Kinetic and label-free, live content imaging assays for neurite outgrowth in primary, iPSC-derived and immortalised neurons

S. L. Alcantara, P. Garay, L. McGillicuddy, T. O'Callaghan, T. Dale, D. Appledorn, V. Groppi, & D.J. Trezise
Essen BioScience, Welwyn Garden City, UK & Ann Arbor, Michigan, 48108 USA

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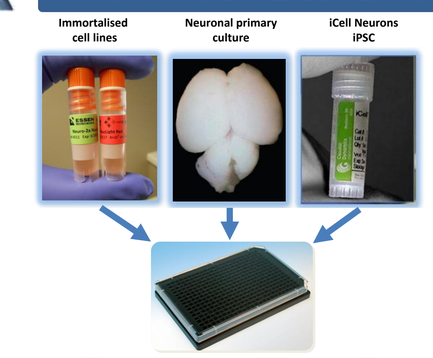
www.essenbioscience.com

Summary & Impact

- The study of neurite dynamics is fundamental to the investigation of neuropathological disorders, neuronal injury, regeneration, differentiation and embryonic development.
- Here we describe an *in vitro* fully kinetic neurite outgrowth assay miniaturised to 96 & 384-well microtitre plate formats based on analysis of time-lapse, phase-contrast images.
- Validation and pharmacology data from a range of cell types including human iPSC-derived neurons (iCell Neurons, CDI), primary neurons (rat cortex) and neuronal-like cell lines (Neuro-2A) are described. Neurite outgrowth (NOG) can be duplexed with cell count and cytotoxicity measurements using fluorescent probes.
- This approach affords a full temporal understanding of neurite outgrowth without the need for complex and expensive Ab-labelling methods (e.g. HCS).
- Miniaturisation of assays with iPSC-derived neurons (iCell Neurons, CDI) to 384-well format is a key step in maximising the value of these cellular reagents.

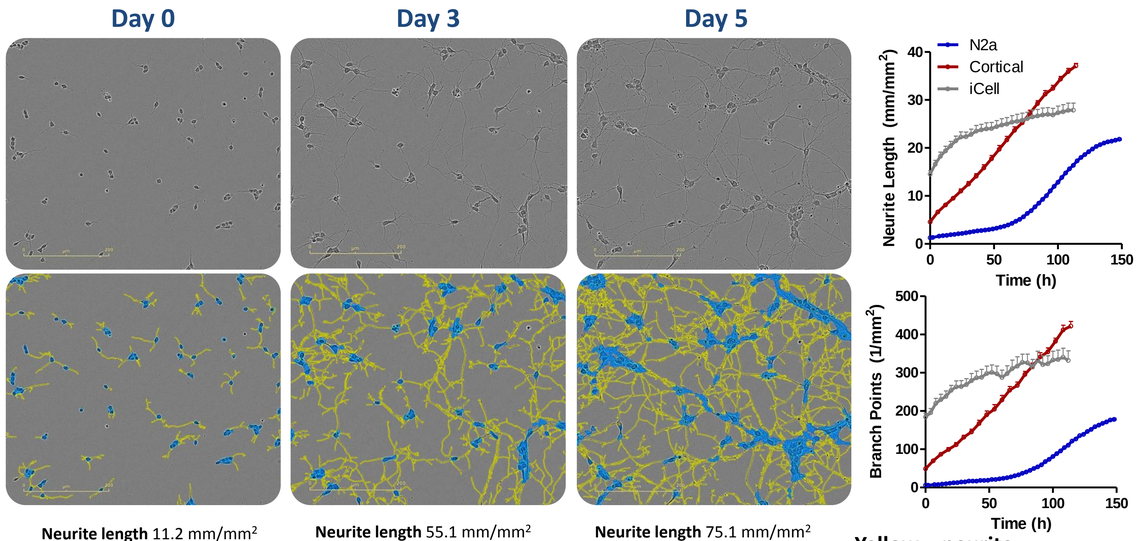
96 & 384-well NeuroTrack Assay – an integrated solution



IncuCyte ZOOM live cell imaging system

1. Seed selected neuronal cell type in the culture microplate (96 or 384-well) and allow adherence for 1h.
2. Treat cells with neuro-degenerative and/or neuro-protective agents.
3. Monitor in the IncuCyte ZOOM live-cell imaging system: capture and analyse phase and fluorescent images every 2-6h for 3 to >12 days.

