AI-enabled automated compound screening for toxicity effects using healthy intestinal organoids

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Introduction

The most common side effect of anti-cancer drugs is their toxicity to intestinal cells which often limits the dose that can be administered to treat patients. In vitro assays using organoids can evaluate toxicity effects on the intestine and provide essential information in the process of drug development. Organoids are three-dimensional (3D) multicellular tissue constructs that originate from human pluripotent stem cells (iPSCs) or adult stem cells. They can recreate the physiological structure and function of human organs through self-assembly in matrix. Studies show that patients and their derived organoids respond similarly to drugs, highlighting the potential benefits of utilizing organoids in screening to improve therapeutic outcomes. Assay automation and artificial intelligence (AI) will greatly increase the productivity and scale of these models, as well as the accuracy of assays that involve complex 3D biology.

The CellXpress.ai™ Automated Cell Culture System can automate complex cell culture process and controls media exchanges, compound additions, imaging, and passaging. The new ImageXpress® HCS.ai High Content Screening System enables high-speed and quality organoid imaging and defines morphological changes using image analysis. Finally, the machine–learning tools in IN Carta® Software automatically determines intact and damaged organoids and provides quantitation of compound effects.

In this study, we show how to automate the compound testing assay for toxicity assessment using 3D mouse intestinal organoids. For compound screening, organoids were automatically plated into a multi-well plate format and treated with seven concentrations of ten compounds known to cause toxicity to intestines. After treatment, organoids were imaged after staining with a mix of dyes for nuclei, mitochondria, cytoskeleton, and other subcellular structures. Images were taken with a confocal, automated imaging system and analyzed using machine learning-enabled software (IN Carta software). Concentration-dependent phenotypic changes in organoids were quantified through multi-parametric feature extraction. These features were then used in a machine-learning classifier to automatically distinguish affected from unaffected phenotypes. This method is suitable for automating toxicity assessment studies which significantly reduces manual cell processing while enhancing productivity and assay scalability. In addition, AI-powered data analysis automates complex analysis steps, enabling more reproducible and efficient compound testing.

Instruments

The CellXpress.ai Automated Cell Culture System and ImageXpress HCS.ai High-Content Screening System

Methods

Organoid culture. Primary mouse intestinal organoids from StemCell Technologies were cultured in Matrigel domes using IntestiCult media according to manufacturers recommended protocols. During organoid culture, automated media exchanges and monitoring by transmitted-light imaging were done every 24h. Organoids self-organized and developed complex crypt structures as expected for intestinal organoid phenotype. For toxicity evaluation, assay organoid domes were seeded into 96-well plates (Ibidi plates) with 50% Matrigel domes, 15 µL per dome. Each dome contained approximately 60 organoids. Organoids were placed manually or placed using automation using the CellXpress.ai system. Compounds were added to organoids after 48h in culture. Compound treatments were applied using 7-point dilution range, 4X dilutions, starting from 200 µM concentration, except staurosporine which was started from 20 µM concentration. Organoids were cultured with compounds for 3 days. After compound treatments, organoids were stained with Hoechst and MitoTracker orange, then fixed with 4% formaldehyde and additionally stained with Alexa488 Phalloidin in the presence of 0.05% of TritonX. All Dyes were from Thermo Scientific.

Imaging organoids. Organoids were imaged using the ImageXpress HCS.ai system with confocal option (60 µm pinhole) in three fluorescent channels DAPI, FITC, TRITC, and at 10X magnification. Then, 3x3 sites-per-well were taken at 10X magnification to cover the entire dome area. Additional images were taken using 4X magnification, in this case, 4 tiled images were used to cover the organoid dome area. Z-stacks of 16 images were taken at 8um interval, covering a z-range of 120 µm. Maximum projection 2D images were used for analysis.

Image analysis. Image analysis was done using IN Carta software's Custom Module Editor (CME) to create a multi-step analysis protocol that defined organoids as blobs using the DAPI channel (nuclear stain) in projection images. Analysis was applied to maximum projection images. Numbers of organoids, average organoid area, and average organoid fluorescent intensities for DAPI (Hoechst stain), FITC (Alexa488 Phalloidin) and TRITC (MitoTracker) were measured. First, a Gaussian filter was used to blur the Hoechst signal to facilitate segmentation of organoids. Next, nuclei were segmented and used to define cells. Cells were scored as positive or negative depending on the signal intensities for Phalloidin (actin cytoskeleton) or MitoTracker (mitochondria). Thresholds for positive and negative cell scoring were set empirically using control (untreated) samples and samples treated with toxic compound (e.g. ailuropodine). Cells that were scored positive for actin or mitochondria were defined as having an intact cytoskeleton or intact mitochondria, respectively. Positive and negative cells per organoid were counted and average area and average intensity of positive cells were measured. Average organoid volumes were evaluated by 3D CME analysis using a custom module that defined organoids in 3D as 3D objects. After analysis, concentration dependencies for different read-outs were plotted as 4-parametric curve fit (for 0.12–200 µM concentration range) to calculate EC50s for compound toxicity effects. SoftMax® Pro Software was used for the curve fit and calculation of EC50s.

