

Assay Name: 3D multicellular tumor spheroid (MCTS) growth inhibition screening assay

Assay ID: Celigo_03_0004



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Experiment: 3D multicellular tumor spheroid (MCTS) growth inhibition screening assay

Purpose	Monitor the effects of a panel of drugs on the growth inhibition of U87MG Glioblastoma MCTS
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 0, 69, 114, 165, 210 hours post-treatment to measurement the growth inhibition effects of the compounds
Hypothesis	Using the bright field imaging, the Celigo will rapidly provide multicellular tumor spheroid images and diameters of treated U87MG MCTS

Celigo Setup

Plate Type	Nexcelom U-bottom Ultra-low Attachment 384-well Plate (Cat# ULA-384U)
Scan Channels	Bright field
Resolution	1 μm /pixel
Scan Area	Whole well
Analysis Method	Tumorsphere 1
Scan Frequency	0, 69, 114, 165, 210 hours post-treatment
Scan Time	~4 min

Assay Protocol and Plate Setup

Goal:

Image and analyze the growth inhibition effect of a panel of drug compounds on U87MG MCTS over time.

Protocol

Cell Preparation

1. Seeded 500 U87MG cells/well in ULA 384-well plates
2. On day 4, added serially diluted different drug compounds at 2x and a vehicle control in media
3. Monitored growth inhibition by imaging and analyzing each 384-well plate at ~4 min/plate on 0, 69, 114, 165, 210 hours post-treatment with the Celigo imaging cytometer
4. Measured the spheroid diameters on 0, 69, 114, 165, 210 hours post-treatment for each drug compound treated MCTS
5. Compared the spheroid diameters 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 μ M	Cmpd 1	Cmpd 2	Cmpd 3	Cmpd 4	Cmpd 5	Cmpd 6	Cmpd 7	CNTL																
C	5 μ M																								
D	2.5 μ M																								
E	1 μ M																								
F	0.5 μ M																								
G	0.1 μ M																								
H	0.05 μ M																								
I	10 μ M	Cmpd 8	Cmpd 9	Cmpd 10	Cmpd 11	Cmpd 12	Cmpd 13	Cmpd 14																	
J	5 μ M																								
K	2.5 μ M																								
L	1 μ M																								
M	0.5 μ M																								
N	0.1 μ M																								
O	0.05 μ M																								
P																									

Data Collection

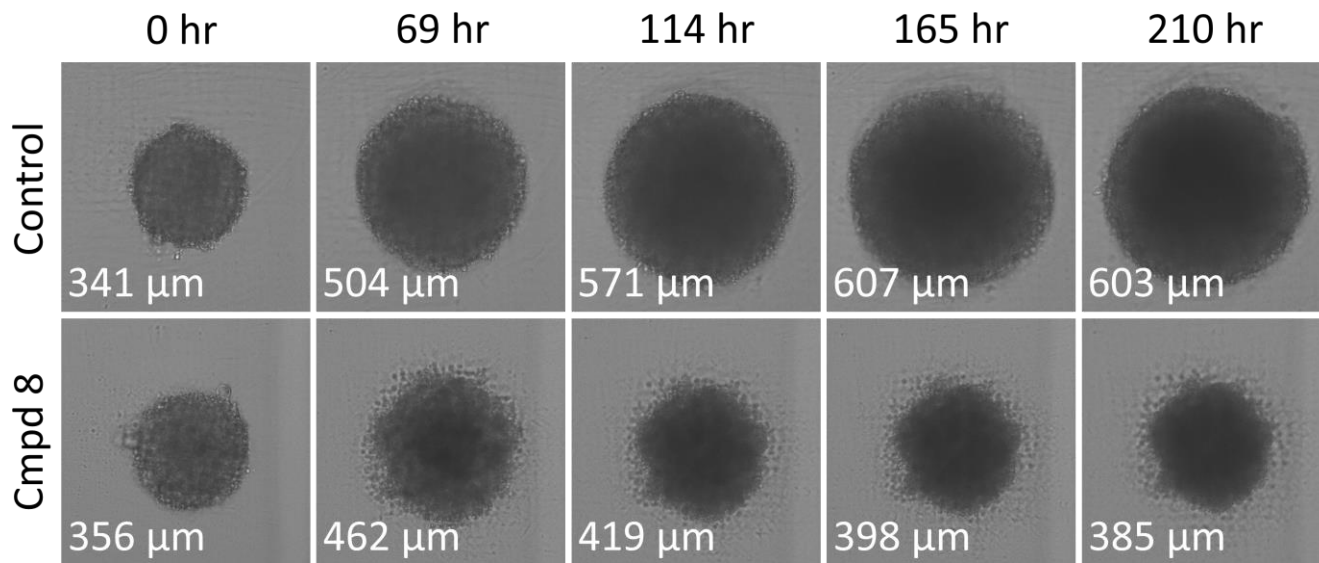
1. After adding the drug at different concentrations on day 4, the plates were imaged and data collected for the entire 384-well plate
2. The plates were again imaged on 0, 69, 114, 165, 210 hours post-treatment in order to perform time course monitoring of tumor spheroid diameter

