Assay Name: Counting of Patient-Derived IDC Organoids

Assay ID: Celigo_03_0006

Table of Contents

Experiment: Counting of Patient-Derived IDC Organoids....2
Celigo Setup....2
Assay Protocol and Plate Setup....3
Results....4
1. Celigo-captured bright field images of organoids....4
2. PDO counting results....5
Conclusion....5

Experiment: Counting of Patient-Derived IDC Organoids

Purpose	To image and count whole well populations of organoids in a rapid manner without having to take multiple, time consuming Z-stacked images
Current Method(s)	Manually, or Confocal Microscopy
Target Cell Type	Patient-derived intestinal differentiated cells (IDC) organoids
Experiment Plan	Organoids are cultivated in 6-well plates embedded in matrigel and imaged in bright field
Hypothesis	Celigo will be able to perform rapid, whole-well imaging and analysis of organoids in a high throughput manner

Celigo Setup

Plate Type	Corning 12-well microplate
Scan Channels	Bright field
Resolution	1 µm/pixel
Scan Area	Whole well
Analysis Method	Tumorsphere 1
Scan Frequency	Once
Scan Time	~8 min

Assay Protocol and Plate Setup

Goal

To image and count whole well populations of organoids in a rapid manner without having to take multiple, time consuming Z-stacked images

Protocol

IDC organoids preparation

1. IDC organoids were cultivated in 12-well microplates and embedded in matrigel following the plate map below
2. Next, the organoids were grown for 20 days from IDC progenitor cells to form the organoids
3. The organoids were also treated with H_2O_2 at time = 0, to inhibit the growth of the organoids

	1	2	3	4
A	Control	H_2O_2		
B	Control	H_2O_2		
C	Control	Matrigel only, no cells		

Data Collection

1. The organoids images were captured using bright field imaging
    a. One of the Control wells was used to focus the organoids to set the focus Z-location
    b. The Celigo scan required approximately 5 min

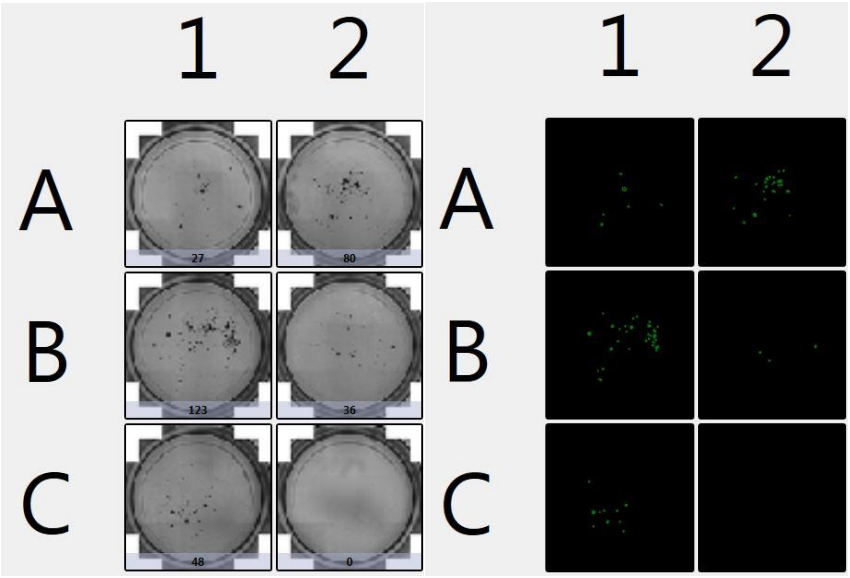
Data Analysis

- The organoids were counted using Celigo application Tumorsphere 1
- The IDC organoids counts were generated, as well as the size of the organoids

