A Novel Imaging Cytometry Method for Immunophenotyping

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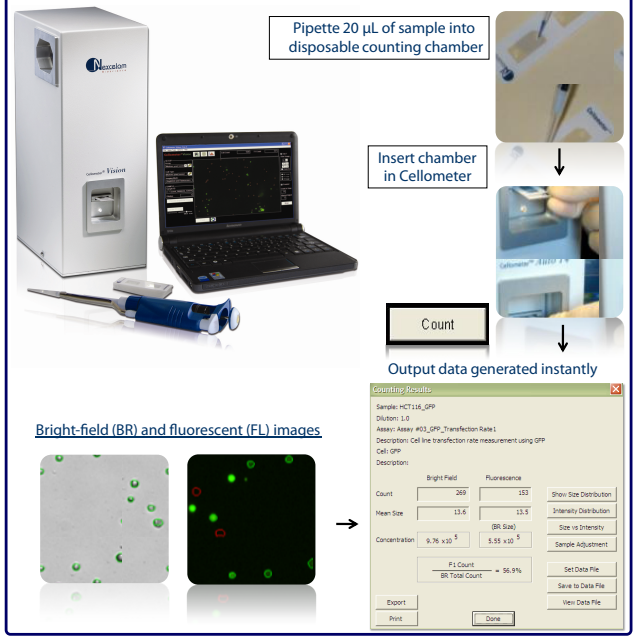
1. ABSTRACT

The ability to detect and enumerate cells with specific surface markers is essential for clinical diagnosis and biomedical research. The traditional cell analysis instrumentation for immunophenotyping involves the use of fluorescence microscopy, confocal fluorescence scanning, and flow cytometry. However, these systems are often expensive to purchase and maintain. In this work, we demonstrate a novel low-cost device, Cellometer® system, which combines both microscopic imaging and cytometry methods with comparable detection sensitivity. This method utilizes both bright-field and fluorescence imaging of a disposable cell counting chamber to quickly provide concentration and percentages of differentially-labeled cell populations. Common fluorescent microspheres used for flow cytometer calibration was used to compare the sensitivity of the Cellometer® system with flow cytometry. The system was also tested to quantify percentages of single- (CD5-FITC, CD4/CD8-PE, and B220-APC) and double-labeled (CD4-APC and CD8-PE) cell population. The Cellometer® was able to detect and enumerate fluorescence microspheres at the lowest intensity. The cell population analysis results were highly consistent with the data generated by FACScalibur flow cytometer. This novel imaging cytometry method provides an efficient tool for cell concentration measurements, as well as surface marker based cell population analysis, especially when flow cytometer is not readily available or a quick test is required.

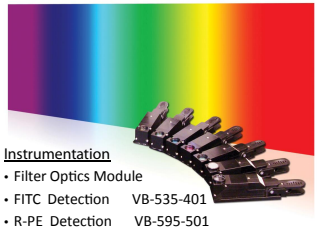
2. INTRODUCTION

Immunophenotyping is one of the key components of clinical diagnosis and biomedical research. Such study is essential for the diagnosis of hematologic malignancy, sub-classifying diseases, monitoring response to treatment, predicting prognosis, detecting rare cell populations and residual malignant cells. In immunological study, cell surface markers have been widely used to identify and classify various immune cells and track their developmental status as well as physiological functions.

3. CELLOMETER® VISION IMAGING CYTOMETRY



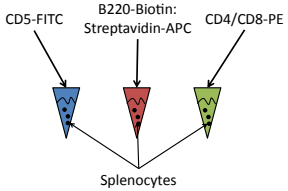
4. MATERIALS AND METHODS



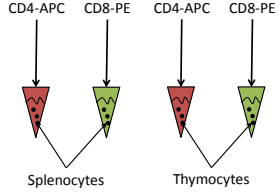
Instrumentation
- Filter Optics Module
- FITC Detection VB-535-401
- R-PE Detection VB-595-501
- APC Detection VB-695-601

