# Raman Spectroscopy – An Overview of its Application and Potential for Life Sciences

*a report by*
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## Introduction

Raman spectroscopy has been used to determine the chemical composition of materials for over 70 years. However, due to spectacular advances in the technology, which gives higher sensitivity and lower cost instruments, Raman spectroscopy is enjoying a strong resurgence in interest. It is already finding application in the life science sector, and increasingly frequent reports of its utility are appearing in the literature. This paper is not intended as a comprehensive review but summarises the core technical features of the method, points to some of the novel and important work that is currently in progress and highlights the potential of the technique for future applications in life science.

## History of Raman Spectroscopy

The 'Raman effect' involves inelastic scattering of light, that is, the radiation scattered by molecules is shifted to a different frequency (energy) than the incident radiation. Although inelastic scattering was first predicted in 1923 by A. Smaekal[1], it was not until 1928 that Sir C.V. Raman carried out the first experiments[2], which confirmed the prediction and led to the award of his Nobel prize in 1930. Due to the fact that it could provide valuable information on the structure of samples at the molecular level, it received considerable attention as a method of non-destructive chemical analysis in the years following its initial discovery. However, its popularity waned after World War Two because technical advances in infrared (IR) absorption spectroscopy, (which also probes molecular vibrations), made that the technique of first choice. It is notable that the move to IR absorption measurements took place because of technological improvements, in this case the development of sensitive IR detectors, which made it easier to obtain the spectra required for chemical analysis. A similar change, but in the opposite direction, towards Raman methods rather than IR, is currently being driven by today's advances in photonics, although the Raman renaissance really started with the onset of the Cold War and the development of lasers in the 1960's. Since that time, the pace of change has accelerated, firstly with the introduction of multichannel detectors (initially photodiode arrays (PDA's), and now with charge coupled detectors (CCDs) designed specifically for spectroscopy). Also more advanced laser sources and high performance optical filters and spectrographs allow the development of ever more sensitive and reliable Raman instruments. This process of constant innovation and improvement in the technology available has pushed back the boundaries and allowed Raman spectroscopy to become a very powerful analytical tool that can be accessed by non-specialists. The technique has always had the potential to find wide application in the health and life science sector but it is only now that the instrumentation has evolved to the stage where this potential can be realised.

## What is Raman?

Raman spectroscopy is based on the detection of light that has been inelastically scattered by a sample; the 'Raman effect'. In the course of this article we will concentrate on the vibrational data that these measurements can give as the overwhelming majority of studies and applications of the technique are vibrational Raman experiments.

In general, when light interacts with a substance it can do so in three main ways: the light may be absorbed, it may be transmitted through the sample unchanged or it may be scattered. Figure 1 illustrates the general principle of the measurement, which is that the light from a powerful monochromatic light source (invariably a laser) is focused onto the sample of interest and as many as possible of the photons that scatter from the sample are collected and dispersed in a spectrometer. The photons that are scattered elastically (i.e without any change in their wavelength) comprise the Rayleigh scattering, which is intense but carries no vibrational information and

