Imaging and Analysis of 3D Patient-Derived Organoids Using the Celigo Automated Image Cytometer

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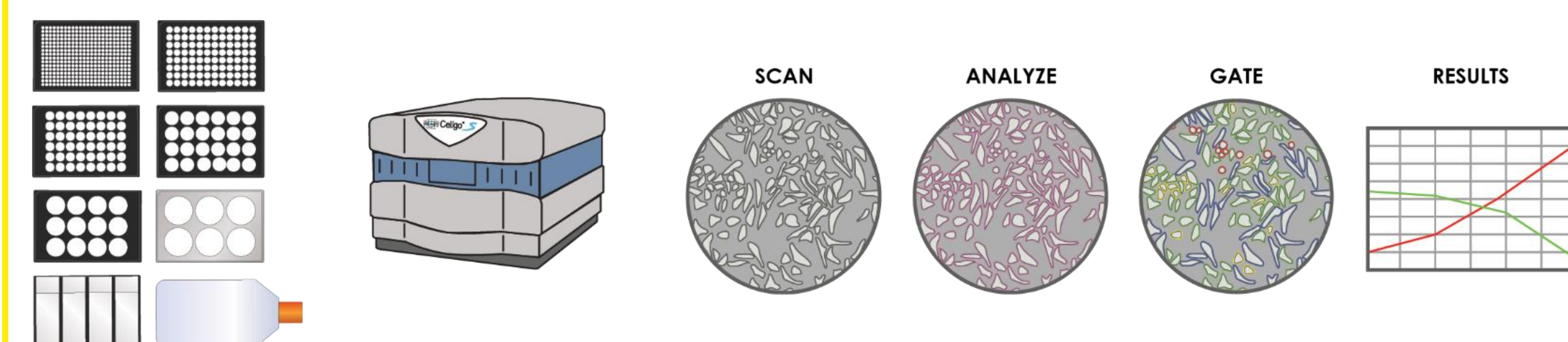
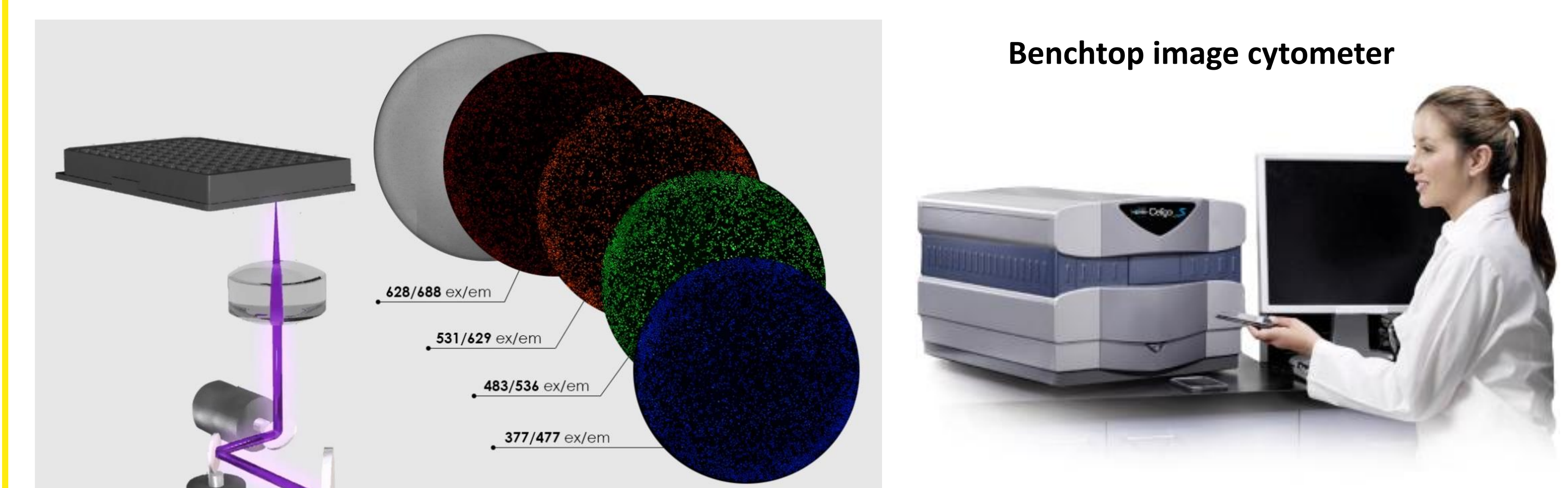
1. ABSTRACT

