Automated selection, isolation and dispensing of standardized organoids, spheroids, and tumoroids, for enhanced 3D-model assay reproducibility and quality

CELLENION
A BICO COMPANY

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Introduction

Complex three-dimensional (3D) *in vitro* models, in particular spheroids, tumoroids and organoids, are now used extensively, from fully fundamental physiology research to pharmaceutical and applied medicine. They allow for instance the development of functional assays for drug discovery, or prediction of patient-dependent response to treatments, yielding highly predictive results and reducing the use of animal models.

However, 3D model handling comes with many challenges:
- 3D models are fragile and difficult to manipulate.
- Imaging 3D models can be tricky (overlaying objects).
- Standard workflows (ULA plates) have low throughput.
- Current workflows generate high spheroid/organoid heterogeneity (size, biomass and functionality), which reduces assay reproducibility, making interpretation difficult.

Here, we present an automated cellular aggregate sorter and dispenser (spheroONE®) that enables rapid and standardized 3D spheroid, organoid and tumoroid sample preparation.

spheroONE workflow example
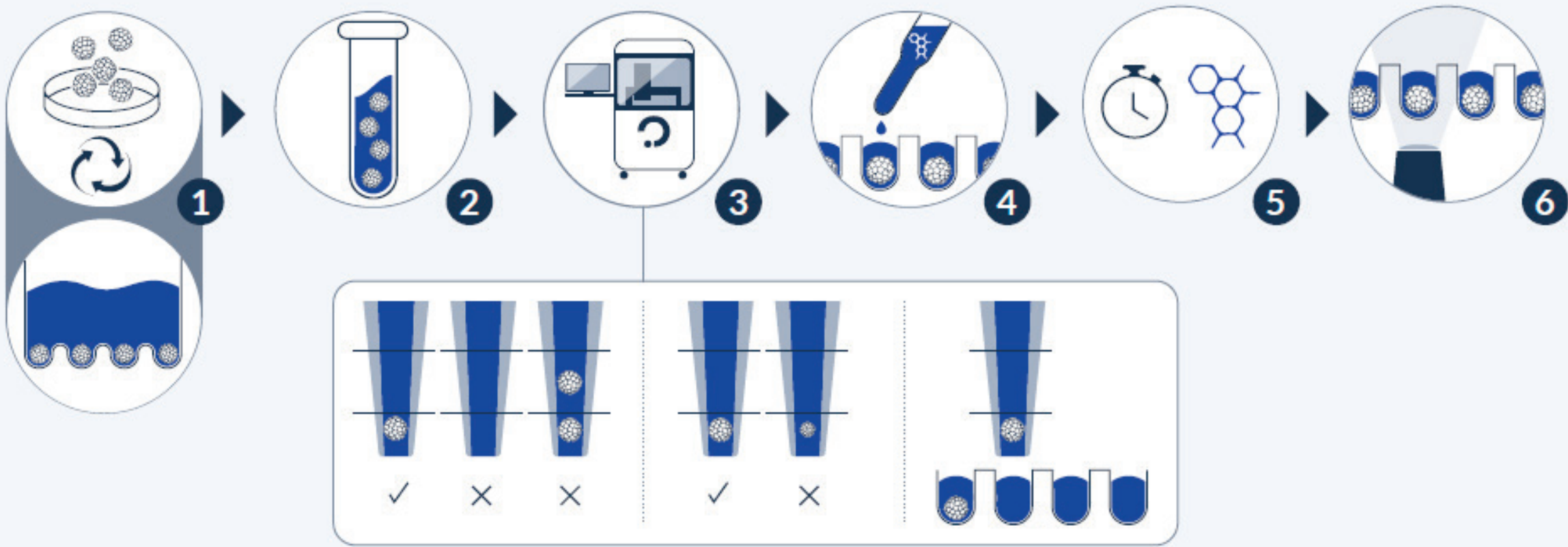
1. 3D model formation in bulk (liquid overlay, microwell array…)
2. Collection of 3D model suspension
3. Loading 0.5-5 mL into the spheroONE: isolation of n object(s) in each well
4. Addition of treatment to individual wells containing standardized 3D models
5. Incubation
6. Assay read-outs

The spheroONE technology

The spheroONE is the combination of:

