Assay Name: 3D multicellular tumor spheroid endpoint apoptosis screening

Assay ID: Celigo_03_0005

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Experiment: 3D multicellular tumor spheroid (MCTS) endpoint apoptosis screening assay

Purpose	Monitor the effects of a panel of drugs on the apoptosis of U87MG Glioblastoma MCTS using Caspase 3/7 and Hoechst fluorescent staining
Current Method(s)	Microscopy
Target Cell Type	U87MG
Experiment Plan	Allow U87MG spheroids to form and treat with a panel of drug compounds, then image on day 13 to measure the apoptotic effects of the compounds
Hypothesis	Using the bright field and fluorescent imaging, the Celigo will rapidly provide multicellular tumor spheroid images, and measure Caspase 3/7 and Hoechst fluorescent intensities of treated U87MG MCTS

Celigo Setup

Plate Type	Nexcelom U-bottom Ultra-low Attachment 384-well Plate (Cat# ULA-384U)
Scan Channels	Green, Blue, and Bright Field
Resolution	1 µm/pixel
Scan Area	Whole well
Analysis Method	Tumorsphere 1 + 2 + Mask
Scan Frequency	Endpoint
Scan Time	~8 min

Assay Protocol and Plate Setup

Goal:

Image and analyze the apoptotic effects of a panel of drug compounds on U87MG MCTS on day 13.

Protocol

Cell Preparation

1. Seeded 500 U87MG cells/well in ULA 384-well plates
2. On day 4, added different serially diluted drug compounds at 2x and a vehicle control in media
3. On day 13, prepared and added Caspase 3/7 and Hoechst for staining the MCTS
4. Incubated the plate at 37 °C and 5% CO_2 for 60 min
5. Imaged and analyzed on Celigo
6. Compared the spheroid Caspase 3/7 fluorescent intensity for each drug compound at each time point to characterize the tested compounds

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	18	20	21	22	23	24
A																								
B	10 µM	Cmpd 1			Cmpd 2			Cmpd 3			Cmpd 4			Cmpd 5			Cmpd 6			Cmpd 7			CNTL	
C	5 µM																							
D	2.5 µM																							
E	1 µM																							
F	0.5 µM																							
G	0.1 µM																							
H	0.05 µM																							
I	10 µM	Cmpd 8			Cmpd 9			Cmpd 10			Cmpd 11			Cmpd 12			Cmpd 13			Cmpd 14				
J	5 µM																							
K	2.5 µM																							
L	1 µM																							
M	0.5 µM																							
N	0.1 µM																							
O	0.05 µM																							
P																								

Data Collection

1. After incubating the spheroids with drugs at different concentrations, the spheroids were stained with Caspase 3/7 and Hoechst
2. The plate with stained spheroids was imaged on day 13
3. The captured images were then analyzed in the Celigo software for the entire 384-well plate

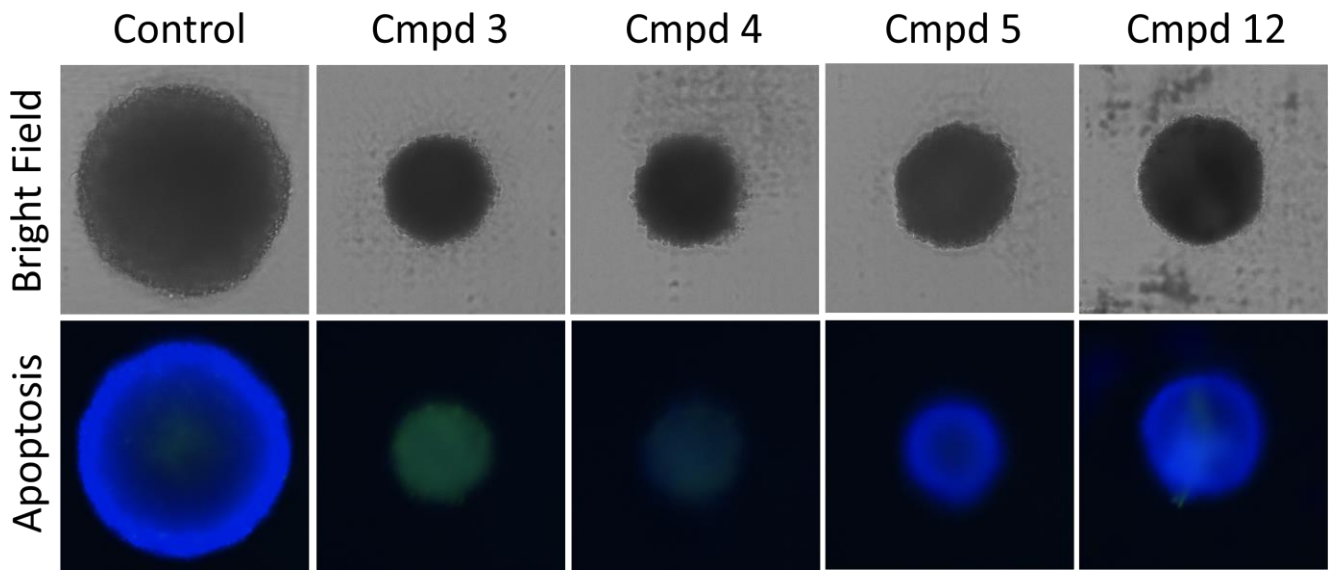
Data Analysis

- The images were analyzed by using Tumorsphere 1 + 2 + Mask application to identify the MCTS in the well
- The fluorescent intensities of Caspase 3/7 were measured for each drug-treated MCTS or control

