Accelerating Your High Content Imaging: A Case Study Using Neuronal Toxicity Screening

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Introduction

By pairing induced pluripotent stem cells (iPSCs) of human origin with an automated high content imaging system, endpoint and live-cell assays can be used to determine whether pharmaceutical drug candidates or environmental contaminants exert neurotrophic, neuroprotective, or neurotoxic effects. High content imaging of neurons allows scientists to both characterize and measure changes in neuronal networks such as neurite number, length, and branching, as well as to determine gross or specific toxicity reactions. We have developed assays using human iPSC-derived iCell® Neurons from Cellular Dynamics International and high content imaging for evaluation of the toxic effects of different compounds on general viability and ability to produce neurite outgrowths.

One assay measures the dose-dependent effects of Antimycin A and Valinomycin on the mitochondrial membrane potential of neurons in a time-dependent manner. High resolution acquisition over a large area is performed with the ImageXpress® Micro XL system and the images are analyzed with MetaXpress® PowerCore™ High Content Image Processing software. Multiple cytotoxic parameters can be evaluated simultaneously in this multiplexed assay.

In a complementary assay model, cells were allowed to form neurite networks in 96 or 384 well plates and then exposed to toxic compounds for 48 hours. Cells were then stained with a live-cell dye and fluorescently imaged. High content analysis was performed to characterize several parameters including number of processes per cell, length of neurite outgrowth, branching, and number of cells. We have shown a dose-dependent disintegration of networks and general neuronal toxicity.

This case study shows that by acquiring the images using the ImageXpress XL System with its larger field of view and analyzing the images using MetaXpress PowerCore software with multi-core processors, we can dramatically cut time to final results.

Materials & Methods

Cell Treatment

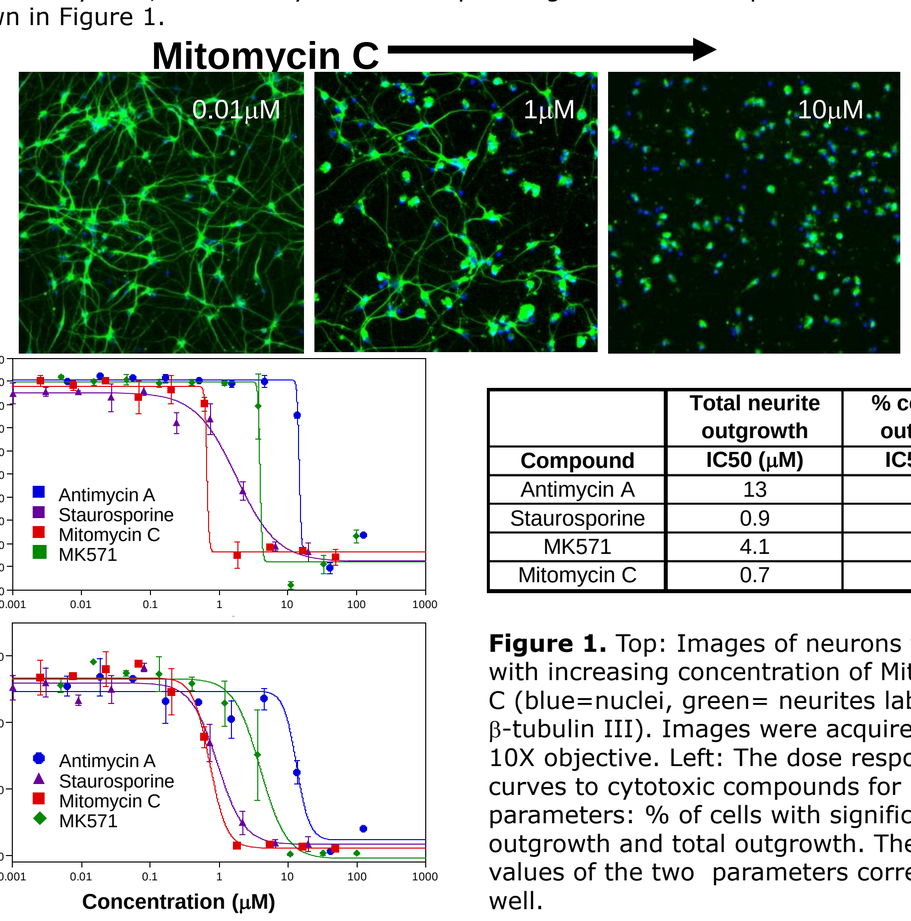
- Aliquots of human iPSC-derived iCell® Neurons from Cellular Dynamics International were thawed and plated into black-walled poly-D-lysine/laminin coated 96 or 384 well microplates according to the provided protocol.
- Cells were cultured for 3-21 days and treated with compounds for up to 2 days
- Live cells were imaged under environmental (CO_2, humidity, and temperature) control
  - Mitochondria were visualized with CellMeter™ JC-10 Assay Kit
  - Outgrowths were visualized with CalceinAM
- Fixed cells were stained with:
  - Hoechst – nuclei
  - Anti-TUJ1-AlexaFluor 488 – TUJ1/microtubule marker

High Content Image Acquisition & Analysis

- Images were acquired with the ImageXpress® Micro XL Widefield System
- Images were analyzed using standard algorithms from MetaXpress® Software including the modules:
  - Neurite Outgrowth – identify number of neurons and number, length or branching of neurite outgrowths
  - Transfluor® – identify up to 2 distinct populations of granules or puncta within cells

Integrity of Neuronal Network

In this toxicity assay, iCell Neurons were allowed to form neurite networks in 96 or 384 well plates and then cultured in the presence of toxic compounds for 48 hours. Neurites and neural networks were visualized with antibodies against β-tubulin (TUJ1) and imaged with the ImageXpress Micro XL system. Several parameters characterizing neuronal networks were analyzed using MetaXpress software. We have shown dose-dependent disintegration of networks and neuronal toxicity using kinase inhibitor Staurosporine, MK 571, Antimycin A, and Mitomycin C. Example images and dose response curves are shown in Figure 1.