Precision liquid dispensing: gentle drop-on-demand dispensing, with highly reproducible volumes (~ 500 nL/drop, CV < 3%), compatible with any kind of target substrates (standard or custom).
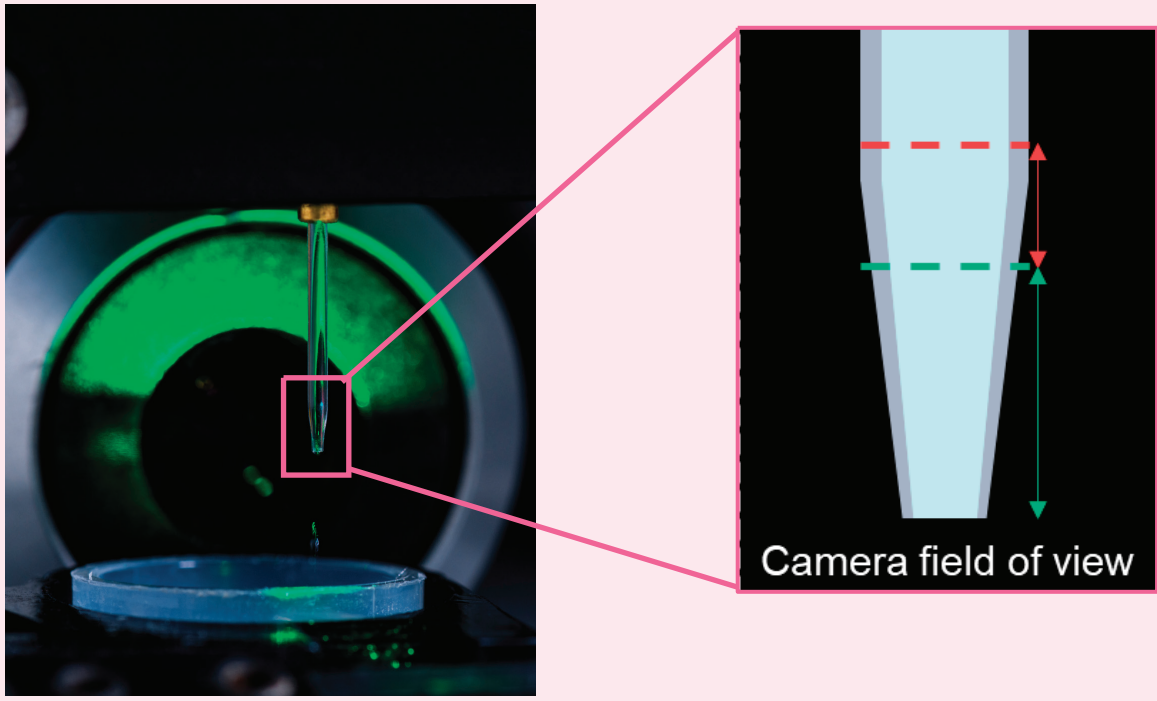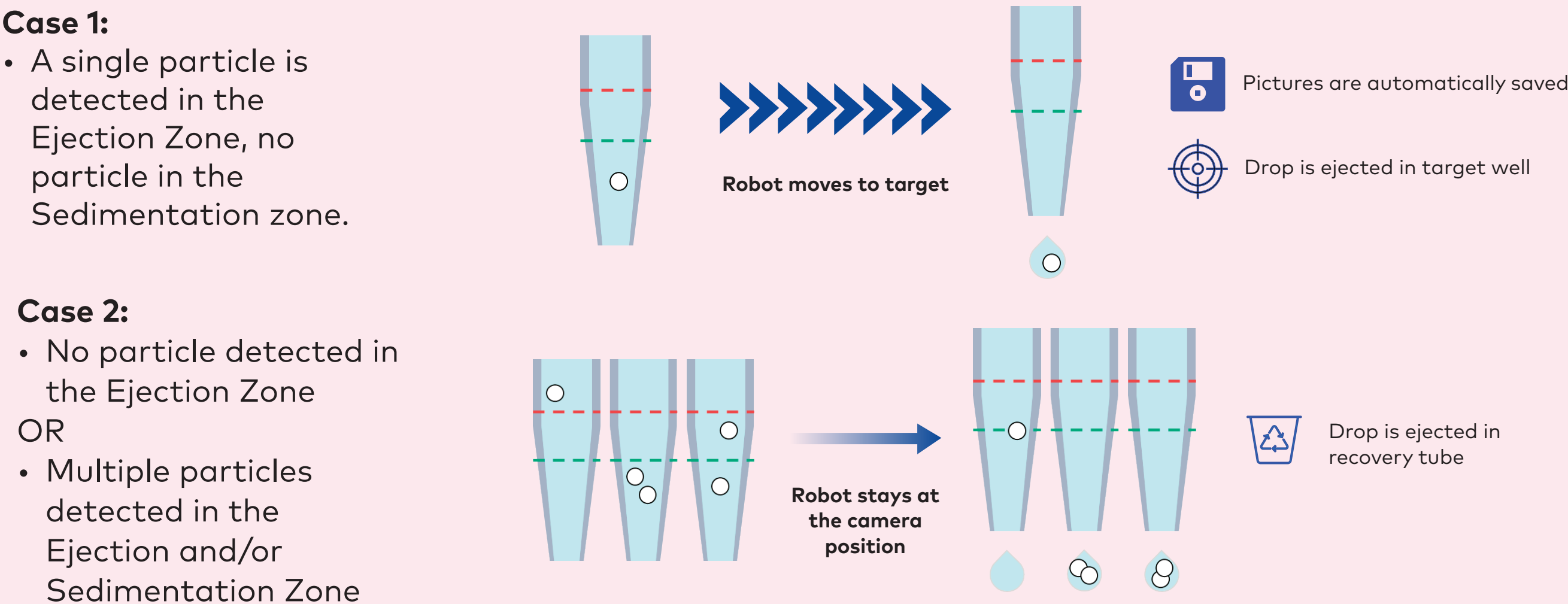

