Application Note

Cell Analysis

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Proliferation and Cell Cycle Analysis Using the NovoCyte Flow Cytometer

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Introduction

The Agilent NovoCyte is a powerful benchtop flow cytometer that can detect up to 13 colors and 15 parameters in total. The system is designed for quantitative and multiparametric analysis of cellular events, such as proliferation, viability, cell cycle, and apoptosis. This application note discusses how the NovoCyte system can be used for automated cell cycle analysis.

Eukaryotic cell division is an evolutionary conserved process where specific cellular components are duplicated to be divided equally among daughter cells. The precise timing and regulation of cell division is essential to many normal physiological processes, and studying the molecular mechanisms regulating cell division is central to understanding both physiological and pathophysiological events, such as cancer. Once a cell becomes committed to undergoing cell division, it progresses through distinct steps of the cell cycle that are regulated by key signaling proteins, which function as gatekeepers. These steps are divided into three phases that can be detected using flow cytometry. Immediately following cell division, the cell enters the G1 phase where cellular growth occurs, including the replication of organelles. This is followed by a transition into the S phase of the cell cycle, in which DNA is duplicated, allowing progression into the G2/M phase. This is a phase of rapid cell growth and protein synthesis to prepare for mitosis, the final step where cellular components are divided into two identical daughter cells. The process repeats as cells continue to proliferate.

Materials and methods

To analyze specific phases of the cell cycle with the NovoCyte flow cytometer, Jurkat T cells were treated with compounds known to arrest growth at G1 and G2/M. After compound treatment, cells were fixed with ice-cold 70% ethanol, and labeled with propidium iodide. This dye binds stoichiometrically to DNA and allows the detection of varying amounts of DNA within cells.

As shown in Figure 1, treatment with 5-fluorouracil (5-FU) increased the population of cells accumulating in the G1 phase of the cell cycle from 52.22% to 70.07%. In contrast, paclitaxel treatment increased the percentage of cells in G2/M phase from 13.86% to 66.58%.

Results and discussion

These results demonstrate that the Agilent NovoCyte system can quantitatively detect cell populations existing in different phases of the cell cycle. The end result of cell cycle progression is cell division, resulting in an increase in cell number, or proliferation. Cell cycle studies are therefore complemented by the analysis of bulk cell proliferation using specific dyes.

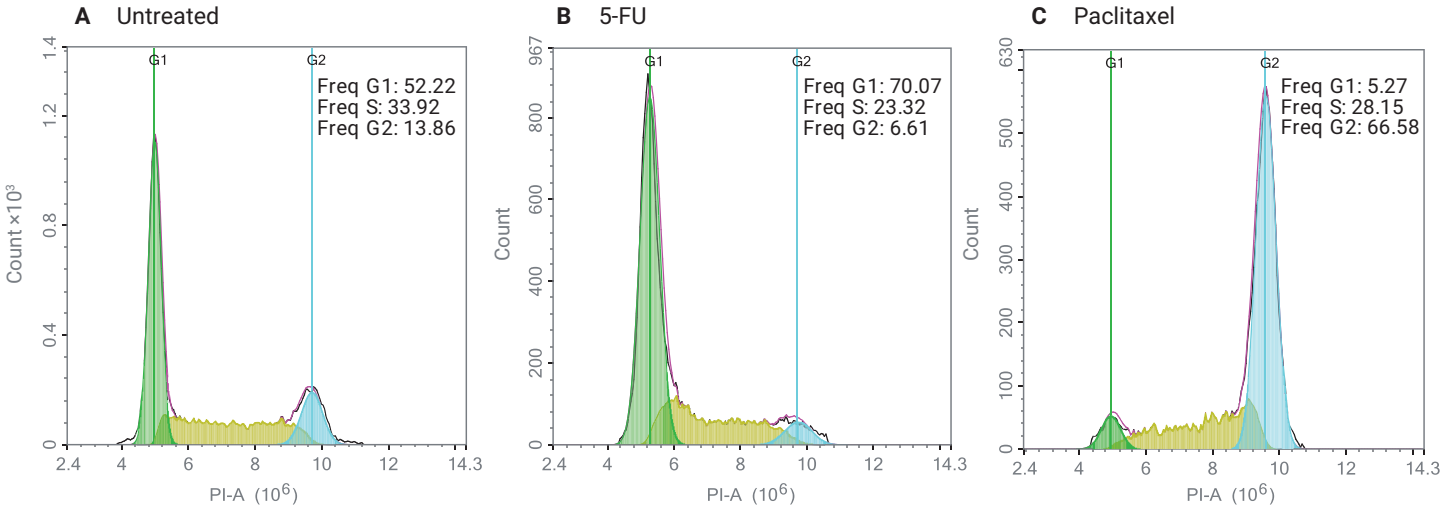

Figure 1. Cell cycle analysis in response to various treatments. Jurkat T cells were left (A) untreated or treated with either (B) 500 µM 5-fluorouracil or (C) 10 nM Paclitaxel for 16 hours. Following treatment, cells were fixed with ethanol and DNA content was measured using propidium iodide. Cell samples were acquired with an Agilent NovoCyte flow cytometer and analyzed using the cell cycle analysis module of the NovoExpress software.

