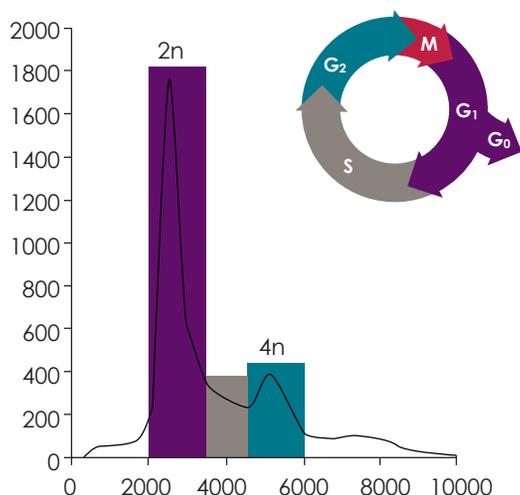


## Introduction: Cell Cycle

The process of DNA replication and cell division is known as the cell cycle. The cell cycle is typically divided into five phases:  $G_0$ , the resting phase,  $G_1$ , the normal growth phase, S, the DNA replication phase,  $G_2$ , involving growth and preparation for mitosis, and M, mitosis.



There are at least two types of cell cycle control mechanisms. The first involves kinase activation to relay a cell from one stage to the next. The second is a type of checkpoint control by which flaws in critical events trigger a delay in cell cycle progression. Because cell cycle events regulate cell proliferation and cancer involves inappropriate cell proliferation, cell cycle and alterations to the cell cycle control mechanisms are at the heart of oncology research<sup>1</sup>.

## Cell Cycle Analysis using PI

Cell cycle analysis is used to determine the proportion of cells in each stage of the cell cycle for a given cell population based on variations in DNA content. Each cell is stained with a fluorescent dye that intercalates with DNA. Propidium iodide (PI) is a nuclear staining dye that is frequently used to measure cell cycle. Because the dye cannot enter live cells, the cells are fixed with ethanol

prior to staining. All of the cells are then stained. Cells preparing for division will contain increasing amounts of DNA and display proportionally increased fluorescence. Differences in fluorescence intensity are used to determine the percentage of cells in each phase of the cell cycle.

<sup>1</sup>Collins, K., et al. (1997). Cell Cycle and Cancer. Proc. Natl. Acad. Sci. V.94, pp2776-2778.

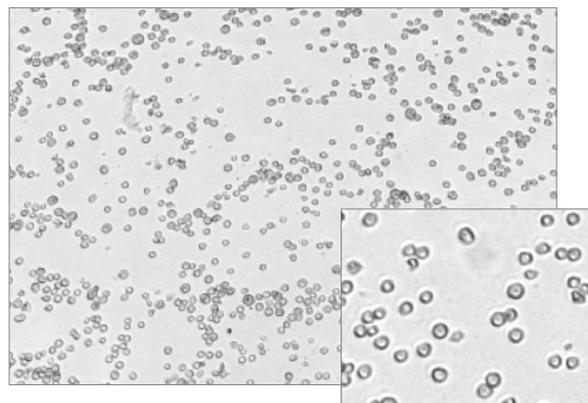
## Experimental Procedure

Jurkat cells were used to analyze cell cycle kinetics following treatment with the cell-cycle-arresting drugs etoposide and nocodazole. Jurkat cells were incubated with media only (control), etoposide (0.12, 0.6, and 3  $\mu$ M), or nocodazole (0.004, 0.02, 0.1  $\mu$ g / mL) for 24 hours. Control and drug-treated cells were fixed then stained with propidium iodide. For each sample, 20  $\mu$ l of cell sample (at  $\sim 4 \times 10^6$  cells / mL) was loaded into a Cellometer Imaging Chamber, inserted into the Vision CBA Analysis System, and imaged in both bright field and fluorescence. The fluorescence intensity for each cell was measured.

## Results

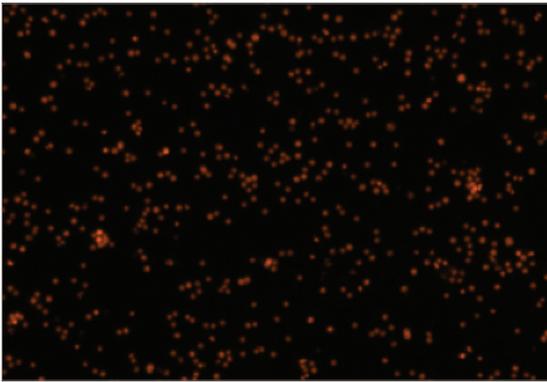
### Bright Field Imaging

The Cellometer instrument acquires a bright field image for each sample tested. The bright field image allows researchers to verify cell morphology, evaluate the degree of homogeneity of the sample, and identify the presence of cellular debris.

