Applications of Laser Raman Spectroscopy

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ABSTRACT

Raman spectroscopy has undergone a technical revolution during the last fifteen years. Following a brief introduction to the physical principles we present recent applications to materials of technological interest. Near-infrared excitation, in particular, offers the ability to measure the Raman spectrum in the absence of undesired absorption and photoreactions. The combination of Raman spectroscopy with microscopic techniques allows the study of materials on the micron scale with high molecular specificity. In the microstructural analysis of waveguide devices Raman spectroscopy employing integrated optical techniques can be extremely sensitive. As an example we review recent results on chalcogenide waveguides.

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INTRODUCTION

Inelastic scattering of light - or Raman scattering - from elementary excitations in a material yields structural and dynamic information on a molecular level. The Raman spectrum can be analyzed in terms of the molecular components or functional groups thus providing a ‘fingerprint’ of the molecule. The Raman effect was first observed in 1928 by C. V. Raman and K. S. Krishnan [1], but wide application was delayed until the development of the laser. The non-destructive nature of the probe, flexibility in sampling arrangements, and a technical revolution [2,3,4,5] in multichannel detection and Rayleigh filters have opened up many new areas where Raman measurements have proven to be very informative [6,7]. Applications in the electronics and chemical industries are increasing and range from process control in semiconductor and polymer production to microanalysis of integrated circuits [8].

The interplay between micro-structure and desirable properties of devices may be illustrated in materials suitable for use in high speed optical communication applications. These require all-optical processing and switching capabilities which must be compatible with current system configurations, possess ultrafast broadband response time, as well as low linear and nonlinear loss. Chalcogenide glasses (ChGs) have shown promise in that they exhibit properties compatible with the above requirements at 1.3 and 1.55 mm wavelengths [9]. Efforts to optimize film properties and device performance have focused on identifying the chemical and structural origin of the linear and nonlinear response in terms of the material processing conditions used in creating the optical element [10]. ChGs are photosensitive when exposed to bandgap energy (Eg ~2.35 eV for As$_2$S$_3$) [11]. Taking advantage of these photosensitive effects (photodarkening and photoexpansion) in ChGs, allows the creation of bulk waveguide structures [12], or the patterning of photoinduced relief gratings and guided wave structures in ChG films [13, 14]. Waveguide structures fabricated in ChG can be combined with proteins such as bacteriorhodopsin with potential applications in spectroscopy and switching of biomolecules on surfaces [15].

Near-infrared Raman spectroscopy affords new opportunities in the non-destructive analysis of materials which are strongly absorbing in the visible. A distinct advantage over the more conventional approach using the visible range of the spectrum is the ability to obtain the Raman spectrum of photosensitive compounds without interference from photoreactions caused by the probe beam. In chalcogenide glasses shifting the excitation wavelength to 840 nm (below the bandgap) allows one to obtain high quality Raman spectra and to correlate the underlying structure with nonlinear optical properties. The higher spatial resolution necessary to characterize planar films can be achieved with a microscope attachment. In the microstructural analysis
of single and multilayer waveguide devices, Raman spectroscopy employing integrated optical techniques can be extremely powerful. The material of interest is cast into a slab waveguide, thereby significantly increasing both the scattering volume and the electrical field intensity within the film. Waveguide Raman spectroscopy (WRS) using guided mode excitation [16] has recently applied to the structural characterization of chalcogenide glasses [17]. We discuss such experiments where Raman spectra were measured in the chalcogenide waveguide itself and in composite structures with protein layers excited by the evanescent field.

Through the incorporation of a microscope in Raman systems spatially-resolved compositional information is obtained. This opens up a wide range of applications where only a minuscule amount of sample (< 10^{-12} cm^3) is available or rely on imaging such as the characterization of internal stresses in semiconductor circuits. Forensic applications and trace detection fall in this category as well. Following recent technological innovations microspectroscopic techniques are now gaining widespread acceptance in research and industry and applications are growing rapidly [18].

There are a number of excellent books and review articles on Raman spectroscopy [2,3,7,19,20] to reference just a few. In this contribution we put some emphasis on recent applications which have been developed in our own laboratory. The article is organized as follows. First a brief background on Raman scattering is presented with examples of current experimental techniques. Experimental aspects of dispersive near-infrared Raman and Fourier-transform infrared Raman spectroscopy are presented. We continue with the use of these technique for novel materials for Raman gain and on investigations of As_{2}S_{3} bulk glasses and films depending on composition and processing conditions. Another application are the use of site specific Raman bands to probe conformational changes in proteins under high pressure. Finally, micro-Raman spectroscopy applications are illustrated.

**SPONTANEOUS RAMAN SCATTERING**

In a typical Raman experiment, the excitation source is a laser, and the scattered light is analyzed by a spectrometer and a detector with sensitivity near the single photon level. The inelastically scattered light contains information on vibrational states of the sample, which manifests itself by a frequency shift from the incident light. The underlying physics is that vibrations (or other excitations) modulate the polarizability tensor and cause the induced dipole moment to radiate at frequencies different from the electric field vector of the incoming light wave. For most applications the spontaneous Raman scattering originating from the linear response to the electric field is measured. The experimental challenge is to detect the weak
Raman signal while rejecting the intense Rayleigh background from elastic scattering.

A classical treatment of Raman scattering illustrates the basic physics. Assuming a simple diatomic molecule the binding effect of the electronic charge distribution is approximated by a spring between its point like nuclei [21,22]. For linear molecules the number of possible vibrations is \((3N-5)\) (for non-linear molecules \((3N-6)\)) where \(N\) is the number of atoms. Accordingly, the nuclei are space and rotation fixed about their equilibrium position but free to vibrate in simple harmonic motion along one normal coordinate \(q\).

