

APPLICATION NOTE

Measuring neurite outgrowth with the SpectraMax MiniMax cytometer and MetaMorph software

Introduction

Neurons create connections via extensions of their cellular body called dendrites or “processes”. This biological phenomena is referred to as neurite outgrowth and is regulated by complex intracellular signaling events¹. Understanding the signaling mechanisms driving neurite outgrowth provides valuable insight for interpreting neurotoxic compound screening data and for elucidating factors influencing neural regeneration.

Neurite outgrowth is assessed by the segmentation and quantification of neuronal processes². These neuronal processes can be imaged using a fluorescence microscope and quantified with manual tracing and counting when throughput is low. However, for samples in a higher-throughput microplate format, an automated imaging system paired with analysis software is a more efficient solution.

In this application note, we demonstrate the benefits of using the SpectraMax® i3x Multi-Mode Microplate Reader with the SpectraMax® MiniMax™ 300 Imaging Cytometer in combination with MetaMorph® Microscopy Automation and Image Analysis Software for performing automated neurite outgrowth imaging and analysis. Specifically, we present neurite outgrowth data from mouse embryonic dorsal root ganglion cells donated by Thong C. Ma, Ph.D and Dianna E. Willis Ph.D. of the Burke Medical Research Institute, an affiliate of Weill Cornell Medicine.

Materials

- SpectraMax i3x Multi-Mode Microplate Reader (Molecular Devices cat. #i3x)
- SpectraMax MiniMax 300 Imaging Cytometer (Molecular Devices cat. #5024062)
- MetaMorph Microscopy Automation and Image Analysis Software (Molecular Devices cat. #5042477)
- Neurite Outgrowth Application Module (Molecular Devices cat. #40154)
- CHO-K1 cells (ATCC cat. #CCL-61)
- Recombinant Nerve Growth Factor (EMD Millipore cat. #GF307)
- Anti-βIII-tubulin antibody (Abcam cat. #52623)
- 5-fluorodeoxyuridine (Sigma cat. #F3503-5MG)
- Neurobasal® Medium (ThermoFisher cat. #21103049)
- B27® Supplement (ThermoFisher cat. #17504044)

Benefits

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

Parameter	Settings
Optical configuration	SpectraMax MiniMax 300 Imaging Cytometer
Plate type	96-well
Read mode	Imaging
Read type	Endpoint
Well area settings	12 sites
Wavelength settings	541 nm (green fluorescence)
Image Acquisition settings	541 exposure: 150 ms 541 focus adjustment: 50 μm

Table 1. MiniMax cytometer Image Acquisition settings.

Methods

Mouse embryonic dorsal root ganglion neurons were isolated from E12.5 embryos and initially cultured in Neurobasal Medium supplemented with B27 Supplement, 50 ng/mL nerve growth factor (NGF), and 10 μ M 5-fluorodeoxyuridine for seven days. The 5-fluorodeoxyuridine was used to kill off any Schwann cells. The dorsal root ganglion cells were then removed from the plate, dissociated, and cultured on a monolayer of control CHO cells.

After 24 hours, the co-cultures were fixed with 4% paraformaldehyde. The cells were then processed for fluorescence immunocytochemistry with an anti- β -tubulin antibody (1:500, rabbit monoclonal) and a green-fluorescent secondary antibody (anti-rabbit). Afterwards, cell cultures were imaged using the MiniMax cytometer using the settings shown in Table 1.

Acquired images were imported into MetaMorph software. Images from each section of the well were loaded into a stack and stitched together into a montage using the software's image processing features. These image montages were then used for subsequent neurite outgrowth analysis.

MetaMorph software's Neurite Outgrowth image analysis module was used to identify and measure neurite outgrowth based on the settings shown in Figure 1. The software automatically calculates and reports measurements such as number of processes and process length. The measurements were then exported into Microsoft Excel for further analysis and visualization.

Results

High-quality images of GFP-expressing neurons and their outgrowths were acquired with the MiniMax cytometer (Figure 2). MetaMorph software's built-in stitching feature was used to create the image montages from the original data generated in SoftMax Pro software (Figure 3). Using MetaMorph software's Neurite Outgrowth module, cell bodies and their outgrowths were identified, and measurements and analysis were reported automatically (Figure 4). Individual neurons could be selected and their neurite outgrowth measurements displayed (Figure 5).

