Flexible Automated Platform for Multimodal Image-Based Neurite Outgrowth Analysis

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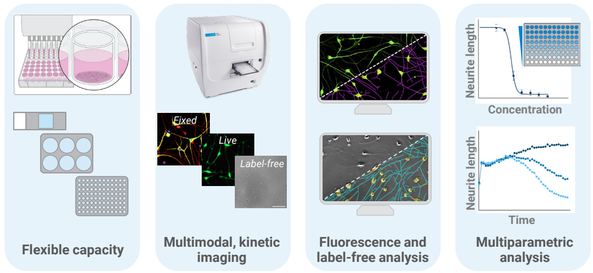
Introduction

- Neurite outgrowth assays provide insights into the key phenotypic changes in neuronal models used across developmental biology, neurotoxicity, degenerative diseases, and neuroregeneration research areas.
- Automated approaches advance neurite outgrowth studies by improving scale, efficiency, and reproducibility.
- Agilent BioTek automated imagers paired with Gen5 software neurite analysis capabilities enable high-throughput investigations across a diverse range of neurite outgrowth assay approaches.
- Multimodal neurite outgrowth assays provide synergistic and complimentary insights into complex profiles of outgrowth effects in response to drug treatment.

Automated solutions for neurite outgrowth



Experimental

Neuron culture

Reagents were sourced from Sigma, unless noted. iPSC-derived neurons (FUJIFILM iCell GlutaNeurons) were cultured according to product recommendations for media composition and culture protocol. For 2D assays, neurons were plated at 10,000 cells/well on poly-ornithine coated 96-well microplates (Greiner). For 3D assays, neurons were plated at 3,000 cells/well in 96-well spheroid plates (Nunc). Cryopreserved mouse cortical neurons (Gibco) were cultured in BrainPhys medium (StemCell Technologies) with media supplement and culture protocol adhering to the media manufacturer recommendations for primary culture and plated at ~ 10,000 cells/well on poly-lysine coated 96-well microplates (Greiner).

Neuron labeling

Paraformaldehyde-fixed neuron cultures were labeled with antibodies detecting β-III-tubulin (Invitrogen), MAP2, and DAPI (Invitrogen) nuclear counterstain. Live neuron spheroid cultures were stained with calcein green AM (Invitrogen) and Hoechst 33342.

Imaging

Kinetic live-cell imaging was performed on the Agilent BioTek Cytation 5 cell imaging multimode reader with the Agilent BioTek BioSpa 8 automated incubator. Fixed-cell imaging was performed on the Cytation 5 imager, and 3D live-cell outgrowth was performed on the Cytation C10 confocal imaging reader.

Analysis

Image acquisition and analysis were performed with the Agilent BioTek Gen5 neurite outgrowth module.

Expanded materials and methods available in the full application notes: IHC-based assay | Label-free kinetic assay

Results and Discussion

Label-free kinetic neurite outgrowth

Assay approach:

- Neurons were cultured in 96-well microplate and imaged every three hours over five days.
- Kinetic live-cell analysis of four treatments across six concentrations

Cytation 5 and BioSpa 8 automated incubator

Automated label-free imaging and analysis

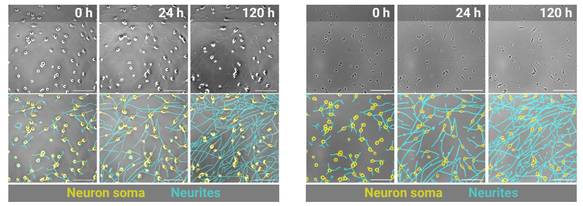


Figure 1. Agilent BioTek imagers support two label-free imaging techniques for live-cell neuron analysis. Example images of iPSC-derived neuron cultures taken in phase contrast and brightfield modes on the Cytation 5 and BioSpa 8 automated system at three culture time points. Automated Gen5 software Image analysis results indicated both transmitted light methods accurately identified neuron soma (yellow fill) and neurite processes (cyan). Scale bars correspond to 200 µm.

