APPLICATION PAPER

An Image-Based Method to Detect and Quantify T Cell Mediated Cytotoxicity of 2D and 3D Target Cell Models

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Abstract

T cell mediated cytotoxicity plays an important role in a suite of new methods being developed with the goal of incorporating a patient's immune system to combat cancer. In order to evaluate and optimize adoptive T cell immunotherapies, sensitive *in vitro* methods must be included in the testing process. In the procedure described here, phenotypic and quantitative assessments of 2D and 3D target cell necrotic induction are made using a walk-away live cell imaging process.

Introduction

CD3+CD8+ cytotoxic T lymphocytes (CTL) are the effector cells responsible for T cell mediated cytotoxicity that can act by cell-to-cell contact either by releasing granzymes and perforin or through Fas ligand mediated toxicity[1]. As part of the adaptive immune system, these cells mount targeted attacks to rid the body of a variety of compromised cells, such as cancer cells, without harming healthy cells. Counteracting this natural defense is the widely known fact that tumors develop multiple methods to avoid immune detection and create a level of tolerance against the immune cells designed to seek out and destroy cells containing foreign antigens[2]. For many years, the development of treatments avoided use of a patient's immune system to kill cancer cells, as immunotherapy-based treatments met with multiple clinical failures. Recently, however, new methods to infuse activated, tumor specific T cells into cancer patients with the goal of recognizing, targeting, and destroying tumor cells[3] is revitalizing work in the field.

The most popular *in vitro* method to monitor CTL effect on target cells is the cell mediated cytotoxicity (CMC) assay where T cells and target cells are added to a microplate well as a co-culture. Traditionally toxicity was measured using chromium (^{51}Cr) release from preloaded target cells. Due to problems with radioactivity disposal, and low sensitivity due to spontaneous release of the isotope from target cells[4], newer methods were developed using microplate-based optical methods generating luminescence or fluorescence. While providing increased sensitivity compared to radioactivity, these techniques were optimized to detect the signal from target cells plated in a uniform two-dimensional (2D) monolayer in microplate wells. With increasing adaptation of cells aggregated into a three-dimensional (3D) configuration to create a more *in vivo*-like model, cells are no longer evenly spread throughout the bottom of a well, decreasing the sensitivity of reader-based detection methods as signal is lost amongst background. In addition, as with ^{51}Cr release, individual CTL: target cellular interactions cannot be monitored. Through the incorporation of microscopic imaging and cellular analysis, sensitive detection of induced cytotoxicity from 2D and 3D plated target cells, as well as visualization of the interplay between CTL and target cells, can be achieved.

Here, we demonstrate an automated method to monitor and measure CTL cell mediated cytotoxicity kinetically using digital widefield microscopy. Co-cultured target MDA-MB-231 breast cancer and fibroblast cells were plated in 2D and 3D format and dosed with a live cell apoptosis/necrosis reagent. T cells, activated using general or directed methods and stained with a far red tracking dye, were then added in ratios of 20, 10, 5, or 0:1 to the target cells. The plates were then added to an automated incubator and shuttled to the digital widefield microscope, using a robotic arm, every four hours where brightfield and fluorescent images were captured for a total of seven days. Visual observation of the kinetic images enabled monitoring of CTL:target cell interactions for 2D and 3D cultured cells. Cellular image analysis also allowed for calculation of CTL induced cytotoxicity during the entire incubation period.

Materials and Methods

Materials

Cells and Media

MDA-MB-231 epithelial breast adenocarcinoma cells (Catalog No. HTB-26) were obtained from ATCC (Manassas, VA). Human Neonatal Dermal Fibroblast cells stably expressing RFP (Catalog No. cAP-0008RFP) were purchased from Angio-Proteomie (Boston, MA). Human purified CD3+ T cells, isolated via negative selection from peripheral blood mononuclear cells (Catalog No. HM-PBMC-TCELLCD3-M) were donated by BioreclamationIVT (Westbury, NY). Advanced DMEM (Catalog No. 12491-015), RPMI 1640 medium (Catalog No. 11875-093), Fetal bovine serum, (Catalog No. 10437-036), and penicillin-streptomycin-glutamine (100X) (Catalog No. 10378-016) were purchased from ThermoFisher Scientific (Waltham, MA).

Assay and Experimental Components

IL-2 Superkine (Fc) (Catalog No. AG-40B-0111-C010), anti-CD3 (human), mAb (UCHT1) (Catalog No. ANC-144-020) and anti-CD28 (human), mAb (ANC28.1/5D10) (Catalog No. ANC-177-020) were donated by AdipoGen Life Sciences (San Diego, CA). SCREENSTAR® 190 µm cycloolefin filmbottom 384-well microplates (GBO Catalog No. 789836), CELLSTAR® µClear 384-well cell-repellent surface microplates (GBO Catalog No. 781976) and the 384-Well BiO Assay Kit (GBO Catalog No. 781846, consisting of 2 vials NanoShuttle-PL, 6-Well Levitating Magnet Drive, 384-Well Spheroid and Holding Magnet Drives (2), 96-Well Deep Well Mixing Plate, 6-Well and 384-Well Clear Cell Repellent Surface Microplates), prototype 384 well Ring Drive, and additional Cell Repellent Surface 6-Well (GBO Catalog No. 657860) were donated by Nano3D Biosciences, Inc., and Greiner Bio-One, Inc., (Monroe, NC). The Kinetic Apoptosis Kit (Microscopy) (Catalog No. ab129817) was donated by Abcam (Cambridge, MA). CellTracker™ Deep Red Dye (Catalog No. C34565) was purchased from ThermoFisher Scientific (Waltham, MA).

Cytation™ 5 Cell Imaging Multi-Mode Reader

Cytation 5 is a modular multi-mode microplate reader combined with an automated digital microscope. Filter- and monochromator-based microplate reading are available, and the microscopy module provides up to 60x magnification in fluorescence, brightfield, color brightfield and phase contrast. The instrument can perform fluorescence imaging in up to four channels in a single step. With special emphasis on live-cell assays, Cytation 5 features shaking, temperature control to 65 °C, CO_2/O_2 gas control and dual injectors for kinetic assays, and is controlled by integrated Gen5™ Microplate Reader and Imager Software, which also automates image capture, processing and analysis. The instrument was used to kinetically monitor CTL:target cell interactions as well as cytotoxicity induction within the 2D and 3D plated target cells.

BioSpa™ 8 Automated Incubator

The BioSpa 8 Automated Incubator links BioTek readers or imagers together with washers and dispensers for full workflow automation of up to eight microplates. Temperature, CO_2/O_2 and humidity levels are controlled and monitored through the BioSpa software to maintain an ideal environment for cell cultures during all experimental stages. Test plates were incubated in the BioSpa to maintain proper atmospheric conditions for a period of seven days and automatically transferred to the Cytation 5 every four hours for brightfield and fluorescent imaging.

