Chemically selective imaging by spectral phase shaping for broadband CARS around 3000 cm\(^{-1}\)

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Chemically selective microscopy based on broadband coherent anti-Stokes Raman scattering (CARS) is demonstrated on a mixed sample of 4 \(\mu\)m diameter polystyrene (PS) and poly (methyl methacrylate) (PMMA) beads. The CARS signal from the PS or the PMMA beads is enhanced or suppressed, depending on the phase profile applied to the broadband spectrum. Using a combination of negative and positive (sloped) \(\pi\)-phase steps the purely nonresonant background signal is removed. © 2009 Optical Society of America

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1. INTRODUCTION

Coherent anti-Stokes Raman scattering (CARS) is a four-photon process, where a pump field at frequency \(\omega_p\), a Stokes field at frequency \(\omega_s\), and a probe field at frequency \(\omega_{pr}\) interact with a molecule to produce an anti-Stokes photon of frequency \(\omega_{as} = \omega_p - \omega_s + \omega_{pr}\). This CARS signal is resonantly enhanced whenever the frequency difference between the pump and Stokes photons \((\omega_p - \omega_s)\) equals the frequency \(\Omega\) of a vibrational active mode of the molecule [1,2].

In broadband CARS [3–7], where several input pulses are broadband, the spectral phase profile of the laser pulses is also of importance as the nonlinear interaction mixes different pathways in the output signal. Selective excitation of Raman levels [8], improved resolution [9], and nonresonant background rejection [10] have been demonstrated by spectral phase shaping of a broadband laser pulse. Previously we demonstrated spectroscopy based on the shaping of pump and probe pulses, to compensate the intrinsic (negative) phase step associated with a vibrational resonance [11]. Here, we demonstrate chemically selective imaging in combination with purely nonresonant background removal.

In our setup there are two broadband pulses—the pump and probe pulse—and multiple combinations of their frequency components can result in the same anti-Stokes frequency, giving rise to interference. The phase of the pump and probe pulse and the phase of the molecular response determine whether the different pathways interfere constructively or destructively. Choosing a suitable phase profile will enhance or suppress CARS signals from certain vibrations, depending on their frequency and linewidths. Compared to imaging based on spectrally resolved signals, this method is potentially faster as the imaging is based on the integrated signal. The approach also scales favorably with the complexity of the spectra; as the spectrum of the target molecule contains more (overlapping) lines, the excitation spectrum is used more efficiently and the shaping increases in specificity for the target molecule. The nonresonant background rejection becomes increasingly simple as the application of the complex phase profile by itself already reduces the nonresonant signal.

For imaging, our method has a few advantages compared to other broadband CARS methods such as single pulse CARS [12] and multiplex CARS [3]. With multiplex CARS a complete spectral measurement is required for each pixel in order to differentiate between two similar substances. Using our method this differentiation can be obtained from the integrated signal instead. Another advantage of our method is the resolution in the phase shaping that can be applied. Single pulse CARS has a reduced resolution at higher wavenumber regions because the applied phase profile mixes with itself to generate the vibrational frequency spectrum. Our method offers a shaping resolution that is nearly constant over the entire frequency range. The phase profile that we apply to the pump spectrum is transfered unaltered to the vibrational frequencies through the interaction with the narrow Stokes. This direct transfer simplifies the identification and application of optimal phase profiles. Compared to narrowband CARS, a shaped broadband approach offers easy switching between the imaging of different materials; only the electronically applied phase profile is changed and (slow) tuning of input frequencies is not required. Furthermore the interaction with multiple resonances allows for selectivity based on multiple lines, which increases the specificity.

2. SIMULATIONS

Numerical simulations are presented to explain the effect of different phase shapes on the CARS signal. For all simulations, the Stokes pulse is considered infinitely spectrally narrow compared to the pump and probe. The pump and probe pulses are assumed to originate from the...
same beam and thus have the same bandwidth, intensity, and spectral phase with a Gaussian spectral amplitude envelope $|P(\omega)|$ in Eq. (2). The third-order nonlinear susceptibility of the sample, consisting of pure polystyrene (PS) or poly(methyl methacrylate) (PMMA), is described using Eq. (1) [13,14], where the nonresonant contribution $\chi_{NR}^{(3)}$ is assumed to be constant for all frequencies. The strength $A_R$, center-frequency $\Omega_R$, and linewidth $\Gamma_R$ of each resonance is determined by a multiple complex Lorentzian fit on the spontaneous Raman spectrum, as shown in Figs. 1(a) and 1(b). The phase step in the pump and probe spectrum is described using the phase profile of Eq. (1) for a single resonance. The CARS response is calculated using Eq. (2).

$$\chi^{(3)} = \chi_{NR}^{(3)} + \sum_k \frac{A_R}{\Omega^2_k - (\omega_p - \omega_s)^2 - 2i(\omega_p - \omega_s)\Gamma_R}$$

$$I_{\text{CARS}} \propto |P(\omega + \omega_p) \cdot e^{i\Phi(\omega + \omega_s)} \cdot [\chi_R^{(3)}(\omega) + \chi_{NR}^{(3)}] \otimes P(\omega) \cdot e^{i\Phi(\omega)}|^2.$$ (2)

