Coherent Raman spectro-imaging with laser frequency combs

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Advances in optical spectroscopy and microscopy have had a profound impact throughout the physical, chemical and biological sciences. One example is coherent Raman spectroscopy, a versatile technique interrogating vibrational transitions in molecules. It offers high spatial resolution and three-dimensional sectioning capabilities that make it a label-free tool1–2 for the non-destructive and chemically selective probing of complex systems. Indeed, single-colour Raman bands have been imaged in biological tissue at video rates3–4 by using ultra-short-pulse lasers. However, identifying multiple, and possibly unknown, molecules requires broad spectral bandwidth and high resolution. Moderate spectral spans combined with high-speed acquisition are now within reach using multichannel detection5 or frequency-swept laser beams6–9. Laser frequency combs10 are finding increasing use for broadband molecular linear absorption spectroscopy11–13. Here we show, by exploring their potential for nonlinear spectroscopy14, that they can be harnessed for coherent anti-Stokes Raman spectroscopy and spectro-imaging. The method uses two combs and can simultaneously measure, on the microsecond timescale, all spectral elements over a wide bandwidth and with high resolution on a single photodetector. Although the overall measurement time in our proof-of-principle experiments is limited by the waiting times between successive spectral acquisitions, this limitation can be overcome with further system development. We therefore expect that our approach of using laser frequency combs will not only enable new applications for nonlinear microscopy but also benefit other nonlinear spectroscopic techniques.

Coherent anti-Stokes Raman spectroscopy (CARS) is a nonlinear four-wave mixing process, which is coherently driven when the energy difference between a pump laser and a Stokes laser is resonant with a Raman active molecular transition. Scattering off the probe beam generates the high-frequency-shifted anti-Stokes signal, which is enhanced by many orders of magnitude relative to spontaneous Raman scattering signals. In our technique of dual-comb CARS, we harness two femtosecond lasers with repetition frequencies \( f + \delta f \) and \( f \) to irradiate a sample. In the time domain (Fig. 1a), a pulse from the first laser coherently excites a molecular vibration of period \( 1/f_{\text{coh}} \) that is longer than the pulse duration and the coherently vibrating molecules give rise15 to an oscillating refractive index modulated at the vibrational frequency (Fig. 1b). A pulse of the second laser probes the sample with a time separation \( \Delta \) that increases linearly from pulse pair to pulse pair. If this second pulse (for simplicity also taken to be short compared with the molecular vibration period16) arrives after a full molecular period \( 1/f_{\text{coh}} \), the vibration amplitude is increased and the back-action on the probe pulse is a spectral shift towards lower frequencies. If it arrives after half a period, the vibration amplitude is damped and the pulse experiences a shift towards higher frequencies. As long as the pulse separation \( \Delta \) remains shorter than the coherence time of the molecular oscillation, an intensity modulation of frequency \( f_{\text{coh}}/2f \) is thus observed in the transmitted probe radiation after a spectral edge filter. The two femtosecond lasers have a symmetrical function: the sign of time separation \( \Delta \) between the pulses changes every \( 1/(2\delta f) \). A theoretical description of such a time-resolved CARS signal can be found in ref. 19.

In the frequency domain (Fig. 1c, d), the two frequency comb generators produce an optical spectrum consisting of several hundred thousand perfectly evenly spaced spectral lines. Their frequencies may be described by

\[
 f^{(1)}_m = m(f + \delta f) + f_{\text{ceo}} \\
 f^{(2)}_{m'} = m'f + f_{\text{ceo}}
\]

where \( m \) and \( m' \) are integers, and \( f_{\text{ceo}} \) and \( f'_{\text{ceo}} \) are the carrier-envelope offset frequencies.

