BECKMAN COULTER
Life Sciences

Matching Cell Counts between Vi-CELL XR and Vi-CELL BLU

When a new instrument technology is introduced there may be the necessity of revalidating protocols on the new platforms however this can be a costly and time consuming exercise.

With this consideration in mind we have designed the Vi-CELL BLU with the flexibility to adjust the Cell Type settings to match results obtained from an equivalent sample when run on the Vi-CELL XR. Complete matching may not be possible in all cases as the performance characteristics between the two instruments is very different, but for many cell types this should be possible to match the performance between machines to acceptable levels.

To demonstrate this, a series of different cell types were run on the Vi-CELL XR using typical cell type parameters for the cell type.

At the same time (to reduce time variability) duplicate samples were run on Vi-CELL BLU instruments using default cell type parameters. The Vi-CELL BLU data was then reanalyzed adjusting the cell type parameters until a new cell type was generated that gave cell concentration within +/-5%* and viable cell density levels within +/-2.5%* of the Vi-CELL XR. Replicate samples were then run using the new cell type to confirm the performance.

Methods

1. The Vi-CELL XR and Vi-CELL BLU instruments were first baselined using manufacturer recommended standard beads and protocols.
2. Instrument performance was then verified using 1M beads/mL BEC concentration controls (catalog number 175478) on Vi-CELL XR and Vi-CELL BLU instruments. (Alternative bead concentrations can be utilized as long as the concentration is determined using another particle counter such as a Multisizer Coulter Counter).
3. Before proceeding the concentration measurements of instruments need to be within 5% of each other.
4. Run samples of cells on Vi-CELL XR using the desired cell types. Cells need to be > 2M/mL and > 50% viability (> 70% is preferred). Export data for later analysis. Replicate samples are recommended to improve statistical confidence.
5. Run samples of the same cells on the Vi-CELL BLU using the nearest equivalent default cell type parameters (typically Mammalian). Export data for later analysis.
6. Use cell type: Reanalysis option (Figure 1) to adjust Vi-CELL BLU cell type parameters to match cell concentration and viability to within 10% of report Vi-CELL XR concentration and within 5% reported viability. Save Adjusted Cell Types.

*Results may vary for different cell lines, concentrations or viability ranges

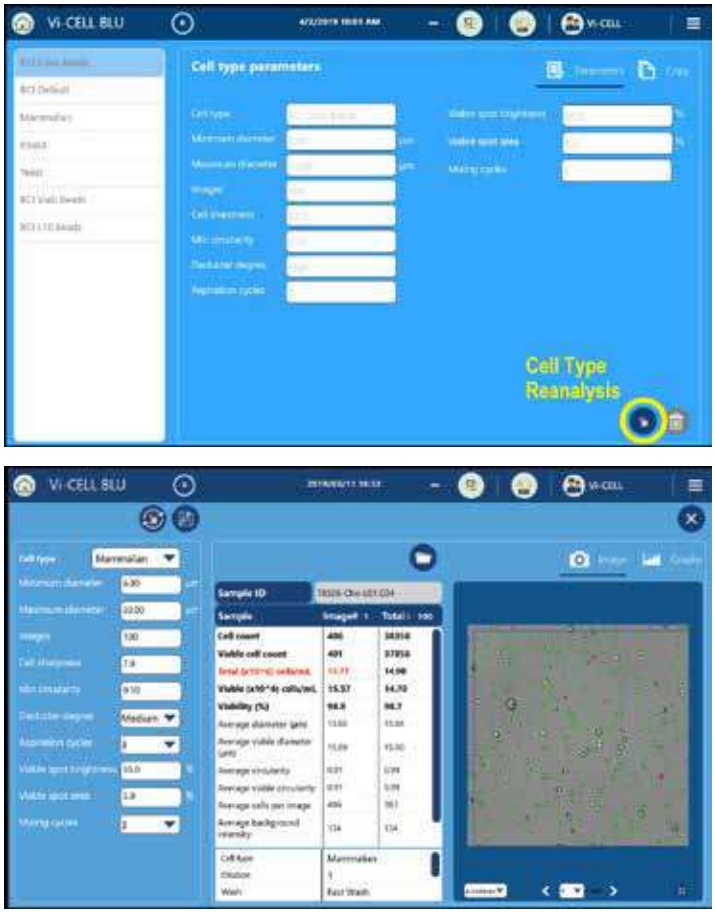


Figure 1. Cell Type Reanalysis

Guidelines for Adjusting Cell Type Parameters

1. Use the annotated images in Vi-CELL XR and Vi-CELL BLU software to determine which parameters to adjust.
2. Min and max diameters and decluster degree will most likely change the cell concentration value and reported average diameter. These parameters have the biggest impact on which objects are included in the overall count (analysis population). Use annotation to adjust if small or large cells are circled blue.
3. Viable spot brightness is adjusted to match viability, use image annotations to adjust if dead cells are circled green or live cells are circled red (see next slide). Note that viable spot brightness is inversely related to viability % as increasing the brightness threshold for what defines a live cell will reduce the number of cells scored as live.
4. Decluster degree can be increased if cells are not accurately counted in clumps or decreased if excess cells are present in clumps. Note that changing decluster will require the full image set to be saved to get accurate results as the images have to be reanalyzed to generate a new object population.
5. Circularity and sharpness can be increased to eliminate debris. Viable spot area can also be used to filter out debris.

