

Evaluating Therapeutic Effects on 3D Cell Models with Live-Cell Confocal Imaging and Quantitative Analysis

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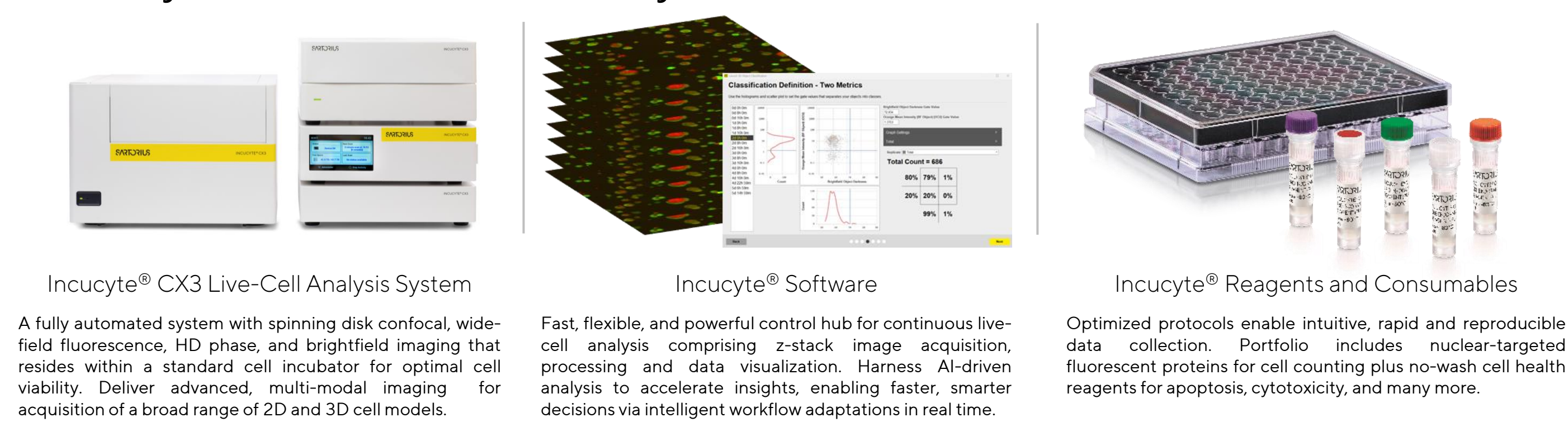
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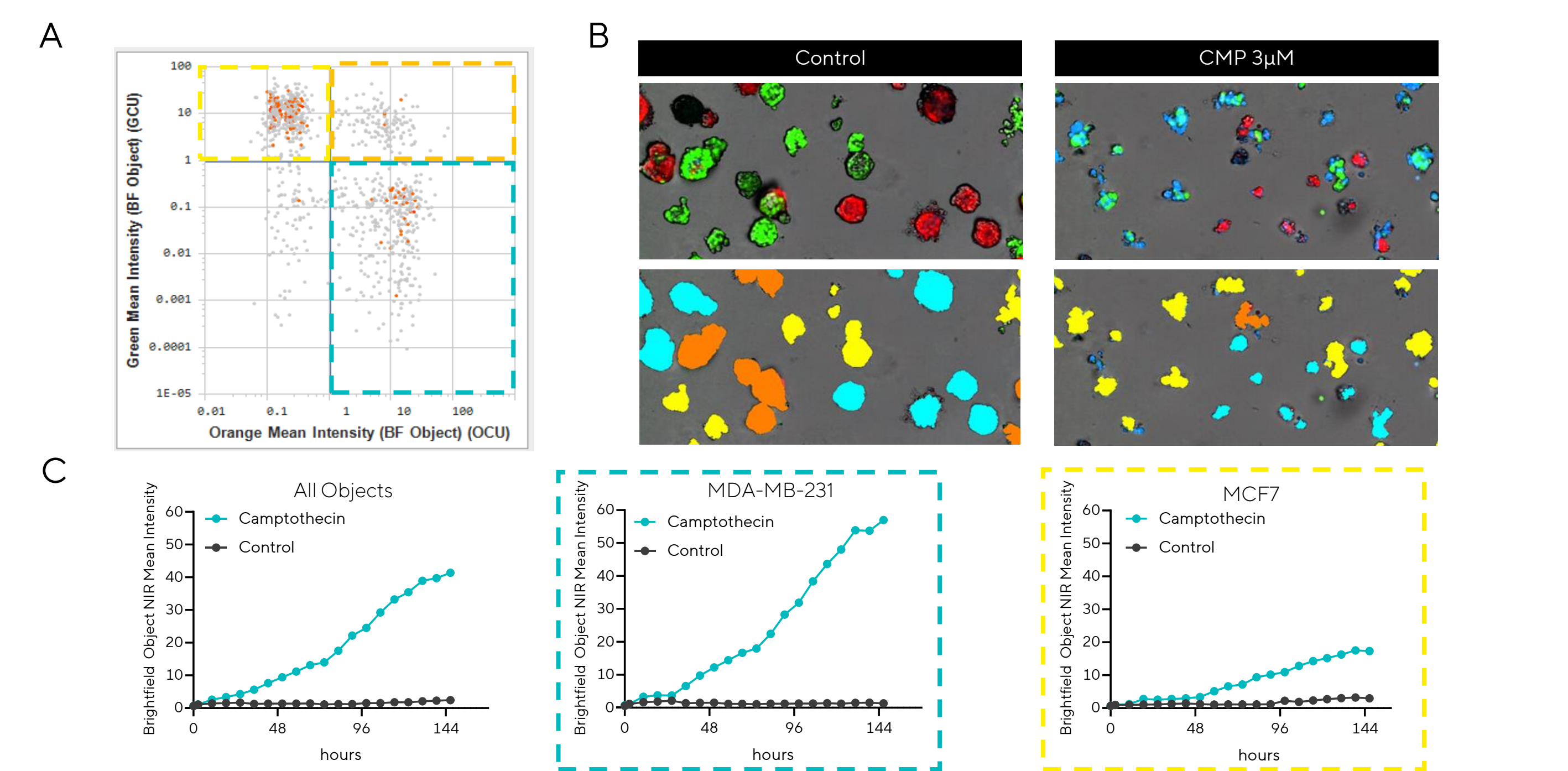
Introduction

