SCIENTIFIC POSTER

# Imaging and analysis of 3D patient-derived organoids using the Celigo image cytometer.


## Authors

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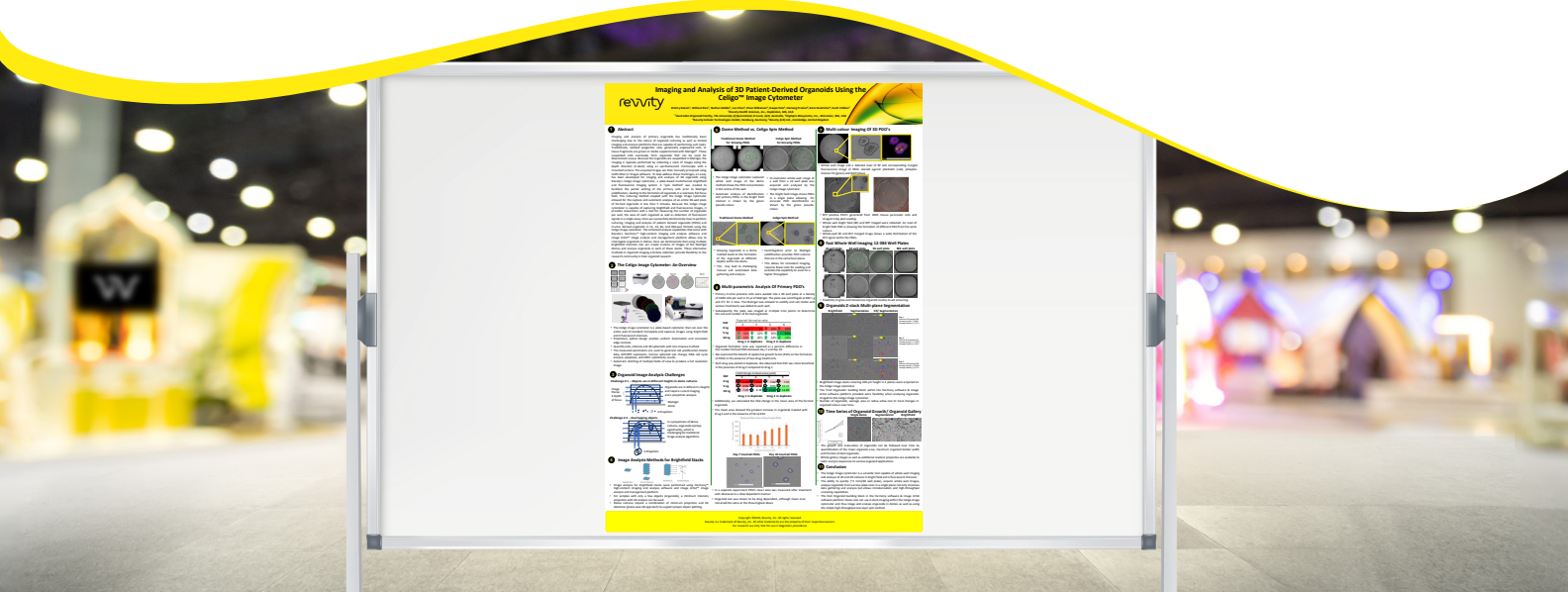
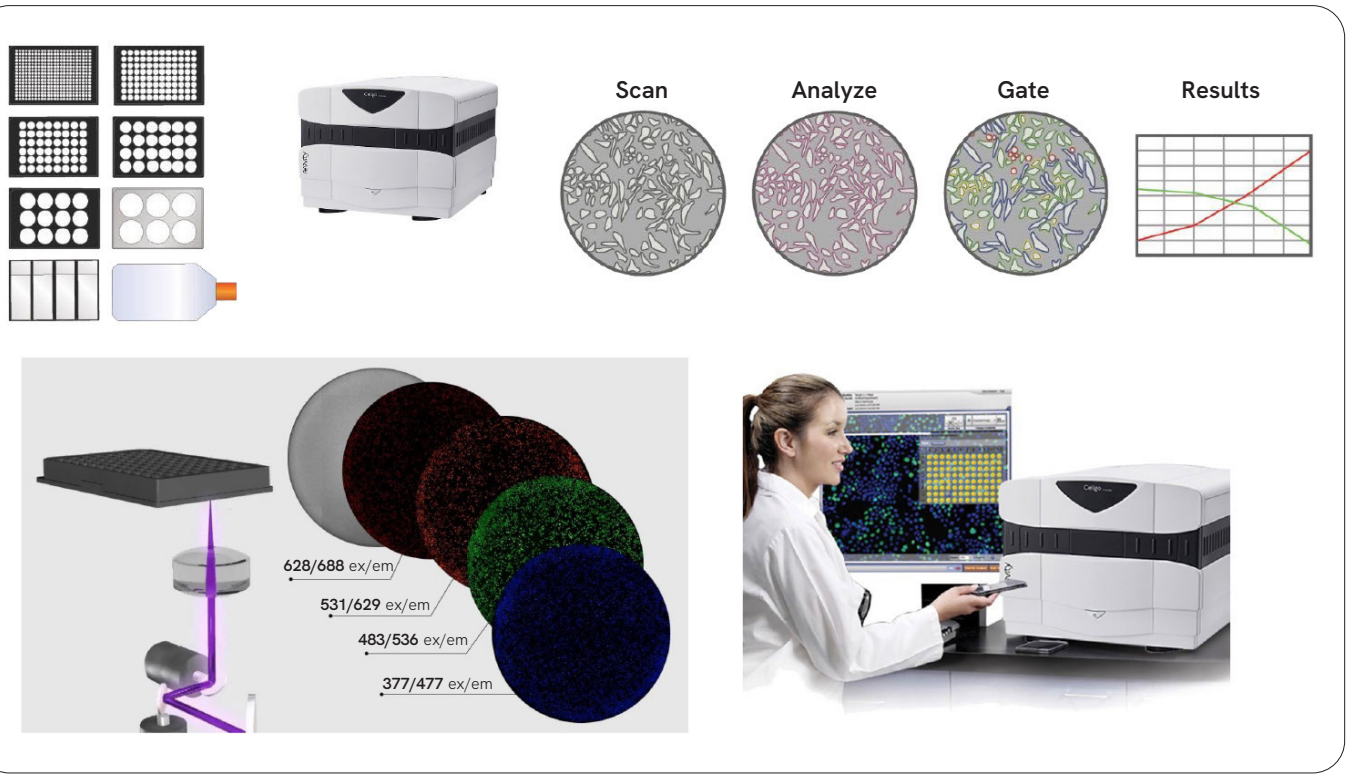
## Abstract