- Sedimentation zone: Safety area to account for particle sedimentation
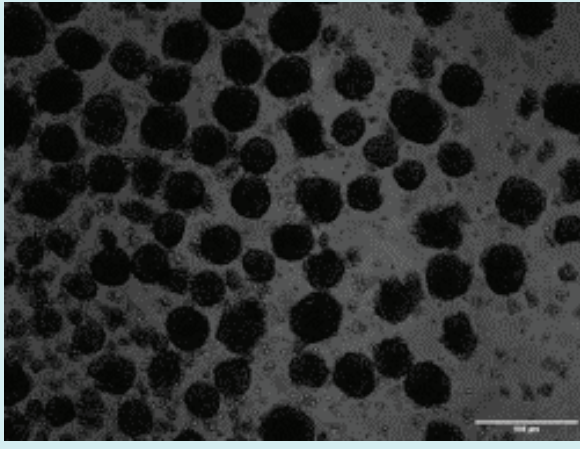- Ejection zone: Area corresponding to the volume of the next drop

Darkfield illumination imaging: differential diffraction patterns according to 3D model morphology.

Dark field diffraction pattern

spheroids

Cystic organoids
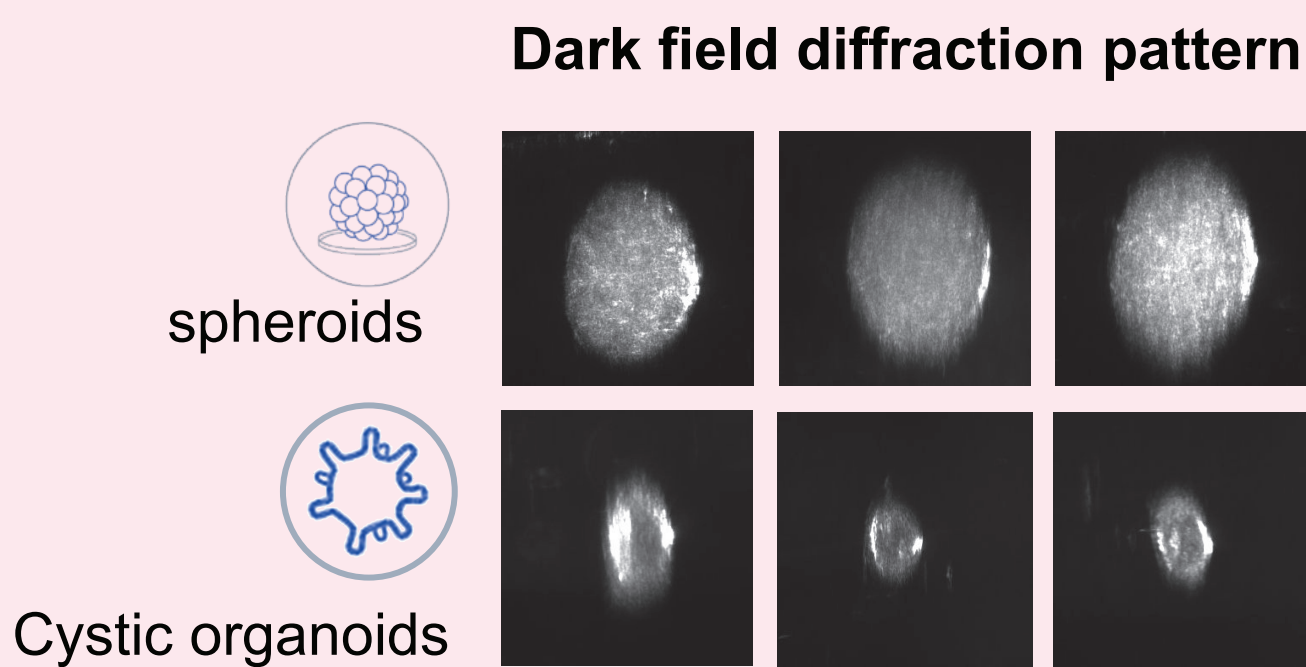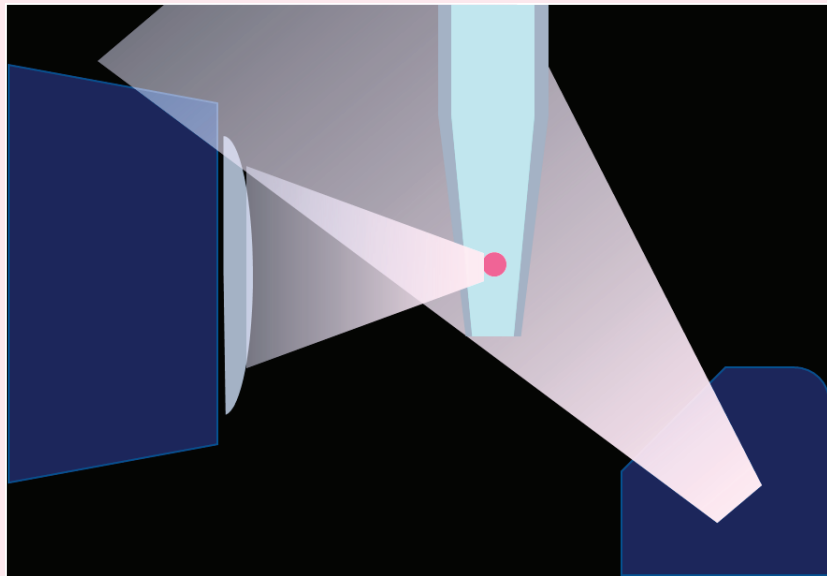
Image-based automated decision logic for isolation, sorting and dispensing. A mapping procedure empirically determines the Ejection Zone (corresponding to the volume of the next drop). A sedimentation Zone is added by the software