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# Tech Note

## Velocyt Large Particle Flow Cytometer

Flow cytometry has long been the standard for many forms of cellular analysis. However, traditional flow cytometry only analyzes one cell stream at an analysis rate of <50K particles/sec and a sample volumetric rate of <250 µL/min. This limitation makes traditional flow cytometers poorly suited for rare cell analysis in high cellular backgrounds, detection of cells/particles in large dilute samples, or rapid analysis of diluted blood samples. In addition, traditional flow cytometers cannot perform analysis of spheroid and organoid models critical to immunology and oncology research[1] , drug screening and clinical applications in oncology, transplantation and artificial organs. Because flow cytometers focus the particles using sheath fluid, the sample is diluted >50-fold after analysis, making sample recovery and kinetic analysis time-consuming or impossible. BennuBio Inc. has developed a novel analytic platform, the Velocyt, that specifically addresses these immunology and oncology cellular analysis pain points, while delivering facile kinetic analyses, analysis of large sample volumes for rare event detection, and even continuous process monitoring.

The Velocyt is a simplified parallel flow cytometer that uses three key components: a multi-node acoustic focusing flow cell; a line-shaped laser excitation profile; and a high-speed imaging detector. The sample flows directly into a 1.5-mm wide by 200 µm deep rectangular flow cell where it encounters an acoustic standing wave that focuses the particles into 10 parallel streams across the flow cell. These streams intersect one or more line-focused laser lines, and the particle-laser intersection regions are imaged using a high-speed cMOS camera. Proprietary software converts these images into conventional flow cytometry data for each stream. Acoustic focusing eliminates hydrodynamic focusing so there is no sample dilution or cell perturbation from high pressure shear. This allows analysis of delicate cells like stem cells, and return of the undiluted sample for reanalysis, kinetic analysis, or repurposing of precious samples to other workflows.

The Velocyt can analyze particles from 3 microns to 150 microns with large particle analysis at a higher spatial resolution than any other flow cytometer. In addition, the volumetric sample delivery rate is as much as 100 times faster than a typical flow cytometer. The Velocyt design also simplifies and shortens the workflow by eliminating washing, lysing, and any enrichment steps. It can analyze samples at 10 mL/min and process data at rates of >100K events/sec, delivering direct high throughput analysis for rare cells without a separation step to provide more accurate and affordable assays.

The Velocyt's unique capabilities address the unmet needs of the global 3D cell culture market which is expected to reach USD 3.2billion by 2027[2]. 3D tissue and tumor models—called

spheroids or organoids—better replicate drug effects *in vivo*[3] by mimicking natural extracellular matrix, cellular physiology, cell-cell interactions and drug transport limitations. In addition, 3D tissue models play an increasingly critical role in cancer research, stem cell biology and drug screening. An expanding application of spheroids is in co-cultures. Spheroids can be produced from a mixture of different cell types, further mimicking the cell-cell and cell-matrix interactions found in tissues. Spheroids have great potential as a drug screening system that fills the gap between single-cell screening and animal models, with potential to reduce the need for animals in drug development. Widespread use of spheroids as a drug screening system has been stymied by the lack of techniques for rapidly analyzing the response of this system to drug exposure. Current systems rely on microscopic analysis of single spheroids held in stationary culture (e.g. on top of, or embedded in, agar). Not only is this microscopy process slow (~1 spheroid analyzed per second in the fastest automated systems) but maintaining spheroids in a stationary culture produces large and irreproducible gradients in inter-spheroid nutrients, waste products, cellular physiology and viability, as well as inconsistent and time-varying drug concentrations in and around the spheroids.

The Velocyt's novel optical analysis platform enables measurement of inter-spheroid fluorescence distributions. Currently, that process can only be done using slow and expensive confocal imaging. With the Velocyt, as a spheroid passes through the line-focused laser, a low-resolution 2D image of the spheroid is produced by combined analysis of the 'scan' of the spheroid in time on one axis, and the distribution of fluorescence across the spheroid structure in the orthogonal dimension on the camera array. Since spheroids are spherically symmetric, these 2D images actually represent the distribution of fluorescence within the 3D spheroid structure. This innovative analysis approach provides the opportunity to develop more sophisticated drug screening assays that involve measuring the distribution of fluorescence intensities within the spheroid structure. This would allow screening based on direct measurement of drug penetration; regional variations in viability/apoptosis/necrosis, alterations in the spatial distribution of cell types in a co-culture; and alterations in the distribution of spheroid physiological and microenvironmental parameters (e.g. proliferation, metabolic activity, hypoxia).

*Keywords: Large particle flow cytometry, acoustic focused flow cytometer, spheroids, organoids, kinetic analysis*

[1] Zanoni, M., Pignatta, S., Arienti, C., Bonafè, M., & Tesei, A. (2019). Anticancer drug discovery using multicellular tumor spheroid models. *Expert Opinion on Drug Discovery, 14*(3), 289-301. doi:10.1080/17460441.2019.1570129

[2] 3D Cell Culture Market Size, Share & Trends Analysis Report By Technology (Scaffold Based, Scaffold Free, Bioreactors), By Application (Cancer, Drug Development), By End Use, By Region, And Segment Forecasts, 2020 – 2027, Grandview Research, 2020.

[3] Kondo, J., & Inoue, M. (2019). Application of Cancer Organoid Model for Drug Screening and Personalized Therapy. *Cells, 8*(5), 470. doi:10.3390/cells8050470