

Assay Name: Label-free Tumor Spheroid Growth Inhibition

Assay ID: Celigo_03_0001



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Experiment: Label-free Tumor Spheroid Growth Inhibition

Purpose	To provide an automated solution that can provide images of multicellular tumor spheroids (MCTS) and report MCTS diameter in a 96-well format
Current Method(s)	Microscopy
Target Cell Type	NCI-H460, MiaPaca
Experiment Plan	Scan two plates daily to measure the size of multicellular tumor spheroids under different drug concentrations in hypoxic or normoxic conditions
Hypothesis	Using the bright field imaging, the Celigo will rapidly provide multicellular tumor spheroid images and diameters of treated MCTS in a 96-well plate

Celigo Setup

Plate Type	Corning™ CLS70007 (ULA plate) - 96 well plate
Scan Channels	Bright field
Resolution	3 micron/pixel
Scan Area	Whole well
Analysis Method	Tumorsphere 1
Scan Frequency	Daily
Scan Time	3 minutes

Assay Protocol and Plate Setup

Goal:

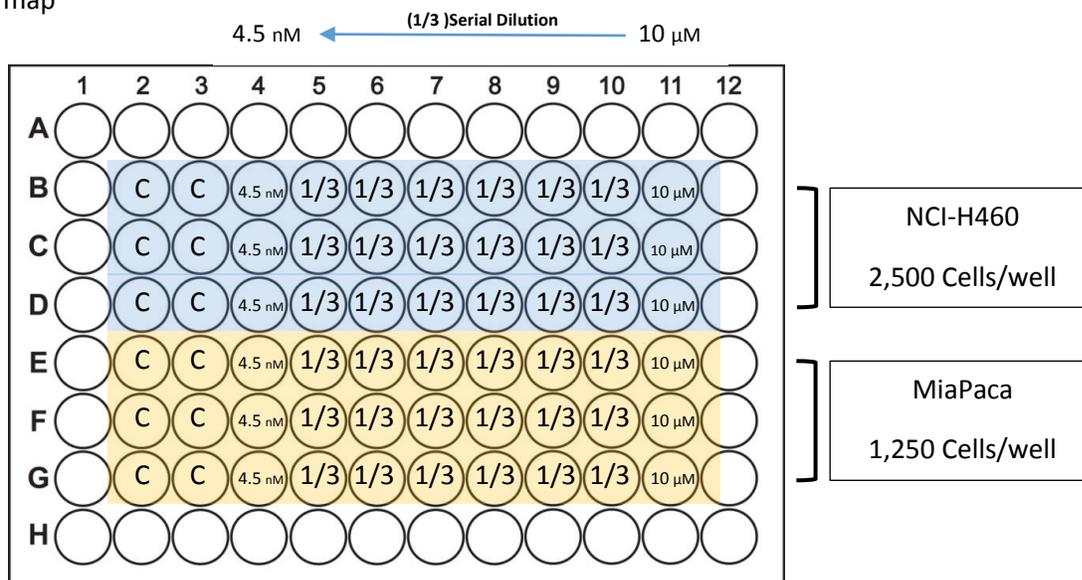
Image and measure the diameter of multicellular tumor spheroids (MTSs) at different drug concentrations that were incubated at either normoxic or hypoxic conditions. Ultimately determine, if incubation conditions (normoxic, and hypoxic) play a role in the reduction of MTS size when treated at different drug concentrations.

Protocol

Cell Preparation

1. Seeded 2,500 NCI-H460 cells/well in a ULA 96-well plates (see plate map below)
2. Seeded 1,250 MiaPaca cells/well in a ULA 96-well plates (see plate map below)
3. On day 4, treated formed MTSs with drug X or vehicle control
 - a. Drug X in [μM] was serially diluted 1/3 from 10 μM to 0.0045 μM concentration
4. Placed one plate in hypoxic conditions and one plate at standard growth conditions

Plate map



Data Collection

1. On day 4, after adding the drug at different concentrations, the plates were imaged and data collected for both the hypoxia and normoxia plates
2. The plates were again imaged 4 days later. Post treatment, one plate was at hypoxic conditions for 4 days and the second plate was normoxic conditions
3. The plates were scanned in Celigo using Tumorsphere 1 (bright field) assay

