

Evaluating antibody-mediated cellular cytotoxicity and potency of antibody-drug conjugates within three-dimensional tumor models

Chris Langsdorf, Bhaskar Mandavilli, Oggie Golub, Dan Cash, Aimei Chen, Yi-Zhen Hu, and Marcy Wickett
Thermo Fisher Scientific

Background

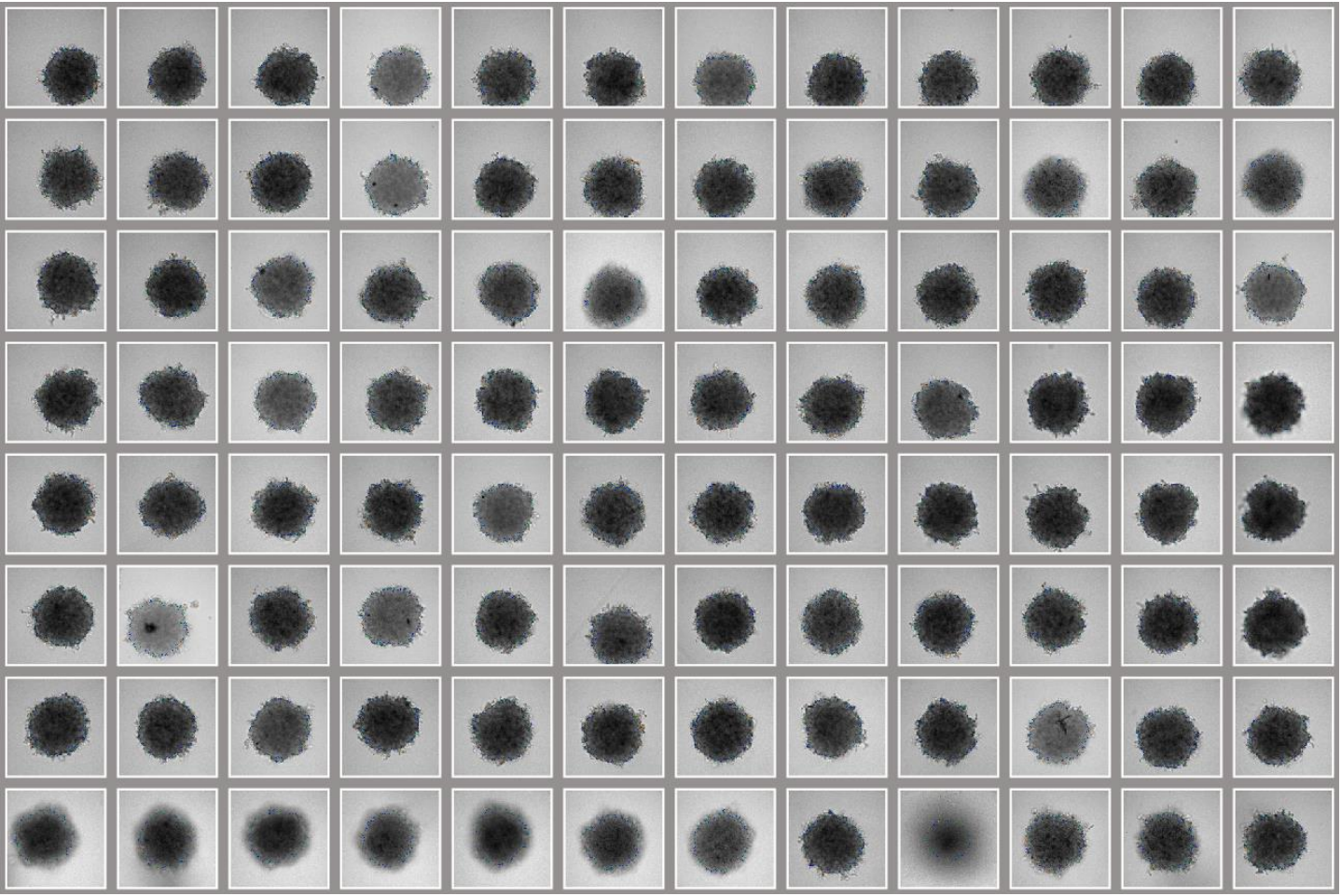
Three dimensional tumor spheroids provide biochemical conditions that closely resemble the tumor microenvironment in an intact organism. Noninvasive approaches such as fluorescence microscopy are highly advantageous as they allow for the study of these 3D systems. Here we investigate the penetration and potency of natural killer cells, cytotoxic T cells, and antibody-drug conjugates in three-dimensional models of breast and lung cancer.

