Femtosecond stimulated Raman spectrometer in the 320-520 nm range

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Abstract: Multi-µJ narrow-bandwidth (≈ 10 cm⁻¹) picosecond pulses, broadly tunable in the visible-UV range (320-520 nm), are generated by spectral compression of femtosecond pulses emitted by an amplified Ti:sapphire system. Such pulses provide the ideal Raman pump for broadband femtosecond stimulated Raman spectroscopy, as here demonstrated on a heme protein.

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References and links

1. Introduction

Femtosecond Stimulated Raman Spectroscopy (FSRS) has been recently introduced as a powerful method for studying chemical and biochemical reaction dynamics [1–3]. By acquiring Stimulated Raman Scattering (SRS) spectra of a molecule as a function of the time delay with respect to an ultrashort pulse (the actinic pump) triggering a photochemical process, FSRS enables to take snapshots of transient vibrational structures with unprecedented combination of temporal (<50 fs) and spectral (<20 cm\(^{-1}\)) resolution. This technique has been already successfully applied to the study of a number of primary photochemical reactions, such as isomerisation in rhodopsin [4] and proton transfer in the green fluorescent protein [5].

FSRS requires a narrowband picosecond pulse (the Raman pump) synchronized to two broadband femtosecond pulses, the actinic pump and the Raman probe. Standard implementations of FSRS synthesize the Raman pump by linear spectral filtering of a femtosecond pulse, typically from a Ti:Sapphire system, using either a narrow bandpass filter or a slit in the Fourier plane of a zero-dispersion 4f pulse shaper. This method has the disadvantage of a high energy loss, at least proportional to the spectral narrowing, and, of a limited tunability around 800 nm. Such wavelength is in many cases not convenient since it can overlap with stimulated emission from the sample, thus masking the desired signal [6]; in addition, it does not allow exploiting resonance enhancement of the Raman response, which is generally obtained at shorter wavelengths. For these reasons, the efficient generation of a narrowband Raman pump with broad tunability in the UV-visible range would be highly desirable and would greatly extend the potential of FSRS.

Several methods for the generation of a tunable Raman pump have been proposed. Narrowband UV pulses at 400 nm have been obtained by sum-frequency generation (SFG), in a thin nonlinear crystal, of two oppositely chirped 800-nm pulses [7, 8]; this approach is however of high experimental complexity, since it requires the use of a pair of carefully balanced pulse stretchers/compressors. Other researchers [9–12] generated tunable picosecond pulses using a two-stage Optical Parametric Amplifier (OPA), pumped by the second harmonic (SH) of Ti:sapphire, in which the output of the first stage is spectrally filtered and used to seed a second narrow-band stage; however, only bandwidths broader than \(\approx 25\ \text{cm}^{-1}\) were obtained, with tuning range from 470 to 780 nm.

Recently, we have demonstrated a simple technique, that we called “spectral compression” (SC), for the efficient synthesis of tunable picosecond pulses starting from femtosecond pulses. SC is based on a second-harmonic-generation (SHG) process in the presence of large group-delay-mismatch (GDM) between the fundamental frequency (FF) and the second harmonic (SH) pulse [12–15], which results in a narrow SH bandwidth given essentially by the inverse of GDM. The high efficiency of this process is explained by the fact that SH photons are generated not only by direct SHG, but also by an intra-pulse SFG between FF spectral components that are symmetric with respect to the SHG phase-matching frequency [13, 16], leading to an efficient energy transfer from the broadband FF to the narrowband SH.
In this paper we apply the SC technique to produce narrowband Raman pump pulses for FSRS spectroscopy, tunable in the visible-UV range from 330 to 520 nm, with approximately 10 cm\(^{-1}\) linewidths and energies reaching the multi-\(\mu\)J level. Such pulses are here used to acquire broadband high-resolution SRS spectra of solvents and heme proteins, exploiting in this latter case the signal enhancement provided by excitation in the Soret absorption band.

2. Experimental

The experimental setup is shown in Fig. 1. The whole system is driven by an amplified Ti:sapphire laser (Coherent Legend) producing 3.6-mJ, 50-fs pulses at 800 nm (28 nm FWHM bandwidth) and 1 kHz repetition rate. In the basic configuration, the narrowband Raman pump is generated by direct SC of the Ti:sapphire output. To understand the choice of the SC crystal it is worth reminding that, in a SHG process in the presence of large GDM between the interacting pulses, the FWHM bandwidth of the SH pulse \(\Delta \nu_{\text{SH}}\) is approximately given by

\[
\Delta \nu_{\text{SH}} = 0.886 \frac{GDM}{L},
\]

