A Novel Imaging Cytometry Method for Quantitative Cell Viability Assay

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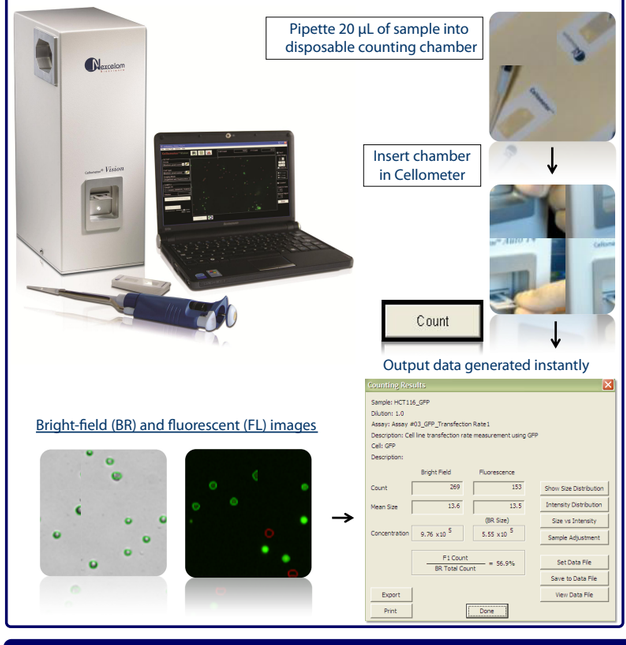
1. ABSTRACT

We demonstrate a rapid and cost-effective method for viability analyses examining various characteristics of cell death using the Cellometer® Vision. This method eliminates many known issues caused by manual hemacytometer and flow cytometer. By using cellometer Vision, the assay time for obtaining viability result is greatly reduced, which is significant for research development in academia and industry.

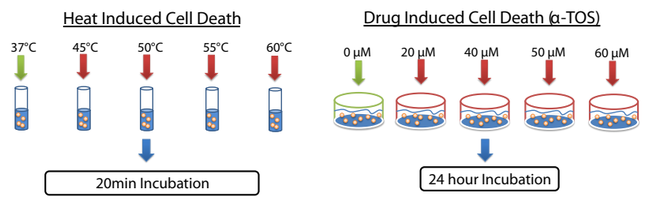
2. INTRODUCTION

Current methods for viability analysis utilizes hemacytometer and standard flow cytometer. Several issues are associated with these methods. Manually counting live and dead cells using hemacytometer under microscopy is tedious and time consuming. It is subject to human error and highly operator dependent. It is often done with trypan blue assay, which on its own has several issues attached. Standard flow cytometer is expensive, large in size and require considerable amount of maintenance. In addition, most of the flow cytometers do not have imaging capabilities, which often generate uncertainties in the fluorescence results obtained. Recently, a novel imaging cytometry platform has been developed by Nexcelom Bioscience (Lawrence, MA). This method utilizes both bright-field and fluorescence imaging of a disposable cell counting chamber to quickly provide concentration and viability measurements of cell populations. This system allows automated cell image acquisition and processing with a novel counting algorithm for accurate and consistent measurement of cell population and viability for a variety of cell types (immunological, cancer, stem, insect, adipocytes, hepatocytes, platelets, algae, and heterogenous cells). This system has been applied to analyze different characteristics of cell death, including mitochondria function assay, apoptosis assay, membrane integrity assay and metabolic assay. In this poster, cell viability after treatment was assessed by apoptosis assay using Annexin-V, membrane integrity assay using trypan blue and propidium iodide, and metabolic assay using CFDA.

