Latest News

December 22, 2008
Volume 86, Number 51
p. 6

Vibrational Microscopy

High-Sensitivity Imaging

Raman-based method maps lipids in cells and drugs in tissue

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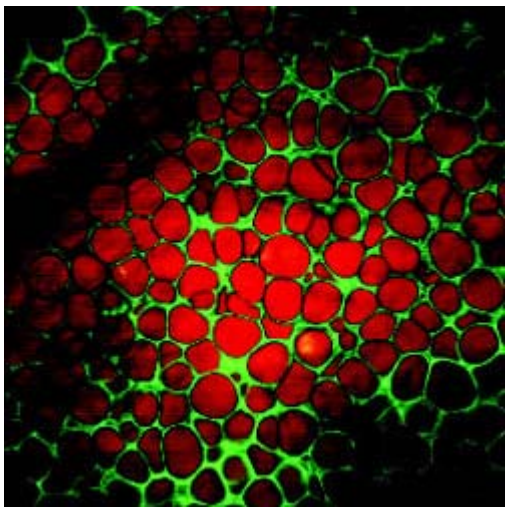
A NEW, HIGH-SENSITIVITY Raman microscopy method allows researchers to distinguish different types of lipids in living cells and monitor drug delivery through the skin. Ultimately, it could become a new method of biomedical imaging.

Harvard University graduate student Christian W. Freudiger, postdoc Wei Min, chemistry professor X. Sunney Xie, and coworkers describe the technique, which is based on a phenomenon called stimulated Raman scattering (SRS), in the latest issue of *Science* (**2008**, *322*, 1857). Unlike fluorescence microscopy methods, Raman methods such as SRS microscopy do not require the addition of bulky labels, which can perturb the biological system.



Brian Saar

A two-color SRS image shows the distribution of dimethyl sulfoxide (green) and lipids (red) in the subcutaneous fat layer under skin.

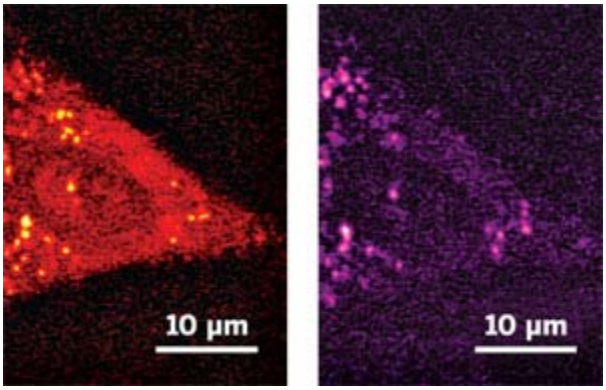
In collaboration with Jing X. Kang's team at Harvard Medical School, Xie's group has used SRS to image lipids and the uptake of topically applied drugs in biological samples. For example, they monitored the uptake of omega-3 fatty acids in cultured human lung cancer cells. And collaborating with Jason C. Tsai of Pfizer Global Medical, Xie and coworkers also have watched the penetration of the acne and antiaging drug retinoic acid into the skin.

SRS microscopy enjoys several advantages over coherent anti-Stokes Raman scattering (CARS) microscopy, another Raman imaging technique developed by Xie and coworkers. For example, unlike the CARS spectrum, the SRS spectrum looks identical to a standard Raman spectrum. The similarity makes the spectrum easier to interpret and means that users can draw on the wealth of literature about standard Raman.

The biggest advantage of SRS, and a key contributor to the method's sensitivity, is the reduced background signal, which goes to zero away from peaks. The stronger background signal in CARS limits it to regions of the Raman spectrum with well-separated spectral peaks, such as those found in the C–H stretching region of lipid spectra. SRS, in contrast, works well even in the region of the Raman spectrum where peaks are crowded together—the "fingerprint" region. These differences mean that SRS microscopy will be able to image a broader range of molecules than CARS microscopy.

Ji-Xin Cheng, a CARS expert at Purdue University, points out that methods exist to suppress the background signal in CARS, but those methods are too complex to use in real biological applications. "Stimulated Raman microscopy allows background-free imaging on a relatively simple platform," he says.

To image omega-3 fatty acids in cancer cells, Xie and coworkers focus on a Raman band that is characteristic of unsaturated lipids. They aim at the sample two laser beams that are tuned such that the difference in their frequencies matches that of the desired Raman band. This frequency-matching amplifies the Raman signal and changes the intensities of the two laser beams. They measure these intensity changes, which are the SRS



Lipids Revealed All lipids have a Raman band at 2,920 cm^{-1} (red), but unsaturated fatty acids can be distinguished by a Raman band at 3,015 cm^{-1} (purple), the intensity of which is proportional to the number of double bonds in the lipid.

signals, by modulating one of the beams and using a phase-sensitive detection scheme. They create images by repeating this process at many spots on the sample.

"We can have all the advantages of Raman, but with much higher sensitivity, acquisition speed, and no damage to biological samples," Xie says.

Wolfgang Zinth, head of the Center for Biomolecular Optics at Ludwig Maximilians University, in Munich, is also developing SRS microscopy. He cautions that generating Raman images with complete spectra using Xie's method will be "very cumbersome" because only one frequency is recorded at a time. Nonetheless, he says, "the present work by the Xie group is an important step toward a 'non laser lab' application of stimulated Raman microscopy."

Chemical & Engineering News
ISSN 0009-2347