Nonlinear interferometric vibrational imaging

Daniel L. Marks

Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign

Stephen A. Boppart

Department of Electrical and Computer Engineering, Bioengineering Program

College of Medicine and

Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, 405 North Mathews Avenue, Urbana, IL 61801

(Dated: November 20, 2018)

Coherent Anti-Stokes Raman Scattering (CARS) processes are “coherent,” but the phase of the anti-Stokes radiation is usually lost by most incoherent spectroscopic CARS measurements. We propose a novel Raman microscopy imaging method called Nonlinear Interferometric Vibrational Imaging, which measures Raman spectra by obtaining the temporal anti-Stokes signal through nonlinear interferometry. With a more complete knowledge of the anti-Stokes signal, we show through simulations that a high-resolution Raman spectrum can be obtained of a molecule in a single pulse using broadband radiation. This could be useful for identifying the three-dimensional spatial distribution of molecular species in tissue.

Functional imaging techniques have been developed to provide insight into biological processes. Optical functional imaging is frequently limited because dyes or markers must be introduced that alter or damage biological tissues. Because it is preferable to use endogenous properties of tissues to identify molecular contents, methods such as infrared and Raman spectroscopy are used. In particular, Coherent Anti-Stokes Raman Scattering (CARS) processes have been successfully integrated with confocal scanning microscopes [1, 2, 3] to achieve three-dimensional molecular images of vibrational resonances. However, existing instruments measure only the total power of the received anti-Stokes radiation. We propose a novel method, Nonlinear Interferometric Vibrational Imaging (NIVI), which utilizes nonlinear interferometry to measure the amplitude and phase of the anti-Stokes light. An experimental demonstration of the principle of this technique has been demonstrated [4]. This additional phase information facilitates the inference of the amplitude and phase of the nonlinear susceptibility of the molecule. By utilizing NIVI with properly designed illuminating radiation, a large region of the amplitude and phase of the Raman spectrum can be sampled in a single brief pulse.

Coherent Anti-Stokes Raman Scattering processes have only recently been used to probe biological specimens. The appeal of CARS is that it can probe the density of molecules with a particular Raman resonance frequency while exposing the specimen to relatively low levels of illumination. A typical CARS process illuminates the specimen with a pump pulse of frequency $\omega_1$, and a Stokes pulse of frequency $\omega_2$, which are separated by the vibrational frequency of interest $\Omega = \omega_1 - \omega_2$. If a molecule with a Raman resonance at frequency $\Omega$ is present, an anti-Stokes pulse at frequency $2\omega_1 - \omega_2$ is produced. In CARS microscopy, tightly focused pump and Stokes beams are scanned through the specimen, and the anti-Stokes photon count is measured at each point. This photon count is proportional to the square of the molecular bond density and the magnitude of the Raman susceptibility. Nonlinear interferometry has been used to characterize the magnitude of the stimulated Raman scattering nonlinearity in liquids [5], and also of two CARS signals [6] in gases.

The model of CARS with broadband pulses used here is similar to that described by [1, 2]. We do not assume that the illuminating radiation is narrowband. However, we stipulate that the molecule does not resonantly interact directly with any of the frequencies inside the illumination bandwidth or the generated anti-Stokes bandwidth of the optical signal. The CARS process is composed of two stimulated Raman scattering processes involving four photons that begins and ends with the molecule in the ground state. To describe CARS, we denote the electric field incident on the molecule as $\tilde{E}_i(\omega)$. The first process is modeled by Eq. [4] and excites the nonlinear dipole polarization of the resonant transition.