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 by collecting a stack of images along the depth direction (Z-stack) using an epi-fluorescent microscope with a mounted camera. The acquired images are then manually processed using CellProfiler™ or ImageJ software. To help address these challenges, an assay has been developed for imaging and analysis of 3D organoids using Revvity's Celigo™ image cytometer, a plate-based multichannel brightfield and fluorescence imaging system. A "spin method" was created to facilitate the partial settling of the primary cells prior to Matrigel® solidification, leading to the formation of organoids in a relatively flat focus field. This culturing method coupled with the 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 image cytometer is capable of capturing brightfield and fluorescence images, it provides researchers with a 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 Celigo image cytometer. The enhanced

analysis capabilities that come with Revvity's Image Artist™ image analysis and management platform allows one to interrogate organoids in domes. Here we demonstrate that using multiple brightfield channels one can create Z-stacks of images of the Matrigel® domes and analyse organoids in each of these stacks. These alternative methods in organoid imaging and data collection provide flexibility to the research community in their organoid research.

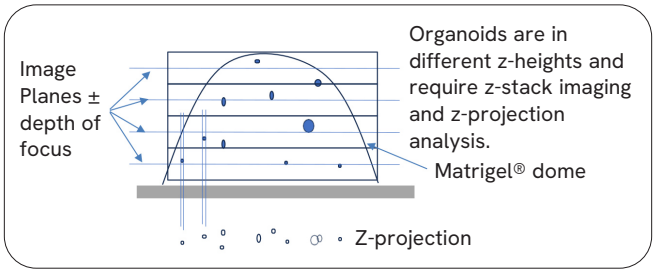
## The Celigo image cytometer: an overview



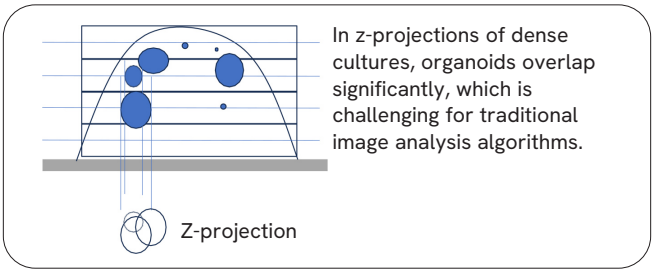

- The Celigo image cytometer is a plate-based cytometer that can scan the entire well of standard microplate and captures images using brightfield and 4 fluorescent channels.
- Proprietary optical design enables uniform illumination and consistent edge contrast.
- Quantify cells, colonies and 3D spheroids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

## Organoid image analysis challenges

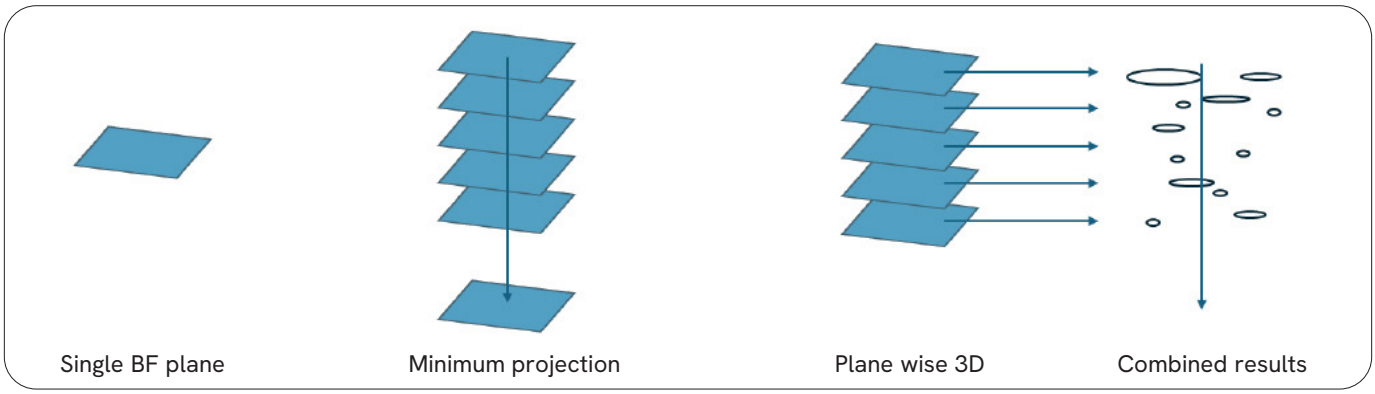
**Challenge # 1 – Objects are in different heights in dome cultures**




**Challenge # 2 – Overlapping objects**



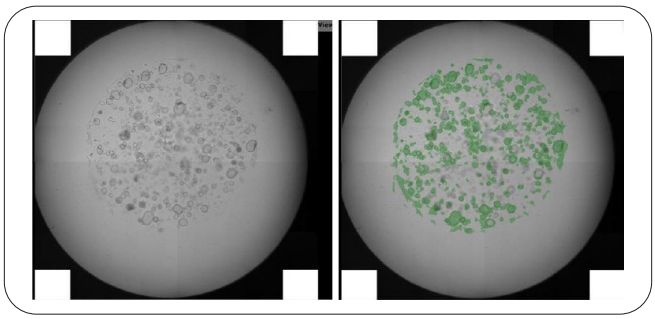

## Image analysis methods for brightfield stacks




- Image analysis for brightfield stacks were performed using Image Artist image analysis and management platform.
- For samples with only a few objects (organoids), a minimum intensity projection with 2D analysis can be used.
- Dense cultures require a combination of minimum projection and 3D detection (plane-wise 3D approach) to support proper object splitting.

