Surface Marker Based Direct Cell Concentration Measurements Using a High Sensitivity Imaging Cytometry Method

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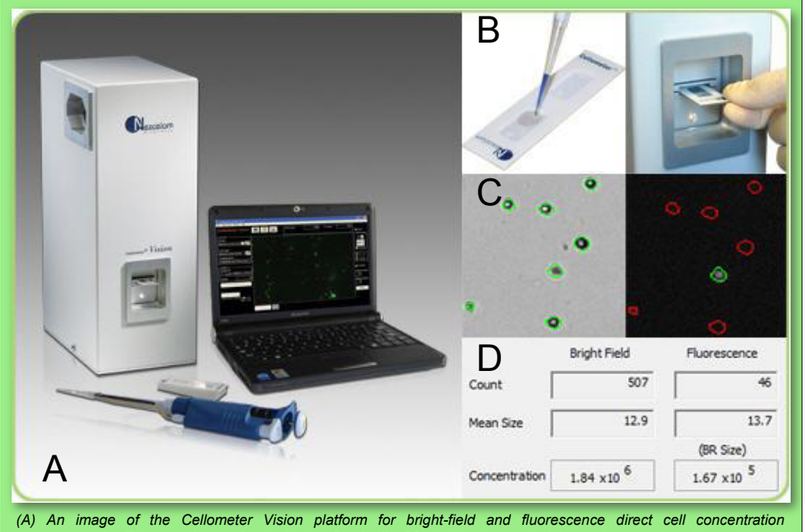
1. ABSTRACT (260)

Cell-based assays routinely employ sensitive fluorescence detection methods [1]. For simple cell counting, manual observation using a fluorescence microscope is time-consuming and prone to human error[2]. High content analysis such as confocal fluorescence microscopy can examine detailed cellular structures, but is not suitable for studying large cell populations. Common cell population analysis such as flow cytometry is fully automated and has high fluorescence sensitivity. Flow cytometers require calibration beads to produce cell concentration results, and careful maintenance is required to eliminate contamination between samples[3].

Recently, a novel imaging cytometry system using inexpensive disposable counting chambers, which require 20 µl of sample, has been developed by Nexcelom Bioscience (Lawrence, MA). Using a combination of bright-field and fluorescent images, the system allows automated cell image acquisition and processing using novel counting algorithms. Cellometer Vision can accurately and consistently measure cell concentration, viability, and percentage of fluorescent cells for a variety of cell types, such as GFP expressed cells, cancer cells, blood components, stem cells, etc. In this work, we demonstrate using the Cellometer Vision for quantifying concentration of splenocytes labeled with specific surface markers.

[1] George et al., *Cytom. p. A*, 59A, 234-245, 2004. [2] Ng et al., *Tiss. Eng.*, 11, 182-191, 2005. [3] Michelson, *Blood,* 87, 4925-4936, 1996

2. CELLOMETER® VISION & CELL COUNTING METHOD

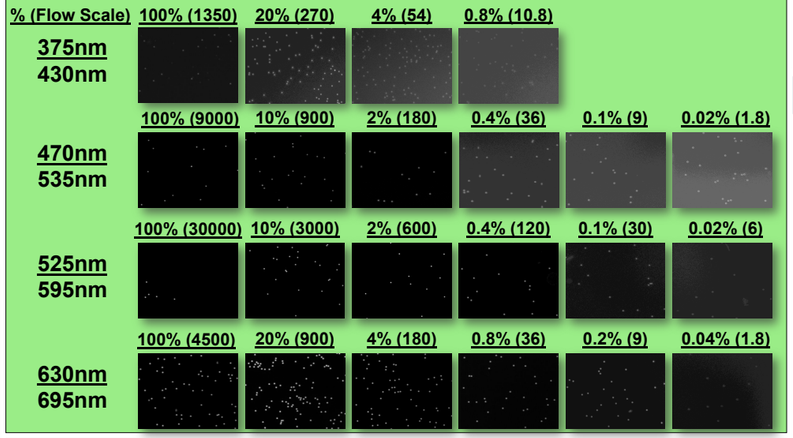


(A) An image of the Cellometer Vision platform for bright-field and fluorescence direct cell concentration measurement. The counting protocol simply (B) pipette 20 l of cell sample into an inexpensive disposable cell counting chamber and place in the Cellometer Vision slide holder. (C) Next, the software automatically outlines and counts the cells by bright-field or fluorescence. (D) The resulting concentrations and cell sizes are calculated depending on the counted cells.

