

Vi-CELL[®] XR Cell Type Tutorial

INNOVATE - SIMPLIFY - AUTOMATE



Purpose of the Tutorial

- Introduce users to the Cell Type Feature in the Vi-CELL XR software.
- Identify the default cell types provided with the software.
- Demonstrate the ability to create, modify and add cell types
- Identify the available features in the Cell Type Dialog.
- Explain the purpose of the various options for defining a cell type.
- Provide scenarios where the default settings may need to be adjusted.



What is a Cell Type?

A Cell Type is a collection of settings that control the sample preparation and analysis.

2.	D1			
	ument Diagnostics Help			
	Cell Types			
Cell Size	🎢 Cell type 🛛 CHO			Imogo
Thresholds	Minimum diameter (microns) 6	Cell brightness (%)	85 Default	Image Analysis
	Maximum diameter (microns) 50	Cell sharpness	100	Settings
	Number of images 50	Viable cell spot brightness (%)		
	Aspirate cycles 1	Viable cell spot area (%)	5 🖉	
	Trypan blue mixing cycles 3	Minimum circularity	0 СНО	
		Decluster degree	Medium	
ample Prep	Created by		Hybridoma	
Frank Frank Frank Frank Frank Frank Frank Frank	Created 25 Apr 2003 4:51	1:11 PM		
	Last modified 25 Apr 2003 4:51	:11 PM	SF-9	
	Comment		8	
			SF-21	
		0-114-0-2	review:	
		Cell type	Vero	
				1
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Where are the Cell Types?

The Cell Types can be viewed by selecting the icon.

	Vi-CELL XR 2.01	
	File View Instrument Diagnostics Help	Previous Run Results
		Sample ID
-	Camera Cell type Conc. Control	Sample ID
	Image Minimum diameter (microns) 5 Cell brightness (%) 85 Image Minimum diameter (microns) 50 Cell brightness (%) 85	
	Autosampler Maximum diameter (microns) 50 Cell sharpness 100	Total
	Number of images 50 Viable cell spot brightness (%) 75	Cell count 0 0
	Aspirate cycles 1 Viable cell spot area (%) 5	Viable cells 0 0
	Cell Tunes	Viability 0.0 % 0.0 %
Select Cell	Trypan blue mixing cycles 3 Minimum circularity 0	Total cells / ml 0.000 × 10 6 0.000 × 10 6
	Decluster degree Low	Viable cells / ml 0.000 x 10 6 0.000 x 10 6
Types Icon	Concentrat Created by	Avg. diam. (microns) 0.00 0.00
	Created 25 Apr 2003 4:50:24 PM	Avg. circularity 0.00 0.00
	Sample_Bi Last modified 25 Apr 2003 4:50:24 PM SF-9	Avg. background intensity 0 0
	Comment	lmages 0
	Comment SF-21	Size distribution 💌 🔲
		300
		250 -
	View Cell Type details	200 · E
	by selecting icons	150 -
	Instrument Status and Control	100-
	Status Instrument not connected Log in sample	50 -
	Position	20 40
	Sample ID Start queue	Diameter (microns)
	Cell type	Image review:
	Operator	Print run



Why Change the Cell Type?

- In most cases, there is no need to change the cell type. The supplied Cell Types - CHO, Hybridoma, SF9, SF21, Vero, and Default - provide good results.
- In cases where the cell line used is not part of the Cell Type list, or customers want to change the sample preparation or results, a new Cell Type can be created or an existing Cell Type can be modified.
- In cases where the cells are the same type as the defaults, but have been treated or genetically altered, the cell type settings may need to be modified.



How Do I Change the Cell Type?

There are several ways to do this.

Add, Modify, or Delete a Cell Type from the "File" menu...





How Do I Change the Cell Type?

Add a Cell Type after Reanalyzing an entire run.

