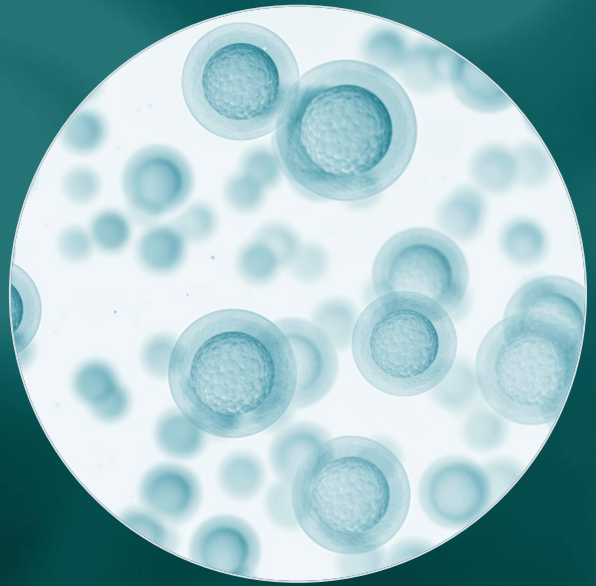
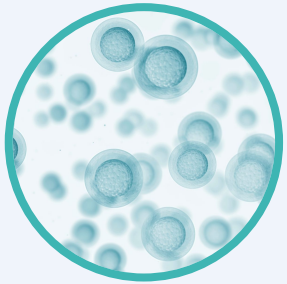
LEVITAS BIO®

Multi-Factorial Organoid Workflows



October 2025

Revolutionize Your Organoid Workflow with the LeviCell® 96 System



Process up to **500,000 cells** per well or sizes of **>500 microns**

Challenges

Variability:

- 3D cultures, even when derived from the same cell source, can display variations in size, shape, and cellular composition. This inconsistency makes it difficult to compare results across different experiments and labs.

Lack of standardized protocols:

- This area is relatively young, and there's a lack of universally accepted, standardized protocols. This makes it hard to ensure consistency and reliability.

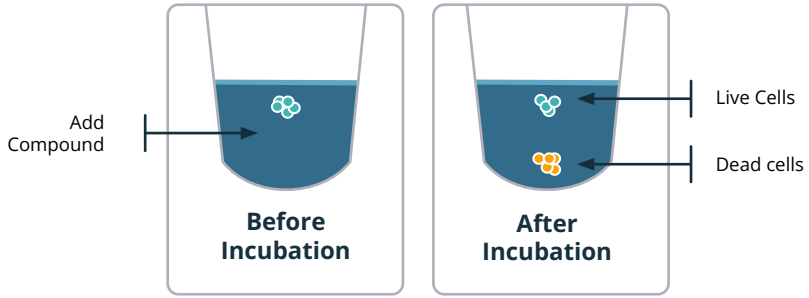
Key Benefits of LeviCell 96

- **Gentle, Label-Free Cell Separation**
- **Debris Removal**
- **High-Throughput Processing**
- **Increased efficiency and faster time to propagation**
  - Whole/dissociated organoids/spheroids

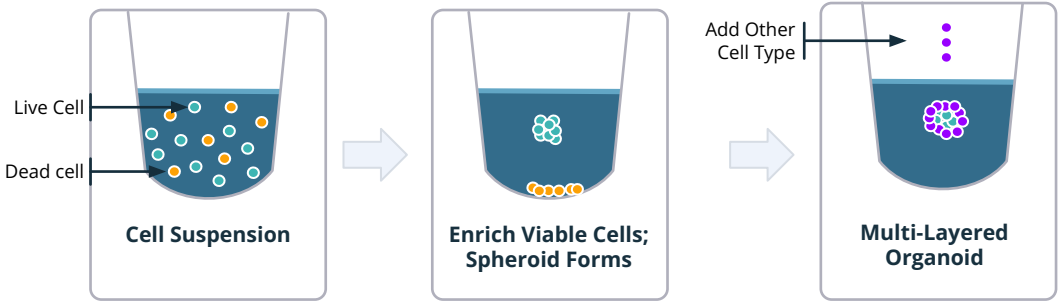
Multi-Factorial Organoid Workflow Capabilities

