Complex Cell Based Assays with a Novel Imaging Cytometry System and Object Recognition Software

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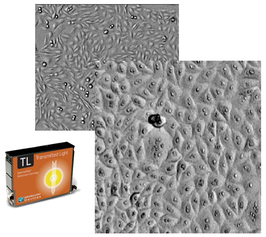
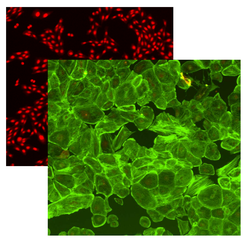
Abstract

There is a need to enable running complex cell-based assays with increased throughput, while maintaining high quality multi-parametric data output. Here we report results from an imaging cytometer integrated into a plate reader platform that provides high-content and high-throughput analysis for cell-based assays. Assays were performed using the SpectraMax® MiniMax™ 300 imaging cytometer using transmitted light, plus 2-channels of fluorescence read-outs. The imager provides the ability for accurate label-free cell count in transmitted light, which enables monitoring of cell proliferation, differentiation, and compound toxicity effects without using any cell staining. The two fluorescence channels make the system useful for a wide variety of assays including monitoring of cell proliferation, transfection efficiency, cell viability/apoptosis, cell differentiation, or cell cycle. The analysis software uses novel algorithms for object recognition that simplify analysis and provide multi-parametric outputs: cell count, cell confluency, average and integrated cell area, averaged and integrated intensities of fluorescent signal. We present several examples of cell-based assays that could be done as kinetic assays. We have demonstrated utility of a new imaging cytometer for biologic research and developed several assay models that will be useful for both academic and bio-pharma environments.

SpectraMax® MiniMax™ Imaging Cytometer

- Combined with SpectraMax® i3 Multimode Plate Reader
- Fluorescence & Transmitted Light Imaging Modes
  - TL Cartridge located in i3
- Robust Design for Trouble-free Operation
  - Proprietary solid state illumination
  - High sensitivity CCD camera
  - Custom laser autofocus
- Excellent Uniformity & Contrast
- Controlled by SoftMax Pro Software





Methods

Hepatotoxicity Assay

•Hela cells were plated 4K/well (96w plate) according to the appropriate protocol. Then cells were treated with anti-cancer compounds for 48 hr. Images were acquired in transmitted light using MiniMax instrument and analyzed by using machine learning cell count and % of covered area algorithms. IC50s calculated using SMP 6.4 software. Assay can be done at different time points.

Hepatotoxicity Assay

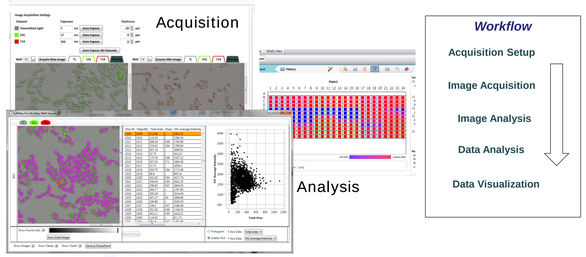
• iPSC-derived hepatocytes (iCell® Hepatocytes) from Cellular Dynamics International (CDI) were plated according to their recommended protocol. Cells were plated at a density of 60K/well (96-well plate) or 15K/well (384-well plate) on collagen coated plates and incubated for 2-3 days. Then cells were treated with appropriate compounds for 72 hr. Cells were stained with Calcein AM (live cells assay, Invitrogen) or AF488 conjugated phalloidin (fixed cells, BD Pharmingen), then images were acquired using MiniMax instrument and analyzed by Cell Proliferation algorithm.

Hematopoietic Differentiation & Toxicity

• Hematopoietic stem cells (CD34+ cells, Lonza) were cultured for 5 days in 96w plates in the presence of indicated cytokines and anti-cancer drugs. Cells were stained with Cell Tracker Green (Invitrogen) or anti-GlycophorinA antibodies (BD Pharmingen), then images were analyzed using Cell Count protocol

Simplified Imaging Cytometry

The new SpectraMax MiniMax Imaging Cytometer has been designed to enable users to easily set up and run their assays. The system is controlled by the industry leading SoftMax Pro software with integrated image analysis capabilities based on MetaMorph™ software. Users can choose from several application protocols and optimize the acquisition and analysis settings for their particular assay cases.

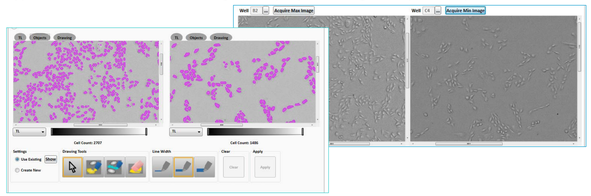


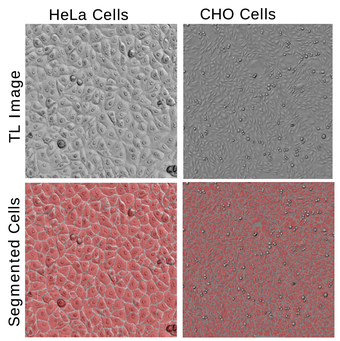
- Intuitive Image Acquisition Setup for 96 and 384 Multi-well Plates
- Standardized Image Analysis Protocols for Cytometry Applications
- Full Data Analysis and Visualization Capabilities of SoftMax Pro Software

Cell Segmentation with TL Imaging

Transmitted light (TL) imaging is very attractive for cell based assays because it is "label-free", can be performed with very short exposures, multiple time-points, without effecting cells. However, segmentation of cells in TL has been challenging due to low cellular contrast and the need for complex processing algorithms.