For cells that deviate significantly between the instruments, check the analysis images and ensure that there are not excessive clumping or for cells are inadequately stained. Some cell types such as adherent cells can be rather clustered whereas yeast and other cell walled organisms may show resistance to trypan blue uptake. To help address this it may be necessary to rerun samples with the following changes.

1. Increasing aspiration cycles can be used to declump cells.
2. Increasing trypan blue mixing cycles can be used to allow for more staining time if dead cells seem faint and are not circled red.

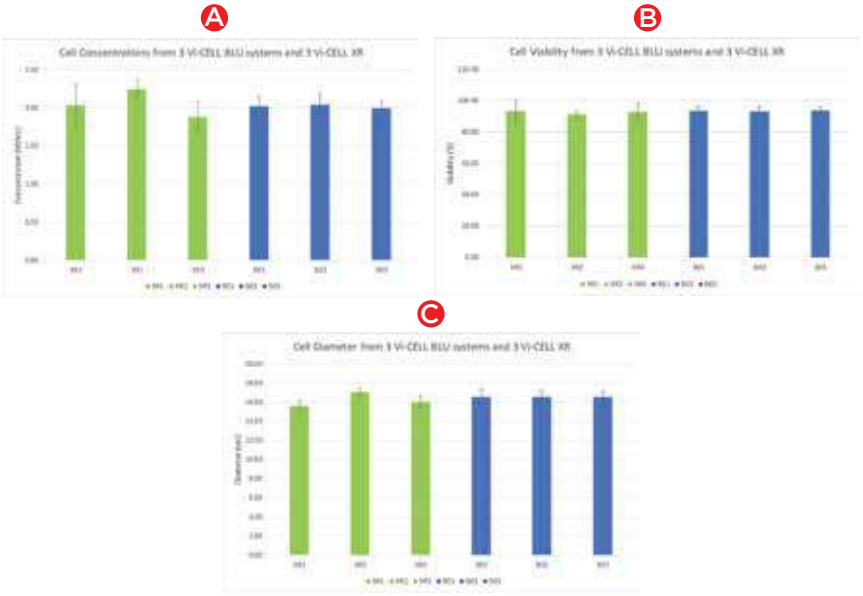
To evaluate the instrument matching approach cells were run on 3 Vi-CELL BLU and 3 Vi-CELL XR systems using the same default cell profiles as outlined below. The goal is to match the systems within +/-5% (10% range) for concentration and diameter and within +/-2.5% (5% range) for Viability. This degree of tolerance was chosen as it falls within the performance criteria for the Vi-CELL BLU instrument. Users can determine their own degree of matching but these general guidelines will typically put the measured values within statistically acceptable limits.

Results

CHO Cells

CHO cells were run on the Vi-CELL XR and Vi-CELL BLU. A default CHO cell profile was used on the Vi-CELL XR and the default mammalian cell profile used on the Vi-CELL BLU.

	Vi-CELL BLU	Vi-CELL XR
Cell type	Mammalian	CHO
Minimum Diameter (µm)	6	6
Maximum Diameter (µm)	30	70
Images	100	50
Cell sharpness	7	100
Minimum circularity	0.1	0
Decluster degree	Medium	Low
Aspiration cycles	3	1
Viable spot brightness (%)	55	75
Viable spot area (%)	5	5
Mixing Cycles	3	3



The Vi-CELL BLU instruments matched the values of the Vi-CELL XR systems within statistical limits. Taking the average of the two systems populations cell concentration, viability and diameter match within the target limit of +/-5%. No further refinement appears necessary in this case as the default mammalian cell type works very well here.

	Concentration +/-5%	Viability +/-2.5%	Average diam. +/-5%
Vi-CELL XR Average	2.05	92.46	16.23
Vi-CELL BLU Average	2.02	93.51	16.52
Difference from XR Average	-1.66%	1.14%	1.79%

HELA Cells

HELA is a widely used cell type for cell biology research but unlike CHO and Jurkat cells HELAs are grown attached to a solid substrate and require trypsinization to release them into suspension. As such they can be prone to more clustering than suspension cells. They also have a different size distribution compared to CHO cells.

