

# Spectroscopy—a technology looking for applications

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Spectroscopy today is, by far, the most extensively and intensively used laboratory technique since its limited application as an analytical tool by Newton in the late 17th century. The extent of its sophistication and applications during the 20th century has grown in leaps and bounds and has given rise to a “brave new world” where, like the laser, it is also a tool looking for more and more new and esoteric applications. It has grown many branches and sub-branches including its applications for understanding the subatomic world, solving many scientific and technical problems on Earth, and analysis of our cosmic Universe. The present article is intended to give a flavour of its potential. Particular emphasis is placed on fluorescence and Raman spectroscopies.

## 1. Introduction

UNWEAVING THE RAINBOW<sup>1</sup> is what spectroscopy is in a nutshell. Isaac Newton started it on a scientific basis in 1672. Until the late 1970s absorption and emission spectroscopies were just common laboratory tools, mostly for routine identifications and limited quantitative analyses of atomic or molecular species in materials. In recent time there have been unprecedented advances in technologies in the fields of lasers, high-resolution dispersive system fabrication, optoelectronic detection and data processing procedures. Applications of these advances in spectroscopy have expanded its sophistication and versatility in leaps and bounds and opened up scope for ever newer and more esoteric research and practical applications. The state-of-the-art of the technology now has reached a stage when it may be said that it is indeed “a technology looking for applications”. This paper is limited to absorption and emission spectroscopies within the UV (ultraviolet)–Vis (visible)–IR (infrared) spectral region, covering a wavelength band from 400 to 700 nm.

In scientific terms, spectroscopy is all to do with the spectral analyses of electromagnetic signals emitted from or absorbed by atoms and molecules in materials. The emission and absorption processes are essentially due to interaction between the electric field of the probing

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<sup>1</sup> The title of a book by Richard Dawkins about the wonder of science.

electromagnetic (EM) radiation directed onto the sample and the electrons of the atoms in the sample materials. Therefore, a concept of these interacting entities is needed for understanding the basis of spectroscopy.

## 2. Electromagnetic radiation

At the end of the 19th century, following a century-long debate on the nature of light, it was concluded from both experimental and theoretical considerations that light behaves like both a particle and a wave in motion, depending on its interaction with itself or with matter. The concept of “wave–particle duality” for the nature of light was accepted as a working model. To explain the correlation between the colour of emitted (optical) radiation and the temperature of a heated (glowing) object (a “black body”), Planck had to make a very fundamental assumption<sup>2</sup>—the heated atoms emit radiation as discrete “wave packets”, each carrying a definite energy  $E$  proportional to the vibration frequency  $\nu$  of the amplitude (strength) of the wave inside the so-called wave packet; i.e.,  $E = h\nu = hc/\lambda$ , where  $h$  is Planck’s constant ( $6.62 \times 10^{-34}$  J s),  $c$  is the velocity of the EM wave ( $2.99 \times 10^8$  m s<sup>-1</sup>) and  $\lambda$  is the wavelength. The wave is just a visual representation of the energy associated with the changing strength (in space and time) of the electric and magnetic fields (energy) emanating from a system as an electromagnetic wave. Faraday discovered that current (flow of electrons) along a metal wire gives rise to a magnetic field across (perpendicular to) its flow and a changing magnetic field generates a current in a metal wire. Clerk Maxwell unified the equations related to these interactions and came out with the startling concept that during rapid changes in electric and magnetic fields in a metal wire, wave-like energy packets with interdependent and at the same time integral oscillating electric and magnetic fields come out and traverse the space (Fig. 1). It turned out that the speed of that wave is the same as the speed of light, which had already been measured quite accurately, implying, as Maxwell propounded, that light must be electromagnetic radiation. It transpired later that the EM spectrum encompasses a wide range of wavelengths, in which visible light falls within a narrow range (Fig. 2).

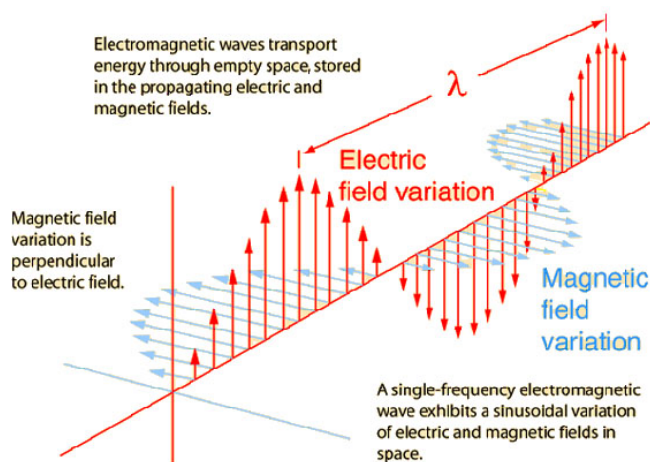
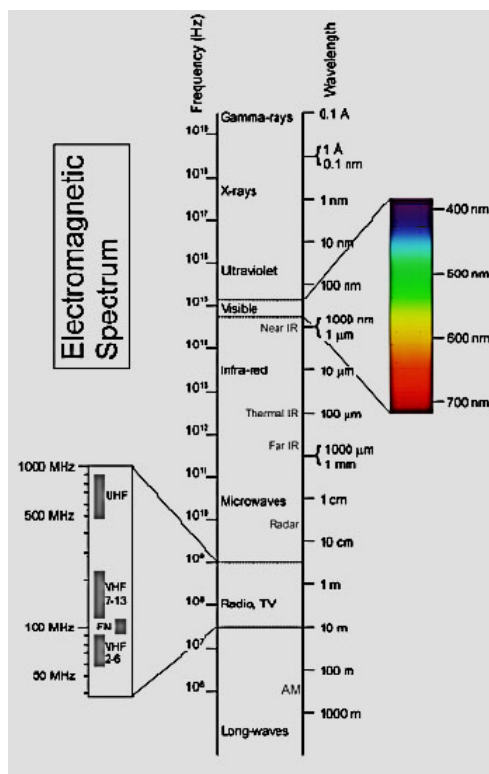


Figure 1. Electromagnetic wave packets (quanta).

