

# Recent Progress in Biosensors for Diagnosis of Cancer Cells, Tissues and Tumors Biomarkers



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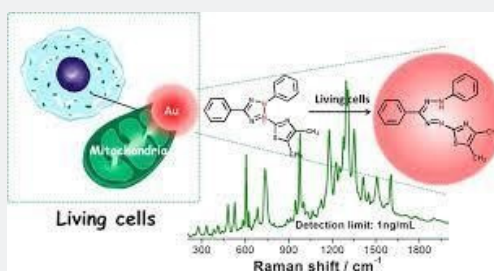
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## Abstract

Raman spectroscopy is an important method for identifying molecules, which is widely used in determining the chemical and structural characteristics of various substances. Many materials have a special Raman spectrum, so that this phenomenon has turned the Raman device into an efficient tool for studying the structural and chemical properties of molecules. Since it is possible to obtain detailed information about the chemical and structural characteristics of biological compounds from Raman spectroscopy, the use of this method is rapidly expanding in the field of life sciences, especially in biological and medical studies. There is no need for special, time-consuming and expensive preparations in the study of materials with the help of a Raman device. In the protein Raman spectrum, distinct bands arise from the vibrational states of the peptide backbone and amino acid side chains. Therefore, based on the position and intensity of the protein's Raman spectrum, it is possible to obtain valuable information about its second, third, and fourth structures. Also, the Raman spectrum of the protein contains information about the orientation and surrounding environment of the amino acid side chains. The correct formation of the disulfide bond in the protein structure can also be studied with the help of the Raman device. In general, the Raman spectrum of proteins contains multiple discrete bands that represent the vibrational states of the molecule and is used as a selective fingerprint to accurately determine the three-dimensional structure of proteins, intramolecular dynamics, and intermolecular interactions (Graphical Abstract).



**Graphical Abstract:** Schematic of leading-edge portable biosensors and biomarkers development for Raman biospectroscopy and imaging in cancer diagnosis.

## Introduction

The application of Raman spectroscopy is basically in the identification of molecules. Today, along with the many advances that have been made in the field of research equipment design,

Raman spectroscopy has become more simple, accessible and affordable. Of course, despite the many advances made, the interpretation of Raman spectra is still a big challenge and requires special skills. Like all spectroscopic methods, the Raman spectrum

contains the information of the electromagnetic waves hitting the sample. After the electromagnetic beam hits the molecule, a part of it is scattered in all directions. Raman spectroscopy is used to observe vibrational, rotational, and other low frequency states in a system. This type of spectroscopy typically provides a specific structural fingerprint that can be used to identify different

molecules. In fact, this type of spectroscopy is based on inelastic scattering called Raman scattering (and the Raman light rays are usually laser light in the visible region, near infrared light or near ultraviolet light [1-38]).

The inelastic scattering of light upon impact with matter was not reported until 1928, while this important physical phenomenon was first predicted by Adolf Schmal<sup>6</sup> in 1923. Also, Chandrasekhara Venkata Raman observed this effect for the first time after studying the passage of sunlight through organic solutions, and in 1930 he was honored to receive the Nobel Prize in Physics due to this important discovery and his other studies in this field. The development and evolution of this physical effect was carried out by George Pleczyk<sup>8</sup> between 1930 and 1934. Also, in 1899, John William Reilly was able to justify the elastic scattering of light while proposing a new hypothesis. This light scattering theory was actually an answer to why the color of the sky is blue. At that time, light scattering studies were also seriously pursued in countries such as Russia, France, India, the United States of America, and Germany. In the early 20<sup>th</sup> century, people like Raman and Krishnan in India and Landsberg and Mendelstam in Russia were pioneers in this field of study. When investigating the change in the frequency of scattered light in different physical conditions, these people achieved results that they had not planned for in advance. Landsberg and Mendelstam also investigated the scattering of light in quartz and some other crystals to find the scattered rays that have undergone a frequency change compared to the incoming light. At the same time, Raman and Krishnan in India and far away from Russian scientists were studying the changes of light in the Compton effect. By publishing three articles in 1928, they recorded the change in the frequency of scattered light while encountering matter, even though the reports of Raman and Krishnan were only slightly earlier than the reports of Russian scientists. Nowadays, extensive studies are carried out on the scattering of light while interacting with matter, and the large volume of studies and the number of published scientific articles about this discovery show the special importance of this issue [39-76].

