Enhancing brightfield high-content analysis: AI-driven segmentation and advanced organoid detection

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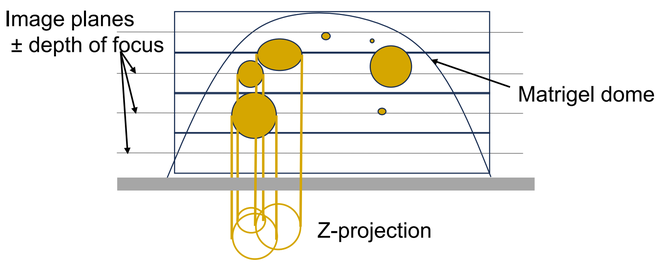
1 Overview

- **Brightfield microscopy** provides rich morphological data without fluorescent labeling, yet its low contrast poses significant challenges for robust automated segmentation. We introduce two innovative approaches that address these challenges and enable reliable, scalable analyses
- **Advanced 3D organoid detection in brightfield -** *Find Organoids* building block in Harmony™ high-content imaging and analysis software delivers up to 2-fold increase in detection via '2.5D' segmentation, robustly handling overlapping objects, variable morphologies, and complex ECM optical properties
- **AI-powered brightfield analysis - Phenologic.AI™** achieves >95% detection accuracy for label-free nuclear and cytoplasm segmentation, and artifact-reduced digital phase contrast reconstruction for reduced phototoxicity in extended time-lapse studies

2 *Find Organoids* addresses challenges in brightfield organoid imaging

Brightfield imaging of organoids poses significant segmentation challenges:

- **Z-height variation** in dome cultures requires z-stack imaging and z-projection analysis.
- **Dense, overlapping cultures** cause under- or over-segmentation.



- **Morphological changes** during maturation demand models adaptable across developmental stages.
- **Inter-line variability** in size, texture, and contrast limits transferability of segmentation approaches.

Robust strategies must address 3D distribution, dynamic morphology, and biological diversity across models and time points.

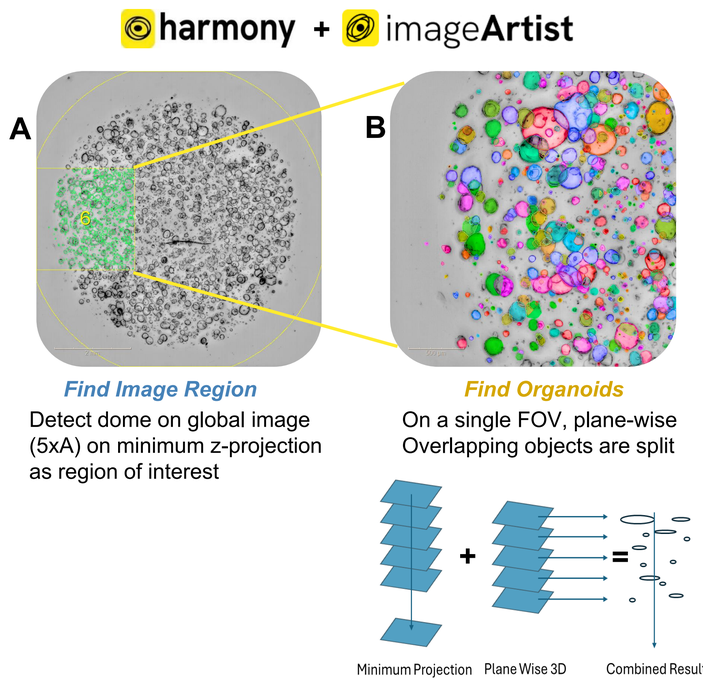


Figure 1: *Find Organoids* enables splitting of overlapping objects

Find Organoids is designed for dense cultures where organoids are distributed across the z-axis and may overlap. It automatically combines minimum z-projection with a plane-wise 3D segmentation approach to ensure accurate object detection and splitting.

A: Region of interest: Potential artifacts surrounding the culture dome can be excluded prior to analysis by applying *Find Image Region* on the global image. This step ensures that only relevant regions are passed to downstream segmentation.