<sup>2</sup> E.L. Grove (ed.), *Applied Atomic Spectroscopy*. New York: Springer (1978).

Figure 2. Electromagnetic spectrum. Within the visible range, red corresponds to 700 nm, orange/yellow to 600 nm, green to 500 nm, and blue/violet to 400 nm.



In this particular conception of light, a light “quantum” (a wave packet) needs to have a mass. This concept is, however, different from the mass of other fundamental particles such as the electron, proton etc. To explain some observed phenomena, such as the photoelectric effect, the light needs to impart momentum to the electron to eject it from its place in an atom. Since by definition light has no rest mass (it only exists in motion), its mass is defined in terms of its momentum  $p$  which, in accordance with the classical concept, is the product of the mass and velocity of a particle. In the case of light (and indeed all EM radiation), Planck attributed a particle-like property to the wave; i.e., a photon having momentum  $p = h/\lambda$  (conversely, de Broglie attributed a wave-like property to the electron, considered to be a particle, and this relation nowadays bears his name).<sup>3</sup>

### 3. Interaction of radiation with matter

Matter is composed of different combinations of either atoms (inorganic) or molecules (organic—made from atoms including carbon). Atoms are essentially made of three stable particles: electrically charged electrons (negative) “orbiting” around a core (nucleus) where

<sup>3</sup> The Nobel Prize for Physics was awarded to Planck in 1918 for the discovery of energy quanta, to Einstein in 1921 for the discovery of the law of the photoelectric effect, and to de Broglie in 1929 for the discovery of the wave nature of electrons.

protons (positive) and neutrons (neutral) exist. EM radiation, essentially a charged entity (electric field), only interacts with atoms by influencing the charged electrons.<sup>4</sup> Each electron orbital corresponds to its inherent energy content (state). Absorption and emission of electromagnetic radiation by electrons cause transitions between energy states (levels) of the electrons. The energy states of the electron in which it can exist is governed by the laws of quantum physics and a graph of the populations of the states versus wavelengths of absorption and emission provides tell-tale spectral “signatures” for the identification of atoms and molecules and their concentrations. Since these signatures are furthermore affected by the environmental conditions of the atoms and molecules, analyses of such spectral signatures will also provide information about the environment. Spectral signatures are complicated by the fact that the electronic states are always associated with some well-defined vibrational sublevels (ignoring the lesser contributions from the associated rotational energy levels), which also participate in transition processes. The concept of vibrational energy levels may be simplified by visualizing the atoms in a molecule as balls connected by springs (bonds) in perpetual vibration. When these are compressed or stretched by external stimuli the overall potential energy of the molecule changes in accordance with the frequency (energy) of the stimuli. The potential energy diagram and the transition scheme in a molecule are typically represented by a potential energy versus internuclear distance diagram (Fig. 3). Vibrational levels (also quantized) are of the bonding energy (also associated with a frequency) of the atoms; transitions between these levels give rise to absorption or emission in the infrared spectral region. When their spectral signatures are very narrow, they are designated and represented by lines (with an associated parameter, linewidth) in intensity (peak value) versus wavelength plots, which constitute the basis of spectroscopy as considered in this article.

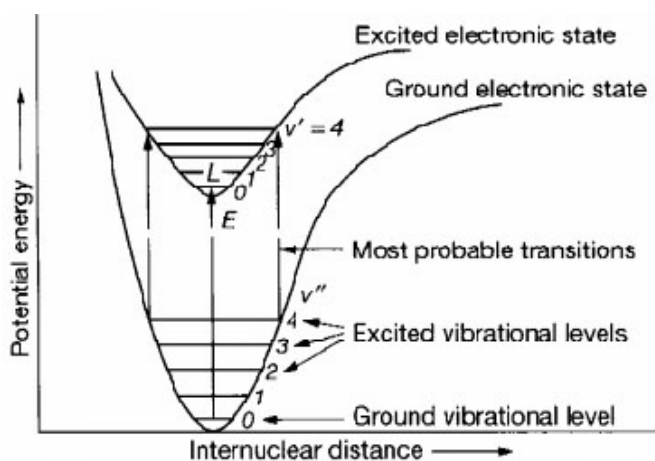


Figure 3. Schematic diagram of energy levels and transitions in a molecule.

<sup>4</sup> In principle the field also interacts with the charged protons in the nucleus, but this is of negligible practical importance. See, e.g., J.M. Blatt and V.F. Weisskopf, *Theoretical Nuclear Physics*, ch. 12 (pp. 583–669). New York: Springer (1979).

Atomic spectroscopy<sup>5</sup> involves complex methods for atomization of the sample and a sophisticated spectral filtering system for the analysis of very narrow-band emission/absorption lines, mainly in the UV-Vis regions. The present article confines its attention to the widely used molecular spectroscopy, in which the transitions take place between electronic states coupled to vibrational levels of atoms of the molecules in different configurations.

