QUICK TECH NOTES

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Development of a High-Throughput Approach for Imaging and Sorting of Organoids and Spheroids using the COPAS VISION™

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Objective

Large particle flow cytometers from Union Biometrica provide automation for the analysis and sorting of organoids and tumor spheroids. Cells growing in clusters communicate with each other and behave differently than cells grown as monolayers or in suspension[1,2]. Research using stem cell clusters, organoids, tumor spheres, and other types of 3D cultures are important biological systems for the discovery of signals responsible for normal development as well as the abnormal disease state of solid tumors[3,4]. There is an enormous interest in studying how cells grow, divide, and differentiate in a more natural context provided by these 3D cell culture models. Many cell types will naturally form cell clusters when given the opportunity[5,6]. Using this research approach allows for cell-cell interactions to occur and provides biological insights otherwise missed when studying flat sheets of cells grown on plastic surfaces or as cells grown in isolation. Here, we demonstrate the analysis, selection, and sorting of organoids and spheroids using a large object imaging flow cytometer, the COPAS VISION™. As models, we used iPSC-derived retinal epithelial organoids and T47D breast cancer tumor spheroids. We were able to discriminate different stages of organoid/spheroid formation and expression levels of fluorescent markers. With this information, we accurately dispensed single, intact spheroids into tubes or multi-well plates, up to 384 well format, quickly and efficiently. In contrast to traditional methods, where organoids are sorted out by hand under a microscope, our approach offers a high-throughput manner to assess quality and sort uniform organoids/spheroids to be used for testing, transplantation, and disease modeling.

Methods

High-throughput analysis and sorting of large and/or fragile objects in biology present a challenge for traditional flow cytometry instruments. Union Biometrica has developed the BioSorter® and COPAS™ platforms to automate the analysis, sorting, and dispensing of cells, seeds, beads, particles, and small model organisms. It records several parameters, such as object size (TOF), optical density (EXT), and the intensity of fluorescent markers. Once analyzed, objects are sorted according to user

selectable criteria and they may be dispensed into stationary bulk receptacles or multi-well plates for high-throughput screening, toxicology, or sequencing. The COPAS VISION, which was used in these experiments, provides a new level of capabilities for laboratories working with large particle samples. Images of the analyzed objects are collected and can be used as surveys of the contents in a sample (population monitoring) or verification of the identity of sorted events (in multi-well plate assays). The COPAS VISION is able to analyze particles with diameters of 10 -750 µm depending on the size of the flow cell in place. The flow cell diameters available are 250 µm, 500 µm, or 1000 µm. The sample is gently carried in a continuously flowing stream at a rate up to 100 objects/second. Using Profiler™ software, an optical profile of each object is graphically displayed showing the location and intensity of all optical parameters.

Key features of the COPAS VISION:

- Real-time brightfield imaging
- Up to 4 excitation lasers, 4 or 8 fluorescence detectors
- Profiler™ graphically displays parameters along the axis of each particle
- Sorting by size, optical density, scatter, fluorescence, and Profiler measurements
- Sorting principle by gentle air diverting, maintaining sample integrity
- Collection in multi-well plates, tubes, and various receptacles

A

B

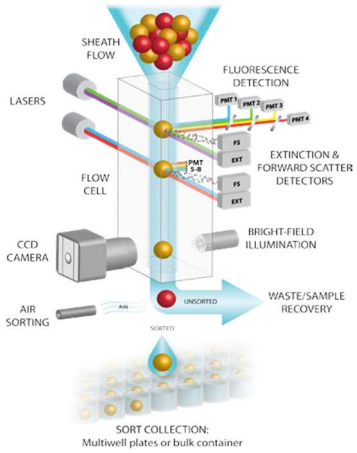

Figure 1. Analysis and sorting of objects inside the flow cell.
A) COPAS VISION instrument and Flow Pilot software interface. Contamination is managed by the enclosed sorting area, built-in UV light and HEPA filter. The small footprint of the instrument also allows easy installation into many conventional biosafety cabinets. B) Objects are carried through the flow cell by a liquid stream while their physical properties are being measured. Convergence of the sheath and sample fluid enables the "hydrodynamic focusing" of the objects, causing them to go through the center of the flow cell along their longitudinal axis. Inside the flow cell objects are illuminated by a 488nm and/or other solid-state lasersto measure the object's optical properties of: Size (TOF), Optical Density (EXT), Scatter (FSC and SSC) and multiple fluorescence (FLU) parameters. Those objects meeting sort criteria determined by the operator are permitted to drop into the collection device of choice, while those that do not are diverted to waste/recovery using a pneumatic sorting device.

