Amnis ImageStream®: Technical Reports & Applications

ImageStream®: Flow Cytometry and Microscopy in a Single Platform

The ImageStream achieves true multispectral Imaging in Flow by combining microscopy and flow cytometry in a single platform. Now the visual information that you obtain with a microscope can be fully integrated with the population statistics you would typically get from a flow cytometer. You can define and quantify cell subpopulations not only by signal intensity, but by the location of the signal in or on the cell. You can use cell and nuclear morphologies to characterize cell populations, taking full advantage of complex visual data to perform robust, multiparameter quantitative analyses.
The ImageStream system truly represents a revolution in cell analysis.

Time Delay Integration: enabling high sensitivity detection for Imaging-in-Flow

Imaging-in-Flow is a concept with long-standing appeal, coupled with a persistent struggle to achieve adequate fluorescence sensitivity. The ImageStream system solves the sensitivity problem by using a unique charge coupled detector (CCD) camera and a powerful image detection technique called time-delay-integration (TDI). The focus of this report is TDI technology and the way it has been implemented in the ImageStream system to enable Imaging-in-Flow with a sensitivity comparable or superior to conventional flow cytometry.

Distinguishing Modes of Cell Death

The ImageStream platform can produce six simultaneous, high resolution images of each cell at rates exceeding 100 cells per second. We have exploited the unique ability of the technology to simultaneously generate brightfield, darkfield, and fluorescence cell imagery to classify cells undergoing apoptotic or necrotic cell death.

A Novel Method to Identify Stem Cells in Cord Blood

Flow cytometry is a standard tool in cell identification and quantification by immunofluorescent staining. However, conventional flow cytometry does not allow direct morphological characterization of cells. The ImageStream® was developed for simultaneous high-speed multispectral imaging of cells in brightfield, darkfield and fluorescence channels. The multimode imagery allows both photometric and morphometric methods to discriminate cell types and features in heterogeneous populations of cells. The aim of the study was to compare the percentage of CD34 positive stem cells in cord blood using conventional flow cytometry and the ImageStream®.

Differential Analysis of Peripheral Blood Leukocytes

The ImageStream cell analysis system provides high resolution, high sensitivity image analysis of cells in flow. The classification presented here is based on analysis of a large number of cells (10,000) and any atypical events can be examined directly without additional procedures. This significantly reduces the subjectivity of the analysis while increasing reliability and reducing test to test variability. The ability to accumulate large data files of thousands of cells also allows greater statistical power when comparing differences between samples as well as discerning the validity of 'rare events'.

Analysis of Intracellular Molecular Trafficking

Amnis' ImageStream® system offers a powerful new approach for the study of intracellular molecular trafficking. Combining the imaging capability of a fluorescence microscope with the population statistics of a flow cytometer, the ImageStream provides fully quantitative analysis of both the amount and location of target molecules in thousands of cells. The experiment presented in this report offers a valuable example of how the ImageStream can be used to develop both qualitative and quantitative pictures of intracellular molecular trafficking.

Simultaneous Measurement of NF-κB Translocation and Apoptosis

The ImageStream, Imaging Flow Cytometer system combines the capabilities of microscopy and flow cytometry in a single platform for quantitative image-based cellular assays in large and heterogeneous cell populations.

Here we demonstrate the simultaneous measurement of drug-induced apoptosis and NF-κB nuclear translocation using a combination of intensity-based and morphometric parameters.

GFP Expression and Subcellular Compartmentalization in Trypanosomes: Similarity Score Analysis

The trypanosomes studied in this report have characteristically small nuclei (3µm) and irreguar morphologies. Even so, the similarity analysis available on the ImageStream was able to distinguish clearly those cells in which GFP was sequestered in the nucleus from cells in which it was found in the cytoplasm. In addition, calculation of mean similarity scores proved to be a sensitive and reliable metric of GFP localization at the population level.

This experiment demonstrates the functionality of the similarity score approach to track GFP expression, and to classify cells based on the staining pattern of the chimeric GFP protein.

Quantitation of Nuclear Translocation Events

The ImageStream offers a uniquely powerful solution for the analysis of NF-κB activation and translocation. In this report, we show how the two central capabilities of the ImageStream system - high resolution quantitative image analysis and flow-based population statistics - can produce a robust quantitative analysis of translocation of NF-κB from the cytoplasm to the nucleus in stimulated cells.