Photons are often reflected, absorbed or scattered while hitting the molecule. In Raman spectroscopy, monochromatic light photons (light of a single wavelength) are scattered in different directions after hitting the sample. In fact, in Raman spectroscopy, photons scattered from the sample are important. Most of the photons that hit the molecule are scattered elastically. This type

of scattering is called Rayleigh scattering, in which the photons scattered from the sample have the same energy or wavelength as the photons that hit the sample. In 1928, Indian physicist Chandra Sekhar Venkata Raman discovered the Raman phenomenon. In this phenomenon, the energy or wavelength of the beam scattered by the molecules is different from the wavelength of the primary beam that hits the sample. This type of scattering of light rays is called inelastic scattering. About one in ten million photons after hitting matter is scattered inelastically. Also, the amount of difference in energy or wavelength of inelastic scattered light depends on the molecular structure of the compound. In fact, Raman spectroscopy was formed based on the analysis of these differences and with the aim of determining the molecular structure of various compounds. The change in the wavelength or the initial radiation energy provides very important information about the molecular movements within the system. In Raman scattering, the photon collides with the material and after scattering its wavelength goes to the longitudinal direction. In these more or less displaced waves, the type of radiation scattering is dominated by the transmission to longer wavelengths, which is called Stokes Raman scattering. Also, the transition to lower wavelengths is called Raman anti-Stokes scattering. It has been reported that the intensity ratio of anti-Stokes to Stokes scattering increases with increasing temperature. In fact, the incoming photon collides with the electron cloud of bonds of functional groups and excites the electrons to a virtual state. Then the electron returns from the virtual state to an excited vibrational or rotational state. This phenomenon causes the photon to lose some of its energy and is revealed in the form of Stokes Raman scattering. The lost energy is directly related to the chemical identity of the functional group, the molecular structure attached to it, the type of atoms in the molecule and its surrounding environment. Therefore, the Raman spectrum of each molecule is specific and can be used like a "fingerprint" to detect the chemical identity of molecular compounds in a liquid, on a surface, or in the air [77-116].

### Leading-Edge Portable Biosensors and Biomarkers Development

The degree of Raman effect is directly related to the polarizability of the electrons of the molecule. The Raman effect is actually the interaction between the electron cloud of the sample and the external electric field of the incoming light rays. This mode creates the formation of which depends on the induced instantaneous dipole polarizability of the sample. Because the laser light does not excite the molecule, no actual transitions between energy levels occur in Raman studies. (with fluctuations, hence the Raman signal is obtained from the collision of the light beam (intermolecular photons (phonons) of the sample). Review and analysis of the information obtained in Raman spectroscopy to determine the structure, qualitative measurement and in some cases, it takes a few molecules. Also, the study of the effect of many

different physical parameters such as temperature, pressure and tension on interatomic and intermolecular oscillations. The Raman scattering spectrum and the infrared absorption spectrum of a molecule have many similarities with each other. In fact, it comes from the similarities of these two methods. Also, despite the great similarity, these two methods are different from each other in the basic principles, so that they are usually used as complementary methods. In infrared absorption, the amount the energy absorbed from the incoming photon corresponds to the energy difference between the initial and final rotational-vibrational states, while in Raman scattering, the amount of energy of the incoming photon is not the same as the outgoing one (usually it is more or less). Also, the dependence of Raman on polarization the acceptability of its electric dipole-dipole species from infrared spectroscopy, which only observes dipole species. E is electric dependent (atomic polar tensor) differentiates. These differences indicate that transitions between rotational-vibrational states may not be active in infrared absorption, but can be studied using Raman spectroscopy. There is also the reverse of this phenomenon, so that infrared absorption spectroscopy is used in cases where Raman spectroscopy is not applicable for the study of molecules. Therefore, transitions that have a high intensity in the Raman spectrum often have weak infrared absorption and vice versa. In other words, a vibration is active in infrared spectroscopy, when a change in the momentary dipole of the molecule can be seen during its occurrence. Likewise, vibration is active in Raman spectroscopy, which changes the polarizability of the molecule as well. For example, molecules with identical nuclei such as N<sub>2</sub>, H<sub>2</sub> and O<sub>2</sub> are active in Raman study, but not active in infrared spectroscopy. In the CO<sub>2</sub> molecule, the symmetric vibrational motion is active in Raman and not active in infrared. On the contrary, asymmetric vibrational motion is not active in Raman, but it is active in infrared. Some vibrations are also active in both infrared and Raman.