Live-cell kinetic neurite outgrowth quantification

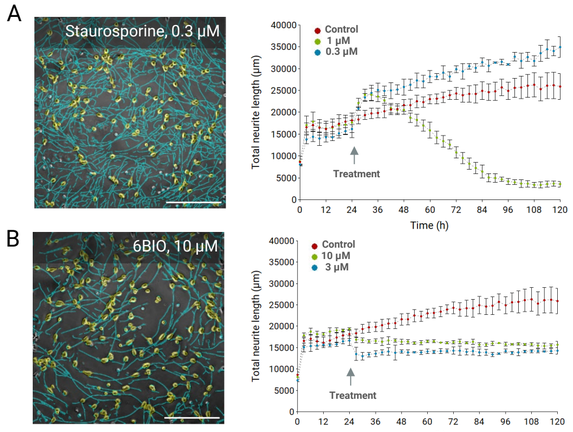


Figure 2. Detailed kinetic analysis of neuron culture outgrowth assessed by total neurite length across five days in culture. (A) Example image of staurosporine-treated neurons after five days in culture (left.) Staurosporine enhances outgrowth (blue trace) compared to control (red trace). At the highest concentration tested (green trace), initial increases in neurite length are followed by cell death and neurite loss. Data points indicate mean and standard deviation of replicates (n = 3). (B) Example image of 6BIO treated neurons after five days in culture (left.) Application of 6BIO rapidly reduces neurite outgrowth and maintains inhibition throughout the culture timeframe. Data points indicate mean and standard deviation of replicates (n = 3). Scale bars correspond to 200 µm.

Drug effect timing: Outgrowth rate-of-change analysis

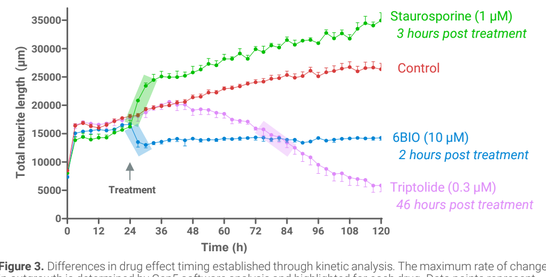


Figure 3. Differences in drug effect timing established through kinetic analysis. The maximum rate of change in outgrowth is determined by Gen5 software analysis and highlighted for each drug. Data points represent mean and SEM for replicate conditions (n = 3).

Results and Discussion

Immunohistochemistry-based neurite outgrowth

Assay approach:

- Neurons were cultured in 96-well microplates and treated with four drugs across six concentrations.
- After five days in culture, cultures were fixed and processed for immunofluorescence labeling of MAP2, β-III-tubulin, and DAPI nuclear counterstain.

Cytation 5 cell imaging multimode reader

Automated multichannel imaging and analysis

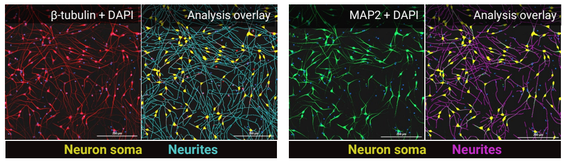


Figure 4. Multichannel image acquisition and automated neurite analysis. Example images of control iPSC-derived neuron culture outgrowth evaluated through β-tubulin (left) and MAP2 (right) antibody staining and corresponding image analysis. Scale bars correspond to 200 µm.

iPSC-derived neuron outgrowth quantification

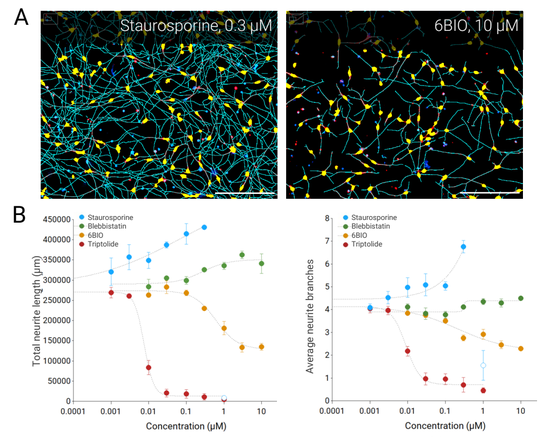


Figure 5. Neurite outgrowth analysis of iPSC-derived cultures across drug treatments. (A) Example images with analysis overlay for treatments that increased (staurosporine, left) and decreased (6BIO, right) outgrowth. Scale bars correspond to 200 µm. (B) Dose-response analysis determined concentration-dependent drug effects across conditions for length and branching related morphology parameters. Data points represent the mean and standard deviation of condition replicates (n = 3).