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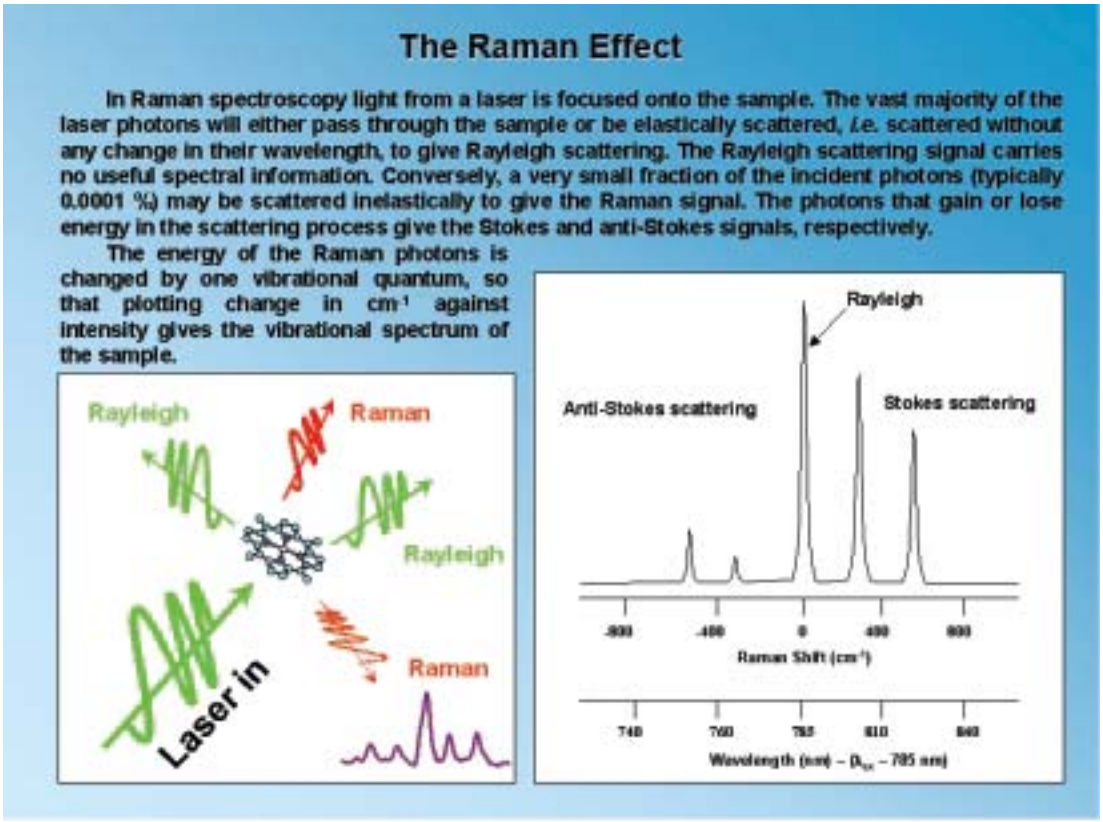
1. *A. Smekal. Naturwissenschaften. 1923; 11: 873.*
2. *C.V. Raman. & K.S. Krishnan. Nature. 1928; 121: 501.*

