BECKMAN COULTER
Life Sciences

Transferring Cell Counting Methods - Recommended Steps to Correlating Results

Introduction

When a new instrument, like the Vi-CELL BLU Cell Viability Analyzer is introduced it may be necessary to conduct a bridge or correlation study in order to migrate from an existing method on the previous instrument.

This document breaks down the Vi-CELL BLU Matching Cell Counts with the Vi-CELL XR application note into a step-by-step process for transferring a cell counting method from a Vi-CELL XR analyzer to a Vi-CELL BLU analyzer.

Tables are included for recording results. The recorded information will be helpful to your Beckman Coulter Support Specialists if you need help during the process.

Materials and Equipment

Description	Beckman Coulter Life Science Part Number if applicable
Vi-CELL XR Concentration Control	175478
Vi-CELL BLU 2M Concentration Control	C09148
Vi-CELL BLU 4M Concentration Control	C09149
Vi-CELL BLU 10M Concentration Control	C09150
Cell culture > 2e6 cells/mL and > 50% viability (enough volume to run samples side by side in triplicates)	N/A
Pipette capable of transferring 200 µL to 1000µL of liquid	N/A
General laboratory Vortex	N/A
General laboratory mini tabletop centrifuge (microfuge)	N/A

Step 1 - Baseline Instruments

It's important to first verify the performance of the instruments that will be used in the comparison study. This ensures all systems are operating to their performance specification before beginning comparison testing.

- Verify instrument performance using manufacturer recommended standard beads and protocols. If any of the tests below fail to meet their acceptance criteria, please contact Beckman Coulter Service for guidance before proceeding further.

Vi-CELL XR Analyzer

Please follow assay sheet instructions to use 1 mL of Vi-CELL concentration control for analysis. This ensures consistent results

1M control	Lot#	Assay Value	-10% of assay value	+ 10% of assay value
Part Number 175478				

Replicates	Total Count	Total cells/mL (x1.0^6)	Within +/- 10% of assay value?
Replicate 1			
Replicate 2			
Replicate 3			

Vi-CELL BLU Analyzer

Please follow sample prep instructions on the assay sheet for all concentration controls and run samples using Normal mode. This will ensure consistent results

2M control	Lot#	Assay Value	-10% of assay value	+ 10% of assay value
Part Number C09148				

Replicates	Total Count	Total cells/mL (x1.0^6)	Within +/- 10% of assay value?
Replicate 1			
Replicate 2			
Replicate 3			

4M control	Lot#	Assay Value	-10% of assay value	+ 10% of assay value
Part Number C09149				

Replicates	Total Count	Total cells/mL (x1.0^6)	Within +/- 10% of assay value?
Replicate 1			
Replicate 2			
Replicate 3			

10M control	Lot#	Assay Value	-10% of assay value	+ 10% of assay value
Part Number C09150				

Replicates	Total Count	Total cells/mL (x1.0^6)	Within +/- 10% of assay value?
Replicate 1			
Replicate 2			
Replicate 3			

Step 2 - Baseline Cell Counting difference

- Compare instrument counting difference by sampling the Vi-CELL concentration control, catalog part number 175478, on both instruments.
    a. For the Vi-CELL XR analyzer, use the same sample volume of concentration control as you would normally use for cell sample analysis.
    b. Use 200µL on Vi-CELL BLU analyzer.

Vi-CELL XR Analyzer (use concentration control cell type)

Replicates	Sample volume used (µL)	Total Count	Total cells/mL (TCD)*	Avg Diameter
Replicate 1				
Replicate 2				
Replicate 3				

Average of TCD =_____

Vi-CELL BLU Analyzer (use BCI Default cell type)

Replicates	Sample volume used (µL)	Total Count	Total cells/mL (TCD)*	Avg Diameter
Replicate 1	200			
Replicate 2	200			
Replicate 3	200			

Average of TCD = _____

c. Calculate the Total cells/mL difference.

$$\frac{\text{Average Vi-CELL XR TCD replicates} - \text{Average Vi-CELL BLU TCD replicates}}{\text{Average Vi-CELL XR TCD replicates}} \times 100$$

Difference = _____ %

Note: If the % Difference is greater than +/- 10% we recommend you contact your Beckman Coulter Service Representative before continuing further.

