

A novel high-throughput solution for screening drugs with a light-crosslinked VersaGel 3D tumor culture model and automated high-content imaging



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INTRODUCTION

In order to screen using a model that closely mimics the *in vivo* tumor microenvironment, but in a microplate, cancer cells and spheroids may be cultured and imaged in a three-dimensional (3D) matrix. In these experiments we paired a novel light-cross linkable 3D cell culture platform, VersaGel® and Symphony®, with high-content imaging to demonstrate a 3D assay amenable to high-throughput screening. It incorporates a straightforward workflow to grow and treat cancer cells or spheroids embedded in a 3D extracellular matrix (ECM) hydrogel, VersaGel, in a standard 96-well format, and subsequently image the culture using the ImageXpress® Micro Confocal High Content Imaging System. Cells may be stained before or after embedding in the gel, and imaged live or after fixing with standard methods. Here we present results of optimized spheroid growth of different cancer cell lines in three different VersaGel stiffnesses and further treatment with chemotherapeutic compounds. In experiments with only 1-4 spheroids/well, QuickID was performed, whereby the entire plate was rapidly scanned at low magnification and then only fields of view containing a spheroid were automatically re-imaged at a higher magnification and with multiple z planes. Alternatively, a 2D projection image was sometimes generated for simple analyses such as viability or apoptosis. Together, VersaGel/Symphony and the ImageXpress Micro Confocal System provide a powerful solution for drug screening in a 3D ECM hydrogel culture system.

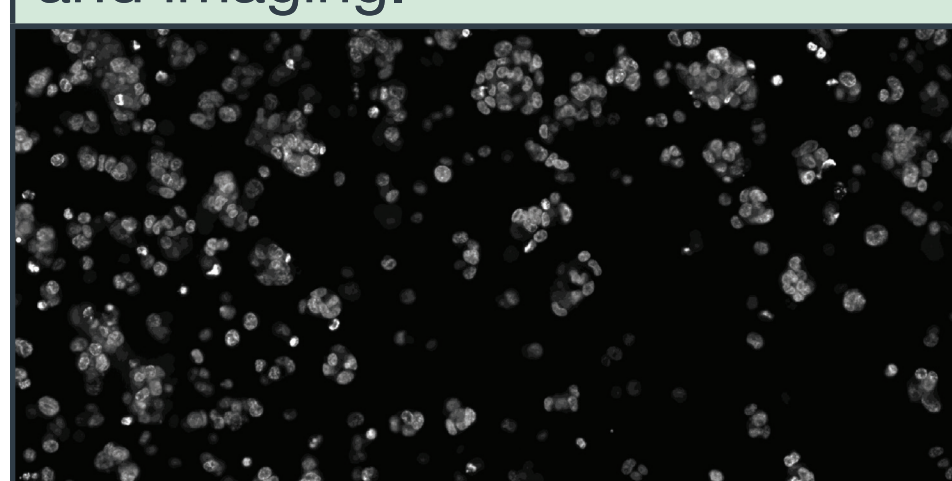
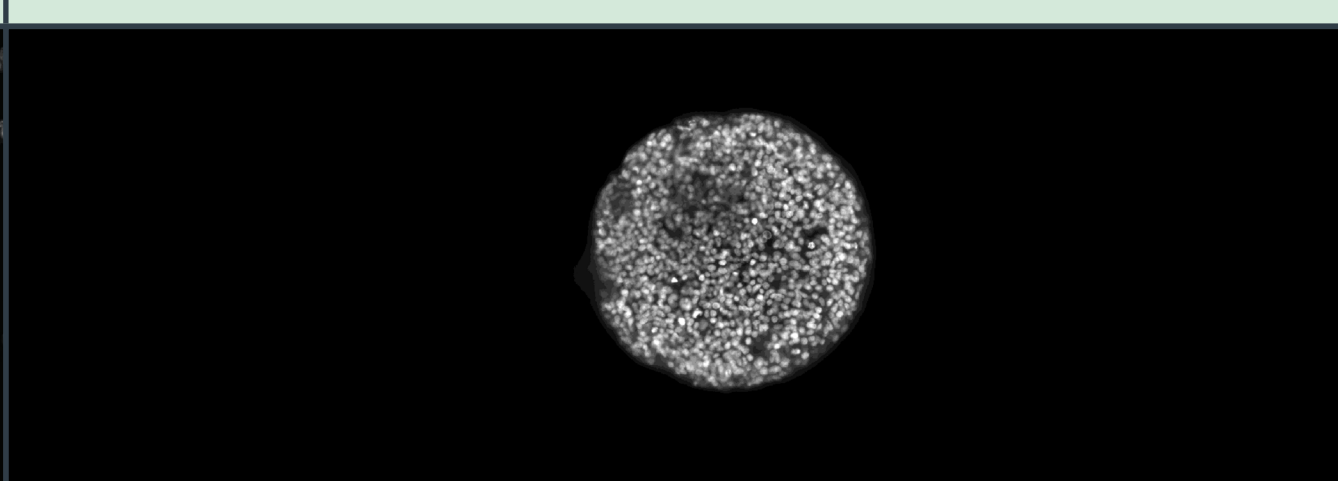
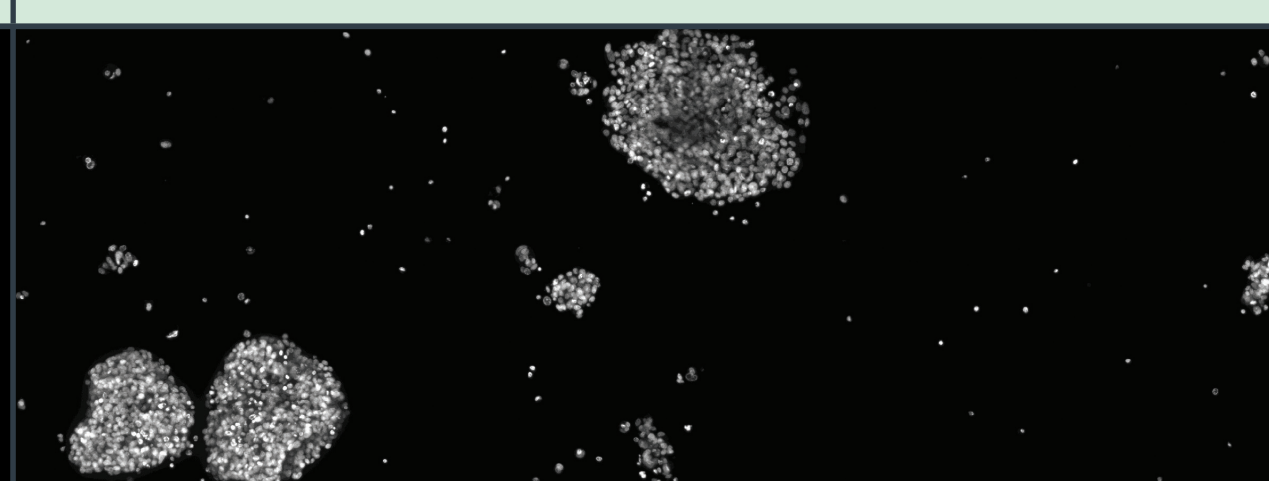
MATERIALS & METHODS