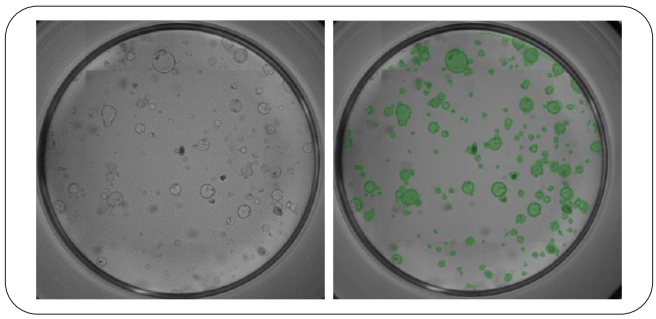
## Dome method vs. Celigo spin method

**Traditional dome method for growing PDOs**



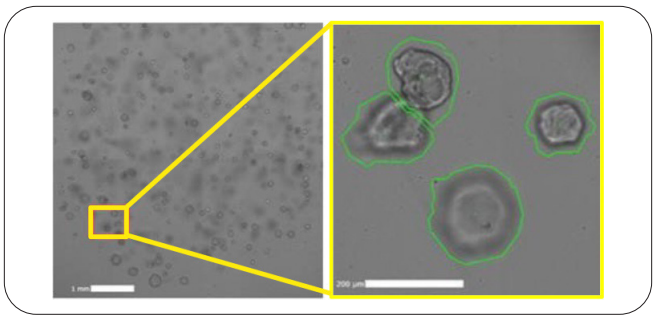
- The Celigo image cytometer captured whole well image of the dome method shows the PDO concentration in the centre of the well.
- Automatic analysis of identification and primary PDOs in the brightfield channel is shown by the green pseudo-colour.

**Celigo spin method for growing PDOs**



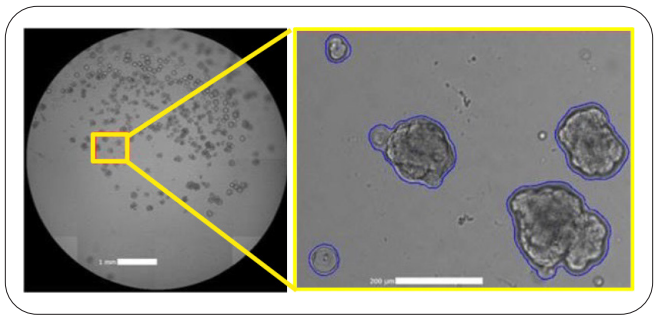
- Hi-resolution whole-well image of a well from a 24 well plate was acquired and analysed by the Celigo image cytometer.
- The brightfield image shows PDOs in a single plane allowing for accurate PDO identification as shown by the green pseudo-colour.

**Traditional dome method**



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

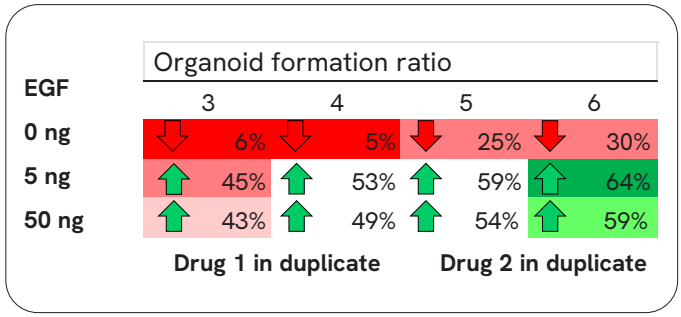
**The Celigo image cytometer spin method**