*Figure 1*



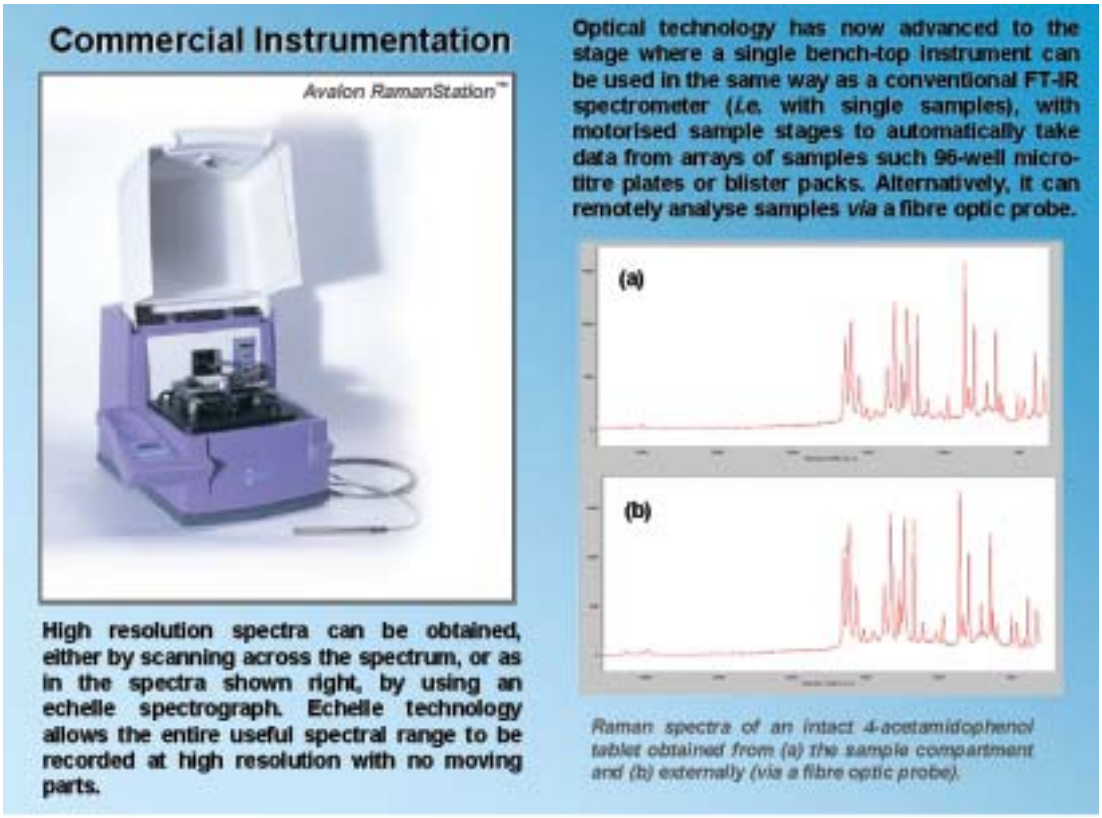

so is filtered out of the signal. In addition to the Rayleigh scattering, Raman scattering, in which the frequency of the incident photons changes due to interaction with the sample, can also be detected. Unfortunately, the Raman scattering comprises a very small fraction of the incident photons, typically 0.0001 %, hence the need for an intense source and sensitive detector.

Not all Raman-scattered photons have the same change in energy. First of all, some of photons lose part of their energy to give the Stokes scattering while others gain energy and generate the anti-Stokes signal. To generate an anti-Stokes signal the photons must encounter a vibrationally excited molecule in the sample, Stokes scattering has no such requirement so the Stokes signals tend to be stronger than the anti-Stokes and spectra are normally recorded only on the Stokes (energy loss) side of the spectrum. The Stokes photons lose energy by depositing into vibrational excitation of the sample so that the pattern of energy loss reflects the vibrational levels within the sample. In practice, plotting the intensity of the Raman scattered photons against frequency difference between incident and scattered radiation maps the vibrational spectrum of the sample. Overall, the Raman and infra-red (IR) signals measure the same vibrational levels (with subtly different selection rules). The major difference is that the presence of a vibrational band is detected in IR measurements directly by the absorption of an infrared photon, while in Raman experiments the same vibration causes the scattering of a photon which retains most of its energy but shows a small loss corresponding to the same energy absorption by the sample as occurs in IR spectroscopy.

Every compound has its own unique Raman spectrum, which can be used for both sample identification and quantification. Raman and IR spectroscopy can be used as complimentary techniques, because, due to differences in the spectroscopic selection rules, each is sensitive to different components of a given sample. As an example, IR spectroscopy is generally more sensitive to polar bonds, for example O-H stretches, whereas Raman is much more sensitive to vibrations of carbon backbone structures and symmetrical bonds, such as those in C = C groups. Using both techniques to characterise a particular substance can provide twice as much information on its chemical composition as would be obtained from using either of the techniques on their own.

Given the fact that inexpensive Fourier-transform infrared absorption spectrometers are widely available there would need to be significant advantages for any user to justify the effort required to move to Raman spectroscopy. There are indeed several advantages for Raman measurements, many of which are significant in the life sciences. Most notable of these are:

*Figure 2*




- Little or no sample preparation is required. Nujol or KBr matrices are not used, the laser is simply directed onto the sample;
- Wet samples or even aqueous solutions can be analysed because water is a particularly poor Raman scatterer;
- In-situ analysis is straightforward; no sample preparation is required, as Raman can analyse samples through glass and plastic;
- Fibre optics of up to 100's of metres in length can be used for remote analysis;
- Raman bands are narrower than those typically observed in mid-IR spectra and can be used more readily for quantitative analysis;
- Overtone and combination bands are generally weaker than in IR absorption spectra;
- The technique is suitable for use with both organic and inorganic materials;
- Raman spectroscopy can measure vibrations from symmetrical bands, which are generally very weak in IR spectra;
- Raman bands can be more easily related to chemical structure due to the fact that fundamental modes are measured.