Imaging and analysis of primary organoids has traditionally been challenging due to the nature of organoid culturing as well as limited imaging and analysis platforms that are capable of performing such tasks. Traditionally, isolated progenitor cells, genetically engineered cells or tissue fragments are grown in media supplemented with Matrigel. These suspended cells eventually form organoids that can be used for downstream assays. Because the organoids are suspended in Matrigel the imaging is typically performed using an epi-fluorescent microscope with a mounted camera. The acquired images are then manually processed using CellProfiler or ImageJ. To address these challenges, a novel assay has been developed for imaging and analysis of 3D organoids using the Celigo, an automated micro-well imaging system. Since organoids grow suspended in medium we created a "spin method" that facilitated the partial settling of the primary cells and led to the formation of organoids in a relatively flat focus field. This culturing method coupled with Celigo image cytometer allowed for the capture and automatic analysis of an entire 96-well plate of formed organoids in less than 5 minutes. Because the Celigo is capable of capturing bright field and fluorescent images it provides researches a new tool for measuring the number of organoids per well, the sizes of each organoid as well as detection of fluorescent signals in a single assay. Here we successfully demonstrate how to perform culturing, imaging and analysis of patient derived organoids (PDOs) and murine derived organoids in 12, 24, 96, and 384-well formats using the Nexcelom Celigo image cytometer. This advancement in organoid imaging and data collection is of significant value to the research community because it provides an efficient method of imaging and analysis of organoids where previously one did not exist.

2. DIRECT CELL COUNTING BY CELIGO IMAGING CYTOMETRY

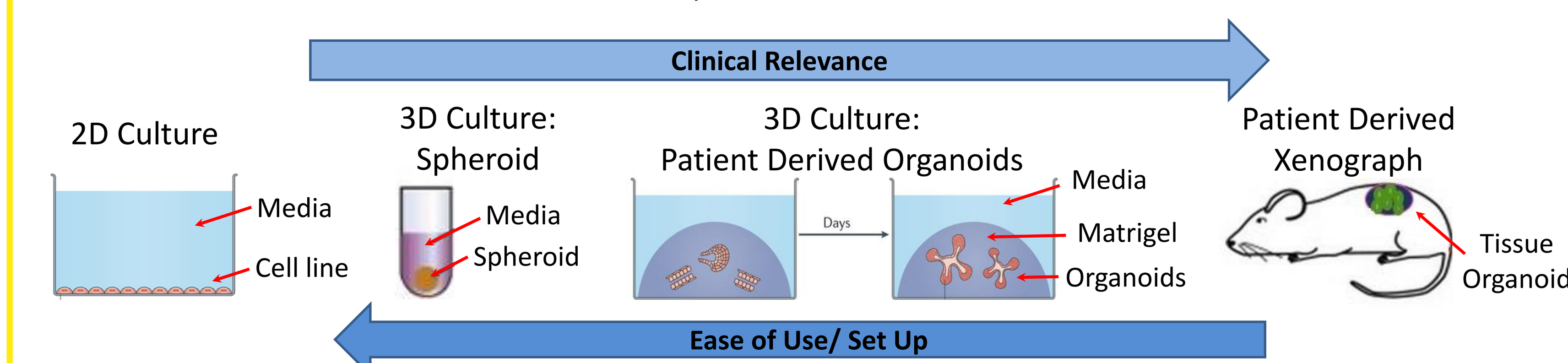




1. Celigo Imaging Cytometer is a plate-based cytometer that can scan the entire well of standard microplate and captures images using bright-field and 4 fluorescent channels
2. Proprietary optical design enables uniform illumination and consistent edge contrast
3. Accurately quantify cells, colonies and 3D spheroids with non-invasive method
4. The measured parameters are used to generate cell proliferation kinetic data, GFP/RFP expression, tumor spheroid size change, DNA cell cycle analysis, apoptosis, and ADCC cytotoxicity results
5. Automatic stitching of multiple fields of view to produce a full resolution image

