# Role of Modified Vibrational Raman Spectra in Detecting Cancerous Cells

**Murei K. Gilbert[1], Khanna M. Kapil[2*]**

[1]Department of Physics, Laikipia University, P.O. Box 1100-20300, Nyahururu, Kenya

[2]Department of Physics, University of Eldoret, P.O Box 1125-30100, Eldoret, Kenya

E-mail, 1gmurei@laikipia.ac.ke: 2*khannak700@gmail.com

***Abstract:*** **Inelastic scattering of light radiation on collision with a molecule is called Raman scattering. The oscillating electric field of the incident electromagnetic radiation creates an oscillating electric dipole which in turn creates an oscillating electric field. It is surmised that this oscillating electric field can affect the vibrational spectra of the molecule on which the incident radiation falls. Thus if $\omega_1$ is the frequency of the incident radiation, and $\omega_2$ is the frequency corresponding the vibrational modes of the molecule, and $\omega_D$ refers to the frequency corresponding to the energy fed into the molecular system of the oscillating electric field created by the oscillating electric dipole, then the Stokes lines can be $(\omega_1 - \omega_2 - \omega_D)$ and $(\omega_1 - \omega_2 + \omega_D)$ when $\omega_2 > \omega_D$; and the anti-Stokes lines will be $(\omega_1 + \omega_2 + \omega_D)$ and $(\omega_1 + \omega_2 - \omega_D)$ for $\omega_2 > \omega_D$.Both $\omega_2$ and $\omega_D$ values will characterize normal and cancerous cells,and by measuring these values and observing the positions of the Stokes and anti-Stokes lines,it should be possible to distinguish healthy cells from cancerous cells. A theory and experimental procedure for such study is proposed in this manuscript.**

***Keywords: electric dipole, Raman lines, stokes lines, anti-stokes lines, vibrational spectra, cancer cells.***

## I. INTRODUCTION

The inelastic scattering of light by molecules such as liquids is known as Raman effect.it was theoretically predicted by Smekel [1] in 1923,and experimentally it was discovered by two groups of scientists in 1928.In India, Raman and Krishnan [2] discovered this phenomena, and in Russia Landsberg and Mandelstam discovered the same [3].Raman showed that each line of incident radiation, and if it had sufficient intensity gave rise to a modified scattered radiation that had different frequency compared to the incident radiation, had modified relative intensities etc. When light falls on a molecule, the electric field of the incident electromagnetic radiation interacts with the charged particles of the molecule or material such that positive charges move in the direction of the electric field, and the negative charges move in the opposite direction to the electric field. This leads to the creation of an oscillating dipole since the incident radiation oscillates at frequencies in the range of $10^3$THz.The dipole oscillates with the frequency of the incident radiation. In Raman scattering, the incident light first creates the dipole that oscillates, and then interacts with it.The oscillating dipole creates an oscillating electric field ,and like the oscillating electric field of the incident radiation, interacts with the incident radiation leading to modified Raman scattering when compared to the Raman scattering of the incident light only. Raman lines created in this manner may have weaker intensity, but it may be observable [4].The practical implications of how Raman lines are obtained is like this. The current source of energy for Raman spectroscopy are lasers. Monochromatic incident light is focused on the sample such that out of $10^8$ incoming photons, only one or single Raman photon gets generated .If the molecule with which the incident photon collides is in an excited state, it can transfer energy to the incident radiation that scatters with a larger energy or the frequency of the scattered radiation increases and such Raman lines are called anti-Stokes lines. Since there is transfer of energy or exchange of energy, the process is called inelastic. If on other hand the molecule is in the ground state before collision with the incident photon, the molecule can absorb energy from the incident photon, and hence the scattered radiation will have lower energy compared to the incident radiation, and such Raman lines are called Stokes lines .This process is also inelastic scattering since there is exchange of energy. If the collision of the incident radiation with molecule is such that there is no exchange of energy and the scattered radiation has the same frequency as that of the incident radiation, then the scattering process is called elastic scattering, and such a process is called Rayleigh scattering. Thus Raman lines are obtained by transferring energy from incident radiation to the molecule with which it collides (Stokes lines). The colliding molecule transfers energy to the incident radiation (anti-Stokes lines).The Stokes and anti- Stokes lines appear symmetrically around the Rayleigh scattering line. Thus Raman Effect is the real absorption of a primary photon followed by the spontaneous emission of a secondary photon.

