

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

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Introduction

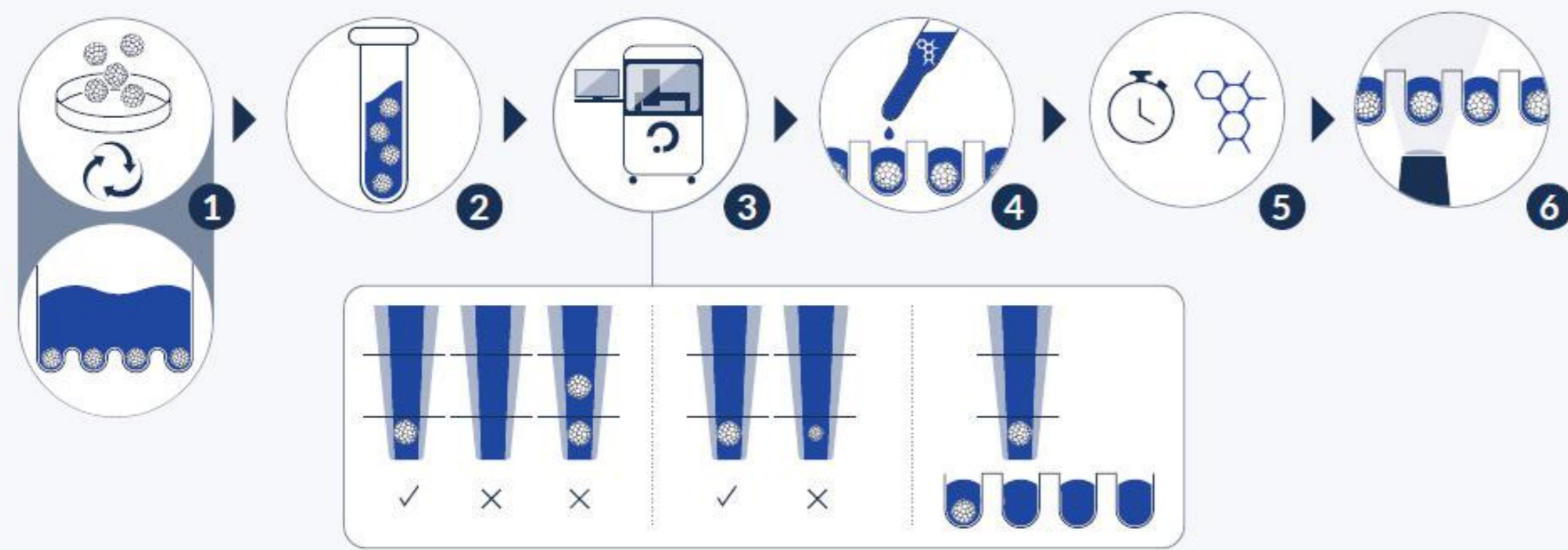
Complex three-dimensional (3D) *in vitro* models, in particular spheroids, tumoroids and organoids, are now used extensively, from fully fundamental physiology research to pharmaceutical and applied medicine. They allow for instance the development of functional assays for drug discovery, or prediction 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 (overlapping 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

