

# Vi-Cell XR Cell Viability Analyzer

## User Instructions

Written by CMCresson 15-Feb-06

The Vi-Cell XR Cell Viability Analyzer is a video imaging system used to analyze yeast, insect and mammalian cells. It automates the trypan blue exclusion protocol, in which dead cells take up the dye while live cells do not, and provides data on percent viability and cell counts. Essentially, the Vi-Cell system is an automated hemocytometer unlike the multisizer, which was simply a particle counter. The Vi-Cell takes up the sample and delivers it to a flow cell and camera for imaging where differences in the grey scale between live and dead cells is determined by the software. For every sample you run, the instrument requires 500  $\mu\text{L}$  of sample volume, which is mixed 1:1 with trypan blue, and takes 50 images to determine the cell concentration and viability. All of this data is output on the computer screen and can also be sent to a printer or excel spreadsheet for further analysis.

### System Specs

Cell Diameter Range = 3 to 70  $\mu\text{m}$

Cell Concentration Range =  $5 \times 10^4$  to  $1 \times 10^7$  cells/mL

Viability Range = 0% - 100%

Counting Accuracy =  $\pm 6\%$

Sample Volume = 0.5 mL to 2.0 mL

Acquisition Time = 2 minutes

### Getting Started with the Vi-Cell

**Everyone** who will be using the Vi-Cell system **must** be trained by the system administrator. In addition, **everyone will be given a user account and password to log on to the instrument.** There will be a main system log in which user activity will be recorded. So, if you allow someone else to use your account, then you will be the responsibility party in the use log if something happens to the instrument. UROPs will not be given user accounts and will be the responsibility of their lab advisor.

The Vi-Cell system default cell type may work for some cell lines in the lab and it is really the best place to start. However, since trypan blue uptake, cell size, and appearance are unique to each cell type you should run some tests to determine the best parameters with your particular cell line. For cell lines that are used by many lab members and that need changes to the default parameters, we will create a cell type that will be specific for those cells. The cell types will appear in every user's menu. The software contains a tutorial for creating your cell lines so this may be a helpful feature for more difficult cell lines. In addition, you can email cell images to Beckman Coulter and they can assist you in determining the best parameters for your particular cell line.

It is also important to be aware of the amount of time your samples will be sitting at room temperature waiting on the carousel. Each sample takes 2 minutes to run and the carousel holds 12 samples. You may not want to prepare more than 12 samples to run at any give time as the

waiting could affect viability counts. However, as sensitivity to temperature and pH will vary for each cell line you may want to run controls before making a large number of samples.

The Vi-Cell user manual will be located on the shelf above the instrument at all times. Please consult the manual or the software help function if you have any questions. If these sources do not provide the info you need, then please contact Catherine (cresson@mit.edu or 8-9488).

This is a very expensive instrument and it will be heavily used by many different lab members so please take good care of the Vi-Cell and follow all of the operating procedures

## The Software

Click on the Vi-Cell icon on the desktop to begin the software.

You will then need to login to the program with your user ID and password provided by the system administrator.

Use the **Navigation Bar** on the left side to determine what is displayed in the main window.



**Camera Image** – allows for the viewing of sample images during and after a run

**Autosampler Queue** – shows the completed samples along with the list of samples in the queue, this is where you can modify/remove samples that are already in the queue

**Cell Types** – displays windows with the information for each specific cell type; this is where you create or remove new cell types

**Bioprocess** – the last icon represents a bioprocess; there can be many of these icons that each represents an ongoing experiment in which data is collected over days or months (for more details of setting up bioprocesses see pg. 49 in the user manual).

## Running a Sample

All reagents including the trypan blue are internal to the instrument so all you need is your cell solution to get started. Sample cups are located on the shelf above the instrument.

**Cell samples can be suspended in any of the following solutions: PBS, isoton (liquid used with old cell counter), or media (even if it contains phenol red).** See system specs to ensure you will be within the necessary volume and cell concentration ranges.

