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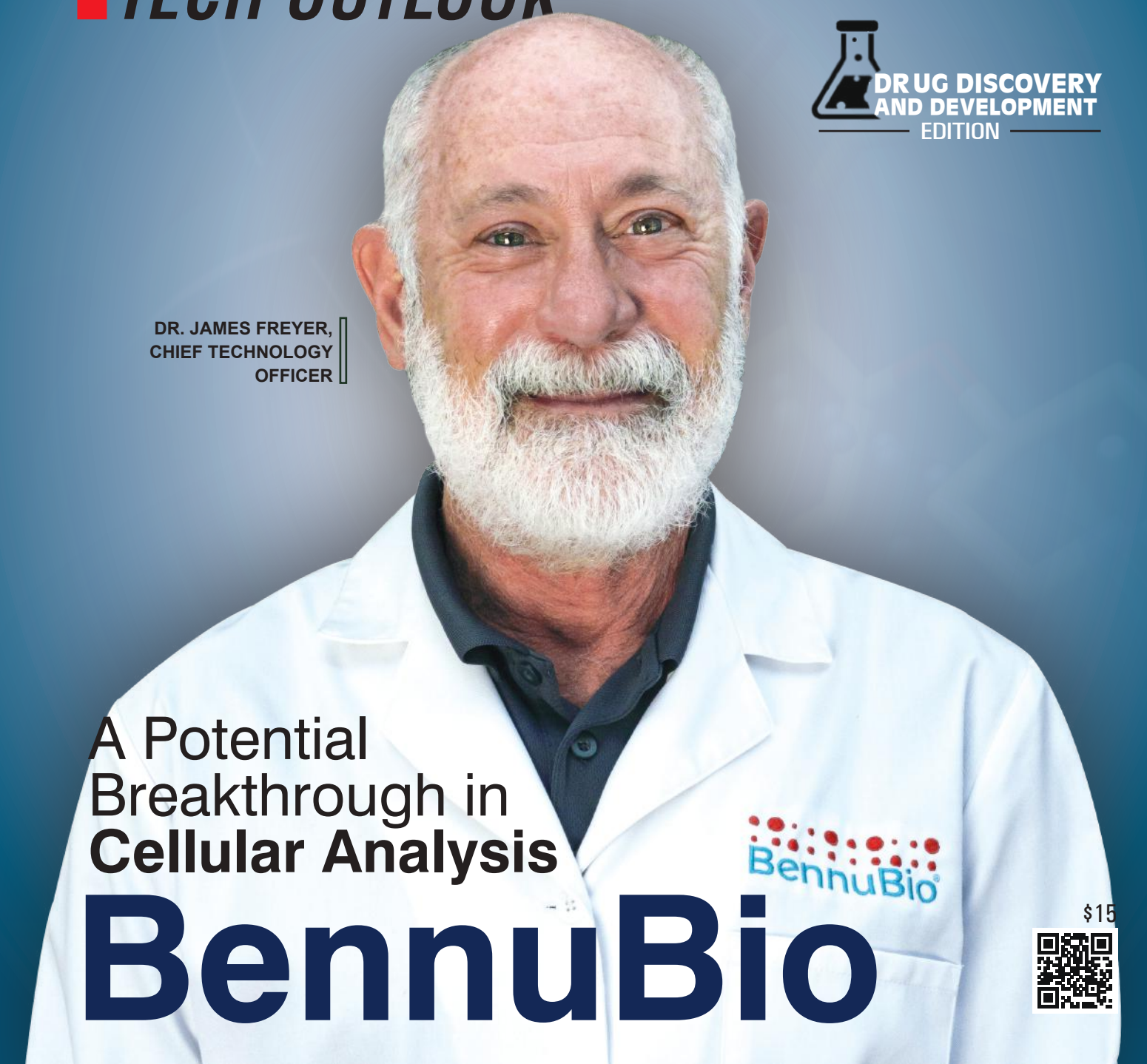
DRUG DISCOVERY AND DEVELOPMENT EDITION



DR. JAMES FREYER, CHIEF TECHNOLOGY OFFICER

A Potential Breakthrough in **Cellular Analysis**

BennuBio

BennuBio

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A Potential Breakthrough in **Cellular Analysis**

By Stacey Smith

The human body's immune system has myriad ways to fend off viral invaders and keep them from returning again. Besides selecting and stockpiling memory B cells that produce antibodies capable of jumping into action if the virus were to return, the immune system also deploys T cells, which patrol the body and destroy infected cells, disrupting the virus's ability to proliferate.

