PURDUE UNIVERSITY

PURDUE NEWS SERVICE
http://news.uns.purdue.edu

NEWS RELEASE

April 17, 2007

New imaging approach promises insights into multiple sclerosis

WEST LAFAYETTE, Ind. — Researchers have developed a way to use three types of microscopic imaging techniques simultaneously to analyze living tissue and learn more about the molecular mechanisms of multiple sclerosis, information that could help lead to earlier detection and new treatments.

The combined imaging method is enabling the researchers to study how multiple sclerosis causes an overproduction of "astroglial filaments," which form bundles between critical nerve fibers and interfere with proper spinal cord functioning. The technique also promises to yield new information about how the disease degrades the myelin sheath, which insulates nerve fibers and enables them to properly conduct impulses in the spinal cord, brain and in the "peripheral nervous system" throughout the body, said Ji-Xin Cheng, an assistant professor in Purdue University's Weldon School of Biomedical Engineering and Department of Chemistry.

The three imaging techniques - called sum frequency generation, two-photon-excitation fluorescence and coherent anti-Stokes Raman scattering - ordinarily are used alone. Purdue researchers have developed a way to combine all three methods in the same platform, promising to reveal new details about the spinal cord and myelin sheath, Cheng said.

"Combining these three methods allows us to conduct more specific and precise molecular analyses," he said. "Ultimately, this work paves the way toward studying the degradation of the myelin sheath as a result of multiple sclerosis and analyzing living tissue to study the mechanisms of disease."

Multiple sclerosis affects more than 350,000 people in the United States and 2 million worldwide.

Findings will be detailed in a paper appearing in May in the Biophysical Journal and is currently online. The paper was authored by biomedical engineering doctoral student Yan Fu and postdoctoral research associate Haifeng Wang; Riyi Shi, an associate professor of basic medical science in Purdue's School of Veterinary Medicine and also an associate professor of biomedical engineering; and Cheng.

… more …

Shi, a member of Purdue's Center for Paralysis Research, specializes in spinal cord and brain trauma and chronic neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease and multiple sclerosis.

"We are using a unique and powerful combination of technologies to uncover the mechanisms of multiple sclerosis," Shi said. "We hope to one day establish an effective intervention to not only slow down, but even possibly reverse the development of this disease, which will potentially have profound economic and social impacts on this nation and the world."

Because the imaging techniques work without using dyes to "label" cells and structures, they can be used to study living tissues, representing a major advantage over conventional microscopic imaging technologies.

Raman microscopy, an imaging technique invented more than three decades ago, cannot be used effectively to study living tissue because the extremely weak "Raman scattering" signals require hours to yield an image, whereas coherent anti-Stokes Raman scattering, or CARS, overcomes this limitation, Cheng said.

"CARS microscopy permits label-free imaging of specific molecules with a speed of one frame per second or even faster," he said.

CARS imaging takes advantage of the fact that molecules vibrate at specific frequencies. In a CARS microscope, two laser beams are overlapped to produce a single beam having a new frequency representing the difference between the original two beams. This new frequency then drives specific molecules to vibrate together "in phase," amplifying the Raman signals from those molecules.

"It's like pushing someone on a swing," Cheng said. "If you push in synch with the upswing, the swing will go higher. That's the same as being in phase."

Sum frequency generation imaging does just the opposite, adding the frequencies of the two original beams, producing a new signal with a frequency that is the sum of the original beams.

The third imaging technique, two-photon excitation fluorescence, provides higher contrast and brighter images than conventional fluorescent imaging methods. Photons are the individual particles that make up light. In two-photon excitation fluorescence, two photons are used to illuminate a target.

The researchers have used the imaging methods to observe living spinal tissue extracted from guinea pigs. The technique of extracting the tissue and then keeping it alive long enough to analyze was developed in Shi's lab.

Conventional microscopic imaging techniques require samples to be labeled with dyes, killing the tissues in the process. Being able to analyze living tissue could allow researchers to determine the molecular mechanisms responsible for multiple sclerosis and other conditions, Cheng said.

The myelin sheath is made of molecules called lipids, which consist of a head and tail segment. The new findings show that images can reveal whether the orientation of the chemical bonds in these lipid molecules is "scrambled," when the myelin sheath is unhealthy and degraded from disease, Cheng said.

"We can see the myelin sheath with CARS, and that's great, but it's not enough," Cheng said. "We also want to study other molecules and see the interactions between cells."