Reagents

- EarlyTox™ Caspase-3/7-D NucView 488 Assay Kit (Molecular Devices PN R8348)
- EarlyTox™ Live/Dead Assay (Molecular Devices PN R8340)
- VersaGel 3D ECM hydrogel (Cypré, Inc.)

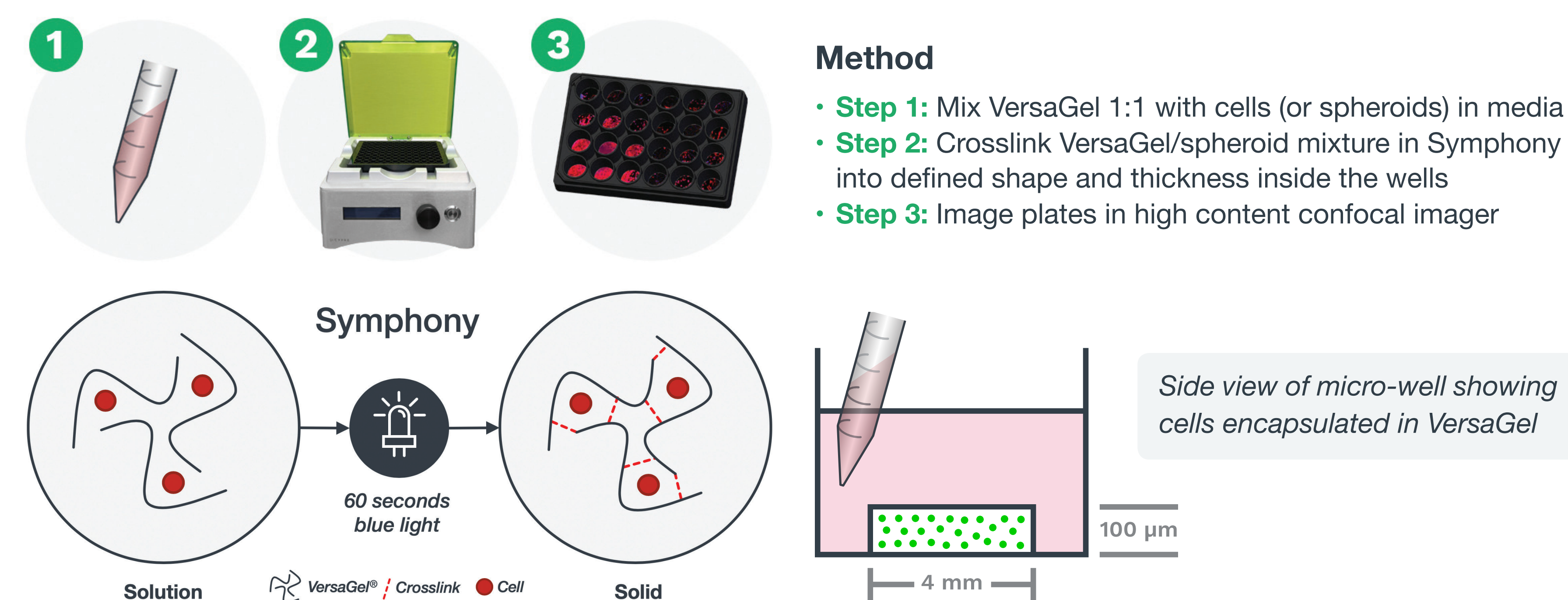
Equipment

- ImageXpress Micro Confocal System with MetaXpress® High-Content Image Analysis Software (Molecular Devices)
- Symphony instrument for patterning VersaGel in 96 well plates (Cypré, Inc.)