These immune cells, which enable a human body to fight harmful pathogens even after the original antibodies have faded, hold the key to vaccine development.

However, discovering these rare cells—which circulate in the blood at a frequency of about 1 cell in 108—has proven to be extremely difficult through conventional cellular analysis methods. One such molecular technique involves staining lymphocytes and their subsets before carrying out fluorescence-based flow cytometry assays to specifically identify cells producing a response to the vaccine. However, current flow cytometers take an impractically long time to analyze enough cells to accurately measure such rare cell types

As researchers work tirelessly to develop a vaccine that can mount a lasting defense against SARS-CoV-2—the virus that causes COVID-19—they are faced with the reality that it is complex to definitively evaluate the human immune response to the virus, which varies by type and degree of response. Also, not all vaccines elicit the antibodies required for functional immunity.

Enter BennuBio

This is where BennuBio, a developer of bioanalytical instruments based in New Mexico, could prove to be a game-changer in the area of cellular analysis and vaccine development. BennuBio's instrument is the Velocyt, an acoustically focused multi-stream flow cytometer based on multiple patents. The Velocyt performs rare cell analysis, rapid analysis of blood cells, and can process large volume samples as well as perform re-analysis of a sample.

"Accurately analyzing rare immune cells is currently arduous and time-consuming. However, if you can discover them reliably, you can tell through a simple test [using our instrument] if the vaccine has been effective or not," explains Dr. James Freyer, the CTO of BennuBio. Dr. Freyer notes that rare cell analysis (including activated immune cells, circulating tumor cells, and fetal cells in maternal blood) is one of many application areas that the Velocyt is poised to tackle.

Another important advantage of the Velocyt versus conventional flow cytometers is its ability to measure particles over a wide size range, from small blood cells to much larger multicellular systems. In the context of COVID-19, this can potentially allow the rapid

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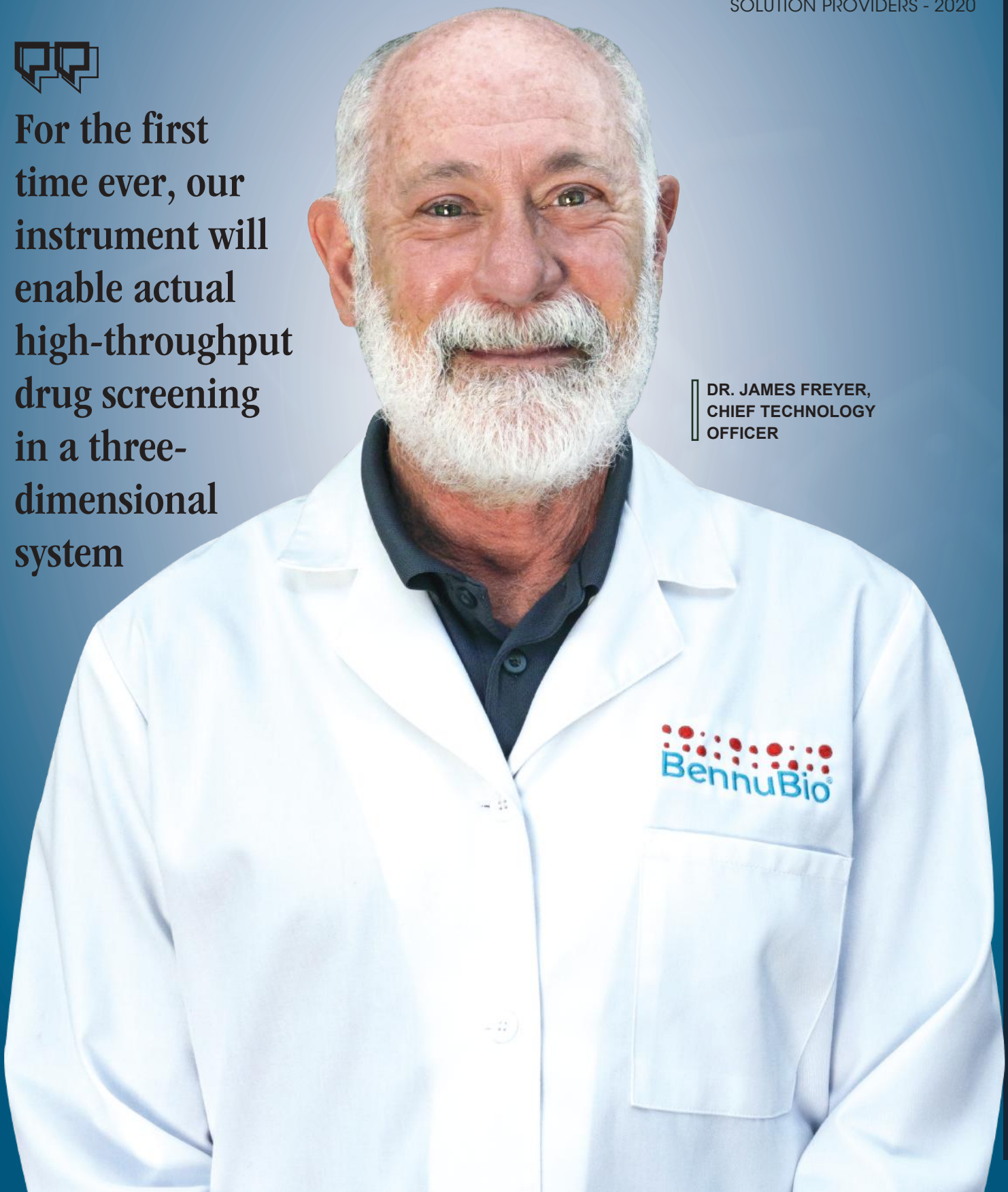

