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Scientific Advances in Cancer Detection Using Raman Spectroscopy

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Abstract

Aim: The present study is focused to investigate the role of Raman spectroscopy (RM) for cancer detection. **Methods:** In this review, we explored a number of scientific databases including PubMed, Web of Science, Embase and Google Scholar for research studies on Raman spectroscopy for diagnosing cancer. We reported key outcomes of research studies conducted involving Raman spectroscopy for diagnosis of cancer and highlighting the potential of novel Raman spectroscopy for diagnosing cancer in our review. **Results:** Based on the available evidence it can be strongly concluded that Raman spectroscopy provide significant information for diagnosing cancer. Numerous comprehensive studies involving living cells in vitro, in-vivo animal models and ex-vivo human tissues pre-clinical Raman experiments are conducted on biological specimens for almost every type of cancer for diagnosing cancer demonstrating the potential for clinical use of Raman spectroscopy. Furthermore, the in-vivo Raman handheld probes are developed and experimentally used for real time detection of human breast and brain tumors with significant sensitivity and specificity. However, it will be important and challenging to explore relevant Raman biomarkers for other types of cancer as well, to distinguish cancer. **Conclusion:** Raman spectroscopy is an effective method and valuable tool in the field of cancer diagnostics. The Raman spectroscopy deserves a place for future in clinics for rapid cancer diagnostics.

Keywords: Cancer- Raman spectroscopy- Diagnosis- Spectrum

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Introduction

Background

Living organisms are immensely complex biological systems. The living biological systems are remarkably ordered and compactly arranged together in a highly efficient way. Living systems store sensitive genetic information for repetitive cellular reproduction, organization, control, and many other functions. However, infection or mutation in the biological mechanisms leads to progression of disease. Amongst all diseases, cardiovascular diseases and cancer are the leading causes of mortality worldwide [1, 2]. There is an urgent need for finding point of care procedures and techniques for the correct diagnosis for diseases with ease of use. Different physical effects are used, and various techniques are applied to achieve diagnostic information that is not directly visible. Raman spectroscopy (RM) has prospective applications for non-invasive use in a broad range of clinical diagnostics. RM has always benefited mankind from its involvement with ever advancing and developing sciences. It is a well-established, well recognized subject; however, the use of RM is recent and fast-growing in detection of cancer [3, 4].

RM has now become an indispensable tool for reporting molecular vibrational frequencies and chemical analyses in industrial and research laboratories. However, the use of RM for biomedical applications was first published in 1970 [5]. Later, the emergence of RM became possible for biomedical applications with the advent of immense advances in light sources and signal detection techniques. Over the last decade, RM has been explored extensively in biomedical applications and gained research interest in clinical laboratories. Molecular substances can be identified, and biochemical vibrations can be monitored with high specificity through distinct Raman spectroscopic patterns. RM is a powerful tool for biomedical applications because of the fact that the laser excitation wavelength in visible and near-infrared range has a high spatial resolution for studying molecular information in biological samples. RM provides information of the composition of individual groups of atoms and the changes occurring in the shape of a macromolecule present in the biological sample. The introduction of novel spectral analysis techniques and advanced Raman instrumentation have considerably improved the clinical usefulness of RM for the wide range of oncological applications. RM has been extensively used in clinical diagnostics of cancer including breast cancer [6-9], lung cancer [10], ovarian cancer [11], cervical

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cancer [12], oral cancer [13], nasopharyngeal carcinomas [14], brain tumors [15], Gastrointestinal Tumors [16], chondrogenic tumors [17], prostate cancer [18], colorectal cancer [19], and skin cancer [20].

Moreover, cancer burden is expected to rise and affecting developing countries where availability of cost-effective point of care cancer detection method is always an issue [21]. Currently available typical diagnostic test for detection of cancer involve the use of histopathology of biological samples and radiological imaging such as ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET). However, each diagnostic method has its own limitations affecting clinical diagnostic decision. Furthermore, some of these diagnostic methods are associated with limitations such as super expensive nature of diagnostic test, time consuming and delay in reporting diagnostic results, unsuitable for intraoperative use, unable to detect small tumor lesions, use ionizing radiation and often fail to characterize cancer completely [22, 23]. Hence, there is an essential requirement for rapid affordable improved cancer detection methodologies. RM is an area of interest and has the potential to overcome the limitations posed by the conventional diagnostic methods for cancer detection. In this review, we explored a number of scientific databases including PubMed, Web of Science, Embase and Google Scholar for research studies published on RM for diagnosing cancer.

