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Application Note

Rapid Assessment of Size Changes Using the Scepter™ Cell Counter can be Predictive of Cell Death

INTRODUCTION

The process of programmed cell death or apoptosis is an essential component in many biological processes and is therefore the focus of much research. Initial studies revealed that reduction in cell volume was an early morphological change during apoptosis[1]. Recent studies have shown that this cell shrinkage is not simply a passive consequence of other apoptotic events, but is a key driver of apoptotic signaling, mediated by the cell's machinery for homeostatic regulation[2-4]. Apoptotic enzymes, including caspases, and membrane depolarization events are activated by changes in intracellular ionic concentrations. The cell actively transports potassium, sodium, calcium, and/or chloride ions to enable apoptosis to occur, and these ionic fluxes cause cell volume changes during apoptosis[4]. The specific ions transported and the directionality of transport depends on the specific cell type and particular apoptotic stimulus.

To examine the relationship between cell volume and camptothecin-induced apoptosis in NIH 3T3 cells and CHO cells, we measured cell size distributions using the new Scepter™ handheld, automated cell counter (Millipore Cat. No. PHCC00000). The Scepter cell counter uses impedance-based particle detection to reliably and precisely count every cell in a sample. Precise volumes of single-cell suspensions are drawn into the sensor at the end of the Scepter device. As cells pass through an aperture in the sensor, the voltage increases. The voltage change reflects the size of the detected cell. Voltage spikes of the same size are binned and presented on a histogram showing a detailed cell size distribution in less than 30 seconds. Scepter counting enables very precise particle volume or diameter measurements. In addition, the Scepter Software 1.2 enables users to perform gating and statistical analysis on chosen cell populations, increasing the instrument's analytical power.

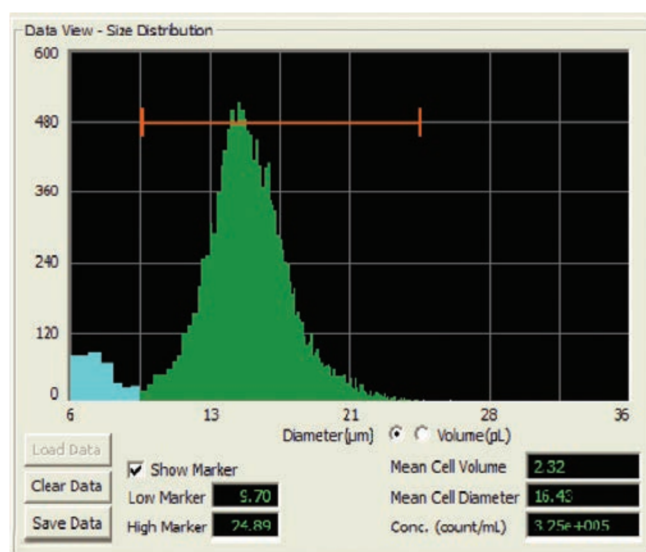

Figure 1. Scepter Software 1.2 provides concentration statistics, on-screen gating, high and low gate displays, and raw and corrected counts.

Cells induced with the camptothecin clearly exhibited an increased percentage of smaller-sized cells, which suggested a higher percentage of cells undergoing apoptosis. This was confirmed by benchtop flow cytometry. Based on this comparison, our data indicate that the distribution of cell sizes within the population accurately reflects both the degree of cell death and the total number of cells in the population as a function of their size.

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RESULTS

Scepter cell counting was used to qualitatively monitor apoptosis events in two different cell lines, NIH 3T3 and CHO. The two cell lines were incubated with camptothecin, an inhibitor of nuclear topoisomerase and known inducer of apoptosis. Both cell lines exhibited an increased number of smaller sized cells and a decreased number of larger sized cells as represented by the shift in the histogram population to the left (Figures 2 and 3) after a 24 hour exposure to camptothecin. For each cell line and for the control and induced cell populations, the concentrations and percentages of presumed apoptotic cells and viable cells were identified by gating the two distinct histogram peaks (Figures 2, 3 and Table 1) using Scepter software version 1.2.

Next, we analyzed the cells using a flow cytometer, which gives highly quantitative data for both early and late apoptotic events. Both untreated and camptothecin-treated NIH 3T3 and CHO cells were analyzed by labeling cells with phycoerythrin (PE)-conjugated Annexin V for flow cytometric analysis (figure 4 (CHO) and data not shown (NIH 3T3).

