PULSE SHAPING STRATEGIES FOR NONLINEAR INTERFEROMETRIC VIBRATIONAL IMAGING OPTIMIZED FOR BIOMOLECULAR IMAGING

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ABSTRACT

Nonlinear Interferometric Vibrational Imaging (NIVI) measures the temporal cross-correlation of anti-Stokes radiation from Coherent anti-Stokes Raman Scattering (CARS) processes to achieve increased sensitivity, stray light rejection, and nonresonant background rejection. Because the intensity of CARS radiation is proportional to the square of the molecular density of a target resonance, it is critical to maximize the recoverable signal for a given illumination level. Especially if one desires to measure several resonances, there can be a sensitivity as well as a speed advantage to measuring them simultaneously rather than serially. We discuss the methods of sample excitation that NIVI allows and their potential sensitivity advantages, as well as present experimental results demonstrating Raman signal recovery using these pulse sequences.

1. INTRODUCTION

Coherent Anti-Stokes Raman Scattering (CARS) microscopy [1, 2, 3, 4, 5] is a molecular imaging technique that has been developed to probe biological samples. It overcomes the disadvantage of most optical molecular imaging methods which frequently require dyes or contrast agents be added to the tissue that alter or damage the tissues being studied. The features probed by CARS are the Raman-active resonances in the tissue, which correspond to vibrational or rotational excitations. CARS typically utilizes picosecond-length pulses on the order of the lifetime of the resonance to maximize the available signal. We propose methods of using very broadband radiation to probe Raman spectra. Such methods which have typically used high-peak-power femtosecond radiation have suffered from a significant nonresonant background problem. We show that by using interferometry, the nonresonant background can be rejected. Furthermore we propose pulse sequences that will allow the Raman spectrum to be recovered to high resolution. Ultimately, ultrabroadband sources may allow great agility for probing vibrational resonances of various frequencies.

Typical CARS processes are stimulated by two pulses, a pump pulse of frequency ω_1, and a Stokes pulse of frequency ω_2, which are separated by the vibrational frequency of interest $\Omega = \omega_1 - \omega_2$. When a Raman-active resonance at frequency Ω is present, an anti-Stokes pulse at frequency $2\omega_1 - \omega_2$ is produced. Because in the past the bandwidth of pulsed sources tended to be much less than the Raman frequencies of interest, two distinct pulses needed to be generated, often the pump pulse acting as the energy source of the Stokes pulse, to ensure they were synchronized. Recently, pulsed sources [6, 7, 8] have become available with bandwidth that span thousands of wave numbers (cm^{-1}), so that a single source can produce a broad band that spans both the pump and Stokes frequencies. To take advantage of this, schemes have been developed that use pulse shapers to stimulate a particular Raman resonance [9, 10, 11, 12, 13, 14, 15]. However, because the pump is now broadband, the anti-Stokes pulse will be broadband also. If multiple resonances are stimulated, their anti-Stokes spectra will overlap, making them difficult to separate. It is desirable to stimulate several resonances simultaneously to differentiate similar molecular species.

2. MODEL OF COHERENT ANTI-STOKES RAMAN SCATTERING

To separate the signals due to different Raman resonances, we proposed [16] using interferometry to measure both the amplitude and phase of the anti-Stokes radiation. When a weak interaction is assumed, the anti-Stokes electric field $\tilde{E}_o(\omega)$ is a linear function of the Raman spectrum $\chi^{(3)}(\Omega)$:

$$P^{(3)}(\Omega) = \chi^{(3)}(\Omega) \int_0^\infty \tilde{E}_i(\omega + \Omega)\tilde{E}_i(\omega)^* \, d\omega \qquad (1)$$

$$\tilde{E}_o(\omega) = \int_0^\omega \tilde{E}_i(\omega - \Omega)P^{(3)}(\Omega) \, d\Omega \qquad (2)$$

Therefore one can use linear estimation to find $\chi^{(3)}(\Omega)$. Note, however, being a nonlinear optical process, it is not a linear function of $\tilde{E}_i(\omega)$. With broadband pulses and interferometry, there is a great deal of flexibility in the choice of $\tilde{E}_i(\omega)$ to reconstruct specific features of $\chi^{(3)}(\Omega)$. By designing pulses and pulse sequences, specific regions of the Raman spectrum can be stimulated and sampled. Reshaping an optical pulse may be a more attractive option than retuning a laser or amplifier becaues it can be done passively outside the oscillator or amplfier, and because it is much more versatile than tuning alone.

