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TRANSFERRING CELL COUNTING METHODS – BEST PRACTICES

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Outline

- Introduction
- Case studies
- Recommendations
- Beckman Coulter Life Sciences

Vi-CELL XR

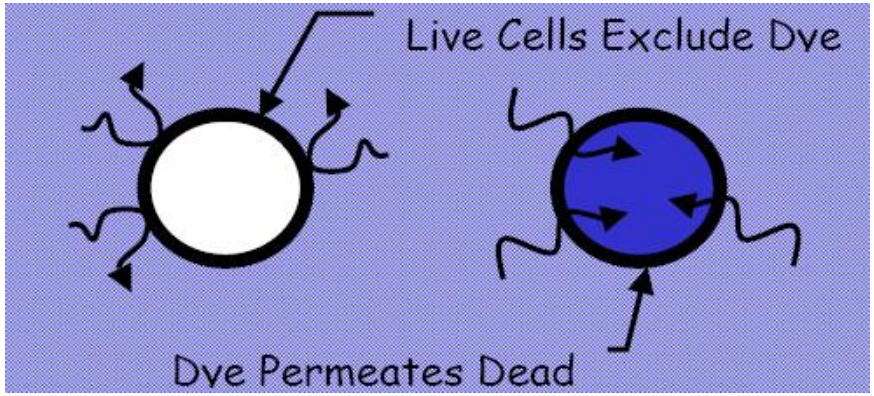
Fully automated, computer-operated image analyzer that uses the Trypan Blue Dye Exclusion Method for Viability, Cell Counting and Total Cell Concentration.

Who are our Vi-CELL XR customers?

- Tissue Culture Facilities: user may be growing many types of cells and needs accurate knowledge of count and viability.
- Biopharma Lab: user performs cell based assays and needs accurate count and viability to quantify results from assays.
- Biopharma Production Facilities: utilizing yeast, insect cells and animal cells to produce biological therapies. User needs to monitor cell health for maximum production and harvest time.
- Clinical Research Labs: isolating cells from human or non-human samples.
  - Blood
  - Bone Marrow (Stem Cells)
  - Spleen
  - Lymph Node

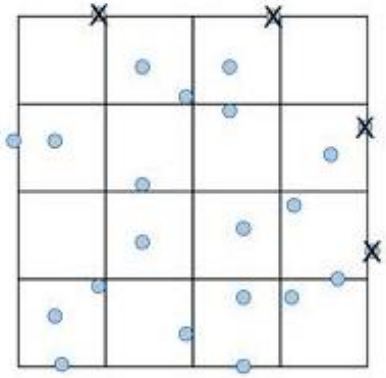
Trypan Blue Dye Exclusion Method

- Trypan blue is a vital stain used to selectively color dead tissues or cells blue.
- The trypan blue dye exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as trypan blue, whereas dead cells do not. A viable cell will be clear in the center whereas a nonviable cell will have a blue center.



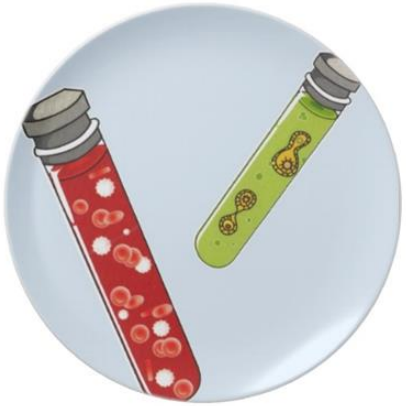
Traditional Method

- 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.
  - Manual method is tedious and quite time consuming for today's busy laboratory environment.



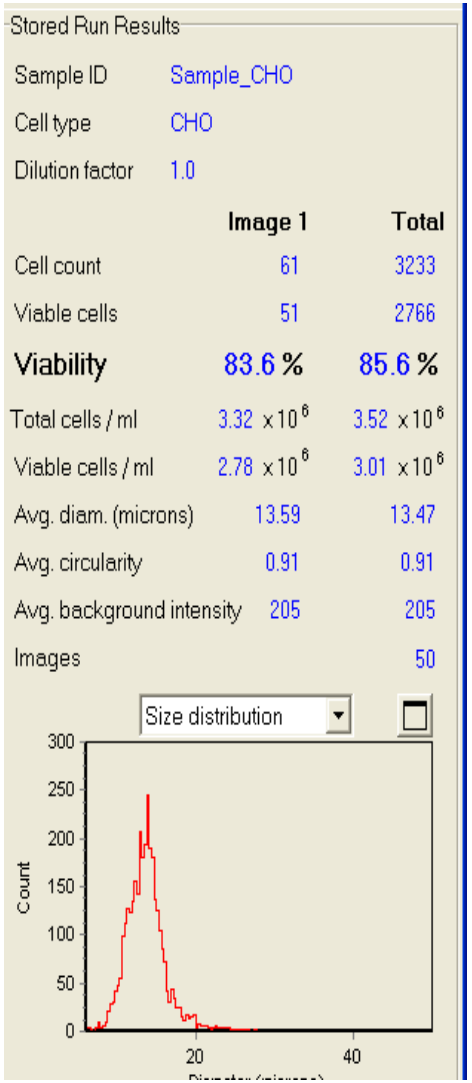
Applications in Research and Manufacturing

- Vi-CELL XR analyzes majority of mammalian cell types, insect cells and yeast.
  - Cells in the range of 2-70 microns.



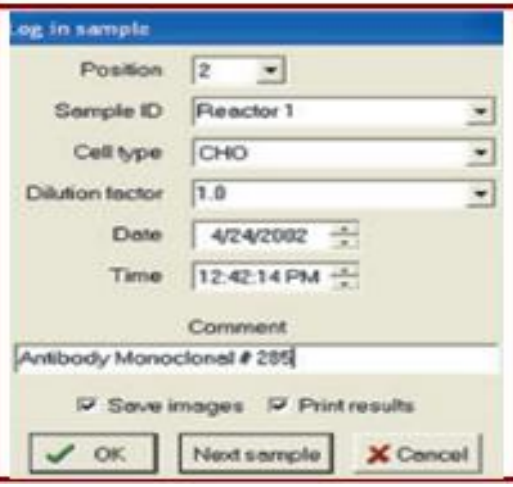
Features

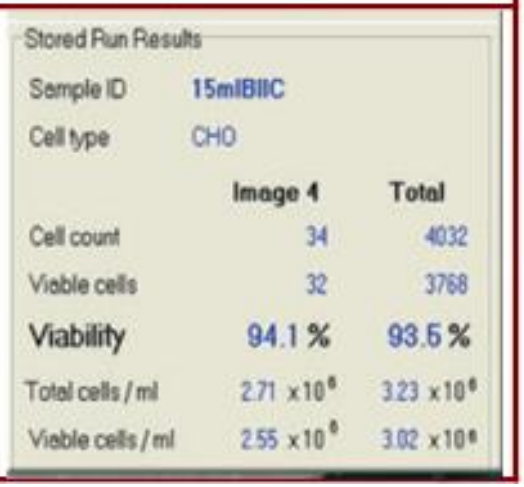
- Automation of the Trypan Blue Assay Method
- % Viability
- Total Cell Concentration
- Total Viable Cell Concentration
- Mean Cell Size
- Real Time Cellular Images
- Calculates Bio-process Growth Rate and Doubling Time
- Convenient Reagent Packs
- Validated Reagents
- Reanalyze data



