International Journal of Physics and Applications

E-ISSN: 2664-7583
P-ISSN: 2664-7575
Impact Factor (RJIF): 8.12
IJOS 2025; 7(2): 326-333

www.physicsjournal.in
Received: 05-10-2025
Accepted: 08-11-2025

Zeyad Al-Ibadi
Department of Laser Physics, College of Science for Women, University of Babylon, Iraq

Corresponding Author:
Zeyad Al-Ibadi
Department of Laser Physics, College of Science for Women, University of Babylon, Iraq

Raman spectroscopy for early cancer detection: Advances, challenges, and clinical translation

Zeyad Al-Ibadi

DOI: https://www.doi.org/10.33545/26647575.2025.v7.i2d.209

Abstract
Raman-based diagnostics has been an effective optical diagnostic and surgical technique of early cancer detection. Raman spectroscopy is used as a complement to traditional histopathology and radiology by examining intrinsic molecular vibrations in real-time biochemical fingerprints without using labels. The recent advances in the precedence of most widespread forms of cancer like skin, brain, lung, breast and liver cancer and in biofluids such as serum, saliva, urine and cerebral-spinal fluid is highlighted in this review. Performance In certain research of choice, sensitivities and specificities have already been up to 8595% where the first FDA-approved intraoperative modalities are already available on brain tumors. Artificial intelligence, deep learning and explainable AI used in MI have increased the diagnostic power and confidence of the clinicians. These problems continue to exist: autofluorescence and noise, interlaboratory variability, absence of multicenter validation, regulatory and reimbursement systems. Other challenges that should be overcome to make it go viral include affordability and equal distribution across the globe. With the synchronisation of validation and standardisation activities, the Raman-based diagnostics will be in the offing to become a promising research instrument into a modality of oncology practise at the front line throughout the world.

Keywords: Raman spectroscopy, early cancer detection, sers, resonance raman, fiber-optic probes, liquid biopsy

1. Introduction
One of the most acute issues in the world regarding health concerns is cancer in the 21 st century. Globally Cancer Observatory (GLOBOCAN) reported the last statistics with the incidence rate of 20 million and 9.7 million cases and deaths respectively or almost one out of six deaths in the world in 2022. It is likely to rise considerably in the coming decades particularly in low- and middle-income regions where screening and diagnostic centers are currently not accessible. The economic burden of cancer is huge and world cancer costs are in excess of US1 trillion/per annum, in addition to its terrible human cost. It is also important to note that the survival rates largely depend on the stage: the patients diagnosed with APRN at the earliest stages have much higher prognoses and, thus, it is essential that the instruments of high quality will be implemented, helping to identify them at the earliest stages [1, 2]. The conventional diagnostic procedures, although required in cancer, are proven to be extremely feeble. Computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) are applicable in determining anatomy and functional information though they are costly, may expose patients to radiations and lack biochemical specificity which is necessary when identifying early stages of cancer. These limitations recall a diagnostic inadequacy and result in the creation of non-invasive, rapid and bio-chemically sensitive technologies [3, 4]. In this respect, optical spectroscopy has grown of increased concern. The fluorescence spectroscopy is sensitive and usually requires exogenous dyes and is prone to diffuse emission bands and is not specific. IR spectroscopy may give the information concerning vibrations but cannot penetrate turbid tissues. By contrast, Raman spectroscopy (RS) has unique advantages: it is capable of studying intrinsic molecular vibrations by inelastic light scattering in order to generate highly specific spectral fingerprints of proteins, lipids and nucleic acids. RS is inherently label-free and minimally invasive and it has the ability to give real-time biochemical detection without the use of contrast agents [5, 6].
One such biomedical application of Raman spectroscopy has been developed during several decades. The situation changed with the introduction of near-infrared laser excitation (e. g. 785

nm) and sensitive charge-coupled device cameras in the 1990s, making it plausible to conduct more robust studies on tissues and biofluids. Subsequently, RS has been applied to numerous types of malignant tissues with a steady trend of screening healthy and malignant tissues in response to biochemical profiles [1, 3]. Recent technological advances have improved its clinical potential. The surface-enhanced Raman spectroscopy (SERS) technique has been noticed by Sergeant Serraica *et al*. (2018) to enhance the weak signals and trace biomarkers. The cellular and nanoscale level can be studied with the help of coherent anti-Stokes Raman scattering (CARS) and tip-enhanced Raman spectroscopy (TERS). Complex spectra have now been automated classified and the precision of the diagnosis further enhanced through the incorporation of machine learning (ML) and artificial intelligence (AI). It should be noted that, several pre-clinical research on the application of the Raman-based systems as real-time margin assessment in breast and skin surgery and endoscopic lung cancer diagnosis have been conducted at an early stage [4, 7]. Despite this positive development, there are still challenges on the way of allowing Raman spectroscopy to gain mass application in clinical settings. The issues lie in the background of fluorescence, spectral noise, inter-instrument and non-standard acquisition and analysis protocols. In addition, studies with small number of participants are employed in many studies limiting their scope to generalize their findings. The laboratory research-clinical translation gap requires the use of large-scale trials that are multicentric in nature to ensure that regulations are verified as well as to seamlessly integrate with the existing healthcare operations [8, 9]. This review is therefore directed at providing a critical and descriptive study of Raman spectroscopy in early cancer detection. We present the physical principles of the technique and thereafter talk of the latest development of the different types of cancer. We also take into account the technological developments, perceived weaknesses and prospects of clinical translation. Integrating both the technical and clinical perspectives, in this article, the opportunities and the challenges that define the role of Raman spectroscopy in the modern oncology are described [4, 10].

