High Order Symmetry Structural Properties of Vibrational Resonances Using Multiple-Field Polarization Coherent Anti-Stokes Raman Spectroscopy Microscopy

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Polarization-resolved coherent anti-Stokes Raman scattering (CARS) is usually applied to measure the depolarization ratio in solutions or evidence orientation effects in anisotropic media. We present an extensive approach based on multiple-field polarization-resolved CARS, in order to unravel the complexity of vibrational resonances up to the fourth-order symmetry, at the microscopic scale in nonisotropic media. The CARS polarized signals measured under a continuous variation of the incident pump and/or Stokes excitation beams are analyzed using a full tensorial picture both in the nonresonant and resonant regimes. This method evidences the strong influence of vibrational symmetries on polarized CARS, and more specifically the occurrence of Kleinman symmetry deviations at the vicinity of the Raman lines frequencies. This technique, illustrated on a cubic symmetry crystal, is general and can be applied to other medium symmetries.

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Polarization-resolved coherent anti-Stokes Raman scattering (CARS) microscopy is a powerful technique to characterize molecular vibrations symmetry properties in isotropic media, quantified by the Raman depolarization ratio of liquids [1,2]. The polarization dependence of CARS has also been used to remove the nonresonant background intrinsically present in CARS spectra [3] and images [4]. More recently, polarization sensitive CARS has been applied to measure molecular orientation in anisotropic samples, such as water molecules in phospholipid bilayers [5], ordered biomolecular assemblies in tissues [6,7], and liquid crystals [8]. In these techniques however, the incident pump and Stokes fields polarizations are kept parallel to each other and only qualitative orientation information is obtained. The full potential of CARS polarization analysis has never been completely explored, most probably because it involves dealing with the complexity of a high order nonlinear tensor of 81 components (57 in degenerate CARS), as well as its spectral specificity near vibrational resonances [9–11]. Deciphering this complexity would bring invaluable structural information on molecular vibrational behaviors in complex environments. Moreover, due to its higher nonlinear order nature (up to four optical fields directions are involved), CARS allows probing high symmetry orders which are not accessible by spontaneous Raman scattering [10]. Implementing this analysis in microscopy imaging would significantly extend the assets of CARS microspectroscopy as a label-free analysis technique, together with unraveling conformational information at the molecular scale.

In this work we investigate the multipolar nature of vibrational resonances in a nonisotropic sample using multiple-field polarization-resolved CARS microscopy, where both pump and Stokes input polarizations are controlled and tuned independently. We focus on a model system which is a crystal of pure fourth-order symmetry that cannot be read out by lower order processes such as polarization-resolved spontaneous Raman. We show that CARS multiple-field control in a nonisotropic medium can reveal not only orientational but also structural information on vibrational resonances, in particular, Kleinman symmetry deviations that are intrinsic to resonant behaviors [12].

In nonisotropic samples, the CARS third-order nonlinear susceptibility tensor has a more complex structure than for isotropic media. First, the nonresonant CARS signal generated by the electronic response of the medium to the incident fields is not isotropic, and therefore possesses its own polarization response [9,11]. Second, each resonance has its own symmetry specificity, which is characterized by a fourth rank tensor whose structure can be different from the nonresonant one, even though it belongs to an irreducible representation of the nonresonant symmetry group [9,10]. The complexity of the susceptibility tensor is further increased at the vicinity of resonances, where Kleinman symmetry conditions do not apply and antisymmetric tensor contributions arise [9].

The sample studied in this work is an octahydrosilasesiquoxane H8Si8O12 crystal, that has cubic symmetry and belongs to the Oh crystallographic point group [13] [Fig. 1(a)]. The investigated crystals are of millimetric size and their in-plane orientation can be easily visualized from their rectangular shape using white light transmission imaging [Figs. 1(b) and 1(c)]. In this work, we explore the polarized CARS responses of a crystal at three spectral positions [Fig. 1(d)]: first, the nonresonant response at \( \approx 1380 \text{ cm}^{-1} \); second, the totally symmetric \( A_{1g} \) vibrational mode (Si-H stretching at 2302 cm\(^{-1}\)), and third, the degenerate \( E_g \) mode (O-Si-H bending at 932 cm\(^{-1}\)).

