

Automated Concentration and Viability Measurement of PBMC using the Cellometer® Vision



Issues related to the current PBMC manual counting method

- Potential judgment error due to residual red blood cells
- Sample loading errors, such as too much liquid, air bubbles, or wear-out hemacytometer
- Operator fatigue due to high background in the PBMC sample, such as platelets
- Calculation error during data conversion after counting
- Potential contamination during washing and cleaning



Cellometer® Vision & Cell Counting Method

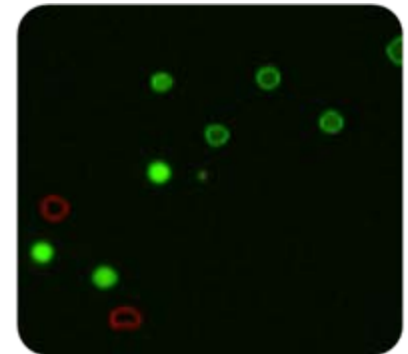
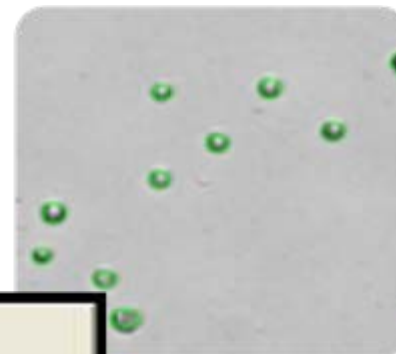
Pipette 20µL of sample into disposable counting chamber



Insert chamber in Cellometer

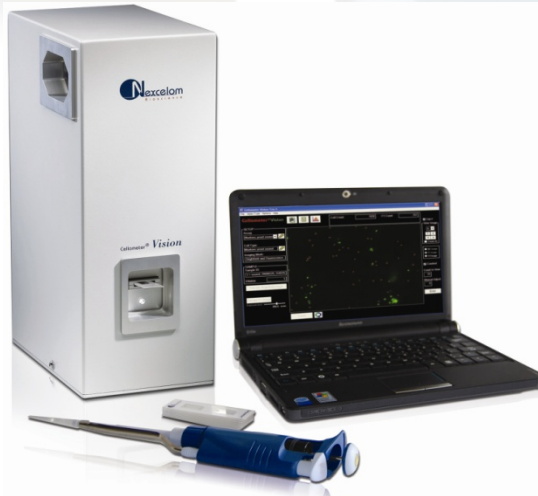


Bright-field (BR) and fluorescent (FL) images acquired and analyzed



Count

Output data generated automatically



Counting Results

Sample: HCT116_GFP
Dilution: 1.0
Assay: Assay #03_GFP_Transfection Rate1
Description: Cell line transfection rate measurement using GFP
Cell: GFP
Description:

	Bright Field	Fluorescence	
Count	269	153	Show Size Distribution
Mean Size	13.6	13.5	Intensity Distribution
		(BR Size)	Size vs Intensity
Concentration	9.76×10^5	5.55×10^5	Sample Adjustment
F1 Count / BR Total Count = 56.9%			
Export			Set Data File
Print			Save to Data File
			View Data File
			Done

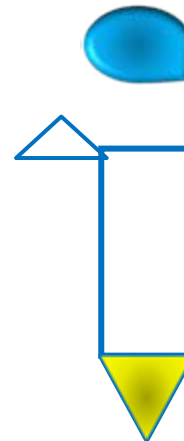


Assay Protocol: SAMPLE PREPARATION

1. Prepare PBMC sample following the standard protocol
2. Mix 20 μ l of PBMCs with 20 μ l of fluorescence dye staining solution
3. Mix well
4. Load 20 μ l of stained PBMC sample into Cellometer[®] slides

