

# BIOPHOTONICS TECHNOLOGY SOLUTIONS

## Getting up to speed with mirrors

For Elizabeth M.C. Hillman, an assistant professor of biomedical engineering at Columbia University in New York, getting the big picture is important. Hillman uses optical brain imaging of living animals to observe, for instance, what happens to blood flow in the brain when a limb is stimulated. For such studies, she and other researchers must get a full yet detailed picture for a variety of reasons, not the least of which is what can be done with the data after the image is captured.

"When full images are acquired, it becomes possible to use image segmentation, spatiotemporal principal component analysis, particulate flow analysis, diameter changes, hematocrit changes, etc.," Hillman said.

Other advantages of the complete picture, she added, include the ability to

more clearly determine when problems, such as movement or image artifacts, occur. Detecting such glitches is more difficult if the image is not complete, such as is the case when acquiring just a line.

Unable to find a commercial system that met her needs and budget, Hillman designed her own custom two-photon microscopy setup. The instrument captures 22 fps of  $180 \times 180$ -pixel images, which translates to 4000 lines per second at a pixel acquisition rate of about 700 kHz. The system can achieve such speed, in part, thanks to the latest galvanometer-based optical scanner technology from Cambridge Technology Inc. of Lexington, Mass. These scanners sweep the laser beam across the viewing area and thereby provide the illumination for the video-rate two-photon microscopy.

According to Michael Thanos, vice pres-

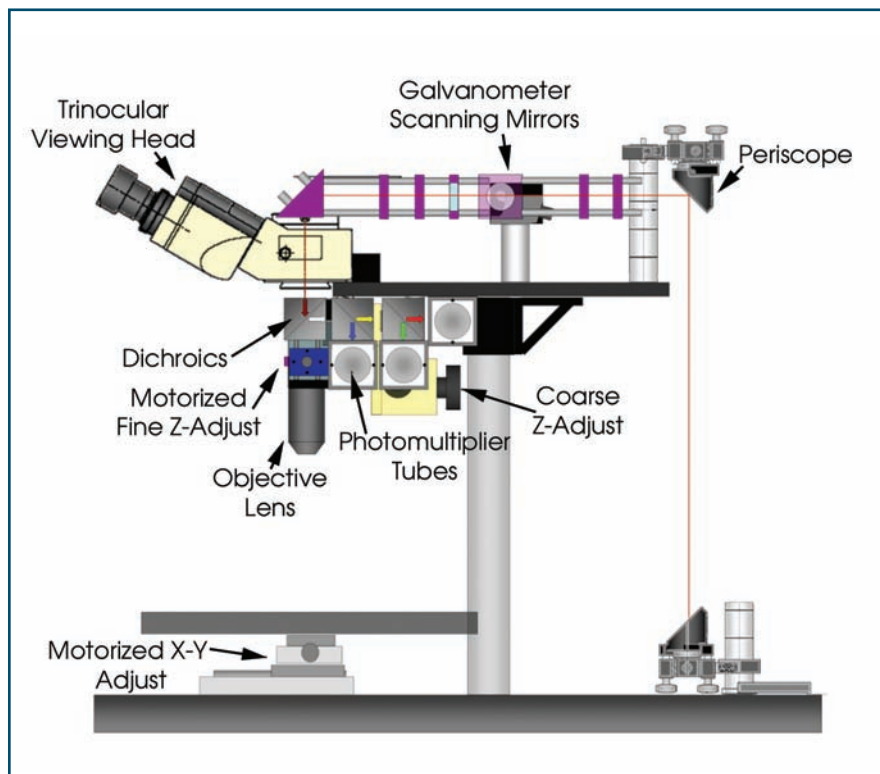
ident of engineering at Cambridge Technology, the scanner, designated 6215HB, is the result of a research and development effort initiated by the company several years ago. Realizing that there was a demand for more speed, the company increased the servo capability of the new scanner substantially, beefed up various parts of the system to handle the resulting increase in heat dissipation, lengthened the motor somewhat as a result and increased the power that the servo could supply fivefold. As a result, the root mean square acceleration went from 1.2 to 4.7 million radians per second squared.

"What that means is you can go four times the amplitude or twice the frequency," Thanos said.

