We report on the first experimental demonstration of the suppression of spontaneous Raman microscopy; (300.6450) Spectroscopy, Raman.

Abstract: We report on the first experimental demonstration of the suppression of spontaneous Raman scattering via ground state depletion. The concept of Raman suppression can be used to achieve sub-diffraction-limited resolution in label-free microscopy by exploiting spatially selective signal suppression when imaging a sample with a combination of Gaussian- and donut-shaped beams and reconstructing a resolution-enhanced image from this data. Using a nanosecond pulsed laser source with an emission wavelength of 355 nm, the ground state of tris(bipyridine)ruthenium(II) molecules solved in acetonitrile was depleted and the spontaneous Raman scattering at 355 nm suppressed by nearly 50%. Based on spectroscopic data retrieved from our experiment, we modeled the Raman image of a scattering center in order to demonstrate the applicability of this effect for superresolution Raman microscopy.

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This is the main advantage of spontaneous and resonance enhanced Raman scattering [5] as well as the coherent Raman methods, e.g., stimulated Raman scattering (SRS) [6] and coherent anti-Stokes Raman scattering (CARS) [7]. However, achieving a spatial resolution comparable to what is possible with fluorescent methods, is still an unsolved task in Raman microscopy.

Several advances have been made in the theory of resolution enhancement for CARS microscopy by numerical investigations using density matrix simulations [8]: the generation of sidebands in the Raman spectrum due to Rabi oscillations driven by a control light beam in the mid-infrared [9], the suppression of the CARS signal by an incoherent population of the vibrational states by irradiation of the sample with mid-infrared light [10] or by stimulated emission pumping (STEP) [11], and the suppression of the CARS signal by depletion of the ground state of the molecules under test [12].

Experimentally, first successes in resolution enhancement were accomplished for CARS setups without injection of an additional beam, but simply by employing structured beams by using vortex phase plates [13] or annular phase masks generated with spatial light modulators [14]. Such methods, however, can only provide a limited resolution enhancement in contrast to the fluorescence superresolution techniques, with which in principle an infinitely high resolution can...
be obtained, provided that control beam intensity (for STED) or imaging duration (for STORM or PALM) are no issues.

Another experimental approach was recently implemented for SRS microscopy, with which a resolution enhancement was demonstrated by employing an additional coherent light beam, which was interpreted by the authors as acting for the destruction of vibrational coherences [15]. As a possible explanation of its physical function, competing four-wave mixing processes are suggested. Although it is known that simultaneously present parametric processes can lead to sidebands in the Raman spectrum and therewith allow to suppress the carrier wave [9], the presented hypothesis seems to be insufficient to predict the general applicability of this method.

For Raman microscopy it is, therefore, still one of the most important goals to develop a superresolution technique, which can provide a sub-diffraction-limited resolution with a theoretically infinite factor of resolution improvement. To allow predictions on the impact and general applicability of a method, it needs to be consistent with a straightforward theoretical description. Furthermore, it is desirable that it can be applied for not only a single but any variant of Raman microscopy, i.e., spontaneous Raman scattering, the different versions of SRS, as well as CARS.

For this goal, the method of ground state depletion (GSD) appears as especially promising. The basic idea is that a depletion of the ground state will suppress the Raman scattering, as little population remains in the state from which the Raman scattering originates. This suppression can be employed to enhance the spatial resolution of a Raman microscopic detection, not only for coherent Raman processes (such as CARS [12]) but also for spontaneous Raman scattering. The latter is most straightforward, requiring only a single excitation wavelength and a simpler setup for the detection of the Raman spectrum, and investigations conducted with spontaneous Raman scattering can easily be transferred to coherent Raman methods.

