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EXECUTIVE SUMMARY OF THE THESIS

Calibration of absolute Stimulated Raman Scattering cross-section for quantitative measurements

LAUREA MAGISTRALE IN PHYSICS ENGINEERING - INGEGNERIA FISICA

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Academic year: 2024-2025

1. Introduction

Spontaneous Raman microscopy is a technique capable of retrieving both morphological details and chemical information about the specimen based on exciting the vibrational levels of the sample. It is a label-free technique with high sensitivity. The Raman signal power is expressed as $P_{Raman} = \sigma_{Raman} I_p$, where I_p is the pump intensity and σ_{Raman} is the spontaneous Raman cross-section [cm^2]. σ_{Raman} represents the material response strength, but due to the incoherent nature of the signal, the numerical values are low. This leads to address Raman process as weak, and to long time for imaging acquisition. Stimulated Raman scattering (SRS), a type of coherent Raman scattering (CRS), solves this problem enhancing Raman signals by sending onto the sample two synchronized (pump and Stokes) laser pulses. In particular it is required that the photons energy difference between pump and Stokes ($h\omega_p - h\omega_s$) matches the vibrational energy level ($h\Omega$). The SRS signal consists in a decrease in the pump intensity (stimulated Raman loss SRL) and an equivalent increase in the Stokes intensity (Stimulated Raman gain SRG). This variation is often expressed as a proportionality $\Delta I \propto I_p I_s$ [1] leading to present the SRS signal as a dimen-

sionless relative intensity variation $\frac{\Delta I}{I}$ or express it in arbitrary units. The problem is that there is not a simple expression of a parameter that can represent the SRS material response strength, and also includes all the physical dimensions and material properties. While σ_{Raman} is not an accurate solution, recently [2, 4] a new parameter has been introduced to resolve this problem. This parameter is inspired by another non-linear effect where two input fields are the involved: the two-photon absorption (TPA) [3]. TPA is governed by the equation $rate_{TPA} = \sigma_{TPA} \Phi^2$, where $rate_{TPA}$ is the rate of photons [$1/s$] emitted via TPA, Φ is the impinging photons flux [$1/cm^2 \cdot s$] and σ_{TPA} is the TPA cross-section. The unit of measure of σ_{TPA} is the Göppert-Mayer (GM), named after her first prediction of the phenomenon. The GM is equal to $10^{-50} cm^4 s$. The authors proposed a similar rate equation for the SRS:

$$rate_{SRS} = n \sigma_{SRS} \Phi_p \Phi_s = [c] V N_a \sigma_{SRS} \Phi_p \Phi_s \quad (1)$$

where $n, [c], V, N_a, \sigma_{SRS}$ are respectively the number of oscillators within the excitation volume, the concentration [mol/L], the excitation volume [L], the Avogadro number and the new SRS cross-section expressed in GM . They also retrieved an equation to directly measure the

σ_{SRS} value. Since the intensities are proportional to the photons fluxes, the relative intensity difference is equal the relative rate of photons generated via SRS

$$\left(\frac{\Delta I}{I}\right)_{SRG} = \frac{rate_{SRS}}{\Phi_s A} = \frac{[c] V N_a \sigma_{SRS} \Phi_p \Phi_s}{\Phi_s A} \quad (2)$$

where A denotes the effective incident area of the laser beam, and we used, without loss of generality, the SRG process. In this equation the Φ_s can be canceled leading to a σ_{SRS} dependent on just the pump flux, and so on the pump power.