- As cellular research continues to evolve towards more complex models, the development of bioanalytical tools is critical to extract clear insights for improving clinical application and treatment.
- The Incucyte[®] CX3 Live-Cell Analysis System's compact spinning disk confocal technology facilitates acquisition and analysis of complex 2D and 3D cell models, such as spheroids and organoids, under physiologically relevant conditions.
- We utilized the Incucyte[®] CX3 to evaluate the effect of therapeutic compounds on multiparametric readouts of cell health and function for various 3D applications.
- The combination of the system's confocal microscopy imaging and integrated, efficient analysis software enabled concurrent evaluation of apoptosis, utilizing dye-based cell health readouts, alongside cell cycle dynamics.
- This data exemplify the value of the Incucyte[®] CX3 to provide fast, simple insights into the cellular mechanisms and enabling quantification across multiple complex cellular models.

Incucyte[®] Live-Cell Analysis Solutions



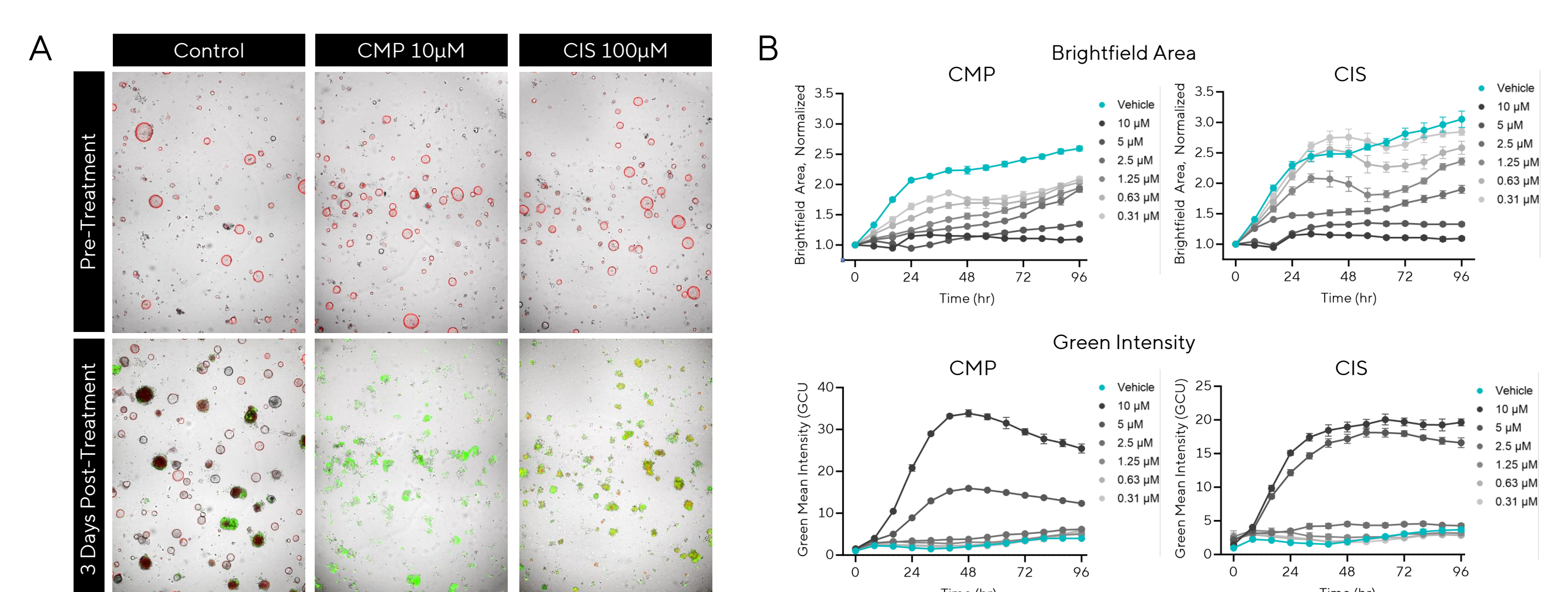
Classification and Analysis of 3D Embedded Spheroids



- MDA-MB-231 Nuclight Green cells (500 cells/well) and MCF7 Nuclight Orange cells (500 cells/well) were mixed prior to seeding, embedded in matrix, and allowed to form for 3 days. Spheroids were treated with camptothecin (CMP) to induced cell death in the presence of Incucyte[®] Annexin V NIR Dye and imaged for an additional 6 days.

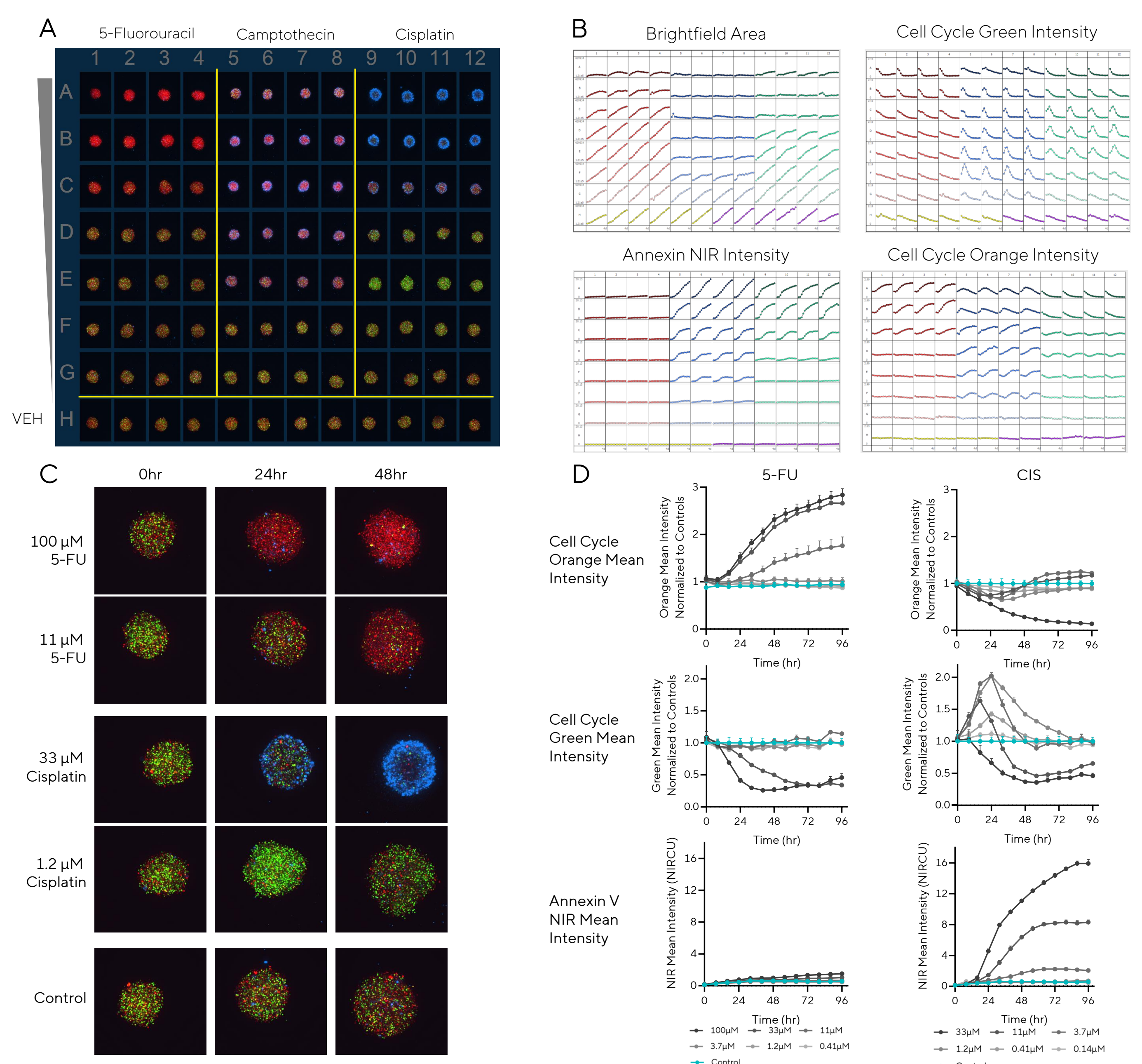
- Using the Incucyte[®] 3D Object Classification Analysis, scatter plots allow for gating of spheroid object populations based on green and orange fluorescent intensity.
- Representative overlaid brightfield and fluorescence images (green, orange and NIR), with and without classification segmentation masks are shown for control and drug-treated wells at 5 d post-treatment.
- Differential apoptosis metrics are graphed for all spheroids (left), MDA-MB-231 (segmented high green fluorescence objects, middle), and MCF7 (segmented high orange fluorescence objects, right) populations.

