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# Development and optimization of matrigel-based multi-spheroid 3D tumor assays using real-time live-cell analysis

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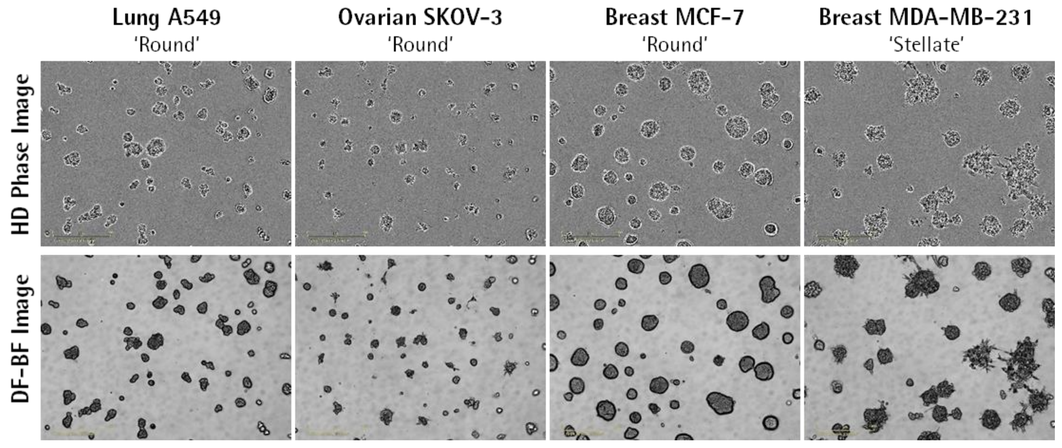
## Summary & Impact

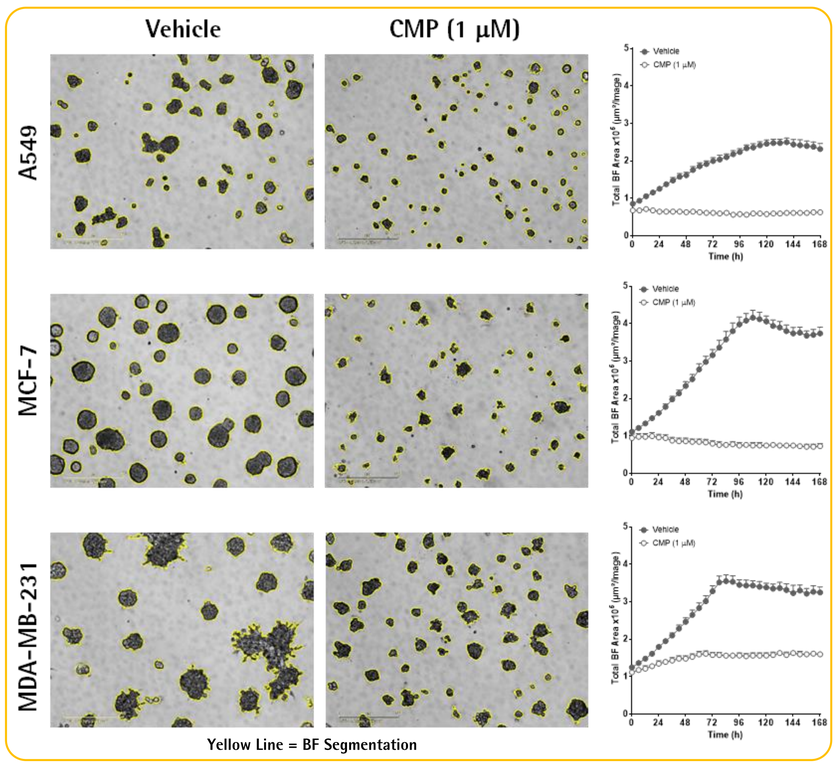

- The tumour-associated extracellular matrix (ECM) micro-environment provides critical biochemical cues as well as an essential structural scaffold for solid tumours to survive and grow.
- Here we describe a robust 3D ECM-based technique for culturing multiple tumour spheroids formed of lung, ovarian or breast cancer cell lines in a 96-well format.
- IncuCyte's DF Brightfield image acquisition tool enables the ability to monitor and quantify changes in spheroid size and morphology (brightfield) as well as viability (fluorescence) using real-time live-cell analysis.
- The use of IncuCyte® Cell Health reagents such as Annexin V to label apoptotic cells elucidates mechanism of action of compound treatments.
- Furthermore, this approach should facilitate more translational investigation of primary- and patient-derived organoid tumours.


