

High Content Analysis of Neural Stem Cell Expansion and Differentiation

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Introduction

Neurogenesis is an important process for nervous system development and maintenance. During neurogenesis, neural stem cells generate neural progenitors that then mature into functional neurons. Pharmacological enhancement of neurogenesis represents a potential therapeutic approach for neuronal loss in neurodegenerative diseases, such as stroke, brain damage, Parkinson's, and Alzheimer's. Accordingly, there is great interest in using neural stem cells as tools for screening neurogenic compounds during early drug development.

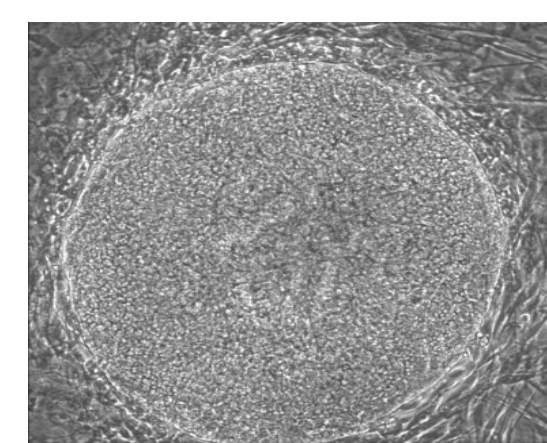
Here we describe automated assay methods for monitoring neural stem cell expansion and differentiation using stem cell derived neural cell lines and high content imaging systems. The assays greatly increase reproducibility and throughput and are suitable for screening of pharmacological agents.

hESC-derived Neural Cell Lines

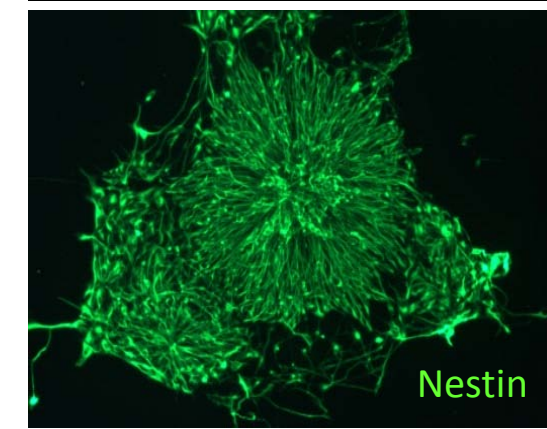
ArunA hNP1™ neural progenitors

- Derived from approved WA09 (H9) hESC cell line
- Derived, maintained and propagated as adherent monolayers using serum- and feeder-free, defined medium
- Possess a stable karyotype for multiple (>10) passages with a doubling time of ~36 hours
- Robust and scalable for HTS format (96-, 384-well) assays
- Proneural: >90% Nestin+; <5% Oct4+; positive for Musashi1, CD133
- Capable of differentiation into multiple neuronal and glial phenotypes