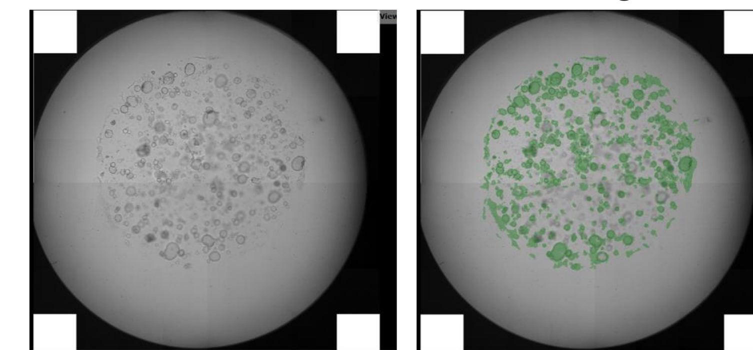
3. TRANSITION FROM 2D TO 3D MODELS WITH CELIGO IMAGE CYTOMETER

- Patient derived organoid models for major solid tumors has proved to be a valuable tool in testing and development of new cancer therapies.
- This functionally relevant system more closely resembles an *in vivo* model compared to 2D culture in its physiology, shape, cell to cell interaction and drug interactions.
- For model development and drug screening the Celigo can image and analyze adherent and suspension cells in 2D culture as well as 3D PDOs and tumor spheroids.



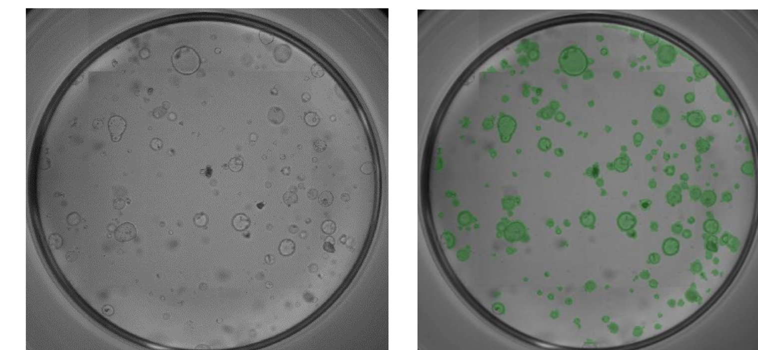
4. DOME METHOD VS CELIGO SPIN METHOD

Traditional Dome Method for Growing PDOs



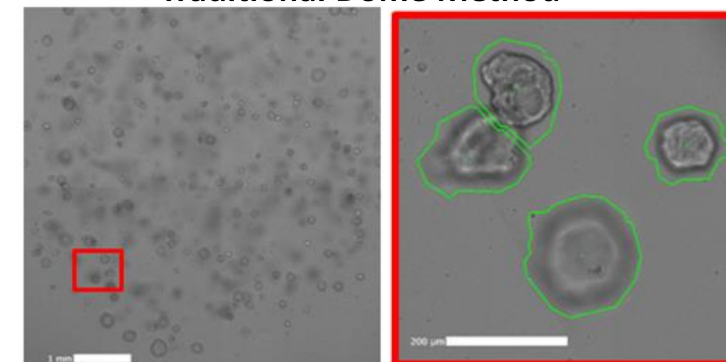
- Celigo captured whole well image of the dome method shows the PDO concentration in the center of the well. Automatic identification and analysis of primary PDOs in the bright field channel is shown by the green pseudo-color.