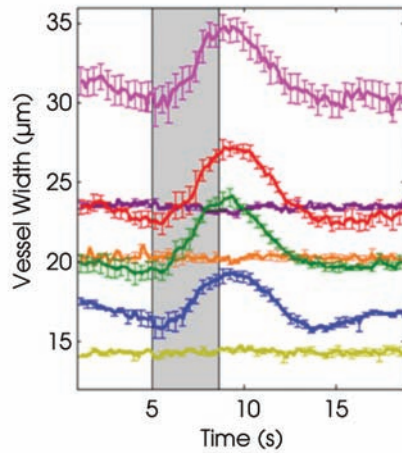
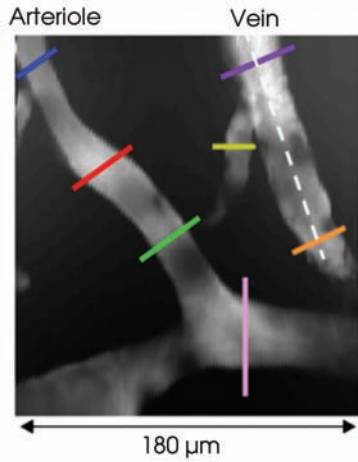
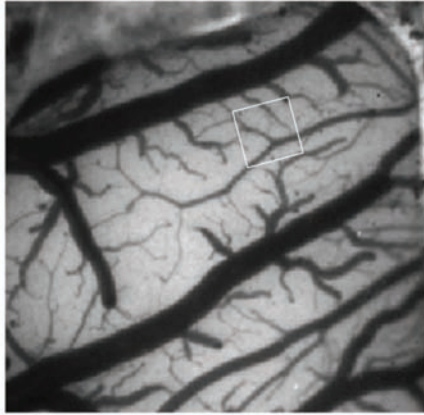
He said an ongoing project may boost the speed by an order of magnitude, but would not go into specifics. However, he noted, attaining the full rated speed of the current crop of scanners requires implementing active cooling via water or some other means. As the scanner moves, heat generated in the drive coil must be dispersed. Otherwise, it builds up and damages the scanner or triggers a shutdown as built-in protective circuitry kicks in. With only air cooling, that thermal barrier is reached at some value below the maximum, a shortfall that typically cuts the acceleration in half.

In Hillman's system, there is no water cooling of the scanner because the scan periods are less than 20 s, and the heat seems to be adequately dissipated with a heat sink. The system uses a Spectra-Physics Ti:sapphire laser that is tunable from 710 to 920 nm. The laser beam is steered by the Cambridge galvanometer mirrors through optics and into the specimen, which is mounted on a motorized X-Y stage. Unlike commercial products, which often are built on inverted systems, the custom system is an upright imaging platform with a fixed stage in the Z and with the objectives, not the stage, moving up and down.

The two-photon fluorescence emerging from the tissue passes through a



Researchers designed a custom two-photon microscopy system that uses galvanometer-based optical scanner technology to achieve a speed of 22 fps for  $180 \times 180$ -pixel images.



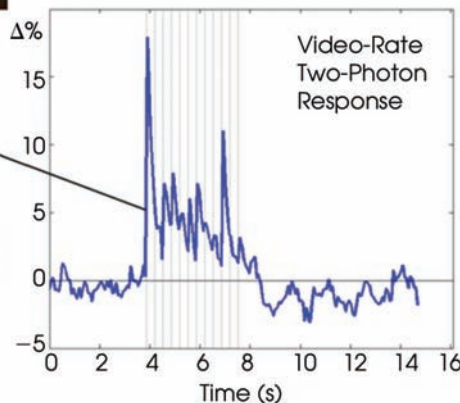
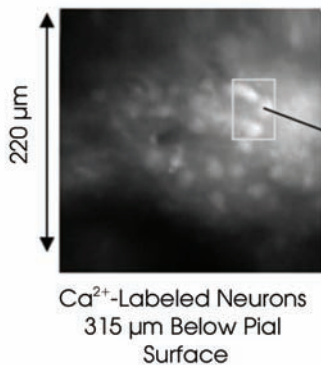
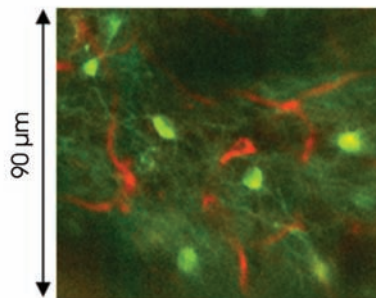
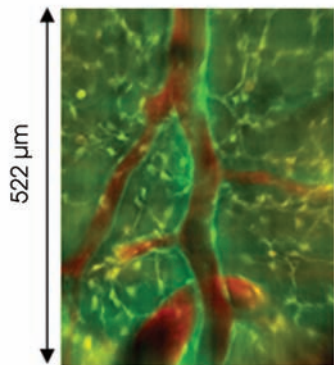
Diameters of arteriole and vein during 4-s (3 Hz) forepaw stimulation were extracted from two-photon image series. Error bars show standard error on four repetitions.

dichroic beamsplitter and is immediately detected by a pair of Hamamatsu photomultiplier tubes. Again, this is different from a commercial system. These often retrofit two-photon capability into existing confocal setups, an approach that reduces the available light. Particularly for in vivo applications where the desire is to image as deep as possible, researchers want to collect as much light as they can. They also have an even better way than a confocal approach to achieve the needed optical sectioning, Hillman noted.

"The beauty of two photon is that you don't need to discard the emitted light because you know that all light that is emerging at the fluorophores' emission wavelengths has come from only the excited spot," she said. "Because of this, the game is just to detect as much of the emitted light as possible."

She added that a custom system can be optimized in other ways, such as in the choice of objectives and data acquisition software. It also helps that the custom system does not start as a confocal microscope and so can be considerably less expensive and complex than its commercial counterpart. The savings, according to Hillman, can run hundreds of thousands of dollars if only the costs of parts are considered.