#### 4. Practical considerations

Pre-laser spectroscopy utilized a broad-band arc lamp (a thermal source) emitting in all directions and over a wide range of wavelengths covering the UV-Vis-IR bands (Fig. 4). Dispersive elements (prism or gratings) were used for wavelength selection for both emission and detection arms by scanning the dispersed beam across a slit or by using a multichannel detector. For absorption spectroscopy the sample under investigation usually needs to be semitransparent; changes in the intensity at selected wavelengths from a broad-band source generates an absorption spectrum (absorbance versus wavelength graph) of the material. For this, the tuning filter and other optical configurations are needed to make the beam of radiation as parallel as possible. The later will allow focusing the beam to the smallest possible width on to the entrance (or exit) slit of the filter, allowing selection of the narrowest possible band of wavelength, hence best resolution. The emission spectroscopy uses a wavelength selector to analyse the emitted broad band source (the sample). In modern spectroscopy the thermal source (including optics and filters) is replaced by a powerful, tunable laser source. Similarly, the conventional lens (or mirrors) in the detection optics is often replaced by efficient fibre-optic cables coupled to highly efficient (shot noise-limited) photodetectors or silicon-based multichannel detectors.

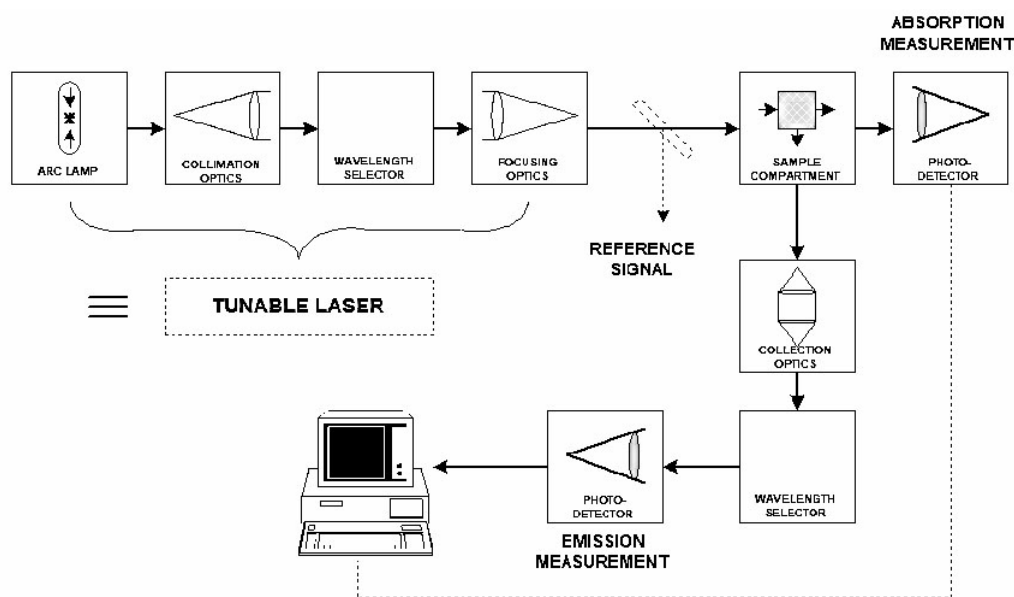


Figure 4. Schematic diagram of a spectroscopy setup.

<sup>5</sup> N.H. Bings and A.C. Broekaert, Atomic spectroscopy: A review. *Anal. Chem.* **82** (2010) 4653–4681.

## 5. Black body radiation spectroscopy

Heated materials emit radiation with a characteristic spectral signature depending on the temperature of the object following Planck's blackbody radiation law.<sup>5</sup> The peak emission wavelength of an incandescent object shifts towards shorter wavelengths; i.e., the UV spectral region, as the temperature increases (Fig. 5).

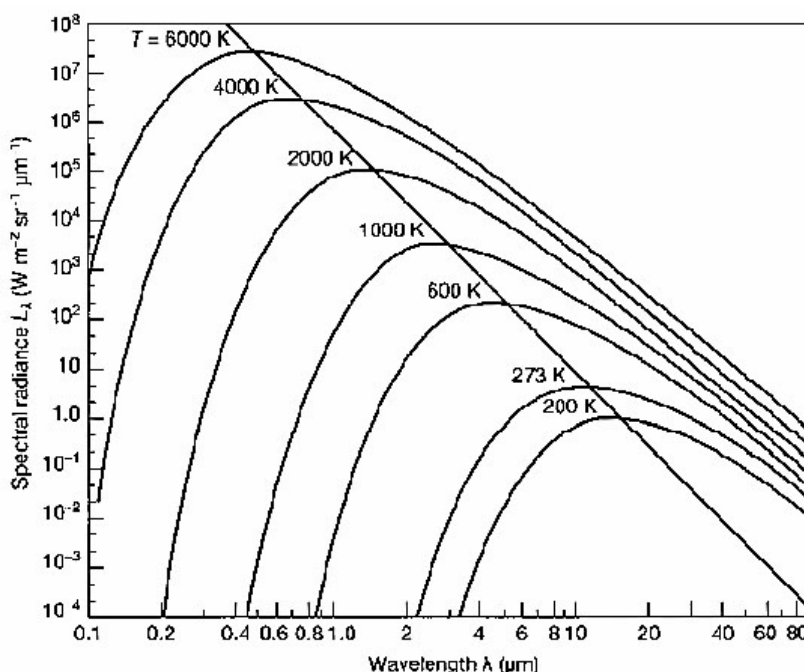


Figure 5. Planck's black body radiation law for objects at different temperatures.

