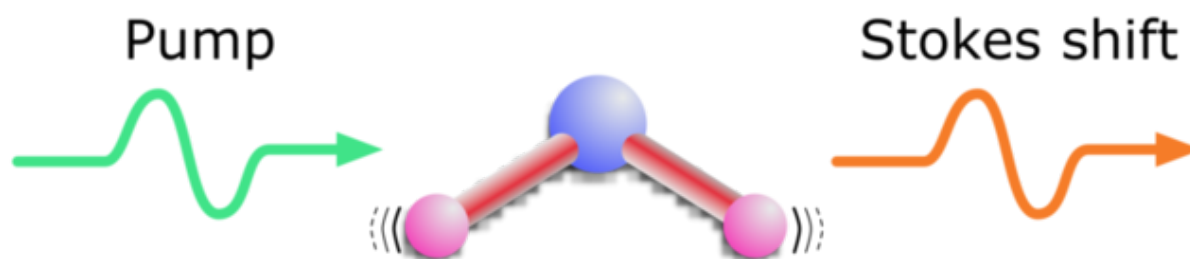


Using interference in a molecule's vibrations to image transparent materials

Researchers show that imaging stuff via their characteristic molecular ...

[Chris Lee](#) - 1/4/2012, 7:34 PM



After a photon hits a molecule, the molecule begins to vibrate. This results in a lower energy photon (orange).

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As many of you know, I am a bit obsessed with imaging techniques. It is not just that I love pretty pictures; I believe that images are absolutely the best way to start understanding something. This is particularly true when that something is complicated, like a living cell.

One of the challenges of imaging live cells (or, in fact, most sorts of organic materials) is contrast. More to the point, there is no contrast. If you take a typical cell, the vast majority of it is water mixed up with oily bits and proteins. These things are all pretty much transparent to visible light and have similar refractive indexes, meaning that the interfaces between the oily and watery bits don't reflect much light either.

A few years back, some researchers suggested there was a way to increase the contrast from biological samples, but only showed that it was possible to do this in theory. Now, some of my compatriots have actually shown that the theory can work, provided you can construct the right equipment.

A couple of years ago, I was made aware of a [theory paper](#) from a pair of researchers at the University of California, Irvine. It showed how to improve the contrast of a particular imaging scheme called coherent anti-Stokes Raman scattering (CARS) by generating interference between two quantum processes in a single molecule. But it was a rather technical paper, and quite difficult to understand the key points—in hindsight, it was really easy to understand how to perform the experiment and really difficult to understand why it would work.

Luckily, some friends of mine from the Optical Sciences group at the University of Twente didn't faint in the face of technical complexity and have managed to [do the experiment](#). They

showed that, yes, if you create interference in the vibrations within a molecule—something they have called vibrational molecular interferometry—you get enhanced image contrast. And, better yet, the enhanced contrast is maintained even in situations where you would normally not be able to use CARS imaging at all. But the cost is a rather complicated experimental setup.

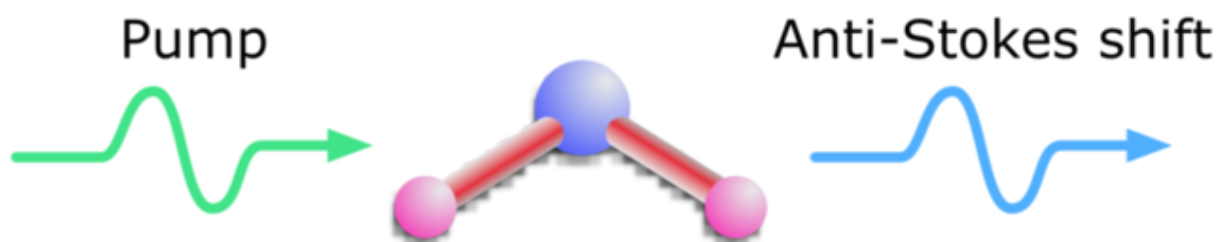
What are these CARS you speak of?

