Saturated Raman scattering for sub-diffraction-limited imaging ^F

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ABSTRACT

We present a scheme for a sub-diffraction-limited Raman microscope. The scheme combines the concept from stimulated depletion microscopy with femtosecond stimulated Raman scattering. The suppression of the Raman signal in a three-beam setup with only two involved wavelength-components was accomplished by the saturation of the Raman scattering. A reduction of the Raman signal of up to 79% could be measured with only a single Raman resonance involved. Based on this signal suppression, a resolution enhancement by a factor of 2 could be verified in a first proof-of-concept measurement, opening up a pathway toward label-free sub-diffraction-limited imaging.

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I. INTRODUCTION

Over the last few decades, Raman scattering has become a powerful method in analytical spectroscopy¹ and label-free imaging² to study the chemical composition of a sample. Background-free Raman spectra with a high spectral quality can be acquired using spontaneous Raman scattering³ but with a poor signal strength, which leads to acquisition times in the order of seconds for a single spectrum.⁴ In contrast, coherent Raman scattering (CRS) techniques such as stimulated Raman scattering (SRS)^{5,6} and coherent anti-Stokes Raman scattering (CARS)⁷ show a signal amplified by multiple orders of magnitude and can therefore even be used for ultra-fast spectroscopy^{8,9} and *in vivo* video-rate imaging of unstained cells and tissues.^{2,10,11}

However, in most cases, the spatial resolution in CRS is still near the diffraction limit, whereas in fluorescence microscopy, super-resolution techniques^{12–16} are widely used and enable, for instance, a lateral resolution down to 20 nm when using stimulated emission depletion (STED) microscopy for the imaging of biological samples.¹⁷

Nevertheless, the need for labeling with fluorescent dyes remains a major downside of fluorescence microscopy, as size,

availability, toxicity, and photobleaching of the used dyes can complicate measurements.^{18–20} Therefore, combining the super-resolution concepts from fluorescence microscopy with the chemical selectivity as well as the label-free measurement in CRS would be very attractive for imaging cells and tissues.

In the recent past, methods to overcome the diffraction limit in Raman microscopy have been investigated.²¹ For instance, the suppression of the Raman signal in the outer region of the focal volume with a STED-like approach shows great potential for future super-resolution CRS imaging. The suppression of the Raman signal can be achieved by the depletion of one of the scattering resources involved in the Raman process: (i) the electrons in the molecular ground state or (ii) the involved photons.

For option (i), different methods have been investigated in the past. Ground state depletion (GSD) to a higher electronic state can be accomplished by using a laser at 355 nm to deplete the electrons in the molecular ground state and to excite the (near-) resonance-enhanced Raman signal at the same time.^{22–25} For laser pulse energies in the order of 1.4 μ J, a reduction of the ground state Raman signal by up to 50% could be shown, but the experiment was fundamentally limited using only a single laser for both Raman scattering and GSD.²⁴

Alternatively, for option (ii), the SRS and CARS signal can be suppressed by using an additional competing SRS process, which leads to photon depletion of the pump beam.^{26–28} In these experiments, the pump photons are depleted via a strong Raman transition, whereby the signal of a second, weak Raman transition could be suppressed by up to 58% using light pulses at three different laser wavelengths, two different molecular resonances, and a depletion pulse energy of 250 nJ.²⁹

Silva *et al.* introduced a so-called decoherence beam to suppress the SRS signal in a doughnut-shaped mode, which led to an improvement in the spatial resolution by a factor of 2, when scanning the edge of a Raman-active diamond.³⁰ The actual suppression mechanism was stated to be an “alternative four-wave mixing pathway.”³⁰

Recently, studies about far-field super-resolution imaging based on saturated CARS and SRS have been demonstrated, enabling a resolution enhancement by a factor of 1.27 for CARS³¹ and 1.41 for SRS^{32,33} via detecting higher-order harmonics of the signal. However, to get an improvement in spatial resolution, the CARS and SRS images have to be acquired by applying several different power levels in the excitation beam in order to extract the nonlinear behavior of the process, which extends the recording time as well as the power load on the sample substantially.