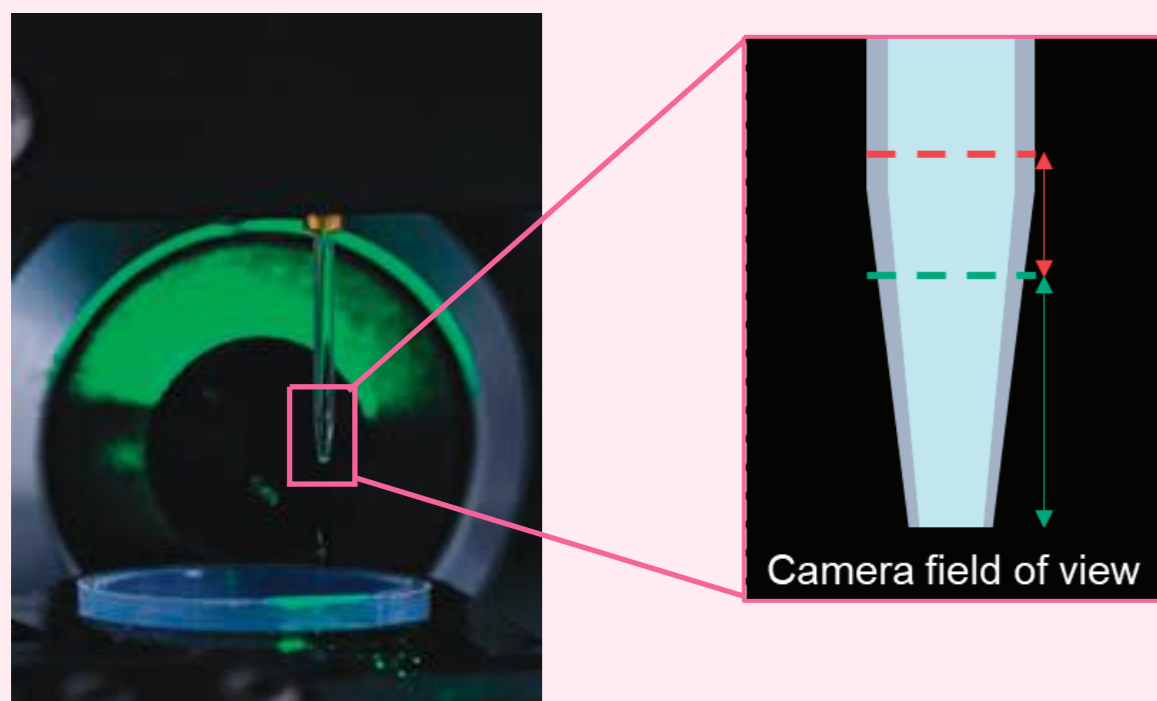


- 3D model formation in bulk (liquid overlay, microwell array...)
- Collection of 3D model suspension
- Loading 0.5-5 mL into the spheroONE: isolation of n object(s) in each well
- Addition of treatment to individual wells containing standardized 3D models
- Incubation
- Assay read-outs

The spheroONE technology

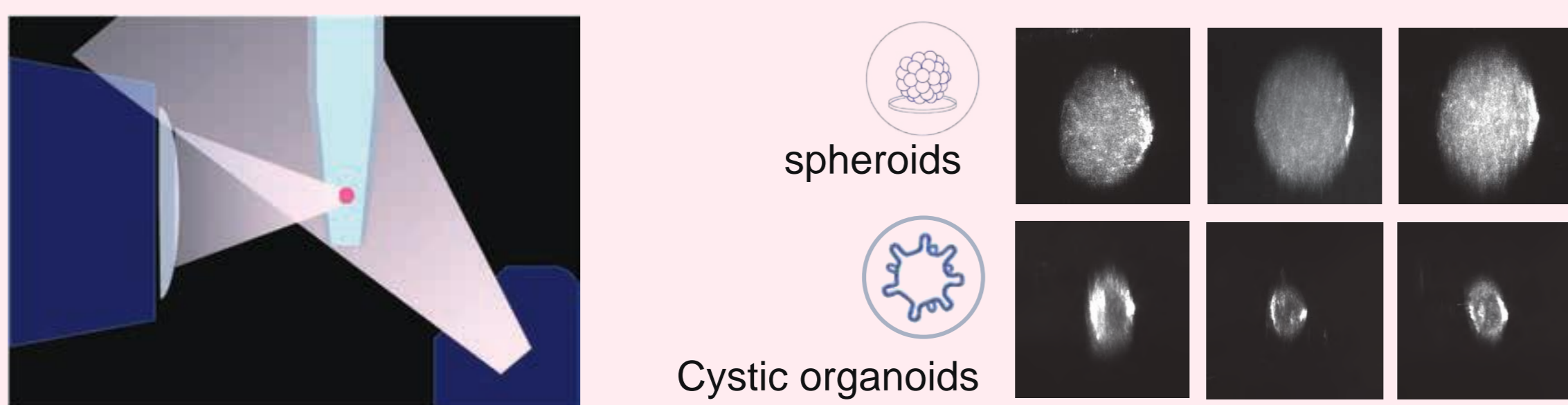
The spheroONE is the combination of:

- 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).