As I said earlier, the contents of a cell are mostly transparent to visible light. However, organic molecules consist of long chains of carbon atoms, mixed with hydrogen, nitrogen, oxygen, and a few other elements. These atoms can all move relative to each other, but because the bonds between the atoms act like springs, the result is a vibration. The frequencies of these vibrational motions are unique to different bond types and can be used to distinguish fats, proteins, and other organic molecules that make up the cell. CARS uses the presence of these vibrational modes as a contrast agent.

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Now, to understand CARS, we have to understand several different processes. Let's start with Raman scattering. This involves illuminating a sample with light that would normally not be absorbed by any of the molecules present. Nevertheless, every now and again, a photon will be absorbed by a molecule anyway. The molecule immediately spits a photon back out, but the second photon will have a slightly longer wavelength—the photon is said to be Stokes-shifted—and the remaining energy goes into vibrational motion.

This is the Raman scattering that is loved by chemists the world over. One shines a laser on a sample and simply looks for light that is Stokes-shifted from the input laser frequency; the difference in energy can be used to map out the vibrational modes of the sample. But if the molecule is already vibrating, the reverse process can also occur—vibrational energy is given up to make a photon more energetic. In this case, the photon that is spat out has a slightly shorter wavelength. We call this anti-Stokes-shifted.



After a photon hits a vibrating molecule, the vibrational energy can be imparted to the photon that is emitted. This results in a higher energy photon (blue).

Adding a C to ARS

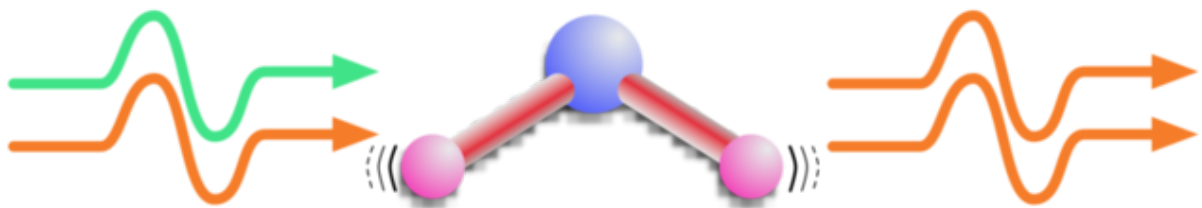
That gets the "ARS" part of CARS out of the way. To add the "C" takes a bit more explanation.

I will focus this explanation on two processes. (In practice, you can do these simultaneously or one after the other.) First, take two lasers, which we will call pump and Stokes. You choose their wavelengths so that the difference in frequency between the two corresponds to the

frequency of a vibrational mode of the sample. When these two light fields are used to illuminate a sample simultaneously, the sample will feel the two fields, as well as their sum and difference frequencies.

The difference frequency corresponds to a vibrational mode, which starts the molecule vibrating. In doing so, energy is transferred from the pump laser field to the Stokes laser field. But because the difference frequency is pushing all the molecules about together, all the molecules start out vibrating together in a coherent fashion. And because vibrations involve shifting electrons around, the vibrational motion has an electric field associated with it.

Now, we follow this up with a third laser. When it hits the sample, it mixes with the electric field of the vibrational mode, creating its own sum and difference frequencies. The sum frequency is the anti-Stokes Raman scattering from the previous paragraph—in this case, the resulting light has a fixed relationship with the lasers involved. This relationship makes it possible, by comparing the anti-Stokes light to a fourth laser, to determine both the phase and amplitude of the vibrational motion (a normal CARS measurement only provides the intensity).