Raman Spectroscopy (Rm)

The Concept Of Raman Spectroscopy

The inelastic scattering of photons of light is known as the Raman effect and named after its experimental discoverer C.V. Raman. The Raman effect was discovered in Calcutta, India in 1928 while investigating the scattering of sunlight [24]. The novel discovery got popularized in such a way that by the end of that year, some 70 research papers had been published on the phenomena. By 1939, over 1800 papers had been reported to be published on the Raman effect, showcased its utility for a wide range of applications. This phenomenon, which later bore Raman's name, was characterized in his own words as "excessive feebleness," due to the low efficiency of inelastic scattering, hence necessitating intense excitation light. This is why it was not until the emergence of lasers in the 1960s that RM was popularized. The Raman techniques most frequently used in characterization of samples in non-destructive way as powerful diagnostic tool. The application of RM in life sciences is more recent [25].

Raman Instrumentation

Laser light is passed through a beam expander and filter to provide monochromatic laser light source. Then excitation laser guided towards set of mirrors to focus and incident onto biological specimen through an objective lens of Raman microscope. Backscattered light is gathered with the help of the same objective lens in the direction of 180°. The collected backscattered light falls onto edge filters to reduce the amount of Rayleigh scattered light. Then the light is passed through an entrance slit to incident onto diffraction grating and thereafter falls onto the detector to produce meaningful Raman signal [26].

The Raman Spectrum

Broadly, the Raman spectrum can be categorized into three main regions: fingerprint (200–1800 cm^{-1}), silent (1,800–2,700 cm−1) and high wavenumber (2,700–3,300 cm−1). The Raman fingerprint region is rich in specific Raman peaks arising from biomolecules such as proteins, lipids, carbohydrates, and nucleic acids. The silent Raman region is generally free of bands and corresponds to molecular vibrations from triple bonds functional groups present in the biological sample, but alkynes are an exception. The high wavenumber region contains predominant information of C–H, CH2, O–H, N–H vibrations of bonds present in the lipids, proteins and water molecules.

Types Of Raman Techniques For Cancer Detection

Spontaneous Raman Spectroscopy (SpRS)

The ground state molecule is excited to a virtual state under beam illumination at a specified frequency. When exited molecule yields remission of a photon of identical frequency and return to its original ground state, the phenomena known as Rayleigh scattering. However, when there is a shift in the frequency of excited photon while returning to its vibrational state and the shift in frequency is proportional to the energy difference among the energy levels of the vibrating molecule. This is known as inelastic Raman scattering. The vibrating molecule is excited to a virtual state and inelastic scattering occurs while returning to the ground state. When the scattered light is of higher or lower frequency than the incident photon during this inelastic Raman scattering, they are known as anti-stoke and stoke transitions respectively. During the conventional spontaneous Raman spectroscopy, the signal from stoke transition are observed under continuous wave illumination by laser. A diode pumped solid state (DPSS) laser is generally used to detect the stoke signal. The optimal optical window of excited wavelength is a balance between sample autofluorescence and Raman signal intensity. Further, the selection of optimal wavelength depends on biological sample and its potential application of interest. Typically, the optimal windows for SpRS are between 500 nm to 1100 nm such as 532 nm 700 nm, 900 nm and around 1064 nm excitation wavelength of laser are commonly used and reported in the literature.

Confocal Raman Spectroscopy

In order to improve the axial and lateral resolutions, conformal configuration was implemented along with SpRS technique. A pinhole collimator or fiber optic is used to eliminate out of focus signal by spatially filtering the collected RS signal. This combination of configuration provides better optical depth sectioning of the sample. However, the acquisition time per point increases with focal depth of interest in the sample. Confocal RS provide a high resolution around 2 μm. Faster acquisition time per point (<1 s) may result in loss of lateral resolution of sample. Confocal RS probes are commonly utilized for in vitro studies with cells and ex-vivo sample studies.

