Neural cell differentiation

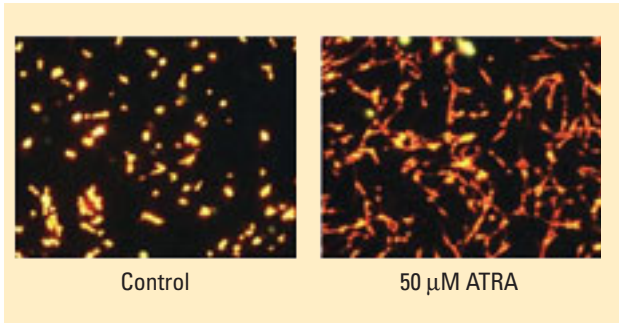
HCS confronts the challenges for high-throughput and secondary screening assays.

BY PAUL WYLIE

High-content screening (HCS) is an emerging technology that has become a reality only in the last few years. By analyzing fluorescently tagged markers, researchers can track cellular events in response to challenge by drugs. HCS also allows multiple cellular effects of a drug to be identified simultaneously, such as the primary drug effect and its toxicity. The technique has the advantage of being performed in the whole-cell environment, where all intracellular components are present in the correct location and in native quantities, which increases the relevance of drug screening.

In neuroscience research, many projects are focused on identifying drugs that affect the growth of neurites. The discovery and characterization of compounds and new chemical entities that promote or suppress neuritogenesis are of great importance in the search for therapies to treat neurodegenerative illnesses, such as Alzheimer's disease and Parkinson's disease, as well as trauma that results in neuropathy and nerve injury, including stroke and spinal cord injuries. In this article, the role of HCS technologies in neuroscience research is discussed, and the functions they can play in both primary and secondary screening assays are highlighted.

Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), and other neurodegenerative diseases are the subject of intensive research and investigation. Research is concentrated in the following areas: (1) development of biomarkers of preclinical disease to identify people at risk for selected environmental toxicants and to identify people who would benefit from neuroprotective drugs, (2) epidemiological studies to identify specific agents and/or combinations of chemicals associated with an increased risk of neurodegenerative disorders, (3) development of models of chronic exposure to environmental agents and potentiating chemical interactions leading to neuronal injury, (4) studies using genetically modified animals to identify increased susceptibility to environmentally induced neurodegeneration, and (5) studies on the effects of aging and of inflammation on toxicant-induced neurodegeneration. With these areas of research key to the potential of providing therapeutic benefits, there is a fundamental need for the ability to screen agents against whole-cell models of neuritogenesis and neurodegeneration.



SHSY-5Y differentiation. Acumen Explorer images of 4-Di-1-ASP-stained control and 7-day ATRA-stimulated cells (ATRA is all-*trans*-retinoic acid). Stimulated cells display the distinct formation of interconnecting neurite outgrowths.

High-content screening

Although yet to be widely adopted in drug discovery, HCS is beginning to have a role in the design of drug screening programs, including those used in neuroscience research. It offers both multiparameter and cell-based information in a screening-feasible format and has been defined as an assay that can provide more than just yes–no answers to multiple questions. HCS assays can investigate multiple cellular responses in a single assay, for example, the inhibition of a binding event can be determined by a compound and, at the same time, the potential cytotoxicity of that compound can be determined (*1*). The main difference between HCS assays and traditional high-throughput screening (HTS) assays is that in HCS, information is gathered on a cell-by-cell basis rather than on a well-by-well basis. This allows the researcher to gain information on variations of response in the cell population rather than just record a mean response from all cells in the well. Therefore, within a screen, each individual cell can be characterized according to the response it exhibits to a compound.

The first major breakthrough in HCS came from Cellomics, which combined automated microscopy techniques with photographic detectors to view and record digitized images requiring computing hardware and software to analyze, store, and retrieve (*2*).

For neuritogenesis assays, different technologies are generally suited to either primary or secondary screens. Two major types of instrumentation have been developed for HCS, fluorescence microscopy and laser-scanning cytometry.

Image-based fluorescence microscopy systems, such as the InCell Analyzer 1000 and 3000 (from GE Amersham), ArrayScan and KineticScan (Cellomics), and Discovery-1 (Molecular Devices) use a charge-coupled device camera to generate cell images. An image is subsequently processed by software algorithms to determine cell morphology, cell number, and changes in subcellular dye localization. These systems offer high-information data at high resolution, such as the identification of neurite outgrowth, but they also introduce a new concern, namely the generation of vast quantities of data (up to terabyte levels) when used in a primary screening environment, and therefore require expensive solutions for data storage and retrieval. However, many such systems cannot run at the speeds necessary to realistically perform screens of over 100,000 compounds.

