Complementarily balanced real-time two-color SRS

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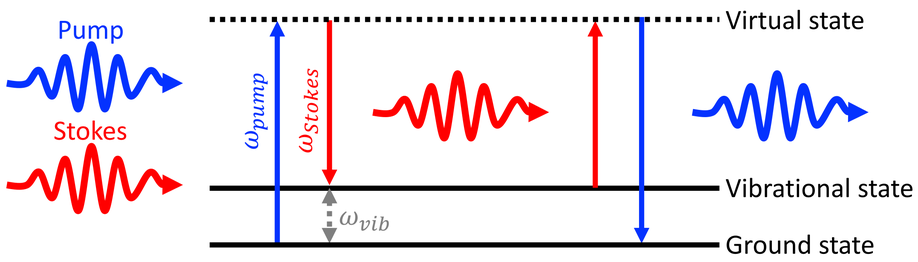
Abstract

The stimulated Raman scattering (SRS) utilizing inherent chemical contrast of sample is applicable to the label-free imaging with enhanced Raman signal intensity and chemical selectivity. It has been widely employed to investigate biomolecules and its dynamics in living organisms without perturbation of exogenous labels. However, to monitor biomolecular dynamics covering whole fingerprint region in biological targets, conventional SRS microscopy has limitation since it targets a single vibration mode of specific chemical structure based on a two-pulse scheme of pump and Stokes pulses. There are various approaches to achieve a broad- or multi-spectral investigation with SRS microscopy by changing the pulse characterization as well as time delay between two pulses. Here, we present a complementarily balanced real-time two-color SRS microscopy using two pairs (pump and Stokes) of chirped laser pulses.

Introduction

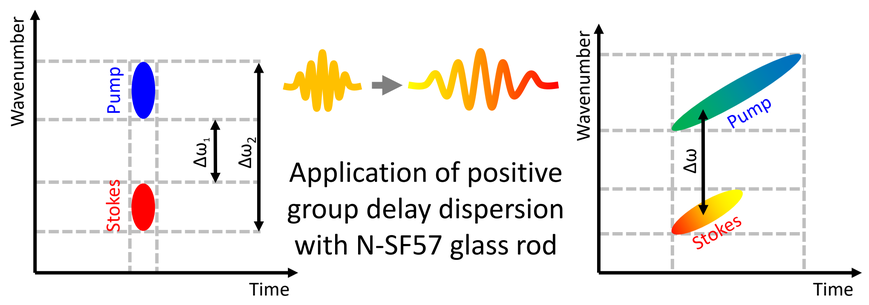
Stimulated Raman scattering



✓ $\Delta I_p \cong -\frac{\omega_p}{\omega_S} \cdot g_R \cdot I_{p,0} \cdot I_{S,0} \cdot \Delta z$ ✓ $\Delta I_s \cong g_R \cdot I_{p,0} \cdot I_{S,0} \cdot \Delta z$

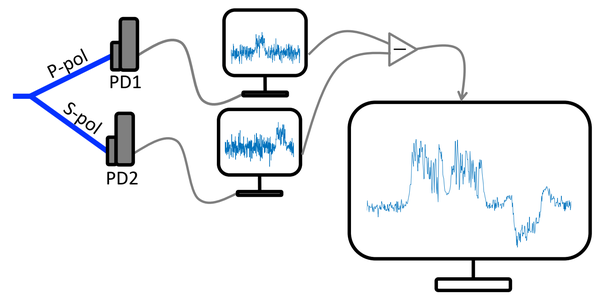
Stimulated Raman scattering as a third-order non-linear phenomenon provides vibrational contrast intrinsic to and characteristic of chemical species. It is quantum mechanically described as a two-photon stimulated process where one pump photon at ω_p is annihilated (stimulated Raman loss: SRL) and one Stokes photon at ω_S is created (stimulated Raman gain : SRG), while the Raman medium makes a transition form the initial electronic ground state to the final vibrationally excited state.

