and
- That the device is not to be disposed via the municipal waste collection system of any member state of the European Union.

For products under the requirement of WEEE directive, please contact your dealer or local Beckman Coulter office for the proper decontamination information and take back program which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of device.

Moving Parts

⚠ WARNING

Risk of personal injury. To avoid injury due to moving parts, observe the following:

- Never attempt to exchange labware, reagents, or tools while the instrument is operating.
- Never attempt to physically restrict any of the moving components of the instrument.
- Keep the instrument work area clear to prevent obstruction of the movement.

Cleaning

Observe the cleaning procedures outlined in this user's manual for the instrument. Prior to cleaning equipment that has been exposed to hazardous material:

- Contact the appropriate Chemical and Biological Safety personnel.
- Review the Chemical and Biological Safety information in the user's manual.

Maintenance

Perform only the maintenance described in this manual. Maintenance other than that specified in this manual should be performed only by service engineers.

IMPORTANT It is your responsibility to decontaminate components of the instrument before requesting service by a Beckman Coulter Representative or returning parts to Beckman Coulter for repair. Beckman Coulter will NOT accept any items which have not been decontaminated where it is appropriate to do so. If any parts are returned, they must be enclosed in a sealed plastic bag stating that the contents are safe to handle and are not contaminated.

RoHS Notice

These labels and materials declaration table (the Table of Hazardous Substance's Name and Concentration) are to meet People's Republic of China Electronic Industry Standard SJ/ T11364-2006 "Marking for Control of Pollution Caused by Electronic Information Products" requirements.

RoHS Caution Label

This label indicates that the electronic information product contains certain toxic or hazardous substances. The center number is the Environmentally Friendly Use Period (EFUP) date, and indicates the number of calendar years the product can be in operation. Upon the expiration of the EFUP, the product must be immediately recycled. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.

RoHS Environmental Label

This label indicates that the electronic information product does not contain any toxic or hazardous substances. The center 'e' indicates the product is environmentally safe and does not have an Environmentally Friendly Use Period (EFUP) date. Therefore, it can safely be used indefinitely. The circling arrows indicate the product is recyclable. The date code on the label or product indicates the date of manufacture.

Safety Symbols

Safety symbols alert you to potentially dangerous conditions. The symbol applies to specific procedures and appears as needed throughout this manual.

Symbol Meanings	
	This symbol indicates the potential of an electrical shock hazard existing from a high-voltage source and that all safety instructions should be read and understood before proceeding with the installation, maintenance, and servicing of all modules. Do not remove system covers. To avoid electrical shock, use supplied power cords only and connect to properly grounded (three-holed) outlets.
	A "CE" mark indicates that a product has been assessed before being placed on the market, and has been found to meet European Union safety, health, and/or environmental protection requirements.
	Wear standard laboratory attire and follow safe laboratory procedures when handling any material in the laboratory.

Contents

Illustrations

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Introduction

Manual Description

This manual is intended to provide the user with information needed to operate and maintain the Vi-CELL XR system safely and effectively.

Conventions

This manual applies the following conventions:

- Menu and dialog items that can be selected or clicked appear in bold type.
- Blue text indicates that you can click on the text to access related information.
- Instrument may be used when referring to the Vi-CELL XR system.
- The terms “screen” and “window” are used interchangeably.

NOTE A Note is used to call attention to notable information that should be followed during installation, use or maintenance of this equipment.

IMPORTANT An IMPORTANT is used for comments that add value to the step or procedure being performed. Following the advice in the IMPORTANT adds benefit to the performance of a piece of equipment or to a process.

Safety

Hazardous Waste Precautions

Always observe local and state regulations regarding the handling and discarding of hazardous waste. Refer to the Material Safety Data Sheet for more information.

⚠ WARNING

Instrument will not be operated without the waste container and used cups receptacle in place; otherwise there is a potential for a hazardous waste spill.

If a hazardous substance such as blood or biological sample is spilled, clean up the spill by using your laboratory decontamination procedure. Then follow your laboratory procedure for disposal of hazardous materials.

WARNING

Risk of chemical injury from bleach. To avoid contact, use barrier protection, including protective eyewear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

Reagent Specific Precautions

Observe warnings on the packaging of Reagents (Vi-CELL Reagent Pak) and other materials as well as Material Safety Data Sheets.

Other Precautions

Warnings

Instrument must not be operated without Syringe Shield in place.

WARNING

If the equipment is used in a manner not specified by Beckman Coulter, Inc., the protection provided by the equipment may be impaired.

NOTE For Safety Data Sheets (SDS/MSDS) information, go to the Beckman Coulter website at www.beckmancoulter.com.

Figure 1 Vi-Cell XR

⚠ WARNING

Risk of personal injury from high voltage. High voltages are present inside the instrument. To prevent personal injury always disconnect the instrument from the power supply before removing the cover.

⚠ WARNING

Risk of biohazard contamination. Toxicity, safety, and proper handling procedures for diluents and reagents used should be adhered to at all times. To prevent biohazard contamination consult appropriate safety manuals, Safety Data Sheets and Material Safety Data Sheets for the items.

Cautions

⚠ CAUTION

Risk of damage to the computer or operating system. Switching off power first will generate a Windows error message at the next computer start up. To prevent damage to the computer or operating system use the Windows Shut Down button to turn the computer off before switching off power.

CHAPTER 1

Introducing the Vi-CELL XR

System Overview

This manual is intended to provide the user with information needed to operate and maintain the Vi-CELL XR system safely and effectively.

The Vi-CELL XR Cell Viability Analyzer is a video imaging system for analyzing yeast, insect and mammalian cells in culture media or in suspension. It automates the widely accepted trypan blue dye exclusion protocol and is designed to analyze a wide variety of cell types. The software includes features to monitor bioreactors and other cell culture processes and is designed to facilitate compliance with the US Food and Drug Administration's (FDA) regulations on electronic records and electronic signatures (21 CFR Part 11).

The main features of the system are:

- Cell Viability reported in percentage, concentration and cell count
- Concentration range of 50,000 to 10,000,000 cells per mL
- Cell size range of 3 microns to 70 microns

  NOTE Although the size range for system performance is 3 microns to 70 microns, the system is capable of detecting particles down to 2 microns.

- 12-position auto-sampler
- User-friendly reagent system
- The values displayed are all non-integer numerical results to a precision of three (3) significant digits on all screen displays, printed reports and spreadsheet files.

Measuring Viability And Cellular Parameters

Why Measure Viability?

The measurement of overall health of cell cultures requires accurate measurements of both cell concentration and percentage of viable or live cells. This data is essential to the decision making process for basic tissue culture cell growth and maintaining optimum culture conditions in bioreactors.

Historical Perspective – The Hemacytometer

Cell viability (Trypan Blue Dye Exclusion Method) determinations traditionally have been performed using a light microscope and hemacytometer. Unfortunately, this technique has

numerous major shortcomings. The hemacytometer has a significant repeatability error. Different technicians analyzing the same cell sample obtain variations in results. In addition, the manual method is tedious and quite time consuming for today's busy laboratory environment.

How Viability is Determined

The Trypan Blue Dye Exclusion Method

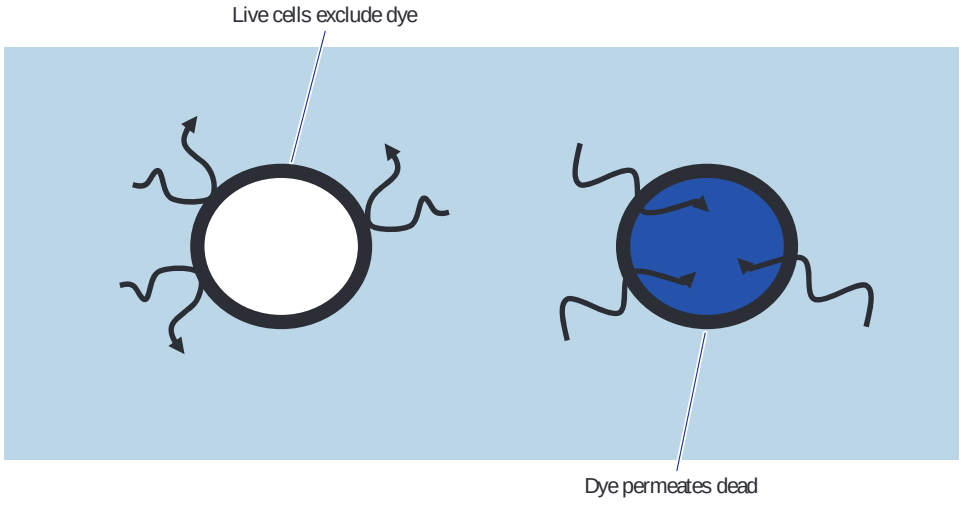
The widely accepted method for cell viability determination is the Trypan Blue Dye Exclusion Method. When cells die, their membranes become permeable allowing for the uptake of the trypan blue dye. As a result, the dead or non-viable cells become darker than the viable cells. This contrast is what is measured in order to determine viability.

An Image Analysis Solution

The Beckman Coulter Vi-CELL XR automates the Trypan Blue Dye Exclusion Method. Utilizing video capture technology and sample handling, the Vi-CELL XR takes the cell sample and delivers it to a flow cell and camera for imaging. The Vi-CELL XR will then capture up to 100 images for its determination of cellular viability.

The software determines which cells have absorbed trypan blue dye and those that have not. Cells absorbing the trypan blue dye appear darker hence have lower gray scale values. Cells with higher gray scale values are considered viable.

Figure 1.1 Trypan Blue Dye Exclusion Method



System Components

The following image describes the main components of the Vi-CELL XR Cell Viability Analyzer.

Figure 1.2 Front View of The Vi-CELL XR Analyzer

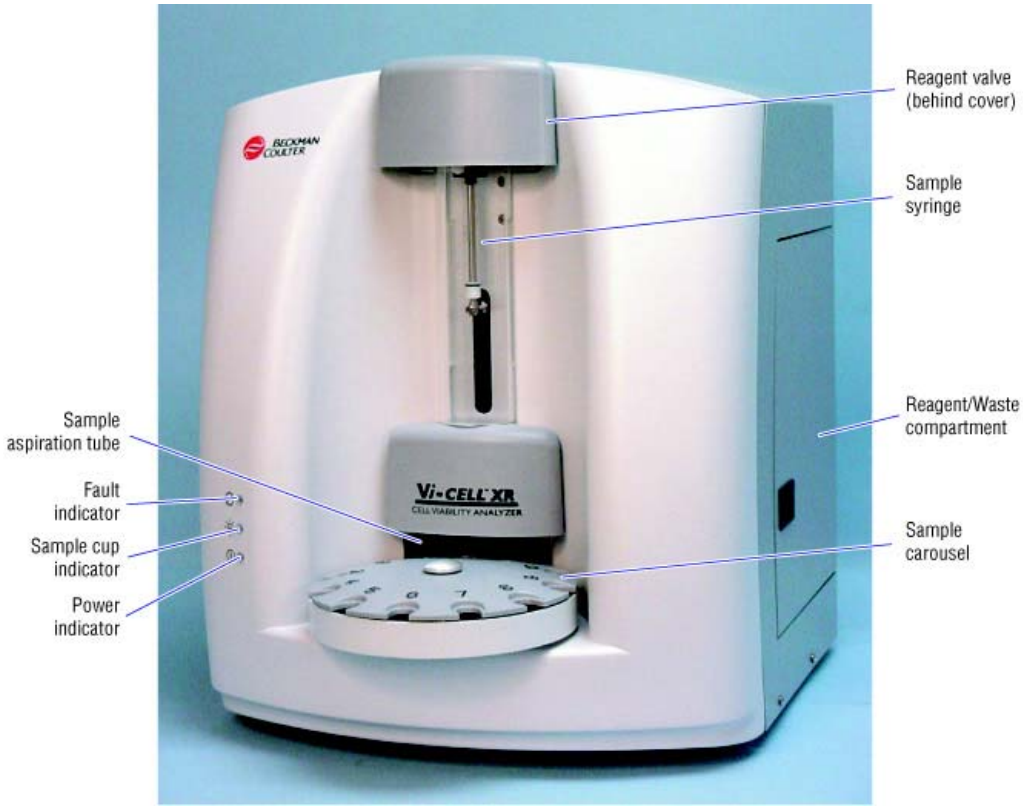
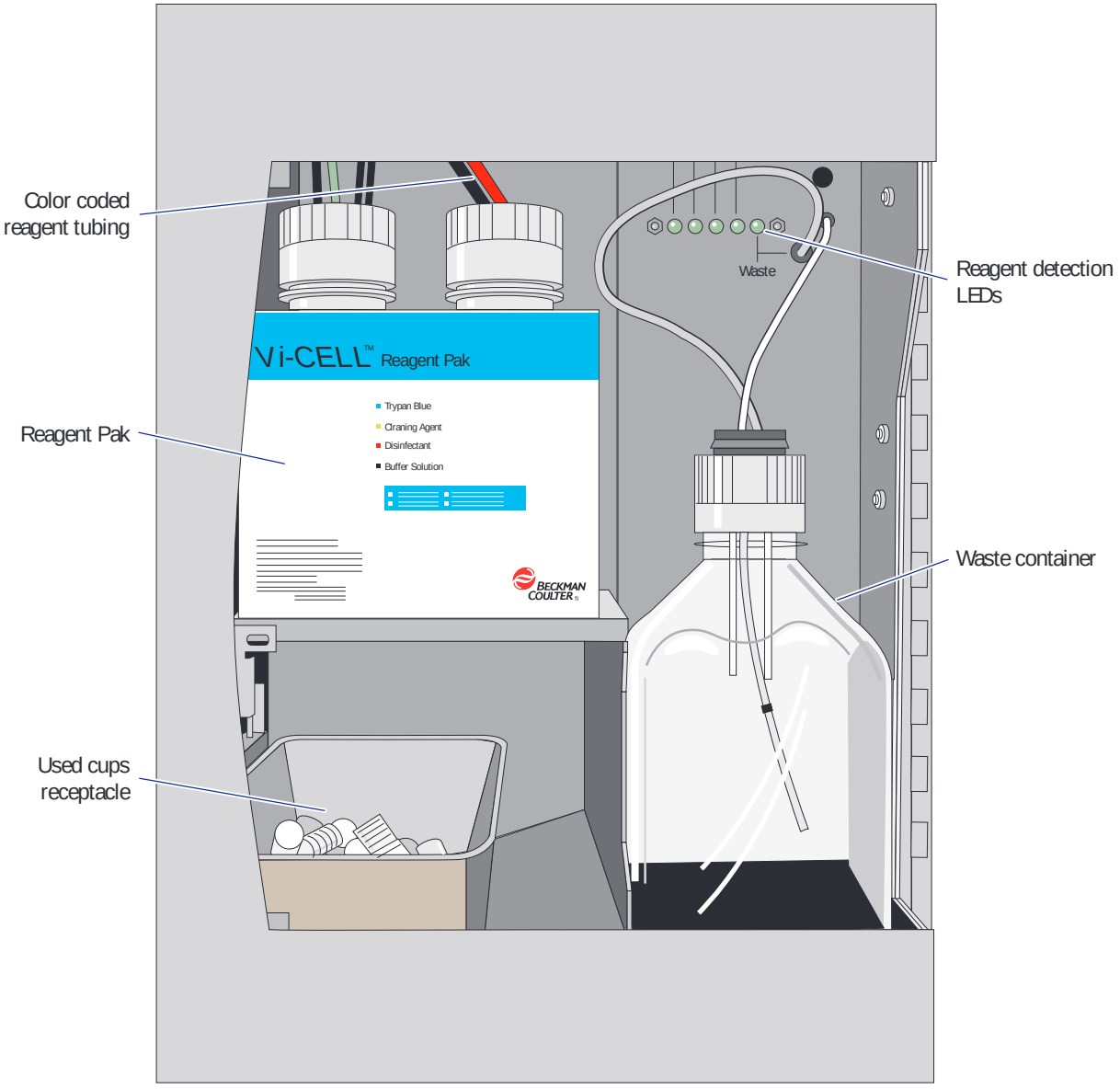


Figure 1.3 Right Side of the Vi-CELL XR Showing Reagent Compartment



Sample Delivery Options

The Vi-CELL XR Cell Viability Analyzer represents the premier model in the Vi-CELL family of products.

Computer System

The computer system requirements used to validate the Vi-CELL XR software application using Windows 7 Operating System are as follows:

- **Operating System**: Windows 7 Professional 32-bit English
- **Ram**: 4 GB
- **Hard Drive**: 500 GB
- **DVD Drive**: DVD Recordable 8X Max Dual Layer
- **Monitor**: XGA
- **Graphics Card**: Intel Graphics Media Accelerator HD, (1280 x 1024)
- **Host Controller**: OHCI compatible IEEE - 1394 FireWire card

Software

Beckman Coulter provides the Vi-CELL XR software and operating system software where PCs are supplied.

CHAPTER 2

Installation and Verification

Introduction

The Vi-CELL XR will be installed only by Beckman Coulter approved and trained installation engineers. Unless otherwise agreed to by Beckman Coulter, DO NOT UNPACK the Vi-CELL XR.

Special Requirements – Pre-installation Checks

Environment

The instrument should be placed on a surface that is not subject to:

1. Excessive airborne dust
2. Strong vibrations
3. Extremes of temperature and humidity

Power Requirements

- Power: 50 watts (65 watts max.)
- Voltages: 100, 120, 220, 240 VAC 50/ 60 Hz

Temperature and Humidity Requirements

- Temperature: 10 to 40°C (50 to 104°F)
- Humidity: 10 to 85%

Starting the Software

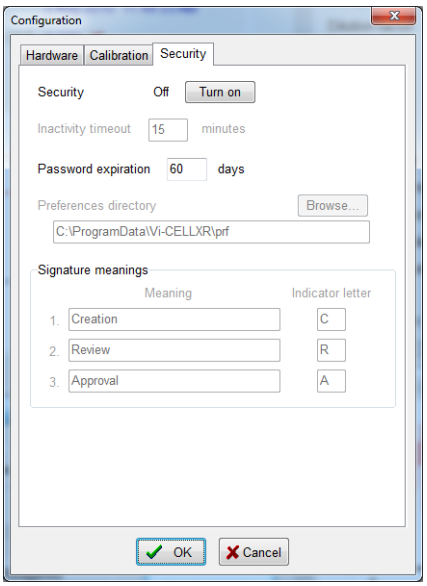
Double-clicking on Vi-CELL XR software icon on the desktop will launch the program. When the Vi-CELL XR program is run for the first time, the configuration and preferences should be verified.

Select File > Configuration to access the Configuration dialog box.

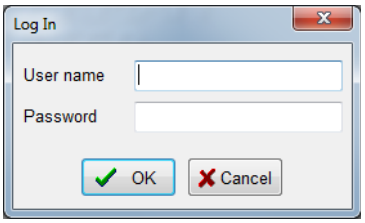
Select File > Preferences to access the Preferences dialog box.

Security Configuration

Selecting the Turn On button in the security configuration dialog box turns on security. A valid Administrator name and password must then be entered to turn on security.



With security on, the instrument has been designed to facilitate compliance with the US Food and Drug Administration's (FDA) regulations on electronic records and electronic signatures (21 CFR Part 11). Vi-CELL XR run files have been designed to meet the requirements for electronic records to be submitted to the FDA in electronic form. The security tab allows for signature meanings to be defined.



Once signed on, select Security > Add New User from the Menu Bar to define users and access levels. The Add New User dialog box is displayed.

Figure 2.1 The Security Options Screen

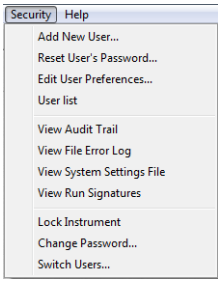
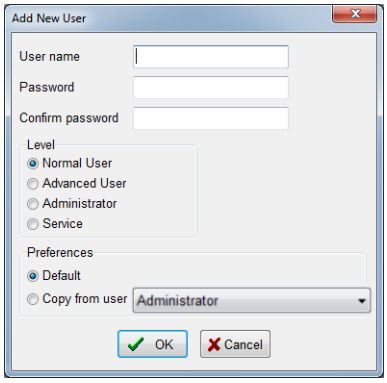


Figure 2.2 Add New User



Refer to Change Password for user name and password requirements.

Setting Up Preferences

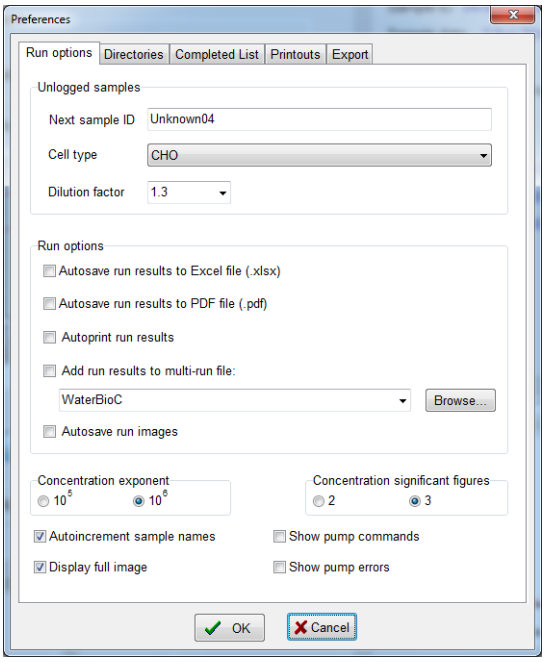
Initial setup of the software is performed within the Preferences dialog box. Directories are where data, images, data mirroring, and export data are to be stored are defined. Saving of images to memory, auto-increment of sample names, auto save of images, auto print and auto save to Excel format are also selectable.

NOTE Auto save of images, auto print and auto save to Excel format options are also available in the Log In Sample dialog box by checking the radio buttons provided.

Run Options Tab

The Run options tab provides information related to Unlogged samples, Run options, Concentration exponent and Concentration Significant figures. Unlogged Samples section allows for the selection of the Cell Type to be used as well as the default dilution factor to be applied to samples that are introduced to the system without logging them in first. There are checkboxes under Run options to Autosave run results to Excel file, Autosave run results to PDF file, Autoprint run results, Add run

results to multi-run file, and to Autosave run images. There are also checkboxes for Autoincrement sample names, Display full image, Show pump commands and Show pump errors.



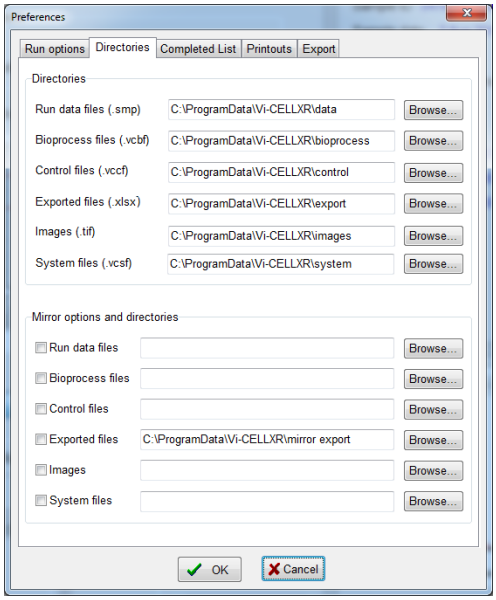
Autoincrement sample names should be selected if the system is required to automatically create new suggested sample names by incrementing the numeric postfix of a previous sample name.

The default printer is defined within the Configuration dialog box. A Beckman Coulter Representative will configure hardware and calibration.

Directories Tab

Select the Directories tab to check that the directory information is acceptable.

NOTE While mirror paths can be changed, it is strongly recommended that default directories are not changed.

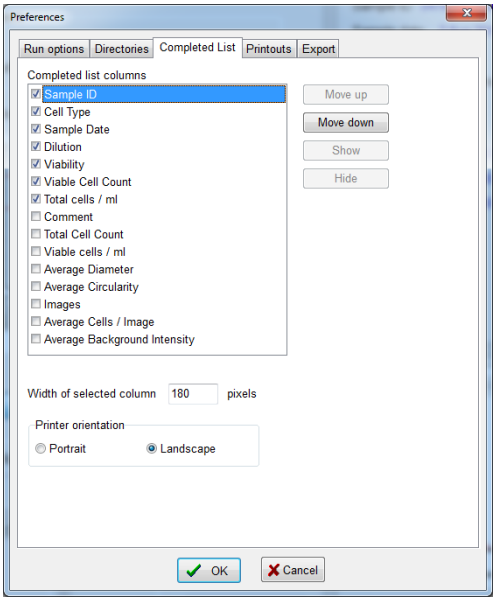


The data directory shows the directory where run, bioprocess and control files are to be stored. The images directory indicates where camera images are to be stored. The export directory shows where the run results in Excel format are to be stored. The System files directory shows where the Vi-Cell system files will be stored.

On this same tab, there is also provision for selecting mirror directories if connected to a server or network. This allows for the safe keeping of data or images by saving information to another location such as a network drive. To select, check off the desired selections and provide a path or address as to where the information is to be sent.