Figure 1. CellInsight CX7 LZR High-Content Analysis Platform



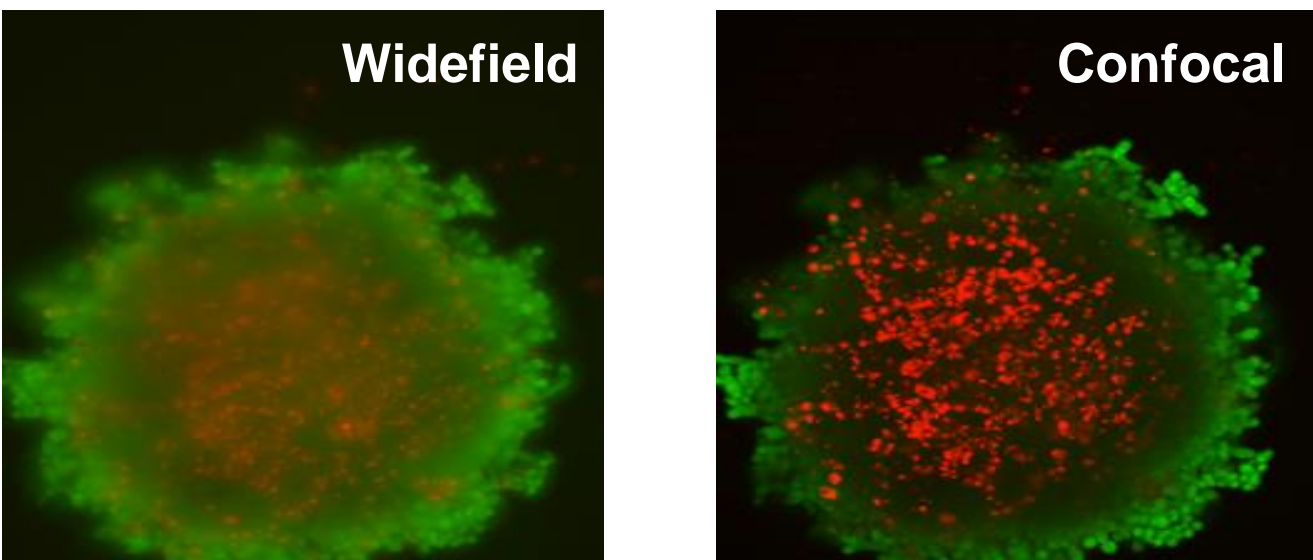
The Thermo Scientific CellInsight CX7 LZR High Content Analysis (HCA) Platform is a fast, laser-based, automated cellular imaging and analysis platform for quantitative microscopy and phenotypic screening designed to provide the sensitivity and speed needed for emerging assays.

