Measuring Mitochondrial Membrane Potential with JC-1 Using the Cellometer Vision Image Cytometer

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1001274 Rev. A

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Introduction

Alterations in apoptosis are relevant to a large number of disease states, including cancer, heart disease, neurological disorders, infectious disease, and more [1]. One of the earliest steps in the apoptosis pathway, the loss of mitochondrial membrane potential, is often an indication of disorder and is therefore a useful target for those investigating the mechanism of disease *in vitro*. Mitochondrial membrane potential can be easily and efficiently monitored using a number of commonly available fluorescent dyes.

Recently, a small desktop imaging cytometry system (Cellometer Vision) has been developed by Nexcelom Bioscience LLC for automated bright-field (BR) and fluorescent (FL) imaging methods [2]. The system can perform rapid cell enumeration and other fluorescent measurements using disposable counting slides. The software utilizes a novel counting algorithm for accurate and consistent measurements on a variety of cell types [3].

By developing fluorescent-based assays to assess mitochondrial membrane potential, the Cellometer imaging cytometry can provide a quick, simple, and inexpensive alternative for biomedical research, which may be beneficial for smaller research laboratories and clinics. In this work, we demonstrate a mitochondrial membrane potential assay using Cellometer imaging cytometry and the JC-1 dye as an alternative to flow cytometry. The data obtained by Cellometer were compared to those from conventional flow cytometry methods.

Materials and Methods

Cellometer Vision and Disposable Counting Chamber

The Cellometer Vision utilizes one bright-field and two fluorescent channels to perform image-based cytometric analysis. Bright-field imaging used a broadband white light-emitting diode (LED) and fluorescence imaging used three different monochromatic LEDs (470, 527, and 630 nm) as the excitation light sources. Each monochromatic excitation was paired with a specific excitation (nm)/emission (nm) filter set (475/ 525, 475/595, 527/595, and 630/695) with a bandwidth of approximately 40 nm.

Cellometer systems were designed to specifically analyze Nexcelom's disposable counting chamber, which holds precisely 20 μL of sample. Four separate areas were imaged and analyzed sequentially by the system, where the target cells were identified and counted by the software. In general, combined image acquisition and cell counting time was approximately 30 seconds.

The Cellometer software used a proprietary algorithm to analyze the captured bright-field and fluorescent images. Parameters such as cell shape circularity and size were gated to count specific population of cells from the bright-field images. Aggregation of cells was included in the total cell count by the use of declustering function, which could distinguish and count individual cells in the cluster. Fluorescent intensity within individually counted cells was measured with sample-dependent fluorescent threshold, based on which a histogram plot was generated to show distribution of fluorescent intensity in the population. Counting and fluorescence measurements were directly exported to FCS Express (De Novo Software) for flexible graph generation Exported data file contained the number, size, and fluorescence intensity of individually counted cells.

Cell Preparation for Membrane Potential Analysis

The MitoProbe^{TM} JC-1 assay kit was purchased for JC-1 mitochondrial membrane potential analysis. The assay measures the depolarization of mitochondrial membrane potential, which has been described as an early indicator of cell death or mitoautophagy [4-7]. The JC-1 fluorescent dye forms red fluorescent J-aggregates (~590 nm) when accumulated in the mitochondria [8]. When the mitochondria membrane potential is disrupted during apoptosis, the emission shifts to a green fluorescence (~529 nm). Jurkat cells were harvested from culture and centrifuged. The pellet was resuspended in PBS at $1x10^6$ cells/mL and separated into three tubes of 1 mL cell sample. One tube is used as the unstained control sample. One tube of Jurkat cells was incubated with CCCP (final concentration at 50 μM) at 37°C to disrupt mitochondrial membrane potential. Both tubes were then stained with JC-1 (final concentration at 2 μM) and incubated for 30 min before performing flow and imaging cytometry analysis with experimental replication n = 2 and 4, respectively. Note that since the Cellometer filter sets were specifically designed to eliminate cross-talk between fluorescent channels, no compensation was required.

