Bolster your confidence with your 3D cellular aggregates assay with automated bulk spheroids and organoids isolation and sorting

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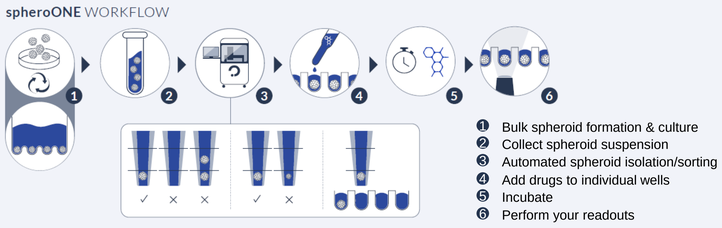
Overview

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

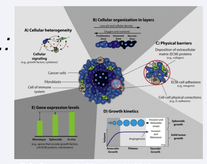


❶ Bulk spheroid formation & culture
❷ Collect spheroid suspension
❸ Automated spheroid isolation/sorting
❹ Add drugs to individual wells
❺ Incubate
❻ Perform your readouts

Introduction

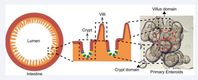
Complex three-dimensional (3D) *in vitro* models, in particular spheroids, tumoroids and organoids, offer unprecedented means to yield highly predictable models of healthy and diseased tissues and organs.

- **Cancer spheroid** mimic *in vitro* several hallmarks of solid tumor, *i.e.:*
  - Drug resistance
  - Differential gene expression
  - Hypoxia/necrotic core



E.C. Costa *et al.*, *Biotech Adv*, 2016

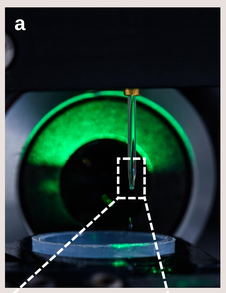
- Stem cell-derived **organoids** replicate native tissue/organ
  - Phenotype
  - Morphology
  - Physiological function

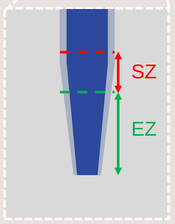


► More predictive models for drug screening and basic research
► Reduced use of animal models

Method

Automated isolation, sorting and dispense of 3D cellular aggregates





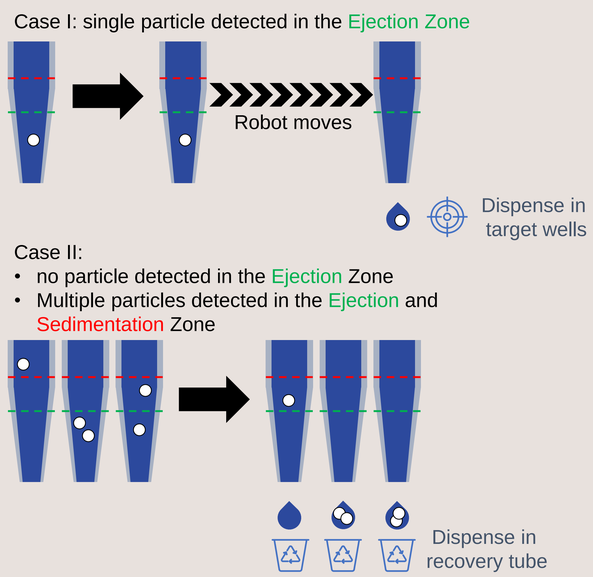


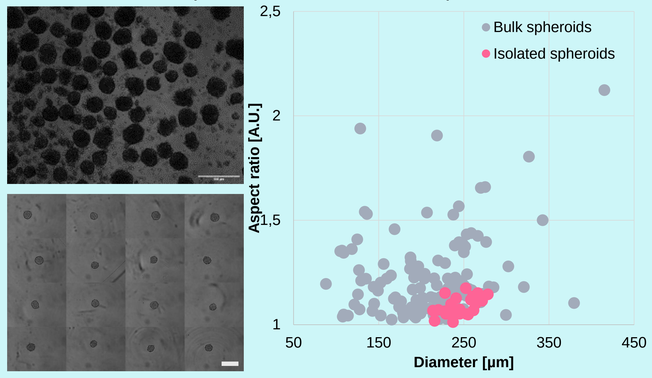
Figure 1: *Image-based automated single large-particle isolation.* ***a****. Picture and schematic representation of the Nano dispensing capillary (NDC) in front of the camera. A Mapping procedure, which tracks objects inside the capillary as droplets are continuously dispensed allows to determine empirically the Ejection Zone (EZ) (ca. Area corresponding to the volume of the next drop). A sedimentation Zone (SZ) is added by the software to account for particle sedimentation.* ***b****. Schematic representation of automated image-based particle isolation, ca. (i) 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, and (ii) if no particle or multiple particles are detected, the next drop is dispensed directly into a recovery vial lying directly below the NDC.*

Cell culture & bulk spheroids formation

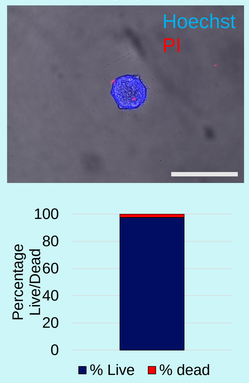
HEK (or HEK-GFP) cells were cultured in DMEM media supplemented with 10% FBS and antibiotics (Penicillin/streptavidin). HEK spheroid were prepared by liquid overlay cell culture on non-adherent surface under constant agitation for 3-7 days. Culture media was exchanged every second day. Before processing on the spheroONE, spheroid suspension. Pre-differentiated intestine organoids (day 4) were prepared on Gri3D plate and kindly provided by SunBiosciences.