Researchers simultaneously took images of astroglial filaments, critical components of structures called astrocyte processes, which provide structural support for the nerve fibers in a spinal column. The sum frequency generation imaging reveals details about the astroglial filaments.

Two-photon excitation fluorescence reveals information about the influx of calcium into cells, which causes damage to nerve fibers.

"So, having all three of these imaging methods in the same platform enables us to study many elements of the disease process simultaneously in the same tissue samples," Cheng said.

Researchers hope one day to use the combined approach to design a "minimally invasive" system for diagnosing patients in the hospital or doctor's office, he said.

"There are two directions of this research," Cheng said. "One is to study the mechanisms of disease, and that should form the foundation for designing new treatments. The other is to keep pushing the technology to make it less and less invasive, which will help in the early detection of multiple sclerosis."

The astroglial filaments also are involved in producing scar tissues following trauma injuries to the central nervous system, so a better understanding of their workings could lead to new treatments for repairing damage caused in accidents.

The research was supported with funds from the National Science Foundation and the state of Indiana. Future work by the same researchers to study multiple sclerosis in rats will be supported by a new three-year, $1 million R01 grant from the National Institutes of Health's National Institute of Biomedical Imaging and Bioengineering.

Writer: Emil Venere, (765) 494-4709, venere@purdue.edu

Sources: Ji-Win Cheng, (765) 494-4335, jcheng@purdue.edu
Riyi Shi, (765) 496-3018, riyi@purdue.edu

Related Web sites:
Ji-Xin Cheng: https://engineering.purdue.edu/BME/People/viewPersonById?resource_id=11620
Riyi Shi: https://engineering.purdue.edu/BME/People/viewPersonById?resource_id=20376
Weldon School of Biomedical Engineering: http://www.purdue.edu/bme

IMAGE CAPTION:

This picture was taken with two microscopic imaging techniques combined in the same platform, enabling researchers to conduct more specific and precise molecular analyses for research regarding multiple sclerosis and other conditions. Combining the techniques offers the hope of

understanding the molecular mechanisms responsible for the impairment of the myelin sheath and overproduction of "astroglial filaments," which form bundles between critical nerve fibers and interfere with proper spinal cord functioning. Purdue researchers have shown how to combine three imaging techniques in one platform to analyze living tissues. This particular image combines pictures taken with two of those imaging techniques. The picture shows the myelin sheath, in red, taken with a technique called coherent anti-Stokes Raman scattering, and astroglial filaments, in green, taken with another imaging technique called sum frequency generation. (Weldon School of Biomedical Engineering, Purdue University)

A publication-quality photo is available at http://news.uns.purdue.edu/images/+2007/cheng-myelin.jpg

ABSTRACT

Second Harmonic and Sum Frequency Generation Imaging of Fibrous Astroglial Filaments in Ex Vivo Spinal Tissues

Yan Fu, Haifeng Wang, Riyi Shi,*[y] and Ji-Xin Cheng*[z]*

**Weldon School of Biomedical Engineering*

[y]Department of Basic Medical Sciences, Institute for Applied Neurology, and Center for Paralysis Research

[z]Department of Chemistry, Purdue University, West Lafayette, Indiana

Sum frequency generation (SFG) and second harmonic generation (SHG) were observed from helical fibrils in spinal cord white matter isolated from guinea pigs. By combining SFG with coherent anti-Stokes Raman scattering (CARS) microscopy, which allows visualization of myelinated axons, these fibers were found to be distributed near the surface of the spinal cord, between adjacent axons, and along the blood vessels. Using 20-mm-thick tissue slices, the ratio of forward to backward SHG signal from large bundles was found to be much larger than that from small single fibrils, indicating a phase-matching effect in coherent microscopy. Based on the intensity profiles across fibrils and the size dependence of forward and backward signal from the same fibril, we concluded that the main SHG signal directly originates from the fibrils, but not from surface SHG effects. Further polarization analysis of the SHG signal showed that the symmetry property of the fibril could be well described with a cylindrical model. Colocalization of the SHG signal with two-photon excitation fluorescence (TPEF) from the immunostaining of glial fibrillary acidic protein demonstrated that SHG arises from astroglial filaments. This assignment was further supported by colocalization of the SHG contrast with TPEF signals from astrocyte processes labeled by a Ca^{2+} indicator and sulforhodamine 101. This work shows that a combination of three nonlinear optical imaging techniques - coherent anti-Stokes Raman scattering, TPEF, and SHG (SFG) microscopy - allows simultaneous visualization of different structures in a complex biological system.