If an experimental evidence of Raman suppression via GSD could be obtained, this effect could be employed for a superresolution technique in the following way: when using a beam of a single wavelength that generates the Raman scattering signal while depleting the ground state of the sample, the detected Raman signal would show a saturation behavior with increasing pulse energy. In this case, a resolution-enhanced image could be obtained by subtracting two images from each other: one acquired by scanning the sample with a donut-shaped beam and one acquired with a beam that is a combination of the donut-shaped beam together with a ten-fold lower intensity Gaussian beam. This procedure would lead to a resolution-enhanced image, because in its regions of highest intensity, the donut-shaped beam would already drive the Raman signal into saturation. In the combined beam, the edges of its Gaussian part would overlap with these regions and, due to the saturation, contribute only to an insignificantly increased amount of Raman scattering compared to the donut-shaped beam alone. Therefore, after subtraction, mainly the Raman signal from the center of the Gaussian beam would remain in the difference image.

Although GSD was successfully used to enhance the resolution in fluorescence microscopy [16] and in transient absorption microscopy [17] its experimental applicability for the resolution enhancement in Raman microscopy yet remained short of a proof.

2. Experimental suppression of Raman scattering

Here, we show the first experimental demonstration of the suppression of spontaneous Raman scattering via ground state depletion (GSD) in a proof-of-principle experiment towards Raman microscopy with sub-diffraction-limited resolution.

For our demonstration, we have chosen the metal complex tris(bipyridine)ruthenium(II) (Ru(bpy)$_3^{2+}$), due to a number of basic and technological advantages. As a result of its highly absorptive electronic transitions in the ultraviolet (UV) (approximately $5.5 \times 10^3$ mol$^{-1}$ cm$^{-1}$ at a wavelength of 355 nm [18]), it should be possible to indirectly populate the first excited state and, as this is a triplet charge transfer state, almost entirely deplete the ground state of Ru(bpy)$_3^{2+}$.
using a conveniently available standard light source such as a frequency tripled Nd:YAG laser at a wavelength of 355 nm. By irradiating the sample with the laser beam, a strong Raman scattering signal is generated simultaneously to the molecular excitation, as the UV transitions resonantly enhance the spontaneously generated Raman scattering, resulting in an increased signal-to-noise ratio (SNR) of the detection.

It is another important advantage of Ru(bpy)$_3^{2+}$ that molecules in the lowest excited state are also responsive to spontaneous Raman scattering. This attribute is due to additional electronic states above the lowest excited state (at wavelengths between 250 nm and 400 nm [18]), which also support a strong, resonance enhanced Raman scattering signal. The Raman spectrum of excited Ru(bpy)$_3^{2+}$, however, exhibits a set of peaks which differ from the ones of the ground state Raman spectrum [19]. This should enable the possibility to observe GSD directly as spectral changes in the Raman emission.

Excited Ru(bpy)$_3^{2+}$ molecules display a long lifetime (890 ns in acetonitrile at room temperature [20]), which enables the excitation of a large number of molecules with nanosecond laser pulses, as the spontaneous decay is small on the time scale of the pulse duration. Upon their decay, Ru(bpy)$_3^{2+}$ molecules emit fluorescence light in the orange-colored spectral region around 600 nm wavelength [21], far apart from the Raman scattered light between 360 and 400 nm, therefore, having no impact on the detection of the Raman spectra. Nevertheless, the fluorescence emission can be observed separately and used to verify that molecules remain intact when irradiated with high pulse energy.

The reasons for the advantageous properties of Ru(bpy)$_3^{2+}$ can be found in its structure. It consists of a Ruthenium ion surrounded by three bipyridine ligands in a hexagonal shape. The lowest excited state results from a metal-to-ligand charge transfer transition [21, 22], which significantly changes the vibrational behavior of the molecule leading to a different Raman spectrum. The molecule has been of interest for various researchers in the past as a convenient sample to study the general behavior of molecules in their excited state [23]. An extensive review on the properties of Ru(bpy)$_3^{2+}$ can be found in [21].

