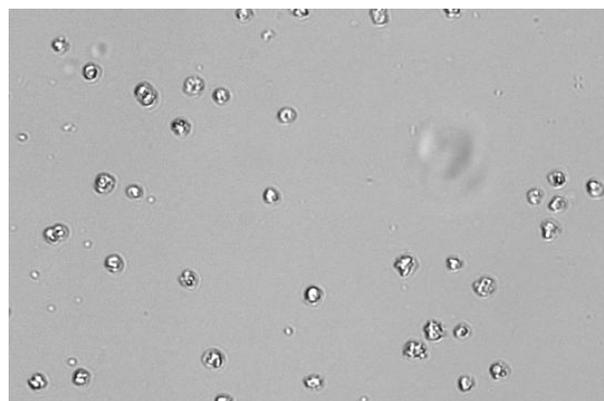
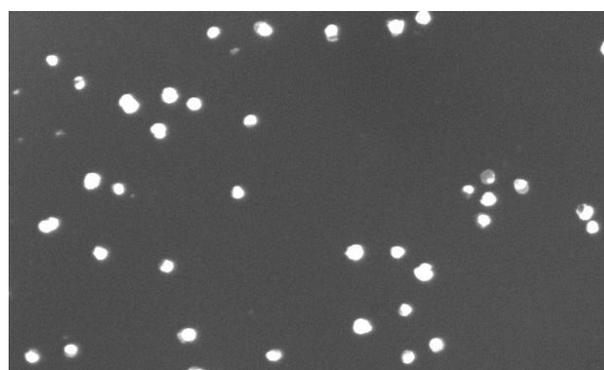


APPLICATION FOCUS: CELLOMETER VISION

Rapid Detection of Apoptosis in Jurkat Cells with FITC Conjugated Annexin-V



Brightfield image of Jurkat cells.



Fluorescent image of Jurkat cells. White spots indicate FITC labeled annexin-V.

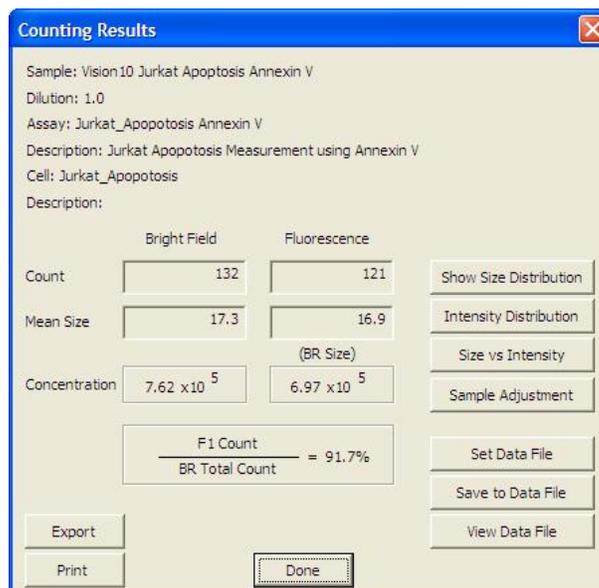
INTRODUCTION

Cellometer Vision incorporates image based cell counting and fluorescence detection in a compact and easy-to-use instrument. With fluorescence detection capabilities, Cellometer Vision is an ideal solution for many complex cell population characterization assays such as rapidly detecting apoptotic cells with FITC conjugated annexin-V.

Apoptosis is programmed cell death that plays a critical role in organism homeostasis and tissue development. Abnormal apoptosis processes have been associated with diseases including various tumors. There are a number of in-vitro and in-vivo assays for detecting apoptosis cells. Fluorescein FITC conjugated annexin-V binding assay is one of the simplest methods to detect the early stage of apoptosis. Traditionally, flow cytometry is used to quantitatively analyze apoptosis, however, due to complexity, cost and availability limitations, they are often not the best solution for rapid and routine cell characterizations.

Cellometer Vision was developed to count total cells and identify FITC-annexin-V positive apoptotic cells based on the microscopic and fluorescence characteristics of the cell sample. Detection typically takes less than 60 seconds and only requires 20µl of cell sample.

Cell images and all analysis data, including cell size distribution histograms, can be saved for documentation. Data can also be easily exported to Microsoft Excel spreadsheets for further analysis.



Counting results box displays total and fluorescence cell count, percentage of apoptotic cells, mean size & concentration.