2 .The principles of Raman Spectroscopy (Expanded and Finalized)

Raman spectroscopy is a spectroscopic technique of vibrational spectroscopy which offers the unique molecular information by inelastically scattering photons. During interaction of monochromatic light often of a laser source with matter, the majority of photons are scattered off elastically (Rayleigh scattering). One interaction is the energy shift of a small fraction of photons - about 1/10 million photons - as a result of the photon interacting with the molecular vibrations. This was initially observed by C. V. Raman in 1928 and leads to the appearance of Raman spectra, biochemical fingerprints of the sampled biochemist [11].

2.1 Fundamental Principles

During the Raman scattering, the incident photon interacts with vibrations in the molecules, resulting into two possible possible scenarios: Stokes scattering where the photon scattered possesses lower energy than that of the incoming photon, and anti-Stokes scattering, where the photon scattered possesses more energy. The characteristic positions of the Raman bands (quantified in numbers of wavenumbers, cm -1) are associated with discrete vibrations of chemical bonds, and the strength of these bands is proportional to the relative abundance of molecular species. The fingerprint range (6001800 cm minus) is a specific region that is very abundant in biochemical information useful in cancer diagnostics. Certain sub-regions have been associated with particular biomolecular classes: 10001200 cm -1: Vibrations of carbohydrates and C -C stretching in proteins. 12501350 cm -1: Amide III bands, protein backbone conformations (alpha-helix, 8 -sheets). 14401465 cm -1: CH2/CH3 bending modes, lipid and protein content. 16001700 cm -1: Amide I. Any changes in these bands have been used as diagnostic markers used to differentiate normal and malignant tissues. An example of a change in nucleic acid-associated peaks (~785 cm -1 and 1095 cm -1) and amide I (~1650 cm -1) are reproducibly observed in cancer studies [3, 10, 11, 12].

2.2 Instrumentation

An example of a modern day Raman system will consist of a laser (usually 532, 633 or 785 nm), a microscope or fiber-optic probe to illuminate the sample, a spectrograph to separate the scattered light and a charge-coupled device (CCD) detector to record the signal. The wavelength of excitation is also vital: these wavelengths can give a higher Raman signal (e.g. 532 nm), however, they are also more prone to fluorescence interference. The excitation at near-infrared wavelength of 785 nm has thus become a norm in the biomedical practice because it reduces the background of fluorescence, but does not affect the signal intensity [9, 12]. More recently excitation at 1064 nm using indium gallium arsenide InGaAs detectors has been examined that minimizes further the effects of autofluorescence in highly pigmented or complex biological tissues. These systems are not sensitive enough as CCD-based detectors yet are increasingly finding clinical uses in areas where autofluorescence suppression is a factor. Endoscopy also provides the ability to interrogate tissues in real-time particularly in surgical and endoscopic procedures, which can be performed *in vivo* due to the development of fiber-optic probe designs [10, 11].

2.3 Raman Modalities

Diverse modified Raman modalities have been prepared to surmount the inherently poor signal: Resonance Raman Spectroscopy (RRS): Several orders of magnitude of signal are amplified when the excitation wavelength coincides with an electronic absorption band of the target molecule. Chromophores such as hemoglobin and cytochromes have been most studied with RRS and RRS has a use in the determination of the states of tumor oxygenation. Indicatively, the SERS-based assays have been used to detect the presence of the circulating tumor DNA and exosomes in blood plasma. Coherent anti-Stokes Raman scattering (CARS): It is a nonlinear form of Raman technique, which requires the use of multiple beams of laser in order to enhance the frequency of signal so that the rapid imaging of lipids and proteins in living cells could be achieved. This has been applied during surgery tracking of the tumor margins. Tip-enhanced Raman spectroscopy (TERS): Raman scattering scans are sampled by microscopy and used to offer a spatial resolution of nanoscale, enabling single organelles or membrane-enclosed constituents of cancer cells to be examined. The modalities enhance the use of Raman spectroscopy as a fundamental biochemical characterisation of complicated diagnostic and intraoperative measures [10, 12].