Evaluation of Cell Death in an Organoid Model of Hepatotoxicity



- Nuclight Orange-expressing murine hepatic organoids were embedded in matrix and cultured for 1 day before treating with a range of camptothecin (CMP) or cisplatin (CIS) in the presence of Incucyte[®] Annexin V Green Dye to monitor apoptosis for an additional 3 days.
- Representative overlaid brightfield and fluorescent images of organoids pre- and post-treatment with the indicated compounds.
 - Graphs demonstrate a reduced brightfield area and an increase in the mean green fluorescence intensity in both CMP and CIS treated organoids, demonstrating concentration-dependent loss of viability and increase in cell death.

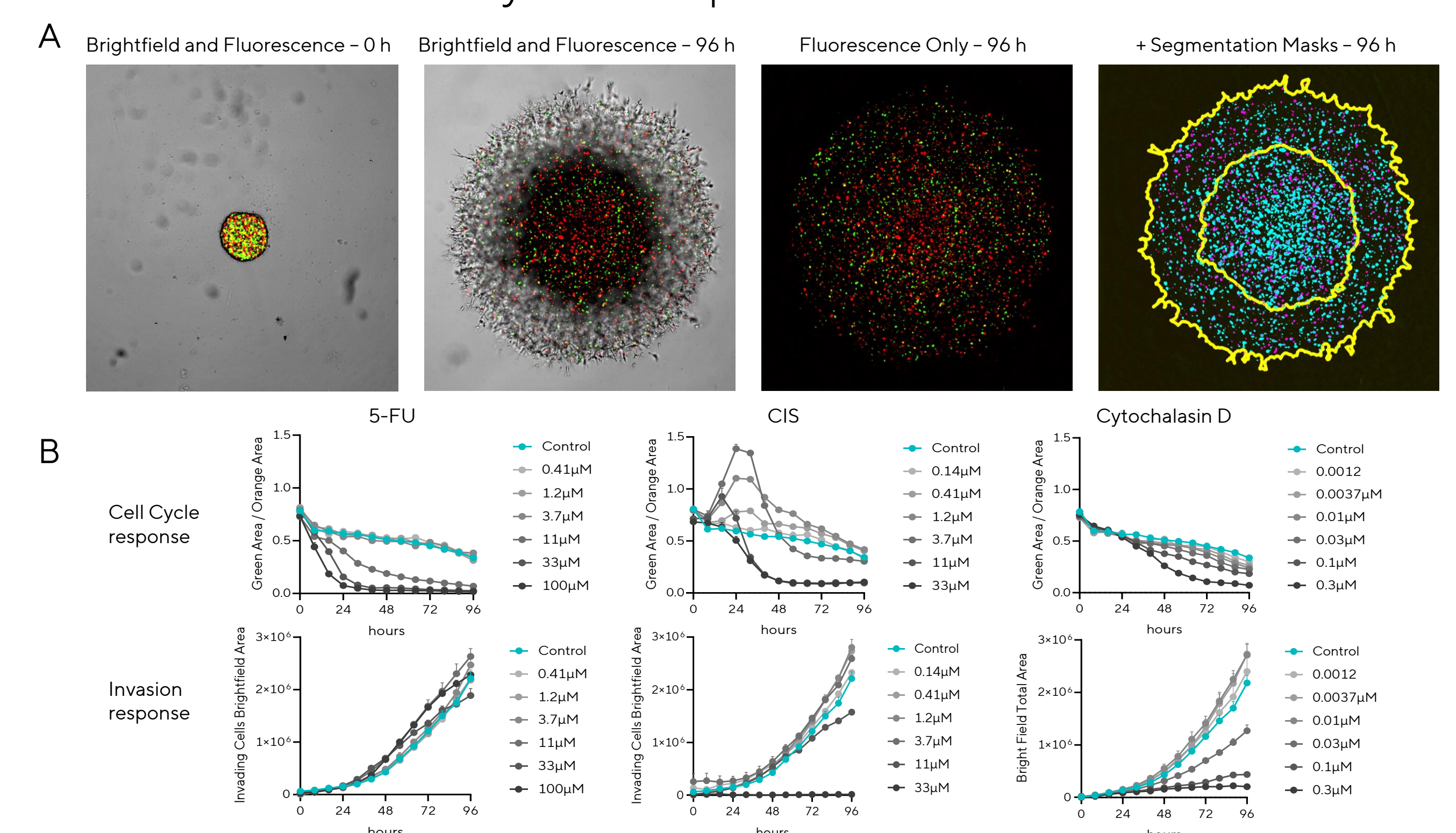
Monitoring Multiplexed Fluorescent Cell Cycle and Cell Health Readouts using Live-Cell Confocal Imaging



- U-87 MG spheroids stably expressing the Incucyte[®] Cell Cycle Green/Orange indicator were treated with 5-FU, CMP, and CIS. Confocal microscopy was employed to image the spheroids over a 96-hour period in the presence of the Incucyte[®] Annexin V NIR Dye.

- Vessel view demonstrates reproducible well-to-well imaging and the ability to observe differential compound trends across the broader set of treatments.
- Microplate graphs demonstrate the ability to track multiparametric kinetic responses from the same spheroids over time.