where \(GDM = \delta L\), \(L\) being the crystal length and \(\delta = \frac{1}{v_{\text{FF}}} - \frac{1}{v_{\text{SH}}}\) the group velocity mismatch (GVM) between FF and SH pulses. To achieve large SC ratios one thus requires long crystals and large values of \(|\delta|\). In addition, since our goal is the generation of a Raman pump with multi-\(\mu\)J energy, the crystal should be able to accept pulses with relatively high energy (of the order of several tens of \(\mu\)Js). Periodically poled crystals, that we previously used for SC [13–15], suffer from photorefractive damage in the visible to near-UV range and, due to their limited thickness, do not allow handling energetic pulses. For these reasons, we have chosen \(\beta\)-barium borate (BBO). Its low nonlinearity \((\approx 2 \text{ pm/V})\) and its large walk-off angle \((\approx 4^\circ)\) are not detrimental to SC due to the large available energy amount of the FF pulses. Its GVM between FF and SH pulses ranges from 320 fs/mm to 90 fs/mm when tuning the FF from 660 to 1040 nm, which implies SH bandwidths narrower than 16 cm\(^{-1}\) in the whole 320-520 spectral range with the chosen length of 25 mm.

We first simulated the SC process in a 20-mm long BBO crystal by numerically solving the coupled nonlinear propagation equations for FF and SH pulses in the plane wave limit [18]. Figure 2(a) displays the results, starting from 800-nm 50-fs FF pulses, for three different
phase-matching conditions. One observes a dramatic spectral compression, with SH bandwidths of 0.24 THz (7.9 cm\(^{-1}\)), in excellent agreement with the calculated GDM of 3.8 ps. It should be noted that, in order to achieve narrow SH bandwidths, one should exploit the whole crystal length, and thus keep the intensities low enough to avoid saturation of the SHG process due to depletion of the FF pulse. In our simulations, this was achieved for peak intensities slightly lower than 1 GW/cm\(^2\).

Experimentally, we frequency-doubled a portion of the Ti:sapphire output (<50 µJ) in a long (20-25 mm) BBO. The incident FF beam spot size was chosen as \(w = 1.0\text{-}3.5\) mm, which is large enough to neglect spatial walk-off. A series of SH spectra, as obtained by tilting the BBO crystal, is shown in Fig. 2(b): they are remarkably narrow (∼8.7 cm\(^{-1}\) after deconvolution of the instrumental response), and in excellent agreement with the numerical simulations. Typical output energies before the onset of SHG saturation (and corresponding broadening of the spectra) were 5-10 µJ.

SC of the FF of Ti:sapphire allows however a limited tuning range for the Raman pump, roughly from 385 to 415 nm. This range can be considerably extended by adding a two-stage optical parametric amplifier (OPA) before the SC crystal. The OPA is pumped by the SH of Ti:sapphire and uses a slightly non-collinear geometry to allow easy rejection of the pump beam. The seed is provided by a White Light Continuum (WLC) generated in a 2-mm-thick sapphire plate. The first stage uses a 1-mm type-I BBO crystal to produce 1-µJ pulses, while the second one uses a 1-mm type-II BBO crystal [19, 20] boosting the energy up to ∼40 µJ.

Fig. 2. (a) Simulated spectra (black solid lines) obtained by SC in BBO, starting from the FF spectrum shown in red dashed line; inset blow up of a spectrum with a FWHM of 7.9 cm\(^{-1}\). (b) Measured SH spectra (black solid lines) and FF spectrum (red dashed line).

Fig. 3. (a) Sequence of tunable narrowband spectra obtained by SC of the visible OPA; (b) measured pulse bandwidths (red circles) after deconvolution with the instrumental response compared to the bandwidths expected from theory (dashed lines) and energy tuning curve (blue squares) for a fixed 12-µJ OPA energy, which provides the best spectral narrowing conditions.
Type II phase matching in the second stage provides narrower gain bandwidths that remain essentially constant over the tuning range, also when approaching the degeneracy point at 800 nm, thus allowing for the desired continuous tunability from 600 to 1040 nm. The OPA output drives the BBO SC stage, for which alternatively we used two different crystals: $L = 25$ mm, $\theta = 27.6^\circ$ to cover the 400-520 nm range or $L = 20$ mm, $\theta = 31.3^\circ$ to cover the 320-400 nm range. Figure 3(a) shows a series of narrowband pulses obtained by SC of the OPA, with energies ranging from 2 to 4 $\mu$J (see Fig. 3(b)) and continuous tunability from 330 nm (3.8 eV) to 520 nm (2.4 eV). The corresponding bandwidths are reported in Fig. 3(b) as red circles and match the theoretical predictions (dashed lines) after deconvolution with the spectrometer response.