Celigo Spin Method for Growing PDOs



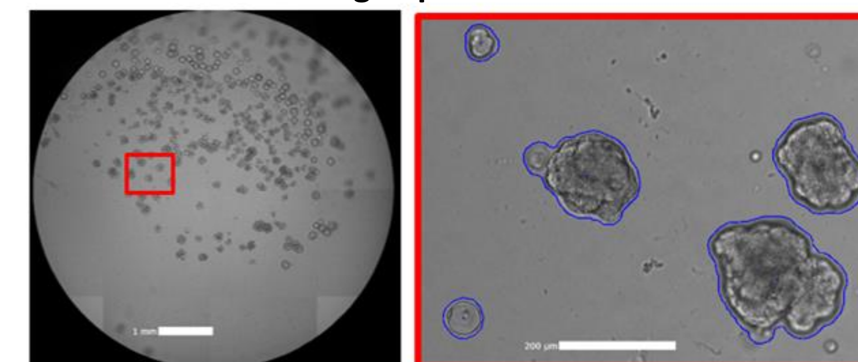
- Hi-resolution whole-well image of a well from a 24 well plate was acquired and analyzed by the Celigo image cytometer. The bright field image shows PDOs in a single plane allowing for accurate PDO identification as shown by the green pseudo-color

Traditional Dome Method



- Growing organoids in a dome method leads to the formation of the organoids at different depths within the dome. This leads to challenging manual and automated data gathering and analysis.

Celigo Spin Method



- Centrifugation prior to Matrigel solidification provides PDO cultures that are in the same focal plane. This allows for consistent imaging, requires fewer cells for seeding and provides the capability to scale for a higher throughput.

5. MUTI-PARAMETRIC ANALYSIS OF PRIMARY PDOs

- Primary murine prostate cells were seeded into a 96 well plate at a density of 1000 cells per well in 15 µl of Matrigel. The plate was centrifuged at 400 *g* and 4°C for 2 mins. The Matrigel was allowed to solidify and cell media with various treatments was added to each well.
- Subsequently the plate was imaged at multiple time points to determine the size and number of formed organoids.

Organoid formation ratio

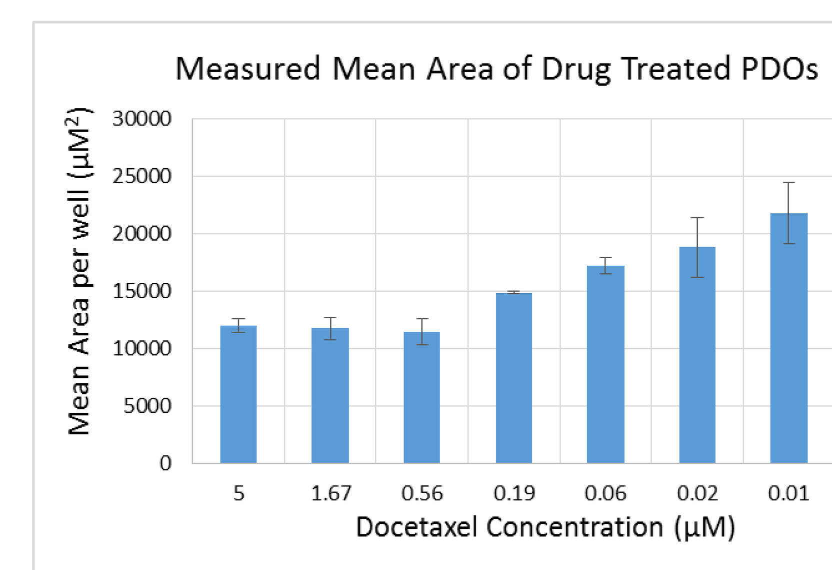
EGF	3	4	5	6
0 ng	↓ 6%	↓ 5%	↓ 25%	↓ 30%
5 ng	↑ 45%	↑ 53%	↑ 59%	↑ 64%
50 ng	↑ 43%	↑ 49%	↑ 54%	↑ 59%
	Drug 1 in duplicate		Drug 2 in duplicate	