Culture spheroids directly in microplate	Transfer individual pre-formed spheroids into microplate	Transfer spheroids in bulk into microplate
Mix 10K-15K cells 1:1 by volume with VersaGel and transfer 50 uL/well	Add 1500 cells/well to round-bottom spheroid plate and culture 3-5 days before combining spheroids in media 1:1 by volume with VersaGel and transferring 50 uL/well.	Add 1.2M cells/well in AggreWell-800 plate and culture 1-2 days before harvesting and mixing 1:1 with VersaGel. Transfer 50 uL/well.
Allow cells to proliferate 3-7 days in VersaGel to form small spheroids before proceeding with drug treatment, staining, and imaging.	Allow spheroids to stabilize overnight in VersaGel before proceeding with drug treatment, staining, and imaging.	Allow spheroids to grow 1-2 days in VersaGel before proceeding with drug treatment, staining, and imaging.
		

VersaGel and Symphony Platform

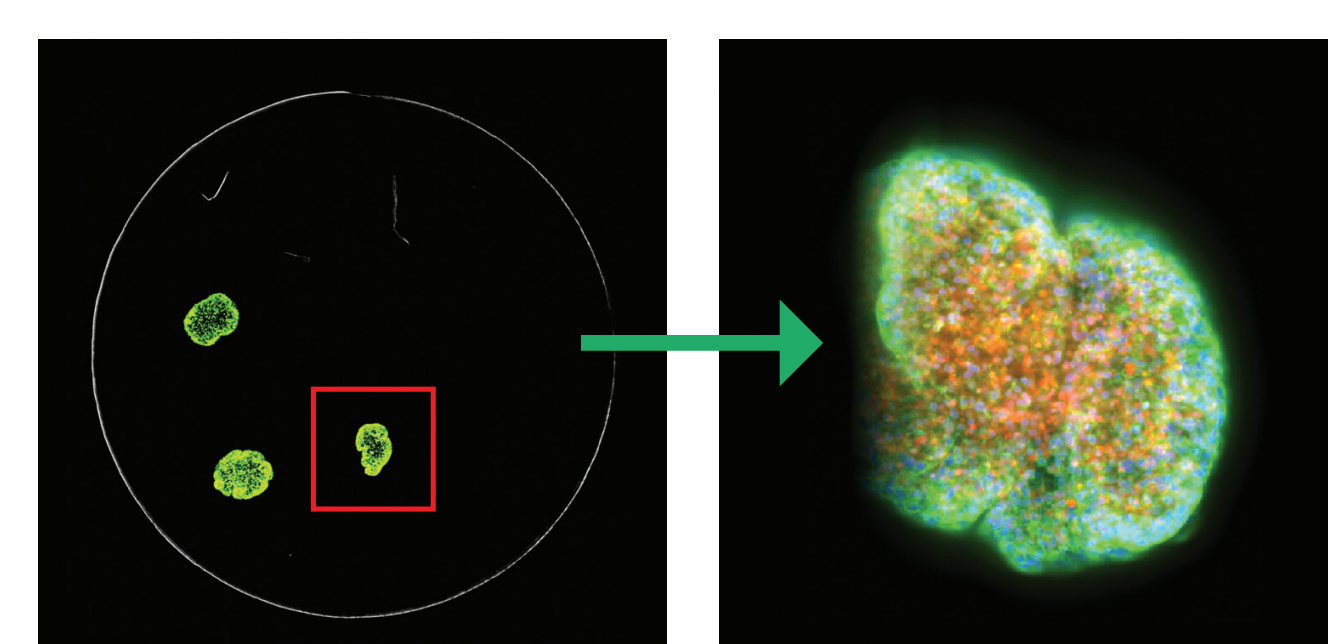
VersaGel is a light-crosslinkable ECM hydrogel for use in 3D *in vitro* & *ex vivo* assays. This tunable ECM hydrogel recapitulates human physiology and supports many cell types. VersaGel allows for cell adhesion through integrin binding sites and is MMP degradable for 3D cell invasion. Symphony uses blue light to polymerize VersaGel across the entire 96 well plate in 60 seconds. Symphony creates thin, flat 3D culture gels centered in the well allowing the gel to stay intact after pipetting.



