High Content Platform For Image Based Screening

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Introduction

Automated microscope based High Content Screening (HCS, or HCA, HCI) has been utilized to study many features simultaneously in complex biology systems in single cell, 3D organoid models or whole organism. HCS can be used all along the preclinical drug discovery pipeline, it has the power to identify and validate new drug targets or lead compounds, to predict *in vivo* toxicity, evaluation of DNA content as an alternative way to evaluate cell cycle and to suggest pathways or molecular targets of orphan compounds. Image-based HCS is a potent drug discovery strategy that characterizes small-molecule effects through the quantification of features that depict cellular changes among or within cell populations, thereby generating valuable data sets for subsequent data analysis. Pharmaron has multiple sets of Operetta® CLS™ and IncuCyte S3 Live cell imaging system to help predict the efficacy of potential drugs in unique cellular niches.

Summary: Pharmaron has established assays to evaluation protein expression or modification in different cellular fraction and substructure formation. HCS is widely used in Pharmaron to support preclinical drug discovery pipeline and made significant milestones for sponsors.

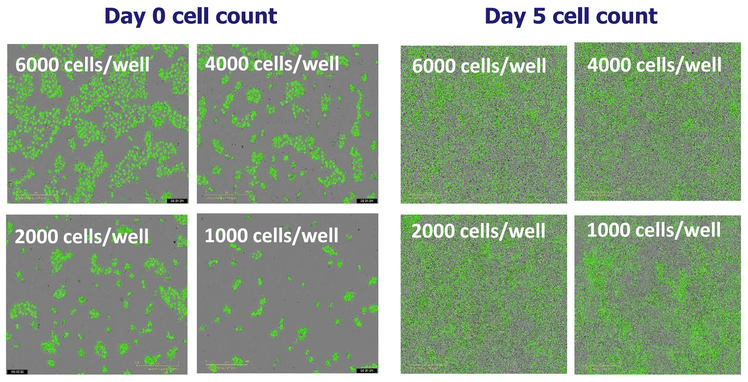
Instruments

IncuCyte S3 Live Cell Analysis Systems
- Temperature, CO_2 and Humidity control
- Realtime and endpoint readout
- Bright Field and Fluorescence
- 96w and 384w
- Up to 20x Image Resolution

Operetta Hight Content Imaging System
- Room environment
- Endpoint readout
- Bright Field and Fluorescence
- 96w and 384w
- Up to 60x water immersion lens

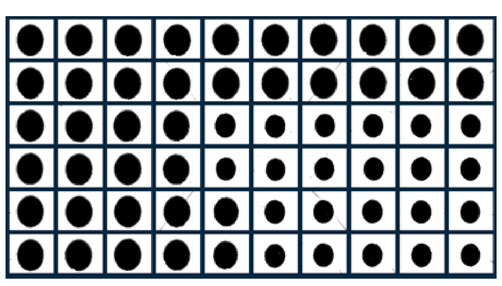
Cell Proliferation (2D & 3D)

Time course detection of cell number using IncuCyte; Demonstrate cell proliferation from different aspect, cell number, cell confluence and cell activity

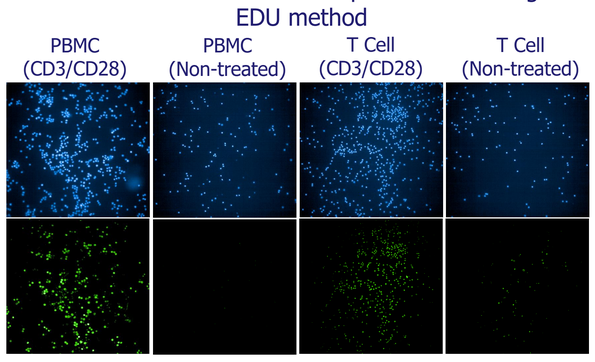


Density (per/ well)	Day0 Object count	Day5 Object count	Growth Fold Cell count	Growth Fold CTG
6000	5893	8875	1.5	1.2
4000	3841	8671	2.3	2.5
2000	2343	8499	3.6	4.2
1000	1056	7943	7.5	9.4
500	415	7265	17.5	16.2

Kinetic monitor of cell spheroid formation in U shape bottom plate

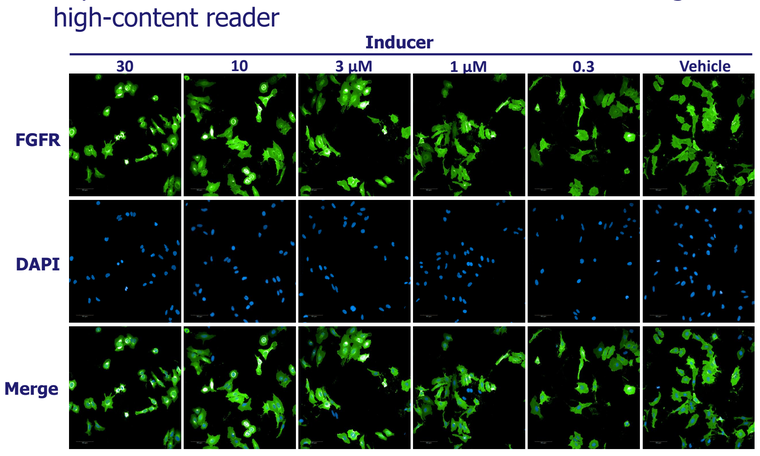


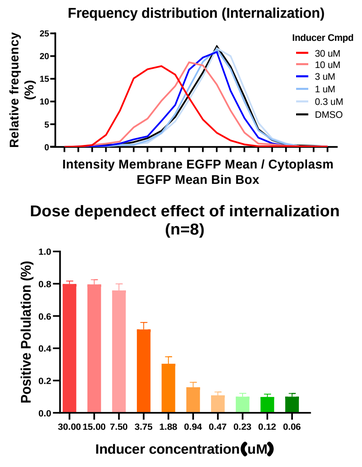
Evaluate hPBMC and T cell proliferation using EDU method



