Cellometer Image cytometry as a complementary analysis tool to flow cytometry for visual verification of gated cell populations

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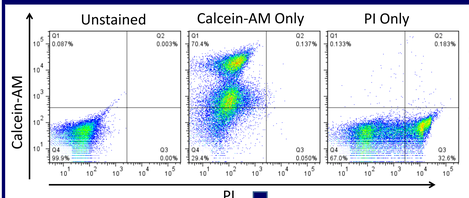
1. ABSTRACT

Traditionally, many cell-based assays that analyze cell populations and functionalities have been performed using flow cytometry. However, flow cytometers remain relatively expensive, and require highly trained operators for routine maintenance and data analysis. Flow cytometer can process and generate a large number of events, but the data gating strategies are often complex and are performed without the visual confirmation of the cells processed, which may lead to an incorrect gating strategy. Recently, a novel image cytometry system (Cellometer) has been developed by Nexcelom Bioscience LLC (Lawrence, MA) for automated cell concentration and viability measurement using bright-field and fluorescent imaging methods. The image cytometer is capable of capturing bright-field and fluorescent images and generates fluorescence intensity data of each analyzed cell. The system can perform gating operations such as fluorescence intensity or cell size similar to flow cytometry on the analyzed cell population. The ability to visually observe the gated cell population allows the elimination of data uncertainties generated using flow cytometry. Here we report, using an enzymatic vitality and viability stain, Calcein AM and propidium iodide, that image cytometry allows for a visual confirmation that the population of cells gated using flow analysis is indeed the population of interest. The image cytometry method offers not only a direct method for performing fluorescence cell-based assays, but also may be utilized as a complementary tool to flow cytometers for aiding the analysis of more complex samples.

2. EXPERIMENTAL OUTLINE

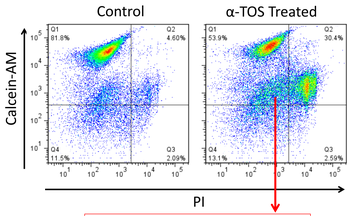
- Viability of control and α-TOS (an apoptosis inducing drug) treated Jurkat cells was assessed by staining the cells with Calcein AM and propidium iodide (PI).
- Treated cells were induced with 10 µM of α-TOS for 24 hours and 1 x PBS was added to the control sample.
- Both treated and control samples were analyzed using flow cytometer (BD LSR II SORP) and image cytometer (Cellometer).
- Direct data comparison was performed between the two systems.

3. FLOW CYTOMETRY DATA ANLYSIS – METHOD 1



Step 1: Fluorescent-based Gating

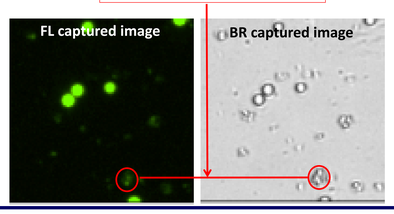
- Initial gating was performed by using FMO (fluorescence minus one) controls to set the low end gates of the positive populations of interest.
- To correct for spectral overlap during multicolor flow cytometry, compensation was also performed



Step 2: Data Acquisition and Interpretation

- Data for control and α-TOS treated samples were acquired based on the gating strategy performed in step one.
- Due to the apoptosis inducing drug α-TOS, the treated samples contain more dead (PI+) cells and cellular debris compared to the control.

What is this population?



BR and FL images captured by Cellometer

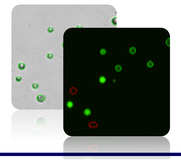
- Image cytometry can be used to examine gated populations
- Based on captured images, the current gating scheme includes dead cells and debris in the viable cell quadrant (Q1).

4. CELLOMETER IMAGE CYTOMETRY PROCEDURE

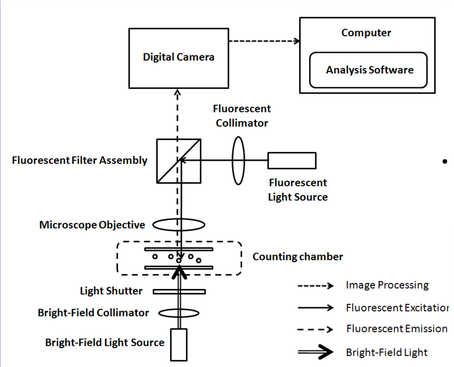
Pipette 20 µL of sample into disposable counting chamber

Insert chamber in Cellometer

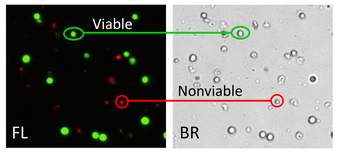
Bright-field (BR) and Fluorescent (FL) images



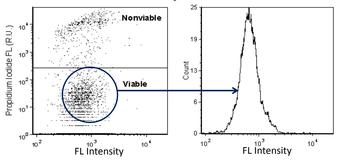
5. CELLOMETER IMAGE CYTOMETRY INSTRUMENTATION



- Cellometer image cytometer utilizes an epi-fluorescence setup for fluorescent image analysis