Spectral focusing



The instantaneous frequency $\omega(t)$ of a linearly chirped pulse with a carrier frequency ω_0 is given at time t by $\omega(t) = \omega_0 + 2bt$. The chirp leads to a prolongation of the Fourier limited pulse duration τ_0 by a stretching factor F to $\tau = F \cdot \tau_0$. The instantaneous spectral bandwidth is then given by $\Delta\omega = {}^{4ln2}/_{\tau}$, which is narrower than the Fourier transform limited spectral bandwidth by a factor of ${}^{1}/_{F}$. In order to spectrally focus the excitation energy, both pulses are required to have the same amount of chirp.

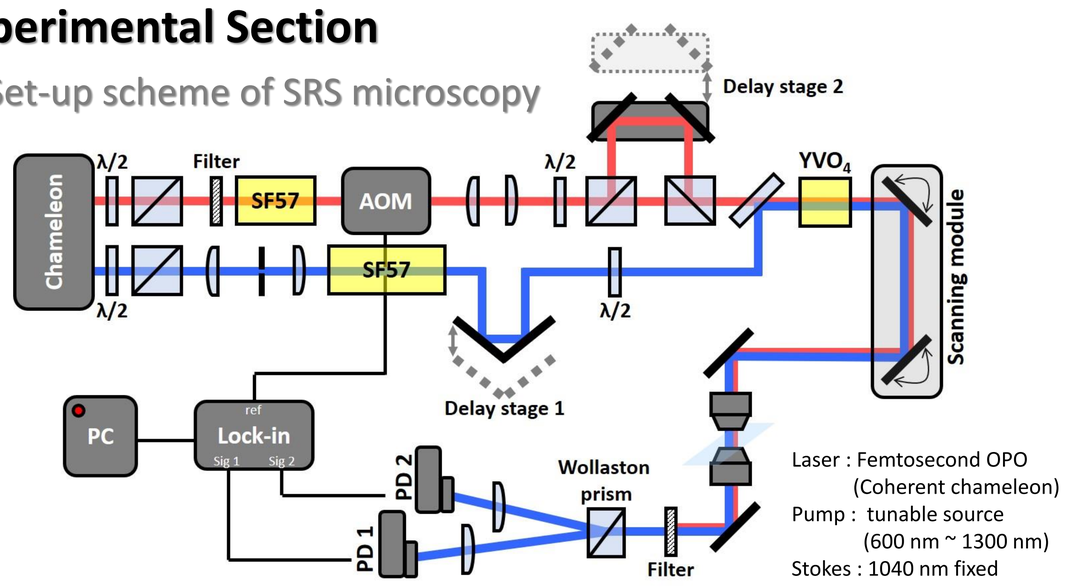
In-line balancing



Since both two pairs of pump and Stokes experience the same environmental condition at the sample position, a simple balancing procedure between SRS processes of two different vibrational modes can decrease background noise level without signal loss. Since two SRL signals are subtracted, they give opposite signs of signal.

