Live-cell analysis of 3D spheroids: label-free & fluorescent cell health reporters

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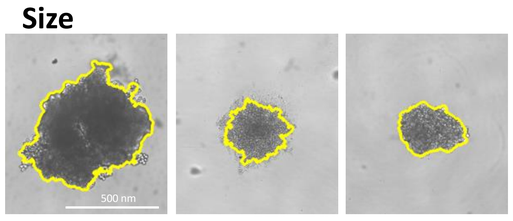
Summary & Impact

- A growing body of evidence suggests that 3D cell models yield more translational biological insight than 2D monolayers.
- Here we describe a simple kinetic live-cell imaging approach based on brightfield, in combination with fluorescent image analysis of spheroids.
- Brightfield analysis enables the monitoring of spheroid size (proliferation) and when combined with cell health markers (cytotoxicity or apoptosis) mechanisms of cell death may be elucidated.
- Expression of fluorescent proteins provides a surrogate for cell viability, where the fluorescence increases during proliferation and decreases following treatment with cytotoxic agents.
- These assays are flexible, simple and provide automated and direct measures of tumour size and health in real-time.

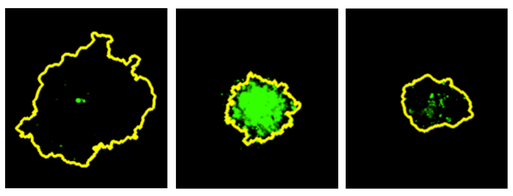
Cell Health Reporters; differentiating MOA

Control Vehicle | **Cytotoxic** CMP | **Cytostatic** CHX | **Cytotoxic** CMP | **Cytostatic** CHX | **CRCs**

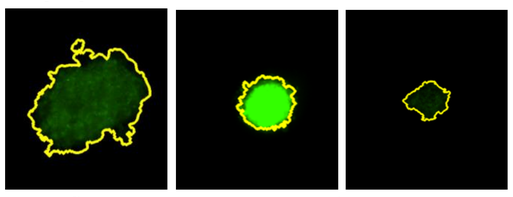
Size



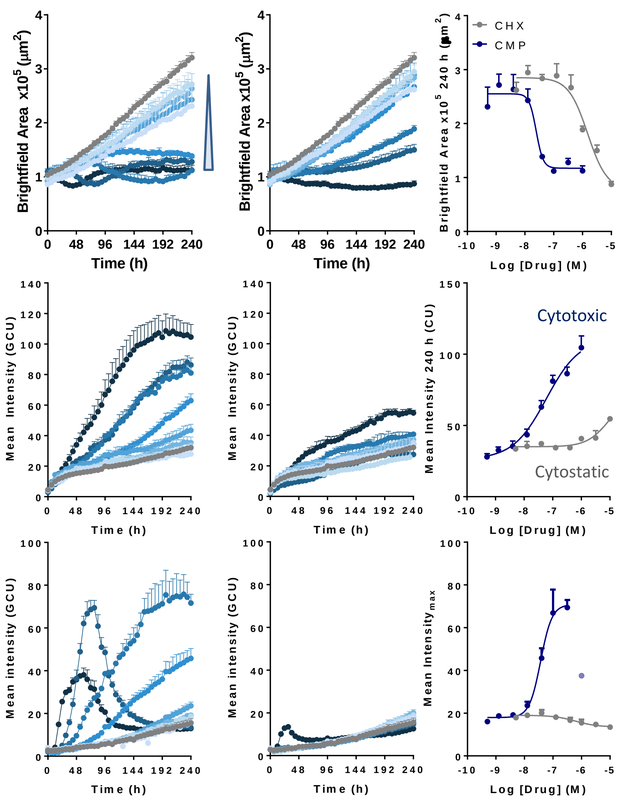
Cytotoxicity



Apoptosis



BF or fluorescence images at 240 h post-treatment. Yellow line represents the BF mask boundary.

