Raman, His Effect and Its Avatars*

V Ramanathan

This article aims to introduce four variants of Raman spectroscopy.

V Ramanathan is a Fulbright Fellow and was a Fellow of the Indian National Young Academy of Science (INYAS) from 2016–2020. He is working as an assistant professor at the Department of Chemistry, IIT (BHU) Varanasi. His areas of research are quantitative Raman spectroscopy, Raman imaging, computational chemistry, Indian scientific heritage and Carnatic musicology.

Introduction

Would it be egregiously judgmental on my part if I were to say that Bharat Ratna, C V Raman would not have imagined the avalanche of research on the effect named after him, and that it would appear in different avatars several tens of years after his discovery? Perhaps yes and perhaps no!

While commemorating the diamond jubilee celebration of the Raman effect through an article titled, 'Research with style: The story of Raman's study of Light Scattering', Prof. S Ramaseshan recollects [1] the remarks made by S Chandrashekhar, the Nobel winning astrophysicist. Chandrashekhar seems to have said, "...on that occasion, someone drew attention to the discovery of the Compton effect a few years earlier, and Raman responded with, 'Ah, but my effect will play a great role for chemistry and molecular structure'." So farsighted was Raman about the impact of his discovery that he had absolutely no qualm in sounding prophetic, which turned out to be absolutely true!

The discovery of laser in the 60s made a paradigm shift in the research related to Raman scattering. Raman scattering is a very weak phenomenon, where only 1 photon in 1–10 million (1 in 1–10 × 10^6) photons is the Raman photon. So the brightness of the optical source is of paramount importance. The discovery of laser, in hindsight, can arguably be said to have not only revived the research in Raman scattering but also opened newer domains

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that I collectively call the 'various avatars of the Raman effect', an aspect that perhaps would have escaped the outlook of C V Raman!

Of the several variants of Raman spectroscopy, in this article, I am going to briefly introduce the following techniques that have had their impact in diverse fields:

a) Resonance Raman Spectroscopy (RRS)
b) Surface Enhanced Raman Spectroscopy (SERS)
c) Coherent Anti-Stokes Raman Spectroscopy (CARS)
d) Raman Microscopy (RM)
e) Resonance Raman Spectroscopy (RRS)

Resonance Raman Spectroscopy (RRS)

Molecules comprise atoms, which in turn are made up of nucleus and electrons. Most of the physical and chemical properties of a molecule are vastly dependent on the electrons and the way energy is distributed amongst them. The structure and energy distribution within the nucleus is mainly studied in the domain of nuclear physics, and it is beyond the scope of this article. The energy of a molecule comprises the electronic energy (energy distributed in the electronic structure), vibration and rotation of the molecule. When this energy of the molecule is perturbed by supplying heat (thermal) or by shining electromagnetic radiation (radiative), the excess energy that is supplied gets distributed to various modes as mentioned above. When the electronic structure is perturbed, it is assumed, by virtue of a nucleus being ~1000 times heavier than the electrons, that with respect to the motion of the electron, the nuclei are nearly stationary. This is called the Born–Oppenheimer approximation, which is of fundamental importance in electronic spectroscopy. In the case of vibrational spectroscopy, it is the motion of the nuclei that is of importance. By studying particularly the way the molecular vibration gets perturbed, one gets to know the functional groups present in a molecule, and thereby, parts of its structure. The way to access the vibration of a molecule

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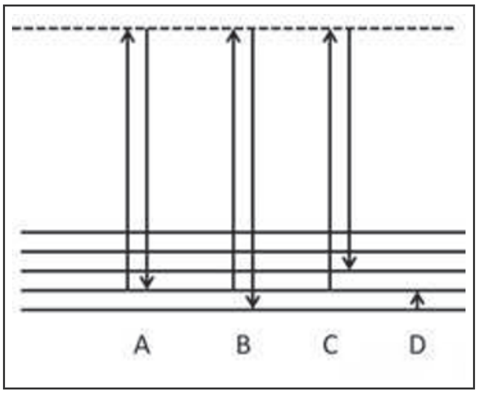

Figure 1. Schematic representation of Rayleigh scattering (A); Raman scattering (anti-Stokes (B) and Stokes (C)), and IR absorption (D).

is either by shining it with infrared light, which the molecule will absorb and result in vibrational excitation or through Raman scattering. The Raman scattered photons carry the vibrational information of the scattering medium or molecules. This can be better understood through the energy level diagram as shown in *Figure* 1.