The frequency differences within each comb form regular combs themselves with vanishing carrier-envelope offset frequencies and line spacings of \( f + \delta f \) and \( f \), respectively. For instance, for comb 1 all pairs of lines with \( m \) and \( n \) contribute to the same difference frequency \( k(f + \delta f) \). Each of the difference frequency combs resonantly excites a molecular level of frequency \( f_{\text{coh}} \) by means of Raman-like two-photon excitation whenever a difference frequency comes close to \( f_{\text{coh}} \) that is, when \( k \sim f_{\text{coh}} \). The excitations by the two combs interfere and modulate the molecular vibration at a beat note frequency \( k\delta f = f_{\text{coh}}\delta f/2f \). The two-photon excitation leads to a resonant enhancement of the third-order nonlinear susceptibility observed by means of the anti-Stokes radiation. The intensity of the generated broadband anti-Stokes radiation is modulated at the beat note frequency \( f_{\text{coh}}\delta f/2f \). When several vibrational levels \( f_{\text{coh}}(1,2, \ldots) \) are excited, the composite modulation contains all the beating frequencies \( f_{\text{coh}}\delta f/2f, f_{\text{coh}}\delta f/2f, \ldots \) representative of the involved levels. The Raman excitation spectrum is revealed by Fourier transformation of the intensity recorded against time. The spectrum is mapped in the radiofrequency domain by the downconversion factor \( \delta f/2f \) (typically of the order of \( 10^{-7} \) to \( 10^{-5} \)). This permits rapid measurement time and efficient signal processing. Absolute calibration of the Raman shifts is achieved by dividing the radiofrequencies by the downconversion factor, which is easy to measure accurately. The carrier-envelope offsets cancel and do not have to be measured or controlled. This notably simplifies the experimental implementation and the calibration procedure. Similar modulation transfer phenomena have been exploited in experiments using a single femtosecond laser and a phase-modulation pulse shaper19 or a Michelson interferometer20–22, but measurement times were fundamentally limited either by the sweep period of the phase modulation or by the mechanical motion in the Michelson interferometer. Our motionless frequency-comb-based technique enables more than 1,000-fold shorter acquisition times (see also Supplementary Information), and a spectral

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resolution and spectral span only limited by the measurement time and the spectral bandwidth of the femtosecond lasers.

Figure 2 sketches the experimental setup (see Methods), which is similar to that used in dual-comb absorption spectroscopy except for dispersion management and spectral filtering to isolate the CARS signal from the comb beams. As the Raman-like two-photon excitation involves virtual energy levels, dispersion decreases both the spectral span and the excitation efficiency. The time-domain interference signal—the interferogram—is periodic. Every $1/(2\Delta f)$, a strong burst mostly contains the non-resonant four-wave mixing signal resulting from the interference between the overlapping pulses of the two combs. A reproducible modulation (Fig. 3a), due to the CARS signal only, follows the burst and has a duration proportional to the coherence time of the sample transitions. A time-windowed portion of the interferogram, which excludes the interferometric non-resonant contribution, is Fourier transformed. The width of the window is chosen according to the desired spectral resolution (see Methods for a detailed explanation of the recording parameters). The resulting spectra (Fig. 3b–d) span Raman shifts from 200 cm$^{-1}$ to 1,400 cm$^{-1}$. The non-resonant background, which strongly lowers the sensitivity of CARS, is entirely suppressed, as in other specific CARS schemes.

We illustrate acquisition times with three spectra at an apodized resolution of 4 cm$^{-1}$ and recorded with $\Delta f = 100$ Hz (Fig. 3b) or 5 Hz (Fig. 3b, 3c, d) for a mixture of hexafluorobenzene, nitrobenzene, nitromethane and toluene in a cuvette 5 mm long. The spectra involve no averaging and were measured in 14.8 s (Fig. 3b, 3c, d); the number of individual spectral elements (defined as the spectral span divided by the resolution) for all three spectra is 300. The signal-to-noise ratio culminates at 1,000 for the most intense blended line of toluene and nitrobenzene in Fig. 3c. Recorded under different experimental conditions, the three spectra show great similarities in line position and relative intensity.

