Deep learning-based image analysis for label-free live monitoring of iPSC 3D organoid cultures

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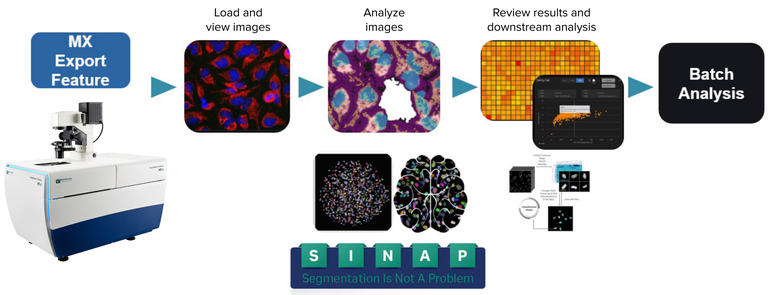
Introduction

Complex 3D biological models such as organoids and patient derived spheroids are gaining popularity in many biomedical research areas because they more closely recapitulate the in vivo tissues. These 3D models offer huge potential in disease modeling, drug screening, toxicity studies, host-microbe interactions, and precision medicine.

In order to use organoids for large scale screens, automation is increasingly used to handle the massive amount of sample, and culture more consistent and reproducible iPSC (induced pluripotent stem cell) lines and their derived organoids.

A major requirement of an automated culture system is the ability to monitor live tissues. Here, we used an artificial intelligence (AI) tool to carry out automated image analysis of iPSC colonies, organoids, and spheroids (imaged in brightfield). The IN Carta™ Image Analysis Software tool provides an intuitive user interface to train models based on user provided images. Deep learning-based (SINAP) enables automatic detection of complex objects of interest (e.g. stem cell colonies or organoids) with minimal human intervention. In contrast to AI-based image analysis, the conventional image analysis approach requires users to provide analysis settings which need to be frequently modified. IN Carta analysis output includes morphological, intensity, and texture measurements. We demonstrate the feasibility of using AI-based object detection and phenotypic characterization for three complex cell models: expansion of iPSCs, development of 3D lung organoids, and effects of anti-cancer drugs on tumoroids. These results support the integration of deep-learning image analysis methods into any high-content automation workflow that will greatly facilitate large scale generation of high-quality iPSCs and organoids for downstream applications.

Methods



IN Carta software workflow

Image acquisition

All images were acquired on the ImageXpress® Micro Confocal High-Content Imaging System (Molecular Devices) using the MetaXpress® High-Content Image Acquisition & Analysis Software. Because iPSC colonies were relatively flat and show little contrast in brightfield, images were acquired with approximately 100 µm offset to increase contrast at the edge of the colonies. For lung organoids, Z-stack images were acquired with the 4X objective with the "best focus" projection selection. For spheroids, Z-stack imaging was carried out with a 10X objective with best focus projection image selected.

IN Carta software was used for all analysis. The "export to IN Carta" function in MetaXpress software was used to import images into IN Carta software. SINAP was used to carry out segmentation of all images. Each model was trained and verified before being used in the analysis protocol. Post analysis, the classifier tool was used to create groups based on specific measurements. For more complex datasets, machine learning-based Phenoglyphs was used to create phenotypic classes.

Cell culture

Lung organoids: 3D lung organoids were derived from primary human lung epithelial cells (ScienCell). Cells were cultured and expanded in 2D according to ScienCell protocol. For 3D organoid culture, the PneumaCult™ Airway Organoid Kit (STEMCELL Technologies) was used according to manufacturer's protocol. Briefly, cells were seeded in 90% Matrigel (Corning) domes in 24-well plate format (1 dome per well) and were fed every second day for two weeks using the PneumaCult Airway Organoid seeding media. Differentiation was carried out for another six weeks using the PneumaCult Airway Organoid differentiation media.

iPSC culture: Human iPSC cells adapted to feeder-free conditions (SC102A-1, System Biosciences) were thawed and cultured in Complete mTeSRTM Plus culture medium (STEMCELL Technologies) in Matrigel coated plates (cat. #354277, Corning). Media was changed every day except once per week when a double volume of media was added to skip changing media on one day. Cells were passaged using the enzyme-free reagent ReLeSRTM (STEMCELL Technologies) at a 1:6-1:10 split ratio.

Spheroid culture: Spheroids were formed from TU-BcX-4IC cells derived from a primary tumor. These cells are classified as metaplastic breast cancer with a TNBC subtype. TU-BcX-4IC cells were seeded at 2000 to 4000 cells per well in 384-well ULA plate (Corning) and incubated for 72 hours. Spheroids were then treated with compounds and monitored on day 1, 3, and 5.

