Label-free Microscopy Using Vibrational Resonance by Stimulated Raman Scattering

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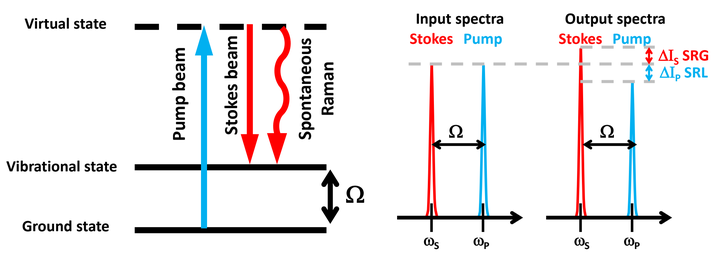
Abstract

Bio-molecular imaging has great importance, since it would be most reliable way to understand the functions and physiology of cells and the pathophysiology of disease. Monitoring cellular process is challenging, for its fast physiological and biochemical dynamics as well as nano-scale spatial dimensions. Moreover, the cellular process, being environmentally sensitive, is easily perturbed (or damaged) by external stimuli such as fluorescent labels and high energy light field. So far, to observe nano-scale bio-complexes and its dynamics, many imaging techniques with high spatio-temporal resolution have been developed and currently under development. Stimulated Raman scattering (SRS) is one of the leading classes of coherent Raman scattering (CRS) technique which uses third-order nonlinear optical property intrinsic to molecule. SRS overcomes many of challenges listed above that other vibrational microscopies are facing. Higher signal level compared to spontaneous Raman scattering by few orders of magnitude permits fast imaging speed, possibly up to video rate for real-time imaging. Unlike coherent anti-Stokes Raman scattering (CARS) and infrared (IR) microscope, SRS is free of non-resonant background which might distort the spectral information and sub-micron spatial resolution is achieved conducting visible light as probe. Our SRS microscope system utilizes femtosecond optical-parametric oscillator (OPO) emitting fixed (1040 nm) and tunable (680-1040 nm) light sources which, in our case will be Stokes and pump beams. Test images for various samples such as polystyrene (PS) beads and C. *Elegans* are achieved, which will be a guide for future experiments.

Stimulated Raman scattering



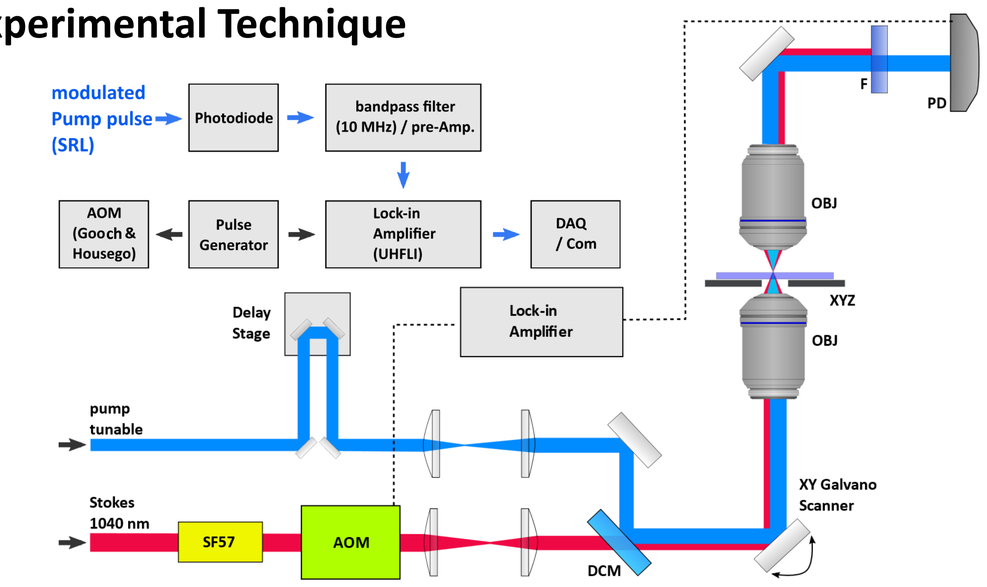
SRS intensity has linear dependence on intensity of both pump and probe beam.

$$I_{SRS}(\Omega) \propto 2Im\left[\chi_R^{(3)}(\Omega)\right] \cdot I_{pump} \cdot I_{probe}$$

