Nonlinear Interferometric Vibrational Imaging:
Efficient Detection of Coherent Anti-Stokes Raman Scattering

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For decades, Raman scattering has been a useful tool for probing the molecular contents of gases, liquids and biological media.[1] Its strength is that it can identify molecules non-invasively based on their resonant vibrational frequencies. The technique uses optical frequency radiation, which is relatively easy to create, manipulate and detect.

Unfortunately, the Raman effect is rather weak: Typically only one in a million photons is converted to the detected Raman radiation, so it is too slow to be used as a clinical diagnostic tool or to monitor fast biological processes. Using pulsed laser illumination, Coherent Anti-Stokes Raman Scattering (CARS) can stimulate the production of many more Raman photons by simultaneously exciting the molecular vibrations and detecting the emitted stimulated Raman scattering.[2,3]

While the use of CARS has vastly improved the available Raman signal, a new method called Nonlinear Interferometric Vibrational Imaging (NIVI) has further increased the efficiency of detection.[4,5] CARS signals are akin to echo pulses from radar or magnetic resonance imaging instruments. However, typical CARS instruments can measure only the average power of the returned signal. NIVI uses interferometry to measure the temporal shape of the return pulse, which can be used to differentiate between multiple resonances and discriminate the CARS signal from the nonresonant background signals.

NIVI uses the heterodyne technique, by which a signal is generated in the sample by the CARS process. A "local oscillator" signal is generated separately, usually by a nonresonant four-wave-mixing process. These are mixed and demodulated by temporal or spectral interferometry, which is the same detection method that is already used to image biological structures with optical coherence tomography.

NIVI can be used in microscopy in a manner similar to typical CARS or two-photon microscopy—by scanning the focus of the illumination in three dimensions throughout the sample.

Because CARS is a nonlinear process, it is most efficient where the beam intensity is highest; therefore, it can resolve features somewhat smaller than the illumination wavelength (typically less than 400 nm with near-infrared illumination).

To demonstrate a preliminary instrument, we present an example of a cuvette partially filled with acetone that was imaged by NIVI. Our NIVI instrument consists of a Ti-sapphire regenerative amplifier producing 808-nm pulses, with 30 nm bandwidth and a 250-kHz repetition rate. These pulses are both used to pump the sample, as well as for an optical parametric amplifier (OPA).

The OPA produces an idler beam at 1056 nm used for the Stokes signal, and a signal beam at 653 nm for the local oscillator reference. The pump and Stokes are focused into the sample cuvette, and the resulting anti-Stokes echo radiation is interfered with the reference. In the figure, (a) shows the cuvette, where the curved bottom is clearly visible, highlighting the boundary of the acetone. The same cuvette is seen in (b) with light microscopy, which does not differentiate between the acetone and the surrounding material. This method will be extended to imaging biological samples to achieve 3-D molecular imaging. ▲

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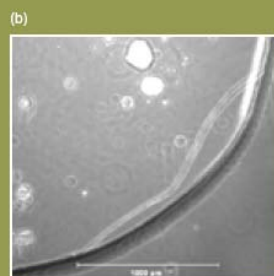
(a) Acetone-filled cuvette with bubbles inside it imaged with NIVI, and (b) the same cuvette imaged with light microscopy.