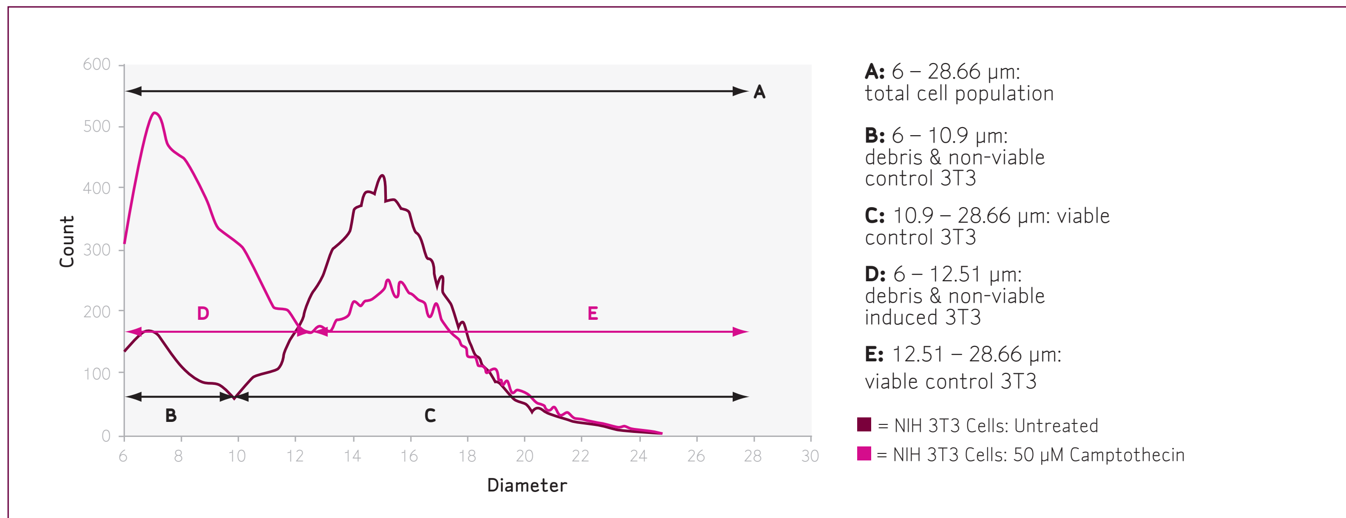

Figure 2. Effect of Apoptosis on NIH 3T3 Size Distribution NIH 3T3 cells (cultured in DMEM (Cat. No. SLM-021-B) with 10% fetal calf serum (Cat. No. ES-009-B), 1% nonessential amino acids, 1% Glutamax™ (Life Technologies Cat. No. 35050), and 1% Penicillin-Streptomycin) were treated with camptothecin (EMD Cat. No. 208925), enzymatically dissociated, washed and resuspended in phosphate-buffered saline (PBS), and counted using a Scepter cell counter. Histograms were generated using averages s (n=3) Scepter cell counts (n=3) of control and camptothecin-treated NIH 3T3 populations. The different peaks were gated and the cell concentrations recorded using Scepter Software 1.2. The results of gating analysis are shown in Table 1.