RESULTS

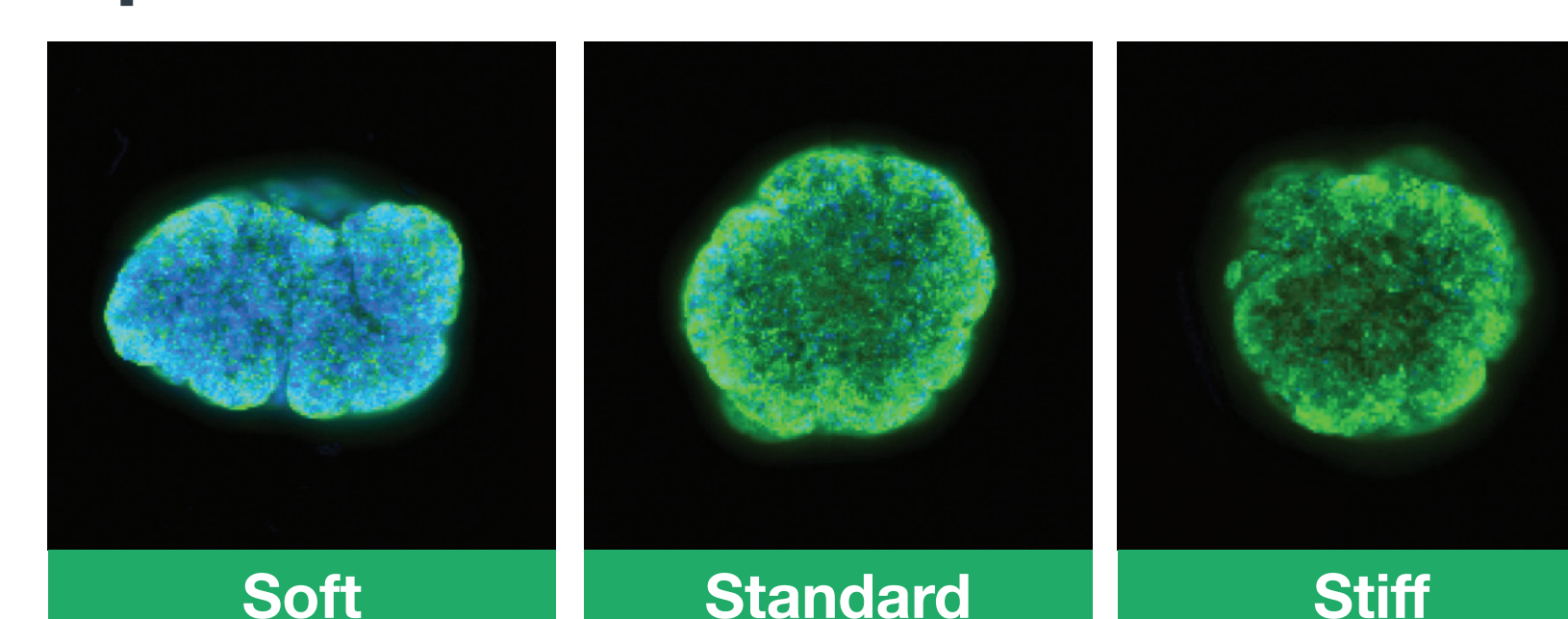
Quick ID and Targeted Imaging of Spheroids in 96 well plates

QuickID was performed with a 2X objective to capture the entire well in one field-of-view. Objects of interest were identified and their X,Y coordinates were used for Targeted Imaging to automatically acquire only sites that contained an object with a higher magnification objective, in 3 wavelengths, and multiple z planes. In this experiment, with the intensive 3D analysis performed on the final images, the entire process took 1/10 the amount of time it would require to image all available spheroids with a high magnification as well as generating only 1/20 the number of images to be stored.



RESULTS (Continued)

