Multifocal Plane Analysis Is Essential for Accurate Cell Viability Assessment Using an Automated Cell Counter

Frank Hsiung, Eli Hefner, Mike Griffin, and Teresa Rubio, Gene Expression Division,
2000 Alfred Nobel Drive, Hercules, CA 94547 USA

Tech Note

Cell Counting

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Introduction

Researchers in the fields of life sciences are often required to determine the concentration of cells they are working with. Calculation of the cell concentration is conventionally done by manually counting cells under a microscope using a hemocytometer. Manual counting is tedious, time-consuming, and error prone, yet it is an essential step prior to many experiments. An accurate cell count ensures consistency in experimental outputs. The TC20™ automated cell counter is a benchtop instrument that counts mammalian cells in one simple rapid step; it provides the total cell count of the sample, as well as assessment of cell viability via trypan blue staining. The accuracy of live vs. dead cell count using the TC20 counter is enabled by an automated multifocal plane analysis.

Similar instruments available on the market all use a single focal plane to assess cell viability, which often leads to miscounting of live cells: light scattering and alignment of cells at different heights in a counting chamber can cause some live cells to appear dead. The TC20 cell counter eliminates this concern by utilizing a multifocal plane analysis strategy to assess cell viability. The instrument engages an auto-focusing process upon insertion of sample slides, and the sharpest focal plane is chosen for calculating the total cell count. The instrument then scores each cell in the counting field across multiple focal planes above the sharpest plane (Figure 1) to precisely determine if a cell is dead or live, and the percentage of live cells in a population is presented.

In this study we demonstrate how single and multiple focal plane analyses can affect live vs. dead cell ratio determination.

Methods

HeLa, Jurkat, Pan T, PBMC, and human fibroblast cells (CCD-1137Sk) were used in the study. Cells were grown in their optimal growth media (as recommended by the American Type Culture Collection) at 37ºC with 5% CO_2 until ~90% confluent. HeLa and CCD-1137Sk cells were trypsinized using 1x trypsin/EDTA, then resuspended in PBS prior to use. Jurkat, Pan T, and PBMC cells were grown in suspension and used straight out of the flask. Cells were stained by mixing cell suspension with trypan blue in a 1:1 ratio.

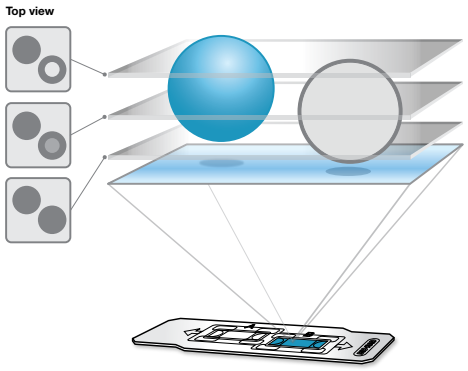

Fig. 1. Schematic of multifocal plane analysis. Top views of two cells (trypan blue stained dead cell and unstained live cell) at different focal planes are illustrated on the left.

Results and Discussion

To illustrate the importance of multifocal plane analysis, an image stack consisting of nine focal planes was acquired (Figure 2). In the example shown in Figure 2, plane 5 had the best contrast between cells and backgrounds, resulting in more cells found than in any other plane and a ratio of 1:1 live to dead cells. The count from plane 2, on the other hand, identified

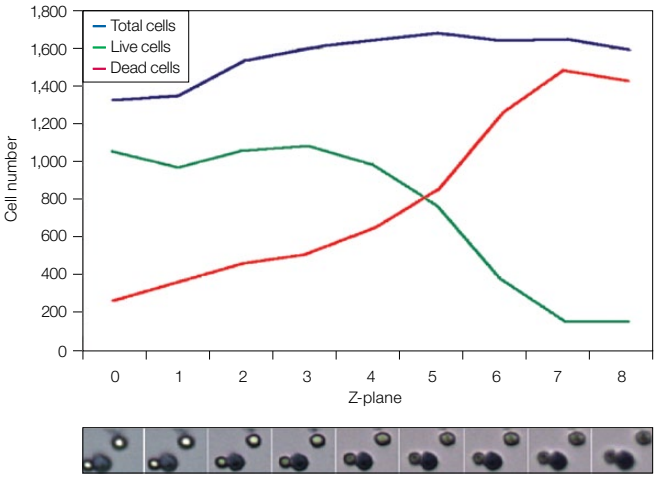

Fig. 2. Effect of focal plane choice on cell count. The bottom panel shows microscopic views of cells at different focal planes. The chart shows the cell count at each plane.

