Two-photon microendoscopy aids brain imaging

Fluorescence microendoscopy is an emerging imaging modality that enables visualization of biological cells within tissues too deep to access by conventional microscopy. Researchers at Stanford University have demonstrated a two-photon fluorescence microendoscope that can capture micron-scale images from deep in the brains of live subjects-all in a package small enough to fit in the palm of your hand. The results of their work appear in the September 1, 2005 issue of *Optics Letters* ("*In Vivo* Brain Imaging Using a Portable 3.9-gram Two-Photon Fluorescence Microendoscope," Benjamin A. Flusberg, Juergen C. Jung, Eric D. Cocker, Erik P. Anderson, and Mark J. Schnitzer).

There is great interest in imaging individual cells inside living subjects because it will provide insight into how cellular behavior gives rise to the properties of organisms as a whole. For instance, the nerve cells of the hippocampus region of the brain give rise to important mental processes such as learning and memory. But imaging living cells below the surface has been difficult to accomplish using conventional techniques. Electron microscopy can't be used on living tissue, and optical microscopy can't penetrate very deeply into tissue because light scatters as it travels through tissue near the surface. Even so, scientists often use some form of fluorescence microscopy to image tissue.

In conventional "one-photon" fluorescence imaging, the scientist injects a dye into tissue and then shines a bright light. The tissue fluoresces, or radiates, light of a different color in response. However, a problem with one-photon fluorescence is that the deep tissue causes the photons to ricochet, or scatter, as they return to the detector. The result is a background haze in the images, almost like viewing the sample through a cloud. However, it is possible to eliminate background haze and reduce the scattering using two-photon

fluorescence imaging. Instead of one higher-energy photon, researchers bombard the molecule with two photons of lower energy. Their combined energies total the energy required to excite the fluorescent-dye molecules used to mark the tissue. This approach gets rid of background haze and reduces scattering because molecules outside the area of interest are much less likely to absorb a pair of photons simultaneously and fluoresce in response.

System design

While two-photon microscopy offers an alternative to traditional one-photon fluorescence microscopy, it still only penetrates brain tissue down to about 500-600 microns. To get at deeper structures, the Stanford researchers opted to use tiny, minimally invasive optical probes that could be inserted deep into living brain tissue. The device combines a compact two-photon fluorescence microendoscope based on a compound gradient refractive index endoscope probe, a DC micromotor for remote adjustment of the image plane, and a flexible photonic bandgap fiber for near distortion-free delivery of ultrashort excitation pulses. The imaging head has a mass of only 3.9 g and provides micrometer-scale resolution. The excitation light was obtained by coupling femtosecond pulses from a Ti:sapphire laser (790-810 nm) into the bandgap fiber.

In the research reported in *Optics Letters,* the Stanford group describes using two-photon microendoscopy to obtain detailed images of the blood vessels in the hippocampus sections of the brains of live mice. The mice were injected with a fluorescein dye that labeled the blood plasma so the vessels in the brain could be clearly seen. To make one group of images, the researchers inserted the microendoscope into the hippocampus, about a millimeter below the mouse brain surface, to image this part of the brain. The two-photon imaging provided an additional 80 microns of depth, below the hippocampal surface. When combined with two-photon fluorescence, the result is a system that brings the power of a cutting-edge imaging technique to the deep tissues of the brain.

“We’re bringing two-photon imaging to endoscopy and we’re putting it all into a miniaturized package,” says Mark Schnitzer. “This is a portable handheld device with the full functionality of a microscope that fits in the palm of your hand.”

Medical Laser Report September, 2005

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