In this article, we present a setup for the suppression of femtosecond stimulated Raman scattering (FSRS). We have chosen an FSRS excitation and detection scheme to show the spectral evolution around the Raman resonance during the suppression process. The suppression scheme is directly transferable to SRS, as only two wavelength-components and three beams are involved. Furthermore, only one molecular vibration is needed and no *a priori* information about the molecular spectrum is necessary. Here, the saturation of the Raman process by the so-called depletion beam leads to a suppression of the FSRS signal of up to 79%. The saturation is accomplished by two related effects: depletion of the pump photons and ground state depletion of the electrons to a vibrational state of the molecule. Moreover, a spatial resolution enhancement by a factor of 2 could be shown in a first proof-of-concept measurement, verifying the potential of the investigated suppression scheme.

II. SUPPRESSION SCHEME

Molecular vibrations are probed within FSRS by the interaction of two incident light fields: These light fields are called the broadband pump field around the center frequency ω_p and the narrowband Stokes field at the frequency $\omega_s < \omega_p$. If the energy difference between these light fields matches the energy of a vibrational resonance $\Omega_{\text{vib}} = \omega_p - \omega_s$, an energy transfer from the pump to the Stokes field can be measured. In general, for a single vibrational resonance, the change in the intensities of the pump and the Stokes fields can be written as

$$\Delta I_p \propto -N\sigma_{\text{Raman}}I_pI_s \quad (1)$$

and

$$\Delta I_s \propto N\sigma_{\text{Raman}}I_pI_s, \quad (2)$$

where N is the number of scattering targets, I_p and I_s the intensity of the pump and Stokes field, respectively, and σ_{Raman} the

specific Raman cross section of the involved molecular resonance. In this work, the combination of a broadband pump beam, subsequently bandpass-filtered around the Raman resonance, and a narrowband Stokes beam was used to detect the stimulated Raman loss (SRL) of the pump beam. In FSRS, femtosecond pulses are used and the wavelength-dependent change in intensity $\Delta I_p(\lambda)$ is typically measured for all wavelengths simultaneously by means of a spectrometer,⁹ resulting in a molecule-specific spectrum. Whereas in SRS, typically, picosecond pulses are used and the intensity change at a certain wavelength is measured by a single photodetector.³⁴

In our case, pump depletion in combination with ground state depletion of molecules to a vibrational state accomplishes saturated FSRS in a three-beam setup with only two wavelength-components involved. The excitation of molecules to a higher electronic state can be neglected, as we use light in the near-infrared and, therefore, are far away from electronic transitions. In the experiments shown later, about 10^4 times more molecules than pump photons are present in the focal volume, from which we conclude that photon depletion is the dominant effect for the saturation of the Raman signal. Figure 1 presents the working principle: (a) In the unsuppressed case, the simultaneous irradiation of the sample with the combination of pump and Stokes beams induces an SRL_S of the pump and a stimulated Raman gain (SRG_S) of the Stokes beam. (b) In the suppressed case, a third, strong beam, working as the depletion beam at the center frequency $\omega_D = \omega_s$, reduces the pump beam energy via SRL_D induced by the depletion beam and the corresponding energy is shifted into the Stokes beam, resulting in a SRG_D. The shortage of pump photons leads to a saturation of the Raman process, as

(a) unsuppressed case

(b) suppressed case

FIG. 1. (a) Stimulated Raman loss (SRL_S) and gain (SRG_S) induced by the combination of the pump and Stokes beams at the frequencies ω_p and ω_s , respectively. In (b), the suppression of SRL_S via the depletion beam at the frequency ω_D is shown. The combination of the depletion beam and the pump beam leads to an SRL_D of the pump and an SRG_D of the depletion beam. The significant shortage of pump photons induced by SRL_D results in a suppressed SRL_{Sup} and SRG_{Sup} of the Stokes beam. The arrows visualize the energy transferred via the Raman process by the Stokes (purple, weak) and the depletion (green, strong) beams, respectively. The shown intensities I are not true to scale.