If the excitation is a vibration, then the dynamical variable is the vibrational displacement \(q\). The polarizability of the molecule can be expanded as a Taylor series:

\[
\alpha = \alpha_0 + \left( \frac{\partial \alpha}{\partial q_k} \right)_0 q_k + \left( \frac{\partial^2 \alpha}{\partial q_k^2} \right)_0 \frac{q_k^2}{2} + \ldots
\]  

(1)

Here \(q_k\) is the displacement of the \(k\)th normal coordinate which can be represented as: \(q_k = q_k^0 \cdot \cos(\omega_m t)\) for a molecule oscillating at frequency \(\omega_m\).

The induced dipole moment can be written as: \(P = \alpha E\), where \(E = E_0 \cos(\omega t)\) is the incoming monochromatic electromagnetic wave. Neglecting the high power terms, we obtain:

\[
P = \alpha_0 E_0 \cos(\omega t) + \left( \frac{\partial \alpha}{\partial q_k} \right)_0 E_0 q_k^0 \cos(\omega t) \cos(\omega_m t)
\]

or

\[
P = \alpha_0 E_0 \cos(\omega t) + \left( \frac{\partial \alpha}{\partial q_k} \right)_0 E_0 q_k^0 \frac{1}{2} [\cos(\omega + \omega_m) t + \cos(\omega - \omega_m) t] \]

(2)

A Hertzian dipole emits electromagnetic radiation. Its intensity \(S\) is proportional to the square of the absolute value of the second time derivative of the induced dipole moment: \(S \propto |P|^2\). Thus, the first of the terms in equ. (2) describes Rayleigh scattering. The second term and third terms concern frequency shifted (i.e. inelastically scattered) light. These are also known as Anti-Stokes and the Stokes Raman scattering. The above equations can be generalized to the case, where the molecular polarizabilities are not isotropic and the induced dipole moment vector points in a different direction than the electric field vector [21]. A quantum mechanical treatment relates the polarizibility tensor to the wave functions and the scattering levels of the scattering system [21]. Similar to Rayleigh scattering, in the absence of nonlinear effects, the intensity of the scattered Raman light increases with the fourth power of the frequency of the exciting radiation. The intensity ratio of
the Anti-Stokes to Stokes line is given by a Boltzmann factor, \( \exp\left(\frac{-\hbar \omega}{kT}\right) \), where \( \hbar \) is Planck’s number and \( k \) the Boltzmann constant. This is illustrated by the Raman spectrum of carbon tetrachloride in Fig. 1. Note the strong polarization dependence of the totally symmetric mode at 13.8 THz (or 459 cm\(^{-1}\)). Shown are both the polarized scattering (vv) and depolarized (vh) scattering intensities. Here, the first v means that the incoming polarization is perpendicular to the scattering plane and that the polarization of the scattered light is selected either in the scattering plane (vh) or perpendicular to it (vv). Due to its higher intensity in spontaneous Raman scattering mostly the Stokes side of the spectrum is recorded.

![Raman spectrum of CCl\(_4\) measured with a double monochromator on the Stokes and Anti-Stokes side for two polarization. Vibrational frequencies are given in Terahertz (0.03 THz \(\approx\) 1 cm\(^{-1}\)). Spectral resolution is 25 GHz.]

In general, excitations modulate the electric susceptibility and consequently the induced polarization through fluctuations in their dynamical variables, \( \xi \). Such dynamical variables can include vibrational displacement for phonons, magnetization for spin waves and spin fluctuations, and electron (quasiparticle) density for electronic (superconducting) excitations.
The modulation of the susceptibility by $\xi$ adds an additional term to the polarization in the original polarization:

$$\mathbf{P} = \mathbf{e}_s (\chi \mathbf{E} + \chi' \xi \mathbf{E})$$

(3)

where $\chi' = d\chi / d\xi$ is the susceptibility derivative with respect to the dynamical variable, $\xi$. The first term in equation (3) drives the polarization at the incident field frequency, and therefore contribute to simple elastic scattering. However, since $\xi$ is itself time dependent, reflecting the characteristic fluctuations of the excitation, the second term modulates the induced polarization at frequencies different from the incident field. This term therefore contributes inelastic features to the spectral response.

Within this framework the differential Raman scattering cross section associated with an elementary excitation can be written as [23]:

$$\frac{d^2 \sigma}{d\Omega \ d\omega_s} = \frac{V \omega_i \omega_s^3}{(4\pi \varepsilon_0)^2 c^4 |E_i|^2} \langle \hat{\mathbf{e}}_s \cdot \hat{\mathbf{P}}_s^* \hat{\mathbf{e}}_s \cdot \hat{\mathbf{P}}_s \rangle_{\omega_i}$$

(4)

where $\langle \hat{\mathbf{e}}_s \cdot \hat{\mathbf{P}}_s^* \hat{\mathbf{e}}_s \cdot \hat{\mathbf{P}}_s \rangle_{\omega_i}$ is the spectral density of polarization fluctuations, $V$ is the light scattering volume, $c$ is the speed of light, and $\mathbf{e}_s$ is the polarization of the scattered light.

The power spectrum due to polarization fluctuations is given by:

$$\frac{d^2 \sigma}{d\Omega \ d\omega_s} = \frac{V \omega_i \omega_s^3}{(4\pi \varepsilon_0)^2 c^4} \left| \mathbf{e}_s \cdot \chi \mathbf{e}_i \right|^2 \langle \xi \xi^* \rangle_{\omega_i}$$

(5)

Here $\mathbf{e}_s$ and $\mathbf{e}_i$ are the scattered and incident polarization directions, and $\langle \xi \xi^* \rangle_{\omega_i}$ is the thermally-averaged correlation function for the dynamical variable $\xi$.