## Novel DF-Brightfield image capture



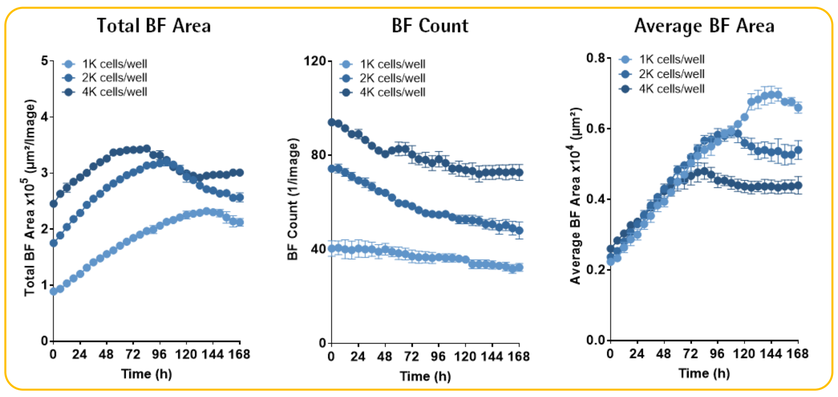

- High quality HD phase and DF-Brightfield (BF) images of multi-spheroids (MS) formed from a range of tumour cell lines (5 days post seeding) on a Matrigel® base.
- IncuCyte's proprietary image acquisition technique, DF-BF for 3D cultures, generates high contrast, extended depth of focus images.
- 3 days post seeding, A549, SKOV-3 and MCF-7 cells formed round aggregates, while MDA-MB-231 MS exhibited stellate branching distinctive of an invasive morphology.

## DF-Brightfield enables label free quantification



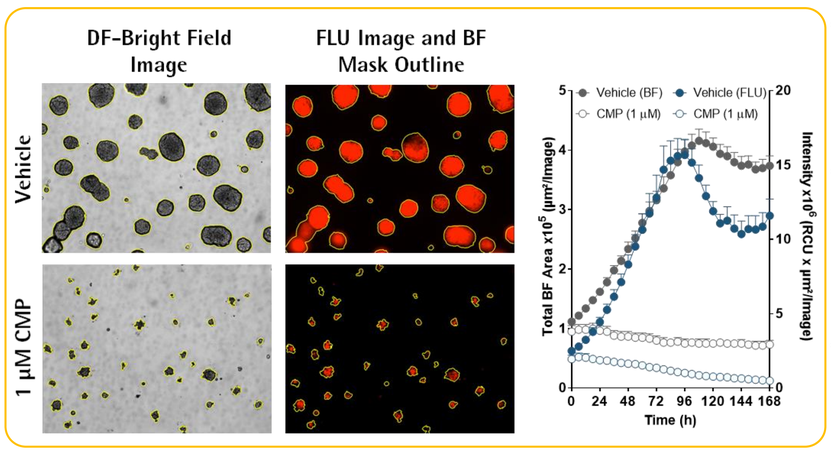

- A549, MCF7, and MDA-MB-231 cells were seeded (2K cells per well) and MS allowed to form for 3 days.
- MS were subsequently treated with the cytotoxic agent camptothecin (CMP, 1 µM) or vehicle control (0.1% DMSO) and images (DF-BF) collected every 6h for 7 days.
- IncuCyte multi-spheroid software enables kinetic quantification of growing and shrinking MS via size measurements (Total BF Area).
- A549, MCF-7 and MDA-MB-231 MS increased 1.9-, 2.0- and 1.8-fold in size over 3 days, respectively.
- Treatment with CMP (1 µM) inhibited growth of all MS.

