GRIN lenses used in microendoscope

Instrument allows two-photon imaging of brain cells in vivo

Researchers based at Stanford University in California have developed a new type of endoscope designed to collect fluorescence images from areas of the live mammalian brain that are too deep to access by conventional microscopy. The method, which they call microendoscopy, depends on gradient index (GRIN) lenses to create endoscopes between 350 and 1000 µm in diameter. Although in its infancy, the technique may have potential as a clinical diagnostic tool.

A variety of fluorescent probes target proteins, calcium ions and other molecular species in tissue, but methods for imaging them in the brains of live mammals have not quite kept pace. In fact, most fluorescence imaging still uses cultured cells in vitro, or tissue slices.

According to Mark J. Schnitzer, principal investigator on research published in the May 5 issue of the *Journal of Neurophysiology*, there are multiple reasons for studying brain cells in vivo. In vitro experiments require preparation, which may produce biochemical and neuromodulatory conditions that can alter cellular properties. Also, neural circuits aren't in their natural intact and connected states, and it is difficult to study how physiological states such as stress, sleep or hunger mutually interact with cellular properties. Lastly, animal behaviors, such as learning and social interaction, also relate ultimately to cellular properties.

However, the brain is difficult to image. Laboratory animals have been imaged by removing enough of the brain to allow a microscope objective to be placed close to the target area. This provides in vivo images, but the trauma caused by surgical resection can upset the delicate balance of brain chemistry. Functional MRI also can do the job, but it doesn't have the resolution to study individual cells.

To tackle this problem, the group developed several tiny endoscopes using GRIN lenses. Instead of using curved surfaces to refract light, a GRIN lens uses various concentrations of a dopant in the glass to change the refractive index. These lenses are often less expensive to make, even in submillimeter sizes.

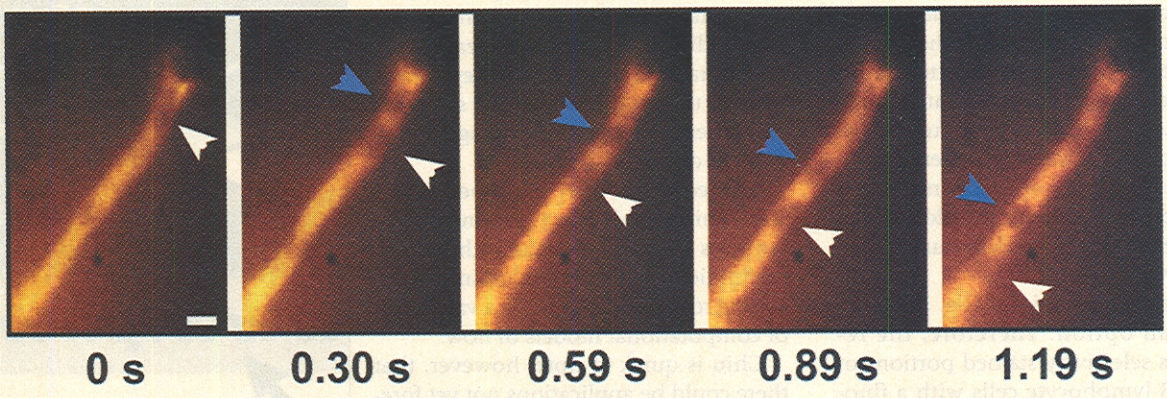
The researchers used thallium-doped GRIN relay lenses from Nippon Sheet Glass Co. Ltd. (NSG) of Osaka, Japan, as well as custom-made thallium-doped or silver-doped objective lenses from NSG and GrinTech GmbH of Jena, Germany, respectively. They created one instrument for epifluorescence imaging and another for laser-scanning two-photon fluorescence imaging.

For the epifluorescence setup, they used a "doublet" lens configuration consisting of a single objective lens with a high numerical aperture and a relay lens. This optical setup allows full-frame imaging at video rates. Although the lack of optical sectioning means that scattering can be a problem, it also means that tissue that moves slightly, such as a blood vessel, doesn't just disappear from view.

