Label-free Coherent Vibrational Imaging by Stimulated Raman Scattering Microscopy

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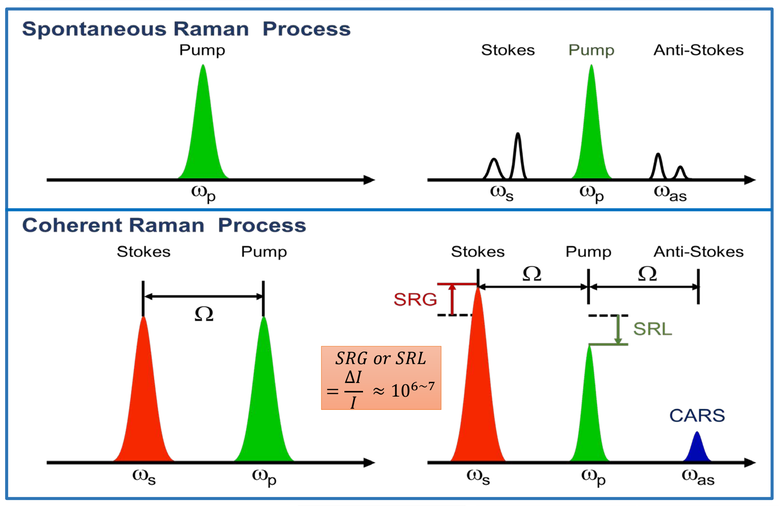
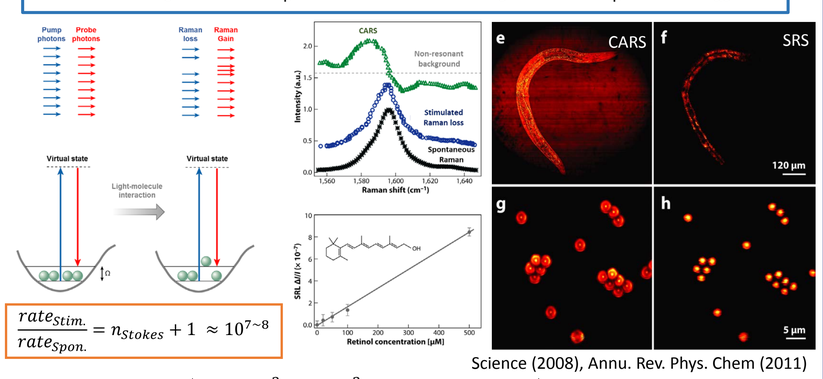
Abstract

We recently have developed a **label-free coherent vibrational imaging** technique, **stimulated Raman scattering** (SRS) microscopy, which obtains chemical-specific information of samples as the label-free imaging manner. The imaging contrast of the SRS microscopy originates from molecular vibrational frequencies intrinsic to chemical compounds without the need for staining or fluorescent labeling. Vibrational microscopies based on infrared absorption and spontaneous Raman processes are limited by low spatial resolution or slow imaging speed. However, in SRS process, the stimulated Raman signal is enhanced by the seven orders of the spontaneous Raman amplitude by virtue of the stimulated excitation of molecular vibrational transitions, allowing for fast imaging acquisition.

Spontaneous vs. Coherent Raman Scattering





$$\frac{rate_{Stim.}}{rate_{spon.}} = n_{Stokes} + 1 \approx 10^{7\sim8}$$

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$$I_{CARS}(\Omega) \propto \left(\left|\chi_R^{(3)}(\Omega)\right|^2 + \left|\chi_{NR}^{(3)}\right|^2 + 2\chi_{NR}^{(3)} Re\left[\chi_R^{(3)}(\Omega)\right]\right) \cdot I_{pump}^2 \cdot I_{probe}$$