Image processing & quantification



Neurite length 11.2 mm/mm² **Cell body cluster** 134 /mm² **Branch points** 259.9 /mm²

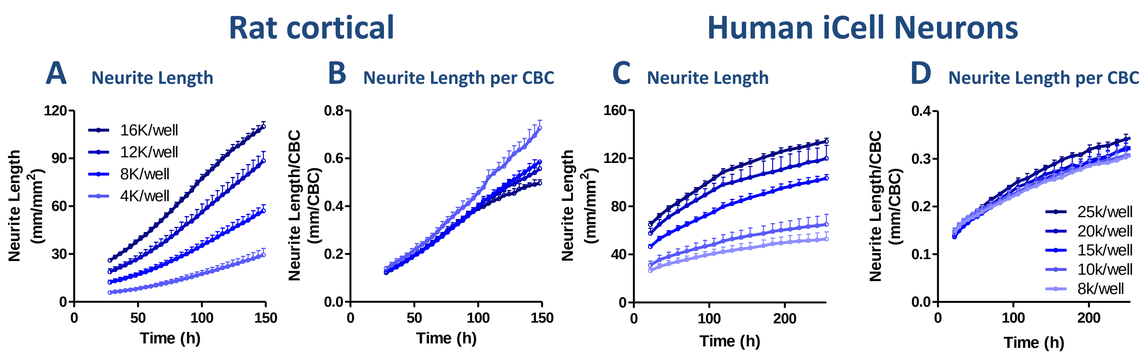
Neurite length 55.1 mm/mm² **Cell body cluster** 130 /mm² **Branch points** 1368 /mm²

Neurite length 75.1 mm/mm² **Cell body cluster** 139 /mm² **Branch points** 2048 /mm²

Yellow = neurite
Blue = cell body

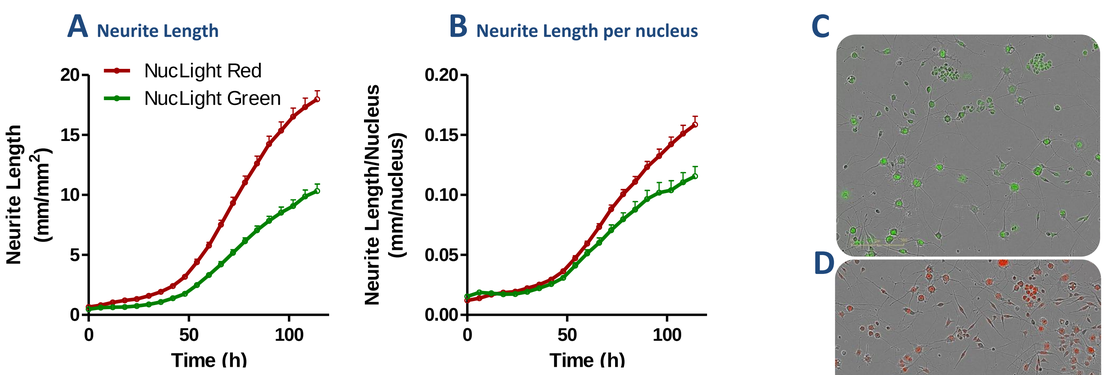
- Phase-contrast images and analysis masks show the kinetics of neurite outgrowth in rat cortical primary neurons.
- Time courses compare mean ± SEM neurite length (mm/mm²) and branch point (1/mm²) values for rat cortical, human iCell Neurons and Neuro-2A cells (n=6, 24 and 48 wells respectively).

Validation: Cell body cluster normalisation



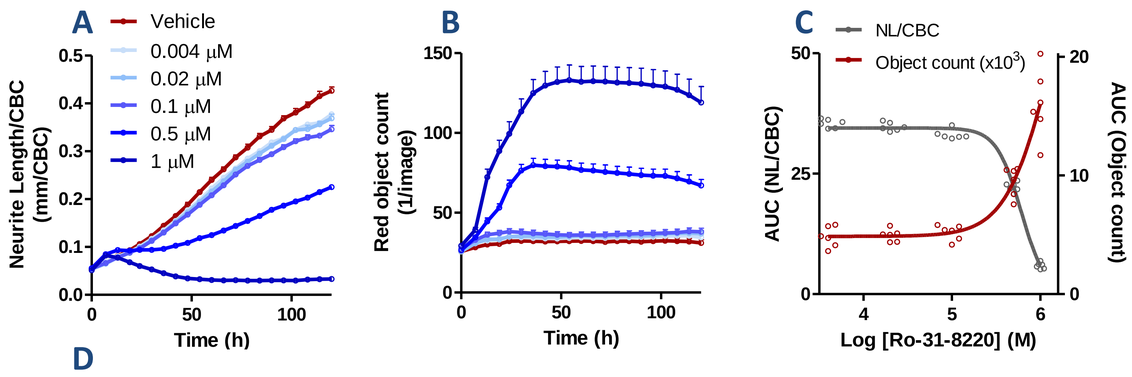
- Data represent mean ± SD values for rat cortical and human iCell Neurons (n=6 wells).
- Note how normalization to cell body cluster allows for data comparison.

