purpose I/O to enable communication with outside devices.

Matrox introduced the Helios family of frame grabbers in 2002 to support higher-performance Camera Link cameras and to offer preprocessing capabilities to help the host CPU. All variations of the board feature a custom-designed chip that performs image formatting and preprocessing tasks.

The university researchers chose the frame grabbers in large part because of the strong integration of the company's products with leading hardware and software providers. Such integration was essential to the development of the digital subtraction angiography system, Sentoni said, because it depends on different off-the-shelf systems talking to one another (using various commercial systems helped to reduce the development costs considerably, relative to the proprietary architectures designed for other commercial systems).

Designing the system with affordability in mind was not without challenges, though. The system is based on the acquisition, multiprocessing, storing and displaying of images at 30 fps. Working with 1024 × 1024 images thus translates to dealing with 300 Mb/s of information — roughly 80 percent of the PCI bandwidth — and medical regulations require that the information be saved to disk without compression. They met these challenges through a well-designed software architecture, Sentoni said, taking advantage of the hardware-software integration of the frame grabber and the company's associated image processing libraries.

The acquisition and processing module, now handled by a company formed by one of the researchers, was released to the market in Argentina in 2006. About 15 systems have been sold thus far, Sentoni noted, and have been deployed in hospitals and in various other health care institutes. Plans are in place to export the systems to several other Latin American countries, after ensuring compliance with local regulations.

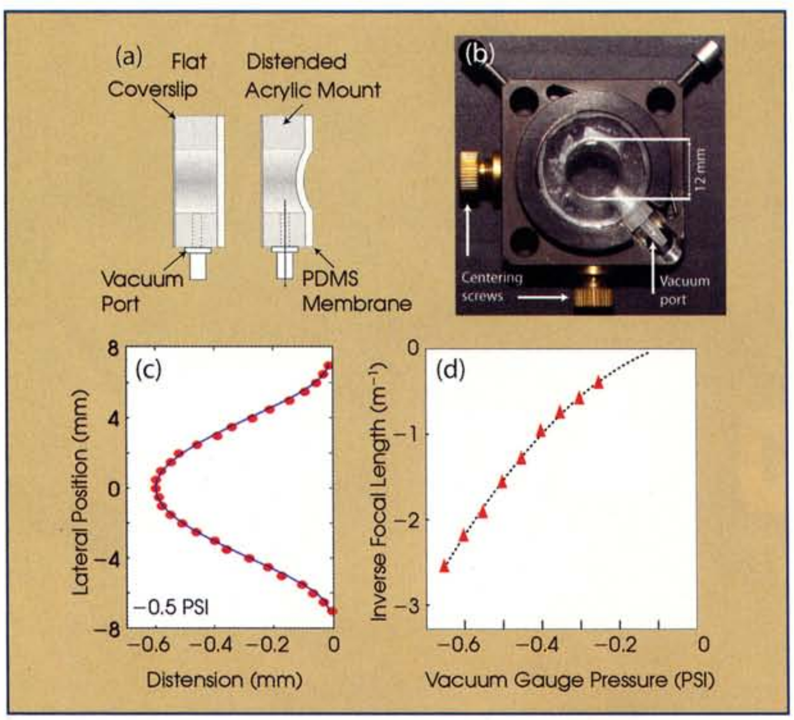
The investigators are working to improve the system, Sentoni said, by adding more functionality for a better diagnosis with digital subtraction angiography. First, they are working to achieve better control over the x-ray generation timing using the embedded PC. Also, upcoming releases will incorporate full Dicom capabilities. Dicom is a Digital Imaging and Communications in Medicine standard for distributing and viewing medical images. □

Gary Boas

Contact: Guillermo B. Sentoni, Universidad Argentina de la Empresa; e-mail: gsentoni@uade.edu.ar; Leonardo Jorge Seminara, Xineus Technology; e-mail: jlseminara@xineus.com; Sarah Sookman, Matrox Imaging; e-mail: ssookman@matrox.com.

An adjustable corrective lens for two-photon microscopy

Those who use two-photon laser scanning microscopy could soon see better performance, thanks to a group of researchers from the University of California, San Diego, and from the Weizmann Institute of Science in Rehovot, Israel. The investigators designed and demonstrated a deformable membrane that improves the axial resolution of two-photon microscopes twofold.



The optical performance issue arises because the water-dipping objectives typically used in two-photon microscopes have a high numerical aperture and a long working distance, two important characteristics. However, because these lenses are designed to image at the surface of a sample and not deep within it, the resolution degrades with increasing depth. For material with an index of refraction

Researchers used a deformable polymer membrane that can be distended via a vacuum (a) for correction of the spherical aberration in two-photon laser microscopy (b). The measured distension averaged over three readings of the membrane as a function of the radial position at a gauge pressure of −0.5 psi is shown(c), as well as the effective focal length of the distended membrane vs. the vacuum pressure (d). The focal length was determined by imaging a filament with the membrane in tandem with a fixed lens. Images reprinted from Applied Physics Letters.

greater than 1.33, which is the case with tissue, the refraction at the water-sample interface induces a negative spherical aberration in the beam. Thus, rays at the margins focus deeper than paraxial rays, and the mismatch becomes larger with increasing depth.