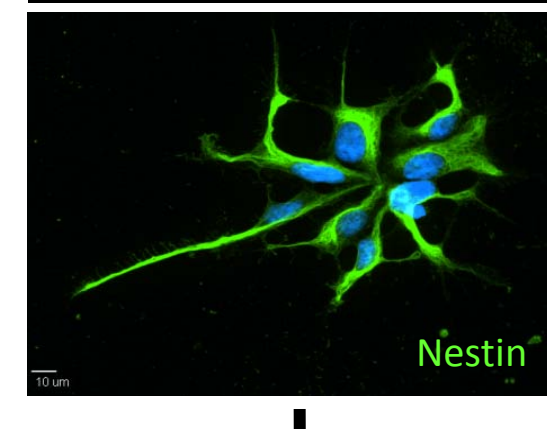
WA09 (H9) hESC colony



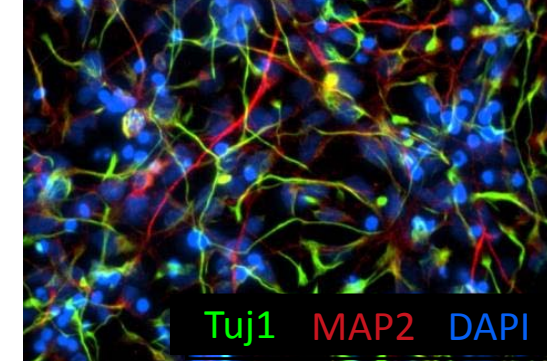
Neural rosette structures



hNP1™ neural progenitors

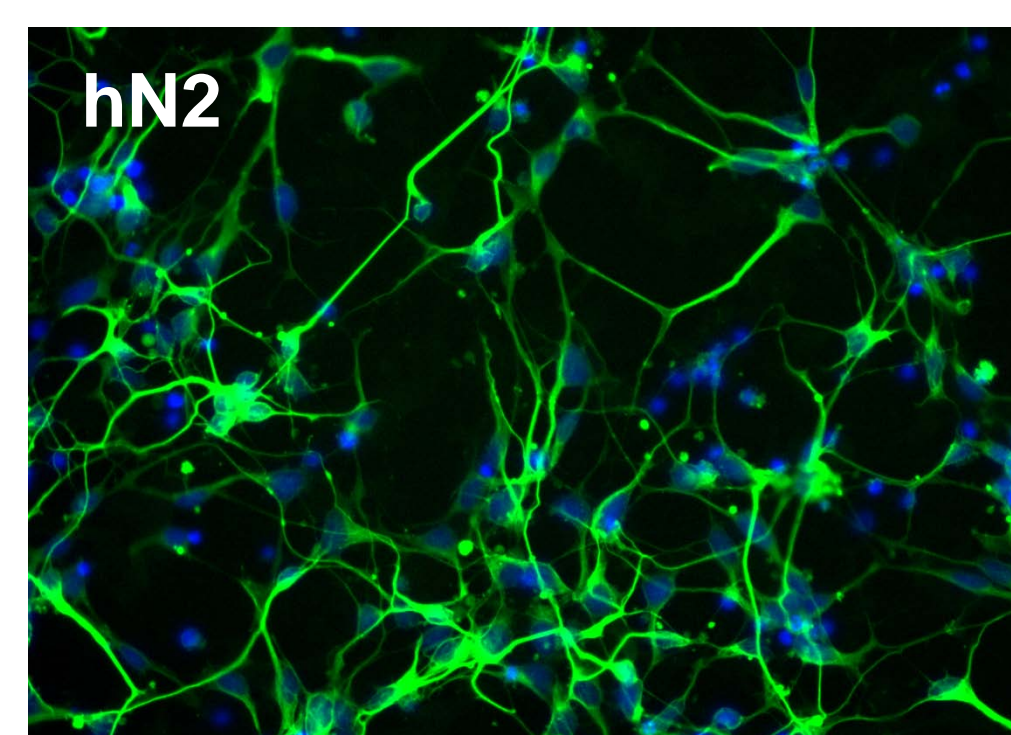
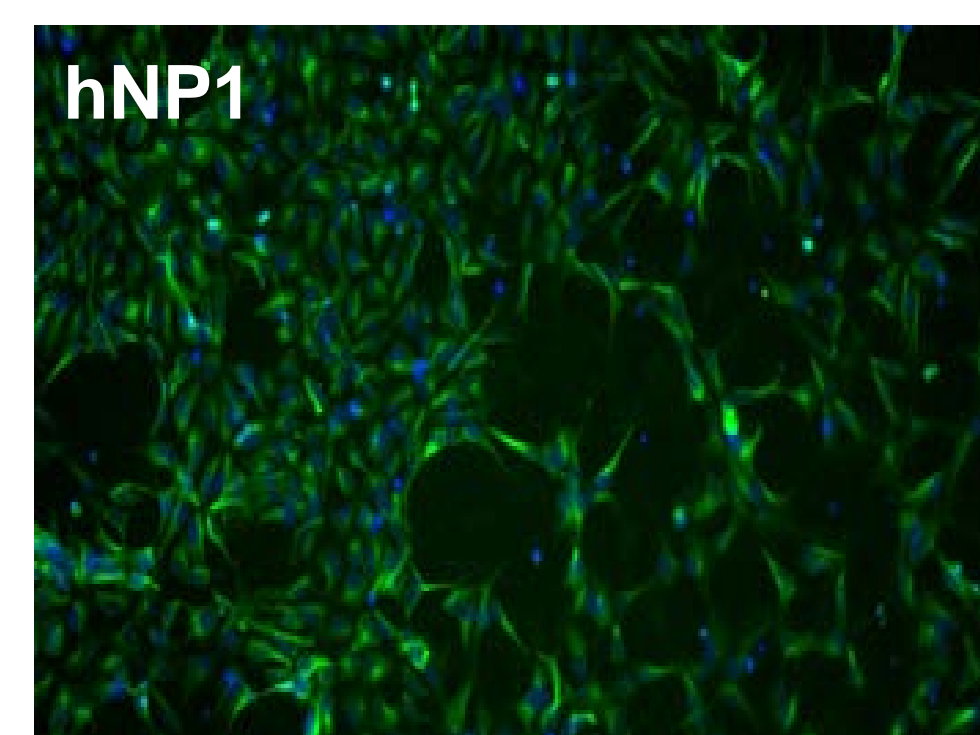


hN2™ differentiated neurons



ArunA hN2™ differentiated neurons

- Differentiated from early stage neural progenitor cells under defined (feeder & serum free) conditions
- Neuronal morphology: >90% β-III tubulin (Tuj1)+, >60% MAP2+, <5% Oct4+; mostly post-mitotic
- Large populations of glutamatergic and GABA-ergic – can be used as a general neuronal model
- Cultured in an adherent 96-well format with defined medium conditions
- Can be cryopreserved for thaw-and-use applications
- Can be maintained in culture for >2 weeks