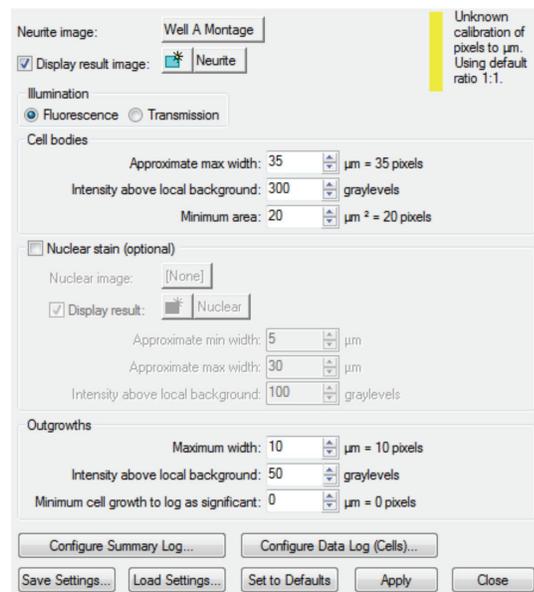


Figure 1. Neurite Outgrowth module settings. These settings were used to select for the size and intensity of the neurons and their processes.

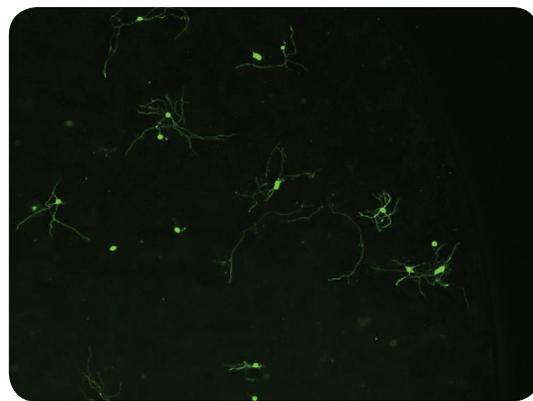


Figure 2. Neuronal cell image. Neurons were imaged using the MiniMax cytometer's green fluorescence channel. This image represents one of twelve sites imaged in the well.

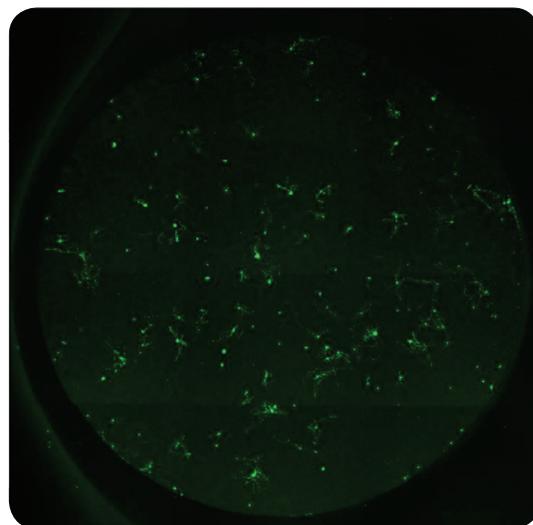


Figure 3. Neuron image montage. Raw images were acquired using the MiniMax cytometer and stitched together using MetaMorph software. This image montage was then used for neurite outgrowth analysis.

Data were exported into Excel for additional analysis. In this example, we explored the relationship between the number of processes and total outgrowth (total area covered by outgrowth, measured in pixels) (Figure 6). This is just one example of data analysis that can be performed; other types include comparing neuronal populations or between individual neurons.

Conclusion

When used together, the MiniMax cytometer and MetaMorph software allow researchers to image neurons and acquire detailed information about neurite outgrowth. The MiniMax cytometer images fluorescently stained neurons with great clarity, while MetaMorph software's suite of image processing and analysis tools enable detailed and quantitative analysis of neurite outgrowth. These two products provide a total solution for researchers to elucidate the mechanisms behind neuron differentiation and regeneration and interpret neurotoxic screening results.

Resources

1. Kiryushko, Darya, Vladimir Berezin, and Elisabeth Bock. "Regulators of neurite outgrowth: role of cell adhesion molecules." *Annals of the New York Academy of Sciences* 1014.1 (2004): 140-154.
2. Meijering, E., et al. "Design and validation of a tool for neurite tracing and analysis in fluorescence microscopy images." *Cytometry Part A* 58.2 (2004): 167-176.