Enabled by LevitasBio's Selective Arrayed Levitation Technology (SALT™)

Compound Screen and Collection



Multi-layered Organoid Development



Culture Time Reduction from 90 days to 2 days

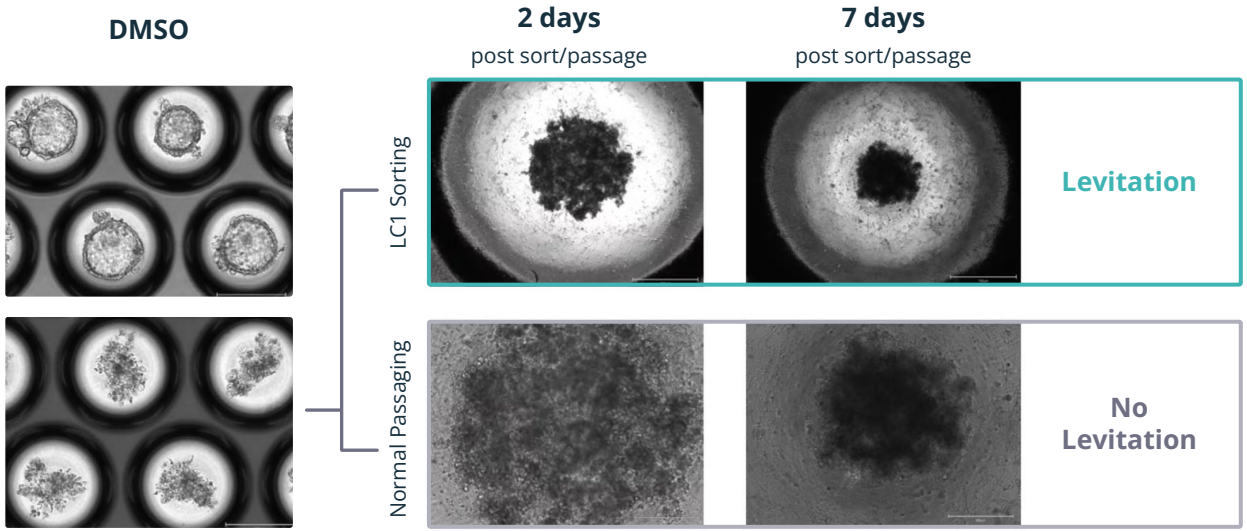
Performance Advantages

1. Yield
Achieve >80% cell yield regardless of input concentration.

2. Viability
Maintain >95% cell viability with low input samples.

3. Debris Removal
Experience >90% debris removal for even the most compromised samples.

4. Reduced Hands-on Time
Requires only 5 minutes of hands-on time with 45 minutes total time.

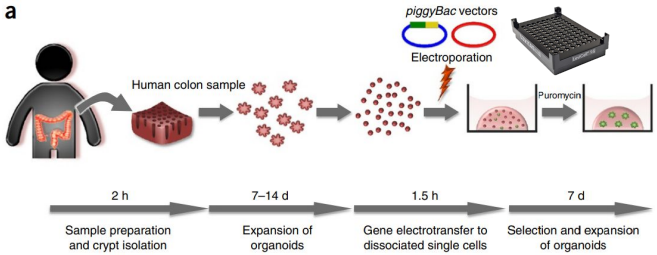


Levitated samples cultured within 2 days of starting culture vs. 90 days for standard samples

Two Ways of Using LeviCell to Enrich for Organoids

LeviCell can enrich for viable input cells

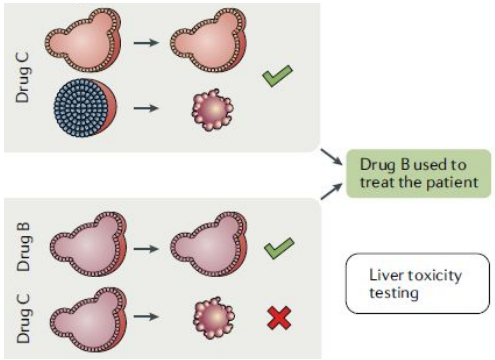
Example:
post-transfection



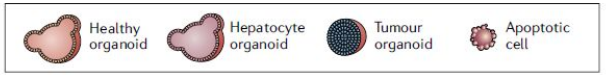
From the human colon sample, genetically modified organoids can be obtained in 3 weeks.

