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Obtaining Consistent and Accurate Cell Counting Results with Cellometer® Automatic Cell Counters

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Abstract

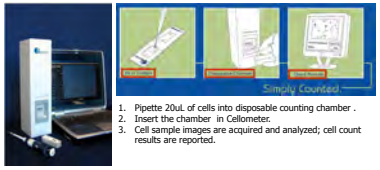
Demand for Cell-based assays are increasing in all biomedical research and drug discovery and development fields. Rapid and accurate characterization of cell population in a sample contributes significantly to assay validity. However, current available automatic cell counting methods often require costly instruments with on-board liquid handling. Daily usage requires special reagent and sample preparation, along with routine calibration and maintenance. Due to these limitations, cell counting remains largely on a traditional, manual process using hemacytometers in most biomedical and pharmaceutical laboratories. We have developed the Cellometer line of automatic cell counting instruments, that aim to replace tedious manual cell counting processes. In comparison with traditional hemocytometers, we found that there is high correlation for measuring cell concentration and trypan blue viability between Cellometer automatic cell counters and hemocytometers (R^2>0.98). Using series diluted red blood cell samples, we found that Cellometer generated reliable results from cell concentration as low as 1.0 X 10^5 to 1.5 X10^7 cells/ml. Experimental results indicated that Cellometer can be used to accurately count clumpy cells and irregularly shaped cells. With proprietary algorithms, the system can specifically analyze subpopulations of cells from heterogeneous biological samples and eliminate cell/tissue debris by size and shape exclusion. We also developed the Cellometer Vision system to automatically analyze fluorescent properties of cells. By combining bright field and fluorescence microscopic characteristics, Cellometer Vision can be applied in many complex cell population characterization assays. Examples include: rapid analysis of GFP transfection rates, direct counting acridine orange stained white-blood-cells from whole blood samples, determining cell viability using propidium iodide (a membrane impermeable dye), specific counting and size measurement of adipocytes with bodipy dye, and quantitative analyzation of Jurkat apoptosis with FITC conjugated annexin-V.

Technology

Cellometer automatic cell counters include a compact instrument with advanced imaging-based cell counting software, which automatically analyzes acquired cell images and measures cell concentration, viability, and cell size. Like a hemacytometer, it requires no special counting buffer or cell manipulation. Cells in growth media, PBS or other solutions can be pipetted directly into disposable counting chambers. The total sampling area in the chamber is equivalent to a hemocytometer's four counting corners. Because it is disposable, there is no washing between each sample and no risk of cross contamination of different samples. Proprietary algorithms have been developed and used to accurately analyze cell images. The user interface of the software is designed to be simple to use, and cell images and analysis data, including cell size distribution histograms, may be saved for research records.

Fluorophores and fluorescence proteins have been widely applied in cell based assays. The fluorescence signals are used as a readout to monitor cell proliferation, differentiations, toxicity, motility, morphology, etc. To quantitatively analyze fluorescent cells, researchers have to utilize a calibrated flow cytometer. However, in addition to the complexity, cost and availability limitations, flow cytometers are often not the best solution for cell population analysis due to the potential of complex samples clogging in the flow system. Time and materials required for analysis can be inhibitive to rapid and routine cell characterizations such as stem cell research and clinical sample analysis. Cellometer Vision was developed to address these needs, by incorporating advanced optical filtering, reliable LED light sources, and cooled CCD camera technologies. Using only 20 µl of cell sample, researchers now can rapidly identify fluorescence positive cells from a sample, analyze individual cell fluorescence intensity, and calculate cell concentration.

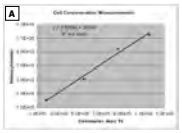
Count and Analyze Cells in 3 steps



1. Pipette 20uL of cells into disposable counting chamber .
2. Insert the chamber in Cellometer.
3. Cell sample images are acquired and analyzed; cell count results are reported.

Cellometer Automatic Cell Counter Performance

Figure 1. Comparison to hemocytometer: Experimental data indicated that Cellometer automatic cell counting results are similar to manual counted concentrations (A) or trypan blue viabilities (data not shown). Cellometer showed good repeatability. Cellometer sample-to-sample variation percentile CVs are 0.39-8.29% vs. manual counting of 8.04-20.65% (B, C).



