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Advancing Drug Discovery: Integrating 3D Spheroid Models and Live-Cell Analysis for Compound Screening

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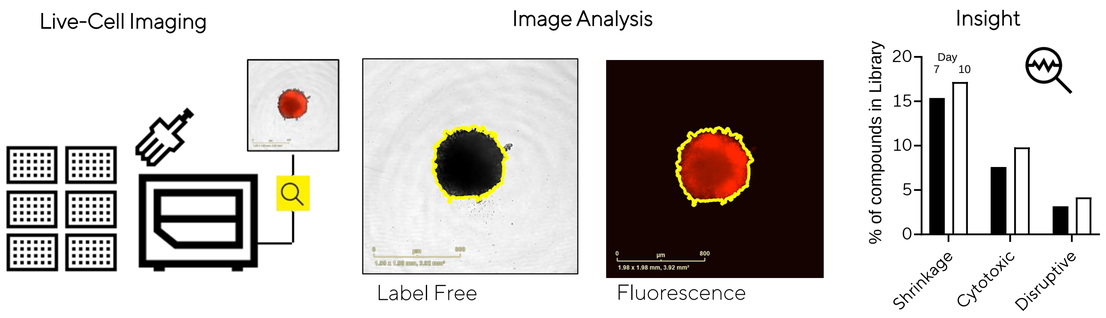
Introduction

- Phenotypic screening, which measures defined changes in cellular phenotype in response to a multitude of compounds, is essential in early-stage drug discovery.
- The study investigates the use of a 3D single spheroid model combined with live-cell analysis for drug screening. A library of 880 FDA-approved drugs was profiled to assess changes in spheroid size and viability.
- Overall, the integration of 3D cellular assays with live-cell analysis offers a powerful and robust tool for phenotypic screening, enhancing drug discovery efforts and ultimately contributing to the development of more effective treatments.

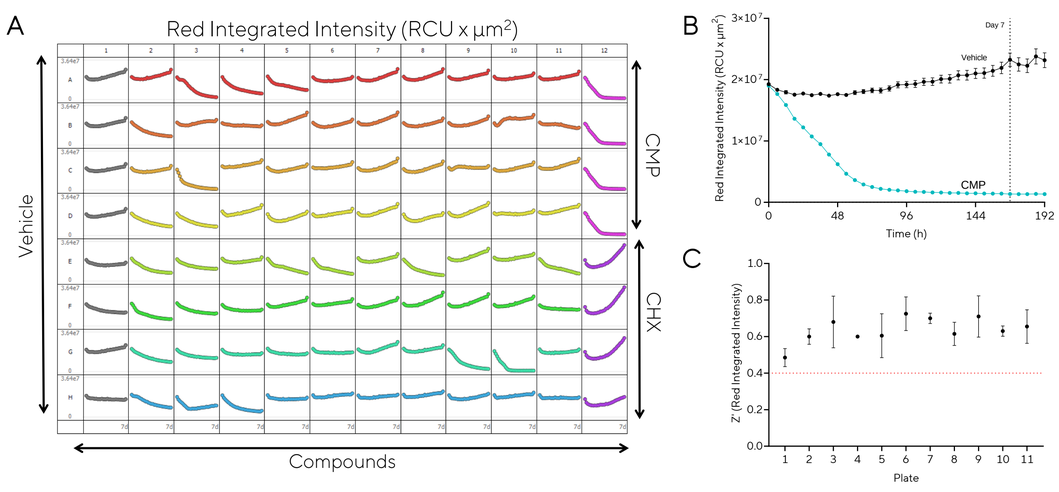
Compound Screen Assay

- A549 Nuclight Red (NR) cells were seeded in 96-well ULA plates. Plates were centrifuged, and spheroid formation was monitored using the Incucyte® Live-Cell Analysis System.
- After 3 days, compounds from a screening library were added to the spheroids. DMSO was used as a vehicle control; Camptothecin (CMP) and Cycloheximide (CHX) served as positive controls.
- 22 plates were treated to assess compound effects on spheroid growth, shrinkage, and viability.



