A Novel Method for Kinetic Measurements of Rare Immune Cell Proliferation using Cellometer Image-Based Cytometry

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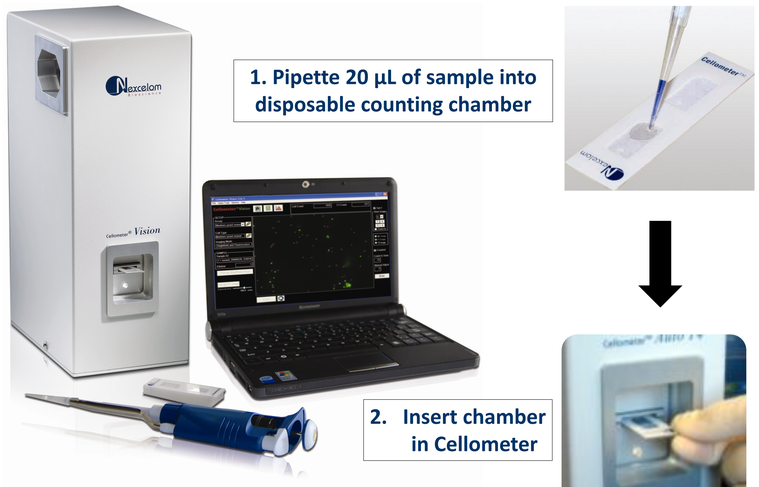
1. ABSTRACT

Measuring cell proliferation is important for studying the effects of various treatments on cultured primary cells or cell lines. Current proliferation analysis methods employ flow cytometry for fluorescence detection of CFSE-labeled cells. However, conventional flow cytometers require a considerable amount of cells per reading, which becomes an issue for kinetic measurements with rare cell population due to the lack of sufficient samples to test at multiple time points during the proliferation period. Here we report the development of a novel cell proliferation kinetic detection method for low cell concentration samples using the new Cellometer Vision image-based cytometry system. Since the Cellometer system requires only 20 µl of sample, cell proliferation can be measured at multiple time points over the proliferation period, whereas typically, flow cytometry is only performed at the end of the proliferation period. To validate the detection method, B1 and B2 B cells also were treated with a B cell mitogen and proliferation was measured on day 1, 3, 5, and 6. To demonstrate the capability, B1 B cells were treated with a panel of TLR agonists and proliferation was measured on day 2, 4, 6, and 7. Cellometer was able to obtain proliferation results on each day that were comparable to flow cytometry. This novel method allows for kinetic measurements of the rare cell samples such as B1 B cell, which has the potential to revolutionize kinetic analysis of cell proliferation.