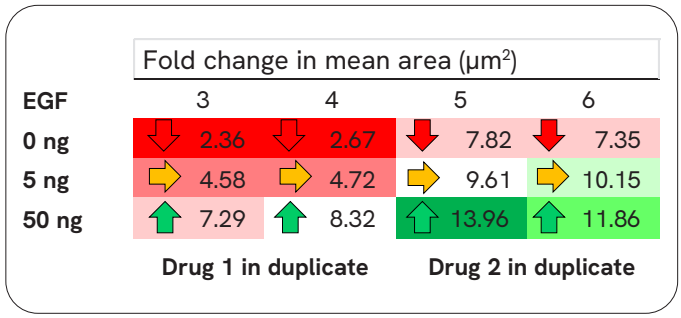
- Centrifugation prior to Matrigel® solidification provides PDO cultures that are in the same focal plane.
- This allows for consistent imaging, requires fewer cells for seeding and provides the capability to scale for a higher throughput.

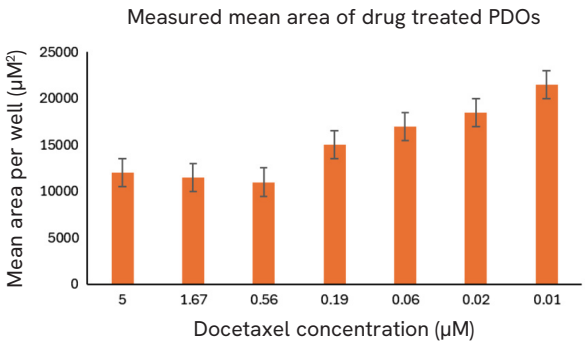
## Multi-parametric analysis of primary PDO's

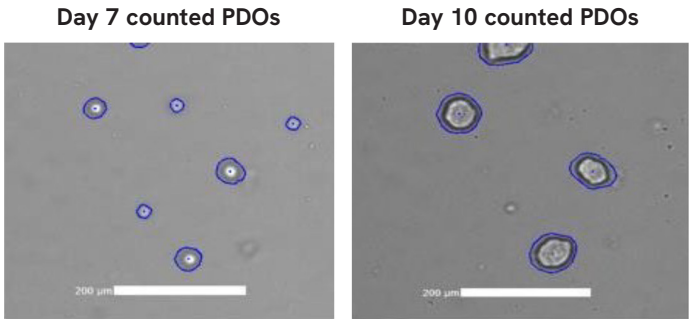
- Primary murine prostate cells were seeded into a 96 well plate at a density of 1000 cells per well in 15 µl of Matrigel®. The plate was centrifuged at 400 × g and 4 °C for 2 mins. The Matrigel® was allowed to solidify and cell media with various treatments was added to each well.
- Subsequently the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



- Organoid formation ratio 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.