Emission spectroscopy is widely used in experimental cosmology for the accurate measurement of temperature of distant stars,<sup>6,7</sup> as they are supposed to behave like black body (idealized emitters). The application of this technique, using the black body radiation law, coupled with atomic spectroscopy, is vital in astronomy and astrophysics as it allows chemical composition, temperature, density, mass, relative motions etc. of distant stars to be determined. It's also widely used for rapid identification of unknown materials using flame tests.

## 6. Optical absorption spectroscopy

### 6.1 Theoretical considerations

In principle, an absorption spectrometer provides a graph of the absorbance  $A$  of a sample of effective length  $L$ , through which the beam has traversed, against the wavelength  $\lambda$  of the light.

<sup>6</sup> G.B. Rybick and A.P. Lightman, *Radiative Processes in Astrophysics*. Wiley (1979).

<sup>7</sup> S.M. Stewart and R.B. Johnson, *Blackbody Radiation*. Boca Raton: CRC Press (2016).



For practical purposes  $A = \log_{10} (1/T)$ , where the transmittance  $T = P_t/P_i$ , where  $P_i$  and  $P_t$  are the incident and transmitted powers of the radiation on and from the sample respectively;  $T$  is also given by the Beer–Lambert–Bouguer law as  $T = \exp(-\mu L)$ , where  $\mu$  is the attenuation coefficient of the medium. In a commercial absorption spectrometer the output is normally a graph of  $A$  versus  $\lambda$ .

In a semitransparent medium the transmission loss is primarily due to surrounding molecules and particulates in the medium. If a molecular species, absorbing at selected wavelengths, is uniformly dispersed in the medium and the attenuation of the beam by the medium is either negligible or compensated by comparing with the spectrum of the medium only, from the Beer–Lambert–Bouguer law we have  $T = \exp(-\alpha c L)$ , where the absorption cross-section  $\alpha$  is defined according to the concentration units.<sup>8</sup> The absorbance at any wavelength is then given as,

$$A = \log_{10} (1/T) = 0.434 \alpha c L. \quad (1)$$

The *linear dependence* between concentration and absorbance is only strictly valid for low concentrations of absorbing molecules in the medium (solid, liquid or gaseous). Variation of the refractive index of the medium at different wavelengths, lack of monochromaticity of the incident light (needs good wavelength selectivity of the filter) in the case of non-laser sources, and the presence of stray light may cause some deviation from equation (1). It should be well noted that the wavelength at the peak of an absorption band of certain chromophores is strongly dependent on the polarity of the solvent, causing shifts in the peak.

In the UV-Vis spectral region, practical absorption spectroscopy often involves chemically unsaturated chromophores (i.e., double or higher bonds). Their absorption bands may occur over the range 200–700 nm, depending upon the complexity of the atomic bonding and vibrational modes.

## 6.2 Applications

We recall that in the infrared spectral region, absorption is attributed to transitions between allowed vibrational levels of electronic manifolds (Figs 3 and 5). This encompasses the wavelength band 0.78–1000  $\mu\text{m}$ , several orders of magnitude wider than the UV–Vis region (0.2–0.7  $\mu\text{m}$ ). For practical reasons the spectrum is divided into three bands: near IR (0.78–2.5  $\mu\text{m}$ ), middle IR (2.5–50  $\mu\text{m}$ ) and far IR (150–1000  $\mu\text{m}$ ). The strongest absorption corresponds to the mid IR band, where the fundamental stretching modes are found. Therefore most analytical equipment is set up for measurements over this wavelength band. Modern IR absorption spectrometers are equipped with tunable diode lasers operating over this band. This, along with developments in other relevant technologies used in the devices, has made this branch of spectroscopy the most widely used in studies in biophysics and molecular biology.<sup>9,10</sup> These

<sup>8</sup> In  $\text{cm}^2$  when  $c$  is expressed as # molecules/ $\text{cm}^3$ ; as absorptivity in litre/(g cm) when  $c$  is in (g/litre) and  $L$  is in cm; as molar absorptivity in litre/(mole cm) when  $c$  is in moles/litre and  $L$  is in cm.

<sup>9</sup> Pranav Kumar, *Fundamentals and Techniques of Biophysics and Molecular Biology*, p. 33. New Delhi: Pathfinder Publications (2018).

<sup>10</sup> K. Langer, A.N. Platonov and G.R. Rossman, *Optical Absorption Spectroscopy*, pp. 109–123. Berlin: Springer-Verlag (1996).

“other relevant technologies” include fibre optics and state-of-the-art “on-chip” data processing, which allow access to other IR spectral bands and enable miniaturization of commercial spectrometers for a plethora of applications, in particular for industrial process control and environmental monitoring.<sup>10,11</sup>

## 7. Optical emission spectroscopy

This branch of spectroscopy includes emission from samples acting as sources over the IR to UV wavelength bands, involving different electronic energy transfer mechanisms in molecules, characterized mainly as fluorescence/phosphorescence, Raman scattering and infrared emission as shown schematically in the energy level diagram in Fig. 6.