1. Sample Prep
  - a. Prepare samples in the TC hood and bring to bench in ependorf or conical tubes.
  - b. Make the necessary dilutions to keep your cell concentration within the correct range.
2. At the lab bench, add 600  $\mu\text{L}$  of solution to sample cup. **Exact volume does NOT matter as the instrument sucks up entire solution and only uses 500  $\mu\text{L}$ .**
3. Place sample cup in next available carousel position.
4. Log in sample on the computer by clicking on the **Log in sample** button: (log in window shown below)
  - a. Select cup position on carousel
  - b. Enter your **Sample ID** (the software is smart and will increment for you with multiple samples)
  - c. Choose a **Cell type** (\*first time choose default)
  - d. Select a dilution factor
  - e. Click **OK** or **Next sample** to enter additional sample data (\*once you start the queue you can continue to enter samples, so it saves time to start the queue first)
5. From the navigation menu, choose **Autosampler queue** to see your samples in the queue. From this screen, you can also edit/remove samples in the queue while a run is in progress.
6. Click on **Start queue** to begin sample analysis. Once the run begins, your sample will disappear from the queue and you will only see it on the main screen. The bottom of the screen will tell you exactly what the instrument is doing (i.e. mixing trypan blue, loading flow cell, etc) and right side displays the run data (i.e. image #, % viable, cell count).

Indicates which position in the carousel you are putting your sample in. NOTE: the current three positions at the back of the carousel do NOT appear in the menu

Each user will be able to set their preferences for the default setting. Also, we will create new cell types for common lab cell lines.

The dilution factor will automatically be added into the result calculation by the instrument. Choices are 1.0 to 20.0

7. Click on **Camera view** in the navigation bar to see images as they are collected. Cells outlined in green are counted as live and cell outlined in red are counted as dead.
8. On the right side of the screen, data is displayed for each image as it is collected and the overall average is updated as the run progresses. There is also a graph that displays detailed data from each parameter such as size distribution, % viability per image, etc.
9. To add additional samples to the queue, click on **Log in Sample** and repeat the process in Step 4 above. You can only add 9 samples to the queue at any given time. But, you can continually add samples as carousel positions become available.
10. When the sample run is complete, the instrument will clean itself. (Note: You cannot review data images until this process is complete and there are no more samples to be counted.)
11. The carousel will then rotate to check for additional samples. At this time, empty sample cups will be knocked into an internal waster container.
12. **Read general system information below to ensure that you follow proper waste guidelines.**

### Analyzing the Sample Run

When the cell analysis is complete, you can flip through the images to check the accuracy of the instrument in identifying live/dead cells. You will also want to look at the size distribution graph to ensure that the correct minimum size value was used.

If you would like to change any of the cell type parameters, choose **instrument** from the top tool bar and scroll down to click on **reanalyze**. At this point, the default cell type window will open up and you can adjust the necessary parameters and click ok. The cell images will then be analyzed using the new parameter values and the data will be displayed on the right side of the screen. Reanalyzing a sample does not overwrite the original data and the new data is not automatically saved. So you need to choose **save run** from the main menu in the top tool bar to save data before closing.

If you would like to reanalyze a previous run, then you can reload the data by clicking **open run** from the main menu. Then, select the **text file** from your data folder and the corresponding images will be automatically loaded as well.

### Saving Data

In the user preferences, everyone will have their own directory in which data is automatically saved. All image files appear as file folders containing tiff files of the individual images and the data is saved as a text file. If selected during the sample log in process, your data will also be output to an excel spreadsheet. There is an example excel sheet with explanations in the Vi-Cell user manual for your reference. **It is recommend that all images are saved during the setup period so that data can easily be reanalyzed to determine the best cell type parameters. However, as the hard drive will quickly fill up you will need to alter your preferences at a later date NOT to automatically save the images.** Please be respectful of how much space you taking up on the computer and remove any unnecessary files. The computer is not currently connected to the internet so data will have to be transferred by floppy disk, CD, or USB memory stick at this time.

## General System Information

It is recommended that the Vi-Cell instrument remains ON at all times. Do NOT turn off the instrument.