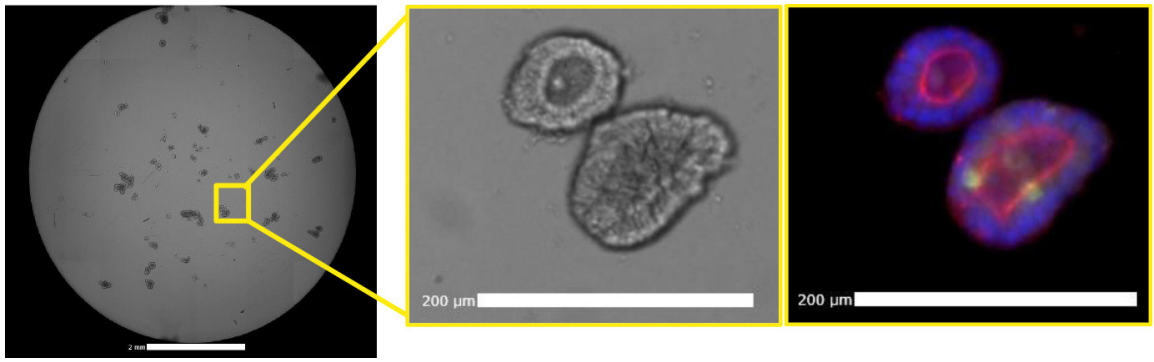


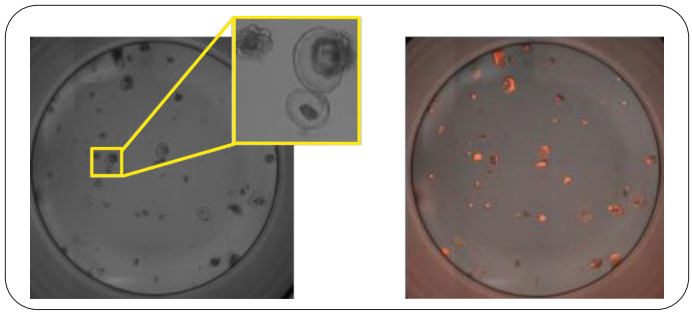

- Additionally, we calculated the fold change in the mean area of the formed organoids.
- The mean area showed the greatest increase in organoids treated with drug 2 and in the presence of 50 ng EGF.
- In a separate experiment PDO's mean area was measured after treatment with docetaxel in a dose dependent manner.
- Organoid size was shown to be drug dependent, although mean area remained the same at the three highest doses.

