

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

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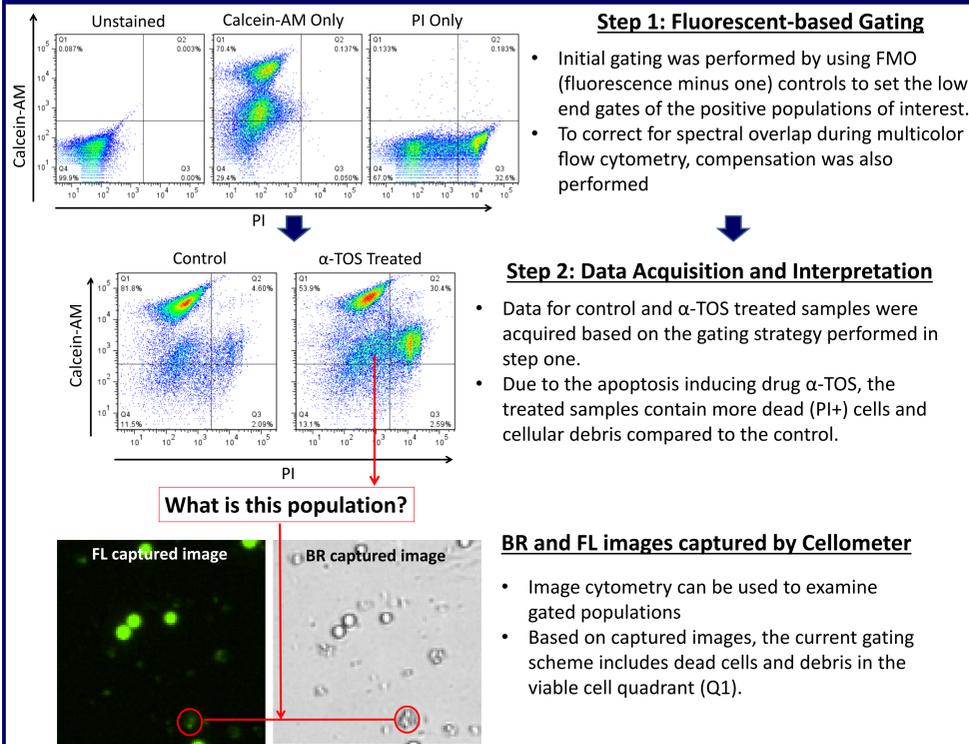
## 1. ABSTRACT

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.

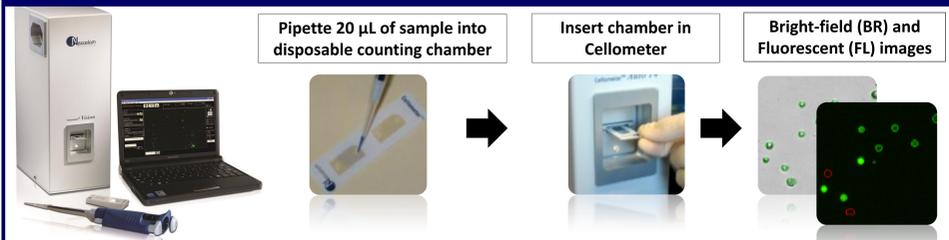
## 2. EXPERIMENTAL OUTLINE

- Viability of control and  $\alpha$ -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  $\mu$ M of  $\alpha$ -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.

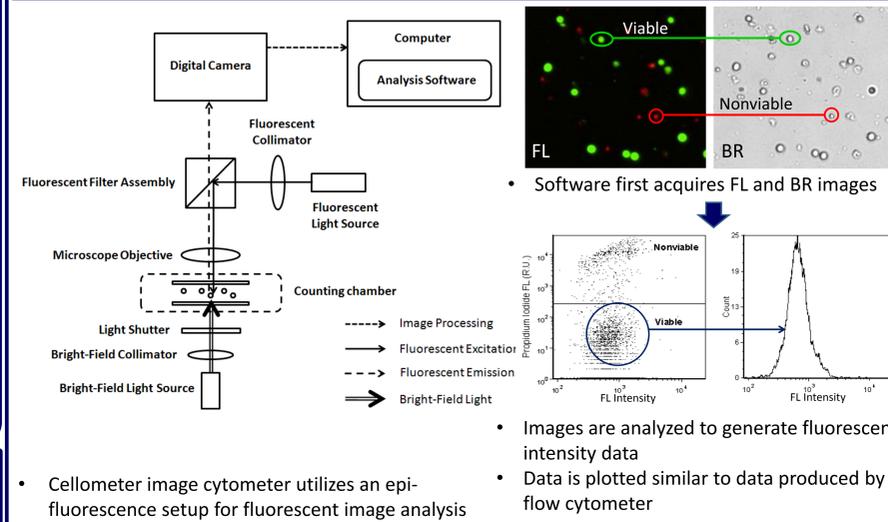
## 3. FLOW CYTOMETRY DATA ANALYSIS – METHOD 1