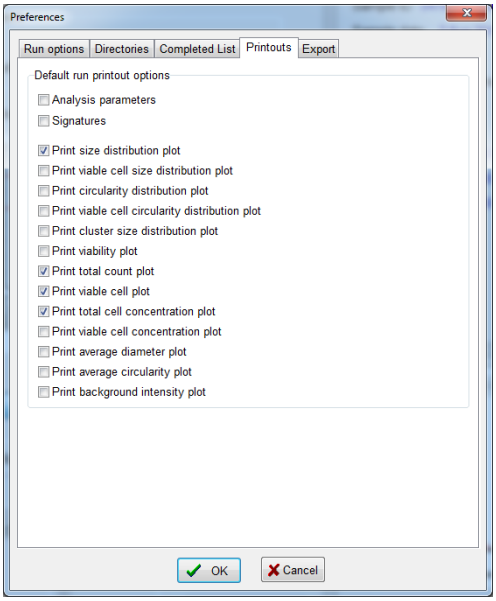
Completed List Tab

Select the Completed List tab to choose which sample results you want to display in the Completed List area of the Auto Sampler Queue. You can also specify Printer Orientation on this tab.



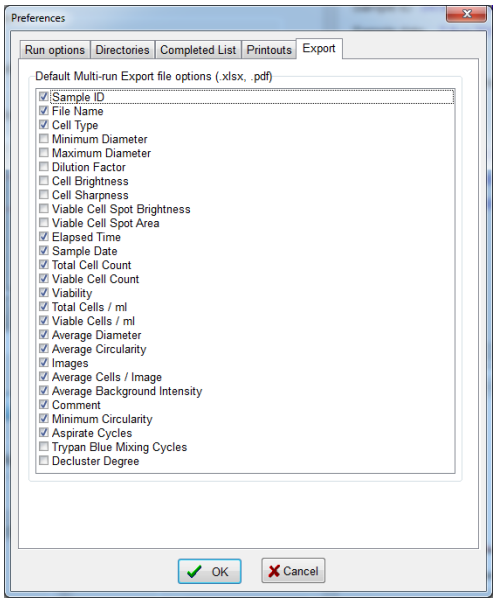
Printouts Tab

The Printout tab allows for the selection of plots to appear on printed reports. These same options may also be selected from any of the print functions available throughout the software.



Export Tab

The Export tab allows for the selection of parameters to be exported in Excel (xlsx) format.



Connecting the Hardware

IMPORTANT Ensure all connections between the computer workstation and the Vi-CELL analyzer are firm and connected.

1 Connect the FireWire video capture cable from the Vi-CELL XR to the computer workstation. See Figure 2.3 for correct connection to the computer.

2 Connect the serial cable from the serial connection on the back of the computer workstation to the serial connection on the Vi-CELL XR instrument.

Figure 2.3 Connections from Computer to Vi-CELL XR

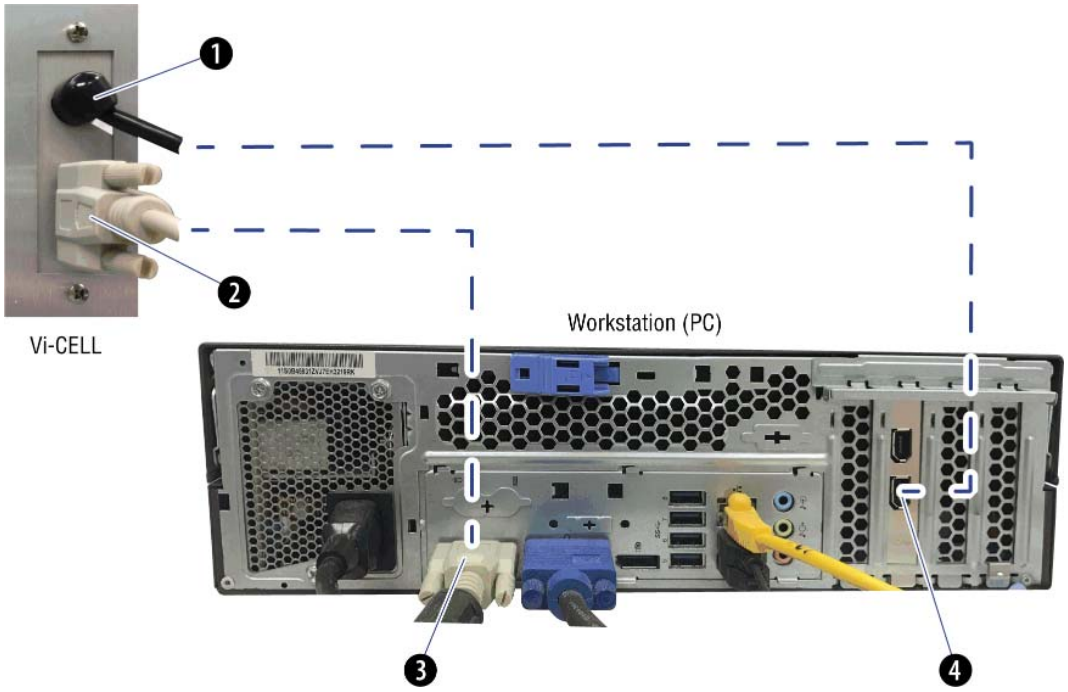


Table 2.1 Hardware connections

1	Vi-CELL FireWire connection	3	Workstation serial connection
2	Vi-CELL serial connection	4	Workstation FireWire connection

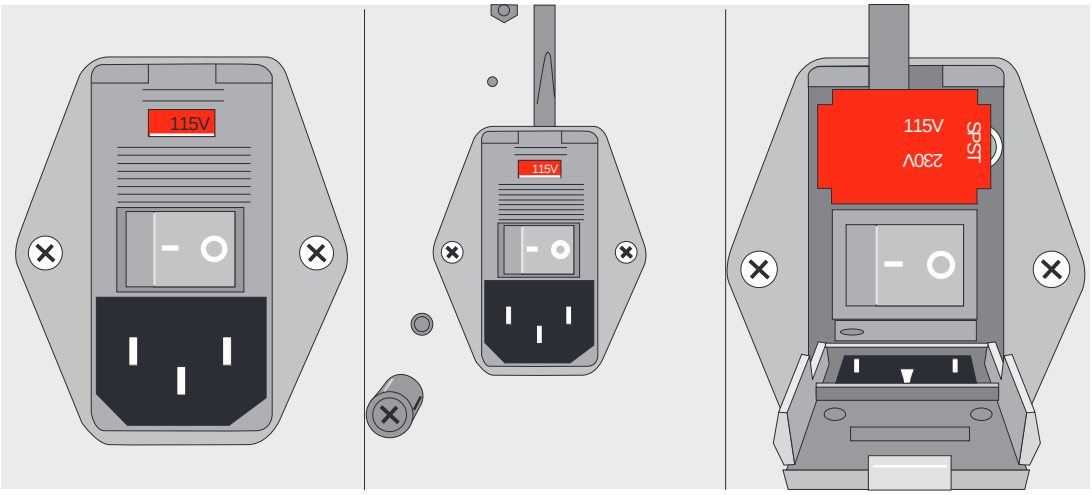
Fuse Installation and Voltage Adjustment

⚠ CAUTION

Always disconnect power from the unit before attempting power adjustment or fuse replacement.

1 Insert a screwdriver at the top of the power switch and fuse holder. With a slight twist of the screwdriver, open the fuse holder exposing the fuse.

Figure 2.4 Power Switch and connection and voltage adjustment



2 Insert the screwdriver at the top of the fuse holder, and again with a slight twist pop out the fuse holder. The voltage selected is the one showing at the top.

a. For 115 Volts, orient the fuse holder with the 115 V at the top.

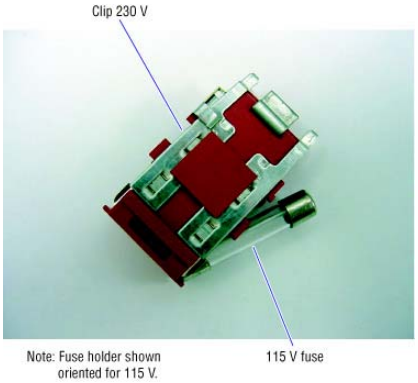
b. Install fuse on the right hand side with appropriate size and rating. (See label on back of Vi-CELL XR or specifications section for fuse rating and quantity).

3 Insert fuse holder and close fuse cover.

4 For 230 Volts power setting, remove fuse holder as in steps 1 and 2.

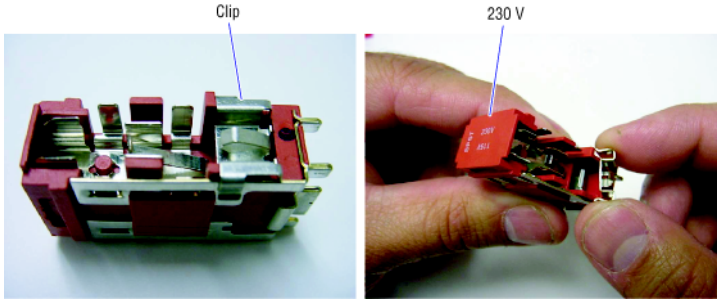
a. Orient the fuse holder with 230 V at the top.

Figure 2.5 Fuse Holder



b. Remove the clip on the right side of the fuse holder (see images in Figure 2.5 and Figure 2.6) towards the back of the fuse holder.

Figure 2.6 Fuse holder and clip and removal of clip



c. Save the clip in a secure place. Insert fuses on both sides of the fuse holder (See label on back of Vi-CELL XR for fuse rating and quantity).

5 Make certain the power cables are installed on both the PC and Vi-CELL XR and the voltage adjustment on the power switch is set according to the power requirements for your country.

Instrument Performance Verification

Post-installation Verification

A Beckman Coulter Representative will perform post installation verification checks.

Daily Verification

A control should be run daily to ensure proper instrument performance. The Beckman Coulter Vi-CELL Concentration Control, PN 175478, has been developed for this purpose.

If the concentration control does not meet the results listed in the Vi-CELL Concentration Control assay sheet:

- Ensure that the concentration control cell type is selected in the Vi-CELL XR software when running concentration control.
- Verify that the concentration control did not freeze at any time. Freezing results in fragmentation and increased counts. See the Vi-CELL Concentration Control assay sheet for the recommended storage temperatures.
- Ensure that the user dispenses enough sample into the sample cup. The Vi-CELL XR requires a minimum of 0.5 mL.
- Ensure that the concentration control is handled properly, as proper mixing is crucial to recovery results. Mix the concentration control per the Vi-CELL Concentration Control assay sheet instructions. Do not leave the bottle uncovered for extended periods time as this affects the concentration.

- If there is debris inside the flow cell, perform the decontamination procedure in APPENDIX E, Maintaining The Vi-CELL XR to ensure that the internal components are clean, as debris can affect results. If the debris does not clear after two decontamination attempts, contact us.
- Try a new, sealed bottle of concentration control. If after two attempts the concentration control does not meet the results specified in the Vi-CELL Concentration Control assay sheet, contact us.

CHAPTER 3

Quick Start Guide

Getting Started

⚠ WARNING

Ensure all doors, covers and panels are closed and secured in place prior to and during instrument operation.

Starting the Instrument and Launching the Software

Once connections have been established and the instrument and computer are powered on, double-click on the Vi-CELL XR software icon on the desktop to launch the software. Upon startup you should hear the pump initialize with the instrument going into "idle" mode.

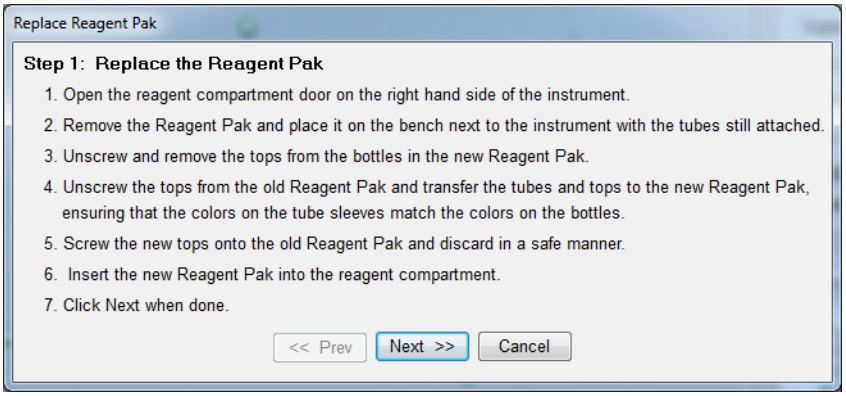
Installing Reagent Pak

1 On the main screen toolbar, select Instrument > Replace Reagent Pak.

IMPORTANT Make certain reagent lines are installed correctly (color coding). Improper installation may result in erroneous results.

2 Open the reagent compartment on the right side of the instrument and install the Vi-CELL Reagent Pak as well as the waste container and cup receptacle. The reagent lines and reagent pack are color coded for easy installation. Follow the instructions in Replace Reagent Pak screen 1 below.

Figure 3.1 Replace Reagent Pak screen 1



3 Attach color-coded tubing to reagent pack.

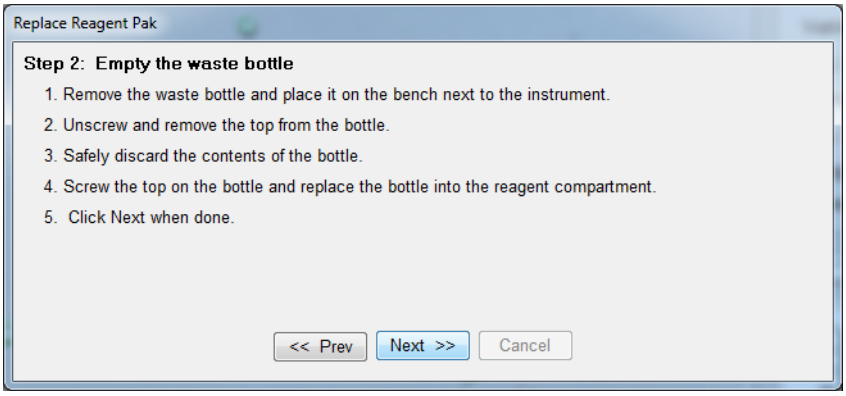
Figure 3.2 Color-coded Tubing

4 Place reagent pack inside the Vi-CELL XR reagent compartment.

Figure 3.3 Reagent Pack Placement

5 Follow the instructions in Replace Reagent Pak screen 2 below to empty and replace the waste bottle.

Figure 3.4 Replace Reagent Pak screen 2



⚠ WARNING

Risk of a hazardous waste spill if you operate the instrument without the waste bottle and used cups receptacle in place. To prevent the potential for a hazardous waste spill, always operate the instrument with the waste container and used cups receptacle in place.

If a hazardous substance such as blood or biological sample is spilled, clean up the spill by using your laboratory decontamination procedure. Then follow your laboratory procedure for disposal of hazardous materials.

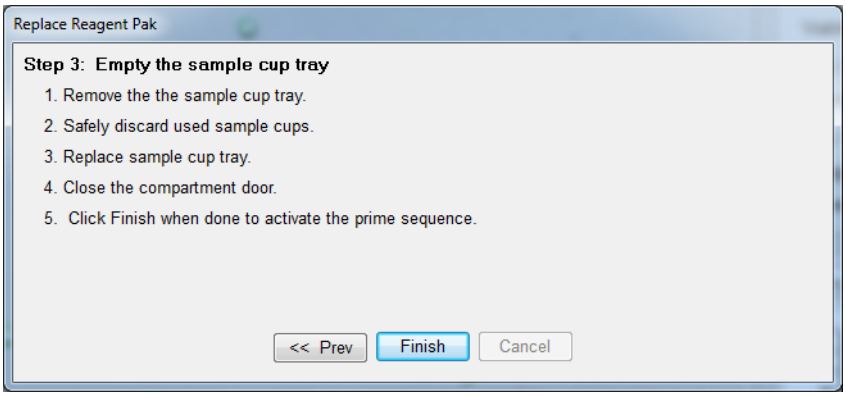
⚠ WARNING

Risk of chemical injury from bleach. To avoid contact with the bleach, use barrier protection, including protective eyewear, gloves, and suitable laboratory attire. Refer to the Safety Data Sheet for details about chemical exposure before using the chemical.

Figure 3.5 Waste Line Attachment

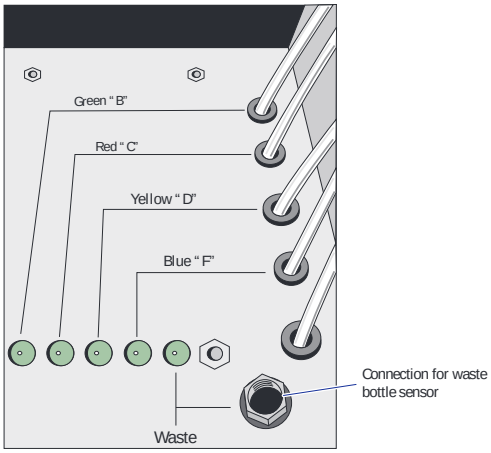
6 Follow the instructions in Replace Reagent Pak screen 3 below to empty the sample cup tray and initiate the prime sequence. This will prime all lines with reagent and prepare the unit for sample analysis. The reagent level meter will reset to maximum number of 218 runs.

Figure 3.6 Replace Reagent Pak screen 3



7 To verify reagent lines are primed and the unit is ready for analysis, check the five LED's inside the reagent compartment. Make certain they are all illuminated. If any of the LED's are not illuminated, select Instrument > Prime from the main menu bar.

Figure 3.7 Reagent LED's



The following describe the LEDs from left to right as they appear on the analyzer.

- Green – Buffer Solution
- Red – Disinfectant
- Yellow – Cleaning Agent
- Blue – Trypan Blue Reagent
- Waste

Log In Sample

Sample cups can be added to the carousel and logged in at any time enabling the instrument to run continuously and maximizing the sample throughput.

While the Log in sample dialog is open, the carousel will not advance to the next position so it is recommended that samples be placed in the carousel while the Log in sample dialog is open.

1 Place a minimum of 0.5 mL (max. 2.5 mL) of sample into a sample cup.

2 Log in samples by selecting the **Log in sample** button.

a. Select sample cup position on the carousel (if applicable).

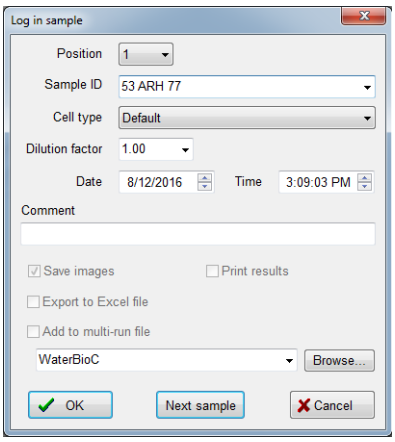
b. Enter **Sample ID**. Sample IDs can be up to 32 characters long and contain any characters except for the following: ([ ]() = * , ./ \ or :).

c. Choose a **Cell type**.

d. Select **Dilution factor** if pre-diluted.

e. Select **OK**.

Figure 3.8 Log in sample dialog



If necessary, you can change the date to the time that the sample was removed from the bioreactor if that would be more appropriate. Changing the date on the Log in sample dialog only applies to the Bioprocess.

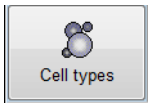
3 Place sample cup in next available carousel position as indicated on the Log In Sample screen.

4 Press **Start queue** to begin the analysis.

Selecting a Cell Type

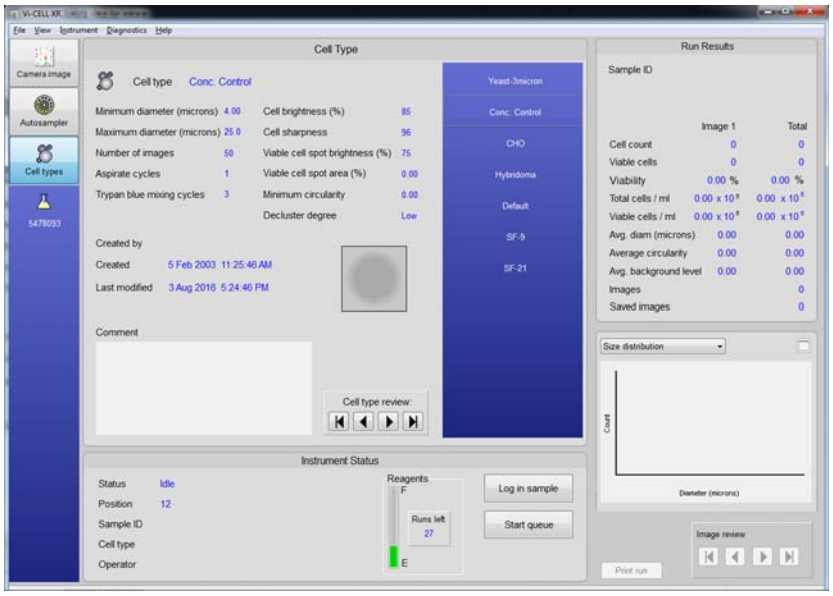
When logging in a sample, select the appropriate cell type. If a cell type does not exist, create a cell type as in Creating And Managing Cell Types in CHAPTER 5, Special Software Features. Cell types may be viewed by selecting the cell type icon on the navigation bar.

Cell Type Icon



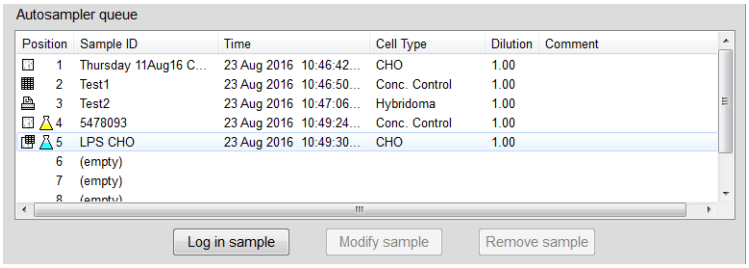
Each cell type provides pre-defined instrument and measurement parameters for ensuring accurate analysis results.

Figure 3.9 Cell Type Screen



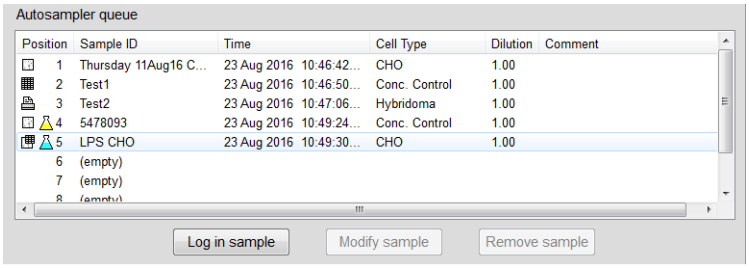
Managing the Autosampler Queue

For Bioprocess and Sample files, you can log in a sample by selecting the Log in sample button, by selecting Instrument > Log in Sample or by double-clicking on a particular sample queue position. There are two Log in sample buttons - one in the instrument status pane, and one at the base of the AutoSampler queue. There is a Log In Run available in the Bioprocess Screen as well. If the analysis is for a Bioprocess, the bioprocess icon appears to the left of the sample position.



NOTE The Sample IDs can be a maximum 32 characters.

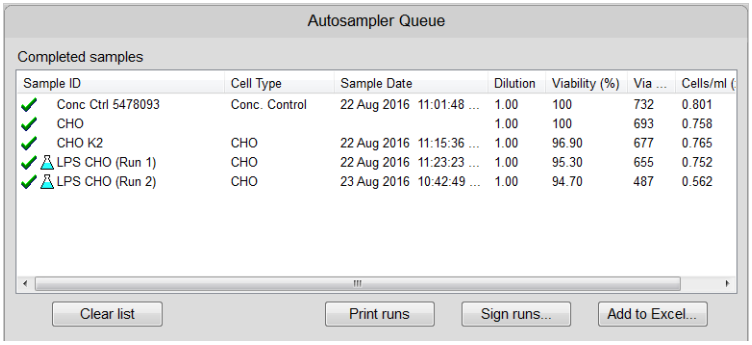
For Control files, you can log in a control by selecting the Log in new run button on the Control screen.



If during log in the check boxes for Save Images, Printing or Exporting to Excel are checked, icons will appear to the left side of the sample position on the sample queue denoting each function.

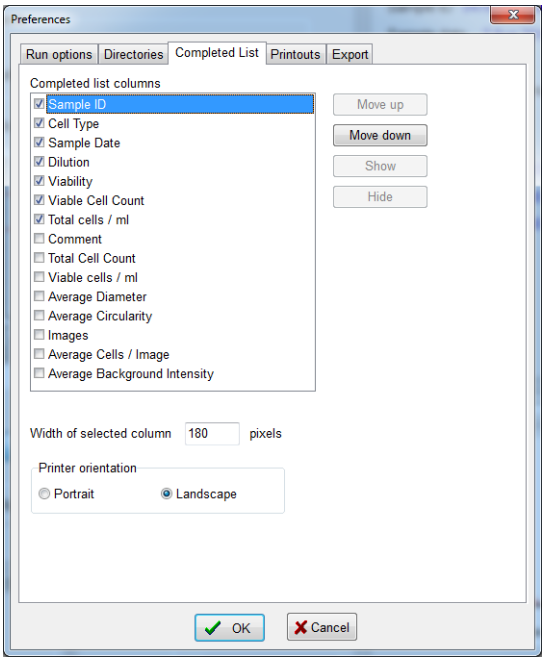
The completed samples window (above the Autosampler queue log in list) displays completed samples along with results. A check mark will appear indicating completion of analysis. A yellow X will indicate either the lower or upper limit of count detection has been reached or exceeded. A red X will indicate the control value is out of range.

