Computational optimization of phase shaped CARS

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ABSTRACT
We explore strategies for optimizing selectivity, specificity, and sensitivity in broadband CARS by precalculating pulse shapes using an evolutionary algorithm. We show the possibility of selective excitation of a single constituent in a test case of a mixture of five resonant compounds. The obtainable contrast ratio for a test case of PMMA in a mixture of five resonant compounds is predicted to be 2000:1, and is related the uniqueness of the complex vibrational response of the compound of interest compared to that of the surrounding molecules. Furthermore we investigate how the effects of homodyne mixing in the focal volume affect the obtainable contrast ratio and how noise affects the optimization. We also show preliminary results of experimental optimization of the CARS signal from PMMA microspheres, resulting in high contrast imaging, free of non-resonant background signal.

Keywords: Coherent anti-Stokes Raman scattering, Coherent control, Phase shaping, Evolutionary algorithms

1. INTRODUCTION
Coherent anti-Stokes Raman scattering (CARS) is a coherent non-linear process that has generated a lot of interest for both spectroscopy and imaging.\(^1\) In CARS, 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 a blue-shifted anti-Stokes photon at frequency \(\omega_{as} = \omega_p - \omega_s + \omega_{pr}\). The CARS signal is resonantly enhanced if the frequency difference between the pump and Stokes beam matches with a vibrational resonance of the molecule.\(^2\) Using broadband excitation, multiple resonances can be excited and detected simultaneously.\(^3\)-\(^5\) It was realized that by tailoring the input pulse(s) using amplitude and/or phase shaping, one can exercise control over the CARS process.\(^6\)-\(^11\) A non-resonant CARS signal is generated along with the resonant CARS signal however, and this non-resonant contribution becomes increasingly dominant as the bandwidth of the excitation pulses increases.

We present a phase-only pulse shaping approach in which phase patterns are used to suppress the non-resonant background contributions as well as to enhance or suppress the signal from resonant compounds in the sample. In our scheme we use a degenerate broadband pump and probe pulse in combination with a narrow-band Stokes pulse, creating multiple pathways to the same anti-Stokes wavelength. This allows us to control the interference between different resonances by controlling the phase of the pump and probe pulse. Both the optimization and the imaging are performed using the integrated output pulse intensity, negating the need for any spectrally resolved detection and increasing imaging speed.

2. THE MOLECULAR VIBRATIONAL PHASE
To accurately model the CARS response generated by a molecule, detailed knowledge of its complex vibrational response, \(\chi^{(3)}(\omega)\), is required. We consider only a classic time invariant model without cross relaxation. The complex vibrational response can be measured using heterodyne CARS,\(^12\) in which case a non-resonant reference signal is required to calibrate the measured phase. A more direct method of measuring the complex vibrational response is vibrational phase contrast CARS (VPC-CARS),\(^13\) in which the reference signal is created on a per pixel basis, using two competing pathways which create an interference on the vibrational level. Other methods...
Figure 1. (a) Spontaneous Raman scattering spectrum of PMMA (black dots) and the associated fit (red line). (b) Obtained vibrational phase profile for PMMA.

for retrieving the vibrational phase have been reported as well, for example by extracting the complex vibrational response from a measured (broadband) CARS spectrum.\textsuperscript{14-17}

Here, we outline our method of retrieving the vibrational phase information from spontaneous Raman scattering spectra.\textsuperscript{18} The spontaneous Raman signal is proportional to the imaginary part of the complex response, and therefore contains both amplitude and phase information. The complex vibrational response of a molecule is a sum of different vibrational resonances, where each vibrational resonance can be considered as a damped driven harmonic oscillator. The complete vibrational response therefore becomes a sum of damped driven harmonic oscillator functions, with different oscillator strengths, damping factors and resonance frequencies, combined with a non-resonant term, as shown in eq.1.

\[
\chi^{(3)}(\omega) = \chi_{NR}^{(3)} + \sum_{R} \frac{A_R}{\omega_R^2 - \omega^2 + 2i\omega\gamma_R} 
\]

We fit the imaginary part of eq.1 to the spontaneous Raman spectrum using an evolutionary algorithm. The specific evolutionary algorithm that we employ is covariance matrix adaptation evolution strategy (CMA-ES), which uses the covariance matrix of the optimization parameters to continuously rotate and adapt the set of candidate solutions. CMA-ES also employs stepsize control, which increases or decreases the stepsize based on the evolution of the searchpath, leading to a fast convergence and preventing the algorithm from getting stuck in a local optimum early on in the optimization. Details of the code implementing CMA-ES can be found in ref.19. An example of a measured spontaneous Raman spectra for PMMA and the corresponding fit and retrieved phase is shown in figure 1.