📓 ¥i-CELL	(R 2.11				
File View	Instrument Diagnostics Log In Sample Clear Completed List	Help D		000	-Stored Run Resi Sample ID
Camera Image	Stop Queue Pause Run Resume Run	0 0 0	o '	° °	Cell type Dilution factor
Queue	Prime Flush Decontaminate	° ° °	Cell type CHO	•	
Cell Type	Drain Replace Reagent Pak Reanalyze	· · · · ·	Min. diameter Max. diameter	6 microns 50 microns	
Sample_B		° °,	Dilution Cell brightness	85 %	
		0	Cell sharpness	100	
	0	• •	Viable cell spot brightness Viable cell spot area	75 % 5 %	
	0	° ° ° °	Minimum circularity Decluster degree	0 Medium	
	-Instrument Status		Add cell type) _ ок	Cancel



How Do I Change the Cell Type?

Add a Cell Type after Reanalyzing a single image.

Open Run						Single Imag
ave Run Sport Run as Excel File AduRun to Excel File	0	o	0	c	0 sik	Sample ID
New Boprocess Open Bioprocess Close Biogrocess	0	•	Reanalyze Cell type Current	Settings 💌	[
Export Biogrocess as Excel File	0 0	0	Min. diameter	6	microns	
Control	Open	8	Max. diameter	50	microns	
Configuration	Close Save As	0	Dilution	1		
Preferences Cell Types •	Reanalyze	, °°	Cell brightness	85	%	
rint			Cell sharpness	100		
xit	0 0 0	-	Viable cell spot brightness	75	%	
		0	Viable cell spot area	5	%	
· * 0			Minimum circularity	0		
° ° 0		0	Decluster degree	Medium	•	
	0	0 1	Add cell type	Apply	🗸 ок	X Cancel



How Do I Change the Cell Type?

To modify an existing Cell Type:

I - Select the Cell Type I (June and the main grade of the main grade		Vi-CELL XR 2.03	~t
Ceil Lype Minimum diameter (microns) 6 Cell brightness (%) 85 Maximum diameter (microns) 50 Cell sharpness 100 Cell count Total Meximum diameter (microns) 50 Cell sharpness 100 Cell count 0 0 Mumber of images 50 Viable cell spot brightness (%) 75 Cell count 0 0 0 Aspirate cycles 1 Viable cell spot area (%) 5 Cell count 0 <		Cell Types CHO Sample ID	ell
Decluster degree Medium 🖉 Viable cells / ml 0.000 x 10 ⁶ 0.000 x 10 ⁶	Icon from the	Minimum diameter (microns) 6 Cell brightness (%) 85 Maximum diameter (microns) 50 Cell sharpness 100 Mumber of images 50 Viable cell spot brightness (%) 75 Cell Types Minimum circularity 0 Cell count 0 0 Trypan blue mixing cycles 3 Minimum circularity 0 Cell count 0.000 x 10 ⁶ 0.000 x 10 ⁶	nain otain Cell
2 - Select Created by Created by Avg. diam. (microns) 0.00 0.00 2 - Select Last modified 25 Apr 2003 451:11 PM SF3 Avg. circularity 0.00 0.00 Last modified 25 Apr 2003 451:11 PM SF3 No 0 Images 0 Type to Comment Cell type review: Cell type review: SF3 Cell type [SF-9] Minimum diameter 8 microns microns	the Cell — Type to be	Sample_BL. Created by Hybridoma Avg. diam. (microns) 0.00 0.00 Created 25 Apr 2003 451:11 PM SF9 SF9 Avg. diam. (microns) 0.00 0.00 Last modified 25 Apr 2003 451:11 PM SF9 SF9 Modify Cell Type SF9 Comment Cell type review: SF21 General Image Analysis SF9 SF9	
Instrument Status and Control Log in sample Status Instrument not connected Log in sample Position Start gueue Cell type Start gueue Operator Operator PN A62133 Rev AB Images	modified	Instrument Status and Control Status Instrument not connected Position Sample ID Cell type Operator	



What are the Functions of the Cell Type Parameters?

- The Cell Type parameters are used to define the following:
 - 1. Sample Preparation Options
 - 2. Cell Size Thresholds
 - 3. Image Analysis Parameters
 - 4. Number of Images to Collect
 - Let's review each area in more detail...