Figure 2. Automated scanning of spheroids



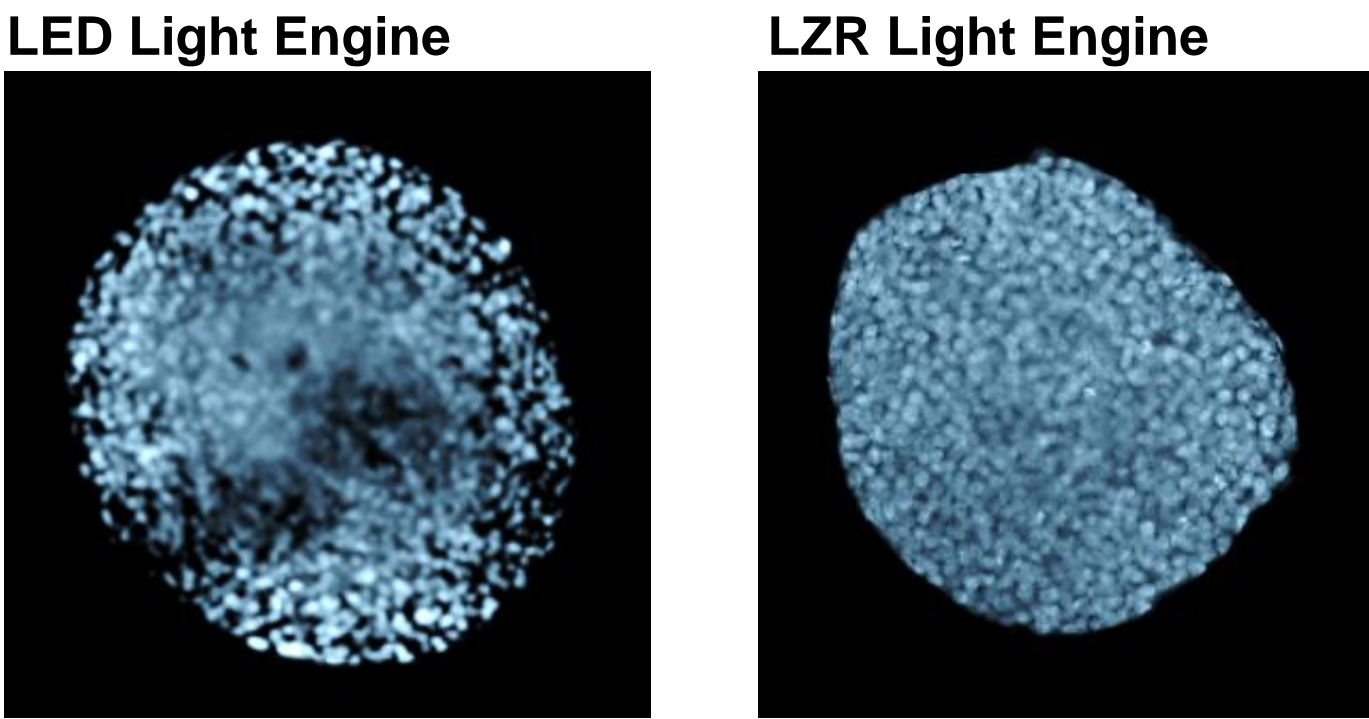
A549 cells plated at 5K/well on a Nunclon™ Sphera™ 96-well plate and incubated 24 hrs. Automatically imaged with 10X objective using brightfield illumination on a CellInsight CX7 LZR HCA instrument.

Figure 3. Improved axial resolution in confocal mode



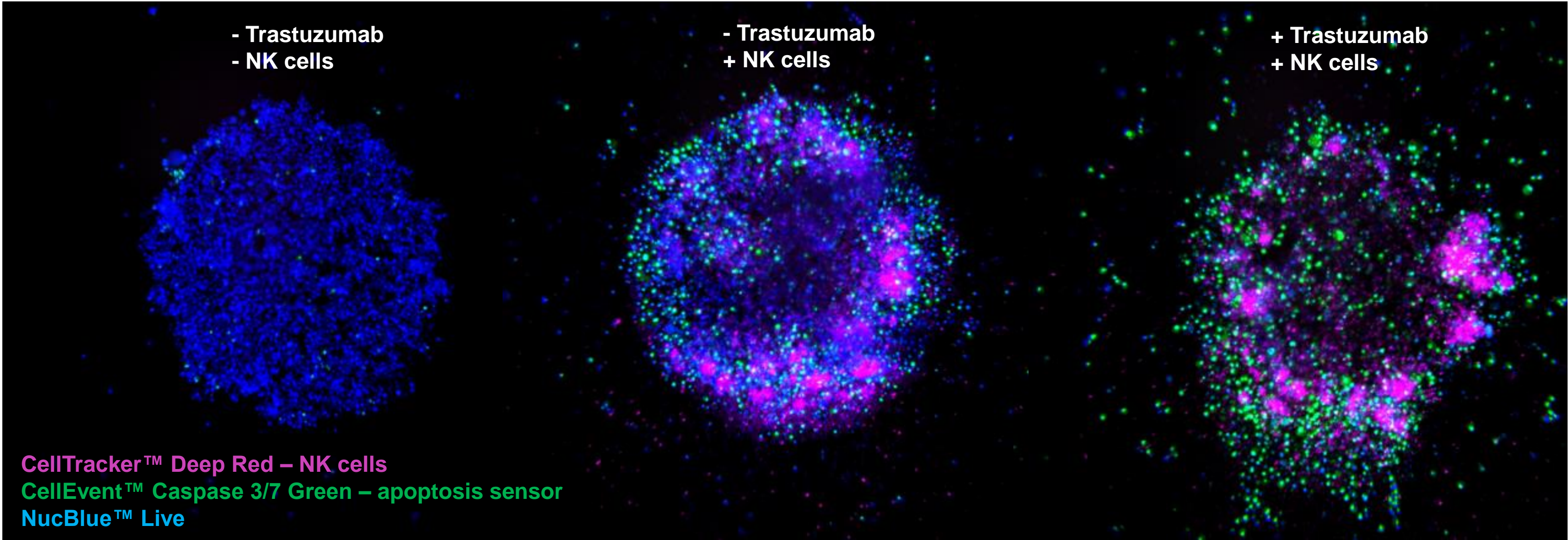
Confocal imaging on CX7 LZR system improves axial resolution of 3D spheroids. A549 cells plated at 5K/well 48 hrs on a U-bottom plate. Imaged with 10X objective using wide field or confocal modes on a CellInsight CX7 LZR system. Maximum intensity projection of 20 optical Z slices of 10 microns each.

