Vi-CELL XR Cell Viability Analyzer

Reference Manual

PN 383674BA (October 2011)

Beckman Coulter, Inc.
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BECKMAN COULTER

WARNINGS AND PRECAUTIONS

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 YOUR BECKMAN COULTER REPRESENTATIVE.

HAZARDS AND OPERATIONAL PRECAUTIONS AND LIMITATIONS

WARNINGS, CAUTIONS, and IMPORTANTS alert you as follows:

WARNING - Can cause injury.
CAUTION - Can cause damage to the instrument.
IMPORTANT - Can cause misleading results.

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 IT IS NOT LIMITED TO, PROTECTIVE EYEWEAR, GLOVES, AND SUITABLE LABORATORY ATTIRE WHEN OPERATING OR MAINTAINING THIS OR ANY OTHER AUTOMATED LABORATORY ANALYZER.

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. Do not defeat safety interlocks and sensors.
- 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 might be compromised and operational failures might 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.

IMPORTANT If you purchased this product from anyone other than Beckman Coulter or an authorized Beckman Coulter distributor, and, if 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.

REVISION STATUS

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.

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

Note: Changes that are part of the most recent revision are indicated in text by a bar in the margin of the amended page.

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

REVISION STATUS

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ILLUSTRATIONS

TABLES

1.1 MANUAL DESCRIPTION

Scope

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

Conventions

Menu and dialog items that can be selected or clicked appear in **bold** type.

Warnings, **Cautions** and **Important** items also appear in **bold** type.

CAUTIONS – indicates a situation or procedure that, if ignored, can cause damage to the instrument.

WARNING – indicates a situation or procedure that, if ignored, can cause personal injury.

1.2 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.

Reagent Specific Precautions

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

1.3 OTHER PRECAUTIONS

Warnings

Instrument shall not be operated without Syringe Shield in place.

Figure 1.1 Vi-Cell XR

1. **Electrical** – High voltages are present inside the instrument. Always disconnect the instrument from the power supply before removing the cover.
2. The instrument must be grounded correctly.
3. Toxicity, safety, and proper handling procedures for diluents and reagents used should be adhered to at all times. Consult appropriate safety manuals and Material Safety Data Sheets for the items.
4. **Mechanical**. – Risk of Operator Injury if:
   - All covers, including syringe shield, reagent valve cover, and aspiration tube are not secured in place prior to and during instrument operation.
   - Contact is made with moving parts.
   - Broken Parts are disposed of incorrectly.
   - Doors, covers, and panels are not opened, closed, removed and/or replaced with care.
   - Improper tools are used for troubleshooting.

Cautions

Electrical – Use the Windows **Shut Down** command to turn the computer off before switching off power. Switching off power first will generate a Windows error message at the next computer start up.

2.1 SYSTEM OVERVIEW

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 comply 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 2 µm to 70 µm
- 12-position auto-sampler
- User-friendly reagent system

2.2 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.

2.3 HOW VIABILITY IS DETERMINED

The Trypan Blue Dye Exclusion Method

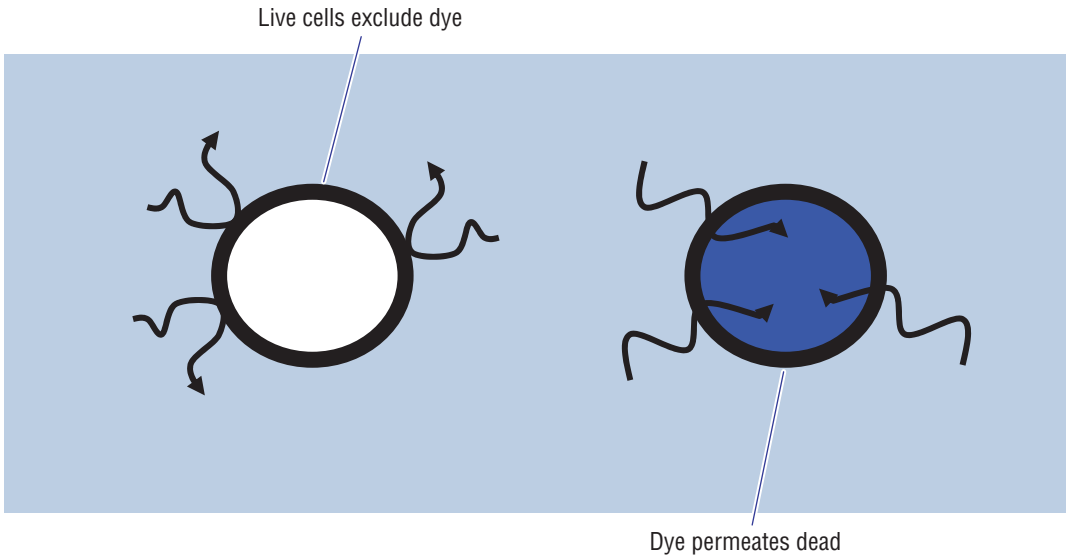
The widely accepted method for cell viability determination is the Trypan Blue Dye Exclusion Method. When cells die, their membranes become permeable allowing for the uptake of the trypan blue dye. As a result, the dead or non-viable cells become darker than the viable cells. This contrast is what is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

An Image Analysis Solution

The Beckman Coulter Vi-CELL XR automates the Trypan Blue Dye Exclusion Method. Utilizing the latest in 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 2.1 Trypan Blue Dye Exclusion Method



2.4 SYSTEM COMPONENTS

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

Figure 2.2 Front view of the Vi-CELL XR Analyzer

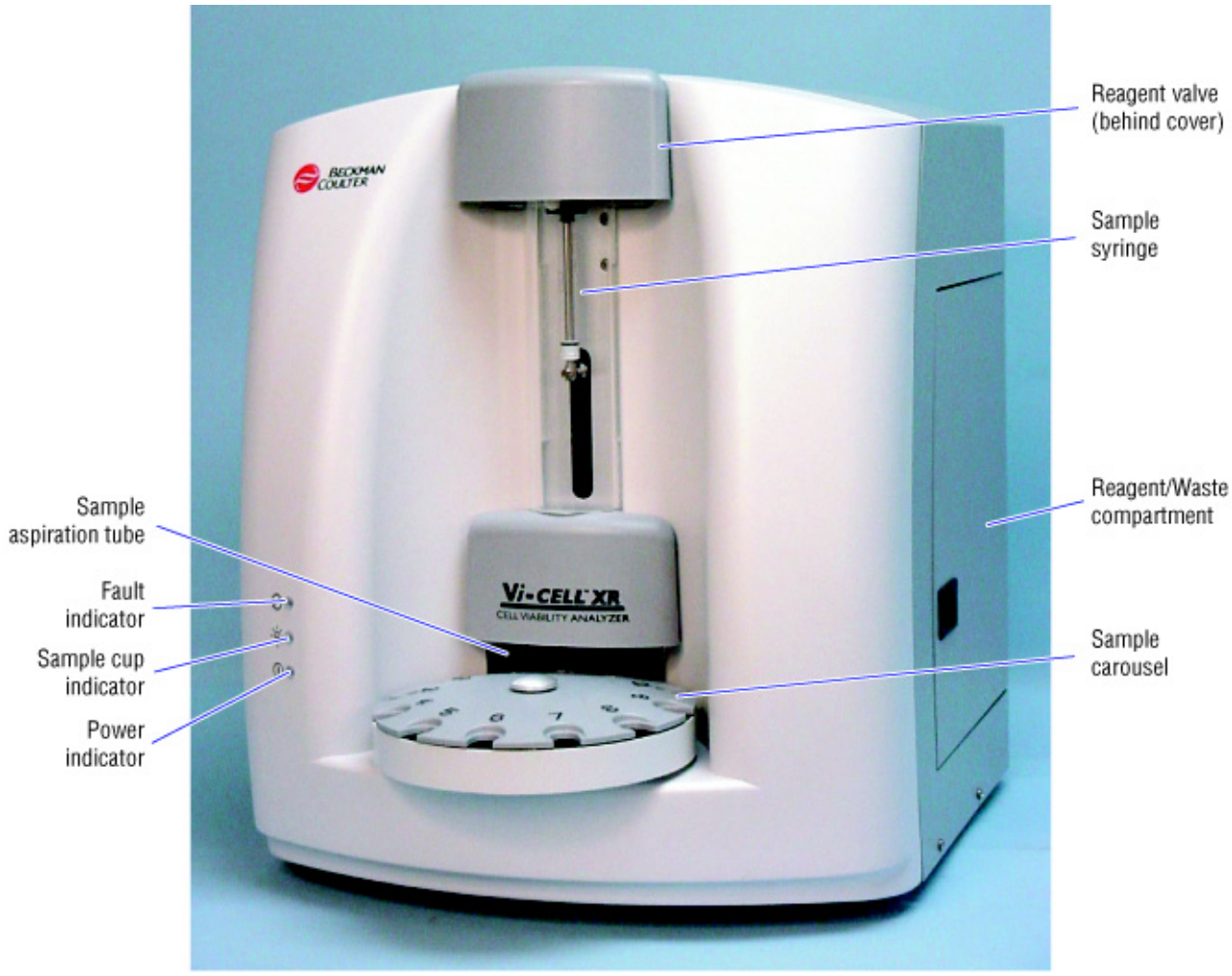
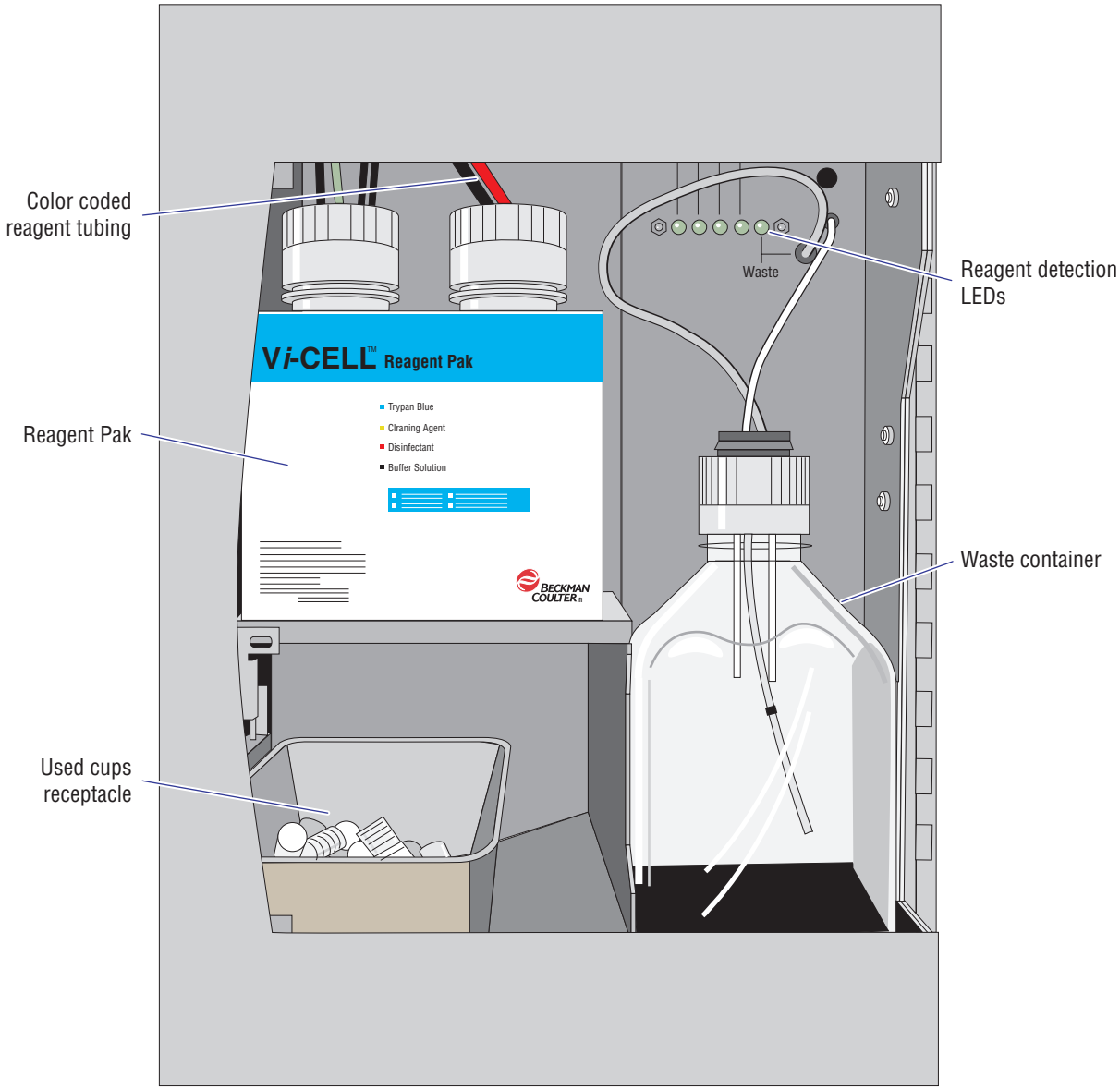


Figure 2.3 Right side of the Vi-CELL XR showing reagent compartment



Sample Delivery Options

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

Computer System

- Windows: 2000 or XP
- Ram: 256 MB DRAM minimum
- Processor speed: Equivalent to Pentium 4, 1.5 GHz or better
- 40 GB hard drive minimum
- CD Rom Drive 12X CD – ROM minimum, CD –RW preferred
- Monitor: XGA or better
- PCI graphics card with minimum 256-color palette. Screen resolution of 1024 x 768
- OHCI compatible IEEE – 1394 FireWire card

Software

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

3.1 INTRODUCTION

The Vi-CELL XR will normally 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.

3.2 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

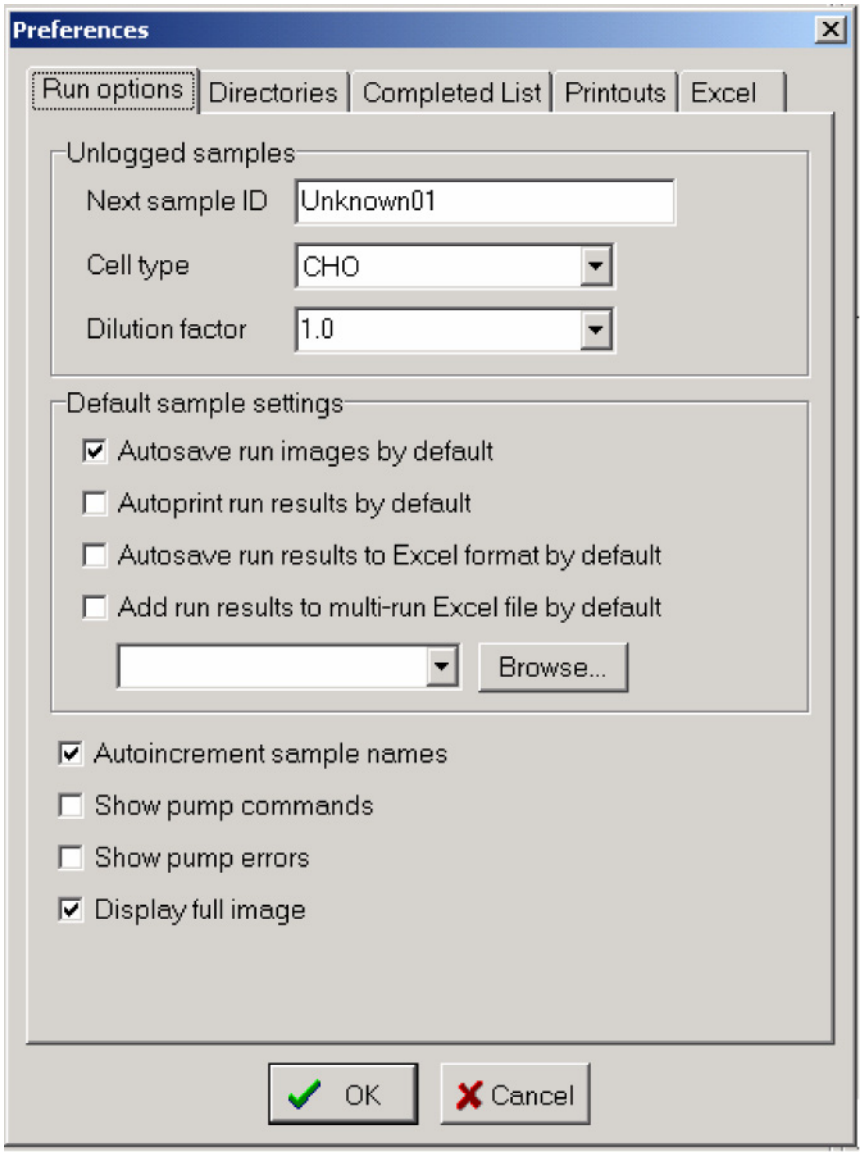
- Temperature: 10 to 40°C (50 to 104°F)
- Humidity: 10 to 85%

3.3 INSTALLING AND STARTING THE SOFTWARE

Once software is installed, an icon will be placed on the desktop. Double clicking on Vi-CELL XR software icon will launch the program.

Select **File, Preferences** to access the Preferences dialog.

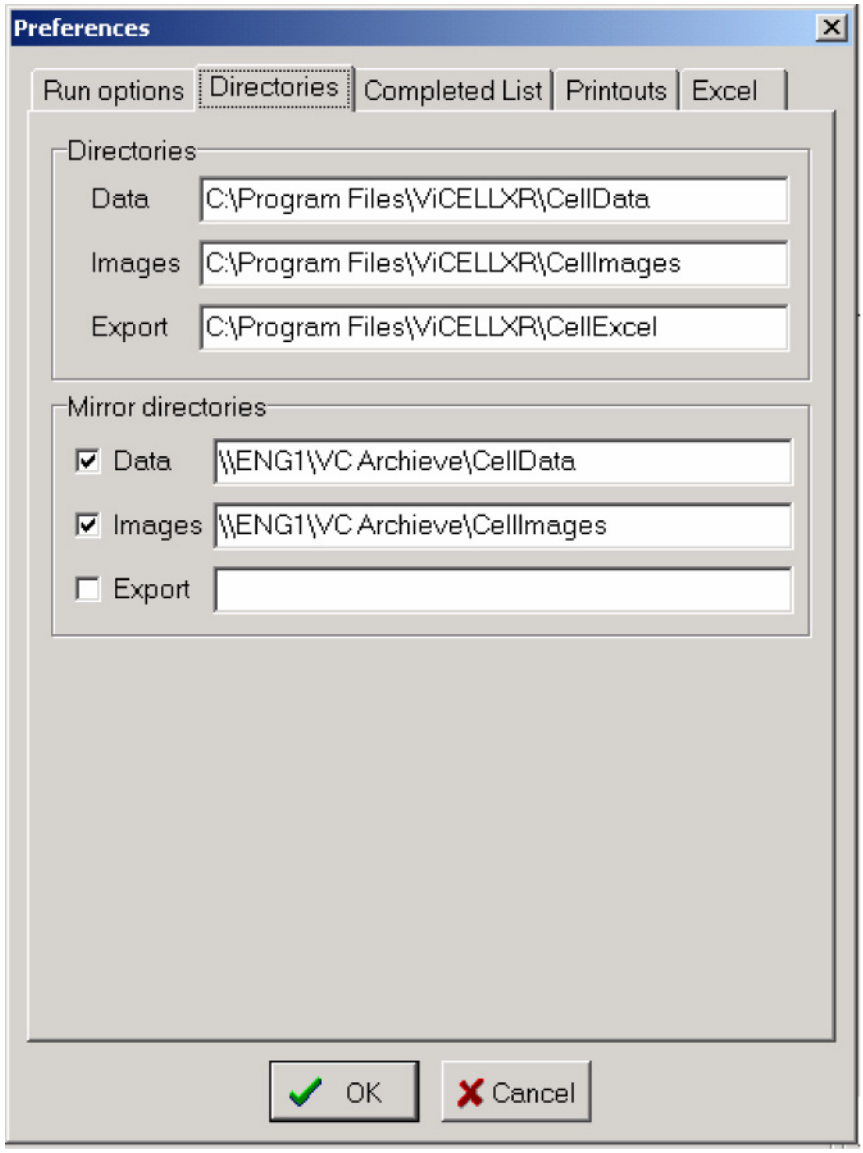
Setting Up Preferences



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

These functions can also be performed from the **Log in sample** dialog by checking the radio buttons provided.

