1272-C

Automation for analysis and handling of cell clusters, tumor spheres and organoid bodies

Rock Pulak[1], Peter McGuirk[1], Pilar Pardo[2], Hilary Sherman[2], Allison Tanner[2]
[1]Union Biometrica, Holliston, MA, [2]Corning Inc., Kennebunk, ME

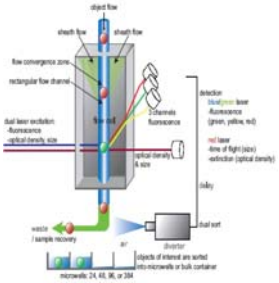
UNION BIOMETRICA
Large Particle Flow Cytometry

Introduction

Large particle flow cytometers from Union Biometrica provide automation for the analysis and dispensing of cell clusters. Cells growing in clusters communicate with each other and behave differently than cells grown as monolayers or in suspension. 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. There is 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. Using this research approach allows for cell-cell interactions to occur and provides biological insights otherwise missed when studying flat sheets of cells growing on plastic surfaces or as cells grow in isolation. Furthermore, there are many methods that enable the introduction of genes for fluorescent proteins expressed from promoters of interest. Researchers can use these to track different cell types and identify when a cell or group of cells has transitioned to a different state.

Methods and Materials

Figure 1: BioSorter® analyzes and sorts cell clusters and large cells (10-1500 µm diameter) in a continuous flow stream at high rate (up to 100 events/sec). The device measures object size (TOF), optical density (EXT) and multiple fluorescent markers.

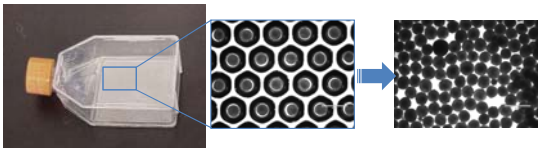


The BioSorter has inter-changeable fluidics and optics core assemblies (FOCAs), allowing the instrument to analyze and dispense objects across a large size range.

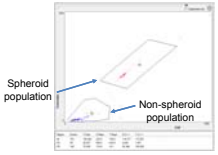
Sorting is accomplished with the use of a pneumatic device located below the flow cell. Furthermore, the fluid pressure through the flow cell (no greater than 6 psi) is significantly lower than in conventional flow cytometers, thus providing gentle sorting conditions.

Sample Preparation and Analysis

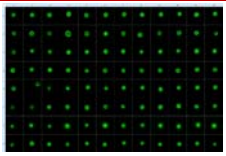
In a collaborative effort we have tested cell clusters produced at Corning Life Sciences (Kennebunk, ME) using their T-25 and T-75 microcavity flasks. We ran various cell clusters on the BioSorter™ flow cytometer and show that the BioSorter instrument can analyze monoculture cell clusters from several different cell types and can dispense these into wells of multiwell plates. We analyzed HT-29/GFP-expressing cells, HEK293/RFP-expressing cells, HCT-116 cells and iPSC.



Results



Gating regions used to sort HT-29 GFP spheroids individually to wells of 96-well plate.



96-well spheroid plate scan (10x) using a CellInsight CX7 High-Content Analyzer

Figure 2: Sorting cell clusters. Single HT-29/GFP spheroids were dispensed individually into wells of 96-w plate. Sample is agitated to prevent settling of the cell clusters. Spheroids remain intact throughout the time required to set and dispense cell clusters to multiwell plates.

Results

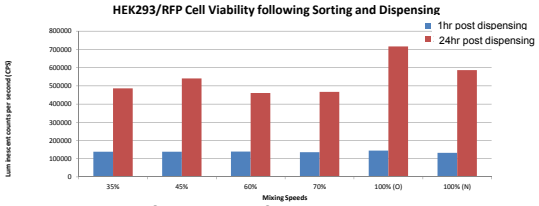
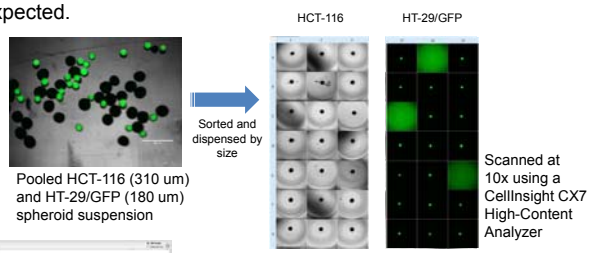


Figure 3: Viability of cells 1 hr after dispensing and at 24 hr post-dispensing. Increased signal at 24 hours indicates the cells continued to grow as expected.



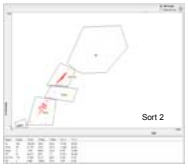


Figure 4: Sorting on the basis of cell size. Single HCT-116 and HT-29/GFP spheroids dispensed into 384-w Spheroid Microplate.

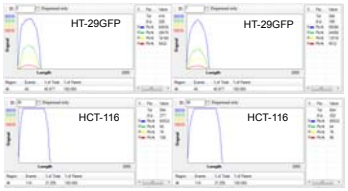


Figure 5: Axial Profiles of cell clusters. HCT-116 profiles show no GFP while HT-29/GFP spheroids display a narrower profile and measureable green fluorescence.

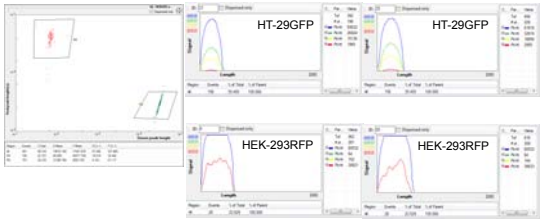


Figure 6: Biosorter data for sorting on fluorescence. Mixture of HEK293/RFP and HT-29/GFP spheroids dispensed into 384-w Spheroid Microplate.

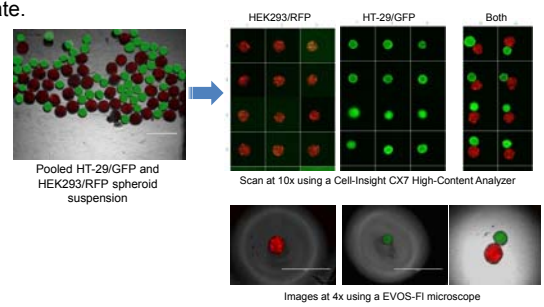


Figure 7: Sorting on fluorescence differences. Single HEK293/RFP and HT-29/GFP spheroids dispensed accurately individually to wells.

Conclusions

The BioSorter is valuable technology for increased throughput of spheroids cultured in bulk. Our data shows that the BioSorter instrument provides automation for unbiased analysis, handling of large numbers of cell clusters, and dispensing of these sample types in a multiwell plate format. This approach can be used to characterize populations of cell clusters, tumor spheres and organoid bodies of various types. Dispensing to wells of multiwell plates provides an approach to using this 3D cultures in large scale biological assays and screens.