

Discovery-1™: Automated Neurite Outgrowth Application Module

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Abstract

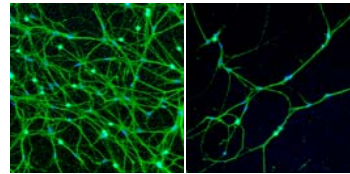
Inhibition or stimulation of neurite outgrowth is implicated in a broad range of CNS disorders or injuries including stroke, Parkinson's disease, Alzheimer's diseases and spinal cord injuries.

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.

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.



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 recognizes 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.

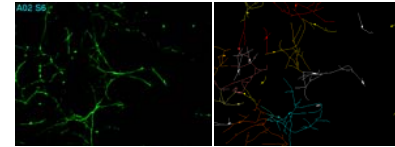
Images were analyzed using version 6.1 of Discovery-1™ software containing the Neurite Outgrowth Application Module.

EC₅₀ 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.

Display Algorithm Results for Visual Inspection

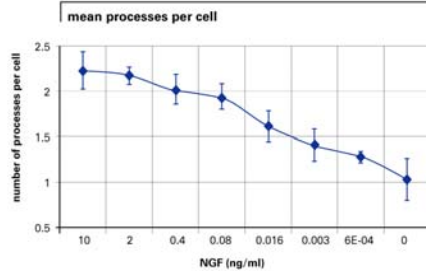
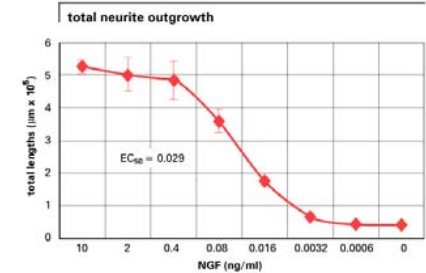


Left: original image, right: the Discovery-1 software identifies and separates every cell.

Save Measurements to a Text File, Spreadsheet, or Database

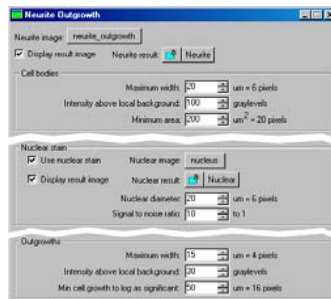
Stage	Label	Number of Cells	Total Outgrowth (µm)	Mean Outgrowth (µm/Cell)	Total Processes	Mean Processes (Per Cell)	Total Branches	Mean Branches (Per Cell)	Total Cell Body Area (µm²)	Mean Cell Body Area (µm²/Cell)	Straightness
AC1	Sta 1	90	6454	71.71	303	3.37	1431	15.74	63977	710.9	0.947
AC1	Sta 2	150	7687	51.25	471	3.14	1953	13.02	69434	463.0	0.900
AC1	Sta 3	194	7482	38.57	751	3.86	326	1.68	11103	57.0	0.942
AC1	Sta 4	85	2407	28.33	199	2.34	302	3.55	7956	93.6	0.916
AC1	Sta 5	114	6465	56.72	380	3.34	1045	9.17	49752	436.4	0.907
AC1	Sta 6	138	6146	44.54	408	2.96	1231	8.92	39044	283.7	0.907
AC1	Sta 7	60	3262	54.37	222	3.70	462	7.70	10300	171.7	0.904
AC1	Sta 8	87	7624	87.63	222	2.55	273	3.14	36934	424.7	0.919
AC1	Sta 9	60	3262	54.37	222	3.70	462	7.70	10300	171.7	0.904
AC1	Sta 10	87	5781	66.33	193	2.22	846	9.72	19137	220.0	0.901
AC1	Sta 11	42	1813	43.17	104	2.48	227	5.42	42162	359.9	0.905
AC1	Sta 12	99	3919	39.59	61	0.61	49	0.26	10336	104.4	0.917
AC1	Sta 1	42	2234	53.19	243	5.80	868	20.41	97371	2317.2	0.926
AC1	Sta 2	75	3180	42.40	147	1.96	437	5.83	5145	68.7	0.921
AC1	Sta 4	206	2121	10.29	44	0.21	22	0.11	9902	38.9	0.922
AC1	Sta 1	111	7516	67.70	306	2.76	1407	12.69	4339	39.0	0.918
AC1	Sta 2	106	2922	27.56	229	2.16	190	1.79	1082	10.2	0.923
AC1	Sta 3	54	1638	30.34	116	2.15	198	3.66	6736	124.7	0.921
AC1	Sta 4	85	2995	35.24	272	3.20	483	5.69	10247	120.6	0.921
AC1	Sta 5	127	6751	53.16	302	2.37	1342	10.57	39007	306.9	0.923
AC1	Sta 6	81	6462	80.00	211	2.60	265	3.28	2044	25.4	0.922
AC1	Sta 7	41	2162	52.73	129	3.15	204	4.99	6152	150.6	0.913
AC1	Sta 8	61	2196	36.00	161	2.64	307	5.03	6679	110.0	0.921
AC1	Sta 9	103	4409	42.81	302	2.93	767	7.39	33306	323.5	0.919
AC1	Sta 10	62	6938	111.90	161	2.60	817	13.19	20996	483.8	0.920
AC1	Sta 11	60	10706	178.44	100	1.66	203	3.36	87914	1466.6	0.911

Inspect Results



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

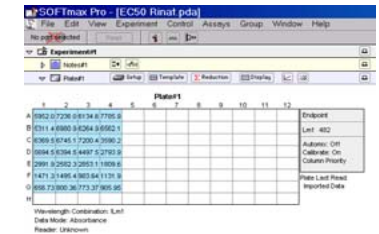


Choose Measurements to Make

Measurements by population or cell by cell (where applicable)

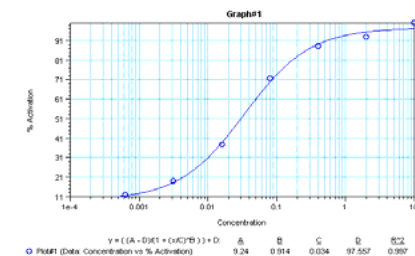
1. Total outgrowth
2. Mean outgrowth per cell
3. Total processes
4. Mean processes per cell
5. Total branches
6. Mean branches per cell
7. Total cell body area
8. Mean cell body area
9. Straightness

Import Data into SoftMax® Pro for Calculation of EC₅₀



Sample	Well	Concentration	Data (M)	Mean Value	% Activation	CV%
Data01	A1	10.000	5952.015	6777.138	100.000	13.0
	A2		7239.913			
	A3		6134.617			
Data02	A4		7785.909			
	B1	2.000	5311.590	6279.826	92.663	11.1
	B2		6980.997			
	B3		6264.903			
Data03	B4		6562.117			
	C1	0.400	6359.451	5676.272	88.183	27.2
	C2		6745.108			
	C3		7200.355			
Data04	C4		3550.174			
	D1	0.080	5694.537	4845.107	71.492	32.5
	D2		6304.481			
	D3		4467.546			
Data05	D4		2763.913			
	E1	0.016	2991.888	2559.233	37.763	20.6
	E2		2562.314			
	E3		2953.095			
Data06	E4		1809.634			
	F1	0.003	1471.207	1270.534	18.747	19.9
	F2		1495.393			
	F3		983.842			
Data07	F4		1131.986			
	G1	0.001	655.734	784.102	11.570	13.1
	G2		800.390			
	G3		773.307			
G4		905.949				

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



Discovery-1™

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