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Ex-CARS: exotic configuration for coherent anti-Stokes Raman scattering microspectroscopy utilizing two laser sources

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Abstract

We propose and experimentally demonstrate a new coherent anti-Stokes Raman scattering setting, which relies on a coherent excitation of Raman vibration using a broadband ultrashort laser pulse and signal read-out using a conventional continuous wave laser radiation. Such an exotic arrangement does not require any synchronization of two laser sources and can be used for direct comparison of amplitudes of nonlinear and spontaneous Raman signals.

Ex-CARS in time- (top panel) and frequency- (bottom panel) domain.

Keywords

Raman; CARS; spectroscopy; microscopy; ultrafast optics

1. Introduction

Nonlinear Raman spectroscopy, based on coherent anti-Stokes Raman scattering and stimulated Raman scattering spectroscopies, are emerging techniques of biomedical optical imaging [1-5]. Under proper excitation conditions, those powerful spectroscopic tools provide a much stronger signal than conventional spontaneous Raman spectroscopy [6-8]. The advantage of Raman spectroscopy, as a non-invasive, chemically specific optical

imaging method, often fades when samples of a relatively large area have to be evaluated. Examples where problems can occur include imaging tissue samples during biopsy, or when there is a very large number of samples continuously flowing through the interrogation volume, as it occurs in flow cytometry, or when fast dynamical processes have to be evaluated in space and time [9]. In many of the above mentioned scenarios, the data acquisition speed becomes essential, and, thus, nonlinear optical methods based on coherent Raman scattering become increasingly important.

Coherent anti-Stokes Raman spectroscopy is a four-wave mixing process (see Figure 1(a)), which involves a coherent excitation of a vibrational oscillation of a molecule using a pair of laser pulses at frequencies ω_1 and ω_2 , which are separated in frequency by a frequency vibration, Ω , of this oscillation, such that $\Omega = \omega_1 - \omega_2$. A third pulse of a frequency ω_3 excites a molecule on the excited state and the generated signal at frequency, $\omega_{\text{CARS}} = \omega_3 + \Omega$, is blue-shifted with respect to the frequency of the third pulse, which avoids interference of the signal with fluorescent background, which occurs often in biological systems.

The obvious complexity of the experimental setup comes from the necessity of generating three different synchronized colours of light and combining them all at the same spatial location to produce the desired signal. The power of spontaneous Raman spectroscopy is in its ability to display the entire vibrational spectrum of the sample under study, which allows the use of sophisticated algorithms based on principal component analysis to extract quantitative information about the sample's chemical content [10]. To achieve the full vibrational spectrum, one has to tune the frequency of the second pulse with respect to the frequency of the first pulse. One solution to the above problem is based on a simultaneous excitation of all vibrational frequencies by using a broadband pulse. This can be achieved by employing a narrow band first pulse and a broadband second pulse [11, 12]. Alternatively, one can utilize a very short, very broadband pulse, which simultaneously serves as the first and the second pulse in such a way, that different spectral components of this pulse stimulates a coherent excitation of vibrational levels of a given molecular system [13-15]. The former approach can be straightforwardly accomplished with a white-light continuum source, and is now widely used to collect broadband CARS spectra in a real time. The second approach requires ultrashort (10 fs or shorter) laser pulses to achieve a broadband excitation ($\sim 1500 \text{ cm}^{-1}$) of molecular vibrations. In both cases, a third, probe pulse is required to interrogate the vibrational population of a molecule. If all the pulses arrive simultaneously, it leads to a non-resonant background in CARS spectroscopy, which is often considered a significant drawback of CARS spectroscopy. There are several ways of reducing this background, and using the proper pulse sequence is one of them. In particular, in one of the proposed approaches, Hybrid-CARS [16, 17], the probe pulse is delayed in time with respect to the pump and Stokes pulses or overlaps only for a brief time with the excitation pulses. This leads to a reduced non-resonant background. However, this scheme still requires a pulse which is temporarily synchronized with the pulses inducing coherent vibrational excitation.

In this report, we introduce a novel scheme for CARS microspectroscopy which utilizes a rather exotic laser combination. We tentatively call this scheme an ExoticCARS, or ex-CARS. We employ a short, ultrabroadband radiation from a Ti : sapphire oscillator to excite the vibration coherence (pump and Stokes pulses), and make use of a continuous wave (cw) laser to probe the induced coherence (see Figure 1(b)). In such a scheme, the cw radiation can be treated as an ultimate limit of a very long laser pulse. A typical life-time of a molecule in solution in the excited vibrational state is on the order of several picoseconds, effectively limiting the time the cw radiation will be utilized in producing a CARS signal. During the time between two consecutive femtosecond laser pulses, no CARS signal will be generated; however, both the Stokes and anti-Stokes spontaneous Raman signal will be

emitted. One can minimize the temporal spacing between adjacent excitation pulses either by using a GHz-rate oscillator [18] or by using a series of beam-splitter, or pulse-shaping apparatus to arrange for a multiple pulse sequence [19, 20]. By doing so, all the energy of the probe radiation can be resourcefully utilized for generation of the CARS signal. The major advantage of the described above scheme is that there is no need for the generation and careful temporal alignment of the probe-pulse beams in CARS spectroscopy, while a straightforward comparison of the amplitude of the CARS and spontaneous Raman signals is readily available as a result of those measurements.

