

Changes in Neurite Outgrowth Detected by the Cellomics Neuronal Profiling BioApplication Are More Sensitive than Traditional Cytotoxicity Assays

Introduction

By the year 2050, nearly 16 million Americans could be affected by Alzheimer's disease, and the current costs associated with caring for and treating those afflicted with the disease equal nearly \$100 billion per yearⁱ. Treatments have focused on blocking the fibrilization of the beta-amyloid₁₋₄₀ (A β) peptide, as research suggests that this is the main neurotoxic event in the disease progressionⁱⁱ. Yet the subtle cascade of effects the neurotoxic peptides and subsequent neuritic plaques have on neuronal cells has been elusive, requiring much time, labor, and expense to determine. With this need in mind, Cellomics[®] (Thermo Fisher Scientific) created the Neuronal Profiling BioApplication to efficiently calculate morphological features of primary neurons, mixed cultures, and neuronal cells lines in response to toxins or therapeutic compound administration. The Neuronal Profiling BioApplication achieves this level of information allowing the most detailed data possible from neuronal cultures.

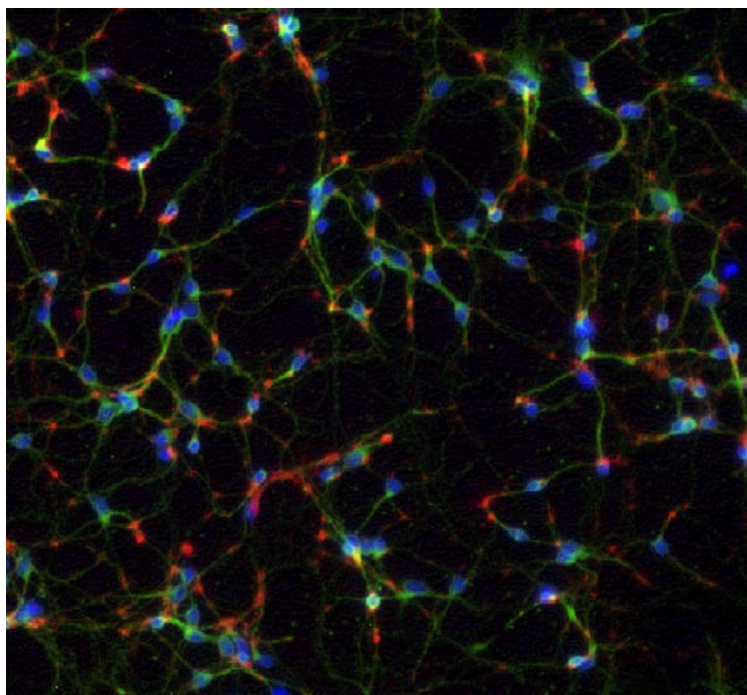


Figure 1: 20x image of primary human cortical culture stained with Hoechst, β 3 tubulin (FITC), and 20 μ M beta-amyloid (TRITC)

Dr. Michael Bova's laboratory at Elan Corporation uses the Cellomics Neuronal Profiling BioApplication to investigate fibrillized and soluble A β 's effects on plates of primary human cortical cultures. To supplement current Alamar Blue viability studies, Dr. Bova's group implemented a High Content Screening approach using the Cellomics ArrayScan[®] V^{TI} HCS Reader and the Neuronal Profiling BioApplication to increase assay sensitivity to A β treatments. Utilizing these tools, the group can make more informed choices when screening compounds for the next agent to combat Alzheimer's disease.

Materials and Methods

Human primary cortical cultures were plated onto poly-D-lysine coated 96-well plates at densities of 25,000 or 50,000 cells per well. The plates were then exposed to

either media alone or media containing 1 μ M fibrillar beta-amyloid₁₋₄₀ (A β) for 1 hour. After the incubation, all media was removed and replaced with media alone or media containing 5, 10, 15, or 20 μ M soluble A β for 1, 2, or 3 days. The cultures were stained with Hoechst 33342 to identify the nuclei, a FITC labeled antibody for human β 3 tubulin labeling both cell bodies and neurites, and a TRITC immunolabeling to identify A β . Cells that were not fixed and stained after treatment were used for Alamar Blue viability testing.