Methods

3D Target Cell Preparation

T-75 flasks of MDA-MB-231 or fibroblast cell cultures were cultured to 80% confluence, then as illustrated in Figure 1, treated with 600 µL NanoShuttle-PL overnight at 37 °C/5% CO_2. After incubation, cells were trypsinized for 3-5 minutes at 37 °C/5% CO_2. Cells were removed from the flasks and added to the 6-well cell repellent plate at a concentration of 1.2x106 cells/well. A 6-well magnet drive was placed atop the well plate to levitate the cells, where aggregation and extracellular matrix (ECM) formation took place during an eight-hour incubation at 37 °C/5% CO_2. After incubation, the cells and ECM were broken up, resuspended, and combined together at equal concentrations in complete advanced DMEM medium.

A total of 2000 cells were added to wells of a 384-well cell repellent microplate intended for 3D spheroid formation previously placed atop the spheroid magnet drive (Figure 1 and 2). The microplate was incubated at 37 °C/5% CO_2 for 48 hours to allow the cells to aggregate into co-cultured tumoroids within each well.

Figure 1. BIO assay kit protocol

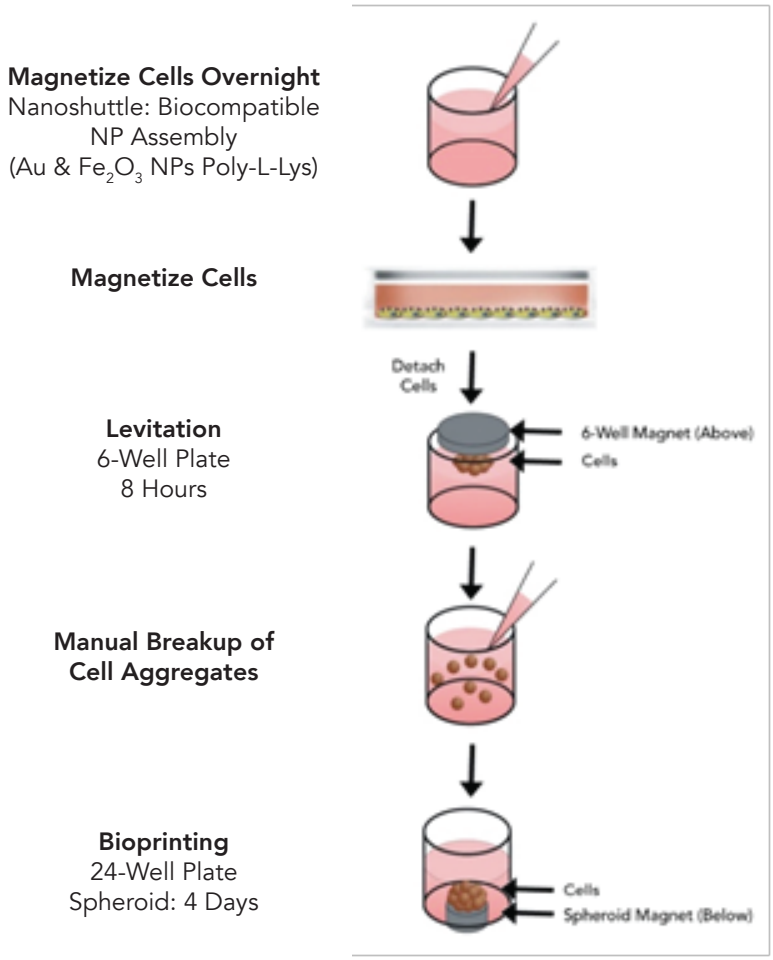
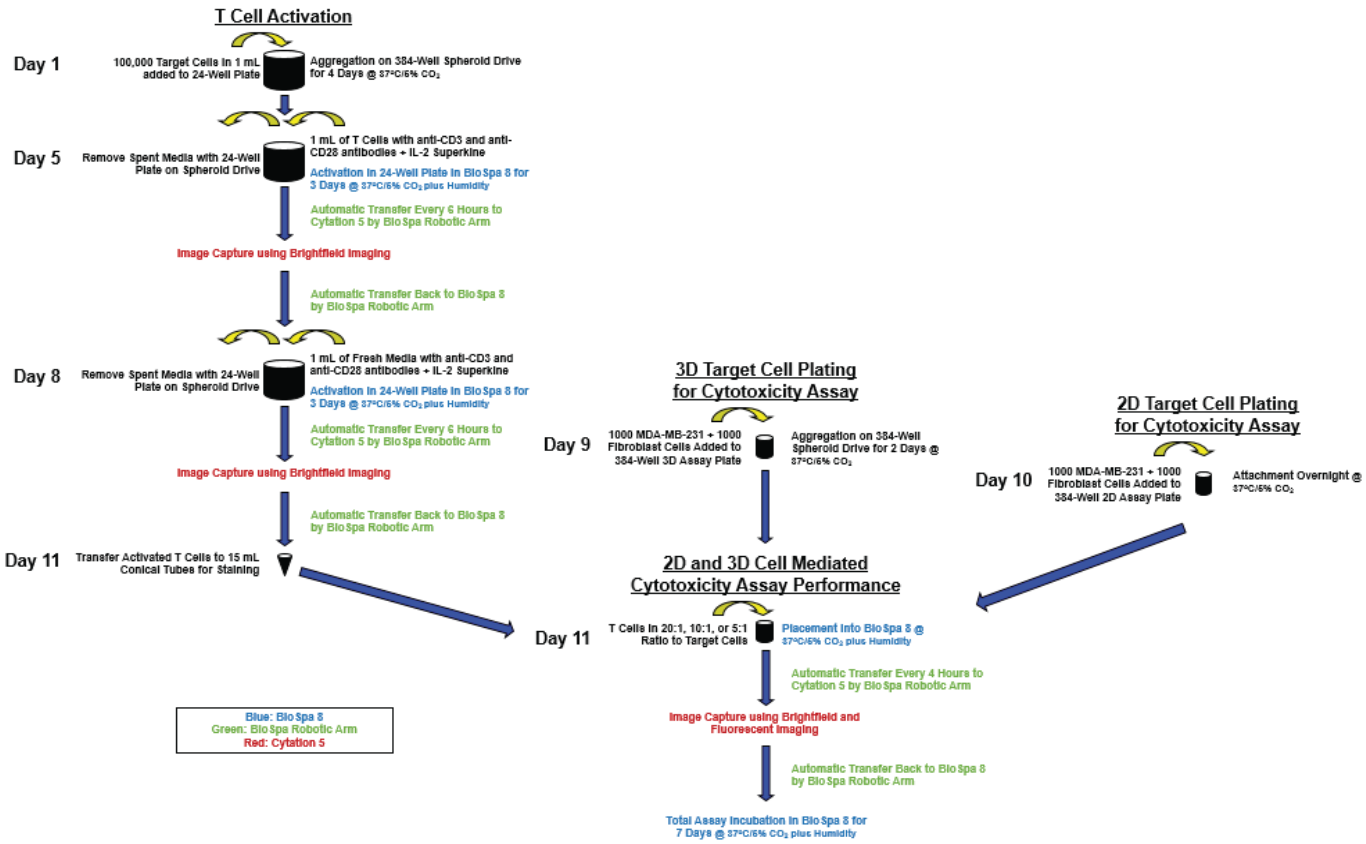