The main components of the Raman device, the system of each Raman device consists of four main parts, including the laser light source, the wavelength selector (sample illumination filter and light collecting lenses. After the light and the detector or spectrometer collide) the laser to the sample and its scattering from its surface, the scattered light is collected by a lens and transmitted to the detector unit by a fiber. Wavelengths close to the laser wavelength (elastic or Rayleigh scattering) are absorbed by a special filter. Only the scattered rays that have changed in terms of energy or wavelength compared to the incoming light are allowed to pass and reach the detector.

The most common sources of laser generation in the Raman device are argon laser with wavelengths of 488 and 514.5 (nm), krypton laser with wavelengths of 530, 568 and 647 (nm), helium/neon laser with a wavelength of 632.8 (nm), diode laser: with 785

and 830 (nm) wavelength and AG Y/d N laser with 1064 (nm) wavelength. The waves that change the frequency (wavelength) after hitting the sample while scattering are the Raman signals that are of special importance. The cross-section of Raman scattering is very small and the most difficult step in this method is to separate the Rayleigh elastic beams from the frequency-shifted Raman beams known as inelastic beams. In the past, holographic gratings and multiple stages were used to obtain a high degree of Raman signal, which made the collection time relatively long. Today, notch filters or edge filters and spectrographs (or spectrometry on the axial transmitter), Zarni-Turner splitter for amplification and detectors of the coupled device based on the Fourier transform of the Raman signal are used.

## Results and Discussion

Raman, as mentioned earlier, Raman spectroscopy is widely used in various fields. In recent years, the use of Raman spectroscopy in medicine, pharmacy, food industry, defense science and other industries has grown significantly. According to the global events of recent years, it is very important to establish methods for rapid detection of biological threats for the military and national security. In the meantime, Raman spectroscopy has received a lot of attention because it provides accurate and fast information about the molecular composition of biological materials in a non-destructive way. Currently, Raman spectroscopy is used to detect explosives, agents of chemical and bacterial warfare, and other dangerous chemical substances. With the help of this method, samples can be checked in a non-contact and non-destructive way inside transparent or semi-transparent packaging. Therefore, drugs and narcotics can be checked through the plastic bag containing them, and in this way, damage to criminal documents and evidence or their contamination can be avoided. It is also possible to equip Raman spectroscopy probe with an optical fiber in order to measure nitrate, nitrite and hydroxide in tanks containing radioactive waste. These three chemicals are often used to display and control tank corrosion. In this way, there is no need to physically remove the sample from the tanks and the risks of transporting it to a fixed laboratory to check them. The accuracy of Raman detection depends on various factors, including the laser wavelength used and the type of material. The detection accuracy of these method variables usually ranges from a few parts per million to a few parts per billion. Raman's ability to display stress and other parameters such as the surface temperature of the component makes it an effective tool in the manufacture of semiconductor components. Also, the ability of this method to provide accurate images of cells allows comparison between healthy and diseased tissues, which is especially important in the study of cancerous tissues (Figures 1-5).