The experimental setup developed to perform polarization-resolved CARS microscopy is detailed...
Fig. 1 (color online). (a) Molecular structure of the $\text{H}_8\text{Si}_8\text{O}_{12}$ crystal. (b) White light transmission image of a crystal in the microscope. (c) Orientation of the crystal in the laboratory frame, defining the three Euler angles ($\theta$, $\phi$, $\psi$) ($E(\alpha)$ is an incident field polarization direction in the ($X$, $Y$) sample plane). (d) Raman spectrum of the crystal. The arrows point the resonances studied in this work ($A_{1g}$ mode $\Omega_R = 2302$ cm$^{-1}$; $E_p$ mode $\Omega_k = 932$ cm$^{-1}$; NR: nonresonant background, 1380 cm$^{-1}$).

Briefly, both pump (at a wavelength $\lambda_p = 724.49$ nm) and Stokes ($\lambda_S$ tuneable) beams are linearly polarized and three different schemes of polarization tuning are used in order to provide a multiple-field polarization analysis: either the Stokes (respectively pump) polarization is fixed along the $X$ axis defined in Fig. 1(c) and the pump (respectively Stokes) polarization rotates by an angle $\alpha_p$ ($\alpha_S$) from $0^\circ$ to $360^\circ$ relatively to $X$, or both pump and Stokes polarizations rotate simultaneously ($\alpha_p = \alpha_S$). The incident beams are focused in the sample through a low numerical aperture objective ($\text{NA} = 0.6$), in order to avoid any contribution from the Z-polarized component of the excitation fields in the experiment and to allow a plane wave approximation. The emitted anti-Stokes signal is detected in the forward direction and split through a polarizing beam splitter in the experiment and to allow a plane wave approximation.

In principle, the independent tuning of both pump and/or Stokes polarizations and projection of the signal over two directions $X$ and $Y$ should allow the determination of 12 independent macroscopic coefficients $X_{ijkl}$, where only the $X$ and $Y$ readout directions are involved. These macroscopic susceptibility coefficients are related to the microscopic (unit-cell) ones by the tensor rotation formulation $X_{ijkl}^{(3)}(\Omega) = \sum_{\alpha} x^{(3)}_{ijkl}(i \cdot J)(j \cdot K)(k \cdot L)(\Omega)$, where $\{i, J\}$ are the rotation matrix components between the macroscopic and microscopic frames, and $\Omega = (\theta, \phi, \psi)$ is the Euler angles set defining the crystal orientation [Fig. 1(c)].

Since the CARS nonlinear radiation from a vibrational resonance is composed of both nonresonant and resonant contributions, a first tensorial analysis of the nonresonant contribution is required. This can be done by setting the incident pump and Stokes wavelengths combination out of resonance. In principle, for samples in which the number of microscopic coefficients is low ($< 12$), this first analysis can bring information on both crystal orientation and microscopic tensorial components $X_{ijkl}^{(3)}$, following the approach already developed for second order nonlinear processes [16]. Here, due to the higher complexity of the CARS process, we chose to target directly the microscopic coefficients. The studied crystal belongs to the $\text{Oh}$ crystallographic point group; therefore its microscopic nonresonant susceptibility tensor $X_{ijkl}^{(3)NR}$ exhibits only two independent components in the unit-cell microscopic frame ($x$, $y$, $z$) [Fig. 1(c)] [12]:

$$X_{xxxx}^{(3)NR} = X_{yyyy}^{(3)NR} = X_{zzzz}^{(3)NR} X_{xxyy}^{(3)NR} = X_{zxyz}^{(3)NR} = X_{zzzz}^{(3)NR}$$

with all index permutations allowed, either from the CARS pump wavelengths degeneracy or from Kleinman symmetry far from resonances [12]. In the following calculations, the data are normalized by $X_{xxxx}^{(3)NR}$. Therefore, the whole microscopic nonresonant tensor is completely characterized by one parameter: the nonresonant depolarization ratio $\rho_{NR} = X_{xxyy}^{(3)NR} / X_{xxxx}^{(3)NR}$. A more complex symmetry would involve more than one depolarization ratio.

