

Stimulated Raman Microscopy Implemented by Three Femtosecond Laser Sources

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Abstract. In this work, the implementation of a femtosecond Stimulated Raman Scattering microscope, equipped with three femtosecond laser sources: a Titanium-Sapphire (Ti:Sa), an optical parametric oscillator (OPO), and a second harmonic generator (SHG); is presented. Our microscope is designed so that it can cover all the regions of Raman spectra, taking advantage of two possible laser combinations. The first, Ti:Sa and OPO laser beams, which cover the C-H region ($>2800\text{ cm}^{-1}$) in stimulated Raman gain (SRG) modality, whereas the second, Ti:Sa and SHG laser beams, covering the C-H region and the fingerprint region in stimulated Raman losses (SRL) modality. The successful realization of the microscope is demonstrated, reporting images of polystyrene beads using both SRL and SRG modalities.

1. Introduction

In spite of the fact that the fluorescence microscopy represents the fundamental pillars for biological imaging, it shows significant limitations: (i) some molecules and/or biological structures cannot be labeled without changing their biological function, such as lipids; (ii) many labels are cytotoxic, which leads to interference with the biological functionality. For these reasons, label-free imaging, obtained by microscopy techniques, is highly desirable, affording high chemical selectivity of unlabeled living cells.

In the last decades, nonlinear optics microscopy has become an attractive tool in both biological and biomedical research to overcome limitations due to fluorescence microscopy. By exploiting nonlinear optical effects, novel microscopy techniques have been developed, which afford high chemical selectivity of unlabeled living cells and implement real-time three-dimensional imaging with high spatial resolution and sensitivity. In multimodal imaging, various nonlinear optics microscopies (CARS, SRS, SHG, THG, and SFG) are combined in a single microscope to image cells or tissue by simultaneous image acquisition. The significant advantage is that the same excitation source can be used for all the nonlinear optics microscopy techniques in this combination of methods [1, 2].

Among novel label-free nonlinear microscopy techniques, Stimulated Raman scattering (SRS) techniques play an essential role. Over the past ten years, SRS has been investigated in nanophotonic [3–6] as well as in biophotonics as an analytical, label-free, non-invasive technique with unique cellular and tissue

imaging capabilities [7–10]. SRS technique is sensitive to the same molecular vibrations, probed in spontaneous Raman spectroscopy, but it exhibits a nonlinear dependence on the incoming light fields and produces coherent radiation. In SRS microscopy, two collinear laser beams, a high-power pump laser and a low-power Stokes laser with different frequencies ($\omega_L > \omega_S$) are focused on a sample to generate an SRS signal. When their difference resonantly excites the vibrational frequency of the molecular bonds of interest, energy is transferred from the pump beam to the probe beam, observable in an increase in probe signal intensity (Stimulated Raman Gain, SRG) and a decrease of the pump signal intensity (Stimulated Raman Loss, SRL) [3,10].

In bio-photonics applications, label-free SRS microscopy allows the image of several molecular species, targeting their intrinsic chemical bonds at the congested cellular fingerprint region ($<1800\text{ cm}^{-1}$) or at the high-frequency C-H region ($>2800\text{ cm}^{-1}$). Moreover, recently, a bio-orthogonal chemical imaging platform has emerged by coupling SRS microscopy with tiny and Raman-active vibrational probes (e.g., alkynes and stable isotopes). Spectroscopically, these Raman active vibration probes exhibit Raman peaks at the cell-silent region where no other peaks from endogenous molecules exist, demonstrating sensitivity, specificity, and biocompatibility for imaging small biomolecules [11, 14].

In the reference [15], nonlinear vibrational bioimaging was demonstrated by taking advantage of femtosecond stimulated Raman loss. It is worth noting that a 12 times more significant SRL loss signal by femtosecond pulse

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excitation was measured with respect to picosecond pulse. Definitely, due to the low sensitivity of Raman-active vibrational probes, femtosecond stimulated Raman microscopy appears as an unavoidable perspective. In addition, interesting biological applications based on femtosecond SRS microscopy have been reported in the literature, extending in the silent region and taking advantage of bio-orthogonal chemical imaging platform [16].