## Cell number dependent multi-spheroid size




- A549 MS were seeded at various densities (1K - 4K cells per well).
- MS size and rate of growth, was proportional to the number of cells seeded.
- The decrease in number of MS over time reflects the merging of larger neighbouring MS.

## FP expression as an alternative measure for cell viability

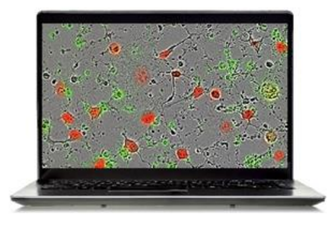



- MCF-7 MS stably expressing nuclear restricted RFP (MCF-7-NucLight Red) were treated with CMP (1 µM) or vehicle (0.1% DMSO) for 7 days.
- IncuCyte analysis software reports both MS size (BF Area) and viability (Fluorescence intensity within BF Area) without the need to mask the fluorescent object.
- Fluorescence (RFP) intensity measurements provide a potential surrogate for MS health.

## IncuCyte® System for Continuous live-cell analysis: Methodology


**IncuCyte® S3 Live-Cell Analysis System**

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



**IncuCyte® Software**

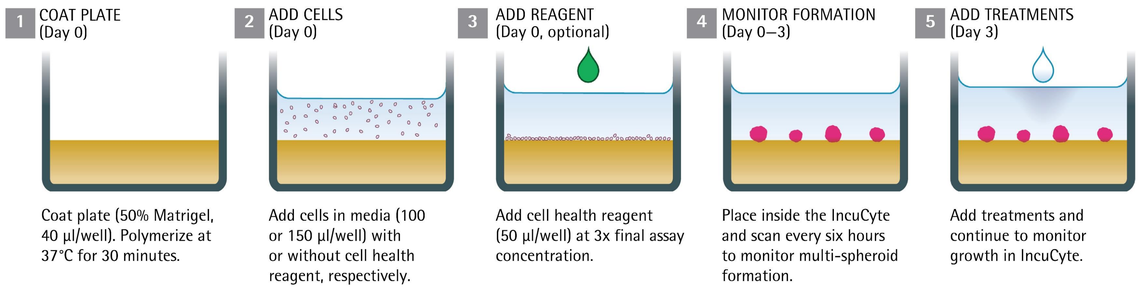
Fast, flexible and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and data visualization.

**New IncuCyte S3 Spheroid software module**

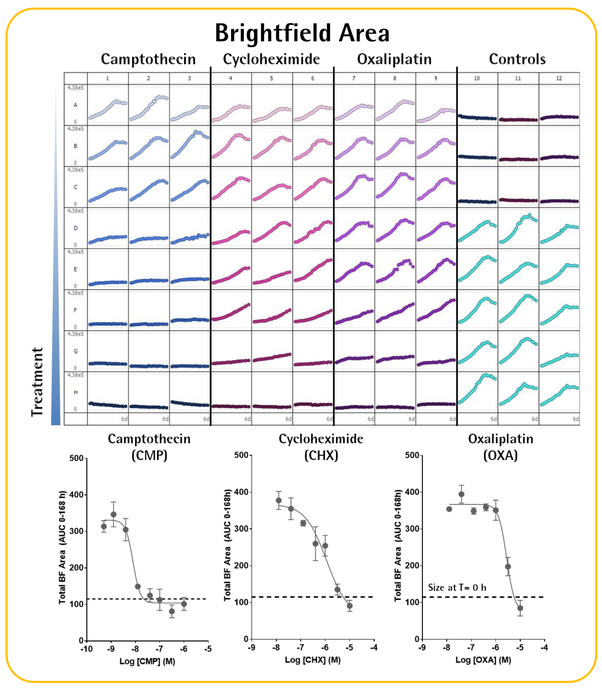
**IncuCyte® Reagents and Consumables**

A suite of non-perturbing cell labeling and reporter reagents. Includes nuclear-targeted GFP and RFPs for cell counting plus no-wash cell health reagents for apoptosis and cytotoxicity.