NOTE The red and yellow X are only available on software version 2.04.



Double-clicking on a given completed sample will show the results under the current results window on the right hand side of the screen and open the linked images, if the images were saved, generated at the time of the run.

Selecting Clear list will clear the Completed Samples List of all runs. For printing a list of completed samples, select the Print list button. In order to print all runs on the completed list, select Print runs. There are also functions for signing runs, Sign runs (21 CFR Part 11) or Add to Excel.



The information displayed in the completed list can be chosen and arranged in a particular order using the Completed List tab in the Preferences dialog box that is accessed by selecting File, Preferences and then Completed list. The list can be reordered by selecting a line item, and using the Move Up and Move Down buttons.

Log In Samples and Performing Sample Analysis

1 Place a minimum of 0.5 mL (max 2.5 mL) of sample into a sample cup and place in the next available carousel position. The volume does not have to be precise.

2 Log in samples by selecting the Log in sample function, or selecting Instrument > Log in Sample, or by selecting the auto-sampler queue icon  on the navigation bar and then selecting Log in sample from the Autosampler Queue.

a. Select sample cup position on the carousel (if applicable).

b. Enter Sample ID. Cannot use these special characters [ ] = . , : / "". The maximum number of characters is 14 numeric or 18 alphabetic.

c. Choose a Cell type. The default cell type may be used to run the sample, and then revised to create a new cell type, if needed.

NOTE If none exists create a cell type as in Creating And Managing Cell Types in CHAPTER 5, Special Software Features. If a bioprocess, select Bioprocess name from Sample ID drop down list. Otherwise create a bioprocess by selecting File > New Bioprocess (see CHAPTER 5, Special Software Features).

d. Enter the correct Dilution factor if pre-diluted.

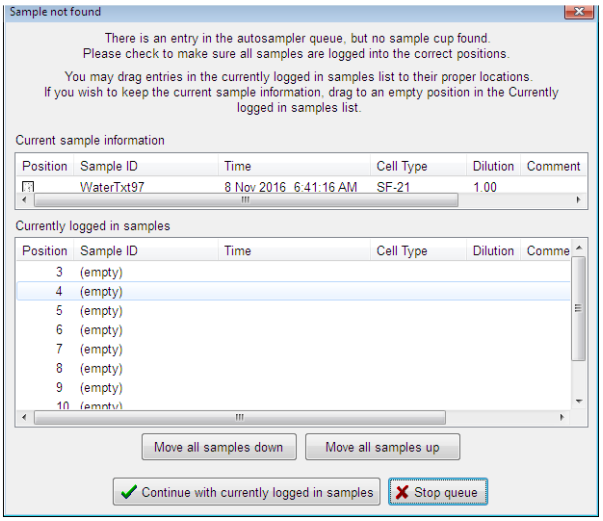
e. Select OK.

3 If logging in sample, using the Log in sample button, go to the auto-sampler queue to check your sample is logged in correctly. Double-click on the sample Position within the auto-sampler queue to modify sample information.

4 Place the sample cup onto the sample carousel at the corresponding position according to the sample queue.

5 Press Start queue to begin the analysis.

If a sample is entered in the queue and the instrument finds that the sample is missing, an audible alarm is emitted, a warning message is displayed, and the queue is stopped.



On this window, there are the options to either move the sample up or down in the queue, continue as logged or stop the queue.

Viewing Data

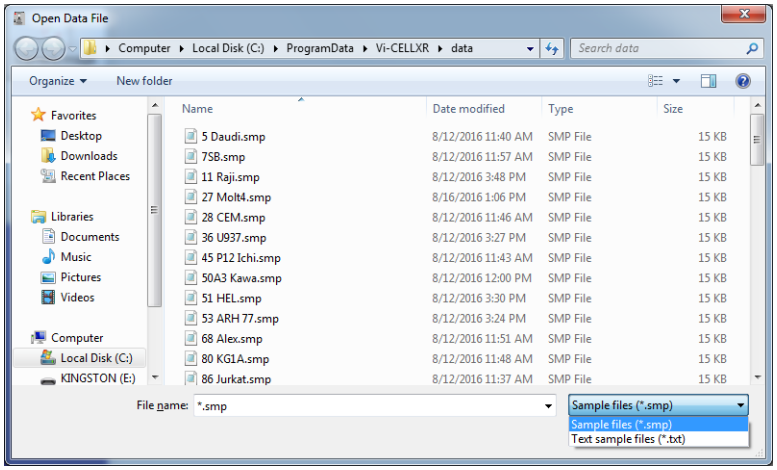
Managing Data Output

Opening a Run

To open previously saved data, on the main menu, select File > Open Run. To open any file, select the .txt or .smp file and select Open.

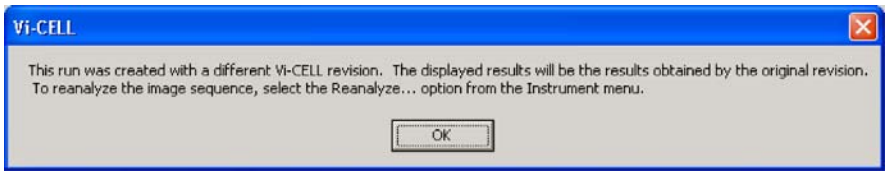
NOTE You must click on the .txt or .smp file to open the run.

NOTE The Vi-CELL XR software can open existing .txt files generated by previous software versions.



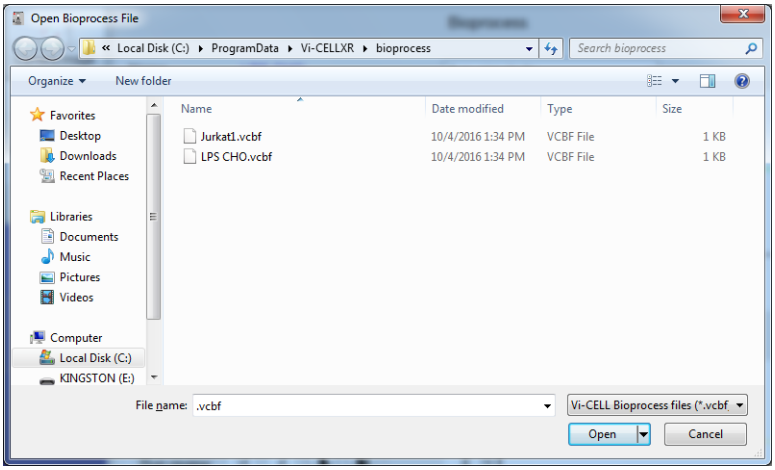
If images exist for the data, the run can reanalyze with a newer version using the Reanalyze function under the instrument menu. The run results will then be updated and images annotated.

NOTE To view images attached to the .txt file, the image folder must be saved in the following folder: C:\Vi-CELL\images.



Opening a Bioprocess file

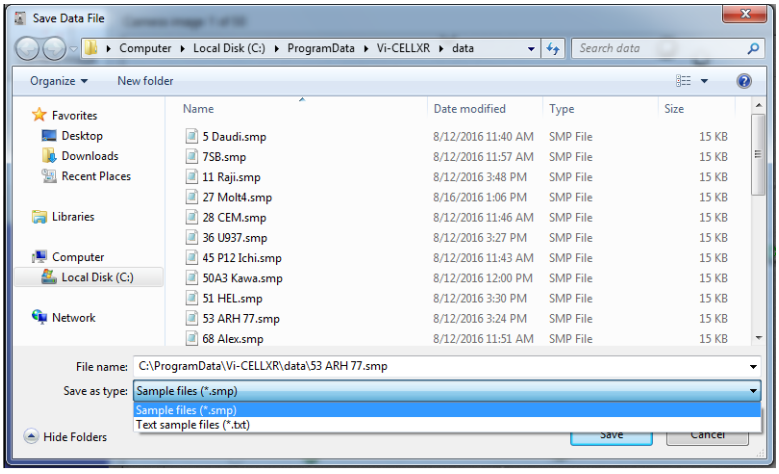
To open a previously saved Bioprocess file, from the main menu, select File > Open Bioprocess. The Open Bioprocess File dialog box is displayed. Select file and select Open.



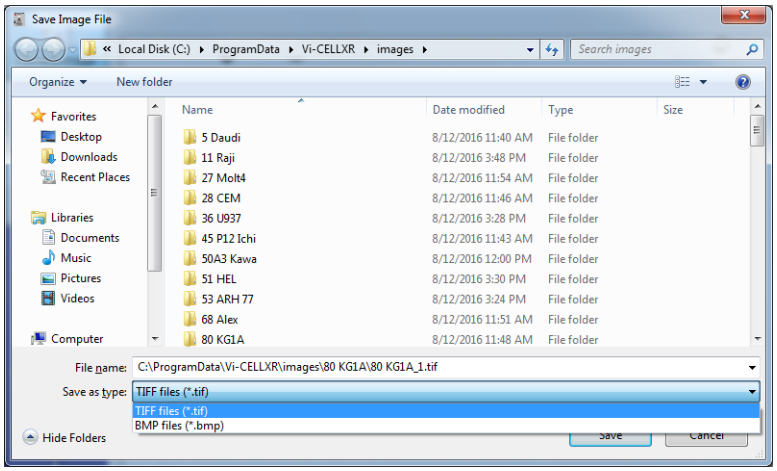
Saving Images and Data

To save a run, from the main menu, select File > Save Run. The Save Data File dialog box will appear. Enter a file name and select Save.

NOTE The data and image files must have the same name or the link between the data and images will be broken, and the system will not be able to open the saved run, link images and results.

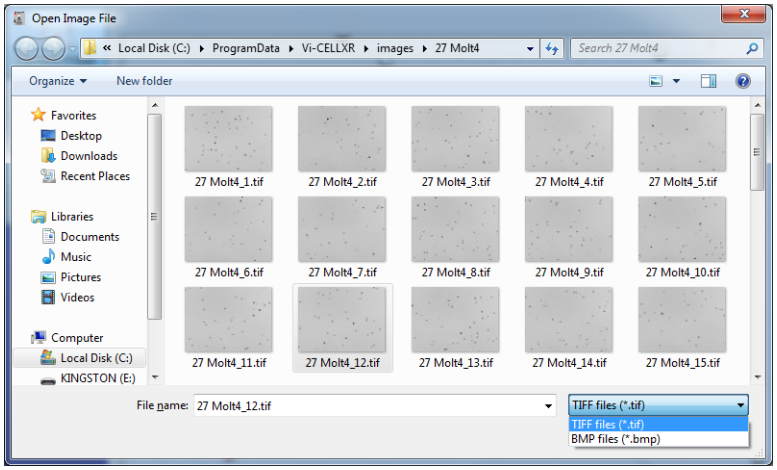


Another dialog box, Save Run Images will appear. You can either select, Save run only, Save run and images or cancel. Directories may also be specified.



Open an Image

To open a single image for review, from the main menu, select File > Image > Open. The Open Image File dialog box will display. Select a folder and double click to access the images. Select an image and select Open. The system is capable of opening both .bmp and .tiff image files.

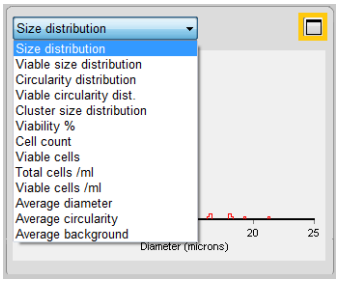


Closing an image

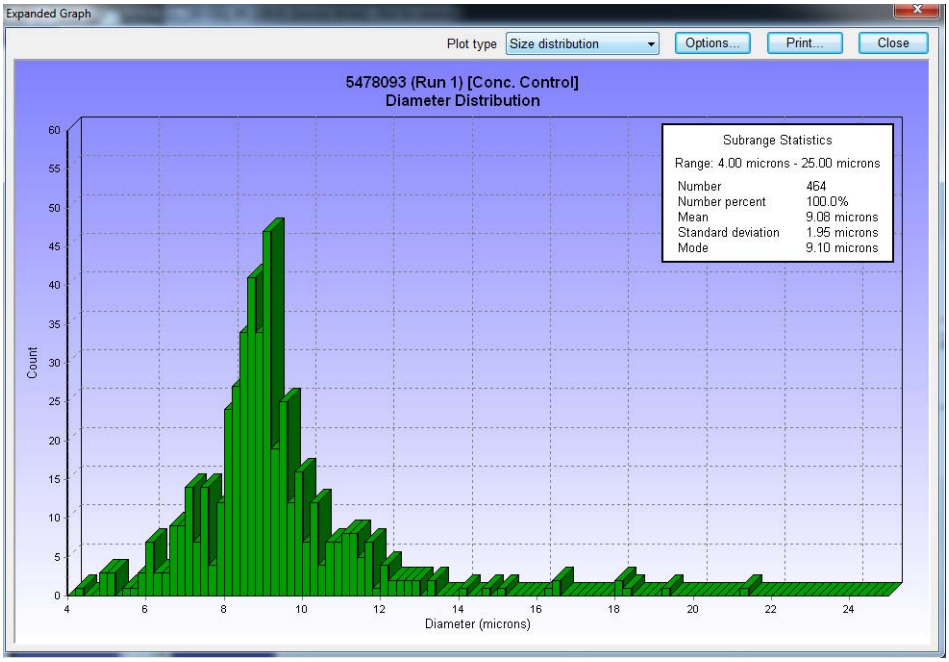
From the main menu, select File > Image > Close.

Data Plots

By selecting from the drop down window on the results section of the main screen, data can be viewed graphically.



Once a plot is selected, selecting the button to the right side of the drop down will expand it. To close the plot, select **Close**. Plots within the **Bioprocess** screen may also be expanded.

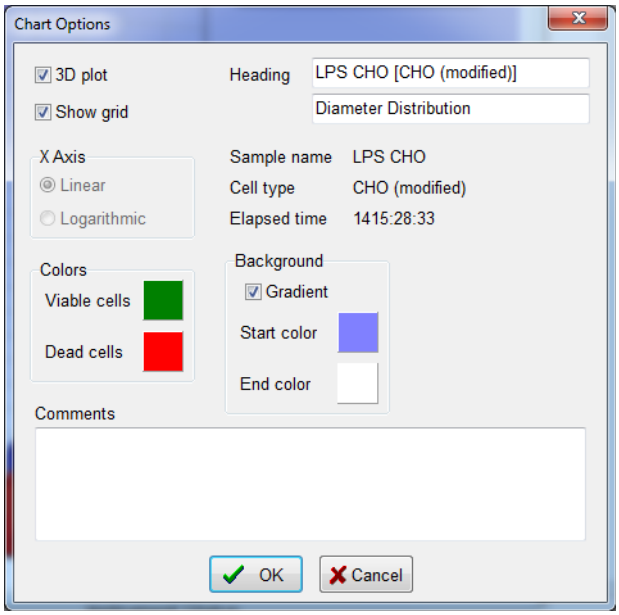


Once a plot has been expanded, statistics can be viewed as well as printed. Various plots can also be viewed from an expanded plot. Just select plot to be displayed from the drop down options.

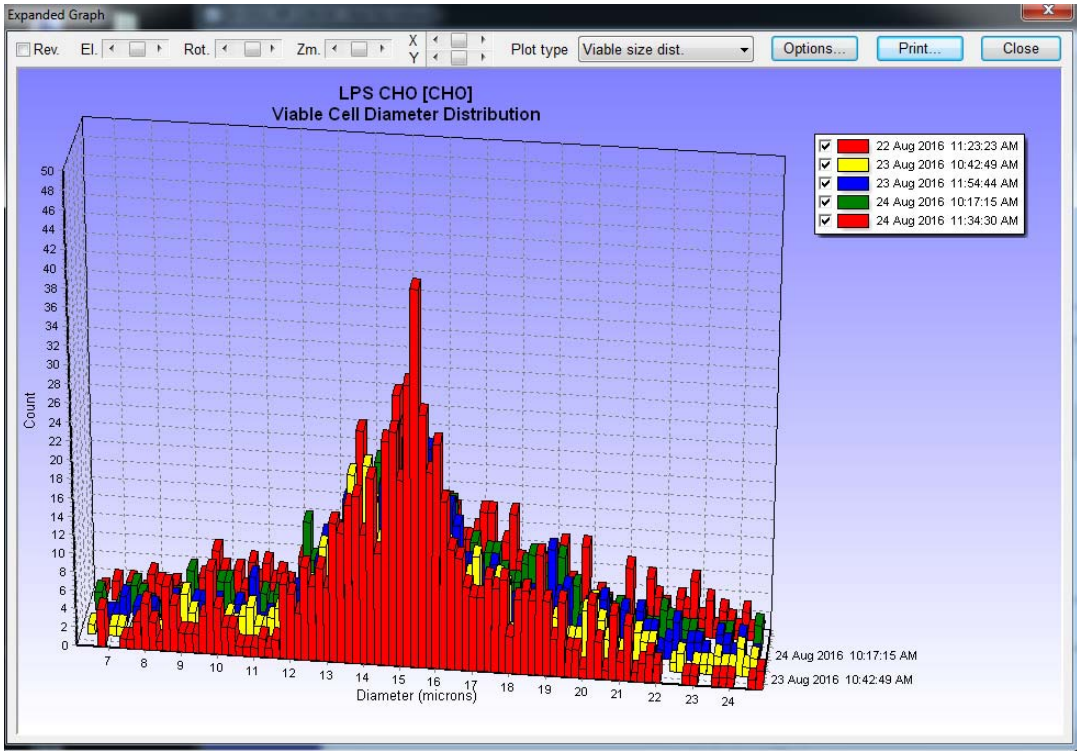
Cursors are provided in order to isolate a particular region of a distribution. Click on the plot at one end of the desired subrange, drag to the other end of the subrange, and release the mouse. The "Subrange statistics" display will reflect the region of interest between the two cursor lines.

Only the Size distribution, viable size, circular distribution and viable circular distributions provide cursors for viewing statistics on a particular region of a plot. All other plots will provide a single cursor for selecting a given image and displays data related to that image.

The Chart option dialog box allows you to change the appearance of the expanded graph and to add a comment that will be appended to the graph print out.

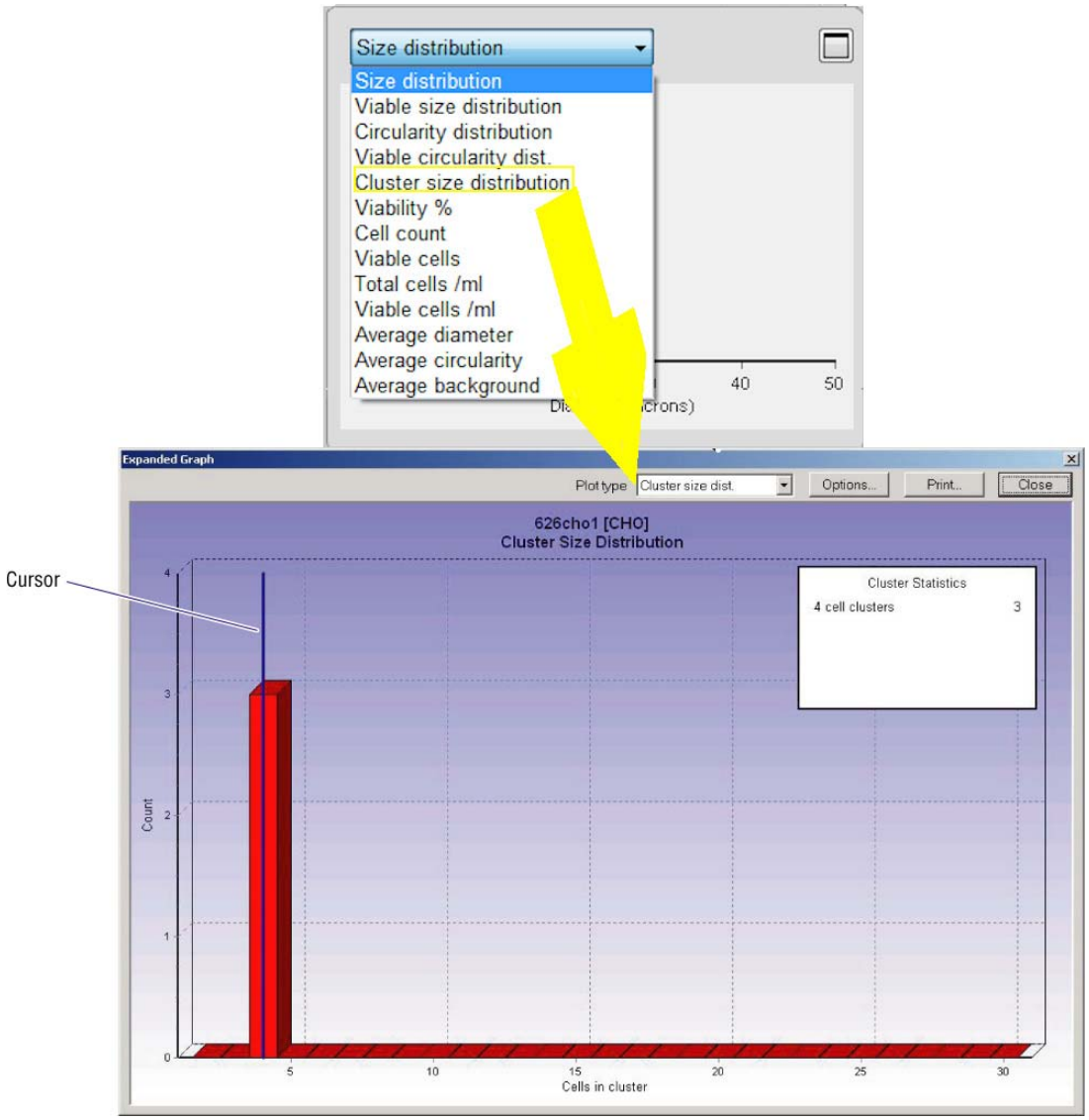


Bioprocess data can be viewed as 3-Dplots. Utilizing the Rot function rotates plots. Utilizing the El function changes the plot elevation. These functions are located on the tool bar at the top of the graph. Azoom function is also provided to modify the size of the graph.



Cluster Size Distribution

The number of cell clusters within a given analysis can be viewed by selecting cluster size distribution from the plot drop down window. From this distribution, the number of cell clusters as well as the number of cells per cluster may be viewed. By clicking on the plot, a cursor appears which allows the viewing of statistics for a given point on the distribution.



Printing

After requesting any print command, a Print Results dialog box appears before printing occurs. Select Cancel or press Esc to Cancel the print command.

Printing a Report

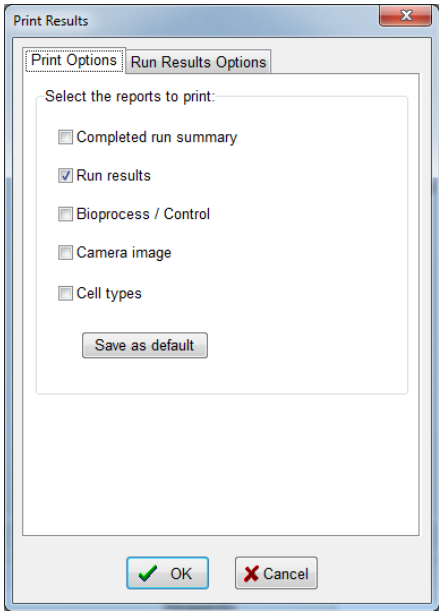
To print a report, on the main menu select File, Print. The Print Results dialog box appears.

Print Options Tab

To select the items to appear on the printed report, check any of the boxes corresponding to the item that is to appear on the printed report.

Selecting the Run results check box will cause another tab to appear on the print results dialog box called Run Results Options. Select Save as default to keep your settings.

Figure 3.10 Run Results Print



Run Results Options

1 To print the Analysis Parameters (instrument settings during analysis), select the Analysis Parameters check box. To print any of the plots or graphs, check any one or all of the available selections. The graphs will appear in condensed form on the printouts.

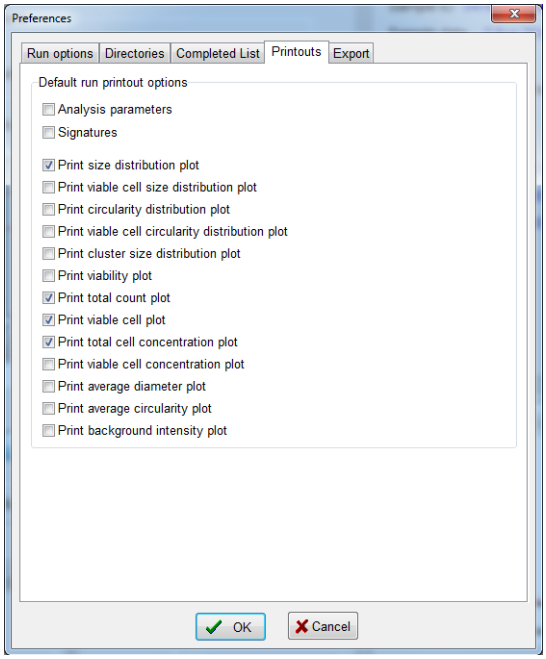
2 Select Save as default to keep your settings.

3 Select OK.

4 Select File > Preferences > Printouts tab and select the printout options (properties options for your printer i.e. portrait, landscape etc).