2. Calibrated SRS cross-section

The aim of this work is to implement in the formula (2) the spatial, temporal, and spectral distribution of the pulses, and then use it to measure σ_{SRS} . We started by imposing the $\left(\frac{\Delta I}{I}\right)_{SRG}$ equal to the relative number of photons **per pulse** generated via SRG. The number of photons generated via SRG in a pulse can be expressed as

$$\int_s rate_{SRS} dt = \int_t \int_x \int_y \int_z [c] N_a \sigma_{SRS} \cdot \beta_p(x, y, z, t) \beta_s(x, y, z, t) dx dy dz dt \quad (3)$$

where $\int_s()dt$ is the temporal integration and $\beta(x, y, z, t)$ is the pointwise value of the photons flux in both space and time. Considering the pointwise function allows us to perform a spatial integral instead of the simple multiplication for the volume. The number of photons per pulse arriving to the sample can be written as $\int_s \int_x \int_y \beta_s(x, y, z, t) dx dy dt$. The value of this expression, thanks to the non-depletion approximation, is constant along z . Therefore, we can write the equivalence

$$\left(\frac{\Delta I}{I}\right)_{SRG} = [c] N_a \sigma_{SRS} \cdot \int_z \left(\frac{\int_t \int_x \int_y \beta_p(x, y, z, t) \beta_s(x, y, z, t) dx dy dt}{\int_t \int_x \int_y \beta_s(x, y, z, t) dx dy dt} \right) dz \quad (4)$$

The main novelty stands here. In this expression the Stokes fluxes can not be canceled. Also there

is no longer a dependence on the pump flux, but there is instead a weighted average of the pump flux where the weight for each x, y, z, t point is the local Stokes flux in that point. Averaging in x, y plane and time, allows us to take into account the shape and overlap between the two beams in both domains. To simplify the expression we can separate the spatial and temporal components in (4). Assuming a gaussian temporal profile we achieve $\beta(x, y, z, t) = \Phi(x, y, z) \cdot \exp(-(t^2/2\tau^2))$ where $\Phi(x, y, z)$ contains all the spatial contribution and τ is the pulse temporal width. It is convenient to introduce a first corrective factor K_1 to enclose the temporal aspect

$$K_1 \equiv \frac{\int_t \exp(-(t^2/2\tau_p^2)) \exp(-(t^2/2\tau_s^2)) dt}{\int_t \exp(-(t^2/2\tau_s^2)) dt} \quad (5)$$

In real scenarios, the spectral distributions of the pump and Stokes beams are Gaussian rather than ideal delta functions, which means frequencies beyond the target $\Omega = \omega_p - \omega_s$ are excited. For this reason what we measure is not precisely $\sigma_{SRS}(\Omega)$ but the integral of the true Raman spectrum multiplied by the correlation between the spectra of the two beams. To take into account this we multiplied the right hand side of equation (4) for another corrective factor

$$K_2 \equiv \int_\theta S(\theta) C(\theta) d\theta \quad (6)$$

where $S(\theta)$ is the intrinsic spontaneous SRS spectrum of the molecule (i.e. ideally measured with an infinite spectral resolution), normalized to unit intensity, and $C(\theta)$ is the correlation function with area normalized to 1. Regardless all those consideration, the standard $\Delta I/I$ is still required. We will now introduce a corrective factor necessary for quantitative measurements.

3. Filling Factor

Typical values of $\Delta I/I$ are in the order of $10^{-4} - 10^{-5}$, therefore lock-in amplifier measurements are necessary. In SRG experiment the pump is modulated at a certain frequency ω while the Stokes is then demodulated at the same frequency. This is done because the Stokes signal after the interaction, is formed by a train of pulses with constant amplitude (**pedestal**), and on top of that there is another train of pulses where their amplitude follows the modulation frequency. The latter is exactly the SRG,