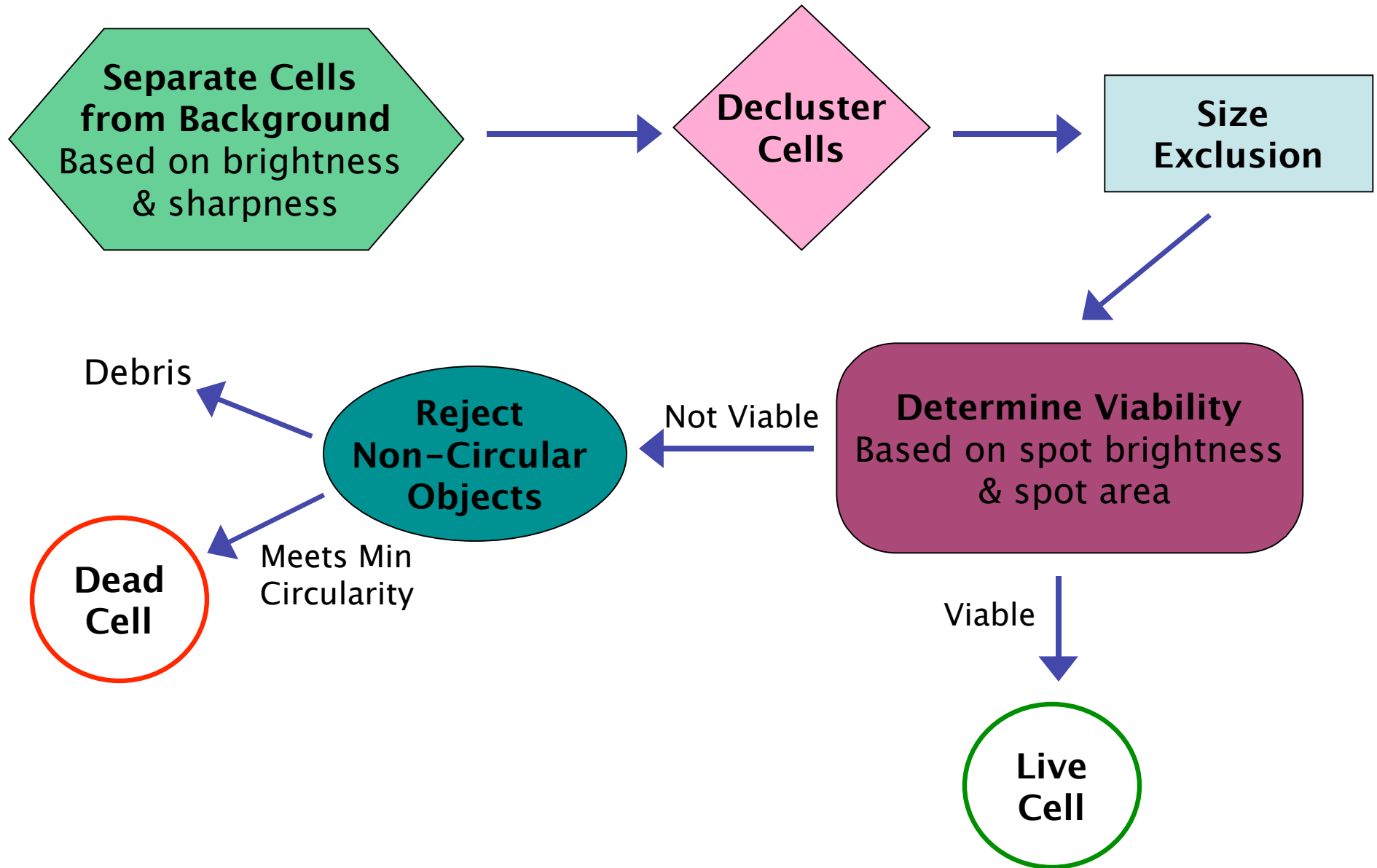
At the end of a run, the sample cup is dumped into a bin inside the machine. The software will alert you when the bin has become full. To empty the bin, open the side door, remove the metal tray, and dispose of the cups in a **biohazard waste** container.