described in Sec. IV A and shown in Fig. 3. Thus, if SRL_S is detected while the depletion beam is turned on, only a small amount of SRL_S induced by the Stokes beam can be measured. Thus, there is only a reduced signal SRL_{Sup} when the depletion beam is turned on, and the Stokes beam experiences a reduced stimulated Raman gain SRG_{Sup} . The energy, transferred via the FSRS process, is visualized for the (weak) Stokes and the (strong) depletion beam by the purple and green arrows, respectively. This method enables the suppression of FSRS without the need for a second molecular vibration, and further on, no *a priori* information about the molecular spectrum is necessary in contrast to the work of Kim *et al.*²⁹

III. EXPERIMENTAL DETAILS

For the experiments presented in this article, a homebuilt FSRS setup was used (Fig. 2). The setup was based on a master-oscillator power-amplifier (MOPA) system with a repetition rate of 1 MHz emitting pulses with a duration of 330 fs at a center wavelength of 1030 nm. The laser pulses were split into three parts: in the first arm, a supercontinuum generation (SCG) was accomplished by focusing (L1, $f = 75$ mm) pulses, each with a pulse energy of 450 nJ, into a 4 mm long gadolinium vanadate crystal (GdVO₄). The resulting pulses, acting as the pump radiation, span the spectral range from 600 to 1600 nm and were bandpass-filtered around the Raman resonances of acetonitrile (780–800 nm) and later of the potassium yttrium tungsten (KYW) crystal (935–955 nm). The filtering was accomplished by a set of short-pass (SP) and long-pass filters (LP).

FIG. 2. Experimental setup for the suppression of FSRS with half-wave plate (HWP), polarizing beam splitter (PBS), supercontinuum generation (SCG), picosecond divided-pulse amplifier (ps-DPA), time delays (Δt), piezoelectric-mirror (PM), dichroic mirror (DM), 50:50 beam splitter (BS), lenses (L#), short-pass filter (SP), long-pass filter (LP), and spectrometer. The sample was mounted on an automated XYZ translation stage. For details, see text.

In order to get a spectral resolution of 12 cm^{-1} within the FSRS spectra, Stokes pulses with a duration of 3.8 ps were generated in a divided-pulse amplification scheme^{35,36} (ps-DPA) within the second arm of the experiment.

In the third arm, the laser pulses were used as the depletion pulses and the optical path length was slightly modulated by a piezomirror with a frequency of around 253 Hz to average over the interference between the Stokes and the depletion beams being both centered at 1030 nm.

Subsequently, the resulting three laser beams were overlapped spatially at a 50:50 beam splitter cube (BS) and a dichroic mirror (DM), and temporally by means of optical delay lines (Δt), and finally focused into the sample with a lens (L3, $f = 25$ mm). A further lens (L4, $f = 25$ mm) collected the transmitted light and guided it to a spectrometer after filtering out the Stokes radiation by means of a short-pass filter (SP).

An FSRS spectrum is defined as the normalized difference between a spectrum $I(\lambda)$ recorded with the pump pulses overlapping with the Stokes pulses in time (Stokes on) and a spectrum recorded with no overlap in time (Stokes off),

$$SRL(\lambda) = \frac{I_{\text{Stokes on}}(\lambda) - I_{\text{Stokes off}}(\lambda)}{I_{\text{Stokes off}}(\lambda)}. \quad (3)$$

In order to keep the thermal influence within the sample constant, we varied the temporal overlap by automated optical delay lines, instead of using a chopper wheel. The overall acquisition time for an FSRS spectrum was equal to the sum of the acquisition time of the spectrometer (1 ms, 100 averages) and the movement of the optical delay line (typically 500 ms).