Data Analysis

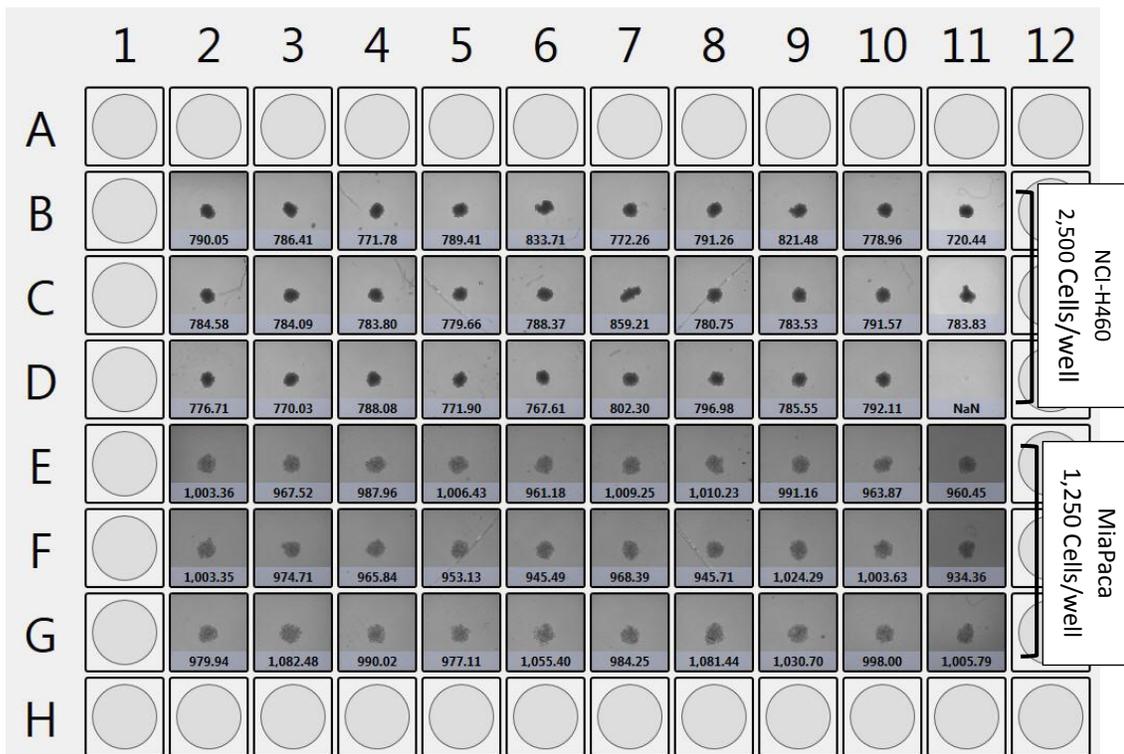
- The images were analyzed by using Tumorsphere 1 application to identify the MCTS in the well
- The well mask was reduced to the 95th percentile to eliminate edge effects
- Several wells had multiple multicellular tumor spheroids. In those instances, each spheroid was identified by the Celigo software