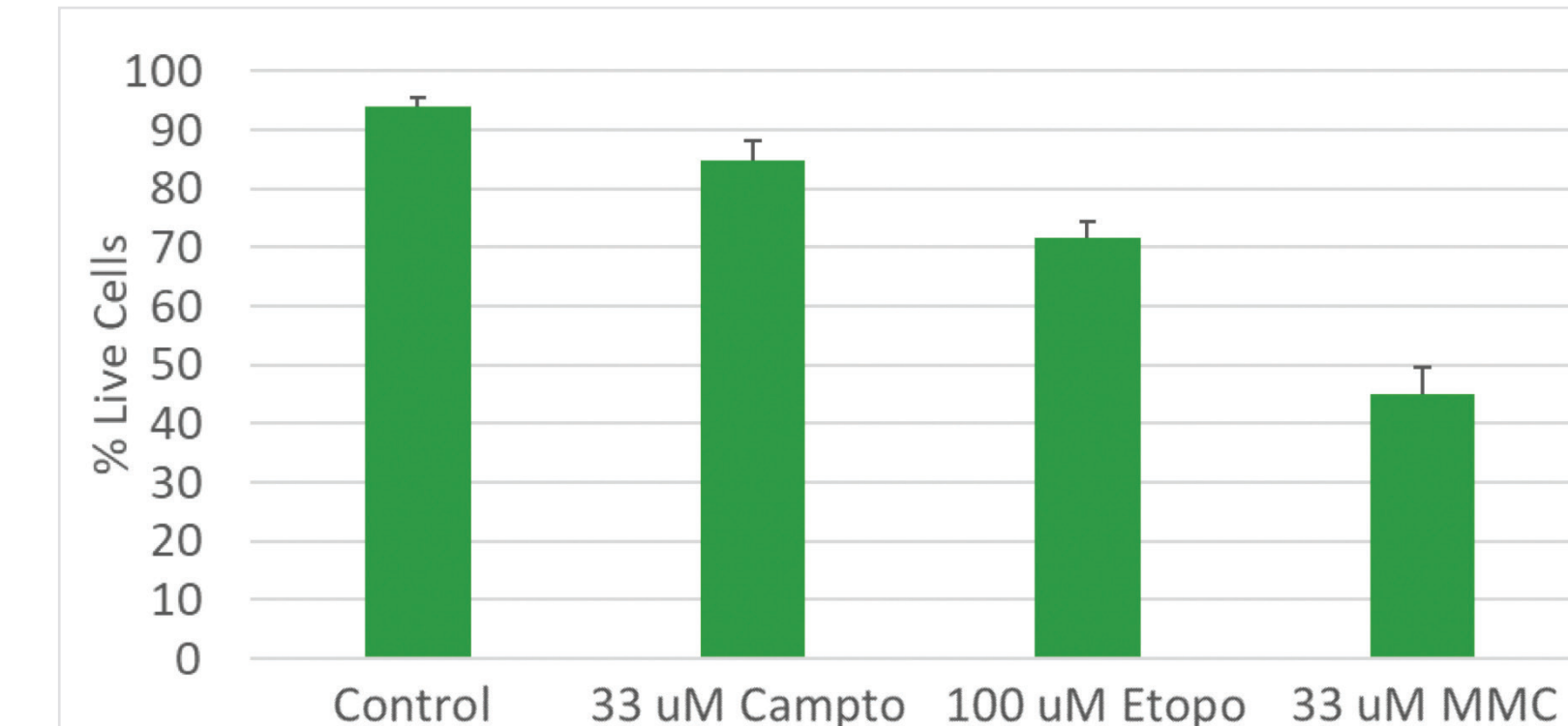
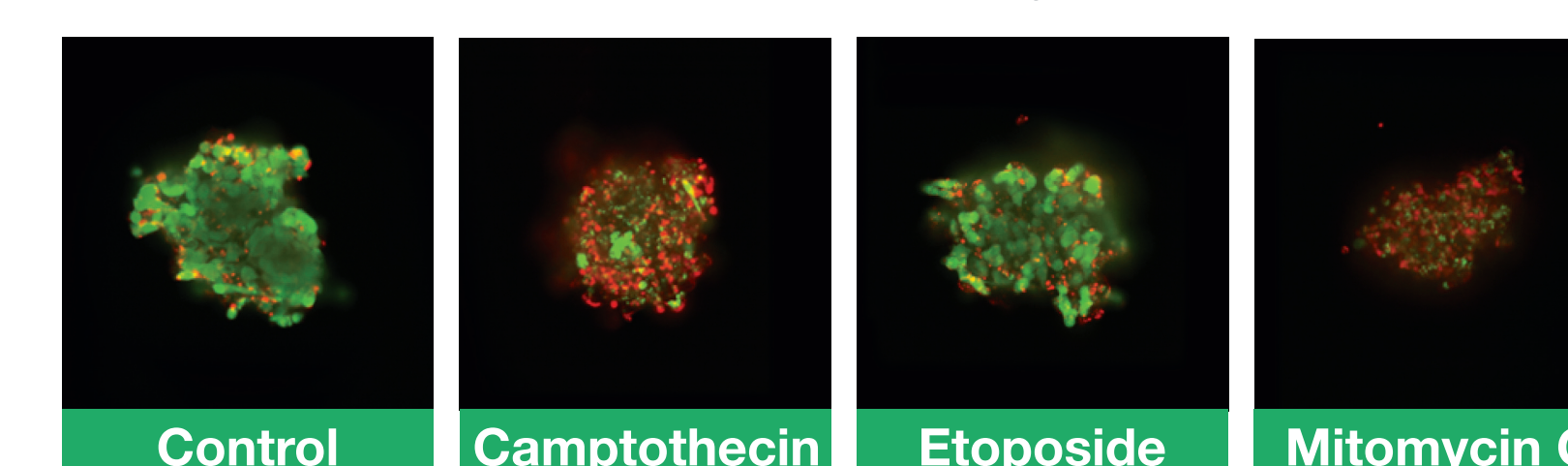
Optimize VersaGel Stiffness for Tumor Cell Type