The light scattering cross section is related both to the correlation function of the relevant dynamical variable, $\xi$, and to the light scattering volume $V$. Furthermore, the light scattering cross section depends on the symmetry of the susceptibility derivative tensor, $\chi$, and indeed one can obtain excitation symmetry information by varying the scattering geometry defined by the polarization directions $\mathbf{e}_i$ and $\mathbf{e}_s$. This is an extremely powerful feature of light scattering techniques, since it allows identification of excitation symmetries.[23].

When light scattering is used to probe relaxations in a liquid the spectrum is mostly presented in the form of the susceptibility $\chi(\omega)$ which is obtained from the scattered intensity (up to a constant) by division with the thermal population factor $n(\nu) + 1$. Here, $n(\nu,T) = [\exp (hv / kT) - 1]^{-1}$, $\nu$ is
the frequency, T the temperature, and h and k are Planck and Boltzmann constants [24].

![Susceptibility of Toluene showing signatures of molecular vibrations, structural relaxation, and the Brillouin line. The horizontal axis displays the frequency of the excitation relative to the Rayleigh on the Stokes side. The excitation wavelength is 514.53 nm. The spectrum shown combines light scattering data measured with a double monochromator and a six-pass tandem Fabry-Perot interferometer.](image)

As an example the light scattering spectrum of toluene over a wide frequency range is shown in Fig. 2 on a double-logarithmic scale, The molecular Raman active vibrations are visible as sharp bands over the frequency range from $5 \times 10^3$ to $10^5$ GHz. Below $5 \times 10^3$ GHz a broad band due to structural relaxation in the liquid is discernible followed by the Brillouin line near 10 GHz. Other spectral representations which are in use for low-frequency Raman spectroscopy, but are related to the susceptibility, are discussed in ref. [25]. For a more extensive treatment of the theoretical background on light scattering the reader is referred to the literature [23,24].

**EXPERIMENTAL APPROACHES**

Over the past fifteen years the development of efficient filters for Rayleigh rejection and the availability of multichannel detectors have
considerably simplified the experimental set up with the additional benefits of increased optical throughput and shorter acquisition time. The spectrometer can be reduced to a single spectrograph stage with a notch [26,27] or sharp cut-off filter [28] selected for high extinction at the Rayleigh line [18]. Thus Raman spectroscopy has become even more accessible as a scientific tool.

From an analytical point of view, it is often desirable to characterize samples in nano- or picogram quantities. These requirements can be met by combining an optical microscope with a Raman system. Then the excitation spot has a dimension in the micron range, and Raman spectroscopy provides molecular compositional information with high spatial resolution. A schematic of a Raman setup is shown in Fig. 3. Raman scattering is excited by either an Ar ion or a Ti: sapphire laser. The scattered light is collected with a low f-number lens and focused with a second lens on the entrance slit of a single grating spectrograph (typical dispersion: 1.2 nm / mm in the focal plane). Multichannel detection at the single photon level is achieved with a backthinned charge-coupled device (CCD) detector.

The CCD itself is a Si based array detector. Absorbed photons are converted to electron-hole pairs. The electrons are collected in potential wells created by a depletion layer. The wells can be addressed individually and digitized with the readout electronics. CCD's are characterized by a high dynamic range (~ $10^5$), high quantum efficiency (~ 90 %), wide spectral range (400 - 1050 nm) and low read-out noise (~ 2-5 e^-/Pixel as seen in Fig. 4).
Traditionally one works in the visible region of the spectrum, sometimes also to exploit resonance enhancement to extract information on the chromophore or the prosthetic group of a protein [29]. At the same time this approach has drawbacks since strongly absorbing samples such as many polymers and biological molecules can degrade or undergo undesired photochemistry during exposure to the laser beam. More importantly, a fluorescence background frequently obscures the Raman signal. One way to overcome these problems is to shift the laser excitation into the near-infrared.

Fourier transform Raman spectroscopy [30,31,20,3] has provided a means of measuring the Raman spectrum of strong visible absorbers in the absence of fluorescence and resonance enhancement. When using the 1064 nm line of a Nd:YAG laser as an excitation source the Stokes shifted Raman spectrum occurs in the near infrared, typically between 6000 and 10000 cm\(^{-1}\). The scattered radiation is focused on the entrance port of a conventional FTIR spectrometer and effectively replaces the internal light source for absorption spectroscopy. The analysis of the Raman spectrum via Fourier Transform benefits not only from the multiplex and throughput advantages but also from the inherently higher wavenumber accuracy of the interferometric method.
In polymer science FT-Raman spectroscopy has been used to probe conformation and side chain packing in polysilanes and nonlinear optical materials [32]. Applications to macromolecules of biological interest concentrated on a synthetic polypeptide [33], polyene antibiotics and lipid bilayers [34]. Of particular interest in biology is the vibrational structure and photochemistry of light harvesting proteins such as bacteriorhodopsin and visual pigments. Due to the photolabile nature of these compounds great care has to be taken to avoid sample deterioration. FT Raman spectroscopy using excitation beyond a wavelength of 1 micron often can overcome these problems. We show that it is feasible to measure high quality spectra of the retinal isomers within minutes in a standard flexible sampling geometry.

