Analysis, imaging and sorting of 3-D tumor spheroids on the COPAS Vision Large Particle Flow Cytometer

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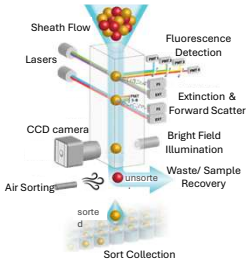
COPAS Vision - Large Particle Flow Cyometer

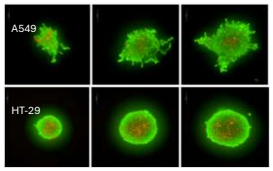


COPAS Vision extends the principles of flow cytometry to large multicellular structures and whole organisms that are **10 to 1500 um in diameter** such as:

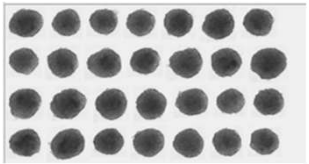
- 3D cell culture models (organoids, spheroids)
- Small model organisms (*C. elegans*, zebrafish)
- Microtissues (pancreatic islets, tissue fragments)

The objects can be selected based on length, optical density and fluorescence and are dispensed intact into the receptacle of choice

3D Models in Cancer Research



Confocal images of A549 and HT-29 spheroids



Images of HT-29 spheroids acquired with the COPAS Vision camera

In-vitro cultured **tumor spheroids** are three-dimensional (3D) cancer cell aggregates that recapitulate key features of the tumor architecture, cell-to-cell interactions and microenvironment of the tumor

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)

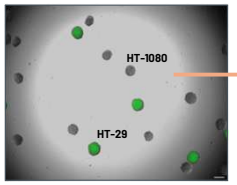
Objectives

- Manual selection of tumor spheroids for downstream analyses results in longer turnaround times and higher variability in spheroids size, compactness and shape
- The COPAS Vision flow cytometer enables the high-throughput analysis and sorting of intact 3D cell aggregates
- Here, we analyzed a 1:1 mixture of human HT-29/GFP colorectal carcinoma and HT-1080 fibrosarcoma spheroids grown into Corning® Elplasia® 12K flasks using a COPAS Vision imaging cytometer with a 1000 µm flowcell
- Individual spheroids were isolated based on size, optical density, and fluorescence and dispensed into 96-well plates for dose-response assays

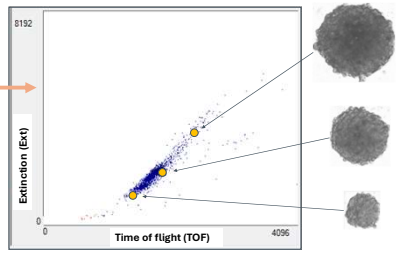
Flow Cytometry Analysis of Intact Tumor Spheroids

HT-1080 and HT-29/GFP tumor spheroids were analyzed on a COPAS Vision equipped with a 1000 um flowcell. Parameters acquired include:

- **Time of flight (TOF):** a correlate of the spheroids' length
- **Extinction (Ext):** measures the opacity of the spheroid
- **Green Fluorescence**

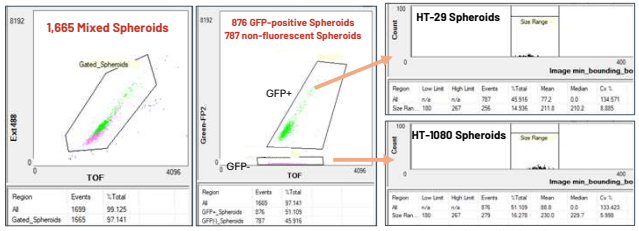


Input: Mixture of HT-1080 and HT-29/GFP Spheroids



In-Flow images of HT-1080 spheroids of different sizes are shown with the corresponding position in the Ext-TOF dot plot

Population-level Batch Analysis Data



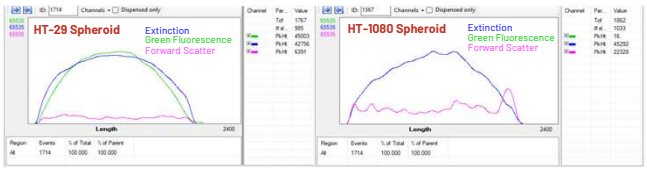
Event count: total number of spheroid based on Ext/TOF

% Labeling: % GFP+/GFP- spheroids

Size Distribution: 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**

Profiler™ : Beyond the integral signal



- Profiler traces each measured parameter displayed across the length (TOF) of the spheroid
- 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

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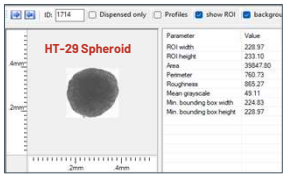
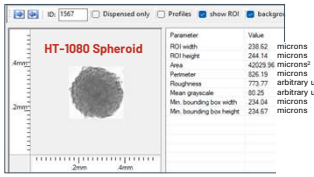
Brightfield Imaging In-Flow





- 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

Precision Plate dispensing of Intact Spheroids

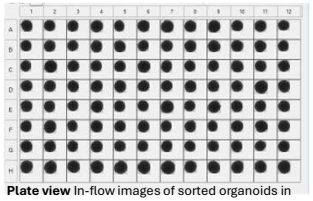
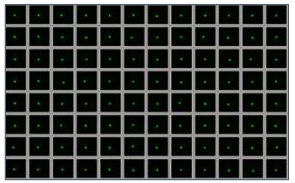


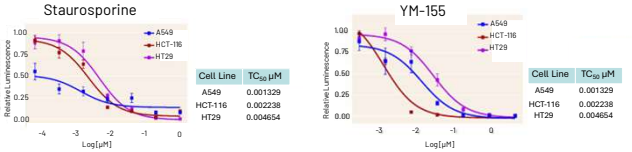
Plate view In-flow images of sorted organoids in each well of a 96 well-plate



Fluorescent microscopy images of HT-29 spheroids sorted in a 96-well plate

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

Dose-Response Tests on Sorted Tumor Spheroids



A549, HCT-116, and HT-29 spheroids were sorted into Corning spheroid microplates with COPAS Vision and exposed to various concentrations of staurosporine and YM-155. Luminescence was analyzed to determine the TC_{50} uM for each compound.

Conclusions

- Large particle flow cytometry enables high-throughput analysis of intact tumor spheroids 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 of selected intact spheroids into multi-well plates is suitable for downstream microscopy and dose-response chemiluminescent assays