 

## II. VIBRATIONAL MOTION OF A MOLECULE

The vibrational motion of a molecule is a periodic motion of the atoms of a molecule relative to each other, in such a manner that the center of mass of the molecule remains at rest or is unchanged (velocity of the center- of-mass $\dot{r}_{cm} = 0$).The frequencies of molecular motion, known as vibrational frequencies, are typically in the range of less than $10^{13}$Hz to $10^{14}$Hz($v < 10^{13} Hz \; to \; v \cong 10^{14} Hz$).These frequencies correspond to wave numbers n of the order 300 to 3000cm$^{-1}$(n=number of waves or wave-lengths in one centimeter distance).A non-linear molecule with N atoms has 3N-6 normal modes of vibration whereas a linear molecules has 3N-5 normal modes of vibration since rotation about its molecular axis cannot be observed[5][6].Thus a diatomic molecule has only one normal mode of vibration. In the case of polyatomic molecules, the normal modes of vibration are independent of each other, but each normal mode involves simultaneous vibrations of different parts of the molecules such as different chemical bonds [7].

## III. VIBRATIONAL SPECTRA

A molecular vibration is created by the absorption of a quantum of energy,E say, and this will correspond to the vibration of frequency $v$ such that $E = hv$(where h=Planck's constant).If a molecule in its ground state absorbs this one quantum of energy $(hv)$, then a fundamental vibration is created in the molecule. If two such quanta are absorbed$(2hv)$,then the first overtone is excited, and similarly with higher such quanta $(3hv, 4hv, - - - -)$,higher overtones are excited in such a manner that the center of mass of the molecule remains stationery such that the velocity of the center of mass is zero, i.e. $\dot{r}_{cm} = 0$.

In the harmonic approximation, the potential energy is a quadratic function of the normal coordinates. Solving the Schrodinger wave equation we can get the energy states for each normal co-ordinate as, [7]

$E_n = \left(n + \frac{1}{2}\right) hv = \left(n + \frac{1}{2}\right) \hbar\omega$ ..... (1)

where the frequency of the photon is equal to $v$ ,$\omega = 2\pi v$ , and the selection rule for the harmonic oscillator is$\Delta\, n = \pm 1$ .

The vibrational energy of an an- harmonic oscillator (A.O) is.

$E_n = \left(n + \frac{1}{2}\right) hv - \left(n + \frac{1}{2}\right)^2 hvx_e + \left(n + \frac{1}{2}\right)^2 hvy_e + \cdots$ ..... (2)

where $x_e, y_e$ etc are anharmonicity constants,

$n = 0,1,2, - - - - - - - - - - - -$

$\Delta n = \pm 1, \pm 2, \pm 3, - - - - - - - -$

$hv > hvx_e > hvy_e - - - - - - - -$

The first term in Eqn (2) corresponds to simple-harmonic oscillation (SHO), and the rest of the terms stand for anharmonicity. It is found that the theoretically calculated frequencies of vibration for vibrational spectra of molecules and the experimentally observed vibrational frequencies do not agree. The difference is mainly due to the neglect of anharmonicity rather than the theoretical method. In addition, it is proposed in this manuscript that the oscillating electric field created by the oscillating dipole may as well affect the vibrational frequencies resulting in the change of the vibrational frequencies or frequencies of the Stokes and Anti-Stokes lines of the Raman spectra [4].

## IV. VIBRATIONAL RAMAN SPECTROSCOPY

Modern Raman spectroscopy uses many non-invasive (non-destructive) reflection techniques for identification of molecules, cells, membranes, nucleic acids, molecular diagnosis of cervical cancer etc. They are all based on the basic character of Raman Effect i.e. monochromatic polarized laser light is inelastically scattered by the molecular sample. Quantum mechanically, the shifted quantum frequencies in the Raman lines are interpreted as shifted quantum states of the molecule during the scattering process. Since the shifted frequencies appear symmetrically around the frequency of the exciting light which is the incident monochromatic laser radiation, it can be concluded that the molecule may have either got excited or de- excited during the scattering process [8] [9].The de-excitation during the scattering process may require that the molecules have been excited either thermally or optically before the scattering event. This scattering is called anti-Stokes scattering; whereas if the molecules are excited during the scattering process (incident light- loses energy to excite the molecule), the scattered radiation is called Stokes scattering or Stokes lines. If the frequency of the incident light is $\omega_i$ and that of Stokes lines is $\omega_s$, then $\omega_s < \omega_i$, whereas in anti- Stokes lines $\omega_S > \omega_I$.Hence in the scattering process, two events take place and the incident light is scatterd.One in which the incident light loses energy to the molecule with which it collides and then gets scattered such that $\omega_s < \omega_i$. In the second case, the molecule is already excited, either thermally or optically, and on collision with the incident light, the molecule de excites leading to increase in the frequency of the scattered radiation such that $\omega_s > \omega_i$ , and we get anti -Stokes lines.

