IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

A review and recent updates on Monitoring of Therapeutic Response of Cancer by Raman Spectroscopy and Applications

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Abstract: Cancer is the foremost cause of death around the globe, almost 10 million deaths in 2020, or appropriately one in six demises. Besides Almost on third of deaths from cancer are due to tobacco use, high body mass index, alcohol consumption, lack of physical activity. Almost all types of cancers can be cured effectively by detecting in cradle stage. Moreover, In Recent days there are huge number of detection techniques are there in the medical field like computed tomography (CT), magnetic resonance imaging (MRI) etc. Nevertheless, advancement in the Raman spectroscopic techniques is also playing a pivotal role in the monitoring and diagnostic purpose. Fundamentally this Raman spectroscopic techniques are showing other auspicious advantages in other backgrounds like polymorphic screening and molecular signature along with the pharmaceutical product's compositional analysis also when compare to all other equipment's. Notably this Raman spectroscopic techniques are very much helpful in the predominant issues like cancer which is world renowned disease.

Key words: Cancer, glioblastoma multiforme (GBM), Raman spectroscopic techniques, SORS, SERS, SOSERS, CLS, MRI, PET, CT, monitoring, detection, Nano particles,

Introduction: Dr. Chandrasekhara Venkata Raman discovered the scattering effect, which bears Raman's name and earned him the Nobel Prize in 1930. Raman spectroscopy gained popularity in the 1970s as lasers became more widely available to researchers, assuming the role of primary source of excitation light and thus replacing mercury-based sources (Ekins *et al* . 2007). Nonetheless, the difficulty of finely adjusting optical systems remained. Second, the sample's fluorescence significantly hampered the detection of Raman scattered photons. Excitation of Raman scattering with light of a visible wavelength may also excite fluorescence from the sample or impurities contained

therein. When the fluorescence intensity is high, the Raman signal is barely visible on top of the incomparably stronger, broad fluorescence signal. The third reason is sample decomposition and denaturation after prolonged exposure to relatively strong laser light due to inefficient detection of scattered radiation. Currently used techniques for imaging (or) identification of cancer:

- Computed tomography (CT)
- Magnetic resonance imaging (MRI)
- Positron emission tomography (PET)
- Ultrasound examination
- Endoscopic examination
- In mammography
- Isotopic diagnostics
- Needle biopsy

But they are associated with at least one of the following limitations: high cost, e.g., MRI and PET; involve the use of ionising radiation, e.g., CT and PET; or provide inadequate molecularly specific information e.g., CT, MRI and ultrasound(Adamson, Kanu *et al*. 2009). Nevertheless, Raman spectroscopy can offer high molecular specificity, rapid application as well as low to moderate cost. Raman spectroscopy is a non-invasive technique for obtaining specific chemical information from various types of samples and for determining the chemical composition of samples solely based on the inelastic scattering of light by molecules. It has also been used in ex vivo biopsies, in vitro biomarker detection, and in vivo analysis to distinguish between cancerous and non-cancerous samples. The detailed molecular information contained in Raman spectra allows for the monitoring of biochemical changes that occur in diseases such as cancer, and can be used for early detection and diagnosis, monitoring treatment, and distinguishing between cancerous and non-cancerous biological samples. The different Raman techniques containing their own advantages that can accommodate the alternative detection formats, allowing the techniques to be applied in several ways for the detection and diagnosis of cancer(Sloan-Dennison, Laing *et al*. 2021).

