

## Utilizing Confocal Live-cell Imaging For Evaluating Therapeutic Efficacy and Toxicity in Complex Oncology Models

Abstract Number: 7608

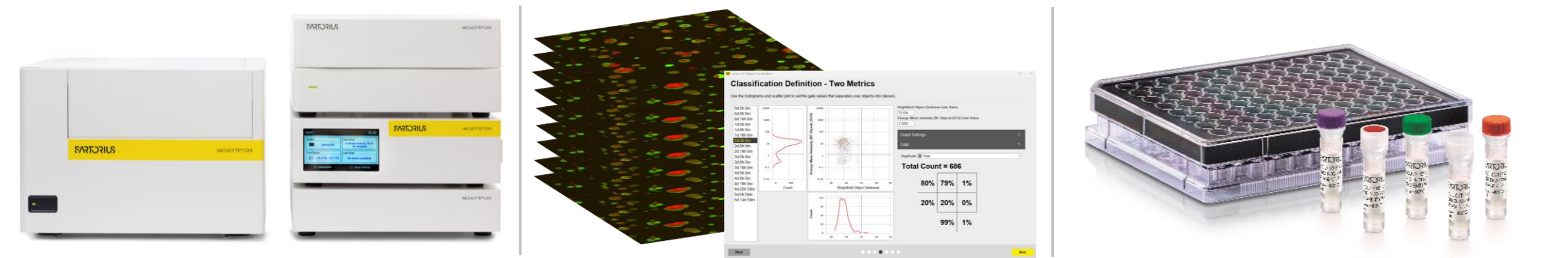
John Rauch<sup>1</sup>, Jasmine Trigg<sup>2</sup>, Kirsty McBain<sup>2</sup>, Jonathan Bezenah<sup>1</sup>, Libuse Oupicka<sup>1</sup>, Richard Lister<sup>1</sup>,

<sup>1</sup>Sartorius BioAnalytical Instruments Inc., <sup>2</sup>Sartorius Royston UK

### Introduction

- Oncology research increasingly relies on complex 3D models (spheroids, organoids, immune co-cultures), driving demand for advanced tools to extract clear, multiparametric insights.
- The Incucyte® CX3 enables multiplane, spinning-disk confocal live-cell imaging with integrated analysis for quantitative assessment of growth, morphology, and cell health under physiological conditions.
- In MCF7 tumor spheroids embedded in an extracellular matrix, camptothecin and cisplatin caused a concentration-dependent decrease in spheroid growth with a complementary increase in apoptosis.
- In mouse hepatic organoids, camptothecin, cisplatin, and acetaminophen elicited concentration-dependent inhibition of growth, accompanied by compound-specific levels of apoptosis.
- In SKOV-3-PBMC co-cultures, multiplane confocal imaging revealed PBMC density-dependent tumor killing and immune cell expansion, illustrating the platform's utility across oncology, immuno-oncology, and organoid-based toxicity models.