Results

Deep learning-based model for image segmentation

Automated image analysis is an integral part of most automated imaging platform. The ability to monitor cells and organoids in real time and extract meaningful information is dependent on robust image analysis of label-free transmitted light images. Challenges associated with analysis of brightfield images include low contrast, high background and imaging artifacts (Figure 1A). A defined set of global parameters would rarely be successful in segmenting objects imaged in brightfield. Recent advances in machine learning is improving image analysis workflow and enabling more robust image segmentation in complex datasets. Here, we show how deep learning models can be used to analyze various biological models such as in stem cell biology, 3D organoids, and spheroids.

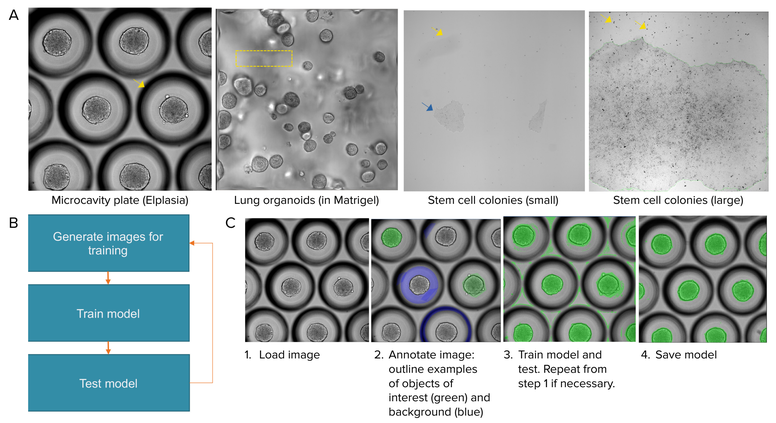


Figure 1. Overcome challenges in image segmentation with machine learning-based models. A) Examples of different biological models presented that are challenging for quantitative analysis. 3D spheroids grown in microcavity plates produce a shadow around each microcavity that interferes with object segmentation (arrow). 3D organoids are grown in Matrigel which often produces non-homogenous background due to distortion from the Matrigel dome and from objects beyond the imaging planes (box). IPSC grow as relatively flat cultures, as a result, the low contrast (blue arrow) and debris (yellow arrow) hampers robust image segmentation of iPSC colonies. B) Overview of the SINAP workflow in IN Carta software. C) Main steps to create model in IN Carta software using SINAP with example images shown. Images are annotated using labeling tools to indicate the object of interest and background. The annotated image, representing ground truth is added to the training set. In the training step, a model is created based on the annotated images and on the selected base model. In the example shown, it is necessary to correct the segmentation mask (step 3), repeating steps 1–3.

Monitoring growth of iPSC culture over time

iPSC culture requires daily feeding and frequent visual inspection of cell morphology to check for spontaneous differentiation. Because culture passaging is determined by colony size and distribution, it is important to monitor the growth of iPSC cultures. Here, the size of individual iPSC colonies are monitored over six days in culture using brightfield imaging and machine learning-based image segmentation for analysis (Figure 2).

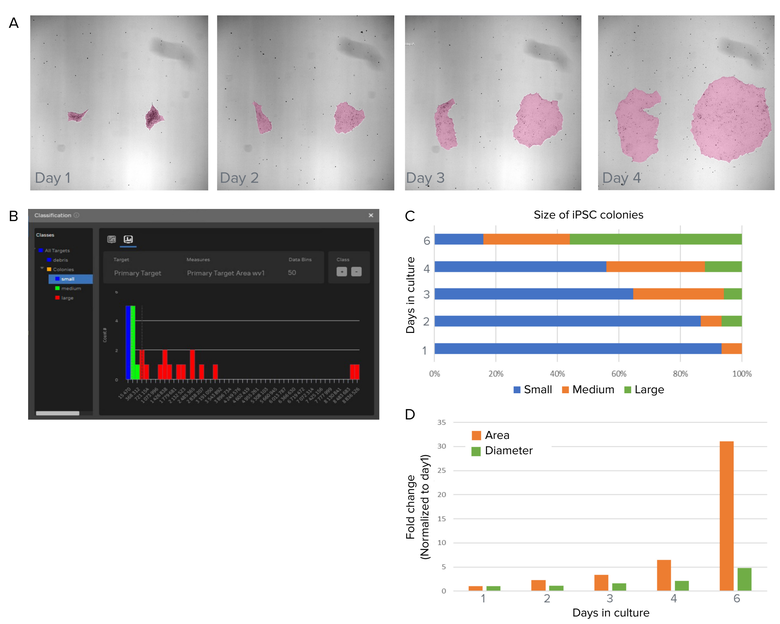


Figure 2. Deep learning-based model used to segment iPSC colonies and monitor growth. A) Example images showing the growth of iPSC in culture over four days. Shown in pink overlay is the segmentation mask from using SINAP. Approximately 12 images were annotated and used to train the deep learning model in SINAP. B) Classifier tool in IN Carta software is used to categorize segmented objects based on user selected measurements and gates. Here, objects are categorized in small, medium, and large classes according to their area. C) Graph shows distribution of iPSC colony sizes over time. As expected, the frequency of large iPSC colonies increased over time in culture. D) Change in average iPSC colony area and diameter over time in culture (normalized values).