Since the Stokes spectrum is very narrow compared to the pump and probe spectrum, the mixing of the pump and Stokes beam is approximated as a spectral shift of the pump. The shifted pump is then multiplied by the spectral response of the molecule (with resonances around

![Fig. 1](image)

Fig. 1. (a) Complex Lorentzian fit (red) of the Raman spectrum of PMMA (black). (b) Complex Lorentzian fit (red) of the Raman spectrum of PS (black). (c) Negative phase step scan simulation on PS. (d) Positive phase step scan simulation on PS. (e) 2D difference spectrum simulation on PS. (f) 2D difference spectrum measurement on PS. (c)–(f) are given as a function of excitation frequency on the lower axis with emission frequency on the vertical axis. The upper axis shows the corresponding vibrational frequency that is addressed by the pump frequency minus the Stokes frequency.
The nonresonant response that is independent of the direction of the phase step; the broad horizontal stripe in Figs. 1(c) and 1(d) is caused by the nonresonant response. When the phase step is near the center of the spectrum the nonresonant response is minimized as the left and right halves of the excitation spectrum are in counterphase. The resonant interaction, however, includes a multiplication with the molecular response that contains a negative phase step for each resonance. When the applied phase step compensates the native phase step, a strong response is again possible, creating a vertical feature in the 2D spectrum. The interaction of the native phase step (with a fixed position) with the swept phase step in the excitation spectrum causes features with a slope of 1 in the 2D spectrum. Interaction of the swept phase step with itself causes a (nonresonant) feature with a slope of 2 in the 2D spectra. Subtracting the positive and negative step intensity spectra removes the purely nonresonant contributions and yields Fig. 1(e). Integration over the difference spectrum provides a simpler representation of the data, shown in Fig. 2(a) together with the same calculation for PMMA. The resonances can still be observed but the effects of different resonances interact. It can be seen that it is possible to obtain zero intensity for either of the two materials separately by choosing the correct frequency position for the \( \pi \)-phase steps. Therefore it is possible to image the materials individually while suppressing the signal from the other material.

3. MEASUREMENTS AND DISCUSSION

A. Setup

A representation of the setup is given in Fig. 3. A neodymium-doped yttrium orthovanadate (Nd:YVO\(_4\)) laser with a center wavelength of 1064.3 nm and a pulse length of 15 ps (1 cm\(^{-1}\), 0.1 nm FWHM, Spectra-Physics Vanguard) is used for the Stokes pulse. The pump and probe pulse is generated by a femtosecond unamplified Ti:sapphire laser (KM-labs) that is tunable around 800 nm (~20 nm FWHM). We use the same pulse for the pump and probe fields. Both lasers have a repetition rate of 80 MHz and the lasers are synchronized by an electronic locking system \[15\].

The shaper contains a liquid crystal device (LCD) with a 1D array of 4096 pixels, spread over a length of 7.4 mm (Boulder Nonlinear Systems). There is an intentional cross talk between adjacent pixels, which smears out the applied phase profile. This effect improves the filling factor at the expense of the number of degrees of freedom (approximately 600), however, the positioning accuracy of the phase profile is unaffected by this cross talk and corresponds to 1 pixel (about 12 GHz or 1 cm\(^{-1}\) \[16\]). A cylindrical mirror with a focal distance of 52 mm is used to focus the negative first-order diffracted beam from a 800 lines/mm grating onto the LCD in a “cylindrical reflective 4-f arrangement” \[17\].

The pump and probe beam from the Ti:sapphire laser is directed through a prism compressor before entering the shaper to compensate for linear dispersion in the system. Residual and/or higher order dispersion is compensated by the shaper. A derandomized adaptation (DR2) algorithm \[18\] is used to optimize the second harmonic signal generated in a 10 \( \mu \)m thick BiBO crystal, which is placed at the sample location. The phase profiles used in the experiments are superimposed on the phase profile required for the Fourier-limited pulse.

The pump, probe (40 mW) and Stokes (100 mW) beams are focused in the sample by a reflective microscope objective with a numerical aperture of 0.65. A reflective objective has been chosen to prevent chromatic aberrations. The CARS signal is collected by a long working distance microscope objective with a numerical aperture of 0.55. The images are obtained using a \( x,y \)-piézo scanner (piezo-system jena) to scan the sample and a photomultiplier tube (Hamamatsu). A chopper in the Stokes beam in combination with a lock-in amplifier is used to reduce noise.

