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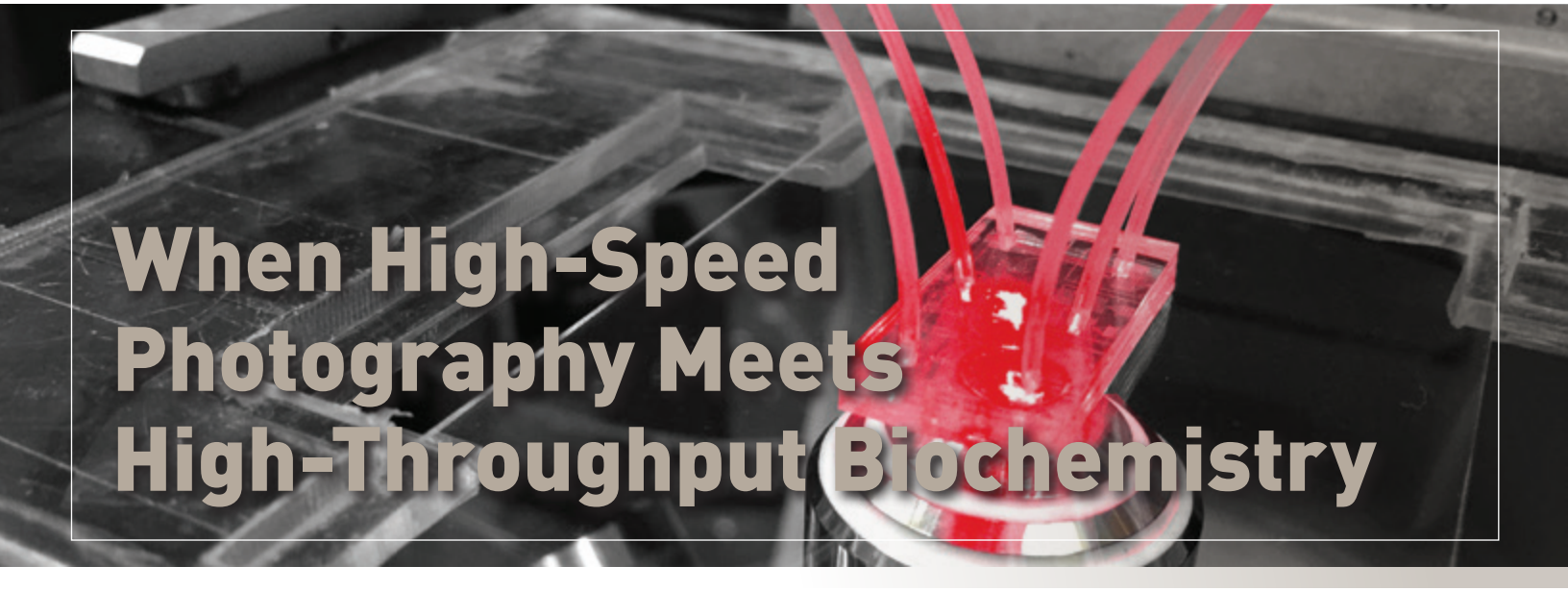
2019
CASE STUDY



When High-Speed Photography Meets High-Throughput Biochemistry

Researchers use a Phantom high-speed camera to observe enzymes during ultra-fast microfluidic experiments—an area of research that has vast implications for protein engineering and the treatment of human disease.

Proteins are the building blocks of life—a fact you probably remember from Biology 101. Because these molecules perform some of the most complex chemical and biological functions, it's no wonder scientists believe they hold the key to solving many human problems related to disease, environmental protection and more. But despite such great promise, our ability to enhance and engineer new proteins is limited by our understanding of these sophisticated molecules. This is the challenge that the biochemistry department at the University of Wisconsin-Madison is hoping to overcome with the help of high-throughput experimentation and high-speed photography.