The differences between the marginal and the paraxial paths can be compensated for by modifying the radial phase profile of the beam. The researchers accomplished this by using a transparent deformable membrane made from the rubbery polymer polydimethyl siloxane (PDMS) constructed by team member Alex Groisman. The membrane was made using a customized formulation that produced PDMS with at least a 70 percent greater extensibility than the standard formula.

It was key to the entire technique that they produce an optically flat layer of silicone prepolymer. To avoid introducing unwanted effects, the membrane had to be of comparable optical quality to the rest of the elements in the microscope and so had to be optically flat. The researchers accomplished this by placing the prepolymer on a flat silicon wafer, illuminating it with a helium-neon laser to create interference fringes and tilting the stage on which the wafer rested. The prepolymer flowed in response. The researchers continued this process until the interference fringes were minimized. Curing produced a 1.5-mm-thick membrane with a thickness variation of less than a wavelength over a 12-mm span.

They placed the membrane on one end of a doughnut-like acrylic mount, with a coverslip affixed to the other end. When they pulled a vacuum through a port in the mount, the membrane bowed inward to form the shape of a meniscus lens. The vacuum allowed control of the membrane distension. Varying the pressure from −0.3 to −0.7 pounds per square inch (psi) led to effective focal lengths from −2000 to −400 mm near the optical axis of the membrane.

They tested this corrective element in a custom two-photon microscope. In one case they imaged 0.2-μm-diameter fluorescent beads suspended in a gel and found that the on-axis resolution with the membrane flat was 2.2 μm at the surface and twice that at a depth of 700 μm. When they applied a pressure of −0.6 psi, they restored the axial resolution to 2.2 μm.

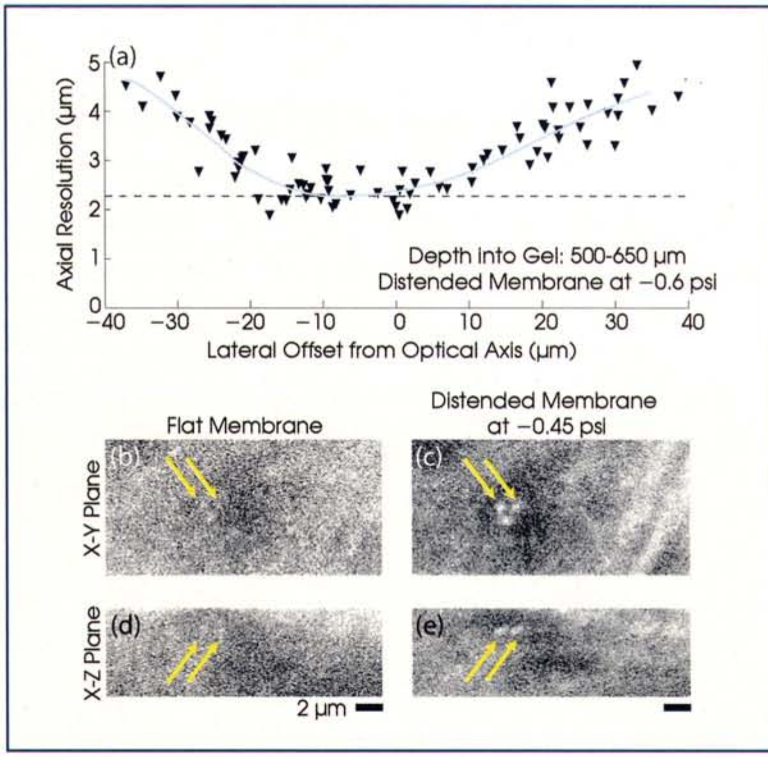
The researchers also tested the deformable membrane by imaging brain tissue from transgenic mice. These mice expressed the cyan fluorescent protein exclusively in their neuronal mitochondria, which are less than 1 μm in diameter. At a depth of 700 μm and with a flat membrane, the scientists had trouble discerning the mitochondria. With the aberration corrected, they were able to clearly resolve the mitochondria in both the lateral and the transverse planes.

Finally, they used the same corrective technique to improve laser-induced plasma-mediated ablation. Setups with uncorrected aberration have an axial elongation of the damage zone and require increasing laser energy to produce visible damage at increasing depth. The researchers found that their corrective membrane reduced the axial elongation by up to 50 percent and allowed them to produce visible damage at depth with lower energy. The work was published in the Nov. 5 issue of *Applied Physics Letters*.

In the future, the investigators may use the technique for a complementary purpose. Recent research has shown that the membrane allows high-speed changes in focus, a capability that can be exploited.

"This will enable us to scan the beam in two-photon microscopy along Z for measurements of blood flow and/or neuronal function," said team member David Kleinfeld. □

Hank Hogan



Shown here is off-axis axial resolution from a two-photon laser scanning microscope, imaging with a 40×, 0.8-NA, water-dipping objective. Resolution vs. lateral position relative to the optical axis at depth in a sample is shown (a), where the spherical dotted line is the diffraction-limited resolution at the surface. Endogenously labeled mitochondria in a sucrose-cleared section of mouse brain were imaged with two-photon laser scanning microscopy at a depth of 700 μm. The X-Z planes are derived from X-Y image stacks. Mitochondria (yellow arrows) that are barely visible with the membrane flat (b and d) are clearly visible with the membrane distended at −0.5 psi gauge pressure (c and e).

Contact: Alex Groisman, University of California, San Diego; e-mail: agroisman@ucsd.edu; David Kleinfeld, University of California, San Diego; e-mail: dk@ucsd.edu.