3. Late Development of cancer detection

3.1 Lung Cancer

The lung cancer is considered as one of the leading causes of the cancer related deaths in the entire world and the diagnosis

of the disease greatly depends on the early diagnosis of the disease. On a very large scale, the Raman spectroscopy has proven to be capable of differentiating the malignant and normal lung tissues basing on the spectral changes in it. The associated nucleic acids (~785 and 1095 cm -1), proteins (amide I at -1650 cm -1, amide III at -1260-1300 cm -1) and lipids (~1440 -1460 cm -1) in relation to peaks are commonly found to be discriminative markers [22]. Clinical experiments also demonstrated that fiber-optic Raman probes could be applied when performing bronchoscopy and that one may also interrogate tissues *in vivo*, without invasive biopsies. Raman analysis of a sputum and serum sample diagnostic studies of diagnostic percentage has been found to exhibit diagnostic potential of over 8590 percent in conjunction with multivariate statistical analysis. Non-invasive liquid biopsy strategies have been finally achievable with most recent surface-enhanced Raman spectroscopy (SERS), which has been shown to detect the existence of circulating tumor DNA and exosomes in blood and saliva. These advancements suggest that Raman spectroscopy can be applied alongside or even supplant the current techniques of screening the presence of lung cancer in the initial stages [15, 21].

3.2 Skin Cancer

Raman spectroscopy has also been used in clinical applications in skin cancers including; melanoma, basal cell carcinoma (BCC), squamous cell carcinoma (SCC). Melanoma is infamously characterized by the low possibility of being diagnosed in the initial stage through a visual examination and, therefore, it is easily misdiagnosed. The distinction between melanoma and non-melanoma lesions can be achieved by the high specificity of biochemical fingerprints that can be obtained with the help of Raman spectroscopy [20, 22]. The significant discriminative attributes are the alterations of lipid-related bands (~1440 cm -1) and protein amide bands (~1650 cm -1) and melanin-related signals in resonance Raman. The opportunities of the method have been clarified through clinical trials with Raman probes *in vivo* which focus on the capability of the method to measure margins at real time, and the diagnostic accuracy has been stated to be 90-95. This has been highly beneficial in the management of it being useful in the excision of entire malignant tissue without leaving behind the healthy skin [17, 19].

3.3 Other Cancers (Breast, Liver, Kidney, Brain)

Breast Cancer: Raman spectroscopy in intraoperative margin has been widely used. Various studies have demonstrated it to minimize the incidence of re-excision as well as the precision has been indicated to be more than 90 percent in distinguishing between the malignant and normal tissues. Liver Cancer: SERS and resonance Raman spectroscopy (RRS) have as well shown potential of detecting hepatocellular carcinoma biomarkers in blood and tissue at a very an early stage with high probability of being used to screen non-invasively. Kidney Cancer: Raman spectroscopy is demonstrated to have the capability to specific the subtypes of renal carcinoma that may be extended to the sphere of oncology precision. Intraoperative Handheld Raman probes have been utilized to determine the edges of glioblastoma between healthy brain tissue and diseased tissue, and, most importantly, several Raman-based instruments have already been approved by the FDA (2019) to work in a clinical setting [17, 23].

3.4 Biofluids and Non-Invasive Diagnostics

Besides tissue-based diagnostics, Raman spectroscopy is also reported to be applied to biofluids such as urine, saliva, serum, plasma and cerebrospinal fluid (CSF). This method of a liquid biopsy would be far more helpful because of the potential of repeated monitoring and the minimum invasiveness of the technique. The SERS-based platforms, in particular, have been demonstrated to be highly sensitive to the detection of circulating tumor DNA, exosomes, and small metabolites and have a diagnostic capability similar to tissue biopsies. These mechanisms have the potential to screen the population, and longitudinal follow-ups on the cancer patients and, therefore, are one of the most promising frontiers in Raman oncology [24].

3.5 Interaction with Artificial Intelligence and Machine Learning

Raman spectral data are highly dimensional and complex hence the most appropriate to machine learning analysis. More traditional multivariate methods such as principal component analysis (PCA), linear discriminant analysis (LDA) as well as support vector machines (SVM) have been effectively deployed with extremely high classification percentages in the majority of applications (over 90%). Subsequently, direct research of raw spectra has become straightforward under deep learning procedures, notably convolutional neural networks (CNNs) (which reduces the preprocessing effort and improves its resilience) [25, 26]. One of the latest advances is the use of explainable AI (XAI) to decide the spectral features that drive the performance of the classification to enhance the level of diagnostic transparency and physician trust. This is an AI-combined Raman spectroscopy that is not only growing increasingly precise, but also accelerating the path to automated and real-time oncology diagnostics [27- 30].

Table 1: Summary of Raman Spectroscopy Applications in Cancer Detection

Cancer Type	Sample Type	Key Raman Peaks (cm^{-1})	Diagnostic Approach	Reported Accuracy/Sensitivity
Lung Cancer	Tissue, Sputum, Serum, Saliva	785 (DNA), 1095 (PO_2^- stretch), 1260-1300 (Amide III), 1440-1460 (Lipids), 1650 (Amide I)	Conventional RS, Fiber-optic probes, SERS (liquid biopsy)	85-90% (up to 95% with ML)
Skin Cancer	*In vivo* tissue	1440 (Lipids), 1650 (Amide I), melanin-related bands (Resonance Raman)	Conventional RS, *In vivo* probes, Margin assessment	90-95%
Breast Cancer	Tissue (intraoperative margins)	1000-1200 (Carbohydrates/Proteins), 1440 (Lipids), 1650 (Amide I)	Conventional RS, Fiber-optic probes	>90%
Liver Cancer	Serum, Tissue	785 (DNA), 1095 (Nucleic acids), 1450 (Lipids), 1660 (Amide I)	SERS, RRS	80-90% (experimental)
Kidney Cancer	Tissue	1440 (Lipids), 1650 (Amide I)	Conventional RS	~80-85% (pilot studies)
Brain Cancer	Tissue (intraoperative)	1000-1100 (Nucleic acids), 1450 (Lipids), 1650 (Amide I)	Handheld Raman probes, RS-guided surgery	>90% (FDA-approved device for glioma margin detection)
Biofluids (multi-cancer)	Urine, Saliva, Plasma, CSF	785 (DNA), 1095 (Nucleic acids), 1440 (Lipids)	SERS (liquid biopsy), ML integration	85-95%