2. Experimental

2.1 Experimental set-up

A schematic diagram of the experimental set-up used for collecting Ex-CARS spectra is shown in Figure 2. In brief, we used a slightly modified commercial Ti : sapphire laser (Tsunami, Newport-Spectra-Physics, Inc.), in which the laser was tuned to the regime, which promotes broadband mode-locking. The output spectrum, being highly asymmetric, and shifted towards 850-nm, where the intracavity dispersion was minimized, was capable of supporting transform-limited laser pulses as short as 9 fs FWHM. As much as 450 mW of average power was generated in this mode. This radiation was further compressed using a series of reflection from the negatively chirped mirrors (CVI Laser, Inc.) and was characterized using a fast scanning interferometric autocorrelator (APE GmbH) and the second-harmonic frequency resolved optical gating (Grenouille, Swamp Optics, Inc.). No attempt was made to compensate for higher order dispersion and to achieve pulse duration shorter than 12 fs. The compressed pulses were collimated using all-reflective Ag mirrors and directed to a reflective, gold-coated microscope objective (36x, NA = 0.52, Newport, Inc.).

To provide a probe excitation, a cw diode-pumped, intracavity-doubled Nd : YVO₄ was used. Its radiation at 532 nm was spectrally filtered from a residual 1064 nm and 808 nm radiation, collimated, collinearly combined with the short pulse near-IR radiation and used for all the described below measurements.

For most of the described experiments, the power of the near-IR beam was set to be several mW at the sample's position and was limited by the parasitic nonlinear optical effects due to a very high peak power of the laser beam. The power of the visible beam (probe beam) was about 10 mW and was limited only by the available laser source.

Several samples were used for our studies. A thin slice of TiO₂ (rutile) of unknown orientation was used as a solid-state target. A microscopic slide with a cavity, filled with a liquid sample and covered with 150-micron-thick cover slip was used to study CARS and Raman signals in several liquids.

The generated CARS signal was collected in the transmission geometry using a high numerical aperture objective (N.A. = 0.55, 50x, Mitutoyo, Inc.) and directed towards a spectrometer with the attached TE-cooled CCD camera (Andor Technology, Inc.). A Raman notch filter was used to block the 532 nm radiation, and a BG-39 filter (Schott Glass, Inc.) or its analogues was used to block the near-IR radiation. Due to some residual transmission of the broadband near-IR radiation, it was not possible to perform accurate measurements in the Stokes part of the spectrum (i.e. coherent Stokes Raman scattering, or CSRS), and all the measurements were done in the anti-Stokes part of the spectrum.

2.2 Experimental results

To collect an ex-CARS spectrum, we first blocked the short-pulse radiation and collected spontaneous Raman spectrum in the anti-Stokes spectral regions (see Figure 3, bottom panel). Then we unblocked the pump/Stokes beam and collected the same spectrum again using the same data acquisition time, which was of the order of several seconds. To achieve a better signal-to-noise ratio, several successive acquisitions were performed and averaged. The ex-CARS signal was calculated as a difference between the spectrum accumulated over the time, when pump/Stokes were “on”, and the spectrum collected over the time, when no femtosecond pulse was applied to the sample. A typical ex-CARS spectrum is shown in Figure 3 (top panel), which shows that the amplitude of this signal is just over 2% of the spontaneous Raman signal. Both spectra appeared to be in a good agreement with each other and with our earlier results on Raman spectra of rutile phase of TiO_2 [21].

To ensure that the signal is, indeed, coming from the nonlinear optical interaction of femtosecond and cw radiation, several tests were performed. Firstly, we slightly misaligned two beams in such a way that they didn't overlap in the focal spot of a microscope objective. The ex-CARS signal disappeared completely. However, the same effect could be also achieved, if a powerful near-IR radiation caused the distortion of the probe beam propagation. Thus, we interrupted the mode-locking and used Ti : sapphire in a cw mode. No ex-CARS signal was observed for any excitation wavelength in the tuning range of the Ti : sapphire oscillator. Clearly, linear thermal effects were not affecting our measurements. We also tried to change the chirp of our femtosecond pulse. In this way, different frequency components of a broadband pulse arrive at different times to the sample, causing no coherent excitation of vibrational levels. That led to a significant degradation of the ex-CARS signal.