To study proliferation, we used the fluorescent dye carboxyfluorescein succinimidyl ester (CFSE). When cells labeled with CFSE divide, the dye is partitioned equally between daughter cells, and the loss of CFSE fluorescence can be measured over time, as the dye is continuously diluted. Jurkat T cells were labeled with CFSE and measurements of CFSE fluorescence were obtained every 24 hours. As shown in Figure 2A, cell proliferation leads to serial dilution of the CFSE dye, which allows the detection of populations that have undergone distinct numbers of cell divisions. The reduction in mean fluorescence intensity (MFI) is inversely correlated to the concentration of cells in the culture (Figure 2B).

The cell cycle analysis module included with the NovoExpress flow cytometry software facilitates quick and easy generation of cell cycle data using the Watson model. All the relevant information is automatically calculated and displayed once the specific single cell population has been identified through gating. This includes percentage of cells in each stage of the cycle, CVs, and G2:G1 ratio. The NovoExpress software now also includes a Cell Proliferation Module for easy, efficient analysis.

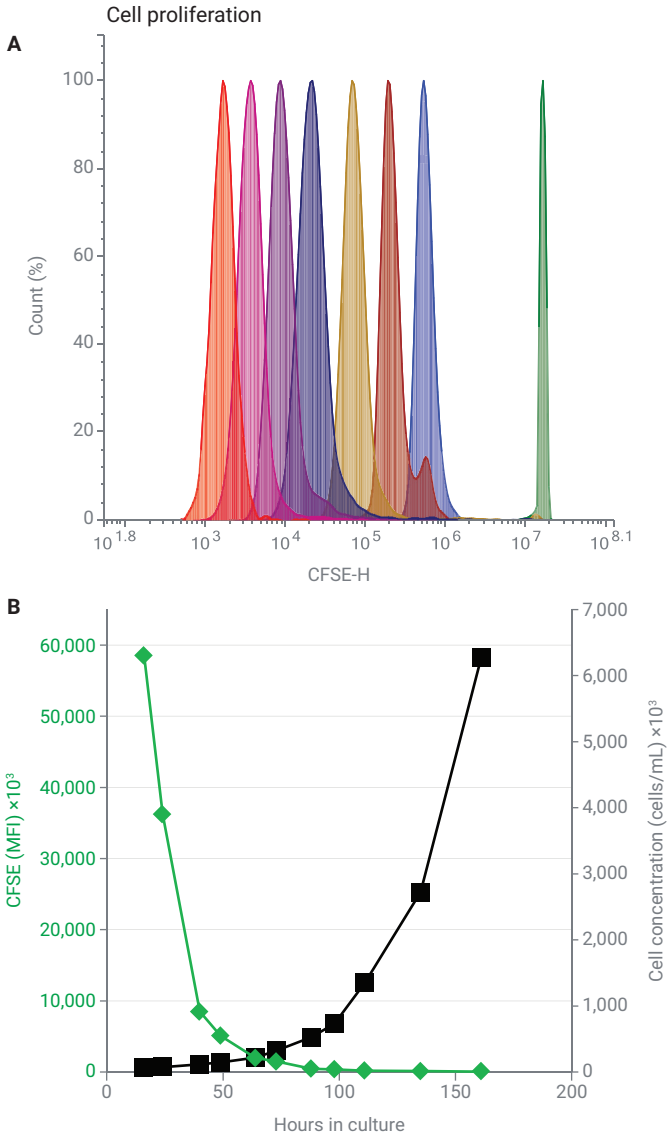

Figure 2. Measurement of proliferation in Jurkat T cells using CFSE. (A) Jurkat T cells were labeled with CFSE and analyzed on an Agilent NovoCyte flow cytometer to measure cell division. Each peak is representative of an individual time point. (B) Absolute cell counts are plotted alongside mean fluorescence intensity (MFI) of CFSE, showing the dilution of signal as cells divide.

References

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2. Lyons, A. B. Analysing Cell Division In Vivo and In Vitro Using Flow Cytometric Measurement of CFSE Dye Dilution. *J. Immunol. Methods* **2000**, *243*, 147–154.

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