RAMAN SPECTROSCOPY

Molecular discrimination imaging

The discrimination of chemical species with overlapping Raman bands is now possible in real time using a stimulated Raman scattering microscope with a fast, bipolar spectral correlator.

Kazuyoshi Itoh and Yasuyuki Ozeki

In recent years, label-free three-dimensional microscopy techniques based on Raman scattering — the absorption and emission of light from the vibrational and rotational states of molecules — have attracted much attention for their promise of molecule specificity and subcellular spatial resolution. However, there exists a severe trade-off between the achievable imaging speed and spectral selectivity of such schemes. One particular example is spontaneous Raman scattering, which, although able to provide information about the entire vibrational spectra of the molecules present, suffers from long imaging times resulting from its weak scattering signal.

Techniques that generate stronger signals, such as coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS)[1–3], have sensitivities covering several orders of magnitude, allowing high-speed imaging up to standard video rates[4,5]. However, SRS and CARS are typically capable of detecting only a single vibrational frequency, making it difficult to discriminate between small spectral differences from biological substances such as lipids, proteins and nuclear acids. Now, writing in *Nature Photonics*, Christian Freudiger and co-workers from Harvard University in the USA report a significant improvement in the spectroscopic selectivity of SRS microscopy through spectrally tailored excitation[6].

In conventional SRS microscopy, excitation is achieved using two differently coloured narrowband pulses tightly focused onto a sample. When the frequency difference between the two pulses matches the vibrational frequency of the molecules in the focal volume, energy in the high-frequency pulse is transferred to the low-frequency pulse through stimulated emission induced by the SRS process. The resulting small intensity change in the output pulse can be detected through the lock-in technique and modulation of the excitation pulses.

The innovative technique of Freudiger *et al.* involves using a broadband pulse with a tailored spectrum as one of the excitation pulses, which allows SRS processes with various frequency differences to occur simultaneously (Fig. 1a). The resulting intensity transfer to the signal (ΔI_s) corresponds to the sum of all the SRS processes from all the molecules present; the challenge is then to find a way of suppressing contributions from spectrally overlapping components (Fig. 1c) to allow different molecules to be discriminated. Shaping the excitation spectrum such that it has negative values (Fig. 1d) would, in principle, make it possible to conduct 'bipolar spectral correlation', which generates a signal relating to the target molecule (Fig. 1b) while rejecting signals from interfering molecules (Fig. 1c). However, because the optical intensity is always positive, it has so far seemed impossible to realize such negative excitation using this crude SRS process.

The experimental method of Freudiger *et al.* achieves bipolar spectral correlation and thus allows the discrimination of different molecules. The two different broadband spectra corresponding to the positive and negative parts of the bipolar correlation spectrum are switched in time and used along with the narrowband pulses for excitation

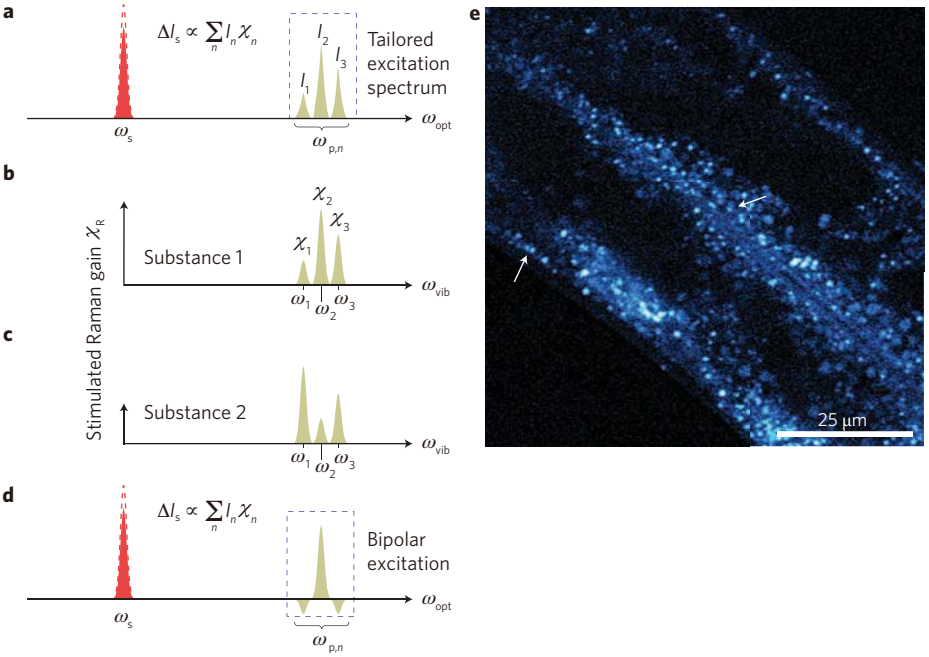

Figure 1 | Spectral correlation imaging with SRS. **a**, Schematic of SRS excitation with spectrally tailored pump pulses, in which the intensity change of the Stokes pulse at ω_s is proportional to the sum of SRS effects caused by multiple molecular vibrations at different frequencies over the range $\omega_{p,n}$ with intensities I_1, I_2 and I_3. **b**,**c**, Stimulated Raman gain (χ) of two fictitious substances. Because the spectral shape of substance 1 is similar to the excitation spectrum in **a**, the correlation signal is large. The spectral shape of substance 2, however, is different from the excitation spectrum, making the correlation signal smaller (but non-zero). **d**, The bipolar excitation spectrum that correlates with substance 1 and is orthogonal to substance 2, allowing the two substances to be distinguished. **e**, Spectral correlation image of *C. elegans*, with arrows showing visualized oleic acid.

