WHAT IS HIGH CONTENT SCREENING (HCS)?

High content screening (HCS), also known as high content analysis, image cytometry, quantitative cell analysis or automated cell analysis, is an automated method that is used to identify substances that alter the phenotype of a cell in a desired manner. This technology is primarily used in biological research and drug discovery and combines fluorescent microscopy, automated cell calculations, and phenotyping using image processing algorithms and informatics tools for the user to make decisions about a treatment.

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Monitoring Neurite Morphology and Synapse Formation in Primary Neurons for Neurotoxicity Assessments and Drug Screening

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Abstract
Synaptogenesis during nervous system development and degeneration in the pathogenesis of human neurological diseases are highly regulated processes. Subtle changes in the environment of the complex neuronal network may cause either breakdown or creation of synaptic connections. Drug discovery screening for neurological diseases and compound neurotoxicity evaluation would benefit from robust, automated, quantitative in vitro assays that monitor neuronal function. We hypothesized that (1) toxic insults to the nervous system will cause neuronal synapses to deteriorate in the early phase of neurotoxicity, eventually leading to neurite degeneration and neuronal cell death if the damage is severe; and (2) an in vitro functional assay for synapse formation and neuronal morphology could be used to monitor and identify such neurotoxic events. We thus developed an automated, functional, high-content screening imaging assay to track and quantify the dynamic changes in neurite morphology and synapses. This assay identifies primary neuronal cells by a neuron-specific marker and detects synapses on the spines of neurites with pre- and postsynaptic markers. The multiplexed targets, including a nuclear marker, are simultaneously detected with four fluorescent colors, and the fluorescent images of the labeled neurons and synapses are acquired by an automated imaging instrument. The phenotypic features of neuronal morphology and the synapse are automatically identified and quantified on-the-fly by specialized image-analysis software. Such features are potential indicators for neuronal development, differentiation and neurotoxicity, and we could quantify changes in these features under different conditions and for different drug treatments. By monitoring changes in these features, we could also quantitatively evaluate compounds involved in developmental neurotoxicity. In summary, this assay facilitates automation and streamlining of a laborious process in drug discovery screening and compound neurotoxicity assessments; it enables quantitative comparisons between compounds in neuronal morphology and function, particularly in neurite and synapse associated events.

Introduction
Neurons in central and peripheral nervous systems function to transmit electric signals from one location to the other to keep the brain and the body functioning properly. One of the critical structures in the neuron to maintain their proper functional network is synapse, which is the junction between a nerve cell and the cell that receives an impulse from the neuron. The molecular network between these synapses controls not just synaptic signal transmission and synaptic plasticity but also regulates neuronal growth, differentiation and death. The microstructure of synaptic junctions has been extensively studied to understand the relationship between synaptic activity and neuropathophysiology, as well as the molecular mechanism involved in synaptogenesis and the regulation of synapse.

Once synaptic function is disrupted by natural or man-made neurotoxic substances, it could lead to long-lasting and often irreversible neuronal damage. Synaptic damage has often been recognized as the first sign of neurodegeneration in many different pathological conditions, including traumatic nerve injury, ischemic stroke, and many neurodegenerative disorders such as Motor Neuron Disease, Alzheimer’s, Parkinson’s and Huntington’s diseases. Many synaptic proteins play an important role in the progression of neurodegenerative diseases. For example, Amyloid beta precursor protein and Presenilin, alpha-synuclein, Huntingtin, Ataxin-1, Frataxin and Prion protein are all involved in pre-synaptic or post-synaptic structure of the neuron and play a role in synaptic damage and neurodegeneration.

To measure the synaptic changes that occur in synaptogenesis or synaptic damage, we needed to develop a reliable, accurate, and efficient method to measure accurate synaptic loss, neurite changes and neuronal death. Here we introduce a new way of measuring synaptic function utilizing the power of automated, quantitative, high-content cell-based imaging and analysis. The Thermo Scientific Synaptogenesis HCS Assay Reagents combined with the Thermo Scientific ArrayScan High Content Screening (HCS) Reader and Neuronal Profiling BioApplication enables the quantitation of neuronal morphology and synapses in vitro. On-the-fly automated image analysis and quantitation accompanying the automated image acquisition is done by the Neuronal Profiling BioApplication, which is an automated image analysis software module on the ArrayScan™ VTI HCS Reader. Using this technology and assay method, we could identify synaptic changes over time and measure synaptic and neurite parameters in an automated manner.