To understand what kind of pulses will be useful for NIVI, we analyze Eqs. 1 and 2. In the time domain, these equations can be restated as

$$P^{(3)}(t) = \int\limits_0^\infty \chi^{(3)}(\tau)\,|E_i(t-\tau)|^2\ d\tau \qquad (3)$$

$$E_o(t) = P^{(3)}(t)E_i(t) \qquad (4)$$

where $E_i(t)$ is the inverse Fourier transform of $\tilde{E}_i(\omega)$, $E_o(t)$ is the inverse Fourier transform of $\tilde{E}_o(\omega)$, $P^{(3)}(t)$ is the inverse Fourier transform of $P^{(3)}(\Omega)$, and $\chi^{(3)}(t)$ is the inverse Fourier transform of $\chi^{(3)}(\Omega)$. Eq. 3 indicates that the nonlinear polarization of the medium is given by the causal convolution of the time-domain Raman response with the instantaneous intensity of the electric field. This convolution indicates that only a Raman frequency will be stimulated that has a corresponding oscillation in the intensity of the excitation. Therefore the Fourier transform of $|E_i(t)|^2$ is a beat frequency spectrum that determines the possible Raman frequencies that can be excited by the pulse. If two narrowband pulses are used, the beat frequency spectrum consists of one frequency only. However, with broadband light we can shape the pulse to excite a narrowband or a broadband of Raman frequencies.

Eq. 4 is the mixing in the time domain of the polarization of the medium and the incoming wave. Therefore the incoming wave also forms a "window" over which the polarization of the medium is observable. If this window is short in time, the resolution with which one is able to separate Raman frequencies will be low due to the uncertainty relationship between time and frequency. Therefore it is necessary to have an illumination wave $E_i(t)$ that is long in duration to establish good Raman frequency resolution. However, if the window is too long in duration, the amount of anti-Stokes radiation (from Eq. 2) will decrease. It is best to have the pulse short enough so that multiple resonances remain excited at the same time so the pulse can interact and generate anti-Stokes from them simultaneously. For this reason, a pulse duration on the order of the resonance lifetime is desired.

Another consideration is that the anti-Stokes signal $\tilde{E}_o(\omega)$ will have to be separated from the illumination signal $\tilde{E}_i(\omega)$. Because the nonlinear process is weak, the rejection must be extremely good (at least one part in 10^4). While using nonlinear processes in microscopy it is usually desirable to focus tightly as possible to maxmimize the peak power as well as achieve the best resolution. However, the output radiation can not be distinguished spatially from the incoming radiation (*e.g.* by angle). It is also possible to use polarization to separate them but in highly scattering media such as most optical tissues the cross-polarization scattering will probably be far too great for this to be useful. The best apparent solution is to use the frequency band to separate the anti-Stokes radiation from the illumination. Therefore a useful pulse will place as much anti-Stokes signal outside the illumination bandwidth as possible.

A further limitation is that while most broadband pulse sources have high power and bandwidth, the power spectral density (power per unit bandwidth) is low. For example, if one filters the broadband pulse for two frequencies to act as pump and Stokes frequencies, the excitation power will be very low. Therefore it is desirable to fully utilize the power spectral bandwidth of the pump source when shaping the pulse.

Finally we note that the ultimate limits to available signal are because biological tissues are unable to tolerate arbitrarily high intensities. There are two basic limits to illumination intensity for samples: average power and instantaneous power. Excess average power typically causes heating and damage to tissues through one-photon absorption processes, and would be the same for any source with the same power spectral density (not just pulsed sources). An additional constraint because of the use of pulsed illumination is limitations in peak power. Excess peak power can damage because of plasma generation, multiphoton ionization, multiphoton absorption, and other nonlinear processes. Therefore it may be optimal to chirp, disperse, or otherwise stretch out in time illumination pulses to minimize peak power. It is conceivable that with very high individual pulse energy and low repetition rate that the generated Raman signal could be made quite large by dispersing the pulse to minimize peak power. This strategy can be made practical by one-shot detection methods that work well at low repetition rates such as spectral interferometry [17] and time-of-flight cross-correlation [18].