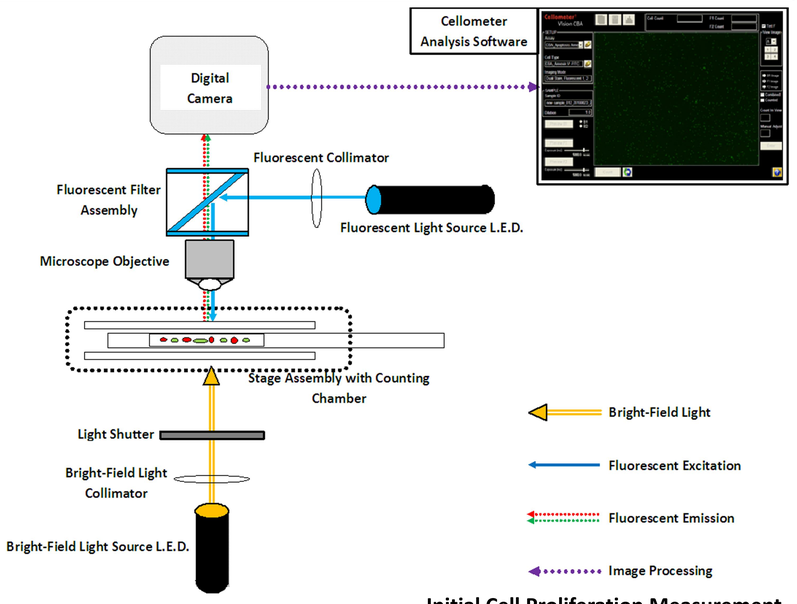
2. INTRODUCTION

Cell proliferation is an important assay for pharmaceutical and biomedical research to test the effects of a variety of treatments on cultured primary cells or cell lines. For immunological studies, the ability to perform rapid cell proliferation analysis allows the identification of potential biological reagents for inducing or inhibiting immune cell proliferation. This novel method allows for kinetic measurement of the same cell sample owing to the low sample volume requirement. In addition, fluorescent images can be used to visually confirm cell proliferation by observing the attenuated CFSE fluorescence,

3. CELLOMETER® VISION IMAGE-BASED CYTOMETRY



4. MATERIALS AND METHODS



Instrumentation

- CFSE Detection → VB-535-401

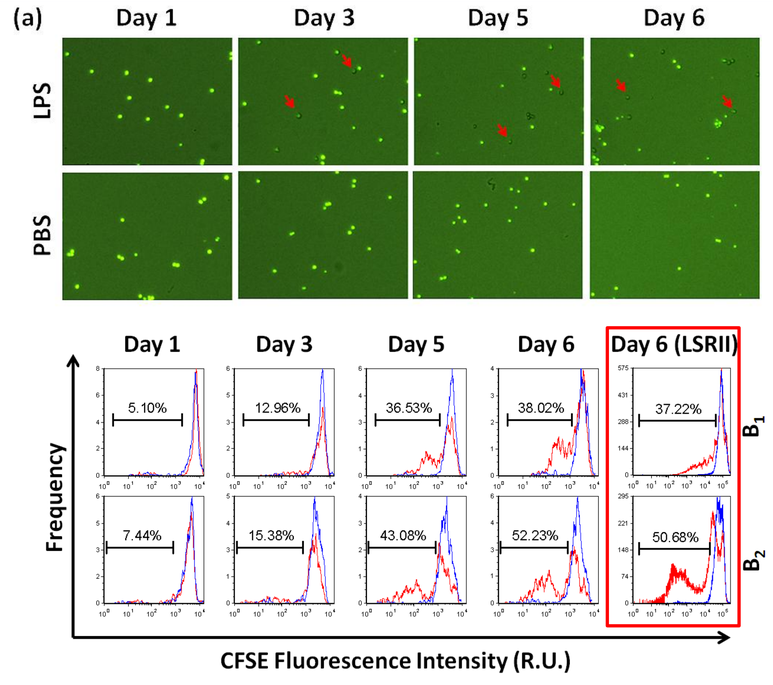
Initial Cell Proliferation Measurement

- B1 and B2 B cells are purified from mice
- Both samples are treated with LPS to show the capability of proliferation detection

Proliferation Compound Screening

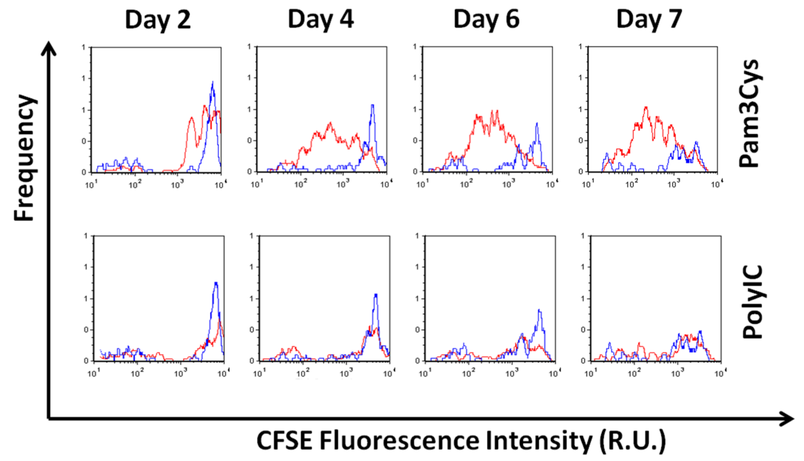
- B1 B cells are used since the cell count is much lower than B2 B cells
- B1 B cells are induced with CpG, CLO97, PolyIC, LPS, Pam3Cys
- Proliferation effect is studied for 7 days