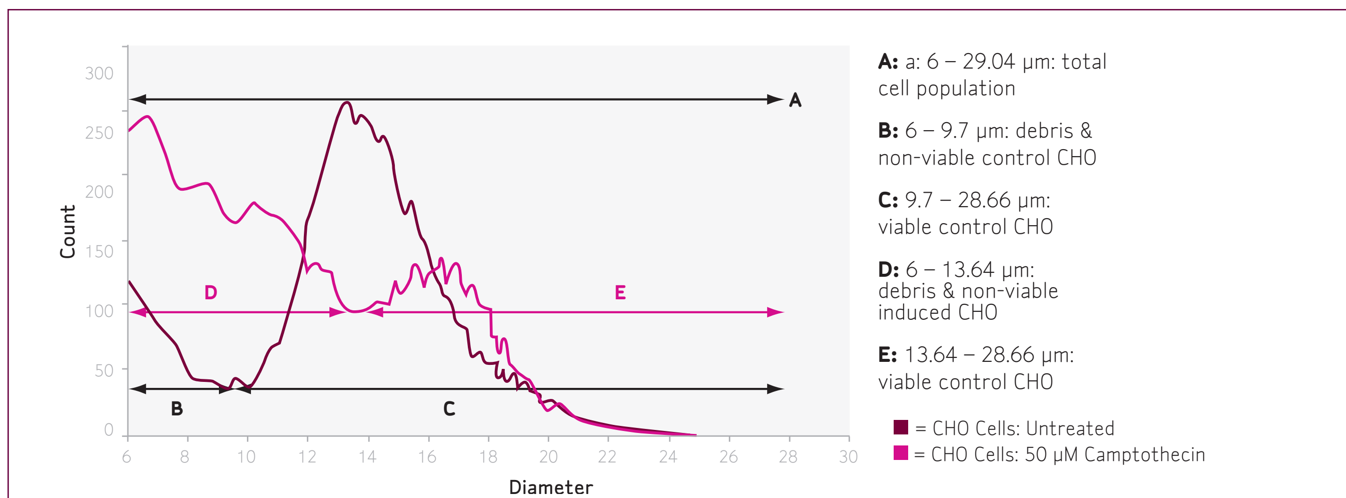

Figure 3. Effect of Apoptosis on CHO Size Distribution CHO cells (cultured in F12 medium with 10% fetal calf serum and 1% Penicillin-Streptomycin) were treated with camptothecin, enzymatically dissociated, washed and resuspended in phosphate-buffered saline (PBS, Cat. No. BSS-1006-A), and counted using a Scepter cell counter. Histograms were generated using average Scepter cell counts (n=3) of control and camptothecin-treated CHO populations. The different peaks were gated and cell concentrations recorded using Scepter Software 1.2. The results of gating analysis are shown in Table 1.

Table 1. Average concentrations (n=3) of different gated populations and percentages of viable and non-viable cells.

	Total		Viable (Scepter)		Non-viable & debris (Scepter)		Viable (flow cytometry)	Non-viable & debris (flow cytometry)
	conc (*E+05)	Size	conc (*E+05)	% of total	conc (*E+05)	% of total	% of total	% of total
3T3 untreated	3.15	16.16	3.00	95	0.17	5	86	14
3T3 + 50 µM camptothecin	3.14	15.92	2.13	68	0.97	31	62	38
CHO untreated	1.82	15.94	1.72	95	0.10	6	89	11
CHO + 25 µM camptothecin	1.49	15.98	0.99	66	0.50	34	59	41
CHO + 50 µM camptothecin	1.80	15.49	1.05	59	0.72	40	54	46

As was anticipated, the percentages of viable and apoptotic cells for each cell type were comparable but not identical to each other and to the results obtained using the Scepter histogram-based quantitation (Figure 4 and Table 1). While the Scepter cell counter cannot quantitatively distinguish stages of apoptosis, it reliably indicates percentages of viable vs. apoptotic/non-viable cells.

Various stages of apoptosis are best distinguished using flow cytometry, because the Scepter cell counter analysis is based strictly on cell volume/size measurements. However, we could distinguish between degrees of apoptosis using Scepter histograms. CHO cells treated with increasing concentrations of camptothecin showed a gradual increase in percentage of apoptotic cells (Figure 5) and were similar to flow cytometry results (Table 1).

CONCLUSION

Because the Scepter device displays high-resolution histograms of entire cell populations, the instrument is useful for differentiating between different cell cycle stages based upon size, as we have shown here with NIH 3T3 and CHO cells. Given recent studies that show that the relationship between apoptosis and cell volume depends on cell type and specific apoptosis-inducing agent, Scepter data may help to elucidate the effects of various agents that effect cell size and volume. In summary, the Scepter handheld automated cell counter is an excellent tool for a fast and qualitative analysis of cells undergoing apoptosis.