LeviCell can enrich for organoids

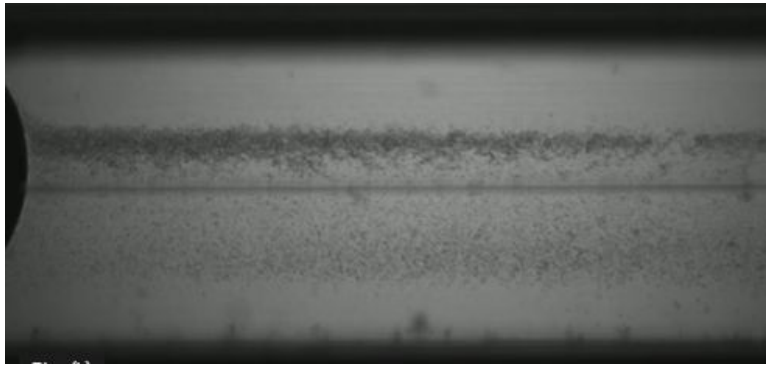
Example:
after drug treatment



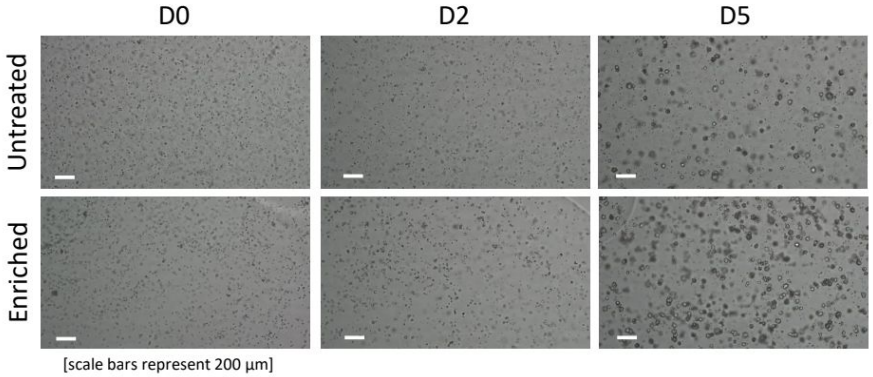
LeviCell can select the live resistant cells after a drug treatment and collect them for further analysis or evaluation of the treatment and toxicity