The tunable Raman pump was applied to SRS experiments using, as a broadband Stokes, the WLC generated by focusing a fraction of the FF in a sapphire or a CaF$_2$ plate. Sapphire provides a higher shot-to-shot stability, but its WLC extends only down to 450 nm. CaF$_2$ requires continuous translation of the plate to prevent photodamage, resulting in larger fluctuations, but its WLC extends down to 340 nm and allows exploiting the whole Raman pump tunability at shorter wavelengths. Raman pump and Stokes are focused (400 mm focal length) non-collinearly on the 0.5-mm-thick sample (see Fig. 1), to easily separate the Stokes from the Raman pump, and the transmitted Stokes is detected, at the full 1-kHz laser repetition rate, by an optical multichannel analyzer (SpectraPro 2500i) equipped with a cooled CCD detector (Acton Instruments) and providing a spectral resolution of 3-5 cm$^{-1}$.

![Fig. 4.](image)

As a first test of the setup, the SRS spectrum of cyclohexane was acquired using a 415-nm, 1-$\mu$J Raman pump and Stokes generated by WLC in CaF$_2$, 10 seconds integration; (b) same as (a) using a 470-nm Raman pump and WLC in sapphire; (c) CW Raman spectrum of cyclohexane.

As a first test of the setup, the SRS spectrum of cyclohexane was acquired using a 415-nm Raman pump (Fig. 4(a)) and compared to the CW Raman response (Fig. 4(c)). The agreement is remarkable, and it demonstrates the ability of the setup to address in a single shot, with high spectral resolution (10 cm$^{-1}$), all the relevant vibrational features in an extremely broad spectral range (300-3200 cm$^{-1}$). Figure 4(b) shows the SRS spectrum when using a 470-nm Raman pump as obtained by SC of the OPA. The spectral resolution is lower due to the smaller GDM at longer FF wavelengths, but all the characteristic spectral features are still observed. The broad tunability of the narrowband Raman pump is however of most interest to obtain enhancement of the Raman response of biological samples through resonant excitation. We demonstrate here such possibility by measuring the SRS spectrum of horse heart myoglobin (Mb) when excited in quasi-resonant conditions around its Soret band. Mb is a
monomeric heme protein found in the skeletal and heart muscles of vertebrates playing a physiological role in intracellular oxygen supply. It is an excellent model system for the investigation of protein structure, function and dynamics [21]. For the SRS experiments Mb (Sigma Chemical Co.) was dissolved in phosphate buffer, pH 7.5 at a concentration of 110 μM. The sample was placed in a flow cell with 0.25 mm thick quartz windows.

The SRS spectrum of Mb, after calibration with a cyclohexane standard is shown in Fig. 5(a) with 2-minutes integration time and with baseline removal. It is in excellent agreement with a resonance Raman spectrum as obtained with cw excitation at 406.7 nm (b). The observed vibrational modes are consistent with those reported in the literature for Soret-excited met Mb [22], namely the oxidation state marker band $v_4$ at 1370 cm$^{-1}$, the $v_3$ band at 1480 cm$^{-1}$ and the $v_2$ band at 1564 cm$^{-1}$ in the high frequency-range, which are characteristic of a ferric six-coordinated high spin heme species. The vinyl stretching band at 1622 cm$^{-1}$ is also present, as well as the $v_{11}$ mode at 1542 cm$^{-1}$ and the $v_{37}$ mode at 1585 cm$^{-1}$. In the low-frequency region, we observed the $v_7$ mode at 678 cm$^{-1}$, the bending modes of the vinyl groups and the propionate groups at 409, 444 and 374 cm$^{-1}$, respectively and the $v_8$ mode at 342 cm$^{-1}$.

3. Conclusion

In this work we have demonstrated a simple approach for the generation of multi-μJ, narrow-bandwidth (∼10 cm$^{-1}$) picosecond pulses broadly tunable in the visible-UV range (320-520 nm), as obtained by SC of femtosecond pulses generated by a Ti:sapphire laser or by an OPA. Such pulses provide the ideal Raman pump for broadband FSRS, enabling to exploit any resonance enhancement of the Raman response in the UV region. We benchmarked our setup by measuring high-quality SRS spectra of a heme protein (Mb) using 40-fs pulses, with a spectral resolution adequate to resolve different ligation states of the heme. This result paves the way to transient FSRS experiments using a femtosecond actinic pulse. Such effort is currently in progress in our group to study ligand binding dynamics with sub-ps resolution [23]. We believe that this source will become a useful tool for the growing community of FSRS users and it will enable to extend considerably the applicability range of this technique to a wide class of biomolecules.

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