to account for particle sedimentation. Only when a single particle is detected does the robot move on top of the next target well, ensuring a single particle is dispensed in it.

Case 1:
- A single particle is detected in the Ejection Zone, no particle in the Sedimentation zone.

Robot moves to target
- Pictures are automatically saved
- Drop is ejected in target well

Case 2:
- No particle detected in the Ejection Zone
OR
- Multiple particles detected in the Ejection and/or Sedimentation Zone

Robot stays at the camera position
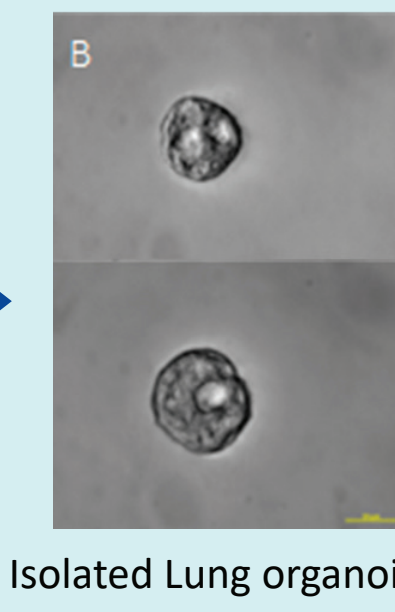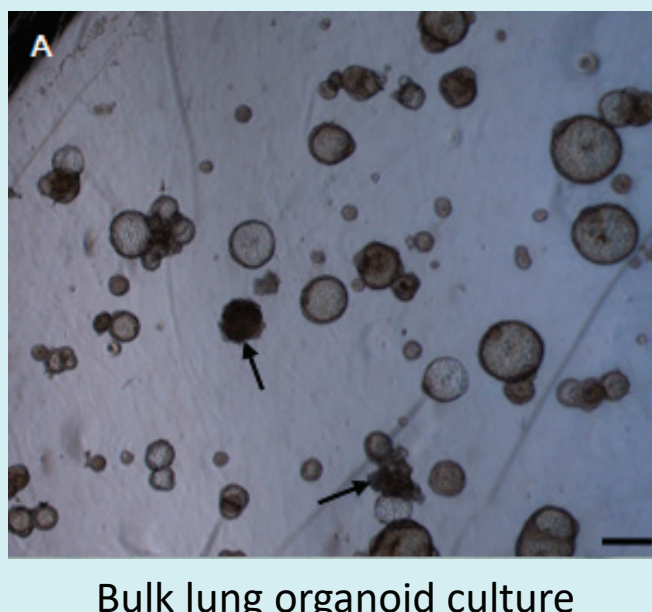- Drop is ejected in recovery tube