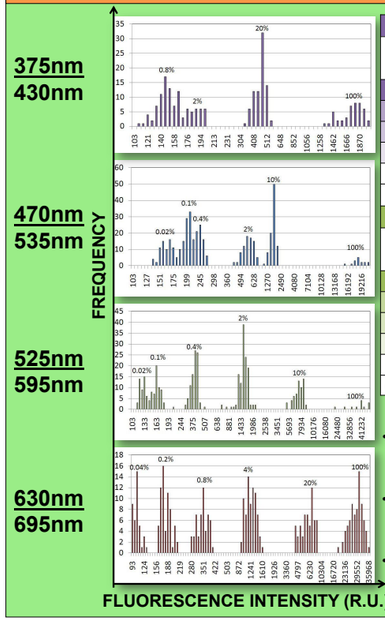
- **Some Application Examples**
  - Total cell concentration for highly viable cell population
  - Live/Dead cell concentration using dual dye fluorescence
  - Total and live nucleated cells without lysing
  - Transfection efficiency by GFP, YFP, and RFP
  - Identify and count cells base on cell size
  - Capture and store cell images
- **Some Cell Type Examples**
  - Cancer cell lines
  - Stem cells
  - Hepatocytes
  - Splenocytes
  - WBCs in whole blood
  - Platelets
  - Insect cells
  - Yeasts and Algae

3. LIMIT OF DETECTION: LOW INTENSITY FLUORESCENT BEAD

- **MATERIALS**
  - Four fluorescent beads (Invitrogen) used to confirm low signal detection of Cellometer Vision
    - Excitation/Emission: 375nm/430nm, 470nm/535nm, 525nm/595nm, 630nm/695nm
    - Each fluorescent bead type has various percentage of fluorescence intensity (100%-0.02%)
- **METHOD**
  - Each set of fluorescent bead is measured and counted individually
  - The various percentage of fluorescence intensity beads are randomly premixed and measured



4. MIXED FLUORESCENT BEAD MEASUREMENTS

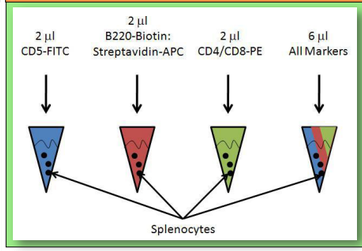


375nm/430nm		470nm/535nm	
Total concentration (particles/ml)	4.95E+06	Total concentration (particles/ml)	9.68E+06
100% (particles/ml)	1.07E+06	100% (particles/ml)	3.59E+05
20% (particles/ml)	1.71E+06	10% (particles/ml)	2.04E+06
4% (particles/ml)	3.86E+05	2% (particles/ml)	1.77E+06
0.8% (particles/ml)	1.78E+06	0.4% (particles/ml)	1.89E+06
		0.1% (particles/ml)	1.95E+06
		0.02% (particles/ml)	1.66E+06
525nm/595nm		**630nm/695nm**	
Total concentration (particles/ml)	8.71E+06	Total concentration (particles/ml)	8.57E+06
100% (particles/ml)	2.65E+05	100% (particles/ml)	1.79E+06
10% (particles/ml)	1.30E+06	20% (particles/ml)	1.37E+06
2% (particles/ml)	2.72E+06	4% (particles/ml)	1.77E+06
0.4% (particles/ml)	2.01E+06	0.8% (particles/ml)	1.23E+06
0.1% (particles/ml)	1.81E+06	0.2% (particles/ml)	1.49E+06
0.02% (particles/ml)	5.97E+05	0.04% (particles/ml)	9.29E+05