Live Dissociated Organoid Cell Enrichment

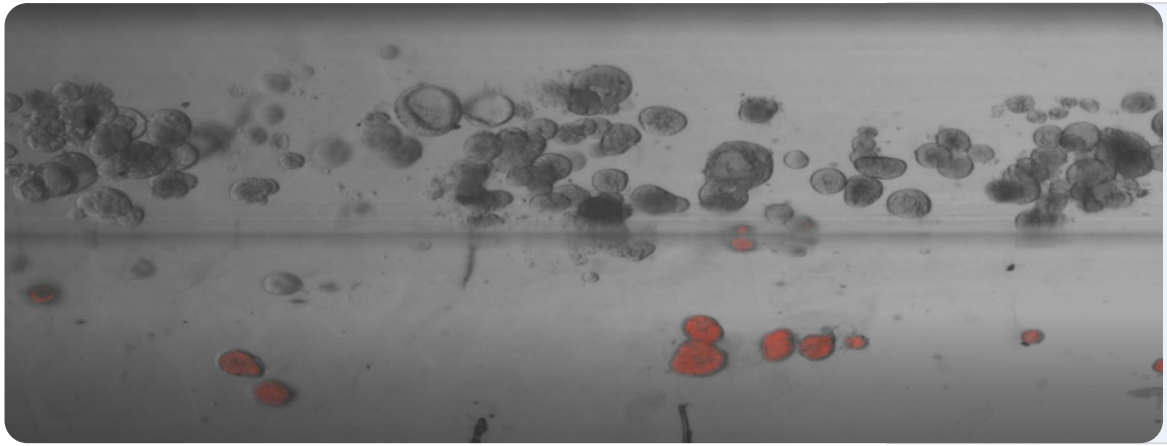


High-viability **(>80%)** colorectal dissociated organoid cells levitating… BUT….



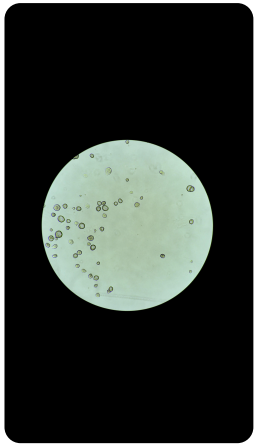
Enriched cells generate substantially more particles!

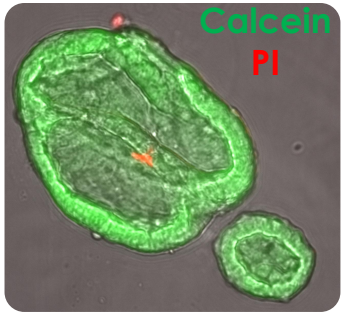
Gentle Separation of 3D Human Gastric Organoids for Viability

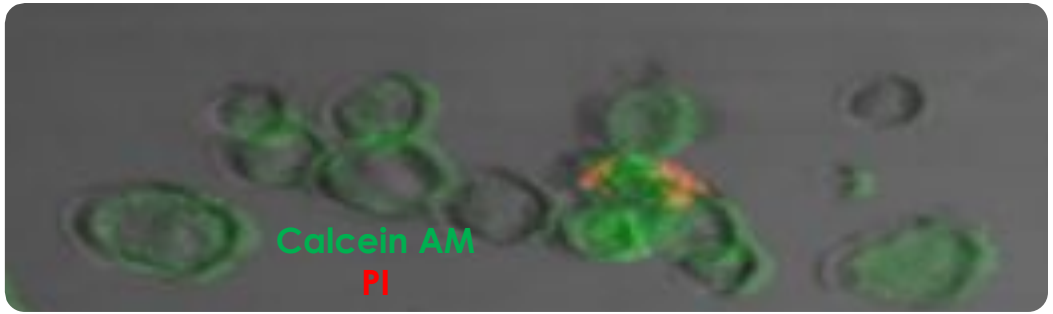


Live Gastric organoids

Killed Gastric organoids







Current Challenges in Spheroid Culture

Spheroid formation take multiple days to weeks

Heterogeneity (shape, size and density is not controlled or uniform)

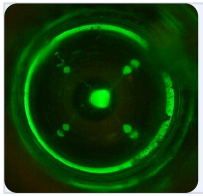
Low reproducibility

Difficult to exchange media

- Often resulting in loss of spheroids
- Time and labor intensive

LevitasBio Technical Advantage

- Able to shape spheroid in 2 hr compared to the usual spheroid culturing methods, 1-5 days (depending on cell type).
- Shape, size, and density can be manipulated by levitation agent concentration and levitation time and magnet array
- **High throughput compatibility with LC96:** "Wash Protocol" can be used to perform a complete media exchange.
- Influence morphology of spheroids by changing magnet configuration.
- Potential to multiplex spheroid formation in each well by modifying magnetic configuration



Example of a 5-cluster well.