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analysis of microemboli in blood samples. These multicellular clusters of different blood cells can be a significant clinical problem in a subset of patients, as they can induce blood vessel blockage in any organ.

Another important application for large particle analysis is the measurement of multicellular spheroids and organoids, which are aggregates of different cells mimicking a tissue. Spheroids are increasingly used in cancer research, stem cell biology, normal tissue biology, and drug screening. Drug screening using spheroids provides a system of intermediate complexity between single cells and in vivo tissues, which has the potential to reduce the use of animals in drug development.

Currently, it is difficult to rapidly analyze spheroid/organoid responses to drugs, presenting a challenge to researchers developing drugs directed against a solid organ or tumor. Most analytical platforms use microscopy of spheroids in stationary culture. This approach has such a low throughput (<1 spheroid analyzed per second) that it is not actually high throughput in the context of drug screening. Another problem is that maintaining spheroids in stationary culture produces unpredictable and time-dependent gradients in inter-spheroid nutrients, waste products, cellular physiology, and viability as well as inconsistent drug concentrations in and around the spheroids. In contrast, drug screening of 3D tissue models with the Velocyt eliminates these limitations in spheroid-based drug screening.

Screening drugs with spheroids has never been high throughput, which makes BennuBio's instrument very disruptive. "For the first time ever, our instrument will enable actual high-throughput drug screening in a three-dimensional system," emphasizes Dr. Freyer, while jesting that modern-day researchers have spheroids "sitting still in a dish," which they look at using a confocal microscope, one at a time. "Despite all that effort, one can barely analyze 10 spheroids per minute, whereas our instrument can analyze over 100 times faster," he says.

Before delving deeper into the wide-ranging benefits of the Velocyt, it is essential to highlight why it is a first-of-its-kind flow cytometer.

No Waste, Faster Analysis and Re-Analysis

Unlike traditional flow cytometers that have several technical constraints— a single analysis stream; analysis rates <50K particles/sec; sample rates of <250 µL/min; and >50-fold sample dilution with sheath fluid—the Velocyt utilizes standing acoustic waves, rather than sheath fluid, to focus a sample directly into 10 parallel particle streams without sample dilution.

