APPLICATION FOCUS

Automated Methods for Counting and Analyzing Stem Cell Samples

INTRODUCTION

The Cellometer line of automated cell counters incorporates image based cell counting and fluorescence detection in a compact and easy-to-use instrument that reliably automates cell counting, viability determination and other fluorescence assays. By simply pipetting 20µL of sample into a disposable chamber, Cellometer acquires cell images that are analyzed to determine cell counts, concentration, cell sizes, and fluorescence intensity, typically in 30 seconds or less.

This combination of features makes it ideal for use in stem cell research. Samples can be counted and viability determined with trypan blue in the Cellometer Auto T4 Plus. Cellometer Vision combines this brightfield functionality with fluorescence detection to also determine stem cell viability using fluorescence stains such as propidium iodide, and being able to accurately determine concentration of nucleated cells in human bone marrow or cord blood. The fluorescence imaging mode can also be used to quantify GFP transfection efficiency when transfecting stem cells.

Accurate counting of stem cells:

Cell images acquired in the brightfield mode are analyzed to determine total cell count. Concentration is automatically calculated, and all data is automatically saved or exported. This eliminates user to user variability and improves throughput over manual counting methods, especially when analyzing stem cells which can be clumpy or irregularly shaped.

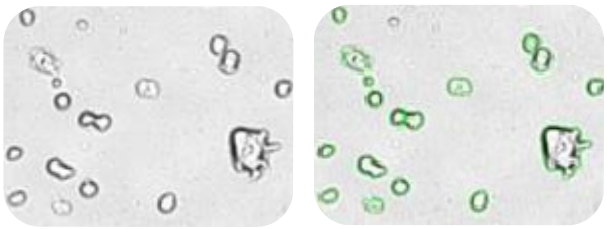

Figure 1: Images of embryonic stem (ES) cells acquired by Cellometer. Green circles indicate cells that have been counted by the software.

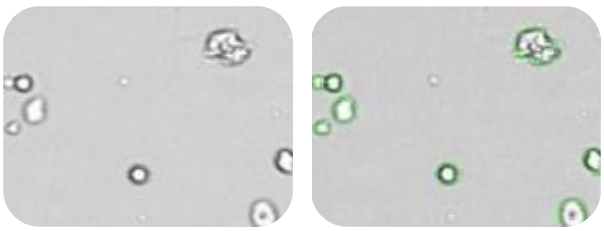

Figure 2: Cell clusters or irregular shaped cells can be accurately counted.

Automated determination of viability:

Stem cell viability can be determined by several methods. Dead cells stained with trypan blue can be analyzed in the brightfield mode (figure 3). Detecting viability via fluorescent dyes, such as propidium iodide (PI) can be performed by acquiring brightfield images for total cell concentration and fluorescent images to count PI stained dead cells

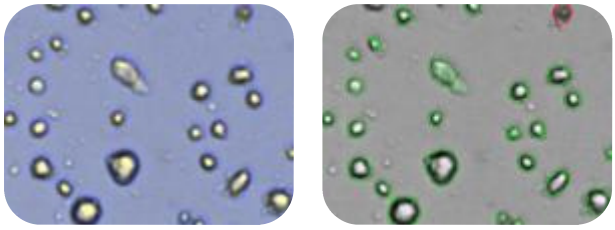

Figure 3: Analyzed brightfield images of HMSC cells show live cells circled in green and trypan blue stained dead cells circled in red.

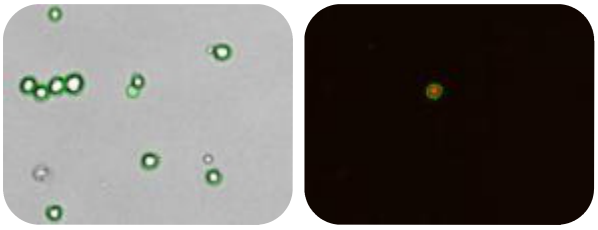

Figure 4. Brightfield image of Mouse Embryonic Feeder (MEF) cells in the brightfield image, PI stained dead cells are visible in the fluorescence imaging mode.

