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...
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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 polydimethylsiloxane (PDMS) constructed by team member Alex Grossman. 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 distention. Varying the pressure from −0.3 to −0.7 pounds per square inch (psig) 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 psig, 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. □

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