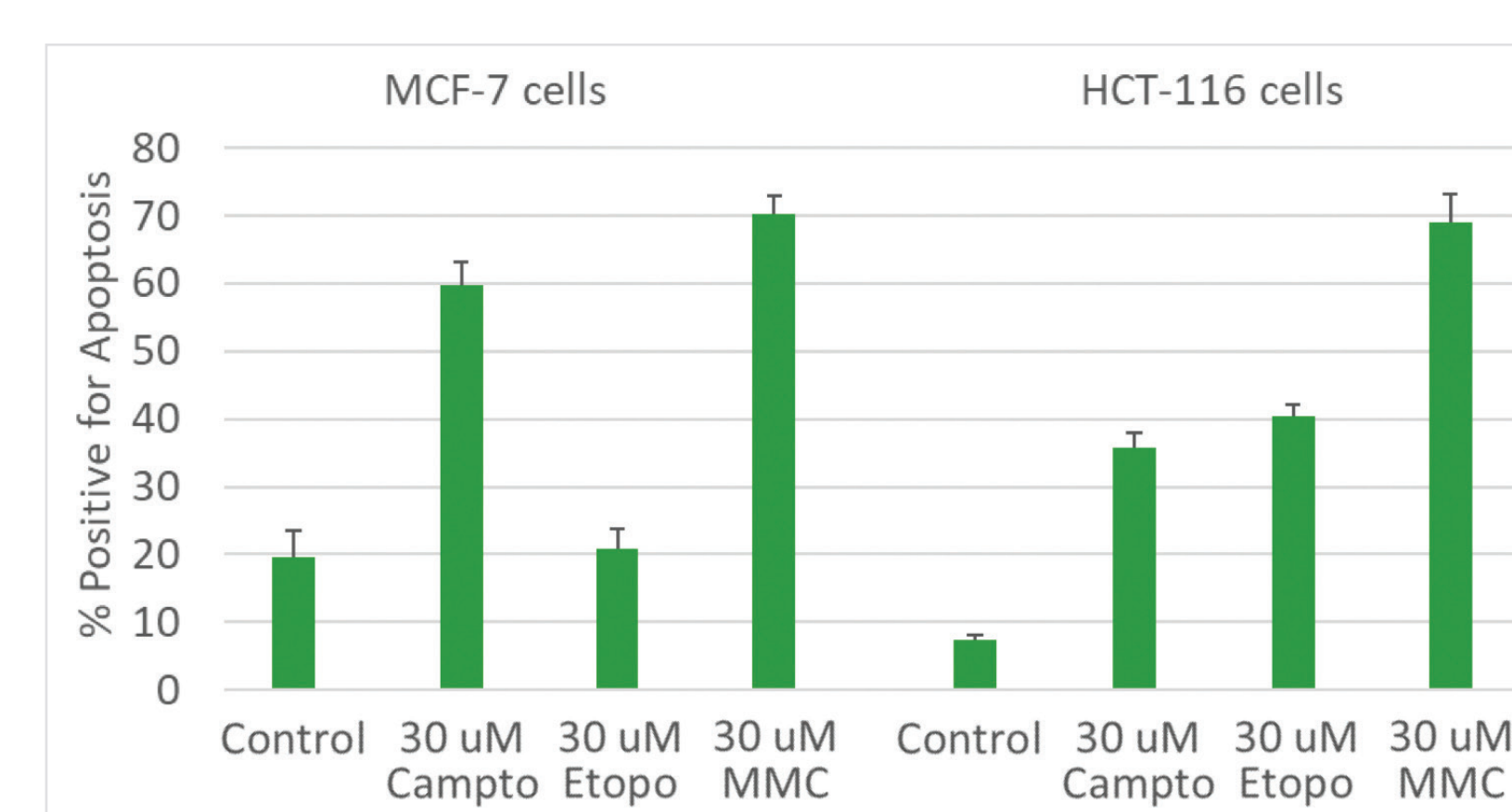
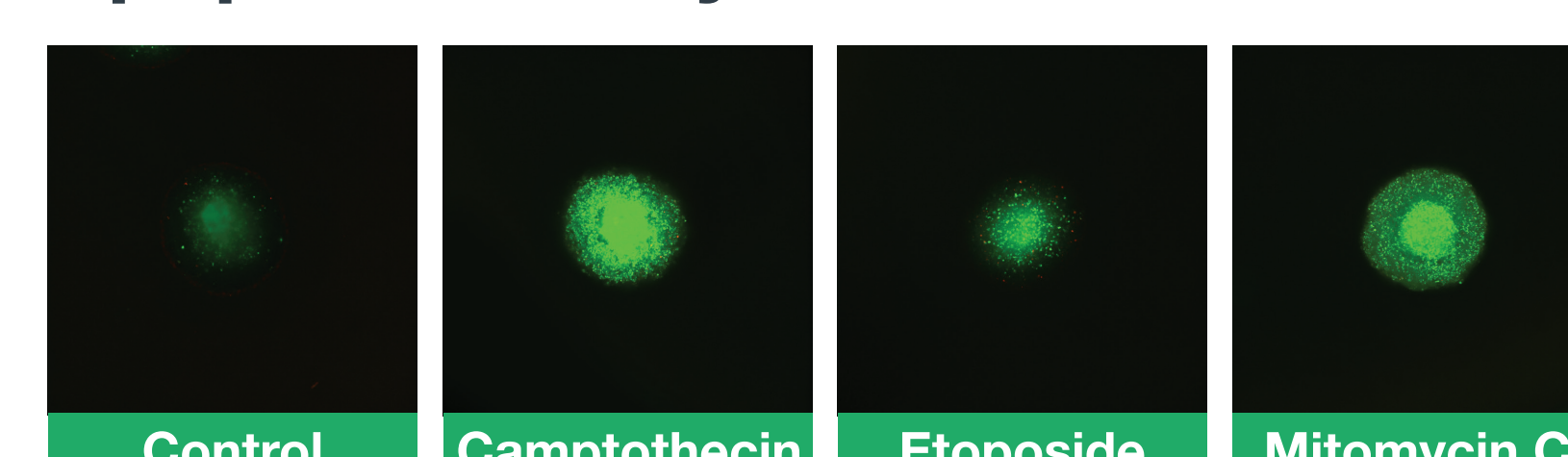
Optimization experiments included determining the best stiffnesses (Soft, Standard, Stiff) of VersaGel to culture spheroids derived from MCF-7 breast cancer cells. Single spheroids were first grown in ultra-low attachment microplates then transferred to VersaGel. 10X images (left) are cells after fixing and staining with Hoechst and Phalloidin-AlexaFluor 546. MCF-7 spheroids generally remained more compact in VersaGel "Stiff" over 3 days culture.