IV. EXPERIMENTAL RESULTS

A. Saturation and suppression of FSRS

The investigated suppression scheme is based on the saturation of FSRS with increasing pulse energies. For this purpose, we measured the spectra of the CH-stretch vibration around 2950 cm^{-1} of acetonitrile and the Raman resonance around 926 cm^{-1} of KYW for different depletion pulse energies. The maximum of the SRL (as shown in Fig. 3) was acquired using a pump and Stokes pulse energy of about 40 pJ and 25 nJ, respectively. Due to the depletion of the pump photons, a nonlinear behavior for increasing pulse energies became visible, leading to a saturation of the FSRS signal. Lines were added to guide the eye and to emphasize the saturation of the SRL signal. For acetonitrile and KYW, an SRL of about 62.5% and 62.2% for depletion pulse energies of 320 nJ and 240 nJ could be measured, respectively. The FSRS signal of KYW runs into saturation earlier, due to the stronger Raman resonance of KYW compared to the CH-stretch resonance of acetonitrile. For KYW, the depletion pulse energy was limited to 240 nJ in order to stay below the damage threshold of the crystal at about 260 nJ. This saturation of the SRL enabled the suppression of the FSRS signal.

In order to determine the suppression efficiency, spectra for different depletion pulse energies (compare Fig. 3) were recorded: selected spectra in the CH-stretch region of acetonitrile around 2950 cm^{-1} are shown in Fig. 4(a) for depletion pulse energies of 0 nJ, 113 nJ, 202 nJ, and 320 nJ. As in the upper case, the pump and Stokes

FIG. 3. Maximum of the SRL around 2950 cm^{-1} of acetonitrile (red circles) and potassium yttrium tungsten (KYW) around 926 cm^{-1} (blue diamonds) for different depletion pulse energies. Lines were added to emphasize the saturation of the SRL signal.

pulse energy was set to 40 pJ (distributed across a spectral spanning from 780 to 800 nm) and 25 nJ, respectively. In the unsuppressed case, i.e., a depletion pulse energy equal to 0 nJ, an SRL_S of 5.4% could be measured, whereas for a depletion pulse energy of 320 nJ,

FIG. 4. Raman spectra in the CH-stretch region of acetonitrile for different depletion pulse energies in (a). On the left y-axis in subfigure (b), the maximum of the stimulated Raman loss (SRL) in the unsuppressed (depletion off, purple triangles) and the suppressed (depletion on, green diamonds) case is shown. In addition, the suppression efficiency η is visualized as black circles on the right y-axis. Lines were added to guide the eye.

the SRL_{Sup} was only 1.17%. Accordingly, a suppression efficiency $\eta = 1 - \text{SRL}_{\text{Sup}}/\text{SRL}_S$ of up to 79% could be determined.

For higher depletion pulse energies, an increased negative SRL, mimicking an SRG, in the shoulders of the Raman resonance was measured, which was caused by cross-phase modulation (XPM).^{37–39} The maximum values of the SRL_{Sup} from Fig. 4(a) (depletion on, green diamonds, dashed-dotted line) are shown in detail as a function of depletion pulse energy in Fig. 4(b). With increasing depletion pulse energy, a reduction of the SRL_{Sup} was measurable, whereas in the reference measurement, with the depletion pulses not overlapping in time with the pump and Stokes pulse in the sample, the SRL_S remained constant at its high value within the experimental errors (depletion off, purple triangles, solid line). In addition, the curves for the depletion beam overlapping in time (depletion on) and the suppression efficiency η (black circles, dashed line, right y-axis) clearly show a nonlinear behavior for depletion pulse energies larger than 150 nJ, which can be explained by the saturation of the FSRS process, induced by the depletion pulses. Lines in Fig. 4(b) were added to guide the eye.