We also measured the power dependence of the ex-CARS signal on the incident power of cw and femtosecond radiation. The result of those measurements is shown in Figure 4, which proves the expected linear power dependence of the ex-CARS signal on the probe (532 nm, cw) radiation and quadratic dependence on the near-IR power (pump and Stokes pulses).

Finally, we changed the sample to one that exhibits substantial Raman lines in the frequency range $>1200\text{ cm}^{-1}$. Due to the limits of our present experimental set-up, which relies on the use of about 1000 cm^{-1} bandwidth of the Ti : sapphire oscillator, excitation of those lines becomes very inefficient. Figure 5 shows, for comparison, spontaneous Raman spectrum (bottom panel) of toluene and its ex-CARS spectrum (top panel). The disappearance of high frequency vibrational lines from the ex-CARS spectrum also supports our hypothesis that the observable spectrum is, indeed, due to nonlinear optical interaction of cw (532 nm) and femtosecond radiation resulting in a non-resonant background-free CARS spectrum.

3. Discussion

The observed ex-CARS spectra do not have a substantial non-resonant background, which is typically a problem in most CARS experiments. This observation can be explained in terms of a so-called “hybrid-CARS”, since the non-resonant background originates only during a short period of time, when all pulses (pump, Stokes, and probe) are present simultaneously. Given the very short pulse duration of our near-IR excitation, providing both the pump and the Stokes pulse for coherent Raman excitation, and a relatively long life-time of molecules in the excited vibrational state, the non-resonant background is greatly reduced. However, a significant background is present as a result of a spontaneous Raman scattering from the anti-Stokes part of the spectrum. Part of the reason is that the efficiency of ex-CARS signal generation is very low, due to a long time delay between two adjacent excitation pulses (see Figure 1(b)). An obvious way to improve on efficiency is to increase the repetition rate of

excitation pulses to 10–100 GHz, which is possible by changing the laser design and using a multiple-pulse excitation scheme. This way, the efficiency of ex-CARS signal can be increased by 2–3 orders of magnitude. Using a higher numerical aperture objective will lead to a higher incident pulse intensity, which will result in a substantial improvement of this nonlinear process (Figure 4).

4. Conclusion

We proposed and experimentally demonstrated a new arrangement for coherent Raman microspectroscopy. It utilizes ultrashort (<10 fs) laser pulses for coherent excitation of vibrational levels and continuous wave radiation to probe this coherent population transfer. This is the first, to our knowledge, nonlinear optical experiment which combines the use of such dramatically distinct and independent laser sources.

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Biography

Vladislav V. Yakovlev is a professor of physics at the University of Wisconsin, Milwaukee. He received his B.S. Degree (Physics) and Ph. D. (Physics, Quantum Electronics) in 1987 and 1990, respectively, from Moscow State University, Moscow, USSR. He worked as a postdoctoral research and assistant project scientist in the Department of Chemistry and Biochemistry, University of California, San Diego, from 1992 till 1998. In 1998 he joined the Department of Physics, University of Wisconsin, Milwaukee, where he was promoted to the rank of Full Professor in 2007. His major research interest is in applying advanced optical spectroscopy to study biomolecular systems both *in vitro* and *in vivo*.

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Corey Harbert received his B. S. in Electrical Engineering from St. Mary's University, San Antonio, TX, in 2010. He works for TASC, Inc. in San Antonio, TX, and is currently investigating the causes of traumatic brain injuries using finite element multi-physics simulations.

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Figure 1. (online color at: www.biophotonics-journal.org) (a) Energy diagram of CARS process, (b) Time- and frequency-presentation of the ex-CARS arrangement. Intensities of all the interacting waves are not shown up to scale.

Figure 2. (online color at: www.biophotonics-journal.org) Schematic diagram of the experimental set-up. CM1(2) are chirped mirrors; OBJR is reflective objective to avoid chromatic aberrations, and OBJ2 is a collecting objective; F1 is a near-IR-blocking glass filter, NF is a Raman notch filter for 532 nm.

Figure 3.
(online color at: www.biophotonics-journal.org) Bottom panel – Raman spectrum of titania (rutile) in the anti-Stokes part of the spectrum, top panel – ex-CARS spectrum of the same sample. Raman frequencies are inverted for convenience.

Figure 4.

(online color at: www.biophotonics-journal.org) Logarithmic plot of the power dependence of the ex-CARS signal on the near-IR power (filled red triangles; dashed red line shows a theoretical quadratic dependence) and the probe power (filled green circles; dashed green line shows a theoretical linear dependence).

Figure 5.
(online color at: www.biophotonics-journal.org) Bottom panel – Raman spectrum of toluene in the anti-Stokes part of the spectrum, top panel – ex-CARS spectrum of the same sample. Raman frequencies are inverted for convenience.