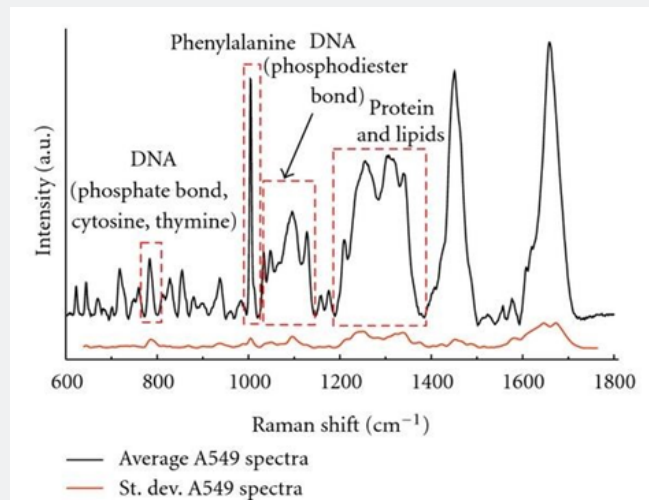


Figure 1: Raman spectroscopy investigation of DNA, protein and lipids in human cancer cells.

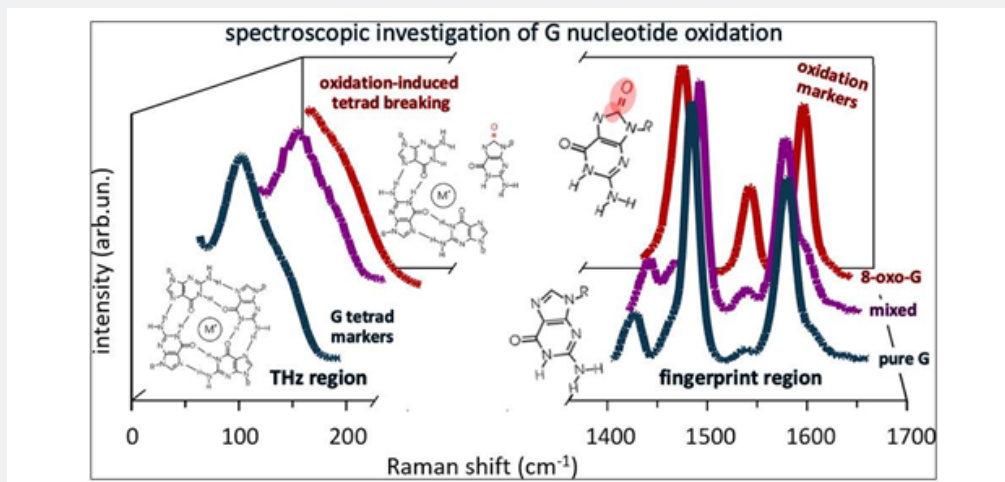


Figure 2: Raman spectroscopy investigation of G nucleotide oxidation in human cancer cells.

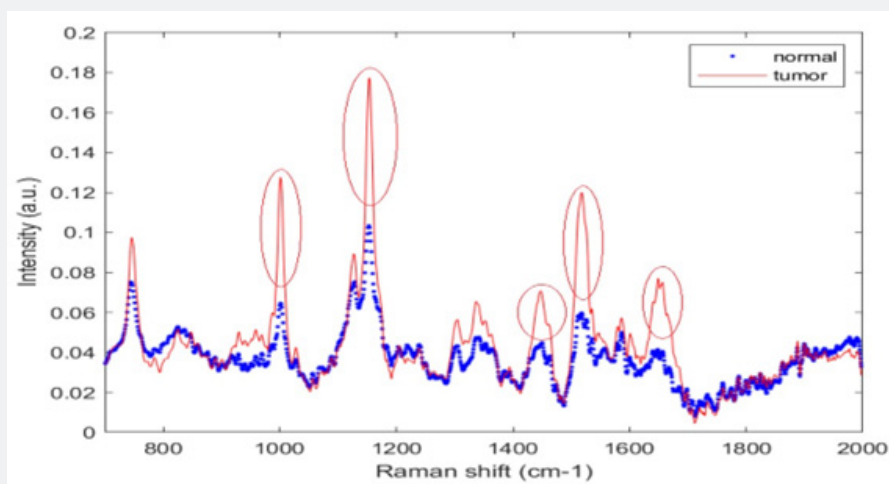


Figure 3: Raman spectroscopy comparison between normal and cancer animal cells.