3. PULSE METHOD

We have designed a pulsing scheme that addresses these points and should be adaptable for many broadband sources. The essence of the method is that it produces a beat pattern that changes in time from a Raman frequency Ω_H to a lower frequency Ω_L. The beat pattern is created by interfering two pulses that have the same frequency spectrum from $\omega_0 - \Delta\omega/2$ to $\omega_0 + \Delta\omega/2$, but have a frequency increasing

with time. Both pulses have normal dispersion applied to them, but the one with less dispersion is delayed with respect to the other. The instantaneous frequency of the two pulses as a function of time is plotted in Fig. 2, with one pulse always having a higher frequency than the other at a given instant when both overlap. The two pulses are dispersed and delayed relative to each other such that when the pulse with the lower frequency starts, the difference in instantaneous frequency between the two is Ω_H, and when the upper frequency pulse ends, the difference in frequency between the two is Ω_L. The total time of overlap, T is set by the maximum lifetime of the resonances being probed, usually 1-10 ps in duration.
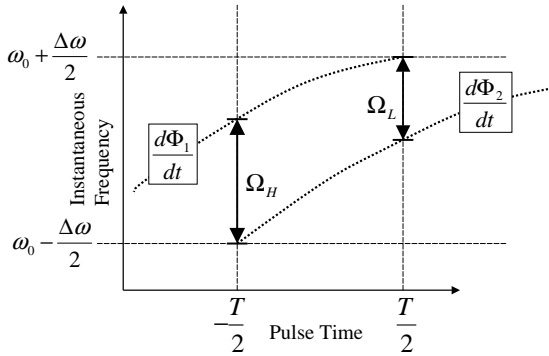

Fig. 1. Frequency vs. time plot of two overlapped pulses. Their frequency difference starts at Ω_H and decreases during the overlap region to Ω_L.

This pulse type has several advantages that make it attractive for NIVI. The entire bandwidth of the pulse can be utilized to stimulate the resonances, so it makes efficient use of the power spectral density. Furthermore, the high Raman frequencies are stimulated when the two pulse frequencies are low, while the low Raman frequencies are stimulated when the two pulse frequencies are high. This helps equalize the anti-Stokes frequencies that are generated and ensures they will end up outside the initial pulse spectrum. Finally the pulses are dispersed in time, so that the Raman frequencies can be sampled to high resolution, and the peak power is kept low and therefore the pulse energy can be high.

In practice, the method could utilize a setup such as Fig. 2 to generate this pulse combination from a single broadband laser with a bandwidth greater than the largest Raman frequency of interest. The pulse is first split by a dichroic beamsplitter into lower and upper frequency component pulses. The upper frequency component acts as a reference pulse $\tilde{R}(\omega)$ for demodulation of the anti-Stokes frequencies,

and so is of the same frequency band. The lower frequency components that comprise the illumination pulse will span a bandwidth $\omega_0 - \Delta\omega/2 < \omega < \omega_0 + \Delta\omega/2$. This pulse is further split into two identical pulses. Each pulse will have a different chirp profile (dispersion) applied to it, and then the two pulses will be recombined with a relative delay.
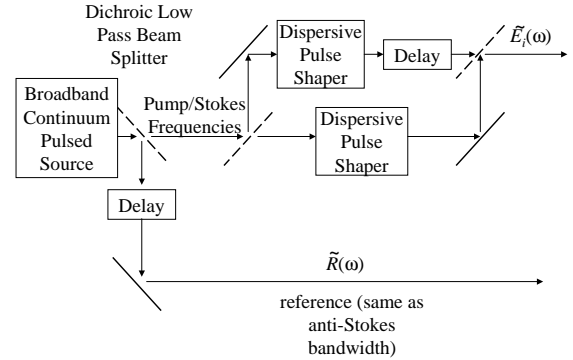

Fig. 2. Proposed pulse shaping mechanism for NIVI

One advantage of this scheme over that previously discussed [16] is that a perfectly linear chirp need not be applied to the pulses. With ultrabroadband pulses, it is much more difficult to achieve a linear chirp. With this method, it is not necessary to do so. This may make it much easier to design an instrument where the dispersions of the two pulses can be changed to dynamically change Ω_L and Ω_H to measure different frequency ranges with the same laser source. Dispersive components such as gratings and mirrors can be translated to achieve varying amounts of dispersion, but higher orders of dispersion are usually difficult to compensate. If the dispersion can be well characterized, then it may not be necessary to correct it well, and just account for it in the inversions of Eqs. 1 and 2.

We have recently demonstrated the interferometric measurement of CARS [19] and characterized the coherent generation of CARS and second-harmonic-generation with interferometry [20]. In addition, using interferometry, the contribution of nonresonant four-wave-mixing processes can be rejected by using interferometry [21]. Clearly the acquisition of temporal or phase information can greatly augment the decoding of anti-Stokes radiation. Like other imaging modalities such as ultrasound or especially Magnetic Resonance Imaging, the pulse shape can be manipulated to acquire specific features of the object or enhance resolution. With coherent acquisition of the anti-Stokes signal, NIVI can be expected to similarly benefit from a well chosen pulse shape. While the methods of optical signal processing are more restrictive than the radio frequency methods used by MRI and ultrasound, there remains many improvements

to be made to the coherent control and detection of Raman processes.

4. REFERENCES

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