Results

1. NCI-H460 multicellular tumor spheroids showed a noticeable decrease in spheroid size at high drug concentration

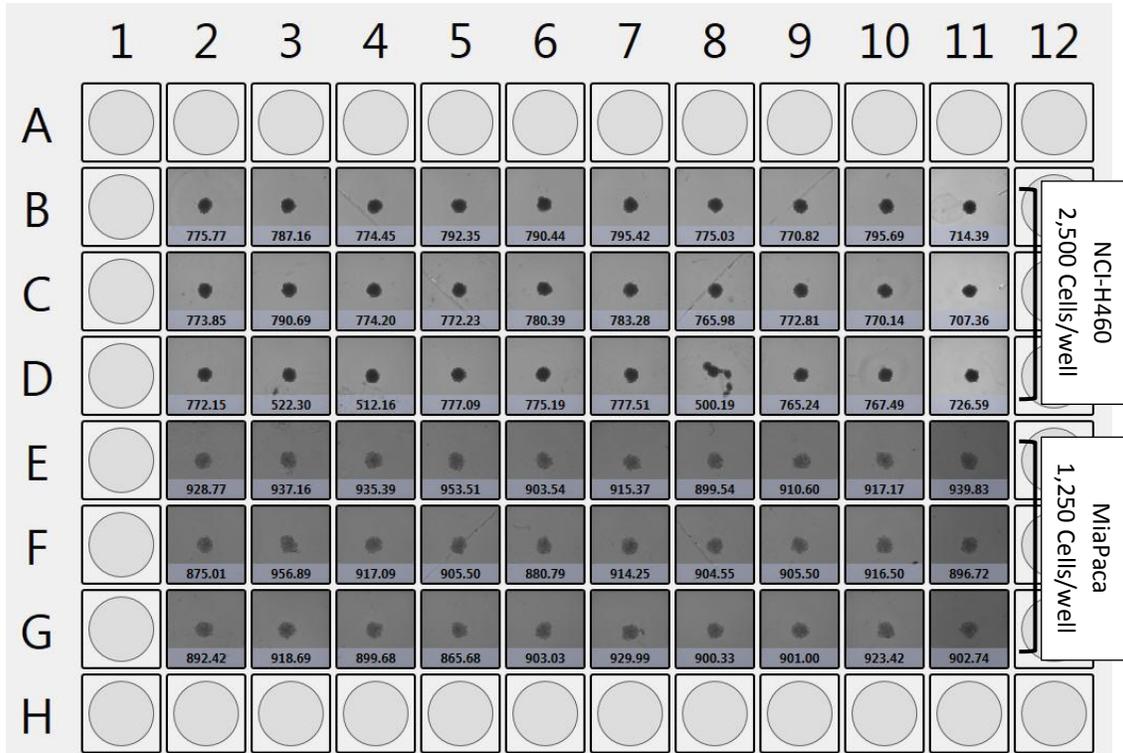
- NCI-H460 multicellular tumor spheroids showed a decrease in spheroid size from 780 μm at 3 μM drug concentration to 750 μm at normoxic and 720 μm at hypoxic conditions at 10 μM .
- MiaPaca multicellular tumor spheroids showed no change in spheroid diameter between hypoxic and normoxic conditions for control or drug treated samples.
- Image and graphed data below represents NCI-H460 and MiaPaca multicellular tumor spheroids treated with Drug X in a dose dependednt manner and incubated at normoxic and hypoxic environmbet for four days before imaging on the Celgio.

Day 4 post treatment with Drug X at **Normoxia** NCI-H640 at 2,500 and MiaPaca at 1,250 cells/well



- The entire plate was scanned in 3 minutes and bright field thumb nails are shown for each well.
- At the bottom of the well picture is the Celigo measured diameter in (microns) for each identified spheroid.

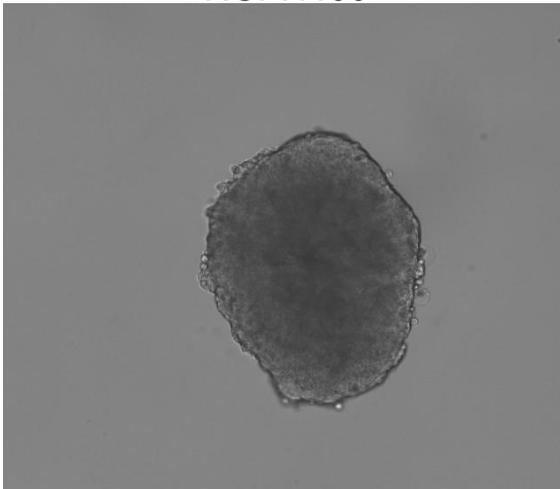
Day 4 post treatment with Drug X at **Hypoxia** NCI-H640 at 2,500 and MiaPaca at 1,250 cells/well