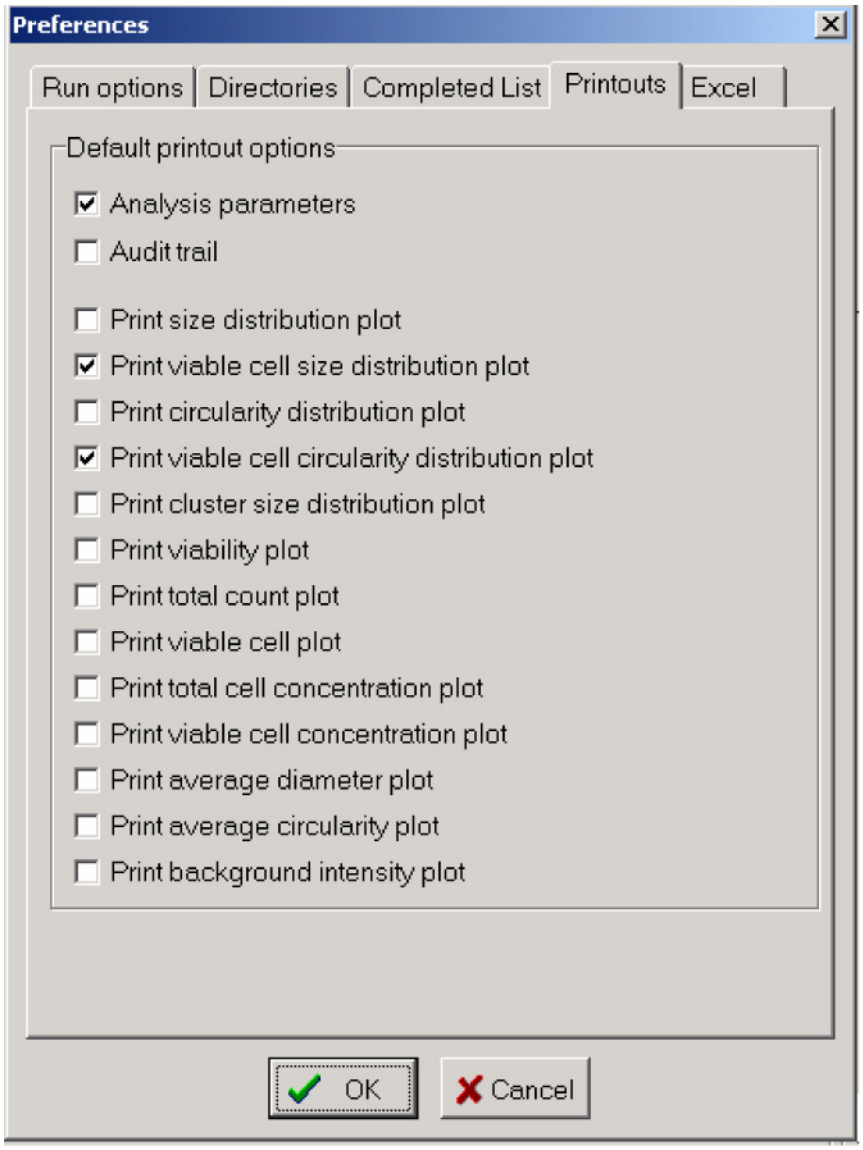
Default Printer may also be defined within the Configuration dialog **Beckman Coulter Personnel will configure hardware and calibration**.



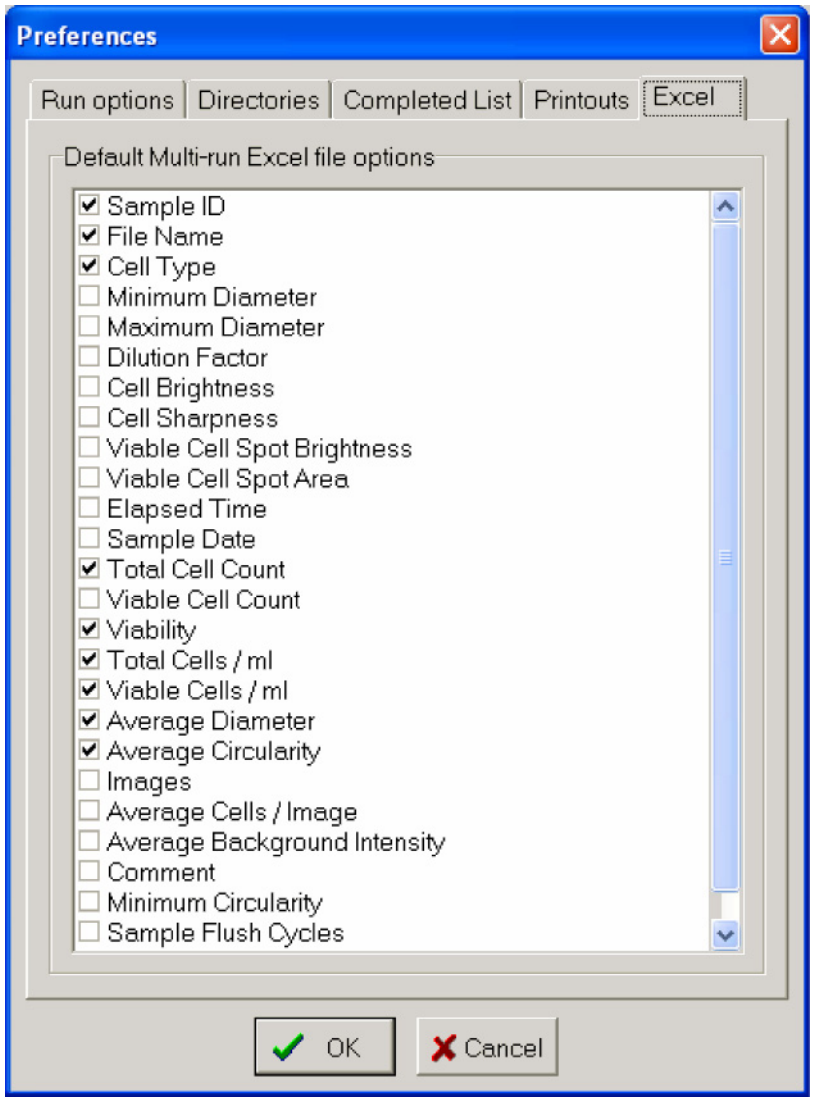
Select the directories tab to check that the directory information is acceptable or change the path accordingly. The data directory shows the directory where run, bioprocess and control files are to be stored. The images directory indicates where camera images are to be stored. The export directory shows where the run results in Excel format are to be stored.

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. Again, this may be a network drive.

The Preferences 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.


Preferences
Run options
Directories
Completed List
Printouts
Excel
Default printout options
Analysis parameters
Audit trail
Print size distribution plot
Print viable cell size distribution plot
Print circularity distribution plot
Print viable cell circularity distribution plot
Print cluster size distribution plot
Print viability plot
Print total count plot
Print viable cell plot
Print total cell concentration plot
Print viable cell concentration plot
Print average diameter plot
Print average circularity plot
Print background intensity plot
OK
Cancel

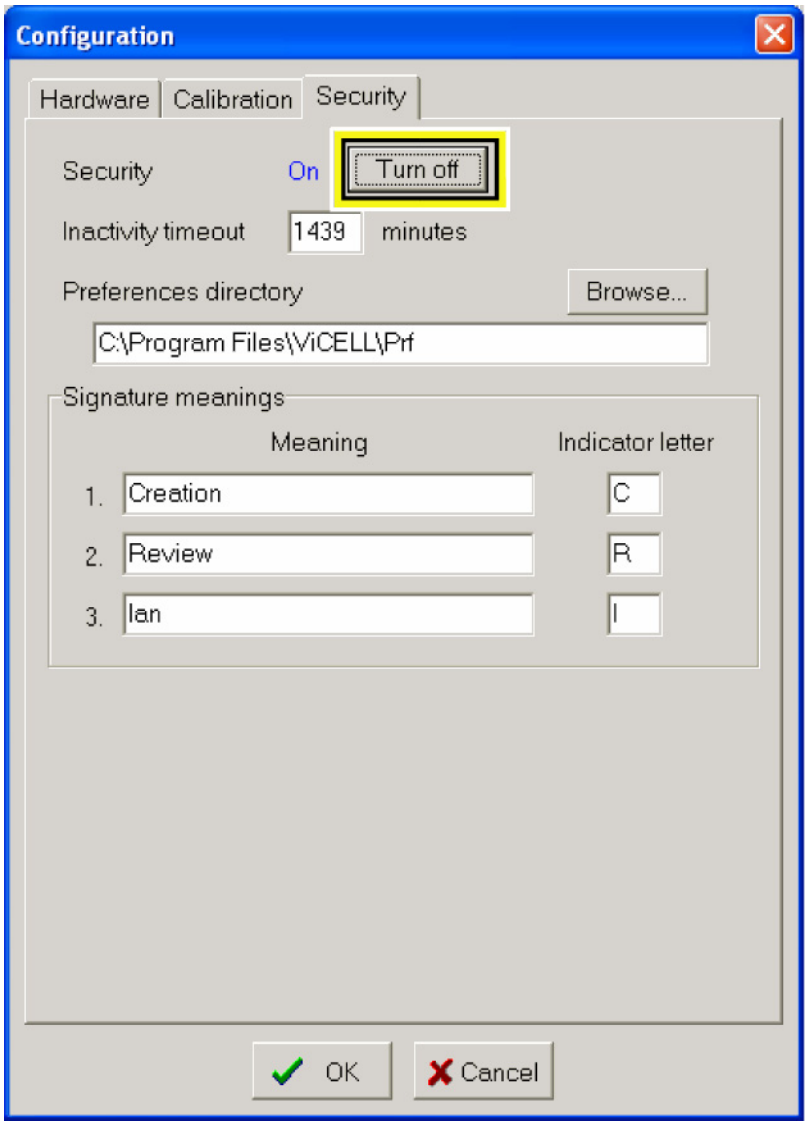
The Excel tabs allows for the selection of parameters to be archived in Excel (xls) format.



Security Configuration

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

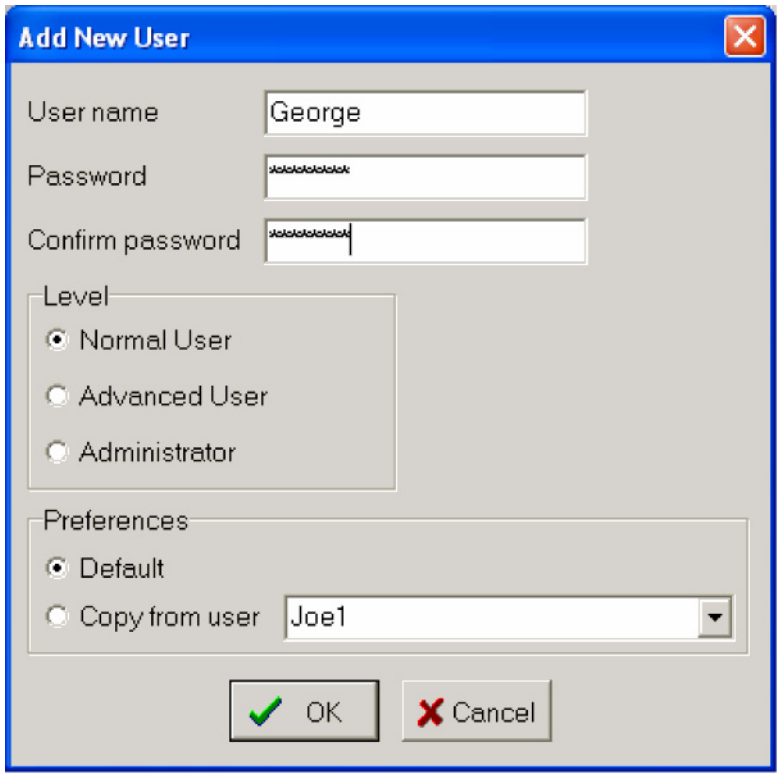
With security on, the instrument has been designed to comply with the US Food and Drug Administration's (FDA) regulations on electronic records and electronic signatures (21 CFR Part 11). Vi-CELL XR run files have been designed to meet the requirements for electronic records and to be submitted to the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



Once signed on, click on **Security**, **Add New User** to define users and access levels. The **Add New User** dialog appears.

Figure 3.1 The Security Options Screen

Figure 3.2 Add New User Dialog



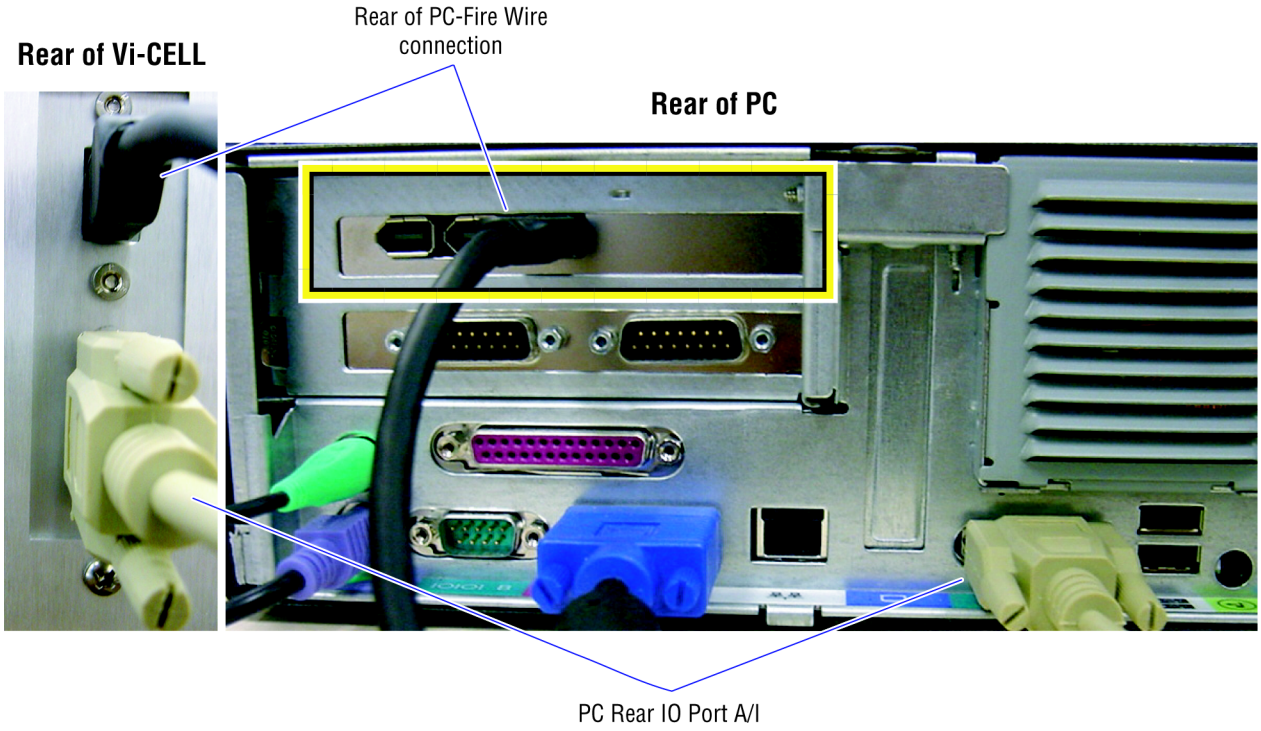
- Passwords must be a minimum of 8 characters
- Password expiry is set at 60 days
- Activity disable is set at 15 minutes with a maximum of 1439 minutes.

3.4 CONNECTING THE HARDWARE

Make certain all connections between the PC and the analyzer are firm and connected as follows:

1. Connect the video capture cable (gray cable) from the FireWire connection on the computer to the Vi-CELL XR (see Figure 3.3). See illustration for correct connection to the PC.
2. Connect the serial cable (beige) cable from IO port number A/1 on the back of the PC to the serial connection on the Vi-CELL XR instrument.

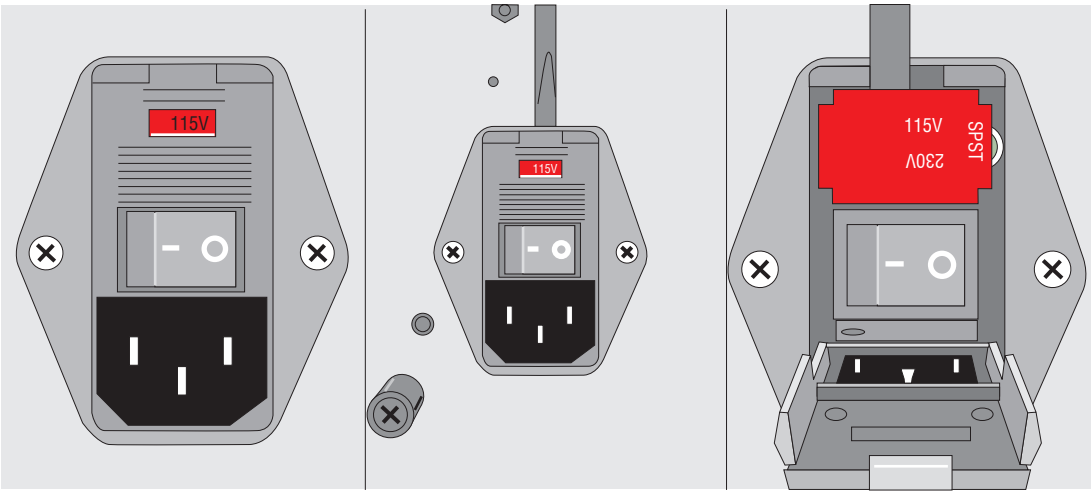
Figure 3.3 Connections from Computer to Vi-CELL XR



Fuse Installation And Voltage Adjustment

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

Figure 3.4 Power Switch and connection and voltage adjustment



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.
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 will be the one showing at the top. **For 115 Volts**, orient the fuse holder with the 115 V at the top. 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.
   Orient the fuse holder with 230 V at the top. Remove clip on the right side of the fuse holder (see images in Figure 3.5 and Figure 3.6) towards the back of the fuse holder. Save 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).

Figure 3.5 Fuse Holder

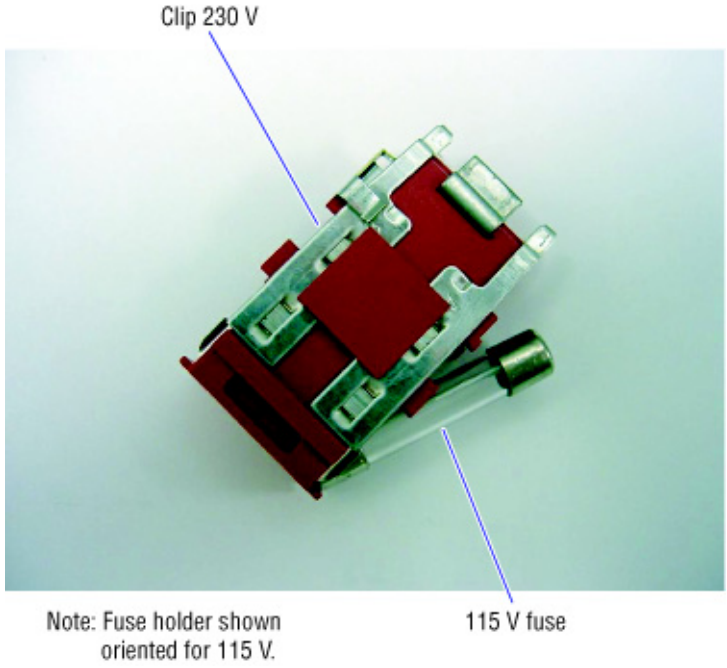
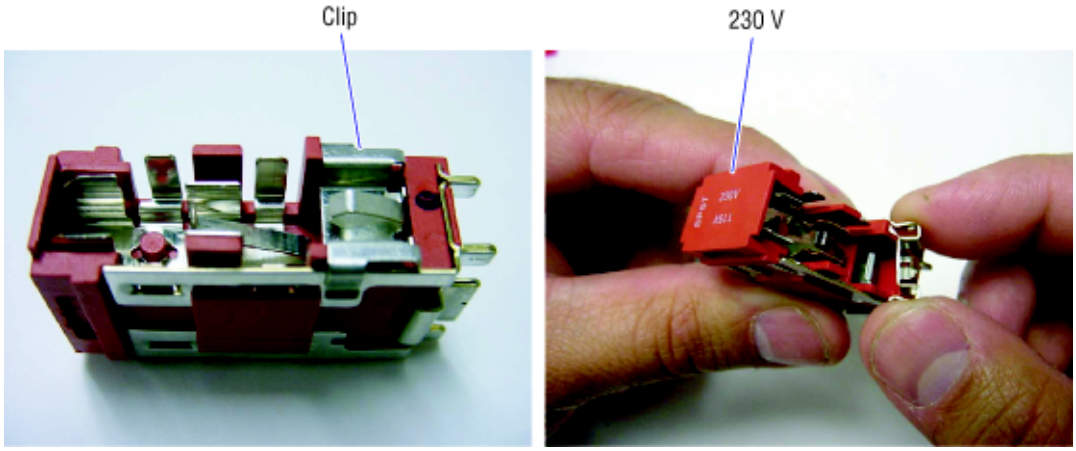


Figure 3.6 Fuse holder and clip and removal of clip



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.

3.5 INSTRUMENT PERFORMANCE VERIFICATION

Post-installation Verification

The Beckman Coulter Service personnel 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.

4.1 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 Its Control Program

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 Pack

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.