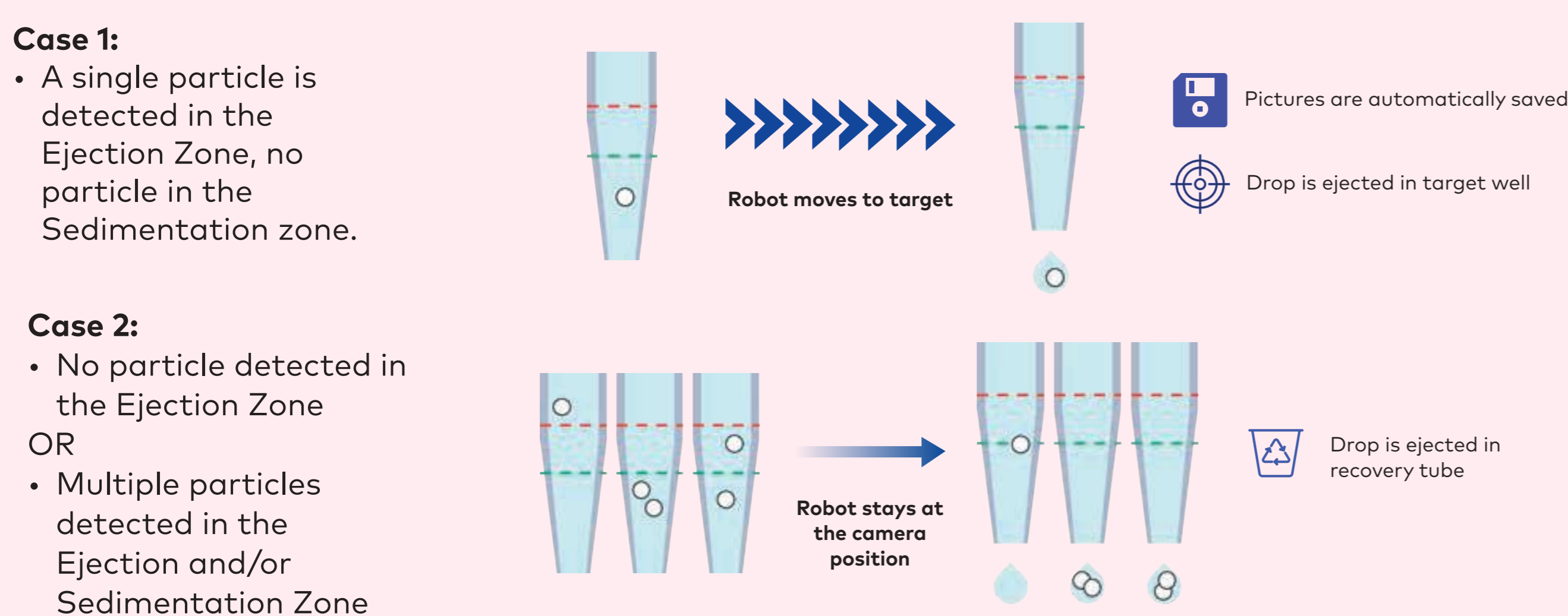


- Sedimentation zone:** Safety area to account for particle sedimentation
- Ejection zone:** Area corresponding to the volume of the next drop

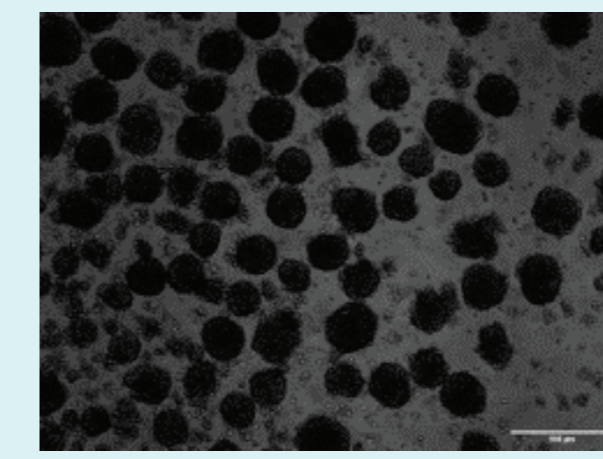
- Darkfield illumination imaging:** differential diffraction patterns according to 3D model morphology.



