A team of Stanford University scientists and engineers has developed a miniature microscope that will allow researchers to observe nerve cells and capillaries deep inside living subjects. The new device, called a two-photon microendoscope, is less than 1.5 inches long and weighs about one-tenth of an ounce. It was designed in the laboratory of Mark Schnitzer, assistant professor of biological sciences and of applied physics.

"Such compact instrumentation should be useful for a broad range of biomedical purposes," write Schnitzer and his colleagues in the Sept. 1 issue of the journal *Optics Letters*. "Fruitful applications might include clinical diagnostics or studies in small animals."

Imaging live cells below the surface is difficult to accomplish with conventional techniques. Electron microscopy cannot be used on living organisms, and optical (light) microscopy cannot penetrate deeply because light scatters as it travels through tissue.

For their device, Schnitzer and his co-authors turned to a newer technology called two-photon fluorescence imaging. This technique reduces scattering and background haze because molecules outside the area of interest are less likely to absorb pairs of photons simultaneously and fluoresce (radiate) in response.

One disadvantage of two-photon microscopy is that it penetrates only about half a millimeter below the tissue surface. To get at deeper structures, the Stanford team combined two-photon imaging with microendoscopy, a technique in which tiny, minimally invasive fiber-optic probes are inserted into living tissue. Probes were placed in the brains of anesthetized laboratory mice to produce detailed images of minute cerebral blood vessels located more than 1 millimeter below the surface. The probes are long enough to reach any portion of the rodent's brain, which is about the size of a lima bean.

"We've designed the world's smallest two-photon microscope," says Schnitzer, an affiliate of Stanford's interdisciplinary Bio-X research program. "This is a portable handheld device with the power of two-photon imaging—the full functionality of a microscope that fits in the palm of your hand."

His next goal is to design a microscope that can be used on unanesthetized mice that are alert and mobile. He and his colleagues also are collaborating with Nikolas Blevins, assistant professor of otolaryngology, who studies the inner ear, and Lawrence Recht,
professor of neurology and neurological sciences, who is using endoscopic probes to image brain tumors in mice. Schnitzer predicts that the microendoscopy technique eventually will have broad applications for imaging human patients as well.

The Optics Letters paper was co-authored by graduate students Benjamin A. Flusberg, Eric D. Cocker and Erik P. Anderson of Stanford, and Juergen C. Jung of Oxford University. The study was supported by the National Science Foundation, the Office of Naval Research and the Beckman Foundation.

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