Data Analysis

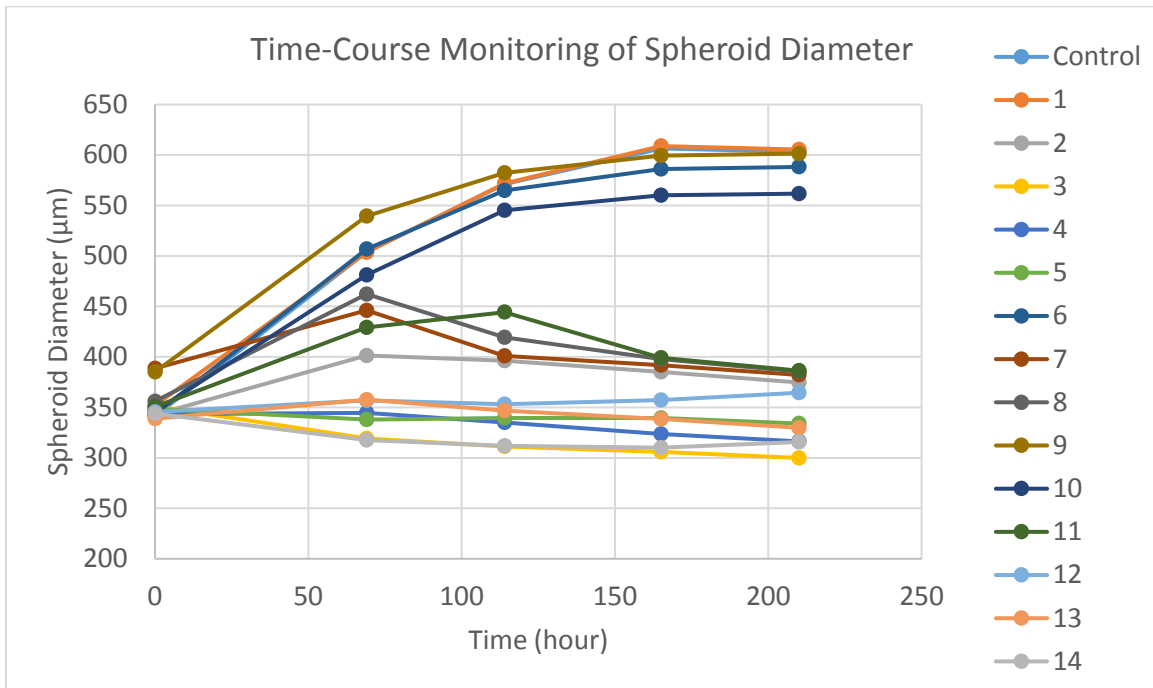
- The images were analyzed by using the Tumorsphere 1 application to identify the MCTS in the well
- The diameter of each MCTS treated with different drug compounds was measured

Results

1. Time-course bright field images and results of MCTS for control and treated sample
 - The bright field images showed the control MCTS increased in diameter over time while the treated sample with compound 8 showed growth inhibition

