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Application Note

Predicting Cell Death by Rapidly Assessing Size Changes with the Scepter™ Cell Counter

Introduction

Apoptosis, the process of programmed cell death, is an essential component of many biological processes. 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 rather 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 resulting in cell volume changes from these ionic fluxes. The identity of ions transported and the directionality of transport depend on the cell type and apoptotic stimulus.

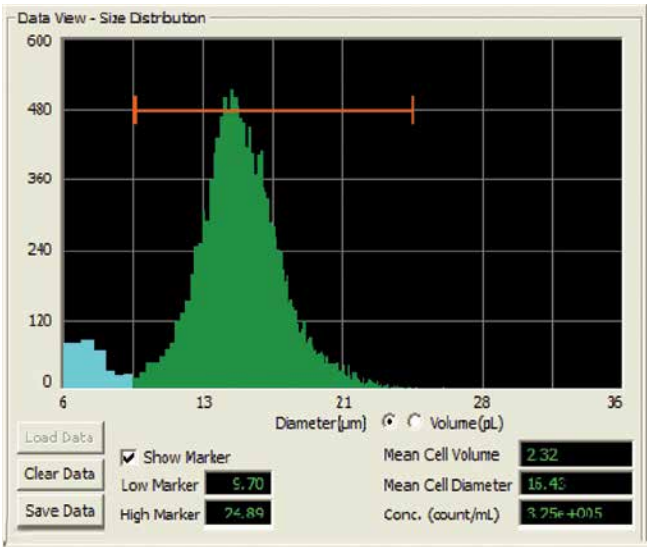


Figure 1.

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

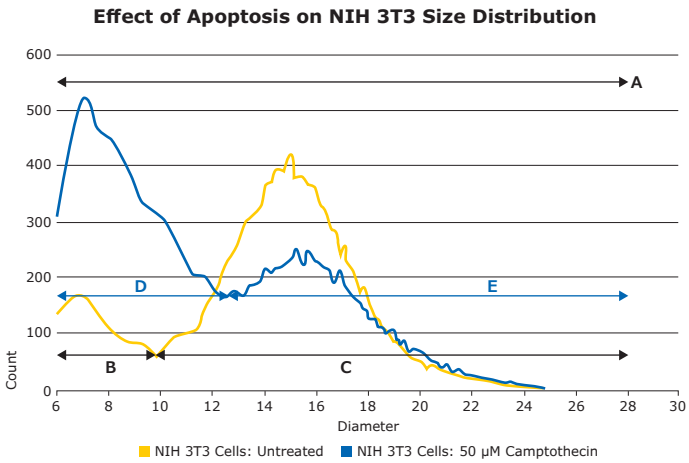
Apoptosis can be distinguished from necrosis biochemically by fragmentation of the genome and cleavage or degradation of multiple cellular proteins. As no single parameter defines cell death, there are many assays for use with microscopy and flow cytometry. However, all current methods can only be used to measure apoptosis after cell death and require access to expensive equipment. In this paper, we will discuss using the Scepter™ Cell Counter to rapidly and accurately predict apoptosis by measuring changes in cell size.

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 Scepter™ handheld, automated cell counter. 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 tip 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 magnitude are binned and presented on a histogram, showing cell size distribution in less than 30 seconds. Compared to results from benchtop flow cytometry, our data indicates 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.

Results

The Scepter™ cell counter 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 percentage of smaller cells, as seen by the shift in the histogram population to the left (Figures 2 and 3) after a 24 hour exposure to camptothecin. 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.

