

Gated heterodyne coherent anti-Stokes Raman scattering for high-contrast molecule-specific imaging

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We demonstrate heterodyne coherent anti-Stokes Raman scattering (CARS) with phase-coherent pulses of different tunable frequencies. With proper timing of phase-locked pulses from three noncollinear optical parametric amplifiers this technique provides efficient background suppression and therefore improves the detection contrast of molecule-specific imaging.

1 Introduction

CARS microscopy is a powerful method for chemical imaging which uses molecular vibrations as a contrast mechanism [1]. A pump and a Stokes laser beam with center frequencies of ν_p and ν_s , respectively, are focused into the sample where the CARS signal is generated at $2\nu_p - \nu_s$. Resonant enhancement of the CARS signal occurs if the frequency difference $\nu_p - \nu_s$ is tuned to a Raman resonance of the molecules under investigation. CARS microscopy is highly suitable to inspect unstained live cells with high spatial resolution.

One of the main problems associated with CARS is the limitation of the detection sensitivity due to resonant and nonresonant background signals. Thus, several techniques have been proposed to increase the signal-to-background ratio in CARS microscopy [2].

In the study reported here, we demonstrate a novel detection scheme for CARS which utilizes the fixed phase relationship between pump, Stokes and CARS fields. The use of an additional local oscillator (LO) field with a fixed phase relation to Stokes and pump fields allows heterodyne detection of the CARS signal. Combined with proper pulse timing this technique, named gated heterodyne CARS (GH-CARS), provides a significantly higher contrast than conventional CARS microscopy.

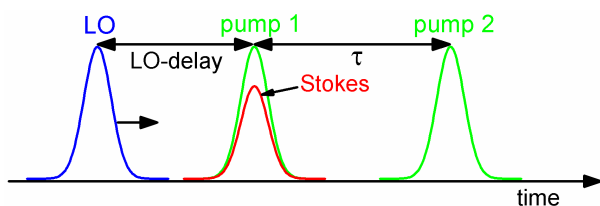


Fig. 1 Pulse timing for gated heterodyne CARS (schematic).

A pump and a Stokes pulse are overlapped in space and time (Fig. 1) in order to create a coherent

Raman excitation. Inelastic scattering of the pump signal yields the CARS signal. This scattering can originate from the exciting pump pulse or a second delayed one (pump 2 in Fig. 1), as long as the macroscopic polarisation has not decayed. A second CARS field is generated at the time of pump 2. Temporal overlap of the LO and the second CARS signal leads to an interference and thus yields a considerable heterodyne signal. For more rapidly dephasing materials, a vanishing heterodyne signal is expected since no second CARS signal is generated. Therefore, different dephasing times of sample and solvent can be exploited to improve the signal-to-background ratio by usage of the GH-CARS method.

2 Experimental setup

To demonstrate GH-CARS, we use three noncollinear optical parametric amplifiers (NOPAs) seeded by a common white-light continuum generated in a sapphire plate [3]. A regenerative Ti:sapphire amplifier system (150 fs pulses at 775 nm, 1 kHz repetition rate) serves as NOPA pump source. The wavelengths of the three output beams can be chosen independently throughout the visible.

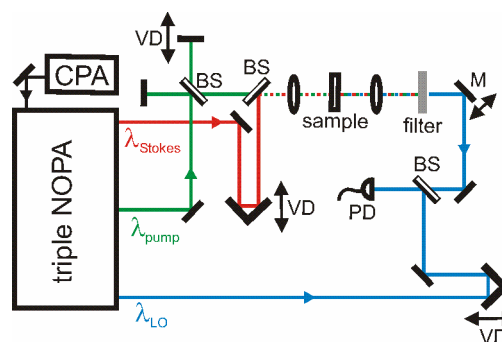


Fig. 2 Experimental setup for gated heterodyne CARS.

Two of the NOPAs serve as pump and Stokes pulse sources. To demonstrate the feasibility of

GH-CARS they are collinearly superimposed and focused into a liquid sample contained in a 1 mm cuvette with a 75 mm lens (Fig. 2). Their temporal overlap is adjusted by a variable delay line. The generated CARS signal is collimated with a second lens and separated from the excitation beams by an appropriate bandpass filter. A Michelson interferometer arrangement in the pump beam allows the generation of a second time-delayed pump pulse (pump 2 in Fig. 1) with adjustable delay τ .