In contrast to the above systems, the

Acumen Explorer, a laser-scanning fluorescence microplate cytometer, can rapidly detect and quantify all fluorescent objects in 96- to 1536-well formats and beyond. The Acumen Explorer differs from fluorescence microscopy systems in that it does not use microscope objectives or autofocusing optics during scanning. Because of focus-free, area-based scanning, the system permits multiplexed, whole-well HCS analysis with exceptionally fast read times, down to 3 min/plate, which is compatible with primary screening timelines. The algorithms use thresholding to identify fluorescent objects in a well, resulting in much smaller file sizes than those obtained by fluorescence microscopy systems.

The role of HCS in drug screening programs has grown rapidly over the last 3–4 years, primarily because of improvements in instrumentation and associated software. Other major advances include the application of fluorescent biomolecules, such as green fluorescent protein, which are used as intracellular protein markers. Labeled antibodies can also be extensively used to determine end-point analyses. Finally, there are growing numbers of fluorescently labeled dyes that stain a plethora of cellular structures, all of which can be used for cellular analysis. These advances let researchers analyze multicolor fluorescence and hence screen for multiple cellular readouts using a single assay in either fixed or live cells.

Neuritogenesis assays

Traditionally, assays for neurite outgrowth have used subjective, labor-intensive methods such as manually counting cells using fluorescence or confocal microscopy, but more recently, automated methods have become available. Automated monitoring and quantification of neurite formation and outgrowth in multiple samples have greatly enhanced therapeutic investigations in the neurobiological sciences, particularly in drug screening and the drug discovery process in general (*3*). However, these methods still offer relatively low throughput in terms of detection when compared with other higher-throughput screens, and they are limited to targeted screens or purely secondary screens. Another disadvantage is that these methods, and the instrumentation used, often require specialist cell lines—for example, Neuroscreen-1 cells for the ArrayScan screening assay—specific vendor-supplied algorithms, and the use of sometimes costly antibodies directed against proteins found in neurites.

The ArrayScan assay utilizes PC12 cells, a rat pheochromocytoma cell line widely used as a standard model system for neurons (*4*). The assay identifies neurites using a primary antibody directed against tubulin and an Alexa Fluor 488 secondary antibody. An algorithm is applied to analyze the images with an option of applying Neuroscreen-1 cells, which are a subclone of the PC12 cells, for higher-throughput screening. The primary advantages of using these cells is that they grow 50–80% faster than wild-type PC12 cells and have a high and accelerated responsiveness to nerve growth factor (measurable neurites appear within 2 days rather than 6–8 days), making them more amenable to higher-throughput screening assays.

The Discovery-1 system uses embryonic mouse day 13.5 trigeminal neurons. The neurons are labeled using anti-PGP9.5, followed by Alexa Fluor 488 secondary antibody. Like the ArrayScan assay, vendor-supplied algorithms are required to analyze the images and determine changes in cell morphology, including straightness and number of branch points.

Finally, the Acumen Explorer-based assay measures neuronal cell differentiation of SH-SY5Y cells, a human neuroblastoma cell line, by the incorporation of a membrane dye (see figure). The major advantage of using a human cell line is the potential to provide data that is more relevant to the likely therapeutic effects of compounds when applied to human biology. Unlike other assays, the Acumen Explorer uses live cells rather than fixed cells, allowing changes to be tracked over several days. The assay does not measure the primary morphological change in neurite formation, but, instead, it utilizes changes in dye intensity as a secondary indicator of cell differentiation. Because of this method of analysis, the Acumen Explorer assay is not as precise as microscope-based systems. However, the assay can rapidly analyze 96- or 384-well plates with plate read times on the order of 8 min/plate, thereby making it possible to run primary screens. Additionally, the open architecture of the Acumen Explorer software offers the potential for creating user-specific assays, for example, by changing cell lines or dyes, without having to purchase new algorithms.

Rapid and slow HCS

The Acumen Explorer offers a rapid, primary screening solution to neuronal cell differentiation with the potential to run up to 20,000 compounds/day. However, the assay gives a less precise determination of neuronal cell differentiation, in contrast to the microscope-based HCS systems, but the Acumen Explorer is able to identify hits from a large compound library primary screen. As stated above, for neuritogenesis assays, different technologies are generally suited to either primary or secondary screens, and in general, two major types of instrumentation have been developed for HCS, fluorescence microscopy and laser-scanning cytometry. The possible role that both these technologies could play in these methodologies has been discussed in this article. Because of their slower read times—it routinely takes in excess of an hour to read and analyze a 96-well plate—they are ideally placed to run secondary screens, where more time is available to gain a greater level of information, rather than primary screening on smaller targeted libraries. Together, these technologies may form a complementary combination to allow neuritogenesis screens in a high-content system.

Therefore, through a combination of currently available HCS technologies, it is possible to run a fast primary screen and a slower secondary neuritogenesis screen using a high-content format in whole cells to determine morphological changes in response to drugs.

References

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