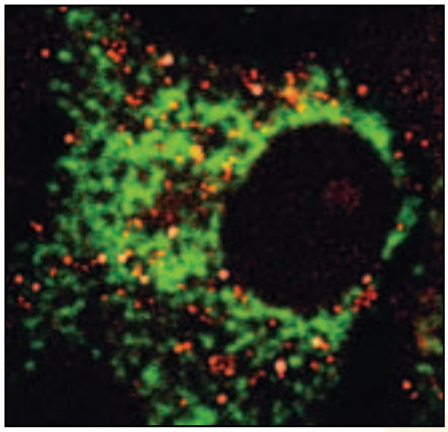
Following cellular traffic with CARS

Researchers use CARS microscopy to watch intracellular movements without damaging living cells.

Observing a live cell in action is one of the thrills of modern biology. Advances in optics and molecular biology have made it possible to track the movements of individual molecules inside the cell in real time. However, methods that yield the most detailed images often require manipulations that can damage the cell or alter the very processes that researchers are trying to investigate. An innovative technique called coherent anti-Stokes Raman scattering (CARS) microscopy may be the ideal solution in some cases. As X. Sunney Xie and colleagues at Harvard University demonstrate in a recent article, CARS can be used to noninvasively capture beautiful images of intracellular lipids (*Biophys. J.* **2006**, *91*, 728–735).

Currently, most researchers rely on fluorescent dyes or protein tags, such as green fluorescent protein (GFP), to visualize molecules inside cells. These fluorescent labeling methods, however, can be problematic in certain situations. Dyes can be toxic, and GFP is so large that it can disrupt the structure and/or function of proteins to which it is attached. Plus, most fluorophores, including GFP, fade in response to the illumination of the microscope, often within a few minutes. CARS, in contrast, can be used to detect molecules on the basis of the characteristic vibrational energy of their chemical bonds, so no dyes or tags are necessary. For example, the technique can distinguish between the C–H bonds that are common in lipids and the amide bonds of proteins.

To detect a specific chemical bond with CARS, researchers illuminate a sample with two lasers, a pump beam and a Stokes beam, whose frequencies differ by an amount equal to the vibrational frequency of the chemical bond of interest. The combination of beams causes all bonds of that type within the field to vibrate in phase, or coherently. "This coherent excitation generates a very strong signal at a new, higher frequency, the anti-Stokes frequency," says Xie. His group has used CARS to detect CH_2, CH_3, OH, amide, phosphate, and carbon–deuterium bonds.



Researchers use CARS to analyze LD movement in mouse adrenal-gland cells. LDs are visualized by CARS (red), and mitochondria are stained with a fluorescent dye (green). (Adapted with permission. Copyright 2006 The Biophysical Society.)

As reported in their recent article, Xie, graduate student Xiaolin Nan, and former postdoctoral fellow Eric Potma used CARS to explore the trafficking of lipid aggregates known as lipid droplets (LDs) in mouse adrenal-gland cells. LDs are present in many cell types and play an important role in lipid metabolism. They have been implicated in a wide range of processes, including fat storage, steroid hormone synthesis, and hepatitis C virus infection. However, the intracellular trafficking of LDs is poorly understood. Because they contain high concentrations of lipids, which are rich in C–H bonds, LDs are ideally suited for visualization with CARS.

Before embarking on their transport studies, Xie and colleagues performed an elegant assay to determine energy and power conditions for the CARS lasers that would produce clear images without damaging the cells. For each set of conditions, they took a series of images over a period of 300 s and overlaid them. A blurry composite image indicated that the cell body had moved during the imaging period; this result is a sign of cellular damage. A sharp composite image indicated that the cells were not reacting adversely to the bombardment of laser light. Michiel Müller at the University of Amsterdam calls these assays "a very valuable addition" to the field.

Using the experimentally derived optimal laser settings, the group obtained images of LDs diffusing passively in the cell and undergoing active transport along microtubules. Cells that were synthesizing steroid hormones had higher levels of active transport than those that were quiescent. The researchers speculate that active transport helps bring LDs, which carry the vital steroid precursor cholesterol, to the mitochondria where steroids are synthesized. "There is an ever-increasing interest in LDs beyond their role as storage compartments, and recent work highlights their growing importance in various topics related to human health," says John McLauchlan at the Medical Research Council Virology Unit (U.K.). "Applications such as CARS microscopy open up new avenues that enable us to explore their dynamic properties and interactions with other cell organelles." However, both Müller and McLauchlan point out that CARS is not the only way to investigate LD transport. Recently, another group reported similar results with third-harmonic-generation microscopy, a technique that is less chemically specific but may be easier to implement than CARS (*Nat. Methods* **2006**, *3*, 47–53).

Xie's group has big plans for CARS, including medical imaging and the study of metabolite and drug distribution in living cells and tissues. CARS "really has matured as a powerful tool," says Xie. ◥

—Karen Ross