Simplified & High Throughput Analysis of Intact 3D Models Using Large Particle Cytometry

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Introduction

The utilization of 3D models, such as organoids, is essential in translational and clinical research, enabling the exploration of intricate biological processes within a physiologically relevant context and supporting the development of cell therapies. However, many existing tools are not conducive to true high-throughput analysis, often confining organoid and spheroid cultures to multiwell plates with a single particle per well. This limitation results in the measurement of only a few 3D particles per treatment, potentially compromising the capture of biological variability among individual organoids. This limitation may lead to compromised data reliability, a lack of statistical robustness, and challenges in detecting subtle differences or trends.

The advantages of growing organoids and iPSC aggregates in suspension cultures are manifold, crucial for various applications, scalability, and adaptability to bioreactors. Nevertheless, the analysis of 3D suspension cultures entails tedious workflows, including the transfer of single particles into multiwell plates for imaging, paraffin embedding of particles for histology, or the dissociation of particles into single cells for flow cytometry analysis.

To overcome these challenges, we used the Velocyt™ cytometer by BennuBio to analyze intact 3D models from suspension and static cultures. The Velocyt uses parallel stream acoustic flow technology along with camera-based optics to combine the imaging power of microscopy with the speed and ease of flow cytometry. The ultra low-pressure fluidics and simplified design can acquire data at up to 10mL/min and return the total sample to the user intact and viable for additional analyses or for kinetic assays.

In the first case study, we assessed the size distribution and scatter parameters of iPSC grown in suspension cultures. Next, we measured apoptosis from pancreatic spheroids grown in microwell dishes after treatment with staurosporine. Finally, we used colon cancer spheroids to analyze immune cell penetrance and cancer cell death in a co-culture setting. These case studies exemplify the flexibility and simplified workflow of the Velocyt and its value for multiple 3D applications.

Methods

iPSC assay – Aggregates were grown in suspension in 6 well dishes. Cultures were transferred to 15 mL tube, settled into a pellet by gravity and media was aspirated. Aggregates were resuspended in 5 mL PBS containing 0.1% BSA and data was acquired on the Velocyt at 3mL/min

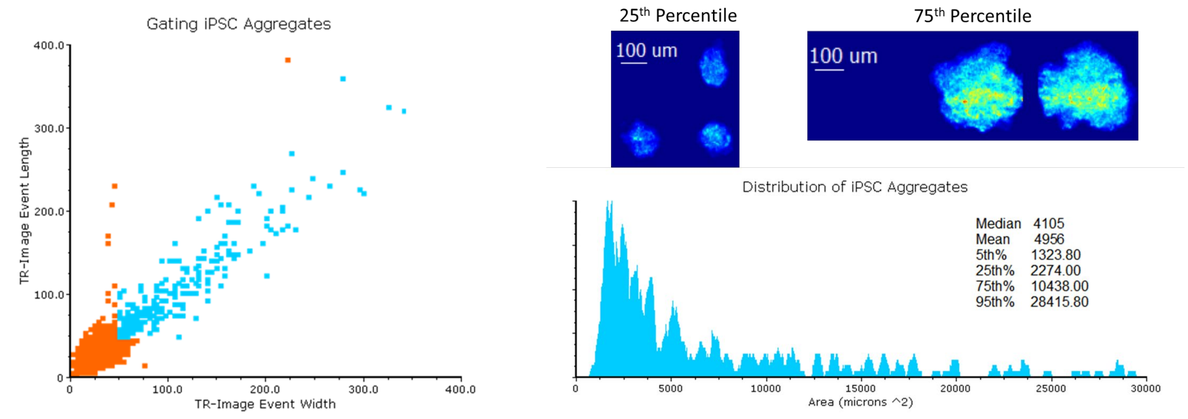
Apoptosis assay – Spheroids from AsPC-1 pancreatic cells were generated using a microwell plate. After 2 days, spheroids were treated overnight with 2.5uM of staurosporine. After treatment, spheroids were stained in well with NucView 488 caspase 3/7 substrate and ethidium homodimer I (both Biotium). Spheroids were harvested and transferred to 15mL tubes. The spheroids were settled into a pellet by gravity, supernatant was aspirated to remove unbound dye and resuspended into PBS with 0.1% BSA. Samples were run on Velocyt at an acquisition rate of 3mL per minute.

