Application note

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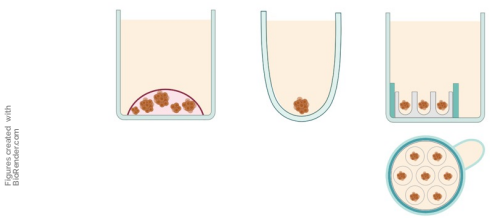
Colorectal cancer (CRC) Organoids Phenotypic Screen on Gri3D®

Summary

Gri3D® is a **ready-to-use platform** for high-throughput and reproducible organoid culture. Here we describe its use to perform an **automated drug screen** of 80 anti-cancer compounds on HCT-116 spheroids and patient-derived colorectal cancer (CRC) organoids. Up to 73 **homogeneous** microtissues are generated in a single well, and their response to treatment is assessed by **high content imaging.** More than 250 phenotypic metrics are extracted from each microtissue. Further analyses reveal a previously unobserved phenotype on organoids grown on Gri3D® during the administration of subtoxic levels of afuresertib. Our system allows **assessment of drug effects at a single-organoid level** in a fully automated high throughput screen workflow.

In vitro 3D model screen: state of the art

The generation of organoids from primary cells enables a range of therapeutic agents to be tested in a more physiologically relevant setting. However, the translation of organoids to the industry for screening applications has so far been hampered by the lack of homogeneity and difficult handling and cumbersome analyses of organoid culture in solid ECM drops. On Gri3D®, organoids are robustly generated in the microwells and are located in the same imaging plane, thus facilitating quantitative analyses in high content image-based phenotypic screens.



Figures created with BioRender.com

In vitro 3D organoid screening methods. From left to right: ECM embedding, non-adherent surface and Gri3D®.

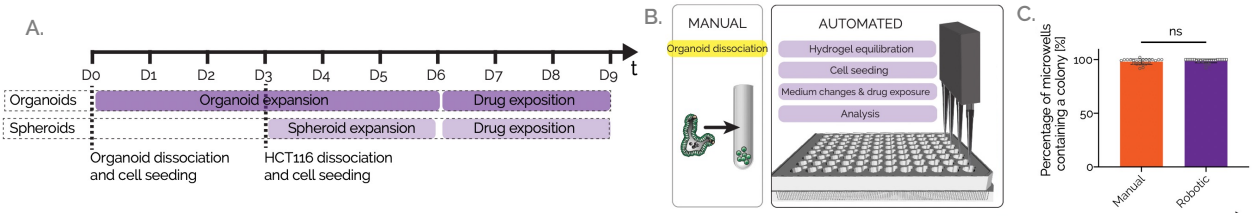
	ECM embedding	Non-adherent surface	Gri3D®
Organoid size homogeneity	■	■■	■■■
Reproducible organoid numbers	■	■■■	■■■
Suitability for organoids	■■	■■	■■■
Throughput	■	■■	■■■
Focal plane location	■	■■■	■■■
Ease of imaging	■	■■	■■■

Comparative table of common in vitro 3D organoid generation and screening platforms and Gri3D®.

Drug screening on Gri3D®

Patient-derived CRC organoids and HCT-116 spheroids are generated in **Gri3D® 96WP imaging-bottom 500 µm microwells** to screen a panel of 80 anti-cancer compounds.

All steps of the screen are executed by an **automatic liquid-handling system**: hydrogel equilibration, cell seeding, medium changes, drug exposure and readout using image-based Live/dead assay. No differences are observed in the organoid-formation efficiency between the manual and automated process.



A. Protocol for the generation of CRC tumoroids and HCT-116 spheroids and compound screen on Gri3D® plastic-bottom 500µm microwells.
B. Schematic of the automated generation of organoids.
C. Percentage of wells containing a colony.