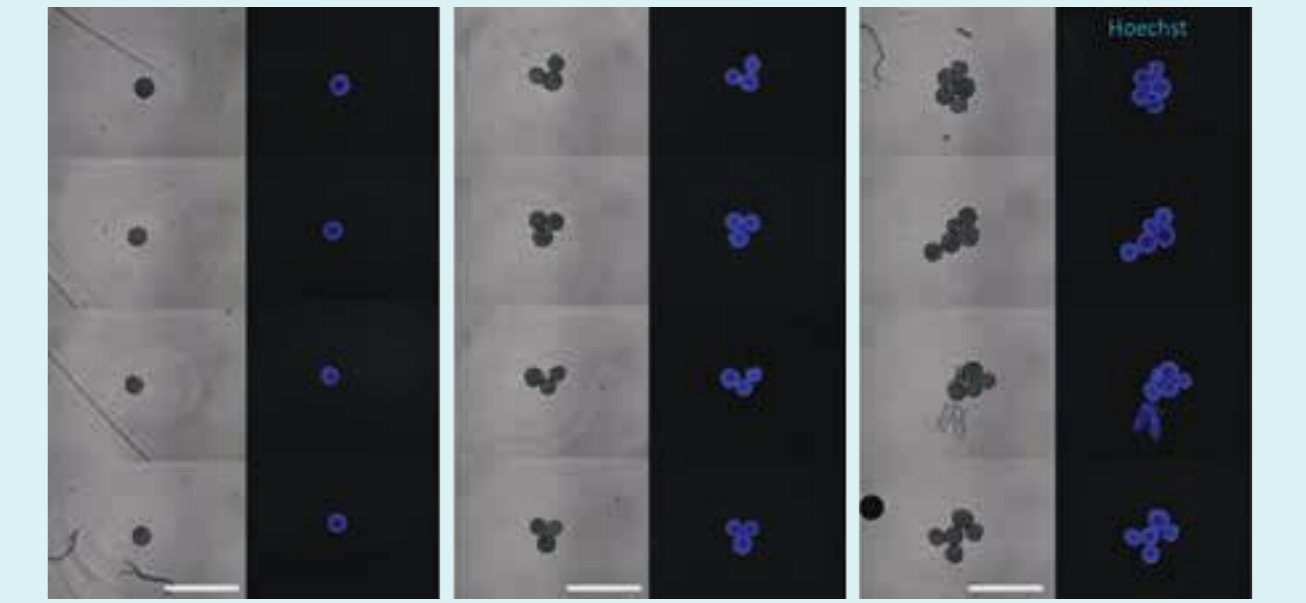
- Image-based automated decision logic** for isolation, sorting and dispensing. A mapping procedure empirically determines the Ejection Zone (corresponding to the volume of the next drop). A sedimentation Zone is added by the software to account for particle sedimentation. Only when a single particle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