MOLECULAR DEVICES

Results

Toxicity assessment using 3D intestinal organoids and high-content imaging

Intestinal organoids were expanded in 24-well plates then plated into 96-well Ibidi plates using the CellXpress.ai system. Matrigel domes contained approximately 60 organoids. Organoids self-organized and developed typical intestinal organoid phenotypes. After 48h in culture organoids were treated with compounds.

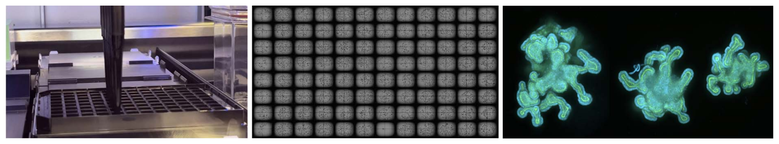


Figure 1. Intestinal organoids were automatically seeded into 96-well plates (Ibidi plates), then treated with compounds. Organoid images shown after staining with Hoechst and AF-488 Phalloidin. Images were obtained with confocal option, 4X magnification.

10 compounds were tested for toxicity effects. Compounds were tested in 4X dilutions, 0–200 µM concentration range, in duplicates or triplicates. After compound treatment for 3 days, organoid domes were fixed, stained, and imaged as described in Materials and Methods. Organoids were imaged using the ImageXpress HCS.ai system with 10X objective, using confocal option. A Z-stack of images was taken 8 µm apart, covering approximately 120 µm range.

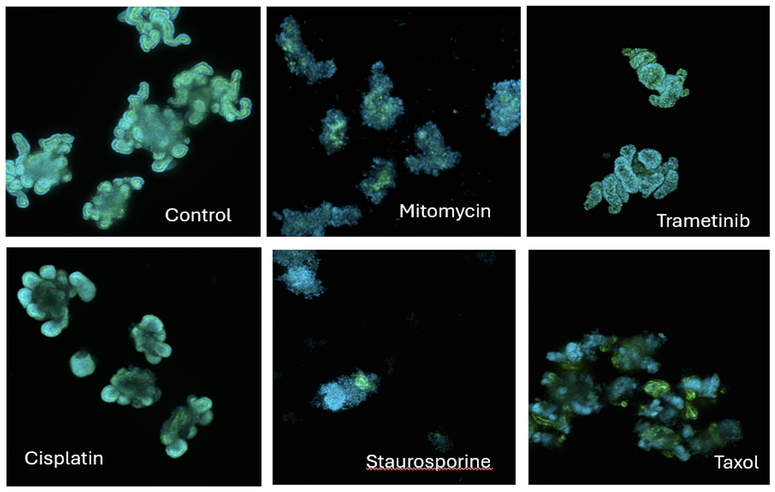


Figure 2. Intestinal organoids were treated with cytotoxic compounds for 72h, then stained with Hoechst, MitoTracker and AF488 phalloidin. Changes in morphology and viability were measured by high-content imaging,automatically seeded into 96-well plates (Ibidi plates), then treated with compounds. Organoid images shown after staining with Hoechst and AF-488 Phalloidin.

High-content imaging analysis determines the number of organoids in the dome and measure the organoid size (area) and fluorescent intensities with different markers. In addition, it allowed the identification of individual cells and counts intact or damaged cells in organoids. To quantify the intact cells, we scored cells as positive—or intact—if they had a high signal for actin or MitoTracker.

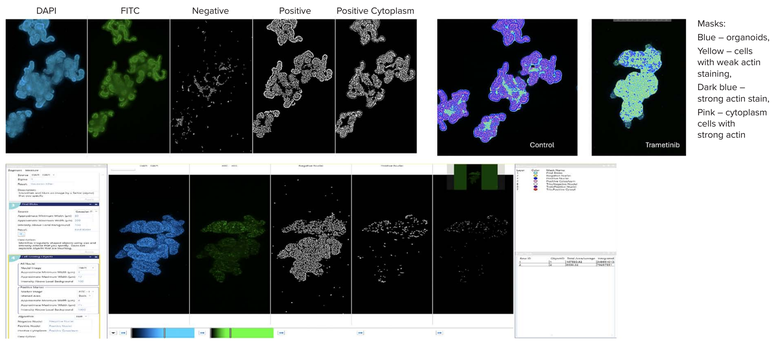
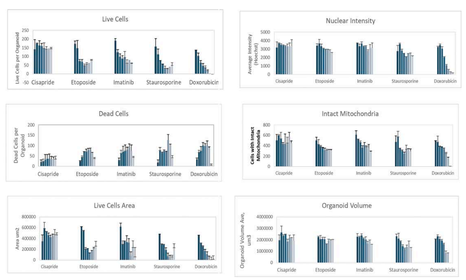


Figure 3. High-content analysis steps: finding organoids then scoring cells as positive or negative based on Phalloidin staining.



