Online News

Raman sensitivity takes a quantum leap

Unlabeled molecules become visible inside cells, thanks to stimulated Raman spectroscopy.

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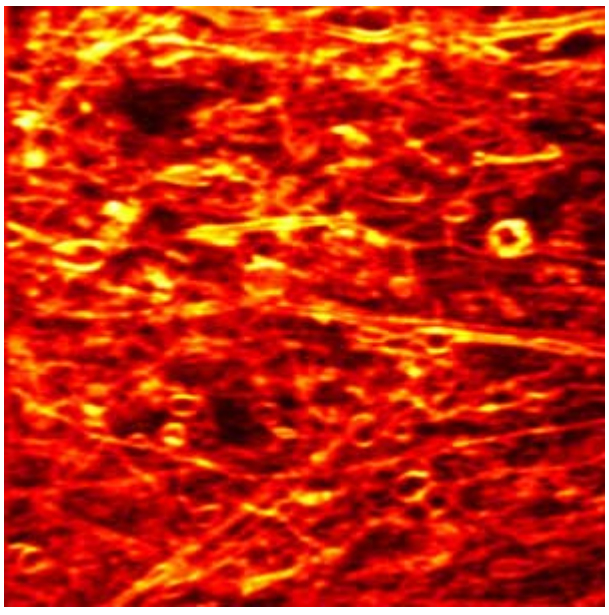
Researchers have imaged small molecules in living cells in real time with a new, more sensitive Raman microscope. “We have developed a nonlethal, highly sensitive imaging technique that is label-free and allows very fast data acquisition,” says X. Sunney Xie of Harvard University, whose group reports this advance in a new *Science* paper (**2008**, DOI 10.1126/science.1165758).

Raman spectroscopy is a nondestructive technique in which a laser beam strikes a sample, causing it to emit photons. Most of the scattered photons retain their original wavelengths, but a tiny fraction emerges with different wavelengths. These shifts in wavelength are characteristic of the sample’s chemical bonds, and researchers use this information to identify the chemical species in the sample. The signals tend to be weak, however, so Raman microscopy requires high laser powers and long data-collection times.



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The researchers also used SRS microscopy of lipids to image neurons in mouse brain tissue.

Xie and colleagues’ new imaging technique is a variation of Raman

Raman scattering (SRS). In SRS, two collinear laser beams (a pump beam and a Stokes beam) are focused on the same spot on a sample. If the difference between the beams' frequencies matches the frequency at which targeted molecules vibrate, an amplified Raman signal emerges. When such stimulated excitation occurs, the pump beam loses intensity (stimulated Raman loss, SRL), and the Stokes beam gains intensity (stimulated Raman gain, SRG). Spectroscopy that detects SRL or SRG is orders of magnitude more sensitive than conventional Raman spectroscopy.

SRS was recently coupled to microscopy, but high-powered lasers were used, which can damage biological materials. Moreover, acquisition speed was slow because of the lasers' low pulse-repetition rate.

In contrast, Xie, graduate student Christian Freudiger, and postdoc Wei Min used low-powered lasers with a high pulse-repetition rate (76 MHz). The SRG and SRL signals were buried by laser noise at first. "But we realized that this noise occurred mostly at lower frequencies, below a kilohertz," Xie says. "So we are now able to extract small SRL or SRG signals by modulating one of the two beams so that the real signal occurs at a high frequency, ~1–2 MHz, and is separated from the laser noise in time." Using a lock-in amplifier, the researchers measured SRL from the pump beam while modulating the intensity of the Stokes beam. By rejecting the unwanted low-frequency noise, they achieved unprecedented sensitivity.

The technique could detect as little as 50 μM—about 3000 molecules—of retinol, a form of vitamin A. "There's no way to do that with CARS," says Xie, referring to coherent anti-Stokes Raman scattering, a sensitive technique that he helped develop during the past decade. Moreover, the CARS spectrum of retinol was shifted to lower wavelengths and contaminated with background noise. In contrast, the SRS spectrum of retinol was identical to that obtained by conventional Raman microscopy. "That is a big advantage, because it allows us to use the wealth of Raman data in the literature to assign chemical species," Xie says.

One of the group's collaborators, Jing Kang at Harvard Medical School, is studying the health benefits of omega-3 fatty acids. So the group used its new technique to monitor the uptake of these lipids into lung cancer cells. The images showed the fatty acids nesting in lipid droplets in the cytoplasm. "SRS is a great approach, because you don't need to label the fatty acids to see them," says Xie, who explains that loading a fluorescent label onto a small fatty acid molecule would perturb the molecule's behavior.

In collaboration with Jason Tsai at Pfizer Global Medical, Xie's group also monitored the passage of retinoic acid, a topical acne drug, into skin. "We wanted to see how fast the drug gets through the upper, protective layer," Xie says. By imaging at 1570 cm^{-1}, the group discovered

researchers followed the movement of DMSO, a solvent that enhances absorption through skin. “Even a couple of years ago, it was hard to imagine how we could determine drug distribution,” Xie says. “But it is now possible to map particular kinds of molecules in three dimensions and follow drug movements into living cells and tissues.”

Several companies appear interested in developing instruments capable of high-sensitivity SRS, Xie says. “There is so much we can do in our lab,” he adds, “but when this machine becomes available to users, I expect a lot of exciting work to occur.”