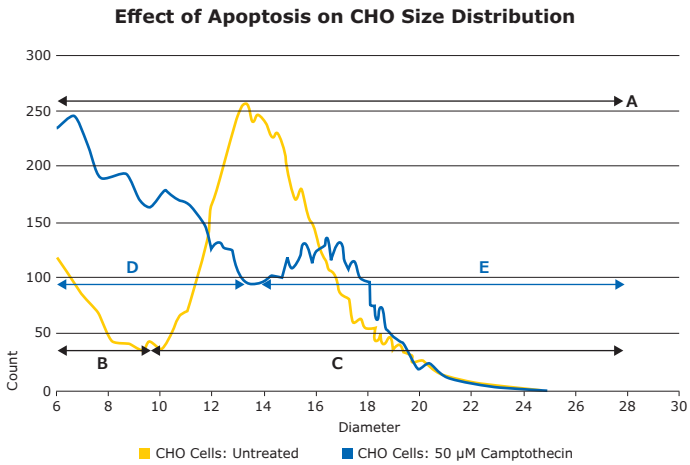
The cells were then analyzed with a flow cytometer, which provides highly quantitative data for early and late apoptotic events. Both untreated and camptothecin-treated NIH 3T3 and CHO cells were analyzed by labeling cells with phycoerythrin (PE)-conjugated Annexin V (CHO, Figure 4, and NIH 3T3, data not shown.)



A: 6 – 28.66 µm: total cell population
B: 6 – 10.9 µm: debris & non-viable control 3T3
C: 10.9 – 28.66 µm: viable control 3T3
D: 6 – 12.51 µm: debris & non-viable induced 3T3
E: 12.51 – 28.66 µm: viable control 3T3

Figure 2.

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 (MilliporeSigma, Cat. No. 208925), enzymatically dissociated, washed and resuspended in phosphate-buffered saline (PBS), and counted using a Scepter™ cell counter. Histograms were generated using average Scepter™ device cell counts (n=3) of control and camptothecin-treated NIH 3T3 populations. The different peaks were gated and the cell concentrations recorded using Scepter™ cell counter software. The results of gating analysis are shown in Table 1.



A: 6 – 29.04 µm: total cell population
B: 6 – 9.7 µm: debris & non-viable control CHO
C: 9.7 – 28.66 µm: viable control CHO
D: 6 – 13.64 µm: debris & non-viable induced CHO
E: 13.64 – 28.66 µm: viable control CHO

Figure 3.

CHO cells (cultured in F12 medium with 10% fetal calf serum and 1% Penicillin-Streptomycin) were treated with camptothecin, enzymatically dissociated, washed and re-suspended in phosphate-buffered saline (PBS, Cat. No. BSS-1006-A), and counted using a Scepter™ cell counter. Histograms were generated using average Scepter™ device 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.

As anticipated, the percentages of viable and apoptotic cells obtained from flow cytometry were comparable to the results obtained using the Scepter™ Software histogram-based quantitation (Figure 4 and Table 1). While the Scepter™ cell counter cannot quantitatively distinguish stages of apoptosis, it indicates percentages of viable versus apoptotic or non-viable cells based on size.

Various stages of apoptosis are best distinguished using flow cytometry, as the Scepter™ cell counter analysis is based strictly on cell size measurements. However, using Scepter™ histograms, we were able distinguish between different degrees of apoptosis, as shown by varying camptothecin concentration. CHO cells treated with increasing concentrations of camptothecin showed a gradual increase in percentage of apoptotic cells (Figure 5) and still aligned with flow cytometry results (Table 1), demonstrating the relative sensitivity of this method.

	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	5	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

Table 1.