and its peak-to-peak **modulated amplitude** is the ΔI we want to retrieve. Since the photodiode bandwidth (BW) is always not sufficient to achieve a temporal resolution of picoseconds, the photodiode will see the Stokes signal as a train of deltas and its output will therefore be a train of its impulse response $h(t)$. To introduce the problem we consider a case where photodiode bandwidth is much lower than the laser repetition rate (RR) (but still higher than ω) and a case where is higher. In the first case the pulsed nature is lost leading to have just the modulation sinusoid raised up by the pedestal, in this case the lock-in will be able to retrieve the amplitude information. In the second case, where the pulsed nature is instead kept, the lock-in return a **smaller** value. The reduction occurs because in the first scenario with a *full* sinusoid, the modulation and demodulation signal align to give a maximal response. Instead in the second case, the lock-in multiplies a modulated pulse train by the reference sinusoid. Since the pulse train amplitude is often less than that of the modulation sinusoid (and can even be zero for extended periods, depending on the shape of $h(t)$), the outcome of this integral will inevitably be smaller than in the *filled signal* case. We introduced a new corrective term: the **filling factor**(FF), named for its function of retrieving the amplitude of the full signal. The filling factor is nothing more than the ratio between the peak-to-peak amplitude modulation, and the lock-in outcome. The most important property of the FF is the independence on the particular pedestal and modulated amplitude, but it depends just on ω and $h(t)$. This means that the FF can be measured **before** the real SRG experiment in a case where the modulated amplitude is big and it can be easily retrieved by other methods, and then use it to calibrate the ΔI when the lock-in is necessary. In this section we will consider the two cases (high and low BW) presented before and an intermediate case, showing how to calculate the FF, how to use it and why it should be used. We prepared an experimental set-up made ad hoc to prepare "dummy signal" where we can separately control the pedestal and modulated amplitudes. In this way we were able to evaluate the FF use for different modulated amplitudes over total amplitudes ratio. In the experimental set-up thanks

to a beam splitter (BS) the beam was separated creating a branch for the pedestal control and another for the modulated part. The photodiode employed has a variable BW allowing to explore the three cases: BW=100MHz (Table 1), 14MHz (Table 2), 1.8MHz (Table 3). The signal is then sent to the lock-in and to a fast oscilloscope, where thanks to a Matlab data analysis the true peak-to-peak amplitude was retrieved. The RR was 40MHz and the modulation was performed by an acusto-optic modulator at 400KHz.

The operative approach was to consider the case with high ratio to measure the FF, as the fraction between the oscilloscope measure and the lock-in measure. Then, lowering the ratio, the lock-in measure was multiplied by the FF to achieve a calibrated value. This value was then compared with the oscilloscope measure.

The first thing we can observe from the data, is that the calculated values closely align with the oscilloscope measurements as expected. Even though ideally the two values should remain the same, a slight error becomes more pronounced as the ratio decreases due to the fact that for smaller ratio the oscilloscope is no longer suitable for this measure. The limit is the real SRG case where it can not be used at all. The second thing we can notice is the fact that for the small BW case the filling factor value is almost just the constant term $2\sqrt{2}$. This value comes always out from the lock-in measurement since it returns the modulation amplitude (not the peak-to-peak, so divided by 2) as RMS value (divided by $\sqrt{2}$). Note that the FF is not precisely $2\sqrt{2}$. This is not a measurement error, but it comes out from the fact that the modulator does not return an ideal sinusoid and also from the fact that even if the BW is small, it is impossible to completely cancel the pulsed nature. In conclusion the FF is always necessary to calibrate the $\Delta I/I$. We can also say that the first case, where the importance of FF is higher, is the most common case in SRS. This because to achieve the best signal-to-noise ratio it is required $\omega = RR/2$, and so it can not be used a low BW in order to not cut away the modulation.

4. SRS cross-section measure

In Fig. 2 it is shown the standard SRG set-up used. The laser (Picus Duo from Refined)

Ratio	Lock-in measure	Oscilloscope measure	FF
96%	20.9 mV	359.3 mV	17.19
Ratio	Lock-in measure	Calculated value	Oscilloscope measure
46%	7.14 mV	122.7 mV	115.2 mV
1.25 %	686.4 μ V	11.8 mV	9.4 mV

Table 1: Data for the 100 MHz photodiode bandwidth

Ratio	Lock-in measure	Oscilloscope measure	FF
95%	40.4 mV	154 mV	3.81
Ratio	Lock-in measure	Calculated value	Oscilloscope measure
32%	57 mV	217.2 mV	216.3 mV
1.23%	2.54 mV	9.68 mV	8.9 mV