![Fig. 1. Experimental setup for the Raman scattering signal suppression via ground state depletion. HWP: half-wave plate; PBS: polarizing beam splitter; BD: beam dump; DM: dichroic mirror; AL: aspherical lens; S: sample (in half-filled cuvette); M: mirror; LP: long-pass filter; L: lens; CCD: charge-coupled device.](image)

The setup for the suppression of Raman scattering via ground state depletion is depicted in Fig. 1. As a light source a frequency-tripled Nd:YAG laser was used, which emitted 13 ns pulses with a wavelength of $\lambda = 355$ nm at a repetition frequency of 500 Hz. The laser beam (diameter $d = 2.7$ mm (FWHM)) was focused into the sample with an aspherical lens (focal length $f = 11$ mm) resulting in a focal spot with a diameter of approximately $1.22 \cdot \lambda/NA = 3.5$ µm (FWHM). Raman scattered light was collected in backwards direction with the same lens and transmitted through a dichroic mirror with an edge wavelength of 358 nm. An additional long-pass filter with the same edge wavelength was used to block remaining laser light. The beam was then focused onto the slit of a 0.5 m Czerny-Turner monochromator (using a grating with 1200 lines mm$^{-1}$ blazed for 300 nm) for scattered light detection with a UV-enhanced silicon CCD camera.
The sample was prepared as a solution of 10 mM Ru(bpy)$_3^{2+}$ hexafluorophosphate in acetonitrile and contained in a half-filled, horizontally lying glass cuvette, which was positioned such that the focus of the laser beam was located directly below the surface of the solution. This design combined the advantages of a high concentration of Ru(bpy)$_3^{2+}$, which is beneficial for a high Raman scattering signal, with a low penetration depth into the sample, which avoids signal loss due to absorption of light outside the focus. The setup furthermore ensured the glass walls of the cuvette to be distant from the focal spot to prevent, e.g., perturbation of the Raman scattering or structural changes within the glass when irradiated with intense UV light.

Spontaneous Raman scattering spectra were recorded in a spectral range from 300 to 2700 cm$^{-1}$ (with a resolution of 6 cm$^{-1}$) with laser pulse energies between 0.06 and 1.48 µJ. The pulse energy was controlled by rotating a half-wave plate (HWP) in front of a polarizing beam splitter (PBS), which avoided changing other pulse parameters such as the duration or shape. The angle of the HWP was calibrated to the average laser power, which was measured with a thermal power sensor at the position of the sample prior to the experiment.

As the Raman scattered light was emitted in the wavelength region between 360 and 400 nm, it was strongly reabsorbed within the sample, which reduced the Raman signal yield. To achieve a high SNR, it was necessary to integrate the Raman spectra over a long time (900 s for the lowest applied pulse energy of 0.06 µJ). This integration time was used for all measurements to ensure equal acquisition properties in terms of linearity and noise of the detection.

![Fig. 2](image_url)

**Fig. 2.** (a) Two examples of spontaneous Raman spectra of Ru(bpy)$_3^{2+}$ in acetonitrile acquired with two different pulse energies (0.06 µJ, straight blue line; and 1.48 µJ, dash-dotted red line), individually normalized to their fluorescence background. Raman peaks of the ground and excited state are marked G$_i$ and E$_i$, respectively. (b) Suppression of the spontaneous Raman scattering of the ground state peak G$_5$ with increasing pulse energy (blue dots, scale on left vertical axis). Simultaneously, occurrence of excited state Raman scattering of excited state peak E$_3$ (red diamonds, scale on right vertical axis). See also Visualization 1.

Figure 2 (a) depicts two of the recorded Raman spectra in the range from 1150 to 1650 cm$^{-1}$. These spectra were acquired at two different pulse energies of the incident light of 0.06 µJ (straight blue line) and 1.48 µJ (dash-dotted red line), respectively. In order to display the spectra comparably to each other in spite of the different pulse energies used, the spectra were individually normalized to their fluorescence background, which results from the excitation of further electronic states and is common for resonance enhanced spontaneous Raman spectroscopy [5].
One normalized unit corresponds to approximately 100 cts for the low-pulse-energy spectrum and approximately 3000 cts for the high-pulse-energy spectrum. The wavenumber axis was calibrated using the peaks of the Rayleigh scattering of the laser at 0 cm\(^{-1}\) and the Raman scattering of the C-N triple bond vibration of acetonitrile at 2256 cm\(^{-1}\) [24].