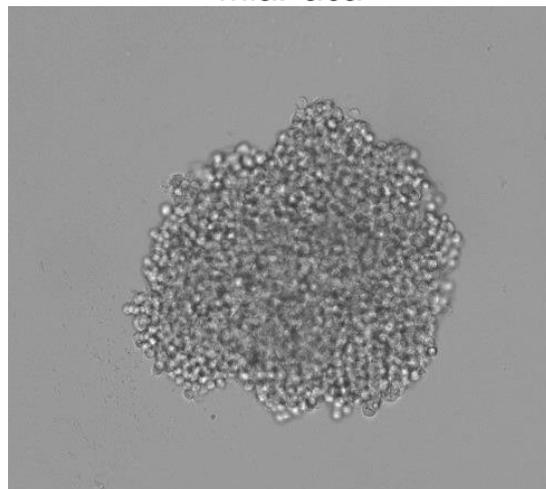
- The entire plate was scanned in 3 minutes and bright field thumb nails are shown for each well.
- At the bottom of the well picture is the Celigo measured diameter in (microns) for each identified spheroid.

Celigo captured representative bright field images of non-drug treated NCI-H460 and MiaPaca multicellular tumor spheroids at normoxic condition on day 0

NCI-H460

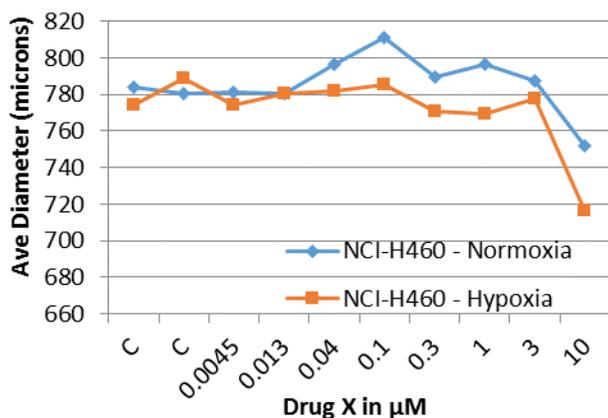


MiaPaca

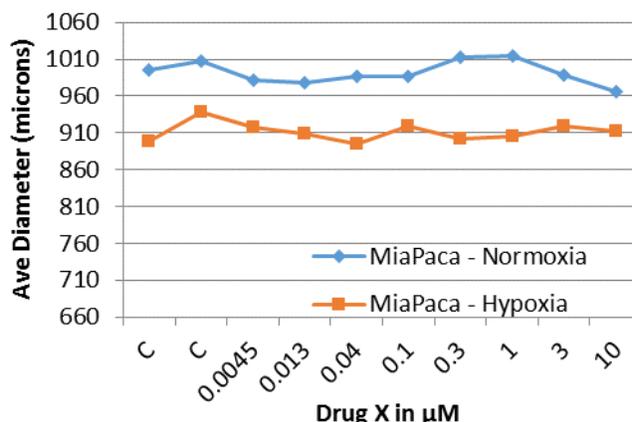


NCI-H460 and MiaPaca multicellular tumor spheroid measurements four days after drug treatment and incubation at normoxic and hypoxic conditions

NCI-H460 cells treated with Drug X at Normoxia vs Hypoxia



MiaPaca cells treated with Drug X at Normoxia vs Hypoxia



- NCI-H460 and MiaPaca were plated at 2,500 and 1,250 cells per well treated with a serially diluted Drug X and incubated for 4 days at either hypoxic or normoxic conditions
- NCI-H460 multicellular tumor spheroids showed a noticeable decrease in spheroid size at 10 μM drug concentration at both normoxic and hypoxic conditions.
- The sizes of MiaPaca multicellular tumor spheroids remained the same throughout the experiment at both normoxic and hypoxic conditions as well as at different drug concentrations.

Conclusion

- Using the 96-well U-bottom ULA plates, we successfully captured images of MCTS and analyzed the data using the Celigo instrument.
- The entire 96-well plate was imaged in 3 minutes. The short scan time significantly increases the throughput during an experiment that has multiple plates.
- After 4 days of drug treatment and incubation at either normoxic or hypoxic conditions, spheroid diameters were measured and automatically reported by the Celigo software. No additional software is required for image processing of multicellular tumor spheroid diameters