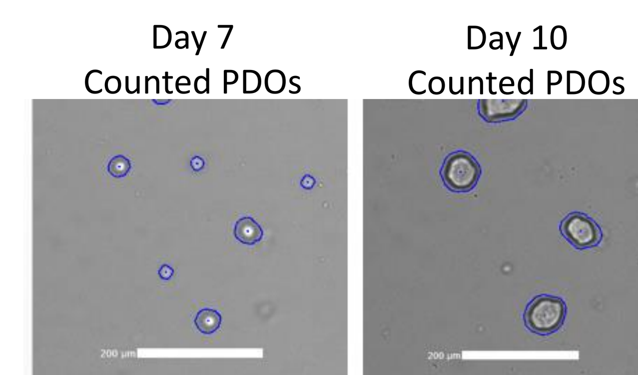
- Organoid formation ratio was reported as a percent difference in the number formed PDOs between day 7 and day 10.
- We examined the benefit of epidermal growth factor (EGF) on the formation of PDOs in the presence of two drug treatments.
- Each drug was plated in duplicate. We observed that EGF was more beneficial in the presence of drug 2 compared to drug 1.

Fold change in mean area (µm2)

EGF	3	4	5	6
0 ng	↓ 2.36	↓ 2.67	↓ 7.82	↓ 7.35
5 ng	⇒ 4.58	⇒ 4.72	⇒ 9.61	⇒ 10.15
50 ng	↑ 7.29	↑ 8.32	↑ 13.96	↑ 11.86
	Drug 1 in duplicate		Drug 2 in duplicate	

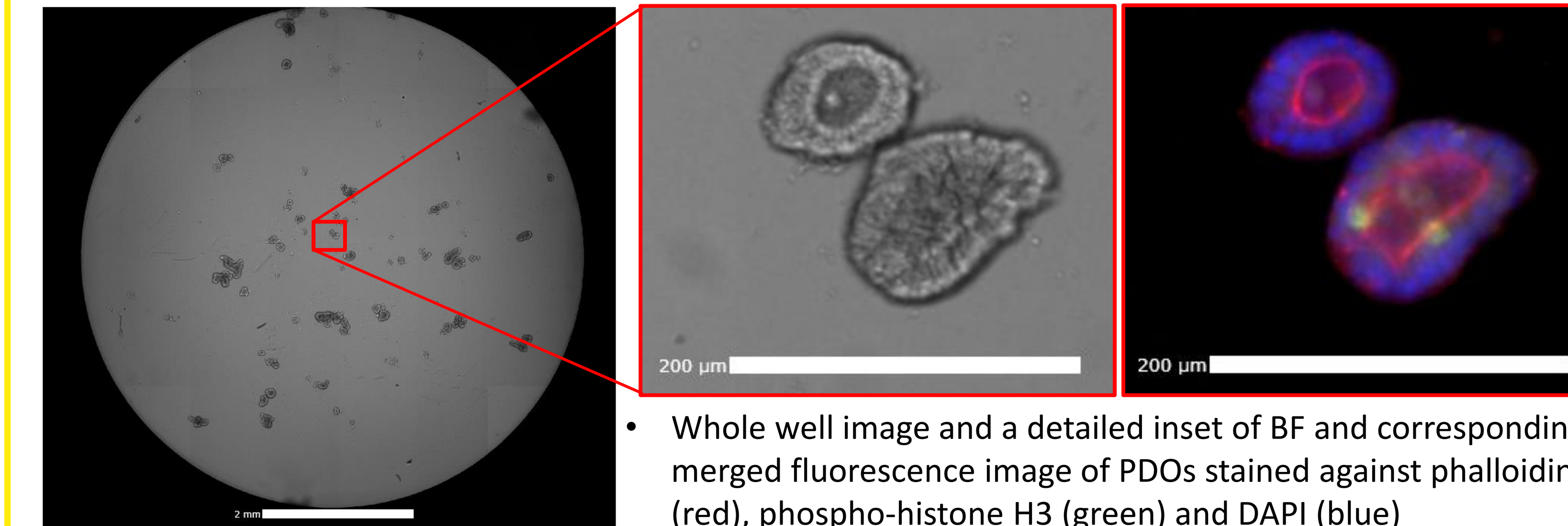
- Additionally, we calculated the fold change in the mean area of the formed organoids.
- The mean area showed the greatest increase in organoids treated with drug 2 and in the presence of 50 ng EGF.