## Multi-colour imaging of 3D PDO's



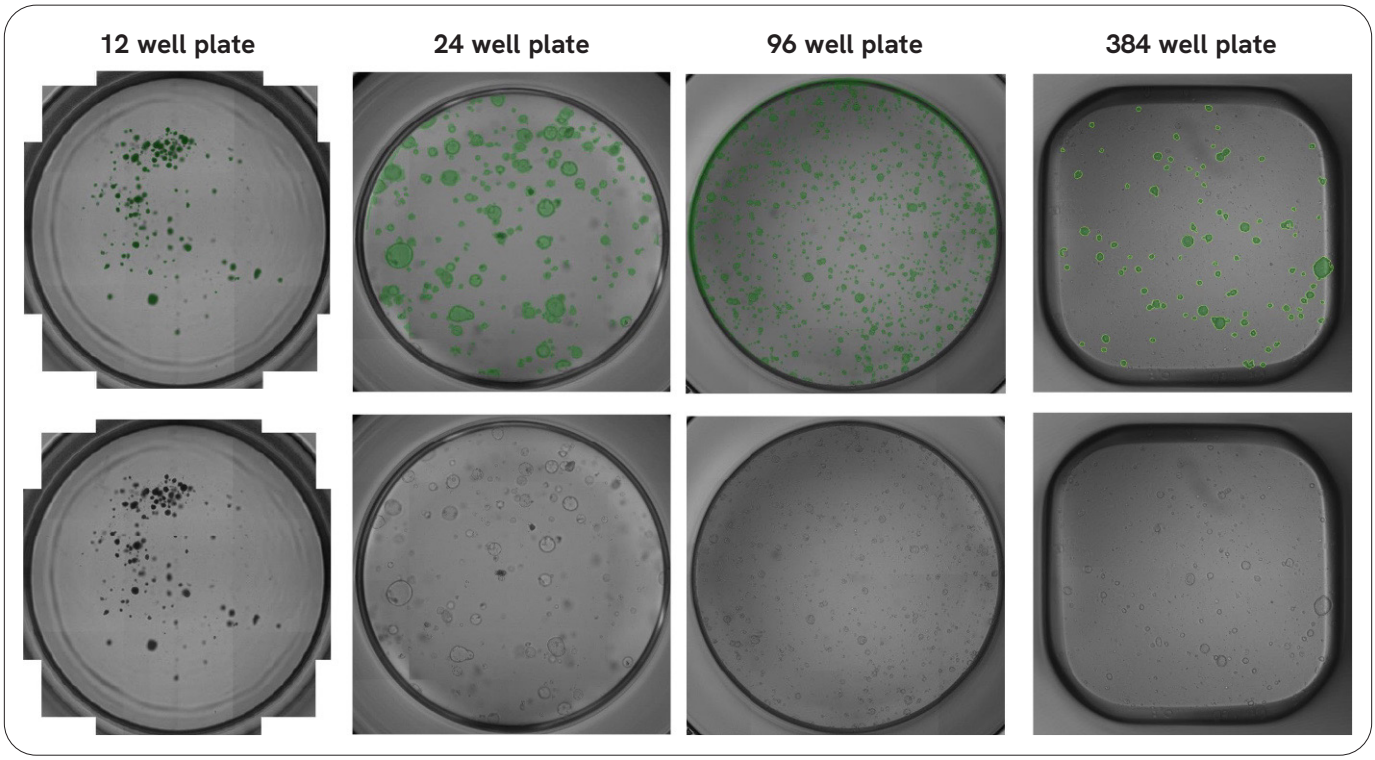

- Whole well image and a detailed inset of BF and corresponding merged fluorescence image of PDOs stained against phalloidin (red), phospho-histone H3 (green) and DAPI (blue).