### Incucyte® Live-Cell Analysis Solutions



Incucyte® CX3 Live-Cell Analysis System

Incucyte® Software

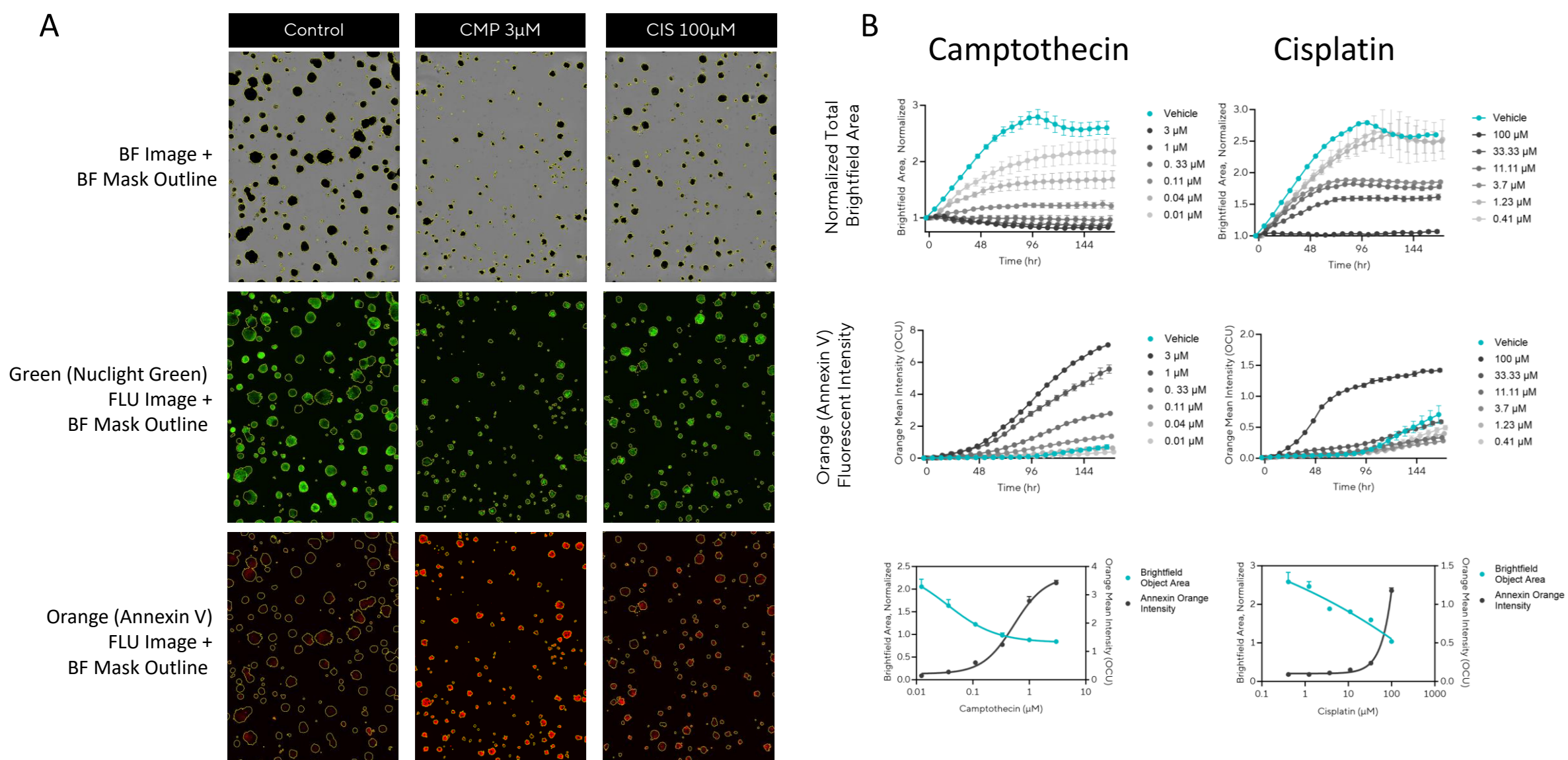
Incucyte® Reagents and Consumables

A fully automated system with spinning disk confocal, wide-field fluorescence, HD phase, and brightfield imaging that resides within a standard cell incubator for optimal cell viability. Deliver advanced, multi-modal imaging for acquisition of a broad range of 2D and 3D cell models.

Fast, flexible, and powerful control hub for continuous live-cell analysis comprising z-stack image acquisition, processing and data visualization. Harness AI-driven analysis to accelerate insights, enabling faster, smarter decisions via intelligent workflow adaptations in real time.