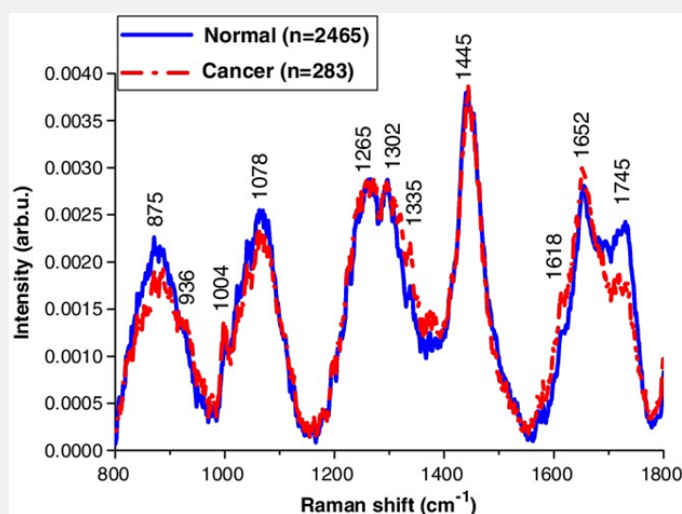


Figure 4: Raman spectroscopy comparison between normal and cancer human cells.

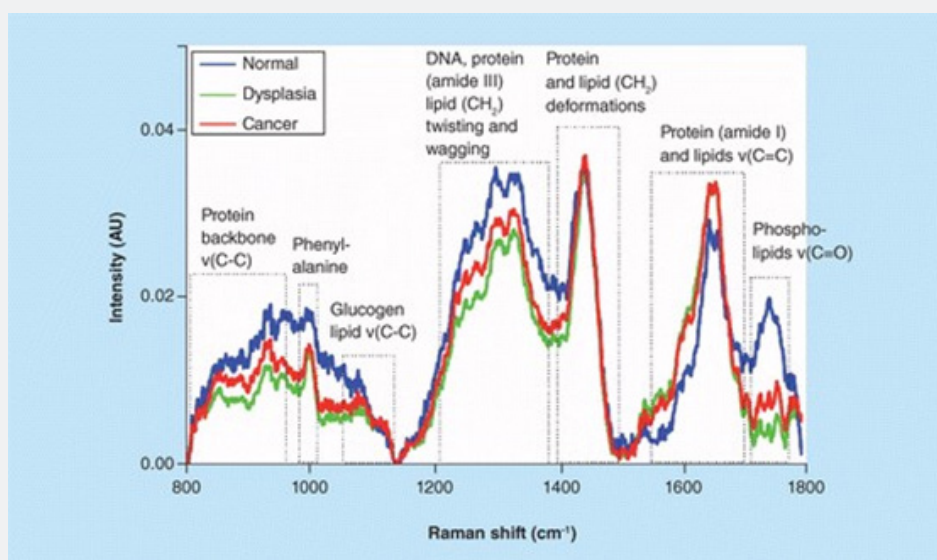


Figure 5: Raman spectroscopy comparison among normal, dysplasia and cancer human cells.

What information can be obtained from examining the Raman scattering spectrum of materials? The vibrational frequencies of a link are very sensitive to the details and the structural features and local environment of the molecule such as crystal phase symmetry, polymer morphology, band position in the Raman spectrum representing the chemical species, crystal phase or substance under study. The composition or compounds that make up the alloy, as well as the intensity of the Raman spectrum, indicates the concentration of the active group present in the composition or substance under investigation. Raman frequency shift indicates the type of functional group and temperature changes in the investigated substance. And finally, the width of the Raman spectrum indicates the presence of disorder or structural disorder in the studied material.