5 Select OK to begin printing or select cancel or Esc to abort printing.

Figure 3.11 Run Results Print Options



Print Run

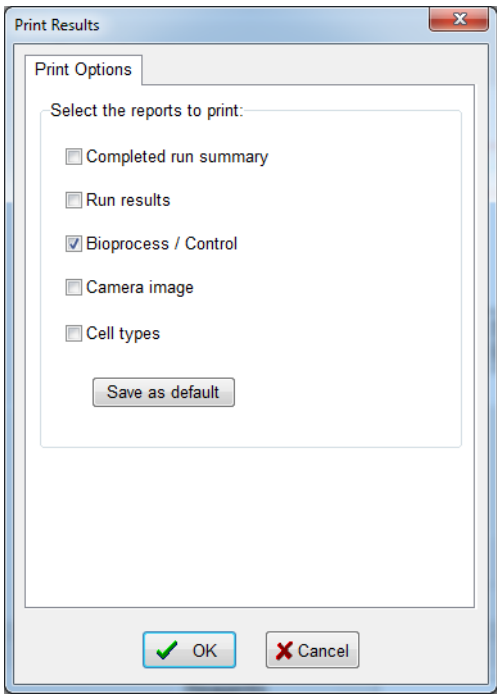
On the lower right hand corner of the main screen, select Print Run. This will generate a quick report showing the current run results as well as the analysis parameters. The report will print out according to the latest saved default settings.

Printing from the Bioprocess/Control Screens

To print a report from either the Bioprocess or the Control screen, select the Print function from the corresponding Bioprocess or Control pane. This will generate a report containing all analysis information as well as the graphs appearing on the screen.

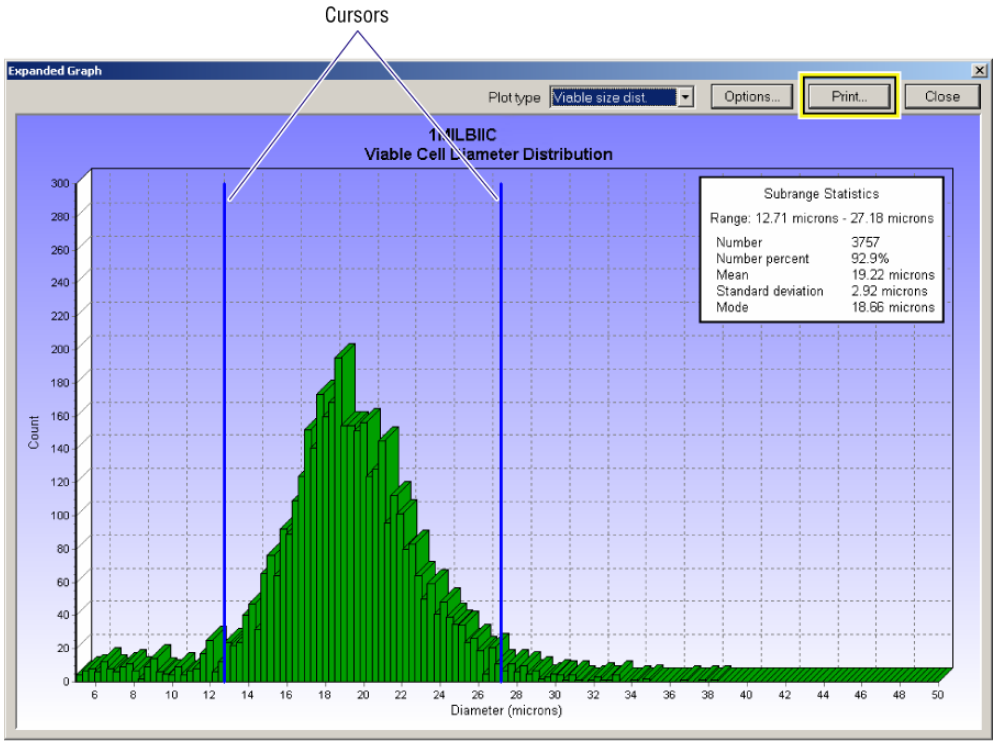
Selecting on the main menu, file, then print and on the print results dialog box Bioprocess / Control will also perform a print of the bioprocess results and/ or control.

Figure 3.12 Print Results Print Options



Printing from an Expanded Plot

To print from an expanded plot, select Print. The plot along with data will print on a single page. If cursors are used, only the statistics for the isolated region will print along with the graph.



Vi-CELL XR Software Menus

The controls contained in the main window are described in the following sections.

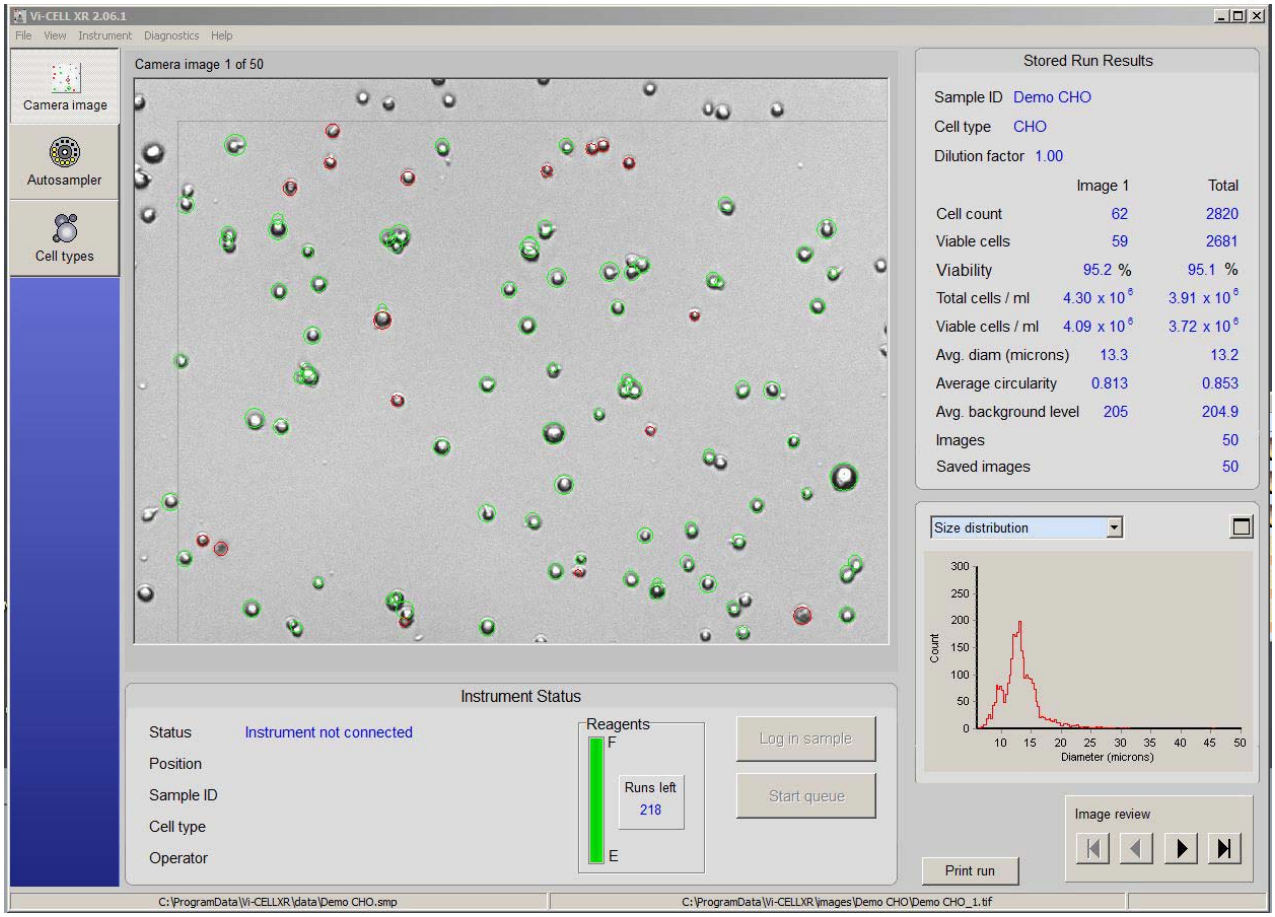


Figure 4.1 Vi-CELL XR main Window

File Menu

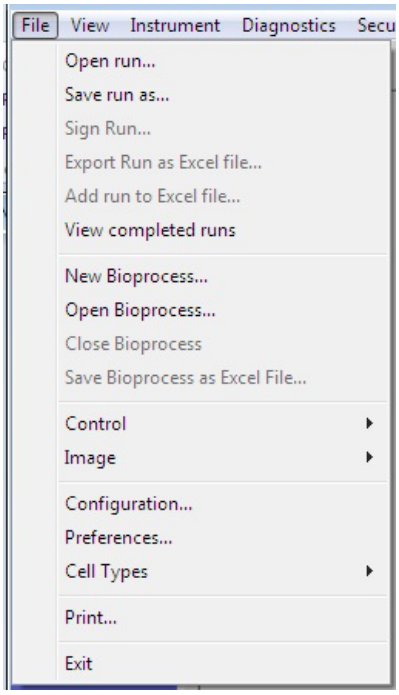
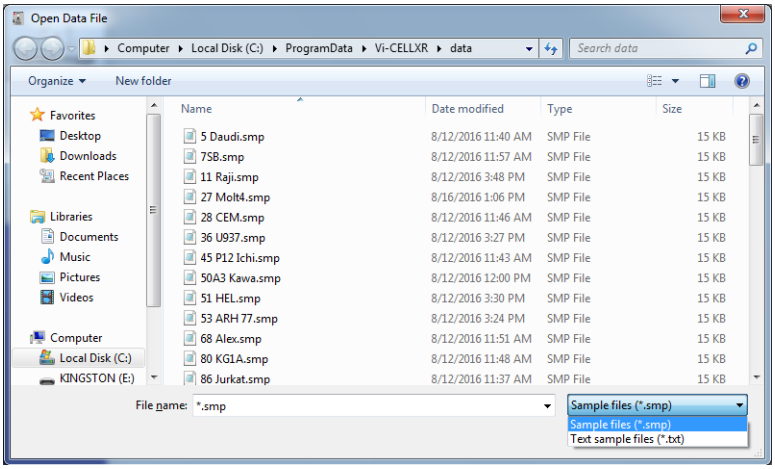


Figure 4.2 File Menu

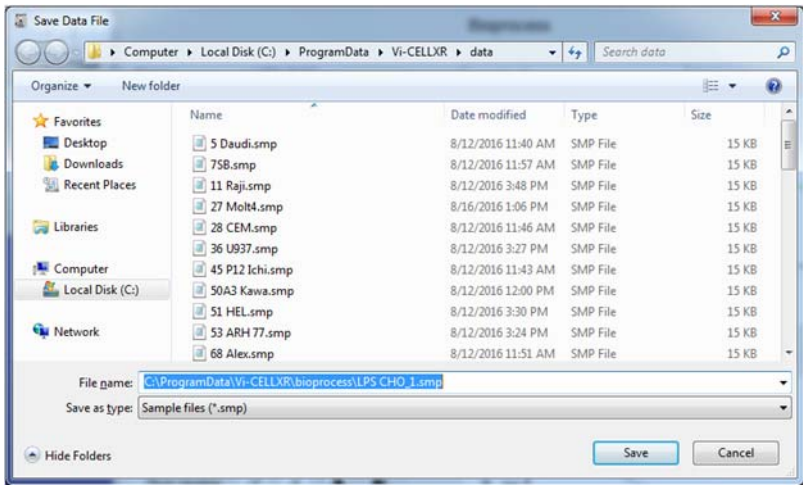
Open Run...

This menu item displays a dialog box to open a previously saved 4.smp or .txt file. If images are linked to the file, they too are opened. You can open existing bioprocess, control, and sample files generated in any Vi-CELL XR software version.



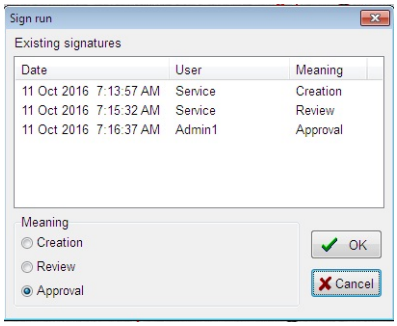
Save Run As...

This menu item saves test data in the directory that is chosen in the dialog box. You can save the filename as .txt or .smp format. Images may also be saved.

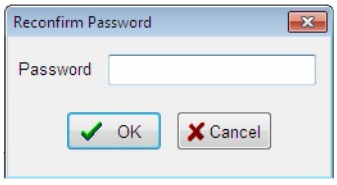


Sign Run

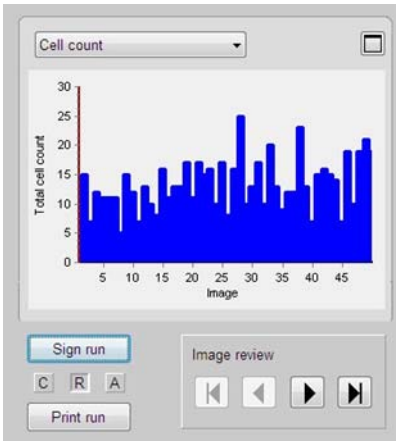
Any logged-in user can add electronic signatures to run files. Run file signatures can be viewed or a new signature added by using the **Sign run** button in the **Run Results** pane (not available during Acquisition), the Autosampler Queue, or selecting **File > Sign Run**. Use the **Meaning** box to select the meaning of the signature for example, **Creation**, **Review**, or **Approval**. Signature meanings can be defined in the **Security: Configuration** tab.



After selecting the **OK** button, you must reconfirm your password.



If the run has at least one signature of a particular meaning, then the appropriate letter below the Sign run button in the Run Results pane will appear depressed. A signature cannot be removed from a run file. Signed runs will maintain the signatures when saved to other locations.

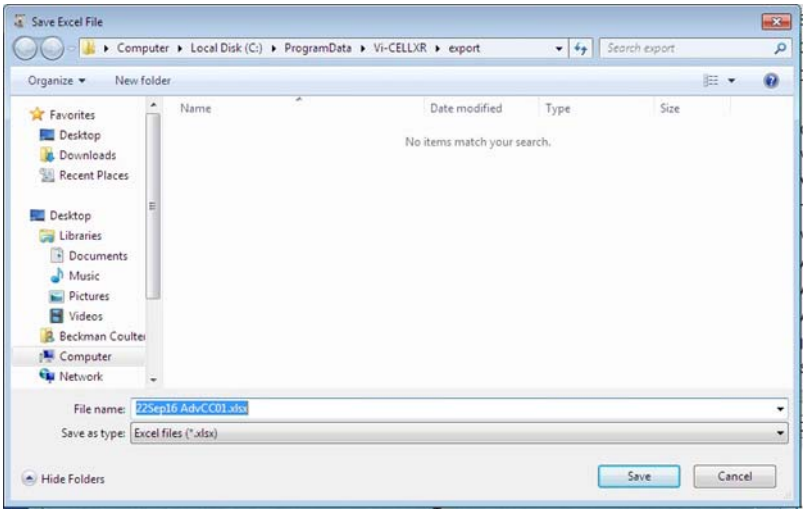


Export Run as Excel file...

This option saves test results as an .xlsx Microsoft Excel file. The run results may also be automatically stored in an Excel file at the end of the run:

- If the Export to Excel check box was selected when the sample information was entered at the Log In screen, or
- If the Autosave run results to Excel format by default was selected in the Preferences > Run Options tab for an unknown sample.

The new file will have the same name as the sample ID with an .xlsx extension. The path of the folder in which the file is stored is indicated by the Exported files path in the Directories tab in the File > Preferences menu. See Directories Tab.

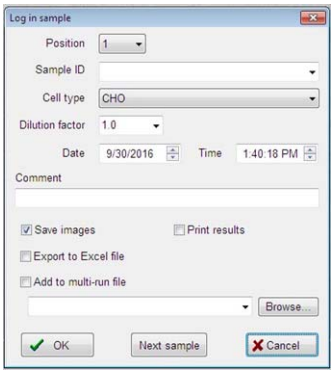


Add run to Excel file

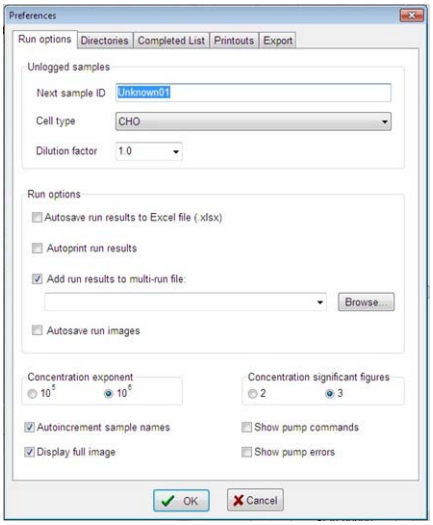
Use this menu item to add run results to an existing multi-run Excel file.

The run results can be automatically stored in a multi-run Excel file at the end of the run:

- If the **Add to multi-run file** check box was selected when the sample information was entered on the **Log In sample** screen.



- If the **Add run results to multi-run Excel file** was selected in the **Run options** box in the **Run options** tab in **File > Preferences** for an unknown sample.



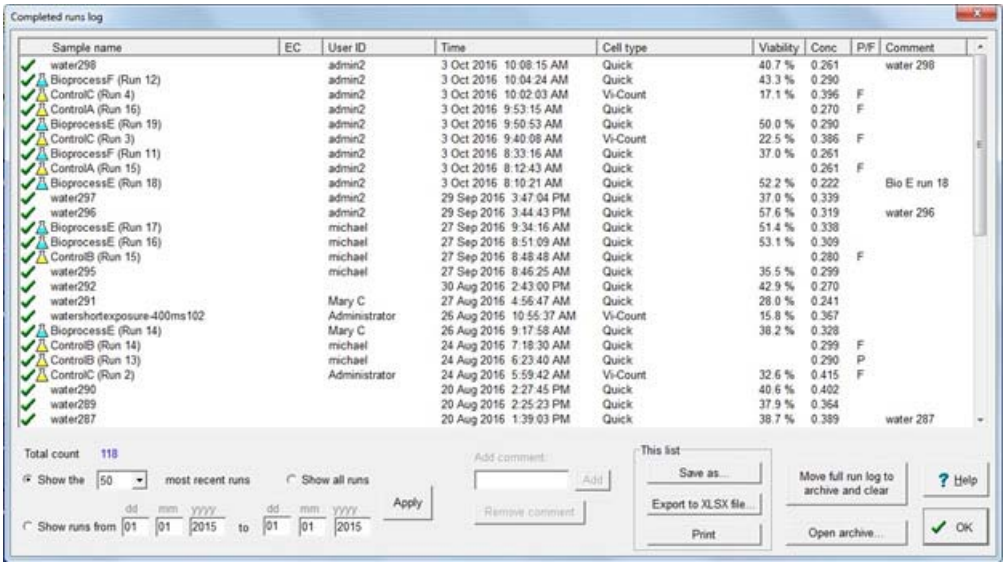
When a new multi-run Excel file is specified in the default sample settings in the **Run options** tab in **File > Preferences**, then a dialog is displayed where the results to be stored are selected.
See Run Options Tab.

View completed runs

The Completed runs log screen creates and maintains a separate list of all completed runs. All completed runs are automatically added to the Completed runs log. Double clicking on a line item from the completed runs log opens the associated data file and loads the linked images to the main screen.

This screen provides the ability to:

- Display the total number of runs
- Show the most recent (n) runs
- Show runs from a specific date range
- Show all runs
- Add or Remove a Comment
- Save the list as…
- Export the list to an xlsx file
- Print
- Move full run log to archive and clear
- Open the archive
- Access the Help - (displays the EC Error codes)



The Completed runs log dialog has a Comments field with a limit of 12 characters. All characters are allowed.

The Save as button will save the list of events selected to an archive file (.vcs3) file.

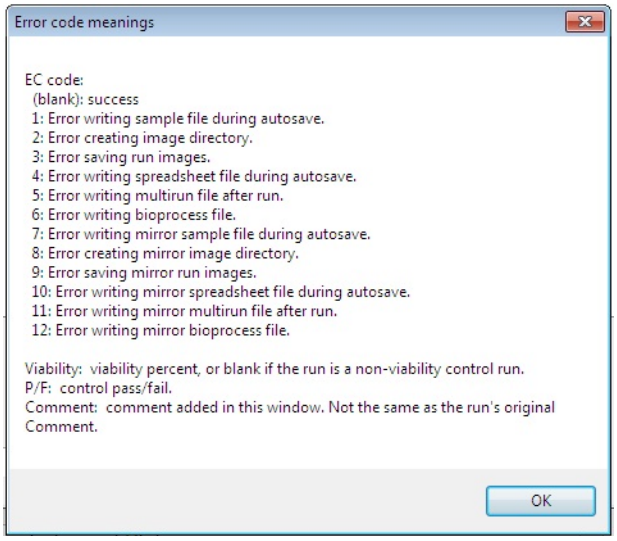
Use the Export to XLSX file button to export the list of runs selected to an Excel file.

Use the Print button to print the list of runs selected.

The Move full run log to archive and clear button moves and archives the whole content (Total runs count) of the run log to a user-specified file and clears the Completed runs log dialog.

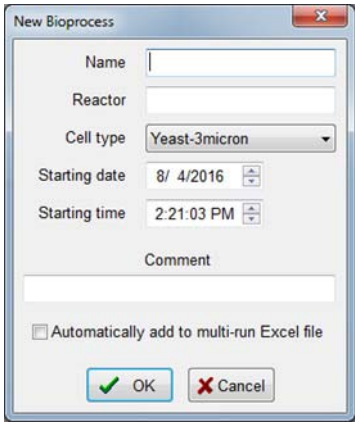
EC Error codes

The error code meanings list is displayed when Help is selected:



New Bioprocess…

Use this menu item to create a new Bioprocess type. A cell type must be selected that is used for analyzing all of the samples of the bioprocess. The starting date and time entered are used to calculate the elapsed time associated with the results for each sample. When OK is selected, the new bioprocess is created and is in the opened state. This will place a new icon on the left hand side of the main screen.



A bioprocess remains open until it is closed using the Close Bioprocess option in the File menu.

Once a bioprocess is closed, it can be reopened using the Open Bioprocess option in the File menu.

If the 6 and restarted, the bioprocesses that were open when the software was exited will still be open when the application is restarted.

When a bioprocess is created, a file is created with the same name as the bioprocess and the .vcbf extension in a folder specified by the bioprocess path in Preferences > Directories.
See Directories Tab.

If Add to multi-run file is selected, then an Excel file is created with the same name as the bioprocess with an .xlsx extension in a folder specified by the Export directory in Preferences > Directories. See Directories Tab.

The parameters stored in the file are determined by the selections in the Preferences > Export tab.

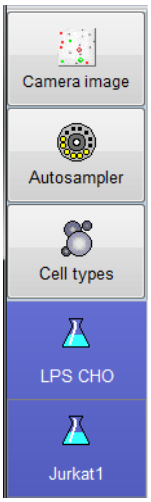
A new line is added to the multi-run Excel file after each run of the bioprocess is completed.

Open Bioprocess…

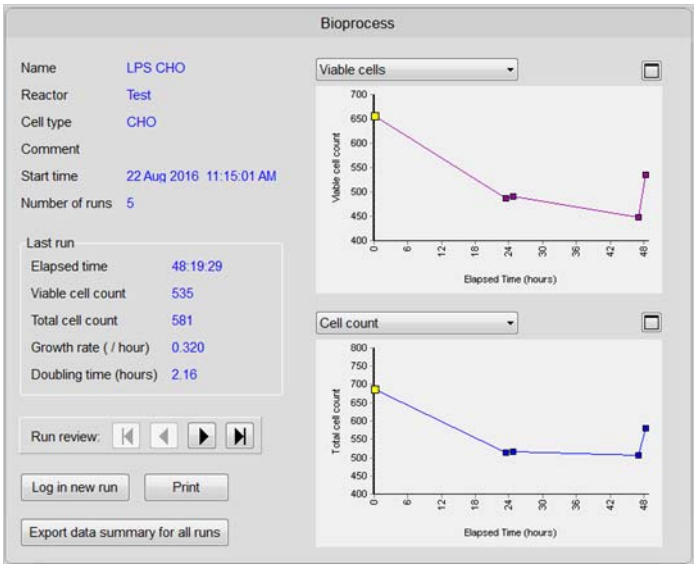
Opens a pre-saved bioprocess.

While in the open state, a bioprocess will have its own icon in the navigational bar. Clicking the left mouse button while selecting the Bioprocess icon will cause the information for the bioprocess to be displayed in the Bioprocess pane.

NOTE The total number of Bioprocesses and/or Controls that can be open at one time is 100.



The Bioprocess pane displays the Bioprocess run results for all runs within the bioprocess, and provides graphical views for 2 result parameters. The displayed parameters can be changed at any time by choosing from drop-down lists.