Principle of Raman spectroscopy: While infrared spectroscopy is based on light absorption, reflection, and emission, Raman spectroscopy is based on light scattering. Scattering occurs in this context due to collisions between photons and molecules (Docherty, Monaghan *et al*. 2004). When a molecule is exposed to light with the frequency v o, it emits a number of photons with the energy E =hv 0. (Fig.1)

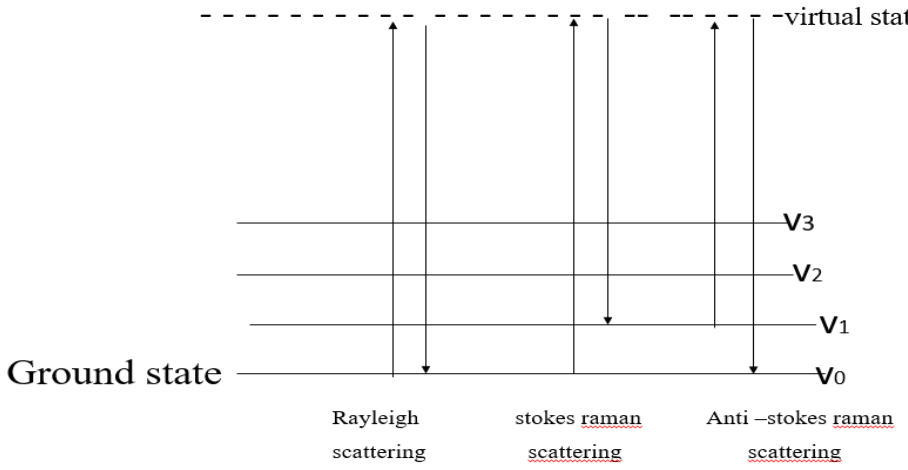

Figure 1: Jablonski diagram of energy transfer

Basic instrumentation of Raman spectroscopy: A Raman analyzer is made up of four basic components: lasers, an optical sampling system, a wavelength separator, and a detector.

Lasers: The most common lasers are NIR lasers with wavelengths of 1064 and 785 nm, and vis lasers with wavelengths ranging from 488 to 638 nm.

Optical sampling: The sampling system may be (containing the laser, spectrometer, and detection system) either by direct coupling or via fibre optics, the latter being particularly preferred for PAT manufacturing settings(Matthews, Jirasek *et al.* 2010).

Spectrometer: The main function of the spectrometer is to divide the wavelengths that comprise the polychromatic Raman signal and present them to the detector

Detector: In scattering spectrographs, the CCD (charge coupled device) detector is nearly universal. It is used because spectral data can be imaged onto the detector. This enables the design of instruments with a small number of moving parts(Matthews, 2012).

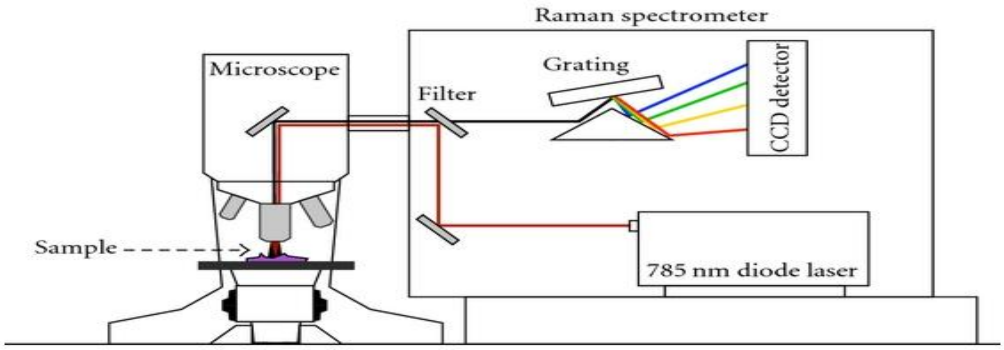

Figure 2: Raman spectroscopy instrument:

Techniques used for the detection of cancer:

When using Raman spectroscopy to monitor radiation treatment response in biological samples such as cells and tissues, specific considerations need to be addressed. The essential considerations addressed in this dissertation include the spectral range for Raman spectra acquisition, the spatial resolution requirements for the Raman microscopy instrument, the laser wavelength and power suitable for biological samples, and the substrates used to hold the biological samples. Raman spectroscopy has been utilised for clinical investigations due to it being non-destructive, non-invasive, and having the ability to monitor changes in molecular composition in a biological sample, which could be indicative of disease

