Discovery-1™: Automated Neurite Outgrowth Application Module

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Abstract

Inhibition or stimulation of neurite outgrowth is implicated in a broad range of CNS disorders or injuries including stroke, Parkinson's disease, Alzheimer's diseases and spinal cord injuries.

The ability to automatically measure neurite outgrowth after compound addition can provide the researcher with a wealth of information. Molecular Devices (MDC) has developed a new software module for the Discovery- 1™ system to analyze neurite images that were acquired on the Discovery-1™ unit.

The new software module Neurite Outgrowth Application Module is one of the out of the box solutions included in the latest version of the Discovery-1™ software. This application module has a user friendly interface to minimize set up efforts, but at the same time provides the flexibility to customize the settings and measurements to the type of cells used.

Materials and Method

Embryonic mouse day 13.5 trigeminal neurons (E13.5 TG) were cultured for 48 hours in the presence of the neurotrophic factor NGF in a 96 well plate.

After 48 hours the cells were fixed, permeabilized, and then fluorescently labelled. Hoechst 33342 was used to label the nuclei and the neurons were labelled with anti-PGP9.5 followed by goat anti-rabbit Alexa 488. PGP9.5 is a neuronal-specific antibody that recognizing neuronal cell bodies and their neurites.

Fluorescent images were acquired by using the Discovery-1™ (Molecular Devices Corporation) using a 4X PlanFluor objective (Nikon). 12 images were collected per well.

Images were analyzed using version 6.1 of Discovery-1™ software containing the Neurite Outgrowth Application Module.

EC_{50} calculations were done using SoftMax® Pro (Molecular Devices Corporation).

Conclusion

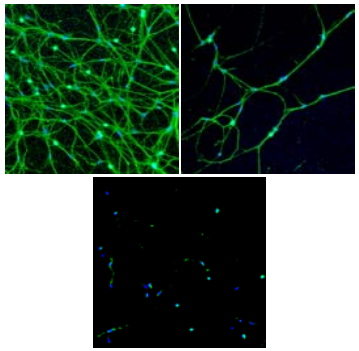
Discovery-1™ provides an automated platform for Neurite Outgrowth experiments including both image acquisition and image analysis.

Discovery-1™

www.moleculardevices.com/d1/

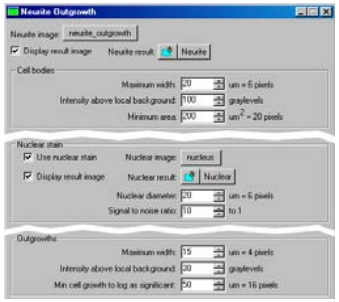
Fluorescent Images Captured by Discovery-1™

Embryonic mouse day 13.5 trigeminal neurons (E13.5 TG) cultured for 48 hours in the presence of the neurotrophic factor NGF. Images at 4x. Courtesy of Kris Poulsen and Davide Foletti, Rinat Neuroscience Corporation.



Software Interface

1. Select images (wavelengths) of interest
2. Configure cell body size
3. Choose whether or not to use a nuclear marker
4. Set classification limits for outgrowths

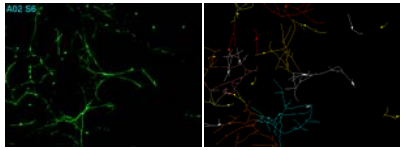


Choose Measurements to Make

Measurements by population or cell by cell (where applicable)

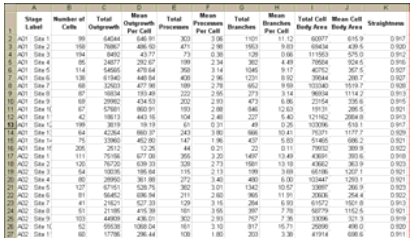
1. Total outgrowth
2. Mean outgrowth per cell
3. Total processes
4. Mean processes per cell
5. Total branches
6. Mean branches per cell
7. Total cell body area
8. Mean cell body area
9. Straightness

Display Algorithm Results for Visual Inspection

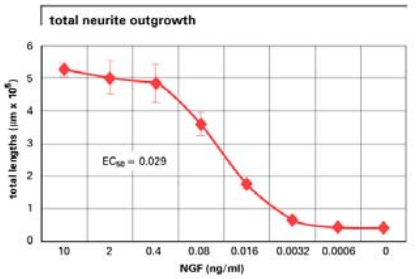


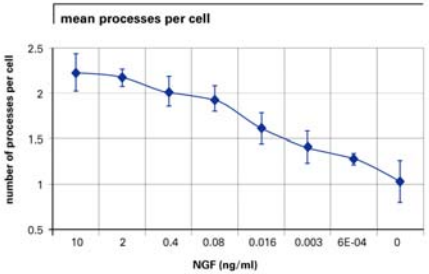
Left: original image, right: the Discovery-1 software identifies and separates every cell.

Save Measurements to a Text File, Spreadsheet, or Database

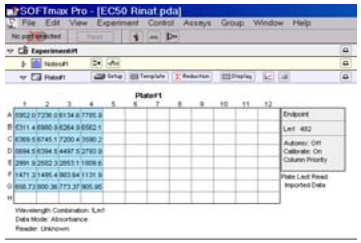


Inspect Results





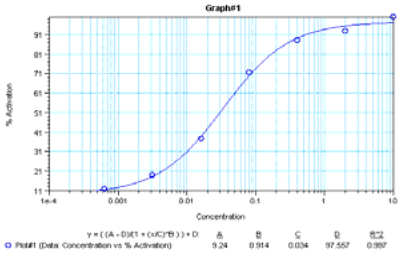
Import Data into SoftMax® Pro for Calculation of EC_{50}



Data (M)

Sample	Wells	Concentration	Values	MeanValue	% Activation	CV%
Data01	A1	10.000	5952.016	6777.138	100.000	13.0
	A2		7236.013			
	A3		6134.617			
	A4		7795.909			
Data02	B1	2.000	5311.388	6279.826	92.662	11.3
	B2		6980.897			
	B3		6264.903			
	B4		6562.117			
Data03	C1	0.400	6369.451	5976.272	88.183	27.2
	C2		6745.108			
	C3		7200.356			
	C4		3590.174			
Data04	D1	0.080	5694.537	4845.107	71.492	32.5
	D2		6394.481			
	D3		4497.496			
	D4		2793.913			
Data05	E1	0.016	2991.888	2559.233	37.763	20.6
	E2		2582.314			
	E3		2853.095			
	E4		1809.634			
Data06	F1	0.003	1471.267	1270.534	18.747	19.9
	F2		1495.383			
	F3		983.642			
	F4		1131.866			
Data07	G1	0.001	656.734	784.102	11.570	13.1
	G2		800.360			
	G3		773.367			
	G4		905.949			

Summary#1 = Maximum Activation = 6777.138
Summary#2 = EC 50% = 0.029



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