Easy to Use

1	2	3
Load Sample *No need for precise pipetting*	Log-in Sample	Obtain Results

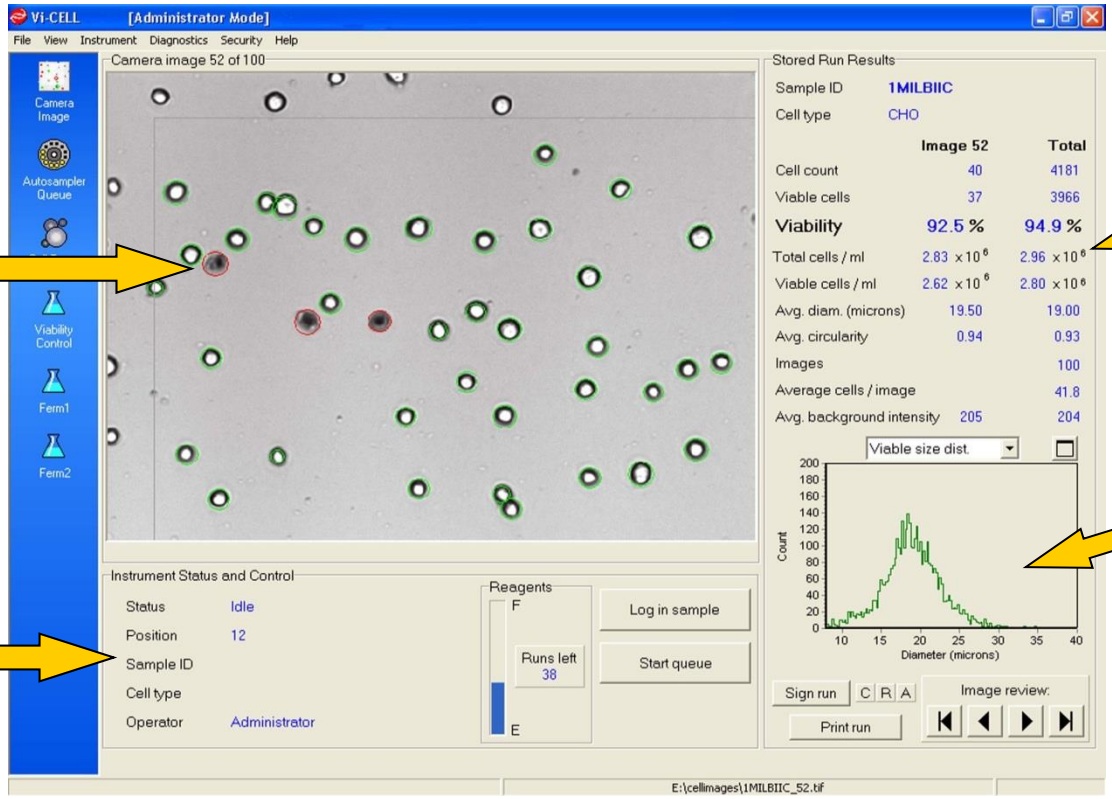




Vi-CELL Software
Main Window

Automatically Classifies Live Cells (Green Circles)

Automatically Classified Dead cells (Red Circles)



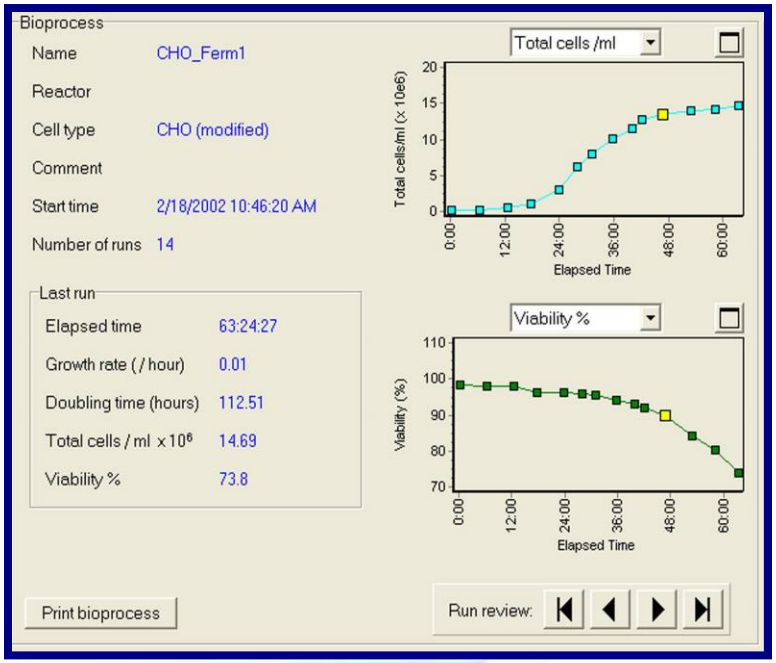
Results

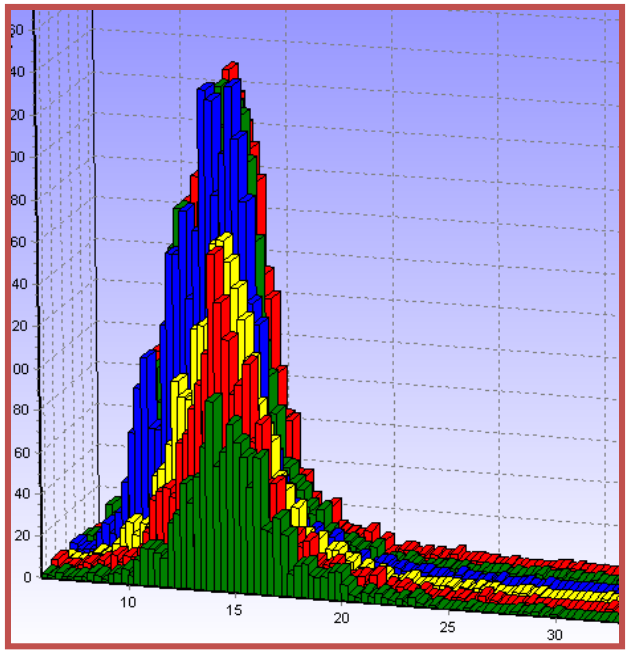
Size Distribut Plot

Instrument Status and Control

Bioprocess Feature

- On the Vi-CELL XR, the user can monitor a bioprocess over time.
  - **Excellent for characterizing growth rate and doubling time**
- Individual runs are automatically appended together.