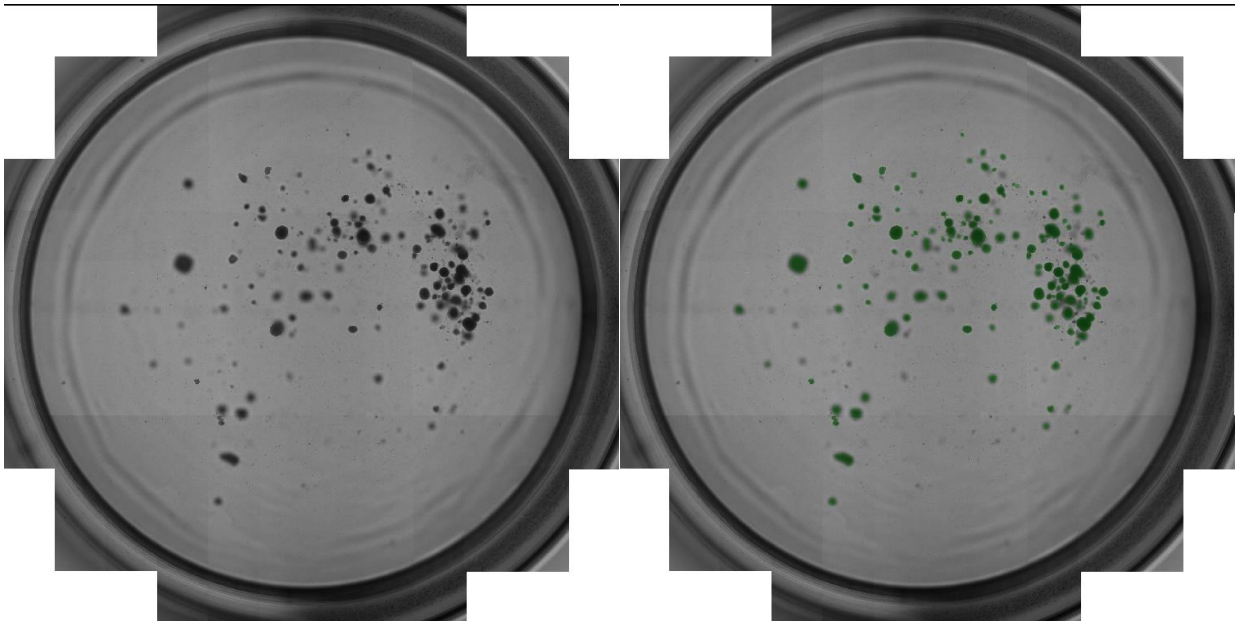
Results

1. Celigo-captured bright field images of organoids

- Celigo captured bright field images in 12-well plates containing organoids in matrigel
- Below is an example whole plate image of the captured data

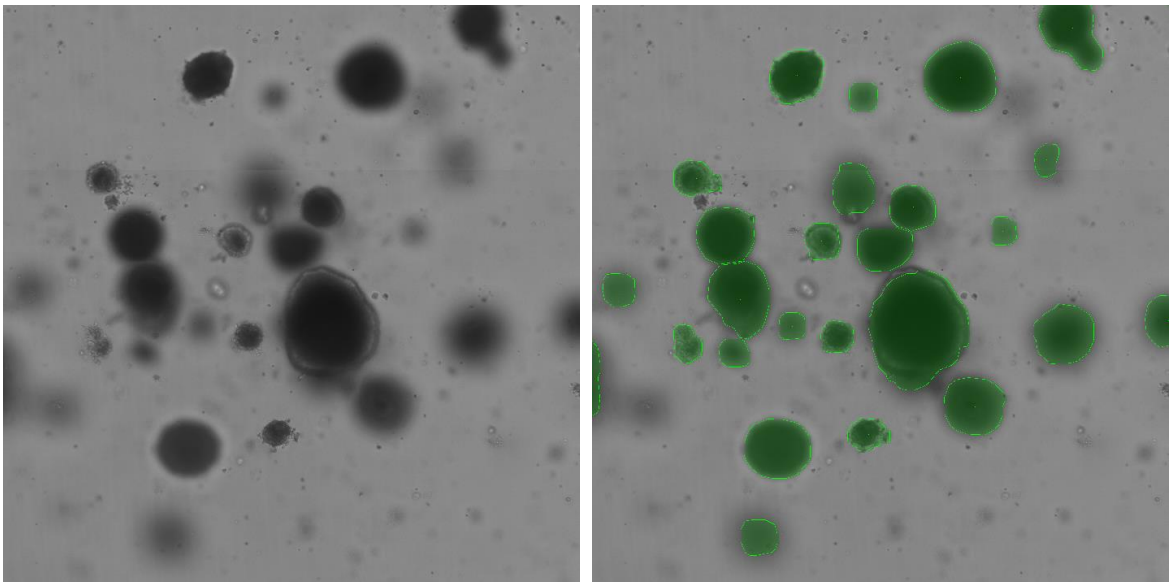


- Celigo captured high resolution images in bright field that can be zoomed-in on from whole well (below)



- The Celigo software accurately counted organoids and declustered them into individual organoids

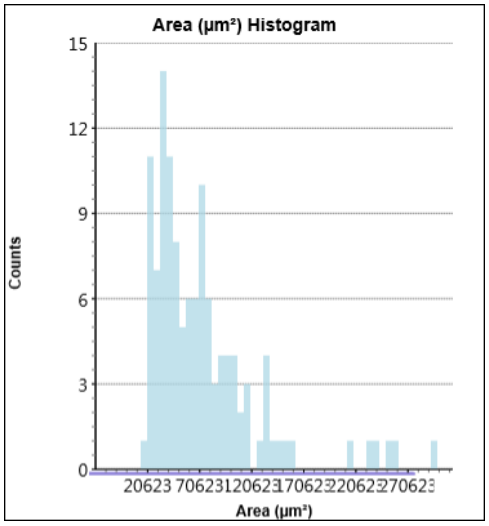
Zoomed in Image **Zoomed in Filled Image**



2. PDO Counting Results

- Celigo was used to count all the organoids in the 12-well plate
- The counted results and the size analysis of organoids are shown below

Tumorsphere Count	1	2
A	27	80
B	123	36
C	48	0
AVG Diameter (µm)	1	2
A	282.5625	311.4015
B	305.0027	261.4917
C	290.1047	NaN



Conclusion

- Celigo was able to count the number of organoids directly in the wells, and measured the diameter of each organoid
- Overall, there was no clear difference between the control and H_2O_2 treated samples
- Celigo software was able to decluster organoids in close proximity, to improve the counting accuracy of the current method