Also, all of the liquid waste is collected by the instrument. When the waste container inside the instrument is full, the software will alert you. First add 50% bleach to the waste, and then you should dispose of the waste in the proper **hazardous waste** container in the hood. If there is no waste container please fill out a new waste disposal card by following the example that is taped inside the door of the Vi-Cell. The waste percentages listed on the card should be the same for all samples: 0.2% trypan blue + 1% sodium hypochlorite + 15% isopropanol. *If you are suspending your cells in a solution that is also hazardous, you need to write that information on the waste card as well – every time you use the cell counter.*

As for the instrument reagents, these will be ordered from Beckman Coulter by the lab manager and stocked near the instrument. *Only the Beckman Coulter reagents are compatible with this instrument. Do NOT use any other brand of trypan blue.* If we are running low on reagents, you will need to place them on the order board in the lab. The instrument contains a reagent fuel tank that displays the relative amount of reagents in the machine and also the remaining number of runs at all times. When the reagents do run out, once again the software will give you an alert. **Do NOT attempt to change the reagents on your own.** This is the most common problem that occurs with the instrument so you will need to locate Catherine to change the reagents for you. If Catherine is not around, then please see Christina, HD, or Lisa to change the reagents.

The instrument will be decontaminated once a month by a 50% bleach cleaning and the person in charge of the Vi-Cell will take care of this process.

# Vi-CellXR Cell Image Analysis Scheme



## Creating and Modifying Vi-Cell Cell Types

When you begin using the instrument, it is best to select the **Default Cell Type** to analyze your sample. Then, you can adjust these parameters to fit the specific cell line you are measuring and create a unique cell type. These cell types will show up in everyone's user account and there should be only **one cell type for each cell line** that we use in the lab. Please talk with other lab members to set the parameters for a given cell line so that everyone can use the same cell type for a given cell line.

For additional details, please see the Vi-Cell User Manual or the cell type power point tutorial, which is saved on the desktop of the Vi-Cell computer with the extension pps.

To access the cell types:

1. choose **File** from toolbar then click on **Cell Type** and you will be given the option to add, modify, or delete a cell type.
2. choose **File** from toolbar then click on **Image-Reanalyze** and you will be given the option to add, modify, or delete a cell type.
3. choose **Instrument-Reanalyze** from toolbar then you will be given the option to add, modify, or delete a cell type.

### Sample Prep Options

Parameter	Range	Default	Details
<b>Aspiration Cycles</b>	1 to 7	3	# of times sample is mixed in syringe; set according to cell sensitivity & clumpiness
<b>Trypan Blue Mixing</b>	1 to 7	3	# of times sample and trypan blue are mixed in syringe

### Image Analysis Parameters

The ranges given below are the normal recommended ranges for each parameter and default value refers to the parameter value in the **Default Cell Type**. The default values are a good starting place and you should tweak individual parameters to determine their effects on the output values.

For the cell brightness parameter, you will need to observe the image in binary mode to see the difference between parameter values. Right click on the image, then **turn off annotation** and then choose **Binary Mode**. Return to normal view for all other parameters.

Parameter	Range	Default	Details
<b>Images</b>	1 - 100	50	Increase value for very low concentration samples
<b>Size</b>	3 - 70 $\mu\text{m}$	10 -40 $\mu\text{m}$	If a particular cell is being problematic, place cursor over cell and size is displayed below image
<b>Brightness</b>	75 - 90	85	Picks out objects from background Increase includes more objects Decrease includes less objects
<b>Sharpness</b>	60 - 200	100	Picks out objects from background Decrease to include more objects Increase to include less objects
<b>Spot Brightness</b>		75 %	Determines cell viability Increase value more cells counted as dead Decrease value more cells counted as live
<b>Spot Area</b>	1 - 20 %	5 %	Ratio of the bright spot to total area Determines cell viability Increase value more cells counted as dead Decrease value more cells counted as live
<b>Minimum Circularity</b>	0-0.5	0	Excludes dead cells so they are considered debris 0 excludes no dead cells 1 excludes all dead cells
<b>Decluster</b>	None, low, med, high	medium	Ability of software to pick individual cells from a cluster of multiple cells None counts a cluster as 1 cell