Table 2: Data for the 14 MHz photodiode bandwidth

Ratio	Lock-in measure	Oscilloscope measure	FF
97%	60.31 mV	166.2 mV	2.75
Ratio	Lock-in measure	Calculated value	Oscilloscope measure
22%	59.07 mV	162.8 mV	163.8 mV
0.53%	2.26 mV	6.22 mV	5.3 mV

Table 3: Data for the 1.8 MHz photodiode bandwidth

is a commercial laser created for CRS. It emits both the pump and the Stokes beam with RR of 40.5Mhz, selecting from a wide range (700 – 3100 cm^{-1}) of wavenumbers. It modulates the pump and owns an internal delay to achieve temporal overlap between the pump and Stokes pulses. We decided to measure the σ_{SRS} of the C-O methanol bond ($\Omega = 1033cm^{-1}$), since it is the one used as reference also in the cited papers[2, 4]. The methanol was placed inside a cuvette with optical path of 1mm. We started by measuring $\Delta I/I = \Delta I' \cdot FF/I = 2.05\mu V \cdot 4.62/83.5mV = 1.13 \cdot 10^{-4}$, Where $\Delta I'$ is the non calibrated value directly from the lock-in. Then we considered the three aspect named before: spatial, temporal and spectral distribution.

1)Spatial distribution

To acquire the spatial profile $\Phi(x, y, z)$ we used a beam profiler, basically a camera that returns a 2D map of the beam profile (Fig. 3). The value in each pixel represents the **relative** weight of the beam power in the space. We mounted the beam profiler on manual translator and scanned all the 1mm (by a steps of $\Delta z = 30\mu m$) where the sample was placed during the SRG exper-

iment. Calling the map $S(x, y, z)$, $\Phi(x, y, z)$ is retrieved by

$$\Phi(x, y, z) = \frac{S(x, y, z) \cdot \frac{P}{h\nu A_{pixel}}}{\sum_{x,y} S(x, y, z)} \quad (7)$$

Where A_{pixel} is the pixel area of $6.7 \times 6.7\mu m^2$.

2)Temporal distribution

For the temporal distribution we used the gaussian temporal width data directly provided by the laser producer. They are $\tau_p = 9.81ps$ and $\tau_s = 2.41ps$. Inserting them into equation (5) we achieved a value of $K_1 = 0.97$.

3)Spectral distribution

In figure Fig. 4 we can clearly see how the correlation of the two pulse spectra does not excite just the peak at $\Omega = 1033cm^{-1}$ but almost all the vibrational peak. The integral of the product between the spontaneous Raman spectrum and the correlation returns a value of $K_2 = 0.548$

Final results

We start from equation (4) and we substitute in a natural manner the integrals with discrete summation, using equation (7) to consider the profiler maps. We substitute the temporal part

