Application Note
Hepatocyte

Development of a Novel Method to Assess Primary Hepatocyte Concentration and Viability

Cellometer®
Cell Viability

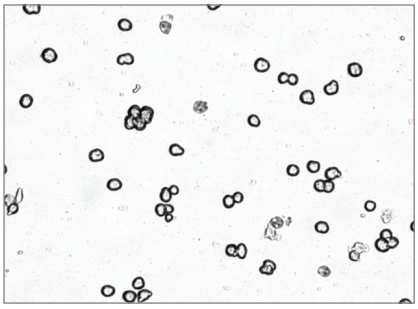
Introduction

The Cellometer® Vision Cell Analyzer incorporates image based cell counting and fluorescence detection in a compact and easy-to-use instrument. With dual fluorescence detection capabilities, Cellometer Vision is an ideal solution for many complex cell population characterization assays, such as reliable counting and viability determination of primary hepatocytes.

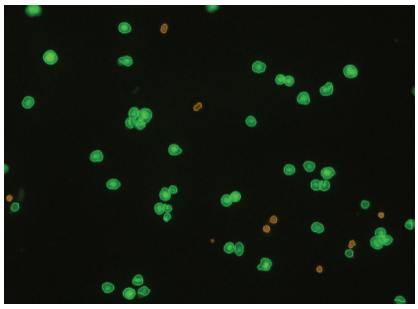
Reliable concentration and viability determination of primary hepatocytes is critical for accurate analysis of compound toxicity in vitro. Due to hepatocytes' variable morphology, fragile nature and tendency to clump, traditional manual counting methods are time-consuming and the subjectivity from operator-to-operator can cause inconsistent results.

Nexcelom's new method incorporates a ready-to-use fluorescent dual staining solution that stains live cells with acridine orange, and dead cells with propidium iodide. Researchers then load 20μL of labeled sample into a disposable counting chamber for analysis. Because the counting chamber is disposable, no washing is required between samples and the risk of cross contamination is eliminated.

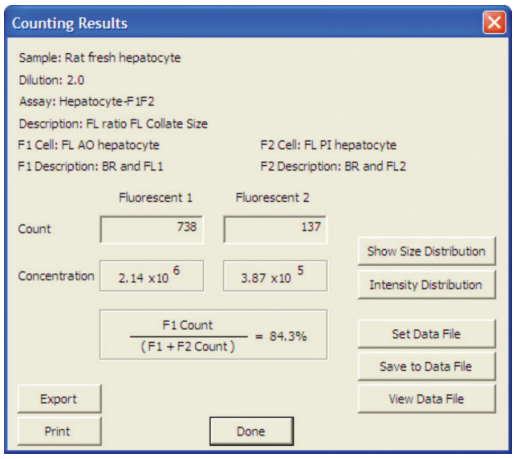
Fluorescent images of the stained cells are captured and analyzed using proprietary algorithms. Cellometer Vision's robust operating software accurately analyzes cell images to generate live cell count, concentration and viability percentage. Total analysis time is typically less than 60 seconds. Cell images and all analysis data, including cell size distribution histograms, can be instantly saved for documentation. Data can also be easily exported to Microsoft Excel spreadsheets for further analysis.



Brightfield image of primary human hepotocytes. Live and dead hepatocytes show different morphology, but are difficult to clearly distinguish resulting in counting variability.



After treating samples with Fluorescent Dual Staining Solution, AO stained live hepatocytes and PI stained dead/dying hepatocytes are easily distinguished.



Cellometer Vision automatically analyzes fluorescent images of dual stained hepatocytes and instantly calculates live cell concentration and viability.