Experimental Section

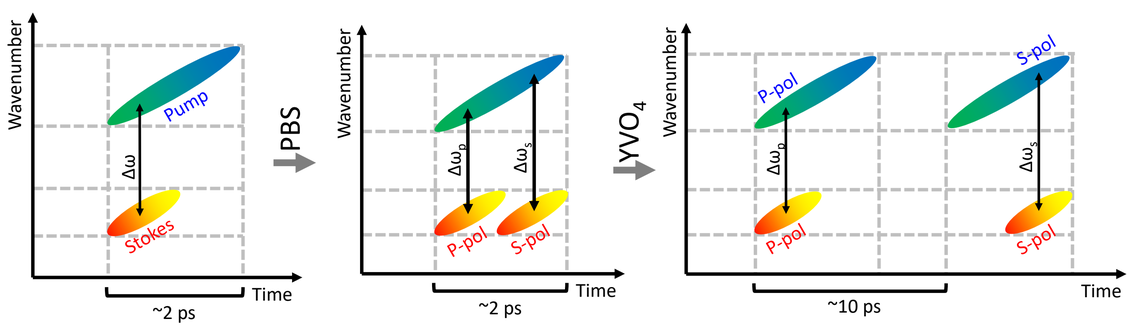
Set-up scheme of SRS microscopy



Laser : Femtosecond OPO (Coherent chameleon)
Pump : tunable source (600 nm ~ 1300 nm)
Stokes : 1040 nm fixed

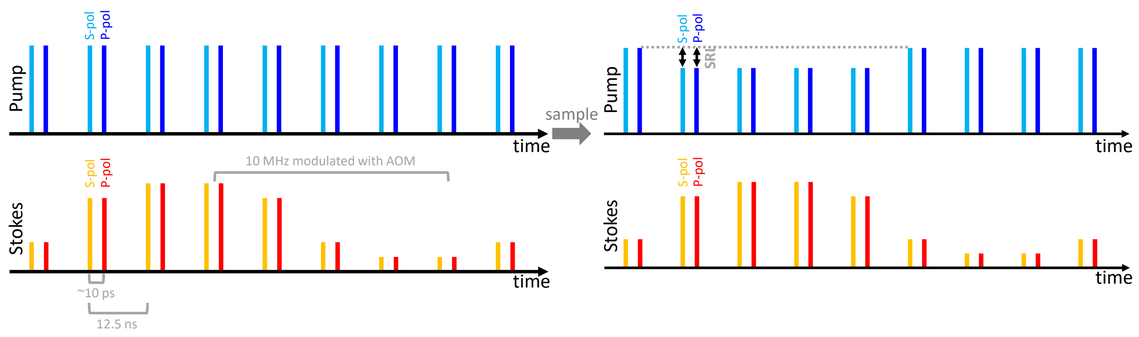
Both pulses are linearly chirped with glass rods (N-SF57) and changing time delay of two pulses enables to target different Raman shift within about 130 cm^{-1} of spectral window. To achieve two-color SRS imaging, resonating with different vibration modes simultaneously without any additional nonlinear process, both pulse-pairs are tailored to 45° polarization with half-wave plate. To avoid any optical interferences between orthogonally polarized pulses, YVO_4 crystal is installed which gives temporal delay about 10 ps.

Schematic drawing of two color SRS



Delay stage 2 containing polarizing beam splitter enables orthogonal polarized pulses to target two different Raman shifts in spectral window of 130 cm^{-1}. Birefringent material YVO4 generates additional time delay about 10 ps. Since two orthogonal pump and Stokes pulses are timely distant, no optical coherence can occur.

