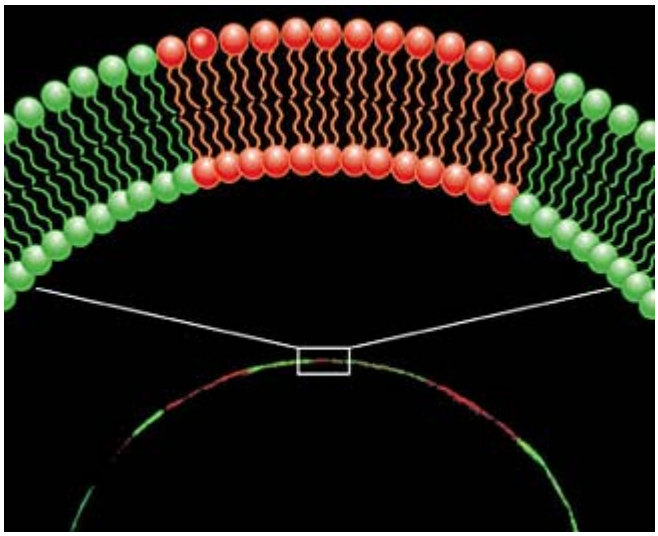
Science & Technology

January 24, 2005
Volume 83, Number 4
p. 36

IMAGING LIPIDS

Technique promises direct observation of lipids in samples ranging from skin to potato chips



DOMAINS The phase segregation of lipids in a giant unilamellar vesicle can be clearly seen with CARS microscopy, as shown in this image of a deuterated lipid (red) overlaid on an image of an undeuterated lipid (green). COURTESY OF X. SUNNEY XIE

CELIA M. HENRY, C&EN WASHINGTON

Visualizing lipid domains is poised to become easier than ever, thanks to recent advances in a technique called CARS microscopy.

Separate lipid domains within membranes--or lipid rafts--are thought to play a role in a variety of biological processes. They are not easy to distinguish visually, and current techniques to image them have drawbacks. The most common method, fluorescence microscopy, requires the addition of fluorescent dyes. Microscopy based on Raman spectroscopy doesn't require bulky tags, but the laser intensities needed to get useful images can damage biological samples. Now, Harvard University chemistry professor X. Sunney Xie and postdoctoral fellow Eric O. Potma have shown that a more sensitive variation of the Raman-based technique, known as coherent anti-Stokes Raman scattering (CARS) microscopy, is a particularly suitable alternative.

CARS is a nonlinear optical technique in which two laser beams at different frequencies, a "pump" beam and a "Stokes" beam, interact with a sample to produce an "anti-Stokes" signal. (Stokes and anti-Stokes refer to the scattering of light at frequencies lower and higher, respectively, than that of the incident light.) The CARS signal is enhanced when the frequency difference between the two beams equals a vibrational frequency of the sample.

Ford Motor Co. developed spectroscopy based on CARS in 1965. Researchers at the Naval Research Laboratory first applied CARS as a visualization technique in 1982. The technique languished for nearly two decades before technical advances began making it attractive for biological applications in 1999. Xie and his coworkers have spent the past five years pushing the limits of CARS microscopy.

CARS microscopy is five orders of magnitude more sensitive than the technique based on normal Raman spectroscopy, but it is still less sensitive than fluorescence microscopy. It has other advantages, however. In fluorescence microscopy, continuous excitation over periods as

short as a few minutes can induce photobleaching, causing the dye molecules to stop fluorescing. CARS does not have this problem. In addition, the labels required in fluorescence microscopy can perturb the system.

In CARS microscopy, lipids are visualized by monitoring the C–H stretching vibration of their acyl chains. Potma and Xie have used it to see separate lipid domains within a single lipid bilayer [*ChemPhysChem,* **6,** 77 (2005)].

For their initial demonstration, they use a model system of giant unilamellar vesicles made of a 50-50 mixture of two lipids, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC). At room temperature, these lipids form two distinct phases. The lipids have only minor differences in the C–H stretching vibrations, which provide only a slight contrast for the image. To improve the contrast, Xie and Potma use deuterated DSPC and regular DOPC. The greater difference between the C–D and the C–H stretching vibrations makes it easy to distinguish the two lipids when they segregate into separate domains.

"Lipids are difficult to study using other techniques," Xie says. "People have been studying phase segregation in giant unilamellar vesicles using dyes, which cause complications. We have a technique that allows in situ observation with no perturbation [and thus is] particularly suitable for live cells."

IN FACT, Xie is already moving beyond the model system and is doing CARS microscopy in living systems. Collaborating with Charles P. Lin at Massachusetts General Hospital, Boston, his group developed a high-speed CARS microscope. With that, the team has imaged the skin of live mice with "contrast that pathologists and dermatologists never saw before," Xie says.

Dermatologists are interested in seeing how the oils from acne and skin care products are distributed in the skin, Xie says. Using CARS microscopy, Xie and his collaborators are able to see the distribution of endogenous lipid-rich cells in the mouse skin, as well as the distribution of externally applied baby oil. Dermatologists "have no other way of telling" how the lipids are distributed "because they cannot use dyes and they cannot use normal microscopes because there's no contrast," Xie says.

Xie believes that applications of CARS microscopy extend beyond biological imaging. For example, it could be used to image the fat distribution in foods such as potato chips. "There's no [other] technique offering such specificity and sensitivity," Xie claims. "If you take just a transmission image, you don't know where the lipids are."

So far, biologists have been more receptive to CARS microscopy than chemists have, but Xie hopes that this will change. For example, CARS could be used to image the distribution of lubricating oils on surfaces. "These applications might inspire chemists to take advantage of the usefulness" of CARS microscopy, he says.

Chemical & Engineering News
ISSN 0009-2347
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