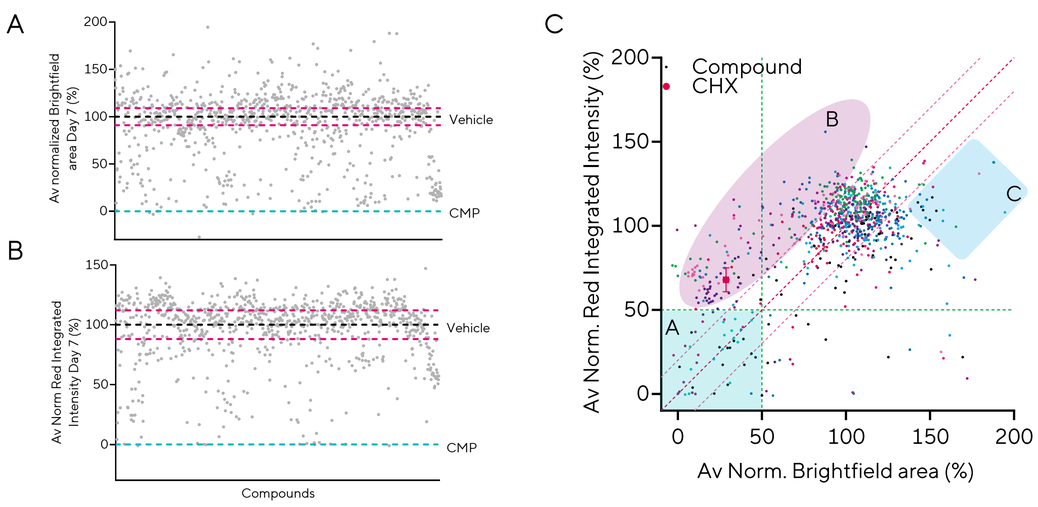
Data Analysis Workflow

- Comprehensive time-course data was collected for all test entities. Incucyte® software's microplate view (A) enabled rapid visualization of the complete 96-well time course.
- Brightfield area indicates spheroid growth or shrinkage, and red integrated intensity (B) indicates cell viability. A clear assay window was observed, increasing over time for both measurements.
- Controls calculated a Z' value (C), values over 0.4 indicate robust assay quality for single-shot screening.



