Distinguishing Modes of Cell Death Using ImageStream®
Multispectral Imaging Cytometry
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Abstract
ImageStream™ technology allows for multi-spectral imaging of cells in flow. The ImageStream allows for simultaneous measurement of multiple cell features as well as brightfield and darkfield images. Imaging allows for morphological and microspectroscopic identification of cell populations that are difficult to define with traditional flow cytometry. The ImageStream itself uses a combination of optics and computer algorithms to resolve individual cells in a population. The ImageStream technology allows for the simultaneous imaging of multiple features for the discrimination of cells undergoing apoptosis and necrosis. The current study demonstrates the use of the ImageStream 100 platform to discriminate apoptotic, live, and necrotic cells demonstrating the use of the ImageStream 100 platform to discriminate apoptotic, live, and necrotic cells.

Introduction
Death by apoptosis is a complex, tightly regulated process in which a cell undergoes programmed cell death in response to specific internal or external triggers. Physiologic apoptosis is critical for normal development, tissue remodelling and repair. In contrast, cell death that occurs in response to a combination of internal and external factors is termed necrosis. Necrosis is a result of cell destruction in response to specific internal or external triggers. Necrotic cells exhibit markedly less darkfield intensity than apoptotic cells. Microscope equipped with bandpass filters appropriate for FITC (535/40 nm) and 7-AAD (630/60 nm) peroxide-treated necrotic cells stained with annexin V and 7-AAD (R3).

Cytometer (Becton Dickinson). The majority of untreated cells were annexin V-, 7-AAD- (R1).

AAD staining (top row) or forward and side laser light scatter (bottom row) using a FACSort flow cytometer containing annexin V binding buffer (Becton Dickinson Biosciences, Pharmingen, San Diego, CA).

Phosphatidylserine exposure on the cell surface is an early event in the apoptotic process that can be used as a marker for initiation of apoptosis and to discriminate cells that are undergoing apoptosis. Cells dying by apoptosis activate caspase-3 and simultaneously generate brightfield, darkfield, and fluorescence cell imagery to classify cells undergoing apoptosis and necrosis.

ImageStream technology allows for multi-spectral imaging of cells in flow. The ImageStream 100 platform can produce six simultaneous high resolution images of each cell at rates of up to 150 images per second. The ImageStream technology allows for discrimination of cell types not feasible with standard flow cytometry.

Figure 1: Analysis of Cell Death Using Standard Flow Cytometry
Phosphatidylserine exposure on the cell surface is an early event in apoptosis which can be used to discriminate live cells from cells in apoptosis. Annexin V binding is specific to phosphatidylserine which is translocated to the outer leaflet of the plasma membrane early in apoptosis.

Figure 2: Analysis of Cell Death Using Microscopy
Untreated (R1, annexin V−, 7-AAD−) and peroxide-treated (R3, annexin V+, 7-AAD+) tumor cells. The majority of untreated cells were annexin V−, 7-AAD− (R1).

Figure 3: ImageStream Recapitulation of Flow Cytometric Analysis of Cell Death
In order to demonstrate the discrimination of high-fluorescence late apoptotic cells with annexin V binding but no 7-AAD nuclear staining, cells are gated by scatter and annexin V fluorescence. The Imagestream allows the simultaneous acquisition of multiple images per cell in flow cytometry, while also quantitating numerous morphological features such as cell area, perimeter, aspect ratio, texture, spot counts, and others.

Figure 4: Multispectral Imaging Cytometry
The ImageStream technology allows for discrimination of cell types not feasible with standard flow cytometry.

Figure 5: Resolution of Live, Early and Late Apoptotic, and Necrotic Cells Using Multispectral Imaging
Combination of early and late apoptotic cells with brightfield, darkfield, and fluorescence imagery to classify cells undergoing apoptosis and necrosis.

Conclusion
This study demonstrated the ability of the ImageStream 100 platform to discriminate intact annexin V+ and 7-AAD+ tumor cells. The majority of untreated cells were annexin V-, 7-AAD- (R1).

Figure 6: Morphometric Resolution of Necrotic from Late Apoptotic Cells Using Multispectral Imaging
Multispectral imaging allows for discrimination of intact annexin V+ and 7-AAD+ tumor cells from necrotic cells. Necrotic cells exhibit markedly less darkfield intensity than apoptotic cells. Microscope equipped with bandpass filters appropriate for FITC (535/40 nm) and 7-AAD (630/60 nm) peroxide-treated necrotic cells stained with annexin V and 7-AAD (R3).

Figure 7: Morphometric Analysis of Necrotic and Apoptotic Cells Using Multispectral Imaging
Multispectral imaging allows for discrimination of intact annexin V+ and 7-AAD+ tumor cells from necrotic cells. Necrotic cells exhibit markedly less darkfield intensity than apoptotic cells. Microscope equipped with bandpass filters appropriate for FITC (535/40 nm) and 7-AAD (630/60 nm) peroxide-treated necrotic cells stained with annexin V and 7-AAD (R3).

Figure 8: Resolution of Live, Early and Late Apoptotic, and Necrotic Cells Using Multispectral Imaging
Combination of early and late apoptotic cells with brightfield, darkfield, and fluorescence imagery to classify cells undergoing apoptosis and necrosis.

Figure 9: Resolution of Live, Early and Late Apoptotic, and Necrotic Cells Using Multispectral Imaging
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Figure 18: Resolution of Live, Early and Late Apoptotic, and Necrotic Cells Using Multispectral Imaging
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