Nuclear-targeted GFP/RFP enables true cell counting

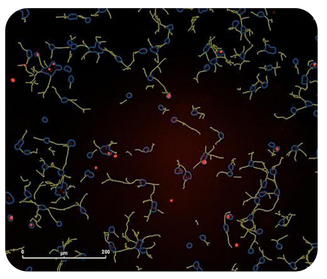


- Time courses for NucLight Green and Red Neuro-2A cells differentiated using atRA (18 µM) showing the mean ± SEM (**A, B**) (n=9 wells).
- Phase and Fluorescence images obtained for NucLight Green and Red Neuro-2A cells (**C, D**) 5 days post-treatment.

Kinetic multiplexed assays : NOG & neurotoxicity



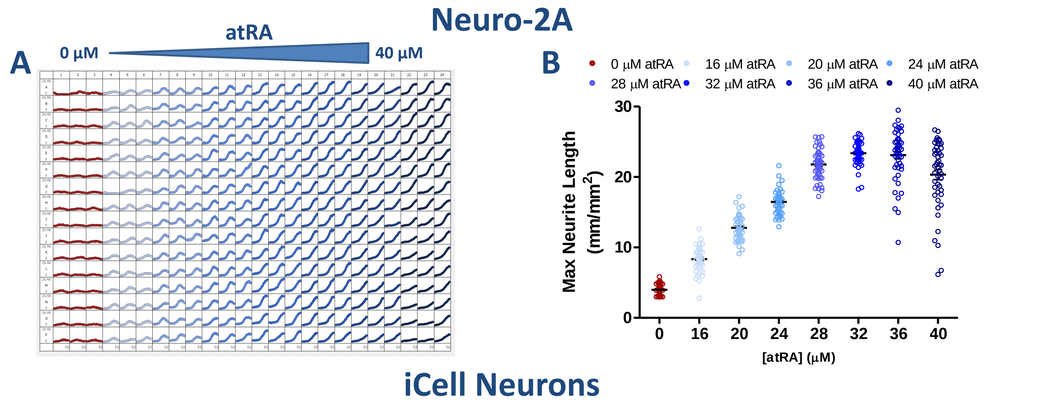
D



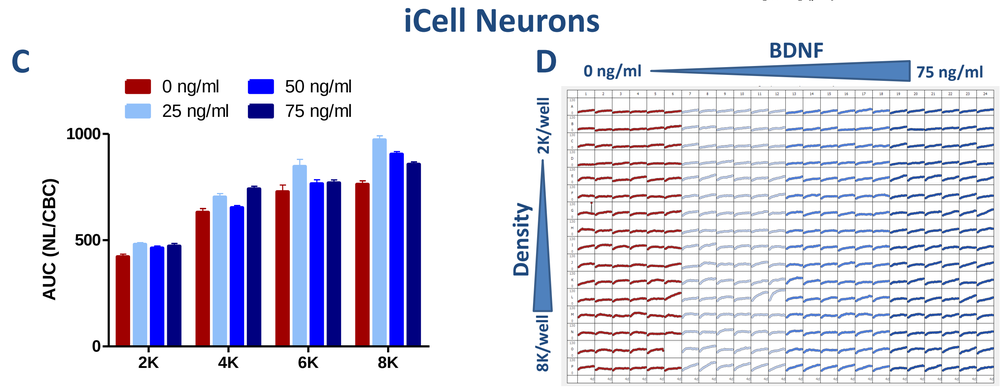
- Primary rat cortical neurons treated with increasing concentrations of PKC inhibitor Ro-31-8220 and fluorescent membrane integrity indicator Yo-Pro3 (150 nM).
- Average time course data from rat primary cortical neurons showing mean ± SEM neurite length/CBC (mm/CBC) (**A**) and red object count per image (1/image) (**B**) (n=6).
- CRCs for Ro-31-8220 showing NOG and cell death (**C**) (n=6).
- Image representing the segmentation mask applied by IncuCyte ZOOM, yellow: neurite, blue: cell body and red: YoPro-3 fluorescence.