We note that typical and widespread commercial laser sources combinations, such as Chameleon compact OPO (Coherent, Inc.) and Ti:Sa, although tailored for multimodal imaging, their minimum Raman shift is 2500 cm^{-1} . This means that by adopting this laser combination, only the CH-OH region of Raman spectra can be explored, while the silent and fingerprint regions are out of emission range [17-22]. Therefore, they cannot accomplish the demand of biorthogonal platforms based on femtosecond stimulated Raman microscopy.

2. Method and Results

In this work, the implementation of a femtosecond SRS microscope, covering all the regions of Raman spectra, is described. The microscope is equipped with three femtosecond laser sources: a Ti:Sapphire (Ti:Sa), a synchronized optical parametric oscillator (SOPO), and a frequency converter for ultrafast lasers, i.e., a second harmonic generator (SHG) optimized for the SOPO, which allows doubling the OPO energy radiation.

This SRS microscope is used to take advantage of two laser combinations. Ti:Sa and OPO laser combination cover the C-H region in SRG modality[17-22]. The latter Ti:Sa and SHG laser combination provide the extension of the microscope to the silent region ($<1800\text{ cm}^{-1}$) and fingerprint region ($1800\text{--}2800\text{ cm}^{-1}$) in the SRL modality [23].

In our experimental setup, a flip mirror (FM) placed after the OPO exit is used in order to switch the Ti:Sa-OPO and Ti:Sa-SHG laser combinations. When the FM is inserted in the optical path, OPO radiation is coupled into SHG, so Ti:Sa-SHG laser combination becomes available, whereas when the FM is flipped, the OPO can go on undisturbed, and Ti:Sa-OPO laser combination becomes available (see Figure 1). Considering the Ti:Sa laser excitation within the wavelength range of $740\text{--}880\text{ nm}$, the corresponding OPO emission range is $1000\text{--}1600\text{ nm}$, while the SHG emission range is $500\text{--}800\text{ nm}$. When the Ti:Sa is used as pump at 830 nm , the largest range of Raman shift is obtained. Indeed, in this case, all the regions of Raman spectra can be covered (from 451 cm^{-1} to 3195 cm^{-1}) using the Ti:Sa-SHG laser combination[23].

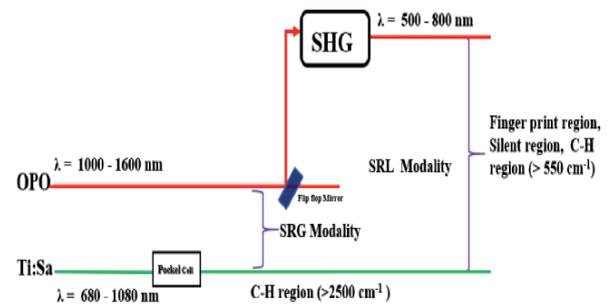


Figure 1. Generation system with combination Ti:Sa-OPO (SRG modality) and Ti:Sa-SHG (SRL modality).

In order to demonstrate its successful realization SRG and SRL images of polystyrene beads with a diameter of $3\text{ }\mu\text{m}$ are reported in figures 2 and 3, respectively, along with transmission image (TI). All the images are single recordings of $512\text{ px} \times 512\text{ px}$, and their acquisition