Effect of solvent on the Raman spectrum of protein. Protein in solution has a wider Raman spectrum than in powder form. The Raman spectrum of lysozyme protein in both powder and solution states. The type III amide bands of the protein in the solution state have a wider spectrum. The effect of chemical reactions on the folding and Raman spectrum of proteins in the presence of concentration different methanol amide bonds of type (I) alpha-synuclein protein have been modified and a detailed examination of this area after separating the sub-spectral surface, it is clear that the structure of alpha-synuclein protein under the influence of methanol gradually deviates from the normal state and in that second structures from alpha helix to structures called beta sheets and structures. The effect of reducing agents on the folding of proteins and its display in the Raman spectrum,

after deconvolution of the protein Raman spectra, the amount of structural changes in the presence of reducing agents is determined. Physics such as reaction rate, free enthalpy and activation energy can be calculated from these data. Interference in protein Raman spectrum by fluorescence emission and signal-to-noise effect. Fluorescence emission can have severe destructive effects on the protein Raman spectrum. Intrinsic fluorescence usually occurs in the presence of aromatic

amino acids, which can be eliminated by choosing the appropriate excitation wavelength in protein Raman studies. Also, transient fluorescence is usually due to impurity, solvent or buffer is created to avoid interference. Transient fluorescence is necessary in Raman studies. Samples can be prepared as pure as possible. Background fluorescence was reduced by quenching or bleaching by emission light. Of course, it should be noted that increasing the temperature in this case may damage the sample (Tables 1-5).

**Table 1:** Role and applications of Raman spectroscopy and techniques in diagnosis of different types of human cancers.

Cancer Type	Technique	Raman	Spot Size (mm)	Power (mW)	Signal Integration Time (s)	Number of	Reference
		Excitation				Skin Lesions	
		Wavelength				Studied and/or	
		(nm)				Patients	
MM, BCC, SCC, actinic keratosis (AK), atypical nevi, melanocytic nevi, blue nevi, and seborrheic keratoses (SK)	Raman	785	3.5	300	1	518 (453 patients)	[36]
BCC, inflammatory scar tissues	Raman + OCT <sup>a</sup>	785	0.044	40	30	1 patient	[15]
MM, BCC, SCC, pigmented nevi	Raman	785	1	150	30	50	[37]
MM, BCC, SCC, pigmented nevi	Raman + OCT	785	1	150	30	23, 50	[38, 39]
MM, BCC, SCC, pigmented nevi	Raman	785	0.1	17	10	137	[40, 41]
BCC, SCC, inflammatory scar tissues	Raman	825	0.005 <sup>b</sup>	40	30	21 (19 patients)	[42]
BCC	Raman	830	1.6	10	30	10 patients	[43]
BCC, SCC	Raman	830	-	200	20 (2 s×10 spectra)	31 (17 patients)	[44]
BCC, SCC, AK	Raman	830	0.17	200	20 (2 s×10 spectra)	49 (25 patients)	[45]
MM, BCC, SCC, actinic keratosis (AK), and non-melanoma pigmented lesions	Raman	830	0.2	100	1	137 (76 patients)	[46, 47]
BCC	Multi Modal	830	0.2	56	4	1 (healthy) <sup>d</sup>	[48]
MM, eczema, psoriatic skin, malignant	Raman	1064	10	-	-	1 (healthy) <sup>d</sup>	[31]
Kaposi sarcomas							

MM, BCC, pigmented nevi	Raman	1064	0.1	120	480	81 (72 patients)	[49]
Carotenoid concentration in BCC and actinic keratosis (AK)	Raman	488	2	10	20	14 patients	[50]
MM	Multi Modal	1064	0.08	-	35	Mice injected with human MM	[51]
						cells	

**Table 2:** Leading-edge portable biosensors and biomarkers data for Raman spectroscopy and imaging in cancer diagnosis.