Synaptogenesis Assay Target Candidates

<table>
<thead>
<tr>
<th>Fluorescence Channel:</th>
<th>Channel 1</th>
<th>Channel 2</th>
<th>Channel 3</th>
<th>Channel 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellular Entity/Targeted:</td>
<td>Nucleus</td>
<td>Cell Body / Neurite Mask</td>
<td>Postsynaptic marker</td>
<td>Presynaptic marker</td>
</tr>
<tr>
<td>Candidates for Cellular Target: (best assay target screened in bold)</td>
<td>DNA</td>
<td>MAP-2, 03-hubulin, Neurillament</td>
<td>PSD95, Syntaxin, synophilin/Neurabin</td>
<td>synaptophysin, synapsin1, synaptotagmin, synaptobrevin</td>
</tr>
<tr>
<td>Fluorescence Dye &amp; Color:</td>
<td>DAPI</td>
<td>Dyl408</td>
<td>Dyl549</td>
<td>Dyl649</td>
</tr>
</tbody>
</table>

Table 1: Potential synaptogenesis HCS assay targets can be detected in four different colors.
The Thermo Scientific HCS Platform Seamless Integration of all the Steps in Cellular Analysis

Automated Measurement of Presynaptic Vesicles and Neurites Using The Thermo Scientific Neuronal Profiling V3.5

Pre-Synaptic Marker, Synaptophysin, Whole Cell Stain and MAP-2

A.

- Mouse cortical neurons 18 DIV
- Thermo Scientific Whole Cell Stain (red)
- Synaptophysin (green)
- Imaged on the ArrayScan VTi HCS Reader

B.

- Rat Hippocampal Neurons 22 DIV
- MAP-2 (green)
- Synaptophysin (red)
- Imaged on the ArrayScan VTi HCS Reader

Figure 1: A. Synaptophysin is a good presynaptic marker. DAPI and Whole Cell Stain is used to detect the nuclei and the fine structure of neurites, respectively (Mouse cortical neuron, 18DIV).

B. Synaptophysin and MAP-2 staining for presynaptic vesicle and neurite detection (Rat hippocampal neuron, 22DIV).

Automated, Simultaneous Measurement of Presynaptic Vesicles, Postsynaptic Structure and Neurites

- Raw Image
  - Presynaptic Marker Synaptophysin (red)
  - Neuronal Marker MAP-2 (green)
- Analyzed Image
  - Branch point (white)
  - Localized Synaptophysin (purple)
  - Neuronal trace (blue)

Figure 2: Mouse cortical neurons (14 DIV) were stained for synaptophysin (red) and MAP-2 (green) (left panel), and analyzed (right panel) with the ArrayScan VTi HCS Reader and the Neuronal Profiling v3.5 BioApplication.

Punctated PSD-95 Stain Increases by Maturation of Neurons

- Figure 3: Rat hippocampal neurons (21 DIV) were stained for synaptophysin, PSD-95 and MAP-2, imaged and analyzed.

  Left panel: Neurite detection with MAP-2 staining.
  Right panel: Postsynaptic marker spot detection with PSD-95 staining (magenta spots) and co-localization with synaptophysin spots (green spot). Co-localized spots (green) represent the location of potential synapses.

- Figure 4: Mouse cortical neurons were cultured for 15 DIV or 21 DIV and stained for synaptophysin, PSD-95 and MAP-2, imaged and analyzed. Only postsynaptic spots stained with PSD-95 antibody increases in 21 DIV neurons compared to 15 DIV neurons (Student’s t-test, p<0.001). Presynaptic spots show no significant change.
Neurite and Synapse Changes as Neurotoxicity Response Against Drug Treatments

Figure 5: Mouse, rat cortical or hippocampal primary neurons were cultured for 21 DIV, and the dose dependent responses of drugs towards various properties of these neurons were investigated. (A) Glutamate with 10 mM glycine in HBSS was treated for 30 min, washed and replaced with culture media. After 24 hr incubation, neurons were fixed, stained and analyzed. (B) Kainate, (C) H2O2, (D) Zinc, (E) U0126 were treated for 24 hrs in culture media. (Student’s t-test, *p<0.05, **p<0.01, ***p<0.001).

Neurite and Synapse Changes as Neurotoxicity Response Against Aβ1-42 Aggregates

Figure 6: Rat hippocampal primary neurons were cultured for 50 DIV. Dose dependent responses of Aβ1-42 aggregates were investigated. 500 mM Aβ1-42 was incubated at 37 °C in media for 3 days to induce oligomerization. Neurons were incubated with the Aβ1-42 oligomers for 48 hrs, and then fixed, stained, and analyzed. Aβ1-42 toxicity leads to synapse loss. (Student’s t-test, *p<0.05, **p<0.01, ***p<0.001).

Summary

Multiparameter Synaptogenesis Assay simultaneously identifies and quantifies neurites, pre- and post-synaptic structures and synapse in an automated manner.

- Neurotoxicity from neurotoxic substances is accurately detected.
- Substances only affecting synapse can be detected.
- Assay works for acute or chronic neurodegenerative disease cell models.