96 Well Organoid Cell Culture

- HepG2 was levitated in LeviCell 96 at 50 and 100 mM Levitation Agent
- Samples were moved into spheroid culture plate after 2 and 24 hr for culturing for 7 days
- Samples were also cultured in LeviCell 96 for 7 days
- No levitation controls were grown on the spheroid culture plate
- No levitation agent controls were also grown in the LeviCell 96

Cell Culture Results

Spheroid structure can be obtained after 2 hrs of levitation

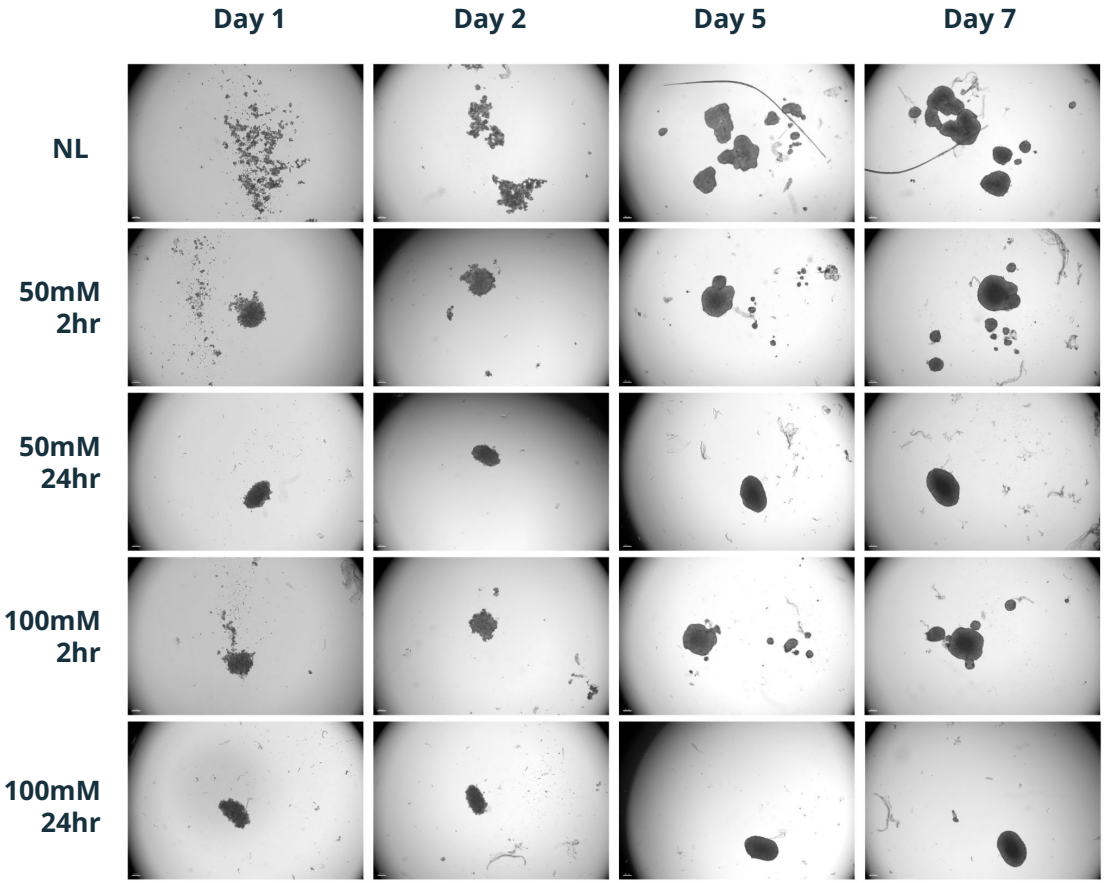
> No Levitation Control shows slower spheroid formation at Day 5

Levitation allows faster and better control of spheroid shape, size and density

> Spheroids are more uniform after levitation in comparison to no levitation control

> Varying the levitation agent concentration can alter the density of the spheroid

Spheroids tested on cell line HepG2



LeviCell 96 Spheroid and Organoid Applications

Cell Culture Spheroid Production

- Rapid production with ‘hands-free’ automation with consistent shape/size/density
- Tumor spheroids and organoids production
- Built-in viability information/monitoring with imaging array (future capability)

