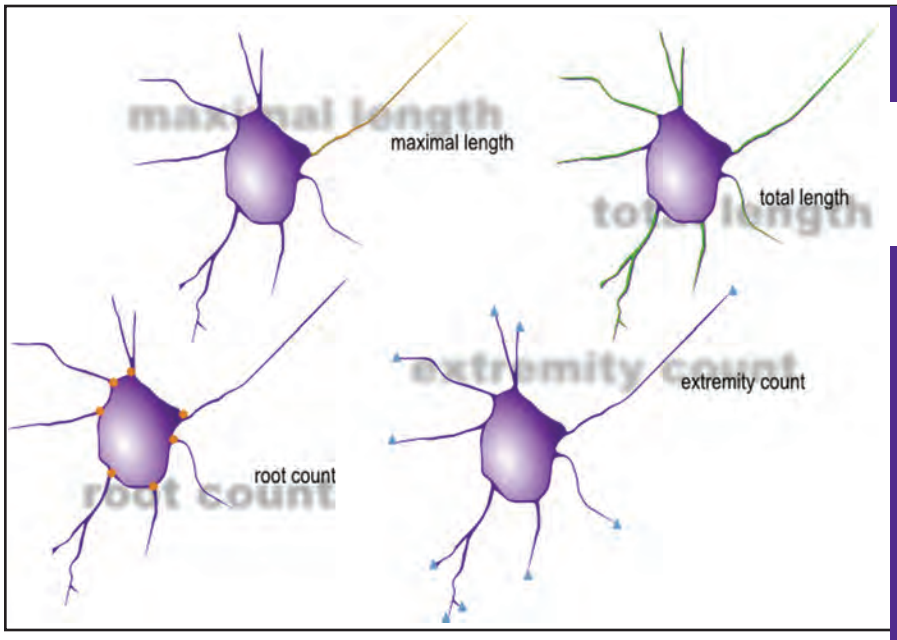

APPLICATION NOTE

Image-based Quantification of Neurite Outgrowth using the Opera

Key Features

- Automated confocal image acquisition using the Opera™ High Content Screening System
- Uses the neuronal-like cell line Neuroscreen™-1
- Image analysis using the versatile Acapella™ **Neurite Outgrowth** script

Neurite Length, Root and Extremity Count

Background

The regeneration of neurons represents a promising strategy for drugs targeted against neurodegenerative injuries and disorders such as Alzheimer's and Parkinson's disease [Geerts *et al*., 2005]. Therefore, the development of new therapies is focused on identifying molecules that affect the differentiation of neurons and neurite outgrowth. For this purpose automated measurement and analysis of neuronal cells is essential for neuroscience research and drug discovery.

Here, a robust and flexible High Content Analysis (HCA) application is presented for the quantification of nerve growth factor (NGF) induced neurite outgrowth of neuronal-like cells. Using the Acapella **Neurite Outgrowth** script, four assay specific read outs of the image analysis are described.

Application

For this high content approach, the neuronal-like cell line Neuroscreen™-1 (NS-1) was used, and neurite outgrowth was stimulated with NGF, which has been shown to play an important role in the growth of neuronal cells in culture [Sofroniew *et al*., 2001]. Cells were seeded at a density of 3500 cells / well in 384 well Collagen I coated CellCarrier™ plates diluted in growth medium containing varying concentrations of NGF (0 – 300 ng/ml). After 4 days incubation in the presence of NGF, cells were fixed, immunofluorescently stained with an anti α-tubulin primary antibody and were visualized using an Alexa Fluor® 488 labeled secondary antibody. The nuclei were counterstained with Hoechst 33342.

For image acquisition the Opera QEHS was used, equipped with the 20x water immersion objective (Figure 1). The images were analyzed with the Acapella **Neurite Outgrowth** script. In this note, four selected read outs are presented; total neurite length, maximum neurite length, number of roots and number of extremities (Figure 2).

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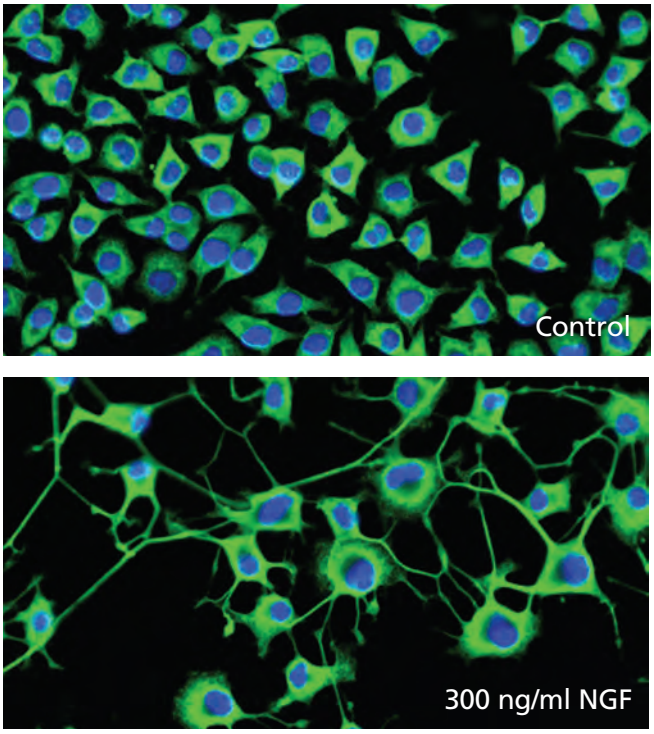

