SCIENTIFIC POSTER

Improved brightfield image segmentation of organoids in Harmony and Image Artist image analysis software.

Authors

Astrid Canivet
David Sagnat

IRSD Organoid Platform, Inserm, University Toulouse, ENVT

Olavi Ollikainen
Kaupo Palo
Hartwig Preckel
Karin Boettcher

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Overview

- Organoid dome cultures require regular quality control imaging in brightfield channel.
- Brightfield images of dome cultures create significant image analysis challenges.
- Here, we present improvements to previous image segmentation methods that lead to detection of approx. 2x more organoids in dome/hydrogel cultures with significantly improved object splitting.

Introduction

Brightfield microscopy serves as the cornerstone for monitoring organoid cultures, offering critical insights into size, morphology, and structural evolution throughout cultivation. In pharmacological research, organoid size variations provide essential readouts for assessing treatment responses and evaluating compound efficacy or toxicity. However, automated brightfield image analysis presents significant technical challenges. Here, we present an enhanced 2.5D image segmentation approach, improving organoid detection and quantification in 3D culture systems.

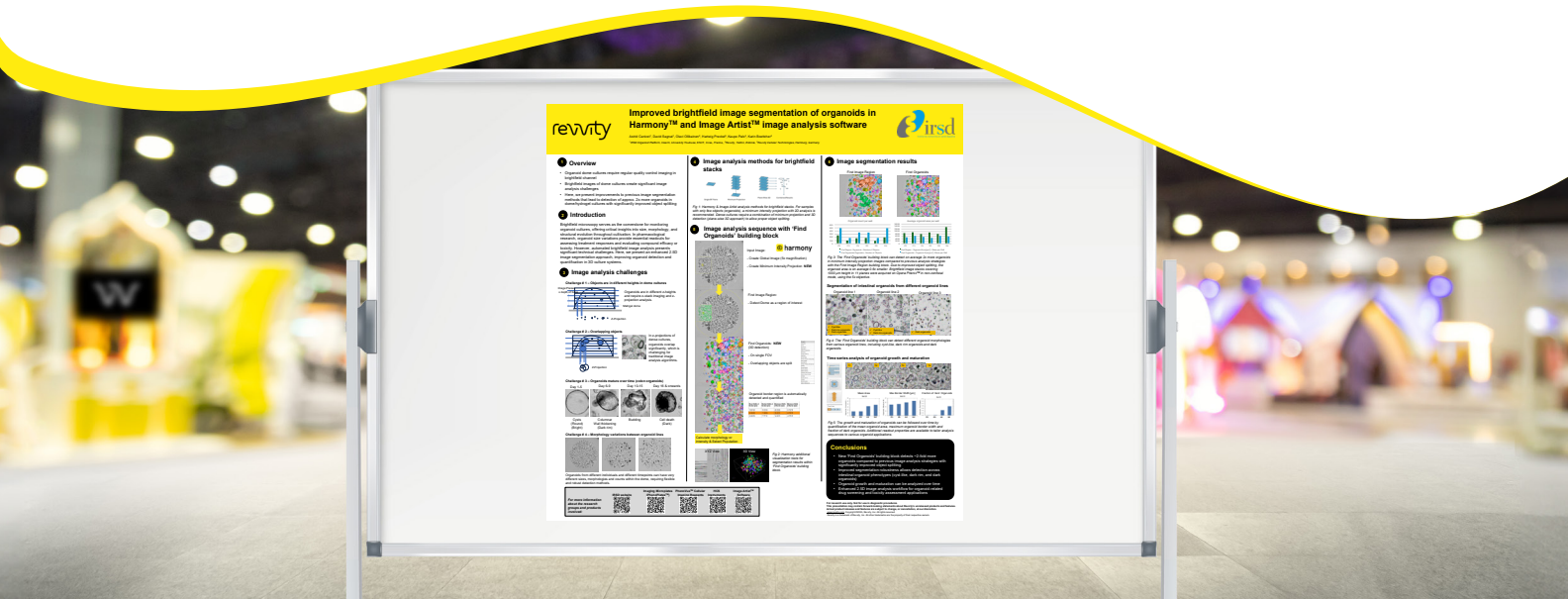
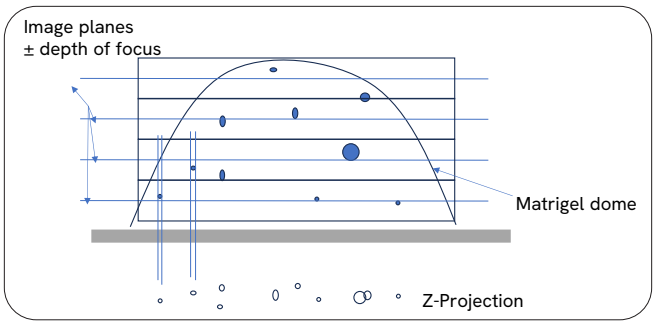


