

Definiens Developer and Definiens Cellenger

Analysis of Neurite Outgrowth in Cultured Primary Neurons

Summary

Treatment of neurodegenerative and neuropathic diseases is likely to involve neurotrophic factors, given their role in neuronal growth, differentiation and plasticity. *In-vitro* evaluation of small molecules for the treatment of such conditions often involves morphological quantification of primary neuronal cultures. These cultures are often heterogeneous and require robust analysis to extract meaningful information from the image data. Here we demonstrate a neurite outgrowth assay on primary cortical neurones, followed by analysis with Definiens Developer and Definiens Cellenger.

Introduction

Neurite outgrowth is a complex process that occurs in three stages: (1) initiation of neurite formation (sprouting), (2) elongation of neurites over long distances and guidance of their growth cones to the appropriate target (path finding) and (3) synapse formation and functional maturation of the newly formed connections. Neurotrophic factors are essential for neuronal survival and play an important role in neuronal growth, differentiation and plasticity. The identification of small, brainpenetrant, neurotrophic molecule mimetics would be a major breakthrough in the treatment of neurodegenerative diseases and psychiatric illnesses as disease modifying agents.

In-vitro analysis of candidate compounds on primary cultures forms an essential part of the development process. Nonetheless, automated analysis of such cultures is a non-trivial exercise, given the heterogeneity of the neurons encountered and their fragility during the staining process (figure 1). In this application note we demonstrate how the objectbased analysis approach of Definiens Developer and Definiens Cellenger was used to analyze outgrowth in primary cortical neurons.

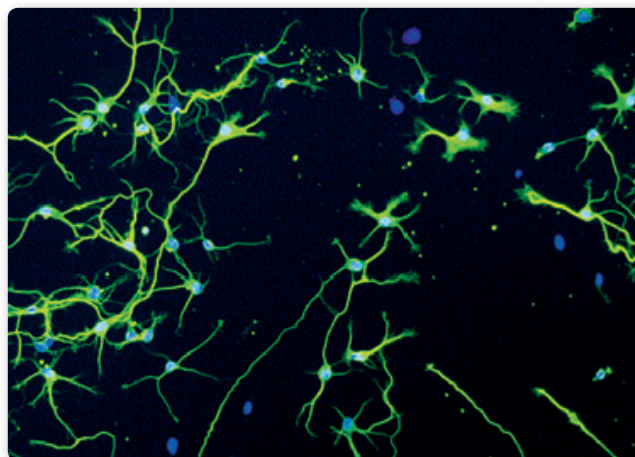


Figure 1 Primary culture of cortical neurons, demonstrating the heterogeneity of structures encountered during a typical assay. Neurons and neurites are labelled green, nuclei blue.

Method

Dissociated E18 embryonic rat cortical neurones were seeded at 2500 cells/well in 96W poly-D-lysine coated culture plates in the absence or presence of compounds. After incubation at 37°C for 48 hours, the cells were fixed in 2% paraformaldehyde containing 5% sucrose and immunostained with an antibody to a marker expressed in the cell bodies and neurites using the NOG HitKit (Cellomics Inc). The plates were then read on an Arrayscan high-content imaging instrument (Cellomics Inc), with at least 4 fields per well captured for analysis. Images were analyzed using a custom module written for the Definiens Cellenger application plug-in using Definiens Developer.

KEY WORDS

Image Analysis – Neurodegeneration – Neurotrophic factors – Primary Neuronal Culture – Neuronal Differentiation – Neuronal Plasticity – Definiens Cellenger – Definiens Developer

Result

The analysis generated a network of image objects which were subsequently used to generate numerical data. At the highest level, entire neuronal objects were generated, which could be used to extract data at the level of individual neurons. Neurons which displayed little or no outgrowth were eliminated from the analysis. Lower levels in the hierarchy identified neuronal subcomponents, such as neurites, branchpoints and nuclei, and were used to generate relational information specific to each neuron (figure 2).