Figure 2. T cell activation and cell mediated cytotoxicity assay workflow



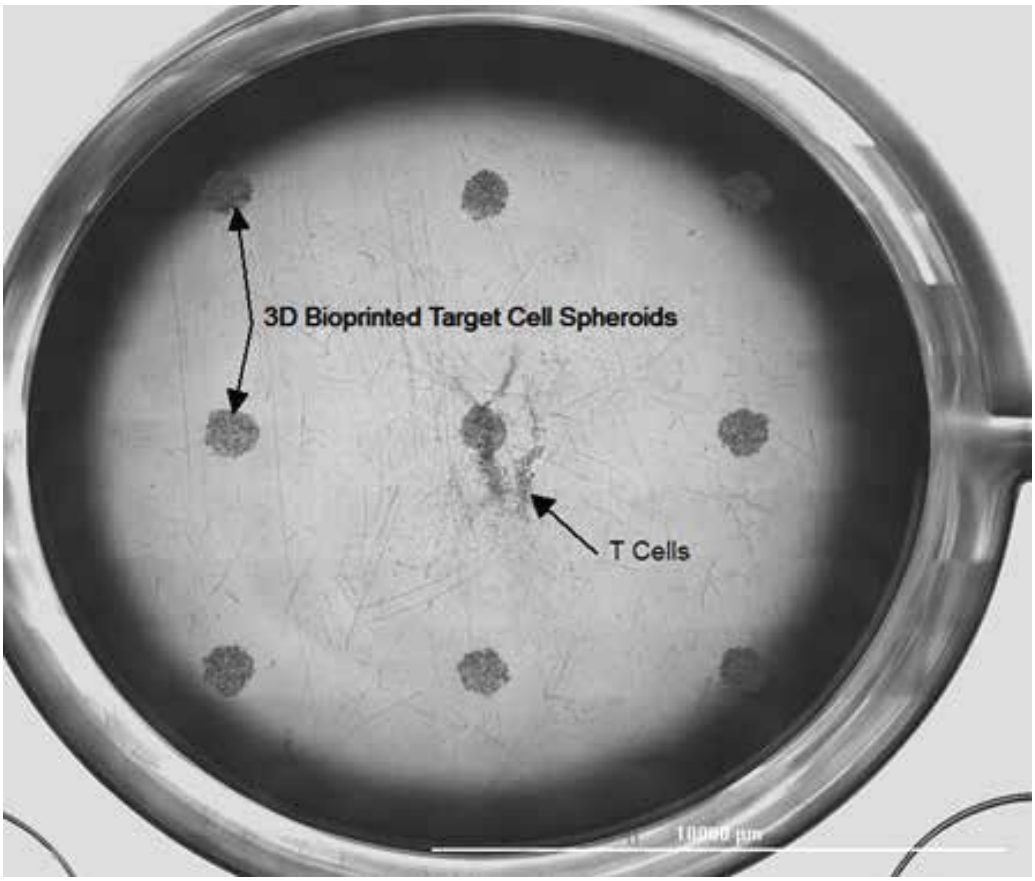
2D Target Cell Preparation

T-75 flasks of MDA-MB-231 or fibroblast cell cultures were cultured to 80% confluence. Cells were then trypsinized for 3-5 minutes at 37 °C/5% CO_2 and removed from the flasks. Following centrifugation, the cells were resuspended and combined together at equal concentrations in complete advanced DMEM medium. A total of 2000 cells were added to wells of a 384-well TC treated microplate intended for 2D cell culture (Figure 2). The microplate was incubated at 37 °C/5% CO_2 overnight to allow the cells to attach to the wells.

Directed and General T Cell Activation

A total of 10,000 target cells and media were added to 24-well cell repellent plate wells for each experimental condition as follows (Figure 2). Directed activation: (A) 100% MDA-MB-231; (B) 75% MDA-MB-231 and 25% fibroblasts; (C) 50% MDA-MB-231 and 50% fibroblasts; general activation: (D) no cells. Total volume was 1 mL for wells in each test condition. The 24-well plate was then placed atop a 384-well spheroid magnet drive and incubated at 37 °C/5% CO_2 for four days where the cells aggregated into multiple 3D spheroids within each well (Figure 3). Note that the magnet drive is designed for 384-well densities, such that the expanded size of a 24-well plate well provides nine (9) separate spheroids/well.

Figure 3. 24-well plate well showing co-culture of T cells and bioprinted magnetized 3D target spheroids prior to commencement of directed activation.
T cells added in a 10:1 ratio to target cells previously aggregated into 3D spheroids (~ 1000 µm ID).



Following spheroid aggregation, T cells were prepared at a concentration of 100,000 cells/mL in RPMI medium containing 100 ng/mL IL-2 Superkine (Fc) (superkine) along with 250 ng/mL each of anti-CD3 and anti-CD28 antibodies. Spent media was then aspirated while the plate remained on the magnet drive to secure the spheroids, and replaced with fresh media containing the T cells, antibodies, and superkine as previously described (Figure 2 and 3). The plate was then placed back into the BioSpa to incubate for six days. The BioSpa was pre-programmed to capture a 12 x 10 image montage from each test well every six hours. Manual exchange of media, IL-2 Superkine, and antibodies was performed after 72 hours. The directed activation procedure over the six days serves to not only activate the T cells, but also teaches them to recognize target cell antigens allowing for targeted cytotoxicity. The general activation procedure uses no target cells, thus there should be no targeted cytotoxicity[5].

T Cell Staining and Addition

Upon completion of the activation process, the 24-well plate containing the T cells and magnetized target cells was placed back on the 384-well magnet drive. The T cells were then removed from each well and transferred to a separate 15 mL conical tube for staining with the CellTracker Deep Red Dye allowing for differentiation from the target cells during the cytotoxicity experiment (Figure 2).

Dye, at a concentration of 1 µM, was added to the tubes and incubated at 37°C/5% CO_2 for 45 minutes. The tubes were then centrifuged for 15 minutes at 200 RCF. Media containing the excess dye was then removed and replaced with fresh RPMI medium. Stained T cells from each activation condition were then diluted in RPMI medium containing 10 µL/mL of the propidium iodide necrosis probe from the Kinetic Apoptosis Kit. The cells were then added to the 384-well 2D or 3D cell culture plates, already containing a total of 2000 target cells, in concentrations equaling 40,000 cells/well, 20,000 cells/well, or 10,000 cells/well (Figure 2). These concentrations created ratios of 20:1, 10:1, or 5:1 T cells to target cells in each well. Untreated negative control wells were also included to examine basal target cell cytotoxicity levels over time. Table 1 illustrates the final plate layout.