Finally, in this nonresonant configuration, the unknown quantities ($\theta$, $\phi$, $\psi$, $\rho_{NR}$) can be considered as data fitting parameters. In the present case, the angles $\theta = 90^\circ$ and $\phi = 49^\circ$ can be deduced from a macroscopic observation of the crystal (which is of millimetric size), and therefore only the third Euler angle $\psi$ is kept as a free parameter. The fitting procedure of the polarization data represented in Fig. 2 consists of finding, for all polarization tuning configurations simultaneously, the couple ($\psi$, $\rho_{NR}$) that minimizes the mean square error for the CARS intensities in the $X$ ($I_X$) and $Y$ ($I_Y$) directions, normalized by the maximum of the total intensity, $I_X + I_Y$. The results show a very good agreement with the crystal structure with a unique solution $\rho_{NR} = 0.38 \pm 0.05$, and with $\psi$ showing a large range of possible values. This angle indetermination is most probably due to the present geometry which investigates a projection of a cubic object in the sample plane. Although the depolarization ratio expression resembles that from an isotropic medium [1], its value differs from the value measured in a water solution ($\rho_{NR} = 0.30 \pm 0.03$, close to the expected 1/3 value).

Note that in the present case of a pure fourth-order
symmetry object, any nonresonant optical contrast of lower order such as polarized transmission would be unable to reveal such information, since a minimum of fourth-order light-matter interaction is required.

In the vicinity of vibrational resonances, the CARS susceptibility tensor takes a more complex expression including a Lorentzian spectral resonant contribution:

$$\chi^{(3)} = \chi^{(3)NR}_{xxxx} \left( \chi^{(3)NR}_{xxxx} A \right) + \left( \omega_p - \omega_S - \Omega_R + i \Gamma \right)^{\chi^{(3)R}}_{xxxx}$$

with $\Omega_R$ the frequency of the addressed vibrational mode, $A = \chi^{(3)R}_{xxxx}/\chi^{(3)NR}_{xxxx}$ the ratio of the strength of the resonant over the nonresonant contributions, and $\Gamma$ the Raman line width. We chose to normalize the whole tensor by the component $\chi^{(3)NR}_{xxxx}$, so the first term in brackets in Eq. (2) corresponds exactly to the nonresonant tensor calculated above. The tensor structure of the resonant contribution $\chi^{(3)R}_{xxxx}$, which can differ from that of $\chi^{(3)NR}_{xxxx}$, depends on the symmetry of the vibrational mode and is deduced from the analysis of the corresponding irreducible representation [9]. It is therefore expected that the CARS wavelength dependence will considerably depend on the polarization settings for both pump and Stokes fields, especially for nontotally symmetric modes that exhibit different symmetry properties than the nonresonant background.

The resonant CARS polarization responses are first analyzed for the totally symmetric vibrational mode $A_{1g}$. These responses, measured at the peak of the CARS spectrum [Figs. 3(a) and 3(b)], resemble slightly the nonresonant responses of Fig. 2, which is expected since an $A_{1g}$ mode exhibits a similar symmetry structure as the crystal point group [9]. The only difference between the nonresonant and resonant cases is the antisymmetric nature of the tensor at resonance, due to the departure from Kleinman symmetry conditions which imposes $X_{ijij} \neq X_{ijji}$ with $i, j = (x, y, z)$. This property is visualized in the behavior of $I_Y(\alpha_S)$ that differs from the nonresonant response [Fig. 2(b)]. Extending the previous approach to this more complex resonant situation, the fit of the experimental data are performed using Eq. (2) on three independent parameters: the depolarization ratios $\rho_{A1}^R = X_{xyxy}/X_{xxxx}$ and $\rho_{A2}^R = X_{xyxy}/X_{yyyy}$, and the Euler angle $\psi$. The $A$ resonant strength factor and the $\Gamma$ vibrational band width are deduced from the CARS spectrum [Fig. 3(b)] and the spontaneous Raman spectrum of the crystal (Fig. 1), respectively.

