AI-enabled hit selection of drug screening on human pancreatic cancer organoids

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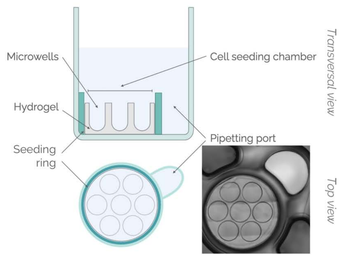
Introduction

Cancer remains one of the leading causes of death in the 21st century. Despite the latest advances in oncology, most cancer patients lack tailored therapeutic approaches with lasting benefit. Measuring the impact of anticancer compounds and their combinations is only possible on ex vivo assays. To this end, patient-derived organoids (PDOs) have been proposed as viable and efficient models for ex vivo testing. PDOs show long-term expansion potential while retaining tumor histopathology as well as cancer gene mutations. However, the translation of PDOs in screening applications has so far been hampered by the lack of organoid homogeneity and difficulties in their handling and automating. Moreover, organoids are typically randomly distributed across the culture which complicates imaging and image analyses.

To overcome these challenges, we set up a compound screening workflow with PDOs using the Gri3D® platform (SUN bioscience), which is comprised of plates with micro-cavities suitable for high-throughput and reproducible organoid culture. Based on a standard 96 microtiter plate, each well contains a microwell array patterned in a cell-repellent hydrogel. On Gri3D, organoids are robustly generated in the microwells and are located in the same imaging plane. This greatly facilitates 3D image acquisition and quantitative analyses in high-content, image-based screens. Furthermore, the pipetting port enables automated cell seeding, media exchange, and compound incubation with liquid handlers, thus increasing assay reproducibility.

In this study, we exposed human pancreatic cancer PDOs to a panel of anti-cancer compounds at different doses and followed their response to the drugs using viability dyes Calcein AM (live stain) and Ethidium Homodimer-1 (dead stain) with high-content confocal imaging. Using an AI-based approach, we efficiently detected each organoid and extracted phenotypic features from all three channels (TL, live stain, and dead stain) which correlate with cytotoxicity. The extracted features (more than 100) were first dimensionally reduced to 3 components using either PCA or UMAP. The treatments were then visualized in 3D scatter plots, ranked, and clustered using machine learning. The data suggests that the compound treatment Palbociclib 50 µM has significant cytotoxicity effects similar to the positive control, which is consistent with the traditional live/dead analysis. This AI-powered method demonstrates the feasibility of performing drug screening using a robust and unbiased data analysis approach.

Labware and instruments



- Gri3D is a ready-to-use platform for high-throughput and reproducible organoid culture. Based on an array of ultra-dense U-bottom microwells in a hydrogel, single organoids are robustly generated in each microcavity and grown in a suspension-like culture without a solid ECM.
- We used the ImageXpress® Micro Confocal High-Content Imaging System equipped with spinning disk confocal and sCMOS camera to capture the 3D structures of whole organoids.

Methods

Standardized pancreatic cancer PDO arrays were generated in Gri3D 96WP plastic-bottom 500 µm microwells and exposed to anti-cancer drugs for 72 hrs. Organoid response to drugs was followed over time with transmitted light (TL) on an ImageXpress Micro Confocal High-Content Imaging System (Molecular Devices). Then, a Live/Dead assay was performed on treated organoids. Images were analyzed using IN Carta® Image Analysis Software (Figure 1). Forty organoids were segmented per well and more than 50 metrics were extracted from each. Finally, the features related to each organoid were analyzed using StratoMineR® data analysis software (Core Life Analytics).