Now a molecule has three distinct energy states. They are called rotational, vibrational and electronic states. Depending on the molecule and the specific experimental conditions, any one or more of these states can be excited, and hence the molecules may be shifted (excited) rotationally, vibrationally or electronically. When the

 

molecules shift in their vibrational states, the Raman scattering is called vibrational Raman spectroscopy. A vibrational Raman spectrum exhibits a highly resolved vibrational signature of the scattering molecule. In general ,Stokes lines are more intense than the anti- Stokes lines since the number of molecules involved in Stokes lines is more than the number of molecules involved in anti - Stokes lines[10].This vibrational technique is performed as a reflection measurement which requires very little or no sample preparation. Investigations of the molecules can be done in their natural environment. It has been successfully used in food industry, medical and environmental applications, chemical analysis etc.

Vibrational spectra are measured by irradiating the sample with polarized laser light with wave frequencies either in the near- infrared (NIR).the visible (VIS), or the ultra- violet (UV), and simultaneously monitoring the reflected light. A vibrational Raman spectrum is obtained by measuring the intensity distribution in the Raman scattered light. The intensity distribution will be a function of the Raman shift $\Delta \upsilon_R$ which is defined as $\Delta\upsilon_R = \upsilon_{laser} - \upsilon_s$ , where $\upsilon_{laser}$ is the frequency of the incident laser light and $\upsilon_s$ is the frequency of the Raman scattered light. For Stokes scattering $\Delta\upsilon_R$ is positive since$\upsilon_{laser} > \upsilon_s$ ; and for anti-Stokes scattering $\Delta\upsilon_R$ is negative since $\upsilon_s > \upsilon_{laser}$ [11].

It is important to know that Raman spectroscopy (especially vibrational Raman spectroscopy) has been very successfully used for the detection of different types of pathologies including cancer[12][13].The potential of Raman spectroscopy, especially vibrational Raman spectroscopy, for disease detection and cancer is well established by now. It has the potential to identify cancerous and precancerous tissue. It can also probe deeper into diseased tissue to display and identify the underlying mechanics leading to the disease ( cancerous cells).Variations of the technique can lead to study of wider range of samples. In this manuscript we have tried to propose that the vibrational Raman spectroscopy could be a potential method in which the oscillating electric field created by the oscillating dipole can affect the vibrational spectra of the molecules under observation to detect the cancerous cells. Actual experimental studies in future may be able to decide how far it will be a reliable technique.

## V. THEORETICAL FORMULATION

Inelastic interaction between light and matter leads to Raman Effect. Depending upon the strength of the applied electric field, and the nature of the sample on which light falls, linear and nonlinear optical phenomena can get generated. The incident oscillating electric field creates an oscillating induced dipole moment, and the oscillating induced dipole moment further creates an oscillating electric field. For some measureable observations, the intensities of the electric field should be of the order of $10^9$v/m or so [14].Giant- pulse lasers can be used to obtain such high electric field intensities. In general, in most of the phenomena, we are interested in linear scattering, and this can be obtained when the frequency,$\omega_0$, of the incident light ( which is a stream of photons) is far away from the molecular electronic absorption frequency $\omega_1$ such that $\omega_\upsilon << \omega_0 << \omega_1$ ,where $\omega_\upsilon$ is the vibrational frequency of the molecule. This corresponds to the restriction of photon wavelength (or energy) which lies in between the visible and near-visible regions and corresponds to vibrational and electronic molecular excitation energies. When this condition is satisfied, the photon transfers its energy $\hbar\omega_0$ to the whole molecule in order to displace the electron and produce an oscillating induce dipole moment. However, the electron remains bound since the large mass of the nucleus does not allow such a transistion.Hence the incident light gets transmitted without change of frequency, the scattering is thus elastic and is known as Rayleigh scattering.

