Robust Cell Cycle Analysis Using the FlowSight Imaging Flow Cytometer with the FlowCellect™ Bivariate Cell Cycle Kit for G2/M Analysis

Abstract

The Flow cytometric analysis of cell DNA content is widely used for the estimation of cell cycle phase distributions. Adding a fluorescently-labeled cell cycle phase-specific antibody to complement the DNA-binding dye typically used for cell cycle analysis allows for a robust bivariate cell cycle assay that can accurately identify mitotic cells. By further running such a bivariate analysis on an imaging flow cytometer, the mitotic population can be visually analyzed to identify and enumerate cells in specific stages of mitosis. This study demonstrates such an approach by pairing the Amnis® FlowSight® imaging flow cytometer with the EMD Millipore FlowCellect™ Bivariate Cell Cycle Kit for G2/M Analysis.

Measuring Cell Cycle

The cell cycle can be divided into two distinct stages. The first stage, Interphase, consists of the G1, S, and G2 phases in which cells are active, growing, and replicating DNA. Cell division takes place in the second, M-phase or mitotic phase.

Researchers have employed various strategies to closely interrogate each of the critical steps of the various phases in the cell cycle. For example, analysis of cells in S-phase provides a direct measurement of newly synthesized DNA, while the ratio of cells in the G2 and M phases is indicative of the number of cells undergoing mitosis. In all, a comprehensive understanding of cell cycle behavior provides useful information which can be important in interpreting the intrinsic nature of cell proliferation and assist in the development of anti-neoplastic agents.

Improving Cell Cycle Analysis

The FlowSight imaging flow cytometer operates like a conventional flow cytometer but provides up to 12 images of every cell, including brightfield, darkfield and up to nine fluorescence channels. This unique combination provides important new capabilities, such as visual verification of each object acquired, and measurement of the spatial location of fluorescence on, within, or between cells. Visual verification removes the guesswork from gating and can accelerate assay development and optimization by providing visual confirmation of assay performance in any individual cell or group of cells. Automated quantitative measurement of fluorescence localization and morphology enables a broad range of applications that would be impossible by traditional flow cytometry with statistically robust, quantitative image analyses that are not available by conventional microscopy.

The FlowCellect Bivariate Cell Cycle Kit for G2/M Analysis (cat. no. FCCHO25103) simplifies the study of the cell cycle by providing a cell cycle phase-specific antibody (Anti-phospho-Histone H3 (Ser10) – Alexa Fluor 488 conjugated monoclonal antibody) in addition to a DNA-binding dye (Propidium Iodide) to yield a robust bivariate analysis in flow cytometry applications. Moreover, the kit includes a BrdU precursor to allow for BrdU measurement by the conjugated antibody and an RNase reagent to ensure that the PI intensity is directly proportional to DNA content only. This method of analysis reveals not only the distribution of cells in particular phases of the cycle, but also provides insight into the molecular and functional mechanisms associated within the cell cycle.
Example Bivariate Analysis

The Amnis IDEAS software, provided with the FlowSight cytometer, allows the user to click on any dot in a bivariate scatter plot and visually verify whether or not it is an object of interest. In figure 2, image parameters such as Area and Aspect Ratio (the minor axis divided by the major axis, i.e. shape of the object) are applied to the bright field imagery, allowing debris and aggregates to be excluded from the analysis with a high degree of confidence that the region for Single Cells has been placed in exactly the right location.

In figure 3, analysis for phosphorylation of Histone H3 at Ser10 enables discrimination between cells in G2 and M phases of the cell cycle. Additionally, figure 4 demonstrates how the particular phases of mitosis can be discerned by simply clicking on the events positive for the phospho-Histone3 marker to visually inspect the Alexa Fluor 488 image as well as the PI image. Hand-tagging populations or performing further morphological measurements on the nuclear imagery allows quantification of each phase of mitosis if desired.

Conclusion

Coupling FlowSight data acquisition and analysis with the EMD Millipore FlowCellect Bivariate Cell Cycle Kit for G2/M Analysis provides a simple and robust method for identifying, discriminating, and quantitating cells between the G2 and M phases of the cell cycle, as well as enumerating cells in various stages of mitosis. The ability to visually and quantitatively distinguish cells undergoing mitosis versus cells remaining in G2 phase of the cell cycle provides a powerful tool to closely monitor cell cycle activity at M-phase which impacts the study of diseases associated with cell proliferation, apoptosis, and cancer.