

## Quantitation of Apoptosis by Nuclear Fragmentation

### BACKGROUND AND SUMMARY

Cell death by apoptosis is a complex, tightly regulated process in which a cell orchestrates its own destruction in response to specific internal or external triggers.

The most reliable distinguishing feature of the apoptotic process is nuclear condensation and DNA fragmentation. Apoptotic nuclei stained with fluorescent DNA intercalating dyes typically produce small, fragmented, highly textured nuclear images. Because of this distinctive morphology, apoptotic nuclei are ideally suited for analysis with the ImageStream<sup>®</sup> system.

In this report, apoptosis was induced in HL60 cells with daunorubicin, a DNA-intercalating agent which inhibits DNA and RNA synthesis and is used as a treatment for acute myeloid leukemia

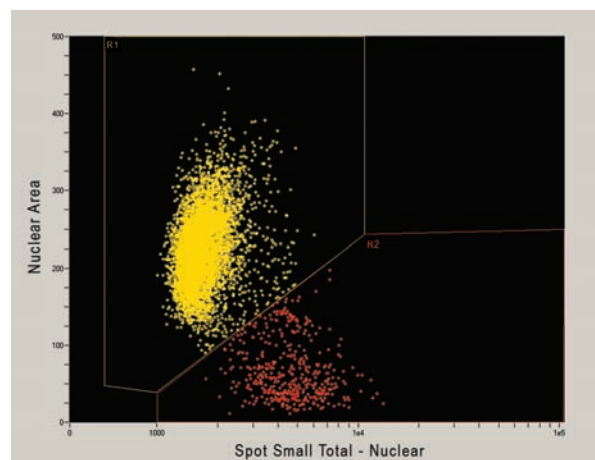
(AML). In this experiment, HL60 cells were exposed to a time course of daunorubicin treatment, and the apoptotic rate was measured directly from nuclear images obtained with the ImageStream system.

The ImageStream imaging flow cytometer produces high resolution brightfield, darkfield, and fluorescence images of cells in suspension at rates up to 300 cells per second. The IDEAS<sup>®</sup> analysis software measures over 200 morphometric and photometric features for each cell based on its imagery. These features offer the ability to measure the sub-cellular location of probes. The ImageStream combines the quantitative power of large sample sizes common to flow cytometry with the high information content of microscopy.

### RESULTS

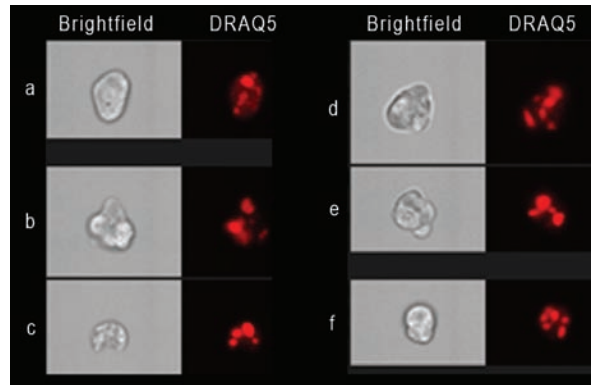
**FIGURE 1.**  
SELECTION OF APOPTOTIC CELLS.

HL60 cells were incubated with daunorubicin, fixed and stained with the fluorescent DNA-binding dye DRAQ5 and imaged on the ImageStream system. Apoptotic cells (R2) were identified using two standard image-based features from the IDEAS package: Area and Spot Small Total. Condensed apoptotic nuclei have lower nuclear area values compared to live cells. Also, images of fragmented apoptotic nuclei exhibit small, bright regions with higher Spot Small Total values compared to the uniform images of normal nuclei. For the time point from which this plot was derived, approximately 10% of the single cell population had become apoptotic.



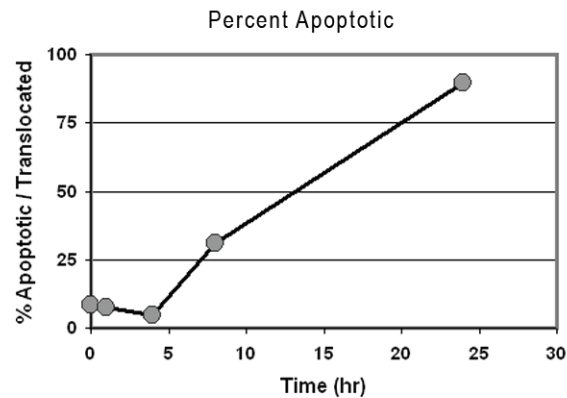
**FIGURE 2.**  
**VERIFICATION OF THE SELECTION STRATEGY BY**  
**VISUAL EXAMINATION**

This figure shows several images taken from the R2 population defined above. Each pair (a - f) shows both the brightfield and nuclear stained images. The characteristically condensed and fragmented nature of the apoptotic nucleus is clearly evident in each of the fluorescent images.



**FIGURE 3.**  
**INCREASE IN APOPTOSIS OVER TIME**

Daunorubicin induces apoptosis in HL60 cells. During the 24-hour time course of this experiment, the apoptotic fraction in the population, measured directly by assessing nuclear fragmentation, increased to over 90%.



## CONCLUSIONS

Visual evidence of nuclear fragmentation has historically been considered the 'gold standard' for identifying apoptotic cells. Nonetheless, nuclear fragmentation is not widely employed to quantitatively assess apoptosis because of the small number of cells that can be examined under the microscope. A number of biochemical tests have, as a result, been adopted in place of the analysis of nuclear fragmentation. The ImageStream system, because of its unique ability to analyze morphologic data for large cell populations, enables the quantitation of apoptosis solely on this accurate and reliable metric.

Advantages of the Nuclear Fragmentation Assay on the ImageStream system:

- Nuclear morphology is the most direct and accurate measure of apoptosis
- The staining protocol is simple
- The assay requires only one color for accurate measurement
- The assay can be multiplexed with other functional assays such as nuclear translocation