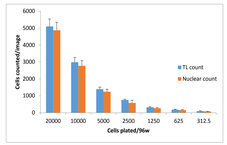
Here we present a novel software program for robustly segmenting multiple cell types that uses machine learning to simplify the image analysis setup.





Cell segmentation in transmitted light for common cell lines

Machine Learning Setup GUI. User draws outlines of wanted objects (e.g., live cells) and selects regions of background or unwanted objects. The SW then calculates the best possible segmentation parameters. A user can then inspect results and further teach the SW program to improve results.

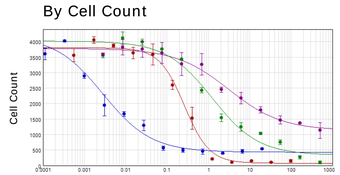


Comparison of the accuracy of cell count by transmitted light segmentation and traditional nuclear count

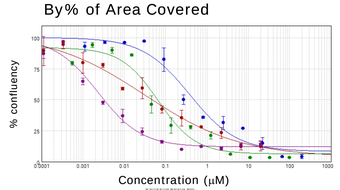
Cell Based Assays

Cell Proliferation / Toxicity Assays
Assessment of efficacy of anti-cancer drugs on proliferation of cancer cells (HeLa)

A cell proliferation assay utilizes time-course assessment of label-free cell count in transmitted light. This allows the building of concentration response curves and measuring IC50s for anti-proliferative drugs or growth factors at selected time points. The assay can be performed at different time points without disturbing cells disturbing cells.

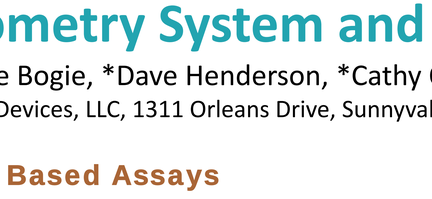


	IC50, μM
Tricostatin	0.25
Etoposide	1.23
Paclitaxel	0.002
Cytarabine	3.3

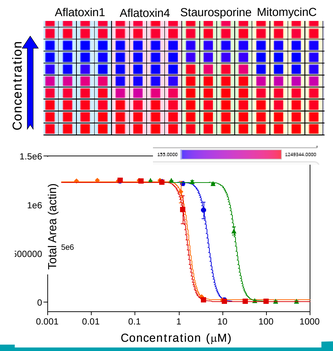


	IC50, μM
Staurosporine	0.020
Paclitaxel	0.002
Mitomycin C	0.403
Doxorubicin	0.063

Hepatotoxicity Assays using iPSC-derived Hepatocytes



Hepatotoxicity was measured using induced pluripotent stem cell (iPSC) derived hepatocytes. Cells were treated with a number of liver toxic drugs for 72 hr. Cell viability was assessed with Calcein AM or AF-488 phalloidin. Toxicity was measured by decrease in cell count or total cell area. IC_{50}s for aflatoxins, staurosporine, etoposide and other hepatotoxic compounds were determined. The assay window was ~200 and Z' values were >0.9.



- Proliferation/toxicity effects were measured by change in cell coverage
- Live cells imaged using cell viability stain
- Assay used to determine IC_{50}s of growth inhibition or toxicity effects by different compounds
- Cells were treated with known toxic compounds and imaged
- Response was measured by Total Area of viable cells

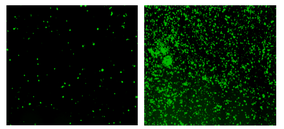
IC_{50}s:	μM
Aflatoxin1	4.7
Aflatoxin4	1.6
Staurosporine	1.7
Mitomycin C	20

The assay window (low concentration value/high concentration value) was ~200 and Z' values were >0.9

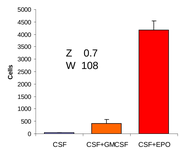
Hematopoietic Stem Cell Differentiation & Toxicity

We have developed an assay suitable for testing the effects of growth factors and anti-cancer drugs on hematopoiesis. Hematopoietic stem cells were cultured in the presence of different growth factors. In addition, effects of several anti-cancer drugs were evaluated. Cells were stained using lineage-specific FITC-conjugated antibodies or cell tracker green. Numbers of cells expressing lineage-specific markers or total cell numbers were detected and IC_{50}s for different factors determined. The assay window was >100 and Z' values were >0.7.

Expansion of Lineage Specific Cells

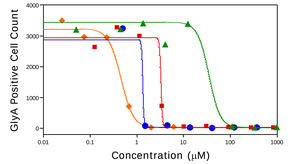


Growth Factor Results



- CD34+ cells were cultured in the presence of different cytokine combinations
- Cells were stained with Cell Tracker Green (total cell count) or GlycophorinA (marker for erythroid progenitors)

Toxicity effects of cancer drugs on erythroid differentiation (anemia)



IC_{50}s:	μM
Fluoroadenin	0.69
Etoposide	2.1
Cytarabine	2.0
Imatinib	100

- CD34+ cells were cultured in the presence of EPO and anti-cancer drugs
- Cell count (expansion/toxicity) was measured

The assay window (high concentration value/low concentration value) was >200 and Z' values were >0.7

Summary

- The SpectraMax MiniMax Imaging Cytometer enables complex phenotypic cell-based assays to aid researchers in drug development and discovery
- Simplified setup and simultaneous image acquisition and analysis speed up users time-to-results for important assays of inflammation response, cell viability, and compound toxicity
- Combined with the SpectraMax i3 Plate Reader, the system allows simultaneous measurements of multiple read-outs combining plate reader and imaging cytometry modes

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