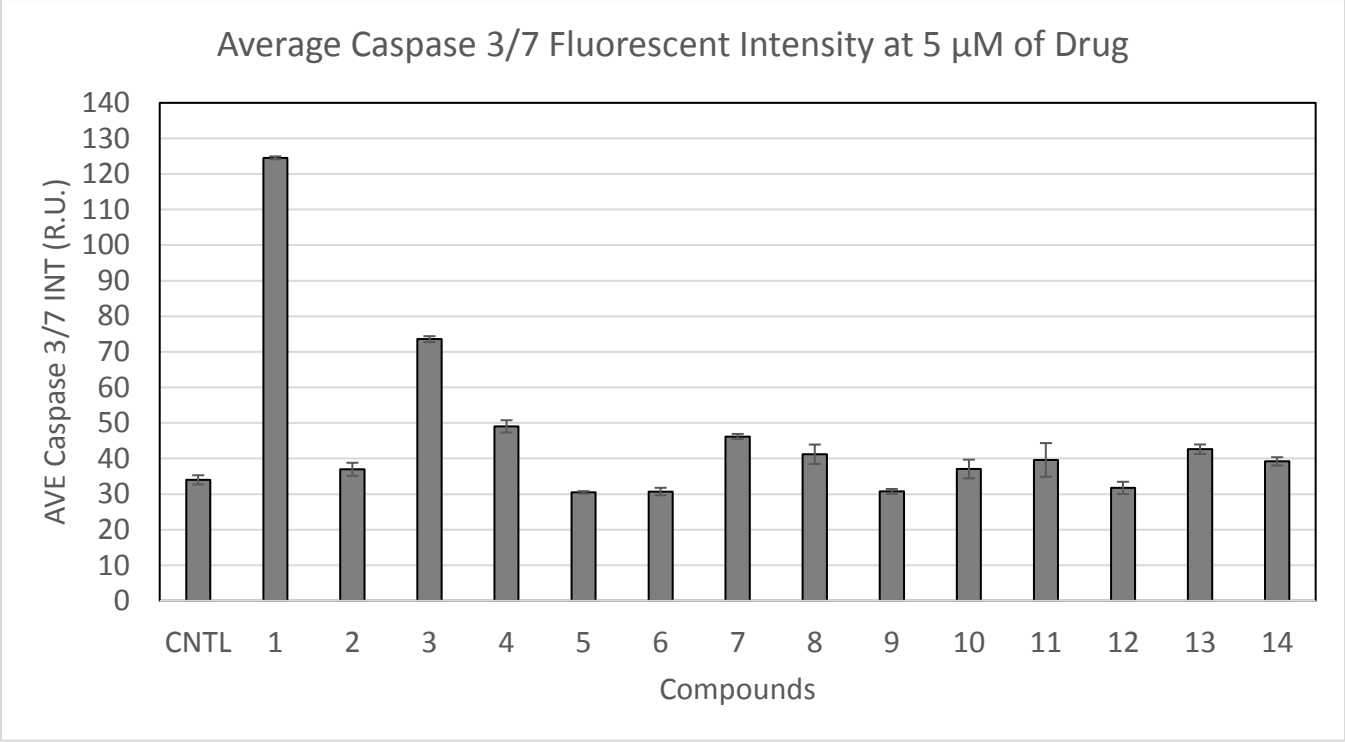
Results

1. Endpoint bright field and fluorescent images and results of MCTS for control and treated sample

- The bright field images were used to identify the spheroids in each well
- The Caspase 3/7 fluorescent intensities were measured from the images



- The plot below is showing the Caspase 3/7 fluorescent intensities, which indicated the apoptosis of each MCTS treated with the different drug compounds
- Only 2 drug compounds induced noticeable apoptosis on the U87MG MCTS, while other drugs had no effects



Conclusion

- Using the 384-well U-bottom ULA plates, we successfully captured images of U87MG MCTS and analyzed the data using the Celigo image cytometer
- The entire 384-well plate was imaged in ~8 min. The short scan time significantly increased the throughput during an experiment that has multiple plates
- After the drug treatments, the spheroid Caspase 3/7 fluorescent intensities were measured and automatically reported by the Celigo software
- No additional software was required for image analysis of MCTS fluorescent intensities