$$P^{(3)}(\Omega) = \chi^{(3)}(\Omega) \int_0^\infty \tilde{E}_i(\omega + \Omega) \tilde{E}_i(\omega)^* d\omega \quad \text{(step 1)} \quad (1)$$

$$\tilde{E}_o(\omega) = \int_0^\infty \tilde{E}_i(\omega - \Omega) P^{(3)}(\Omega) d\Omega \quad \text{(step 2)} \quad (2)$$
Each pair of frequencies that are separated by a resonance of the molecule at frequency $\Omega$ produces a nonlinear polarization in the molecule. Another way to look at Eq. 1 is that, in the time domain, the molecule has a nonlinear polarization that is driven not by the electric field but by the instantaneous intensity envelope of the signal. In this formulation, we are neglecting any changes in $\chi^{(3)}$ that are dependent on the “carrier” envelope frequency on which the beats are imposed. Therefore any pulse train with intensity beats at the resonance frequency will stimulate the nonlinear polarization. Examples of this are pulses that are modulated periodically in the spectral domain with period $\Omega$, and interfering two relatively delayed chirped pulses to achieve a beat frequency proportional to time delay. The second step creates anti-Stokes radiation by mixing the incoming radiation field with the polarization in the time domain, and is modeled by Eq. 2. The Eqs. 1 and 2 allow one to calculate the emitted CARS radiation $E_o(\omega)$ for a given $E_i(\omega)$ and $\chi^{(3)}(\Omega)$. While these relations do not constitute a linear relationship between $E_i(\omega)$ and $E_o(\omega)$, there is a linear dependence of $E_o(\omega)$ on $\chi^{(3)}(\Omega)$ given an input field $E_i(\omega)$. This suggests that with knowledge of the complex $E_o(\omega)$, one can do linear estimation to find $\chi^{(3)}(\Omega)$. The advantage of NIVI over incoherent detection is that nonlinear interferometry enables the recovery of the complex $E_o(\omega)$. With a properly designed input pulse $E_i(\omega)$, the nonlinear susceptibility can be found in a particular frequency range.

NIVI takes advantage of the coherent nature of the CARS process to allow the phase-sensitive measurement of the anti-Stokes radiation. Conventional linear interferometry involves splitting a source light beam into two parts, each of which scatters linearly in the field, and which are then recombined and detected. NIVI differs in that a CARS process occurs to one of the split beams.

NIVI can be implemented with the setup detailed in Fig. 1. We start with a broadband, phase-locked source of light pulses, such as those from a mode-locked laser. Sources that can produce such light are ultra-broadband mode-locked Ti:sapphire oscillators, and supercontinuum sources. Because the source is phase-locked, there is a deterministic relationship between phases at various frequencies. This deterministic relationship will be preserved by coherent processes such as CARS. To utilize this determinacy, the source bandwidth is split into higher and lower frequency bands, as shown in Fig. 1 with a dichroic beam splitter. The higher frequency band will be a reference signal $\tilde{R}(\omega)$, and will correspond to the bandwidth of the anti-Stokes frequencies produced by a sample. The lower frequency band is temporally shaped to stimulate CARS in the sample, a signal we denote by the frequency spectrum $\tilde{E}_i(\omega)$. Some of the illumination signal will be converted to anti-Stokes radiation by the sample. Because CARS processes are usually rather weak, we will assume that any new anti-Stokes radiation created in the same bandwidth as the illumination will be inseparable from the illumination. Therefore we will discard all anti-Stokes light inside the illumination bandwidth with a high-pass frequency filter. The remaining anti-Stokes light that passes through the filter, which we denote by the spectrum $\tilde{E}_o(\omega)$, corresponds to frequencies in the reference signal. We combine the reference signal $\tilde{R}(\omega)$ and the anti-Stokes spectrum $\tilde{E}_o(\omega)$ with a 50:50 beam splitter, and utilize balanced detection to measure the interference component on two photodetectors. There is a delay of time $\Delta t$ placed in the reference path to facilitate measuring the temporal cross-correlation between the reference and anti-Stokes signals. The difference in the two intensities $\Delta I(\Delta t)$ as a function of delay between the signal and reference will be:

$$\Delta I(\Delta t) = I_+ - I_- = \int_0^{\infty} 4 \text{Re} \left\{ \tilde{E}_o(\omega)^* \tilde{R}(\omega) \exp(i\omega \Delta t) \right\} d\omega$$

(3)

If we call $I(\omega)$ the Fourier transform of $\Delta I(\Delta t)$ with respect to $\Delta t$, we find that $\Delta I(\omega) = 4 \tilde{E}_o(\omega)^* \tilde{R}(\omega)$. Thus the measured data retains its linear relationship with respect to the anti-Stokes spectrum $\tilde{E}_o(\omega)$ and therefore the nonlinear susceptibility $\chi^{(3)}(\Omega)$.