Figure 4. Laser illumination improves resolution



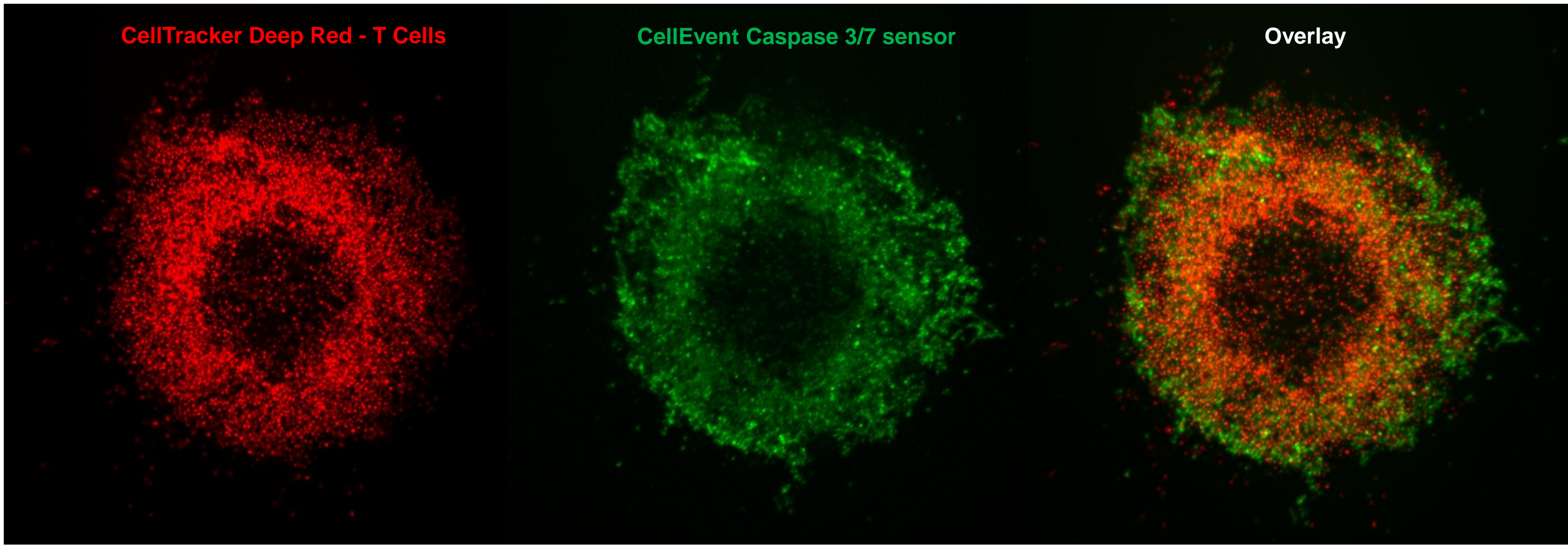
Confocal laser-based illumination improves axial resolution when imaging 3D specimens compared to confocal LED-based illumination.

Figure 5. NK cell ADCC assay in breast cancer spheroids



Natural killer cells isolated from human PBMCs using DynaBeads™ Untouched NK cells were added to SKBR3 breast cancer spheroids with or without trastuzumab. NK cell penetration and tumor cytotoxicity were evaluated using live-cell whole-spheroid imaging on the CellInsight CX7 LZR High Content Analysis system. Addition of NK cells induced moderate cytotoxicity, while addition of NK cells and trastuzumab resulted in substantial apoptosis and degradation of spheroid structure

Figure 6. T cell penetration and killing of lung cancer spheroids



T cells isolated from human PBMCs using Dynabeads Human T-Expander CD3/CD28 were activated for 72 hours and labeled with CellTracker Deep Red before adding to lung cancer spheroids for four hours. Cells were labeled with CellEvent Caspase 3/7 sensor. T cell penetration and tumor cytotoxicity were evaluated using live-cell whole-spheroid imaging on the CellInsight CX7 LZR High Content Analysis system. Activated T cells penetrated and induced apoptosis in target cells throughout the spheroids.