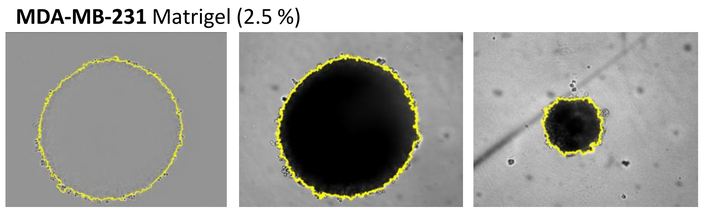


- SKOV3 spheroids treated with cytotoxic agent camptothecin (CMP) or the cytostatic agent cycloheximide (CHX) and loaded with IncuCyte Cytotox Green (25 nM) or IncuCyte Caspase 3/7 Green (2.5 µM) cell health reagents.
- Time-courses show temporal response to each agent using BF or green channels. CRCs compare the mechanism of action (cytotoxic vs cytostatic).

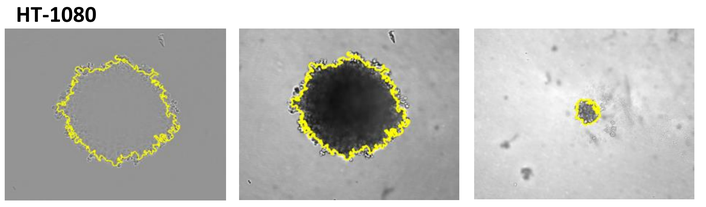
Brightfield enables label free quantification of spheroids

Phase | **BF (vehicle)** | **BF (CMP 1 µM)**

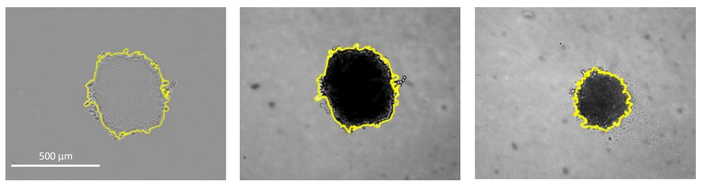
MDA-MB-231 Matrigel (2.5 %)

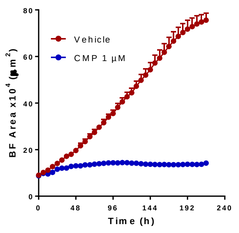


HT-1080



A549

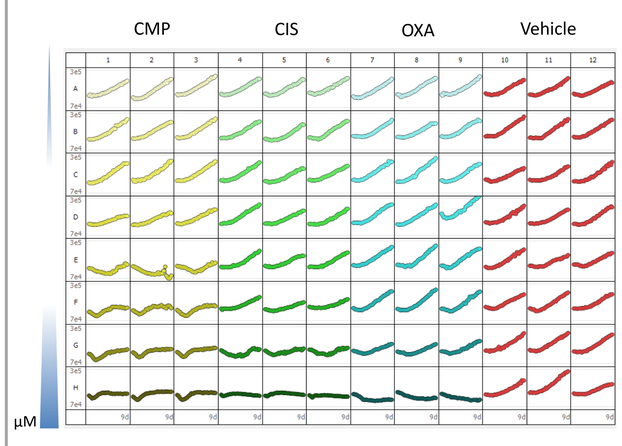




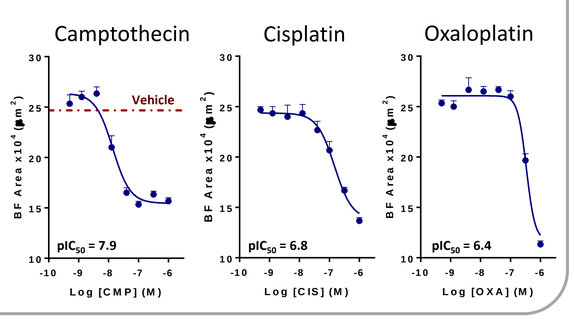
- Label free BF analysis enables accurate kinetic quantification of spheroids growth.
- Spheroids formed for 72h (2,500 cells per well), followed by treatment with vehicle or CMP (1 µM).
- Each data point represents mean ±SEM, n=4

BF images at 240 h post-treatment. Yellow line is the mask boundary.