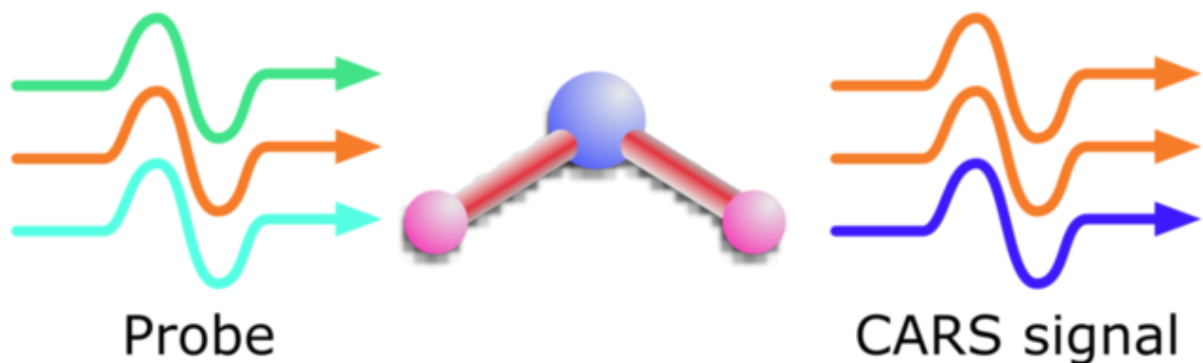
A pump (green) and Stokes (orange) photon combine to resonantly excite a vibrational mode of a molecule. The pump photon is absorbed and a second Stokes photon is emitted.

In principle, this provides an imaging technique that's sensitive to chemical composition, because only those molecules that have the right vibrational modes will respond to the excitation lasers. But it's not always possible to get a clean image.

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There is a second process (which I am not going to explain) that doesn't rely on a vibrational mode. This process, called four-wave mixing, takes the pump, Stokes, and probe lasers, and combines them in such away as to provide a signal that is exactly at the anti-Stokes frequency, which is one of the things you're trying to measure. Everything, including the vacuum, allows four-wave mixing to occur. The result of the four wave mixing is that CARS images come with a background that distorts the image. Indeed, in some cases, the background is stronger than the signal, leaving you with no image at all.

Finally, there is another process that can cause additional confusion, in which two photons are absorbed simultaneously, placing the sample in an excited electronic state. If this happens, all bets are off, because the sample is going to spew light all over the anti-Stokes signal. You might as well set your sample on fire and retire to the pub.



Three photons hit the molecule, the pump (green) and Stokes (orange) resonantly excite a vibrational mode, absorbing the pump photon and generating an additional Stokes photon. The probe (light blue) is absorbed by the vibrating molecule and emits an anti-Stokes photon (dark blue).

Get rid of that background through interference

To avoid some of these background problems, we need to add a fourth laser to the setup. With that, we have pairs of two lasers: the pump and the Stokes, plus the probe, and a laser that is at exactly the frequency we expect the anti-Stokes signal. With the two pairs of lasers, we are running two CARS processes at the same time, and both of them involve exciting the same vibrational mode.

The two CARS processes interfere with each other via the vibrations of the molecule. The four-wave mixing processes (which provide the unwanted background signal), however, don't interfere with each other.

Of the three processes that are going on, two (CARS and four-wave mixing) simply transfer power from one laser beam to another. The last (two-photon absorption) subtracts power from all the beams. So, if we carefully measure the power in each laser beam after it has passed through the sample, the background can be subtracted by looking at how the power in each laser beam change with respect to each other.

That is what the researchers did. In practice, since the power changes are really small, they also needed to play some pretty serious signal processing games to be able to observe anything. The final results, however, were background-free, although the authors noted that there are other techniques that can be used to eliminate the background from CARS images.

To show that vibrational molecular interferometry is substantially better than these other options, they also performed spectroscopy on a dye solution. Under ordinary circumstances, the Raman signal and the CARS signal would both be swamped by the dye's fluorescence signal. However, because the fluorescence subtracts energy from all beams, they found that they could also obtain a high quality Raman spectrum. This cannot be achieved with any other current background elimination techniques, so it shows that vibrational molecular interferometry's has a distinct utility.

It must be admitted that this is a rather complicated imaging technique, involving, as it did, three lasers—the researchers cheated by re-using the same laser as the pump and the probe. It also required some rather fancy signal processing. But because you obtain both the amplitude and phase of the vibrational resonance, and can avoid fluorescence, this will be very good for

imaging samples that contain materials that have very similar vibrational modes and hard-to-avoid backgrounds.

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