## Assay Workflow

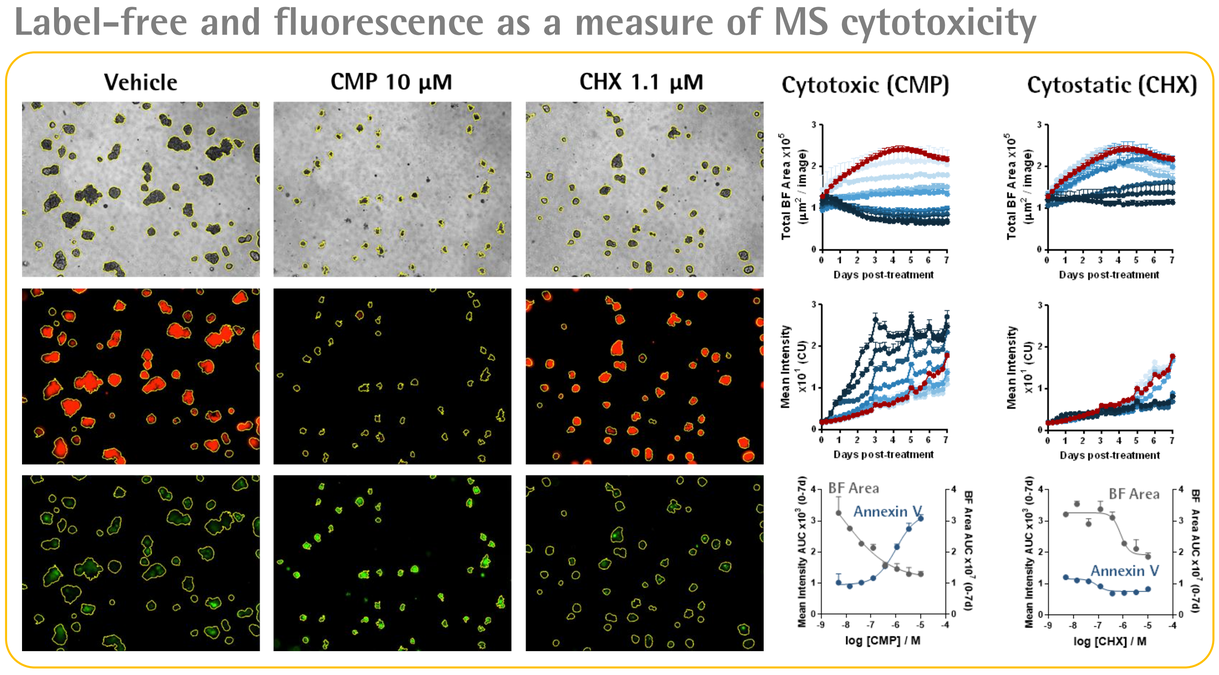



## Quantitative pharmacology using label free and fluorescent readouts




- MCF-7-NucLight Red MS were allowed to form for 3 days prior to treatment (7 days) with known cytotoxic compounds.
- Time-course plate-views enable rapid visualisation of treatment effects on both MS size (Total BF Area) and viability (FLU Intensity within BF Boundary).
- Concentration response curves represent area under curve (AUC) analysis of the time-course data.
- All compounds caused a concentration dependent inhibition of growth and viability with rank order of potency CMP > CHX ≥ OXA.

## Label-free and fluorescence as a measure of MS cytotoxicity




- A549-NR cells (2K cells per well) were seeded in the presence of IncuCyte Annexin V Green reagent (1%) and MS allowed to form for 3 days.
- MS were treated with CMP, CHX or vehicle control and images (DF-BF, red and green fluorescence) acquired every 6h for 7 days.
- Both CMP (cytotoxic) and CHX (cytostatic) caused a concentration-dependent inhibition of MS growth (Total BF time courses).
- A loss of RFP signal, lack of growth and a simultaneous increase in Annexin V green fluorescence intensity (apoptosis) was observed in CMP treated MS.
- Despite CHX inhibiting MS growth, RFP expression remained high, whilst little or no increase in Annexin V fluorescence was observed, suggesting minimal cell death. These observations are consistent with the cytostatic properties of CHX.
- CRCs compare the cytotoxic vs cytostatic mechanisms of CMP and CHX respectively.