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The solid horizontal lines in the *Figure* 1 depict the vibrational energy levels of the scattering medium, whereas the vertical arrows depict the different optical processes taking place. Raman scattering, which is the combination of both Stokes and anti-Stokes signals are shown in B and C, which carry the vibrational information of the scattering medium. The small arrow shown in D is the absorption process where, in the context of vibrational energy levels, the vertical arrow would correspond to the infrared (IR) photon. The dashed horizontal line, popularly referred to in the literature as a ‘virtual state’ for want of a better term, is in fact not a ‘bound state’, which means that there exists no corresponding mathematical equation in whose solution one can locate such a state. Many experts, e.g., Prof. Mukamel, use the seemingly sophisticated density matrix formalism and nonlinear susceptibility (χ) of third-order to explain the spontaneous Raman scattering without resorting to this popular term, namely the virtual state [2]. But I will restrict myself to the use of the so-called virtual state for the sake of simplicity and its intuitive appeal. Now, in resonance Raman spectroscopy, if the incident photon’s energy is

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very close to a bound electronic state of the scattering medium, it results in a large enhancement of the Raman signal. This technique is particularly useful for studying larger molecules like proteins as only the functional group of the protein can now be perturbed by the near electronic transition while accessing the vibrational states with relatively higher resolution. Metalloproteins have been extensively studied using this technique [3]. One of the major drawbacks of this technique is that it lays restrictions on the wavelength of the light source to be used for the Raman studies.

Surface Enhanced Raman Spectroscopy (SERS)

SERS combines the intrinsic advantages of Raman spectroscopy with the sensitivity required to detect analytes at relevantly low concentrations.

Accidental discoveries and inventions are nothing new in the scientific field, and the invention of this technique may be regarded as one such. Fleischmann and co-workers were primarily interested in studying the role of adsorption on electrodes using Raman spectroscopy. To their surprise, they observed that the Raman spectrum of pyridine, adsorbed on a roughened silver electrode, showed elevated intensities of the peaks [4]. And this report by them opened an avalanche of research as gleaned from the ~6700 citations it has received. SERS combines the intrinsic advantages of Raman spectroscopy with the sensitivity required to detect analytes at relevantly low concentrations. The otherwise relatively weak signal strength of Raman scattering is overcome through SERS which increases the Raman signal by 106–108 times.

In this technique, the molecule is adsorbed on a metallic surface. By virtue of this adsorption, two possible interactions may happen between the molecule and the metal surface: (a) charge transfer (also referred to as chemical) and (b) physical (also known as electromagnetic). Consequently, SERS has been observed in both the instances of chemi- and physi-soprtion of the molecule on the metal surface.

In the physical or electromagnetic mechanism, the enhancement is due to the excitation of plasmons on the surface of the metal.

Surface plasmons are the collective oscillations of the conduction electrons against the background of the metal ionic cores. They are caused by the time-varying electric fields induced by the incident light [5]. There are dipolar as well as multipolar plasmons on the metal surface. If the particle size of the metal, where these plasmons are generated, is much smaller than the wavelength of light, then all but the dipolar plasmons can be ignored. When the excitation light is resonant with these plasmons, light is emitted which is characteristic of dipolar radiation. Surface enhancement is a near-field phenomenon which means that both the generation of surface plasmons and the enhancement of the field caused by them are restricted to only within a few nm (nanometres) of the metal surface.