1) Surface Enhanced Raman Spectroscopy (SERS)

2) Spatially Offset Raman Scattering (SORS)

3) Surface Enhanced Spatially Offset Raman Spectroscopy (SESORS)

1) Surface enhanced Raman spectroscopy for detection and monitoring of cancer:

SERS is an effective method for studying living intact cell cultures. A monochromatic laser source irradiates the sample and excites local molecular vibrations in the basic Raman micro spectroscopy setup. The bands in the spectra can be assigned to specific biomolecules and provide information about the cell's overall condition. Within minutes, differences in the chemical composition of the observed sample can be collected. The SERS analysis scheme is depicted. The primary goal of bringing the sample into close contact with signal-amplifying nanostructured materials is to obtain repeatable results (Grünfelder, Herbinger et al. 2022).

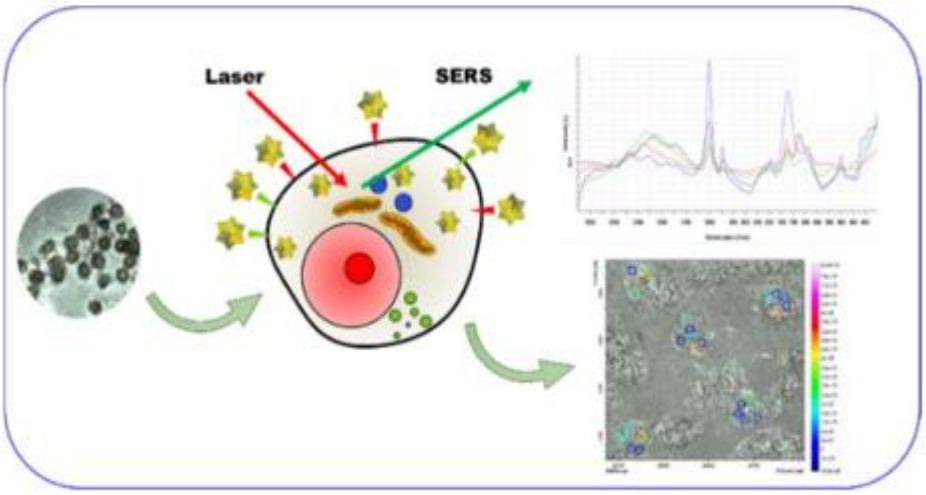

Figure 3: Scheme of SERS Analysis

Signal-enhancing colloidal metallic nanoparticles (NPs) can be prepared in a variety of shapes (stars, pyramids, shares), materials (silver or gold), and sizes (10 to 100). This variety is achieved by combining several reducing agents (e.g., ethanolamine, citrate, sucrose) and a metal salt. The reagent ratio is also important for preparation (Grünfelder, Herbinger et al. 2022).

Biomarker detection by using SERS analysis:

Cancer biomarkers detected in body fluids eliminate the need for more invasive procedures such as tissue biopsies. Furthermore, biomarker detection is more sensitive and specific than traditional morphological characterisation, potentially allowing early detection of cancer, increasing the PPV and thus improving patient prognosis. SERS has higher sensitivity than competing techniques for biomarker detection and can detect multiple biomarkers at the same time, allowing for more accurate cancer classification. Additionally, this method can be used to study biomolecular interactions, yielding significant information that may be useful in understanding cancer pathways. For example, the tumour suppressor protein, p53, plays a key role in many cancers and is regulated by mouse double minute (MDM2) protein. Therefore, understanding the interaction between these proteins could be invaluable in cancer therapeutics. MDM2 interactions were studied in solution using a nanoparticle assembly approach with SERS detection, allowing monitoring of the entire protein rather than just one binding interaction. To demonstrate the state of MDM2 in solution while maintaining the protein's biological activity, a p53-mimicking peptide was used. The group has also investigated SERS-based sandwich assays that use capture antibodies bound to a surface and detection antibodies functionalized to a SERS nanotag to detect low concentrations of clinically relevant biomolecules. This format has been used to detect cancer biomarkers such as MUC4 expressed in pancreatic cancer and the multiplexed detection of breast and prostate cancer biomarkers (Robson, Hupp *et al.* 2012).