To evaluate the default mammalian cell profile against another mammalian cell line HELAs were grown in flasks and then released and suspended at a concentration of approximately 6M/mL.

The cells were run on 3 Vi-CELL BLU and 2 Vi-CELL XR systems using the same default profiles as outlined above. The averages for the systems were used for the matching exercise. The goal is to match the systems within +/-5% (10% range) for concentration and diameter and within +/-2.5% (5% range) for viability. This degree of tolerance was chosen as it falls within the performance criteria for the Vi-CELL BLU instrument. Users can determine their own degree of matching but these general guidelines will typically put the measured values within statistically acceptable limits.

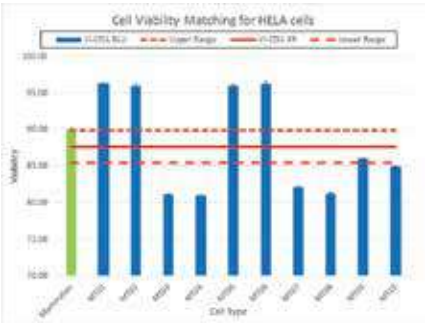
	Vi-CELL BLU	Vi-CELL XR
Cell type	Mammalian	CHO
Minimum Diameter (µm)	6	6
Maximum Diameter (µm)	30	70
Images	100	50
Cell sharpness	7	100
Minimum circularity	0.1	0
Decluster degree	Medium	Low
Aspiration cycles	3	1
Viable spot brightness (%)	55	75
Viable spot area (%)	5	5
Mixing Cycles	3	3

In addition to the default a combination of different variants of cell profiles was used to reanalyze the data to define a range of settings based on the default mammalian profile to determine if a more precise match between the Vi-CELL BLU and Vi-CELL XR could be found. These are summarized below.

Cell type	MT01	MT02	MT03	MT04	MT05	MT06	MT07	MT08	MT09	MT10
Min Diameter (µm)	6	6	6	6	6	6	6	6	5	5
Max Diameter (µm)	20	40	20	40	20	40	20	40	20	20
Images	100	100	100	100	100	100	100	100	100	100
Cell sharpness	7	7	7	7	7	7	7	7	7	7
Minimum circularity	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Decluster degree	High	High	High	High	Low	Low	Low	Low	Low	None
Aspiration cycles	3	3	3	3	3	3	3	3	3	3
Viable spot brightness (%)	40	40	90	90	40	40	90	90	75	75
Viable spot area (%)	5	5	5	5	5	5	5	5	5	5
Mixing cycles	3	3	3	3	3	3	3	3	3	3

A

B



C

The table below shows the percentage difference between the Vi-CELL BLU values for the different cell profiles used compared to the Vi-CELL XR.

Cell Profile	Concentration +/- 5%	Viability +/-2.5%	Average diam. +/-5%
Mammalian	-0.01%	2.73%	-2.53%
MT01	4.82%	9.84%	-2.84%
MT02	5.00%	9.52%	-1.85%
MT03	4.34%	-7.42%	-2.46%
MT04	2.23%	-7.63%	-1.58%
MT05	5.35%	9.54%	-1.08%
MT06	5.92%	9.82%	-0.08%
MT07	5.26%	-6.30%	-0.68%
MT08	6.40%	-7.31%	0.25%
MT09	2.32%	-1.81%	-3.19%
MT10	-13.43%	-3.04%	-1.67%

From the table above we can see that the default Mammalian profile provided a good match for Concentration, viability and average diameter. However Cell Profile MT09 appears to be a stronger cell type candidate as its results are a closer match for all 3 parameters

Further refinement of the MT09 profile could be applied if a closer match was desired. Altering the cell profile as show below improves the match for Viability and Diameter even further.

Cell type	MT09	MT09*
Min Diameter (µm)	5	5
Max Diameter (µm)	20	25
Images	100	100
Cell sharpness	7	7
Minimum circularity	0.1	0.1
Decluster degree	Low	Low
Aspiration cycles	3	3
Viable spot brightness (%)	75	70
Viable spot area (%)	5	5
Mixing cycles	3	3

Cell Profile	Concentration +/– 5%	Viability +/–2.5%	Average diam. +/–5%
MT09	2.32%	–1.81%	–3.19%
MT09*	2.58%	–1.25%	–1.71%

Conclusion

To match values between a Vi–CELL XR and Vi–CELL BLU the recommendation is to start with the most appropriate default Cell Profile in the Vi–CELL BLU software for the cell type being sampled. Only if the result deviates more than the desired value (5% or 10% for example) should further fine tuning be necessary.

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