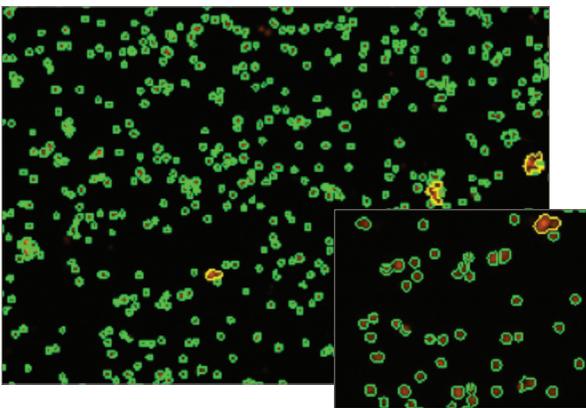


## Fluorescent Imaging

Because all of the cells have been fixed, all of the cells are stained with propidium iodide and appear in the fluorescent image.



The fluorescent counted image can be used to confirm that cells are counted correctly. Individual counted cells are outlined in green. Uncounted cells are outlined in yellow. Cellometer software uses proprietary algorithms to accurately count individual cells within clumps.



## Cell Cycle Histogram

A cell cycle histogram is automatically generated for each sample using the optimized Nexcelom cell cycle data layout in FCS Express 4 Flow Software. Gating can be manually optimized directly on the histogram with automatic update to the associated data table. Histograms for control and experimental samples are shown in the following experimental data section.

### Experimental Data

For the control sample (Figure 1, Table 1), over half of the cell population is in the G<sub>0</sub>/G<sub>1</sub> phase. There are also distinct populations of cells in the S phase (more than 20%) and the G<sub>2</sub>/M phase (more than 12%). The Sub-G<sub>1</sub> group in the following histograms includes cellular debris and very late-stage apoptotic and necrotic cells. Gating was set for the control sample and applied to histograms for the four experimental conditions presented.

Figure 1. Control

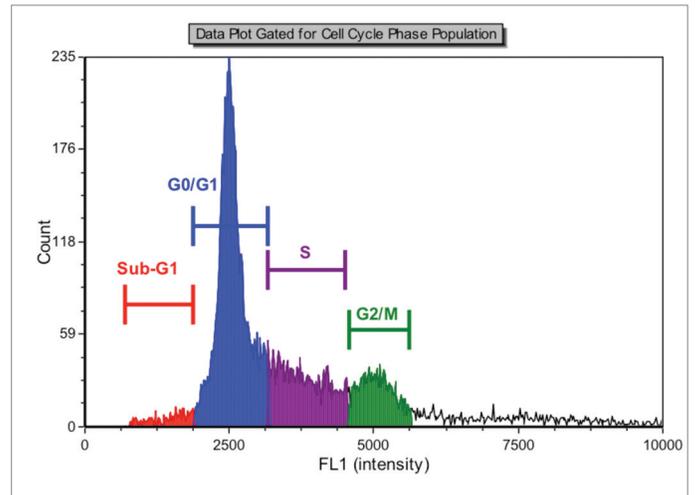


Figure 1 Population histogram for the control sample generated with FCS Express 4 software. Sub-G<sub>1</sub> population is indicated in red, G<sub>0</sub>/G<sub>1</sub> phase population is in blue, S phase population is in purple, and G<sub>2</sub>/M phase population is in green.

Table 1.

Phase Population	% of Total Cells	Concentration (x 10 <sup>6</sup> Cells/ml)
Total	100.00	14.77
Sub-G <sub>1</sub>	2.80	0.41
G <sub>0</sub> /G <sub>1</sub>	51.40	7.61
S	20.02	2.96
G <sub>2</sub> /M	12.62	1.87

Addition of increasing concentrations of the cell-arresting drug etoposide caused a shift in the cell population from the G<sub>0</sub>/G<sub>1</sub> phase to the S phase (Figures 2 and 3).