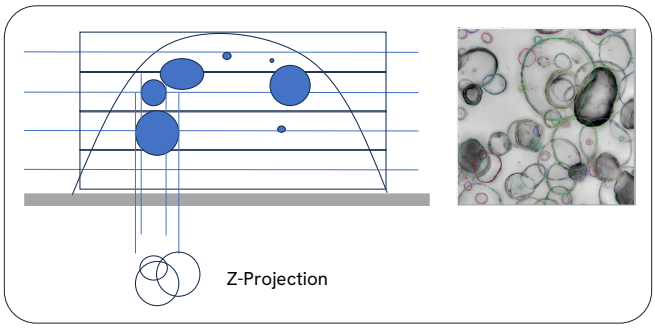
Image analysis challenges

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



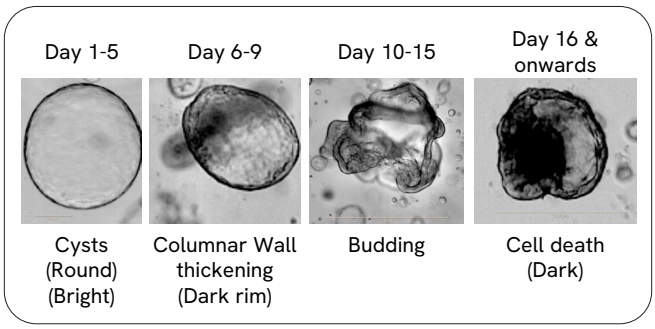
Organoids are in different z-heights and require z-stack imaging and z-projection analysis.

Challenge # 2 – Overlapping objects

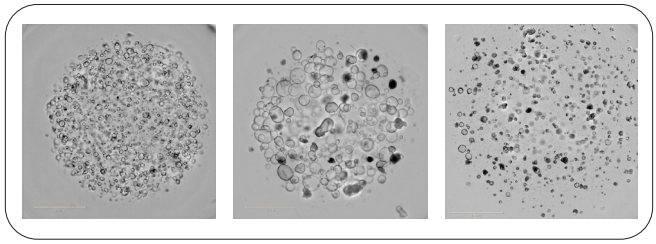


In z-projections of dense cultures, organoids overlap significantly, which is challenging for traditional image analysis algorithms.

Challenge # 3 – Organoids mature over time (colon organoids)



Challenge # 4 – Morphology variations between organoid lines



Organoids from different individuals and different timepoints can have very different sizes, morphologies and counts within the dome, requiring flexible and robust detection methods.

Image analysis methods for brightfield stacks

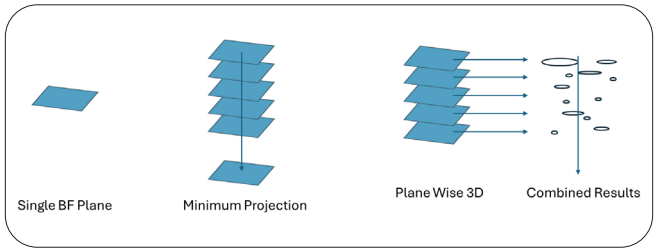
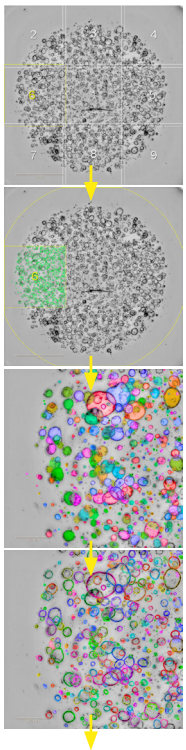


Figure 1: Harmony™ and Image Artist™ analysis methods for brightfield stacks. For samples with only few objects (organoids), a minimum intensity projection with 2D analysis is recommended. Dense cultures require a combination of minimum projection and 3D detection (plane-wise 3D approach) to allow proper object splitting.