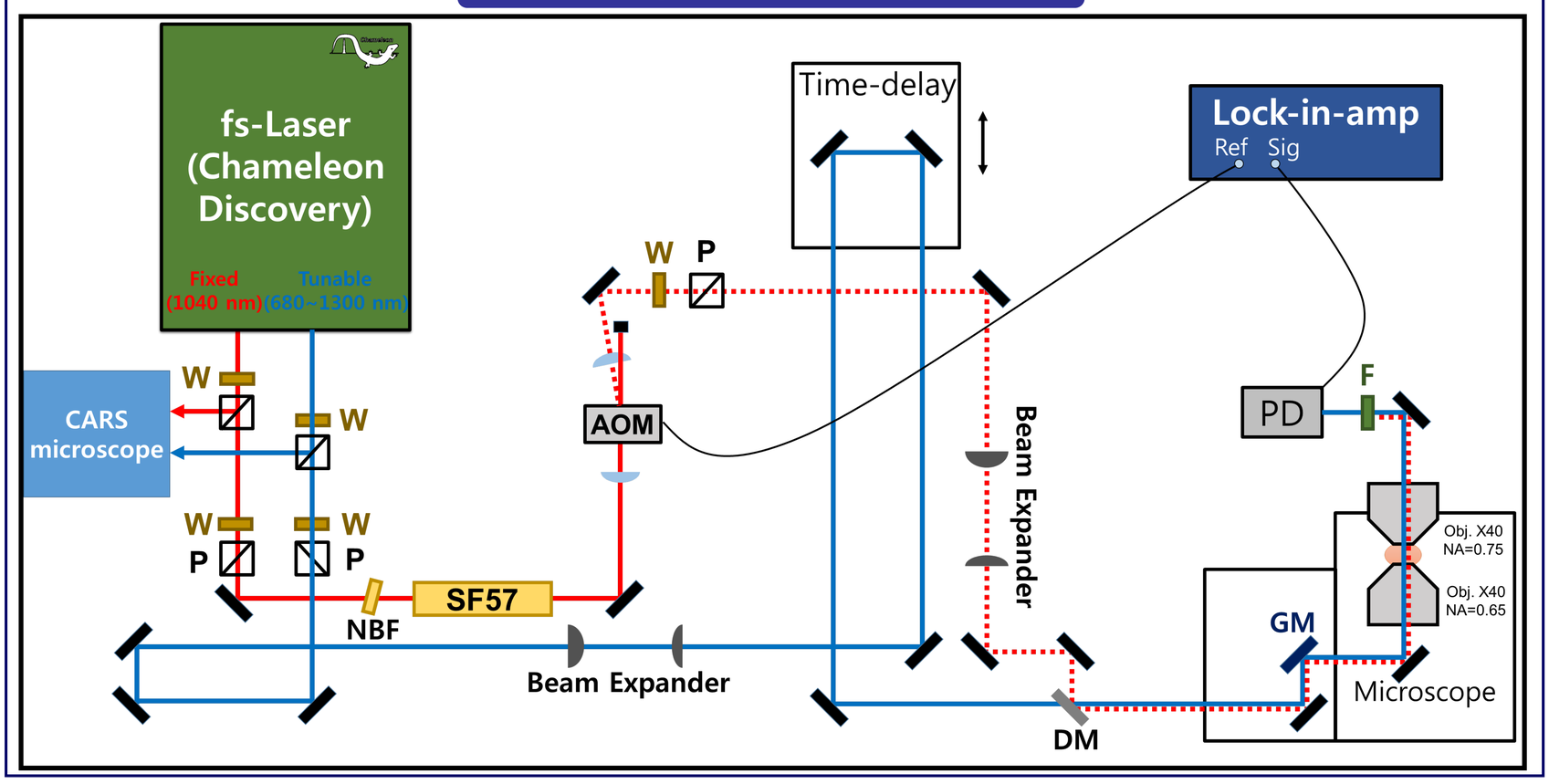
$$I_{SRS}(\Omega) \propto 2\,Im\left[\chi_R^{(3)}(\Omega)\right] \cdot I_{pump} \cdot I_{probe}$$

Why interested in SRS imaging?