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

Figure 4.1 Attach color-coded tubing to reagent pack

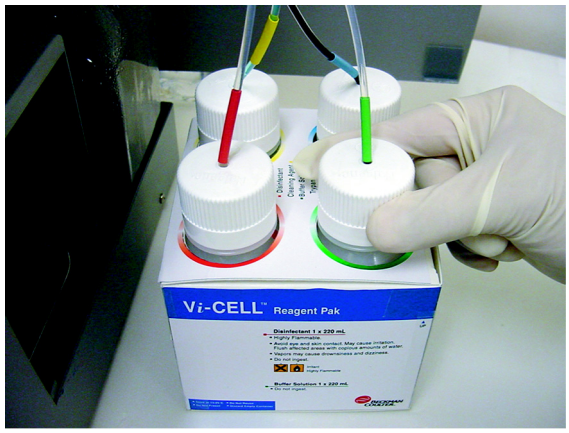


Figure 4.2 Place reagent pack inside the Vi-CELL XR reagent compartment

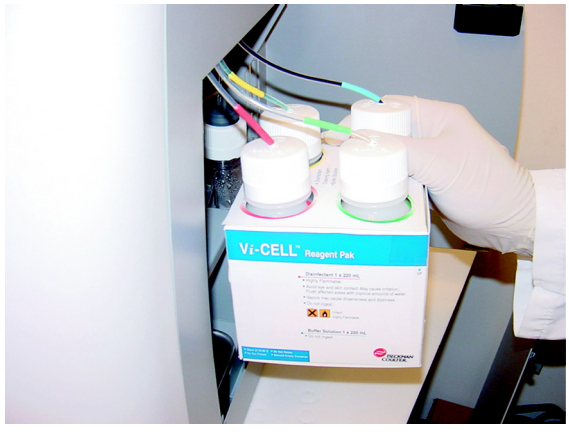
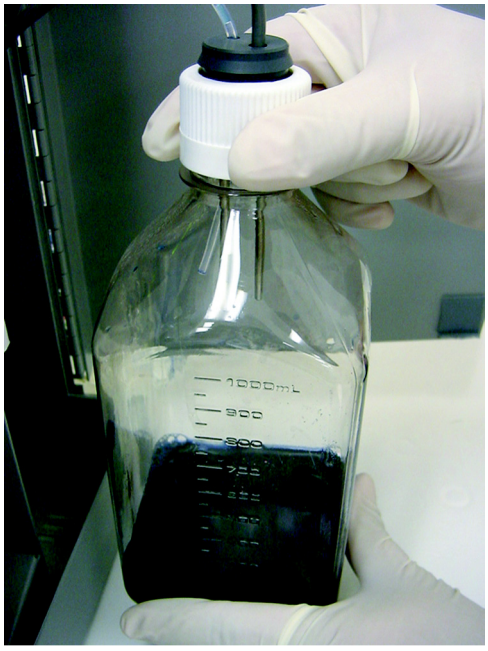


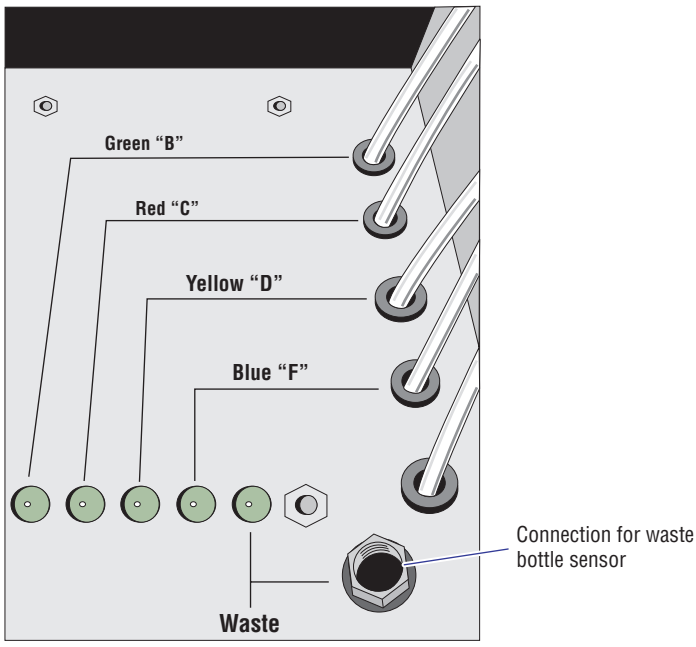
Figure 4.3 Attach waste line to waste container and place inside the Vi-CELL XR



Once reagents have been installed, click on the main screen toolbar, **Instrument** and **Replace reagent pack**. This will prime all lines with reagent and prepare the unit for sample analysis. The reagent level meter will reset to maximum number of runs.

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 is not lighted, do a **Prime** under **Instruments**.

Figure 4.4 Reagent LED's



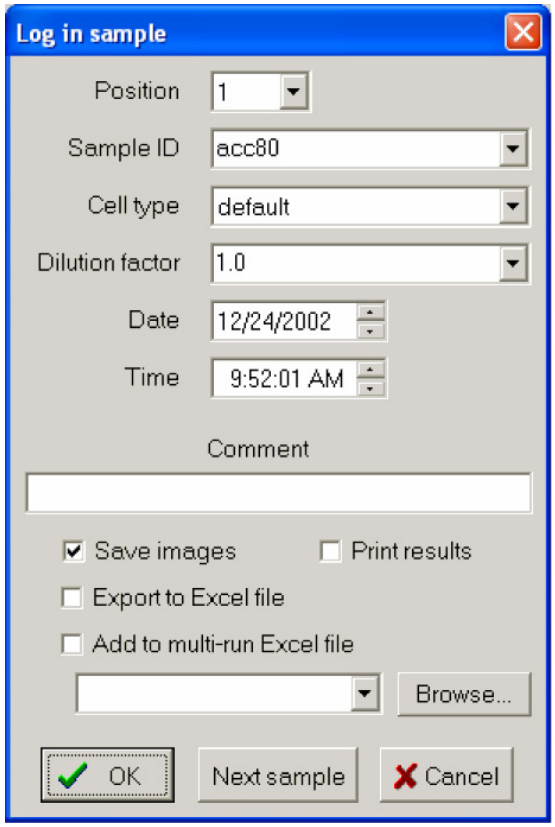
The following describe the LEDs from left to right as they appear on the analyzer

1. **Green – Buffer Solution**
2. **Red – Disinfectant**
3. **Yellow – Cleaning Agent**
4. **Blue – Trypan Blue Reagent**
5. **Waste**

Log In Sample

1. Place a minimum of **0.5 mL (max. 2.5 mL)** of sample into a sample cup.
2. Place sample cup in next available carousel position.
3. Log in samples by clicking on the **Log in sample** button.
   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**.
   d. Select **Dilution factor** if pre-diluted.
   e. Click **OK**.
4. Press **Start queue** to begin the analysis.

Figure 4.5 Log in sample dialog

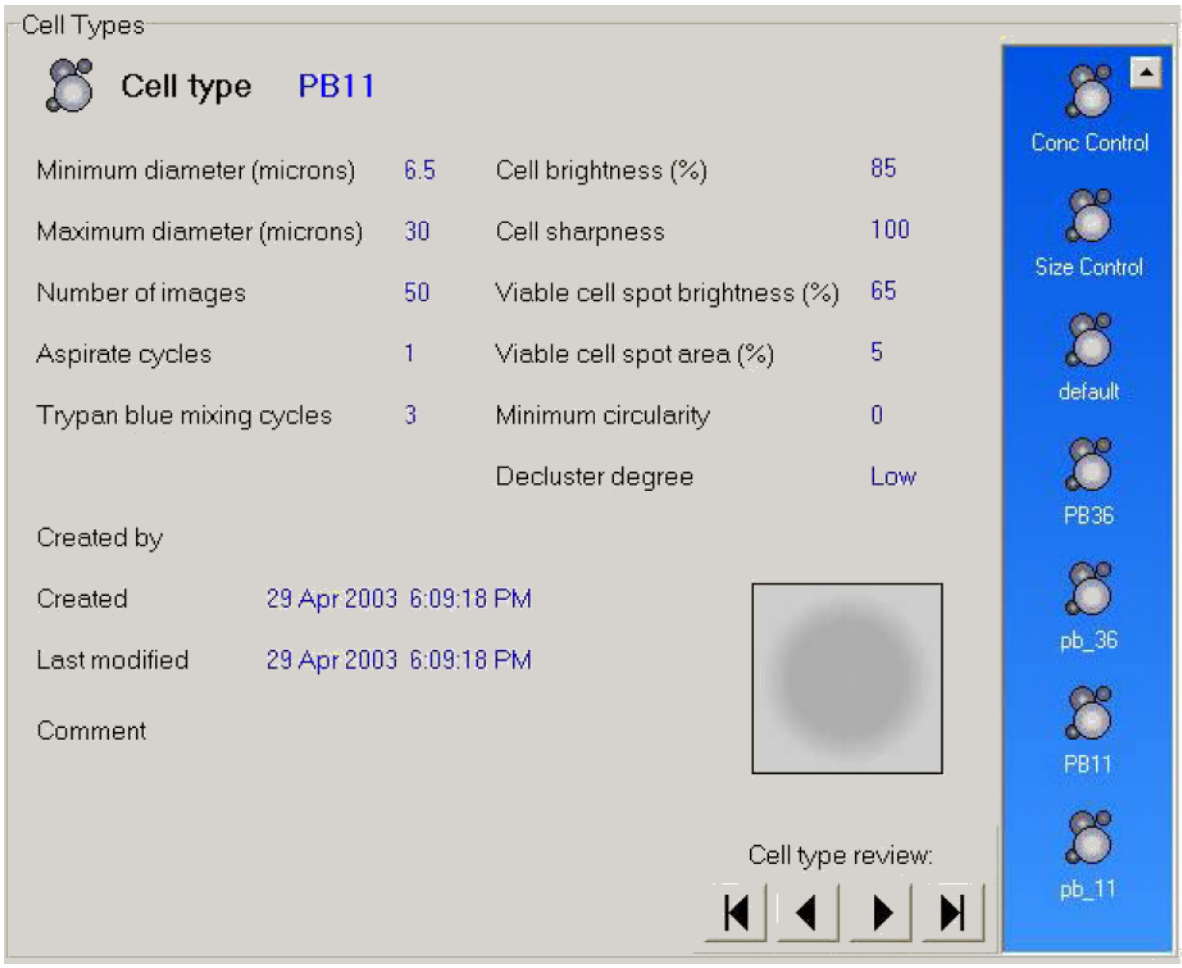


Selecting A Cell Type

When logging in a sample, select the appropriate cell type. If a cell type does not exist, create a cell type as in Heading 6.3. Cell types may be viewed by clicking on the cell type icon on the navigation bar.

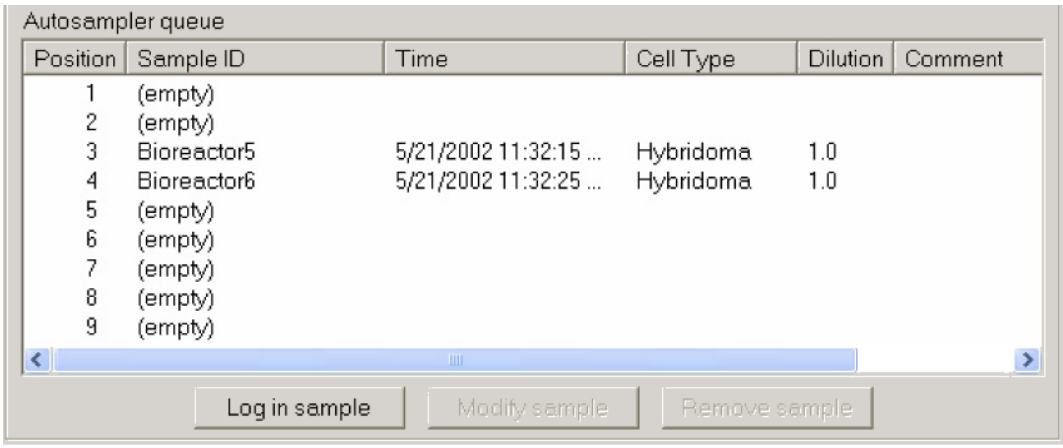
Cell Type Icon 

Figure 4.6 Cell Type Screen



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

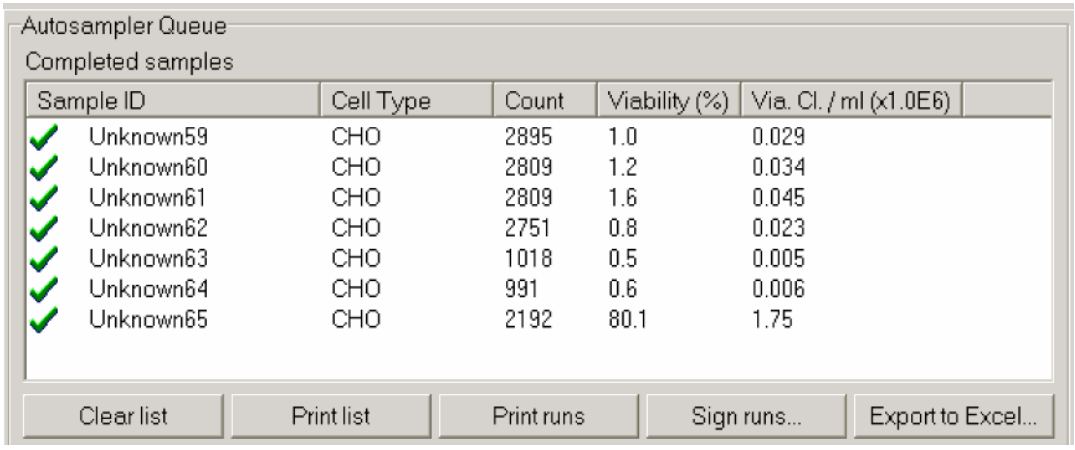
4.2 MANAGING THE AUTOSAMPLER QUEUE



You can log in a sample by clicking on the **Log in sample** button or by double clicking on a particular sample queue position. If the analysis is for a Bioprocess, the bioprocess icon appears to the left of the sample position. Note: The maximum number of characters is 14 numeric or 18 alphabetic. Sample IDs longer than this will not display or print correctly from the Completed Run Summary.

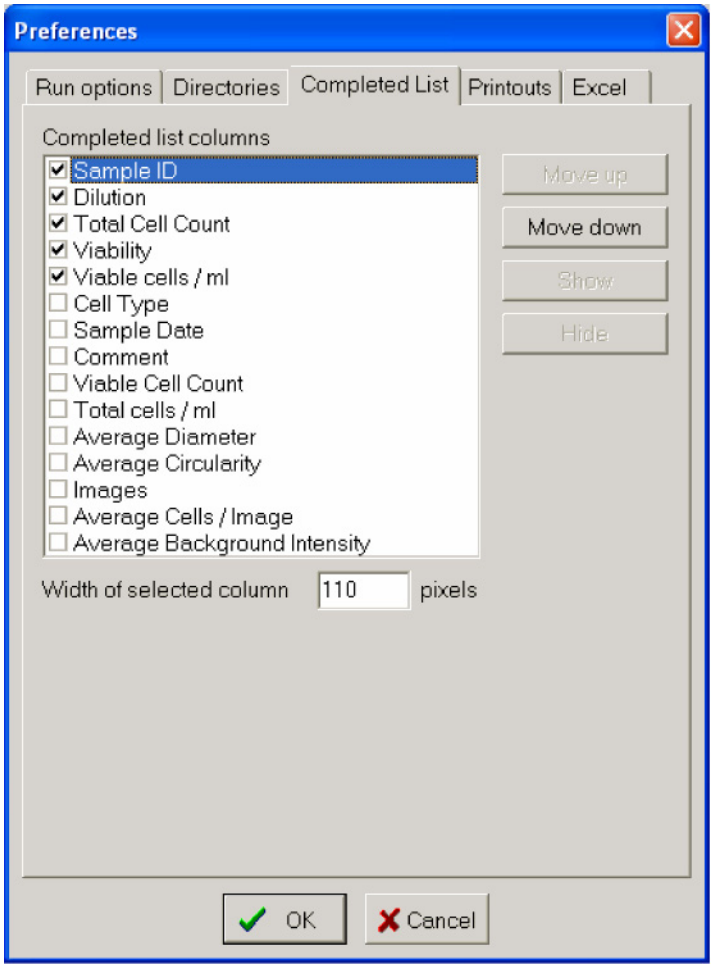
If during log in the radio button for **Save Images**, **Printing** or **Exporting to Excel** is checked, icons will appear to the left side of the sample position on the sample queue denoting each function.

The completed samples window (above the auto-sample queue log in screen) displays completed samples along with results. A check mark will appear indicating completion of analysis.



Double clicking on a given completed sample will show the results under the current results window on the right hand side of the screen.

Clicking on **Clear list** will clear the window of all runs. For printing a list of completed samples, click the **Print list** icon. In order to print all runs on the completed list, simply click on Print runs. There are also functions for signing runs, **Sign runs** (21 CFR Part 11) or **Export 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 that is accessed by selecting **File, Preferences** and then **Completed list**.

Log In Samples and Performing Sample Analysis

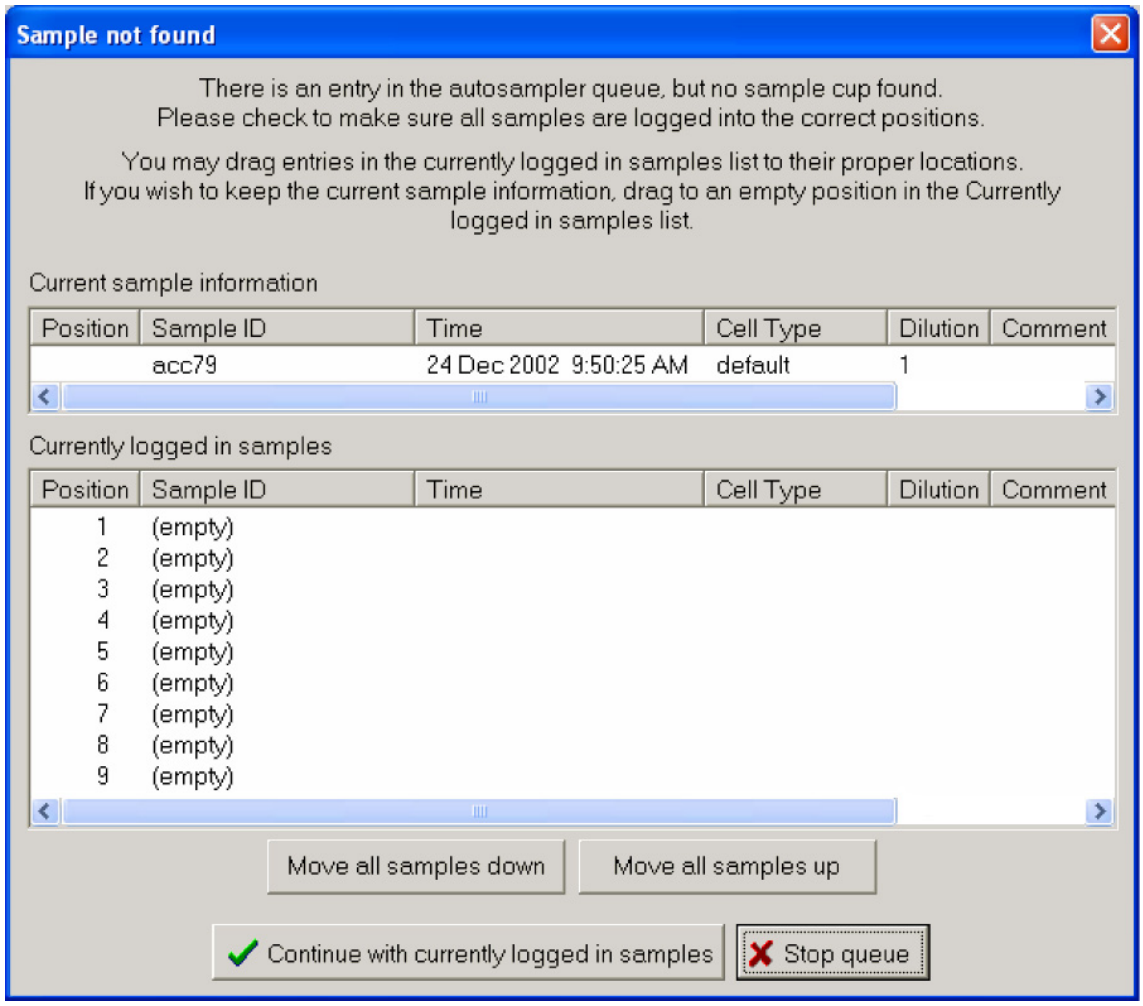
1. Place a minimum of **0.5 mL (max 2.5 mL)** of sample into a sample cup and place in the next available carousel position. The volume does not have to be precise.
2. Log in samples by clicking on the **Log in sample** function or by clicking on the auto-sampler queue icon  on the navigation bar.
   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**.
   (If none exists create a cell type as in Heading 6.3). If a bioprocess, select Bioprocess name from **Sample ID** drop down list. Otherwise create a bioprocess by clicking on **File, New Bioprocess** (see Chapter 6).

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

e. Click **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 will sound, a warning message displayed and the queue stopped.