Spatially Offset Raman Spectroscopy (Sors)

When the Raman signal collected from deep sections of sample by spatially offsetting excitation and detection fibers. Then, the emitted photons elastically scattered multiple times for the detection. This enables collection of Raman intensity from different layers and offsets in the sample. Hence, SORS is a modified technique to SpRS in terms of introducing slight offset in the detection and excitation method. Conventional RS measures Raman spectra beneath several hundred microns whereas SORS effectively measure Raman signal to a depth of few millimeters in the surface of medium. Raman scattered photons produced at deeper depths move in the media and diffuse scattering of photons occur when they come out from the sample surface. Higher Raman signal produced and collected at greater depths of sample with increasing offset between laser excitation and collection points, at the loss of Raman intensity.

Coherent Raman Spectroscopy

Generally, nonlinear Raman scattering is known as coherent Raman spectroscopy (CRS). It is divided into two processes: Coherent Anti-Stokes Raman Scattering (CARS) and Stimulated Raman Scattering (SRS). Basically, Raman intensities are produced using spontaneous Raman scattering. However, CARS involve pump, probe and Stokes fields that interact with specimen in combination. Thus, CARS reduces the limitation of spontaneous Raman scattering by measuring weak anti-Stokes Raman signals efficiently. CARS provides higher signal to noise ratio (SNR) compared to SpRS since all the vibrating molecules are coherently moved to a stable vibrational eigenstate prior coming to ground level. However, the primary limitations of CARS are that it requires multiple pulsed lasers with complex instrumentation and specialized spectral preprocessing methodologies. Moreover, the intensities of pulsed lasers depend as a quadratic function and significant non resonant background signal added to the useful resonant signal. The major drawback of this method is with tissue because water produces a high non resonant background in tissue samples. CARS spectrum is different from SpRS due to non resonant background signal leading to dependency of CARS signal with molecular concentrations in a nonlinear fashion. Further, it is challenging task for researchers to integrate CARS for in-vivo translations in form of simple handheld tool or contact probe to miniaturize optical components and achieving high efficiency of laser delivery and collection using CARS.

Stimulated Raman Scattering

SRS method facilitates rapidly acquiring Raman spectroscopy information by using two laser pulses including pump and stoke frequency. Since the SRS signal is proportional to the difference in the pump and Stokes beams intensities. Accordingly, the amplification of Raman signal for a vibrational mode occurs when difference in frequency between the two beams is equivalent to the frequency of molecular vibration. Unlike CARS, the modulation of laser intensity is linearly proportional to molecular concentration, and it helps increase signal amplitude. The experimental setup of SRS provides great liberty for preprocessing, chemometric analysis and data interpretation. SRS also have a main disadvantage similar to CARS that both techniques can acquire a limited range of Raman shifts over time. However, SRS is advantageous in practices where a narrow spectral range of interest has to be probed to characterize the specimen.

Surface-Enhanced Raman Spectroscopy

One of the major limitations of RM is very low signal intensity. To overcome this limitation, an alternative approach for coherent amplification of Raman signal is performed by using nanoparticles and chemical amplification. This type of RM technique is known as SERS. The mechanism involved in SERS is metal nanoparticle that generate a localized surface resonance through the amplification of electromagnetic field and enhances the pump frequency for illumination and Raman signal at Stoke frequency. Typically, the enhancement is 10 to 12 orders of magnitude. Further, the chemical amplification is comparatively weaker and is attributed to the creation of charge transfer states between the molecules and SERS main active platform i.e. nanoparticle. SERS is particularly useful in the quantitative analysis and identification of biochemical molecules at ultra-low concentrations.

Generally, two elementary methods have been used to SERS for applications in biomedicine. In the first approach, fiber optic probe is coated with metallic nanoparticles and enhances the intrinsic Raman spectroscopic signature during SERS of a biological specimen. In the second approach, metallic nanoparticles are administered in the biological sample and these nanoparticles are coated with concerned molecule of interest with known reference Raman spectrum. Further, the choice of nanoparticles depends on the targeting of a particular biomarker employing peptides or antibodies functioning as nanotags. There are several drawbacks of SERS for biomedical applications such as availability of targeted biomarkers, investigation of potential toxicity profile in biological system, understanding of disease specific sensitive biomarkers and the requirement for regulatory approval of the nanoparticle material. However, SERS displays promising results for diagnosis of disease from Raman sensitive biomarkers utilizing blood and body fluids available in human body to evolve point of care testing [27].

