# Non-destructive, longitudinal organoid analysis to reduce reliance on CellTiter-Glo3D, derive deeper drug insights, and improve translational fidelity

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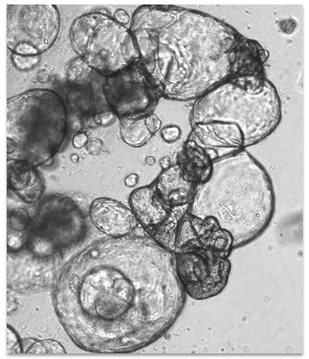
## INTRODUCTION



### Patient-derived organoids (PDOs)

3D human-relevant tissue models and key *New Approach Methodologies* (NAMs) to reduce reliance on animal models

### *Analytical Challenges*

Biochemical assays are **endpoint & destructive**

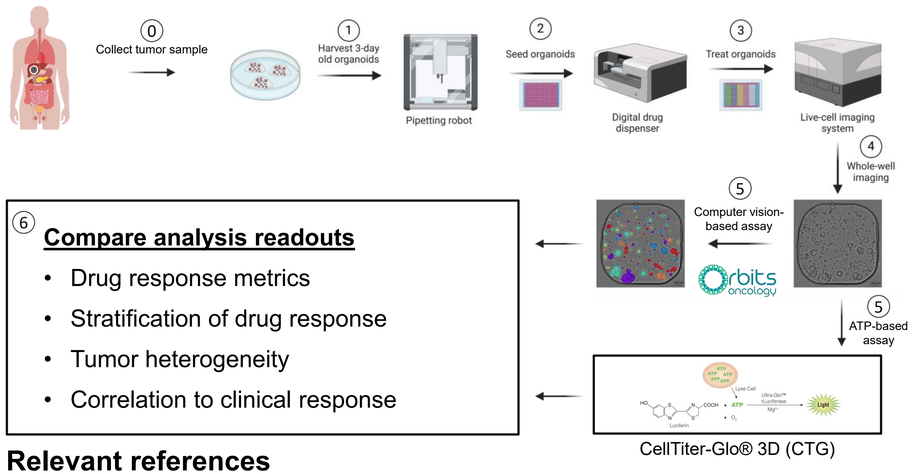
Current live-imaging software is **inflexible & laborious**

Existing readouts miss **dynamic, phenotypic behavior**, limiting translation

*We hypothesized that **live-imaging with AI-driven computer vision analysis** will enable extraction of rich therapeutic insights from PDOs at a scale.*

## AIMS & METHODS

We developed a **standardized imaging-based framework** for PDO drug response analysis and benchmarked our readouts with the widely used CTG assay. Our framework was applied to a PDO panel from pancreatic cancer patients (n=8). PDO drug responses were matched to retrospective progression-free survival following SOC treatment.

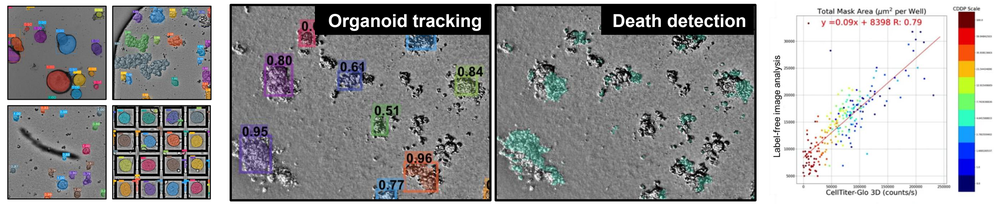



### Relevant references

Le Compte et al. *JoVE* (2022), Deben et al. *Cellular Oncology* (2022), Le Compte et al. *npj Precision Oncology* (2023), Deben et al. *Communications* Biology (2024), Deben et al. *J Exp Clin Cancer Res* (2024)

