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Simplifying Progress

AI-Driven Image Analysis for Label-Free Quantification of Chemotherapeutic Cytotoxicity in Glial Cells

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Introduction

- Glioblastoma multiform (GBM) is a malignant brain tumour associated with poor prognosis. To progress effective chemotherapies there is a need to develop more translational models and advanced quantitative methods which enable increased insight into cytotoxic effects in a non-perturbing manner.
- Here we demonstrate a robust *in vitro* assay using the Incucyte® Live-Cell Analysis System and integrated software to assess cytotoxic effects of clinically relevant chemotherapeutics in glial cell types.
- We utilized the Incucyte® AI Cell Health Analysis Software Module, which is driven on pre-trained neural networks (CNN), and is a robust solution for label-free cell segmentation and Live/Dead classification.
- We observed differential time- and concentration-dependent effects across glial cell types.
- These data exemplify that Incucyte® Live-Cell Analysis System, alongside advanced AI-driven analytics, is a powerful approach for assessing cytotoxicity in glial cell types and is amenable to screening of therapeutic candidates.

Incucyte® Live-Cell Analysis Solutions

Incucyte® Live-Cell Analysis System

A fully automated HD phase-contrast and multi-color fluorescence system that resides within a standard cell incubator for optimal cell viability. Designed to scan plates and flasks repeatedly over time.

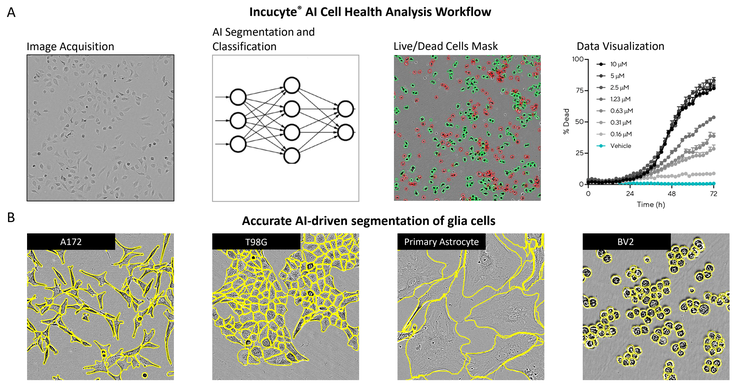
Incucyte® Software

Fast, flexible, and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and data visualization.

Incucyte® Reagents and Consumables

A suite of non-perturbing cell labelling and reporter reagents. Includes nuclear-targeted fluorescent proteins for cell counting plus no-wash cell health reagents for apoptosis, cytotoxicity, and many more.

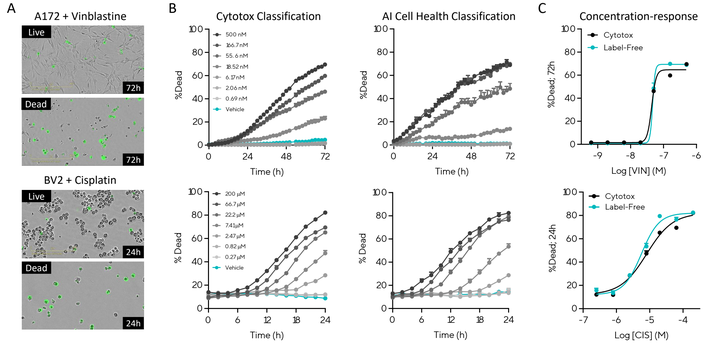
Assay Principle



A. Incucyte® AI Cell Health Analysis workflow. HD phase-contrast images are acquired and processed using pre-trained neural networks to automatically segment and classify cells as live or dead. This AI-driven analysis is applied to all wells and timepoints, providing robust data and visualization of live or dead masks.

B. The AI Cell segmentation is highly accurate even in confluent images and adapts to a multitude of cell morphologies. Shown is the AI segmentation applied to glial cell types, including GBM cell lines A172 and T98G, primary rat cortical astrocytes, and semi-adherent BV2 microglia.

AI-driven Live/Dead Classification Validation



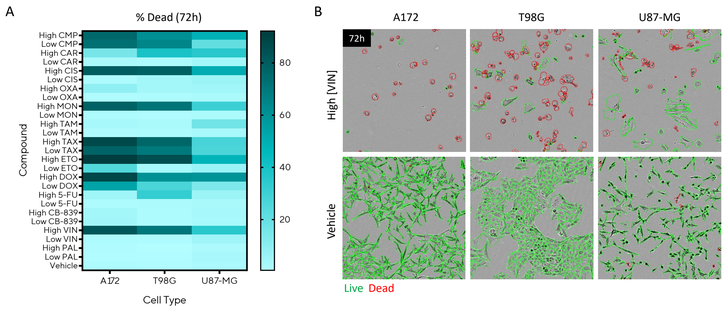
- A172 or BV2 cells seeded into 96-well plates (2 and 8K cells/well, respectively) were treated with a concentration-range of cytotoxic compounds Vinblastine or Cisplatin in the presence of Incucyte® Cytotox Green Dye.