B: Automated plane-wise segmentation: ***Find Organoids*** automatically segments and splits organoids on a field-by-field and plane-by-plane basis. Plane-wise analysis of the full 3D stack and combination of the results into a single 2D image ('2.5D') allows robust object detection and accurate splitting of overlapping objects. At the end, results are reported for the whole well, including z-height of each organoid.

3 Organoid segmentation results

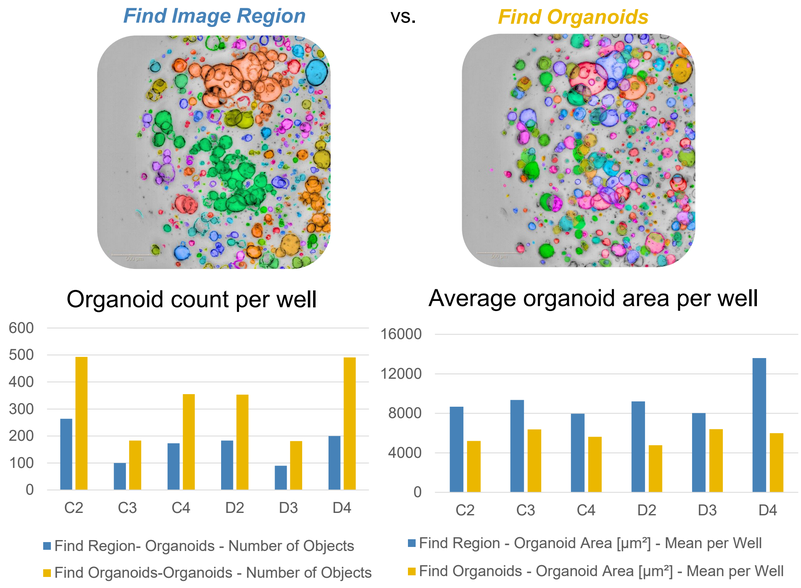


Figure 2: Improved organoid segmentation: *Find Organoids* can detect on an average 2x more organoids in minimum z-projection images compared to previous analysis strategies using the ***Find Image Region*** building block on a minimum z-projection. Due to improved object splitting, the organoid area 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.

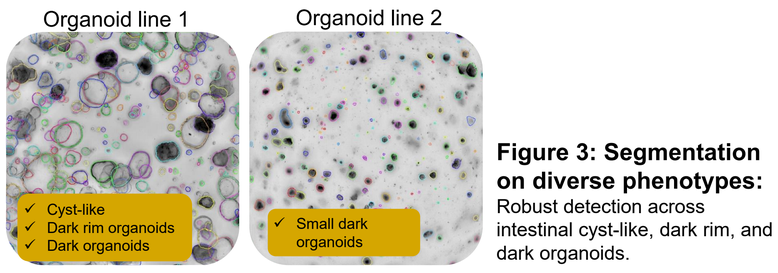


Figure 3: Segmentation on diverse phenotypes: Robust detection across intestinal cyst-like, dark rim, and dark organoids.

4 Phenologic.AI: AI-powered label-free cell segmentation

Pre-trained Phenologic.AI models integrate directly into Harmony®, Opera Phenix® Plus, Operetta® CLS™, and Image Artist™ - delivering accurate cell, cytoplasm, and nuclear segmentation via digital phase reconstruction, nuclei detection, and cell segmentation, without the need for user-training or parameter tuning.