The Raman spectrum that was obtained at low pulse energy (straight blue line) showed peaks at 1175 cm\(^{-1}\) (G\(_1\)), 1320 cm\(^{-1}\) (G\(_2\)), 1493 cm\(^{-1}\) (G\(_3\)), 1564 cm\(^{-1}\) (G\(_4\)), and 1608 cm\(^{-1}\) (G\(_5\)), which resulted from the vibrational states of the electronic ground state. The spectrum at high pulse energy (dash-dotted red line) displayed additional peaks at 1210 cm\(^{-1}\) (E\(_1\)), 1284 cm\(^{-1}\) (E\(_2\)), 1427 cm\(^{-1}\) (E\(_3\)), 1506 cm\(^{-1}\) (E\(_4\)), and 1550 cm\(^{-1}\) (E\(_5\)), which resulted from the vibrations of the lowest excited state. The small peak around 1376 cm\(^{-1}\) (S) originated from the solvent acetonitrile. The acquired Raman spectra were comparable (peak positions mostly within a range of less than 7 cm\(^{-1}\)) to other studies in which the structure and behavior of Ru(bpy)\(_2^2^+\) was analyzed [19, 23].

Figure 2 (b) shows the suppression of the Raman scattering due to GSD of the ground state peak G\(_5\) (blue dots) with increasing pulse energy of the incident light. Additionally, an increasing excited state Raman scattering is indicated by the excited state peak E\(_2\) (red diamonds). The horizontal axis displays the incident pulse energy \(E_P\). The vertical axis displays the Raman response

\[
\frac{S_R}{S_{bg}} = \frac{(S_{tot} - S_{bg})}{S_{bg}},
\]

with \(S_{tot}\) being the total spectral power density of a peak, \(S_R\) the spectral power density of the Raman scattering, and \(S_{bg}\) the spectral power density of the fluorescence background in the vicinity of the respective peak. The latter was verified to increase linearly with the pulse energy (average detection rate approximately 2000 cts \(\mu J^{-1}\)).

It can be seen that, while the signal of the excited state Raman scattering increased with higher pulse energy, the ground state Raman scattering significantly decreased in comparison to the background due to GSD and approached a value of nearly 50% of its initial value at a pulse energy of 1.5 \(\mu J\). Note that it is in the nature of our measurement approach that the signal was acquired as an integral over the entire transverse and longitudinal light distribution in the focus and that the suppression should be even stronger in the center of the focus. In comparison to this result, STED microscopy routinely reaches a fluorescence suppression of nearly 100% [1]. However, our measurement is the first experimental evidence of the suppression of Raman scattering by GSD, which is a prerequisite to enhance the spatial resolution of Raman microscopy to below the diffraction limit.