Figure 7. Antibody-drug conjugate penetration and killing in breast cancer spheroids

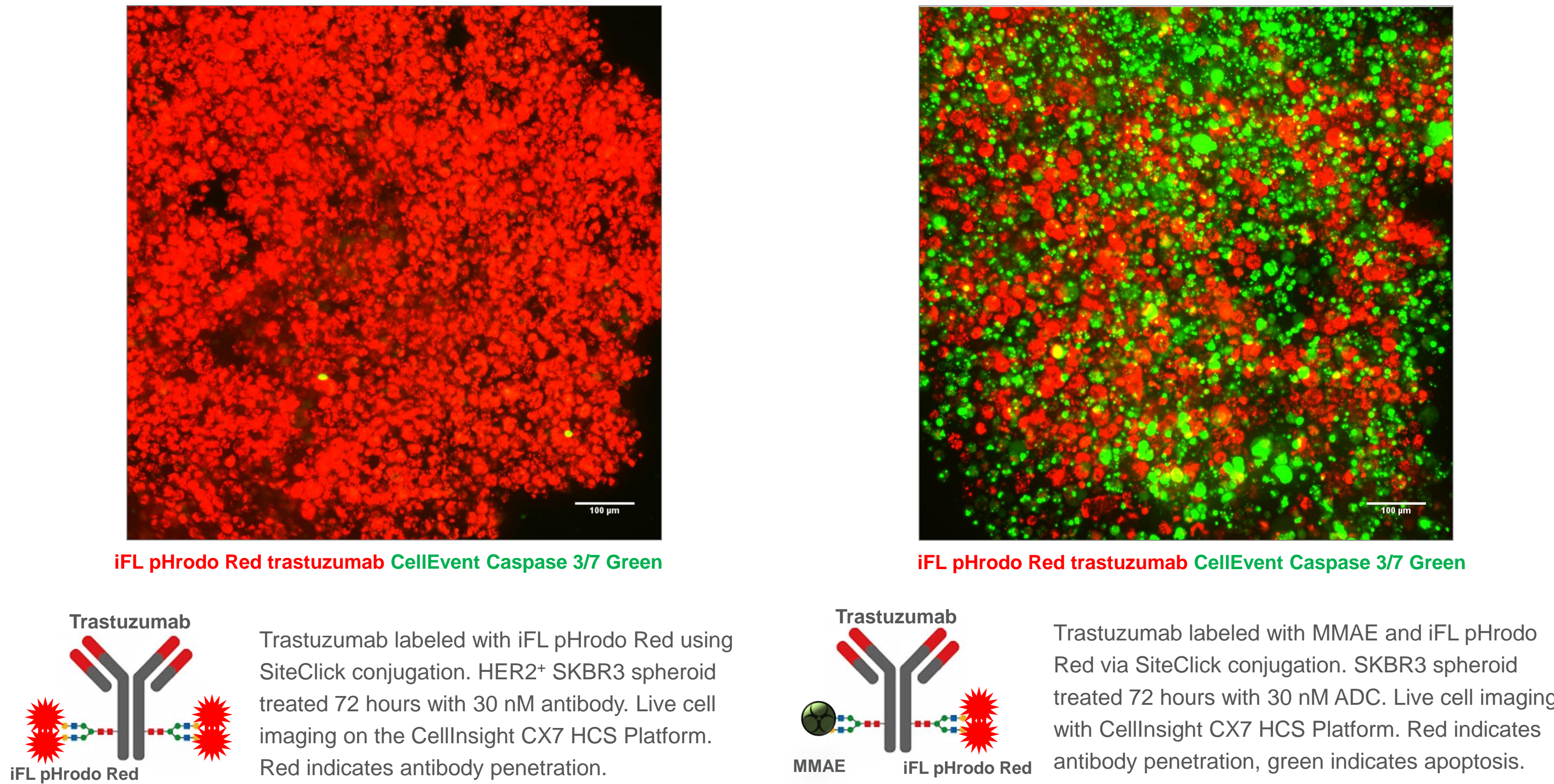
