CARS Moving Beyond the Lab

Kevin Robinson

New research promises to take a technique that combines Raman spectroscopy and multiphoton three-dimensional microscopy beyond the research lab.

Pioneered by Sunney Xie's group at Pacific Northwest National Laboratory in Richland, Wash., in 1999, the method uses coherent anti-Stokes Raman scattering (CARS) as the contrast in a microscope image. As in two-photon fluorescence microscopy, the nonlinear power dependence of CARS generates a signal only at the focal volume where the intensities are the highest, enabling 3-D imaging with diffraction-limited resolution.

Xie, now a professor of chemistry at Harvard University in Cambridge, Mass., and postdoctoral fellows Andreas Volkmer and Ji-Xin Cheng have refined the optical setup for the technique so that it works with a standard epifluorescence microscope -- a great simplification of the previous approach, which required two identical microscope objective lenses. In addition, the new setup uses destructive interference to overcome the background signal received from the solvent.

"We are now convinced more than ever that this will be useful in biological studies and complementary to two-photon fluorescence microscopy," Xie said. The method yields a high-contrast image without chemical dyes or fluorescent markers and does not damage the sample, he said.

The CARS technique amplifies the Raman effect for spectroscopy by employing two laser beams. If the difference between their frequencies equals a vibrational frequency, all of the molecules with that vibrational frequency vibrate together. The interaction of the beams with the sample generates a large anti-Stokes signal. This coherent vibration also means that constructive interference magnifies an anti-Stokes signal in the same direction as the laser beam. Conversely, destructive interference destroys an anti-Stokes signal moving against the flow of photons.

The initial experiments, therefore, were set up for forward detection, which is problematic because the constructive interference also magnifies the background signal. Despite this, Xie's previous group relied on conventional wisdom and set up a CARS microscope for forward detection. The Harvard researchers, however, after some study, thought that conventional wisdom might not hold, and they decided to employ backward detection.

This meant using only one objective lens to focus the two collinear laser beams and to collect the anti-Stokes signal. They used a Nikon TE 300 inverted optical microscope with a Nikon 60x oil-immersion lens. To get the two laser beams required for coherent anti-Stokes Raman scattering microscopy, they split the output of a Coherent Inc. femtosecond Ti:sapphire laser, using one beam to pump a Coherent optical parametric amplifier.

During the experiment, they discovered that the destructive interference filtered out the background but
did not affect the signal. "If the scatterer is very small," Xie explained, "the destructive interference in the backward direction is negligible. If the size of the scatterer is much smaller than the wavelength of light, the molecules radiate like an individual-point dipole." The solvent in the excitation volume is large enough to create destructive interference in the backward direction, automatically eliminating the background.

To quickly generate images for the study of processes, the researchers raster scan the focal spot instead of the sample. "We can do very fast scanning, about 10 s, to get an image," Xie said. "So we can follow dynamic change. For example, by tuning to the vibrational frequency of lipids, we can watch the changes of the mitochondria as a cell goes through apoptosis."

**Potential for biology**

Because CARS microscopy can be tuned to a variety of molecules, Xie said that the technique has great potential in cell biology. It can provide high-contrast images of lipids, proteins or DNA in live cells without fluorescent dyes. The method also can be coupled with simultaneous differential-interference contrast, fluorescence or two-photon fluorescence microscopy.

The researchers also recently added a forward-detection method that uses polarization-sensitive detection to further expand the range of molecules the method can detect. Eventually, Xie said, the method may prove highly useful in a variety of practical applications, particularly in hospital laboratories. "It can provide a point-by-point chemical map in vivo, which is very promising for many medical applications. We haven't had a chance to experiment with all of them yet."

from photonics.com: 11/01/2001