Accurate 3D Model Isolation

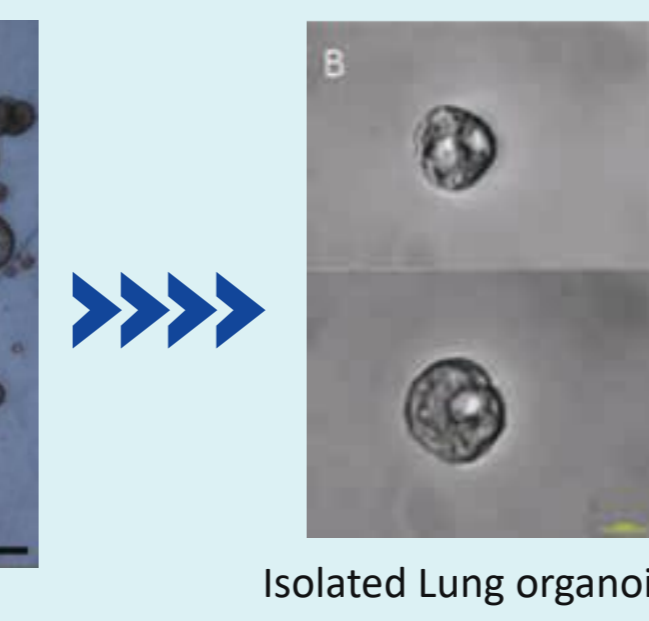


Brightfield and DAPI images of n = 1, 3 or 5 HEK spheroid(s) per well. Scale bar = 500 µm.

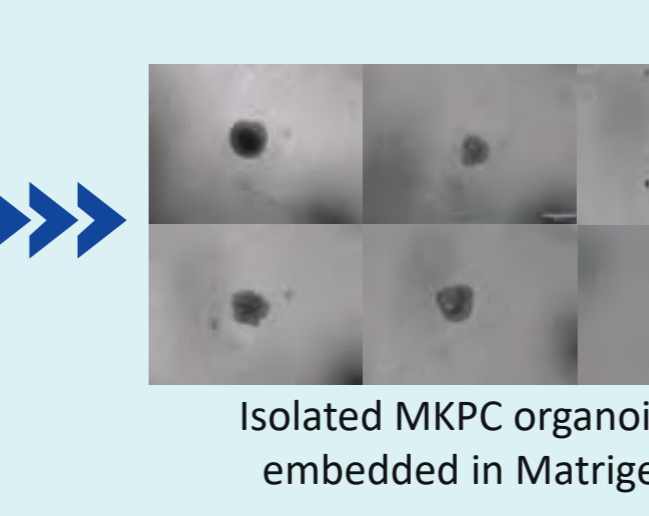


- From bulk culture to 1 -> n 3D models per well
- Accuracy: >90%** accurate isolation guaranteed

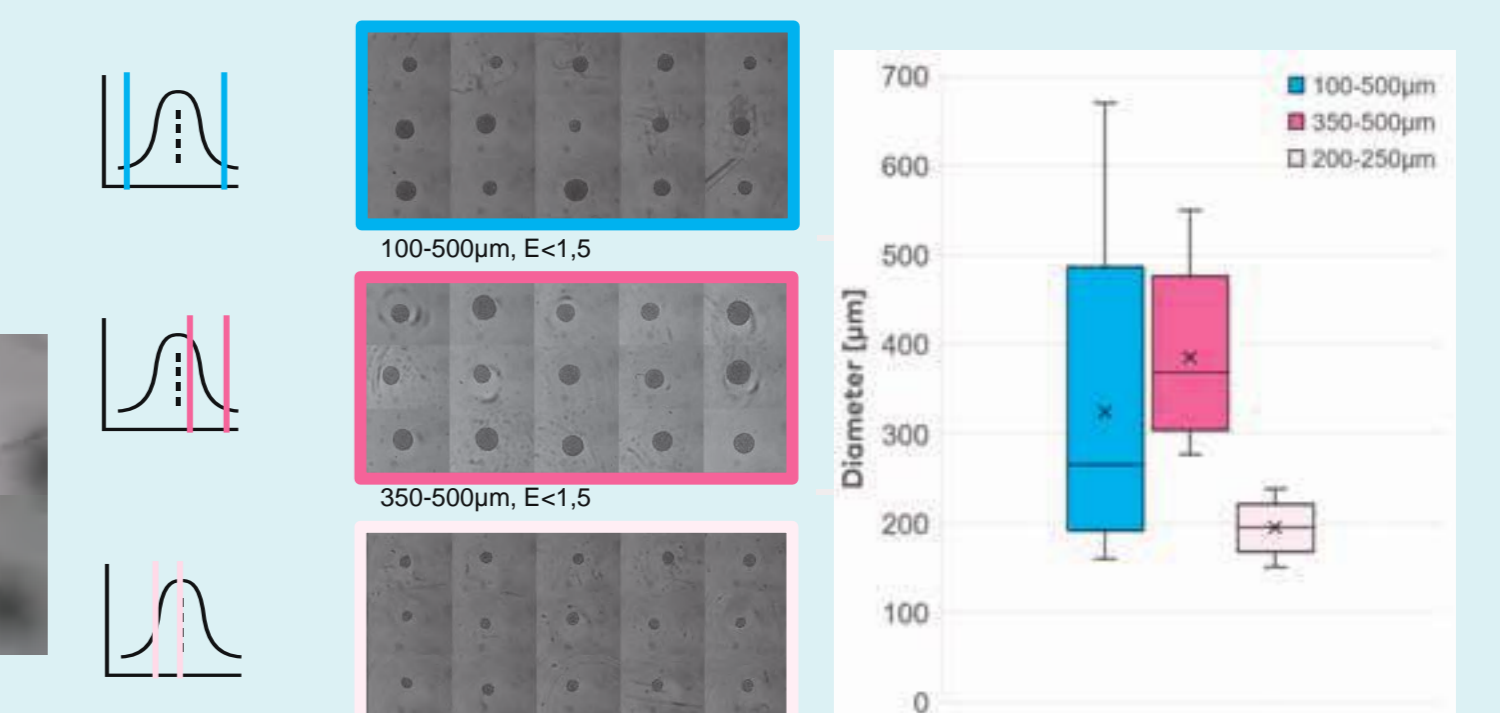
From highly heterogeneous sample to standardized plates