Sample Preparation Options

Aspirate Cycles

Add New Cell Type			×
General Image Analysis			
Cell type			
Minimum diameter	5	microns	
Maximum diameter	50	microns	
Images	50		
Aspirate cycles	1		
Trypan blue mixing cycles	3 💌		
Comment			
 Image: A second s	ок 🗶	Cancel	

- Controls the number of sample mixing cycles before the precise volume is dispensed and Trypan Blue is added.
- This is important to ensure sample is re-suspended in the sample cup prior to sampling. It ensures the Vi-CELL is presented with a representative sample of the cell suspension.
- Aspirate Cycles are also useful in de-clustering clumps of cells, so increasing this value can often improve the analysis of CHO and other "clumpy" cells.
- The default value and recommended minimum number of aspirate cycles is 1, but this number can be increased to help break up cell clusters.



Add New Cell Type

General Image Analysis

Cell type

Minimum diameter

Maximum diameter

Aspirate cycles

Trypan blue mixing cycles

Images

Comment

Sample Preparation Options

Trypan Blue Mixing Cycles

x

microns

microns

X Cancel

50

150

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- Controls the number of sample mixing cycles after the addition of Trypan Blue.
- For mammalian cells, the default value is 3 cycles.
- For insect cells, the default value is 2 cycles, as these cells are generally more fragile.
- Since increasing the number of mixing cycles also increases the staining time prior to analysis, this value can also be increased if customers would like longer staining time.



Cell Size Thresholds

Minimum and Maximum Diameter

Modify Cell Type				×
General Image Analysis				
Cell type CHO		[
Minimum diameter	6	microns		
Maximum diameter	50	microns	(\cdot)	
Images	50			
Aspirate cycles	1 💌			
Trypan blue mixing cycles	3 💌			
Comment				
✓	ок 🔀	Cancel]		

- Excludes live and dead cells and also debris from the analysis that is less than the minimum diameter or greater than the maximum diameter.
- The maximum diameter value should be set to 40 um or higher for the best cell de-clustering performance for mammalian cells.
- The minimum diameter value can be adjusted to help exclude small debris or apoptotic bodies from the analysis.
- To provide help in setting these values, size information can be obtained for every cell found in an image. The following slide provides details...



Viewing Size of Individual Cells

The size, viability, and circularity determination of individual cells can be viewed in the software as shown below.





Image Analysis Parameters

Modify Cell Type	×
General Image Analysis	
Cell type CHC)
Cell brightness	85 %
Cell sharpness	100
Viable cell spot brightness	75 %
Viable cell spot area	5 %
Minimum circularity	0
Decluster degree	Medium
	Use defaults
	OK X Cancel

- Cell Brightness (% of background)
- Cell Sharpness (unit less)
- Viable Cell Spot Brightness (% of background)
- Viable Cell Spot Area (% of total cell area)
- Minimum Circularity (1 = perfect circle)
- Decluster Degree
- Let's review each parameter in detail...



Image Analysis Flowchart

Vi-CELL XR Image Analysis Flowchart (simplified)





- Adjusting the Cell Brightness value changes the threshold at which dark pixels are included as objects.
- Higher values of Cell Brightness include light grey pixels in the object separation.
- Lower values include only very dark pixels in object separation.
- Use Binary Image mode to see the changes.
- Let's practice making some changes...







Then, right click the mouse on the image to bring up this menu. Turn "Annotate" off and turn "Binary" on to get a black and white image.







0

Notice the difference in the binary image...





With Cell Brightness = 50, notice how those areas are gone. This shows how Cell Brightness can be used to remove debris or faint cells.



- Although we used Cell Brightness of 50 for illustration, you will most likely never want to use such a low value.
- A value of 85 is best, and the normal range of values is 75 (to exclude debris and apoptotic bodies) to 90 (to include all objects in the analysis)
- Remember, normal values are 75 90!



- Cell Sharpness is also used to include or exclude objects from the analysis.
- Cell Sharpness describes how quickly an object boundary transitions from light to dark.
- Lower Cell Sharpness values will exclude "fuzzy" edged objects or cells from the analysis.
- Higher values include more objects.
- Let's try it...



Large fuzzy cell and small apoptotic body are included in this image, to exclude them...