Advances in the Identification of Apoptotic Cells

The powerful capabilities of the ImageStream Flow Imaging system were used in the experiment presented to measure both BRDU staining and nuclear fragmentation in a TUNEL assay, a traditional assay for apoptosis.

Using the ImageStream system, we were able to identify a common cell preparation artifact that leads to false-positive results in non-imaging systems and thus enhance the utility of the TUNEL assay. We also independently identified apoptotic cells by their unique nuclear morphologies as an independent validation of the assay. These results offer one example of the unique power of analytical morphometry.

Quantitation of Apoptosis by Nuclear Fragmentation

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® 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 daunorubucin treatment, and the apoptotic rate was measured directly from nuclear images obtained with the ImageStream system.

Multiplexed Analysis of Beads and Cells

The acquisition of greater amounts of information from a biological sample leads to an increased probability of understanding the underlying biological process. The adoption of cytometric bead arrays (CBA) to simultaneously assess the concentrations of multiple cytokines in a single sample has been a significant technological advance in the field of cytokine biology.

With the use of ImageStream imaging cytometer, we demonstrate the combined capability of imaging cells in flow as well as measuring cytokine concentrations using a commercial cytometric bead array kit. Both cytokine levels and cell classification can be performed simultaneously using the ImageStream cytometer.

Analysis of Complement 3b deposition on Rituximab opsonized cells

The ImageStream cell analysis system provides high resolution, high sensitivity image analysis of cells in flow. Using a similarity algorithm, we were able to quantify the degree of similarity between distributions of two molecules – RTX and C3b – on the surfaces of large populations of Raji and B cells.

The results provided a valuable method for measuring the degree of co-localization of these molecules on both individual cells and cell populations that is not possible by subjective visual inspection techniques. Quantitative analysis of the population of cell images significantly improves the scope and reliability of the conclusions in contrast to the analysis of a few individual cell alone.

Quantitative Analysis of Pseudopod Formation

In the experiment presented here, we used the capabilities of the ImageStream system to quantitate changes in cell morphology during the process of pseudopod formation in a cytokine-dependent cell line and to correlate the morphological changes with the distribution of a marker protein. Using only measurements of cell morphology we were able to follow the process of pseudopod formation in the cell population during recovery from cytokine deprivation. Adding measurements of molecular distribution allowed us to create a comprehensive classification scheme to separate three distinct cell types and identify one atypical cell group. These results offer one striking example of the unique power of analytical morphometry offered by the ImageStream system.

Analysis of the Cellular Fate of a Drug-Conjugated Antibody

This report demonstrates the use of the ImageStream to study the effects of binding of a drug conjugated to a monoclonal antibody (a drug-conjugated antibody, or DcA) specific for a cell-surface molecule expressed on the RAMOS B-lymphoblastoid line, and shows time-dependent internalization and intracellular processing of the mAb. The high optical resolution achieved by the ImageStream system combined with novel image correlation algorithms built into the IDEAS™ data analysis software allow accurate intracellular co-localization of the mAb to endosomes and lysosomes over multiple time points in a large population of treated cells, thereby permitting statistically valid analysis.

Creating New Features using the IDEAS™ Image Analysis Software Feature Manager

The IDEAS software package calculates over 30 standard features for every channel in an ImageStream run -- over 200 features in all. In this report, we look at an example of how a new feature is defined and created in the IDEAS environment with an experiment that involved analysis of apoptotic and necrotic cells.

The IDEAS™ Similarity Score: A Powerful Tool for Comparative Image Analysis with the ImageStream® System

Comparative measurement is a powerful tool in cell biology. Amnis has developed a method of quantitative comparison of multiple images of the same cell, called the similarity score. The algorithm is used, for example, in comparing cytoplasmic vs. nuclear location of NFkB in a nuclear translocation assay. It is also used to assess co-localization of cell surface markers and to evaluate the association of fluorescently labeled probes with components of the endocytic pathway and for many other applications. Overall, the similarity algorithm is one of the most powerful and useful tools in the IDEAS package.

Copies of Amnis Technical and Application Notes can be obtained from Cronus Technologies.

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