Automated spheroid isolation and dispense

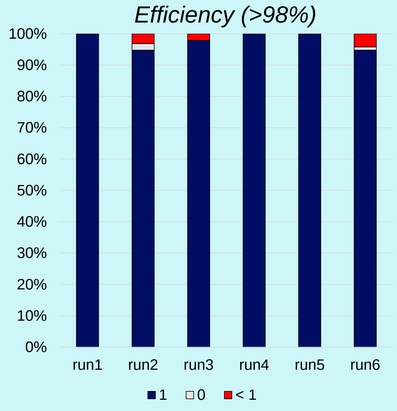
Bulk spheroid isolation and dispense



Viability (>97%)

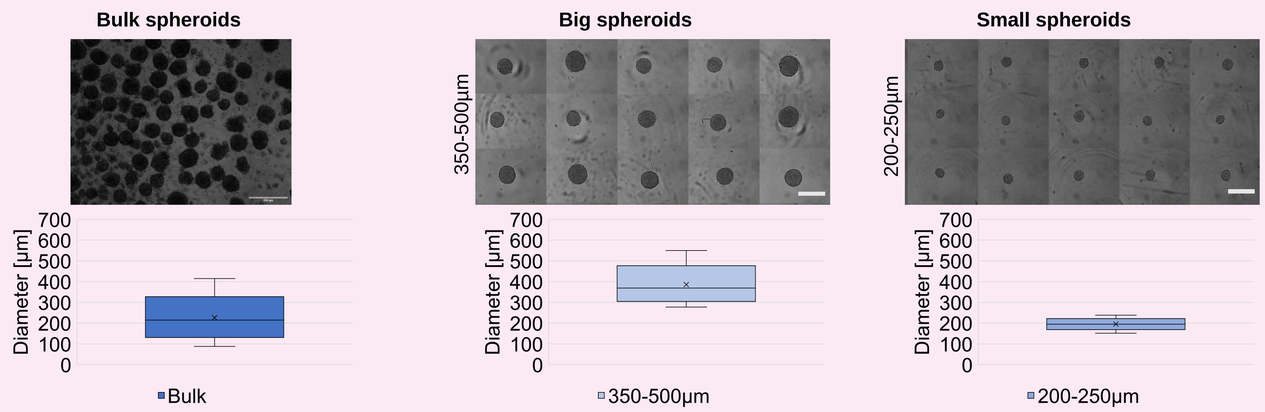


Efficiency (>98%)



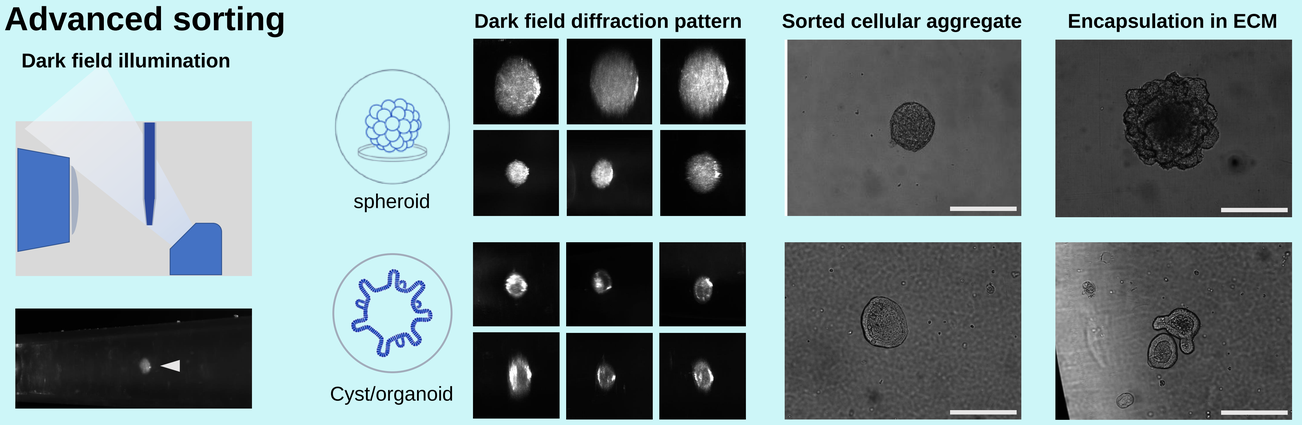
► Accuracy: up to 100% single spheroid isolation
► Gentle dispensing maintains aggregate integrity and viability

Cellular aggregate sorting by size



► User-defined sorting by size
► Achieve enhanced well-to-well sample homogeneity

Advanced sorting



► Sort aggregates based on diffraction pattern to specifically select cysts and lumenized organoids (Intestine)
► Re-encapsulate aggregates in ECM using pre-filled target plate placed on chilled (4°C) plate holder

Conclusion

Accuracy
- Up to 100% single spheroid per well

Homogeneity
- User-defined sorting of spheroids by size and shape
- Enables to get highly homogeneous spheroids in each wells

Advanced sorting
- Selection of cysts or lumenized organoids based on diffraction pattern

Compatibility with organoid workflow
- Encapsulation of cellular aggregates in ECM gel

Versatility
- Open-platform
- Any standard well plates (*i.e.* 96, 384) or specialty/custom designed labware
- Temperature of target labware and environmental humidity control

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