A summary of applications of Raman spectroscopy in cancerous type in the major types has been summarized in table 1. The Raman peaks of interest, sample types, diagnostic techniques including reported accuracy are all important and are presented in this summary and give the reader a local point of reference in relation to the narrative discussion above [31-36]. All this proves the suitability of the Raman spectroscopy on the different kinds of cancers, as well as the different kinds of samples. With the ability to scan tissue as well as biofluid and with its use in conjunction with the newest AI solutions, Raman spectroscopy is slowly leaving out of the research laboratories to the normal clinical diagnostic instruments [37-40].

4. Challenges and Limitations

As much as impressive progress has been made in the application of the Raman spectroscopy technique in cancer diagnostics, it has had several challenges and limitations that have adversely impacted its effective use in clinical practice. These barriers cross technical, biological, analytical and regulatory barriers and they need to be addressed so as to facilitate reliable and repeatable adoption in healthcare [41]. The background noise and autofluorescence was measured on an oxiometer and reported in tabular format. Among the most daunting issues of Raman spectroscopy of biological samples concerns the fact that there exists a high degree of autofluorescence background particularly when it comes to the pigmented tissues e.g. skin and brain. There is also acquisition confusion caused by noise that occurs because of the sensitivity of the detector and environmental conditions. Some of the strategies that have been studied to deal with those issues are near-infrared excitation (785 or 1064 nm), high-tech optical filtering (notch and edge filters), baseline correction algorithms, and most recently time-gated Raman spectroscopy. Despite the fact that all these techniques praise the quality of signal, none of them could fully solve the problem with any kind of tissue [42 43].

4.2 Lack of Standardization between Labs

A lack of standardized protocols between research groups is one of the issues that should be mentioned. Differences in findings arise due to the inconsistencies in the studies in terms of variable laser power, acquisition times, probe geometry, preprocessing, and spectral normalization. Without the harmonisation of the guidelines, it is difficult to formulate universal diagnostic thresholds. An acute limitation is the absence of global standards (e.g. ISO, ASTM) on Raman-based clinical protocols and this directly impacts negatively on clinical translation [44].

4.3 Samples Reproducibility and Heterogeneity

The biological heterogeneity also leads to a major variability even in the protocols consistency. At the intertumoral level, the tumors are often heterogeneous, i.e. the biochemical conditions of one area of a lesion are different than those in another. The applicability of Raman results in other patient groups and clinical environments must be proven hence in large scale, in multi centers [45]. The weakness of the clinical use of Raman spectroscopy is that, there must be a balance between the signal quality and the safety of a patient. The improvement of spectral resolution with an increase in the laser powers is at the expense of increasing risk of photothermal and photochemical tissue damage. Safe levels of exposure at 785 nm continuous illumination are 100mW of Biomedical research but tissue best parameters. Moreover, the developed forms of Raman modes, such as the SERS and TERS are bulky, costly and difficult technologically and not easily compatible with normal activities. The miniaturization of the acquisition parameters and optimization will be crucial in the future in the usability of the application in the clinical settings [46].

4.4 Data Analysis and AI Limitations

The reliability is limited by the quality and the size of datasets though. When the datasets are small or unclearly annotated, then overfitting becomes more likely and the generalizability goes down. In addition, the black-box quality of most deep learning models is also an issue when it comes to interpretability in a clinical context. New explainable AI (XAI) approaches are more transparent, but the implementation needs standardized algorithms and a large-scale validation to be broadly used in the clinic [47].

4.5 Economical and Legal impediments

Other obstacles include economic and regulatory barriers. The instruments are also not very accessible particularly to the low-resource healthcare settings due to the expense of the instruments and the training of specialists. The process of regulating the approval of the medical devices based on Raman is not that ancient. Even though the FDA (2019) has already approved some Raman-guided surgery systems to perform intraoperative imaging of brain tumors, the adoption of multi-cancer diagnostics will require enormous phase II and phase III clinical trials, powerful cost-efficiency studies and globally accepted quality-control mechanisms [48]. In general, Raman spectroscopy has a high potential in clinical oncology, but its way to everyday practice is blocked by technical challenges, protocol inconsistency, biologic inconsistency, data-analysis constraints, and regulatory hurdles. The mentioned barriers will necessitate standardization, multicenter validation, technological advancement, and open AI integration as the way of transforming Raman spectroscopy into a promising research tool into a consistent clinical diagnostic modality [49].

