Quantitate neuron potential

Understanding the signaling mechanisms driving neurite outgrowth provides valuable insight for interpreting neurotoxic compound screening data and for elucidating factors influencing neural regeneration. Inhibition or stimulation of neurite outgrowth is implicated in a broad range of CNS disorders or injuries including stroke, Parkinson’s disease, Alzheimer’s disease, and spinal cord injuries.

Our ImageXpress® automated imaging systems, SpectraMax® i3x reader with MiniMax™ cytometer, and Axon® instruments patch-clamp systems are designed to capture and quantify neuronal activities quickly and accurately.

For more information, visit www.moleculardevices.com
Neurite outgrowth using human iPSC-derived neurons

- Utilize human induced pluripotent stem cells in developmental neurotoxicity and neurotoxicity screening
- Screen compounds for their ability to inhibit neurite outgrowth
- Identify, rank, and prioritize compounds for further *in vivo* testing

Fluorescently stained neurons with red mask overlay generated using MetaXpress software.
Identify compound-specific effects on neurite outgrowth

- Evaluate compound-specific effects on neurite outgrowth
- Quantitate characterization of the extent and complexity of neural networks by multiplexed measurements
- Utilize assay to study neuronal development and neuronal degeneration in vitro

XCell® neurons were treated with compounds for three days and were then fixed and stained with AF488-conjugated anti-ß-tubulin antibodies. Images were taken by the ImageXpress™ Pico system using the 10X Plan Fluor objective and FITC and DAPI channels. Images were processed using the Neurite Tracing analysis protocol. Analysis masks show the outgrowths (green), cell bodies (blue), and branching points (yellow).
Visualizing subcellular vesicles to quantitate autophagy in neuronal cells

- Segment unlabeled neurites accurately
- Gain deeper insights into mechanisms of neurodegenerative disease
- Discriminate against similar subcellular structures with precision
- Generate rich data by measuring multiple biologies in one well

Visualizing autophagy. The process of autophagy begins when an internal or external signal promotes the formation of a phagophore, a spherical double-membrane sequestering structure. Microtubule-associated protein 1 light chain 3 alpha (LC3) stimulates elongation of the phagophore, which begins to engulf cytoplasmic targets. In a process mediated by autophagy-related (ATG) proteins, the phagophore closes around the targets to become an autophagosome. The autophagosome then fuses with a lysosome, exposing its contents and its own membrane to degradation by hydrolytic enzymes. Reference for Figure: Mizushima, Yoshimori and Levine (2010) Methods in Mammalian Autophagy Research. Cell 140:313-326.
• Better predict neural physiology and neurotoxicity earlier in drug discovery

• Evaluate compound effects on neuronal development

• Learn how to scale up high-content assays to screening

Red overlay shows outgrowths and cell bodies identified by the MetaXpress® software Neurite Outgrowth module.
Measuring neurite outgrowth with the SpectraMax MiniMax cytometer

- Acquire neuronal images easily using the SpectraMax MiniMax cytometer
- Accelerate time to results using MetaMorph software’s automated image processing and analysis tools as compared to manual microscopy
- Automatically generate detailed neurite outgrowth data such as number of processes, process length, and total measured outgrowth

**Neuron image montage.** Raw images were acquired using the MiniMax cytometer and stitched together using MetaMorph® software. This image montage was then used for neurite outgrowth analysis.
Signaling dynamics on neural stem cell differentiation

- Gain further insights into the biology of adult neurogenesis
- Identify the effects of continuous vs. high frequency signaling on neuronal differentiation
- Understand why the overexpression or CRISPR/Cas9 mediated knockdown of one cell cycle regulatory factor alters cell differentiation and survival

Optogenetically-induced neurogenesis in a Greiner black-walled 96-well plate, 488 nm channel taken at 10X objective on the ImageXpress® Micro system, courtesy of Alyssa Rosenbloom, UC Berkeley.
Accelerate mechanistic study in reception and transmission using electrophysiological studies

- Elucidate the signaling mechanism differences in the circadian system between the central and peripheral nervous systems
- Use long-term potentiation studies and field electrophysiology to study the mechanisms of long-term memory and their effects in neuropsychiatric and neurological disorders in rats