- Confocal images of spheroids treated as indicated. D. Graphs of fluorescence intensity changes across the various metrics. 5-FU caused an increase in orange mean fluorescence, indicative of an elevated number of cells in the G1 phase. In contrast, CMP and CIS induced apoptosis at higher concentrations. At lower concentrations, CIS caused a transient increase in green mean fluorescence at early time points, indicating an accumulation of cells in S/G2/M.

Quantification of Compound Effects on Cancer Spheroid Invasion and Cell Cycle Responses



- U-87 MG spheroids expressing Incucyte[®] Cell Cycle Green/Orange indicator were embedded in ECM and treated with 5-FU, CIS, and cytochalasin D. Confocal microscopy images were captured every 8 hours over 96 hours.

- Control images show brightfield segmentation in yellow, and fluorescence segmentation in purple (green fluorescence) and blue (orange fluorescence). Graphs show a reduced brightfield area and an increase in mean green intensity in both CMP and CIS treated organoids, demonstrating concentration-dependent loss of viability and increase in cell death.
- Cell cycle response was assessed by the ratio of Green to Orange fluorescence areas. 5-FU increased relative orange fluorescence, indicating arrest in phase G1. Cisplatin transiently increased the green/orange ratio at lower concentrations, indicating a block in S/G2/M phases. Cisplatin and cytochalasin D reduced the Invading cells brightfield object area, suggesting decreased spheroid invasion, while 5-FU had minimal impact.