The third output beam of the triple NOPA is tuned to the center frequency of the CARS signal and used as LO. The CARS and LO pulses are combined via a beamsplitter. If they overlap in time they can interfere constructively or destructively depending on their mutual phase difference. A four step phase-shifting interferometry algorithm [4] is used to extract the interference amplitude A which represents the amplitude of the CARS field. This amplitude will be discussed as heterodyne signal below. The value of A is inferred from quadruplets of intensities ($I_1 \dots I_4$) which are taken with a Si photodiode for CARS signal phase shifts of 0 , $\lambda/4$, $2\lambda/4$, and $3\lambda/4$ using the relation

$$A = ((I_1 - I_3)^2 + (I_2 - I_4)^2)^{1/2} \quad (1).$$

The phase shifts are accomplished by the piezo-driven mirror M (Fig. 2).

3 Experimental results

We tune the NOPAs to 565 nm (pump) and 649 nm (Stokes), respectively in order to excite the C-D stretching vibration of deuterated benzene (C_6D_6) at 2293 cm^{-1} . The third NOPA serving as LO is tuned to 500 nm, i.e. the resulting CARS wavelength. Heavy water (D_2O) is used as second sample representing a possible solvent which would contribute a strong background signal in the case of conventional CARS microscopy.

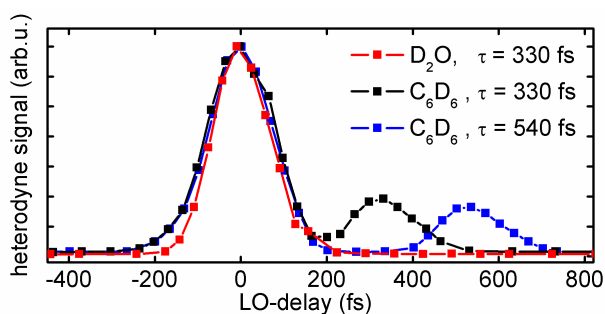


Fig. 3 Gated heterodyne CARS measurement.

Fig. 3 shows the heterodyne signal of C_6D_6 and D_2O versus the LO-delay. The delay τ between the two pump pulses is set to either 330 fs or 540 fs. A strong heterodyne signal is found for both samples if pump 1, Stokes and LO pulse overlap in time (LO-delay equals 0 fs). With increasing LO-delay

the signal for C_6D_6 first decreases but then rises again to reach a second maximum at the temporal position of pump 2 (330 or 540 fs, respectively). This clearly indicates a considerable remaining coherence due to the sufficiently long dephasing time of C_6D_6 . In contrast to that, D_2O shows no second maximum due to its extremely fast dephasing behavior. Normalizing the maximum amplitudes of the heterodyne signals from C_6D_6 and D_2O at 0 fs LO-delay, their ratio is >100 for a LO-delay of 330 fs ($\tau = 330$ fs).

For the experiments described so far, pulse energies on the order of 100 nJ were found to be optimum. This is a good compromise for a strong CARS signal and negligible higher order contributions to the measurement.

The setup can be converted to a GH-CARS scanning microscope by replacing the focusing and collimating lens by microscope objectives (40X, NA = 0.75) and mounting the sample on a piezo-driven scanner. Due to the much smaller focus the pulse energies have to be reduced to just a few nJ. Even with such low energies a well detectable CARS signal is generated. The use of a sufficiently sensitive detector also allows the heterodyne detection of the second CARS signal.

In a first trial run we have already observed microscopic images of polystyrene beads in water. These show the desired suppression of the water background signal despite the overlapping Raman bands of polystyrene and water. The results will be published elsewhere in detail.

4 Conclusion

In conclusion, we have exploited the mutual phase coherence of three NOPAs seeded with a common supercontinuum to accomplish gated heterodyne CARS. It was shown that appropriately delayed probing and heterodyning significantly reduces solvent background signals and thus can clearly improve the detection contrast of molecule-specific imaging.

References

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