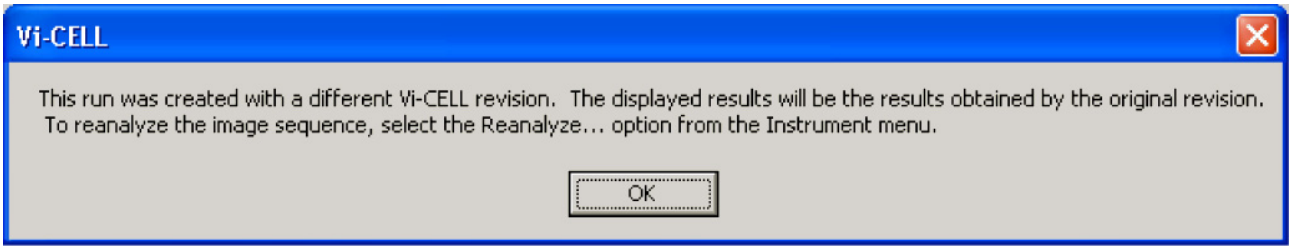
4.3 VIEWING DATA

Managing Data Output

Opening a Run:

To open previously saved data, on the main menu, click **File**, then **Open Run**. To open any file, select the txt file and click **Open**. **Note**: you must click on the text file, not image folder to open the run.

If a recalled run has been calculated with an earlier version of software the images will be displayed but will not be annotated. A warning message will be displayed. If images exist for the data, the run can be reanalyzed with the new software using the Reanalyze function under the instrument menu. The run results will then be updated and images annotated.

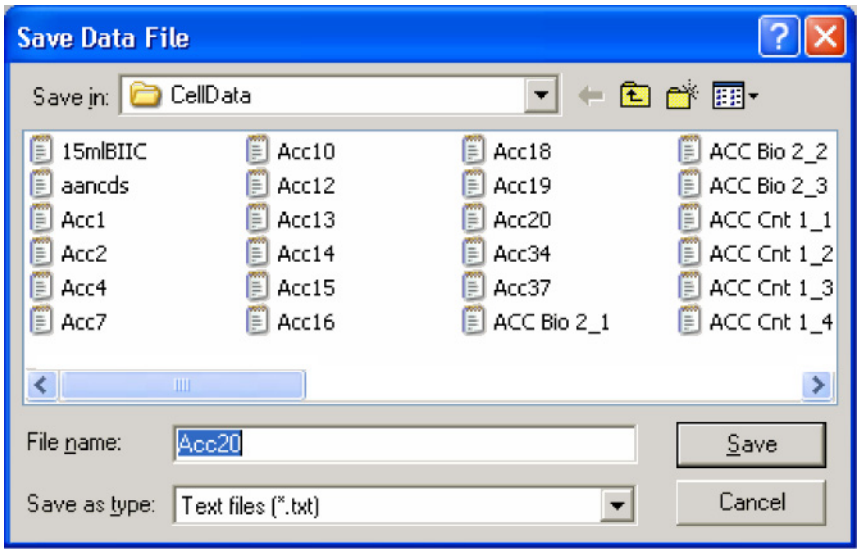


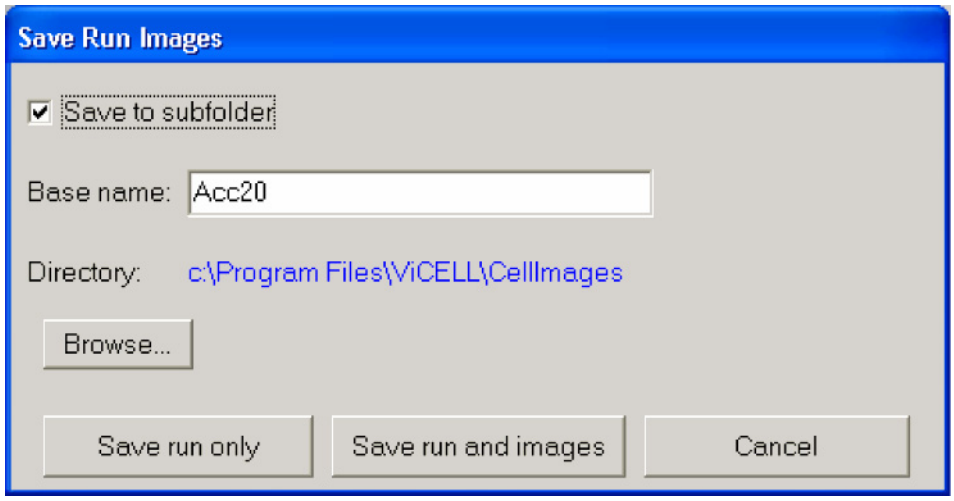
Opening a Bioprocess file:

To open a previously saved file, on the main menu, click **File**, then **Open Bioprocess**. The **Open Bioprocess File** dialog will be displayed. Select file and click **Open**.

Saving Images and Data:

To save a run, on the main menu, click **File**, and then **Save Run**. The **Save Data File** dialog will appear. Enter a file name and click **Save**. Another dialog, **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:

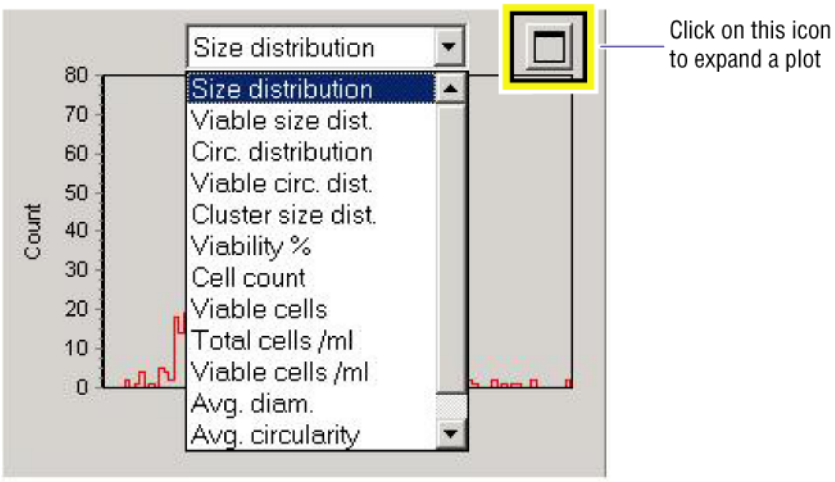
To open a single image for review, on the main menu, click **File**, then **Image**. Select **Open**. The **Open Image File** dialog will display. Select an image and Click **Open**.

Closing an image:

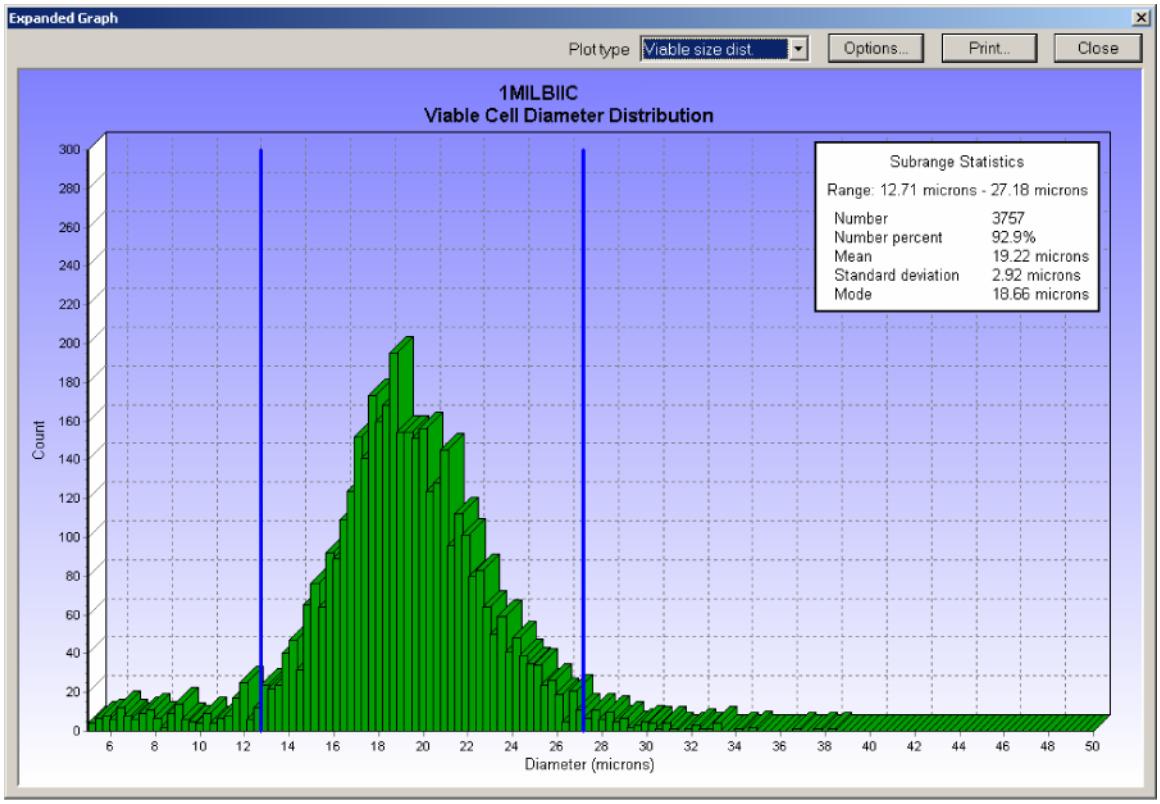
On the main menu, click **File**, then **Image** and **Close**.

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, clicking on the button to the right side of the drop down will expand it. To close the plot, click on **Close**. Plots within the **Bioprocess** screen may also be expanded.

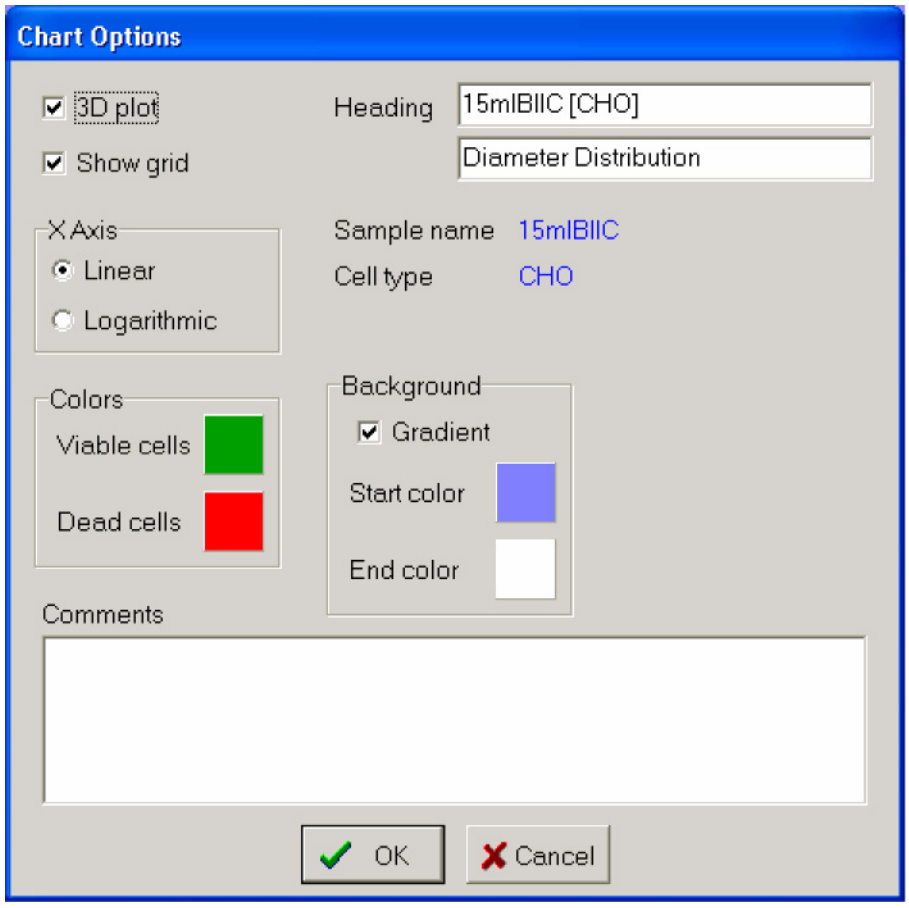


Once a plot has been expanded, statistics can be viewed as well as printed. Various plots can also be viewed from an expanded plot. Just select from the drop down a plot to be displayed.

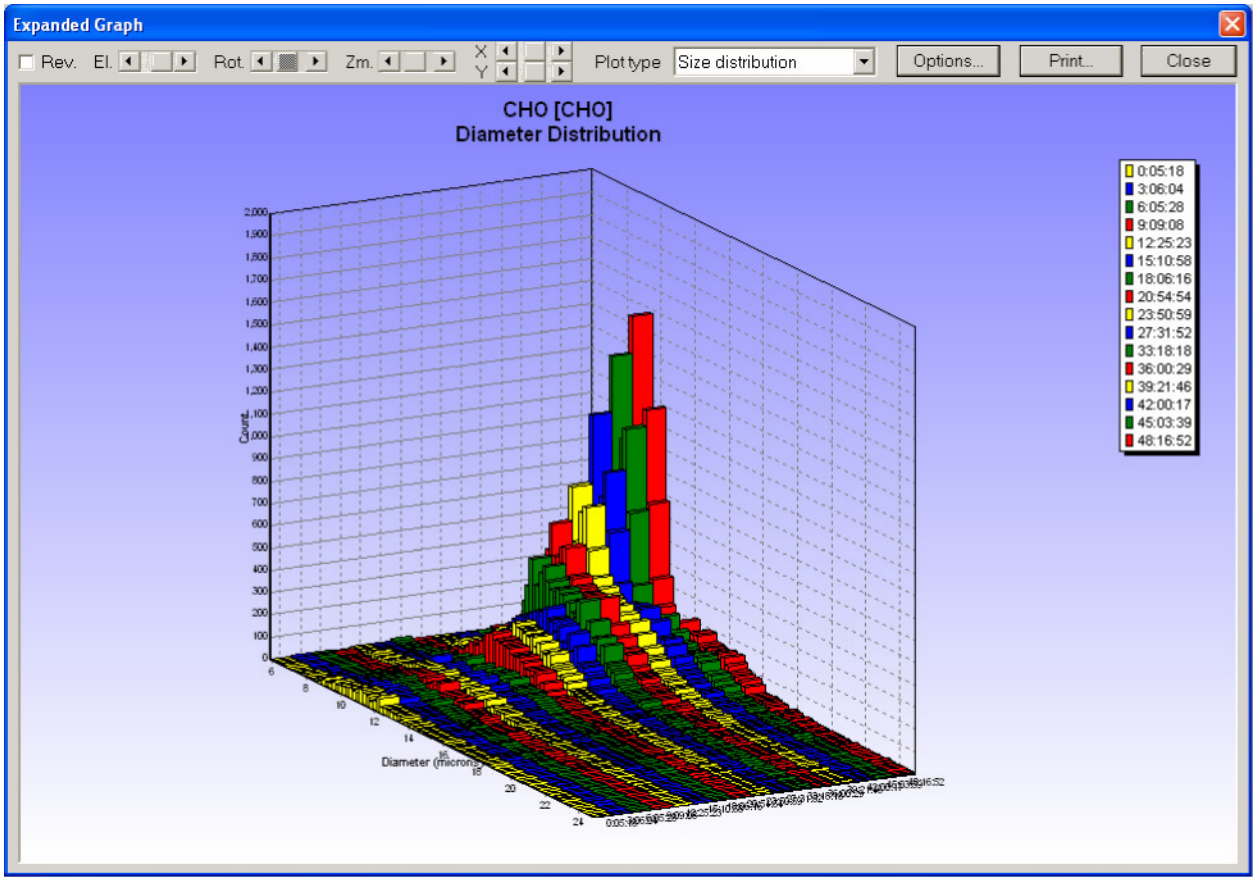
Cursors are also provided in order to isolate a particular region of a distribution. Simply double click on the plot and cursors will appear. Just drag the cursors around the region of interest. The statistics will automatically reflect the region of interest.

Only the Size distribution, viable size, circ. 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.

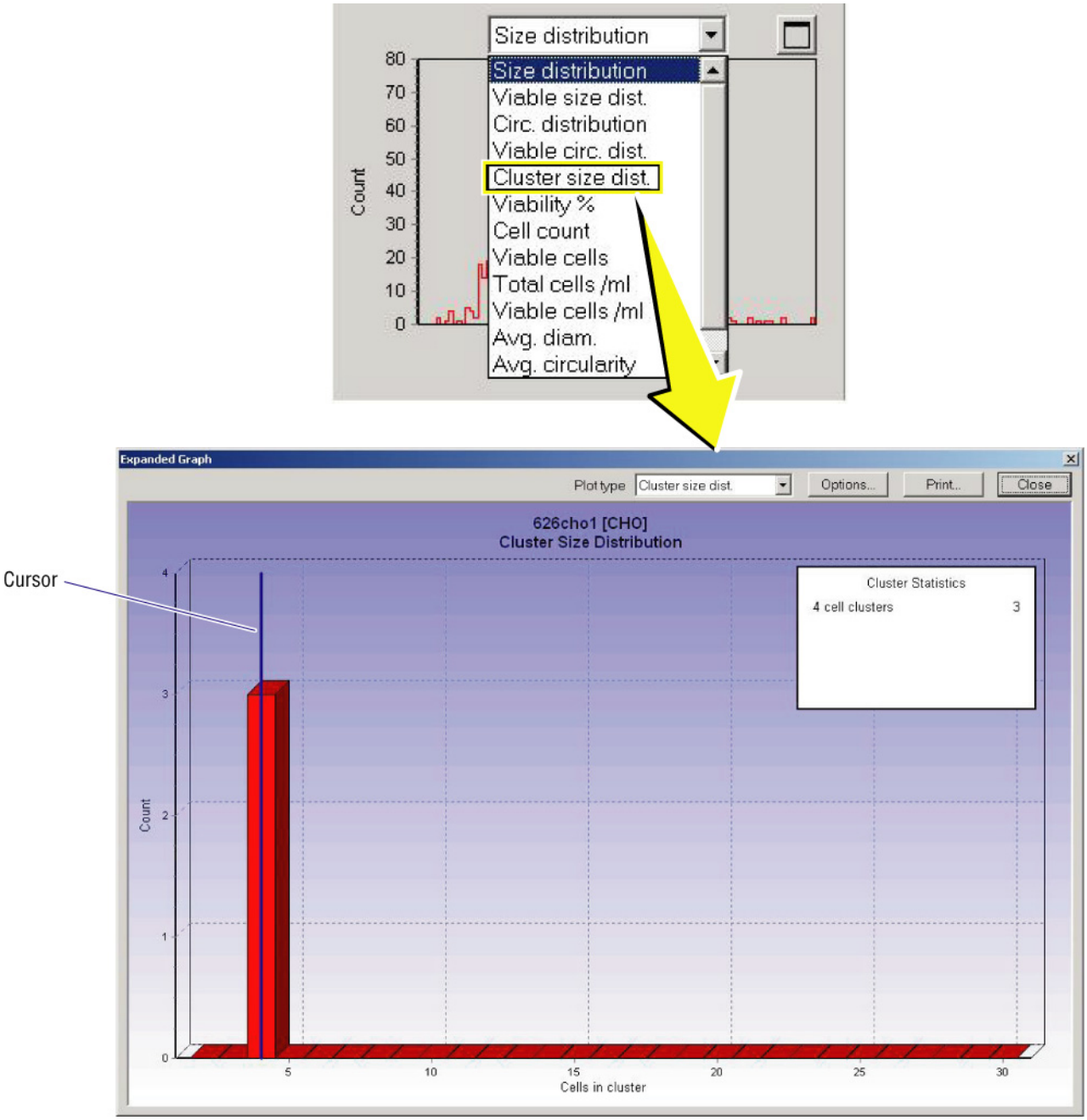
The Chart option dialog allows the user to change the appearance of the expanded graph and to add a comment that will be appended to the graph print out.



Bioprocess histograms can be viewed as 3-D plots. 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. A zoom function is also provided to increase the size of the graph.


Expanded Graph
Rev.
El.
Rot.
Zm.
Plot type
Size distribution
Options...
Print...
Close
CH0 [CH0]
Diameter Distribution
Count
Diameter (microns)
0:05:18
3:06:04
6:05:28
9:09:08
12:25:23
15:10:58
18:06:16
20:54:54
23:50:59
27:31:52
33:18:18
36:00:29
39:21:46
42:00:17
45:03:39
48:16:52

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 allowing to view statistics for a given point on the distribution.