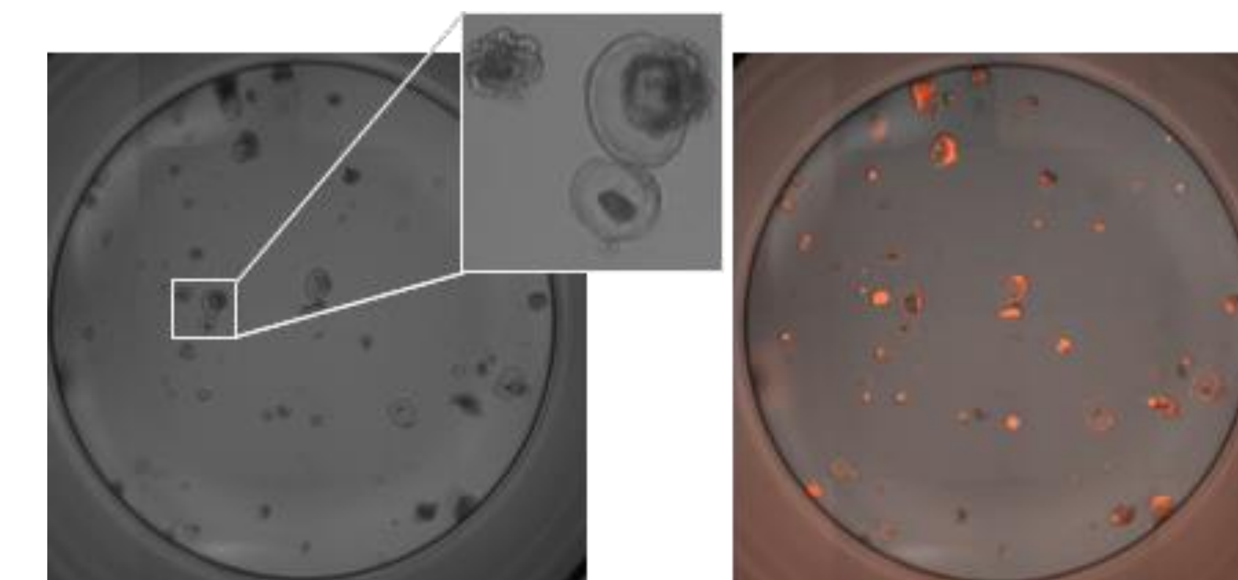


- In a separate experiment PDO's mean area was measured after treatment with docetaxel in a dose dependent manner.
- Organoid size was shown to be drug dependent, although mean area remained the same at the three highest doses.