Accurate 3D Model Isolation

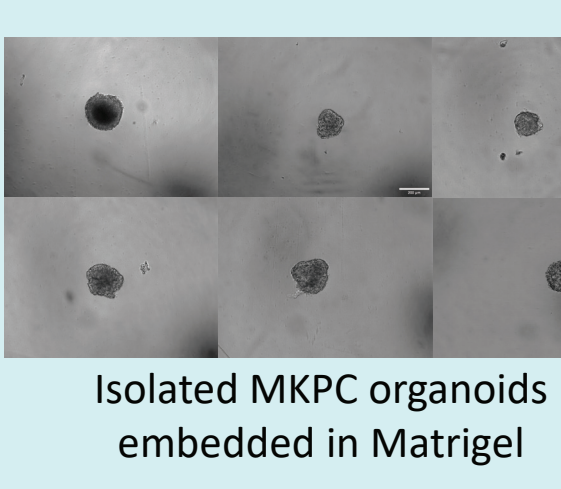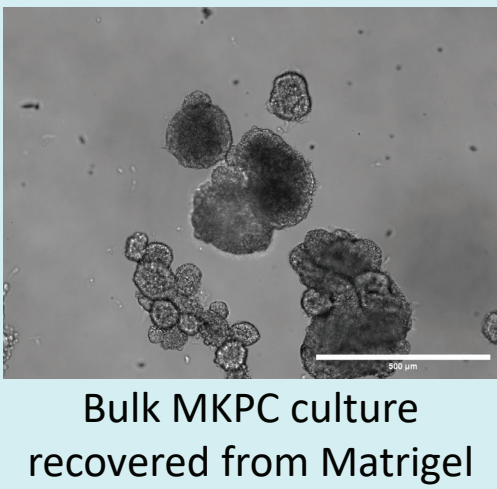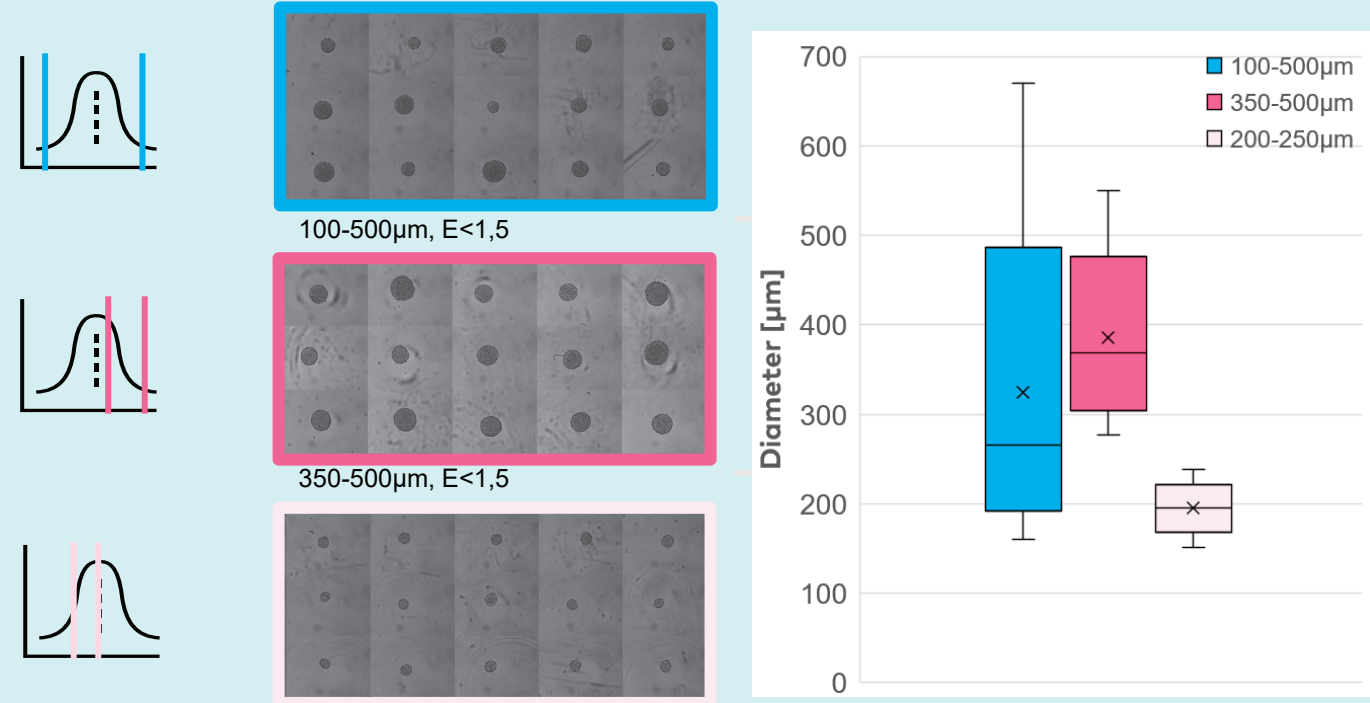Brightfield and DAPI images of n = 1, 3 or 5 HEK spheroid(s) per well. Scale bar = 500 µm.
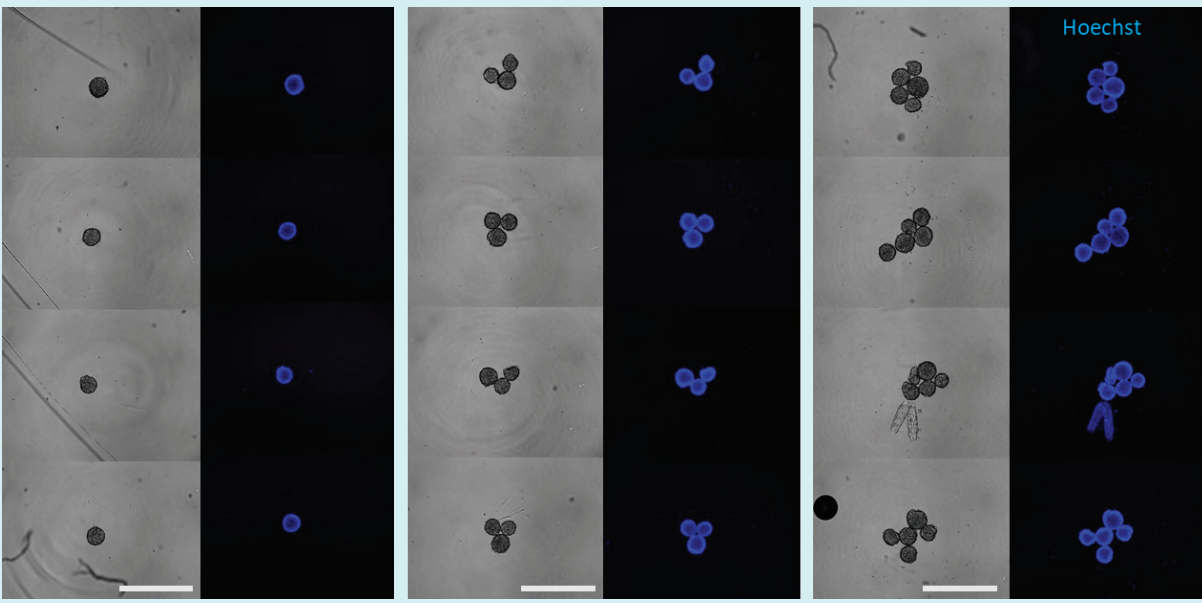
- From bulk culture to 1 -> *n* 3D models per well
- Accuracy: >90% accurate isolation guaranteed

From highly heterogeneous sample to standardized plates

Bulk lung organoid culture → Isolated Lung organoids

Bulk MKPC culture recovered from Matrigel → Isolated MKPC organoids embedded in Matrigel

- User-defined sorting by size and shape
- Removal of debris and dead cells

Size Gating

100-500µm, E<1,5
350-500µm, E<1,5
200-250µm, E<1,5

Maximized 3D model functionality for high-quality and reproducible 3D model assays

Generation of assay-ready plates containing one single HepaRG™ spheroid per well.

