A Rapid Alternative Method for Cell Cycle Analysis Using Cellometer Vision

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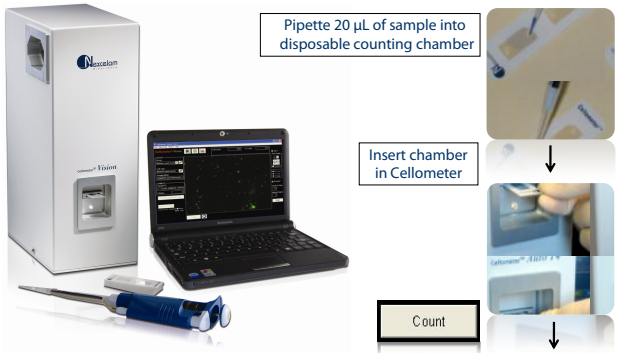
1. ABSTRACT

Traditional cell cycle analysis instrumentation involves the use of fluorescence microscopy, laser scanning cytometry, and flow cytometry. In this work, we demonstrate new applications for the Cellometer Vision, which utilizes an imaging cytometry method for cell cycle analysis. This method employs both bright-field and fluorescence imaging of a disposable cell counting chamber to quickly provide concentration and percentages of cell subpopulations. Signal measurement and data analysis could be performed less than five min. Cell cycle assay was demonstrated by detection of changes in phase percentage within a cell population when Jurkat cells were induced with three different drugs, nocodazole, etoposide, and aphidicolin that arrest cell cycle at G2/M, S, and G0/G1 phase, respectively. Jurkat cells were fixed with 75% ethanol and stained with propidium iodide. Individual cells were identified by the software and the fluorescence intensity was measured from within the cell. The imaging cytometry method was able to detect and analyze fluorescence from the Jurkat cells, which generated cell cycle data plot for each drug at three different concentrations. The cell cycle analysis results were highly consistent with the results obtained from FACScalibur flow cytometer. This imaging cytometry method provides a rapid and cost-effective tool for cell concentration measurements, as well as cell cycle analysis, which may benefit smaller research laboratories, where flow cytometer is not readily available.

2. INTRODUCTION

Cell cycle analysis is one of the key components of clinical diagnosis and biomedical research. Cell cycle analysis distinguishes cells in different phases of cell cycle and is often used to determine cellular response to drugs and biological stimulations. The assay is based on measuring DNA content of cells to determine if the cells are behaving normally under certain perturbation to the environment such as drug induction. By using a nucleic acid stain, Propidium Iodide, one could use fluorescence to accurately analyze cell cycle.

3. CELLOMETER® VISION IMAGING CYTOMETRY



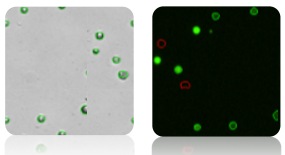
Pipette 20 µL of sample into disposable counting chamber

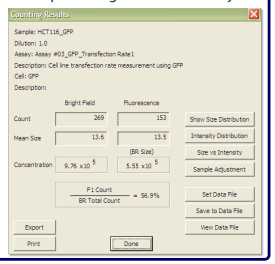
Insert chamber in Cellometer

Count

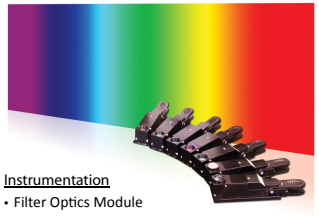
Output data generated instantly

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





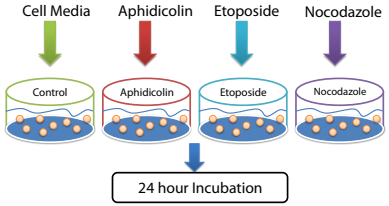
4. CELL CYCLE DOSE RESPONSE EXPERIMENT



Instrumentation
- Filter Optics Module
- Propidium Iodide Detection
  - VB-595-501