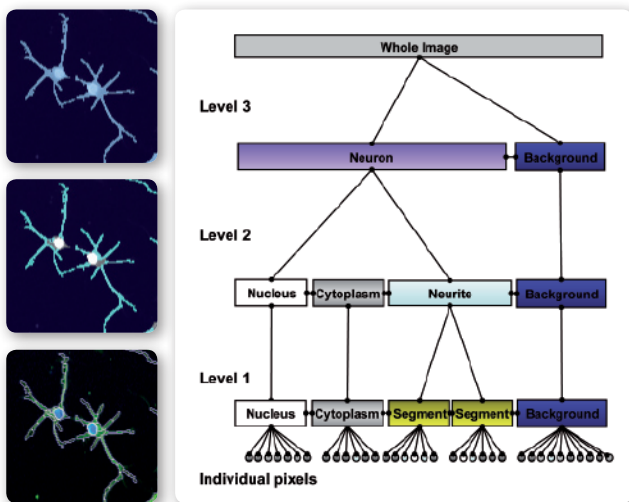


Figure 2 Image object hierarchy

Network of image objects generated by the analysis: whole neurons are represented at the top level; neurites and cellular components on the level below and sub-components of the neurites at the lowest level.

Cells were treated with two different compounds, and a number of metrics were selected to evaluate neurite extension, including total neurite length, mean branch length per neuron, mean number of branches per neuron and mean area of the largest neuron per field.

Figure 3 shows a platemap of the data, along with representative images; the plate map shows a response with treatment by compound A (row A, wells 1-10) showing this compound promotes neurite extension in cortical neurons, whereas compound B (row B, wells 1-10) is inactive.

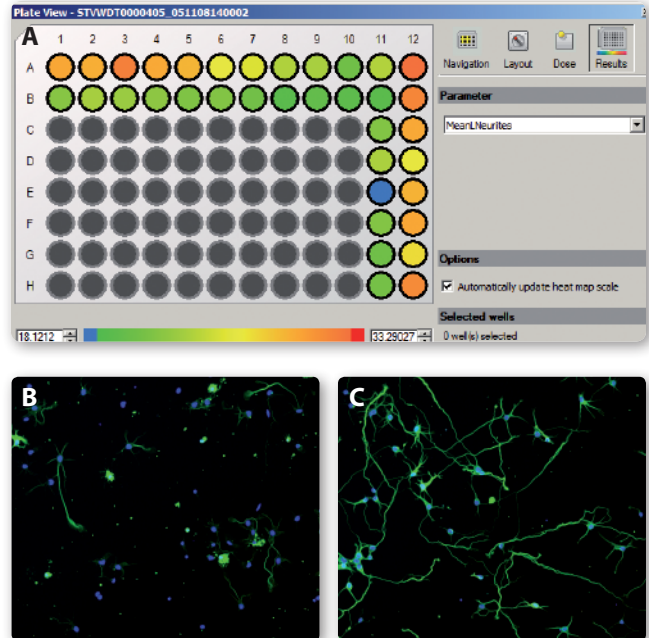


Figure 3 Platemap and representative images

A) Platemap showing mean number of branches per neuron for the wells illustrated. Row A, 1-10 shows cells which respond to treatment with compound in a dose-dependent manner (A1, high dose; A10 low dose). Cells in Row B, 1-10 do not respond to treatment with a different compound. Wells in columns 11 and 12 are negative and positive controls, respectively. Representative images of negative (B) and positive (C) controls are shown.

Figure 4 shows dose-response curves for the readouts of total neurite length, mean branch length and mean number of branches per neuron. It is clear from this data that the largest effect of the compound is observed with the metric made across the entire image (total neurite length) as opposed to the data points averaged across the individual neurons. This may be due to the relatively low numbers of neurons per field in this dataset.