Because the spontaneous Raman spectrum is proportional to the imaginary part of the vibrational response, any purely real contributions to the vibrational response will not show up in the spontaneous Raman spectrum. In CARS, the purely real contribution to the vibrational phase response shows up as a nonresonant background signal. Since this non-resonant contribution can not be obtained from the spontaneous Raman spectrum, it has to be estimated. In our case we use a non-resonant background that has an amplitude of 20\% of the maximum amplitude of the resonant vibrational response and we consider the nonresonant background to be constant with respect to frequency.
3. COMPUTATIONAL OPTIMIZATION

3.1 Single Compound Optimization

After the complex vibrational response, \(\chi^{(3)}(\omega)\), has been obtained, it can be used to model the CARS response of a molecule for a given set of input pulses. In our time-invariant model, the CARS signal can be described by a series of convolutions between the input fields and a multiplication with the complex vibrational response (eq. 2).

\[
I_{CARS}(\omega) \propto \left[ \left| \langle E_{\text{pump}}(\omega) \rangle e^{i\phi_{\text{pump}}(\omega)} \otimes |E_{\text{Stokes}}^*(\omega) e^{-i\phi_{\text{Stokes}}(\omega)} \rangle \cdot \chi^{(3)}(\omega) \right| \otimes |E_{\text{probe}}(\omega) e^{i\phi_{\text{probe}}(\omega)} \rangle^2 \right]
\]

In the case of a CARS process with a broadband pump and probe pulse and a narrowband Stokes pulse, this equation can be simplified. Due to the very narrow bandwidth of the Stokes pulse, it is approximated by a delta function at the center frequency of the pulse. As a result, the only effect resulting from the convolution with the Stokes pulse is a frequency shift of the pump pulse, equal in magnitude to the center frequency of the Stokes pulse. Equation 3 shows the simplified equation for the generated CARS signal.

\[
I_{CARS}(\omega) \propto \left| \langle |E_{\text{pump}}(\omega + \omega_{\text{Stokes}}) e^{i\phi_{\text{pump}}(\omega + \omega_{\text{Stokes}})} \cdot \chi^{(3)}(\omega) \rangle \otimes |E_{\text{probe}}(\omega) e^{i\phi_{\text{probe}}(\omega)} \rangle^2 \right|
\]

We use a degenerate pump and probe pulse, centered at 12420 cm\(^{-1}\) (805.2 nm), with a bandwidth of 216 cm\(^{-1}\) (14 nm). The Stokes pulse is assumed to be infinitely narrow with a center frequency of 9395.85 cm\(^{-1}\) (1064.3 nm). The spectrum of the pump and probe pulse is defined over 4096 points and the corresponding phase profile is extended to the same 4096 points by cubic spline interpolation. The complex molecular response is also defined over 4096 points, with a 1 cm\(^{-1}\) resolution.

The calculated CARS signal can be used to evaluate the effect of differently phase-shaped pulses. We use this calculated CARS signal as a feedback criterium in a closed loop optimization to find an optimal excitation phase. Our goal is to find an excitation phase that maximizes the amount of resonant CARS signal from a substance and simultaneously minimizes any purely non-resonant contributions. To optimize only the resonant signal, the non-resonant contribution to the CARS signal needs to be discarded. Therefore we look at the difference in CARS signal generated by a phase profile \(\Phi(\omega)\) and the inverse phase profile \(-\Phi(\omega)\), which is free of non-resonant background. This CARS difference signal still contains some non-resonant contributions due to the homodyne mixing of the CARS process, but since this term is dependent on the amount of resonant contribution to the CARS signal as well, it does not conflict with our optimization goal. We apply CMA-ES\(^{19}\) to numerically optimize the spectral excitation phase, where we use the aforementioned spectrally integrated CARS difference signal as our fitness value. Here, we choose to run the algorithm for 1000 generations with 20 parents and a population size of 40 per generation.

The optimization of the phase profile is performed over 60 parameters. These parameters are evenly spaced along the frequency axis over a 574 cm\(^{-1}\) region and describe the absolute phase offset at these points. The phase profile is interpolated to 4096 points by cubic spline interpolation to achieve better accuracy in calculating the CARS response. The results of an optimization for pure PMMA are shown in figure 2, where it can be seen that the optimal excitation phase resembles the molecular phase profile. Furthermore it can be seen that the algorithm converges in about 100 generations.
Figure 2. Optimized excitation of pure PMMA. (a) shows the pump/probe intensity envelope (solid black), the molecular phase of PMMA (dashed red) and the optimal excitation phase (dotted purple). (b) shows the fitness of the laser pulse as a function of generation of the evolutionary optimization.

Figure 3. Selective excitation of PMMA in a sample of PMMA, polystyrene, polyethylene, toluene, and ethanol. (a) shows the pump/probe intensity envelope (solid black), the molecular phase of PMMA (dashed red) and the optimal excitation phase (dotted purple). (b) shows the fitness of the laser pulse for PMMA (red), polystyrene (green), polyethylene (blue), toluene (cyan), and ethanol (yellow) as a function of generation of the evolutionary optimization.