* TCD is total cell density

Step 3 - Create a New Vi-CELL BLU Cell Type

- Create a new Vi-CELL BLU Cell Type to correlate with the Vi-CELL XR Cell Type.

  Use the nearest equivalent default Cell Type. (for example, Mammalian for CHO, HEK or Insect for SF-9.

  Or Create a new Cell Type using the suggested conversion table below:

Parameter	XR setting	BLU Setting Starting value
Minimum diameter	>2	Same as XR
Maximum diameter	<70	Same as XR to <60
Images	50 to 100	Same as XR
Aspirate Mix	1 to 9	3 or higher*
Trypan Mix	1 to 9	3 or higher*
Cell brightness	50-90	N/A
Cell sharpness	100	7
	80	15
	60	20
Viable spot brightness	85	65
	75	50
	65	45
	40	40
Viable spot area	0 to 95	Same as XR
Min circularity	0 to 1	Same as XR
Decluster	None, Low, High, Med	Same as XR

* Cells with tendency to aggregate, increase Aspirate and Trypan Mix cycles to help break up clusters.

Record Cell Type Settings:

Parameter	XR Cell Type	BLU Cell Type
Minimum diameter		
Maximum diameter		
Images		
Aspirate		
Trypan Mix		
Cell brightness		N/A
Cell sharpness		
Min circularity		
Viable spot area		
Min circularity		
Decluster		

Step 4 - Compare Sample Results

- Run samples side by side on both Vi-CELL XR and Vi-CELL BLU instruments
    a. Prepare samples with cell concentration > 2e6 cells/mL and > 50% viability (>70% is preferred).

    b. Replicate samples are recommended to improve statistical confidence.

    c. For Vi-CELL BLU use the cell type created in step 3 for analysis.

    d. Save images for Cell Type optimization with the Reanalysis function.

Note: Export results to append to a .xlsx file on the Vi-CELL XR analyzer and to .csv file on the Vi-CELL BLU analyzer for easier data analysis.

    e. Review results and optimize Vi-CELL BLU cell type settings if results are not within your expectation.

Guidance: We recommend you start with the following criteria for variation differences between instruments:

Cell concentration +/- 10% difference and % Viability +/- 5%.
At concentrations below 2x10^6 cells/mL the differences can be greater than 10% because fewer objects are analyzed per image which increases variability of the sample.

	Vi-CELL XR Analzyer			
Replicate	**Sample volume used**	**Total Count**	**Total cells/mL (TCD)**	**% Viability**
1				
2				
3				

	Vi-CELL BLU Analzyer			
Replicate	**Sample volume used**	**Total Count**	**Total cells/mL (TCD)**	**% Viability**
1				
2				
3				

Step 5 - Optimizing Vi-CELL BLU Cell Type

For some cell lines, parameters will need to be changed to optimize the analysis of the cells. Use the Cell Type submenu in the Vi-CELL BLU application to create, reanalyze data and refine parameters to reach the desired target.

The table below and appendix A helps guide you through the Cell Type optimization process.