RESULTS

- Cellometer Vision was able to detect and measure the concentration of fluorescent beads at each intensity
- The limit of detection thus far showed 10.8, 1.8, 6, and 1,8 on a flow cytometric scale for UV, blue, green, and red excitation, respectively
- By calibrating to the exposure time during image acquisition, the histogram of each fluorescent intensity can be plotted together

5. SPLENOCYTE SURFACE MARKER MEASUREMENT PROTOCOL



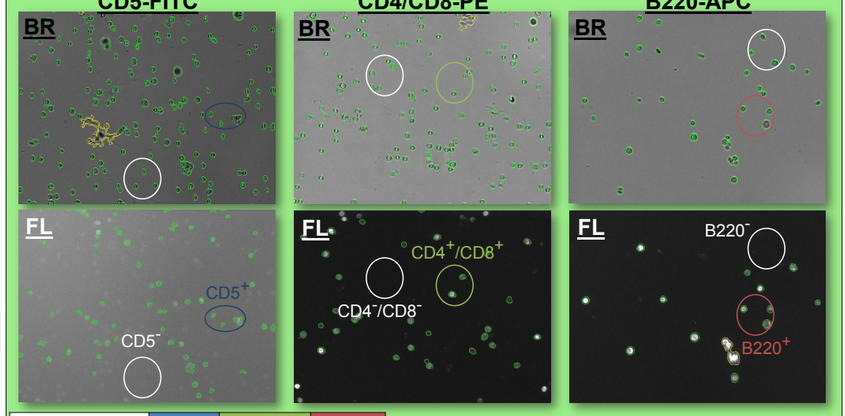
Assay Protocol

Step 1: Add 1 l of FcBlocker to each tube on ice ~ 15 min
Step 2: Add 2 l of surface marker to respective tube
Step 3: Incubate for ~30 min
Step 4: Wash with 500 l of buffer for 2 times
Step 5: Add 100 l of FACS buffer to 4 tubes

Instrumental Setup

470nm/535nm Detecting CD5-FITC
525nm/595nm Detecting CD4/CD8-PE
630nm/695nm Detecting B220-APC

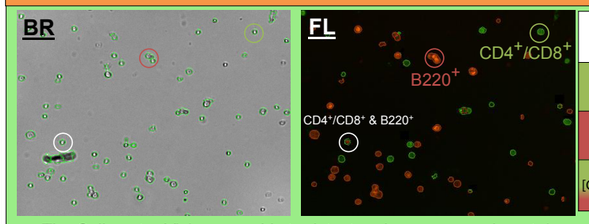
6. SURFACE MARKER DETECTION & MEASUREMENTS



	CD5-FITC	CD4/CD8-PE	B220-APC
[BR] (particles/ml)	7.51E+06	8.73E+06	3.27E+06
[FL] (particles/ml)	3.90E+06	2.43E+06	1.56E+06
FL percentage	48.0%	27.8%	47.5%

- Each surface marker was detected and measured
- PE and APC had the highest signal
- Percentages of protein expression were consistent with flow cytometry measurements

7. DUAL LABEL DETECTION & MEASUREMENTS



[BR] (particles/ml)	5.67E+06	
[CD4/CD8] (particles/ml)	1.50E+06	26.3%
[B220] (particles/ml)	3.02E+06	53.1%
[CD4/CD8/B220] (particles/ml)	1.13E+05	2.0%

- The Cellometer Vision was able to detect and measure each protein expression and overlay images
- Concentrations of splenocytes with a single or both protein expressions were quantified
- Future work will be utilizing three or more filter sets for multi-surface marker measurement

8. CONCLUSION & ACKNOWLEDGEMENT

We have demonstrated surface marker-based direct cell concentration measurement with a sensitivity comparable to flow cytometry using Cellometer® Vision. The authors would like to thank Dr. Xuemei Zhong for providing the splenocytes and performing surface marker labeling protocol