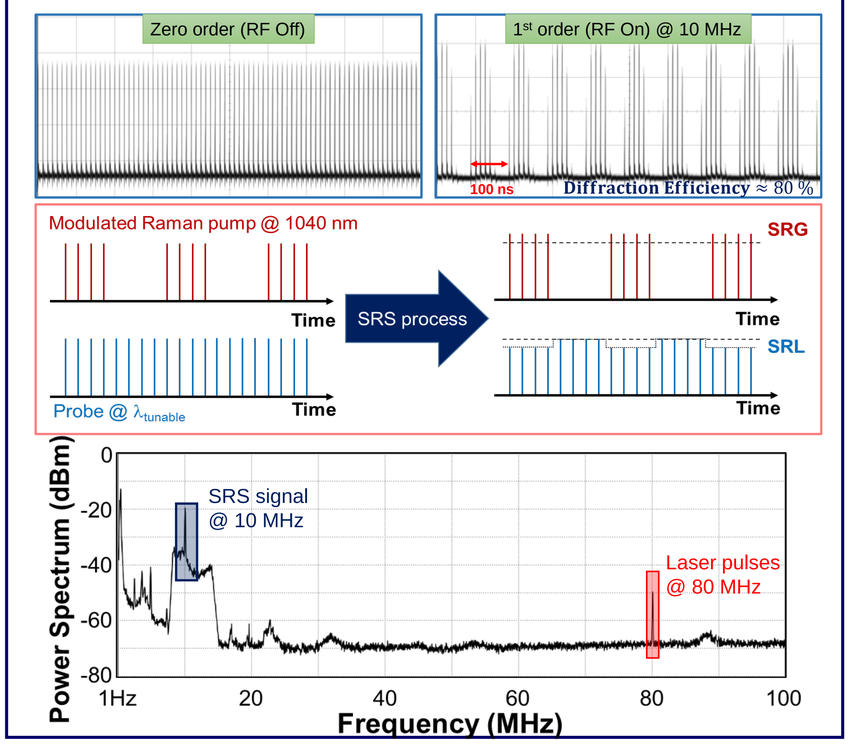
- ✓ *Identical Raman spectrum*
- ✓ *Linear concentration dependence*
- ✓ *Shot noise limited sensitivity*
- ✓ *Free non-resonant background*
- ✓ *Free imaging artifact due to PSF*

"only being good for the imaging of lipids by CARS"