Mouse cortical culture outgrowth analysis

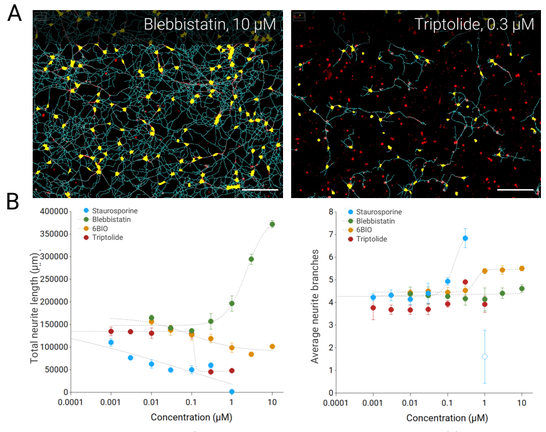


Figure 6. Neurite outgrowth analysis of mouse cortical cultures across drug treatments. (A) Example images with analysis overlay for treatments that increased (blebbistatin, left) and decreased (triptolide, right) outgrowth. Scale bars correspond to 200 µm. (B) Dose-response analysis determined concentration-dependent drug effects across conditions for length and branching related morphology parameters. Data points represent the mean and standard deviation of condition replicates
(n = 3).

Results and Discussion

Neurite outgrowth in live-cell 3D neuron spheroids

Assay approach:

- Neuron spheroids were formed in round-bottom ULA 96-well microplates and transferred to ECM-coated 96-well plate for outgrowth evaluation.
- 48 hours post transfer, calcein AM was applied for live-cell outgrowth analysis.

Cytation C10 confocal imaging reader

Live-cell outgrowth in iPSC-derived neuron spheroids

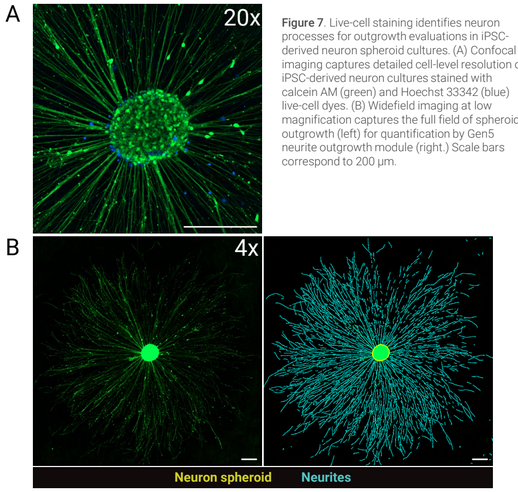


Figure 7. Live-cell staining identifies neuron processes for outgrowth evaluations in iPSC-derived neuron spheroid cultures. (A) Confocal imaging captures detailed cell-level resolution of iPSC-derived neuron cultures stained with calcein AM (green) and Hoechst 33342 (blue) live-cell dyes. (B) Widefield imaging at low magnification captures the full field of spheroid outgrowth (left) for quantification by Gen5 neurite outgrowth module (right.) Scale bars correspond to 200 µm.

Drug effect evaluation in 3D neuronal outgrowth model

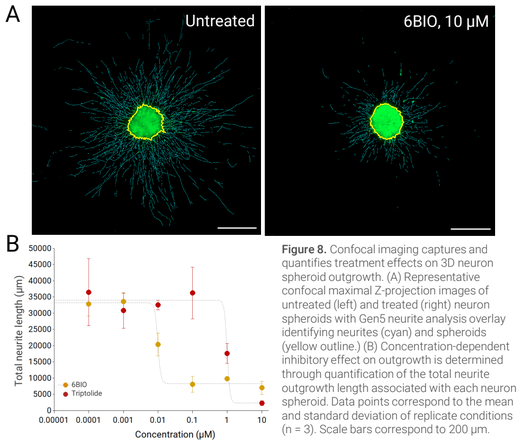


Figure 8. Confocal imaging captures and quantifies treatment effects on 3D neuron spheroid outgrowth. (A) Representative confocal maximal Z-projection images of untreated (left) and treated (right) neuron spheroids with Gen5 neurite analysis overlay identifying neurites (cyan) and spheroids (yellow outline.) (B) Concentration-dependent inhibitory effect on outgrowth is determined through quantification of the total neurite outgrowth length associated with each neuron spheroid. Data points correspond to the mean and standard deviation of replicate conditions (n = 3). Scale bars correspond to 200 µm.

Conclusion

- The Agilent BioTek instrument and software platform demonstrated sensitive and robust quantification of neurite outgrowth across a spectrum of assay approaches.
- Gen5 Neurite Outgrowth analysis software automatically provided key morphology parameters for both label-free and fluorescence outgrowth analysis.
- Powerful downstream software analysis tools yielded quantitative insights into both time-dependent and concentration-dependent treatment effects.