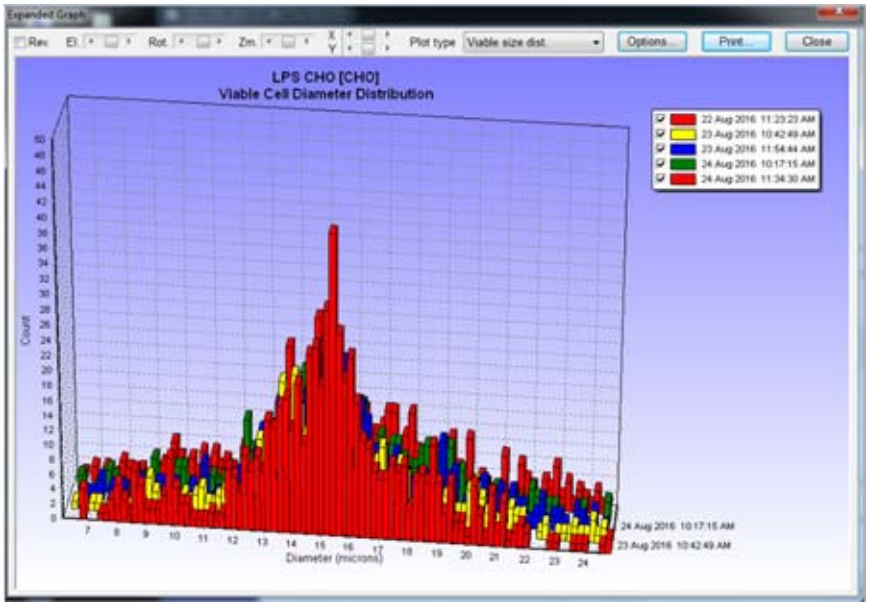
The Last run area provides the most updated information about the bioprocess. It shows the most updated values of the bioprocess together with the growth rate and doubling time of viable cells.

The values are calculated from the results of the last two runs using the formulae:

- Growth rate per hour = (ln V2 - ln V1)/ (t2 - t1)
- Doubling time in hours = ln 2 / Growth rate per hour

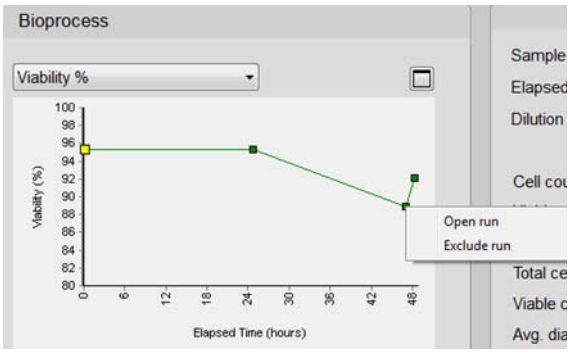
Where:
V1 = Viable cell concentration in cells/ mL at elapsed time t1 in hours
V2 = Viable cell concentration in cells/ mL at elapsed time t2 in hours

An expanded version of the graphs can be displayed by selecting ☐ in the top right hand corner of the graph.



Graphs are either provided with populations for the different runs in the bioprocess indicated in different colors, with a legend provided, or as a single line graph.

When the instrument is the idle state, stored run results can be accessed using the Run review arrows or by double clicking a particular point on one of the two graphs with the left mouse button.

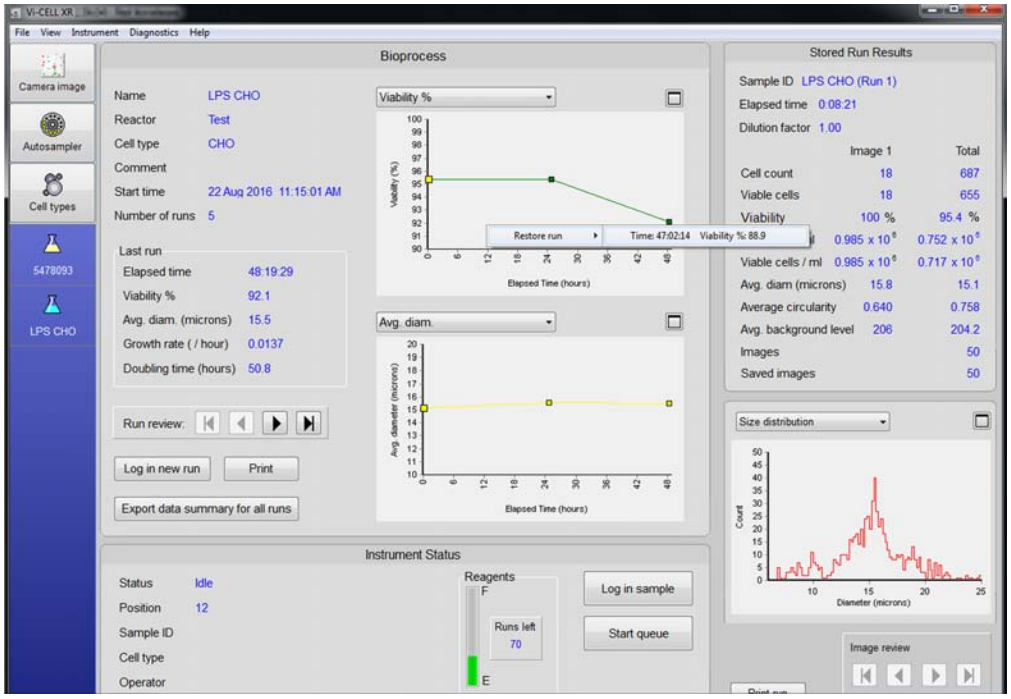


The selected runs are indicated by slightly larger yellow squares.

A run can be excluded from the displayed bioprocess result by selecting the run on one of the graphs, clicking the right mouse button and selecting the Exclude run menu item.

The number of runs and the run numbering will remain the same and the run file of the excluded run will still be retained, but the graphs and last run values will no longer include data from the excluded run

The process can be reversed by clicking the right mouse button while pointing at some part of the graph other than a specific point, selecting Restore runs and then left clicking on the run to be restored, which will be identified via the file name.



Logging in a Bioprocess Sample

In order to log in a bioprocess sample, the bioprocess must be created and be in the open state.

The bioprocess sample's name is displayed in the drop-down list of sample names in the Log in Sample dialog.

When a bioprocess is selected as the sample name, the cell type is set automatically to the type specified when the bioprocess was created.

NOTE The name provided for the bioprocess at the time it was created must not be changed. If changed, the sample results will not be linked to the bioprocess in the software.

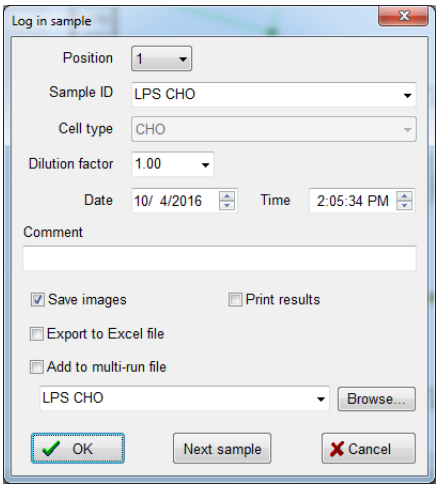
The time and date associated with the sample is set to the current time and date.

This time is used to calculate the elapsed time that is associated with the sample.

If necessary, you can change the date to the time that the sample was removed from the bioreactor if that would be more appropriate. Changing the date on the Log in sample dialog only applies to the Bioprocess.

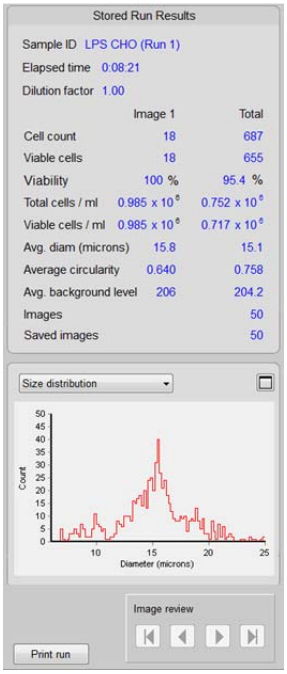
A bioprocess sample may also be logged in by double-clicking its icon in the blue navigation bar, then selecting "Log In New Run" from the Bioprocess pane, by clicking the Log in Sample menu item

in the Instrument menu or the Instrument Status area of the software. The Log in sample dialog is then displayed with the sample name and cell type already set.



Bioprocess Run Results

Access run results using the Run review arrows or by double clicking a particular point on one of the two graphs with the left mouse button. The results are displayed in the Stored Run Results pane.



The Sample IDshows the name of the bioprocess together with the bioprocess run number and the elapsed time from the start of the bioprocess.

The run results are automatically stored in a run file whose name will consist of the bioprocess name and the bioprocess run number.

The run file is given the .smp extension and is stored in the folder specified in the File > Preferences > Directories tab, in the Run data files area. See Directories Tab.

The results of a single bioprocess run can be opened, printed, exported and saved.

Close Bioprocess…

A Bioprocess remains in the open state until it is closed using File > Close Bioprocess. The complete bioprocess file is stored in the Bioprocess folder, with the file extension of .vcbf.

Save Bioprocess as Excel file…

This option saves test results as an .xlsx Microsoft Excel file.

Control…

New —Create a control file for monitoring instrument performance.

Modify —Modify an existing control file.

Open —Opens an existing control file.

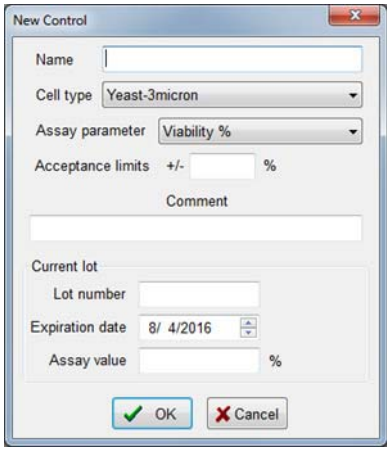
NOTE The total number of Bioprocesses and/or Controls that can be open at one time is 100.

Close —Closes any opened control files.

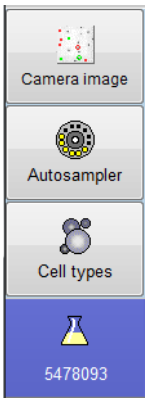
Export —Exports data as an Excel file.

Creating a New Control

The Vi-CELL Concentration Control is the only validated control for use on the system. The cell type should be the Conc. Ctrl cell type, and the assay parameter and acceptance limits are provided in the product assay sheet. The assay parameter and acceptance limits must be entered together with information about the current lot.



When the dialog is accepted, the new control is created and is in the opened state. An icon with the control name will be present in the Navigation Pane.



A control remains in the open state until you select File > Control > Close.

If the software is closed and restarted, the controls that were open when the software was exited will still be open when the software is restarted.

When a control is created, a file is created with the same name as the control and a .vccf extension in a folder specified by the Control files path in the Directories box of the Directories tab in the File > Preferences menu. See Directories Tab.

While in the open state, a control will have its own icon or list entry in the navigational bar. Clicking the left mouse button while pointing at this icon or list entry will cause the inform9ation for the control to be displayed in the Control pane.

For an open control, the pane can be accessed by pointing at the control entry icon in the navigational bar.

The Control pane is automatically displayed when the control is opened and when the control is created.

The Control pane contains a graph that shows how the measured value of the assay parameter has changed with time. The acceptance limits are shown on the graph as two lines displaying the upper and lower limits of the assay range.

An expanded version of the graph can be displayed by selecting  in the top right hand corner of the graph.

The Last check box provides the most updated information about the control, showing the date when the control was last run and the measured value of the assay parameter.

If it was within the acceptance range, then a green check mark is shown. If the values are not within the acceptance range, a red cross is displayed. The selected run is shown as a yellow square on the graph below the Last Check area.

A run may be excluded from the displayed control results by pointing at the point from the run on the graph, clicking the right mouse button and selecting Exclude run.

The number of runs and the run numbering will remain the same and the run file of the excluded run will still be retained, but the graphs and last run values will no longer include data from the excluded run.

The process can be reversed by clicking the right mouse button while pointing at some part of the graph other than a specific point, and selecting Restore runs, then selecting the run entry to be restored.

When the instrument is the idle state, detailed run results can be accessed using the Run review buttons or by pointing at a particular point on the graph and clicking the left mouse button. This prompts the system to load the linked images for the selected point, and displays the results in the Stored Run Results area.

Logging in a Control Sample

In order to log in a control sample, the control must be created and be in the open state.

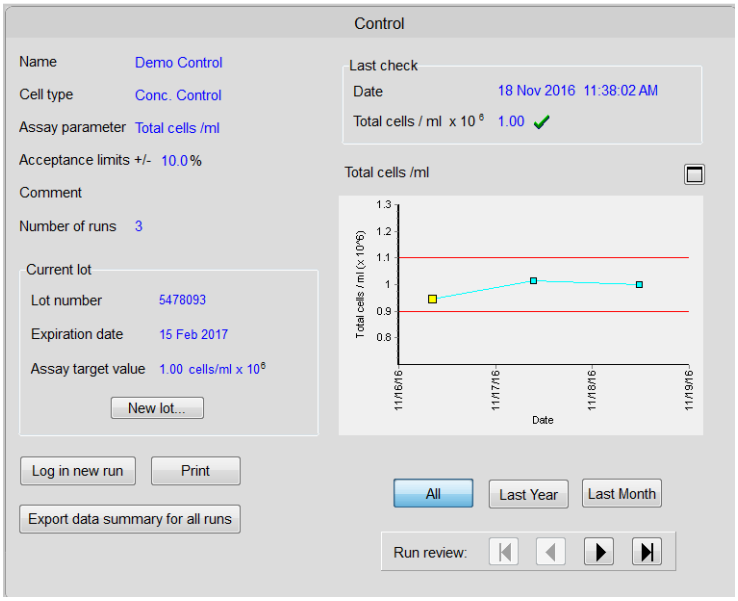
The control sample's name will then appear in the drop-down list of sample names in the Log in sample dialog.

When a control is selected as the sample name, the cell type is set automatically to the type specified when the control was created. The time and date associated with the sample is set to the current time and date.

A control sample may also be logged in by selecting Log in new run from the control pane, or by selecting Instrument > Log In sample from the main menu bar, or selecting Log in sample from the Instrument Status pane.

Control Run Results

When a sample from a control is analyzed, the results are shown in the Run results pane.



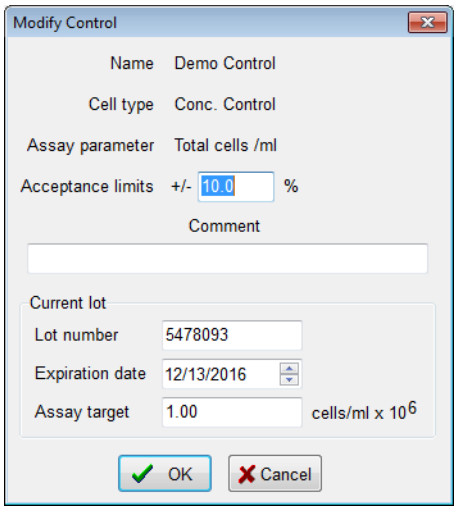
The run results is automatically stored in a run file whose name will consist of the control name and the control run number.

The run file is given a .smp extension and be stored in the folder specified by the control path in the Preferences > Directories. See Directories Tab.

The results of a single control run can be opened, printed, exported and saved.

Modifying a Control Lot

When you change the lot of the control sample, the information about the new lot can be entered in the Modify control dialog found in File > Control > Modify, or the New Control can be created in File > Control > New.



The graph in the Control pane automatically shows the appropriate acceptance range for each sample that takes account of the lot from which it was taken.

The system displays a warning when the current lot has passed its expiration date.

When you select the Log in new run button on the control pane, the Log in sample dialog appears with the sample name automatically shown as the control name and the Concentration Control cell type should be displayed in the Control pane. Select OK to close the Log in sample dialog, load the sample in the appropriate location in the carousel and select Start queue.

The run results are automatically added to the control file information and be displayed in the last check box and on the graph in the Control pane.

Image...

Opens —Opens a stored image file.

Close —Closes an image being viewed.

Save as —Allows for the saving of images. Files are saved as .tiff files.

Reanalyze —This option “reanalyzes” a saved image.

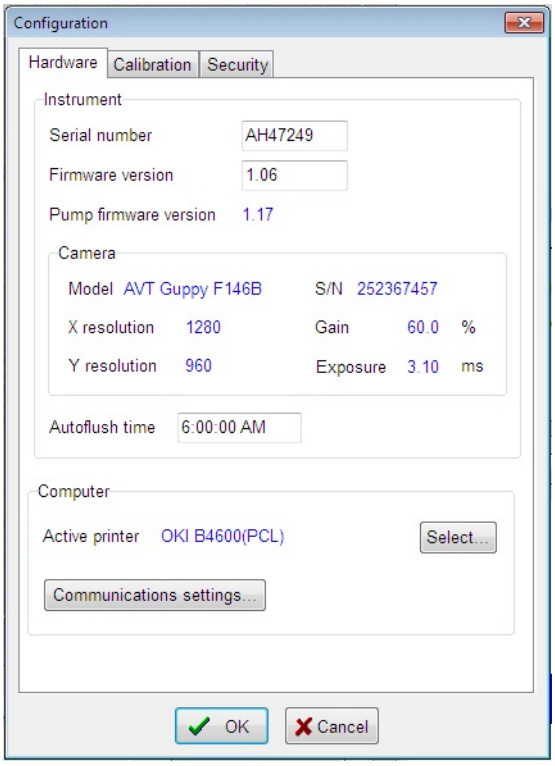
The effect of changing individual cell type settings can also be investigated by reanalyzing single images. This is much faster than reanalyzing all of the images of a run and is a good way to experiment and observe the effects of changing the various settings. Once approximate settings have been found, they can then be checked and optimized by reanalyzing all of the images.

Configuration...

Is where hardware such as printers are defined. Also, calibration and security information are defined. The Instrument Serial Number is specified here so that it can be included in the saved runs and reports.

Hardware Tab

Select the Hardware tab to access the hardware configuration information



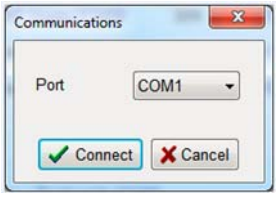
This dialog provides the following information:

- Instrument - Serial number, Firmware version, Pump firmware version
- Camera - Model, S/N, X resolution, Y resolution, Gain%, Exposure%

  NOTE The Instrument and Camera values can only be modified by service engineers.

- Autoflush time
- Computer - Active printer, an option to select the printer

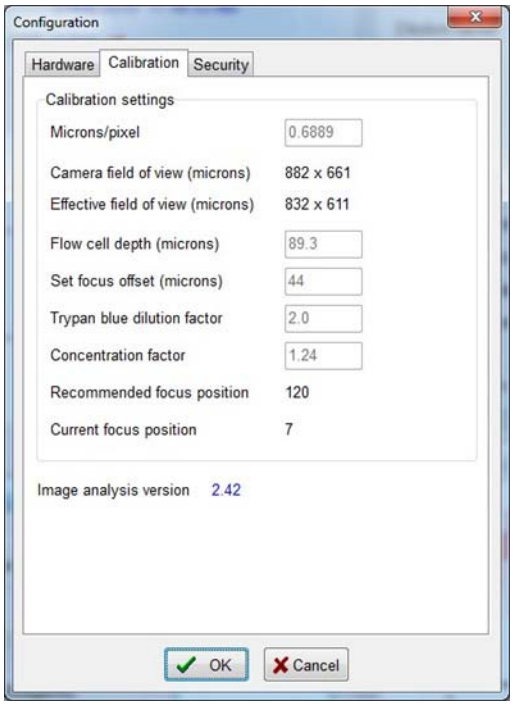
- Communications settings - When selected and connected to the unit, the following message is displayed:



Calibration Tab

Select the Calibration tab to access the calibration settings.

The Effective Field of View value in the configuration or calibration screens permits you to calculate concentration to verify them against reported values



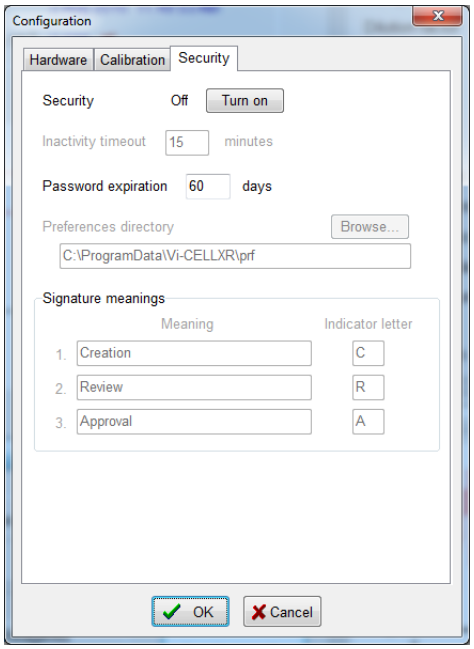
This dialog provides the following calibration settings for the system:

- Microns/ pixel
- Camera field of view (microns)
- Effective field of view (microns)
- Flow cell depth (microns)
- Set focus offset (microns)
- Trypan Blue dilution factor
- Concentration Factor
- Recommended focus position

- Current focus position
- Image Analysis Version

Security Tab

Select the Security tab to access the security settings.



This dialog provides the following settings for facilitating 21-CFR Part 11 compliance with the Vi-CELL XR software:

- Turn Security on/ off
- Inactivity timeout time - Minimum 1 minute to a maximum of 1439 minutes
- Password expiration - See Change Password for password requirements.
- Preferences directory location and the ability to browse for different locations
- Signature meanings which are User defined

Preferences...

Preferences are configured during software installation. Refer to Setting Up Preferences to define various parameters such as Run options, Directories where data is to be saved to, what auto-sampler Completed List items to show, options and parameters on Printouts and the items to Export on Excel spreadsheets. Auto-increment file name, Auto-save run images, Auto-print and Auto-save run results to Excel are other options available. For detailed information on preferences, refer to,

- Run Options Tab
- Directories Tab
- Completed List Tab
- Printouts Tab
- Export Tab

Cell Types...

Add —Allows for the creation of cell types.

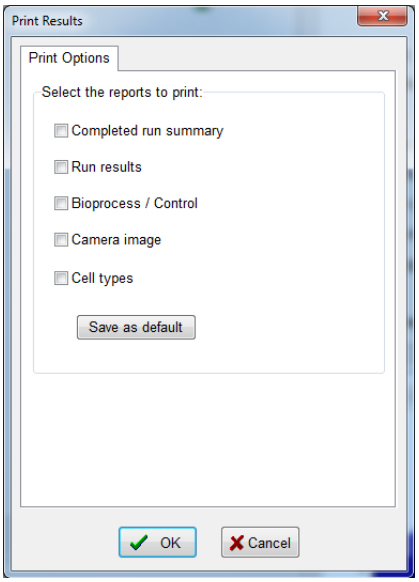
NOTE The maximum number of Cell Types is 100.

Modify —If a cell type requires the changing of certain parameters, use the 'modify' option to perform this task.

Delete —Allows for the deletion of cell types.

Print...

Use the Print Options dialog box to choose which elements of the report to print.



Exit...

Exits the program. Upon exit, if any test results remain open, a dialog box will appear asking whether or not you wish to save your results.

View

Camera Image —Provides 'real time' camera images.

Auto-sample Queue —Changes over to the Sample Queue screen.

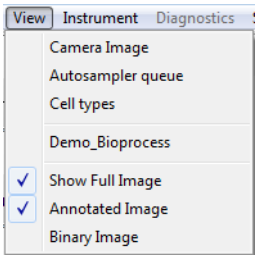
Cell Types —Changes over to the Cell Types Screen.

Show Full Image —The image is scaled so that the full image is shown within the working pane.

Annotated Image —Turns on the red and green circles around cells on the images, which denote dead and live respectively.

Binary Image —Will convert images to black and white.

Also, any control or bioprocess files that are open will also be listed on the **View** menu.



Instrument

Log In Sample —A dialog box appears for entering sample information.

Clear Completed List —Clears the completed runs list.

Start Queue —Begins an analysis.

Stop Queue —Will halt analysis on samples already in the auto-sampler queue.

Pause Run —Will pause analysis.

Resume Run —Will continue with a run if paused.

Cancel Run —Cancels a run.

Prime —Will prime reagents through the lines ensuring no bubbles are in the system.

Flush —Flushes the flow cell.

Decontaminate —Takes you through a step-by-step decontamination procedure.

Drain —Empties the reagent lines back into the reagent containers.

NOTE If the instrument is to be left unused for an extended period of time, if it is to be transported to another location or is likely to be exposed to temperatures below or close to freezing, then it should be drained.

If the instrument has been exposed to potentially hazardous samples you should decontaminate it before draining.

Replace Reagent Pak —Provides instructions on how to properly replace reagents and also empty the waste container.

Reanalyze —Re-calculates data on saved runs.

Diagnostics

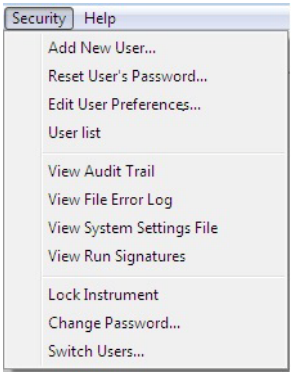
Set Focus —If the system requires re-focusing, this option takes you through a step-by-step procedure on performing an auto-focus routine using the Vi-CELL focus control (PN 175474).

Set Reagent Level —Will set the reagent levels based on a percentage specified by the end user.

Live Image —Shows real-time images.

Gray Level Histogram —This option shows a gray-scale histogram. This is for checking the quality of the light source.