Most of the disadvantages of Raman methods arise directly from the fact that it is a weak effect, which leads to the need for intense laser excitation sources and sensitive detectors. In the past this, in turn, led to relatively high costs for Raman instrumentation, which was one of the main obstacles to the widespread application of Raman spectroscopy for routine chemical and biological analysis. However, this situation is now changing, as laser and detector costs have fallen significantly, whilst performance has steadily improved.

There are numerous variations on the general experimental Raman methodology but the most commonly encountered variants are dispersive and Fourier-transform (FT) Raman methods, so we will confine discussion to these most important approaches. In general, Raman instruments, whether interferometric (FT) or dispersive consist of three basic components: the laser excitation source, the spectrometer (or energy analyser) and the detector. FT-Raman typically employs a 1064 nm excitation laser, an inferometer (which encodes the unique frequencies of the Raman spectrum into a single scan), and a single channel near infrared (NIR) detector. In comparison, dispersive systems use visible or near-red excitation lasers (488, 532, 633 and ca. 785 nm are most commonly employed), a grating for dispersion and a multichannel CCD detector. Overall, the dispersive systems are much more sensitive than those based on 1064 nm laser/inferometer combinations,

primarily because of the exceptional quantum efficiency of modern CCD detectors and the enhancement in Raman signal that occurs at shorter wavelengths. This increased sensitivity allows data to be acquired in shorter times, which has significant implications for high-throughput screening (HTS) applications. The dispersive instruments are also less expensive than FT instruments.

A significant factor in considering any Raman experiment on unpurified samples is the possibility that the sample may give a broad optical emission signal. In Raman spectroscopy such background fluorescence is problematic because the Raman signal is relatively weak so that any other emission coming from the sample can drown it out. Problem levels of background fluorescence can arise not only from samples that contain known flurorophores but also from adventitious fluorescent impurities that may be present at relatively low (sub-millimolar) concentrations. The problem of sample luminescence has been recognised for a considerable time and many different strategies have been employed, with reasonable success, to overcome it. These include: quenching the luminescence by using Surface Enhanced Raman Spectroscopy (SERS) and shifting the excitation wavelength to one which does not generate luminescence or gives luminescence that lies at a different wavelength range to the Raman signal[3]. Up to the 1990's the only widely used method was to turn to FT Raman instruments, which had long wavelength excitation sources and therefore did not excite the fluoresecence that was observed when using visible excitation. Recently, dispersive instruments with long wavelength (near-red) excitation has begun to find favour as a compromise which reduces sample fluorescence in much the same way as FT instruments but gives the sensitivity advantages inherent with multichannel CCD detection. Typically these use ca. 785 nm excitation and a classical spectrograph, although the advantages of using an echelle spectrograph, which allows the entire useful spectral range to be recorded at high resolution with no moving parts, are now being recognised and exploited.

The final major choice to be made in specifying a Raman spectrometer is whether to opt for a microscope-based system or to use 'macroscopic' sampling. A wide range of commercial instruments based on both sampling protocols is available and the choice of a microscope-based system is easy if very small routine sampling (< 10 _m diameter) is required. If this is not the case, the choice is much more complex as it becomes necessary to balance the increased cost and complexity of a microscope-based system (with its potential for sample damage from the highly-focussed laser source) against typical macroscopic systems which are simpler and less costly but only allow sampling of regions ca. 50 _m diameter. This is clearly a case where there is no single best option but where the individual user's requirements determine the optimum choice.