Accurate counting of bone marrow and cord blood:

Samples containing stem cells have large amounts of red blood cells (RBCs) or platelets present and can be difficult to count via manual methods. Because nucleated cells cannot be visually identified amongst these other cells, a fluorescent dye such as Acridine Orange (AO) can be used to positively identify nucleated cells.

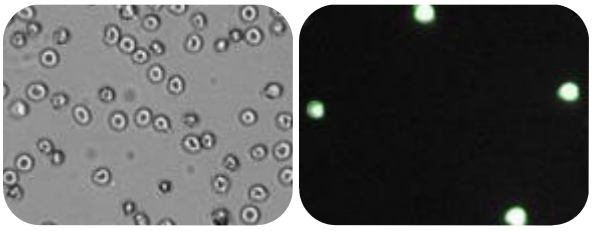

Figure 5: AO stained nucleated cells in human bone marrow are identified and counted in the fluorescence image (right).

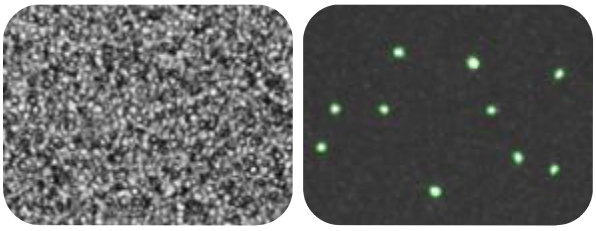

Figure 6: Cord blood samples treated with AO, positively identifies nucleated cells amongst RBCs without need to lyse or perform ficol separation

Quantifying GFP transfection efficiency:

The unique brightfield/fluorescence imaging capabilities can also be used to quantify transfection efficiency when using reporter proteins such as GFP, without having to use complex methods, such as flow cytometry. A total count is acquired in the brightfield mode, and GFP positive cells are counted in fluorescent mode to calculate the percentage of cells that are expressing GFP.

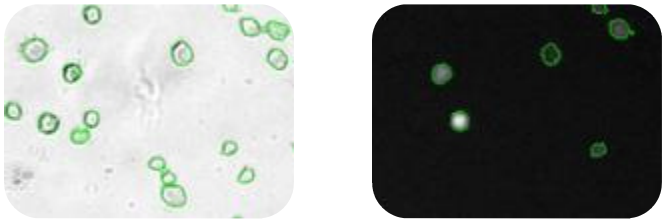

Figure 7: Mouse embryonic feeder (MEF) cells that have undergone transfection. Total cell count is determined in the brightfield image (left), GFP positive cells are indicated by green circles in the fluorescent image (right).

Example results of stem cells analyzed on Cellometer:

Cell Type	Cell Concentration	Treatment
CD 34 +	4.81 x 10^6/ml	PI viability: 74.3%
Mouse embryonic feeder cell (MEF)	5.18 x 10^5/ml	AO assisted
Mouse embryonic feeder cell (MEF)	2.48 x 10^6/ml	Trypan blue viability: 92.1%
Mouse embryonic feeder cell (MEF)	1.06 x 10^6/ml	PI viability: 91.0%
Transfected Mouse embryonic feeder cell (MEF)	Total: 3.52 x 10^6/ml GFP: 8.24 x 10^5/ml	GFP
Human mesenchymal stem cells (HMSC)	6.49 x 10^6/ml	Trypan blue viability: 91.4%
Induced pluripotent stem cell (IPS)	7.51 x 10^5/ml	Trypan blue viability: 37.9%
Human cordblood	1.40 x 10^7/ml	AO assisted
Human bone marrow	7.32 x 10^7/ml	AO assisted
Adult cardiac stem cell	3.30 x 10^5/ml	Trypan blue viability: 85.3%
Human embryonic stem cell (HES)	2.38 x 10^6/ml	None
Transfected human embryonic stem cell (HES)	1.23x 10^6/ml RFP: 8.32x10^5/ml	RFP