Live/Dead Assay in VersaGel

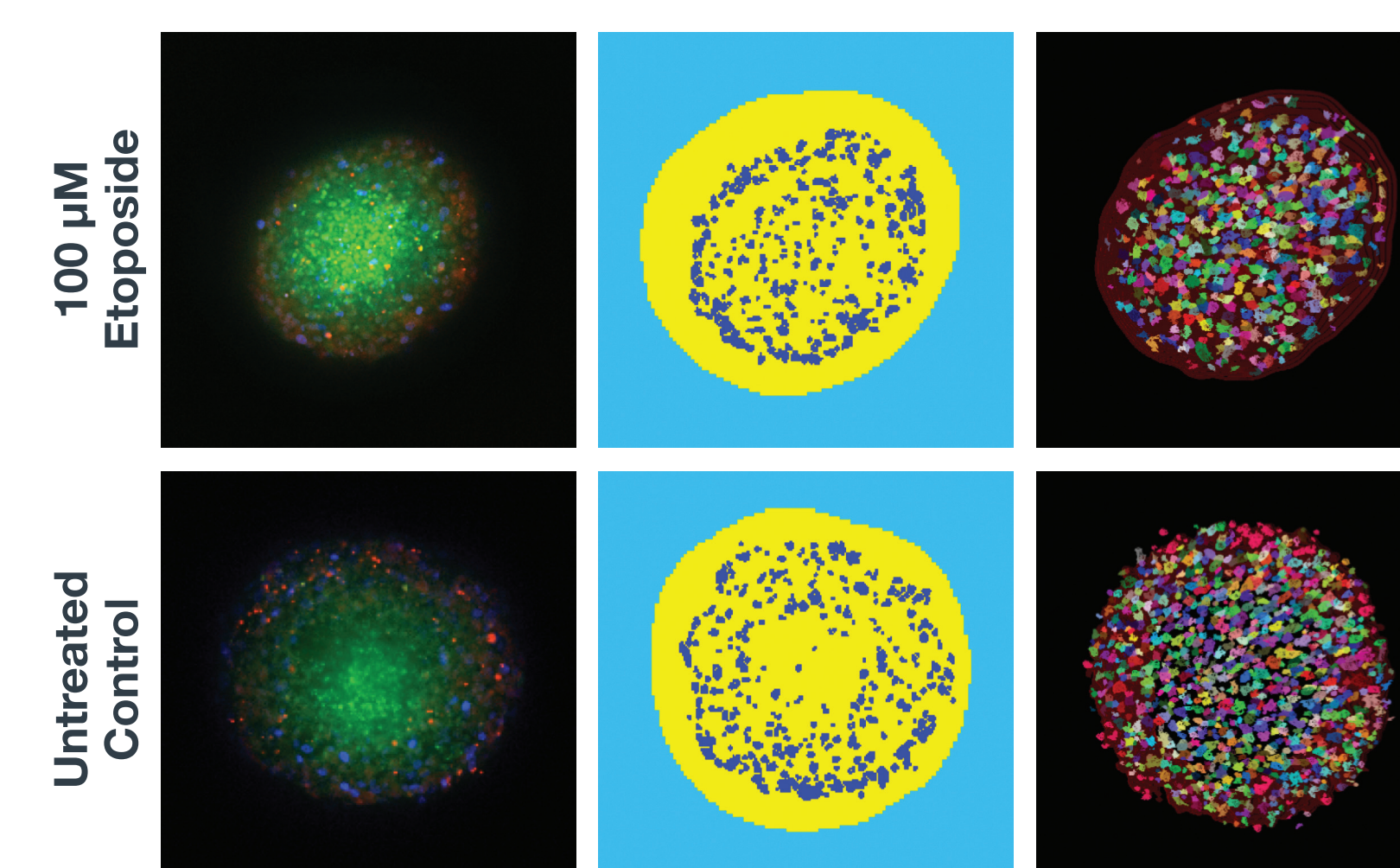
Pre-formed U2OS bone cancer spheroids were cultured in VersaGel "Standard" and treated with 3 different compounds for 48 hours. Toxicity was measured using the EarlyTox Live/Dead Assay and Targeted Imaging to measure presence of live stain (green) compared to dead stain (red). In a separate plate, a live/dead assay was conducted in cells that had been seeded as a suspension in VersaGel for 3 days before commencing treatment and the results of analysis (graph to the right) show expected responses to the compounds.