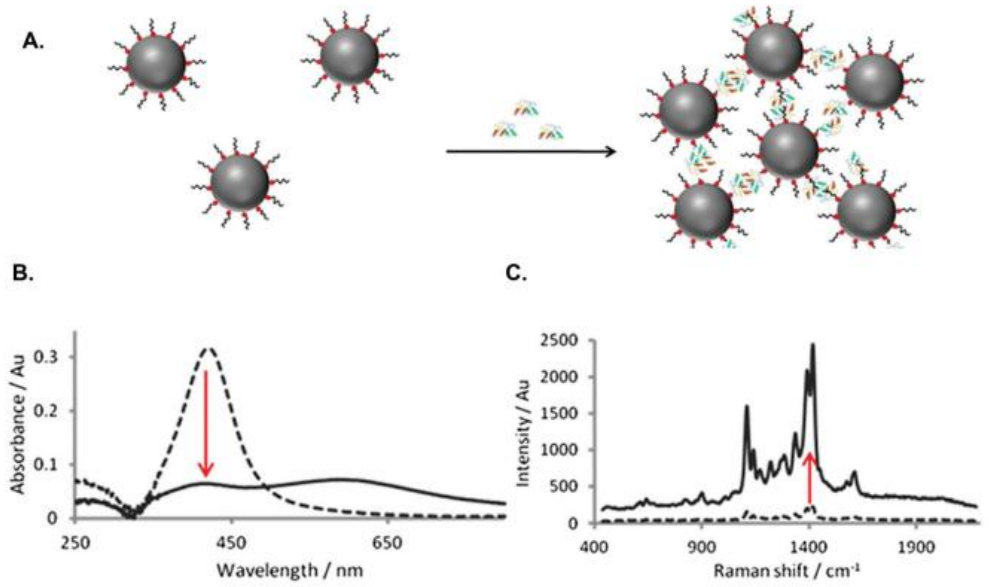

Figure 4: (A) schematic of assay for the MDM2 detection showing specific interactions between MDM2 and peptide on silver nanoparticle surface, resulting in nanoparticle assembly. (B) change in extension after nanoparticle assembly due to aggregation of particle

Table 1: SERS optimised parameters:

Parameter	**Optimised value**
Nano particles	Gold / ascorbic acid and sucrose reduced
Laser wavelength	785nm
Resolution	3-5 $^{cm-1}$
Integration time	4 seconds
Co-additions	20

2) Spatially offset Raman spectroscopy (SORS): Breast conserving therapy, for example, is a viable treatment option in the case of breast cancer (BCT). The surgical component of BCT consists of a partial mastectomy, also known as a lumpectomy, to remove only the primary lesion and a small amount of surrounding normal tissue(Been ken, WangerJr et al. 2004). Positive margins are a major predictor of local tumour recurrence, so a second operation is sometimes required. Existing intraoperative margin evaluation tools, such as simple visual examination, ultrasound, cytological examination ("touch prep"), and frozen section analysis, all have significant limitations in terms of accuracy and/or time required, indicating the need for an automated, real-time method to accurately evaluate surgical margins during BCT. This technique has the ability to detect spectral contributions from breast tumours buried under 0.5 to 2 mm of normal breast tissue(Mahadevan-Jansen 2011). In addition, a SORS Monte Carlo (MC) code was developed to quantify signals obtained from layered constructs of normal breast tissue overlying breast tumours Spatially offset Raman spectroscopy (SORS) to enable non-invasive probing of living tissue through depths of up to 5 cm(Wilke, Brown *et al*. 2009) . Common SORS excitation wavelengths used are 785 nm, 808 nm and 830 nm; these are used with the intention of generating a spectrum within the sensitive range of silicon-based CCDs, which extends up to around 1,100nm. The advent of ‘‘Spatially Offset Raman

Spectroscopy'' (SORS) and the development of its variants, now permits Raman imaging at up to an order of two magnitudes deeper than previously possible using conventional Raman techniques.

