2020
CASE STUDY

Cytometry Gets Fast

High-speed imaging enables real-time, single cell analysis.

From cancer research to drug development, flow cytometry plays an increasingly important role in cell analysis. Modern flow cytometry analyzers even allow researchers to characterize the image of single cells, rather than sole spectral data, to gain insights into a variety of key cellular phenotypes. These analyzers have traditionally relied on lasers, but recent breakthroughs in high-speed streaming cameras are creating new approaches to flow cytometry.

One such effort comes from the machine vision experts at Spica Technologies (Spicatek). The company's engineers have developed an image-based flow cytometry capability that can track and analyze single cells in real time as they travel through a microfluidic channel.

Imaging microscale objects, such as cells, as they pass through a microfluidic channel is no easy task. Depending on the velocity of the cell, the required frame rates will range from hundreds, to hundreds of thousands of frames per second. The cell velocity also raises motion blur concerns—resulting in the need for camera exposure times on the order of a few microseconds. Unless the motion blur is addressed, fast-moving cells will appear as amorphous streaks, rather than as well-defined shapes. (*See sidebar, Understanding Microfluidics Imaging.*)

When it's too fast to see, and too important not to.®

CELLULAR HIGH-SPEED IMAGING

To meet these challenging imaging requirements, Spicatek based its flow cytometry technique on high-speed machine vision cameras from Vision Research, an off-the-shelf frame grabber and a computer processor. "Most of the setup is commercial off-the-shelf hardware," says Mike Brommels, one of Spicatek's principals. This commercial-off-the-shelf (COTS) approach differs from other commercial flow cytometry analyzers, which tend to use proprietary pieces of equipment.

Phantom S640

Spicatek chose to work with a Phantom S640 camera, which offers highly detailed 12-bit imaging in a streaming camera with a 4-megapixel sensor and 10-micrometer pixel size. It can stream up to 6 gigapixels per second of data directly into frame grabbers, and you can choose to use either 4, 8 or 16 CoaXpress (CXP) channels from the camera. The S640, like the other Phantom streaming cameras, can easily be integrated with compatible laboratory microscopes via C-Mount or F-Mount adapters.

The rest of the setup includes other readily available hardware components—including a syringe pump, a transparent microfluidic chip and an LED fiber optic light.

In operation, the system uses the syringe pump to force cell-containing liquid into the inlet of the microfluidic channel, providing one-cell-in, one-cell-out flow control. The camera images the cells though the microscope, with LED fiber optic lights set up to backlight the scene. "The lighting setup is very important," notes Vision Research field applications engineer Dr. Kyle Gilroy, explaining that intense backlighting overcomes potential light starvation that can arise when running cameras at high frame rates and ultra-short microsecond exposure times.

REAL-TIME PROCESSING OF HIGH-BANDWIDTH IMAGING DATA—AND WHY IT MATTERS

A recent demo of this image cytometry technique mimicked a eukaryotic cell—a 25-micrometer sphere of polystyrene—moving through a microfluidic channel at velocities up to 230 millimeters per second. Setting the Phantom S640 to capture 3,300 frames per second, the company was able to precisely and accurately track the position and velocity of individual spheres (*see Figure 1*).

While most of the hardware components for this imaging flow cytometry technique were off-the-shelf, Spicatek engineers developed custom image processing software—running on a Windows PC with some processing help from an additional computer processor—to ingest and analyze the high-bandwidth imaging data in real time. "Even at high frame rates, our algorithms can perform an analysis on the current frame before the next frame arrives," says Brommels, explaining that Spicatek's cell tracking algorithms are looking a couple frames behind the existing frame and making a forward prediction even with data streaming from the camera at up to 6 gigapixels per second. "The software is highly efficient," he says.

Spicatek's demonstration has implications for a variety of single-cell flow cytometry applications. Building on the ability to track individual cells through the channel, the imaging algorithms can also characterize a single cell's morphological characteristics—including size, shape, opacity and granularity.