Consumable
- 20 µL sample volume
- Concentration range: 10^5 – 10^7 cells/ml

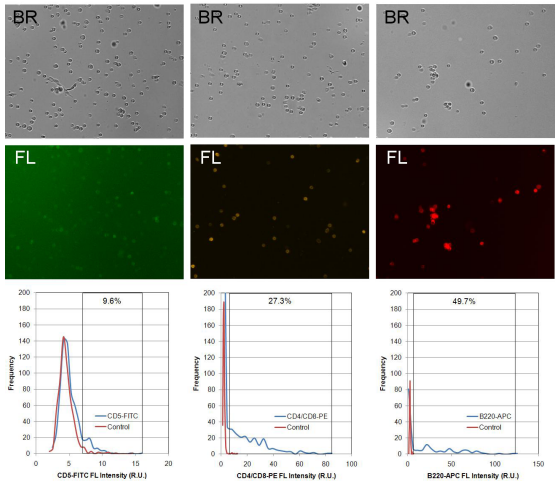
Initial ImmunophenotypingMeasurement



CD4 CD8 Measurement Validation

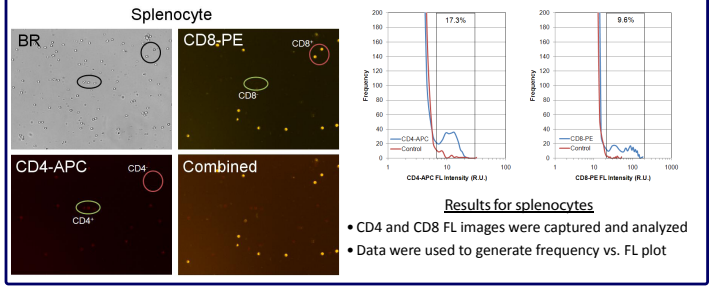


5. INITIAL IMMUNOPHENOTYPING MEASUREMENT



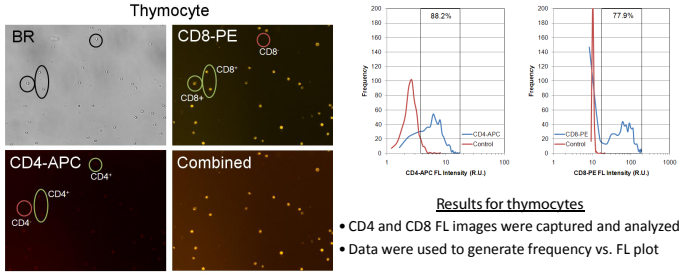
Results
- Cellometer was capable of detecting of CD5-FITC, CD4/CD8-PE, and B220-APC
- Concentration and percentages of specific surface marker positive cells were measured
- Each measurement was complement with a control sample

6. SPLENOCYTE CD4 CD8 MEASUREMENT



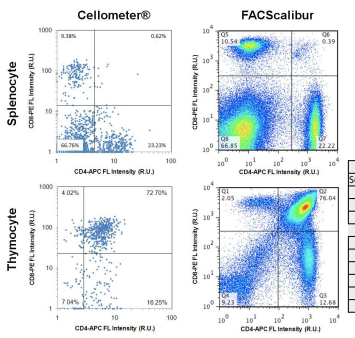
Results for splenocytes
- CD4 and CD8 FL images were captured and analyzed
- Data were used to generate frequency vs. FL plot

7. THYMOCYTE CD4 CD8 MEASUREMENT



Results for thymocytes
- CD4 and CD8 FL images were captured and analyzed
- Data were used to generate frequency vs. FL plot

8. RESULTS VALIDATION USING FLOW CYTOMETRY



Results
- CD4-APC and CD8-PE stained splenocytes and thymocytes were counted
- FL data was used to generate a scatter plot of CD4-APC vs. CD8-PE
- Flow cytometry also generated similar results which validated the Cellometer method

Single Label	Percentage
Surface Marker	(%)
CD5	9.60%
CD4/CD8	27.31%
B220	49.69%

Dual Label	FACScalibur	Cellometer	Dual Label	FACScalibur	Cellometer
Splenocyte	(%)	(%)	Thymocyte	(%)	(%)
$CD4^-CD8^-$	66.85%	66.76%	$CD4^-CD8^-$	9.23%	7.04%
$CD4^+CD8^-$	22.22%	23.23%	$CD4^+CD8^-$	12.68%	16.25%
$CD4^-CD8^+$	10.54%	9.38%	$CD4^-CD8^+$	2.05%	4.02%
$CD4^+CD8^+$	0.39%	0.62%	$CD4^+CD8^+$	76.04%	72.70%

9. CONCLUSION & ACKNOWLEGEMENT

We have demonstrated new immunophenotyping application for the Cellometer imaging cytometry method, which is of great importance to a variety of clinical diagnosis and biomedical research. The ability to rapidly and effectively perform cell population analysis assays, as well as cell concentration and viability measurement, may improve research productivity, especially where flow or laser scanning cytometers are not readily available.

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