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In the chemical mechanism, the enhancement occurs when molecules form a covalent bond with the metal resulting in the formation of a metal-adsorbate complex [6, 7]. Due to the complex formation, molecular orbitals of the adsorbate gets broadened by interacting with the conduction bands of the metal. Consequently, this leads to a ready transfer of electronic charge and excitation from the metal to the adsorbate and vice versa.

This chemical enhancement, due to the formation of charge complexes, is relatively difficult to observe experimentally. Beyond the first layer of adsorbate, there is no exchange of electrons between the metal and adsorbate. On the other hand, the electromagnetic mechanism continues to enhance the signals beyond the monolayer. Though this extension only lasts for a few nanometers (nm), it is enough to dominate the enhancement due to chemical mechanisms completely. Thus, to study the chemical enhancement mechanism, one would have to devise a surface with a smooth geometry, where the electromagnetic effects are well studied and understood. The major advantage of this technique is that detection of very low levels of an analyte can be easily accomplished without any fluorescent label. Hence, this technique has also been used in several biological applications and in vivo studies [8, 9].

Coherent Anti–Stokes Raman Spectroscopy (CARS)

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CARS is a very different variant of Raman spectroscopy compared to its congeners and is possible only using laser as an optical source. This is fundamentally a non-linear optical technique where more than one laser beam is used. Before getting into the details of this technique, let us look at the term coherent in this technique. In optics, there are two types of coherence—spatial and temporal. What is coherence? A train of waves or a field is said to be coherent if there exists a fixed phase relation between the electric field at different locations (spatial) or instances of time (temporal). This translates in mathematical terms to the cross-correlation of the field at two points in space or time. The concept of coherence can further be understood in simpler terms if we ask the question—why do we use a near monochromatic source in Young's double-slit experiment. Recollect that in Young's experiment, the requirement was of a point source of monochromatic light. The point source was achieved by making use of a pinhole, and the monochromaticity was ensured by either using a mercury (Hg) lamp or using a suitable wavelength filter. This is because only coherent light can result in vivid bright and dark fringes due to interference. Whereas incoherent light, although will undergo interference, will not result in clear bright and dark fringes but more or less a band-like pattern. The perfectly monochromatic light will have temporal coherence but lacks spatial coherence, and this is brought about by the pinhole. Now, most importantly, what is the advantage of this coherence? This technique of CARS is an immediate albeit indirect answer to this question.

But we need to get introduced to yet another concept of nonlinear polarizabilities before describing the technique of CARS. Simply speaking, polarizability is an intrinsic property of every matter. In the context of optics, this is a parameter, which indicates the extent to which the electrons in a system, typically a molecule, gets displaced from their equilibrium position due to the incident electric field. This displacement induces a dipole moment. The magnitude of this dipole moment depends on the extent of displacement caused by the incident field. In the case of a relatively