Reagent Pack

- Contains all reagents required to run samples and clean system.
- System monitors reagent consumption.

Green:	Buffer Solution
Red:	Disinfectant
Yellow:	Cleaning Agent
Blue:	Trypan Blue Reagent

Reagent Pack

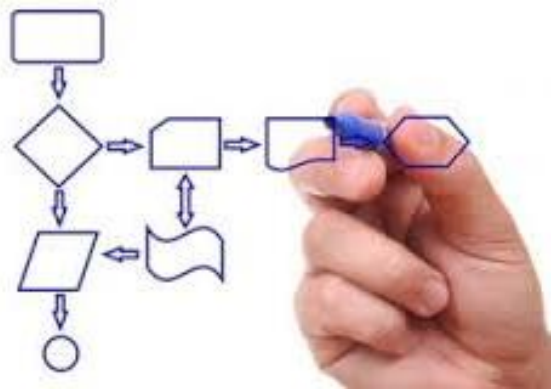
Concentration Control

- Beckman Coulter Vi-CELL concentration control standards are beads used to confirm the overall system performance.
  - Control is recommended to be run daily

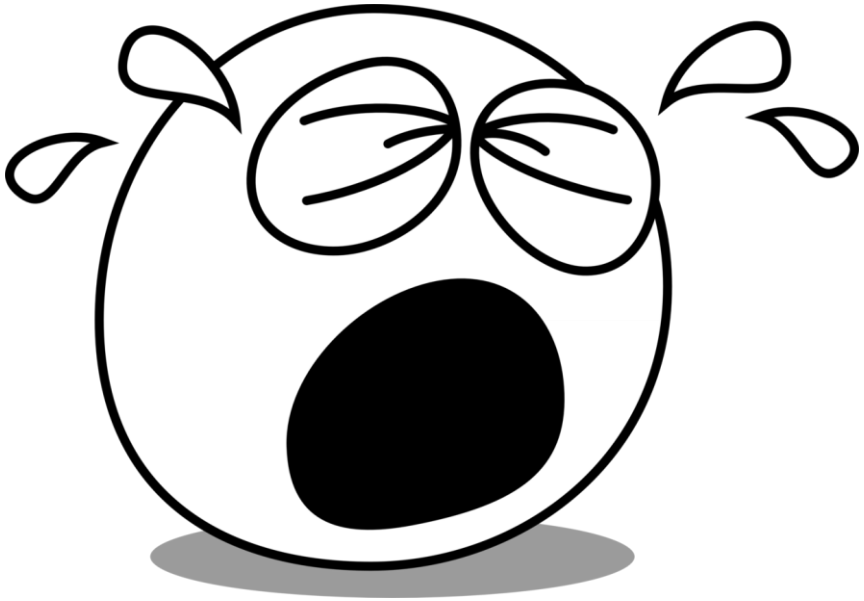
- Note: Viability standards are available through Bangs Laboratory

Reasons to Change

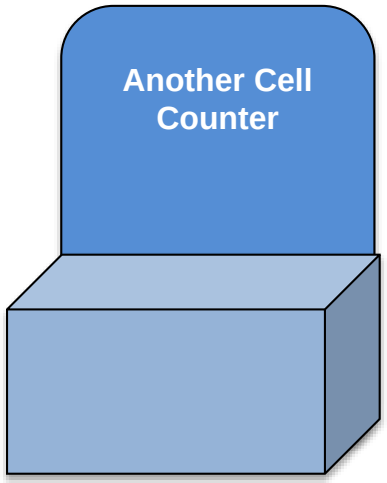
- Processes rarely remain the same over time.
- Many factors drive change
  - Obsolescence of equipment or materials
  - Increased throughput
  - Need to decrease variation
  - Process Improvement



Change sometimes requires Studies and Validation



Changing the Cell Counting Process



Move to Vi-CELL

Typical Criteria for new Method

- Correlate to previous method
  - More important to have the same answer than an accurate answer
- Reliability
- Repeatability

Case Study

- Dr. Iveta Bottova is a Process Development Specialist at SOTIO, a biotechnology company developing a next generation Active Cellular Immunotherapy drug.

- Share SOTIO's Validation study to change from the manual counting process of dendritic cells to an automated counting process using the Vi-CELL XR.

Dendritic Cells (DC)

- Dendritic cells are antigen-presenting cells of the mammalian immune system. Their main function is to process antigen material and present it on the cell surface to the T cells of the immune system.
- SOTIO develops new medical therapies using an immunotherapy platform based on activated dendritic cells
- The correct cell count and adequate viability of DC are one of the quality control criteria for the final product release.

Validation Study

- Evaluated Accuracy and Precision against manual method using beads.
  - Two Operators
  - Concentration Control (latex beads) 1x10^6 beads/mL
  - Triplicate measurements
  - Side by Side

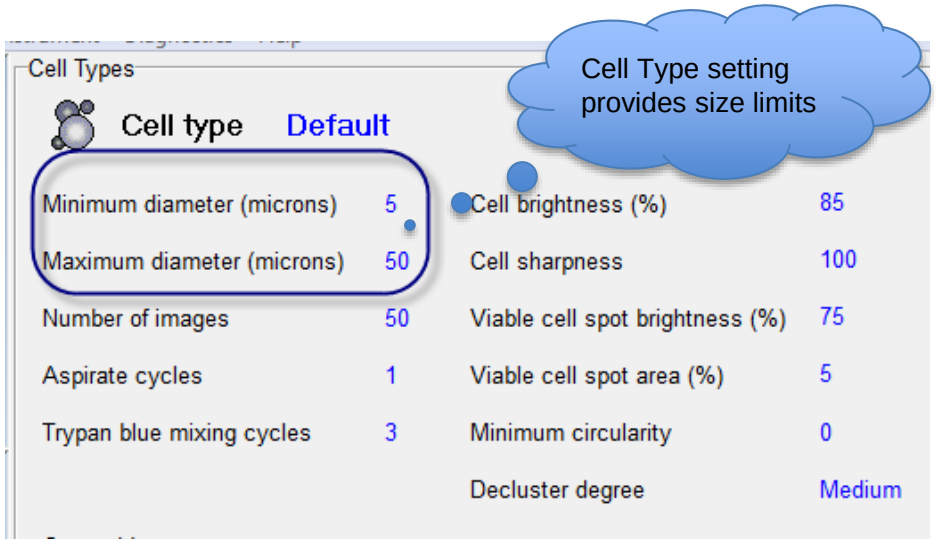
BURKER	Number of beads [x10^6]	CV % intra
Op1 average	1.03	1.12
Op2 average	0.99	6.44
Op1-2 average	1.01	3.78
SD Op1-2	0.028	
CV % inter Op1-2	2.81	

Vi-CELL	Number of beads [x10^6]	
Op1 average	1.03	
Op2 average	1.02	
Op1-2 average	1.02	2.89
SD Op1-2	0.005	
CV % inter Op1-2	0.46	