Besides the ability to find the complex-valued $\chi^{(3)}(\Omega)$, interferometry eliminates the need for photon-counting detectors. Another advantage is that interference will only occur when the anti-Stokes light and the reference light arrive at the beam splitter at the same time. Because of this, temporal gating can be used to produce three-dimensional vibrational images in a manner analogous to Optical Coherence Tomography. Coherent detection is also far less sensitive to stray light than photon counting. Because of this, NIVI may be
more adaptable to various scanning configurations and environments outside the laboratory.

To show that NIVI can measure intervals of the Raman spectrum in a single pulse, a pulse must be designed that can stimulate molecules in a broad Raman spectrum. The approach we take creates beats that instead of being of a constant frequency [10], will be themselves chirped. This can be accomplished by combining two chirped pulses with a relative delay, but with different chirp rates. If we have a transform-limited pulsed source of center frequency \( \omega_0 \) and bandwidth \( \Delta \omega \), and we wish to sweep the beat frequency from \( \Omega_L \) to \( \Omega_H \) in time \( T \), we can design a pulse \( \tilde{E}_i(\omega) \) such that:

\[
\tilde{E}_i(\omega) = E_0 \cos \left( \frac{\pi(\omega-\omega_0)}{\Delta \omega} \right) \left\{ \frac{1+\kappa}{2} \exp \left( \frac{-i(\omega-\omega_0)\tau}{2} \right) - \frac{i(\omega-\omega_0)^2}{2(\alpha-\beta)} \right\} + \frac{1-\kappa}{2} \exp \left( \frac{i(\omega-\omega_0)\tau}{2} \right) \right\} \text{ for } \omega - \frac{\Delta \omega}{2} < \omega < \omega_0 + \frac{\Delta \omega}{2}
\]

\[
\tilde{E}_i(\omega) = 0 \text{ otherwise}
\]

where \( \alpha = \frac{2 \Delta \omega - \Omega_L - \Omega_H}{2T}, \beta = \frac{\Omega_L - \Omega_H}{2T}, \text{ and } \tau = \frac{T}{2} \left( \frac{\Omega_L}{\Delta \omega - \Omega_H} + \frac{\Omega_H}{\Delta \omega - \Omega_L} \right) \]

The variable \( \alpha \) is the common chirp to both pulses, \( \beta \) is the difference chirp, \( \tau \) is the time delay between the two pulses, and \( \kappa \) is the difference in field magnitude between the two pulses. The pulse bandwidth has been apodized with a cosine window because in practice it seems to help the stability of the inversion. Note that the bandwidth of the source \( \Delta \omega \) must exceed \( \Omega_H \) so that beats can be formed at all Raman frequencies. When creating the pulse, the chirp time \( T \) will control the resolution with which one will be able to resolve frequencies in the Raman spectrum. The largest practical \( T \) is determined by the dephasing time of the resonances, which in most liquids is on the order of picoseconds.

To demonstrate the feasibility of NIVI, we simulate the illumination of a target molecule with the broadband pulse of Eq. [1] and use the returned signal to estimate the complex susceptibility \( \chi^3(\Omega) \). We will show two simulations: one that is able to probe a wide bandwidth of Raman resonances in a single pulse, and the other which is able to distinguish between two nearby resonances. We take as our hypothetical laser source a mode-locked Ti:sapphire laser that can produce a pulse with a uniform bandwidth from 700–1000 nm, and the setup of Fig. [1]. The bandwidth from 800–1000 nm will be reserved for stimulating CARS, with the remainder used as the reference signal. For the first simulation, the CARS excitation bandwidth will be shaped such that \( \Omega_L = 700 \text{ cm}^{-1}, \Omega_H = 1300 \text{ cm}^{-1}, \text{ and } T = 5 \text{ ps}. \) To show that the system can reconstruct several simultaneous resonances over the entire bandwidth, we create a hypothetical \( \chi^3(\Omega) \) with several Lorentzian resonances centered at 800 cm\(^{-1}\), 900 cm\(^{-1}\), 1000 cm\(^{-1}\), and 1100 cm\(^{-1}\). These frequencies are in the Raman “fingerprint” region and would be useful for practical molecular identification.