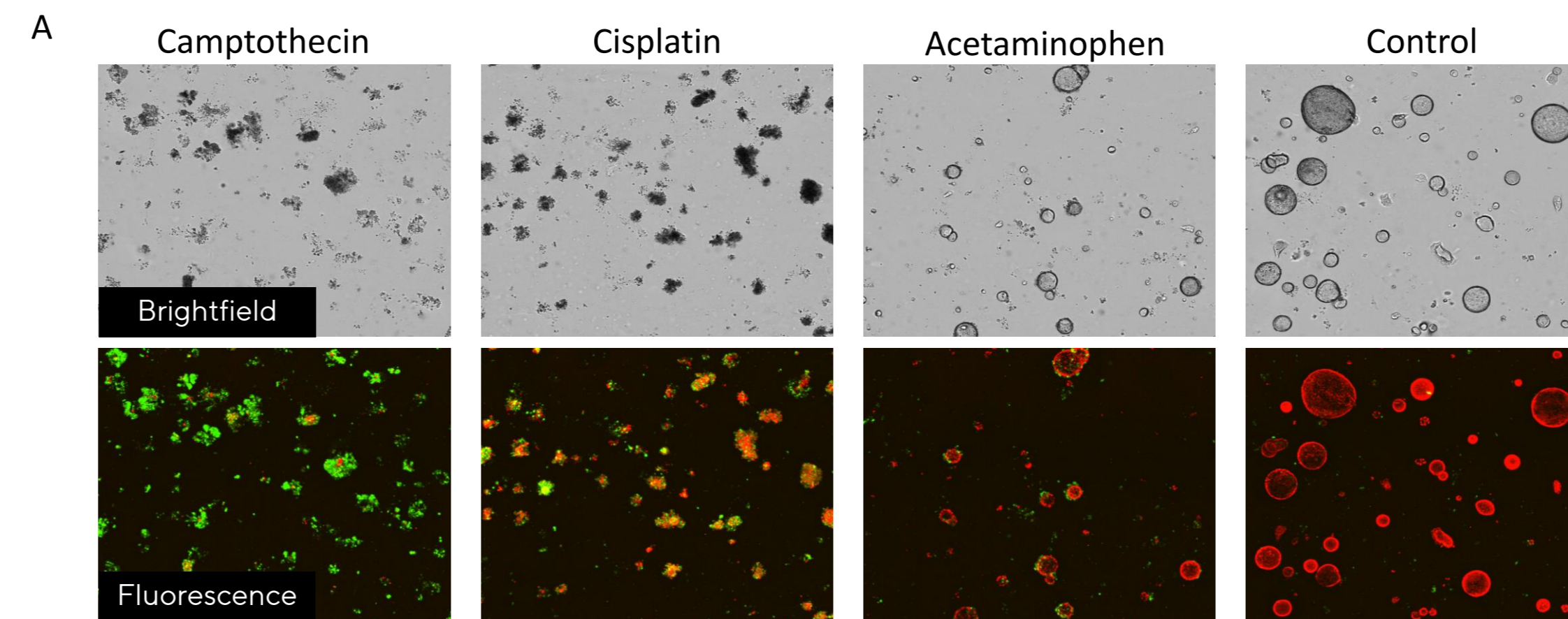
Optimized protocols enable intuitive, rapid and reproducible data collection. Portfolio includes nuclear-targeted fluorescent proteins for cell counting plus no-wash cell health reagents for apoptosis, cytotoxicity, and many more.