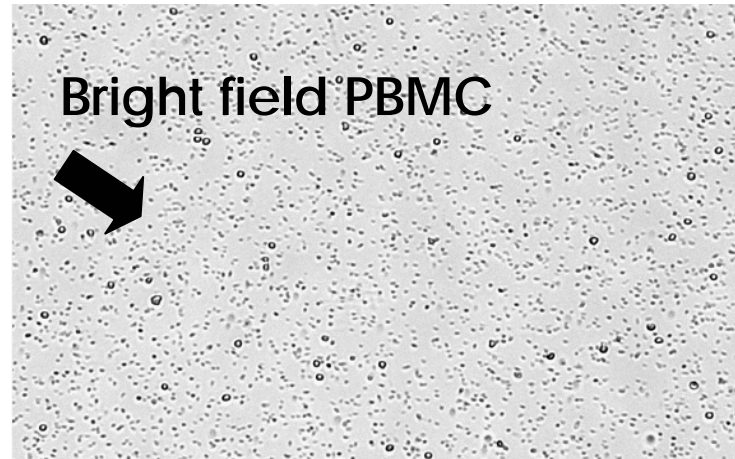
Take 20 μ L PBMCs
sample

Add to 20 μ L
fluorescent dye
solution preloaded
in small microtube

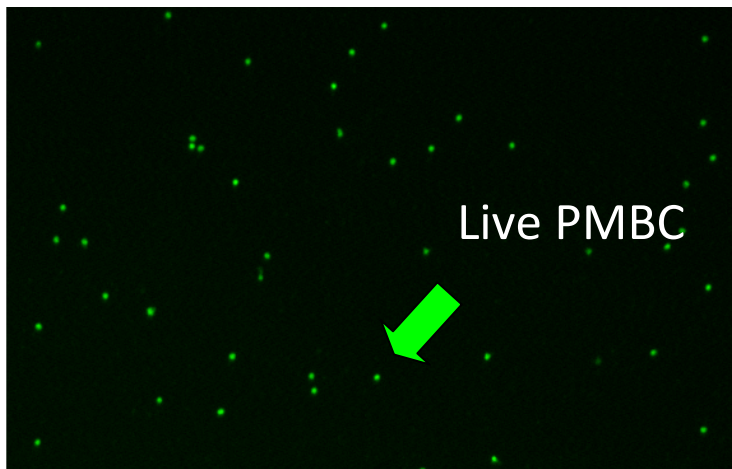


Assay Protocol: PBMC Cellometer Images

Bright field image



Fluorescent image 1 (AO)

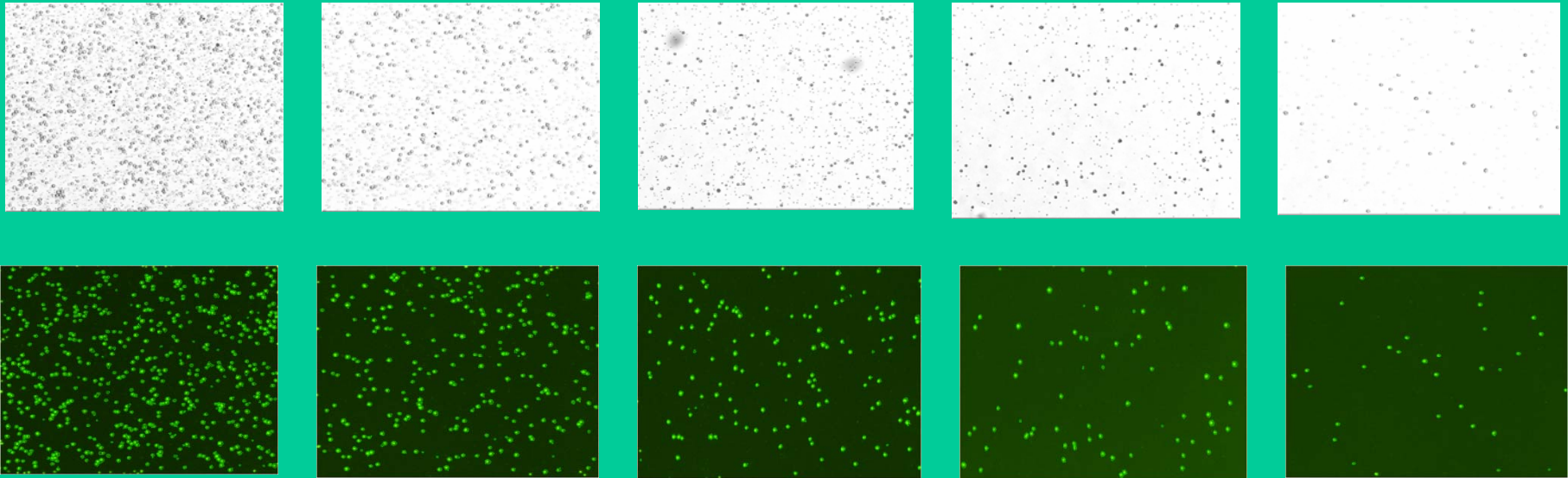


Fluorescent image 2 (EB)