Figure 1. Confocal images of control (top panel) and NGF treated (lower panel) NS-1 cells. Hoechst 33342 stained nuclei are shown in blue. α-tubulin was labeled by indirect immunofluorescence with Alexa Fluor® 488 and is shown in green. The figure shows the impact of NGF on neurite outgrowth of NS-1 cells. Images were acquired on the Opera using the 20x water immersion objective.

Conclusions

A robust and flexible HCA application for the measurement and analysis of neuronal cells using the Opera imaging platform is presented here. It was shown that the Acapella **Neurite Outgrowth** script provides reliable results for NGF induced neurite outgrowth of NS-1 cells. The calculated dose-response curves and EC_{50} values (Figure 2) clearly demonstrate that the algorithms in this script can precisely estimate the total neurite length, the maximum neurite length, the number of roots and the number of extremities.

References

Geerts H, Trojanowski JQ, Lee VMY (2005): Drug Discovery in Neurodegenerative Diseases. Science of Aging Knowledge Environment 6, 4.

Sofroniew MV, Howe CL, Mobley WC (2001): Nerve Growth Factor signaling, neuroprotection, and neural repair. Annual Review of Neuroscience 24, 1217-1281.

Authors

Judith Lucke
Eleni Mumtsidu

PerkinElmer
Cellular Technologies Germany GmbH
Cellular Imaging & Analysis
Hamburg, DE

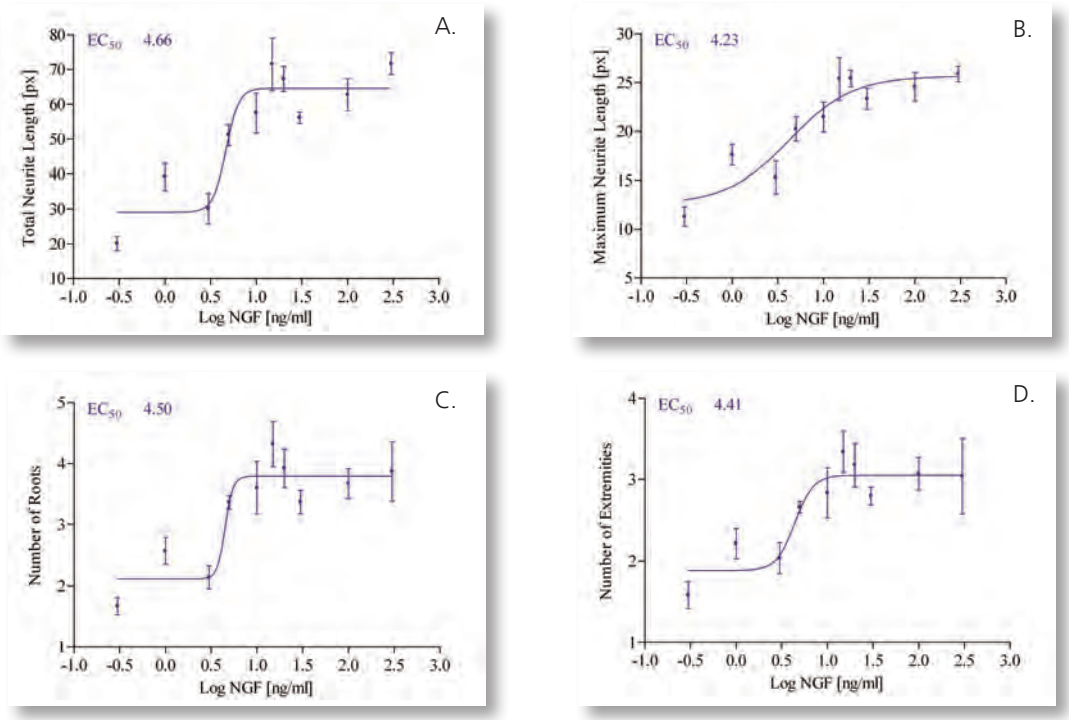

Figure 2. NGF-generated dose-response curves deduced from the total neurite length (A), maximum neurite length (B), number of roots (C) and number of extremities (D). After 4 days treatment with varying concentrations of NGF the NS-1 cells exhibited an increase in the number and length of neurites with increasing concentrations of NGF. N = 5 wells

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

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