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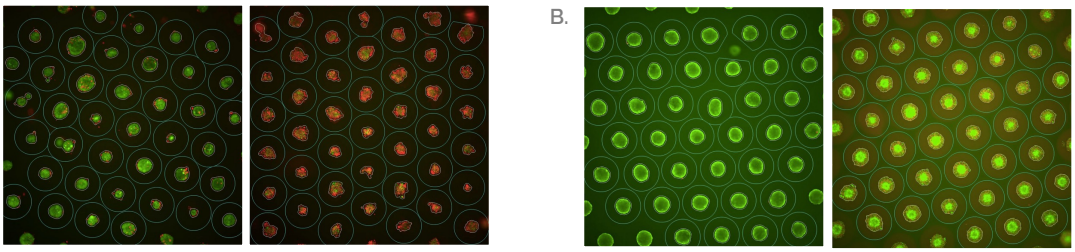
Spheroid and organoid screen

After drug exposure, we assess the viability of the microtissues and investigate their phenotype with high-content image analyses. 40 microtissues are segmented per well and more than 250 metrics are extracted from each. Using the positive and negative controls, compound efficacy can be classified. Replicate screens show high reproducibility (R^2=0.9 for spheroids and 0.79 for organoids).

A. B.

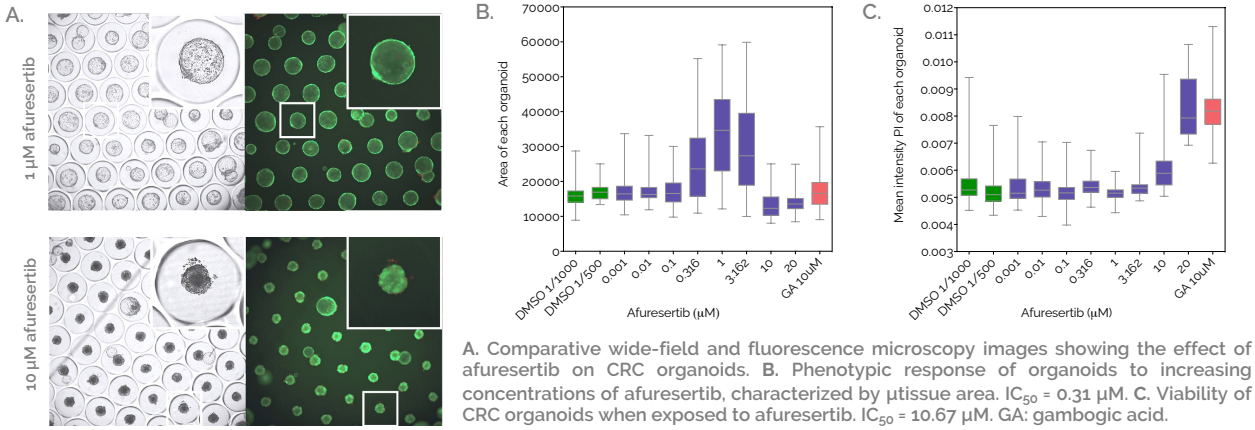


Fluorescence microscopy images of a single field of view of the positive (left; gambodgic acid) and negative (right; DMSO) controls of the automated imaging pipeline for **A.** HCT-118 spheroids and **B.** CRC organoids.

In depth analysis: afuresertib

Analyses on the 80 compounds uncover kinase inhibitor afuresertib as a strong phenotypic outlier. It induces major swelling on organoids (but not spheroids) at subtoxic concentrations, a phenotype that would go missed on classical viability analyses.

A. B. C.

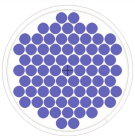


A. Comparative wide-field and fluorescence microscopy images showing the effect of afuresertib on CRC organoids. **B.** Phenotypic response of organoids to increasing concentrations of afuresertib, characterized by µtissue area. IC_{50} = 0.31 µM. **C.** Viability of CRC organoids when exposed to afuresertib. IC_{50} = 10.67 µM. GA: gambogic acid.

Highlights of the screen

- **Fully-automated:** Gri3D® is a front-to-end solution and all steps from seeding to readouts are automated
- **Scalable:** the use of Gri3D® 96 500 µm microwells allows the generation of more than 70 organoids per well, of which 40 are segmented for high-content image analyses in a single field of view
- **Single-organoid analyses:** instead of population-based readouts, thus revealing phenotypes that would otherwise be unnoticed
- **Relevant:** patient-derived tumoroids closely mimic their corresponding ***in vivo*** tumour and can be used to study patient-specific differences in response to therapy

Materials



Gri3D® 96 wellplate 500µm microwells imaging bottom were used.
Order your plates here!

500 µm, 73 µwells

References

The data reported were generated at SUN bioscience. For more information you can read the original publication: Brandenberg, N. et al. High-throughput automated organoid culture via stem-cell aggregation in microcavity arrays. Nat. Biomed. Eng. 4, 863–874 (2020) doi.org/10.1038/s41551-020-0565-2.

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