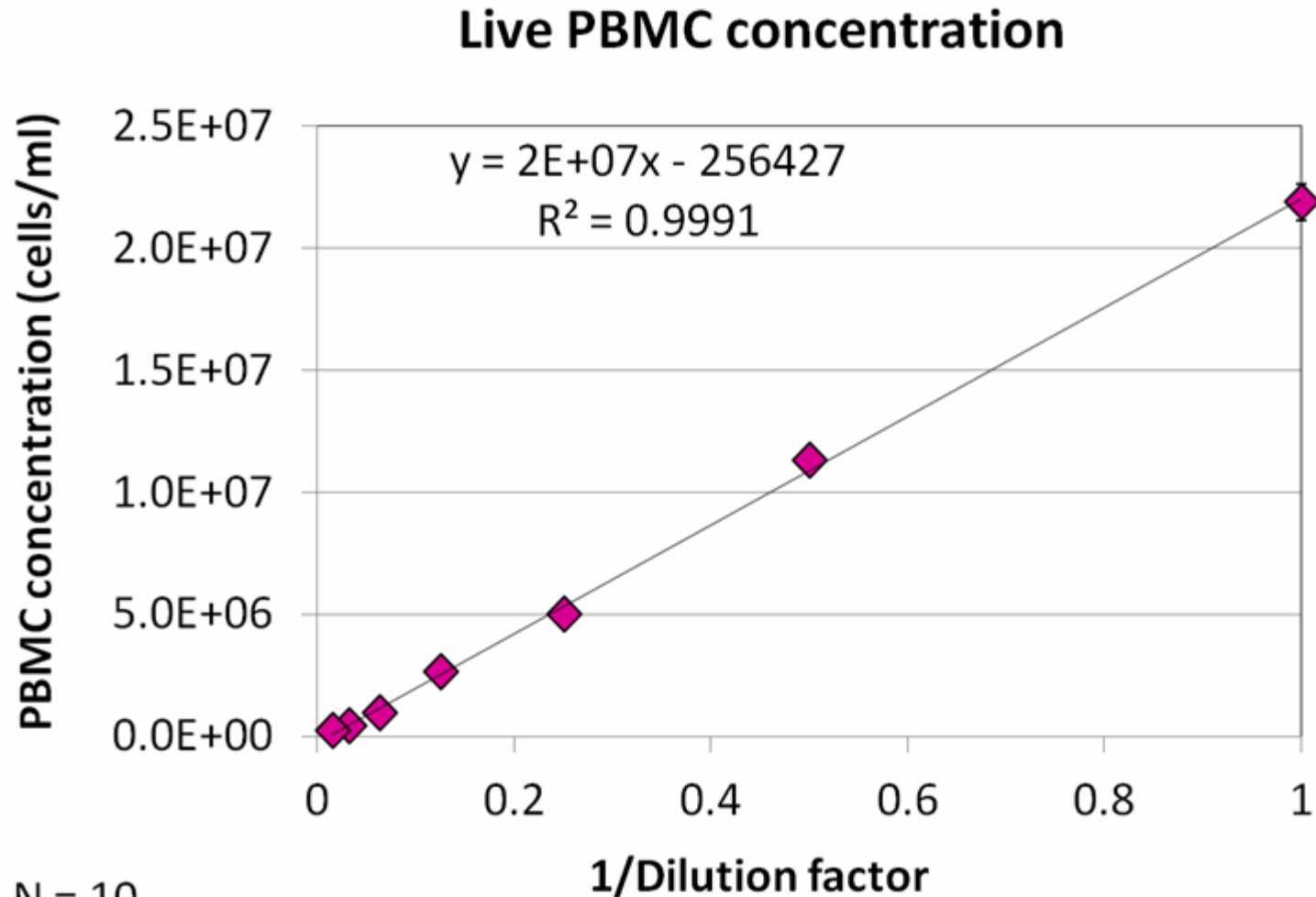


Linearity and Consistency for PBMC Concentration Measurement

Make Series PBMC Dilution in PBS



Linearity for PBMC Concentration Measurement



N = 10



PBMC Concentration Linearity and Consistency

Dilution factor	Live PBMC (cells/ml)	Stdev (n=10)	%CV
1	2.2E+07	7.6E+05	3.5
0.5	1.1E+07	3.4E+05	3.0
0.25	5.0E+06	2.4E+05	4.8
0.125	2.6E+06	1.6E+05	6.2
0.0625	9.7E+05	5.3E+04	5.4
0.03125	4.1E+05	5.6E+04	13.9
0.015625	2.1E+05	2.9E+04	14.1

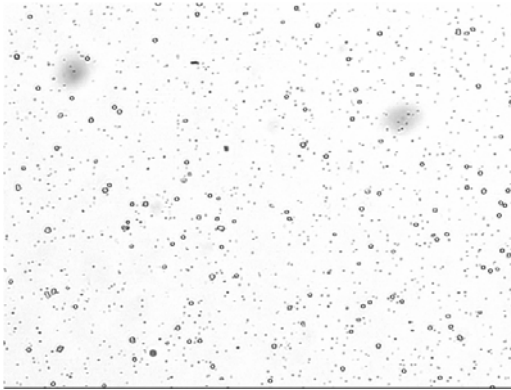
Results

- Viable and nonviable PBMCs are specifically stained with AOEB
- PBMCs dilution series showed linearity of $R^2 = 0.9991$
- Consistently measure PBMC from concentration of 2×10^5 to 2×10^7 cells/ml
- %CV calculated ranges from 3.5% to 14.1%, which are in acceptable range