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

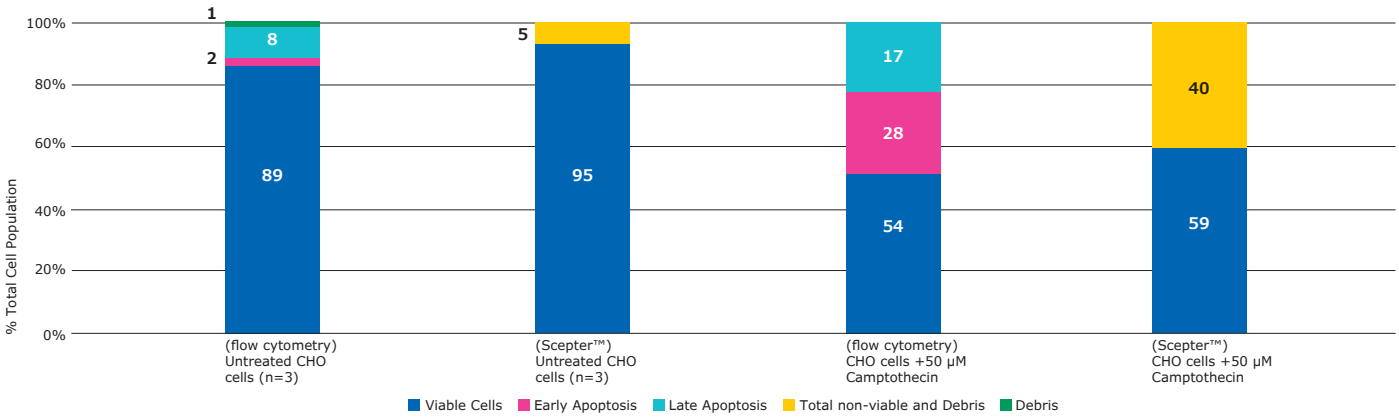


Figure 4.

Comparison of the Scepter™ cell counter 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 a Scepter™ cell counter. 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/were not 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.

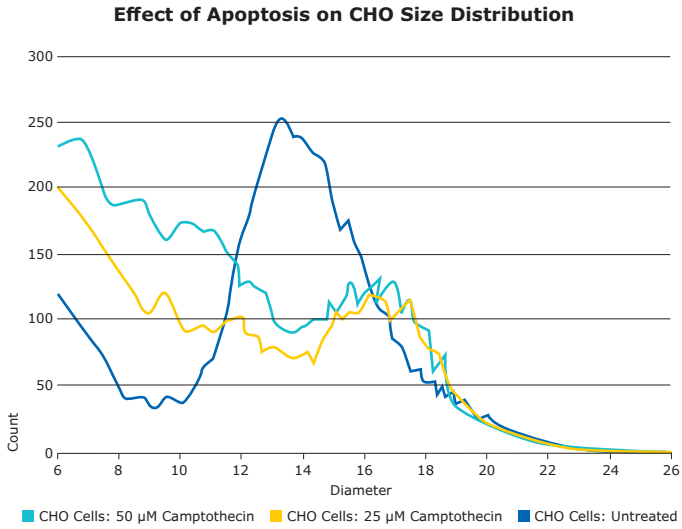


Figure 5.

Histograms were generated using averages (n=3) of cell counts for CHO populations treated for 24 hours with 25 or 50 µM camptothecin, as measured by the Scepter™ device.

Ordering Information

Description	Qty/Pk	Cat. No.
Scepter™ Handheld Automated Cell Counter	1	**PHCC00000**
Includes:		
Scepter™ Cell Counter	1	
Scepter™ Software CD	1	
O-Rings	2	
Scepter™ Test Beads	1	**PHCCBEADS**
Scepter™ USB Cable	1	**PHCCCABLE**
Accessories		
Scepter™ Sensors, 60 µm	50	**PHCC60050**
	500	**PHCC60500**
Universal Power Adapter	1	**PHCCP0WER**
Scepter™ O-Ring Kit Contains two O-rings and one filter cover	1 kit	**PHCC0CLIP**

Conclusion

The Scepter™ cell counter can be used to differentiate between different cell cycle stages based upon cell size. The high-resolution histograms of entire cell populations can be processed with the Scepter™ Software to rapidly estimate the number of cells undergoing apoptosis. Recent studies have shown that the relationship between apoptosis and cell volume depends on cell type and the apoptosis-inducing agent. Using the Scepter™ counter, scientists may be able to elucidate the effects of various agents that affect cell size and volume. In summary, the Scepter™ handheld automated cell counter is a useful tool for fast, qualitative analysis of cell apoptosis.

References

1. Kerr JF et al. *Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics*. **Br J Cancer**. 1972 Aug; 26(4):239-57.
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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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