Security



When security is turned on, the Vi-CELL XR software has been designed to facilitate compliance with the US Food and Drug Administration's (FDA) regulations on electronic records and electronic signatures (21 CFR Part 11). Vi-CELL XR run files have been designed to meet the requirements for electronic records to be submitted to the FDA in electronic form.

When security is turned off, the Vi-CELL XR software gives all users access to all features except for a few service and diagnostics capabilities that might damage or affect the accuracy of the instrument, and certain security-related features.

Add New User —Administrator function. See Add New User.

Reset User's Password —Reset the password to create a new password.

Edit User Preferences —Edit User Preferences for Run options, Directories, Completed List, Printouts, Export. Refer to Preferences...

User List —Displays a list of users and contains information on when the user was created, by whom, at what level, and when it was created. Administrator can change user levels, can remove a user, enable a user and disable a user.

View Audit Trail —The system audit trail is displayed by selecting **Security > View Audit Trail**. See View Audit Trail for details.

View File Error Log —Maintains a list of all file open/ read/ write errors encountered anywhere in the software. See View File Error Log for details.

View System Settings File —The System settings file maintains the system settings for reagents and the system parameters. See View System Settings File.

View Run Signatures —The View existing run signatures shows the existing signatures for the selected run. See View Run Signatures.

Lock Instrument —Locks out a user from attempting to utilize the instrument. In order to gain access to the system, a user name and password must be entered.

Change Password —Create a new password. See Change Password.

Switch Users —Switch to another operator.

View Audit Trail

Select **Security > View Audit Trail**. The Audit trail log can be archived, saved to a different file, exported to an Excel file, and printed.

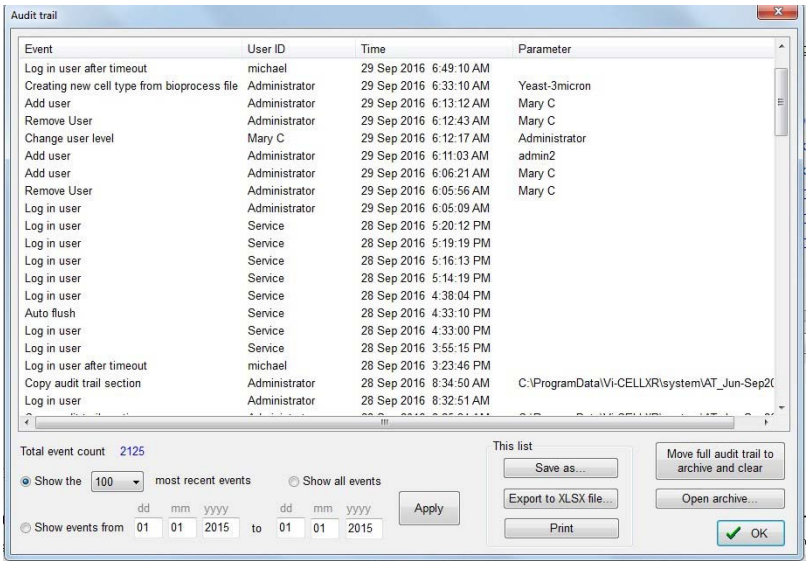
Use the Save as button to save the list of audit trail events selected to a file (*.vcs1).

Use the **Export to XLSX file** button to export the list of selected events to an Excel file.

Use the Print button to print the list of selected events.

Use the **Move full audit trail to archive and clear** button to move and archive the whole content (Total event count) of the audit trail to a user-specified file and clear the Audit trail dialog.

Use the Open archive button to view a saved archive file.



The View Audit Trail screen displays the time and details of the following events.

- Log in user
- Login Failed
- Checksum error
- Switch Users
- Change Password
- Reset Password
- Archive audit trail
- Archive Error Log
- Archive completed run log
- Copy audit trail section to file
- Copy error log section to file
- Copy run log section to file
- Security On
- Security Off
- Add User
- Enable User
- Disable User
- Change User Level
- Lock Instrument
- Unlock instrument
- Login User after Time Out
- Autoflush
- New Bioprocess

- New Control
- New Cell Type
- Cell Type Deleted
- Cell Type Modified
- Creating new cell type from bioprocess file
- Flush
- Prime
- Drain
- Decontaminate
- Replace Vi-pak
- Memory allocation error

View File Error Log

Select Security > View File Error Log.

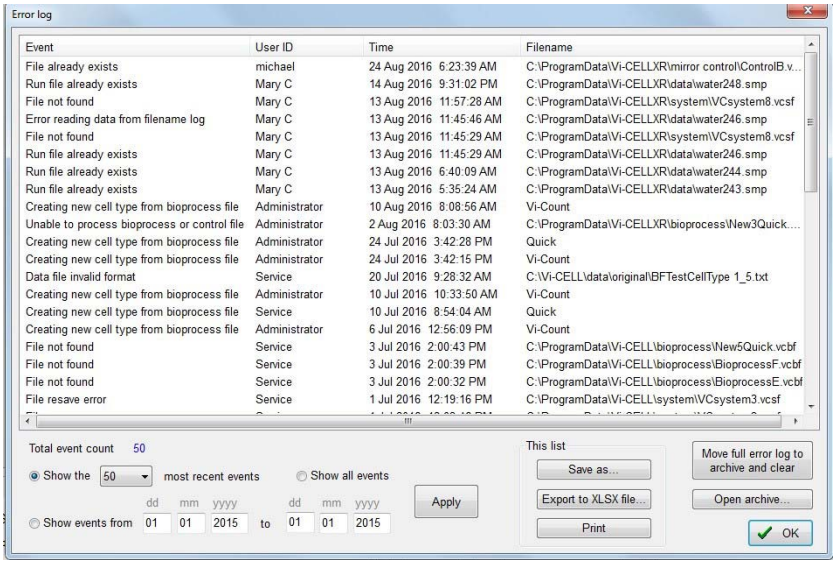
Use the Save as button to save the list of selected events selected to a file (*.vcs2).

Use the Export to XLSX file button to export the list of selected events to an Excel file.

Use the Print button to print the list of selected events.

Use the Move full error log to archive and clear button to move and archive the whole content (Total event count) of the error log to a user-specified file and clear the error log dialog.

Use the Open archive button to view a saved error log file.



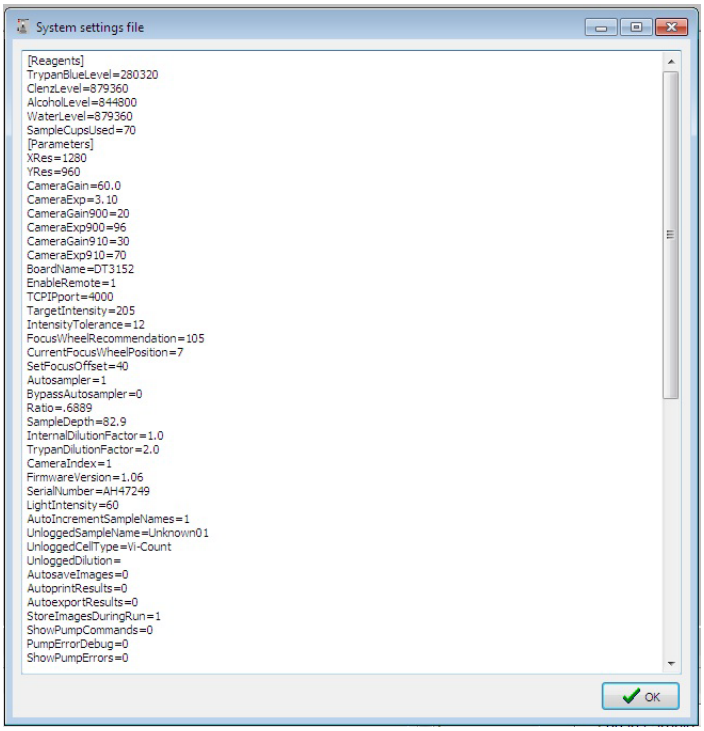
The *Error log* dialog maintains a list of all file open/ read/ write errors encountered anywhere in the software and details of the following error.

- Error creating file
- File not found
- File already exists
- Run file already exists
- Run series file already exists
- Data file open error
- Data file read error
- Error writing data file
- Unknown data file error
- Data file invalid format
- File does not have the correct format
- Invalid file type
- Error in decryption
- Image save error
- Error creating Excel file
- Error opening Excel file
- Error writing Excel file
- Error appending to Excel file
- Error writing image file
- Excel file: no data exists
- Error appending to Excel file
- File is not a control file
- Max number of open bioprocesses is 32
- Unable to process bioprocess or control file
- File resave error
- Error writing sample file during autosave
- Error writing mirror sample file during autosave
- Error writing spreadsheet file during autosave
- Error writing mirror spreadsheet file during autosave
- Error creating image directory
- Error creating mirror image directory
- Error saving run images
- Error saving mirror run images
- Error writing multirun file after run
- Error writing mirror multirun file after run
- Error writing bioprocess file

Error writing mirror bioprocess file

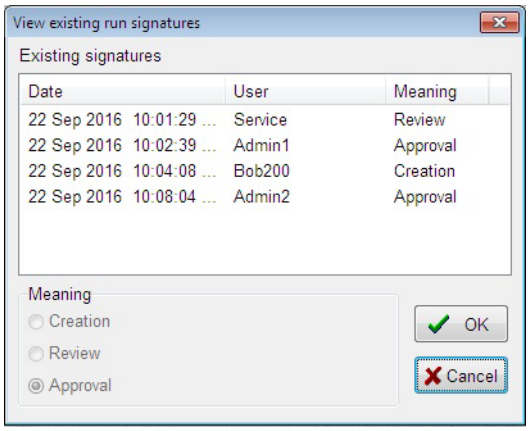
View System Settings File

The System settings file maintains the system settings for reagents and the system parameters.



View Run Signatures

The View existing run signatures shows the existing signatures for the selected run.



Types of Users

The following are the default conditions for the different user access levels:

The types of users are: Normal, Advanced and Administrator, with the administrator assigning access levels.

Table 4.1 User Types and Access Levels

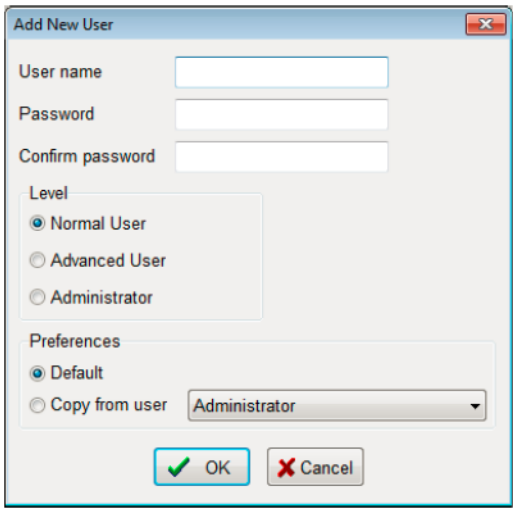
Menu Item	Security Off	Normal	Advanced	Administrator
File Menu				
File/Save run as	Enabled	Enabled	Enabled	Enabled
File/Sign Run	Not Displayed	Enabled	Enabled	Enabled
File/Export Run as Excel file	Enabled	Enabled	Enabled	Enabled
File/Add run to Excel file	Enabled	Enabled	Enabled	Enabled
File/Image	Enabled	Disabled	Enabled	Enabled
File/Cell Types	Enabled	Disabled	Enabled	Enabled
File/Configuration	Enabled	Disabled	Enabled	Enabled
File/Configuration/Hardware	Enabled	Not Accessible	Enabled	Enabled
File/Configuration/Calibration	Read Only	Not Accessible	Read Only	Read Only
File/Configuration/Security	Read Only	Not Accessible	Read Only	Enabled
File/Configuration/Security/Turn On	Enabled	Not Accessible	Read Only	Enabled
File/Configuration/Security/Turn Off	Not Displayed	Not Accessible	Disabled	Enabled
File/Preferences	Enabled	Disabled	Enabled	Enabled
Instrument Menu				
Instrument/Log in sample/Save images	Enabled	Disabled	Enabled	Enabled
Instrument/Log in sample/Print results	Enabled	Disabled	Enabled	Enabled
Instrument/Log in sample/Export to Excel file	Enabled	Disabled	Enabled	Enabled
Instrument/Log in sample/Add to multi-run file	Enabled	Disabled	Enabled	Enabled
Instrument/Reanalyze	Enabled	Disabled	Enabled	Enabled
Diagnostics Menu				
Diagnostics	Enabled	Disabled	Enabled	Enabled
Diagnostics/Set Focus	Enabled	Not Accessible	Disabled	Enabled
Diagnostics/Repetitive Test	Not Displayed	Not Accessible	Not Displayed	Not Displayed
Diagnostics/Low Level Control	Not Displayed	Not Accessible	Not Displayed	Not Displayed
Diagnostics/Load Nudge Expel	Not Displayed	Not Accessible	Not Displayed	Not Displayed
Diagnostics/Get Image and Analyze	Not Displayed	Not Accessible	Not Displayed	Not Displayed
Diagnostics/Continuous Analysis	Not Displayed	Not Accessible	Not Displayed	Not Displayed
Diagnostics/Clear Diagnostic Data	Not Displayed	Not Accessible	Not Displayed	Not Displayed
Diagnostics/Diagnostic Analysis Parameters	Not Displayed	Not Accessible	Not Displayed	Not Displayed

Table 4.1 User Types and Access Levels

Menu Item	Security Off	Normal	Advanced	Administrator
Security Menu				
Security/Add New User	Not Accessible	Not Displayed	Not Displayed	Enabled
Security/Add New User/Service	Not Accessible	Not Displayed	Not Accessible	Not Displayed
Security/Reset User's Password	Not Accessible	Not Displayed	Not Displayed	Enabled
Security/Reset Password/Service	Not Accessible	Not Accessible	Not Accessible	Disabled
Security/Edit User's Preferences	Not Accessible	Not Displayed	Not Displayed	Enabled
Security/User list	Not Accessible	Not Displayed	Not Displayed	Enabled
Security/View Audit Trail	Not Accessible	Not Displayed	Not Displayed	Enabled
Security/View File Error Log	Not Accessible	Enabled	Enabled	Enabled
Security/View System Settings File	Not Accessible	Not Displayed	Enabled	Enabled
Security/View Run Signatures	Not Accessible	Enabled	Enabled	Enabled
Security/Lock Instrument	Not Accessible	Not Displayed	Enabled	Enabled
Security/Change Password	Not Accessible	Not Displayed	Enabled	Enabled
Security/Switch User	Not Accessible	Enabled	Enabled	Enabled

Add New User

Select Security > Add New User.



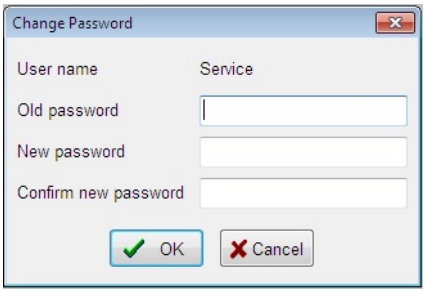
- User name can be any character except for =*, . \ or :
- User name must be a minimum of 8 characters and a maximum of 64 characters

For password requirements, see Change Password.

Once a user has been established, define the Level of access.

Change Password

Select Security > Change Password.



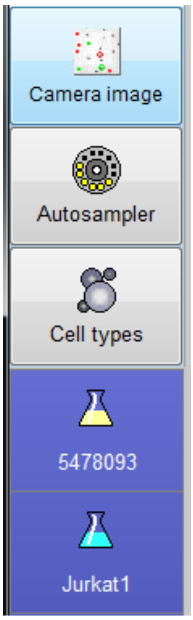
- The previous three passwords cannot be used
- Passwords must be a minimum of 8 characters and a maximum of 64 characters
- Passwords can be any keyboard characters
- Password expiration can be set to 30 days up to 365 days

Help

Provides documentation related to the software version and access to the operator's Instructions for Use.

Navigation Bar

Determines what is displayed in the main window.



Camera Image: When selected allows for the viewing of the analysis images during and after a run.

Auto-sampler Queue: Opens the sample queue for logging in of samples and verifying samples, which have been logged-in. This window also shows the completed sample list.

Cell Types: Opens the Cell Types window. This is where all pre-defined cell types are stored. This is also where new cell types can be created and/ or removed. Cell types can be added up to a maximum of 100.

Bioprocess: This icon represents Bioprocesses. Multiple bioprocess icons are possible.

Controls: Control file icons also appear on the Navigation window for easy access.

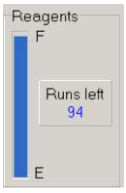
NOTE The total number of Bioprocesses and/or Controls that can be open at one time is 100.

Instrument Control

Log in Sample —Opens the login dialog box for entering sample and run information.

Stop/Start Queue —Begins an analysis. If clicked a second time, carousel will not continue to next position.

Pause Queue —Pauses an analysis (only displayed when system is running an analysis).

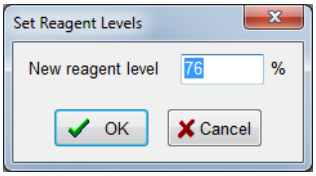


Reagent Level Meter: Monitors reagent levels.

Manual Reset of the Reagent Level Meter

Select Diagnostics > Set Reagent level to set the Reagent level meter. This is useful for when a reagent pack has been removed that still has reagent left in it. In the event a partly filled reagent pack must be removed, it is a good idea to record the Runs left before removing a reagent pack. When re-attaching the pack, enter the runs left in the New reagent level field.

Set Reagent Levels —Allows for manual reset of the reagent meter.

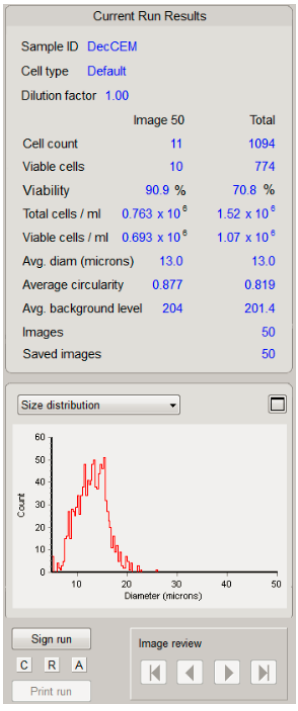


Run Results

This window contains the results for a given sample run. Results can either be obtained by opening a saved run (.txt or .smp) file or by clicking on a run within the completed runs list within the auto-sampler queue.

The Run Image review function allows for scrolling through images and their associated results. Also, within the Run Results screen is the Sign Run function (for 21 CFR Part 11 compliance).

There are of two types of images: Size distribution and Graphs. Size distributions are derived from all of the data points, while Graphs show the results with one data point for each image.



CHAPTER 5

Special Software Features

Bioprocess Feature

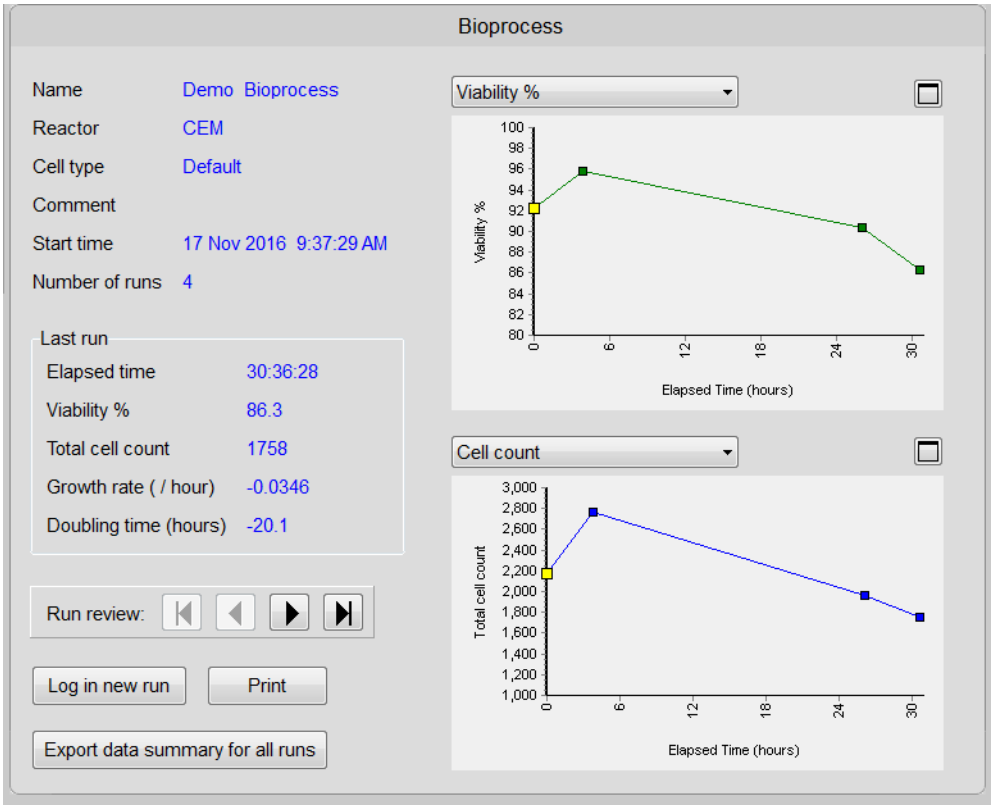
What Is A Bioprocess?

A bioprocess consists of the analysis results of a number of samples taken from the same source over a period of time. The source might be a bioreactor, a fermentation vessel or even a laboratory flask. A source may be associated with many bioprocesses as when one bioprocess finishes (for example when a bioreactor is harvested) then another separate bioprocess can be started. The measurements associated with a single bioprocess can be manipulated (opened and closed) as if they were a single entity. The Vi-CELL XR is designed to make it easy to monitor multiple, asynchronous, bioprocesses at the same time. Changes in selected analysis parameters over time can be displayed making it easy for example to check the progress of a bioreactor and to choose the optimum time for harvest.

The Vi-CELL XR bioprocess feature allows convenient, automated "tracking" of any of the measured cell culture parameters and calculates growth rate, doubling time, all essential to optimum bioreactor productivity. Data points are recorded and stored, eliminating the necessity for manual recording of the cell culture measurements. See Figure 5.1.

Figure 5.1 contains two graphs that show how selected bioprocess parameters have changed over time. This enables the state of the bioprocess to be easily monitored and helps the operator to make better-informed decisions such as the optimum time for harvest.

Figure 5.1 The Vi-CELL XR Bioprocess Screen, Example



The Bioprocess plots can be expanded for a more detailed view. Select the icons above each plot to expand them accordingly. Selecting Print bioprocess will print bioprocess data.

The most up to date information about the bioprocess is given in the Last run box. It shows the most up to date values of the two selected parameters together with the growth rate and doubling time of viable cells.

Growth Rate and Doubling times are calculated from the results of the last two runs using the formulas:

Growth rate per hour = (ln V2 - ln V1) / (t2 - t1)

Doubling time in hours= ln 2 / Growth rate per hour

Where

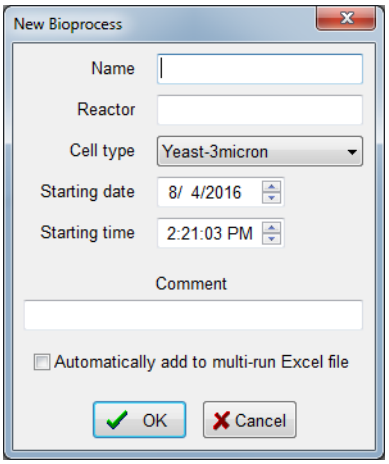
V1 = Viable cell concentration in cells/ mL at elapsed time t1 in hours

V2 = Viable cell concentration in cells/ mL at elapsed time t2 in hours

Creating A Bioprocess File

1 Select File > New Bioprocess.

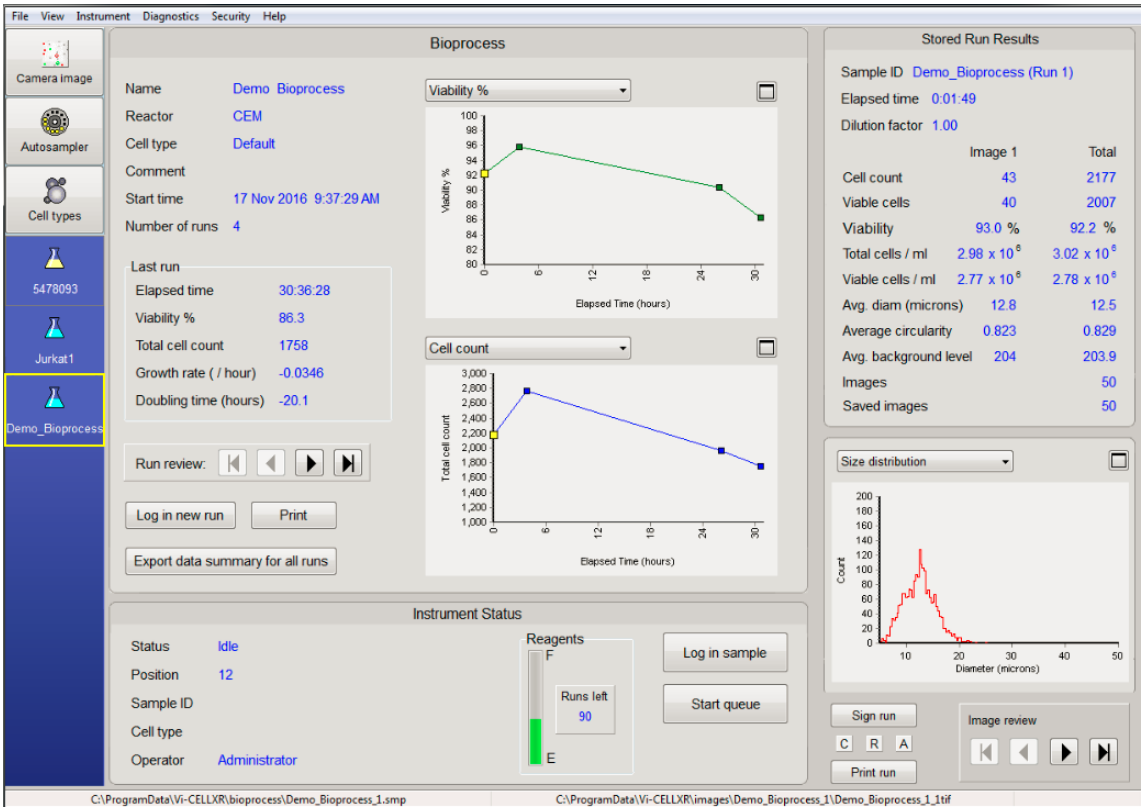
2 Enter the information on the New Bioprocess dialog box.