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MEET THE ROMERO LAB

Headed by Assistant Professor Phillip Romero, the university's Romero Laboratory studies the design principles of natural proteins, including how these proteins perform their biological functions and how that information can be applied to engineer new proteins. This research holds particular promise for enzymes—catalytic proteins that accelerate the rate of specific chemical reactions in animals, plants and microorganisms. This ability to design enzymes with tailor-made chemical functions has far-reaching consequences for a number of industries, including medicine, agriculture and industrial chemistry.

As part of their research, Romero and his research team utilize high-throughput experimentation, which enables rapid, large-scale repetition of experiments aimed at dissecting protein function on a molecular basis. This interdisciplinary process combines techniques from biochemistry, molecular biology, applied physics, engineering and computer science—to name a few.

"Recent advances in high-throughput technologies have resulted in an explosion of new structural, genomic and functional protein data," Romero says. The lab utilizes this data to develop machine learning-based tools that infer the relationship between protein sequence and function. In addition, the team is developing high-throughput technologies of their own using microfluidics, or the manipulation of the flow of very small volumes of fluid within micrometer-sized channels.

MILLIONS OF MICROSCOPIC EXPERIMENTS

Romero and his team utilize microfluidics to perform biological assays in water-in-oil microemulsions. "These techniques allow us to conduct millions of biological experiments at once, which is impossible to do any other way," Romero says. "Running large numbers of experiments like this is very advantageous."

Each picoliter-sized droplet can be made to house reactions involving different cells, biomolecules or chemical combinations. For example, droplets are loaded with single cells, incubated over a range of times and temperatures, injected with additional reagents and sorted based on their optical properties. These droplet operations can be performed with full automation at kilohertz frequencies, providing a general platform for high-throughput biochemistry. The results provide a comprehensive view of the enzyme function landscape, which plays a role in the evolution of enzymes, DNA synthesis and mapping of protein sequence-function relationships.

"Thanks to these high-throughput techniques, we can screen millions of proteins at once," Romero says. "After we mutate them, we flow the variants through the small channels to sort the ones we're looking for—including variants that will potentially respond to a specific drug molecule." Identifying these target proteins is the first step towards developing new drugs to combat disease. In fact, Romero and his team are currently applying microfluidic techniques to screen caspase enzymes, which play a significant role in the treatment of cancer and the Zika virus.

"The flow through the channels happens very fast—too fast for normal cameras to pick up," Romero says.

SEEING THE UNSEEABLE WITH PHANTOM

Observing microscopic particles during microfluidic experimentation is beyond what the human eye can handle. In addition to the small size of the microfluidic droplets, "the flow through the channels happens very fast—too fast for normal cameras to pick up," Romero says. These droplet operations, performed at full automation, occur at kilohertz frequencies—or thousands of times per second. That's where the camera comes in.

The Romero Lab utilizes a Phantom Miro C110 high-speed digital camera, which can record at speeds up to 900 frames per second (fps) at full 1,280 x 1,024 resolution and over 52,400 fps at smaller resolutions. This camera is well suited for microfluidic applications, thanks to its flexible form factor, high magnification and C-mount lens that attaches to microscopes. For their experiments, Romero and his team recorded at speeds between 20,000 and 30,000 fps, enabling them to record the microscopic droplets as they moved quickly through the channels.

The Phantom Miro C110 records at 900 fps at full 1,280 x 1,024 resolution.