Final lesion diagnosis	Subjects			Number of lesions	Number biopsied (%)	Head and neck	Location		
	Mean age, y (range)	Male	Female				Trunk	Upper limb	Lower limb
<b>MM</b>									
LM	69 (51-88)	12	8	20	20 (100)	19	1	0	0
LMM	67 (42-85)	7	1	8	8 (100)	8	0	0	0
SS	60 (22-77)	6	8	14	14 (100)	3	3	7	1
MM other	61 (60-62)	2	0	2	2 (100)	1	1	0	0
<b>BCC</b>									
Superficial	63 (34-86)	10	13	28	28 (100)	10	9	5	4
Nodular	66 (39-94)	34	29	73	73 (100)	52	10	9	2
Pigmented	67 (46-83)	2	4	6	6 (100)	2	4	0	0
Other BCC	68 (60-75)	1	1	2	2 (100)	1	1	0	0
<b>SCC</b>									
In situ	70 (56-88)	12	5	18	18 (100)	7	4	5	2
Invasive	66 (39-94)	16	10	28	28 (100)	16	1	5	6
Other SCC	78	0	1	1	1 (100)	1	0	0	0
Actinic keratosis (AK)	66 (43-92)	13	14	32	10 (31.3)	28	0	3	1
Atypical nevus	48 (20-75)	22	26	57	24 (42.1)	3	39	8	7
Junctional nevus	43 (18-70)	12	17	34	4 (11.8)	5	11	15	3
Compound nevus	35 (18-67)	13	15	30	6 (20)	9	8	9	4

Intradermal nevus	50 (28–83)	9	26	38	12 (31.6)	21	8	7	2
Blue nevus	37 (18–66)	4	9	13	4 (30.8)	4	1	6	2
Seborrheic	64 (25–89)	49	42	114	31 (27.2)	47	47	14	6

**Table 3:** Comparison between Mid-FTIR spectroscopy and Raman spectroscopy in cancer diagnosis.

	Mid-FTIR	Raman
Diagnostic criteria	Objective (based on biochemical spectral fingerprint)	
Wavenumber range	800–4000 cm <sup>-1</sup>	400–4000 cm <sup>-1</sup>
Type of spectroscopic detection	Mid-infrared light absorption using a polychromatic light source	Inelastic light scattering using a monochromatic laser excitation (usually 785 or 830 nm but visible lasers also used)
	Changes in the dipole moment	Changes in polarizability
Conditions for Raman/FTIR activation (selection rules) Molecular bond sensitivities		Non-polar bonds including C-C double and triple bonds including aromatic rings
	Strong polar bonds including hydroxyl (OH), carbonyl (CO) and amide bonds	Weaker Raman cross-section of biological material results in lower SNR for normal Raman spectra. Higher spatial resolution (~ 1 μm) due to diffraction limit
SNR	Generally higher SNR in similar timescales	Point raster mapping, line and rapid synchronous readout mapping, Fast Raman imaging, ultrafast confocal Raman imaging, Wide-field imaging,
		LCTF Raman imaging
Spatial resolution	Lower spatial resolution due to diffraction limit	SORS: the Raman scatter is collected from regions laterally offset from laser excitation, leading to significantly lower contributions from the surface layer, enabling depth probing.
	(~ 10 μm). Synchrotron sources (2–5 μm)	CARS: two laser beams are used to generate a coherent anti-Stokes frequency beam, which can be enhanced by resonance.
Imaging/mapping modes	Rapid scan imaging using focal plane or linear array detectors. ATR imaging can improve spatial resolutions and be applied to thicker samples	SERS: enhancements over normal Raman scattering of typically 10 <sup>3</sup> –10 <sup>6</sup> due to electromagnetic and chemical enhancement effects, with fluorescence quenching. Requires close proximity/adsorption onto a roughened metal surface, a colloidal solution or a roughened electrode (usually Ag or
		Au). Can tune to a specific chromophore for
	ATR (attenuated total reflection): IR direct sample analysis by contact with an ATR crystal.	
	Penetration is within the evanescent field which can be controlled and allows measurement from aqueous body fluids or non-dried tissue samples	
Enhanced techniques		