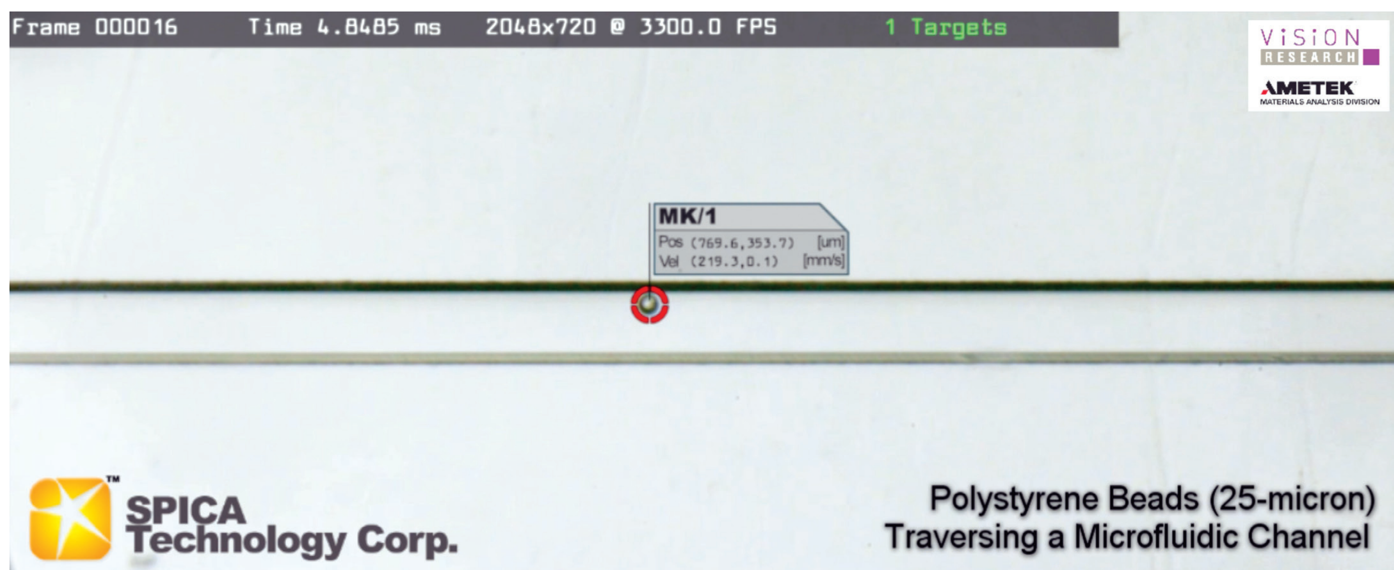
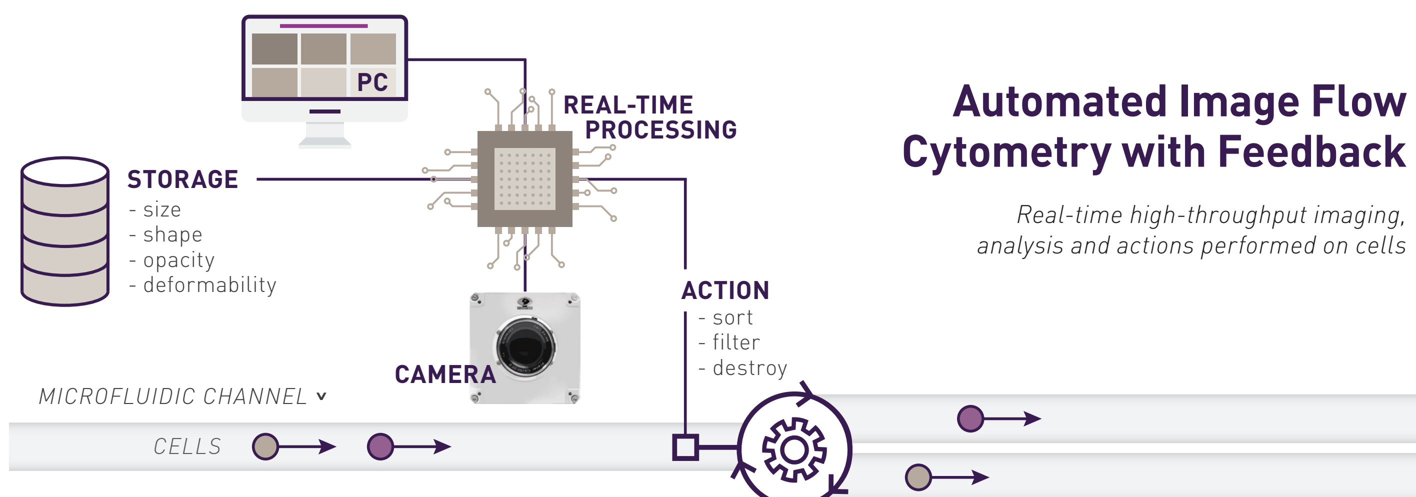


Figure 1: Spicatek's cytometry rig accurately tracks the position of 25µm polystyrene beads in real time as they travel through a microfluidic channel. The bead size and velocity are similar to the requirements for tracking and classifying individual biological cells in flow cytometry applications.

High-speed imaging can also shine a light on how individual cells deform under applied forces—with the elasticity of individual cells providing information about cell health. The speed of Phantom cameras and Spicatek's software together can potentially allow the filtering of individual cells based on their morphology. "With real-time analysis of cell characteristics, you can start to make well-informed decisions about single cells in real-time," says Gilroy.