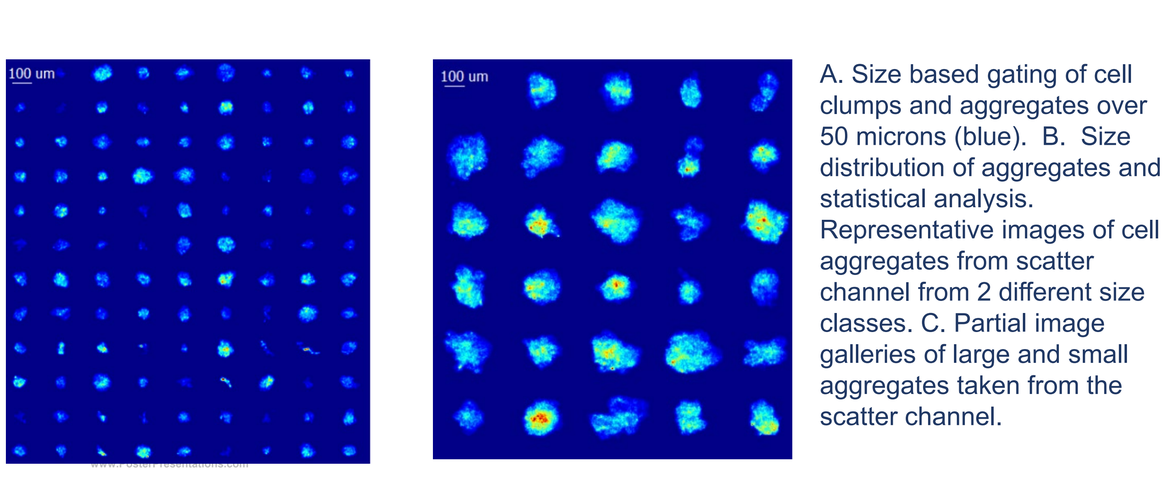
Co-culture assay – Spheroids from HCT116 colon cancer cells were generated using microwell plates. PBMCs were cultured for 3-days in media containing IL-2, IL-15 and activated using ImmunoCult (Stem Cell Technologies). Prior to co-culture, PBMCs were labeled with CytoTell UltraGreen (AAT Bioquest). Labeled PBMCs were co-cultured overnight with various E;T ratios. Co-cultures were stained with ethidium homodimer I and spheroids were purified from PBMC using filtration. Additional washes were performed to remove loosely bound PBMCs

Data analysis – Samples were acquired on the Velocyt and initial gates and parameters were set using Kytos™ software and image analysis was performed using VeloView™ software (BennuBio). Additional analysis was done using FCS Express software (Dotmatics) using FCS 3.0 files generated during data acquisition on the Velocyt.