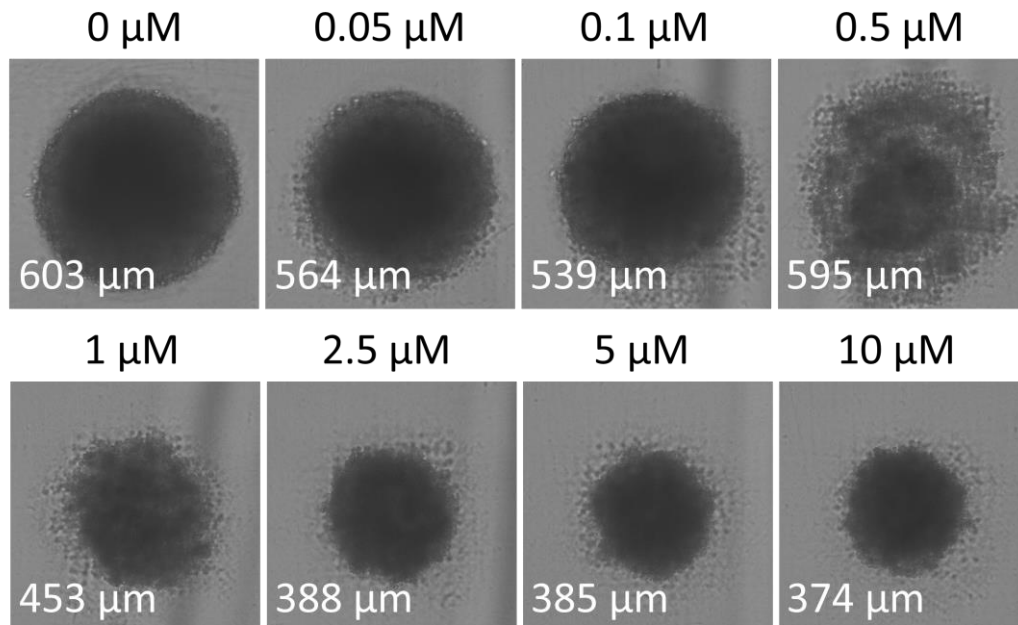


- The time-course results showed that some drugs inhibited the growth in the beginning of the treatment, and some drugs inhibited several days after the treatment
- Some drug compounds did not induce inhibition, similar to the control