SORS Variants: There are two types of variants are there.

- Point -like SORS
- Ring – collection SORS

SORS technique for compositional identification of liquid explosives:

Raman spectroscopic method for the non-invasive detection of liquid explosives within bottles, and other packaging, of substantially higher sensitivity and wider applicability than that currently available via conventional Raman spectroscopy. The approach uses a modification of the spatially offset Raman spectroscopy (SORS) concept, which permits the interrogation of a wide range of containers, including transparent, coloured, and diffusely scattering plastic and glass beverage, medicine, and cosmetic bottles, with no change in experimental geometry. The enhanced sensitivity is achieved by the technique's inherent ability to effectively suppress fluorescence and Raman contributions originating from the wall of the container. The application is demonstrated on the non-invasive detection of hydrogen peroxide solution, a critical component of a number of liquid explosives. In contrast to conventional Raman spectroscopy, the modified SORS concept enables the detection of concealed hydrogen peroxide.

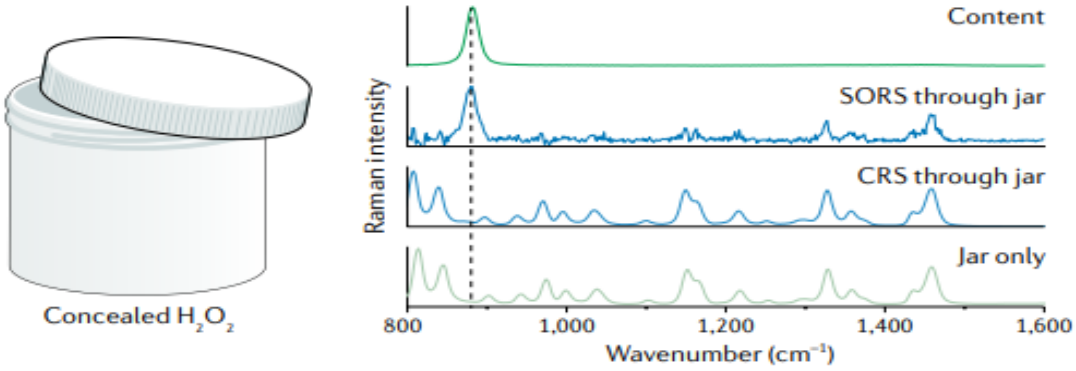

Figure 5: Analysing the content of plastic jar

Spectra were collected using conventional Raman spectroscopy (CRS) and spatially offset Raman spectroscopy (SORS) on a white plastic 1.2mm thick polypropylene jar filled with a 30% H2O2 in water solution. The conventional Raman spectrum of the H2O2 -containing jar (CRS through jar) is essentially identical to a reference Raman spectrum of an empty jar (Jar only), with no observable Raman signature of H2O2 (dashed line).The SORS spectrum of the H2O2-containing jar (SORS via jar) has a significant Raman component that matches the reference spectrum of the aqueous H2O2 solution (Content). Raman bands are caused by imperfect subtraction of container Raman signal, it self-caused by self-absorption of the spatially offset signal propagating through the probed object (Eliasson, Macleod et al. 2007).

3. Surface enhanced spatial offset Raman spectroscopy (SESORS):

Use of SESORS In – *vivo* analysis: The combination of SERS and spatially offset Raman spectroscopy (SORS) techniques, referred to as SESORS. Multiplexed surface enhanced Raman scattering (SERS) signals were recovered noninvasively from tissues at a depth of 20 mm and reconstructed to produce a false colour image for the first time. Until recently, it was figured that signals from these NPs could only be measured at maximum depths of around 5 mm (Stone, Kerssens *et al.* 2011).