(Fig. 1d). The resulting intensity change in the two successive narrowband pulses is detected by the lock-in technique, which measures the difference in SRS effects caused by the positive and negative spectra, thereby performing a bipolar correlation. If all of the components' spectra are known, we can readily calculate a set of bipolar excitation spectra beforehand in such a way that every excitation spectrum has a certain correlation with its target spectrum while being orthogonal to all other spectra. Thus, the bipolar spectral correlator can discriminate one species while suppressing all interfering signals from other substances. Based on this technique, the researchers demonstrated quantitative detection of cholesterol, oleic acid and ethanol in test solutions, as well as *in vivo* visualization of proteins and lipids in *C. Elegans* (Fig. 1e). Note that this technique works only when the spectra of all the constituents are known, with unknown components contributing to background noise.

This technique is powerful because it allows a specific substance of interest to be discriminated and quantified at reasonably fast imaging speeds. In Raman microscopy, C–H stretching vibrations at around 3,000 cm^{-1} are often studied because of their strong scattering signals. However, almost all organic materials have vibrations in C–H stretching bands, and the resulting spectral overlap often makes it difficult to discriminate between different substances.

Note that this enhanced discrimination capability is realized at the expense of a slight decrease in imaging speed (~30 s per frame). Because the spectra of a sample's constituents usually overlap, the signal detected by the bipolar excitation spectrum is small and must be integrated by a lock-in amplifier to obtain a reasonable signal-to-noise ratio. More intense pump pulses of course provide stronger signals, but the optical power of the broadband pump pulses is limited by the onset of sample damage. The answer is instead to use the intrinsic high sensitivity of SRS microscopy to provide a good compromise between imaging speed and spectroscopic selectivity.

Efforts are also underway to improve both spontaneous Raman microscopy and CARS microscopy. The spontaneous Raman imaging of biological samples is increasing in speed through the use of line-scanning geometry[7], with multiplex CARS microscopy providing spectroscopic information[8]. Post-processing of the spectral data obtained using these techniques allows samples to be analysed in a pixel-by-pixel manner. The molecular spectral correlator of Freudiger *et al.* is advantageous because it provides strong molecular discrimination capability at high imaging speeds. Competition between these different Raman imaging techniques will stimulate further technological developments in the field.

Although Raman scattering and coherent Raman techniques were first demonstrated more than 30 years ago, rapid progress in photonic technology and the continued demands for efficient biomedical imaging techniques drive the development of new and improved forms of imaging. The technique of Freudiger *et al.*[6] and the recent demonstration of video-rate SRS microscopy[5] seem to be quite powerful for label-free three-dimensional imaging. The wealth of activity in this research field suggests that new modalities reaching next-generation performance levels may be just around the corner. ❐

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PHOTONIC CRYSTALS

Lasing woodpiles

A new woodpile-type photonic crystal nanocavity with a three-dimensional bandgap that supports lasing from embedded quantum dots paves the way for three-dimensional integrated photonic circuits and highly efficient nanolasers.

Stefan Strauf

Photonic crystals provide researchers with the ability to control, bend, trap, switch, slow, reflect and efficiently extract light emitted from embedded or adjacent semiconductors. They are made from dielectric materials with refractive indices that change periodically on a length scale comparable to the wavelength of light[1], consequently forming photonic bandgaps that effectively prohibit the propagation of light in certain directions and at certain wavelengths. Breaking the periodicity in a controlled way creates nanocavities that confine light to extremely small volumes in which the light–matter interaction is dominated by cavity quantum electrodynamic effects.

Photonic crystal nanocavities containing quantum dots have gained tremendous interest from the research community in recent years[2]. This interest is largely driven by potential applications that include the use of nanolasers for optical communications, on-chip interconnects and beam steering, biochemical sensing and quantum information processing. Most work in this field so far has utilized planar two-dimensional (2D) photonic crystal slab cavities that confine light in-plane by distributed Bragg reflection and out-of-plane by total internal reflection. Within this platform, photonic crystal nanolasers with small footprints[3], ultralow lasing thresholds[4], low power consumption and fast signal modulation in excess of 100 GHz (ref. 5) have been demonstrated.

Writing in *Nature Photonics*, Aniwat Tandaechanurat *et al.* now report the first realization of a quantum dot nanolaser made from a 3D photonic crystal