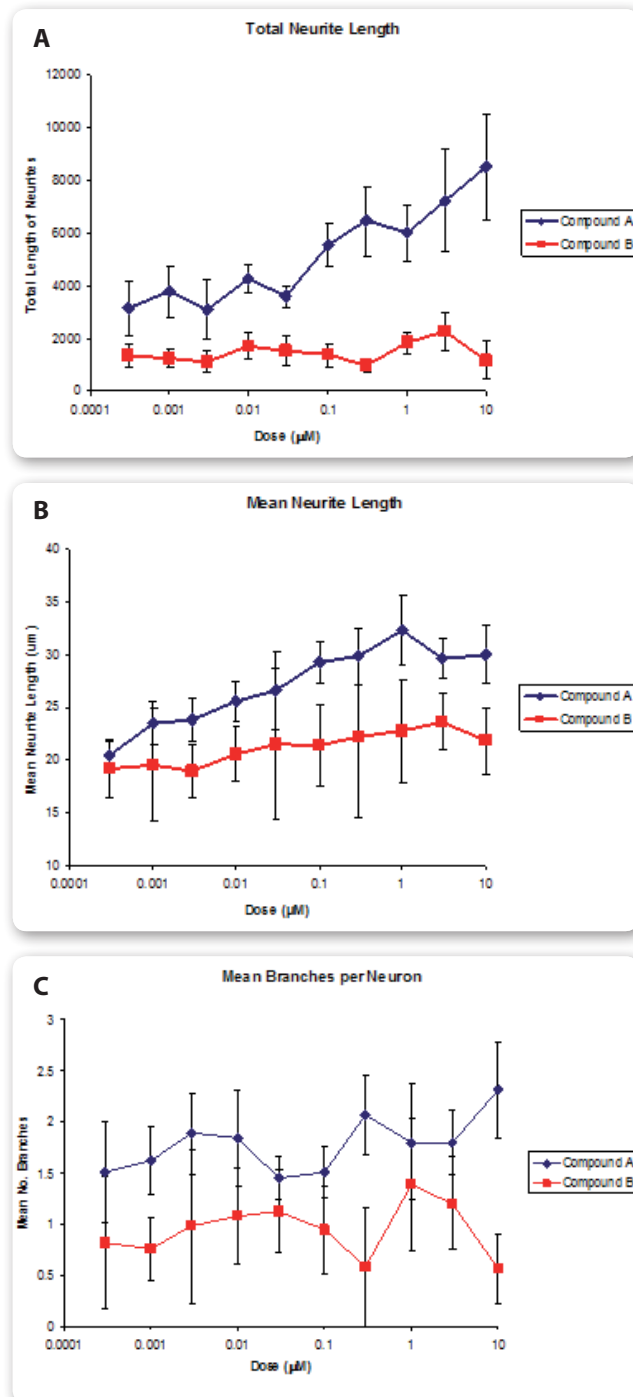


Figure 4 Dose response curves

Dose response curves for A) total neurite length per scene, B) mean neurite length per neuron and C) mean number of branch points per neuron. Error bars are \pm standard deviations.

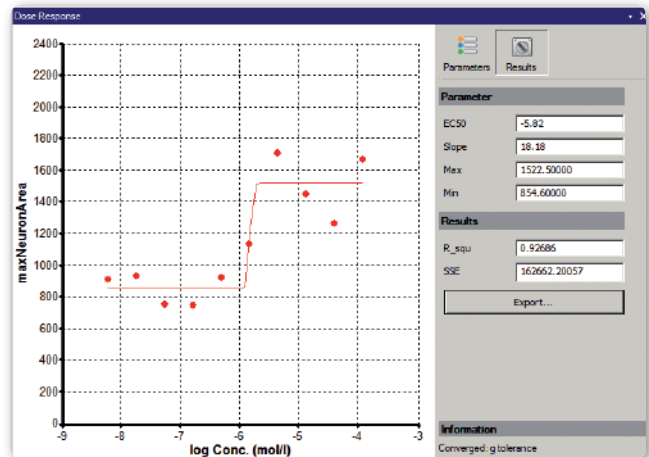


Figure 5 Dose response curve for mean maximum neurite area per field for Compound A, generated in Definiens Cellenger.

We also looked at the mean of the maximum neuronal area per field as well. This data is shown in Figure 5, along with a dose-response curve generated with the Definiens Cellenger application plug-in.

The EC50 calculated is 1.51nM, which is general agreement with the value generated for total neurite length (1.56nM).

Conclusions

In-vitro models of neurite outgrowth are highly valuable tools for the development of neurotrophic agents to treat central nervous system illnesses. Neuronal cultures, however, can be heterogeneous and robust analysis techniques are required to extract meaningful information. Here we have treated cortical neuronal cultures with compounds and analyzed the images using Definiens Cellenger. The hierarchical analysis quickly exposes a large number of morphometric parameters relevant to the assay, which can be subsequently subjected to statistical analysis.

Acknowledgements

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