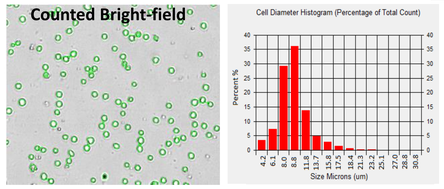


- Software first acquires FL and BR images



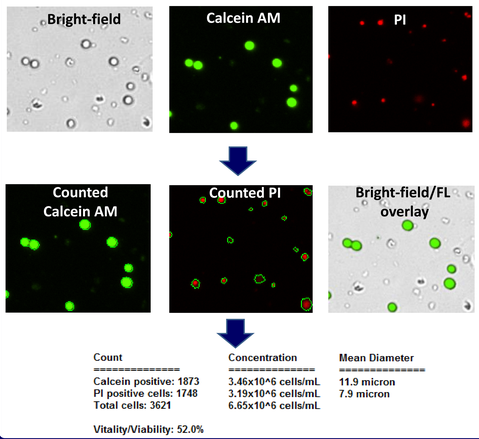
- Images are analyzed to generate fluorescent intensity data
- Data is plotted similar to data produced by flow cytometer

6. CELLOMETER IMAGE CYTOMETRY GATING STRATEGY



Step 1: Establish Size-Gating Parameters

- Load a control sample
- Preview and count cells in bright-field
- Confirm that the counted cells (outlined in green) are in fact the cells of interest
- Use cell diameter histogram to determine the upper and lower boundary for cell size gating



Step 2: Acquire Image Data

- Load α-TOS-treated sample
- Focus on cells in bright-field
- Preview cells in FL1 channel to examine Calcein AM positive cells
- Preview cells in FL2 channel to visualize PI positive cells
- Acquire images

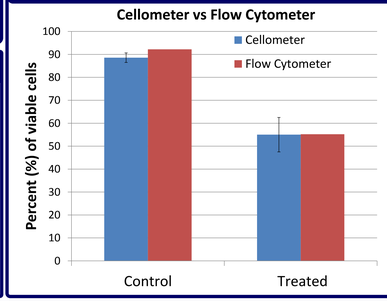
Step 3: Review Captured Images

- Once images are acquired they are automatically counted based on the determined size parameters
- Examine both the Calcein AM and PI counted populations to confirm proper gating.

Step 4: Examine Results

- Automatic data readout is produced after cell counting is completed

7. IMAGE VS FLOW CYTOMETRY COMPARISON



Examining the viability of control and drug-treated Jurkat cells using image and flow cytometry

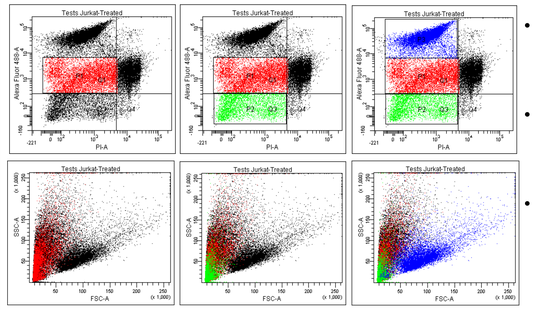
- Advanced flow cytometry gating was performed to eliminate dead cells and debris from live cell analysis
- Size gating was used in Cellometer analysis to eliminate the contaminating dead cells and debris from live cell analysis.

8. FLOW CYTOMETRY DATA ANLYSIS – ADVANCED METHOD

Step 1: Fluorescence-base Gating

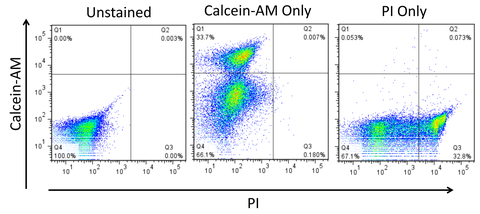
- Use FMO to set the low end gates of the positive populations of interest.
- To correct for spectral overlap during multicolor flow cytometry compensation was also performed.

Step 2: Forward and Side Scatter Analysis



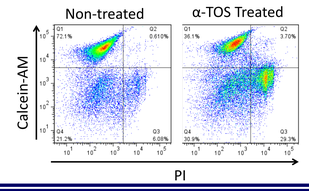
- Further data analysis was performed in order to determine the three distinct Calcein AM positive populations: weak, medium, and strong fluorescing
- Each fluorescing population was gated in the FL intensity dot plot and displayed in forward and side scatter plot (weak=green P2, medium=red P1, and strong=blue P3)
- Both medium and weakly fluorescing particles are shown to be either dead cells or cellular debris in the forward/side scatter plot

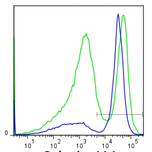
Step 3: Manual Re-gating



- To exclude the debris and dead cells from viable cell analysis manual re-gating was performed
- Based on the advanced gating strategy only Calcein AM Hi cells were considered viable

Step 4: Data acquisition and Interpretation



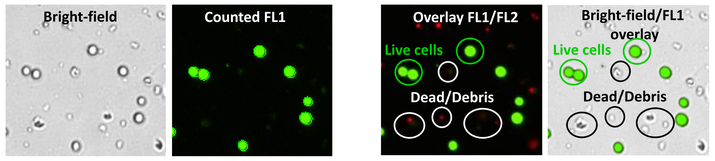


Sample	Calcein AM Hi %	Calcein AM Hi MFI
Non-Treated	72.7%	31,900
Treated	39.7%	43,300

Non Treated Jurkat
Treated Jurkat

9. CONCLUSION

- Cellometer image cytometry offers researchers the ability to review and identify populations of interest in bright-field while simultaneously performing fluorescence-based cellular analysis.
- Measures cell viability in complex samples without the need to perform additional data analysis.
- Produces results that closely correlate to flow cytometer.



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- Lo Surdo J, Bauer SR (2012) Quantitative Approaches to Detect Donor and Passage Differences in Adipogenic Potential and Clonogenicity in Human Bone-Marrow-Derived Mesenchymal Stem Cells. *Tissue Engineering Part C, Methods* 18:11.