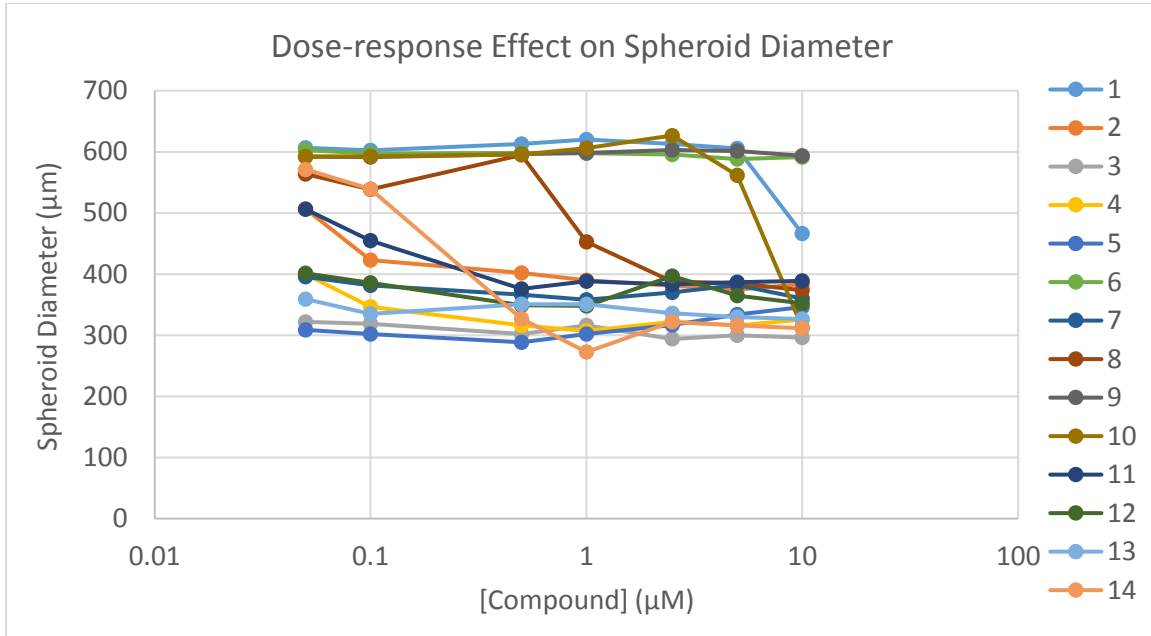


2. Dose-response bright field images and results of MCTS for control and treated sample

- The bright field images showed the dose response of compound 8 growth inhibition of MCTS
- As the concentration increased, the spheroid size decreased

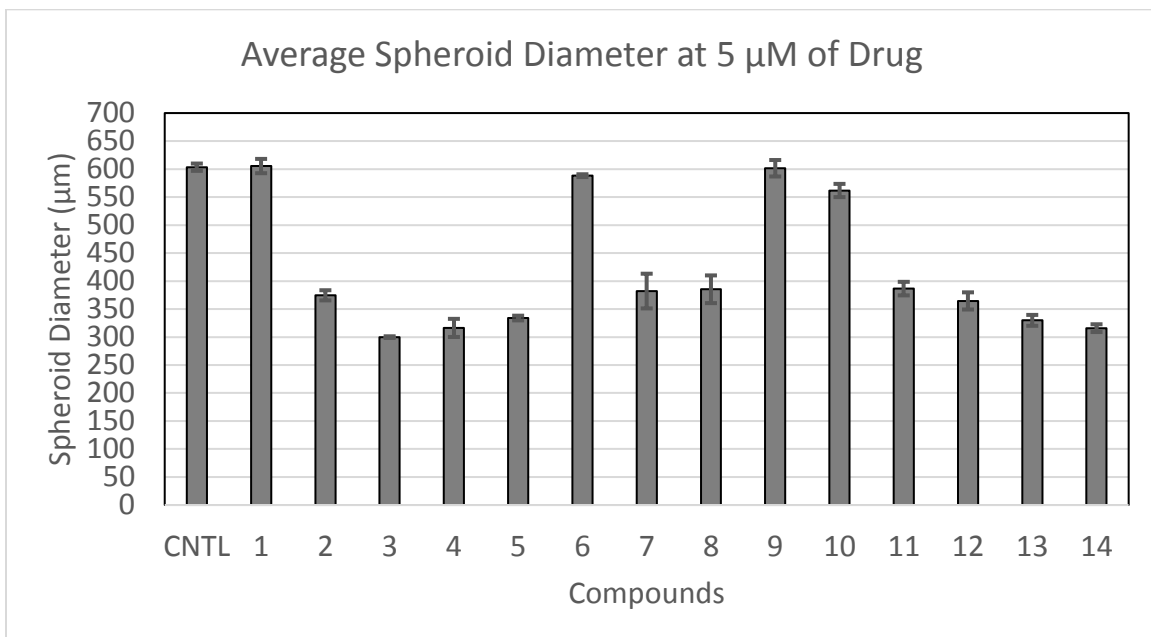


- The dose-response results showed that some drugs generated great dose response, some drugs inhibited growth at every concentration, and some drug did not have any effect



3. Endpoint results of MCTS for control and treated sample

- The endpoint results showed that drug compounds 2, 3, 4, 5, 7, 8, 11, 12, 13, and 14 inhibited the growth of U87MG MCTS
- Drug compounds 1, 6, 9, and 10 did not inhibit growth



Conclusion

- Using the 384-well U-bottom ULA plates, we successfully captured images of U87MG MCTS and analyzed the data using the Celigo imaging cytometer
- The entire 384-well plate was imaged in ~4 min. The short scan time significantly increases the throughput during an experiment that has multiple plates
- After the drug treatments, spheroid diameters were measured and automatically reported by the Celigo software
- No additional software was required for image analysis of MCTS diameters