Notice large "fuzzy" cell and small object. We can adjust the cell sharpness parameter to exclude them from the analysis.

Change Cell Sharpness value to 60 and select "Apply" to view the change.



Notice the cells are no longer annotated as live cells and the small 'x' to the left of the cell, indicating sharpness rejection.



Fuzzy cell and apoptotic bodies now excluded from analysis with small 'x' indicating rejection for Cell Sharpness.



- Cell Sharpness is an effective way to exclude "fuzzy" objects and apoptotic bodies because they generally do not have a well defined cell membrane.
- Be careful to check that a lower setting does not exclude normal cells.
- Default setting is 100, typical settings can go as low as 60.
- Normal range of settings is 60 200.



Parameters – Viable Cell Spot Brightness

- After the cells are separated from the background by the Cell Brightness and Cell Sharpness values, and the declustering and size thresholds are applied, the cell viability is determined using the Viable Cell Spot Brightness and Viable Cell Spot Area settings.
- Viable Cell Spot Brightness defines how 'white' the cell center spot must be to be classified as viable.
- It is a percentage of the full scale white value.
- Let's look at an example...

Parameters – Viable Cell Spot Brightness

File View Inst	rument Diagnostics	Help			Ois statistics as	
Camera Image	Saved Image	D		•	- Single Image- Sample ID	No ha spo wit
Autosampler Queue					Cell count	Се
Ő					Viable cells	
Cell Types	0.	00	Reanalyze		Vishiliti X	va
Δ	~0	~ 0	Cell type Current S	Settings 🔽) /	l pre ∕∕Re
			Min. diameter	6 microns		
<u> </u>			Max. diameter	50 microns		
Sample_Bi			Dilution	1	Les la	
	0	0	Cell brightness	85 %		Ch
			Cell sharpness	100		90
			Viable cell spot brightness	75 %		
			Viable cell spot area	5 %		vie
	Instrument Status	and Control	Minimum circularity	0		
	Status	Instrument not connected	Decluster degree	Low		
	Position					
	Sample ID		Add cell type	Apply 🗸 OK	X Cancel	

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Notice this cell, which has a light grey center spot, is classified as live with the default Viable Cell Spot Brightness value of 75. Notice the preview image in the Reanalyze dialog

Change the value to 90 and select Apply to view the change.



Parameters – Viable Cell Spot Brightness

File View Inst Camera Image Autosampler Queue Cell Types	Saved Image	0	Reanalyze Cell type Current S	Settings •		Sample ID Cell count Viable cells	With a value of 90%, this cell is now classified as dead. The center spot of the cell is not bright enough to meet the viability criteria.
Concentrat			Min. diameter	6	microns		
Sample Bi			Max. diameter	50	microns		
compo_orm			Dilution	1			Notice the image
	0	0	Cell brightness	85	%		preview in the
			Cell sharpness	100			Reanalyze dialog
	1		Viable cell spot brightness	90	%		
			Viable cell spot area	5	%		
	-Instrument Status	and Control	Minimum circularity	0			
	Status	Instrument not connected	Decluster degree	Low	•		
	Position			1			
	Sample ID		Add cell type	Apply	🗸 ОК	X Cancel	



- Viable Cell Spot Brightness and Viable Cell Spot Area work together to classify cells as dead or alive.
- Viable Cell Spot Area is the required size of the center bright spot to meet the viability threshold.
- Default value is 5% of total cell area, but values of 1% to 20% could be used.
- Be careful though. Let's take a look...





With a 2% center spot area setting, these 20 um beads are classified as live.

Notice the preview graphic, and the size of the cell spot area here









With a 5% center spot area requirement, notice that this small cell is classified as live

Changing the value to 10%, will make this cell dead









A value of 30% causes these cells to be classified as dead, as well as the small cell!



Parameters – Viability Review

- Remember, the viability determination is made after the cells are separated from the background, the declustering is applied, and the size thresholds are applied.
- Viable Cell Spot Brightness and Viable Cell Spot Area work together to determine viability.
- Increase both values to reduce viability, decrease both values to increase viability.
- Default values are generally the best.