FT-Raman spectra were measured in a 90° scattering degree with excitation by the 1064 nm line from a cw Nd:YAG laser [35]. The scattered light was analyzed with a Bomem model DA3.02 Fourier transform interferometer, which was equipped with a cooled (-35 ℃) indium-gallium-arsenide photodiode detector.
Fig. 6 displays the FT-Raman spectrum of bacteriorhodopsin between 500 and 3500 cm\(^{-1}\) [35]. The sample was in solution and no smoothing or baseline correction has been performed. Quite differently, to obtain the resonance enhance Raman spectrum with excitation in the visible rather tedious sampling handling methods like molecular flow or spinning cells are required [36]. The CH stretch mode is near 2900 cm\(^{-1}\). The most intense band at 1531 cm\(^{-1}\) can be assigned to the C=C due to the polyene part of the retinal. The chromophore vibrations dominate the spectrum. This may be caused by the high polarizability of the conjugated bonds in the retinal. The FT-Raman technique references the measured frequencies to the frequency of an internal He:Ne laser. Therefore the absolute frequency can be determined to better than 0.01 cm\(^{-1}\) and the band positions reported are limited by the collection parameters. Fig. 7 displays the spectra in the ethylenic mode region of bacteriorhodopsin, 13-cis, and all-trans retinal. Some bands of the isomers differ by frequency shifts of a few wavenumbers only, but are clearly resolved. The interaction with the protein causes a frequency shift of the chromophore bands in bacteriorhodopsin.
The examples presented above have demonstrated the potential of FT-Raman spectroscopy as a nondestructive technique for molecular characterization of photolabile chromophores and biopolymers. Among the limitations of FT Raman spectroscopy are the loss in signal intensity due to the dependence of the scattering cross section on the 4th power of the wavenumber and the need for Rayleigh line rejection filters. By employing a tunable Ti:sapphire laser in the range between 700 and 1000 nm the excitation can be shifted to shorter wavelengths, yet the excitation wavelength can be chosen long enough to avoid fluorescence or undesired photochemistry. An excellent long pass filter can be realized by a semiconductor single crystal with a band gap in this energy range [37]. The alignment can be performed in the visible before the Ti:sapphire laser is tuned to the desired excitation wavelength in the near infrared.

Another approach is to use a near-infrared laser source (most commonly a diode laser at 785 nm or a tunable Ti:sapphire laser) and a back-thinned CCD detector in combination with a dispersive instrument [38, 28, 2]. Some applications using this technique are included in the following section.

Fig. 7  The ethylenic mode in bacteriorhodopsin, all-trans, and 13-cis retinal.
APPLICATIONS

In the following we illustrate recent applications of Raman spectroscopy to materials of current technological interest. To begin with we discuss recent examples of advanced materials for Raman gain applications. The elucidation of structural properties in chalcogenides glass and the use of waveguide Raman spectroscopy follows. Then, we describe experiments which use Raman spectroscopy to probe conformational changes in heme proteins. Finally, we touch on a few applications of micro-Raman spectroscopy.

Glasses for Raman gain

One the most crucial components in optical communication systems is the optical amplifier. Recent progress in the fabrication of glass fibers have significantly increased the available transmission window for optical communication [39]. The increase in bandwidth has caused great interest in employing Raman amplifiers due to their potentially much larger bandwidth as compared with Erbium doped fiber amplifiers (EDFA). It is important, therefore, to find a material with wide bandwidth for Raman gain. On theoretical grounds, the coefficient for Raman gain depends linearly on the spontaneous Raman scattering cross-section [40]. Experimentally, a direct comparison between spontaneous and Raman gain spectra in two TeO$_2$ based glasses has recently shown a peak gain thirty times that of fused silica and twice its spectral bandwidth. It was also demonstrated that the Raman gain profile and intensity mimics that of the spontaneous Raman spectrum.[41].

The Raman spectrum of a tellurite based glass is shown over an extended frequency range from 6 – 1500 cm$^{-1}$ in Fig. 8, together with the spectrum of fused silica (SiO$_2$). The spontaneous Raman spectra were measured using 514.53 nm excitation and a double monochromator. The top spectrum is that of the glass with composition 85%TeO$_2$-15%WO$_3$. Note that the fused silica spectrum has been multiplied by a factor 17. Fused silica is employed as a standard material to quantify Raman gain. The high intensity and large bandwidth of the tellurite glass compared fused silica predicts favorable properties for Raman gain application.

Raman bands in the high frequency region originate from the vibrations of the molecular bonds. In the TeO$_2$ system increasing TeO$_2$ and decreasing PbO concentrations are determinants for the intensity of the main peaks. The intensity of the bands between 610 and 670 cm$^{-1}$ associated with trigonal bipyramids increases with TeO$_2$ content. In the borophosphate compositions containing tungsten oxide significant features in the Raman spectra are bands at 770 and 950 cm$^{-1}$ attributable to vibrations of distorted WO$_3$ units. This
band is highly polarized indicating that WO$_3$ are preserved with small intermolecular coupling.

The depolarization ratio is indicative of the symmetry of the vibrations involved in the scattering process. A highly symmetric vibration will have a depolarization ratio close to 0. In the low frequency region large Raman scattering is observed. The intense band near 40 cm$^{-1}$ (Fig. 8) is attributed to the Boson peak. The larger depolarization ratio in the frequency region below 400 cm$^{-1}$ suggests that in this range Raman amplification is much less polarization dependent.

In the low frequency range the scattered intensity needs to be corrected for the thermal population factor, $n(\nu) + 1$ where $n(\nu,T) = \exp (h\nu / kT) - 1$,$^4$, $\nu$ is the frequency, $T$ the temperature, and $h$ and $k$ are Planck and Boltzmann constants $^2$. The vv polarized spectra divided by $n(\nu) + 1$ are shown in Fig. 9 $^{[42]}$. These correspond to the predicted Raman gain curves and they show a very broad band in the low frequency region (50-400cm$^{-1}$). This indicates that in the Tellurite glasses a flat Raman gain profile down to very low
wavenumber can be obtained while the gain is more than ten times that of fused silica. The bandwidth of both glasses are almost twice as wide as that of fused silica, and the Raman intensity (which is proportional to the Raman scattering cross-section) of the telluride glass is several times higher than that of the fused silica [42].