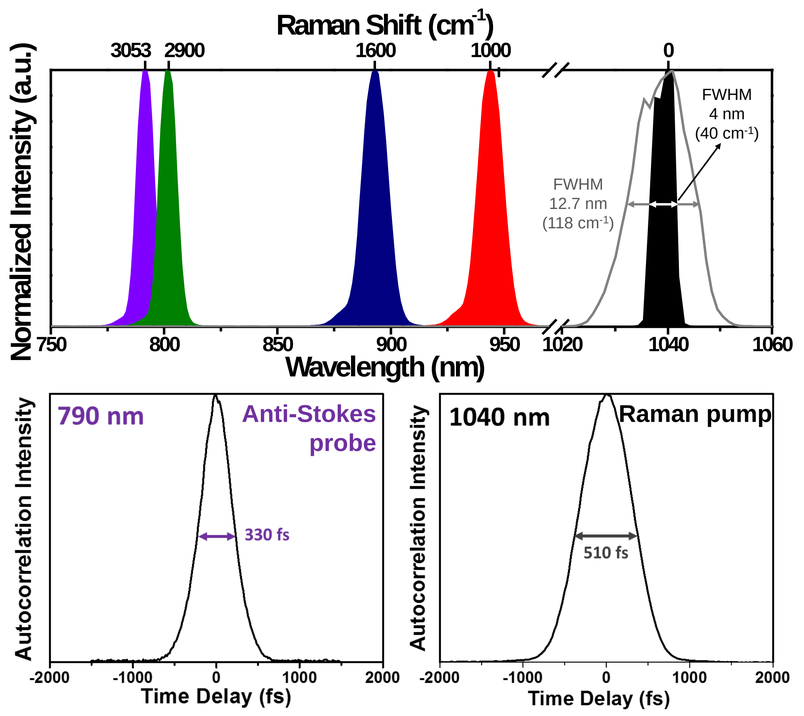
Experimental Setup



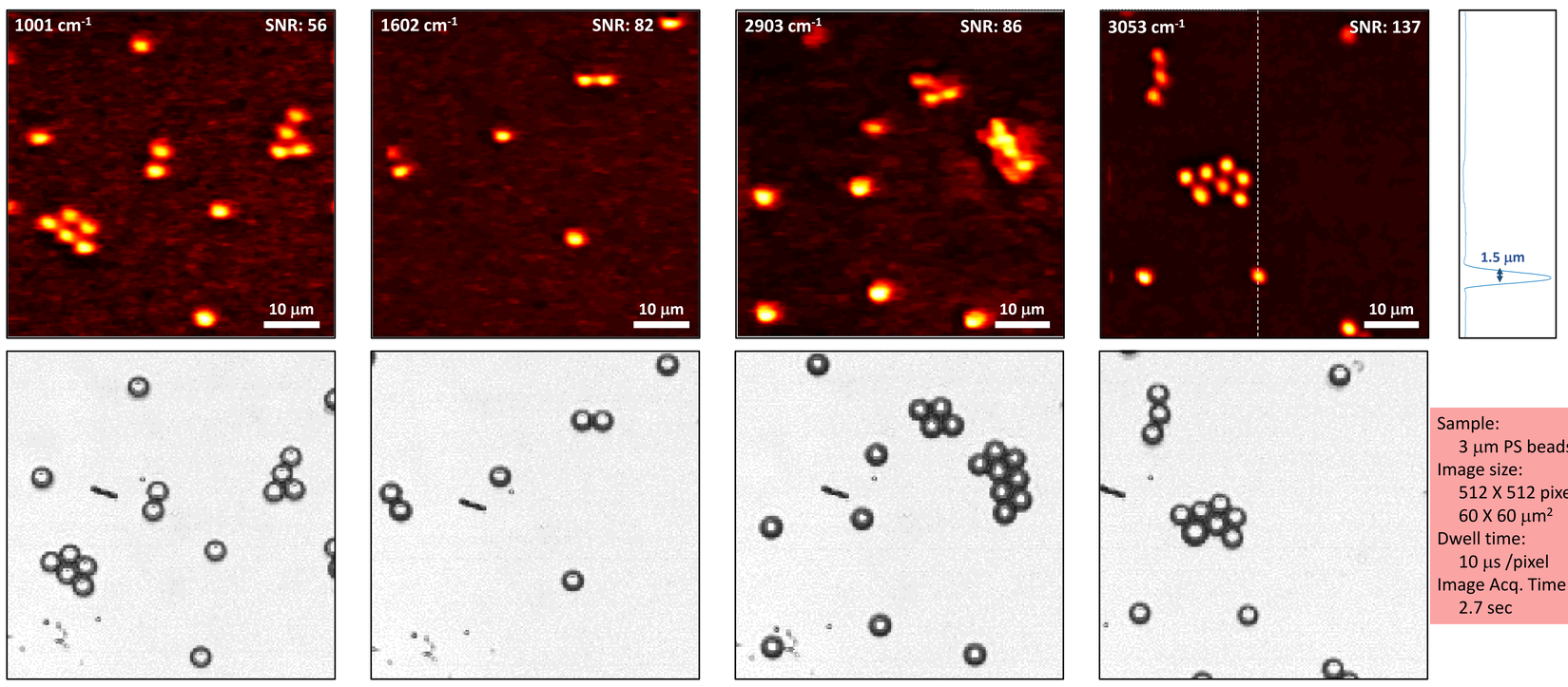
Fast Amplitude-modulated Pulse train



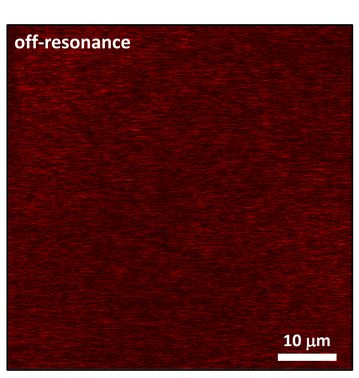
Characterization of Laser Pulses



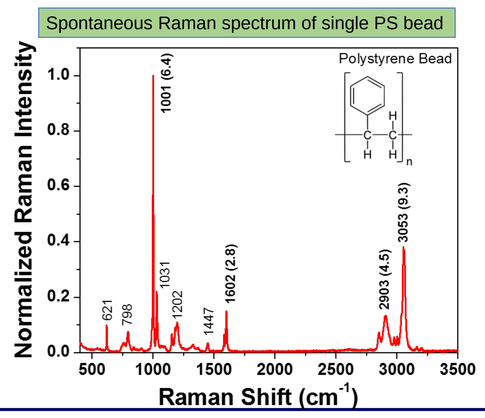
SRS & Bright-field Images of 3 μm PS Beads & Yeast

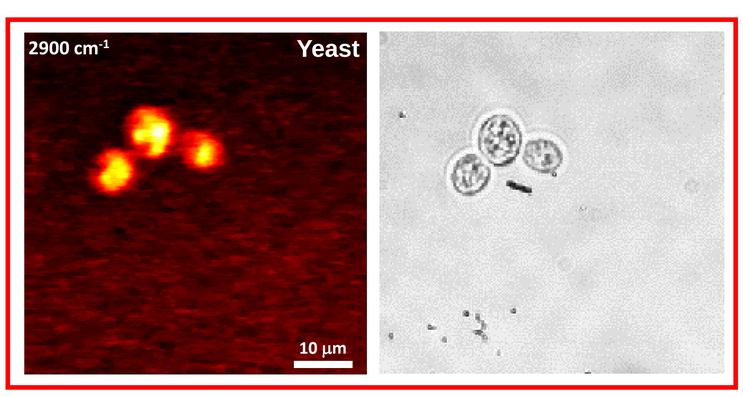


Sample:
3 μm PS beads
Image size:
512 X 512 pixels
60 X 60 μm²
Dwell time:
10 μs /pixel
Image Acq. Time:
2.7 sec



Spontaneous Raman spectrum of single PS bead





Conclusion

We have developed a label-free stimulated Raman scattering microscopy using a femtosecond optical parametric oscillator synchronously pumped a fiber-based femtosecond oscillator. The fundamental output at 1040 nm is used as a Raman pump for the SRS microscopy, whereas the OPO output, which has a wide tuning range from 680 nm to 1300 nm, is served a probe beam to detect the stimulated Raman signal. The Raman energy of the specimens would match the energy difference with the fixed Raman pump and the signal from the OPO. The broad tuning range of the OPO also allows the entire range of the SRS imaging with the range of the Raman shift from 1000 cm^{-1} to 3200 cm^{-1}. The intensity of the Raman pump is modulated by an acousto-optic modulator with approximately 80 % of the diffraction efficiency and with 10 MHz high frequency to reach over the shot noise limit. The SRL signals are detected by a photodiode, and then sent to a fast pre-amplifier & a fast lock-in amplifier with 10 μs time constant. The lateral resolution of our SRS microscope is about 1 μm, measured by an intensity profile of 100 μm PS bead (not shown here). We can observe the four SRS images of the PS beads from 1001 cm^{-1} (ring breathing), 1602 cm^{-1} (ring str.), 2903 cm^{-1} (aliphatic CH str.), and 3053 cm^{-1} (aromatic CH str.),respectively. We also demonstrate the SRS image of the yeast in the CH stretching mode of 2900 cm^{-1} in order to check a feasible application to biological samples.

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