Application Of Raman Spectroscopy For Cancer Detection

As a compilation of work carried out by the authors worldwide in the field of RM and the potential uses of RM in various diagnostic applications with especial emphasis on cancer diagnostics, are discussed below:

Skin

Zhao et al. [28] studied in vivo Raman spectra for 9 different types of lesions incorporating basal cell carcinoma and squamous cell carcinoma among 289 patients and showed 91% sensitivity and 75% specificity for discriminating cancer from benign tumor and reported 97% sensitivity and 78% specificity for differentiating

Table 1. An Overview of Published Data Utilizing Raman Spectroscopy for Detection of Breast Cancer

Sample type	Substrate	Excitation power (mW)	Excitation wavelength (nm)	Microscope magnification (x objective)	Exposure time (ET) and Integration time (IT) in seconds	Reference
Frozen section	Not defined	10	532	40	IT: 0.3-0.5	Abramczyk et al. [9]
Frozen section	Not defined	10	532	50	Not available	Brozek-Pluska et al. [62]
Frozen section	Not required	100-150	830	63	IT: 10-30	Haka et al. [63]
Formalin-fixed paraffin-embedded (FPPE)	Glass slides	Low value, not specified	786	Not specified	Not specified	Rehman et al. [64]
FPPE	MgF2 slides	100-150	830	63	IT: 60	Haka et al. [65]
Cell lines	Glass slides	10	532	50	IT: 50	Talari et al. [66]
Cell lines	MgF2 coverslips	28	785	50	IT: 25 and 15	Chaturvedi et al. [67]
No preparation In vivo tissue examination	Not applicable	82-125	830	Not applicable	ET: 1	Haka et al. [60]
Tissue microarray	Not specified	10	532	50	Not specified	Lazaro-Pacheco et al. [68]
Tissue stored in saline	Not specified	150	785	Not specified	IT: 30	Abramczyk et al. [69]
Mouse breast cancer	Not required	50	785	20	ET: 10	Kast et al. [70]

malignant melanoma from pigmented nevi. On the contrary, Schleusener et al. [29] were unable to distinguish cancer from benign lesion. However, they achieved 87% sensitivity and 94% specificity for distinguishing malignant melanoma from pigmented benign lesion. Commercially available skin cancer detection instrument (Verisante Technology, Inc.).

Gynecology

Cervical cancer is one of the most common reasons of mortality in females in low- and middle-income countries. There are several research studies evident of use of RM in detection of gynecological cancers. Krishna et al. [30] investigated formalin-fixed ovarian tissue samples for normal, benign, and cancerous tissue. They reported that RM of normal and benign tissue samples are identical and distinctly showed discrimination from the RM of cancerous tissue. In another study, 72 Raman spectra were obtained from freshly resected ovaries among 15 patients by Maheedhar et al. [31] and claimed 100% sensitivity and specificity for differentiating ovarian cancer. Boca-Farcau et al. [32] showed the use of distinct markers as silver nanoparticles. These nanoparticles were both SERS labeled and folic-acid conjugated. It is well known that foliate receptors are overexpressed among the majority of epithelial tumors of ovarian origin and hence, these nanotriangles are promising markers with higher specificity for detecting ovarian malignancies. Borel et al. [33] conducted spectrally-resolved confocal Raman microscopy on serous epithelial tumor cells for ovarian malignancy. They achieved 92% sensitivity and 85% specificity for differentiating ovarian malignant cells to normal cells.

Oral

Head and neck cancer is the most common cancer in male in developing countries. Oral cancers encompass cancerous tissue that appear in the region of the floor of mouth, tongue, and hard palate. RM has been explored for diagnosis of head and neck cancers and steering freshly

Table 2. The Table Below Lists the Advantages and Limitations of Raman Spectroscopy for Detection of Cancer.

Advantages	Limitations
*Nature: Non-invasive, versatile, non-destructive, relatively rapid, inexpensive	*Raman signal intensity: Weak
*Sample preparation: Minimal	*Autofluorescence: High and sample dependent
*Dependency: Mostly power and wavelength dependent	*Acquisition time: Large due to weak Raman signals
*Specificity: High	*Signal detection: Challenging
*Detection: Simultaneous detection of macromolecules	*Sensitivity: Low
*Technology type: Label free method, no dyes and toxic waste products	*Raman imaging: Slow due to point scanning
*Compatibility: Physiological measurements because of weak water interactions	*Video rate imaging: Nearly impossible because of very low scattering efficiency and large acquisition times.
*Applications: In vivo surgical guidance, In vivo fiber optic application for natural body cavities and blood vessels, portable point of care diagnostic tool.	*Trade off parameters: Balance between spatial resolution, spectral resolution, field-of-view and acquisition time
*Other applications: Feasibility for quantification, classification, chemical analysis and imaging of biological specimens.	*Add-ons: Often, advanced sophisticated software/ tools are required for analysis of complex spectral data to serve the desired purpose.

resections of tissue during surgery [34].