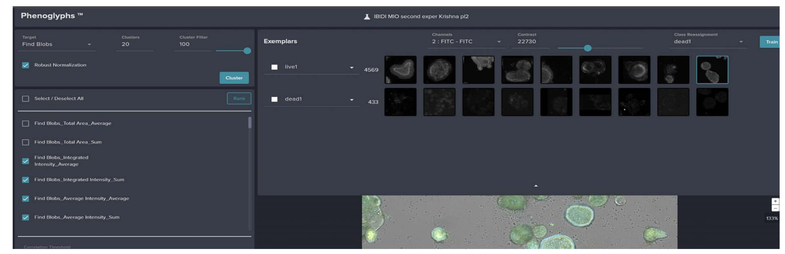
EC50, µM	Positive (intact) cells	Nuclear intensity	Cytoplasm Area Sum	Mitochondria Integrity
Cisapride	nd	nd	nd	nd
Staurosporine	0.62	0.61	0.58	0.52
Etoposide	5.5	nd	5.2	6.1
Doxorubicin	5.2	10.8	5.5	inconclusive
Imatinib	4.8	nd	7.6	12.1
Mitomycin	5.3	5.1	7.3	5.6
Cytarabin	4.8	4.3	8.4	5.8
Cisplatin	18.1	nd	22.8	30.8
Trametinib	4.6	20.1	18.2	20.3

Figure 4. Concentration-dependencies and effective concentrations for various readouts that reflect various phenotypic changes caused by toxic compounds. Concentration for indicated compounds were in the range from 0–200mM, increasing from left to right.

Results

Machine learning-based classification of compound toxicity effects

Machine learning and AI show promise to eliminate time-consuming and labor-intensive tasks from scientists by providing automated approaches to image analysis with automated reasoning, unbiased image analysis, and greater consistency of data. Image analysis was done using IN Carta software. First, high-content analysis was done using the Custom Module Editor software module to find organoid objects. These objects were then characterized using images captured in three fluorescent channels plus a Transmitted Light channel to extract a variety of measurements including fluorescent intensities, areas, diameters, etc. Then nuclei and individual cells were identified inside the organoids and characterized using various measurements featuring intensities, areas, and linear measurements. Sixty measurements total were extracted from each image.



After un-supervised clustering there were initially 8 clusters determined. We consolidated classes into just two: Live (intact) organoids, and Dead (damaged) organoids, then initiated training. After the training run was complete, we reviewed the classifications and found just a few errors in classification which we corrected by manual re-assignment of images into either Live or Dead category. After some corrections, we repeated the training, and after 2 cycles shifted the classification score to 1. As a result, we obtained good classification of objects in the wells and extracted value indication % of Live or Dead organoids. The % of Live organoid numbers were decreasing with increasing concentrations of compounds and were showing the trends expected for effects of the tested compounds.

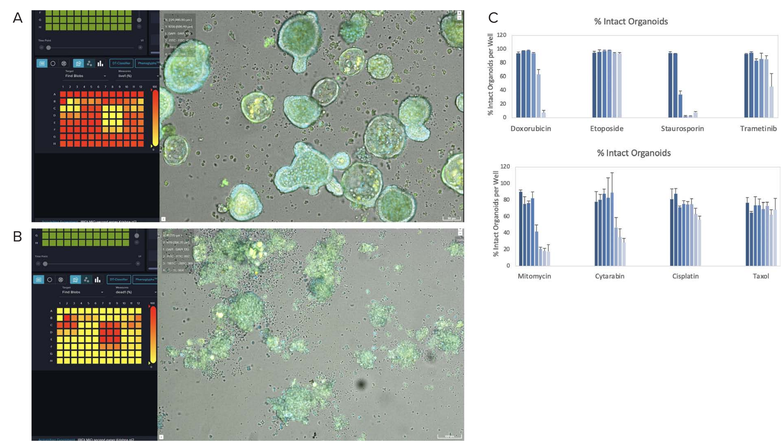


Figure 5. Shows results of analysis by wells (samples) based on percentage of organoids classified as Live (A) or Dead (B). Representative images of each shown. C. Concentration-dependencies of the % of classified Live (Intact) organoids.

Summary

- Demonstrated an automated protocol for the toxicity assay using intestinal organoids.
- Described the workflow for evaluating compound toxicity effects using healthy intestinal organoids and imaging approaches for phenotypic analysis
- Developed a machine learning-based method for quantitation of phenotypic effects caused by compounds
- Demonstrated benefits of machine learning in image analysis for detection and evaluation of toxicity-related phenotypic changes in organoids
- Automated key processes to greatly reduce manual labor and effort spent by scientist on the cell culture process, showing promise for more reproducible and scalable cell assay experiments.

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