3 Select OK to close the dialog box.

A new icon will appear on the left side of the main screen with the name of your bioprocess. Select the icon to access the Bioprocess screen.

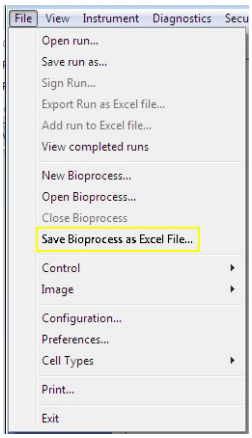
Figure 5.2 Bioprocess Screen and Navigation Bar Icons



Managing A Bioprocess

Once a bioprocess file is created, to analyze subsequent bioprocess samples, simply double-click on the bioprocess icon on the navigation bar and the bioprocess and the log in dialog box appears. The cell type and sample ID are automatically selected as defaults. Simply place the sample in the indicated position in the carousel and select OK. Then select Start Queue.

Exporting A Bioprocess File To Excel



Bioprocess data may be exported in Excel format via the 'Save Bioprocess as Excel File' feature.

Select File > Save Bioprocess as Excel File and data is saved as an Excel file.

Control Feature

What Is The Control Feature?

The control feature monitors Vi-CELL XR performance. The accuracy of concentration measurements (Total Cells/ mL) can be checked using Vi-CELL Concentration control (PN 175478) that is supplied with the instrument or can be obtained from Beckman Coulter.

The instrument contains special software that makes it very easy to run the control sample and to store and review the results obtained.

A collection of results from the same control sample are grouped together and can be saved, exported, printed as a single entity called a control (opened, closed etc). The Vi-CELL XR is supplied with a control already created to check concentrations.

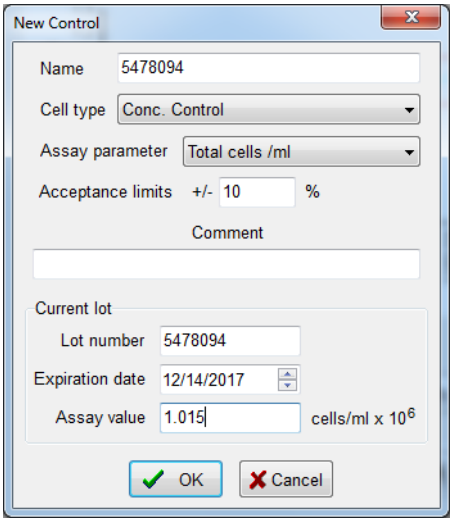
To run a control sample, first check that the lot number and assay value of the Vi-CELL Concentration Control that you are going to use is correct. Then double right click the navigational bar icon for the identified lot, check the information in the log in or new sample dialog and put the control in a sample cup in the specified position.

A collection of results from the same control sample are grouped together and can be saved, exported, printed as a single entity called a control (opened, closed etc).

Creating A Control Chart – For Total Concentration/mL

To setup a control file, go to File then select Control then New. The New Control dialog box will appear.

Figure 5.3 New Control



Enter the name and control information from the Vi-CELL concentration control assay sheet. Select OK. Once completed, a control icon will appear on the Navigation bar on the left side of the main screen as well as the control screen.

Upon run completion, the data is stored automatically within the control file.

Managing Controls

To log in a control sample, click on the control icon, which will display the Control Pane for the identified lot. Select Log in new run to log in the sample and select OK. Place the sample in the indicated location on the carousel, and press Start.

Upon completion, the data will automatically be stored within the control file.

As in the bioprocess screen, the plot within the control screen can be expanded for a more detailed view. Selecting the icon above the plot will expand the view of the graph.

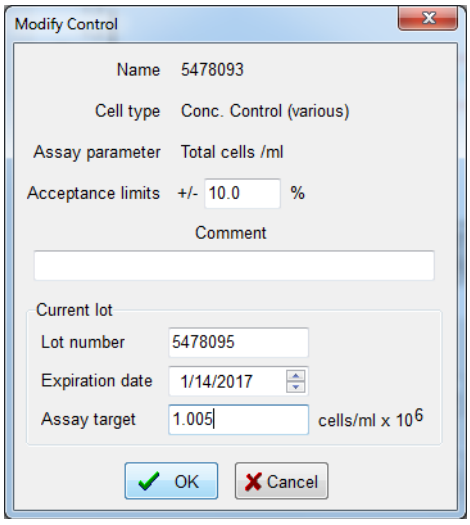
Printing of control data can be achieved by selecting Print.

Exporting Data To Excel

Control data may be exported as an excel file via the export function available by selecting File, Control, and Export.

Modifying A Control File

To modify control file information, such as lot number or values, select File, Control and Modify.



Creating And Managing Cell Types

What is a Cell Type?

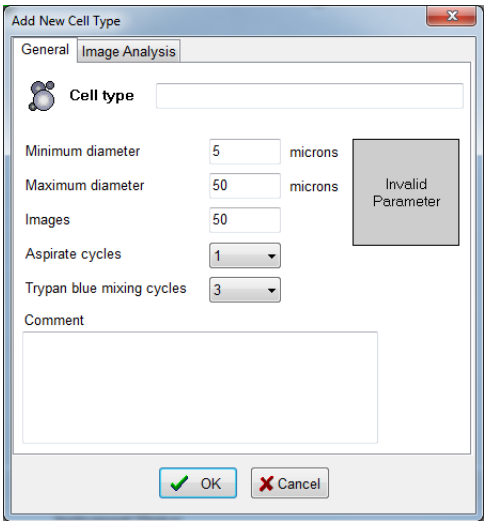
Cell types are files that store the optical settings required to correctly identify and quantify viable versus non-viable cells. Cells will vary in their optical characteristics and understanding how to establish the correct settings is important.

For many cell types, the default cell type values are suitable. In the event any of the parameters must be changed for a given sample, a new cell type may be created or an existing one modified. This section provides the instructions for creating new cell types for use with the software. The Vi-CELL XR software has 8 cell types predefined, and intended to be used as starting points for additional customer-defined cell types.

Creating a New Cell Type

1 On the menu bar select File > Cell Types > Add.

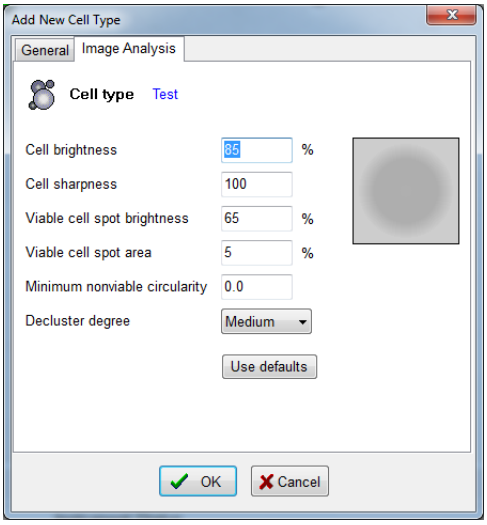
2 The Add New Cell Type dialog box appears.



NOTE Cell types can be added up to a maximum of 100.

3 Under the General tab, enter the appropriate information for your cell type, the minimum and maximum cell diameter, the number of images (max. 100) to acquire for the particular cell type and dilution factor if necessary. Use the default settings as a starting point if necessary. Use the minimum diameter parameter for excluding cellular debris or unwanted cells. Use the trypan blue mixing cycle to adjust for cell lines that tend to shear under the stress of mixing. For insect cell lines, a mixing cycle of 1 is found to be suitable.

4 Under Image Analysis set the parameters to match the type of cells expected. Utilize the default settings as a starting point. The first two parameters control the image recognition portion of the Vi-CELL XR software. The second two control cell recognition whether the cell is viable or non-viable. The minimum circularity parameter is for eliminating debris such as dead cell fragments. The range is 0 to 1, with 1 representing a perfect circle. If you find viability results a bit low, and there is debris, begin with a setting of about 0.7 and adjust accordingly. Refer to Image Analysis Tab for detailed descriptions of the settings.

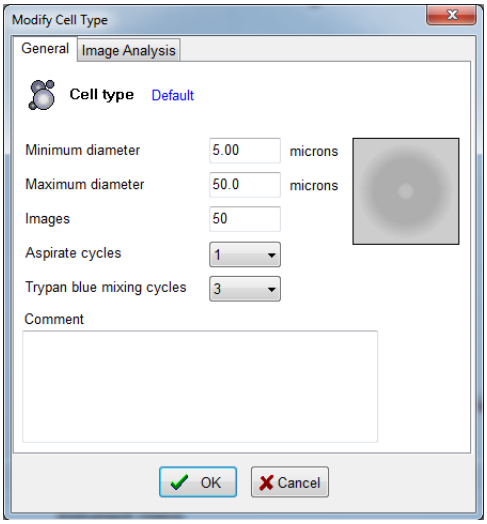


NOTE For steps 3 and 4, perform an initial analysis to get an approximation as to cell size.

5 If changes to your new cell type are required, use the Modify Cell Type under Cell Types to edit the cell type. To delete a cell type simply select Delete Cell Type

Modify Cell Type

To modify cell type, use the Modify Cell Type under Cell Types.



General Tab

Minimum and Maximum diameter (3 microns to 70 microns) —The minimum and maximum size of cells within the sample. Any cells that fall outside of this range are ignored. They will appear within the camera image but will not be annotated and will not be included in any of the numerical run results. The minimum diameter can be used to exclude debris and/ or unwanted cells. The size range should be used only to specify the expected range of cell sizes within the sample and should not be used to identify sub ranges.

NOTE Although the size range for system performance is 3 microns to 70 microns, the system is capable of detecting particles down to 2 microns.

Number of images (1 to 100) —Up to 100 images can be captured and analyzed in each run. The default setting is 50. A larger number will give a statistically better result. However a smaller number can be used if cycle time and image storage requirements are more important than absolute accuracy.

Trypan Blue Mixing Cycles (1 to 9) —The trypan blue and sample are mixed by sending the mixture back and forth between the sample cup and syringe. This parameter determines the number of times that the mixture is returned to the cup. Normally three times is sufficient but if the sample is immiscible with trypan blue then a higher value may be necessary to achieve good mixing and even background intensities.

This feature is especially useful for cell types, which may shear due to excessive mixing.

Lowering the number of mixing cycles will alleviate this situation. For insect cell lines, it has been determined that 1 mixing cycle is most suitable.

Aspirate Cycles (0 to 9) —In order to ensure that all of the cells are equally dispersed some of the sample is aspirated and then returned to the cup. One cycle is normally sufficient but if the cells are

difficult to keep in suspension and have a tendency to attach themselves to the walls of the cup then additional aspirate cycles may be beneficial.

NOTE Cells must be in single cell suspension prior to placement on the system.

IMPORTANT When modifying or deleting a cell type, the current active cell type is selected. Verify this is the cell type for modification or deletion.

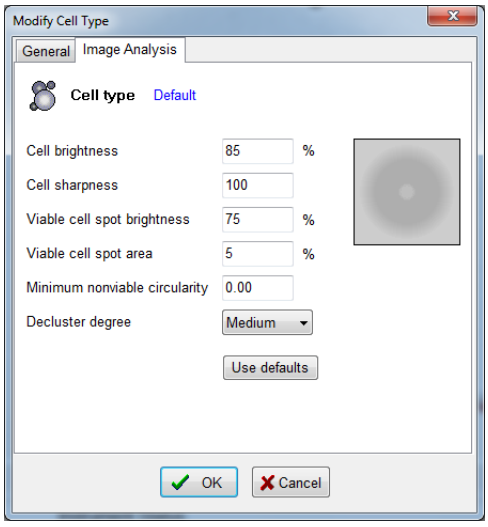


Image Analysis Tab

The image in the top right hand corner of the Cell Types pane is an attempt to show in graphical form the worst case of a cell that would be considered as viable (i.e. the least sharp, lightest cell with the minimum size, darkest spot to be considered a viable cell).

Cell Brightness (Darkest =50%, Brightest=90%) —Is the brightness of the cell boundary within a given image. Different cell types will have varying cell 'brightness' settings. The software detects the transition from dark (cell boundary pixels) to light (background of the image). This parameter separates cell pixels from background pixels. It represents a percentage of the grayscale range. A lower value will generally result in a higher pixel count for cells and means a dark boundary is required for cell identification but it must remain high enough to bypass all background pixels. A higher value means a lighter boundary. Pixels that are darker are treated as being part of the cell and those that are lighter as being outside of the cell.

Cell Sharpness (Sharpest=I, Least Sharp=200) —Represents the "clarity" of an image and specifies the minimum sharpness at the outer edge of a cell. This setting affects the focus discrimination algorithm. A higher value will require a more sharply-defined cell, to be accepted. Enter a range from 1 to 200. 1 represents sharpest, 200 unclear. This value also affects the transition from cell boundary (dark) to light (background).

Viable Cell Spot Brightness (Darkest=O%, Lightest=100%) —Is the brightness of the center spot of the cell. This is a percentage of the grayscale range. Cell centers must be higher (lighter) than this percent to be counted as viable. This parameter is expressed as a percentage of the background intensity. 75% is a typical value. Pixels in the center of a cell that are brighter than this setting are

considered to be part of the center spot. For latex beads the viable cell spot brightness should not be set less than 75%.

NOTE The cell brightness and sharpness help determine whether or not the boundary "dark" pixels belong to a cell or are part of the background. The cell spot brightness and area determine whether or not a cell is viable or non-viable.

Viable Cell Spot Area (Smallest=0% Largest-100%) —The cell spot area is a percentage of the total area of the Cell. The bright center spot must be larger than this, as a percent of the total area, to be considered viable. 5 to 10% are typical values. Any extremes in this value will either make the cells all viable or non-viable. If the area of the center spot (as determined from the viable cell spot brightness) is greater than this percentage of the total area contained within the outer edge of the cell (as determined from the cell sharpness), then the cell is considered to be viable.

Minimum Circularity (Least Circular=O, Perfectly Circular=l) —(Least Circular=0, Perfectly Circular=1). This parameter can be used to reject debris that exceeds the minimum cell diameter and are too irregularly shaped to be treated as a real cell. All objects with original (pre-fit) circularity less than this value will be ignored in the analysis. Raise this value to bypass debris more easily.

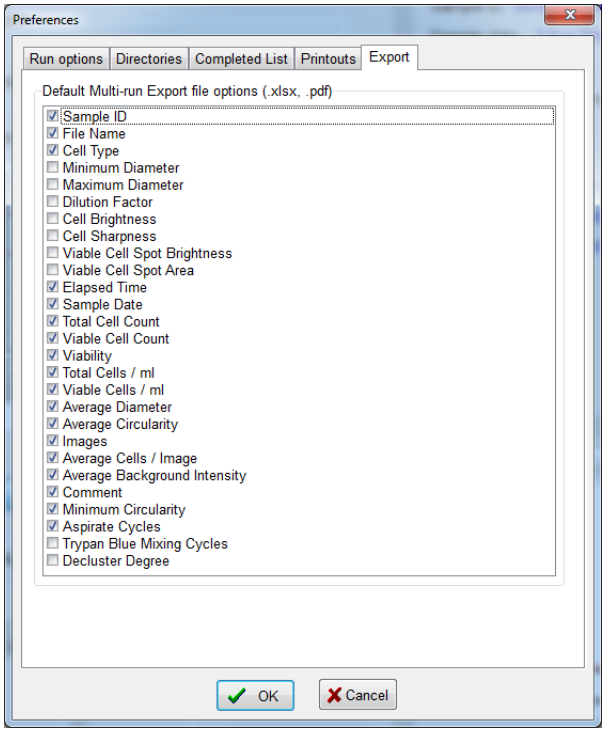
Decluster Degree (None, Low, medium or High) —The amount of 'de-cluster' applied to the sample. The default setting is medium. This function increases the ability of the software to detect cells that are clumped together. Set the de-cluster degree according to how well the cell clusters appear within the images (if not de-clustered properly). Generally, a higher value will identify more cells within a cluster, but a higher value may also split a single non-circular cell into more than one cell. If the cells in a sample are circular, a higher value can be safely used. High will perform the most aggressive declustering; Low will perform the least aggressive declustering. If individual cells are being split into 1 or more cells by the declustering, set this value to Medium or Low.

CHAPTER 6

Exporting Results

Exporting Data

Data can be exported in Microsoft Excel file format (xlsx) for archival or data manipulation. To setup export format (parameters to be included within the Excel file) select File, Preferences and check off the desired parameters.



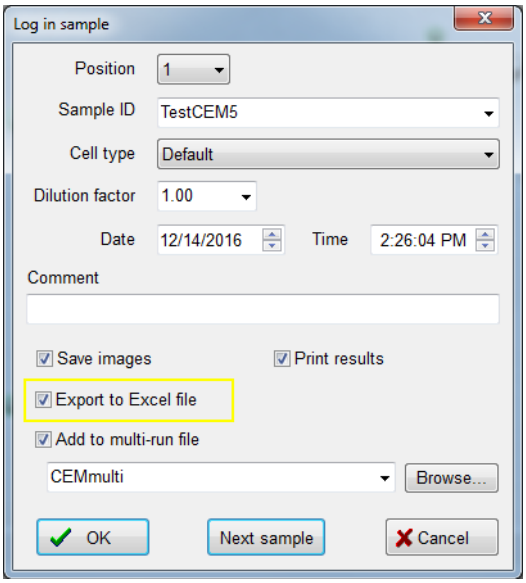
On the main menu tool bar, select File, then Export Run as Excel file.

Another way of exporting as an Excel file is to select the Export to Excel radio button on the Log In dialog box.

For saving data to the same Excel file, select the Add to multi-run Excel file.

The Multi-Run file must be defined in the Preferences > Run Options Tab. Either select a pre-existing multi-run file from the drop down menu, or enter a new name. It will be listed as one of the drop-down multirun options in the sample login dialog. When logging in a sample, ensure to check the Add to multi-run file in the Log In sample screen, and select the desired multifile for the data to be added to.

The default value for the check box is determined by the Auto-save run results to Excel format by default setting in configuration.



Exporting the Control Data

Exporting of the control data is a simple process. Select File, Control, and Export. The data is exported as an Excel file.

CHAPTER 7

Regulatory Compliance – 21 CFR Part 11

21 CFR Part 11

The Electronic Records and Electronic Signatures Rule (21 CFR Part 11) was established by the FDA to define the requirements for submitting documentation in electronic form and the criteria for approved electronic signatures. This rule, which has been in effect since August 20, 1997, does not stand in isolation; it defines the standards by which an organization can use electronic records to meet its record-keeping requirements. Organizations that choose to use electronic records must comply with 21 CFR Part 11. It is intended to improve an organization's quality control while preserving the FDA's charter to protect the public. Since analytical instrument systems such as the Vi-CELL XR Cell Viability Analyzer, generate electronic records, these systems must comply with the Electronic Records Rule.

This section describes the relevant portions of the 21 CFR Part 11 regulations and their implementation using the Vi-CELL XR control software. The implementation and compliance of 21 CFR Part 11 remains the responsibility of the organization or entity creating and signing the electronic records in question. Proper procedures and practices, such as GLP and GMP, are as much part of the overall compliance with these regulations as are the features of the Vi-CELL XR control software.

Electronic Records

Per Section 11.3 subpart A of 21 CFR Part 11, an electronic record is 'any combination of text, graphics, data, audio, pictorial, or other information representation in digital form that is created, modified, maintained, archived, retrieved or distributed by a computer system'. This refers to any digital computer file submitted to the FDA, or any information not submitted but that needs to be archived. Public docket No. 92S-0251 of the Federal Register (Vol. 62, No. 54) identifies the types of documents acceptable for submission in electronic form and where such submissions may be made.

FDA Requirements

The general comments section of the ruling states that 'The agency emphasizes that these regulations do not require, but rather permit, the use of electronic records and signatures'. The introduction to the final ruling states that 'The use of electronic records as well as their submissions to FDA is voluntary'.

If electronic submissions are made, Section 11.2 explains that 'persons may use electronic records in lieu of paper records or electronic signatures in lieu of traditional signatures provided that: (1) The requirements of this part are met; and (2) The document or parts of a document to be submitted have been identified in public docket No. 92S-0251'.

The Vi-CELL XR control software has been designed to allow users to comply with the electronic records and signatures rule. Any organization deciding to employ electronic signatures must declare to the FDA their intention to do so.

Implementing Electronic Records and Signatures

Section 11.3 Subpart A describes two classes of systems:

Closed Systems

A closed system is one 'in which system access is controlled by persons who are responsible for the content of electronic records'. In other words, the people and organization responsible for creating and maintaining the information on the system are also responsible for operating and administering the system.

Open Systems

An open system is one 'in which system access is not controlled by persons who are responsible for the content of electronic records'.

The Vi-CELL XR control software is designed to ensure the proper operation, maintenance and administration for system security and data integrity. Anyone who interacts the Vi-CELL XR, from administrators to users, must abide by these procedures. Therefore the ultimate responsibility is with the organization generating electronic records and signatures. The Vi-CELL XR software is a component, albeit a vital one, of the overall process.

Controls for Electronic Records

Subpart B, Section 11.10 describes the controls to be applied to a "closed system". Section 11.30 describes the controls for an "open system", which include "those identified in Section 11.10, as appropriate, and additional measures such as document encryption and use of appropriate digital signature standards". Since a typical Vi-CELL XR system can be regarded as a closed system, additional controls for open systems will not be discussed in this document. The primary thrust of these controls is "to ensure the authenticity, integrity, and, when appropriate, the confidentiality of electronic records, and to ensure that the signer cannot readily repudiate the signed record as not genuine". In other words, to protect the data and to make it difficult for someone to say that this is

not their "signature". Many of the controls described in Section 11.10 refer to written procedures (SOPs) required of an organization by the agency, for the purpose of data storage and retrieval, access control, training, accountability, documentation, record keeping, and change control. The other controls are addressed either by the Vi-CELL XR software itself, or in combination with end-user procedures.

Of the other controls, perhaps the foremost is described in Section 11.10 Paragraph (a): "Validation of systems to ensure accuracy, reliability, consistent intended performance, and the ability to discern invalid or altered records." It is the complete and overall validation of the system, as developed by the organization, which ensures the integrity of the system and the data within. It is to this end that the features of the Vi-CELL XR software comply with the specifications of these regulations.

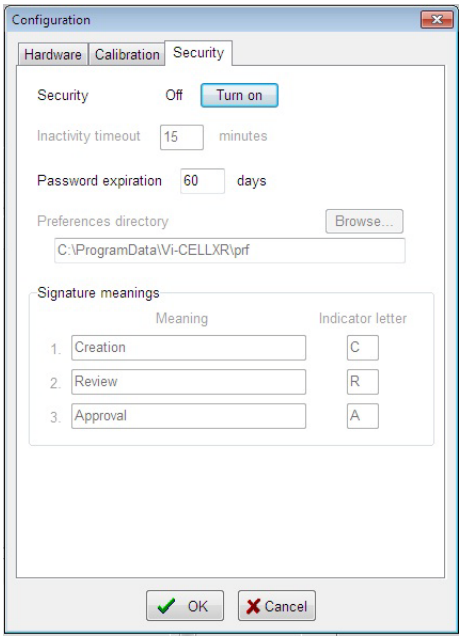
Establishing an Electronic Record

The Vi-CELL XR software employs a system of usernames and passwords, consistent with the specifications of Subpart C, Section 11.300, "to ensure that only authorized individuals can use the system, electronically sign a record, access the operation or computer system input or output device, alter a record, or perform the operation at hand".

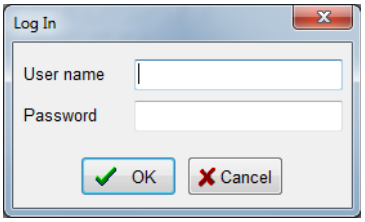
21 CFR Part 11 Security

To turn on the security option, select File > Configuration, select the Security tab and Turn on.

NOTE Inactivity timeout is set to prevent unofficial access to the system, as when the system is left unattended directly after starting the queue.



The system will prompt you to log in. On the Log In dialog, enter your user name and password.



Once signed on, select File > Security > Add New User to define users and access levels. See Figure 7.1.
The Add New User dialog box appears. See Figure 7.2.