3. Verification of ground state depletion by rate equation theory

For the significance of our results it was important to ensure that the measured suppression of the Raman signal originated from the GSD and not from any other effect. Such an effect would, e.g., be the destruction of the molecules under test at high pulse energies. In order to exclude the presence of such effects, we observed the excited state Raman scattering and the fluorescence emission of Ru(bpy)\(_2^2^+\), which both only occur with intact molecules. As the excited state Raman scattering increased with increasing pulse energy as expected (see Fig. 2 (b) and theory below), it can be concluded that the molecules under test remained intact. The fluorescence emission of Ru(bpy)\(_2^2^+\) was observed from pulse to pulse as well as over long time scales (> 12 h) in a separate measurement with the spectrometer replaced by a fast photodiode. We only detected fluctuations in the order of ±10% but no depletion, which we again took as an indicator that the molecules under test remained intact. To reduce thermal influence on the sample and to ensure that high laser powers did not affect the structure of the glass of the cuvette, we employed the above described setup and restricted our measurement to pulse energies below 1.5 \(\mu J\).
In order to verify the physical explanation that the Raman suppression is a result of GSD and to obtain a quantitative description of the Raman signal emission, which can be used to calculate the possible resolution enhancement, the data from Fig. 2 (b) was compared with a rate equation theory. A simple three-level scheme can be assumed (based on a state diagram of Ru(bpy)$_2^{3+}$ as it can be found, e.g., in [21]): The molecules are excited from the ground state (energy level 1) to an upper excited electronic state (level 3) that is located above the lowest excited state and is in resonance with the wavelength of 355 nm. From there, the population is rapidly transferred to the lowest excited state (level 2). Then the molecules relax back to the ground state with the decay time $\tau = 1/A_{12}$. As the transition from the upper to the lowest excited state is fast (approximately 10 ps) in comparison to the pulse duration ($>10$ ns), it can be assumed that, after excitation, the population is instantly transferred into the lowest excited state and stimulated emission from the upper excited state into the ground state can be neglected. The corresponding rate equation for the relative populations $n_1$ and $n_2$ of the ground state and the lowest excited state is

$$\frac{dn_2}{dt} = B_{13} n_1 p - A_{12} n_2,$$

with $A_{12}$ and $B_{13}$ being the Einstein coefficients of the transitions from the excited state to the ground state and from the ground state to the upper excited state, respectively. The variable $p$ is the spectral power density of the laser in the focus, which is proportional to the pulse energy $E_p$. The relative populations follow from the steady state solution of this rate equation and the assumption $n_1 + n_2 = 1$, i.e., that all population stays either in the ground state or in the excited state, as justified by the short lifetime of the upper excited state.

For a comparison of the predictions of the rate equation model with the experimental data we note that the detected signals of the ground state Raman scattering $S_{gs}$ and the excited state Raman scattering $S_{es}$ are proportional to the relative populations $n_1$ and $n_2$ of the ground and lowest excited state, respectively, and to the laser pulse energy $E_p$. The background fluorescence signal $S_{bg}$ is also proportional to the laser pulse energy $E_p$. Therefore, the following equations represent the theoretically expected functional relation that should match the measured experimental decrease of the ground state Raman signal as well as the increase of the excited state Raman signal from Fig. 2 (b):

$$\frac{S_{gs}}{S_{bg}} = \frac{\alpha_1}{1 + \delta E_p} \quad \text{and} \quad \frac{S_{es}}{S_{bg}} = \frac{\alpha_2 \delta E_p}{1 + \delta E_p},$$

with $\alpha_1$ and $\alpha_2$ being the proportionality factors of the Raman scattering and $\delta \propto B_{13}/A_{12}$.

Using the parameters $\alpha_1$, $\alpha_2$ and $\delta$ as fit coefficients, Eq. (3) was fitted to the data from Fig. 2 (b), leading to the depicted, so-called saturation curves, with the resulting fit values being $\alpha_1 = 1.6$, $\alpha_2 = 9.7$ and $\delta = 0.6$. The rate equation theory is in good agreement with the experimental data (within the uncertainties of all individual data points), which proves that GSD was the mechanism that suppressed the Raman signal. Furthermore, the rate equation theory provides a straightforward model from which predictions can be derived. It can, e.g., be expected that the suppression of peak G$_5$ would approach a 90 %-level at a pulse energy of approximately 15 $\mu$J.