B. Verification of spatial resolution improvement

Following the suppression measurements in Sec. IV A, we measured the lateral resolution of our microscope by scanning across the edge of a Raman-active KYW crystal. We have chosen a KYW crystal to have a temporally and thermally stable sample with a sharp edge. The Raman resonance of KYW around 926 cm^{-1} was investigated and could be suppressed by 75% for a depletion pulse energy of about 150 nJ. At first, we measured the spatial resolution with the depletion pulses not overlapping in time with the pump and Stokes pulses, and subsequently, with the depletion pulses overlapping in time. In the latter case, the depletion beam suppressed the Raman signal partly in the focal plane and correspondingly narrowed its effective point spread function [see Fig. 5(a)].

We used a lens ($L3, f = 25\text{ mm}$) instead of a microscope objective to avoid increasing XPM artifacts, damage of the sample, and to benefit from the higher transmission in the near-infrared. Subsequently, spectra across the edge of the KYW crystal were acquired, while scanning the sample with a piezoelectric-stage. In Fig. 5(b), the maximum of the SRL signal of the molecular vibration around 926 cm^{-1} with the depletion pulses not overlapping in time with the Stokes pulses in the sample is shown as purple triangles. In this case, the resolution of the system could be calculated to be $7.03 \pm 1.20\text{ }\mu\text{m}$ for this experiment with the low numerical aperture of lens L3.

In order to show a spatial resolution enhancement using the depletion pulses, the Stokes and depletion pulses were overlapping in time with the pump pulses. Subsequently, we spatially separated the Stokes against the depletion beam step by step in the sample as shown in Fig. 5(a), like Klar and Hell did in their first STED experiments.⁴⁰ Then, the best spatial resolution enhancement was achieved for a distance between the Stokes and the depletion beam of approximately $0.6\text{ }\mu\text{m}$. As both the Stokes and the depletion beam had a Gaussian-shaped beam profile in the sample, the Raman signal was only suppressed to the right of the center, resulting in a narrower full width at half maximum (FWHM) of the resulting point spread function only in the horizontal direction. The results for scanning the edge of the KYW crystal with the depletion beam overlapping in time and slightly separated in space are shown with green

FIG. 5. Raman imaging across the edge of a KYW crystal with the depletion beam overlapping in time with the pump and the Stokes pulses (depletion on) and not overlapping in time (depletion off), respectively. (a) shows the bright field image of the sample with a schematic not-to-scale visualization of beam positions of pump, Stokes, and depletion beam. In (b), the normalized Raman signal for different lateral positions is shown at a beam separation of approximately $0.6 \mu\text{m}$ between the centers of Stokes and depletion beam.

diamonds in Fig. 5(b). In this case, the calculated resolution was $3.58 \pm 1.60 \mu\text{m}$, which represents a spatial resolution enhancement by a factor of about 2. The shown results were acquired with a pump pulse energy of about 40 pJ (935–955 nm) and a Stokes pulse energy of about 50 nJ. The depletion pulse energy was 150 nJ to take advantage of the saturation and to stay well below the damage threshold. The resolution of our microscope was calculated by following the method introduced by Curtin *et al.*⁴¹ Therefore, we fit

$$y = \frac{A}{1 + \exp((x_0 - x)/\sigma)} \quad (4)$$

to the position-dependent Raman intensity of the edge scan. For a perfectly sharp edge, a multiplication of the value σ from the fit by a factor of 3.33 will lead to the resolution of the system.⁴¹ For a non-perfectly sharp edge, the calculated value gives an upper bound for the resolution of our microscope.^{30,41}

With the chosen beam configuration, a first proof-of-concept resolution enhancement could be shown. We used the calculation procedure from Ref. 24 to estimate the resolution enhancement when using a doughnut-shaped depletion beam. For a suppression efficiency of about 80%, we expect a resolution enhancement by a factor of about 4–6 in future experiments.