To avoid damaging or altering fragile samples, a gentle pneumatic device, located after the flow cell, is used for sorting (Fig. 1). The fluid pressurization of the instrument (up to 5 PSI) is also significantly lower than those of traditional flow cytometers reducing any potential damage from shear forces.
In this report, we describe tests with the COPAS VISION configured with a 500 µm (organoids) and a 1000 µm (spheroids) flow cell for the analysis and dispensing of spheroids on the basis of size, optical density, and fluorescent intensity.

COPAS VISION Analysis, Sorting, and Imaging

We demonstrate the function of COPAS VISION for data collection and high-throughput assay setup using retinal pigment epithelial organoids and T47D breast cancer tumor spheroids.

iPSC-derived retinal epithelial organoids were used as a representative case study in this technical note. These cells are transformed with a GFP-reporter from a neuronal promoter (Thy1 gene) expressed in mature neurons. After harvesting the spheroids from Matrigel, the organoids were pooled together in PBS buffer into a 50 ml conical sample cup and attached to the COPAS VISION. As optical measurements of the organoids were collected with the 488 nm laser, a dual parameter dot plot of TOF (size) vs EXT (optical density) was generated where every organoid is represented as a dot in the plot. Regions were then drawn around the signals (dots on the graph) representing organoids of different relative sizes. Representative organoids from each trapezoidal region is shown in Figure 2.

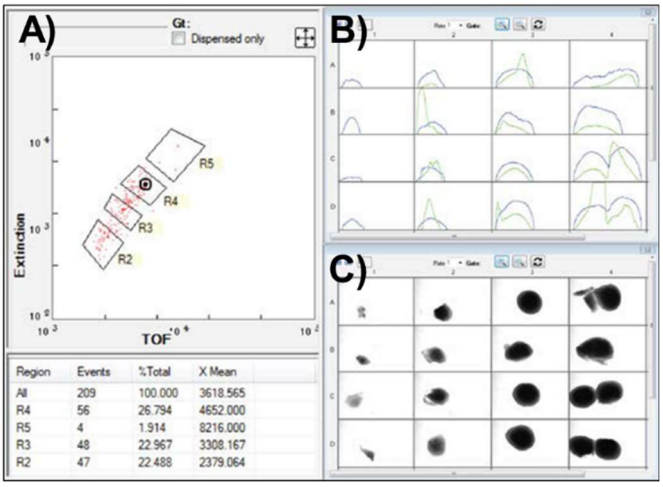

Figure 2. A.) Dot plot of four populations of organoids sorted based on size and optical density, B.) Profiler data identifies GFP fluorescence intensity and position, C.) Brightfield images identify morphology

The profiles of the sorted organoids are shown in panel B) and the corresponding images in panel C). The profiles identify the fluorescence distribution across each organoid. After data collection, the images were processed and information about

the surface area, perimeter, roughness, and mean greyscale was determined. Another example of image analysis is given in Figure 3. T47D breast cancer spheroids were collected and analyzed on the COPAS VISION 1000. Spheroids of the larger size (region R1 in Figure 3) were dispensed and the images analyzed. Figure 3C shows the results from one of the sorted tumor spheroids. The parameters of roughness and mean grayscale can be use to characterize the sample objects.