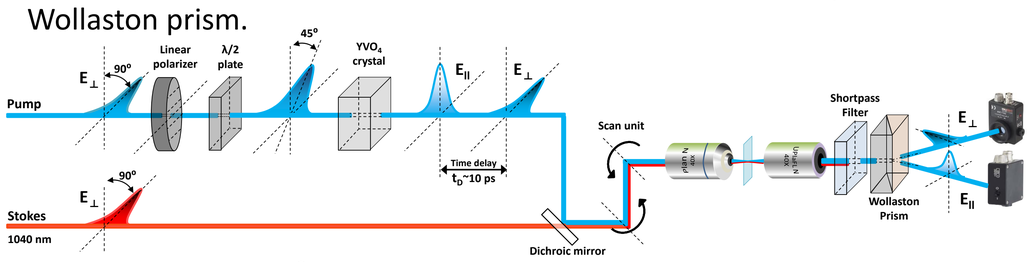
I_{SRS}: Intensity of SRS
I_{pump}: pump beam intensity
$\chi_R^{(3)}$: third-order susceptibility
I_{probe}: probe beam intensity

- Stimulated Raman scattering (SRS) is nonlinear optical phenomena with third-order susceptibility where Stokes beam gain photons (SRG) and pump beam loses photons (SRL).
- SRG and SRL is about 10^{-7}-10^{-4} of intensity of pump and Stokes beam.
- SRS is free of non-resonant back-ground and spectrum matches that of spontaneous Raman which is beneficial when acquiring spectral information.

Experimental Technique

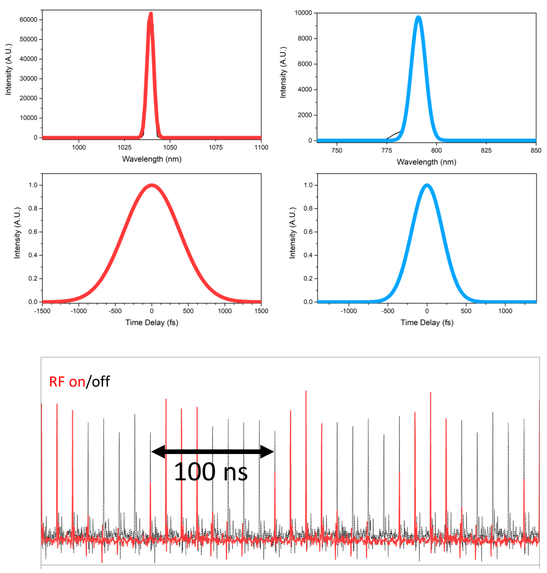


- A femtosecond optical-parametric oscillator (fs-OPO) with fiber based source (Chameleon Discovery) has two output beam lines of fundamental at 1040 nm and widely tunable output ranging from 680 nm to 1300 nm.
- Stokes beam is modulated with acousto-optic modulator at 10 MHz.
- Modulated SRL signal is detected by photodiode, then amplified with pre-amplifier and demodulated by lock-in amplifier.
- In-line balanced detection scheme is possible with YVO_4 crystal and Wollaston prism.



- Pump beam is 45° polarized before YVO_4 crystal, then temporally separated as p-pol and s-pol with time delay about 10 ps.
- After shortpass filter, Wollaston prism splits SRL and reference pump beams spatially for subtraction by lock-in amplifier's software.

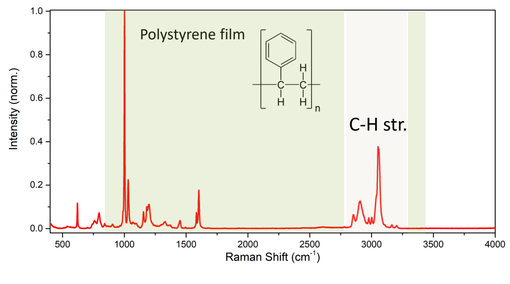
Characterization of Laser Pulses

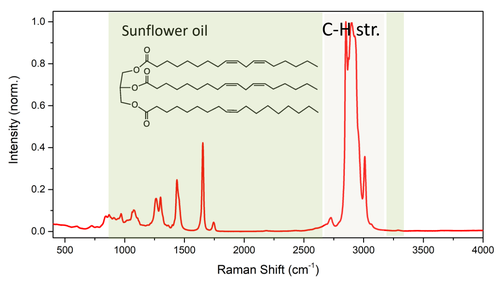


- Pulses with high repetition rate of 80 MHz comes from OPO (12.5 ns).
- For Stokes beam, fixed laser pulse (1040 nm), FWHM of 4 nm and pulse-width of 900 fs is adjusted with narrow bandpass filter and 10 cm SF57 glass.
- For pump beam, tunable output tuned to 790 nm, FWHM is 9 nm and the pulse-width is 400 fs.
- Amplitude modulation at 10 MHz is applied as radio frequency from function generator to AOM with 50% duty cycle.
- The 1st order diffraction efficiency is about 80% fulfilling its specification.
- Modulation frequency above 1 MHz is recommended to avoid 1/f noise.