Results

Analysis of 3D lung organoids

3D organoids are powerful models with many biomedical applications. Because they can be derived from any patient, organoids hold huge potential in drug discovery and in personalized therapeutics. Organoids may be cultured up to months thus the ability to monitor organoid status is instrumental for quality control and time lapse studies. Here, we show an example of lung organoids cultured and imaged in Matrigel domes over time in brightfield (Figure 3). Measurements such as area, diameter, shape factor, texture, and intensity may be used to monitor organoid growth and differentiation.

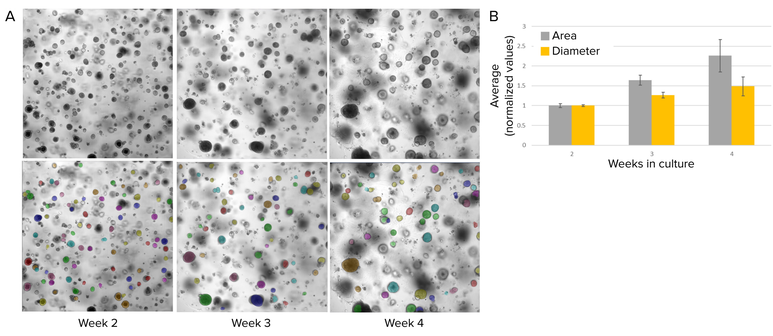


Figure 3. Applying AI-based method to analyze lung organoids. A) Images of lung organoids grown in Matrigel dome. These images usually have high, non-homogenous background which prevents robust object segmentation. Using SINAP, a model was created to segment lung organoids (mask shown in colored overlay). B) Graph showing change in average lung diameter and area over 2, 3 and 4 weeks in culture (normalized, error bars represent standard deviation between replicate wells).

Compound treated patient-derived spheroids

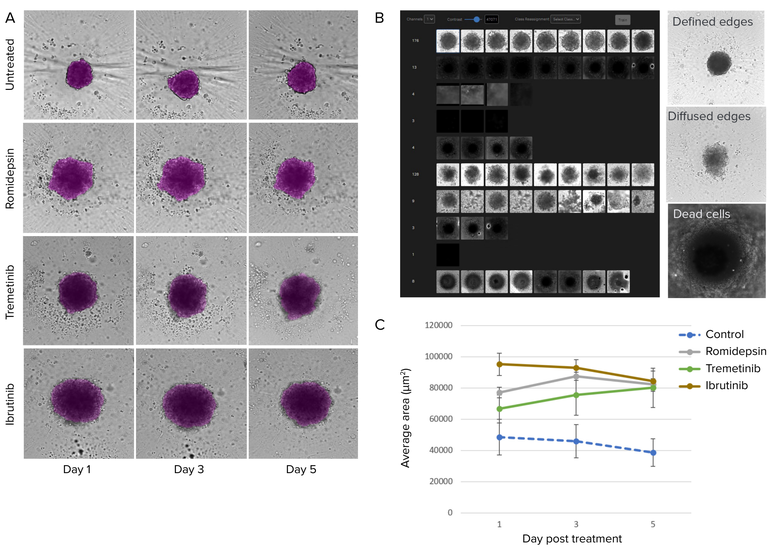


Figure 4. Morphometric analysis of compound treatment on patient derived spheroids over time. A) Spheroids were monitored using brightfield imaging on days 1, 3, and 5 post-treatment. Images were segmented using SINAP in IN Carta software (magenta overlay). B) IN Carta™ Phenoglyphs™ was used to detect the different phenotypes. Untreated control spheroids show tightly packed cells with defined edges while treated spheroids show a variety of phenotypes. The edges of the treated spheroids are less defined, have a diffused edge, and cells are loosely packed. C) Graphs showing average area of spheroids over time post-treatment. Treated spheroids show larger area which indicates the loss of spheroid integrity. Error bars represent standard deviation for four replicates. (Romidepsin 200 nM, Tremetinib 200 nM, Ibrutinib, 62.5 µM)

Conclusions

- AI-based approaches in analysis of complex images and datasets offer highly robust results for high-content imaging workflows.
- SINAP is a user-friendly tool to create custom deep learning models for robust segmentation of label-free biological models.
- Phenoglyphs enables user friendly machine learning-based phenotypic profiling that can be applied to complex datasets such as patient derived spheroids.
- SINAP and Phenoglyphs are complementary AI-tools in IN Carta software that offers an end-to-end solution, bridging the gap between complex biological questions and AI-driven answers.

MOLECULAR DEVICES