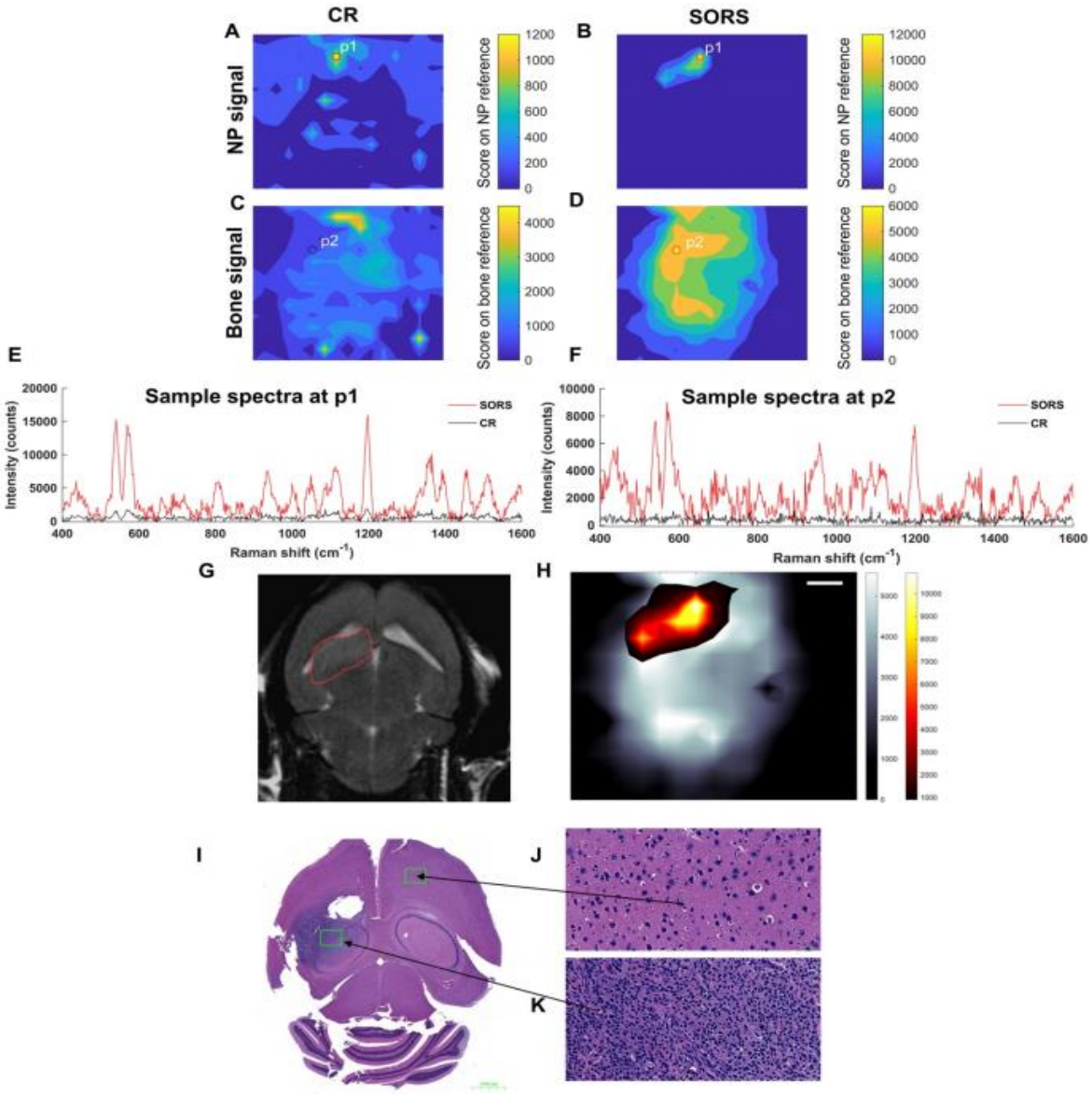

Figure 6: Detection of glioblastoma multiforme (GBM) using SESORS method:(A)detection and CLS contribution of SERRS NPs through skull by CR imaging (B) SORS approach (C) detection and contribution of CLS of bone through skull by CR imaging technique (D) SORS approach (E) A spectrum collected at the point of maximum intensity (P1) that replaces to the greatest CLS contribution from SERRS NPs using SORS (RED) and CR (black) respectively (F) a spectrum collected at the point of maximum intensity (P2) that replaces to the greatest CLS contribution from skull using by CR imaging (G) 2D axial T2 – weighed MRI taken 4 weeks post injection of DF-1 cells conforms that presence left frontal tumour (H) SORS heatmap showing that superimposed on to SORS bone heat map (I) H and E strained 5 micron section of brain (J) arrow represent area of slide normal tissue (k) arrow represents area of slide cancerous tissue

Summary table of various imaging techniques of cancer:

Name of the Technique	Benefits	Drawbacks
Conventional techniques like CT, MRI, PET.	MRI will provide better soft tissue contrast and no exposure of ionising radiation and CT will provide better imaging with bones.	MRI technique little bit expensive, produce loud noise, difficult for claustrophobia persons.
Surface enhanced Raman spectroscopy (SERS)	Excessive collection of signals and enables greater laser power used. imaging is the multiplexing potential achieved by bio conjugating SERS nanotags.	Cannot generate Raman signal from depth, SERS effect of other metals not ideal.
Spatial offset Raman spectroscopy (SORS)	It enables non-invasive probing of living tissue through depths of up to 5 cm, accuracy of measurement.	Due to using a near-infrared laser excessively high laser intensities can induce undesirable sample damage
Surface enhanced spatially offset Raman spectroscopy (SESORS)	Exceptional sensitivity, high accessible depth (up to 50mm) and more ever chemical specificity.	Requires nanoparticles or SERS substrate inside sample.

Numerous other applications of Raman spectroscopy other than cancer monitoring:

