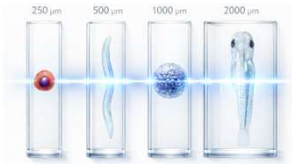
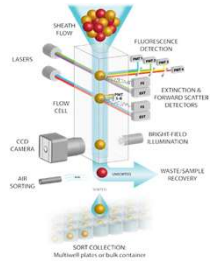
Image-assisted Large Particle Flow Cytometry and Plate-Based Sorting of Intact 3D Models to Support Reproducible New Approach Methodologies

Megan S. Harrison[1], Deborah R. Frenkel[1], Yongwoon Kim[1], Rock Pulak[1], Hilary Sherman[2], Ann Ferrie[2]
1 Union Biometrica, Inc., Holliston, MA USA, 2 Corning Life Sciences, Kennebunk, ME USA

Introduction

Flow Cells for Every Scale





Union Biometrica, Inc. develops instrumentation that extends the principles of flow cytometry to large multicellular structures and whole organisms.

These platforms enable researchers to quantitatively analyze and selectively isolate intact biological systems including:

- 3D cell culture models (organoids, spheroids)
- Small model organisms (*C. elegans*, zebrafish)

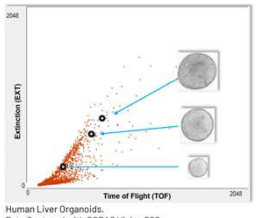
Spheroids: multicellular 3D aggregates that recapitulate key aspects of tissue architecture and gradients
Organoids: stem cell-derived systems that self-organize into structures resembling native tissue

Why 3D models matter:

- More physiologically relevant than traditional 2D culture
- Increasingly used in drug discovery, disease modeling, and regenerative medicine
- 3D models are central to the shift towards human-relevant, non-animal models and new approach methodologies (NAMs)
- FDA draft guidance mandates that NAMs—including organoids and other 3D models—must meet four criteria: fit-for-purpose, biological relevance, technical characterization, and reproducibility

Materials and Methods

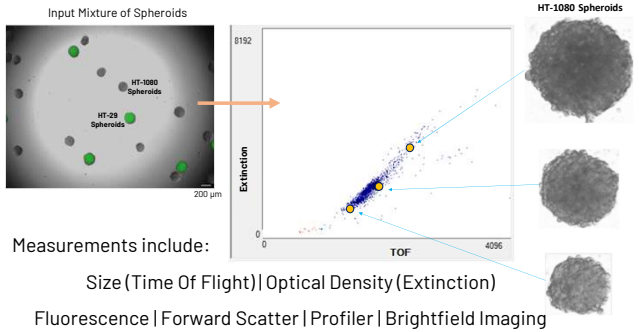
COPAS Vision™: Large Particle Imaging Flow Cytometer



Human Liver Organoids.
Data Captured with COPAS Vision 500

Center Panel - GFP-positive HT-29 and non-fluorescent HT-1080 cell lines were cultivated as roughly uniform, ~200-micron spheroids using Corning® Elplasia® 12K flasks. HT-29 spheroids and HT-1080 spheroids were mixed 1:1, then data were acquired from mixtures of heterogeneous, intact spheroids using the COPAS Vision equipped with a 1000-micron flow cell. HT-29 spheroids were distinguished from HT-1080 spheroids based on Green Fluorescence. GFP-positive HT-29 spheroids of uniform size were automatically dispensed singly into the wells of a 96-well plate.
Right Panel - A549, HCT-116, and HT-29 cells were seeded into Corning® Elplasia® 12K flasks. After 72 hours, spheroids were collected and resuspended in CryoStor CS10. Spheroids (~1000 spheroids/vial) were slow-frozen at -80ºC using Corning CoolCell® containers. Spheroids were thawed into DMEM containing 10% FBS, then resuspended in Dulbecco's Phosphate- Buffered Saline for data analysis and dispensing into Corning Spheroid microplates. Sorted spheroids were exposed to various concentrations of small molecule drugs (Panobinostat, YM-155, and Staurosporine) or matched DMSO control for 72 hours. Luminescence was detected using CellTiter-Glo 3D. Relative luminescence was calculated based on a ratio of drug response compared to DMSO control response.