A. Phase HD and fluorescence images were acquired in an Incucyte® Live-Cell Analysis System and representative live and dead images are shown.

B. Cell death was quantified using Incucyte® AI Cell Health Analysis Live/Dead classification (label-free) and fluorescence classification of Cytotox positive cells. Time courses show comparable time- and concentration-dependent increase in the % dead cells.

C. Corresponding concentration-response curves show comparable EC_{50} values are obtained using these classification models.

Chemotherapeutic Cytotoxicity Screen

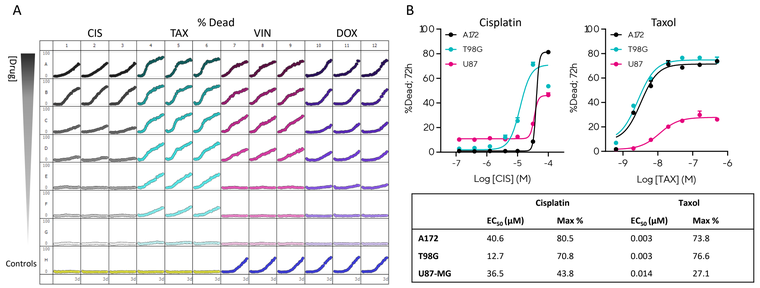


- A172, T98G and U87-MG GBM cells (2K cells/well) were treated with a 96-well throughput screen consisting of high and low concentrations of 13 chemotherapeutics. Images were acquired in the Incucyte® over 3 days and quantified using Incucyte® AI Cell Health Analysis Software Module.

A. Heatmap shows % dead cells at 72h for all compounds and cell types. Observed that U87-MG shows reduced sensitivity for most compounds.

B. Representative phase images show Live/Dead classification masks for high vinblastine (VIN) treated cells and vehicle and highlight adaptability of segmentation and classification across different live and dead morphologies. Data indicates differences in potency for vinblastine across glioblastoma types.

Comparing Concentration-dependent Cytotoxicity in GBM Cells



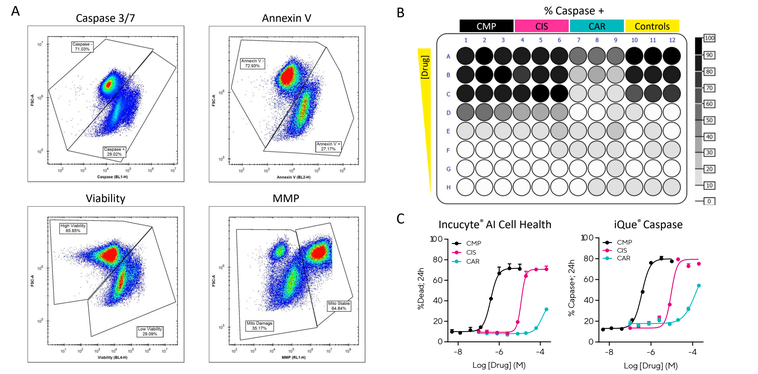
	Cisplatin		Taxol	
	EC_{50} (µM)	Max %	EC_{50} (µM)	Max %
A172	40.6	80.5	0.003	73.8
T98G	12.7	70.8	0.003	76.6
U87-MG	36.5	43.8	0.014	27.1

- A172, T98G, and U87-MG cells were seeded (2K cells/well) and treated with concentration-ranges of 4 chemotherapeutics.

A. Microplate graph displays % dead over 3 days for T98G cells, with varying kinetic concentration-dependent effects observed.

B. Concentration-response curves shown for Cisplatin (CIS) and Taxol (TAX) which reveal a cell-type dependent differences in compound sensitivities with U87-MG showing reduced potency compared A172 and T98G cells for both compounds. EC_{50} values and maximal % cell death are shown in table.

Combined Approach to Quantify Cell Death in Microglia



- BV2 microglia cells were seeded onto a PLO coated 96-well plate (8K cells/well) and once adhered treated with concentration ranges of camptothecin (CMP), cisplatin (CIS) and carboplatin (CAR). Images were acquired every 2 hours in the Incucyte® over 24 hours and cell death quantified using Incucyte® AI Cell Health Analysis Software Module. At 24 hours, cells were harvested, labeled using the iQue® 4-Plex Apoptosis Kit and quantified on the iQue®3, a high-throughput screening (HTS) cytometer.

A. Dot plots show gating strategy used for each apoptosis readout.

B. Heat map of 96-well plate shows caspase positive cells expressed as a percentage of single cells.

C. Transformed data shows EC_{50} curves for Incucyte® quantifying the percentage of dead cells and iQue® showing the percentage of caspase positive cells at 24 hours.

Data presented as mean ± SEM, 3 – 6 replicates