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



Size Gating



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

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

In collaboration with:



- HepaRGTM cells:**
- Adult-phenotype human hepatic cell line (HPR116)
 - Fully functional, differentiated
 - Ideal model for hepatotoxicity tests

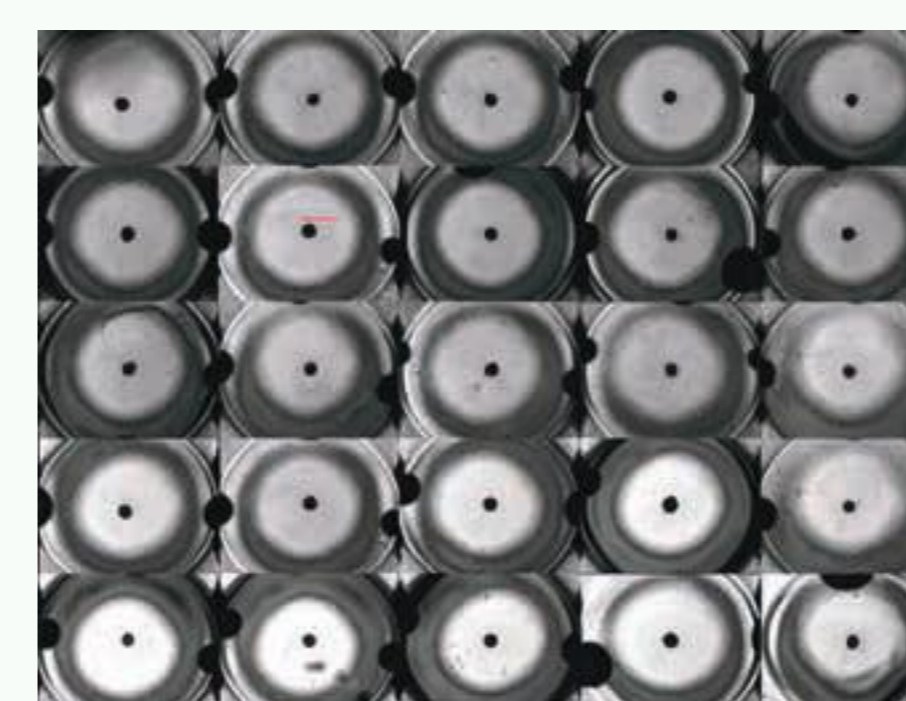


Plating HPR116 cells in MIL610 medium (Biopredic) in Elplasia plates and incubation 4 days at 37°C.