With the video-rate two-photon setup, Hillman and a team of researchers have simultaneously imaged neurons and vascular networks during single-trial stimulation experiments. For example, they repeatedly imaged together a rat brain



Researchers performed in vivo imaging of neurons stained with calcium-sensitive Oregon Green 488 BAPTA-1 AM, blood plasma labeled with Texas dextran red (top left). On the top right, neurons are expressing green fluorescent protein, with blood plasma in capillaries labeled with Texas dextran red. The bottom image shows that neurons stained with calcium-sensitive dye exhibit fast responses to forepaw stimuli delivered at 3 Hz. (NPY-GFP mouse supplied by Dr. Jeffrey M. Friedman, Rockefeller University, New York.)

arteriole and a vein while stimulating the animal's forepaw. After using wide-field imaging to determine where in the brain the blood flow responded, the investigators injected dextran conjugated with fluorescein to label the blood plasma and did two-photon imaging. They measured vessel width and blood flow as a function of time after the stimulation. They found that the arterioles dilated considerably, and they saw no change in vein diameter. Previously, it had been assumed that most of the hemodynamic changes were the result of veins ballooning. The researchers published work on this in the

March issue of *Neuroimage*.

By loading smooth muscle cells and neurons with a calcium-sensitive dye and imaging rat neurovascular structures using the same two-photon setup, the scientists began the process of understanding how communication between neurons and the circulatory system takes place. That quest is helped by the use of multispectral detection in which different emission bands are tracked simultaneously.

As for the future, Hillman is building a system that will detect at least three emission bands and that will be incrementally faster than the old, with the limit a result

of the speed of the galvanometer mirrors. Its capabilities will have to be balanced against what must be imaged and how often the images must be updated.

"It's a trade-off between how long the line is versus how many lines per second you can go," Hillman noted. □

*Hank Hogan*

*Contact: Elizabeth M.C. Hillman, department of biomedical engineering, Columbia University; e-mail: eh2245@columbia.edu.; Michael Thanos, Cambridge Technology Inc., Lexington, Mass.; e-mail: davidf@camtech.com.*

# Scanner Sets for Microscopy

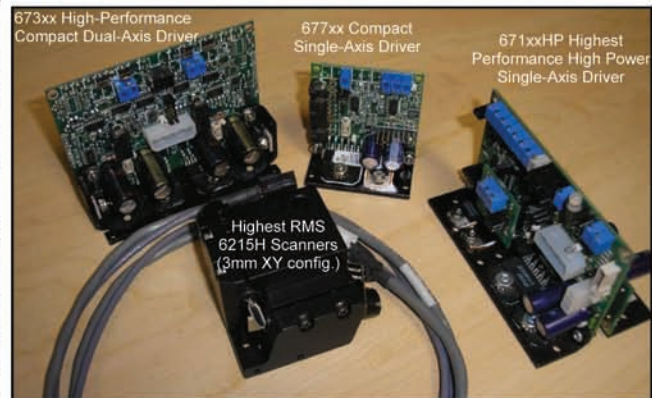
## Family of High-Performance Galvo Sets Optimized for Scanning Laser Microscopy Applications Achieve ~2kHz Scanning Speeds

**Cambridge Technology Inc.** is the world's leading supplier of galvanometer solutions for laser microscopy applications such as Confocal Laser Scanning Microscopes, OCT, multi-photon microscopy, LSOs, etc. Our patented galvanometer technology and servo driver architectures provide high-speed smooth, linear, low-jitter, constant velocity line sweeps enabling clear, crisp accurate images from your application. Our 6210H/6215H products have been optimized for high-speed raster and peak acceleration demands of emerging microscopy applications and has enabled scan speeds up to 2kHz\* making Cambridge Technology the choice supplier among leading microscope companies and research institutes.

**The Cambridge Technology Advantage** is the result of years of technological development to reach the highest speeds without sacrificing image quality or lifetime. High torque galvos were developed along with low-inertia position detector technology to achieve the highest scanning speeds. To unleash this speed, materials were developed to improve the thermal management and servo technology was developed to increase the power driven into the galvo. In parallel, bearing technology was developed to substantially increase the lifetime so this new speed performance can be enjoyed without negatively impacting product lifetime. Together, these technology developments result in the highest performance galvo-based scanning solutions available in the market.

**Cambridge Technology** has been supplying quality products to the biomedical and scientific community for over 25 years. We are fully committed to bringing you the latest technology scanning solutions to enhance your products, and to enable new areas of research. Visit our website for further information.

**Affordable solutions** are the result of Cambridge Technology's high volume, mass-customization of the configuration optimized for your application. We support several different aperture sizes; from 3mm to 75mm, galvo sizes, and servo capabilities to precisely meet your application requirements. Contact Cambridge Technology scanning professionals today for assistance in configuring your scanning system.



Example Products for Laser Scanning Microscopy

**Experienced Applications Assistance** is provided by Cambridge Technology to support the unique demands of microscopy applications. Cambridge Technology engineers will provide applications help to optimize tuning for maximum speed, constant velocity, minimized jitter, maximized duty cycle, increased product lifetime, and any other parameter of interest in your application.

\* scan angle dependent