Sub-diffraction Limited CARS Microscopy— a Theoretical Investigation

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Abstract: The possibility of obtaining sub-diffraction limited spatial resolution with label-free imaging, based on coherent anti-Stokes Raman (CARS) microscopy, is investigated numerically. Like STED, CARS emission is strongly suppressed by applying an additional light field.

OCIS codes: (350.5730) Resolution; (300.6230) Spectroscopy, coherent anti-Stokes Raman scattering

1. Introduction

Optical microscopy is the modern workhorse of biology, providing high contrast images, often in real time of biological processes at sub-cellular distance scales. Nevertheless, improved understanding of biological processes is difficult to achieve with diffraction-limited resolution as is offered by standard optical microscopy techniques. Sub-diffraction limited resolution has been achieved by two notable techniques: stimulated emission depletion (STED) microscopy [1], and stochastic optical reconstruction microscopy (STORM) [2], but a significant disadvantage of both approaches is that they require chemically attached fluorescent labels in the sample.

Label-free imaging modalities, such as CARS microscopy, have demonstrated high contrast levels and video rate image acquisition times [3]. However, current CARS implementations are unable to achieve sub-diffraction limited lateral resolution. In this paper we show via density matrix calculations that CARS emission can be suppressed controllably through the use of an additional saturation laser, in analogy to the working principle of STED microscopy, thus opening the possibility for label-free chemical imaging well below the diffraction limit.

2. Theoretical framework

The CARS process has been investigated using the density matrix approach for the 4-level system as shown in Fig. 1(a), where we calculate the dynamics of the matrix elements with the Liouville equation [4] $d\rho/dt = -\{\rho,H\}-\Gamma_{ij}\rho_{ij}$. The Hamiltonian, H (in dipole approximation), contains the standard set of laser fields (ω_p: pump laser frequency, ω_S: Stokes, ω_{Pr}: probe) that generate emission at the CARS frequency, ω_{CARS}. However, an additional laser field at ω_{sat}, is also applied in order to investigate whether CARS emission can be deliberately suppressed (saturated) at a reasonable intensity, I_{sat}. The diagonal elements of the density matrix, ρ, represent the normalized population density of each state, while the off-diagonal elements represent the coherence between states. Spontaneous emission on the dipole-allowed transitions $|3\rangle$ - $|1\rangle$ and $|3\rangle$ - $|2\rangle$ are described by relaxation rates Γ_{ij}, ($i > 1$) while decoherence rates between states i and j are represented by Γ_{ij} ($i \neq j$). The input laser pulses are represented by fields of the form $E(t) = A\exp(-((t-t_0)/\tau)^2)\exp(i\omega t)$, where A is the amplitude, t_0 the delay of the pulse, τ the pulse duration, and ω the carrier frequency. The detuning of laser fields from the transition (Bohr) frequencies are given by Δ_i.

We choose energy levels, detunings and pulse durations that are typical for CARS emission processes from molecules, and in view of the complexity of the system, we use numerical solutions (fourth-order Runga-Kutta algorithm with a fixed step size). The $|1\rangle$ - $|3\rangle$ transition frequency is set to 1000 THz, and the $|1\rangle$ - $|2\rangle$ frequency to 47 THz. Likewise, we chose a $|1\rangle$-$|4\rangle$ transition frequency of 97 THz. The detuning for the pump, Δ_p, and Stokes, Δ_S light fields are both taken as -300 THz to provide two-photon resonance with the $|1\rangle$ - $|2\rangle$ transition. The total lifetime of state $|3\rangle$ is of the order of nanoseconds, while the decoherence rates between states is of the order of ps. The laser pulses are 3 ps ($1/e^2$) in duration, while the simulations extend over 10 ps in steps of 2.5 as.

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3. Results

The calculations were first used to confirm the stability and accuracy of the numerical solution by obtaining well-known features. For example, as expected, CARS emission can be approximated as two processes. First, the population difference between the states $|1\rangle$ and $|2\rangle$ leads to a build-up of the coherence ρ_{12} via the pump and Stokes lasers. Second, such coherence induces an optical sideband on the probe laser frequency at the CARS frequency. The nature of this sequence also indicates how to suppress CARS emission: if the system is prepared with equal initial populations in the $|1\rangle$ and $|2\rangle$ states, this suppresses the build-up of coherence on that transition, thus suppressing CARS emission as well. In our approach it is the purpose of the additional laser at ω_{sat} to achieve this by populating state $|4\rangle$, which then increases the population in $|2\rangle$ via rapid, non-radiative mechanisms, before the arrival of the pump, Stokes, and probe pulses.

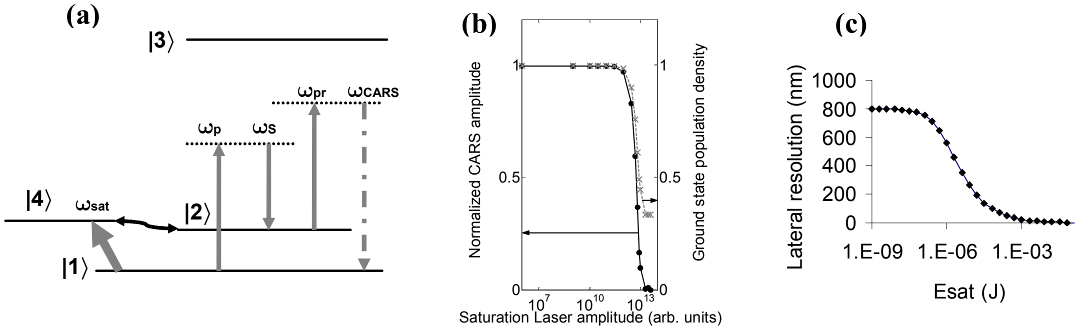

Fig. 1 Saturated CARS level system **(a)**. Normalized CARS emission amplitude and ground state population density as a function of saturation laser amplitude **(b)**. Spatial resolution as a function of saturation laser pulse energy for diffraction limit of 800 nm **(c)**.

Using this scenario, Fig. 1(b) shows the results of the CARS emission amplitude as a function of the additional laser intensity. One can see that CARS can indeed be suppressed to a large extent with sufficient intensity. Our calculations indicate that near the full saturation, the CARS signal is suppressed by 95% compared to the normal strength of CARS emission.

We found that the saturation intensity, defined as when the CARS signal drops to one half of its maximum value [6], corresponds to a reduction of the ground state population density of 0.75. This should be readily achievable with only moderate laser intensities. The laser pulse energy required to saturate an intracavity saturable absorber (see ref [5]) has been used to estimate the improvement in resolution below the diffraction limit, as shown in Fig. 1(c). Since a donut beam shape can be used to excite the $|1\rangle$-$|4\rangle$ transition, the CARS signal from a large portion of the focal volume can be substantially reduced, similar to the process employed in STED microscopy.

In conclusion, we have presented density matrix calculations that demonstrate how the CARS signal can be saturated by populating coupled vibrational levels. These results provide a concept for sub-diffraction limited CARS microscopy.

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