Images were acquired using the Cellomics ArrayScan V^{TI} HCS Reader (Thermo Fisher Scientific, Pittsburgh, PA) and were analyzed with the Neuronal Profiling BioApplication to investigate neuronal morphology after A β administration (Figure 2, left). The Neuronal Profiling BioApplication algorithm created an overlay to measure the cell bodies and neurites within the sample (Figure 2, right). The algorithm assigns neurites to each valid cell body and calculates a variety of outputs to measure neuronal morphology at both the well and individual cell levels.

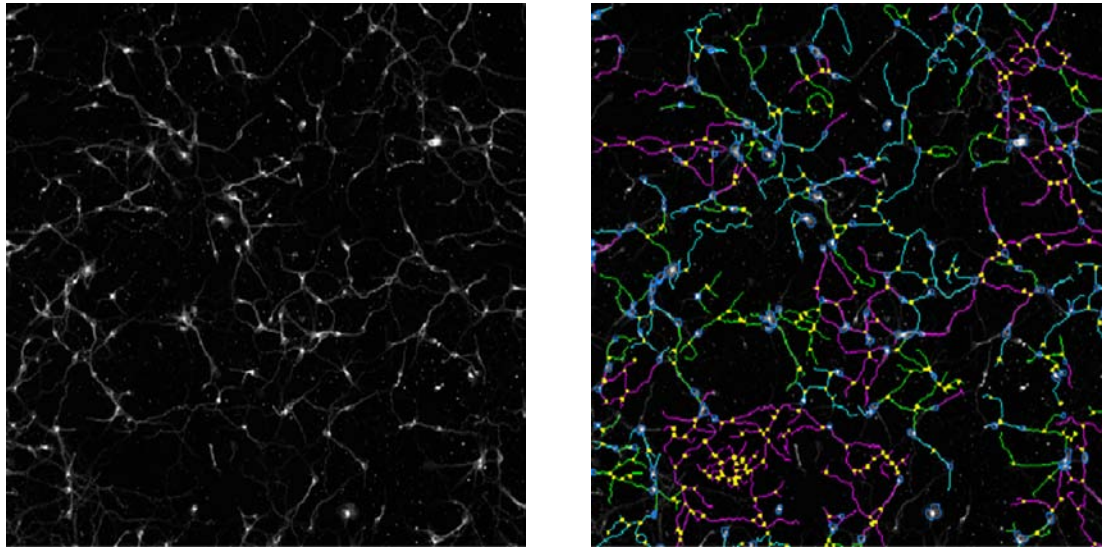


Figure 2. Primary cortical neurons images acquired on the Cellomics ArrayScan V^{TI} HCS Reader at 10x magnification (LEFT) FITC labeled β 3 tubulin gray scale image representing the neuronal cell bodies and neurites. (RIGHT) Overlays created and analyzed by the Neuronal Profiling BioApplication

Results

Abeta toxicity—Human Cortical Neurons (50,000 cells/well)