## Applications of Raman Spectroscopy

Raman spectroscopy was first employed for the analysis of biological samples as far back as the 1930's, however, due to the difficulties in obtaining high quality data its application in this area remained largely under-utilised. Generally, the study of intact biological samples using Raman spectroscopy only became widespread with the introduction of NIR FT-Raman instruments in the late 1980's, when there was a dramatic upsurge of activity in this field. The introduction of near-red diode laser-based dispersive instruments in the past few years has fuelled the growth in application of Raman methods most notably in the pharmaceutical and semi-conductor industries. Examples of cases where Raman spectroscopy has been applied in the investigation of biological samples include[4]:

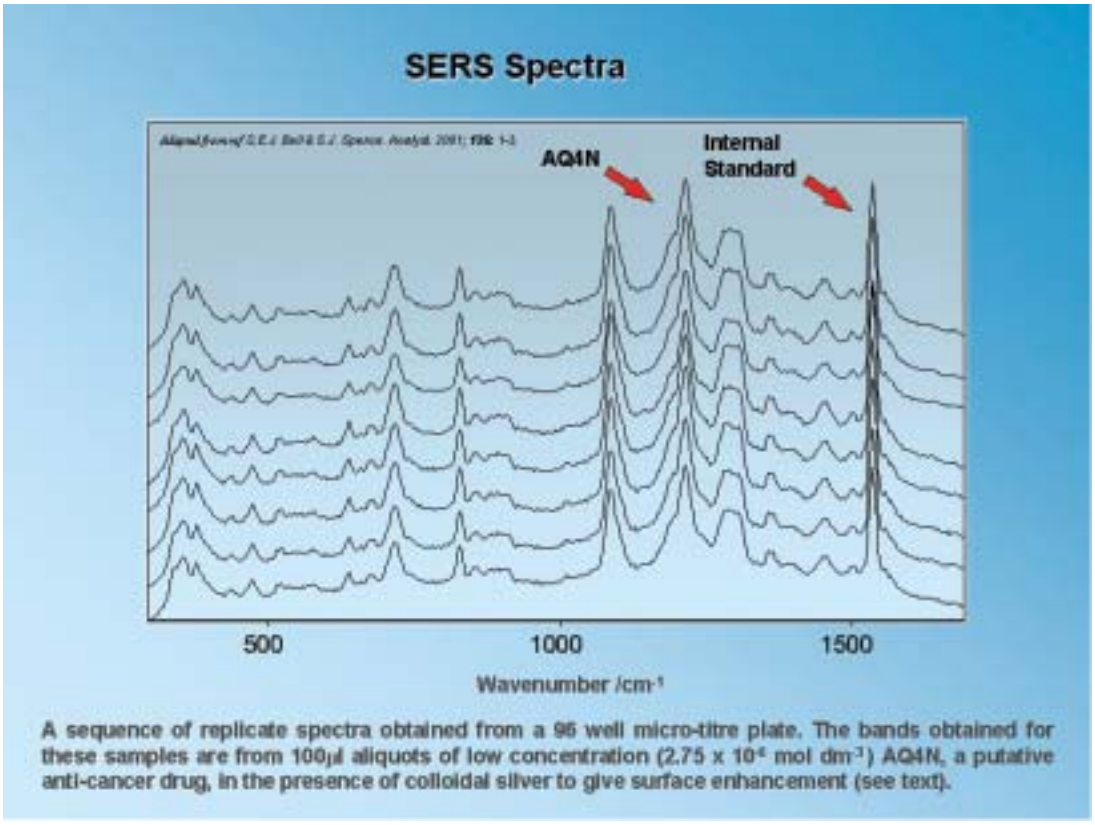
- Quantitative histochemical analysis of human arteries;
- Cancer diagnosis;
- Characterisation of gallstones and kidney stones;
- Diagnosis of metabolic disorders by taking Raman spectra of hair and nails;
- Hard tissue implant biocompatibility and in vivo recovery characteristics;
- Corneal dehydration in relation to impaired visual acuity;
- Imaging of cells, e.g. carotenoids in lymphocytes;
- Diagnosis of Alzheimers disease;
- Analysis of the stratum corneum in human skin in relation to the administration of therapeutic agents.