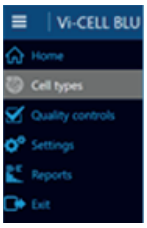
	Cell Type Parameters	Typical Range	Full Range	Increase Value	Decrease Value	When/Why You Would Want to Change This	Warnings
Defines whether there is a cell present or not	Minimum Diameter (µm)	5 to 12	1 to 60	Fewer cells identified	More cells identified	Software is excluding small cells (circled blue) Debris included in analysis	Too low, may be including debris Too high, cells excluded
	Maximum Diameter (µm)	15 to 50	1 to 60	More cells identified	Fewer cells identified	Software is excluding large cells (circled blue) Clumps of cells are included	Too low, may be excluding cells Clumps of cells included
	Cell Sharpness (no units)	5 to 25	0 to 100	Fewer cells identified	More cells identified	Software is excluding "fuzzy" cells Unwanted debris is being picked up	Set too high may cause dead cell to be excluded and alter the percent viability.
	Minimum Circularity (no units)	0 to 0.60	0 to 1.00	More irregular shaped dead cells will be excluded	Fewer irregular shaped dead cells will be excluded	Unwanted irregular shaped debris are being picked up	Too low, may be capturing unwanted debris Too high, may start excluding cells
	Decluster Degree	None, Low, Medium or High	N/A	More cells identified	Fewer cells identified	Cells in clusters are being missed Single cells are being identified as 2 or more cells	Too low, may be missing cells in clusters Too high, may over decluster by annotating single cells as 2 or more cells
Defines whether identified cells are viable or not	Viable Cell Spot Brightness	40 to 60%	0 to 95%	More cells identified as dead	More cells identified as viable	Viable cells are identified as dead Dead cells are identified as viable	Too low, may start identifying dead cells as live Too high, may start identifying live cells as dead
	Viable Cell Spot Area	3 to 12%	0 to 95%	More cells identified as dead	More cells identified as viable	Viable cells are identified as dead Dead cells are identified as viable	Too low, may start identifying dead cells as live Too high, may start identifying live cells as dead
In Vi-CELL BLU version 1.4 and above	Concentration-Adjustment-Factor	0 to ±10%	0 to ±20%	Allows specific cell type to be adjusted to match legacy instrument results. Adjustment range is limited to ±20%. It is recommended to use the following formula to determine the value: (Target Concentration – Measured Concentration) / Measured Concentration = Concentration adjustment factor			

We have designed the Vi-CELL BLU analyzer with the flexibility to adjust the Cell Type settings to match results obtained from an equivalent sample when run on the Vi-CELL XR analyzer. Complete matching may not be possible in all cases as the performance characteristics between the two instruments are very different - but for many cell types it should be possible to match the performance between machines to acceptable levels. Please reach out to your local customer support representative for further assistance and troubleshooting.

Appendix A:

The flow charts presented here are intended to assist you with a systematic approach to modifying the parameters available in the cell type.

Cell Type Menu>Reanalysis



In the Vi-Cell BLU software main menu select the Cell Types submenu

Select the Reanalysis Icon

Copy and rename the cell type used in the sample runs

Open one of the sample result files

Is % Viability within +/- 5%?

Yes

Is total cell concentration within +/- 10%?