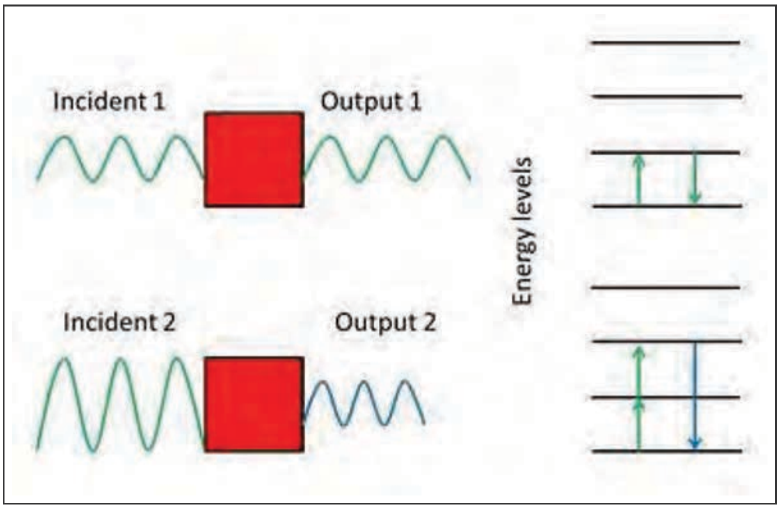


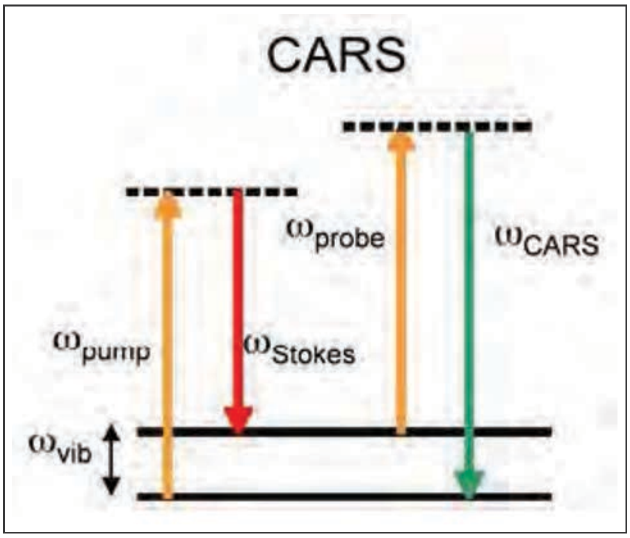
Figure 2. Depiction of linear and nonlinear interaction of light and matter.

weak incident field, the magnitude of the induced dipole moment varies linearly with the incident electric field. On the contrary, when stronger fields are incident, the extent of displacement is larger. In such cases, the relation between the magnitude of the induced dipole moment and the incident electric field ceases to be linear, giving rise to the nonlinear behaviour of the material. Without resorting to the mathematical aspect, the basic information is conveyed pictorially in *Figure* 2. Note that in the figure, the amplitude of incident 2 is much larger than the amplitude of incident 1, and thus the outputs are different in the two cases. In the linear domain, that is the weak field case, both the input and output energies are similar. This is not so in the case where amplitude becomes larger, giving rise to nonlinear responses. The same is depicted through energy levels and transition in the figure.

Having given a very brief introduction to coherence and nonlinear polarization, we can now take up CARS. In this technique, a minimum of two laser beams are used, although the same technique can also be carried out using three beams. But for the sake of a simple introduction, let us restrict the discussion to only two beams. The interaction of these two laser beams with the medium is summarized in *Figure* 3.

The wavelengths of the pump and Stokes beam are chosen such

Figure 3. Transitions in CARS (Image reproduced from *Wikipedia*).



that their difference, $\omega_{\text{pump}} - \omega_{\text{Stokes}}$ matches the frequency of a vibrational mode of the medium, ω_{vib}. Carefully compare the transitions in *Figure* 1 and 3. You may note that in *Figure* 1 (B), (C) and (D), the initial and final states are different, and this difference in energy is carried by the Raman photon as the vibrational signature of the molecule. But in *Figure* 3, the initial and the final states are the same, indicating that the molecule effectively does not gain or lose any energy. It merely acts like a medium where the photons mix and exchange energies. Even though there is no net change of energy in the molecule, for the sake of a simple understanding of CARS, we can still use transitions, strictly in a metaphorical sense. Here, the pump photon excites the molecule to the virtual state. Then the Stokes photon, which is also simultaneously present, drives the molecules to coherence. In other words, the molecules behave as efficient oscillators, or oscillating dipoles, which in turn modulates the refractive index in a periodic manner. This periodic modulation can be examined using a third beam, often called the probe beam, which, propagating through this medium, is modulated due to these oscillators or dipoles. When only two beams are used, the pump beam itself plays the role of the probe as well. A part of this probe beam gets modified to $\omega_{\text{pump}}+\omega_{\text{probe}}-\omega_{\text{Stokes}}$ (in the case of only two beams as discussed below, this becomes: $2\omega_{\text{pump}} - \omega_{\text{Stokes}}$) and is emitted as the anti-Stokes beam. Even though the molecule is not vibrationally excited, this anti-Stokes beam still carries the vibrational signature of the molecule. This anti-Stokes beam is coherent and several orders of magnitude stronger than the conventional Raman signal. Since it is a coherent technique, the signals, as mentioned above, are much higher compared to conventional Raman spectroscopy. For this reason, CARS has been extensively used in a very wide range of applications—from detecting the temperatures of the flame to identifying tumours in tissues of the human body [10]. One of the main disadvantages of CARS is that it requires two lasers, along with other sophisticated optical tools. In addition, the data handling becomes more involved compared to the other variants. Despite these challenges, CARS has been extensively exploited, as evident from the number of publications in the literature.