Flow Cytometry of Intact Spheroids



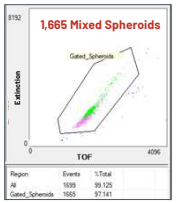
Measurements include:

Size (Time Of Flight) | Optical Density (Extinction)

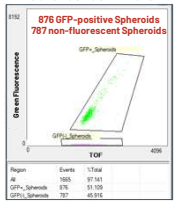
Fluorescence | Forward Scatter | Profiler | Brightfield Imaging

Population-level Batch Analysis Data

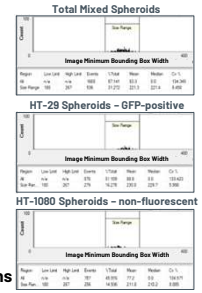
Event Counts: Report out counts of spheroids



% Labeled Objects: % of spheroids with fluorescence vs. total



Size Distribution: Report out the size range of spheroids in microns

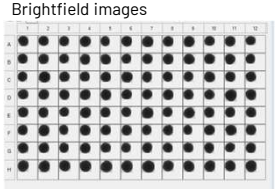


- HT-29 spheroids and HT-1080 spheroids make up 51.1% and 45.9% of all spheroids respectively
- HT-29 spheroids have a mean diameter of **230 microns**
- HT-1080 spheroids have a mean diameter of **211.8 microns**

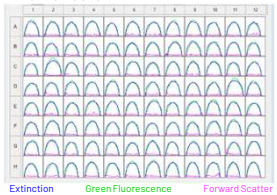
Precision Plate Dispensing of Intact Spheroids

Index Sorting

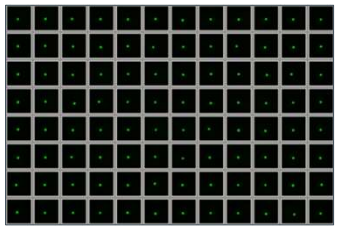
Brightfield images



Profiler data



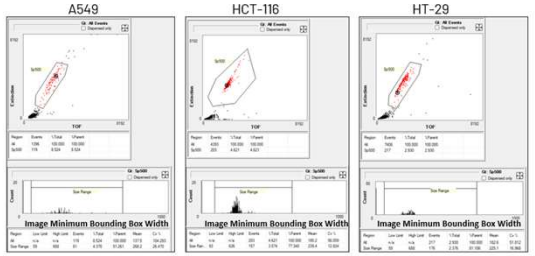
Sorted Spheroids



Imaged with CellularInsight CX7

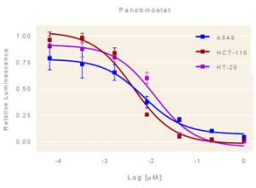
- Dispensed one GFP+ HT-29 spheroid per well of a 96-well plate
- Filled plate in **~2 minutes** with **100% accuracy**

Cryopreserved Spheroids – Analysis



- Cryopreserved A549, HCT-116, HT-29 Spheroids are distinguished from cell debris based on Extinction and Size
- Image data are used to report size range in microns
- Spheroids were dispensed singly into 96 well plates for drug response studies

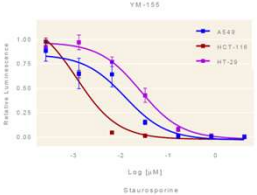
Drug Response in Cryopreserved Spheroids



Cell Line	TC_{50} µM
A549	0.006749
HCT-116	0.004081
HT29	0.01193

Response to Panobinostat

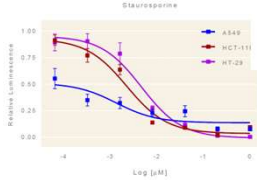
Concentration-response curves generated using automatically dispensed, cryopreserved spheroids following treatment with increasing concentrations of Panobinostat. Data average from 3 microplates shown with standard error. N=10 wells for A549, 18 wells for HCT-116, and 16 wells for HT-29 per drug concentration.



Cell Line	TC_{50} µM
A549	0.0122
HCT-116	0.001159
HT29	0.02541

Response to YM-155

Concentration-response curves generated using automatically dispensed, cryopreserved spheroids following treatment with increasing concentrations of YM-155. Data average from 3 microplates shown with standard error. N=9 wells for A549, 17 wells for HCT-116, and 15 wells for HT-29 per drug concentration.



Cell Line	TC_{50} µM
A549	0.001329
HCT-116	0.002238
HT29	0.004654

Response to Staurosporine

Concentration-response curves generated using automatically dispensed, cryopreserved spheroids following treatment with increasing concentrations of Staurosporine. Data average from 3 microplates shown with standard error. N=7 wells for A549, 18 wells for HCT-116, and 17 wells for HT-29 per drug concentration.

Summary

- Large particle imaging flow cytometry enables quantitative analysis and precision dispensing of intact 3D models while preserving biological context.
- Integrated cytometric, imaging, and Profiler-based measurements support reproducible characterization of spheroid populations without dissociation.
- Cryopreserved spheroids can be distinguished from debris, analyzed at population scale, and dispensed into assay-ready multi-well plate formats.
- Drug response assays performed on intact cryopreserved spheroids demonstrate the utility of this platform for standardized and scalable 3D model workflows.
- These capabilities support the growing need for reproducible, high-throughput workflows aligned with NAM initiatives.

Contact: sales@unionbio.com Visit: www.unionbio.com