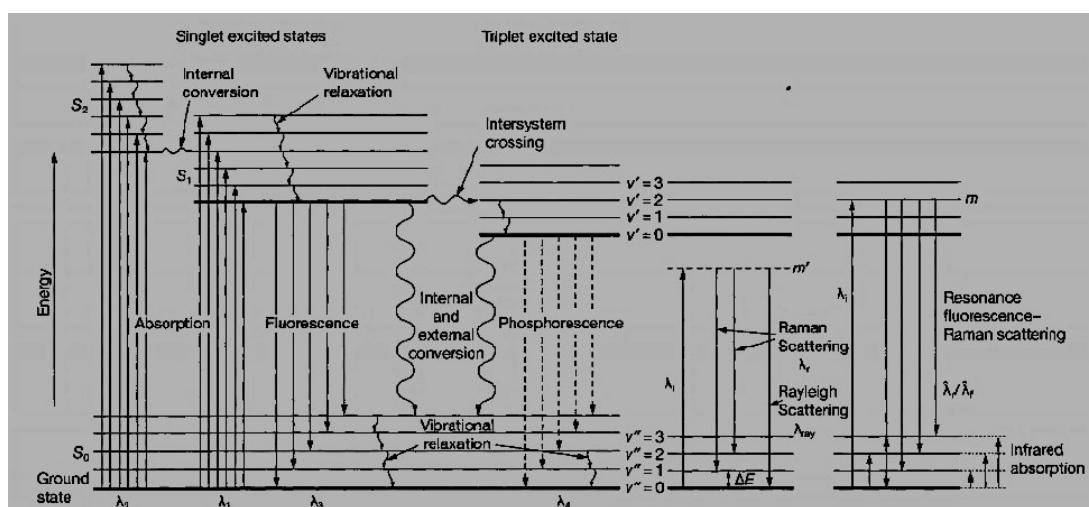


Figure 6. Absorption and emission scheme in a molecular system.

### 7.1 Fluorescence

#### 7.1.1 Principle

Energy absorbed by a molecule may raise its potential energy from a stable (ground) electronic state to an allowed excited (higher) electronic state. As is shown in Figs 3 and 6, these electronic states are split into discrete allowed vibrational levels. The excited molecule reverts back to its stable state by dissipating the energy through nonradiative processes (vibrational relaxation, internal and external conversions) and a plethora of other radiative processes. Depending upon the excitation wavelength, molecules will either absorb the energy to make a transition to a metastable allowed singlet ( $S_1$ ) manifold (lifetime  $\sim$ nanoseconds) or simply scatter the energy instantly, reverting back to the ground state and emitting radiation without any change of the wavelength of the incident radiation (Rayleigh scattering). There is a probability (depending

<sup>11</sup> Rafi Ahmad, Michael Cartwright and Frank Taylor, *Analytical Methods for Environmental Monitoring*. London: Pearson Education (2001).



upon the type and the environment of the molecules) that some of the excited molecule will change their energy state to another singlet state ( $S_2$ ) through a vibrational relaxation (nonradiative) process. In this state the last level has a relatively longer lifetime (tens of nanoseconds). Transitions from this level to the vibrational levels of the ground electronic state give rise to fluorescence. Further intersystem crossing, depending upon any contribution from the electronic spin-state, to a relatively stable (lifetime  $\sim$ microseconds) state may ensue. Transitions from such levels to the ground electronic manifolds will give delayed emission (phosphorescence). If the excitation energy is wavelength-specific, such as light from a tunable laser source, the molecule can be excited to a specific vibrational level of an excited electronic state. Transitions from these levels to other vibrational levels of the ground state give rise to resonance fluorescence. The quantum yield for such emission is higher than that of the normal Raman or fluorescence emissions (*vide infra*). From practical considerations, fluorescence and Raman spectroscopies are the most widely and effectively utilized for many applications, and are therefore dealt with in some detail in the following.

### 7.1.2 Fluorescence quantum yield

Molecules exhibiting strong fluorescence when excited by UV-Vis radiation are known as chromophores. The spectral signature of such emission identifies the molecules. However, for most practical applications of this spectroscopic technique the target molecules are surrounded by other molecules, such as when suspended in a solution, or embedded in a solid. Therefore their strength (efficiency) and spectral signatures will be affected not only by their structure within the molecule (bonds) but also by the properties of the surrounding molecules and also on the temperature and polarity of the medium. In general, the fluorescent efficiency of a chromophore is characterized by *quantum yield*  $\eta_f$ :

$$\eta_f = (\text{N}^\circ \text{ fluorescent molecules}) / (\text{N}^\circ \text{ excited molecules}). \quad (2)$$

Equivalently, it is the ratio of numbers of emitted and absorbed photons. Clearly, the quantum yield of fluorescence must lie between 0 and 1. Since fluorescence is invariably associated with many nonfluorescent deactivation processes, any theoretical analysis must take account of these. Therefore, the quantum yield needs to be expressed as:

$$\eta_f = k_f / k_t, \quad (3)$$

where  $k_f$  and  $k_t$  are the intrinsic rate constant of the fluorescing molecule in isolation and the combined rate constants of all the other deactivation processes, respectively. The rate constant is defined simply as the ratio of the number of events per absorbed photon to the total number of all deactivation processes; the total rate constant will then be expressed as (see Fig. 6):

$$k_t = k_f + k_{ic} + k_{ec} + k_{ix} + k_d + k_p, \quad (4)$$

where  $k_{ic}$ ,  $k_{ec}$ ,  $k_{ix}$ ,  $k_d$  and  $k_p$  are the rate constants of internal conversion, external conversion, intersystem crossing, (pre)dissociation and phosphorescence, respectively. It is noted that the parameters,  $k_f$ ,  $k_d$  and  $k_p$  are dependent on the intrinsic molecular structure of the fluorescing molecule and the others on its chemical and physical environment. Therefore, molecules which exhibit higher values for  $k_f$  and lower ones for  $k_t$  are expected to provide stronger fluorescence. For example, under ideal conditions  $\eta_f$  is approximately 1 for fluorescein and 0 for nitrobenzene.