Now in this manuscript, the objective is to study the effect of the oscillating electric field, E, produced by the oscillating dipole on the vibrational spectra of the molecule on which the incident radiation falls. The energy associated with the oscillating electric field, E, is given by [4], U, i.e.

$$U = \frac{qp_0\omega^2}{4\pi\varepsilon_0 C^2} \quad \ldots\ldots(3)$$

where

$q = electron\ charge$

$p_0 = Ql (Q = charge\ of\ one\ end\ of\ the\ electric\ dipole)$

$, l\ is\ the\ distance\ between\ the\ two\ opposite\ charges, and\ it$

Or

$p_0 = maximum\ value\ of\ the\ electric\ dipole\ moment$

$\omega =$ frequency of the oscillating dipole moment

$\varepsilon_0 = Electric\ permitivity\ in\ free\ space$

$C = velocity\ of\ light$

The frequency,$\omega_D$,associated with the energy U due to the oscillating electric field produced by the induced oscillating dipole, is given by,

$$\omega_D = \frac{U}{\hbar} = \frac{qp_0\omega^2}{4\pi\epsilon_0 C^2\hbar} \quad \ldots\ldots(4)$$

It is this frequency $\omega_D$ that can alter the Stokes and anti-Stokes lines in the vibrational Raman spectroscopy.

Now if $\omega_1$ is the frequency of the incident monochromatic laser radiation, and $\omega_2$ is the vibrational frequency of the molecule on which the incident laser radiation falls, and $\omega_D$ is the frequency corresponding to

 

the energy fed into the system due to the oscillating electric field created by the oscillating induced electric dipole, then the Stokes lines and anti-Stokes lines may have the following frequencies depending on the relative magnitudes of $\omega_2$ and $\omega_D$.

Stokes lines $\begin{vmatrix}\omega_1 - \omega_2 - \omega_D \\ \omega_1 - \omega_2 + \omega_D\end{vmatrix} (\omega_2 > \omega_D)$

…….. (5)

Anti-Stokes lines $\begin{vmatrix}\omega_1 + \omega_2 + \omega_D \\ \omega_1 + \omega_2 - \omega_D\end{vmatrix} (\omega_2 > \omega_D)$

Eqns (3), (4) and (5) are the fundamental equations that are to be used to obtain the vibrational Raman lines (Stokes, and anti-Stokes lines) of the molecule under study. The skill lies in calculating and or measuring the values of $\omega_2$and $\omega_D$ , since $\omega_1$will be known for the incident polarized laser radiation. For a given molecule, the Stokes and anti-Stokes will be given by Eqn (5).

## VI. DISCUSSION AND SUMMARY

Vibrational Raman spectra or lines are a consequence of the collision between the incident light photons and molecules. In this scattering process, it is the characteristics vibrational frequency $\omega_2$ of the molecule that will determine the frequencies of the Stokes and anti-Stokes lines. In this manuscript, it is proposed that the frequency $\omega_D$ will also affect the frequencies of the Stokes and anti-Stokes lines. This adds a new dimension to the experimental observations of the vibrational Raman Spectra, i.e,the vibrational Raman lines will be altered when the effect of $\omega_D$ is taken into account since the value of $\omega_D$ will be different for different molecules, especially when the vibrational Raman spectroscopy is used to distinguish normal cells from cancerous cells. It is proposed that intense electric field laser can lead to larger values of $\omega_D$, and larger number of photons in the laser beam will lead to larger intensity of Raman lines, and thus it can more effectively distinguish one type of cells from the other.

Raman lines, both Stokes and anti-Stokes lines will be displaced with respect to the central line that corresponds to the frequency $\omega_1$ depending on how $\omega_D$ varies with the magnitude of disease of the diseased cell. Thus this kind of vibrational Raman spectroscopy can be used in biomedical studies since it could be capable of identifying and characterizing the tissues, cells and structure of the molecules involved. This method can be profitably used in identifying cervical cancer, and it should be able to detect pre-malignancy and early malignancy stages. Raman lines differ between normal and malignant biopsy samples [15][8][16][17][18][19][20][21][22][23].

The concept in this paper is that the vibrational modes may as well be excited by the oscillating electric field created by the oscillating electric dipole. We have to point out that we have not been able to find any theoretical or experimental observations on these lines in the literature. Our theory predicts new method of getting Stokes and anti-Stokes lines with modified frequencies that could facilitate detection of all kinds of cancerous cells. Lasers with typical high intensity electric field of the order of $10^{10}$V/m or more may be needed to observe the phenomena.

Experiments have shown [24], [25] that a single cancer cell is about 70% softer than the normal healthy cell. Thus the force constant of the cancer cell will differ from the force constant of the healthy cell, and this will result in a different vibrational frequency of the cancer cell when compared to the normal healthy cell. Hence the Stokes and anti -Stokes lines will be different for the two type of cells, and by measuring their frequencies, we could calculate the vibrational frequencies of the cancer and healthy cells. By knowing the exact values of the natural frequencies of vibration of the cancer cell, it should be possible to devise a method of killing the cancer cells, without disturbing the healthy cells.