Image analysis sequence with ‘Find Organoids’ building block



harmony

Input Image:

- Create Global Image (5x magnification)
- Create Minimum Intensity Projection **NEW**

Find Image Region:

- Detect Dome as a region of interest

Find Organoids: **NEW**
(3D detection)

- On single FOV
- Overlapping objects are split

Organoid border region is automatically detected and quantified

Calculate morphology or intensity & Select Population

Mean width of border [µm]	Median width of border [µm]	Maximum width of border [µm]	Minimum width of border [µm]
18.9169	19.2324	40.0595	5.75278
8.30028	7.14864	15.2151	4.76576
8.82258	7.71116	14.2973	4.76576

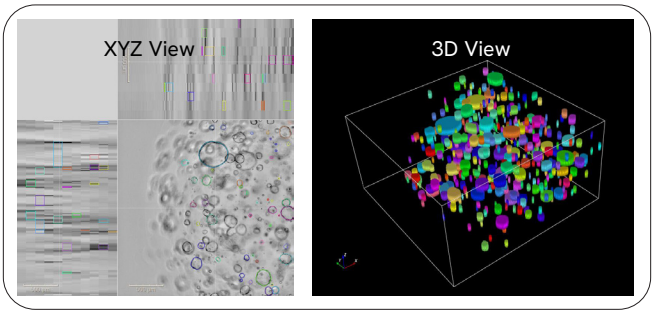


Figure 2: Harmony additional visualization tools for segmentation results within ‘Find Organoids’ building block.

Image segmentation results

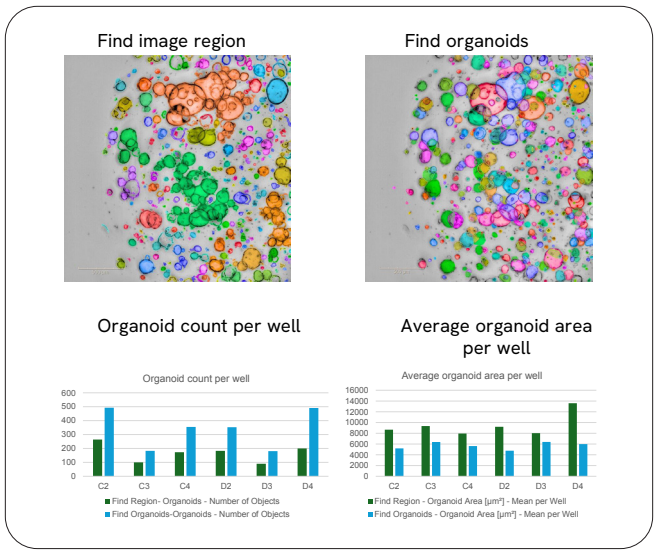


Figure 3: The ‘Find Organoids’ building block can detect on average 2x more organoids in minimum intensity projection images compared to previous analysis strategies with the Find Image Region building block. Due to improved object splitting, the organoid area is on average 0.6x smaller. Brightfield image stacks covering 1000 µm height in 11 planes were acquired on Opera Phenix™in non-confocal mode, using the 5x objective.

Segmentation of intestinal organoids from different organoid lines

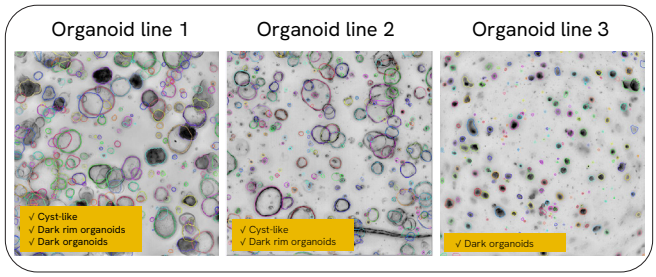


Figure 4: The ‘Find Organoids’ building block can detect different organoid morphologies from various organoid lines, including cyst-like, dark rim organoids and dark organoids.

Time series analysis of organoid growth and maturation

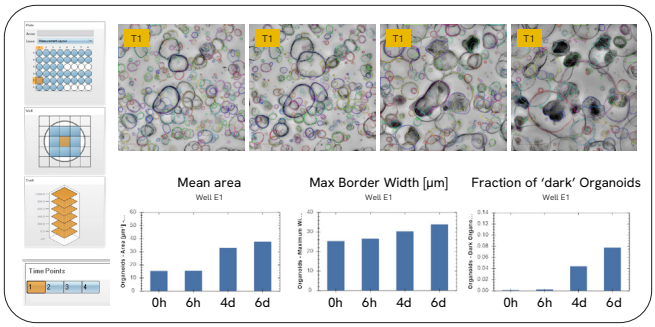


Figure 5: The growth and maturation of organoids can be followed over time by quantification of the mean organoid area, maximum organoid border width and fraction of dark organoids. Additional readout properties are available to tailor analysis sequences to various organoid applications.

Conclusions

- New 'Find Organoids' building block detects ~2-fold more organoids compared to previous image analysis strategies with significantly improved object splitting
- Improved segmentation robustness allows detection across intestinal organoid phenotypes (cyst-like, dark rim, and dark organoids)
- Organoid growth and maturation can be analyzed over time
- Enhanced 2.5D image analysis workflow for organoid-related drug screening and toxicity assessment applications

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