These streams intersect line-focused lasers, and these intersections are imaged using a high-speed camera. Proprietary software converts the images into conventional flow cytometry measurements for each sample stream. The Velocyt's design also simplifies workflows by eliminating washing, lysing, and concentration steps, and analyzes samples at up to 10 mL/min and >100K events/sec. Because the sample is returned undiluted after analysis, the Velocyt offers the novel ability to preserve precious samples for other analyses or to perform kinetics on a single sample.

By returning the sample unchanged after analysis, the Velocyt opens new possibilities in the realm of kinetics. Dr. Freyer elaborates, "You can expose just one sample to a drug, measure it, wait a while, measure it again, and thereby obtain kinetic responses from just one sample." In a world that predates the Velocyt, a researcher would have to run a sample in a flow cytometer, perform the analysis, and the sample would be deposited in the waste bottle. "The sample after your analysis is diluted by a factor of 50-100 in sheath fluid.

Therefore, it becomes very tough to do anything with it," adds Dr. Freyer. These limitations also mean multiple samples are required to do a kinetic blood assay or cytometry measurement, which results in lower throughput, slower analysis rate, and fewer analysis points. Such studies are extremely difficult for clinical samples since it can be hard to obtain enough cells for a multi-sample kinetic study.

In contrast, the Velocyt analyzes 10 streams of cells simultaneously, making it essentially 10 flow cytometers in one instrument. And, while a typical flow cytometer analyzes

particles up to 30 microns in diameter, at a speed of <100 microliters per minute, the Velocyt's flow cell is 200 micrometers deep and 1.5 mm wide, allowing the instrument to analyze samples 100 times faster. According to Dr. Freyer, you can run the sample faster, experience 100 times the analysis rate of a single stream instrument, and most of all, push the sample through the instrument and get it back exactly the way it went in—which allows simple re-analysis of the same sample.

The unique capability of performing kinetics with one sample and running large volumes of samples through the instrument can be a boon for researchers. For example, BennuBio is engaged in talks with a team that is working on a drug screening system to screen for tumor drugs. Rather than screening thousands of drugs for a particular tumor, this group handpicks ~20 drugs that have been effective with some, but not all, patients. "What they're developing are methods to use each patient's own cells to do a drug screen against those 20 drugs," says Dr. Freyer. "Before this approach, it was a 'throw darts and see what sticks' method, not knowing whether a patient is going to respond until you give them the drug and they respond, or not."

> **Cell-cell interactions are involved in essentially all diseases, so screening drugs on the basis of enhancing or abrogating a specific cell-cell interaction would be a unique and powerful tool**

En Route to Unveiling the HTSSP by 2022

Already disrupting the market with its Velocyt model V1 cytometer, BennuBio promises to usher in more possibilities in cellular analysis through its high-throughput spheroid screening platform (HTSSP), which is on track for a 2022 launch.

An entirely new tool to study cell-cell interactions, the HTSSP is a powerful multicellular spheroid 3D tissue/tumor analysis system with potential applications in 3D drug screening and other true high-throughput screening (HTS) applications. The HTSSP, which will be integrated with a modified version of the Velocyt flow cell and analysis software, will maintain spheroids in a stirred suspension prior to, during, and after analysis, providing reproducible spheroids and consistent drug exposures. The spheroids will be gently pushed through the flow cell for analysis and returned, undiluted, to a second chamber for further culture or re-analysis, notes Dr. Freyer.

In addition to advantages in spheroid sample handling, the HTSSP will also provide unique benefits for spheroid analysis. For example, whole spheroid parameters (size, cell count, morphology, apoptosis, and viability) can be rapidly measured on thousands of spheroids. One can also analyze spheroids composed of co-cultures of two or more different cell types: using fluorescence labelling, whole spheroid measurements can be used to derive changes in the ratios of different cell types within the spheroid.

Importantly, the HTSSP will allow screening assays that target a specific cell-cell interaction, which is not possible through existing technology. "Cell-cell interactions are involved in essentially all diseases, so screening drugs on the basis of enhancing or abrogating a specific cell-cell interaction would be a unique and powerful tool," adds Dr. Freyer.