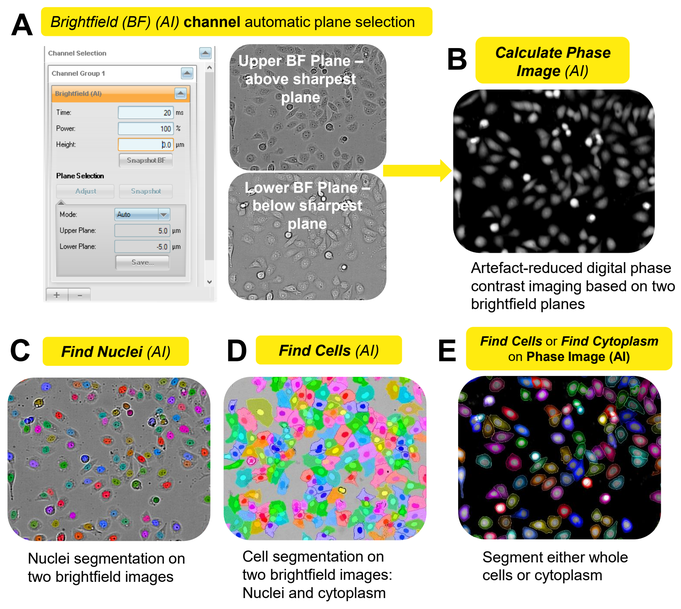


Figure 4: Phenologic.**AI-based segmentation options**

A: *Brightfield (AI)* acquisition channel automatically selects two z-planes (below and above sharpest plane) which are used in AI building blocks. **B:** AI-based reconstruction of a digital phase contrast. **C:** *Find Nuclei (AI)* finds nuclei. **D:** *Find Cells (AI)* segments cell, cytoplasm and nuclei in a single building block. **E:** Digital phase contrast image allows segmentation of whole cells (not AI); cytoplasm detection requires separate nuclei detection.

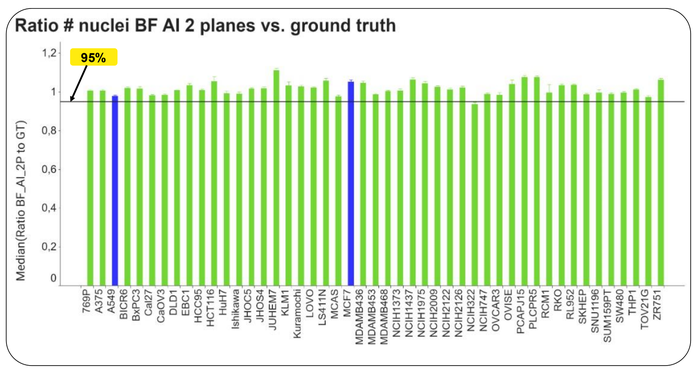


Figure 5: Nuclei detection rate across 45 cell lines

The detection rate (ratio of nuclei found using *Find Nuclei (AI)* to number of Hoechst-stained nuclei detected by the standard *Find Nuclei* building block (serving as ground truth) - consistently reached approximately 95% across all 45 cell lines tested. **Green** bars represent cell lines not previously encountered by the AI model (i.e., unknown during training), while **blue** bars indicate the two cell lines included in the model's training. THP1 is a suspension cell line. (Opera Phenix, 20x water immersion objective, widefield mode, 9 fields of view, two cell densities per cell line.)

5 Live cell application: PMA-induced effects on proliferation and morphology of MCF7-cells

Live-cell applications stand to benefit greatly from AI-based brightfield analysis. To explore this, we investigated a live-cell assay in which MCF7 (breast cancer) cells were treated with PMA (phorbol 12-myristate 13-acetate) across a six-point concentration range (0, 0.3, 3, 10, 30, 100, and 300 nM) (6 wells per concentration). Brightfield images were acquired on the Opera Phenix using a 10x air objective in widefield mode, capturing one field of view (FOV) per well at 30-minute intervals over 20 hours in 41 time points.