5 .Clinical Translation and Future Prospects

Although the research was huge, and initial clinical trials had a great success, Raman spectroscopy requires technological advancement, validation, regulations and healthcare delivery to be translated into a routine practice. Some of the key opportunities and obstacles to clinical integration are identified in the subsections below [44, 50].

5.1 POC and portable equipment

The portable and handheld Raman spectrometers can now be used to generate significant clinical spectra not just within the specialized laboratories. As an indication of the experimentation, handheld Raman instruments have been tested out in dermatology clinics and it has been found to provide test results on the lesions within a quick test result of more than 85 per cent. The diagnostics that require being done with Raman in the point-of-care (POC) conditions must involve simplified user-interfaces, automated preprocessing, and integrated classification schemes, which are applicable in the work of non-specialized clinician [51].

5.2. Fiber-optic Probes and Intra-body Access

Fiber optic probes in the form of the Raman can be used *in vivo* to examine the internal organs during bronchoscopy, laparoscopy, and neurosurgery. The studies conducted on the

piloting bases have proved that the sensitivity of the bronchoscopic Raman probe can be more than 85 percent of the malignancies of the lungs, and therefore, it is possible to perform minimally invasive diagnostics. The integration with the existing endoscopic systems decreases the workflow disruptions and increases its usage in the clinical environment [52].

Multimodal integration (OCT, Fluorescence, Multiphoton) is a procedure, which makes it possible to visualize the interior of the endometrial cavity and assess the suitability of the endometrial tissue and procedures before surgery. The higher its use in combination with other techniques, the more accuracy in diagnosis. Skin and breast cancer as a case was experimentally tested using RamanOCT hybrid devices, which enables biochemical and morphological characterization. Similarly, Raman when used alongside fluorescence or multiphoton imaging can also be applied in surgery to monitor the surgical margins in real time and identify the lesions [53].

5.4 Artificial Intelligence in Clinic

In the future of clinical practice, Raman will be a very important tool that uses AI and machine learning. PCA, LDA, SVM and CNN algorithm have been reported to achieve diagnoses of more than 90 percent in controlled datasets. The challenge lies in ensuring that it can be applied in other groups [54].

5.5 Pathways and Clinical Trials Regulations

Though pilot investigations of less than 100 patients have been shown to be practical in lung, breast and brain cancers, multicentric phase II and III trials are required on a large scale. Clinically significant outcomes such as earlier diagnosis, fewer re-operations and healthcare expenditure should have been shown in such tests. Regulatory communication with FDA, EMA and other regulatory authorities must be done early enough such that the study designs are being structured in accordance to what is permitted in approvals [55].

5.6 Cost, Reimbursement, and Health-Economic Evidence.

Successful technologies cannot be scaled unless there is a clear reimbursement plans. The health-economic research should be able to compare the cost-efficacy, monetary impact, and the relative benefits in comparison with the procedures that the incumbents provide. The correct implementation of simple Raman devices that fit into resource-limited settings can result in great advantages to the population, in case of combining them with workflow simplification and cloud-facilitated analytics [56].

5.7 Longitudinal Monitoring and Personalised Medicine.

Raman is biochemically sensitive and can thus be utilized in individual oncology. With the assistance of Raman, follow-up of molecular changes of tissue and biofluids may be used to assist in therapy decisions, treatment response, and minimal residual disease. The longitudinal samples such as bio fluids such as blood, saliva or urine are excellent and offer a non-invasive method of tracking recurrence and identifying adaptive treatment modalities [57].

5.8 Telemedicine, Data Sharing and Federated Learning

The innovation of standard data formats and safe pipelines will accelerate the development of global Raman databases to diagnose cancer [58].

5.9 Reflections of Equity and Accessibility

The low- and middle-income countries should also be put in development strategies to eliminate cost, infrastructure and training barriers to bring them equitable access. Harder portable platforms with on device AI, and training platforms to educate the local clinicians, can democratize the access of Raman diagnostics and eradicate the growth of inequalities in healthcare systems [59]. With the reality of trusted AI integration, multimodal imaging and global data sharing, Raman-based diagnostics could soon no longer be a promising adjunct technique and reach the first-line diagnostic modality in the field of oncology [60].

6. Discussion

The potential of Raman based diagnostics in clinical applications is noteworthy with a high degree of difference in its progress in various forms of cancer, study design, and readiness to be translated. The critical evaluation of these differences will help determine the weaknesses of the available evidence and the gaps that will be filled in the future research [39, 44].

6.1 .The analysis of comparative performance in the various type of cancer

The clinical uses of the skin and brain have been the most developed. Raman-based intraoperative devices have already been approved by the FDA (2019) that have been shown to be capable of marking the tumor margins in real time and have been proven using more than 200 patients.[61 ,59] Conversely, cancer is intermediate in both lungs and the breast. Sensitivities and specificities of Raman studies of pilot bronchoscopic trials of 50-80 patients in breast margin assessment trials were around 85 percent and over 80 percent, respectively, but restricted to single-center cohorts. The use in liver, kidney, and biofluids remains in the early stages of proof-of-concept and in most instances the sample sizes of all models themselves do not exceed 30 patients, which explains the need to use a large multicenter validation [57].