Raman Microscopy

Raman microscopy is a rather clever combination of Raman spectroscopy and microscopy. Microscopy offers spatial information, and when this is combined with Raman spectroscopy, we have chemical information as well. In a Raman microscope, several thousand Raman spectra are measured at various predefined locations. Subsequently, these numerous spectra are processed, and an image is recreated, which reveals both the morphology and the chemical information of the sample under investigation [11]. This is depicted in a picture form in *Figure* 4.

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In *Figure* 4, the entire process of collecting a Raman image is summarized. Briefly, the object that is to be imaged is placed under a virtual grid (*Figure* 4(a)). The Raman spectra are collected from each of these grid points as in *Figure* 4(b), which results in a data cube (*Figure* 4(c)). The data cube has the spatial information as well as the Raman spectral information in it. From thousands of Raman spectra, after sufficient processing, one can generate the image. There are several ways to generate the image, and one

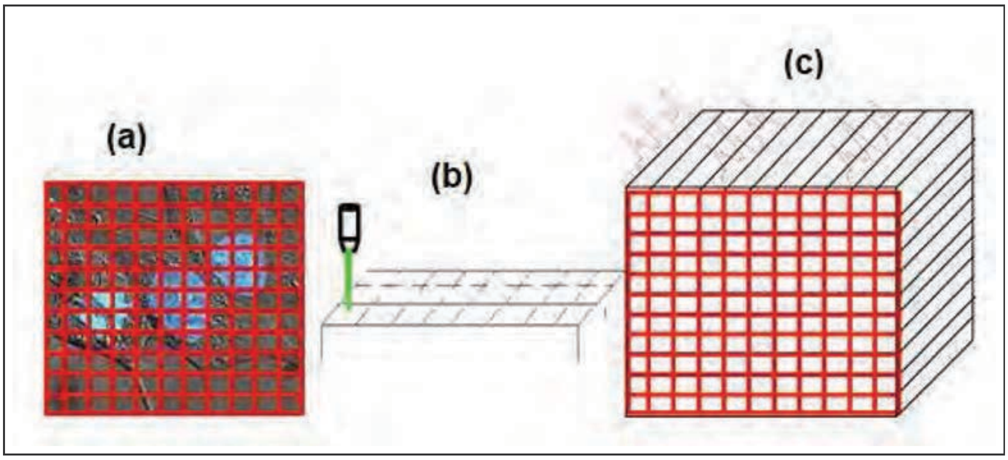

Figure 4. Steps involved in the construction of Raman image. (Note: (a), (b) and (c) are explained in the text).

of them is called the univariate method, where a single frequency neighbourhood is selected, and the corresponding intensity in all the spectra is converted to the image. This is illustrated in *Figure* 5, where the univariate Raman image of adipocyte cells is generated.

In *Figure* 5(a), the conventional bright field image of the cell is shown. This image was divided into 24000 grids, and the Raman spectrum was measured from each one of these grid points. *Figure* 5(b) has two such Raman spectra, and in fact, these two spectra are measured from the spots marked as A (bottom spectrum) and B (top spectrum) in *Figure* 5(a). Two small windows highlighting two peaks in the spectra in *Figure* 5(b) indicate the intensity of all the 24000 Raman spectra, and when these intensities were correlated with the spatial information, the image was generated. Please note that the images generated from the two spectra windows are quite different, where one corresponds to the nucleus, and the other to the cytoplasm of the cell. But just to recollect, these are merely Raman intensity plots only. Raman imaging has tremendous application in biology, for it can give chemical information without resorting to any sort of invasion or labelling. This has been used in several in vivo imaging to detect tumours, lesions and other abnormalities in the human body.