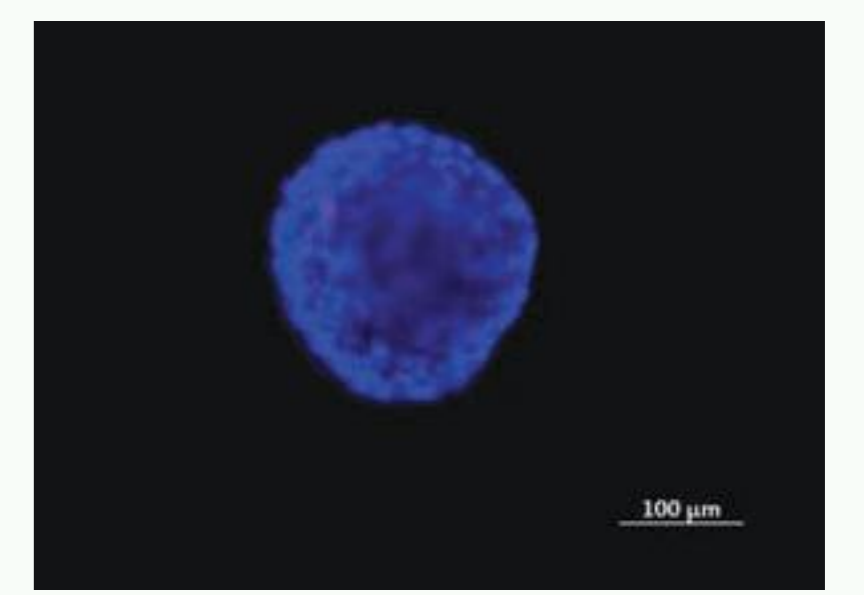
Collection of HepaRGTM spheroids, resuspension in PBS and loading into spheroONE reservoir.

Single HepaRGTM spheroids isolation into 384-well ULA plates pre-filled with MIL620 medium (Biopredic), gating size 200-250 µm.

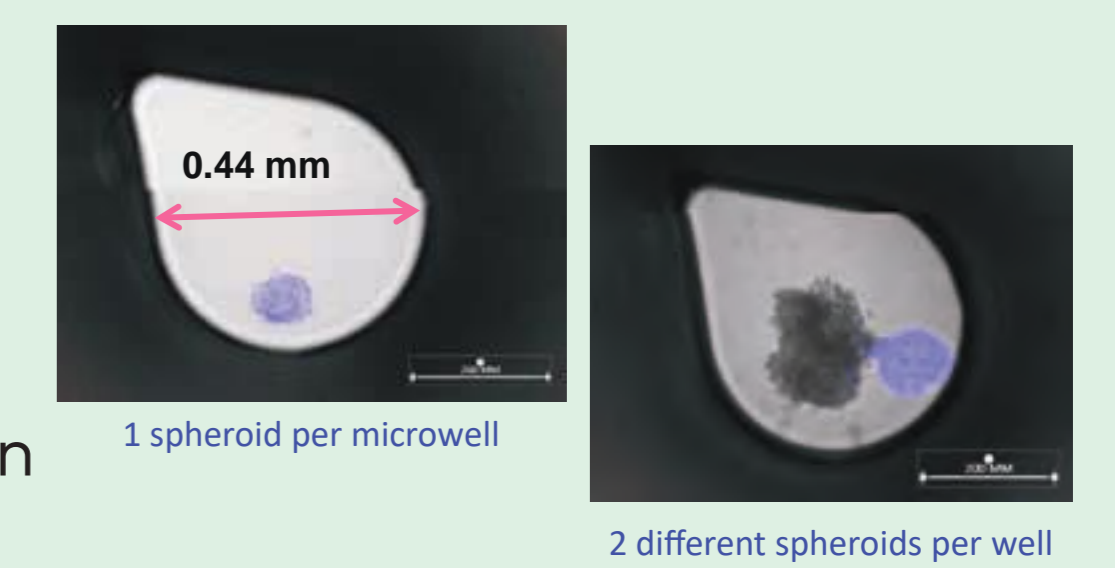
4. Functionality Testing



- Single HepaRGTM 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)



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.