Guze et al. [35] studied in vivo laser Raman spectral datasets of normal oral mucosa among 7 specified oral sites within the mouth of 51 healthy subjects with signal acquisition time of 1 s for the entire 1500-3100 cm^{-1} region. The spectral band of 2800-3100 cm^{-1} indicates the best results for classification with significant accuracy. A study was carried out by Krishna et al. [36] who investigated in vivo Raman spectra for differentiating normal oral mucosa, oral leukoplakia, submucous fibrosis and squamous cell carcinoma claiming accuracies of 85%, 82%, 85% and 89% respectively. The overall sensitivity was reported 94% for discriminating normal to abnormal change in tissue by combining other three types of tissue. Recently, a review article by Faur et al. [37] reported accuracy of 94% for detection of oral cavity and oropharyngeal cancer using in vivo mucosa examination and showed great potential for oral cancer detection.

Brain

Gliomas are the most frequently diagnosed adult malignant brain tumors and account for almost 80% of malignant brain tumors in adults [38]. Visual check with a neurosurgical microscopic examination and navigational guidance from MRI are the standards for surgical resection. As a result, the full degree of tumor infiltration is missed frequently and resulting in residual tumor and recurrence of disease. Furthermore, the removal of healthy tissue may result in long-term neurological impairments. With the advent of RM for in vivo neurosurgical guiding interventions indicating the improved residual tumor detection and extension of safe excision. Several studies [39, 40] have compared the Raman spectral characteristics of healthy and malignant tissue utilizing ex vivo human tissue or rodent glioma models, and they have found significant biochemical variations. A portable RM system was designed and demonstrated by Desroches et al. [41] for intraoperative application during removal of brain cancer with 0.2 s of integration time. A set of measurements from 17 patients producing a total of 161 Raman spectra with grade 2-4 gliomas were collected by Jermyn et al. [42] in vivo using the method. Results showed 93% sensitivity and 91% specificity for differentiating between healthy brain from dense malignancy and low-density tumor invasion.

Gastrointestinal (GI) Tract

The stomach, intestines, esophagus, pancreas, and other organs constitute the GI tract. Early diagnosis is essential for lowering fatality rates in many GI malignancies. Several research studies have been reported to design endoscopic RM instruments because endoscopy is frequently used to diagnose GI illnesses. However, some of the main limitations to deploying RM in this way include prolonged acquisition time and the requirement to miniaturise fiber-optic components. Although, a significant amount of in vitro research work has been performed by several researchers [43-45] using endoscopy RM probe for GI tract cancers. In vivo RM endoscopy systems with less than 1 s integration time were developed by Bergholt et al. [46] and they were able to distinguish adenocarcinoma in gastric lesion in vivo with 85% sensitivity and 96% specificity. In another study, this group [47] has also been able to discriminate between normal mucosa, benign and cancerous tissue in the stomach with sensitivities of 91%, 85%, 82% and specificities of 94%, 95%, 95%, respectively. For the purpose of differentiating between adenomas and hyperplastic polyps, the Wilson group [48] evaluated both in vivo and ex vivo tissue from the colon, with 100% sensitivity and 89.0% specificity for in vivo samples with a 30 s integration time. They [49] also achieved a sensitivity and specificity of about 90% for diagnosing high-grade dysplasia in Barrett's esophagus. Wang et al. [50] used integration times of 0.1–0.5 s to combine high-wavenumber with fingerprint RS in vivo to diagnose esophageal squamous cell cancer with a sensitivity and specificity of 97%. This in vivo research has demonstrated strong classification performance and suggested that endoscopic RM in the GI tract has the potential to be a diagnostic tool. The feasibility of endoscopic RM in vivo was also tested by McGregor et al. [51] for early diagnosis of lung carcinoma. With an integration time of 1 s, they were able to diagnose high grade dysplasia and cancerous lung tissues in 280 samples from 80 patients with 90% sensitivity and 65% specificity.