METHOD

Labeling of cell surface with FITC conjugated annexin-V

1. Harvest Jurkat cells both induced with anti-CD95L antibody (EOS9, Biolegend, San Diego, CA) and uninduced.
2. Spin down at 1800 rpm for 5 minutes.
3. Wash the cells one time with apoptosis wash buffer (AWB).
4. Discard the supernatant.
5. Resuspend the cells in AWB buffer at concentration of $\sim 5 \times 10^6$ /ml.
6. Label reaction tubes & add 0, 50, 25, and 12.5 μ L of FITC conjugate annexin-V (40 μ g/ml) into each tube and apply 50, 0, 25, and 37.5 μ L of AWB buffer into each tube accordingly.
7. Add 100 μ L of Jurkat cells into each tube and mix well by briefly vortexing.
8. Incubate the tubes in the dark at room temp for 15 min.
9. Wash the cells twice with AWB.
10. Resuspend cells with 0.25ml of AWB and mix gently.

Running Assay:

1. Load 20 μ L of labeled sample into the disposable counting chamber.
2. Insert counting chamber into Cellometer Vision
3. Select assay from drop-down menu and enter sample ID
4. Preview cell images and click 'Count' to begin analyzing sample
5. Review images and counting results.
6. Save or export images and/or report data.

RESULTS

Total counted Jurkat cells are indicated by green circles in the brightfield image (Figure 1). Apoptotic cells are indicated as fluorescent positive, green circled cells in the fluorescence image (Figure 2). Total and fluorescence positive cells counted, concentration, mean size, and percentage are displayed in the results box (Figure 3).

Cell & fluorescent size distribution histograms, scatter plots (figure 5) & data files can be instantly generated and exported to an Excel spreadsheet or saved in a data table.

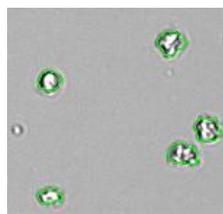


Figure 1. Total counted cells are indicated by green circles

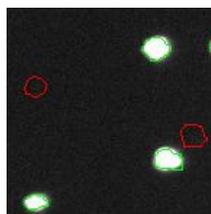
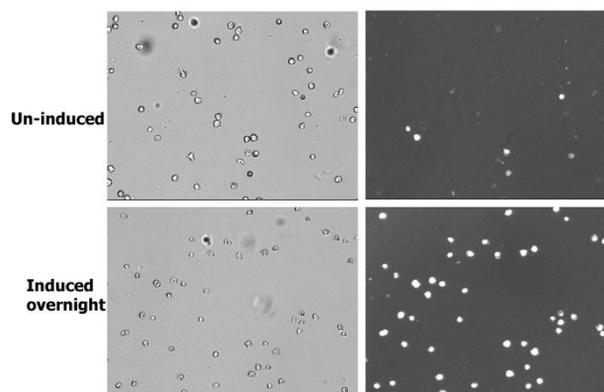


Figure 2. Apoptotic cells are indicated by green circles.

	Bright Field	Fluorescence
Count	132	121
Mean Size	17.3	16.9
		(BR Size)
Concentration	7.62×10^5	6.97×10^5
$\frac{\text{F1 Count}}{\text{BR Total Count}} = 91.7\%$		

Figure 3. Total Concentration (Brightfield), Apoptotic cell concentration (Fluorescence) and % positive are displayed immediately after image analysis



Sample	Cell Concentration	Fluorescence Positive %
Un-induced	3.6×10^6 /ml	16%
Induced overnight	3.0×10^6 /ml	92%

Figure 4. Changes in fluorescent positive cells can be easily monitored over different time points.

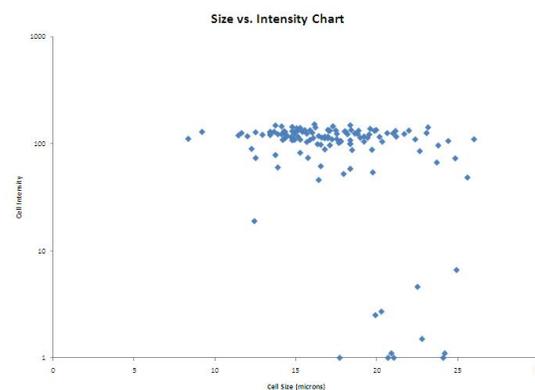


Figure 5. Scatter plot of cell size versus fluorescence intensity

CELLOMETER Vision Trio SPECIFICATIONS:

Imaging Modes: Brightfield & 2 Fluorescence Channels

Filter Set 101: Excitation/Emission Peak: 475nm/535nm

Filter Set 202: Excitation/Emission Peak: 525nm /595nm

Dimensions: 6" x 8.5" x 14" (15cm x 22cm x 36cm)

Weight: 25lbs (11kg)

PC Specs: WinXP/1.8+GHz/1GB RAM/laptop included