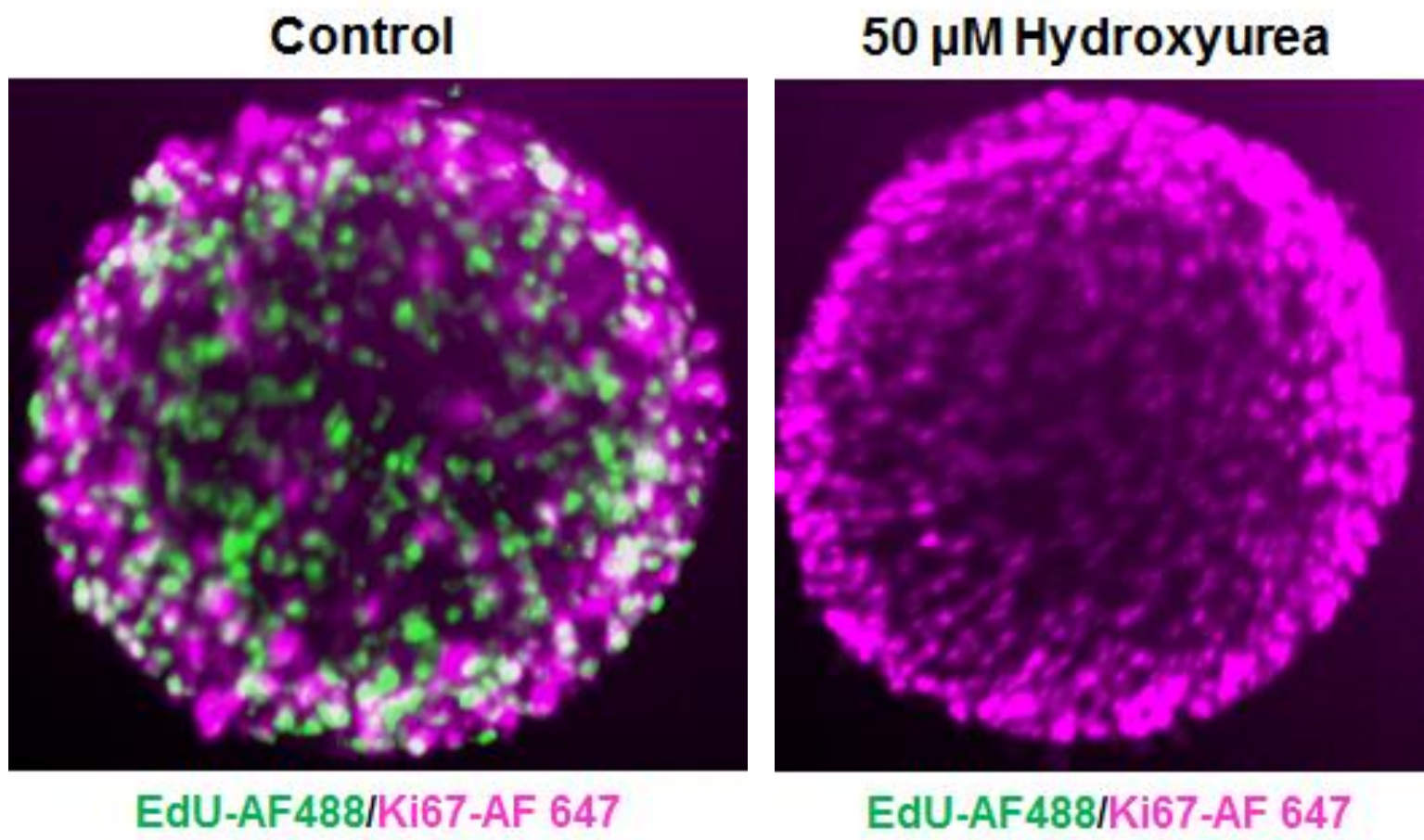
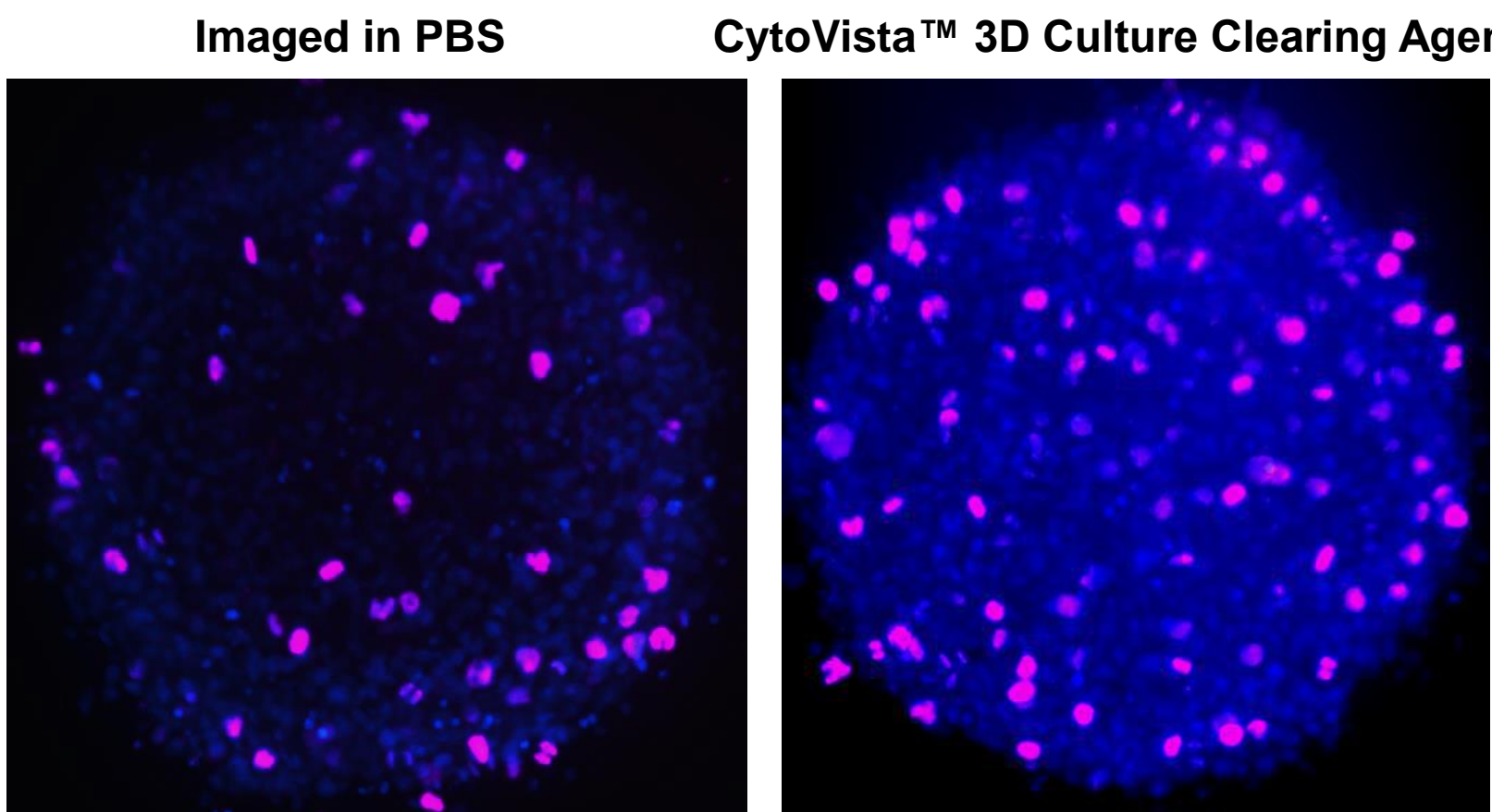