Printing

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

Printing a Report:

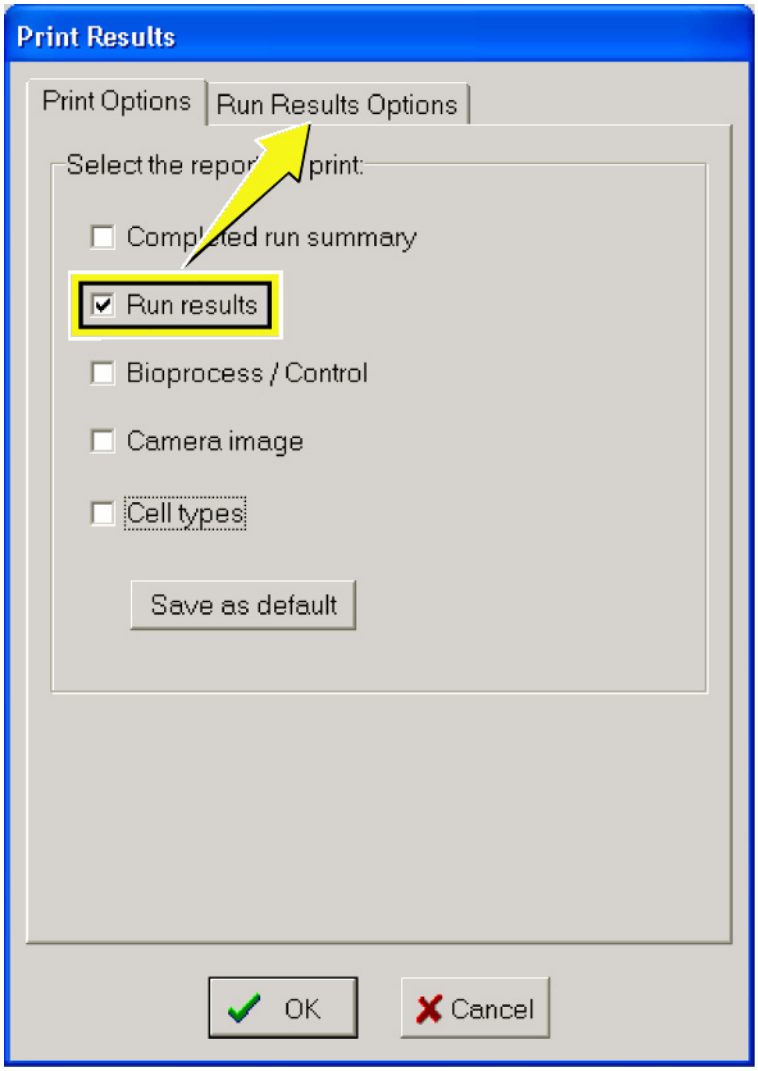
To print a report, on the main menu select **File**, **Print**. The **Print Results** dialog box appears.

Print Options Tab:

To select the parameters 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 called **Run Results Options**. Select **Save as default** to keep your settings.

Figure 4.7 Run Results Print Dialog



Run Results Options:

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.

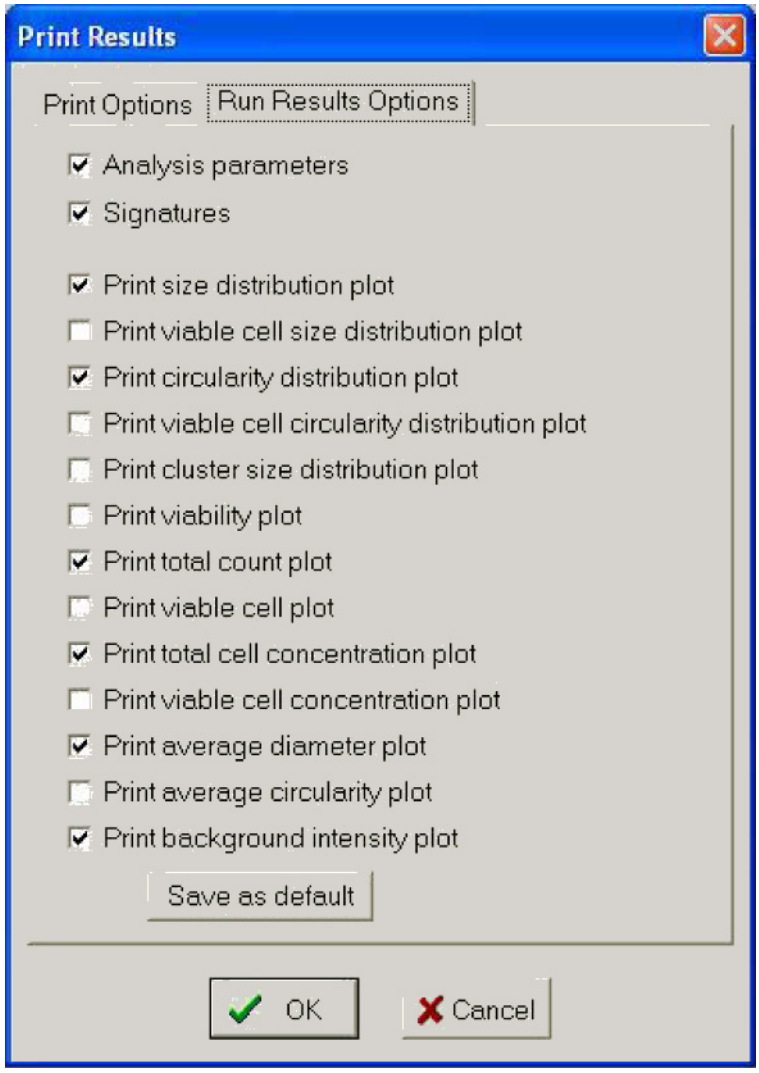
Select **Save as default** to keep your settings.

Select **OK**.

Select Print options (properties options for your printer i.e. portrait, landscape etc).

Click **OK** to begin printing or select **cancel** or **Esc** to abort printing.

Figure 4.8 Run Results Print Options Dialog



Print Run:

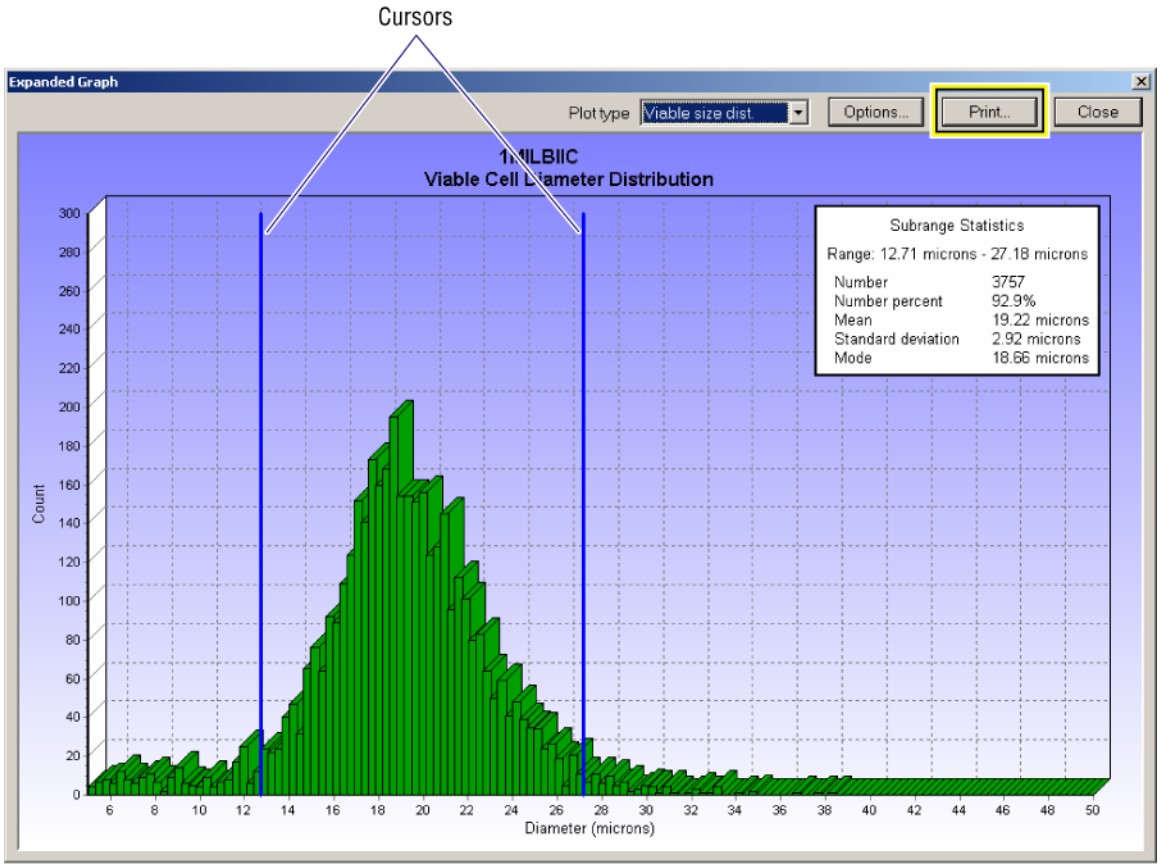
On the lower right hand corner of the **main screen**, select **Print Run**. This will generate a quick report showing the current run results as well as the analysis parameters. The report will print out according to the latest saved default settings.

Printing from the Bioprocess/Control Screens:

To print a report from either the **Bioprocess** or the **Control screen**, click the **Print Bioprocess** function or in the case of a control, the **Print control** function. This will generate a report containing all analysis information as well as the graphs appearing on the screen.

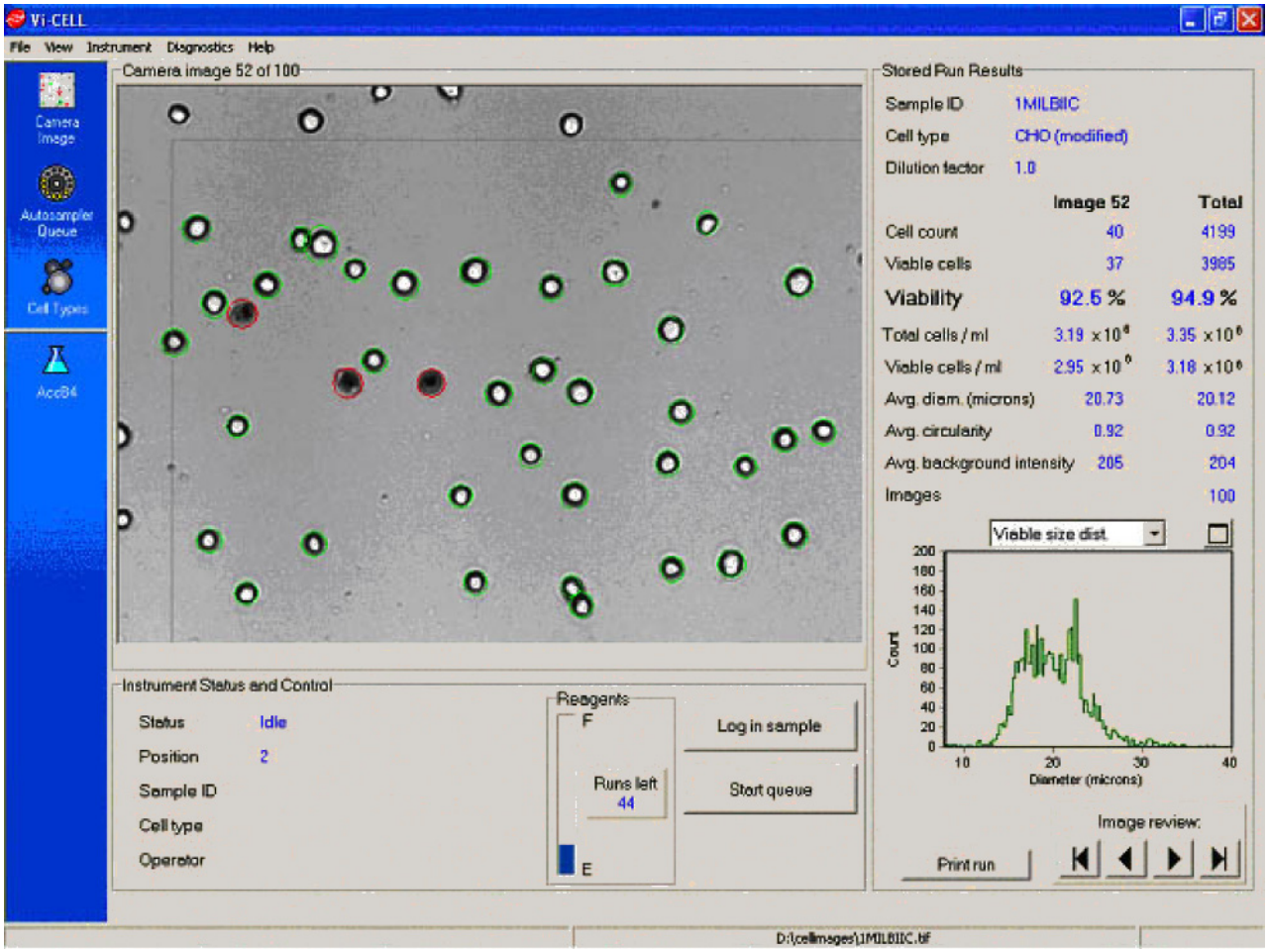
Selecting on the main menu, **file**, then **print** and on the **print results** dialog box **Bioprocess / Control** will also perform a print of the bioprocess results and/or control.

Printing from an Expanded Plot



To print from an expanded plot, click on **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.

The controls contained in the main window will be described in the following sections.



5.1 FILE

Open Run…

This command brings up a dialog box to open a previously saved .txt file. If images are linked to the file, they too will be opened.

Save Run…

This option saves test data in the directory that is chosen in the dialog box. The filename will always end in .TXT. If you do not enter this extension, it will be added automatically. Images may also be saved.

Export Run as Excel file…

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

New Bioprocess…

Allows for the creation of a new Bioprocess type. This will place a new icon on the left hand side of the main screen.

Open Bioprocess…

Opens any number of pre-saved bioprocesses.

Close Bioprocess…

Closes a Bioprocess.

Export Bioprocess as Excel file…

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

Control…

New: Allows creation of a control file for monitoring instrument performance.

Modify: For modifying a control file.

Open: Opens an existing control file.

Close: Closes any opened control files.

Export as Excel file: Exports data as an Excel file.

Image…

Open: 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.

Cell Types…

Add: Allows for the creation of cell types.

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.

Configuration…

Is where hardware such as printers are defined. Also, calibration and security information are defined.

Preferences…

Defines various parameters such as run options, directories where data is to be saved to, what auto-sampler completed list items to show, to appear on printouts and the items to appear on Excel spreadsheets. Auto-increment file name, Auto-save run images, Auto-print and Auto-save run results to Excel are other options available.

Print…

The Print Options dialog allows one to choose which elements of the report to print. Information in the Text window may be edited before printing (these changes do not affect the stored TXT file).

Exit…

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

5.2 VIEW

Camera Image: Provides 'real time' camera images.

Auto-sampler Queue: Changes over to the Sample Queue screen.

Cell Types: Changes over to the Cell Types Screen.

Binary Image: Will convert images to black and white.

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

5.3 INSTRUMENT

Log in Sample: A dialog appears for entering sample information.

Clear Completed List: Clears the completed runs Queue.

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.

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

Reanalyze: Re-calculates data on saved images.

5.4 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.

5.5 SECURITY

Turn Security Off: Disables the security option.

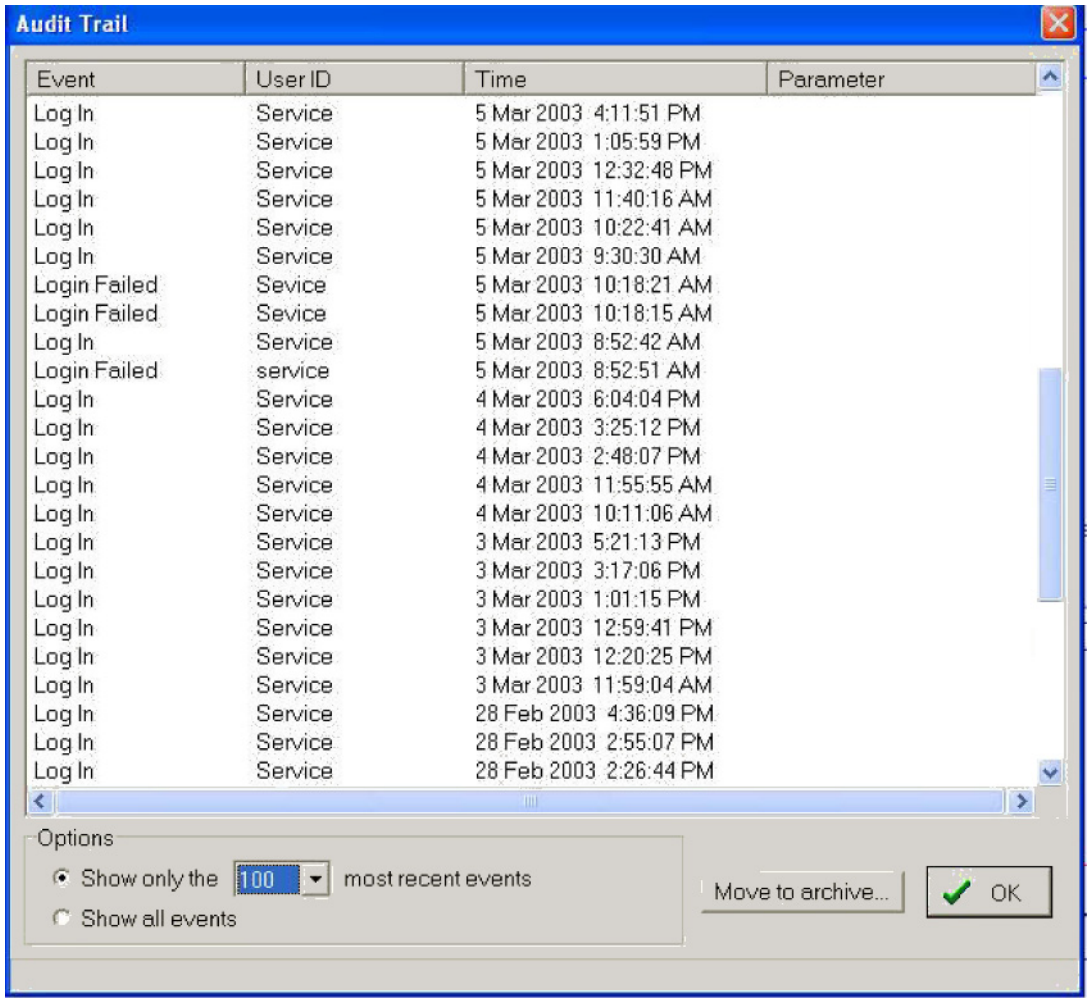
Add New User: The addition of operators to the system.

Reset User's Password: If a password is forgotten, allows for the creation of another password.

View Audit Trail: The system audit trail is displayed by selecting **Audit Trail** from the **Security** menu.

The system audit trail is displayed by selecting the **Audit Trail** menu item from the **security** menu. It displays the time and details of the following events. Audit trail information may be archived using the **Move To Archive** feature.

- Log In
- Login Failed
- Switch Users
- Security On
- Security Off
- Add User
- Enable User
- Disable User
- Change Password
- Reset Password
- Checksum Failed



Lock Instrument: Will lock out a user from attempting to utilize the instrument. In order to gain access to the system, a password must be entered.

Change Password: Allows for the creation of a new password.

Switch Users: Will allow change to another operator.

- Passwords must be a minimum of 8 characters
- Password expiry is set at 60 days
- Activity disable is set at 15 minutes with a maximum of 1439 minutes.

5.6 TYPES OF USERS

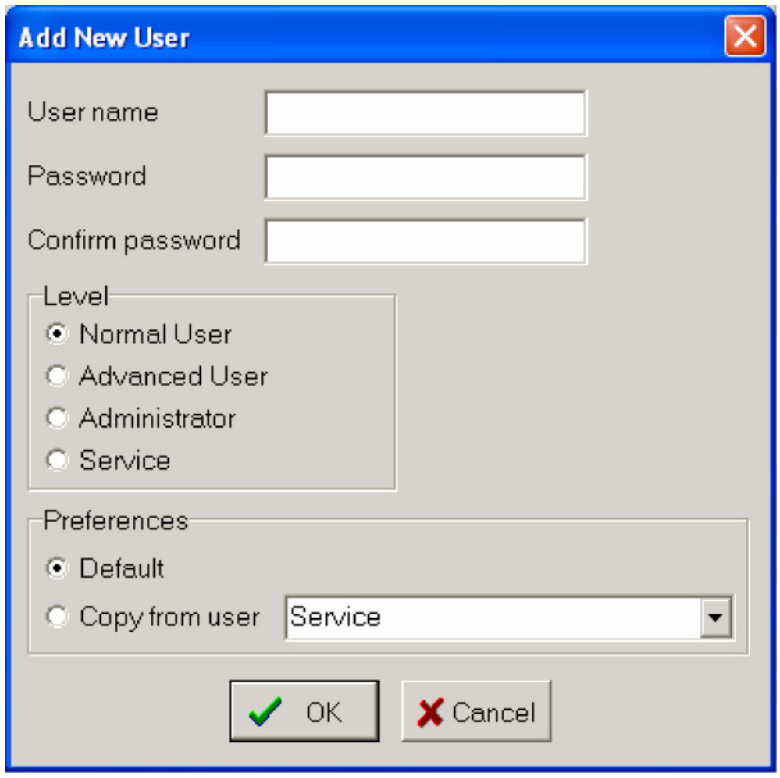
The following are the default conditions for the different user access levels:

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

Table 5.1 User Types and Access Levels

Menu Item	Security Off	Normal	Advanced	Administrator
File / Save Run	Enabled	Disabled	Enabled	Enabled
File / Export Run as 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 / Calibration parameters	Disabled	Not Accessible	Read Only	Read Only
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	Enabled	Disabled	Enabled	Enabled
Instrument / Reanalyze	Enabled	Disabled	Enabled	Enabled
Diagnostics	Enabled	Disabled	Enabled	Enabled
Diagnostics / Set Focus	Enabled	Disabled	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
Security / Turn Security On	Enabled	Not Displayed	Not Displayed	Not Displayed
Security / Turn Security Off	Not Displayed	Not Displayed	Not Displayed	Enabled
Security / Add New User	Not Displayed	Not Displayed	Not Displayed	Enabled
Security / Add New User / Service	Not Displayed	Not Accessible	Not Accessible	Disabled
Security / Reset Users Password	Not Displayed	Not Displayed	Not Displayed	Enabled
Security / Reset Password / Service	Not Displayed	Not Accessible	Not Accessible	Disabled

The first step is to setup a user via **Add New User** dialog.