6. MULTI-COLOR IMAGING OF 3D PDOs

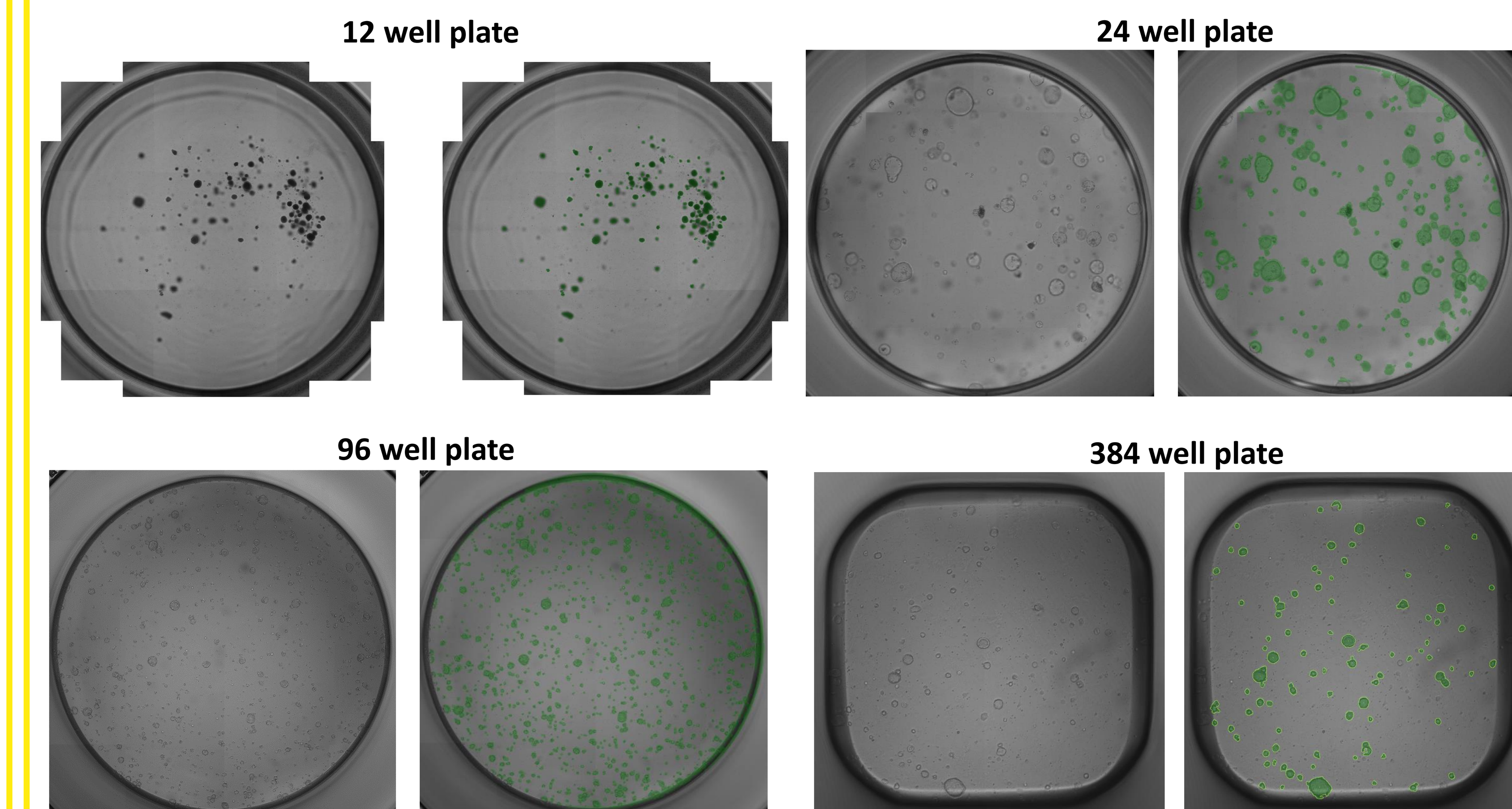


- Whole well image and a detailed inset of BF and corresponding merged fluorescence image of PDOs stained against phalloidin (red), phospho-histone H3 (green) and DAPI (blue)



- RFP positive PDO's generated from 2000 mouse pancreatic cells and imaged 3 day post seeding.
- Whole well bright field (BF) and RFP imaged were collected. An inset of bright field PDO is showing the formation of different PDO from the same culture
- Whole-well BF and RFP merged image shows a wide distribution of the RFP signal within the PDOs

7. FAST WHOLE WELL IMAGING AND ANALYSIS: 12 WELL – 384 WELL PLATES



- The ability to quickly (~3 mins/96 well plate) acquire whole well images and analyze (counted PDOs shown in green pseudo-color) for any plate size drastically improves data gathering and analysis.

8. CONCLUSION

- Celigo image cytometer is a versatile tool capable of whole-well imaging and analysis of 2D and 3D cultures in bright field and 4 fluorescent channels.
- Celigo successfully images and performs automated analysis based on size, number, area and fluorescent intensity of formed patient derived organoids.

References:

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