### Multiplexed growth and health measurements in embedded multi-spheroids

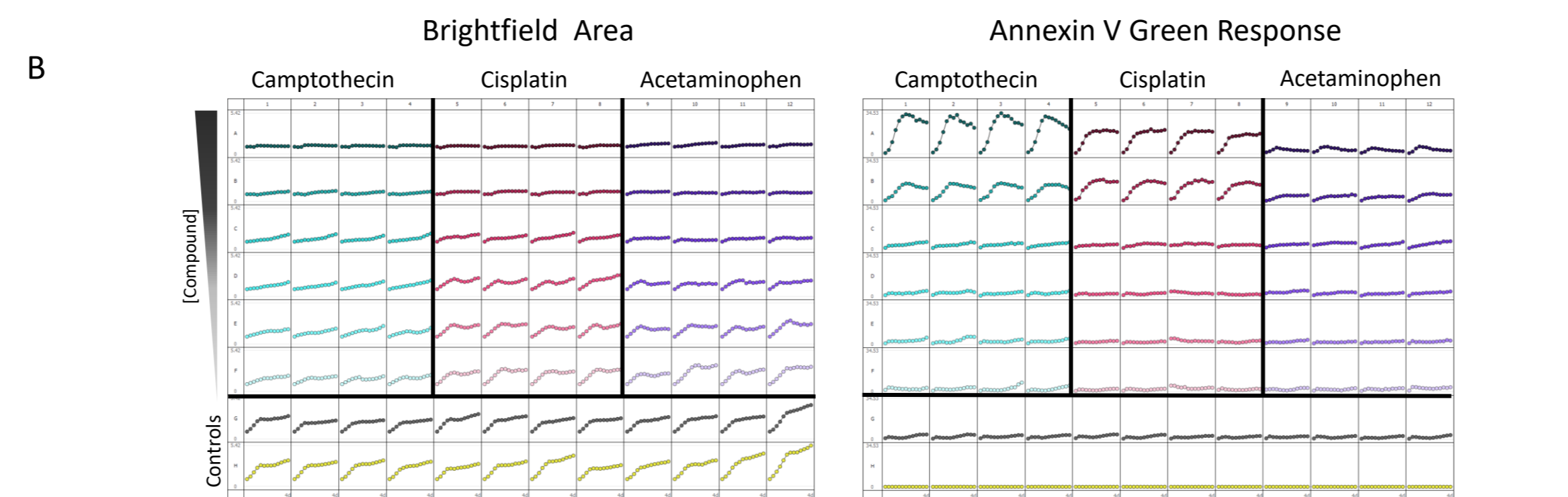


MCF7-NuLight Green cells (1K cells/well) embedded in an extracellular matrix were seeded in the presence of Incucyte® Annexin V Orange Dye (1%). Spheroids were allowed to form for 3 days. Spheroids were treated with a range of camptothecin (CMP) or cisplatin (CIS) concentrations and imaged for an additional 7 days. (A) Brightfield (BF, top row), Incucyte® NuLight Green fluorescent protein (green fluorescence, middle row), and Incucyte® Annexin V Orange (orange fluorescence, bottom row) images are compared 6 days post-treatment. (B) A lack of growth (BF Area time-course) and increase in the Mean Orange Fluorescence Intensity was observed in both CMP and CIS treated spheroids. CMP and CIS EC<sub>50</sub> from 4 days post treatment shows concentration-dependent loss of viability and increase in cell death.