Figure 2. 0.12 μm Etoposide

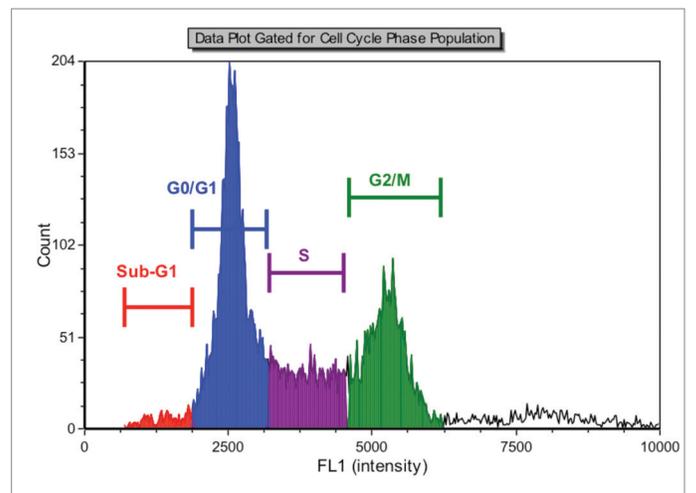
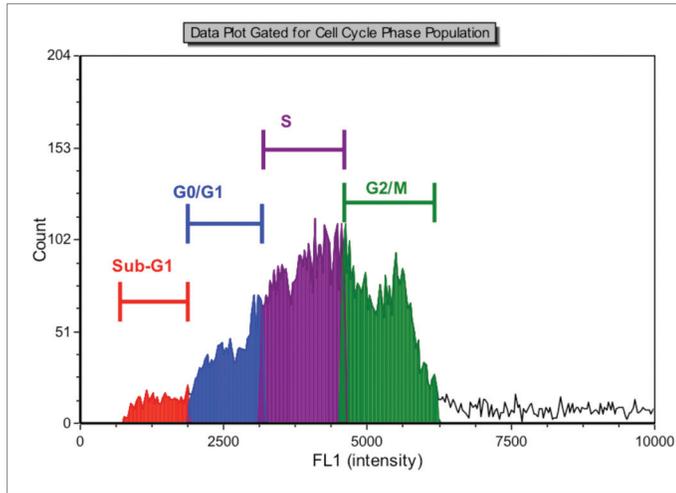


Figure 2 Population histogram for the 0.12 μm etoposide sample generated with FCS Express 4 software. Sub-G<sub>1</sub> population is indicated in red. G<sub>0</sub>/G<sub>1</sub> population is in blue, S phase population is in purple, and G<sub>2</sub>/M phase population is in green.

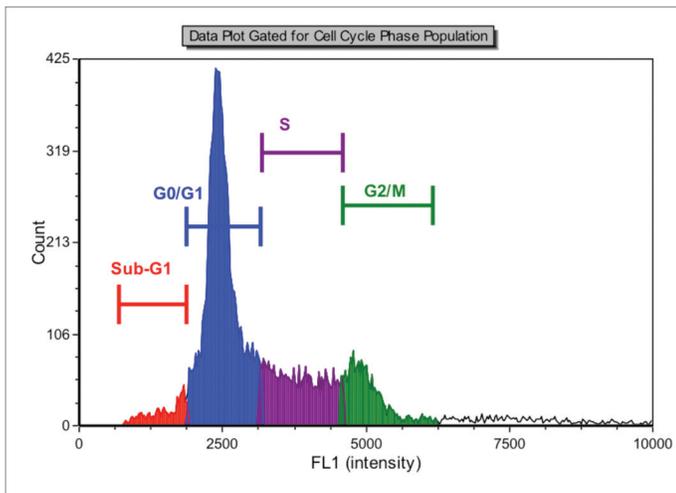
**Figure 3. 3  $\mu\text{m}$  Etoposide**



**Figure 3** Population histogram for the 3 $\mu\text{m}$  etoposide sample generated with FCS Express 4 software. Sub-G<sub>1</sub> population is in red, G<sub>0</sub>/G<sub>1</sub> population is indicated in blue, S population is in purple, and G<sub>2</sub>/M population is in green.

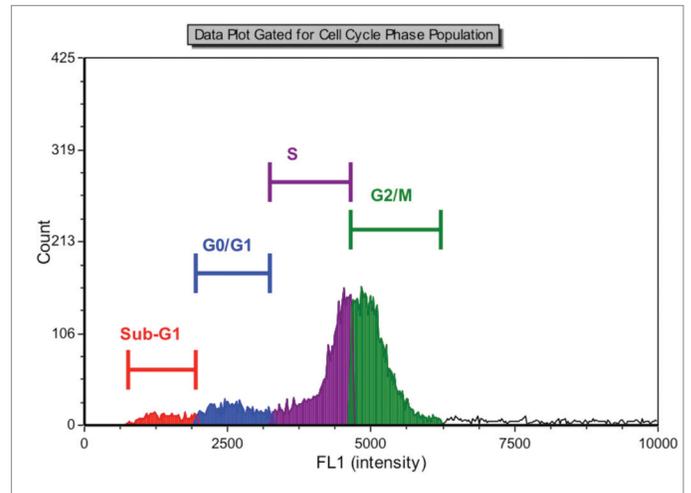
Addition of increasing amounts of nocodazole, a mitotic inhibitor, caused a shift from the G<sub>0</sub>/G<sub>1</sub> and S phases to the G<sub>2</sub>/M phase (Figures 4 and 5).