3.2 Multiple Compound Mixtures

Instead of optimizing the phase for maximum signal from a compound, optimizing for minimum signal is also possible. Considering a sample which contains multiple resonant compounds, an optimization for maximum signal of one compound can be performed, while simultaneously aiming for minimum signal from any other compounds. By actively suppressing the CARS signals from unwanted compounds, the contrast with regards to the compound of interest is enhanced.20 We consider a test case with five different resonant compounds, namely PMMA, polystyrene, ethanol, toluene, and ethanol. We apply the same optimization parameters as in section 3.1, but instead of just optimizing the CARS difference signal from PMMA, we aim for simultaneous optimization of the (difference) signal for PMMA and suppression of the signals from the other resonant compounds. The results of the optimization for selective detection are shown in figure 3.

It can be seen from figure 3 that the optimal phase deviates significantly from the molecular phase of PMMA to suppress the signal of the other resonant compounds. Furthermore the algorithm converges more slowly, due to the increased complexity of the optimization goal. The absolute amount of CARS difference signal from the PMMA has decreased by 48% when compared to the optimization of a pure PMMA sample (without suppression).
Figure 4. Homodyne mixing effects in a sample of PMMA, polystyrene (PS) and polyethylene (PE) with a pulse optimized for selective detection of PMMA. In the ternary plots the CARS difference signal for different ratio blends is shown on (a) a linear scale and (b) a logarithmic scale. (c) shows the signal for PMMA (red) as a function of relative concentration on a log/log scale. The dashed black line indicates an ideal linear dependence. The points in (c) are taken along the black dashed line in (b).

<table>
<thead>
<tr>
<th>Noise Level (σ)</th>
<th>Contrast Ratio</th>
<th>Norm. CARS signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2000 : 1</td>
<td>1</td>
</tr>
<tr>
<td>0.01π</td>
<td>215 : 1</td>
<td>0.97</td>
</tr>
<tr>
<td>0.02π</td>
<td>93 : 1</td>
<td>0.94</td>
</tr>
<tr>
<td>0.05π</td>
<td>36 : 1</td>
<td>0.86</td>
</tr>
<tr>
<td>0.1π</td>
<td>22 : 1</td>
<td>0.81</td>
</tr>
<tr>
<td>0.2π</td>
<td>10 : 1</td>
<td>0.41</td>
</tr>
<tr>
<td>0.3π</td>
<td>5 : 1</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Table 1. CARS signal and contrast ratios for selective excitation of PMMA, in a sample containing PMMA, polystyrene, polyethylene, toluene, and ethanol, in the presence of various levels of noise. The normalized signal is relative to the signal obtained in the case of no noise.

3.3 Noise and Homodyne Mixing

We investigate the robustness of our optimization approach by considering the effect of phase noise and homodyne mixing. In case of a sample with multiple resonant compounds, it is possible for several resonant compounds to be present in the focal volume simultaneously, leading to homodyne mixing between the CARS signals generated by the different compounds, which is not taken into account in the optimization. The effects of homodyne mixing in a sample of PMMA, polystyrene and polyethylene is shown in figure 4 for the case where selective PMMA imaging is desired. It can be seen from figure 4 that the effects of homodyne mixing are minimal and the intensity dependence is close to linear, except at very low relative concentrations in the order of a few percent.

The effect of (phase) noise on the optimization is modeled to gain insight in the robustness of the optimization approach to, for example, fluctuations in the spatial light modulator or jitter between the different laser sources. We simulate the effect of noise by adding random noise to each individual phase profile in the optimization, such that \( \phi_{\text{total}} = \phi_{\text{orig}} + \Phi_{\text{noise}}(\mu, \sigma) \), where \( \phi_{\text{total}} \) is the resulting phase profile with noise, \( \phi_{\text{orig}} \) is the original noiseless phase profile and \( \Phi_{\text{noise}} \) is a random noise function with a normal distribution with mean \( \mu = 0 \) and variance \( \sigma \). The results are presented in table 1.