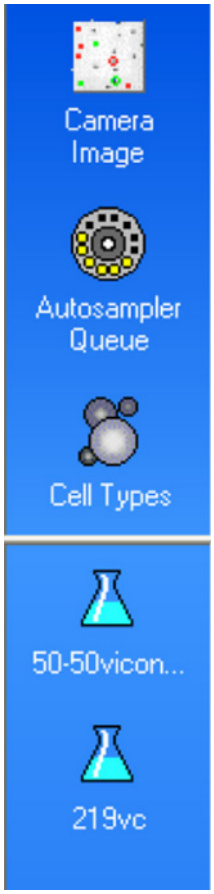
Once a user has been established, the level of access is defined as described in Table 5.1.

5.7 HELP

Provides documentation related to the software version and access to the operator's **reference manual**.

5.8 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.

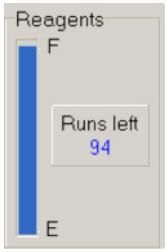
Bioprocess: This icon represents Bioprocesses. Multiple bioprocess icons are possible.

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

5.9 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.



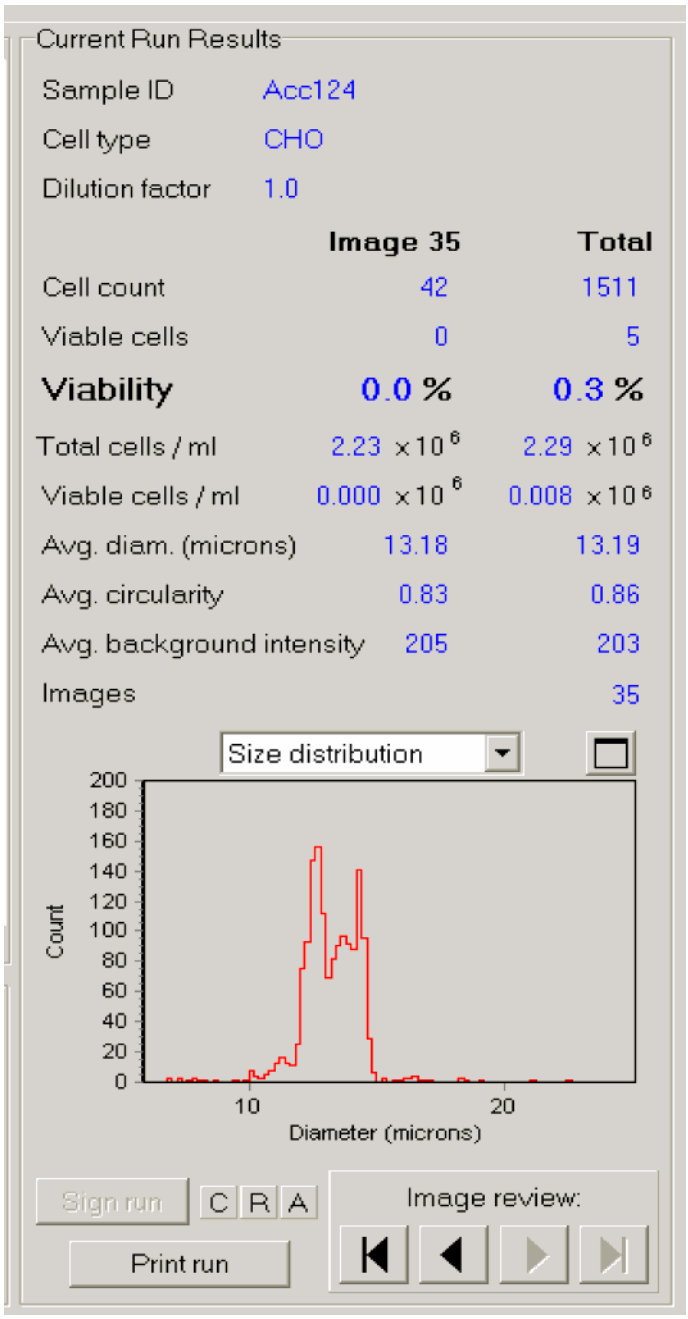
Reagent Level Meter: Monitors reagent levels.

Manual Reset Of The Reagent Level Meter

Clicking on **Diagnostics**, then **Set Reagent level**, will 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.

5.10 RUN RESULTS



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

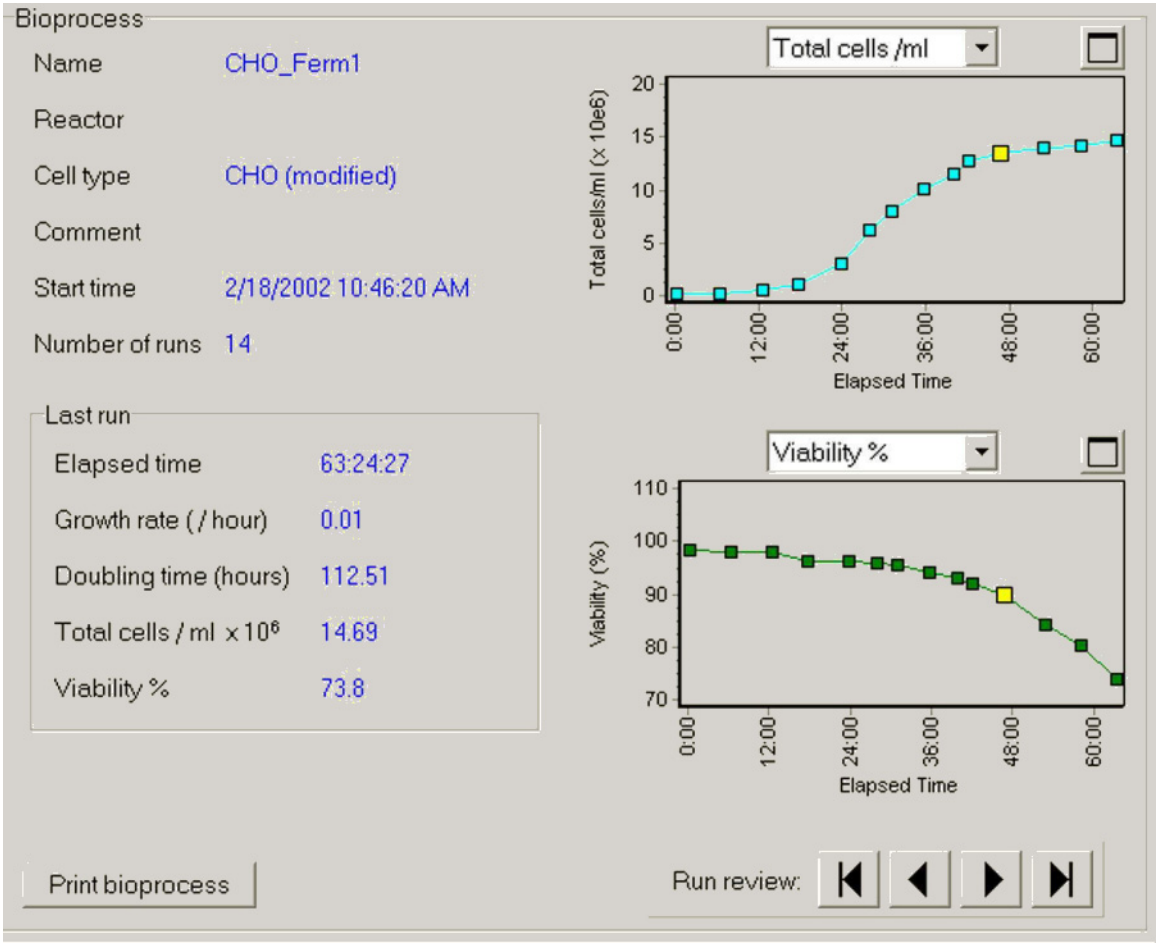
The Run **Image review** function allows for scrolling through images and their associated results. Also, within the Run Results screen is the **Sign Run** function (for 21 CFR Part 11 compliance).

6.1 BIOPROCESS FEATURE

What Is A Bioprocess?

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.

Figure 6.1 The Vi-CELL XR Bioprocess Screen, Example



The Bioprocess plots can be expanded for a more detailed view. Click on 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)

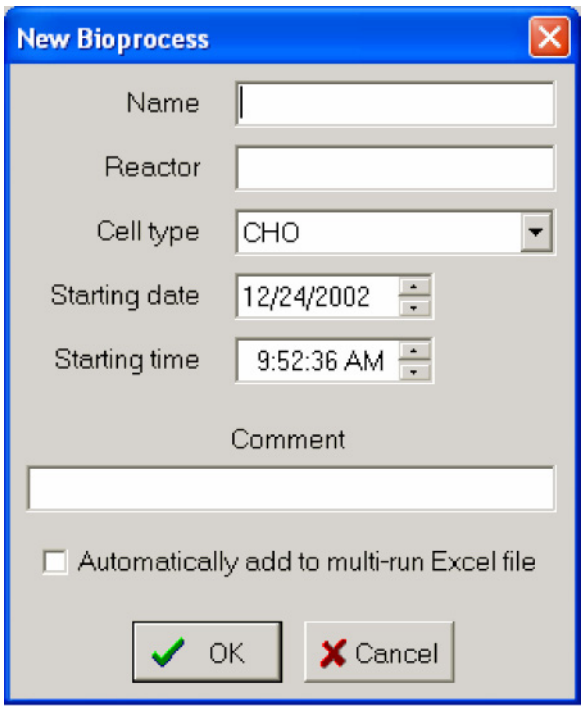
Doubling time in hours= ln 2 / Growth rate per hour

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

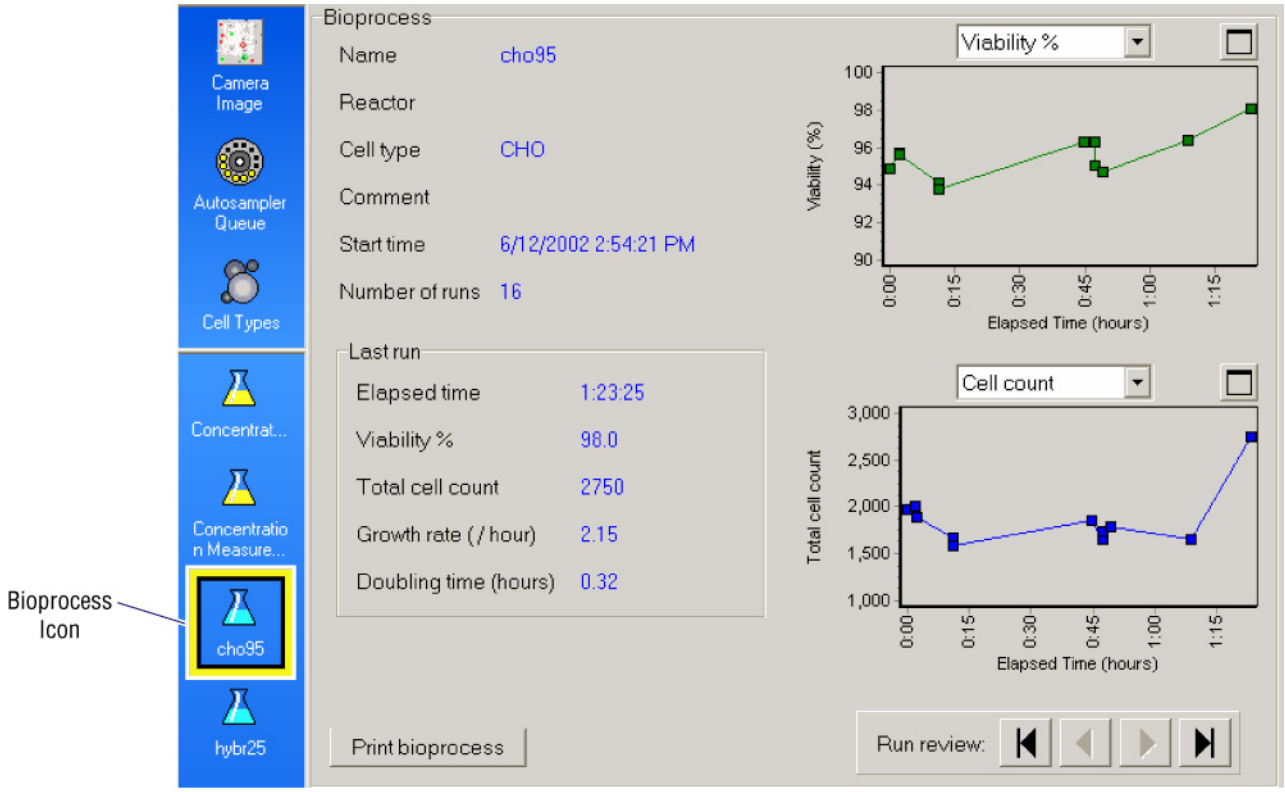
Creating A Bioprocess File



1. Open **File** on the menu bar.
2. Select **New Bioprocess**.
3. Enter the information on the New Reaction dialog box.
4. Click **OK** to close the dialog.

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

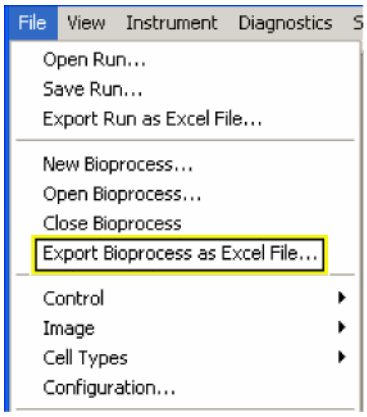
Figure 6.2 Bioprocess Screen and Navigation Bar Icons



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 **log in** dialogs appears. The cell type and sample ID are automatically selected as defaults. Simply place the sample on the carousel and select **OK**. Then click **Start Queue**.

Exporting A Bioprocess File To Excel



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

Select **File**, **Export Bioprocess as Excel File** and data is saved as an Excel file.

6.2 CONTROL FEATURE

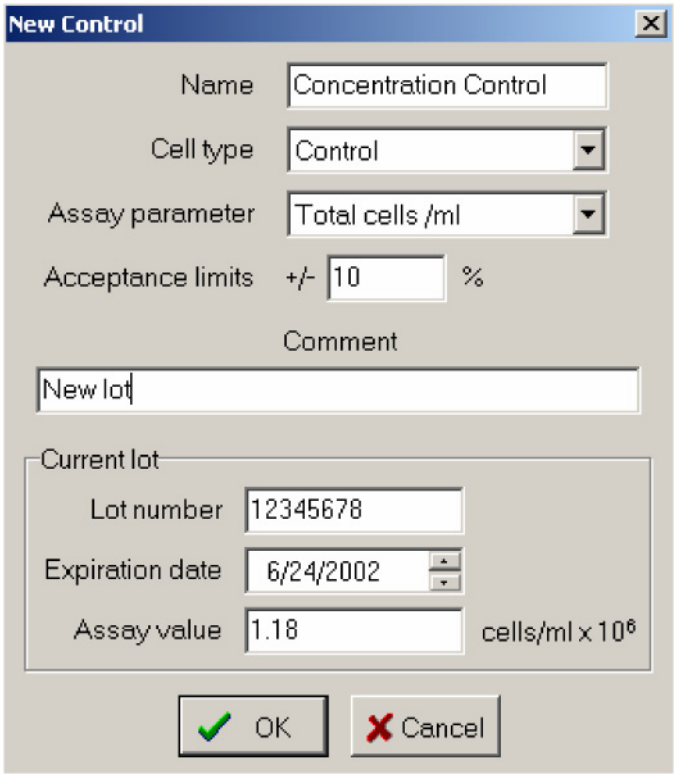
What Is The Control Feature?

The control feature monitors Vi-CELL XR performance. Beckman Coulter provides Vi-CELL Concentration Control (PN 175478) for the monitoring of Total Cells/mL.

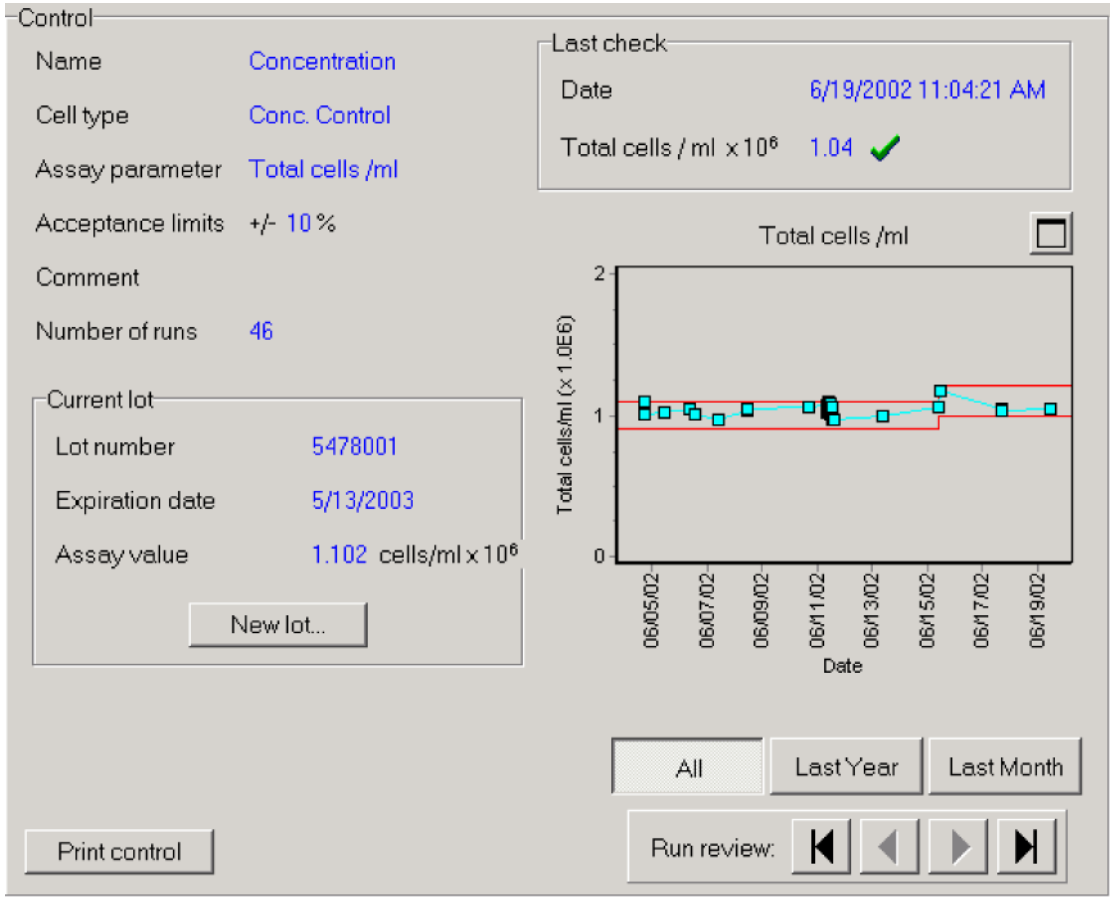
Creating A Control Chart – For Total Concentration/mL

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

Figure 6.3 The New Control dialog box



Enter the name and control information from the Vi-CELL concentration control assay sheet. Click **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 will be stored automatically within the control file.

Managing Controls

To log in a control sample, double click on the **control icon**, which will display the log in screen. Log in the sample and click **OK**. Press **Start**.

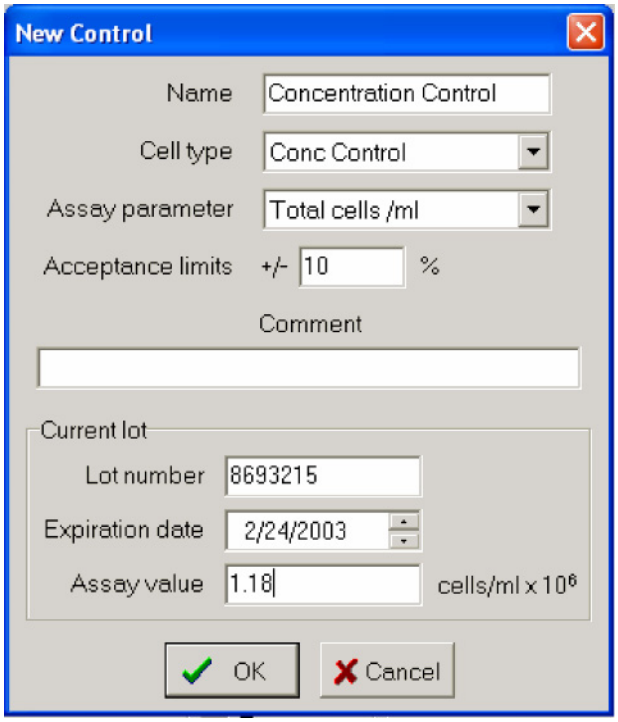
Upon completion, the data will automatically be stored within the control file.