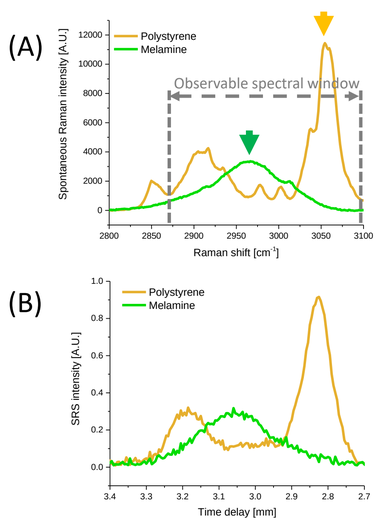
Pulse train of two color SRS



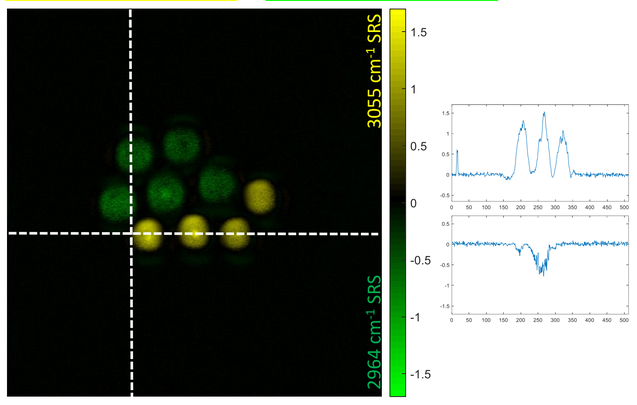
S-pol and p-pol pump pulses are targeted at different Raman shift. SRL signals are collected with separated photodiodes then transferred to different signal-in channel of lock in amplifier.

Result & Discussion

Polystyrene & melamine beads



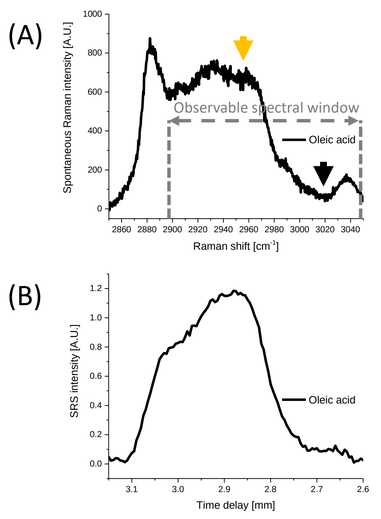
(C) 3055 cm^{-1} targeted – 2964 cm^{-1} targeted



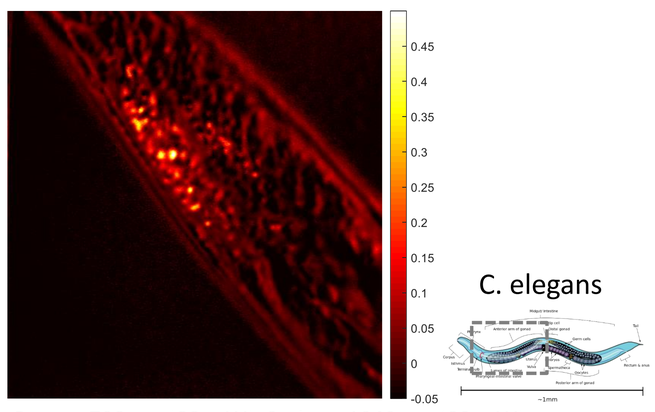
Pump : 792 nm (80 mW), Stokes : 1040 nm (80 mW)

(A) Measured spontaneous Raman spectra of polystyrene and melamine. (B) Corresponding SRS spectra of polystyrene and melamine. (C) Real-time two-color SRS image of 9 μm polystyrene beads and 10 μm melamine beads mixture. Calculated signal to noise ratio is 51.28 and 28.45 each.

Lipid targeted C-elegans image



(C) 2960 cm^{-1} targeted – 3020 cm^{-1} targeted



Pump : 798 nm (80 mW), Stokes : 1040 nm (80 mW)

(A) Measured spontaneous Raman spectra of oleic acid. (B) Corresponding SRS spectra of oleic acid. (C) Real-time two-color SRS image of C-elegance at 2960 cm^{-1} as on resonance and 3010 cm^{-1} as off resonance.

Conclusion

Detection of two distinct Raman shift at once is apparent merit of this two-color SRS set-up, moreover comparable signal to noise ratio with conventional SRS microscopy. Due to pulse character, it has limited Raman spectral window (about 130 cm^{-1}). Since lipid and DNA have Raman peaks around at 2900 cm^{-1}, we expect to explore cellular dynamics with simultaneously monitoring lipid and DNA compositions in bio-assays.

Acknowledgements

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[4] Francesco Crisafi, Vikas Kumar, Tullio Scopigno, Marco Marangoni, Giulio Cerullo, and Dario Pollo, "In-line balanced detection stimulated Raman scattering microscopy", Scientific Reoprts 2017, 7, 10745.