Biofluids

RM has also witnessed a surge in application for disease diagnosis and as a prognostic indication for treatment monitoring using biofluids such as blood, saliva, urine, sperm and vaginal secretions. Recently, the extensive application of RM is driven by the abundance of chemical information in biofluids. Generally, standard of care disease tests frequently lacks the required specificity for efficient screening. For instance, although the prostate-specific antigen (PSA) test is frequently used for screening of prostate cancer but only ~25% of men who undergo biopsy owing to increased PSA level have prostate cancer [52]. In order to supplement or replace current techniques, RM has the significant potential to provide a reliable, inexpensive, and non-invasive optical diagnostic. The vibrational spectroscopy has expanded to SERS to provide biomolecule specific spectroscopic information for rapid diagnostic of disease, especially using biofluids. SERS is frequently used for biofluid analysis because of its large surface coverage and enhanced capacity for signal detection. In a study conducted by Feng et al. [53] and SERS was utilised to examine blood plasma for adenomatous polyps and colorectal cancer, reporting an 86% sensitivity and an 80% specificity. Moreover, this group [54] employed SERS on saliva to distinguish between healthy participants and those with benign and malignant breast cancers, claiming sensitivity values of 75%, 72%, and 74% and specificities of 94%, 81, and 86%. Using RM based on urine samples with a 90s acquisition time, Elumalai et al. [55] were able to detect oral cancer with a 99% sensitivity and an 87% specificity. Ryzhikova et al. [56] has applied RS to blood serum-based diagnosis of Alzheimer's disease using two 10 s acquisitions per spectrum and reporting >95% sensitivity and specificity. Generally,

chemical tests are costly, laborious and require pre sample preparation. Handheld RM systems are now routinely used for forensic investigation due to their non-destructive nature [57]. A review article published by Polykretis et al. [58] emphasized prospective preclinical and clinical diagnosis of the most common neurodegenerative disorder Alzheimer's disease (AD) by identifying specific Raman biomarkers present in the biofluids. Although the claimed sensitivities and specificities of biofluid-based RM diagnosis are still rather limited for numerous applications, however, the standard of care for cancer screening has a lot of scope for improvement, especially utilizing RM as a supplementary tool for current diagnostic techniques.

Breast

Female breast cancer is the leading cancer worldwide. The evaluation of standard margins for surgical resection utilizing frozen tissue sections is laborious and produces delays in procedure. A number of research studies are published employing Raman spectroscopy for diagnosis of breast cancer predominantly using human tissue specimens and animal experiments. In vivo RM was investigated in mice model transcutaneously with a 15 s integration time and claimed a discrimination accuracy of 99% by Bhattacharjee et al. [59]. Haka et al. [60] was the first to employ in vivo RM among 9 breast cancer patients during mastectomy procedure with less than 1 s integration time and achieved an accuracy of 100% in differentiating cancer to benign lesion. But the study has limited statistical level of significance. Later, the prospective ex vivo research study was conducted by the same group [22] reported 83% sensitivity and 93% specificity. Further, the potential of SERS has been explored for intraoperative use for delineating tumor margins by Jiang et al. [61]. The outcomes of published studies strongly suggest that RM has potential for point of care diagnostics and rapid assessment of surgical margin for intraoperative applications. The comprehensive details of RM research studies explored for breast cancer detection are summarized and compared in Table 1.

From the observations presented in Table 1, the fact can be easily pointed out that a wide range of heterogeneity exist in the parameters of Raman techniques used for differentiating normal breast tissue to malignant breast tissue. Therefore, the better understanding of Raman techniques applied to biological samples and standardization of procedure is of utter importance. There is an immediate need for standardization of RS procedures involving human subjects. Jain et al. [71] has attempted to investigate Raman spectroscopy for standardization of procedure based on best known global bioethical principles for providing the best outcomes for detection of cancer in real world settings. The scope of this information is not limited to RM and can be used for performing other research studies involving biological samples. In another study carried out by the same group [6] focused on identifying the features and parameters of RM for discriminating human breast cancerous tissue from normal human breast tissue. They observed that the differences between Raman profiles of cancerous tissue were more prominent compared with normal breast tissues. There is noticeable spectral differences observed in both the absolute and relative intensities of the peaks in the Raman spectra between cancerous and normal tissues. Correlating the differences between cancerous and normal tissues, these results consistent with the studies of investigators Haka et al. [63], Gebrekidan et al. [72] and Chowdary et al. [73] utilized breast samples such as FFPE tissue, routine fresh surgical excision and procured in ice-cold saline respectively.