NOG assay miniaturisation to 384-well format

Neuro-2A

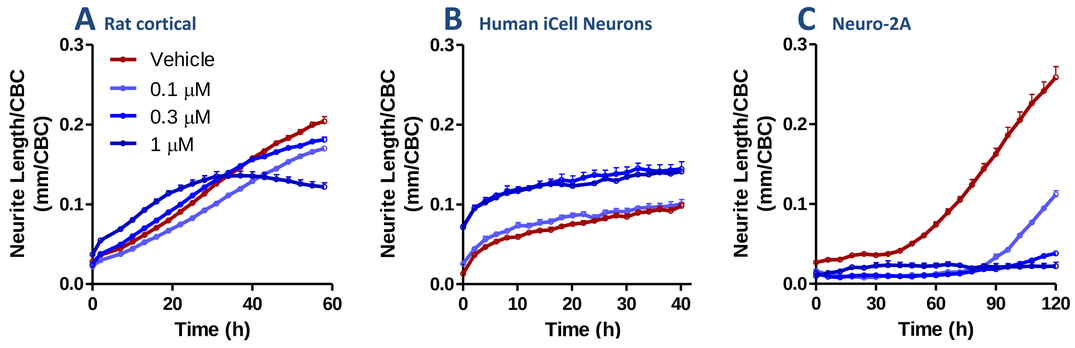


iCell Neurons



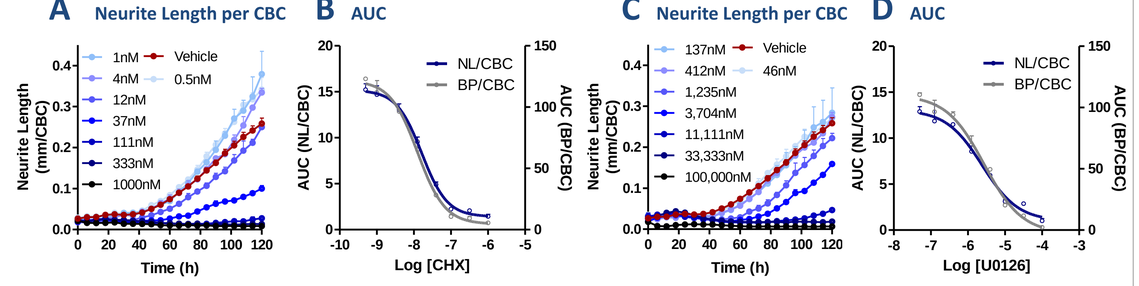
- Cells treated with increasing concentrations of atRA (Neuro-2A) or BDNF (iCell Neurons) 1h post-plating and monitored for 5 and 4 days respectively. Increasing cell densities (iCell Neurons) in a 384-well plate format.
- Plate views show the kinetics of neurite length (mm/mm²) for Neuro-2A cells (**A**) or iCell Neurons (**D**).
- Inter-well variability plot; maximal neurite length ± SEM (mm/mm²) for Neuro-2A (CV<7%, n=48 wells) (**B**).
- Bar graph shows the AUC of increasing iCell Neurons cell densities treated with increasing BDNF concentrations (CV=8%) (**C**) (n=24 wells).

Differential effects of Cytochalasin D across cell types



- Cells treated with increasing concentrations of the cytoskeleton modulator cytochalasin D.
- Time courses showing mean ± SEM neurite length/CBC (mm/CBC) for rat cortical (**A**), human iCell Neurons (**B**), and Neuro-2A cells (**C**) (n=6, 4, and 2 wells respectively).
- Different kinetic profiles appear dependant on drug concentration and cell type.

Quantitative pharmacology – Kinetic profiles



- Neuro-2A cells treated with increasing concentrations of the protein biosynthesis inhibitor cycloheximide and the MEK inhibitor U0126.
- Time courses show mean ± SEM neurite length/CBC (mm/CBC) (**A, C**) (n=2 wells).
- Area under the curve analysis show CRCs for neurite length/CBC (mm/CBC) and branch points (1/CBC) (**B, D**) (n=2 wells).

Acknowledgement
We thank Cellular Dynamics International for technical support with iCell Neurons.