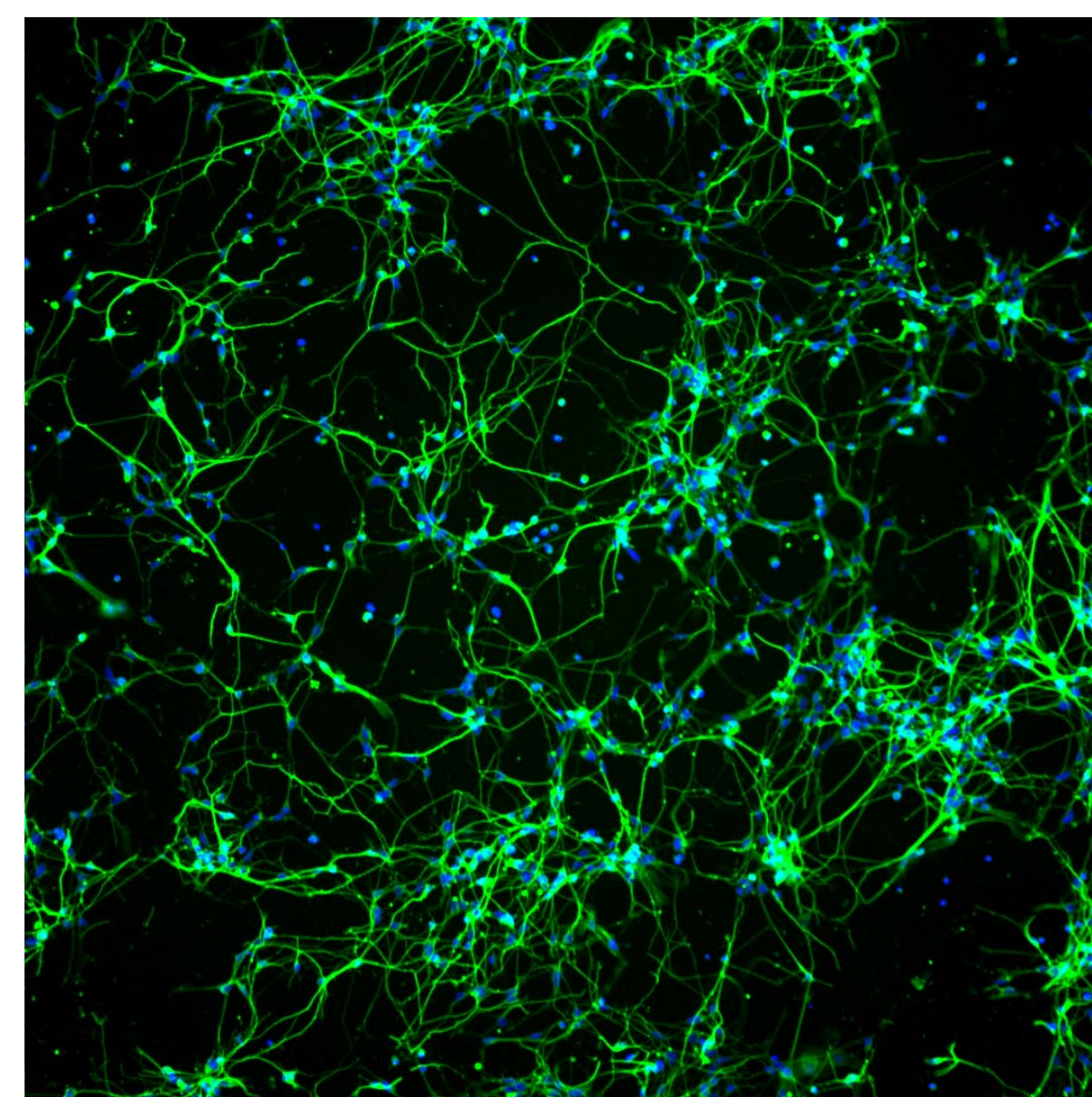
Overlaid images of hNP1 and hN2 cells. β-III tubulin + DAPI.

Neural Progenitor Proliferation and Differentiation

Neuronal progenitors hNP1 expanded with leukemia inhibitory factor (LIF) and basic fibroblast growth factor (bFGF) on matrigel coated dishes. We have tested 2 assays using hNP1 cell line:

- Proliferation of neural progenitors:** the effect of different agents on neural progenitor proliferation can be tested in 3-5 day assay in 96-well format.
- Differentiation of neural progenitors:** hNP1 neuronal progenitors can be differentiated into cells with a neuronal phenotype. Effects of different growth factors can be evaluated in a 14 day assay in 96-well format.