Table 1. 2D and 3D Cell Mediated Cytotoxicity Assay Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
B	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b
C	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
D	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d

100% Directed Activation
75% Directed Activation
50% Directed Activation
General Activation
Unactivated

a 20:1 T Cell:Target Cell Ratio
b 10:1 T Cell:Target Cell Ratio
c 5:1 T Cell:Target Cell Ratio
d 0:1 T Cell:Target Cell Ratio

Cell Mediated Cytotoxicity Assay Automated Procedure

Figure 4. BioSpa Live Cell Imaging System, including BioSpa8 and Cytation 5

2D and 3D assay plates, containing T cells and target cells, were added to the BioSpa 8 (Figure 2), as part of the BioSpa Live Cell Imaging System (Figure 4), with atmospheric conditions previously set to 37°C/5% CO_2. Water was also added to the pan to create a humidified environment, which was monitored. The BioSpa software was set such that the plates were automatically transferred to the Cytation 5 for brightfield and fluorescent imaging of the test wells every four hours for a total of seven days. Table 2 explains the imaging carried out with each channel. For 2D plated cells, a single 4x magnification image was taken with each channel to capture a representative population of cells per well. Laser autofocus was incorporated to ensure proper focusing on the target cell layer as well as the most efficient focusing procedure. For 3D plated cells, since the cells within the 3D target cell spheroids existed on multiple z-planes, a z-stack consisting of five slices was captured with each channel. Laser autofocus was again incorporated. Two images each were taken below and above the decided upon focal plane.

Table 2. Cells imaged per imaging channel

Imaging Channel Target	
Brightfield	All Cells
PI	Necrotic Cells
CY5	T Cells

2D and 3D Image Processing

Following capture, 2D and 3D images were processed prior to analysis. 2D images underwent preprocessing to remove background signal from each channel using the settings in Table 3.

Table 3. 2D image preprocessing parameters

2D Image Preprocess ing Parameters			
Channel	Apply Image Preprocessing	Background	Rolling Ball Diameter
Brightfield	No		
PI	Yes	Dark	Auto
CY5	Yes	Dark	Auto

For 3D images, first a z-projection of the images captured in the z-stack was carried out to create a final image containing only the most in-focus information (Table 4).

Table 4. 3D z-projection criteria

3D Image Stitching Parameters	
Method	Focus Stacking
Size of Max. Filter	11 pixels
Top Slice	0 µm from focal plane
Bottom Slice	-53.8 µm from focal plane

Preprocessing of the projected image was then performed to again remove background signal from each channel (Table 5).

Table 5. 3D image preprocessing parameters

3D Image Preprocess ing Parameters			
Channel	Apply Image Preprocessing	Background	Rolling Ball Diameter
Brightfield	No		
PI	Yes	Dark	Auto
CY5	Yes	Dark	Auto

Cellular Analysis of 2D and 3D Processed Images

Cellular analysis was carried out on the processed images to determine the total signal emanating from necrotic target cells using the criteria in Table 6.

Table 6. Necrotic cell identification criteria

Necrotic Cell Identification Criteria		
	2D Analysis	**3D Analysis**
Channel	Tsf[Propidium Iodide]	Tsf(ZProj[Propidium Iodide])
Threshold	Auto (-6)	5000
Background	Dark	Dark
Split Touching Objects	Checked	Checked
Fill Holes in Masks	Checked	Checked
Min. Object Size	10 µm	10 µm
Max. Object Size	4000 µm	100 µm
Include Primary Edge Objects	Unchecked	Unchecked
Analyze Entire Image	Checked	Checked
Advanced Detection Options		
Rolling Ball Diameter	1000 µm	75 µm
Image Smoothing Strength	20	0
Evaluate Background On	5% of Lowest Pixels	5% of Lowest Pixels
Analysis Metric		
Metric of Interest	Cell Count	Object Sum Int[Tsf[ZProj[Propidium Iodide]]]

An additional image analysis step was performed on the 3D images to determine the extent to which target cell spheroids disintegrated following T cell treatment (Table 7).

Table 7. Spheroid disintegration criteria

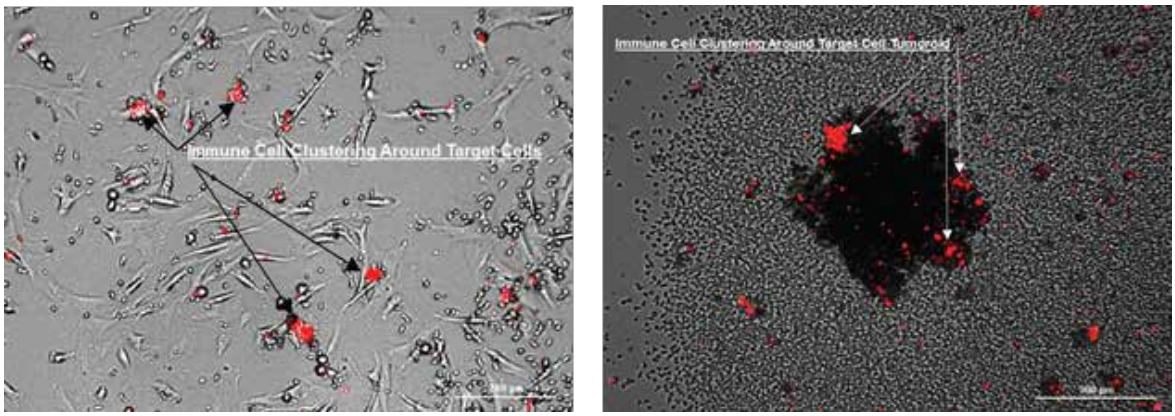
3D Image Analysis Parameters	
Data In	Tsf(ZProj[Brightfield])
Threshold (Lower Value)	Unchecked
Threshold (Upper Value)	Checked (2500)
Analysis Metric	
Metric of Interest	Confluence

Results and Discussion

T cells, activated using direct and general activation procedures, were added to the target cells in concentrations equaling 20:1, 10:1, 5:1 and 0:1 to start the cell mediated cytotoxicity assay. To monitor the interaction of the co-cultured cells, plates were imaged immediately following T cell addition and every four hours subsequent throughout the entire seven-day incubation period.

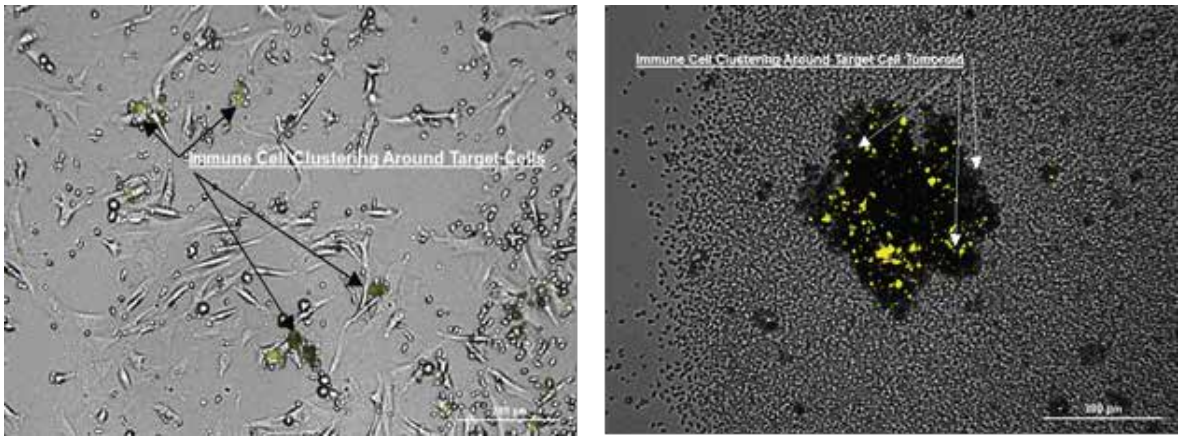
As assay incubation times increase, it is apparent that activated T cells (red fluorescence) seek out and cluster around the antigen presenting target cells through antigen-receptor binding in both 2D and 3D formats (Figure 5A). This T cell aggregation is in marked contrast to the more even distribution of red fluorescence at time 0.