V. CONCLUSION

The suppression of femtosecond stimulated Raman scattering (FSRS) was investigated. We presented a scheme with only two

wavelength-components, three beams, and only a single molecular vibration being involved. The scheme is directly transferable from FSRS to SRS, and in the experiment, a reduction of the SRS signal by 79% could be verified. Kim *et al.*²⁹ showed the suppression of SRS with another competing SRS process but therefore needed three different wavelength-components with pulse energies comparable to the ones used in our investigations. In their experiment, they showed the reduction of a Raman resonance by 58% when using an additional stronger Raman resonance for the competing SRS process. However, the energy difference of the three used wavelength-components had to match the energy difference of two molecular vibrations in the sample under investigation. In contrast to ground state depletion (GSD),^{22–25} the saturation of the FSRS process enabled the suppression of the Raman signal using pulse energies which are at least one order of magnitude lower.

Furthermore, in a proof-of-concept measurement, a resolution enhancement by a factor of 2 was measured, providing strong evidence that the investigated scheme will enable an even more significant resolution improvement in label-free imaging of high damage threshold samples in, for example, materials science and mineralogy, when using a doughnut-shaped depletion beam. Silva *et al.* also showed a resolution enhancement by a factor of about 2 using a doughnut-shaped decoherence beam,³⁰ with the hypothesis that the depletion mechanism has been CARS. Our results can be solely explained by the saturation of SRS. Recently, Gong and Wang³² and Gong *et al.*³³ demonstrated a spatial resolution enhancement by a factor of $\sqrt{2}$ in biological samples when calculating the first higher harmonic of the saturated SRS signal. The resolution enhancement scales by a factor of about $\sqrt{n}$, where n is the order of the higher harmonic. However, to further improve the spatial resolution, even higher harmonics have to be measured, which would lead to higher pulse energies and extend the acquisition time.

In conclusion, our investigated scheme could open the pathway for routine sub-diffraction-limited Raman imaging, limited by the high pulse energies used. Nevertheless, the spatial resolution enhancement will be further increased by using a doughnut-shaped depletion beam to suppress the Raman signal in the outer region of the focal spot.

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REFERENCES

- A. Kudelski, "Analytical applications of Raman spectroscopy," *Talanta* **76**, 1–8 (2008).
- C. L. Evans, E. O. Potma, M. Puoris-haag, D. Cote, C. P. Lin, and X. S. Xie, "Chemical imaging of tissue in vivo with video-rate coherent anti-Stokes Raman scattering microscopy," *Proc. Natl. Acad. Sci. U. S. A.* **102**, 16807–16812 (2005).
- C. V. Raman, "A classical derivation of the Compton effect," *Indian J. Phys.* **369**, 357–364 (1928).
- L.-P. Choo-Smith, H. G. M. Edwards, H. P. Endtz, J. M. Kros, F. Heule, H. Barr, J. S. Robinson, H. A. Bruining, and G. J. Puppels, "Medical applications of Raman spectroscopy: From proof of principle to clinical implementation," *Biopolymers* **67**, 1–9 (2002).

