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

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 cytometry can process and generate 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 incorrect gating strategy. Recently, a novel image cytometry system (Cellometer) has been developed by Nexcelom Biosciences LLC (Lawrence, MA) for automated cell concentration and viability measurement using bright-field and fluorescent imaging methods. The image cytometry 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 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 cytometry 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 µPBS 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 ANALYSIS – METHOD 1

Step 1: Fluorescence-based Gating
- Initial gating was performed by using FMO (fluorescence minus one) controls to set the low and high 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.

Step 3: Review Captured Images
- 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 (BF) and Fluorescent (FL) images captured by Cellometer.

5. CELLOMETER IMAGE CYTOMETRY INSTRUMENTATION

- Software first acquires FL and BF 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 4: Data interpretation
- Automatic data readout is produced after cell counting is completed.

7. IMAGE VS FLOW CYTOMETRY COMPARISON

Examing 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 to perform the analysis.
- Size gating was used in Cellometer analysis to eliminate the contaminating dead cells and debris from live cell analysis.

8. FLOW CYTOMETRY DATA ANALYSIS – ADVANCED METHOD

Step 1: Fluorescence-base Gating
- Use FMO to set the low end gates of the positive populations of interest.

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 dead cells 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
- Dead or non-viable cells are visualized PI positive cells.
- Acquire images.

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.

References:

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