Nexcelom Bioscience

Method

Treat cell sample with Nexcelom's Fluorescence Dual Staining Solution:

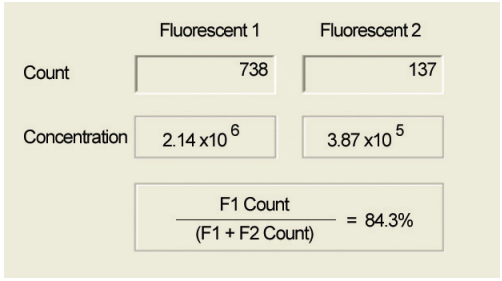
1. Take 20µl of freshly isolated hepatocyte sample or freeze-thaw cryopreserved cell sample in a small microtube.
2. Apply 20µl of ready-to-use dual staining solution (acridine orange/propidium iodide cocktail).
3. Gently mix. Sample is ready to count.

Running Assay:

1. Load 20µl of labeled sample into the Cellometer disposable counting chamber.
2. Insert 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

AO stained live hepatocytes are clearly visible in the fluorescent image obtained from Filter Set 101 (Figure 1). The software indicates counted cells with a green circle (enlarged to show detail) while ignoring cellular debris. The software can also recognize and discretely count clumpy cells. PI stained dead cells are visible in the image obtained from Filter Set 202 (Figure 2). The software then accurately calculates total cell count, concentration and viability (below). By using this method, live and dead cells are clearly distinguished and automatically counted for improved accuracy. By combining a ready to use staining solution and imaging based system results can be obtained much easier and faster compared to other methods.



Counting results are displayed on-screen instantly. Fluorescent 1 indicates AO stained live cells, Fluorescent 2 indicates PI stained dead cells. Viability percentage is calculated automatically. Results can be saved to a data table (below)

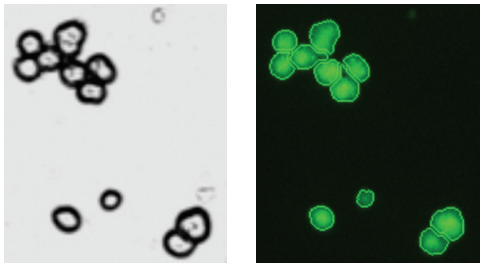


Figure 1 The fluorescent image from Filter Set 101(R) shows counted AO stained live hepatocytes. Cellular debris seen in the brighttield image (L) are not counted in the fluorescence image.

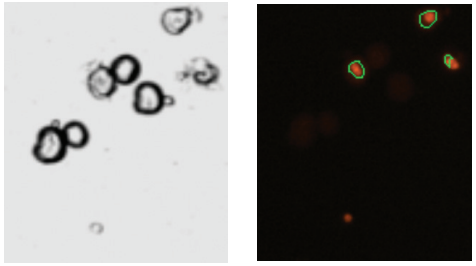


Figure 2 The fluorescent image (R) from Filter Set 202 shows counted PI stained dead hepatocytes (Circled with green). Free nuclei from lysed dead cells are not counted.

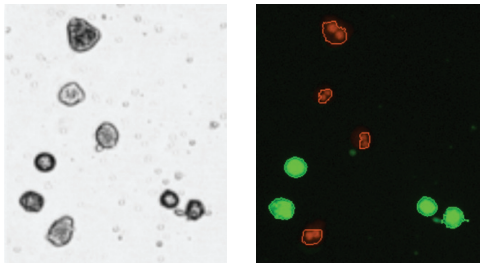


Figure 3 By overlaying AO/live and PI/dead hepatocyte images, counting results can be visually confirmed. Live hepatocytes are circled in green and dead hepatocytes are circled in orange (R).

Sample ID	Image	Cell counts	Dilution factor	Conc. (cell/ml)	Viability	Mean diameter (mm)
Rat fresh hepatocyte	F1 live F2 dead	738 137	2 2	2.1E+06 3.9E+05	84.3%	19.3
Rat fresh diluted	F1 live F2 dead	405 98	2 2	1.2E+06 2.8E+05	80.5%	21.0
Dog cryopreserved	F1 live F2 dead	352 162	2 2	1.0E+06 4.5E+05	68.5%	16.4
Mouse cryopreserved	F1 live F2 dead	163 295	2 2	4.7E+05 8.4E+05	35.6%	19.3

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