A novel approach for label-free drug efficacy analyses of pancreatic cancer organoids using high-content imaging

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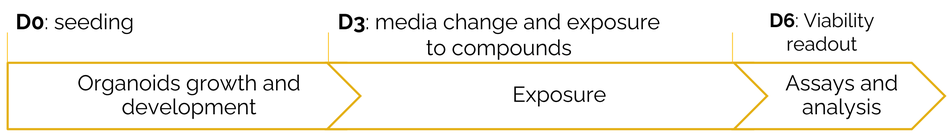
INTRODUCTION

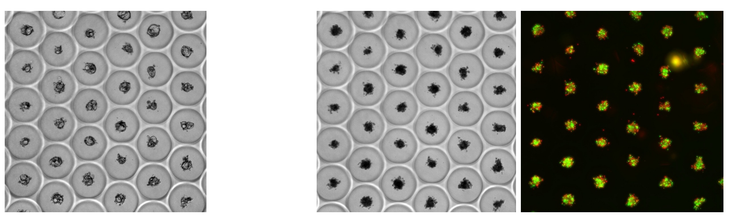
Patient-derived organoids (PDOs) have been proposed as viable and efficient alternatives for *in vitro* testing in oncology[1]. PDOs show long-term expansion potential while retaining tumor histopathology as well as the tumor's original genetic make-up.[2] However, the translation of organoids in screening applications has so far been hampered by the lack of homogeneity and difficult imaging, handling and automatability[3]. To overcome these challenges, we set up a screening workflow on PDOs using Gri3D®, a ready-to-use platform for high-throughput and reproducible organoid culture[4]. On Gri3D®, organoids are robustly generated in the microwells and are located in the same imaging plane. Furthermore, the pipetting port enables automation of cell seeding, media exchange and compound incubation with liquid-handlers, thus increasing assay reproducibility. In the presented work, we expose pancreatic cancer PDOs to a panel of anti-cancer compounds at different doses and follow their response to the drugs with single-plane brightfield imaging. Using an AI-based approach, we efficiently detect each single organoid and extract phenotypic features which correlate with cytotoxicity. We further validate the approach by comparing the obtained results to traditional multi-plane fluorescence-based Live/Dead assay.

METHODS

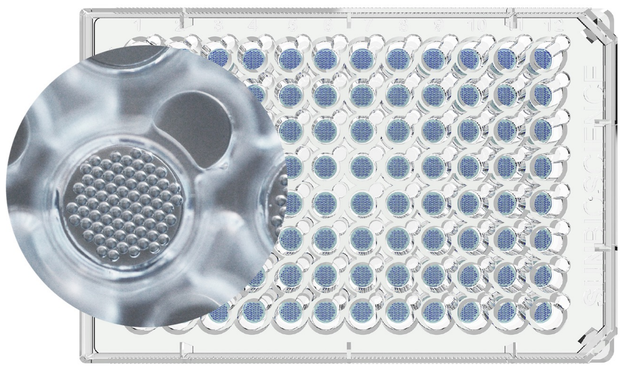
Standardized pancreatic cancer PDOs arrays are generated in Gri3D® 96WP plastic-bottom 500 µm microwells and exposed to anti-cancer drugs. Organoid response to drugs is followed over time with transmitted light (TL) on an ImageXpress® Micro Confocal system (Molecular Devices). Finally, Live/Dead assay is performed on treated organoids. Images are analyzed using IN-Carta® Image Analysis Software. 40 organoids are segmented per well and more than 50 metrics are extracted from each.

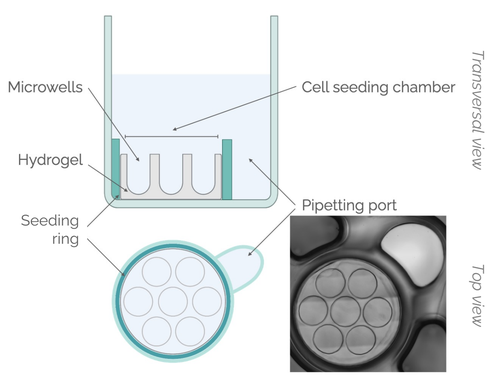


Compounds:
- Alpelisib
- Infigratinib
- Palbociclib
- Trametinib



GRI3D® TECHNOLOGY





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 suspension-like culture without a solid ECM.