The multiple-field polarization data fitting shows that again the $\psi$ angle is not a crucial factor, with an angle range ($\psi \leq 15^\circ$) providing acceptable solutions. A fit solution, depicted in Fig. 3(c), shows a good agreement with the experimental data. The cartography of the mean square error between theory and experiment as a function of the two depolarization ratios $\rho_{A1}^R$ and $\rho_{A2}^R$ [Fig. 3(d)] shows that a large range of reliable solutions follow a linear dependence of slope 0.53, which is well below 1 (as it would be in the nonresonant case). This experiment, in addition to quantifying microscopic depolarization ratio relations for this vibrational band, provides also an estimation of Kleinman symmetry conditions departure at the specific wavelength of measurement.

The degenerate vibrational mode $E_g$ is finally investigated. The polarization responses at the peak of the CARS spectrum strongly differ from the previous cases [Fig. 4(a)]. In addition, the CARS spectrum itself is seen to be strongly dependent on the incident polarization settings [Fig. 4(b)]. This is essentially due to the very different tensorial structure of the $E_g$ susceptibility as compared to the nonresonant CARS tensor, where the $z$ dependent components become nondegenerate [9]:

$$X_{zzzz}^{(3)R} = -(X_{xxxx}^{(3)R} + X_{yyyy}^{(3)R}) \quad \text{with} \quad X_{xxxx}^{(3)R} \neq X_{yyyy}^{(3)R}$$
$$X_{xyxy}^{(3)R} = -(X_{xxzz}^{(3)R} + X_{yyzz}^{(3)R}) \quad \text{with} \quad X_{xxzz}^{(3)R} \neq X_{yyzz}^{(3)R}$$

In order to include this spectral dimension in the CARS polarization data fit, we chose to fit principally the $I_Y(\alpha_p)$ dependence (which is the most sensitive to wavelengths...
that the whole CARS spectral region around the band in its vicinity [Fig. 4(b)]. Overall, this analysis shows slightly perturbed by the appearance of another vibrational ensemble typical nonresonant responses, although resonance effects. The surrounding points are consistent with the model [Fig. 4(d)]. Between the peak and dip position tuning experiments also show a relatively good agreement with the data only in some spectral regions, in particular, at the spontaneous Raman band [point C in Fig. 4(b)], at the peak of the CARS spectrum, and at the pump intensity tuning experiments, indicating essentially that polarization-resolved CARS responses are not solely sensitive to the crystal symmetry ($\chi^{(3)NR}$), but also to both the vibrational band structure and the Kleinman symmetry deviation rule that involves antisymmetric susceptibility tensors [12]. This method is furthermore directly transposable to microscopy imaging, opening new prospective investigations based on not only chemical selectivity, but also structural imaging, dedicated to materials or biological diagnostics down to the nanoscale.

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We have proposed a new methodology able to approach the full complexity of multipolar symmetry vibrational bands up to the fourth-order symmetry at the microscopic scale, and therefore distinguish features that are not accessible via spontaneous Raman polarized microscopy. We show on a model system with cubic symmetry, that polarization-resolved CARS responses are not solely sensitive to the crystal symmetry ($\chi^{(3)NR}$), but also to both the vibrational band structure and the Kleinman symmetry deviation rule that involves antisymmetric susceptibility tensors [12]. This method is furthermore directly transposable to microscopy imaging, opening new prospective investigations based on not only chemical selectivity, but also structural imaging, dedicated to materials or biological diagnostics down to the nanoscale.

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