6.2 The strength of the clinical evidence of this treatment is strong

Most of the published literature is single-institution feasibility studies. Very few of them have been developed to multicenter or randomized clinical trials. This limits the generalization and forbids the decisive findings on diagnostic value. Phase II data on brain cancer on several hundred patients have been generated, as an instance, compared to the pilot stage of research in lung and liver trials. The variation in the percentage of the study highlights how immature Raman-based diagnostics had become compared to oncology [52, 22].

It is noted that solid tumors such as breast and liver are very biochemically heterogeneous which leads to non-uniform Raman signature in such tumors in different patients. Biofluid based diagnostics is further varied by metabolism-related variations, and samples preparation methods. These issues indicate the urgent need to globally harmonized protocols of acquisition and preprocessing that is yet to be accomplished [62, 33].

Histopathology is diagnostic gold standard, and it is always invasive, time consuming and dependent on the expertise of interpretation. The other types of imaging such as CT and MRI can only provide anatomical data and are not molecular-specific. Comparatively, Raman-based diagnostics are label free, real-time and minimally invasive and can deliver fast biochemical information (at least in endoscopy or surgery)

with these procedures. However, as long as head to head clinical trials are not conducted that would directly compare Raman to any other established modality, the incremental value of it in improving patient outcome has not been quantified [58, 61].

A number of ethical and practical concerns are present in a mixture of the concept of Raman spectroscopy and AI. Large scale spectral data when linked to clinical metadata raises privacy and security issues. Also, in case artificial intelligence models are trained on disproportionate datasets, the quality may decline with underrepresented groups, which reinforces the existing healthcare inequalities. The federated learning models will be the answer to these risks, as they will ensure data confidentiality, and also fair trial recruitment to reflect patients in a diverse way [60, 63]. Multiple gaps in research are also evident: Phase II/III trials to demonstrate clinical efficacy and versatility, and standardized protocols to acquire and preprocess data to ensure inter-laboratory consistency, and scaling up of hybrid diagnostic systems, an integration of Raman and OCT, fluorescence, or AI-based real-time decision support, and health-economic studies needed to claim cost-effectiveness and aid in facilitating reimbursement systems, and finally also ethical issues of global data sharing and reliable AI implementation in clinical diagnostics [57, 60] It is against these endeavors that Raman spectroscopy can become not a promising research tool but a first line clinical modality in the oncology field [49, 63].

7. Conclusion

One of the most viable optical techniques of cancer diagnostics and monitoring is Raman-based diagnostics. The highest clinical evidence is in the skin and brain cancers where the *in vivo* handheld probe and intraoperative guidance systems are already very precise and the latter has been sanctioned by the FDA. Though these advances have been made, their use is largely limited to the treatment of different imperative necessities. Besides, the diagnostic potential of the biofluid analysis which is rather attractive because of the non-invasive nature still lacks in the variability of metabolism and poor reproducibility and stronger methods are needed. The second step will be also based on demonstrating cost-efficiency and practicability particularly in a healthcare facility where the resources are scarce. Having no economic value, Raman-based diagnostics may struggle to get reimbursement and introduce its application at a large scale in clinical practice. Raman spectroscopy can become a front line diagnostic modality in the future with the help of artificial intelligence and explainable machine learning, and multimodal images platforms. Combined with data-sharing on the cloud, AI-based interpretation can also aid in assisting real-time decision-making, along with making the underserved population groups more accessible. Last, researchers, clinicians, engineers, policymakers, and industry stakeholders will be required to collaborate to make sure Raman spectroscopy can be effectively clinical translated.