Compound	Total neurite outgrowth IC50 (µM)	% cells with outgrowth IC50 (µM)
Antimycin A	13	14
Staurosporine	0.9	1.7
MK571	4.1	3.9
Mitomycin C	0.7	0.7

Figure 1. Top: Images of neurons treated with increasing concentration of Mitomycin C (blue=nuclei, green= neurites labeled for β-tubulin III). Images were acquired with 10X objective. Left: The dose response curves to cytotoxic compounds for 2 parameters: % of cells with significant outgrowth and total outgrowth. The IC50 values of the two parameters correlate well.

Integrity of Mitochondrial Membrane

Mitochondrial depolarization has been shown to be an early sign of hypoxic damage or cell toxicity. We have monitored mitochondria membrane potential using the dye JC-10. In healthy cells, JC-10 accumulates in mitochondria as orange "J-aggregates." As the inner membrane potential is lost, the monomeric form of JC-10 is released into the cytoplasm where it fluoresces green. iCell Neurons were treated with JC-10 and exposed to Antimycin A and Valinomycin for 30 minutes (compounds cause interruption of oxidative respiration and $Ca2^+$ overload) and imaged on the ImageXpress Micro XL system. Images were analyzed using the Transfluor module of MetaXpress software.

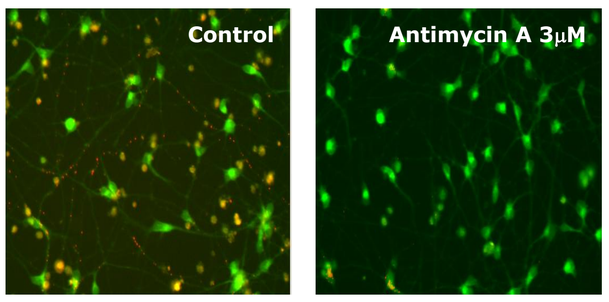


Figure 2. Top: Images of Control neurons and cells treated for 30 mins. with Antimycin A, causing block of oxidative respiration, for and then stained with JC-10. Left: Dose response as measured by total mitochondria JC-10 aggregates.

Compound	IC50 (nM)
Antimycin A	46
Valinomycin	0.15

Improvements to Throughput

Several sites are often acquired and tiled to better visualize neuronal networks. By using a large field-of-view camera, more cells can be captured in each image, requiring the acquisition of fewer sites, and leading to much faster plate read times.

Images in neuronal toxicity studies are often very large (up to 8.9MB/image) and the analysis is computer-intensive, meaning it takes a significant amount of time before the results are available. Simply using faster algorithms in the MetaXpress software modules can improve analysis time by up to 7.3 times (Table 1).

Analysis Module	Speed Improvement
Cell Cycle	3.6X
Live Dead	2.1X
Cell Scoring	4.0X
Transfluor	2.7X
Multi Wavelength Cell Scoring	4.9X
Mitotic Index	5.6X
Count Nuclei	7.3X

Table 1. Measured speed improvements by using the fast algorithm in a sampling of the MetaXpress software modules.

By using the parallel processing capabilities of the PowerCore system, the analysis time could be decreased by over 90%. Two different processor configurations were compared, the highest with 16 physical cores utilizing hyperthreading for the processing capability of 24 cores.

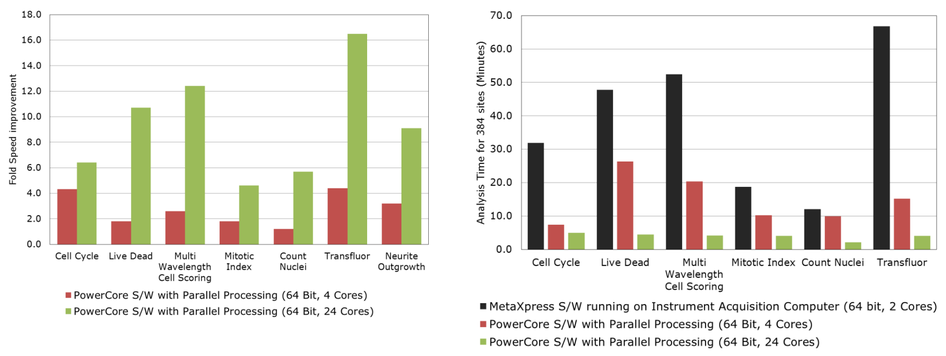


Figure 3. Left: Decreases in overall analysis time are shown for 6 relevant software modules. Right: Analysis speed in increased by up to 16.3 fold using a computer system with 24 Processors.

Summary

- We have demonstrated two high content imaging methods, using iPSC-derived human neurons, that allow evaluation of neuronal toxicity in a screening environment:
  - Neuronal network integrity
  - Mitochondrial membrane integrity
- To maximize high-content screening efficiency:

  1. Acquire images with large field-of-view optics so more cells can be sampled with fewer sites per well, leading to dramatically faster plate acquisition times.

  2. Use new, faster modules in MetaXpress Imaging Software.

  3. Use MetaXpress® PowerCore™ High Content Distributed software with dedicated analysis computers and efficient parallel processing.

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