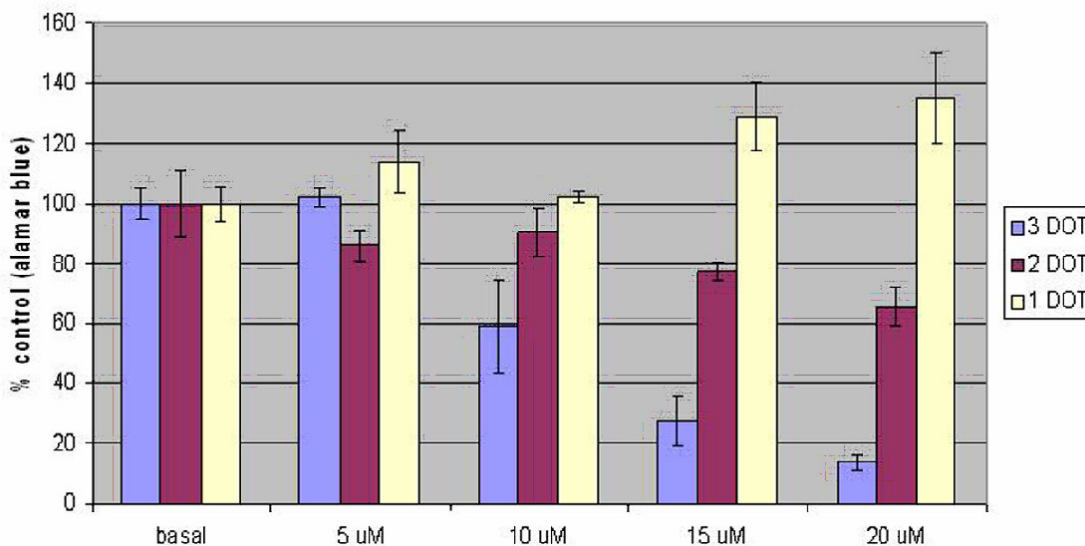


Figure 3. Alamar Blue assay to assess cell viability. This histogram represents viable cells from the Alamar Blue assay on primary cortical cultures treated with 0, 5, 10, 15, or 20 μ M A β for 1, 2, or 3 days of treatment (DOT). The data are represented as % control of media alone (control) wells.

The Alamar Blue test, for the first two days of treatment in the lower concentrations (0 - 10 μ M A β) showed only small changes in live cell count compared to controls (Figure 3). After one day of treatment, no significant cell death was observed at any concentration of A β . However, Alamar Blue did detect a significant decrease in viable cell count at the highest A β concentrations (10 to 20 μ M A β) beyond two days of treatment.

The High Content Screening approach allowed for a more in-depth analysis of the extent of neurite development. The Neuronal Profiling BioApplication calculated morphological features of neurite cross points (Figure 4), neurite average length, neurite maximum length with branches, average branch point count, and average distance of branch point from cell body as percents of control. These morphological measurements showed considerable downward trends after 1 day's time, indicating less-complex neuronal networks with increasing A β doses. In contrast, the Alamar Blue's results hovered close to the 100% of control value across all doses of A β (Figure 4).