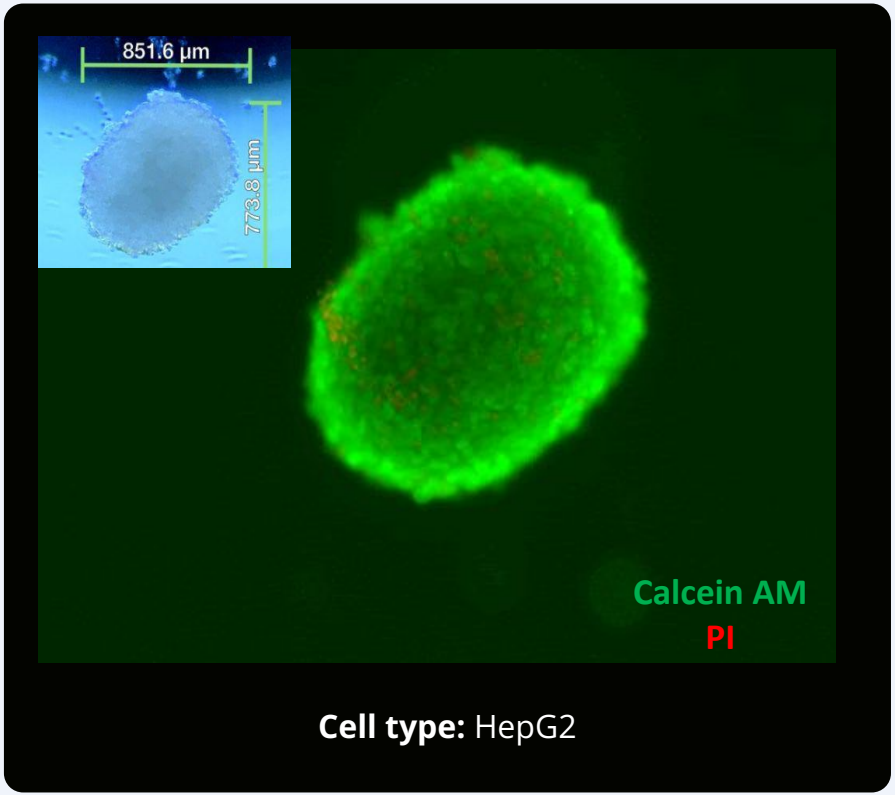
Spheroid Shape Engineering and “Assembloids”

- Elongated shape will benefit studies of intestinal diseases
- Potential customized shape by request
- Assembloids from multiple cell types

In Vitro Drug Study

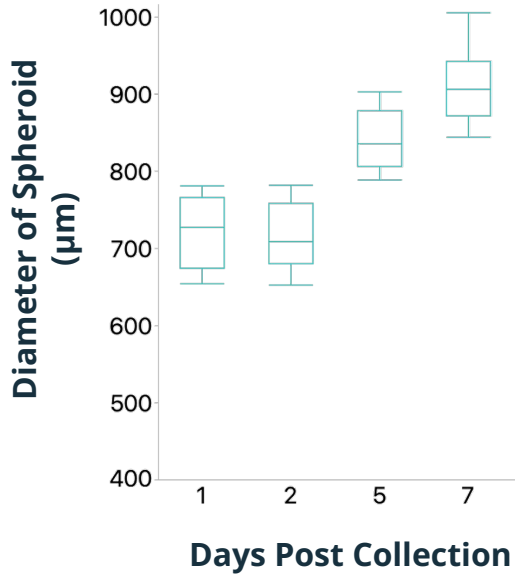
- In vitro drug studies are faster and more efficient
- Viability enrichment of starting cells, or already-formed organoids