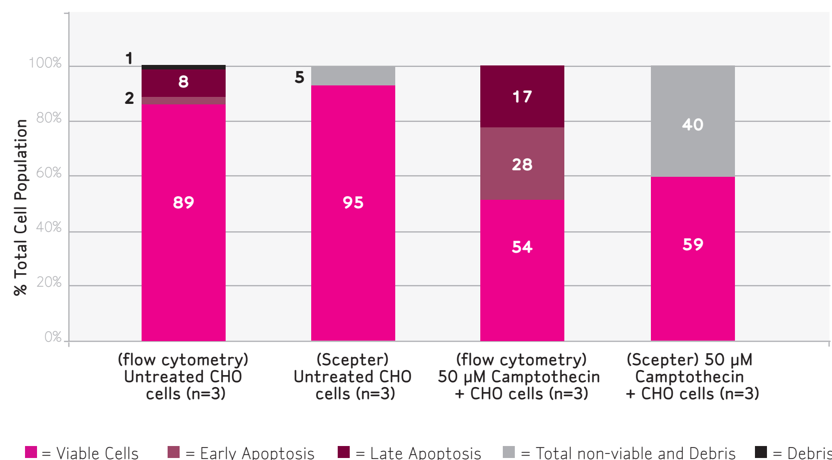

Figure 4. Comparison of Scepter counting with a flow cytometer in measuring apoptotic and non-apoptotic cell populations. Percentages of viable, early and late apoptotic CHO cells determined using flow cytometry, and compared with viable and non-viable/debris populations determined using Scepter cell counting. Cells were enzymatically dissociated, washed and resuspended in PBS. Each well of a 6-well plate was seeded with 20,000 cells and incubated until cells reached confluency. Cells were incubated with camptothecin for another 24 hours, harvested, and analyzed with flow cytometry or the Scepter cell counter, following manufacturer's instructions. Apoptosis in NIH 3T3 cells was measured in the same way, and the corresponding percentages are shown in table 1.

ORDERING INFORMATION

Description	Quantity	Catalog No.
Scepter 2.0 Handheld Automated Cell Counter		
with 40 µm Scepter Sensors (50 Pack)	1	PHCC20040
with 60 µm Scepter Sensors (50 Pack)	1	PHCC20060
Includes:		
Scepter Cell Counter	1	
Downloadable Scepter Software	1	
O-Rings	2	
Scepter Test Beads	1	PHCCBEADS
Scepter USB Cable	1	PHCCCABLE
Scepter Sensors, 60 µm	50	PHCC60050
	500	PHCC60500
Scepter Sensors, 40 µm	50	PHCC40050
	500	PHCC40500
Universal Power Adapter	1	PHCCPOWER
Scepter O-Ring Kit, includes 2 O-rings and 1 filter cover	1	PHCCOCLIP

Are you an existing Scepter user interested in upgrading your device to Scepter 2.0?

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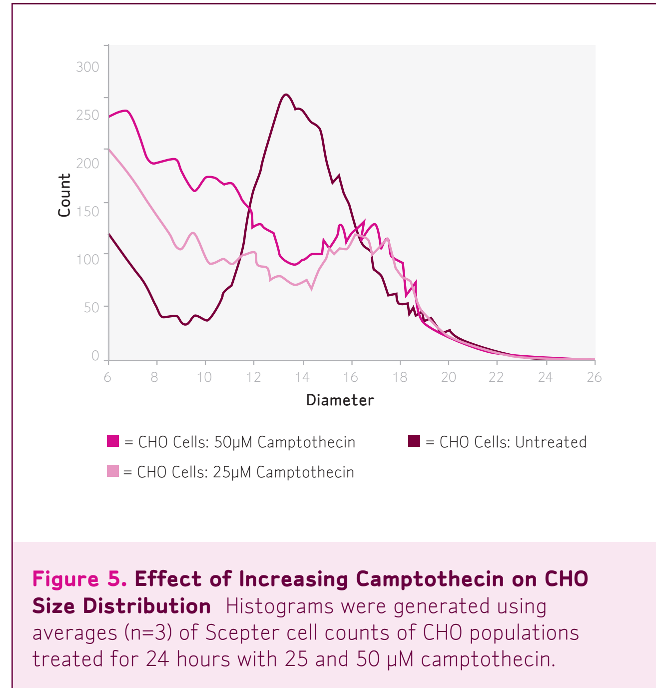

Figure 5. Effect of Increasing Camptothecin on CHO Size Distribution Histograms were generated using averages (n=3) of Scepter cell counts of CHO populations treated for 24 hours with 25 and 50 μM camptothecin.

References

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2. Panayiotidis MI. *Ouabain-induced perturbations in intracellular ionic homeostasis regulate death receptor-mediated apoptosis.* **Apoptosis.** 2010 Jul;15(7):834-49.

3. Franco R et al. *Glutathione depletion and disruption of intracellular ionic homeostasis regulate lymphoid cell apoptosis.* **J Biol Chem.** 2008 Dec 26;283(52):36071-87.

4. Bortner CD, Cidlowski JA. *Cell shrinkage and monovalent cation fluxes: role in apoptosis.* **Arch Biochem Biophys.** 2007 Jun 15;462(2):176-88.

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