RESULTS

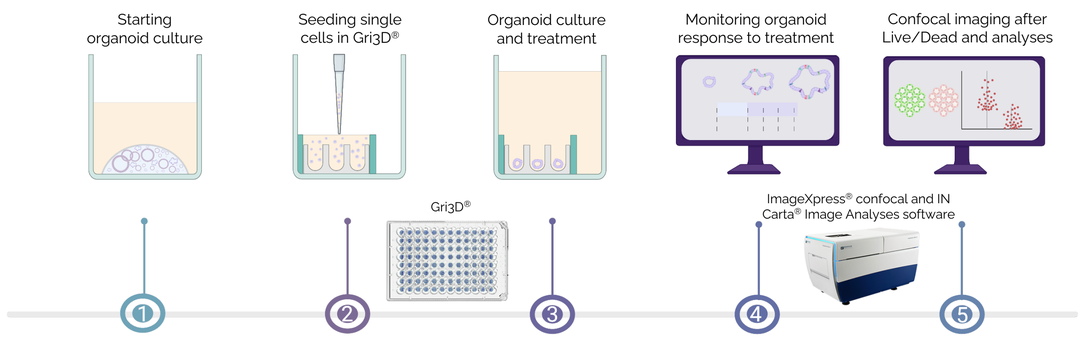


Figure 1. Schematic of the organoid drug efficacy workflow. Using Gri3D® in combination with a high-content imager and an AI-based image analysis software (Molecular Devices).

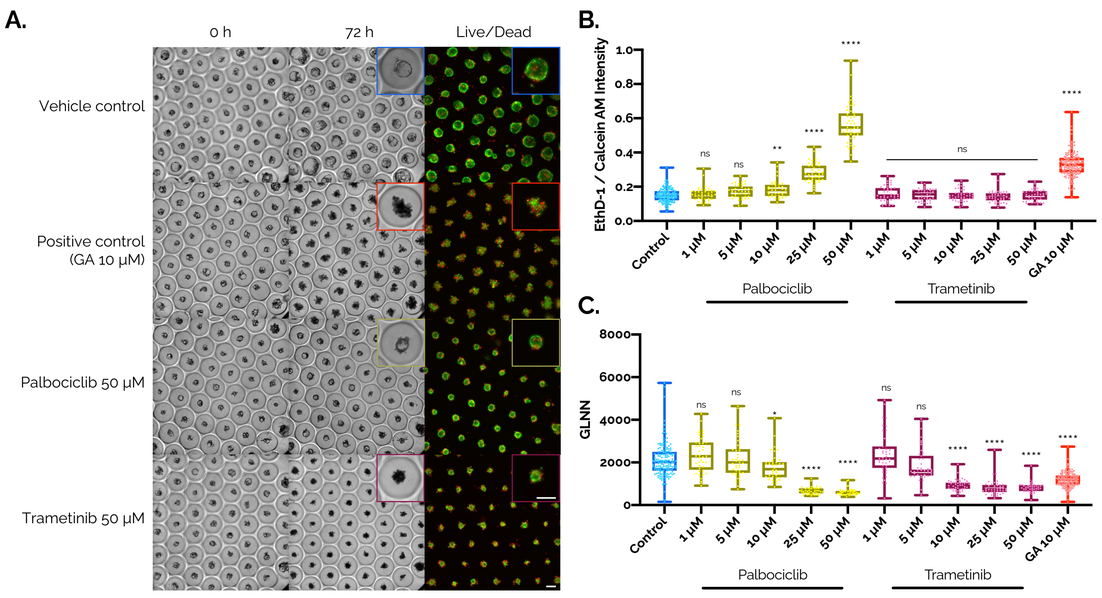


Figure 2. Response of pancreatic cancer PDOs exposed to anti-cancer compounds for 72 hours. A. TL images before (0 h) and after (72 h) exposure and maximum projection images of organoids after Live/Dead assay at 72h. Green: Calcein AM, live; red: EthD-1, dead. **B.** Ethidium homodimer-1 (EthD-1) to 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.