Spheroids Formed on the Levitation Array are Highly Viable

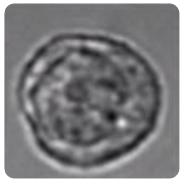


Cell type: HepG2

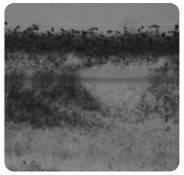
Cells were levitated for just 2 hrs



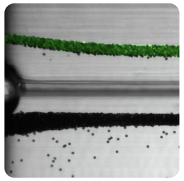
Seeing the Effects of a Cell Based Assay in Real Time



Record cell to cell interactions or cell activity without any loss of information

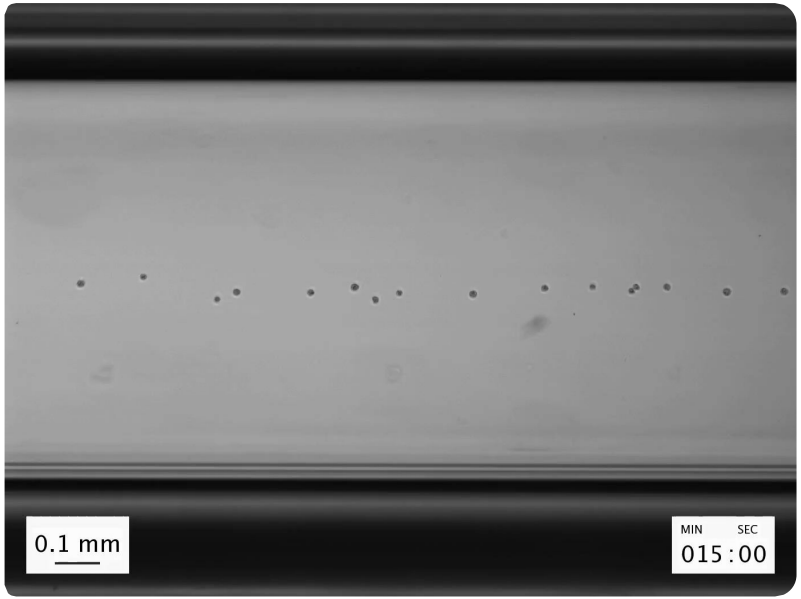


Visualize the entire sample in real-time