Figure 7.1 The Security Options Screen

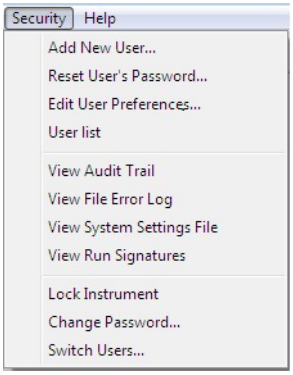
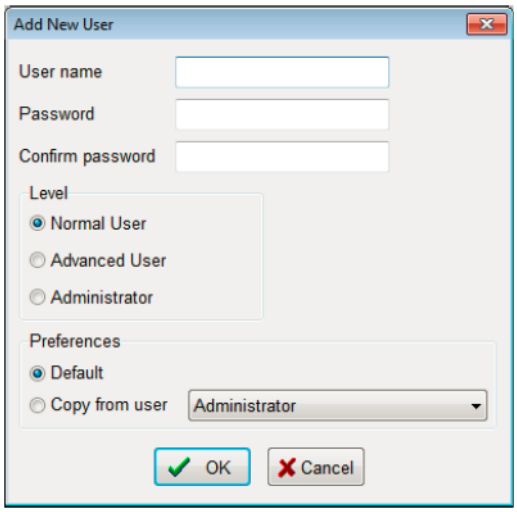


Figure 7.2 Add New User



New users can only be created and passwords reset by users with Administrator rights. This file is protected with a checksum and for each user name, contains information on when the user was created, by whom, at what level, the user's password in encrypted form and the user's file paths. If this file does not exist or if the checksum is missing or invalid then access to the system will only be possible to a limited number of special users.

File History

The Vi-CELL XR software also performs data input and "operational checks", as specified in Subpart B, Section 11.10, "to determine, as appropriate, the validity of the source of data input or operational instruction", and "to enforce permitted sequencing of steps and events". These two features ensure that, as much as possible, valid data are being entered into the system, and all required steps have been completed to perform the task at hand.

The purpose of all such data checking and validation is described in Section 11.10, Paragraph (b): "The ability to generate accurate and complete copies of records in both human readable and electronic form suitable for inspection, review, and copying by the agency". Vi-CELL XR software data files are all automatically saved upon creation and protected with a checksum. Vi-CELL XR software also allows for the capability for backing up data to mirror directories.

Section 11.10, paragraph (e) requires “use of secure computer-generated, time-stamped audit trails to independently record the date and time of operator entries and actions that create, modify, or delete electronic records. Such audit trail documentation must be retained for a period at least as long that required to for the subject electronic records and must be available for agency review and copying.” The Vi-CELL XR software complies with this rule by generating an audit trail which records the time a user was logged on. The audit trail is encrypted and checksummed for added security. The audit trail also will record and time-stamp: failed login attempts, switching users, turning security on or off, adding new user, enable/ disable user, change password, reset password, lock instrument and failed checksums.

When a data file is created, the Vi-CELL XR system software provides a computer-generated time-stamped record that documents actions taken to create a record. This information is stored within the actual data file itself, not in the Audit Trail file. Each data file contains a computer-generated time-stamped record, the date and time of operator entries, and the actions taken to create the data file.

The system software does not allow a data record to be modified or deleted within the normal operation of the system software.

If the integrity of a data file is compromised in some way, the file is rendered unusable by the system and it can no longer be used by the Vi-CELL XR software. Each data file contains an embedded checksum that is used to check the integrity of the file each time the file is loaded. If the data file is compromised, an error message is displayed and the file does not load.

Electronic Signature

In Subpart A, Section 11.3, an electronic signature is defined as "a computer data compilation of any symbol or series of symbols executed, adopted, or authorized by an individual to be the legally binding equivalent of the individual’s handwritten signature". Subpart C, Section 11.100 of the regulation defines the general requirements of such a manifestation. Paragraph (a) states that "each electronic signature must be unique to one individual and must not be reused by, or reassigned to, anyone else". These two paragraphs, taken together, mean that an electronic signature is some computer representation of a user’s identity, developed to ensure the distinct and unique identity of that user. The procedural aspect of Section 11.100 requires that before any such electronic representation is applied, the organization first must "verify" the identity of that individual. The subsequent use of electronic signatures as the "legally binding equivalent of traditional handwritten signatures" then must be "certified" to the agency in writing.

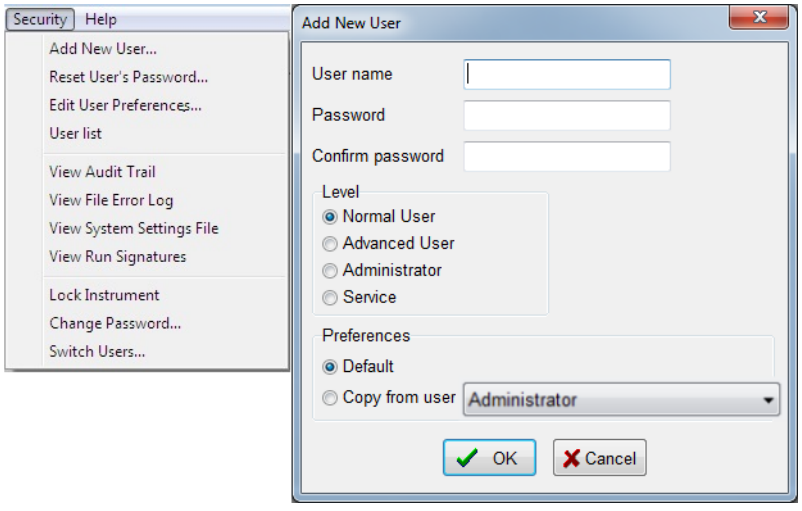
Subpart C, Section 11.200, refers to biometric and non-biometric forms of electronic signature. Biometric signatures are defined in Subpart A, Section 11.3 as a "a method of verifying an individual's identity based on measurement of the individual's physical feature(s) or repeatable action(s) where those features and/ or actions are both unique to that individual and measurable". Biometrics are generally regarded as techniques such as fingerprints or retinal scans, which are considered to be totally unique to each individual and require specific forms of scanning devices to read and interpret. Non-biometric signatures are those that are computer generated and, as per Section 11.200, "Employ at least two distinct identification components such as an identification code and password". It is this form of electronic signature that is supported by the Vi-CELL XR software.

Generating Electronic Signatures

The Vi-CELL XR software employs User IDs and passwords to verify the identification of each user logging into the system. When using this technique, Subpart C, Section 11.300 of the regulation requires "maintaining the uniqueness of each combined identification code and password, such that no two individuals have the same combination of identification code and password". This section also requires that the "identification code and password issuances are periodically checked, recalled, or revised". Vi-CELL XR software supports both of these provisions.

The administration of the system requires that individuals are added to the list of valid Vi-CELL XR users via the Add a New User dialog box. The "identification code" or username of each Vi-CELL XR user must be unique. No two users on the same Vi-CELL XR system can have the same user name. It is also required that these users supply a password to access the Vi-CELL XR software, thus satisfying the requirement to "employ at least two distinct identification components such as an identification code and password". Passwords can be controlled to prohibit the use of duplicates and to force the selection of new passwords after a prescribed period of time.

By the implementation of these features, the Vi-CELL XR software can satisfy the requirement that "identification code and password issuances are periodically checked, recalled, or revised".



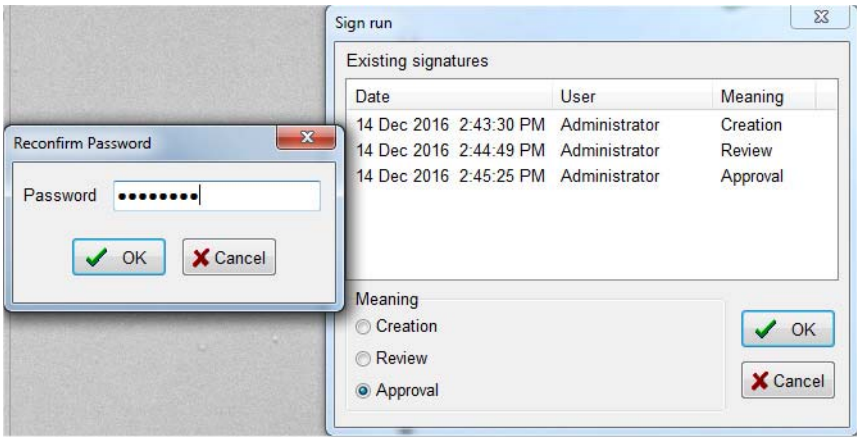
Signing A Run

Once users have been created and access levels assigned, users given signing rights are able to sign a run upon completion and review of analysis data. To generate the electronic signature, open a file as described in Viewing Data in CHAPTER 4, Software Menus. Once the file is either opened, created or reviewed select Sign Run on the lower right side of the screen to approve a run.

The Sign Run dialog box will appear. Select Creation, Review or Approval (these options are defined by the Administrator). A Reconfirm Password dialog box will appear. Enter the password.

The results will then be electronically signed and saved. All Completed Run log files are encrypted and checksummed for security.

Figure 7.3 Sign Run



Meanings

- Creation - Generating a file
- Review - Checking of data
- Approval - Acceptance of data

NOTE Within the Sign Run dialog, the users can select the meaning of the signature. These are recommendations only, and the meanings of the signature can be defined in Configuration > Security.

Applying Electronic Signatures

Subpart C, Section 11.200 stipulates several requirements for the control of electronic signatures. Procedurally, the regulations require that electronic signatures "be used only by their genuine owners" and that they "be administered and executed to ensure that attempted use of an individual's electronic signature by anyone other than its genuine owner requires collaboration of two or more individuals". Through the application of Vi-CELL XR user and password configuration procedures, the system can be configured to "ensure" that inappropriate use of these identifiers can be performed only by the intentional divulgence of security information.

Section 11.200 further specifies the use of electronic signature components during a period "when an individual executes a series of signings during a single, continuous period of controlled system access", and "when an individual executes one or more signings not performed during a single, continuous period of controlled system access". This section of the document represents the "heart" of electronic signature application. To comply with these provisions, the Vi-CELL XR software uses the application of the username and password to authenticate the user making and saving the changes, in conjunction with file history.

APPENDIX A

System Specifications

Data Acquisition

- Operating principle: analysis of video images
- Sample type: spatial data
- Cell size range: 3 microns to 70 microns

  NOTE Although the size range for system performance is 3 microns to 70 microns, the system is capable of detecting particles down to 2 microns.

- Analysis rate: up to 50 Images in 2.5 minutes
- Digitizing resolution: 1.45 mega-pixels

Cell Viability/Concentration/Cell Count

- Concentration Range: 5×10^4 to 1×10^7 Cells/ mL
- Viability Range: 0 to 100%
- Mean Recovery Value of 20 replicates of Concentration Control on Vi-CELL XR compared to the lot assay value used are ±6%.

  NOTE The results for samples at the low end of the concentration range will not be as statistically accurate due to the low number of measured cells. The accuracy at the high end of the concentration range is affected by the difficulty of declustering groups of cells particularly if the cells are large. The sample should be diluted to bring its concentration into range or to improve the accuracy of the results

Physical Requirements

- Power 50 watts (65 watts max.)
- Voltages 100, 120, 220, 240 VAC 50/ 60 Hz
- Temperature 10 to 40°C (50 to 104°F)
- Fuses 1 - 120 V 1 A SLO-BLO, 2 - 240 V 2.5 A SLO-BLO

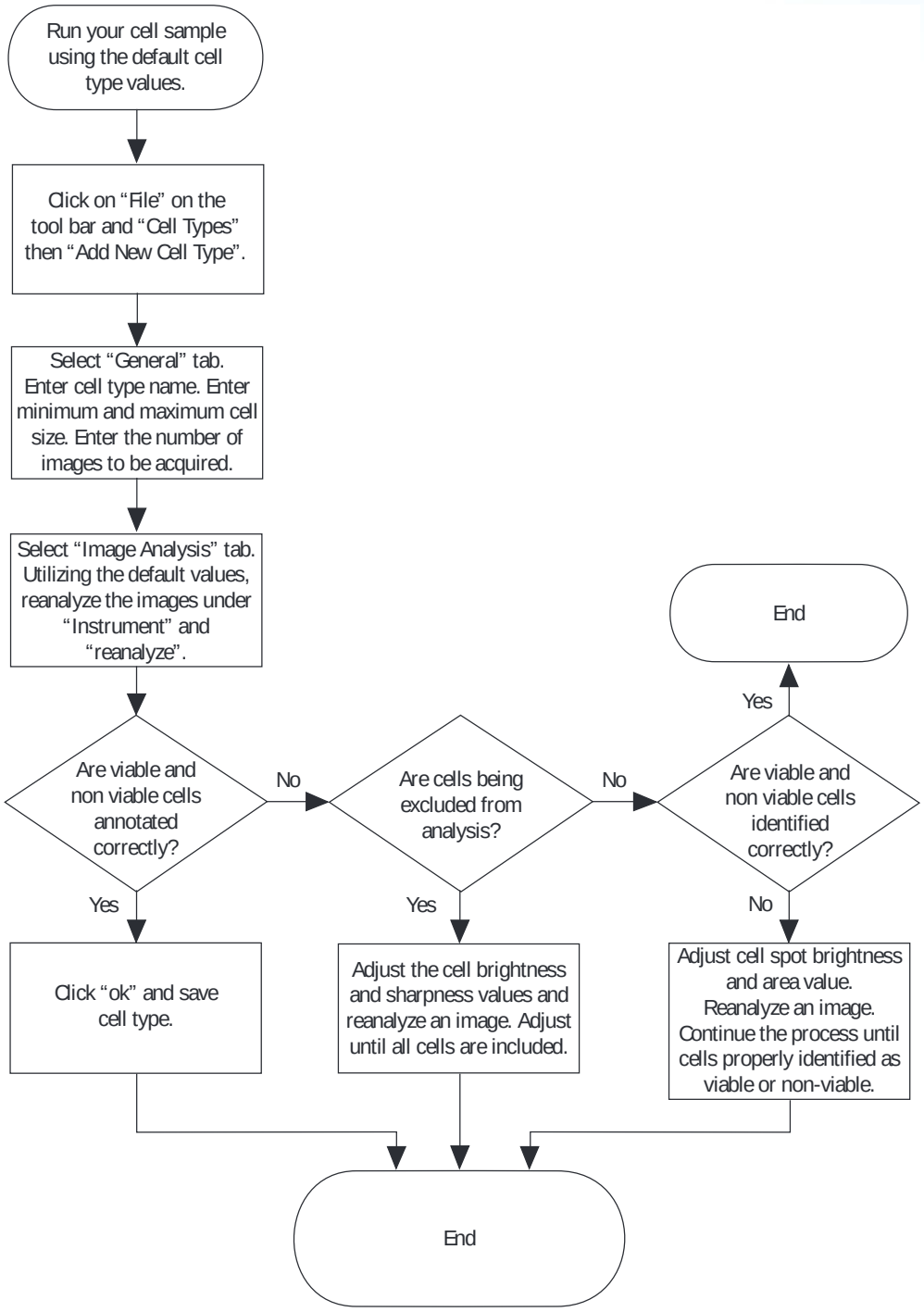
Unit Dimensions

- Analyzer: 17.5 in. (44.5 cm) x 15.0 in. (38 cm) x 16.0 in. (41 cm)
- Weight: 25 lbs. (11.3 kg.)

APPENDIX B

Cell Types – Flowchart

Creating and Modifying a Cell Type



APPENDIX C

Statistics

Circularity

A value from 0 to 1, with 1 representing a perfect circle. Computed as Da/Dp, where Da = square root (4 A/ p), Dp = P / p ; A = pixel area, P = pixel perimeter.

The circularity distribution is based on individual cells, not cells that are part of clusters.

System Performance

Run Statistics

Cell Count — The actual number of cells recorded per frame and for the total number of frames.

Viable Cells —The number of viable cells per image and for the total number of images.

Viability —The percentage of viable cells per image and for the total number of images.

Total Cells/mL — The concentration of cells per mL.

Viable Cells/mL —The concentration of viable or "live" cells per mL.

Avg. Diameter —The average size of cells per image and for total images.

Avg. Circularity —The average "roundness" of the cells.

Images —The total number of images analyzed.

Average Cells/Image —The number of cells captured per image.

Background Intensity —The average pixel value, from 0 to 255, of the image background.

Calibration

Micron/Pixel Ration —The micron distance that a linear pixel represents.

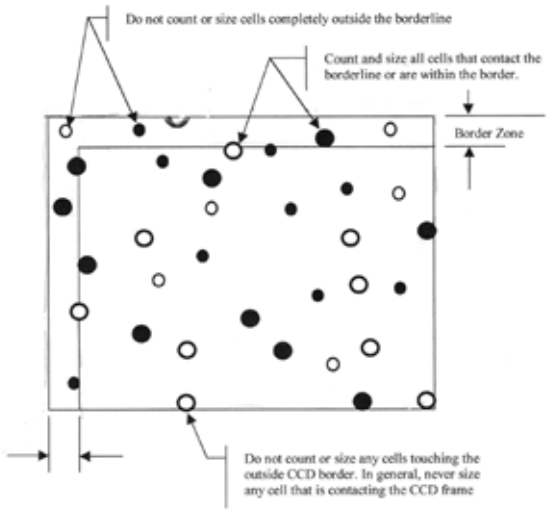
Magnification —The increase in size, as a factor, from an actual object to its image on the CCD array.

Image Size —The area in square cm that each image encompasses.

Effective Field of View

"Effective Field of View" is the size of the area inside the reduced measurement frame, and it most accurately represents the actual counting area. It is the area that should be used in computing cell concentrations.

The Effective Field of View value in the configuration or calibration screens permits you to calculate concentrations to verify them against reported values.



The border zone indentations on the top and left border of the image are used to correct for the effect whereby larger objects are more likely not to be because of contact with an edge of the image. That effect introduces a bias into size distributions and concentration results, and the indents are a way to correct for it.
Objects intersecting the bottom or right edge are not counted.
Objects that are partially in the main counting area and partially in the border zone are counted.
Objects that are entirely within the border zone are not counted.
With this method, larger objects that would have gotten rejected are now counted.
Smaller objects are not affected (the correction varies in proportion to particle size).
The border indent is 50 microns, which is large enough to handle all actual cell sizes that are encountered.

APPENDIX D

Troubleshooting

Black Image

Possible causes are:

- Light source failure
- Camera failure or camera cable problem
- Digitizer board problem
- Light completely obstructed by an object or extremely high cell density
- Board or camera not present
  - Check that the FireWire board and associated driver have been correctly installed and that the FireWire cable is correctly plugged into the PC and instrument.

Error Messages During Program Start

Instrument not connected.

Possible causes are:

- an instance of Vi-CELL XR is already running on the computer
- there is a problem with the board driver
- the serial cable is not connected
- check the communications settings on the hardware tab of the configuration dialog to ensure that the Port is the same as the port that the connection cable is plugged into and that the Baud rate is set to 9600.

Erratic or Delayed Image Capture

Possible causes are:

- Interrupt conflict
- Network connection

  In this case, the network driver must be disabled when running Vi-CELL XR (simply disconnecting from the network or removing cables will not work).

Prime Or Replace Reagent Pak Message and One or More Reagent LEDs Off

Possible causes are:

- Out of one or more reagents
- Reagent tubing coming from Vi-CELL XR to the Reagent pack has been pulled, causing optical sensor to not see fluid in the line. To remedy, first ensure there is indeed reagent in the lines and the reagent pack then grab the tubing near the opening where the tubing exits the instrument and press in the tubing slightly until the LED comes on.

APPENDIX E

Maintaining The Vi-CELL XR

Decontamination Procedure

WARNING

All instrument decontamination must be conducted under universal precautions for blood-borne pathogens. Instrument effluents should be regarded as a biohazard. Special care is required when opening pressurized fluid lines.

WARNING

Risk of personal injury or contamination. To prevent possible injury or biohazardous exposure, always wear proper laboratory attire, including gloves, a laboratory coat, and eye protection.

IMPORTANT In the Vi-CELL XR software, there is a Decontamination wizard, which guides the operator through this standard procedure (for the internal components). Select Instrument and, from the drop-down box, Decontaminate. The Vi-CELL XR performs the procedure via the embedded wizard.

1 Perform the decontamination cycle as instructed by the decontamination wizard embedded in the software.

2 Decontaminate the external aspects of the system by washing with 0.5% Sodium hypochlorite solution (10% bleach, prepared by mixing 1 part household bleach with 9 parts water).

3 Remove all dried blood or cell culture media from the instrument surface before disinfection.

a. To remove these substances and prevent scattering potentially biohazardous material, the blood or culture media should be wetted and softened with the 0.5% sodium hypochlorite solution.

b. After removal of the dried substances, decontaminate the surface of the Vi-CELL XR with the bleach solution again.

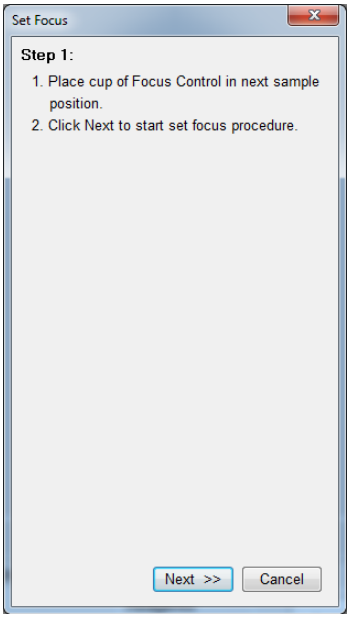
c. If complete removal is not possible, expose the instrument surface to the 10% bleach solution for 20-30 minutes. Rinse the surface with water to remove the bleach.

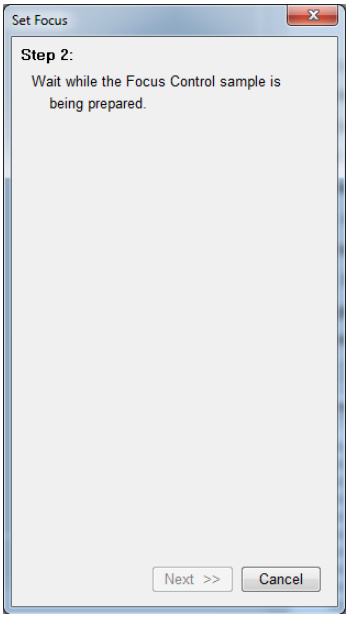
APPENDIX F

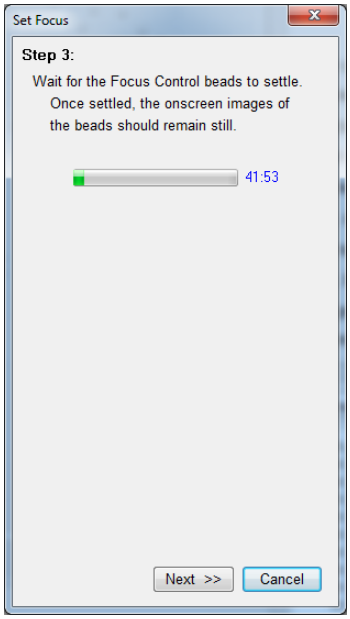
Autofocusing Procedure

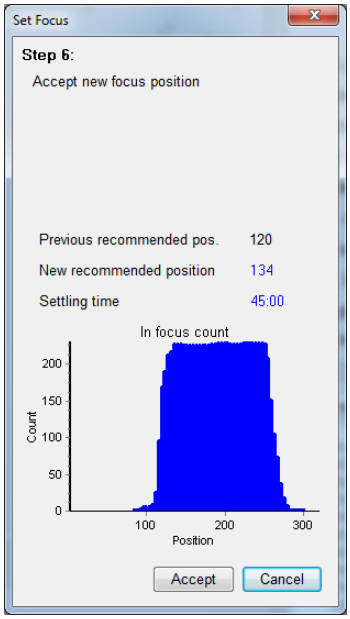
Focusing Wizard

A focusing wizard is provided that automatically checks and if necessary adjusts the focus. Selecting the set focus item in the diagnostic menu and using the Vi-CELL XR Focus Control that is supplied with the instrument (or can be supplied by Beckman Coulter) begins the process. It is especially important to run the wizard after the instrument has been physically moved to ensure optimum results.






Set Focus
Step 3:
Wait for the Focus Control beads to settle.
Once settled, the onscreen images of
the beads should remain still.
41:53
Next >>
Cancel


Set Focus
Step 6:
Accept new focus position
Previous recommended pos. 120
New recommended position 134
Settling time 45:00
In focus count
Count
200
150
100
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Abbreviations

CD —compact disc

CD-RW —compact disc - rewritable

DVD —digital video disc

GB —gigabyte (1024 megabytes)

IEEE —Institute of Electrical and Electronics Engineers

HD —hard drive

OHCI —open host controller interface

PCI —peripheral component interconnect

PN —part number

RoHS —Restriction of Use of Hazardous Substances

X —times

RAM —random access memory

WEEE —Waste Electrical and Electronic Equipment Directive

XR —extended range

XGA —extended graphics adapter

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Related Documents

Vi-CELL XR Cell Viability Analyzer, Instructions for Use
PN 383674

- Introduction
- Introducing the Vi-CELL XR
- Installation and Verification
- Quick Start Guide
- Software Menus
- Special Software Features
- Exporting Results
- Regulatory Compliance - 21 CFR Part 11
- Appendices
- References

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