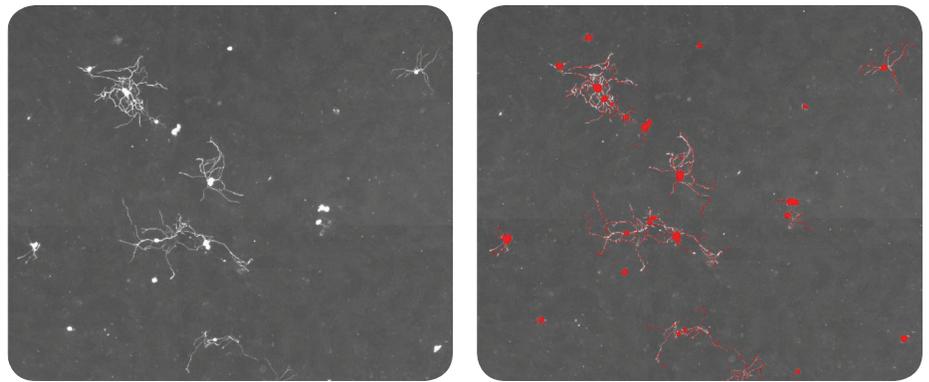


Figure 4. MetaMorph software object overlay. The MetaMorph software's neurite outgrowth module used the grayscale neuronal image montage (left) to identify neuronal cell bodies and their processes, which are indicated by a red image overlay (right).

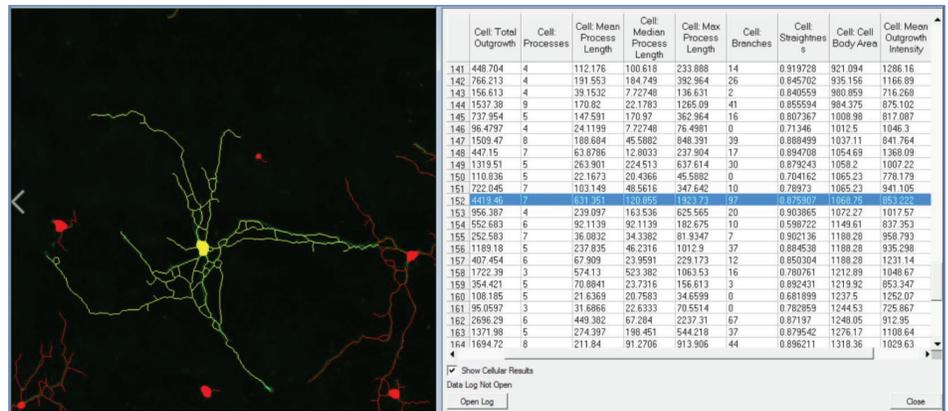


Figure 5. Neurite processes measurements. MetaMorph software automatically calculates a variety of neurite outgrowth measurements. Each identified neuron has a number associated with it. Here, a specific neuron is selected (yellow overlay) and its associated measurements are highlighted (blue in the table).

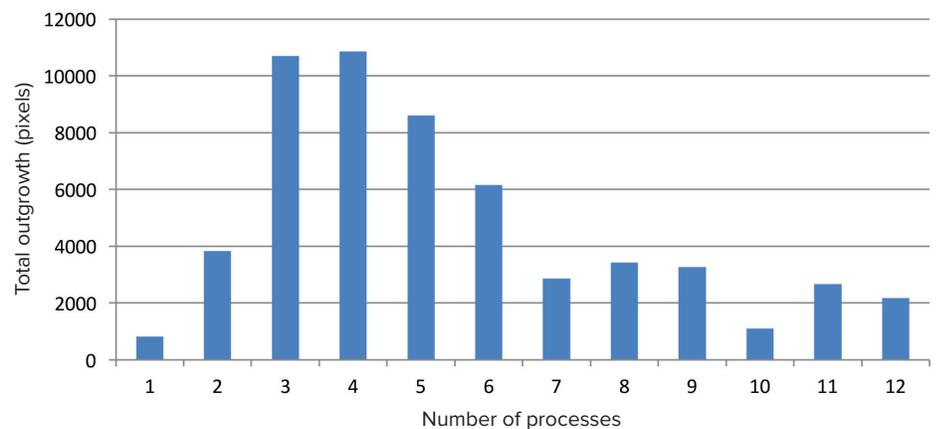


Figure 6. Relationship between total neurite outgrowth and number of processes. Neurons were sorted based on their number of processes, and each group's total outgrowth was averaged. The relationship between number of processes and total outgrowth was displayed as a bar graph using Microsoft Excel. The graph seems to indicate that the total outgrowth is maximized at around three or four processes with total outgrowth decreasing with increased processes.

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