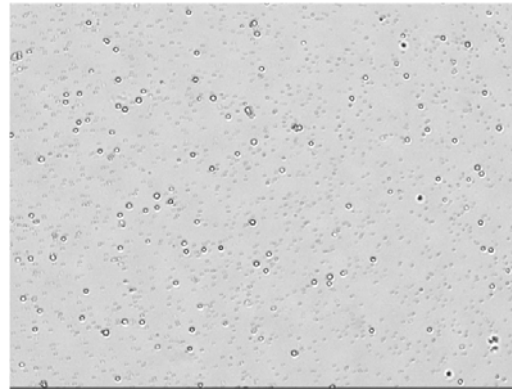


Determine PBMC Viability

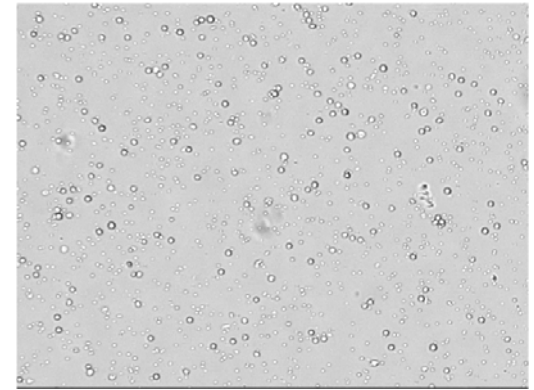
Day 1



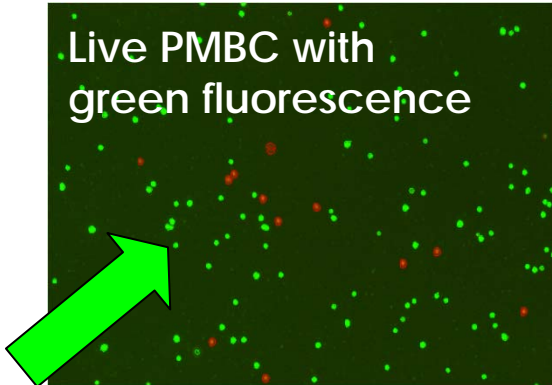
Day 4



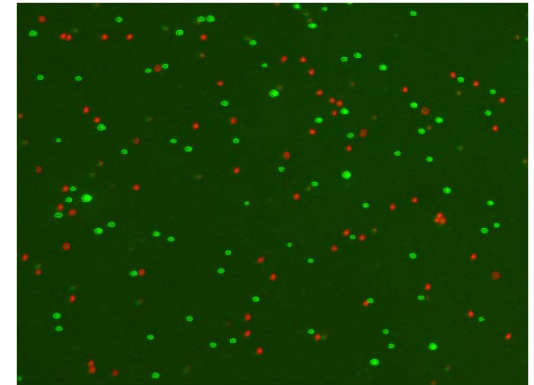
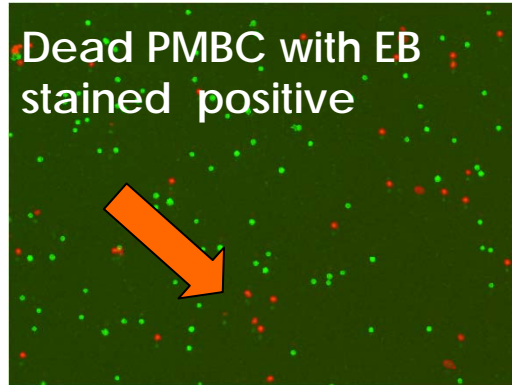
Day 7