Pretty good repeatability

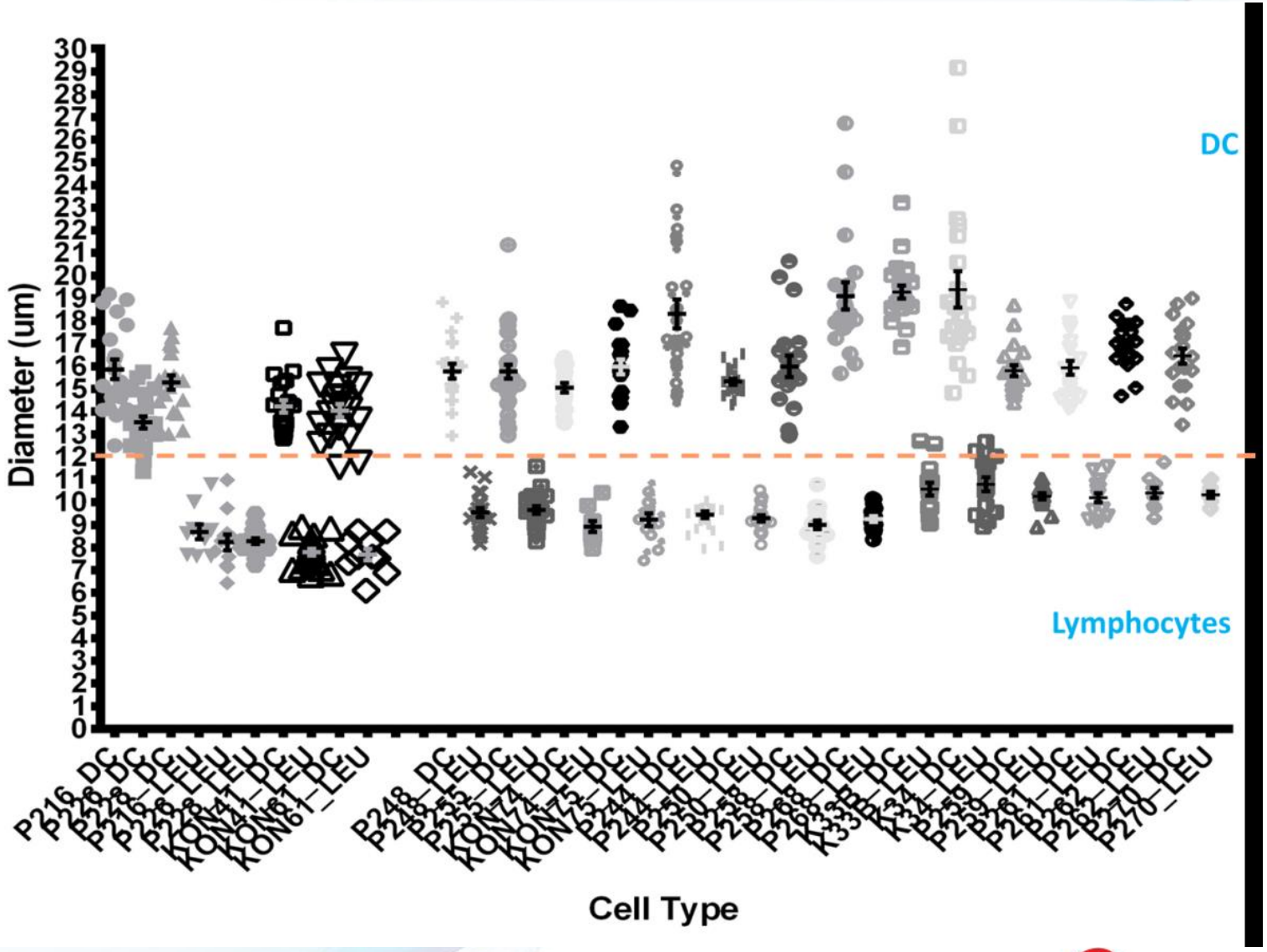
Validation Study

- Assessed the cell diameter for optimal DC measurement
  - SOTIO product contains DC and lymphocytes.
  - Evaluate whether Vi-CELL can use size to identify DC and ignore lymphocytes
    - DC size 11 – 30 μm and lymphocytes 5 – 12.5 μm, partial overlapping but only ~4%
  - 19 Lots used in study



Note: Size determined on 20X microscope and Vi-CELL

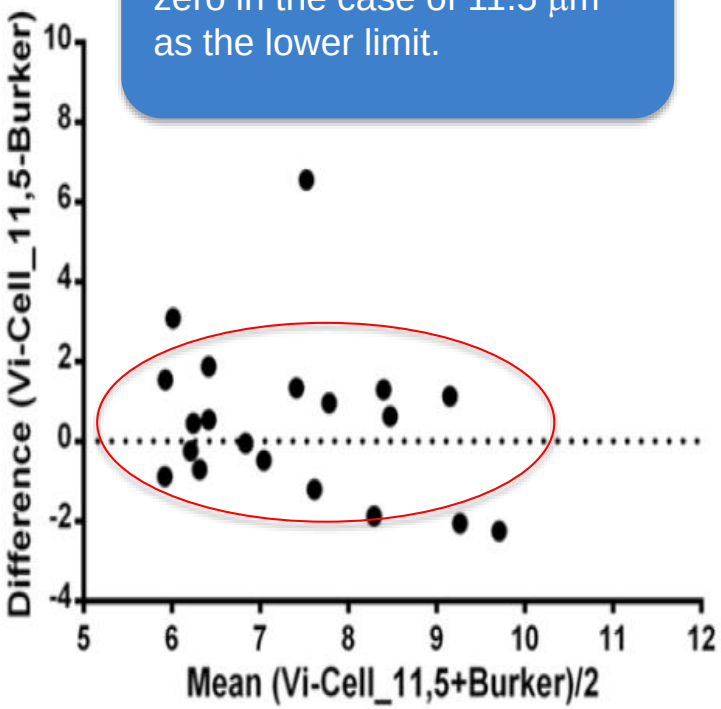
Size plot of 40 analyses with different size cut-off

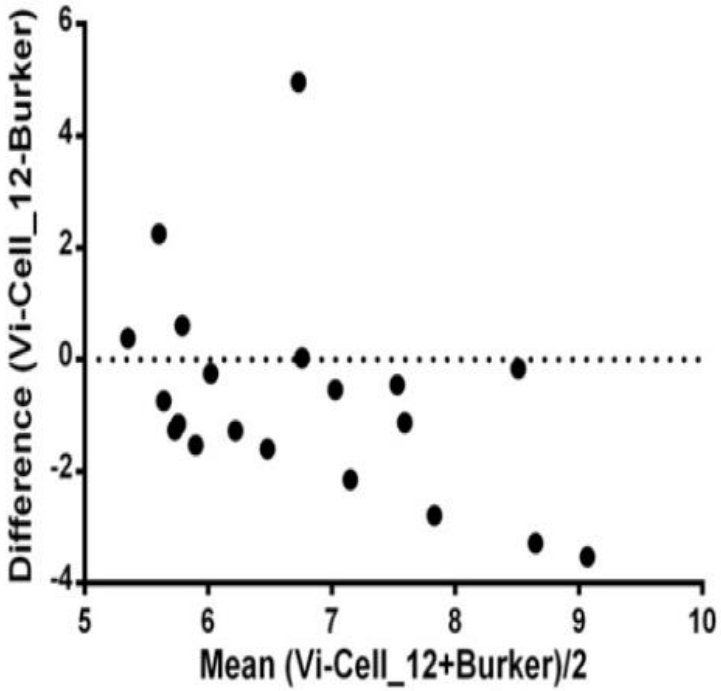


Comparison against manual method with DC

- Compared Vi-CELL analysis of 11.5 or 12 μm as the lower size limit against manual counts.