Figure 5. Brightfield/CY5 imaging of cellular interaction
4x brightfield and CY5 images showing T cell clustering and binding to (A) 2D or; (B) 3D target cells. Time = 24 hours.



When images from the PI channel are overlaid with those from the brightfield channel one can observe that yellow fluorescent signal from the propidium iodide necrotic cell probe originates from the same target cells with bound T cells (Figure 6). This confirms the downstream cytotoxic effect of T cell binding to the target cells.

Figure 6. Brightfield/PI imaging of cellular interaction.
4x brightfield and PI images showing necrotic (A) 2D or; (B) 3D target cells in response to T cell binding. Time = 24 hours.



Kinetic Imaging of T Cell Mediated Target Cell Cytotoxicity Induction

In order to determine the kinetics of cytotoxicity induction within the target cells, imaging must be carried out at regular intervals throughout the entire incubation period. As the full cytotoxic effect may not be reached until days after T cell addition, it is also essential that cells be allowed to interact for multiple days. The environmental controls of the Cytation 5 and BioSpa 8, as well as automatic transfer of test plates from incubator to imager, allow kinetic analysis to be completed without compromising cell health. In the experiments performed here, brightfield and fluorescent images were captured every four hours for a total of seven days. Figures 7 and 8 demonstrate the iterative cytotoxic effect that T cells, directly activated in the presence of 100% MDA-MB-231 cells and added at a 20:1 ratio, have on 2D and 3D cultured target cells, respectively.

Figure 7. CY5/PI imaging of 2D cytotoxic target cell induction
4x overlaid CY5 and PI images showing stained T cells and signal from propidium iodide necrotic cell probe following (A) 0; (B) 48; (C) 96; and (D) 168 hour co-culture incubation periods

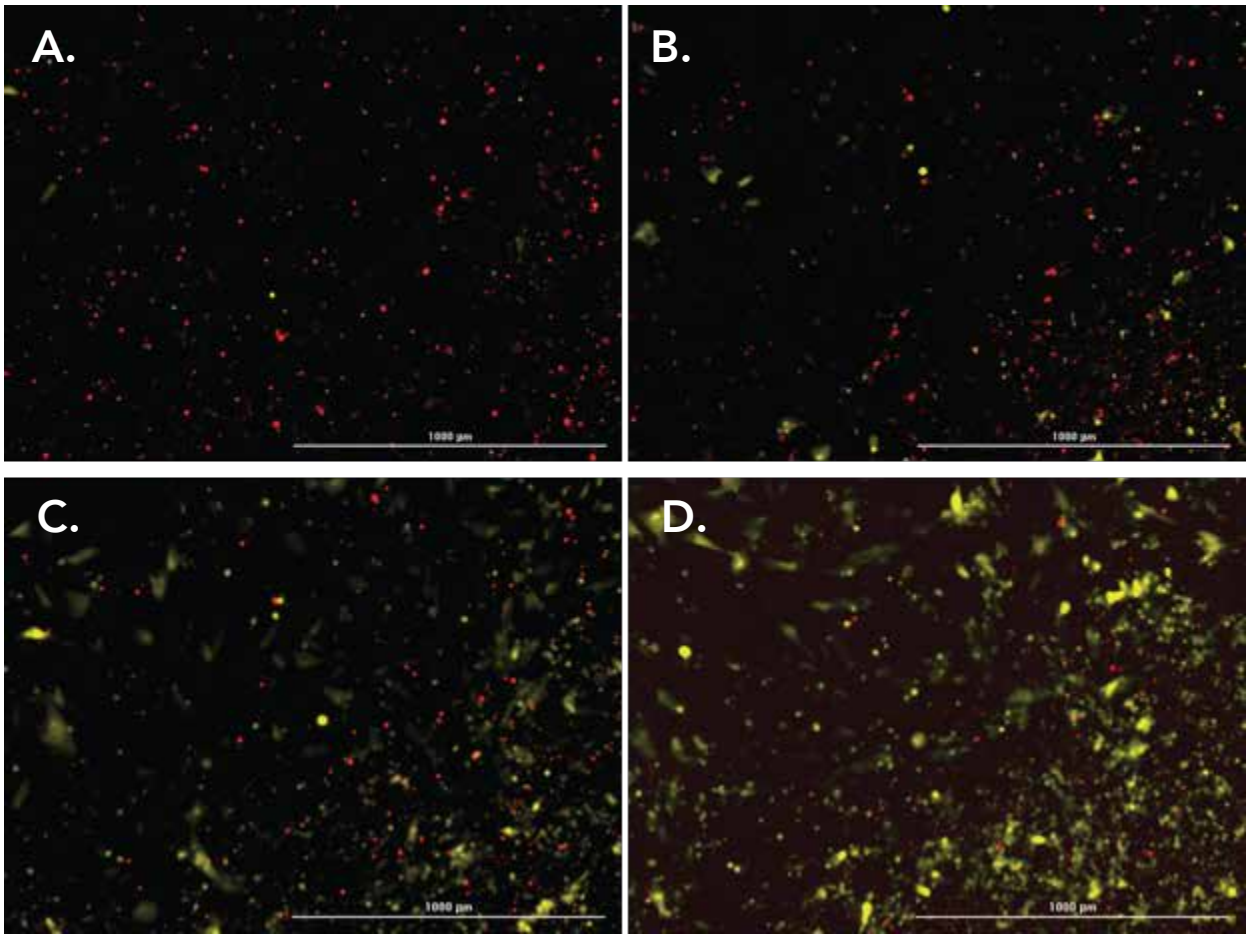
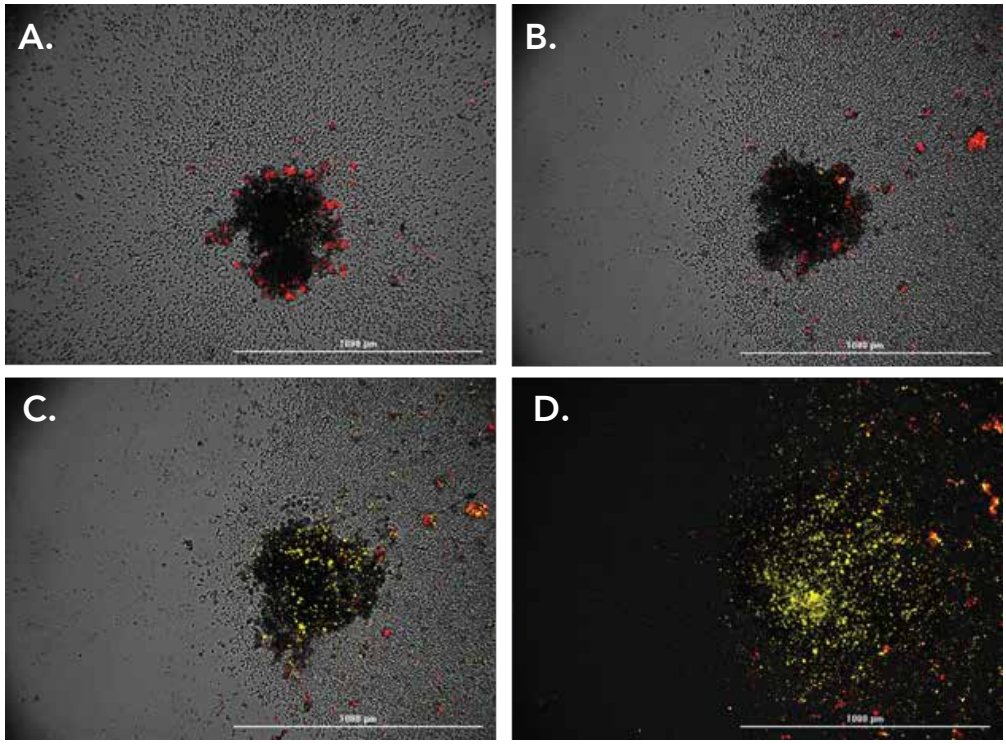