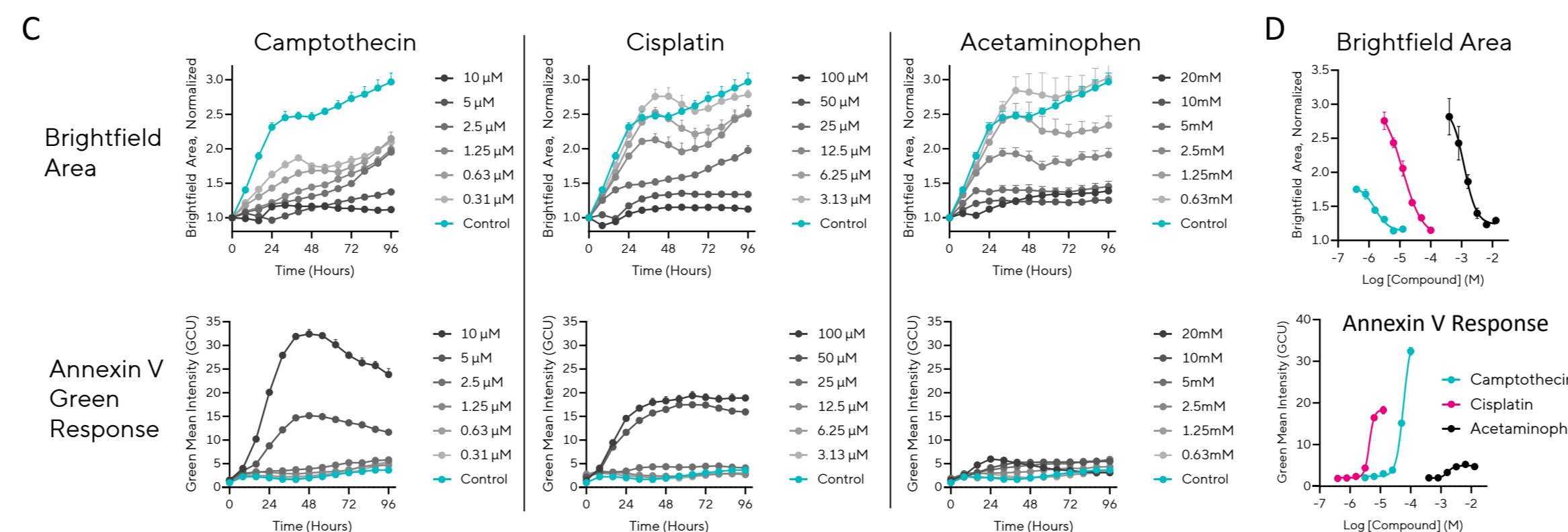
### Evaluation of cell death in an organoid model of hepatotoxicity



NuLight Orange Lentivirus-expressing murine hepatic organoids were embedded in matrix and cultured for 1 day before treating with a range of camptothecin, cisplatin, or acetaminophen in the presence of Incucyte® Annexin V Green Dye to monitor apoptosis for an additional 4 days. (A) Representative brightfield or max projection confocal fluorescent images of organoids 40 hours post-treatment with the indicated compounds.

