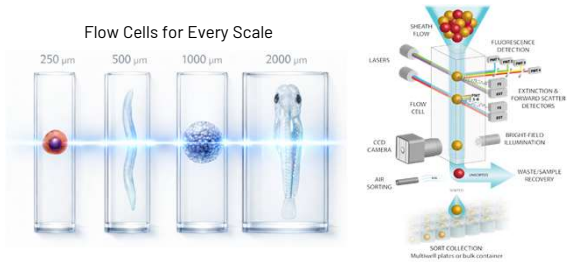
Large Particle Flow Cytometry Platform for High-Throughput Analysis, Brightfield Imaging, and Precision Dispensing of Intact HT-29 and HT-1080 Spheroids

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



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

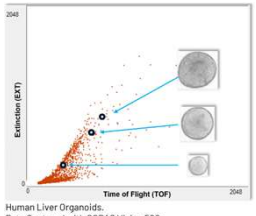
These platforms enable researchers to quantitatively analyze and selectively isolate intact biological systems including:
- 3D cell culture models (organoids, spheroids)
- Small model organisms (*C. elegans*, zebrafish)

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- FDA draft guidance mandates that NAMs—including organoids and other 3D models—must meet four criteria: fit-for-purpose, biological relevance, technical characterization, and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

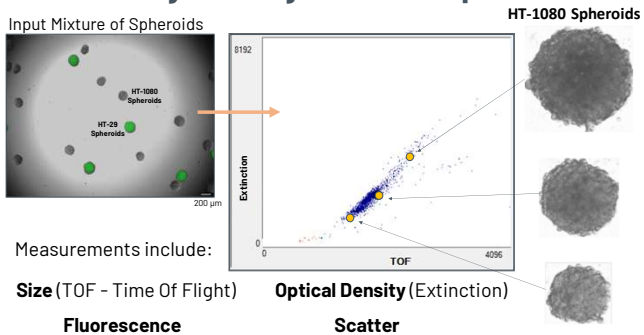
COPAS Vision™: Large Particle Imaging Flow Cytometer



Human Liver Organoids.
Data Captured with COPAS Vision 500

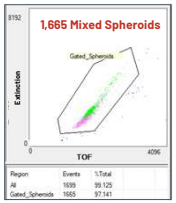
GFP-positive HT-29 and non-fluorescent HT-1080 cell lines were cultivated as roughly uniform, ~200-micron spheroids using Corning® Elplasia® 12K flasks. HT-29 spheroids and HT-1080 spheroids were mixed 1:1, then data were acquired from mixtures of heterogeneous, intact spheroids using the COPAS Vision 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. Brightfield imaging, Profiler, and Forward Scatter provide additional means to distinguish HT-29 from HT-1080 spheroids.

Flow Cytometry of Intact Spheroids

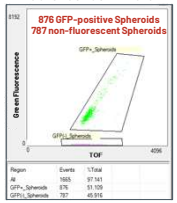


Population-level Batch Analysis Data

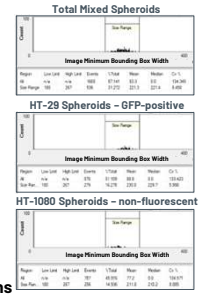
Event Counts: Report out counts of spheroids



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

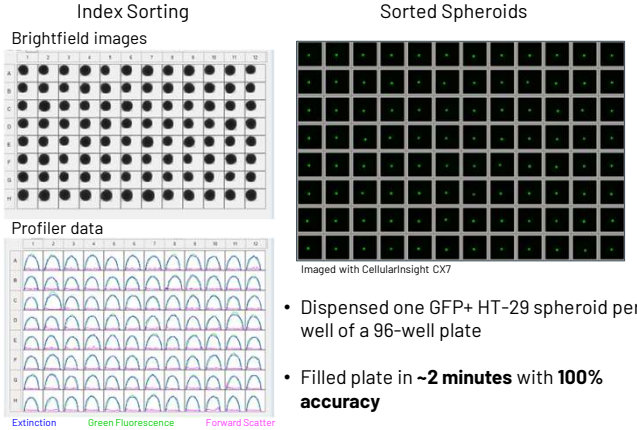


Size Distribution: Report out the size range of spheroids in microns



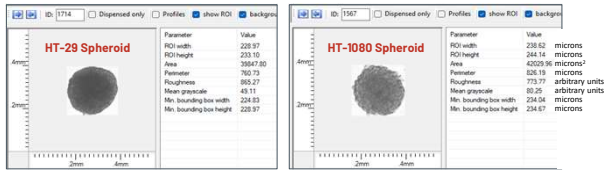
- HT-29 spheroids and HT-1080 spheroids make up 51.1% and 45.9% of all spheroids respectively
- HT-29 spheroids have a mean diameter of **230 microns**
- HT-1080 spheroids have a mean diameter of **211.8 microns**

Precision Plate Dispensing of Intact Spheroids



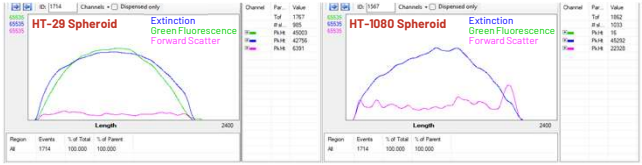
- Dispensed one GFP+ HT-29 spheroid per well of a 96-well plate
- Filled plate in **~2 minutes** with **100% accuracy**

Brightfield Imaging in-flow



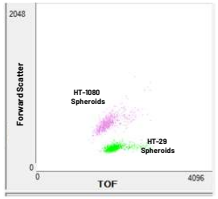
- HT-29 and HT-1080 spheroids can be distinguished based on the appearance of brightfield images
  - HT-29 spheroids appear dark, compact, and smooth
  - HT-1080 spheroids appear light, loose, and rough
- Image analysis provides information on spheroid size in microns or microns^2

Profiler™: Beyond the Integral Signal



- Profiler displays values for each measured parameter as they vary
- The Peak Height, Peak Width, and Integral signal (area under the curve) are captured for each parameter
- The Green Fluorescence profile for HT-29 spheroids is consistent with uniform localization of signal throughout the spheroid

Label-free Parameters Distinguish Spheroids



Researchers may avoid fluorescent labeling in cases where viability or biological activity may be impacted

- HT-29 spheroids can be distinguished from HT-1080 spheroids based on differences in Forward Scatter

Summary

- Large particle flow cytometry enables high-throughput analysis of intact 3D cellular models without dissociation.
- Combined cytometric measurements, Profiler data, and in-flow brightfield imaging provide orthogonal characterization of spheroid populations in a single workflow
- Population-level analysis reveals heterogeneity in size, morphology, fluorescence, and optical density across intact spheroid models.
- Precision dispensing enables rapid isolation of selected intact spheroids into multi-well plates for downstream assays and workflow automation.
- These capabilities support scalable, information-rich analysis and handling of complex 3D biological structures.

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