Results

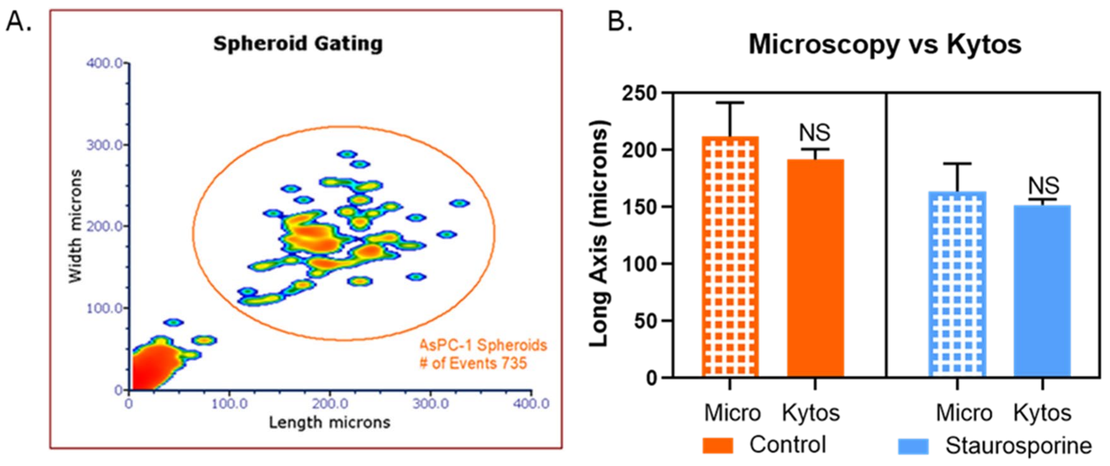
Figure 1. iPSC Aggregates: Morphology, Scatter and Size Distribution



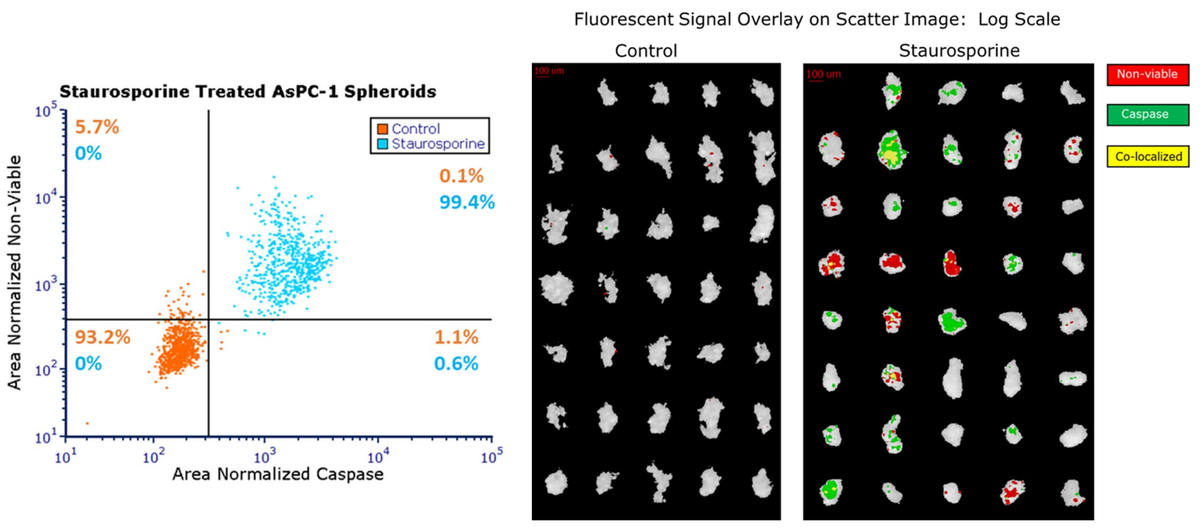


A. Size based gating of cell clumps and aggregates over 50 microns (blue). B. Size distribution of aggregates and statistical analysis. Representative images of cell aggregates from scatter channel from 2 different size classes. C. Partial image galleries of large and small aggregates taken from the scatter channel.