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70% live cells but only 85% of the total cells found on plane 5 could be counted. Results from the analysis shown in Figure 2 strongly indicate that the focal plane chosen can dramatically bias the accuracy of total and live:dead cell counts.

Multifocal Plane Analysis

The TC20 cell counter measures total cell count by collecting the cell count from the plane with the highest contrast. Live:dead cell count is then assessed by checking and comparing images from several planes neighboring the one used for total counts. Three cell types were tested and the results (Table 1) confirm that the accuracy of live:dead cell count determination can vary dramatically if measured using only a single focal plane, with the larger cells being the easiest to assess (for example, CCD-1137Sk cells show no difference between single and multiple focal plane analyses). The discrepancy in results between single and multiple focal plane analyses varies between cell types, presumably due to differences in their shapes and sizes, though a correlation has not been established. In the case of HeLa cells, 30% of cells were misidentified as dead when only a single focal plane was used, strongly indicating that multifocal plane analysis is essential for an accurate live:dead assessment.

Table 1. Effect of single and multiple focal plane analyses on cell viability assessment.

	Live Cells, %		
Cell Viability Assessment	Jurkat Cells	HeLa Cells	CCD-1137Sk Cells
Single best focal plane	50	35	22
Multiple focal planes	56	65	22
Cells misidentified as dead by single focal plane analysis, %	6	30	0

Automated vs. Manual Live Cell Counting

In a comparative analysis (Figure 3), a random population of Pan T cells mixed with trypan blue (1:1) were counted with a hemocytometer, a TC20 automated cell counter, and a commercially available automated cell counter that does not apply multifocal plane analysis. The results obtained from the TC20 cell counter and a hemocytometer for both live and dead cells showed no statistically significant differences, while the results obtained with the automated counter using only single focal plane analysis showed a significantly lower number of live cells, resulting in a much lower total cell count. Multifocal plane analysis, coupled with the TC20 cell counter's upgraded optical system and improved algorithm, enables users to obtain the percentage of live cells in small cell populations, such as Pan T and PBMC.

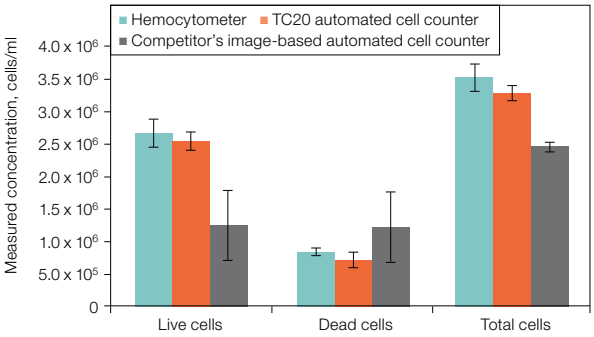

Fig. 3. The TC20 cell counter demonstrates accurate counts of viable cells. Pan T cells mixed with trypan blue (1:1) were counted with a hemocytometer, a TC20 automated cell counter, and a competitor's image-based automated cell counter. The TC20 counter and hemocytometer cell counts showed no statistically significant differences. Precision is indicated by the standard deviations; error bars represent average standard deviations. Cell counts on the TC20 counter were performed on four different instruments with five sample replicates.

Conclusions

Using just a single focal plane for analysis not only increases the risk of mistaking live cells for dead ones, but also the likelihood of severely undercounting the total number of cells, presumably due to the fact that cells outside the chosen single focal plane are difficult to recognize as such. Therefore, having the capability of assessing each cell across several focal planes is truly necessary in order to provide an accurate report of live, dead, and total cell counts. The TC20 automated cell counter from Bio-Rad Laboratories, Inc. is the only cell counting instrument that allows automatic multifocal plane analysis, therefore providing more accurate results than other instruments of its kind currently available on the market.

For more information, visit www.bio-rad.com/web/TC20MPA.

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