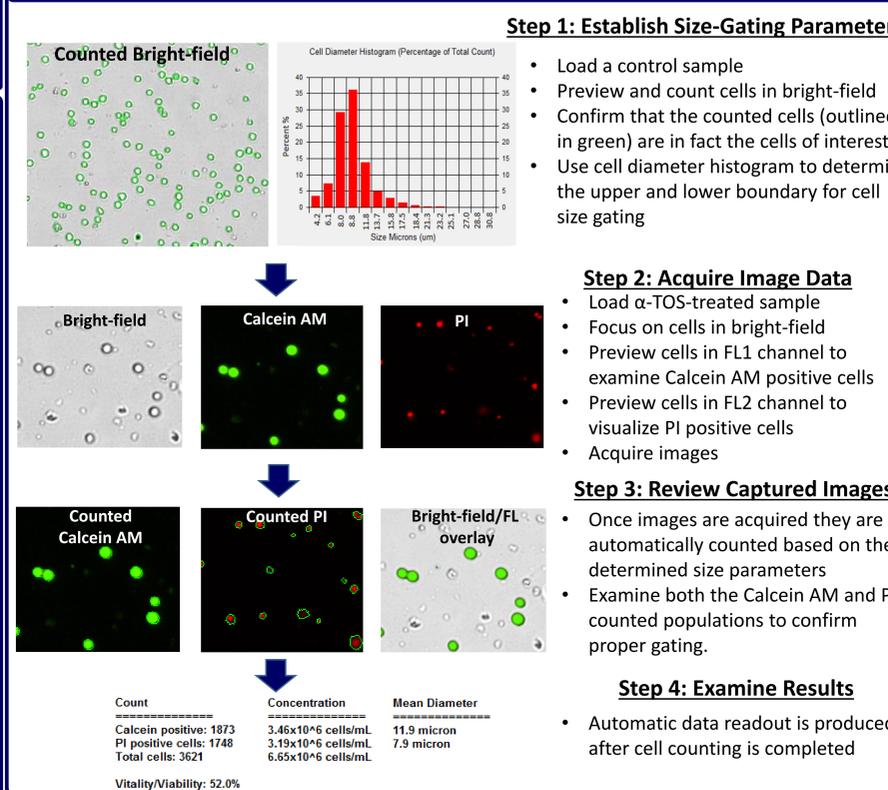
## 4. CELLOMETER IMAGE CYTOMETRY PROCEDURE



