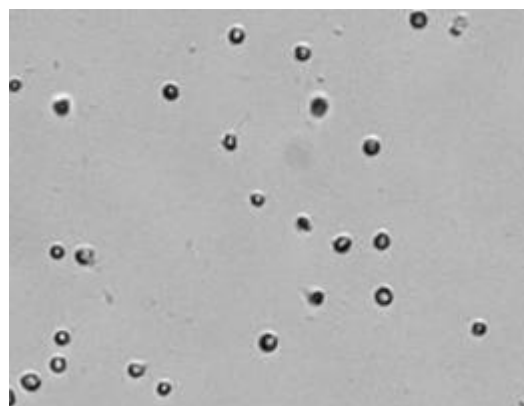# Simple, Fast Determination of Viability by Staining Cells with Propidium Iodide

Brightfield Image of Jurkat cells.

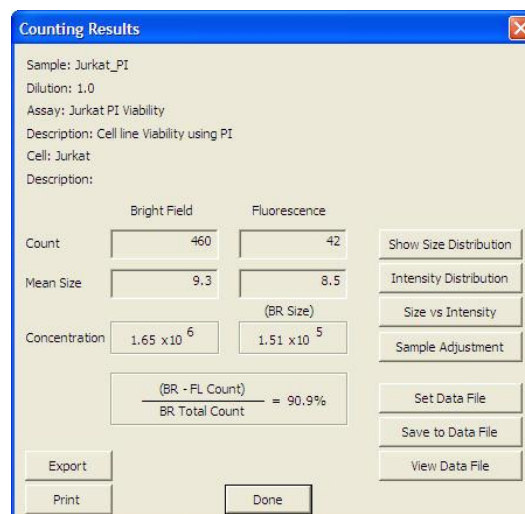Fluorescent image indicating PI stained dead Jurkat cells.

INTRODUCTION

Cellometer Vision incorporates image based cell counting and fluorescence detection in a compact and easy-to-use instrument. Cellometer Vision is ideal for many cell population characterization assays such as rapidly determining viability by staining cells with propidium iodide.

Cell viability is routinely tested in cell culture laboratories and used in many assays such as cell proliferation, cytotoxicity, and tumor killing activity. In the traditional viability method of trypan blue exclusion, live cells exclude the dye as the membrane is impermeable, but dead cells uptake the dye due to the lack of an intact cell membrane. Viability is then determined by manually counting the stained sample in a hemacytometer, a time consuming and labor intensive process. More importantly, trypan blue stained samples have to be analyzed within 3-5 minutes, as longer incubation will increase blue colored cells. Trypan blue staining may result in an overestimate of viable cells especially for low viability samples.

Reliable and accurate viability testing can also be done using propidium iodide (PI), a fluorescent dye. Cellometer Vision was developed to automatically count total cells and propidium iodide fluorescence labeled dead/dying cells for fast, simple PI viability determination.

Using proprietary algorithms, Cellometer Vision's robust operating software accurately analyzes cell images, and generates this data in less than 60 seconds typically. Cell images and all analysis data, including cell size distribution histograms, can be saved or exported to Excel for further analysis.

Counting Results

Sample: Jurkat_PI
Dilution: 1.0
Assay: Jurkat PI Viability
Description: Cell line Viability using PI
Cell: Jurkat
Description:

	Bright Field	Fluorescence	
Count	460	42	Show Size Distribution
Mean Size	9.3	8.5	Intensity Distribution
		(BR Size)	Size vs Intensity
Concentration	1.65×10^{6}	1.51×10^{5}	Sample Adjustment

$$\frac{(BR - FL\ Count)}{BR\ Total\ Count} = 90.9\%$$

Set Data File
Save to Data File
View Data File
Export
Print
Done

Counting results box displays brightfield and fluorescence cell count, mean size, concentration & viability percentage.

METHOD

Direct PI labeling
1. Harvest cell from a 75 cm^2 flask and wash cell pellet with appreciated culture media.
2. Losen cell pellet then resuspend cell sample with 10 ml of culture media.
3. Take 20μl of cell sample and mix cell with 20μl of PI solution.
4. Mix well by gently vortexing sample.
5. Load 20μl of sample into the disposable counting chamber.

Running Assay:

1. Load 20μl of labeled sample into the disposable counting chamber.
2. Insert chamber into Cellometer Vision.
3. Select assay from drop-down menu.
4. Enter Sample ID for this sample.
5. Preview cell images and click 'Count' to begin analyzing sample.
6. Review Images and counting results.
7. Save or Export images and/or report data.

Figure 1. Total counted cells are indicated by green circles

Figure 2. Fluorescent image showing live Jurkat (red circles) and dead (green cricles).

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

RESULTS

Total counted Jurkat cells are indicated by green circles in the brightfield image (Figure 1). Dead cells stained with PI 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 viability are displayed in the results box (Figure 3).

Cell and fluorescent size distribution histograms, scatter plots and data files can be instantly generated. All experimental data can be instantly transferred to an Excel spreadsheet or saved in a data table.
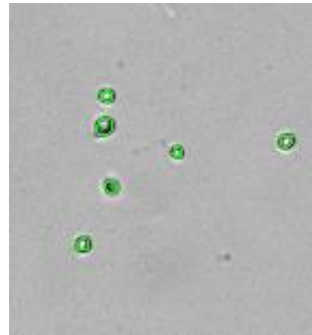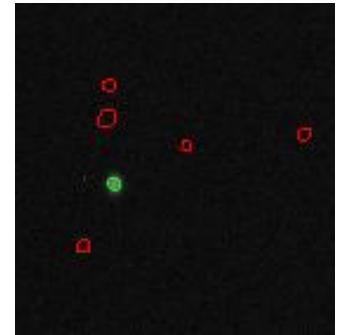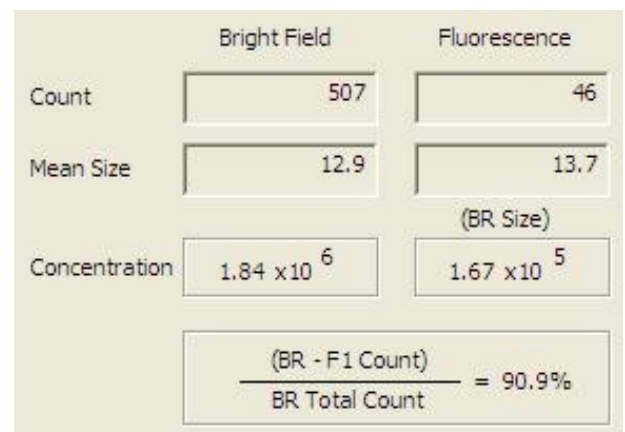
Sample	Cell Concentration	Viability %
Jurkat	1.7 x 10^6/ml	91%
Mouse thymocyte	1.6 x 10^6/ml	81%
Mouse splenocyte	1.1 x 10^6/ml	85%
Mouse bone marrow	0.96 x 10^6/ml	81%

Figure 4. PI Viability results for various cell types.

CELLOMETER Vision Trio SPECIFICATIONS:

Imaging Modes: Brightfield and 2 Fluorescence Channels
  Filter Set 101: Excitation/Emission Peak: 475nm/535nm
  Flter 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

To learn more or request a demo, call: (978)327-5340   **email:** info@nexcelom.com   **or visit:** www.nexcelom.com

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