In collaboration with:
Biopredic INTERNATIONAL

HepaRG™ cells:
- Adult-phenotype human hepatic cell line (HPR116)
- Fully functional, differentiated
- Ideal model for hepatotoxicity tests
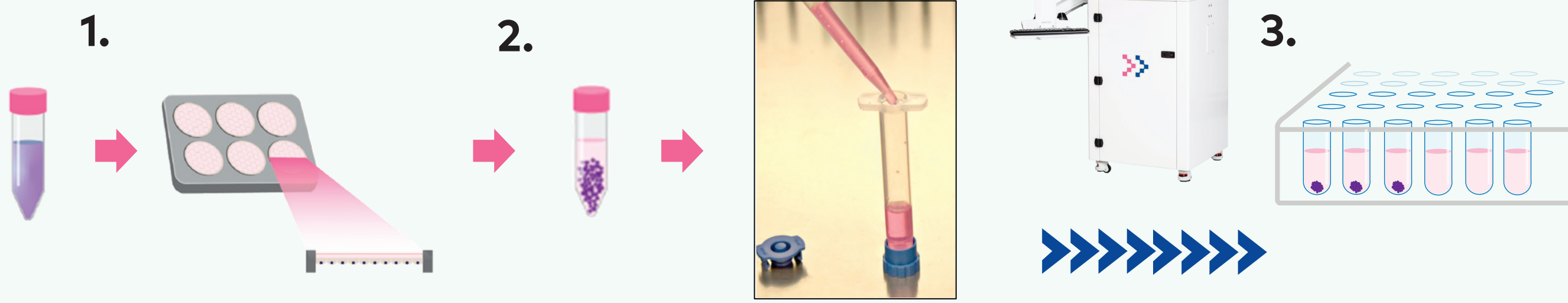
1. Plating HPR116 cells in MIL610 medium (Biopredic) in Elplasia plates and incubation 4 days at 37°C.

2. Collection of HepaRG™ spheroids, resuspension in PBS and loading into spheroONE reservoir.

3. Single HepaRG™ spheroids isolation into 384-well ULA plates prefilled with MIL620 medium (Biopredic), gating size 200-250 µm.