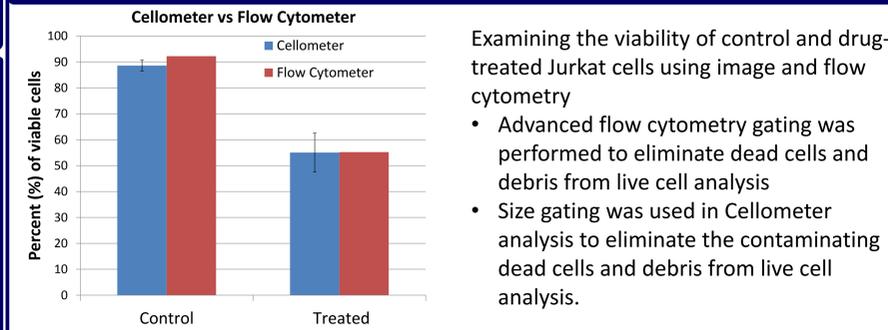
## 5. CELLOMETER IMAGE CYTOMETRY INSTRUMENTATION



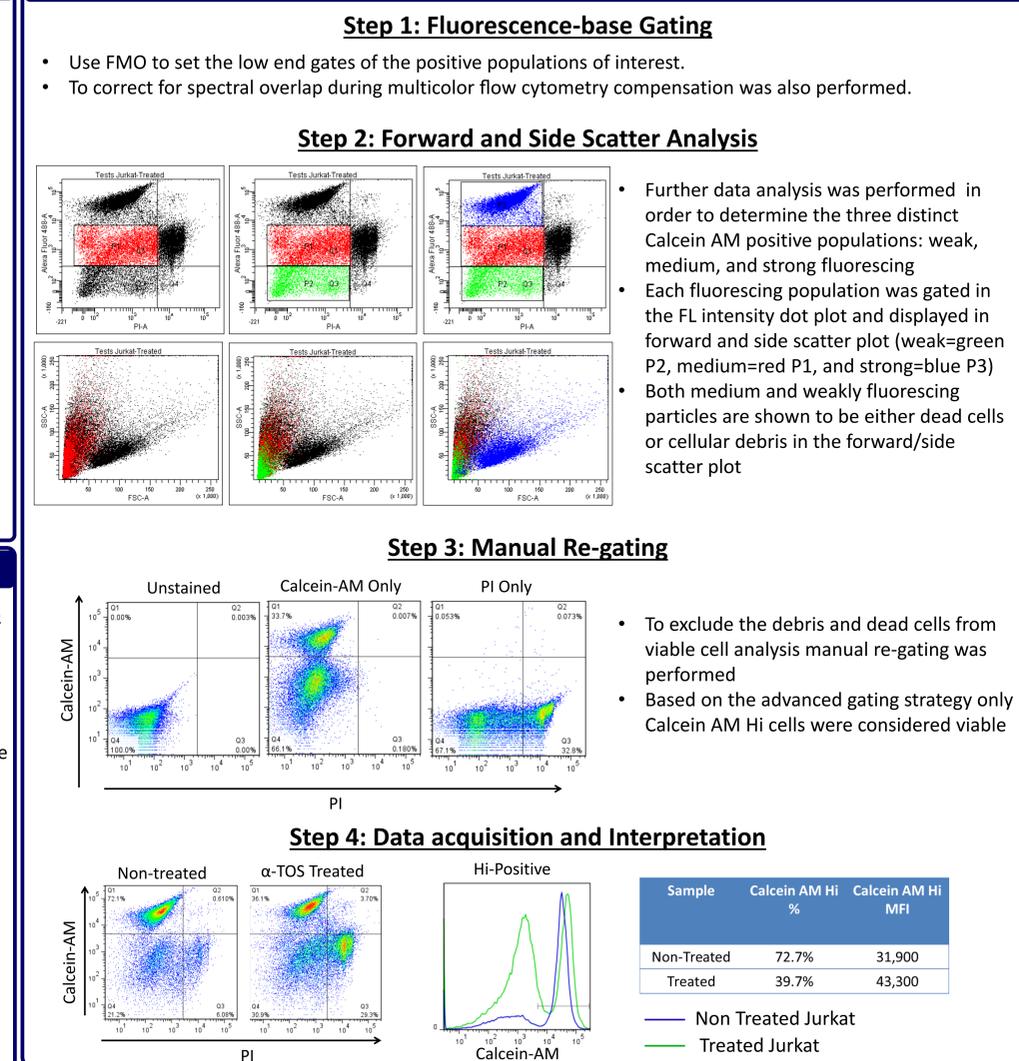
## 6. CELLOMETER IMAGE CYTOMETRY GATING STRATEGY



## 7. IMAGE VS FLOW CYTOMETRY COMPARISON

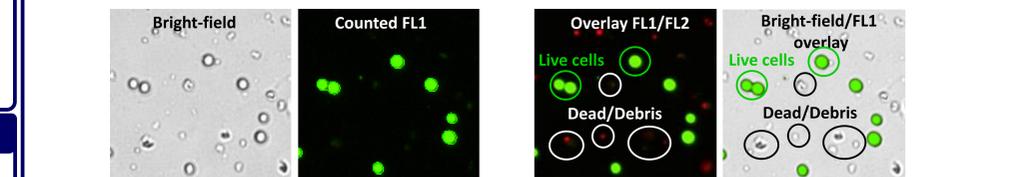


## 8. FLOW CYTOMETRY DATA ANALYSIS – ADVANCED METHOD



## 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:

- Chan LL, Lai N, Wang E, et al. (2011) A rapid detection method for apoptosis and necrosis measurement using the Cellometer imaging cytometry. *Apoptosis*, 16:12.
- Chan LL, Zhong X, Qiu J, et al. (2011) Cellometer Vision as an Alternative to Flow Cytometry for Cell Cycle Analysis, Mitochondrial Potential, and Immunophenotyping. *Cytometry Part A* 79:5.
- Lugli E, Gattinoni L, Roberto A, et al. (2012) Identification, isolation and in vitro expansion of human and nonhuman primate T stem cell memory cells. *Nature Protocols* 8:33.
- 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.