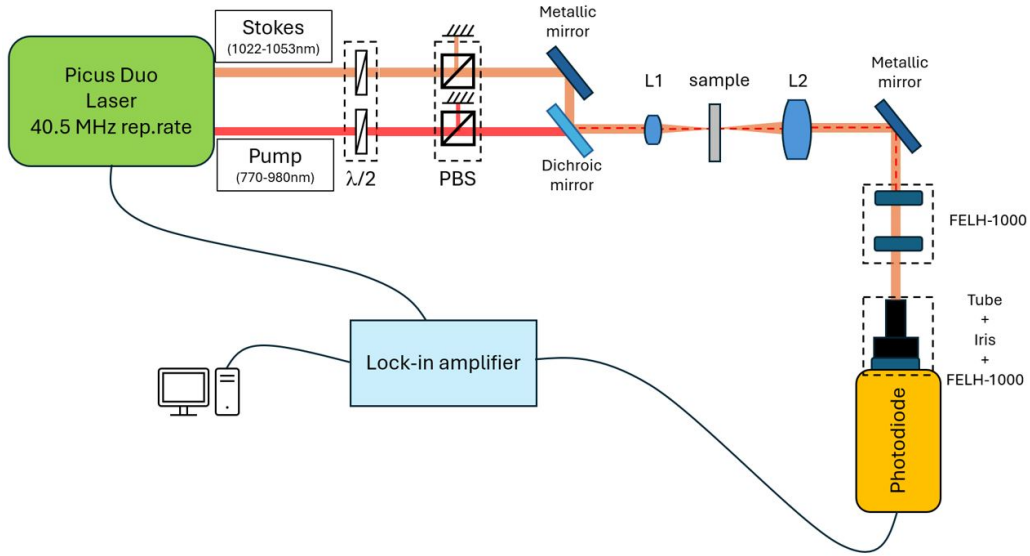


Figure 1: Set-up implied for the SRS measurements. $\lambda/2$ = half wave plate, PBS = Polarized Beam Splitter, FELH-1000 = High performance long-pass filter from 1000 nm, L = lens.

with K_1 and we insert also K_2 . All the constants relative to the Stokes power can still be canceled from numerator and denominator. As in the previous formulation the Stokes power is not influent, but what is relevant is spatial distribution. Regarding the pump power we have to do some consideration. First of all what one typically measure, for example with a powermeter, is the average power P_{ave} . Instead we should consider the real power value arriving within the pulse, the peak power P_{peak} . The two are linked by $P_{peak} = P_{ave}/\tau RR$, where τRR can be seen as a duty cycle. We also used the equation $\nu = c/n_{mat}\lambda$. Finally we should also consider the transmittance between air and cuvette glass ($T_1 = 0.96$ and the transmittance between glass

and methanol $T_2 = 0.996$. The complete and final equation can therefore be written as

$$\left(\frac{\Delta I}{I}\right)_{SRG} = \left(\frac{[c]N_a\sigma_{SRS}P_{p,ave}T_1T_2\lambda_p n_{mat}K_1K_2}{\tau_p RR hc A_{pixel}A_{pixel}}\right) \cdot \sum_z \sum_{x,y} \left(\frac{S_p(x,y,z)}{\sum_{xy} S_p(x,y,z)}\right) \cdot \left(\frac{S_s(x,y,z)}{\sum_{xy} S_s(x,y,z)}\right) A_{pixel} \Delta z \quad (8)$$