Imaging capabilities are illustrated with a capillary plate (25-μm diameter holes, thickness 500 μm) filled with a mixture of hexafluorobenzene, nitromethane and toluene. For each pixel, we measure an interferogram within 12 μs to obtain a spectrum at an apodized resolution of...
The multiplex nature: all the spectral elements are measured at the same time on a single photodetector, which ensures consistency of the spectra. Moreover, the frequency combs guarantee the reproducibility and accuracy of the wavenumber scale. However, a major limitation in the present proof-of-principle experiments is the low duty cycle (the ratio between the time it takes to measure an interferogram and the time before the next interferogram is measured), which for the spectro-imaging experiments shown in Fig. 4 is only $6 \times 10^{-4}$. The interferogram refresh time is the inverse of the difference of the laser repetition frequencies $1/\delta f$, whereas the spectral information is only collected when the delay between the pairs of pulses is shorter than the coherent molecular ringing time (see Supplementary Information for more detailed discussion). One solution would be to use combs with a larger line spacing, which could allow the duty cycle of interferogram acquisition to approach unity while keeping measurement times and signal-to-noise ratios similar to those in Figs 3 and 4. Frequency comb generators based on solid-state lasers with a short cavity or on chip-scale microring resonators might offer a route towards realizing such high duty cycle experiments. For microscopy experiments, scanning the laser beam with a galvanometric mirror rather than the sample stage provides a straightforward way to speed up the mapping process. High-speed cameras (more than $10^8$ frames s$^{-1}$) could even allow real-time hyperspectral dual-comb CARS imaging. There is also scope for improvements that would affect the signal itself. For example, a more sophisticated dispersion management, particularly of third-order dispersion, would enhance the signal-to-noise ratio. This could be complemented with fast synchronous or differential detection schemes that might further decrease the noise level. Finally, few-cycle oscillators or spectral broadening with nonlinear fibres will expand the spectral span of the setup.
Several schemes exploiting coherent Raman scattering for novel spectroscopy and microscopy applications have recently emerged, and we expect that their combination with our method will deliver techniques with improved performance and utility. For example, our dual-comb approach could benefit surface-enhanced26,27 CARS measurements or studies of Raman optical activity28. Moreover, exciting imaging capabilities might arise when extending our method to exploit either near-field effects (for example at a metal tip29) or far-field effects (for example state depletion30) to achieve sub-wavelength spatial resolution.

METHODS SUMMARY

Two titanium–sapphire lasers (Synergy20 UHP; Femtolasers) emit 20-fs pulses centred at 12,580 cm\(^{-1}\) with energies up to 13 nJ. Both have adjustable repetition frequencies of about 100 MHz. The beams of the two lasers (Fig. 2) are combined on a beamsplitter, and a chirped mirror compressor (Layertec) compensates for the second-order dispersion induced by the optical components of the setup. Spectral filtering is applied to improve the signal-to-background ratio. A low-pass-depletion30) to achieve sub-wavelength spatial resolution.

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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METHODS
Detailed experimental setup. Two titanium–sapphire lasers (Synergy20 UHP; Femtolasers) emit 20-fs pulses centred at 12,580 cm$^{-1}$ with energies up to 13 nJ. Titanium–sapphire lasers are chosen because of their capabilities to generate ultrashort pulses in a spectral region where most samples have no or only weak absorption and where advanced photonic tools are available. Both oscillators have repetition frequencies of about 100 MHz, which can be adjusted by moving a cavity mirror mounted on a motorized translation stage and a piezoelectric transducer. The repetition frequencies are monitored with fast silicon photodiodes connected to frequency counters (53131A; Agilent). To prevent long-term drifts, the repetition frequency of each laser comb is stabilized against a radiofrequency clock by means of a mirror of the laser’s cavity mounted on a piezoelectric transducer. This does not affect the quality of an individual spectrum but improves the reproducibility of the wavenumber scale of a sequence of spectra. The laser beams are linearly polarized. The pulse energy available for the spectroscopy experiments is adjusted for each laser beam individually with a combination of a half-wave plate and a polarizer. The beams of the two lasers are combined (Fig. 2) on a pellicle beamsplitter, and a chirped mirror compressor (Layertec) compensates for the second-order dispersion induced by the optical components of the setup. Spectral filtering is applied to improve the signal-to-background ratio. A low-frequency-pass optical filter (ET750LP, cutoff 13,330 cm$^{-1}$; Chroma Technology) before the sample and a high-frequency-pass optical filter (3RD740SP, cutoff 13,510 cm$^{-1}$; Omega Optical Inc.) after the sample isolate the CARS signal that is generated by the sample after proper focusing with a lens or a microscope objective. The spectral span is thus limited on the low-energy side by the optical filters and on the high-energy side by the spectral bandwidth of the femtosecond lasers. The anti-Stokes radiation is limited on the low-energy side by the optical filters and on the high-energy side by the spectral bandwidth of the CARS signal that is generated by the sample after proper focusing with a lens or a microscope objective. The spectral span is thus limited on the low-energy side by the optical filters and on the high-energy side by the spectral bandwidth of the CARS signal that is generated by the sample after proper focusing with a lens or a microscope objective. The spectral span is thus limited on the low-energy side by the optical filters and on the high-energy side by the spectral bandwidth of the CARS signal that is generated by the sample after proper focusing with a lens or a microscope objective. The spectral span is thus limited on the low-energy side by the optical filters and on the high-energy side by the spectral bandwidth of the CARS signal that is generated by the sample after proper focusing with a lens or a microscope objective.