Cue-based amygdala synaptic pathway.
Investigations of the effects of amyloid-beta proteins on hSlo1.1, a BK channel, in a xenopus oocyte model

- Investigate how various Aβ conformations interact with hSlo 1.1 Ion Channels
- Learn how two-electrode-voltage-clamp is used to quantify the effects of both extracellular and intracellular Aβ conformations on hSlo 1.1 mediated currents

Diagram illustrating the cleavage of Aβ from the amyloid precursor proteins.
Functional and mechanistic neurotoxicity profiling using 3D neural cultures

- Evaluate the neurotoxicity profiling of a library of environmental chemicals and drugs
- Characterize calcium oscillation profiles in neurospheroids with multi-parametric analysis
- Assess cellular and mitochondrial toxicity using high-content imaging

Neural spheroids respond to glutamate agonist and antagonist, GABAergic agonists, lidocaine, and the antipsychotic medications.
Morphological characterization of neuronal development in 3D matrix

- Develop 3D neurotoxicity assays for compound screening
- Leverage hydrogels and high-content imaging for high-throughput 3D neurite outgrowth assays
- Generate quantitative measurements that can be used to define IC$_{50}$ values and compare toxicities of various compounds

CNS.4U® cells were seeded on 3DProSeed® hydrogels from Ectica Technologies and imaged at 14 days in confocal mode. Nuclei were stained with Hoechst (red) and tubulin –TUJ-1 (green). Four panels represent different focal planes. The network of neurites extends several hundreds of micrometers into the hydrogel.
Morphological characterization of 3D neuronal networks in a microfluidic platform

- Establish a high-throughput 3D neurite outgrowth assay using iPSC-derived neurons
- Generate more in vivo-like results using the microfluidic OrganoPlate platform
- Optimize high-content imaging for evaluation of treatment effects on neuronal networks

Live cell staining of neurons. iCell® neurons plated into OrganoPlate® capillary wells, in Matrigel. After 5 days, live cells were stained and imaged using the ImageXpress® Micro Confocal system in confocal mode using the 60 µm pinhole at 20x magnification. 25 z plane images 6 µm apart. Maximum projection composite images are shown here for the DAPI and FITC channels.
StemoniX® microBrain® 3D Assay Ready Plates for HTS

- Easily integrate 3D spheroids for high-throughput screening
- Generate consistent results with minimal variability across the plate
- Enable scalable, high-content, image-based 3D assays

**Human iPSC-derived neural spheroids.** Human iPSC-derived neural spheroids cultured to 600 µM in diameter. Low magnification transmitted light image (top left) as well as 20X magnification fluorescent images were acquired using ImageXpress Micro Confocal Imaging System. The spheroids are composed of a co-culture of active cortical neurons (identified by MAP2; green) and astrocytes (GFAP; red).
Customer Breakthrough:
MIMETAS uses the ImageXpress Micro Confocal system to develop tissue models for their OrganoPlates

Challenge
Need for more predictive tissue models for the pharmaceutical industry to speed up and improve screening and development of new medicines and personalized therapies.

Solution
MIMETAS offers the OrganoPlate, a unique 3D organ-on-a-chip platform.

Technology
ImageXpress Micro Confocal system

Results
A variety of miniaturized organ models has been developed which can be further combined with other tissue cultures to establish vascularized tissue models.

iPSC-derived neurons growing in an OrganoPlate®. At seeding, cells are globules. Within 24 hours, we observe a fully functional connected neural network.
Customer Breakthrough: 
StemoniX uses three of our solutions to validate their microBrain 3D Assay Ready Plates

Challenge
Current models available to neuroscience researchers have limitations that hamper the development of new medicines.

Solution
Scientists at StemoniX tackled this gap by developing the microBrain 3D platform.

Technology
ImageXpress Micro Confocal system, FLIPR Tetra system, SpectraMax i3x plate reader

Results
Integration of microBrain 3D in multiple platforms resulted in extensive characterization of the toxicological profile of a targeted library and demonstrated the feasibility of integrating this platform for in vitro investigations.

3D neurospheroid grown on the StemoniX® microBrain® Assay Ready Plate; imaged using the ImageXpress Micro Confocal system.