The simulation was implemented by sampling the spectra of \( \chi^3(\Omega), \tilde{E}_i(\omega), \text{ and } \tilde{E}_o(\omega) \) with 20,000 points spaced at equal intervals from 0 cm\(^{-1}\) to 20000 cm\(^{-1}\) in 1.0 cm\(^{-1}\) steps. The cross-correlations of Eqs. [1] and [2] were computed using the Fast Fourier Transform. These two equations form a “forward” CARS linear operator computing \( \chi^3(\Omega) \) from \( \tilde{E}_o(\omega) \) which we call \( \mathbf{A}(\omega, \Omega). \) To find the inverse of this operator, we used the Tikhonov-regularized least-squares inversion operator, which is formally denoted by \( \mathbf{A}^\dagger = (\mathbf{A}^\dagger \mathbf{A} + \epsilon \mathbf{I})^{-1} \mathbf{A}^\dagger \). The Tikhonov regularization was included to improve the stability of the inverse and to account for potential noise sources such as thermal and photon noise in a practical experiment.

The constant \( \epsilon \) is chosen to account for the magnitude of additive white Gaussian noise in a realistic experiment. In practice, \( \mathbf{A}^\dagger \) was computed using the pre-conditioned conjugate gradient method to avoid the very computationally expensive direct matrix inversion. While we do not model a real noise source here, Tikhonov regularization adjusts the inverse operator such that features of the estimated spectrum \( \chi^3(\Omega) \) that would be unstable due to insufficient information for reconstruction would tend towards zero.

The left column of Figure [2] shows the temporal and spectral shapes of the input pulse. Part (a) shows two chirped pulses that partially overlap, producing a beat pattern that stimulates the resonance. Part (b) shows the power spectrum of the input pulse. Part (c) shows the anti-Stokes radiation spectrum, that is calculated using Eqs. [1] and [2]. Because the excitation light is assumed to be much more powerful that the anti-Stokes light, we filter out all of the excitation bandwidth and utilize only wavelengths shorter than 800 nm for the inverse. The right column of Figure [2] shows the original and reconstructed \( \chi^3(\Omega) \). Part (d) is the magnitude of the spectrum of the intensity of the original pulse, i.e. the beat frequencies of the pulse. It shows the possible measurable Raman frequencies with this pulse. Part (e) shows the original \( \chi^3(\Omega) \) Raman spectrum magnitude. Finally, part (f) is the Tikhonov-regularized least-squares recon-
constructed $\chi^{(3)}(\Omega)$ based on only the anti-Stokes frequencies from 700–800 nm. In simulation, all of the spectral lines can be recovered. The minimum discernible separation in Raman frequencies tends to increase as the Raman frequency decreases because the anti-Stokes radiation created by lower frequency resonances tends to overlap the original spectrum more.

As a second demonstration with two closely spaced Raman lines, we consider deoxyribonucleic acid (DNA), which would be contained in the nucleus of a cell, and ribonucleic acid (RNA) located throughout the cell. Both macromolecules have PO$_2$ phosphodiester resonances, but the resonance occurs in DNA at 1094 cm$^{-1}$ and in RNA at 1101 cm$^{-1}$. To show that a properly designed pulse can recover both resonances distinctly, we design a $\chi^{(3)}(\Omega)$ that has resonances both at 1094 cm$^{-1}$ and 1101 cm$^{-1}$, that could be created by mixing DNA and RNA. To probe this mixture, we create a pulse using Eq. 4 with $\Omega_L = 1070$ cm$^{-1}$, $\Omega_H = 1130$ cm$^{-1}$, and $T = 5$ ps. The results of this simulation are shown in Figure 3. Figure 3 shows the beat frequency spectrum, and original and reconstructed $\chi^{(3)}(\Omega)$ Raman spectra. While the reconstructed lines are broadened, they are still quite distinct and would be useful for discerning the two molecules.