The differences were smaller and more centered around zero in the case of 11.5 μm as the lower limit.





Validation Conclusion

- The Vi-CELL method was found to be accurate and suitable for DC counting and comparable to the currently used quality control method Bürker chamber (manual method).
- There was no significant difference between DC counts values obtained by Vi-CELL and by Bürker chamber, moreover the size range 11.5-30µm is important for DC recognition in the Vi-CELL.

OTHER REAL EXAMPLES

Dilution Study

- Dilution Study to move from Manual to Vi-CELL
  - Customer was concerned about high variance from expected result.

Volume of concentration control stock (uL)	Volume of diluent (uL)	Reported Total cells/ml (x 10^6)	Diff from expected conc	Reported Total cells/ml (x 10^6)	Diff from expected conc	Dilution factor
		Set 1		Set 2		
20	980	1.7135	**69.8%**	1.4772	**42.4%**	50
50	950	1.5363	**52.2%**	1.489	**43.5%**	20
100	900	1.1935	**18.3%**	1.4063	**35.5%**	10
500	500	1.1038	9.4%	1.0612	2.3%	2
500	0	1.0092		1.0376		1

Dilution Study

- Dilution Study Problem
  - Concentration of starting material is too low for the dilution study or dilution factors are not appropriate for the sample being used.

Volume of concentration control stock (uL)	Volume of diluent (uL)	Reported Total cells/ml (x 10^6)	Diff from expected conc	Reported Total cells/ml (x 10^6)	Diff from expected conc	Dilution factor	**Estimated total bead count**	**Count per image**
		Set 1		Set 2				
20	980	1.7135	**69.8%**	1.4772	**42.4%**	50	20	0.4
50	950	1.5363	**52.2%**	1.489	**43.5%**	20	50	1
100	900	1.1935	**18.3%**	1.4063	**35.5%**	10	100	2
500	500	1.1038	9.4%	1.0612	2.3%	2	500	10
500	0	1.0092		1.0376		1	1000	20

Bridge Study

- Count Comparison to move from Manual to Vi-CELL
  - Customer was concerned that Vi-CELL VCD had high variance with their manual method.

Summary					
Vi-Cell (N Std)		Manual		Vi-Cell vs manual	
Viability (%)	Viable cells / ml (x10^6)	Viability (%)	Viable cells / ml (x10^6)	Difference in Viab	Difference in VCD
98.3	3.98	98	3.16	0.3%	21%
97.7	4.20	96	3.01	1.7%	28%
71.9	2.40	73.5	1.78	-2.2%	26%
56.6	1.75	53	1.10	6.4%	37%
41.2	1.21	37.5	0.70	8.9%	42%

Bridge Study

- Count Comparison

Operator	Viability Manual	VCD Manual
A	62	1.12
B	70	1.31
C	62	1.42
D	64	1.51
E	55	0.97
F	51	1.43
G	53	1.2
H	53	0.97
I	60	1.59
Average	59	1.28
SD	6.3	0.228
RSD	10.7%	17.8%
Min	51	0.97
Max	70	1.59

Sample	Vi-CELL: Viability (%)	Total cells / ml (x10^6)	Viable cells / ml (x10^6)
1	58.1	3.85	2.24
2	60.1	3.99	2.40
3	60.6	3.98	2.41
4	61.6	4.12	2.54
5	62.3	3.63	2.26
6	63.4	3.75	2.38
7	62.9	4.04	2.54
8	63.1	3.61	2.27
9	62.4	3.70	2.31
Average	62	3.85	2.37
SD Std	1.7	0.19	0.11
RSD	2.8%	4.9%	4.7%
Min	58	3.6	2.2
Max	63	4.1	2.5

Vi-CELL is more Repeatable

Which process is more repeatable?