4. Envisioned resolution enhancement via ground state depletion

In order to underline the important implications of our results, we present in the following the according resolution enhancement for a Raman microscopic detection of a scattering center that has similar properties to Ru(bpy)$_2^{3+}$. We simulated an imaging process as described in Sec. 1, using the saturation curve of the ground state Raman response of resonance G$_5$ from Fig. 2 (b) as a basis to determine the emitted Raman signal of a sample that was irradiated with excitation light of a specific intensity.
In doing so, we accounted for the fact that the Raman signal of our measurement was a result of the entire lateral and axial light distribution integrated over the focus. The lateral structure of the light field in the focus was assumed to be Gaussian-shaped, which means that the local intensity in the center was by a factor of two higher than the average intensity. Therefore, the intensity axis of the fit curve that was gained from the experimental data was rescaled by a factor of two in order to calculate the correct Raman scattering signal for each local intensity value of the incident light distribution. As the focusing was loose (Rayleigh length $z_R \approx 80 \, \mu m$), the axial structure of the beam was neglected.

As a virtual test sample we chose a single scattering center of 50 nm diameter (see Fig. 3 (a)) on a surface (size $2 \times 2 \, \mu m^2$, numerical resolution 10 nm). The sample was numerically scanned with a diffraction-limited Gaussian (TEM$_{00}$) beam, with a donut-shaped (TEM$_{01}$*) beam, and with a combination of the donut-shaped beam together with a ten-fold lower intensity Gaussian beam. For each scanning position the Raman emission was calculated (as described above) individually for each sample pixel that overlapped with the beam, taking the local intensity of the beam into account. The resulting Raman emission was calculated as a sum of the individual scattering signals emitted by the sample pixels and plotted versus the scan coordinates (see Fig. 3 (b-d)). For both, the Gaussian and the donut-shaped beam, a beam waist (half width at $1/e^2$) of 350 nm was assumed. The peak intensity was set to 5.3 kW cm$^{-2}$, which corresponds to the peak intensity of the beam with the highest pulse energy of 1.5 $\mu$J that was used in our experiment to suppress the Raman signal by nearly 50 % and (according to the beam shape correction described above) should lead to a suppression of 65 % in the center of the focus.

A resolution enhanced image (see Fig. 3 (e)) was obtained by exploiting the saturation behavior of the Raman signal by subtracting the image obtained with the donut-shaped beam (see Fig. 3 (c)) from the one obtained with the above mentioned beam combination (see Fig. 3 (d)). The resulting image shows a point spread function (PSF) of 175 nm FWHM, which is by a factor of three narrower in comparison to the PSF (536 nm FWHM) in the image generated by the diffraction-limited Gaussian beam only (see Fig. 3 (b)). If, in a less-than-ideal imaging situation, the Raman suppression in the center of the focus would be lower, e.g., 50 % or even only 25 %, the resolution-enhancement would still reach a factor of 2 or 1.4, respectively.

The above results demonstrate that GSD should enable to significantly enhance the resolution in Raman microscopy. However, the described method, using only a single laser for both Raman scattering and GSD, is fundamentally limited in the maximally achievable Raman suppression. This is due to the leading edge of the pulse that will always generate ground state Raman scattered...
light before having transferred a major part of the molecules into their excited state. The Raman
signal generated by the trailing edge of the pulse is subsequently suppressed. As a fixed amount
of energy is needed for the excitation of the molecules, higher pulse energies could be used to
raise the ratio between these two parts of the pulse, if possible damaging of the sample is no
issue. Besides, the remaining ground state signal could be further reduced by only detecting
in the trailing edge of the pulse, i.e., by employing a fast optical switch, e.g., an electro-optic
modulator synchronized to the laser pulse emission, to filter out the Raman signal of the leading
pulse edge by a time window for signal transmission that is adjustable in starting time and width.

Another possibility to increase the Raman suppression gained by GSD is the application of
two synchronized pulsed lasers. At first, a (donut-shaped) UV beam for GSD is irradiated into
the sample, followed by a (Gaussian) beam with a wavelength above the fluorescence emission
of \( \text{Ru(bpy)}_2^3^+ \) to perform the Raman scattering. By this way, the reabsorption of the Raman
scattered light and the background fluorescence, which both are high for UV Raman scattering,
could be circumvented and therefore the SNR improved. The additional laser beam could be
generated by building an UV seeded optical parametric amplifier or by synchronizing the UV
laser to another laser with the desired wavelength. A potential setup could be either arranged
using spontaneous Raman scattering or – by involving a third wavelength – as a SRS or CARS
setup. Additionally, by using a coherent Raman method, one of the main obstacles on the way
to implement this concept into a scanning Raman microscope could be overcome, as the SNR
could be improved by a factor of \( 10^5 \) and, therefore, the integration time of currently 900 s being
reduced and the detection inherently confocal.