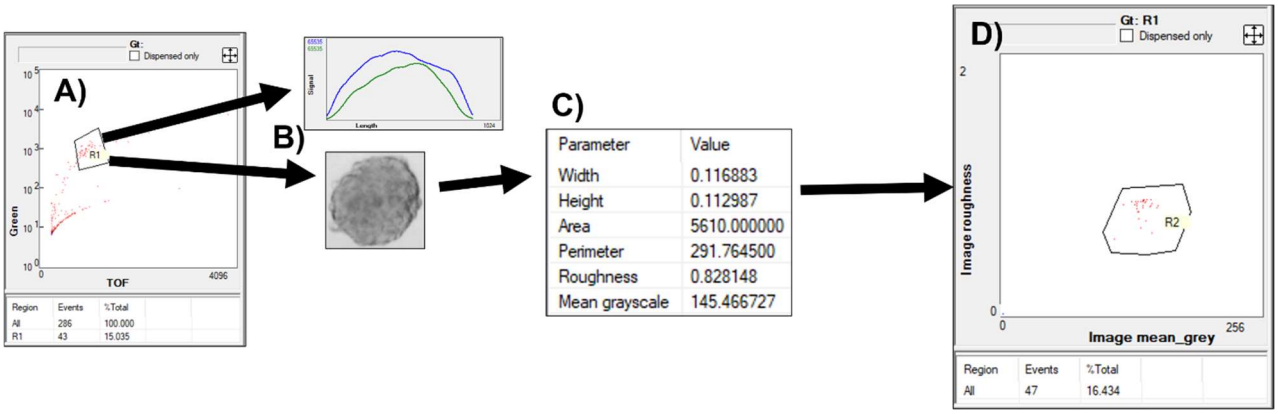

Figure 3. FlowPilot image showing processed/analyzed single T47D Breast Cancer Tumor Spheroid.
A.) Selection of spheroids of uniform size and GFP expression, B.) representative Profiler data and brightfield image of one spheroid from the selected population, C.) Image processing = Area, Perimeter, Roughness, Mean Grayscale, D.) Plot of image roughness and mean greyscale measurements.

A plate view is generated in FlowPilot software in which images of sorted objects are organized by plate well. Bright field images are matched with Profiler data for each object sorted. In Fig 4 we show a plate view of iPSC-derived retinal epithelial organoids which were sorted into a 96 well plate. The corresponding profile for each organoid is also displayed. Viability of sorted organoids was confirmed 24 hours post sorting.

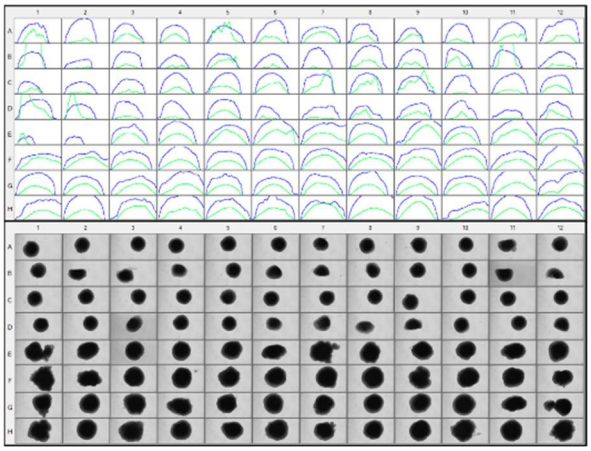

Figure 4. FlowPilot plate view representation: A summary view of all the images and profiles of a set of dispensed organoids.

Conclusions

These experiments demonstrate that the COPAS VISION is well suited for the handling of organoids and tumor spheroids. We demonstrated that sorting organoids and spheroids is fast, reproducible, and maintains the integrity of the sorted objects. The automated analysis and sorting process is gentle and does not influence morphology or viability.

The COPAS VISION provides a level of automation to the process of handling cell tumor spheroids or organoids, allowing for increased throughput and eliminates biases that might be introduced by the researcher. The integrated image processing software allows for accurate determination of surface area, perimeter, and other parameters of every dispensed spheroid for easy data interpretation in high throughput screening assays. The data is collected and analyzed electronically and made available for easy incorporation into presentations and reports.

The COPAS VISION instrument brings the method of flow cytometry to the analysis and sorting of spheroids which are otherwise too large and fragile for analysis on conventional single-cell flow cytometers. This instrument brings the advantages of flow cytometry's statistically meaningful data, large unbiased data sets, multiparametric analysis and imaging to experiments using 3D biology.

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