**Figure 4. 0.004  $\mu\text{g}/\text{mL}$  Nocodazole**



**Figure 4** Population histogram for the 0.004  $\mu\text{g}/\text{mL}$  nocodazole sample generated with FCS Express 4 software. Sub-G<sub>1</sub> population is indicated in red. G<sub>0</sub>/G<sub>1</sub> population is in blue, S population is in purple, and G<sub>2</sub>/M population is in green.

**Figure 5. 0.1  $\mu\text{g}/\text{mL}$  Nocodazole**

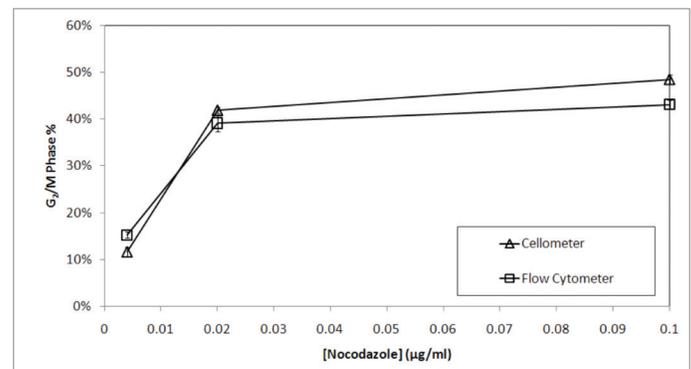


**Figure 5** Population histogram for the 0.1  $\mu\text{g}/\text{mL}$  nocodazole sample generated with FCS Express 4 software. Sub-G<sub>1</sub> population is indicated in red. G<sub>0</sub>/G<sub>1</sub> population is in blue, S population is in purple, and G<sub>2</sub>/M population is in green.

**Correlation to Flow Cytometry**

The graph below shows the percentage of cells arrested at the G<sub>2</sub>/M phase following incubation with nocodazole. There was excellent correlation between the Cellometer Vision CBA Analysis System and the FACS-Calibur flow cytometer. Similar correlation data for etoposide is presented in the Nexcelom publication referenced on the following page.

**Figure 6.**



**Figure 6** Percent population of cells at the G<sub>2</sub>/M phase for the control sample, cells treated with 0.02  $\mu\text{g}/\text{mL}$  nocodazole, and cells treated with 0.1  $\mu\text{g}/\text{mL}$  nocodazole are plotted above. The graph includes data for the Vision CBA and the FACS-Calibur flow cytometer.

## Conclusion

Incubation with an increasing concentration of etoposide generated a shift in population from the G<sub>0</sub>/G<sub>1</sub> phase to the S phase. Incubation with an increasing concentration of nocodazole resulted in a population shift from the G<sub>0</sub>/G<sub>1</sub> phase to the G<sub>2</sub>/M phase. Cellometer Vision CBA data showed the same population shifts as the data generated using a flow cytometer.

The Cellometer Vision CBA Analysis System is a simple, accurate, image-based platform for cell cycle analysis using only 20  $\mu$ l of sample. The small sample volume and simple imaging procedure enabled analysis of the dose response for several compounds using a limited number of cells. Image cytometry is a simple, accurate method for cell cycle analysis with proven correlation to flow cytometry.

Please see the complete published article for more details:

Chan, L., et al. (2011). Cellometer Vision as an Alternative to Flow Cytometry for Cell Cycle Analysis, Mitochondrial Potential, and Immunophenotyping. *Cytometry Part A*. 79A: 507-517. Doi: 10.1002/cyto.a.21071.

To learn more about the Cellometer Vision CBA Analysis System or request an in-lab demonstration, call 978-327-5340, e-mail [info@nexcelom.com](mailto:info@nexcelom.com), or visit [www.nexcelom.com](http://www.nexcelom.com).

Vision CBA



Nexcelom Bioscience  
360 Merrimack Street  
Building 9  
Lawrence, MA 01843, USA

T: +1.978.327.5340  
F: 978.327.5341

[www.nexcelom.com](http://www.nexcelom.com)

1001125 Rev.A 03/12