B

	Auto T4			Hemacytometer		
	Concentration	n	CV	Concentration	CV	n
Jurkat	1.17 x 10^6	4	2.27%	1.11 x 10^6	8.04%	3
YAC-1	1.38 x 10^6	4	3.17%	1.41 x 10^6	11.89%	3
K562	6.36 x 10^5	4	8.29%	5.58 x 10^5	9.66%	3
H460	1.32 x 10^6	4	5.57%	1.16 x 10^6	20.65%	3
SW620	3.81 x 10^5	4	2.79%	3.70 x 10^5	19.49%	3
Hut 102	9.18 x 10^5	4	0.39%	7.68 x 10^5	10.60%	3

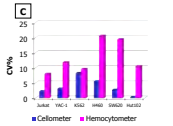
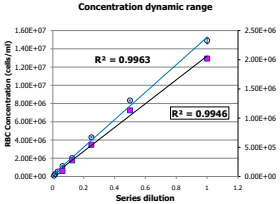


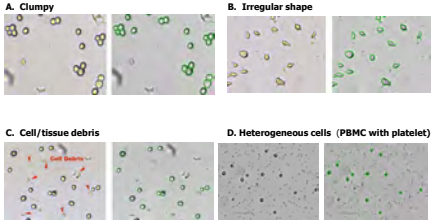
Figure 2. Determine dynamic range for measuring cell concentration (cells/ml):

Human RBC	Series dilutions	Test 1	Test 2	Test 3	Average Concentration (cells/ml)	%CV
Sample 1	1	1.53E+07	1.46E+07	1.48E+07	1.49E+07	2.42%
Sample 2	2	8.33E+06	8.29E+06	8.39E+06	8.34E+06	0.60%
Sample 3	4	4.36E+06	4.28E+06	4.25E+06	4.30E+06	1.32%
Sample 4	8	1.96E+06	2.05E+06	2.05E+06	2.02E+06	2.57%
Sample 5	16	1.09E+06	1.14E+06	1.17E+06	1.13E+06	3.57%
Sample 6	32	5.48E+05	5.12E+05	5.68E+05	5.43E+05	5.23%
Sample 7	64	2.88E+05	3.00E+05	2.43E+05	2.77E+05	10.85%
Sample 8	128	9.32E+04	9.06E+04	9.89E+04	9.42E+04	4.51%



Human red blood cells were diluted in PBS and series dilutions were made to define the dynamic range. As shown in here, there is good linerialty for tested concentration range 9.4 X 10 ^{4} to 1.5 X 10^7 cells/ml (open circle R^2=0.9963). Even at the low concentration range 9.4 X10^4 to 2 X 10^6 cell/ml, the linearity showed R^2=0.9946.

Figure 3. The Cellometer robust operating software uses proprietary algorithms to accurately analyze cell images for counting and viability determination. Cells which are counted are circled on the cell images for visual confirmation. In addition, it has strong capabilities to count individual cells in a clumpy sample (A) or irregular shaped cells (B). It automatically eliminates debris by size and shape exclusion (C) and can specifically count PBMC from platelets (D).



Cellometer Applications

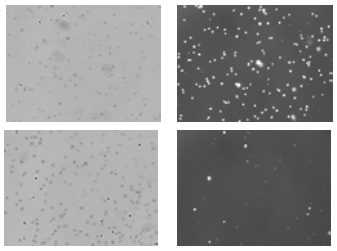
Figure 4. Determine GFP transfection rate and quantify GFP fluorescence intensity using Cellometer Vision system.

Cell Name	GFP Positve Rate	Cell Concentration	GFP Positive Concentration
HCT116	58.6%	9.3 x 10^5 /ml	5.4 x 10^5 /ml
Hela	71.9%	7.0 x 10^5 /ml	5.0 x 10^5 /ml
K562	50.5%	7.0 x 10^5 /ml	3.5 x 10^5 /ml
HEK293-1	23.4%	5.0 x 10^5 /ml	1.6 x 10^5 /ml
HEK293-2	4.3%	6.3 x 10^6 /ml	2.7 x 10^5 /ml
HEK293-3	25.7%	4.4 x 10^6 /ml	1.1 x 10^6 /ml
HEK293-4	20.2%	7.0 x 10^5 /ml	1.4 x 10^5 /ml

Figure 5. Detect Jurkat apoptosis with FITC-conjugated annexin-V. Jurkat cells were incubated with (A) or without (B) anti-CD95L antibody. To observe the apoptosis, FITC conjugated annexin-V was used to labeled surface phophatidylserine. Take 20 µl of each sample to quantitatively analyze induced apoptosis in Jurkat cells (c).