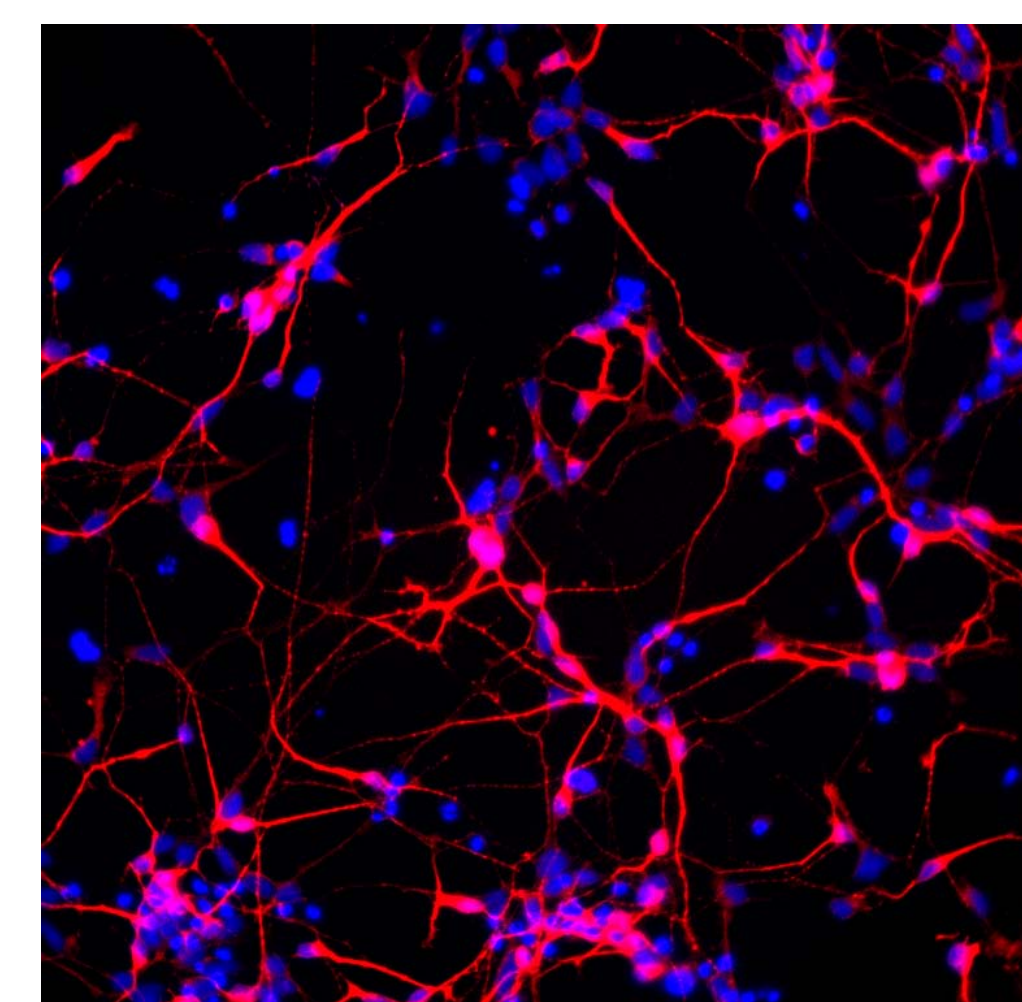
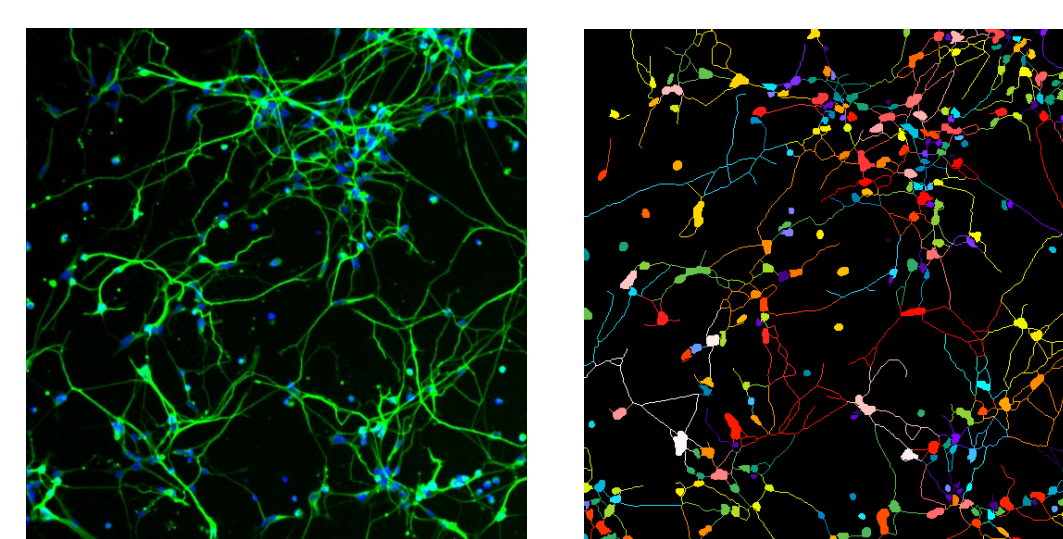
High Content Analysis



β-III tubulin + DAPI, 10x, hN2 cells

High content imaging provides an efficient tool to monitor and evaluate neuronal expansion and differentiation. The ImageXpress® XL Micro System provides a fast, automated way to acquire high resolution images. The MetaXpress® Software package then analyzes the images to characterize cell phenotype, quantify extent of differentiation based on marker expression.