Brightfield analysis; a spheroid growth assay



- SKOV3 cells (5,000 cells per well), 72 h formation, followed by treatment.
- Plate view shows BF area (µm²) over time.
- Concentration response curves represent the BF area (µm²) at 204 hours post-treatment. Each data point represents mean ±SEM, n=8



Continuous Live-Cell Analysis: Methodology

IncuCyte® S3 Live-Cell Analysis System

A flexible assay platform that sits inside a standard tissue culture incubator. IncuCyte automatically and continuously acquires and analyzes HD phase and fluorescent images of living cells cultured in microplates, dishes, or flasks.

IncuCyte® Software

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

IncuCyte® Reagents & Consumables

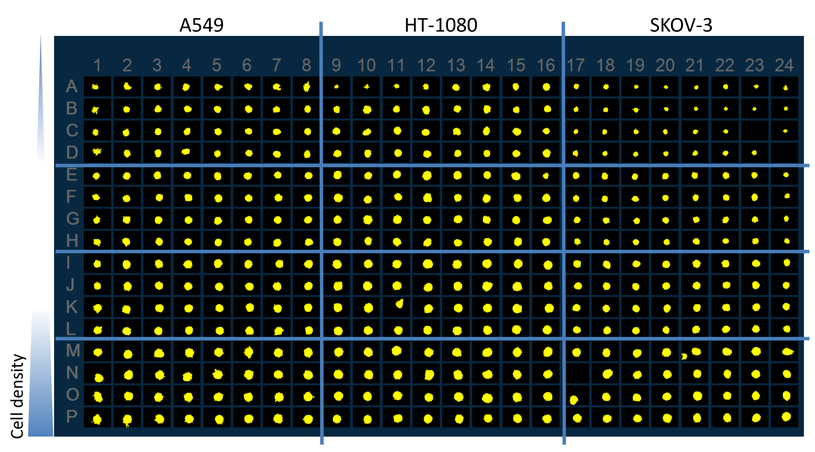
A suite of non-perturbing cell labeling and reporter reagents. Includes nuclear-targeted GFP and RFPs for cell counting, no-wash caspase 3/7 substrate for apoptosis, and cell kits for angiogenesis.

3D Spheroid assay principle

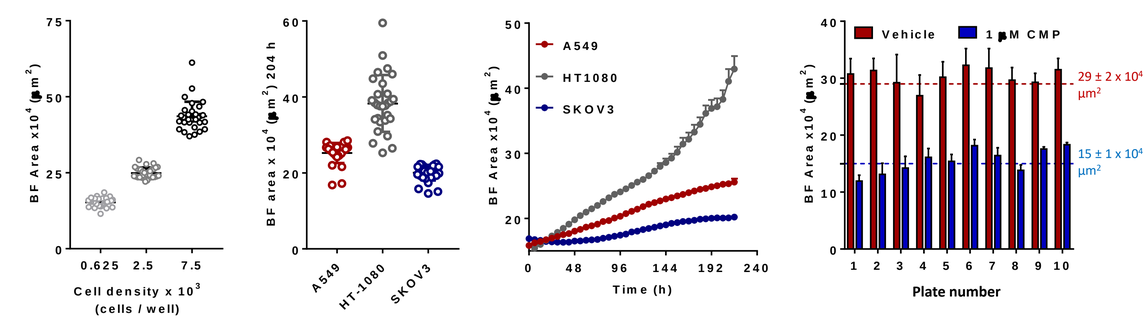
KEY ATTRIBUTES

- Automatic image capture
- Simple mix & read
- 96- & 384-assay formats
- Fully kinetic
- Flexible assay platform
- Multiplexing