B. Spectroscopy

Sweeping a \( \pi \)-phase step is first applied to a bulk PS sample. A \( \pi \)-phase step with a width of 0.6 nm (10 cm\(^{-1}\)) is scanned through the Ti:sapphire (pump and probe) spectrum while measuring the CARS spectrum. The width was chosen to match the vibrational transitions in
this spectral region (on average). We measure the CARS spectrum for the positive step, negative step, and Fourier-limited pulse. Spectra are integrated for several hundred milliseconds and a complete 2D spectrum is measured in a few minutes. The difference spectrum for PS obtained from these measurements can be found in Fig. 1(f). A spectral scan of PMMA has not been measured, but data from literature has been used [19].

Both the sloped and vertical contributions from the Raman vibration at 3052 cm\(^{-1}\) are visible at 373.5 THz (3062 cm\(^{-1}\)). A second, weaker resonance is also revealed. The two vertical lines indicate the crossing point of the resonant line features of both resonances with the non-resonant line. The second, weaker resonance is located at 369.6 THz (or 2930 cm\(^{-1}\)). This feature can be assigned to a Raman resonance of PS (2910 cm\(^{-1}\)). Furthermore, there is a signal evident from an even weaker resonance at 367.5 THz (or 2862 cm\(^{-1}\)). This signal is assigned to the weak resonance at 2850 cm\(^{-1}\) observed in the Raman spectrum of PS [20].

**C. Microscopy**

A sample consisting of a mixture of 4 \(\mu\)m PS and PMMA beads dried on a glass surface is used for imaging. Selective imaging is demonstrated by applying a positive and negative \(\pi\)-phase step near the main resonance for PMMA or PS. By looking at the integrated CARS signal from a \(\pi\)-phase step sweep, as presented in Fig. 2(b), a suitable phase step location can be chosen. Measurements are taken with a 10 ms/pixel integration time. Phase patterns are switched per line as the liquid crystals in the LCD require roughly 200 ms to completely stabilize. Each line was measured three times (positive step, negative step, Fourier limited) so that the total image takes some 30 min to acquire. This speed is limited by the relatively low signal level. The low signal level is due to the fact that both PMMA and PS have a limited set of resonances in this area, which is useful for the purpose of this demonstration, but also means that a large part of the excitation spectrum is wasted. Using a denser part of the spectrum would allow for higher scanning speeds. Scanning speeds can then be further improved by using the full switching speed of the LCD (2 kHz) with compensation for the crystal relaxation and setting the lock-in detection directly to the pattern modulation. This would allow for the imaging of a frame every few minutes.

Measurements with positive and negative \(\pi\)-phase steps at 370.2 THz, which is near the main resonance of PMMA, are shown in Figs. 4(a) and 4(b), respectively. For an image with a Fourier-limited pulse, shown in Fig. 4(c), the difference in intensity between the PMMA and PS beads is largely due to the larger overall scattering cross section of PS compared to PMMA. For the positive phase step at 370.2 THz an increase in relative intensity of the PMMA beads compared to the PS beads is observed when compared to the Fourier-limited case. For the negative phase step the relative intensity of the PMMA beads is observed to be roughly the same as in the Fourier-limited case.

The intensity difference (between signals for a positive and negative phase step) for the PMMA and PS beads varies greatly from positive to negative as a function of the step position (see Fig. 2). The difference intensity images for the \(\pi\)-phase step centered near the main resonance of PMMA (370.2 THz) and near the main resonance of PS (373.2 THz) are presented in Fig. 5.

In the difference intensity image for the phase step positioned near the main resonance of PMMA [Fig. 5(a)] the PMMA beads are visible, while the PS beads are suppressed. For the phase step positioned near the main resonance of PS [Fig. 5(b)] the PS beads are visible, while the PMMA beads are mostly suppressed. The PMMA beads are still somewhat visible, but with a negative (difference) intensity as opposed to the positive intensity of the PS beads.
4. CONCLUSION AND OUTLOOK
Broadband CARS imaging around 3000 cm$^{-1}$ is demonstrated with phase shaped pump and probe pulses on a mixed sample of dried 4 μm PS and PMMA beads. We show suppression of either the PS or the PMMA signal, depending on the location of the π-phase step in the pump and probe pulses. Additionally we show nonresonant background removal in the images. No optical amplifiers are required for these measurements.

The generation of background free CARS images of PMMA and PS beads in itself is not a major achievement and substantially easier methods are available for this. However, the possibility to image different constituents based only on the applied phase shape promises the possibility of imaging many different components in quick succession without changes in filter sets or realignment. Also the favorable scaling for increasingly dense spectra depending on the location of the π-phase steps suggest the possibility to image different constituents in quick succession without changes in filter sets or realignment. The generation of background free CARS images of PMMA and PS beads in itself is not a major achievement and substantially easier methods are available for this. However, the possibility to image different constituents based only on the applied phase shape promises the possibility of imaging many different components in quick succession without changes in filter sets or realignment.

Preliminary simulations on 2π-phase steps suggest even greater contrast in the integrated signal when compared to π-phase steps. Furthermore the vibrational resonances may be more easily identified in the resulting CARS spectra. This topic will be the subject of further research.

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