- ⁵N. Bloembergen, "The stimulated Raman effect," *Am. J. Phys.* **35**, 989–1023 (1967).
- ⁶R. Hellwarth, "Theory of stimulated Raman scattering," *Phys. Rev.* **130**, 1850–1852 (1963).
- ⁷W. M. Tolles, J. W. Nibler, J. R. McDonald, and A. B. Harvey, "Review of the theory and application of coherent anti-Stokes Raman spectroscopy (CARS)," *Appl. Spectrosc.* **31**, 253–272 (1977).
- ⁸J. Dasgupta, R. R. Frontiera, K. C. Taylor, J. C. Lagarias, and R. A. Mathies, "Ultrafast excited-state isomerization in phytochrome revealed by femtosecond stimulated Raman spectroscopy," *Proc. Natl. Acad. Sci. U. S. A.* **106**, 1784–1789 (2009).
- ⁹P. Kukura, D. W. McCamant, and R. A. Mathies, "Femtosecond stimulated Raman spectroscopy," *Annu. Rev. Phys. Chem.* **58**, 461–488 (2007).
- ¹⁰B. Levine and C. Bethea, "Ultrahigh sensitivity stimulated Raman gain spectroscopy," *IEEE J. Quantum Electron.* **16**, 85–89 (1980).
- ¹¹J.-X. Cheng and X. S. Xie, "Vibrational spectroscopic imaging of living systems: An emerging platform for biology and medicine," *Science* **350**, aaa8870 (2015).
- ¹²B. Huang, M. Bates, and X. Zhuang, "Super-resolution fluorescence microscopy," *Annu. Rev. Biochem.* **78**, 993–1016 (2009).
- ¹³L. Schermelleh, R. Heintzmann, and H. Leonhardt, "A guide to super-resolution fluorescence microscopy," *J. Cell Biol.* **190**, 165–175 (2010).
- ¹⁴S. W. Hell and J. Wichmann, "Breaking the diffraction resolution limit by stimulated emission: Stimulated-emission-depletion fluorescence microscopy," *Opt. Lett.* **19**, 780–782 (1994).
- ¹⁵M. J. Rust, M. Bates, and X. Zhuang, "Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM)," *Nat. Methods* **3**, 793–796 (2006).
- ¹⁶E. Betzig, G. H. Patterson, R. Sougrat, O. W. Lindwasser, S. Olenych, J. S. Bonifacio, M. W. Davidson, J. Lippincott-Schwartz, and H. F. Hess, "Imaging intracellular fluorescent proteins at nanometer resolution," *Science* **313**, 1642–1645 (2006).
- ¹⁷G. Donnert, J. Keller, R. Medda, M. A. Andrei, S. O. Rizzoli, R. Luhrmann, R. Jahn, C. Eggeling, and S. W. Hell, "Macromolecular-scale resolution in biological fluorescence microscopy," *Proc. Natl. Acad. Sci. U. S. A.* **103**, 11440–11445 (2006).
- ¹⁸R. Alford, H. M. Simpson, J. Duberman, G. C. Hill, M. Ogawa, C. Regino, H. Kobayashi, and P. L. Choyke, "Toxicity of organic fluorophores used in molecular imaging: Literature review," *Mol. Imaging* **8**, 341–354 (2009).
- ¹⁹Q. Zheng, S. Jockusch, Z. Zhou, and S. C. Blanchard, "The contribution of reactive oxygen species to the photobleaching of organic fluorophores," *Photochem. Photobiol.* **90**, 448–454 (2014).
- ²⁰J. W. Lichtman and J.-A. Conchello, "Fluorescence microscopy," *Nat. Methods* **2**, 910–919 (2005).
- ²¹C. T. Graefe, D. Punihaole, C. M. Harris, M. J. Lynch, R. Leighton, and R. R. Frontiera, "Far-field super-resolution vibrational spectroscopy," *Anal. Chem.* **91**, 8723–8731 (2019).
- ²²W. P. Beeker, C. J. Lee, K. J. Boller, P. Groß, C. Cleff, C. Fallnich, H. L. Offerhaus, and J. L. Herek, "A theoretical investigation of super-resolution CARS imaging via coherent and incoherent saturation of transitions," *J. Raman Spectrosc.* **42**, 1854–1858 (2011).
- ²³C. Cleff, P. Groß, C. Fallnich, H. L. Offerhaus, J. L. Herek, K. Kruse, W. P. Beeker, C. J. Lee, and K.-J. Boller, "Stimulated-emission pumping enabling sub-diffraction-limited spatial resolution in coherent anti-Stokes Raman scattering microscopy," *Phys. Rev. A* **87**, 033830 (2013).