Parameters – Minimum Circularity

- Minimum circularity excludes **only dead** cells from the analysis.
- Dead cells or debris that are oblong in shape can be excluded using this parameter.
- Default value is 0 which includes all dead cells and oblong debris.
- Be careful using this parameter because a value of 1 will exclude all dead cells!!
- Let's have a look...


Parameters – Minimum Circularity





Parameters – Minimum Circularity

	nimeri	trument Diagnosti Saved Image						Single Image
→	Camera Image	00	00	(-	Sample ID
	Autosampler Queue		0	I	Reanalyze Cell type Current S	Settings 🔽		×
	Cell Types	-			Min. diameter	6	microns	
	Δ				Max. diameter	50	microns	(\bullet)
	Concentrat		0)	Dilution	1		
	Sample_Bi	e.	•	-	Cell brightness	85	%	
	oumpio_bi			0	Cell sharpness	100		
					Viable cell spot brightness	75	*	
		٠		C	Viable cell spot area	5	%	
		4	-		Minimum circularity	0.75	\mathbf{b}	
					Decluster degree	Medium	•	
		Instrument Sta	tus and Control			1		
		Status	Instrument not co	nnected	Add cell type	Apply	🗸 ОК	X Cancel

With a Minimum Circularity value of 0.75, this cell is no longer included in the analysis.

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Parameters – Minimum Circularity

- Remember, minimum circularity excludes only dead cells from the analysis.
- The Default value is 0 which includes all dead cells and oblong debris.
- Normal values are in the range of 0 to 0.5.
- Be careful using this parameter because a value of 1 will exclude all dead cells!!



- Decluster Degree includes 4 settings for how aggressively clumps of cells should be declustered.
 - Setting options are:
 - None Clumps are ignored (Version 2.01) or counted as one large cell (Version 2.02)
 - Low Analysis looks for arcs in the clumps and closes larger radius arcs.
 - Medium Analysis looks for arcs in the clumps and closes large and small radius arcs
 - **High** Analysis looks for arcs in the clumps and closes even smaller radius arcs (note: sometimes cells in clusters get excluded by size with High degree.
- Setting applies to live cells and dead cells, although Version 2.02 of Vi-CELL XR has much improved declustering of dead cell clumps.
- Let's give it a spin...





With Decluster Degree = None, clumps are classified as one big cell. Note that the Max. Diameter setting must be large enough not to exclude these clumps





With Decluster Degree = Low, clumps are declustered. Notice the large dead cell clumps are classified as 4 and 4 dead cells respectively.





With Decluster Degree = Medium, clumps are declustered more aggressively. The large dead cell clumps are classified as 4 and 6 dead cells respectively.



- Decluster Degree selection should be based on how the user typically counts cell clumps manually. Review a sample of images and decide on the most appropriate setting.
- 'Low' setting is typically used for Hybridoma and insect cells, as these are not cluster prone.
- 'Medium' setting is typically used for CHO and Vero cells as these tend to be clumpy.
- 'None' setting is useful for myocytes or other none circular cells (Version 2.02 software and later).



Image Analysis Review

Vi-CELL XR Image Analysis Flowchart (simplified)





Number of Images Setting

• Number of Images

dd New Cell Type				x				
General Image Analysis								
Cell type								
Minimum diameter	5	microns		1				
Maximum diameter	50	microns						
Images	50							
Aspirate cycles	1			נ				
Trypan blue mixing cycles	3 💌							
Comment								
V OK X Cancel								

- The number of images collected can be adjusted between 1 and 100, depending on speed and accuracy requirements.
- Increasing images to 100 helps improve statistical accuracy of low concentration samples.
- Decreasing number of images reduces sample analysis time by approximately 1 second per image.
- Default value is 50.



Cell Types – Recommendations

- Start with the supplied Cell Types or Default values.
- To exclude debris or other objects, first try to adjust the size thresholds to eliminate.
- Don't get frustrated on problems in a single image, look at the overall run result accuracy.
- When in doubt, call your Beckman Coulter Rep.
- Practice re-analyzing runs and images!!



Good Luck

and

Enjoy your Vi-CELL™ XR Cell Viability Analyzer