Figure 8. Cell proliferation studies in spheroids



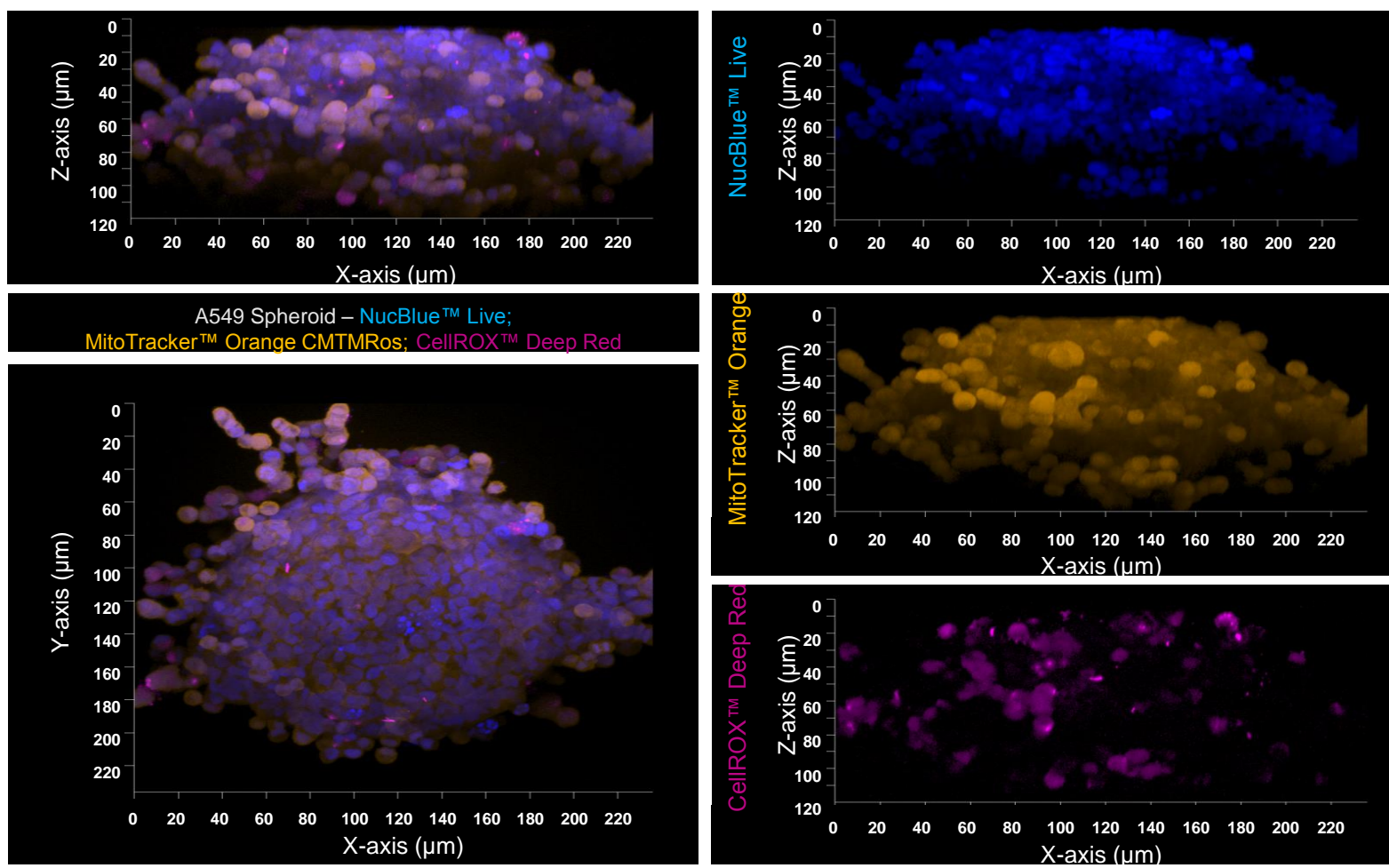
HeLa spheroids 50 μM hydroxyurea 24 hrs. Fixed and labeled with Alexa Fluor™ 488 Click-iT EdU and Alexa Fluor™ 647 Ki-67 antibody. Imaged at 4X using confocal mode on CellInsight CX7 LZR. Maximum intensity projection from 15 slices of 10 microns each.

Figure 9. CytoVista™ 3D Culture Clearing Agent



NucBlue™ Live; anti-Ki-67 Alexa Fluor 647 conjugate
CytoVista™ 3D Culture Clearing Agent provides superior optical transparency enabling visualization inside thick samples of fixed cells.

Figure 10. Improved Axial Resolution with ProLong™ Glass



Prolong Glass mountant has a refractive index of 1.52 after curing, similar to that of glass coverslips, compatible immersion oil, and oil-immersion microscope optics, enabling superior resolution and sensitivity when imaging fixed cells.

Conclusions

- Confocal imaging with laser-based illumination on the CX7 LZR system improves axial resolution of 3D spheroids.
- Penetration and potency of immune effector cells and ADCs can be evaluated using live-cell whole-spheroid imaging.
- Seven fluorescence detection channels enable multiplexed cell analysis. Phenotypic, structural, and functional probes can be combined to answer multiple biological questions simultaneously.

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