CONCLUSIONS

- Organoids grown in Gri3D® plates show high reproducibility
- Tessellated positions of organoids enable enhanced imaging and monitoring of individual organoid
- Robust image analysis can be accomplished with advanced tools such as deep learning-based segmentation
- Phenotypic features extracted from brightfield images correlate with fluorescence-based cytotoxicity
- Advanced label-free analyses require minimal manipulation and have the potential to significantly accelerate organoid screening processes
- Present a complementary approach using label-free drug efficacy analyses combining microwell plates, high-content imaging and AI-based algorithms

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Pancreatic cancer PDOs are cultured in Gri3D® and exposed to anti-cancer drugs for 72 hours. After imaging with an ImageXpress® Micro Confocal, results are analyzed with IN Carta® (Fig.1). Upon Palbociclib exposure, Live/Dead assay shows a viability decrease of organoids with increasing drug concentrations, whereas no response is observed in trametinib-treated organoids (Fig. 2B). Using a machine learning image-based approach on TL images, we efficiently detect each single organoid and quantify different parameters over time. Grey-levels non normality factor (GLNN) is an indicator of similarity of grey values within an organoid. This value decreases with increasing dose of Palbociclib and Trametinib (Fig. 2C), indicating organoid growth defects. Analyses over time of other factors such as organoid area and form factor indicate that Palbociclib exerts a cytotoxic effect while Trametinib is cytostatic, stopping growth (Fig. 3). Thus, TL analyses reveal mechanisms unnoticed on Live/Dead.

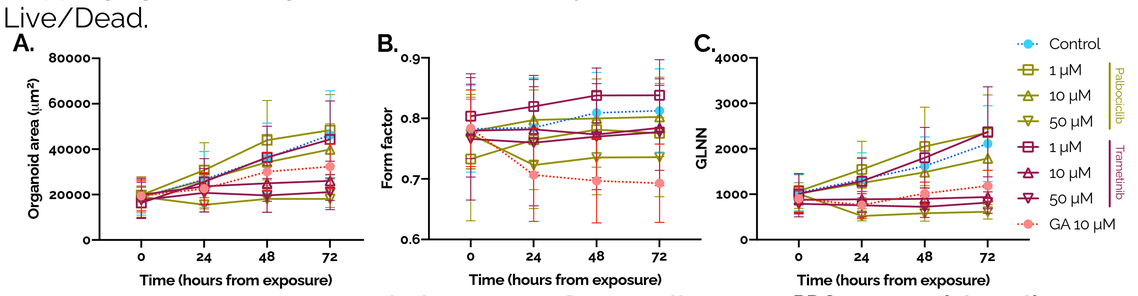


Figure 3. Time and dose-dependent response of pancreatic cancer PDOs exposed to anti-cancer compounds for 72 hours from TL imaging. Organoids response to treatment is monitored during 72 hours exposure with TL imaging and features are extracted: **A.** organoid area, **B.** form factor, **C.** grey levels non normality factor (GLNN) over time. Error bars show standard deviation.

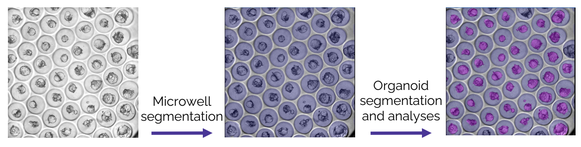
Live/Dead assay

Opportunities
- Allows extraction of quantitative fluorescence data from individual organoids
- Is a very robust assay for toxicity assessment
- Obtains multiple phenotypic parameters
- Investigates the effect of compounds in 3D

Challenges
- 3D fluorescence imaging is slow and generates large data volume
- Long-term dye toxicity
- Dynamics of response missing
- Irregular cell dye uptake

Label-free approach

- Faster, simpler workflow
- Small data volume generated
- Monitor live cells over time
- Works on all cell types
- No dye incubation
- Dynamics of response assessed



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