Development of a 3-color assay for mitosis and apoptosis using the laser-scanning IsoCyte™ and comparison with the microscope-based INCell Analyzer 1000

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Abstract

High Content Screening (HCS) has become increasingly popular for compound profiling in support of lead optimization because it allows quantification of multiple markers at the cellular level, providing information-rich analysis of complex biological systems. However, this information often comes at the price of slower assay speed. Here we describe the evaluation of the IsoCyte™ from Blueshift Biotechnologies, a new instrument which has the capability to combine the best of both High Throughput Screening (HTS) and HCS.

The IsoCyte™ is a fast laser scanning fluorimeter capable of imaging all cells in a single well at a rate closer to HTS requirements, while still providing enhanced cell-by-cell analysis. Using the IsoCyte™ equipped with one 488 nm laser we developed a three-color fluorescence cellular assay with detection of apoptosis by terminal deoxynucleotide transferase dUTP nick end labeling (TUNEL) and detection of mitosis via quantification of phospho-histone H3 levels. Data from the IsoCyte was compared with data obtained using the INCell Analyzer 1000 (GE Healthcare), a traditional automated microscopy-based imager. Despite the different methods and modes of detection, the results from both instruments were very comparable. Our results demonstrate that the IsoCyte™system provides multiplexed intensity measurements with similar accuracy, yet requires significantly less imaging time compared to the INCell Analyzer.

Introduction

Modulation of the cell cycle and processes leading to apoptosis are common targets for pharmacological interventions in drug discovery. Applying high content screening technology, we developed a rapid assay to simultaneously measure these important cellular events using the Blueshift Isocyte™ and validated it against the INCell Analyzer 1000, a more traditional high content imaging platform.

We measured the mitotic index by monitoring levels of the mitotic marker phospho-histone H3 (phospho-Ser28), while apoptotic cells were identified using the TUNEL method to detect single-strand DNA breaks. At the same time, a nuclear stain allowed cell counting as an indication of proliferation.

Method and Detection

Day 1 Plate HeLa cells in media containing 0.5% FBS @ 3000 cells/well in 384-well plates, incubate overnight at 37°C, 5% CO_2

Day 2 Treat cells with compounds, incubate overnight at 37°C, 5% CO_2

Day 3 Fix cells with 1% paraformaldehyde for 30min. @ 4°C
Wash and permeabilize with 70% ethanol

Treat with TUNEL reagents for 1hr

Block with 5% normal goat serum for 1hr

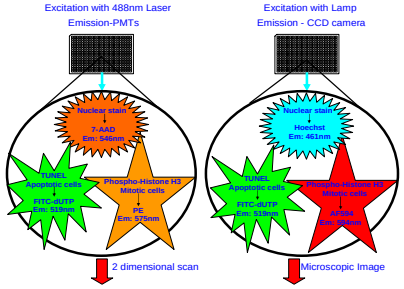
Incubate with primary anti-pHistoneH3 for 1hr

Incubate with secondary phycoerythrin Ab & 7-aminoactinomycin D (7AAD) nuclear stain for 1hr

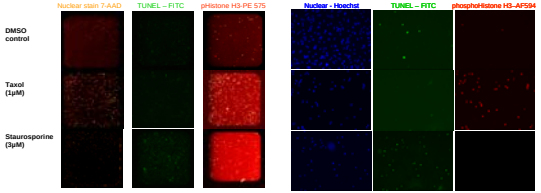
Detection on IsoCyte™

Incubate with secondary Alexa Fluor 594 Ab & Hoechst nuclear stain for 1hr

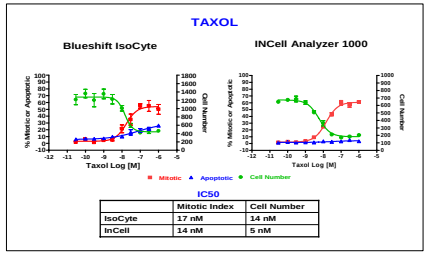
Detection on INCell Analyzer 1000



IsoCyte – Full Well Scan **IN Cell – CCD Camera Field of View**

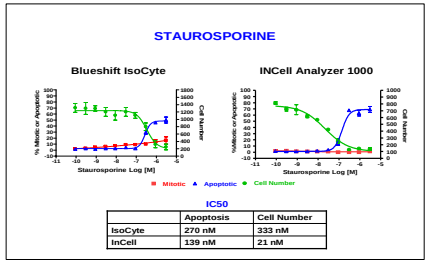


Results



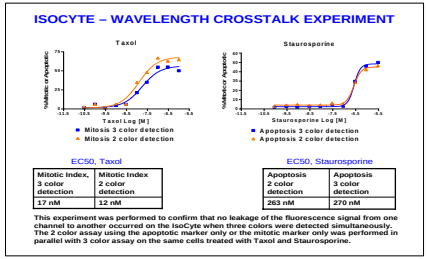
IC50

	Mitotic Index	Cell Number
IsoCyte	17 nM	14 nM
InCell	14 nM	5 nM



IC50

	Apoptosis	Cell Number
IsoCyte	270 nM	333 nM
InCell	139 nM	21 nM



EC50, Taxol

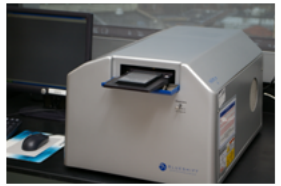
Mitotic Index, 3 color detection	Mitotic Index 2 color detection
17 nM	12 nM

EC50, Staurosporine

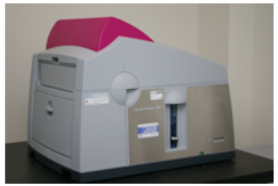
Apoptosis 2 color detection	Apoptosis 3 color detection
263 nM	270 nM

This experiment was performed to confirm that no leakage of the fluorescence signal from one channel to another occurred on the IsoCyte when three colors were detected simultaneously. The 2 color assay using the apoptotic marker only or the mitotic marker only was performed in parallel with 3 color assay on the same cells treated with Taxol and Staurosporine.

BlueShift IsoCyte



InCell Analyzer 1000



INSTRUMENT COMPARISON

	IsoCyte™	INCell Analyzer 1000
Acquisition Speed	~ 2 min / 384 well plate	~ 105 min / 384 plate (3 image fields/well)
Acquisition Area	Whole well in 1 scan	Fraction of a well per image field
Analysis	Simultaneous with acquisition	Separate analysis required
Applications	Best suited for intensity-based measurements	Best suited for measuring morphology changes and subcellular events

Conclusion

We have developed a rapid multi-parametric high content assay for simultaneous detection of apoptosis, mitotic arrest and proliferation in a single microplate well using the IsoCyte laser-based fluorimeter. Our data demonstrates that the IsoCyte is comparable in accuracy to traditional microscope-based imaging system for cell fluorescence intensity measurements, but has the added advantages of fast acquisition with concurrent image analysis. It also images an entire well, collecting data on a much larger population of cells. Based on our evaluation, this type of high content assay on the IsoCyte can be utilized not only for lead optimization but meets the needs of high throughput primary screening. Together the IsoCyte and INCell 1000 are two complementary instruments that allow an HTS group to perform a broad range of high content experiments -- from detecting subcellular features to rapidly analyzing large populations of cells.

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Campbelj, 7/28/2004