THE PHANTOM MIRO C110: PERFECT FOR MICROFLUIDICS

High-speed cameras used in microfluidic applications must be fast and light-sensitive, while producing high-quality images with little noise. Because capturing microfluidic images requires a microscope, using a small, lightweight camera is also key. Here are some of the ways the Phantom Miro C110 stacks up:

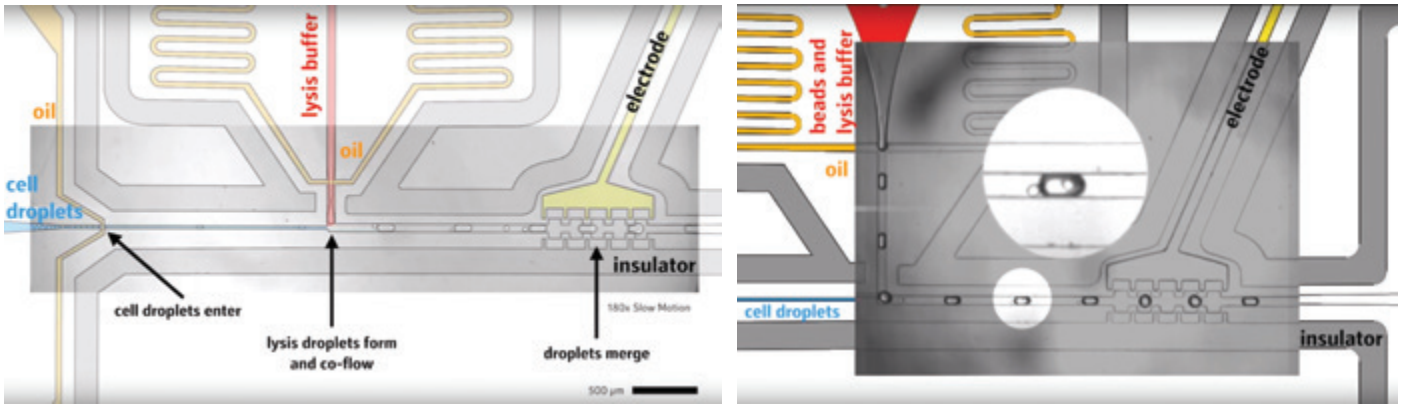
- **Small and flexible.** The Phantom Miro C110 weighs only 1.2 pounds and is housed in a compact 2.9 x 3.65 x 3.25-inch frame, making it a flexible, easy-to-use tool in laboratory settings.
- **High magnification.** The camera features a powerful 1.3-megapixel, 12-bit CMOS sensor with 5.6-µm pixels. Camera sensors with small pixels are capable of higher magnification and therefore higher resolution—resulting in higher measurable image detail for microscopic subjects.
- **Superior speed and image quality.** The Phantom Miro C110 has 1.2-gigapixels of throughput, providing 915 fps at full resolution with very low noise to capture critical details.
- **Light-sensitive.** Available in color or monochrome, the camera makes the most out of available light and includes the following light sensitivity ratings, which were measured according to the ISO 122326:2006 system: mono 5,000 (T), mono 2,500 (D), color 640 (T) and color 640 (D).

CASE EXAMPLE: **MAPPING A GLYCOSIDASE ENZYME**

In one experiment, the researchers applied ultra high-throughput microfluidics to map the sequence-function relationships of a glycosidase enzyme. They encapsulated the enzyme variants in microfluidic droplets, which also contained lysis reagents and a fluorogenic enzyme substrate, and injected the droplets into a microfluidic sorting device. Upon lysis, or cell membrane disintegration, variants were released into the droplets, causing them to interact with the substrate. Droplets that contained active variants accumulated fluorescent product, which the researchers measured using a fluorescence detection system.

Thanks to the camera, the researchers slowed down the droplets and observed their position in the capillary channel. It also generated microscopy images of the enzyme assay—including images of bright green droplets and dark droplets, which contained the active and inactive enzyme variants, respectively.

Over 1 million of the active variants were then recovered using high-throughput DNA sequencing and statistical analysis, creating a sequence-function dataset for the glycosidase enzyme. From this map, the researchers evaluated the enzyme's tolerance to mutation—discovering sites within the protein that cannot tolerate mutations and therefore play an important role in enzyme function. Having this kind of knowledge about a protein is critical for determining where and how the enzyme can be engineered—and this experiment provides a successful new method that can potentially be applied to other enzymes.



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