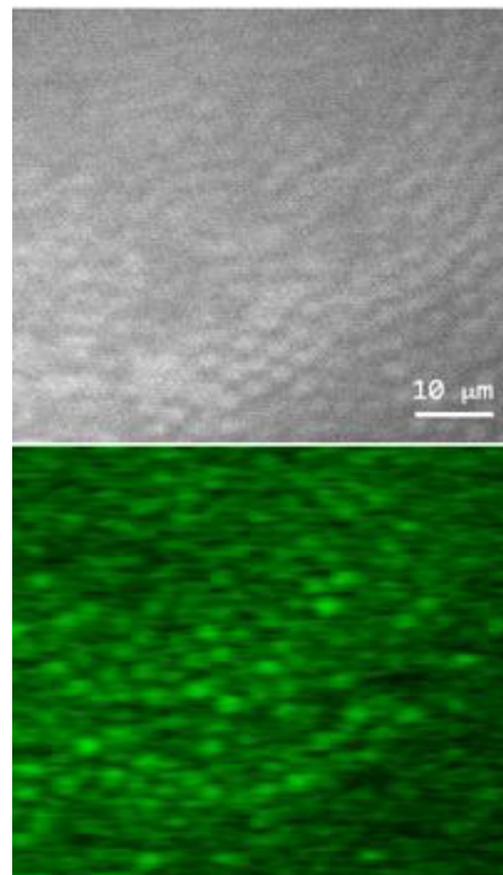


Figure 2. On the top transmission image, the bottom SRG image of polystyrene beads sample with a diameter of $3\text{ }\mu\text{m}$.

time is 16 s . The Lock-in amplifier (LIA) time constant was set to 100 s with an 18 dB/oct slope and a sensitivity of 10 V . For SRG images, the pump signal generated by Ti:Sa was set at 808 nm , while the probe signal generated by OPO was set at 1072 nm . For both of them, the average focused power was about 10 mW . For SRL

imaging, the pump signal generated by the SHG unit was set at 650 nm, while the probe signal generated by Ti:Sa was set at 811 nm, for Ti:Sa, the average focused power was 8 mW, while for SHG, the average focused power was of 6 mW.

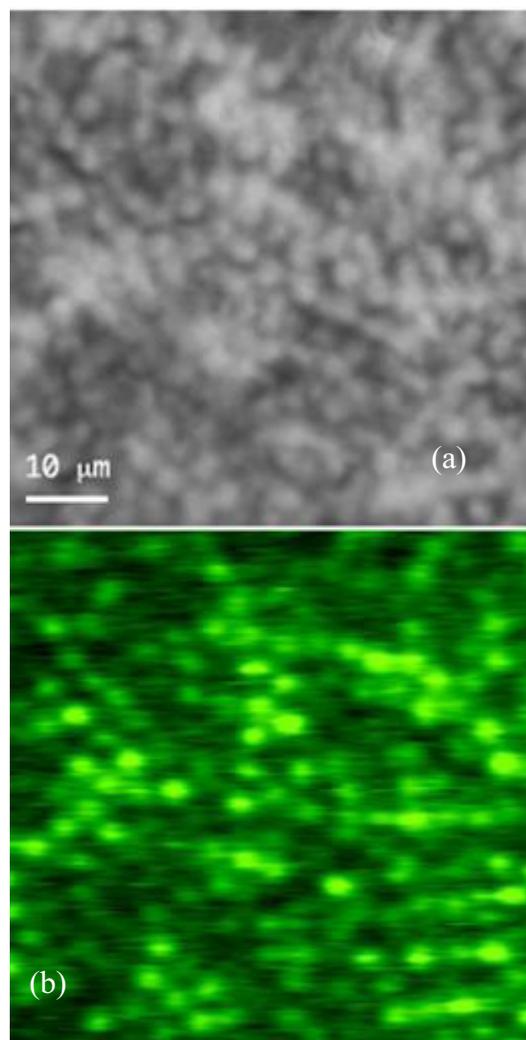


Figure 3 (a) Transmission image and (b) SRL image of a polystyrene beads sample with a diameter of 3 μm .

CONCLUSIONS

In conclusions, we have demonstrated the implementation of a femtosecond stimulated Raman microscope, extending its working range in fingerprint and in silent regions. Our microscope has been designed in order to allow the coexistence of SRL and SRG optical modalities without modifying optical path, i.e. without adding, removing or moving components.

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