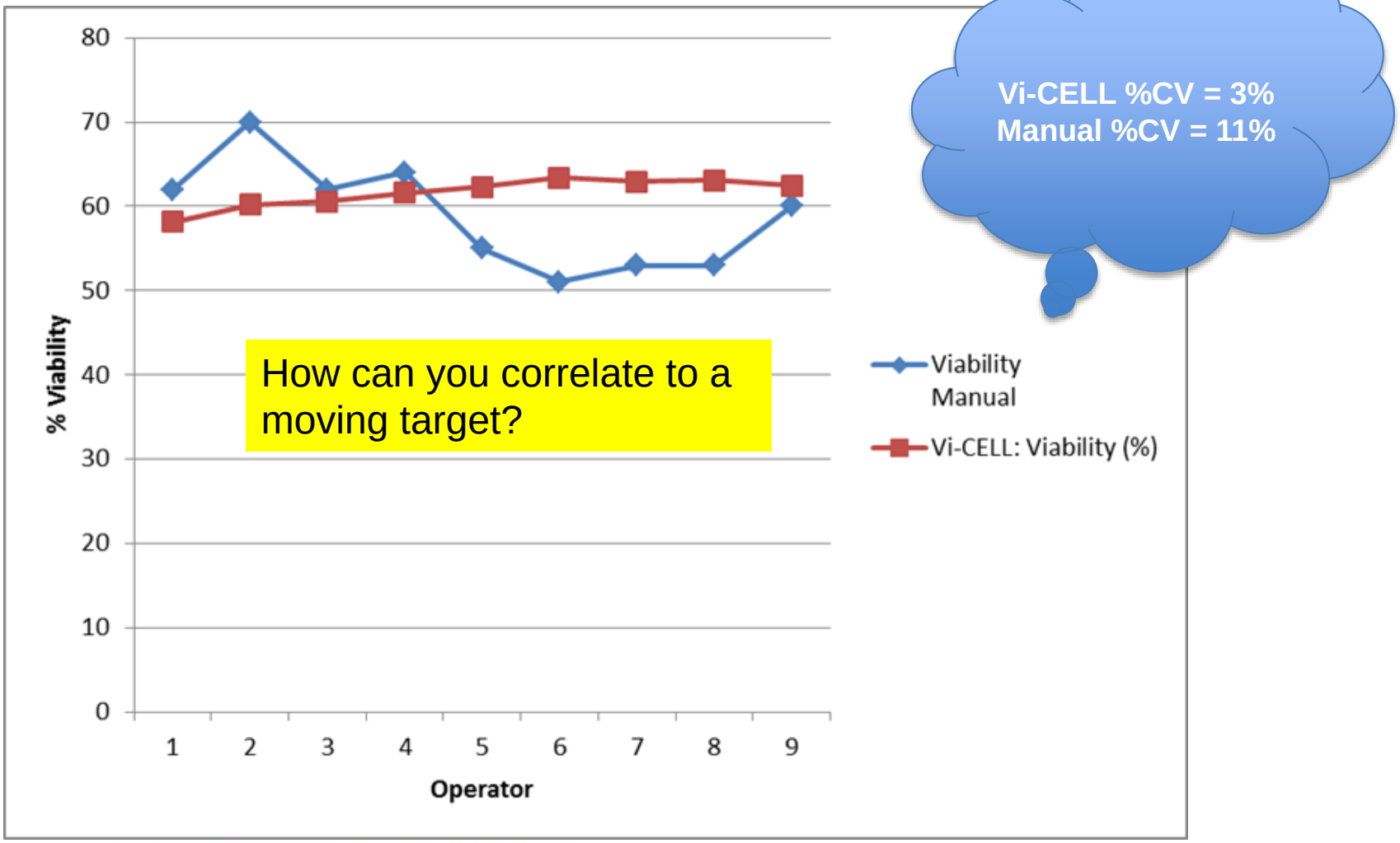
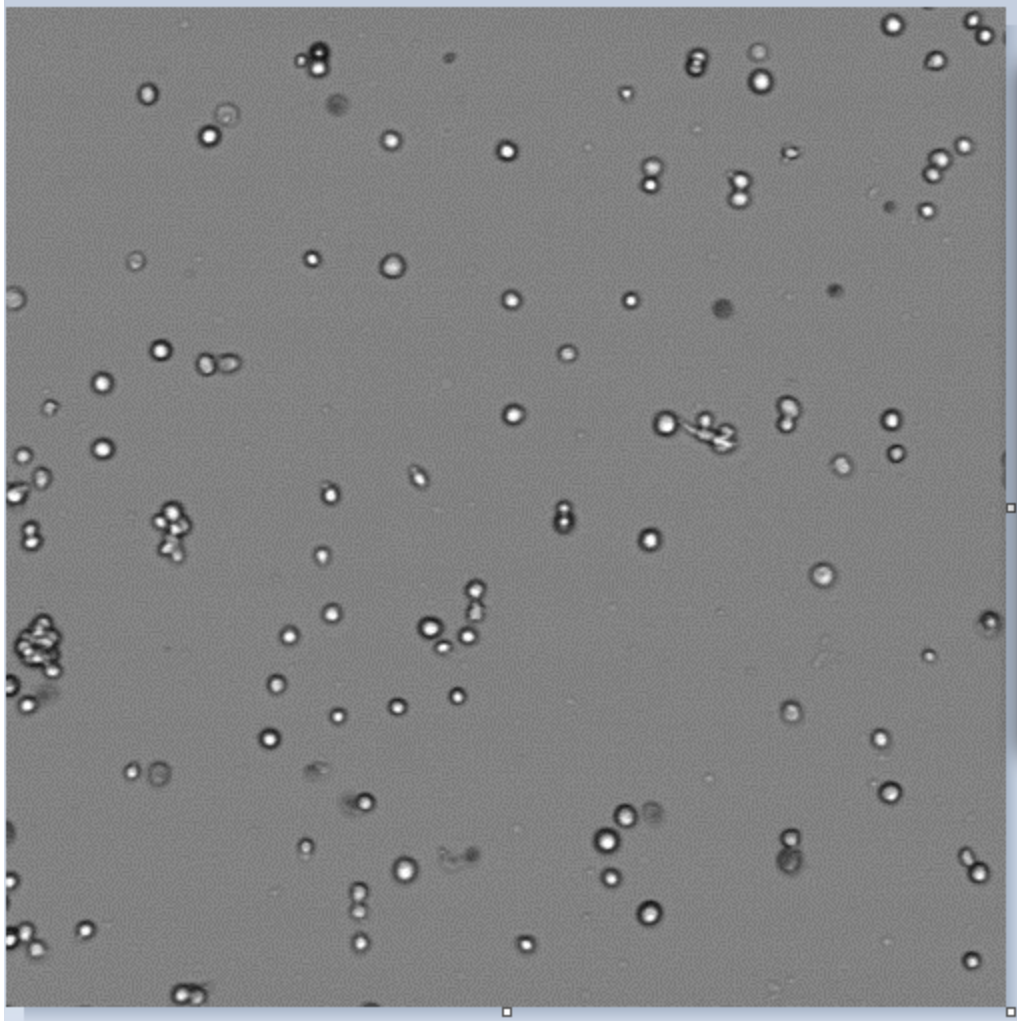
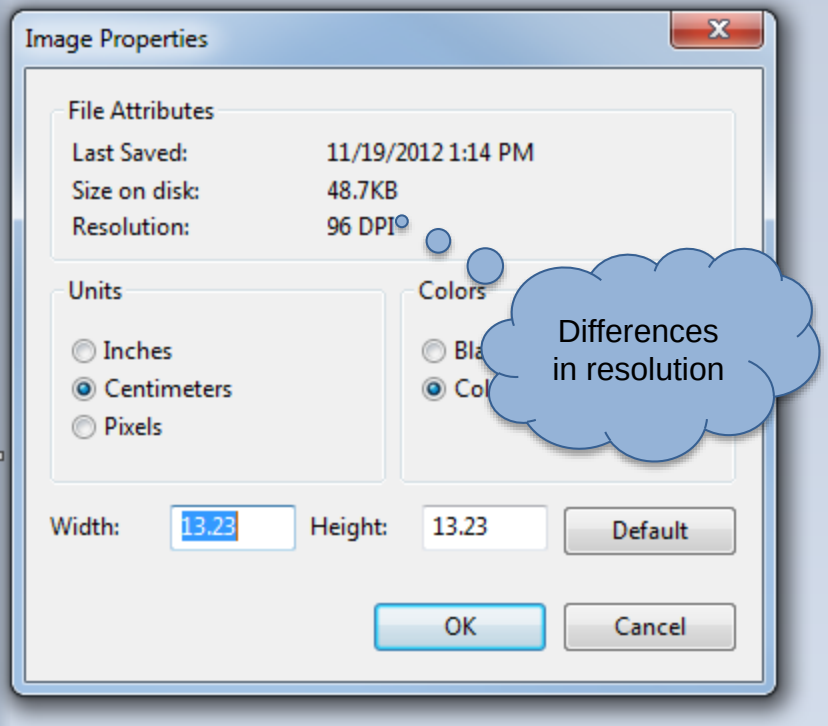