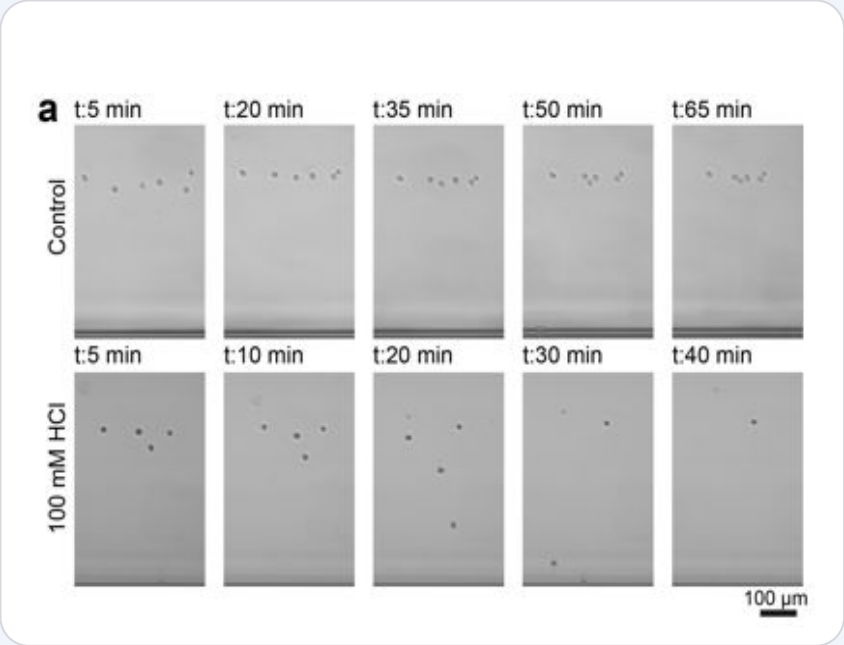


Collect population of interest after analysis

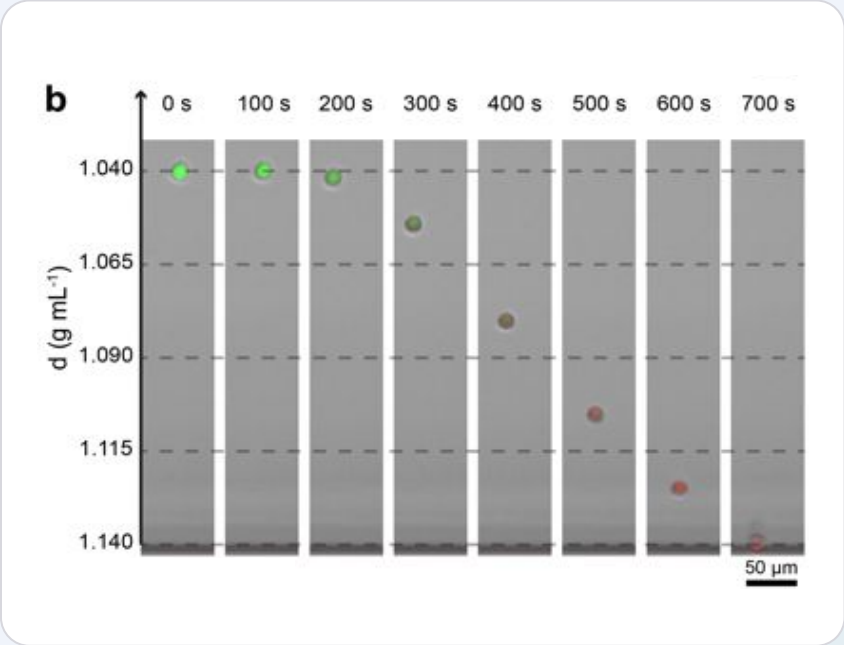
Real-Time



Fast, Real-time Drug Screening Response

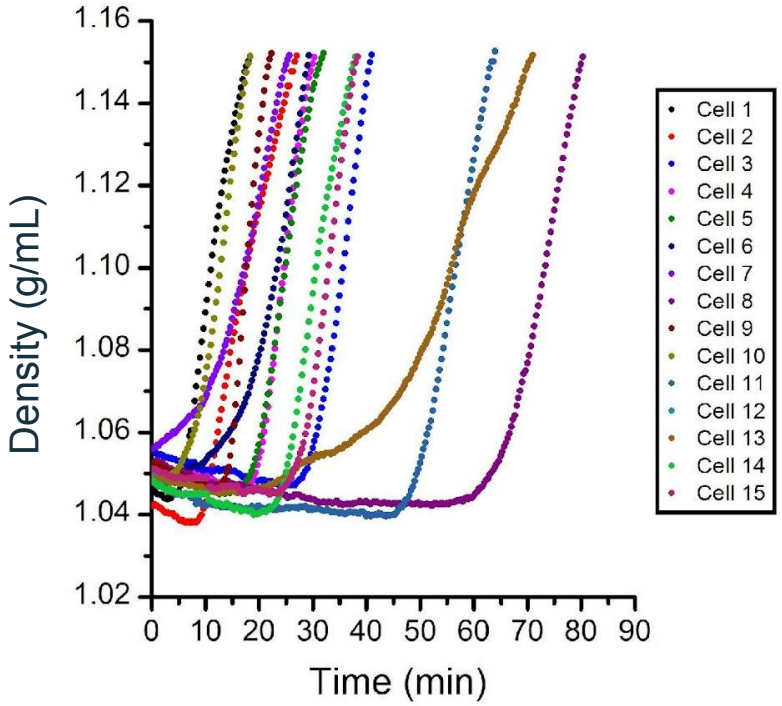


Durmus et al., PNAS, 2015.



Green: Calcein (Live Cell)
Red: Propidium Iodide (Dead Cell)

Cell Death is Detected in Real-Time at Single-Cell Level



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Thank You.

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