Experimental Result

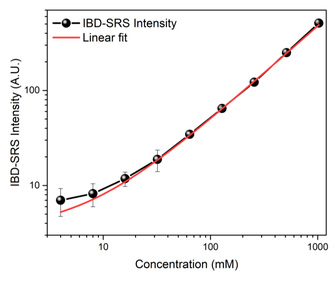
Raman shift range



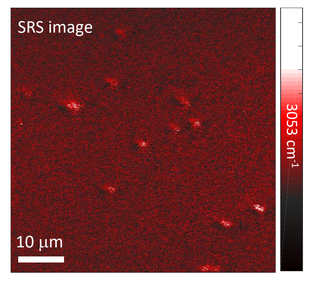


- Aliphatic C-H stretch mode at 2900 cm^{-1} and aromatic C-H stretch mode at 3050 cm^{-1}.

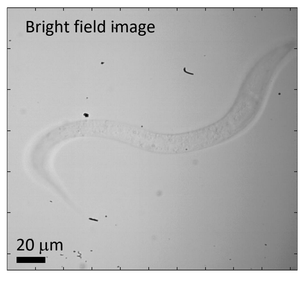
Sensitivity

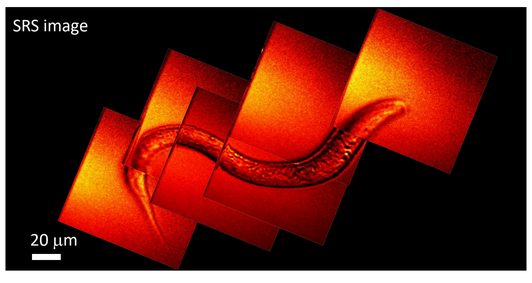


DMSO-d_6 (4 -1024 mM)
Pump power : 40 mW
Stokes power : 40 mW
Raman shift : 2125 cm^{-1}
Time constant : 1 ms

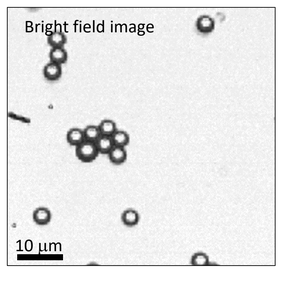


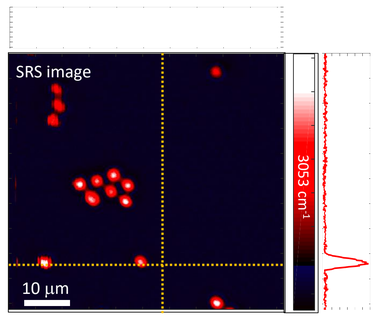
1 μm PS bead
Pump power : 60 mW
Stokes power : 40 mW
Raman shift : 3053 cm^{-1}
Time constant : 10 μs

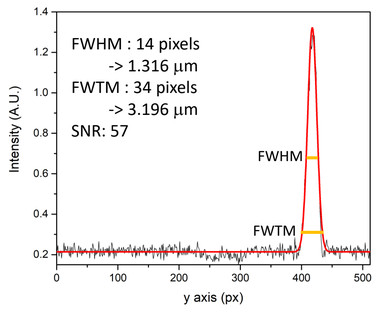




C. *Elegans*
Pump power : 10 mW
Stokes power : 30 mW
Raman shift : 3053 cm^{-1}
Time constant : 10 μs







3 μm PS bead
Pump power : 30 mW
Stokes power : 30 mW
Raman shift : 3053 cm^{-1}
Time constant : 10 μs

Conclusion

Our SRS microscope uses femtosecond optical parametric oscillator with fundamental output at 1040 nm as probe and tunable output ranging from 680 nm to 1300 nm as Raman pump , thus being able to perform SRS imaging for Raman shift from 1000 cm^{-1} to 3200 cm^{-1}. In-line balanced detection, with polarization sensitive SRS signal, suppresses most of background issues. Concentration dependence of SRS signal intensity opens up the opportunity to quantify subcellular composition of biomolecules in cells. Images of PS beads and C. *Elegans* can be a guide for our future experiment regarding biological samples.

Reference [1] Freudiger, C. W. *et al.* Science 2008, 322(5909), 1857-1861. [2] Crisafi, F.. *et al.* Scientific Reports 2017, 7, 10745.