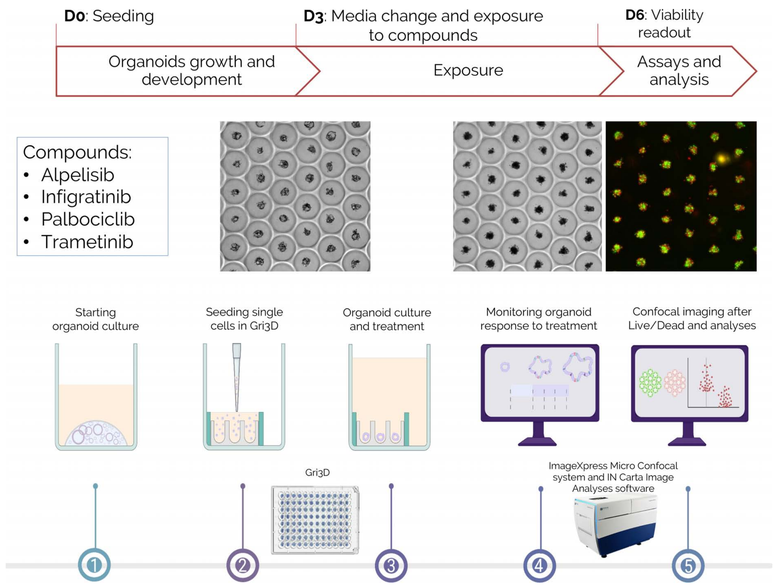


Figure 1. Schematic of the organoid drug efficacy workflow. This workflow uses Gri3D in combination with a high-content imager and AI-based image analysis software (Molecular Devices).

Phenotypic effects of compounds

Upon Palbociclib exposure, the Live/Dead assay showed a viability decrease of organoids with increasing drug concentrations, whereas no response was observed in trametinib-treated organoids (Fig. 2B). Using a deep learning image-based approach on TL images, we efficiently detected each single organoid and quantified different parameters over time. Grey-levels non normality factor (GLNN) is an indicator of the similarity of grey values within an organoid. This value decreases with increasing doses of Palbociclib and Trametinib (Fig. 2C), indicating organoid growth defects.

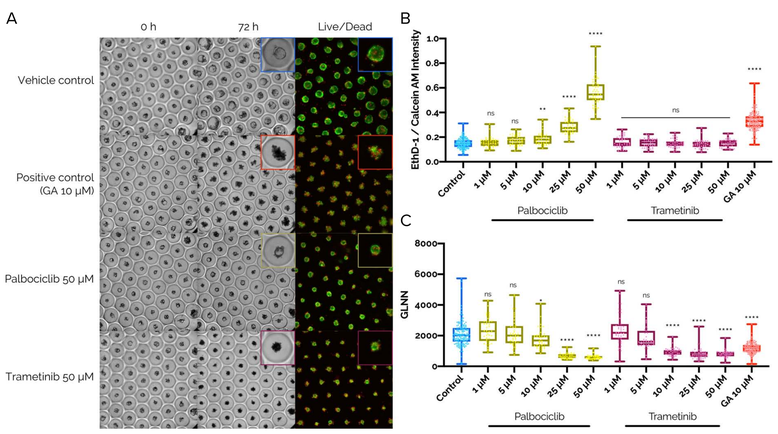
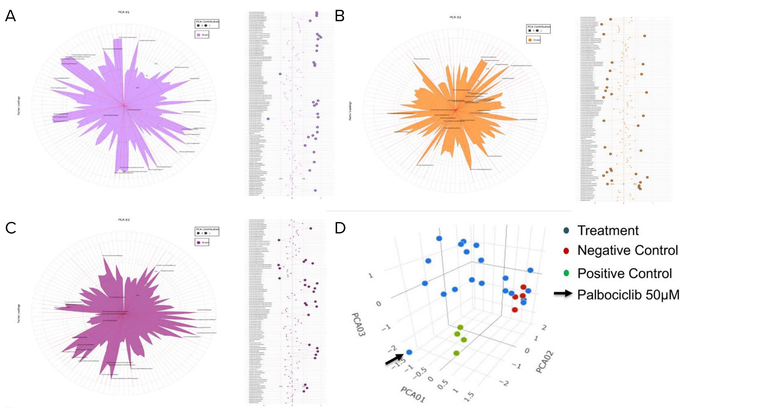


Figure 2. Response of pancreatic cancer PDOs exposed to anti-cancer compounds for 72 hours. A. TL images before (0h) and after (72h) exposure and maximum projection images of organoids after Live/Dead assay at 72h. Green: Calcein AM, live; red: EthD-1, dead. B. Ethidium homodimer-1 (EthD-1) to Calcein AM intensity ratio. C. Grey levels non-normality factor (GLNN) from TL images. Error bars show standard deviation. Each dot represents an organoid. One-way ANOVA Dunnett's multiple comparisons, **P < 0.01, P**** < 0.0001, ns: non-significant. Scale bar: 250 µm.