NIVI is a flexible tool utilizing ultrafast pulses that can measure small or large portions of a Raman spectrum of a molecule in a single pulse. It does so by interferometrically measuring the anti-Stokes radiation from a molecule, stimulated by beats in intensity of an excitation field. From this anti-Stokes field, the complex Raman susceptibility can be estimated. It is especially suited to biological imaging because while the pulse energy can be large, the peak power can remain small by chirping the pulse. For these reasons, we believe that NIVI can be a general tool for noninvasively probing the molecular content of biological tissues.

ACKNOWLEDGEMENTS

We acknowledge the scientific contributions and advice from Jeremy Bredfeldt, Selezion Hambir, Claudio Vinegoni, Martin Gruebele, Dana Dlott, Amy Wiedemann, and Barbara Kitchell from the University of Illinois at Urbana-Champaign. This research was supported in part by the National Aeronautics and Space Administration (NAS2-02057), the National Institutes of Health (National Cancer Institute), and the Beckman Institute for Advanced Science and Technology.
[1] M. D. Duncan, J. Reintjes, and T. J. Manuccia, Opt. Lett. 7, 350 (1982).
[2] E. O. Potma, D. J. Jones, J.-X. Cheng, X. S. Xie, and J. Ye, Opt. Lett. 27, 1168 (2002).
[3] J.-X. Cheng, L. D. Book, and X. S. Xie, Opt. Lett. 26, 1341 (2001).
[4] J. S. Bredfeldt, D. L. Marks, C. Vinegoni, S. Hambir, and S. A. Boppart, Coherent anti-stokes raman scattering heterodyne interferometry (2003). E-print@arxiv.org/physics/0311057, URL http://www.arxiv.org/abs/physics/0311057.
[5] A. Owyoung and P. S. Peercy, J. Appl. Phys. 48, 674 (1977).
[6] J. W. Hahn and E. S. Lee, J. Opt. Soc. Am. B 12, 1021 (1995).
[7] D. Oron, N. Dudovich, D. Yelin, and Y. Silberberg, Phys. Rev. Lett. 88, 063904 (2002).
[8] D. Oron, N. Dudovich, D. Yelin, and Y. Silberberg, Phys. Rev. A 65 (2002).
[9] N. Dudovich, D. Oron, and Y. Silberberg, Nature 418, 512 (2002).
[10] E. Gershgoren, R. A. Bartels, J. T. Fourkas, R. Tobey, M. M. Murnane, and H. C. Kapteyn, Opt. Lett. 28, 361 (2003).
[11] K. G. Purchase, D. J. Brady, and K. Wagner, Opt. Lett. 18, 2129 (1993).
[12] W. Drexler, U. Morgner, F. X. Kartner, C. Pitris, S. A. Boppart, X. Li, E. P. Ippen, and J. G. Fujimoto, Opt. Lett. 24, 1221 (1999).
[13] W. J. Wadsworth, A. Ortigosa-Blanch, J. C. Knight, T. A. Birks, T.-P. Martin Man, and P. S. J. Russell, J. Opt. Soc. Am. B 19, 2148 (2002).
[14] D. L. Marks, A. L. Oldenburg, J. J. Reynolds, and S. A. Boppart, Opt. Lett. 27, 2010 (2002).
[15] D. Huang, E. A. Swanson, C. P. Lin, J. S. Schuman, W. G. Stinson, W. Chang, M. R. Hee, T. Flotte, K. Gregory, C. A. Puliafito, et al., Science 254, 1178 (1991).
[16] S. A. Boppart, B. E. Bouma, C. Pitris, J. F. Southern, M. E. Brezinski, and J. G. Fujimoto, Nature Medicine 4, 861 (1998).
[17] B. E. Bouma and G. J. Tearney, eds., Handbook of Optical Coherence Tomography (Marcel Dekker, Inc., 2001).
[18] G. H. Golub and C. F. Van Loan, Matrix Computations (Johns Hopkins University Press, Baltimore, MD, 1996).