Apoptosis Assay in VersaGel



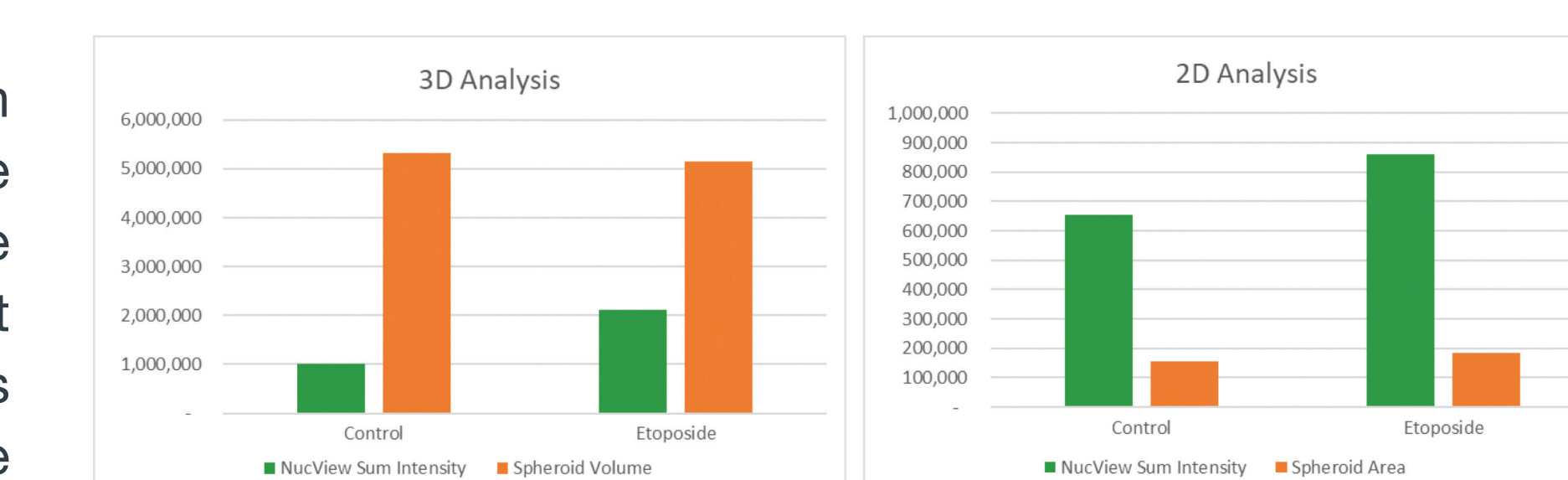
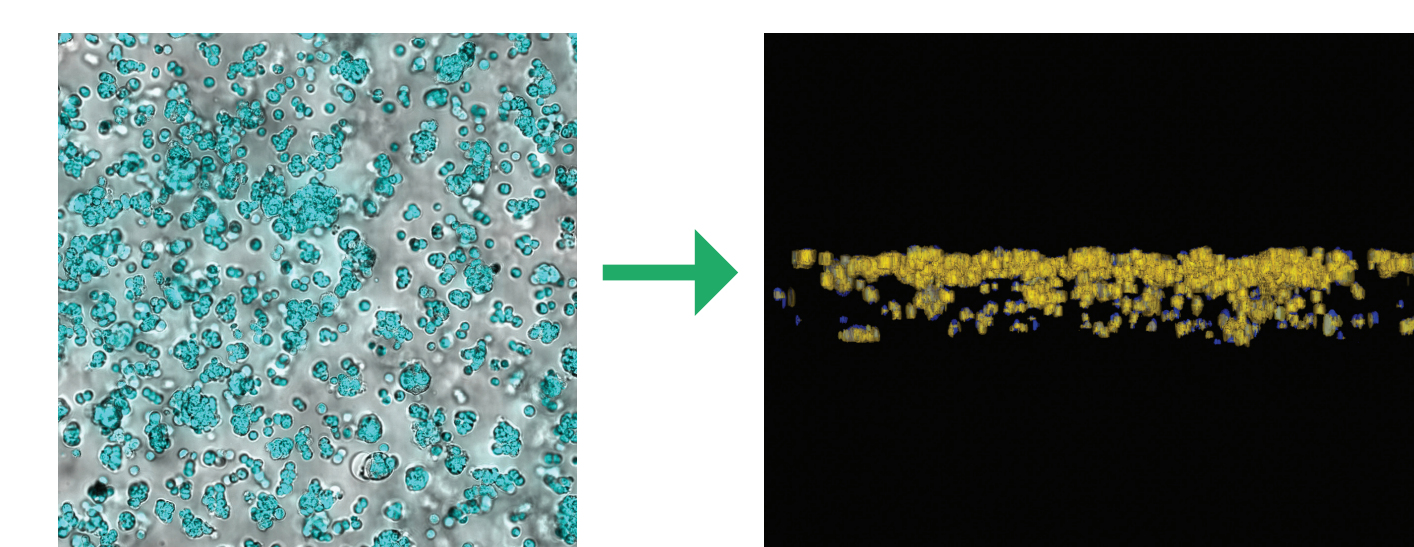
Pre-formed HCT-116 colon cancer spheroids were cultured in VersaGel "Standard" and treated with 3 different compounds for 48 hours. Apoptosis was measured using the EarlyTox Caspase 3/7 Assay and Targeted Imaging to measure presence of apoptotic nuclei (green). In a separate experiment, an apoptosis assay was conducted in 2 different cell types that had been seeded as a suspension in VersaGel for 3 days before commencing treatment. The results of analysis (graph to the left) show expected responses to the compounds.



2D vs. 3D Analysis

Cells or spheroids suspended in 100 µm or 250 µm thick VersaGel were imaged using multiple z planes to sample very small spheroids or to collect images spanning the spheroids' depth. The resulting images (right) were saved to construct a 3D object or collapsed into a single 2D projection for analysis.

Pre-formed HCT-116 colon cancer spheroids (right) in VersaGel "Standard". Apoptosis was measured using the EarlyTox Caspase 3/7 NucView 488 Assay. Analysis of the 2D projection was compared to analysis of the 3D object (segmentation masks represent NucView 488 positive cells within an identified spheroid). Graphs of results from these representative images is shown to the right.



HCT-116 colon cancer cells were encapsulated in VersaGel "Standard" and allowed to proliferate in the incubator for 7 days before staining with Hoechst nuclear stain. Wells were imaged with a 20X PA objective and 60 µm pinhole confocal setting. Overlay of cells is shown on left and MetaXpress High Content Analysis software generated a 3D rendering of the segmented cells in ~100 µm thickness and is shown on the left.

CONCLUSION

- High-throughput assays are possible for screening cells or spheroids in 96 well plates embedded in VersaGel ECM hydrogel
- Quick ID can be used to target only areas of the well that contain spheroids or other objects of interest for a high magnification acquisition.
- MetaXpress software can segment and measure cells or entire objects in either 2D or 3D images.
- VersaGel/Symphony platform simplifies 3D culture and provides thin, optically clear gels for excellent imaging quality for 3D assays.

We would like to recognize Spirochrome Inc. for their collaboration on this project by providing SiR-DNA Kit for staining of live spheroids.