## REFERENCES


[1]. Smekal. A, Naturwissenschaftene 16 (1923) 612

[2]. Raman C. V. and Krishnan K.S: Nature 121 (1928) 501

[3]. Landsberg. G and Mandelstam. L: Naturwissenschaften 16(1928) 557

[4]. Khanna. K and Murei, K. G.(2019) *Effect of the Oscillating Electric Field Due to the Oscillating Electric Dipole on Raman Lines*. East European Journal of physics (EEJP) 4 47-57

[5]. Landau L.D and Lifshitz. E.M (1976) *Mechanics* pergamon press. New York.

[6]. Wilson E.B, Decius. J.C and Cross. P.C (1995), Molecular Vibrations, New York.

[7]. Gans P. (1971) Vibrating molecules. Chapman and Hall. New York.

[8]. Mortensen O.S, Hassing S, (1980) Polarization and interference phenomena in resonance Raman Scattering. Advances in Infrared and Raman spectroscopy. Vol 6. New York, Willey.

[9]. Hassing S. Khan. M. (2017) Raman Spectroscopy and Applications, Rijeka, Croatia: In Tech

[10]. Long, D. A. (2002) The Raman Effect, John Wiley & sons. ISBN: 978-0-471-49028-9.

[11]. Diem M, Griffiths, P, Chalmers. J.M, (2008) Vibrational Spectroscopy foe Medical diagnosis. John Wiley & sons

[12]. Krafft.C,(2004) Bio analytical Applications of Raman Spectroscopy doi: 10.1007/500216-003-2266-6(Pub-Med)

[13]. Kandel C, Isabelle M, Bazant-Hegemark. F, (2009) Vibrational Spectroscopy: a clinical tool


 

for Cancer diagnostics. Analyst. 134(6); 1029-1045(Pub.Med)

[14]. Kiefer.W. and Long. D. A, (1982) Non –linear Raman Spectroscopy and its chemical applications, D. Reidel.u.k.

[15]. Ines Requel Martins Ramos, Alison Malkin and Fiona Mary Lyng; Current Advances in the Application of Raman Spectroscopy of molecular diagnosis of Cervical Cancer.

[16]. R.R Jones, D.C Hooper, Liwu Zhang, D. Wolverson, and V.K Valev, (2019) Nanoscale. Res Lett 14, 231 https;//dx.doi.org/10.1186/02FS11671-019-3039-2

[17]. K.E Sundling and A.C Lowe,(2019), Advance Anat. Pathology and Experiments 26(1) ,56-63

[18]. A. Mahadevan- Jansen, M.F. Mitchell, N. Ramanujam, A. Malpica, S. Thomsen, U Ut-zingers and R.Richards-Kortum.(1998) Photochemistry and photobiology 68 (1) 123-132

[19]. A. Mahadevan- Jansen, M.F. Mitchell, N. Ramanujam, A. Malpica, S. Thomsen, U Ut-zingers and R.Richards-Kortum.(1998) Photochemistry and photobiology 68(3), 426-431

[20]. J.L. Gonzalez – Solis *et al* (2014) .Lasers in medical science 29(3)979-985.

[21]. Skoeg et.al;(2019) Theory of Raman Spectroscopy

[22]. Yukihiro Ozaki, Malgorzata Baranska,Kamilla Malek, Igor Lednev, Beydeu. R. Wood. (2020) *Vibrational Spectroscopy in protein Research*. Academic press.

[23]. Raman Imaging Highlights. Biochemical Heterogeneity of Human eosinophils versus human eosinophilic Leukaemia cell line by Anna Rygula, Refaella. F.Fernaudes, Marek Grosicki, Bozena Kukla, Kamila Malek, Adrian Cernescu, Aleksandra Dorosz, Malgorzata Baranska. 28 May 2019, British Journal of Hematology/Vol 186, Issue 5.

[24]. Fraldi.M, Cugno.A, Deseri.L, Dayal.K and Pugno.N.M.A Frequency-based Hypothesis for mechanically targeting and selectively attacking Cancer cells.J.R.soc.Interface 12(2015)0656.

[25]. Savavana.K Jaganathan *et.al* .Natural Frequency of Cancer cells as a starting point in Cancer treatment. Current Science Vol.110, No.9, 10 May 2016.



 