- Images acquired with ImageXpress Micro System using 10x or 20x objective and 2-3 exposures
- AF488 anti-β-tubulinIII: 488nm Ex, 520nm Em
- AF647 anti-MAP2: 628nm/692nm
- DAPI label for nuclei: 405nm Ex, 450nm Em



MAP2 + DAPI, 20x, hN2 cells

Image analysis: Neurite Outgrowth module



ImageXpress® Micro XL System

- Images were analyzed using standard algorithms from MetaXpress Software
- Cell Scoring – identify number of Neurons
- Neurite Outgrowth – Identify total outgrowth, number of neurons, neuron length, branching, number of processes, etc.
- Cell cycle- identifies number of cells undergoing different phases of mitosis

Assay for Neural Progenitor Proliferation

hNP1 cells were cultured in the presence of different growth factors for 5 days. Image analysis was done using the Cell Scoring, Cell Cycle, and Neurite Outgrowth modules in MetaXpress Software, and data visualization and analysis was done using AcuityXpress™ Software. The Cell Scoring module determines a "positive" cell by presence of both nuclear and β-III tubulin stains. Statistics on number and phenotype of cells in each well are then calculated.

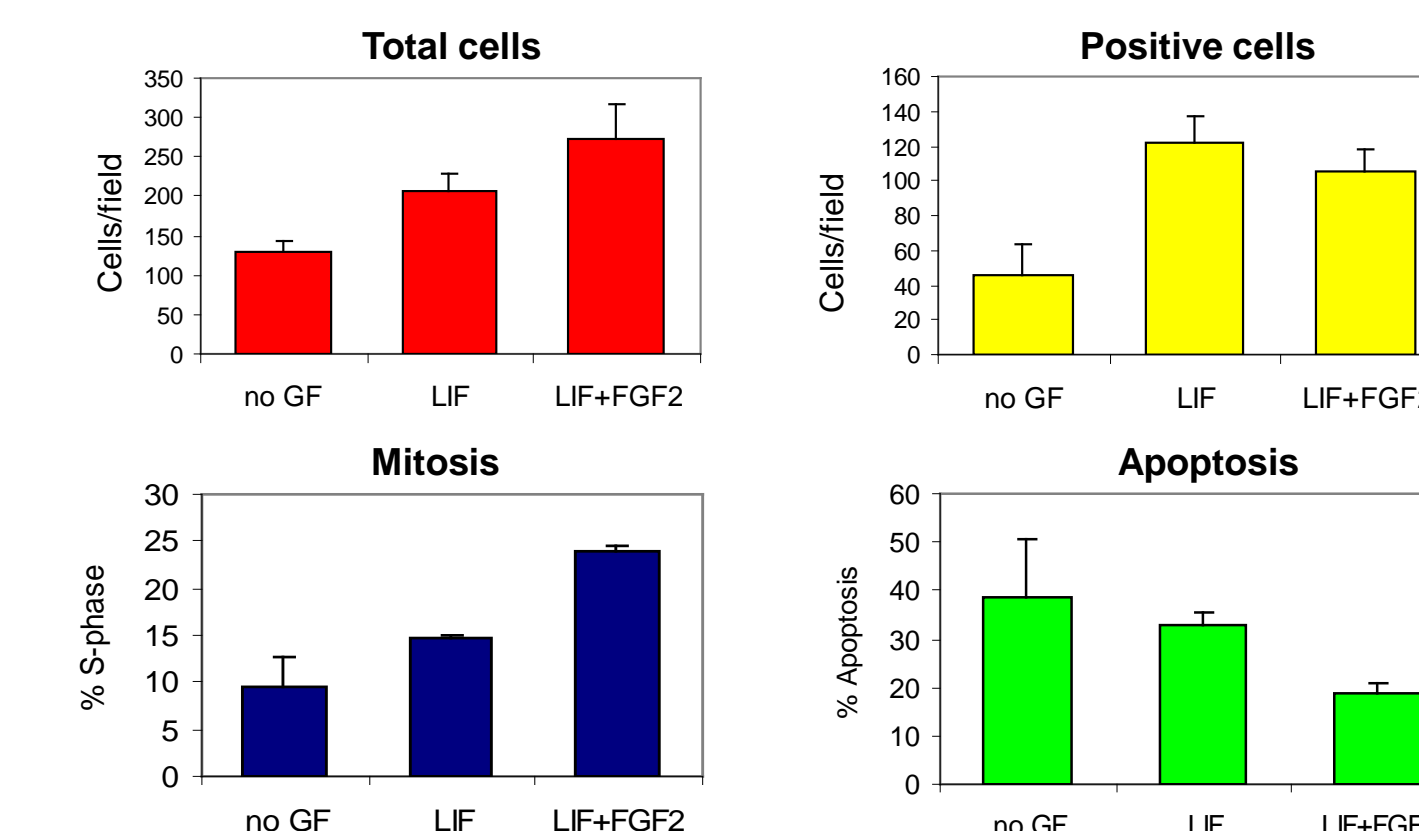
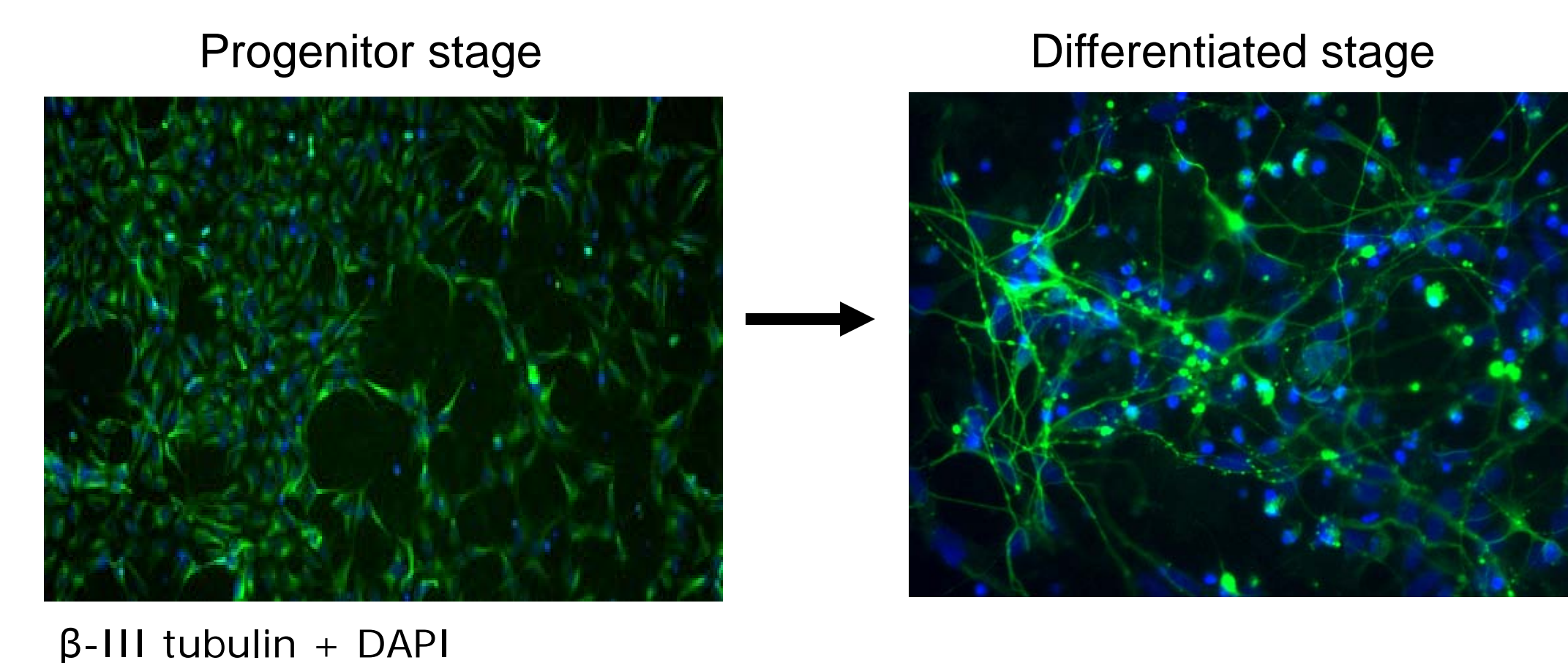