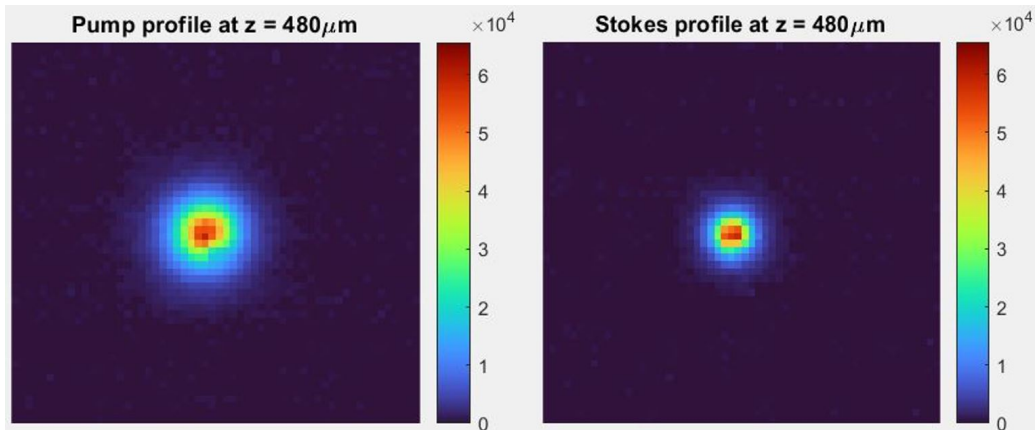


Figure 2: 2D map acquired of both the beams with the beam profiler

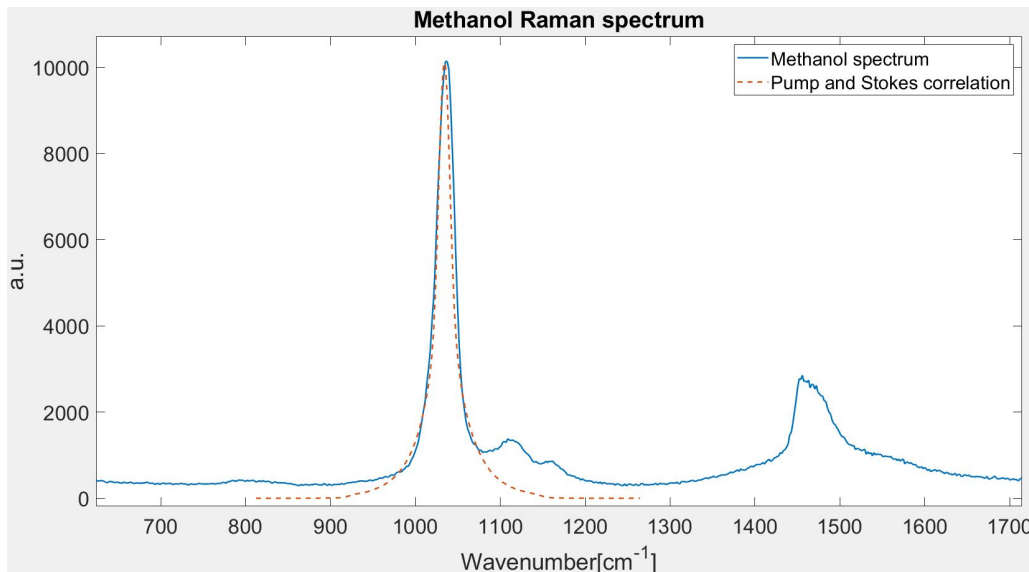


Figure 3: The methanol spontaneous Raman spectrum (blue) and the correlation between pump and Stokes spectra (dashed orange)

Where $[c]$ is 24.71 mol/L , $n_{mat} = 1.32$ and $\lambda_p = 949.3 \text{ nm}$. We decided to not cancel out A_{pixel} to explicitly show the correct unit of measure to use. At the numerator A_{pixel} is expressed in dm^2 to achieve a volume in L , while at denominator they are expressed in cm^2 .

The measured value for σ_{SRS} was 0.367 GM while the measured value in the papers[2, 4] was 0.04 GM . This difference should not really surprise us since the implicit assumption performed by not considering the three distribution are those:

- In the spatial domain the pulses are uniform squares: canceling of Φ_s
- In the temporal domain the pulses are uniform squares: $K_1 = 1$
- Beam spectra are perfect deltas: $K_2 = 1$

Naturally if we put those assumption into equation (8) we would retrieve equation (2). In fact, we calculated also a value of σ'_{SRS} using the simple formula from (2), achieving a value of 0.055 GM . In this case the slight variation is probably due to our use of a simple cylindrical volume in the calculation.

4.1. Conclusions

The aim of this work was to perform a further step towards quantitative SRS measurement. In particular we focused on a developing a process that can be followed in order to calibrate the most precisely possible the σ_{SRS} . In particular the significance of this work lies in the measure

of a universal parameter capable of describing the material SRS response, considering all the perturbations introduced by the measurement process itself. Regarding the first part of the work, we can say that is fundamental to consider the FF when dealing with quantitative SRS experiment. It is important to observe that FF should also be used in all the cases where the lock-in and pulsed laser are employed, for example during the calibration of the relative intensity noise (RIN). Regarding the second part instead we are satisfied from the fact that when we used the same formula we retrieved almost the same value, instead with our new proposed procedure we obtained an higher value as expected.

References

- [1] Ji-Xin Cheng, Wei Min, Yasuyuki Ozeki, and Dario Polli. *Stimulated Raman scattering microscopy: Techniques and applications*. Elsevier, 2021.
- [2] Xin Gao et al. Absolute stimulated raman cross sections of molecules. *The Journal of Physical Chemistry Letters*, 2023.
- [3] Maria Goppert-Mayer. Uber elementarakte mit zwei quantensprungen. *Ann. Phys.*, 1931.
- [4] Wei Min and Xin Gao. The duality of raman scattering. *Accounts of Chemical Research*, 2024.