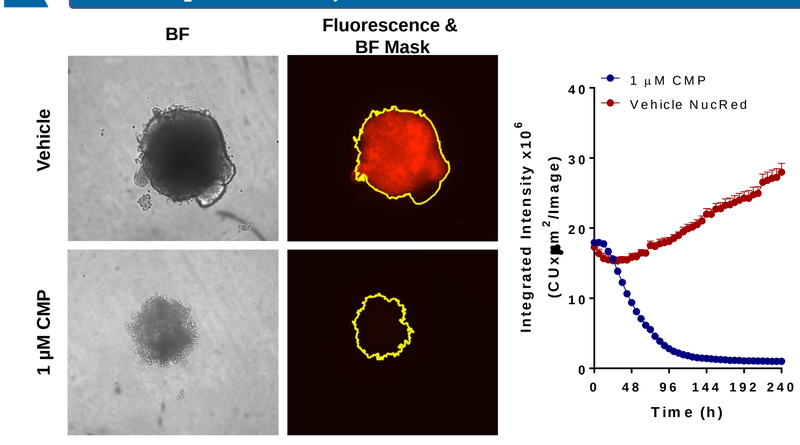
Robust intra- and inter-plate reproducibility



- Intra-plate variability analysis; seeding density of 310 to 7,500 cells/well of 384-well plate.
- Spheroid size is seeding density-dependent.
- Profiles of A549, HT-1080 and SKOV3 cells; consistent, differential growth kinetics.
- Inter-plate variability analysis; seeding density of 2,500 cells per well.
- Reproducible BF area metrics across plates for vehicle- and CMP-treated spheroids.

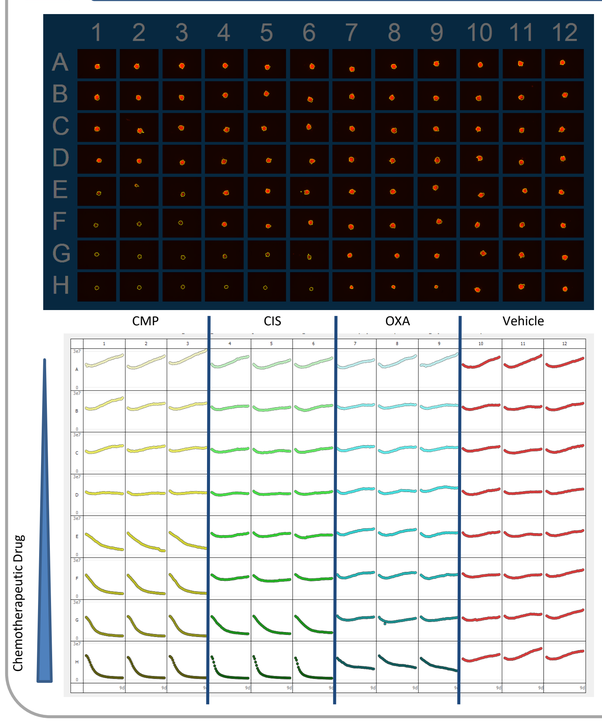


FP expression; an alternative for cell viability measurement



- Cells stably expressing fluorescent proteins (e.g. NucLight or CytoLight) enable robust kinetic quantification of spheroid size and viability.
- Viability of spheroids formed from SKOV3 cells stably expressing a NucLight Red fluorescent protein following treatment with vehicle or CMP (1 µM).
- Quantification; integrated intensity of the pixels within the BF boundary.

FP expression; pharmacology of cell viability



- SKOV3-NucLight spheroids formed by seeding 2,500 cells per well for 72 h, then treatment with the chemotherapeutics camptothecin, cisplatin or oxaloplatin.
- Whole plate visualisation of fluorescence with the BF boundary shown (240 h post-treatment) enables rapid assessment of the effects of the test compounds.
- Plate view of timecourses highlight the reproducibility of the triplicate data sets.
- The data shows a concentration-dependent inhibition by all chemotherapeutics illustrating their effect on cell health.