Image analysis results of MetaXpress Software from Cell Scoring and Cell Cycle modules

LIF and FGF2 increases hNP1™ proliferation by promoting mitosis and reducing apoptosis

Assay for Neural Progenitor Differentiation

hNP1 cells were differentiated for 14 days in the presence of different growth factors. The Neurite Outgrowth module finds neuronal cells and then characterizes β-III tubulin labeled neurites. Output parameters include number of neurites, length, branches, etc. per cell or per field.

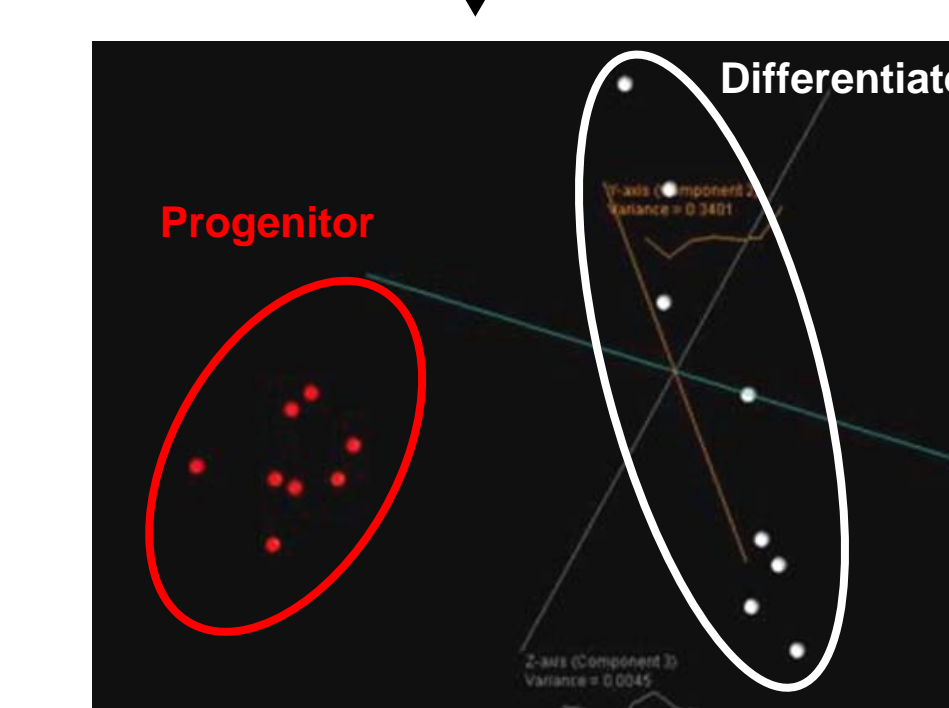


β-III tubulin + DAPI

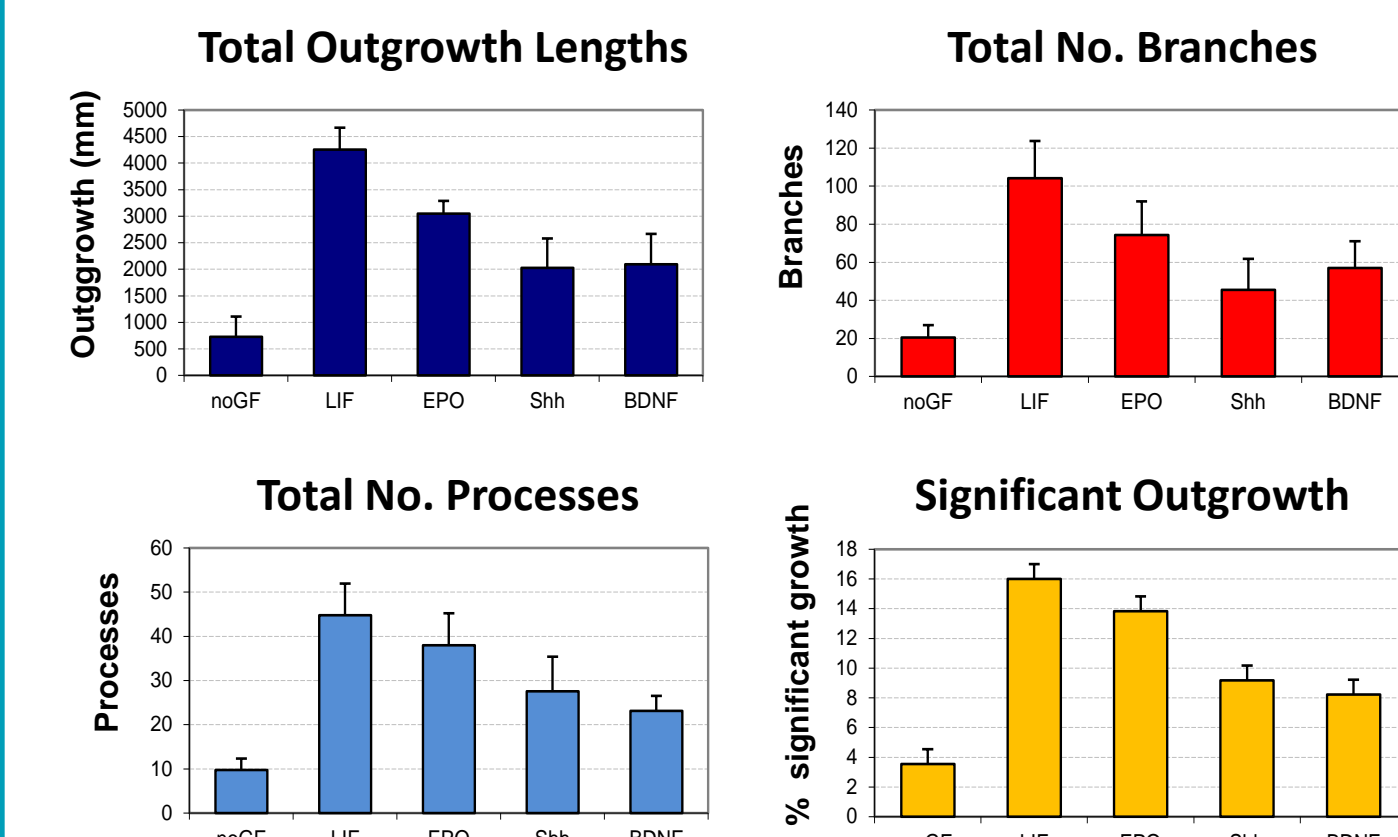
Component	Total	Total	Total	Total	Total	Total	Total	Total	Total
Phase	Cells	Area	Per Cell	Area	Per Cell	Area	Per Cell	Area	Per Cell
1	1000	10000	10	10000	10000	10000	10000	10000	10000
2	2000	20000	10	20000	20000	20000	20000	20000	20000
3	3000	30000	10	30000	30000	30000	30000	30000	30000
4	4000	40000	10	40000	40000	40000	40000	40000	40000
5	5000	50000	10	50000	50000	50000	50000	50000	50000
6	6000	60000	10	60000	60000	60000	60000	60000	60000
7	7000	70000	10	70000	70000	70000	70000	70000	70000
8	8000	80000	10	80000	80000	80000	80000	80000	80000
9	9000	90000	10	90000	90000	90000	90000	90000	90000
10	10000	100000	10	100000	100000	100000	100000	100000	100000