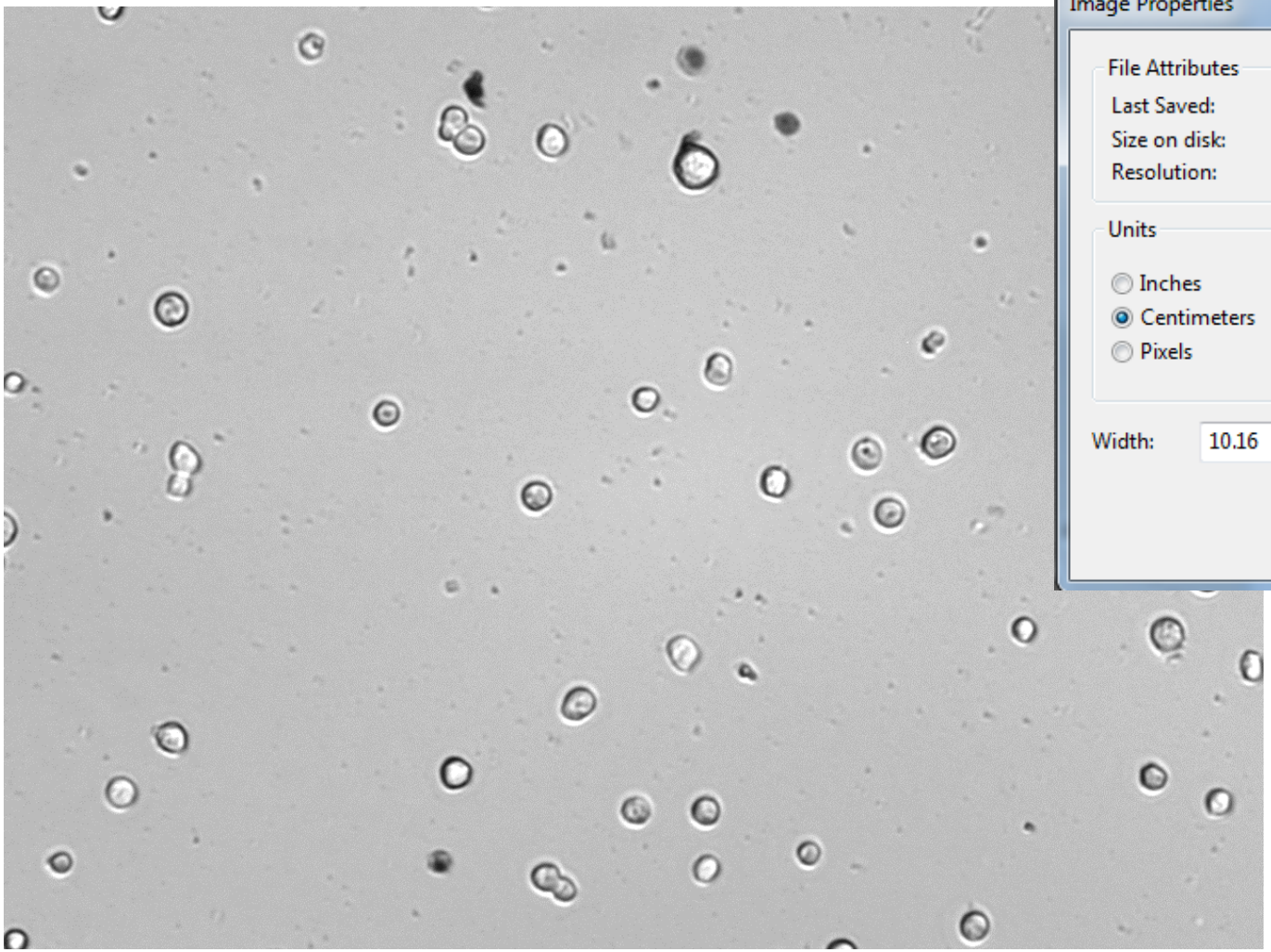
IMAGE FROM ANOTHER CELL COUNTER

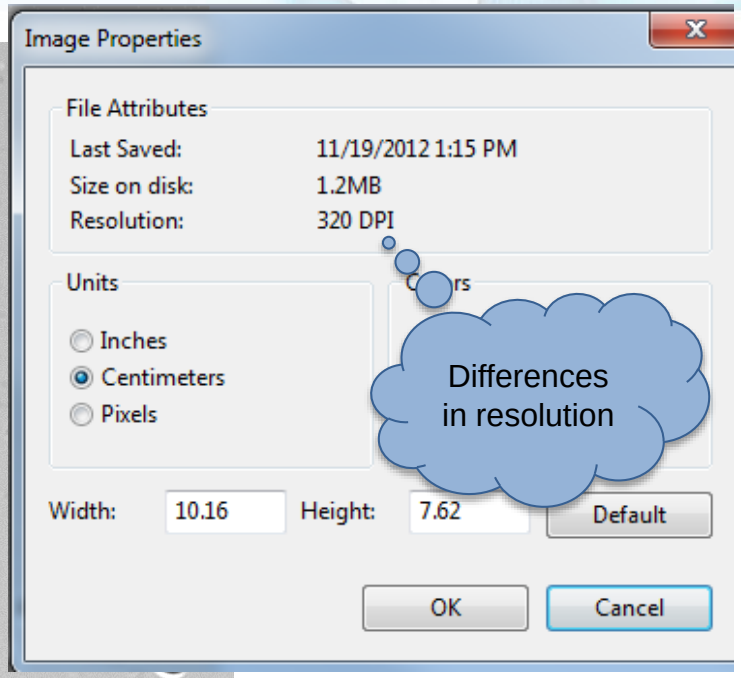




Magnification 4x

VI-CELL IMAGE (1280 X 960 PIXELS)