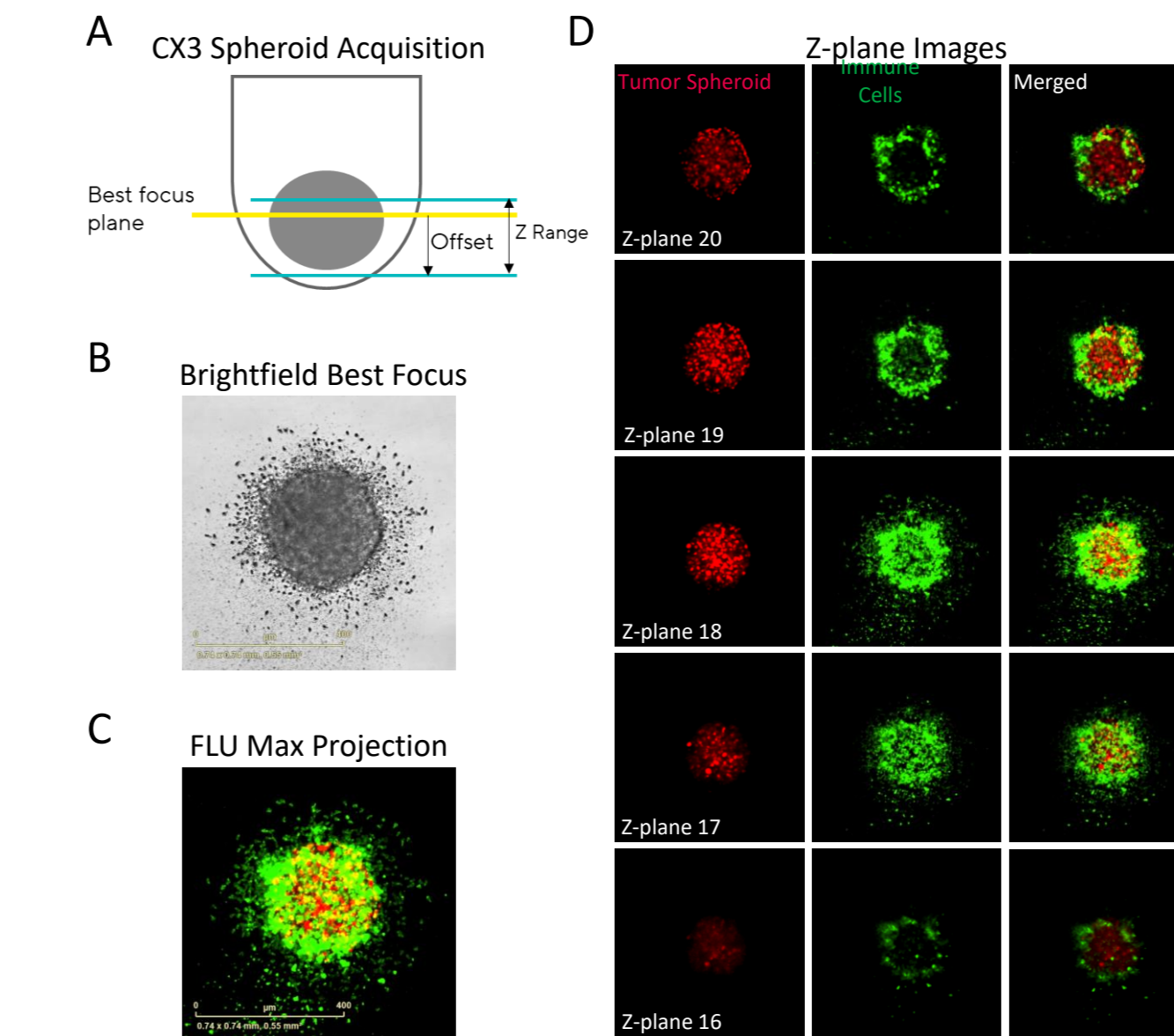


(B) Microplate graphs show 96 individual kinetic plots of Total Brightfield Area or Annexin V Green response (Green Mean Intensity) over 4 days.



(C) Kinetic graphs depict a concentration-dependent decrease in brightfield (BF) organoid area accompanied by an increase in mean green fluorescence intensity in camptothecin- and cisplatin-treated hepatic organoids, consistent with reduced viability and increased apoptosis. In contrast, acetaminophen treatment results in a concentration-dependent decrease in BF area with minimal change in green fluorescence intensity, indicating a reduction in organoid growth with limited apoptotic response. (D) Concentration response curves generated from the 48hr time point for Brightfield Area and Annexin V response (green intensity).

### Confocal imaging enables multiplexed readouts to spatially resolve immune cells and tumor spheroids



SKOV3-NuLight Orange spheroids were co-cultured with activated PBMCs which were labeled with Incucyte® Fabfluor-488-CD45 (green).

Brightfield and dual color fluorescence images were acquired at 10x using confocal multiplane acquisition.