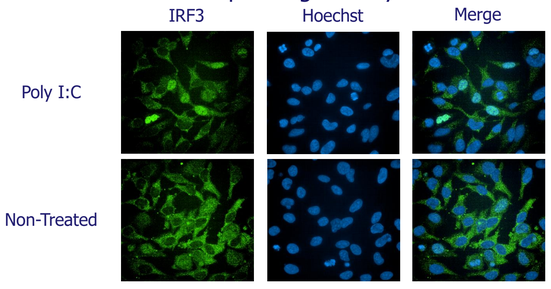
Target Protein Translocation

EGFR fusion protein is overexpressed in HEK293 and dose-dependent membrane internalization is evaluated using high-content reader

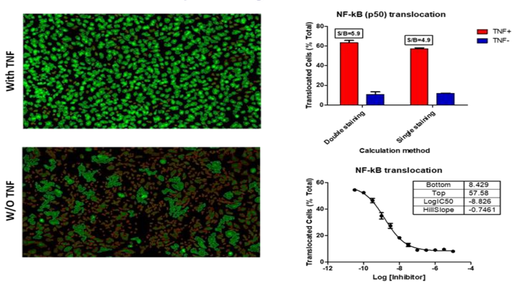




Immunofluorescence evaluation of nuclear internalization pending on Poly I:C stimulation



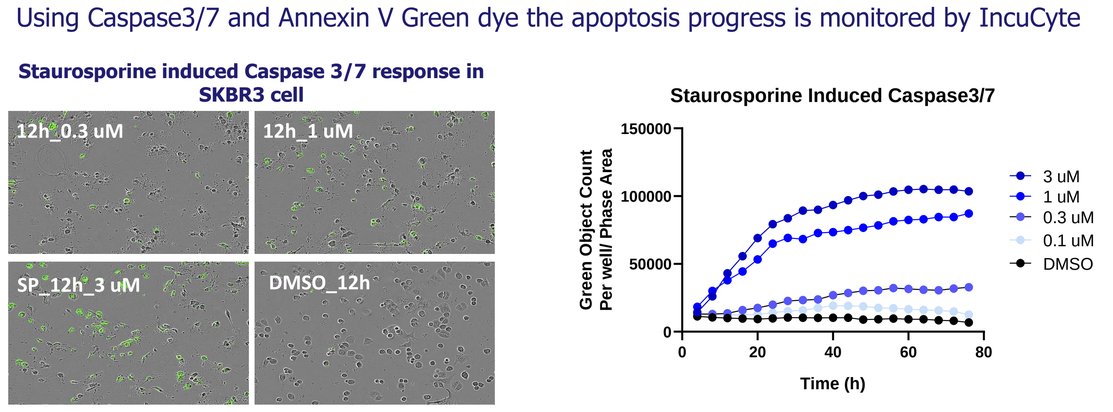
Immunofluorescence evaluation of NFkB Translocation pending on TNFa stimulation



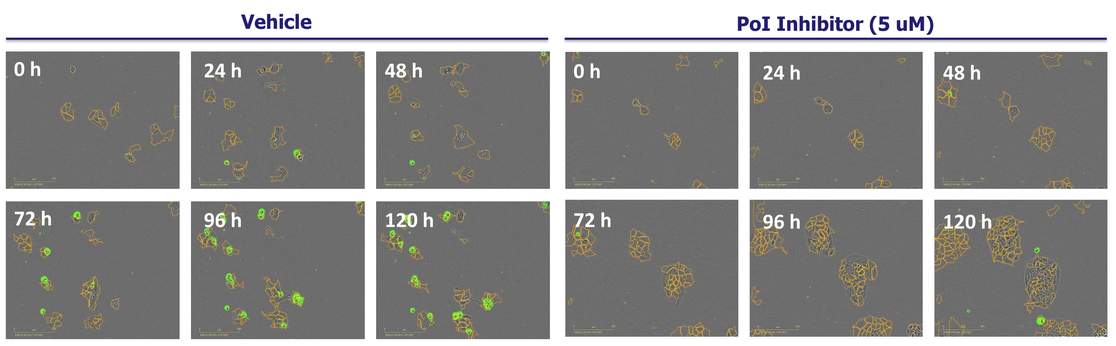
Category	Readout/Event	Realtime
Cell Growth	Confluence	✔
	3D Spheroid formation	✔
	Cell count	✔
	DNA synthesize	-
	Cell Cycle	-
Cell	DNA Damage	-
	Apoptosis	✔
Neurite outgrowth	Neurite outgrowth	✔
Cell functional	Protein expression/modification	-
	Translocation	✔

Realtime Reading of Apoptosis Response

Using Caspase3/7 and Annexin V Green dye the apoptosis progress is monitored by IncuCyte



PoI inhibitor induced Annexin V response in RT112 cell



DNA Damage & Foci Detection

To evaluate DNA damage, gH2AX expression is determined by Immunofluorescence. gH2AX positive cell population correlated well to inducer compound. The gH2AX foci is counted using high resolution high content reader