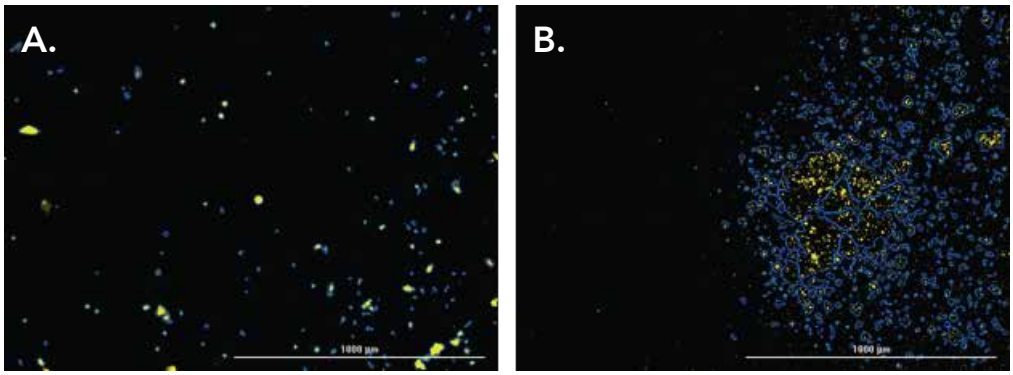
Figure 8. Brightfield/CY5/PI imaging of 3D cytotoxic target cell induction
4x overlaid brightfield, CY5 and PI images showing stained T cells and signal from propidium iodide necrotic cell probe following (A) 0; (B) 48; (C) 96; and (D) 168 hour co-culture incubation periods.



Quantification of Target Cell Cytotoxicity

Following image capture, the level of T cell induced target cell cytotoxicity was then quantified.

Figure 9. Cellular analysis of target cell cytotoxicity.
4x images showing fluorescence from propidium iodide necrotic cell probe following 96-hour incubation. Object masks (in blue) placed around (A) 2D and; (B) 3D cultured target cells meeting cellular analysis criteria.



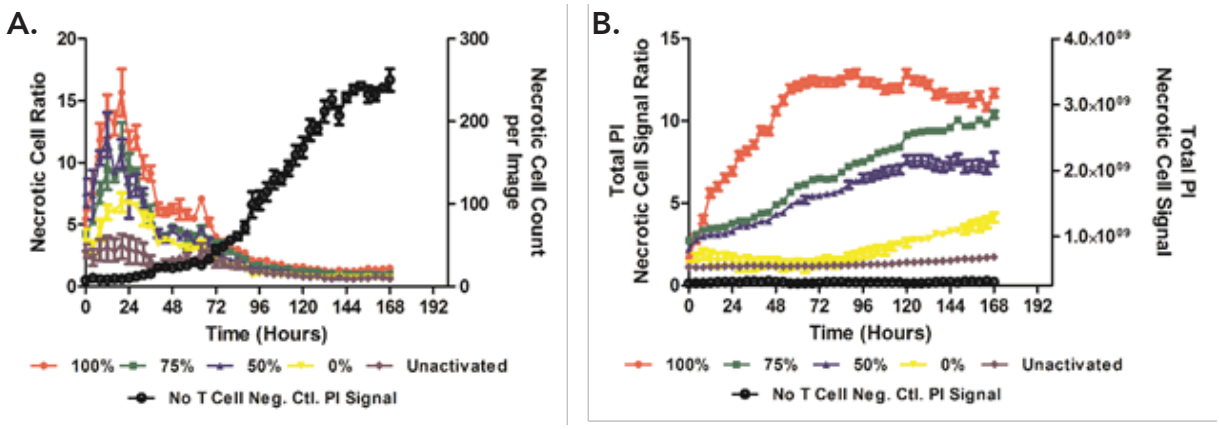
Using the optimized image analysis criteria described in Table 6, object masks were placed around cells meeting minimum threshold signal criteria from the PI necrotic cell probe (Figure 9). As T cells have a smaller size compared to the target cells in either 2D or 3D format, the minimum object size cutoff value was set such that single necrotic T cells were not included in the analysis. This can be seen in Figures 9A and B.

A phenomenon also observed in the kinetic images of the 3D CMC assay is that the tumoroid began to disintegrate in response to increasing cytotoxicity, releasing groups of cells into the surrounding media. While smaller than the intact tumoroid body, these aggregates remain larger than individual T cells and also emit signal from the PI necrotic cell probe, therefore are included in the final analysis (Figure 9B).

From the analysis performed, the number of necrotic cells per image was calculated for 2D cultured target cells. When cultured in 3D, cells within the tumoroid and smaller aggregates exist on multiple z-planes. Therefore, to quantify induced cytotoxicity with the greatest level of accuracy, the total PI signal within all object masks per image was quantified. The values (cell count or total PI signal) calculated at each timepoint were then automatically divided by the value calculated at time 0 in Gen5 software. In this way small variances between replicates were normalized. Following analysis, the results were plotted to evaluate whether differences were seen in induced target cell cytotoxicity between test conditions. The graphs in Figure 10 show the calculated data for T cells added to test wells in a 20:1 ratio, activated in the presence of 100%, 75%, 50% or 0% MDA-MB-231 cells, compared to unactivated T cells.