1.Raman spectroscopy used for finger printing of compounds (molecular signature).

2.It was widely used in solid state analysis of compounds like crystalline or non-crystalline (polymorphic screening).

3.It was also used to analyse the content (or) composition of the pharmaceutical substances.

4. This technique not only applicable in pharmaceutical field but also in the food science for to identify counterfeit products(Ghita, Matousek *et al*. 2018).

5.Nevertheless it was used for cancer diagnosis it also used for diagnosis of dementia.

6.Besides it was also useful in bacterial identification, and plant disease diagnostic purpose.

7.This spectroscopy mainly reaction monitoring, chromatographic detection, environmental monitoring, and materials chemistry(Nicolson, Andreiuk *et al*. 2019)

Conclusion:

The suitability of conventional SORS and transmission Raman for non-invasive disease analysis has advanced to an extremely exciting level in recent years. These Phenomenal techniques are using for ex vivo analysis, in vivo tumour detection, and invitro biomarker detection. The translation of SESORS into the clinical setting is highly dependent on regulatory bodies such as the Food and Drug Administration (FDA) approving SE(R)RS NPs; I am believing that advances in SESO(R)RS imaging will aid in achieving such approval. Furthermore, I am anticipating a greater shift in the field toward the use of SORS over conventional Raman for preclinical and clinical imaging applications. This will be greatly helped by advancement as well as modernisation in Raman instrumentation and, in the case of SESO(R)RS, the creation of brighter and safer SER(R)S NPs. This Raman spectroscopy not only in case of medical diagnosis, treatment but also it will make technical revolution in case of the analysis of historical species and forensic, food science sectors, and the further development of SORS instrument components are likely to lead to further downsizing of Raman spectrometer technology that can be implemented in mobile phones or even smaller devices in future generations.

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