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

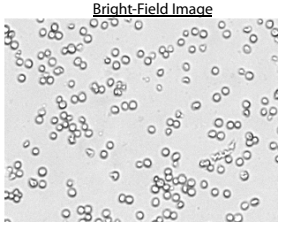
Drug Induced Cell Cycle Phase Arrest



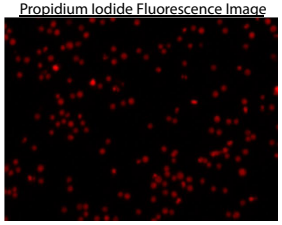
Experimental Protocol
- Jurkat cells are cultured under 37°C and 5% CO_2
- 4 separate flasks are induced with various concentrations of Aphidicolin, Etoposide, and Nocodazole for 24 h.
- Aphidicolin (1.2, 6, 30 µg/ml)
- Etoposide (0.12, 0.6, 3 µM)
- Nocodazole(.004, .02, .1 µg/ml)

5. CELL CYCLE ANALYSIS AND THEORETICAL RESULTS

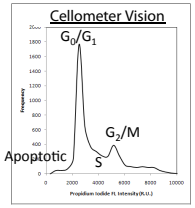
Bright-Field Image



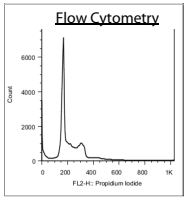
Propidium Iodide Fluorescence Image



Cellometer Vision



Flow Cytometry



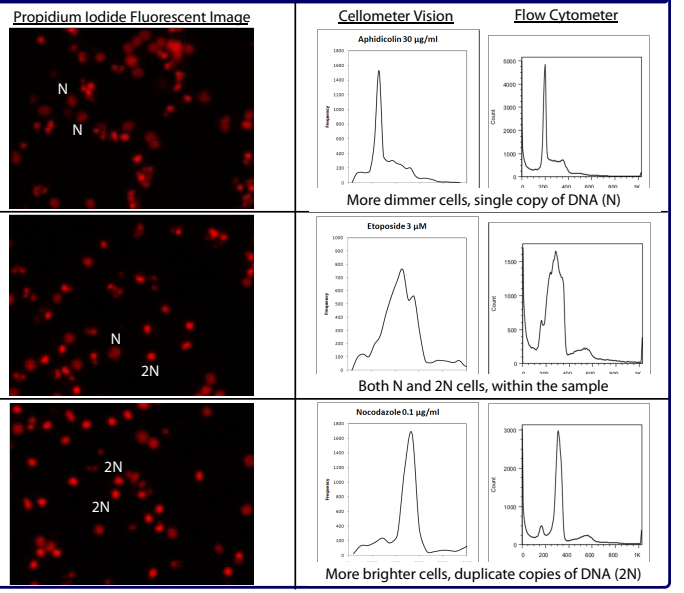
Cell Cycle Analysis
- The Jurkat cells induced with the 3 drugs are spun down at 1500 rpm for 5 min
- The supernatant is replaced with 66% ethanol and kept on ice for 15 min
- The cells are again spun down and replaced with 50 µg/ml Propidium Iodide
- The cells are allowed to incubate with stain for 30 min before Cellometer Vision and flow cytometry analysis

Aphidicolin
- Aphidicolin induced Jurkat cells should arrest in the G_0/G_1 and S phase

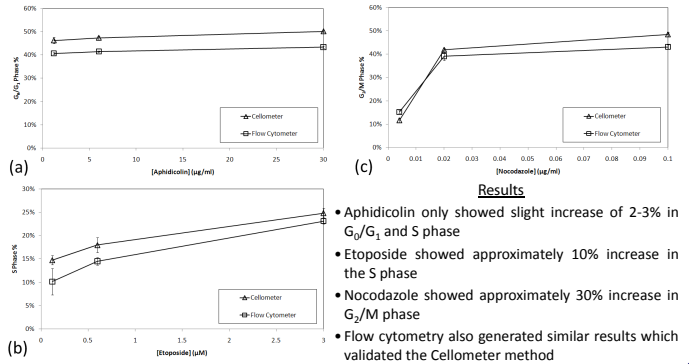
Etoposide
- Etoposide induced Jurkat cells should arrest in the S phase

Nocodazole
- Nocodazole induced Jurkat cells should arrest in the G_2/M phase

6. CELL CYCLE PHASE ARREST RESULTS



8. CELLOMETER VISION AND FLOW CYTOMETER COMPARISON



Results
- Aphidicolin only showed slight increase of 2-3% in G_0/G_1 and S phase
- Etoposide showed approximately 10% increase in the S phase
- Nocodazole showed approximately 30% increase in G_2/M phase
- Flow cytometry also generated similar results which validated the Cellometer method

9. CONCLUSION & ACKNOWLEGEMENT

We have demonstrated new cell cycle analysis 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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