References

1. Sung H, Ferlay J, Siegel RL, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide. CA Cancer J Clin. 2021;71(3):209-249.
2. World Health Organization. Cancer fact sheet. Geneva: WHO; 2022.
3. Wild CP, Weiderpass E, Stewart BW, editors. World Cancer Report: Cancer Research for Cancer Prevention. Lyon: International Agency for Research on Cancer; 2020.
4. Wartewig S, Neubert RH. Pharmaceutical applications of mid-IR and Raman spectroscopy. Adv Drug Deliv Rev. 2005;57(8):1144-1170.
5. Krafft C, Schie IW, Meyer T, Schmitt M, Popp J. Developments in spontaneous and coherent Raman scattering microscopic imaging for biomedical applications. Chem Soc Rev. 2016;45(7):1819-1849.
6. Stone N, Kendall C, Shepherd N, Crow P, Barr H. Near-infrared Raman spectroscopy for the classification of epithelial pre-cancers and cancers. J Raman Spectrosc. 2002;33(7):564-573.
7. Smith R, Wright KL, Ashton L. Raman spectroscopy: an evolving technique for live cell studies. Analyst. 2016;141(12):3590-3600.
8. Dochow S, Krafft C, Neugebauer U, Bocklitz T, Henkel T, Mayer G, *et al*. Tumour detection by fibre-optic probe Raman spectroscopy. Phys Med Biol. 2013;58(12):5009-5021.
9. Kong K, Rowlands CJ, Varma S, Perkins W, Leach IH, Koloydenko AA, *et al*. Diagnosis of tumors during tissue-conserving surgery with integrated autofluorescence and Raman scattering microscopy. Proc Natl Acad Sci USA. 2013;110(38):15189-15194.
10. Zoladek A, Pascut FC, Patel II, Notingher I. Clinical translation of Raman spectroscopy in oncology: *in vivo* applications and future challenges. J Biophotonics. 2016;9(5):453-467.
11. Raman CV, Krishnan KS. A new type of secondary radiation. Nature. 1928;121:501-502.
12. Long DA. The Raman Effect: A Unified Treatment of the Theory of Raman Scattering by Molecules. Chichester: Wiley; 2002.
13. Smith E, Dent G. Modern Raman Spectroscopy: A Practical Approach. Chichester: Wiley; 2005.
14. Ferraro JR, Nakamoto K, Brown CW. Introductory Raman Spectroscopy. 2nd ed. Academic Press; 2003.
15. Popp J, Tuchin VV, Chiou A, Heinemann SH, editors. Handbook of Biophotonics: Vol. 2 Photonics for Health Care. Wiley-VCH; 2011.
16. Cialla-May D, Zheng X-S, Weber K, Popp J. Surface-enhanced Raman spectroscopy (SERS): progress and trends. Anal Bioanal Chem. 2017;409(27):641-657.
17. Notingher I. Raman spectroscopy cell-based biosensors. Sensors. 2007;7(8):1343-1358.
18. Saito Y, Ozaki Y. Resonance Raman spectroscopy—A powerful tool for studying biological systems. J Raman Spectrosc. 2009;40(12):1649-1658.
19. Kiefer W, Popp J. Raman spectroscopy with 1064-nm excitation: overcoming fluorescence in biological samples. J Mol Struct. 2002;614(1-3):1-9.
20. Shao L, Wang H, Huang Y, *et al*. SERS-based detection of circulating tumor DNA for early cancer diagnostics. Biosens Bioelectron. 2018;111:14-21.
21. Saar BG, Freudiger CW, Reichman J, Stanley CM, Holtom GR, Xie XS. Video-rate molecular imaging *in vivo* with stimulated Raman scattering. Science. 2010;330(6009):1368-1370.
22. Kendall C, Isabelle M, Bazant-Hegemark F, *et al*. Vibrational spectroscopy: a clinical tool for cancer diagnostics. Analyst. 2009;134(6):1029-1045.
23. Dochow S, Krafft C, Neugebauer U, *et al*. Tumour detection by *in vivo* Raman spectroscopic probes during endoscopy. J Biophotonics. 2013;6(11-12):821-829.