3. CELLOMETER® VISION IMAGING CYTOMETRY



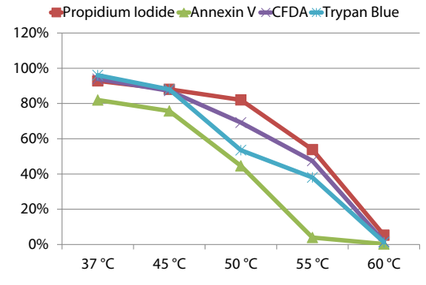
4. JURKAT CELLS PREPARATION & STAINING PROTOCOL



Experimental Protocol

- Jurkat cells are cultured under 37°C and 5% CO_2
- 5 separate tubes are incubated with agitation in water baths that have been heated to 37, 45, 50, 55 and 60 °C for 20 min.
- 5 separate flasks were treated with cytotoxic apoptosis inducing reagents (a-TOS) at different concentration (0, 20, 40, 50, 60 µm) for 24 h.
- Trypan Blue Staining: cell sample is mixed at 1:1 (v/v) ratio with 0.2% (v/v) trypan blue for 5-10 sec.
- Propidium Iodide (PI) Staining: cell sample is mixed at 1:1 (v/v) ratio with 200 µg/ml PI for 5-10 sec.
- Annexin V – FITC staining: Annexin-V Binding Buffer and Annexin V-FITC are from Biolegend (San Diego, CA). After centrifugation, Jurkat cells are resuspended in Annexin V Binding buffer at approximately 2x10^6 cells/ml. 100ul of cells are mixed with 5ul of Annexin V - FITC and incubated at room temperature for 15-30 min at dark.
- CFDA staining: cell sample is mixed at 1:1 (v/v) ratio with 10µM CFDA and incubated at room temperature for 15 min at dark .

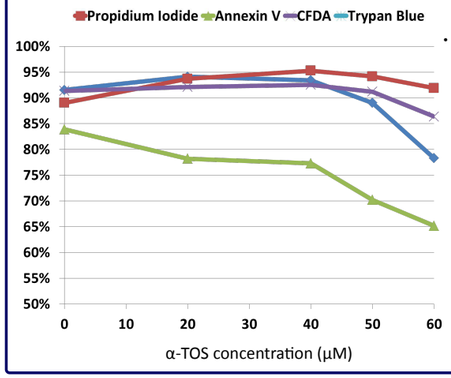
6. VIABILITY RRSULTS OF HEAT TREATED CELLS



Results

- Comparable viabilities were obtained from different assay types for 37 and 60°C samples.
- Large differences in cell viabilities were obtained from different assay types for 45, 50 and 55°C samples.
- Different assays test for different characteristics of cells death. Therefore, for cells in intermediate stages of cell death, viability measurements may vary a lot depending on the assay type.

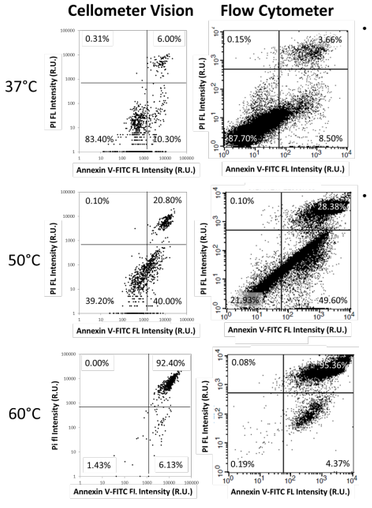
7. VIABILITY RESULTS OF α-TOS TREATED CELLS



Results

- For apoptosis induced cells, results indicate apoptosis assay - Annexin V to detect cell death earlier, and measure viabilities significantly lower than other assays.

8. COMPARISON WITH FLOW CYTOMETER RESULTS



Results

- Annexin V and Propidium Iodide were analyzed simultaneously with Cellometer Vision and a BD FACSCalibur flow cytometer. Jurkat cells were incubated with continual agitation in water baths that were heated to 37, 50 and 60°C for 20min.
- Cellometer Vision and flow cytometer give comparable results for Annexin V – PI dual staining analysis.

9. CONCLUSION & ACKNOWLEGEMENT

Obtaining accurate viability measurements is essentail for many cell based research. Different viability assays examine different characteristics of cell death. We have demonstrated here Cellometer Vision is a rapid and cost-effective method for a variety of viability assays, including mitochondria function assays, apoptosis indicating assays, membrane integrity assays, metabolic activity assays, etc. Here we tested different assays' behaviour under two different stresses. The results indicate that for cells in intermediate stages of cell death, viability measurements may vary a lot depending on the assay type

ACKNOELEDGEMENT

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