A universal feature of the Raman spectrum of glasses is the so-called Boson peak. We can see from Fig. 9 that after correction for the thermal occupation factor there is broad and intense Raman scattering in the low frequency region due to excess vibrational excitations.

We know that, for crystals, the density of states follows the Debye’s law [e.g. $g(\omega) \propto \omega^2$] in the low frequency region, but there are deviations for amorphous materials. For amorphous materials, there is an additional contribution to the density of states as compared with the Debye’s law. This excess density of states is characteristic for the Boson peak. Sometimes the representation $g(\omega)/\omega^2$ is chosen which peaks at a frequency $\omega_{BP}$.

Fig. 9 Predicted Raman gain curves after correcting for the thermal population factor of tellurite glass (85 TeO$_2$-15 WO$_3$) and fused silica. The fused silica spectrum has been multiplied by a factor 10 to allow better comparison with the spectrum of 85 TeO$_2$-15 WO$_3$. 
In amorphous materials the density of states $g(\omega)$ and the Raman spectrum can be connected using a relation by Shuker and Gammon [43]:

$$I(\omega) = C(\omega) \frac{g(\omega) [n(\omega) + 1]}{\omega}$$  \hspace{1cm} (6)

where $I(\omega)$ denotes the Raman intensity for the Stokes side of the spectrum, $[n(\omega) + 1]$ the Bose-Einstein factor, and $C(\omega)$ is the light-vibration coupling coefficient. The density of states can be independently measured by neutron scattering. Based on such combined data it has been suggested that in many glasses the coupling coefficient varies nearly linearly with frequency [44].

The origin of the Boson peak or excess of density of states in amorphous materials is still under debate, and several models including localized vibrational states and medium range disorder effects have been proposed [45]. Excess density of states due to disorder can be considered to arise from the atomic positions or a distribution of force constants [46]. Computer simulations have shown that force constant disorder alone can give rise to the boson peak [47].

### Chalcogenide glasses

Two characteristics of As-S-(Se) compounds - a large glass forming region and a wide optical transmission band, with potentially low loss for the 1.3 - 1.55 mm telecommunications window - make them excellent candidates for infrared guiding configurations. The availability of these glasses in substantial quantities and the capability of fabricating good optical quality thin films by thermal evaporation and other deposition techniques enables the realization of relatively low cost As-S-(Se) integrated optical devices. Another attractive feature capability to create integrated components with one- and two-photon laser writing [48,49].

Fig. 10 illustrates the near-infrared Raman spectra (incident and scattered polarization resolved along the z-axis) for a series of binary and ternary compounds. The spectra were obtained at a spectral resolution of 1.5 cm$^{-1}$. The increased spectral resolution of the bulk spectra clearly shows that each of the dominant bands consist of several overlapping components [50,51]. The dominant feature in the binary sulfide and selenide compounds are bands at 345 cm$^{-1}$ ($As_2S_6$) and 230 cm$^{-1}$ ($As_2Se_6$), respectively. These spectra are in good agreement with other studies [52,53], and the strong, broad band is attributed to an anti-symmetric As-(S,Se) -As stretching vibration in the As(S,Se)$_3$ pyramids. According to the analysis of Lucovsky and Martin [54], the normal modes of the bulk glasses (e. g. clusters of As(S,Se)$_3$ molecules with weak intermolecular coupling) are obtained by treating the molecular
pyramid modes \((\text{As(Se)}_3)\) and bridging chain modes \((\text{As-(Se)}_3-\text{As})\) independently.

![Raman spectra of a series of bulk chalcogenide glasses obtained with near-infrared excitation.](image)

**Fig. 10** Raman spectra of a series of bulk chalcogenide glasses obtained with near-infrared excitation.

In the ternary compounds with \(\text{S/Se} = 1\) molar ratio and decreasing As content, a progressive decrease of these broad bands is observed, indicative of a decrease in the number of As-containing pyramidal sites. New bands appearing around \(255\ \text{cm}^{-1}\) and \(440 - 480\ \text{cm}^{-1}\) form in the now chalcogen-rich glasses, and are attributed to Se-Se and S-S homo-polar bonds. These may be correlated with the enhancement of nonlinear optical properties \((n_2)\) in ternary compounds with \(\text{S/Se} = 1\) molar ratio and decreasing As content [10]. These units serve as chalcogen chains connecting the remaining pyramidal units. The small number of S-S bonds indicated by a weak band near \(495\ \text{cm}^{-1}\) for equal concentrations of S and Se, suggests that the S stays with the remaining pyramids, and that it is the Se which dominates the connecting chain units.
Chalcogenide thin films - waveguide Raman

Waveguide Raman spectroscopy (WRS), using guided mode excitation has been applied to thin organic and polymeric films, to probe spontaneous [55,16] and coherent [56] scattering; and very recently, to sol-gel derived planar germano-silicate [57] waveguides and lead titanate [58] films. The relative low refractive index n of these organic and oxide materials allows the use of glass prisms such as LaSF5 (n ~ 1.8) for coupling a range of propagation vectors into the waveguide structure. In spite of its sensitivity WRS has not been applied to the structural characterization of chalcogenide glasses until recently [17], most likely due to their high index (n ~ 2.45), the lack of suitable prism couplers, and difficulties associated with working in the near-infrared.

Cleaved silicon substrates were employed for high efficiency endface coupling of the near-infrared laser beam into a single layer channel waveguide structure [17]. The wave guides were nominally 1.75 - 2 µm thick. Fig. 11 displays the Raman spectrum of As$_2$S$_3$ and Fig. 12 the spectrum obtained from bacteriorhodopsin layered on the waveguide substrate [15]. As shown in Fig. 13, As$_2$S$_3$ has Raman active vibrational bands below 500 cm$^{-1}$. The vibrational frequencies below 300 cm$^{-1}$ are attributed to S-S interactions and the vibrational frequencies between 300-400 cm$^{-1}$ are attributed to AsS$_3$ pyramidal units and their interactions [52].