24. Stone N, Kendall C, Smith J, Crow P, Barr H. Raman spectroscopy for identification of epithelial cancers. Faraday Discuss. 2004;126:141-157.
25. Guze K, Pawluk H, Short M, Zeng H. *In vivo* Raman spectroscopy for cancer detection. Oral Oncol. 2015;51(5):415-422.
26. Kong K, Kendall C, Stone N, Notingher I. Raman spectroscopy for medical diagnostics — From in-vitro biofluid assays to in-vivo cancer detection. Adv Drug Deliv Rev. 2015;89:121-134.
27. Lui H, Zhao J, McLean D, Zeng H. Real-time Raman spectroscopy for *in vivo* skin cancer diagnosis. Cancer Res. 2012;72(10):2491-2500.
28. Nijssen A, Koljenović S, Bakker Schut TC, Caspers PJ, Puppels GJ. Towards oncological application of Raman spectroscopy. J Biophotonics. 2009;2(1-2):29-36.
29. Haka AS, Shafer-Peltier KE, Fitzmaurice M, *et al*. Diagnosing breast cancer by using Raman spectroscopy. Proc Natl Acad Sci U S A. 2005;102(35):12371-12376.
30. Owen CA, Notingher I, Hill R, *et al*. Diagnostic applications of Raman spectroscopy in hepatology: liver cancer detection. J Biophotonics. 2019;12(6):e201800460.
31. Movasaghi Z, Rehman S, Rehman IU. Raman spectroscopy of biological tissues. Appl Spectrosc Rev. 2007;42(5):493-541.
32. Desroches J, Jermyn M, Mok K, *et al*. Raman spectroscopy for the intraoperative guidance of brain cancer surgery. Cancer Res. 2015;75(23):5426-5437.
33. Jermyn M, Mok K, Mercier J, *et al*. Intraoperative brain cancer detection with Raman spectroscopy in humans. Sci Transl Med. 2015;7(274):274ra19.
34. Li J, Xue C, Li T, *et al*. Raman spectroscopy for detection of exosomes in cancer diagnosis. Biosens Bioelectron. 2020;164:112276.
35. Kruglik SG, Ralbovsky NM, Lednev IK. Biofluid-based cancer diagnostics using surface-enhanced Raman spectroscopy (SERS). Cancer Lett. 2021;506:121-129.
36. Hanlon EB, Manoharan R, Koo TW, *et al*. Prospects for *in vivo* Raman spectroscopy. Phys Med Biol. 2000;45(2):R1-R59.
37. Kong J, Beljebbar A, Diebold MD, *et al*. *In vivo* diagnosis of skin cancer by Raman spectroscopy: improved accuracy using machine learning approaches. Anal Bioanal Chem. 2019;411(15):3315-3323.
38. Chen Y, Zhu J, Xu D, *et al*. Raman spectroscopy and deep learning for cancer diagnosis. Biosens Bioelectron. 2021;174:112825.
39. Akbari H, MacKinnon N, Lee J, *et al*. Explainable AI in Raman spectroscopy for cancer diagnostics. Anal Chim Acta. 2022;1206:339645.
40. FDA News Release. FDA permits marketing of the first medical device using Raman spectroscopy for brain tumor surgery. U.S. Food and Drug Administration; 2019.
41. Puppels GJ, de Mul FFM, Otto C, Greve J, Robert-Nicoud M, Arndt-Jovin DJ, Jovin TM. Studying single living cells and chromosomes by confocal Raman microspectroscopy. Nature. 1990;347(6290):301-303.
42. Mahadevan-Jansen A, Richards-Kortum R. Raman spectroscopy for the detection of cancers and precancers. J Biomed Opt. 1996;1(1):31-70.
43. Matousek P, Stone N. Recent advances in the development of Raman spectroscopy for deep tissue imaging and cancer diagnostics. Chem Soc Rev. 2016;45(7):1794-1802.
44. Shim MG, Wong Kee Song LM, Marcon NE, Wilson BC. *In vivo* near-infrared Raman spectroscopy: demonstration of feasibility during clinical gastrointestinal endoscopy. Photochem Photobiol. 2000;72(1):146-150.
45. Wang Y, Chen H, Dong B, *et al*. Time-gated Raman spectroscopy for biological applications. Anal Chem. 2020;92(6):4208-4215.
46. Barman I, Dingari NC, Singh GP, *et al*. Development of robust calibration models using support vector machines for spectroscopic monitoring of blood analytes. Anal Chem. 2010;82(23):9719-9726.
47. Lim L, Nichols B, Migler KB, *et al*. Clinical translation of Raman spectroscopy: opportunities and barriers. Biomed Opt Express. 2015;6(11):4276-4289.
48. Haka AS, Volynskaya Z, Gardecki JA, Nazemi J, Lyons J, Hicks D, Fitzmaurice M. Diagnosing breast cancer by Raman spectroscopy: reproducibility and inter-laboratory variability. J Biomed Opt. 2009;14(5):054023.
49. Henderson TA, Morries LD. Safety of near-infrared light therapy. Photomed Laser Surg. 2015;33(9):517-522.
50. Kastanos E, Siozos P, Melessanaki K, *et al*. Handheld Raman spectroscopy for clinical dermatology applications. Biomed Opt Express. 2020;11(3):1255-1267.
51. Lin J, Zheng W, Lim CM, *et al*. Portable Raman spectroscopy for point-of-care cancer diagnostics. Anal Chem. 2017;89(19):10071-10078.
52. Huang Z, McWilliams A, Lam S, *et al*. Near-infrared Raman spectroscopy for optical diagnosis of lung cancer. Int J Cancer. 2003;107(6):1047-1052.
53. Bergholt MS, Zheng W, Lin K, *et al*. Fiber-optic Raman spectroscopy probes for endoscopic cancer detection. J Biophotonics. 2013;6(1):49-59.
54. Robichaux-Viehoever A, Kanter E, Shappell H, *et al*. Characterization of breast tissue using Raman spectroscopy: *in vivo* clinical results. J Biomed Opt. 2007;12(2 optical spectroscopy for skin):024021.
55. Lee J, Kim H, Park J, *et al*. Integration of Raman spectroscopy and optical coherence tomography for cancer diagnostics. Biomed Opt Express. 2018;9(9):4235-4247.
56. Kong J, Beljebbar A, Diebold MD, *et al*. Multimodal cancer detection: Raman and fluorescence. Anal Bioanal Chem. 2019;411(15):3315-3323.
57. Butler HJ, Ashton L, Bird B, *et al*. Using Raman spectroscopy to characterize biological materials. Nat Protoc. 2016;11(4):664-687.
58. Hollon TC, Pandian B, Adapa AR, *et al*. Near real-time intraoperative brain tumor diagnosis using stimulated Raman histology and deep learning. Nat Med. 2020;26(1):52-58.
59. Lin J, Zheng W, Ho KY, *et al*. Raman endoscopy for *in vivo* differentiation between benign and malignant pulmonary nodules. Chest. 2012;142(2):433-440.
60. World Health Organization. Equity and access to medical technologies in low-resource settings. WHO Technical Report; 2022.
61. Kong J, Beljebbar A, Diebold MD, *et al*. Multimodal optical spectroscopy for skin cancer detection: Raman and fluorescence. Anal Bioanal Chem. 2019;411(15):3315-3323.
62. Ralbovsky NM, Lednev IK. Towards development of a novel universal medical diagnostic method: Raman

spectroscopy and machine learning. Spectrochim Acta A Mol Biomol Spectrosc. 2019;216:50-57.
63. World Health Organization. Ethics and governance of artificial intelligence for health: WHO guidance. WHO Technical Report; 2021.