Hit selection via dimensionality reduction

Data analysis performed using IN Carta software returned hundreds of features associated with each organoid from the TL, EthD-1, and Calcein AM channels. To visualize and cluster the treated and control wells, we first performed dimensionality reduction through principal component analysis to three components, i.e., PCA01, PCA02, and PCA03. We then visualized the treated and control wells in a 3D scatter plot, where the negative controls and positive controls form their own clusters, respectively (Figure 3), and Palbocilib 50 µM is the only treatment that clusters closer to the positive controls (Figure 3). This observation is aligned with the Live/Dead analysis (Figure 2). In addition, the ranking of each treatment (Chebyshev Maximum distance) from the average negative control shows that Palbociclib 50 µM ranks No. 1 while the four positive control wells rank the top 6. Thus, Palbociclib 50 µM has the most compound effect on the human pancreatic cancer organoids.



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	PlateID	WellLocation	replicateIdNumber	ReagentClass	ReagentID	PCA01	PCA02	PCA03	DistanceScore	pvalue
	All	All	All	All	All	All	All	All	All	All
1	1	g07	1	PALBOCICLIB50UM		-1.527646033920830	-2.487392503958	-2.561180595284790	3.870168016178910	1.098917517568810e-39
2	1	f04	1	GA10UM		0.862462318754492	-2.318515212438940	-1.043112753967340	3.701290723222050	2.696334287856180e-36
3	1	g02	1	GA10UM		0.480809835351206	-2.244246195491100	-1.869711030969320	3.627021706274210	6.616096698685870e-35
4	1	f05	1	GA10UM		0.345455679419856	-1.962112615594480	-1.227348525557770	3.344888122637580	2.117184754417500e-29
5	1	g06	1	PALBOCICLIB25UM		-0.807954677983391	-1.899336100573440	0.472136934623852	3.282111611356550	2.588404271187690e-28
6	1	f06	1	GA10UM		0.339016158226025	-1.763044682793	-1.057843834833220	3.145820193576100	6.591017551268420e-26
7	1	g11	1	ALPELISIB25UM		-0.888444621203697	-0.931063706952482	1.784690746230710	2.810160983339080	2.353508097888180e-20
8	1	h04	1	INFIGRATINIB25UM		-1.480002818354410	-0.293394256208730	-0.800320059839896	2.513839840401560	4.832241422190930e-16
9	1	g12	1	ALPELISIB50UM		-1.443749315091910	-0.955974476043284	0.902242904820249	2.477586337139060	1.213356875794450e-15
10	1	h08	1	TRAMETINIB10UM		-0.105021909921140	-0.008868383116060950	1.453328490288290	2.478798729196660	1.213356875794450e-15

Figure 3. Clustering and ranking via the principal component analysis. A., B., C. The three PCA components with weighted association with original features. D. 3D scatter plots of the three PCA components. E. Ranking of treatments and controls through the Chebyshev Maximum distance from the average negative control.

UMAP

To validate our analysis, we used Uniform Manifold Approximation and Projection (UMAP) to reduce the dimensions of features. With all features considered, the clustering plot also shows Palbociclib 50 µM clustered close to the positive controls. To determine if we can distinguish phenotypic changes with only brightfield images, we ran the analysis using only features derived from the TL channel. The resulting 3D scatter plot shows no obvious clustering characteristics, which suggests the need to for additional features.

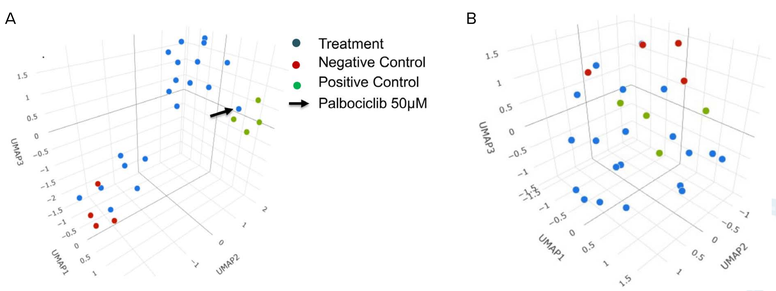

Figure 4. UMAP dimensionality reduction and clustering. A. 3D scatter plot of UMAP components of all features. B. 3D scatter plot of UMAP components of the features of TL channel only.

Summary

- Gri3D plate is a platform for high-throughput and reproducible organoid culture.
- The ImageXpress Micro Confocal Imaging System captures organoid 3D structures in high-throughput and at high-resolution.
- IN Carta image analysis software generates masks for each organoid and measures hundreds of features that are associated with each organoid.
- StratoMineR data analysis software uses AI to cluster and rank each drug treatment.

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