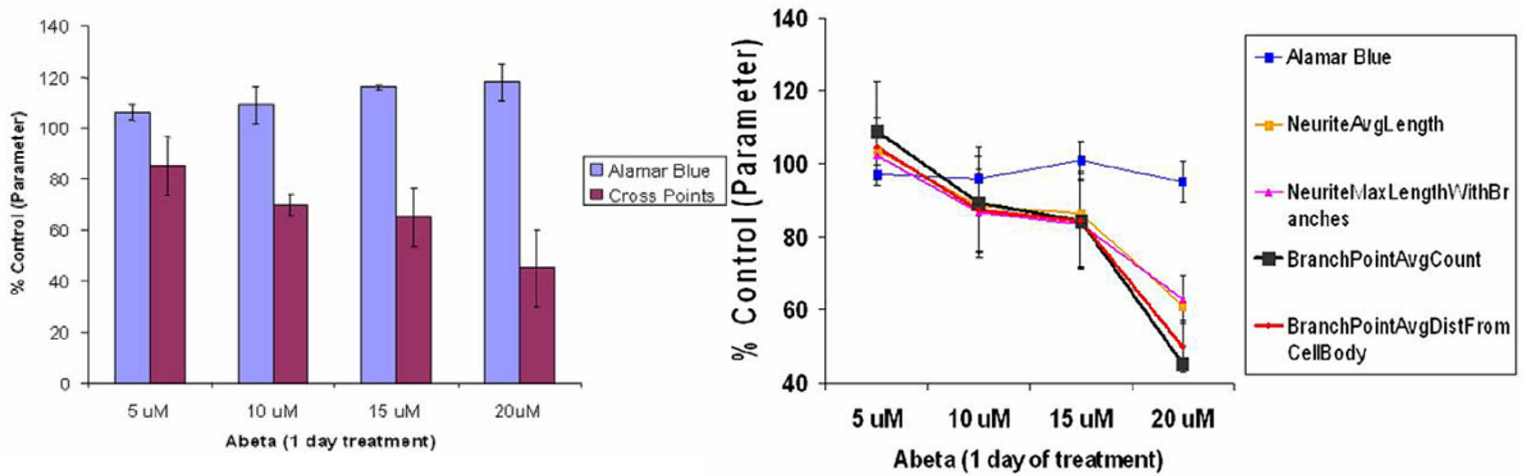


Figure 4. Comparison of Alamar Blue assay to the Neuronal Profiling BioApplication outputs. (Left) This histogram contrasts percentage of Alamar Blue positive neurons compared to mean number of neurite cross points per well, both displayed as % of control, across increasing A β concentrations after 1 day's treatment. (Right) This graph illustrates the differences between morphological changes neurite average length, neurite maximum length with branches, average branch point count, and average distance of branch point from cell body measured by the Neuronal Profiling BioApplication compared to the results achieved by the Alamar Blue assay. All outputs are reported as % of control across increasing A β concentrations over 1 day of treatment.

Similar dose dependent trends were seen over a 3 day treatment of A β (data not shown). The data obtained from the High Content Screening assay clearly show advantages over simple live-dead assays, demonstrating earlier, more subtle, changes in neuronal morphology and health in response to the toxin.

Conclusions

Dr. Bova's research team at Elan Corporation used the Cellomics ArrayScan V^{TI} HCS Reader and the Neuronal Profiling BioApplication to extract data from primary human neuronal cultures exposed to A β . The Neuronal Profiling BioApplication calculated in-depth morphological measurements on thousands of individual primary cortical neurons including: neurite average length, neurite maximum length with branches, average branch point count, and average distance of branch point from cell body. By using a high content screening approach, the group created a more complete picture of neuronal cell health supplementing standard viability assays, permitting future screening for potential therapeutic compounds.

Product	Catalog Numbers
Neuronal Profiling BioApplication	S50-2026-1 (for ArrayScan V ^{TI} HCS Reader, with 0.63x coupler) S50-5026-1 (for KineticScan® HCS Reader and ArrayScan HCS Readers version X.5 or higher)
Neurite Outgrowth BioApplication	S50-2007-1 (for ArrayScan V ^{TI} HCS Reader) S50-5007-1 (for KineticScan HCS Reader) S50-0007-2 (for ArrayScan HCS Readers 3.1/4.0)
ArrayScan® V ^{TI} HCS Reader	N01-0002A (115v) N01-0002B (230v)
cellWoRx™ High Content Cell Analysis System	N01-3001
Neurite Outgrowth HitKit® HCS Reagent Kit	K07-0001-1 (5-plate size) R01-0513-1 (50-plate size)
Neuroscreen™-1 Cells	R04-0001-AP (Academic) R04-0001-CI (Commercial)
SlidePort™ slide insert	N01-0111-1 (1 pack size) N01-0111-3 (3 pack size) N01-0111-5 (5 pack size)

ⁱ <http://www.alz.org/AboutAD/statistics.asp> "Statistics about Alzheimer's Disease." 27 November 2006.

ⁱⁱ Wogulis, M, et al. (February 2, 2005) "Nucleation-Dependent Polymerization is an Essential Component of Amyloid-Mediated Neuronal Cell Death." *Neurobiology of Disease*. **25(5)**: 1071-1080.



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