- RFP positive PDO's generated from 2000 mouse pancreatic cells and imaged 3 day post seeding.
- Whole well brightfield (BF) and RFP imaged were collected. An inset of brightfield 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.



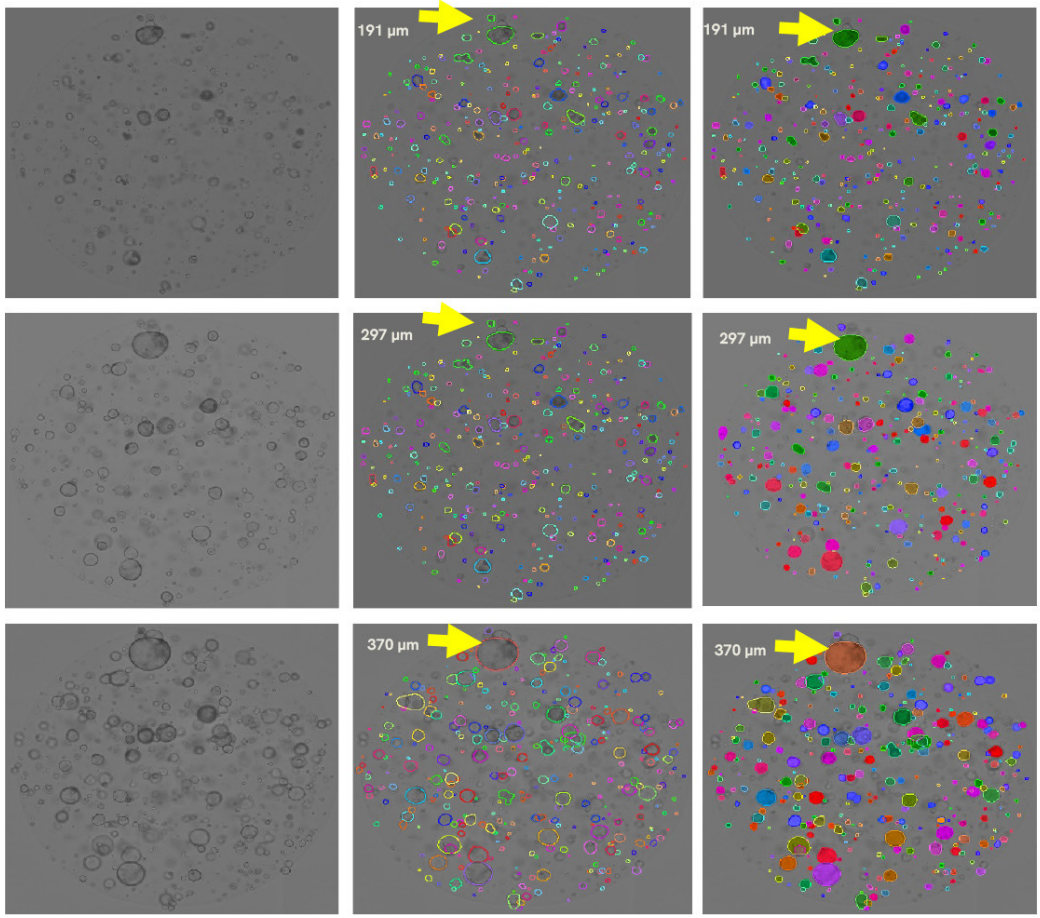
## Fast whole well imaging 12-384 well plates