**Table 4:** Cancer type of interest and classification analysis groups of type of Raman system in cancer diagnosis.

Cancer type of interest	Classification analysis groups	Type of Raman system (HF/LF)	Authors (year)	Number of spectra	Analysis method Sensitivity: specificity	Reference
				(number of patients)		
Skin cancer	Malignant + premalignant vs benign and normal	Macro-Raman (LF)	Zeng group (2001-2012)	518 (453)	PCA-GDA and	[20, 40-43]
					PLS	
					90%: 66%	
Skin cancer	Nonmelanoma cancers vs normal lesions	Macro-Raman (LF)	Mahadevan-Jansen group (2008)	42 (19)	MRDF-SMLR 100%: 91%	[44]
Lung cancer	Malignant + premalignant vs benign and normal	Macro-Raman (LF)	Zeng group (2008-2011)	129 (26)	PCA-LDA 90%: 91%	[21, 47]
Breast cancer	Tumor vs normal (tumor	Macro-Raman (LF)	Feld group (2006)	30 (9)	Model fitting 100%: 100%	[49]
	tissue margin detection)					
Colorectal cancer	Adenomatous tissue vs	Macro-Raman (LF)	Wilson group (2003)	19 (3)	PCA-LDA	[55]
	hyperplastic polyps				100%: 89%	
Cervical cancer	Squamous	Macro-Raman (LF)	Richards-Kortum group (2001)	27 (13)	Intensity ratios	[63]
	dysplasia vs normal,				NA	
	inflammation and metaplasia					
Cervical cancer	High-grade preneoplastic lesions vs normal	Macro-Raman (LF)	Mahadevan-Jansen group (2007)	172 (66)	Logistic regression	[64]
					89%:>81%	
Cervical cancer	1. Dysplasia vs normal	Macro-Raman (LF)	Huang group	1. 92 (46)	PCA-LDA	[65-67]
	2. High-grade dysplasia vs		1. (2009)	2. 105 (29)	1. 94%: 98%	
			2. (2011)	3. 476 (44)	2. 73%: 89%	
			3. (2012)		3. 85%: 82%	

**Table 5:** Raman spectroscopy application, experimental set up and analysis method in cancer diagnosis.

Raman application		Experimental setup				Analysis method	Reference
		Wavelength (nm)	Power (mW) (sec)	Exposure time	Other		
Cancer detection	Breast cancer	830	100-150	10-30		Nonnegative least square (NNLS)	[8]
		830	82-125	1	Probe	NNLS	[31]
		786	N/A	4		Raman shifts	[7]

		830	65	10		PCA-LDA	[34]
		514.5	8	N/A		Hierarchical cluster	[42]
	Cervical cancer	785	80	5-15	Probe	Logistic regression	[45]
		785	80	5	Probe	Maximum representation and discrimination feature (MRDF)	[47]
						Sparse multinomial logistic regression (SMLR)	
		785	15	60	Probe	PCA-LDA	[49]
		785	10	60		Raman shifts	[43]
		785	100	30		PCA	[50]
	Colorectal cancer	785	300	5	Probe	PCA-Support vector machines (SVMs)	[51]
		785	70	N/A		PCA	[35]
						Hierarchical cluster	
						Multiple least squares (MLS)	
		782.5	11.5	60	LTRS	PCA- Logistic regression	[52]
		782.5	11.5	60	LTRS	PCA-Artificial neural network (ANN)	[53]
Allograft rejection	Cardiac rejection	785	100	10		PCA	[54]
	Renal rejection	785/514.5	8-12	10		PCA-Discriminant function analysis (DFA)	[56]
SERS for		785	100	20		NNLS	[65]

## Conclusion

High signal-to-noise ratio in the presence of a higher signal-to-noise ratio, the Raman spectrum of the sample is more accurate. This ratio can be increased by increasing the number of scans or increasing the scan time. In addition, it should be noted that an excessive increase in the number of scans as well as a change in the time interval of the scan can cause serious damage to the sample. This method has many applications in various research fields. Also, this method provides important information about the structure of molecules, so that Raman bands can be considered as a kind of fingerprint of a compound. The similarities and differences between the Raman light scattering method and the infrared absorption spectrometry method have made these two methods to be used for a more detailed structural study of a compound and complement each other. Raman spectroscopy provides valuable information on secondary, tertiary and even

quaternary structures of proteins. With the help of this method, it is possible to check the correct formation of disulfide bonds in the protein structure. Also, the amount of information that can be obtained from the protein Raman spectrum is far more than other conventional spectroscopic methods. Therefore, Raman spectroscopy is suggested as a useful tool to study the protein structure more precisely.

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