A. Induced overnight

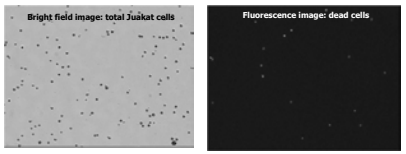
B. Un-induced



C. Data analysis

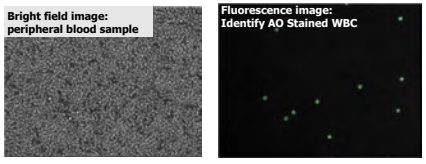
Sample	Cell Concentration	Fluorescence Positive (%)
Un-induced	3.6 x 10^6/ml	16%
Induced overnight	3.0 x 10^6/ml	92%

Figure 6. Test cell viability by staining cell with propidium idodium fluorescence dye.



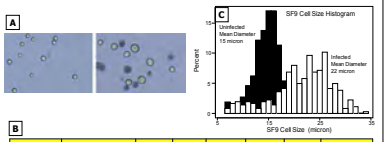
Sample	Cell Concentration	Viability %
Jurkat	1.7 x 10^6/ml	91%
Mouse thymucyte	1.6 x 10^6/ml	81%
Mouse splenocyte	1.1 x 10^6/ml	85%
Mouse bone marrow	0.95 x 10^6/ml	81%

Figure 7. Directly count white blood cells from peripheral blood without lysing of red blood cells



More Applications

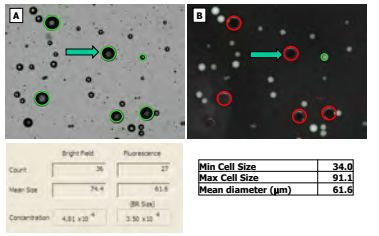
Figure 8. The user interface of the software is designed to be simple to use. All images can be saved for research records or reanalysis (A). And image analysis data report (B), including a cell size distribution histogram (C) are generated.



B

Date Time	Cell Type	Sample Name	Total Cell Count	Mean Diameter (µm)	Viability	Dilution Factor	Concentration (cells/ml)
3/9/2006 15:37	Spleen_mouse	1	388	6.48	92.2	10	1.04E+07
3/16/2006 13:34	Jurkat	J2-1	276	9.48	N/A	1	7.40E+06
3/16/2006 13:34	Jurkat	J2-2	276	9.33	N/A	1	7.40E+06
3/16/2006 13:35	Jurkat	J2-3	275	9.37	N/A	1	7.38E+06
3/17/2006 13:22	PBMC_Human_Frozen	PBMC1	1092	7.59	97.3	20	5.86E+07
3/17/2006 13:24	PBMC_Human_Frozen	PBMC2	1094	8.01	98.6	20	5.87E+07

Figure 9. Specifically detect primary mouse adipocytes from lipid droplets by staining the cell sample with bodipy fluorescence dye and estimate the size of mouse adipocytes. As shown here, bright field image (A) identified 36 objects but only 27 of them are adipocytes (B: green circled cells). The rest of them are lipid droplets (B: red circled particles).



Min Cell Size	34.0
Max Cell Size	91.1
Mean diameter (µm)	61.6

Summary

Experimental results shown here indicate that the non-flow, image based Cellometer automatic cell counter can be used to replace manual hemocytometers to rapidly and accurately measure cell concentration, viability, and cell size using just 20 µl of cell sample in less than 30 seconds. By using the plastic disposable counting chamber, all washing steps can be eliminated.

Sophisticated algorithms were developed to process cell sample images. More than 300 different type of cells have been counted on Cellometer systems. Utilizing the user friendly software interface, researchers can also be applied to quantitatively determine specific cell concentration from heterogeneous biological samples, such as PBMC from platelet background, primary adipocytes from lipid droplets, etc.

Cellometer Vision can be applied to various cell sample analyses and fluorescent cell-based assays. Multiple fluorescent filters are available for detecting commonly used fluorophores or fluorescent proteins.

In conclusion, Cellometer automatic cell counting systems provide a novel tool to standardize cell counting processes consequently eliminating variations inherent in manual cell counting methods used in cell-based assays today.