Fig. 11 Raman spectrum of a chalcogenide thin film obtained with waveguide excitation and a power of 20 mW.
For the integration of waveguide structures with photo-sensitive proteins bacteriorhodopsin (bR) is of particular interest, since there are applications in molecular electronic devices and optical switching [59,60]. As with all proteins and organic assemblies, the characteristic vibrational frequencies of bR are in the 1000-4000 cm⁻¹ range. This allows for no interference in the protein’s signal from that of the waveguide. The bR spectrum in Fig. 12 is indicative of the light-adapted state [61]. The light adapted form of bR is the initial state for the proton pumping cycle. At 785 nm the Raman spectrum is still associated with the vibrations of the atoms that comprise the chromophore. Resonance Raman, nuclear magnetic resonance (NMR), and chemical extraction studies have established that the chromophore in bR 568 is a C₁₃=C₁₄ trans, C₁₅=NHR trans protonated Schiff base of retinal. As shown in Fig. 13, bR has several vibrational frequencies that are identifiable. The band at 1012 cm⁻¹ is assigned to the rocking vibrations of a C-CH₃ group of the bR molecule. The 1100-1300 cm⁻¹ region of the Raman spectrum is the fingerprint region for C-C bonds and is very sensitive to isomerization. The intense band at 1528 cm⁻¹ is attributed to the ethylenic stretching of C=C bonds [61]. The data demonstrate that evanescently excited near-infrared Raman spectra can be measured with high signal-to-noise ratio providing an in-situ probe of the native state of the protein.
ChG waveguides are optimized for near-infrared excitation allowing to obtain the Raman spectra of biological compounds at minimal background fluorescence. This feature of the substrates can be a useful tool in the study of cells and microorganisms. Obtaining the Raman spectra at wavelengths less than 1 \( \mu \)m allows for the resolution of small spatial features compared to mid-infrared absorption wavelengths of 5-10 \( \mu \)m. Integrated optical components fabricated with ChG can be combined with proteins such as bR. Evanescent wave excitation may be employed for optical switching and spectroscopy of bio-assemblies on patterned ChG semiconductors. There are also potential applications involving bio-molecular sensors.

**High-pressure Raman spectroscopy of proteins**

Resonance Raman spectroscopy is one of the few techniques which can probe the local environment of the active site inside a large biological system. The laser excitation wavelength is chosen near an electronic transition of the chromophore, and the Raman scattering cross sections for vibrational modes which couple to this transition are selectively enhanced [29]. Raman spectra of heme proteins reveal a number of bands that have been well characterized and yield information on the spin state, coordination, and environment of the heme [29]. The small, globular heme protein myoglobin has served as a model system for extensive experimental and theoretical studies of protein dynamics. During the process of reversibly binding small ligands such as O\(_2\), CO, or NO to the heme iron, both the chromophore and the protein undergo conformational changes. The bond between the iron and the proximal histidine imidazole nitrogen is the only covalent linkage between the heme group and protein. Resonance Raman studies at ambient pressure support the view that modulation of the iron by the protein through the proximal histidine exerts control at the level of reactivity [62,63,64].

In vivo, functional properties of proteins are affected by environmental parameters such as viscosity, pH, temperature and pressure. For instance, sea animals survive over a wide range of pressure, from sea level to extreme depths. On the other hand, pressure can deactivate enzymes and kill bacteria [65]. For a description on a molecular level the effect of high pressure on prototype reactions of isolated proteins must be understood [66,67]. The approach to combine high pressure and vibrational spectroscopy is motivated by the following observations: Spectral band parameters (frequencies, intensities, line shapes and widths) are sensitive to dynamic and structural changes of biomolecules [68] at the sub-Angstrom level, a length scale where small, yet significant conformational changes for enzyme activity occur. From changes in the Raman spectra, pressure effects on protein function can be
correlated with structural changes, for instance at the chromophore-protein interface [69] and compared with theoretical models [70]. Deoxymyoglobin is used as a reference structure since the reaction process is absent, and pressure induced changes of the conformation can be separated from those along the reaction coordinate.

Resonance Raman scattering was excited with the frequency doubled output of a Ti:sapphire laser tunable from 441 to 425 nm or by the 457.9 nm line of an Ar ion laser. Detection of the backscattered Raman radiation is accomplished using a thin back-illuminated charge-coupled in conjunction with a single-grating spectrograph and a Rayleigh line rejection filter. The pressure cell is constructed of beryllium-copper that combines the ability to resist high pressure (up to 400 MPa) with good thermal conductivity. Sapphire windows allow measurements from the near UV to the near infrared region. The high-pressure Raman setup has been described in more detail in refs. [71] and [72].

![Fig. 13](image)

**Fig. 13** Low frequency region of the Raman spectra of deoxygenated myoglobin at ambient and high pressure. Samples are in aqueous solution or glycerol-water mixtures at pH 7. Note the shift of the band near 220 cm\(^{-1}\) to higher frequency with increasing pressure.

The resonance Raman spectra of horse deoxy myoglobin (Mb) in the frequency range from 150 to 600 cm\(^{-1}\) are shown for ambient and high pressure in Fig. 13. The band at 220 cm\(^{-1}\) has been assigned to the iron-histidine (Fe-His) stretching mode [73,74,75]. The other lines have been classified as follows [75]: The band near 241 cm\(^{-1}\) is a pyrole ring tilting
mode. The modes from 250 to 420 cm\(^{-1}\) all involve peripheral substituents, and those from 420 to 520 cm\(^{-1}\) are attributed to out-of-plane distortions of the pyrole rings \[76,75\].