In conclusion, the true value of RM unquestionably lies in its versatility and non-destructive nature. When applied on biological samples, RM can reveal a rich data of biomolecules that can be utilised to identify and measure novel intrinsic Raman biomarkers linked to disease. Cancer diagnosis using RM is being increasingly studied in recent decades. More RM studies have been published in recent years reporting promising results that suggest it may aid in the diagnosis of cancer. The RM based technologies is gradually entering into clinics for disease diagnostics as its potential is investigated and realized. As a result, a variety of portable equipment have already been developed and established and currently, a number of handheld tools are being explored in pre-clinical phase, especially for in vivo Raman spectroscopy. The recent extensive research work in this direction strongly suggests that RS will find its well-deserved place for disease detection into clinics in coming decade. Table 2 summarizes the main advantages and limitations of RM for cancer diagnostics.

There many scientific advances in cancer detection using RM in recent decades. Moreover, the application of SERS is rapidly increasing in life sciences. However, the cost effectiveness of nanomaterials and understanding of biomolecule specific Raman biomarkers are the main challenges for implementation of SERS for point of care diagnostics.

The power of RM lies in the distinct biochemical information present in the Raman spectral profile that is often displayed in Raman peaks linked to certain biochemical bonds present in the biological sample. This feature allows differentiating cancerous tissue from normal tissue with high precision. RM has potential to develop as diagnostic tools for real time for in vivo interventional applications.

Future perspectives

For in vivo interventional or surgical applications, the capacity to acquire real-time measurements is of utter importance. Interestingly, RM has shown great potential for clinical decision making with the advancement of instrumentation and improvements in chemometric methods for spectral analysis. However, the balance between the spatial resolution, spectral resolution, field-of-view, and integration time are frequently necessary when deciding on the most useful and precise diagnostic method. The trade-off between spatial and spectral resolution is typically present, necessitating more thorough analyses that determine the data that is most important to various applications.

One of the key obstacles to using RM for clinical diagnostics is signal detection. The comparatively weak

Raman signal is frequently dominated by inherent auto-fluorescence background in tissue, and additional variables like noise, instrument response, and ambient light makes it much more complicated. With advanced spectral analysis and pre-processing methods can reduce some of the confounding elements, however, signal detection still remains the main limitation. Furthermore, this limitation is being addressed by several Raman techniques, including CARS, SRS, and SERS. To enable clinical translation, it will be essential to assess the balance between these techniques in the context of various oncology applications, particularly with regard to acquisition time, spectral/spatial resolution and cost effectiveness.

RM in combination with other clinical diagnostic methods enables multimodal approaches with high degree of precision. For better diagnosis or treatment guidance, complementary information from other clinical diagnostic modalities might be coupled with the molecular information obtained with RM. Although the in vivo sensitivities and specificities obtained with RM may be adequate in several situations for clinical translation, there are many disease detection applications that could benefit from complementary Raman biomarkers. There has been significant advancement in the use of spectroscopy and multimodal imaging to support the molecular identification of RM.

Recently, the surge in machine learning research has benefited RM by enabling the development of sophisticated classification algorithms that can make the best use of Raman spectral information. Although surgical guidance is one of the primary clinical applications for RM because of its optimal precision, ease of use, and cost effectiveness also make it increasingly practical for screening and point-of-care applications, opening up Raman methods to a wider range of clinical use. The key for effective RM spectral analysis is combining it with machine learning and artificial intelligence for extraction of important features from spectral information to support accurate clinical decision making.

In order to successfully implement the RM methods, interdisciplinary support from engineers, mathematicians, and physicists as well as clinicians, biologists, and other scientists are needed. The use of RM for cancer detection is motivated by the fact that disease progression is frequently correlated with molecular changes. As our knowledge of the biomolecules causes of disease deepens, this knowledge can be included into the ideal Raman system design. For disease diagnosis, RM can be utilised alone or along with other practiced clinical diagnostics methods such as histopathology, biopsy and radiological image guided surgical procedure, depending on the application.

Author Contribution Statement

All authors contributed equally in this study.

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Approval

This work is part of an approved Ph.D. thesis.

Ethical Clearance

Ethical approval was granted by the institution ethical committee at the Sawai Man Singh Medical College, Jaipur, India (3095/MC/EC/12/04/2017).

Availability of data

All the relevant data is presented in the article, and sources have been cited. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors have no conflict of interest to declare.

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