In addition to whole spheroid analysis, the HTTSP also provides the opportunity to develop more sophisticated drug screening assays that involve measuring distributions of fluorescence within the spheroid structure. This is possible due to the Velocyt's optical design, which measures inter-spheroid fluorescence distributions, a task that is currently (and painstakingly) done using confocal microscopy. As spheroids transit Velocyt's line-focused laser, a 2D image of each spheroid in each stream, and each fluorescence color, is produced. Since spheroids are spherically symmetric, these 2D images represent the spatial distribution of fluorescence within the 3D structure.

This innovative analysis approach will allow screening based on drug penetration, spatial variations in viability/apoptosis/necrosis, spatial distributions of cell types, and distributions of microenvironmental parameters (e.g., proliferation, metabolic activity, hypoxia)—another benefit that is not possible in current flow cytometers.

A Tale of Three Academics Coming Together

Dr. Freyer met Dr. Steve Graves and Mr. Travis Woods during his time as the head of the National Flow Cytometry and Sorting Resource at the Los Alamos National Laboratory in the early 2000s. Using this NIH funding to develop instrumentation and applications for flow cytometry, the group began making serious headway developing a new paradigm for particle focusing in flow cytometry, which had not evolved much since the 1970s. By 2010, Graves and his team had designed novel acoustic-focusing technology [as opposed to hydrodynamic-focusing] and were responsible for the focusing technology behind the popular Attune NxT Acoustic flow cytometers.

In 2018, the trio got together, founded BennuBio Inc., and received first-round funding from VCs to establish the new company.

To Dr. Freyer, BennuBio's Velocyt marks the culmination of two disciplines that he has spent his entire adult life working on: multicellular spheroids and flow cytometry.

"As a graduate student in 1978 [at University of Rochester, NY], I was growing spheroids and analyzing single cells using a flow cytometer. Today, towards the end of my career, two of my fundamental scientific interests have finally come together through one instrument. Now, I can do flow cytometry on spheroids, something I never thought was possible," reflects Dr. Freyer, a Ph.D. biophysicist by training, who is admittedly getting accustomed to life as an entrepreneur. "Steve, Travis, and I have worked together for 20 years. We are collectively motivated to commercialize technologies that can address serious limitations in flow cytometery," he adds.

> **We see ourselves as a company that can expand the boundaries of what people can do with flow cytometry**

Just Getting Started...

BennuBio recently secured a Round B funding of $5M to go with another $1M SBIR grant from the National Institute of General Medical Sciences to develop the HTSSP. While the one-laser Velocyt model V1 is currently for sale, the two-laser model will reach the market by Q2 of 2022, and a beta version of the HTSSP will be made available by late 2022.

Having hardened its original Velocyt for commercial use, BennuBio is presently ramping up its marketing efforts, visiting trade shows and cytometry meetings, where the initial response to the Velocyt has been nothing short of overwhelming. "The basics of flow cytometry haven't changed in over 30 years. However, the feedback we keep receiving is, 'this is the first fundamental innovation in this field in over a decade,'" says a proud Dr. Freyer.

Clearly, a favorable future awaits BennuBio, whose founders truly believe that a lot of the Velocyt's benefits are yet to be tapped. For example, the instrument can be used as an in-line measuring device to monitor the contents inside a fermenter by continuously passing a stream of the contents through a Velocyt. Likewise, the Velocyt can be used to develop assays to analyze blood samples quickly, with fewer preparative steps. Currently, flow cytometric blood cell analysis requires separate concentration, staining and washing steps, and standard instruments are practically restricted to only analyzing a small subset of the cells in the sample. "We can take 1mL of blood, add fluorescence stains to label particular cells in a few minutes, dilute the sample ~10-fold and run the entire sample through the instrument to analyze every single blood cell. This is unheard of with current methods," states Dr. Freyer.

Moving forward, BennuBio plans to sell its instruments through its own marketing channels. However, the company is open to strategic partnerships in the future, especially once the technology generates significant interest. "We are all idea guys here, so having someone else manufacture and sell the Velocyt under license will allow us to move on to our next innovation."

In conclusion, Dr. Freyer says BennuBio will continue to push the boundaries of what flow cytometry can do, stating, "Is your instrument unable to discover rare cells? We'll fix that. Is it not good enough to accommodate big particles? We'll fix that. Can't do facile kinetic analysis? Talk to us. We see ourselves as a company that can expand the boundaries of what people can do with flow cytometry." Ph