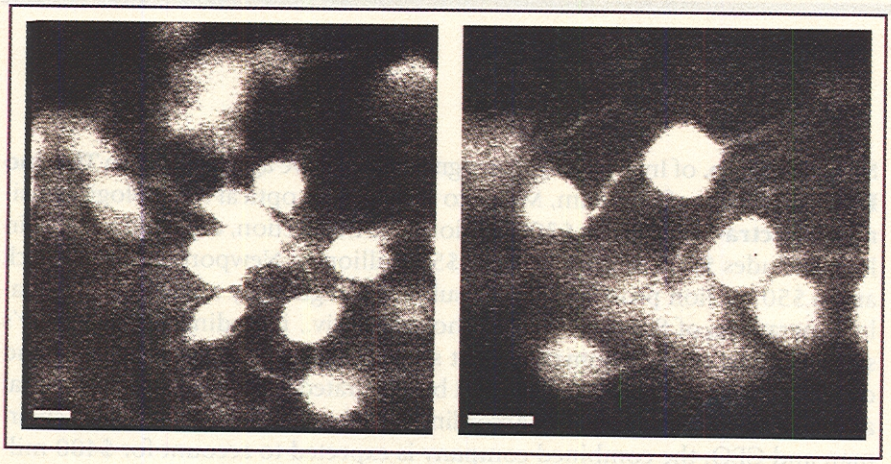
The group's illumination and collection setup consists of a mercury arc lamp coupled to an optical fiber, delivered through various filters and an optical train that includes a binocular and eyepieces from Carl Zeiss GmbH of Jena, Germany. A microscope objective couples the excitation light into the endoscope as well as coupling fluorescence emissions collected by the endoscope to the imaging train, where a dichroic mirror and filter reject the excitation light and send the fluorescence image to a cooled CCD camera from Roper Scientific Inc. of Tucson, Ariz.

Two-photon imaging

In two-photon imaging, a laser focal spot is scanned over a sample to excite fluorescence, which is collected serially and which can be employed to construct an image. The group tried several



Using a microendoscope based on a GRIN lens, researchers capture epifluorescence images of single red blood cell dynamics in the laterodorsal thalamic nucleus of a live rat in vivo and at video rate. Courtesy of Mark J. Schnitzer and reprinted with permission of the Journal of Neurophysiology.



The 1000-µm-diameter instrument enabled researchers to capture in vivo images of pyramidal cell bodies in the hippocampus using two-photon excitation (left). The technique also can image pyramidal cell bodies with the 500-µm-diameter instrument (right). Scale bars are 10 µm.

optical configurations. One design used a GRIN objective, a relay lens and an additional coupling lens to bring the fluorescence to a weak focus at the top face of the microendoscope, where it is collected by a microscope objective for imaging. The two-photon setup inherently produces optical sections, so scattering doesn't affect the image as much.

In earlier work, the researchers introduced the triplet lens, but at present they prefer doublet lenses because they are less expensive, offer similar fields of view, have greater magnification and permit a lower numerical aperture on the microscope objective that couples the excitation laser light.

For illumination, they used a Ti:sapphire laser pumped by an Nd:YVO_4 laser, both from Spectra-Physics of Mountain View, Calif. The Ti:sapphire laser produced 100-fs pulses of light in the near-IR region. They used galvanometer-mounted mirrors for scanning the laser. In this setup, a dichroic mirror separates the fluorescence from the excitation light, which is detected by a photomultiplier tube from Hamamatsu of Japan.

Test images of a 1-µm grid and of 170-nm fluorescent beads from Molecular Probes Inc. of Eugene, Ore., produced images with a resolution within about a factor of two of the diffraction limit, Schnitzer said. The researchers also tested the endoscopes' functionality in vivo. They imaged individual neurons and dendrites in the hippocampus and thalamus of live Sprague-Dawley rats. They also studied the same brain regions in mice that were genetically engineered to produce yellow fluorescent protein (YFP). Schnitzer explained that these areas are of great interest to neuroscientists and are deeper in the brain than conventional instruments can comfortably reach.

Using epifluorescence microendoscopy, the scientists imaged hippocampal pyramidal cells expressing YFP and individual dendrites labeled with Di-I. They also used the video-rate capability to image cerebral blood flow and red blood cell dynamics, with fluorescein dye injected into the bloodstream. Using the two-photon microendoscope, they successfully imaged hippocampal neurons and dendrites labeled with Di-I, as well as apical dendritic trunks of hippocampal cells expressing YFP, a feat made possible by the inherent optical sectioning of the two-photon method.

Schnitzer said that the group plans to continue working on a number of improvements to the microendoscopes and had been considering making them smaller. In previous work at Lucent Technologies, he developed GRIN lenses as small as 125 µm. However, making the instruments smaller comes at the cost of a smaller field of view. A more likely path is to improve the resolution of the existing sizes and designs. □

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