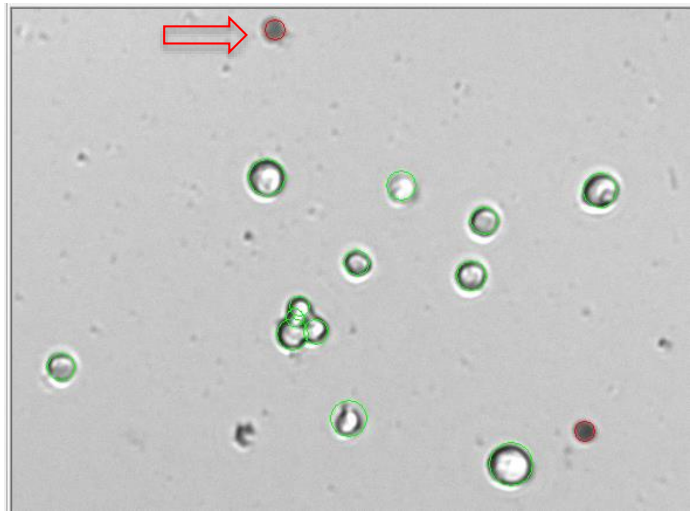


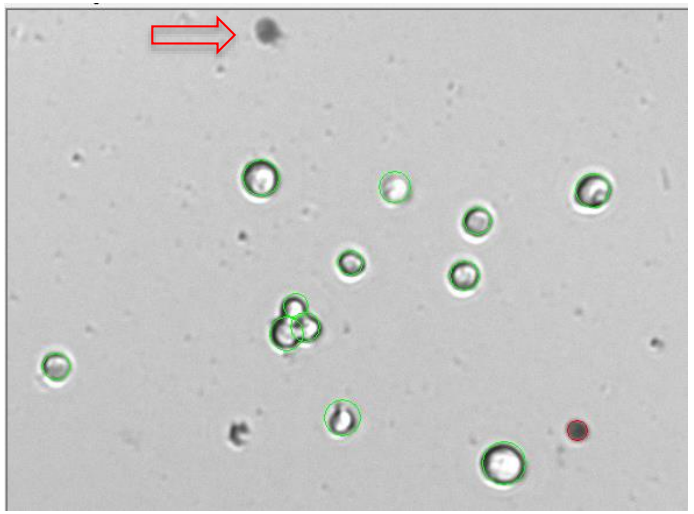


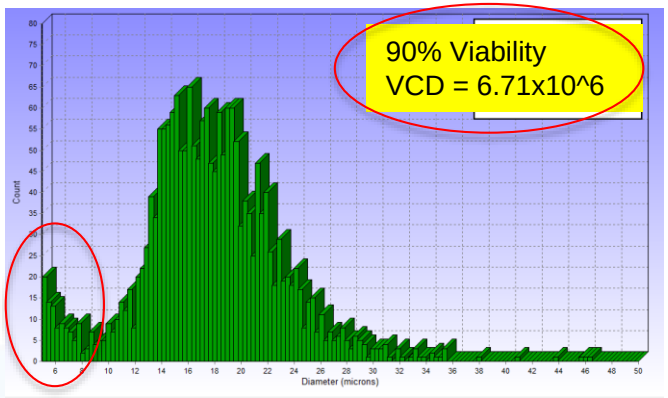
Magnification 6.75x

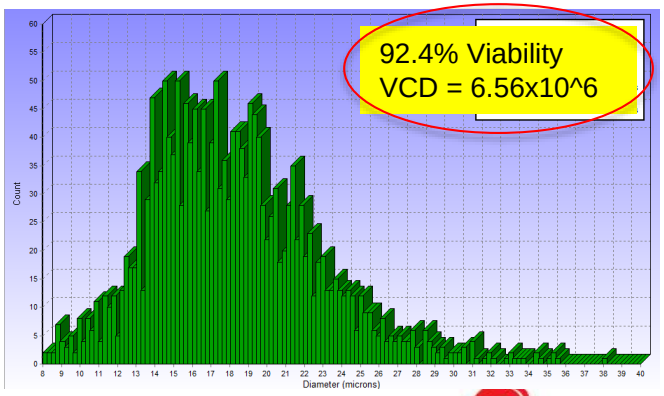
Recommendations for Correlation Study

- Optimize instrument settings as close to previous method (size, brightness, cluster, ignore debris)

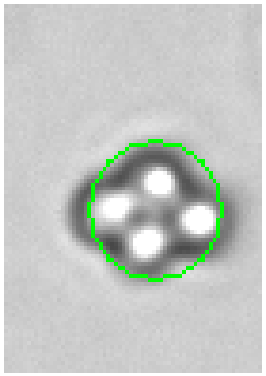




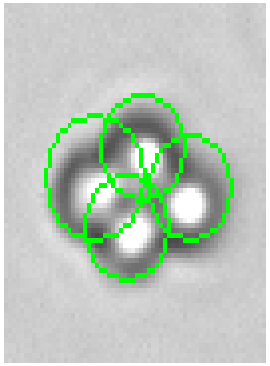




APPLYING DECLUSTERING



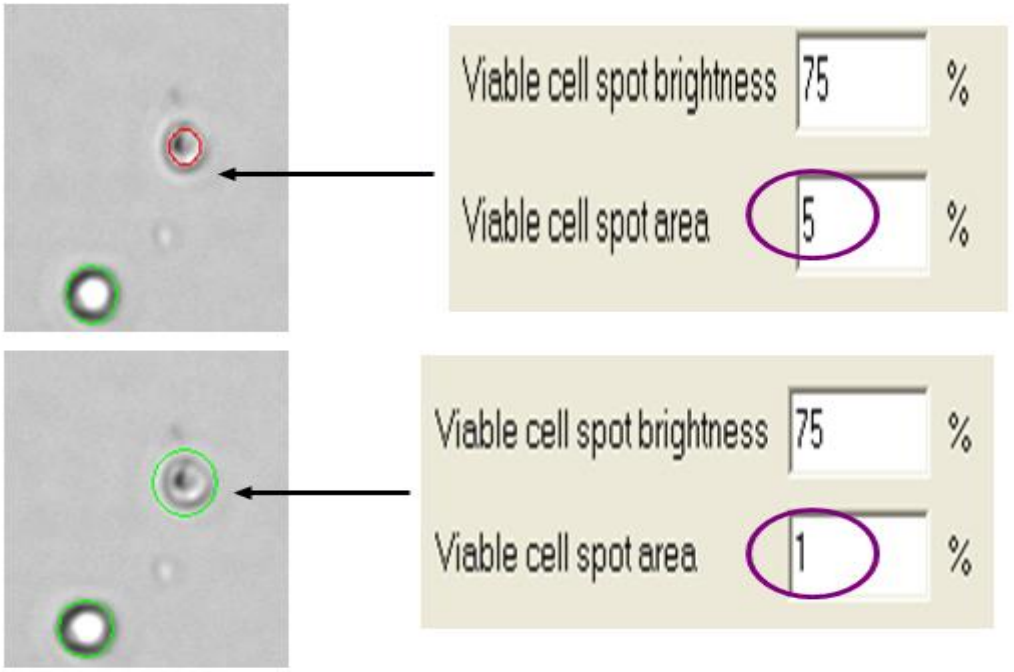
Decluster degree None



Decluster degree Medium

Same CHO Cell clump BUT due to the Medium Declustering, each cell within the clump is differentiated thus increasing cell count

CHANGING VIABLE CELL SPOT AREA



For cells on the border of live/dead, adjust viable spot area to increase or decrease % viability

Recommendations for Correlation/Bridge Study

- Use concentration and viability standards (latex beads) to start study
- Practice good sample handing(mixing, pipetting, time, temp)
- Test side by side (same sample, same time)
- Practice good statistics – Ensure a representative sample
- Account for variables in the process – realistic tolerance ranges
  - Some processes inherently have high variability
- Account for differences in Technology (Resolution, parameters)

Thank you

Questions?

Contact me:

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