Yes

Save Cell Type

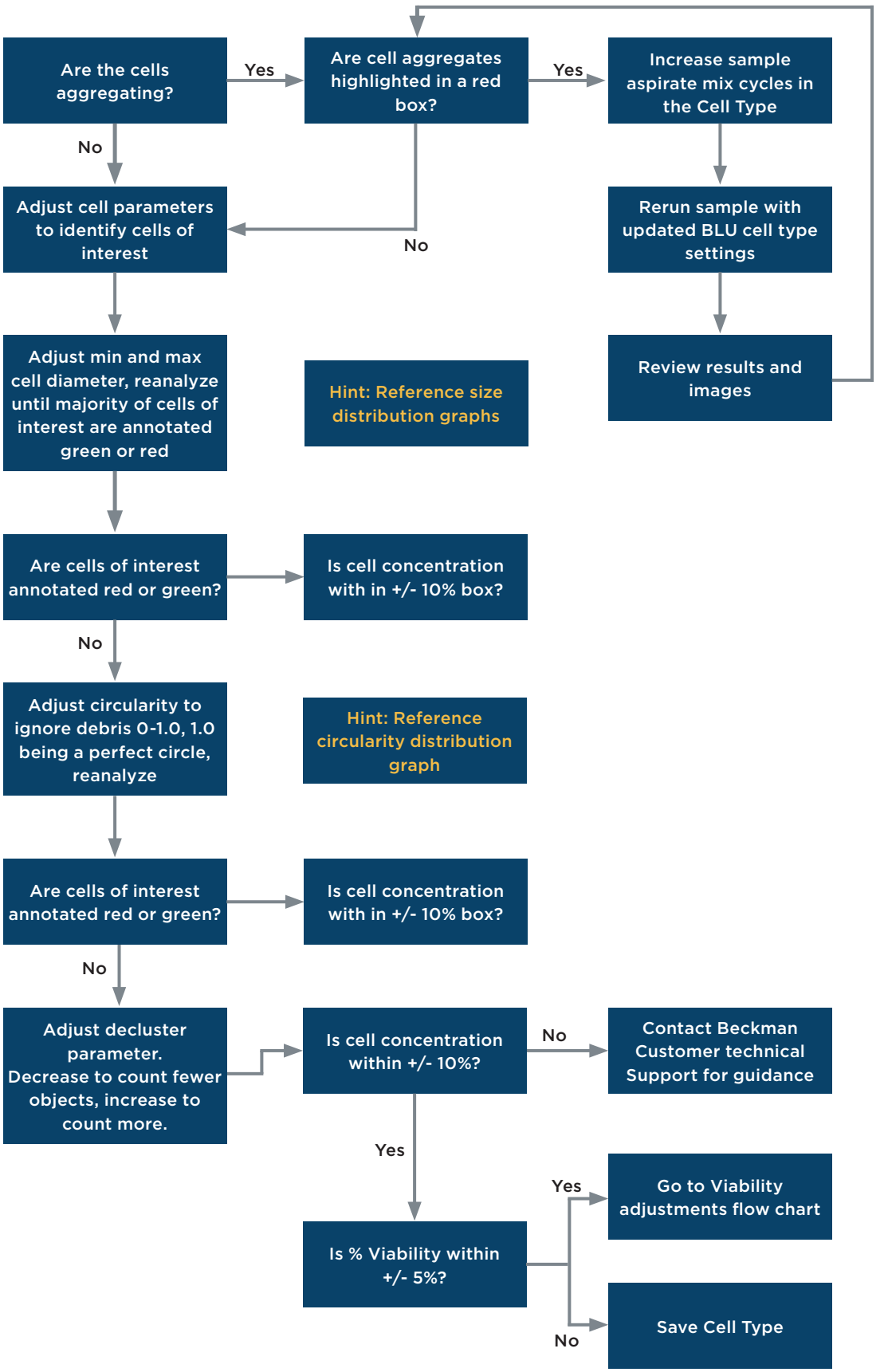
No

Go to Viability Adjustment flowchart

No

Go to Cell Count Adjustment flowchart

Cell Count Adjustment Flowchart



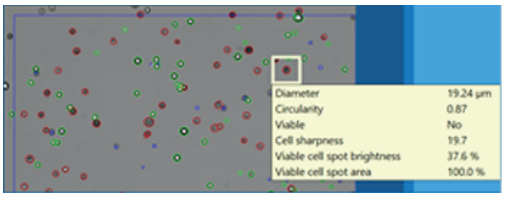
Viability Adjustment Flowchart

Open the Vi-Cell BLU menu and select Cell Types

Click on the reanalysis icon located at the lower right corner of the application

Click on the folder icon to open images from a completed run

Click through the images and use the cursor to select objects that you would determine to be non-viable



The object attributes will appear.
Note the average viable cell spot brightness and spot area values and record here:

Viable Spot Brightness _____
Viable Spot Area _____

Round up values:
Example 53% = 55% or 57% = 60%
Viable Spot Brightness _____

Example 4% = 5% or 7% = 10%
Viable Spot Area _____

In the Cell Type list, select the recently copied /created Cell Type

Adjust viable cell spot brightness and viable area to the roundup values

Reanalyze the data

Is % Viability within +/- 5%?

No

Change viable spot brightness:
Decrease viability, add another 5 increments
Increase viability, substract another 5 increments

Yes

Save Cell Type

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For Beckman Coulter's worldwide office locations and phone numbers, please visit Contact Us at beckman.com

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