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

Printing of control data can be achieved by selecting **Print control**.

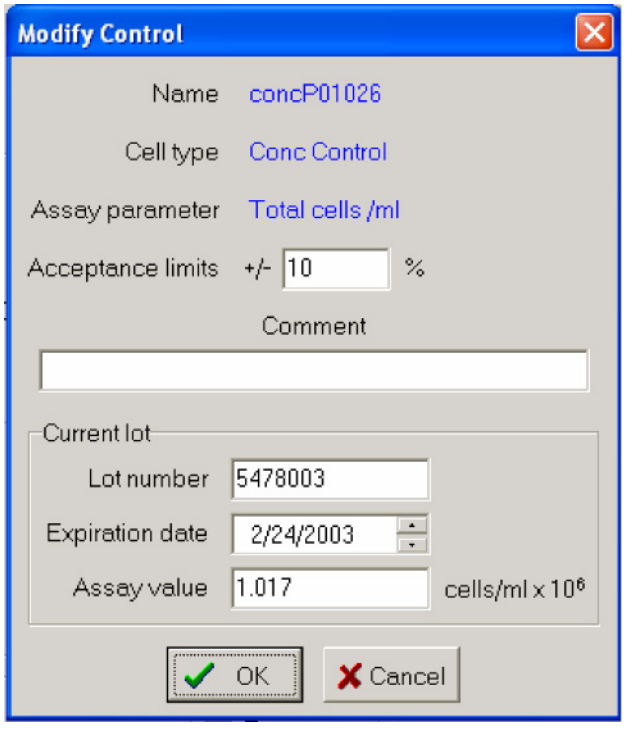
Exporting Data To Excel

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**.



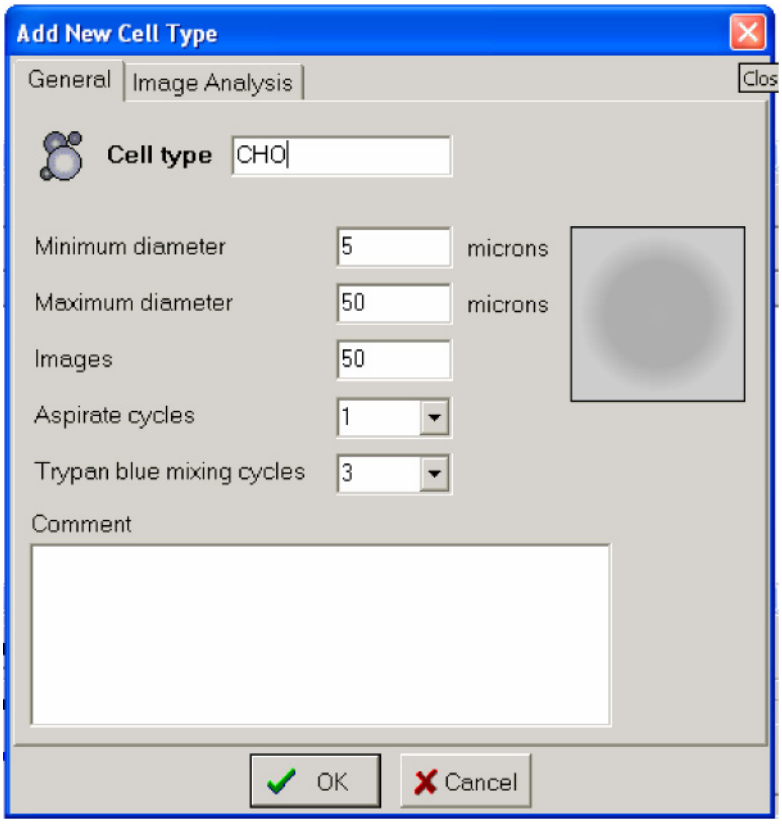
6.3 CREATING AND MANAGING CELL TYPES

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 will be 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 is an attempt to help you better understand how to setup the Vi-CELL XR for a given cell type.

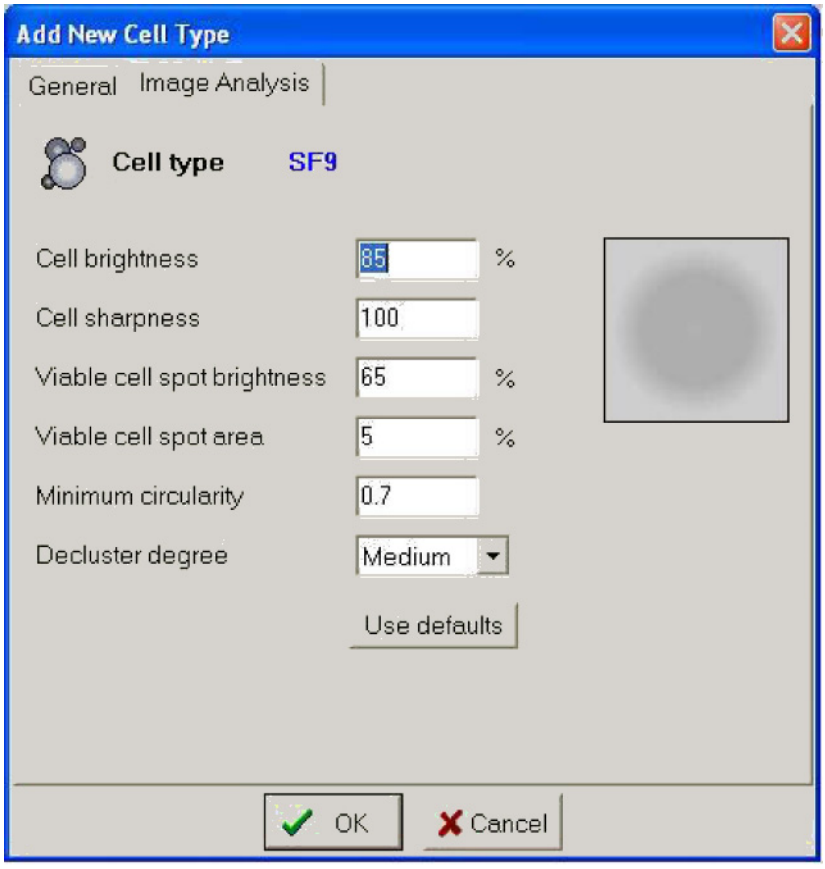
Creating a New Cell Type



1. On the menu bar click on **File**, **Cell Types** then **Add**.
2. The **Add New Cell Type** dialog (as above) should appear.
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.

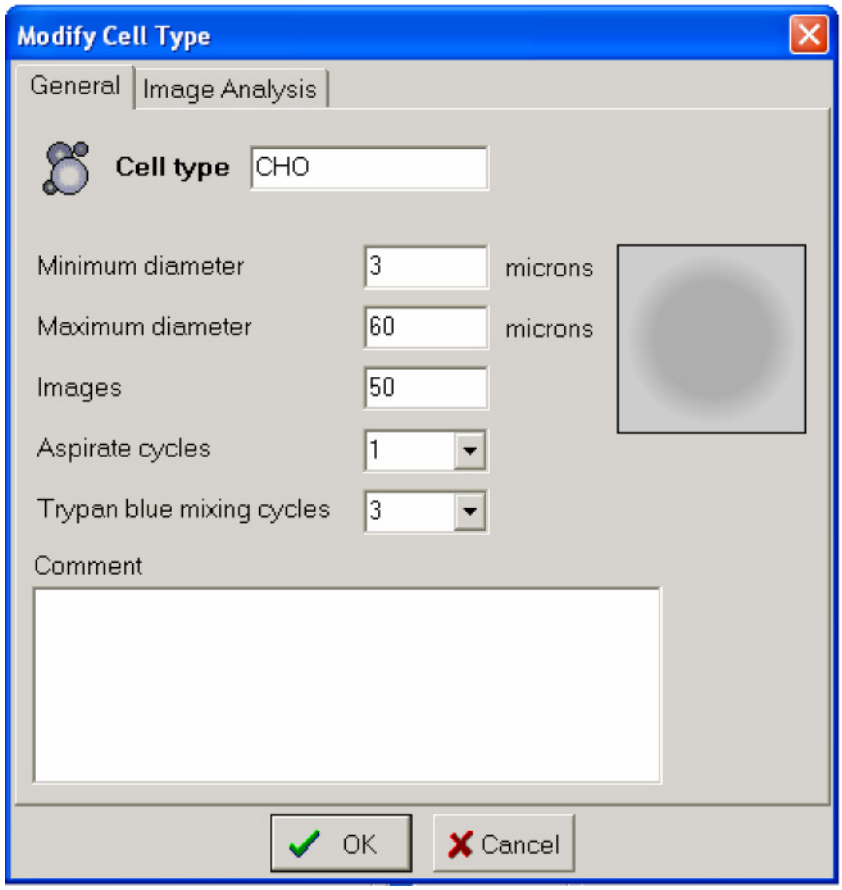
4. Under **Image Analysis** set the parameters accordingly. Utilize the default settings as a starting point.
   The first four parameters control the image recognition portion of the Vi-CELL XR software. The first two control cell recognition whether viable or not, and the second set of parameters control whether a cell is viable or non-viable.
   The minimum circularity parameter is for eliminating debris such as dead cell fragments. This parameter only works on dead cells or debris. 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.

   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



Minimum Diameter: The minimum diameter of the cell type. 2 is the minimum diameter allowed. The minimum diameter can be used to exclude debris and/or unwanted cells.

Maximum Diameter: The maximum diameter of the cell type. 70 is the maximum diameter allowed.

Images: The minimum or maximum number of images to capture for a given Cell type. 100 images is the maximum allowed.

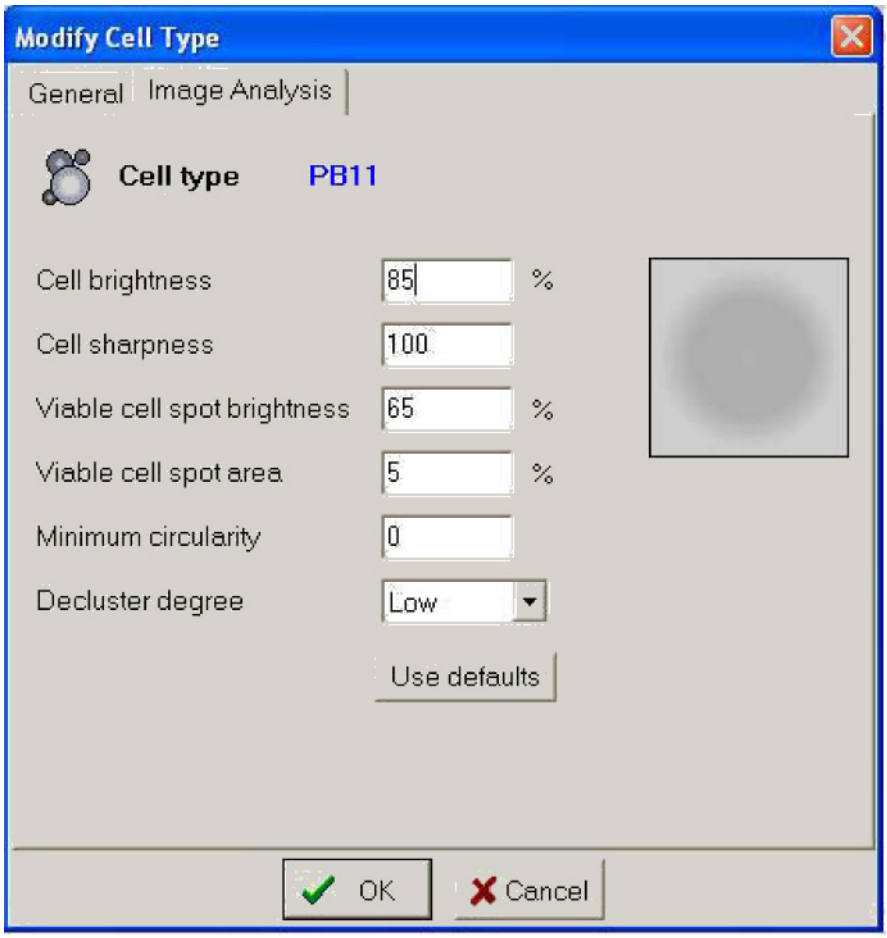
Aspirate Cycles: In order to ensure that all of the cells are equally dispersed some of the sample is aspirated and then returned to the cup. One cycle is normally sufficient but if the cells are difficult to keep in suspension and have a tendency to attach themselves to the walls of the cup then additional aspirate cycles may be beneficial. The range of values for this parameter is 0 to 9.

Trypan Blue Mixing Cycles: The trypan blue and sample are mixed by sending the mixture back and forth between the 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. The range of values allowable is 1 to 9.

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.

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.



Cell Brightness: Is the brightness of the cell boundary within a given image. The range is 50% (darkest) to 90% (lightest). 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). A lower value means a dark boundary will be required for cell identification. A higher value means a lighter boundary.

Cell Sharpness: Represents the "clarity" of an image. 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: Is the brightness of the center spot of the cell. The range for spot brightness is 0 to 100%. 75% is a typical value.

Viable Cell Spot Area: The cell spot area will be a percentage of the total area of the Cell. 5 to 10% are typical values. Any extremes in this value will either make the cells all viable or non-viable.

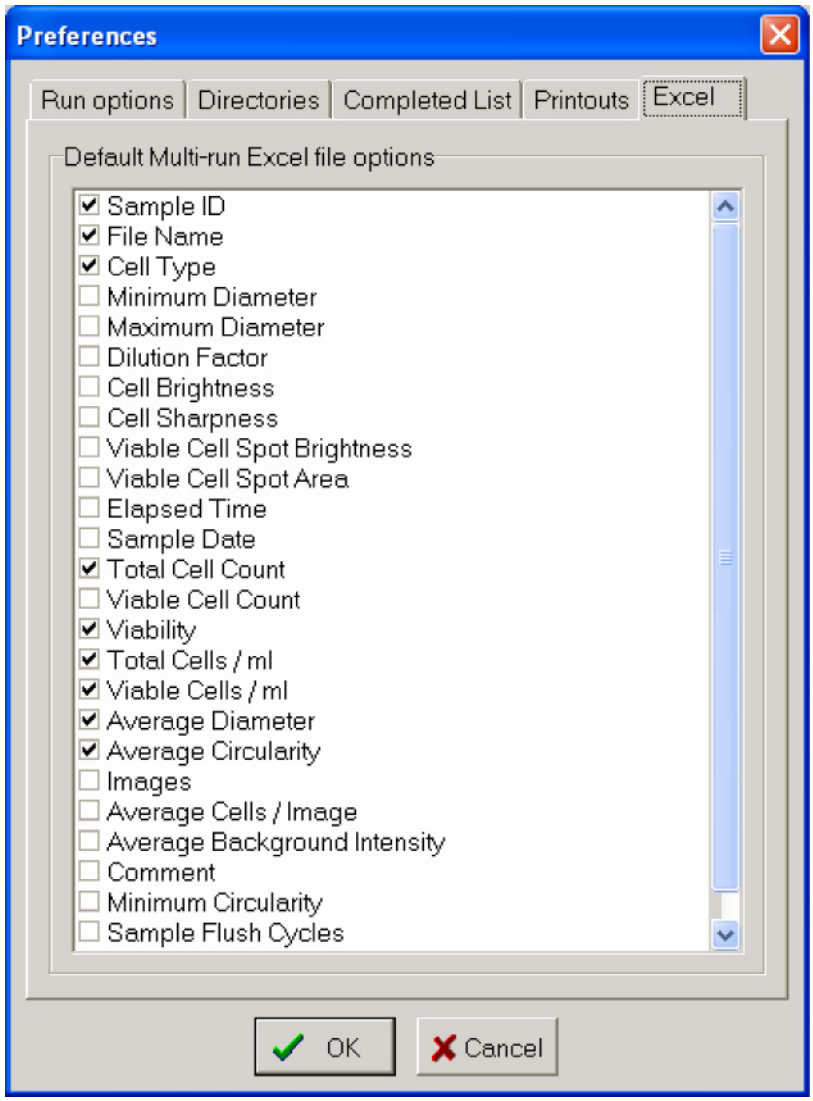
Minimum Circularity: (Least Circular=0, Perfectly Circular=1) A non-viable cell will only be accepted if its circularity is greater than or equal to the minimum circularity. This parameter can be used to reject debris that exceeds the minimum cell diameter and are too irregularly shaped to be treated as a real cell. This parameter only affects dead cells.

Decluster Degree: The amount of 'de-cluster' applied to the sample. The selections are none, low, medium and high de-cluster. 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).

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.

7.1 EXPORTING DATA

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

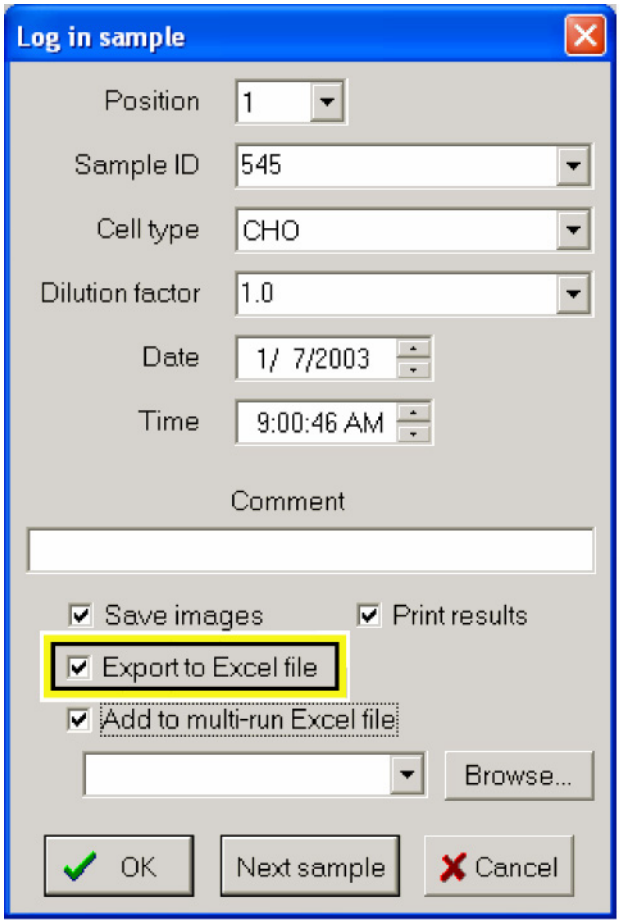


On the main menu tool bar, select **File**, then **Export run as Excel file**.

Another way of exporting as an Excel file is to click the **Export to Excel** radio button on the **Log in** dialog screen.

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

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 Bioprocess Data

Exporting of the bioprocess data is possible through the 'Export Bioprocess as Excel file' feature found by selecting **File**, **Export Bioprocess as Excel file**. All bioprocess data is exported to Excel and represented in Excel (xls) format.

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.

8.1 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.

Here are described the relevant portions of the 21 CFR Part 11 regulations and their implementation using the Vi-CELL XR control software explained. It is important to realize that implementation and compliance of the rule 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 overall compliance with these regulations as are the features of the Vi-CELL XR control software.

8.2 ELECTRONIC RECORDS

Section 11.3 subpart A of 21 CFR Part 11 deals with the definitions assigned to electronic records, 'electronic record means 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'. In reality this refers to any digital computer file submitted to the agency, or any information not submitted but which is necessary to be maintained. 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.

8.3 FDA REQUIREMENTS

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

If electronic submissions are made, Section 11.2 subpart A comes into play: '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.

8.4 IMPLEMENTING ELECTRONIC RECORDS AND SIGNATURES

Section 11.3 Subpart A describes two classes of systems: “closed systems” and “open 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. In contrast, an open system is one "in which system access is not controlled by persons who are responsible for the content of electronic records".

A typical Vi-CELL XR installation will need to have a procedure designed to ensure proper operation, maintenance and administration for system security and data integrity. Anyone who interacts with the system, 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.

8.5 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.

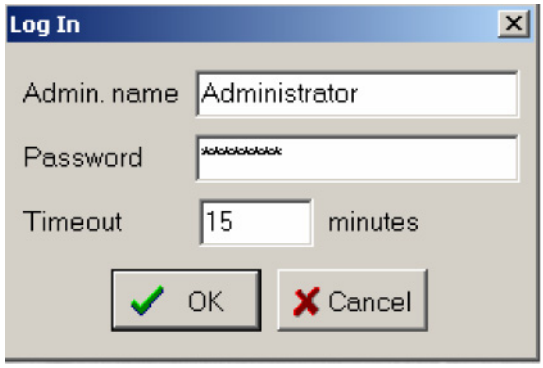
Of the other controls, perhaps the foremost is described in Section 11.10 Paragraph (a): "Validation of systems to ensure accuracy, reliability, consistent intended performance, and the ability to discern invalid or altered records." It is the complete and overall validation of the system, as developed 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.

8.6 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

If you select the security option (**Security**, **Turn Security On**) a dialog box appears requiring you to enter a user name and password and set the time in minutes for instrument timeout.