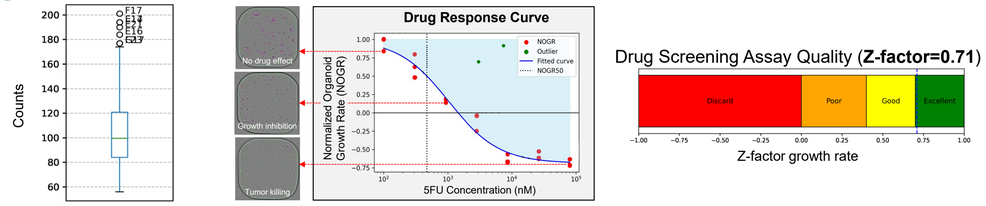
## RESULTS

### 1 *Validated label-free models for diverse phenotypes*



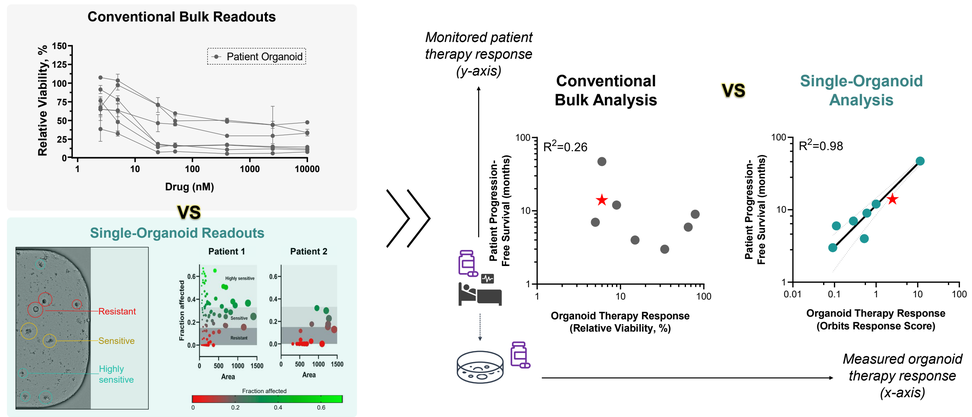

Computer vision models for live-organoid monitoring was robust to diverse organoid morphologies and avoids artifacts, debris, and complex plate formats (left panel). Vision models also quantified dead regions within organoids (teal), classified dead organoids, and tracked viable-dead transitions without fluorescence dyes (middle panel). Label-free analysis of Cisplatin-treated (CDDP) organoids highly correlated with CellTiter-Glo3D analysis (right panel).

### 2 *Standardization for seeding and growth variation*




Lab automation enabled consistent organoid seeding and wells with higher organoid counts were also identified (left). We pioneered the Normalized Organoid Growth Rate (NOGR) metric, derived from longitudinal analysis and normalization to each organoid growth rate, to capture cytostatic (0<NOGR<1) and cytotoxic (-1<NOGR<0) drug effects in a single metric – visually validated from the images (middle). An excellent Z-factor (0.71) was achieved in a high-throughput screen (6000+ drugs) with lab automation and our analysis (right).

### 3 *Improved translational fidelity – human study*




## CONCLUSIONS

**Sophisticated biological models need equally advanced analysis to ensure improved reproducibility & translational.**

- **Label-free organoid tracking & death detection models** outperformed CTG assays.
- Lab automation enabled **standardized** organoid seeding and experimentation
- Analysis automation enabled **high quality, high-throughput** image data processing and analysis
- Live-phenotypic analysis at single-organoid resolution derived **clinically relevant insights**.
- Lab and analysis automation must complement each other for **a complete screening solution**

***Case Study***




**37,000 images**

**High-throughput screening facility**
- 6000+ drug combinations screened
- Twenty 384-well plates
- 921 GBs image data

**Automated analysis software**
- Images analyzed in under 8 hrs
- Saved $2160+ on CTG reagents

## CONTACT

**Interested in organoid image analysis?**

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