Figure 2 Pancreatic Spheroids: Small Molecule Treatment

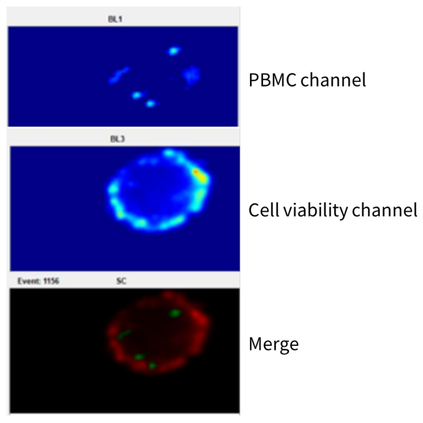


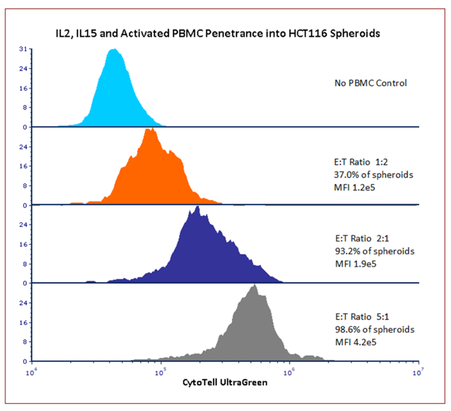
A. Size based spheroid gating. B. Validating size measurements generated on Velocyt compared to microscopy.

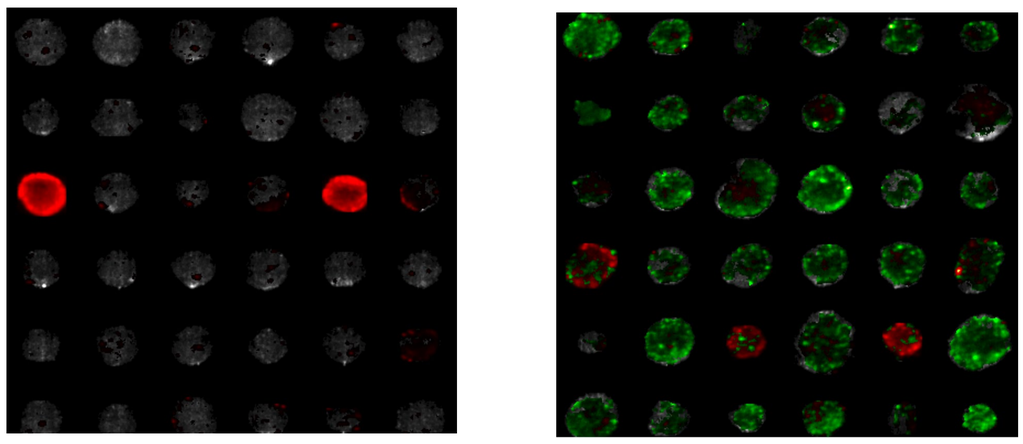


A. Apoptosis detection with spheroids stained in –well using both caspase 3/7 substrate and ethidium homodimer. B. Partial overlay image gallery generated in VeloView software. Green are cells with activated caspase 3/7 while the red signal are cells within the spheroid with compromised membranes (non-viable).

Figure 3 Colorectal Spheroids: Immune Cell Killing







A. Single spheroid showing the individual green and red fluorescent channels and merged image. B. Histogram showing increasing PBMC penetrance (green +) into spheroids with increasing E:T ratios. C&D. Partial image gallery of spheroid immune cell killing without PBMC co-culture (C) or with an E:T ratio of 5 to 1. PBMCs are green and non-viable cells in spheroid are red.

Conclusions

In this poster, the use of the Velocyt was exemplified using three different applications. Importantly, these data illustrate 3 key values of the Velocyt over current analysis tools:

1) The flow-based imaging technology maximizes workflow flexibility to generate 3D models as best required for the study, whether that is suspension cultures, microwells technology, scaffold embedded cultures or even *in vivo* 3D models
2) Generate higher quality data and data confidence by imaging hundreds to thousands of intact 3D particles in 5 minutes or less.
3) Enhance productivity and reduce sample costs by reclaiming the sample after analysis, intact and unadulterated allowing for additional downstream analysis, or kinetic studies…..do more with less.

For more information, stop by booth 18.
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