AI-based analysis options were used to calculate number of nuclei and cell area (see also Figure 4 B, C, E):

A. Number of nuclei: *Find Nuclei (AI)*
B. Cell Area: *Find Nuclei (AI)* → *Find Cells (AI)* vs. *Find Nuclei (AI)* → *Find Cells on Phase Image (AI)*

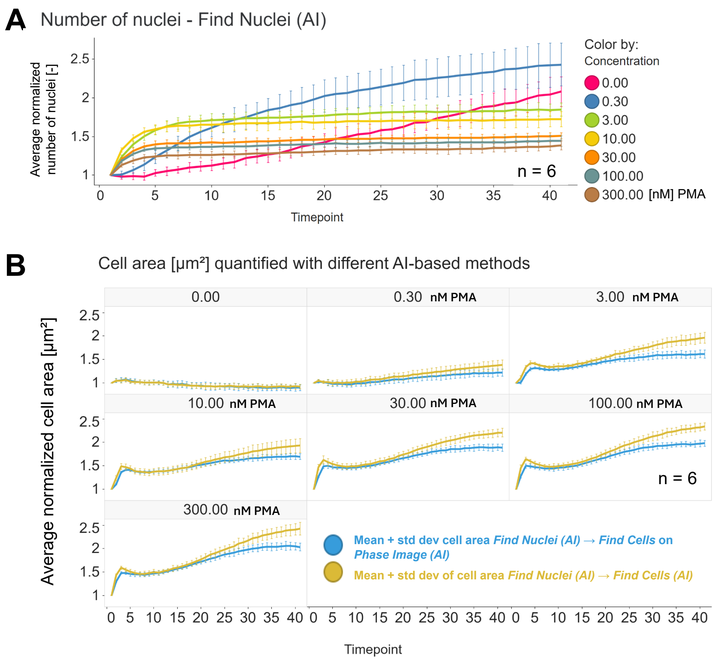
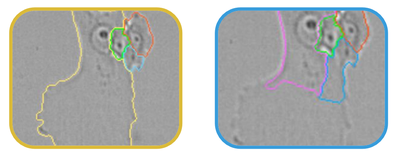


Figure 6: Effect of PMA on proliferation and cell area of MCF7 cells over 20 hours

A: In the untreated control, MCF7 cell numbers increase steadily over time. At 0.3 nM PMA, proliferation is further enhanced, exceeding control levels - indicating a pro-proliferative effect at this dose. At all higher concentrations, proliferation is enhanced in the first hours but then remains constant and below 0.3 nM on a concentration-dependent level.

B: In the untreated control, mean cell area remains stable over time. In PMA-treated wells, cell area increases progressively in a concentration-dependent manner, reflecting PMA-induced morphological changes. Across all PMA-treated conditions, cell area values derived from the brightfield channel (yellow) consistently exceed those from the phase contrast image (blue), indicating a channel-dependent difference in segmentation.

PMA is a potent activator of protein kinase C (PKC) that induces concentration-dependent changes in the proliferation and morphology of MCF7 cells. At low concentrations, PMA promotes cell differentiation and adhesion, accompanied by cytoskeletal reorganization and the adoption of a more elongated, morphology. At higher concentrations, PMA progressively inhibits proliferation and drives a transition toward a flatter, more spread cellular morphology. As cells enlarge and flatten, their optical contrast in phase contrast imaging is reduced, leading to less distinct cell borders and an underestimation of cell area when segmentation is based on phase contrast images:



This likely accounts for the consistently larger area values obtained from brightfield-based segmentation compared to phase contrast-based measurements observed in Figure 6B.

Conclusions

- Our innovations establish a comprehensive next-generation screening platform that unites the speed and cost-effectiveness of **brightfield imaging** with AI-driven analytical power and enhanced for 3D organoid detection.
- The new ***Find Organoids*** building block detects approximately 2-fold more organoids than previous image analysis strategies, with markedly improved object splitting performance - significantly increasing detection accuracy in complex 3D models.
- Pre-trained Phenologic**.AI** models enable versatile and highly differentiated segmentation of brightfield images, expanding analytical options without the need for fluorescent labeling.
- **Label-free analysis** eliminates phototoxicity concerns, making it ideally suited for long-term time-lapse studies, while preserving fluorescent channels for target-specific markers - delivering a flexible, non-invasive solution for modern cell and organoid imaging workflows.

Find more information about the research groups and products involved:

IRSD website | Imaging Microplates (PhenoPlates™) | PhenoVue™ Cellular Imaging Reagents | HCS Instruments | Image Artist™ Software