Figure 10. Activation protocol cytotoxicity induction analysis

Comparison of cytotoxic target cell induction by T cells activated in the presence of anti-CD3 and anti-CD28 antibodies, superkine, and 100% MDA-MB-231 cells, 75% MDA-MB-231/25% fibroblast cells, 50% MDA-MB-231/50% fibroblast cells, or no cells. Data for unactivated T cells also included and plotted using left y-axis. Necrotic cell count or total PI signal over time from untreated negative control target cells plotted on right y-axis. Results shown for T cells incubated with (A) 2D cultured target cells; or (B) 3D cultured target cells for seven days



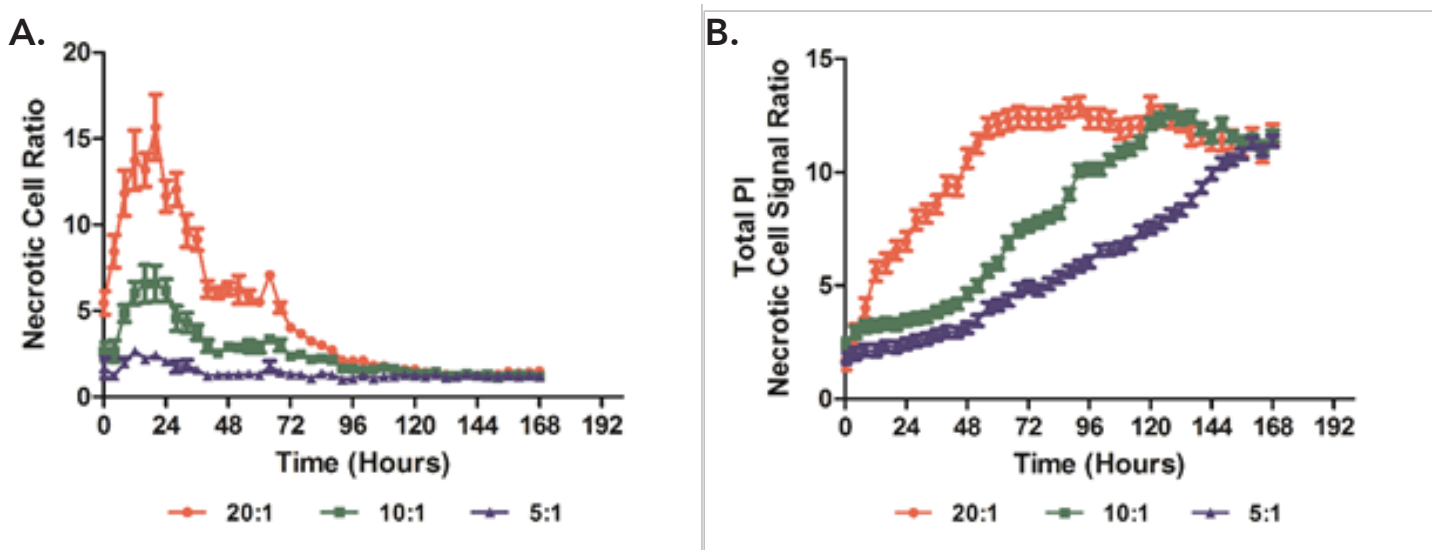
From Figure 10 it is evident that T cell induced cytotoxicity increases in terms of the degree of directed cell activation in both 2D and 3D cell models. T cells activated in the presence of 100% MDA-MB-231 cells elicit the highest level of cytotoxicity, while those activated only in the presence of antibodies and superkine elicit the lowest increase in necrotic cell numbers per image over basal necrotic cell numbers. The models differ in their kinetic responses, however. In the 2D model (Fig 10A), T cell-mediated cytotoxicity peaks at about 24 hours after addition of the activated T cells, as witnessed by the ratio of necrotic cells from wells containing T cells to necrotic cell numbers from negative control wells. Any further necrosis beyond about 3 days is due to the limitations of the 2D model as noted by the increased necrosis over time evident in the negative control. Conversely, in the 3D model (Figure 10B), necrotic ratios of total signal

(Figure 10B), necrotic ratios of total signal from the PI probe continue to increase or plateau over the course of the kinetic run due to the fact that cell health is much better retained in the untreated 3D cell model.

Analysis of necrotic cell induction was then performed on wells containing T cells directly activated in the presence of 100% MDA-MB-231 cells and then added to 2D and 3D plated target cells for the CMC assay in ratios of 20:1, 10:1, 5:1. A negative control was also included where target cells were untreated.

Figure 11. Effect of T cell concentration

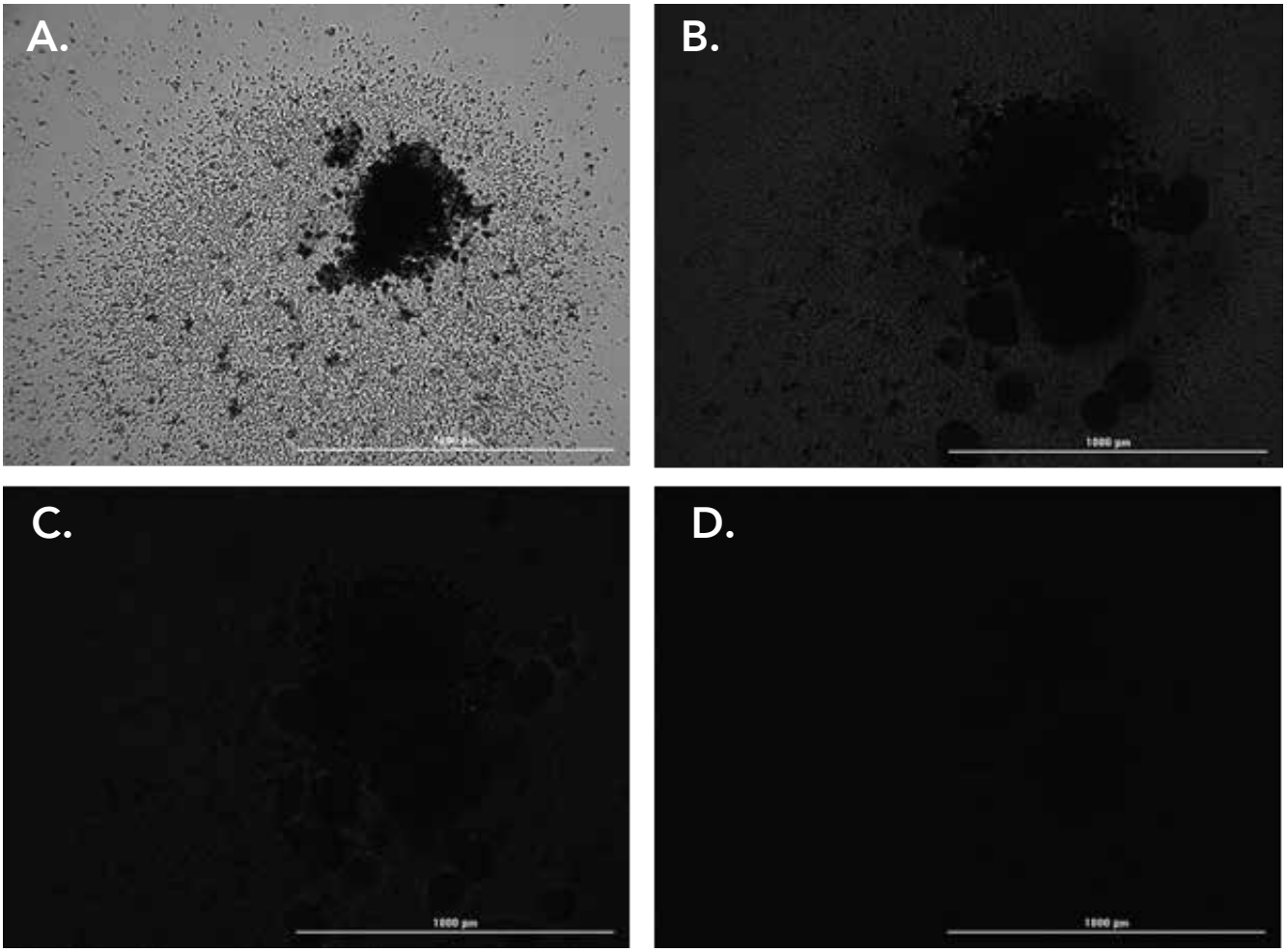
Comparison of cytotoxic target cell induction by T cells added to wells at concentrations of 40,000 cells/well (20:1 ratio), 20,000 cells/well (10:1 ratio), 10,000 cells/well (5:1 ratio), and 0 cells/well (negative control). Results shown for T cells incubated with (A) 2D; or (B) 3D cultured target cells for seven days.