The most significant spectral change with pressure is a shift of the peak frequency \(\nu_{\text{Fe-His}}\) of the iron histidine mode. \(\nu_{\text{Fe-His}}\) shifts to higher wavenumber by \(\sim 3\) cm\(^{-1}\) between 0.1 and 175 MPa. The shift of the Fe-His mode has been observed in different solvents (75% gly / H\(_2\)O) and in Mb from sperm whale. The peak position of \(\nu_{\text{Fe-His}}\) as a function of pressure is plotted in Fig. 14. The error bars correspond to a precision of \(0.6\) cm\(^{-1}\).

A smaller shift is apparent in the band near 343 cm\(^{-1}\). Choi and Spiro \[76\] have assigned this band to out-of-plane modes of propionate porphyrin macrocycle substituent groups. Since the hydrogen bonding partner of the carboxyl group of the propionic acid attached to the D pyrole \[77\] is Arg 45 (CD3), changes in the 343 cm\(^{-1}\) band may indicate motion of the protein helices.

![Fig. 14 Pressure dependence of the peak frequency of the iron-histidine mode in myoglobin in aqueous solution and 75% gly / H\(_2\)O.](image)

We attribute the observed frequency shift to a conformational change, which alters the tilt angle between the heme plane and the proximal histidine and the out-of-plane iron position. The geometry of the proximal histidine influences both the frequency and the intensity of the Fe-His stretch band \[74\]. An important factor affecting the global protein conformation, the heme pocket structure, and the iron-histidine mode is water activity \[78\]. The altered water activity in a glycerol / H\(_2\)O mixture causes a 2.6 cm\(^{-1}\) downshift of \(\nu_{\text{Fe-His}}\) as compared to aqueous solution. Apparently glycerol influences the protein-water interaction with a possible release of bound water molecules from the surface, though this perturbation of the Fe-His frequency is opposite to that from pressure.
The main conclusion from the shift of the iron-histidine mode is that pressure causes global conformational changes in the protein as well as rearrangements of the active site environment. Indeed, a very recent high-pressure crystallographic study [79] of myoglobin confirms that the change in protein structure due to pressure is not purely compressive but involves conformational changes. Large collective displacements are observed in six regions including sliding of the F-helix towards the E-helix [79].

In the case of ligand bound myoglobin (MbCO, MbO₂) photolysis by the laser beam during the acquisition of a Raman spectrum creates a stationary mixture of bound and photolyzed molecules. Photostationary experiments demonstrate a significant pressure dependence of the ligand rebinding rate in myoglobin [80]. The photolysis of ligated myoglobin by the laser beam during the acquisition of a Raman spectrum (100 seconds) creates a stationary mixture of bound and photolyzed molecules (Fig. 15).

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Fig. 15  Resonance Raman spectra of MbCO (top) and MbO₂ (bottom) in aqueous solution as a function of pressure at 295 K. The sample is in photostationary equilibrium. As the pressure is increased the intensity of the ν₂ band of MbCO (1374 cm⁻¹) increases relative to that of Mb (1356 cm⁻¹).
This is evident from the oxidation state marker band (n₄) which appears at 1354 cm⁻¹ for deligated Mb and 1372 cm⁻¹ for MbCO [62]. The spectrum at high pressure (keeping all other parameters fixed) shows a significant increase in intensity of the peak at 1372 cm⁻¹ relative to that at 1354 cm⁻¹ reflecting an increase in population of the bound state [71]. This population increase is due to a speedup of the overall rebinding. Consistent changes between bound and unbound states are also seen in the core size marker band n₂ (1560 and 1582 cm⁻¹) and the vinyl modes (1618 and 1632 cm⁻¹). The lower amount of photolysis at high pressure is also indicated by the reduced intensity of the iron-histidine mode at 220 cm⁻¹ [71].

**Micro-Raman spectroscopy**

For the measurement of a minuscule amount of sample or when spatial resolution is required (in addition to spectral resolution) various combinations of a Raman spectrometer with an optical microscope have been developed [81,18]. This is known as Raman microscopy or Micro-Raman spectroscopy. A typical setup is shown in Fig. 16. The excitation laser is reflected by a beam splitter, goes through a microscope objective, and is focused on the sample. The backscattered Raman is then collected with the same objective. The major part of the collimated beam is reflected from the beam splitter and is focused on entrance slit of the monochromator. The small sample area which is at the focusing point is thus imaged through the entrance slit. It is important to understand that a narrow slit width will determine a well defined sharp image, and thus result in a high resolution.

Fig. 16 Schematics of micro-Raman spectrometer. The sample is located on an 3-d positioning stage.
Applications in an industrial environment or for routine testing of samples require an experimental technique which requires no specialized sample preparation. Raman spectroscopy is a non-destructive probe and spectra can be measured on materials in solid or liquid form. Using a Raman microscope the sample can simply be placed on a slide and the area of interest is selected optically by using a viewing system. Fig. 17 shows the Raman spectrum of a piece of silicon and an aspirin sample obtained with a commercial micro-Raman instrument. With micro-Raman vibrational spectra can be measured from micron-sized particles which makes it well suited as an analytical tool in chemistry and biotechnology.

![Raman spectra of aspirin and silicon](image)

**Fig. 17** Raman spectra of aspirin and silicon obtained with a commercial micro-Raman instrument (LabRam HRUV). Integration time is 1 sec, HeNe laser power 10 mW, and slit width 100 µm.