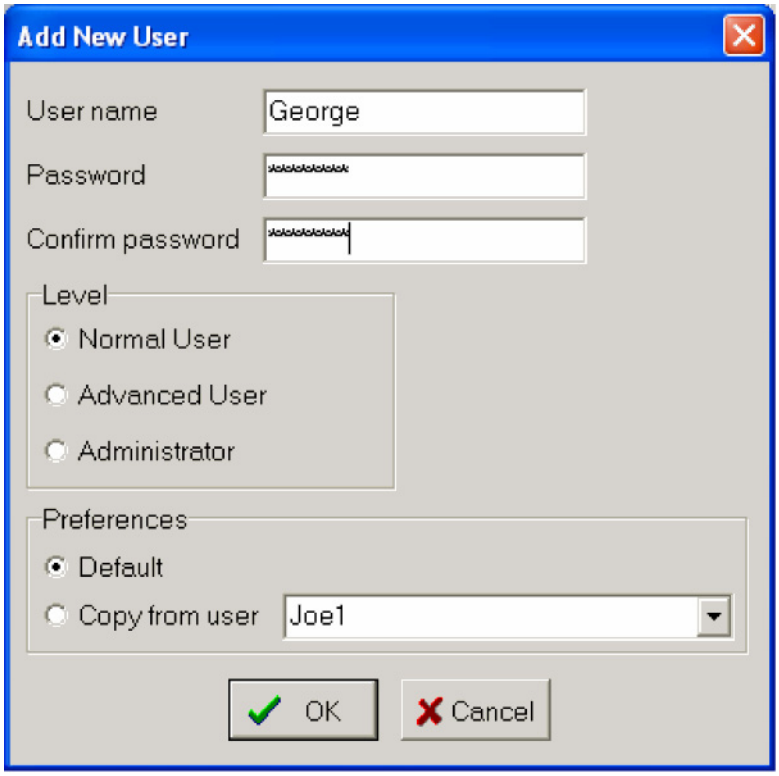


Once signed on, click on **Security**, **Add New User** to define users and access levels. The **Add New User** dialog appears.

Figure 8.1 The Security Options Screen

- Passwords must be a minimum of 8 characters
- Password expiry is set at 60 days
- Activity disable is set at 15 minutes with a maximum of 1439 minutes.

Figure 8.2 The Add New User dialog screen



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 shall be retained for a period at least as long that required to for the subject electronic records and shall 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 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, and failed checksums.

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 shall be unique to one individual and shall 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.

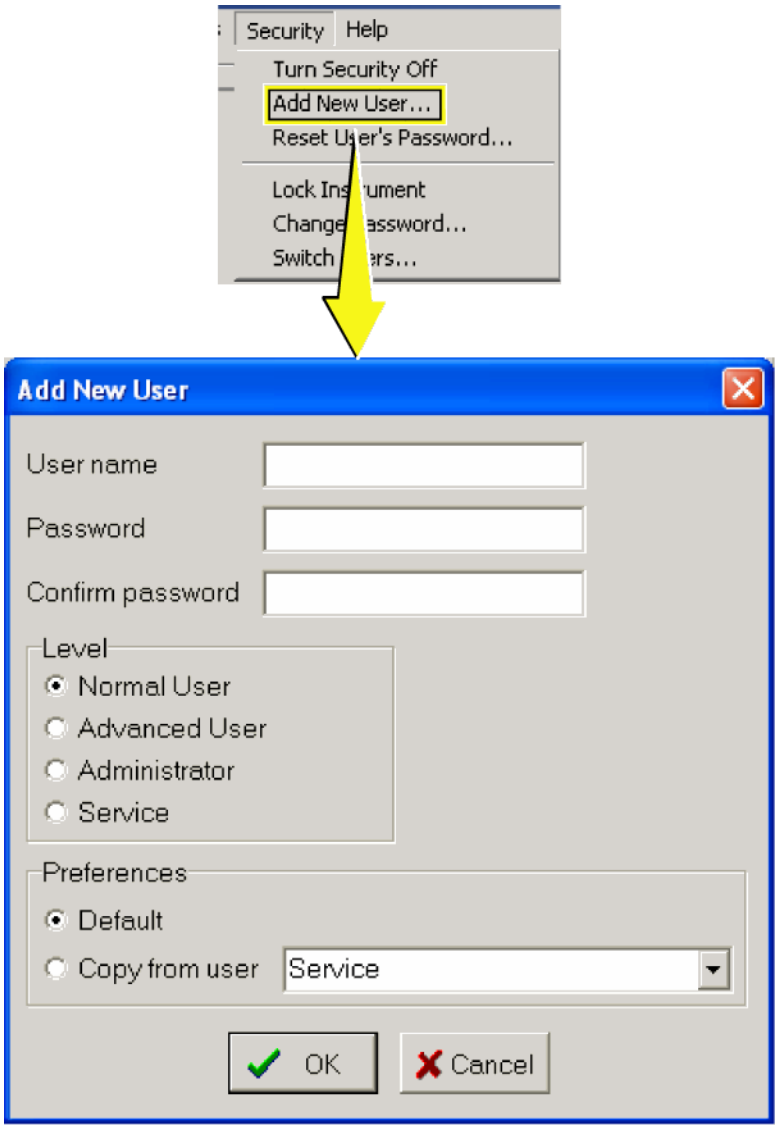
Subpart C, Section 11.200, refers to biometric and non-biometric forms of electronic signature. Biometric signatures are defined in Subpart A, Section 11.3 as a "a method of verifying an individual's identity based on measurement of the individual's physical feature(s) or repeatable action(s) where those 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 issuance's 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 screen. 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 issuance's are periodically checked, recalled, or revised".



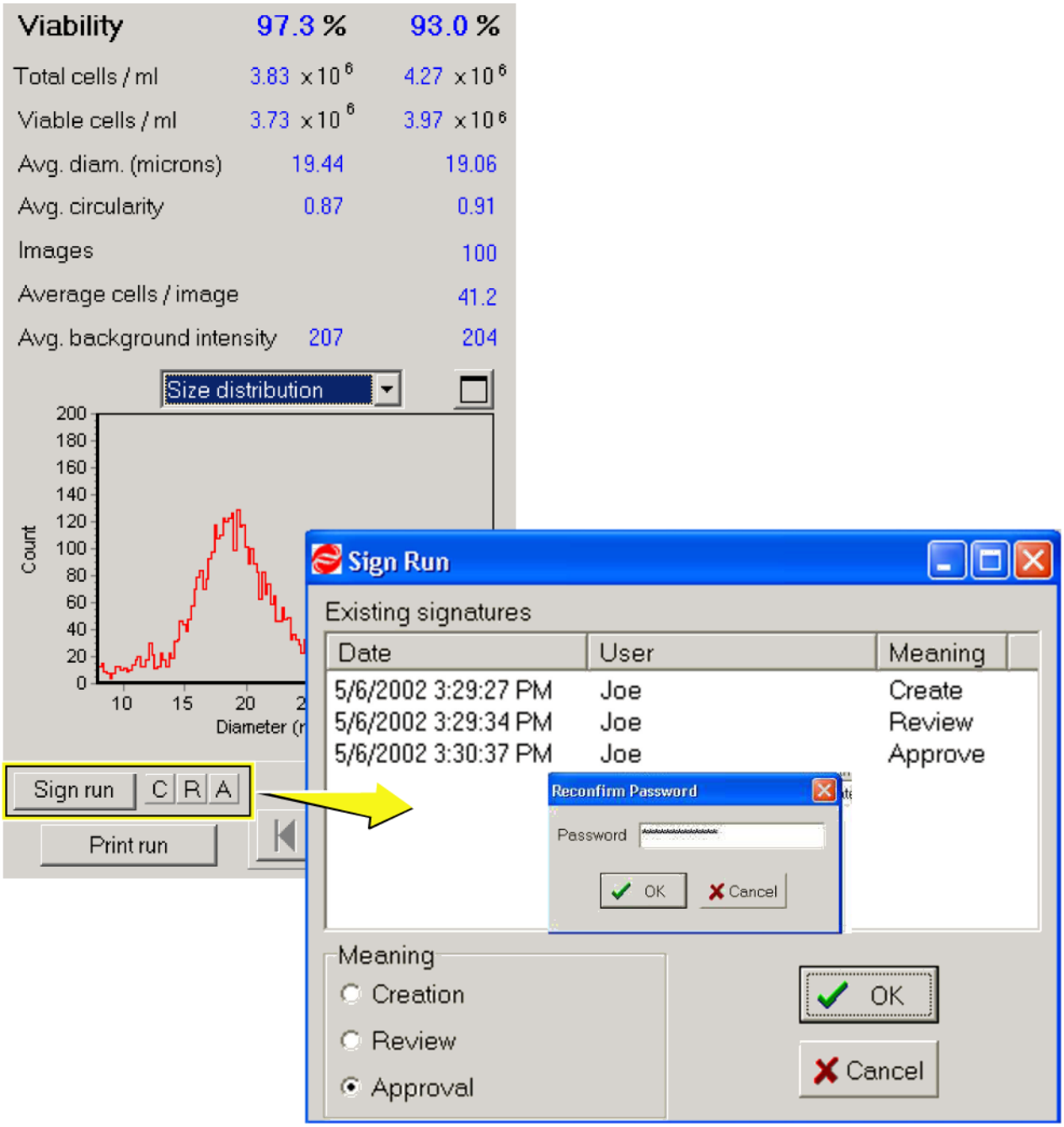
Signing A Run

Once users have been created and access levels assigned, users given signing rights will be able to sign a run upon completion and review of analysis data. To generate the electronic signature, open a file as described in Heading 4.3. Once the file is either opened, created or reviewed click on **Sign Run** on the lower right side of the screen to approve a run.

The **Sign Run** dialog will appear. From the list of user names, select the appropriate user ID and select **Creation**, **Review** or **Approval**. A **Reconfirm Password** dialog will appear. Enter the password.

The results will then be electronically signed and saved.

Figure 8.3 The Sign Run Dialog Screens



Meanings

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

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 and audit trailing, "to independently record the date and time of operator entries and actions that create, modify, or delete electronic records".

A.1 DATA ACQUISITION

- Operating principle: analysis of video images
- Sample type: spatial data
- Overall diameter range: 2 microns to 70 microns
- Analysis rate: up to 50 Images in 2.5 minutes
- Digitizing resolution: 1.45 mega-pixels

A.2 CELL VIABILITY/CONCENTRATION/CELL COUNT

- Concentration Range: 5×10^4 to 1×10^7 Cells/mL
- Viability Range: 0 to 100%
- Concentration Average Accuracy: Average Accuracy (n=20) within ±3% of Z2 Analyzer or other reference COULTER COUNTER measurement.

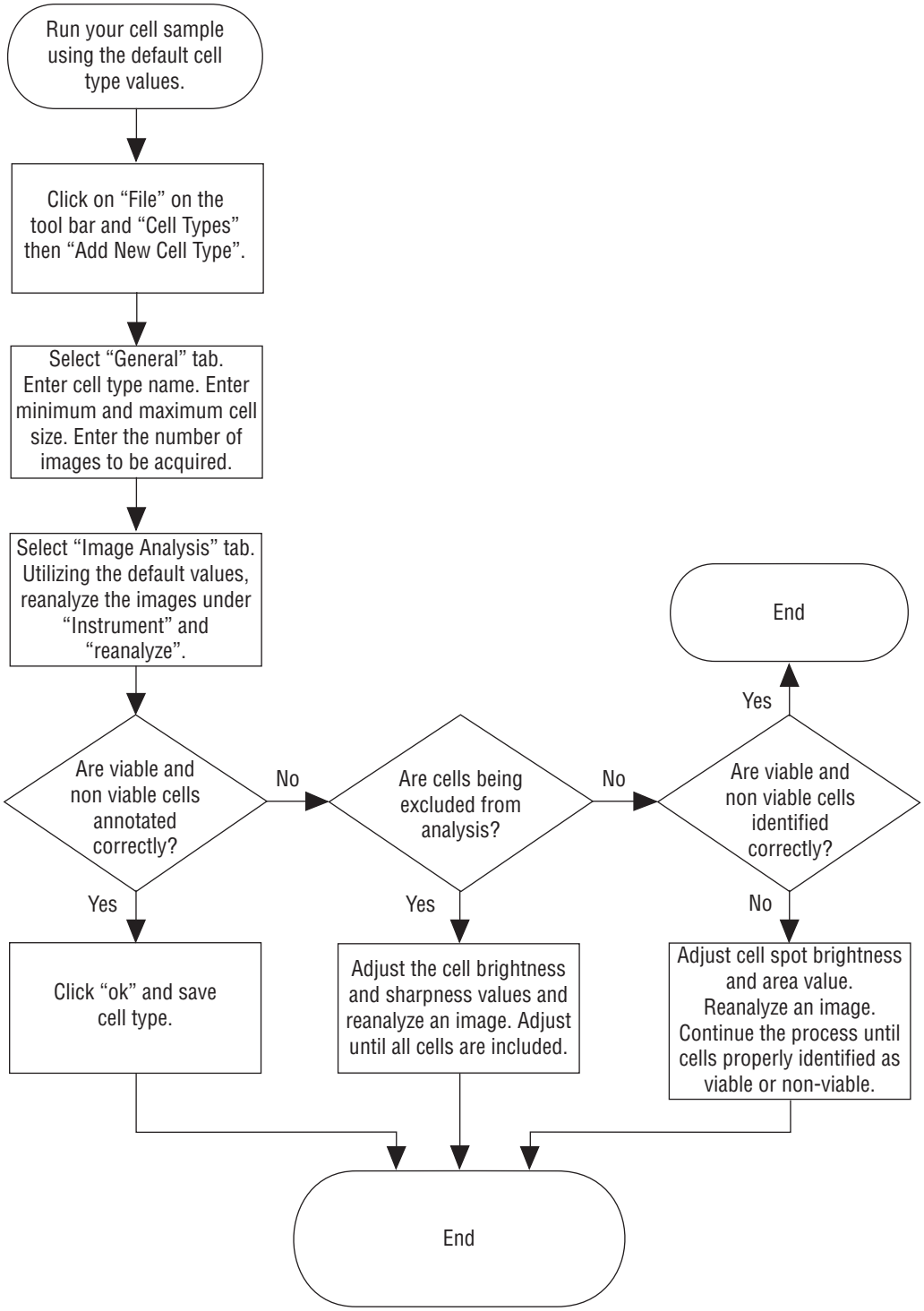
A.3 PHYSICAL REQUIREMENTS

- 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

A.4 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.)

Figure B.1 Creating and modifying a cell type



C.1 CIRCULARITY

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

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

C.2 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.

C.3 CALIBRATION

Micron/pixel ratio: 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.

D.1 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

D.2 ERROR MESSAGES DURING PROGRAM START

"Instrument not connected": an instance of Vi-CELL XR is already running on the computer, there is a problem with the board driver, or the serial cable is not connected.

D.3 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).

D.4 "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.

The Vi-CELL XR performs the following procedure via the on-board wizard.

Should the system require decontamination, perform the following procedure.

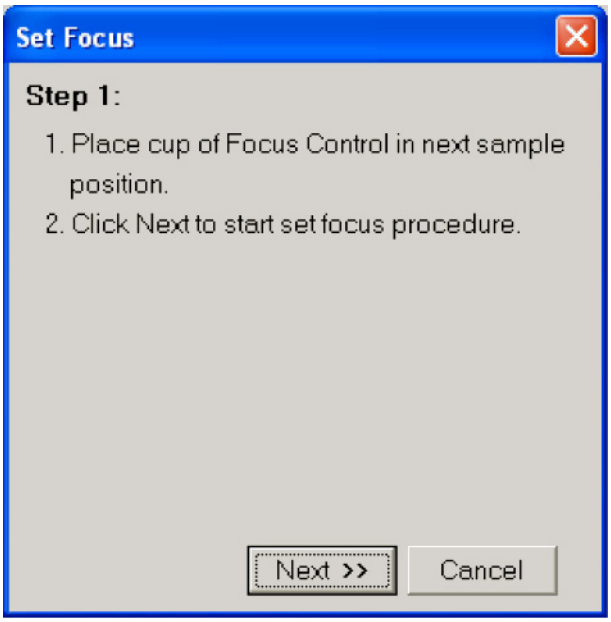
WARNING All instrument decontamination must be conducted under universal precautions for blood-borne pathogens. Instrument effluents should be regarded as a biohazard. Special care is required when opening pressurized fluid lines. Appropriate safety equipment must be worn (eye protection, latex gloves, and lab coat).

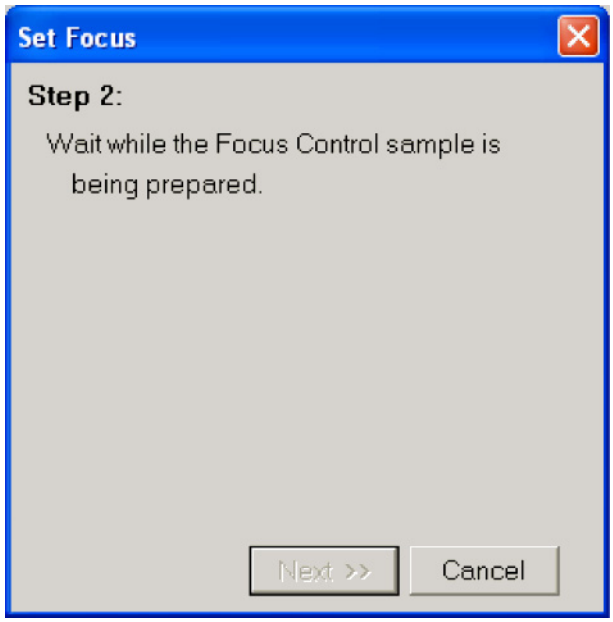
1. Purge the system for 15 minutes with a solution of 0.5% sodium hypochlorite (10% bleach, prepared by mixing 1 part household bleach with 9 parts water).
2. Flush the system with water for at least 5 minutes to completely wash the Bleach from the system. Purge rinse water from the system. Ensure system is thoroughly drained.
3. Decontaminate the external aspects of the system by washing with 0.5% Sodium hypochlorite solution (10% bleach, prepared as in step 1 above).

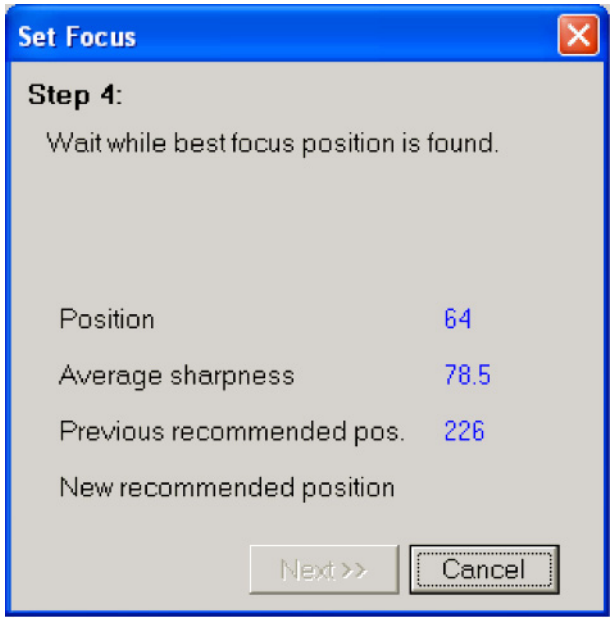
   Remove all dried blood or cell culture media from the instrument surface before disinfection. To remove these substances and prevent scattering potentially biohazardous material, the blood or culture media should be wetted and softened with the 0.5% sodium hypochlorite solution. After removal of the dried substances, decontaminate the surface of the Vi-CELL XR with the bleach solution again. 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.

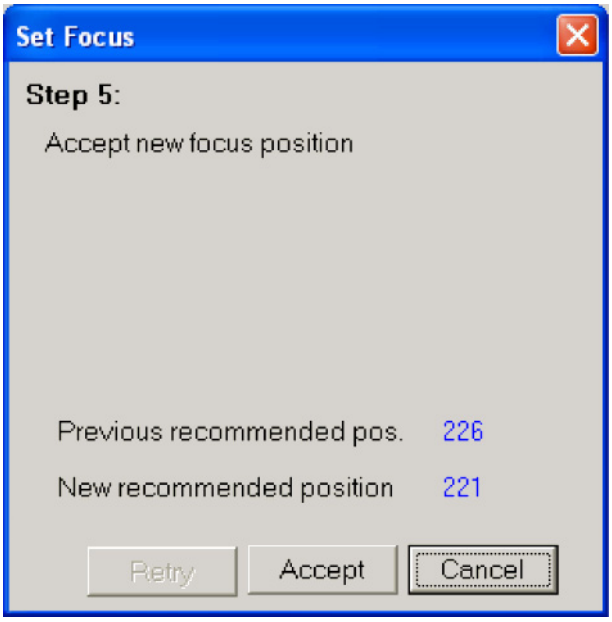
   In the Vi-CELL XR software, there is a Decontamination wizard, which “walks” the operator through this standard procedure (for the internal components). Select **Instrument** and, from the dropdown box, **Decontaminate**.

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 5:
Accept new focus position
Previous recommended pos. 226
New recommended position 221
Retry
Accept
Cancel


Set Focus
Step 6:
Wait while focus is moved to new position
and the cell is flushed.
Finish
Cancel


Set Focus
Step 7: Set focus complete
Finish
Cancel

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