Flow Cytometry Analysis

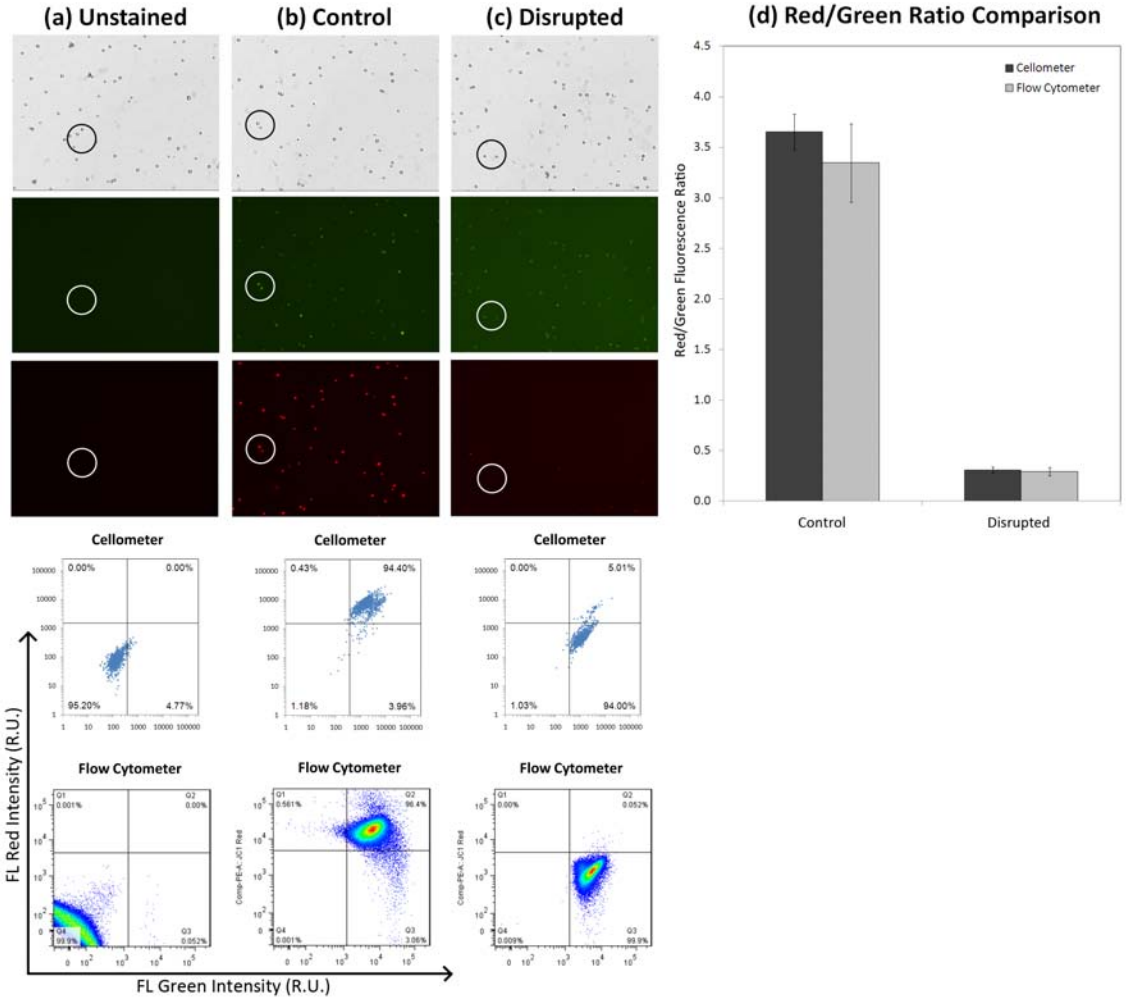
JC-1 mitochondrial potential measurement was analyzed on a LSRII flow cytometer where a 488 nm excitation laser was used in combination with FITC and PE emission channels to measure green and red fluorescence of the JC-1 stain, respectively. The measured results were analyzed using the FlowJo software and compared with that of Cellometer imaging cytometry.

Results

Figure 1. Mitochondrial membrane potential analysis.

Bright-field, fluorescent images, and scatter plots generated by Cellometer (top) and flow cytometer (bottom) of (a) unstained Jurkat cells, (b) control Jurkat cells, and (c) cells with induced with CCCP.

- Mitochondrial membrane potential experiment required two fluorescence detection wavelengths for JC-1. The Cellometer Vision was able to generate fluorescent images in both channels without optical crosstalk with an optimized filter set.
- The fluorescent Cellometer images clearly visualized the reduction of red fluorescence that represents a decrease in polarization of mitochondrial membrane potential, an indication of early cell death [4-7].
- Analysis of fluorescence also revealed a large decrease in red/ green fluorescence ratio (d), which was comparable to the flow cytometry results.
- Since detection of JC-1 required two fluorescent channels, it would be possible to silmultaneously measure cell death using trypan blue staining in bright-field imaging because the red fluorescence of JC-1 overlaps PI staining.
- It must be noted that cell circularity was only used as a gating parameter to include or exclude certain cells and cannot be measured as a read out in the current software.



Conclusions

- The ability to rapidly and cost-effectively perform imaged-based mitochondrial membrane potential assays may improve research efficiency, especially where a flow or laser scanning cytometer is not available or in situations where a rapid analysis of data is critical.
- Cellometer Vision performed the mitochondrial membrane potential assay outlined here and achieved results consistent with those of the conventional flow cytometry method.
- Besides the compatibility, Cellometer Vision method also has several advantages over conventional flow cytometry:
    - In comparison to the 300 μL of sample for flow cytometry, only 20 μL of sample is required for the Cellometer Vision. It can immediately provide both concentration and percentage of each cell population, whereas further post-harvest analysis is usually required to obtain flow cytometry results and indirect calculation is needed to obtain cell concentration.
    - In addition, the ability to record both BR and FL images of cell sample allows visualization of cell detection and image analysis, which cannot be done by conventional flow cytometry.
    - Also, the counting algorithm enables declustering of clumpy cells, which improves accuracy and consistency of population analysis.
    - Furthermore, the lack of high power lasers or photo-multiplying tubes in the Cellometer systems eliminates the need for precise optical alignment, where the simple epifluorescence setup does not require daily user maintenance.
- Further improvement in instrument sensitivity, counting volume, and higher throughput will make it more versatile in the future.

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