### 7.1.3 Structural effects

Absorption of (optical) energy by a molecule, raising it to a metastable energy state, is a prerequisite for fluorescence (Fig. 6). Unsaturated aromatic hydrocarbon molecules with, typically, allowed absorption bands in the UV-Vis range and low rate constants for other deactivation processes generally exhibit high quantum yields for fluorescence. Because of the complex and high degree of interactions between the various levels of vibro–electronic states the absorption and fluorescence spectra are usually broad bands, mirror images of each other, with a shift in the peak positions. To obtain the best possible quantum yield the excitation wavelength should therefore coincide with the peak of the absorption band. Structural rigidity (i.e., a fused ring structure such as in fluorescein or anthracene) favours fluorescence, whereas compounds with little or no substitutions like pyridine, biphenyl etc., are practically nonfluorescent.

Substitution with heavy atoms usually shifts the peak of the fluorescence (and absorption) band. This enables tuning the fluorescence maximum (peak). However, the heavier the substitution the lower the quantum yield (the heavy atom effect).

### 7.1.4 Environmental effects

Fluorescence of a molecule is greatly affected by its environment, including the concentration of the molecule in the medium, temperature, polarity of the solvent etc. At higher temperatures the collision rates of the molecule with other surrounding molecule both in the liquid and gaseous state and also mobility in a solid increases. This increases the nonradiative external conversion rate and reduces the fluorescence quantum yield. Solvents containing heavy atoms promote intersystem crossing, resulting in a similar effect. The effect of solvent pH on the fluorescence of dissolved molecules is rather complex, arising from the dissociation (protonation) of acidic and basic functional groups, thereby affecting rate constants (eqn 4) and shifting the fluorescence peak. For example, the protonation of electron-withdrawing groups results in a shift to longer wavelengths, whereas protonation of electron-donating groups shifts the peak to a shorter wavelength. It is, therefore, necessary to closely monitor and control solvent pH in quantitative analytical fluorescence spectroscopy.

### 7.1.5 Concentration effect

Fluorescence intensity ( $I_f$ ) from a species is directly proportional to the intensity of the absorbed ( $I_a$ ) light:  $I_f = \eta_f I_a$ . Hence (cf. eqn 1):

$$I_f = \eta_f I_i \propto cL. \quad (5)$$

Therefore, fluorescence intensity for low  $c$ , with molecules uniformly distributed in a relatively short path length  $L$ , will be linear for most practical purposes. For quantitative analyses, data must be recorded using a path length and concentration optimized for linearity.

Quenching (decrease) of fluorescence by other interacting molecules present in the medium may be an important consideration in analytical spectroscopy. Molecular oxygen, acrylamide, nitrobenzene, iodine ions etc. are known to substantially quench fluorescence, the analysis of which may provide information on the location of chromophores within the fluorescing molecule.

### 7.1.6 Applications

Thanks to the relative simplicity of its implementation (emission mode in Fig. 3), fluorescence spectroscopy is a versatile technique suitable for a wide range of applications, including organic chemistry, biochemistry, medicine, forensic science, pollution monitoring etc. Fluorescent microscopy is often used to image specific features of small specimens such as microbes. Its applications for tracing, identification and authentication are widespread, including automatic identification of tracer-doped waste plastic for separation and recycling, authentication of banknotes and documents, early diagnostics of tracer-doped carcinoma, and even of undoped tissue,<sup>12,13</sup> etc. Applications are so widespread that tailor-made chromophoric materials (“tracer dyes” with useful emission criteria and polarity, lifetime, quantum yield etc.) are available commercially from many companies throughout the world.<sup>14,15</sup>

## 7.2. Raman spectroscopy

### 7.2.1 Principle

In any optical interaction, if the absorbed energy doesn't coincide with an allowed energy level, most molecules will instantly release the energy, causing Rayleigh scattering with no change between the absorbed and emitted energy (wavelength). Some excited molecules may revert to excited levels of the ground electronic manifold (Fig. 5). In this case the scattered radiation will have a lower energy ( $h\nu_s$ ) than that of the absorbed radiation ( $h\nu_a$ ). Molecules thus excited to vibrational levels of the ground electronic state deactivate to a stable vibrational level through vibrational relaxation. The difference in energy, expressed in terms of a parameter called the Raman shift,<sup>16</sup>  $\Delta\nu = (h\nu_a - h\nu_s)$ , is typically given in wavenumber ( $\text{cm}^{-1}$ ) units. Various well-defined emission lines in a Raman spectrum correspond to the various vibrational modes observed in IR absorption spectroscopy. It is to be noted that Raman spectroscopy involves excitation and emission in the UV-Vis wavelength bands, allowing in effect IR spectroscopy of molecules to be carried out without using infrared radiation at all. This offers the advantage of avoiding problems with water vapour absorption in direct IR spectroscopy.<sup>17</sup> Besides, optical sources, light delivery and collection optics and optoelectronic detectors are more efficient and superior for UV-Vis wavelengths compared to those for IR wavelengths.

<sup>12</sup> Z.V. Jaliashvili, T.D. Medoidze, Z.G. Melikishvili, K.M. Mardaleishvili and J.J. Ramsden, Laser-excited fluorescence from normal and abnormal human thyroid cells: a pilot study. *Laser Phys. Lett.* **1** (2004) 521–524.

<sup>13</sup> Z.V. Jaliashvili, T.D. Medoidze, K.M. Mardaleishvili, J.J. Ramsden and Z.G. Melikishvili, Laser-induced fluorescence model of human goiter. *Laser Phys. Lett.* **5** (2008) 217–219.

<sup>14</sup> A. Sharma and S.G. Schulman, *Introduction to Fluorescence Spectroscopy*. Wiley (1999).