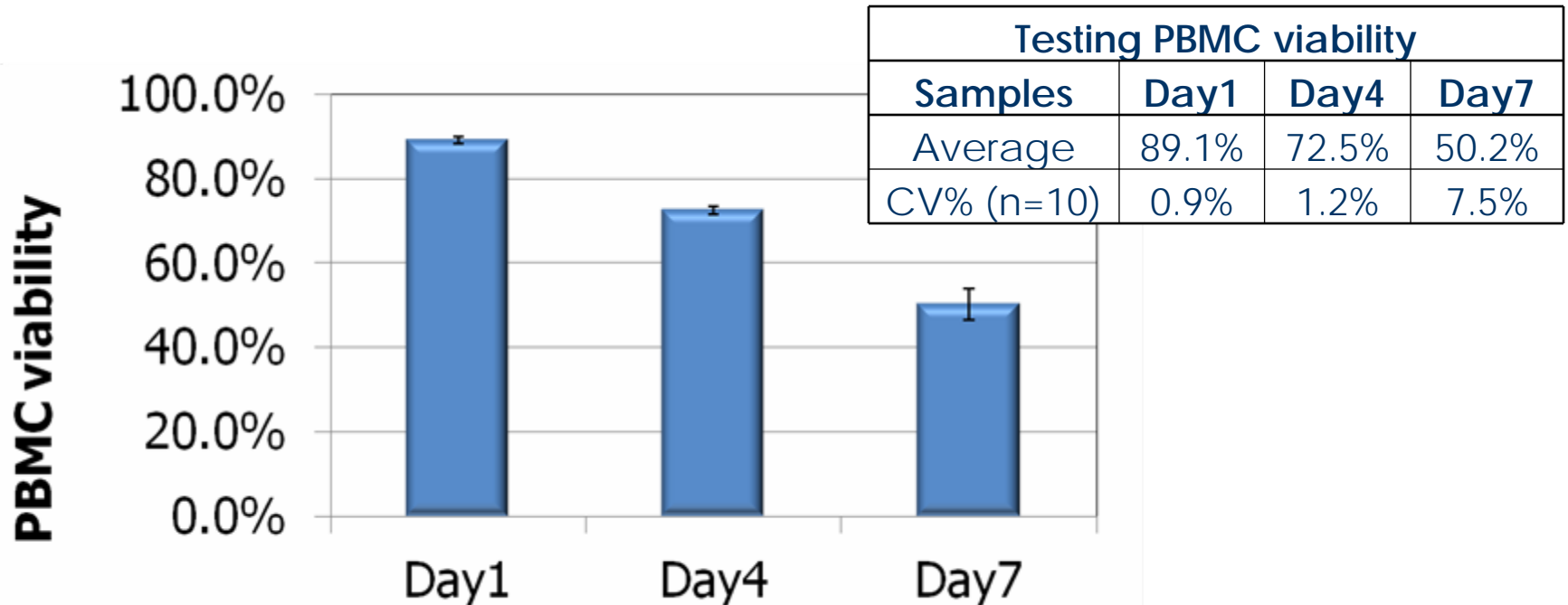
Live PMBC with
green fluorescence



Dead PMBC with EB
stained positive



PBMC Viability Results

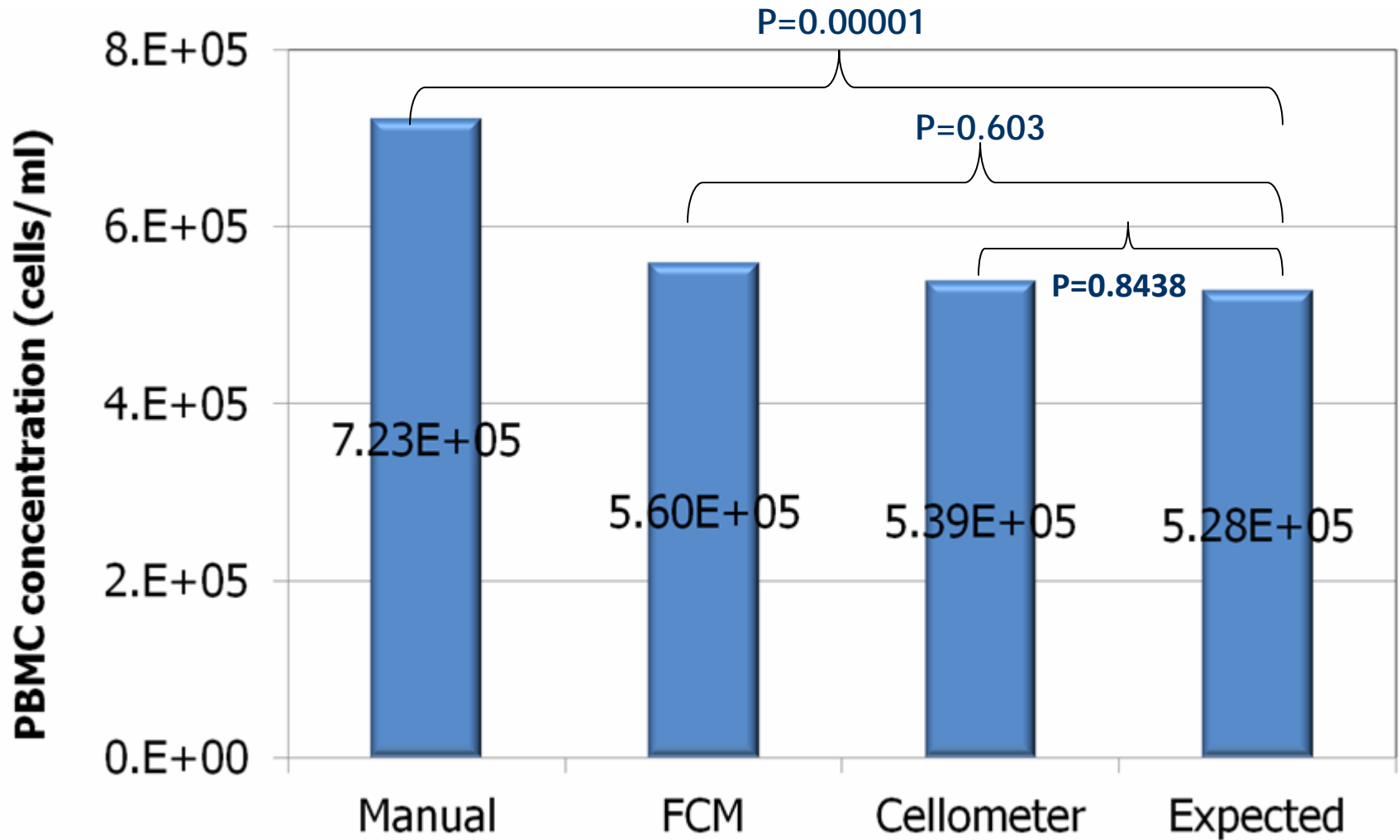


Results

- PBMCs sample stored in 4°C condition for 1-7 days
- Viability of PBMCs reduced by ~40% after one week incubation
- Measurement stays consistent in various concentrations