It is also an important question, for which types of molecules superresolution GSD Raman
microscopy could be used. We note that the excited state Raman scattering signal as it is emitted
by \( \text{Ru(bpy)}_2^3^+ \) in our experiment is not a prerequisite for a sample that is suitable for GSD Raman
microscopy. Indeed, molecules that do not emit a measurable excited state Raman signal because
the transitions from the lowest to higher excited states are weak could be equally well used
as a sample as it is only necessary for the ground state Raman scattering signal to decrease
when the ground state of the molecules is depleted. It is, therefore, sufficient for a sample to
feature a state that can receive population from the ground state and that has a life time which is
longer than the duration of the pulses that are used to generate the Raman scattering. From the
work described in this article, it can be concluded that GSD Raman microscopy should be well
applicable for samples such as \( \text{Ru(bpy)}_2^2^+ \), which feature a long lived excited state that can be
indirectly populated and, therefore, receive nearly the entire population of the ground state. It
is, however, a reasonable assumption that GSD can also be employed using directly populated
excited states, which can be seen as follows. In a simple two-state system, the GSD could reach
at least a value of 50 %, which was estimated to be sufficient for a potential twofold resolution
enhancement. The effect could be increased by utilizing the simultaneous population of more
than one excited state at the same time, which is an idea that was numerically investigated for
GSD CARS microscopy in the past, with the result, that by using two instead of one excited state,
the minimally achievable ground state population could be lowered from a half to a third [12].
To verify these predictions, future investigations should follow to assess the applicability of
GSD on other molecules with similar properties to \( \text{Ru(bpy)}_2^3^+ \) as well as molecules without a
long-living fluorescence state, which better resemble samples that are typically examined with
Raman microscopy.

Another relevant parameter to study is the necessary laser power or intensity for the GSD.
The average power and the maximum focal intensity that were used in our experiment were
0.75 mW and 5.3 kW cm\(^{-2}\), respectively, which are in the range of what was previously reported
for biological imaging, e.g., the SRS setup from [6] uses less than 40 mW with 30 MW cm\(^{-2}\)
per beam and the CARS setup from [7] employs 50 – 100 \( \mu \)W per beam. However, as our setup
uses a 500 Hz repetition rate, the pulse energy is considerably higher (1.5 \( \mu \)J corresponding to
5.3 J cm\(^{-2}\)) in comparison to setups, which feature a kilo to megahertz repetition rate (0.5 nJ \([6]\) and 0.2 – 0.4 nJ \([7]\) per beam). Nonetheless, GSD should be possible also with a far lower pulse energy, as it is known that for certain samples such as graphite nanoplatelets an equal population distribution of ground and excited state can be reached with a pulse energy fluence of only 15 mJ cm\(^{-2}\) \([17]\). Also our previous numerical studies on GSD as well as STEP for CARS suggested that Raman suppression should be possible with by up to several orders of magnitude lower pulse energy \([11, 12]\). Therefore, future research may show a way to implement GSD in Raman microscopy.

5. Conclusion

In conclusion, we reported the suppression of spontaneous Raman scattering by nearly 50\% using ground state depletion. The observed effect is in good agreement with theoretical predictions based on simple rate equations. We simulated an application of this effect in Raman microscopy on the basis of the experimental data that we gained with the metal complex tris(bipyridine)ruthenium(II) and obtained a theoretical resolution enhancement in lateral dimensions by a factor of three below the diffraction-limit. Therefore, our results demonstrate the general applicability of ground state depletion in the achievement of sub-diffraction-limited spatial resolution in label-free microscopy.

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