<sup>15</sup> A. Romani, C. Clementi and F. Gianna, Fluorescence spectroscopy: A powerful technique for the non-invasive characterization of artwork. *Acc. Chem. Res.* **43** (2010) 837–846

<sup>16</sup> Named after Chandrasekhara Venkata Raman for his work (with Kariamanikkam Srinivasa Krishnan) on the topic, for which he was awarded the Nobel Prize for Physics in 1930. The effect was discovered independently at about the same time as by Raman and Krishnan in India by Leonid Isaakovich Mandelstam and Grigory Samuilovich Landsberg in the USSR, where it was usually referred to as combinatorial light scattering.

<sup>17</sup> It should, however, be noted that not every line in a Raman scattering spectrum is replicated in an IR absorption spectrum. For the latter, the molecular dipole moment must change during the vibration.

A typical Raman spectrum of a molecular species would normally (low resolution spectroscopy) have sharp, well-defined lines on the longer side of the excitation wavelength. These correspond to the energies of its vibrational levels as shown schematically in Fig. 7. Even at room temperature a few molecules will occupy higher vibrational levels, according to the Boltzmann distribution law. These will also participate in Raman scattering, some making a direct transition to the lower vibrational levels of the ground state, hence emitting light at higher energies (i.e., shorter wavelengths) than those of the incident radiation. These relatively much weaker lines (corresponding to the anti-Stokes shifts) will be observed on the shorter side of the excitation wavelength:  $\Delta\nu = (1/\lambda_0 + 1/\lambda_r)$ , where  $\lambda_0$  and  $\lambda_r$  are the wavelengths (at the peak) of excitation and the Raman line, respectively. As the temperature increases the higher vibrational levels become more populated and the anti-Stokes lines become stronger.

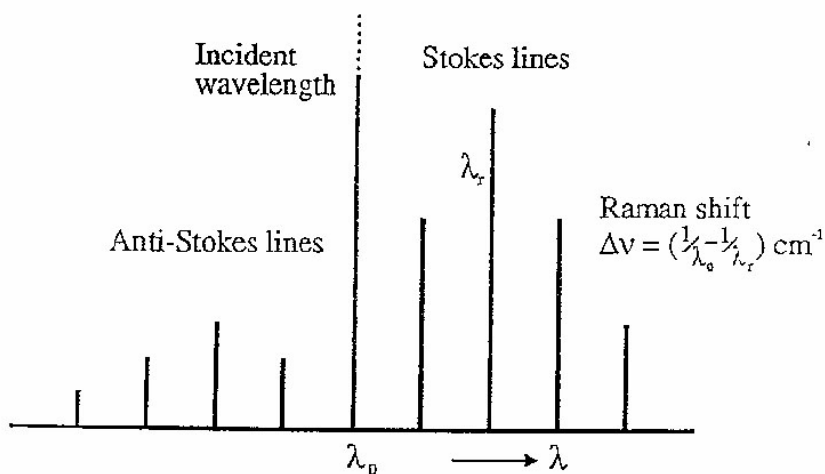


Figure 7. Raman lines in a wavelength versus intensity plot.

### 7.2.2 Theory

The theory of Raman scattering is exceedingly complex,<sup>18</sup> involving parameters of the participating molecules and their environment. Detailed analysis relates the incident and emitting energies involved in the interaction. The strength of the process is parametrized by a quantity called the scattering cross-section  $\sigma_{p,q}$  pertaining to a transition between two vibrational levels  $p$  and  $q$  and defined as  $\sigma_{p,q} = \sigma_r = P_r/P_0$ , where  $P_r$  and  $P_0$  are total Raman scattered power in all directions (i.e., over a solid angle of  $4\pi$  steradians) and incident power per unit area  $A$  and per molecule, respectively. For practical purpose it is expressed per solid angle  $\Omega$  of detection of Raman signal as a differential scattering cross-section (typical units are  $\text{cm}^2/\text{steradian}$ ):

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A useful rule is that if a molecule has an inversion centre (i.e., is centrosymmetric), then no vibration can be both IR- and Raman-allowed. The fundamental reason for the different selection rules is that infrared absorption is a 1-photon process governed by a dipole moment vector whereas Raman scattering is a 2-photon process governed by a polarizability tensor.

<sup>18</sup> B.S. Shastry and B.I. Shraiman, Theory of Raman scattering in Mott–Hubbard systems. *Phys. Rev. Lett.* **65** (1990) 1068–1071.

$$d\sigma_{p,q}/d\Omega = k (v_0 - v_e)^4 [(v_e^2 + v_0^2)/(v_e^2 - v_0^2)^2]^2 \quad (6)$$

where  $k = [2^4 \pi^4 / 3^2 c^4] G f(T) M_e^2$ ,  $v_0$  is the frequency (typically in Hz) of the exciting radiation,  $v_e$  is the frequency of the resonance state, that is the frequency of the vibrational transition pertaining to the Raman scattering,  $G$  is a degeneracy factor of the initial state,  $f(T)$  is the Boltzmann weighting factor (i.e., the thermal occupancy of the vibrational states) and  $M_e$  is the amplitude of the electronic transition moment.<sup>19</sup>

Raman scattering is a relatively very weak interaction process, yielding roughly 1 photon per  $10^7$ – $10^8$  incident photons. From the above expression (eqn 6) for Raman scattering cross-section it is evident that as the frequency of the excitation energy approaches that of an allowed isolated vibrational level of an excited electronic state ( $v_e$ ), the Raman scattered intensity (power/area) increases drastically, providing as much as three orders of magnitude enhancement close to resonance ( $v_0 \sim v_e$ ). For nonresonant excitations, however, the magnitude of  $v_e$  will be negligible in comparison to that of  $v_0$  and for all intents and purposes the Raman scattered intensity may simply be considered to be dependent on the fourth power of the frequency of the incident radiation; i.e.,  $d\sigma_{p,q}/d\Omega \propto (v_0)^4$ , or  $\propto (1/\lambda_0)^4$ .