- Flexibility to grow and miniaturise organoid studies to aid screening.



## Organoids Z-stack multi-plane segmentation

- Brightfield image stacks covering 200 µm height in 4 planes were acquired on the Celigo image cytometer.
- The '*Find Organoids*' building block within the Image Artist software platform provided extra flexibility when analysing organoids imaged on the Celigo image cytometer.
- Number of organoids, average area or radius allow one to track changes in organoid culture over time.



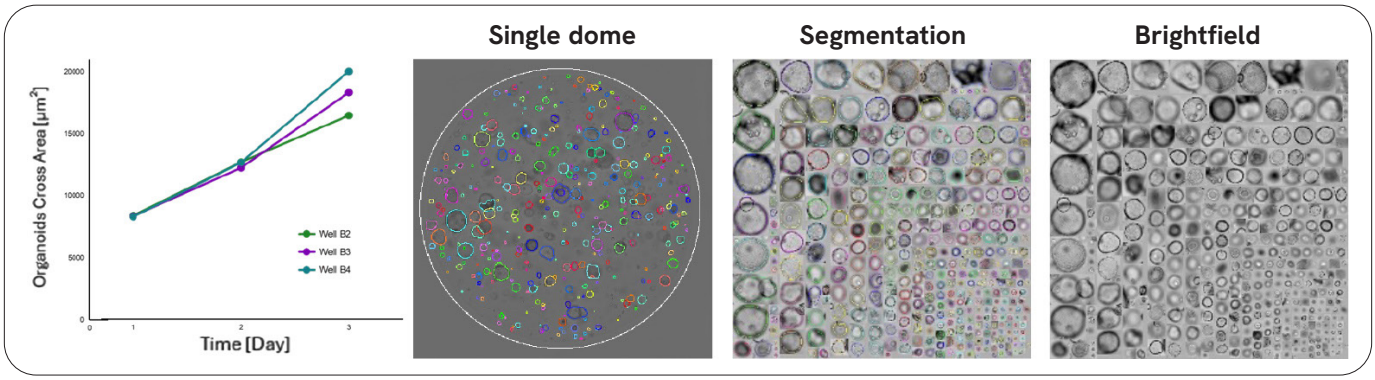

**Day 1**
Number of organoids: 329
Average area [µm$^2$]: 8290
Average radius [µm]: 37.9

**Day 2**
Number of organoids: 313
Average area [µm$^2$]: 12675
Average radius [µm]: 45.5

**Day 3**
Number of organoids: 305
Average area [µm$^2$]: 20038
Average radius [µm]: 57.2

## Time series of organoid growth/organoid gallery

- The growth and maturation of organoids can be followed over time by quantification of the mean organoid area, maximum organoid border width and fraction of dark organoids.
- Whole gallery images as well as additional readout properties are available to tailor analysis sequences to various organoid applications.




## Conclusion

- The Celigo image cytometer is a versatile tool capable of whole-well imaging and analysis of 2D and 3D cultures in brightfield and 4 fluorescent channels.
- The ability to quickly (~3 mins/96 well plate), acquire whole well images, analyze organoids from various plate sizes in a single plane not only improves data gathering and analysis but allows miniaturisation and high-throughput screening capabilities.
- The *Find Organoid* building block in the Image Artist software platform means one can use Z-stack imaging within the Celigo image cytometer and thus image and analyse organoids in domes as well as using the simple high-throughput one layer spin method.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