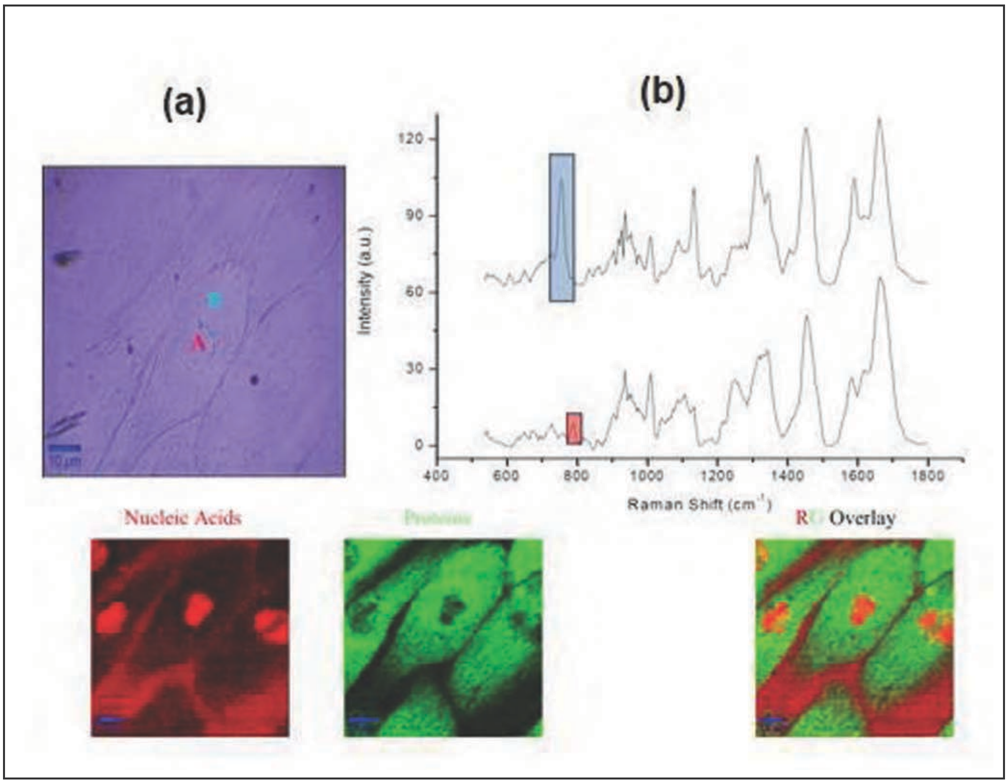

Figure 5. **(a)** Bright field image of adipocytes **(b)** Spectra measured from spots A and B shown in **(a)**. The Red (R) and Green (G) colour are respectively used to depict the images of nucleic and proteins which correspond to the intensities in the spectral windows shown in **(b)**. (Note: These images are a part of a hitherto unpublished study and is primarily work of the author carried out at the 3rd Institute of Physics, University of Stuttgart, Germany)).

Summary

In this article, the fundamental principle of Raman scattering was briefly introduced, and we discussed how the Raman scattered photons carry the information of vibrational modes of a molecule. A major lacuna of Raman scattering, i.e, its low signal, was highlighted, and the impact of lasers on Raman spectroscopy was mentioned. Various versions of Raman spectroscopy, resulting from the advent of lasers, like the resonance Raman (RR) spectroscopy, surface-enhanced Raman scattering (SERS) spectroscopy, coherent anti-Stokes Raman scattering (CARS) and Raman microscopy, were briefly introduced along with their broad areas of applications in different scientific domains. Thus, we have seen a sample of the avatars of Raman spectroscopy, and it is only a matter of time before newer avatars are announced.

Suggested Reading

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Address for Correspondence
V Ramanathan
Department of Chemistry
IIT (BHU), Varansi 221 005,
India.
Email:
vraman.iitk@gmail.com