### 7.2.3 Practical considerations

Most molecules participate in Raman scattering process, emitting their characteristic Raman lines. Hence, the Raman lines from various vibrational modes are expected to be overcrowded and overlap with each other.<sup>20</sup> Therefore, in practical Raman spectroscopy the instrumental setup is the same as shown in Fig. 3 except that the broadband source is replaced with an appropriate laser. Raman scattered intensity is linearly proportional to the incident intensity (below saturation level) and lasers usefully provide highly intense incident radiation in a narrow band in the near-UV region. This enables Raman emission in a narrow band (line), facilitating filtering from associated optical noise; weak Raman signals (strength  $S$ ) are usually buried in electronic and optical noise (strength  $N$ ). The  $S/N$  (ratio) parameter determines the detectability of the Raman signal. For background thermal or electronic noise the ratio can be drastically improved by signal averaging (needing a repetitively pulsed excitation source) or phase-sensitive detection methods for continuously emitting lasers. The optical noise, arising from the Rayleigh scattered signal and the broadband background fluorescence, can largely be removed by state-of-the-art wavelength filters (monochromators). However, the portion of fluorescent background (as almost everything fluoresces under UV excitation) and the strong Rayleigh signal within the wavelength gate (albeit very narrow) may limit the  $S/N$  ratio. Since Raman scattering is practically instantaneous, a time-gating method may be implemented to remove the inevitably slightly delayed contribution from fluorescence (§7.1) within the Raman detection window. Although the intensities of anti-Stokes lines are 1 or 2 orders of magnitude lower than those of the Stokes lines, the signals appear at the shorter wavelength side of the excitation line (Fig. 7) and, therefore, are free from any contribution from the background fluorescence (as that

<sup>19</sup> See also D.J. Ulness, J.C. Kirkwood and A.C. Albrecht, Raman Spectroscopy. In: *Encyclopaedia of Chemical Physics and Physical Chemistry* (eds J.H. Moore & N.D. Spencer), vol. II, ch. B1.3, pp. 1017–1062. Philadelphia: IOP (2001).

<sup>20</sup> P. Vandenabeele, *Practical Raman Spectroscopy: An Introduction*. Wiley (2013).

appears only over the longer wavelength side of the exciting radiation). In some applications the trade-off in  $S/N$  may favour the use of anti-Stokes rather than Stokes lines.

#### 7.2.4 Applications

For over a century since the discovery of Raman scattering, spectroscopes based on Raman scattering have remained a useful laboratory tool for routine analyses of materials, primarily for identification and characterization purposes. Drastic developments in recent years in the fields of lasers and electrooptics technologies, coupled with unprecedented advances in data acquisition and processing techniques, have transformed Raman spectroscopy to a state-of-the-art technology that, on its own merit, may be taken as a tool “looking for applications”.<sup>21</sup> Fields of application include almost all branches of science, not only for the identification of substances but also for remote sensing of temperature and pollution, real time process control in industry, early diagnosis of carcinoma, authentication and tracing and many more industrial, commercial and medical applications. Effective Raman spectroscopy requires a sample excitation volume of approximately  $10^{-5}$  mm<sup>3</sup>, allowing practically nondestructive measurements on anything and anywhere using a hand-portable Raman spectrometer. Such a tool will doubtless be equipped with miniaturized fibre-optic light delivery and collection systems, small diode lasers operating at UV-Vis wavelengths and “on-chip” solid-state small dispersive devices as effective multichannel optical dispersive systems (filters).<sup>22</sup> No wonder modern Raman spectrometers are so versatile and compact as to find their applications to be essential on board in space exploration.

Raman spectroscopy diversified into many subbranches for more or less esoteric applications in research and development: surface-enhanced Raman spectroscopy (SERS); Raman microscope spectroscopy (RMS); Raman imaging technique (RIT); Fourier transform Raman spectroscopy (FTRS) are just a few that could be mentioned. When molecules are dispersed in colloidal form or on a specially designed metallic microstructured surface, SERS offers a drastic enhancement in the Raman signal (up to  $10^{11}$  times). In RMS, incorporation of a Raman system in a specially adapted microscope for collection and delivery of signals enables identification of materials from an extremely small sample volume, hence allowing nondestructive analyses for identification and authentication in unique, valuable art and cultural objects, and rendering the remote detection of explosives practically possible. RIT allows scanning samples for high-resolution hyperspectral imaging and analyses of even single molecules. In FTRS, time-domain measurements use an interferometer and the data are converted into the form of conventional spectra using a well-known computer algorithm, which allows the use of near-IR lasers for excitation, thereby drastically decreasing fluorescence noise with a consequent improvement in the  $S/N$  ratio of the Raman spectra.

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<sup>21</sup> J. Lindon, G.E. Tranter and D. Koppenaal (eds), *Encyclopaedia of Spectroscopy and Spectrometry*, 3rd edn. Academic Press (2016).

<sup>22</sup> Cf. the interesting paper “Proposal for an all semiconductor combined spectrometer and detector” by M. Haines and B.C. Cavenett, *Phys. Rev. Lett.* **64** (1990) 48–51.