AcuityXpress™ High Content Informatics

- Uses Principal Component Analysis
- Analyzes various parameters at once
- Determines specific phenotypes



LIF and EPO Promote Neuronal Differentiation



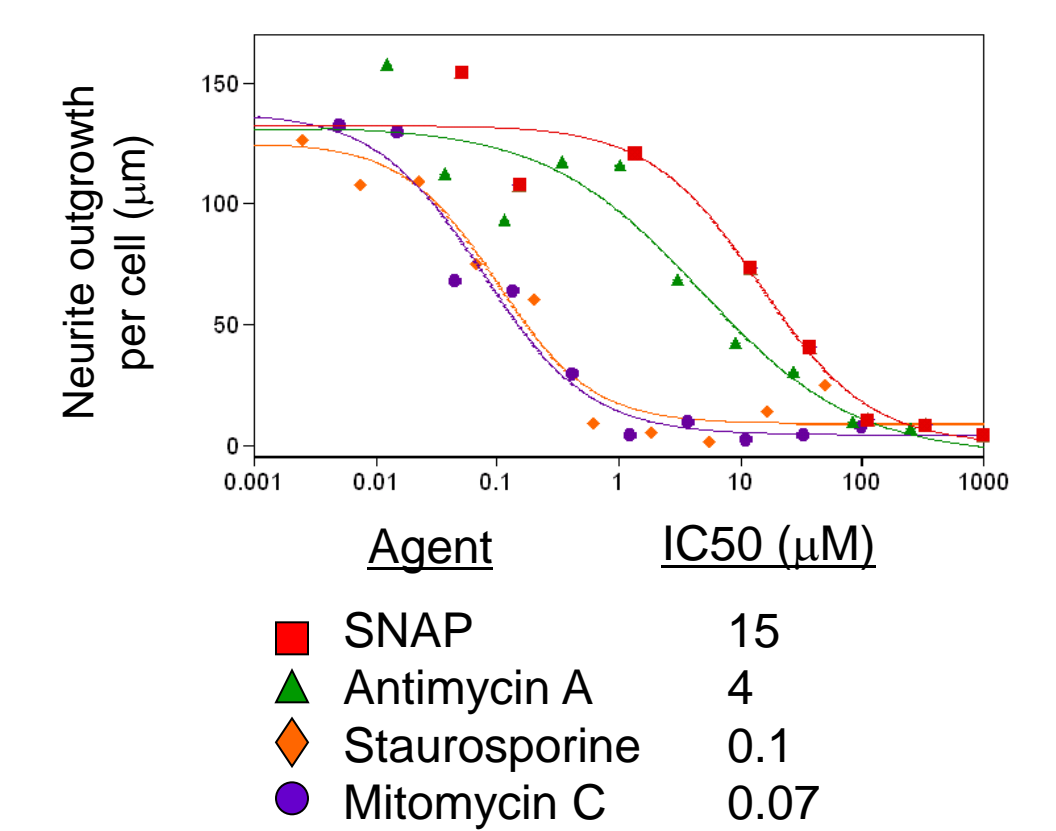
hNP1 neuronal progenitors were differentiated into cells with neuronal phenotype. Effects of different growth factors were evaluated in 14 day assay in 96w format.

Growth factors promote neural differentiation as measured by neurite outgrowth

Output parameters from Neurite Outgrowth analysis: Total outgrowth per field, number of neuronal branches and processes, significant growth (>6µm). Error bars: SEM, N=4

Disintegration of Neuronal Networks by Cytotoxic Agents

The hN2 cells can be used for evaluation of potential toxic effects on neuronal development. Nitric Oxide (NO) was found to contribute to neuronal death and brain damage in neurological diseases. Inhibition of neuronal development by NO inducer (SNAP) and several other cytotoxic compounds was evaluated using various output parameters after visualizing cells with β-III tubulin.



Summary

We have developed high content imaging methods that allow automatic evaluation of proliferation and differentiation of hESC derived neuronal progenitors

We have demonstrated the effect of positive and negative factors on neuronal development:

- EPO promotes differentiation of human neural progenitors toward neurons
- NO inducer SNAP, Antimycin A and staurosporine inhibit neuronal development

These methods can be used to automate assays for:

- Testing biologics or chemical compounds on neuronal development
- Screening and validation of drug candidates

Stem cell derived neurons and high content imaging provide powerful platform for neurobiology research and drug screening