Comparison to Hemacytometer and Flow Cytometer



Comparison to Hemacytometer and Flow Cytometer

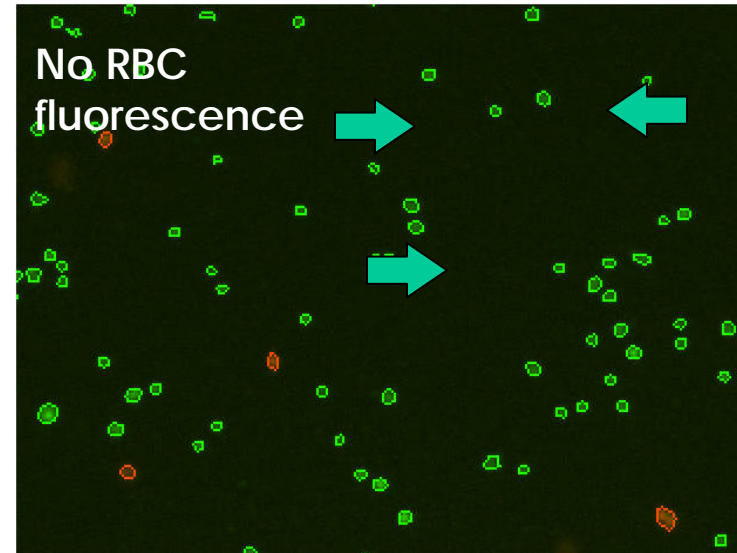
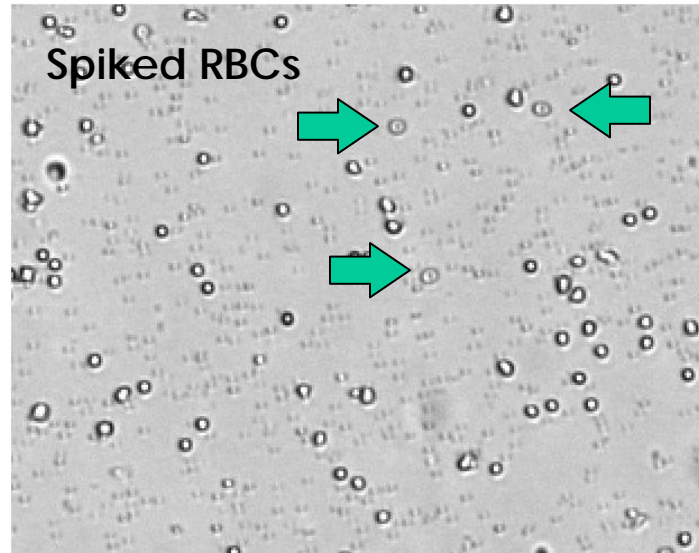
TTEST	Manual	FCM	Cellometer	Expected
Manual	1.00	0.03	0.01	0.00
FCM	0.03	1.00	0.80	0.60
Cellometer	0.01	0.80	1.00	0.84
Expected	0.00	0.60	0.84	NA

Results

- PBMC count compared between hemacytometer, flow cytometer and Cellometer®
- Cellometer® showed results closest to expected value
- Hemacytometer showed results with a $P = 0.00001$



Analyze PBMC sample with spiked red blood cell (RBC)



- Concentration: cells/ml
- Total nucleated cells measured by fluorescence (green + red).
- All cells measure by bright field only



Specifically identify PBMC from RBC Contamination samples

Sample	Measurement	Total nucleated	All cells	Difference	% RBC	N
PBMC+RBC	Average	1.26E+07	1.39E+07	1.23E+06	8.9%	10
	STD	7.88E+05	1.22E+06			
	CV	6.2%	8.8%			
PBMC+1/2RBC	Average	1.21E+07	1.27E+07	5.82E+05	4.6%	10
	STD	5.85E+05	6.81E+05			
	CV	4.8%	5.4%			
PBMC+1/4RBC	Average	1.22E+07	1.24E+07	2.27E+05	1.8%	10
	STD	9.69E+05	9.02E+05			
	CV	7.9%	7.3%			



Conclusions

- Cellometer Vision provides a rapid and cost-effective method for concentration and viability measurement of PBMCs
- The method uses very small amount of sample: less than 20 micro-liter
- Residual red blood cell concentrations do not influence PBMC concentration and viability measurements
- The method has a large linearity and good consistency