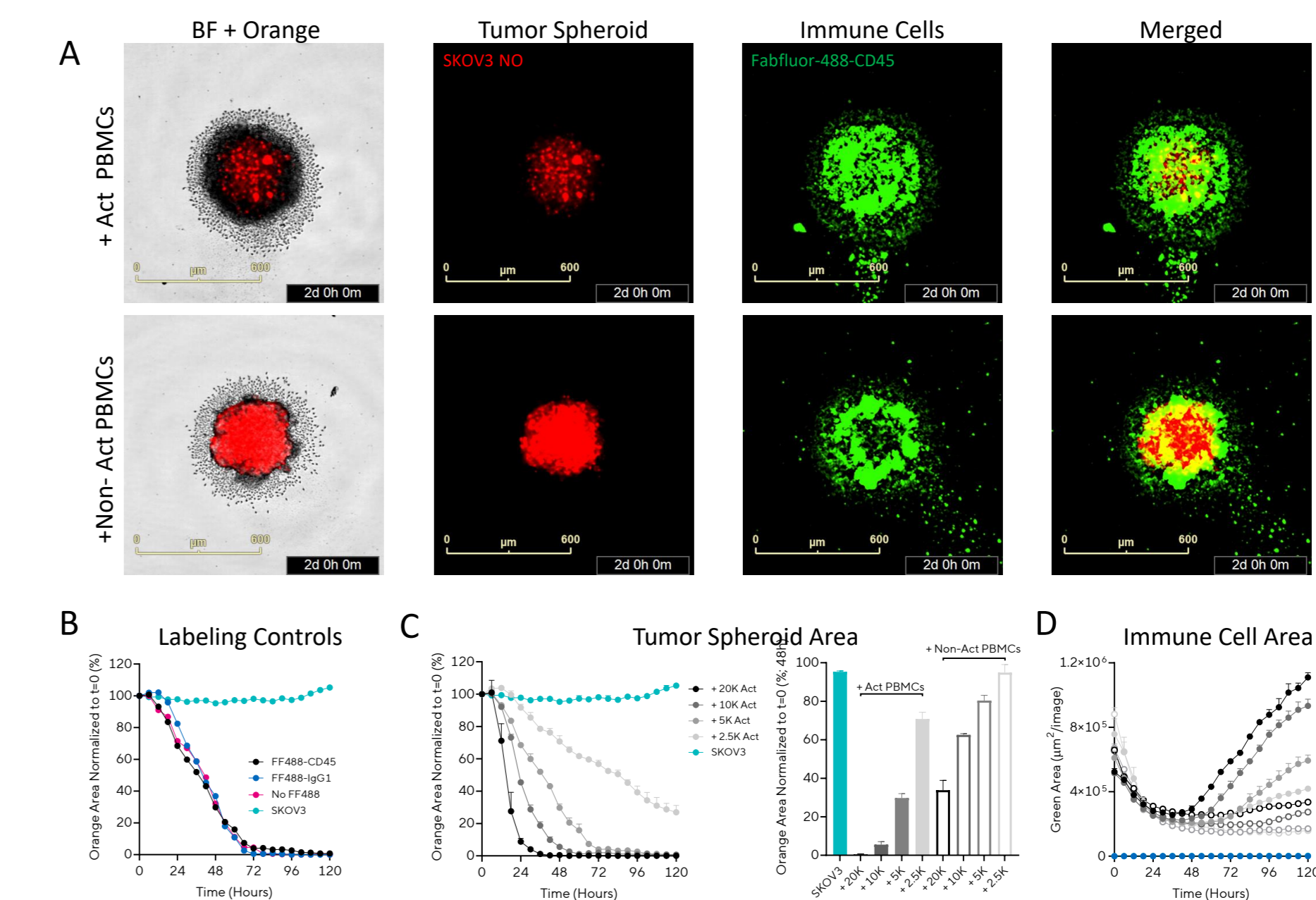
(A) Schematic of confocal spheroid acquisition using the Incucyte® CX3.

(B) Representative brightfield best focus image used for defining confocal fluorescence Z-range.

(C) Representative fluorescence (FLU) max projection image.

(D) Z-plane images for individual or merged fluorescence channels, sweeping down through the spheroid co-culture from Z-plane 20 to 17 (5 out of 21 Z-planes shown).

### Measure tumor death and effector cell proliferation using 2-color ICK assay



SKOV3-NuLight Orange spheroids were co-cultured with a density range of activated or non-activated PBMCs in the presence of Fabfluor-488-CD45 or Fabfluor-488-IgG1. Co-cultures were imaged using confocal multiplane acquisition at 4x. A) Max projection images shown at 2 days for E:T 2.5:1. (B) Time course of normalized orange area for pre-activated PBMCs (2.5:1 E:T) in the presence or absence of Fabfluor-488 conjugated to CD45 or IgG1 control. (C) Time course and bar graph (2 days) of normalized orange area for density range of pre-activated and non-activated PBMCs. (D) Time course of green area. Data presented as mean + SEM, n = 3 replicates.