5. INITIAL CELL PROLFERATION MEASUREMENT



- The captured fluorescent images of CFSE stained B cells were clearly visible, with diminishing fluorescence signals observed at longer duration
- Cellometer was able to measure B1, B2 proliferation comparable to flow cytometry
- Results showed the feasibility of the method to measure kinetic cell proliferation
- In this work, the number of B1 B cells are in much lower quantity than the B2 B cells, thus it is important to note that at lower quantity (~2000 cells), Cellometer was able to generate comparable results as flow cytometry
- In addition, flow cytometry was only able to collect data for the last day due to the high number of cells required for flow analysis

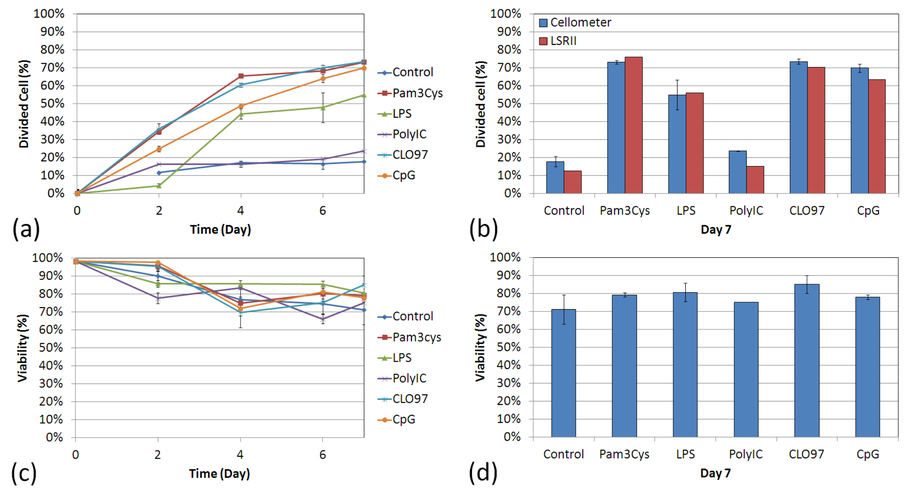
6. PROLIFERATION COMPOUND SCREENING



% Proliferation	Day 2	Day 4	Day 6	Day 7
PBS	11.6%	17.1%	16.5%	17.8%
Pam3Cys	34.6%	65.6%	68.4%	73.2%
LPS	4.4%	44.4%	48.0%	55.0%
PolyIC	16.4%	16.4%	19.2%	23.6%
CLO97	36.0%	60.5%	70.1%	73.6%
CpG	24.8%	48.9%	64.2%	70.0%

- Histogram obtained from Cellometer showed various effect of compounds on the proliferation of B1 B cells, shown in the table above
- B1 B cells induced with CpG, CLO97, LPS and Pam3Cys showed significant proliferation during 7-day incubation. Only PolyIC did not show a proliferation effect
- Cellometer was able to measure cell proliferation with under 1000 cells counted for each sample

7. END-POINT COMPARISON TO FLOW CYTOMETRY



- Measuring proliferation on day-by-day basis can produce a kinetic plot to better characterize the proliferation effect of a chemical compound
- In addition, viability of cells can be determined simultaneously with propidium iodide

8. CONCLUSION & ACKNOWLEGEMENT

We have demonstrated a novel method for kinetic measurement of cell proliferation using the Cellometer image-based cytometry system, which is of great importance to a variety of cell-based assays where cell samples are in low volume. 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.