By combining high-speed imaging with off-the-shelf frame grabbers in a single, easy-to-use package, Spicatek has made single-cell flow cytometry more accessible to other researchers. This turnkey technique is also highly flexible, providing the building blocks for others to tailor their own cytometry experiments with algorithms that can characterize, measure and analyze cells in real time.

Spicatek's image cytometry technique is just one of many advances in the use of high-speed imaging in biomedical microfluidics applications. To learn more about this fast-growing field, including camera usage tips and case studies, download Gilroy's paper, "High-Speed Imaging in Biomedical Microfluidic Applications: Principles & Overview."



Automated Image Flow Cytometry with Feedback

Real-time high-throughput imaging, analysis and actions performed on cells

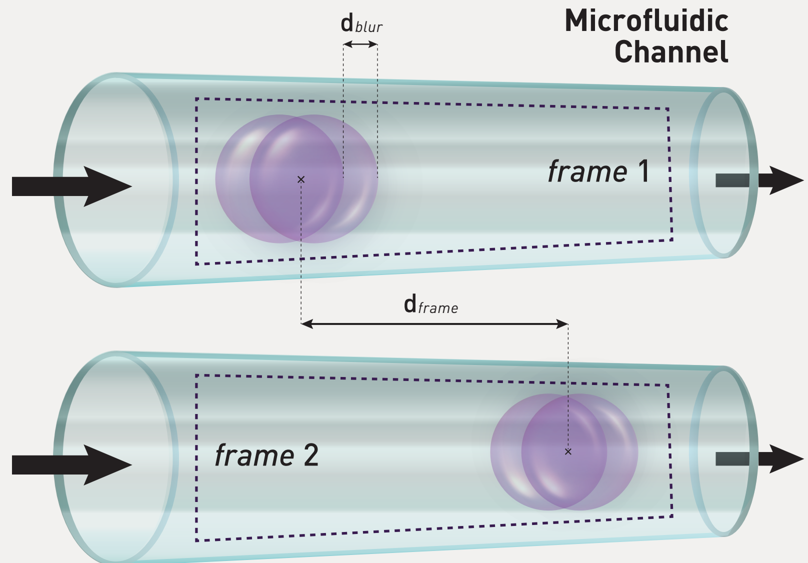
UNDERSTANDING MICROFLUIDICS IMAGING

Successfully imaging microfluidic experiments requires a basic understanding of microfluidic-flow physics and high-speed imaging principles. What sets microfluidics apart from other applications is a small field of view (FOV) combined with extremely small subject sizes. Due to the unique combination of these challenges, it's important to minimize motion blur by employing a high frame rate and low exposure time when working with fast-moving micro-objects.

MICROSPHERES & MOTION BLUR

Consider two sequential frames of a microsphere moving through a microchannel. The microsphere, moving at a velocity (v_{sphr}), is imaged at a frame rate of FR and with an exposure time of t_{exp}. Using the frame rate, we can calculate the distance (d_{frame}) the microsphere will travel between frames:

$$d_{frame} = v_{sphr} \times FR^{-1}$$



A typical microfluidics experiment may require imaging a cell moving linearly at a speed of 1 meter per second—a seemingly simple task. Your natural inclination might be to mount a standard camera on top of a microscope with a 10x objective lens, which has a round, 2-millimeter FOV. Based on this setup, it wouldn't take long before you realized imaging the cell would be difficult. Why?

Lengthy exposure times around 100 microseconds create motion blur, causing cells to appear like a streak and eliminating valuable shape information. According to the following equation, the cell's blur-length (d_{blur}) is directly related to its velocity (v_{sphr}) and exposure time (t_{exp}):

$$d_{blur} = v_{sphr} \times t_{exp}$$

Using this value, we can then calculate the percent motion blur (%blur). For a 10-micrometer cell moving at 1 meter per second with a 10-microsecond exposure time, this value would be 100 percent in the direction of motion:

$$\%blur = 100 \times d_{blur} \times \text{cell size}^{-1}$$

KEY TAKEAWAYS

Before you conduct an experiment, it's helpful to use these calculations to estimate the frame rate and exposure time you'll need based on your microsphere's approximate size and velocity, FOV and minimum distance you want the microsphere to travel between frames.

For applications that require high frame rates above 7,000 fps and maximum light sensitivity, 1-megapixel cameras may be the best solution due to their large pixel area. For applications that require lower frame rates and less light sensitivity, 4- and 9-megapixel cameras can provide more detail and higher potential resolution. In the context of lighting, because bright-field microscopy employs backlighting of the sample, it is rare that sensitivity, and thus pixel size, is ever an issue.

To learn more about Vision Research high-speed expertise and equipment, visit ***www.phantomhighspeed.com***

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