The unprecendented growth that has taken place in the biomedical and life sciences sector over the past decade is now providing a new challenge to spectroscopists. In particular, the sequencing of the human genome has marked a watershed in mankind's continued development. However, it is clear that instrumentation has been a limiting factor to the continued exploitation of the achievements to date. Despite the success of Raman methods in analysing bulk materials such as those listed above, investigations involving single molecules with low concentrations of DNA were prohibited by the low sensitivity of normal Raman methods. However, the development of surface-enhanced Raman spectroscopy (SERS), which involves the analysis of samples that have been

*3. S.E.J. Bell, E.S.O. Bourguignon & A. Dennis. Analyst. 1998; 123: 1729-1734.*

*4. E.E. Lawson, B.W. Barry, A.C. Williams & H.G.M. Edwards. Jn. Raman Spec. 1997; 28: 111-117.*

*Figure 3*




A sequence of replicate spectra obtained from a 96 well micro-titre plate. The bands obtained for these samples are from 100µl aliquots of low concentration (2.75 x $10^{-6}$ mol $dm^{-3}$) AQ4N, a putative anti-cancer drug, in the presence of colloidal silver to give surface enhancement (see text).

adsorbed to, or interact with, metal surfaces (typically a roughened metal electrode or a metal colloid) has allowed trace analysis by Raman methods to be carried out[5,6]. For example, it can be used for high-throughput screening (HTS) and detection of low concentrations of drug compounds, such as in the example shown in Figure 3[7]. In addition, under optimised conditions, SERS can provide spectral enhancements of 1014 or even 1015 compared to normal Raman scattering[8]. With these enhancement levels it is possible to envisage adsorbing a gene sequence onto an enhancing metal surface detecting minute quantities of specific DNA. Applications may include gene identification, gene mapping, DNA sequencing, medical diagnostics and nucleic acid identification. Further application may also be found within the nanotechnology sector, which involves work on the nanometer scale (10-9m). As we move into the 21st Century, advanced nanotechnology and biotechnology applications will become increasingly common. This emerging field of nano-biotechnology has the potential to significantly enhance healthcare in the future. It also represents a new challenge for methods such as Raman spectroscopy, especially in relation to the sensitivity of the instruments, as we move from the micro to the nano-level. Raman spectroscopy has now become a very important and powerful analytical tool in many different areas of industry and academia. Its utility within the health and life science sector is now of particular significance, because it can provide selective, rapid (HTS) and highly sensitive methods of analysis.

However, with regard to the biotechnology sector, a lot of work remains to be done, especially in relation to gene sequencing. It should be remembered that the human genome project involved the sequencing of the genes of only a few individuals. As yet, a lot of work has yet to be carried out in order to understand how the gene sequence relates to protein function and, indeed, how this can be used to provide effective disease treatment. If the potential of the human genome project is to be realised, in that individuals can receive personalised healthcare, there will be an increased need for fast, sensitive and low cost screening methods. It is envisaged that in the coming years genomic-based medicine will have a huge impact on our healthcare industry, and ultimately, the quality of life. Certainly, Raman spectroscopy has the potential to play a key role, and will undoubtedly make an increasing impact in this area in the coming years. ■

*5. A.C. Dennis, J.J. McGarvey & S.E.J. Bell. Proc. of the XVIIth Int. Conf. on Raman Spec. Beijieng. 2000; 682-683.*
*6. Y. Ye, J. Hu, L. He & Y Zeng. Vib. Spec. 1999; 20: 1-4.*
*7. S.E.J. Bell & S.J. Spence. Analyst. 2001; 126: 1-3.*
*8. A.M. Michaels, J. Jiang & L. Brus. Jn. Phys. Chem. B. 2000; 104: 11965-11971.*