4. Functionality Testing
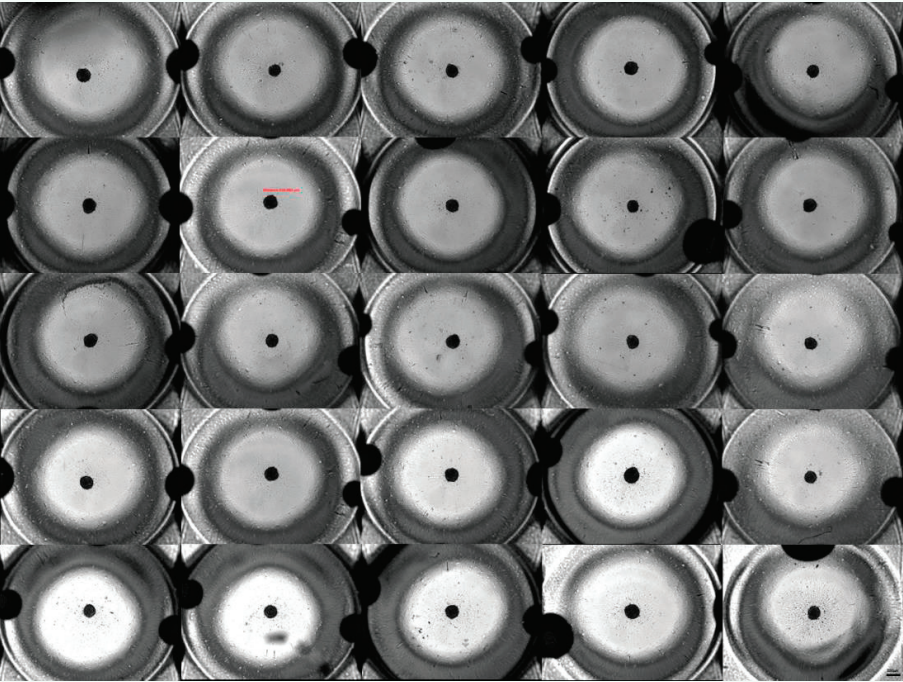
Single HepaRG™ spheroids had:
- Expected size and good integrity (tightly packed, uniform).
- Albumin secretion: 55 +/- 4 ng/mL
- ATP production: 1032 +/-23 nM
- Excellent viability (97% "live" in live/dead assays)
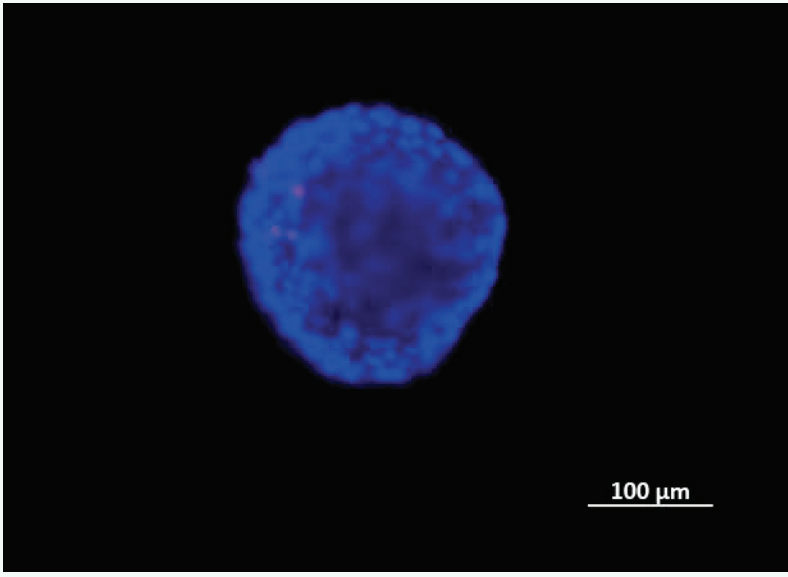
- High post-isolation integrity, viability and functionality
- Direct embedding into Matrigel (target temperature control)
- Less debris and dead cells
- Improved imaging and readouts (all particles on same focal plane + high precision positioning)

0.44 mm
1 spheroid per microwell
2 different spheroids per well
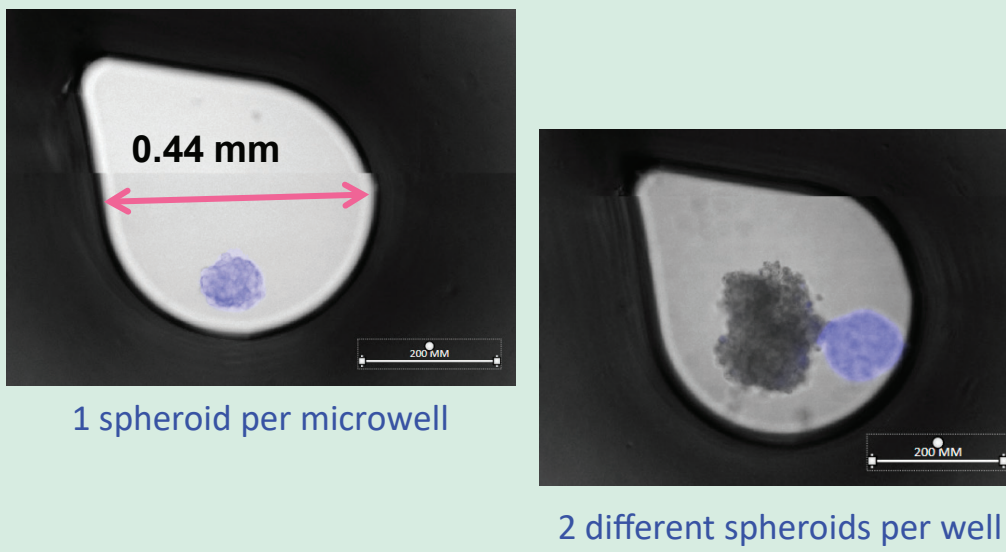
Conclusion

- Sorting and isolation of aggregates of interest for highly homogenous populations, defined bio-assemblies, and biomass control.
- Gentle dispensing: functional 3D models, no waste.
- Imaging made easy by spheroid/organoid isolation and precision positioning.
- Throughput: up to 20 spheroids / min.
- Enhanced 3D-model assay reproducibility and quality.