Raman spectroscopy has proven to be an informative and nondestructive technique in III-V material characterization including local structure determination and stress analysis [82]. One of the key issues for the performance of wide bandgap semiconductor based device structures is the control of growth-induced defects and their impact on optoelectronic and transport properties. Despite the impressive progress in device applications a deeper understanding of defect and impurity issues is necessary for continued rapid development in the areas of LEDs, laser diodes, UV detectors, and high voltage unipolar and bipolar [83].

In the following we present an example where micro-Raman spectroscopy was used to probe optical phonons in gallium nitride close to the
GaN / sapphire substrate interface [84]. Frequency shifts in vibrational modes correlate with independently obtained data on the dislocation density and are connected to the strain due to lattice mismatch at the interface. Micro-Raman spectra were measured in a backscattering geometry using the 514.53 nm line of an Ar$^+$ ion laser. An infinity corrected microscope objective focused the laser beam to a spot size of about 1 µm in diameter. A very narrow slit width (5 µm) in combination with binning in the vertical direction of the CCD chip acts as a confocal aperture which leads to a 1 – 2 µm depth resolution. A translation stage with submicron sensitivity was used for laser beam positioning at the predetermined distance from the GaN/sapphire interface. The excitation beam was parallel to the surface of the interface and perpendicular to grows direction of the GaN layers.

![Raman spectra](image)

**Fig. 18** Raman spectra of a 64 mm thick GaN film measured with 514.5 nm excitation. Insert shows $E_1$(TO) and $E_2$(high) modes for various distances from the GaN interface.

Fig. 18 depicts the Raman spectra at various distances from the GaN/sapphire interface over a range of 60 µm. The data were measured with a backscattering geometry corresponding to $x(\ldots)x$ configuration in Porto notation. The propagation direction of the laser beam was perpendicular to the c-axis but not along one of the principle axes so the $A_1$(TO), $E_1$(TO), $E_1$(LO), and $E_2$(high) modes [85] are observed at 535 cm$^{-1}$, 562 cm$^{-1}$, 745 cm$^{-1}$ and 569 cm$^{-1}$, respectively. Due to limitations of the Rayleigh filter the $E_2$(low) mode
(144 cm\(^{-1}\)) was not investigated. The spectral resolution of 1 cm\(^{-1}\) was achieved by recording the Raman spectrum in second order. The raw data presented in Fig. 18 show a shift to lower frequency with increasing distance from the sapphire substrate. The precision in the determination of the frequency shifts could be further increased by fitting Lorentzian lines to the Raman peaks. Since the lineshape remains constant we estimate that spectral shifts of 0.1 cm\(^{-1}\) can be reliably detected [86].

![Graph showing peak frequencies of the Raman E\(_2\)(high) and E\(_1\)(TO) modes as a function of distance to the substrate interface. The solid lines represent exponential fits to the data points with asymptotic values of 567.2 and 558.3 cm\(^{-1}\), respectively. The open symbols show results from a second experiment on a sample grown under the same conditions.](image)

The peak frequency of the Raman modes are displayed as a function of the distance from the GaN / sapphire interface in Fig. 19. To verify the
reproducibility measurements were made in both directions: from the interface to the surface and then backwards. For the A₁(TO) mode no shift could be observed. Also there were no significant changes in full width at half maximum (FWHM) or intensity of the spectral bands. The E₂ Raman mode is known to be shifted by stress. In GaN films large compressive stresses causing frequency shifts of +4.5 cm⁻¹ compared to single crystals have been observed [87,88]. The small shifts in the data presented here indicate the good quality of our films. The absence of a shift in the A₁ phonon can be explained by the direction of the optical phonon eigenvectors with respect to the c-axis. Among the Raman active modes A₁ is the only one with displacements along the c-axis as opposed to the E₂(high) and E₁(TO) vibrations [82,85]. Thus it appears that the stresses are perpendicular to the c-axis.

Raman microscopy enables measurement of spectra from a single “point” defined by the optical resolution limit of the microscope. It thus allows point-to-point mapping whereby the spectroscopic information from different points of a relatively large sample can be detected and compared. Raman imaging allows to non-invasively visualize chemical heterogeneity through the integration of microscopy and spectroscopy. It provides information which is useful in the fabrication of new materials, evaluation of the performance of existing materials, and control of product quality.

Fig. 20 shows an optical image of an integrated circuit and a Raman intensity image of the region indicated by the square. The stepsize was 1.75 µm at an integration time of 10 s per point spectrum. From the spectra the Raman intensity map on the right of Fig. 20 was constructed. Different gray levels are used to label different intensity of the 520 cm⁻¹ silicon mode.

Fig. 20  Optical image (left) and Raman intensity map of the region indicated by the square (right) of an integrated circuit.
The frequency of the Si phonon is sensitive to local stress and thus Raman microscopy is employed in strain analysis of silicon materials [89]. Raman imaging is an rapidly developing field which extends into cell imaging and biomedical diagnostics. We refer to some excellent reviews in the literature [6,90,91] which describe the development and detailed applications of this technique.

**CONCLUSIONS AND OUTLOOK**

Raman spectroscopy combined with flexible sampling arrangement such as a microscope attachment has developed into powerful analytical technique providing molecular information on materials with high spatial resolution. Changes in chemical bonding as well as structural variations depending on processing and illumination conditions can be characterized. The micro-Raman configuration provides depth resolved data suitable for probing interfacial structural differences within multilayer structures, whereas the waveguide Raman technique enables longitudinal sampling.

Small, all-solid-state excitations sources are likely to continue to have an impact on extremely compact, portable microspectroscopy systems for material analysis. This applies particularly to near-infrared diode or miniaturized Ti:sapphire lasers. Another recent development is to combine two of the principal probes of molecular structure - Raman scattering and infrared absorption - in a united instrument [92]. Because their selection rules complement one another, the two vibrational spectrosocopies provide a powerful tool in combination.

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