It is evident from Figure 11 that kinetic responses of T cell-mediated cytotoxicity for different ratios of T cell to target cell for both 2D and 3D models are obtained over time. These findings are consistent with the previous results from the activation protocol comparison (Fig 10), as well as results reported with *in vivo* testing[6].

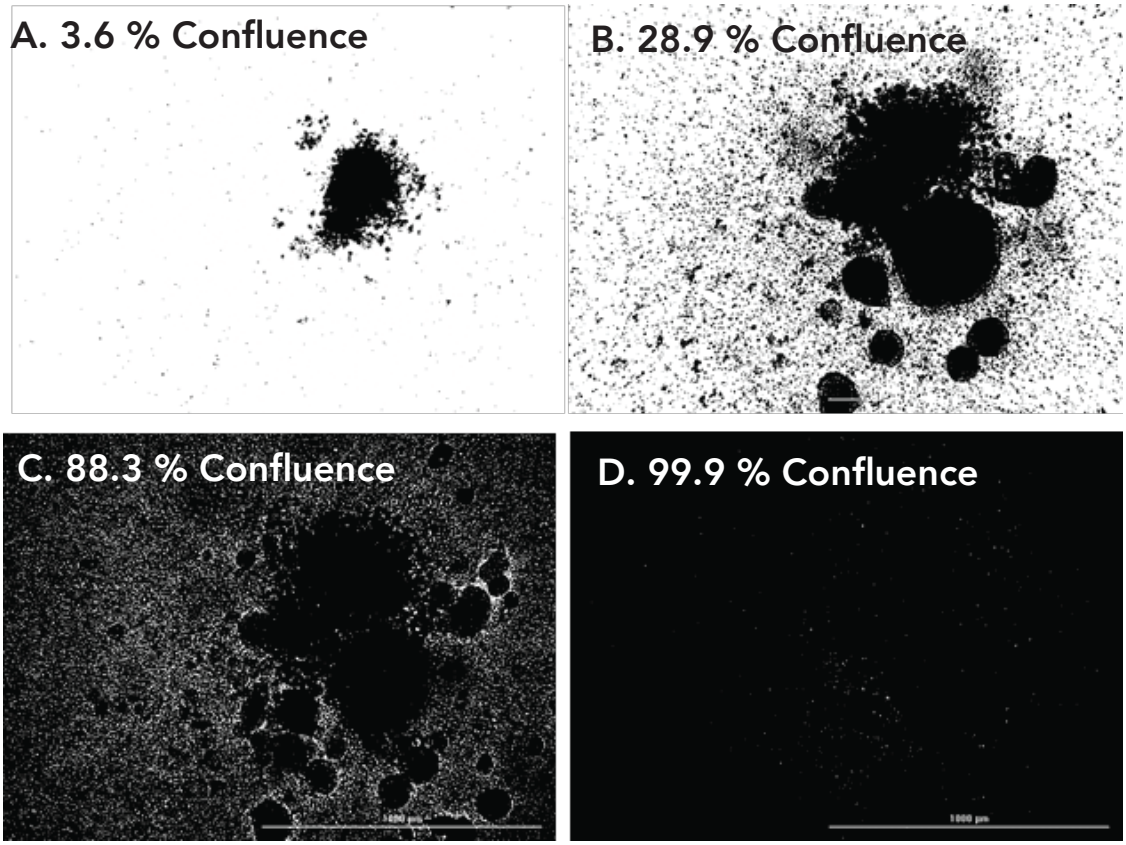
Finally, the effects of directed activation can also be measured using the brightfield channel when target cells are cultured in 3D. This is due to the fact that in response to the cytotoxic T cell effect, tumoroids break apart over time, or explode, releasing cells and ECM within the well. When viewed with the brightfield channel, images appear black from the tumoroidal cells and matrix now suspended within the media (Figure 12).

Figure 12. Brightfield imaging of T cell mediated target cell tumoroid disintegration
4x brightfield images showing tumoroid disintegration following cell interaction and binding with a 10:1 T cell to target cell ratio for (A) 72; (B) 116; (C) 136; and (D) 168 hour incubation periods.



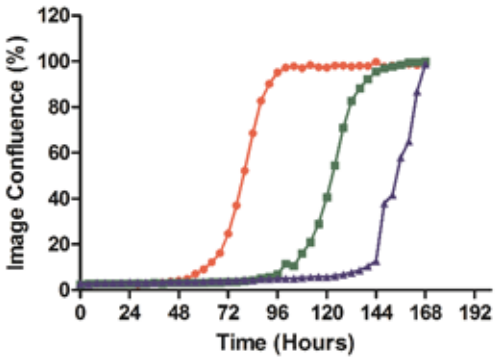
Using the confluence measurement capabilities of Gen5 and the optimized metrics in Table 7, the extent of tumoroid disintegration can then be quantified. Only pixels within each image with a brightfield signal below the upper threshold criteria are included in the percent confluence calculation. When viewed in Gen5, outlier pixels are seen as white (Figure 13).

Figure 13. Image confluence determination using brightfield signal
4x brightfield images following image analysis and % confluence determination. Pixels not included in confluence calculation appear white. Images shown after cell interaction and binding with a 10:1 T cell to target cell ratio for (A) 72; (B) 116; (C) 136; and (D) 168 hour incubation periods.



Percent confluence values can then be plotted over time to visualize the kinetics of tumoroid disintegration in response to increasing T cell to target cell ratios.

Figure 14. Kinetic percent image confluence quantification
Plot of kinetic brightfield image percent confluence due to 3D tumoroid disintegration.



Conclusions

Through the use of the Cytation 5, kinetic imaging of T cell mediated cytotoxicity can be carried out. Incorporation of the BioSpa 8 also allows experiments to be performed using long-term incubations in a walk-away manner. Quantification of induced cytotoxicity is then completed with the addition of a live cell fluorescent necrosis probe and Gen5 cellular analysis. When 3D tumoroid target cell models are included, accurate results from extended incubation studies can be generated without risking inclusion of non-induced necrotic cell numbers. The combination creates a sensitive, easy to use method to visualize phenotypic changes and quantify the effect of T cell mediated cytotoxicity for immuno-oncology applications.

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