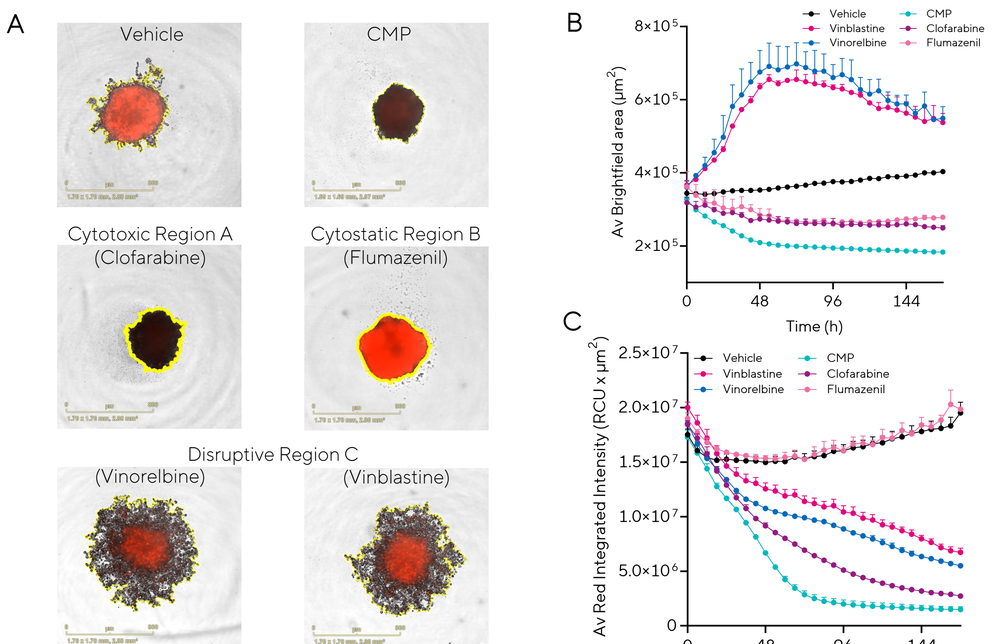
Comparative Analysis Across Plates

- Analysis used the average of vehicle wells to define the no-effect response (100% spheroid size) and CMP wells for the positive cytotoxic effect (0% spheroid shrinkage). The compound library targets various mechanisms of action, and normalized values were plotted across all plates to overview spheroid size (A) and viability responses (B).
- The two readouts are compared directly, regions of interest can be identified (C):
  - Region A: <50% response, indicating cytotoxicity with reduced viability and spheroid shrinkage.
  - Region B: Affects spheroid size, limited viability impact; includes cycloheximide as a control.
  - Region C: Spheroid Disruption Compounds increase spheroid size (>150%) with minimal viability effect, suggesting disruption.



Investigation of Mechanisms of Action

- For all plates, the entire time course of effect was collected, complete with images of responses, both of which can be used to provide more biological insights.
- Images are shown at 7 days post-spheroid formation for various treatment groups (A). Time courses shown for both size and viability measurements (B and C).
- Clofarabine mimics the CMP cytotoxic response (<50% for both parameters, purple line).
- Flumazenil, targeting GABAA receptors, shows a cytostatic profile with reduced spheroid size like clofarabine but no viability effect (pink line, Region B).
- Vinorelbine and vinblastine, targeting microtubule processing, cause spheroid disruption (Region C), leading to increased spheroid size and partial fluorescence drop.

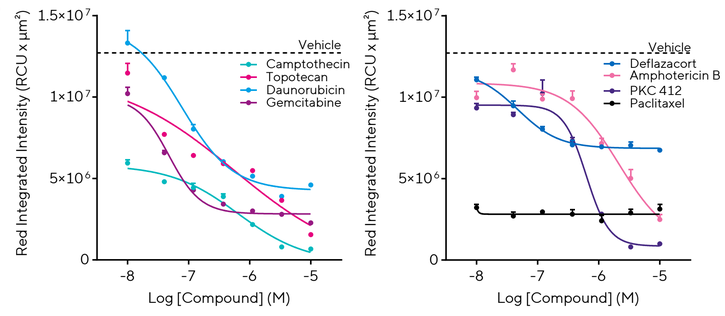


In-Depth Compound Profiling

- Fourteen DNA interference compounds were identified as cytotoxic hits (<50% for either readout) in the single-shot screen. Selected compounds were tested across a wide concentration range (0.01–10 µM) in a 384-well plate. Compounds tested and log values are shown in table (A).
- Concentration response curves for compounds shown at 7 days (B). Black dotted line presents average vehicle response.
- Other active compounds showed potent effects across various targets.

A

Compound	Target	Log IC_{50}
Camptothecin	Topoisomerase I	-6.2
Topotecan	Topoisomerase I	-6.2
Daunorubicin	Topoisomerase II	-7.1
Gemcitabine	DNA intercalating agent	-7.2
Paclitaxel	Tubulin	>-8
PKC 412	Kinases-Multiple	-6.2
Amphotercin B	Ergosterol Biosynthesis	-5.6
Deflazacort	Glucocorticoid receptor	-7.3



Enhanced Data with Next Generation Incucyte ® CX3

- The New Incucyte ® CX3 supports multi-colour single spheroid imaging and quantification, enabling both brightfield and fluorescence imaging with the use of confocal max projection images.
- A549 Nuclight Orange cells were treated with various compounds in combination with Incucyte® Cytotox green over 5 days.
- Microplate view at 5 days shows both the cytotox response in green and the cells in the spheroid in red. (A) The zoomed in images highlight that cell death starts peripherally.
- Quantification of the CMP response (B), shows an increase in green and a decrease orange fluorescence over time.