- ²⁴S. Rieger, M. Fishedick, K.-J. Boller, and C. Fallnich, "Suppression of resonance Raman scattering via ground state depletion towards sub-diffraction-limited label-free microscopy," *Opt. Express* **24**, 20745 (2016).
- ²⁵S. Rieger, T. Würthwein, K. Sparenberg, K.-J. Boller, and C. Fallnich, "Density matrix study of ground state depletion towards sub-diffraction-limited spontaneous Raman scattering spectroscopy," *J. Chem. Phys.* **148**, 204110 (2018).
- ²⁶M. Cho, "Three-beam double stimulated Raman scatterings," *J. Chem. Phys.* **148**, 014201 (2018).
- ²⁷B. J. Rao and M. Cho, "Three-beam double stimulated Raman scatterings: Cascading configuration," *J. Chem. Phys.* **148**, 114201 (2018).
- ²⁸B. J. Rao, D. S. Choi, and M. Cho, "Selective suppression of CARS signal with two competing stimulated Raman scattering processes," *J. Chem. Phys.* **149**, 234202 (2018).
- ²⁹D. Kim, D. S. Choi, J. Kwon, S.-H. Shim, H. Rhee, and M. Cho, "Selective suppression of stimulated Raman scattering with another competing stimulated Raman scattering," *J. Phys. Chem. Lett.* **8**, 6118–6123 (2017).
- ³⁰W. R. Silva, C. T. Graefe, and R. R. Frontiera, "Toward label-free super-resolution microscopy," *ACS Photonics* **3**, 79–86 (2016).
- ³¹Y. Yonemaru, A. F. Palonpon, S. Kawano, N. I. Smith, S. Kawata, and K. Fujita, "Super-spatial- and -Spectral-Resolution in vibrational imaging via saturated coherent anti-Stokes Raman scattering," *Phys. Rev. Appl.* **4**, 014010 (2015).
- ³²L. Gong and H. Wang, "Breaking the diffraction limit by saturation in stimulated-Raman-scattering microscopy: A theoretical study," *Phys. Rev. A* **90**, 013818 (2014).
- ³³L. Gong, W. Zheng, Y. Ma, and Z. Huang, "Saturated stimulated-Raman-scattering microscopy for far-field superresolution vibrational imaging," *Phys. Rev. Appl.* **11**, 034041 (2019).
- ³⁴C. W. Freudiger, W. Min, B. G. Saar, S. Lu, G. R. Holtom, C. He, J. C. Tsai, J. X. Kang, and X. S. Xie, "Label-free biomedical imaging with high sensitivity by stimulated Raman scattering microscopy," *Science* **322**, 1857–1861 (2008).
- ³⁵S. Zhou, F. W. Wise, and D. G. Ouzounov, "Divided-pulse amplification of ultrashort pulses," *Opt. Lett.* **32**, 871–873 (2007).
- ³⁶M. Brinkmann, S. Dobner, and C. Fallnich, "Light source for narrow and broadband coherent Raman scattering microspectroscopy," *Opt. Lett.* **40**, 5447–5450 (2015).
- ³⁷K. Ekvall, P. van der Meulen, C. Dhollande, L. E. Berg, S. Pommeret, R. Naskrecki, and J. C. Mialocq, "Cross phase modulation artifact in liquid phase transient absorption spectroscopy," *J. Appl. Phys.* **87**, 2340–2352 (2000).
- ³⁸S. Lim, B. Chon, H. Rhee, and M. Cho, "Spectral modulation of stimulated Raman scattering signal: Beyond weak Raman pump limit," *J. Raman Spectrosc.* **49**, 607–620 (2018).
- ³⁹G. Batignani, G. Fumero, E. Pontecorvo, C. Ferrante, S. Mukamel, and T. Scopigno, "Genuine dynamics vs cross phase modulation artifacts in femtosecond stimulated Raman spectroscopy," *ACS Photonics* **6**, 492–500 (2019).
- ⁴⁰T. A. Klar and S. W. Hell, "Subdiffraction resolution in far-field fluorescence microscopy," *Opt. Lett.* **24**, 954–956 (1999).
- ⁴¹A. E. Curtin, R. Skinner, and A. W. Sanders, "A simple metric for determining resolution in optical, ion, and electron microscope images," *Microsc. Microanal.* **21**, 771–777 (2015).