From table 1 it can be seen that the presence of (phase) noise has a large influence on the optimization. At low levels of noise it is primarily the contrast ratio that is reduced, as the noise makes it increasingly difficult to suppress the signal from unwanted compounds. At higher noise levels the absolute signal from the optimized compound also starts to decrease significantly. It should be noted however that even at large amounts of noise, the evolutionary algorithm still converges to a solution.
4. EXPERIMENTAL OPTIMIZATION

4.1 Experimental setup

Our experimental setup uses two oscillators. The first is a Ti:Sapphire oscillator (KM-Labs) that generates the pump and probe beam, with a center wavelength around 805 nm, a pulse length of 70 femtoseconds and an average output power of 400 mW at a 80 MHz repetition rate. This oscillator is pumped by a continuous wave 532 nm laser (Spectra Physics Millennia VI). The second oscillator is a Nd:YVO$_4$ oscillator (Spectra Physics Vanguard), which generates the Stokes beam. This laser has a center wavelength of 1064.3 nm and a pulse length of about 10 picoseconds at a 80 MHz repetition rate and 1.2 W average power. The repetition rate of both lasers is synchronized by a two-stage electronic and optical feedback system.$^{21}$

The phase shaping setup consists of a zero-dispersion reflective 4-f arrangement,$^{22}$ using a blazed grating with 830 lines/mm and a cylindrical mirror with f=597mm. The spatial light modulator (SLM) that is used is a 640-pixel dual-mask linear liquid crystal array (CRi Inc.), which is operated in phase-only mode. The pixel pitch is 100 μm with an interpixel gap of 2 μm, resulting in a frequency spread of 97 GHz/pixel (0.2 nm/pixel) on the SLM.

The excitation objective is a 0.65 numerical aperture (NA) infra-red air objective (Nachet Plan Fluor) and the collection objective is a 0.75 NA air objective (Zeiss Plan-Neofluar 40x). The average power incident on the sample is 30 mW of pump/probe and 100 mW of Stokes. A chopper wheel is used to modulate the Stokes beam at a frequency of 327 Hz. The CARS signal is separated from the pump/probe and Stokes beams by a set of filters and is collected using a silicon photodiode. The signal from the photodiode is amplified using a transimpedance amplifier and filtered using a low-pass filter with a cut-off frequency of 3.37 kHz. The amplified and filtered signal is subsequently sent to a lock-in amplifier (EG&G Princeton Applied Research Model 5210) for detection. A 2-dimensional piezoscanner is used for sample scanning.

4.2 Results

The sample used in the experimental optimization consists of 20 μm diameter poly(methyl methacrylate) (PMMA) microspheres, which are dried on a glass coverslide (170 μm thickness) and are subsequently covered with a thin layer (±200 μm) of water and another coverslide.

The phase profile is optimized on 60 points spread evenly over 60 nm. The phase profile is interpolated to obtain the phase values for the individual pixels of the SLM. We once again use CMA-ES for the optimization and we choose 150 generations with 5 parents and a population size of 10 per generation. For every trial phase profile $\Phi(\omega)$, the difference in CARS signal generated by a pulse with phase $\Phi(\omega)$ and by a pulse with phase $-\Phi(\omega)$ is measured for both the PMMA microspheres and the non-resonant background (water). The fitness is defined as the difference intensity obtained from the PMMA minus twice the difference intensity from the non-resonant background. We subtract the background in the fitness to suppress unwanted effects caused by, for example, slight errors in the calibration or crosstalk in the SLM.

After the optimal phase profile $\Phi_{opt}(\omega)$ has been determined, this phase profile is used for imaging (see figure 5). An image is made with a pulse with phase $\Phi_{opt}(\omega)$ (figure 5(a)) and another image with phase $-\Phi_{opt}(\omega)$ (figure 5(b)). The difference between these images (figure 5(c)) gives a non-resonant background free image with improved contrast. An image with an unshaped pulse is recorded for comparison (figure 5(d)).

From figure 5(d) it can be seen that the contrast for an unshaped pulse is low due to the large non-resonant background. The amount of CARS signal from the PMMA microspheres is similar to that of the non-resonant background and the microspheres are visible largely due to the diffraction at the edges of the spheres. When looking at the difference image (figure 5(c)) it can be seen that the non-resonant background has been suppressed and that the contrast has been strongly enhanced, making the PMMA microspheres clearly visible. The contrast ratio is in the order of 10:1.
5. CONCLUSIONS

We have shown the retrieval of the vibrational phase information from a spontaneous Raman scattering spectrum, using an evolutionary algorithm. By using this vibrational phase information, the complex vibrational response can be reconstructed, which can be used to numerically calculate the CARS response for an arbitrary set of input pulses. We have shown numerical optimization of the CARS signal by use of an evolutionary algorithm. The optimization is feasible for imaging of a single compound as well as selective excitation of a compound of interest in a mixture of several resonant compounds. The obtainable contrast ratio for a test case of PMMA in a mixture of five resonant compounds is predicted to be 2000:1, where the contrast ratio depends on the uniqueness of the complex vibrational response of the compound of interest compared to that of the surrounding molecules. We have also shown a study of the effects of phase noise and homodyne mixing on our optimization approach. Furthermore we have shown the feasibility of our optimization approach under experimental conditions with the optimization of the CARS signal from PMMA microspheres, resulting in high contrast non-resonant background free imaging capability.

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REFERENCES


